CHEMICAL DERIVATIZATIONS OF MAJOR CONSTITUENT OF CUMIN (*Cuminum cyminum*) AND THEIR BIOLOGICAL ACTIVITY

Dissertation

Submitted to the Punjab Agricultural University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY
in
CHEMISTRY
(Minor Subject: Physics)

By

Pardeep Kaur
(L-2016-BS-82-D)

Department of Chemistry
College of Basic Sciences and Humanities
© PUNJAB AGRICULTURAL UNIVERSITY
LUDHIANA - 141 004

2021

CERTIFICATE I

This is to certify that the dissertation entitled, **“Chemical derivatizations of major constituent of cumin *(Cuminum cyminum*) and their biological activity”** submitted for the degree of Ph.D., in the subject of **Chemistry** (Minor subject: **Physics**) to the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Ms. Pardeep Kaur (L-2016-BS-82-D)** under my supervision and that no part of this dissertation has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

(Dr. (Mrs.) Sunita Sharma)
Major Advisor
Principal Analyst (Cereals and Pulses)
Department of Plant Breeding and Genetics
Punjab Agricultural University
Ludhiana -141004

CERTIFICATE II

This is to certify that the dissertation entitled, **"Chemical derivatizations of major constituent of cumin *(Cuminum cyminum*) and their biological activity"** submitted by **Ms. Pardeep Kaur** (**L-2016-BS-82-D**) to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of **Ph.D.** in the subject of **Chemistry** (Minor subject: **Physics**) has been approved by the Student's Advisory Committee along with the Head of the Department after an oral examination of the same in collaboration with External Examiner.

5/3/2021

(Dr. (Mrs.) Sunita Sharma)
Major Advisor

(Dr. Sudhir Kumar)
External Examiner
Professor
Department of Mol. Biology, Biotechnology and Bioinformatics
CSS Haryana Agricultural University
Hisar

(Dr. (Mrs.) Manpreet Kaur)
Head of the Department

(Dr. (Mrs.) Gurinder Kaur Sangha)
Dean, Postgraduate Studies

ACKNOWLEDGEMENTS

First and foremost, I bow my head to 'God' who gave me courage, patience and strength to fulfill this work and brought a change in my life.

With great pleasure, I extend my deep sense of gratitude, obligation and sincere thanks to my esteemed Major Advisor, ***Dr. (Mrs.) Sunita Sharma****, Principal Analyst (Cereal and Pulses), Department of Plant Breeding and Genetics, PAU for her keen interest, unceasing encouragement, guidance, patience and ever available help, which brought the work to a successful completion.*

I am thankful to other members of my advisory committee ***Dr. (Mrs.) Anjali****, Nanotechnologist (Chemistry), Department of Soil Science,* ***Dr. Paramjit Singh****, Professor of Physics, Department of Maths, Statistics and Physics,* ***Dr. (Mrs.) Poonam Sharma,*** *Principal Microbiologist (Pulses), Department of Plant Breeding and Genetics and* ***Dr. (Mrs.) Manpreet Kaur****, Assistant Professor (Dean PGS Nominee), Department of Chemistry for extending their suggestions and reviews in the work.*

I find no words, to complementary to acknowledge in so formal manner the selfless sacrifice, unending patient, encouraging, moral support, blessings and affection rendered by my parents ***S. Palwinder Singh*** *and* ***Smt. Ranjit Kaur****. I can never repay the ever-willing and whole hearted constant support and guidance of my dear brother* ***Harjit Singh*** *and sisters* ***Harpreet Kaur*** *and* ***Ramandeep Kaur*** *who always kept me in high spirits and for making the atmosphere friendly and motivating always.*

I would be inappropriate if I omit to mention the names of my seniors ***Jyoti Gaba*** *and* ***Sukesha Joshi****, juniors* ***Tanvi Sahni, Diksha Verma, Ravneet Singh, Gurinderjit Kaur*** *and* ***Kirandeep Kaur****. I give my special thanks to my dear friends* ***Nikki, Prachi, Jasmeen, Manjit, Dechen, Padma, Dolma, Kunzang, Narmada.***

I owe my special thanks to ***Mr. Makhan Singh, Mr. Naresh Kumar, Mrs. Sunita, Mrs. Harjinder Kaur*** *and* ***Mr. Manjit*** *for providing all possible help during the research work.*

I feel proud to be a part of PAU, Ludhiana where I learnt a lot and spent unforgettable moments of my life. I also wish to thanks CIL and SAIF, Panjab University for providing FT-IR and ^{1}H NMR spectral data.

Last but not the least, I wish to offer my thanks to all those who love and care for me. Every name may not be mentioned but none is forgotten.

Dated:

Place: Ludhiana ***(Pardeep Kaur)***

Title of Dissertation	:	Chemical derivatizations of major constituent of cumin *(Cuminum cyminum)* and their biological activity
Name of the Student and Admission No.	:	Pardeep Kaur (L-2016-BS-82-D)
Major Subject	:	Chemistry
Minor Subject	:	Physics
Name and Designation of Major Advisor	:	Dr. (Mrs.) Sunita Sharma Principal Analyst (Cereals and Pulses)
Degree to be Awarded	:	Ph.D.
Year of award of Degree	:	2021
Total Pages in Dissertation	:	181 + Annexures (xxix) + VITA
Name of University	:	Punjab Agricultural University, Ludhiana – 141 004, Punjab, India

ABSTRACT

Cuminum cyminum is an annual herbaceous plant belonging to the family Apiaceae genus *Cuminum.* In the present study, chemical composition of essential oil and different solvent extracts of cumin of variety GC-4 was determined by GC-MS analysis. Cuminaldehyde was found to be the major constituent present in the essential oil and second major compound present in the different solvent extracts (except chloroform). The proximate analysis of cumin seeds showed that it contains 8.58±0.20, total minerals 8.62±0.05, crude protein 14.43±0.02, crude fibre 11.06±0.01, fats 15.00±0.04, total sugars 2.30±0.21 and total carbohydrates 40.01±0.06. Elemental investigation of cumin seeds showed that it contains micronutrients like Iron: 181.33, copper: 14.25, manganese: 25.75, zinc: 31.25, chromium: 10.15, nickel: 2.38, cobalt: 2.05, cadmium: 2.03, lead: 58.75, arsenic: 118.50 in parts per million concentrations. Phytochemical study revealed that various phytochemicals *viz* alkaloids, saponins, tannins, coumarins, amino acids, flavonoids, proteins, phenols and carbohydrates were present in different solvent extracts. Aqueous extract of cumin seeds was used as a bio-reductant for the synthesis of silver nanoparticles. Different derivatives *i.e.* Schiff Bases, hydrazones, thiosemicarbazones, chalcones and pyrazolines of major constituent of essential oil namely cuminaldehyde (isolated from essential oil by column chromatography) were synthesized. Characterization of synthesized compounds was done using UV-visible, FT-IR, ^{1}H and ^{13}C NMR. Essential oil, different solvent extracts, silver nanoparticles, cuminaldehyde and its derivatives were screened for their antioxidant (*via* DPPH assay), microbial (against *Pseudomonas* sp., *Klebsiella* sp. and *Enterobacter* sp.) and fungicidal potential (against *Fusarium wilt*, *Ascochyta blight* and *Bortrytis gray mould*. Cuminaldehyde derivatives were found more effective as compared to cumin essential oil, its major constituent, different solvent extracts of cumin seeds and silver nanoparticles synthesized from aqueous cumin seed extract.

Keywords: *Cuminum cymminum*, Phytochemicals, Proximate composition, GC-MS, Cuminaldehyde, Antioxidant, Antimicrobial, Fungicidal activity

Signature of Major Advisor **Signature of the Student**

ਖੋਜ ਗ੍ਰੰਥ ਦਾ ਸਿਰਲੇਖ	:	ਜ਼ੀਰੇ (*ਕੁਮੀਨਮ ਸਿਮੀਨਮ*) ਦੇ ਪ੍ਰਮੁੱਖ ਸੰਘਟਕਾਂ ਦੀ ਰਸਾਇਣਕ ਡੇਰੀਵੀਟਾਈਜੇਸ਼ਨ ਅਤੇ ਉਨ੍ਹਾਂ ਦੀਆਂ ਜੈਵਿਕ ਗਤੀਵਿਧੀਆਂ।
ਵਿਦਿਆਰਥੀ ਦਾ ਨਾਂ ਅਤੇ ਦਾਖਲਾ ਨੰਬਰ	:	**ਪਰਦੀਪ ਕੌਰ** **(ਐਲ-2016-ਬੀ.ਐਸ.-82-ਡੀ)**
ਪ੍ਰਮੁੱਖ ਵਿਸ਼ਾ	:	ਰਸਾਇਣ ਵਿਗਿਆਨ
ਸਹਿਯੋਗੀ ਵਿਸ਼ਾ	:	ਭੌਤਿਕੀ ਰਸਾਇਣ
ਮੁੱਖ ਸਲਾਹਕਾਰ ਦਾ ਨਾਂ ਅਤੇ ਅਹੁਦਾ	:	**ਡਾ: (ਮਿਸਿਜ) ਸੁਨੀਤਾ ਸ਼ਰਮਾ** **ਪ੍ਰਿੰਸੀਪਲ ਐਨਾਲਿਸਟ (ਸਿਰੀਅਲ ਅਤੇ ਪਲਸਿਸ)**
ਡਿਗਰੀ	:	**ਪੀ.ਐਚ.ਡੀ.**
ਡਿਗਰੀ ਮਿਲਣ ਦਾ ਸਾਲ	:	2021
ਖੋਜ ਪੱਤਰ ਵਿੱਚ ਕੁੱਲ ਪੰਨੇ	:	181 + ਅੰਤਿਕਾਵਾਂ (**xxix**)+ ਵੀਟਾ
ਯੂਨੀਵਰਸਿਟੀ ਦਾ ਨਾਮ	:	ਪੰਜਾਬ ਖੇਤੀਬਾੜੀ ਯੂਨੀਵਰਸਿਟੀ, ਲੁਧਿਆਣਾ-141 004 ਪੰਜਾਬ, ਭਾਰਤ ।

ਸ਼ਾਰ ਅੰਸ਼

ਕੁਮੀਨਮ ਸਿਮੀਨਮ ਇੱਕ ਸਾਲਾਨਾ ਜੜ੍ਹੀ ਬੂਟੀ ਹੈ ਜੋ ਅਪੇਸੀਐਸੀ ਪਰਿਵਾਰ ਦੇ ਜੀਨਸ ਕੁਮੀਨਮ ਨਾਲ ਸੰਬੰਧਤ ਹੈ। ਮੌਜੂਦਾ ਅਧਿਐਨ ਵਿੱਚ, ਜੀ ਸੀ4 ਕਿਸਮ ਦੇ ਜ਼ੀਰੇ ਦੇ ਜ਼ਰੂਰੀ ਤੇਲਾਂ ਅਤੇ ਵੱਖਰੇ ਸੌਲਵੈਂਟ ਐਕਸਟ੍ਰੇਕਾਂ ਦੀ ਰਸਾਇਣਕ ਰਚਨਾ ਨੂੰ ਜੀ ਸੀ ਐੱਮ ਐੱਸ ਵਿਸ਼ਲੇਸ਼ਣ ਦੁਆਰਾ ਨਿਰਧਾਰਤ ਕੀਤਾ ਗਿਆ ਸੀ। ਕੁਮੀਨਮੈਲਡੀਹਾਈਡ ਜ਼ਰੂਰੀ ਤੇਲਾਂ ਵਿੱਚ ਪ੍ਰਮੁੱਖ ਸੰਘਟਕ ਪਾਇਆ ਗਿਆ ਅਤੇ ਵੱਖਰੇ ਸੌਲਵੈਂਟ ਐਕਸਟ੍ਰੇਕਾਂ ਵਿੱਚ ਦੂਜਾ ਪ੍ਰਮੁੱਖ ਮਿਸ਼ਰਣ (ਕਲੋਰੋਫਾਰਮ ਨੂੰ ਛੱਡ ਕੇ) ਸੀ। ਜ਼ੀਰੇ ਦੇ ਬੀਜ਼ਾਂ ਦੇ ਅਨੁਮਾਨਿਤ ਵਿਸ਼ਲੇਸ਼ਣ ਤੋਂ ਪਤਾ ਚੱਲਿਆ ਕਿ ਇਸ ਵਿੱਚ 8.58±0.20, ਕੁੱਲ ਖਣਿਜ 8.62±0.05, ਕੱਚਾ ਪ੍ਰੋਟੀਨ 14.43±0.02, ਕੱਚਾ ਫਾਈਬਰ 11.06±0.01, ਚਰਬੀ 15.00±0.04, ਕੁੱਲ ਸ਼ੂਗਰ 2.30±0.21 ਅਤੇ ਕੁੱਲ ਕਾਰਬੋਹਾਈਡ੍ਰੇਟ 40.01±0.06 ਹਨ। ਜ਼ੀਰੇ ਦੇ ਬੀਜ਼ਾਂ ਦੀ ਮੁੱਢਲੀ ਜਾਂਚ ਤੋਂ ਪਤਾ ਚੱਲਿਆ ਕਿ ਇਸ ਵਿੱਚ ਲੋਹਾ 181.33, ਤਾਂਬਾ 14.25, ਮੈਂਗਨੀਜ਼ 25.75, ਜ਼ਿੰਕ 31.25, ਕਰੋਮੀਅਮ 10.15, ਨਿੱਕਲ 2.38, ਕੋਬਾਲਟ 2.08, ਕੈਡਮੀਅਮ 2.03, ਲੈੱਡ 58.75, ਆਰਸੈਨਿਕ 118.50, ਵਰਗੇ ਸੂਖਮ ਤੱਤ ਪ੍ਰਤੀ ਮਿਲੀਅਨ ਇਕਾਗਰਤਾ ਦੇ ਹਿੱਸਿਆਂ ਵਿੱਚ ਸ਼ਾਮਲ ਸਨ। ਫਾਈਟੋ-ਕੈਮੀਕਲ ਅਧਿਐਨ ਨੇ ਖੁਲਾਸਾ ਕੀਤਾ ਕਿ ਵੱਖਰੇ ਸੌਲਵੈਂਟ ਐਕਸਟ੍ਰੇਕਾਂ ਵਿੱਚ ਵੱਖੋ-ਵੱਖਰੇ ਫਾਈਟੋ-ਕੈਮੀਕਲ ਜਿਵੇਂ ਕਿ ਐਲਕਲਾਈਡ, ਸੈਪੋਨੀਨ, ਟੈਨਿਨ, ਕੋਮਰਿਨ, ਐਮੀਨੋ ਐਸਿਡ, ਫਲੇਵੇਨੋਈਡ, ਪ੍ਰੋਟੀਨ, ਫੀਨੋਲ ਅਤੇ ਕਾਰਬੋਹਾਈਡ੍ਰੇਟ ਮੌਜੂਦ ਸਨ। ਜ਼ੀਰੇ ਦੇ ਬੀਜ਼ਾਂ ਦੇ ਐਕੁਅਸ ਐਬਸਟ੍ਰੇਕਟ ਸਿਲਵਰ ਦੇ ਨੈਨੋ ਪਾਰਟੀਕਲਾਂ ਦੇ ਸੰਸਲੇਸ਼ਣ ਲਈ ਜੈਵਿਕ-ਰੀਡਕਟੈਂਟ ਵਜੋਂ ਵਰਤੇ ਜਾਂਦੇ ਹਨ। ਅਲੱਗ-ਅਲੱਗ ਡੈਰੀਵੇਟਿਵ ਅਰਥਾਤ ਸਕਪਿ ਬੇਸ, ਹਾਈਡ੍ਰਾਜੋਨ, ਥਾਈਓਸੈਮੀਕਾਰਬਾਜੋਨ, ਚਲਕੋਨ ਅਤੇ ਪਾਈਰਾਜੋਲਾਈਨ ਜ਼ਰੂਰੀ ਤੇਲਾਂ ਦੇ ਪ੍ਰਮੁੱਖ ਹਿੱਸੇ ਅਰਥਾਤ ਕੁਮੀਨਮੈਲਡੀਹਾਈਡ (ਕਾਲਮ ਕ੍ਰੋਮੈਟੋਗ੍ਰਾਫੀ ਦੁਆਰਾ ਜ਼ਰੂਰੀ ਤੇਲਾਂ ਤੋਂ ਅਲੱਗ ਕੀਤੇ) ਦਾ ਸ਼ੰਸਲੇਸ਼ਣ ਕੀਤਾ ਗਿਆ। ਸ਼ੰਸਲੇਸਿਤ ਕੀਤੇ ਮਿਸ਼ਰਣਾਂ ਦਾ ਗੁਣਾਂਕ ਯੂ ਵੀ ਵੀਸੀਵਲ, ਐਫ ਟੀ ਆਈ ਆਰ, 1 ਐੱਚ ਅਤੇ 13 ਸੀ ਐੱਨ ਐੱਮ ਆਰ ਦੀ ਵਰਤੋਂ ਕਰਕੇ ਕੀਤਾ ਗਿਆ। ਜ਼ਰੂਰੀ ਤੇਲਾਂ ਵੱਖਰੇ ਸੌਲਵੈਂਟ ਐਕਸਟ੍ਰੇਕਾਂ ਸਿਲਵਰ ਦੇ ਨੈਨੋ ਪਾਰਟੀਕਲਾਂ ਕੁਮੀਨਮੈਲਡੀਹਾਈਡ ਅਤੇ ਇਸਦੇ ਡੈਰੀਵੇਟਿਵਾਂ ਨੂੰ ਉਨ੍ਹਾਂ ਦੇ ਐਂਟੀਆਕਸੀਡੈਂਟ (ਡੀ ਪੀ ਪੀ ਐੱਚ ਦੁਆਰਾ), ਮਾਈਕ੍ਰੋਬੀਅਲ (ਸੂਡੋਮੋਨਸ ਐੱਸ.ਪੀ., ਕਲੇਬੀਸੀਲਾ ਐੱਸ.ਪੀ. ਅਤੇ ਐਂਟਰੋਬੈਕਟਰ ਐੱਸ.ਪੀ. ਵਿਰੁੱਧ) ਅਤੇ ਉੱਲੀਨਾਸ਼ਕ ਸਮਰੱਥਾ (ਫੁਸੇਰਿਅਮ ਵਿਲਟ, ਐਸਕੋਕਾਈਟਾ ਬਲਾਇਟ ਅਤੇ ਬੋਟ੍ਰਾਈਟਸ ਗ੍ਰੇ ਮੋਲਡ ਵਿਰੁੱਧ) ਪਰਖਿਆ ਗਿਆ। ਜ਼ੀਰੇ ਦੇ ਜ਼ਰੂਰੀ ਤੇਲਾਂ, ਇਸਦੇ ਪ੍ਰਮੁੱਖ ਹਿੱਸੇਦਾਰਾਂ, ਜ਼ੀਰੇ ਦੇ ਬੀਜ਼ਾਂ ਦੇ ਵੱਖਰੇ ਸੌਲਵੈਂਟ ਐਕਸਟ੍ਰੇਕਾਂ ਅਤੇ ਜ਼ੀਰੇ ਦੇ ਬੀਜ਼ਾਂ ਦੇ ਐਕੁਅਸ ਐਬਸਟ੍ਰੇਕਟ ਤੋਂ ਸੰਸਲੇਸ਼ਿਤ ਸਿਲਵਰ ਦੇ ਨੈਨੋ ਪਾਰਟੀਕਲਾਂ ਦੇ ਮੁਕਾਬਲੇ ਕੁਮੀਨਮੈਲਡੀਹਾਈਡ ਡੈਰੀਵੇਟਿਵ ਵਧੇਰੇ ਪ੍ਰਭਾਂਵਸ਼ਾਲੀ ਪਾਏ ਗਏ ।

ਮੁੱਖ ਸ਼ਬਦ: *ਕੁਮੀਨਮ ਸਿਮੀਨਮ*, ਜੀ ਸੀ ਐੱਮ ਐੱਸ, ਕੁਮੀਨਮੈਲਡੀਹਾਈਡ, ਅਨੁਮਾਨਿਤ ਵਿਸ਼ਲੇਸ਼ਣ, ਐਂਟੀਆਕਸੀਡੈਂਟ, ਮਾਈਕ੍ਰੋਬੀਅਲ, ਉੱਲੀਨਾਸ਼ਕ ਸਮਰੱਥਾ

(ਮੁੱਖ ਸਲਾਹਕਾਰ ਦੇ ਹਸਤਾਖਰ) **(ਵਿਦਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ)**

CONTENTS

CHAPTER	TOPIC	PAGE NO.
I	INTRODUCTION	1-5
II	REVIEW OF LITERATURE	6-28
III	MATERIAL AND METHODS	29-61
IV	RESULTS AND DISCUSSION	62-162
V	SUMMARY	163-166
	REFERENCES	161-181
	ANNEXURES	xxix
	VITA	

LIST OF TABLES

Table No.	Title	Page No.
1.	Physical properties of essential oil of cumin seeds	44
2.	Different fractions of essential oil after Column Chromatography	46
3.	Physical properties of Schiff bases (II-XII), thiosemicarbazones (XIII & XIV) and hydrazones (XV-XVIII) of cuminaldehyde	56
4.	Physical properties of chalcones (XIX-XXIV) and pyrazolines (XXV-XXXVI) of cuminaldehyde	57
5.	Proximate composition of cumin seeds	62
6.	Elemental investigation of cumin seeds	63
7.	Qualitative phytochemical analysis of cumin seeds in different solvent extracts	64
8.	Quantitative phytochemical analysis of cumin seeds in different solvent extracts	66
9.	GC-MS analysis of different solvent extracts of cumin seeds	67-68
10.	Chemical composition of essential oil of cumin seeds determined by GC-MS analysis	70
11.	Optical properties of AgNPs (nm) from cumin seed extract at different concentrations	109
12.	DPPH radical scavenging activity of cumin oil and cuminaldehyde along with standard ascorbic acid at different concentrations	111
13.	DPPH radical scavenging activity of different solvent extracts of cumin seeds along with standard ascorbic acid at different concentrations	114
14.	DPPH radical scavenging activity of synthesized Schiff bases, thiosemicarbazones and hydrazones	116
15.	DPPH radical scavenging activity of synthesized chalcones (XIX-XXIV) and pyrazolines (XXV-XXXVI)	119
16	Antioxidant activity of silver nanoparticles from aqueous extract of cumin	121
17.	Microbial activity of cumin oil and cuminaldehyde along with standard ampicillin on the growth of *Pseudomonas sp*. at different concentrations	122
18.	Microbial activity of cumin oil and cuminaldehyde along with standard ampicillin on the growth of *Klebsiella sp*. at different concentrations (µg mL^{-1})	123
19.	Microbial activity of cumin oil and cuminaldehyde along with standard ampicillin on the growth of *Enterobacter sp*. at different concentrations (µg mL^{-1})	124

Table No.	Title	Page No.
20.	Microbial activity of different solvent extracts of cumin seeds on the growth of *Pseudomonas sp*., at different concentrations	125
21.	Microbial activity of different solvent extracts of cumin seeds on the growth of *Klebsiella sp*. at different concentrations	126
22.	Microbial activity of different solvent extracts of cumin seeds on the growth of *Klebsiella sp*., at different concentrations	126
23.	Microbial activity of Schiff bases, thiosemicarbazones and hydrazones on the growth *Pseudomonas* sp.	129
24.	Microbial activity of Schiff bases, thiosemicarbazones and hydrazones on the growth of *Klebsiella sp.*	130
25.	Microbial activity of Schiff bases, thiosemicarbazones and hydrazones on the growth of *Enterobacter sp.*	131
26.	Microbial activity of chalcones and pyrazolines on the growth *Pseudomonas* sp.	136
27.	Microbial activity of chalcones and pyrazolines on the growth *Klebsiella* sp.	137
28.	Microbial activity of chalcones and pyrazolines on the growth *Enterobacter* sp.	138
29.	Microbial activity of AgNPs from aqueous extract of seed on the growth of *Pseudomonas* sp. at different concentrations (mg/ml)	141
30.	Microbial activity of AgNPs from aqueous extract of leaf on the growth of *Klebsiella* sp. at different concentrations (mg/ml)	142
31.	Microbial activity of AgNPs from aqueous extract of seed on the growth of *Enterobacter* sp. at different concentrations (mg/ml)	142
32.	Minimum Inhibitory Concentration (MIC) of AgNPs from seed extract on the growth different bacteria's	143
33.	Per cent inhibition of cumin oil and cuminaldehyde against *Fusarium wilt* at different concentrations (µg ml^{-1})	144
34.	Per cent inhibition of cumin essential oil and cuminaldehyde against *Bortrytis gray mould*	145
35.	Per cent inhibition of different solvent extracts against *Fusarium wilt* at different concentrations (µg ml^{-1})	147
36.	Per cent inhibition of different solvent extracts against *Bortrytis gray mould* at different concentrations (µg ml^{-1})	149
37.	Per cent inhibition of synthesized Schiff bases (II-XII), thiosemicarbazones (XIII & XIV) and hydrazones (XV-XVIII) against *F. wilt* at different concentrations (µg ml^{-1})	152

Table No.	Title	Page No.
38.	Per cent inhibition of synthesized Schiff bases (II-XII), thiosemicarbazones (XIII & XIV) and hydrazones (XV-XVIII) against *Bortrytis gray mould*	154
39.	Per cent inhibition of synthesized chalcones (XIX-XXIV) and pyrazolines (XXV-XXXVI) against *F. wilt* at different concentrations (μg ml^{-1})	157
40.	Per cent inhibition of synthesized chalcones (XIX-XXIV) and pyrazolines (XXV-XXXVI) against *Bortrytis gray mould*	159
41	Per cent inhibition of silver nanoparticles of cumin seeds against *Fusarium wilt* at different concentrations (mg ml^{-1})	161
42	Per cent inhibition of silver nanoparticles of cumin seeds against *Bortrytis gray mould* at different concentrations (mg ml^{-1})	161

LIST OF FIGURES

Figure No.	Title	Page No.
1.	Standard curve plotted between concentration of gallic acid and absorbance	40
2.	Standard curve plotted between concentration of quercetin and absorbance	41
3.	Standard curve plotted between concentration of saponin and absorbance	42
4.	Standard curve plotted between concentration of tannic acid and absorbance	43
5.	Per cent yield of various solvent extracts	64
6.	Composition (%) of essential oil of cumin seeds as per GC-MS analysis	71
7.	Comparison of %RSA of cumin oil and cuminaldehyde	112
8.	Comparison of IC_{50} of tested compounds	112
9.	Per cent RSA of methanolic and aqueous extrat	113
10.	Comparison of IC_{50} of methanol and aqueous extract with standard ascorbic acid	115
11.	Comparison of IC_{50} of Schiff Bases, thiosemicarbazones and hydrazones with ascorbic acid	117
12.	Comparison of IC_{50} of chalcones and pyrazolines with standard ascorbic acid	120
13.	MIC (Minimum inhibition concentration) of cumin oil and cuminaldehyde along with ampicillin against *Pseudomonas sp*., *Klebsiella sp*., *Enterobacter sp*.	124
14.	MIC (Minimum inhibition concentration) of different solvent extracts of cumin seeds against *Pseudomonas sp*., *Klebsiella sp*. and *Enterobacter sp*.	127
15.	MIC (Minimum inhibition concentration) of Schiff bases (II-XI), thiosemicarbazones (XII & XIII) and hydrazones (XIV-XVIII) along with ampicillin against *Pseudomonas sp*.	132
16	MIC (Minimum inhibition concentration) of Schiff bases (II-XI), thiosemicarbazones (XII & XIII) and hydrazones (XIV-XVIII) along with ampicillin against *Klebsiella sp*.	133
17.	MIC (Minimum inhibition concentration) of Schiff bases (II-XI), thiosemicarbazones (XII & XIII) and hydrazones (XIV-XVIII) along with ampicillin against *Enterobacter sp*.	134

Figure No.	Title	Page No.
18.	MIC (Minimum inhibition concentration) of chalcones and pyrazolines along with ampicillin against *Pseudomonas sp*.	139
19.	MIC (Minimum inhibition concentration) of chalcones and pyrazolines along with ampicillin against *Klebsiella sp*.	140
20.	MIC (Minimum inhibition concentration) of chalcones and pyrazolines along with ampicillin against *Enterobacter sp*.	140
21.	ED_{50} of cumin oil and cuminaldehyde against *Fusarium wilt*	144
22.	ED_{50} of cumin oil and cuminaldehyde against *Bortrytis gray mould*	145
23.	ED_{50} of different solvent extracts against *Fusarium wilt*	148
24.	ED_{50} of different solvent extracts against *Bortrytis gray mould*	150
25.	ED_{50} of Schiff bases (II-XII), thiosemicarbazones (XIII & XIV) and hydrazones (XV-XVIII) against *Fusarium wilt*	153
26.	ED_{50} of Schiff bases (II-XII), thiosemicarbazones (XIII & XIV) and hydrazones (XV-XVIII) against *Bortrytis gray mould*	155
27.	ED_{50} of chalcones (XIX-XXIV) and pyrazolines (XXV-XXXVI) against *Fusarium wilt*	158
28.	ED_{50} of chalcones (XIX-XXIV) and pyrazolines (XXV-XXXVI) against *Fusarium wilt*	160

CHAPTER-I

INTRODUCTION

In traditional systems, richest bio-resource of drugs is the medicinal plants (Ncube *et al* 2008). It is well-known fact that spices not only add flavor and color to the food but also impart medicinal value to them. All over the world, many researches have been carried out by the scientists to study the pharmacological activities of the spices. Different parts of the plant like fruit, seed, bark, leaves, bud and bulb that are used as a flavoring agents and are source of many medicines. The word ‘spice’ comes from the Latin term ‘species’, which means of specific kind. Spices play different functions in food, primarily they are used not only to add flavor in the food products but also for the preservation of food and provision of their nutritional as well as their health benefits (Denre 2014). The volatile and non-volatile components *i.e.* the flavor components of the spice are protected within a matrix of cell components. Whenever the spice is crushed or powdered, the cell matrix of spice will break down and there is a release of the volatile components (Nadeem and Riaz 2012). In ancient times, the Egyptian, the Roman and the Arab used spices as drugs, incenses, stimulants and aphrodisiac agents.

Plants of Apiaceae family possess a wide range of compounds with numerous biological activities. Cumin (*Cuminum cyminum*) is an annual herbaceous plant belonging to the family of Apiaceae (Gohari and Saeidnia 2011) genus *Cuminum.* After black pepper, it is the second most popular spice in the world (Hasan *et al* 2016). In India, it is mainly cultivated in Rajasthan and Gujarat. Moderately cool and dry climate is ideal for its cultivation. High humidity and heavy rainfalls are not suitable for the cultivation of cumin. Well-drained, loamy soils that are rich in organic matter are best for cumin cultivation. Ideal time for sowing cumin seeds is November-December. The seeds are sown 10 cm deep. According to Spice Board India (Rawal *et al* 2014), 70% of world production of cumin is produced in India while the major consumers are China, Indonesia, Singapore, Malaysia, Bangladesh, Nepal and India itself. Apart from India it is also grown in Syria (7%), Iran (6%) and Turkey (6%). The area and production of cumin in the states of Gujarat and Rajasthan collectively accounts to 0.70 M ha and 0.37 M tonnes, respectively (Dubey *et al* 2018).

It is a spice plant which is 20-30 cm tall and has a slender branched stem. The leaves of the plant are either pinnate or bipinnate and are 5 to 10 cm long. The fruit is 4-5 mm long. The flowers are small and are pink or white in color. The fruit is 4-5 mm long, lateral fusiform or ovoid achene and containing only a single seed. Cumin seed resembles anise and fennel seeds in appearance but not in size as it is smaller in size than these two. The seeds are also known as Jeera, Jira, Jirn, Safeed zoor, Zirah, Sheeragam, Dhalajeera and Cumin seeds. It has slightly bitter, pungent, nutty and hot in taste. It is the popular flavouring spice used in

Indian kitchens. Volatile oil in cumin seeds is responsible for the flavour of cumin seeds. It constitutes an important part of ayurvedic medicines as it is used to treat many health problems like obesity, stomach pain and dyspepsia (Srinivasan 2018).

Cumin seeds are highly nutritious due to the presence of oil canals in seed carpel. The nutritional value of cumin seeds per 100 g includes: carbohydrates 44.24 g, energy 370 kcal, fiber 10.5 g, protein 17.81 g, moisture 8.06 g, thiamin (Vit. B1) 0.628 mg, riboflavin 0.327 mg, niacin 4.579 mg, vitamin B6 0.435 mg, vitamin C 7.7 mg, vitamin E 3.33 mg, Ca 931 mg, Fe 66.36 mg and other trace elements (Nadeem and Riaz 2012).

In the domestic as well as in international market, there is excessive demand of value added products of cumin such as seed, powder, essential oil and oleoresin etc. (Kumar *et al* 2015). The seeds contain 3.0-4.5% essential oil (Dubey *et al* 2017). The flavor in cumin is judged by its volatile oil content (Hirasa and Takemasa 1998). The reason behind the high demand of volatile oil in international market is that it is 100 times more concentrated than the spice powder and thus required in very less quantity. The odour and flavor is due to the presence of aldehydes (Nadeem and Riaz 2012). Cuminaldehyde (45–54%) is major compound of cumin essential oil is cuminaldehyde and possess many health benefits (Varo and Heinz 1970, Li and Jiang 2004). Cuminaldehyde content in cumin seeds collected from different geographical regions of Rajasthan and Gujarat *i.e.* Jodhpur (Rajasthan), Nagaur (Rajasthan) and Patan (Gujarat) is was 44.5, 44.2 and 40.3%, respectively. The essential oil has antioxidant, antispasmodic, diuretic, carminative and antibacterial activities (Bettaieb *et al* 2010). Organic acids such as malic, tartaric, propionic, aspartic, citric, ascorbic, oxalic and fumaric acids are isolated from seeds of cumin (Hashum and Al-Hashemi 2014). Cumin fruits contain 2.5 to 4.5% volatile oil and 10.0% fixed oil (Al-Snafi 2016). It is noted that the constituents and their percentage in *Cuminum cyminum* essential oil differ according to the area. Number of various environmental factors such as weather, altitude, rainfall and other conditions may affect the growth of plants which in turn affect the quality of herbal ingredients present in a particular species even when it is produced in the same country. These conditions may produce major variations in the bioactive compounds present in the plants (Santhi and Sengottuvel 2016). Cuminaldehyde in the Turkish cumin seed oil is (19.25-27.02%), in Egypt (35.25%) and Tunisian variety of cumin contains cuminlaldehyde (39.48%) (Al-Snafi 2016).

Depending on the regional variations and age of cumin plant, the color of essential oil varies from pale to colorless. Cumin oil mainly contains monoterpene aldehyde. Cuminaldehyde is the major constituent of the cumin oil (26-35%). Other constituents include pinene, camphene, sabinene, myrcene, limonene, cymene and cumin alcohol (Beis *et al* 2000). Chemical analysis of essential oil of cumin seeds showed that it contains cumin aldehyde as a major constituent (35%), terpinene (30%), α- and β-pinene (21%), *p* -cymene

(8.5%), *p* -mentha-1,3-dien-7-al (5.6%), cuminyl alcohol (2.8%) and β-farnesene (1.1%). Other compounds such as perilla aldehyde, cuminol, β-phellandrene and dipentene are also present in cumin (Rong and Jiang 2004).

Phytochemical studies of cumin seeds revealed that it contains alkaloid, anthraquinone, coumarin, flavonoid, glycoside, protein, resin, saponin, tannin and steroid etc. (Rai *et al* 2012). Secondary metabolites or phytochemicals are naturally occurring biologically active molecules present in plants and plays an important role in defence system of plant (Lako *et al* 2007). In the past decades, these phytochemicals have been used as pharmaceuticals, flavoring agents, dyes and agrochemicals (Rathore et al 2013). *In vitro* studies reported that phytochemicals such as phenolic compounds have potential role against different diseases and used as anti-inflammatory, anti-mutagenic, antiviral and antibacterial agents (AL Juhaimi and Ghafoor 2013 and Senevirathne *et al* 2006). Seed extracts of cumin are reported to possess antiallergic, antioxidant, antiplatelet aggregation and hypoglycaemic activities (Singh *et al* 2002, Lee *et al* 2005, Allahghadri *et al* 2010). Ethanolic extract of cumin seeds showed antimicrobial potential against biofilm *Escherichia coli* (Bameri *et al* 2013). Now a days, solvent extracts of different parts of the plant are used as a reducing agents for the synthesis of metallic nanoparticles.

Metallic nanoparticles can be synthesized by reduction of metal ion *via* some chemical molecules. Plants are the richest bio-resources of free radical scavenging molecules namely phenolic compounds, nitrogen compounds, vitamins, reducing sugar, terpenoids and some other metabolites that have rich antioxidant potential (Salam *et al* 2012). All the plants used to synthesize nanoparticles are known to be rich in polyols. Hydroxyl and carboxylic groups present may act as reducing and stabilizing agents in the synthesis of nanoparticles (Vilchis-Nestor *et al* 2008). According to Amin *et al* 2012, functional groups such as phenolics and alkaloids are responsible for capping and stabilizing of nanoparticles reduced.

In modern materials science, the nanotechnology field is most active areas of research. Nanotechnology is being applied in various fields like chemistry, biology, catalysis, medicine, photonics and electronics. Nanoparticles of silver metal have been synthesized using different plant extracts of *Argemone maxicana* (Singh *et al* 2010), *Ocimum* (Mallikarjun *et al* 2011), *Cleome Viscosa*, *Trigonella foenum-graecum*, *Cycas* (Anal and Prasad 2010), *Eucalyptus hybrida* (Dubey *et al* 2009), *Iresine herbstii* (Dipankar and Murugan 2012), *Avena sativa* (Armendariz *et al* 2004), *Lantana camara*, *Citrus limon*, *Calotropis gigantean*, *Achillea wilhemsii* (Andeani *et al* 2011), *Carica papaya* (Joshi 2020) and *Tagetes erecta*. Kudle *et al* (2012) reported the synthesis of silver nanoparticles using *Cuminum cyminum via* microwave irradiation method. The synthesized nanoparticles were also screened for their antimicrobial potential and showed a significant activity against microbes.

Presently, numerous techniques are existing for the preparation of nanoparticles *i.e.*

reduction in solutions, thermal decomposition, chemical and photochemical reactions in reverse micelles, radiation assisted, electrochemical, sonochemical, microwave assisted process *via* green chemistry routes. Green synthesis has an advantage over physical and chemical procedures as these are highly cost effective, ecofriendly and there is no need to control the temperature, pressure and energy. Eco-friendly, green synthesis of nanoparticles of some common indian spices were carried out by Sinha and Paul (2014).

Now a days, there is increase in the number of food-borne diseases caused by some pathogens and their enterotoxins which is a topic of concern for food safety researchers, food processors and regulatory agencies. Some microorganisms such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella spp*., *Yersinia spp*. and *Clostridium spp*. causes food spoilage and leads to economic losses. For many cases, they are also responsible of intestinal syndromes, causing diarrhoea and vomiting (Demirci *et al* 2008 and Friedman *et al* 2002). Increased and over-prescribing of the same antibiotics and poor patient compliance, is the major cause of bacterial resistance to antibiotics. Therefore, now a days, there is lot of interest in the synthesis of novel, non-toxic and effective antimicrobial compounds. Burt (2004) has reported that in the past decade natural products such as essential oils have received considerable attention due to their antimicrobial potential. Tepe *et al* (2004) studied the antimicrobial activities of various natural products, such as essential oils and plant extracts. Essential oil can be obtained from the different parts of the plant such as root, seed or stem which is aromatic oily liquids and possess a broad range of biological activities.

Literature survey revealed that essential oil not only possess antibacterial and antiviral effects but also exhibit antifungal, insecticidal (Gaba 2018), acaricidal, cytotoxic and antioxidant potential (Sacchetti *et al* 2005). Plants belonging to the Apiaceae family exhibit a wide range of compounds that are responsible for their biological activities. The essential oil of cumin exhibited antifungal, antimicrobial (Tavakoli 2015), antioxidant (Bettaieb *et al* 2010), antiestrogenic, antidiabetic, antitussive, anti-inflammatory (Bhat *et al* 2014), analgesic, hepatoprotective, anticancer, antiinfertility (Priya *et al* 2012), antistress, alzhiemer, antiulcer and antiasthamatic (Singh *et al* 2015) activities. The biological activities of the essential oils may be due the presence of major compound which was responsible for their antagonistic or synergistic action. Sometimes, the biological potential was also enhanced due to the presence of minor components. The antibacterial potential of *cuminum cyminum* essential oil may be due to presence of high level of cuminaldehyde, which was a compound with antimicrobial potential (Farag *et al* 1989 and Helander *et al* 1998).

Different solvent extracts of cumin seeds are also known to possess various biological activities. It contains alkaloids, anthraquinones, coumarins, flavonoids, glycosides, proteins, resins, saponins, tannins and steroids etc. These naturally occurring biologically active chemical compounds play significant role in defence mechanism of plants against predation

by microorganisms. Various studies reported that phenolic phytochemicals compounds are effective against diverse diseases and shows anti-inflammatory, anti-mutagenic, antiviral and antibacterial activities (Al Juhaimi and Ghafoor 2013 and Senevirathne *et al* 2006). Cumin extracts possess wide range of biological activities such as antiallergic, antioxidant, antiplatelet aggregation and hypoglycaemic (Singh *et al* 2002, Lee *et al* 2005, Allahghadri *et al* 2010). Ethanol extract of seed exhibited antimicrobial activity against *Escherichia coli* (Bameri *et al* 2013). Keskin and Toroglu (2011) studied the antimicrobial potential of different solvent extracts namely ethyl acetate, acetone and methanol of *Cuminum cyminum* against eight bacterial species *i.e. Klebsiella pneumonia* 13883, *Bacillus megaterium* NRS, *Pseudomonas aeroginosa* ATCC 27859, *Staphylococcus aureus* 6538 P, *Escherichia coli* ATCC 8739, *Enterobacter cloaca* ATCC 13047, *Corynebacterium xerosis* UC 9165, *Streptococcus faecalis* DC 74. Results revealed that the different solvent extracts displayed significant antimicrobial activity against all eight bacterial strains.

Cuminaldehyde derived from essential oil of cumin seeds possess wide range of biological activities such as antimicrobial, antifungal, anti-inflammatory, antimalarial, antioxidant, antifibrillation, anticarcinogenic and antidiabetic. Derakhshan *et al* (2008) and Derakhshan *et al* (2010) reported the synthesis and antimicrobial activity of Schiff base obtained from cuminaldehyde and 4-aminoantipyrine against *E. coli, S. aureus, Klebsiella pneumonia, Proteus vulgaris, C. albicans and A. niger*, using agar-well diffusion method. Newly synthesized Schiff base showed significant antimicrobial activity.

Keeping in view the above potential biological activities of cumin and cuminaldehyde, the present study was conducted with following objectives:

i. To study the chemical composition of cumin seeds and extraction of essential oil.
ii. To isolate and derivatize the major constituent of essential oil of cumin.
iii. To synthesize metal nanoparticles (NPs) of *Cuminum cyminum.*
iv. To screen the major constituent, its derivatives and synthesized nanoparticles for their fungicidal, microbial and antioxidant activity.

CHAPTER II

REVIEW OF LITERATURE

Among all the food ingredients, spices are the richest source of the bio-nutrients. From the ancient times, spices have been extensively used as food additives in order to enhance the taste as well as the flavor of food. Apart from their use as a food additive, spices also possess many medicinal properties and are used for the treatment of many disorders that increases their importance in the field of Ayurvedic Pharmacopoeia. In Indian recipes, they played a significant role in food to increase its flavor and add bactericidal, bacteriostatic, fungistatic, antifertility, antihelminthic and other medicinal properties. They also improve digestion (Nazeem 1995). Cumin (*cuminum cyminum*) is one of the most popular spice and is used as culinary spice because of its special aromatic effects. In the past decades, India, China and Mediterranean countries are the major cultivars of the cumin. Now a days, it is extensively cultivated in Turkey, India, China, Iraq, Libya and Palestine. The major production of cumin came from India (states of Rajasthan and Gujarat) (Parashar *et al* 2014).

Cumin is an annual herbaceous plant having an erect, round, slender, branched stem which is about a twelve inch high (Rebey *et al* 2012). The color of cumin seeds is brownish-gray in color and is elongated shape having various biological activities. The cumin seeds are carminative, aromatic, stomachic, stimulant, astringent and synergistic (Hanif *et al* 2012) in effect. Well drained sandy loam to loamy soil with pH 6.8-8.3 is ideal for growth of cumin (Sheikh *et al* 2010). Optimal growth conditions include temperature ranging from 25-30°C and relative humidity between 65-70%. The crop is highly sensitive to rain and raining during the harvesting time of crop will reduce its yield and quality (Sheikh *et al* 2010).

Taxonomical classification

Kingdom: *Plantae*

Subkingdom: *Viridiplantae*

Infrakindom: Streptophyta

Order: Apiales

Family: Apiaceae

Division: *Tracheophyta*

Super division: *Embryophyta*

Sub division: Spermatophytins

Class: *Magnoliopsida*

Genus: *Cuminum*

Species: *Cyminum*

Common names

Cumin, Zeera, Comino, Kmin, Cumino etc.

At present, numerous techniques are available for extraction of volatile oil. One of the most common technique is hydrodistillation through Clevenger's apparatus. Extraction is defined as the process used for the separation of medicinally effective compounds from different parts of plant using standardized procedures. There are certain techniques for extraction of volatile oil used in different areas such as pharmaceutical, herbal industries and research laboratories include maceration, infusion, percolation, digestion, decoction, hot continuous extraction (Soxhlet), aqueous-alcoholic extraction by fermentation, counter-current extraction, microwave-assisted extraction, ultrasound extraction (sonication), supercritical fluid extraction, and phytonic extraction (with hydrofluorocarbon solvents). Preparation of aromatic plant extract could be done by other extraction methods such as hydro distillation techniques, hydrolytic maceration followed by distillation, expression and effleurage (cold fat extraction). Headspace trapping, solid phase micro-extraction, protoplast extraction, microdistillation, thermo-microdistillation and molecular distillation are some of the modern extraction methods for aromatic plants (Tiwari *et al* 2011).

2.1 Proximate, phytochemical and chemical composition of cumin

Cumin (*Cuminum cyminum*) has been used for many medicinal and culinary purposes from the ancient times in various countries. It is a popular spice in the world and used as a flavoring agent in many products such as cheese, pickle, soup, bean dishes or liqueurs. Essential oil of the seeds are also used as a flavor or in aromatherapy. The flavor of cumin is judged by its volatile oil content. The advantage of use of volatile oil is that it is 100 times more concentrated then the spice powder and hence is required in very less quantity. The essential oil is responsible for the characteristic cumin odor. Many pharmacological effects have been reported from this spicy plant such as anti-diabetic, Immunologic, anti-epileptic, anti-tumor and antimicrobial activities.

Gupta (2013) analyzed different spices used in Indian kitchen for their total phenolic (TPC), total flavonoid content (TFC) and antioxidant activity (using DPPH assay and FRAP assay). The spices used were cinnamon (*Cinnamamum verum*), black pepper (*P. nigrum*), ajwain (*T. ammi*), cumin (*C. cyminum*), black cumin (*N. sativa*), coriander (*Coriander sativum*), fennel (*Foeniculum vulgare*), staranise (*I. verum*) and clove (*S. aromaticum*). The methanolic extracts of the above spices were used for analysis. The total phenolic content showed the following order; cloves > cinnamon > ajwain > staranise > cumin > blackpepper > fennel > coriander > black cumin. The highest total flavonoid content obtained was in clove > cinnamon > ajwain > black cumin > star anise > fennel > coriander > black pepper.

Zhang *et al* (2014) studied the phytochemical evaluation of different solvent extracts (methanol, ethanol, acetone, ethyl-acetate and n-hexane) of *Cuminum cyminum* including total polyphenols, flavonoids and tannins, as well as antioxidant activities were investigated. Acetone extract contained highest content of polyphenols, flavonoids and tannins and thus

exhibited stronger antioxidant activity.

Al-Rubaye and his co-workers (2017) reported the chemical composition of methanolic extract of cumin seed, collected from local market of Hilla, Iraq. Total of twenty compounds were detected and cuminaldehyde was the major component.

Essential oil is one of the most important chemical components of cumin fruits, ranged between 2.5% to 4.5% and pale to colorless depending on age and regional variation (Nadeem and Riaz 2012). The differences in the chemical composition of oil may be related to distinct environmental and climatic conditions (Bisht *et al* 2009) such as light (Johnson *et al* 1999), precipitation, growing site (Satta *et al* 1999), temperature (Santos-Gomes and Fernandes-Ferreira 2001; Mallavarapu *et al* 1999; Farhat *et al* 2001), soil (Pala-Paul *et al* 2008), seasonal sampling periods, geographic origins, plant populations (Barra 2009), vegetative plant phases (Santos-Gomes and Fernandes-Ferreira 2001; Mallavarapu *et al* 1999; Farhat *et al* 2001) extraction and quantification methods (Figiel *et al* 2010 and Russo *et al* 1998). Therefore, quality and quantity of oil is highly sensitive to air, light, moisture, metals and to alkali (Beis *et al* 2000). The percentage of various components of volatile oil from seed was terpenenoids like α-pinene (0.50%) and β-pinene (13.00%), myrcene (0.30%), limonene (0.50%), 1-8cineole (0.20%), caryophyllene (0.80%), β-bisaboline (0.90%) (Nadeem and Riaz 2012), *p*-cymene (8.50%), β-phellandrene (0.30%), β-terpenine (29.50%), cuminaldehyde (32.40%), cuminyl alcohol (2.80%), β -farnesene (1.10%), linalool (1.06%), α-terpenieol (2.75%), benzyl cinnamate (4.47%), γ-terpinene, *p*-mentha-1,4-dien-7-al, *p*-mentha-1,3-dien-7-al and perillaldehyde, flurocoumarin, chromocoumarin, saponins, resin and alkaloids (El-Said and El-Hady 2014). The leaves contained flavonoids, glycosides (containing quercetin, kaempferol), *p*-coumaric, rosmarinic, trans-2-dihydrocinnamic acids and resorcinol (Tabbasum *et al* 2010).

Bettaieb *et al* (2010) investigated different parts of cumin *i.e.* roots, stems, leaves and flowers for their essential oil, total phenolics, flavonoids and tannin content and individual phenolic compounds. Essential oil yields were 0.03% in roots, 0.10% in stem and leaves and 1.70% in flowers. Bornyl acetate (23.0%), R-terpinene (34.0%), and γ-terpinene (51.0%) in roots, stems and leaves, and flowers, respectively were the major constituents of oils. Total phenolic content in all *C. cyminum* organs, ranged between 11.80 to 19.20 mg of GAE/g of DW. Thirteen, seventeen and fifteen polyphenols were identified in roots, stem and flowers, respectively. The roots also contained quercetin, stem contained *p*-coumaric, rosmarinic, trans-2-dihydrocinnamic acids and resorcinol and flowers contained vanillic acid.

Gohari and Saeidnia (2011) studied the photochemistry and antimicrobial properties of cumin. Cuminaldehyde (I) was the main active compound which may be responsible for its antimicrobial activity reported by Derakhshan *et al* (2008) and Derakhshan *et al* (2010). Limonene (II), eugenol (III), α and β-pinenes (IV, V) were the minor constituents that were

found in cumin oil and responsible for its antimicrobial potential (Johri 2011, Dorman and Deans 2000).

(I) (II) (III) (IV) (V)

(VI) (VII)

(VIII) R = CH_3

(IX) R = $-CH_2CH_2OH$

(X) R_1 = Glc, R_2 = R_3 = H

(XI) R_1 = R_3 = H, R_2 = Glc

(XII) R_1 = R_2 = H, R_3 = Glc

Literature review on phytochemistry revealed the presence of secondary metabolites in the cumin seeds (Takayanagi *et al* 2003 and Kitajima *et al* 2003). Cuminoside A (VI) and Cuminoside B (VII) are two sesquiterpenoid glucosides compounds VIII and IX are alkyl glycosides which were isolated from the methanolic extract of cumin seeds. Kitajima *et al* (2003) reported the isolation of 1-O-β-D-glucopyranoside (X), 3-O-β-D-glucopyranoside (XI) and 4-O-β-D-glucopyranoside (XII) from the cumin seeds.

(XIII)

A previously unreported aldehyde, 1,4-p-mentha-dien-7-al, was isolated from fresh,

whole cumin seeds by Varo and Heinz (1970). Infrared, ultraviolet, nuclear magnetic resonance, and mass spectra were used to characterize the compound.

Cumin oil was extracted either by steam distillation or by hydrodistillation. Distillation was performed immediately after grinding of the seeds to prevent decomposition and oil loss. Steam distillation of cumin seed was studied by Beis and his co-workers (2000) to show effect of particle size on the recovery of essential oil. It was found that when the size of cumin seeds has reduced from 0.710 to 0.177 mm then oil yield increases from 1.40 to 2.80%. The essential oil obtained from cumin seed extracted by steam distillation was also subjected for GC-MS analysis in order to study its chemical composition. As far as, the composition of the oil was concerned, the major constituents were cuminaldehyde (27.60%), γ-terpinene (17.25%), *p*-mentha-l.3-dien-7-al (15.18%), α-pinene (10.22%), and *p*-mentha-l,4-dien-7-al (9.48%).

Eikani and his co-workers (2007) conducted an experiment of superheated water extraction *via* two conventional methods namely hydrodistillation and Soxhlet extraction for isolation of essential oil from cumin (*Cuminum cyminum* L.). At 20 bar, the effect of some superheated water parameters such as temperature, seed size and flow rates, on extraction of essential oil were investigated. Separation and identification of the components was done by gas chromatography using flame ionization detector and gas chromatography-mass spectrometry, respectively. The ideal conditions of the superheated water extraction were 150 °C temperature, 0.50 mm mean size of seed and 4 ml/min flow rate. The SWE method resulted in a more valuable essential oil with respect to the oxygenated components.

Zhao *et al* (2016) reported the three-stage microwave extraction of cumin (*Cuminum cyminum* L.) seed essential oil with natural deep eutectic solvents (NADESs). When the natural deep eutectic solvent was composed of choline chloride and L-lactic acid (1:3 molar ratio) with 40% (w/w) of water, maximum yield of essential oil was obtained. NADESs had a major impact on extraction of essential oil with higher yield, premium quality, and more quantity, especially when it was combined with microwave technology. A total of 45, 48, and 58 volatile components were identified using gas chromatography-mass spectrometry with microwave hydrodistillation (MHD), ultrasound-assisted natural deep eutectic solvents pretreatment combined with microwave hydrodistillation (UA-NADES-MHD) and microwave-assisted natural deep eutectic solvents pretreatment coupled with microwave hydrodistillation (MA-NADES-MHD), respectively.

Cumin is an annual herb and has been used since ancient times as medicine and spices in food (Patil *et al* 2017). AL Juhaimi and Ghafoor (2013) reported the extraction of phenolic compounds from cumin (*Cuminum cyminum* L.) seed was optimized using three process variables *i.e.* temperature (35-50°C), time (1-4 hr) and ethanol concentration (40-70%). Cumin seed extracts showed good antiradical (34.25-39.25%) and antioxidant (8.25-

11.24 mg/ml) activities determined *via* DPPH radical scavenging and phosphomolybdenum complex methods, respectively.

Rebey and his co-workers (2012) evaluated the effect of drought on total and individual polyphenol contents as well as the antioxidant activity of cumin (*Cuminum cyminum* L.) seeds collected from two geographic origins namely Tunisia (TCS) and India (ICS). Plants were treated with different levels of water deficit. Moderate water deficit (MWD) improved the number of umbels per plant and the seed yield in both varieties as compared to the control. Drought increased the level of total and individual polyphenols in TCS than in ICS. Antioxidant activities of the extracts were determined by 2,2-diphenyl-1-picrylhydrazyl, β-carotene/linoleic acid chelating and reducing power assays and showed promising results.

Miri and Djanane (2018) conducted an experiment to inhibit the growth of *Aspergillius flavus* E73 (*A. flavus* E73) and production of aflatoxin B_1 (AFB_1) by *Cuminum cyminum* and *Coriandrum sativum* essential oils along with their antioxidant and phytotoxicity activities. Major components of essential oil of *Cuminum cyminum* and *Coriandrum sativum* were cuminaldehyde (65.98 %) and linalool (78.86 %), respectively. Percent inhibition of both the oils against *A. flavus* E73 ranged between 24.27-84.90% (*C. cyminum*) and 15.09-65.00% (*C. sativum*).

The essential oil of cumin seeds (*Cuminum cyminum* L.) collected from Bulgaria, stored for more than 35 years and were analyzed by physico-chemical methods, GC, GC-MS and olfactometry and its antimicrobial activity tested using different strains of microorganisms (Jirovetz *et al* 2005). More than sixty constituents of cumin oil were identified as essential volatiles, responsible for the pleasant fresh, clean, spicy (typical cumin-like) odour of a high quality product. Cumin aldehyde (36%), β-pinene (19.3%), p-cymene (18.4%) and γ-terpinene (15.3%) were the principal compounds. Antimicrobial testing showed high activity of the essential *C. cyminum* oil against the mold *Aspergillus niger*, the Gram (+) bacteria *Bacillus subtilis* and *Staphylococcus epidermidis* as well as the yeast *Saccharomyces cerevisiae* and *Candida albicans*.

Hajlaoui *et al* (2012) extracted essential oil from Tunisian variety of Cuminum cyminum by hydrodistillation and was characterized using GC and GC-MS. Twenty-one components were identified and *C. cyminum* contained cuminlaldehyde (39.48%), γ-terpinene (15.21%), *o*-cymene (11.82%), β-pinene (11.13%), 2-caren-10-al (7.93%), trans-carveol (4.49%) and myrtenal (3.5%).

Li and Jiang (2004) extracted essential oil from seeds of *Cuminum cyminum* L. by hydrodistillation collected from China. Percent yield of extracted essential oil was 3.8%. The chemical composition of the essential oil was examined by GC and GC–MS. Total of 37 components representing 97.97% of the oil were identified. Cuminal (36.31%), cuminic

alcohol (16.92%), *γ* -terpinene (11.14%), safranal (10.87%), *p* -cymene (9.85%) and *β* -pinene (7.75%) were the major components.

Rahimi *et al* (2013) noted the effect of salicylic acid and methyl jasmonate on growth, yield, essential oil quantity and quality of cumin. For this purpose, the plants were sprayed with different concentration of essential oil and 1 mM of *salicylic acid* (*SA*) and *Methyl jasmonate* (MeJa). Total of 22 compounds were identified in cumin essential oil and the major compounds were γ-terpinene-7-al, cuminaldehyde, α-terpinene-7-al, *p*-cymene and β-pinene respectively. α-Terpinene-7-al was more affected by treatments, and it considerably reduced by 1 mM SA and MeJa.

Saha *et al* (2014) reported the comparative study on extraction of essential oil from same raw material of cumin seed with hydrodistillation and super critical fluid extraction (SCFE). The composition of the oil was β-pinene, p-cymene, γ-terpinene, cuminaldehyde, phellandral, carenal (2-caren-10-al), cuminic alcohol, carvacrol, γ-cadinene, β-farnesene, α-cubebene, γ-curcumene, α-logipinene, tricosane, eicosane and docosane as determined by gas chromatography-mass spectrometry. Yield of essential oil was more in the SCFE method but percentage of cuminaldehyde was found to be higher by hydrodistillation method which contained higher percentage of cuminaldehyde (52.6%) than by SCFE (37.3%). Both the oils were also screened for their antioxidant potential and it was found that hydrodistilled volatile oil showed better antioxidant activity measured by DPPH and FRAP assay.

Estimation of essential oil for its yield, composition, antimalarial, and antioxidant capacity of cumin (*Cuminum cyminum* L.) seed by steam distillation experiment was reported by Zheljazkov *et al* 2015. The parameters were recorded at ten steam distillation time durations. The major oil constituents were α-pinene, β-pinene, γ-cymene, γ-terpinene, cumin aldehyde, α-terpinen-7-al and β-terpinen-7-al. The concentrations of α-pinene, β-pinene, γ-cymene, γ-terpinene in the oil increased with the increase of the duration of the distillation time. Extracted essential oil was also screened for its antioxidant and antimalarial activity.

Fragrance and flavor profile of essential oil of *Cuminum cyminum* collected from the Algerian market, Northwest Africa was reported by Boughendjioua (2019). The essential oil from the cumin seeds was isolated by hydro-distillation and the chemical composition was determined by GC-MS. A total of eighteen compounds constituting 91.10% composition of essential oil were identified. Major components present in essential oil were cuminaldehyde (50.50%), β-pinene (9.50%), γ-terpinene (10.00%) and p-cymene (11.80%). Physicochemical properties of the essential oil were also studied like refractive index (20 °C): (1.48), density (0.91 at 20 °C), aldehyde percentage: 50%, acidity: 1.0, alcohol percentage: 3.5%, carbonyl index: 9.32 and steric index: 19.24.

The study on essential oil extraction from mature seeds of *Cuminum cyminum* was carried out by Chaudhry *et al* (2012). The percentage yield of essential oil extracted through

hydro distillation was 2.8%. Physical characteristics were recorded according to standard procedures. Chemical composition of extracted essential oil was α-pinene (0.98%), β-pinene (16.29%), limonene (0.23%), γ-terpinene (5.65%), *p*-cymene (15.55%), dipentene (0.21%), 1,8-cineol (2.24%), cuminaldehyde (25.87%), cuminyl alcohol (30.02%), perillaldehyde (0.63%) and γ-terpineol (0.19%) and (2.13%) remain unidentified. The essential oil was also screened for its antimicrobial potential against four gram positive and two gram negative bacteria.

Although the seeds of cumin (*C. cyminum* L.) are widely used as the spice for their distinctive aroma, they are also commonly used in traditional medicine to treat a variety of diseases, including chronic diarrhoea and dyspepsia, acute gastritis, diabetes, and cancer. The literature presents ample evidence for the biological activities of cumin, which have generally been ascribed to its bioactive constituents such as terpenes, phenols, and flavonoids (Srinivasan 2018).

Cumin seeds are nutritionally rich as they contain high amount of fats, protein and dietary fibre. The major volatile components of cumin seeds are cuminaldehyde, cymene and terpenoids (Bettaieb *et al* 2011). Cumin has a distinctive strong flavor and warm aroma due to cuminaldehyde and cuminic alcohol. Other important aroma compounds of roasted cumin are the substituted pyrazines, 2-ethoxy-3-isopropylpyrazine, 2-methoxy-3-sec-butylpyrazine and 2-methoxy-3-methylpyrazine (Srinivasan 2018).

Abbdellaoui *et al* (2019) studied the chemical composition and antioxidant activities of the essential oil of cumin (*Cuminum cyminum*) derived from the seeds of three local cumin populations that were grown in an oasis environment. The organically cultivated cumin seeds had a strong, distinctive aroma due to an abundance of essential oil (3.8-5.27 %). The main components present in all essential oils were cuminaldehyde (30.42-33.24 %), γ-terpinen-7-al (20.54-28.36 %), α-terpinen-7-al (about 13.00 %), γ-terpinene (6.15-12.60 %), β-cymene (4.19-5.38 %), β-pinene (3.10-5.36 %) and *p*-mentha-1,4-dien-7-ol (0.71-0.99 %). Essential oils were also screened for their antioxidant potential *via* DPPH scavenging ability and ferric reducing power FRAP.

Variability in physical and chemical composition of *Cuminum cyminum* seeds from arid and semiarid India was reported by Dubey *et al* (2016). Cumin seed samples were collected from 7 districts of Rajasthan and 5 districts of Gujarat classified under Agro-Ecological Sub Regions (AESR) of India. Quantity of essential oil from various districts ranged between 28.4 to 39.1 g/kg. In Rajasthan, highest essential oil content was recorded from Nagaur (38.5 ± 0.37), followed by Jaisalmer (37.4 ± 0.31) and was least in Ajmer (28.6 ± 0.27) whereas, in Gujarat, samples from Patan possessed highest essential oil content (39.1± 0.17) with least values in Amreli (28.4 ±1.08). The average flavonoid content expressed as mg QE/g seeds was highest in Nagaur (39.72), followed by Amreli (36.03) and lowest in

Ajmer district (23.71), similarly the estimated phenol content (mg GAE/g seeds) was maximum in Jalore (73.61), followed by Nagaur (63.77) and least in Amreli district (41.50). The carbohydrate content and total protein content ranged between 15.86-28.88 and 19.30-21.89 per cent respectively.

Li *et al* (2009) studied the essential oil extraction from cumin seeds by steam distillation. They also studied the effect of different parameters *i.e.* particle size, temperature and extraction time on the yield of essential oil. It was noted that yield of the extract (oleoresin) was most affected by the temperature followed by extraction time and particle size. In order to compare the different extraction methods, essential oil of cumin seeds obtained by different methods *i.e.* by supercritical fluid extraction (SFE), hydrodistillation (HD), combination technology of organic solvent with low boiling point and steam distillation (OS-SD) was subjected to GC-MS analysis. A total of forty-five compounds were identified.

From the literature survey, it has been revealed that essential oil of cumin mainly contained monoterpene aldehydes. Major constituents of cumin were: cuminaldehyde (*p*-isopropylbenzaldehyde, 25.0 to 35.0%), terpinene (29.5%), α and β-pinene (21.0%), *p*-cymene (8.5%), *p*-mentha-1,3-dien-7-al (5.6%), cuminyl alcohol (2.8%) and β-farnesene (1.1%). Perilla aldehyde, cuminol, dipentene and β-phellandrene were also present (El-Hamidi and Ahmed 1996).

Characterization of essential oil components of Iranian cumin seed was reported by Jalali-Heravi *et al* (2007) using Gas chromatography-mass spectrometry. A total of 19 compounds were identified by GC-MS. With the help of chemometric techniques, this number of compounds was extended to 49 components. Major constituents present in cumin seed essential oil were 2-methyl-3-phenyl-propanal (32.27%), gamma-terpinene (15.82%) and myrtenal (11.64%).

Cumin (*Cuminum cyminum* L.) is used for the treatment of stomach disorders. Mehdizadeh and his co-workers (2017) studied the effect of storage temperature *i.e.* −20 °C, 4 °C and 25 °C on the quality of cumin essential oil. The main constituents of the essential oil were cumin aldehyde, p-cymene and β-pinene. Results indicated that at room temperature, the proportions of compounds with lower boiling temperatures such as β-pinene (1.57–10.03%) and *p*-cymene (14.93-24.9%) were decreased; however, cumin aldehyde (45.45–64.31%) increased during cumin oil storage.

Cumin oil samples (Cuminum cyminum L.) from four different geographical origins were characterized using GC-MS and GC-FID for their qualitative and quantitative composition (Wanner *et al* 2010). The major compounds in all cumin oils were the monoterpenes β-pinene, p-cymene and γ-terpinene and the terpenoid aldehydes cuminic aldehyde and the isomeric menthadien carboxaldehydes. All essential oils, and cuminic aldehyde, were tested, using agar diffusion and serial dilution methods, against different

Gram-positive and Gram-negative bacteria isolated from different sources of food (pork fillet, minced meat and sausages) and clinical isolates, as well as three different *C. albicans* isolates. All cumin oils and cuminaldehyde exhibited a considerable inhibitory effect against all the organisms tested, except *Pseudomonas spp*.

Esmaeili (2015) studied the chemical composition of the essential oil *Cuminum cyminum* collected from Ilam, was determined by GC-MS. In total, 25 components (83.36%) of essential oil were identified. Major constituents were Isobutyl isobutyrate (0.45%), α-thujene (0.5%), α-pinene (30.12%), sabinene (1.11%), myrcene (0.34%), γ-3-carene (0.21%), p-cymene (0.6%), limonene (10.11%), 1,8-cineole (11.54%), (E)-ocimene (0.1%), γ-terpinene (3.56%), terpinolene (0.32%), linalool (10.3%), α-campholenal (1.76%), terpinene-4-ol (0.6%), *trans*-carveole (0.7%), geraniol (1.0%), linalyl acetate (4.76%), α-terpinyl acetate (1.8%), neryl acetate (1%), methyl eugenol (0.2%), β-caryophyllene (0.42%), α-humulene (0.3%), spathulenol (0.56%) and humulene epoxide II (1%).

El-Ghorab *et al* (2010) studied the chemical composition of the volatile oil of cumin and ginger, analyzed by GC-MS. Major chemical components present in cumin essential oil were cuminal (27.70%), γ-terpinene (23.70%), pinocarveol (11.40%), 1-methyl-2-(1-methylethyl)benzene (7.70%), copaene (6.00%), (5R)-5-methyl-2-(1-methylethylidene)cyclohexanone (5.50%), carotol (4.40%), 2-ethylidene-6-methyl-3,5-heptadienal (2.80%) and sabinene (1.20%). El-Ghoran and his co-workers also studied the nutritional composition of cumin seeds. The seeds contain moisture (6.10%), crude fat (11.50%), crude fibre (37.20%), ash (9.30%), crude protein (15.70%) and carbohydrates (20.10%).

Oroojalian *et al* (2010) extracted the essential oil of three Apiaceae species, namely *Bunium persicum*, *Cuminum cyminum* and *Carum copticum* were analyzed by GC and GC-MS. The major constituents of *C. cyminum* were cuminaldehyde (30.20%), *p*-cymene (14.10%), γ-terpinene (12.80%), and safranal (9.40%), while those of *C. copticum* were thymol (48.40%), *p*-cymene (21.80%) and γ-terpinene (21.30%). The antibacterial effects of the essential oils were assessed on several food-borne pathogens, namely *S. aureus*, *Bacillus cereus*, *Escherichia coli* O157:H7, *Salmonella enteritidis* and *Listeria monocytogenes*. The ranges of minimum inhibitory concentration (MIC) of the oils were 0.03-0.50, 0.18-3.00, and 0.37-3.00 mg/ml, respectively, for *C. copticum*, *B. persicum* and *C. cyminum*. Moreover, the combination of *B. persicum* and *C. cyminum* essential oils confirmed synergistic and additive activities against the pathogens.

Rebey *et al* (2012) examined yield components, fatty acid, essential oil compositions, the total phenolic amounts as well as the antioxidant activities of cumin (*Cuminum cyminum* L.) seeds under drought. For this purpose, plants were treated with three levels of water deficit: control (C), moderate water deficit (MWD) and severe water deficit (SWD). Fatty

acid composition of cumin was petroselinic acid (55.90%) followed by palmitic (23.82%), linoleic (12.40%) acids, palmitoleic acid (2.12%), caprylic acid (1.63%), tridecanoic acid (1.20%), capric acid (0.92%), oleic acid (0.32%), α-linolenic acid (0.20%), lauric acid (0.16%) and myristic acid (0.15%). The essential oil yield was 1.64% based on the dry weight and increased by 1.40 folds under MWD. Antioxidant activities of the extracts were determined by four different test systems, namely DPPH, β-carotene/linoleic acid chelating and reducing power assays and showed that treated seeds (MWD and SWD) exhibited the highest activity.

2.2 Chemical derivatization of cuminaldehyde

Arish and Nair (2010) reported the synthesis of 2-(2-(4-isopropylbenzylideneamino) acetamido) acetic acid. Synthesis of this Schiff base was afforded by the condensation of cuminaldehyde with glycylglycine in the presence of potassium hydroxide. The synthesis of Schiff base was confirmed by UV-vis spectra, IR, ^{1}H and ^{13}C NMR, mass and elemental analysis.

Scheme 1

Derakhshan *et al* (2008) and Derakhshan *et al* (2010) reported that cuminaldehyde (I) was the major compound present in cumin and also responsible for its numerous biological potentials. A Schiff base obtained from *p*-isopropylbenzaldehyde and 4-aminoantipyrine had been synthesized and characterized by Bennie *et al* (2014). The structure of the synthesized compound was established on the basis of UV-visible, IR, mass and ^{1}H NMR spectral studies. Synthesized Schiff base was also evaluated for its antimicrobial activity against *E. coli, S. aureus, Klebsiella pneumonia, Proteus vulgaris, C. albicans and A. niger*, employing agar-well diffusion method. Newly synthesized Schiff base showed significant antimicrobial activity.

Scheme 2

Morshedi *et al* (2015) reported the synthesis of new Schiff base, synthesized by the condensation of cuminaldehyde and amino acid lysine. Characterization of the synthesized Schiff base was done by UV-vis, IR, ^{1}H and ^{13}C NMR, mass spectra and

elemental analysis.

Scheme 3

1-Isopropyl-4-(2-methoxybut-1-enyl)benzene was synthesized by reacting cuminaldehyde with methyl propyl ketone, reported by Fuson and Bull (1934). The synthesized product could also be reconverted into starting aldehyde by its treatment with sodium hypochlorite. Characterization of the synthesized compound was done by IR and ^1H NMR spectroscopic techniques.

Scheme 4

Krishna and Reddy (2016) reported the synthesis of Schiff bases derived from N-substituted thiosemicarbazides and cuminaldehyde. The prepared compounds were characterized using various physical and spectral techniques *viz.* elemental analysis, molar conductance, magnetic susceptibility measurements, IR, electronic absorption spectral studies and cyclic voltammetry.

5% CH_3COOH

Reflux

R = H, $-CH_3$, $-C_2H_5$, $-C_6H_5$

Scheme 5

Nine odorant Schiff bases were prepared by condensation of anthranilic acid with corresponding naturally occurring carbonyl compounds namely anisaldehyde, benzaldehyde, cinnamaldehyde, citral, citronellal, cuminaldehyde, veratraldehyde, acetophenone, and α-

ionone employing conventional and microwave irradiation methods. These compounds were characterized using FT-IR, ^{1}H NMR and ^{13}C NMR. Synthesized Schiff bases were screened for antimicrobial activity against *A. niger, Penicillium chrysogenum, S. aureus* and *E. coli*.

CHO + O OH NH$_2$ —MW→ O OH N

Scheme 6

Saraf (1983) reported the reaction of cuminaldehyde and thionyl bromide at room temperature resulted in the conversion of CHO group into $CHBr_2$. Synthesized product was characterized by elemental analysis and ^{1}H NMR.

CHO + HBr ⇌ OH Br H —$SOBr_2$→ OSOBr Br —HBr→ Br Br

Scheme 7

2.3 Nanoparticles of plant extracts

Nanotechnology is a modern area of research due to vast applications in the field of science and technology for manufacturing new materials at nanoscale. There are enormous benefits of nanotechnology which includes enhancement of agricultural productivity by nanoporous zeolites (for controlled release and efficient dosage of water and fertilizer) (Sekhon 2014), nanocapsules (for herbicide delivery and pest management) (Radhika *et al* 2011) and nanosensors (for pest detection) (Ragaei and Sabry 2014). These are used as nanofertilizers as they allowed controlled release of the agrochemical due to high surface area of the particles (Raja *et al* 2017). These are increasingly used to improve crop production leads to the decrease in usage of harmful agents in crop protection (Ragaei and Sabry 2014).

Numerous methods are available for the synthesis of nanoparticles such as chemical reduction of metal ions in aqueous solution with or without stabilizing agents, thermal decomposition in organic solvents, chemical reduction and photo-reduction in reverse micelles and radiation chemical reduction (Rane *et al* 2018). Most of these methods are not

cost-effective and involves the use of toxic, hazardous chemicals, which may result in potential environment and biological risks. Biological methods of nanoparticles synthesis using microorganisms (Joerger *et al* 2000), enzymes, fungus (Shankar *et al* 2003), sugars, biodegradable polymers (chitosan, etc.) (Ahmed *et al* 2014) and plants or plant extracts (Chandran *et al* 2006; Gardea-Torresdey *et al* 2002; Shankar *et al* 2003; Logeswari *et al* 2015: Joshi 2020) have been suggested as successful alternatives of chemical and physical methods (Ahmed and Ikram 2015; Kharissova *et al* 2013).

In the synthesis of nanoparticles, plant extracts may act reducing agents as well as stabilizing agents (Kumar and Yadav 2009). Characteristics of the synthesized nanoparticles were directly influenced by the source of the plant extract (Kumar and Yadav 2009). This is because different extracts contain different concentrations and combinations of organic reducing agents (Mukunthan and Balaji, 2012). Synthesis of metallic nanoparticles of noble metals like gold, silver, and platinum has gained evoked interest due to its vast biological activities.

In recent time, synthesis of silver nanoparticles has gained a considerable interest due to its vast applications in the field of catalysis (Khalil *et al* 2013), agriculture, home water purification systems, medical devices, cosmetics, electronics and household appliances (Wijnhoven *et al* 2009). Silver serves as nutrient for the plants and increase the nutrient uptake efficiency of the plants. It also acts as a potent inhibitor of microbes present in medical and industrial processes. Duhan and his co-workers (2017) reported that silver nanoparticles increase the seed germination efficacy of plants. Silver nanoparticles (AgNPs) are synthesized using a reduction of aqueous Ag^{+} ion by various biological agents *i.e.* micro-organisms or plant extracts (Ahmed *et al* 2016). It plays an important role in medical industry such as topical ointment which is used to prefect infection due to burn and open wounds. Silver nanoparticles are also reported to possess versatile applications such as antimicrobial (Khan *et al* 2014; Kumar *et al* 2014), antitumor effect (Jeyaraj *et al* 2013), sensitivity to detect the presence of various pollutants such as metals (Balavigneswaran *et al* 2014), dyes (Kumar *et al* 2013), antibiotics (Singh *et al* 2012) etc. Recent literature reveals the use of seed or leaf extract from various plants such as *Jatropha curcas* (Bar *et al* 2009), carob leaves (Awwad *et al* 2013), *Olea europaea* (Awwad *et al* 2012), mulberry (Awwad and Salem 2012), *Ficus benghalensis* (Saxena *et al* 2012), *Arbutus Unedo* (ouvaris *et al* 2012), *Ocimum tenuiflorum*, *Solanum tricobatum*, *Syzygium cumini*, *Centella asiatica* and *Citrus sinensis* (Logeswari *et al* 2015), *Nicotiana tobaccum* (Parasad *et al* 2011), *Stevia rebaudiana* (Yilmaz *et al* 2011), mangosteen (Veerasamy *et al* 2011), *Ocimum sanctum* (Singhal *et al* 2011), *Macrotyloma uniflorum* (Vidhu *et al* 2011), *Murraya koenigii* (Christensen *et al* 2011), *Sesuvium portulacastrum* L. (2010), Basil (Ahmad *et al* 2010), *Trianthema decandra* (Geethalakshmi and Sarada 2010), *Rosa rugosa* (Dubey *et al* 2010), banana peel (Bankar *et al* 2009),

Acalypha indica (Krishnaraj *et al* 2010), cycas (Jha and Prasad 2010), *Azadirachta indica* (Nazeruddin *et al* 2014), *Delonix elata* (Sathiya and Akilandeswari, 2014), *Tephrosia purpurea* (Ajitha *et al* 2014), *Melia dubia* (Kathiravan *et al* 2014), *Tribulus terrestris* (Ashokkumar *et al* 2014), *Artemisia nilagirica* (Vijayakumar *et al* 2013), *Boerhaavia diffusa* (Kumar *et al* 2014), *Ficus religiosa* (Antony *et al* 2013), *Piper pedicellatum* (Tamuly *et al* 2013) and *Carica papaya L.* (Joshi 2019) as sources for synthesis of silver nanoparticles.

Mittal *et al* (2013) reported a green method for the synthesis of nanoparticles in which biomolecules, which were present in plant extracts to reduce metal ions to nanoparticles in a single-step reaction. This reduction of metal ion to base metal was quite rapid and readily conducted at room temperature and pressure. Synthesis mediated by plant extracts was environment friendly and agents involved in this process of reduction were water soluble plant metabolites (*e.g.* alkaloids, phenolic compounds, terpenoids) and co-enzymes. Silver (Ag) and gold (Au) nanoparticles had been the particular focus of plant-based synthesis. Extracts of a diverse range of plant species had been successfully used in making nanoparticles.

Sharma *et al* (2016) reported the green synthesis of gold nanoparticles of cumin and fennel seeds. Characterization of the synthesized nanoparticles was done by UV-visible spectroscopy. The UV-visible spectrum of cumin and fennel-gold nanoparticles showed absorption maxima in the range of 535 and 540 nm respectively.

Shalaby *et al* (2015) reported the green synthesis of gold nanoparticles using cumin seeds along with gum Arabic which is used as a non-toxic and eco-friendly ‘green material’. Characterization of nanoparticles was done by UV-Visible and Fourier Transform Infrared Spectroscopy along with electron microscopic techniques *i.e* Transmission Electron Microscopy (TEM) and X-ray Diffraction (XRD.. The TEM analysis of Cumin-Au NPs showed the formation of spherical nanoparticles with an average size of 5.5 nm.

Sneha *et al* (2011) investigated cumin seed for preparation of gold nanoparticles. The effect of pH and temperature on the synthesis of nanoparticles has also been evaluated. Polydispersed particles of size 1-10 nm were obtained at pH 3 and 30 °C. TEM and XPS analysis were used for the characterization of the synthesized nanoparticles.

The green synthesis of silver nanoparticles with the twelve different spices extract has been done by Sinha and Paul (2014). In the present study, the nanoparticles were synthesized by mixing silver nitrate ($AgNO_3$) with different spices extracts. Synthesis of nanoparticles was confirmed by UV-visible and also by the change in the color of the extracts.

Synthesis of silver nanoparticles from the aqueous extract of cumin under microwave irradiation was reported by Kudle *et al* (2012). Color change from pale yellow to reddish brown indicated the reduction of aqueous Ag+. Synthesized nanoparticles were characterized by UV-visible Spectroscopy, Fourier Transform Infrared spectroscopy, Scanning Electron

Microscopy (SEM) and Transmission electron Microscopy (TEM). The synthesized silver nanoparticles were also screened for their antifungal activity activity and showed promising results.

Rajesh *et al* (2018) reported the single-step green synthesis of copper nanoparticles (CuNPs) using *Cuminum cyminum* seed extract. Synthesized CuNPs have been characterized by studying the structural, morphological, optical and antimicrobial properties. Crystal structure, morphology and shape of synthesized CuNPs were studied by X-ray diffraction (XRD), field emission scanning electron microscopy (FESEM) and transmission electron microscopy (TEM) techniques, respectively. Absorption peak at 590 nm using UV-Vis spectroscopy confirms the formation of CuNPs. Synthesized nanoparticles were also screened for their antimicrobial potential and showed the better inhibitory activity against *Pseudomonas spp*. and *Penicillium spp*.

Katti *et al* (2009) reported the synthesis of gold nanoparticles of *Cuminum cyminum* using sodium tetrachloroaurate. The antioxidant characteristics of cumin due to the presence of various phytochemicals are helpful in reducing sodium tetrachloroaurate. Spherical gold nanoparticles were synthesized by mixing of cumin to an aqueous solution of sodium tetrachloro aurate. *Cuminum cyminum* mediated gold nanoparticles did not aggregate suggesting that the cocktail of phytochemicals including proteins serve as excellent coatings on nanoparticles. Characterization of synthesized nanoparticles was done using UV-visible and transmission electron microscopy (TEM) techniques.

Karamian and Kamalnejad (2019) synthesized silver nanoparticles using *Cuminum cyminum* leaf extract as bioreducer. Characterization of nanoparticles was done using UV-vis, FT-IR spectroscopy, Scaning electron microscopy and XRD. Synthesized nanoparticles were also screened for their antibacterial and antifungal activities against four gram positive and gram negative bacteria using agar disc diffusion method and fungus *Fusarium oxysporum*, respectively. Leaf extract and silver nanoparticles of *Cuminum cyminum* showed promising results against gram positive and negative bacteria, respectively.

Choudhary and his co-workers reported the biogenic synthesis of silver nanoparticles using *Cuminum cyminum* seed extract. Phytochemicals present in seeds extract are able to reduce silver ions, because of their strong anti-oxidant potential and also stabilize the newly formed silver (Ag^0) nuclei. Choudhary and his co-workers also noted the effect of pH, concentration of extract, reaction time and stability of nanoparticles by UV-visible spectroscopy. The synthesized nanoparticles were further investigated by XRD, HR-TEM and FT-IR spectroscopy. Synthesized AgNPs act as excellent nanocatalysts towards borohydride reduction of various anthropogenic dye pollutants *viz.* methylene blue (MB), methyl red (MR), rhodamine-B (Rh-B) and 4-nitrophenol (4-NP).

Nanoparticles bearing antimicrobial potential have emerged as a new class of

biomedical materials. Zare *et al* (2017) conducted green synthesis of zinc oxide nanoparticles of *Cuminum cyminum* using zinc nitrate [$Zn(NO_3)_2$] as Zn^{2+} source. It was observed that the factors like concentration, time, temperature and pH have a direct impact on the synthesis of zinc nanoparticles and change in any one of these causes the variation in the process of synthesis. The characteristics of nanoparticles were examined by UV-visible spectroscopy, X-ray diffraction spectroscopy and transmission electron microscopy (TEM). The antimicrobial potential of silver nanoparticles against gram positive and gram negative bacteria was determined using disc diffusion method.

Alphonse and Thanaraj (2019) reported green synthesis of *Cuminum cyminum* mediated nickel oxide nanoparticles. Characterization of synthesized nanoparticles was done using UV-vis, FT-IR, XRD, SEM, EDAX. The shape and particle size was found using Debye Scherrer formula from x-ray diffraction pattern.

The biosynthesis and anticancer activities of *Cuminum cyminum L.* (Cumin) seed extract on human breast adenocarcinoma cell line (MCF-7) and human breast adenocarcinoma metastatic cell line (AU565) was reported by Dinparvar *et al* 2020. The synthetized nanoparticles were characterized by dynamic light scattering (DLS), UV–visible spectroscopy, X-ray diffraction, Fourier-transform infrared spectroscopy and Scanning electron microscopy. The cytotoxic and anticancer effects of AgNPs and Bio-AgNPs were determined by MTT assay.

Keerthiga *et al* (2019) reported the synthesis and antioxidant activity of cumin seed oil mediated silver nanoparticles. Silver nanoparticles were characterized by UV-visible and TEM. Synthesized AgNPs were subjected to DPPH assay to determine the antioxidant activity. Cumin oil mediated AgNPs showed good antioxidant activity.

2.4 Biological activities

Allahghadri *et al* (2010) screened the antioxidant potential of cumin oil and displayed higher antioxidant activity as compared to butylated hydroxytoluene and butylated hydroxyanisole. Oil (5.4 µg) was enough to scavenge 50% of DPPH radicals/ml. The antioxidant capacity of cumin by ABTS and DPPH assays was 3.26 ± 0.29 and 2.16 ± 0.06 (mmol Trolox equivalents/g DW) respectively as reported by Vallverdu-Queralt *et al* (2014). Radical inhibition of *Cuminum cyminum* essential oils by DPPH radical scavenging assay was 83.59% (Romeilah *et al* 2010). The radical scavenging activity was increased with the increase in essential oil concentration.

Rebey *et al* (2012) reported that polyphenols and condensed tannin content was higher in full ripe seeds than unripe ones and exhibited higher antioxidant potential. Unripe cumin seeds had a higher total flavonoid content as compared to half and full ripe cumin seeds.

The antioxidant potential of β-pinene, p-cymene, γ-terpinene, cuminaldehyde and

cumin oils [cumin oleoresin (COR), cumin essential oil (CEO), distillation residue (DR) and distillate condensed from cold trap (CT)] were reported by Chen *et al* (2013). IC_{50} values and kinetics rates were used to evaluate the efficiency of tested samples in scavenging the alkyl peroxyl radical generated in the β-carotene-linoleic acid system and the 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH). The antioxidant potential of cumin seed oils follows the order: DR = COR > CEO > CT. γ-Terpinene appeared the most effective antioxidant compound in cumin seed oil.

Evaluation of lipid peroxidation of *Cuminum cyminum* extract *via* thiobarbituric acid reactive substances (TBARS) was done by Koppula and Choi (2011). The extract showed promising lipid peroxidation inhibition as compared to ascorbic acid in both rat liver and brain.

Atrooz (2013) assessed the antioxidant activity of methanolic and acetonic seed extracts of cumin and caraway *via* the DPPH free radical scavenging and the β-carotene bleaching assay. Both cumin and caraway seed extracts were able to neutralize free radicals over a period of 60 mins in the DPPH assay. The methanolic and acetonic extract of cumin showed slightly higher neutralization ability than caraway extracts.

Prajapati *et al* (2019) conducted the phytochemical screening and antioxidant activity of *Cuminum cyminum* L. and *Nigella sativa* L. Qualitative analysis of cumin extract revealed the presence of alkaloids, flavonoids, saponins, steroids, terpenoids phenol, tannin and absence of protein, glycoside, cardic glycoside, fixed oil, fats and carbohydrates. Evaluation of antioxidant potential *via* DPPH method shows highest activity for cumin seed than black cumin.

Plants of Apiaceae family possess a wide range of compounds with numerous biological activities. Cumin seeds are extensively used in various ayurvedic medicines also especially for the conditions like obesity, stomach pain and dyspepsia (Srinivasan 2018). Phytochemical analysis of cumin seeds showed that it contains alkaloids, anthraquinones, coumarins, flavonoids, glycosides, proteins, resins, saponins, tannins and steroids etc. (Rai *et al* 2012). Secondary metabolites or phytochemicals, are biologically active naturally occurring chemical compounds and plays an important role in defence system of plants against predation by microorganisms, insects and herbivores (Lako *et al* 2007; Santhi and Senogottuvel 2016). These phytochemicals have historically been used as pharmaceuticals, fragrances, flavor compounds, dyes, and agrochemicals (Rathore *et al* 2013). *In vitro* studies reported that phytochemicals such as phenolic compounds have potential role against different diseases and used as anti-inflammatory, anti-mutagenic, antiviral and antibacterial agents (Al Juhaimi and Ghafoor 2013 and Senevirathne *et al* 2006). Cumin extracts are reported to possess antiallergic, antioxidant, antiplatelet aggregation and hypoglycaemic properties (Singh *et al* 2002, Lee 2005, Allahghadri *et al* 2010). Ethanol extract of seed

exhibited antimicrobial activity against biofilm *E. coli* (Bameri *et al* 2013).

Prevention of many diseases is associated with ingestion of different parts of the plants which are rich in natural antioxidants (Atrooz 2009). It was observed that higher intake of these compounds lowers the risk of mortality from different diseases (Ajih and Janardhanan 2002, Lim *et al* 2002; McCune and Johns 2002; Tziveleka *et al* 2002). There are many phytochemicals which may act as a powerful antioxidants such as polyphenols (Shahidi *et al* 1992; Sanchez-Moreno *et al* 1998; Argolo *et al* 2004), flavonoids (Zhang and Wang, 2002) etc. by neutralizing free radicals or by removing their power to create damage. Antioxidants can interfere with the oxidation process by reacting with free radicals, chelating catalytic metals, acting as oxygen scavengers (Shahidi *et al* 1992) and prevent lipid auto oxidation (Brand-Williams *et al* 1995; Bondet *et al* 1997). The antimicrobial properties of essential oil of plants and its extracts have been assessed (Lis-Balchin and Deans 1997), reviewed (Janssen *et al* 1987; Rios *et al* 1987) and it was found that phytochemicals have potential applications in medical procedures, cosmetics, pharmaceuticals and food industries (Baratta *et al* 1998; Baratta *et al* 1998; Iocabellis *et al* 2005; Lo Cantore *et al* 2004; Youdin *et al* 1999).

El-Ghorab *et al* (2010) studied the antioxidant activity of different extracts of *cuminum cyminum.* Methanol extract of the fresh cumin contained highest total phenolic content while registered lowest in hexane extract of cumin (10.6 mg/g dry extract). Cumin essential oil showed highest antioxidant activity by DPPH method. Screening of antioxidant potential *via* DPPH method showed the highest antioxidant activity for cumin essential oil (85.44 ± 0.50%) followed by dried ginger essential oil (83.87 ± 0.50%) and fresh ginger essential oil (83.03 ± 0.54%).

Essential oil and solvent extracts are reported to possess wide range of biological activities. Volatile oil and aqueous extract of cumin exhibited antimicrobial activity as reported by Dorman and Deans (2000). Cumin seed oil and alcoholic extract were effective against *K. pneumonia.* Cumin oil is reported to possess high antioxidant potential due to the presence of monoterpene alcohols (De Martino *et al* 2009). Significant reduction in urinary calcium excretion and augmentation of calcium content and mechanical strength of bones in animals by methanolic extract of cumin was reported by Shirke *et al* (2008). Aqueous extract of cumin seeds showed the protective effect against gentamycin-induced nephrotoxicity (Mahesh *et al* 2010). Antimicrobial potential of methanolic, hydroalcoholic and aqueous extracts of cumin seeds was less than its essential oil (Chaudhary *et al* 2014).

Bettaieb *et al* (2010) studied the antioxidant potential of different parts of cumin. 1,1-Diphenyl-2-picrylhydrazyl (DPPH), β-carotene/linoleic acid, reducing power and chelating power assays were used to determine the antioxidant potential of essential oil of cumin and three acetone extracts of different parts of cumin. The acetone extract of flowers was found to

be highly effective as a DPPH radical scavenger, lipid peroxidation inhibitor and reducing agent with IC_{50} values of 4, 32, and 8 µg ml^{-1}, respectively. Tavakoli *et al* (2015) reported the antimicrobial activity by combined use of essential oil of cumin seed with nisin against *Salmonella typhimurium* and *S. aureus.* The growth of *S. typhimurium* was significantly decreased by the concentration of essential oil ≥ 30 µL/100 ml in combination with nisin ≥ 0.5 µg/ml. From the above results, it has been concluded that the use of essential oil along with nisin was effective against *S. typhimurium* and *S. aureus* bacteria and they could also be used as a substitutes of the chemicals that were used for preservation of food. *Cyminum cuminum* were tested for antimicrobial activity *in vitro* by the micro dilution method. Ethanol extract of seed exhibited antimicrobial activity against *E. coli* (Bameri *et al* 2013).

Cuminaldehyde is the major compound of all the solvent extracts and essential oil of the cumin seeds. It possess wide range of biological activities such as antimicrobial (Oroojalian *et al* 2010; Rasheehan *et al* 2013; Suleimana *et al* 2009), antifungal (Sekine *et al* 2007), anti-inflammatory (Philipe *et al* 2005; Woguem *et al* 2014), antimalarial (Tamura *et al* 2010), antioxidant (Barakat and Mohamed 2011), antifibrillation (Gambin *et al* 2011; Burre *et al* 2003), anticarcinogenic (Tsai *et al* 2016; Shu-meiang *et al* 2016) and antidiabetic (Lee 2005).

In cumin, cuminaldehyde was identified as a potent inhibitor of mushroom tyrosinase inhibitor. Kubo and Kinst-Hori (1998) reported that cuminaldehyde inhibited the oxidation of L-3,4-dihydroxyphenylalanine (L-DOPA) by mushroom tyrosinase with an ID_{50} of 7.7 µg ml^{-1} (0.05 mM). Its oxidized analogue *i.e.* cumic acid (*p*-isopropylbenzoic acid) was also found to inhibit this oxidation with an ID_{50} of 43 µg ml^{-1} (0.26 mM).

Antimicrobial potential of extracted essential oil was tested by Farag *et al* (1989) against G(-) bacteria (*P. fluorescens*, *E. coli* and *Serratia marcescens*), G(+) bacteria (*S. aureus*, *Micrococcus spp*., *Sarcina spp*. and *Bacillus subtilis*), acid fast bacterium (*Mycobacterium phlei*) and yeast (*Saccharomyces cerevisiae*) using the filter paper disc agar diffusion method. Cumin oil possessed very strong antimicrobial activity.

Essential oil of *Cuminum cyminum* exhibited strong antimicrobial potential against *E. coli*, *S. aureus* and *L. monocytogenes*. Complete death of *E. coli*, *S. aureus* and *L. monocytogenes* took place in 20, 180 and 90 mins, respectively (Farag *et al* 1989).

Cumin essential oil showed activity against *E. coli*, *P. aeruginosa* and *Salmonella sp*. and their inhibitory zones were 18, 10 and 23 mm respectively (Stefanini *et al* 2003). Iacobellis *et al* (2005) studied the antibacterial activity of *cuminum cyminum* against gram positive and gram negative bacteria. Cumin was found to be effective against the genera *Clavibacter, Curtobacterium, Rhodococcus, Erwinia, Xanthomonas, Ralstonia* and *Agrobacterium*. It was found to be least effective against the bacteria belonging to genus *Pseudomonas*.

The effectiveness of the essentials oil from cumin (Cuminum cyminum) was tested on the growth of some bacteria commonly used in the food industry, *Lactobacillus curvatus*, *Lactobacillus sakei*, *S. carnosus* and *S. xylosus* or related to food spoilage *Enterobacter gergoviae*, *Enterobacter amnigenus* by agar disc diffusion method. It showed an inhibitory effect against all tested bacteria (Manuel *et al* 2008).

The volatile oil of *Cuminum cyminum* was active against *S. epidermidis*, *S. aureus*, *S. haemolyticus, Propionibacterium acnes*, *Corynebacterium diphtheriae*, *Erysipelothrix rhusiopathiae*, *Bacillus cereus, Clostridium tetani, C. difficile, E. coli, Salmonella typhi, K. pneumoniae, Vibrio cholerae, Aeromonas hydrophila, Mycobacterium tuberculosis*, *Neisseria gonorrhoeae*, *Asperigillus niger, Saccharomyces cerevisae and Colletrotrichum gloeosporioides* (Al-Snafi 2016).

Essential oil from seeds of *Cuminum cyminum*, exerted antifungal activity against *A. flavus* was reported by Dwivedi and Dubey (1993). Aspergillus species produced Aflatoxin B_1 (AFB_1) which was highly toxic and hepatocarcinogenic metabolite. Some natural products were used to kill fungi and also destroy the toxins produced by them along with their toxin-producing agents. Mohammadpour *et al* (2012) studied the antifungal potential of the *Cuminum cyminum* L. essential oil against fungi *A. flavus* PICC-AF39, *A. flavus* PICC-AF24, *A. parasiticus* NRRL-2999 and *A. niger.* Sixty per cent of the spores of four *Aspergillus* were killed by *C. cyminum* L. oil within 12 hrs.

Effectiveness of cumin essential oil against *Botrytis cinerea*, *Rhizopus stolonifer* and *Aspergillus niger* was reported by Hadian *et al* (2008). The incorporation of 750 µl/1 from *cuminum cyminum* oil to PDA medium completely inhibited the growth of *B. cinerea*, *R. stolonifer* and *A. niger*. The fungicidal activities of cuminaldehyde and *p*-isopropyl benzoic acid extracted from *Cuminum cyminum* were studied against *Alternaria solani*, *Verticillium dahliae*, *Rhizoctonia cerealis*, *Alternaria alternata*, *Gaeumannomyces graminis*, *Sclerotinia sclerotiorum*, *Phytophthora capsici*, *Thanatephorus cucumeris*, *Blumeria graminis* and *Botrytis cinerea*. The results showed that both compounds had fungicidal activities. Cuminaldehyde and *p*-isopropyl benzoic acid had better inhibitory effects against *Sclerotinia sclerotiorum.*

Essential oils obtained from *Hyssopus officinalis*, *Cuminum cyminum* and *Thymus vulgaris* were tested for their antifungal potential against *A. flavus*. Essential oils were applied in five different percentages (0.000, 0.125, 0.25, 0.375 and 0.500%). The results showed that the essential oil of *Cuminum cyminum* was more effective in comparison with other essential oils (Karbin *et al* 2009). Essential oil of cumin (*Cuminum cyminum*) significantly increased the storage life of the strawberry fruits because they inhibited the growth of *Botrytis cinerea* (Marjanlo *et al* 2009).

Cuminum cyminum oil possessed higher antifungal and antibacterial activities against

Vibrio spp. with a diameter of inhibition zones ranging from 11 to 23 mm and their minimum bactericidal concentration (MBC) and minimum inhibition concentration (MIC) values ranged between 0.078-0.31 mg ml^{-1} to 0.31-1.25 mg ml^{-1} respectively (Hajlaoui *et al* 2010). A great inhibition of *Cuminum cyminum* essential oil was recorded on *Pseudomonas syringae* (Mahmoudi *et al* 2010).

The antifungal potential of the volatile components of the essential oil of fruits of *Cuminum cyminum* was tested on dermatophytes and phytopathogens, fungi, yeasts and some new *Aspergilli* by Romagnol *et al* (2010). Antifungal activity of *Cuminum cyminum* showed that it was effective on all fungi particularly on the dermatophytes. It was most effected on the *Trichophyton rubrum i.e.* it had inhibited at lowest dose *i.e.* at 5 µl. Phytopathogens were less effective to this treatment.

Essential oil of the *Cuminum cyminum* possessed antifungal activity against different pathogens of the Candida species (Naeini *et al* 2014). The inhibition zone of the fungi against the tested organisms ranged between 7 to 50 mm. *Cuminum cyminum* oil was found to be effective against *C. albicans* and *C. dubliniensis* with minimal inhibitory concentration (MIC) of 289 mg L^{-1}.

Anjaum and Akhtar (2012) evaluated the antifungal potential of different essential oils extracted from cinnamon buds, clove buds, cumin seeds against *Penicillium italicum* causing agent of blue mold disease on citrus plant. Shayegh *et al* (2008) reported the antimicrobial potential and biofilm-formation preventive properties of *Cuminum cyminum* essential oil and chlorhexidine against *Streptococcus mutans* and *Streptococcus pyogenes*. *Cuminum cyminum* induced mild antibacterial and *in vivo* biofilm preventive effects.

Screening of antimicrobial activity of essential oil by Toroglu (2011) against *Micrococcus luteus* LA 2971, *Bacillus megaterium* NRS, *Bacillus brevis* FMC 3, *Enterococcus faecalis* ATCC 15753, *Pseudomonas pyocyaneus* DC 127, *Mycobacterium smegmatis* CCM 2067, *E. coli* DM, *Aeromonas hydrophila* ATCC 7966, *Yersinia enterocolitica* AU 19, *S. aureus* Cowan 1, *Streptococcus faecalis* DC 74 bacteria, and *Saccharomyces cerevisiae* WET 136 and *Kluvyeromyces fragilis* DC 98 fungi revealed that *Cuminum cyminum* essential oil (2 µl) exerted antibacterial effect against all the tested microorganisms with MIC ranged from 10- 60mm. While the inhibition zone was higher in the bacteria *E. faecalis*, it was lowest in *E. coli* and *P. pyocyaneus*. Among the fungi, the inhibition zone against *K. fragilis* was higher than *S. cerevisiae*.

The antimicrobial effects of cumin essential oil was studied against *Listeria monocytogenes* AUFE 39237, *E. coli* ATCC 25922, *Salmonella enteritidis* ATCC 13076, *Proteus mirabilis* AUFE 43566, *Bacillus cereus* AUFE 81154, *Saccharomyces uvarum* UUFE 16732, *Kloeckera apiculata* UUFE 10628, *C. albicans* ATCC 10231, *Candida oleophila* UUPP 94365, and *Metschnikowia fructicola* UUPP 23067 (Irkin and Korukluoglu 2009).

Cumin oils actively inhibited the growth of yeasts.

Derakhshan *et al* (2008) reported the activity of cumin seed essential oil and alcoholic extract against *K. pneumoniae* ATCC 13883 and clinical *K. pneumonia* isolates by evaluating their effect on cell morphology, capsule expression and urease activity. Growth of *K. pneumoniae* strains exposed *Cuminum cyminum* extracts resulted in cell elongation and repression of capsule expression.

Awan *et al* (2013) studied antibacterial activity of chloroformic and isoamyl alcohol extracts of *Cuminum cyminum* against six human bacterial pathogens namely *Streptococcus pyogenes, S. epidermidis, K. pneumonia, S. aurues, Serratia marcesnces* and *Pseudomonas aeruginosa* using agar disc diffusion method. Both the solvent extracts of cumin had promising activity against *P. aeruginosa, S. marcesnces* and *S. pyogenes*.

Kedia *et al* (2014) reported the potential of essential oil (EO) of cumin seed (as a plant based shelf life enhancer) against fungal, aflatoxin contamination and lipid peroxidation. Essential oil showed efficiency as a preservative in stored wheat and chickpeas. MIC and minimum aflatoxin inhibitory concentration of essential oil were 0.6 and 0.5 µl/ml respectively. It displayed toxicity against several food borne fungi. Effect of cumin essential oil on the mycelia growth of 90 fungal isolates was reported by El-Said and El-Hady (2014). Cumin oil was highly effective against all the isolates of tested fungi.

CHAPTER-III

MATERIAL AND METHODS

This chapter provides information regarding the materials used and the experimental procedures employed during the course of investigation. The various chemicals used, essential oil extraction method, thin layer chromatography, column chromatography for fractionation of cumin oil, isolation of major constituent *i.e.* cuminaldehyde, chemical derivatization of cuminaldehyde and testing of extracted essential oil, cuminaldehyde and derivatized cuminaldehyde for their antioxidant, antimicrobial and antifungal activities are reported. All the melting points were determined in open capillaries and are uncorrected. GC-MS analysis was recorded at Advanced Instrumentation Research Facility (AIRF), Jawaharlal Nehru University, New Delhi. UV spectra were recorded on UV 2600 spectrophotometer of Techcomp company. IR spectra were got scanned from Central Instrument Laboratory (CIL). ^{1}H-NMR and ^{13}C-NMR spectra were got scanned from Sophisticated Analytical Instrumentation Facility (SAIF), Panjab University, Chandigarh. IR spectra were recorded on Perkin-Elmer, Model RX-1 FT-IR Spectrophotometer with wavenumber (ν) in cm^{-1}. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker Avance II 400 MHz and Bruker Avance II 400 MHz spectrophotometers using TMS as internal reference. The chemical shifts were expressed in δ (ppm) values and the abbreviations 's', 'd', 't', 'dd', 'bs' and 'm' stand for singlet, doublet, triplet, double doublet and multiplet, respectively.

3.1 MATERIALS

The adsorbents and various reagents used are given below:

3.1.1 Silica gel

1. Silica gel - Qualigens Fine Chemicals, Mumbai
   (for column chromatography)
   Pore size: 60-120 mesh
   pH (10 percent aqueous suspension): 7
   Chloride max: 0.02 per cent
   Iron max: 0.03 percent
2. Silica gel-G - Thermo Fisher Scientific India Private Limited, Mumbai
   (for thin layer chromatography)

3.1.2 Various reagents used

1. 2-Aminopyridine - Sisco Research Laboratories Private Limited, Mumbai
2. 4-Amino phenazone - S.D. Fine Chemicals Limited, Mumbai
3. 4-Amino triazole - Loba Chemie Private Limited, Mumbai
4. 2-Amino-3-methylpyridine – HiMedia Laboratories Private Limited, Mumbai
5. *p*-Aminophenol – Central Drug House Private Limited, Mumbai

6. 2-Aminopyrimidine - HiMedia Laboratories Private Limited, Mumbai
7. 4-Aminopyridine - Central Drug House Private Limited, New Delhi
8. Acetone - Thermo Fisher Scientific India Private Limited, Mumbai
9. Acetophenone - Loba Chemie Private Limited, Mumbai
10. Agar-agar - Central Drug House Private Limited, New Delhi
11. Aluminium chloride - Thermo Fisher Scientific India Private Limited, Mumbai
12. Ammonia solution - Thermo Fisher Scientific India Private Limited, Mumbai
13. Aniline - Loba Chemie Private Limited, Mumbai
14. Anisaldehyde - Sisco Research Laboratories Private Limited, Mumbai
15. *m*-Anisidine - Loba Chemie Private Limited, Mumbai
16. Benzylamine - Loba Chemie Private Limited, Mumbai
17. Boric acid - Thermo Fisher Scientific India Private Limited, Mumbai
18. *p*-Bromoacetophenone - Loba Chemie Private Limited, Mumbai
19. Bromocresol green - Thermo Fisher Scientific India Private Limited, Mumbai
20. *p*-Chloroacetophenone - Loba Chemie Private Limited, Mumbai
21. Chloroform - Thermo Fisher Scientific India Private Limited, Mumbai
22. Copper sulfate - Qualigens Fine Chemicals, Bombay
23. Cuminaldehyde - HiMedia Laboratories Private Limited, Mumbai
24. Dichloromethane - Thermo Fisher Scientific India Private Limited, Mumbai
25. Diethyl ether - Loba Chemie Private Limited, Mumbai
26. Dimethyl sulfoxide - Thermo Fisher Scientific India Private Limited, Mumbai
27. 2,4-Dinitrophenyl hydrazine - Loba Chemie Private Limited, Mumbai
28. Ethanol - S.D. Fine Chemicals Limited, Mumbai
29. Ethyl acetate - Thermo Fisher Scientific India Private Limited, Mumbai
30. Ferric chloride - Thermo Fisher Scientific India Private Limited, Mumbai
31. *p*-Fluoroacetophenone - Loba Chemie Private Limited, Mumbai
32. Follin-Ciocalteu reagent - Sisco Research Laboratories Private Limited, Mumbai
33. Gallic acid - S.D. Fine Chemicals Limited, Mumbai
34. Glacial acetic acid - Sisco Research Laboratories Private Limited, Mumbai
35. Hydrazine hydrate - Thermo Fisher Scientific India Private Limited, Mumbai
36. Hydrochloric acid - Thermo Fisher Scientific India Private Limited, Mumbai
37. Iodine - S.D. Fine Chemicals Limited, Mumbai
38. Methanol - Sisco Research Laboratories Private Limited, Mumbai
39. *m*-Methoxyacetophenone - Loba Chemie Private Limited, Mumbai
40. *p*-Methyacetophenone - Loba Chemie Private Limited, Mumbai
41. Ninhydrin reagent - Thermo Fisher Scientific India Private Limited, Mumbai
42. *p*-Nitrophenyl hydrazine - Loba Chemie Private Limited, Mumbai

43. Orthophosphoric acid - Thermo Fisher Scientific India Private Limited, Mumbai
44. Petroleum ether - Thermo Fisher Scientific India Private Limited, Mumbai
45. Picric acid- Central Drug House Private Limited, New Delhi
46. *o*-Phenylenediamine - Loba Chemie Private Limited, Mumbai
47. Phenyl hydrazine - Loba Chemie Private Limited, Mumbai
48. Phenylisothiocyanate - Loba Chemie Private Limited, Mumbai
49. Potassium acetate - Thermo Fisher Scientific India Private Limited, Mumbai
50. Potassium iodide - Qualigens Fine Chemicals, Bombay
51. Potato Dextrose Agar - Central Drug House Private Limited, New Delhi
52. Quercetin - S.D. Fine Chemicals Limited, Mumbai
53. Sodium chloride - Thermo Fisher Scientific India Private Limited, Mumbai
54. Sodium carbonate - Thermo Fisher Scientific India Private Limited, Mumbai
55. Sodium hydroxide - Thermo Fisher Scientific India Private Limited, Mumbai
56. Sodium nitroprusside - Sisco Research Laboratories Private Limited, Mumbai
57. Sodium sulphate - Sisco Research Laboratories Private Limited, Mumbai
58. Sodium tungstate - Thermo Fisher Scientific India Private Limited, Mumbai
59. Sulfuric acid - Thermo Fisher Scientific India Private Limited, Mumbai
60. Thiosemicarbazide - Sisco Research Laboratories Private Limited, Mumbai
61. Triethylamine - Thermo Fisher Scientific India Private Limited, Mumbai

3.1.3 Instruments

1. Digestor- Pelican Equipments, India
2. Hot-air oven- MAC, India
3. Hot Plate- MAC, India
4. Ice maker- Blue star Limited, India
5. Incubator-cum-shaker- MAC,India
6. Kjeltec^{TM}2100- Foss, Sweden
7. Magnetic stirrer with hot plate- Tarsons, New Delhi
8. Melting point apparatus- Relitech, Haryana
9. Mixer grinder- Gopi, Ambala, India
10. Muffle furnace- MAC,India
11. Refrigerator - LG, New Delhi
12. Rotary vaccum evaporator- Perfit, India
13. Socs Plus- Pelican Equipments, India
14. UV-visible spectrophotometer- Systronics India Limited, Naroda, Ahmedabad
15. Vortex shaker - REMI, New Delhi
16. Water bath - Narang Scientific Works, New Delhi
17. Weighing balance - Mettler Toledo, New Delhi

3.1.4 Glassware and Plastic ware

1. Clevenger-type apparatus- LABCO, Delhi, India
2. Glass columns (1.5 cm i.d x 40 cm long)- LABCO, India
3. Petridishes- Tarsons Products Private Limited, Kolkata, India
4. Plastic containers of 6.5 cm height and 10.0 cm diameter- Tarsons Products Private Limited, Kolkata, India
5. Thin layer chromatographic equipment
   TLC plates 5 x 10 cm: 20 x 10 cm glass plates- LABCO, India
   Development tank: LABCO, India

3.2 CHROMATOGRAPHIC TECHNIQUES

Chromatography is defined as a procedure of separation in which the components to be separated are distributed between two phases, one is stationary (stationary phase) while the other (the mobile phase) moves in a definite direction. The different components of the mixture travel at different rates, which leads them to separate. The separation of the components is based on differential partitioning between the two phases *i.e.* mobile and stationary phase. Subtle differences in a partition coefficient of the compound cause differential retention on the stationary phase and thus affect the separation.

The rate of migration of compound on an adsorbent depends upon the solvent used. The solvents in order of their increasing polarity (increasing eluting powers) are:

Petroleum ether < cyclohexane < carbon tetrachloride < toluene < benzene < dichloromethane < chloroform < ether < acetone < alcohol.

Since chromatography is the most useful technique for identification and separation of components from mixture, therefore, the brief description of various chromatographic techniques used during research work is as follows:

3.2.1 Column Chromatography

Column chromatography involves the separation of components from a mixture by eluting the column with solvent of increasing polarity in a step wise manner and the collection of fractions according to the sequence regarding the eluted products being monitored by thin layer chromatography. For column chromatography, column was packed with silica gel having 60-120 mesh size activated at 110 ºC for one hr. Clean-up of column was carried out using hexane as the solvent. Firstly, the material to be chromatographed was dissolved in minimum quantity of hexane and adsorbed on silica gel for 30 min. The solution was allowed to flow down in a vertical column. Separation of different compounds was carried out by eluting the column with hexane followed by solvents of increasing polarity. For the recovery of the material, solvent was distilled using rotary vacuum evaporator.

3.2.2 Thin Layer Chromatography (TLC)

It is a technique used to separate mixtures. In this technique, a thin layer of adsorbent

i.e. silica gel, alumina or cellulose is coated on a chromatoplate and this thin layer of adsorbent is called stationary phase. Silica gel G (containing gypsum as binder) is most commonly used adsorbent. After the application of given sample on the plate, a solvent or solvent mixture (known as mobile phase) is drawn up the plate *via* capillary action.

3.2.2.1 Preparation of Thin Layer Chromatographic plates

The plates were coated with adsorbent silica gel G containing $CaSO_4$ as binder by dipping a pair of plates held back to back in slurry (10 g of silica gel per 100 ml of water), then these chromatograph plates were air dried for about 4 hrs at room temperature and finally activated at 110 ºC for 45 mins. The spotting of plates was done with the help of capillary tubes after drying of plates for 10-15 mins. The spot was applied 1.50 cm upward from the lower end of chromatographic plate. The plates were placed inside the developing chamber after the initial spotting. When the chromatoplate was fully developed, it was removed from the developing chamber and air dried. After the plate was fully dried, the spots were visualised in iodine chamber or by spraying with spray reagents. The chromatoplates after spraying well were immediately placed in the oven maintained at 110 ºC for 5-10 mins in order to reveal the spots.

3.2.2.2 Spray Reagents

The chromatoplates were developed in suitable solvent and visualization of spots was done by spraying the plates with methanol: sulfuric acid (19: 1) as the spray reagent.

3.3 TEST MATERIAL

Cumin seeds of variety GC-4 were taken from Rajasthan State Seed and Organic Production Certification Agency, Jaipur. The sample was cleaned and washed to remove extraneous matter, dried in open air and then in oven at 50 ºC. The dried seeds were grinded using mixer grinder and kept in air tight containers at 4 ºC for further use.

3.4 PROXIMATE COMPOSITION OF CUMIN SEEDS

Analysis for moisture, crude protein, fat, crude fibre, total minerals and total carbohydrates or nitrogen free extract (NFE) of grinded seeds of cumin was carried out according to A.O.A.C. (2000) methods. All the tests were carried out in triplicates.

3.4.1 Moisture content

Pre-weighed crucible was filled with 5.0 g of cumin seeds and was kept in a hot air temperature controlled oven at 45 ºC to obtain constant weight. The crucibles were immediately transferred to a desiccator and weighed when these attained room temperature. Per cent moisture was calculated from loss in weight of the sample.

$$\text{Moisture (\%)} = \frac{\text{Loss in wt. of sample (g)} \times 100}{\text{Wt. of sample}}$$

3.4.2 Total Minerals

Weighed sample (5 g) was taken in triplicate in pre weighed silica crucible and was burnt on hot plate until charred. The crucible and its content were placed in the muffle furnace at 600° C until greyish white residue was obtained. From the weight of the residue, the percentage of total minerals was calculated.

$$\text{Total minerals (\%)} = \frac{\text{Wt. after ignition (g) x 100}}{\text{Wt. of sample}}$$

3.4.3 Crude Protein

Determination of crude protein was done by macro Kjeldahl method. Protein content was calculated by multiplying nitrogen content with a factor of 6.25 (Yeoh and Wee 1993).

Principle

Cumin seeds were oxidised with concentrated H_2SO_4 in the presence of oxidising agent *i.e.* mixture of potassium sulfate and copper sulfate (K_2SO_4: $CuSO_4$; 9: 1) to form CO_2 and H_2O and nitrogen was released as ammonia. Ammonia exists in H_2SO_4solution as ammonium sulphate. The known volume of ammonium sulfate solution formed was steam distilled with NaOH to liberate ammonia which was absorbed in excess of boric acid. The amount of ammonia absorbed in boric acid was determined by titrating it with 0.01N HCl using methyl red-bromocresol green mixed indicator. End point was indicated by change in color from blue to pink.

Reagents

- Digestion mixture (1 part of $CuSO_4$: 9 parts of K_2SO_4)
- Concentrated H_2SO_4
- Saturated NaOH (40%)
- 0.01 N HCl
- Methyl red-bromo cresol green mixed indicator (0.5 g bromo cresol green + 0.1 g methyl red), volume made to 100 ml with 95% absolute alcohol
- 4% Boric acid

Procedure

In Kjeldahl's digestion tubes, 100 mg of powdered cumin seeds was digested on digestor (Pelican Equipments) with 10 ml concentrated sulfuric acid. To this mixture, add a pinch of the digestion mixture in order to obtain a clear solution and the solution was diluted with distilled water to make total volume 25 ml. A measured aliquot (5 ml) was distilled with 10 ml of 40% NaOH solution using Kjeldahl distillation unit (Kjeltec™2100). About 140-150 ml of the distillate was collected in 25 ml of 4% boric acid solution using methyl red-bromo cresol green mixed indicator and titrated with 0.01N HCl till light pink color was obtained as end point. Nitrogen content of the sample was calculated from the formula:

$$\text{Nitrogen (\%)} = \frac{\text{Vol. of 0.01N HCl (Sample- blank)} \times 0.0014 \times \text{Vol. of dilution (ml)} \times 100}{\text{Wt. of sample (g)}}$$

Crude protein (%) = Nitrogen (%) x 6.25

3.4.4 Crude Fibre

Principle

Crude fibre is the organic residue which remains after food sample has been treated under standardized conditions with boiling dilute H_2SO_4 and boiling dilute NaOH solution. Crude fibre consists largely of cellulose and hemicellulose together with lignin.

Procedure

Digest fat free sample (1.00 g) firstly with 1.25% H_2SO_4 for 30 mins and then with 1.25% NaOH solution in 600 ml spoutless beaker. While refluxing, the round bottom flask filled with cold water was placed on top of spoutless beaker as condenser. Afterwards, sample was filtered through pre-weighed sintered glass crucible and washed with distilled water to remove NaOH and then dried in hot air temperature controlled oven. The weight was recorded after cooling in a desiccator. The residue was then charred on heater and ignited in furnace at 600 ºC for 3 to 4 hr. The crucible was cooled in a desiccator and again weight was recorded. The loss in weight after ignition represented the crude fibre in the sample.

$$\text{Crude Fibre (\%)} = \frac{\text{(Wt. of crucible + dried wt. of residue before ashing)} - \text{(Wt. of crucible + dried wt. of residue after ashing)} \times 100}{\text{Wt. of sample (g)}}$$

3.4.5 Fat

Oil content was estimated by solvent extraction method. The instrument used was Socs Plus AS Series 4.

Principle

Soxhlet is used to extract oil or fat from the sample using solvent. The extraction is fast as compared to conventional method, because sample is immersed in boiling solvent. Generally solvent extraction is carried out in three stages.

- Boiling
- Rinsing
- Recovery

The homogenised sample is taken in a cellulose thimble. During boiling stage, the thimble with sample is immersed in the solvent. The solvent is heated near its boiling point. Most of the oil or fat is extracted in this stage. The temperature of solvent is further increased, so that the solvent is evaporated and collected in solvent compartment. Recovered solvent is allowed to flow through the thimble by opening the Teflon knob. During this stage, the oil or fat sticking to the walls of the thimble will be washed and collected in the beaker. The recovered

solvent can be drained out and used for subsequent samples.

Procedure

Empty beaker was weighed (W_1 g). The sample (1.0 g) was weighed and transferred to the thimble. Petroleum ether 60-80 ºC (80 ml) was taken in beaker and thimble holder was placed into beaker and then thimble was placed in thimble holder. The beaker was placed between heater and extractor. Perfect sealing of the beaker against the Teflon ring was ensured. The 'POWER' switch and temperature controller in the control panel was switched on. The boiling temperature in the temperature controller was set. The boiling temperature was selected near the boiling point of the solvent used, to ensure no condensation of solvent during this stage, so that the given time of contact between solvent and sample can be fully utilized. 'START' key was pressed to initiate the boiling time. When level of solvent in the beaker was below the thimble, the Teflon valve was opened to flow the recovered solvent through the thimble to remove the oil or fat sticking to the walls of the thimble to the beaker. The solvent from the beaker was removed. The beaker was removed along the fat and kept inside the oven for some time and weight of the beaker was taken (W_2 g). Fat (%) was calculated by the following calculation:

$$\text{Fat (\%)} = \frac{(W_2 - W_1) \times 100}{\text{Wt. of sample (g)}}$$

3.4.6 Total carbohydrates

Total carbohydrates of cumin seeds were calculated using following equation:

Total Carbohydrates (%) = 100 - (Crude Protein % + Crude Fibre % + Crude Fat % + Total minerals %+ Moisture %).

3.5 MINERAL COMPOSITION

Powdered cumin seeds were dry ashed at 550 ºC. The ash was boiled with 10 ml of 20% hydrochloric acid in a beaker and then filtered into a 100 ml standard flask. It was made up to the mark with deionised water. The minerals were determined from the resulting solution using Atomic Absorption Spectroscopy (AAS) on Perkin Elmer Analyst 200. The burner system provides the thermal energy necessary to dissociate the chemical compounds, providing free analyte atoms so that absorption occurs. The spectrometer measures the amount of light absorbed at a specific wavelength using a hollow cathode lamp as the primary light source, a monochromator and a detector.

3.6 QUALITATIVE PHYTOCHEMICAL ANALYSIS

For extraction of phytochemicals from different solvent extracts, dried powdered seeds (5.00 g) were mixed separately with 25 ml acetone, petroleum ether, water, methanol, ethanol, dichloromethane, chloroform and ethyl acetate and kept in refrigerator (4-5 °C) for 3-4 days with occasional stirring. After that extracts were filtered and solvents were removed by rotary vacuum evaporator and the concentrated extract was further evaporated to get dry

powder. The dried powder was preserved in air tight glass bottle. For each extract percentage yield was calculated. It was then used for qualitative analysis of secondary metabolites by dissolving them in respective solvents (50 ml). Yields of extracts obtained were calculated as follows and the data is presented in Table 8:

$$\text{Yield (\%)} = \frac{\text{Wt. of extract x 100}}{\text{Wt. of sample}}$$

3.6.1 Identification Tests

The different solvent extracts of cumin seeds were analyzed for the presence of various phytoconstituents such as alkaloids, saponins, tannins, coumarins, amino acids, flavonoids, proteins, phenols and carbohydrates. Phytochemical tests were carried out by adopting standard procedures as reported by Sawant and Godghate (2013) and Santhi and Sengottuvel (2016).

Alkaloids

Extract (3.00 ml) was taken in a test tube. 1 ml of hydrochloric acid was added and mixture was subjected to gentle heating for atleast 20 min. The mixture was then cooled, filtered and the filtrate was used to proceed following tests:

a) Wagner test: Filtrate (3.00 ml) was treated with 1.00 ml of Wagner's reagent (2.00 g iodine and 8.00 g KI in 100 ml of water); brown reddish colored precipitates were formed that indicated the presence of alkaloids.

b) Hager's test: Filtrate (3.00 ml) was treated with 1.00 ml of Hager's reagent (1.00 g of picric acid in 100 ml of water), formation of yellow colored precipitates confirmed the presence of alkaloids.

Saponins

Extract (5 ml) was mixed with 20 ml of distilled water then shaked on vortex shaker in graduated cylinder for 15 mins. Presence of saponins was confirmed by the formation of stable foam.

Tannins

Equal volume of extract and $FeCl_3$ *i.e.* 4.00 ml were mixed in a test tube; appearance of blue-green color showed the presence of condensed tannins.

Anthocyanins

Equal volume *i.e.* 2.00 ml of extract, 2 N HCl and NH_3 was added in test tube, vortex the contents, appearance of pink red turning to blue violet confirmed the presence of anthocyanins.

Emodins

In a test tube containing 3.00 ml of extract, add 2.00 ml of ammonium hydroxide and 3.00 ml of benzene, vortex the contents, appearance of red color indicated presence of

emodins.

Coumarins

In 2.00 ml of extract in a test tube, 3.00 ml of 10% aqueous NaOH was added. Formation of yellow color indicated presence of coumarins.

Amino acids

Ninhydrin test: To the 2 ml extract and equal volume of ninhydrin reagent was added. Vortex and boil the contents for two mins, formation of blue colour indicates the presence of amino acid.

Flavonoids

a) Alkaline reagent test: Extract (3.00 ml) was treated with 10 % 2 ml of aqueous sodium hydroxide solution;

intense yellow color indicated the presence of flavonoids.

b) NH_4OH test: Extract (3 00 ml) was treated with 2 ml of 10 % ammonium hydroxide solution and development of yellow fluorescence indicated positive test for flavonoids.

Proteins

Xanthoproteic test: Solvent extract (1.00 ml) was treated with few drops of concentrated nitric acid and formation of yellow indicates the presence of proteins.

Total phenols

Ferric chloride test: 3.00 ml of extract was treated with four drops of alcoholic ferric chloride solution. Appearance of bluish black color indicated the presence of phenols.

Carbohydrates

Extracts was dissolved in a 5 ml of distilled water, filtered and then filtrate was used to carry out the following test:

a) Molisch's Test: Filtrate were treated with 2 drops of alcoholic α-naphthol solution, formation of violet ring at the junction indicates the presence of carbohydrate.

b) Iodine Test: Extract (2.00 ml) was treated with 5 drops of alcoholic iodine solution, blue color indicates the positive test.

3. 7 QUANTITATIVE PHYTOCHEMICAL ANALYSES

Total phenolics, total flavonoids, saponins and tannins will be determined by Ebrahimzadeh *et al* (2008); Calabro *et al* (2004); Fenwick and Oakenfull (1983); Swain and Hillis (1959) methods, respectively.

3.7.1 Extraction

For quantitative estimation of phytochemicals present in different solvent extracts, dried powdered seeds (5.00 g) were refluxed separately with 25 ml acetone, petroleum ether, water, methanol, ethanol, dichloromethane, chloroform and ethyl acetate. The refluxed material was filtered and methanol was evaporated. Total volume was made 50 ml for different solvent extracts, separately. The extracts thus prepared was used further for

quantitative estimation of phytochemicals namely total flavonoids, total phenols and tannins present in different solvent extracts and the results are presented in Table 9.

3.7.2 Total phenolic content

Total phenolic content in different solvent extracts was determined according to Follin-Ciocalteu method (Ebrahimzadeh *et al* 2008).

Principle

All phenolic compounds present in the different solvent extracts of cumin seeds are oxidized by Folin-Ciocalteu reagent *i.e.* a mixture of phosphotungstic acid and phosphomolybdic acid. After oxidation of all phenolic compounds, the reagent gets reduced to a mixture of blue oxides of tungsten and molybdenum. The blue colouration produced has a maximum absorption at 730 nm and is directly proportional to the quantity of phenolic compounds present.

Reagents

- Methanol
- Gallic acid
- Sodium carbonate (2%)
- Folin-Ciocalteu reagent (100 µl)

Estimation of total phenolic content

Total phenolic content was determined according to Follin-Ciocalteu method (Ebrahimzadeh *et al* 2008). For this purpose, 0.10 ml of Folin-Ciocalteu reagent was added to 0.10 ml of extract followed by the addition of 2.00 ml of 2% Na_2CO_3 solution. The resulting mixture was then allowed to stand at room temperature for half an hr and the absorbance was measured at 765 nm using UV-visible Spectrophotometer against blank containing water and both the reagents without extracts. A calibration curve was constructed using gallic acid solutions as a standard (Figure 2) and results were expressed in terms of milligrams of gallic acid equivalent per gram (mg GAE g^{-1}).

Preparation of standard curve of total phenols

A standard calibration curve of gallic acid was obtained by preparing the solutions of 10, 20, 40, 60 and 80 µg ml^{-1} concentrations. Stock solution (80 µg ml^{-1}) of volume 10 ml was made in methanol and desired concetrations were made by dilution. Each concentration (0.10 ml) of gallic acid solution was mixed separately with 0.10 ml of Folin-Ciocalteu reagent and 2.00 ml of 2% sodium carbonate (Na_2CO_3). The resulting mixture was allowed to stand at room temperature for 30 min and the absorbance was noted at 765 nm using UV-visible Spectrophotometer against a blank prepared similarly but containing distilled water instead of standard solution of gallic acid. A standard curve was obtained by plotting absorbance against different concentrations of gallic acid (Figure 1).

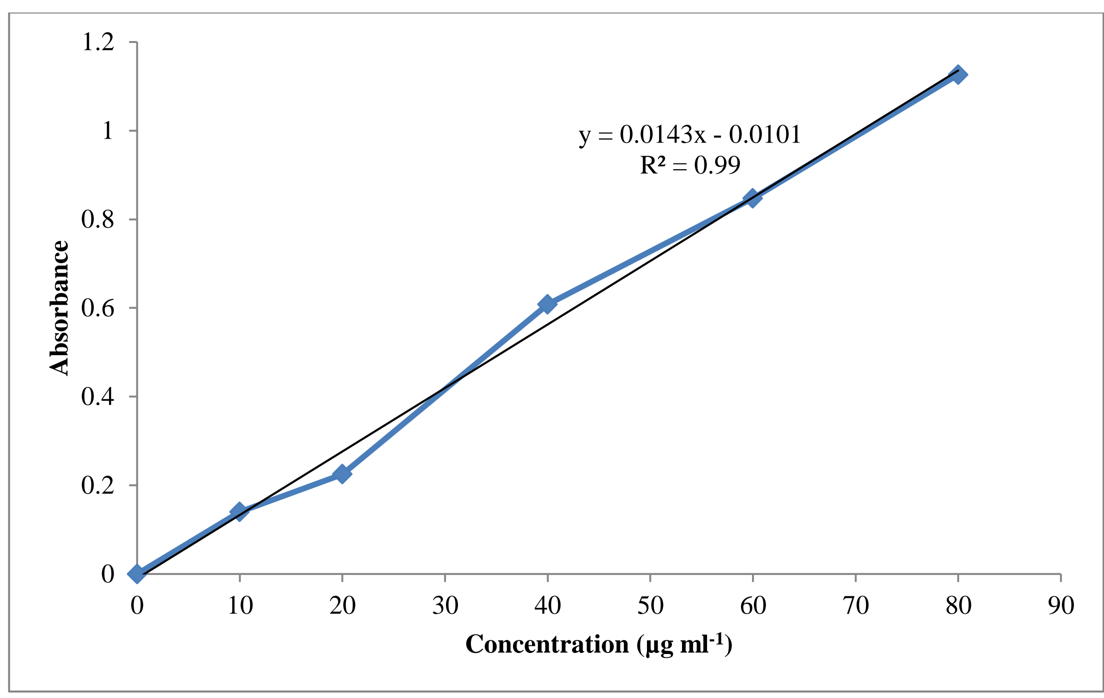
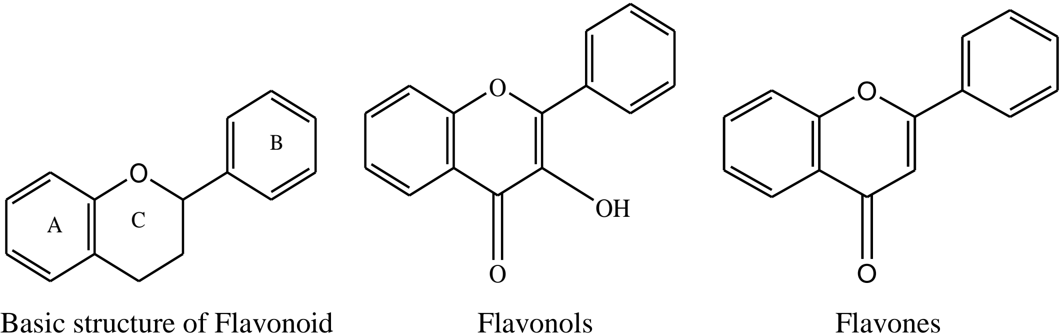


Figure 1: Standard curve plotted between concentration of gallic acid and absorbance

3.7.3 Flavonoids

The flavonoid content was determined according to Calabro *et al* (2004).



Basic structure of Flavonoid   Flavonols   Flavones

Principle

The basic principle of flavonoids involves the formation of stable complex between aluminium chloride with the keto group and hydroxyl group of flavones and flavonols. In addition, aluminium chloride also forms acid labile complexes with the orthodihydroxyl groups in the A or B ring of flavonoids.

Reagents

- Quercetin
- Aluminium chloride (10%)
- Potassium acetate (1M)

Estimation of total flavonoids content

The flavonoid content was determined according to Calabro *et al* (2004). For estimation of total flavonoids in different solvent extracts of cumin seeds, Ten per cent aluminium chloride (0.10 ml) and 0.10 ml of potassium acetate (1M) was added in 0.50 ml of

extract. Eighty per cent (4.30 ml) methanol was added to resulting mixture in order to make total volume 5.00 ml. The absorbance of different solvent extracts was measured at 415 nm using UV-visible spectrophotometer against blank containing respective reagents. The amount of total flavonoids present in the extracts was calculated from the standard curve (Figure 3) and results were expressed as milligrams of quercetin equivalent per gram (mg QE g^{-1}).

Preparation of standard curve of total flavonoids

Different concentations *i.e.* 10, 20, 40, 60, 80 and 100 µg ml^{-1} were prepared to draw a standard calibration curve of quercetin. Stock solution of 100 µg ml^{-1} was made in 10 ml of methanol and further dilutions were prepared. Each concentration of standard solution (0.50 ml) was mixed with equal volume *i.e.* 0.10 ml of aluminium chloride (10%) and potassium acetate (1 M) each. the total volume was made 5.00 ml by adding 4.30 ml of methanol (80%) in it. The absorbance of resulting solutions was noted at 415 nm using UV-visible spectrophotometer against blank containing distilled water. A standard curve was obtained by plotting absorbance against different concentrations of quercetin (Figure 2).

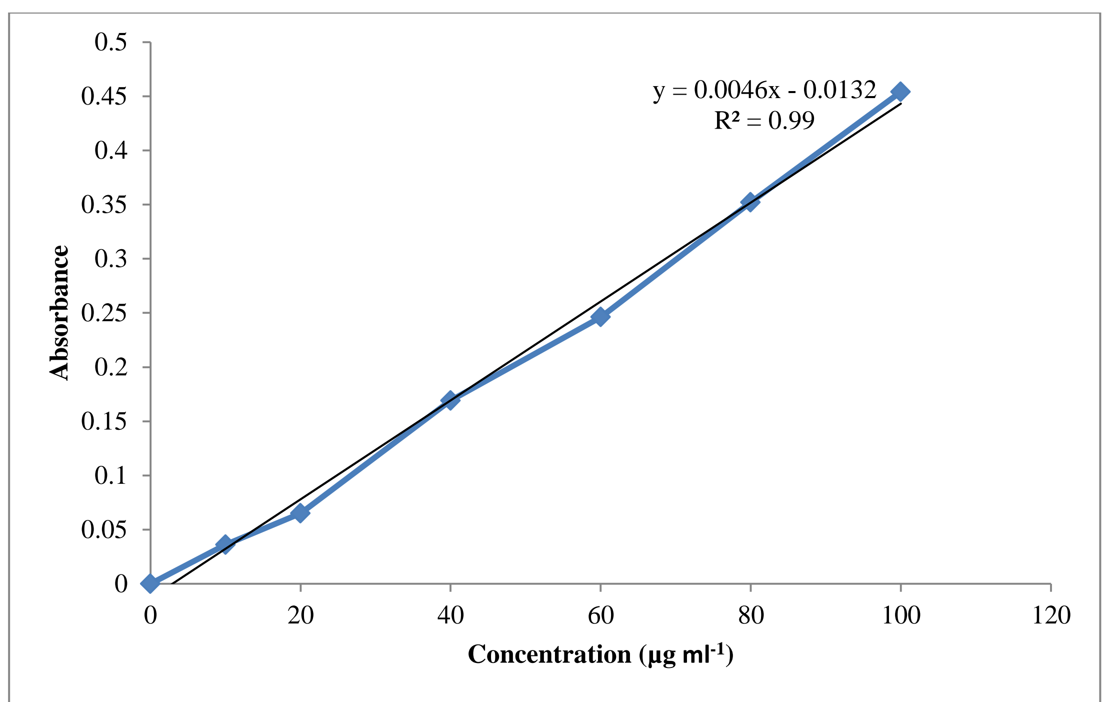


Figure 2: Standard curve plotted between concentration of quercetin and absorbance

3.7.4 Saponins

Saponins were determined using method given by Fenwick and Oakenfull (1983).

Reagents

- Reagent A: 0.5 ml anisaldehyde and 99.5 ml ethyl acetate
- Reagent B: concentrated sulphuric acid

Estimation of saponins: Saponins were determined using method given by Fenwick and Oakenfull (1983). In a test tube, take 1.00 ml of extracted sample and kept in boiling water

bath in order to remove alcohol. After cooling, the resulting mixture 2.00 ml of ethyl acetate was added in each test tube. To this, equal volume *i.e.* 1.00 ml of reagent A (0.5 ml anisaldehyde and 99.5 ml ethyl acetate) and reagent B (concentrated sulfuric acid) were added. After vortex, the test tubes were kept at room temperature for 10 mins. The intensity of red color developed was measured at 430 nm. A standard curve was obtained by plotting absorbance against amount of saponin (Figure 4).

Preparation of standard curve of saponins: A standard calibration curve was drawn by preparing different concentrations such as 10, 20, 30, 40, 50, 100 and 200 µg ml^{-1} of saponin. Stock solution having concentration 200 µg ml^{-1} was prepared in 10 ml of methanol and then further concentrations were made. Each concentration of standard (1.00 ml) was kept in boiling water bath to remove alcohol. After cooling, 2.00 ml of ethyl acetate, 1.0 ml of reagent A and 1.0 ml of reagent B was added. Vortex the contents and kept at room temperature for 10 mins. Absorbance was measured at 430 nm using UV-visible spectrophotometer against a blank prepared similarly but containing distilled water. A standard curve was obtained by plotting absorbance against amount of saponin (Figure 3).

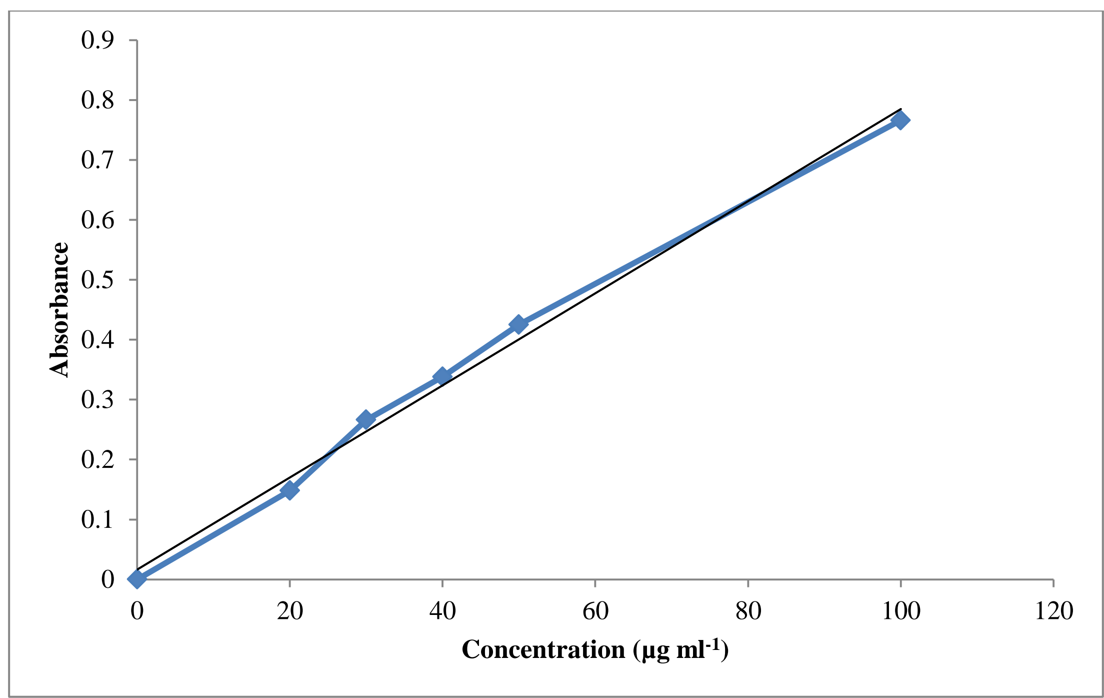


Figure 3: Standard curve plotted between concentration of saponin and absorbance

3.7.5 Tannins

Tannins were determined using method given by Swain and Hillis (1959).

Principle

Estimation of tannins is based on the determination of blue color intensity formed by reduction of phosphotungstomolybdic acid by tannin like compounds in alkaline solution.

Reagents

- 10% methanol

- Folin Denis Reagent-Dissolved 100 g of sodium tungstate in 750 ml of distilled water. 20 g phosphomolybdic acid and 50ml orthophosphoric acid was added to resulting mixture. Reflux the contents for 2 hrs, cooled and diluted to 1 litre with distilled water
- Saturated Na_2CO_3 solution

Estimation of tannins

In a test tube, 1.00 ml of extract, 8.50 ml of distilled water and 0.50 ml of Folin Denis reagent was added. After 3 mins, 1 ml of saturated solution of sodium carbonate was added. Vortex the contents and absorbance was measured at 760 nm.

Preparation of standard curve of tannins

A standard calibration curve was made with solutions of 20, 30, 40, 50 and 100 μg ml^{-1}concentrations of tannic acid. Stock solution (100 μg ml^{-1}) was made in 10 ml of methanol and then further dilutions were made. In 1.00 ml of each concentration of standard solution, 8.50 ml of distilled water and 0.50 ml of Folin Denis reagent was added. After 3 mins, 1.00 ml of saturated sodium carbonates solution was added. The contents were mixed well and absorbance was measured at 760 nm using UV-visible Spectrophotometer against a blank prepared similarly but containing distilled water instead of standard solution of tannic acid. A standard curve was obtained by plotting absorbance against amount of tannic acid (Figure 4).

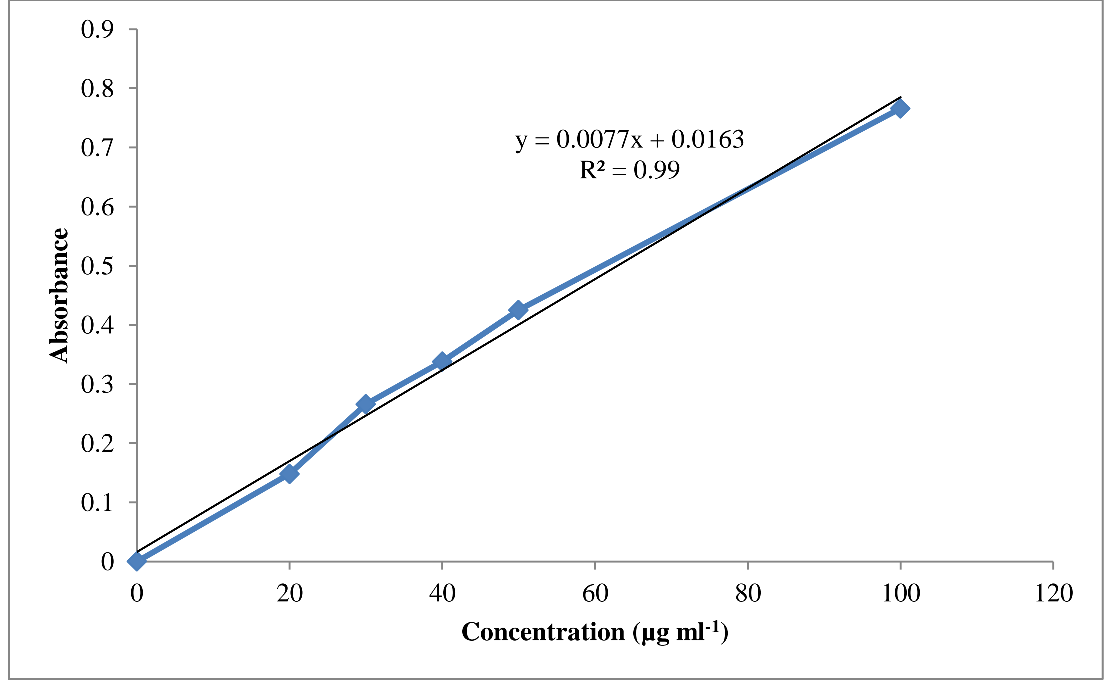


Figure 4: Standard curve plotted between concentration of tannic acid and absorbance

3.8 EXTRACTION OF ESSENTIAL OIL

Cumin seeds were grinded in mixture cum grinder and were used for the extraction of essential oil by hydro-distillation method.

3.8.1 Hydrodistillation

Extraction of essential oil from cumin seeds was done by hydro distillation using Clevenger-type apparatus. Seeds (500 g) were dipped in a 500 ml round bottomed flask and kept overnight. The contents were transferred in 1 litre round bottomed flask, mixed thoroughly and subjected to hydro-distillation for four hrs using a Clevenger type apparatus (Boughendjioua 2019). The contents were refluxed for 10 hrs (until no more oil was obtained). The layer containing a mixture of essential oil and little water was collected in conical flask. **Partitioning of essential oil layer**

The collected oil was extracted with diethylether as oil was soluble in it and was then dried and dehydrated over anhydrous sodium sulphate.

Essential oil layer obtained by hydro distillation was transferred into 250 ml separatory funnel. The collected oil was extracted with diethylether as oil was soluble in it and was partitioned thrice using diethyl ether (100, 50 and 50 ml). The diethyl ether layer (upper layer) containing oil was passed over anhydrous sodium sulfate to remove traces of water present if any. Diethyl ether was removed using rotary vacuum evaporator and the essential oil was stored in a refrigerator at 4 °C. Per cent yield of extracted essential oil was calculated using following equation:

$$\text{Yield (\%)} = \frac{\text{Wt. of essential oil (g) x 100}}{\text{Wt. of sample taken (g)}}$$

3.9 THIN LAYER CHROMATOGRAPHY OF CUMIN ESSENTIAL OIL

Essential oil of cumin seeds was dissolved in a diethyl ether and applied 1.50 cm from the base on thin layer chromatoplates (20 x 10 cm). The chromatoplates were developed for 30 mins using benzene:chloroform (7:3) as the solvent system. The chromatoplates were removed from the developing chamber and air dried. The spots were then sprayed with methanol: sulfuric acid spray (19:1) reagent and the chromatoplates were kept in oven operating at 80 ºC to visualize the spots. Four spots were observed having R_f values 0.48, 0.63, 0.78 and 0.92.

3.10 PROPERTIES OF CUMIN ESSENTIAL OIL

Essential oil of cumin seeds was collected and various properties were recorded such as colour, state and odour (Table 1).

Table 1: Physical properties of essential oil of cumin seeds

Property	Essential oil
Color	Pale Yellow
State	Liquid
Odor	Strong and Stringent

3.11 GAS CHROMATOGRAPHY-MASS SPECTROMETRY

3.11.1 GC-MS of essential oil

Gas chromatography-mass spectroscopy (GC-MS) analyses of essential oil of cumin seeds was carried out on QP2010 Plus, Shimadzu, Japan, equipped with an Rtx-5 MS capillary column (30.0 m x 0.20.25 mm i.d., 0.25 µm film thickness) for the separation of its components. The injector was maintained at 260°C and operated in split injection mode (split ratio 50) with the split valve closed for one min and injection volume was 6 µL. Helium gas was used as the carrier gas at a constant pressure of 69 kPa. The column oven was initially maintained at 50°C for two mins, raised to 210°C at 3°C/min, then to 250°C at 6°C/min. The interface temperature was 270°C and the ionization mode was electron impact (70 eV). The mass selective detector was operated in the scan mode between 40 and 650 m/z. Data acquisition was started 3.0 mins after injection. MS parameters used were; Ionisation Voltage (EI) 70 eV, peak width 2s, mass range 40-650 amu and detector voltage 1.5 V. Peak identification was done by matching the mass spectra with mass spectra data and retention indices available on database of NIST08, WILEY8, Perfumery and Flavor and Fragrance libraries.

3.11.2 GC-MS of different solvent extracts

Different solvent extracts *i.e.* acetone, petroleum ether, water, methanol, ethanol, dichloromethane, chloroform and ethyl acetate of cumin seeds were analysed using Shimadzun Nexis GC-2030 gas chromatograph (GC) coupled with Shimadzu TQ8050 NX mass spectrometer (Shimadzu Corporation, Japan). Samples were injected using Shimadzu AOC-20i plus auto-injector and AOC-20s plus auto-sampler unit. The capillary column used was SH-Rxi-5Sil MS (5% diphenyl/95% dimethylpolysiloxane, Shimadzu) (30m × 0.25mm × 0.25µm). Helium was the carrier gas at a flow rate of 1.17 ml/min. Temperature program: initial temperature 50 ºC for 2 mins; ramp at 3 ºC/min to 210 ºC and held for 2 min followed by next increase at the rate of 10 ºC/min to reach a temperature of 280 ºC which was held for 6 mins. Injection temperature: 260 ºC, injection mode: split, sample injection volume: 1 µL, split ratio: 20, Mass detector conditions, ion source temperature: 230 ºC, ionization mode: Electron impact (EI), 70 eV, interface temperature: 270 ºC, start time: 3 mins and end time: 70 mins. The optimization of retention times and chromatographic resolution were conducted in scan mode for identification of different solvent extracts.

3.12 ISOLATION OF CUMINALDEHYDE FROM ESSENTIAL OIL BY COLUMN CHROMATOGRAPHY

3.12.1 Isolation of pure compound from cumin seed essential oil

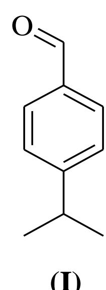
Cumin seed essential oil (5.0 g) was chromatographed over silica gel. Column was packed with silica gel (600 g) for column chromatography with 60-120 mesh size activated at 110˚C for one hr. Essential oil was dissolved in hexane (2.0 ml) and then adsorbed on silica

gel for 30 mins. Column was eluted with hexane and polarity was increased using chloroform. Each fraction was monitored using thin layer chromatography and compared R_f value with cuminaldehyde (0.78).

Table 2: Different fractions of essential oil after Column Chromatography

S. No.	Eluent (ml)	Weight (g)	TLC based remarks
1.	Hexane (12 × 100)	1.87	Mixture
2.	Hexane: Chloroform (99 mL:1mL) (8 × 100)	0.78	Mixture
3.	Hexane: Chloroform (98 mL:2 mL) (8 × 100)	0.29	Mixture
4.	Hexane: Chloroform (95 mL:5 mL) (8 × 100)	1.25	Cuminaldehyde
5.	Hexane:Chloroform(90 mL:10 mL) (12 × 100)	0.81	Mixture

Fraction 4 (Table 2) was identified as cuminaldehyde on the basis of R_f and spectral analyis:



(I)

Spectral data of cuminaldehyde (I)

Boiling point: 235-237 ºC

In UV-visible spectroscopy, cuminaldehyde (I) absorbed at 255 nm.

IR (KBr, ν, cm^{-1}) spectrum showed prominent peaks at 3290 (OH stretching), 3061 (aromatic C-H stretching), 2964 (ν_{as} CH_3 stretching), 2869 (ν_{sym} CH_3 stretching), 2809 (C-H stretching of aldehyde), 1677 (C=O stretching), 1646, 1592 and 1424 (aromatic C=C stretching), 1466 (ν_{as} CH bending), 1365 (C-H bending of gem-dimethyl), 1255 (C-O stretching), 933 and 808 (C-H bending), 735 (C-C bending) (Bennie *et al* 2014).

^{1}H NMR spectrum ($CDCl_3$, 'δ', ppm) of cuminaldehyde (I) showed a doublet of six protons of gem-dimethyl group in the range of 1.27-1.28 (*J*= 6.96 Hz). A multiplet corresponding to CH group of isopropyl was observed in the range of 2.94-3.01, For aromatic protons displayed as two doublets in the range 7.31-7.33 (*J*= 8.00 Hz) and 8.04-8.06 (*J*= 8.00 Hz). Aldehydic proton of cuminaldehyde gave a singlet at 10.20 (s, 1H, -CHO).

^{13}C NMR spectrum ($CDCl_3$, 'δ', ppm) recorded a peak at 23.78 due to two equivalent carbon atoms of gem-dimethyl group. Peak of CH of isopropyl group was displayed at 36.30. Four aromatic carbons were observed at 126.70, 129.32, 134.30 and 154.25. Aldehydic

carbon was recorded at 191.24.

3.13 PREPARATION OF SEED EXTRACT FOR SYNTHESIS OF METAL NANOPARTICLES

Cumin seed powder was weighed (10 g) and dipped in distilled water (100 ml). Keep it as such for 24 hrs in the refrigerator. Filter the contents through Whatmann No. 1 filter paper to obtain the homogeneous aqueous extract. The obtained aqueous extract was rich in natural antioxidants and was used as a reducing agent for the reduction of silver metal in order to synthesize silver nanoparticles (AgNPs). Different concentrations (1mM, 3mM and 5mM) of silver nitrate solution were prepared in double distilled water. 5 ml, 10 ml and 15 ml of prepared extract were added in each 10 ml of different prepared silver nitrate solutions. All these reactions were carried out in 30 ml culture tube. Culture tubes were subjected for heating in a water bath for 30 mins at 80°C (Balavijayalakshmi and Ramalakshmi 2017). Color change of the plant extract from light yellow to dark brown indicated the formation of the nanoparticles. Once the color intensities of the solutions were developed, the tubes were removed from heating and were stored in a dark place at room temperature to prevent agglomeration of the nanoparticles. The optical properties of the synthesized AgNPs were studied with the help of UV-Vis spectra. Transmission Electron Microscopic (TEM) analysis was used to determine the size of the synthesized nanoparticles.

3.13.1 PURIFICATION AND CONCENTRATION OF SILVER NANOPARTICLES FOR TESTING BIOLOGICAL ACTIVITIES

The purification and concentration of silver nanoparticles from the final reaction mixture was transferred to pre-weighed sterile 50 ml centrifuge tubes. The preparations were then centrifuged at 4000 rpm for 2 hrs, at 4 °C. Supernatants were discarded and the pellet was washed in 10 ml of distilled water to remove any contaminating plant material before centrifugation for one hr. This wash step was repeated twice to remove water soluble biomolecules such as proteins and cellular metabolites. Then they were dried in an oven at 37 °C for 24 hrs to determine the dry mass of the metal nanoparticles (difference between mass of tube with nanoparticles and mass of tube).

3.14 EXPERIMENTAL

Chemical derivatizations of cuminaldehyde

Schiff bases (II-XVIII), Chalcones (XIX-XXIV) and pyrazolines (XXV-XXXVI), were synthesized using following procedures.

3.14.1 Schiff bases of cuminaldehyde

3.14.1.1 *N*-(4'-isopropylbenzylidene)benzenamine (II)

Equimolar amounts of cuminaldehyde (0.01 mole, 1.51 ml) and aniline (0.01 mole,

0.91 ml) were taken in a 250 ml round bottom flask using ethanol as a solvent. Add catalytic amount of glacial acetic acid to the reaction. Reflux the reaction mixture for 4 hrs. Progress of the reaction was monitored by thin layer chromatography. Brown colored product was obtained in good yield (70.00%). Recrystallization resulted in formation of *N*-(4'-isopropylbenzylidene)benzenamine (II) having melting point 133-135 °C.

3.14.1.2 4-(4'-Isopropylbenzylideneamino)phenol (III)

Schiff base (III) was prepared by condensation of cuminaldehyde (0.01 mole, 1.51 ml) with *p*-aminophenol (0.01 mol, 1.09 g) in the presence of catalytic amount of glacial acetic acid using ethanol as a solvent and the mixture was refluxed for 4 hrs. The progress of reaction was monitored by TLC. On completion of reaction the product was separated as light brown crystalline solid which was filtered, dried, and recrystallized from chloroform yielded (84.03%) pure Schiff base (III) having melting point 93-95 °C.

3.14.1.3 *N*-(4'-isopropylbenzylidene)-3-methoxybenzenamine (IV):

A mixture of cuminaldehyde (0.01 mol, 1.51 ml) and *m*-anisidine (0.01 mol, 1.12 ml) were dissolved in ethanol. The contents were subjected to refluxing for 4 hrs after adding catalytic amount of glacial acetic acid. Thin layer chromatography is used to check the progress of reaction. After the completion of the reaction, light brown colored solid product was obtained after pouring the hot reaction mixture in ice cold water. The solid product was then obtained was filtered and obtained in 77.05% having melting point 207-209 °C.

3.14.1.4 *N*-(4'-isopropylbenzylidene)(phenyl)methanamine (V)

Equimolar amount of cuminaldehyde (0.01 mole, 1.51 ml) and benzylamine (0.01 mole, 1.09 ml) was dissolved in ethanol. Add 5 drops of glacial acetic acid in the reaction mixture and reflux for 8 hrs. Thin layer chromatographic technique was used to check the reaction progress. Brown colored solid product (melting point: 192-194 °C) was obtained in good yields (87.48%) after the recrystallization process.

3.14.1.5 *N*-(4'-isopropylbenzylidene)pyridin-2-amine (VI)

2-Aminopyridine (0.01 mole, 0.94 g) was dissolved in ethanol in the presence of glacial acetic acid. A solution of cuminaldehyde (0.01 mole, 1.51 ml) was added dropwise and refluxed for 4 hrs. Monitoring of reaction progress was done by TLC. Brown colored solid product was then recrystallized from chloroform. Yield and melting point of synthesized product was 71.13% and 121-123 °C, respectively.

3.14.1.6 *N*-(4'-isopropylbenzylidene)pyridin-4-amine (VII)

An equimolar methanolic solution of 4-aminoantipyrine (5 mmol) and cuminaldehyde (5 mmol) were mixed and gently heated for 2 hrs with constant stirring. The characteristic

pale yellow precipitate obtained was filtered out and recrystallized from methanol. Fine pale yellow crystals were obtained upon slow evaporation at room temperature. It was washed with alcohol, ether and dried in vacuum desiccator over anhydrous calcium chloride.

An equimolar ethanolic solution of 4-aminopyridine (0.01 mole, 0.94 g) and cuminaldehyde (0.01 mole, 1.51 ml) were mixed completely in the presence of catalytic amount of glacial acetic acid and refluxed for 4 hrs. White colored crystalline product was filtered, recrystallized in chloroform and was optained in 72.11% having melting point 110-112 °C. A single spot on a chromatographic plate confirmed the purity of product.

3.14.1.7 *N*-(4'-isopropylbenzylidene)-3-methylpyridin-2-amine (VIII)

N-(4-isopropylbenzylidene)-3-methylpyridin-2-amine was synthesized by refluxing an equimolar amount of cuminaldehyde (0.01 mol, 1.51 ml) and 2-amino-3-methylpyridine (0.01 mol, 1.08 g) in the presence of glacial acetic acid (5 drops) using ethanol as a solvent. The resulting mixture was subjecte to refluxing for 4 hrs. The progress and purity of the Schiff base was checked by TLC. The synthesized product (m.p. 105-107 °C) was obtained in 75.26%.

3.14.1.8 *N*-(4'-isopropylbenzylidene)pyrimidin-2-amine (IX)

A mixture of cuminaldehyde (0.01 mol, 1.51 ml) and 2-aminopyrimidine (0.01 mol, 0.95 g) and catalytic amount of glacial acetic acid was refluxed for 4 hrs with continuous monitoring by TLC. Then the reaction mixture was filtered and the white crystalline product recovered by recrystallization with chloroform. The obtained yield of the product (IX) was 82.19% having melting point 97-99 °C.

3.14.1.9 4-(4'-Isopropylbenzylideneamino)-2,3-dimethyl-1-phenyl-1,2-dihydropyrazol-5-one (X)

Condensation of cuminaldehyde (0.01 mol, 1.51 ml) with 4-aminophenazone (0.01 mol, 2.03 g) yielded 4-(4'-isopropylbenzylideneamino)-2,3-dimethyl-1-phenyl-1,2-dihydropyrazol-5-one (X) in ethanol. The reaction proceeds in the presence of glacial acetic acid. Refluxed the reaction mixture for 4 hrs and product was obtained in 80.53% yield having melting point 144-146 °C. Completion of reaction was confirmed using thin layer chromatography.

3.14.1.10 *N*-(4'-isopropylbenzylidene)-4*H*-1,2,4-triazol-4-amine (XI)

Cuminaldehyde (0.01 mol, 1.51 ml) and 4-amino-1,2,4-triazole (0.01 mol, 0.84) were dissolved in ethanol (20 ml) and refluxed for 4 hrs, cooled and poured into crushed ice. The precipitate obtained was recrystallized using ethanol. Monitoring of reaction progress was done using thin layer chromatography. *N*-(4'-isopropylbenzylidene)-4*H*-1,2,4-triazol-4-amine (m.p. 138-140 °C) was obtained in 86.13% yield.

3.14.1.11 (N^1E,N^2E)-N^1,N^2-bis(4'-isopropylbenzylidene)benzene-1,2-diamine (XII)

Cuminaldehyde (0.02 mole *i.e.* 3.02 ml) was mixed with *o*-phenylenediamine (0.01 mole, 1.08 g) in ethanol in the presence of catalytic amount of glacial acetic acid. Reflux the reaction content for four hrs. Check the progress the reaction using TLC. A light brown colored solid product having melting point 154-156 °C was obtained in 82.17% yields.

3.14.1.12 1-(4'-Isopropylbenzylidene)thiosemicarbazide (XIII)

Cuminaldehyde (0.01 mol, 1.51 ml) and thiosemicarbazide (0.01 mol, 0.75 g) were taken in a round bottom flask using ethanol as a solvent. Add 5 drops of glacial acetic acid and reflux the contents for 3 hrs. On cooling, white colored product was obtained which was filtered, dried and the yield was 89.33%. Completion of reaction was confirmed using TLC. The solid product was obtained after recrystallization from ethanol. The melting point of synthesized compound was 146-148 °C.

3.14.1.13 1-(4'-Isopropylbenzylidene)-4-phenylthiosemicarbazide (XIV)

1-(4-Isopropylbenzylidene)-4-phenylthiosemicarbazide (XIV) was synthesized by reacting phenylthiosemicarbazide with cuminaldehyde. Phenylthiosemicarbazide was synthesized by reacting equimolar amounts of phenylisothiocyanate (0.01 mole, 1.20 ml) and hydrazine hydrate (0.01 mole, 0.48 ml). The reaction gets completed in 5 mins and a white colored solid product was obtained. Wash the product with water in order to remove excess hydrazine hydrate. Filter and dry the product and use it for the synthesis of thiosemicarbazones.

In 250 ml rounded bottomed flask, equimolar amount of cuminaldehyde (0.01 mole, 1.51 ml) and phenylthiosemicarbazide (0.01 mole, 1.67 g) was added in ethanol. Add 5 drops of glacial acetic acid. Refluxed the reaction mixture for 3 hrs. Progress of reaction was calculated using TLC. White colored solid product (XIV) (melting point: 140-142 °C) was obtained in 90.42% after recrystallization from ethanol.

3.14.1.14 1,2-Bis(4'-isopropylbenzylidene)hydrazine (XV)

A quick reaction took place on double moles of cuminaldehyde (0.02 mole, 3.02 ml) and hydrazine hydrate (0.01 mole, 0.48 ml) in a round bottomed flask in the presence of glacial acetic acid. Refluxed the reaction mixture for one hr. Progress of reaction was checked by thin layer chromatography. White colored solid product (85.01%, melting point: 122-124 °C) was obtained after recrystallization from ethanol.

3.14.1.15 1-(4'-Isopropylbenzylidene)-2-phenylhydrazine (XVI)

In the round bottom flask of 250 ml, cuminaldehyde (0.01 mole, 1.51 ml) was dissolved in an ethanol. Added equimolar amount of phenylhydrazine (0.01 mole, 0.98 ml) along with catalytic amount of glacial acetic acid in it. Reflux the reaction mixture for one hr.

Progress of reaction was monitored by TLC. Pour the hot reaction mixture on crushed ice. Brown color precipitates were formed, filtered and dried. Yield and melting point of synthesized product was 70.08% and 110-112 °C, respectively.

3.14.1.16 1-(4'-Isopropylbenzylidene)-2-(4''-nitrophenyl)hydrazine (XVII)

Synthesis of 1-(4-isopropylbenzylidene)-2-(4-nitrophenyl)hydrazine was done by dissolving cuminnaldehyde (0.01 mole, 1.51 ml) with *p*-nitrophenylhdrazine (0.01 mol, 1.53 g) in ethanol. Subject the reaction mixture for refluxing after adding catalytic amount of glacial acetic acid. Advancement of reaction was checked by TLC. An orange colored crystalline solid compound having melting point 135-137 °C, was obtained in good yields (87.18%).

3.14.1.17 1-(4'-Isopropylbenzylidene)-2-(2'',4''-dinitrophenyl)hydrazine (XVIII)

0.76 ml (0.005 mol) of cuminaldehyde was dissolved in 5 ml of 2, 4-dinitrophenylhydrazine solution (2, 4 DNP) which was made by dissolving 2,4-dinitrophenylhydrazine (0.005 mol, 1.0 g) in 5 ml of concentrated H_2SO_4 and then slowly added this solution to a solution of 7.0 ml water in 25 ml 95% ethanol. In a 100 ml beaker, dissolved cuminaldehyde (0.74g) in ethanol and added above prepared 2,4 DNP solution to it. Dark orange colored precipitates were obtained. Wash the precipitates with excess of water, then filtered and dried the precipitates. Single spot on the chromatographic plate confirmed the formation of product. Yield and melting point of synthesized product was 92.37% and 140-142 °C, respectively.

3.14.2 Chalcones of cuminaldehyde (XIX-XXIV)

3.14.2.1 (*E*)-3-(4'-Isopropylphenyl)-1-phenylprop-2-en-1-one (XIX)

In a 250 ml round bottom flask, 0.01 mole of acetophenone (1.16 ml) was dissolved in methanol (10 ml) and 5 ml of 33% aqueous sodium hydroxide was added to it. In another flask, a solution of cuminaldehyde (0.01 mole, 1.51 ml) in methanol (10 ml) was prepared and drop-wise added to above prepared solution over an interval of 15-20 mins with continuous stirring. The reaction mixture was then stirred for 24 hrs at low temperature in order to get good yield of product. Reaction progress was monitored with TLC. After completion of reaction, it was poured over crushed ice and filtered off. Filtrate was neutralised with dilute HCl (3 N) and kept at 3-4 ºC till the formation of white colored precipitates. The precipitates were filtered, dried and washed with petroleum ether to remove unreacted acetophenone and cuminaldehyde. Synthesized product was obtained in semi-solid state having yield 70.16%.

3.14.2.2 (*E*)-1-(4'-Fluorophenyl)-3-(4''-isopropylphenyl)prop-2-en-1-one (XX)

(*E*)-1-(4'-Fluorophenyl)-3-(4''-isopropylphenyl)prop-2-en-1-one (XX) was synthesized by base catalyzed condensation of a cuminaldehyde (0.01 mole, 1.51 ml) with *p*-

fluoroacetophenone (0.01 mole, 1.20 ml) in methanol. 33% solution of sodium hydroxide (5 ml) was added drop wise with continuous stirring. The reaction mixture was stirred at low temperature for 24 hrs. Progress of the reaction was checked by TLC. The reaction mixture was diluted with crushed ice and neutralized with dilute HCl (3 N). The reaction mixture was kept at 3-4 ºC till the formation of white colored precipitates. The precipitates were then filtered, dried and washed with petroleum ether. Pure white colored solid product (XX) was obtained (85.41%, m.p. 95-97 °C) after recrystallization with ethanol.

3.14.2.3 (*E*)-1-(4'-Chlorophenyl)-3-(4''-isopropylphenyl)prop-2-en-1-one (XXI)

p-Chloroacetophenone (0.01 mole, 1.33 ml) was dissolved in a methanol and add 5 ml of 33% aqueous solution of NaOH to it. In 100 ml beaker, cuminaldehyde (0.01 mole, 1.51 ml) was dissolved in 10 ml methanol. Dropwise added methanolic solution of cuminaldehyde to the reaction mixture. Stir the contents for 24 hrs. Monitoring of reaction progress was done using TLC. Pour the contents over crushed ice and neutralize it by 3 N HCl. Kept the reaction mixture at low temperature (3-4 ºC) and white colored precipates were obtained which was filtered and dried. Recrystallization of synthesized product was done in ethanol. Yield and melting point of synthesized product was 77.30% and 110-112 °C, respectively.

3.14.2.4 (*E*)-1-(4'-Bromophenyl)-3-(4''-isopropylphenyl)prop-2-en-1-one (XXII)

In 100 ml beaker, prepare a methanolic solution of cuminaldehyde (0.01 mole, 1.51 ml). 0.01 mole of *p*-bromoacetophenone (1.21 ml) was dissolved in methanol in the presence of 33% NaOH (aqueous). Dropwise mixed both the solutions and the reaction mixtute was subjected to stirring for 24 hrs. Reaction progress was monitored using TLC. Pour the contents over crushed ice and neutralization was done using 3 N HCl. Reaction mixture was placed in refrigerator at a low temperature (3-4 ºC). White colored precipites were obtained. The precipitates were filtered and dried. Recrystallization of product was done in ethanol and thr product was obtained in 72.27% yield having melting point 133-135 °C.

3.14.2.5 (*E*)-3-(4'-Isopropylphenyl)-1-*p*-tolylprop-2-en-1-one (XXIII)

The condensation between *p*-methylacetophenone (0.01 mole, 1.98 ml) and cuminaldehyde yielded α, β-unsaturated compound namely (*E*)-3-(4'-Isopropylphenyl)-1-*p*-tolylprop-2-en-1-one (XXIII). Reaction took place in the presence of aqueous solution of 33% NaOH and subjected to stirring for 24 hrs. Monitoring of reaction was done using TLC. Cool the reaction mixture and pour on crushed ice. White colored solid precipates were formed after neutralizing the reaction mixture with 3 N HCl. Filtered the reaction mixture to get white colored product. Synthesized product was obtained in semi-solid state having yield 81.43%.

3.14.2.6 (*E*)-3-(4''-Isopropylphenyl)-1-(3'-methoxyphenyl)prop-2-en-1-one (XXIV)

(*E*)-3-(4''-Isopropylphenyl)-1-(3'-methoxyphenyl)prop-2-en-1-one (XXIV) was

synthesized by reacting equimolar amount of cuminaldehyde (0.01 mole, 1.51 ml) with *m*-methoxyacetophenone (0.01 mole, 1.37 ml) using 33% aqueous solution of sodium hydroxide as a catalyst. Subjected the reaction mixture to conventional stirring for 24 hrs. Monitoring of reaction progress was carried out using thin layer chromatographic technique. White colored product was obtained after pouring the reacting mixture over crushed ice followed by neutralization done by 3 N HCl. Synthesized product was obtained in semi-solid state having yield 82.30%.

3.14.3 Pyrazolines of cuminaldehyde (XXV-XXXVI)

3.14.3.1 1-(5'-(4''-Isopropylphenyl)-3'-phenyl-4',5'-dihydropyrazol-1'-yl)ethanone (XXV)

In a 250 ml round bottomed flask, an equimolar amount of (*E*)-3-(4'-Isopropylphenyl)-1-phenylprop-2-en-1-one (XIX) (0.01 mole, 2.50 g) and hydrazine hydrate (0.01 mole, 0.48 ml) were dissolved in glacial acetic acid and refluxed for 8 hrs. Reaction progress was monitored by TLC. After reaction gets completed, the contents were brought to room temperature and then poured into ice cold water. The obtained solid product was filtered off, washed with petroleum ether and obtained in 50.13% having melting point 99-101 ºC.

3.14.3.2 1-(3'-(4''-Fluorophenyl)-5'-(4'''-isopropylphenyl)-4',5'-dihydropyrazol-1'-yl)ethanone (XXVI)

An equimolar amount of (*E*)-1-(4'-fluorophenyl)-3-(4''-isopropylphenyl)prop-2-en-1-one (XX) (0.01 mole, 2.68 g) and hydrazine hydrate (0.01 mole, 0.48 ml) were mixed using glacial acetic acid as a solvent. Reaction was subjected to refluxing for 8 hrs. A single spot on the chromatoplate confirmed the completion of reaction. The reaction mixture was poured on ice cold water. The solid obtained was filtered off, washed with petroleum ether and obtained in 52.29% having melting point 105-107 ºC.

3.14.3.3 1-(3'-(4''-Chlorophenyl)-5'-(4'''-isopropylphenyl)-4',5'-dihydropyrazol-1'-yl)ethanone (XXVII)

Synthesis of 1-(3'-(4''-Chlorophenyl)-5'-(4'''-isopropylphenyl)-4',5'-dihydropyrazol-1'-yl)ethanone was carried out by cyclization of the (*E*)-1-(4'-chlorophenyl)-3-(4''-isopropylphenyl)prop-2-en-1-one (XXI) (0.01 mole, 2.84 g) with hydrazine hydrate (0.01 mole, 0.48 ml) in the glacial acetic acid. The reaction mixture was refluxed for 8 hrs and poured over crushed ice after the completion of reaction. The product was filtered and washed with petroleum ether. Yield and melting point of synthesized product was 50.31% and 119-121 °C, respectively.

3.14.3.4 1-(3'-(4''-Bromophenyl)-5'-(4'''-isopropylphenyl)-4',5'-dihydropyrazol-1'-yl)ethanone (XXVIII)

In a 250 ml round bottomed flask containing glacial acetic acid, dissolved (*E*)-1-(4'-

bromophenyl)-3-(4''-isopropylphenyl)prop-2-en-1-one (XXII) (0.01 mole, 3.28 g) and hydrazine hydrate (0.01 mole, 0.48 ml) in it. Refluxed the resulting mixture for 8 hrs and added crushed ice to the mixture. The solid obtained was filtered off and washed with petroleum ether. Yield and melting point of synthesized product was 59.07% and 127-129 °C, respectively.

3.14.3.5 1-(5'-(4''-Isopropylphenyl)-3'-*p*-tolyl-4',5'-dihydropyrazol-1'-yl)ethanone (XXIX)

Equal moles of (*E*)-3-(4'-Isopropylphenyl)-1-*p*-tolylprop-2-en-1-one (XXIII) (0.01 mole, 2.64 g) and hydrazine hydrate (0.01 mole, 0.48 ml) were taken in glacial acetic acid (20 ml) in a 250 ml round bottomed flask. Refluxed the contents for 8 hrs. After completion of reaction, the contents were kept at room temperature and then poured into ice cold water. The precipitates formed were filtered off, washed with petroleum ether and obtained in 55.60% yield having melting point 96-98 ºC.

3.14.3.6 1-(5'-(4'''-Isopropylphenyl)-3'-(3''-methoxyphenyl)-4',5'-dihydropyrazol-1'-yl)ethanone(XXX)

A mixture of (*E*)-3-(4''-Isopropylphenyl)-1-(3'-methoxyphenyl)prop-2-en-1-one (XXIV) (0.01mol, 2.80 g) and hydrazine hydrate (0.01 mole, 0.48 ml) in 20 ml glacial acetic acid was refluxed for 24 hrs. After completion of reaction (monitored by TLC), it was poured into crushed ice. The solid obtained was washed with petroleum ether and recrystllized from ethanol. Yield and melting point of synthesized product was 54.10% and 101-103 °C, respectively.

3.14.3.7 5-(4'-Isopropylphenyl)-1,3-diphenyl-4,5-dihydro-1*H*-pyrazole (XXXI)

In round bottomed flask of a 250 ml, an equal quantity of (*E*)-3-(4'-Isopropylphenyl)-1-phenylprop-2-en-1-one (XIX) (0.01 mole, 2.50 g) and phenylhydrazine (0.01 mole, 0.98 ml) were mixed. Add 20 ml of in glacial acetic acid and refluxed for 12 hrs. Reaction progress was checked by TLC. After reaction gets completed, the reaction were brought to room temperature and then poured into ice cold water. The solid product obtained (55.12%) was filtered off and washed with petroleum ether. Melting point of product was 57-59 °C.

3.14.3.8 3-(4'-Fluorophenyl)-5-(4''-isopropylphenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole (XXXII)

An equimolar amount of (*E*)-1-(4'-fluorophenyl)-3-(4''-isopropylphenyl)prop-2-en-1-one (XX) (0.01 mole, 2.68 g) and phenylhydrazine (0.01 mole, 0.98 ml) were mixed in glacial acetic acid. Reaction was subjected to refluxing for 12 hrs. A single spot on the chromatographic plate confirmed the completion of reaction. The reaction mixture was poured on ice cold water. The solid obtained was filtered off, washed with petroleum ether

and obtained in 59.31% yield having melting point 60-62 °C.

3.14.3.9 3-(4'-Chlorophenyl)-5-(4''-isopropylphenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole (XXXIII)

Synthesis of 3-(4-chlorophenyl)-5-(4-isopropylphenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole were carried out by cyclization of the (*E*)-1-(4'-chlorophenyl)-3-(4''-isopropylphenyl)prop-2-en-1-one (XXI) (0.01 mole, 2.84 g) with phenylhydrazine (0.01 mole, 0.98 ml) in the glacial acetic acid. Refluxed the reaction mixture for 12 hrs and poured over crushed ice after the completion of reaction. The product was filtered and washed with petroleum ether. Yield and melting point of synthesized product was 56.14% and 64-66 °C, respectively.

3.14.3.10 3-(4'-Bromophenyl)-5-(4''-isopropylphenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole (XXXIV)

In round bottomed flask of 250 ml containing glacial acetic acid, mix (*E*)-1-(4'-bromophenyl)-3-(4''-isopropylphenyl)prop-2-en-1-one (XXII) (0.01 mole, 3.28 g) and phenylhydrazine (0.01 mole, 0.98 ml) in it. Refluxed the reaction mixture for 12 hrs and added crushed ice to it. The solid obtained was filtered off and washed with petroleum ether in order to obtain a pure product having melting point 72-74 °C in 51.35% yield.

3.14.3.11 5-(4'-Isopropylphenyl)-1-phenyl-3-*p*-tolyl-4,5-dihydro-1*H*-pyrazole (XXXV)

Equal moles of (*E*)-3-(4'-Isopropylphenyl)-1-*p*-tolylprop-2-en-1-one (XXIII) (0.01 mole, 2.64 g) and phenylhydrazine (0.01 mole, 0.98 ml) were mixed in glacial acetic acid (20 ml). Refluxed the contents for 8 hrs. After completion of reaction, the contents were kept at room temperature and then poured into ice cold water. The precipitates formed were filtered off and washed with petroleum ether. Yield and melting point of 5-(4'-Isopropylphenyl)-1-phenyl-3-*p*-tolyl-4,5-dihydro-1*H*-pyrazole was 50.43% and 98-100 °C, respectively.

3.14.3.12 5-(4''-Isopropylphenyl)-3-(3'-methoxyphenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole (XXXVI)

(*E*)-3-(4''-Isopropylphenyl)-1-(3'-methoxyphenyl)prop-2-en-1-one (XXIV) (0.01mol, 2.80 g) and phenylhydrazine (0.01 mole, 0.98 ml) dissolved in 20 ml glacial acetic acid, was refluxed for 24 hrs. After completion (monitored by TLC), the reaction was poured into crushed ice. The solid obtained was washed with petroleum ether in order to get pure product. Yield and melting point of synthesized product was 53.16% and 80-82 °C, respectively.

3.15 PHYSICAL PROPERTIES OF CUMINALDEHYDE DERIVATIVES

Physical data of cuminaldehyde derivatives is presented in Table 3 and 4. All the melting points were determined in open capillaries in an electric melting point apparatus and are uncorrected.

Table 3: Physical properties of Schiff bases (II-XII), thiosemicarbazones (XIII & XIV) and hydrazones (XV-XVIII) of cuminaldehyde

Comp. No.	Molecular formula	Molar mass	Color	Yield (%)	R_f	Melting point (°C)
Schiff Bases						
II	$C_{16}H_{17}N$	223	Brown	70	0.60	133-135
III	$C_{16}H_{17}NO$	239	Light brown	84	0.71	93-95
IV	$C_{17}H_{19}NO$	253	Light brown	77	0.65	207-209
V	$C_{16}H_{18}N_2$	238	Brown	87	0.68	192-194
VI	$C_{17}H_{19}N$	237	Brown	71	0.52	121-123
VII	$C_{15}H_{16}N_2$	224	White	72	0.66	110-112
VIII	$C_{15}H_{16}N_2$	224	White	75	0.69	105-107
IX	$C_{16}H_{18}N_2$	238	White	82	0.58	97-99
X	$C_{14}H_{15}N_3$	225	White	80	0.61	144-146
XI	$C_{21}H_{23}N_3O$	333	Pale-yellow	86	0.73	138-140
XII	$C_{12}H_{14}N_4$	214	Off-white	82	0.63	154-156
Thiosemicarbazones						
XIII	$C_{11}H_{15}N_3S$	221	White	89	0.65	146-148
XIV	$C_{17}H_{19}N_3S$	297	White	90	0.67	140-142
Hydrazones						
XV	$C_{10}H_{14}N_2$	162	White	85	0.58	122-124
XVI	$C_{16}H_{18}N_2$	238	Brown	70	0.70	110-112
XVII	$C_{16}H_{17}N_3O_2$	283	Orange	87	0.55	135-137
XVIII	$C_{16}H_{16}N_4O_4$	328	Red	92	0.57	140-142

*All the cuminaldehyde derivatives (II-XVIII) were obtained in solid form

*Solvent system for TLC was pure ethanol

Table 4: Physical properties of chalcones (XIX-XXIV) and pyrazolines (XXV-XXXVI) of cuminaldehyde

Chalcones						
Comp. No.	**Molecular formula**	**Molar mass**	**Color**	**Yield (%)**	**R_f**	**Melting point (°C)**
XIX	$C_{18}H_{18}O$	250	White	70	0.63	-
XX	$C_{18}H_{17}FO$	268	White	85	0.61	95-97
XXI	$C_{18}H_{17}ClO$	284	White	77	0.55	110-112
XXII	$C_{18}H_{17}BrO$	328	White	72	0.52	133-135
XXIII	$C_{19}H_{20}O$	264	White	81	0.66	-
XXIV	$C_{19}H_{20}O_2$	280	White	82	0.63	-
Pyrazolines						
XXV	$C_{20}H_{22}N_2O$	306	White	50	0.54	99-101
XXVI	$C_{20}H_{21}FN_2O$	324	Light pink	52	0.57	105-107
XXVII	$C_{20}H_{21}ClN_2O$	340	Light brown	50	0.63	119-121
XXVIII	$C_{20}H_{21}BrN_2O$	384	Off-white	59	0.69	127-129
XXIX	$C_{20}H_{24}N_2O$	320	White	55	0.70	96-98
XXX	$C_{21}H_{24}N_2O_2$	336	White	54	0.57	101-103
XXXI	$C_{24}H_{24}N_2$	340	Brown	55	0.63	57-59
XXXII	$C_{24}H_{23}FN_2$	358	Brown	59	0.68	60-62
XXXIII	$C_{24}H_{23}ClN_2$	374	Pale yellow	56	0.64	64-66
XXXIV	$C_{24}H_{23}BrN_2$	418	Light brown	51	0.52	72-74
XXXV	$C_{25}H_{26}N_2$	354	Brown	50	0.56	98-100
XXXVI	$C_{25}H_{26}N_2O$	370	Brown	53	0.60	80-82

*All the cuminaldehyde derivatives (XX-XXII, XXV-XXXVI) were obtained in solid form and XIX, XXIII, XXIV were semisolids.

*Solvent system for TLC was pure ethanol

3.16 TESTING OF BIOLOGICAL ACTIVITIES

Essential oil, different solvent extracts of cumin seeds, silver nanoparticles of cumin (AgNPs), cuminaldehyde and its derivatives were screened for biological activities *viz.* antioxidant, antimicrobial and antifungal activity.

3.16.1 Antioxidant Activity

Antioxidant assays

The essential oil, different solvent extracts, silver nanoparticles, major constituent (cuminaldehyde) of cumin seeds and its derivatives were evaluated for antioxidant activity in terms of free radical scavenging activity (RSA) using methanol as the control and ascorbic acid as standard.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radicals scavenging activity: The antioxidant activity of the compounds was tested by their ability to bleach the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), as reported (Blois 1958). DPPH gives a strong absorption band at 517 nm in the visible region because of its odd electronic structure. DPPH solution (0.1mM) was prepared by dissolving 4.00 mg in minimum quantity of methanol and further the volume was made to 250 ml. Different concentrations (3.00, 2.00, 1.00, 0.50, 0.25, 0.10, 0.05 and 0.025 mg ml^{-1}) of test compounds (40 μl) and ascorbic acid (40 μl), dissolved in methanol, were added to 3.96 ml of the DPPH solution. The absorbance at 517 nm was noted after 30 mins at room temperature, and the scavenging activity was calculated as percentage of the radical reduction. Each experiment was performed in triplicate. Methanol was used as a control and ascorbic acid as a reference compound. The radical scavenging activity was obtained as follows:

$$\text{Radical Scavenging Activity (\%)} = \frac{O.D._{control} - O.D._{sample}}{O.D._{control}} \times 100$$

Where $O.D._{control}$ and $O.D._{sample}$ is the absorbance of blank (methanol) and test compounds, respectively at 517 nm. IC_{50} values were also calculated.

Statistical analysis

The significant difference in the antioxidant activity of cumin essential oil and cuminaldehyde was calculated using Factorial Complete Randomized Design.

3.16. 2 Evaluation of antimicrobial activity

Procurement of microbial cultures

Pure cultures of three gram negative *viz Pseudomonas sp. Klebsiella sp.* and *Enterobacter sp.* were procured from Pulses Microbiology Laboratory, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana.

Preparation of culture media

Culture medium provides the minimal nutrients for the growth of bacteria and are

essential for isolation and maintenance of the microorganism. It can be in the form of gel like agar media or liquid broths.

Procedure for preparation of Nutrient Agar Broth and Nutrient Agar Media

Pure cultures of three gram negative bacteria were maintained on nutrient agar medium slants, subcultured once in a month throughout the period of investigation and the slants were stored at 4 °C in refrigerator. Medium was sterilized in autoclave at 15 psi (121°C) for 20 mins, pH of the medium was 7.2.

The composition of nutrient agar medium is given below (Arora 2007).

Ingredients	g/l
Protease peptone	5.0
Beef extract	3.0
NaCl	5.0
Agar	20.0
Distilled water	1 litre

Evaluation of antimicrobial activity

Antimicrobial activity was assessed against all the three bacteria by bacterial sensitivity - filter paper disc method (Bauer *et al* 1966). Essentila oil, different solvent extracts *i.e.* acetone, petroleum ether, water, methanol, ethanol, dichloromethane, chloroform and ethyl acetate of cumin seeds, AgNPs, cuminaldehyde and its derivatives along with the standard Ampicilin were screened for their antimicrobial potential at different concentrations (3000, 2000, 1000, 500, 250, 100, 50 and 25 µg ml^{-1}) against three gram negative bacteria *viz. Pseudomonas sp*. *Klebsiella sp., Enterobacter sp.* by disc plate method on nutrient agar medium. Nutrient broth containing test bacteria was inoculated on petri plates having solidified nutrient agar medium. The culture of bacteria was spread properly. Sterile discs having diameter 6 mm were treated with different solvent extracts of different concentrations and then placed on a petri plate having solidified nutrient agar medium. The plates were incubated for 24 hrs in an incubator at 37 ºC and the inhibition zone around the disc were measured in mm. Each treatment was replicated thrice. Sterilized filter paper discs dipped in dimethyl sulphoxide (DMSO) served as control. Ampicillin was used as standard antibiotic for the three test organisms.

Statistical Analysis

The significant difference in the biological activities was calculated at 5% level of significance using Completely Randomized Design (CRD).

3.16.3 Antifungal Activity

Procurement of fungus

Cultures of *Fusarium wilt* (FW), *Ascochyta blight* (AB) and *Bortrytis gray mould*

(BGM) were procured from Pulses Laboratory, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana.

Preparation of culture media

Culture media is necessary for isolation and sub-culturing. Potato Dextrose Agar (PDA) media was used for maintaining them either in test tubes or in Petri plates.

PDA	
Potato dextrose broth	24 g
Agar agar	24 g
Distilled water	1000 ml

Procedure for preparation of PDA

In 500 ml of distilled water, dissolved 24 g potato dextrose broth. The solution was then subjected to boiling in a cooker. After 20 mins, 24 g agar agar was added to that solution and boiled the mixture with continuous stirring until the agar agar was completely dissolved in the solution. Then total volume was made to one litre with distilled water. PDA solution (99 ml) was poured into sterilized conical flasks (250 ml) and plugged with non-absorbent cotton wool. The flasks were arranged, covered with a paper sheet and were sterilized in an autoclave at 15 psi (121 ºC) for 20 mins. The sterilized flasks were taken out after releasing the steam and were cooled at room temperature.

Poisoned food technique

A culture of the test fungi was grown in Petri plates on PDA media and kept in incubator for seven days. Stock solution of test compounds (100 mg ml^{-1}) was prepared in dimethyl sulfoxide and stored at 4 ºC for further use. PDA supplemented with different prepared solutions at six concentrations (1000, 500, 250, 100, 50 and 25 µg ml^{-1}) was poured in the Petri plates under aseptic conditions. After solidification, small disc (0.5 cm diameter) of the test fungi was cut with a sterile cork borer and transferred aseptically upside down in the centre of Petri dish. Suitable checks were maintained, where the culture discs were grown under same conditions on PDA. Petri plates were incubated at 25 ± 1 ºC and growth of fungi was recorded at regular intervals. Three fungi were selected for bioassay *viz. Fusarium wilt* (FW), *Ascochyta blight* (AB) and *Bortrytis gray mould* (BGM). Growth of test fungi colony was measured after every 24 hrs till the fungus in the control plates completely occupied it. Each treatment was replicated thrice. The antifungal activity was evaluated by measuring the relative growth of fungus in each treatment (Grover and Moore, 1962). The per cent growth inhibition over control was worked out using the formula.

$$I = \frac{(C\text{-}T) \times 100}{C}$$

Where, I is inhibition per cent,

C is colony diameter in control (cm) and

T is colony diameter in treatment (cm)

Statistical analysis

The significant difference in the antifungal activity of compounds was calculated using Factorial Complete Randomized Design.

CHAPTER-IV

RESULTS AND DISCUSSION

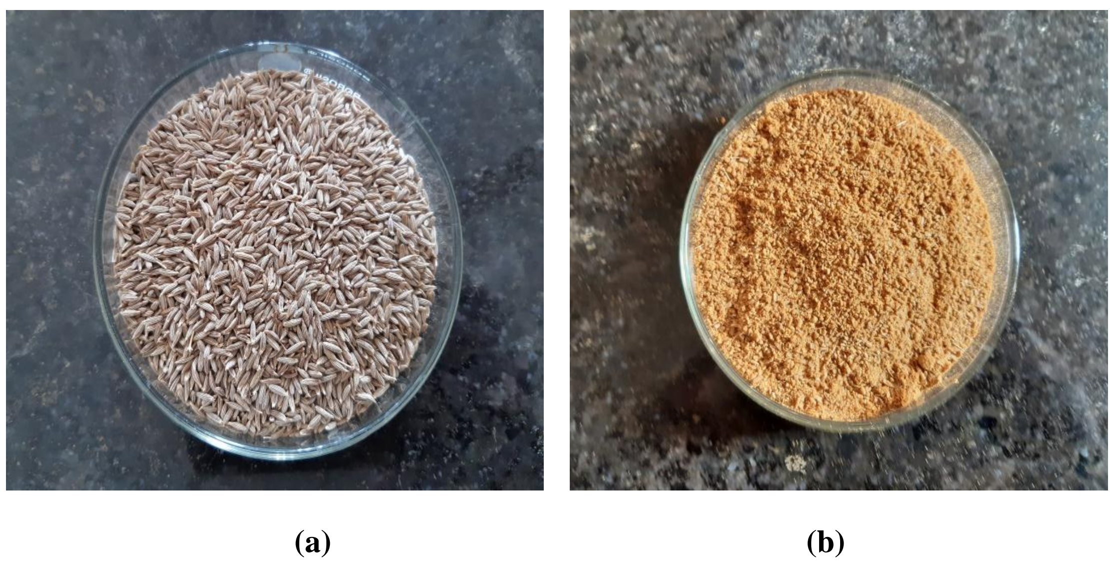
Cumin (*Cuminum cyminum*) is an annual herbaceous plant belonging to the family of Apiaceae (Gohari and Saeidnia 2011) genus *Cuminum.* It is the second most popular spice after black pepper (Hasan *et al* 2016). Aroma chemicals present in spices have wide application in aromatherapy since ancient times suggesting that they have some beneficial health effects in addition to pleasant flavour. The most utilized part of cumin is fruit, which is particularly popular in Indian savory recipies, savory pastries, and snacks. In Ayurvedic medicines, it is used as a medicinal plant for its antispasmodic, stimulant, tonic, and carminative properties (Singh *et al* 2002). Cumin extracts are reported to possess antiallergic, antioxidant, antiplatelet aggregation and hypoglycaemic properties (Singh *et al* 2002, Lee 2005, Allahghadri *et al* 2010). Ethanol extract of seed exhibited antimicrobial activity against *Escherichia coli* (Bameri *et al* 2013). The main components of essential oil of cumin were cuminadehyde, terpinene, α- and β-pinene, *p* -cymene, *p* -mentha-1,3-dien-7-al, cuminyl alcohol and β-farnesene. The essential oil of cumin exhibited antifungal, antimicrobial (Tavakoli 2015), antioxidant (Bettaieb *et al* 2010), antiestrogenic, antidiabetic, antitussive, anti-inflammatory (Bhat *et al* 2014), analgesic, hepatoprotective, anticancer, antiinfertility (Priya *et al* 2012), antistress, alzhiemer, antiulcer and antiasthamatic (Singh *et al* 2015) activities. Therefore, in the present study, work on essential oil, isolation of cuminaldehyde from essential oil, different solvent extracts, derivatives of cuminaldehyde, synthesis of silver nanoparticles using aqueous extract as a reducing agent and their antioxidant, antimicrobial and antifungal activities was carried out.

4.1 PROXIMATE COMPOSITION OF CUMIN SEEDS

Proximate composition of cumin seeds was studied and data is presented in Table 5. Cumin seeds variety GC-4 was taken from Rajasthan State Seeds and Organic Production Certification Agency, Jaipur. The sample was cleaned and washed to remove extraneous

Table 5: Proximate composition of cumin seeds

Characters	Proximate composition (%)
Moisture content	8.58
Total minerals	8.62
Crude protein	14.43
Crude fibre	11.06
Fat	15.00
Total sugars	2.30
Total carbohydrates	40.01



(a) **(b)**

(c)

Plate 1: Cumin seeds (a), Cumin seeds powder (b) and different solvent extracts (c)

matter, dried in open air and then in oven at 50 ºC, kept in air tight glass containers at 3 ºC. Proximate composition was determined by A.O.A.C. method (2000).

According to the nutritional profile, cumin is a rich source of carbohydrates, proteins, fats and crude fibers. Similar results were reported by Singh *et al* (2017).

4.2 MINERAL COMPOSITION

Few elements are essential to the body as nutrients called minerals. Knowledge of type and concentration of minerals present in food products is of vital importance to food industry. Mineral analysis using Atomic Absorption Spectroscopy (AAS) showed the concentration of different elements present in small and bold seeded ajwain and data for macro and micronutrients is given in Table 6.

Table 6: Elemental investigation of cumin seeds

Minerals	Concentration (μg ml^{-1})
Manganese (Mn)	25.75
Iron (Fe)	181.33
Copper (Cu)	14.25
Zinc (Zn)	31.25

Elemental investigation of cumin seeds showed that it contains micronutrients like Iron, copper, manganese, zinc in parts per million concentrations. Among all the elements, iron was maximum present in maximum quantity. Iron is important in maintaining the good health of human being. Manganese, zinc and copper were found in small amounts. The results are inaccordance with the results reported by Singh *et al* (2017).

Cumin seeds are a very good source of iron and minerals that plays many vital roles in the body. Iron is an integral component of hemoglobin, which transports oxygen from the lungs to all body cells, and is also part of key enzyme systems for energy production and metabolism. So, cumin can be a nutritious additive to daily diet for anemic people. Additionally, iron is instrumental in keeping your immune system healthy.

4.3 QUALITATIVE PHYTOCHEMICAL ANALYSIS

The per cent yield of different solvent extracts namely acetone, petroleum ether, water, methanol, ethanol, chloroform, dichloromethane and ethyl acetate was calculated and is presented in Figure 5 and follows the order: water > dichloromethane > chloroform > methanol > ethyl acetate > acetone > ethanol > petroleum ether. Yield was found highest in aqueous extract (14.6%) and lowest in petroleum ether extract (4.2%).

Phytochemicals are secondary metabolites produced by plants that plays diverse roles in plants such as defense against animal predators, insect's invasion, provides strengths to plants and attracts the insects for pollination. In the present study, different solvent extracts of

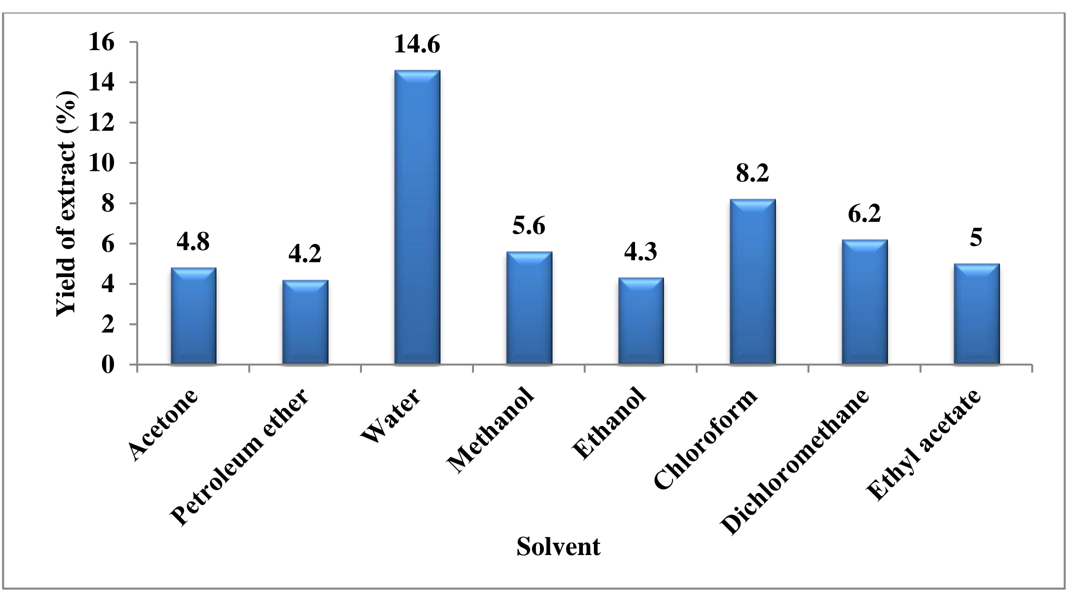


Figure 5: Per cent yield of various solvent extracts

cumin seeds were evaluated for the presence or absence of some phytochemicals such as alkaloids, saponins, tannins, anthocyanins, emodins, coumarins, amino acids, flavonoids, proteins, phenols and carbohydrates (Table 7). Present study revealed that aqueous, methanolic and ethanolic extracts are rich in phytochemicals *i.e.* they contain saponins, tannins, coumarins, amino acids, flavonoids, proteins, phenols and carbohydrates. However, none of the above mentioned phytochemicals are detected in ethyl acetate.

Table 7: Qualitative phytochemical analysis of cumin seeds in different solvent extracts

S.No.	Phytochemical	Pet-ether	Water	Methanol	Ethanol	Dichloro-methane	Chloroform
1	Alkaloids	-	+	-	-	-	-
2	Saponins	-	+	+	+	-	+
3	Tannins	-	+	+	+	-	-
4	Anthocyanins	-	-	-	-	-	-
5	Emodins	-	-	-	-	-	-
6	Coumarins	-	+	+	+	-	-
7	Amino acids	-	+	+	+	-	-
8	Flavonoids	-	+	+	+	+	+
9	Proteins	-	+	+	+	-	-
10	Phenols	-	+	+	+	-	-
11	Carbohydrates	+	+	+	+	-	-

+ present; - absent

*Acetone and ethyl acetate extracts did not contained above phytochemicals

Some of the extracts contained only one or two phytochemicals such as petroleum ether (carbohydrates), dichloromethane (flavonoids) and chloroform (saponins and flavonoids). Alkaloids were present only in aqueous extract and there about 30 alkaloids reported in literature that are of commercial interest primarily due to their medicinal and flavoring properties (Bribi 2018). It has been found that none of the solvent extract contained anthocyanins as they are mostly present in fruits and vegetables only. Similar results were reported by Malik *et al* (2020). These phytochemicals possessed wide range of biological activities such as anti-inflammatory, antioxidant and antimicrobial (Bozin *et al* 2008; Dimitrios 2006; Fu *et al* 2017; Lopez-lutz *et al* 2008). Phenols, tannins and flavonoids are considered as natural antioxidants and protect plants from oxidative stress. They have the ability to chelate metals and inhibit oxidases reactions.

4.4 QUANTITATIVE PHYTOCHEMICAL ANALYSIS

Quantitative phytochemical analysis was done for the estimation of phenolics, flavonoids, saponins and tannins contents in different solvent extracts of cumin seeds and the results are presented in Table 8. Results revealed that phenolics, flavonoids and saponins contents were higher in methanolic extract, however tannin content was higher in water extract. The total phenolic content in different solvent extracts of cumin seeds is presented in Table 8 and follows the order: methanol > water > ethanol > ethyl acetate > petroleum ether > dichlromethane > chloroform > acetone. Results were expressed as mg GAE g^{-1} dwb ± SD using the gallic acid standard curve (Figure 1). Phenolic compounds have oxidation-reduction properties which play a substantial role in inhibition and termination of free radicals, quenching singlet and triplet oxygen or degrading peroxides (Yingming *et al* 2004) that accounts for its antioxidant potential (Soobrattee 2005).

Flavonoids, inclusive of flavones and flavanols are phytochemicals, the antioxidant potential of which is governed by the presence of free hydroxyl groups. Functional hydroxyl groups in flavonoids mediate their antioxidant effects by scavenging free radicals and/or by chelating metal ions (Cook and Samman 1996; Kumar *et al* 2013; Kumar and Pandey 2013). Flavonoids are known to possess *in vitro* and as well as *in vivo* antioxidant activity (Shimoi *et al* 1996, Geetha *et al* 2003). Total flavonoids determined in different solvent extracts of cumin seeds are expressed as mg QE g^{-1} dwb ± SD using quercetin as standard (Table 8) and follows the trend: methanol > water > ethanol > chloroform > dichlromethane > ethyl acetate > acetone > petroleum ether.

Saponins are glucosides with foaming characteristics. They possess wide range of biological activities such as anitidiabetic, antifungal, antitumor activity, antiviral, antiparasitic, immunomodulatory, synthesis of hormones, acting on the cardiovascular system, acting on the central nervous and endocrine systems (Barbosa 2014; Milgate and Roberts 1995, Francis *et al* 2002). Tannins are polyphenolic plant metabolites that form

complexes with alkaloids and proteins and protect plants from the attack of predators (Khanbabaee and Van Ree 2001). Data for quantitative estimation of saponins and tannins in different solvent extracts of cumin seeds is expressed as mg SE g^{-1} dwb ± SD and mg TAE g^{-1} dwb ± SD, respectively (Table 8). The present study revealed that saponins and tannins contents were higher in methanolic and water extract, respectively. Saponins and tannins content in solvent extracts follows the order: methanol > water > ethanol > chloroform > acetone > ethyl acetate > petroleum ether > dichloromethane and water > methanol > ethanol > ethyl acetate > dichloromethane > chloroform > petroleum ether > acetone, respectively.

Table 8: Quantitative phytochemical analysis of cumin seeds in different solvent extracts

S. No.	Solvent extract	Total Phenolics[a]	Total Flavonoids[b]	Saponins[c]	Tannins[d]
1	Acetone	1.97±0.008	2.43±0.036	25.19±0.035	0.35±0.011
2	Petroleum ether	4.83±0.016	2.15±0.014	15.45±0.045	0.82±0.069
3	Water	8.08±0.011	3.43±0.013	38.73±0.027	3.95±0.041
4	Methanol	8.25±0.021	4.65±0.045	47.66±0.055	3.86±0.042
5	Ethanol	6.93±0.022	3.24±0.039	35.46±0.055	3.63±0.024
6	Chloroform	2.19±0.049	3.12±0.022	30.33±0.026	0.99±0.107
7	Dichloromethane	3.90±0.023	2.95±0.048	11.08±0.046	1.05±0.044
8	Ethyl acetate	5.10±0.053	2.64±0.046	22.72±0.023	1.74±0.038

Data are expressed as Mean ± Standard deviation

[a]mg gallic acid equivalent g^{-1} dwb ± SD; [b]mg quercetin equivalent g^{-1} dwb ± SD; [c]mg saponin equivalent g^{-1} dwb ± SD; [d]mg tannic acid equivalent g^{-1} dwb ± SD; dwb: dry weight basis.

4.5 CHEMICAL COMPOSITION OF DIFFERENT SOLVENT EXTRACTS

Total of thiry one compounds were identified from GC-MS of *Cuminum cyminum* in different solvent extracts namely acetone, petroleum ether, methanol, ethanol, chloroform, dichloromethane and ethyl acetate (Table 9), accounting 99.6-100.0% of the total composition. Different solvent extracts contains different chemical compounds that are enlisted in Table 9. Minimum number of compounds was identified in ethyl acetate and ethanolic extracts. These extracts were mainly composed of monoterpene hydrocarbons (22.43-27.28%), oxygenated monoterpenes (63.60-68.81%), acids and esters (0.65-6.41%), aldehydes and ketones (1.17-4.81%), sesquiterpene hydrocarbons (0.41-8.76%) and saturated hydrocarbons (0.74-1.55%). Additionally, Sarverogenin (6.08-6.79%) represented a substantial fraction of different solvent (acetone, methanol, ethanol) extracts. Al-Rubaye and his co-workers (2017) reported the chemical composition of methanolic extract of cumin seed, collected from local market of Hilla, Iraq. Methanolic extract of cumin seeds contained twenty compounds and it was found that its composition varied to greater extent from the

Table 9: GC-MS analysis of different solvent extracts of cumin seeds

Compound	Mol. wt.	Retention index	Area (%)						
			Acetone	Pet. ether	Methanol	Ethanol	Chloro-form	Dichloromethane	Ethyl acetate
4-hydroxy-4-methyl pentan-2-one	116	4.78	1.17	-	-	-	-	-	-
3,7-dimethyl octa-1,3,7-triene	136	7.67	0.60	-	-	-	-	0.40	-
α-Pinene	136	7.67	-	0.46	-	-	0.58	-	-
α-Thujene	136	9.16	-	0.51	-	-	0.65	-	-
3-isopropyl-6-methyl enecyclohex-1-ene	136	9.16	0.60	-	-	-	-	-	-
β-pinene	136	9.33	10.68	11.46	12.33	10.59	12.14	9.92	12.62
β-Myrcene	136	9.87	0.60	0.50	-	-	0.49	0.38	-
p-Cymene	134	11.30	3.66	3.71	4.41	3.91	3.83	3.29	4.12
γ-Terpinene	136	12.80	9.13	9.47	9.34	9.39	9.59	8.44	10.28
Sabinene hydrate	154	14.72	0.53	-	-	-	-	-	-
4-isopropylcyclohex-3-enecarbaldehyde	152	19.05	0.85	0.75	-	-	0.93	0.66	-
Cuminaldehyde	148	21.13	26.85	26.98	28.51	28.55	30.34	28.17	28.24
4-isopropyl cyclohexa-1,3-dienecarbaldehyde	150	23.11	3.65	3.98	2.98	3.55	3.85	3.04	4.19
4-isopropyl cyclohexa-1,4-dienecarbaldehyde	150	23.34	34.49	36.05	29.66	36.71	29.07	34.96	35.75
1-(4-(2-hydroxy propan-2-yl)phenyl) ethanone	174	26.61	-	-	2.45	-	-	-	-

Ethyl 2-isopropyl phenyl oxalate	236	28.28	-	-	4.25	-	-	-	-
1-(dimethoxymethyl) benzene	152	28.29	-	-	-	-	-	0.43	-
2,4-di-tert-butyl phenol	206	32.64	-	-	-	-	0.41	-	-
Diisobutyl phthalate	278	45.51	-	-	-	0.65	-	-	-
Sarverogenin	418	58.56	6.79	-	6.08	6.65	-	-	-
Hydroxydehydrostevic acid	316	58.57	-	5.24	-	-	6.41	-	-
(E)-2-methyl-4-(2,6,6-trimethyl cyclohex-1-enyl)but-2-enal	206	58.57	-	-	-	-	-	-	4.81
Alloaromadendrene	208	58.58	-	-	-	-	-	8.32	-
(E)-8-hydroxy-8a-methyl-3,5-dimethylene-2-oxo-dodecahydronaphtho[2,3-b]furan-4-yl 2-methylbut-2-enoate	346	64.83	-	-	-	-	0.41	0.44	-
n-Pentacosane	352	64.69		0.19	-	-	-	-	-
n-Eicosane	282	65.75	-	-	-	-	0.79	-	-
n-Nonacosane	408	65.75	-	0.23	-	-	-	-	-
1-iodo-triacontane	548	66.97	-	0.32	-	-	-	-	-
n-Tetratetracontane	618	66.97	-	-	-	-	0.49	0.37	-
n-Tetracontane	562	66.97	-	-	-	-	-	0.70	-
n-Dotriacontane	450	68.40	-	-	-	-	-	0.48	-

composition of methanolic extract reported in the present study. The differences in the chemical composition of extracts may be related to distinct environmental and climatic conditions (Bisht *et al* 2009) such as Light (Johnson *et al* 1999), precipitation, growing site (Satta *et al* 1999), temperature (Santos-Gomes and Fernandes-Ferreira 2001; Mallavarapu *et al* 1999; Farhat *et al* 2001) and soil (Pala-Paul *et al* 2008); seasonal sampling periods, geographic origins, plant populations (Barra 2009), vegetative plant phases (Santos-Gomes P C and Fernandes-Ferreira 2001; Mallavarapu *et al* 1999; Farhat *et al* 2001) extraction and quantification methods (Figiel *et al* 2010 and Russo *et al* 1998). Light (Peer and Langenheim 1998) and water (Pala-Paul *et al* 2001; Taveria *et al* 2003) was responsible for the increasing concentration of monoterpenes and phenyl propanes and hence resulted in increasing the concentration of monoterpenes.

4.6 EXTRACTION AND CHARACTERIZATION OF CUMIN SEED ESSENTIAL OIL

Essential oil of cumin seeds was extracted by hydrodistillation method using Dean-Stark apparatus. There is great demand of value added products of cumin namely seed, powder, essential oil and oleoresin etc. in the domestic as well as in international market (Kumar *et al* 2015). Cumin seeds have been reported to contain 3.0-4.5% essential oil (Dubey *et al* 2017). The aroma of cumin is due to the presence of aromatic volatile compounds. The flavor in cumin is judged by its volatile oil content (Hirasa and Takemasa 1998). Now a day's demand for volatile oil in international market is increasing. The advantage in use of volatile oil is that it is 100 times more concentrated than the spice powder and hence is required in very less quantity. The essential oil is responsible for the characteristic cumin odor. This odor and flavor is due to the presence of aldehydes (Nadeem and Riaz 2012). Major constituent of cumin essential oil is cuminaldehyde, present to an extent of 45.0–54.0%. Percentage of cuminaldehyde in cumin samples collected from Rajasthan and Gujarat was 44.5, 44.2 and 40.3% in Jodhpur, Nagaur (Rajasthan) and Patan (Gujarat) respectively (Nadeem and Riaz 2012).

Yield of cumin essential oil obtained was 2.52 %. It was insoluble in water and completely soluble in organic solvents such as hexane, benzene, chloroform, diethyl ether, methanol and acetone. Thin layer chromatography of essential oil showed four coloured spots having R_f values of 0.48, 0.71, 0.78 and 0.94. The R_f value of cuminaldehyde was 0.71 which confirmed its presence in essential oil.

4.6.1 GC-MS analysis of essential oil

Gas chromatography- mass spectrometry (GC-MS) of oil showed the presence of 38 compounds which account for 99.89 % of total composition (Table 10). The identification of the compounds was done by matching their retention times, retention indices and mass spectra with the library and literature data. Cuminaldehyde (36.46%) was found to be the

Table 10: Chemical composition of essential oil of cumin seeds determined by GC-MS analysis

Peak No.	Compound	Molecular formula	m/z	Retention index	Area (%)
1	α-Thujene	$C_{10}H_{16}$	136	7.43	0.19
2	1-isopropylcyclo hex-1-ene	C_9H_{16}	124	7.55	0.37
3	α-Pinene	$C_{10}H_{16}$	136	7.69	0.69
4	5-Ethylcyclohexa-1,3-diene	C_8H_{12}	108	8.98	0.16
5	Sabinene	$C_{10}H_{16}$	136	9.21	0.36
6	β-pinene	$C_{10}H_{16}$	136	9.47	7.92
7	Myrcene	$C_{10}H_{16}$	136	9.90	0.98
8	*p*-Cymene	$C_{10}H_{14}$	134	11.51	7.96
9	Limonene	$C_{10}H_{16}$	136	11.61	0.46
10	Eucalyptol	$C_{10}H_{18}O$	154	11.70	0.38
11	γ-Terpinene	$C_{10}H_{16}$	136	12.95	5.64
12	Linalool	$C_{10}H_{18}O$	154	14.84	0.19
13	3,6,6-trimethylbicyclo[3.1.1]heptan-2-one	$C_{10}H_{16}O$	152	16.47	0.22
14	6,6-dimethyl-2-methylenebicyclo[3.1.1] heptan-3-ol	$C_{10}H_{16}O$	152	16.60	0.23
15	1-(2-Propenyl)-cyclohexene	C_9H_{14}	122	16.83	0.16
16	2,5-dimethyloctahydropentalene	$C_{10}H_{18}$	138	17.04	2.30
17	1-(3-Isopropenyl-2,2-dimethylcyclopropyl)-2-methylpropan-1-one	$C_{12}H_{20}O$	180	17.81	0.73
18	1-(1-Methylethyl)-4-methyl-3-cyclohexen-1-ol	$C_{10}H_{18}O$	154	18.47	1.24
19	4-isopropylcyclohex-3-enecarbaldehyde	$C_{10}H_{16}O$	152	19.18	3.71
20	1-(1-Ethyl-2,3-dimethyl-2-cyclopenten-1-yl)ethanone	$C_{11}H_{18}O$	166	20.49	1.89
21	Cuminaldehyde	$C_{10}H_{12}O$	148	22.00	36.46
22	Phellandral	$C_{10}H_{16}O$	152	22.99	1.27
23	5-Methyl-2-propylphenol	$C_{10}H_{14}O$	150	23.86	9.10
24	γ-Terpinen-7-al	$C_{10}H_{14}O$	150	24.10	7.74
25	2-Methyl-5-(propan-2-ylidene)cyclohexane-1,4-diol	$C_{10}H_{18}O_2$	170	24.84	0.33
26	4-Hydroxycryptone	$C_9H_{14}O_2$	154	25.39	2.56
27	4-Isopropylphenylacetic acid	$C_{11}H_{14}O_2$	178	25.68	0.97
28	3,7-dimethyl-octa-2,6-dien-1-al	$C_{10}H_{16}O$	152	26.65	1.46
29	2,4(10)-Thujadiene	$C_{10}H_{14}$	134	24.48	0.44
30	*p*-Butyryloxybenzaldehyde	$C_{11}H_{12}O_3$	192	27.92	0.44
31	But-2-enyl hexanoate	$C_{10}H_{18}O_2$	170	28.78	0.74
32	β-Farnesene	$C_{15}H_{24}$	204	30.43	0.42
33	(1R,4R,5S)-1,8-Dimethyl-4-(prop-1-en-2-yl)spiro[4.5]dec-7-ene	$C_{15}H_{24}$	204	31.23	0.33
34	2-(4-methyl-3-(prop-1-en-2-yl)-4-vinylcyclohexyl)propan-2-yl acetate	$C_{17}H_{28}O_2$	264	32.24	0.22
35	*p*-Mentha-2,8-dien-1-ol	$C_{10}H_{16}O$	152	32.45	0.27
36	Caryophyllene oxide	$C_{15}H_{24}O$	220	35.46	0.35
37	Carotol	$C_{15}H_{26}O$	222	36.28	0.65
38	Dihydroterpineol	$C_{10}H_{20}O$	156	36.63	0.48

major compound in cumin seeds. The characteristic flavour of cuminaldehyde is attributed to the presence of such high per cent of cuminaldehyde. Other minor compounds present were 5-methyl-2-propylphenol (9.10 %), β-pinene (7.92 %), *p*-cymene (7.96), phellandral (1.27 %), γ-terpinen-7-al (7.74 %), γ-terpinene (5.64%), 4-isopropylcyclohex-3-enecarbaldehyde (3.71 %), 4-hydroxycryptone (2.56 %), 2,5-dimethyloctahydropentalene (2.30 %), 1-(1-Ethyl-2,3-dimethyl-2-cyclopenten-1-yl)ethanone (1.89 %), 3,7-dimethyl-octa-2,6-dien-1-al (1.46 %), and. Apart from these, many other compounds having area less than 1.00 % were also detected.

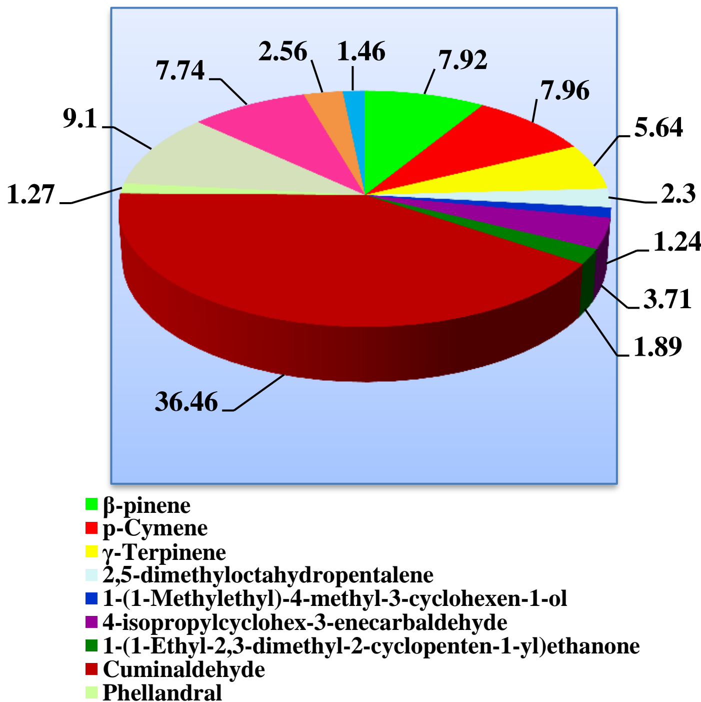


Figure 6: Composition (%) of essential oil of cumin seeds as per GC-MS analysis

Essential oil cumin seeds contain monoterpene hydocarbons 27.31%, oxygenated monoterpenes 63.05%, sesquiterpene hydocarbons (0.75%), oxygenated sesquiterpenes (1.00%), aldehyde and letones (5.62), acids and esters (1.93%).

The essential oil of cumin seeds (*Cuminum cyminum* L.) collected from Bulgaria contained more than sixty constituents of cumin oil were identified as essential volatiles, responsible for the pleasant fresh, clean, spicy (typical cumin-like) odour of a high quality product (Jirovetz *et al* 2005). Cumin aldehyde (36.0%), β-pinene (19.3%), p-cymene (18.4%) and γ-terpinene (15.3%) were the principal compounds. Hajlaoui *et al* (2012) extracted essential oil from Tunisian variety of *Cuminum cyminum* by hydrodistillation and was characterized using GC and GC-MS. Twenty-one components were identified like cuminlaldehyde (39.48%), γ-terpinene (15.21%), *o*-cymene (11.82%), β-pinene (11.13%), 2-caren-10-al (7.93%), trans-carveol (4.49%) and myrtenal (3.5%). These findings are in

agreement with the results presented but of different concentrations. The essential oil has antioxidant, antispasmodic, diuretic, carminative and antibacterial activities (Bettaieb *et al* 2010).

4.7 CHEMICAL DERIVATIZATIONS OF CUMINALDEHYDE

4.7.1 Schiff bases of cuminaldehyde (II-XVIII)

4.7.1.1 *N*-(4'-isopropylbenzylidene)benzenamine (II)

N-(4'-isopropylbenzylidene)benzenamine (II) was synthesized by reaction of cuminaldehyde with aniline in presence of glacial acetic acid. Monitoring of reaction progress was done by TLC. A single spot on thin layer chromatographic plate revealed the purity of compound (Scheme 1). The product obtained was characterized by UV-visible, IR, ^{1}H NMR and ^{13}C NMR spectroscopic techniques.

CHO NH$_2$ + CH_3COOH Reflux C=N H **(II)**

Scheme 1

The synthesized compound (II) showed absorption maxima at 302 nm in UV-visible region. IR (KBr, ν, cm^{-1}) spectrum showed prominent peaks at 3053 (aromatic CH stretching), 3024 (=CH stretching), 2961 (ν_{as} CH_3 stretching), 2871 (ν_{sym} CH_3 stretching), 1622 (C=N stretching), 1502, 1461, 1418 (aromatic C=C stretching), 1364 (C-H bending of gem-dimethyl), 1314 (C-N stretching), 979 and 830 (C-H bending), 749 (C-C bending).

3' 2' 4' 1' C=N H 5' 6' 1 2 3 4 6 5 **(II)**

^{1}H NMR spectrum ($CDCl_3$, 'δ', ppm) showed a doublet at 1.25-1.27 (*J*=6.96 Hz) due to six hydrogens of geminal dimethyl. Proton attached to the carbon (of isopropyl group group) directly attached to the aromatic ring of cuminaldehyde was recorded as a multiplet in the range of 2.91-2.97. A multiplet was observed in the range of 7.13-7.83 due to nine aromatic protons. A singlet due to proton attached to iminium carbon was observed at 8.39.

^{13}C NMR spectrum ($CDCl_3$, 'δ', ppm) recorded peaks at 28.90 due to two carbon atoms of gem-dimethyl. Peak at 39.09 was recorded due to carbon of isopropyl group (CH) directly attached to aromatic ring of cuminaldehyde. Eight aromatic carbons showed peaks at 123.34, 126.73, 127.70, 128.60, 131.31, 134.33, 147.63 and 149.99. Signal corresponding to iminium carbon appeared downfield at 166.64.

Appearance of band, singlet and peak due to (CH=N) in IR, ^{1}H NMR and ^{13}C NMR, respectively confirmed the formation of the product.

4.7.1.2 4-(4'-isopropylbenzylideneamino)phenol (III):

Treatment of cuminaldehyde with *p*-aminophenol in the presence of catalytic amount of glacial acetic acid using ethanol as a solvent afforded 4-(4'-isopropylbenzylideneamino)phenol (III) (Scheme 2). A single spot on thin layer chromatographic plate revealed the purity of synthesized product. Characterization was done using UV-visible, IR, ^{1}H NMR and ^{13}C NMR spectroscopic techniques.

CHO NH$_2$ + OH $\xrightarrow[\text{Reflux}]{CH_3COOH}$ C=N (H) OH **(III)**

Scheme 2

The synthesized compound (III) showed absorption maxima at 310 nm in UV-visible region. IR (KBr, ν, cm^{-1}) spectrum showed prominent peaks at 3223 (OH stretching), 2955 (ν_{as} CH_3 stretching), 2868 (ν_{sym} CH_3 stretching), 1670 (C=N stretching), 1532, 1475, 1433 (aromatic C=C stretching), 1389 (C-N stretching), 1358 (C-H bending of gem-dimethyl, 992 and 873 (C-H bending), 782 (C-C bending)

3' 2' 3 2 4' 1' C=N 4 1 OH H 5' 6' 5 6 **(III)**

^{1}H NMR spectrum ($CDCl_3$, 'δ', ppm), a doublet was observed at 1.24-1.26 (*J*= 6.96 Hz) due to six hydrogens of two equivalent methyl groups. A multiplet corresponding to one hydrogen atom of isopropyl group was observed in the range of 2.52-2.53. In the aromatic ring of *p*-aminophenol four aromatic protons were recorded as a set of two double doublets. Out of two double doublet, one double doublet corresponding to two protons attached to C_2, C_6 appeared in the range of 7.13-7.15 (*J*= 6.60, 1.96 Hz) and another double doublet of two equivalent protons attached to C_3, C_5 was observed in the range of 6.78-6.80 (*J*= 6.60, 1.96 Hz). Two doublets corresponding to two hydrogens each, were observed at 7.31-7.33 (*J*= 8.16 Hz) and 7.79-7.81 (*J*= 8.16 Hz) due to aromatic hydrogen atoms of cuminaldehyde. Proton attached to iminium carbon (CH=N) displayed at 8.50. Hydroxyl proton was highly deshielded and observed at 9.32.

^{13}C NMR spectrum ($CDCl_3$, 'δ', ppm) displayed peaks at 23.80 due to two equivalent carbon atoms of gem-dimethyl group. Carbon of isopropyl group (CH) directly attached to aromatic ring of cuminaldehyde displayed a peak at 39.09. Peaks corresponding to eight aromatic carbons were recorded at 116.13, 122.33, 127.17, 128.77, 130.05, 145.11, 153.24 and 157.11. Signal corresponding to iminium carbon appeared downfield at 160.16.

4.7.1.3 *N*-(4'-isopropylbenzylidene)-3-methoxybenzenamine (IV):

N-(4'-isopropylbenzylidene)-3-methoxybenzenamine (IV) was prepared from cuminaldehyde and *m*-anisidine using glacial acetic acid in ethanol (Scheme 3). The progress of reaction was monitored by thin layer chromatography. Synthesized product was characterized using UV-visible, IR, ^{1}H NMR and ^{13}C NMR spectroscopy.

CHO + NH$_2$ OCH$_3$ —(CH_3COOH, Reflux)→ C=N, H, OCH$_3$ **(IV)**

Scheme 3

The synthesized compound (IV) showed absorption maxima at 312 nm in UV-visible region. IR (KBr, ν, cm^{-1}) spectrum showed prominent peaks at 3086 (aromatic CH stretching), 3048 (=CH stretching), 2959 (ν_{as} CH_3 stretching), 2870 (ν_{sym} CH_3 stretching), 1614 (C=N stretching), 1507, 1462, 1414 (aromatic C=C stretching), 1384 (C-H bending of gem-dimethyl), 1362 (C-N stretching), 919 and 828 (C-H bending), 713 (C-C bending).

3' 2' 4' 1' C=N H 5' 6' 2 OCH$_3$ 3 1 4 6 5 **(IV)**

^{1}H NMR spectrum ($CDCl_3$, 'δ', ppm) showed a doublet at 1.32-1.34 (J = 6.92 Hz) due to six hydrogen of isopropyl group. Proton attached to carbon (CH) atom of isopropyl group was appeared as a multiplet in the range of 2.81-2.90. A singlet due to three protons of methoxy group was observed at 3.68. A multiplet corresponding to eight aromatic protons was appeared in the range of 6.95-7.47. Proton attached to iminium carbon appeared as singlet at 9.31 which confirmed the formation of product.

^{13}C NMR spectrum ($CDCl_3$, 'δ', ppm), gem-dimethyl group displayed peaks at 22.65 due to two equivalent carbon atoms. Carbon of isopropyl group (CH) directly attached to aromatic ring of cuminaldehyde displayed a peak at 39.55. Carbon of methoxy group displayed a peak at 49.17. Peaks corresponding to ten aromatic carbons displayed their peaks at 105.79, 112.20, 121.27, 125.57, 127.25, 133.65, 135.74, 147.85, 149.64 and 161.51. Peak corresponding to iminium carbon appeared downfield at 165.92.

4.7.1.4 *N*-(4'-isopropylbenzylidene)(phenyl)methanamine (V)

Treatment of equimolar amount of cuminaldehyde and benzylamine in ethanol results in the formation of *N*-(4'-isopropylbenzylidene)(phenyl)methanamine (V) (Scheme 4). Thin layer chromatographic technique was used to check the reaction progress. Formation of product was confirmed using UV-visible, IR, ^{1}H NMR and ^{13}C NMR spectroscopic techniques.

(V)

Scheme 4

The synthesized compound (V) showed absorption maxima at 306 nm in UV-visible region. IR (KBr, ν, cm^{-1}) spectrum showed prominent peaks at 3051 (aromatic CH stretching), 3024 (=CH stretching), 2959 (ν_{as} CH_3 stretching), 2871 (ν_{sym} CH_3 stretching), 1645 (C=N stretching), 1509, 1456, 1418 (aromatic C=C stretching), 1361 (C-H bending of gem-dimethyl), 1287 (C-N stretching), 916 and 890 (C-H bending), 733 (C-C bending).

(V)

^{1}H NMR spectrum ($CDCl_3$, 'δ', ppm) exhibited a doublet at 1.34-1.36 (J = 6.92 Hz) due to six equivalent hydrogens of gem-dimethyl group. A multiplet corresponding to one proton attached to carbon (CH) atom of isopropyl group was recorded in the range of 2.80-2.86. A singlet due to two protons attached to carbon C_1 was observed at 4.47. A multiplet due to nine aromatic protons was displayed in the range of 7.16-7.65. A singlet was observed downfield at 8.38 due to proton attached to iminium carbon which in turn confirmed the formation of product.

^{13}C NMR spectrum ($CDCl_3$, 'δ', ppm) exhibited a peak at 23.49 due to two carbons of gem-dimethyl group. Peak at 36.17 was observed due CH carbon of isopropyl group which was directly attached to the aromatic ring. A Signal at 58.96 was recorded corresponding to C_1. Eight aromatic carbons showed peaks at 122.61, 123.65, 131.28, 134.66, 138.30, 145.81, 149.14 and 151.11. Peak due to iminium carbon was appeared downfield at 166.16.

4.7.1.5 *N*-(4'-isopropylbenzylidene)pyridin-2-amine (VI)

N-(4'-isopropylbenzylidene)pyridin-2-amine (VI) was synthesized by condensation of 2-aminopyridine with cuminaldehyde in the presence of glacial acetic acid (Scheme 5). Monitoring of reaction progress was done by TLC. Product formation was confirmed using different spectroscopic techniques *viz.* UV-visible, IR, ^{1}H NMR and ^{13}C NMR.

(VI)

Scheme 5

The synthesized compound (VI) showed absorption maxima at 315 nm in UV-visible region. IR (KBr, ν, cm^{-1}) spectrum showed prominent peaks at 3061 (aromatic CH stretching), 2964 (ν_{as} CH_3 stretching), 2869 (ν_{sym} CH_3 stretching), 1646 (C=N stretching), 1466, 1424, 1400 (aromatic C=C stretching), 1365 (C-H bending of gem-dimethyl), 1326 (C-N stretching), 933 and 881 (C-H bending), 774 (C-C bending).

3' 2' 1 4' 1' 2 C=N N H

(VI)

^{1}H NMR spectrum ($CDCl_3$, 'δ', ppm) exhibited a doublet at 1.30-1.32 (J = 6.92 Hz) due to six equivalent hydrogens of gem-dimethyl group. A multiplet corresponding to one proton attached to carbon (CH) atom of isopropyl group was recorded in the range of 2.80-2.86. Eight protons of aromatic rings were recorded as a multiplet in the range of 6.95-7.47. A singlet at 8.31, was recorded downfield due to proton attached to iminium carbon confirmed the formation of product.

^{13}C NMR spectrum ($CDCl_3$, 'δ', ppm) exhibited a peak at 23.32 due to two equivalent carbons of gem-dimethyl group. Peak at 35.92 was observed due CH carbon of isopropyl group which was directly attached to the aromatic ring. Nine aromatic carbons showed peaks at 120.00, 125.54, 127.00, 129.67, 137.24, 156.55, 158.88, 162.22 and 163.83. Iminium carbon displayed a peak at 193.40.

4.7.1.6 *N*-(4'-isopropylbenzylidene)pyridin-4-amine (VII)

An equimolar methanolic solution of 4-aminoantipyrine (5 mmol) and cuminaldehyde (5 mmol) were mixed and gently heated for 2 hrs with constant stirring. The characteristic pale yellow precipitate obtained was filtered out and recrystallized from methanol. Fine pale yellow crystals were obtained upon slow evaporation at room temperature. It was washed with alcohol, ether and dried in vacuum desiccator over anhydrous calcium chloride.

Reaction between an equimolar ethanolic solution of 4-aminopyridine and cuminaldehyde in the presence of catalytic amount of glacial acetic acid yielded *N*-(4'-isopropylbenzylidene)pyridin-4-amine (VII) (Scheme 6). A single spot on a chromatographic plate confirmed the purity of product. UV-visible, IR, ^{1}H NMR and ^{13}C NMR spectroscopic techniques were used for the identification of the product.

CHO + H_2N N $\xrightarrow[\text{Reflux}]{CH_3COOH}$ C=N N H

(VII)

Scheme 6

The synthesized compound (VII) showed absorption maxima at 308 nm in UV-visible region. IR (KBr, ν, cm^{-1}) spectrum showed prominent peaks at 3096 (aromatic CH stretching), 3048 (=CH stretching), 2961 ($ν_{as}$ CH_3 stretching), 2837 ($ν_{sym}$ CH_3 stretching), 1596 (C=N stretching), 1458, 1425 (aromatic C=C stretching), 1362 (C-H bending of gem-dimethyl), 1333 (C-N stretching), 937 and 860 (C-H bending), 795 (C-C bending).

(VII)

^{1}H NMR spectrum ($CDCl_3$, 'δ', ppm) a doublet was observed at 1.54-1.56 (*J* = 6.92 Hz) due to six equivalent hydrogens of gem-dimethyl group. A multiplet was displayed at 2.71-2.74 due to one proton attached to carbon (CH) atom of isopropyl group. Eight protons of aromatic rings were recorded as a multiplet in the range of 7.20-7.45. A singlet at 8.38, was recorded downfield due to proton attached to iminium carbon confirmed the formation of product.

^{13}C NMR spectrum ($CDCl_3$, 'δ', ppm), a peak was displayed at 28.74 due to two equivalent carbons of gem-dimethyl group. Peak of carbon (CH) of isopropyl group was recorded at 38.72 was observed due CH carbon of isopropyl group which was directly attached to the aromatic ring. Nine aromatic carbons showed peaks at 123.34, 129.16, 136.64, 144.02, 148.87, 152.79 and 157.00. A peak at 161.06 appeared due to iminium carbon.

4.7.1.7 *N*-(4'-isopropylbenzylidene)-3-methylpyridin-2-amine (VIII)

N-(4'-isopropylbenzylidene)-3-methylpyridin-2-amine (VIII) was synthesized by refluxing an equimolar amount of cuminaldehyde and 2-amino-3-methylpyridine in the presence of glacial acetic acid using ethanol as a solvent (Scheme 7). The progress and purity of the Schiff base was checked by TLC. Characterization of the product was done UV-visible, IR, ^{1}H NMR and ^{13}C NMR spectroscopy.

CH_3COOH / Reflux

(VIII)

Scheme 7

The synthesized compound (VIII) showed absorption maxima at 314 nm in UV-visible region. IR (KBr, ν, cm^{-1}) spectrum showed prominent peaks at 3075 (aromatic CH stretching), 3046 (=CH stretching), 2911 ($ν_{as}$ CH_3 stretching), 2850 ($ν_{sym}$ CH_3 stretching), 1576 (C=N stretching), 1497, 1461, 1432 (aromatic C=C stretching), 1371 (C-H bending of gem-dimethyl), 1303 (C-N stretching), 995 and 886 (C-H bending), 774 (C-C bending).

(VIII)

^{1}H NMR spectrum ($CDCl_3$, 'δ', ppm) a doublet was observed at 1.35-1.36 (J = 6.92 Hz) due to six equivalent hydrogens of gem-dimethyl group. A singlet was observed at 2.31 due to methyl group attached to carbon C_3. A multiplet was displayed at 2.12-2.13 due to one proton attached to carbon (CH) atom of isopropyl group. Seven aromatic protons of both rings were displayed as a multiplet in the range of 7.09-7.35. Proton attached to iminium carbon displayed a singlet at 9.02.

^{13}C NMR spectrum ($CDCl_3$, 'δ', ppm), a peak was observed due to carbon of methyl group attached to carbon C_3. Two equivalent carbons of gem-dimethyl group displayed a peak at 22.82. Carbon (CH) of isopropyl group *i.e.* carbon atom directly attached to aromatic ring, displayed a peak at 33.71 Nine aromatic carbons showed peaks at 110.94, 126.24, 127.00, 129.65, 134.30, 155.57, 157.83, 161.10 and 163.47. Iminium carbon displayed a peak at 192.28.

4.7.1.8 *N*-(4'-isopropylbenzylidene)pyrimidin-2-amine (IX)

A mixture of cuminaldehyde (0.01 mol, 1.51 ml) and 2-aminopyrimidine (0.01 mol, 0.95 g) and catalytic amount of glacial acetic acid was refluxed to yield *N*-(4'-isopropylbenzylidene)pyrimidin-2-amine (1X) with continuous monitoring by TLC (Scheme 8). Different chromatographic techniques namely UV-visible, IR, ^{1}H and ^{13}C NMR spectroscopy were used for characterization of product.

CHO + H_2N– (2-aminopyrimidine) —CH_3COOH, Reflux→ (IX)

Scheme 8

The synthesized compound (IX) showed absorption maxima at 324 nm in UV-visible region. IR (KBr, ν, cm^{-1}) spectrum showed prominent peaks at 3060 (aromatic CH stretching), 3016 (=CH stretching), 2964 (ν_{as} CH_3 stretching), 2872 (ν_{sym} CH_3 stretching), 1620 (C=N stretching), 1453 (aromatic C=C stretching), 1380 (C-H bending of gem-dimethyl), 1267 (C-N stretching), 986 and 897 (C-H bending), 726 (C-C bending).

(IX)

^1H NMR spectrum ($CDCl_3$, 'δ', ppm), six equivalent hydrogens of gem-dimethyl group displayed a doublet was observed at 1.28-1.29 (J = 6.92 Hz). A proton attached to carbon (CH) atom of isopropyl group displayed a multiplet at 2.88-2.99. Seven aromatic protons of both rings were displayed as a multiplet in the range of 6.99-7.48. Singlet due to iminium carbon was displayed at 9.22.

^{13}C NMR spectrum ($CDCl_3$, 'δ', ppm), a peak at 23.78 was observed due to two equivalent carbons of gem-dimethyl group. Carbon (CH) of isopropyl group *i.e.* carbon atom directly attached to aromatic ring, displayed a peak at 34.11. Seven aromatic carbons displayed peaks at 112.70, 118.99, 126.09, 129.70, 137.46, 139.56 and 156.40. A peak due to iminium carbon was displayed at 177.72.

4.7.1.9 4-(4'-Isopropylbenzylideneamino)-2,3-dimethyl-1-phenyl-1,2-dihydropyrazol-5-one (X)

The analysis of Scheme 10 mentioned the preparation of 4-(4'-Isopropylbenzylideneamino)-2,3-dimethyl-1-phenyl-1,2-dihydropyrazol-5-one (X) from cuminaldehyde with 4-aminophenazone in the presence of catalytic amount of glacial acetic acid. A single spot on thin layer chromatographic plate depicted the purity of synthesized Schiff base (Scheme 9). Characterization of synthesized product was done using UV-visible, IR, ^1H and ^{13}C NMR spectroscopy.

CHO + H_3C N CH_3 N NH_2 O — CH_3COOH / Reflux → H_3C N CH_3 N O N=C H

(X)

Scheme 9

The synthesized compound (X) showed absorption maxima at 316 nm in UV-visible region. IR (KBr, ν, cm^{-1}) spectrum showed prominent peaks at 3053 (aromatic CH stretching), 3026 (=CH stretching), 2959 (ν_{as} CH_3 stretching), 2873 (ν_{sym} CH_3 stretching), 1689 (C=O stretching), 1652 (C=N stretching), 1494, 1460, 1421 (aromatic C=C stretching), 1380 (C-H bending of gem-dimethyl), 1292 (C-N stretching), 1132 (N-N stretching), 909 and 879 (C-H bending), 757 (C-C bending).

H_3C CH_3 1 N 2 N 3 5 4 O N=C H 1' 2' 3' 4' 5' 6'

(X)

^1H NMR spectrum ($CDCl_3$, 'δ', ppm), two geminal methyl groups displayed a doublet at 1.26-1.29 (J = 6.92 Hz). A singlet corresponding to three hydrogens of methyl group

(attached to C_3) was recorded at 2.48. Carbon (CH) atom of isopropyl group which was directly attached to aromatic ring displayed a multiplet in the range of 2.85-2.94. A singlet corresponding to three equivalent hydrogens of methyl group bonded with nitrogen atom of the pyrazolone ring was observed at 3.14. Nine aromatic protons of both rings were recorded as a multiplet in the range of 7.28-7.80. Singlet due to hydrogen atom attached to iminium carbon was displayed at 9.72.

^{13}C NMR spectrum ($CDCl_3$, 'δ', ppm), a peak due to carbon atom of methyl group, attached to carbon C_3 of pyrazolone ring was observed at 10.13. Peak of two equivalent carbon atoms of gem-dimethyl was observed at 23.71. Carbon (CH) of isopropyl group *i.e.* carbon atom directly attached to aromatic ring, displayed a peak at 34.16. A peak corresponding to carbon atom of methyl group directly attached to nitrogen atom of pyrazolone ring was appeared at 35.92. Eight aromatic carbons displayed peaks at 118.90, 124.34, 126.50, 126.64, 126.83, 127.87, 129.16, 151.37 and 151.88. A peak due to iminium carbon was displayed at 177.72. Carbonyl carbon (C_5) of pyrazolone ring showed a peak at 160.96.

4.7.1.10 *N*-(4'-Isopropylbenzylidene)-4*H*-1,2,4-triazol-4-amine (XI)

Refluxing of cuminaldehyde and 4-amino-1,2,4-triazole in the presence of glacial acetic acid yielded *N*-(4'-isopropylbenzylidene)-4*H*-1,2,4-triazol-4-amine (XI) (Scheme 10). Monitoring of reaction progress was done using thin layer chromatography. The product obtained was characterized by UV-visible, IR, ^1H NMR and ^{13}C NMR spectroscopic techniques.

CHO + H_2N-N (triazole) $\xrightarrow[\text{Reflux}]{CH_3COOH}$ **(XI)**

Scheme 10

The synthesized compound (XI) showed absorption maxima at 292 nm in UV-visible region. IR (KBr, ν, cm^{-1}) spectrum showed prominent peaks at 3113 (aromatic CH stretching), 3034 (=CH stretching), 2966 (ν_{as} CH_3 stretching), 2874 (ν_{sym} CH_3 stretching), 1608 (C=N stretching), 1492, 1464, 1401 (aromatic C=C stretching), 1364 (C-H bending of gem-dimethyl), 1288 (C-N stretching), 1159 (N-N stretching), 937 and 883 (C-H bending), 772 (C-C bending).

(XI)

^{1}H NMR spectrum ($CDCl_3$, ‘δ’, ppm) showed a doublet at 1.23-1.25 (J = 6.92 Hz) due to six hydrogens attached to two methyl groups at germinal position. Proton of isopropyl group appeared as a multiplet in the range of 2.89-2.94. Four aromatic protons of cuminaldehyde ring displayed a doublet each corresponding to two protons in the range of 7.33-7.35 (J= 8.20 Hz) and 7.67-7.69 (J= 8.20 Hz). Two equivalent protons of triazole ring were recorded as a singlet at 8.39. Proton attached to iminium carbon appeared as singlet at 11.96.

^{13}C NMR spectrum ($CDCl_3$, ‘δ’, ppm), peak at 23.67 was observed due to two equivalent carbons of gem-dimethyl group. Another carbon atom (CH) of isopropyl group displayed a peak at 34.27. Aromatic carbons showed peak at 127.16, 128.87, 129.33 and 138.69. Two equivalent carbons of triazole ring displayed their peak at 154.28. Iminium carbon was observed at 157.67.

4.7.1.11 (*N^1E,N^2E*)-*N^1,N^2*-Bis(4'-isopropylbenzylidene)benzene-1,2-diamine (XII)

Reaction of cuminaldehyde with *o*-phenylenediamine in the presence of catalytic amount of glacial acetic acid results into the formation of (*N^1E,N^2E*)-*N^1,N^2*-Bis(4'-isopropylbenzylidene)benzene-1,2-diamine (XII) (Scheme 11). The progress of reaction was checked by thin layer chromatography. Characterization of Schiff base was done using UV-visible, IR, ^{1}H NMR and ^{13}C NMR spectroscopy.

Scheme 11

The synthesized compound (XII) showed absorption maxima at 375 nm in UV-visible region. IR (KBr, ν, cm^{-1}) spectrum showed prominent peaks at 3085 (aromatic CH stretching), 3030 (=CH stretching), 2968 (ν_{as} CH_3 stretching), 2840 (ν_{sym} CH_3 stretching), 1672 (C=N stretching), 1467, 1452, 1423 (aromatic C=C stretching), 1363 (C-H bending of gem-dimethyl), 1332 (C-N stretching), 909 and 880 (C-H bending), 783 (C-C bending).

^{1}H NMR spectrum ($CDCl_3$, ‘δ’, ppm) showed a doublet at 1.27-1.29 (J = 6.88 Hz) due to twelve hydrogens attached to gem-dimethyl group. Two equivalent protons attached to isopropyl group (CH) appeared as a multiplet in the range of 2.53-2.99. Twelve aromatic protons were observed as multiplet in the range of 7.14-7.72. Two protons attached to iminium carbons appeared as singlet at 12.71.

(XII)

In ^{13}C NMR spectrum ($CDCl_3$, 'δ', ppm), four methyl groups of two equivalent isopropyl groups displayed a peak at 23.27. Remaining two protons attached to two equivalent carbon atoms of isopropyl groups were displayed their peak at 38.52. Aromatic carbons displayed peak at 110.22, 114.49, 124.25, 127.97, 131.83, 134.33 and 159.27. Two equivalent iminium carbons showed peak at 167.61.

SYNTHESIS OF THIOSEMICARZONES OF CUMINALDEHYDE

4.7.1.12 1-(4'-Isopropylbenzylidene)thiosemicarbazide (XIII)

Treatment of cuminaldehyde and thiosemicarbazide using glacial acetic acid as a catalyst results into the formation of 1-(4'-Isopropylbenzylidene)thiosemicarbazide (XIII). Completion of reaction was confirmed using TLC (Scheme 12). Identification of synthesized compound was done using UV-visible, IR, ^{1}H NMR and ^{13}C NMR spectroscopy.

CH_3COOH, Reflux

(XIII)

Scheme 12

The synthesized compound (XIII) showed absorption maxima at 305 nm in UV-visible region. IR (KBr, ν, cm^{-1}) spectrum showed prominent peaks at 3413 (NH_2 stretching), 3044 (aromatic CH stretching), 3014 (=CH stretching), 2961 (ν_{as} CH_3 stretching), 2869 (ν_{sym} CH_3 stretching), 1590 (C=N stretching), 1511, 1471, 1419 (aromatic C=C stretching), 1369 (C-H bending of gem-dimethyl), 1285 (C-N stretching), 1232 (C=S stretching), 1090 (N-N stretching), 933 and 875 (C-H bending), 732 (C-C bending).

(XIII)

^{1}H NMR spectrum ($CDCl_3$, 'δ', ppm), six hydrogen atoms of two methyl groups at geminal position were recorded as a doublet at 1.25-1.27. A multiplet corresponding to one

hydrogen atom (CH) of isopropyl group was recorded in the range of 2.90-2.95. Four aromatic protons were recorded at 7.26-7.28 (*J*= 6.64 Hz) and 7.57-7.59 (*J*= 6.64 Hz) as two doublet each corresponding to two protons. Two protons (-NH_2) displayed as a broad singlet at 6.38. Proton attached to the nitrogen (-NH-) atom was displayed downfield as a singlet 7.84. Proton attached to iminium carbon appeared as singlet at 9.57.

^{13}C NMR spectrum ($CDCl_3$, 'δ', ppm) exhibited peak at 23.75 corresponding to two equivalent carbons of isopropyl group. A peak corresponding to remaining carbon atom (CH) of isopropyl group was displayed at 34.16. Aromatic carbons displayed peaks at 127.02, 127.63, 130.59 and 144.29. Iminium carbon displayed peak at 152.19 (CH=N). Carbon (C=S) was absorbed downfield at 178.21.

4.7.1.13 1-(4'-Isopropylbenzylidene)-4-phenylthiosemicarbazide (XIV)

The reaction of equimolar amount of cuminaldehyde and phenylthiosemicarbazide afforded 1-(4'-Isopropylbenzylidene)-4-phenylthiosemicarbazide (XIV) (Scheme 13). Progress of reaction was checked using TLC. Synthesis of product was confirmed using UV-visible, IR, ^{1}H NMR and ^{13}C NMR spectroscopy.

CHO + H_2N-NH-C(=S)-NH-Ph $\xrightarrow[\text{Reflux}]{CH_3COOH}$ (XIV)

(XIV)

Scheme 13

The synthesized compound (XIV) showed absorption maxima at 323 nm in UV-visible region. IR (KBr, ν, cm^{-1}) spectrum showed prominent peaks at 3420 (NH stretching), 3062 (aromatic CH stretching), 3032 (=CH stretching), 2967 (ν_{as} CH_3 stretching), 2871 (ν_{sym} CH_3 stretching), 1664 (C=N stretching), 1491, 1464, 1410 (aromatic C=C stretching), 1363 (C-H bending of gem-dimethyl), 1311 (C-N stretching), 1212 (C=S), 1143 (N-N stretching), 913 and 883 (C-H bending), 769 (C-C bending).

(XIV)

^{1}H NMR spectrum ($CDCl_3$, 'δ', ppm) showed a doublet at 1.26-1.28 (*J*=6.96 Hz) due to six hydrogens of geminal dimethyl. Proton attached to the carbon (of isopropyl group group) directly attached to the aromatic ring of cuminaldehyde was recorded as a multiplet in the range of 2.92-2.97. A multiplet was observed in the range of 7.25-7.44 due to five aromatic protons (phenyl ring of phenylthiosemicarbazide). Aromatic protons of cuminaldehyde displayed as a two doublets at 7.60-7.62 (d, 2H, ArH) (*J*= 8.00 Hz) and 7.66-7.68 (d, 2H, ArH) (*J*= 8.00 Hz). Proton attached to nitrogen atom displayed as a singlet at

7.91. Similarly, a singlet corresponding to another nitrogen atom appeared at 9.21. A singlet due to proton attached to iminium carbon was observed at 9.88.

^{13}C NMR spectrum ($CDCl_3$, 'δ', ppm) recorded peaks at 23.88 due to two carbon atoms of gem-dimethyl. Peak at 39.17 was recorded due to carbon of isopropyl group (CH) directly attached to aromatic ring of cuminaldehyde. Eight aromatic carbons showed peaks at 113.69, 116.33, 118.99, 123.88, 130.82, 135.08, 139.49 and 152.82. Iminium carbon displayed a peak at 164.53. Carbon (C=S) was absorbed downfield at 176.63. Appearance of band, singlet and peak due to (CH=N) in IR, ^{1}H NMR and ^{13}C NMR, respectively confirmed the formation of the product.

4.7.1.14 1,2-Bis(4'-isopropylbenzylidene)hydrazine (XV)

A quick reaction took places on mixing cuminaldehyde and hydrazine hydrate in the presence of glacial acetic acid resulted in the formation of 1,2-bis(4'-isopropylbenzylidene)hydrazine (XV). Progress of reaction was checked by thin layer chromatography. Identification of synthesized product was confirmed using different spectroscopic techniques such as UV-visible, IR, ^{1}H NMR and ^{13}C NMR.

CHO

2 + NH_2NH_2 $\xrightarrow[\text{Reflux}]{CH_3COOH}$ C=N−N=C (H, H)

(XV)

Scheme 14

The synthesized compound (XV) showed absorption maxima at 313 nm in UV-visible region. IR (KBr, ν, cm^{-1}) spectrum showed prominent peaks at 3060 (aromatic CH stretching), 3016 (=CH stretching), 2964 (ν_{as} CH_3 stretching), 2872 (ν_{sym} CH_3 stretching), 1620 (C=N stretching), 1509, 1453, 1380 (aromatic C=C stretching), 1308 (C-H bending of gem-dimethyl), 1267 (C-N stretching), 1180 (N-N stretching), 986 and 897 (C-H bending), 726 (C-C bending).

3' 2' 1 2 2' 3'
4' 1' C=N−N=C 1' 4'
H H
5' 6' 5' 6'

(XV)

^{1}H NMR spectrum ($CDCl_3$, 'δ', ppm), a doublet was observed at 1.26-1.27 (*J*= 6.96 Hz) due to six hydrogens of two equivalent methyl groups of gem-dimethyl. A multiplet corresponding to two hydrogen atom of isopropyl group was observed in the range of 2.86-2.99. In the aromatic ring, eight aromatic protons were recorded as a multiplet in the range of 7.26-7.86. A singlet corresponding to two equivalent hydrogen atoms attached to iminium carbon (CH=N) was observed at 9.96.

^{13}C NMR spectrum ($CDCl_3$, 'δ', ppm) displayed peaks at 23.77 due to two equivalent carbon atoms of gem-dimethyl group. Carbon of isopropyl group (CH) directly attached to aromatic ring of cuminaldehyde displayed a peak at 34.23. Aromatic carbons displayed peaks at 127.46, 145.66, 150.10 and 152.47. Signal corresponding to iminium carbon appeared downfield at 161.78.

4.7.1.15 1-(4'-Isopropylbenzylidene)-2-phenylhydrazine (XVI)

Treatment of cuminaldehyde with phenylhydrazine along with catalytic amount of glacial acetic acid afforded 1-(4'-isopropylbenzylidene)-2-phenylhydrazine (XVI). Progress of reaction was monitored by TLC. Identification of synthesized compound was confirmed using different spectroscopic techniques such as UV-visible, IR, ^{1}H NMR and ^{13}C NMR.

CHO + $NHNH_2$ —(CH_3COOH, Reflux)→ **(XVI)**

Scheme 15

The synthesized compound (XVI) showed absorption maxima at 369 nm in UV-visible region. IR (KBr, ν, cm^{-1}) spectrum showed prominent peaks at 3410 (NH stretching), 3036 (aromatic CH stretching), 2960 (ν_{as} CH_3 stretching), 2866 (ν_{sym} CH_3 stretching), 1617 (C=N stretching), 1454, 1428 (aromatic C=C stretching), 1394 (C-H bending of gem-dimethyl), 1341 (C-N stretching), 1151 (N-N stretching), 902 and 861 (C-H bending), 746 (C-C bending).

(XVI)

^{1}H NMR spectrum ($CDCl_3$, 'δ', ppm) a doublet was observed at 1.25-1.27 (J = 6.92 Hz) due to six equivalent hydrogens of gem-dimethyl group. A multiplet was displayed at 2.88-2.94 due to one proton attached to carbon (CH) atom of isopropyl group. Proton attached to nitrogen atom was displayed as a singlet at 4.01. Nine aromatic protons of both rings were displayed as a multiplet in the range of 6.86-7.59. Proton attached to iminium carbon displayed a singlet at 7.67.

^{13}C NMR spectrum ($CDCl_3$, 'δ', ppm), two equivalent carbons of gem-dimethyl group displayed a peak at 23.89. Carbon (CH) of isopropyl group *i.e.* carbon atom directly attached to aromatic ring, displayed a peak at 34.02. Aromatic carbons showed peaks at 112.72, 119.93, 126.25, 126.71, 129.24, 132.98, 137.54 and 144.86. Iminium carbon displayed a peak at 149.49.

4.7.1.16 1-(4'-Isopropylbenzylidene)-2-(4''-nitrophenyl)hydrazine (XVII)

Synthesis of 1-(4'-isopropylbenzylidene)-2-(4''-nitrophenyl)hydrazine was done by reacting cuminaldehyde with *p*-nitrophenylhdrazine in ethanol. Advancement of reaction was checked by TLC. Different spectroscopic techniques *i.e.* UV-visible, IR, ^{1}H NMR and ^{13}C NMR were used for characterization of product.

CHO + $NHNH_2$ / NO_2 —(CH_3COOH, Reflux)→ (XVII)

Scheme 16

The synthesized compound (XVII) showed absorption maxima at 373 nm in UV-visible region. IR (KBr, ν, cm^{-1}) spectrum showed prominent peaks at 3421 (NH stretching), 3086 (aromatic CH stretching), 2957 (ν_{as} CH_3 stretching), 2866 (ν_{sym} CH_3 stretching), 1687 (C=N stretching), 1524 and 1344 (NO_2 stretching), 1435, 1410 (aromatic C=C stretching), 1344 (C-H bending of gem-dimethyl), 1313 (C-N stretching), 1169 (N-N stretching), 942 and 892 (C-H bending), 749 (C-C bending).

(XVII)

^{1}H NMR spectrum ($CDCl_3$, 'δ', ppm), six equivalent hydrogens of gem-dimethyl group displayed a doublet showed at 1.24-1.26 (J = 6.92 Hz). A proton attached to carbon (CH) atom of isopropyl group displayed a multiplet at 2.91-2.94. Singlet due to hydrogen atom attached to nitrogen (NH) atom appeared at 4.12. Aromatic protons of both rings exhibited as a multiplet in the range of 7.25-7.53. Singlet due to iminium carbon was displayed at 8.40.

^{13}C NMR spectrum ($CDCl_3$, 'δ', ppm), a peak at 23.22 was observed due to two equivalent carbons of gem-dimethyl group. Carbon (CH) of isopropyl group *i.e.* carbon atom directly attached to aromatic ring, displayed a peak at 37.73. Aromatic carbons displayed peaks at 118.87, 124.66, 129.77, 139.97, 144.29, 146.88, 150.50 and 157.87. A peak due to iminium carbon was displayed at 160.60.

4.7.1.17 1-(4'-Isopropylbenzylidene)-2-(2'',4''-dinitrophenyl)hydrazine (XVIII)

1-(4'-Isopropylbenzylidene)-2-(2'', 4''-dinitrophenyl) hydrazine (XVIII) was synthesized by condensation of cuminaldehyde and 2, 4-dinitrophenylhydrazine using sulphuric acid. Single spot on the chromatographic plate confirmed the formation of product (Scheme 17). UV-visible, IR, ^{1}H NMR and ^{13}C NMR spectroscopic techniques were used for characterization of product.

Scheme 17

The synthesized compound (XVIII) showed absorption maxima at 390 nm in UV-visible region. IR (KBr, ν, cm^{-1}) spectrum showed prominent peaks at 3314 (NH stretching), 3071 (aromatic CH stretching), 2964 (ν_{as} CH_3 stretching), 2868 (ν_{sym} CH_3 stretching), 1651 (C=N stretching), 1592 and 1399 (NO_2 stretching), 1467, 1423 (aromatic C=C stretching), 1366 (C-H bending of gem-dimethyl), 1327 (C-N stretching), 1162 (N-N stretching), 933 and 881 (C-H bending), 774 (C-C bending).

(XVIII)

^1H NMR spectrum ($CDCl_3$, 'δ', ppm) a doublet was observed at 1.28-1.30 (*J* = 6.92 Hz) due to six equivalent hydrogens of gem-dimethyl group. A multiplet was displayed at 2.96-2.99 due to one proton attached to carbon (CH) atom of isopropyl group. Two doublets corresponding to two hydrogens each, were observed at 7.32-7.35 (*J*= 8.00 Hz) and 7.69-7.71 (*J*= 8.00 Hz) due to aromatic hydrogen atoms of cuminaldehyde. Three aromatic protons appeared as a multiplet in the range of 8.08-8.37. Proton attached to iminium carbon displayed a singlet at 9.15. A singlet was observed downfield at 11.30 due to hydrogen atom attached to nitrogen atom.

^{13}C NMR spectrum ($CDCl_3$, 'δ', ppm), two equivalent carbons of gem-dimethyl group displayed a peak at 23.76. Carbon (CH) of isopropyl group *i.e.* carbon atom directly attached to aromatic ring, displayed a peak at 34.23. Aromatic carbons displayed peaks at 116.77, 123.54, 127.18, 127.78, 129.29, 130.01, 130.77, 138.13, 144.87 and 148.09. Iminium carbon displayed a peak at 152.53.

4.7.2 Chalcones of cuminaldehyde (XIX-XXIV)

4.7.2.1 (*E*)-3-(4'-Isopropylphenyl)-1-phenylprop-2-en-1-one (XIX)

Acetophenone and cuminaldehyde was reacted using sodium hydroxide as a catalyst gives (*E*)-3-(4'-isopropylphenyl)-1-phenylprop-2-en-1-one (XIX) (Scheme 18). Reaction progress was monitored with TLC. UV-visible, IR, ^1H NMR and ^{13}C NMR spectroscopic techniques were used for identification of product.

CHO COCH$_3$ 33% NaOH O

(XIX)

Scheme 18

The synthesized compound (XIX) showed absorption maxima at 328 nm in UV-visible region. IR (KBr, ν, cm^{-1}) spectrum showed prominent peaks at 3136 (aromatic CH stretching), 3075 (=CH stretching), 2944 (ν_{as} CH_3 stretching), 2838 (ν_{sym} CH_3 stretching), 1658 (C=O stretching), 1576 (aliphatic C=C stretching), 1466, 1453, 1431 (aromatic C=C stretching), 1332 (C-H bending of gem-dimethyl), 1223 (C-O stretching), 1047 (H-C=C bending), 973 and 840 (C-H bending), 736 (C-C bending).

(XIX)

In ^{1}H NMR spectrum ($CDCl_3$, 'δ', ppm), a doublet was observed at 1.27-1.28 (*J*= 6.92 Hz) due to six hydrogens attached to carbons of geminal dimethyl group. Proton attached to the carbon (of isopropyl group) directly attached to the aromatic ring of cuminaldehyde was recorded as a multiplet in the range of 2.92-2.99. Two olefinic protons attached to C_2 and C_3 appeared as doublet from 7.83-7.79 (*J* = 15.7 Hz) and 8.00-8.04 (*J* = 15.7 Hz) due to trans coupling with their adjacent protons (Aksoz and Ertan 2012, Gaba 2018). A multiplet corresponding to eight aromatic protons was observed in the range of 7.26-7.60.

^{13}C NMR spectrum ($CDCl_3$, 'δ', ppm) recorded peaks at 23.64 due to two carbon atoms of gem-dimethyl. Peak at 34.89 was recorded due to carbon of isopropyl group (CH) directly attached to aromatic ring of cuminaldehyde. Eight aromatic carbons and two olefinic carbons showed peaks at 109.48, 115.26, 117.33, 124.50, 127.06, 133.39, 145.50, 147.36, 152.68 and 155.71. Carbonyl carbon (C_1) was observed at 188.38.

4.7.2.2 (*E*)-1-(4'-Fluorophenyl)-3-(4''-isopropylphenyl)prop-2-en-1-one (XX)

(*E*)-1-(4'-Fluorophenyl)-3-(4''-isopropylphenyl)prop-2-en-1-one (XX) was synthesized by base catalyzed condensation of a cuminaldehyde with *p*-fluoroacetophenone in methanol using sodium hydroxide as a catalyst. Progress of the reaction was checked by TLC. Identification of product was done using UV-visible, IR, ^{1}H NMR and ^{13}C NMR spectroscopic techniques.

CHO COCH$_3$ + 33% NaOH → (XX)

Scheme 19

The synthesized compound (XX) showed absorption maxima at 352 nm in UV-visible region. IR (KBr, ν, cm^{-1}) spectrum showed prominent peaks at 3059 (aromatic CH stretching), 3008 (=CH stretching), 2924 (ν_{as} CH_3 stretching), 2852 (ν_{sym} CH_3 stretching), 1656 (C=O stretching), 1599 (aliphatic C=C stretching), 1493, 1459, 1434 (aromatic C=C stretching), 1348 (C-H bending of gem-dimethyl), 1223 (C-O stretching), 1193 (C-F stretching), 1042 (H-C=C bending), 960 and 872 (C-H bending), 770 (C-C bending).

(XX)

^{1}H NMR spectrum ($CDCl_3$, 'δ', ppm) a doublet appeared at 1.26-1.28 due to six hydrogens attached to two methyl groups of isopropyl substituent. One proton of isopropyl group appeared as a multiplet in the range of 2.91-2.98. Two olefinic protons attached to C_2 and C_3 were recorded as doublets at 7.45-7.49 (J=15.64 Hz) and 7.77-7.82 (J=15.64 Hz). Eight aromatic protons were observed as multiplet in the range of 7.07-8.07.

^{13}C NMR spectrum ($CDCl_3$, 'δ', ppm) recorded peak at 23.92 due to two equivalent carbons of gem-dimethyl of isopropyl side chain. Peak at 36.22 was observed due CH carbon of isopropyl group. Aromatic carbons and olefinic carbons displayed peaks at 117.16, 123.12, 133.84, 138.94, 140.73, 145.13, 146.80, 151.20, 155.93 and 158.43. A peak corresponding to carbonyl carbon (C_1) was observed at 189.18.

4.7.2.3 (*E*)-1-(4'-Chlorophenyl)-3-(4''-isopropylphenyl)prop-2-en-1-one (XXI)

p-Chloroacetophenone was reacted with cuminaldehyde using NaOH as a catalyst to give (*E*)-1-(4'-chlorophenyl)-3-(4''-isopropylphenyl)prop-2-en-1-one (XXI). Monitoring of reaction progress was done using TLC (Scheme 20). Characterization of product was done using UV-visible, IR, ^{1}H NMR and ^{13}C NMR spectroscopic techniques.

CHO COCH$_3$ + 33% NaOH → (XXI)

Scheme 20

The synthesized compound (XXI) showed absorption maxima at 346 nm in UV-visible region. IR (KBr, ν, cm^{-1}) spectrum showed prominent peaks at 3055 (aromatic CH stretching), 2996 (ν_{as} CH_3 stretching), 2835 (ν_{sym} CH_3 stretching), 1677 (C=O stretching), 1572 (aliphatic C=C stretching), 1487, 1463, 1451 (aromatic C=C stretching), 1366 (C-H bending of gem-dimethyl), 1228 (C-O stretching), 1146 (H-C=C bending), 998 and 836 (C-H bending), 772 (C-C bending) and 757 (C-Cl stretching).

(XXI)

^{1}H NMR spectrum ($CDCl_3$, 'δ', ppm), a doublet was observed at 1.27-1.28 (*J*= 7.00 Hz) due to six hydrogens of two equivalent methyl groups. A multiplet corresponding to one hydrogen atom of isopropyl group was observed in the range of 2.92-2.98. Two multiplets, each corresponding to two hydrogen atoms of aromatic ring of *p*-chloroacetophenone were recorded at 7.16-7.19 and 8.04-8.07. Two doublets corresponding to two hydrogens each, were observed at 7.28-7.30 (*J*=8.0 Hz) and 7.58-7.59 (*J*=8.0 Hz) due to aromatic hydrogen atoms of cuminaldehyde. Two doublets corresponding to olefinic protons attached to C_2 and C_3 were observed at 7.45-7.48 (*J*=16.0 Hz) and 7.79-7.82 (*J*=16.0 Hz).

^{13}C NMR spectrum ($CDCl_3$, 'δ', ppm) showed peak due to two equivalent carbon atoms of gem-dimethyl group at 23.65. Carbon of isopropyl group (CH) directly attached to aromatic ring of cuminaldehyde displayed a peak at 35.88. Peaks corresponding to aromatic carbons and olefinic carbons were displayed at 109.54, 115.26, 117.34, 125.88, 129.09, 133.42, 145.52, 147.38, 152.65 and 155.78. Signal corresponding to carbonyl carbon (C_1) was appeared downfield at 189.84.

4.7.2.4 (*E*)-1-(4'-Bromophenyl)-3-(4''-isopropylphenyl)prop-2-en-1-one (XXII)

Cuminaldehyde and *p*-bromoacetophenone were reacted in ethanol using NaOH (aqueous) as a catalyst to give (*E*)-1-(4'-bromophenyl)-3-(4''-isopropylphenyl)prop-2-en-1-one (XXII). Reaction progress was monitored using TLC (Scheme 21). UV-visible, IR, ^{1}H NMR and ^{13}C NMR spectroscopic techniques were used for characterization of product.

33% NaOH

(XXII)

Scheme 21

The synthesized compound (XXII) showed absorption maxima at 342 nm in UV-visible region. IR (KBr, ν, cm^{-1}) spectrum showed prominent peaks at 3065 (aromatic CH

stretching), 2914 (ν_{as} CH_3 stretching), 2848 (ν_{sym} CH_3 stretching), 1680 (C=O stretching), 1600 (aliphatic C=C stretching), 1586 (aliphatic C=C stretching), 1519, 1441, 1427 (aromatic C=C stretching), 1348 (C-H bending of gem-dimethyl), 1288 (C-O stretching), 1171 (H-C=C bending), 933 and 851 (C-H bending), 772 (C-C bending) and 653 (C-Br stretching).

(XXII)

^{1}H NMR spectrum ($CDCl_3$, 'δ', ppm) exhibited a doublet at 1.26-1.28 (J = 6.92 Hz) due to six equivalent hydrogens of gem-dimethyl group. A multiplet corresponding to one proton attached to carbon (CH) atom of isopropyl group was recorded in the range of 2.89-2.99. A multiplet corresponding to eight aromatic protons was observed in the range of 7.21-7.82. Protons attached to two olefinic carbons C_2 and C_3 were observed as a doublet in the range of 7.42-7.46 (J=15.7 Hz) and 7.87-7.91 (J=15.7 Hz).

In ^{13}C NMR spectrum ($CDCl_3$, 'δ', ppm), two carbons of gem-dimethyl group exhibited a peak at 23.30. Peak at 34.46 was observed due CH carbon of isopropyl group which was directly attached to the aromatic ring. Signal corresponding to aromatic carbons were observd at 117.44, 124.93, 125.84, 127.70, 129.46, 131.42, 134.06, 134.88, 141.61 and 149.40. Peak due to carbonyl carbon was appeared downfield at 190.81.

4.7.2.5 (*E*)-3-(4'-Isopropylphenyl)-1-*p*-tolylprop-2-en-1-one (XXIII)

The condensation between *p*-methylacetophenone and cuminaldehyde yielded α, β-unsaturated compound namely (*E*)-3-(4'-isopropylphenyl)-1-*p*-tolylprop-2-en-1-one (XXIII). Monitoring of reaction progress was done using TLC (Scheme 22). Characterization of product was done using UV-visible, IR, ^{1}H NMR and ^{13}C NMR spectroscopy.

Scheme 22

The synthesized compound (XXIII) showed absorption maxima at 382 nm in UV-visible region. IR (KBr, ν, cm^{-1}) spectrum showed prominent peaks at 2986 (aromatic CH stretching), 2874 (ν_{as} CH_3 stretching), 2847 (ν_{sym} CH_3 stretching), 1687 (C=O stretching), 1586 (aliphatic C=C stretching), 1538, 1483, 1419 (aromatic C=C stretching), 1353 (C-H bending of gem-dimethyl), 1277 (C-O stretching), 1073 (H-C=C bending), 972 and 888 (C-H bending) and 788 (C-C bending).

(XXIII)

^{1}H NMR spectrum ($CDCl_3$, ‘δ’, ppm) a doublet was observed at 1.29-1.31 (*J* = 6.92 Hz) due to six equivalent hydrogens of gem-dimethyl group. A singlet corresponding to three equivalent protons of methyl group (attached to aromatic ring) was recorded at 2.46. A multiplet was displayed at 2.94-3.01 due to one proton attached to carbon (CH) atom of isopropyl group. A multiplet of four aromatic protons of benzene ring was observed at 7.25-7.34. Two doublets corresponding to two hydrogens each, were observed at 7.52-7.56 (*J*= 8.0 Hz) and 7.60-7.62 (*J*= 8.0 Hz) due to aromatic hydrogen atoms of cuminaldehyde. Protons of olefinic carbons C_2 and C_3 displayed doublet in the range of 7.52-7.56 (*J*=15.7 Hz) and 7.82-7.86 (*J*=15.7 Hz).

^{13}C NMR spectrum ($CDCl_3$, ‘δ’, ppm), two equivalent carbons of gem-dimethyl group displayed a peak at 22.17. A signal corresponding to carbon of methyl group was observed at 26.45. Carbon (CH) of isopropyl group *i.e.* carbon atom directly attached to aromatic ring, displayed a peak at 36.79. Aromatic carbons displayed peaks at 116.04, 119.86, 122.91, 125.44, 133.05, 136.21, 138.69, 143.59, 149.15 and 155.79. Carbonyl carbon displayed peak at 190.43.

4.7.2.6 (*E*)-3-(4''-Isopropylphenyl)-1-(3'-methoxyphenyl)prop-2-en-1-one (XXIV)

(*E*)-3-(4''-isopropylphenyl)-1-(3'-methoxyphenyl)prop-2-en-1-one (XXIV) was synthesized by reacting equimolar amount of cuminaldehyde with *m*-methoxyacetophenone using sodium hydroxide as a catalyst (Scheme 23). Monitoring of reaction progress was done using TLC. Formation of product was confirmed using different spectroscopic techniques namely UV-visible, IR, ^{1}H NMR and ^{13}C NMR.

(XXIV)

Scheme 23

The synthesized compound (XXIV) showed absorption maxima at 350 nm in UV-visible region. IR (KBr, ν, cm^{-1}) spectrum showed prominent peaks at 3032 (aromatic CH stretching), 3003 (=CH stretching), 2977 (ν_{as} CH_3 stretching), 2838 (ν_{sym} CH_3 stretching), 1682 (C=O stretching), 1590 (aliphatic C=C stretching), 1512, 1462, 1419 (aromatic C=C stretching), 1302 (C-H bending of gem-dimethyl), 1252 (C-O stretching), 1102 (H-C=C

bending), 974 and 889 (C-H bending) and 791 (C-C bending).

(XIV)

In ^{1}H NMR spectrum ($CDCl_3$, 'δ', ppm), a doublet was observed at 1.23-1.25 (*J*= 6.92 Hz) due to six hydrogens attached to carbons of geminal dimethyl group. Proton attached to the carbon (of isopropyl group group) directly attached to the aromatic ring of cuminaldehyde was recorded as a multiplet in the range of 2.92-2.95. A singlet of three equivalent protons of methoxy group was displayed at 3.85. Two olefinic protons attached to C_2 and C_3 appeared as doublet from 7.76-7.80 (J = 15.7 Hz) and 8.01-8.05 (J = 15.7 Hz) due to trans coupling with their adjacent protons (Aksoz and Ertan 2012, Gaba 2018). A multiplet corresponding to eight aromatic protons was observed in the range of 7.26-7.78.

^{13}C NMR spectrum ($CDCl_3$, 'δ', ppm) recorded peaks at 22.85 due to two carbon atoms of gem-dimethyl. Peak at 36.11 was recorded due to carbon of isopropyl group (CH) directly attached to aromatic ring of cuminaldehyde. Peak corresponding to carbon of methoxy substituent was displayed at 55.02. Aromatic carbons and two olefinic carbons showed peaks at 112.78, 121.84, 123.91, 125.72, 127.92, 133.63, 135.74, 140.21, 141.26, 147.84, 155.12 and 159.51. Carbonyl carbon (C_1) was observed at 189.92.

4.7.3 Synthesis of pyrazolines of cuminaldehyde (XXV-XXXVI)

4.7.3.1 1-(5'-(4''-Isopropylphenyl)-3'-phenyl-4',5'-dihydropyrazol-1'-yl)ethanone (XXV)

Reaction of an equimolar amount of (*E*)-3-(4'-isopropylphenyl)-1-phenylprop-2-en-1-one (XIX) and hydrazine hydrate in glacial acetic acid yielded 1-(5'-(4''-Isopropylphenyl)-3'-phenyl-4',5'-dihydropyrazol-1'-yl)ethanone (XXV). Reaction progress was monitored by TLC (Scheme 24). Identification of synthesized product was done using UV-visible, IR, ^{1}H NMR and ^{13}C NMR.

The synthesized compound (XXV) showed absorption maxima at 326 nm in UV-visible region. IR (KBr, ν, cm^{-1}) spectrum showed prominent peaks at 3135 (aromatic CH stretching), 3116 (=CH stretching), 2931 (ν_{as} CH_3 stretching), 2855 (ν_{sym} CH_3 stretching), 1676 (C=O stretching), 1603 (C=N stretching), 1516, 1470, 1456 (aromatic C=C stretching), 1384 (C-H bending of gem-dimethyl), 1319 (C-N stretching), 1259 (C-O stretching), 1154 (N-N stretching), 972 and 853 (C-H bending) and 760 (C-C bending).

^{1}H NMR spectrum ($CDCl_3$, 'δ', ppm) showed a doublet at 1.20-1.21 (*J*=7.0 Hz) due to six hydrogens of geminal dimethyl. Singlet corresponding to three protons of attached to C_2 was observed at 2.41. Proton attached to the carbon (of isopropyl group group) directly attached to the aromatic ring of cuminaldehyde was recorded as a multiplet in the range of

2.84-2.89. Two protons attached to methylenic of pyrazoline ring were recorded as double doublet in range of 3.00-3.14 (J = 17.5, 4.0 Hz) and 3.66-3.71 (J = 17.5, 12.0 Hz). Similar pattern of double doublets for pyrazoline ring were reported by Gaba *et al* (2015) and Gaba (2020). A double doublet due to proton of pyrazoline ring appeared in the range of 5.54-5.57 (dd, 1H, $C_{5'}H$) (J = 11.5, 4.0 Hz). A multiplet was observed in the range of 7.08-7.71 due to eight aromatic protons.

(XIX) + $NH_2NH_2H_2O$

CH_3COOH, Reflux

(XXV)

Scheme 24

^{13}C NMR spectrum ($CDCl_3$, 'δ', ppm) recorded peaks at 21.27 due to two carbon atoms of gem-dimethyl. Peak at 22.87 was observed due to carbon C_2. Peak at 32.22 was recorded due to carbon of isopropyl group (CH) directly attached to aromatic ring of cuminaldehyde. Carbon C4' and C5' of pyrazoline rings displayed their peaks at 42.24 and 60.19, respectively. Peaks corresponding to aromatic carbons and $C_{3'}$ carbon of pyrazoline ring was observed at 112.84, 123.32, 126.62, 134.94, 136.63, 146.44, 150.03, 154.31 and 159.98. Carbonyl carbon (C_2) was observed at 178.87, respectively.

(XXV)

4.7.3.2 1-(3'-(4''-Fluorophenyl)-5'-(4'''-isopropylphenyl)-4',5'-dihydropyrazol-1'-yl) ethanone (XXVI)

The analysis of Scheme 25 mentioned the preparation of 1-(3'-(4''-fluorophenyl)-5'-(4'''-isopropylphenyl)-4',5'-dihydropyrazol-1'-yl)ethanone (XXVI) from (*E*)-1-(4'-

fluorophenyl)-3-(4"-isopropylphenyl)prop-2-en-1-one (XX) and hydrazine hydrate in glacial acetic acid. A single spot on thin layer chromatographic plate depicted the purity of synthesized product. Formation of product was confirmed using different spectroscopic techniques namely UV-visible, IR, ^{1}H NMR and ^{13}C NMR.

(XX) + $NH_2NH_2H_2O$

CH_3COOH | Reflux

(XXVI)

Scheme 25

The synthesized compound (XXVI) showed absorption maxima at 324 nm in UV-visible region. IR (KBr, ν, cm^{-1}) spectrum showed prominent peaks at 3058 (aromatic CH stretching), 2958 (=CH stretching), 2921 (ν_{as} CH_3 stretching), 2861 (ν_{sym} CH_3 stretching), 1687 (C=O stretching), 1568 (C=N stretching), 1522, 1490 (aromatic C=C stretching), 1387 (C-H bending of gem-dimethyl), 1347 (C-N stretching), 1206 (C-O stretching), 1159 (N-N stretching), 1079 (C-F stretching), 948 and 855 (C-H bending) and 746 (C-C bending).

(XXVI)

^{1}H NMR spectrum ($CDCl_3$, 'δ', ppm), a doublet was observed at 1.20-1.21 (*J*= 7.0 Hz) due to six hydrogens of two equivalent methyl groups. Singlet corresponding to three protons attached to C_2 was observed at 2.41. A multiplet corresponding to one hydrogen atom of isopropyl group was observed in the range of 2.52-2.53. Two protons attached to $C_{4'}$ of pyrazoline ring were recorded as double doublet in range of 3.11-3.16 (*J* = 17.5, 4.0 Hz) and 3.67-3.73 (*J* = 17.5, 12.0 Hz). Another double doublet corresponding to proton attached to $C_{5'}$ was observed in the range of 5.57-5.60 (*J* = 11.5, 4.0 Hz). A multiplet corresponding to

aromatic protons was observed in the range of 7.08-7.73.

^{13}C NMR spectrum ($CDCl_3$, 'δ', ppm) displayed peaks at 21.98 due to two equivalent carbon atoms of gem-dimethyl group. A signal corresponding to C_2 was observed at 23.95. Carbon of isopropyl group (CH) directly attached to aromatic ring of cuminaldehyde displayed a peak at 33.78. Carbon $C_{4'}$ and $C_{5'}$ of pyrazoline rings displayed their peaks at 42.24 and 60.19, respectively. Peaks corresponding to eight aromatic carbons were recorded at 115.98, 125.49, 126.97, 127.81, 128.60, 139.08, 148.25, 152.92 and 164.94. Signal corresponding to carbonyl carbon appeared downfield at 168.84.

4.7.3.3 1-(3'-(4''-Chlorophenyl)-5'-(4'''-isopropylphenyl)-4',5'-dihydropyrazol-1'-yl)ethanone (XXVII)

Synthesis of 1-(3'-(4''-chlorophenyl)-5'-(4'''-isopropylphenyl)-4',5'-dihydropyrazol-1'-yl)ethanone (XXVII) was done by cyclization of the (*E*)-1-(4'-chlorophenyl)-3-(4''-isopropylphenyl)prop-2-en-1-one (XXI) with hydrazine hydrate in glacial acetic acid. Monitoring of reaction progress was done using TLC. Formation of product was confirmed using different spectroscopic techniques namely UV-visible, IR, ^{1}H NMR and ^{13}C NMR.

(XXI) + $NH_2NH_2H_2O$

CH_3COOH Reflux

(XXVII)

Scheme 26

The synthesized compound (XXVII) showed absorption maxima at 330 nm in UV-visible region. IR (KBr, ν, cm^{-1}) spectrum showed prominent peaks at 3036 (aromatic CH stretching), 2918 (ν_{as} CH_3 stretching), 2863 (ν_{sym} CH_3 stretching), 1681 (C=O stretching), 1604 (C=N stretching), 1527, 1483, 1443 (aromatic C=C stretching), 1346 (C-H bending of gem-dimethyl), 1318 (C-N stretching), 1202 (C-O stretching), 1161 (N-N stretching), 968 and 857 (C-H bending), 791 (C-Cl stretching) and 742 (C-C bending).

^{1}H NMR spectrum ($CDCl_3$, 'δ', ppm) showed a doublet at 1.20-1.21 (J = 7.0 Hz) due to six hydrogen of isopropyl group. A singlet due to three protons of $-COCH_3$ group observed at 2.41. Proton attached to carbon (CH) atom of isopropyl group appeared as a multiplet in the

range of 2.81-2.90. Two double doublets each of one proton attached to methylene carbon was observed in the range 3.11-3.16 (J = 17.5, 4.0 Hz) and 3.67-3.73 (J = 17.5, 12.0 Hz). Another proton of pyrazoline ring was observed as a double doublet in the range of 5.57-5.60. A multiplet corresponding to eight aromatic protons appeared in the range of 7.08-7.73.

(XXVII)

In ^{13}C NMR spectrum ($CDCl_3$, 'δ', ppm), peaks at 22.30 (gem-dimethyl), 26.21 (CH_3), 35.46 (CH), 42.61 ($C_{4'}$), 59.40 ($C_{5'}$) were observed. Aromatic carbons and carbon $C_{3'}$ showed peaks at 117.44, 125.84, 127.70, 129.46, 131.42, 134.88, 141.61, 149.40 and 163.58. A downfield signal corresponding to carbonyl carbon was at 170.81.

4.7.3.4 1-(3'-(4''-Bromophenyl)-5'-(4'''-isopropylphenyl)-4',5'-dihydropyrazol-1'-yl)ethanone (XXVIII)

Reaction of (E)-1-(4'-bromophenyl)-3-(4''-isopropylphenyl)prop-2-en-1-one (XXII) and hydrazine hydrate yielded 1-(3'-(4''-bromophenyl)-5'-(4'''-isopropylphenyl)-4',5'-dihydropyrazol-1'-yl)ethanone (XXVIII) Progress of reaction was checked using TLC (Scheme 27). Characterization of compound was done using UV-visible, IR, ^{1}H NMR and ^{13}C NMR spectroscopic techniques.

(XXII) + $NH_2NH_2H_2O$ → (CH_3COOH, Reflux) → **(XXVIII)**

Scheme 27

The synthesized compound (XXVIII) showed absorption maxima at 304 nm in UV-visible region. IR (KBr, ν, cm^{-1}) spectrum showed prominent peaks at 3198 (aromatic CH stretching), 3086 (=CH stretching), 2957 (ν_{as} CH_3 stretching), 2866 (ν_{sym} CH_3 stretching), 1687 (C=O stretching), 1612 (C=N stretching), 1524, 1435, 1410 (aromatic C=C stretching), 1344 (C-H bending of gem-dimethyl), 1313 (C-N stretching), 1263 (C-O stretching), 1169 (N-N stretching), 942 and 851 (C-H bending), 749 (C-C bending) and 690 (C-Br stretching).

(XXVIII)

^{1}H NMR spectrum ($CDCl_3$, 'δ', ppm) exhibited a doublet at 1.22-1.23 (J = 7.0 Hz) due to six equivalent hydrogens of gem-dimethyl group. A singlet corresponding to –$COCH_3$ group was observed at 2.44. A multiplet corresponding to one proton attached to carbon (CH) atom of isopropyl group was recorded in the range of 2.82-2.89. Two protons attached to methylenic carbon were appeared as double doublets in the range 3.12-3.16 (J = 17.5, 4.5 Hz) and 3.67-3.73 (J = 17.5, 11.5 Hz). Another double doublet of proton attached to pyrazoline ring was observed in the range of 5.54-5.57 (J = 12.0, 4.5 Hz). A multiplet of four aromatic protons was observed in the range of 7.13-7.21. Two doublets (of aromatic protons of cuminaldehyde), each corresponding to two protons was observed in the range 7.23-7.26 (J= 8 Hz) and 7.63-7.66 (J= 8 Hz).

^{13}C NMR spectrum ($CDCl_3$, 'δ', ppm) recorded peaks at 22.17 due to two carbon atoms of gem-dimethyl. Peak at 26.45 was observed due to carbon C_2. Peak at 33.05 was recorded due to carbon of isopropyl group (CH) directly attached to aromatic ring of cuminaldehyde. Carbon C4' and C5' of pyrazoline rings displayed their peaks at 42.59 and 59.11, respectively. Peaks corresponding to aromatic carbons and $C_{3'}$ carbon of pyrazoline ring was observed at 119.86, 122.91, 125.44, 133.05, 136.21, 138.69, 143.59, 149.15 and 155.79. Carbonyl carbon (C_2) was observed at 160.90, respectively.

4.7.3.5 1-(5'-(4''-Isopropylphenyl)-3'-*p*-tolyl-4',5'-dihydropyrazol-1'-yl)ethanone (XXIX)

Treatment of equal moles of (*E*)-3-(4'-isopropylphenyl)-1-*p*-tolylprop-2-en-1-one (XXIII) and hydrazine hydrate in glacial acetic acid afforded 1-(5'-(4''-Isopropylphenyl)-3'-*p*-tolyl-4',5'-dihydropyrazol-1'-yl)ethanone (XXIX). Progress of reaction was monitored using TLC (Scheme 28). Characterization of compound was done using UV-visible, IR, ^{1}H NMR and ^{13}C NMR spectroscopic techniques.

$NH_2NH_2H_2O$

(XXIII)

CH_3COOH Reflux

(XXIX)

Scheme 28

The synthesized compound (XXIX) showed absorption maxima at 320 nm in UV-visible region. IR (KBr, ν, cm^{-1}) spectrum showed prominent peaks at 3198 (aromatic CH stretching), 3075 (=CH stretching), 2954 ($ν_{as}$ CH_3 stretching), 2867 ($ν_{sym}$ CH_3 stretching), 1691 (C=O stretching), 1602 (C=N stretching), 1464, 1433, 1403 (aromatic C=C stretching), 1357 (C-H bending of gem-dimethyl), 1288 (C-N stretching), 1256 (C-O stretching), 1170 (N-N stretching), 929 and 855 (C-H bending) and 747 (C-C bending).

(XXIX)

In ^1H NMR spectrum ($CDCl_3$, 'δ', ppm) six equivalent hydrogens of gem-dimethyl group displayed a doublet at 1.20-1.21 (*J* = 7.0 Hz). A singlet corresponding to $–COCH_3$ group was observed at 2.39. A multiplet corresponding to one proton attached to carbon (CH) atom of isopropyl group was recorded in the range of 2.83-2.88. Two protons attached to methylenic carbon were appeared as a double doublet in the range 3.13-3.17 (*J* = 17.5, 4.5 Hz) and 3.67-3.73 (*J* = 17.5, 11.5 Hz). Another double doublet of proton of pyrazoline ring was observed in the range of 5.55-5.58 (*J* = 12.0, 4.5 Hz). A multiplet of four aromatic protons was observed in the range of 7.13-7.17. Two doublets (of aromatic protons of cuminaldehyde), each corresponding to two protons was observed in the range 7.21-7.23 (*J*= 8 Hz) and 7.62-7.63 (*J*= 8 Hz).

^{13}C NMR spectrum ($CDCl_3$, 'δ', ppm) recorded peaks at 21.51 due to two carbon atoms of gem-dimethyl. Peaks at 22.50 and 23.95 were observed due to carbon C_2 and methyl

group. Peak at 33.77 was recorded due to carbon of isopropyl group (CH) directly attached to aromatic ring of cuminaldehyde. Carbons of pyrazoline rings displayed their peaks at 42.38 and 59.61, respectively. Peaks corresponding to aromatic carbons and $C_{3'}$ carbon of pyrazoline ring was observed at 125.54, 126.56, 126.91, 128.73, 129.43, 139.27, 140.61, 148.12 and 154.07. Carbonyl carbon was observed at 168.81, respectively.

4.7.3.6 1-(5'-(4'''-Isopropylphenyl)-3'-(3''-methoxyphenyl)-4',5'-dihydropyrazol-1'-yl) ethanone(XXX)

Treatment of (*E*)-3-(4''-isopropylphenyl)-1-(3'-methoxyphenyl)prop-2-en-1-one (XXIV) and hydrazine hydrate in glacial acetic acid results 1-(5'-(4'''-Isopropylphenyl)-3'-(3''-methoxyphenyl)-4',5'-dihydropyrazol-1'-yl)ethanone(XXX). Progress of reaction was checked by TLC (Scheme 29). Characterization of compound was done using UV-visible, IR, ^{1}H NMR and ^{13}C NMR spectroscopic techniques.

O
OCH_3
(XXIV)
+ $NH_2NH_2H_2O$
CH_3COOH Reflux
O
N-N CH_3
OCH_3
(XXX)

Scheme 29

The synthesized compound (XXX) showed absorption maxima at 316 nm in UV-visible region. IR (KBr, ν, cm^{-1}) spectrum showed prominent peaks at 3104 (aromatic CH stretching), 3016 (=CH stretching), 2997 (ν_{as} CH_3 stretching), 2841 (ν_{sym} CH_3 stretching), 1668 (C=O stretching), 1579 (C=N stretching), 1470, 1444, 1406 (aromatic C=C stretching), 1330 (C-H bending of gem-dimethyl), 1253 (C-N stretching), 1215 (C-O stretching), 1167 (N-N stretching), 972 and 837 (C-H bending) and 732 (C-C bending).

^{1}H NMR spectrum ($CDCl_3$, 'δ', ppm) exhibited a doublet at 1.24-1.25 (J = 7.0 Hz) due to six equivalent hydrogens of gem-dimethyl group. Singlets corresponding to $-COCH_3$ and $-OCH_3$ group were observed at 2.42 and 3.87. A multiplet corresponding to one proton attached to carbon (CH) atom of isopropyl group was recorded in the range of 2.83-2.89. Two protons attached to methylenic carbon were appeared as a double doublet in the range 3.13-

3.17 (J = 17.5, 4.5 Hz) and 3.68-3.74 (J = 14.0, 11.5 Hz). Another double doublet of proton of pyrazoline ring was observed in the range of 5.56-5.59 (J = 12.0, 4.5 Hz). A multiplet of eight aromatic protons was observed in the range of 6.96-7.34.

(XXX)

^{13}C NMR spectrum ($CDCl_3$, 'δ', ppm) recorded peaks at 22.66 due to two carbon atoms of gem-dimethyl. Peak at 21.99 was observed due to carbon C_2. Peak at 33.77 was recorded due to carbon of isopropyl group (CH) directly attached to aromatic ring of cuminaldehyde. Carbons of pyrazoline rings displayed their peaks at 42.42 and 55.38, respectively. Signal corresponding to methoxt group was observed at 59.76. Peaks corresponding to aromatic carbons and carbon of pyrazoline ring was observed at 111.59, 116.19, 119.26, 125.53, 126.93, 129.76, 132.83, 139.16, 148.19, 153.90 and 163.58. Carbonyl carbon (C_2) was observed at 168.91, respectively.

4.7.3.7 5-(4'-Isopropylphenyl)-1,3-diphenyl-4,5-dihydro-1*H*-pyrazole (XXXI)

Equal quantity of (*E*)-3-(4'-isopropylphenyl)-1-phenylprop-2-en-1-one (XIX) and phenylhydrazine reacts to yield 5-(4'-Isopropylphenyl)-1,3-diphenyl-4,5-dihydro-1*H*-pyrazole (XXXI). Reaction progress was checked by TLC (Scheme 30). Formation of product was confirmed using different spectroscopic techniques namely UV-visible, IR, ^{1}H NMR and ^{13}C NMR spectroscopic techniques.

(XIX)

CH_3COOH Reflux

(XXXI)

Scheme 30

The synthesized compound (XXXI) showed absorption maxima at 339 nm in UV-visible region. IR (KBr, ν, cm^{-1}) spectrum showed prominent peaks at 3195 (aromatic CH stretching), 3002 (=CH stretching), 2946 (ν_{as} CH_3 stretching), 1651 (C=N stretching), 1492, 1456, 1411 (aromatic C=C stretching), 1366 (C-H bending of gem-dimethyl), 1302 (C-N stretching), 1140 (N-N stretching), 961 and 810 (C-H bending) and 756 (C-C bending).

(XXXI)

^{1}H NMR spectrum ($CDCl_3$, 'δ', ppm) showed a doublet at 1.20-1.21 (*J*=7.0 Hz) due to six hydrogens of geminal dimethyl. Proton attached to the carbon (of isopropyl group) directly attached to the aromatic ring of cuminaldehyde was recorded as a multiplet in the range of 2.87-2.94. Two protons attached to methylene carbon of pyrazoline ring were recorded as double doublet in range of 3.31-3.36 (*J* = 17.5, 4.5 Hz) and 3.70-3.75 (*J* = 17.0, 13.5 Hz). Similar pattern of double doublets for pyrazoline ring were reported by Gaba *et al* (2015) and Gaba (2020). Remaining proton of pyrozoline ring showed a double doublet in the range of 5.70-5.74 (*J* = 12.0, 4.5 Hz). A multiplet was observed in the range of 6.76-7.70 due to fourteen aromatic protons.

^{13}C NMR spectrum ($CDCl_3$, 'δ', ppm) recorded peaks at 22.17 due to two carbon atoms of gem-dimethyl. Peak at 33.45 was recorded due to carbon of isopropyl group (CH) directly attached to aromatic ring of cuminaldehyde. Carbons of pyrazoline rings displayed their peaks at 42.03 and 59.55, respectively. Peaks corresponding to aromatic carbons and C=N carbon of pyrazoline ring was observed at 117.44, 119.76, 121.11, 125.47, 126.21, 129.59, 133.45, 136.05, 138.69, 143.59, 145.55, 149.05 and 156.86.

4.7.3.8 3-(4'-Fluorophenyl)-5-(4''-isopropylphenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole (XXXII)

Reaction of an equimolar amount of (*E*)-1-(4'-fluorophenyl)-3-(4''-isopropylphenyl)prop-2-en-1-one (XX) and phenylhydrazine in glacial acetic acid gives 3-(4'-Fluorophenyl)-5-(4''-isopropylphenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole (XXXII). A single spot on the chromatographic plate confirmed the completion of reaction (Scheme 31). Product formation was confirmed using different spectroscopic techniques *i.e.* UV-visible, IR, ^{1}H NMR and ^{13}C NMR spectroscopic techniques.

(XX)

CH_3COOH Reflux

(XXXII)

Scheme 31

The synthesized compound (XXXII) showed absorption maxima at 334 nm in UV-visible region. IR (KBr, ν, cm^{-1}) spectrum showed prominent peaks at 3103 (aromatic CH stretching), 3073 (=CH stretching), 2993 (ν_{as} CH_3 stretching), 2881 (ν_{sym} CH_3 stretching), 1596 (C=N stretching), 1520, 1405 (aromatic C=C stretching), 1348 (C-H bending of gem-dimethyl), 1291 (C-N stretching), 1170 (N-N stretching), 1105 (C-F stretching), 975 and 859 (C-H bending) and 784 (C-C bending).

(XXXII)

^1H NMR spectrum ($CDCl_3$, 'δ', ppm), a doublet was observed at 1.24-1.26 (*J*= 7.0 Hz) due to six hydrogens of two equivalent methyl groups. A multiplet corresponding to one hydrogen atom of isopropyl group was observed in the range of 2.90-2.95. Two protons attached to methylene carbon of pyrazoline ring were recorded as double doublet in range of 3.14-3.19 (*J* = 17.5, 4.5 Hz) and 3.70-3.75 (*J* = 17.0, 13.5 Hz). Another double doublet corresponding to CH-N proton of pyrazoline ring was observed in the range of 5.57-5.61 (*J* = 11.5, 4.0 Hz). A multiplet corresponding to thirteen aromatic protons was observed in the range of 7.06-7.88.

^{13}C NMR spectrum ($CDCl_3$, 'δ', ppm) displayed peaks at 23.83 due to two equivalent carbon atoms of gem-dimethyl group. Carbon of isopropyl group (CH) directly attached to aromatic ring of cuminaldehyde displayed a peak at 33.87. Carbons of pyrazoline ring displayed their peaks at 44.87 and 60.01, respectively. Peaks corresponding to aromatic

carbons and C=N moiety of pyrazoline ring were recorded at 115.63, 120.69, 125.37, 126.59, 127.14, 127.55, 128.65, 131.10, 132.43, 134.70, 145.23, 152.16 and 166.57.

4.7.3.9 3-(4'-Chlorophenyl)-5-(4''-isopropylphenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole (XXXIII)

Synthesis of 3-(4'-Chlorophenyl)-5-(4''-isopropylphenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole (XXXIII) was done by cyclization of the (*E*)-1-(4'-chlorophenyl)-3-(4''-isopropylphenyl)prop-2-en-1-one (XXI) with phenylhydrazine in the glacial acetic acid (Scheme 32). Single spot on chromatoplate confirmed the formation of product and identification of product was done using UV-visible, IR, ^{1}H NMR and ^{13}C NMR spectroscopic techniques.

(XXI)

CH_3COOH Reflux

(XXXIII)

Scheme 32

The synthesized compound (XXXIII) showed absorption maxima at 354 nm in UV-visible region. IR (KBr, ν, cm^{-1}) spectrum showed prominent peaks at 3179 (aromatic CH stretching), 3077 (=CH stretching), 2974 (ν_{as} CH_3 stretching), 2850 (ν_{sym} CH_3 stretching), 1649 (C=N stretching), 1514, 1431 (aromatic C=C stretching), 1348 (C-H bending of gem-dimethyl), 1275 (C-N stretching), 1170 (N-N stretching), 961 and 842 (C-H bending) and 796 (C-Cl stretching).

(XXXIII)

^{1}H NMR spectrum ($CDCl_3$, 'δ', ppm) showed a doublet at 1.99-1.22 (*J* = 6.8 Hz) due to six hydrogen of isopropyl group. Proton attached to carbon (CH) atom of isopropyl group

was appeared as a multiplet in the range of 2.82-2.88. Two double doublets corresponding to two methylenic protons of pyrazoline ring were appeared in the range of 3.10-3.16 (J = 17.6, 4.6 Hz), 3.66-3.73 (J = 17.6, 11.8 Hz). Proton of pyrazoline ring (directly attached to aromatic ring of cuminaldehyde) was observed as a double doublet in the range of 5.55-5.69 (J = 11.8, 4.6 Hz). A multiplet corresponding to thirteen aromatic protons was appeared in the range of 7.26-7.87.

In ^{13}C NMR spectrum ($CDCl_3$, 'δ', ppm), peaks at 22.81 (gem-dimethyl), 33.02 (CH of isopropyl group), 42.61 (methylenic carbon of pyrazoline ring), 59.40 (C-N of pyrazoline ring) were observed. Aromatic carbons and C_3 showed peaks at 104.89, 117.66, 123.78, 126.60, 127.21, 128.69, 129.98, 131.81, 139.65, 140.10, 143.99, 149.31 and 160.22.

4.7.3.10 3-(4'-Bromophenyl)-5-(4"-isopropylphenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole (XXXIV)

3-(4'-Bromophenyl)-5-(4"-isopropylphenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole (XXXIV) was prepared by treatment of (*E*)-1-(4'-bromophenyl)-3-(4"-isopropylphenyl)prop-2-en-1-one (XXII) with phenylhydrazine in glacial acetic acid. The purity of prepared compound (Scheme 33) was determined by single spot on TLC plate. Characterization of synthesized product was done using UV-visible, IR, ^{1}H NMR and ^{13}C NMR spectroscopic techniques.

O
NHNH$_2$
Br
+
(XXII)
CH_3COOH Reflux
N-N
Br
(XXXIV)

Scheme 33

The synthesized compound (XXXIV) showed absorption maxima at 349 nm in UV-visible region. IR (KBr, ν, cm^{-1}) spectrum showed prominent peaks at 2961 (aromatic CH stretching), 2941 (ν_{as} CH_3 stretching), 2864 (ν_{sym} CH_3 stretching), 1615 (C=N stretching), 1516, 1455, 1428 (aromatic C=C stretching), 1347 (C-H bending of gem-dimethyl), 1291 (C-N stretching), 1148 (N-N stretching), 994 and 864 (C-H bending), 797 (C-C bending), 665 (C-Br stretching).

(XXXIV)

^{1}H NMR spectrum ($CDCl_3$, 'δ', ppm) showed a doublet at 1.24-1.26 (J = 7.0 Hz) due to six hydrogen of isopropyl group. Proton attached to carbon (CH) atom of isopropyl group was appeared as a multiplet in the range of 2.85-2.92. Two protons attached to C_4 of pyrazoline ring were appeared as double doublets in the range of 3.08-3.13 (J = 17.0, 7.0 Hz) and 3.75-3.81 (J = 17.0, 12.5 Hz). Proton attached to carbon C_5 was observed as a double doublet in the range of 5.24-5.28 (J = 12.5, 7.5 Hz). A multiplet corresponding to thirteen aromatic protons was appeared in the range of 6.77-7.80.

In ^{13}C NMR spectrum ($CDCl_3$, 'δ', ppm), peaks at 23.83 (gem-dimethyl), 33.75 (CH of isopropyl group), 43.36 (C_4), 64.37 (C_5) were observed. Aromatic carbons and carbon C_3 showed peaks at 104.87, 113.41, 119.26, 125.36, 126.60, 127.69, 128.93, 128.95, 131.67, 139.65, 139.65, 145.59, 148.27 and 150.81.

4.7.3.11 5-(4'-Isopropylphenyl)-1-phenyl-3-*p*-tolyl-4,5-dihydro-1*H*-pyrazole (XXXV)

Equal moles of (*E*)-3-(4'-isopropylphenyl)-1-*p*-tolylprop-2-en-1-one (XXIII) and phenylhydrazine were reacted in glacial acetic acid gives 5-(4'-Isopropylphenyl)-1-phenyl-3-*p*-tolyl-4,5-dihydro-1*H*-pyrazole (XXXV). Monitoring of reaction progress was done using TLC (Scheme 34). Characterization of synthesized compound was done using UV-visible, IR, ^{1}H NMR and ^{13}C NMR spectroscopic techniques.

(XXIII)

CH_3COOH Reflux

(XXXV)

Scheme 34

The synthesized compound (XXXV) showed absorption maxima at 346 nm in UV-visible region. IR (KBr, ν, cm^{-1}) spectrum showed prominent peaks at 3107 (aromatic CH stretching), 2966 (=CH stretching), 2923 (ν_{as} CH_3 stretching), 2864 (ν_{sym} CH_3 stretching), 1578 (C=N stretching), 1511, 1439, 1415 (aromatic C=C stretching), 1345 (C-H bending of gem-dimethyl), 1260 (C-N stretching), 1173 (N-N stretching), 962 and 817 (C-H bending) and 730 (C-C bending).

(XXXV)

In ^{1}H NMR spectrum ($CDCl_3$, 'δ', ppm) six equivalent hydrogens of gem-dimethyl group displayed a doublet at 1.21-1.23 (J = 7.0 Hz). A singlet corresponding to $-CH_3$ group was observed at 2.37. A multiplet corresponding to one proton attached to carbon (CH) atom of isopropyl group was recorded in the range of 2.86-2.88. Two protons attached to C_4 carbon were appeared as a double doublet in the range 3.10-3.15 (J = 17.0, 7.0 Hz) and 3.77-3.83 (J = 17.0, 12.5 Hz). Another double doublet of proton attached to $C_{5'}$ was observed in the range of 5.20-5.24 (J = 12.0, 7.0 Hz). A multiplet of thirteen aromatic protons was observed in the range of 6.75-7.62.

^{13}C NMR spectrum ($CDCl_3$, 'δ', ppm) recorded peaks at 21.91 due to two carbon atoms of gem-dimethyl. Peak at 23.94 was observed due to $-CH_3$ methyl group. Peak at 33.34 was recorded due to carbon of isopropyl group (CH) directly attached to aromatic ring of cuminaldehyde. Carbon C_4 and C_5 of pyrazoline rings displayed their peaks at 42.42 and 59.25, respectively. Peak corresponding to aromatic carbons and C_3 carbon of pyrazoline ring was observed at 112.42, 116.15, 119.12, 125.53, 126.93, 128.76, 132.83, 139.11, 141.29, 142.87, 148.19, 153.4 and 159.89.

4.7.3.12 5-(4''-Isopropylphenyl)-3-(3'-methoxyphenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole (XXXVI)

(*E*)-3-(4''-isopropylphenyl)-1-(3'-methoxyphenyl)prop-2-en-1-one (XXIV) and phenyl-hydrazine were reacted to yield 5-(4''-Isopropylphenyl)-3-(3'-methoxyphenyl)-1-phenyl-4,5-dihydro-1*H*-pyrazole (XXXVI) in glacial acetic acid (Scheme 35). Progress of reaction was noted using thin layer chromatography. Different spectroscopic techniques such as UV-visible, IR, ^{1}H NMR and ^{13}C NMR were used for characterization of synthesized product.

Scheme 35

The synthesized compound (XXXVI) showed absorption maxima at 335 nm in UV-visible region. IR (KBr, ν, cm^{-1}) spectrum showed prominent peaks at 3035 (aromatic CH stretching), 2962 (=CH stretching), 2927 (ν_{as} CH_3 stretching), 2869 (ν_{sym} CH_3 stretching), 1619 (C=N stretching), 1516, 1456, 1426 (aromatic C=C stretching), 1360 (C-H bending of gem-dimethyl), 1286 (C-N stretching), 1159 (N-N stretching), 943 and 855 (C-H bending) and 735 (C-C bending).

(XXXVI)

In ^1H NMR spectrum ($CDCl_3$, 'δ', ppm) six equivalent hydrogens of gem-dimethyl group displayed a doublet at 1.24-1.25 (J = 7.0 Hz). A multiplet corresponding to one proton attached to carbon (CH) atom of isopropyl group was recorded in the range of 2.87-2.93. Two protons attached to C_4 carbon were appeared as a double doublet in the range 3.15-3.20 (J = 17.5, 4.5 Hz) and 3.70-3.76 (J = 17.5, 12.5 Hz). Singlet corresponding to three protons of methoxy group was observed at 3.84. Another double doublet of proton attached to C_5 was observed in the range of 5.58-5.61 (J = 12.5, 4.5 Hz). A multiplet of thirteen aromatic protons was observed in the range of 6.88-7.49.

^{13}C NMR spectrum ($CDCl_3$, 'δ', ppm) recorded peaks at 23.84 due to two carbon atoms of gem-dimethyl. Peak at 33.87 was recorded due to carbon of isopropyl group (CH) directly attached to aromatic ring of cuminaldehyde. Carbon C_4, C_5 and carbon of methoxy

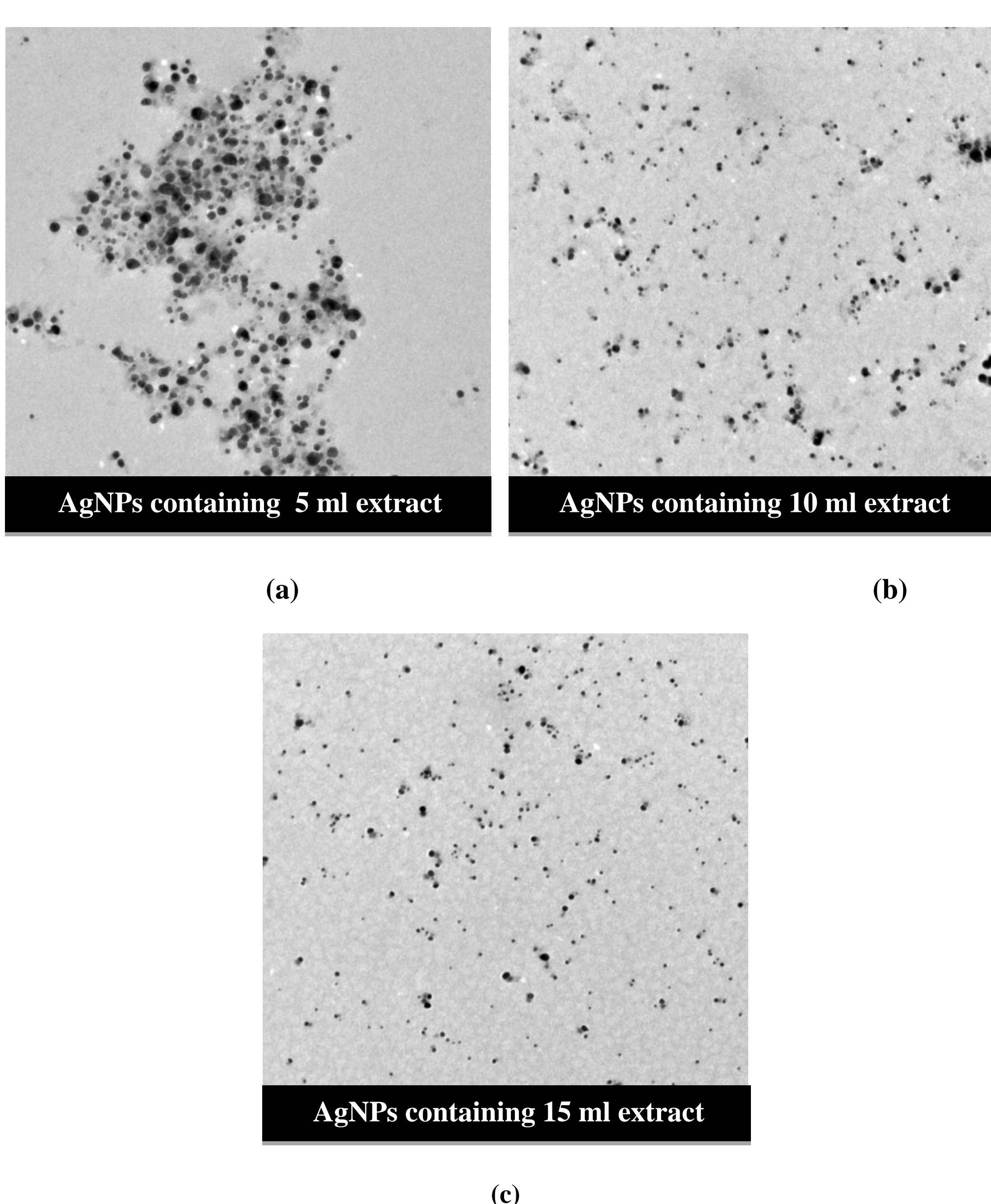


Plate 2: For 1 mM $AgNO_3$, 5 ml of seed extract (a), 10 ml of seed extract (b), 15 ml of seed extract (c)

group displayed their peaks at 40.10, 55.37 and 62.38. Peak corresponding to aromatic carbons and C_3 carbon of pyrazoline ring was observed at 105.18, 110.86, 114.15, 118.49, 125.42, 126.56, 127.44, 128.64, 129.64, 134.46, 140.19, 144.56, 149.19, 151.78 and 159.96.

4.8 PREPARATION OF SILVER NANOPARTICLES (NPs) USING AQUEOUS EXTRACT OF CUMIN SEEDS

Fixed volume (10 ml) of different concentration of silver nitrate *i.e.* 1mM, 3mM and 5mM solution was added to 5 ml, 10ml and 15ml of prepared cumin seed aqueous extract. The reaction mixture was subjected to heating for 30 mins at 80 °C. Color change of the reaction mixture from yellow to dark brown indicated the completion of reaction. The optical properties of the synthesized AgNPs were studied with the help of UV-Vis spectra and Transmission Electron Microscopic (TEM) analysis

The optical properties of synthesized nanoparticles were presented in Table 11. It was observed that the color intensity was increased with increase in the concentration of both seed extract and silver nitrate solution. The maximum color intensity (deep brown) was observed for the 15 ml concentration of extract and 5mM of $AgNO_3$ solution with smallest size of nanoparticles *i.e.* 5-30 nm (Joshi 2020). It was observed that if the concentration of plant extract was kept constant and the concentration of silver nitrate solution was increased from 1mM to 3mM and 5mM then the size of the silver nanoparticles was also increased. The results were in accordance with previous work reported by Ajitha et al (2013) and Janardhanan et al (2009) and Htwe *et al* (2019). They reported a direct relationship between the initial concentration of metal ions with the average nanoparticle size where increasing of initial concentration of metal ions results in increased size of nanoparticles produced.

Table 11: Optical properties of AgNPs (nm) from cumin seed extract at different concentrations

Silver nanopaticles (AgNPs)						
Concentration of $AgNO_3$ (mM)	**Plant extract volume**					
	5ml		**10ml**		**15ml**	
	λ max	**Size (nm)**	**λ max**	**Size (nm)**	**λ max**	**Size (nm)**
1.00	430	9-20	434	7-20	440	6-18
3.00	433	11-25	437	9-20	444	7-15
5.00	440	15-30	443	10-25	448	8-20

The formation and stability of the synthesized nanoparticles of *Cuminum cyminum* in colloidal solution was monitored by UV-vis spectroscopy. UV-vis spectra of synthesized nanoparticles showed maximum absorbance in the range of 430-448 nm. Absorbance of the

synthesized nanoparticles around 430 nm is a characteristic band for the synthesis of silver nanoparticles (Logeswari *et al* 2015). It was noted that the size of the synthesized nanoparticles decreased as increase the amount of aqueous seed extract. It was due to the fact that the seed extract act as a reducing agent *i.e.* helps in reduction of Ag^+ ions to Ag and there was increase in wavelength upto 448 nm as shown in Table 11. The UV-vis spectra were recorded after 24 hrs after the initiation of reaction with varying amount of aqueous seed extract as well as silver nitrate solution.

The intensity of the color was found to increase with the increase in the seed extract concentration. The appearance of brown color was primarily due to the excitation of surface plasmon vibrations and this color change is an indicator of synthesis of nanoparticles (Kumar *et al* 2014). Similarly, the shallow brown and light color of the reaction mixtures indicated the formation of less number of silver nanoparticles. It was also observed that the absorption peak wavelength of AgNPs shifted to the higher wavelength side as the concentration of the extract increased from 5 to15 ml, corresponding to the red shift. This red shift indicated a gradual increment in the mean diameter of the nanoparticles. It was further observed that, as the concentration of extract and metal salt decreased to 5 ml, the absorption wavelength of nanoparticles shifted to lower wavelength side corresponding to blue shift. This blue shift indicated a gradual reduction in the mean diameter of the nanoparticles. The average particle size of silver nanoparticles was found to be from 5 nm to 40 nm.

The UV spectroscopy is a useful study to confirm the bio-reduction of nanoparticles. Bio-reduction of nanoparticles in the presence of plant extract was confirmed from UV–Vis spectral measurements. Nanoparticles contain free electrons which have the probability to give rise to a SPR (surface Plasmon resonance) absorption band (Sindhura *et al* 2014), and this may due to the combined vibration of electrons of metal nanoparticles in resonance with the light wave.

Heat plays a key role in providing energy to the reaction system. The process continues until activation of the capping agent from the plant extracts, which will ultimately arrest the growth of high-energy atomic growth planes. This results in the formation of NPs. Generally, during the synthesis, the reducing agents donate electrons to the metal ions and convert them to NPs. These NPs exist at a high-surface energy state and tend to convert to their low-surface energy conformations by aggregating against each other. Thus, the presence of higher amounts of reducing agents and stabilizing agents prevents the aggregation of nanoparticles and promotes production of smaller NPs. The participation of sugars, terpenoids, polyphenols, alkaloids, phenolic acids, tannins, saponins, carboxylic acids and proteins in the reduction of metal ions into NPSs and in supporting their subsequent stability has also been postulated (Makarov *et al* 2014). The green routes of synthesis for NPs have additional advantages such as eco-friendliness, cost effectiveness, inexpensive, safer, and fast

and provide natural capping and stabilization agents.

Size and morphology of the produced nanoparticles was based on several factors like the plant extract concentration and their metal salt concentration (Ahmed *et al* 2016). Synthesis of the nanoparticles by the usage of plant extract has several benefits due to their eco-friendly, rapid and being economical.

4.9 EVALUATION OF ANTIOXIDANT ACTIVITY

4.9.1 Evaluation of antioxidant activity of cumin oil and cuminaldehyde

Cumin essential oil and its major compound cuminaldehyde were evaluated for antioxidant activity in terms of free radical scavenging activity (RSA) using dimethyl sulphoxide (DMSO) as the control and ascorbic acid as standard.

Table 12: DPPH radical scavenging activity of cumin oil and cuminaldehyde along with standard ascorbic acid at different concentrations

Concentration (µg ml^{-1})	Per cent Radical scavenging activity (RSA)		
	Cumin oil	**Cuminaldehyde**	**Ascorbic acid**
3000	54.95±0.57	59.95±0.57	100±0.00
2000	45.83±0.49	51.36±0.15	100±0.00
1000	38.52±0.42	42.38±0.46	100±0.00
500	29.98±0.84	36.45±0.36	100±0.00
250	26.06±0.23	26.89±1.13	100±0.00
100	21.23±0.37	23.40±0.55	100±0.00
50	16.38±0.14	19.56±0.20	95.56±0.20
25	12.51±0.40	15.52±0.19	88.25±0.40
Particulars		**CD (5%)**	
Compounds		0.33	
Concentrations		0.08	
Compounds × concentrations		0.09	

The free radical scavenging activity was scanned using DPPH as substrate. This assay is based on the stabilization of radical reactant. It gives strong absorption maximum at 517 nm due to the presence of odd electron of DPPH. As the odd electron of the radical becomes paired off in the presence of a hydrogen donor, which is a free radical scavenging antioxidant, the absorbance decreases. The radical scavanging efficiency has been calculated in per cent and further IC_{50} values (µg ml^{-1}) have been presented (Table 12). Out of cuminaldehyde and cumin oil, former was found to be more effective at all the tested concentrations (Figure 7).

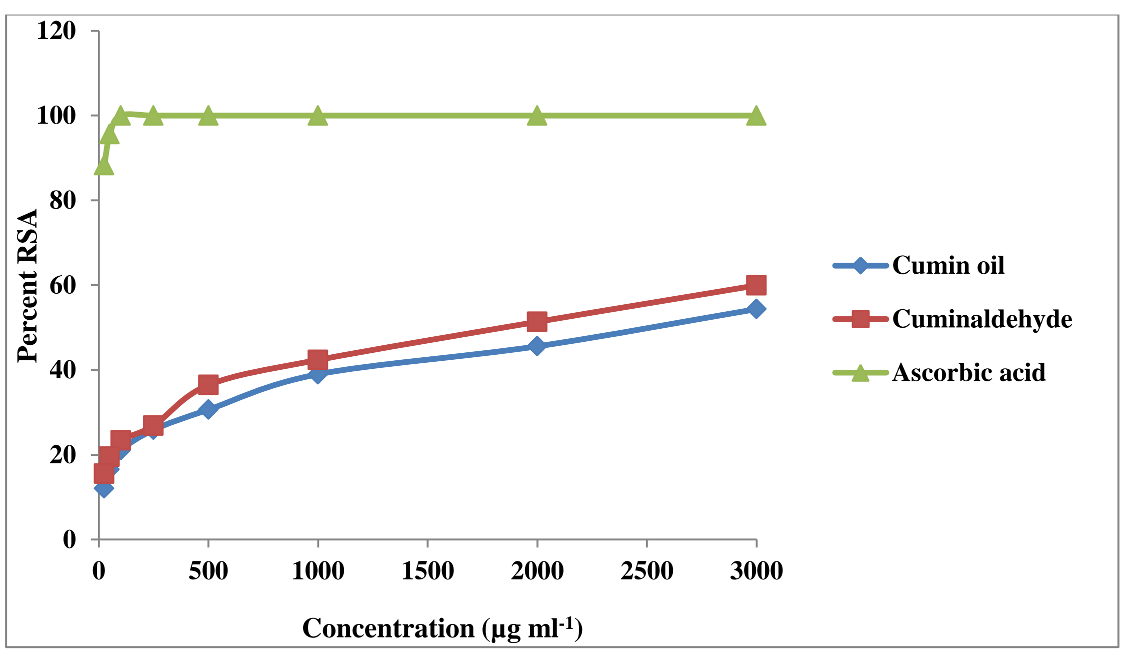


Figure 7: Comparison of %RSA of cumin oil and cuminaldehyde

Therefore, activity of oil may be due to presence of cuminaldehyde as active ingredient in it. Electron donating group *i.e.* isopropyl group at the para position of cuminaldehyde may be responsible for the antioxidant potential. Per cent RSA varied directly with concentration for both oil and cuminaldehyde. It is evident from Table 12 and Figure 7 that cuminaldehyde had more antioxidant potential as compared to cumin oil. IC_{50} of oil, cuminaldehyde and ascorbic acid were found to be 2466, 1960 and 10 µg ml^{-1}, respectively (Figure 8). Einafshar *et al* (2012) also assessed the antioxidant potential of cumin essential oil and recorded similar results. Significant differences were observed between the antioxidant potential of cumin oil and cuminaldehyde. Both cumin oil and cuminaldehyde registered less per cent RSA than ascorbic acid at all the concentrations.

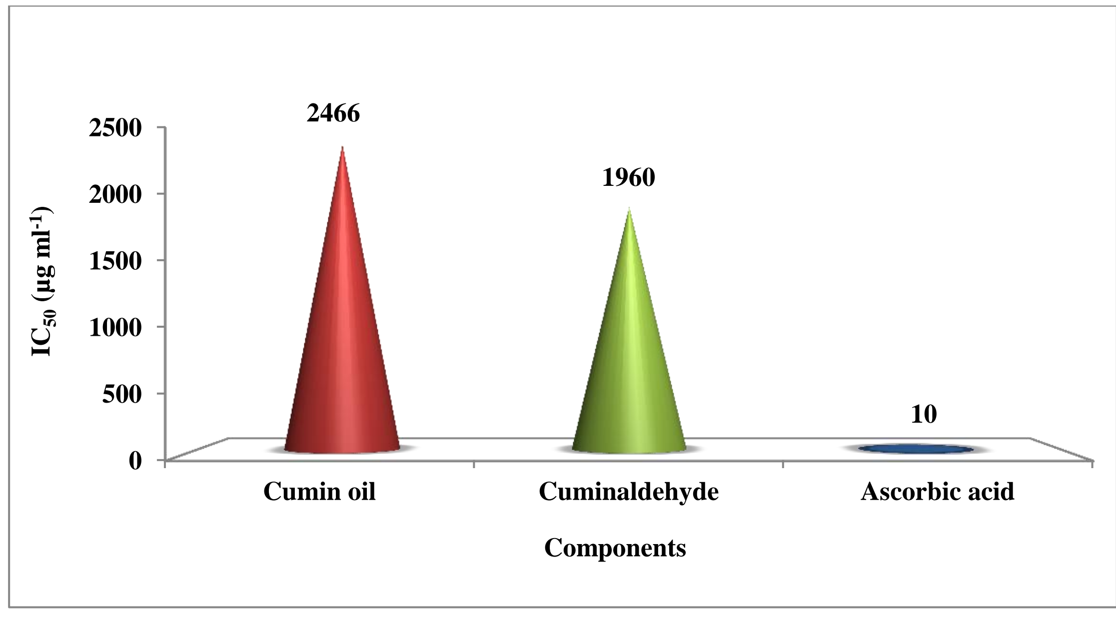


Figure 8: Comparison of IC_{50} of tested compounds

4.9.2 Evaluation of antioxidant activity of different solvent extracts of cumin seeds

Different solvent extracts *i.e.* acetone, petroleum ether, water, methanol, ethanol, chloroform, dichloromethane and ethyl acetate of *Cuminum cyminum* (cumin) seeds were evaluated for their antioxidant potential *via* DPPH assay using dimethyl sulphoxide (DMSO) as the control and ascorbic acid as standard. It was evident from the Table 13 that methanol and aqueous extract possessed more antioxidant potential as compared to other solvent extract. Phenolics and flavonoids are naturally occurring compounds produced in plants from aromatic amino acid phenylalanine, tyrosine and malonate. In plants they acts as antioxidants, antimicrobials and photoreceptors. They are considered as natural antioxidants (Nadeem and Riaz 2012; Pietta 2000; Panche *et al* 2016). The antioxidant potential of the flavonoids is due to their ability to reduce the formation of free radicals and to scavenge the free radicals. According to Halliwell and Gutteridge (1998) mechanisms of antioxidant action can include suppression of reactive oxygen species formation either by inhibition of enzymes (cyclooxygenase, lipoxygenase, microsomal monooxygenase, glutathione S-transferase, mitochondrial succinoxidase, and NADH oxidase) (Brown 1998) or chelating trace elements involved in free radical production, by scavenging reactive oxygen species and upregulating or protecting antioxidant defenses (Hanasaki *et al* 1994; Ursini *et al* 1994). The antioxidant potential of the phenolic compounds is due to their ability to terminate free radicals (Shahidi and Wanasundara 1992; Pourmorad *et al* 2006). They are regarded as free radical terminators. They react with high-energy lipid radicals to convert them to thermodynamically more stable products (Wanasundara and Shahidi 1998). Therefore, antioxidant potential can be directly correlated with the amount of flavonoids and phenolics present in the plant extracts. Present study revealed that methanolic extracts bears highest antioxidant potential (at all the concentrations) among all the solvent extracts of cumin seeds (Table 13, Figure 9).

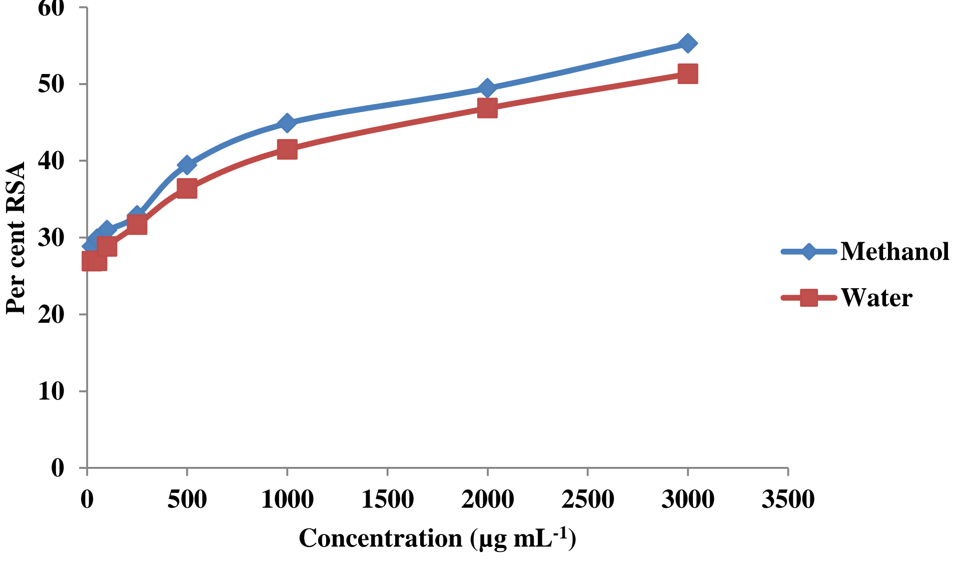


Figure 9: Per cent RSA of methanolic and aqueous extrat

Table 13: DPPH radical scavenging activity of different solvent extracts of cumin seeds along with standard ascorbic acid at different concentrations

Concentration (µg mL^{-1})	Per cent Radical Scavenging Activity								Ascorbic acid
	Acetone	Petroleum ether	Water	Methanol	Ethanol	Dichloro methane	Chloroform	Ethyl acetate	
3000	33.39±1.67	33.50±0.50	51.28±0.49	55.24±0.25	44.58±0.52	31.17±0.76	29.29±0.62	36.46±0.50	100±0.00
2000	28.38±0.33	30.05±1.40	46.83±0.76	49.43±0.51	38.53±0.50	28.23±0.40	28.15±0.13	32.90±0.18	100±0.00
1000	26.25±0.23	30.07±0.39	41.47±0.46	44.87±0.23	34.87±0.23	26.15±0.26	25.88±0.34	31.58±0.52	100±0.00
500	24.81±0.17	29.07±0.73	36.36±0.35	39.40±0.37	32.06±0.36	24.32±0.55	24.28±0.49	30.09±0.87	100±0.00
250	22.66±0.57	25.56±0.82	31.63±0.30	32.88±0.83	30.88±0.21	22.10±0.10	23.10±0.18	26.56±0.51	100±0.00
100	21.63±0.55	22.14±1.03	28.83±0.29	30.95±0.48	28.62±0.33	21.51±0.88	21.21±0.36	23.19±0.73	100±0.00
50	17.64±0.56	18.11±0.54	26.95±0.62	29.83±0.29	26.40±0.48	16.76±0.41	16.07±0.12	20.23±0.68	95.75±0.29
25	17.02±0.48	17.77±0.25	25.56±0.96	28.82±0.28	24.83±0.76	16.37±0.32	15.22±0.38	19.42±0.52	89.01±0.31

Particulars	CD (5%)
Compounds	0.21
Concentrations	0.47
Compounds × concentrations	0.32

It is evident from Table 13 that methanolic extract contains highest amount of phenolics and flavonoids which are responsible for its antioxidant potential (Teixeira *et al* 2013). Aqueous extract also bears comparable antioxidant potential and bears highest amount of phenolics and flavonoids after methanolic extract. IC_{50} value of methanolic and aqueous extract (calculated from Figure 10) is 2100 and 2400, respectively.

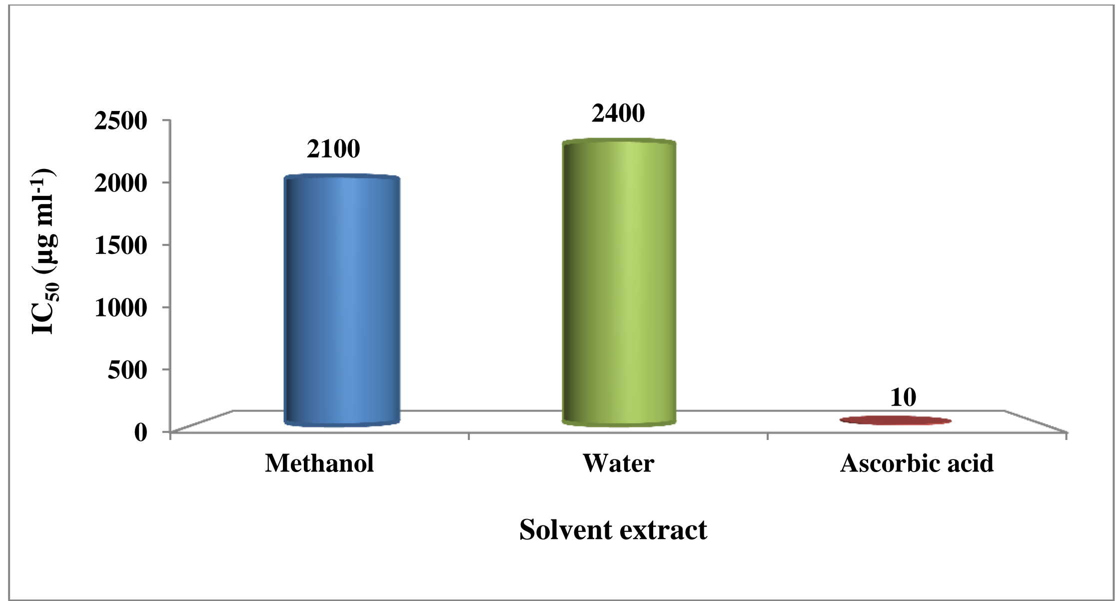


Figure 10: Comparison of IC_{50} of methanol and aqueous extract with standard ascorbic acid

Antioxidant potential of different solvent extracts follows the order: Methanol > water > ethanol > ethyl acetate > petroleum ether > acetone > dichloromethane > chloroform. This order is in accordance with the combination of amount of phenolics and flavonoids present in the different solvent extracts. Significant differences were observed between antioxidant potential of different solvent extracts at different concentrations. None of the plant extracts registered more activity than the standard ascorbic acid.

4.9.3 Evaluation of antioxidant activity of Schiff bases, thiosemicarbazones and hydrazones

Different synthesized derivatives of cuminaldehyde *i.e.* Schiff bases, thiosemicarbazones and hydrazones were screened for their antioxidant potential by DPPH assay. Antioxidant activity was tested by varying the concentration of the compounds and this variation is shown in Table 14 and the IC_{50} values, determined from log dose response curve, are shown in Figure 11.

Among all the synthesized compounds, Schiff bases possess highest antioxidant potential as compared to different substituted thiosemicarbazone and hydrazones. Out of all Schiff bases, compounds III and VIII exhibited highest antioxidant potential. Azomethine group increase the antioxidant potential of the synthesized products. Effectiveness of compound III is may be due to the presence of free OH group. Gaikwad and his workers

Table 14: DPPH radical scavenging activity of synthesized Schiff bases, thiosemicarbazones and hydrazones

Conc. ($\mu g\ mL^{-1}$) / Compounds	Per cent Radical Scavenging Activity							
	3000	2000	1000	500	250	100	50	25
II	26.53 ± 0.61	24.26 ± 1.15	22.42 ± 0.52	21.49 ± 0.50	21.25 ± 0.25	20.61 ± 0.35	14.56 ± 0.41	10.28 ± 0.25
III	89.67 ± 0.49	85.57 ± 0.40	71.16 ± 0.15	67.38 ± 0.54	57.12 ± 0.13	54.70 ± 0.61	49.07 ± 0.40	41.19 ± 0.27
IV	54.70 ± 0.27	49.52 ± 0.48	44.20 ± 0.27	36.87 ± 0.15	33.88 ± 0.16	29.60 ± 0.36	25.31 ± 0.17	18.17 ± 0.29
V	54.99 ± 0.01	50.66 ± 0.34	44.53 ± 0.36	37.18 ± 0.28	34.34 ± 0.14	29.60 ± 0.40	26.61 ± 0.35	18.20 ± 0.26
VI	51.08 ± 0.14	47.37 ± 0.16	41.20 ± 0.07	34.58 ± 0.10	32.87 ± 0.13	27.36 ± 0.13	19.61 ± 0.35	15.78 ± 0.26
VII	55.14 ± 0.12	52.34 ± 0.57	45.35 ± 0.13	39.40 ± 0.10	35.41 ± 0.09	29.93 ± 0.11	26.73 ± 0.24	19.52 ± 0.50
VIII	87.38 ± 0.14	80.79 ± 0.18	70.54 ± 0.50	66.45 ± 0.51	56.78 ± 0.26	54.73 ± 0.25	48.64 ± 0.32	40.93 ± 0.06
IX	53.50 ± 0.50	48.75 ± 0.25	42.24 ± 0.23	36.27 ± 0.25	33.34 ± 0.14	28.96 ± 0.04	22.71 ± 0.25	16.15 ± 0.05
X	64.35 ± 0.13	54.12 ± 0.07	48.66 ± 0.30	39.71 ± 0.31	36.36 ± 0.13	30.68 ± 0.29	27.41 ± 0.16	21.10 ± 0.13
XI	68.44 ± 0.07	57.11 ± 0.13	49.58 ± 0.38	41.72 ± 0.26	38.36 ± 0.13	31.70 ± 0.27	28.27 ± 0.20	22.11 ± 0.10
XII	69.12 ± 0.10	61.04 ± 0.04	54.20 ± 0.06	42.02 ± 0.08	38.62 ± 0.35	32.30 ± 0.26	30.91 ± 0.09	24.41 ± 0.12
XIII	26.15 ± 0.05	24.23 ± 0.25	20.23 ± 0.25	19.39 ± 0.14	18.34 ± 0.14	15.37 ± 0.12	13.37 ± 0.15	9.95 ± 0.05
XIV	42.36 ± 0.13	38.32 ± 0.18	34.10 ± 0.13	31.31 ± 0.16	29.85 ± 0.15	23.72 ± 0.30	18.71 ± 0.26	14.38 ± 0.11
XV	25.05 ± 0.01	21.13 ± 0.27	19.96 ± 0.25	17.30 ± 0.14	15.37 ± 0.14	12.30 ± 0.56	10.36 ± 0.15	8.85 ± 0.02
XVI	69.40 ± 0.53	62.09 ± 0.10	56.11 ± 0.10	42.25 ± 0.25	39.15 ± 0.15	33.36 ± 0.15	31.12 ± 0.33	25.06 ± 0.05
XVII	67.97 ± 0.05	56.92 ± 0.08	49.18 ± 0.28	40.13 ± 0.11	37.61 ± 0.35	31.08 ± 0.10	28.17 ± 0.28	21.34 ± 0.14
XVIII	50.12 ± 0.13	45.33 ± 0.15	40.19 ± 0.17	34.37 ± 0.13	31.50 ± 0.50	26.74 ± 0.25	19.09 ± 0.14	15.10 ± 0.13
Ascorbic acid	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	95.75±0.29	89.01±0.31

At 5% level of significance CD (Compounds) = 0.33, CD (Concentrations) = 0.38, CD (Compounds×Concentrations) = 0.65

Antioxidant potential of different synthesized compounds could be easily predicted from their IC_{50} values as given in Figure 11.

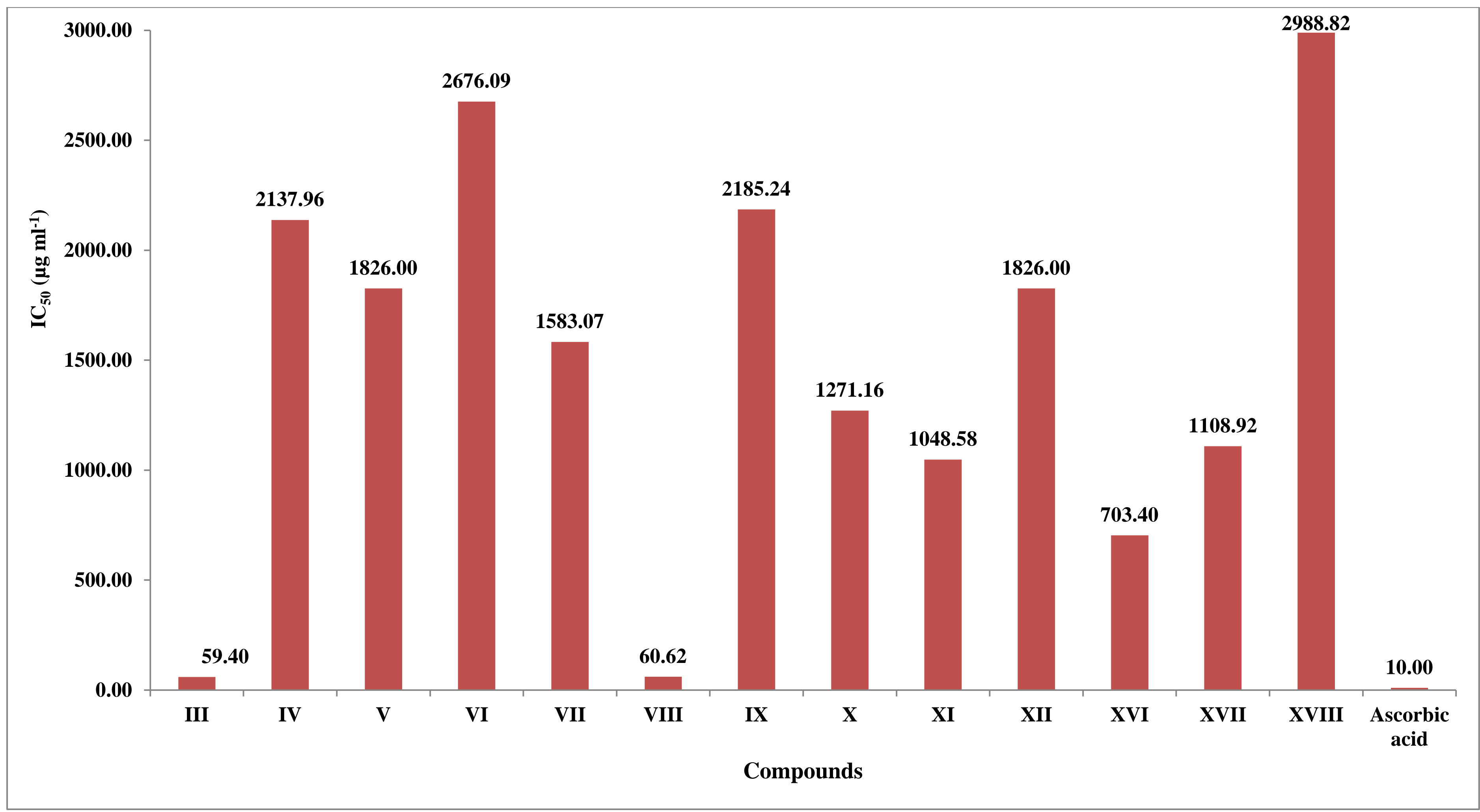


Figure 11: Comparison of IC_{50} of Schiff Bases, thiosemicarbazones and hydrazones with ascorbic acid

reported the similar results. Compound VIII contains phenolic moiety which may made it more active than other compounds. Due to the lower bond dissociation energies (BDE) of O–H, it loses H atom comparatively easier to than other compounds (Bendary *et al* 2013). BDE is an important factor in determining the efficacy of an antioxidant since the weaker the OH bond, the faster will be the reaction with the free radicals (Kumari *et al* 2019). The antioxidant potential of the phenolic compounds is also because of its ability to terminate free radicals (Pourmorad *et al* 2006). These are regarded as free radical terminators. They react with high-energy lipid radicals to convert them to thermodynamically more stable products. Compound VIII containing azomethine moiety along with substituted pyridine ring also showed comparable activity as compound III. The antioxidant activity of synthesized compounds is presented in Table 14 and follows the trend: III > VIII > XVI > XII > XI > XVII > X > VII > V > VI > IX > VI > XVIII > XIV > II > XIII at all tested concentrations. Significant differences were observed between all the synthesized compounds at all the concentrations. No compound registered more activity than the standard ampicillin.

4.9.4 Evaluation of antioxidant activity of chalcones and pyrazolines of cuminaldehyde

Chalcones (XIX-XXIV) and pyrazolines (XXV-XXXVI) were evaluated for their antioxidant potential *via* DPPH assay. The variation of percent radical scavenging activity (% RSA) of synthesized compounds at different compounds was enlisted in Table 15. Compound XXXII was found effective among all the synthesized compounds. It was found that the compounds containing electron withdrawing groups displayed better activity as compared to compounds containing electron donating substituent. Pyrazoline being heterocyclic compounds exhibited higher activity as compared to chalcones. It was evident from Table 15 that pyrazolines (XXV-XXX) which were synthesized from condensation of chalcones and phenyl hydrazine exhibited better activity than pyrazoline (XXXI-XXXVI) synthesized from chalcones and hydrazine hydrate. The better activity of the pyrazolines than chalcones was may be due to the presence of heterocyclic moiety in the pyrazolines. In case of chalcones, electron withdrawing groups displayed better activity. As the electronegativity of the halogen atom decreases antioxidant potential decreases. Similar trends were observed in case of pyrazolines. Therefore, antioxidant potential of synthesized compounds was directly proportional to the electronegativity of the halogen atom attached to the aromatic ring. It was found that para substituted products were more effective than meta substituted product. Thus, order of effectiveness of synthesized chalcones followed the order: XX>XXI>XXII>XXIII>XIX>XXIV. Pyrazolines synthesized by reacting chalcones with phenyl hydrazine (XXXI-XXXVI) were found to be more effective as compared to pyrazolines synthesized from substituted chalcones and hydrazine hydrate (XXIV-XXX). Additional phenyl ring present in compounds (XXXI-XXXVI) may enhance the antioxidant potential of synthesized compounds (Sapnakumari *et al* 2015). Order of effectiveness of

Table 15: DPPH radical scavenging activity of synthesized chalcones (XIX-XXIV) and pyrazolines (XXV-XXXVI)

Compounds \ Conc. (μg mL^{-1})	Per cent Radical Scavenging Activity							
	3000	**2000**	**1000**	**500**	**250**	**100**	**50**	**25**
XIX	31.23±0.74	27.72±0.46	26.96±0.03	22.79±0.24	22.04±0.98	20.28±0.95	19.77±0.92	18.39±1.42
XX	50.41±0.19	48.61±0.53	45.49±0.45	42.37±0.70	40.91±0.11	38.68±0.29	34.42±0.37	28.93±0.29
XXI	47.17±0.37	45.05±0.43	42.06±0.86	39.94±0.09	37.77±0.30	34.77±0.21	30.13±0.84	25.56±0.51
XXII	33.18±0.34	30.93±0.66	29.85±0.26	28.39±0.53	26.51±0.50	25.95±0.08	23.16±0.29	21.81±0.72
XXIII	31.26±0.65	28.61±0.35	28.21±0.70	27.99±0.63	26.60±0.53	26.33±0.45	22.93±0.60	21.63±0.54
XXIV	31.06±0.11	27.40±0.34	26.81±0.27	22.57±0.51	20.31±0.27	19.39±0.53	17.46±0.44	16.29±0.26
XXV	43.27±0.28	41.30±0.29	38.17±0.39	33.22±0.41	30.63±0.55	27.48±0.45	24.18±0.32	23.16±0.66
XXVI	56.25±0.46	52.40±0.42	49.27±0.31	44.10±0.22	43.33±0.31	40.42±0.53	36.45±0.50	30.03±0.18
XXVII	53.25±0.32	51.56±0.51	47.19±0.17	41.26±0.28	38.23±0.29	36.13±0.59	32.05±0.48	28.50±0.48
XXVIII	50.43±0.46	48.41±0.45	45.20±0.17	40.18±0.16	35.17±0.71	31.24±0.25	30.08±0.15	27.37±0.44
XIX	46.24±0.25	43.15±0.15	40.19±0.13	36.19±0.18	32.11±0.18	29.36±0.15	26.35±0.33	25.30±0.39
XXX	41.43±0.37	39.61±0.66	37.52±0.50	32.18±0.32	28.25±0.38	25.19±0.43	23.04±0.37	21.29±0.42
XXXI	52.21±0.17	50.20±0.17	45.42±0.44	42.50±0.48	38.92±0.62	34.02±0.47	29.52±0.46	27.19±0.32
XXXII	64.42±0.51	61.41±0.52	59.27±0.34	53.83±0.24	49.51±0.48	45.44±0.50	39.94±0.34	32.39±0.35
XXXIII	61.18±0.35	58.39±0.35	56.26±0.28	51.49±0.44	45.30±0.35	42.29±0.26	37.52±0.50	30.27±0.27
XXXIV	57.34±0.57	52.33±0.31	49.41±0.35	46.22±0.19	43.61±0.26	39.22±0.29	35.00±0.01	29.18±0.27
XXXV	54.30±0.35	51.37±0.46	47.32±0.30	44.20±0.27	41.19±0.34	37.48±0.44	31.99±0.42	28.22±0.38
XXXVI	50.35±0.34	45.45±0.62	43.30±0.35	38.86±0.25	35.53±0.48	31.45±0.40	27.40±0.34	25.49±0.42
Ascorbic acid	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	95.75±0.29	89.01±0.31

At 5% level of significance: CD (Compounds) = 0.57, CD (Concentrations) = 0.24, CD (Compounds×Concentrations) = 0.27

synthesized pyrazolines (derived by reacting chalcones and phenyl hydrazine followed the order: XXXII> XXXIII >XXXIV>XXXV> XXXI>XXXIV.

IC_{50} of synthesized chalcones and pyrazolines was also determined and presented in Figure 12. It was evident from IC_{50} values that the pyrazolines synthesized using chalcones and phenyl hydrazine showed better antioxidant potential as compared to substituted chalcones and pyrazolines synthesized by reacting chalcones and hydrazine hydrate.

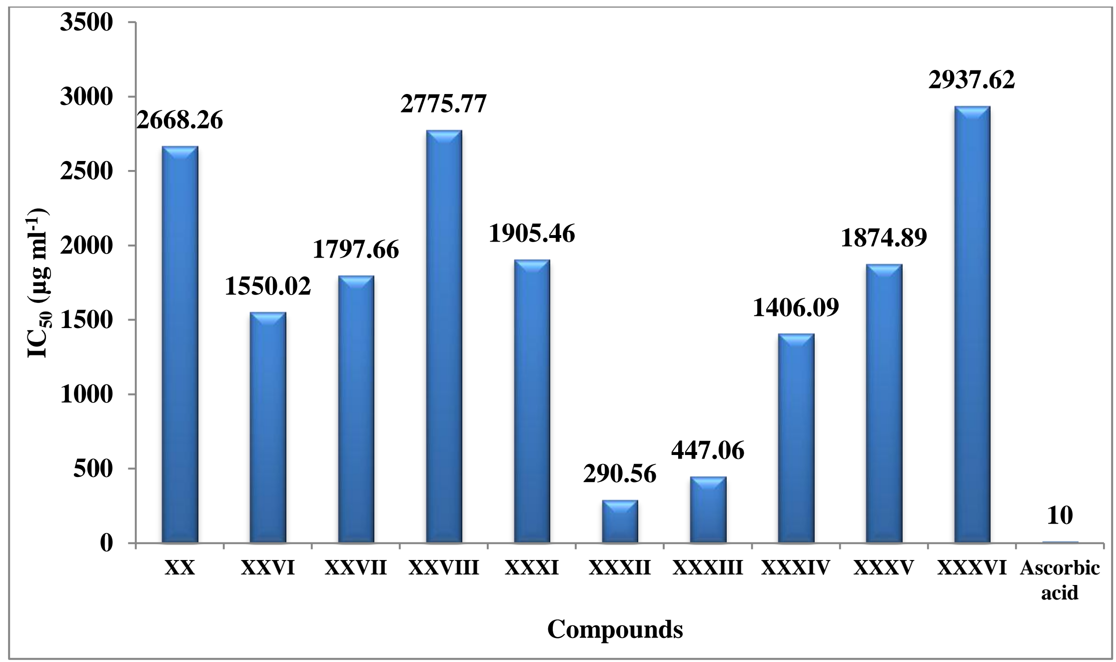


Figure 12: Comparison of IC_{50} of chalcones and pyrazolines with standard ascorbic acid

It was clear from above Figure 12 that pyrazolines (XXXII and XXXIII) were more effective than the remaining pyrazolines and chalcones. Chalcone and pyrazolines having Fluorine atom at para position were found more effective than remaining chalcones and pyrazolines, respectively.

4.9.5 evaluation of antioxidant activity of silver nanoparticles

Different synthesized silver nanoparticles of cumin seeds were screened for their antioxidant potential by DPPH assay. DPPH was used as the free radical source, since it simulates reactive oxygen and nitrogen species affecting biological systems. Antioxidant activity was tested by varying the concentration of both plant extracts (5 ml, 10 ml and 15 ml) and silver nitrate solution (1mM, 3mM and 5mM) and this variation is shown in Table 16 and the IC_{50} values were not determined as no synthesized nanoparticles showed radical scavenging activity more than 50%. It was found that at a particular concentration, the synthesized nanoparticles showed better activity than solvent extracts of cumin seeds. Similar results were reported by Abdel-Aziz *et al* (2014). The better activity of silver nanoparticles than solvent extracts was may be due to higher phenolic and flavonoid content in AgNPs-containing seed extract compared to the plant extract.

Table 16: Antioxidant activity of silver nanoparticles from aqueous extract of cumin

Concentration of $AgNO_3$ (mM)	Volume of plant extract (ml)								
	5ml			10ml			15ml		
	Conc. of NPs (mg ml^{-1})								
	0.10	**0.20**	**0.40**	**0.10**	**0.20**	**0.40**	**0.10**	**0.20**	**0.40**
	Per cent Radical Scavenging Activity								
1.00	29.03	31.26	35.46	32.29	35.36	38.17	35.65	37.51	40.88
3.00	33.79	35.06	39.06	36.78	38.15	40.86	39.69	42.45	45.96
5.00	39.58	40.75	42.98	41.09	43.23	45.27	43.63	45.54	49.51
Ascorbic acid	100.00	100.0	100.0	100.0	100.00	100.00	100.00	100.00	100.00

Particulars	**CD (5%)**
Compounds	0.58
Concentrations	0.24
Compounds × concentrations	0.41

It was evident from Table 16 that antioxidant potential of silver nanoparticles increased with increase in the concentration of plant extract as well as the concentration of silver nitrate solution. The increased activity of silver nanoparticles with the increased concentration of seed extract may be due to presence of higher content of phenolics and flavonoids. Phenolics and flavonoids are considered as a natural antioxidants, therefore enhanced the antioxidant potential of AgNPs-containing cumin seed extract. Significant differences were observed between the antioxidant potential of different synthesized nanoparticles at different concentrations.

4.10 EVALUATION OF MICROBIAL ACTIVITY

4.10.1 Evaluation of microbial activity of cumin oil and cuminaldehyde

Cumin oil, cuminaldehyde, different solvent extracts of cumin seeds, silver nanoparticles from aqueous extract of and derivatives of cuminaldehyde were evaluated for *in vitro* microbial activity against *Pseudomonas* sp., *Klebsiella* sp. and *Enterobacter* sp. by filter paper disc diffusion method. Cumin oil was found more effective as compared to cuminaldehyde against all three tested bacteria. Since all the three tested bacteria were grm negative bacteria therefore mode of action of cuminaldehyde was same in all the bacteria. Higher bioactivity of cumin oil was due to mechanism of action of cumin oil in bacterial cells *i.e.* cell elongation, repression of capsule expression in bacteria and inhibition of urease activity (Abdul Jabbar 2013).

Pseudomonas sp.

Evaluation of microbial activity of cumin oil, cuminaldehyde and ampicillin against *Pseudomonas* sp. was presented in Table17. It was evident from Table 17 that cumin oil exhibited more activity as compared to cuminaldehyde which was due to inhibition of urease activity in bacteria by cumin oil. Cumin oil was more effective as compared to cuminaldehyde which may be due to the lipophilic constituents present in cumin oil that penetrates into the cell walls of bacteria and cause elongation or inhibit urease activity (Abdul Jabbar 2013). Cumin oil was found to be more effective than cuminaldehyde against *Pseudomanas sp.* at concentration above 250 µg ml^{-1}. No inhibition zone was detected at lower concentrations. Significant differences were observed between the microbial activity of cumin oil and cuminaldehyde. Neither cumin oil nor cuminaldehyde registered more activity than standard ampicillin.

Table 17: Microbial activity of cumin oil and cuminaldehyde along with standard ampicillin on the growth of *Pseudomonas sp*. at different concentrations

Concentration (µg ml^{-1})	Inhibition zone (in mm)		
	Cumin oil	**Cuminaldehyde**	**Ascorbic acid**
3000	11.56±0.26	10.50±0.56	24.00±0.58
2000	9.87±0.06	8.95±0.11	20.00±0.26
1000	8.01±0.36	7.52±0.32	18.31±0.21
500	6.50±0.11	6.21±0.14	16.30±0.33
250	6.22±0.26	ND	15.10±0.11
100	ND	ND	8.20±0.26
50	ND	ND	6.60±0.21
25	ND	ND	6.10±0.25
Particulars		**CD (5%)**	
Compounds		0.21	
Concentrations		0.02	
Compounds × concentrations		0.11	

Klebsiella sp.

Evaluation of microbial activity of cumin oil, cuminaldehyde and ampicillin against *Klebsiella* sp. was presented in Table 18. Among all the three tested bacteria cumin oil was most effective against *Klebsiella* sp. It was evident from Table 18 that cumin oil exhibited more activity as compared to cuminaldehyde. Cumin oil was found to be more effective than

cuminaldehyde against *Klebsiella* sp. at concentration above 100 µg ml^{-1}. No inhibition zone was detected at lower concentrations. Significant differences were observed between the microbial activity of cumin oil and cuminaldehyde against *Klebsiella* sp. Neither cumin oil nor cuminaldehyde registered more activity than standard ampicillin.

Table 18: Microbial activity of cumin oil and cuminaldehyde along with standard ampicillin on the growth of *Klebsiella sp*. at different concentrations (µg mL^{-1})

Concentration (µg ml^{-1})	Inhibition zone (in mm)		
	Cumin oil	**Cuminaldehyde**	**Ascorbic acid**
3000	16.26±0.26	15.23±0.65	30.00±0.12
2000	13.52±0.03	12.36±0.74	23.00±0.02
1000	10.56±0.59	9.56±0.23	19.00±0.03
500	8.20±0.44	7.89±0.58	17.00±0.06
250	6.9±0.23	6.58±0.02	16.00±0.08
100	6.1±0.36	ND	8.60±0.52
50	ND	ND	6.80±0.21
25	ND	ND	6.20±0.10
Particulars		**CD (5%)**	
Compounds		0.33	
Concentrations		0.21	
Compounds × concentrations		0.11	

***Enterobacter sp*.**

Screening of microbial activity of cumin oil and cuminaldehyde against *Enterobacter sp*. at different concentrations revealed that cumin oil was more effective than cuminaldehyde. Variation of bioactivity of cumin oil and cuminaldehyde was given in Table 19. Effectiveness of cumin oil against *Enterobacter sp*. was may be due to the inhibition of urease activity. Both cumin oil and cuminaldehyde registered less activity than standard ampicillin. Significant differences were observed between the bioactivity of cumin oil and cuminaldehyde at tested concentrations.

It was evident from the above Tables 17, 18 and 19 that cumin oil registered more activity against all three tested bacteria. Minimum inhibition concentration of cumin oil and cuminaldehyde was also determined and presented in Figure 13. MIC values of cumin oil was less than cuminaldehyde against all three tested bacteria that in turn showed the effectiveness of cumin oil.

Table 19: Microbial activity of cumin oil and cuminaldehyde along with standard ampicillin on the growth of *Enterobacter sp*. at different concentrations (µg mL^{-1})

Concentration (µg ml^{-1})	Inhibition zone (in mm)		
	Cumin oil	**Cuminaldehyde**	**Ascorbic acid**
3000	13.02±0.12	11.20±0.56	24.00±0.02
2000	11.85±0.01	10.01±0.02	22.00±0.03
1000	9.23±0.02	8.21±0.21	18.50±0.00
500	7.05±0.40	6.55±0.18	16.50±0.19
250	6.10±0.12	ND	15.50±0.10
100	ND	ND	8.30±0.03
50	ND	ND	6.10±0.01
25	ND	ND	6.10±0.25

Particulars	**CD (5%)**
Compounds	0.32
Concentrations	0.12
Compounds × concentrations	0.36

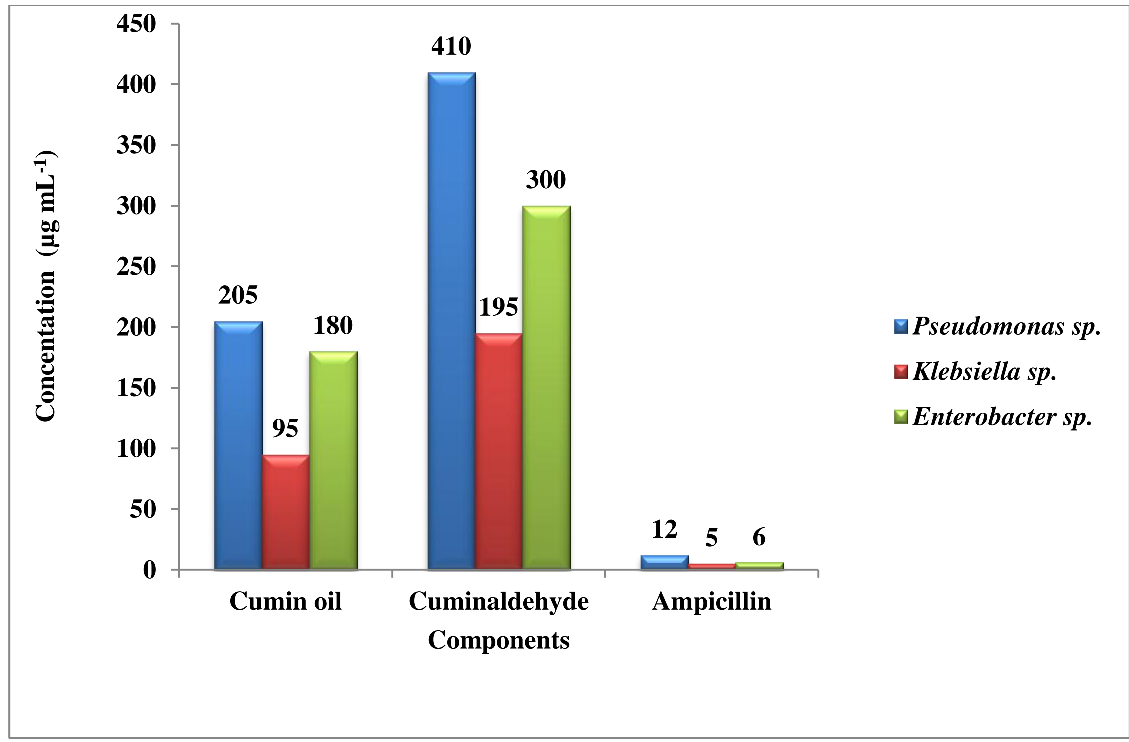


Figure 13: MIC (Minimum inhibition concentration) of cumin oil and cuminaldehyde along with ampicillin against *Pseudomonas sp*., *Klebsiella sp*., *Enterobacter sp*.

4.10.2 Evaluation of microbial activity of different solvent extracts of cumin seeds

The different solvent extracts of cumin seeds namely acetone, petroleum ether, water, methanol, dichloromethane, chloroform and ethyl acetate of cumin seeds were subjected to antimicrobial evaluation against three gram negative bacteria *Pseudomonas sp. Klebsiella sp.* and *Enterobacter sp.* The results obtained *i.e.* values of inhibition zones and MIC value of different solvent extracts against three gram negative bacteria are graphically represented in Tables 20, 21, 22 and Figure 14, respectively. It is evident from the results that the plant extracts does not display any significant microbial activity. All extracts are ineffective in inhibiting the growth of the three tested bacteria. Similar results are also reported by Shaik *et al* 2018. Out of all solvent extracts, aqueous extract displays maximum antimicrobial activity at all the tested concentrations against three tested bacteria. Dichloromethane and ethylacetate extracts are least effective among all the extracts against all the bacteria. Significant differences were observed between the bioactivity of different solvent extracts of cumin seeds at different concentrations. None of the extract was found to be as effective as standard ampicillin at any of test concentrations.

Table 20: Microbial activity of different solvent extracts of cumin seeds on the growth of *Pseudomonas sp*., at different concentrations

Conc. (μg mL^{-1}) / Solvent extract	Inhibition zone (in mm)							
	3000	**2000**	**1000**	**500**	**250**	**100**	**50**	**25**
Acetone	9.39	8.31	ND	ND	ND	ND	ND	ND
Petroleum ether	10.03	8.39	ND	ND	ND	ND	ND	ND
Water	13.36	10.73	9.71	6.72	ND	ND	ND	ND
Methanol	12.31	9.61	8.72	6.60	ND	ND	ND	ND
Ethanol	9.03	7.39	ND	ND	ND	ND	ND	ND
Chloroform	10.39	9.31	8.67	ND	ND	ND	ND	ND
Dichloro-methane	7.65	6.36	ND	ND	ND	ND	ND	ND
Ethyl acetate	7.34	6.32	ND	ND	ND	ND	ND	ND
Ampicillin	24.00	20.00	18.30	16.30	15.10	8.20	6.60	6.10
Particulars				**CD (5%)**				
Compounds				0.32				
Concentrations				0.51				
Compounds × concentrations				0.69				

*ND stands for inhibition zone not detected

Table 21: Microbial activity of different solvent extracts of cumin seeds on the growth of *Klebsiella sp*. at different concentrations

Conc. ($\mu g\ mL^{-1}$) / Solvent extract	Inhibition zone (in mm)							
	3000	**2000**	**1000**	**500**	**250**	**100**	**50**	**25**
Acetone	8.30	7.34	ND	ND	ND	ND	ND	ND
Petroleum ether	8.32	7.36	ND	ND	ND	ND	ND	ND
Water	9.74	9.34	8.75	ND	ND	ND	ND	ND
Methanol	8.79	8.36	7.73	ND	ND	ND	ND	ND
Ethanol	7.74	7.35	ND	ND	ND	ND	ND	ND
Chloroform	8.39	7.74	ND	ND	ND	ND	ND	ND
Dichloro-methane	7.75	6.76	ND	ND	ND	ND	ND	ND
Ethyl acetate	7.32	6.71	ND	ND	ND	ND	ND	ND
Ampicillin	30.00	23.00	19.00	17.00	16.00	8.60	6.80	6.20
Particulars			**CD (5%)**					
Compounds			0.21					
Concentrations			0.31					
Compounds × concentrations			0.06					

*ND stands for inhibition zone not detected

Table 22: Microbial activity of different solvent extracts of cumin seeds on the growth of *Klebsiella sp*., at different concentrations

Conc. ($\mu g\ mL^{-1}$) / Solvent extract	Inhibition zone (in mm)							
	3000	**2000**	**1000**	**500**	**250**	**100**	**50**	**25**
Acetone	8.33	7.34	ND	ND	ND	ND	ND	ND
Petroleum ether	8.30	7.31	ND	ND	ND	ND	ND	ND
Water	12.32	10.71	9.31	7.61	ND	ND	ND	ND
Methanol	11.30	9.70	8.36	6.32	ND	ND	ND	ND
Ethanol	8.34	7.34	ND	ND	ND	ND	ND	ND
Chloroform	10.70	8.33	6.34	ND	ND	ND	ND	ND
Dichloro-methane	7.64	6.60	ND	ND	ND	ND	ND	ND
Ethyl acetate	7.70	6.72	ND	ND	ND	ND	ND	ND
Ampicillin	24.00	22.00	18.50	16.50	15.50	8.30	6.70	6.10
Particulars			**CD (5%)**					
Compounds			0.02					
Concentrations			0.32					
Compounds × concentrations			0.61					

*ND stands for inhibition zone not detected

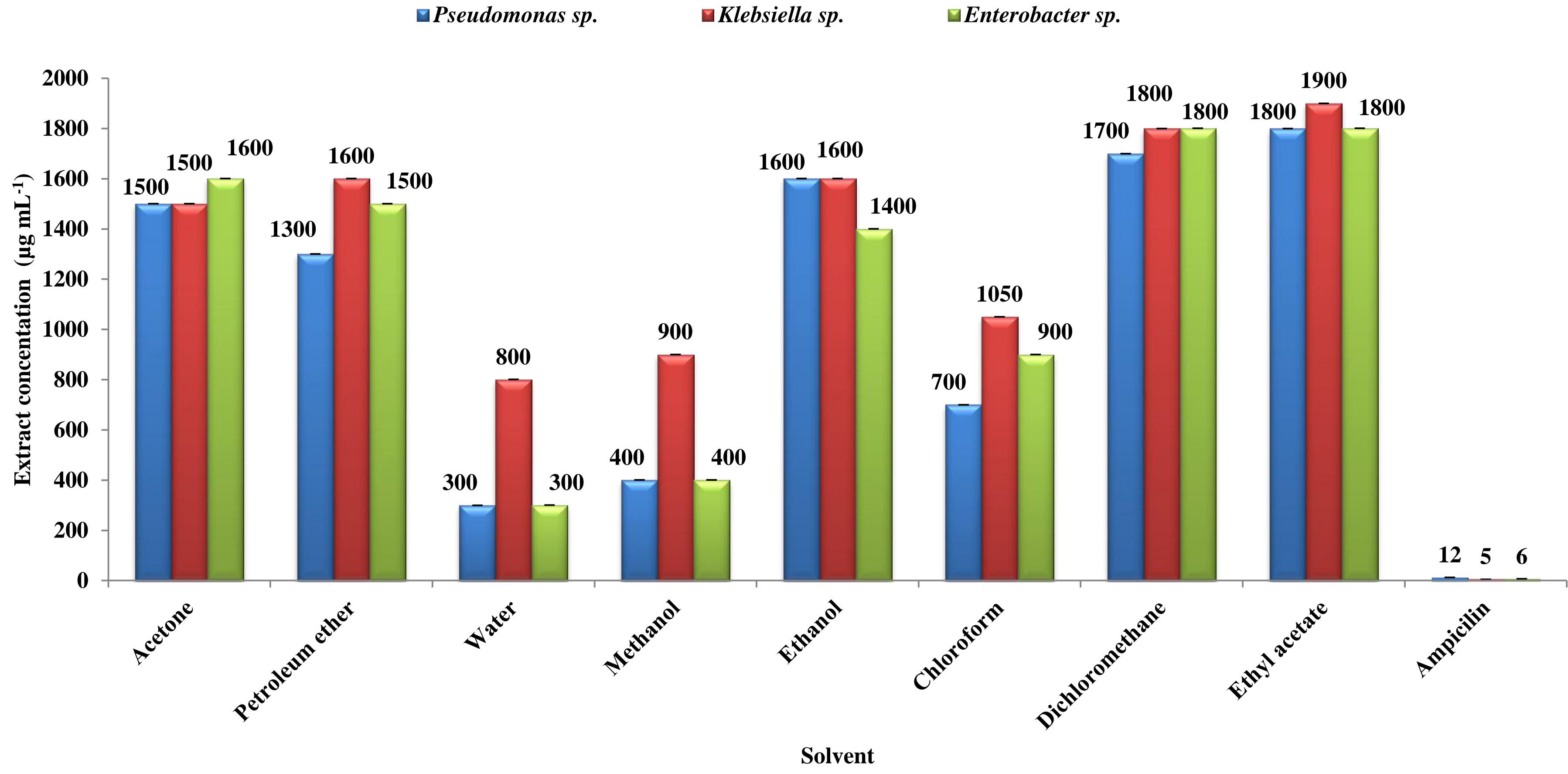


Figure 14: **MIC (Minimum inhibition concentration) of different solvent extracts of cumin seeds against *Pseudomonas sp*., *Klebsiella sp*. and *Enterobacter sp*.**

4.10.3 Evaluation of microbial activity of Schiff bases, thiosemicarbazones and hydrazones of cuminaldehyde

The newly synthesized derivatives of cuminaldehyde **(II-XVIII)** were evaluated for their *in vitro* antimicrobial potential against Gram-negative bacteria *viz. Pseudomonas sp*., *Klebsiella sp*. and *Enterobacter sp.* at different concentrations (3000, 2000, 1000, 500, 250, 100, 50 and 25 µg mL^{-1}). The results obtained *i.e.* values of inhibition zones and MIC values are graphically represented in Table 23, 24, 25 and Figures 15,16, 17, respectively. Inhibition zone data was analyzed statistically by factorial CRD to determine the significant differences among the activities of synthesized compounds against three bacteria. The bioactivity of the cuminaldehyde derivatives varied significantly 5% level of significance. All synthesized cuminaldehyde derivatives exhibited moderate to good antimicrobial potential against the tested bacteria. Antimicrobial potential of synthesized compounds was also assessed by their MIC using bacterial sensitivity-filter paper disc method. Ampicillin was used as the standard drug against all the tested bacteria.

***Pseudomonas* sp.**

Synthesized derivatives of cuminaldehyde namely Schiff bases, thiosemicarbazones and hydrazones were evaluated for their antimicrobial potential against *Pseudomonas sp.* at different concentrations *i.e.* 3000, 2000, 1000, 500, 250, 100, 50 and 25 µg mL^{-1}. Screening of antimicrobial potential of synthesized derivatives of cuminaldehyde against *Pseudomonas sp.* revealed that compound IX possessed higher antimicrobial potential (Table 23, Figure 15). The greater potential of compound IX against *Pseudomonas sp.* may be due to the combination of azomethine group and pyrimidine ring (Kaur *et al* 2019; Rostom *et al* 2009; Holla *et al* 2005). Inhibition zones of prepared compounds followed the order: IX > III > XIV > IV > X > XVIII > XIII > XI > V > XII > VII > VIII > XVII > II > XVI > VI > XV.

Klebsiella sp.

Concerning the antibacterial activity against *Klebsiella sp.*, the results revealed that different synthesized compounds displayed variable inhibitory effects on the growth of the tested Gram-negative bacteria and followed the order: XIV > XI > XVIII > X > III > IV > II > V > VIII > XII > VII > XVIII > VI > XVII > XVI > IX > XV (Table 24, Figure 16). Electron delocalization in thiosemicarbazide moiety (Nandi *et al* 1984) along with azomethine group may be responsible for higher antimicrobial potential of compound XIV.

Enterobacter sp.

Bioactivity of different synthesized derivatives of cuminaldehyde against *Enterobacter sp.* follows the trend: XVIII > X > VIII > XIV > III > V > VII > II > IV > XII > XIII > VI > XVII > IX > XI > XVI > XV. Effectiveness of compound XVIII against *Enterobacter sp.* may be due to the presence of the electron withdrawing groups on the aromatic ring (Ortiz *et al* 2016) along with the azomethine group (Table 25, Figure 17).

Table 23: Microbial activity of Schiff bases, thiosemicarbazones and hydrazones on the growth *Pseudomonas* sp.

Conc. ($\mu g\ mL^{-1}$) / Solvent extract	Inhibition zone (in mm)							
	3000	**2000**	**1000**	**500**	**250**	**100**	**50**	**25**
II	11.72	9.7	8.3	7.0	6.3	ND	ND	ND
III	15.32	12.3	10.7	8.7	7.3	6.2	ND	ND
IV	14.76	12.31	10.32	8.33	7.01	ND	ND	ND
V	13.31	11.70	9.73	7.70	6.70	ND	ND	ND
VI	10.33	9.33	7.71	6.32	ND	ND	ND	ND
VII	12.74	11.31	9.35	7.34	6.32	ND	ND	ND
VIII	12.71	10.76	8.35	7.03	6.32	ND	ND	ND
IX	17.36	14.33	12.01	9.02	7.73	6.70	ND	ND
X	14.30	12.70	10.33	8.31	7.01	ND	ND	ND
XI	13.76	11.70	9.74	7.73	6.72	ND	ND	ND
XII	13.34	11.30	9.32	7.31	6.33	ND	ND	ND
XIII	13.77	11.72	10.34	8.00	7.01	ND	ND	ND
XIV	14.73	12.30	10.70	8.72	7.33	6.11	ND	ND
XV	10.10	9.20	7.53	6.21	ND	ND	ND	ND
XVI	11.04	9.70	8.30	6.73	ND	ND	ND	ND
XVII	12.35	10.32	8.30	7.02	6.30	ND	ND	ND
XVIII	13.70	11.70	10.31	8.01	6.71	ND	ND	ND
Ampicillin	24.00	22.00	18.50	16.50	15.50	8.30	6.70	6.10
Particulars			**CD (5%)**					
Compounds			0.26					
Concentrations			0.54					
Compounds × concentrations			0.21					

*ND stands for inhibition zone not detected

Table 24: Microbial activity of Schiff bases, thiosemicarbazones and hydrazones on the growth of *Klebsiella sp.*

Conc. ($\mu g\ mL^{-1}$) / Solvent extract	Inhibition zone (in mm)							
	3000	**2000**	**1000**	**500**	**250**	**100**	**50**	**25**
II	14.72	12.73	11.04	9.30	6.72	ND	ND	ND
III	15.31	13.05	11.70	10.31	7.08	6.2	ND	ND
IV	14.73	12.76	11.31	9.73	6.74	ND	ND	ND
V	13.32	11.77	10.30	8.31	6.32	ND	ND	ND
VI	10.31	8.35	7.74	6.75	ND	ND	ND	ND
VII	11.02	9.36	8.33	7.33	6.31	ND	ND	ND
VIII	12.77	10.76	8.75	7.76	6.31	ND	ND	ND
IX	9.36	8.32	7.34	6.74	ND	ND	ND	ND
X	15.38	14.32	11.70	10.72	7.70	6.3	ND	ND
XI	15.72	15.85	12.34	11.33	8.33	6.3	ND	ND
XII	12.03	10.32	8.75	7.71	6.32	ND	ND	ND
XIII	15.71	14.74	12.33	11.36	8.05	6.3	ND	ND
XIV	23.32	18.70	15.72	11.39	8.70	6.7	6.3	ND
XV	9.14	8.01	7.04	6.14	ND	ND	ND	ND
XVI	9.33	8.32	7.33	6.34	ND	ND	ND	ND
XVII	9.72	8.36	7.70	6.70	ND	ND	ND	ND
XVIII	10.70	9.00	8.31	7.33	6.31	ND	ND	ND
Ampicillin	30.00	23.00	19.00	17.00	16.00	8.60	6.80	6.20

Particulars	CD (5%)
Compounds	0.23
Concentrations	0.65
Compounds × concentrations	0.15

*ND stands for inhibition zone not detected

Table 25: Microbial activity of Schiff bases, thiosemicarbazones and hydrazones on the growth of *Enterobacter sp.*

Conc. ($\mu g\ mL^{-1}$) / Solvent extract	Inhibition zone (in mm)							
	3000	**2000**	**1000**	**500**	**250**	**100**	**50**	**25**
II	11.71	9.72	8.30	6.70	ND	ND	ND	ND
III	11.72	10.31	8.72	7.31	ND	ND	ND	ND
IV	11.30	9.30	8.33	6.70	ND	ND	ND	ND
V	11.73	10.30	8.78	7.01	ND	ND	ND	ND
VI	11.04	8.75	7.76	6.31	ND	ND	ND	ND
VII	11.71	9.77	8.31	7.02	ND	ND	ND	ND
VIII	12.32	10.34	9.03	7.30	6.31	ND	ND	ND
IX	9.73	8.73	7.35	6.10	ND	ND	ND	ND
X	12.31	10.70	9.39	7.72	6.32	ND	ND	ND
XI	9.36	8.31	7.34	6.10	ND	ND	ND	ND
XII	11.31	9.35	8.03	6.71	ND	ND	ND	ND
XIII	11.04	9.34	7.79	6.32	ND	ND	ND	ND
XIV	12.06	10.30	9.01	7.30	ND	ND	ND	ND
XV	8.99	7.15	6.92	6.10	ND	ND	ND	ND
XVI	9.04	8.01	7.33	6.10	ND	ND	ND	ND
XVII	9.72	8.72	7.74	6.19	ND	ND	ND	ND
XVIII	14.71	11.35	9.30	7.71	6.62	ND	ND	ND
Ampicillin	24.00	22.00	18.50	16.50	15.50	8.30	6.70	6.10

Particulars	CD (5%)
Compounds	0.25
Concentrations	0.29
Compounds × concentrations	0.36

*ND stands for inhibition zone not detected

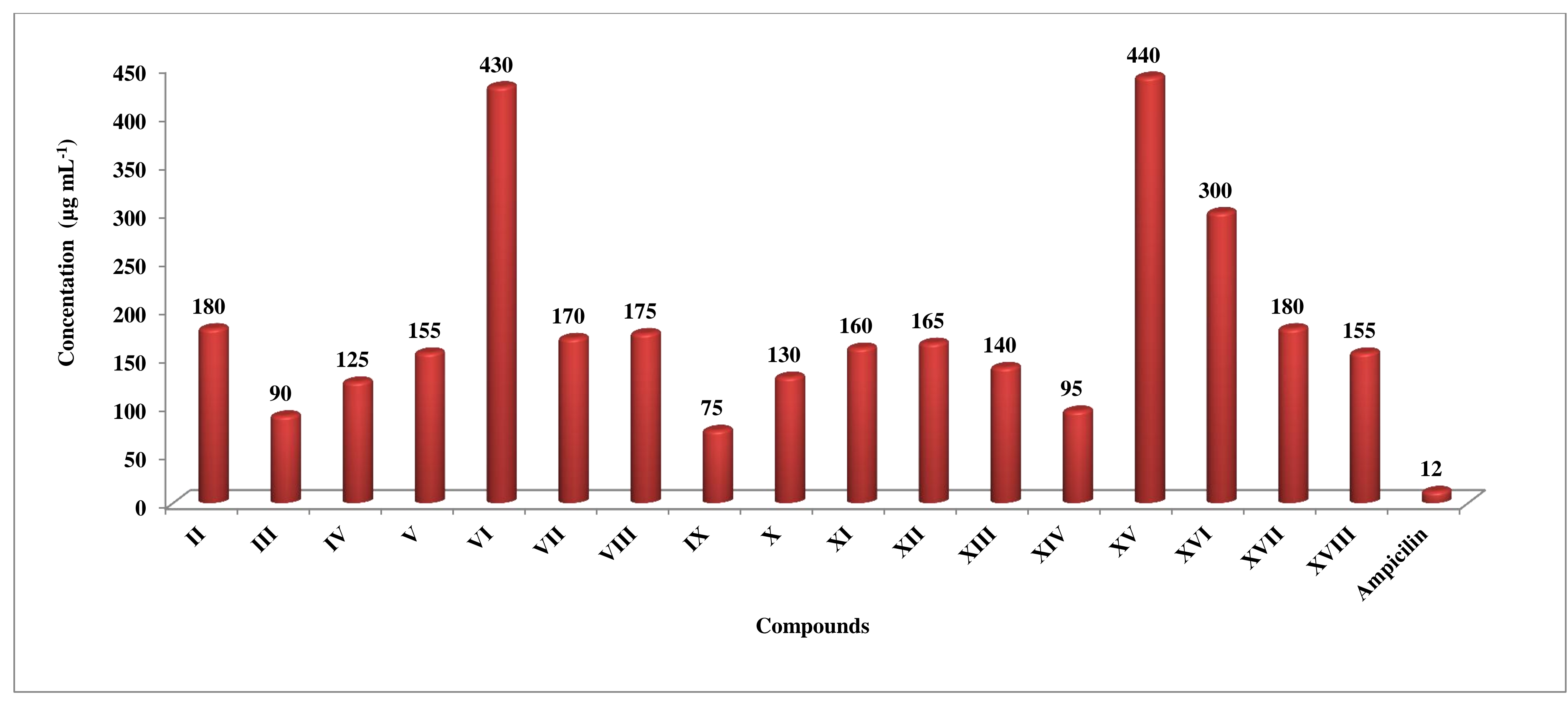


Figure 15: **MIC (Minimum inhibition concentration) of Schiff bases (II-XI), thiosemicarbazones (XII & XIII) and hydrazones (XIV-XVIII) along with ampicillin against *Pseudomonas sp*.**

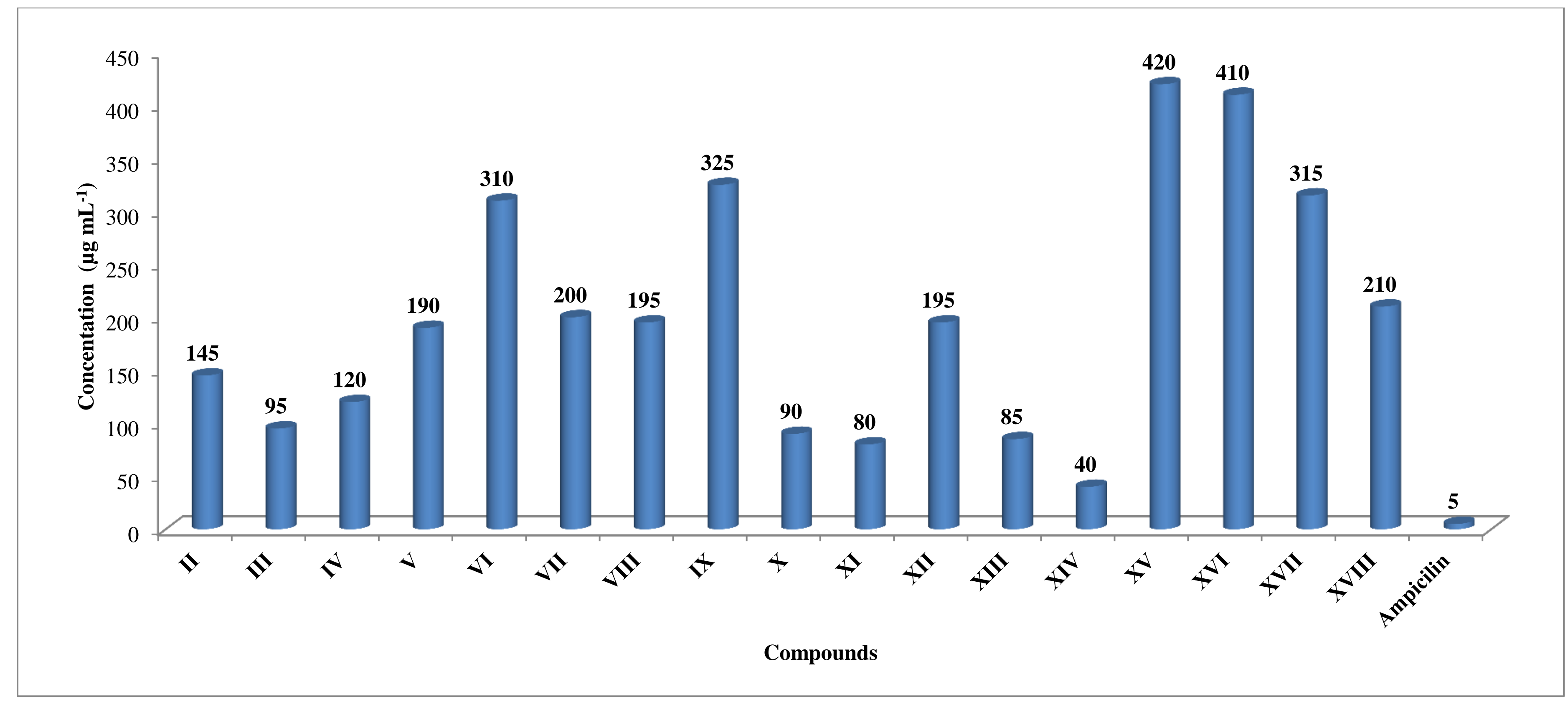


Figure 16: **MIC (Minimum inhibition concentration) of Schiff bases (II-XI), thiosemicarbazones (XII & XIII) and hydrazones (XIV-XVIII) along with ampicillin against *Klebsiella sp*.**

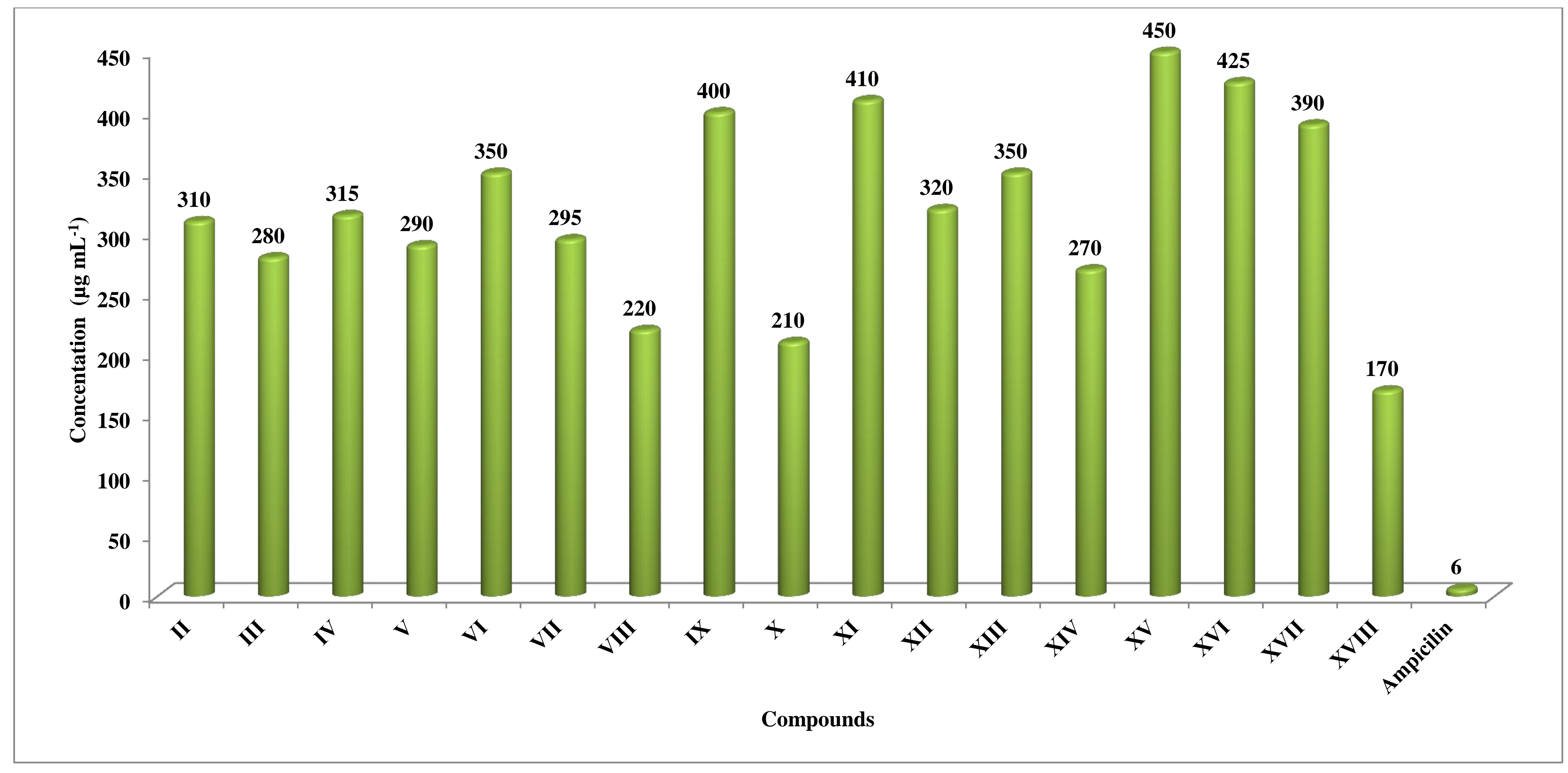


Figure 17: MIC (Minimum inhibition concentration) of Schiff bases (II-XI), thiosemicarbazones (XII & XIII) and hydrazones (XIV-XVIII) along with ampicillin against *Enterobacter sp*.

Presence of aromatic ring and electron withdrawing groups played an important role in the bioactivity of synthesized thiosemicarbazones and hydrazones against all the three tested bacteria. It has been found that in all tested bacteria, thiosemicarbazone XIV possessed more inhibition potential as compared to XIII. In case of hydrazones, bioactivity of compounds varied directly with the number of the electron withdrawing groups. As the number of electron withdrawing groups increased antimicrobial potential of synthesized hydrazones increased. Significant differences were observed between different synthesized compounds against all the three tested fungi at five percent level of significance.

Compound IX, XIV, XVIII are showed maximum activity against *Pseudomonas sp., Klebsiella sp.,* and *Enterobacter sp.,* respectively. In case of hydrazones, compounds containing more electron withdrawing groups showed better biological activity.

4.10.4 Evaluation of microbial activity of chalcones and pyrazolines of cuminaldehyde

Different synthesized chalcones (XIX-XXIV) and their pyrazolines (XXV-XXXV) were evaluated for their antimicrobial potential against three tested gram negative bacteria namely *Pseudomonas* sp., *Klebsiella* sp. and *Enterobacter* sp. at different concentrations (3000, 2000, 1000, 500, 250, 100, 50 and 25 µg mL^{-1}). The results obtained *i.e.* values of inhibition zones and MIC values are graphically represented in Tables 26, 27, 28 and Figures 18, 19, 20 respectively. Inhibition zone data was analyzed statistically by factorial CRD to determine the significant differences among the activities of synthesized compounds against three bacteria.

***Pseudomonas* sp.**

Different substituted chalcones and pyrazolines were sreened for their antimicrobial potential against *Pseudomonas* sp. Compound XX displayed maximum activity against *Pseudomonas* sp. at all the concentrations as compared to other compounds (Table 26, Figure 18). Effectivenss of synthesized compound was may be due to the presence of most electronegative fluorine element. Overall it was found that chalcones and pyrazolines were somewhat equally biologically active against *Pseudomonas* sp. Order of effectiveness of synthesized compounds against *Pseudomonas* sp.: XX > XXXI > XXXII > XXIV > XXXV >XXI > XXX > XXXIV> XXIX > XXIII > XXII > XXV> XXXVI > XXVI > XXVIII > XXXIII > XXVII > XIX. This order could be justified from their MIC values.

***Klebsiella* sp.**

Evaluation of synthesized compounds for their antimicrobial potential revealed that the synthesized compounds displayed significant activity against *Klebsiella* sp. Compound XX exhibited higher activity as compared to other compoundsagainst *Klebsiella* sp.at all concentrations. Effectiveness of compound XX was may be due to presence of electronegative fluorine atom in a highly conjugated system (Table 27, Figure 19).

Table 26: Microbial activity of chalcones and pyrazolines on the growth *Pseudomonas* sp.

Conc. ($\mu g\ mL^{-1}$) / Solvent extract	Inhibition zone (in mm)							
	3000	**2000**	**1000**	**500**	**250**	**100**	**50**	**25**
XIX	11.33	9.33	8.13	6.20	6.03	ND	ND	ND
XX	14.67	12.67	10.67	8.67	7.33	6.67	ND	ND
XXI	14.33	11.67	8.36	6.33	6.10	ND	ND	ND
XXII	12.67	11.33	9.33	7.33	6.03	ND	ND	ND
XXIII	13.33	11.33	9.67	7.67	6.07	ND	ND	ND
XXIV	14.67	11.33	8.50	6.80	6.30	ND	ND	ND
XXV	12.67	11.00	8.67	7.33	6.10	ND	ND	ND
XXVI	12.67	10.67	8.33	7.00	ND	ND	ND	ND
XXVII	11.00	10.33	8.17	6.63	ND	ND	ND	ND
XXVIII	12.33	10.67	8.23	6.90	ND	ND	ND	ND
XXIX	13.67	11.67	9.67	7.67	6.07	ND	ND	ND
XXX	13.67	11.67	8.33	6.30	6.10	ND	ND	ND
XXXI	14.67	12.33	10.67	8.67	7.33	ND	ND	ND
XXXII	14.67	12.33	9.33	7.67	6.30	ND	ND	ND
XXXIII	11.33	10.33	8.20	6.80	ND	ND	ND	ND
XXXIV	13.67	11.67	10.33	8.00	ND	ND	ND	ND
XXXV	14.33	11.30	8.40	6.67	6.20	ND	ND	ND
XXXVI	12.67	10.67	8.63	7.33	6.00	ND	ND	ND
Ampicillin	24.00	22.00	18.50	16.50	15.50	8.30	6.70	6.10

Particulars	CD (5%)
Compounds	0.36
Concentrations	0.25
Compounds × concentrations	0.06

Table 27: Microbial activity of chalcones and pyrazolines on the growth *Klebsiella* sp.

Solvent extract \ Conc. ($\mu g\ mL^{-1}$)	Inhibition zone (in mm)							
	3000	**2000**	**1000**	**500**	**250**	**100**	**50**	**25**
XIX	12.00	9.33	7.24	6.20	ND	ND	ND	ND
XX	13.67	11.33	9.67	7.80	6.70	ND	ND	ND
XXI	12.67	11.33	9.67	7.63	6.67	ND	ND	ND
XXII	12.00	9.20	7.10	6.33	ND	ND	ND	ND
XXIII	12.33	9.67	8.67	7.33	6.33	ND	ND	ND
XXIV	10.33	8.33	7.27	6.17	ND	ND	ND	ND
XXV	11.67	9.00	7.33	6.33	6.33	ND	ND	ND
XXVI	12.33	9.73	8.13	7.33	6.33	ND	ND	ND
XXVII	9.67	8.33	7.13	6.07	ND	ND	ND	ND
XXVIII	12.33	9.33	8.33	6.67	ND	ND	ND	ND
XXIX	10.67	8.97	7.33	6.10	ND	ND	ND	ND
XXX	11.33	10.33	9.00	7.33	ND	ND	ND	ND
XXXI	11.67	9.20	7.67	6.33	ND	ND	ND	ND
XXXII	9.33	8.17	7.09	6.03	ND	ND	ND	ND
XXXIII	9.67	8.33	7.20	6.17	ND	ND	ND	ND
XXXIV	11.67	9.00	7.17	6.20	ND	ND	ND	ND
XXXV	12.33	9.33	7.33	6.33	ND	ND	ND	ND
XXXVI	12.33	9.67	8.33	6.67	ND	ND	ND	ND
Ampicillin	30.00	23.00	19.00	17.00	16.00	8.60	6.80	6.20

Particulars	CD (5%)
Compounds	0.09
Concentrations	0.21
Compounds × concentrations	0.11

Table 28: Microbial activity of chalcones and pyrazolines on the growth *Enterobacter* sp.

Conc. ($\mu g\ mL^{-1}$) / Solvent extract	Inhibition zone (in mm)							
	3000	**2000**	**1000**	**500**	**250**	**100**	**50**	**25**
XIX	11.67	9.67	8.33	6.67	ND	ND	ND	ND
XX	13.60	10.33	8.67	7.33	6.20	ND	ND	ND
XXI	11.20	9.30	7.33	6.20	ND	ND	ND	ND
XXII	11.33	9.33	7.67	6.17	ND	ND	ND	ND
XXIII	10.67	9.16	7.60	6.13	ND	ND	ND	ND
XXIV	10.30	8.67	7.50	6.10	ND	ND	ND	ND
XXV	11.67	9.67	8.33	6.60	ND	ND	ND	ND
XXVI	12.33	10.67	8.33	7.27	6.20	ND	ND	ND
XXVII	9.67	8.67	7.30	6.07	ND	ND	ND	ND
XXVIII	12.33	10.33	8.00	7.23	ND	ND	ND	ND
XXIX	9.33	8.33	7.33	6.07	ND	ND	ND	ND
XXX	11.00	9.33	7.67	6.33	ND	ND	ND	ND
XXXI	12.00	10.33	8.00	7.33	6.20	ND	ND	ND
XXXII	9.00	8.10	7.30	6.03	ND	ND	ND	ND
XXXIII	9.67	8.67	7.33	6.13	ND	ND	ND	ND
XXXIV	11.67	9.67	7.67	6.33	ND	ND	ND	ND
XXXV	11.09	9.10	7.30	6.07	ND	ND	ND	ND
XXXVI	12.00	9.67	7.13	6.67	ND	ND	ND	ND
Ampicillin	24.00	22.00	18.50	16.50	15.50	8.30	6.70	6.10

Particulars	CD (5%)
Compounds	0.25
Concentrations	0.39
Compounds × concentrations	0.57

Effectiveness of synthesized compounds followed the trend: XX > XXI> XXII > XXVI > XXXVI > XXVIII > XXXV > XIX > XXII > XXXI > XXV > XXXIV > XXX > XXIX > XXIV > XXXIII > XXVII. > XXXII. No inhibition zone was observed at low concentrations.

***Enterobacter* sp.**

Evalaution of antimicrobial potential of synthesized compounds against three gram negative bacteria revealed that chalcone (XX) synthesized by condensation of cuminaldehyde and *p*-chloroacetophenone was more effective against *Enterobacter* sp (Table 28). Effectiveness of synthesized compound was to greater extent of conjugation and presence of most electronegative element *i.e.*fluorine atom at the para position of the aromatic ring. Bioactivity of synthesized compounds against *Enterbacter sp.*followed the trend: XX > XXVI > XXVIII > XXXI > XXXVI > XIX > XXV > XXXIV > XXII > XXI > XXXV > XXX > XXIII > XXIV > XXXIII > XXVII > XXIX > XXXII.

Compound XX was found effective against all the three tested gram negative bacteria. Significant differences were observed between different synthesized compounds against all the three tested fungi at five percent level of significance. MIC values of synthesized chalcones and pyrazolines were also calculated and were presented in Figure 20.

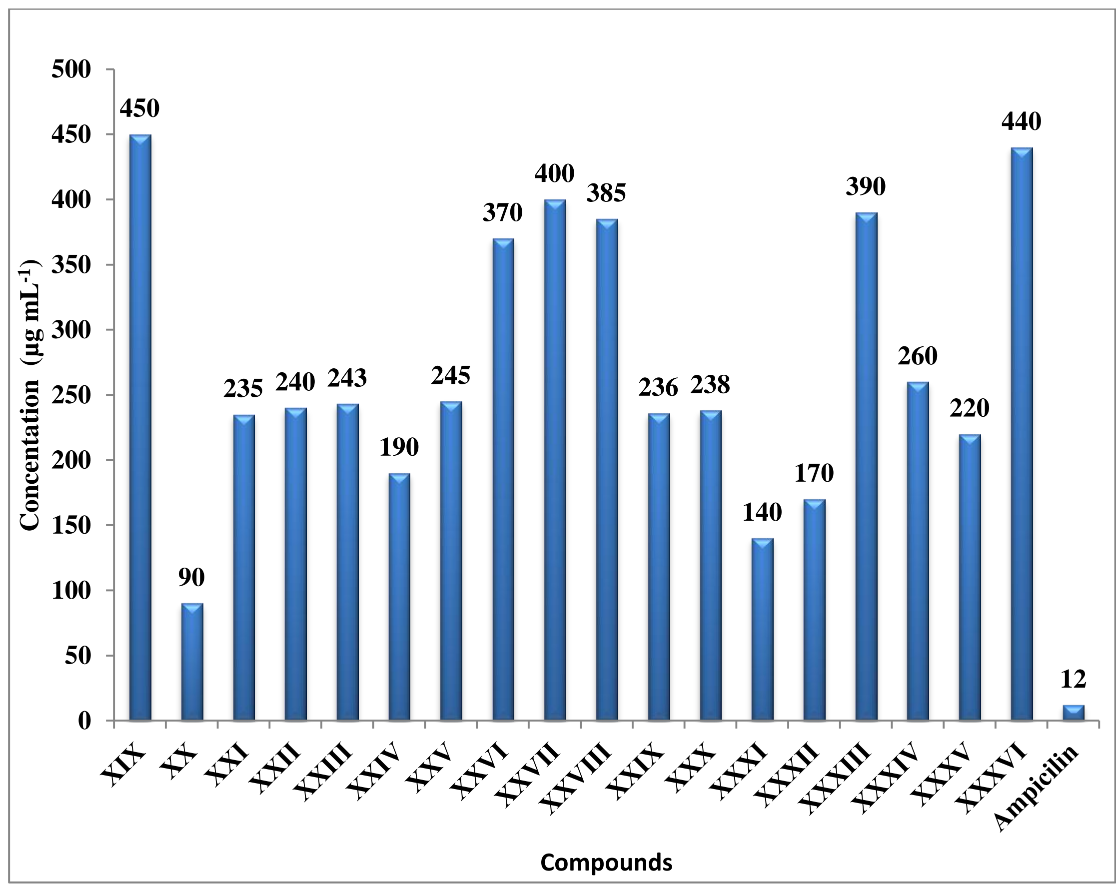


Figure 18: MIC (Minimum inhibition concentration) of chalcones and pyrazolines along with ampicillin against *Pseudomonas sp*.

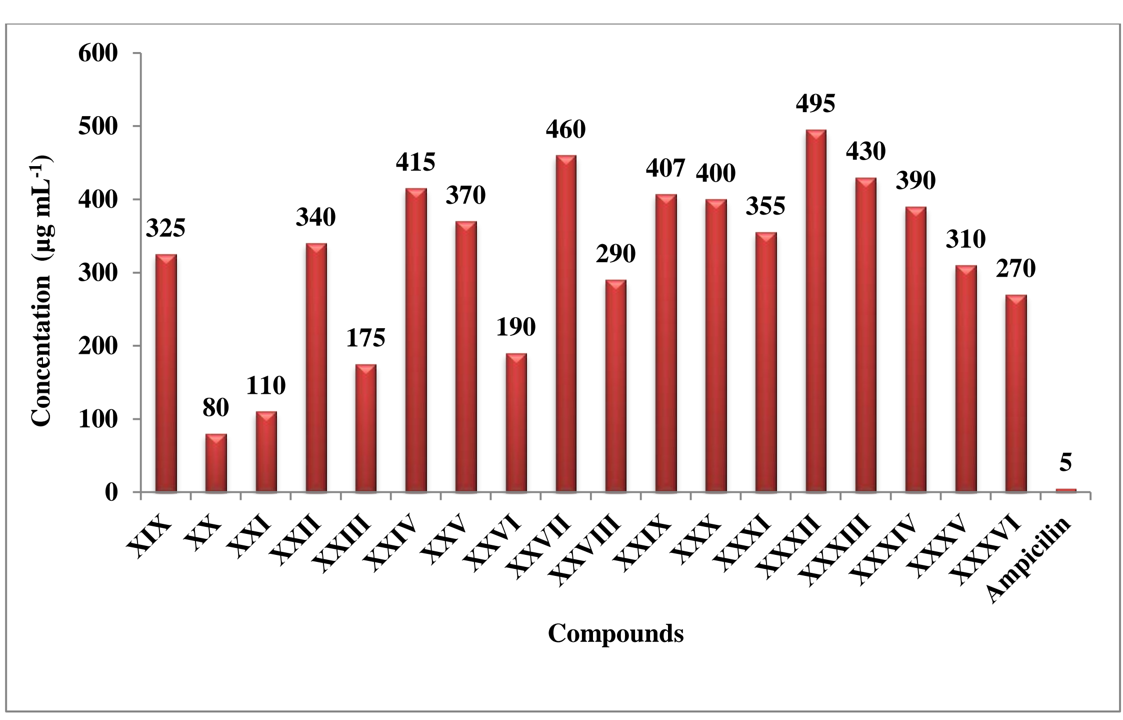


Figure 19: MIC (Minimum inhibition concentration) of chalcones and pyrazolines along with ampicillin against *Klebsiella sp*.

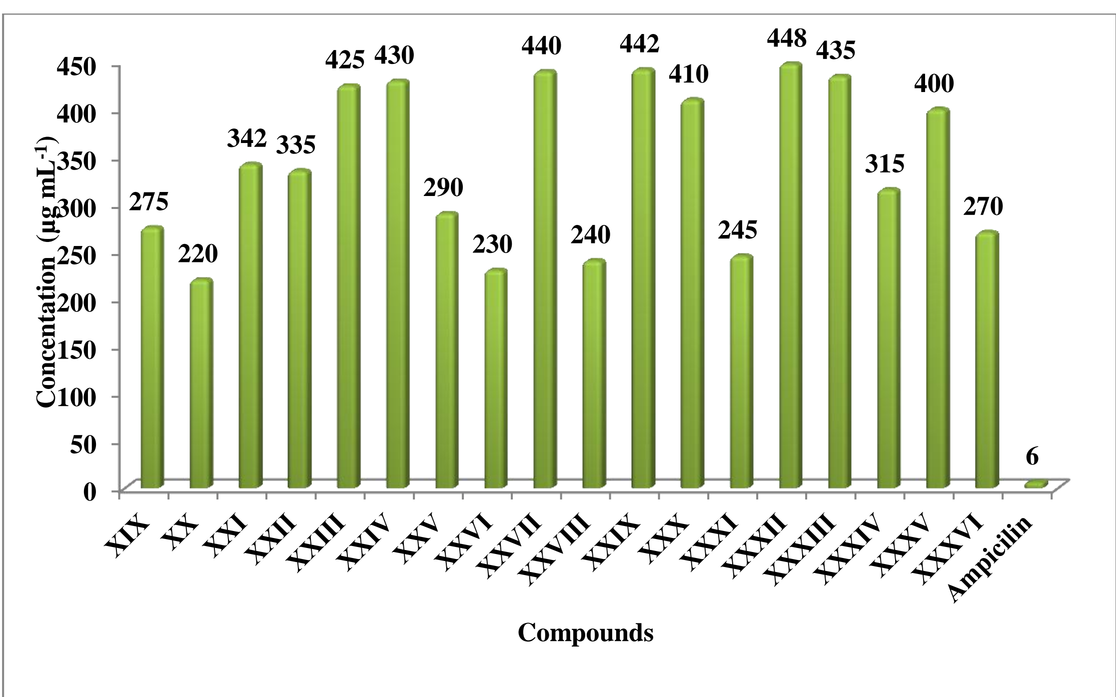
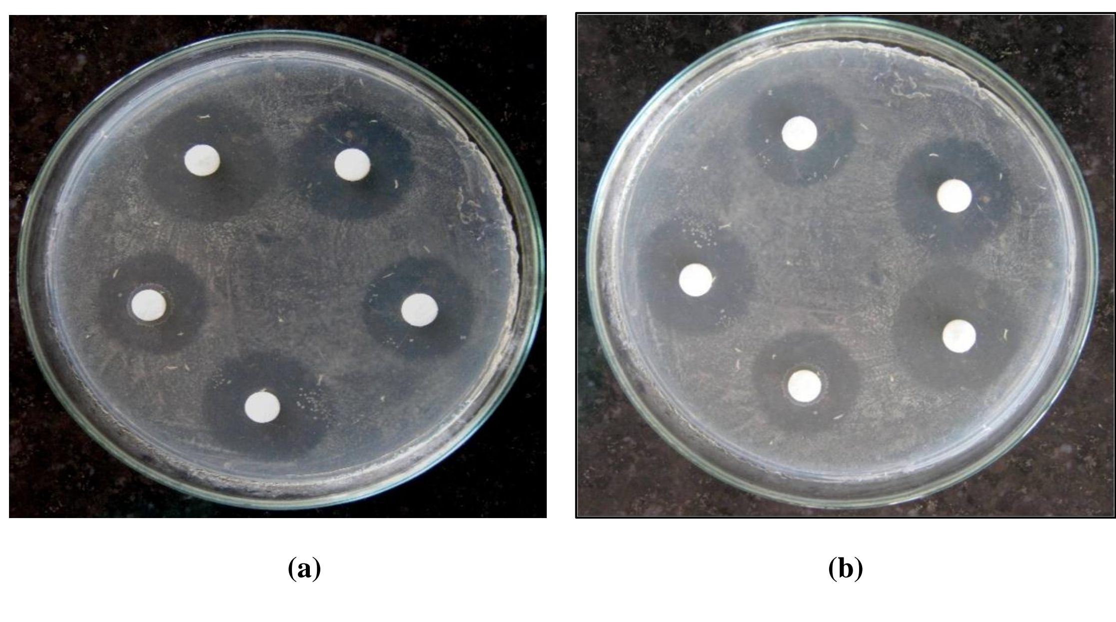


Figure 20: MIC (Minimum inhibition concentration) of chalcones and pyrazolines along with ampicillin against *Enterobacter sp*.

4.10.5 Microbial activity of silver nanoparticles from aqueous extract of cumin

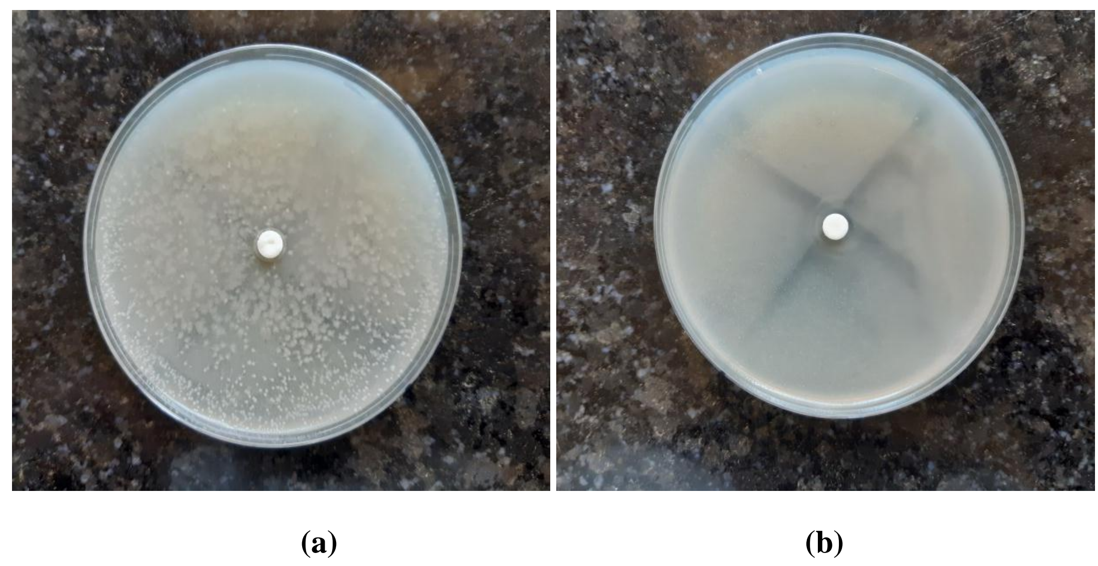
Evaluation of microbial activity of AgNPs (Table 29-31) against different bacteria's namely *Pseudomonas* sp., *Klebsiella* sp. and *Enterobacter sp.* at different concentrations *i.e.* 0.10, 0.20 and 0.40 mg ml^{-1} of seed extract (5,10 and 15 ml) and 10 ml $AgNO_3$ solution (1mM, 3mM and 5mM) has been presented.



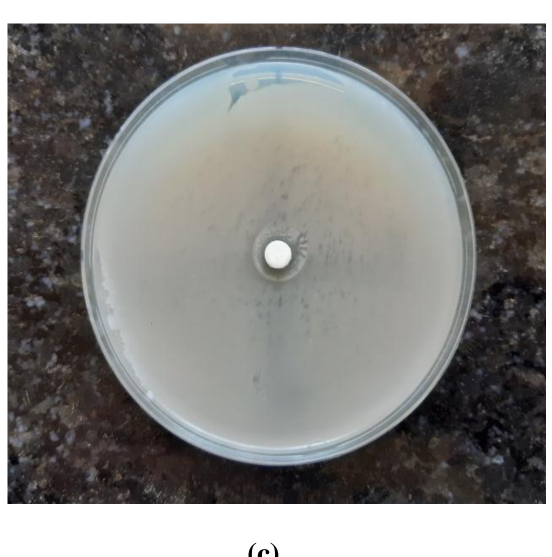
(a) **(b)**

(c)

Plate 3: Zones of inhibition formed by Ampicillin against *Pseudomonas* sp. (a), *Klebsiella* sp. (b) and *Enterobacter* sp. (C) at different concentrations

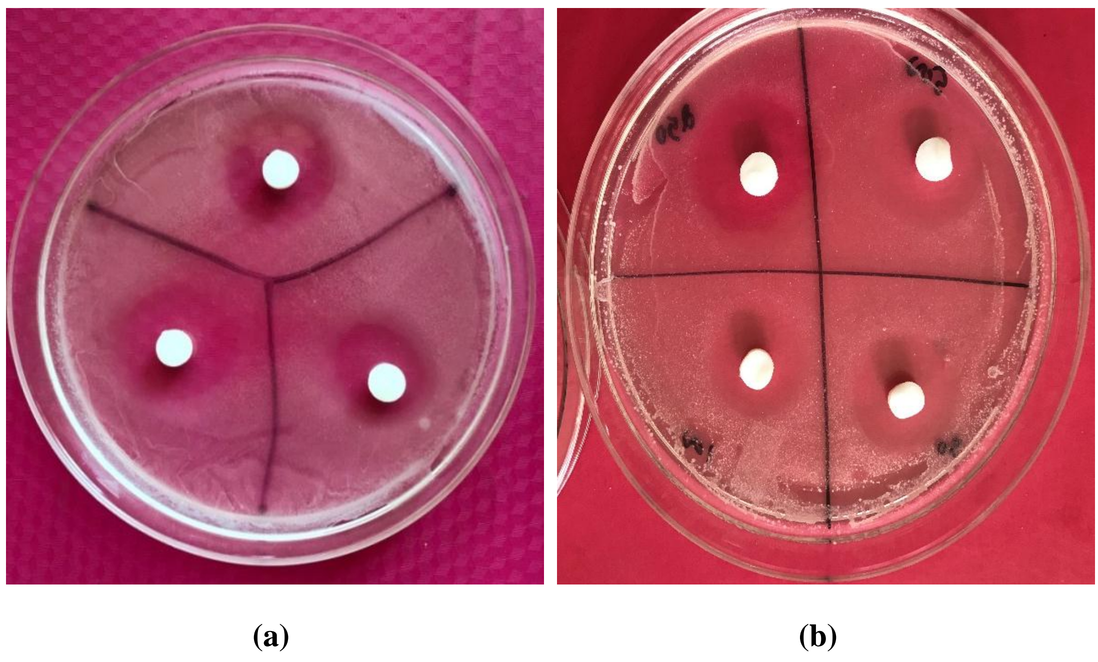


(a) **(b)**



(c)

Plate 4: Growth of *Pseudomonas* sp. (a), *Klebsiella* sp. (b) and *Enterobacter* sp. (C) in control



(a) **(b)**

(c)

Plate 5: Zones of inhibition formed by Compound IX against *Pseudomonas* sp. (a), Compound XIV against *Klebsiella* sp. (b) and Compound XVIII against *Enterobacter* sp. (c) at different concentrations

***Pseudomonas* sp.**

It was evident from above Table 29 that the silver nanoparticles did not inhibit the growth of *pseudomonas* sp. at lower concentration of seed extract as well as of silver nitrate solution. Non-significant differences were observed between different synthesized nanoparticles at different concentrations against *Pseudomonas* sp. None of the synthesized nanoparticles showed better activity than standard ampicillin. It was found that the antimicrobial potential of synthesized nanoparticles increased with increase either in the concentration of seed extract or silver nitrate solution.

Table 29: Microbial activity of AgNPs from aqueous extract of seed on the growth of *Pseudomonas* sp. at different concentrations (mg/ml)

Concentration of $AgNO_3$ (mM)	Volume of plant extract (ml)								
	5ml			10ml			15ml		
	Conc. of NPs (mg ml^{-1})								
	0.10	0.20	0.40	0.10	0.20	0.40	0.10	0.20	0.40
	Inhibition zone (in mm)								
1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND
3.00	ND	6.56	8.04	ND	7.06	8.55	6.26	7.95	9.29
5.00	6.13	8.55	9.03	6.44	8.73	9.14	6.51	9.06	9.49
Ampicillin	12.00	13.00	15.00	12.00	13.00	15.00	12.00	13.00	15.00

CD (5%) = Non-significant

***Klebsiella* sp.**

Bioactivity of synthesized nanoparticles against *Klebsiella* sp. increased with increase in the concentration of seed extract as well as silver nitrate solution (Table 30). Synthesized nanoparticles were not capable for inhibition of *Klebsiella* sp. at lower concentration of seed extracts. No synthesized nanoparticles showed better activity against *Klebsiella* sp. at any concentration as compared to standard ampicillin. Non-significant differences were observed between different synthesized nanoparticles at different concentrations against *Klebsiella* sp.

Enterobacter sp.

Synthesized silver nanoparticles were not effective against *Enterobacter* sp. at lower concentration of silver nitrate solution. It was evident from above Table 31 that the effectiveness of nanoparticles increased with increase in concentration of silver nitrate solution as well as seed extract. Therefore, bioactivity of AgNPs was directly proportional to concentration of seed extract and $AgNO_3$ solution. Non-significant differences were observed between different synthesized nanoparticles at different concentrations against *Enterobacter* sp.

Table 30: Microbial activity of AgNPs from aqueous extract of leaf on the growth of *Klebsiella* sp. at different concentrations (mg/ml)

Concentration of $AgNO_3$ (mM)	Volume of plant extract (ml)								
	5ml			10ml			15ml		
	Conc. of NPs (mg ml^{-1})								
	0.10	0.20	0.40	0.10	0.20	0.40	0.10	0.20	0.40
	Inhibition zone (in mm)								
1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND
3.00	ND	ND	6.20	ND	ND	6.75	ND	6.39	7.24
5.00	ND	ND	7.16	ND	7.57	7.91	ND	8.56	9.01
Ampicillin	13.51	14.03	15.58	13.57	14.0	15.5	13.57	14.05	15.54

CD (5%) = Non-significant

Table 31: Microbial activity of AgNPs from aqueous extract of seed on the growth of *Enterobacter* sp. at different concentrations (mg/ml)

Concentration of $AgNO_3$ (mM)	Volume of plant extract (ml)								
	5ml			10ml			15ml		
	Conc. of NPs (mg ml^{-1})								
	0.10	0.20	0.40	0.10	0.20	0.40	0.10	0.20	0.40
	Inhibition zone (in mm)								
1.00	ND	ND	ND	ND	ND	ND	ND	ND	ND
3.00	ND	ND	7.01	ND	ND	7.55	ND	ND	8.25
5.00	6.10	7.40	9.36	6.63	7.91	9.93	7.24	8.76	11.29
Ampicillin	13.00	14.50	16.1	13.50	14.59	16.18	13.00	14.57	16.1

CD (5%) = Non-significant

Most effective treatment in all cases was highest concentration of 0.40 mg/ml with 15 ml of seed extract and 5mm $AgNO_3$ solution. No zone of inhibition was observed at lowest concentration of silver nitrate solution. Minimum inhibition concentration of synthesized nanoparticles were also determined and presented in Table 32.

Table 32: Minimum Inhibitory Concentration (MIC) of AgNPs from seed extract on the growth different bacteria's

Concentration of $AgNO_3$ (mM)	Volume of extract (ml)	*Pseudomonas* sp.	*Klebsiella* sp.	*Enterobacter* sp.
3mM	5 ml	0.15	0.35	0.25
	10 ml	0.11	0.32	0.30
	15 ml	0.08	0.28	0.32
5 mM	5 ml	0.09	0.25	0.08
	10 ml	0.07	0.15	0.07
	15 ml	0.05	0.12	0.06
Ampicillin		0.012	0.005	0.006

*At 1.00 mM concentration, MIC was not found using 5, 10 and 15 ml of aqueous extract of cumin seeds.

4.11 EVALUATION OF ANTIFUNGAL ACTIVITY

4.11.1 Evaluation of fungicidal activity of cumin essential oil and cuminaldehyde

Essential oil, different solvent extracts of cumin, major constituent cuminaldehyde, its synthesized derivatives and silver nanoparticles of cumin were evaluated for antifungal activity against *Fusarium wilt, Ascochyta blight* and *Bortrytis gray mould* using poisoned food technique at different concentrations. Essential oil, different solvent extracts, cuminaldehyde, its derivatives and silver nanoparticles of cumin did not registered any activity against *Ascochyta blight i.e.* they have no effect on the growth of these two fungi. Therefore, no percent inhibition was calculated.

Fusarium wilt

Cumin oil was found to be more effective than cuminaldehyde at all tested concentration (Table 33) against *F. wilt.* Essential oil components have the capability to alter cell permeability by entering between the fatty acyl chains making up membrane lipid bilayers and disrupt the lipid packing. Due to this, the membrane properties like membrane fluidity/permeability and functions may get changed. This may also affect the regulation and function of the membrane bound enzymes that alter the synthesis of many cell wall polysaccharide components (i.e. β-glucan, chitin, and mannan) and alter the cell growth and morphogenesis. Moreover, essential oil can cause extensive cellular damage due to better penetration and contact. The major components of *C. cyminum* are terpenes, which have the capability to inhibit the respiration of fungi, and may have adverse effects on mitochondria. It may be the cause of cell death and other morphological changes (Naeini *et al* 2014).

Table 33: Per cent inhibition of cumin oil and cuminaldehyde against *Fusarium wilt* at different concentrations (µg ml^{-1})

Concentration (µg ml^{-1})	Per cent Inhibition		
	Cumin oil	Cuminaldehyde	Carbendazim 50 WP
3000	100±0.00	100±0.00	100±0.0
2000	100±0.00	100±0.00	100±0.0
1000	67.58±0.26	65.20±0.65	100±0.0
500	50.53±0.65	44.46±0.45	100±0.0
250	41.89±0.66	37.36±0.95	100±0.0
100	24.89±0.67	22.55±0.58	100±0.0
50	19.23±0.69	18..23±0.55	100±0.00
25	17.23±0.54	16.89±0.23	100±0.00
Particulars	**CD (5%)**		
Compounds	0.59		
Concentrations	0.24		
Compounds × concentrations	0.67		

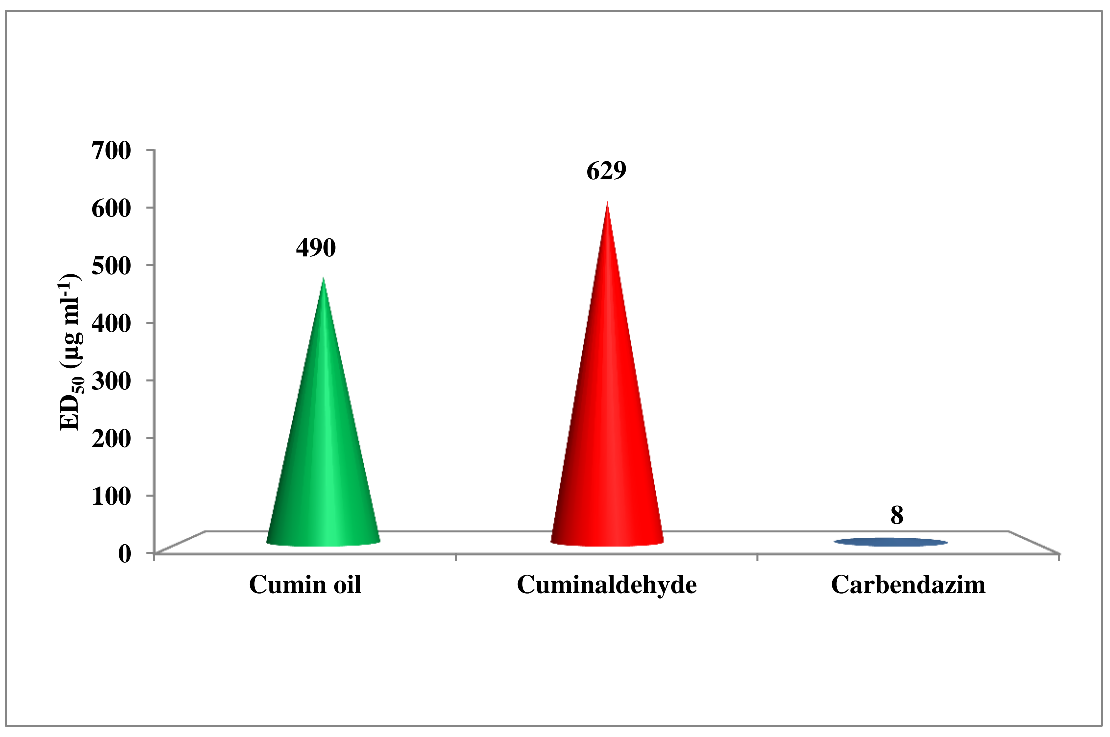


Figure 21: ED$_{50}$ of cumin oil and cuminaldehyde against *Fusarium wilt*

Table 34: Per cent inhibition of cumin essential oil and cuminaldehyde against *Bortrytis gray mould*

Concentration ($\mu g\ ml^{-1}$)	**Per cent Inhibition**		
	Cumin essential oil	Cuminaldehyde	Carbendazim 50 WP
3000	100±0.00	100±0.00	100±0.0
2000	100±0.00	100±0.00	100±0.0
1000	100±0.00	100±0.00	100±0.0
500	57.25±0.25	100±0.00	100±0.0
250	42.75±0.32	72.25±0.15	100±0.0
100	26.25±0.89	49.28±0.46	100±0.0
50	19.75±0.47	29.48±0.59	100±0.00
25	18.26±0.35	21.54±0.13	100±0.00
Particulars		**CD (5%)**	
Compounds		0.46	
Concentrations		0.19	
Compounds × concentrations		0.64	

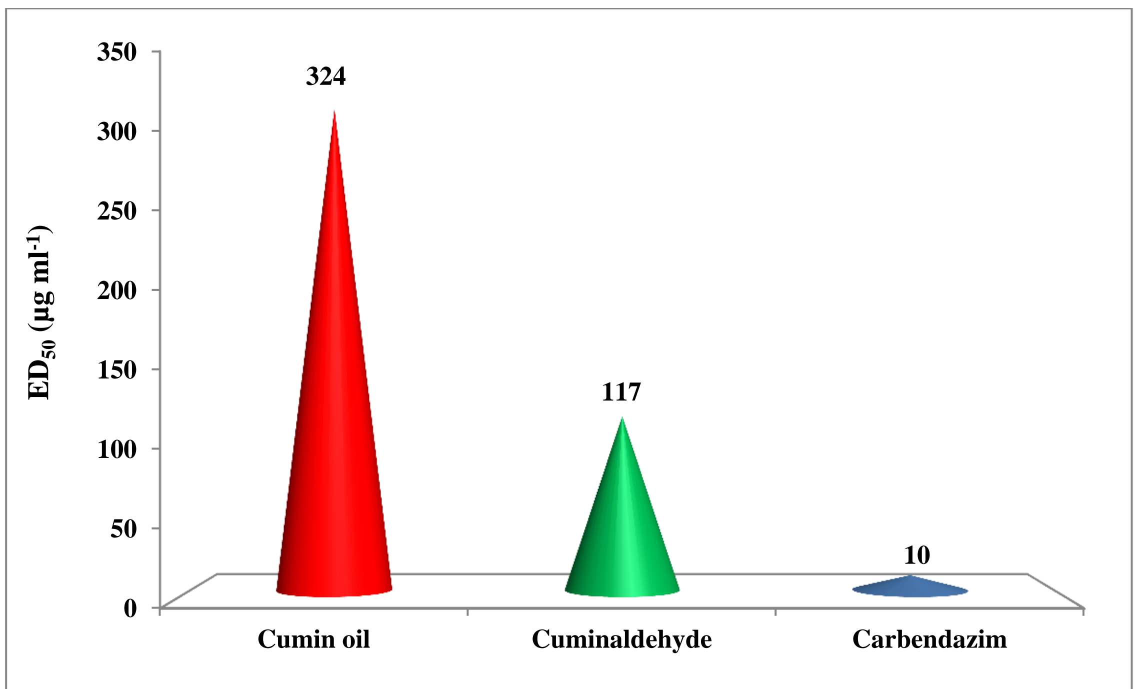


Figure 22: ED_{50} of cumin oil and cuminaldehyde against *Bortrytis gray mould*

Cumin oil displayed better activity as compared to cuminaldehyde at all the tested concentrations. Significant differences were observed between antifungal potential against cumin oil and cuminaldehyde at all the different concentrations. Neither cumin oil nor cuminaldehyde showed better activity than standard. ED_{50} of cumin oil, cuminaldehyde and standard Carbendazim 50 WP was also evaluated and presented in Figure 21.

Bortrytis gray mould

Out of cumin essential oil and cuminaldehyde, the latter displayed better activity at all tested concentration (Table 34) against *Bortrytis gray mould.* Cuminaldehyde is the major constituent of essential oil and has an abilty to interfere with the cell wall of fungal pathogens which may leads to disfunction of membrane bound enzymes. Cumin essential oil and cuminaldehyde displayed ED_{50} 324 and 117 µg ml^{-1}, respectively. Both cumin essential oil and cuminaldehyde displayed complete growth inhibiton of *Bortrytis gray mould* at concentration above 1000 and 500 µg ml^{-1}, respectivrly. Significant differences were observed between antifungal potential against cumin oil and cuminaldehyde at all the different concentrations. Neither cumin oil nor cuminaldehyde showed better activity than standard. ED_{50} of cumin oil, cuminaldehyde and standard Carbendazim 50 WP was also evaluated and presented in Figure 22.

4.11.2 Evaluation of fungicidal activity of different solvent extracts of cumin against *Fusarium wilt* and *Bortrytis grey mould*

Aqueous and methanol extract were found to be more effective as compared to other extracts at all concentrations against *Fusarium wilt* and *Bortrytis grey mould.* Percent inhibition of aqueous extract against *Fusarium wilt* and *Bortrytis grey mould* at 3000 µg ml^{-1} was 81.32 and 83.54%, respectively. Different solvent extracts displayed similar bioactivity against both the fungi. Acetone extract was least effective among all extracts, its percent inhibition at 3000 µg ml^{-1} was 62.23 and 62.48% against *Fusarium wilt* and *Bortrytis grey mould*, respectively. However, none of the extract was found to be more effective than standard Carbendazim 50 WP (Table 35 and 36, Figure 23 and 24). All the extracts varied significantly among themselves at all the concentrations. Naeini *et al* (2014) reported the antifungal activity of *Cuminum cyminum* and *Salvadora persica* herbs extracts against pathogenic *Candida strains.*

Antifungal potential of cumin solvent extracts was may due to the presence the presence of different phytochemicals. Higher content of phenolics and flavonoids in aqueous and methanolic extracts was responsible for their bioactivity (Al Aboody and Mickymaray 2020).

Antifungal potential of different solvent extracts against *Fusarium wilt* and *Bortrytis grey mould* followed the trend: aqueous > methanol > ethanol > ethyl acetate > petroleum ether > dichloromethane > chloroform > acetone.

Table 35: Per cent inhibition of different solvent extracts against *Fusarium wilt* at different concentrations (µg ml^{-1})

Concentration (µg mL^{-1})	Per cent Radical Scavenging Activity								Carbendazim 50 WP
	Acetone	Petroleum ether	Water	Methanol	Ethanol	Dichloro methane	Chloroform	Ethyl acetate	
3000	62.23±0.15	65.26±0.36	81.32±0.23	80.34±0.24	78.11±0.54	63.96±0.35	62.33±0.07	70.16±0.26	100±0.00
2000	50.26±0.62	55.69±0.26	74.26±0.85	71.65±0.22	69.31±0.59	55.26±0.65	53.95±0.56	58.23±0.28	100±0.00
1000	42.27±0.95	47.59±0.11	60.26±0.36	58.98±0.24	54.32±0.26	46.65±0.51	45.95±0.26	49.11±0.69	100±0.00
500	26.32±0.05	32.33±0.23	44.54±0.56	42.26±0.95	38.15±0.65	30.15±0.06	28.48±0.07	35.32±0.36	100±0.00
250	21.45±0.59	27.26±0.66	38.26±0.58	36.36±0.24	32.45±0.02	24.69±0.26	22.48±0.05	30.16±0.33	100±0.00
100	17.69±0.56	18.50±0.54	23.89±0.16	22.95±0.35	20.96±0.11	18.44±0.56	17.34±0.06	20.66±0.59	100±0.00
50	14.21±0.06	16.26±0.26	20.62±0.69	19.45±0.78	17.11±0.02	16.26±0.33	15.96±0.03	17.95±0.36	100±0.00
25	9.39±0.65	10.95±0.96	14.21±0.26	14.26±0.36	12.36±0.65	10.36±0.25	10.36±0.36	11.19±0.95	100±0.00

Particulars	CD (5%)
Compounds	0.14
Concentrations	0.25
Compounds × concentrations	0.36

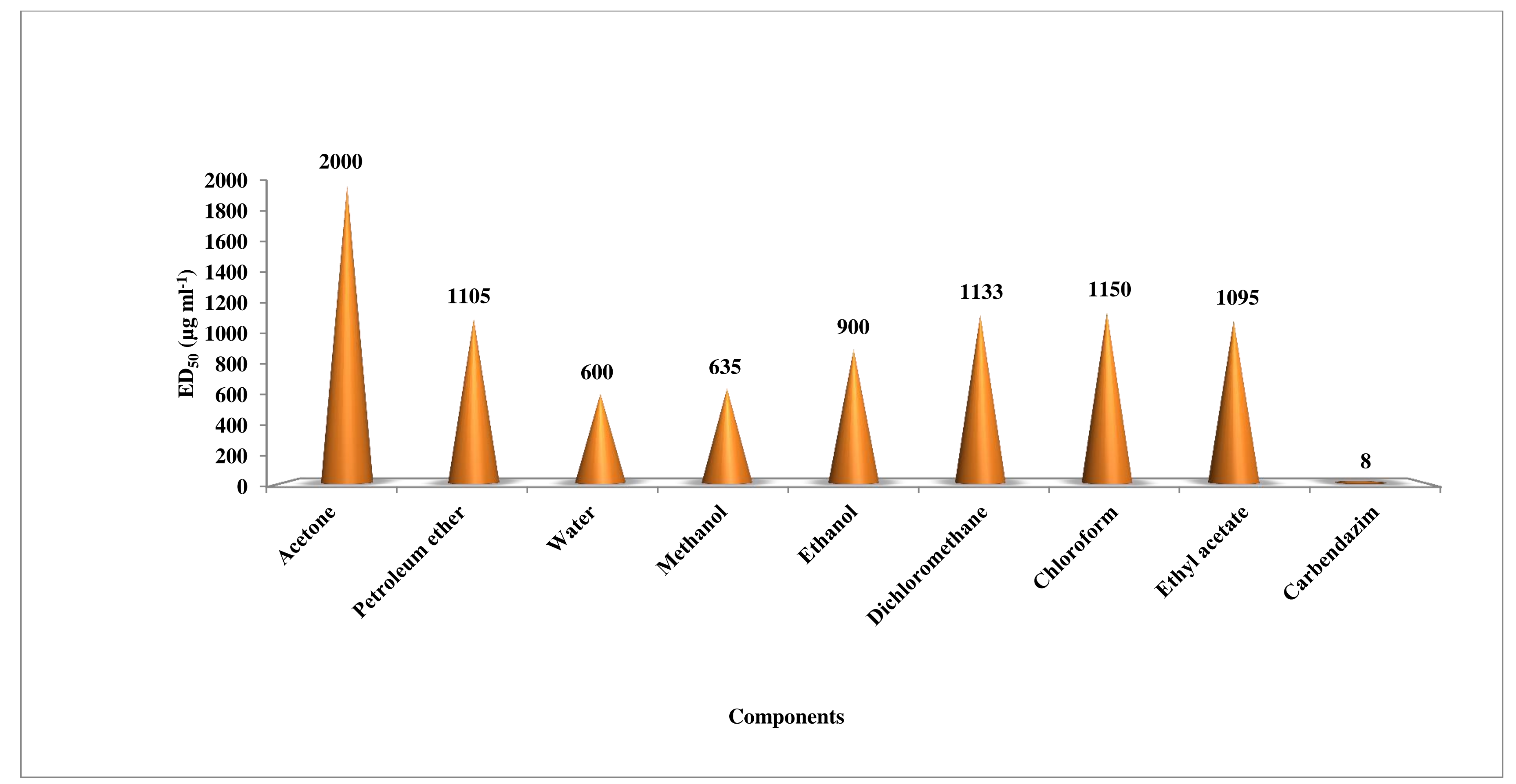


Figure 23: ED_{50} of different solvent extracts against *Fusarium wilt*

Table 36: Per cent inhibition of different solvent extracts against *Bortrytis gray mould* at different concentrations (µg ml^{-1})

Concentration (µg ml^{-1})	Per cent Radical Scavenging Activity								Carbendazim 50 WP
	Acetone	Petroleum ether	Water	Methanol	Ethanol	Dichloro methane	Chloroform	Ethyl acetate	
3000	62.48±0.25	65.89±0.07	83.54±0.11	81.44±0.77	79.65±0.87	64.36±0.54	63.49±0.07	71.23±0.16	100±0.00
2000	50.95±0.34	56.71±0.02	78.31±0.48	73.24±0.53	69.77±0.23	54.48±0.42	54.25±0.15	59.04±0.58	100±0.00
1000	43.98±0.48	48.62±0.53	64.46±0.16	59.84±0.21	55.61±0.35	45.26±0.51	45.12±0.84	50.02±0.66	100±0.00
500	26.78±0.45	35.15±0.46	47.35±0.69	43.58±0.89	40.26±0.65	31.35±0.06	28.58±0.07	36.32±0.28	100±0.00
250	21.11±0.95	27.86±0.71	39.78±0.88	38.62±0.41	35.23±0.17	23.75±0.48	22.36±0.69	29.96±0.43	100±0.00
100	17.24±0.24	18.94±0.09	25.28±0.25	24.47±0.05	21.05±0.86	18.67±0.65	17.98±0.46	19.52±0.61	100±0.00
50	14.85±0.18	16.76±0.43	22.87±0.69	20.65±0.52	18.65±0.25	16.53±0.04	15.52±0.25	17.65±0.27	100±0.00
25	9.55±0.26	10.88±0.33	16.47±0.33	14.26±0.36	13.85±0.61	10.50±0.41	10.78±0.49	11.04±0.56	100±0.00

Particulars	CD (5%)
Compounds	0.25
Concentrations	0.47
Compounds × concentrations	0.32

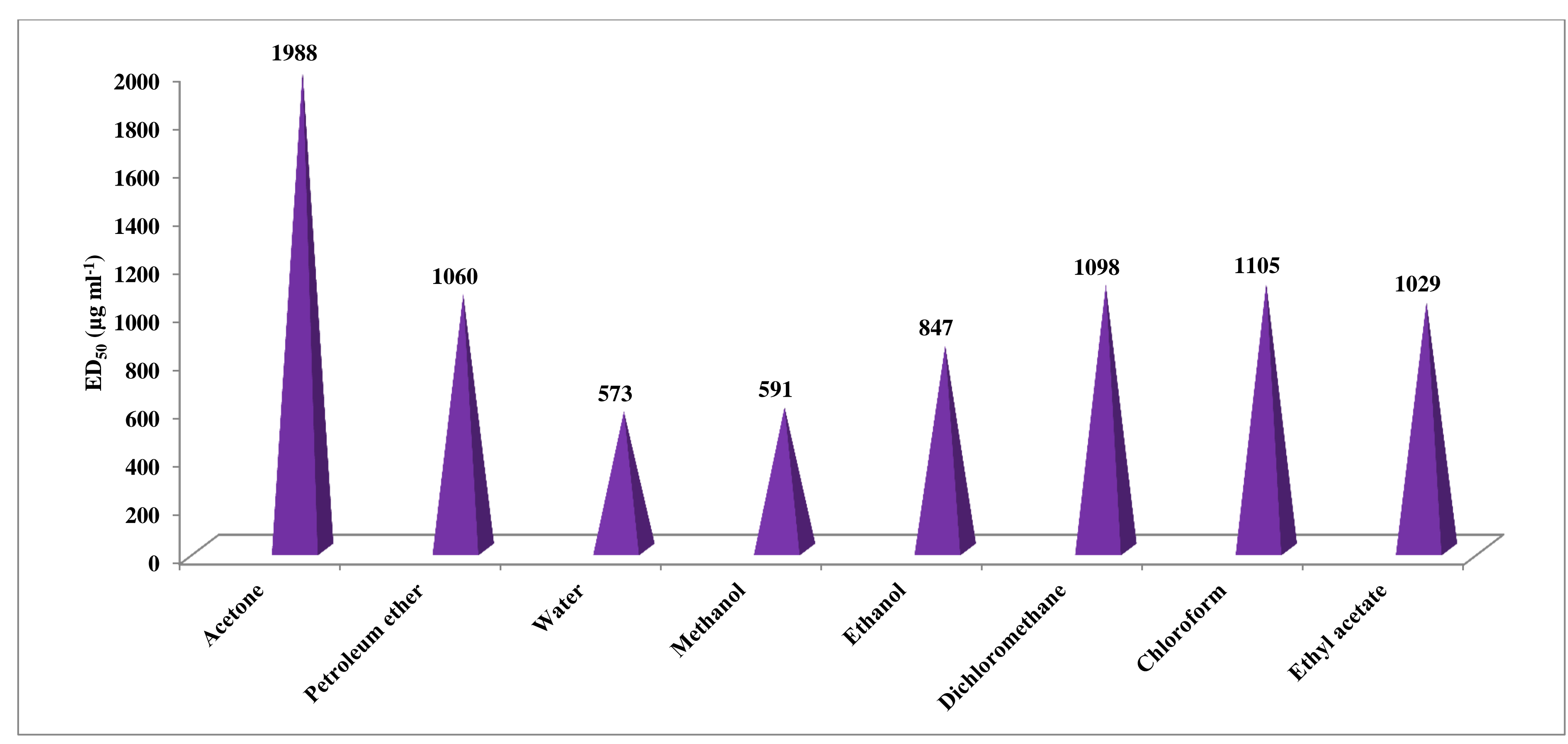


Figure 24: ED_{50} of different solvent extracts against *Bortrytis gray mould*

4.11.3 Evaluation of fungicidal activity of Schiff bases, thiosemicarbazones and hydrazones of cuminaldehyde

Different substituted derivatives of cuminaldehyde *i.e.* Schiff bases, thiosemicarbazones and hydrazones were evaluated for their fungicidal potential againt *F. wilt* and *Bortrytis gray mould.*

Fusarium wilt

It was evident from Table 37 that compound X exhibited maximum percent inhibition against *F. wilt.* Compound X displayed better activity than other compounds. The better activity compound X was may be due to the presence of heterocyclic ring along with azomethine group (Kaur *et al* 2019; Rostom *et al* 2009; Holla *et al* 2005). It was found that Schiff bases containing heterocyclic moiety displayed better activity as compared to synthesized thiosemicarbazones and hydrazones. Out of thiosemicarbazones and hydrazones of cuminaldehyde, thiosemicarbazones exhibited better activity than hydrazones. Significant differences were observed between the different synthesized compounds at different concentrations.

In case of hydrazones, it was found that as the number of electron withdrawing groups on benzene ring increases bioactivity of synthesized hydrazones increases. ED_{50} of all the compounds were determined and presented in Figure 25.

Bortrytis gray mould

Screening of fungicidal activity of synthesized products against *Bortrytis gray mould* revealed that compound XI displayed better activity as compared to the other compounds (Table 38, Figure 26). The better activity of compound XI may be due to the presence azomethine moiety along with the heterocyclic triazole ring. Triazoles are the commericialy available fungicide. Compound XI exhibited complete inhibition of fungal pathogens at and above 2000 ppm. At 1000 ppm, Compound XI registered 81.26% inhibition. Triazoles inhibit the production of C14- demethylase which plays important role in sterol production which inturn inhibits the development of functional cell walls. Compound X-XIV did not display any growth of fungi at and above 2000 ppm. Significant differences were observed between the different synthesized compounds at different concentrations. Out of thiosemicarbazones, Compound XIV *i.e.* the compound synthesized *via* condensation of cuminaldehyde with phenylthiosemicarbazide displayed better activity as compared to the compound XIII. The better activity of compound XIV may be due to the presence of azomethine group alog with the phenyl ring.

In case of hydrazones (XV-XVIII), it was observed that as the number of electron withdrawing groups on benzene ring increases bioactivity of synthesized hydrazones increases. ED_{50} of all the compounds were determined and presented in Figure 26. It was evident from Figure that compound XI displayed maximum activity.

Table 37: Per cent inhibition of synthesized Schiff bases (II-XII), thiosemicarbazones (XIII & XIV) and hydrazones (XV-XVIII) against *F. wilt* at different concentrations (μg ml^{-1})

Conc. (μg mL^{-1}) / Compounds	Per cent Radical Scavenging Activity							
	3000	**2000**	**1000**	**500**	**250**	**100**	**50**	**25**
II	100±0.00	70.23±0.22	52.01±0.85	33.23±0.54	23.09±0.63	19.48±0.52	15.25±0.66	10.12±0.16
III	100±0.00	76.26±0.63	54.69±0.74	35.23±0.36	26.59±0.32	20.48±0.03	15.48±0.21	11.96±0.56
IV	100±0.00	72.89±0.74	53.95±0.54	36.32±0.22	26.23±0.11	20.23±0.69	16.23±0.23	12.56±0.39
V	100±0.00	71.98±0.88	50.23±0.09	34.36±0.05	25.56±0.65	20.19±0.48	15.84±0.62	13.85±0.49
VI	100±0.00	79.45±0.58	60.29±0.52	40.84±0.09	31.11±0.26	21.55±0.33	18.78±0.63	15.65±0.48
VII	100±0.00	75.15±0.54	55.10±0.96	37.56±0.58	23.02±0.23	21.59±0.85	15.69±0.59	13.45±0.01
VIII	100±0.00	90.21±0.66	68.27±0.23	50.23±0.02	36.03±0.51	29.55±0.23	25.06±0.64	18.50±0.56
IX	100±0.00	65.29±0.29	47.23±0.54	33.56±0.66	23.23±0.65	17.23±0.96	14.23±0.33	9.56±0.65
X	100±0.00	100±0.00	80.98±0.36	56.25±0.15	42.56±0.33	35.12±0.32	27.32±0.95	20.23±0.35
XI	100±0.00	100±0.00	70.56±0.95	50.96±0.33	39.56±0.84	31.22±0.48	25.69±0.24	18.89±0.06
XII	100±0.00	100±0.00	72.50±0.51	53.55±0.08	40.20±0.63	31.25±0.66	26.30±0.39	19.20±0.05
XIII	100±0.00	100±0.00	66.23±0.74	47.03±0.03	35.50±0.09	28.48±0.05	21.36±0.84	17.96±0.98
XIV	100±0.00	100±0.00	70.10±0.25	49.25±0.16	36.33±0.21	30.23±0.09	24.66±0.21	18.56±0.96
XV	100±0.00	81.26±0.62	56.23±0.95	35.07±0.11	25.23±0.46	19.22±0.03	16.05±0.36	10.65±0.36
XVI	100±0.00	82.56±0.26	57.63±0.77	39.22±0.78	29.55±0.65	21.85±0.09	17.04±0.85	12.25±0.52
XVII	100±0.00	86.26±0.03	61.36±0.35	41.60±0.57	33.12±0.95	22.56±0.36	18.75±0.95	15.50±0.36
XVIII	100±0.00	89.02±0.65	65.89±0.84	45.52±0.12	35.69±0.31	26.23±0.44	19.05±0.36	17.44±0.58
Carbendazim 50 WP	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00

At five percent level of significance CD (Compounds) = 0.72, CD (Concentrations) = 0.09, CD (Compounds×Concentrations) = 0.48

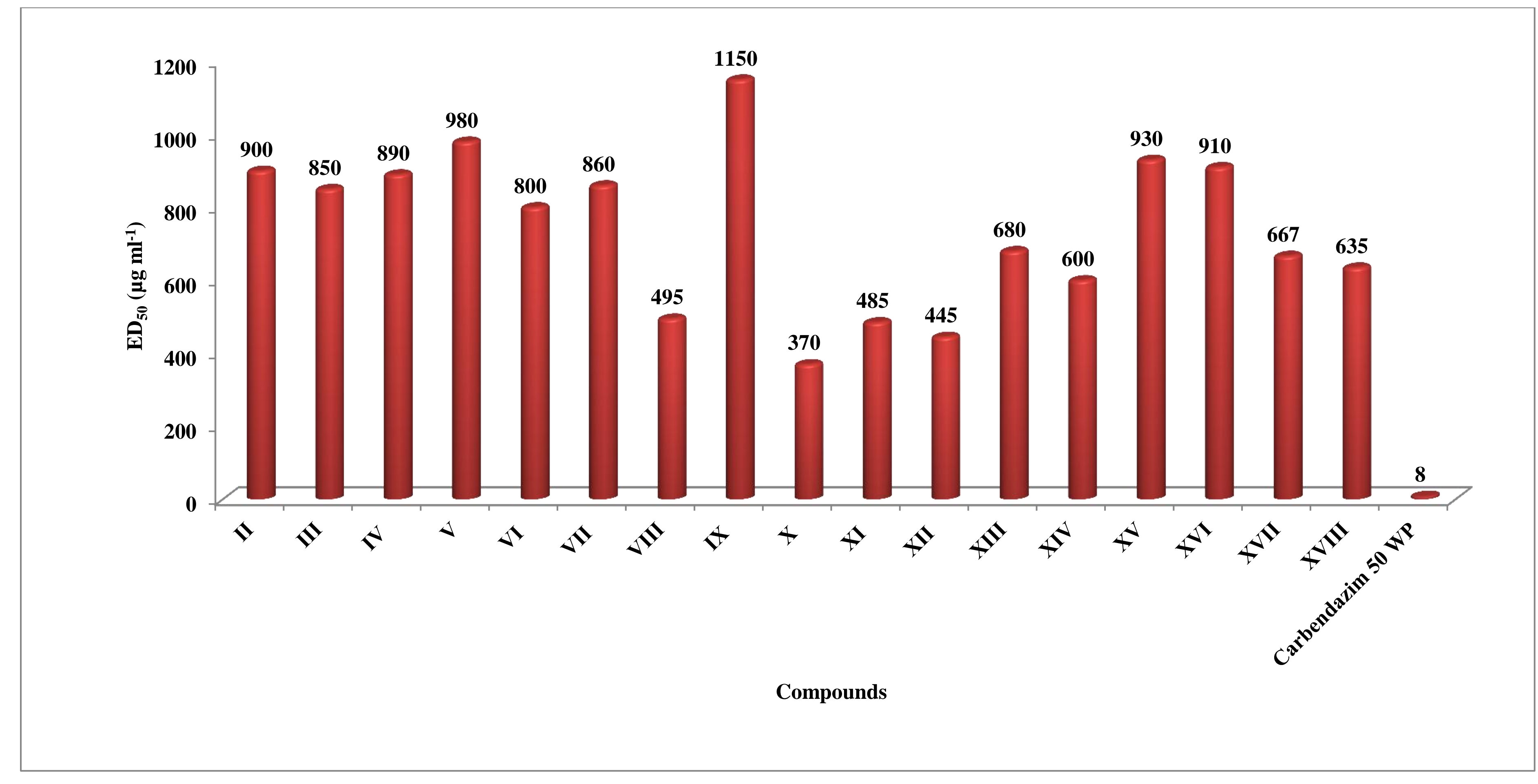


Figure 25: ED_{50} of Schiff bases (II-XII), thiosemicarbazones (XIII & XIV) and hydrazones (XV-XVIII) against *Fusarium wilt*

Table 38: Per cent inhibition of synthesized Schiff bases (II-XII), thiosemicarbazones (XIII & XIV) and hydrazones (XV-XVIII) against *Bortrytis gray mould*

Conc. (μg ml^{-1}) / Compounds	Per cent Radical Scavenging Activity							
	3000	**2000**	**1000**	**500**	**250**	**100**	**50**	**25**
II	100±0.00	70.78±0.63	52.67±0.62	33.59±0.14	23.09±0.63	19.48±0.52	15.25±0.66	10.11±0.16
III	100±0.00	80.27±0.56	57.54±0.48	36.25±0.84	27.25±0.41	21.02±0.62	16.21±0.18	11.18±0.24
IV	100±0.00	75.56±0.63	55.36±0.96	35.05±0.12	26.94±0.52	20.78±0.31	15.99±0.65	10.98±0.89
V	100±0.00	72.63±0.26	54.66±0.02	34.85±0.91	25.62±0.61	19.98±0.47	15.52±0.12	10.75±0.44
VI	100±0.00	81.65±0.26	60.08±0.89	37.25±0.41	28.01±0.56	21.15±0.45	16.25±0.65	11.25±0.16
VII	100±0.00	69.18±0.95	50.26±0.23	32.65±0.78	22.58±0.86	15.63±0.73	13.69±0.15	9.89±0.81
VIII	100±0.00	91.26±0.85	68.02±0.24	50.51±0.35	37.96±0.95	28.06±0.45	21.45±0.48	17.50±0.51
IX	100±0.00	68.66±0.56	49.49±0.43	33.99±0.66	22.95±0.78	18.56±0.62	15.06±0.15	10.09±0.95
X	100±0.00	100±0.00	68.33±0.48	51.29±0.15	36.06±0.10	28.48±0.05	23.59±0.26	17.99±0.59
XI	100±0.00	100±0.00	81.26±0.57	58.48±0.25	44.75±0.15	36.33±0.57	28.85±0.62	20.66±0.23
XII	100±0.00	100±0.00	71.06±0.24	52.85±0.35	40.74±0.24	32.91±0.27	26.77±0.35	18.99±0.41
XIII	100±0.00	100±0.00	69.24±0.47	52.65±0.08	37.48±0.04	31.06±0.58	26.36±0.84	18.96±0.08
XIV	100±0.00	100±0.00	73.25±0.11	54.48±0.07	41.26±0.21	33.51±0.75	27.38±0.89	19.15±0.04
XV	100±0.00	82.56±0.03	59.28±0.54	38.17±0.09	28.03±0.13	21.56±0.25	16.95±0.78	12.15±0.15
XVI	100±0.00	83.11±0.02	60.23±0.78	39.88±0.59	29.55±0.18	23.75±0.35	17.04±0.85	12.36±0.72
XVII	100±0.00	87.95±0.54	62.65±0.56	42.62±0.31	33.95±0.22	25.54±0.29	18.75±0.32	16.05±0.62
XVIII	100±0.00	89.63±0.94	67.04±0.48	46.45±0.75	37.25±0.37	27.56±0.54	20.05±0.35	16.25±0.89
Carbendazim 50 WP	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00

At five percent level of significance CD (Compounds) = 0.65, CD (Concentrations) = 0.27, CD (Compounds×Concentrations) = 0.51

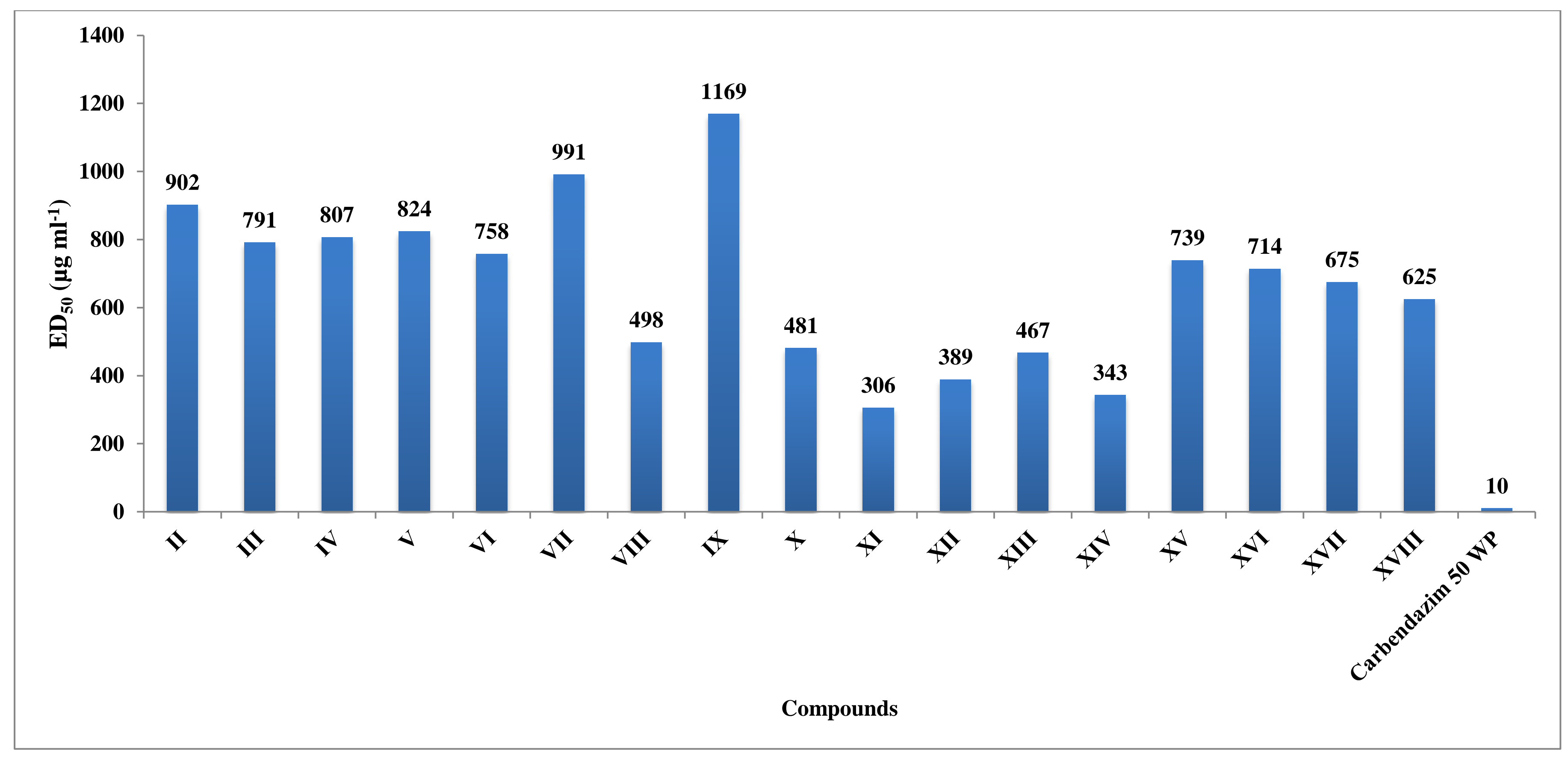


Figure 26: ED$_{50}$ of Schiff bases (II-XII), thiosemicarbazones (XIII & XIV) and hydrazones (XV-XVIII) against *Bortrytis gray mould*

4.11.4 Evaluation of fungicidal activity of chalcones and pyrazolines of cuminaldehyde

Different substituted chalcones (XIX-XXIV) and pyrazolines (XXV-XXXVI) were evaluated for their fungicidal potential against *F. wilt* and *Bortrytis gray mould* at different concentrations (µg ml^{-1}). at higher concentrations *i.e.* 2000 and 3000 µg ml^{-1} synthesized compounds showed 100% inhibition against *Fusarium wilt*.

F. wilt

It was evident from Table 39 that pyrazolines exhibited better fungicidal potential than substituted chalcones. Similar results were also reported by Banewar (2002). From all the synthesized chalcones and pyrazolines, pyrazoline synthesized by reacting chalcone (XXI) and phenyl hydrazine exhibited better activity than remaining compounds. The effectiveness of compound (XXXIII) was may be due to presence of heterocyclic ring. It was found that the out of all the synthesized chalcones (XIX-XXIV), chalcone (XXIII) containing chloro group was found most effective. Similar trend was observed in case of pyrazoline. Pyrazoline XXVII (synthesized from chalcone and hydrazine hydrate) and XXXIII (synthesized from chalcone and phenyl hydrazine), both containing chloro group at the para position of aromatic ring exhibhited maximum than their corresponding compounds.

Significant differences were observed between the different synthesized compounds at different concentrations. All the synthesized derivatives displayed lesser activity as compared to the standard. ED_{50} values of substituted chalcones and pyrazolines was determined and presented in Figure 27.

Bortrytis gray mould

Screening of antifungal activity of synthesized chalcones and pyrazolines revealed that the synthesized chalcones displayed better activity as compared to pyrazolines. Compound XX displayed maximum antifungal potential (Table 40). Higher bioactivity of compound XX was may be due to the presence of most electronegative atom on the benzene ring. Highly conjugated system increases the buiactivity of the compound. ED_{50} of compound XX was 317 ppm. Activity of synthesized compound against synthesized compound followed the trend XX > XXI > XXVI > XXII > XXVII > XXVIII > XXXII> XXIV > XXXIII> XXVIII > XXIX > XIX > XXXIV > XXX > XXXV > XXXVI > XXV > XXXI.

Halogen substituted chalcones displayed better activity as compaed to remaining chalcones. Activity of chalcones varies directly as a electronegativity of halogen atom increases. Chalcones (XIX - XXIV) possessed ED_{50} values 603, 317, 345, 435, 487, 498, 498 ppm (Figure 28).

Table 39: Per cent inhibition of synthesized chalcones (XIX-XXIV) and pyrazolines (XXV-XXXVI) against *F. wilt* at different concentrations (μg ml^{-1})

Compounds \ Conc. (μg mL^{-1})	Per cent inhibition							
	3000	**2000**	**1000**	**500**	**250**	**100**	**50**	**25**
XIX	100±0.0	100±0.0	74.23±0.26	46.25±0.26	35.15±0.21	26.55±0.222	18.15±0.36	10.91±0.55
XX	100±0.0	100±0.0	69.11±0.56	40.23±0.11	30.85±0.47	23.36±0.36	16.23±0.11	10.88±0.38
XXI	100±0.0	100±0.0	85.23±0.25	55.01±0.57	44.22±0.36	35.75±0.17	24.36±0.95	18.15±0.42
XXII	100±0.0	100±0.0	77.85±0.73	50.55±0.54	38.46±0.98	27.56±0.24	19.45±0.42	12.85±0.06
XXIII	100±0.0	100±0.0	79.15±0.22	52.35±0.12	41.33±0.54	29.85±0.98	21.75±0.54	15.69±0.04
XXIV	100±0.0	100±0.0	80.23±0.28	53.75±0.25	42.25±0.35	32.11±0.63	23.23±0.09	16.45±0.19
XXV	100±0.0	100±0.0	76.36±0.51	50.26±0.69	36.48±0.53	27.88±0.15	20.11±0.08	11.69±0.03
XXVI	100±0.0	100±0.0	72.32±0.95	48.55±0.25	31.26±0.03	24.48±0.84	18.28±0.04	10.96±0.58
XXVII	100±0.0	100±0.0	86.02±0.11	57.36±0.56	48.23±0.52	36.89±0.18	26.21±0.06	19.20±0.35
XXVIII	100±0.0	100±0.0	79.01±0.17	51.26±0.02	40.83±0.34	29.45±0.25	20.06±0.24	14.69±0.15
XXIX	100±0.0	100±0.0	80.26±0.31	52.95±0.21	42.85±0.26	32.88±0.11	23.75±0.45	16.96±0.29
XXX	100±0.0	100±0.0	82.95±0.22	54.22±0.77	44.36±0.59	34.26±0.09	24.01±0.95	17.09±0.32
XXXI	100±0.0	100±0.0	78.32±0.56	52.26±0.65	37.66±0.04	28.90±0.23	22.45±0.24	12.36±0.33
XXXII	100±0.0	100±0.0	73.26±0.25	50.23±0.11	33.59±0.11	26.20±0.84	20.56±0.82	11.03±0.07
XXXIII	100±0.0	100±0.0	88.25±0.57	58.75±0.25	49.13±0.74	37.00±0.25	27.23±0.74	20.63±0.36
XXXIV	100±0.0	100±0.0	80.55±0.31	53.56±0.52	43.68±0.36	31.78±0.66	23.05±0.51	16.08±0.05
XXXV	100±0.0	100±0.0	81.36±0.22	54.23±0.11	43.56±0.22	33.96±0.36	24.09±0.65	17.06±0.28
XXXVI	100±0.0	100±0.0	84.02±0.84	55.89±0.25	45.69±0.15	35.98±0.02	26.03±0.23	18.32±0.49
Carbendazim 50 WP	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00

At five percent level of significance CD (Compounds) = 0.56, CD (Concentrations) = 0.44, CD (Compounds×Concentrations) = 0.59

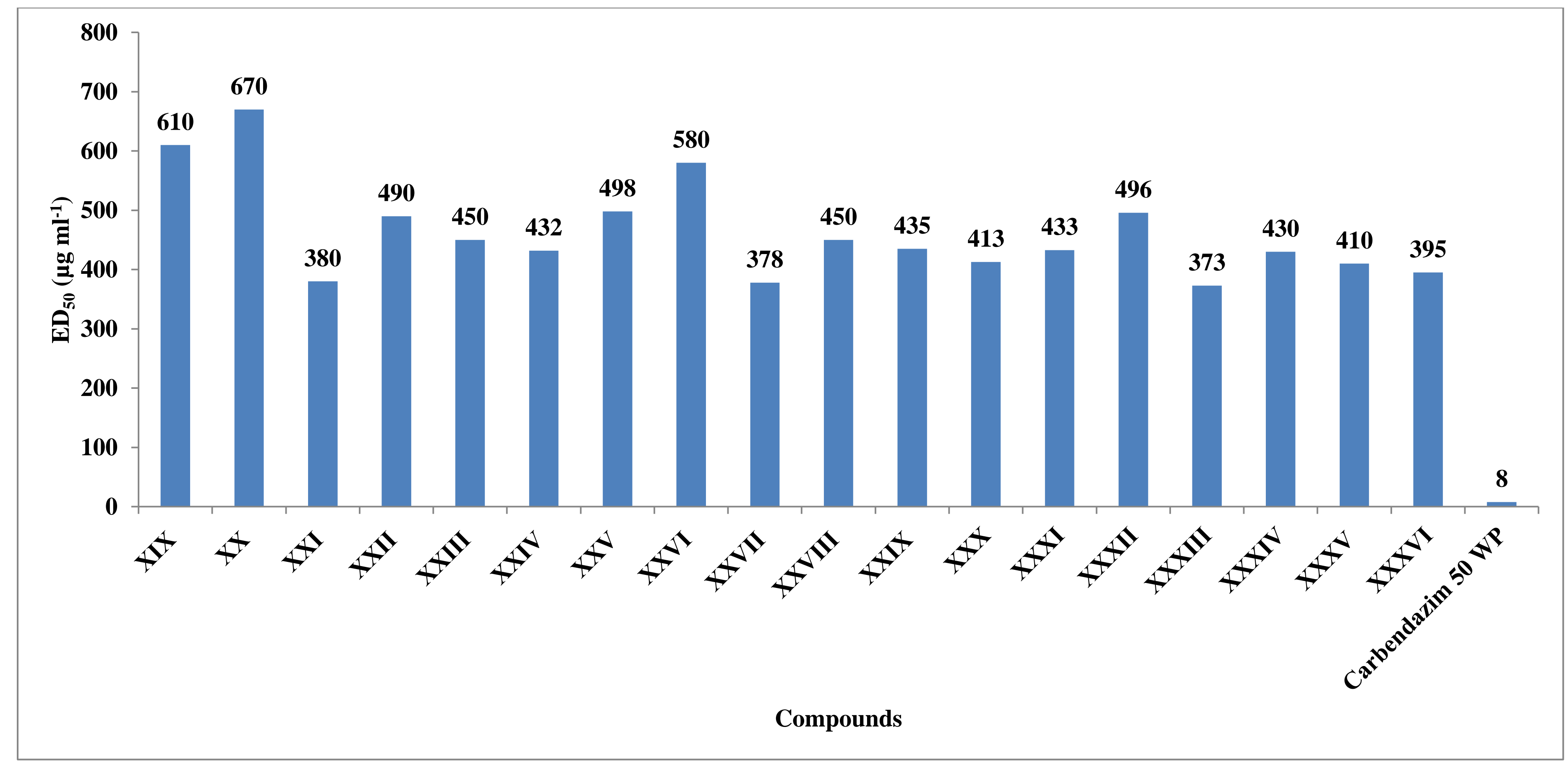


Figure 27: ED_{50} of chalcones (XIX-XXIV) and pyrazolines (XXV-XXXVI) against *Fusarium wilt*

Table 40: Per cent inhibition of synthesized chalcones (XIX-XXIV) and pyrazolines (XXV-XXXVI) against *Bortrytis gray mould*

Compounds \ Conc. (μg ml^{-1})	Per cent inhibition							
	3000	**2000**	**1000**	**500**	**250**	**100**	**50**	**25**
XIX	100±0.0	100±0.0	76.43±0.63	44.71±0.24	33.61±0.52	28.16±0.57	21.24±0.93	14.30±0.51
XX	100±0.0	100±0.0	83.07±0.26	57.08±0.73	41.78±0.01	36.44±0.21	29.31±0.54	20.67±0.61
XXI	100±0.0	100±0.0	81.97±0.16	54.26±0.13	41.46±0.53	36.01±0.16	28.13±0.61	19.64±0.84
XXII	100±0.0	100±0.0	80.97±0.61	52.91±0.27	38.11±0.05	34.79±0.85	25.66±0.26	18.17±0.08
XXIII	100±0.0	100±0.0	80.13±0.67	51.08±0.47	37.16±0.44	33.18±0.81	24.84±0.94	17.29±0.14
XXIV	100±0.0	100±0.0	79.64±0.88	50.11±0.25	36.05±0.25	32.19±0.35	23.82±0.19	16.26±0.12
XXV	100±0.0	100±0.0	73.14±0.65	39.64±0.84	28.18±0.32	25.31±0.44	18.76±0.01	12.78±0.04
XXVI	100±0.0	100±0.0	81.68±0.10	53.16±0.27	39.16±0.09	35.63±0.08	26.01±0.14	19.17±0.52
XXVII	100±0.0	100±0.0	80.75±0.13	51.90±0.23	37.42±0.12	34.09±0.18	25.10±0.23	17.86±0.74
XXVIII	100±0.0	100±0.0	78.16±0.42	47.43±0.12	35.13±0.34	31.06±0.55	22.15±0.74	15.04±0.14
XXIX	100±0.0	100±0.0	76.64±0.33	45.81±0.82	34.78±0.28	30.91±0.17	21.63±0.45	14.69±0.28
XXX	100±0.0	100±0.0	75.87±0.12	42.95±0.77	31.81±0.63	27.06±0.59	19.21±0.63	13.81±0.68
XXXI	100±0.0	100±0.0	71.64±0.24	38.36±0.67	25.46±0.14	23.78±0.62	17.05±0.44	11.67±0.37
XXXII	100±0.0	100±0.0	79.71±0.61	50.73±0.11	36.87±0.51	32.92±0.62	24.03±0.87	16.83±0.63
XXXIII	100±0.0	100±0.0	78.96±0.28	49.68±0.74	35.78±0.44	31.78±0.15	23.19±0.85	15.95±0.41
XXXIV	100±0.0	100±0.0	76.33±0.54	43.52±0.25	32.17±0.34	27.11±0.66	20.61±0.46	14.03±0.05
XXXV	100±0.0	100±0.0	74.79±0.28	41.07±0.13	30.16±0.85	26.88±0.63	19.03±0.86	13.07±0.52
XXXVI	100±0.0	100±0.0	73.46±0.96	40.56±0.26	29.87±0.45	26.09±0.05	18.91±0.23	12.94±0.45
Carbendazim 50 WP	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00	100±0.00

At five percent level of significance CD (Compounds) = 0.29, CD (Concentrations) = 0.52, CD (Compounds×Concentrations) = 0.38

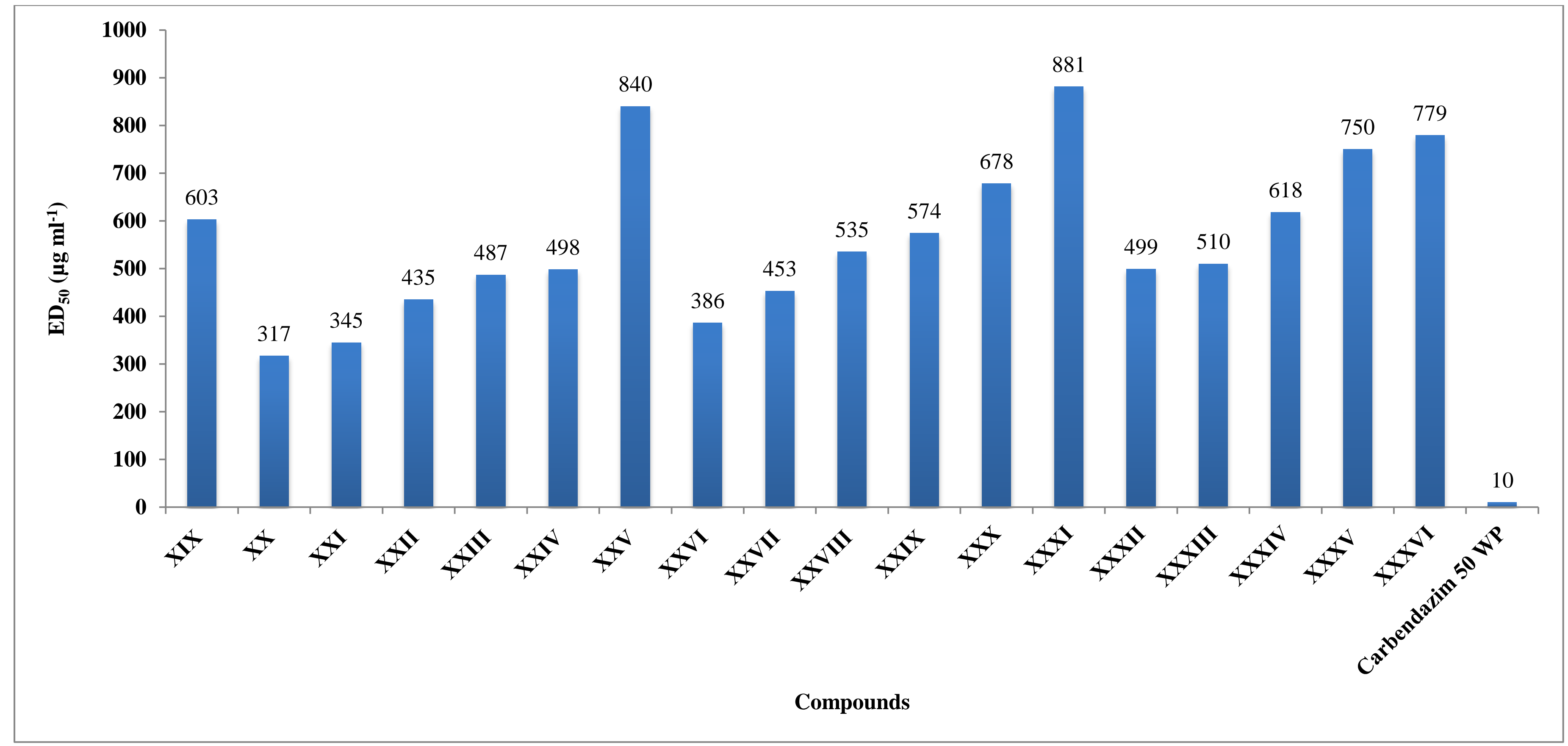
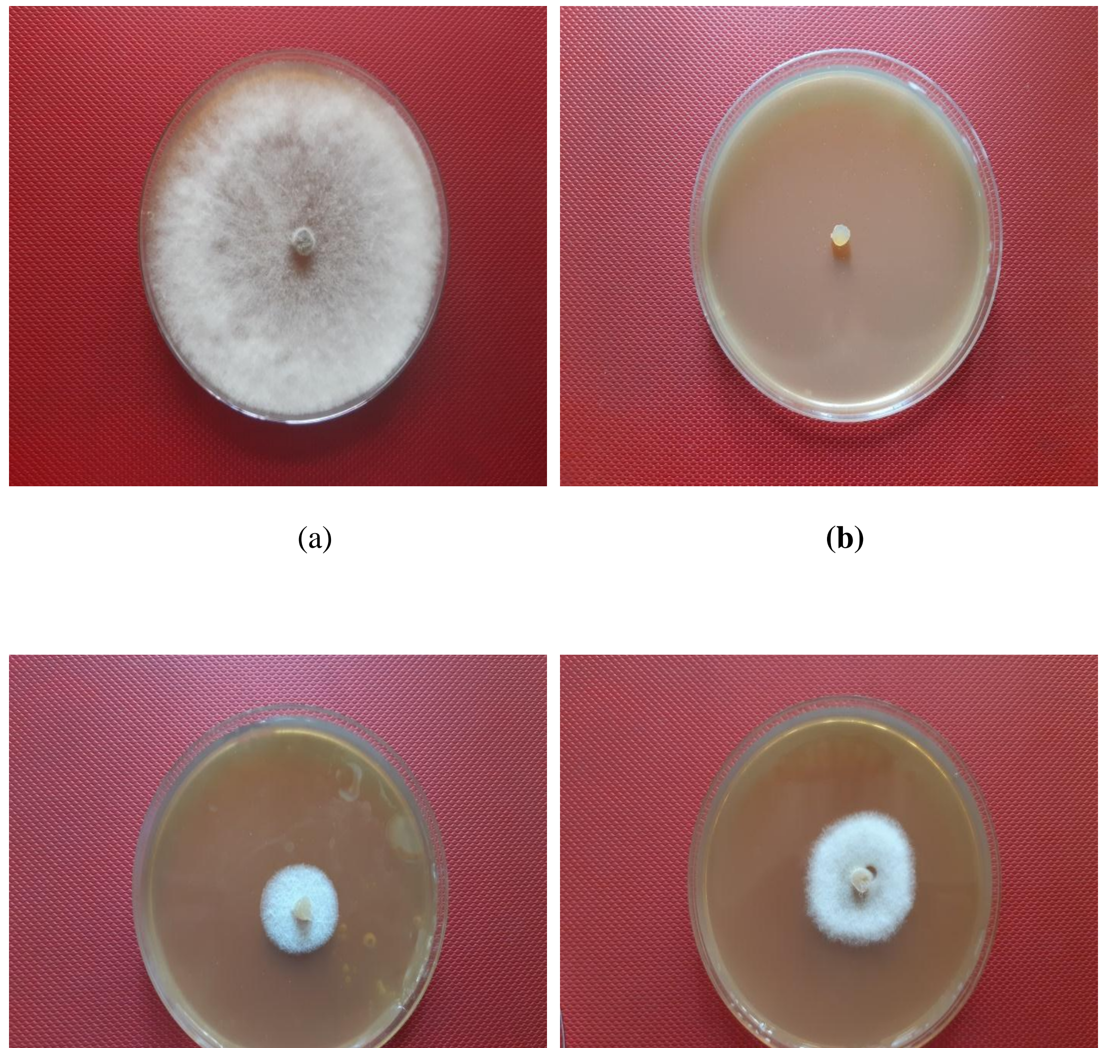


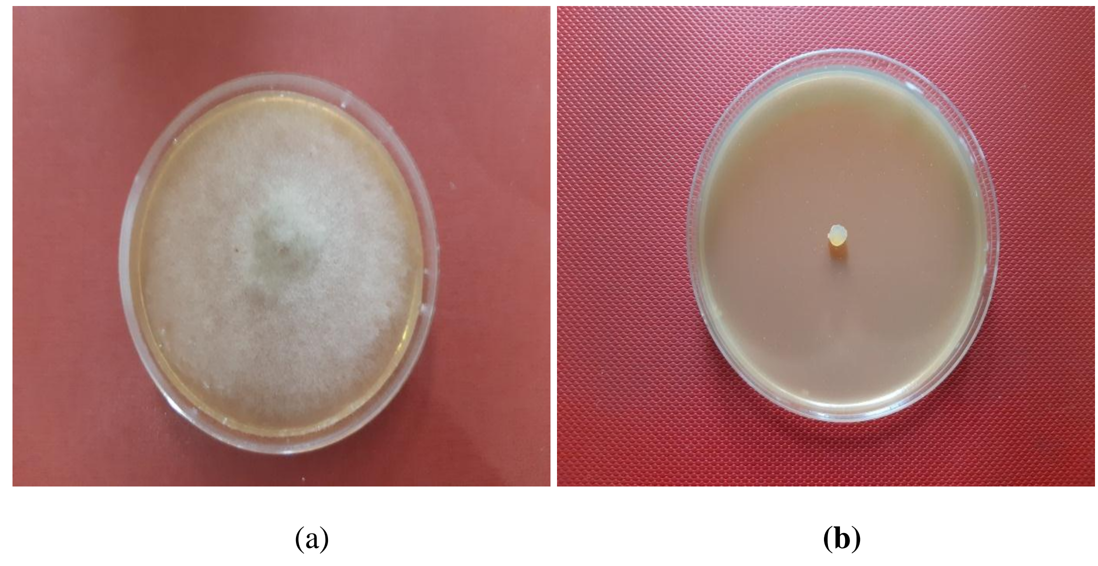
Figure 28: ED_{50} of chalcones (XIX-XXIV) and pyrazolines (XXV-XXXVI) against *Bortrytis gray mould*



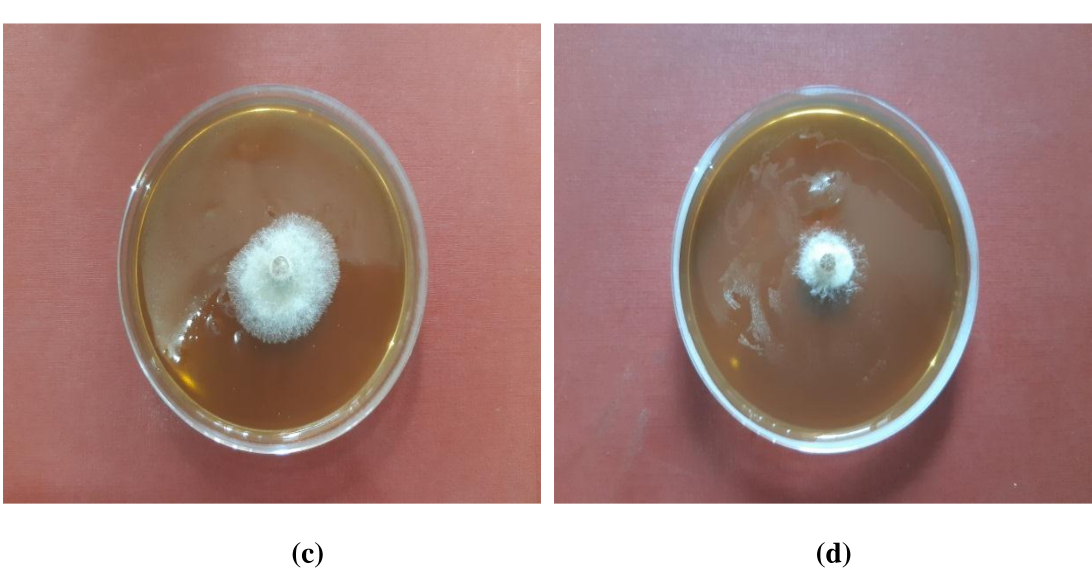
(a) **(b)**

(c) **(d)**

Plate 6: Mycelial growth inhibition shown by control (a), carbendazim 50 WP (standard) (b), Compound XXXIII at 1000 µg ml^{-1} (c) and compound XII at 1000 µg ml^{-1} concentration against *Fusarium wilt* (d)



(a) **(b)**



(c) **(d)**

Plate 7: Mycelial growth inhibition shown by control (a), carbendazim 50 WP (standard) (b), Compound XI at 1000 µg ml^{-1} (c) and compound XX at 1000 µg ml^{-1} concentration against *Bortrytis gray mould* (d)

`

4.11.5 Evaluation of fungicidal activity of silver nanoparticles of cumin seeds

Different synthesized silver nanoparticles of cumin seeds were screened for their antifungal potential by poison food technique against *Fusarium wilt and Bortrytis gray mould.* Similar trends were observed of synthesized nanoparticles against both the tested fungi. Fungicidal potential of synthesized nanoparticles was tested by varying the concentration of both plant extracts (5 ml, 10 ml and 15 ml) and silver nitrate solution (1mM, 3mM and 5mM) and this variation is shown in Table 41, 42 and the ED_{50} values were not determined as no synthesized nanoparticles showed percent inhibition more than 50%.

Table 41: Per cent inhibition of silver nanoparticles of cumin seeds against *Fusarium wilt* at different concentrations (mg ml^{-1})

Conc. of $AgNO_3$ (mM)	Volume of plant extract (ml)								
	5ml			**10ml**			**15ml**		
	Conc. of NPs (mg ml^{-1})								
	0.10	0.20	0.40	0.10	0.20	0.40	0.10	0.20	0.40
	Per cent inhibition								
1.00	0.00	8.52	15.23	0.00	10.23	16.02	0.00	12.26	18.26
3.00	9.19	11.59	25.45	10.21	12.99	28.15	12.99	14.85	29.23
5.00	11.52	24.56	35.23	13.56	26.44	38.26	15.26	28.45	40.26
Carbendazim 50 WP	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Table 42: Per cent inhibition of silver nanoparticles of cumin seeds against *Bortrytis gray mould* at different concentrations (mg ml^{-1})

Conc. of $AgNO_3$ (mM)	Volume of plant extract (ml)								
	5 ml			**10 ml**			**15 ml**		
	Conc. of NPs (mg ml^{-1})								
	0.10	0.20	0.40	0.10	0.20	0.40	0.10	0.20	0.40
	Per cent inhibition								
1.00	0.00	8.64	16.02	0.00	10.74	16.98	0.00	13.04	19.52
3.00	10.23	11.75	27.16	10.79	13.25	29.12	13.24	14.95	30.59
5.00	11.85	25.62	36.28	13.75	27.15	39.84	14.95	30.58	45.26
Carbendazim 50 WP	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

It was evident from Table 41 and 42 that as we increase the concentration of seed extracts and silver nanoparticles, the bioactivity of silver nanoparticles increases. The better activity of silver nanoparticles with increase in concentration of seed extract was may be due to higher phenolic and flavonoid (Abdel-Aziz *et al* 2014). None of the seed extract displayed better activity than standard carbendazim 50 WP. Non significant differences were observed between different synthesized compounds at different concentrations.

4.12. Conclusion

Cumin seeds variety GC-4 was taken from Rajasthan state seed and organic production certification agency, Jaipur. Proximate analysed revealed that cumin seeds contains total sugars (2.30%), moisture content (8.58%), total minerals (8.62%), crude fibre (11.06%), crude protein (14.13%), fats (15.00%) and carbohydrates (40.01 %). Elemental investigation of cumin seeds revealed that it contains several biologically important elements (in ppm) such as copper (14.25), Manganese (25.75), zinc (31.25) and iron (181.33). Essential oil of cumin seeds was extracted by hydrodistillation method using Clevenger apparatus and yield was 2.52%, respectively. Cuminaldehyde (36.46%) was found to be the major compound in cumin seeds. The characteristic flavour of cuminaldehyde is attributed to the presence of such high per cent of cuminaldehyde. Other minor compounds present were 5-methyl-2-propylphenol (9.10 %), β-pinene (7.92 %), *p*-cymene (7.96), phellandral (1.27 %), γ-terpinen-7-al (7.74 %), γ-terpinene (5.64%), 4-isopropylcyclohex-3-enecarbaldehyde (3.71 %), 4-hydroxycryptone (2.56 %), 2,5-dimethyloctahydropentalene (2.30 %), 1-(1-Ethyl-2,3-dimethyl-2-cyclopenten-1-yl)ethanone (1.89 %) and 3,7-dimethyl-octa-2,6-dien-1-al (1.46 %). Different derivatives of cuminaldehyde *viz.* Schiff bases (II-XII), thiosemicarbazones (XIII & XIV), hydrazones (XV-XVIII), chalcones (XIX-XXIV) and pyrazolines (XXV-XXXVI) were synthesized. Cumin oil, its major constituent cuminaldehyde, different solvent extracts, synthesized derivatives of cuminaldehyde were evaluated for antioxidant, microbial and fungicidal potential. Cuminaldehyde derivatives were found more effective as compared to cumin essential oil, its major constituent, different solvent extracts of cumin seeds and silver nanoparticles synthesized from aqueous cumin seed extract. Compound III registered maximum antioxidant potential by DPPH assay. Compound IX, XIV, XVIII are showed maximum activity against *Pseudomonas sp., Klebsiella sp.,* and *Enterobacter sp.,* respectively. Compound XXXIII and XX exhibited maximum fungicidal potential against *Fusarium wilt* and *Bortrytis gray mould*, respectively. All the synthesized compounds were found less effective than standard.

CHAPTER – V

SUMMARY

Cuminum cyminum L. commonly called cumin, is a annual herbaceous plant from Apiaceae family. Other common name of cumin seeds are zira, jeera, comino and kmin. It is the second most popular spice after black pepper commonly used in Indian kitchens. In India, it is mainly cultivated in the states of Gujrat and Rajasthan. It constitutes an important part of ayurvedic medicines and used to treat many health issues such as obesity, stomach pain and dyspepsia (Srinivasan 2018). Whenever the spice is crushed or powdered, the cell matrix of spice will break down and there is a release of the volatile components (Nadeem and Riaz 2012). In domestic and international markets, there is an excessive demand of essential oil of cumin seeds because it is 100 times more concentrated than the spice powder and thus required in very small quantity. Volatile oil present in cumin seeds is respondible for flavour and aroma of cumin seeds. The essential oil has antioxidant, antispasmodic, diuretic, carminative and antibacterial activities (Bettaieb *et al* 2010).

Cumin seed essential oil showed a significant effect in inhibiting DPPH as reported by Einafshar *et al* (2012). Jirovetz *et al* (2005) reported the high activity of the essential *C. cyminum* oil against the mold *Aspergillus niger*, the Gram (+) bacteria *Bacillus subtilis* and *Staphylococcus epidermidis* as well as the yeast *Saccharomyces cerevisiae* and *Candida albicans*. Fungitoxic behavior of *Cuminum cyminum* essential oil against the growth of *Aspergillus flavus* PICC-AF39, *Aspergillus flavus* PICC-AF24, *Aspergillus parasiticus* NRRL-999 and *Aspergillus niger* was reported by Mohammadpour *et al* (2012). Different derivatives of cuminaldehyed was synthesized such as Schiff bases, hydrazones and thiosemicarbazones, chalcones and pyrazolines and assessed for their biological potential against different fungi and bacteria. Therefore, it was anticipated that heterocyclic derivatives of cuminaldehyde may enhance antimicrobial and fungicidal activity and will help to explore alternative for pathogens control.

The work incorporated in the present dissertation reports the proximate composition and chemical composition of cumin seeds, phytochemical and chemical composition of different solvent extracts, derivatizations of cuminaldehyde, synthesis of silver nanoparticles from aqueous seed extract and evaluation for their antioxidant (by DPPH assay), antimicrobial (against *Pseudomonas* sp. *Enterobacter* sp. and *Klebsiella* sp.) and fungicidal potential (*Fusarium wilt*, *Ascochyta blight* and *Bortrytis gray mould*).

Cumin seeds variety GC-4 was taken from Rajasthan state seed and organic production certification agency, Jaipur. Proximate analysed revealed that cumin seeds contains total sugars (2.30%), moisture content (8.58%), total minerals (8.62%), crude fibre (11.06%), crude protein (14.13%), fats (15.00%) and carbohydrates (40.01 %). Elemental

investigation of cumin seeds revealed that it contains several biologically important elements (in ppm) such as copper (14.25), Manganese (25.75), zinc (31.25) and iron (181.33). Cumin seeds are rich source of iron which is essential for lactating women and proper growth of new borns.

Different solvent extracts (acetone, petroleum ether, water, methanol, ethanol, dichloromethane, chloroform and ethyl acetate) were subjected to the qualitative phytochemical screening for the presence of different phytochemicals such as alkaloids, saponins, tannins, anthocynanins, emodins, coumarins, amino acids, flavonoids, proteins, phenols and carbohydrate. None of the phytochemicals were detected in aetone and ethyl acetate. Percent yield of extracts was registered higher in aqueous ectract (14.60%). Different solvent extracts were also subjected for quantitative phytochemical screening for phenolics, tannins, saponins and flavonoids. Quantitative phytochemical analyses revealed that the amount of total phenols, tannins, saponins and flavonoids was found to be more in aqueous and methanol extracts. GC-MS analysis of different solvent extracts was also carried out and it was found that cuminaldehyde was one of the major component present in all the solvent extracts which may responsible for biological activity of different solvent extracts.

Essential oil of cumin seeds was extracted by hydrodistillation method using Dean-Stark apparatus and yield was 2.52%, respectively. Thin layer chromatography of pale yellow coloured essential oil showed four spots having R_f values of 0.48, 0.63, 0.78 and 0.92 which corresponded with R_f of cuminaldehyde (0.71). Further GC-MS also confirmed the presence of cuminaldehyde. Gas Chromatography- Mass Spectrometry (GC-MS) of oil exhibited the presence of 38 identified compounds which account for 99.89% of total composition. Cuminaldehyde (36.46%) was found to be the major compound in cumin seeds. The characteristic flavour of cuminaldehyde is attributed to the presence of such high per cent of cuminaldehyde. Other minor compounds present were 5-methyl-2-propylphenol (9.10 %), β-pinene (7.92 %), *p*-cymene (7.96), phellandral (1.27 %), γ-terpinen-7-al (7.74 %), γ-terpinene (5.64%), 4-isopropylcyclohex-3-enecarbaldehyde (3.71 %), 4-hydroxycryptone (2.56 %), 2,5-dimethyloctahydropentalene (2.30 %), 1-(1-Ethyl-2,3-dimethyl-2-cyclopenten-1-yl)ethanone (1.89 %), 3,7-dimethyl-octa-2,6-dien-1-al (1.46 %), and. Apart from these, many other compounds having area less than 1.00 % were also detected.

Different derivatives of cuminaldehyde *viz.* Schiff bases (II-XII), thiosemicarbazones (XIII & XIV), hydrazones (XV-XVIII), chalcones (XIX-XXIV) and pyrazolines (XXV-XXXVI) were synthesized. Reaction of cuminaldehyde with different substituted amines, thiosemicarbazides and hydrazines in the presence of glacial acetic acid yielded Schiff bases, thiosemicarbazones and hydrazones, respectively. Reaction of cuminaldehyde with different substituted acetophenones in the presence of sodium hydroxide yielded different substituted chalcones which were futhur reacted with hydrazine hydrate and phenyl hydrazine using

glacial acetic acid as a solvent to give different substituted pyrazolines. All the synthesized compounds were purified by recrystallization purity of synthesized compounds was checked by thin layer chromatography technique (TLC). Physical data *i.e.* yield, melting point, R_f and colour of all the synthesized compounds was recorded. UV-visible, IR, ^{1}H NMR and ^{13}C NMR spectroscopic techniques were used for characterization of synthesized cuminaldehyde derivatives.

Synthesis of silver nanoparticles of cumin seed aqueous extract was done using 10 millilitres of 1mM, 3mM and 5mM solution of silver nitrate ($AgNO_3$) was added to 5 ml, 10ml and 15ml of prepared cumin seed extract under constant stirring. Mixtures were heated for 30 mins at 80°C. Color change of the reaction mixtures determined nanoparticle formation which was indicated by dark brown color. The optical properties of the synthesized AgNPs were studied with the help of UV-Vis spectra and Transmission Electron Microscopic (TEM) analysis. UV-Visible spectra of synthesized nanoparticles showed maximum absorbance in the range of 430-448 nm, which is specific for AgNPs with size varied from 5-40 nm. It was noted that the size of the synthesized nanoparticles decreased as increase the amount of aqueous seed extract and decrease the concentration of silver nanoparticles.

Cumin oil, its major constituent cuminaldehyde, different solvent extracts, synthesized derivatives of cuminaldehyde were evaluated for antioxidant activity in terms of free radical scavenging activity (RSA) using dimethyl sulphoxide (DMSO) as the control and ascorbic acid as standard. Out of cuminaldehyde and cumin oil, former was found to be more effective at all the tested concentrations. IC_{50} of oil, cuminaldehyde and ascorbic acid were found to be 2466, 1960 and 10.00 µg ml^{-1}, respectively. Methanol and water extracts exhibited maximum antioxidant activity among all the solvent extracts with IC_{50} 2100 and 2400, respectively. Among all the synthesized compounds, Schiff bases possess highest antioxidant potential as compared to different substituted thiosemicarbazone and hydrazones. Out of all Schiff bases, compounds III and VIII exhibited highest antioxidant potential with IC_{50} 59.40 and 60.62, respectively. Azomethine group increase the antioxidant potential of the synthesized products. Compound XXXII was found effective among chalcones and pyrazolines. It was found that the compounds containing electron withdrawing groups displayed better activity as compared to compounds containing electron donating substituent. Pyrazoline being heterocyclic compounds exhibited higher activity as compared to chalcones. Out of all the synthesized derivatives of cuminaldehyde, Schiff bases (III and VIII) displayed highest antioxidant activity with IC_{50} 87 µg ml^{-1}.

Cumin oil, cuminaldehyde, different solvent extracts and newly synthesized derivatives of cuminaldehyde were evaluated for their *in vitro* antimicrobial potential against Gram-negative bacteria *viz. Pseudomonas sp*., *Klebsiella sp*. and *Enterobacter sp.* at different concentrations (3000, 2000, 1000, 500, 250, 100, 50 and 25 µg mL^{-1}).Cumin oil registered

more activity against all three tested bacteria. Minimum inhibition concentration of cumin oil against *Peudomonas* sp., *Klebsiella* sp. and *Enterobacter* sp. was 205, 95 and 180, respectively. Different solvent extracts does not display any significant microbial activity. All extracts are ineffective in inhibiting the growth of the three tested bacteria. Similar results are also reported by Shaik *et al* 2018. In case of Schiff bases, thiosemicarbazones and hydrazones, compound IX, XIV, XVIII are showed maximum activity against *Pseudomonas sp., Klebsiella sp.,* and *Enterobacter sp.,* respectively. Out of synthesized chalcones and pyrazolines, compound XX was found effective against all the three tested gram negative bacteria.

Essential oil, different solvent extracts of cumin, major constituent cuminaldehyde, its synthesized derivatives and silver nanoparticles of cumin were evaluated for antifungal activity against *Fusarium wilt* (FW), *Ascochyta blight* (AB) and *Bortrytis gray mould* (BGM) using poisoned food technique at different concentrations. Cumin oil displayed better activity as compared to cuminaldehyde at all the tested concentrations against *Fusarium wilt*. However, in case of *Bortrytis gray mould* cuminaldehyde displayed better activity as compared to cumin essential oil at all the tested concentration. Neither cumin oil nor cuminaldehyde showed better activity than standard. Aqueous and methanol extract were found to be more effective as compared to other extracts at all concentrations against *F. wilt and Bortrytis gray mould.* Out of all the synthesized Schiff bases, thiosemicarbazones and hydrazones, compound X and XI displayed better activity against *F. wilt and Bortrytis gray mould*, respectively than other compounds. From all the synthesized chalcones and pyrazolines, pyrazoline synthesized by reacting chalcone (XXI) and phenyl hydrazine exhibited better activity than remaining compounds against *F. wilt*. Compuond XX displayed highest activity among all the synthesized chalcones and pyrazolines against *Bortrytis gray mould*.

REFERENCES

A.O.A.C. (2000) Official methods of analysis. Association of Official Analytical Chemists. Washington, DC, USA.

Abbdellaoui M, Bouhlali E T and El Rhaffari L (2019) Chemical composition and antioxidant activities of the essential oils of cumin (*Cuminum cyminum*) conducted under organic production conditions. *J Essent Oil Bear Pl* **22**(6): 1500-08.

Abdel-Aziz M S, Shaheen M S, El-Nekeety A A and Abdel-Wahhab M A (2014) Antioxidant and antibacterial activity of silver nanoparticles biosynthesized using *Chenopodium murale* leaf extract. *J Saudi Chem Soc* **18**(4): 356-63.

Abdul Jabbar R A (2013) Chemical analysis and antimicrobial activity of cumin seeds extracted oil against some bacterial and fungal isolates. *J Thi-Qar Sci* **3**(4): 65-73.

Ajih T A and Janardhanan K K (2002) Antioxidant and antihepato-toxic activities of *Phellinus rimosus* (Berk) pilat. *J Ethnopharmacol* **81**: 387-91.

Ajitha B, Divya A, Harish G S and Sreedhara Reddy P (2013) the influence of silver precursor concentration on size of silver nanoparticles grown by soft chemical route. *Res J Physical Sci* **1**(7): 11-14.

Al Aboody M S and Mickymaray S (2020) Anti-fungal efficacy and mechanisms of flavonoids. *Antibiotics (Basel)* **9**(2): 45-86.

Al Juhaimi F Y and Ghafoor K (2013) Extraction optimization and *in vitro* antioxidant properties of phenolic compounds from Cumin (*Cuminum cyminum* L.) seed. *Int Food Res J* **20**(4): 1669-75.

Allahghadri T, Rasooli I, Owlia P, Nadooshan M J, Ghazanfari T and Taghizadeh M (2010) Antimicrobial property, antioxidant capacity and cytotoxity of essential oil from cumin produced in Iran. *J Food Sci* **75**: 54-61.

Alphonse R and Thanaraj C (2019) *Cuminum cyminum* extract mediated green synthesis and characterization of nickel oxide nanoparticles. *IJRAR* **6**(1): 712-16.

Al-Rubaye A F, Kadhim M J and Hameed I H (2017) Phytochemical profiles of methanolic seeds extract of *Cuminum cyminum* using GC-MS technique. *Int J Curr Pharm Rev Res* **8**(2): 114-24.

Al-Snafi A E (2016) The pharmacological activities of *Cuminum cyminum* - A review. *J Pharm* **6**(6): 46-65.

Anal K and Prasad J K (2010) Green synthesis of silver nanoparticles using cycas leaf. *Int J Green Nanotechnol: Phy Chem* **1**: 110-17.

Andeani J K, Kazemi H, Mohsenzadeh S and Afavi A (2011) Biosynthesis of gold nanoparticles using dried flowers extract of *Achillea wilhelmsii* plant. *Digest J Nanomat Biostruct* **6**(3): 1011-17.

Anjaum T and Akhtar N (2012) Antifungal activity of essential oils extracted from clove, cumin and cinnamon against blue mold disease on citrus fruit. *ISALS* **10**: 321-26.

Argolo A C, Ana A E S, Pletsch M and Coelho L C (2004) Antioxidant activity of leaf extracts from *Bauhinia monandra*. *Bioresour Technol* 95: 229-33.

Arish D and Nair M S (2010) Synthesis of some Schiff base metal complexes involving para substituted aromatic aldehydes and glycylglycine: Spectral, electrochemical, thermal and surface morphology studies. *J Mol Struct* **983**: 112-21.

Armendariz V, Herrera I and Peralta-Videa J R (2004) Size controlled gold nanoparticle formation by *Avena sativa* biomass: use of plants in nanobiotechnology. *J Nanopart Res* **6**(4): 377-82.

Atrooz O M (2009) The antioxidant activity and polyphenolic contents of different plant seeds extracts. *Pak J Biol Sci* **12**(15): 1063-68.

Atrooz O M (2013) The effects of *Cuminum cyminum* L and *Carum carvi* L seed extracts on human erythrocyte hemolysis. *Int J Biol* **592**: 57-63.

Awan U A, Andleeb S, Kiyani A, Zafar A, Shafique I, Riaz N, Azhar M T and Uddin H (2013) Antibacterial screening of traditional herbal plants and standard antibiotics against some human bacterial pathogens. *Pak J Pharm Sci* **26**(6): 1109-16.

Bameri Z, Amini-Boroujeni N, Saeidi S and Bazi S (2013) Antimicrobial activity of *Cuminum cyminum* against biofilm *E. coli*. *Int Res J Basic Appl Sci* **5**(10): 1232- 34.

Banewar V (2013) Green synthesis and *in vitro* biological evaluation of heteroaryl chalcones and pyrazolines of medicinal interest. *J chem* **13**: 1-4.

Barakat L A A and Mohamed M M (2011) Ginger, cumin and mustard seeds modulate acetaminophen-induced acute hepatic injury in rats. *J Appl Sci Res* **7**(9): 1368-74.

Baratta T M, Dorman D H J and Deans S G (1998) Chemical composition antimicrobial and antioxidative activity of Laurel, Sage, Rosemary, Oregano, and Coriander essential oils. *J Essent Oil Res* **10**: 618-27.

Baratta T M, Dorman D H J, Deans S G, Figueiredo A C, Barroso J C and Ruberto G (1998) Antimicrobial and antioxidant properties of some commercial essential oils. *Flavour Frag J* **13**: 235-44.

Barbosa A D P (2014) An overview on the biological and pharmacological activities of saponins. *Int J Pharm Pharm Sci* **6**(8): 47-50.

Barra A (2009) Factors affecting chemical variability of essential oils: a review of recent developments. *Nat Prod Commun* 4(8): 1147-54.

Beis S H, Azcan N, Ozek T, Kara M and Baser K H C (2000) Production of essential oil from Cumin seeds. *Chem Nat Compd* **36**(3): 265-68.

Bendary E, Francis R R, Ali H M G, Sarwat M I and El Hady S (2013) Antioxidant and structure-activity relationships (SARs) of some phenolic and anilines compounds. *Ann Agric Sci* **58**(2): 173-81.

Bennie R B, David S T, Sivasakthi M, Mary S A, Seethalakshmi M, Abraham S D, Joel C and Antony R (2014) Synthesis, spectral characterization and antimicrobial studies of Schiff base transition metal complexes derived from cuminaldehyde and 4-aminoantipyrine. *Chem Sci Trans* **3**(3): 937-44.

Bettaieb I, Bourgou S, Wannes W A, Hamrouni , Limam F and Marzouk B (2010) Essential oils, phenolics, and antioxidant activities of different parts of cumin (*Cuminum cyminum* L.). *J Agric Food Chem* **58**: 10410-418.

Bhat S P, Rizvi W and Kumar A (2014) Effect of *Cuminum cyminum* L. seed extracts on pain and inflammation. *J Nat Rem* **14**(2): 186-92.

Bisht D, Chanotiya C S, Rana M and Semwal M (2009) Variability in essential oil and bioactive chiral mono terpenoid compositions of Indian oregano (*Origanum vulgare* L.) populations from northwestern Himalaya and their chemotaxonomy. *Ind Crops Prod* **30**: 422-26.

Blois M S (1958) Antioxidant determinations by the use of stable free radical. *Nature* 181: 1199-200.

Bondet V, Brand-Williams W and Berset C (1997) Kinetics and mechanisms of antioxidant activity using the DPPH free radical method. *Food Sci Technol* **30**: 609-15.

Boughendjioua H (2019) Characterization of aroma active compounds of cumin (*Cuminum cyminum* L.) seed essential oil. *Mod Appl Bioequiv Availab* **4**(2): 1-5.

Bozin B, Mimica-Dukic N, Samojlik I, Goran A and Igic R (2008) Phenolics as antioxidants in garlic (*Allium sativum* L., Alliaceae). *Food Chem* **111**: 925-29.

Brand-Williams W, Cuvelier M E and Berset C (1995) Use of a free radical method to evaluate antioxidant activity. *Food Sci Technol* 28: 25-30.

Bribi N (2018) Pharmacological activity of alkaloids: A review. *Asian J Bot* 1: 1-7.

Brown J E, Khodr H, Hider R C and Rice-Evans C (1998) Structural dependence of flavonoid interactions with Cu^{2+} ions: implications for their antioxidant properties. *Biochem J* 330: 1173-78

Burre J, Sharma M and Sudhof T C (2003) Definition of molecular pathway mediating alphasynuclein neurotoxicity. *J Neurosci* **35**: 5221-32.

Burt S (2004) Essential oils: Their antibacterial properties and potential applications in foods-A review. *Int J Food Microbiol* **94**: 223-53.

Calabro M L, Galtieri V, Cutroneo P, Tommasini S, Ficarra P and Ficarra R (2004) Study of the extraction procedure by experimental design and validation of a LC method for determination of flavonoids in *Citrus bergamia* juice. *J Pharm Biomed Anal* **35**:349-63.

Chaudhary N, Husain S S and Ali M (2014) Chemical composition and antimicrobial activity of cumin oil (Cuminum cyminum, Apiaceae). *J Pharm Pharma Sci* **3**(7): 1428- 41.

Chaudhry A H, Tanveer A, Shar A, Akhtar M S, Shahid M K, Ashfaq M, Malik T A and Siddiqui R H (2012) Physico-chemical investigation and antimicrobial activity of essential oil of *Cuminum cyminum* L. *World Appl Sci* J **19**(3): 330-33.

Chen Q, Gan Z, Zhao J, Wang Y, Zhang S, Li J and Ni Y (2013) In vitro comparison of antioxidant capacity of cumin (Cuminum cyminum L) oils and their main components. *Food Sci Technol* **55**(2): 632-37.

Choudhary M K, Kataria J and Sharma S (2018) Evaluation of the kinetic and catalytic properties of biogenically synthesized silver nanoparticles. *J Clean Prod* **198**: 882-90.

Cook N C and Samman S (1996) Flavonoids: Chemistry, metabolism, cardio protective effects and dietary sources. *Nutr Biochem* **7**:66-76.

De Martino L, De Feo V, Fratianni F and Nazzaro F (2009) Chemistry, antioxidant, antibacterial and antifungal activities of volatile oils and their components. *Nat Prod Commun* **4**: 1741-50.

Demirci F, Guven K, Demirci B, Dadandi M Y and Baser K H C (2008) Antibacterial activity of two phlomis essential oils against food pathogens. *Food Control* **19**: 1159-64.

Denre M (2014) The determination of vitamin C, total phenol and antioxidant activity of some commonly cooking spices crops used in West Bengal. *Int J Plant Physiol Biochem* **6**(6): 66-70.

Derakhshan S, Sattari M and Bigdeli M (2008) Effect of sub-inhibitory concentrations of cumin (*Cuminum cyminum* L.) seed essential oil and alcoholic extract on the morphology, capsule expression and urease activity of *Klebsiella pneumoniae*. *Int J Antimicrob Agents* **32**: 432-36.

Derakhshan S, Sattari M, Bigdeli M (2010) Effect of cumin (*Cuminum cyminum* L.) seed essential oil on biofilm formation and plasmid integrity by *Klebsiella pneumoniae*. *Pharmacog Mag* **6**: 57-61.

Dimitrios B (2006) Sources of natural phenolic antioxidants. *Trends Food Sci Technol* **17**(9): 505-12.

Dinparvar S, Bagirova M, Allahverdiyev A M, Abamor E S, Safarov T, Aydogdu M and Aktas D (2020) A nanotechnology-based new approach in the treatment of breast cancer: Biosynthesized silver nanoparticles using *Cuminum cyminum* L. seed extract. *J Photoch Photobio B*: https://doi.org/10.1016/j.jphotobiol.2020.111902.

Dipankar C and Murugan S (2012) The green synthesis, characterization and evaluation of the biological activities of silver nanoparticles synthesized from *Iresine herbstii* leaf aqueous extracts. *Colloids* **98**: 112-19.

Dorman H J D and Deans S G (2000) Antimicrobial agents from plants: Antibacterial activity of plant volatile oils. *J Appl Microbiol* **88**: 308-16.

Dubey M, Bhadauria S and Kushwah B S (2009) Green synthesis of nanosilver particles from extract of *Eucalyptus hybrida* (safeda) leaf. *Digest J Nanomat Biostruct* **4**(3): 537-43.

Dubey P N, Saxena S N, Mishra B K, Aishwath O P, Solanki R K, Singh B and Lal G (2016) Assessment of variability in physical and chemical composition of *Cuminum cyminum* seeds from arid and semiarid India. *Indian J Agric Sci* **86**(10): 1366-70.

Dwivedi S K and Dubey N K (1993) Potential use of the essential oil of *Trachyspermum ammi* against seedborne fungi of guar (*Cyamopsis tetragonoloba* L. Taub). *Mycopathologia* **121**(2): 101-04.

Ebrahimzadeh M A, Pourmorad F and Hafezi S (2008) Antioxidant activities of Iranian corn silk **32**:43-49.

Eikani M H, Golmohammad F, Mirza M and Rowshanzamir S (2007) Extraction of volatile oil from cumin (*Cuminum cyminum* L.) with superheated water. *J Food Process Eng* **30**(2): 255-66.

Einafshar S, Poorazrang H, Farhoosh R and Seiedi S M (2012) Antioxidant activity of the essential oil and methanolic extract of cumin seed (*Cuminum cyminum*). *Eur J Lipid Sci Technol* **114**(2): 168-74.

El-Ghorab A H, Nauman M, Anjum F M, Hussain S and Nadeem M (2010) A Comparative Study on Chemical composition and antioxidant activity of ginger (*zingiber officinale*) and cumin (*cuminum cyminum*). *J Agric Food Chem* **58**: 8231-37.

El-Hamidi A and Ahmed S S (1966) The effect of plant age on content and composition of dill essential oil *Anethum graveolens* L. *Pharamazie* **21**: 438-39.

El-Said A H M and El-Hady G (2014) Antifungal activities of *Cuminum cyminum* and *Pimpinella anisum* essential oils. *Int J Curr Microbiol App Sci* **3**(3): 937-44.

Esmaeili F (2015) Composition of essential oil of *Cuminum cyminum*. *J Essent Oil Bear Plan* **18**(2): 507-509.

Farag R S, Daw Z Y and Abo-Raya S H (1989) Influence of some spice essential oils on *Aspergillus parasiticus* growth and production of aflatoxin in a synthetic medium. *J Food Sci* **54**: 74-76.

Farag R S, Daw Z Y, Hewedi F M and El-Baroty G S A (1989) Antimicrobial activity of some Egyptian spice essential oils. *J Food Prot* **52**(9): 665-67.

Farhat G N, Affara N I and Gali-Muhtasib H U (2001) Seasonal changes in the composition of the essential oil extract of East Mediterranean sage (*Salvia libanotica*) and its toxicity in mice. *Toxicon* **39**: 1601-05.

Fenwick D E and Oakenfull D (1983) Saponin content of food plants and some prepared foods. *J Sci Food Agric* **34**(2):186-91.

Figiel A, Szumny A, Gutierrez-Ortiz A and Carbonell-Barrachina A A (2010) Composition of oregano essential oil (*Origanum vulgare*) as affected by drying method. *J Food Eng* **98**: 240-47.

Francis G, Kerem Z, Makkar H P S and Becker K (2002) The biological action of saponins in animal systems: A review. *Brit J Nutr* **88**:587-605.

Friedman M, Henika P R, and Mandrell R E (2002) Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenese*, and *Salmonella enterica*. *J Food Prot* **65**: 1545-60.

Fu M, Xu Y, Chen Y, Wu J, Yu Y, Zou B, An K and Xiao G (2017) Evaluation of bioactive flavonoids and antioxidant activity in *Pericarpium Citri Reticulatae* (*Citrus reticulata* 'Chachi') during storage. *Food Chem* **230**: 649-56.

Fuson R C and Bull B A (1934) The Haloform reaction. *Chem Rev* **15**(3): 275-309.

Gaba J, Sharma S, Arora G, Joshi S and Goyal A (2015) Synthesis and microbial activity of pyrazolines. *J Ind Chem Soc* **92**:1587-93.

Gaba J, Sharma S, Joshi S and Gill P (2018) Gas Chromatography-Mass Spectrometric analysis of essential oil, nutritional and phytochemical composition of ajwain seeds (*Trachyspermum ammi*. L.). *J Essent Oil Bear Pl* 21 (4): 1128-37.

Gaikwad P, Barik A, Priyadarsini K I and Rao B S M (2012) Antioxidant activities of phenols in different solvents using DPPH assay. *Res Chem Intermed* **36**(9): 1065-72.

Gambin Y, VanDelinder V, Ferreon A C, Lemke E A, Groisman A and Deniz A A (2011) Visualizing a one-way protein encounter complex by ultra-fast single-molecule mixing. *Nat Methods* **8**: 239-241.

Geetha S, Sai-Ram M, Mongia S S, Singh V and Ilavazhagan G (2003) Evaluation of antioxidant activity of leaf extract of sea buckthorn (*Hippophae rhamnoides* L.) on chromium (VI) induced oxidative stress in albino rats. *J Ethnopharmacol* **87**: 247-51.

Gohari A R and Saeidnia S (2011) A review on phytochemistry of *cuminum cyminum* seeds and its standards from field to market. *PHCOG J* **3**(25): 1-5.

Grover R K and Moore J D (1962) Toxicometric studies of fungicides against brown rot organisms *Sclerotonia fructicola* and *Sclerotonia laxa*. *Phytopathology* **52**: 876-80.

Hadian J, Ghasemnezhad M, Ranjbar H, Frazane M and Ghorbanpour M (2008) Antifungal potency of some essential oils in control of postharvest decay of strawberry caused by *Botrytis cinerea*, *Rhizopus stolonifer* and *Aspergillus niger*. *J Essent Oil Bear Pl* **11**(5): 553-62.

Hajlaoui H, Mighri H, Noumi E, Snoussi M, Trabelsi N, Ksouri R and Bakhrouf A (2010) Chemical composition and biological activities of Tunisian *Cuminum cyminum* L. essential oil: a high effectiveness against *Vibrio spp*. strains. *Food Chem Toxicol* **48**(9): 2186-92.

Halliwell B and Gutteridge J M C (1998) In free radicals in biology and medicine; Oxford University Press: Oxford.

Hanasaki Y, Ogawa S and Fukui S (1994) The correlation between active oxygens scavenging and antioxidative effects of flavonoids. *Free Rad Biol Med* **16**: 845-850.

Hanif C, Ayesha T, Adila S, Saeed M, Tanveer A and Ashfaq M (2012) Physico-chemical investigation and antimicrobial activity of essential oil of *cuminum cyminum* L. *World app Sci J* **19**(3): 330-33.

Hashum F and Al-Hashemi (2014) Chromatographic separation and identification of some volatile oils, organic acids and phenols from the seeds of *Cuminum cyminum* growing in Iraq. *IJRRAS* **19** (1): 80-90.

Helander I M, Alakomi H L, Latva-Kala K, Sandholm T M, Pol I, Smid E J, Gorris L G M and Wright A V (1998) Characterization of the action of selected essential oil components on gram-negative bacteria. *J Agric Food Chem* **46**: 3590-95.

Holla B S, Mahalinga M, Karthikeyan M S, Akberali P M and Shetty N S (2005) Synthesis of some novel pyrazolo[3,4-*d*]pyrimidine derivatives as potential antimicrobial agents. *Bioorgan Med Chem* **14**(6): 2040-47.

Htwe Y Z N, Chow W S, Suda Y and Mariatti M (2019) Effect of silver nitrate concentration on the production of silver nanoparticles by green method. *Materials Today:*

Proceedings **17**: 568-73.

Iacobellis N S, Cantore P L, Capasso F and Senatore F (2005) Antibacterial activity of *Cuminum cyminum L.* and *Carum carvi L.* essential oils. *J Agric Food Chem* **53**: 57-61.

Irkin R and Korukluoglu M (2009) Growth inhibition of pathogenic bacteria and some yeasts by selected essential oils and survival of *L. monocytogenes* and *C. albicans* in apple-carrot juice. *Foodborne Pathog Dis* **6**(3): 387-94.

Jalali-Heravi, Zekavat M B and Sereshti H (2007) Use of gas chromatography-mass spectrometry combined with resolution methods to characterize the essential oil components of Iranian cumin and caraway. *J Chromatography A* **1143**: 215-26.

Janardhanan R, Karuppaiah M, Hebalkar N, Rao T N (2009) Synthesis and surface chemistry of nano silver particles. *Polyhedron* **28**: 2522-30.

Janssen M A, Scheffer J J C and Svendsen A B (1987) Antimicrobial activities of essential oils: A 1976-86 literature review on possible applications. *Pharmaceutische Weekblad (Scientific Edition)* **9**: 193-97.

Jirovetz L, Buchbauer G, Stoyanova A S, Georgiev E V and Damianova S T (2005) Composition, quality control and antimicrobial activity of the essential oil of cumin (*Cuminum cyminum* L.) seeds from Bulgaria that had been stored for up to 36 years. *Int J Food Sci Tech* **40**: 305-10.

Johnson C B, Kirby J, Naxakis G, Pearson S (1999) Substantial UV-mediated induction of essential oils in sweet basil (*Ocimum basilicum* L.). *Phytochemistry* **51**: 507-10.

Johri R K (2011) *Cuminum cyminum* and *Carum carvi*: An update. *Phcog Rev* **5**: 63-72.

Karamian R and Kamalnejad J (2019) Green synthesis of silver nanoparticles using *Cuminum cyminum* leaf extract and evaluation of their biological activities. Journal of Nanostructures 9: 74-85.

Karbin S, Rad A B, Arouiee H and Jafarn S (2009) Antifungal activities of the essential oils on post-harvest disease agent *Aspergillus flavus*. *Adv Environ Biol* **3**(3): 219-25.

Katti K, Chanda N, Shukla R, Zambre A, Suibramanian T, Kulkarni R R, Kannan R and Katti KV (2009) Green nanotechnology from cumin phytochemicals: Generation of biocompatible gold nanoparticles. *Int J Nanotechnolog: Biomed* **1**(1), 39–52.

Kaur G, Sharma S, Kaur P and Gaba G (2019) Synthesis of some Schiff bases of *o*-phenylenediamine and their antimicrobial activity. *Pestic Res J* **31**(1): 74-80.

Kedia A, Prakash B, Mishra P K and Dubey N K (2014) Antifungal and antiaflatoxigenic properties of *Cuminum cyminum* (L.) seed essential oil and its efficacy as a preservative in stored commodities. *Int J Food Microbiol* **168-169**: 1-7.

Keerthiga N, Anitha R, Kumar S R, Lakshmi T (2019) The aim of the study was to employ cumin oil in the synthesis of silver nanoparticles and check the antioxidant activity of the cumin oil mediated silver nanoparticles. *Pharmacogn J* **11**(4):787-89.

Khanbabaee K and van Ree T (2001) Tannins: Classification and definition. *Nat Prod Rep* **385**(18):641-649.

Kitajima J, Ishikawa T, Fujimatu E, Kondho K and Takayanagi T (2003) Glycosides of 2-C-methyl-D-erythritol from the fruits of anise, coriander and cumin. *Phytochemistry* **62**:115-20.

Koppula S and Choi D K (2011) *Cuminum cyminum* extract attenuates scopolamine-induced memory loss and stress-induced urinary biochemical changes in rats: a noninvasive biochemical approach. *Pharm Biol* **49**(7): 702-08.

Krishna M P and Reddy K H (2016) Synthesis, Structural Characterization and DNA Studies of Nickel (II) Complexes of (2*E*)-^{4}*N*-Substituted-2-[4-(propan-2-yl) Benzylidene]Hydrazinecarbothioamide Schiff's Bases. *J Chem Pharm Res* **8**(10): 61-68.

Kubo I and Kinst-Hori I (1998) Tyrosinase inhibitors from cumin. *J Agric Food Chem* **46**: 5338-41.

Kudle K R, Donda M R, Alwala J, Koyyati R, Nagati V, Merugu R, Prashanthi Y, Rudra1 M P P (2012) Biofabrication of silver nanoparticles using *cuminum cyminum* through microwave irradiation. *Int J Nanomater Biostruct* **2**(4): 65-69.

Kumar P A, Reddy P Y, Srinivas P and Reddy G B (2009) Delay of diabetic cataract in rats by the antiglycating potential of cumin through modulation of α-crystallin chaperone activity. *J Nutr Biochem* **20**: 553−62.

Kumar S and Pandey A K (2013) Phenolic content, reducing power and membrane protective activities of *Solanum xanthocarpum* root extracts. *Vegetos* **26**: 301-07.

Kumar S, Mishra A and Pandey A K (2014) Antioxidant mediated protective effect of *Parthenium hysterophorus* against oxidative damage using *in vitro* models. *BMC Compl Alternative Med***13**: 1-9.

Kumari M, Gupta R, Jain Y (2019) Preparation of a simple biocompatible magnetite@citric acid: An efficient reusable solid acid catalyst for the rapid synthesis of antipyrine Schiff's bases and study of their radical scavenging potential. *Synth Commun* **49**(4): 529-38.

Lako J, Trenerry V, Wahlqvist M, Wattanapenpaiboon N, Sotheeswaran S and Premier R (2007) Phytochemical Flavonols, Carotenoids and the antioxidant properties of a wide selection of fijian fruit, vegetables and other readily available foods. *J Food Chem* **102**: 777-83.

Lee H S (2005) Cuminaldehyde: aldose reductase and α-glucosidase inhibitor derived from *Cuminum cyminum* L. seeds. *J Agric Food Chem* **53:** 2446-50.

Li R and Jiang ZT (2004) Chemical composition of the essential oil of *Cuminum cyminum* L. from China. *Flav Fragr J* **19**: 311-13.

Li X M, Tian S L, Pang Z C, Shi J Y, Feng Z S and Zhang Y M (2009) Extraction of *Cuminum cyminum* essential oil by combination technology of organic solvent with low boiling point and steam distillation. *Food Chem* **115**: 1114-19.

Lim K T, Hu C and Kitts D D (2002) Antioxidant activity of a *Rhus verniciflua* stokes ethanol extract. *Food Chem Toxicol* **39**: 229-37.

Lis-Balchin M and Deans S G (1997) Bioactivity of selected plant against *Listeria*

monocytogenes. J Appl Microbiol 82: 759-62.

Lo Cantore P, Iacobellis N S, De Marco A, Capasso F and Senatore F (2004) Antibacterial activity of *Coriandrum sativum* L. and *Foeniculum vulgare* Millar Var. *Vulgare* (Millar) essential oils. *J Agric Food Chem* **52**: 862-66.

Lopes-Lutz D, Alviano D S, Alviano C S and Kolodziejczyk P P (2008) Screening of chemical composition, antimicrobial and antioxidant activities of *Artemisia* essential oils. *Phytochemistry* **69**: 1732-38.

Mahesh C M, Gowda K P S, Gupta A K (2010) Protective action of Cuminum cyminum against gentamicin- induced nephrotoxicity. *J Pharmacy Res* **3**:753-57.

Mahmoudi H, Rahnama K and Arabkhani M A (2010) Antibacterial effect essential oil and extracts of medicinal plant on the causal agents of bacterial canker and leaf spot on the stone fruit tree. *J Med Plants* **9**(36): 34-42.

Mallavarapu G R, Kulkarni R N, Baskaran K, Rao L and Ramesh S (1999) Influence of plant growth stage on the essential oil content and composition in Davana (*Artemisia pallens* Wall.). *J Agric Food Chem* 47: 254-58.

Mallikarjun K, Narsimha G, Dillip G R, Praveen B, Shreedhar B, Lakshmi S, Reddy V S and Raju D P (2011) Green synthesis of silver nanoparticles using *ocimum* leaf extract and their characterization. *Digest J Nanomat Biostruct* **6**(1): 181-86.

Manuel V, Ruiz-Navajas Y, Fernandez-Lopez J and Perez-Alvarez J A (2008) Antibacterial activity of different essential oils obtained from spices widely used in Mediterranean diet. *Int Food Sci Tech* **43**(3): 526-31.

Marjanlo A A, Mostofi Y, Shoeibi S assnd Fattahi M (2009) Effect of cumin essential oil on postharvest decay and some quality factors of strawberry. *J Med Plants* **8**(31): 25-43.

McCune L M and Johns T (2002) Antioxidant activity in medicinal plants associated with the symptoms of diabetes mellitus used by the indigenous peoples of the North American boreal forest. *J Ethnopharmacol* **82**: 197-205.

Milgate J, and Roberts D C K (1995) The nutritional and biological significance of saponins. *Nutr Res* **15**:1223-49.

Miri Y B and Djenane D (2018) Evaluation of protective impact of Algerian *Cuminum cyminum* L. and *Coriandrum sativum* L. essential oils on *Aspergillus flavus* growth and Aflatoxin B_1 production. *Pak J Biolog Sci* **21**: 66-77.

Mittal A K, Chisti Y and Banerjee U C (2013) Synthesis of metallic nanoparticles using plant extracts. *Biotechnol Adv* **31**:346-56.

Mohammadpour H, Moghimipour E, Rasooli I, Fakoor M H, Astaneh S A, Moosaie S S and Jalili Z (2012) Chemical composition and antifungal activity of *cuminum cyminum* essential oil from alborz mountain against *aspergillus* species. *Jundishapur J Nat Pharm Prod* **7**(2): 50-55.

Morshedi D, Aliakbari F, Tayaranian-Marvian A, Fassihi A, Pan-Montojo F and Sanchez H P (2015) Cuminaldehyde as the major component of *cuminum cyminum*, a natural aldehyde with inhibitory effect on alpha-synuclein fibrillation and cytotoxicity. *J Food Sci* **80**: 1-10.

Nadeem M and Riaz A (2012) Cumin (*cuminum cyminum*) as a potential source of antioxidant. *Pak J Food Sci* **22**(2): 101-07.

Naeini A, Jalayer Naderi N and Shokri H (2014) Analysis and *in vitro* anti-Candida antifungal activity of *Cuminum cyminum* and *Salvadora persica* herbs extracts against pathogenic *Candida strains. J Med Mycol* **24**: 13-18.

Nandi A K, Chaudhuri S, Mazumdar S K and Ghosh S (1984) Effect of chlorine substitution on the structure and activity of 4-phenylthiosemicarbazide: crystal and molecular structure of 4-(4-chlorophenyl)thiosemicarbazide. *J Chem Soc Perkin Trans 1* **2**(11): 1729-33.

Nazeem P A (1995) The spices of India. *The herb, spice, and medicinal plant digest* **13**(1): 1-5.

Ncube N S, Afolayan A J and Okoh A I (2008) Assessment techniques of antimicrobial properties of natural compounds of plant origin: Current methods and future trends. *African J Biotech* **7**: 1797-806.

Oroojalian F, Kasra-Kermanshahi R, Azizi M and Bassami M R (2010) Phytochemical composition of the essential oils from three Apiaceae species and their antibacterial effects on food-borne pathogens. *Food Chem* **120**(3): 765-70.

Ortiz S, Nelson R, Kesternich V, Pérez-fehrmann M, Christen P and Marcourt L (2016) Synthesis and antifungal activity of diaryl hydrazones from 2,4-dinitrophenylhydrazine. *J Chil Chem Soc* **61**(3): 3081-84.

Pala-Paul J, Perez-Alonso M J, Velasco-Neguerela A, Pala-Paul R, Sanz J, Conejero F C O (2001) Seasonal variation in chemical constituents of *Santolina rosmarinifolia* L. ssp. *rosmarinifolia*. *Biochem Syst Ecol* **29:** 663-72.

Pala-Paul J, Usano-Alemany J, Soria A C, Perez-Alonso M, Brophy J J (2008) Essential oil composition of *Erygium campestre* L. growing in different soil types. A preliminary study. *Nat Prod Commun* **3**: 1121-26.

Panche A N, Diwan A D and Chandra S R (2016) Flavonoids: an overview. *J Nutr Sci* **5**: 1-15.

Parashar M, Jakhar M L and Malik C P (2014) A review on biotechnology, genetic diversity in cumin (*Cuminum cyminum*). *Life Sci* **4**(4): 17-34.

Patil A K, Baghel R P S, Nayak S, Malapure C D, Govil K, Kumar D and Yadav P K (2017) Cumin (*Cuminum cyminum*): As a feed additive for livestock. *J Entomol Zool Stud* **5**(3): 365-69.

Peer W A and Langenheim J H (1998) Influence of phytochrome on leaf monoterpene variation in *Satureja douglasii*. *Biochem Syst Ecol* **26:** 25-34.

Pietta P G (2000) Flavonoids as antioxidants. *J Nat Prod* **63**(7): 1035-42.

Pourmorad F, Hosseinimehr S J and Shahabimajd N (2006) Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *Afr J Biotechnol* **5** (11): 1142-45.

Prajapati M, Jayswal S and Maitreya B (2019) Phytochemical screening and comparative

study of antioxidant activity of *Cuminum cyminum* L. and *Nigella sativa* L. *IJSRR* **8**(2): 1356-64.

Priya G, Saravanan K and Renuka C (2012) Medicinal plants with potential antifertility activity- A review of sixteen years of herbal medicine research (1994-2010). *Int J Pharm Tech Res* **4**(1): 481-94.

Rahimi A R, Rokhzadi A, Amini S and Karami E (2013) Effect of salicylic acid and methyl jasmonate on growth and secondary metabolites in *Cuminum cyminum* L. *J Bio Env Sci* **3**(12): 140-49.

Rai N, Yadav S, Verma A K, Tiwari L and Sharma R K (2012) A monographic profile on quality specifications for a herbal drug and spice of commerce- *Cuminum cyminum* L. *Int J Adv Herb Sci Techol* **1**(1): 1-12.

Rajesh K M, Ajitha B, Reddy A K, Suneetha Y, Reddy P S and Ahn C W (2018) A facile bio-synthesis of copper nanoparticles using *Cuminum cyminum* seed extract: antimicrobial studies. *Adv Nat Sci Nanosci Nanotechnol* **9**: 1-7.

Rasheehan N, Iftikhar H, Abdul T, Muhammad T, Moazur R, Sohail H, Mahmood M S, Siddique A B and Iqbal M (2013) Antimicrobial activity of the bioactive components of essential oils from Pakistani spices against Salmonella and other multi-drug resistant bacteria. *BMC Complement Altern Med* **13**: 265-70.

Rathore S S, Saxena S N and Singh B (2013) Potential health benefits of major seed spices. *International J Seed Spices* **3**: 1-12.

Rebey I B, Jabri-Karoui I, Hamrouni-Sellami I, Bourgou S, Limam F and Marzouk B (2012) Effect of drought on the biochemical composition and antioxidant activities of cumin (*cuminum cyminum* L.) seeds. *Ind Crops Prod* **36**: 238-45.

Rebey I B, Zakhama N, Karoui I J and Marzou B (2012) Polyphenol composition and antioxidant activity of cumin (*Cuminum cyminum* L.) seed extract under drought. *J Food Sci* **77**(6): C734-C39.

Rios J L, Recio M C and Villar A (1987) Antimicrobial activity of selected plants employed in the Spanish Mediterranean area. *J Ethnopharmacol* **21**: 139-52.

Romagnol C, Andreotti E, Maietti S, Rai M and Mares D (2010) Antifungal activity of essential oil from fruits of Indian *Cuminum cyminum*. *Pharm Biol* **48**(7): 834-38.

Romeilah R M, Fayed S A and Mahmoud G I (2010) Chemical compositions, antiviral and antioxidant activities of seven essential oils. *Res J Appl Sci* **6**(1): 50-62.

Rostom S A F, Ashr H M A and Abd El Razik H A (2009) Synthesis and biological evaluation of some novel polysubstituted pyrimidine derivatives as potential antimicrobial and anticancer agents. *Arch Pharm Chem Life Sci* **342**: 299-310.

Russo M, Galletti G C, Bocchini P and Carnacini A (1998) Essential oil chemical composition of wild populations of Italian oregano spice (*Origanum vulgare* ssp. *hirtum* (Link) Ietswaart): A preliminary evaluation of their use in chemotaxonomy by cluster analysis. 1. Inflorescences. *J Agric Food Chem* **46**: 3741-46.

Sacchetti G, Maietti S, Muzzoli M, Scaglianti M, Manfredini S and Radice M (2005) Comparative evaluation of 11 essential oils of different origin as functional

antioxidants, antiradicals and antimicrobials in foods. *Food Chem* **91**: 621-32.

Saha S, Walia S, Kundu A, Sharma K, Singh J, Tripathi B and Raina A (2014) Compositional and functional difference in cumin (Cuminum cyminum) essential oil extracted by hydrodistillation and SCFE. *Cogent Food Agric* **2**(1): 1143166-74.

Sanchez-Moreno C, Larrauri J A and Saura-Calixto F (1998) A procedure to measure the antiradical efficiency of polyphenols. *J Sci Food Agric* 76: 270-76.

Santhi K and Sengottuvel R (2016) Qualitative and quantitative phytochemical analysis of *Moringa concanensis* Nimmo. *Int J Curr Microbiol App Sci* **5**(1): 633-40.

Santos-Gomes P C and Fernandes-Ferreira M (2001) Organ and season-dependent variation in the essential oil composition of *Salvia officinalis* cultivated at two different sites. *J Agric Food Chem* **49**: 2908-16.

Sapnakumari M, Narayana B, Gurubasavaraj swamy P M and Sarojin B K (2015) Design, synthesis and pharmacological evaluation of new pyrazoline derivatives. Monatsh Chem **146**: 1015-24.

Saraf S D (1983) Reaction of thionyl bromide with aromatic aldehydes. *Synth Commun* **13**(1): 7-14.

Satta M, Tuberoso C I G, Angioni A, Pirisi F M and Cabras P (1999) Analysis of the essential oil of *Helichrysum italicum* G. Don. ssp. *microphyllum* (willd.) Nym. *J Essent Oil Res* **11**: 711-15.

Sawant R S and Godghate A G (2013) Qualitative phytochemical screening of rhizomes of *Curcuma longa* Linn. *Int J Sci Env Technol* **2(**4):634-41.

Sekine T, Sugano M, Majid A and Fujii Y (2007) Antifungal effects of volatile compounds from black zira (*Bunium persicum*) and other spices and herbs. *J Chem Ecol* **33**: 2123-32.

Senevirathne M, Kim S, Siriwardhana N, Ha J, Lee K and Jeon Y (2006) Antioxidant potential of Ecklonia cava on reactive oxygen species scavenging metal chelating, reducing power and lipid peroxidation inhibition. *Food Sci Technol Int* **12**: 27-38.

Shahidi F, Janitha P K and Wanasundara P D (1992) Phenolic antioxidants. *Crit Rev Food Sci Nutr* **32**(1): 67-103.

Shaik M R, Khan M, Kuniyil M, Al-Warthan A, Alkhathlan H Z, Siddiqui M R H, Shaik J P, Ahamed A, Mahmood A, Khan M and Adil A F (2018) Plant extract assisted green synthesis of silver nanoparticles using *Origanum vulgare* L.extract and their microbicidal activities. *Sustainability* **10**: 1-13.

Shalaby T I, Shams El-Dine R S and Abd El-Gaber S A (2015) Green synthesis of gold nanoparticles using cumin seeds and gum arabic: studying their photothermal efficiency. *Nanosci Nanotechnol* **5**(4): 89-96.

Sharma M, Pathak M, Roy B, Chand A, Dhanda G, Abbasi N and Panchal G (2016) Green synthesis of gold nanoparticles and their characterization. *J Mat Nanosci* **3**(1): 8-10.

Shayegh S, Rasooli I, Taghizadeh M and Astaneh S D (2008) Phytotherapeutic inhibition of supragingival dental plaque. *Nat Prod Res* **22**(5): 428-39.

Sheikh M I, Islam S, Rahman A, Rahman M, Rahman M, Raman M and Rahim A (2010) Control of some human pathogenic bacteria by seed extracts of cumin (*Cuminum cyminum* L.). *Agric Conspec Sci* **75**: 39-44.

Shimoi K, Masuda S, Shen B, Furugori M and Kinze N (1996) Radioprotective effects of antioxidative plant flavonoids in mice. *Mutat Res Fund Mol* **350**:153-61.

Shirke S S, Jadhav S R and Jagtap A G (2008) Methanolic extract of *Cuminum cyminum* inhibits ovariectomy-induced bone loss in rats. *Exp Biol Med* **233**: 1403-10.

Shu-meiang, Tsai K, Wong H, Liu Y, Chen T, Cherng J, Hsu K, Ang Y and Cherng J (2016) Molecular mechanism of *Cinnamomum verum* component cuminaldehyde inhibits cell growth and induces cell death in human lung squamous cell carcinoma NCI-H520 cells *in vitro* and *in vivo*. *J Cancer* **7**(3): 251-61.

Singh A, Jain D, Upadhyay M K, Khandelwal N and Verma H N (2010) Green synthesis of silver nanoparticles using argemone mexicana leaf extract and evaluation of their antimicrobial activities. *Dig J Nanomater and Biostruct* **5**(2): 483-89.

Singh G, Kapoor I P S, Pandey S K, Singh U K, Singh R K (2002) Antibacterial activity of volatile oils of some spices. *Phytother Res* **16**: 680-82.

Singh S K, Patel J R, Dubey P K and Thakur S (2015) A review on antiasthmatic activity of traditional medicinal plants. *Int J Pharma Sci Res* **15**: 1-11.

Sinha S N and Paul D (2014) Eco-friendly green synthesis and spectrophotometric characterization of silver nanoparticles synthesized using some common indian spices. *IJGHC* **3**(2): 401-08.

Sneha K, Sathishkumar M, Lee S Y, Bae M A and Yun Y S (2011) Biosynthesis of Au nanoparticles using cumin seed powder extract. *J Nanoscie Nanotechnol* **11**: 1811-14.

Soobrattee M A, Neergheen V S, Luximon-Ramma A, Aruoma O I and Bahorun O T (2005) Phenolics as potential antioxidant therapeutic agents: Mechanism and actions. *Mutat Res Fund Mol* **579**:200-13.

Srinivasan K (2018) Cumin (*Cuminum cyminum*) and black cumin (*Nigella sativa*) seeds: traditional uses, chemical constituents, and nutraceutical effects. *Food Qual Saf* **2**(1): 1-16.

Stefanini M B, Figueiredo R O, Ming L C and Junior A F (2003) Antimicrobial activity of the essential oils of some spice herbs. *Acta Horticulturae* **597**: 215-16.

Suleimana M M, McGaw L J, Naidoo V and Eloff J N (2009) Detection of antimicrobial compounds by bioautography of different extracts of leaves of selected South African tree species. *Afr J Tradit Complement Altern Med* 7(1): 64-78.

Swain J and Hillis E (1959) The phenolic constituents of *Prunus domestica*. The quantitative analysis of phenolic constituents. *J Sci Food Agric* **10**:63-68

Tabbasum I, Siddiqui Z N and Rijvi SJ (2010) Effect of *Ocimum sanctum* and *Camellia sinensis* on stress induced anxiety and depression in male albino *Rattus norvegicus*. *Ind J Pharm* **42**: 283-88.

Takayanagi T, Ishikawa T and Kitajima J (2003) Sesquiterpene lactone glucosides and alkyl

glycosides from the fruit of cumin. *Phytochemistry* **63**: 479-84.

Tamuly C, Hazarikaa M, Borah S, Das M R and Boruah M P (2013) *In situ* biosynthesis of Ag, Au and bimetallic nanoparticles using *Piper pedicellatum* C.DC: green chemistry approach. *Colloids Surf., B Biointerfaces* **102**: 627-34.

Tamura S, Kubata B K, Itagaki S, Horii T, Taba M K and Murakami N (2010) New anti-malarial phenylpropanoid conjugated iridoids from Morinda morindoides. *Bioorg Med Chem Lett* **20**: 1520-23.

Tavakoli H R, Mashak Z, Moradi B and Sodagan H (2015) Antimicrobial activities of the combined use of *cuminum cyminum* L. essential oil, nisin and storage temperature against *Salmonella typhimurium* and *Staphylococcus aureus in vitro. Jundishapur J Microbiol* **8**(4): 1-12.

Teixeira B, Marques A, Ramos C, Serrano C, Matos O, Neng N R, Nogueira J M F, Saraiva J A and Nunes M L (2013) Chemical composition and bioactivity of different oregano (*Origanum vulgare*) extracts and essential oil. *J Sci Food Agric* **93**(11): 2707-14.

Tepe B, Donmez E, Unlu M, Candan F, Daferera D and Vardar-Unlu G (2004) Antimicrobial and antioxidative activities of the essential oils and methanol extracts of *Salvia cryptantha* and *Salvia multicaulis*. *Food Chem* **84**: 519-25.

Tiwari P, Kumar B, Kaur M, Kaur G and Kaur H (2011) Phytochemical screening and extraction: A review. *International Pharmaceutica Sci* **1**:98-106.

Toroglu S (2011) *In-vitro* antimicrobial activity and synergistic/antagonistic effect of interactions between antibiotics and some spice essential oils. *J Environ Biol* **32**(1): 23-29.

Tsai K, Liu Y, Chen T, Yang S, Wong H, Cherng J, Chou K S and Cherng J M (2016) Cuminaldehyde from *Cinnamomum verum* induces cell death through targeting topoisomerase 1 and 2 in human colorectal adenocarcinoma COLO 205 cells. *Nutrients* **8**(6): 318-35.

Tziveleka L A, Kourounakis A P N, Rousis V and Vagias C (2002) Antioxidant potential of natural and synthesized polyprenylated hydroquinone. *Bioorganic Med Chem* **10**: 935-39.

Ursini F, Maiorino M, Morazzoni P, Roveri A and Pifferi G (1994) A novel antioxidant flavonoid (IdB 1031) affecting molecular mechanisms of cellular activation. *Free Rad Biol Med* **16**: 547-53.

Vallverdu-Queralt A, Regueiro J, Martínez-Huelamo M, Rinaldi Alvarenga J F, Leal L N and LamuelaRaventos R M (2014) A comprehensive study on the phenolic profile of widely used culinary herbs and spices: rosemary, thyme, oregano, cinnamon, cumin and bay. *Food Chem* **154**: 299-07.

Varo P T and Heinz D E (1970) Identification and characterization of 1,4-*p*-Menthadien-7-a1 isolated from cumin seeds. *J Agr Food Chem* **18**(2): 239-42.

Wanasundara U N and Shahidi F (1998) Antioxidant and pro-oxidant activity of green tea extracts in marine oils. *Food Chem* **63**(3): 335-42.

Wanner J, Bail S, Jirovetz L, Buchbauer G, Schmidt E, Gochev V, Girova T, Atanasova T

and Stoyanova A (2010) Chemical composition and antimicrobial activity of cumin oil (*Cuminum cyminum*, Apiaceae). *Nat Prod Commun* **5**(9): 1355-58.

Woguem V, Fogang H P, Maggi F, Tapondjou L A, Womeni H M, Quassinti L, Bramucci M, Vitali L A, Petrelli D, Lupidi G, Papa F, Vittori S and Barboni L (2014) Volatile oil from striped African pepper (*Xylopia parviflora*, Annonaceae) possesses notable chemopreventive, anti-inflammatory and antimicrobial potential. *Food Chem* **149**: 183-89.

Yingming P, Ping L, Hengshan W and Min L (2004) Antioxidant activities of several chinese medicinal herbs. *Food Chem* **88**: 347-50.

Youdin K A, Dorman H J D and Deans S G (1999) The antioxidant effectiveness of thyme oil, α-tocopherol and ascorbyl palmitate on evening primrose oil oxidation. *J Essent Oil Res* **11**: 643-48.

Zare E, Pourseyedi S, Khatami M and Darezereshki E (2017) Simple biosynthesis of zinc oxide nanoparticles using nature's source, and it's *in vitro* bio-activity. *J Mol Struct* **1146**: 96-103.

Zhang H Y and Wang L F (2002) Theoretical elucidation on structure-antioxidant activity relationships for indolinonic hydroxylamines. *Bioorg Med Chem Lett* **12**: 225-27.

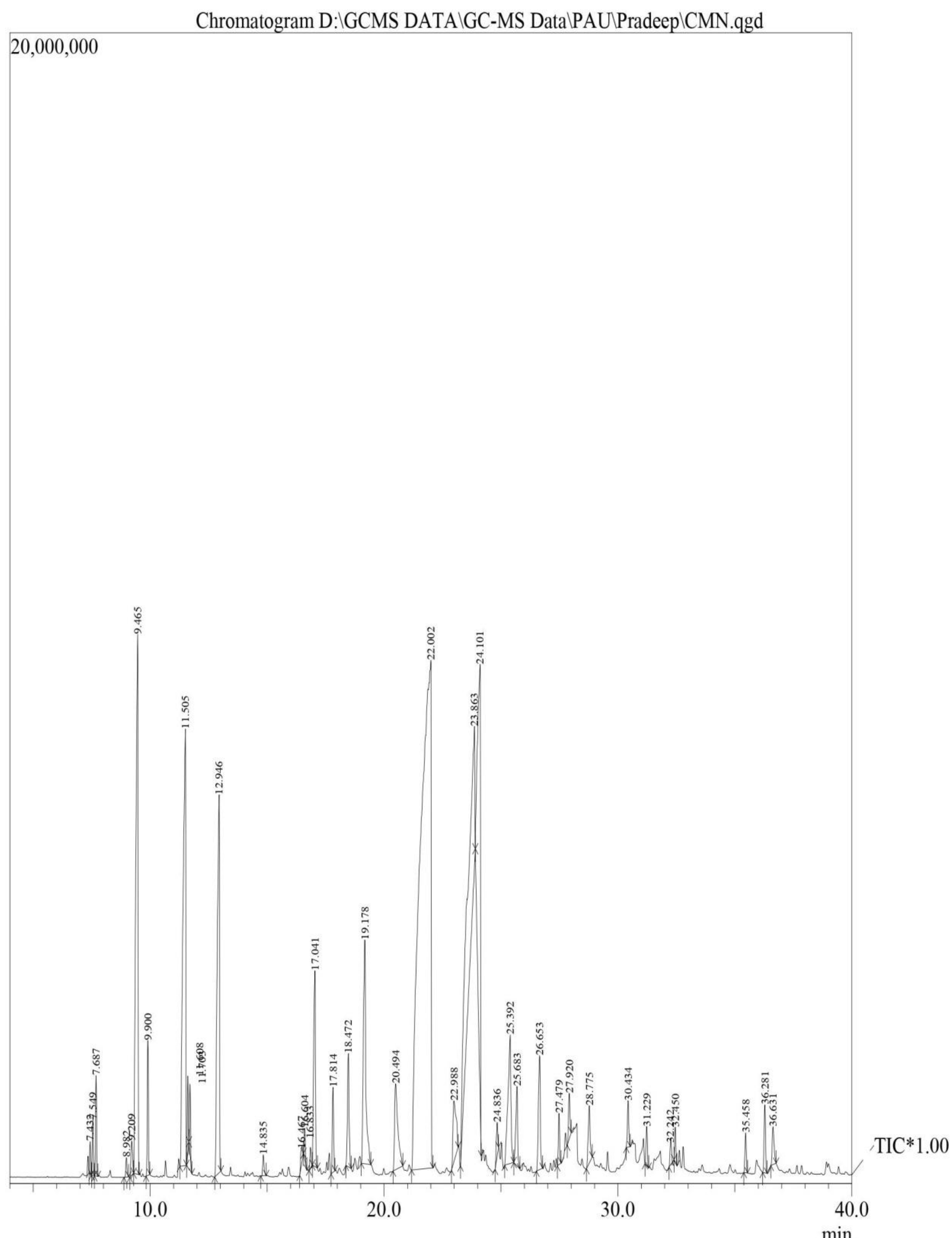
Zhang L L, Feng S and Xu J (2014) Profile of phytochemicals and antioxidant activities of different solvent extracts of cumin seeds. *Appl Mech Mater* **675-677**: 1612-1616.

Zhao Y, Wang P, Zheng W, Yu G, Li Z, She Y and Lee M (2016) Three-stage microwave extraction of cumin (*Cuminum cyminum* L.) Seed essential oil with natural deep eutectic solvents. *Ind Crop Prod* **140**: 111660-70.

Zheljazkov V D, Gawde A, Cantrell C L, Astatkie T and Schlegel V (2015) Distillation time as tool for improved antimalarial activity and differential oil composition of cumin seed oil. *Plos One* **10**(12): e0144120-32.

Sample Informatior

Analyzed by	: $Admn.$
Analyzed	: 9/27/2017 1:44:01 PM
Sample Type	: $Organic$
Sample Name	: CMN
Method File	: D:\GCMS Method\Method\Essential oil.qgn

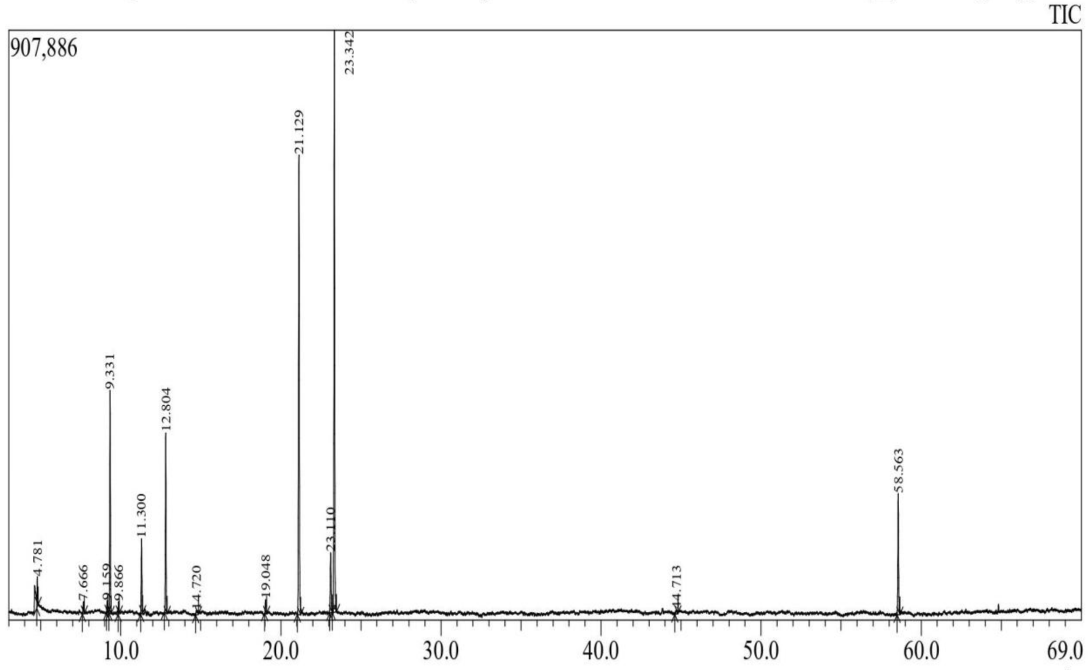


GC-MS of essential oil

Sample Information

Analyzed by	: Admin
Analyzed	: 03-Feb-20 9:36:06 PM
Sample Type	: Unknown
Level #	: 1
Sample Name	: CMNACETONE
Sample ID	:
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 18
Injection Volume	: 1.00
Data File	: C:\Users\hp\Desktop\GCMS data 2020\feb 2020\CMNACETONE__03-Feb-20_12.qgd
Org Data File	: C:\Users\hp\Desktop\GCMS data 2020\feb 2020\CMNACETONE__03-Feb-20_12.qgd
Method File	: C:\Users\hp\Documents\EO.qgm
Org Method File	: C:\Users\hp\Documents\EO.qgm
Report File	:
Tuning File	: C:\Users\hp\Desktop\Toshvin_Training2020\Training_F1_CID ON_06012020.qgt
Modified by	: Admin
Modified	: 28-Feb-20 10:18:15 AM

Chromatogram CMNACETONE C:\Users\hp\Desktop\GCMS data 2020\feb 2020\CMNACETONE__03-Feb-20_12.qgd

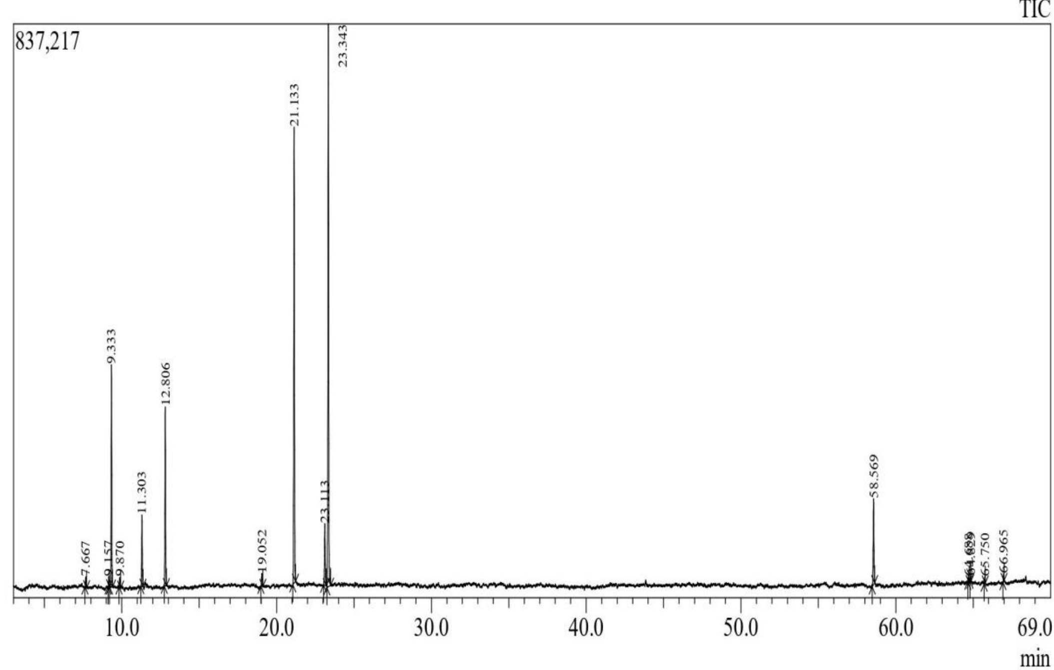


GC-MS of acetone extract

Sample Information

Analyzed by	: Admin
Analyzed	: 04-Feb-20 1:33:15 AM
Sample Type	: Unknown
Level #	: 1
Sample Name	: CMNPETETHER
Sample ID	:
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 21
Injection Volume	: 1.00
Data File	: C:\Users\hp\Desktop\GCMS data 2020\feb 2020\CMNPETETHER__03-Feb-20_15.qgd
Org Data File	: C:\Users\hp\Desktop\GCMS data 2020\feb 2020\CMNPETETHER__03-Feb-20_15.qgd
Method File	: C:\Users\hp\Documents\EO.qgm
Org Method File	: C:\Users\hp\Documents\EO.qgm
Report File	:
Tuning File	: C:\Users\hp\Desktop\Toshvin_Training2020\Training_F1_CID ON_06012020.qgt
Modified by	: Admin
Modified	: 04-Feb-20 11:30:24 AM

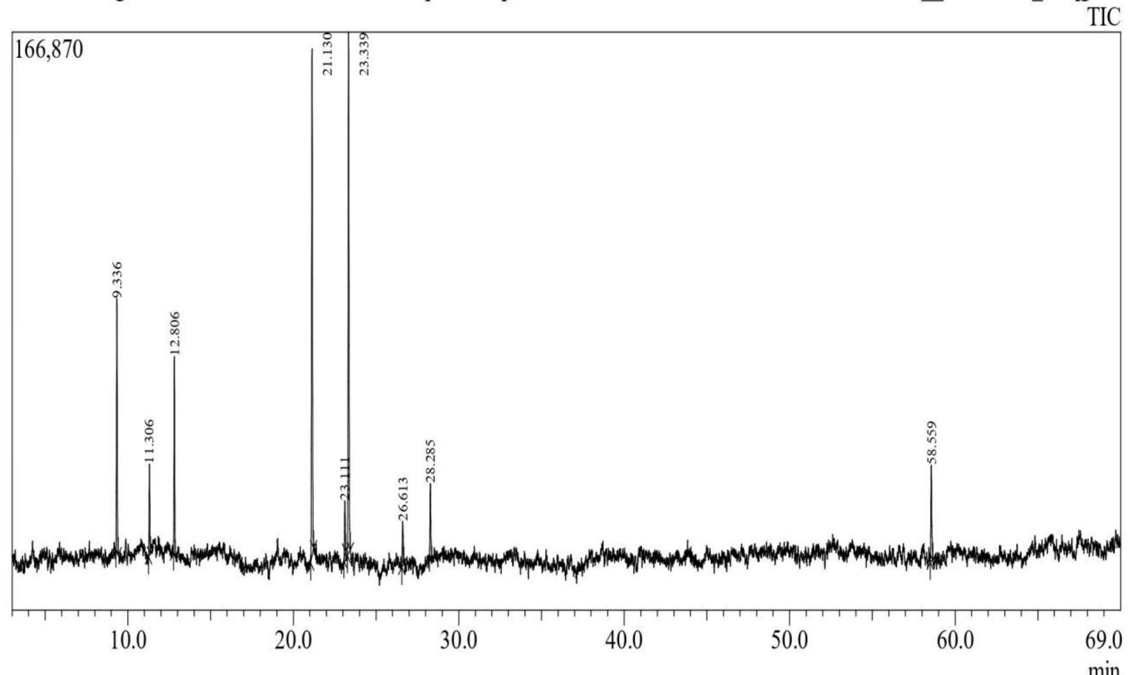
Chromatogram CMNPETETHER C:\Users\hp\Desktop\GCMS data 2020\feb 2020\CMNPETETHER__03-Feb-20_15.qgd



GC-MS of petroleum ether

Sample Information

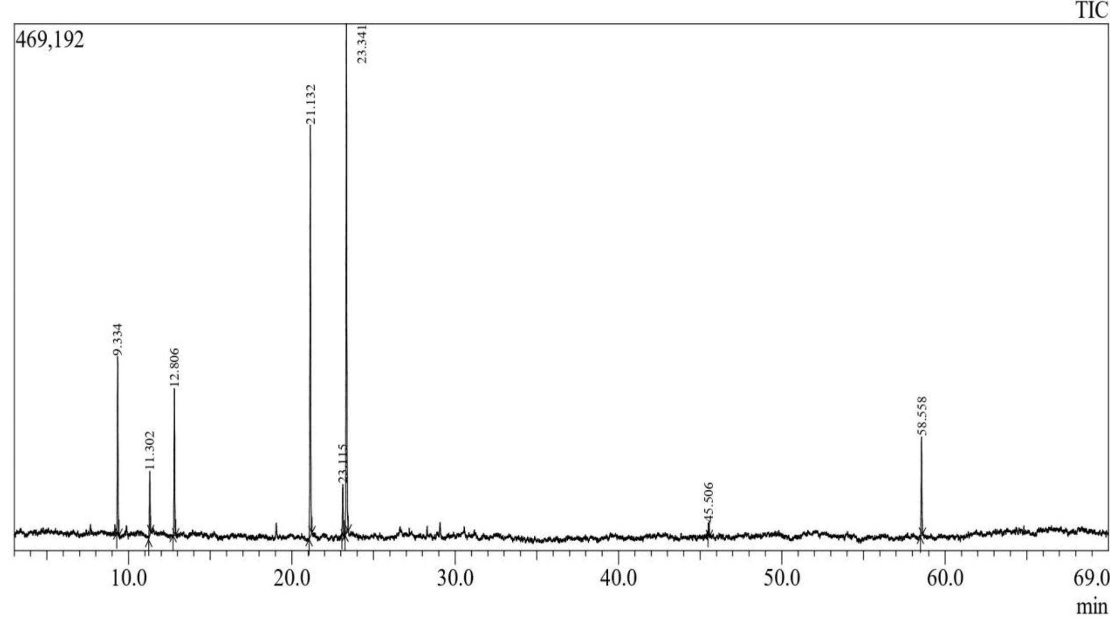
Analyzed by	: Admin
Analyzed	: 04-Feb-20 12:14:13 AM
Sample Type	: Unknown
Level #	: 1
Sample Name	: CMNMETHANOL
Sample ID	:
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 20
Injection Volume	: 1.00
Data File	: C:\Users\hp\Desktop\GCMS data 2020\feb 2020\CMNMETHANOL__03-Feb-20_14.qgd
Org Data File	: C:\Users\hp\Desktop\GCMS data 2020\feb 2020\CMNMETHANOL__03-Feb-20_14.qgd
Method File	: C:\Users\hp\Documents\EO.qgm
Org Method File	: C:\Users\hp\Documents\EO.qgm
Report File	:
Tuning File	: C:\Users\hp\Desktop\Toshvin_Training2020\Training_F1_CID ON_06012020.qgt
Modified by	: Admin
Modified	: 04-Feb-20 11:33:17 AM



GC-MS of methanol

Sample Information

Analyzed by	: Admin
Analyzed	: 03-Feb-20 10:55:08 PM
Sample Type	: Unknown
Level #	: 1
Sample Name	: CMNMETHNOL
Sample ID	:
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 19
Injection Volume	: 1.00
Data File	: C:\Users\hp\Desktop\GCMS data 2020\feb 2020\CMNMETHNOL__03-Feb-20_13.qgd
Org Data File	: C:\Users\hp\Desktop\GCMS data 2020\feb 2020\CMNMETHNOL__03-Feb-20_13.qgd
Method File	: C:\Users\hp\Documents\EO.qgm
Org Method File	: C:\Users\hp\Documents\EO.qgm
Report File	:
Tuning File	: C:\Users\hp\Desktop\Toshvin_Training2020\Training_F1_CID ON_06012020.qgt
Modified by	: Admin
Modified	: 04-Feb-20 11:35:42 AM

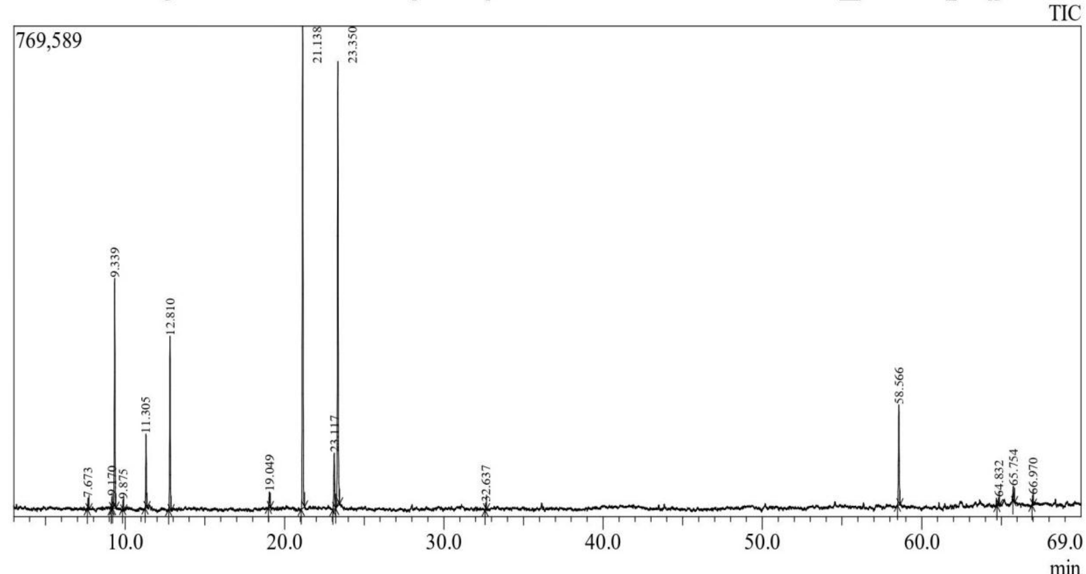


GC-MS of ethanol

Sample Information

Analyzed by	: Admin
Analyzed	: 04-Feb-20 5:30:20 AM
Sample Type	: Unknown
Level #	: 1
Sample Name	: CMNCHCL3
Sample ID	:
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 24
Injection Volume	: 1.00
Data File	: C:\Users\hp\Desktop\GCMS data 2020\feb 2020\CMNCHCL3__03-Feb-20_18.qgd
Org Data File	: C:\Users\hp\Desktop\GCMS data 2020\feb 2020\CMNCHCL3__03-Feb-20_18.qgd
Method File	: C:\Users\hp\Documents\EO.qgm
Org Method File	: C:\Users\hp\Documents\EO.qgm
Report File	:
Tuning File	: C:\Users\hp\Desktop\Toshvin_Training2020\Training_F1_CID ON_06012020.qgt
Modified by	: Admin
Modified	: 04-Feb-20 11:25:35 AM

Chromatogram CMNCHCL3 C:\Users\hp\Desktop\GCMS data 2020\feb 2020\CMNCHCL3__03-Feb-20_18.qgd

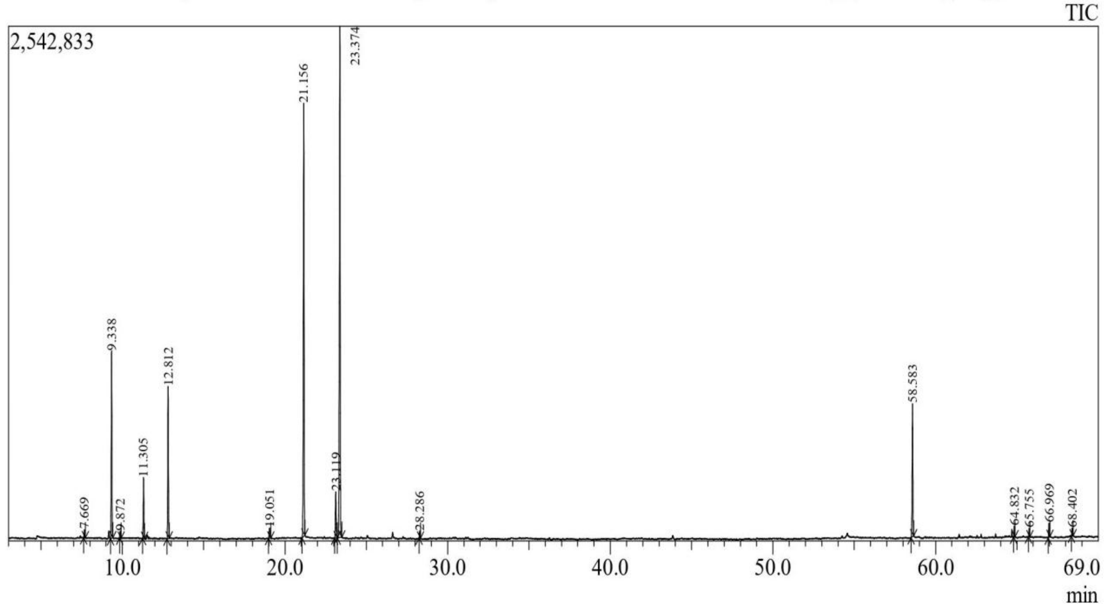


GC-MS of chloroform

Sample Information

Analyzed by	: Admin
Analyzed	: 04-Feb-20 4:11:21 AM
Sample Type	: Unknown
Level #	: 1
Sample Name	: CMNDCM
Sample ID	:
IS Amount	: [1]=1
Sample Amount	: 1
Dilution Factor	: 1
Vial #	: 23
Injection Volume	: 1.00
Data File	: C:\Users\hp\Desktop\GCMS data 2020\feb 2020\CMNDCM__03-Feb-20_17.qgd
Org Data File	: C:\Users\hp\Desktop\GCMS data 2020\feb 2020\CMNDCM__03-Feb-20_17.qgd
Method File	: C:\Users\hp\Documents\EO.qgm
Org Method File	: C:\Users\hp\Documents\EO.qgm
Report File	:
Tuning File	: C:\Users\hp\Desktop\Toshvin_Training2020\Training_F1_CID ON_06012020.qgt
Modified by	: Admin
Modified	: 04-Feb-20 11:22:57 AM

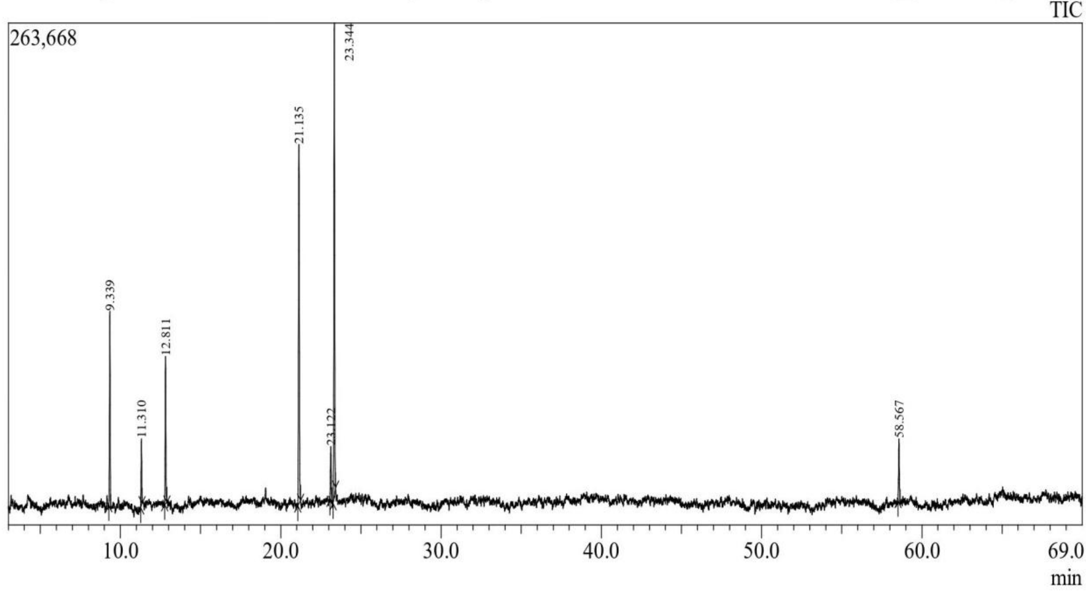
Chromatogram CMNDCM C:\Users\hp\Desktop\GCMS data 2020\feb 2020\CMNDCM__03-Feb-20_17.qgd



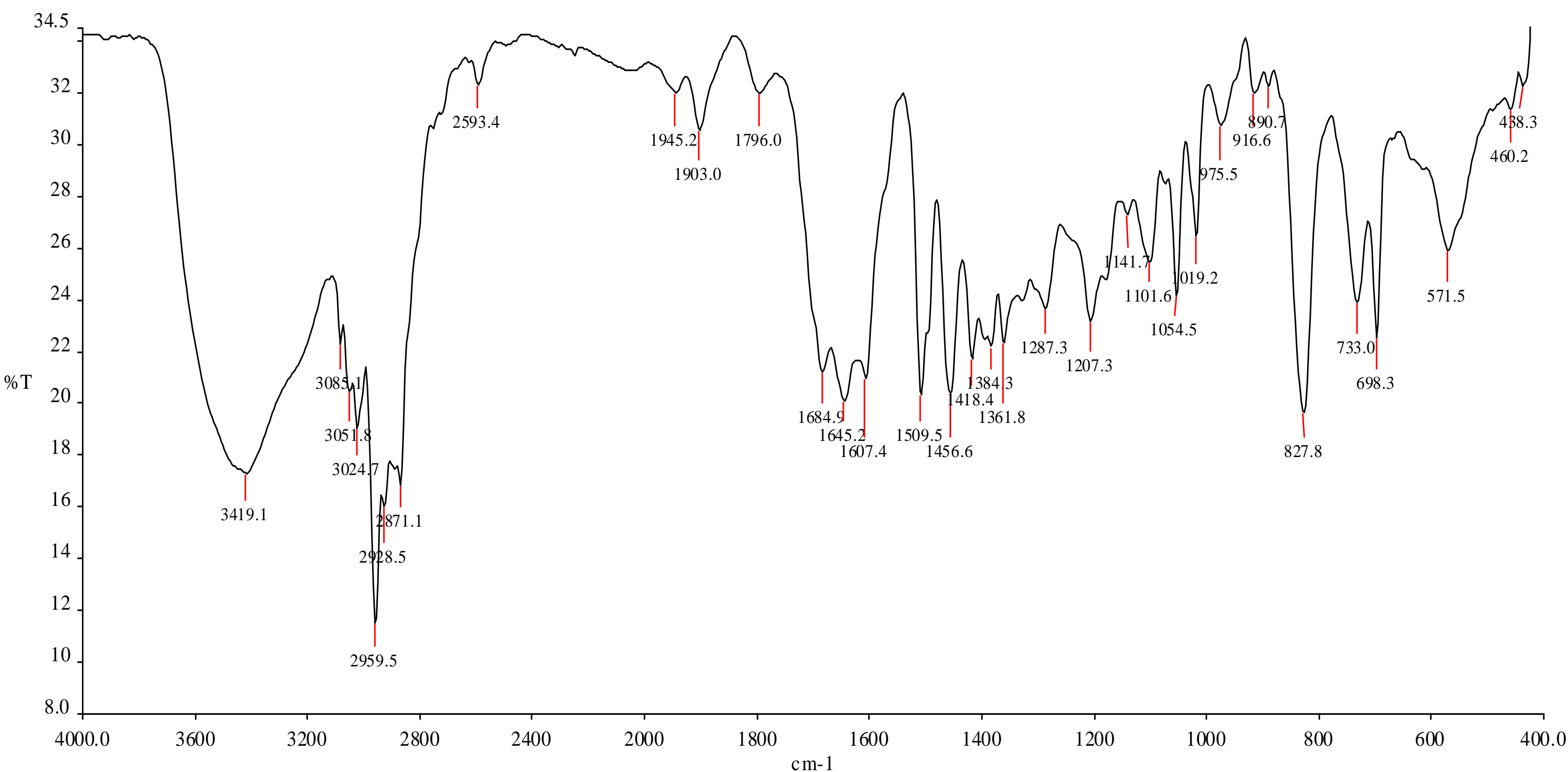
GC-MS of dichloromethane

Sample Information

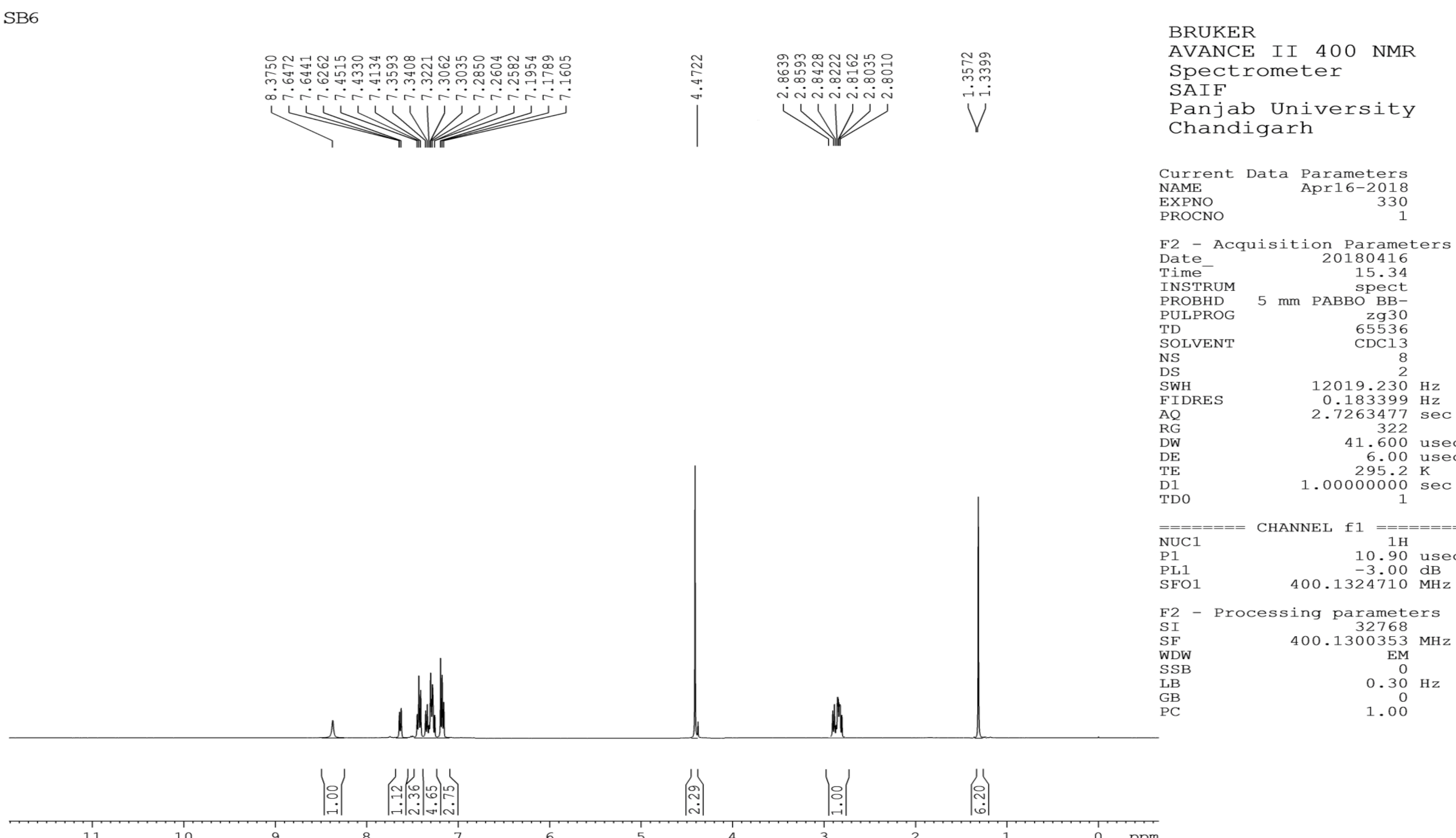
Analyzed by : Admin
Analyzed : 04-Feb-20 2:52:16 AM
Sample Type : Unknown
Level # : 1
Sample Name : CMNETHYLACET
Sample ID :
IS Amount : [1]=1
Sample Amount : 1
Dilution Factor : 1
Vial # : 22
Injection Volume : 1.00
Data File : C:\Users\hp\Desktop\GCMS data 2020\feb 2020\CMNETHYLACET__03-Feb-20_16.qgd
Org Data File : C:\Users\hp\Desktop\GCMS data 2020\feb 2020\CMNETHYLACET__03-Feb-20_16.qgd
Method File : C:\Users\hp\Documents\EO.qgm
Org Method File : C:\Users\hp\Documents\EO.qgm
Report File :
Tuning File : C:\Users\hp\Desktop\Toshvin_Training2020\Training_F1_CID ON_06012020.qgt
Modified by : Admin
Modified : 04-Feb-20 11:20:47 AM



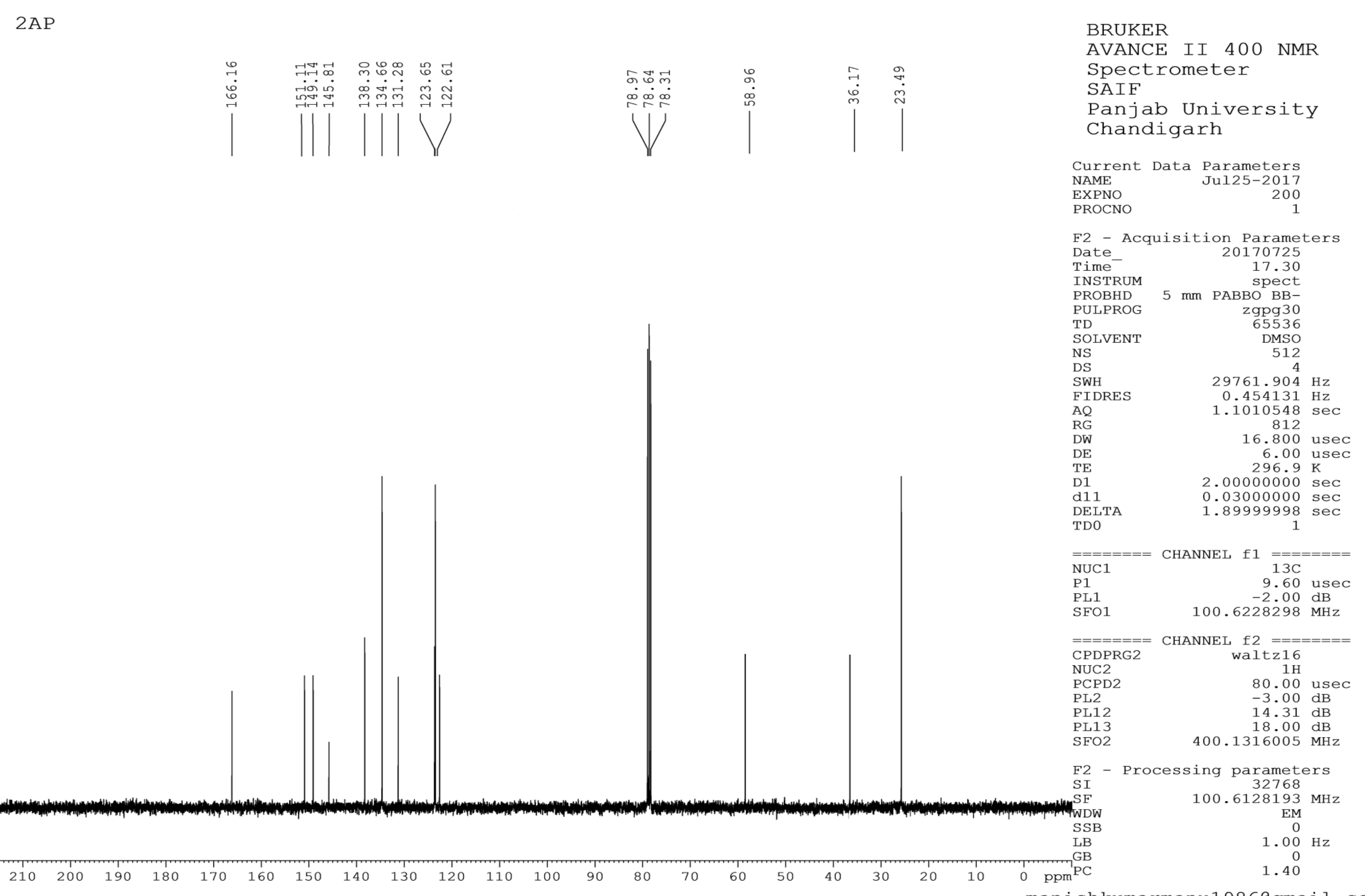
GC-MS of ethylacetate



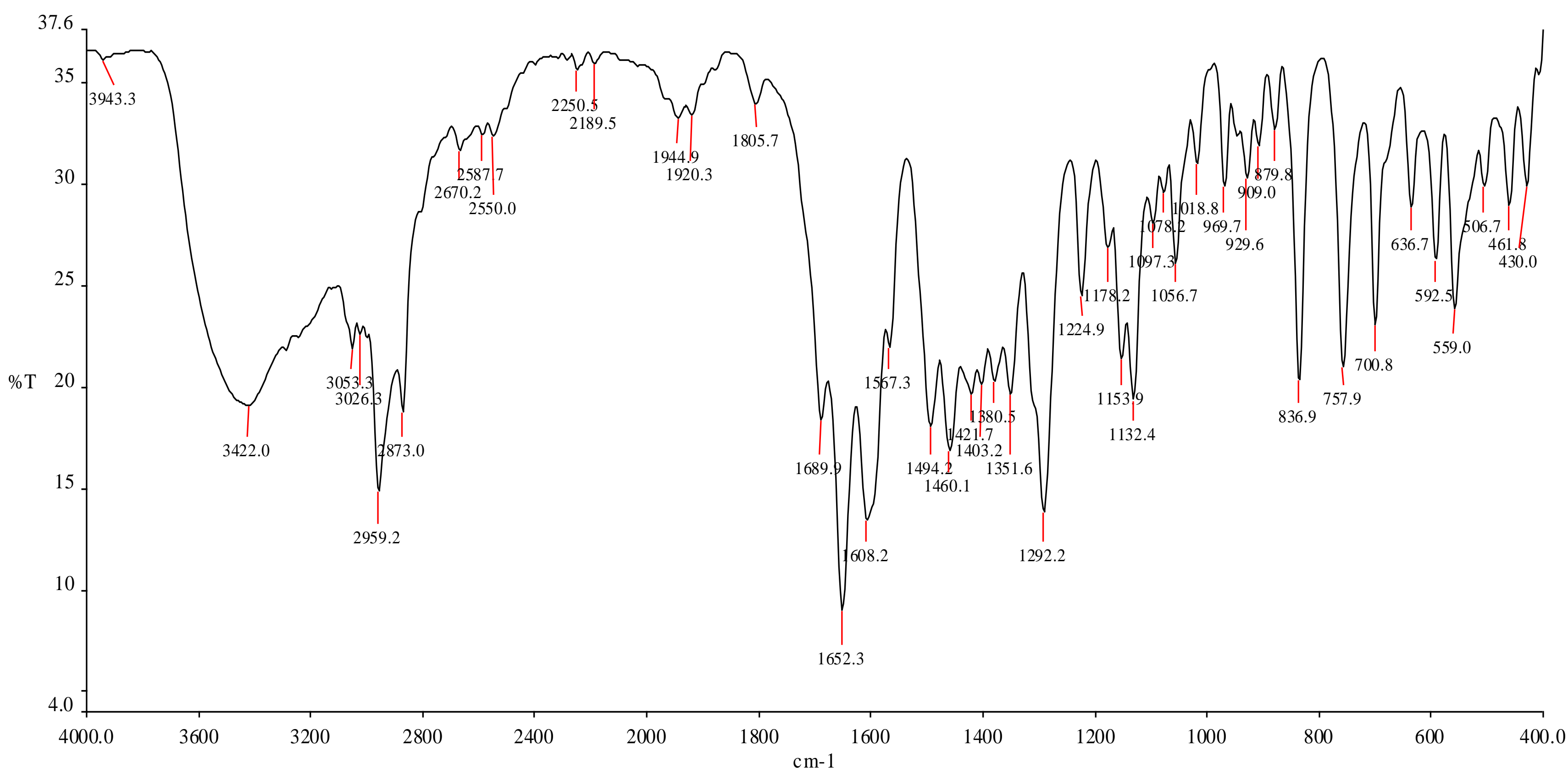
FT-IR of compound V



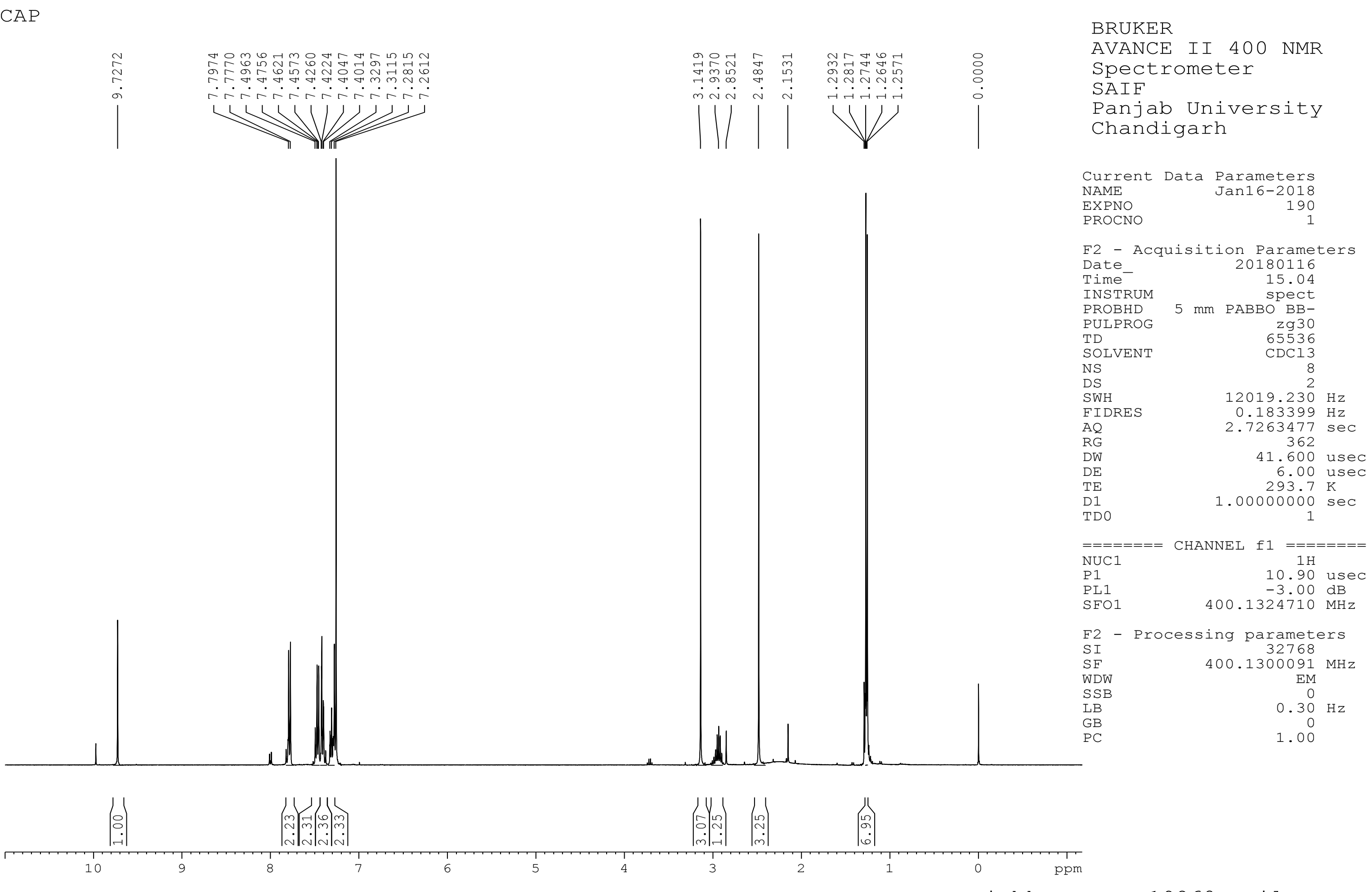
^{1}H NMR of compound V



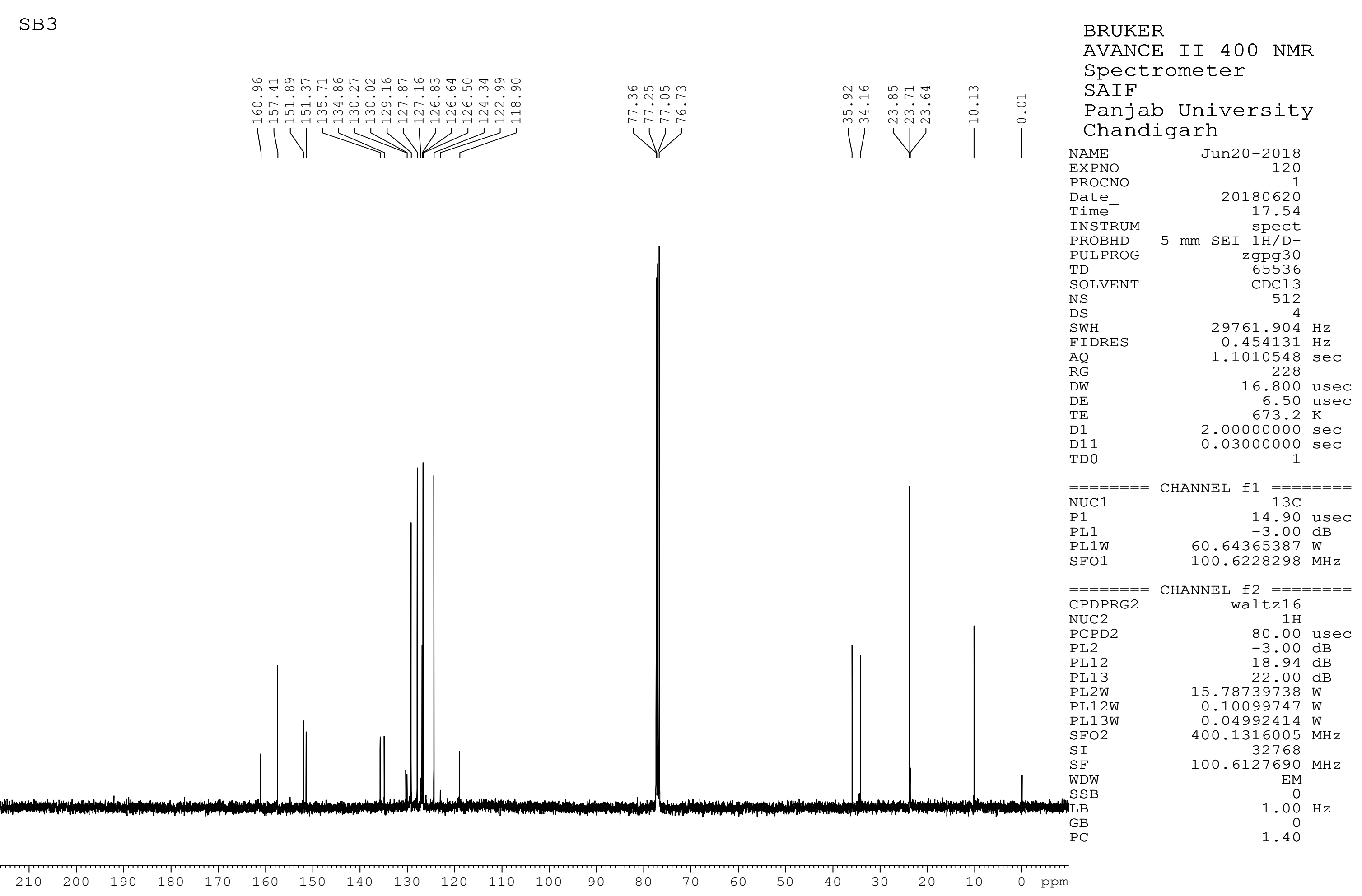
^{13}C NMR of compound V



FT-IR of compound X

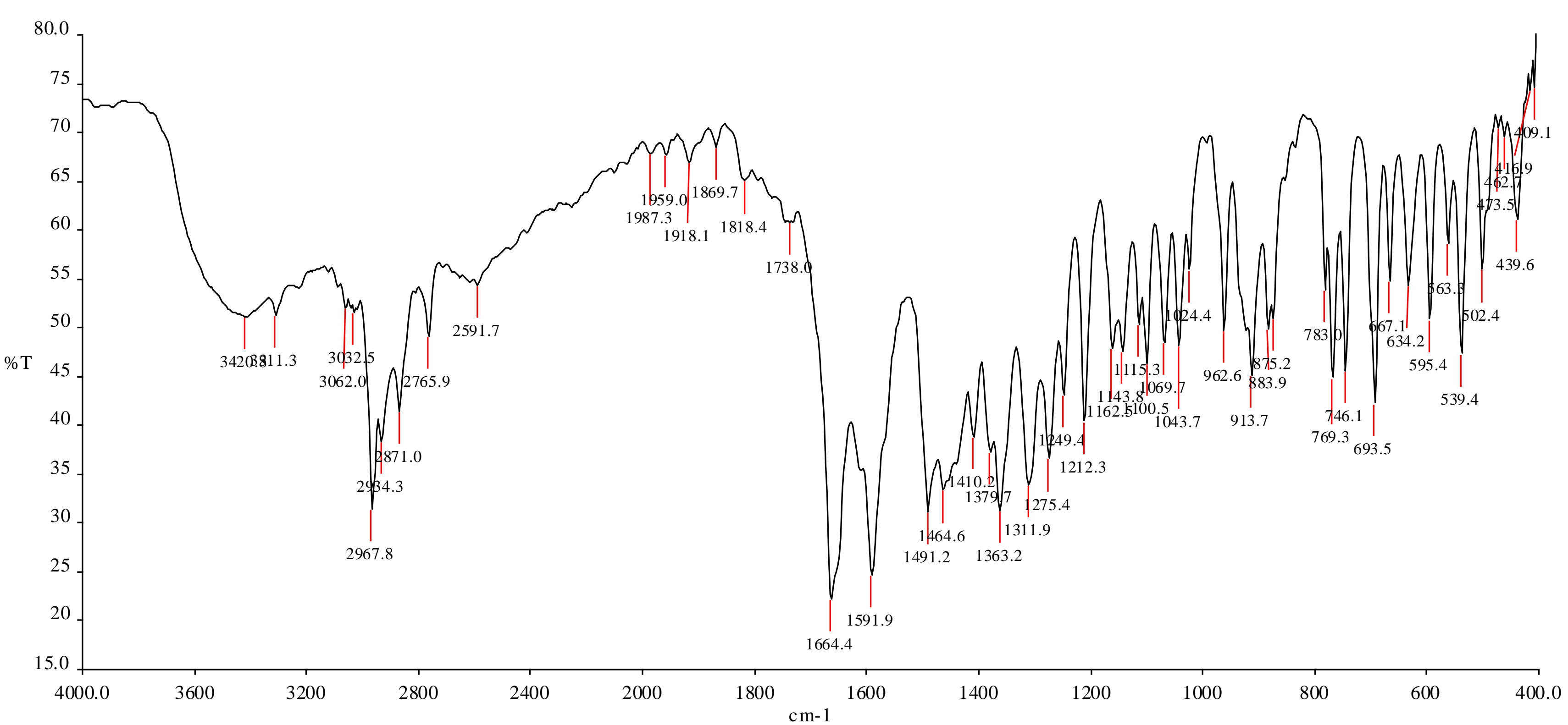


^{1}H NMR of compound X

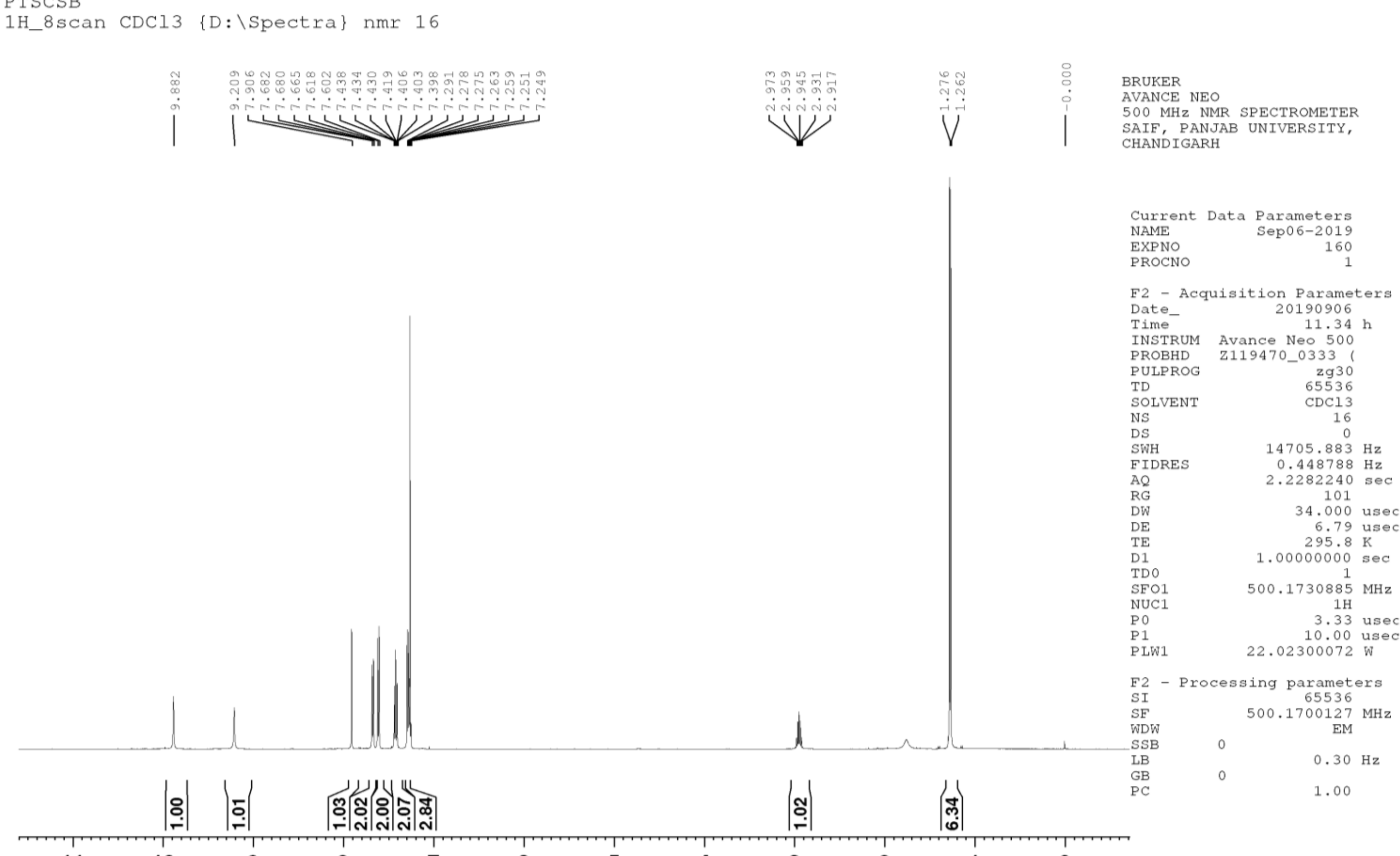


^{13}C NMR of compound X

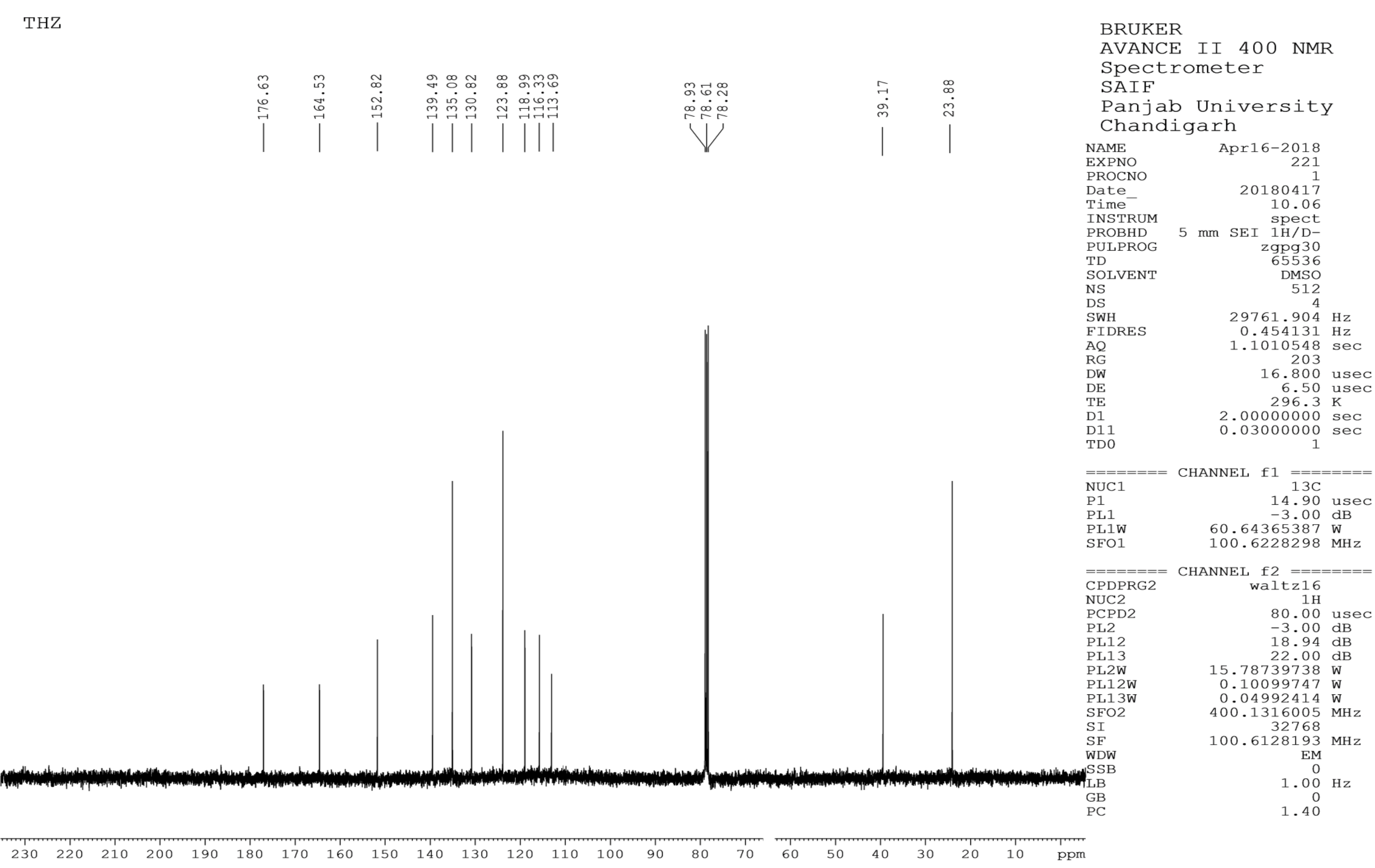
RC SAIF PU, Chandigarh



FT-IR of compound XIV

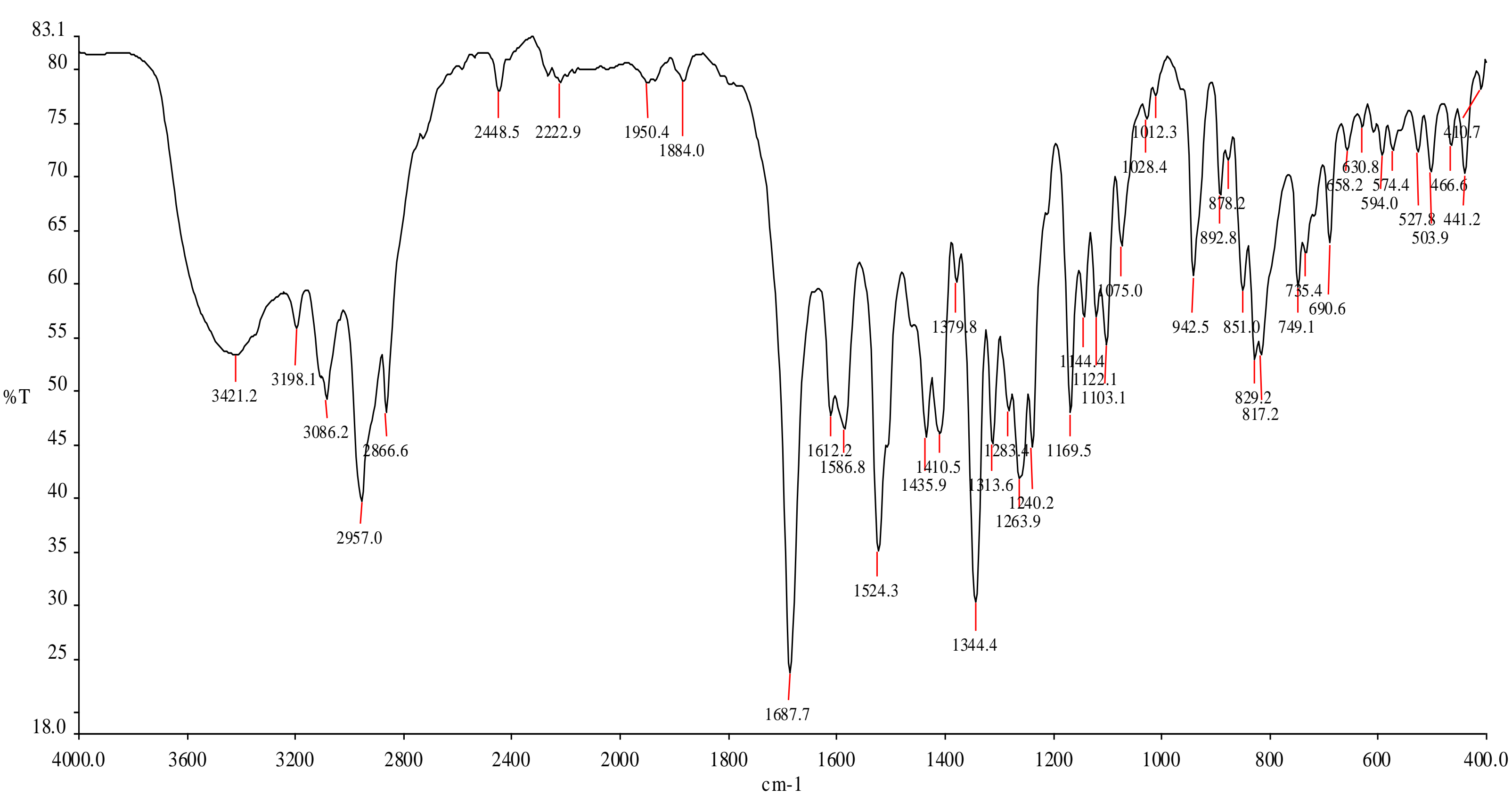


^{1}H NMR of compound XIV

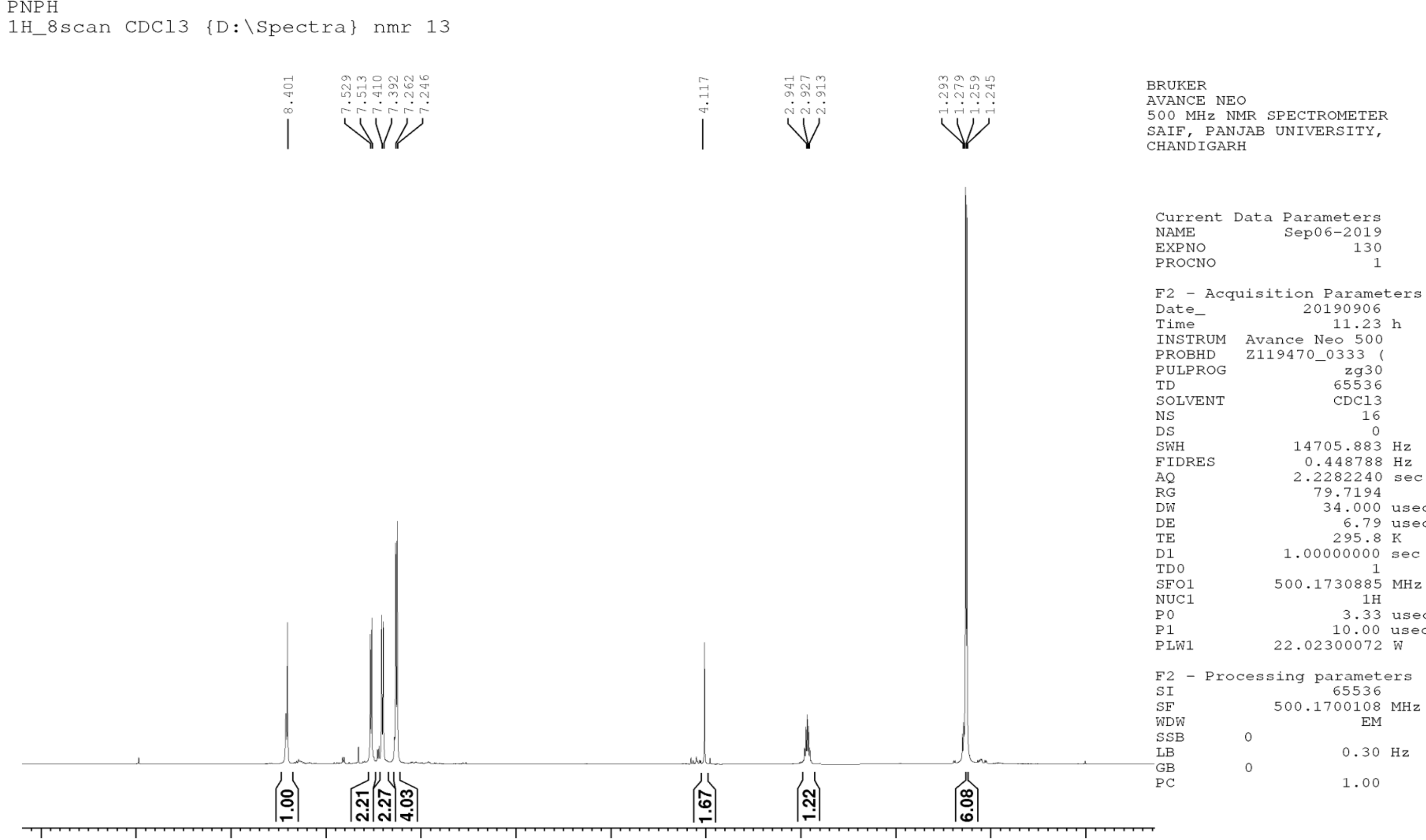


^{13}C NMR of compound XIV

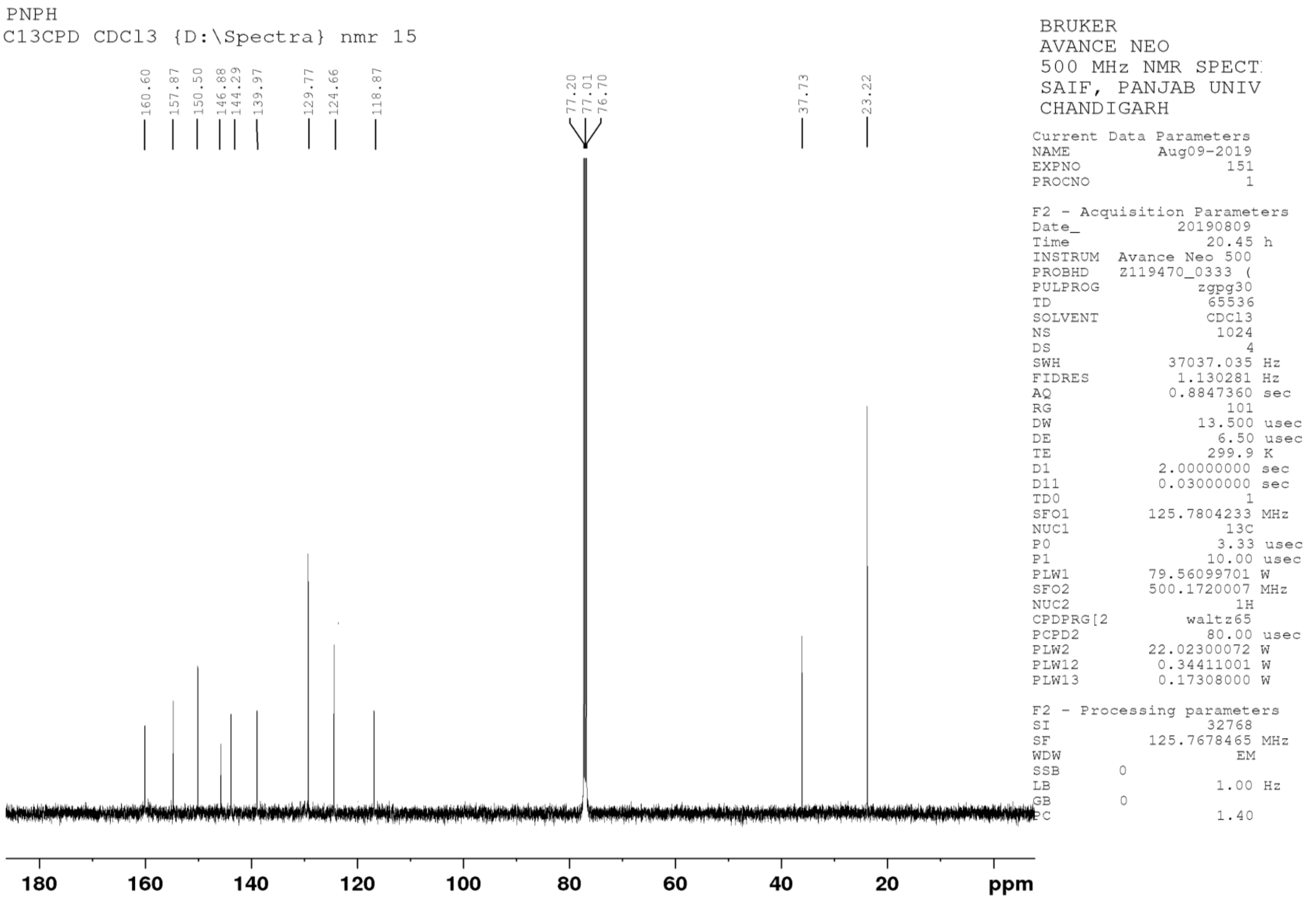
RC SAIF PU, Chandigarh



FT-IR of compound XVII

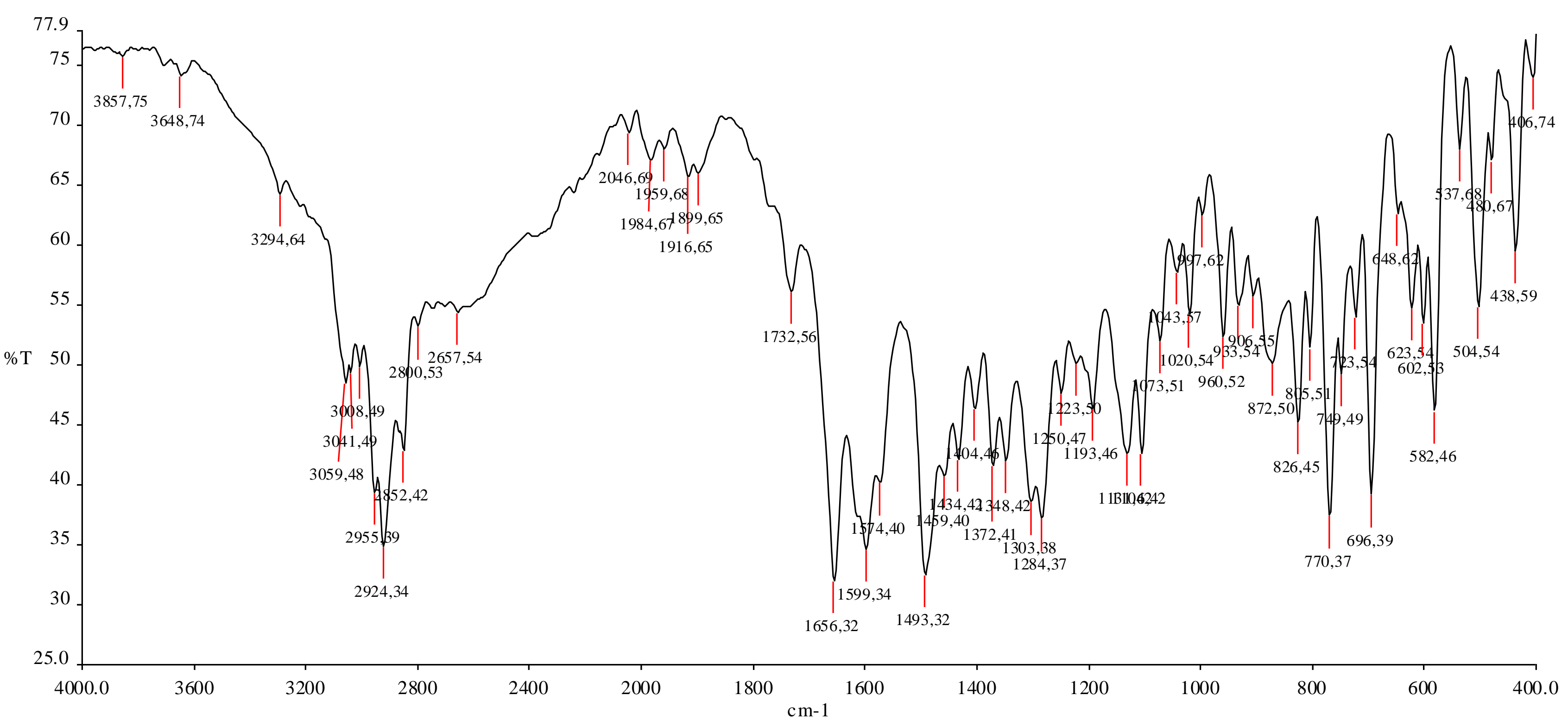


^{1}H NMR of compound XVII

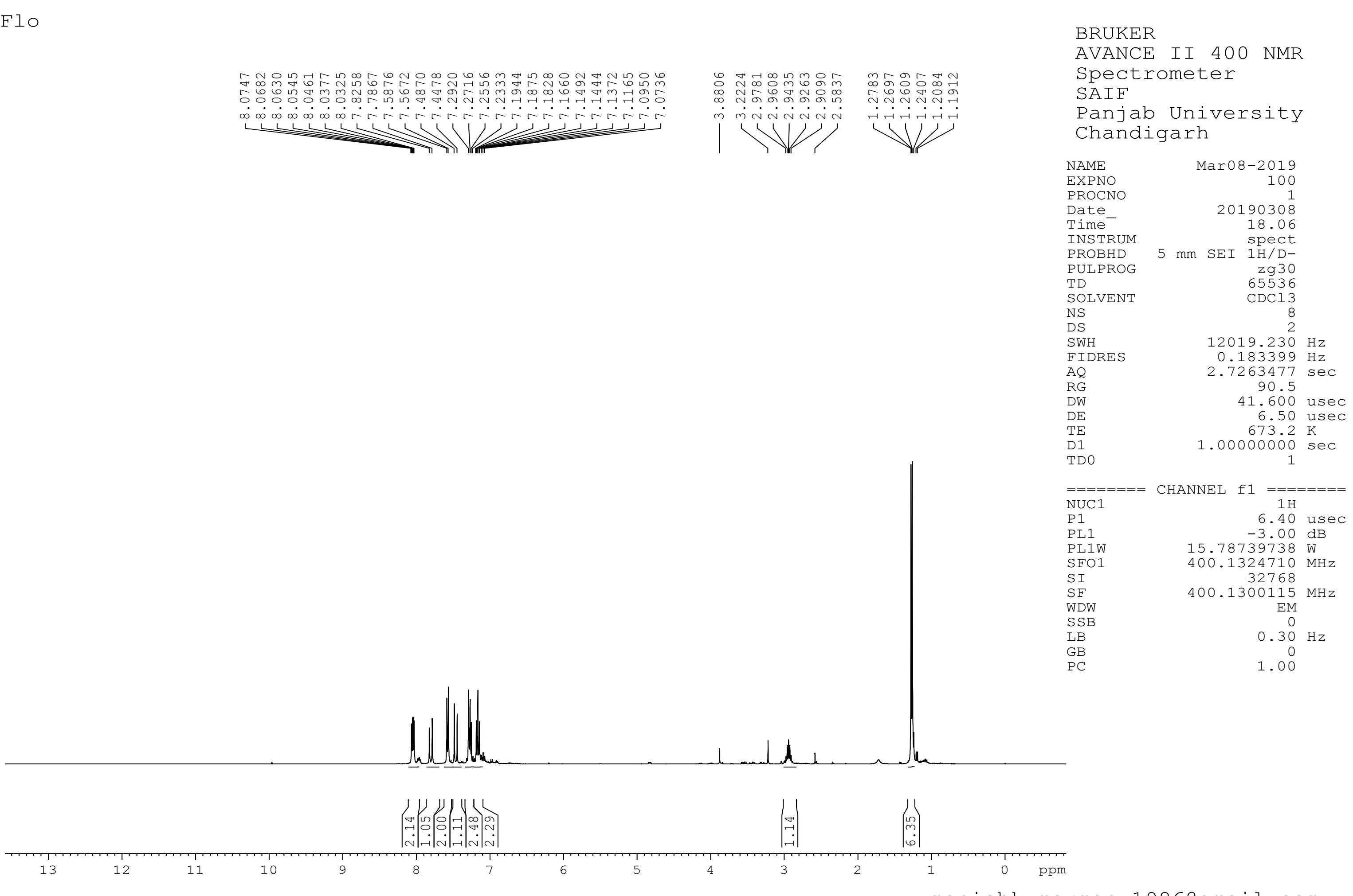


^{13}C NMR of compound XVII

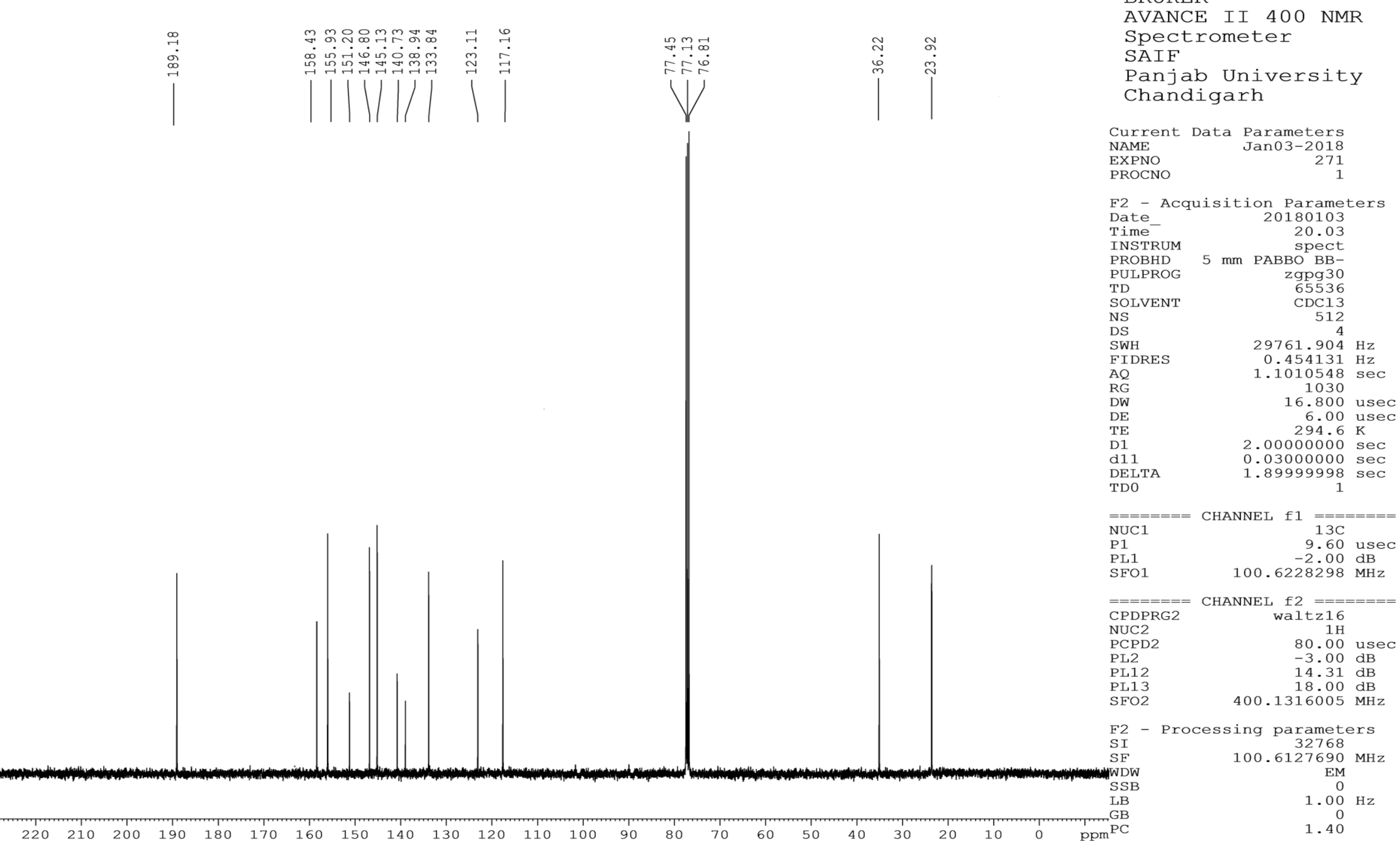
RC SAIF PU, Chandigarh



FT-IR of compound XX

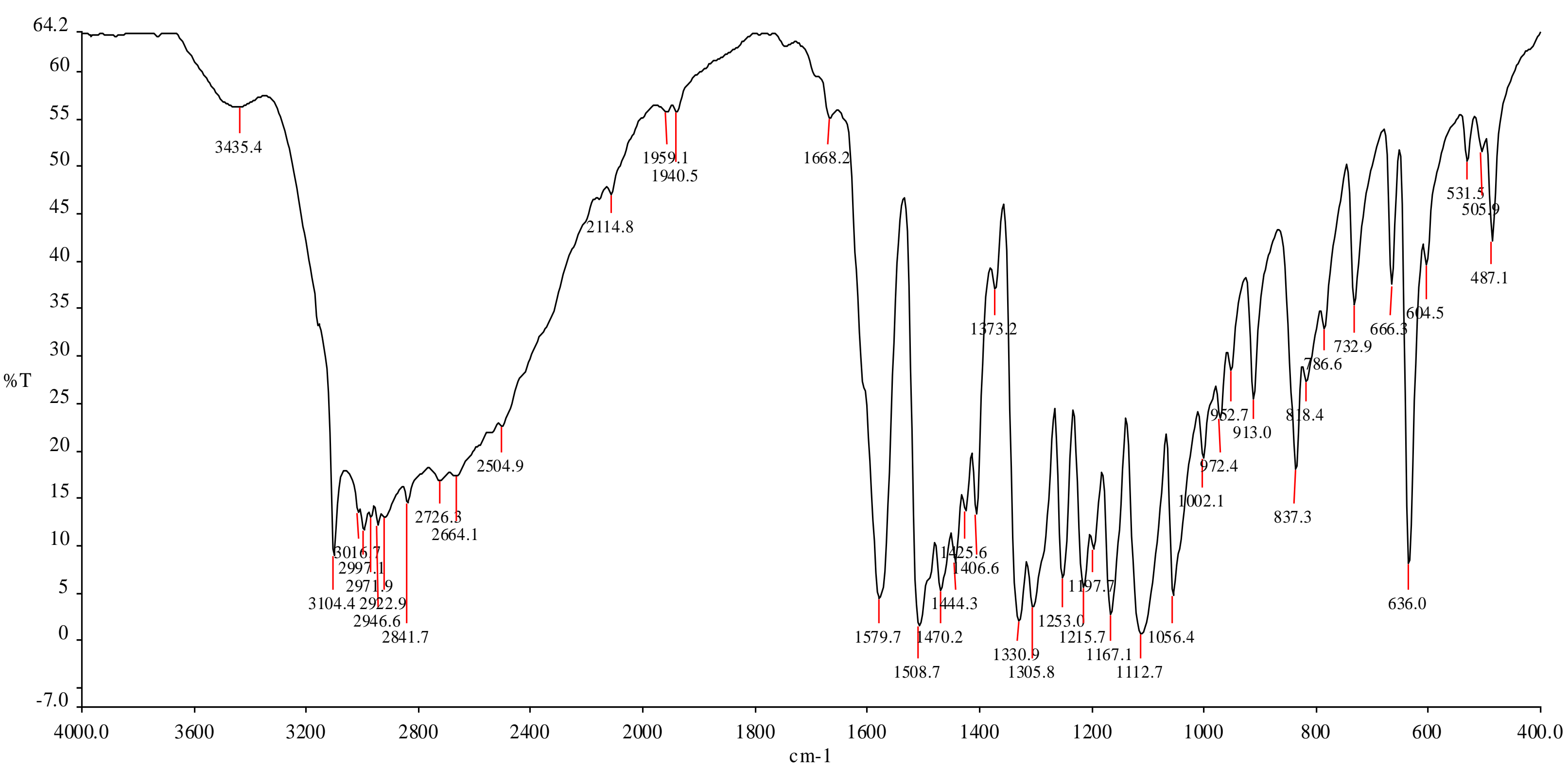


^{1}H NMR of compound XX

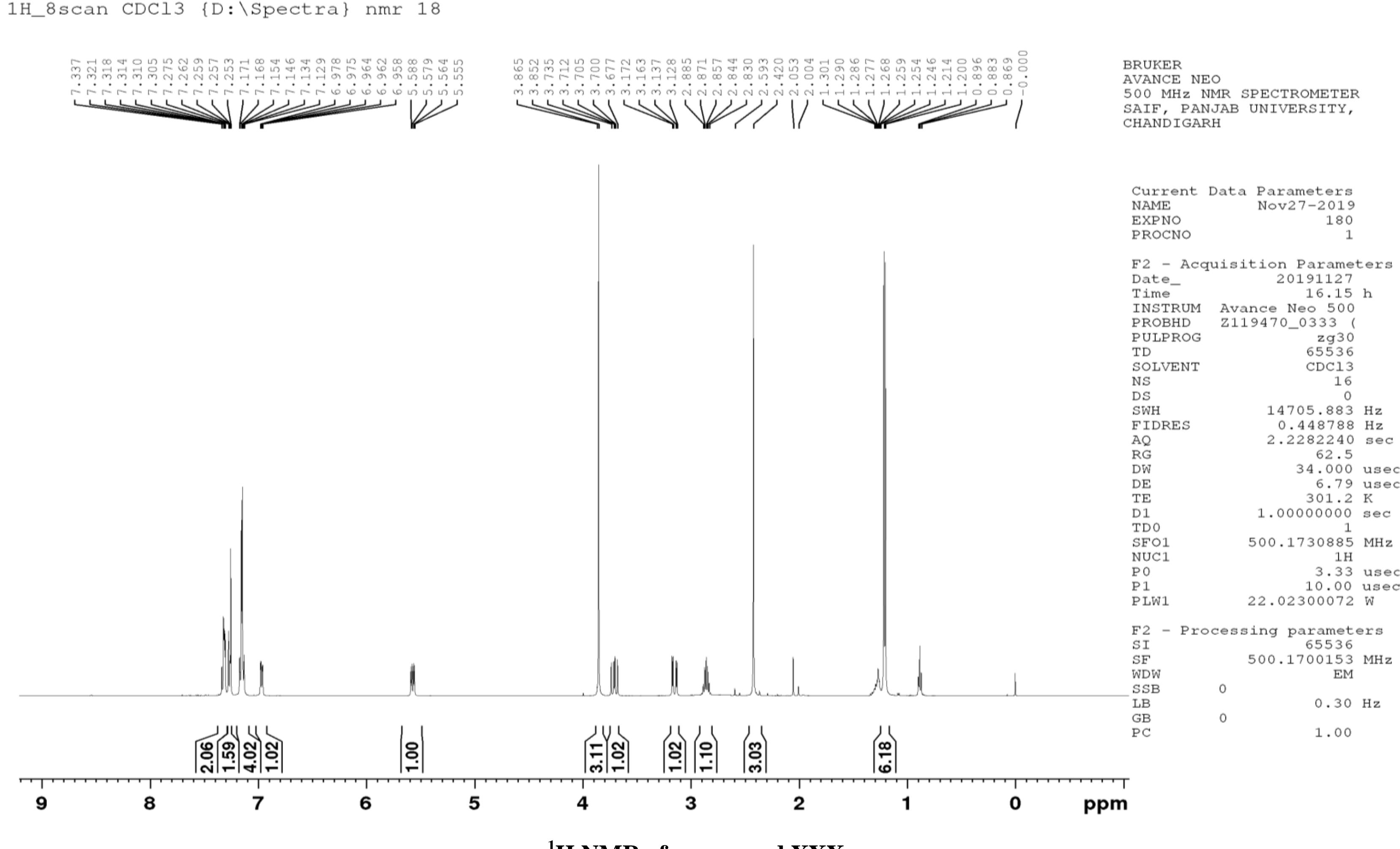


^{13}C NMR of compound XX

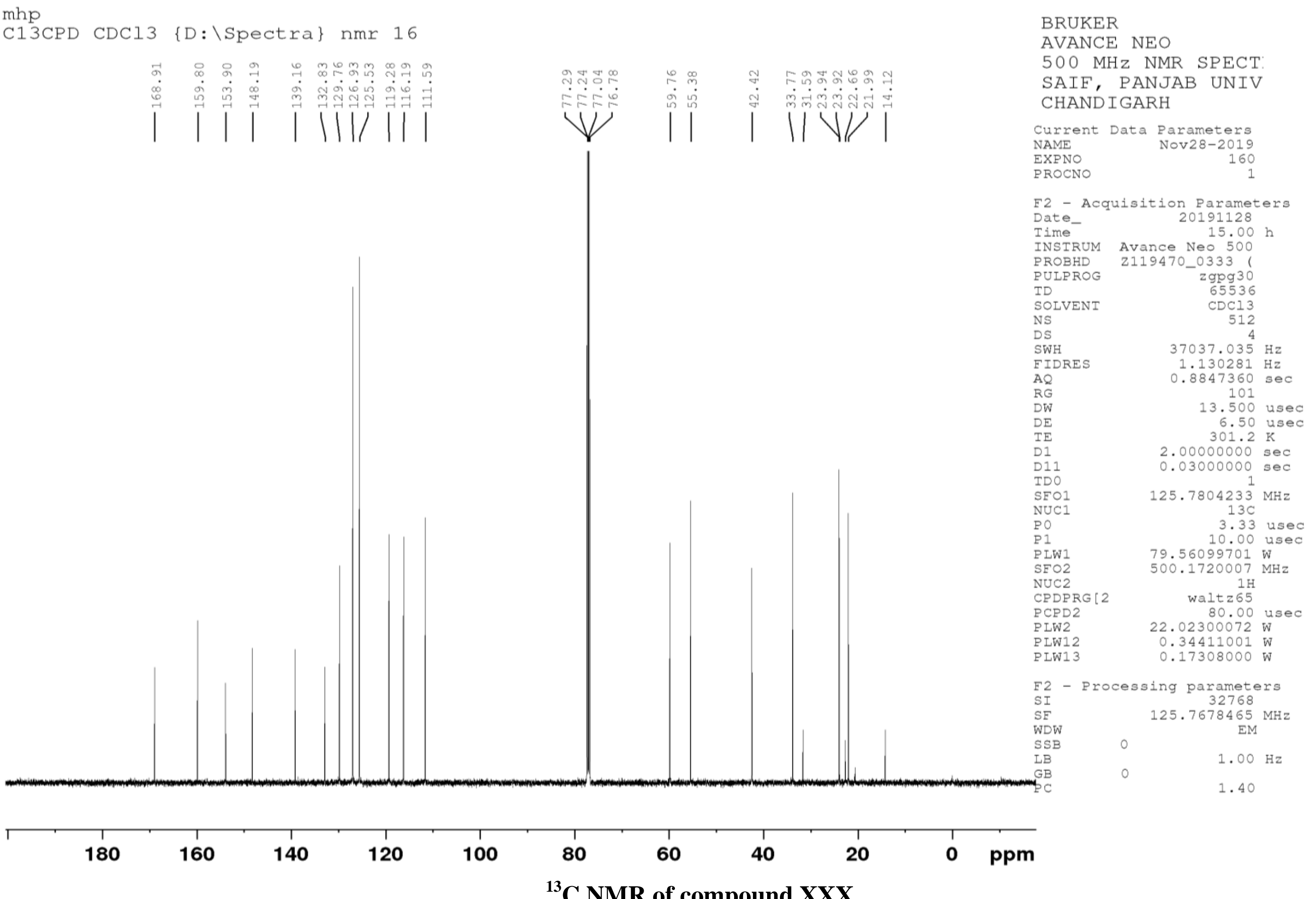
RC SAIF PU, Chandigarh



FT-IR of compound XXX

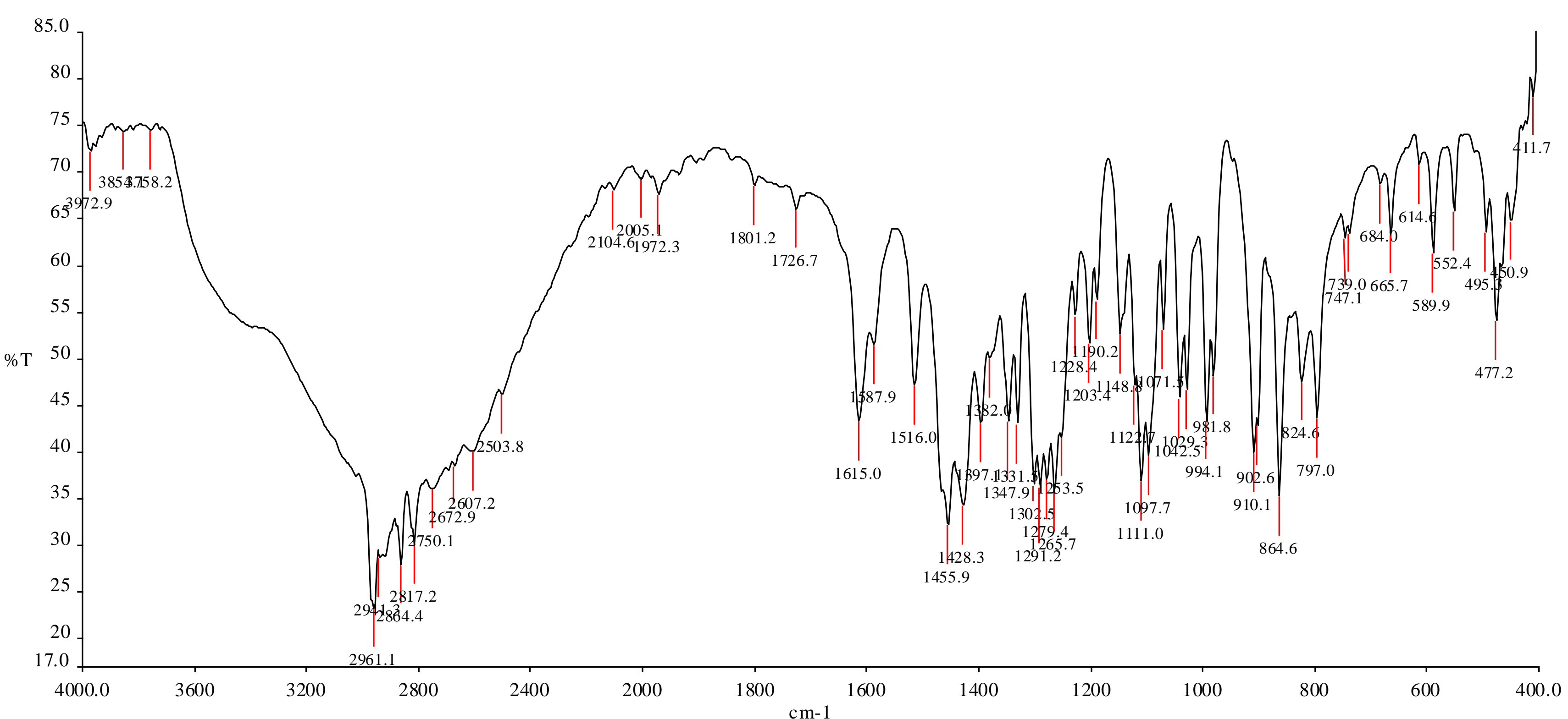


^{1}H NMR of compound XXX

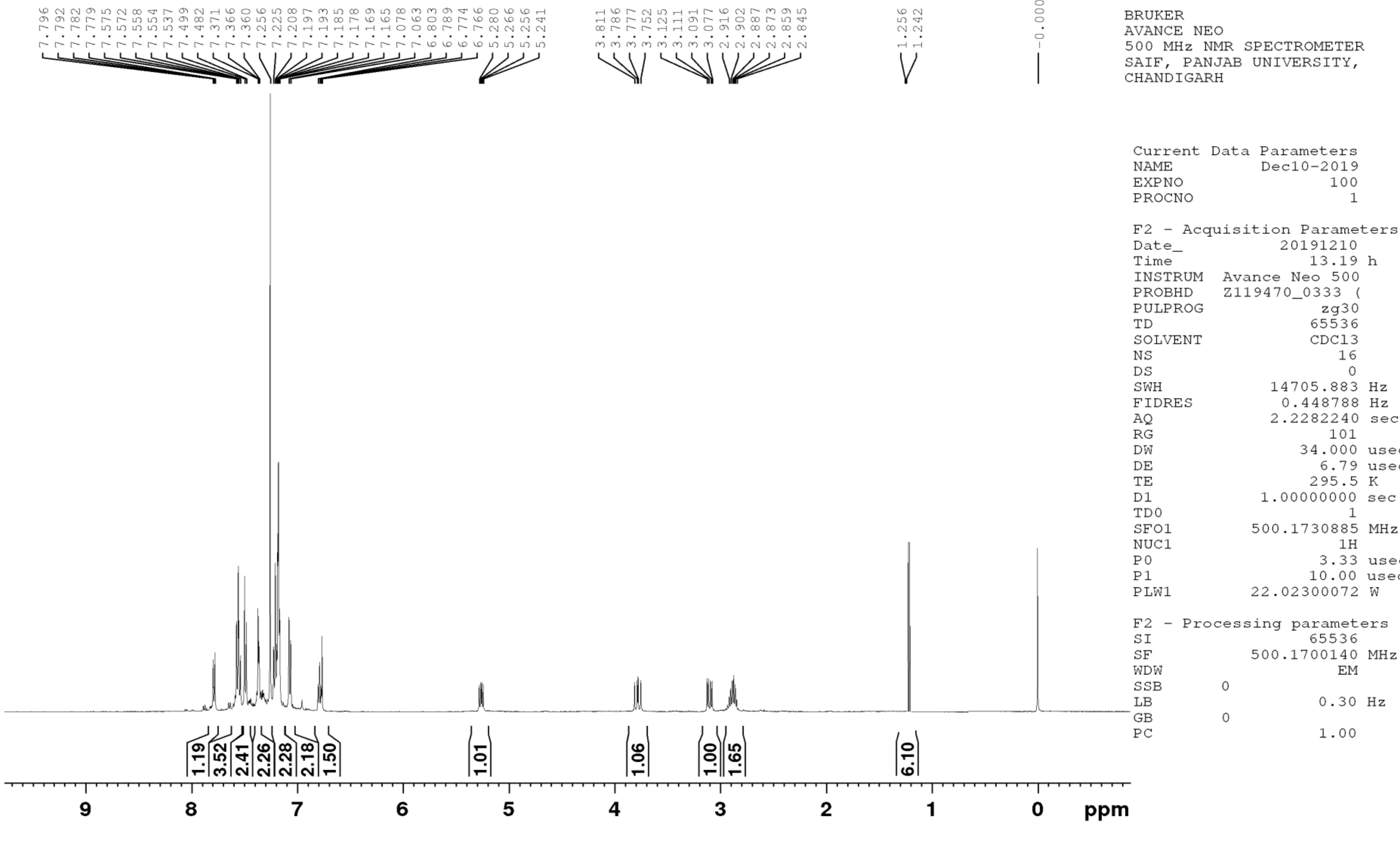


^{13}C NMR of compound XXX

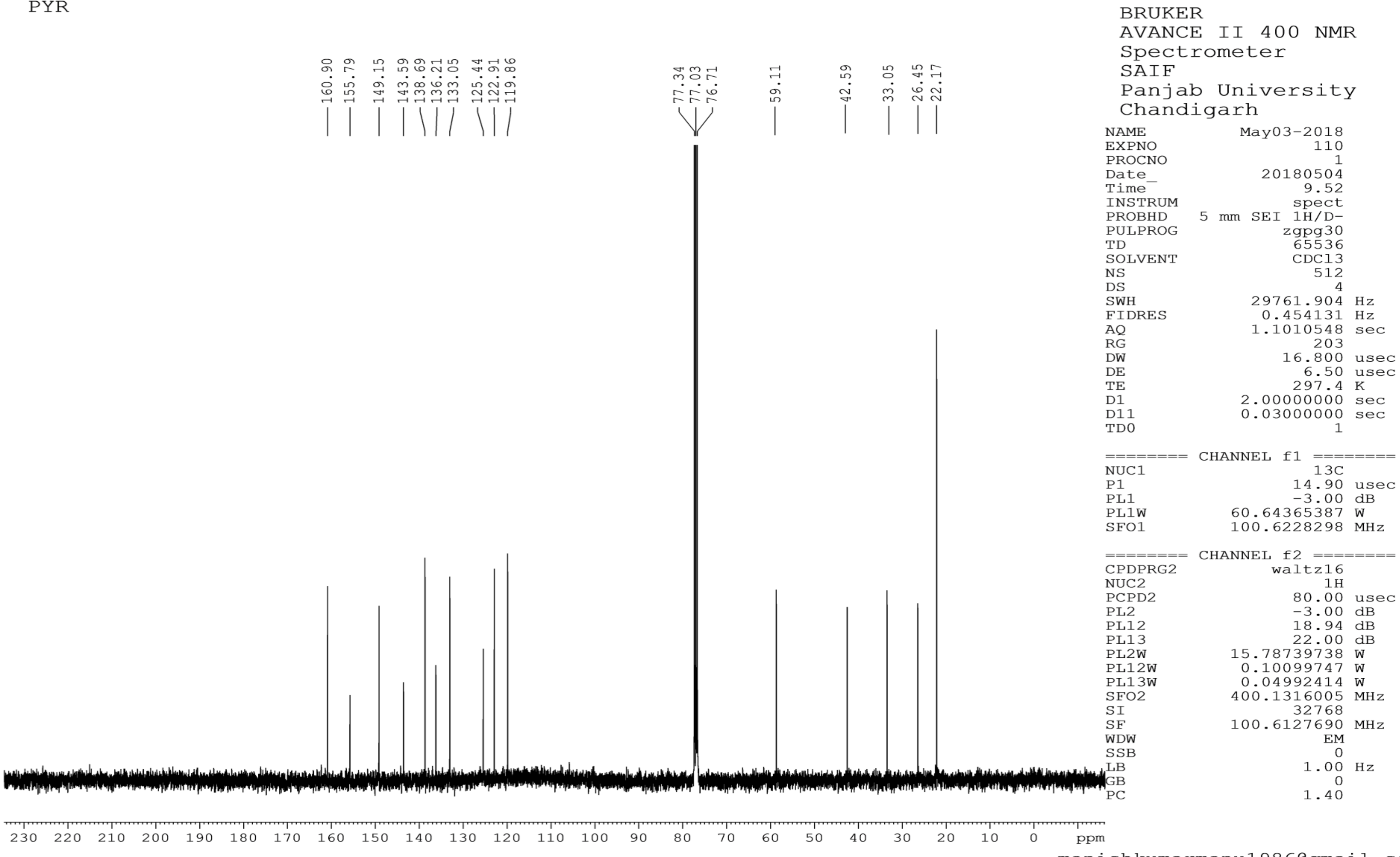
RC SAIF PU, Chandigarh



FT-IR of compound XXXIV



^{1}H NMR of compound XXXIV



^{13}C NMR of compound XXXIV

LIST OF PUBLISHED/ACCEPTED/SUBMITTED RESEARCH ARTICLES

S.No	Title	Journal	NAAS rating	Status
1	**Synthesis, characterization of some novel Schiff bases, hydrazones and thiosemicarbazones of cuminaldehyde and their biological activity**	Organic Preparations and Procedures International	7.52	Accepted
2	**Phytochemical profile of different solvent seed extracts of *Cuminum cyminum* using GC-MS and their bioactivity**	Natural Product Research	8.00	Submitted
3	**Chemical composition, nutritional profile and bioactivity of *Cuminum cyminum* essential oil and its major compound**	Jounal of Food Science and Technology	7.85	Submitted

Ekta Computers <ektacomputersldh@gmail.com>

Fwd: (Organic Preparations and Procedures International) Your submission has been accepted

---

pardeep kaur <pardip2493@gmail.com> Sun, Jan 10, 2021 at 12:41 PM
To: ektacomputersldh@gmail.com

---------- Forwarded message ---------
From: **Michael M. J. Hearn** <em@editorialmanager.com>
Date: Thu, Sep 3, 2020 at 7:46 PM
Subject: (Organic Preparations and Procedures International) Your submission has been accepted
To: Pardeep Kaur <pardip2493@gmail.com>

Ref.: Ms. No. UOPP-2020-0064R2

Preparation and Biological Activities of Novel Cuminaldehyde Derivatives
Organic Preparations and Procedures International

Dear Miss Kaur,

We have now received the necessary reviewer reports, and they are appended below. In the opinion of the Editors, the manuscript has been substantially strengthened through the peer review process. As you will see, both reviewers are recommending acceptance, one with no revisions and one with minor revisions. Because the latter were minor, we Editors have been able to handle them right here in the editorial office.

Your manuscript for *Organic Preparations and Procedures International* has been accepted for publication.

Although publication schedules are somewhat difficult to predict, we hope to include your article in a spring issue of the journal. However, the electronic version is likely to appear online considerably in advance. The next time you hear from us about this manuscript will be for the final correction of the galley proofs. They will come to you directly from the production editor (Mr. Nathan Clark) at our publishing house, Taylor and Francis; but you can also contact me at any time.

We thank you for your interest in publishing with OPPI. We would look forward to your future manuscript contributions.

With kind regards,

Prof. M. J. Hearn
Executive Editor
Organic Preparations and Procedures International

****

Taylor & Francis strives to be compliant with the open access (OA) policies of the major national and international funding bodies. Information about funder compliance and OA policies can be found on our Author Services website. Details of our Green OA policy, including deposit in institutional and subject repositories, can be found on the Sharing your work page. We recommend that you retain your Accepted Manuscript for this purpose.

For more information regarding the versions of your article that can be used to meet Green OA requirements, and the embargo period that applies, please see the open access journal finder page.

****

Reviewer #1: In this manuscript entitled "Preparation and Biological Activities of Novel Cuminaldehyde Derivatives" Sunitha Sharma and co-workers reported the synthesis and biochemical evaluation of fifteen novel derivatives of cuminaldehyde. All the products synthesized are thoroughly characterized by the authors using UV-Vis, IR, 1H NMR, and 13C NMR. The authors made all the necessary changes suggested by the editors and reviewers. This current revised version (second revision) of the manuscript is improved and ready to be published in the OPPI journal.

Reviewer #2: This manuscript reports the preparation of cuminaldehyde Schiff bases, thiosemicarbazones and hydrazones. The information on their biological activities is provided. Those information might be interesting to the people who are working on this field. This manuscript looks OK and is acceptable. Two minor issues: (1) In Scheme 1, structure 2i looks odd, please draw it in formal format; (2) In Tables 1 and 2, the compound numbers should be Bold.

Synthesis, characterization of some novel Schiff bases, hydrazones and thiosemicarbazones of cuminaldehyde and their biological activity

Pardeep Kaur[a], Sunita Sharma[b*], and Jyoti Gaba[a]

[a]*Department of Chemistry, Punjab Agricultural University, Ludhiana-141004, India*

[b]*Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana-141004, India*

[*]Corresponding author. Email: sunita_sharma@pau.edu

Synthesis, characterization of some novel Schiff bases, hydrazones and thiosemicarbazones of cuminaldehyde and their biological activity

In modern era, development of non-hazardous chemicals from natural resources is one of the biggest challenges for organic chemists.[1] Derivatization of bioactive compounds isolated from natural resources such as spices has become a great field of interest.[2] Spices are the richest bio-resource of bioactive compounds such as phenolics,[3] flavonoids,[4] saponins,[5] tannins,[6] alkaloids[7] etc., which exhibit antioxidant,[8] anti-inflammatory,[9] antifungal,[10] antifertility,[11] antidiabetic,[12] antihyperglycemic,[13] antihyperlipidemic,[14,15] antiproliferative and antimicrobial[16] potentials. Plants of Apiaceae family possess a wide range of compounds with numerous biological activities.[17] Cumin (*Cuminum cyminum*) is an annual herbaceous plant belonging to the family of Apiaceae[18] genus *Cuminum.* It is the second most popular spice in the world after black pepper.[19] In India, it is mainly cultivated in states of Rajasthan and Gujarat.[20] It is traditionally used to relieve abdominal colic,[21] treat heartburn,[22,23] diarrhea and jaundice.[24] It also has antibacterial,[25] antioxidant,[26] antifungal[27] and anticonvulsant activity.[28]

The natural compounds especially containing carbonyl groups have gained an evoked interest in their derivatization to yield biologically active molecules for the control fungal pathogens. One such carbonyl compound namely cuminaldehyde derived from essential oil of cumin seeds possess wide range of biological activities such as antimicrobial,[29,30,31] antifungal[32] anti-inflammatory,[33,34] antimalarial,[35] antioxidant,[36] antifibrillation,[37,38] anticarcinogenic[39,40] and antidiabetic[41]. It is a natural monoterpenoid and commonly found in commelinid plants such as *Cuminum cyminum*, *Cinnamomum verum*, *Carum carvi*, *Cinnamomum cassia* and *Bunium persicum*[42].

Numerous synthetic derivatives can prepared from cuminaldehyde such Schiff bases, chalcones, thiosemicarbazones and hydrazones that may act as a starting material for many biological active compounds such lactums, pyrazolines, isoxazolines, triazoles and thiazoles etc. Over the past decades, they have made significant contribution in the field of both organic and inorganic chemistry. The presence of the imino nitrogen atom of the azomethine group (-HC=N-) made them more significant chemically and biologically.[43] A facile reaction takes place due to the good electrophilic and nucleophilic characteristic properties of the carbonyl and amine groups respectively. These play a significant role in industries with several biological applications that include antibacterial, antifungal and antitumor activities.

Therefore, keeping in view the need and biological importance of non-hazardous compounds, the present study was designed to synthesize different derivatives of cuminaldehyde derived from spice namely *C. cyminum*. Since limited work has been done on the derivatization of cuminaldehyde,[44,45,46,47,48] the current study was done with the objectives to synthesize different substituted Schiff bases (contains only azomethine group), thiosemicarbazides (azomethine group along with sulfur and nitrogen atoms) and

hydrazones (azomethine group in addition to nitrogen atoms) comparative screening of different biological activities of synthesized products *i.e.* Schiff bases, thiosemicarbazides and hydrazones. The synthesized compounds were screened for their antioxidant potential *via* DPPH assay and antimicrobial activity against three gram-negative bacteria such as *Pseudomonas sp.*, *Klebsiella sp.* and *Enterobacter sp.* was also studied.

The synthetic protocol employed to synthesize the compounds is outlined in Scheme 1. Condensation reaction of cuminaldehyde with different substituted primary amines, thiosemicarbazides and hydrazines in the presence of catalytic amount of glacial acetic acid yielded substituted Schiff bases, thiosemicarbazones and hydrazones, respectively. The structure of the products was elucidated with the help of UV-visible, IR, ^{1}H and ^{13}C NMR spectroscopy. In all the synthesized products formation of new C=N bond took place which was confirmed using IR, ^{1}H and ^{13}C NMR spectroscopy. IR spectra of synthesized derivatives of cuminaldehyde showed absorption band in the range of 1567-1651 cm^{-1} due to C=N stretching of the azomethine group. ^{1}H and ^{13}C NMR spectra of compounds **(2a-2k, 3a-3b & 4a-4c)** exhibited a singlet at δ 7.67-12.71 and δ 149.49-193.40 respectively, due to newly formed CH=N. Spectroscopic data of compounds **2i**[46], **3a**[44], **3b**[44] found similar to previously reported data. A facile reaction took place due to the good electrophilic and nucleophilic characteristic properties of the carbonyl and amine groups respectively. It was observed that more reaction time is required to synthesize Schiff bases (4 hrs) followed by thiosemicarbazones (3 hrs) and hydrazones (1 hr). Hydrazones took lesser reaction time for its completion due to strong reducing potential of hydrazines.[49] The presence of the imino nitrogen atom of the azomethine group (-HC=N-) makes them more significant chemically and biologically.

Different synthesized derivatives of cuminaldehyde were screened for their antioxidant potential by DPPH assay. DPPH was used as the free radical source, since it simulates reactive oxygen and nitrogen species affecting biological systems. Antioxidant activity was tested by varying the concentration of the compounds and this variation is shown in Table 1 and the IC_{50} values, determined from log dose response curve, are shown in Figure 1. Among all the synthesized compounds, Schiff bases possess highest antioxidant potential as compared to different substituted thiosemicarbazone and hydrazones. Out of all Schiff bases, compounds **2b** and **2g** exhibited highest antioxidant potential. Azomethine group increase the antioxidant potential of the synthesized products. Effectiveness of compound **2b** is may be due to the presence of free OH group. Gaikwad and his workers reported the similar results.[50] Compound 2b contains phenolic moiety which may made it more active than other compounds. Due to the lower bond dissociation energies (BDE) of O–H, it loses H atom comparatively easier to than other compounds.[51] BDE is an important factor in determining the efficacy of an antioxidant since the weaker the OH bond, the faster will be the reaction with the free radicals.[52] The antioxidant

potential of the phenolic compounds is also because of its ability to terminate free radicals.[53] These are regarded as free radical terminators. They react with high-energy lipid radicals to convert them to thermodynamically more stable products. Compound **2g** containing azomethine moiety along with substituted pyridine ring also showed comparable activity as compound **2b**. The antioxidant activity of synthesized compounds is presented in Table 1 and follows the trend: 2b > 2g > 4a > 2k > 2j > 4b > 2i > 2f > 2d > 2c > 2h > 2e > 4c > 3b > 2a > 3a at all tested concentrations.

The newly synthesized derivatives of cuminaldehyde **(2a-2k, 3a-3b, 4a-4c)** were evaluated for their *in vitro* antimicrobial potential against Gram-negative bacteria *viz*. *Pseudomonas sp*., *Klebsiella sp*. and *Enterobacter sp.* at different concentrations (3000, 2000, 1000, 500, 250, 100, 50 and 25 $\mu g\ mL^{-1}$). The results obtained *i.e.* values of inhibition zones and MIC values are graphically represented in Table 2 and Figure 2, respectively. Inhibition zone data was analyzed statistically by single factor analysis of variance to determine the significant differences among the activities of synthesized compounds against three bacteria. From Table 3, it is evident that, the bioactivity of the cuminaldehyde derivatives varied significantly at 1 and 5% level of significance. All synthesized cuminaldehyde derivatives exhibited moderate to good antimicrobial potential against the tested bacteria. Antimicrobial potential of synthesized compounds was also assessed by their MIC using bacterial sensitivity-filter paper disc method. Ampicillin was used as the standard drug against all the tested bacteria. Screening of antimicrobial potential of synthesized derivatives of cuminaldehyde against *Pseudomonas sp.* revealed that compound **2i** possessed higher antimicrobial potential. The greater potential of compound **2i** against *Pseudomonas sp.* may be due to the combination of azomethine group and pyrimidine ring.[54,55,56] Inhibition zones of prepared compounds followed the order: 2h > 2b > 3b > 2c > 2i > 3a > 4c > 2j > 2d > 2k > 2f > 2g > 4b > 2a > 4a > 2e. Concerning the antibacterial activity against *Klebsiella sp.*, the results revealed that different synthesized compounds displayed variable inhibitory effects on the growth of the tested Gram-negative bacteria and followed the order: 3b > 2j > 3a > 2i > 2b > 2c > 2a > 2d > 2g > 2k > 2f > 4c > 2e > 4b > 2h > 4a. Electron delocalization in thiosemicarbazide moiety[57] along with azomethine group may be responsible for higher antimicrobial potential of compound **3b**. Bioactivity of different synthesized derivatives of cuminaldehyde against *Enterobacter sp.* follows the trend: 4c > 2i > 2g > 3b > 2b > 2d > 2f > 2a > 2c > 2k > 3a > 2e > 4b > 2h > 2j > 4a. Effectiveness of compound **4c** against *Enterobacter sp.* may be due to the presence of the electron withdrawing groups on the aromatic ring [58] along with the azomethine group.

Presence of aromatic ring and electron withdrawing groups played an important role in the bioactivity of synthesized thiosemicarbazones and hydrazones against all the three tested bacteria. It has been found that in all tested bacteria, thiosemicarbazone **3b** possessed more inhibition potential as compared to **3a**. In case of hydrazones, bioactivity of compounds varied directly with the number of the

electron withdrawing groups. As the number of electron withdrawing groups increased antimicrobial potential of synthesized hydrazones increased.

In conclusion, synthesis of some novel cuminaldehyde derivatives namely Schiff bases, thiosemicarbazones and hydrazones was done and the products were obtained in moderate to good yields. Compounds **2b** and **2g** shows promising antioxidant activity at higher concentration. Compound **2h, 3b, 4c** are showed maximum activity against *Pseudomonas sp., Klebsiella sp.,* and *Enterobacter sp.,* respectively. In case of hydrazones, compounds containing more electron withdrawing groups showed better biological activity.

Experimental

Cuminaldehyde was purchased from Hi-media Laboratories Private Limited (Mumbai, India) and used as such without purification. All other chemicals and solvents were purchased from Loba Chemie Private Limited (Mumbai, India), S.D. Fine Chemicals Limited (Mumbai, India) and Sisco Research Laboratories Private Limited (Mumbai, India). Nutrient broth and agar agar used for evaluating the antimicrobial potential was also purchased from Hi-media Laboratories Private Limited (Mumbai, India). Melting points of the solid samples were determined in open capillaries using melting point apparatus (Relitech, Haryana, India) and were uncorrected. The purity of compounds was checked by thin layer chromatography, and the visualization was done in iodine chamber. Electronic spectra (λ_{max} in nm) were recorded on Techcomp UV 2600 spectrophotometer. Fourier transform infrared (FT-IR), ^{1}H and ^{13}C NMR spectra were got scanned from, Central Instrument Laboratory, sophisticated analytical instrumentation facility, Punjab University, Chandigarh. FT-IR spectra recorded on Perkin-Elmer FT-IR spectrophotometer in the range of 4000-400 cm^{-1}. The ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance II 400 MHz spectrophotometer and Bruker Avance Neo 500 MHz spectrophotometer using tetramethylsilane as internal standard. The chemical shifts were expressed in δ (ppm) values and the abbreviations used for ^{1}H NMR signals are: s = singlet, d = doublet, t = triplet, q = quartet and m = multiplet. CHN analysis was performed on a Vario El III Elementor analyzer.

General procedure and spectral data of the synthesized compounds

Synthesis of Schiff bases (2a-2k)

N-(4-isopropylbenzylidene)benzenamine (2a)

Equimolar amounts of cuminaldehyde (0.01 mole, 1.51 mL) and aniline (0.01 mole, 0.91 mL) were taken in a 250 mL round bottom flask using ethanol as a solvent. Add catalytic amount of glacial acetic acid to the reaction. Reflux the reaction mixture for 4 hrs. Progress of the reaction was monitored by thin layer chromatography. Brown colored product was obtained (2a) in good yield. Brown crystals, mp 133-135 °C, Yield (70%). UV-vis (λ_{max}): 302 nm. FT-IR (KBr, cm^{-1}) ν_{max} 1622 (C=N). ^{1}H NMR (500 MHz, $CDCl_3$) ppm: δ 1.25-1.26 (d, 6H, gem-dimethyl), 2.90-2.97 (m, 1H, CH), 7.13-7.38 (m, 7H, ArH), 7.77-7.83 (m,

2H, ArH), 8.39 (s, 1H, CH=N). ^{13}C NMR (400 MHz, $CDCl_3$) ppm: δ 28.90 (gem-dimethyl), 39.09 (CH), 123.34, 126.73, 127.70, 128.60, 131.31, 134.33, 147.63, 149.99 (Aromatic carbons), 166.64 (CH=N).

Anal. Calcd for $C_{16}H_{17}N$: C, 86.05; H, 7.67; N, 6.27. Found: C, 86.09; H, 7.69; N, 6.22.

4-(4-Isopropylbenzylideneamino)phenol (2b)

Schiff base was prepared by condensation of cuminaldehyde (0.01 mole, 1.51 mL) with *p*-aminophenol (0.01 mol, 1.09 g) in the presence of catalytic amount of glacial acetic acid using ethanol as a solvent and the mixture was refluxed for 4 hrs. The progress of reaction was monitored by TLC. On completion of reaction the product was separated as light brown crystalline solid which was filtered, dried, and recrystallized from chloroform. Light brown crystals, mp 93-95 °C, Yield (84%). UV-vis (λ_{max}): 310 nm. FT-IR (KBr, cm^{-1}) ν_{max} 1609 (C=N). ^{1}H NMR (400 MHz, $CDCl_3$) ppm: δ 1.25-1.26 (d, 6H, gem-dimethyl), 2.52-2.53 (m, 1H, CH), 6.78-8.18 (m, 8H, ArH), 8.50 (s, 1H, CH=N), 8.50 (s, 1H, CH=N), 9.32 (s, 1H, OH). ^{13}C NMR (400 MHz, $CDCl_3$) ppm: δ 23.80 (gem-dimethyl), 34.22 (CH), 116.13, 122.33, 127.17, 128.77, 130.05, 145.11, 153.24, 157.11 (Aromatic carbons), 160.16 (CH=N).

Anal. Calcd for $C_{16}H_{17}NO$: C, 80.30; H, 7.16; N, 5.85. Found: C, 80.27; H, 7.19; N, 5.90.

N-(4-isopropylbenzylidene)-3-methoxybenzenamine (2c)

A mixture of cuminaldehyde (0.01 mol, 1.51 mL) and *m*-anisidine (0.01 mol, 1.12 mL) were dissolved in ethanol. The contents were subjected to refluxing for 4 hrs after adding catalytic amount of glacial acetic acid. Thin layer chromatography is used to check the progress of reaction. After the completion of the reaction, light brown colored solid product was obtained after pouring the hot reaction mixture in ice cold water. The solid product was then obtained was filtered and recrystallized from chloroform.

Light brown crystals, mp 207-209 °C, Yield (77%). UV-vis (λ_{max}): 312 nm. FT-IR (KBr, cm^{-1}) ν_{max} 1614 (C=N). ^{1}H NMR (400 MHz, $CDCl_3$) ppm: δ 1.32-1.33 (d, 6H, gem-dimethyl), 2.81-2.90 (m, 1H, CH), 3.68 (s, 1H, OCH_3), 6.95-7.47 (m, 8H, ArH), 9.31 (s, 1H, CH=N). ^{13}C NMR (400 MHz, $CDCl_3$) ppm: δ 22.65 (gem-dimethyl), 39.55 (CH), 49.17 (OCH_3), 105.79, 112.20, 121.27, 125.57, 127.25, 133.65, 135.74, 147.85, 149.64, 161.51 (Aromatic carbons), 165.92 (CH=N).

Anal. Calcd for $C_{17}H_{19}NO$: C, 80.60; H, 7.56; N, 5.53. Found: C, 80.65; H, 7.60; N, 5.48.

N-(4-isopropylbenzylidene)(phenyl)methanamine (2d)

Equimolar amount of cuminaldehyde (0.01 mole, 1.51 mL) and benzylamine (0.01 mole, 1.09 mL) was dissolved in ethanol. Add 5 drops of glacial acetic acid in the reaction mixture and reflux for 8 hrs. Thin layer chromatographic technique was used to check the reaction progress. Brown colored solid product was obtained in good yields after the completion of reaction. Brown crystals, mp 121-123 °C, Yield (71%). UV-vis (λ_{max}): 306 nm. FT-IR (KBr, cm^{-1}) ν_{max} 1607 (C=N). ^{1}H NMR (400 MHz, $CDCl_3$) ppm: δ 1.34-1.36 (d, 6H, gem-dimethyl), 2.80-2.86 (m, 1H, CH), 4.47 (s, 2H, CH_2), 7.16-7.65 (m, 9H, ArH), 8.38

(s, 1H, CH=N). ^{13}C NMR (400 MHz, $CDCl_3$) ppm: δ 23.49 (gem-dimethyl), 36.17 (CH), 58.96 (CH_2), 122.61, 123.65, 131.28, 136.66, 138.30, 145.81, 149.14, 151.11 (Aromatic carbons), 166.16 (CH=N).

Anal. Calcd for $C_{17}H_{19}N$: C, 86.03; H, 8.07; N, 5.90. Found: C, 86.07; H, 8.13; N, 5.80.

N-(4-isopropylbenzylidene)pyridin-2-amine (2e)

2-Aminopyridine (0.01 mole, 0.94 g) was dissolved in ethanol in the presence of glacial acetic acid. A solution of cuminaldehyde (0.01 mole, 1.51 mL) was added dropwise and refluxed for 4 hrs. Monitoring of reaction progress was done by TLC. White colored solid product was then recrystallized from chloroform. White crystals, mp 110-112 °C, Yield (72%). UV-vis (λ_{max}): 315 nm. FT-IR (KBr, cm^{-1}) ν_{max} 1592 (C=N). ^{1}H NMR (400 MHz, $CDCl_3$) ppm: δ 1.30-1.31 (d, 6H, gem-dimethyl), 2.78-2.86 (m, 1H, CH), 6.95-7.02 (m, 3H, ArH), 7.27-7.47 (m, 5H, ArH), 8.31 (s, 1H, CH=N). ^{13}C NMR (400 MHz, $CDCl_3$) ppm: δ 23.32 (gem-dimethyl), 35.92 (CH), 120.00, 125.54, 127.00, 129.67, 137.24, 156.55, 158.88, 162.22, 163.83 (Aromatic carbons), 193.40 (CH=N).

Anal. Calcd for $C_{15}H_{16}N_2$: C, 80.32; H, 7.19; N, 12.49. Found: C, 80.40; H, 7.17; N, 12.43.

N-(4-isopropylbenzylidene)pyridin-4-amine (2f)

An equimolar ethanolic solution of 4-aminopyridine (0.01 mole, 0.94 g) and cuminaldehyde (0.01 mole, 1.51 mL) were mixed completely in the presence of catalytic amount of glacial acetic acid and refluxed for 4 hrs. White colored crystalline product was filtered and recrystallized in chloroform. A single spot on a chromatographic plate confirmed the purity of product. White crystals, mp 105-107 °C, Yield (75%). UV-vis (λ_{max}): 308 nm. FT-IR (KBr, cm^{-1}) ν_{max} 1596 (C=N). ^{1}H NMR (400 MHz, $CDCl_3$) ppm: δ 1.54-1.56 (d, 6H, gem-dimethyl), 2.71-2.74 (m, 1H, CH), 7.20-7.45 (m, 8H, ArH), 8.38 (s, 1H, CH=N). ^{13}C NMR (400 MHz, $CDCl_3$) ppm: δ 28.74 (gem-dimethyl), 38.72 (CH), 123.34, 129.16, 136.64, 144.02, 148.87, 152.79, 157.00 (Aromatic carbons), 161.06 (CH=N).

Anal. Calcd for $C_{15}H_{16}N_2$: C, 80.32; H, 7.19; N, 12.49. Found: C, 80.38; H, 7.23; N, 12.39.

N-(4-isopropylbenzylidene)-3-methylpyridin-2-amine (2g)

N-(4-isopropylbenzylidene)-3-methylpyridin-2-amine was synthesized by refluxing an equimolar amount of cuminaldehyde (0.01 mol, 1.51 mL) and 2-amino-3-methylpyridine (0.01 mol, 1.08 g) in the presence of glacial acetic acid (5 drops) using ethanol as a solvent. The resulting mixture was subjecte to refluxing for 4 hrs. The progress and purity of the Schiff base was checked by TLC. White crystals, mp 97-99 °C, Yield (82%). UV-vis (λ_{max}): 314 nm. FT-IR (KBr, cm^{-1}) ν_{max} 1590 (C=N). ^{1}H NMR (400 MHz, $CDCl_3$) ppm: δ 1.35-1.36 (d, 6H, gem-dimethyl), 2.31 (s, 3H, CH_3), 2.12-2.13 (m, 1H, CH), 7.09-7.35 (m, 7H, ArH), 9.02 (s, 1H, CH=N). ^{13}C NMR (400 MHz, $CDCl_3$) ppm: δ 15.93 (CH_3), 22.82 (gem-dimethyl), 33.71 (CH), 110.94, 126.24, 127.00, 129.65, 134.30, 155.57, 157.83, 161.10, 163.47 (Aromatic carbons), 192.28 (CH=N).

Anal. Calcd for $C_{16}H_{18}N_2$: C, 80.63; H, 7.61; N, 11.75. Found: C, 80.60; H, 7.67; N, 11.73.

N-(4-isopropylbenzylidene)pyrimidin-2-amine (2h)

A mixture of cuminaldehyde (0.01 mol, 1.51 mL) and 2-aminopyrimidine (0.01 mol, 0.95 g) and catalytic amount of glacial acetic acid was refluxed for 4 hrs with continuous monitoring by TLC. Then the reaction mixture was filtered and the white crystalline product recovered by recrystallization with chloroform. White crystals, mp 144-146 °C, Yield (80%). UV-vis (λ_{max}): 324 nm. FT-IR (KBr, cm^{-1}) ν_{max} 1587 (C=N). ^{1}H NMR (400 MHz, $CDCl_3$) ppm: δ 1.29-1.28 (d, 6H, gem-dimethyl), 2.88-2.99 (m, 1H, CH), 6.99-7.48 (m, 7H, ArH), 9.22 (s, 1H, CH=N). ^{13}C NMR (400 MHz, $CDCl_3$) ppm: δ 23.78 (gem-dimethyl), 34.11 (CH), 112.70, 118.99, 126.09, 129.70, 137.46, 139.56, 156.40 (Aromatic carbons), 177.72 (CH=N).

Anal. Calcd for $C_{14}H_{15}N_3$: C, 74.64; H, 6.71; N, 18.65. Found: C, 74.58; H, 6.75; N, 18.67.

4-(4-Isopropylbenzylideneamino)-2,3-dimethyl-1-phenyl-1,2-dihydropyrazol-5-one (2i)[46]

Condensation of cuminaldehyde (0.01 mol, 1.51 mL) with 4-aminophenazone (0.01 mol, 2.03 g) yields 4-(4-isopropylbenzylideneamino)-2,3-dimethyl-1-phenyl-1,2-dihydropyrazol-5-one in ethanol. The reaction proceeds in the presence of glacial acetic acid. Reflux the reaction mixture for 4 hrs. Completion of reaction was confirmed using thin layer chromatography. Pale-yellow crystals, mp 138-140 °C, Yield (86%). UV-vis (λ_{max}): 316 nm. FT-IR (KBr, cm^{-1}) ν_{max} 1567 (C=N), lit FT-IR (KBr, cm^{-1}) ν_{max} 1605 (C=N)[46]. ^{1}H NMR (400 MHz, $CDCl_3$) ppm: δ 1.26-1.29 (m, 6H, gem-dimethyl), 2.48 (s, 3H, CH_3), 2.85-2.94 (m, 1H, CH), 3.14 (s, 3H, N-CH_3), 7.28-7.50 (m, 7H, ArH), 7.77-7.80 (m, 2H, ArH), 9.72 (s, 1H, CH=N), lit ^{1}H NMR (400 MHz, $CDCl_3$) ppm: δ 1.21-1.23 (m, 6H, gem-dimethyl), 2.50 (s, 3H, CH_3), 2.88-2.97 (m, 1H, CH), 3.34 (s, 3H, N-CH_3), 7.31-7.88 (m, 9H, ArH), 9.56 (s, 1H, CH=N).[46] ^{13}C NMR (400 MHz, $CDCl_3$) ppm: δ 10.13 (CH_3), 23.71 (gem-dimethyl), 34.16 (CH), 35.92 (N-CH_3), 118.90, 124.34, 126.50, 126.64, 126.83, 127.87, 129.16, 151.37, 151.88 (Aromatic carbons), 157.41 (CH=N), 160.96 (C=O).

Anal. Calcd for $C_{21}H_{23}N_3O$: C, 75.65; H, 6.95; N, 12.60. Found: C, 75.62; H, 6.93; N, 12.63.

N-(4-isopropylbenzylidene)-4H-1,2,4-triazol-4-amine (2j)

Cuminaldehyde (0.01 mol, 1.51 mL) and 4-amino-1,2,4-triazole (0.01 mol, 0.84) was dissolved in ethanol (20 ml) and refluxed for 4 hrs, cooled and poured into crushed ice. The precipitate obtained was recrystallized using ethanol. Monitoring of reaction progress was done using thin layer chromatography. Off-white crystals, mp 154-156 °C, Yield (82%). UV-vis (λ_{max}): 292 nm. FT-IR (KBr, cm^{-1}) ν_{max} 1608 (C=N). ^{1}H NMR (400 MHz, $CDCl_3$) ppm: δ 1.23-1.25 (m, 6H, gem-dimethyl), 2.89-2.94 (m, 1H, CH), 7.32-7.34 (d, 2H, ArH), 7.67-7.69 (m, 2H, ArH), 8.37-8.39 (d, 2H, triazole ring), 11.95 (s, 1H, CH=N). ^{13}C NMR (400 MHz, $CDCl_3$) ppm: δ 23.67 (gem-dimethyl), 34.27 (CH), 127.16, 128.87, 129.33, 128.60, 138.69 (Aromatic carbons), 154.28 (triazole ring), 157.67 (CH=N).

Anal. Calcd for $C_{12}H_{14}N_4$: C, 67.27; H, 6.59; N, 26.15. Found: C, 67.24; H, 6.63; N, 26.13.

(N^1E,N^2E)-N^1,N^2-bis(4-isopropylbenzylidene)benzene-1,2-diamine (2k)

0.02 Mole *i.e.* 3.02 mL of cuminaldehyde was mixed with *o*-phenylenediamine (0.01 mole, 1.08 g) in ethanol in the presence of catalytic amount of glacial acetic acid. Reflux the reaction content for four hrs. Check the progress the reaction using TLC. A light brown colored solid product was obtained in good yields. Brown crystals, mp 192-194 °C, Yield (87%). UV-vis (λ_{max}): 375 nm. FT-IR (KBr, cm^{-1}) ν_{max} 1586 (C=N). ^1H NMR (500 MHz, $CDCl_3$) ppm: δ 1.17-1.29 (m, 12H, gem-dimethyl), 2.53-2.99 (m, 2H, CH), 7.14-7.72 (m, 10H, ArH), 8.10-8.15 (m, 2H, ArH), 12.71 (s, 2H, CH=N). ^{13}C NMR (400 MHz, $CDCl_3$) ppm: δ 23.27 (gem-dimethyl), 38.52 (CH), 110.22, 114.49, 124.25, 127.97, 131.83, 134.33, 159.27 (Aromatic carbons), 167.61 (CH=N).

Anal. Calcd for $C_{26}H_{28}N_2$: C, 84.74; H, 7.66; N, 7.60. Found: C, 84.70; H, 7.64; N, 7.66.

Synthesis of thiosemicarbazones (3a-3b)

1-(4-Isopropylbenzylidene)thiosemicarbazide (3a)

Cuminaldehyde (0.01 mol, 1.51 mL) and thiosemicarbazide (0.01 mol, 0.75 g) were taken in a round bottom flask using ethanol as a solvent. Add 5 drops of glacial acetic acid and reflux the contents for 3 hrs. On cooling, white colored product was obtained which was filtered and dried. Completion of reaction was confirmed using TLC. White crystals, mp 146-148 °C, lit mp 144-147 °C, Yield (89%). UV-vis (λ_{max}): 305 nm. FT-IR (KBr, cm^{-1}) ν_{max} 1590 (C=N), lit FT-IR (KBr, cm^{-1}) ν_{max} 1590.[44] ^1H NMR (400 MHz, $CDCl_3$) ppm: δ 1.25-1.27 (d, 6H, gem-dimethyl), 2.90-2.95 (m, 1H, CH), 6.38 (bs, 2H, NH_2), 7.23-7.28 (m, 2H, ArH), 7.56-7.59 (m, 2H, ArH), 7.84 (s, 1H, NH), 9.57 (s, 1H, CH=N). ^{13}C NMR (400 MHz, $CDCl_3$) ppm: δ 23.75 (gem-dimethyl), 34.16 (CH), 127.02, 127.63, 130.59, 144.29 (Aromatic carbons), 152.19 (CH=N), 178.21 (C=S).

Anal. Calcd for $C_{11}H_{15}N_3S$: C, 59.69; H, 6.83; N, 18.99; S, 14.49. Found: C, 59.65; H, 6.80; N, 19.05; S, 14.50, lit Anal. Calcd for $C_{11}H_{15}N_3S$: C, 59.5; H, 6.2; N, 18.9; S, 14.4. Found: C, 59.7; H, 6.8; N, 18.9; S, 14.5.[44]

1-(4-Isopropylbenzylidene)-4-phenylthiosemicarbazide (3b)

1-(4-isopropylbenzylidene)-4-phenylthiosemicarbazide was synthesized by reacting phenylthiosemicarbazide with cuminaldehyde. Phenylthiosemicarbazide was synthesized by reacting equimolar amounts of phenylisothiocyanate (0.01 mole, 1.20 mL) and hydrazine hydrate (0.01 mole, 0.48 mL). The reaction gets completed in 5 mins and a white colored solid product was obtained. Wash the product with water in order to remove excess hydrazine hydrate. Filter and dry the product and use it for the synthesis of thiosemicarbazones.

In 250 mL rounded bottomed flask, equimolar amount of cuminaldehyde (0.01 mole, 1.51 mL) and phenylthiosemicarbazide (0.01 mole, 1.67 g) was added in ethanol. Add 5 drops of glacial acetic acid.

Reflux the reaction mixture for 3 hrs. Rate of reaction was calculated using TLC. White colored solid product was obtained after recrystallization from ethanol.
White crystals, mp 140-142 °C, lit mp 140-142 °C, Yield (90%). UV-vis (λ_{max}): 323 nm. FT-IR (KBr, cm^{-1}) ν_{max} 1591 (C=N). ^{1}H NMR (500 MHz, $CDCl_3$) ppm: δ 1.26-1.28 (d, 6H, gem-dimethyl), 2.92-2.97 (m, 1H, CH), 7.25-7.29 (m, 2H, ArH), 7.40-7.44 (m, 2H, ArH), 7.60-7.90 (m, 5H, ArH), 9.21 (s, 1H, NH), 9.88 (s, 1H, CH=N). ^{13}C NMR (400 MHz, $CDCl_3$) ppm: δ 23.88 (gem-dimethyl), 39.17 (CH), 113.69, 116.33, 118.99, 123.88, 130.82, 135.08, 139.49, 152.82 (Aromatic carbons), 164.53 (CH=N), 176.63 (C=S).

Anal. Calcd for $C_{17}H_{19}N_3S$: C, 68.65; H, 6.44; N, 14.13; S, 10.78. Found: C, 68.68; H, 6.46; N, 14.17; S, 10.69, lit Anal. Calcd for $C_{17}H_{19}N_3S$: C, 68.5; H, 6.2; N, 13.9; S, 10.7. Found: C, 68.6; H, 6.4; N, 14.1; S, 10.8.[44]

Synthesis of hydrazones (4a-4c)

1-(4-Isopropylbenzylidene)-2-phenylhydrazine (4a)

In the round bottom flask of 250 ml, cuminaldehyde (0.01 mole, 1.51 mL) was dissolved in an ethanol. Add equimolar amount of phenylhydrazine (0.01 mole, 0.98 mL) along with catalytic amount of glacial acetic acid in it. Reflux the reaction mixture for 1 hr. Progress of reaction was monitored by TLC. Pour the hot reaction mixture on crushed ice. Brown color precipitates were formed, filtered and dried. Brown crystals, mp 110-112 °C, Yield (70%). UV-vis (λ_{max}): 369 nm. FT-IR (KBr, cm^{-1}) ν_{max} 1617 (C=N). ^{1}H NMR (500 MHz, $CDCl_3$) ppm: δ 1.25-1.29 (m, 6H, gem-dimethyl), 2.88-2.94 (m, 1H, CH), 4.01 (s, 1H, NH), 6.86-7.59 (m, 9H, ArH), 7.67 (s, 1H, CH=N). ^{13}C NMR (500 MHz, $CDCl_3$) ppm: δ 23.89 (gem-dimethyl), 34.02 (CH), 112.72, 119.93, 126.25, 126.71, 129.24, 132.98, 137.54, 144.86 (Aromatic carbons), 149.49 (CH=N).

Anal. Calcd for $C_{16}H_{18}N_2$: C, 80.63; H, 7.61; N, 11.75. Found: C, 80.58; H, 7.63; N, 11.78.

1-(4-Isopropylbenzylidene)-2-(4-nitrophenyl)hydrazine (4b)

Synthesis of 1-(4-isopropylbenzylidene)-2-(4-nitrophenyl)hydrazine was done by dissolving cuminaldehyde (0.01 mole, 1.51 mL) with *p*-nitrophenylhdrazine (0.01 mol, 1.53 g) in ethanol. Subject the reaction mixture for refluxing after adding catalytic amount of glacial acetic acid. Advancement of reaction was checked by TLC. An orange colored crystalline solid compound was obtained in good yields. Orange crystals, mp 135-137 °C, Yield (87%). UV-vis (λ_{max}): 373 nm. FT-IR (KBr, cm^{-1}) ν_{max} 1612 (C=N). ^{1}H NMR (500 MHz, $CDCl_3$) ppm: δ 1.24-1.26 (d, 6H, gem-dimethyl), 2.91-2.94 (m, 1H, CH), 4.12 (s, 1H, NH), 7.25-7.26 (m, 4H, ArH), 7.39-7.41 (m, 2H, ArH), 7.51-7.53 (m, 2H, ArH), 8.40 (s, 1H, CH=N). ^{13}C NMR (500 MHz, $CDCl_3$) ppm: δ 23.22 (gem-dimethyl), 37.73 (CH), 118.87, 124.66, 129.77, 139.97, 144.29, 146.88, 150.50, 157.87 (Aromatic carbons), 160.60 (CH=N).

Anal. Calcd for $C_{16}H_{17}N_3O_2$: C, 67.83; H, 6.05; N, 14.83. Found: C, 67.79; H, 6.10; N, 14.86.

1-(4-Isopropylbenzylidene)-2-(2,4-dinitrophenyl)hydrazine (4c)

0.76 mL (0.005 mol) of cuminaldehyde was dissolved in 5 mL of 2, 4-dinitrophenylhydrazine solution. 2, 4 DNP solution was made by dissolving 1 g of 2,4-dinitrophenylhydrazine (0.005 mol) in 5 mL of concentrated H_2SO_4 and then slowly add this solution to a solution of 7.0 mL water in 25 mL 95% ethanol. In a 100 mL beaker, dissolve 0.74g of cuminaldehyde in ethanol and add above prepared 2,4 DNP solution to it. Dark orange colored precipitates were obtained. Wash the precipitates with excess of water, then filter and dry the precipitates. Single spot on the chromatographic plate confirmed the formation of product. Red crystals, mp 140-142 °C, Yield (92%). UV-vis (λ_{max}): 390 nm. FT-IR (KBr, cm^{-1}) ν_{max} 1651 (C=N). ^1H NMR (500 MHz, $CDCl_3$) ppm: δ 1.28-1.30 (d, 6H, gem-dimethyl), 2.96-2.99 (m, 1H, CH), 7.32-7.34 (m, 2H, ArH), 7.69-7.71 (m, 2H, ArH), 8.08-8.11 (m, 2H, ArH), 8.34-8.37 (m, 1H, ArH), 9.15 (s, 1H, CH=N), 11.30 (s, 1H, NH). ^{13}C NMR (500 MHz, $CDCl_3$) ppm: δ 23.76 (gem-dimethyl), 34.23 (CH), 116.77, 123.54, 127.18, 127.78, 129.29, 130.01, 130.77, 138.13, 144.87, 148.09 (Aromatic carbons), 152.53 (CH=N).

Anal. Calcd for $C_{16}H_{16}N_4O_4$: C, 58.53; H, 4.91; N, 17.06. Found: C, 58.57; H, 4.93; N, 17.08.

CHO
R-NH_2 CH_3COOH
NH_2 NH_2 CH_3COOH
H_2N S R' N H N H CH_3COOH
H_2N-N(H)-R" CH_3COOH
HC=N-R
(2a-2j)
N N
(2k)
HC=N-NH-C(=S)-NH-R'
(3a-3b)
HC=N-NH-R"
(4a-4c)

Biological activities

Antioxidant activity

The free radical scavenging activity of the compounds was estimated by their capacity to bleach the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH).[59] DPPH gives a strong absorption band at 517 nm in the visible region because of its odd electronic structure. Different concentrations (3000, 2000, 1000, 500, 250, 100, 50 and 25 µg mL^{-1}) of test compounds (40 µL) and ascorbic acid (40 µL), dissolved in methanol, were added to 3.96 mL of the DPPH solution. After 30 min, the absorbance was read at 517 nm at room temperature, and the scavenging activity was calculated as percentage of the radical reduction.

Each experiment was executed thrice. Methanol was used as control and ascorbic acid was used as standard. The radical scavenging activity was obtained as follows:

$$\text{Radical Scavenging Activity (\%)} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

Where $A_{control}$ and A_{sample} were the absorbances of blank (methanol) and test compounds, respectively at 517 nm.

Antimicrobial activity

Authentic pure cultures of bacteria namely *Pseudomonas sp*. *Klebsiella sp.*and *Enterobacter sp.* was procured from Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. Different synthesized compounds along with standard Ampicilin were screened for their antimicrobial potential at different concentrations (3000, 2000, 1000, 500, 250, 100, 50 and 25 μg mL^{-1}) against three mentioned gram negative bacteria by bacterial sensitivity - filter paper disc method on nutrient agar medium.[60] The nutrient broth medium (Himedia, 24 g, Mumbai, India) was suspended in distilled water (1000 mL) and heated until it dissolved completely. The medium was autoclaved at a pressure of 15 Pascal (Pa) for 20 min and poured in petri plates. After the setting of nutrient agar medium, nutrient broth containing test bacteria was inoculated on petri plates. The culture of bacteria was spread properly. Sterile discs having diameter 6 mm were treated with different synthesized compounds of different concentrations and then placed on a petri plate having solidified nutrient agar medium. The plates were incubated for 24 hrs in an incubator at 37 ºC and the inhibition zone around the disc was measured in mm. Each treatment was replicated thrice. MIC (Minimum Inhibition Concentration) values of synthesized products were determined.

Acknowledgement

The authors are thankful to Pulses microbiology laboratory, Department of plant breeding and genetics, Punjab Agricultural University, Ludhiana for providing the pure cultures of bacteria.

References:

1. S. Patil, S. D. Jadhav and U. P. Patil, *Arch. Appl. Sci. Res.*, **4**, 1074 (2012).
2. D. A. Dias, S. Urban and U. Roessner, *Metabolites*, **2**, 303 (2012).
3. B. Bozin, N. Mimica-Dukic, I. Samojlik, A. Goran and R. Igic, *Food Chem.*, **111**, 925 (2008).
4. A. N. Panche, A. D. Diwan and S. R.Chandra, *J. Nutr. Sci.*, **5**, 1 (2016).
5. D. E. Fenwick and D. Oakenfull, *J. Sci. Food Agric.*, **34**, 186 (1983).
6. B. S. N. Rao and T. Prabhavathi, J. Sci. Food Agric., **33**, 89 (1982).
7. N. Bribi, *Asian J. Bot.*, **1**, 1 (2018).
8. M. Nadeem and A. Riaz, *Pak. J. Food Sci.*, **22**, 101 (2012).
9. M. Sellamia, B. Ghariania, H. Louatia, N. Mileda and Y. Gargouri, *Int. J. Curr. Eng. Technol.*, **3**, 1051 (2015).
10. Q. Liu, X. Meng, Y. Li, C. Zhao, G. Tang and H. Li, *Int. J. Mol. Sci.*, **18**, 1283 (2017).
11. M. S. Al-Said, K. I. Al-Khamis, M. W. Islam, N. S. Parmar, M. Tariq and A. M. Ageel, *J. Ethnopharmacol*, **21**, 165 (1987).

12. R. S. Beji, S. Khemir, W. A. Wannes, K. Ayari and R. Ksouri, *Braz. J. Pharm. Sci.*, **54**, e17576 (2018).
13. M. Anitha, K. Rajalakshmi, S. Muthukumarasamy and V. R. Mohan, *Int. J. Pharm. Pharm. Sci.*, **4**, 490 (2012).
14. V. Chithra and S. Leelamma, *Plant. Foods. Hum. Nutr.*, **51**, 167 (1977).
15. N. Rekha, R. Balaji and M. Deecaraman, *J. Appl. Biosci.*, **28**, 1718 (2010).
16. S. Nanasombat and P. Wimuttigosol, *Food Sci. Biotechnol.*, 20, 45 (2011).
17. M. S. Amiri and M. R. Joharchi, *Avicenna J. Phytomed.*, **6**, 621 (2016).
18. A. R. Gohari and S. Saeidnia, *PHCOG J.*, **3**, 1 (2011).
19. A. M. Hasan, A. A. Sakure, R. S. Fougat and S. Kumar, *International J. Seed Spices*, **6**, 96 (2016).
20. R. Johri, *Pharmacogn. Rev.*, **5**, 63 (2011).
21. D. Kumar, K. Ganguly, H. V. Hegde, P. A. Patil and S. D. Kholkute, *J. Ayurveda Integr. Med.*, **6**, 194 (2015).
22. A. Nostro, L. Cellini, S. Di Bartolomeo, E. Di Campli, R. Grande, M. A. Cannatelli, L. Marzio, V. Alonzo, *Phytother Res.*, **19**, 198 (2005).
23. N. Vador, A. G. Jagtap, A. Damle, *Indian J Pharm Sci.*, **74**, 387 (2012).
24. S. Dhandapani, V. R. Subramanian, S. Rajagopal and N. Namasivayam, *Pharmacol Res.*, **46**, 251 (2002).
25. H. Hajlaoui, H. Mighri, E. Noumi, M. Snoussi, N. Trabelsi, R. Ksouri and A. Bakhrouf, *Food Chem. Toxicol.*, **48**, 2186 (2010).
26. T. Allahghadri, I. Rasooli, P. Owlia, M. J. Nadooshan, T. Ghazanfari, M. Taghizadeh, S. D. A. Astaneh, *J. Food Sci.*, **75**, H54 (2010).
27. A. Kedia, B. Prakash, P. K. Mishra, N. K. Dubey, *Int. J. Food Microbiol.*, **168**, 1 (2014).
28. M. Janahmadi, F. Niazi, S. Danyali and M. Kamalinejad, *J. Ethnopharmacol*, **104**, 278 (2006).
29. F. K. R. Oroojalian, M. Azizi and M. R. Bassami, *Food Chem.*, **120**, 765 (2010).
30. N. Rasheehan, H. Iftikhar, T. Abdul, T. Muhammad, R. Moazur, H. Sohail, M. S. Mahmood, A. B. Siddique and M. Iqbal, *BMC Complement Altern. Med.*, **13**, 265 (2013).
31. M. M. Suleimana, L. J. McGaw, V. Naidoo, J. N. Eloff, *Afr. J. Tradit Complement Altern. Med.*, **7**, 64 (2009).
32. T. Sekine, M. Sugano, A. Majid and Y. Fujii, *J. Chem. Ecol.*, **33**, 2123 (2007).
33. G. Philippe, L. Angenot, P. D. Mol, E. Goffin, M. P. Hayette, M. Tits and M. Frederich, *J. Ethnopharmacology*, **97**, 535 (2005).
34. V. Woguem, H. P. Fogang, F. Maggi, L. A. Tapondjou, H. M. Womeni, L. Quassinti, M. Bramucci, L. A. Vitali, D. Petrelli, G. Lupidi, F. Papa, S. Vittori, L. Barboni, *Food Chem.*, **149**, 183 (2014).
35. S. Tamura, B. K. Kubata, S. Itagaki, T. Horii, M. K. Taba and N. Murakami, *Bioorg. Med. Chem. Lett.*, **20**, 1520 (2010).
36. L. A. A. Barakat, *J. Appl. Sci. Res.*, **7**, 1368 (2011).
37. Y. Gambin, V. VanDelinder, A. C. Ferreon,; E. A. Lemke, A. Groisman and A. A. Deniz, *Nat Methods*, **8**, 239 (2011).
38. J. Burre, M. Sharma and T. C. Sudhof, *J. Neurosci.*, **35**, 5221 (2003).
39. K. Tsai, Y. Liu, T. Chen, S. Yang, H. Wong, J. Cherng, K. S. Chou and J. M. Cherng, *Nutrients*, **8**, 318 (2016).
40. Shu-meiang, K. Tsai, H. Wong, Y. Liu, T. Chen, J. Cherng, K. Hsu, Y. Ang, J. Cherng, *J. Cancer*, **7**, 251 (2016).

41. H. S. Lee, *J. Agric. Food Chem.*, **53**, 2446 (2005).
42. D. Kannan and L. Pari, *Int. J. Multidiscip. Res. Dev.*, **4**, 201 (2017).
43. K. S. Girisha and B. Kalluraya, *Synth. Commun.*, **42**, 3097 (2012).
44. M. P. Krishna and K. H. Reddy, *Mediterr. J. Chem.*, **6**, 88 (2017).
45. D. Arish, and M. S. Nair, *J. Mol. Struct.*, **983**, 112 (2010).
46. R. B. Bennie, S. T. David, M. Sivasakthi, S. A. Mary, M. Seethalakshmi, S. D. Abraham, C. Joel, R. Antony, *Chem. Sci. Trans.,* **3**, 937 (2014).
47. P. K. Kaushik, V. K. Varshney, P. Kumar, P. Bhatia and S. V. Shukla, *Synth. Commun.*, **46**, 2053 (2016).
48. S. D. Saraf, *Synth. Commun.*, **13**, 7 (1983).
49. S. Rollas, *J. Res. Pharm.*, **14**, 41 (2010).
50. P. Gaikwad, A. Barik, K. I. Priyadarsini and B. S. M. Rao, *Res. Chem. Intermed.*, **36**, 1065 (2010).
51. E. Bendary, R. R. Francis, H. M. G. Ali, M. I. Sarwat and S. El Hady, *Ann. Agric. Sci.*, **58**, 173 (2013).
52. M. Kumari, R. Gupta and Y. Jain, *Synth. Commun.*, **49**, 529 (2019).
53. F. Pourmorad, S. J. Hosseinimehr and N. Shahabimajd, *Afr. J. Biotechnol.,* **5**, 1142 (2006).
54. G. Kaur, S. Sharma, P. Kaur, G. Gaba, *Pestic. Res. J.*, **31**, 74 (2019).
55. S. A. F. Rostom, H. M. A. Ashr, H. A. Abd El Razik, *Arch. Pharm. Chem. Life Sci.,* **342**, 299 (2009).
56. B. S. Holla, M. Mahalinga, M. S. Karthikeyan, P. M. Akberali and N. S. Shetty, *Bioorgan. Med. Chem.*, **14**, 2040 (2005).
57. A. K. Nandi, S. Chaudhuri, S. K. Mazumdar and S. Ghosh, *J. Chem. Soc. Perkin Trans. 1*, **2**, 1729 (1984).
58. S. Ortiz, R. Nelson, V. Kesternich, M. Pérez-fehrmann, P. Christen and L. Marcourt, *J. Chil. Chem. Soc.*, **61**, 3081 (2016).
59. M. S. Blois, *Nature*, **181**, 1199 (1958).
60. S. Joshi, S. Sharma, J. Gaba and P. Kaur, *Indian J. Heterocyc. Chem.*, **28**, 459 (2018).

Ekta Computers <ektacomputersldh@gmail.com>

Fwd: Natural Product Research - Your Manuscript ID is GNPL-2020-2373

pardeep kaur <pardip2493@gmail.com> Sun, Jan 10, 2021 at 12:39 PM
To: ektacomputersldh@gmail.com

---------- Forwarded message ---------
From: **Natural Product Research** <onbehalfof@manuscriptcentral.com>
Date: Thu, Aug 27, 2020 at 5:21 PM
Subject: Natural Product Research - Your Manuscript ID is GNPL-2020-2373
To: <pardip2493@gmail.com>
Cc: <pardip2493@gmail.com>, <sunita_sharma@pau.edu>, <jyotgcw@gmail.com>, <tanvisahni1994@gmail.com>

*******This is an automated email generated by the Natural Product Research ScholarOne Manuscripts site*******

27-Aug-2020

Dear Miss Kaur:

Thank you for submitting your manuscript entitled "Comprehensive phytochemical profile of <i>Cuminum cyminum</i> seeds and their bioactivity" to our ScholarOne Manuscripts site.

Your manuscript ID is GNPL-2020-2373.

This manuscript ID should be used in all future correspondence with the journal office and editors. Please do not hesitate to contact the journal office if you have any queries about your paper.

You can view the status of your manuscript at any time by checking your Author Centre after logging in to https://mc.manuscriptcentral.com/gnpl.

Thank you for submitting your manuscript to Natural Product Research.

Sincerely,
Natural Product Research Editorial Office

* **Author-Centre-ManuscriptStatus.docx**
112K

Phytochemical profile of different solvent seed extracts of *Cuminum cyminum* using GC-MS and their bioactivity

Running title: Phtochemical profile of cumin seeds extracts and their bioactiviy

Pardeep Kaur[a], Sunita Sharma[b], Jyoti Gaba[a] and Tanvi Sahni[a]

[a]Department of Chemistry, Punjab Agricultural University, Ludhiana-141004, India
[b]Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana-141004, India
[#]Corresponding author. Email: sunita_sharma@pau.edu

Abstract

Due to hazardous effects and lack of biodegradation by micro-organisms, there is a growing interest of scientists to replace synthetic chemicals by natural bioactive molecules. As far as cumin is concerned, studies are lacking addressing chemical composition, phytochemical profile and bioactivity of different extracts of the cumin. The present study was conducted to determine the GC-MS profile, qualitative, quantitative study of different solvent extracts *i.e.* acetone, petroleum ether, water, methanol, ethanol, chloroform, dichloromethane and ethyl acetate of *Cuminum cyminum* (cumin) seeds. Total of 31 compounds were identified in solvent extracts. The qualitative phytochemical analysis showed that different solvent extracts contained Alkaloids, saponins, tannins, coumarins, amino acids, flavonoids, proteins, phenols and carbohydrates. Quantitative analysis was also conducted to determine the amount of alkaloids, flavonoids, phenols and tannins present in different extracts. Solvent extracts were evaluated for their antioxidant potential *via* DPPH assay. Antimicrobial activity against three gram-negative bacteria was also studied.

Keywords: *Cuminum cyminum,* GC-MS, phytochemicals, antioxidant, antimicrobial

1. Introduction

Traditionally used medicinal plants have recently attracted the attention of the pharmaceutical and scientific communities (Taylor et al. 2001). According to World Health Organization (WHO), medicinal plants are the richest bio-resource to obtain a variety of drugs (Ncube et al. 2008). Sawant and Godghate (2013) reported that in developed countries, about 80% of individuals used traditional medicines, that contains compounds derived from medicinal plants. The evaluation of all the drugs is based on phytochemical and pharmacological approaches which leads to the drug discovery referred as natural product screening (Prajapati et al. 2019). These active compounds (phytochemicals) are the chemicals produced by various parts of the plants such as bark, leaves, flowers, roots and seeds (Devanaboyina et al. 2013).

Prevention of many diseases is associated with ingestion of different parts of the plants which are rich in natural antioxidants (Atrooz 2009). It was observed that higher intake of these compounds lowers the risk of mortality from different diseases (Ajih and Janardhanan 2002). There are many phytochemicals which may act as a powerful antioxidants such as polyphenols (Argolo et al. 2004), flavonoids (Zhang and Wang 2002) etc. by neutralizing free radicals or by removing their power to create damage. Antioxidants can interfere with the oxidation process by reacting with **free radical**s, chelating catalytic metals, acting as oxygen scavengers and prevent lipid auto oxidation (Bondet et al. 1997). The antimicrobial properties of essential oil of plants and its extracts have been assessed (Lis-Balchin and Deans 1997), reviewed (Rios et al. 1987) and it was found that phytochemicals have potential applications in medical procedures, cosmetics, pharmaceuticals and food industries (Iocabellis et al. 2005; Youdin et al. 1999).

Plants of Apiaceae family possess a wide range of compounds with numerous biological activities (Ghasemi et al. 2018). Cumin (*Cuminum cyminum*) is an annual herbaceous plant belonging to the family

of Apiaceae genus *Cuminum* (Gohari and Saeidnia 2011). Cumin is a spice and in India, it is mainly cultivated in Rajasthan and Gujarat. It's seeds are widely consumed as aromatic food spice having antiglycative and antidiabetic properties (Patil et al. 2013; Zhang et al. 2015). Cumin seeds are extensively used in various ayurvedic medicines also especially for the conditions like obesity, stomach pain and dyspepsia (Srinivasan 2018). Phytochemical analysis of cumin seeds showed that it contains alkaloids, anthraquinones, coumarins, flavonoids, glycosides, proteins, resins, saponins, tannins and steroids etc (Rai et al. 2012). Secondary metabolites or phytochemicals, are biologically active naturally occurring chemical compounds and plays an important role in defence system of plants against predation by microorganisms, insects and herbivores (Santhi and Sengottuvel 2016). These phytochemicals have historically been used as pharmaceuticals, fragrances, flavor compounds, dyes, and agrochemicals (Rathore et al. 2013). *In vitro* studies reported that phytochemicals such as phenolic compounds have potential role against different diseases and used as anti-inflammatory, anti-mutagenic, antiviral and antibacterial agents (Al Juhaimi and Ghafoor 2013). Cumin extracts are reported to possess antiallergic, antioxidant, antiplatelet aggregation and hypoglycaemic properties (Allahghadri et al. 2010). Ethanol extract of seed exhibited antimicrobial activity against biofilm *Escherichia coli*. Therefore in this way, qualitative phytochemical analysis will help us to understand a variety of chemical compounds produced by plants and quantification of those metabolites will help to extract, purify and identify the bioactive compounds for useful aspects to human beings.

Keeping in view the importance of chemical compounds present in different parts of the plant, the present study was conducted to study chemical composition, qualitative and quantitative estimation of different solvent extracts along with estimation of their potential to scavenge DPPH free radical. Different solvent extracts were also evaluated for their antimicrobial potential against three gram negative bacteria *i.e. Pseudomonas sp.*, *Klebsiella sp.* and *Enterobacter sp.* by bacterial sensitivity - filter paper disc method.

2. Results and discussion

2.1 Chemical composition of different solvent extracts

Total of thirty one compounds were identified in different solvent extracts of *Cuminum cyminum* (Table 1), accounting 99.6-100.0% of the total composition. Minimum number of compounds were identified in ethyl acetate and ethanolic extracts. These extracts were mainly composed of monoterpene hydrocarbons (22.43-27.28%), oxygenated monoterpenes (63.60-68.81%), acids and esters (1.17-4.81%), aldehydes and ketones (1.17-4.81%), sesquiterpene hydrocarbons (0.41-8.76%) and saturated hydrocarbons (0.74-1.55%) (Table 2). Additionally, Sarverogenin (6.08-6.79%) represented a substantial fraction of different solvent (acetone, methanol, ethanol) extracts. Al-Rubaye and his co-workers (2017) reported the chemical

composition of methanolic extract of cumin seed, collected from local market of Hilla, Iraq (Al-Rubaye et al. 2017). The differences in the chemical composition of extracts may be related to distinct environmental and climatic conditions (Bisht et al. 2009) such as light (Johnson et al. 1999), precipitation, growing site (Satta et al. 1999), temperature (Farhat et al. 2001; Santos-Gomes and Fernandes-Ferreira 2001) and soil (Pala-Paul et al. 2008); seasonal sampling periods, geographic origins, plant populations (Barra 2009), vegetative plant phases (Farhat et al. 2001; Santos-Gomes and Fernandes-Ferreira 2001) extraction and quantification methods (Figiel et al. 2010). Light (Peer and Langenheim 1998) and water (Taveira et al. 2003) are responsible for the increasing concentration of monoterpenes and phenyl propanes and resulted in increasing the concentration of monoterpenes.

2.2 Phytochemical analysis

Qualitative and quantitative analysis of different solvent seed extract was carried out and the results are presented in Table 3 and 4, respectively. In addition to this, per cent yield of the different solvent extracts is also calculated. Percent yield of different extracts is represented in Figure 1 and follows the order: Water > Dichloromethane > Chloroform > Methanol > Ethyl acetate > Acetone > Ethanol > Petroleum ether. Yield was found to be highest in aqueous extract (14.6%) and lowerest in petroleum ether extract (4.2%). methanol followed by in all the solvents used for extraction (Figure 1).

2.2.1 Qualitative phytochemical analysis

Phytochemicals are secondary metabolites produced by plants that plays diverse roles such as defense against animal predators, insect's invasion, provides strengths to plants and attracts the insects for pollination. In the present study, different solvent seed extracts were evaluated for the presence or absence of some phytochemicals such as alkaloids, saponins, tannins, anthocyanins, emodins, coumarins, amino acids, flavonoids, proteins, phenols and carbohydrates (Table 3). Present study revealed that aueous, methanolic and ethanolic extracts are rich in phytochemicals *i.e.* they contain saponins, tannins, coumarins, amino acids, flavonoids, proteins, phenols and carbohydrates. However, none of the above mentioned phytochemicals were detected in acetone and ethyl acetate extracts. Some of the extracts contained only one or two phytochemicals such as petroleum ether (carbohydrates), dichloromethane (flavonoids) and chloroform (saponins and flavonoids). Alkaloids are present only in aqueous extract and there about 30 alkaloids reported in literature that are of commercial interest primarily due to their medicinal and flavoring properties (Bribi 2018). It has been found that none of the solvent extract contains anthocyanins as they are mostly present in fruits and vegetables only. Similar results were reported by Malik et al. 2020. These phytochemicals possess wide range of biological activities such as anti-inflammatory, antioxidant and antimicrobial (Bozin et al. 2008; Dimitrios 2006; Fu et al. 2017). Phenols, tannins and flavonoids are considered as natural antioxidants and protect plants from oxidative stress. They have the ability to chelate metals and inhibit oxidases reactions.

2.2.2 Quantitative phytochemical analysis

Quantitative phytochemical analysis was done for the estimation of phenolics, flavonoids, saponins and tannins content in different solvent seed extracts and the results are presented in Table 4. Analysis of data revealed that phenolics, flavonoids and saponins contents were higher in methanolic extract, however tannin content was higher in aqueous extract. The total phenolic content in different solvent seed extracts follows the order: Methanol > water > ethanol > ethyl acetate > petroleum ether > dichloromethane > chloroform > acetone (Table 4). Results were expressed as mg GAE g^{-1} dwb ± SD using the gallic acid as standard. Phenolic compounds have oxidation-reduction properties which play a substantial role in inhibition and termination of free radicals, quenching singlet and triplet oxygen or degrading peroxides (Yingming et al. 2004) that accounts for its antioxidant potential (Soobrattee 2005).

Flavonoids, inclusive of flavones and flavanols are phytochemicals, the antioxidant potential of which is governed by the presence of free hydroxyl groups. Functional hydroxyl groups in flavonoids mediate their antioxidant effects by scavenging free radicals and/or by chelating metal ions (Kumar et al. 2013; Kumar and Pandey 2013). Flavonoids are known to possess *in vitro* as well as *in vivo* antioxidant activity (Geetha et al. 2003). Total flavonoids determined in different solvent extracts of cumin seeds are expressed as mg QE g^{-1} dwb ± SD using quercetin as standard (Table 4) and follows the trend: methanol > water > ethanol > chloroform > dichlromethane > ethyl acetate > acetone > petroleum ether.

Saponins are glucosides with foaming characteristics. They possess wide range of biological activities such as anitidiabetic, antifungal, antitumor activity, antiviral, antiparasitic, immunomodulatory, synthesis of hormones, acting on the cardiovascular system, acting on the central nervous and endocrine systems (Barbosa 2014). Tannins are polyphenolic plant metabolites that form complexes with alkaloids and proteins and protect plants from the attack of predators (Khanbabaee and van Ree 2001). The present study revealed that saponins and tannins contents were higher in methanolic and aqueous extract, respectively. Saponins and tannins content in seed solvent extracts follows the order: methanol > water > ethanol > chloroform > acetone > ethyl acetate > petroleum ether > dichloromethane and water > methanol > ethanol > ethyl acetate > dichloromethane > chloroform > petroleum ether > acetone, respectively . Data for quantitative estimation of saponins and tannins in different solvent extracts of cumin seeds is expressed as mg SE g^{-1} dwb ± SD and mg TAE g^{-1} dwb ± SD, respectively (Table 4).

2.3 Antioxidant activity

Phenolics and flavonoids are naturally occurring compounds produced in plants from aromatic amino acid phenylalanine, tyrosine and malonate. In plants they act as antioxidants, antimicrobials and photoreceptors. They are considered as natural antioxidants (Nadeem and Riaz 2012; Panche et al. 2016;

Pietta 2000). The antioxidant potential of the flavonoids is due to their ability to reduce the formation of free radicals and to scavenge the free radicals. Mechanisms of antioxidant action include suppression of reactive oxygen species formation either by inhibition of enzymes (cyclooxygenase, lipoxygenase, microsomal monooxygenase, glutathione S-transferase, mitochondrial succinoxidase, and NADH oxidase) (Halliwell and Gutteridge 1998; Brown et al. 1998) or chelating trace elements involved in free radical production, by scavenging reactive oxygen species and upregulating or protecting antioxidant defenses (Hanasaki et al. 1994; Ursini et al. 1994). The antioxidant potential of the phenolic compounds is due to their ability to terminate free radicals (Pourmorad et al. 2006). These are regarded as free radical terminators. They react with high-energy lipid radicals to convert them to thermodynamically more stable products (Wanasundara and Shahidi 1998). Therefore, antioxidant potential can be directly correlated with the amount of flavonoids and phenolics present in the plant extracts. Present study revealed that methanolic extracts bears highest antioxidant potential (at all the concentrations) among all the solvent extracts of cumin seeds (Table 5). It is evident from Table 4 that methanolic extract contains highest amount of phenolics and flavonoids which are responsible for its antioxidant potential (Teixeira et al. 2013). Antioxidant potential of the different solvent extracts is analyzed statistically by arc sine transformation using CPCS1 and significant differences are found between the activities of prepared extracts. Aqueous extract also exhibited comparable antioxidant potential and contained highest amount of phenolics and flavonoids after methanolic extract. RS value of methanolic and ethanolic extract (calculated from Figure 2) is 2100 and 2400, respectively. Antioxidant potential of different solvent extracts follows the order: methanol > water > ethanol > ethyl acetate > petroleum ether > acetone > dichloromethane > chloroform. This order is in accordance with the combination of amount of phenolics and flavonoids present in the different solvent extacts. None of the plant extracts registered more activity than the standard ascorbic acid.

2.4 Antimicrobial activity

Different solvent extracts of cumin seeds namely acetone, petroleum ether, water, methanol, dichloromethane, chloroform and ethyl acetate were subjected to antimicrobial evaluation against three gram negative bacteria *Pseudomonas sp. Klebsiella sp.* and *Enterobacter sp.* Statistical analyses revealed that the antimicrobial potential of different cumin seed extract differ significantly from each other. Data was analyzed by Factorial CRD using CPCS1. The results obtained *i.e.* values of inhibition zones and MIC values are graphically represented in Table 6 and Figure 3, respectively. It is evident from the results that the plant extracts does not display any significant microbial activity. All extracts are ineffective in inhibiting the growth of the three tested bacteria. Similar results are also reported by Shaik et al. 2018. Out of all solvent extracts, aqueous extract displayed maximum antimicrobial activity at all the tested concentrations against three tested bacteria. Dichloromethane and ethylacetate extracts were least

effective among all the extracts against all the bacteria. None of the extract was found to be as effective as standard ampicillin at any of test concentrations.

3. Experimental

3.1 Collection of cumin seeds

One kilogram cumin seeds of variety GC-4 were taken from Rajasthan State Seed and Organic Production Certification Agency, Jaipur. The sample was cleaned and washed to remove extraneous matter, dried in open air and then in oven at 50 ºC, kept in air tight glass containers at 4 ºC for further use.

3.2 Extraction procedure

For extraction of phytochemicals from different solvent extract, 5 g of dried powdered seeds were mixed separately with 25 mL acetone, petroleum ether, water, methanol, ethanol, dichloromethane, chloroform and ethyl acetate and kept in refrigerator (4-5 °C) for 3-4 days with occasional stirring. After that extracts were filtered and solvents were removed by rotary vacuum evaporator and the concentrated extract was further evaporated to get dry powder. The dried powder was preserved in air tight culture tubes. The crude extracts thus obtained were used for further investigation of chemical composition, phytochemical screening, antioxidant and antimicrobial evaluation. Per cent yield of each extract was calculated and is presented in Figure 1.

3.3 Gas chromatography-Mass spectrometry (GCMS) of different solvent seed extracts (Makkar et al. 2018)

Different solvent seed extracts *i.e*. acetone, petroleum ether, water, methanol, ethanol, dichloromethane, chloroform and ethyl acetate of cumin seeds was analysed using Shimadzun Nexis GC-2030 gas chromatograph (GC) coupled with Shimadzu TQ8050 NX mass spectrometer (Shimadzu Corporation, Japan). Samples were injected using Shimadzu AOC-20i plus auto-injector and AOC-20s plus auto-sampler unit. The capillary column used was SH-Rxi-5Sil MS (5% diphenyl/95% dimethylpolysiloxane, Shimadzu) (30m × 0.25mm × 0.25µm). Helium was the carrier gas at a flow rate of 1.17 mL/min. Temperature program: initial temperature 50 ºC for 2 min; ramp at 3 ºC/min to 210 ºC and held for 2 min followed by next increase at the rate of 10 ºC/min to reach a temperature of 280 ºC which was held for 6 mins. Injection temperature: 260 ºC, injection mode: split, sample injection volume: 1 µL, split ratio: 20, Mass detector conditions, ion source temperature: 230 ºC, ionization mode: Electron impact (EI), 70 eV, interface temperature: 270 ºC, start time: 3 min and end time: 70 min. The optimization of retention times and chromatographic resolution were conducted in scan mode for identification of different solvent extracts. For peak identification, mass spectra were compared with mass spectra data available in NIST08, WILEY8, Perfumery and Flavor and Fragrance libraries.

3.4 Phytochemical identification test

3.4.1 Qualitative phytochemical analysis

Different solvent seed extracts were analyzed for the presence of various phytoconstituents such as alkaloids, saponins, tannins, coumarins, amino acids, flavonoids, proteins, phenols and carbohydrates. Phytochemical tests were carried out by adopting standard procedures (Sawant and Godghate 2013; Gaba et al. 2018).

3.4.2 Quantitative phytochemical analysis

Quantitative phytochemical analysis was also conducted to determine the amount of alkaloids, total flavonoids, total phenols and tannins present in different solvent extracts. The total phenolics, total flavonoids, saponins and tannins were determined by reported methods (Calabro et al. 2004; Ebrahimzadeh et al. 2008; Fenwick and Oakenfull 1983; Gaba et al. 2019).

3.5 Determination of antioxidant activity by DPPH radical scavenging method:

The free radical scavenging activity of the compounds was estimated by their capacity to bleach the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Blois 1958). DPPH gives a strong absorption band at 517 nm in the visible region because of its odd electronic structure. Different concentrations (3000, 2000, 1000, 500, 250, 100, 50 and 25 μg mL^{-1}) of test compounds (40 μL) and ascorbic acid (40 μL), dissolved in methanol, were added to 3.96 mL of the DPPH solution. After 30 min, the absorbance was read at room temperature, and the scavenging activity was calculated as percentage of the radical reduction. Each experiment was executed thrice. Methanol was used as control and ascorbic acid was used as standard. The radical scavenging activity was obtained as follows:

$$\text{Radical Scavenging Activity (\%)} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

Where $A_{control}$ and A_{sample} are the absorbances of blank and test compounds, respectively.

3.6 Determination of antimicrobial activity by filter paper disc method

Authentic pure cultures of bacteria namely *Pseudomonas sp. Klebsiella sp.*and *Enterobacter sp.* were procured from Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. Different solvent extracts *i.e.* acetone, petroleum ether, water, methanol, ethanol, dichloromethane, chloroform and ethyl acetate of cumin seeds along with the standard Ampicilin were screened for their antimicrobial potential at different concentrations (3000, 2000, 1000, 500, 250, 100, 50 and 25 μg mL^{-1}) against three mentioned gram negative bacteria by bacterial sensitivity - filter paper disc method on nutrient agar medium. Nutrient broth containing test bacteria was inoculated on petri plates having solidified nutrient agar medium. The culture of bacteria was spread properly. Sterile discs having diameter 6 mm were treated with different solvent extracts of different concentrations and then placed on a petri plate having solidified nutrient agar medium. The plates were incubated for 24 hrs in an incubator at 37 ºC and the inhibition zone around the disc was measured in mm. Each treatment was replicated thrice. MIC (Minimum Inhibition Concentration) values of different solvent extracts were determined.

4. Conclusion

Cuminaldehyde and 4-isopropyl cyclohexa-1,4-dienecarbaldehyde were the major compounds present in all the solvent extracts. Phytochemical study revealed that various phytochemicals *viz* alkaloids, saponins, tannins, coumarins, amino acids, flavonoids, proteins, phenols and carbohydrates were present in different solvent extracts. Methanolic and aqueous extracts were rich in flavonoids and phenolics which may be responsible for their antioxidant and antimicrobial potential.

5. Data analysis

All experiments were performed in triplicates. Antioxidant activity was expressed as the means with standard deviations (±SD) and data was analysed statistically by arc sine transformation using CPCS1. Statistical analysis of antimicrobial activity was done by Factorial CRD using CPCS1. Graphics were drawn using MS Office Excel 2010.

Acknowledgements

The authors are thankful to Rajasthan State Seed and Organic Production Certification Agency, Jaipur for providing the seeds of GC-4 variety and Pulses microbiology laboratory, Department of plant breeding and genetics for providing the pure cultures of bacteria.

Disclosure statement

The authors declare no conflicts of interest.

References:

Ajih TA, Janardhanan KK. 2002. Antioxidant and antihepato-toxic activities of *Phellinus rimosus* (Berk) pilat. J Ethnopharmacol. 81:387-391.

Al Juhaimi FY, Ghafoor K. 2013. Extraction optimization and *in vitro* antioxidant properties of phenolic compounds from Cumin (*Cuminum cyminum* L.) seed. Int Food Res J. 20(4):1669-1675.

Allahghadri T, Rasooli I, Owlia P, Nadooshan MJ, Ghazanfari T, Taghizadeh M. 2010. Antimicrobial property, antioxidant capacity and cytotoxity of essential oil from cumin produced in Iran. J Food Sci. 75:54-61.

Al-Rubaye AF, Kadhim MJ, Hameed IM. 2017. Phytochemical profiles of methanolic seeds extract of *Cuminum cyminum* using GC-MS technique. Int J Pharm Sci Rev Res. 8(2):114-124.

Argolo AC, Ana AES, Pletsch M, Coelho LC. 2004. Antioxidant activity of leaf extracts from *Bauhinia monandra*. Bioresour Technol. 95:229-233.

Atrooz OM. 2009. The antioxidant activity and polyphenolic contents of different plant seeds extracts. Pak J Biol Sci. 12(15):1063-1068.

Barbosa ADP. 2014. An overview on the biological and pharmacological activities of saponins. Int J Pharm Pharm Sci. 6(8):47-50.

Barra A. 2009. Factors affecting chemical variability of essential oils: a review of recent developments. Nat Prod Commun. 4(8): 1147-1154.

Bisht D, Chanotiya CS, Rana M, Semwal M. 2009. Variability in essential oil and bioactive chiral mono terpenoid compositions of Indian oregano (*Origanum vulgare* L.) populations from northwestern Himalaya and their chemotaxonomy. Ind Crops Prod. 30:422-426.

Blois MS. 1958. Antioxidant determinations by the use of stable free radical. Nature. 181:1199-1200.

Bondet V, Brand-Williams W, Berset C. (1997) Kinetics and mechanisms of antioxidant activity using the DPPH free radical method. Food Sci Technol. 30:609-615.

Bozin B, Mimica-Dukic N, Samojlik I, Goran A, Igic R. 2008. Phenolics as antioxidants in garlic (*Allium sativum* L., Alliaceae). Food Chem 111: 925-929.

Bribi N. 2018. Pharmacological activity of alkaloids: A review. Asian J Bot. 1: 1-7.

Brown JE, Khodr H, Hider RC, Rice-Evans C. 1998. Structural dependence of flavonoid interactions with Cu^{2+} ions: implications for their antioxidant properties. Biochem J. 330:1173-1178.

Calabro ML, Galtieri V, Cutroneo P, Tommasini S, Ficarra P, Ficarra R. 2004. Study of the extraction procedure by experimental design and validation of a LC method for determination of flavonoids in *Citrus bergamia* juice. J Pharm Biomed Anal. 35:349-363.

Devanaboyina N, Ramalakshmi N, Satyanarayana B, Sudeepthi P, Hemachakradhar K, PavankumarRaju N. 2013. Preliminary phytochemical screening, quantitative estimation and evaluation of antimicrobial activity of *Alstoniamacrophylla* stem bark. Int J Sci Innovs Today. 2(1):31-39.

Dimitrios B. 2006. Sources of natural phenolic antioxidants. Trends Food Sci Technol. 17(9): 505-512.

Ebrahimzadeh MA, Pourmorad F, Hafezi S. 2008. Antioxidant activities of Iranian corn silk. 32:43-49.

Farhat GN, Affara NI, Gali-Muhtasib HU. 2001. Seasonal changes in the composition of the essential oil extract of East Mediterranean sage (*Salvia libanotica*) and its toxicity in mice. Toxicon. 39:1601-1605.

Fenwick DE, Oakenfull D. 1983. Saponin content of food plants and some prepared foods. J Sci Food Agric. 34(2):186-191.

Figiel A, Szumny A, Gutierrez-Ortiz A, Carbonell-Barrachina AA. 2010. Composition of oregano essential oil (*Origanum vulgare*) as affected by drying method. J Food Eng. 98:240-247.

Fu M, Xu Y, Chen Y, Wu J, Yu Y, Zou B, An K, Xiao G. 2017. Evaluation of bioactive flavonoids and antioxidant activity in *Pericarpium Citri Reticulatae* (*Citrus reticulata* 'Chachi') during storage. Food Chem. 230:649-656.

Gaba J, Sharma S, Joshi S and Gill P. 2018. Gas Chromatography-Mass Spectrometric analysis of essential oil, nutritional and phytochemical composition of ajwain seeds (*Trachyspermum ammi*. L.). J Essent Oil Bear Pl. 21(4):1128-1137.

Gaba J, Sharma S, Kaur P, Joshi S. 2019. Essential oil and thymol extracted from ajwain as effective antioxidant agents. J *Spices* Aromat Crops. 28(2): 141-146.

Geetha S, Sai-Ram M, Mongia SS, Singh V, Ilavazhagan G. 2003. Evaluation of antioxidant activity of leaf extract of sea buckthorn (*Hippophae rhamnoides* L.) on Chromium (VI) induced oxidative stress in albino rats. J Ethnopharmacol. 87:247-251.

Ghasemi G, Fattahi M, Alirezalu A. 2018. A new source of oxygenated monoterpenes with phytotoxic activity: essential oil of *Cuminum Cyminum* L. from Iran. Nat Prod Res. 1-4.

Gohari AR, Saeidnia S. 2011. A review on phytochemistry of *cuminum cyminum* seeds and its standards from field to market. PHCOG J. 3(25):1-5.

Halliwell B, Gutteridge JMC. 1998. In free radicals in biology and medicine; Oxford University Press: Oxford.

Hanasaki Y, Ogawa S, Fukui S. 1994. The correlation between active oxygens scavenging and antioxidative effects of flavonoids. Free Rad Biol Med. 16:845-850.

Iocabellis NS, Lo Cantore P, Capasso F, Senatore F. 2005. Antibacterial activity of *Cuminum cyminum* L. and *Carum carvi* L. essential oils. J Agric Food Chem. 53:57-61.

Johnson C B, Kirby J, Naxakis G, Pearson S. 1999. Substantial UV-mediated induction of essential oils in sweet basil (*Ocimum basilicum* L.). Phytochemistry. 51:507-510.

Khanbabaee K, van Ree T. 2001. Tannins: Classification and definition. Nat Prod Rep**.** 385(18):641-649.

Kumar S and Pandey AK. 2013. Phenolic content, reducing power and membrane protective activities of *Solanum xanthocarpum* root extracts. Vegetos. 26:301-307.

Kumar S, Mishra A, Pandey AK. 2013. Antioxidant mediated protective effect of *Parthenium hysterophorus* against oxidative damage using *in vitro* models. BMC Compl Alternative Med.13: 1-9.

Lis-Balchin M, Deans SG. 1997. Bioactivity of selected plant against *Listeria monocytogenes.* J Appl Microbiol. 82:759-762.

Makkar MK, Sharma S, Kaur H. 2018. Evaluation of *Mentha arvensis* essential oil and its major constituents for fungitoxicity. J Food Sci Technol. 55(9):3840–3844.

Mallik S, Sharangi AB, Sarkar T. 2020. Phytochemicals of coriander, cumin, fenugreek, fennel and black cumin: A preliminary study. https://doi:10.1007/s40009-020-00884-5.

Nadeem M, Riaz A. 2012 Cumin (*cuminum cyminum*) as a potential source of antioxidant. Pak J Food Sci. 22(2):101-107.

Ncube NS, Afolayan AJ, Okoh AI. 2008. Assessment techniques of antimicrobial properties of natural compounds of plant origin: Current methods and future trends. African J Biotech. 7:1797-1806.

Pala-Paul J, Usano-Alemany J, Soria AC, Perez-Alonso M, Brophy JJ. 2008. Essential oil composition of *Erygium campestre* L. growing in different soil types. A preliminary study. Nat Prod Commun 3:1121-1126.

Panche AN, Diwan AD, Chandra S R. 2016. Flavonoids: an overview. J Nutr Sci. 5:1-15.

Patil SB, Takalikar SS, Joglekar MM, Haldavnekar VS, Arvindekar AU. 2013. Insulinotropic and β-cell protective action of cuminaldehyde, cuminol, and an inhibitor isolated from *Cuminum cyminum* in streptozotocin-induced diabetic rats. Br J Nutr 110: 1434-1443.

Peer W A, Langenheim J H. 1998. Influence of phytochrome on leaf monoterpene variation in *Satureja douglasii*. Biochem Syst Ecol. 26:25-34.

Pietta PG. 2000. Flavonoids as antioxidants. J Nat Prod. 63(7):1035-1042.

Pourmorad F, Hosseinimehr SJ, Shahabimajd N. 2006. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. Afr J Biotechnol. 5(11):1142-1145.

Prajapati M, Jayswal, Maitreya B. 2019. Phytochemical screening and comparative study of antioxidant activity of *Cuminum Cyminum* L. and *Nigella Sativa* L. Int J Sci Basic Appl Res. 9(5):1303-1315.

Rai N, Yadav S, Verma AK, Tiwari L, Sharma RK. 2012. A monographic profile on quality specifications for a herbal drug and spice of commerce- *Cuminum cyminum* L. Int J Adv Herb Sci Techol. 1(1):1-12.

Rathore SS, Saxena SN, Singh B. 2013. Potential health benefits of major seed spices. International J Seed Spices. 3: 1-12.

Rios JL, Recio MC, Villar A. 1987. Antimicrobial activity of selected plants employed in the Spanish Mediterranean area. J Ethnopharmacol. 21:139-152.

Santhi K, Sengottuvel R. 2016. Qualitative and quantitative phytochemical analysis of *Moringa concanensis* Nimmo. Int J Curr Microbiol App Sci. 5(1):633-640.

Santos-Gomes PC, Fernandes-Ferreira M. 2001. Organ and season-dependent variation in the essential oil composition of *Salvia officinalis* cultivated at two different sites. J Agric Food Chem. 49:2908-2916.

Satta M, Tuberoso CIG, Angioni A, Pirisi FM, Cabras P. 1999. Analysis of the essential oil of *Helichrysum italicum* G. Don. ssp. *microphyllum* (willd.) Nym. J Essent Oil Res. 11:711-715.

Sawant RS, Godghate AG. 2013. Qualitative phytochemical screening of rhizomes of *Curcuma longa* Linn. Int J Sci Env Technol. 2(4):634-641.

Shaik MR, Khan M, Kuniyil M, Al-Warthan A, Alkhathlan H Z, Siddiqui MRH, Shaik JP, Ahamed A, Mahmood A, Khan M, Adil AF. 2018. Plant extract assisted green synthesis of silver nanoparticles using *Origanum vulgare* L.extract and their microbicidal activities. Sustainability 10:1-13.

Soobrattee MA, Neergheen VS, Luximon-Ramma A, Aruoma OI, Bahorun OT. 2005. Phenolics as potential antioxidant therapeutic agents: Mechanism and actions. Mutat Res Fund Mol. 579: 200-213.

Srinivasan K. 2018. Cumin (***Cuminum cyminum***) and black cumin (***Nigella sativa***) seeds: traditional uses, chemical constituents, and nutraceutical effects. Food Qual Saf. 2(1):1-16.

Taveira FSN, de Lima WN, Andrade EHA, Maia JGS. 2003. Seasonal essential oil variation of *Aniba canelilla*. Biochem Syst Ecol. 31:69-75.

Taylor JLS, Rabe T, McGaw LJ, Jager AK, van Staden J. 2001. Towards the scientific validation of traditional medicinal plants. Plant Growth Regul. 34(1):23-37.

Teixeira B, Marques A, Ramos C, Serrano C, Matos O, Neng NR, Nogueira JMF, Saraiva JA, Nunes M L. 2013. Chemical composition and bioactivity of different oregano (*Origanum vulgare*) extracts and essential oil. J Sci Food Agric. 93(11):2707-2714.

Ursini F, Maiorino M, Morazzoni P, Roveri A, Pifferi G. 1994. A novel antioxidant flavonoid (IdB 1031) affecting molecular mechanisms of cellular activation. Free Rad Biol Med**.** 16:547-553.

Wanasundara UN, Shahidi F. 1998. Antioxidant and pro-oxidant activity of green tea extracts in marine oils. Food Chem. 63(3):335-342.

Yingming P, Ping L, Hengshan W, Min L. 2004. Antioxidant activities of several chinese medicinal herbs. Food Chem. 88:347-350.

Youdin KA, Dorman HJD, Deans SG. 1999. The antioxidant effectiveness of thyme oil, α-tocopherol and ascorbyl palmitate on evening primrose oil oxidation. J Essent Oil Res. 11:643-648.

Zhang HY, Wang LF. 2002. Theoretical elucidation on structure-antioxidant activity relationships for indolinonic hydroxylamines. Bioorganic Med Chem Lett. 12:225-227.

Zhang Y, Ma H, Liu W, Yuan T, Seeram NP. 2015. New antiglycative compounds from cumin (*Cuminum cyminum*) spice. J Agric Food Chem. 63(46):10097-10102.

Ekta Computers <ektacomputersldh@gmail.com>

Fwd: Journal of Food Science and Technology - Submission Notification to co-author - [EMID:028eefa7bad3108e]

---

pardeep kaur <pardip2493@gmail.com> Sun, Jan 10, 2021 at 12:38 PM
To: ektacomputersldh@gmail.com

---------- Forwarded message ---------
From: **Journal of Food Science and Technology** <em@editorialmanager.com>
Date: Wed, Dec 16, 2020 at 12:28 PM
Subject: Journal of Food Science and Technology - Submission Notification to co-author - [EMID:028eefa7bad3108e]
To: Pardeep Kaur <pardip2493@gmail.com>

Re: "Chemical composition, nutritional profile and bioactivity of Cuminum cyminum essential oil and its major compound"
Full author list: Pardeep Kaur; Sunita Sharma; Jyoti Gaba; Poonam Sharma

Dear Miss Pardeep Kaur,

We have received the submission entitled: "Chemical composition, nutritional profile and bioactivity of Cuminum cyminum essential oil and its major compound" for possible publication in Journal of Food Science and Technology, and you are listed as one of the co-authors.

The manuscript has been submitted to the journal by Dr. Dr. Sunita Sharma who will be able to track the status of the paper through his/her login.

If you have any objections, please contact the editorial office as soon as possible. If we do not hear back from you, we will assume you agree with your co-authorship.

Thank you very much.

With kind regards,

Springer Journals Editorial Office
Journal of Food Science and Technology

Our flexible approach during the COVID-19 pandemic

If you need more time at any stage of the peer-review process, please do let us know. While our systems will continue to remind you of the original timelines, we aim to be as flexible as possible during the current pandemic.

This letter contains confidential information, is for your own use, and should not be forwarded to third parties.

Recipients of this email are registered users within the Editorial Manager database for this journal. We will keep your information on file to use in the process of submitting, evaluating and publishing a manuscript. For more information on how we use your personal details please see our privacy policy at https://www.springernature.com/production-privacy-policy. If you no longer wish to receive messages from this journal or you have questions regarding database management, please contact the Publication Office at the link below.

In compliance with data protection regulations, you may request that we remove your personal registration details at any time. (Use the following URL: https://www.editorialmanager.com/jfst/login.asp?a=r). Please contact the publication office if you have any questions.

Chemical composition, nutritional profile and bioactivity of *Cuminum cyminum* essential oil and its major compound

Pardeep[a] Kaur; Sunita Sharma[b]; Jyoti Gaba[a]; Poonam Sharma[b]

[a]Department of Chemistry, Punjab Agricultural University, Ludhiana-141004, India
[b]Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana-141004, India
[#]Corresponding author. Email: pardip2493@gmail.com

Abstract

Medicinal plants are the richest bio-resource of drugs and played vital role in the human civilization. *Cuminum cyminum* is the most popular culinary spice known for its special aromatic effects. The characteristic aroma of the cumin is judged by its volatile oil content. In the present study, essential oil of *Cuminum cyminum* of variety GC-4 was extracted using hydro-distillation method. Essential oil was then further subjected for GC-MS analysis in order to determine its chemical composition. Thirty eight compounds representing 99.98% composition of essential oil. Cuminaldehyde was found to be the major compound of oil *i.e.* 36.00%, which was further isolated from it using column chromatography. Proximate analysis and elemental analysis of commercial variety GC-4 was also carried out. Essential oil and its major compound was also screened from their antioxidant potent *via* DPPH assay, antimicrobial potential against *Pseudomonas* sp., *Klebsiella* sp., *Enterobacter* sp. and fungicidal potential against *Fusarium wilt* along with their corresponding standard.

Keywords: *Cuminum cyminum*, Essential oil, GC-MS, Nutritional profile, Biological activities

Introduction

India is the most recognized country because of richest source of spices and medicinal plants. A spice is a dried seed, fruit, root, bark or flower of a plant or herb used in small quantities for flavor, color or as a preservative (Sachan et al. 2018). These are the excellent bio-nutrient supplement that not only enhance flavor and aroma in the food but also imparts medicinal values. They exhibit wide range of medicinal properties such as bactericidal, bacteriostatic, fungistatic, antifertility, antihelminthic antioxidant, anti-inflammatory, antitumorigenic, anticarcinogenic, lowering of glucose- and cholesterol (Jiang 2019); and used to treat numerous ailments that form an significant part of the Ayurvedic Pharmacopoeia. There is a great demand of Indian spices in the global market due to their rich aroma, texture, taste and biological activities. India has the largest domestic market for spices in the world. In world, India is at number three and constitute 8.8% share in the trade of spices (Dubey 2017).

Spices of Apiaceae family possess a wide range of physiological and pharmacological properties. Cumin is a popular culinary spice that belongs to the family Apiaceae, in the genus; *Cuminum*. It bears small, brownish gray, elongated seeds with vertical ridges on their outer surface. India is the largest

producer and consumer of cumin seeds. In India, it is mainly cultivated in Gujrat and Rajasthan. Cumin seeds are nutritionally rich as they provide high amounts of fat (especially monounsaturated fat), protein and dietary fiber. Vitamin B, E and several dietary minerals, especially iron, are also considerable in cumin seeds.

There is great demand of value added products of cumin namely seed, powder, essential oil and oleoresin etc. in the domestic as well as in international market (Kumar et al. 2015). Cumin seeds have been reported to contain 3-4.5% essential oil (Dubey et al. 2016). The aroma of cumin is due to the presence of aromatic volatile compounds. Now a day's demand for volatile oil in international market is increasing. The advantage in use of volatile oil is that it is 100 times more concentrated than the spice powder and hence is required in very less quantity. The essential oil is responsible for the characteristic cumin odor. This odor and flavor is due to the presence of aldehydes (Nadeem and Riaz 2012). Major constituent of cumin essential oil is cuminaldehyde, present to an extent of 45–54% and considered to be as an important phytochemical possessing some cumin growing areas of Rajasthan and Gujarat was 44.5, 44.2 and 40.3% in Jodhpur, Nagaur (Rajasthan) and Patan (Gujarat) respectively. The essential oil specifically have antioxidant, antispasmodic, diuretic, carminative and antibacterial properties (Bettaieb et al. 2010). Organic acids such as malic, tartaric, propionic, aspartic, citric, ascorbic, oxalic and fumaric acids are isolated from seeds of cumin (Hashum and Al-Hashemi 2014). Cumin fruits contain 2.5 to 4.5% volatile oil and 10% fixed oil (Al-Snafi 2016). It is noted that the constituents and their percentage in *Cuminum cyminum* essential oil were differ according to the area. Number of various environmental factors such as weather, altitude, rainfall and other conditions may affect the growth of plants which in turn affect the quality of herbal ingredients present in a particular species even when it is produced in the same country. These conditions may produce major variations in the bioactive compounds present in the plants (Santhi and Sengottuvel 2016).

Medicinal plants are used for the treatment of many diseases such as cancer, diabetes, neurodegenerative and cardiovascular diseases which are caused by oxidative stress *i.e.* an imbalance between the production of reactive species and antioxidant defense activity. Nowadays, there is increase in the number of food-borne diseases caused by some pathogens (bacteria, fungi and viruses) and their enterotoxins which is a topic of concern for food safety, researchers, food processors and regulatory agencies. In plants, fungal pathogens are the primary infection agents leads to significant economic losses in agro-chemistry. Traditionally, these pathogens have been treated with different synthetic fungicides but excessive use of these fungicides leads to fungi resistance in plants. Natural products are the best alternative of these synthetic fungicides as they are not injurious to health and are

also environment friendly. Moreover, synthetic compounds are not readily bio-degradable, therefore, remain in the soil for several years which leads to decrease in the fertility of soil.

Currently, more emphasis is given in the development of plant extracts, essential oils or their active compounds as natural fungicides. The present study is designed with an objective to study the chemical, proximate, mineral composition and biological activities (antioxidant, antimicrobial and antifungal) of essential oil of commercial variety GC 4 of cumin seeds.

Materials and Methods

Collection of cumin seeds

One kilogram cumin seeds of variety GC-4 were taken from Rajasthan State Seed and Organic Production Certification Agency, Jaipur. The sample was cleaned and washed to remove extraneous matter, dried in open air and then in oven at 50 ºC, kept in air tight glass containers at 4 ºC for further use.

Proximate composition

Determination of crude protein, total mineral, moisture, fat, fibre, total sugars and carbohydrate content of *Cuminum cyminum* was done as described by Association of Official Analytical Chemists (AOAC). The nitrogen content was determined by Kjeldahl method (Foss KjeltecTM 2100) and multiplied by factor 6.25 to find the protein content9. The weight difference method was used to find moisture and total mineral content. Solvent extraction method with Soxhlet apparatus (Pelican equipments Socs Plus SCS 4 AS) was used for fat content determination, using n-hexane as solvent. Crude fibre content was estimated by determining loss in weight of treated sample (with boiling 1.25 % H_2SO_4 and boiling 1.25 % NaOH) during ignition. The total carbohydrates were determined by difference method [100 - (protein + total mineral + moisture + fat + fibre)].

Mineral composition

Powdered cumin seeds were dry ashed at 550 ºC. The ash was boiled with 10 ml of 20% hydrochloric acid in a beaker and then filtered into a 100 ml standard flask. It was made up to the mark with deionised water. The minerals were determined from the resulting solution using Atomic Absorption Spectroscopy (AAS) on Perkin Elmer Analyst 200. The burner system provides the thermal energy necessary to dissociate the chemical compounds, providing free analyte atoms so that absorption occurs. The spectrometer measures the amount of light absorbed at a specific wavelength using a hollow cathode lamp as the primary light source, a monochromator and a detector.

Extraction of essential oil from cumin seeds

The seeds were ground in electric grinder and were used for the extraction of essential oil by hydro distillation method. 50 g of seeds were dipped in a 500 mL round bottomed flask. The contents were transferred in 1 L round bottomed flask, mixed thoroughly and subjected to hydro-distillation for fours using a Clevenger type apparatus (Boughendjioua 2019). The layer containing a mixture of essential

oil and little water was collected in conical flask. The collected oil was extracted with diethylether as oil was soluble in it and was then dried and dehydrated over anhydrous sodium sulphate. Diethyl ether was removed using rotary vacuum evaporator and the essential oil was stored in a refrigerator at 4 °C. Per cent yield of extracted essential oil was calculated using following equation:

$$\text{Volatile oil (\%)} = \frac{\text{Weight of volatile oil recovered Volatile oil (\%) in the receiver}}{\text{Weight of sample}} \times 100$$

Gas chromatography-Mass spectrometry (GCMS) of cumin oil

Gas chromatography-mass spectroscopy (GC-MS) analyses of essential oil of cumin seeds was carried out on QP2010 Plus, Shimadzu, Japan, equipped with an Rtx-5 MS capillary column (30.0 m x 0.20.25 mm i.d., 0.25 µm film thickness) for the separation of its components. The injector was maintained at 260°C and operated in split injection mode (split ratio 50) with the split valve closed for one min and injection volume was 6 µL. Helium gas was used as the carrier gas at a constant pressure of 69 kPa. The column oven was initially maintained at 50°C for two min, raised to 210°C at 3°C/min, then to 250°C at 6°C/min. The interface temperature was 270°C and the ionization mode was electron impact (70 eV). The mass selective detector was operated in the scan mode between 40 and 650 m/z. Data acquisition was started 3.0 min after injection. MS parameters used were; Ionisation Voltage (EI) 70 eV, peak width 2s, mass range 40-650 amu and detector voltage 1.5 V. Peak identification was done by matching the mass spectra with mass spectra data and retention indices available on database of NIST08, WILEY8, Perfumery and Flavor and Fragrance libraries.

Determination of antioxidant activity by DPPH radical scavenging method:

The free radical scavenging activity of the compounds was estimated by their capacity to bleach the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), as reported (Blois 1958). DPPH gives a strong absorption band at 517 nm in the visible region because of its odd electronic structure. Different concentrations (3000, 2000, 1000, 500, 250, 100, 50 and 25 µg mL^{-1}) of test compounds (40 µL) and ascorbic acid (40 µL), dissolved in methanol, were added to 3.96 mL of the DPPH solution. After 30 min, the absorbance was read at 517 nm at room temperature, and the scavenging activity was calculated as percentage of the radical reduction. Each experiment was executed thrice. Methanol was used as control and ascorbic acid was used as standard. The radical scavenging activity was obtained as follows:

$$\text{Radical Scavenging Activity (\%)} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

Where $A_{control}$ and A_{sample} are the absorbances of blank (methanol) and test compounds, respectively at 517 nm.

Determination of antimicrobial activity by disc plate method

Authentic pure cultures of bacteria namely *Pseudomonas sp*. *Klebsiella sp., Enterobacter sp.* were procured from department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. Essential oil and major constituent of oil *i.e.* cuminaldehyde along with the standard Ampicilin were screened for their antimicrobial potential at different concentrations (3000, 2000, 1000, 500, 250, 100, 50 and 25 µg mL^{-1}) against three gram negative bacteria *viz. Pseudomonas sp*. *Klebsiella sp., Enterobacter sp.* by disc plate method on nutrient agar medium. Nutrient broth containing test bacteria was inoculated on petri plates having solidified nutrient agar medium. The culture of bacteria was spread properly. Sterile discs having diameter 6 mm were treated with different solvent extracts of different concentrations and then placed on a petri plate having solidified nutrient agar medium. The plates were incubated for 24 hrs in an incubator at 37 ºC and the inhibition zones around the disc were measured in mm. Each treatment was replicated thrice. MIC (Minimum Inhibition Concentration) values of different solvent extracts against *Pseudomonas sp*. *Klebsiella sp., Enterobacter sp.*were presented in Figure 1 respectively.

Determination of antifungal activity by poisoned food technique

A culture of the test fungi *i.e. Fusarium wilt* (FW), *Ascochyta blight* (AB) and *Bortrytis gray mould* (BGM) was grown separately in Petri plates on potato dextrose agar media and kept in incubator for 7 days. Stock solution of essential oil and cuminaldehyde (300 mg ml^{-1}) was prepared in dimethyl sulfoxide and stored at 4 ºC for further use. PDA supplemented with different prepared solutions at eight different concentrations (3000, 2000, 1000, 500, 250, 100, 50 and 25 µg ml^{-1}) was dispensed in the Petri plates under aseptic conditions. After solidification, small disc (0.5 cm diameter) of the test fungi was cut with a sterile cork borer and transferred aseptically upside down in the centre of Petri dish. Petri plates were incubated at 25 ± 1 ºC and growth of fungi was recorded at regular intervals. Growth of test fungi colony was measured after every 24 hrs till the fungus in the control plates completely occupied it. Each treatment was replicated thrice. The antifungal activity was evaluated by measuring the relative growth of fungus in each treatment (Grover and Moore 1962). The per cent growth inhibition over control was worked out using the formula.

$$I = \frac{(C-T) \times 100}{C}$$

Where, I is inhibition per cent,

C is colony diameter in control (cm) and

T is colony diameter in treatment (cm)

Results and Discussion

Cuminum cyminum was an annual herbaceous plant belonging to family Apiaceae. Classification of cumin, according to APG system III 2009; **Division:** Magnoliophyta; **Class:** Magnoliophyta; **Order:** Apiaceae; **Genus:** Cuminum and **Species:** *Cuminum cyminum* L. Essential oil was pale yellow in color with a very strong and stringent odour. Yield of cumin oil obtained was 2.52 %

Proximate composition of cumin seeds

Nutritional composition of *Cuminum cyminum* seeds of variety GC-4 was taken from Rajasthan State Seeds and Organic Production Certification Agency, Jaipur. The sample was cleaned and washed to remove extraneous matter, dried in open air and then in oven at 50 ºC, kept in air tight glass containers at 3 ºC. Proximate composition was determined by A.O.A.C. method (2000). The proximate analysis of cumin seeds showed that it contains 8.58±0.20, total minerals 8.62±0.05, crude protein 14.43±0.02, crude fibre 11.06±0.01, fats 15.00±0.04, total sugars 2.30±0.21 and total carbohydrates 40.01±0.06. It was evident from nutritional profile; cumin is a rich source of carbohydrates, proteins, fats and crude fibers. Similar results were reported by Singh et al. 2017.

Mineral composition

Few elements are essential to the body as nutrients called minerals. Knowledge of type and concentration of minerals present in food products is of vital importance to food industry. Mineral analysis using Atomic Absorption Spectroscopy (AAS) showed the concentration of different elements present in cumin seeds. Elemental investigation of cumin seeds showed that it contains micronutrients like Iron: 181.33, copper: 14.25, manganese: 25.75, zinc: 31.25, chromium: 10.15, nickel: 2.38, cobalt: 2.05, cadmium: 2.03, lead: 58.75, arsenic: 118.50 in parts per million concentrations. Among all the elements, iron was maximum present in maximum quantity. Iron is important in maintaining the good health of human being. Manganese, zinc and copper were found in small amounts. The results are inaccordance with the results reported by Singh et al. 2017.

Cumin seeds are a very good source of iron and minerals that plays many vital roles in the body. Iron is an integral component of hemoglobin, which transports oxygen from the lungs to all body cells, and is also part of key enzyme systems for energy production and metabolism. So, cumin can be a nutritious additive to daily diet for anemic people. Additionally, iron is instrumental in keeping your immune system healthy.

GC-MS analysis of essential oil

Gas chromatography- mass spectrometry (GC-MS) of oil showed the presence of 38 compounds which account for 100.00 % of total composition (Table 1 and 2). The identification of the compounds

was done by matching their retention times and mass spectra with the library and literature data. Cuminaldehyde (36.46%) was found to be the major compound in cumin seeds. The characteristic flavour of cuminaldehyde is attributed to the presence of such high per cent of cuminaldehyde. Other minor compounds present were 5-methyl-2-propylphenol (9.10 %), β-pinene (7.92 %), *p*-cymene (7.96), phellandral (1.27 %), γ-terpinen-7-al (7.74 %), γ-terpinene (5.64%), 4-isopropylcyclohex-3-enecarbaldehyde (3.71 %), 4-hydroxycryptone (2.56 %), 2,5-dimethyloctahydropentalene (2.30 %), 1-(1-Ethyl-2,3-dimethyl-2-cyclopenten-1-yl)ethanone (1.89 %), 3,7-dimethyl-octa-2,6-dien-1-al (1.46 %), and. Apart from these, many other compounds having area less than 1.00 % were also detected. Essential oil cumin seeds contain monoterpene hydocarbons 27.31%, oxygenated monoterpenes 63.05%, sesquiterpene hydocarbons (0.75%), oxygenated sesquiterpenes (1.00%), aldehyde and ketones (5.62), acids and esters (1.93%).

Isolation of cuminaldehyde from essential oil by column chromatography

Essential oil (5 g) from seeds of *Cuminum cyminum* was subjected to column chromatography to isolate its major constituent namely cuminaldehyde. For column chromatography, column was packed with silica gel having 60–120 mesh size which was activated at 110 °C for about 1 h. Essential oil was firstly dissolved in n-hexane and then adsorbed on silica gel for 30 min. Column was eluted with hexane and polarity was increased using chloroform. Each fraction was monitored using thin layer chromatography and isolation of pure products was confirmed by UV-visible, IR, ^{1}H and ^{13}C NMR analysis.

CHO

UV-vis (λ_{max}): 255 nm

IR: (cm^{-1}): 2809 (C-H stretching of aldehyde), 1700 (C=O stretching), 1255 (C-O stretching)

^{1}H NMR ($CDCl_3$): δ 1.27-1.28 (d, 6H, gem-dimethyl) (*J*= 6.96 Hz), 2.94-3.01 (m, 1H, CH), 7.31-7.33 (d, 2H, ArH), 8.04-8.06 (d, 2H, ArH), 10.20 (s, 1H, -CHO).

^{13}C NMR ($CDCl_3$): δ 23.42, 36.30, 126.70, 129.32, 134.30, 154.25 and 191.24

Evaluation of antioxidant activity of cumin oil and cuminaldehyde

Cumin essential oil and its major compound cuminaldehyde were evaluated for antioxidant potential in terms of free radical scavenging activity (RSA) using dimethyl sulphoxide (DMSO) as the control and ascorbic acid as standard. The free radical scavenging activity was scanned using DPPH as substrate. This assay is based on the stabilization of radical reactant. It gives strong absorption maximum

at 517 nm due to the presence of odd electron of DPPH. As the odd electron of the radical becomes paired off in the presence of a hydrogen donor, which is a free radical scavenging antioxidant, the absorbance decreases. The radical scavenging efficiency has been calculated in per cent (Table 3) and further IC_{50} values (µg ml^{-1}) were also determined. Out of cuminaldehyde and cumin oil, former was found to be more effective at all the tested concentrations.

Per cent RSA varied directly with concentration for both oil and cuminaldehyde. It is evident from Table 3 that cuminaldehyde had more antioxidant potential as compared to cumin oil. Electron donating group *i.e.* isopropyl group at the para position of cuminaldehyde may be responsible for the antioxidant potential. IC_{50} of oil, cuminaldehyde and ascorbic acid were found to be 2466, 1960 and 10 µg ml^{-1}, respectively. Einafshar et al. 2012 also assessed the antioxidant potential of cumin essential oil and recorded similar results. Significant differences were observed between the antioxidant potential of cumin oil and cuminaldehyde. Both cumin oil and cuminaldehyde registered less per cent RSA than ascorbic acid at all the concentrations.

4.10.1 Evaluation of microbial activity of cumin oil and cuminaldehyde

Evaluation of microbial activity of cumin oil, cuminaldehyde and ampicillin against *Pseudomonas* sp., *Klebsiella* sp. and *Enterobacter* sp. was presented in Table 4. It was evident from Table 4 that cumin oil exhibited more activity as compared to cuminaldehyde against all the three tested bacteria which was due to inhibition of urease activity in bacteria by cumin oil. The lipophilic constituents present in cumin oil that penetrates into the cell walls of bacteria and cause elongation or inhibit urease activity (Abdul Jabbar 2013). Cumin oil was found to be more effective than cuminaldehyde against all three tested bacteria at concentrations above 250 µg ml^{-1}. No inhibition zone was detected at lower concentrations. Significant differences were observed between the microbial activity of cumin oil and cuminaldehyde. Both cumin oil and cuminaldehyde registered less activity than standard ampicillin. Significant differences were observed between the bioactivity of cumin oil and cuminaldehyde at tested concentrations.

Minimum inhibition concentration of cumin oil and cuminaldehyde was also determined and presented in Figure 1. MIC values of cumin oil were less than cuminaldehyde against all three tested bacteria that in turn showed the effectiveness of cumin oil.

Evaluation of fungicidal activity of cumin essential oil and cuminaldehyde against *Fusarium wilt*

Essential oil and major constituent cuminaldehyde were evaluated for antifungal activity against *Fusarium wilt* using poisoned food technique at different concentrations. Cumin oil was found to be more effective than cuminaldehyde at all tested concentration (Table 5) against *F. wilt.* Essential oil components have the capability to alter cell permeability by entering between the fatty acyl chains

making up membrane lipid bilayers and disrupt the lipid packing. Due to this, the membrane properties like membrane fluidity/permeability and functions may get changed. This may also affect the regulation and function of the membrane bound enzymes that alter the synthesis of many cell wall polysaccharide components (*i.e.* β-glucan, chitin, and mannan) and alter the cell growth and morphogenesis. Moreover, essential oil can cause extensive cellular damage due to better penetration and contact. The major components of *C. cyminum* are terpenes, which have the capability to inhibit the respiration of fungi, and may have adverse effects on mitochondria. It may be the cause of cell death and other morphological changes (Naeini et al. 2014).

Cumin oil displayed better activity as compared to cuminaldehyde at all the tested concentrations. Significant differences were observed between antifungal potential against cumin oil and cuminaldehyde at all the different concentrations. Neither cumin oil nor cuminaldehyde showed better activity than standard. ED_{50} of cumin oil, cuminaldehyde and standard Carbendazim 50 WP was 490, 629, 10 μg ml^{-1} .

Acknowledgment

We are thankful to Panjab University, Chandigarh for providing IR, ^{1}H and ^{13}C NMR spectral data of isolated compounds and Jawaharlal Nehru University, New Delhi for providing GC–MS data.

References

A.O.A.C. (2000) Official methods of analysis. Association of Official Analytical Chemists. Washington, DC, USA.

Abdul Jabbar RA (2013) Chemical analysis and antimicrobial activity of cumin seeds extracted oil against some bacterial and fungal isolates. J Thi-Qar Sci 3:65-73.

Acimovic M G, Tesevic V, Mara D, Mirjana Cvetkovic M, Stankovic J, Filipovic V (2016) The analysis of cumin seeds essential oil and total polyphenols from postdestillation waste material. Advan Technol 5:23-30.

Ali F, Wang J, Yu J (2018) Effect of cumin essential oil (*Cuminum cyminum* L.) on milk fat globule membrane stability and micro structure properties after heat treatment. Int J Dairy Sci 13:22-29.

Al-Snafi A E (2016) The pharmacological activities of *Cuminum cyminum* - A review. J Pharm 6: 46-65.

APG III (2009) An update of the Angiosperm phylogeny group classification for the orders and families of flowering plants. Bot J Linn Soc 161:105-121.

Beis SH, Azcan N, Ozek T, Kara M, Baser KHC (2000) Production of essential oil from Cumin seeds. Chem Nat compd 36:265-268.

Benrejdal A, Dridi F, Nabiev M (2012) Extraction and analysis of essential oil of cumin. Asian J Chem 24:1949-1951.

Bettaieb I, Bourgou S, Wannes WA, Hamrouni , Limam F, Marzouk B (2010) Essential oils, phenolics, and antioxidant activities of different parts of cumin (*Cuminum cyminum* L.). J Agric Food Chem 58:10410-10418.

Blois M S (1958) Antioxidant determinations by the use of stable free radical. Nature 181:1199-200.

Boughendjioua H (2019) Characterization of aroma active compounds of cumin (*Cuminum Cyminum* L.) seed essential oil. Mod Appl Bioequiv Availab 4:1-5.

Dubey PN, Saxena SN, Mishra BK, Aishwath OP, Solanki RK, Singh B, Lal G (2016) Assessment of variability in physical and chemical composition of *Cuminum cyminum* seeds from arid and semiarid India. Indian J Agric Sci 86:1366-1370.

Dubey S (2017) Indian Spices and their Medicinal Value. Indian J Pharm Educ Res 51: S330-S333.

Einafshar S, Poorazrang H, Farhoosh R, Seiedi SM (2012) Antioxidant activity of the essential oil and methanolic extract of cumin seed (*Cuminum cyminum*). Eur J Lipid Sci Technol 114:168-174.

EL-Manylawi MA, Ali HFM (2009) Gas Chromatography-Mass Spectroscopy and evaluate cumin seeds and their essential oil as growth promoters of New Zealand white rabbits. Int J Agric Res 4:107-115.

Gotmare SR, Tambe EA (2018) Chemical characterization of cumin seed oil (*Cuminum cyminum*) by GC-MS and its comparative Study. Int J Sci *Res* 5:36-45.

Grover RK, Moore JD (1962) Toxicometric studies of fungicides against brown rot organisms *Sclerotonia fructicola* and *Sclerotonia laxa*. Phytopathology 52:876-880.

Hashum F and Al-Hashemi (2014) Chromatographic separation and identification of some volatile oils, organic acids and phenols from the seeds of *Cuminum cyminum* growing in Iraq. IJRRAS 19:80-90.

Jiang TA (2019) Health benefits of culinary herbs and spices. J AOAC Int 102:395-411.

Khan IU, Mehriya ML, Rathore BS, Kumhar SR, Singh B (2017a) Evaluation of volatile phytochemical constituents in cumin (*Cuminum cyminum*) genotypes by Gas Chromatography-Mass Spectroscopy. *J Pharmacog Phytochem* 6:768-773.

Khan IU, Rathore BS, Mehriya ML, Singh B (2017b) Evaluation, estimation and identification of essential oil constituents in cumin (*Cuminum cyminum*) genotypes grown in western Rajasthan. J Essent Oil Bear Pl 20:7690-7778.

Kumar S, Saxena SN, Mistry JG, Fougat RS, Solanki RK, Sharma R (2015) Understanding *Cuminum cyminum*: An important seed spice crop of arid and semi-arid regions. Int J Seed Spices 5:1-19.

Li R, Jiang Z (2004) Chemical composition of essential oil of *Cuminum cyminum* L. from China. Flavour Fragr J 19:311-313.

Nadeem M, Riaz A (2012) Cumin (*cuminum cyminum*) as a potential source of antioxidant. Pak J Food Sci 22:101-107.

Naeini A, Jalayer Naderi N, Shokri H (2014) Analysis and *in vitro* anti-candida antifungal activity of *Cuminum cyminum* and *Salvadora persica* herbs extracts against pathogenic *Candida strains.* J Med Mycol 24:13-18.

Ravi R, Prakash M, Bhat KK (2013) Characterization of aroma active compounds of cumin (*Cuminum cyminum* L.) by GC-MS, E-Nose, and sensory techniques. Int J Food Prop 16:1048-1058.

Sachan AKR, Kumar S, Kumari K, Singh D (2018) Medicinal uses of spices used in our traditional culture: World wide. JMPS 6:116-122.

Santhi K, Sengottuvel R (2016) Qualitative and quantitative phytochemical analysis of *Moringa concanensis* Nimmo. Int J Curr Microbiol App Sci 5:633-640.

Singh RP, Gangadharappa HV, Mruthunjaya K (2017) *Cuminum cyminum*-A popular spice: an updated review. Pharmacogn J 9:292-301.

Table 1: GC-MS analysis of essential oil of cumin seeds

Peak No.	Compound	Structure	Molecular formula	Mol. weight	Retention time (min)	Area (%)	References
1	α-Thujene		$C_{10}H_{16}$	136	7.43	0.19	Khan et al. 2017a; Khan et al. 2017b; Ali et al. 2018; Acimovic et al. 2016; Benrejdal et al. 2012; EL-Manylawi and Ali 2009; Li and Jiang 2004; Beis et al. 2000
2	1-isopropylcyclo hex-1-ene		C_9H_{16}	124	7.55	0.37	-
3	α-Pinene		$C_{10}H_{16}$	136	7.69	0.69	Gotmare and Tambe 2018; Khan et al. 2017a; Khan et al. 2017b; Ali et al. 2018; Acimovic et al. 2016; Benrejdal et al. 2012; EL-Manylawi and Ali 2009; Li and Jiang 2004
4	5-Ethylcyclohexa-1,3-diene		C_8H_{12}	108	8.98	0.16	-
5	Sabinene		$C_{10}H_{16}$	136	9.21	0.36	Acimovic et al. 2016; Benrejdal et al. 2012; EL-Manylawi and Ali 2009; Beis et al. 2000

6	β-pinene		$C_{10}H_{16}$	136	9.47	7.92	Gotmare and Tambe 2018; Khan et al. 2017a; Ali et al. 2018; Ravi et al. 2013; Acimovic et al. 2016; Benrejdal et al. 2012; EL-Manylawi and Ali 2009; Li and Jiang 2004; Beis et al. 2000
7	Myrcene		$C_{10}H_{16}$	136	9.90	0.98	Khan et al. 2017a; Khan et al. 2017b; Ali et al. 2018; Ravi et al. 2013; Acimovic et al. 2016; Benrejdal et al. 2012; EL-Manylawi and Ali 2009; Li and Jiang 2004; Beis *et al* 2000
8	*p*-Cymene		$C_{10}H_{14}$	134	11.51	7.96	Gotmare and Tambe 2018; Ali et al. 2018; Ravi et al. 2013; Acimovic et al. 2016; Benrejdal et al. 2012; EL-Manylawi and Ali 2009; Li and Jiang 2004
9	Limonene		$C_{10}H_{16}$	136	11.61	0.46	Gotmare and Tambe 2018; Benrejdal et al. 2012; Li and Jiang 2004; Beis et al. 2000

10	Eucalyptol		$C_{10}H_{18}O$	154	11.70	0.38	Gotmare and Tambe 2018; Acimovic et al. 2016; Benrejdal et al. 2012; Li and Jiang 2004
11	γ-Terpinene		$C_{10}H_{16}$	136	12.95	5.64	Gotmare and Tambe 2018; Khan et al. 2017a; Acimovic et al. 2016; Benrejdal et al. 2012; EL-Manylawi and Ali 2009; Li and Jiang 2004; Beis et al. 2000
12	Linalool	OH	$C_{10}H_{18}O$	154	14.84	0.19	Khan et al. 2017b; Li and Jiang 2004; Beis et al. 2000
13	3,6,6-trimethylbicyclo[3.1.1]heptan-2-one	O	$C_{10}H_{16}O$	152	16.47	0.22	-
14	6,6-dimethyl-2-methylenebicyclo[3.1.1] heptan-3-ol	HO	$C_{10}H_{16}O$	152	16.60	0.23	-
15	1-(2-Propenyl)-cyclohexene		C_9H_{14}	122	16.83	0.16	-
16	2,5-dimethyloctahydropentalene		$C_{10}H_{18}$	138	17.04	2.30	-

17	1-(3-Isopropenyl-2,2-dimethylcyclopropyl)-2-methylpropan-1-one		$C_{12}H_{20}O$	180	17.81	0.73	-
18	1-(1-Methylethyl)-4-methyl-3-cyclohexen-1-ol		$C_{10}H_{18}O$	154	18.47	1.24	-
19	4-isopropylcyclohex-3-enecarbaldehyde		$C_{10}H_{16}O$	152	19.18	3.71	Gotmare and Tambe 2018; Ali et al. 2018
20	1-(1-Ethyl-2,3-dimethyl-2-cyclopenten-1-yl)ethanone		$C_{11}H_{18}O$	166	20.49	1.89	-
21	Cuminaldehyde		$C_{10}H_{12}O$	148	22.00	36.46	Gotmare and Tambe 2018; Khan et al. 2017a; Khan et al. 2017b; Ali et al. 2018; Ravi et al. 2013; Acimovic et al. 2016; Benrejdal et al. 2012; Li and Jiang 2004; Beis et al. 2000
22	Phellandral		$C_{10}H_{16}O$	152	22.99	1.27	Gotmare and Tambe 2018; Khan et al. 2017b; Li and Jiang 2004

23	5-Methyl-2-propylphenol		$C_{10}H_{14}O$	150	23.86	9.10	-
24	γ-Terpinen-7-al		$C_{10}H_{14}O$	150	24.10	7.74	Gotmare and Tambe 2018; Acimovic et al. 2016
25	2-Methyl-5-(propan-2-ylidene)cyclohexane-1,4-diol		$C_{10}H_{18}O_2$	170	24.84	0.33	-
26	4-Hydroxycryptone		$C_9H_{14}O_2$	154	25.39	2.56	Gotmare and Tambe 2018
27	4-Isopropylphenylacetic acid		$C_{11}H_{14}O_2$	178	25.68	0.97	-
28	3,7-dimethyl-octa-2,6-dien-1-al		$C_{10}H_{16}O$	152	26.65	1.46	-
29	2,4(10)-Thujadiene		$C_{10}H_{14}$	134	24.48	0.44	-
30	*p*-Butyryloxybenzaldehyde		$C_{11}H_{12}O_3$	192	27.92	0.44	-

31	But-2-enyl hexanoate		$C_{10}H_{18}O_2$	170	28.78	0.74	-
32	β-Farnesene		$C_{15}H_{24}$	204	30.43	0.42	Gotmare and Tambe 2018; Khan et al. 2017b; EL-Manylawi and Ali 2009; Li and Jiang 2004; Beis et al. 2000
33	(1R,4R,5S)-1,8-Dimethyl-4-(prop-1-en-2-yl)spiro[4.5]dec-7-ene		$C_{15}H_{24}$	204	31.23	0.33	-
34	2-(4-methyl-3-(prop-1-en-2-yl)-4-vinylcyclohexyl)propan-2-yl acetate		$C_{17}H_{28}O_2$	264	32.24	0.22	-
35	*p*-Mentha-2,8-dien-1-ol		$C_{10}H_{16}O$	152	32.45	0.27	Gotmare and Tambe 2018
36	Caryophyllene oxide		$C_{15}H_{24}O$	220	35.46	0.35	Khan et al. 2017b; EL-Manylawi and Ali 2009; Li and Jiang 2004

37	Carotol		$C_{15}H_{26}O$	222	36.28	0.65	Khan et al. 2017b; Acimovic et al. 2016; EL-Manylawi and Ali 2009; Li and Jiang 2004
38	Dihydroterpineol		$C_{10}H_{20}O$	156	36.63	0.48	Acimovic et al. 2016

Table 2: Chemical composition of volatiles in the *Cuminum cyminum* essential oil

Peak No.	Compound	Area (%)
Monoterpene hydocarbons		**27.31**
1	α-Thujene	0.19
2	1-isopropylcyclo hex-1-ene	0.37
3	α-Pinene	0.69
4	Sabinene	0.36
5	β-pinene	7.92
6	Myrcene	0.98
7	*p*-Cymene	7.96
8	Limonene	0.46
9	γ-Terpinene	5.64
10	2,5-dimethyloctahydropentalene	2.30
11	2,4(10)-Thujadiene	0.44
Oxygenated monoterpenes		**63.08**
12	Eucalyptol	0.38
13	Linalool	0.19
14	3,6,6-trimethylbicyclo[3.1.1]heptan-2-one	0.22
15	6,6-dimethyl-2-methylenebicyclo[3.1.1] heptan-3-ol	0.23
16	1-(1-Methylethyl)-4-methyl-3-cyclohexen-1-ol	1.24
17	4-isopropylcyclohex-3-enecarbaldehyde	3.71
18	Cuminaldehyde	36.46
19	Phellandral	1.27
20	5-Methyl-2-propylphenol	9.10
21	γ-Terpinen-7-al	7.74

22	2-Methyl-5-(propan-2-ylidene)cyclohexane-1,4-diol	0.33
23	3,7-dimethyl-octa-2,6-dien-1-al	1.46
24	Dihydroterpineol	0.48
25	*p*-Mentha-2,8-dien-1-ol	0.27
Sesquiterpene hydocarbons		**0.75**
26	β-Farnesene	0.42
27	(1R,4R,5S)-1,8-Dimethyl-4-(prop-1-en-2-yl)spiro[4.5]dec-7-ene	0.33
Oxygenated sesquiterpenes		**1.00**
28	Caryophyllene oxide	0.35
29	Carotol	0.65
Aldehyde and ketones		**5.62**
30	*p*-Butyryloxybenzaldehyde	0.44
31	1-(3-Isopropenyl-2,2-dimethylcyclopropyl)-2-methylpropan-1-one	0.73
32	1-(1-Ethyl-2,3-dimethyl-2-cyclopenten-1-yl)ethanone	1.89
33	4-Hydroxycryptone	2.56
Acid and esters		**1.93**
34	4-Isopropylphenylacetic acid	0.97
35	But-2-enyl hexanoate	0.74
36	2-(4-methyl-3-(prop-1-en-2-yl)-4-vinylcyclohexyl)propan-2-yl acetate	0.22
Others		**0.32**
37	5-Ethylcyclohexa-1,3-diene	0.16
38	1-(2-Propenyl)-cyclohexene	0.16

Table 3: DPPH radical scavenging activity of cumin oil, cuminaldehyde and ascorbic acid

Concentration ($\mu g\ ml^{-1}$)	Per cent Radical scavenging activity (RSA)		
	Cumin oil	Cuminaldehyde	Ascorbic acid
3000	54.95±0.57	59.95±0.57	100±0.00
2000	45.83±0.49	51.36±0.15	100±0.00
1000	38.52±0.42	42.38±0.46	100±0.00
500	29.98±0.84	36.45±0.36	100±0.00
250	26.06±0.23	26.89±1.13	100±0.00
100	21.23±0.37	23.40±0.55	100±0.00
50	16.38±0.14	19.56±0.20	95.56±0.20
25	12.51±0.40	15.52±0.19	88.25±0.40
Particulars		**CD (5%)**	
Compounds		0.33	
Concentrations		0.08	
Compounds × concentrations		0.09	

Table 4: Microbial activity of cumin oil, cuminaldehyde and ampicillin against *Pseudomonas sp*., *Klebsiella sp*. and *Enterobacter sp*.

Concentration (µg ml^{-1})	Inhibition zone (in mm)								
	Pseudomonas sp.			***Klebsiella sp.***			***Enterobacter sp.***		
	Cumin oil	Cumin-aldehyde	Ampicillin	Cumin oil	Cumin-aldehyde	Ampicillin	Cumin oil	Cumin-aldehyde	Ampicillin
3000	11.56±0.26	10.50±0.56	24.00±0.58	16.26±0.26	15.23±0.65	30.00±0.12	13.02±0.12	11.20±0.56	24.00±0.02
2000	9.87±0.06	8.95±0.11	20.00±0.26	13.52±0.03	12.36±0.74	23.00±0.02	11.85±0.01	10.01±0.02	22.00±0.03
1000	8.01±0.36	7.52±0.32	18.31±0.21	10.56±0.59	9.56±0.23	19.00±0.03	9.23±0.02	8.21±0.21	18.50±0.00
500	6.50±0.11	6.21±0.14	16.30±0.33	8.20±0.44	7.89±0.58	17.00±0.06	7.05±0.40	6.55±0.18	16.50±0.19
250	6.22±0.26	ND	15.10±0.11	6.9±0.23	6.58±0.02	16.00±0.08	6.10±0.12	ND	15.50±0.10
100	ND	ND	8.20±0.26	6.1±0.36	ND	8.60±0.52	ND	ND	8.30±0.03
50	ND	ND	6.60±0.21	ND	ND	6.80±0.21	ND	ND	6.10±0.01
25	ND	ND	6.10±0.25	ND	ND	6.20±0.10	ND	ND	6.10±0.25

Particulars	**CD (5%)**		
Compounds	0.21	0.33	0.32
Concentrations	0.02	0.21	0.12
Compounds × concentrations	0.11	0.11	0.36

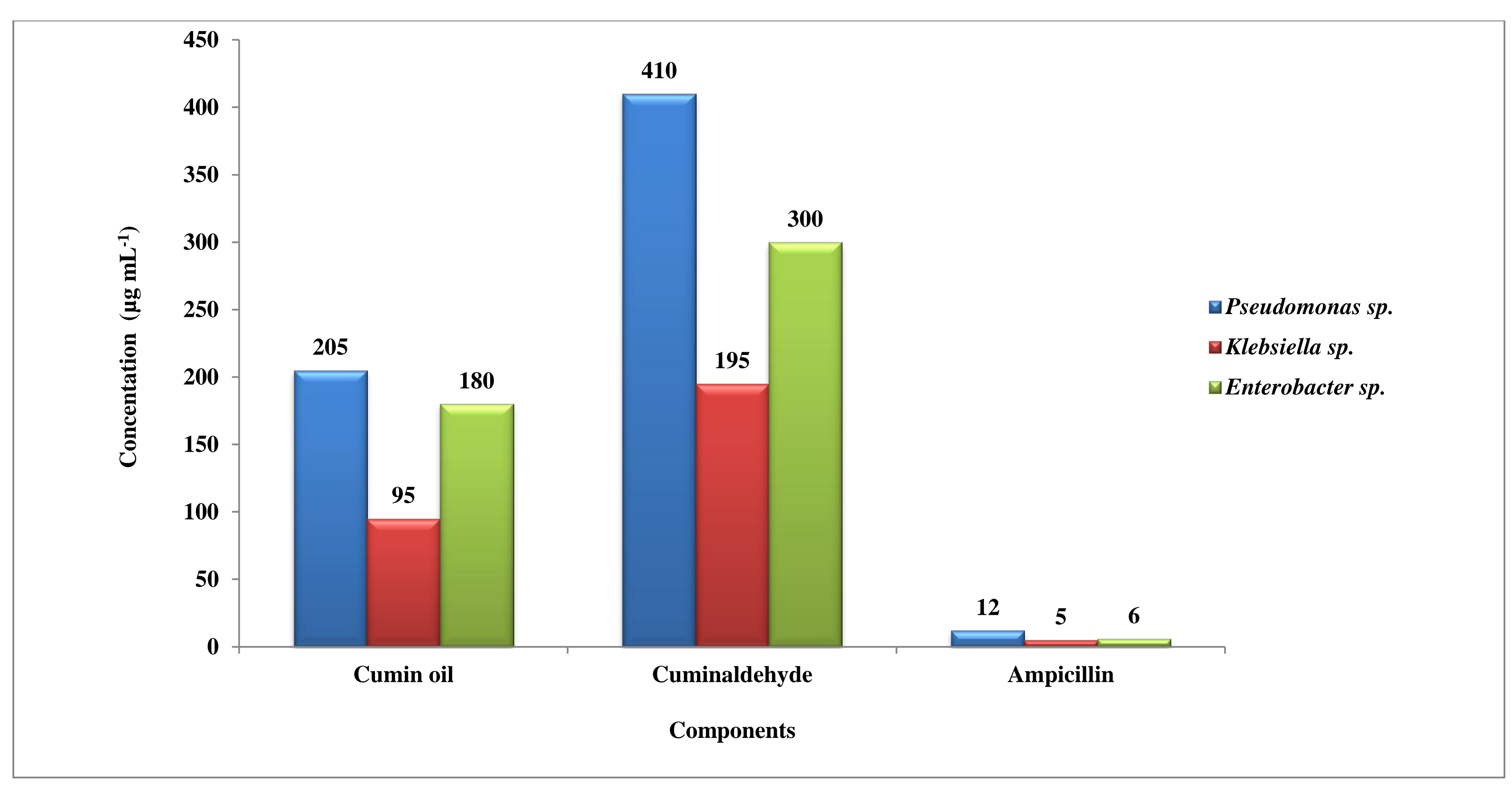


Figure 1: MIC of cumin oil, cuminaldehyde and ampicillin against *Pseudomonas sp*., *Klebsiella sp*. and *Enterobacter sp*.

Table 5: Per cent inhibition of cumin oil, cuminaldehyde and Carbendazim 50 WP against *Fusarium wilt*

Concentration (µg ml^{-1})	Per cent Radical scavenging activity (RSA)		
	Cumin oil	**Cuminaldehyde**	Carbendazim 50 WP
3000	100±0.00	100±0.00	100±0.0
2000	100±0.00	100±0.00	100±0.0
1000	67.58±0.26	65.20±0.65	100±0.0
500	50.53±0.65	44.46±0.45	100±0.0
250	41.89±0.66	37.36±0.95	100±0.0
100	24.89±0.67	22.55±0.58	100±0.0
50	19.23±0.69	18..23±0.55	100±0.00
25	17.23±0.54	16.89±0.23	100±0.00

Particulars	CD (5%)
Compounds	0.59
Concentrations	0.24
Compounds × concentrations	0.67

VITA

Name of the student	:	Pardeep Kaur
Father's name	:	S. Palwinder Singh
Mother's name	:	Smt. Ranjit Kaur
Nationality	:	Indian
Date of birth	:	May 20, 1994
Permanent home address	:	VPO Boparai, Teh. Bholath Distt. Kapurthala

EDUCATIONAL QUALIFICATIONS

Bachelor's degree	:	B.Sc. (Non-Med.)
University	:	Guru Nanak Dev University, Amritsar
Year of award	:	2014
%age	:	68.83%
Master's degree	:	M.Sc. Chemistry
University	:	Punjab Agricultural University, Ludhiana
Year of award	:	2016
OCPA	:	7.73/10.00
Title of Master's Thesis	:	Comparative studies on the synthesis of amides and their herbicidal activity
Ph.D degree	:	Ph.D Chemistry
University	:	Punjab Agricultural University, Ludhiana
Year of award	:	2021
OCPA	:	7.64/10.00
Title of Ph.D Dissertation	:	Chemical derivatizations of major constituent of cumin *(Cuminum cyminum)* and their biological activity