Seasonal Variation in Chemical Composition and Biological Activities of Essential Oils from *Artemisia annua* L.

Thesis

Submitted to the

G. B. Pant University of Agriculture & Technology
Pantnagar- 263145, Uttarakhand, India

By

Akanksha Malhotra
Id. No. 55503

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

Master of Science
(Chemistry)

October, 2021

ACKNOWLEDGEMENT

First and foremost, I would like to praise and thank God, the almighty, who has granted me countless blessings, knowledge, patience and opportunity so that I could finally complete my thesis.

Apart from my efforts, the success of this thesis depends largely on the encouragement and guidelines of many others. I take this opportunity to express my gratitude to the people who have been instrumental in the successful completion of this thesis.

It is hard to determine from where to start expressing the gratitude but for sure the gratitude goes to all those who have assisted me in the process of completing this thesis.

It would be impossible to list all the names but several people deserve my sincere and special thanks.

There are no proper words to convey my deepest gratitude, indebtedness and respect for my advisor Dr. Om Prakash, Professor, Department of Chemistry for his suggestive, inspiring, valuable guidance, intellectual inputs, motivation, patience throughout the entire research work.

My sincere thanks regards and gratitude goes to the esteemed members of my advisory committee Dr. R.M. Srivastava, Professor, Department of Entomology, Dr. D. S. Rawat, Assistant Professor, Department of Biological Sciences, Dr. Ravendra Kumar, Assistant Professor, Department of Chemistry for all the support and valuable suggestions and comments towards improving my work.

I wish to express my sincere thanks to Dr.Vivekanand, Professor and Head of the Department, for his encouragement and support during my research work.

I gratefully acknowledge the open doors of the faculty members of the Chemistry department Dr. MGH ZaidiProfessor, Dr. Virendra Kasana, Professor Dr. Anjana Srivastav, Professor Dr. SameenaMehtab, Assistant Professor , Dr. Shishir Tandon JRO in which I ran into, whenever I encountered trouble or had any query regarding my research work. Thanks are also due to the non-teaching staff of the department namely, Mr. Anil Joshi, Mr. Gaurav Sharma, Mr. Vazeer Ahmad, Mr. Shakeel Ahmad, Mr. Deepak Fulara, Mrs. Munnietc for their direct or indirect help during my thesis work.

I also owe great thanks to Dean, PGS and Dean, CBSH for providing necessary facilities during the pursuit of my research work.

They consistently allowed this research to be my work by their constructive criticisms at times but steered me in the right direction whenever I needed it, which helped me to develop a broader perspective to my thesis.

I would like to acknowledge all the teachers I learnt from since my childhood, I would not have been here without their guidance, blessing and supports.

I deeply thank my parents Mr. Manoj Kumar Malhotra and Mrs. Seema Malhotra, and my whole family wholeheartedly for their endless patience, unconditional trust and timely encouragement. It was their love and support that raised me up to this stage whenever I got demotivated. I have special words of thanks from the core of my heart to my brother Madhav Malhotra and sister Neha talwarfor their love, care, affection and for making me feel better here.

I would also like to thank my labmates,Stuti, Tanuja, Pringal, Avneesh sir, Kirti ma'am ,Sushila ma'am, Himani ma'am and Shailja ma'am for their kind cooperation and their time which they offer gratefully.

Special gratitude and respect for my seniors Himanshi ma'am and Avneesh sir to be a constant motivator and emotional supporter.

I would like to express my special thanks to my senior sir Chenesh Patel for his tremendous support, patience and advice during my research work.

I am thankful to my colleagues Shristi, Pratibha, Stuti, Tanuja, Moin, Megha, Arun, Kamal, Pooja, Pringal and Rahul .I am also thankful to my roommate Aparna Dixit for her constant support and help.

I thank with love to my all dear friends Aparna, Shristi, Aabha, Deepak, Pratibha, hema, Kanchan, Vaishali, Pragya, Monica, Himanshu, kamal, Riya, Divya who helped me endure the frequent frustration. They had been my best friends, great companions as they loved, supported, encouraged, entertained and helped me to get through this period in a most positive way.

"There are friends, there is family, and then there are friends that become family"

It would be difficult to find adequate words to convey how much I owe to all the people involved directly or indirectly.

Thanks to all of you.

Pantnagar
October, 2021

(Akanksha Malhotra)
Authoress

CERTIFICATE-I

This is to certify that the thesis entitled **"Seasonal Variation in Chemical Composition and Biological Activities of Essential Oils from *Artemisia annua* L."** submitted in partial fulfillment of the requirements for the degree of **Master of Science** with major in **Chemistry,** of the College of Post Graduate Studies, G.B. Pant University of Agriculture and Technology, Pantnagar, is a record of bonafide research carried out by **Ms. Akanksha Malhotra,** Id. No. **55503,** under my supervision, and no part of the thesis has been submitted for other degree or diploma.

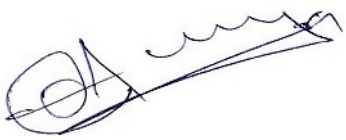
The assistance and help received during the course of this investigation have been duly acknowledged.

Pantnagar
October, 2021

(Om Prakash)
Chairman
Advisory Committee

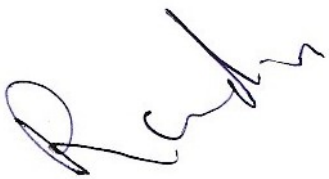
CERTIFICATE-II

We, the undersigned, members of the Advisory Committee of **Ms. Akanksha Malhotra,** Id. No. **55503,** a candidate for the degree of **Master of Science** with major in **Chemistry,** agree that the thesis entitled **"Seasonal Variation in Chemical Composition and Biological Activities of Essential Oils from *Artemisia annua* L."** may be submitted in partial fulfilment of the requirements for the degree.



(Om Prakash)
Chairman
Advisory Committee

(R.M. Srivastava)
Member



(Ravendra Kumar)
Member

(D.S. Rawat)
Member

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

%	Percentage
µL	Microliter
AAREO	*Artemisia annua* rainy EssentialOil
AAWEO	*Artemisia annua* winter EssentialOil
A_o	Absorption value of control sample
A_t	Absorption value of test sample
BHT	Butylated hydroxytoluene
cm	Centimeter
DH	Diterpenoid hydrocarbons
DPPH	2,2-diphenyl-1-picrylhydrazyl
EOs	Essential oils
g	Gram
GC	Gas chromatography
GC-MS	Gas chromatography-Mass Spectrometry
IB_{50}	The half maximal inhibition of protein denaturation
IC_{50}	The half maximal inhibitory concentration
IPP	Isopentyl pyrophosphate
M	Meter
MH	Monoterpenoid hydrocarbons
Min	Minute
MS	Mass spectrometry
Na_2EDTA	Sodium salt of ethylenediaminetetraacetic acid
OD	Oxygenated Diterpenoids
OM	Oxygenated monoterpenoid

OS	Oxygenated Sesqueterpenoid
pH	Potential of hydrogen
ppm	Parts per million
SEm=	Standard error
SH	Sesqueterpenoid hydrocarbons
Sq	Square
STD	Standard deviation
v/v	Percentage by volume
w/w	Percentage by mass

Introduction

Chapter 1 INTRODUCTION

Throughout human history, plant products have always played an important role in medicine .The use of herbal medicine is becoming increasingly popular around the world. Medicinal plants as we know are known for playing vital role in betterment of human health from time immemorial. India is thought to have a long history of using plants as medicines. Approximately 2500 plant species have been reported to have medicinal value, with 750 of these species known to be the active ingredients in 14,000 published species of Ayurvedic, Siddha, and Unani medicines **(Chauhan, 1999).**

Amongst 1,75,000 flowering plants which are believed to be found in India about 3000 of them are known for their medicinal uses **(Sharma *et al.*, 1997).** People have been using compounds obtained from plants for treating variety of diseases since prehistoric times. over 50,000 plant species are used for medicinal purposes like Analgesic (e.g., morphine), antitussive (e.g., codeine), antihypertensive (e.g., reserpine), cardiotonic (e.g., digoxin), or antimalarial (e.g., artemisinin) compounds, are only a few of the several irreplaceable medicinal products derived from plants **(Nessler, 1994**). About 25% of the new drugs sanctioned in the past 30 years are based on a molecule of plant origin, and about 50% of the top selling chemicals obtained from study on the plant secondary metabolism **(Terryn *et al*., 2006).**

Majority of civilizations in developing countries like India have traditionally relied on products obtained from plants, especially from forests, for treatment of various human and livestock ailments. Variety of aromatic plants are popular for domestic and commercial uses. Collectively they are called medicinal and aromatic plants (MAPs). Out of 4,22,000 plant species reported worldwide, 12.5% are reported to have medicinal values; but only a few of them are known to be in cultivation **(Rao *et al*., 2004).** The isolation of the hypertensive alkaloid from sarpgandha (*Rouwolfia serpentina*), priced for the treatment of insomnia, hypertension, and insanity, was the first and most notable contribution from ayurvedic medicine **(Dev, 1999).**

With new concepts like phytotherapy and veterinary medicinal uses, aromatherapy, nutraceuticals, cosmeceuticals, and animal welfare uses, MAPs have now evolved into "industrial products."**(Máthé *et al.*, 2015).** MAPs are of utmost importance in curing various diseases. These plants are the resources of traditional medicines and several modern medicines have been produced indirectly from plants. Over the last 2,500 years, there have been very strong traditional systems of medicine such as Chinese, Ayurvedic, and the Unani, born and practiced, more in the eastern continent **(Rashid *et al.*, 2014).**

Aromatic plants, also called as herbs and spices, have been used since ages as folk medicine and also as preservatives in foods. The best known aromatic plants, originated from the Mediterranean area constitute many biologically active compounds, like polyphenolics, which have been found to possess antimicrobial, antioxidant, antiparasitic, antiprotozoal, antifungal, and anti-inflammatory properties. In aromatic and scented plants, the volatile organic compounds originate from three categories of chemicals including phenolic compounds, fatty acid derivatives, and isoprenoids **(Caissard *et al.*, 2004*)*.**

About 120 plant-derived chemical compounds have been developed into modern pharmaceuticals. Natural essential oils and their fragrance are among the most exceptional products of plant metabolism, and they have had an impact on human thoughts and emotions since the dawn of civilization. India is well known for its plant resources from a long time, and is one of the world’s 12 bio-diversity centers sheltering over 45,000 different plant species. The diversity of India is remarkable due to the presence of 16 different agro climatic zones, 10 vegetation zones, 25 biotic provinces and 426 biomes. Out of these, about 15,000-20,000 species have good medicinal value **(Thomas *et al.*, 1999).**

The medicinal properties of plants are primarily due to the production of secondary metabolites in the plants **(Dar *et al.*, 2017).**The study of medicinal plants continues primarily for the purpose of discovering secondary metabolites, as secondary metabolites are responsible medicinal activity of the plants. Plant products have been used in phytomedicines. These can be derived from any part of the plant,

such as the bark, leaves, flowers, seeds, and so on; in other words, any part of the plant can contain active components **(Savithramma *et al*., 2011).**

The secondary metabolites of plants have an immense application in human health and have commercial importance in pharmaceuticals, food additives and other industrial materials **(Naik and Khayri, 2016).** The secondary compounds of plants are generally grouped on the basis of their biosynthetic pathways. An excellent example of a metabolite family is given by phenolics: because these molecules are involved in the synthesis of lignin, they are common to all higher plants**.** Due to their biological activities; plant secondary metabolites have been used for centuries in traditional medicine **(Bourgaud *et al*., 2001)**. They provide plants with a distinct aroma, colour, flavour, and medicinal properties, as well as protection from biotic and abiotic stresses **(Weisshaaar and Jenkins, 1998).**The basis of the classification of secondary metabolites is chemical structure, composition, their solubility in different solvents, or the pathway from which they are synthesized. The main classification system is comprised of three major groups: terpenoids, alkaloids and phenolics. Phenolic compounds from plants are considered as one of largest group of secondary metabolites synthesized by fruits, vegetables, teas, cocoa and other plants that have specific health benefits. They have antioxidant, anti-inflammatory, anti-carcinogenic and other biological properties, and may protect from oxidative stress and some otherdiseases. Simple phenolics possess bactericidal, antiseptic and anthelmintic properties **(Kabera *et al*., 2014).**The plant secondary metabolites includes terpenes, phenolics,nitrogen containing compounds, sulphur containing compounds etc as shown in **figure 1.1.**

The literature studies revealed that certain derivatives of nitrogenous terpenes have the potent anti- hypertensive property and may indicate a new era in the field of medicine, where as due to some insecticidal and anti-microbial activities in other terpenoids, they are able to be used as fungicides and pesticides in agriculture **(Kataev *et al*., 2011; Bohme *et al* ., 2014).**

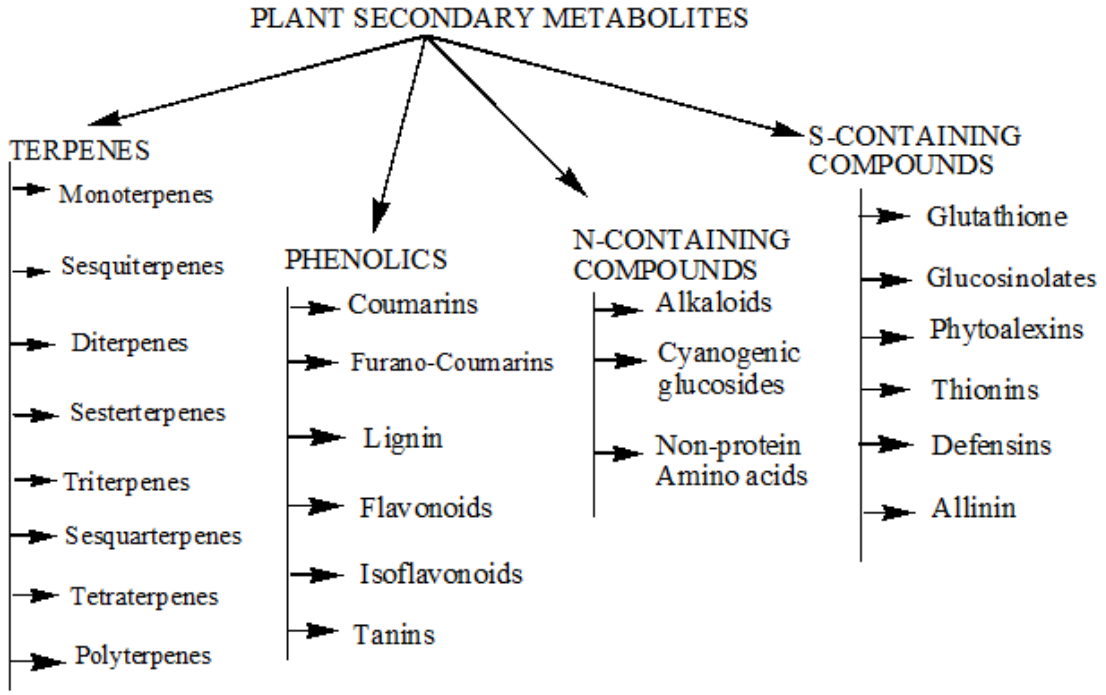


Fig. 1.1 Secondary metabolites of plants

Essential oils are a complex mixture of terpenoids formed from the secondary metabolism of plants and are responsible for their unique aroma. They are basically complex mixtures, comprised by terpenoid hydrocarbons, oxygenated terpenes and sesquiterpenes. These oils are extracted by the following methods like steam distillation, extractions with solvents and expression, of which steam distillation is the most common method as it permits the separation of slightly volatile, water immiscible substances due to low temperature distillation **(Chamorro *et al.*, 2012).** Essential oils generally help plants in protection against diseases and predators due to their strong fragrance. They are known to stimulate certain responses in the body for example lavender helps in relaxation, and *Mentha piperita* boosts exercise performance where Eucalyptus relieves helps in over coming congestion **(Meamarbashi, 2014 ; Tan and Johnston 2014).** They are volatile, natural and known to have a strong odour. They are liquid, volatile, limpid and rarely coloured, lipid soluble and soluble in organic solvents with a density less than that of water. They can usually be synthesized by all plant organs viz.. buds, flowers, leaves, stems, twigs, seeds, fruits, roots, wood or bark, and are stored in secretory cells, cavities,

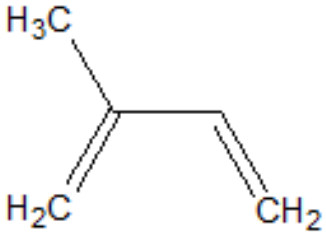
canals, epidermic cells or glandular trichomes. Essential oils are generally known for their antiseptic i.e. bactericidal, virucidal, fungicidal, and medicinal properties and their fragrance, they are often used in preservation of foods and as antimicrobial, analgesic, sedative, anti-inflammatory, and locally anesthesic remedies **(Bakkali *et al.*, 2008).**

Chemical composition of essential oils

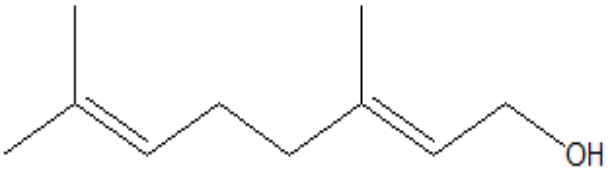
Terpenoid and phenylpropanoid derivatives are the two main components found in essential oils. Terpenoids account for about 80% of the terpenoids in most plant's essential oils. However, essential oils have significant flavour, odour, and are piquant due to the presence of phenylpropanoid derivatives. These two groups of compounds are derived from two different pathways from different primary metabolites **(Sangwan *et al.*, 2001).** Classification of terpenoids on the basis of number of isoprene units which are important for biosynthetic pathway is given in **table 1.1.**

Table 1.1 The main classes of plant terpenoids

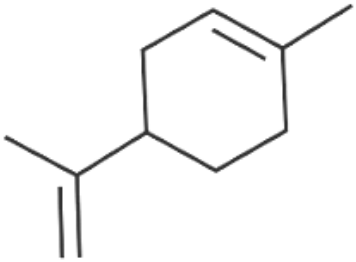
S No.	Classification	No. of carbon atoms	No. of isoprene units	Example
1.	Isoprene	5	1	Isoprene **[1]**
2.	Monoterpenes	10	2	Geraniol **[2],** Limonene **[3]**
3.	Sesquiterpenes	15	3	Farnesol **[4]**
4.	Diterpenes	20	4	Taxadiene **[5]**, cafestol **[6]**
5.	Sesterterpesnes	25	5	Ophiobolin **[7]**
6.	Triterpenes	30	6	β-amyrin **[8]**, squalene **[9]**
7.	Sterols	27-30	5-6	Sitosterol **[10]**
8.	Tetraterpenes	40	8	β-carotene [**11],**lycopene **[12]**
9.	Polyterpenes	≥ 40	≥8	Gutapercha **[13]**



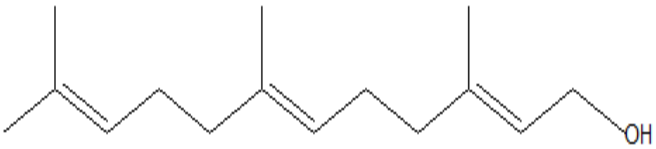
[1]



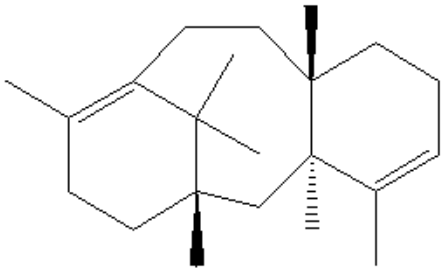
[2]



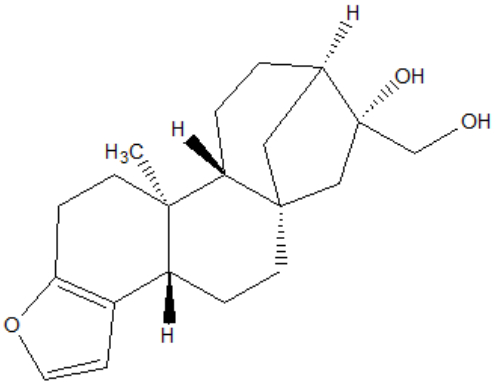
[3]



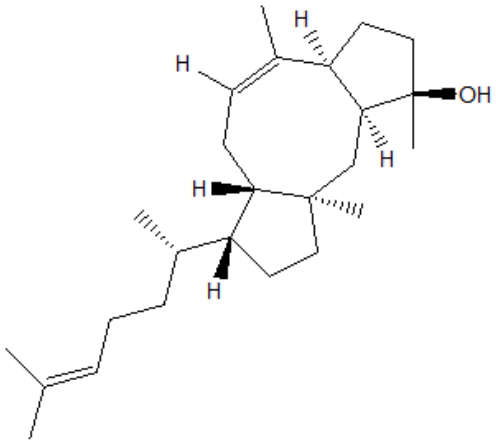
[4]



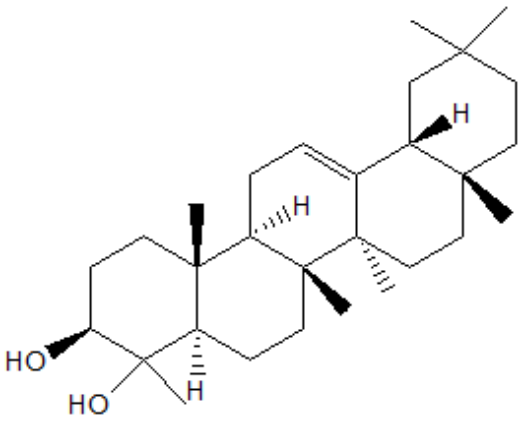
[5]



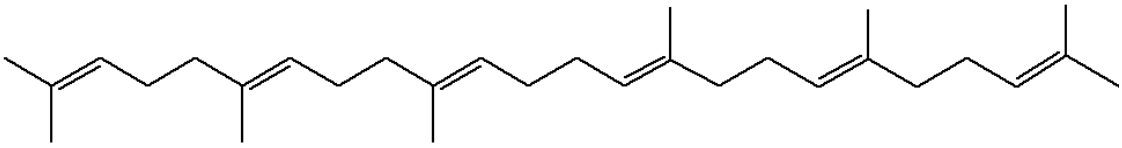
[6]



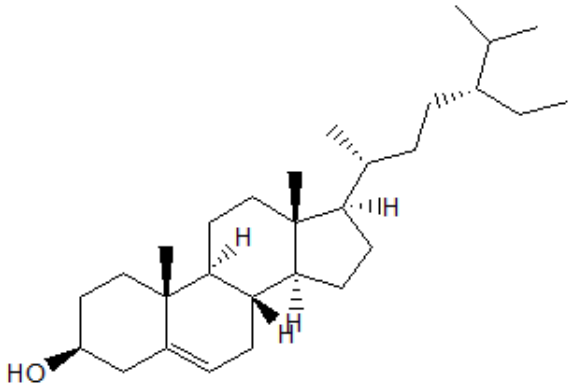
[7]



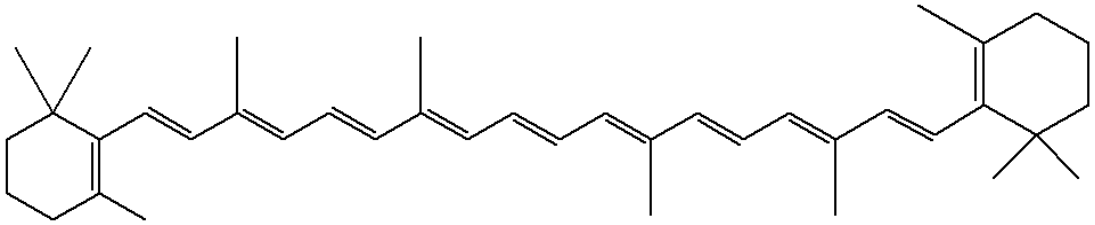
[8]



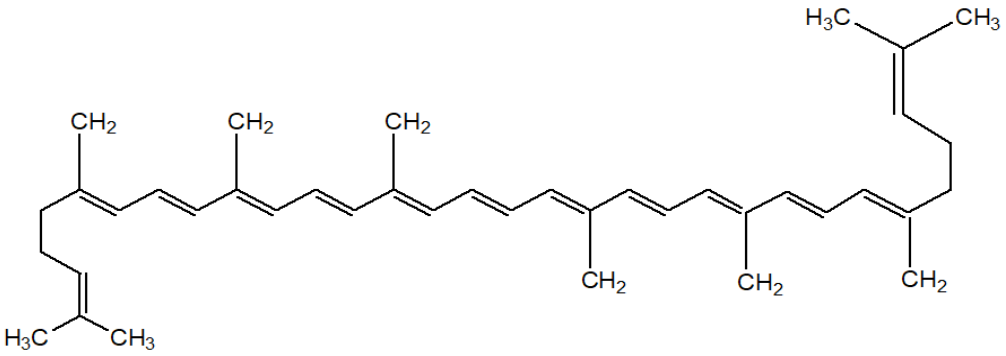
[9]



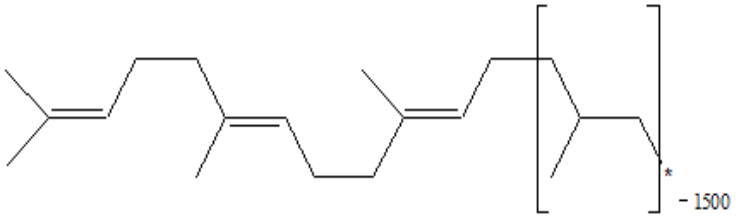
[10]



[11]



[12]



[13]

Inspite of the huge structural differences between terpenoids, they all have been derived from the same C_5 skeleton of isoprene [1]. The backbone of the terpenoids are formed from two precursors: isopentenyl pyrophosphate (IPP) and dimethyl allyl pyrophosphate (DMAPP) via a series of repeats, rearrangement, and cyclization reactions. Two distinct biosynthetic pathways for the synthesis of these universal precursors have been identified, the classical mevalonate (MVA) pathway and the most recently characterized 2*C*-methyl-D-erythritol-4-phosphate (MEP) pathway, also known as the 1-deoxy-D-xylulose- 5-phosphate (DXP) pathway. The MVA pathway is present in eukaryotes (all mammals, the cytosol and mitochondria of plants, fungi), archaea, and some eubacteria while the non-mevalonate pathway is found to occur in eubacteria, algae, cyanobacteria, and the chloroplasts of plants. The MVA pathway consists of mainly seven enzymatic reactions to convert the precursor acetyl-CoA to IPP and DMAPP, whereas while the MEP pathway converts the starting materials, pyruvate and glyceraldehyde-3-phosphate, to IPP and DMAPP via eight enzymatic reactions **fig.1.1 (Abdallah and Quax, 2017)**. There are two pathways that lead to the synthesis of isoprene. These two pathways occur in the different parts of the specialized cell. The mevalonic acid pathway occurs in the cytosol, whereas the MEP pathwayoccurs in the plastid **(Fokou *et al*., 2020).**

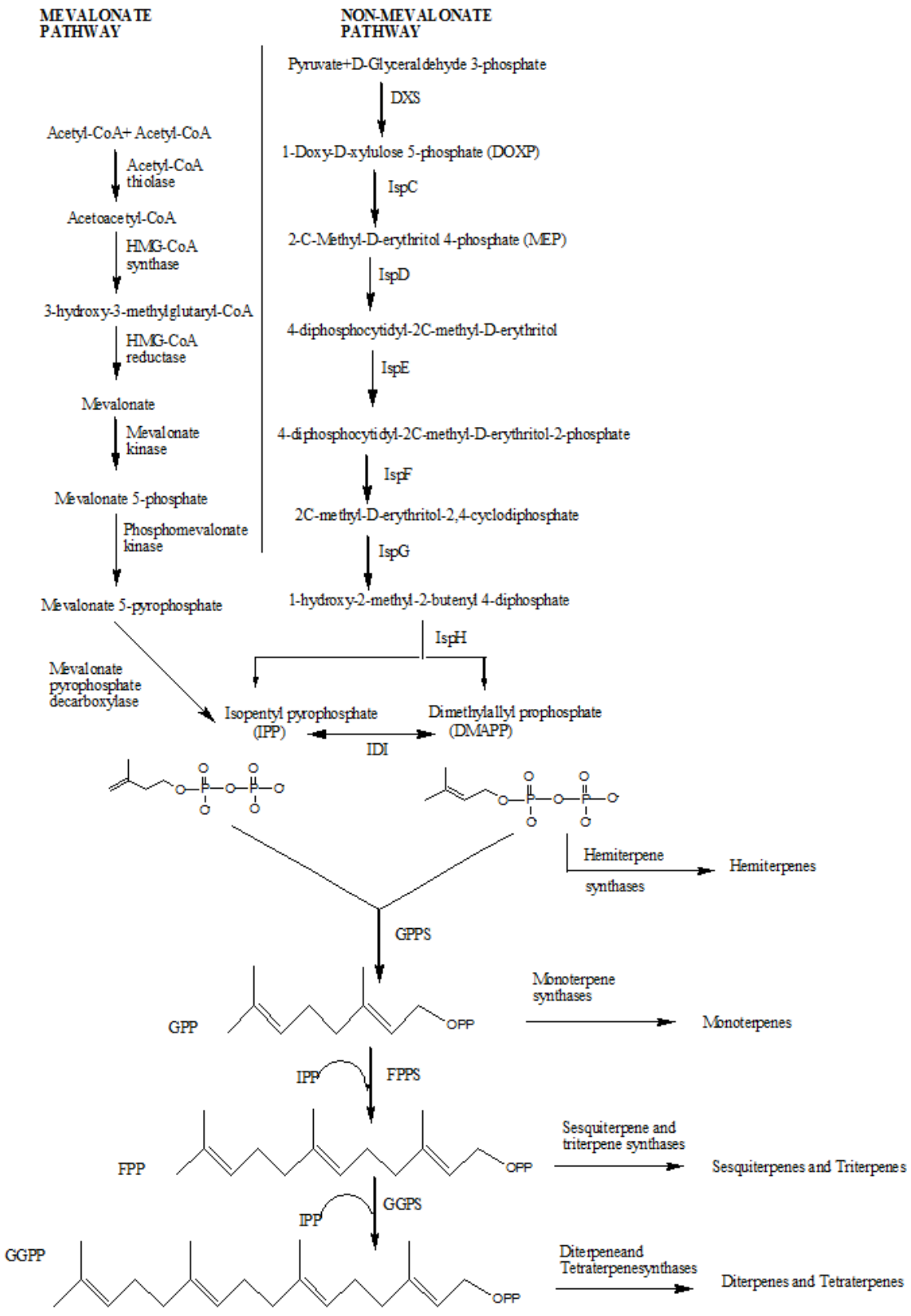


Fig. 1.2 Biosynthetic pathways for terpenoid production

The Essential oils also posess the following significant biological activities:

Oil repellent efficiency- Plant essential oils and their individual metabolites have a high potential for repellent activity against a variety of insect and arthropod species. It has been reported that among the various metabolites isolated from essential oils, The ones with the best repellent activity are oxygenated, being the hydroxyl group part of the molecules with better activities **(Nerio *et al*, 2010).**

Culinary use- Due to the presence of several chemical compounds like cinnamaldehyde, eugenol, chavicol, safrole etc, the essential oils provide aroma to the food items **(Bakkali *et al.*, 2008).**

In Perfumery- Some constituents of essential oils like D-limonene, geranyl acetate or D-carvone are used in perfumes, creams, soaps, as fragrances for cleaning products in household and as industrial solvents **(Bakkali *et al*., 2008).**

In Aroma therapy- Aroma therapy is one of the commendatory therapies which utilize essential oils as the major therapeutic factor in treatment of various diseases.This therapy has a calming effect on person's mind and the body. **(Ali *et al*., 2015).**

As a food Preservative- Several EOs have been used as a food preservative for meat and meat products, vegetables and fruits as well as for dairy products **(Falleh *et al*., 2020).**

Allelopathic affects- Allelopathy is mechanism of interference inwhich plants produce certain chemical compounds that effect other plants **(Gholami *et al*., 2011).** It has been reported from various studies that chemicals found in essential oils of plants have allelopathic effects on germination of seeds **(Rahimi *et al*., 2013).**

As pheromones- Several essential oil components are utiliised by insects as major or minor components of their pheromones and thus attract conspecifics to them. Certain species of termites *Reticulitermes* were found to posess (−)-α-Pinene, (−)-β-pinene, (−)-camphene, myrcene, (−)-limonene and both (Z)- and (E)-ocimene, (+)-γ-cadinene,

, (−)-germacrene A and B, γ-himachalene and β-bisabolene in their pheromones **(Muller and Buchbauer, 2011).**

Apart from the above mentioned activities, the essential oils have been known to possess many more biological activities as depicted in **fig.1.3**

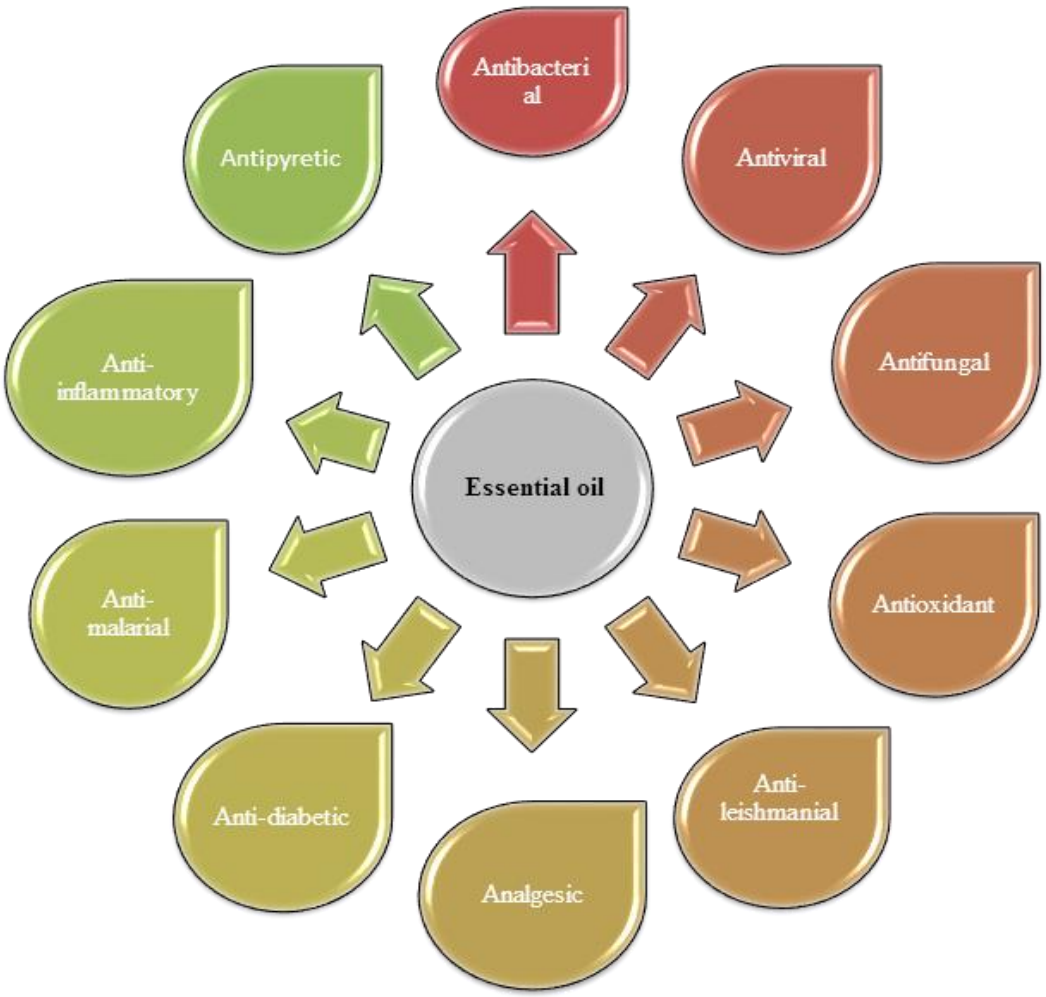


Fig 1.3 Different biological activities of essential oils

Based on the thorough search of literature and ethanobotanical applications, its traditional applications, it becomes necessary to generate more data based on phytochemical analysis and various biological potential of essential oil from aerial parts of *A.annua*, I have taken this herb for the study of its seasonal variation in chemical composition of essential oils and determination of different biological activities with following objectives:

OBJECTIVES

1. Collection of fresh plant material from its natural habitat.
2. Taxonomic identification of collected plant material and maintainence of herbarium.
3. Extraction of essential oil by using hydrodistillation/steam distillation through a Clevenger type apparatus.
4. Phytochemical analysis of essential oil using gas chromatography and gas chromatography- mass spectrometry.

   Different biological activities (anti-inflammatory, anti-oxidant, anti-diabetic, antifeedant and nematicidal activities will be studied using standard methods reported and generally are being practiced by various researchers).

Review of Literature

2.1 Family: Asteraceae

The Asteraceae Bercht. & J. Presl (= compositae Giseke) family is also known as 'daisy', 'aster' or 'composite' family **(Xu and Chang, 2017)**.The Asteraceae family is broadly divided into two large subfamilies: Cichorioideae (syn.Lactucoideae, multisieae, Cardueae) and Asteroideae (Inuleae, Astereae, Anthemideae). It is one of the most numerous plant families. This family is found all over the world, but it is especially prevalent in the Mediterranean, Eastern Europe, and Asia Minor. There are approximately 25,000 species in the family under about 1,568 genera. **(Mabberley, 2017)**. The majority of the species are herbaceous, but some are shrubs or even trees, and they are mainly found in the tropical regions of North and South America, Africa, and Madagascar, as well as on the islands of the Atlantic and Pacific Oceans. Generally the species of Asteraceae have a capitulum or head infloroscence, an inferior, unilocular ovary with one ovule, and with few exceptions fused anthers surrounding the style **(Harris, 1995).** Some of these species, such as Chamomile (*Matricariarecutita* L.), Yarrow (*Achillea millefolium* L.) and Wormwood (*Artemisia nilagirica* (C.B Clarke Pamp.)) are reported to have medicinal properties. Essential oils extracted from the Asteraceae species have been reported to be used against several ailments and sufferings.

The majority of percentage of this family is herbaceous, while about 2 percent are trees or shrubs **(Lawrence, 1973).** Asteraceae members typically have leaves that are alternate, rarely opposite, exstipulate, and rarely stipulate; inflorescence capitulum or head surrounded by involucre of bracts; ray and disc florets, flower tubular or ligulate, flowers bisexual or unisexual or outer male or female, pentamerous, actinomorphic or zygomorphic, calyx modified topappus, corolla gamopetalous, petallobes 5, stamens 5, epipetalous, usually dithecous, filament free and anthers united i.e. syngenesious, introrse, ovary unilocular, inferior with solitary ovule in basal placentation, style slender stigma bifid; fruit cypsela.The stem can beerect, or prostrate, herbaceous or woody (*Artemisia*), hairy, sometimes with latex. Stem tubers are also

present (*Helianthus*); tubers are edible (*H. tuberosus*); cylindrical, solid or fistular, stem may be leaf-like (*Baccharis*).The anatomical features that can be usually seen in Asteraceae are: (a) presence of different types of glandular or covering trichomes; (b) papillae (c) anomocytic, anisocytic and rarely heliocytic stomata; (d) presence of hydathodes; (e) presence of hypoderm; (f) homogeneous or heterogeneous mesophyll; and (g) vascular bundles with parenchymatic sheath composed by large cells **(Metcalfe and Chalk, 1950)**. This family contains species that are extremely important in the fields of nutrition, cosmetics and pharmacy due to the production of essential oils **(Milan *et al*, 2006).** The systematic position of family Asteraceae is given in **table 2.1** and the diversity of the Family Asteraceae is depicted in **table 2.2** respectively.

Table 2.1 Systematic position of family Asteraceae

Kingdom	Plantae
Sub-Kingdom	Tracheobionta
Division	Magnoliophyta
Sub-division	Angiospermae
Class	Dicotyledons
Sub-class	Asteridae
Order	Asterales
Family	Asteraceae

Table 2.2 Diversity of the family Asteraceae

SN.	Distribution	No. of genera	No. of species	References
1.	World	1568-1,911	25,000-32,913	**The Plant List (2013); Christenhusz and Byng (2016); Mabberely (2017)**
2.	India	193	999	**Karthikeyan *et al.*, (2009)**
3.	Uttarakhand	125	376	**Uniyal *et al*., (2007)**

2.2 Genus *Artemisia* L.

Artemisia L. is a large, diverse and economically important genus of the family Asteraceae. It has more than 540 species in the world **(Mabberley, 2017).** *Artemisia* is a wind pollinated cosmopolitan genus that is primarily found in temperate areas of the northern hemisphere's mid to high latitudes, colonising arid and semiarid landscapes, with just a few representatives in the southern hemisphere. Its centre of diversification is Central Asia, with the Mediterranean region and North West America serving as secondary areas of speciation **(Hayat *et al*., 2009)**.The geographical distribution of genus *Artemisia* illustrated in the **fig. 2.1.**

The species of *Artemisia* are generally perennial, biennial and annual herbs or small shrubs. The genus *Artemisia* is characterized by a wide range of morphological and phytochemical variability, which is associated with different geographical origins of the samples. The genus displays a huge ecological plasticity, with species occurring from sea level to high mountains and from arid zones to wetlands. Phytochemical analysis of the genus *Artemisia* revealed that the *Artemisia* species consists mainly terpenoids, flavonoids, coumarins, caffeoylquinic acids, sterols and acetylenes. Amongst numerous species of *Artemisia*, *A. absinthium*, *A. afra* Jacq., *A.annua* L., *A. maritima* L. and *A. scoparia* Waldst. & Kit. are principally rich in terpenoids. A new class of highly effective antimalarials has been discovered using artemisinin, which is an endoperoxide sesquiterpene lactone isolated from the Chinese medicinal plant *A. annua*. Combination therapies based on artemisinin are now widely regarded as the best treatment against *Plasmodium falciparum* malaria **(Bora and Sharma, 2011).**

Species of the genus *Artemisia* are mainly known by the common names such as mugwort, wormwood and sagebrush. Due to the presence of terpenoids and sesquiterpene lactones, most of the species possess strong aromas and bitter tastes, which discourage herbivory, and may have had a selective advantage. Major components consisted of several terpenes, terpenoids and phenolic compounds, where as 1, 8-cineole, beta-pinene, thujone, artemisia ketone, camphor, caryophyllene, camphene and germacrene-D were reported dominant in several species of *Artemisia*. The different essential oils from the genus *Artemisia* and their compounds have been reported as effective antimicrobial, insecticidal and antioxidant agents. Antioxidant activity found in oils is basically due to presence of phenolic compounds **(Pandey and Singh, 2017).**

The genus *Artemisia*, which belongs to the Anthemideae tribe, contains important medicinal plants that are currently the focus of phytochemical research due to their biological and chemical diversity, as well as the production of essential oils. The strong and aromatic odour of some *Artemisia* species is caused mainly by high levels of volatile terpenes, which are constituents of their essential oils, notably in leaves and flowers. The chemical composition of essential oils derived from the *Artemisia* genus has been extensively researched in a wide range of species from around the world. Many studies have shown that *Artemisia* species show significant intraspecific variations in the terpene constituents of their essential oils. It has been reported that the quality and yield of essential oils obtained from *Artemisia* species is generally due to the influence of the harvesting season, fertilizer and pH of soils, the choice and stage of drying conditions, the geographic location, chemotype or subspecies, choice of plant part or genotype, or extraction method **(Abad *et al*., 2012).** The genus *Artemisia* L. is one of the most important genera of the Asteraceae, as it is used all over the world in traditional medicine, as a source for spices (e.g., *Artemisia dracunculus* L.), ingredient in liquors (e.g., *Artemisia absinthium* L.), and as a source for artemisinin (*Artemisia annua* L.) **(Panda *et al*., 2019). Joshi (2013)** reported that the chemical composition of various species of *Artemisia* from different origins exhibited the presence of 1, 8- cineole, α-thujone, β-thujone, chamazulene, davanone, artemisia ketone, germacrene-D, β-caryophyllene and caryophyllene oxide. The systematic classification of genus *Artemisia* is depicted in **table 2.3** where as **table 2.4** contains the *Artemisia* Species that are reported from India and Uttarakhand.

Table 2.3 Systematic classification of genus *Artemisia* L.

Kingdom	Plantae
Clade	Tracheophytes
Clade	Angiosperms
Clade	Eudicots
Clade	Asterids
Order	Asterales
Family	Asteraceae
Subfamily	Asteroideae
Supertribe	Asterodae
Tribe	Anthimideae
Genus	*Artemisia* L.

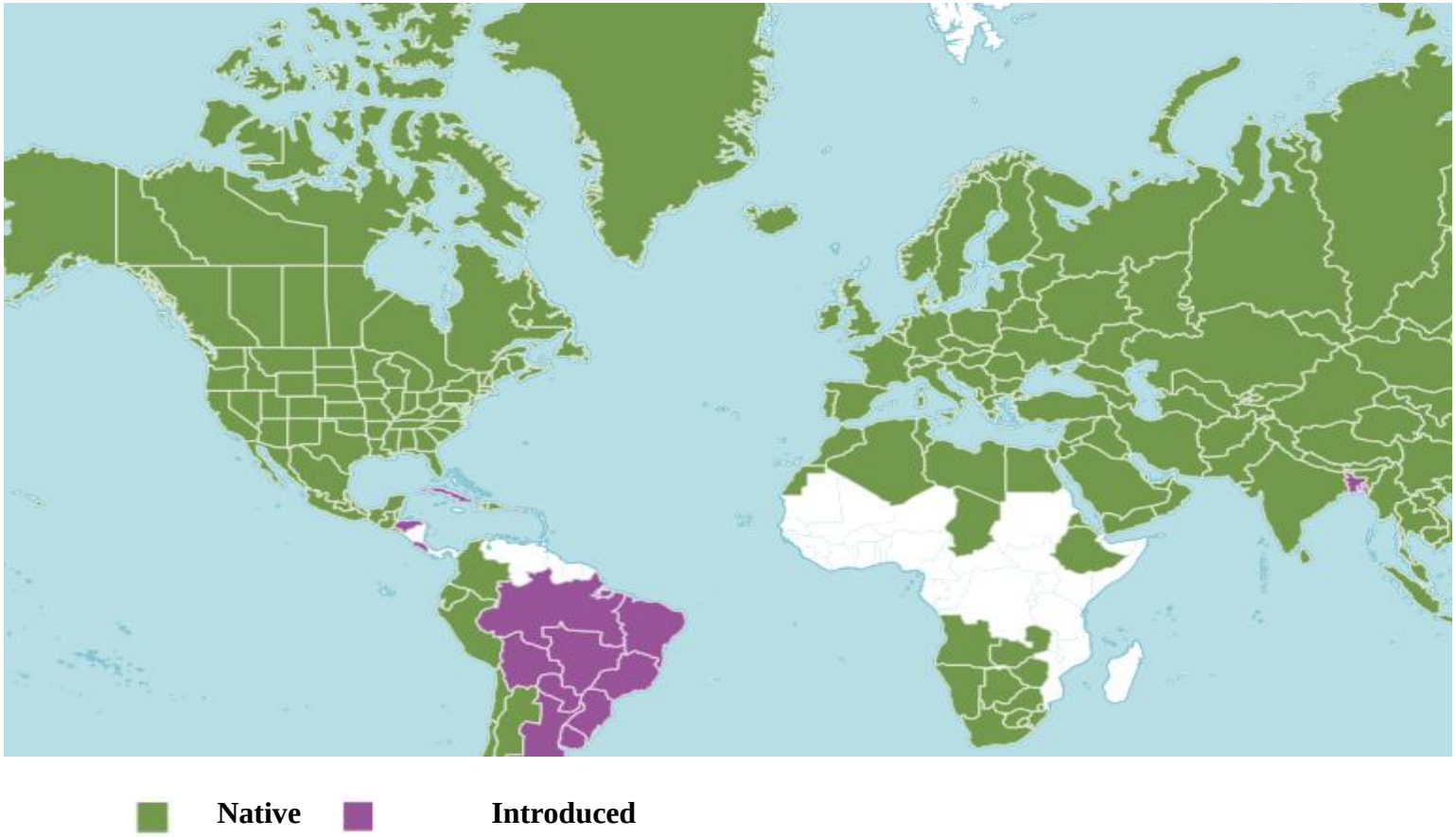


Fig. 2.1 Map showing distribution of genus *Artemisia* across the world (POWO, 2019)

Table 2.4 *Artemisia* species reported from India and Uttarakhand (Karthikeyan *et al*., 2009; Uniyal *et al*., 2007)

SN.	Species	SN.	Species
1.	**Artemisia absinthium* L.	2.	**Artemisia annua* L.
3.	**Artemisia austrohimalyana* Ling &Puri	4.	**Artemisia biennis* Willd.
5	**Artemisia capillaris* Thunb.	6.	**Artemisia caruifolia* Buch.-Ham.ex Roxb.
7.	**Artemisia dracunculus* L.	8.	**Artemisia dubia* Wall.
9.	**Artemisia edgeworthii* N.P. Balakr.	10.	**Artemisia elegantissima* Pamp.
11.	**Artemisia eriocephala* Pamp.	12.	**Artemisia filiformilobulata* Y.R Ling &Puri
13.	**Artemisia gmelinii* Weber	14.	**Artemisia hedinii* Ostenf. & Paulson
15.	**Artemisia incia* pamp.	16.	**Artemisia indica* Willd.
17.	**Artemisia japonica* Thunb	18.	**Artemisia macrocephala* Jacquem.
19.	**Artemisia maritima* L.	20.	**Artemisia myriantha* Wall.
21.	**Artemisia nilagirica* (C.B. Clarke) Pamp.	22.	**Artemisia roxburghiana* Wall.
23.	**Artemisia scoparia* Waldst. and Kit	24.	*Artemisia abrotanum* L.
25.	*Artemisia amygdalina* Decne.	26.	*Artemisia austroyunnanensis* Ling & Y.R. Ling
27.	*Artemisia brunnea* Pamp.	28.	*Artemisia campbellii* Hook.f.
29.	*Artemisia demissa* Krasch.	30.	*Artemisia demissa* Krasch.
31.	*Artemisia desertorum* Spreng.	32.	*Artemisia dolichocephala* Pamp.
33.	*Artemisia dubia*Wall.ex Besser	34.	*Artemisia falconeri* C.B. Clarke
35.	*Artemisia glauca*sensu Hook.f.	36.	*Artemisia grandis* Pamp.
37.	*Artemisia hypoleuca* Edgew.	38.	*Artemisia jacquemontiana* Besser
39.	*Artemisia kohatica* (Klatt) Pamp	40.	*Artemisia laciniata* Willd.
41.	*Artemisia lactiflora* Wall.	42.	*Artemisialancea* Vaniot.
43.	*Artemisia lavandulaefolia* DC.	44.	*Artemisia leptostachya* DC.

45.	*Artemisia macrocephala* Jacquem.	46.	*Artemisia minor* Jacquem.
47.	*Artemisia mollissima* DC.	48.	*Artemisia moorcroftiana* Wall. ex DC
49.	*Artemisia nepalensis* Nees	50.	*Artemisia pamirica* C.Winkl.
51.	*Artemisia pallens* Wall.	52.	**Artemisia persica* Boiss.
53.	*Artemisia parviflora* Roxb.	54.	*Artemisia princeps* Pamp.
55.	*Artemisia pleiocephala* Pamp.	56.	*Artemisia revolute* Edgew.
57.	*Artemisia purpurascens* Jacquem.	58.	**Artemisia roxburghiana* Bess.
59.	***Artemisia robusta* (Pamp.) Ling & Y. R.	60.	**Artemisia roxburghiana* var. hypoleuca Pamp.
61.	**Artemisia roxburghinia* var. acutilobaPamp.	62.	*Artemisia royleana* DC.
63.	**Artemisia roxburghinia* var. purpurascens (Jacq.) Hook.f.	64.	**Artemisia salsoloides* Willd.
65.	*Artemisia sacrorum* Ledeb.	66.	*Artemisia schmidtiana*
67.	*Artemisia santolinifolia* Turcz.	68.	*Artemisia simplicifolia* Pamp.
69.	*Artemisia sericea* (Besser) Weber	70.	*Artemisia stewartii* C.B. Clarke
71.	**Artemisia sieversiana* Ehrh.	72.	**Artemisia strongylocephala* Pamp
73.	*Artemisia stelleriana* Besser.	74.	*Artemisia tainingensis* Hand. – Mazz
75.	*Artemisia stricta* Edgew.	76.	*Artemisia tenufolia* Y.R. Ling &Puri
77.	*Artemisia subdigitata* Mattf.	78.	*Artemisia thomsonii* C.B. Clarke
79.	*Artemisia tainingensis* var. nitida (Pamp)Y.R. Ling	80.	*Artemisia tridentata* (USA)
81.	*Artemisia thellungiana* Pamp.	82.	*Artemisia velutina* Pamp.
83.	*Artemisia tournefortiana* Rhcb.	84.	**Artemisia vestita* Wall.
85.	*Artemisia tukuchaensis* Kitam.	86.	*Artemisia vulgaris* T.
87.	**Artemisia verlotiorum* Lamotte	88.	*Artemisia wallichiana* Bess.
89.	*Artemisia vulgaris* Hook.f.	90.	*Artemisia wadei* Edgew.
91.	*Artemisia wellbyi* Hemsl. & H.Pearson	92.	**Artemisia elegantissima* var. *Kumaunensis* Pamp

*species reported from Uttarakhand

2.3 *Artemisia annua* L.

Artemisia annua L., also known as sweet wormwood, sweet sagewort and annual wormwood (Chinese: qinghao), is a common type of wormwood that is native to temperate Asia, but naturalized throughout the world. *A. annua* is a large shrub often reaching more than 2.0 m in height, usually single-stemmed with alternate branches. The leaves are aromatic with deeply dissected lamina and range from 2.5 to 5 cm in length. Leaves and flowers contain both 10-celled biseriate trichomes and 5 cell filamentous trichomes. *A. annua* is well-known medicinal plant for being as a source of antimalarial compound artemisinin (Qinghaosu), which is a cadinane-type sesquiterpene lactone with an endoperoxide bridge that currently is the most potent and effective compound against chloroquine and quinine-resistant *Plasmodium falciparum* and other malaria-causing parasites **(Das, 2012).**

A.annua grows naturally as a part of steppe vegetation in northern parts of Chatar and Suiyan province in China at 1,000–1,500 m above sea level.. The stem is generally cylindrical and branched. Leaves can be seen as alternate, dark green, or brownish green. Odour is characteristic and aromatic while the taste is bitter. The Chinese name of the plant is Qinghao (or Qing Hao or Ching-hao which means green herb **(Dhingra *et al*., 1999)**. The plant is cropped on a large scale in China, Vietnam, Turkey, Iran, Afghanistan, and Australia. In India, it is cultivated on an experimental basis in the Himalayan regions, as well as temperate and subtropical conditions **(Bhakuni *et al*., 2001).**

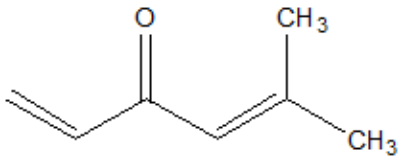
Artemisia annua is a potent antimalarial plant and used in folks, Homeopathic and Ayurvedic system of medicine. According to the literature search, in China, aqueous preparation of the dried herb was applied against fever, malaria, skin diseases, jaundice and haemorrhoids **(Brisibe *et al*., 2008; Nofal *et al*., 2009; Rezelman and Goris, 2008)**. Leaves of *Artemisia annua* are widely used for the treatment of malaria and as anti-inflammatory in most part of the world **(Das, 2012).**

According to reports, the essential (volatile) oil of *A. annua* can yield up to 85 kg/ha. It is produced by secretory cells, particularly those in the plant's uppermost foliar portion, which has nearly twice the number of cells as the lower leaves. According to reports, capitate glands, which contain terpenoidic volatile constituents, cover 35% of the mature leaf surface. Essential oil from *A. annua* is distributed, with 36% of the total from the upper third of the foliage, 47% from the middle third, and 17% from the lower third, with only trace amounts in the main stem side shoots and roots. The yield of the oil generally ranges between 0.3 and 0.4% but it can reach 4.0% (V/W) from selected genotypes. Several studies have concluded that the *A. annua* crop can be harvested much before flowering to obtain high artemisinin yields and must be allowed to mature to obtain high essential oil yields. **(Bilia *et al*., 2014)**. Whole flowering plant of *A.annua* have been reported to be antihelminthic, antipyretic, antiseptic, antispasmodic, carminative, stimulant, tonic, and stomachic **(Sadiq *et al*., 2014).** It is considered as an economically important crop. Due presence of Artemisinin in medicinal plant *Artemisia annua,* It has proved to be an effectual anti-malarial agent **(Miraj and Alesaeidi, 2016).**

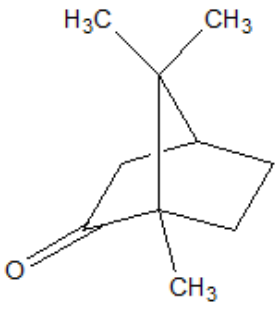
2.3.1 Phytochemical analysis

A detailed updated review of literature on chemical composition of *A. annua* is being given chronologically as under:

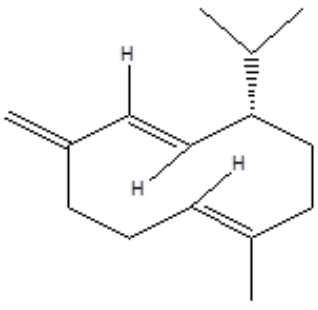
The volatile constituents like artemisia ketone **[1]**, camphor **[2]** and germacrene-D **[3]** have reported in *A.annua* from China and Vietnam **(Woerdenbag *et al.*, 1993).**



[1]

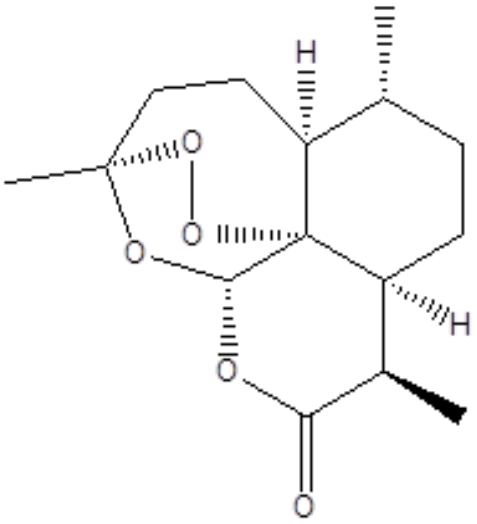


[2]



[3]

The phytochemistry of *A. annua* has been reported to be dominated by terpenoids flavonoids, coumarins and other shikimate metabolites. The sesquiterpene, artemisinin **[4]**, is however unique to *A. annua* **(Klayman *et al*., 1984; Balachandran *et al*., 1987).**



[4]

Brown (2010) reported that the main intermediate in the biosynthesis of artemisinin **[4]** was the bicyclic sesquiterpene-amorpha-4,11-diene, which is formed from FPP (farnesyl di phosphate) by the action of the sesquiterpene cyclase, amorpha-4,11-diene synthase (ADS). Two molecules of isopentenyl phosphate (IPP) and one dimethyl allyl phosphate (DMAPP) are condensed by farnesyl di phosphate synthase (FPPS/FPS) into farnesyl di phosphate (FPP), the C_{15} Sesquiterpenoid precursor **(Weathers *et al*., 2006; Brown, 2010; Wen and Yu, 2011).** FPP is converted to amorpha-4,11-diene by amorpha-4,11-diene synthase (ADS) via carbocation formation and cyclization **(Ikram and Simonsen, 2017).**

The biosynthetic pathway of artemisnin, the major constituents present in *Artemisia annua* is being depicted in **fig 2.2.**

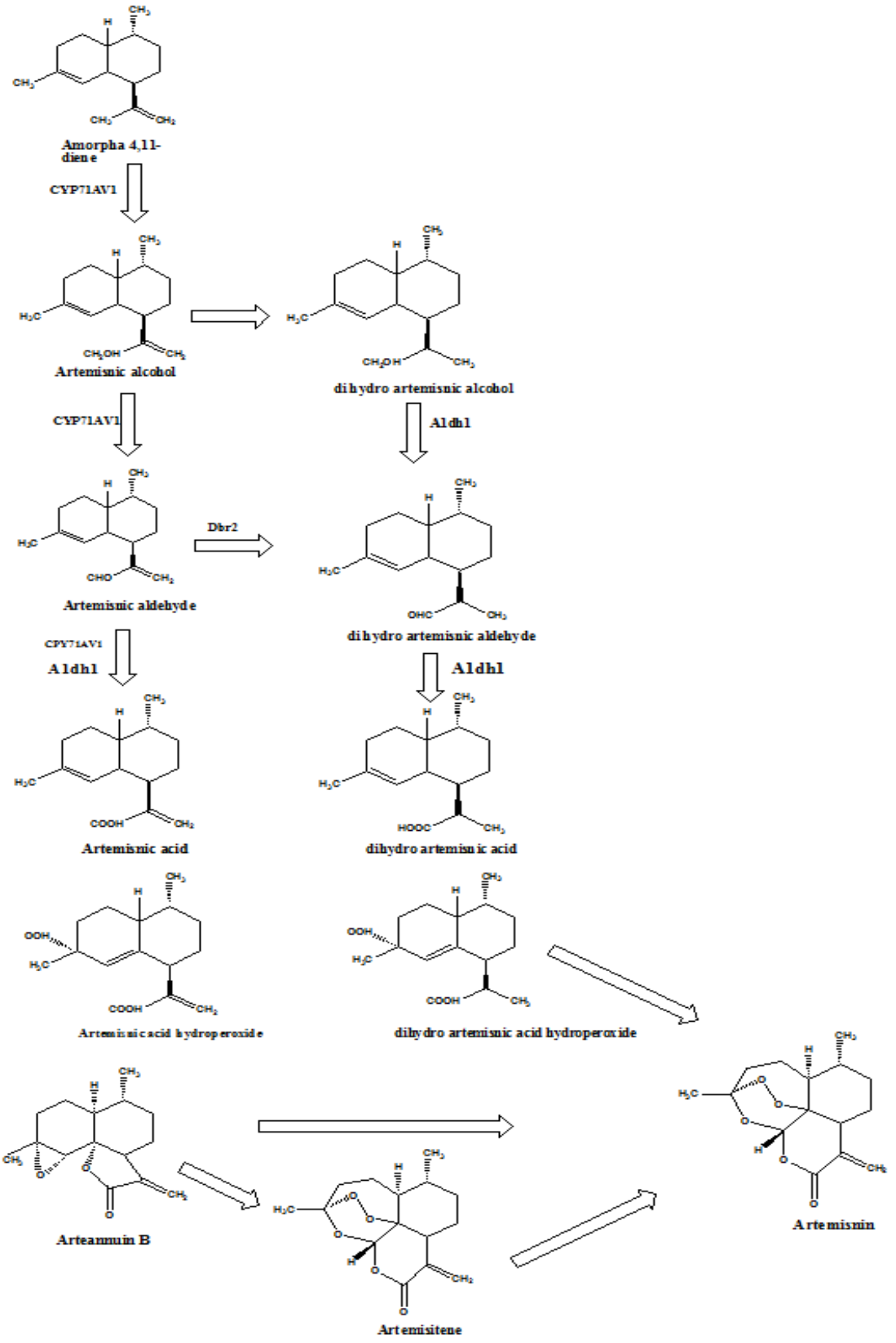
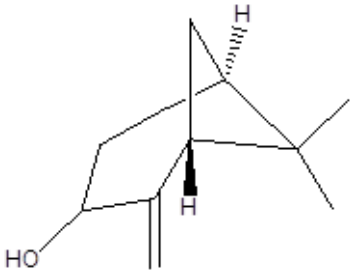


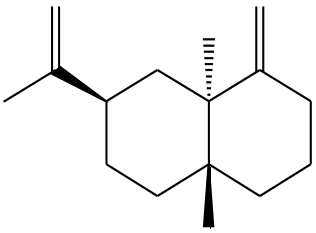
Fig 2.2 The biosynthetic pathway of artemisnin

A cDNA for (3R)-linalool synthase, which converts geranyl pyrophosphate (GPP) to (3R)-linalool (250) by ionization of the pyrophosphate group, has been reported from *A. annua* **(Jia *et al*, 1999)**.

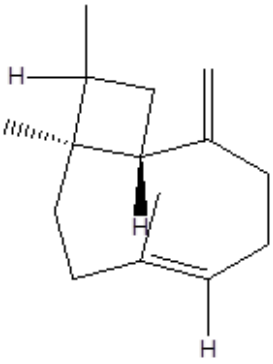
The phytochemical investigation of essential oil composition of *A.annua* revealed the presence camphor **[2]** (44%), germacrene D **[3]** (16%), *trans*-pinocarveol **[5]** (11%), β-selinene **[6]** (9%), β-caryophyllene **[7]** (9%) and artemisia ketone **[1]** (3%) **(Juteau *et al*., 2002).**



[5]

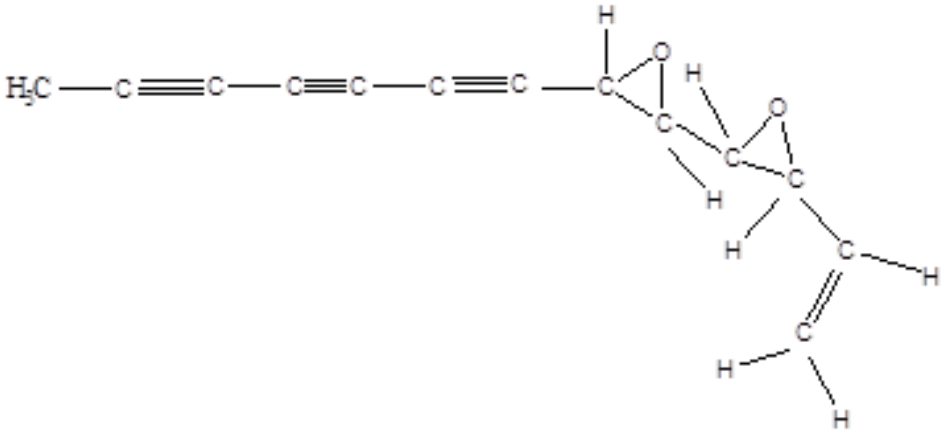


[6]

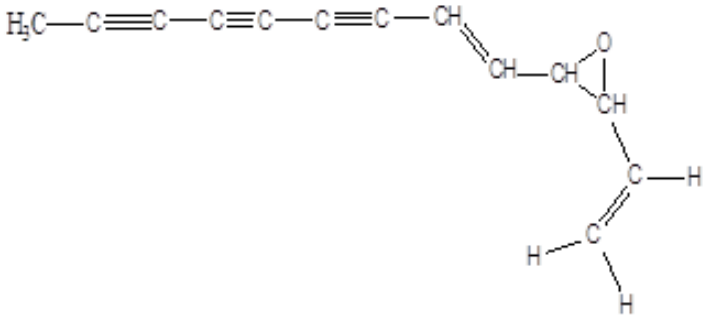


[7]

Two compounds like annuadiepoxide **[8]** and ponticepoxide **[9]** from leaves and seeds of *A.annua* have been reported. Few polyacetylenes have also been reported from *A. annua* **(Manns and Hartmann, 1992; Brown *et al*., 2003).**



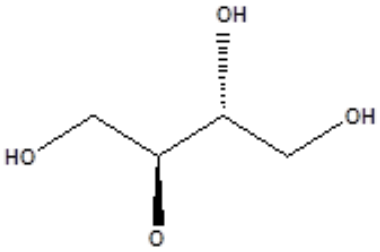
[8]



[9]

Literature search revealed that most phytochemical investigations of *A. annua* have been reported on the aerial parts (leaves and/or stems - sometimes also including the flowers) although some report revealed the analysis on the seeds **(Brown *et al*., 2003).**

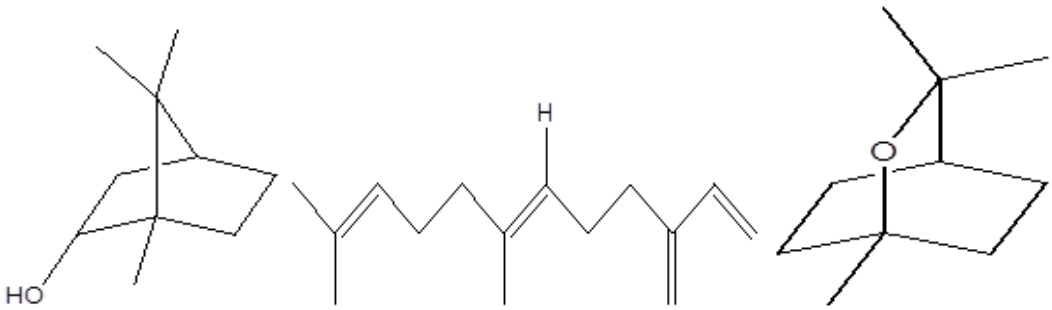
Erythritol (50.30 %) **[10]**, camphor (7.25 %) **[2]**, pinocarveol (4.13 %) **[5]**, and diethoxyethane (2.18 %) **[11]** have reported from the non-volatile components of *A.annua* **(Haghighian *et al*., 2008).**



[10]

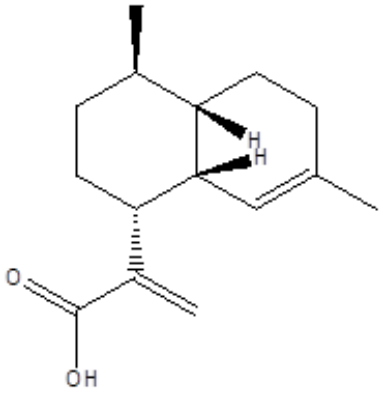
[11]

It has been reported that *A. annua* from China contained borneol (15.9%) **[12]**, and (Z)-β-farnesene (12.9%) **[13]** while same species cultivated in Iran has been reported to be very rich in camphor (36.7–48.0%) **[2]** and 1,8-cineole (9.4–13.9%) **[14] (Mohammadreza, 2008).**



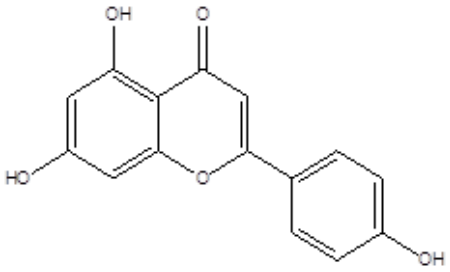
[12] **[13]** **[14]**

Sesquiterpenoids, including artemisinin **[4]**, artemisinin I, artemisinin II, artemisinin III, artemisinin IV, artemisinin V, artemisnic acid **[15]**, artemisilactone, artemisinol and epoxyarteannuinic acid have been reported as the main chemical constituents of *Artemisia annua* **(Squires and Ferreira, 2009).**



[15]

Ivanescu *et al*., (2010) reported that flavonoid apigenin **[16]** has been reported in *A. annua*.



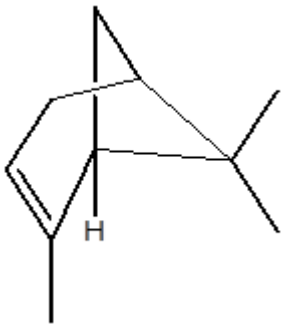
[16]

Reports suggested that the terpenoids is by far the largest group of metabolites from *A. annua*, which are biosynthetically derived from a branched isoprenoid unit (C5).); and higher terpenoids and flavonoids, which are biosynthetically derived from this same C6C3 precursor, are considered as the second largest group of metabolites from *A. annua* **(Brown, 2010).**

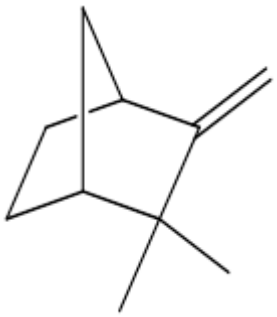
The phytochemical studies on the *A.annua* from different countries like France, Finland, Hungary, Romania, Kazakhstan, Iran, India, China and Vietnam has

been reported. It has also been reported that *A.annua* contains large group of secondary metabolites biosynthesized from (C5) units **(Brown, 2010).**

Bora and Sharma (2011) reported the constituents like α-pinene **[17]**, camphene **[18]**, camphor **[2]**, germacrene D **[3]**, artemisia ketone **[1]**, borneol **[12]**and cineole**[14]**in the essential oil of *Artemisia annua* along with the higher yield of essential oil.

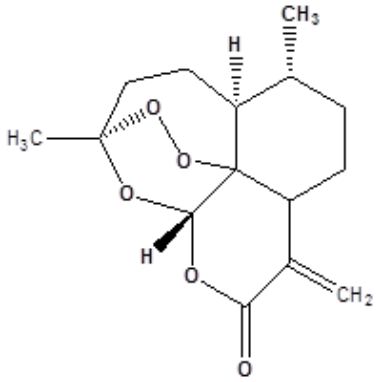


[17]



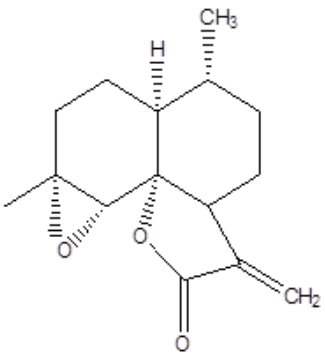
[18]

Carbonara *et al.*, (2012) reported that the genus *Artemisia* represents a high reservoir of terpenes, phenols and acetylenes, but coumarins and flavonoids have also been identified. The most peculiar compounds are however the artemisinin related sesquiterpenes such as artemisitene **[19]**, dehoxyartemisinin, arteannuin B **[20]**, artemisinic acid **[15]** and dihydroartemisinic acid **[21]**

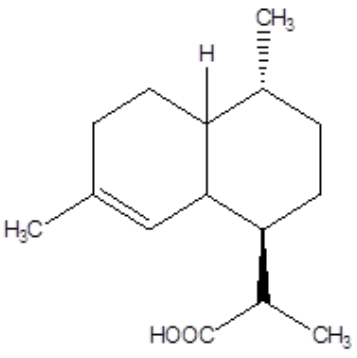


.

[19]

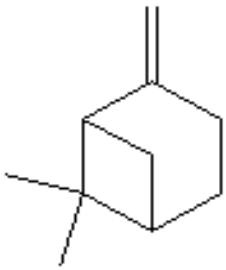


[20]

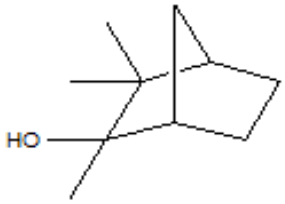


[21]

The documentation revealed that the chemical composition of *Artemisia annua* comprises of volatile and non-volatile constituents. The main compounds, which account for about 70% of the essential oils, are reported to be camphene **[18]**,isoartemisia ketone **[1],** camphor**[2],** β-caryophyllene **[7]** and β-pinene **[22]**. In addition, other minor ingredients, such as artemisia ketone **[1],** 1, 8- cineole **[14],** and camphene hydrate **[23]** have also been reported in volatile parts of *Artemisia annua* **(Das, 2012).**

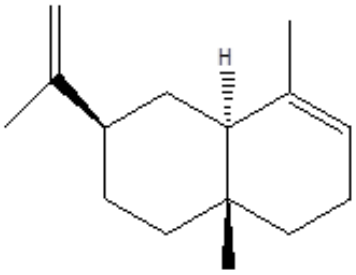


[22]



[23]

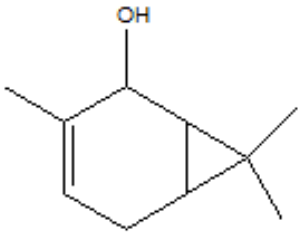
Camphor (16.30 %) **[2],** β-selinene (10.41 %)**[6],** artemisia ketone (8.79 %) **[1]**, germacrene-D (7.14 %) **[3]**, α-pinene (4.79 %) **[17],** 1,8-cineole (4.38 %)**[14]** and α-selinene (4.09 %) **[24]** have been reported as the major components in the essential oil hydrodistilled from aerial part of *A.annua*. Whereas α-pinene (12.03 %) **[17]**, artemisia ketone (11.24 %), β-selinene (8.73 %) **[6]**, 1,8-cineol (6.76 %)**[14],** camphene (4.90 %) **[18]** and α-selinene (4.85 %) **[24]** have been reported in the essential oil extracted by HS-SPME (head space-solid phase microextraction) technique (**Nekoei *et al*., 2012).**



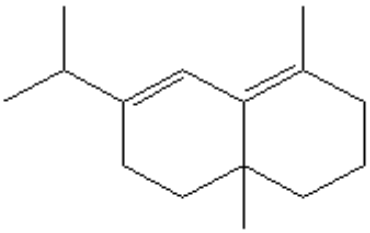
[24]

Saponins, phytosterols, carbohydrates, proteins, amino acid, and flavonoids have been reported in the alcoholic extract of *Artemisia annua* **(Ashok and Upadhyaya, 2013).**

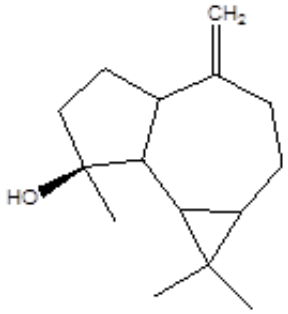
Trans-3(10)-caren-4-ol (22.3%) **[25],** artemisia ketone (18.6%) **[1],** 1,8-cineole (14.9%)**[14]**, δ-selinene (13.0%) **[26]** and α-pinene (8.2%) **[17]**, the major compounds have been reported in the seeds of essential oils of *A. annua*. where as camphor (48.00%)**[2]**, 1,8-cineole (9.39%)**[14],** camphene (6.98%)**[18]** and spathulenol (4.89%) **[27]** have been reported as the major components in the essential oil derived from the dried flowering aerial parts of *A. annua* **(Habibi *et al*., 2013).**



[25]



[26]

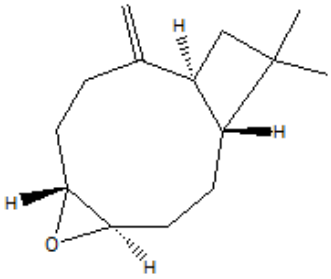


[27]

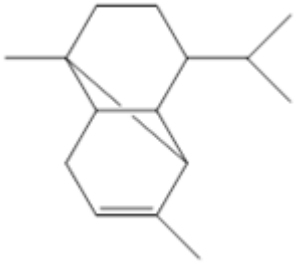
Artemisia ketone (35.7%) **[1]**, alpha-pinene (16.5%) **[17],** 1,8-cineole (5.5%) **[14]** have been reported from aerial parts of *A.annua* from Serbia **(Radulovic *et al*., 2013).**

Ogbole *et al.*, (2014) reported the chemical composition as ethanolic extract from *A. annua* with the presence of contained terpenes, steroids, cardiac glycosides, carbohydrates and flavonoids.

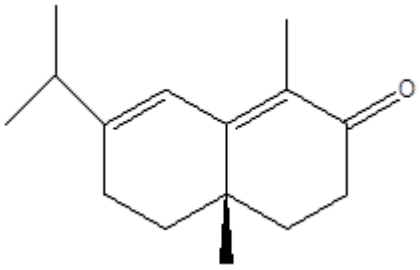
Islamuddin *et al*., (2014) reported that the major chemical constituent identified was represented in *A.annua* by camphor (52.06%) **[2]**. The other constituents present in the essential oil were β-caryophyllene (10.95 %) **[7]**, 1,8-cineole (5.57 %) **[14]**, β-caryophyllene oxide (4.21 %) **[28]**, β-farnesene (3.83 %) **[13]**, α-copaene (2.91 %) **[29]**, β-cyperone (1.93 %) **[30]**, α-selinene (1.54 %) **[24]**, and *trans*-pinocarveol **[5]** (1.22 %).



[28]



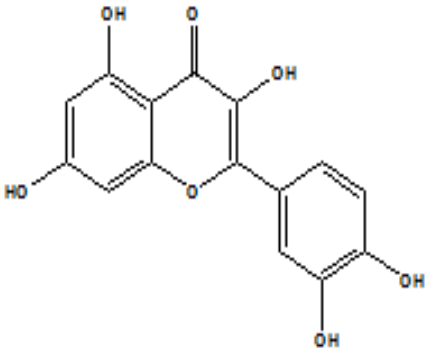
[29]



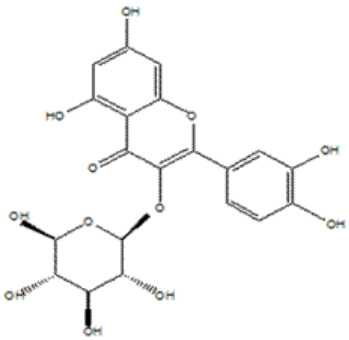
[30]

Camphor (17.74%) **[2]**, α-pinene (9.66%), germacrene D (7.55%) **[3]**, 1,8-cineole (7.24%) **[14]**, β-caryophyllene (7.02%) **[7]**, artemisia ketone (6.26%) **[1]** have been reported in aerial parts *A.annua* from Romania **(Marinas *et al*., 2015).**

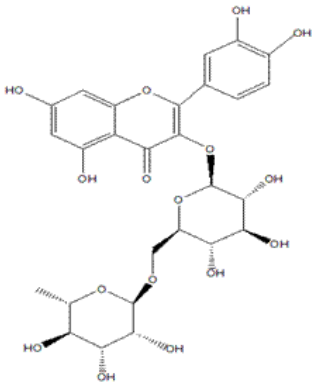
Flavonoids like quercetin (0.01 mg/g DW) **[31]**, isoquercetin (0.107 mg/g DW) **[32]** , rutin (0.31 mg/g DW) **[33]** and caffeic acid (0.03 mg/g DW) **[34]** have also been reported in *A.annua* using HPLC methods **(Dilshad *et al*., 2016).**



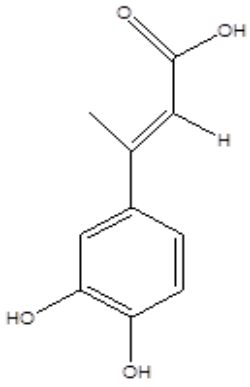
[31]



[32]



[33]

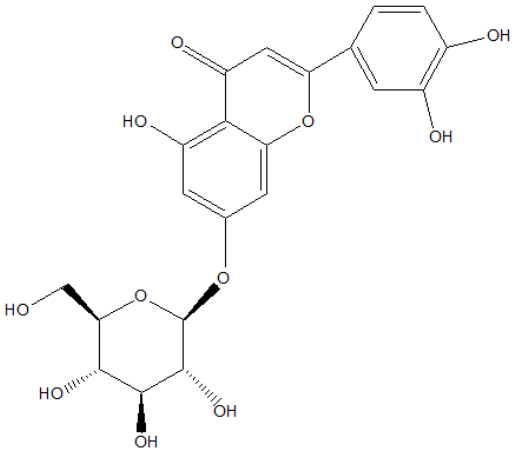


[34]

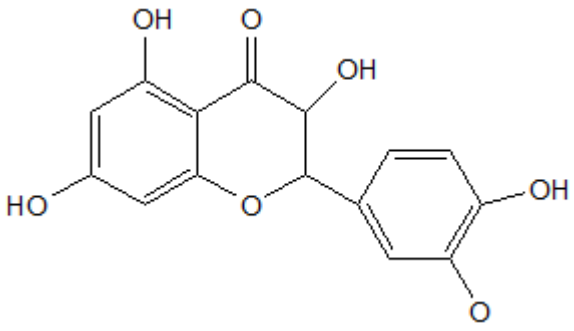
The chemical derivatives of artemisinin **[4]**, a sesquiterpene lactone produced by *Artemisia annua*, was found to be the active ingredient in the most effective treatment for malaria. Detailed phytochemical analysis of two contrasting chemotypesa low-artemisinin production (LAP) chemotype and a high-artemisinin production (HAP) chemotype of *A. annua* resulted in the characterization of over 80 natural products by NMR **(Czechowski *et al.*, 2018).**

Artemisia annua has been declared as a therapeutic herb by the World Health Organisation (WHO), for its artemisinin content, an active ingredient against malarial infections. This drug is used by over 300 million people worldwide . In context to low artemisinin content in past few years work has been reported for the optimization of new extraction methods for better yield of artemisnin**[4] (Zarrelli *et al.*, 2019).**

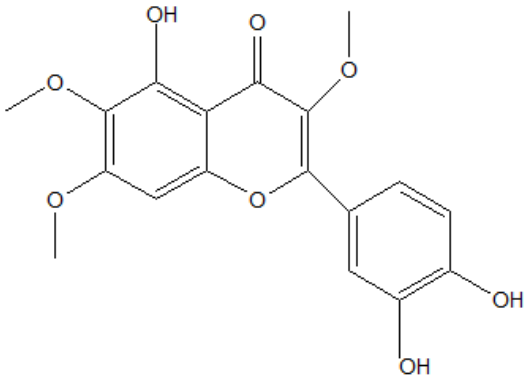
Fu *et al.*, (2020) reported the presence of eleven bioactive components, including five flavonoids i.e. rutin **[33]**, cynaroside **[35]**, isorhamnetin **[36]**, chrysosplenol D **[37]** and casticin **[38],** four sesquiterpenes viz.. arteannuin B **[20]**, dihydroartemisinic acid **[21]** artemisinic acid **[15]** and artemisinin **[4]** and two coumarins scopoletin **[39]**and scopolin **[40]** in *A.annua*.



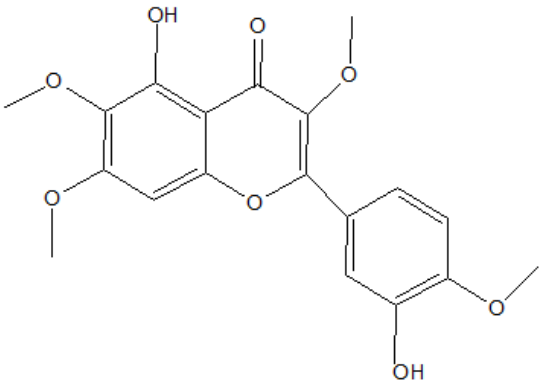
[35]



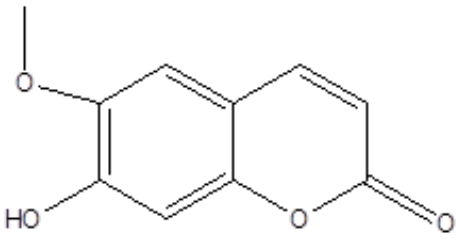
[36]



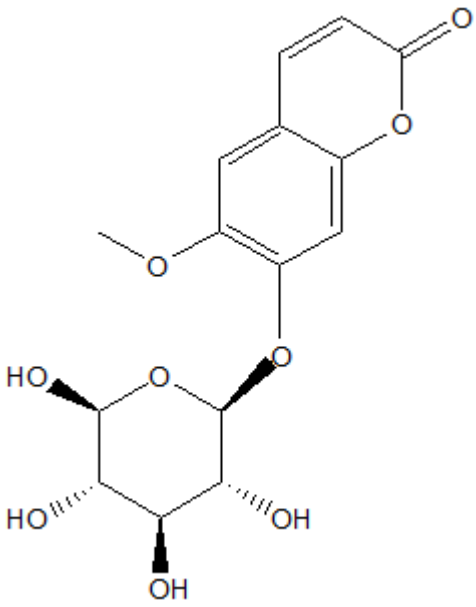
[37]



[38]



[39]



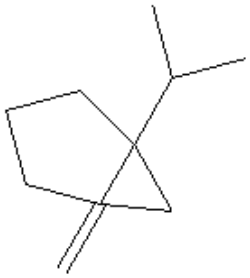
[40]

A brief review of literature on phytochemical composition in different species of *Artemisia* besides *A. annua* reported from Uttarakhand is being given in **table 2.5.**

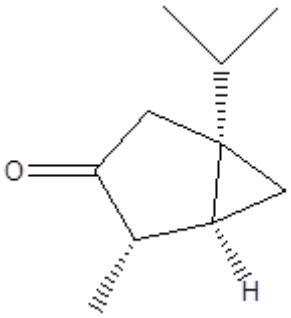
Table 2.5 Phytochemical composition of different Species of *Artemisia* reported in Uttarakhand

SN.	Plant Species	Major essential oil components	Reference
1.	*Artemisia gmelinii* Weber ex Stechm.	artemisia ketone (28.2%)**[1]**, 1,8-cineole (13.0%)**[14]**, sabinene (6.6%)**[41]**	**Mathela *et al*., (1994)**
		α-thujone (9.9%)[**42]**, ar-curcumene (8.5%)**[43]**artemisia ketone (53.3%)**[1]**, 1,8-cineole (6.6%)**[14]**	**Haider *et al*., (2012)**
2.	*Artemisia japonica* Thunb.	linalool (27.5%) **[44]**, germacrene-D**[3]** (11.2%), (E)-β-ocimene (6.5%)**[45]**, 1,8-cineole (5.5%)**[14]**, (Z)-β-ocimene (5.5%)**[46]**	**Joshi (2015)**
3.	*Artemisia maritima* L.	α-thujone (63.3%)**[42]**, sabinene (7.8%) **[41]**, 1,8-cineole (6.5%) **[14]**	**Mathela *et al*., (1994)**
		1,8-cineole (23.6%)**[14]**, chrystanthenone (25.7%)**[47]**, germacrene-D (6.7%)**[3]**	**Sah *et al*., (2010)**
4.	*Artemisia nilagirica* (C.B. Clarke) Pamp.	α-thujone (36.4%)**[42]**, β-thujone (9.4%) **[48]**,germacrene-D (6.3%) **[3],**terpinen-4-ol (6.3%) **[49]**	**Sati *et al*., (2013)**
		α-thujone (36.9%)**[42]**, β-thujone (8.2%)**[48]**, terpinen-4-ol (7.1%)**[49]**, β-eudesmol (12.4%)**[50]**, β-caryophyllene (7.4%)**[7]**,sabinene (6.6%) **[41]**	**Badoni *et al*., (2009)**
		artemisia ketone (55.07%)**[1]**, germacrene-D (5.8%) **[3]**, perillene (3.2%)**[51]**,camphene (5.47%)**[18]** and borneol (4.12%)**[12]**	**Joshi (2020)**
5.	*Artemisia scoparia* Waldst. and Kit	capillene (42.1%)**[52]**, β-caryophyllene (12.5%)**[7]**, myrcene (9.2%)**[53]**, β-pinene (8.6%)**[22]**, p-cymene (6.8%)**[54]**, γ-terpinene (5.3%)**[55]**, 1-phenyl-2,4-pentadiyne (1.1%) **[56].**	**Semwal *et al*., (2015)**

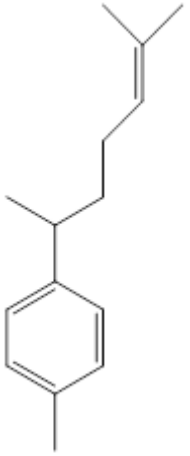
6.	*Artemisia indica* Willd.	davanone (30.8%)**[57]**, β-pinene (15.3%)**[22]**, β-pinene (15.30%)---**[22]**, germacrene-D(5.82%)**[3]**, β-elemene (4.93%), cymene (4.30%)**[54]**, *trans*-caryophyllene (3.81%)**[7]**, and linalool (3.60%)	**Haider *et al*., (2014)**
7.	*Artemisia roxburghiana* Bess.	β-thujone (65.3%)**[48]**	**Haider *et al*., (2009)**
		borneol (21.2%)**[12]**, linalyl acetate (7.4%), α-humulene (6.7%)**[58]**, β-caryophyllene (16.3%)**[7]**, α-thujone (12.0%)**[42]**, eugenol (16.2%)	**Mathela *et al*., (1994)**
8.	*Artemisia vulgaris* L.	artemisia ketone (6.77–8.64 %)**[1]**, *trans*-caryophyllene (6.22–6.94 %)**[7]** and 1,8-cineole (4.75–5.13 %)**[14]**	**Lohani *et al*., (2016)**
		chrysanthenone (0.1-26.6 %)**[47]**, vulgarole (0.1-20.6 %)**[59]**, artemisia ketone (0.01-19.8 %)**[1]**, α-thujone (0.01-19.0 %)**[42]**, 1,8-cineole (1.6-13.5 %)**[14]**, β-thujone (0.2-13.2 %)**[48]**, caryophyllene oxide (1.4-11.2 %) **[28]** and camphor (0.9-11.1 %) **[2]**	**Haider *et al*., (2019)**
9.	*Artemisia parviflora* Buch.-Ham. ex D. Don	germacrene D (41.01%)**[3]**, α-humulene (7.86%) **[58],** β-caryophyllene (10.58%) **[7]**	**Tewan *et al*., (2015)**
		β-caryophyllene (15.3%) **[7]** camphor (11.4%)**[2]**, artemisia ketone (7.8%)**[1]**, 1,8-cineole (5.8%)**[14],**germacrene D (14.7%) **[3]**	**Rana *et al*., (2003)**
10.	**Artemisia elegantissima* var. *Kumaunensis* Pamp	davanone (5.5%)**[57]**	**Shah (2010)**



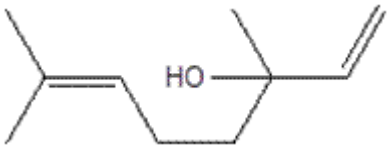
[41]



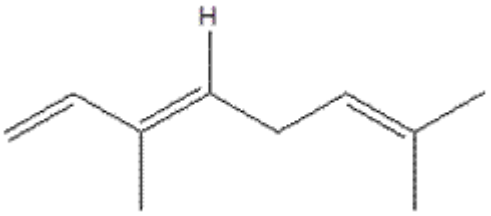
[42]



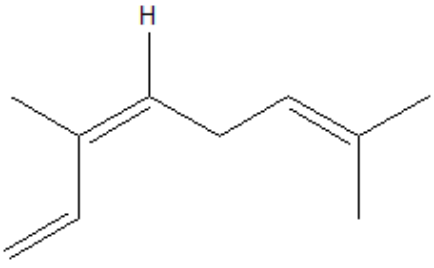
[43]



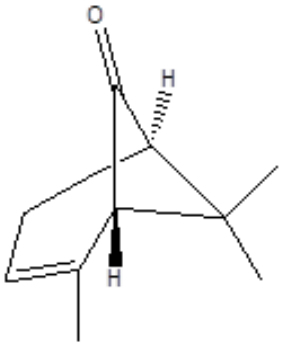
[44]



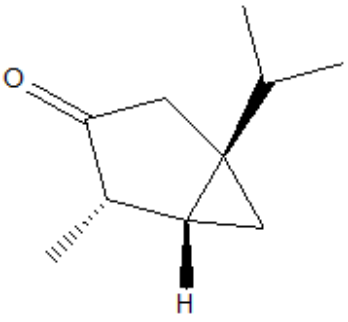
[45]



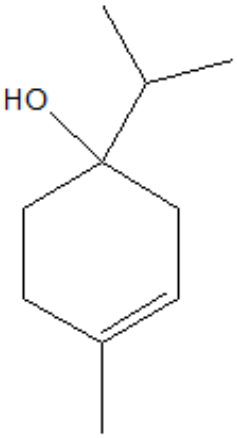
[46]



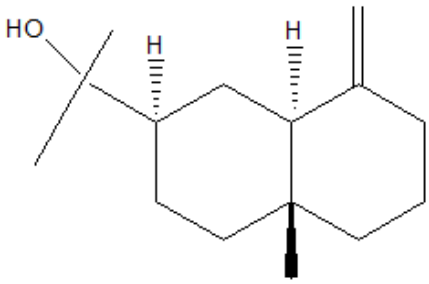
[47]



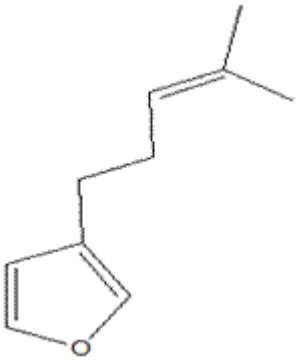
[48]



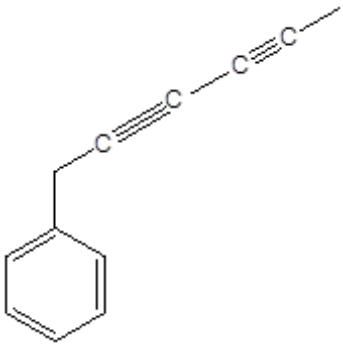
[49]



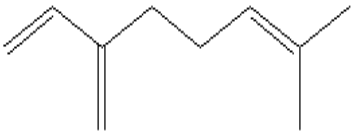
[50]



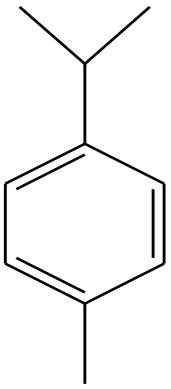
[51]



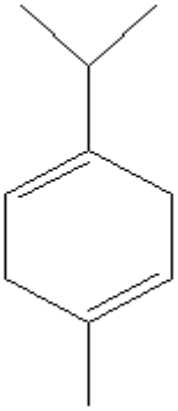
[52]



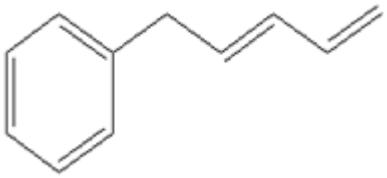
[53]



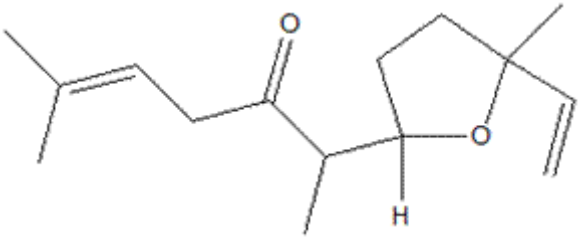
[54]



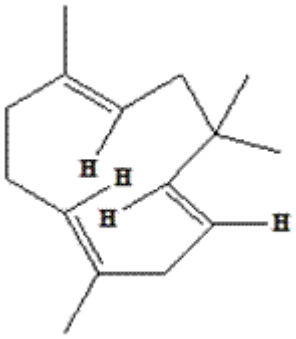
[55]



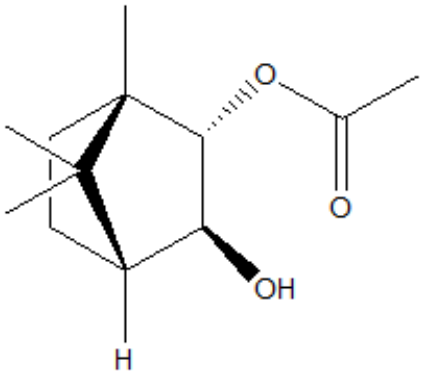
[56]



[57]



[58]



[59]

2.3.2 Biological activities

A detailed updated review of literature on biological activities of *A. annua* is being given in chronological order as under:

It has been reported that flavonoids and essential oil content present in *A. annua* are known to impart antioxidant activity. These studies ranked *A. annua* among those medicinal plants which are at the top of list, based on their highest antioxidant potential **(Juteau *et al*., 2002).**

The documentation revealed antifeedant activity in crude extracts of *A. annua*. Due to deterrency, growth regulatory effect and ovicidal potential it has also been recommended it as a good antifeedant herb **(Haghighian *et al*., 2008).**

Due to notable activity of artemisnin, the anti-leishmanial activity of *A. annua* has been reported **(Malebo *et al*., 2009).**

Ryu *et al.*, (2011) reported thatthe anti-oxidant activity of the *A. annua* essential oil has been evaluated by five testing methods, DPPH, ABTS, reducing power, ORAC, and metal-chelating assays.

Anticancerous activity of various organic extracts of *A. annua* has been analysed by determining their cytotoxic potential in *Trypanosoma b. brucei* (TC221 cells) and HeLa cancer cells **(Efferth *et al*., 2011).**

The antimicrobial activity of *A. annua* essential oil have been evaluated against gram-positive, gram-negative bacteria, and one fungus.**(Cavar *et al.*, 2012).**

Lubbe *et al.*, (2012) documented in the literature, the first *in vitro* evidence of anti-HIV activity of the *Artemisia annua* tea infusion.

Artemisinic acid was reported to be the first ever found component obtained from *A. annua* . The compound has been which known to possess *in vitro* anti-adipogenic activities **(Lee *et al*., 2012).**

It has been reported that essential oil of *A.annua* had a good antibacterial activity against gram-positive and gram-negative bacteria **(Massiha *et al*., 2013).**

It has been documented in the literature that *A. annua* contains essential component known as artemisinin which provides structural chemical base for combinatorial treatment therapy for world antimalarial program **(Sadiq *et al*., 2014).**

It has been reported that artemisinin and its derivatives from *A.annua* exhibits anti-cancerous effect by causing cancer cell growth cycle arrest, promoting apoptosis, and preventing angiogenesis and tissue invasion of tumour **(Ho *et al*., 2014).**

It has been reported that the essential oil from *A.annua* posess exciting antibacterial and antifungal activities. Studies show that artemisia ketone is the component of the oil that posess the maximum antimicrobial activity. **(Bilia *et al.*, 2014).**

The camphor-rich essential oil of A. annua has been reported to exhibit a dose dependent antileishmanial activity against the promastigotes as well as the intracellular amastigotes of *L. donovani* **(Islamuddin *et al*., 2014).**

The antimicrobial activity of artemisinin and precursor derived from the *in vitro* plantlets of *A. annua* has been reported to be antimicrobial in nature similar to that of the commercially available antibiotic, streptomycin. **(Appalasamy *et al*., 2014).**

Artemisinin, the active component found in *A. annua* has been reported to have pronounced anti-malarial activity. It has also been reported that artemisinin and its derivatives could inhibit *T. gondii* infection **(Ho *et al*., 2014).**

Kim *et al.*, (2015) evaluated the anti-inflammatory, antioxidant, and antimicrobial properties of artemisinin derived from water, methanol, ethanol, or acetone extracts of *Artemisia annua* L.

The anti-fungi activity of *A. annua* essential oil has been reported against *C. albicans* and *C. dubliniensis* and *C. parapsilosis* **(Santomauro *et al*., 2016).**

It has been reported that the synergistic effects of artemisinin and its flavonoids compounds and their biological interaction between malaria and cancer can be a rich source to anti-malarial, immunosuppressive, anti-inflammatory, and anti-cancer properties of this plant **(Alesaeidi and Miraj, 2016).**

Huang *et al*., (2017) reported the *in vitro* anti-asthmatic activities of *A. annua* by using tracheal rings (TRs) and acute isolated airway smooth muscle cells (ASMCs) of mice.

The *in vitro* and *in vivo* anti leishmanial activity and cytotoxicity of *A. annua* L. has been reported **(Mesa *et al*., 2017).**

The published data revealed the antifungal activity of *Artemisia annua* synthesized AgNPs against three *Candida* species (*C. albicans* ATCC 90028, *C. tropicalis* ATCC 750 and *C. glabrata* ATCC 90030) **(Khatoon *et al.*, 2019).**

The *in vivo* hepatoprotection, antihyperlycemic and antioxidant activities of the polar fraction of the 70% ethanolic extract of *A. annua* leaves in model rats has been reported **(El-Askary *et al.*, 2020).**

It has been reported that casticin and chrysosplenol D,the flavonoid components present in *A .annua* possess anti-cancerous activities **(Fu *et al*., 2020).**

Artemisia extracts have been reported to have a strong bactericidal activity against Mtb (*Mycobacterium tuberculosis*), suggesting that *A. annua* extracts kill Mtb through a combination of Artemisnin and other additional compounds **(Martini *et al*., 2020).**

The leaves powder extraction and the crude extraction of the whole plant of *A.annua* have been reported to show anti-microbial activities, making *A. annua* a promising source of new anti-microbial agents **(Feng *et al*., 2020).**

Studies revealed that artemisinin and arteannuin B which are found in *A. annua*, posessed anti-osteoporotic activities by blocking receptor activator of nuclear factor kappa-B ligand (RANKL)-induced osteoclast differentiation by reducing the expression of the two transcription factors, c-Fos and NFATc1**(Feng *et al*., 2020).**

Rolta *et al*., (2021) reported that the methanolic extract of *A.annua* showed higher antioxidant potential (IC_{50}= 37 $0.75 \pm 0.34\ \mu g\ mL^{-1}$) in comparison to petroleum ether extract. Where as in antimicrobial analysis, methanolic and petroleum ether extracts of *A. annua* have been reported to possess strong inhibitory activity against the strains of *Candida* as compared to bacterial strains.

Materials and Methods

Chapter 3 MATERIALS AND METHODS

The present investigation was implemented in Govind Ballabh Pant University of Agriculture and Technology, Pantnagar (Uttarakhand). Extraction and isolation of essential oils from the plant, Evaluation of its different activites like antioxidant, anti-inflammatory, anti-diabetic, insect-repellent was performed in phytochemistry Laboratory of Department of Chemistry, College of Basic Science and Humanities, Pantnagar. The insect repellant activity was performed in Department of Entomology, College of Agriculture, G.B.P.U.A.T, Pantnagar. Chemical Investigation of Essential Oil was done with the help of GC and GC/MS analysis in Advanced Instrument Research Facility (AIRF), Jawahar Lal Nehru University, New Delhi.

3.1 Materials

3.1.1 Collection of Plant material

The herb *Artemisia annua* was collected from Pantnagar, Uttarakhand in the month of September, 2020 and January 2021. The collected plant was identified by Taxonomist Dr. D.S. Rawat, (Assistant professor). Department of Biological Sciences, College of Basic Sciences and Humanities, Pantnagar. The herbarium specimen of *Artemisia annua* L. with the herbarium number GBPUH-1033 was deposited at herbarium of Department of Biological Sciences, C.B.S&H., G. B. Pant University of Agriculture & Technology, Pantnagar.

3.1.2 Chemicals

The chemicals and solvents used for the study were of laboratory grade and analytical grade and were acquired from Merk, Himedia and SD fine. Chemicals such as ferrozine, DPPH, BHT, acarbose, catechin used were of sigma Aldrich and standard drug diclofenac sodium was purchased from the shop.

3..1.3 Glass wares and plastic wares

The glass wares and plastic wares used during the whole work were either Borosil or Thermo Fisher Scientific made.

3.1.4 Source of insect anti-feedant activity

The egg mass **(fig 3.1)** of *Spodoptera litura* was collected from the castor field which was grown in Pantnagar and was acclimatized until 3rd instar larvae, in the laboratory condition of department of Entomology, College of Agriculture, G.B. Pant University and Techonology, Pantnagar.

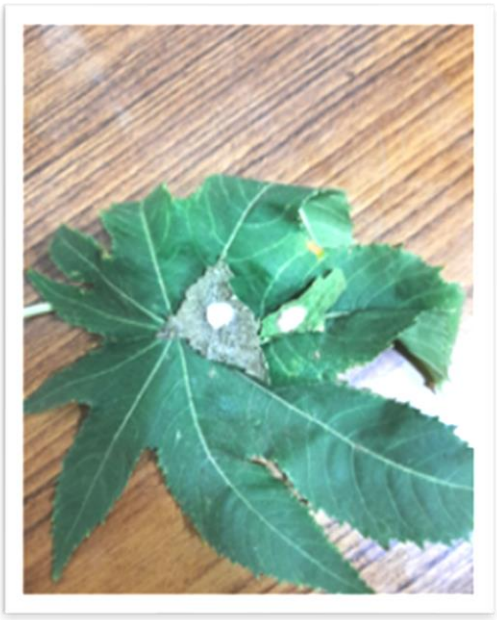


Fig 3.1 Egg masses of *Spodoptera litura*

3.1.5 Isolation and Identification of the Root-Knot nematode (*Meloidogyne incognita*)

3.1.5.1 Collection of Root knot nematode infected sample

Capsicum was taken as the Root knot infected plant collected from the Vegetable Research Center, G. B. Pant University of Agriculture and Technology, Pantnagar. The sample was collected on the basis of the visual symptoms of Root-knots or galls formed in the capsicum plant.

3.2 Phytochemical analysis

Essential oils extraction from the aerial parts of *A.annua* were isolated by hydro-distillation method in a Clevenger apparatus **(Clevenger,1928)** as **fig.3.2(a) and (b).**The plant was cut into small pieces, crushed and hydrodistilled for 3-4 hours.

The whole process of hydrodistillation was repeated for three times in order to obtain desirable amount of essential oil. The oil was extracted with hexane and desiccated over anhydrous sodium sulphate. The solvent was evaporated under vacuum and the essential oils so obtained were stored at low temperature (4^{o} C in refrigerator) for further use.

The % yield of (v/w) is being record in **table 3.1** below:

Table 3.1 Yield of essential oil from *A.annua* from two seasons i.e *Artemisia annua* rainy essential oil (AAREO) and *Artemisia annua* winter essential oil (AAWEO).

SN.	Plant part	Method of extraction	Amount of plant material	Duration of heating	Yield % (V/W)
1.	AAREO	Hydrodistillation	600g	3-4 hours	0.7%
2.	AAWEO	Hydrodistillation	500g	3-4 hours	0.5%

(a) **(b)**

Fig. 3.2 *Artemisia annua* from two different seasons

3.3 GC-MS Analysis

For the phytochemical composition of essential oil, an analytical technique GC-MS was carried out using GC MS-QP 2010 plus equipment with following experimental conditions:

3.3.1 GC-MS parameters

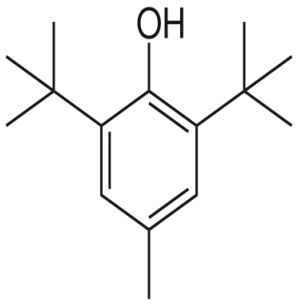
Parameters	Conditions
Carrier gas	Helium
Column flow rate	1.21 mL/min
Temperature programme: -	
	Initial temperature at 50°C
	RAMP@ 3°C/min upto 210°C (isotherm for 2 min) then 6°C/min upto 280°C (isotherm for 2 min), finally hold for 11 min
Injection volume	1 μL
Injection temperature	260°C
Injection mode	Split
Split ratio	22.0
Flow control mode	Linear Velocity
Pressure	69.0 kPa
Total flow	30.8 mL/min
Linear velocity	39.9 cm/sec
Purge flow	3.0 mL/min
High Pressure Injection	OFF
Carrier Gas Saver	OFF
Splitter Hold	OFF

Identification of the chemical constituents of the essential oils were done by comparing their mass spectral fragmentation pattern and their RI with that of the MS library (NIST14.lib, FFNSC2.lib, WILEY8.LIB) and comparing the spectra with literature data **(Adams, 2007).**

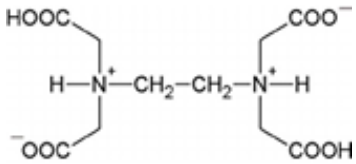
3.4 Biological activities

3.4.1 Evaluation of antioxidant activity:

Investigation of the *in-vitro* antioxidant potential of the essential oils of *A.annua* (**AAREO & AAWEO**) was done by generally practiced and reported methods. Standard antioxidants used were BHT (butylated hydroxyl toluene) and Na_2-EDTA (disodium salt of ethylenediaminetetraacetic acid) **fig 3.3.**



BHT



Na_2EDTA

Fig 3.3 Structures of standard antioxidants

3.4.2 Metal chelating activity

The metal chelating activity of $Fe^{2+}$was determined by spectrophotometric method. Based on the principle of Fe^{2+} chelating ability of the antioxidant by measuring the absorbance of the ferrous ion-ferrozine complex formed at 560 nm **(Kunwar *et al.*, 2013).** 0.1 mL of 2mM $FeCl_2.4H_2O$, 0.2 mL of 5mM ferrozine and 4.7 mL of methanol was added to different concentrations (20-100µl/mL). the solutions were mixed and incubated for about 10 min. The absorbance of test sample was measured at 562 nm using UV Visible spectrophotometer. Na_2EDTA (0.01 mM) was used as the standard. The metal-chelating activity of samples, was calculated using the following formula:

$$IC\ \% = \frac{(A_0 - A_t)}{A_0} \times 100$$

where, $A_{0=}$ absorbance value of control sample

A_t= absorbance value of test sample

The percent of chelating activity was plotted against concentrations and the standard curve was drawn using standard antioxidant (Na_2EDTA) to calculate the IC_{50} values for standard and different samples of essential oil, column fraction and solvent extracts. A lower IC_{50} values valued indicates greater metal chelating ability.

3.4.3 Hydrogen peroxide (H_2O_2) scavenging activity

Hydrogen peroxide activity of the essential oils was evaluated by method given **(Leyton *et al.*, 2015)**. The reaction mixture comprised of 0.4 ml of different concentration of essential oils in methanol (20-100µl/mL) and 0.6 ml of 40mM hydrogen peroxide in phosphate buffer pH (7.4). After keeping the reaction mixture at room temperature for 10 minutes. , the absorbance was taken at 230 nm against the blank, i.e., methanol. Here BHT was taken as a positive control.

The percentage of H_2O_2 scavenging was determined by using the given formula:

$$IC\ \% = \frac{(A_0 - A_t)}{A_0} \times 100$$

where, A0= Absorbance value of control sample

At= Absorbance value of test sample

IC = Inhibitory concentration

IC_{50} values of all the samples were calculated with regression method analysis.

3.4.4 DPPH (2,2-diphenyl-2-picrylhydrazyl)radical scavenging activity

Estimation of DPPH radical scavenging activity was based on the method given by **(Prakash *et al.*, 2011).** Various concentrations of essential oil (20-100µl/mL) were mixed with 5 mL of freshly prepared methanol solution of DPPH (0.004%). The reaction mixture was thoroughly mixed then their absorbance was measured by using UV-Visible spectrophotometer at 517 nm. All observations were taken in triplicate and the standard antioxidants used were butylated hydroxytoluene (BHT). The ability to scavenge DPPH radical **(fig 3.4)** was calculated by the following equation:

$$IC\ \% = \frac{(A_0 - A_t)}{A_0} \times 100$$

where, A_0 = absorbance value of control sample

A_t = absorbance value of test sample

IC = inhibitory concentration

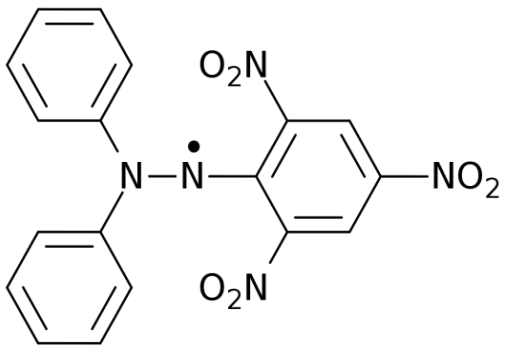


Fig 3.4 DPPH (free radical)

3.4.5 *In-vitro* anti-inflammatory activity

The in-vitro anti-inflammatory activity of the essential oil was estimated by using inhibition of albumin denaturation technique **(Singh *et al*., 2020)**. The sample consisted of 200µL of egg albumin, 3 mL of saline phosphate buffer solution (PBS) (pH= 6.4) and different amount of essential oil (20-100µl/mL). Double distilled water was used as control. The prepared mixtures were incubated at 37±2°C (for 15 min and then heated for 5 min at 72°C in water bath) . After the cooling of the mixture, the absorbance was recorded at 660nm. Diclofenac sodium **(fig 3.5**) was used as the standard.

The percentage inhibition of protein denaturation was calculated using the formula:

$IC\% = (A0 – At)/A0 \times 100$

A0 = absorbance value of control sample

At = absorbance value of test sample

IC = inhibitory concentration

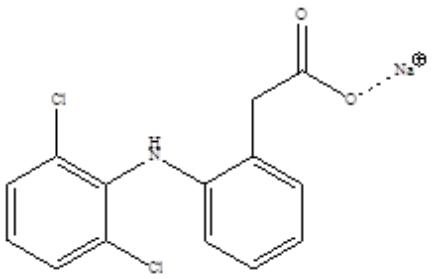


Fig 3.5 Diclofenac sodium

3.4.6 *In-vitro* antidiabetic activity

The antidiabetic activity of the essential oil was evaluated by using α-amylase assay as given by **Nazir *et al*., (2021)** with slight modifications. Different concentrations of the oil and the standard drug, acarbose (20-100µl/mL) **(fig 3.6)** were added to a reaction mixture containing 100 µL of 2mM phosphate buffer (pH 6.9) and α-amylase (200 µL). The reaction mixture was then incubated at 25ºC for 20 min.

After that 100µL of 1starch solution was added to the above solution and then incubated at room temperature for 5 min. Then 1 mL of 3,5- Dinitrosalicylic acid (DNSA) solution was added to the reaction mixture and placed in a boiling water bath for 5 min and cooled at room temperature. Further the absorbance was recorded at 540nm using a UV spectrophotometer.

The percentage inhibition was calculated using the following formula:

Inhibition of α-amylase activity (%) = (A0 – At)/A0 × 100

Where,

A0 = absorbance value of control sample

At = absorbance value of test sample

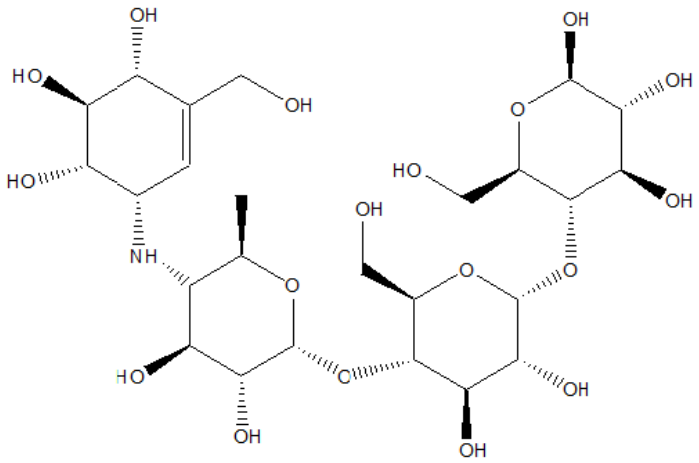


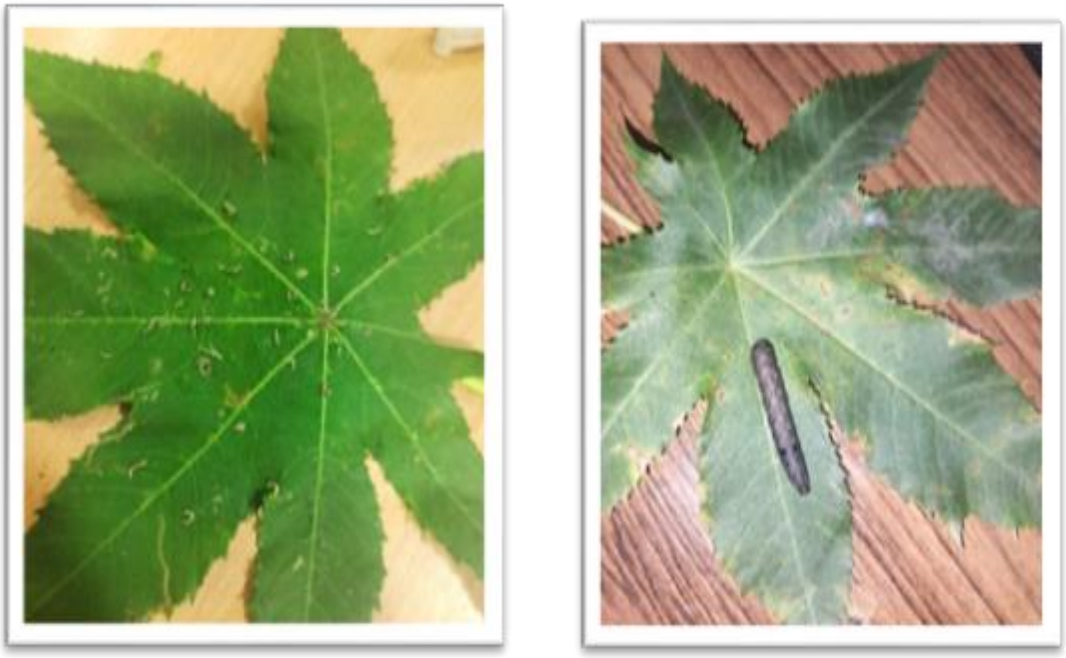
Fig 3.6 structure of acarbose

3.4.7 Evaluation of anti-feedent activity

3.4.7.1 Test insect

The antifeedant activity of the essential oils was tested against the second instar larvae of *Spodoptera litura* **Fig 3.7 (a)**, *Spodoptera litura* (Fab). (Lepidoptera: Noctuidae) is one of the major polyphagous pest which attacks economically essential crops. viz., cotton, groundnut, chilly, tobacco, castor and pulses in India, China and Japan thereby causing extensive damage **(Arivoli and Tennyson, 2012).** In severe

infestation, this pest can cause severe damage to the plant which results into stunted growth and reduced yield of the plant **(Javier *et al*., 2017)**.



(a) Second instar larvae of *Spodoptera litura* (b) Third instar larvae of *Spodoptera litura*

Fig 3.7 Picture showing (a) secondand (b) third instar larvae of *Spodoptera litura*

3.4.7.2 Collection of Larvae and its maintenance

The egg mass of *Spodoptera litura* **(Fig. 3.1)** was collected from the castor crop (*Ricinus communis*) from C.R.C. (Crop Research Center), G.B.P.U.A.T. Pantnagar, Uttarakhand, India in the month of April. The rearing of insect was done in laboratory conditions maintained at 27^{o}C temperature in a glass jar packed with muslin cloth from the top. The insect larvae were fed on fresh castor leaves collected on daily basis. The fully grown 2nd instar larvae kept starved for 2-3 hours in insect growth chamber **(Fig 3.8)** and were then tested for the study of anti-feedant activity.

Fig 3.8 Second instar larvae kept in Insect growth chamber

3.4.7.3 Experimental procedure Of Anti-feedant activity

The experiment was conducted as per the developed protocol. The efficacy was tested against *Spodoptera litura* using leaf dipping method. Essential oils (**AAREO & AAWEO)** in 0.5% concentration were prepared by dissolving different concentrations of oils (2µL, 4µL, 8µL) in the solvent which is 0.5 mL of tween 20 (stabilizing agent) in 99.5 mL of water i.e., 0.5% Tween 20 (V/V). Leaf discs (4×4 sq cm) of castor leaves were then dipped in each concentration of oils for few seconds and then were air dried. The leaf discs were then kept in glass petri dishes and one 2nd instar larvae (starved for 2-3 hours) of test insect was then released in each petri dish. Solvent without oil was taken as control. Three replications were maintained for each

oil concentration. The consumed area of leaf after 24 and 48 hours was then observed and the percent antifeedant activity was then calculated using the following formula:

$$\% \text{ Antifeedant activity} = \frac{\text{Leaf disc consumed in control-Leaf disc consumed in treated}}{\text{Leaf disc consumed in control+Leaf disc consumed in treated}} \times 100$$

3.4.8 Evaluation of nematicidalactivity

3.4.8.1 Identification of the Root-knot nematode species

- Infected capsicum roots with root-knot nematode were washed and stained with acid fuschin lactophenol solution.
- Then after the roots were allowed to cool at room temperature followed by destaining via acidified glycerine.
- Females were dissected out from the well-developed galls of root under stereobinocular microscope and planced on lactophenol solution. To get the perineal section, the posterior portion of the female was cut and the body contents were cleaned off.
- The cleaned posterior portion of female was trimmed and transferred to drop of lactophenol on a microscopic slide.
- The confirmation of species was done by examining the pattern of posterior section as described by **Eisenback, 1985**.

3.4.8.2 Preparation of Acid Fuschin stain and procedure for root staining

Acid Fuschin stain: 3.5g of acid Fuschin added in 250 mL of acetic acid, later dissolved in 750mL of distilled water and mixed thoroughly. This can be used as stock stain by storing.

Staining of roots **(Eisenback, 1985)**

- The infected roots were soaked in 2% chlorine bleach solution for 2 min followed by rinsing in running water for 45 seconds and soaking in tap water for 15 min to remove the residual bleach that interferes with stain.

- ❖ The drained roots were then transferred to a beaker containing 30-45mL of tap water added with 1ml of acid Fuschin stain.
- ❖ The roots in solution were boiled for 30 seconds on hot plate and cooled to cooled to room temperature followed by draining and rinsing with water.
- ❖ Stained roots were then transferred to 20-30ml of acidified glycerin (few drops of 5N HCl were added) and heat to destain.
- ❖ After staining, the roots were stored in acidified glycerin until their examination.

3.4.8.3 Pathogenicity test

The seedling of 10-12 days old cucumber seedlings raised in autoclaved soil in pots were inoculated with the fresh second stage juveniles (J2) emerged from the egg mass of *Meloidogyne incognita* maintained in pure culture. Inoculation was done by removing 1-2cm of top soil surrounding the seedlings and the nematode juveniles in water were poured on the exposed root system. Exposed roots after inoculation were covered with soil and lightly watered. After 45-50 days of inoculation, roots were examined with the presence of root galls and microscopically studied by preparing perineal pattern of female for the confirmation of pathogen (*Meloidogyne incognita*).

3.4.8.4 Nematode culture preparation

The pure culture of nematode to be used in the experiment was obtained from a single egg mass of *M. incognita* infected roots.

- ❖ A single egg mass was transferred on to a petri plate containing a little amount of water and the incubated at room temperature of 25-27^{o}C for 3 days for emergence of second stage juveniles (**Den Ouden, 1958**).
- ❖ 3 weeks old transplanted tomato seedlings raised in autoclaved soil were inoculated with second stage juveniles. Inoculation was done by removing top soil of 1-2 cm around the seedlings and the juveniles in water were poured on partly exposed root system.

- Nematodes were then allowed to multiply in the pot for upto 2-month duration and inoculum for the experiment was obtained by extracting eggs and second stage juveniles from it using 2% sodium hypochlorite solution as described by **Hussy and Baker, 1973**.

3.4.8.5 *In vitro* evaluation of AAREO and AAWEO on egg hatching of *Meloidogyne incognita*

In vitro experiment was conducted to evaluate the efficacy of different essential oils on egg hatching of *M. incognita.* The eggs of *M. incognita* were obtained from the nematode pure culture.

Gridded Petri dishes were used for carrying out the experiment. Total 3 treatments were there, each one replicated three times and 3 different doses respectively. Out of the three treatments, 2 were oils of *A.annua* collected in two different seasons and the rest was distilled water treated as control. 2 egg masses of *M. incognita* were suspended in 2, 6 and 10 µl/mL concentration of essential oils taken in gridded Petri dishes. All the treatments were arranged in completely randomized design and kept at ambient temperature of 27±1^{o}C in BOD incubator. Observations on egg hatchability of eggs were recorded at 24hr, 48hr and 72hr exposer period by counting the number of eggs hatched, under microscope at 40x magnification.

3.4.8.6 *In vitro* evaluation of essential oil samples on mobility of second stage larvae of *M. incognita*

The experiment was conducted under *in vitro* condition to evaluate the efficacy of essential oils on the mobility of second stage larvae of *M. incognita.* The second stage larvae were isolated from the galled roots with egg masses from pure culture of nematodes. After removal of galled roots from pot, they were washed in tap water to remove the soil adhered to it. Then after it was cut into small pieces of 2 cm and transferred into a 2% sodium hypo chloride solution and shaken for two minutes to separate the organic debris from eggs. The suspension was poured through series of sieves and the eggs sieved on 38µm- pore were collected and washed cautiously in the tap water. Egg suspension was then poured and incubated at 28±1^{o}C, so that the juveniles of second stage from the hatched eggs were collected within 48 hrs.

100 second stage juveniles were counted and placed on the gridded Petri dishes containing stock solution of essential oils. In total, there were 3 treatments replicated three times and three different doses of 1, 3 and 5µl/mL. The juveniles placed in distilled water were treated as control. All the treatments were arranged in randomized manner. Observation on the count of dead juveniles was done at different time periods of 24hrs, 48hrs and 72hrs using stereo-binocular microscope. The nematodes appearing almost straight in position (not showing any movement) were trapped by picking instrument to check the aliveness of larvae and when they were completely not responding, they were picket out of the Petri dish and placed in distilled water to confirm the larval death.

3.5 Statistical analysis

All the experiments were conducted in three replicates and the data were expressed in terms of mean±standard deviation. Data illustrated in the tables and the graphs were subjected to ANOVA at 1% level of significance.

Results and Discussion

Chapter 4 RESULTS AND DISCUSSION

This chapter deals with the result of the present study in accordance to the objectives mentioned in chapter one. The plant *Artemisia annua* was collected widely from Tarai Region of Pantnagar, Uttarakhand, India having altitude of 270 m, For the study of seasonal variation in essential oil components. The plant material was collected in the month of September 2020 and January 2021. The essential oils were studied by GC/MS and various biological activities were carried out in EO's. The results are being described as follows.

4.1 GC/MS analysis of AAREO

The essential oils isolated in rainy and winter season for their chemical composition and various biological activities were designated as AAREO & AAWEO respectively. Seventy five constituents contributing 91.7% of the total oil were identified in AAREO. Camphor (14.1%) was found to be the major component of the oil followed by germacrene-D (9.0%), *trans*-β-caryophyllene (8.7%), eucalyptol (4.2%), *cis*-cadin-4en-7-ol (3.9%), isoborneol (3.2%), p-cymene (2.9%), (-)-aromadendrene (2.6%), lavandulyl acetate (2.1%), α –cadinol (2.0%), camphene (2.0%), (-)-globulol (1.8), ϒ-cadinene (1.7%), spathulenol (1.5%), amyl vinyl carbinol (1.5%), germacra-4 (15), 5,10 (14)-trien-1-α–ol (1.3%), bicyclogermacrene (1.3%), selina-4,11-diene (1.2), (+)-α-terpineol (1.2%), isogermacrene- D (1.1%), α–humulene (1.0%), dihydro-β-ionone (1.0%), β- *trans*- farnesene (1.0%), *trans*-3-hexenyl acetate (1.0%), and β-pinene (1.0%). The minor components (<1.0%) were identified as α –copaene (0.9%), naphthalene (0.8%), bornyl angelate (0.8%), phytol (0.8%), benzyl 2-methylbutyrate (0.7%), *trans*-carveol (0.6%), 2-δ-carene (0.5%), epicedrol (0.5%), α-Selinene (0.5%), sobrerol 8-acetate (0.5%), β- costol (0.5%), (+)-spathulenol (0.4%), sabinene (0.4%), α-hydroxy-terpenyl acetate (0.4%), *cis*-carvyl acetate (0.4%), *cis*-jasmone (0.4%), cis-3-hexen-1-ol (0.3%), thymol (0.3%), bicycloelemene (0.3%), *neo*-alloocimene (0.3%), ϒ-gurjunenepoxide- (2) (0.2%), isogeranial (0.2%), caryophyllene oxide (0.2%), eugenol (0.2%), *cis*-carveol (0.2%),

pinocarvone (0.2%), ethanone (0.2%), linalool (0.2%), and *cis*- sabinene hydrate (0.2%). The compound which was present in trace amount was myrtenal i.e its composition was (<0.1%). The detailed representation of chemical composition of aerial parts of AAREO along with their chemical formula and Kovatt index (KI) value are being recorded in **table 4.1.** The class composition of essential oil constituents has been depicted in **table4.2** and **fig.4.2**, and its gas chromatogram in **fig. 4.1.** The structures of major compounds of AAREO are given in **fig. 4.5.**

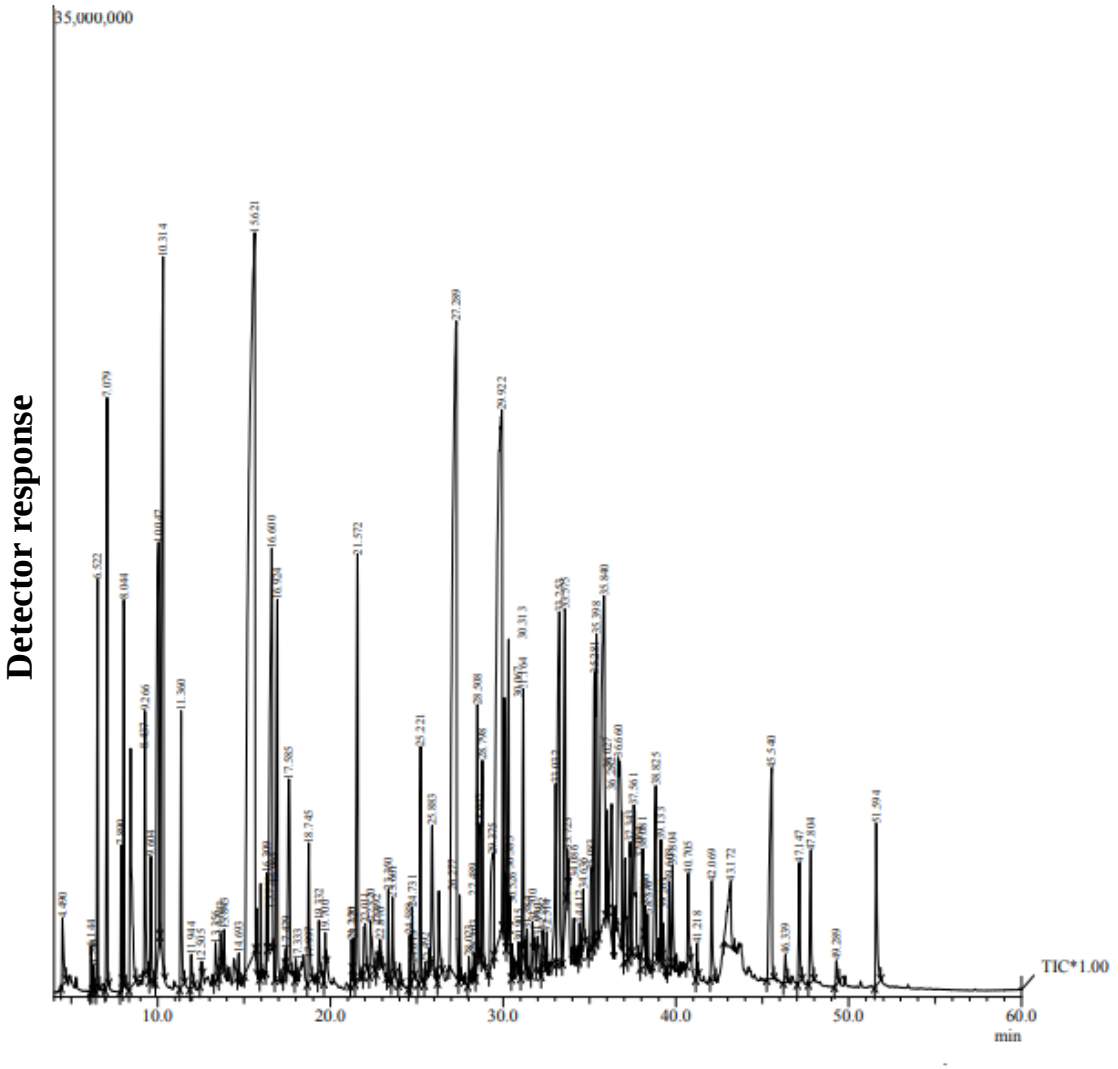


Fig 4.1 Gas Chromatogram of AAREO

Table 4.1 Chemical composition of AAREO

S.N.	Compound name	K.I	% Composition	Molecular Formula	Method of Identification (MS) (NIST-MS; Adams, 2007)
1.	*cis*-3-Hexen-1-ol	868	0.3	$C_6H_{12}O$	M^+= 100, m/z; 100,82,67,55,41 (100%), 27
2.	tricyclene (MH)	923	0.1	$C_{10}H_{16}$	M^+= 136, m/z; 136,121,105,93 (100%), 79,67
3.	α-thujene (MH)	927	0.1	$C_{10}H_{16}$	M^+= 136, m/z; 136,121,105,93 (100%), 77,65
4.	α-pinene (MH)	933	1.2	$C_{10}H_{16}$	M^+= 136, m/z; 136,121,105,93 (100%), 92,91,77,67,41
5.	camphene (MH)	954	2.0	$C_{10}H_{16}$	M^+= 136, m/z; 136,121,107,93 (100%), 79,67,53,41
6.	amyl vinyl carbinol (OM)	969	1.5	$C_{10}H_{16}O$	M^+= 128, m/z; 127,99,85,72,57 (100%), 43,29,27
7.	sabinene (MH)	972	0.4	$C_{10}H_{16}$	M^+= 136, m/z; 136,121,105,93 (100%), 77,69,41
8.	*β*-pinene (MH)	978	1.0	$C_{10}H_{16}$	M^+= 136 m/z; 136,121,107,93 (100%), 79,69,41
9.	2-δ-carene (MH)	1000	0.5	$C_{10}H_{16}$	M^+= 136, m/z; 136,121,105,93 (100%), 79,77,65
10.	*trans*-3-hexenyl acetate	1001	1.0	$C_8H_{14}O_2$	M^+= 142, m/z; 112,98,82,67,43 (100%), 41
11.	p-cymene (MH)	1025	2.9	$C_{10}H_{14}$	M^+= 134, m/z; 134,119 (100%), 91,77,65
12.	**eucalyptol (OM)**	**1031**	**4.2**	**$C_{10}H_{18}O$**	**M^+= 154, m/z; 154,139,108,84,81,69,43 (100%), 41**

13.	ϒ-terpinene (MH)	1058	1.0	$C_{10}H_{16}$	M^+= 136, m/z; 136,121,,105,93 (100%), 77,43,41
14.	*cis*- sabinene hydrate (OM)	1069	0.2	$C_{10}H_{18}O$	M^+= 154, m/z; 154,136,121,111,93,71,59,43 (100%), 41
15.	hotrienol (OM)	1072	0.1	$C_{10}H_{16}O$	M^+= 152, m/z; 119,107,91,83,71 (100%), 67,43
16.	linalool (OM)	1096	0.2	$C_{10}H_{18}O$	M^+= 154, m/z; 136,121,93,79,71,69,43 (100%), 41
17.	(-)-terpinen-4-ol (OM)	1137	1.8	$C_{10}H_{18}O$	M^+= 154, m/z; 154,136,111,93,71 (100%), 69,43,41
18.	p-menth-2-en-1-ol (OM)	1140	0.1	$C_{10}H_{18}O$	M^+= 154, m/z; 154,140,139,121,111,94,79,71,69,43 (100%), 41
19.	**camphor (OM)**	**1149**	**14.1**	**$C_{10}H_{16}O$**	**M^+= 152, m/z; 152,137,108,95 (100%), 81,69,55,41**
20.	camphene hydrate (OM)	1156	0.1	$C_{10}H_{18}O$	M^+= 154, m/z; 136,121,111,96,71 (100%), 69,43
21.	ethanone (OM)	1161	0.2	$C_{10}H_{16}O$	M^+= 152, m/z; 152,137,109 (100%), 93,81,67
22.	Pinocarvone (OM)	1164	0.2	$C_{10}H_{14}O$	M^+= 150, m/z; 150,135,122,108,81,69,53 (100%), 41
23.	isoborneol (OM)	1165	3.2	$C_{10}H_{18}O$	M^+= 154, m/z; 121,110,95 (100%), 81,67,41
24.	naphthalene (SH)	1181	0.8	$C_{15}H_{24}$	M^+= 204, m/z;204,189,175,162,147,133,119 (100%), 105,93,79,67,55,41
25.	(+)-α-terpineol (OM)	1188	1.2	$C_{10}H_{18}O$	M^+= 154, m/z; 136,121,93,81,59 (100%), 43,41
26.	myrtenal (OM)	1197	t	$C_{10}H_{14}O$	M^+= 150, m/z; 135,107,91,79 (100%), 66,53,41

27.	*trans*-carveol (OM)	1223	0.6	$C_{10}H_{16}O$	M^+= 152, m/z; 152,137,119,109 (100%), 84,83,69,55
28.	*cis*-Carveol (OM)	1232	0.2	$C_{10}H_{16}O$	M^+= 152, m/z; 134,119,109,84 (100%), 83,69,55,41
29.	sobrerol 8-acetate (OM)	-	0.5	$C_{12}H_{20}O_3$	M^+= 212, m/z; 152,137,109 (100%), 95,69,43
30.	isogeranial (OM)	-	0.2	$C_{10}H_{16}O$	M^+= 152, m/z; 152,137,119,109,94 (100%), 81,67,53,43,41
31.	carvone (OM)	1246	0.1	$C_{10}H_{14}O$	M^+= 150, m/z; 150,108,93,82 (100%), 58,54,41,40
32.	lavandulyl acetate (OM)	1284	2.1	$C_{12}H_{20}O_2$	M^+= 196, m/z; 136,121,107,93,80,69 (100%), 43,41,40
33.	bornyl acetate (OM)	1285	0.1	$C_{12}H_{20}O_2$	M^+= 196, m/z; 196,136,121,108,95 (100%), 80,43,41
34.	thymol (OM)	1293	0.3	$C_{10}H_{14}O$	M^+= 150, m/z; 150,135 (100%), 115,91,77,65,51,41
35.	4-vinyl Guaiacol	1309	0.1	$C_9H_{10}O_2$	M^+= 150, m/z; 150 (100%), 135,107,77,51,40
36.	*cis*-carvyl acetate (OM)	1346	0.4	$C_{12}H_{18}O_2$	M^+= 194, m/z; 152,134,119,109,84,79,67,43 (100%), 41
37.	α –Copaene (SH)	1375	0.9	$C_{15}H_{24}$	M^+= 204,m/z; 204,189,161,147,133,119,105 (100%), 93,81,
38.	(-)-cis-beta Elemene (SH)	1390	0.1	$C_{15}H_{24}$	M^+= 204, m/z; 189,175,161,147,121,107,93 (100%), 68,55,41
39.	eugenol (OM)	1392	0.2	$C_{10}H_{12}O_2$	M^+= 164, m/z; 164 (100%), 149,131,121,103,91,77,65,55,40
40.	benzyl 2-methylbutyrate	1394	0.7	$C_{12}H_{16}O_2$	M^+=192, m/z; 192,174,108,91 (100%), 57,41

41.	*cis*-jasmone (MH)	1394	0.4	$C_{11}H_{16}O$	M^+= 164, m/z; 164,149,135,122,110,93,79 (100%), 67,55,41,40
42.	*trans*-β-caryophyllene (SH)	1419	8.7	$C_{15}H_{24}$	M^+= 204, m/z;204,189,175,161,147,,120,105,93,79,55,41 (100%)
43.	ε-Muurolene (SH)	1430	0.1	$C_{15}H_{24}$	M^+= 204, m/z; 204,176,161 (100%), 148,133,105,91,81,67,41
44.	β-Copaene (SH)	1433	0.3	$C_{15}H_{24}$	M^+= 204, m/z; 204,189,161 (100%), 147,133,119,105,91,81,67,55
45.	dihydro-β-ionone (OS)	1436	1.0	$C_{13}H_{18}O$	M^+= 190, m/z; 175,147,131,105,91,77,43 (100%)
46.	β-trans-Farnesene (SH)	1440	1.0	$C_{15}H_{24}$	M^+= 204, m/z; 161,133,120,93,79,69 (100%), 55,41
47.	(-)-aromadendrene (SH)	1441	2.6	$C_{15}H_{24}$	M^+= 204, m/z; 161,147,133,119,105,91,81,55,41 (100%)
48.	isogermacrene- D (SH)	1447	1.1	$C_{15}H_{24}$	M^+= 204, m/z; 204,161 (100%), 119,105,91,81,41
49.	α -humulene (SH)	1454	1.0	$C_{15}H_{24}$	M^+= 204, m/z; 204,189,161,147,121,107,93 (100%), 80,67,41,40
50.	δ-Cadinene (SH)	1469	0.1	$C_{15}H_{24}$	M^+= 204, m/z; 204,189,161,145,119,105 (100%), 91,81,55,41
51.	α-Selinene (SH)	1474	0.5	$C_{15}H_{24}$	M^+= 204, m/z; 204,189,175,161,147,135,121,107,105,93,79,67,53,43,41 (100%)
52.	selina-4,11-diene (SH)	1476	1.2	$C_{15}H_{24}$	M^+= 204, m/z; 204,189 (100%), 175,161,147,133,119,105,91,41
53.	Germacrene-D (SH)	1485	9.0	$C_{15}H_{24}$	M^+= 204, m/z; 204,161 (100%), 147,133,119,105,91,81,67,41

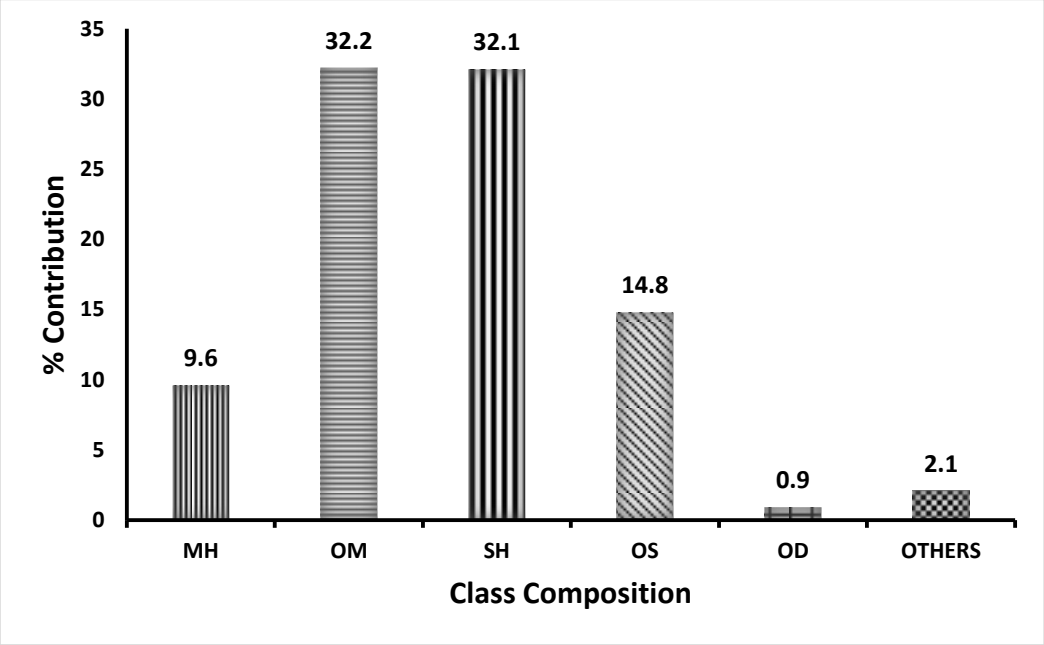
54.	(-)-β-Selinene (SH)	1489	1.0	$C_{15}H_{24}$	M^+= 204, m/z; 204,189,175,161,147,133,121,93 (100%), 67,55,41
55.	bicyclogermacrene (SH)	1497	1.3	$C_{15}H_{24}$	M^+= 204, m/z; 204,189,161,147,136,121 (100%), 107,93,67,41
56.	ϒ-cadinene (SH)	1512	1.7	$C_{15}H_{24}$	M^+= 204, m/z; 161 (100%), 147,133,119,105,91,79,67,55,41
57.	bicycloelemene (SH)	-	0.3	$C_{15}H_{24}$	M^+= 204, m/z; 189,161,136,121 (100%), 107,93,79,67,43,41
58.	epicedrol (OS)	-	0.5	$C_{15}H_{26}O$	M^+= 222, m/z; 222,207,189,177,150,135,121,109,95 (100%), 81,69
59.	neoalloocimene (SH)	-	0.3	$C_{15}H_{24}$	M^+= 204, m/z; 204,189,161 (100%), 147,133,119,105,81,69,55,
60.	α- Hydroxy-terpenyl acetate (OM)	1521	0.4	$C_{12}H_{20}O_3$	M^+= 212, m/z; 119,109,91,79,43 (100%), 41
61.	himbaccol (OS)	1530	0.1	$C_{15}H_{26}O$	M^+= 222, m/z; 189,147,133,121,109,95,81,69,43 (100%), 41
62.	(-)-globulol (OS)	1530	1.8	$C_{15}H_{26}O$	M^+= 222, m/z; 204,161,147,133,121,109,93,81,69,67,43 (100%), 41
63.	α-Cadinene (SH)	1538	0.1	$C_{15}H_{24}$	M^+= 204, m/z;204,289,161,147,133,119,105 (100%), 81,69,41
64.	ϒ-Gurjunenepoxide- (2) (OS)	1558	0.2	$C_{15}H_{24}O$	M^+= 220, m/z; 220,189,161,147,133,119,107,91,81 (100%), 67,55,41
65.	bornyl angelate (OS)	1569	0.8	$C_{15}H_{24}O_2$	M^+= 236, m/z; 236,136,109,93,83 (100%), 69,55,41
66.	spathulenol (OS)	1576	1.5	$C_{15}H_{24}O$	M^+= 220, m/z; 187,159,147,131,105,93,79,67,43 (100%), 41

67.	α –Cadinol (OS)	1580	2.0	$C_{15}H_{26}O$	M^+= 222, m/z; 204,161,137,121,95,79,69,43 (100%), 41
68.	caryophyllene oxide (OS)	1587	0.2	$C_{15}H_{24}O$	M^+= 220, m/z; 149,135,121,109,107,96,79,69,55,41 (100%)
69.	*cis*-cadin-4en-7-ol (OS)	1638	3.9	$C_{15}H_{26}O$	M^+= 222, m/z; 222,204,179,161,147,134,119 (100%), 105,93,71,43,41
70.	germacra-4 (15), 5,10 (14)-trien-1- α –ol (OS)	1683	1.3	$C_{15}H_{24}O$	M^+= 220, m/z; 220,177,159,145,133,121,109,91,79,67,43,41 (100%)
71.	8-α-11- elemodiol (OS)	1740	0.9	$C_{15}H_{26}O_2$	M^+= 238, m/z; 162,147,133,121,107,93,59 (100%), 43,41
72.	β- costol (OS)	1763	0.5	$C_{15}H_{24}O$	M^+= 220, m/z; 220,202,189,145,133,121 (100%), 105,91,79,67,55,41
73.	deoxyqinghaosu (OS)	1794	0.1	$C_{15}H_{22}O_4$	M^+= 266, m/z; 195,165,151,135,124,107,93,81,43 (100%), 41
74.	phytol (OD)	2045	0.8	$C_{20}H_{40}O$	M^+= 296, m/z; 126,123,111,95,71 (100%), 57,43,41
75.	thunbergol (OD)	2211	0.1	$C_{20}H_{34}O$	M^+= 290, m/z; 161,147,135,123,121,107,93,81,67,43 (100%), 41
	Total				

t= trace >0.1%, MS= Mass Spectometry, KI= Kovatt Index, AAREO= *Artemisia annua* rainy Essential oil, AAWEO= *Artemisia annua* winter Essential oil, - = not present, MH= Monoterpenoids Hydrocarbons, SH= Sesquiterpenoids Hydrocarbons, OM=Oxygenated monoterpenoid, OS=Oxygenated sesquiterpenoids, OD= Oxygenated diterpenoid

Table 4.2 Classes of compounds present in essential oil of AAREO

S. No.	Classes of composition	% Composition
1.	Monoterpenoid hydrocarbon (MH)	**9.6**
2.	Oxygenated monoterpenoid (OM)	**32.2**
3.	Sesquiterpenoid hydrocarbon (SH)	**32.1**
4.	Oxygenated sesquiterpenoid (OS)	**14.8**
5.	Oxygenated diterpenenoid (OD)	**0.9**
6.	others	**2.1**
	Total	**91.7%**



MH= monoterpenoids hydrocarbons, SH= sesquiterpenoids hydrocarbons, OM= oxygenated monoterpenoid, OS= oxygenated sesquiterpenoids, OD= oxygenated diterpenoids

Fig 4.2 Class composition of AAREO

4.2 GC/MS analysis of AAWEO

A total of fifty nine components accounting for 94.1% of the total oil composition have been identified in AAWEO as presented in **Table 4.3** with structures of major constituents in **fig 4.5 .**and the gas chromatogram in **Fig4.3** respectively.

Camphor (17.9%) was found to be the major component of the oil followed by eucalyptol (14.6%), (*E*,*E*)-2,6-dimethyl-3,5,7-octatrien-2-ol (6.3%), (*5E*)-2,6-dimethyl-1,5,7-octatrien-3-ol (5.2%) artemisia alcohol (3.5%), terpinene-4-ol (3.4%) isoborneol (3.2%), p-cymene (3.0%), caryophyllene oxide (3.1%), *trans* β-caryophyllene (2.7%), mycrene (2.6%), (+)-α-terpineol (2.5%), artemisia ketone (2.5%), camphene (2.1%), (-)β- farnesene (2.0%), yomogi alcohol (1.8%), longifolenaldehyde (1.5%), intermedeol (1.3%), sabinene (1.3%), α- pinene (1.3%) and *cis*-sabinene hydrate (1.1%) etc. The minor components (<1.0%) were α-terpenine (0.7%), α-copaene (0.7%), β-selinene (0.7%), (*3Z*)-2-Methyl-4- (2,6,6-trimethyl-1-cyclohexen-1-yl)-3-butenal (0.7%), germacra-4 (15), 5,10 (14)-trien-1-α-ol (0.6%), isoledene (0.5%), β-pinene (0.5%), santolinatriene (0.4%), *neo* intermedeol (0.4%), 8- α-11- elemodiol (0.4%), *cis* -carvyl angelate (0.4%), *trans*-carveol (0.4%), p-menth-2-en-1-ol (0.3%), sobrerol 8-acetate (0.3%), (-)-spathulenol (0.3%), α-selinene (0.3%), camphene hydrate (0.3%), sorbyl butyrate (0.3%), α-humulene (0.2%), eugenol (0.2%), carvacrol (0.2%), (-)-carvone (0.2%), phellandral (0.2%), nerolidol (0.2%), t-butyl isobutyl ketone (0.2%), benzyl valerate (0.2%), (-)-β-bourbonene (0.2%), 3-furanpropanol (0.1%), *cis*-linalool oxide (0.1%), phytone (0.1%), alloaromadendrenoxide- (1) (0.1%), epoxymyrcene (0.1%), α- thujene (0.1%), tricyclene (0.1%) etc. The compounds which were present in trace amount (<0.1%) were filifolone and indipone . In term of their class composition the identified constituents in AAWEO have been categorized as mono, sesqui and diterpenoids and are presented in **table 4.4** and **fig. 4.4** respectively.

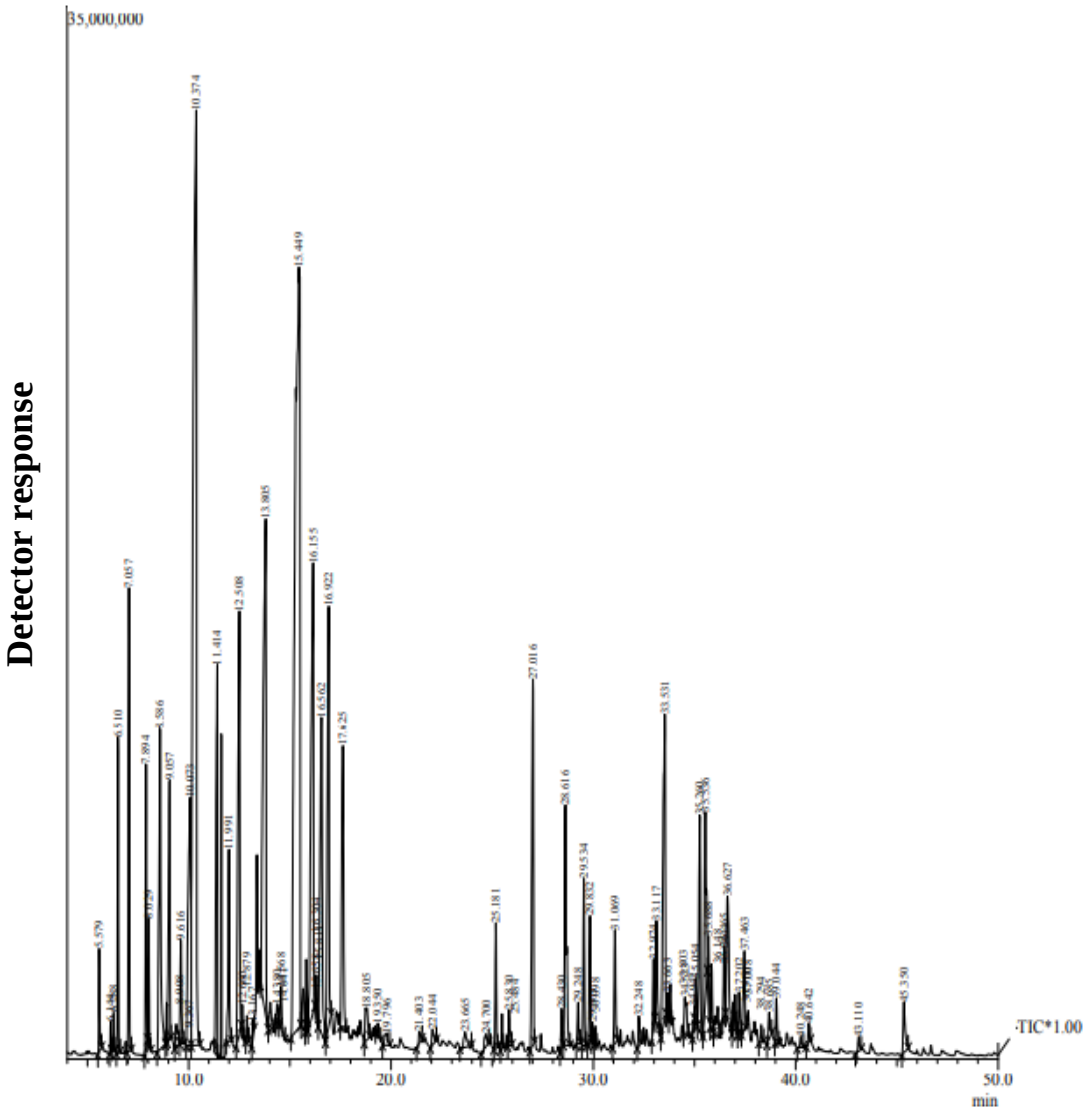


Fig 4.3: Gas chromatogram of AAWEO

Table 4.3 Chemical composition of AAWEO

S.N.	Compound Name	K.I	% Composition	Molecular Formula	Method of Identification (MS) (NIST-MS; Adams, 2007)
1.	santolinatriene (MH)	902	0.4	$C_{10}H_{16}$	M^+= 136, m/z; 136,121,105,93 (100%), 79,67
2.	t-Butyl isobutyl ketone	903	0.2	$C_9H_{18}O$	M^+ = 142, m/z; 142,85,57 (100%), 43,41,27
3.	tricyclene (MH)	923	0.1	$C_{10}H_{16}$	M^+ = 136, m/z; 136,121,105,93 (100%), 79,67,43,41
4.	α- thujene (MH)	927	0.1	$C_{10}H_{16}$	M^+ = 136, m/z; 136, 121,105,93 (100%), 77
5.	α- pinene (MH)	933	1.3	$C_{10}H_{16}$	M^+ = 136, m/z; 136, 121,105,93 (100%), 77
6.	camphene (MH)	954	2.1	$C_{10}H_{16}$	M^+ = 136, m/z; 136,121,107,93 (100%), 79,67,53,41
7.	sabinene (MH)	972	1.3	$C_{10}H_{16}$	M^+ = 136, m/z; 136,121,105,93 (100%), 77,69
8.	*β*-pinene (MH)	978	0.5	$C_{10}H_{16}$	M^+ = 136, m/z; 136,121,107,93 (100%), 79,69
9.	**mycrene** (MH)	**991**	**2.6**	$C_{10}H_{16}$	**M^+ = 136, m/z;136,121,107,93,79,69,53,41 (100%)**
10.	yomogi alcohol (OM)	999	1.8	$C_{10}H_{18}O$	M^+ = 154, m/z; 139,121,111,85,81,59,43 (100%), 41

11.	α-terpenine (MH)	1018	0.7	$C_{10}H_{16}$	M^+= 136, m/z; 136,121,107,93 (100%), 77,65,43,41
12.	p-cymene (MH)	1025	3.0	$C_{10}H_{14}$	M^+ = 134, m/z; 134,119 (100%), 103,91,77,65
13.	**eucalyptol** (OM)	**1031**	**14.6**	**$C_{10}H_{18}O$**	**M^+ = 154,** **m/z; 154,139,108,84,81,69,43 (100%), 41,39**
14.	artemisia ketone (OM)	1056	2.5	$C_{10}H_{16}O$	M^+ = 152, m/z; 152,136,121,105,93,83 (100%), 55
15.	artemisia alcohol (OM)	1068	3.5	$C_{10}H_{18}O$	M^+ = 154, m/z; 139,121,85 (100%), 70,67,55,41
16.	cis-sabinene hydrate (OM)	1069	1.1	$C_{10}H_{18}O$	M^+= 154, m/z; 154,121,111,93,71,69,43 (100%), 41
17.	*cis*-linalool Oxide (OM)	1072	0.1	$C_{10}H_{18}O_2$	M^+ = 170, m/z; 155,112,11,94,81,59 (100%), 43,41
18.	(*E*,*E*)-2,6-dimethyl-3,5,7-octatrien-2-ol (OM)	1090	6.3	$C_{10}H_{16}O$	M^+ = 152, m/z; 152,137,109,91,81,67,43 (100%), 41
19.	epoxymyrcene (OM)	1096	0.1	$C_{10}H_{16}O$	M^+ = 152, m/z; 132,117,109,85,79 (100%), 59,43,41
20.	filifolone (OM)	-	t	$C_{10}H_{14}O$	M^+ = 80, m/z; 150,122,107,91,80 (100%)
21.	(*5E*)-2,6-Dimethyl-1,5,7-octatrien-3-ol (OM)	1120	5.2	$C_{10}H_{16}O$	M^+ = 152, m/z; 152,137,119,105,84,71,67,41 (100%), 39

22.	terpinene-4-ol (OM)	1137	3.4	$C_{10}H_{18}O$	M^+ = 154, m/z; 154,136,111,93,71 (100%), 69,43
23.	p-Menth-2-en-1-ol (OM)	1140	0.3	$C_{10}H_{18}O$	M^+ = 154, m/z; 154,139,121,111,93,71,69,43 (100%)
24.	**camphor (OM)**	**1149**	**17.9**	**$C_{10}H_{16}O$**	**M^+ = 152, m/z; 152,108,95 (100%), 81,69,55,41**
25.	camphene hydrate (OM)	1156	0.3	$C_{10}H_{18}O$	M^+ = 154, m/z; 136,121,111,96,71 (100%), 69,43
26.	sabine ketone	1159	0.4	$C_9H_{14}O$	M^+ = 138, m/z; 138,123,96,81 (100%), 67,55,41
27.	isoborneol (OM)	1165	3.2	$C_{10}H_{18}O$	M^+ = 154, m/z; 136,121,110,95 (100%), 67,43,41
28.	(+)-α-terpineol (OM)	1188	2.5	$C_{10}H_{18}O$	M^+ = 154, m/z; 136,121,93,81,59 (100%), 43
29.	(-)-carvone (OM)	1190	0.2	$C_{10}H_{14}O$	M^+ = 150, m/z; 150,135,108,93,82 (100%), 54,39
30.	*trans*-carveol (OM)	1223	0.4	$C_{10}H_{16}O$	M^+ = 152, m/z; 152,137,119,109,84,83,69,55,43 (100%), 41
31.	sorbyl butyrate (OM)	1252	0.3	$C_{10}H_{16}O_2$	M^+ = 168, m/z; 168,139,135,97,71 (100%), 43,41
32.	phellandral (OM)	1277	0.2	$C_{10}H_{16}O$	M^+ = 152, m/z; 152,137,109 (100%), 95,79,67,55,41

33.	carvacrol (OM)	1299	0.2	$C_{10}H_{14}O$	M^+ = 150, m/z; 150,135 (100%), 115,107,91,77
34.	(-)-β-bourbonene (SH)	1339	0.2	$C_{15}H_{24}$	M^+ = 204, m/z; 204,189,161,123,81 (100%), 79,41
35.	eugenol (OM)	1357	0.2	$C_{10}H_{12}O_2$	M^+ = 164, m/z;164 (100%), 149,131,121,103,91,77,65,55
36.	cis Carvyl angelate (OM)	-	0.4	$C_{15}H_{22}O_2$	M^+ = 234, m/z; 191,152,134,119,107,93,83 (100%)
37.	longifolenaldehyde (OS)	-	1.5	$C_{15}H_{24}O$	M^+ = 220, m/z; 220,205,187,176,163,135,109,95,81,67,55,43 (100%)
38.	α-copaene (SH)	1375	0.7	$C_{15}H_{24}$	M^+ = 204, m/z; 161,119,105 (100%), 93,81,69,55,41
39.	isoledene (SH)	1376	0.5	$C_{15}H_{24}$	M^+ = 204, m/z; 204,189,161 (100%), 147,119,105,91,81
40.	*trans* β-caryophyllene (SH)	1419	2.7	$C_{15}H_{24}$	M^+= 204, m/z; 204,189,161,133,120,105,93,79,69 (100%), 41
41.	β-farnesene (SH)	1452	2.0	$C_{15}H_{24}$	M^+ = 204, m/z; 204,161,133,120,93,69 (100%), 41
42.	α-humulene (SH)	1454	0.2	$C_{15}H_{24}$	M^+ = 204, m/z; 204,161,147,121,107,93 (100%), 80

43.	benzyl valerate	1458	0.2	$C_{12}H_{16}O_2$	M^+ = 192 m/z; 192,174,145,108,91 (100%), 57,44
44.	β-Selinene (SH)	1469	0.7	$C_{15}H_{24}$	M^+ = 204, m/z; 204,189,175,161,147,133,105 (100%), 93,79,67
45.	germacrene-D (SH)	1485	0.1	$C_{15}H_{24}$	M^+ = 204, m/z; 204,161 (100%), 133,119,105,91,81,67
46.	indipone (OM)	1492	T	$C_{15}H_{24}O$	M^+ = 220, m/z; 149 (100%), 121,107,93,81,69,41
47.	sobrerol 8-acetate (OM)	-	0.3	$C_{12}H_{20}O_3$	M^+ = 212, m/z; 152,137,119,109 (100%), 95,43
48.	3-furanpropanol (OM)	-	0.1	$C_{10}H_{14}O_2$	M^+ = 166, m/z; 166,148.133,119,105,95,82 (100%)
49.	α-selinene (SH)	1501	0.3	$C_{15}H_{24}$	M^+= 204, m/z;204,189 (100%), 175,161,147,133,119,107,93
50.	caryophyllene oxide (OS)	1507	3.1	$C_{15}H_{24}O$	M^+ = 220, m/z; 220,187,177,161,149,138,121,109,93,79,69,43 (100%)
51.	(3Z)-2-methyl-4- (2,6,6-trimethyl-1-cyclohexen-1-yl)-3-butenal	1545	0.7	$C_{14}H_{22}O$	M^+ = 206, m/z; 206,191,163,135,123,107,95,81 (100%), 69,55
52.	nerolidol (SH)	1564	0.2	$C_{15}H_{26}O$	M^+ = 222, m/z;136,121,107,93,71,69 (100%), 43,41

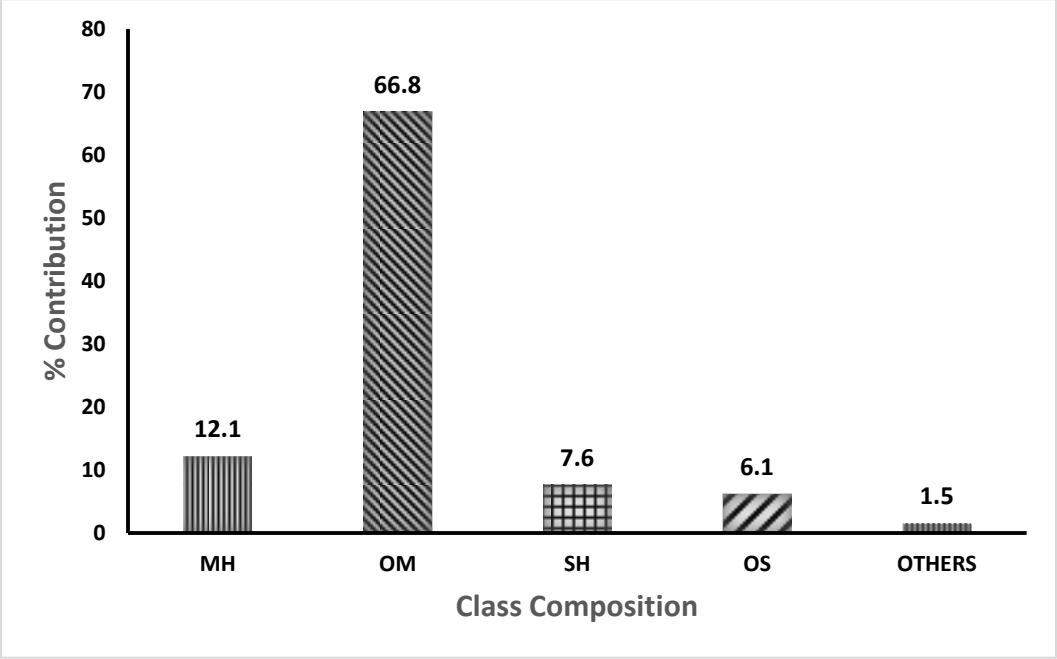
53.	(-)-spathulenol (OS)	1578	0.3	$C_{15}H_{24}O$	M^+ = 220, m/z; 205,187,159,147,131,119,91,79,43 (100%), 41
54.	alloaromadendrenoxid- (1) (OS)	1641	0.1	$C_{15}H_{24}O$	M^+ = 220, m/z; 220,189,177,159,147,133,119,105,94 (100%), 81
55.	neo intermedeol (OM)	1661	0.4	$C_{15}H_{26}O$	M^+ = 222, m/z; 204,189,161,147,135,121,109,93,81,67,43 (100%)
56.	intermedeol (OM)	1668	1.3	$C_{15}H_{26}O$	M^+ = 222, m/z; 204,189,161,133,123,107,93,81,67,43 (100%)
57.	germacra-4 (15), 5,10 (14)-trien-1- α –ol (OS)	1683	0.6	$C_{15}H_{24}O$	M^+ = 220, m/z; 220,177,159,145,133,121,109,91,79,41 (100%)
58.	8- α-11- elemodiol (OS)	1740	0.4	$C_{15}H_{26}O_2$	M^+ = 238, m/z; 162,147,121,107,93,79,59,43 (100%)
59.	phytone (OS)	1841	0.1	$C_{18}H_{36}O$	M^+ = 268, m/z; 250,179,124,109,85,71,58,43 (100%)
	94.10%				

t= trace >0.1%, MS= Mass Spectometry, KI= Kovatt Index, AAREO= *Artemisia annua* rainy Essential oil, AAWEO= *Artemisia annua* winter Essential oil, - = not present, MH= monoterpenoids hydrocarbons, SH= sesquiterpenoids hydrocarbons, OM= oxygenated monoterpenoid, OS= oxygenated sesquiterpenoids, OD= oxygenated diterpenoid

Table 4.4 Classes of compounds present in AAWEO

S. N.	Classes of composition	% composition
1.	Monoterpenoid hydrocarbon (MH)	**12.1**
2.	Oxygenated monoterpenoid (OM)	**66.8**
3.	Sesquiterpenoid hydrocarbon (SH)	**7.6**
4.	Oxygenated sesquiterpenoid (OS)	**6.1**
5.	Others	**1.5**
	Total	**94.1%**

MH= monoterpenoids mydrocarbons, SH= sesquiterpenoids hydrocarbons, OM= oxygenated monoterpenoid, OS= oxygenated sesquiterpenoids, OD= oxygenated diterpenoids



MH= monoterpenoids mydrocarbons, SH= sesquiterpenoids hydrocarbons, OM= oxygenated monoterpenoid, OS= oxygenated sesquiterpenoids, OD= oxygenated diterpenoids

Fig. 4.4 Class composition of AAWEO

4.3 Comparative study of AAREO and AAWEO

Comparing the identified constituents of AAREO and AAWEO by GC-MS analysis, It was observed that the monoterpenoid hydrocarbon in AAREO contributed 9.6 % while in AAWEO, there contribution was 12.1%, being mainly represented by α-pinene, camphene, sabinene, p-cymene, myrcene, ϒ-terpinene etc. The oxygenated monoterpenoids in both the oils contributed 32.2% and 66.8 % respectively which dominated overall composition in both the oils. The major oxygenated monoterpenoids identified in AAREO & AAWEO were eucalyptol (1,8 cineole), *cis*-sabinene hydrate, yomogi alcohol, artemisia ketone, artemisia alcohol, amyl-vinyl carbinol, linalool, hotrineol etc. However the major constituent identified was camphor with 14.1 % and 17.9 % contribution in AAREO and AAWEO respectively. The sesquiterpenoid hydro carbon were dominated in AAREO with 32.1% while 7.6 % in AAWEO. The constituents identified as sesquiterpenoid hydrocarbons were *trans*-β-caryophyllene, α-hummulene, germacrene-D, β-selinene, aromadendrene etc. besides other minor and trace constituents. Similarly the oxygenated sesquiterpenoids were dominated in AAREO with 14.8% contribution over AAWEO with 6.1% contribution only. The major representative oxygenated sesquiterpenoids identified were caryophyllene oxide, longifolenaldehyde, spathulenol, *cis*-cadin-4en-1ol, α-cadinol, globulol etc. besides other minor and trace constituents.The contribution of oxygenated diterpenoid identified in AAREO contributed only 0.9% being represented by phytol (0.8%) and thunbergol (0.1%). However none of the oxygenated diterpenoid could be identified could be identified in AAWEO. The other constituents identified in both the oils contributed 2.1 and 1.5% in AAREO & AAWEO respectively. In terms of constituents identified in AAREO & AAWEO both the oils showed different qualitative and quantitative makeup viz..santolinatriene (0.4%), t-Butyl isobutyl ketone (0.2%), myrcene (2.6%), yomogi alcohol (1.8%), α-Terpenine (0.7%), artemisia ketone (2.5%) artemisia alcohol (3.5%), *cis*-linalool Oxide (0.1%), (*E*,*E*)-2,6-dimethyl-3,5,7-octatrien-2-ol (6.3%), epoxymyrcene (0.1%), (*5E*)-2,6-dimethyl-1,5,7-octatrien-3-ol (5.2%), sorbyl butyrate (0.3%), phellandral (0.2%), carvacrol, (0.2%), (-)-β-bourbonene (0.2%), isoledene (0.5%), benzyl valerate (0.2%), (3Z)-2-methyl-4- (2,6,6-trimethyl-1-cyclohexen-1-yl)-3-butenal (0.7%),

alloaromadendrenoxid- (1) (0.1%), *neo*-intermedeol (0.4%), intermedeol (1.3%), phytone (0.1%), *cis*-carvyl angelate (0.4%), longifolenaldehyde (1.5%), filifolone (t>0.1%) and indipone (t>0.1%) were identified in AAWEO and were missing in AAREO, Similarly the constituents like *cis*-3-Hexen-1-ol (0.3%), amyl vinyl carbinol (1.5%), 2-δ-carene (0.5%), *trans*-3-hexenyl acetate (1.0%), ϒ-terpinene (1.0%), hotrienol (0.1%), linalool (0.2%), ethanone (0.2%), pinocarvone (0.2%), naphthalene (0.8%), *cis*-Carveol (0.2%), lavandulyl acetate (2.1%), bornyl acetate (0.1%), thymol (0.3%), 4-vinyl guaiacol (0.1%), *cis*-carvyl acetate (0.4%), (-)-*cis*-β elemene (0.1%), benzyl 2-methylbutyrate (0.7%), *cis*-jasmone (0.4%), ε-muurolene (0.1%), β-Copaene (0.3%), dihydro-β-ionone (1.0%), (-)-aromadendrene (2.6%), isogermacrene- D (1.1%), δ-cadinene (0.1%), selina-4,11-diene (1.2%), bicyclogermacrene (1.3%), ϒ-cadinene (1.7%), α- hydroxy-terpenyl acetate (0.4%), (-)-globulol (1.8%), himbaccol (0.1%), ϒ-gurjunenepoxide- (2) (0.2%), bornyl angelate (0.8%), α –Cadinol (2.0%), *cis*-cadin-4en-7-ol (3.9%), β- costol (0.5%), deoxyqinghaosu (0.1%), phytol (0.8%), bicycloelemene (0.3%), epicedrol (0.5%), isogeranial (0.2%), and neoalloocimene (0.3%) were identified in AAREO and were found to be missing in AAWEO respectively.

The detailed comparative composition in terms of identified constituents is being depicted in **table 4.5** and the class composition in **table 4.6 & fig 4.6** respectively and their mass spectra of major compounds in **appendix -1.**

It has been reported that the chemical composition of the essential oil of different species of aromatic plants might differ mainly due to harvesting season and geographical conditions arising from seasonal variations **(Verma *et al*., 2010).**

Padalia *et al*., (2011) have reported the presence of camphor (22.8%-42.6%), 1,8 cineole (3.7- 8.4%), β-caryophyllene (2.0-9.2%), germacrene-D (0.5-7.3%) in essential oils of *A.annua* collected at different stages.

Camphor (48.00%), 1,8 – cineole (9.39%), camphene (6.98%) and spathulenol (4.695%) have been reported as major constituents in the essential oils of *A.annua* **(Mohammadreza, 2008).**

Presence of artemisia ketone (68.5%) as a major component along with camphor (27.5%), 1,8-cineole (22.8%), α-pinene (16.0%), camphene hydrate (12.0%), and artemisia alcohol (5.2%) have been reported by **(Charles *et al*., 1991).** Artemisia ketone (35.7%). α-pinene (16.5%), 1,8-cineole (5.5%) have been reported in the aerial parts of *A.annua*from Serbia (**Radulovic *et al*., 2013).** Camphor (48%) including 1,8 cineole (9.39%), camphene (6.98%) have been reported in essential oil of *A.annua* **(Verdian-rizi, 2009).**

Islamuddin *et al*., (2014) reported the chemical constituent identified in essential oil of *A.annua*with camphor (52.06%).The study also revealed the presence of the other constituentslike β-caryophyllene (10.95 %), 1,8-cineole (5.57 %), β-caryophyllene oxide (4.21 %), β-farnesene (3.83 %), α-copaene (2.91 %), β-cyperone (1.93 %), α-selinene (1.54 %), and *trans*-pinocarveol (1.22 %). **Bagchi *et al*., (2003)** reported the major changes in the chemical constituents of *A.annua* due to the change in growing season. It has been reported that camphor (10.5–44.4%) tends be the major constituent of oil instead of usually dominated constituent i.e artemisia ketone. Artemisia ketone (35.7%), The major constituent followed by1,8-cineole (31.5%), alpha-pinene (11.2%), artemisia alcohol (5.2%), and myrcene (4.6%) have been reported in the essential oils of *A.annua* **(Libbey and Sturtz, 1989). Omer *et al*., (2013)** have reported 1-camphor, camphene, β-camphene, isoartemisia ketone, β-caryophyllene and α-pinene in the essential oils of *A.annua***.**

Comparing the chemical composition of essential oils isolated from *A.annua* in two different seasons with that of previous reports, It was observed that AAREO and AAWEO have vastly different qualitative and quantitative variations. It has also been observed that camphor and 1,8 cineole, germacrene-D with different quantitive make up has been reported as the major constituent in earlier as well as in the present study along with other constituents. Under stress conditions, plants biosynthesise secondary metabolites such as terpenoids, alkaloids, polyphenolics, and other compounds in response to their need for defence. It could be possible that the qualitative and quantitative differences in chemical composition observed in AAREO and AAWEO are due to changing weather conditions.

Table 4.5 Comparative chemical composition of AAREO and AAWEO:

S.N.	Name of compound	% Contribution		KI	Method of Identification (MS) (NIST-MS; Adams, 2007)
		AAREO	AAWEO		
1.	*cis*-3-hexen-1-ol	0.3	-	868	M^+= 100, m/z; 100,82,67,55,41 (100%), 27
2.	santolinatriene (MH)	-	0.4	902	M^+= 136, m/z; 136,121,105,93 (100%), 79,67
3.	t-butyl isobutyl ketone	-	0.2	903	M^+ = 142, m/z; 142,85,57 (100%), 43,41,27
4.	tricyclene (MH)	0.1	0.1	923	M^+= 136, m/z;136,121,105,93 (100%), 79,67
5.	α-thujene (MH)	0.1	0.1	927	M^+= 136, m/z; 136,121,105,93 (100%), 77,65
6.	α-pinene (MH)	1.2	1.3	933	M^+= 136, m/z; 136,121,105,93 (100%), 92,91,77,67,41
7.	camphene (MH)	2.0	2.1	954	M^+= 136, m/z; 136,121,107,93 (100%), 79,67,53,41
8.	amyl vinyl carbinol (OM)	1.5	-	969	M^+= 128, m/z; 127,99,85,72,57 (100%), 43,29,27
9.	sabinene (MH)	0.4	1.3	972	M^+= 136, m/z; 136,121,105,93 (100%), 77,69,41
10.	β-pinene (MH)	1.0	0.5	978	M^+= 136 m/z; 136,121,107,93 (100%), 79,69,41

11.	myrcene (MH)	-	2.6	**991**	M^+ = 136, m/z;136,121,107,93,79,69,53,41 (100%)
12.	yomogi alcohol (OM)	-	1.8	999	M^+ = 154, m/z; 139,121,111,85,81,59,43 (100%), 41
13.	2-δ-carene (MH)	0.5	-	1000	M^+= 136, m/z; 136,121,105,93 (100%), 79,77,65
14.	*trans*-3-hexenyl acetate	1.0	-	1001	M^+= 142, m/z; 112,98,82,67,43 (100%), 41
15.	α-terpenine (MH)	-	0.7	1018	M^+= 136, m/z; 136,121,107,93 (100%), 77,65,43,41
16.	p-cymene (MH)	2.9	3.0	1025	M^+= 134, m/z; 134,119 (100%), 91,77,65
17.	eucalyptol (OM)	4.2	14.6	1031	M^+= 154, m/z; 154,139,108,84,81,69,43 (100%), 41
18.	artemisia ketone (OM)	-	2.5	1056	M^+ = 152, m/z; 152,136,121,105,93,83 (100%), 55
19.	ϒ-terpinene (MH)	1.0	-	1058	M^+= 136, m/z; 136,121,,105,93 (100%), 77,43,41
20.	artemisia alcohol (OM)	-	3.5	1068	M^+ = 154, m/z; 139,121,85 (100%), 70,67,55,41
21.	*cis*-sabinene hydrate (OM)	0.2	1.1	1069	M^+= 154, m/z; 154,121,111,93,71,69,43 (100%), 41
22.	hotrienol (OM)	0.1	-	1072	M^+= 152, m/z; 119,107,91,83,71 (100%), 67,43

23.	*cis*-linalool Oxide (OM)	-	0.1	1072	M^+ = 170, m/z; 155,112,11,94,81,59 (100%), 43,41
24.	(*E*,*E*)-2,6-dimethyl-3,5,7-octatrien-2-ol (OM)	-	6.3	1090	M^+ = 152, m/z; 152,137,109,91,81,67,43 (100%), 41
25.	linalool (OM)	0.2	-	1096	M^+= 154, m/z; 136,121,93,79,71,69,43 (100%), 41
26.	epoxymyrcene (OM)	-	0.1	1096	M^+ = 152, m/z; 132,117,109,85,79 (100%), 59,43,41
27.	(*5E*)-2,6-dimethyl-1,5,7-octatrien-3-ol (OM)	-	5.2	1120	M^+ = 152, m/z; 152,137,119,105,84,71,67,41 (100%), 39
28.	p-menth-1-en-4-ol/ terpinen-4-ol (OM)	1.8	3.4	1137	M^+ = 154, m/z; 154,136,111,93,71 (100%), 69,43
29.	p-menth-2-en-1-ol OM)	0.1	0.3	1140	M^+= 154, m/z; 154,140,139,121,111,94,79,71,69,43 (100%), 41
30.	camphor (OM)	14.1	17.9	1149	M^+= 152, m/z; 152,137,108,95 (100%), 81,69,55,41
31.	camphene hydrate (OM)	0.1	0.3	1156	M^+= 154, m/z; 136,121,111,96,71 (100%), 69,43
32.	sabine ketone	-	0.4	1159	M^+ = 138, m/z; 138,123,96,81 (100%), 67,55,41
33.	ethanone (OM)	0.2	-	1161	M^+= 152, m/z; 152,137,109 (100%), 93,81,67
34.	Pinocarvone (OM)	0.2	-	1164	M^+= 150, m/z; 150,135,122,108,81,69,53 (100%), 41

35.	isoborneol (OM)	3.2	3.2	1165	M^+= 154, m/z; 121,110,95 (100%), 81,67,41
36.	naphthalene (SH)	0.8	-	1181	M^+= 204, m/z;204,189,175,162,147,133,119 (100%), 105,93,79,67,55,41
37.	(+)-α-terpineol (OM)	1.2	2.5	1188	M^+= 154, m/z; 136,121,93,81,59 (100%), 43,41
38.	myrtenal (OM)	t	-	1197	M^+= 150, m/z; 135,107,91,79 (100%), 66,53,41
39.	*trans*-carveol (OM)	0.6	0.4	1223	M^+ = 152, m/z; 152,137,119,109,84,83,69,55,43 (100%), 41
40.	*cis*-carveol (OM)	0.2	-	1232	M^+= 152, m/z; 134,119,109,84 (100%), 83,69,55,41
41.	carvone (OM)	0.1	0.2	1246	M^+ = 150, m/z; 150,135,108,93,82 (100%), 54,39
42.	sorbyl butyrate (OM)	-	0.3	1252	M^+ = 168, m/z; 168,139,135,97,71 (100%), 43,41
43.	phellandral (OM)	-	0.2	1277	M^+ = 152, m/z; 152,137,109 (100%), 95,79,67,55,41
44.	lavandulyl acetate (OM)	2.1	-	1284	M^+= 196, m/z; 136,121,107,93,80,69 (100%), 43,41,40
45.	bornyl acetate (OM)	0.1	-	1285	M^+= 196, m/z; 196,136,121,108,95 (100%), 80,43,41

46.	thymol (OM)	0.3	-	1293	M^+= 150, m/z; 150,135 (100%), 115,91,77,65,51,41
47.	carvacrol (OM)	-	0.2	1299	M^+ = 150, m/z; 150,135 (100%), 115,107,91,77
48.	4-vinyl guaiacol	0.1	-	1309	M^+= 150, m/z; 150 (100%), 135,107,77,51,40
49.	(-)-β-bourbonene (SH)	-	0.2	1339	M^+ = 204, m/z; 204,189,161,123,81 (100%), 79,41
50.	*cis*-carvyl acetate (OM)	0.4	-	1346	M^+= 194, m/z; 152,134,119,109,84,79,67,43 (100%), 41
51.	α-copaene (OM)	0.9	0.7	1375	M^+= 204, m/z; 189,161,147,133,119,105 (100%), 93,81,69,55,41
52.	isoledene (SH)	-	0.5	1376	M^+ = 204, m/z; 204,189,161 (100%), 147,119,105,91,81
53.	(-)-*cis*-beta Elemene (SH)	0.1	-	1390	M^+= 204, m/z; 189,175,161,147,121,107,93 (100%), 68,55,41
54.	eugenol (OM)	0.2	0.2	1392	M^+ = 164, m/z;164 (100%), 149,131,121,103,91,77,65,55
55.	benzyl 2-methylbutyrate	0.7	-	1394	M^+=192, m/z; 192,174,108,91 (100%), 57,41
56.	*cis*-jasmone (MH)	0.4	-	1394	M^+= 164, m/z; 164,149,135,122,110,93,79 (100%), 67,55,41,40
57.	*trans* β-caryophyllene (SH)	8.7	2.7	1419	M^+= 204, m/z; 204,189,161,133,120,105,93,79,69 (100%), 41

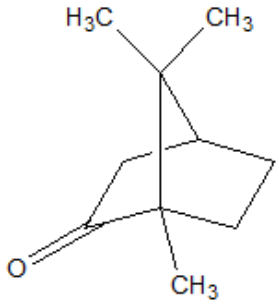
58.	ε-Muurolene (SH)	0.1	-	1430	M^+= 204, m/z; 204,176,161 (100%), 148,133,105,91,81,67,41
59.	β-copaene (SH)	0.3	-	1433	M^+= 204, m/z; 204,189,161 (100%), 147,133,119,105,91
60.	dihydro-β-ionone (OS)	1.0	-	1436	M^+= 190, m/z; 175,147,131,105,91,77,43 (100%)
61.	(-)-aromadendrene (SH)	2.6	-	1441	M^+= 204, m/z; 161,147,133,119,105,91,81,55,41 (100%)
62.	isogermacrene- D (SH)	1.1	-	1447	M^+= 204, m/z; 204,161 (100%), 119,105,91,81,41
63.	β-farnesene (SH)	1.0	2.0	1452	M^+ = 204, m/z; 204,161,133,120,93,69 (100%), 41
64.	α-humulene (SH)	1.0	0.2	1454	M^+ = 204, m/z; 204,161,147,121,107,93 (100%), 80
65.	benzyl valerate	-	0.2	1458	M^+ = 192 m/z; 192,174,145,108,91 (100%), 57,44
66.	δ-cadinene (SH)	0.1	-	1469	M^+= 204, m/z; 204,189,161,145,119,105 (100%), 91,81,55,41
67.	α-selinene (SH)	0.5	0.3	1474	M^+= 204, m/z;204,189 (100%), 175,161,147,133,119,107,93
68.	selina-4,11-diene (SH)	1.2	-	1476	M^+= 204, m/z; 204,189 (100%), 175,161,147,133,105,91,41
69.	germacrene-d (SH)	9.0	0.1	1485	M^+= 204, m/z; 204,161 (100%), 147,133,119,105,91,81,67,41

70.	β-Selinene (SH)	1.0	0.7	1489	M^+= 204, m/z; 204,189,175,161,147,133,121,93 (100%), 67,55,41
71.	indipone (OM)	-	t	1492	M^+ = 220, m/z; 149 (100%), 121,107,93,81,69,41
72.	bicyclogermacrene (SH)	1.3	-	1497	M^+= 204, m/z; 204,189,161,147,136,121 (100%), 107,93
73.	ϒ-cadinene (SH)	1.7	-	1512	M^+= 204, m/z; 161 (100%), 147,133,119,105,91,79,67,55,41
74.	α- hydroxy-terpenyl acetate (OM)	0.4	-	1521	M^+= 212, m/z; 119,109,91,79,43 (100%), 41
75.	(-)-globulol (OS)	1.8	-	1530	M^+= 222, m/z; 204,161,147,133,121,109,93,81,69,67,43 (100%),
76.	himbaccol (OS)	0.1	-	1530	M^+= 222, m/z; 189,147,133,121,109,95,81,69,43 (100%), 41
77.	spathulenol (OS)	1.5	0.3	1536	M^+ = 220, m/z; 205,187,159,147,131,119,91,79,43 (100%), 41
78.	α-cadinene (SH)	0.1	-	1538	M^+= 204, m/z;204,289,161,147,133,119,105 (100%), 81,69,41
79.	(3Z)-2-methyl-4- (2,6,6-trimethyl-1-cyclohexen-1-yl)-3-butenal	-	0.7	1545	M^+ = 206, m/z; 206,191,163,135,123,107,95,81 (100%), 69,55
80.	ϒ-gurjunenepoxide- (2) (OS)	0.2	-	1558	M^+= 220, m/z; 220,189,161,147,133,119,107,91,81 (100%), 67,55,41

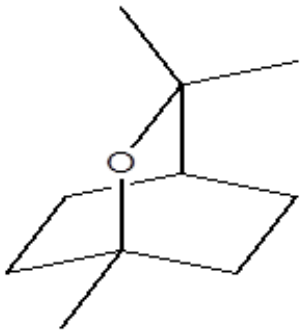
81.	bornyl angelate (OS)	0.8	-	1569	M^+= 236, m/z; 236,136,109,93,83 (100%), 69,55,41
82.	α –cadinol (OS)	2.0	-	1580	M^+= 222, m/z; 204,161,137,121,95,79,69,43 (100%), 41
83.	caryophyllene oxide (OS)	0.2	3.1	1587	M^+ = 220, m/z; 220,187,177,161,149,138,121,109,93,79,69,43 (100%)
84.	*cis*-cadin-4en-7-ol (OS)	3.9	-	1638	M^+= 222, m/z; 222,204,179,161,147,134,119 (100%), 105,93,71,43,41
85.	alloaromadendrenoxid- (1) (OS)	-	0.1	1641	M^+ = 220, m/z; 220,189,177,159,147,133,119,105,94 (100%), 81
86.	*neo* intermedeol (OM)	-	0.4	1661	M^+ = 222, m/z; 204,189,161,147,135,121,109,93,81,67,43 (100%)
87.	intermedeol (OM)	-	1.3	1668	M^+ = 222, m/z; 204,189,161,133,123,107,93,81,67,43 (100%)
88.	germacra-4 (15), 5,10 (14)-trien-1- α –ol (OS)	1.3	0.6	1683	M^+ = 220, m/z; 220,177,159,145,133,121,109,91,79,41 (100%)
89.	8-α-11- elemodiol (OS)	0.9	0.4	1740	M^+ = 238, m/z; 162,147,121,107,93,79,59,43 (100%)
90.	β- costol (OS)	0.5	-	1763	M^+= 220, m/z; 220,202,189,145,133,121 (100%), 105,91,79,67,55,41

91.	deoxyqinghaosu (OS)	0.1	-	1794	M^+= 266, m/z; 195,165,151,135,124,107,93,81,43 (100%), 41
92.	phytone (OS)	-	0.1	1841	M^+ = 268, m/z; 250,179,124,109,85,71,58,43 (100%)
93.	phytol (OD)	0.8	-	2045	M^+= 296, m/z; 126,123,111,95,71 (100%), 57,43,41
94.	thunbergol (OD)	0.1	-	2211	M^+= 290,m/z; 161,147,135,123,107,93,67,43 (100%), 41
95.	*cis*-carvyl angelate (OM)	-	0.4	-	M^+ = 234, m/z; 191,152,134,119,107,93,83 (100%)
96.	longifolenaldehyde (OS)	-	1.5	-	M^+ = 220, m/z; 220,205,187,176,163,135,109,95,81,67,55,43 (100%)
97.	sobrerol 8-acetate (OM)	0.5	0.3	-	M^+ = 212, m/z; 152,137,119,109 (100%), 95,43
98.	3-furanpropanol (OM)	-	0.1	-	M^+ = 166, m/z; 166,148.133,119,105,95,82 (100%)
99.	bicycloelemene (SH)	0.3	-	-	M^+= 204, m/z; 189,161,136,121 (100%), 107,93,79,67,43,41
100.	epicedrol (OS)	0.5	-	-	M^+= 222, m/z; 222,207,189,177,150,135,121,109,95 (100%), 81,69,41
101.	filifolone (OM)	-	t	-	M^+ = 80, m/z; 150,122,107,91,80 (100%)
102.	isogeranial (OM)	0.2	-	-	M^+= 152, m/z; 152,137,119,109,94 (100%), 81,67,53,43,41
103.	neoalloocimene (SH)	0.3	-	-	M^+= 204, m/z; 204,189,161 (100%), 47,133,119,105,81,69,55,41
	Total	91.7%	94.10%		

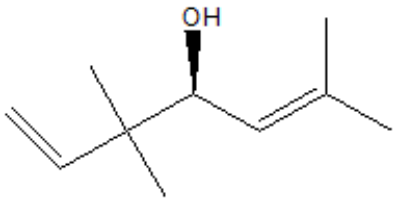
t= trace >0.1%, MS= Mass Spectometry, KI= Kovatt Index, AAREO= *Artemisia annua* rainy Essential oil, AAWEO= *Artemisia annua* winter Essential oil, - = not present, MH= monoterpenoids hydrocarbons, SH= sesquiterpenoids hydrocarbons, OM= oxygenated monoterpenoid, OS= oxygenated sesquiterpenoids, OD= oxygenated diterpenoids



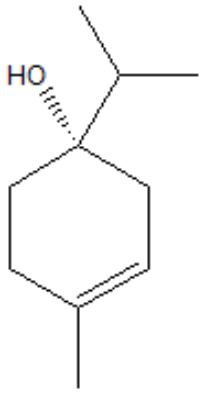
camphor



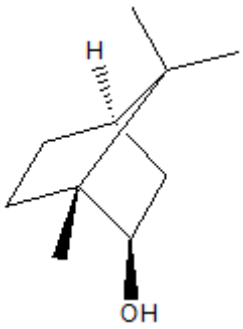
eucalyptol



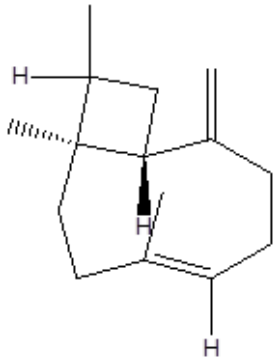
artemisia alcohol



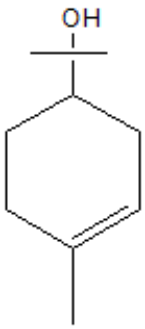
terpinene-4-ol



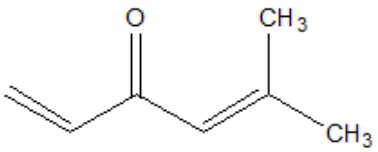
isoborneol



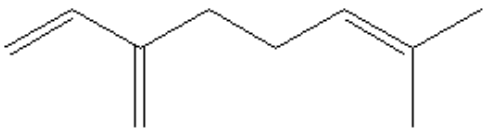
***trans* β-caryophyllene**



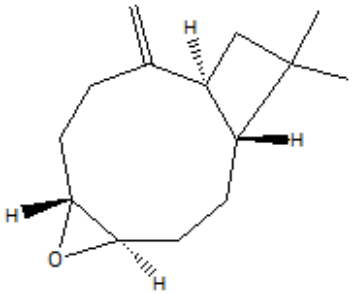
(+)- α-terpineol



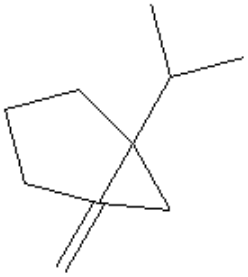
artemisia ketone



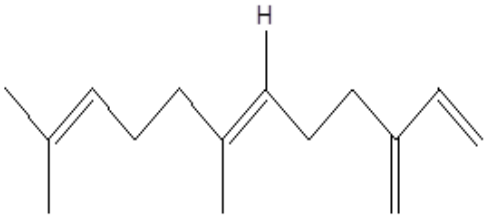
myrcene



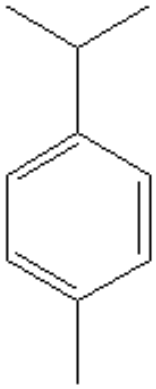
caryophyllene oxide



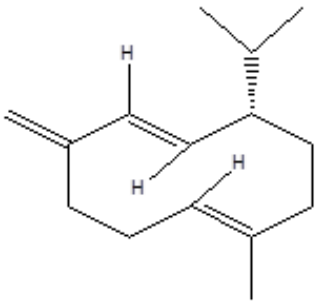
sabinene



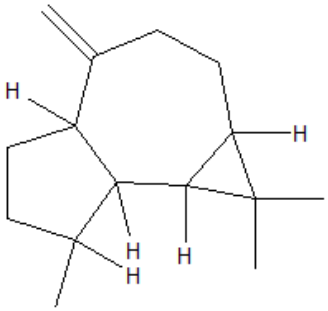
β-Farnesene



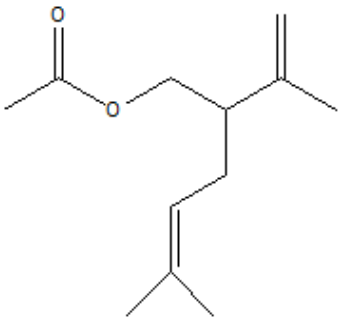
p-cymene



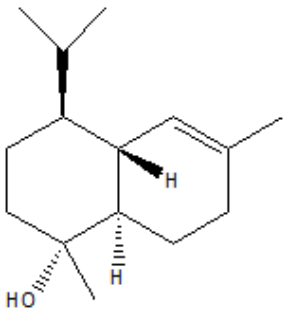
germacrene-D



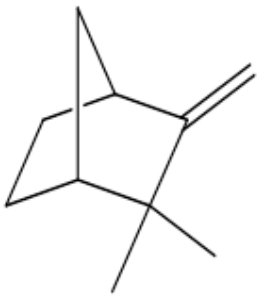
(-)-aromadendrene



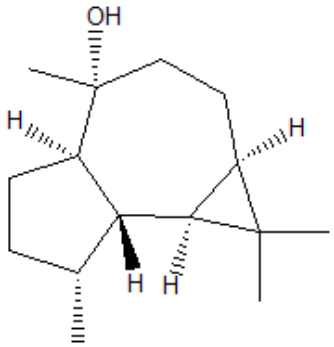
lavandulyl acetate



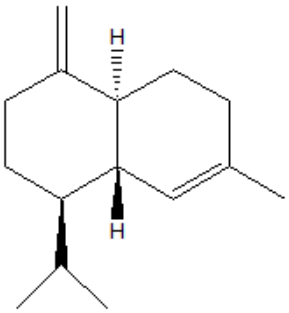
α –Cadinol



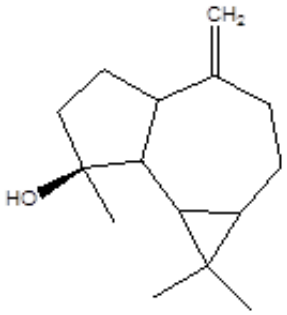
camphene



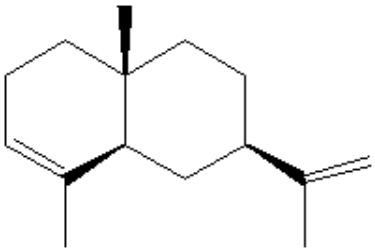
(-)-globulol



ϒ-cadinene



spathulenol



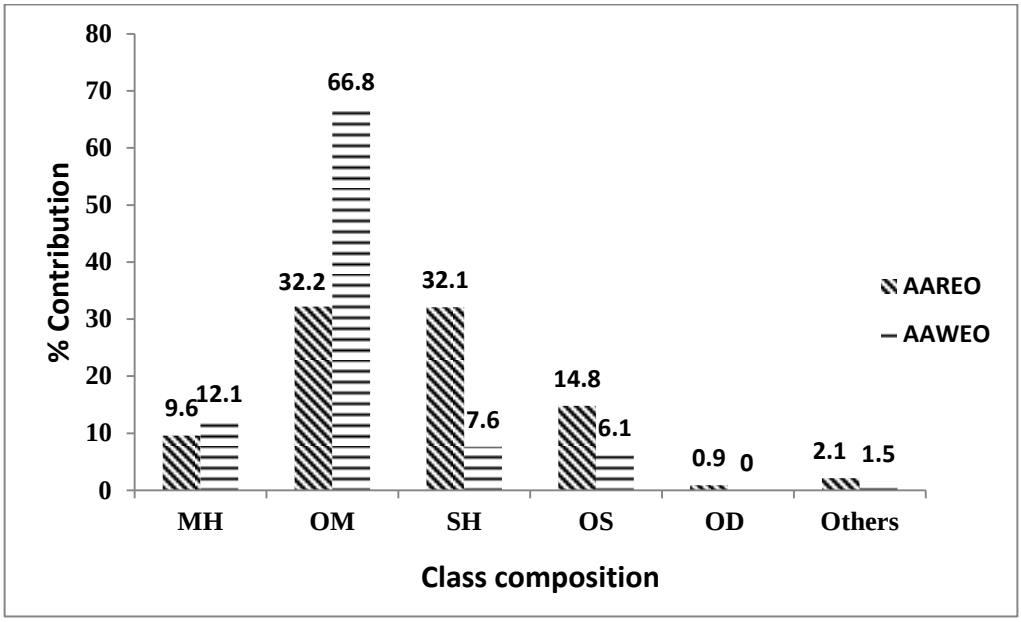
selina-4,11-diene

Fig 4.5 Structures of major compounds present in AAREO & AAWEO

Table: 4.6 Comparison of classes of compounds present in AAREO and AAWEO

S.N.	Classes of composition	% Composition	
		AAREO	AAWEO
1.	monoterpenoid hydrocarbon (MH)	9.6	12.1
2.	oxygenated monoterpenoid (OM)	32.2	66.8
3.	sesquiterpenoid hydrocarbon (SH)	32.1	7.6
4.	oxygenated sesquiterpenoid (OS)	14.8	6.1
5.	oxygenatedDiterpenenoid (OD)	0.9	-
6.	Others	2.1	1.5
	Total	**91.7%**	**94.10%**

MH= monoterpenoids hydrocarbons, SH= sesquiterpenoidshydrocarbons, OM= oxygenated monoterpenoid, OS= oxygenated sesquiterpenoids, OD= oxygenated diterpenoids



MH= monoterpenoids hydrocarbons, SH= sesquiterpenoidshydrocarbons, OM= oxygenated monoterpenoid, OS= oxygenated sesquiterpenoids, OD= oxygenated diterpenoids

Fig 4.6 : Comparative class composition of AAREO and AAWEO

4.4 Biological activities

Various biological activities like antioxidant, *in-vitro* anti-inflammatory, *in-vitro* antidiabetic, anti-feedant, nematicidal activity anti bacterial activity and anti fungal activity of AAREO & AAWEO are being presented as follows.

4.4.1 Antioxidant activity

Antioxidants are the compounds that are known to be capable of retarding the oxidation of an oxidizable material, despite used in very moderate amount. Most common synthetic antioxidants which are found to be damaging to human health are butylated hydroxyl anisole (BHA) or butylated hydroxyl toluene (BHT) **(Amorati *et al.*, 2013).** Natural antioxidant compounds posess antioxidant activity through different mechanisms which includes chain breaking by donation of hydrogen atoms or electrons which convert free radicals into stable species (**Iqbal *et al.*, 2013).** Biological redox reactions leads to the production of reactive oxygen and nitrogen free radicals, which can be triggered by a variety of environmental factors such as pollution, smoke, and sunlight. Reactive oxygen and nitrogen species (ROS/RNS) are

formed when electron acceptors, such as molecular oxygen and nitrogen, react readily with free radicals to become radicals themselves**.** There's a lot of evidence that these free radicals produce oxidative stress by causing oxidative damage to biomolecules like lipids, proteins, and nucleic acids **(Anthony *et al.*, 2012).** Oxidative stress influences the development and of many diseases, such as cardiovascular diseases, inflammation, neurodegenerative diseases and aging processes also arterial hypertension can be developed from oxidative stress and is believed to result from systemic damage in different target tissues by oxygen free radicals **(Khaleel and Buchbauer 2018).**

Plants produce a plethora of secondary products with potent antioxidant properties. Some natural ingredients, such as essential oils, have strong biological, antioxidant, therapeutic, and pharmaceutical applications **(Mata *et al.*, 2007).**

Fig. 4.7 depicts the generation of free radicals and their scavenging by antioxidants where as mechanism of hydrocarbon autoxidation and antioxidant protection is shown in **Fig.4.8.**

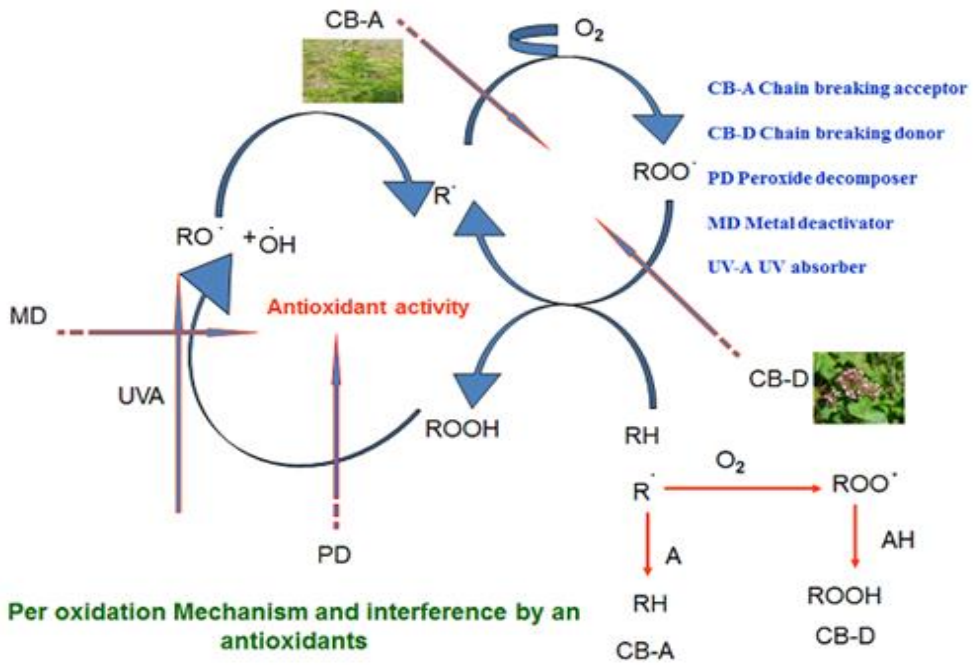


Fig. 4.7 Generation of free radicals and their scavenging by antioxidants

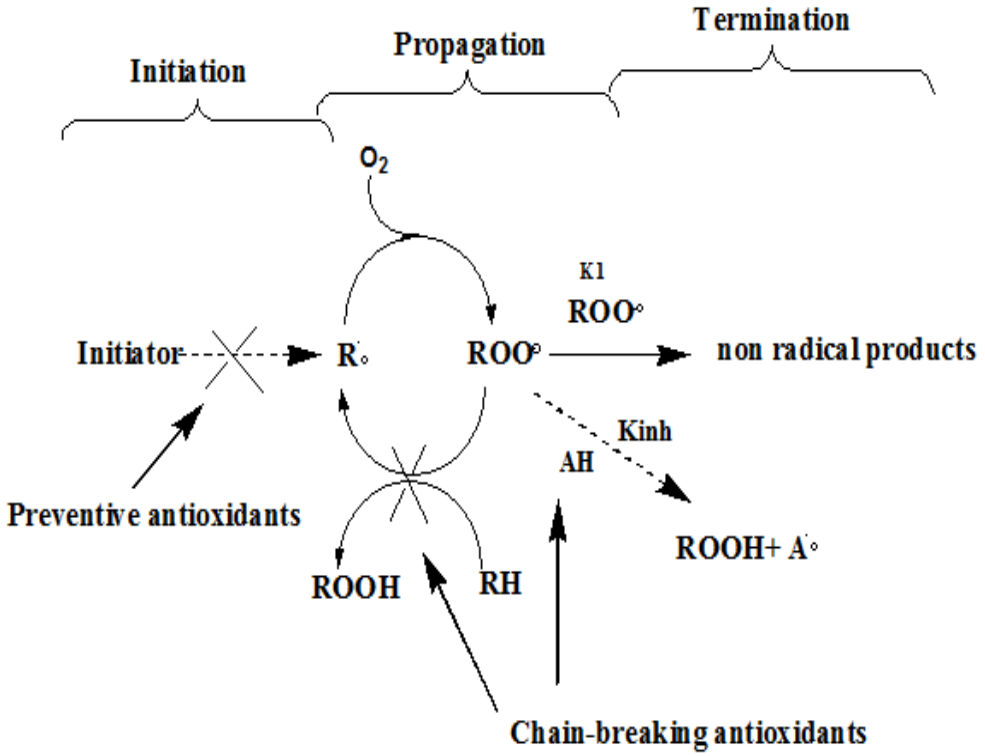


Fig. 4.8 Mechanism of hydrocarbon autoxidation and antioxidant protection

In present study, the *in vitro* antioxidant activity of AAREO and AAWEO was evaluated by DPPH radical scavenging method, H_2O_2 radical scavenging activity and metal chelating activity in comparison to standard antioxidants, BHT, Na_2-EDTA. The results obtained are being described as under.

4.4.1.1 DPPH free radical scavenging activity

DPPH radical scavenging assay is the most renowned and widely used for the of antioxidant activity of essential oils. DPPH is known as a stable radical in solution and appears purple colour absorbing at 515 nm in methanol. This assay is based on the principle that DPPH on accepting a hydrogen (H) atom from the scavenger molecule i.e. antioxidant, resulting into reduction of DPPH to 1,1-diphenyl-2-picrylhydrazine (DPPH-H) or DPPH2 and characterized by changing colour from purple to yellow with decrease in absorbance **fig 4.9** .Hence DPPH provides a simple way to analyse anti oxidant activity spectrophotometrically **(Mishra *et al*., 2012).**

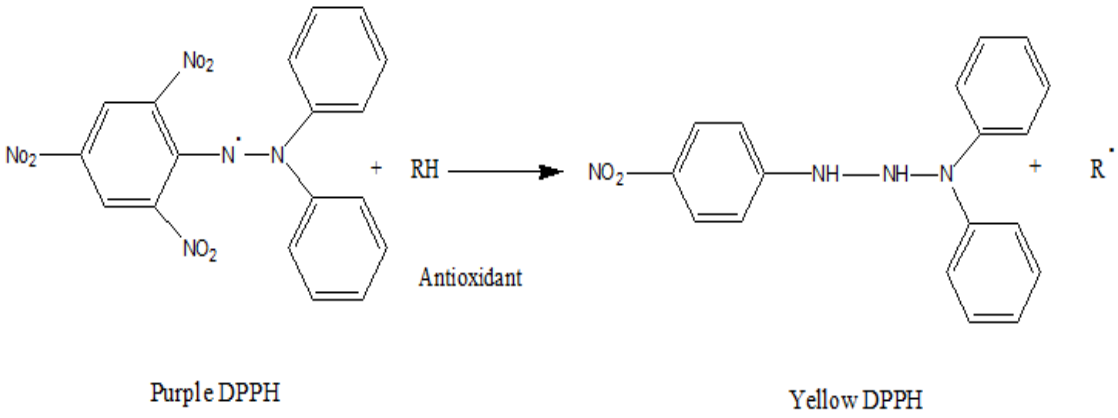


Fig 4.9 DPPH radical scavenging reaction mechanism

The DPPH free radical activity of AAREO and AAWEO was observed in the order of AAWEO (IC_{50}=45.588±0.334) > AAREO (IC_{50}=77.87±0.528) compared to BHT (IC_{50}=43.48±1.55) which indicated that AAWEO possess maximum DPPH free radical scavenging activity with minimum IC_{50} value followed by AAREO. The detailed presentation of radical scavenging activities observed spectrophotometrically in terms of OD (optical density), percent inhibition and IC_{50} values of AAREO and AAWEO are being presented in **Table 4.7** and **Fig. 4.10** while the IC_{50} values of AAREO and AAWEO are being shown in **Fig 4.11** As the concentration of essential oils increases, the increase in the radical free scavenging of both the oils was observed i.e the anti-oxidant activity in terms of DPPH radical scavenging assay was concentration dependent.

Table 4.7 DPPH radical scavenging antioxidant activity of AAREO and AAWEO

S.N	Sample Name	R	20µl/mL		40 µl/mL		60 µl/mL		80 µl/mL		100 µl/mL		IC50
			O.D	%I	O.D	%I	O.D	%I	O.D	%I	O.D	%I	
1.	AAREO	R1	0.385 0.386 0.387	**20.24**	0.292 0.290 0.295	**39.66**	0.278 0.276 0.281	**42.56**	0.242 0.240 0.245	**50**	0.197 0.195 0.200	**59.29**	**77.29**
		Avg	**0.386±0.001**		**0.292 ±0.002**		**0.278±0.002**		**0.242 ±0.002**		**0.197±0.002**		
		R2	0.382 0.383 0.382	**24.63**	0.298 0.296 0.301	**38.42**	0.272 0.270 0.275	**43.80**	0.248 0.246 0.251	**48.76**	0.199 0.197 0.202	**58.88**	**78.01**
		Avg	**0.382±0.005**		**0.298±0.002**		**0.272±0.002**		**0.248±0.002**		**0.199±0.002**		
		R3	0.385 0.386 0.387	**20.24**	0.298 0.296 0.301	**38.42**	0.282 0.280 0.285	**41.7**	0.244 0.242 0.247	**49.58**	0.197 0.195 0.200	**59.29**	**78.32**
		Avg	**0.385±0.001**	**21.27±2.534**	**0.298±0.002**	**39.12±0.715**	**0.282±0.002**	**47.01±1.055**	**0.244±0.002**	**57.085±0.630**	**0.197±0.002**	**69.36±0.236**	**77.87±0.528**
2.	AAWEO	R1	0.303 0.302 0.302	**37.60**	0.289 0.288 0.287	**40.49**	0.208 0.207 0.208	**57.23**	0.107 0.107 0.108	**77.89**	0.077 0.076 0.077	**84.29**	**45.471**
		Avg	**0.302±0.0005**		**0.288±0.001**		**0.207±0.0005**		**0.107±0.005**		**0.076±0.005**		
		R2	0.302 0.301 0.302	**37.80**	0.287 0.287 0.288	**40.70**	0.209 0.208 0.207	**57.02**	0.106 0.105 0.106	**77.89**	0.075 0.074 0.076	**84.50**	**45.328**
		Avg	**0.301±0.0005**		**0.287±0.0005**		**0.208±0.001**		**0.107±0.005**		**0.075±0.001**		
		R3	0.303 0.302 0.302	**37.60**	0.287 0.286 0.287	**40.90**	0.209 0.207 0.209	**57.02**	0.108 0.109 0.108	**77.68**	0.079 0.078 0.078	**83.88**	**45.966**
		Avg	**0.302±0.0005**	**37.33±0.642**	**0.286±0.0005**	**40.69±0.205**	**0.208±0.001**	**57.09±0.121**	**0.108±0.005**	**77.82±0.121**	**0.078±0.005**	**84.22±0.315**	**45.588±0.334**
3.	BHT	R1	0.299 0.297 0.302	**38.22**	0.232 0.230 0.235	**52.06**	0.211 0.209 0.214	**56.40**	0.186 0.184 0.189	**61.5**	0.168 0.166 0.171	**65.28**	**45.23**
		Avg	**0.299±0.0025**		**0.232±0.0025**		**0.211±0.0025**		**0.186±0.005**		**0.168±0.005**		
		R2	0.292 0.290 0.295	**39.66**	0.225 0.223 0.227	**53.51**	0.215 0.213 0.217	**55.57**	0.181 0.179 0.184	**62.60**	0.164 0.162 0.167	**66.11**	**42.28**
		Avg	**0.292±0.0025**		**0.225±0.0025**		**0.215±0.0025**		**0.181±0.005**		**0.164±0.005**		
		R3	0.292 0.290 0.295	**39.66**	0.230 0.228 0.233	**52.47**	0.209 0.207 0.212	**56.81**	0.186 0.184 0.189	**61.5**	0.168 0.166 0.171	**65.28**	**42.93**
		Avg	**0.292±0.0025**	**34.38±0.831**	**0.230±0.0025**	**49.51±0.715**	**0.209±0.747**	**57.19±0.631**	**0.186±0.0025**	**66.40±0.635**	**0.168±0.005**	**74.16±0.479**	**43.48±1.55**

R= Replica, O.D= optical density, %I= percent inhibition AAREO= *Artemisia annua* rainy essential oil, AAWEO= *Artemisia annua* winter essential oil

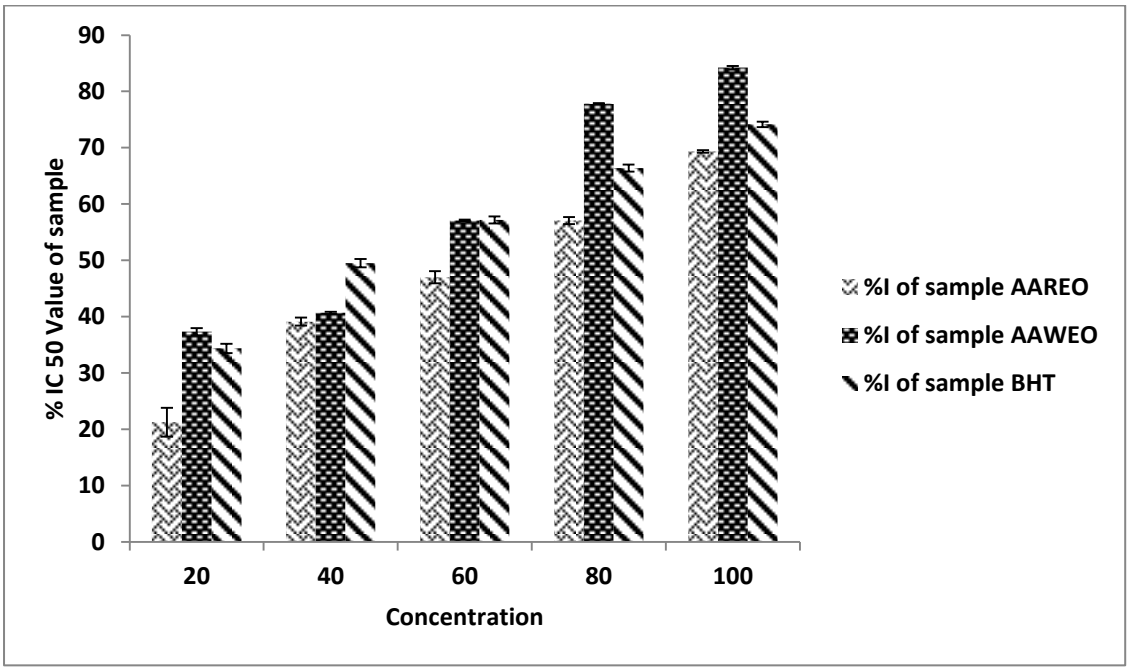


Fig. 4.10 DPPH radical scavenging activity of AAREO and AAWEO

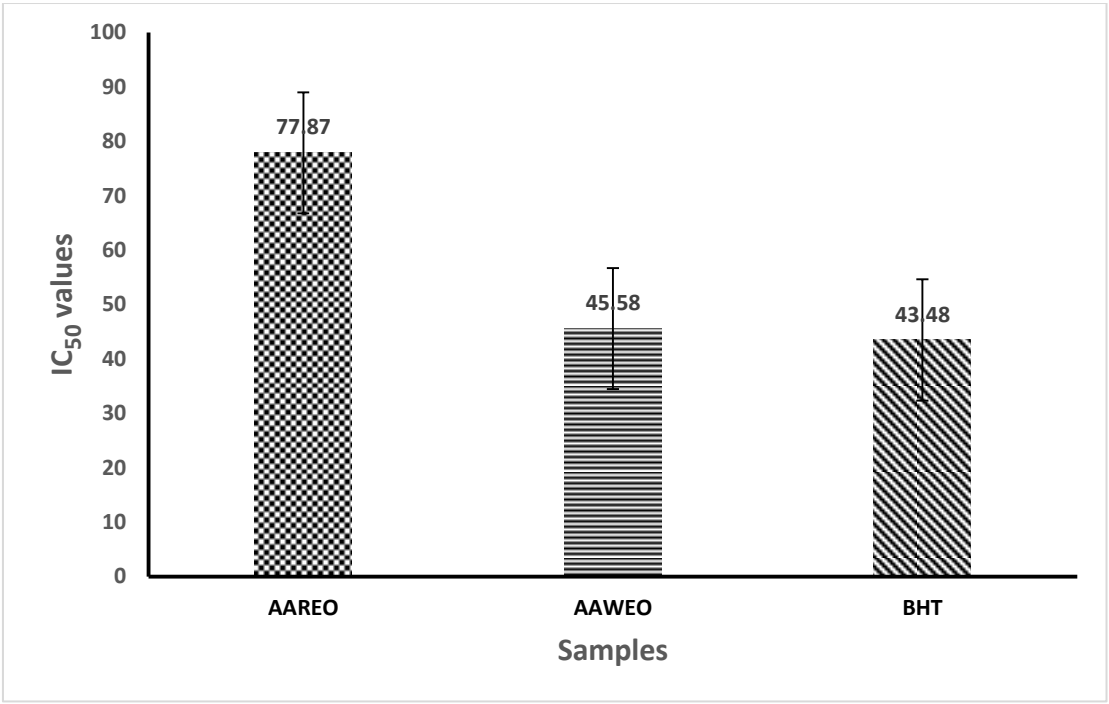


Fig. 4.11 IC_{50} Values of AAREO &AAWEO

4.4.1.2 Metal chelating activity

Chelation of transition metals has been reported to be one of the important mechanisms of antioxidative action. Transition metal ions can promote lipid peroxidation through generation of initiating species, and accelerating peroxidation by decomposing lipid hydroperoxides into other components which are capable of abstracting hydrogen (**Miguel, 2010)**.

The transition metal ion, Fe^{2+} exhibits the ability to move single electrons by virtue of which it can allow the formation and propagation of many radical reactions even starting with relatively non-reactive radicals. The main strategy to avoid generation of reactive oxygen species is associated with redox active metal catalysis involves chelating of the metal ions. Ferrozine can quantitatively chelate with Fe^{2+} and form a complex with a red color. This reaction is limited in the presence of other chelating agents and results in a decrease of the red color of the ferrozine–Fe^{2+} complexes. Measurement of the color reduction estimates the chelating activity to compete with ferrozine for the ferrous ions **(Ćavar *et al*., 2012).**

The effect on metal chelating activity of Fe^{2+} by AAREO and AAWEO was evaluated in presence of $FeCl_2$ and ferrozine in dose dependent manner. The metal chelating activity of AAREO and AAWEO was observed in the following order of AAWEO (IC_{50}=49.50±0.234**)**>AAREO (IC_{50} =51.40±0.158) compared to EDTA (IC_{50} =41.68±0.396) which indicated that AAWEO possess maximum metal chelating activity with minimum IC_{50} value followed by AAREO. The detailed data of percent metal chelating activity and IC_{50}values of oils has been depicted in **table 4.8 and fig 4.12** while the IC_{50} values of AAREO and AAWEO are being presented in **Fig 4.13**

Table 4.8 Metal chelating activity of AAREO and AAWEO

S.N	Sample	R	20µl/mL		40 µl/mL		60 µl/mL		80 µl/mL		100 µl/mL		
			O.D	**%I**	**O.D**	**%I**	**O.D**	**%I**	**O.D**	**%I**	**O.D**	**%I**	**IC50**
		R1	1.285		0.826	45.37	0.586	61.24	0.324	78.57	0.184	87.83	51.49
			1.283		0.824		0.584		0.322		0.187		
			1.288	15.01	0.829		0.589		0.327		0.182		
		Avg	1.285±0.0025		0.826±0.0025		0.586±0.0025		0.324±0.0025		0.184±0.0025		
		R2	1.279	15.41	0.819	45.83	0.586	61.24	0.321	78.76	0.186	87.69	51.22
			1.282		0.817		0.584		0.319		0.184		
			1.277		0.822		0.589		0.324		0.189		
1.	**AAREO**	**Avg**	**1.279±0.0025**		**0.819±0.0025**		**0.586±0.0025**		**0.321±0.0025**		**0.186±0.0025**		
		R3	1.282	15.34	0.826	45.37	0.589	61.04	0.324	78.57	0.192	87.30	51.5
			1.28		0.824		0.592		0.322		0.19		
			1.285		0.829		0.587		0.327		0.195		
		Avg	**1.282±0.0025**	**16.44±0.213**	**0.826±0.0025**	**44.14±0.265**	**0.589±0.0025**	**60.88±0.115**	**0.324±0.0025**	**78.97±0.109**	**0.192±0.0025**	**90.70±0.274**	**51.40±0.158**
		R1	1.342	11.24	0.624	58.73	0.548	63.75	0.418	72.35	0.176	88.35	49.41
			1.345		0.622		0.551		0.416		0.174		
			1.34		0.627		0.546		0.421		0.179		
		Avg	**1.342±0.0025**		**0.624±0.0025**		**0.548±0.0025**		**0.418±0.0025**		**0.176±0.0025**		
		R2	1.348	10.84	0.626	58.59	0.552	63.49	0.422	72.08	0.171	88.69	49.77
			1.346		0.624		0.55		0.42		0.169		
2.	**AAWEO**		1.351		0.629		0.555		0.425		0.174		
		Avg	**1.348±0.0025**		**0.626±0.0025**		**0.552±0.0025**		**0.422±0.0025**		**0.465±0.0025**		
		R3	1.342	11.24	0.621	58.92	0.548	63.75	0.416	72.48	0.176	88.35	49.33
			1.345		0.619		0.551		0.414		0.174		
			1.34		0.624		0.546		0.419		0.179		
		Avg	**1.342±0.0025**	**11.10±0.23**	**0.621±0.0025**	**58.74±0.165**	**0.548±0.0025**	**63.66±0.150**	**0.416±0.0025**	**72.30±0.204**	**0.176±0.0025**	**88.46±0.196**	**49.50±0.234**
			1.306	13.69	0.466	69.24	0.391	74.14	0.301	80.09	0.273	81.94	41.23
		R1	1.305		0.465		0.392		0.301		0.274		
			1.306		0.464		0.390		0.302		0.272		
		Avg	**1.305±0.0005**		**0.465±0.001**		**0.391±0.001**		**0.301±0.0005**		**0.273±0.001**		
		R2	1.316	13.02	0.478	68.45	0.394	74.00	0.301	80.09	0.275	81.81	41.94
			1.314		0.477		0.393		0.301		0.276		
3.	**Na_2EDTA**		1.316		0.478		0.393		0.303		0.275		
		Avg	**1.315±0.001**		**0.477±0.0005**		**0.393±0.0005**		**0.301±0.001**		**0.275±0.0005**		
		R3	1.312	13.22	0.479	68.38	0.396	73.87	0.302	80.09	0.275	81.81	41.89
			1.311		0.478		0.395		0.301		0.276		
			1.313		0.479		0.395		0.302		0.274		
		Avg	**1.312±0.001**	**13.31±0.343**	**0.478±0.005**	**68.69±0.477**	**0.395±0.0005**	**74.00±0.135**	**0.301±0.0005**	**80.09±0**	**0.275±0.001**	81.85**±0.075**	**41.68±0.396**

R= Replica, O.D= optical density, %I= percent inhibition AAREO= *Artemisia annua* rainy essential oil, AAWEO= *Artemisia annua* winter essential oil

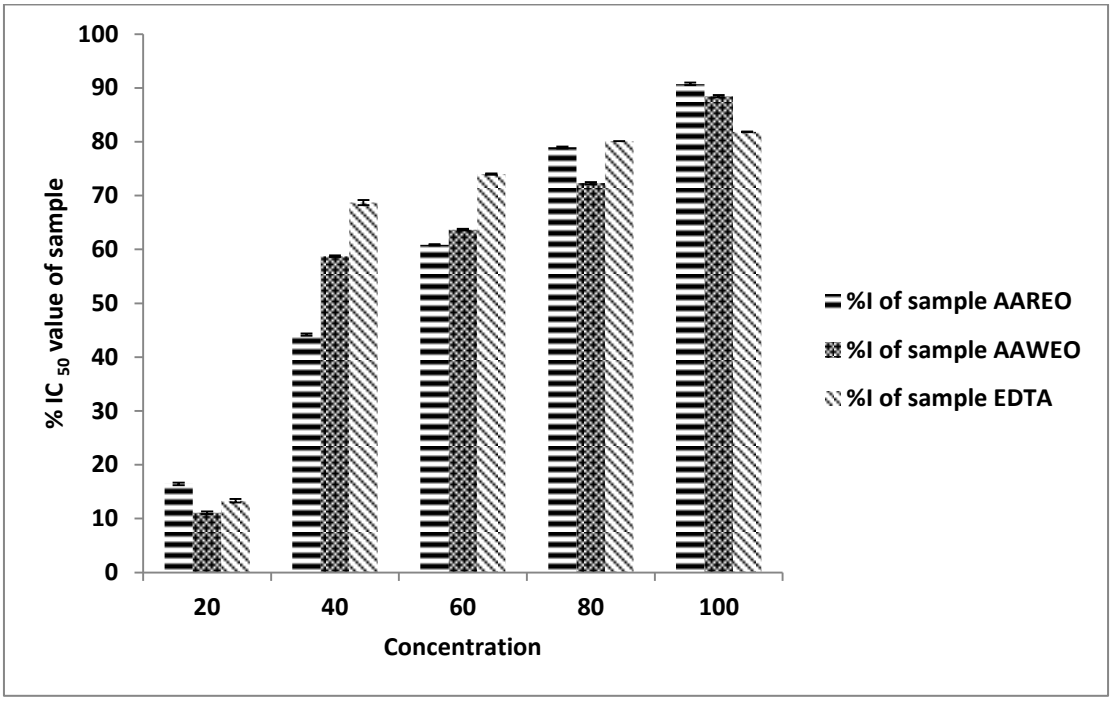


Fig.4.12 Metal chelating activity of AAREO and AAWEO

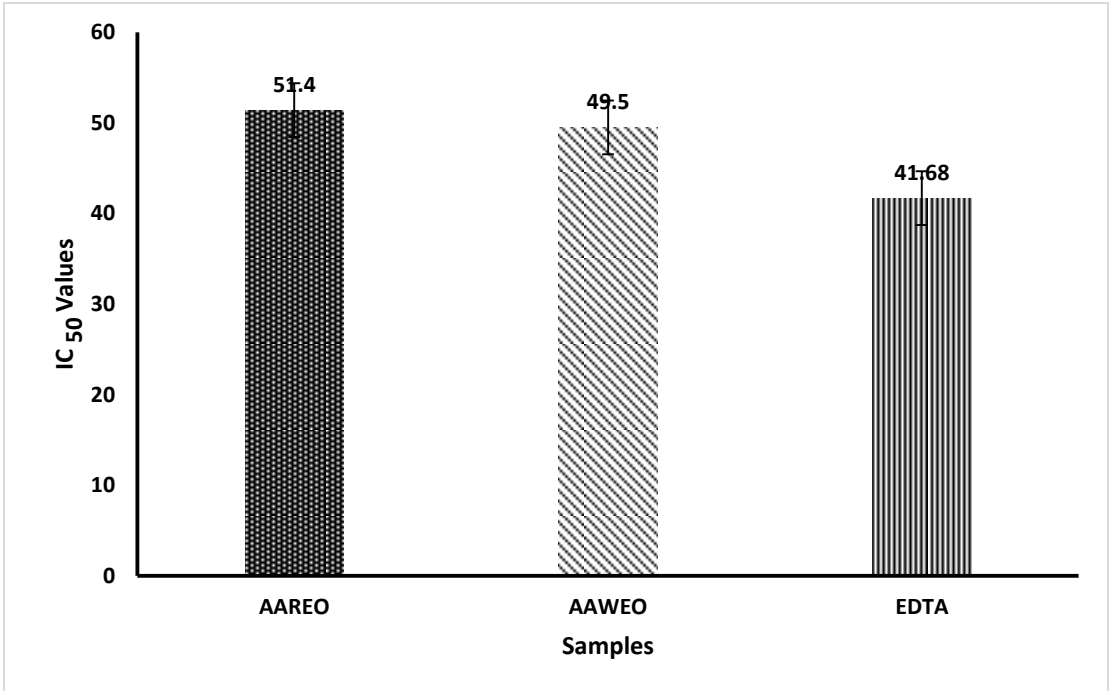


Fig. 4.13 IC_{50} Values of AAREO and AAWEO

4.4.1.3 H_2O_2 radical scavenging activity

Free radical generation has a direct relation with oxidation in foods and biological systems. Biological systems may produce hydrogen peroxide (H_2O_2) . The generation of hydrogen peroxide (H_2O_2) through the activation of phagocytes is known to play an important part in the killing of various bacterial and fungal strains **(Sánchez-Moreno, 2002).**Hydrogen peroxide (H_2O_2) belongs to the reactive oxygen species (ROS), known as oxidants that can react with various cellular targets thereby causing cell damage or even cell death. Therefore it is necessary to remove H_2O_2radicals from the body as it can attack cellular energy producing systems. The antioxidant compound has the ability to scavenge H_2O_2 through the donation of $e^-$and neutralizes it to a water molecule.

$$H_2O_2 + RH^+ + 2e^- \xrightarrow{230nm} 2H_2O$$

The ability of oil to scavenge hydrogen peroxide was determined spectrophotometrically and compared with that of BHT as a standard. The H_2O_2 free radical scavenging activity of AAREO,AAWEO and standard was observed in dose dependent manner (20µg/mL,40 µg/mL,60 µg/mL,80 µg/mL and 100 µg/mL) The order of activity was observed as AAWEO (IC_{50} =34.79±0.419) > AAREO (IC_{50} =44.83±1.304compared to BHT (IC_{50} =27.96±1.195). The detailed % H_2O_2 radical scavenging activity with IC_{50} values at 230nm of AAREO and AAWEO are being presented in **Table 4.9** and **Fig 4.14** and the IC_{50} values of AAREO and AAWEO are being presented in **Fig 4.15.**

Table 4.9 H_2O_2 radical scavenging activity of AAREO and AAWEO

S.N	Sample	R	20 µl/mL		40 µl/mL		60 µl/mL		80 µl/mL		100 µl/mL		
			O.D	%I	O.D	%I	O.D	%I	O.D	%I	O.D	%I	IC_{50}
1.	AAREO	R1	0.429 0.427 0.432	43.10	0.398 0.396 0.401	47.21	0.328 0.326 0.331	47.21	0.262 0.265 0.26	65.25	0.184 0.182 0.187	75.59	46.33
		Avg	**0.429±0.0025**		**0.398±0.005**		**0.398±0.0025**		**0.262±0.025**		**0.184±0.0025**		
		R2	0.448 0.446 0.451	40.15	0.406 0.404 0.409	46.15	0.316 0.314 0.319	58.09	0.261 0.264 0.259	65.38	0.179 0.177 0.182	76.25	44.23
		Avg	**0.448±0.0025**		**0.406±0.0025**		**0.316±0.0025**		**0.261±0.0025**		**0.179±0.0025**		
		R3	0.452 0.450 0.455	40.05	0.406 0.404 0.409	46.15	0.310 0.308 0.313	58.88	0.262 0.265 0.26	65.25	0.188 0.186 0.191	75.06	43.94
		Avg	**0.452±0.0025**	**41.1±1.732**	**0.406±0.0025**	**46.50±0.611**	**0.310±0.0025**	**54.72±6.521**	**0.262±0.0025**	**65.29±0.075**	**0.188±0.0025**	**75.63±0.596**	**44.83±1.304**
2.	AAWEO	R1	0.443 0.441 0.447	41.24	0.324 0.322 0.327	57.02	0.310 0.308 0.313	58.88	0.298 0.296 0.301	60.47	0.252 0.255 0.250	66.57	34.72
		Avg	**0.443±0.003**		**0.324±0.0025**		**0.310±0.0025**		**0.298±0.0025**		**0.252±0.003**		
		R2	0.444 0.442 0.447	41.11	0.323 0.321 0.327	57.16	0.309 0.307 0.312	59.01	0.292 0.290 0.295	61.27	0.253 0.251 0.255	66.31	34.41
		Avg	**0.444±0.0025**		**0.324±0.0025**		**0.309±0.0025**		**0.292±0.0025**		**0.253±0.002**		
		R3	0.445 0.448 0.443	40.98	0.325 0.323 0.328	56.89	0.309 0.307 0.312	59.01	0.298 0.296 0.301	60.44	0.251 0.249 0.254	66.71	35.24
		Avg	**0.443±0.0025**	**41.11±0.130**	**0.324±0.0025**	**57.02±0.135**	**0.309±0.0025**	**58.96±0.075**	**0.298±0.0020**	**60.72±0.470**	**0.251±0.0025**	**66.53±0.202**	**34.79±0.419**
3.	BHT	R1	0.438 0.436 0.441	41.90	0.315 0.318 0.312	58.22	0.272 0.27 0.275	63.92	0.218 0.216 0.221	71.08	0.196 0.194 0.199	74.00	29.31
		Avg	**0.438±0.0025**		**0.315±0.0025**		**0.272±0.0025**		**0.218±0.0025**		**0.196±0.0025**		
		R2	0.432 0.436 0.429	42.70	0.308 0.306 0.311	59.12	0.27 0.268 0.273	64.19	0.212 0.21 0.215	71.88	0.198 0.196 0.201	73.74	27.02
		Avg	**0.432±0.0035**		**0.308±0.0025**		**0.270±0.0025**		**0.212±0.0025**		**0.198±0.0025**		
		R3	0.438 0.436 0.441	41.90	0.315 0.318 0.312	58.22	0.272 0.27 0.275	63.92	0.218 0.216 0.221	71.08	0.196 0.194 0.199	74.00	29.31
		Avg	**0.438±0.0025**	**42.43±0.461**	**0.315±0.0025**	**58.75±0.478**	**0.272±0.0025**	**63.92±0.265**	**0.218±0.0025**	**71.61±0.461**	**0.196±0.0025**	**73.82±0.150**	**27.96±1.195**

R= Replica, O.D= optical density, %I= percent inhibition, AAREO= *Artemisia annua* rainy essential oil, AAWEO= *Artemisia annua* winter essential oil

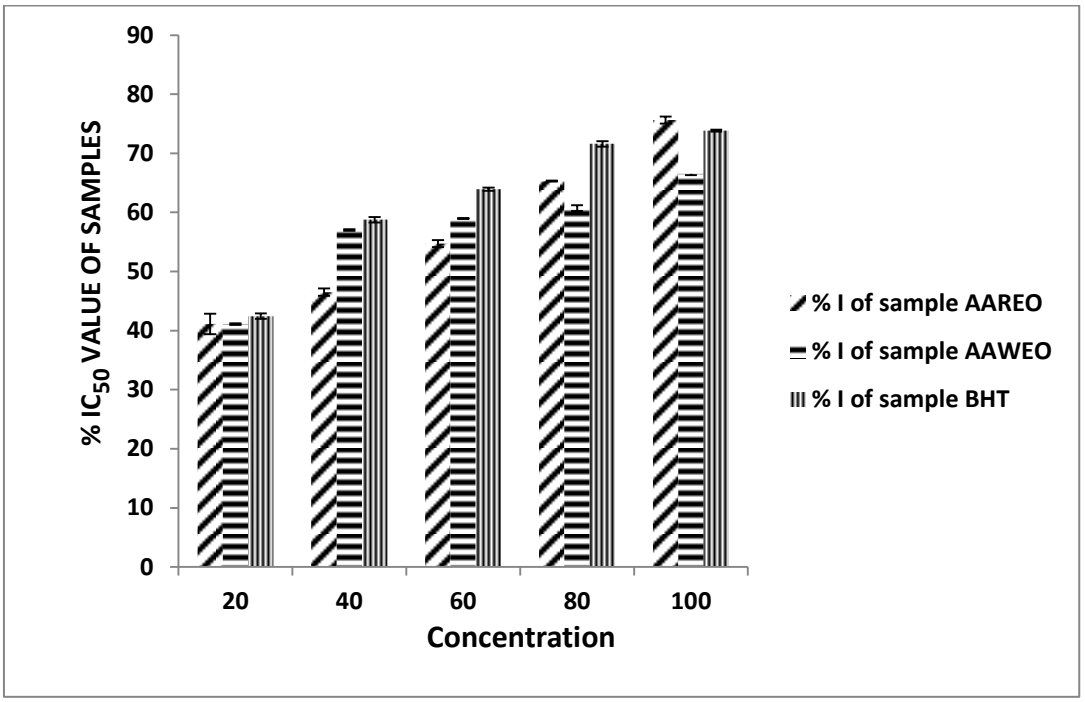


Fig.4.14 H_2O_2 radical scavenging activity of AAREO and AAWEO

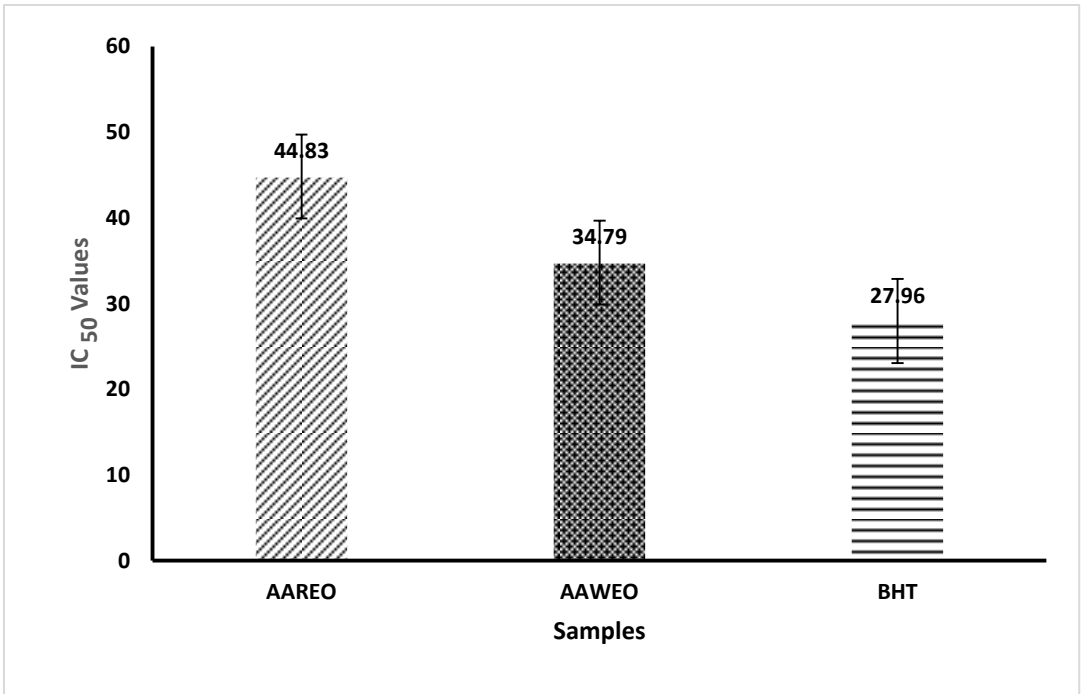


Fig. 4.15 IC_{50} Values of AAREO and AAWEO

Cavar *et al*., (2012) reported the antioxidant activity of the *A. annua* essential oil by five testing methods viz. DPPH, ABTS, reducing power, ORAC, and metal-chelating assays. The oil sample was capable of reducing the stable violet DPPH radical to the yellow DPPH-H, re aching 50% of reduction also the essential oil of *A. annua* interrupted with the process of formation of ferrous and ferrozine complex, concluding that it has chelating activity and captures ferrous ion before ferrozine.

Wang *et al.*, (2008) have analysed the DPPH scavenging activity of 1,8-cineole, α-pinene, β-pinene, camphene. **Da Silva *et al.*, (2014)** have also reported the anti-oxidant activity evaluated by means of DPPH free radical scavenging activity because of the presence of β-selinene, α-selinene, α-humulene, germacrene-D, spathulenol, α-pinene, α-copaene, α- terpineol, *neo*-intermedol.

Keskes *et al*., (2014) reported that due incorporation of terpenes there is high antioxidant activity in alloxan-induced diabetic rats. α-terpinene and p-cymene have been reported to possess antioxidant activity **(Masotti *et al*., 2003).** It has been reported that artemisia ketone exhibited higher anti oxidant activity than camphor and 1,8-cineole in DPPH assay **(Zhigzhitzhapova *et al*., 2020).**

A thorough search of literature revealed that essential oils obtained from plants belonging to the *Artemisia* genus are markedly rich in non-phenolic constituents therefore *A. annua* essential oil is found to be a relatively weak anti oxidant **(Juteau *et al*., 2002; Lopes-Lutz *et al*., 2008).**

However in the present study of AAREO & AAWEO revealed the notable antioxidant activity which might be due to presence of 1,8-cineole, α-pinene, β-pinene, camphene, artemisia ketone, germacrene-D, spathulenol etc as the previous reports support the scientific rational antioxidant activity of *A.annua* essential oil or due to synergic effects of other major, minor or trace constituents of the essential oils.

4.4.1 *In-vitro* anti-inflammatory activity

Inflammation is defined as a complex biological response of vascular tissues against following agents viz pathogens, irritants, or damaged cells. It can be classified as either acute or chronic, and involves a surge of biochemical processes consisting of

the local vascular system, the immune system, and different cell types found in the injured tissue (**Andrade and De Sousa, 2013)**. In an inflammatory infection, macrophages and mast cells release inflammatory mediators, such as cytokines (e.g., interleukin-1β (IL-1β), IL-6, IL-12, and the chemokine IL-8), tumor necrosis factors (e.g., TNF-α and TNF-β) or interferons (e.g., IFN-γ) **(Andrade *et al.*, 2014).** It has been inferred from the reported that medicinal plants and their isolated compounds are employed worldwide in folk herbal medicine for the treatment of different inflammatory conditions, such as lung and skin inflammations. In the continuous search for natural products against inflammation, essential oils are increasingly being regarded to as a rich source of such products. This activity was based on the inhibition percent of denaturation of the albumin proteins, as a measure of *in vitro* anti-inflammatory activity. In presence of saline salt phosphate buffer solution (at physiological pH), the denaturation reaction of albumin protein (Hen's egg) takes place. The inhibition of denaturation of albumin protein is because of the presence of compounds having anti-inflammatory property, which is measured spectrophotometrically by change in absorbance at 660nm (**Kar *et al.*, 2012**).

In present study, the *in vitro* anti-inflammatory activity of AAREO and AAWEO was investigated. Both the essential oils exhibited dose-dependent anti-inflammatory activity. The order in which AAREO and AAWEO possessed anti-inflammatory activity was observed as AAWEO (IC_{50}=66.90±0.040) > AAREO (IC_{50}=76.55±0.218) compared to the standard anti-inflammatory drug diclofenac sodium tablets (IC_{50}=63.41±0.037). AAREO was found to exhibit maximum activity with minimum IC_{50} value followed by AAWEO with higher IC_{50} value. The detailed presentation of in- vitro anti-inflammatory activity observed spectrophotometrically in terms of OD (optical density), percent inhibition and IC_{50}valuesof AAREO and AAWEO are being presented in **Table 4.10** and **Fig.4.16** while the IC_{50} values of AAREO and AAWEO are being shown in **Fig 4.17.**

Camphor has been reported to possess a potent anti-inflammatory activity **(Sharma, 2021).**

Essler *et al.*, (2013) reported that eucalyptol (1,8-cineole), camphor, β-caryophyllene exhibit an effective *in vitro* and *in vivo* anti inflammatory activity.

Table 4.10 *In vitro* anti-inflammatory activity of AAREO and AAWEO

S.N	Sample	R	20 µl/mL		40 µl/mL		60 µl/mL		80 µl/mL		100 µl/mL		
			O.D	%I	O.D	%I	O.D	%I	O.D	%I	O.D	%I	IC50
1.	AAREO	R1	1.276		1.151		0.934		0.892		0.464		
			1.274		1.149		0.932		0.889		0.462		
			1.279	19.03	1.154	26.96	0.937	40.73	0.895	43.40	0.46	70.55	76.51
		Avg	**1.276±0.0025**		**1.151±0.0025**		**0.934±0.0025**		**0.892±0.003**		**0.464±0.0025**		
		R2	1.278		1.146		0.942		0.896		0.465		
			1.281		1.144		0.94		0.899		0.463	70.49	76.79
			1.276	18.9	1.149	27.28	0.945	40.22	0.894	43.14	0.468		
		Avg	**1.278±0.0025**		**1.146±0.0025**		**0.942±0.0025**		**0.896±0.0025**		**0.465±0.0025**		
		R3	1.276		1.142		0.934		0.891		0.464		
			1.274	19.03	1.14	27.53	0.932	40.73	0.889	43.46	0.462	70.55	76.36
			1.279		1.145		0.937		0.894		0.467		
		Avg	**1.276±0.0025**	**18.98±0.0750**	**1.142±0.0025**	**27.25±0.285**	**0.934±0.0025**	**40.56±0.294**	**0.891±0.0025**	**43.33±0.170**	**0.464±0.0025**	**70.53±0.034**	**76.55±0.218**
2.	AAWEO	R1	1.307		1.198		0.885		0.594		0.382		
			1.305		1.196		0.888		0.592		0.38		
			1.31	17.06	1.201	23.98	0.882	43.84	0.597	62.3	0.385	75.76	66.95
		Avg	**1.307±0.0025**		**1.198±0.0025**		**0.885±0.03**		**0.594±0.0025**		**0.382±0.0025**		
		R2	1.306		1.195		0.883		0.585		0.391		
			1.304		1.193		0.881		0.583		0.389	75.19	
			1.309	17.13	1.198	24.17	0.886	43.97	0.588	62.88	0.394		66.88
		Avg	**1.306±0.0025**		**1.195±0.0025**		**0.883±0.0025**		**0.585±0.0025**		**0.391±0.0025**		
		R3	1.3		1.198		0.885		0.591		0.385	75.57	
			1.298	17.51	1.196	23.98	0.888	43.84	0.589	62.5	0.383		66.88
			1.303		1.201		0.882		0.594		0.388		
		Avg	**1.300±0.0025**	**17.23±0.242**	**1.198±0.0025**	**24.04±0.109**	**0.885±0.03**	**43.88±0.075**	**0.591±0.0025**	**62.56±0.294**	**0.385±0.0025**	**75.50±0.290**	**66.90±0.040**
3.	Diclofenac	R1	1.423		1.085		0.767		0.556		0.338		
			1.421		1.083		0.765		0.554		0.336		
			1.425	9.70	1.088	31.15	0.77	51.33	0.559	64.72	0.341	78.55	63.39
		Avg	**1.423±0.0025**		**1.085±0.0025**		**0.767±0.0025**		**0.556±0.0025**		**0.338±0.0025**		
		R2	1.422		1.090		0.761		0.554		0.342		
			1.420		1.093		0.759		0.552		0.345		
			1.425	9.77	1.088	30.83	0.764	51.71	0.557	64.84	0.34	78.29	63.40
		Avg	**1.422±0.0025**		**1.090±0.0025**		**0.767±0.0025**		**0.554±0.0025**		**0.342±0.0025**		
		R3	1.421		1.081		0.767		0.554		0.348		
			1.419	9.83	1.084	31.40	0.765	51.33	0.552	64.84	0.346	77.91	63.46
			1.424		1.079		0.77		0.557		0.351		
		Avg	**1.421±0.0025**	**9.76±0.065**	**1.081±0.0025**	**31.12±0.285**	**0.767±0.0025**	**51.45±0.219**	**0.554±0.0025**	**64.80±0.069**	**0.348±0.0025**	**78.25±0.321**	**63.41±0.037**

R= Replica, O.D= optical density, %I= percent inhibition AAREO= *Artemisia annua* rainy essential oil, AAWEO= *Artemisia annua* winter essential oil

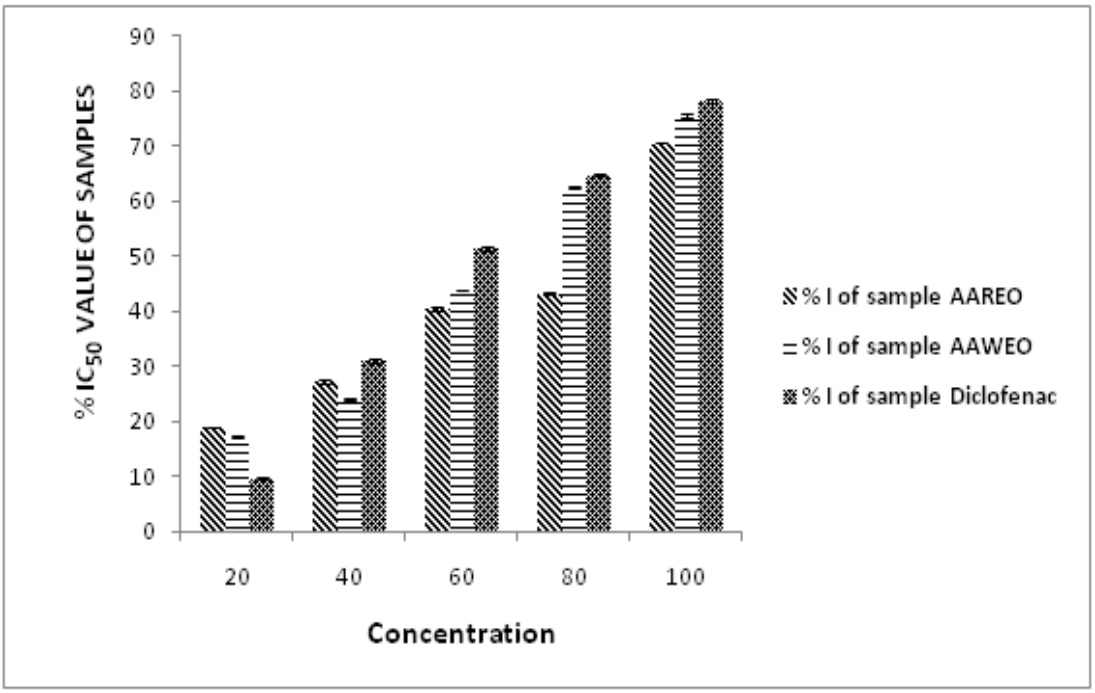


Fig.4.16*In- vitro* anti-inflammatory **activity of AAREO and AAWEO**

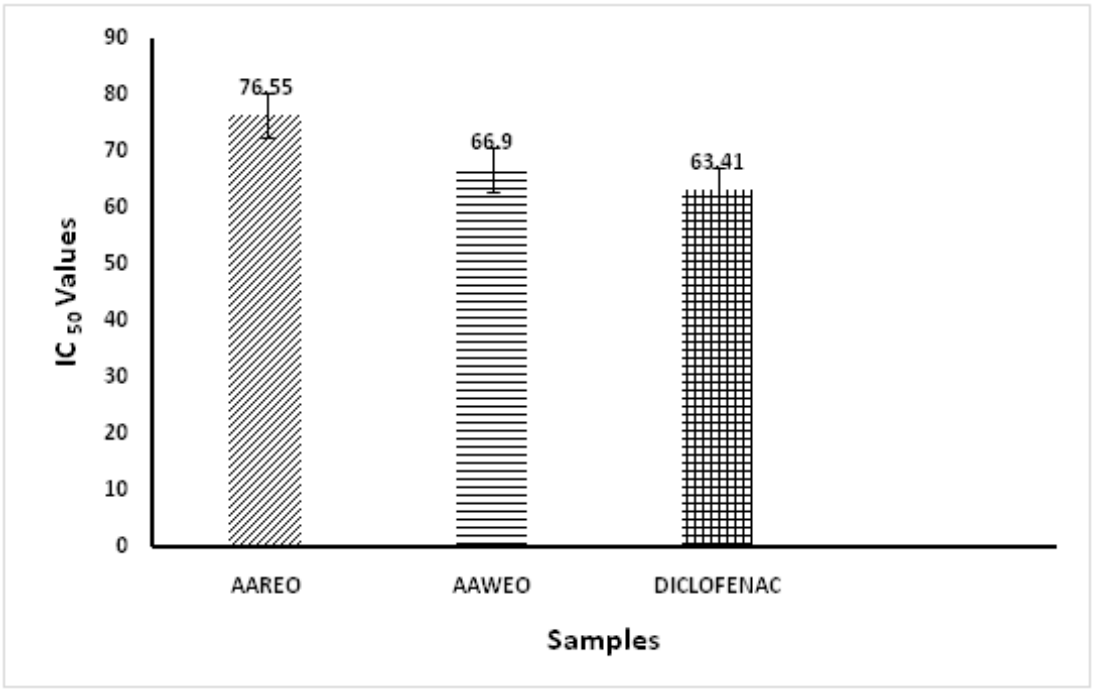


Fig. 4.17 IC_{50} Values of AAREO and AAWEO

The major components of *Artemisia annua* like artemisinin and scopoletin, have been reported to possess potent anti-inflammatory effects **(Verma *et al*., 2020).**The aqueous methanolic extract of *Artemisia annua* has been reported to possess anti-inflammatory activity when studied using carrageenan and egg albumin induced rat paw edema in acute, and cotton pellets and grass pith induced chronic inflammation models **(Das, 2012).**

The terpenoids *viz.,* 1,8-cineole, spathulenol, α-cadinol, γ-cadinene, α-pinene, terpinen-4-ol, borneol, β-pinene, sabinene, camphene etc have been reported to exhibit anti-inflammatory activity (**Martins *et al.*, 2008; E Sa *et al.*, 2013; Ludwiczuk *et al.*,2017**).

Compunds like borneol α - β-pinenes, have been previously reported to possess anti-inflammatory properties (**Asanova *et al*., 2003). Rungqu *et al*., (2016)** reported that Artemisia ketone and δ-cadinene could be associated with the anti-inflammatory effect and to reduce pain and inflammation.

β-caryophyllene, a bicyclic sesquiterpene has been reported to exhibit anti-inflammatory action through the inhibition of the main inflammatory mediators, like inducible nitric oxide synthase (iNOS), Interleukin 1 beta (IL-1β), Interleukin-6 (IL-6), tumor necrosis factor-alfa (TNF-α), nuclear factor kapp a-light-chain-enhancer of activated B cells (NF-κB), cyclooxygenase 1 (COX-1), cyclooxygenase 2 (COX-2) **(Francomano *et al*., 2019).**

Most of the compounds reported above like camphor, 1,8 cineole, camphor, Artemisia ketone, α – cadinol, ϒ-cadinene, β-caryophyllene, caryophyllene oxide, α-pinene, terpinen-4-ol, borneol, β-pinene and sabinene etc have been identified both in AAREO & AAWEO with different quantity hence the results are in support that the *in-vitro* anti-inflammatory activity of AAREO & AAWEO might be possibly due to these constituents or synergistic effects of other major/minor constituents present in he essential oils. Based on the observed data it can be inferred that the herb, *Artemisia annua* can be a good source for the herbal drug development program against inflammation after proper clinical trials.

4.4.2 *In vitro* Anti diabetic activity

Diabetes mellitus or type-2 diabetes, is known to be one of the most common endocrine metabolic disorders has caused significant morbidity and mortality due to

microvascular (retinopathy, neuropathy, and nephropathy) and macrovascular (heart attack, stroke and peripheral vascular disease) complications .Human bodies under go enzymatic and non-enzymatic antioxidative mechanisms which reduces the formation of reactive oxygen species, responsible for many degenerative diseases including diabetes **(Patel *et al.*, 2011).** This disease is rapidly increasing worldwide and affecting all parts of the world. deficiency of the insulin leads to increase in blood sugar levels **(Ponnusamy *et al.*, 2011).** Some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically in antidiabetic and anti-hyperlipidemic remedies. It has been reported that, plant showing hypoglycemic potential mainly belongs to the family Asteraceae, Leguminoseae, Lamiaceae, Liliaceae, Cucurbitaceae, Moraceae, Rosaceae and Araliaceae. Antihyperglycemic activity of the plants is basically due to their capabaility to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes **(Malviya *et al.*, 2010).** The cause of diabetes might be because of the increase in oxidative stress which results in irregular production of free radicals. This irregular formation of free radicals and sudden degradation in the mechanism of anti-oxidant defence in the body leads to the destruction of enzymes and different cell organelles, increase in lipid per oxidation and irregular production of insulin **(Maritim *et al.*, 2003)** and thus; there is a need of herbal and medicinal anti-diabetic agent to inhibit the action of oxidative stress.

Thus; there is need of herbal and medicinal anti-diabetic agent to inhibit the action of oxidative stress. In present study, the *in vitro* anti-diabetic activity of AAREO and AAWEO has been investigated. The observation shows that both the oils possess anti-diabetic activity in a dose dependent manner. As the concentration of essential oils increases, %inhibition also increases with increase in absorbance. The order of anti-diabetic activity of AAREO and AAWEO have been observed in the order of AAWEO (47.45±0.195) > AAREO (74.26±3.706) compared to the standard acarbose (38.21±0.03) while the detailed results of anti-diabetic activity observed spectrophotometrically in terms of OD (optical density), percent inhibition and IC_{50}valuesof AAREO and AAWEO are being presented **Table 4.11** and **Fig 4.18 and Fig. 4.19.**

Table 4.11 *In vitro* anti-diabetic activity of AAREO and AAWEO

S.N.	Sample	R	20 µl/mL		40 µl/mL		60 µl/mL		80 µl/mL		100 µl/mL		IC50
			O.D	%I	O.D	%I	O.D	%I	O.D	%I	O.D	%I	
1.	AAREO	R1	1.198 1.196 1.201	17.54	0.942 0.94 0.945	35.16	0.896 0.894 0.899	38.33	0.718 0.716 0.721	50.58	0.418 0.416 0.421	71.23	78.54
		Avg	**1.198±0.0025**		**0.942±0.0025**		**0.896±0.0025**		**0.718±0.0025**		**0.418±0.0025**		
		R2	1.192 1.19 1.195	17.96	0.939 0.937 0.942	35.37	0.891 0.889 0.894	38.67	0.721 0.719 0.724	50.37	0.422 0.425 0.42	70.95	72.12
		Avg	**1.192±0.0025**		**0.939±0.0025**		**0.891±0.0025**		**0.721±0.0025**		**0.422±0.0025**		
		R3	1.194 1.192 1.197	17.82	0.942 0.94 0.945	35.16	0.892 0.89 0.895	38.60	0.718 0.716 0.721	50.58	0.422 0.425 0.42	70.95	72.12
		Avg	**1.192±0.0025**	**17.77±0.213**	**0.942±0.0025**	**35.23±0.121**	**0.892±0.0025**	**38.53±0.179**	**0.718±0.0025**	**50.51±0.121**	**0.422±0.0025**	**71.04±0.161**	**74.26±3.706**
2.	AAWEO	**R1**	0.917 0.915 0.921	36.88	0.722 0.725 0.72	50.3	0.659 0.657 0.662	52.37	0.542 0.54 0.545	62.69	0.344 0.342 0.347	76.32	47.47
		Avg	**0.917±0.003**		**0.722±0.0025**		**0.659±0.0025**		**0.542±0.0025**		**0.344±0.0025**		
		R2	0.922 0.92 0.925	36.54	0.726 0.724 0.729	50.03	0.661 0.659 0.664	54.5	0.545 0.548 0.543	62.49	0.345 0.343 0.348	76.25	47.64
		Avg	**0.922±0.0025**		**0.726±0.0025**		**0.661±0.0025**		**0.545±0.0025**		**0.345±0.0025**		
		R3	0.921 0.919 0.924	36.61	0.722 0.725 0.72	50.3	0.661 0.659 0.664	54.5	0.562 0.56 0.565	61.32	0.353 0.351 0.356	75.70	47.25
		Avg	**0.921±0.0025**	**36.67±0.179**	**0.722±0.0025**	**50.21±0.155**	**0.661±0.0025**	**53.79±1.229**	**0.562±0.0025**	**62.16±0.740**	**0.353±0.0025**	**76.09±0.339**	**47.45±0.195**
3.	acarbose	R1	0.976 0.975 0.977	32.82	0.607 0.607 0.606	58.27	0.512 0.513 0.512	64.76	0.345 0.346 0.344	76.25	0.121 0.123 0.121	91.67	38.42
		Avg	**0.976±0.001**		**0.606±0.0005**		**0.512±0.0005**		**0.345±0.001**		**0.121±0.001**		
		R2	0.975 0.975 0.977	32.89	0.606 0.607 0.607	58.29	0.513 0.514 0.513	64.69	0.346 0.345 0.345	76.25	0.122 0.123 0.122	91.60	38.21
		Avg	**0.975±0.001**		**0.606±0.0005**		**0.513±0.0005**		**0.345±0.0005**		**0.122±0.0005**		
		R3	0.976 0.975 0.974	32.89	0.606 0.605 0.607	58.29	0.512 0.511 0.512	64.83	0.346 0.347 0.347	76.18	0.121 0.122 0.122	91.67	38.18
		Avg	**0.975±0.001**	**32.86±0.040**	**0.606±0.001**	**58.29±0**	**0.511±0.0005**	**64.76±0.07**	**0.346±0.0005**	**76.22±0.040**	**0.121±0.0005**	**91.64±0.040**	**38.21±0.03**

R= Replica, O.D= optical density, %I= percent inhibition AAREO= *Artemisia annua* rainy essential oil, AAWEO= *Artemisia annua* winter essential oil

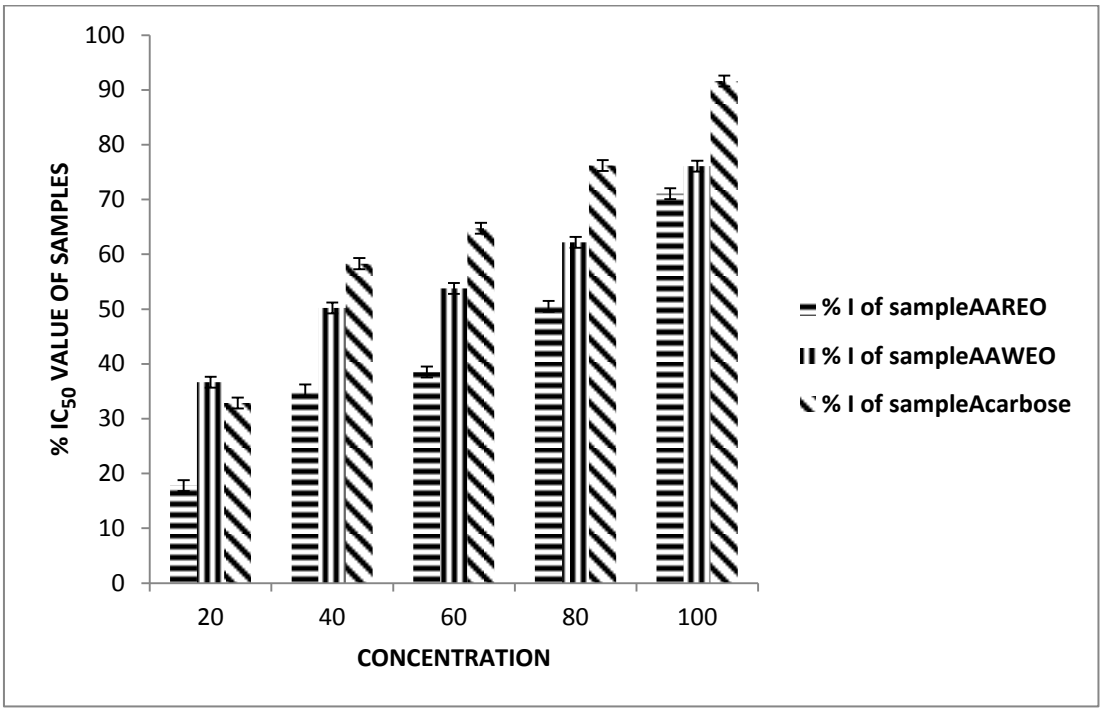


Fig.4.18 *In- vitro* anti-diabetic activity of AAREO and AAWEO

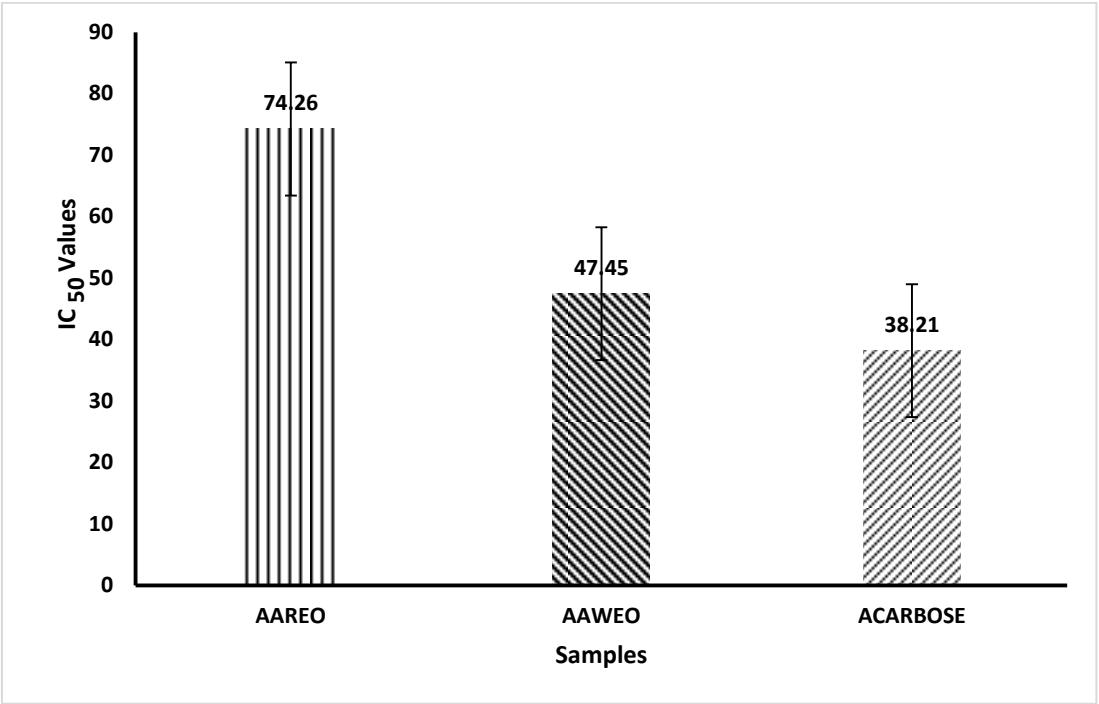


Fig. 4.19 IC_{50} Values of AAREO and AAWEO

It has been documented in the literature that the diabetic rats when treated with aqueous Extract of *A. annua*, exhibited significant anti-hyperglycemic activity in diabetic animals **(Verma *et al*., 2020).**

Camphor has been reported to exhibit a significant antidiabetic activity **(Sharma, 2021).**

The terpenoids *viz.*, α-pinene, caryophyllene oxide, β-pinene, 1,8-cineole, terpinen-4-ol, α-humulene, β-selinene, β-caryophyllene oxide, borneol and γ-cadinene have been reported to possess anti-diabetic activity (**Tahir *et al.*, 2016; Bouyahya *et al.*, 2020**).

Literature search revealed that *Artemsia annua* extracts play a crucial role in inhibiting hyperglycemia and improving metabolic abnormalities induced by diabetes through its antioxidant advantage. It has also been reported in the studies that essential oils components such as (linalool, carvacrol, eugenol,, sabinene hydrate, α-terpineol, camphor, germacrene-D, artemisia ketone and 1,8-cineole) have a modulatory effect on the values of HOMA_IR, which may be attributed to enhanced peripheral uptake of glucose and clarify its hypoglycemic effect in addition to the increase in serum insulin level **(Helal *et al*., 2014).**

It has been reported that germacrene-D and eucalyptol (1,8 cineole) had a higher α-glucosidase inhibition activity suggesting that these compounds are responsible for a significant anti-diabetic effect in EO of *Artemisia gmelinii* weber ex Stechm **(Xu *et al*., 2021).**

Keskes *et al*., (2014) reported that the terpene such as α-pinene which exist in *Juniperus phoenicea* L. essential oil might inhibited key enzymes related to Type 2 diabetes principally α-amylase. It was reported that due administration of terpenes to diabetic exhibits blood glucose lowering effect in alloxan-induced diabetic rats.

On the basis of the above mentioned literature, it can be concluded that the anti-diabetic activity of AAREO and AAWEO might be possibly due to the presence of eucalyptol (1,8-cineole), germacrene-D, camphor, Artemisia ketone, terpinen-4-ol,isoborneol α-humulene, β-selinene, β-caryophyllene oxide, etc or synergetic effects of other major, minor or trace constituents of essential oils.

4.4.3 Anti-feedant activity

Human beings suffer extensively due to havoc created by insect populations in agriculture as well as in health. In agriculture, an insect directly affect the crop and causes severe damage to the growing crop which results in revenue loss. Crop loss due to insect pests has been estimated between 10-30% percent for major crops **(Arivoli and Tennyson, 2013).**

To control the increasing population of pests in the crop fields, due to which the use of synthetic insecticides is drastically increasing to protect the crops from getting damaged by pests (**Montenergo *et al.*, 2018**). Excessive utilization of synthetic insecticides has resulted in to various problems such as the resurgence of primary pests, secondary pest outbreaks, resistance development, insecticide residue, health hazards, environmental contamination, and increased costs of insect control **(Roy and Mukhopadhyay, 2010).**

Plants' bioactive molecules are being used now a days for controlling pests. As plants produce phytochemicals in the environment, including secondary metabolites that are used by the plant in defense against phytophagous insects. These secondary metabolites are basically alkaloids, polyphenols, terpenoids, polyacetylenes, flavonoids, unusual amino acids, sugars, phenylpropanoids, quinines, essential oils, etc., which usually have a wide variety of insecticidal properties, including, repellent, antifeedant, and insect growth inhibitory activities **(Ahmad, 2007; Dhaliwal and Koul 2011).**

Amongst the different group of agricultural pest, *Spodoptera litura* (Fab). (Lepidoptera: Noctuidae) is known as the one of the major polyphagous pest attacking economically important crops thereby causing major damage. Various economically important crops such as cotton, groundnut, chilly, tobacco, castor and pulses are being damaged on a large scale by *Spodoptera litura*. commanly known as tobacco bud worm **(Ayyangar and Rao, 1989).** In context to these facts in the present study, the anti-feedant activity was evaluated based on no-choice experiment **(Zandi-Sohani *et al.*, 2012).**It was observed that both AAREO and AAWEO possessed significant anti-feedant activity in a dose dependent manner as a function of concentration. At 8µl/mL

concentration both AAREO and AAWEO showed 90.85% and 87.92% anti-feedant activity respectively. The detailed results of AAREO and AAWEO exhibiting the anti-feedant activity are being presented in **Table 4.12 & Table 4.13** respectively and **fig 4.20 & fig 4.21** while the comparison of antifeedant activity between AAREO & AAWEO is being presented in **fig 4.22.** The photographs of the present experimental data layout are being shown in **fig 4.23 & fig 4.24.**

Tripathi *et al.*, (2001) reported that the compound 1,8-cineole drastically reduced the hatching of *T. castaneum* eggs and the successive survival rate of the larvae. Adult emergence was also reported to be hampered by 1,8-cineole.

It has been inferred from the earlier study that the methanolic extract *A. annua* critically affects feeding pattern in larva by acting as an antifeedant **(Khosravi *et al.*, 2010).**

The essential oil of *Artemisia annua* L., has been studied for its toxicity and physiological aspects on 4th instar larva of the cotton bollworm *Helicoverpa armigera*. It has been suggested that essential oil of *A. annua* can be used as an active agent in controlling important pest of field crops **(Mojarab-Mahboubkar *et al.*, 2015).**

The compounds like α-pinene, *β*-pinene, camphene, sabinene, 1,8-cineole, α-copaene, germacrene-D, borneol, α-selinene, and, γ-cadinene, have been reported to exhibit an effective larvicidal and anti-feedant activity **(Mondal *et al.*, 2009; Espinoza *et al.*, 2018).**

α-pinene, β-caryophyllene, has been reported to possess insect repellent activity against *S. oblique* **(Kumar *et al.*, 2019).** α-pinene, camphene, sabinene, D-camphor and eucalyptol have been reported to exhibit antifeedant and insecticidal activity against the 4th larval instar of *S. littoralis* **(Ali and Ibrahim, 2018).**

on the basis of afore mentioned facts it can be concluded that the anti-feedant activity of AAREO and AAWEO might be possibly due to the presence of major constituents like camphor, 1,8 cineole, germacrene-D, β-caryophyllene or due to the synergic effect of other major or minor/trace components of essential oils which show both qualitative and quantitative variations in their make-up.

Table 4.12 Antifeedant activity of AAREO

Conc. µL/mL	24 hrs	48 hrs	MLAC	Feeding %	Antifeedant activity	Feeding inhibition	Preference index	Antifeedant category
2µl/mL	1.61	2.19	1.9±0.410	11.875	47.36842	31.03448	0.689655	Slightly antifeedant
4µl/mL	0.59	0.96	0.77±0.261	4.8125	78.67036	64.84018	0.351598	Moderately antifeedant
8µl/mL	0.2	0.46	0.33±0.183	2.0625	90.85873	83.24873	0.167513	Extremely antifeedant
Control	2.02	5.21	3.61±2.255	22.5625	0	0	1	
CD1	0.67		**CD2**	0.96		**CD3**	1.35	
CV	47.10							

CD1=critical difference with respect to treatment CD2= critical difference with respect to concentration, CD3= critical difference with respect to interaction between concentration and treatment, CV= Cofficient of variation AAREO= *Artemisia annua* rainy essential oil MLAC= mean leaf square area

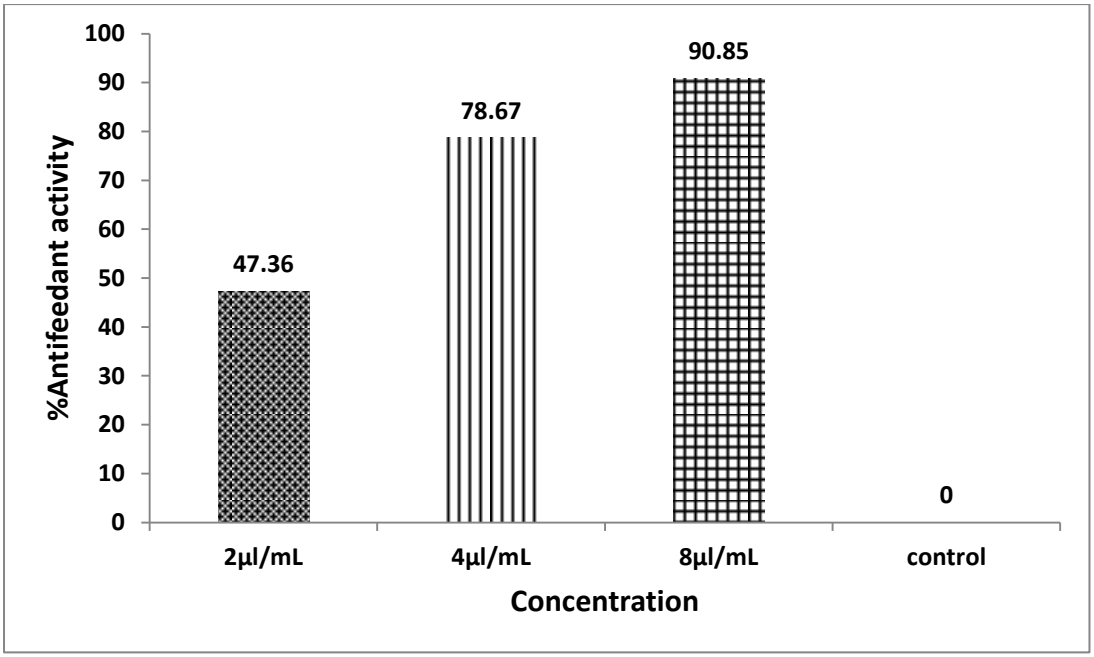


Fig. 4.20 Antifeedant activity of AAREO

Table. 4.13 Antifeedant activity of AAWEO

Conc. µl/mL	24 hrs	48 hrs	MLAC	Feeding %	Antifeedant activity	Feeding inhibition	Preference index	Antifeedant category
2µl/mL	3.20	1.67	2.435±1.081	15.21875	31.60112	18.76564	0.812344	Slightly antifeedant
4µl/mL	1.63	1.24	1.435±0.275	8.96875	59.69101	42.54254	0.574575	Moderately antifeedant
8µl/mL	0.24	0.62	0.43±0.268	2.6875	87.92135	78.44612	0.215539	Extremely antifeedant
Control	3.84	3.28	3.56±0.395	22.25	0	0	1	
CD1	0.65		**CD2**	0.92		**CD3**	1.30	
CV	37.87							

CD1=critical difference with respect to treatment, CD2= critical difference with respect to concentration, CD3= critical difference with respect to interaction between concentration and treatment, CV= Cofficient of variation AAWEO= *Artemisia annua* winter essential oil MLAC= mean leaf square area

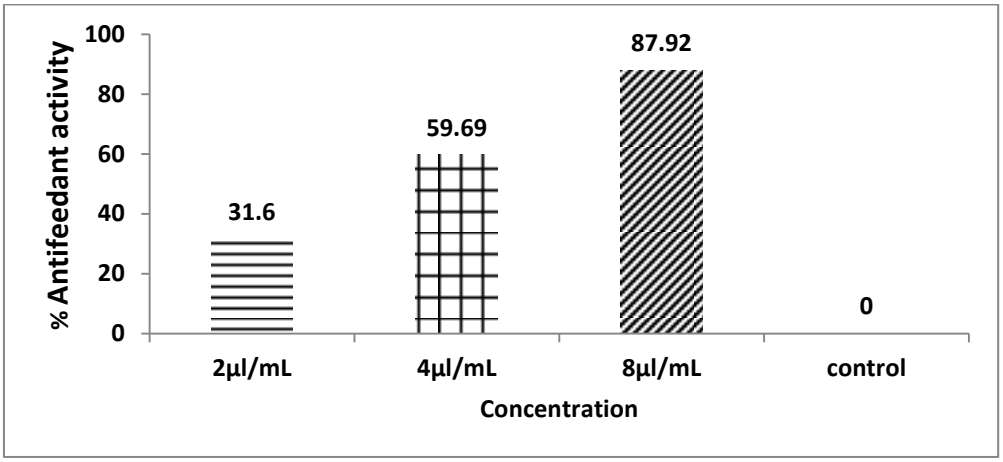


Fig. 4.21 Antifeedant activity of AAWEO

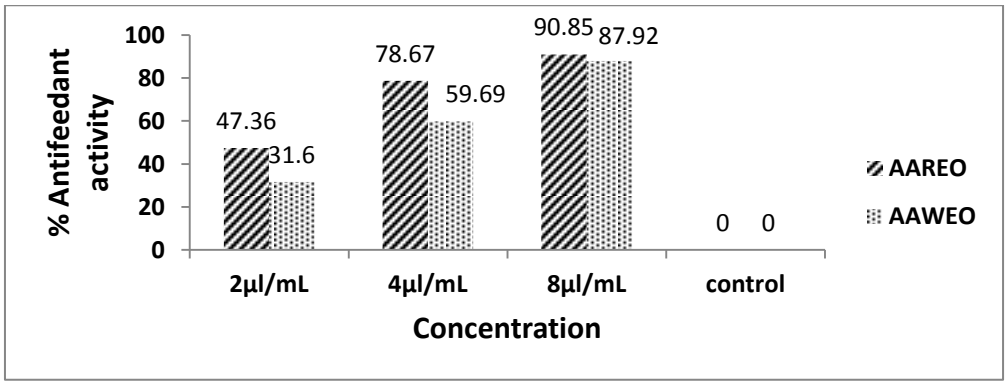


Fig. 4.22 Comparison between antifeedant activity of AAREO & AAWEO

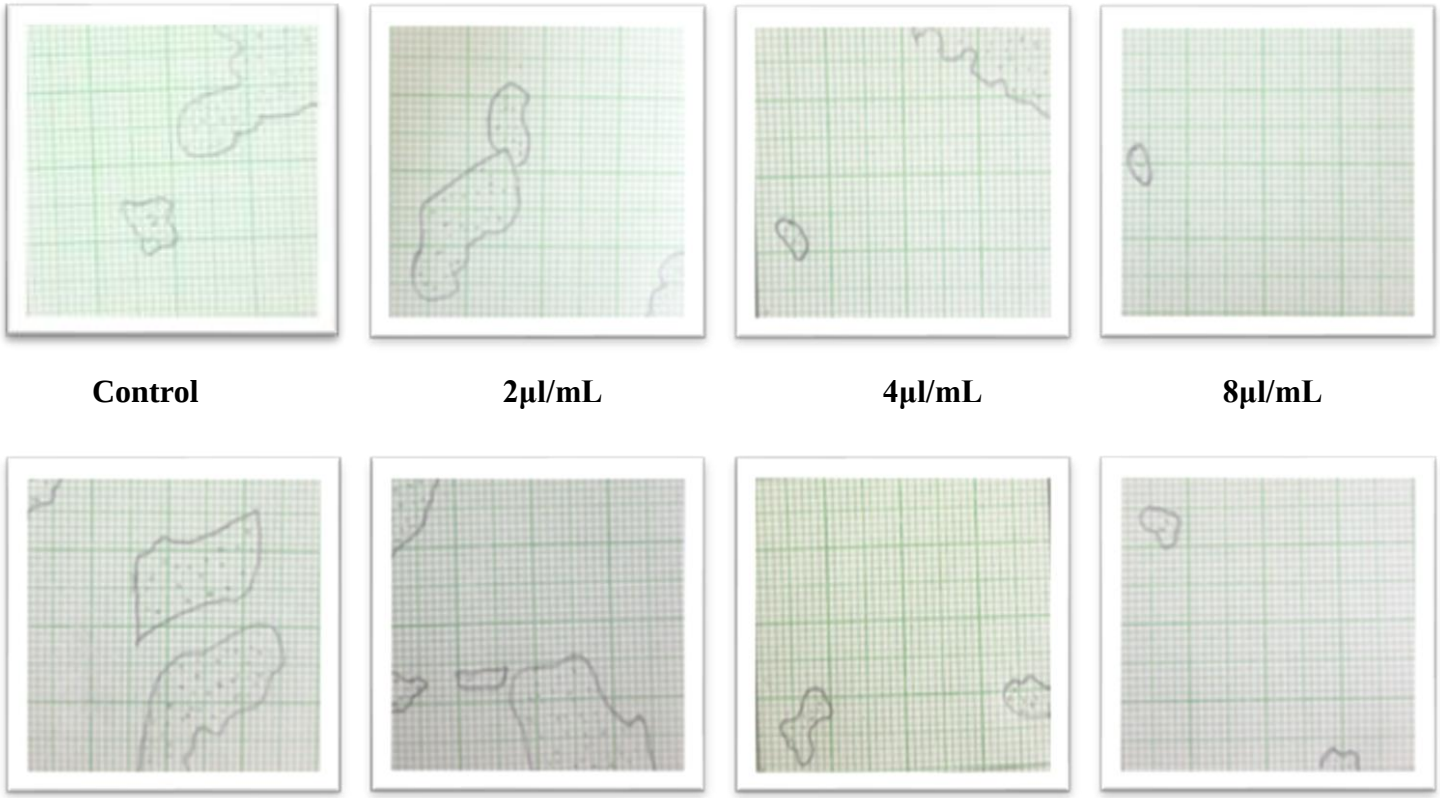
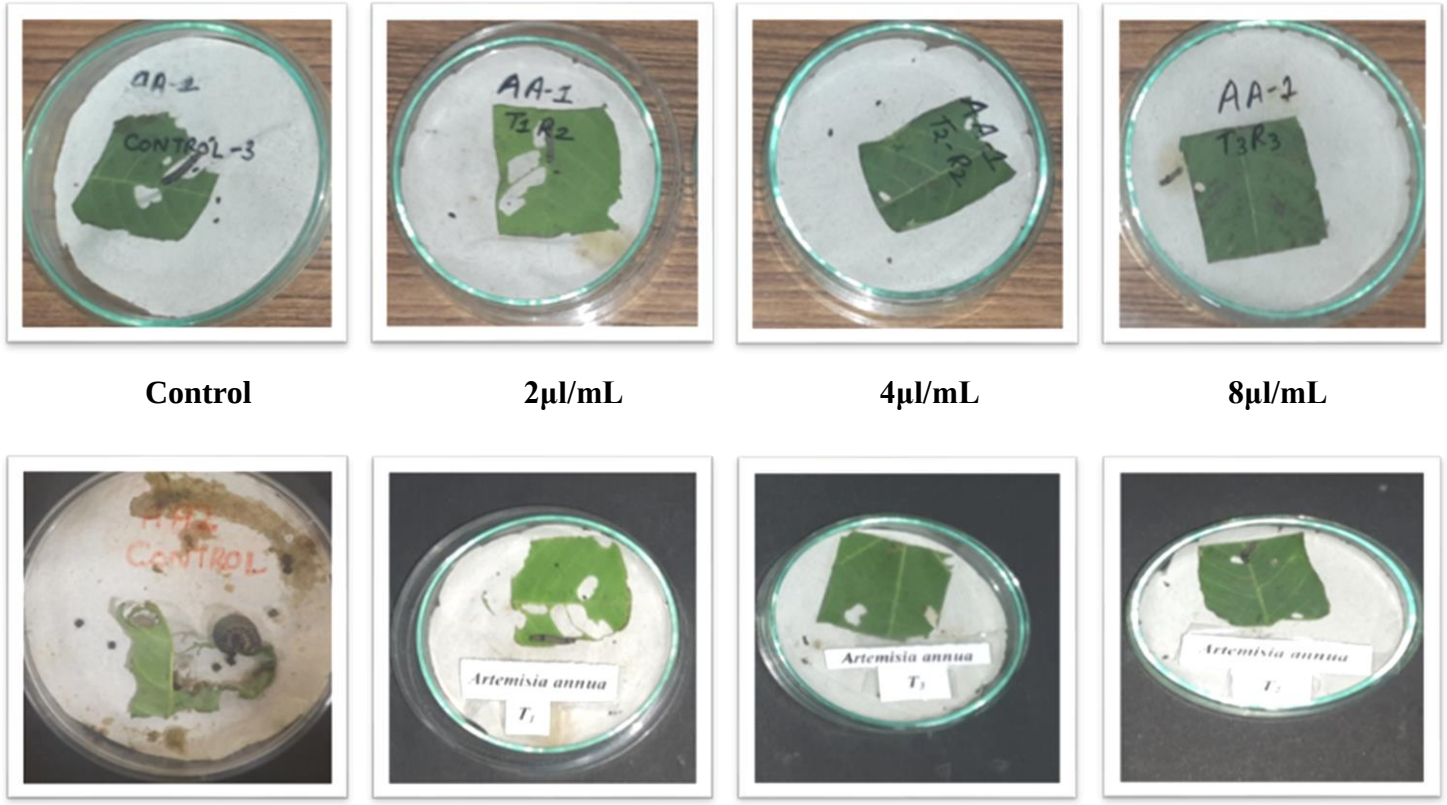


Fig 4.23 Graph paper method analysis of anti-feedantactivity of AAREO & AAWEO against *S. litura*.



Control **2µl/mL** **4µl/mL** **8µl/mL**

Control **2µl/mL** **4µl/mL** **8µl/mL**

Fig.4.24 Experimental layout of antifeedant activity in (a) AAREO & (b) AAWEO against *Spodoptera litura*

4.4.4 NEMATICIDAL ACTIVITY

Andrés *et* a*l*., (2012) reported that plant parasitic nematodes are the known to be one of the most destructive group of plant pathogens all over the world and their control is highly challenging. Plant parasitic nematodes mainly attack theirs host by means of various strategies. They can be ectoparasites, which feed on the plant's outer tissues, or endoparasites, which feed and live within the plant's tissues. Endoparasites include a number of important plant parasitic nematodes. *Meloidogyne* spp., root-knot nematodes, are one of the most economically damaging genera of plant parasitic nematodes on horticultural and field crops. Root-knot nematodes. *Meloidogyne* sp., attacking over 2,000 different plant species are widely distributed. Root-knot nematodes spend a portion of their lives in the soil as eggs or as second-stage larvae. The latter enter the roots and develop feeding sites in susceptible hosts, causing roots to expand and take on a distinctive "knotty" appearance. Root galling limits water and nutrient uptake, resulting in serious symptoms such as malnutrition, chlorosis, and stunting, leading to significant quantitative and qualitative losses in crop plants. In tropical and subtropical environments, four species, *M. incognita*, *M. javanica*, *M. arenaria*, and *M. hapla*, have been reported to be responsible for nearly 90% of the agronomical damages (**Echeverrigaray *et al*., 2010).** Phytochemicals present in essential oils have been reported to possess great potential in nematode control **(Chitwood, 2002).**

Essential oils from various families, such as Asteraceae, Lamiaceae, and Rutaceae, have thouroughly been examined for nematicidal activity against *Meloidogyne* sp.. with special reference to genus *Artemisia, Lavandula, Mentha* etc **(Andrés *et al*., 2012).** The presence of volatile monoterpenes in essential oils have been reported to provide an important defense strategy to the plant against insect pests and pathogenic organisms. These terpenoids have also been reported to play a role in plant parasitic interactions, acting as signaling molecules **(Batish *et al*., 2008).**

4.4.4.1 Effect of AAREO & AAWEO on mobility of second stage larvae of *Meloidogyne. incognita*

In present investigation both AAREO & AAWEO have been examined against second stage juvenile larvae of *M. incognita* (root knot nematode). Both the samples

were tested at three concentration (1µl/mL, 3 µl/mL,5 µl/mL) and were observed for the duration of 12,48 and 72 hour.

A dose dependent and time dependent mortality/immobility of second instar larvae of *M. incognita* was observed both in AAREO & AAWEO with respect to negative control. AAREO was observed to be more effective than AAWEO . The immobility of larvae at a dose level of 5µl/mL was observed 21.37%, 24.08% and 27.71% respectively after the time interval of 24, 48 & 72 hours respectively. In AAREO with final average value of 24.38% at the end of the experiment. Similarly the immobility in AAWEO was observed in the order of 18.16%, 23.84% and 27.25% with the time interval of 24,48 & 72 hours respectively at a dose level of 5µl/mL. However the order of activity was observed as AAREO (24.38±3.181)> AAWE) (23.08±4.591%)> Control (7.91±4.902) respectively. The detailed experimental observation in terms of percentage mortality of *M. incognita* 2nd instar larvae has been depicted in **table 4.14.**

4.4.4.2 Effect of AAREO & AAWEO on egg hatching of *M.incognita*

A concentration dependent and time dependent hatching of egg masses of *M. incognita* was observed in AAREO & AAWEO with respect to negative control. It was observed that AAWEO was more effective than AAREO on the inhibition of egg hatching. The rate of egg hatching was directly proportional to the exposure of time period and was inversely proptional to the concentration of oil samples. The maximum inhibitory effect on egg hatching at a concentration of 2µl/mL was observed as 23.79% in AAWEO after a time interval of 72 hour while the minimum inhibitory effect was observed as 5.38% at a concentration of 10 µl/mL after 72 hours. Similarly in AAREO the maximum rate of hatching 18.31% was observed at a concentration of 2µl/mL while the minimum 11.41% was observed at 10 µl/mL concentration after 72 hours. However the order of activity was observed as: AAWEO (5.38±1.814)> AAREO (18.31±7.495)>control (32.29±16.61) respectively. The experimental layout with the observation in terms of percentage egg hatching of *M. incognita* egg masses are being depicted in **table 4.15** and **fig 4.25 w**hile the morphological characters of *M.incognita* in different stages are being shown in **fig. 4.26.**

Table 4.14 Nematicidal activity of AAREO and AAWEO against 2nd instar larvae of*M.incognita*

Sample	Dose	Observation												Mean
		Percentage mortality of *M.incognita* 2nd instar larvae												
		24 hour				48 hour				72 hour				
		R1	R2	R3	Avg	R1	R2	R3	Avg	R1	R2	R3	Avg	
AAREO	1 µl/mL	14.85	16.82	17.92	**16.53±1.555**	17.82	20.56	19.81	**19.39±1.415**	21.78	26.16	22.64	**23.52±2.320**	**19.81±3.514**
	3 µl/mL	18.44	19.62	20.90	**19.65±1.230**	22.33	21.49	24.54	**22.78±1.575**	28.15	27.1	27.1	**27.45±0.606**	**23.29±3.925**
	5 µl/mL	20.37	21.62	22.12	**21.37±0.901**	24.07	23.42	24.77	**24.08±0.675**	28.7	26.12	28.31	**27.71±1.390**	**24.38±3.181**
AAWEO	1 µl/mL	5.76	8.33	5.88	**6.65±1.450**	14.42	16.34	11.53	**14.09±2.421**	25.3	20.37	15.68	**20.45±4.810**	**13.73±6.907**
	3 µl/mL	12.72	13.27	15.25	**13.74±1.330**	16.36	16.81	20.33	**17.83±2.173**	20.9	22.12	23.72	**22.24±1.414**	**17.93±4.251**
	5µl/mL	15.51	20.33	18.64	**18.16±2.44**	20.68	24.57	26.27	**23.84±2.865**	25	27.96	28.81	**27.25±2.00**	**23.08±4.591**
control	In water	2.12	1.42	1.17	**1.57±0.492**	9.75	8.21	10.36	**9.44±1.108**	10.06	9.21	12.44	**10.57±1.674**	**7.19±4.902**
					CD (5%)				SEm±					
Hours (H)					1.13				0.387					
Concentration (C)					1.30				0.447					
Interaction (H×C)					2.26				0.775					
CV 7.19														

CD=critical difference at 5% CV= Cofficient of variation AAWEO= *Artemisia annua* winter essential oil

Table 4.15 Nematicidal activity of AAREO and AAWEO against *M. incognita* egg hatching from egg masses

Sample	Dose	Observation												Mean
		Percentage egg hatching of *M.incognita* from egg masses												
		24 hour				48 hour				72 hour				
		R1	R2	R3	Avg	R1	R2	R3	Avg	R1	R2	R3	Avg	
AAREO	2 µl/mL	17.85	12.65	8.48	**10.24±4.694**	23.33	27.36	25.96	**19.66±2.046**	34.53	33.65	30.02	**25.05±2.390**	**18.31±7.495**
	6 µl/mL	10.85	9.44	6.50	**8.19±2.219**	13.53	15.64	13.31	**12.12±1.286**	21.94	20.68	19.02	**16.91±1.464**	**12.40±4.367**
	10 µl/mL	7.00	5.21	6.50	**7.17±0.923**	11.76	13.96	12.87	**12.14±1.100**	17.66	15.44	16.66	**14.94±1.111**	**11.41±3.935**
AAWEO	2 µl/mL	11.76	13.53	15.23	**13.50±1.735**	25.32	26.12	23.84	**25.09±1.156**	35.39	33.26	29.73	**32.79±2.858**	**23.79±9.710**
	6 µl/mL	7.00	7.50	10.85	**8.45±2.093**	17.46	24.17	18.63	**20.08±3.584**	25.48	23.50	23.16	**24.04±1.252**	**17.52±8.103**
	10 µl/mL	3.89	3.36	4.43	**3.89±0.535**	4.95	5.73	3.89	**4.85±0.923**	7.50	8.97	5.73	**7.40±1.622**	**5.38±1.814**
control	In water	13.56	12.29	16.15	**14.00±1.606**	35.12	35.15	39.11	**36.46±1.873**	45.12	43.12	51.09	**46.42±3.385**	**32.29±16.61**
					CD (5%)				SEm±					
Hours (H)					2.04				0.699					
Concentration (C)					2.35				0.808					
Interaction (H×C)					4.08				1.399					
CV					11.755									

CD=critical difference at 5% CV= Cofficient of variation AAWEO= *Artemisia annua* winter essential oil

The *in-vitro* toxicity of *Artemisia annua* essential oil has been reported against J_2 of *M. incognita* with 100 percent mortality of the nematode *M. incognita* at the dose levels of in 500 and 250 ppm concentrations respectively which gradually decreased with the lower concentrations respectively **(Shakil *et al*., 2004).** Nematotoxic effect of an aqueous extract of *Artemisia annua* and its components caffeic acid, chlorogenic acid (5-caffeoylquinic acid, 5-CQA), artemisinin and the related semi-synthetic artesunate, have been reported on the root-knot nematode *Meloidogyne incognita* (**D'Addabbo *et al*., 2013).**

The effect of major compounds in the oils of AAREO &AAWEO cannot be neglected for attributing the nematicidal activity. Since the major compunds of both the oils are 1,8- cineole and camphor. These compounds have been reported to possess significant nematicidal activity in previous study against root knot nematode *M.incognita.* Compounds like 1,8-cineole, L-camphor, L-borneol, caryophyllene oxide α- and β-pinene, p-cymene, terpinen-4-ol, β-caryophyllene and carvacrol have been reported to posess a potent nematicidal activity against the *M.incognita* **(Ntalli *et al*., 2011).** Nematicidal activity due to synergistic interaction of carvone with 1,8-cineole an D-menthol **(Andrés *et al*., 2012).** Similarly anti-egg hatching and mortality of nematode larvae in α-terpineol and camphor has been reported **(Echeverrigaray *et al*., 2010).**

Kumar and Singh, (2021) reported that camphene, pinene, myrcene, terpinene, terpinen- 4-ol, thymol and carvacrol) showed nematicidal activity against PWN32 (pine wood nematode).Camphor, 1,8- cineole, camphene from *A.sieberi* have been reported to possess nematicidal activity against *M. incognita***,** *C. maculatus, S. oryzae, T. castaneum, B. tabaci and M. javanica* **(Mahboubi, 2014).** The chemical constituents identified in AAREO & AAWEO correlates with the above mentioned constituents. It can be thus inferred that the nematicidal activity of AAREO & AAWEO with respect to immobility of second instar larvae of *M.incognita* and egg hatching could be possibly due to the presence of camphor, p-cymene, 1,8-cineole, terpinen-4-ol, *β*-caryophyllene, carvacrol, L-borneol, caryophyllene oxide and α- and β-pinene or due to the synergistic effects of other major or minor/trace components of essential oils.

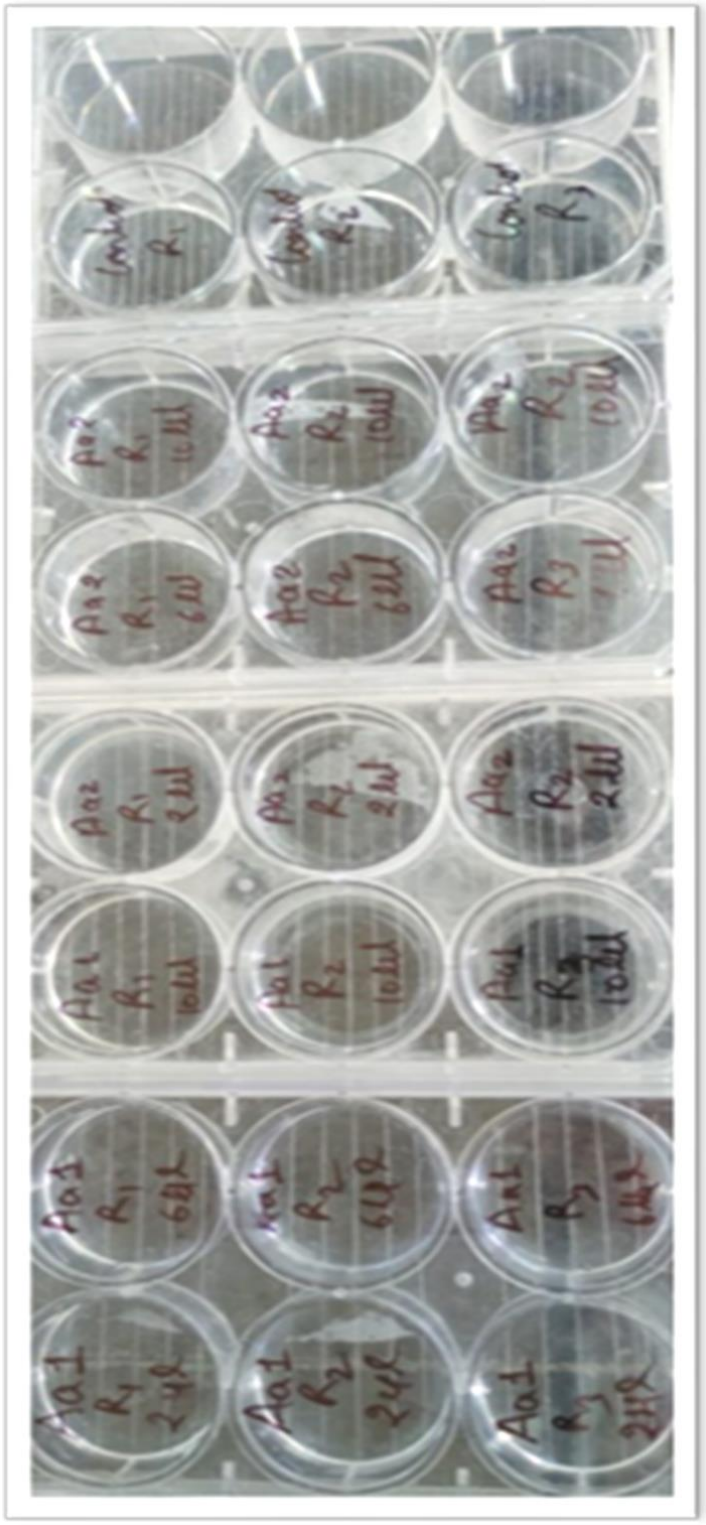
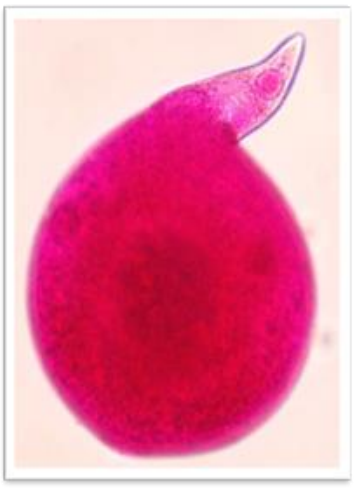


Fig.4.25 Experimental layout of egg hatching of *M.incognita*

(a) Second stage larvae of root knot (*Meloidogyne* spp.) nematode

(b) Posterior region of second stage larvae root knot (*Meloidogyne* spp.) nematode



(c) Adult female of root knot (*Meloidogyne* spp.) nematode

(d) Anterior region of second stage larvae of root knot (*Meloidogyne* spp.) nematode

Fig. 4.26 Morphological characters of *M. incognita* in different stages

Summary and Conclusions

Chapter 5 SUMMARY AND CONCLUSION

The present research was investigated with the objective "Seasonal Variation in Chemical Composition and Biological Activities of Essential Oils from *Artemisia annua* L." which was collected wildely from Tarai region of Kumaon viz., Pantnagar ,Uttarakhand ,India with an altitude of 243m. The plant material was collected in the month of September 2020 and January 2021 respectively. The essential oils isolated through hydrodistillation were studied by GC/MS and various biological activities were carried out .The essential oils isolated in rainy and winter season for their chemical composition and various biological activities were designated as AAREO & AAWEO respectively

. The chemical composition of AAREO revealed the presence of seventy five essential oil constituents which contributed 91.7 % of the total oil. The presence of oxygenated monoterpenoids with 32.2% composition dominated over sesquiterpenoids hydrocarbon with 32.1% composition. Camphor (14.1%) was found to be the major component of AAREO followed by germacrene –D (9.0%), *trans*-β-caryophyllene (8.7%), eucalyptol (4.2%), *cis*-cadin-4en-7-ol (3.9%), isoborneol (3.2%), p-cymene (2.9%), (-)-aromadendrene (2.6%), lavandulyl acetate (2.1%), α –cadinol (2.0%), camphene (2.0%), The chemical composition of AAWEO revealed the presence of fifty nine chemical constituents which contributed 94.1% of the total essential oil. Oxygenated monoterpenoids with 66.8% dominated over Monoterpenoid hydrocarbon and Sesquiterpenoid hydrocarbon with 12.1% and 7.6% respectively. The major chemical constituents of AAWEO were Camphor (17.9%) followed by eucalyptol (14.6%), (E,E)-2,6-dimethyl-3,5,7-octatrien-2-ol (6.3%), (5E)-2,6-dimethyl-1,5,7-octatrien-3-ol (5.2%) artemisia alcohol (3.5%), terpinene-4-ol (3.4%) isoborneol (3.2%), p-cymene (3.0%), caryophyllene oxide (3.1%), *trans* β-caryophyllene (2.7%), mycrene (2.6%), (+)-α-terpineol (2.5%), artemisia ketone (2.5%), camphene (2.1%), (-) β-farnesene (2.0%), besides other constituents which were present in minor and trace amounts.

In present study, AAREO and AAWEO were studied for anti-oxidant activity via following three methods viz..H_2O_2, DPPH scavenging and metal chelating activity. Both AAREO and AAWEO exhibited DPPH radical scavenging activity at 515nm absorbance in dose dependent manner. The DPPH free radical scavenging activity of AAREO and AAWEO was observed in the order of AAWEO (IC_{50}=45.588±0.334) > AAREO (IC_{50}=77.87±0.528) compared to BHT (IC_{50}=43.48±1.55) which indicated that AAWEO possess maximum DPPH free radical scavenging activity with minimum IC_{50} value followed by AAREO.

The effect on metal chelating activity of Fe^{2+} by AAREO and AAWEO was evaluated in presence of $FeCl_2$ and ferrozine in a dose dependent manner. The metal chelating activity of AAREO and AAWEO was observed in the following order of AAWEO (IC_{50}=49.50±0.234)> AAREO (IC_{50} =51.40±0.158) compared to Na_2EDTA (IC_{50} = 41.68±0.396)which indicated that AAWEO possess maximum metal chelating activity with minimum IC_{50} value followed by AAREO.

H_2O_2 radical savenging activity of AAREO and AAWEO was conducted by measuring absorbance at 230nm in dose dependent manner. The H_2O_2 free radical scavenging activity of AAREO, AAWEO and standard was observed in dose dependent manner (20µl/mL, 40 µl/mL,60 µg/mL,80 µl/mL and 100 µg/mL respectively). The order of activity was observed as : AAWEO (IC_{50} =34.79±0.419) > AAREO (IC_{50} =44.83±1.304compared to BHT (IC_{50} =27.96±1.195).

Both AAREO and AAWEO were investigated for their *in-vitro* anti-inflammatory activity based on inhibition percentage of the Hen's egg albumin protein denaturation measured by absorbance at 660nm in a dose dependent manner. AAWEO possess maximum anti-inflammatory activity with minimum IC_{50} value followed by AAREO with higher IC_{50} value. The order in which both AAREO and AAWEO showed anti-inflammatory activity was observed as AAWEO (IC_{50}=66.90±0.040) > AAREO (IC_{50}=76.55±0.218) compared to the standard anti-inflammatory drug diclofenac sodium tablets (IC_{50}=63.41±0.037).

A study was conducted to investigate *in-vitro* anti-diabetic potential of AAREO and AAWEO spectrophotometrically by measuring absorbance at 540nm in dose dependent manner. AAWEO exhibited maximum activity with minimum IC_{50} value followed by AAREO with higher IC_{50} value in comparison to the standard acarbose with $IC_{50=}$ 38.21±0.03.The order in which both AAREO and AAWEO exhibited anti-diabetic activity was observed as AAWEO (47.45±0.195) > AAREO (74.26±3.706).

The essential oils were studied their anti-feedant activity. The activity was based on 'No-Choice Experiment' against *spodopteralitura*. Both AAREO and AAWEO were observed to show strong anti-feedant activity in a dose dependent manner. AAREO exhibited 90.85 % anti-feedant activity while AAWEO exhibited 87.92% anti-feedant activity at a dose level of 8µl/mL.

The present investigation of both AAREO & AAWEO were examined for nematicidal activity against second stage juveniles of *M. incognita* (root knot nematode). It was observed that , Between the two samples, AAREO was found to be most effective at 5 µl/mL concentration with 24.38% inhibition in mobility of larvae, followed by AAWEO (23.08%) inhibition. So from this analysis it was observed that AAREO is exhibiting more activity over AAWEO at different concentration (1µl/mL, 3µl/mL,5µl/mL) respectively with the exposure time of 12, 48 and 72 hour, where as in the case of egg inhibition process AAWEO was found to be more effective than AAREO as it exhibited minimum inhibition (5.38%) of egg hatching at a concentration of 10µl/mL, followed by AAREO with minimum (11.41%) inhibition respectively.

From various reports it has been observed that Camphor and 1,8 cineole is a strong bioactive natural compound and possess various biological activities. In present investigation, camphor has been found to be a major compound with 14.1% and 17.4% composition both in AAREO and AAWEO respectively, and the oil yield has been observed as 0.70-0.50% in both the collections. Hence, it can be inferred that *Artemisia annua* grown in wild and cultivated in various part of Uttarakhand can also

be a good source of camphor and 1,8 cineole with revenue generation for local people of the state.

Similarly the essential oil components *viz.*.camphor, α-pinene, 1,8-cineole, artemisia ketone, *trans*-β-caryophyllene, isoborneol, p-cymene , terpinen-4-ol, α, sabinene, germacrene-D, camphene etc. have been reported to exhibit strong to moderate anti-feedant, anti-inflammatory, anti-diabetic and nematicidal activities, In present investigation AAREO & AAWEO have been observed to possess diversified chemical composition with varying chemical make-up along with the above mentioned compounds. Hence, it can be inferred that the essential oil of *Artemisia annua* can be used as a natural anti-feedant, nematicidal, anti-inflammatory or food preservative to protect it from larval stage of insects and oxidative deterioration, respectively and can also be used as a natural anti-diabetic agent in pure form or via formulation after proper clinical trials.

Literature Cited

LITERATURE CITED

Abad, M. J., Bedoya, L. M., Apaza, L. and Bermejo, P. 2012. The *Artemisia* L. genus: a review of bioactive essential oils. *Mol*., 17(3): 2542-2566.

Abdallah, I.I. and Quax, W.J., 2017. A glimpse into the biosynthesis of terpenoids. *KnE Life Sci.*, pp. 81-98.

Adams, R. P. 2007. *Identification of essential oil components by gas chromatography/mass spectrometry*. Carol Stream, IL: Allured publishing Corporation. pp. 542-544.

Ahmed F, Shahi IZ, Biswas UK, Roy BA, Das AK, Choudhuri MSK .2007. . Anti inflammatory, antinociceptive and neuro pharmacological activities of *Clerodendron infortunatum* . *Pharm. Biol.*, 45 (7): 587 – 593.

Ali, A.M. and Ibrahim, A.M., 2018. Castor and camphor essential oils alter hemocyte populations and induce biochemical changes in larvae of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). *J. Asia. Pac. Entomol*., 21(2): 631-637.

Ali, B., Al-Wabel, N. A., Shams, S., Ahamad, A., Khan, S. A., and Anwar, F. 2015. Essential oils used in aromatherapy: A systemic review. *Asian Pac. J. Trop. Biomed*., 5(8): 601-611.

Amorati, R., Foti, M. C., and Valgimigli, L. 2013. Antioxidant activity of essential oils. *J.agric. food.chem*., 61(46): 10835-10847.

Andrade, L.N. and De Sousa, D.P., 2013. A review on anti-inflammatory activity of monoterpenes. *Mol.*, 18(1): 1227-1254.

Andrade, L.N., Dos Reis Barreto de Oliveira, R. and De Sousa, D.P., 2014. A review on anti-inflammatory activity of phenylpropanoids found in essential oils. *Mol.*, 19(2): 1459-1480.

Andrés, M. F., González-Coloma, A., Sanz, J., Burillo, J. and Sainz, P. 2012. Nematicidal activity of essential oils: a review. *Phytochem. Rev.*, 11(4): 371-390.

Anthony, K.P., Deolu-Sobogun, S.A. and Saleh, M.A., 2012. Comprehensive assessment of antioxidant activity of essential oils. *J. Food Sci.*, *77*(8): 839-843.

Appalasamy, S., Lo, K. Y., Ch'ng, S. J., Nornadia, K., Othman, A. S. and Chan, L. K. 2014. Antimicrobial activity of artemisinin and precursor derived from in vitro plantlets of *Artemisia annua* L. *Biomed. Res. Int.*, 7(5):24-56.

Arivoli, S. and Tennyson, S., 2012. Antifeedant activity of plant extracts against *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae). *Am. Eurasian. J. Agric. Environ Sci*., 12(6): 764-768.

Arivoli, S. and Tennyson, S., 2013. Antifeedant activity, developmental indices and morphogenetic variations of plant extracts against *Spodoptera litura* (Fab) (Lepidoptera: Noctuidae). *J. Entomol. Zool. Stud.*, 1(4): 87-96.

Asanova, Z.K., Suleimenov, E.M., Atazhanova, G.A., Dembitskii, A.D., Pak, R.N., Dar, A. and Adekenov, S.M., 2003. Biological activity of 1, 8-cineole from levant wormwood. *Pharm. Chem. J.*, 37(1): 28-30.

Ashok, P. K. and Upadhyaya, K. 2013. Preliminary Phytochemical Screening and Physico-Chemical Parameters of *Artemisia absinthium* and *Artemisia annua. J. Pharmacogn. Phytochem.*, 1(6):229-235.

Ayyangar GSG. and Rao PJ. 1989. Neem (*Azadirachta indica* A. Juss) extracts as larval repellents and ovipositional deterrents to *Spodoptera litura* (Fab.). *Indian J. entomol.*, 51:121-124.

Badoni, R., Semwal, D.K. and Rawat, U. 2009. Altitudinal variation in the volatile constituents of *Artemisia nilagirica*..*Int. J. Essent. Oil Ther.*, 3: 66–68.

Bagchi, G.D., Haider, F., Dwivedi, P.D., Singh, A. and Naqvi, A.A., 2003. Essential oil constituents of *Artemisia annua* during different growth periods at monsoon conditions of subtropical north Indian plains. *J. Essent. Oil Res.*, 15(4): 248-250.

Bakkali, F., Averbeck, S., Averbeck, D., and Idaomar, M. 2008. Biological effects of essential oils–a review. *Food Chem. Toxicol*., 46(2): 446-475.

Balachandran, S., Vishwakarma, R.A. and Popli, S. P. 1987. Chemical investigation of some *Artemisia* species: Search for artemisinin or other related sesquiterpene lactones with a peroxide bridge. *Ind. J. Pharm. Sci*., 49:152-154.

Batish, D.R., Sing, H.P., Kohli, R.K., Kaur S. 2008. Eucalyptus essential oil as a natural pesticide. *For. Ecol .Manag.,* 256: 2166–2174.

Bhakuni, R. S., Jain, D. C., Sharma, R. P. and Kumar, S. 2001. Secondary metabolites of *Artemisia annua* and their biological activity. *Curr. Sci.,* 80 (1): 35-48.

Bilia, A. R., Santomauro, F., Sacco, C., Bergonzi, M. C. and Donato, R. 2014. Essential oil of *Artemisia annua* L.: an extraordinary component with numerous antimicrobial properties. *Env. Comp. Alt. Med.*, 7:124-153.

Böhme, K. Velázquez, J. B., and Calo-Mata., P. 2014. "Antibacterial, Antiviral and Antifungal Activity of Essential Oils: Mechanisms and Applications." In Antimicrobial Compounds. Berlin: Springer. 51-81 p.

Bora, K. S. and Sharma.A. 2011. The genus *Artemisia*: a comprehensive review. *Pharm. Biol.,* 49 (1):101-109.

Bourgaud, F., Gravot, A., Milesi, S., and Gontier, E. 2001. Production of plant secondary metabolites: a historical perspective. *Plant.Sci.,* 161(5): 839-851.

Bouyahya, A., Lagrouh, F., El Omari, N., Bourais, I., El Jemli, M., Marmouzi, I., and Bakri, Y. 2020. Essential oils of Mentha viridis rich phenolic compounds show important antioxidant, antidiabetic, dermatoprotective, antidermatophyte and antibacterial properties.*Biocatal. Agric. Biotechnol*., 23: 101471.

Brisibe, E. A., Umoren, U. E., Owai, P. U. and Brisibe, F. 2008. Dietary inclusion of dried *Artemisia annua* leaves for management of coccidiosis and growth enhancement in chickens. *Afr. J. Biotech.,* 7(22): 255-352.

Brown, G. D. 2010. The biosynthesis of artemisinin (Qinghaosu) and the phytochemistry of *Artemisia annua* L. (Qinghao). *Mol.*, 15: 7603–7698.

Brown, G. D., Liang, G. Y., and Sy, L. K. 2003. Terpenoids from the seeds of *Artemisia annua*. *Phytochem.*, 64(1): 303-323.

Caissard, J.C., Joly, C; Bergougnoux, V; Hugueney, P.; Mauriat, M.and Baudino, S. 2004. Secretion mechanisms of volatile organic compounds in specialized cells of aromatic plants. *Res. Dev. Cell Biol.*, 2:1–15.

Carbonara, T., Pascale, R., Argentieri, M. P., Papadia, P., Fanizzi, F. P., Villanova, L. and Avato, P. 2012. Phytochemical analysis of a herbal tea from Artemisia annua L. *J. Pharm. Biomed*., 62:79-86.

Ćavar, S., Maksimović, M., Vidic, D. and Parić, A. 2012. Chemical composition and antioxidant and antimicrobial activity of essential oil of *Artemisia annua* L. from Bosnia. *Ind. Cr. Pro.*, 37(1): 479-485.

Chamorro, E. R., Zambón, S. N., Morales, W. G., Sequeira, A. F., and Velasco, G. A. 2012. Study of the chemical composition of essential oils by gas chromatography. *Gas chromatography in plant science, wine technology, toxicology and some specific applications*, 1: 307-324.

Charles, D.J., Cebert, E. and Simon, J.E., 1991. Characterization of the essential oil of *Artemisia annua* L. *J. Essent. Oil Res*., 3(1): 33-39.

Chauhan, N. S. 1999. Medicinal and aromatic plants of Himachal Pradesh. Indus publishing. pp. 342-368.

Chitwood DJ 2002.Phytochemical based strategies for nematode control. *Ann. Rev. Phytopathol.*, 40: 221–249.

Christenhusz, M. J. N. and Byng, J. W. 2016. The number of known plant species in the world and its annual increase. *Phyto. tax.*, 261(3):201-217.

Clevenger, J. F. 1928. Apparatus for the determination of volatile oil. *J. Am. Pharm. Assoc*., 17(4): 345-349.

Czechowski, T., Larson, T. R., Catania, T. M., Harvey, D., Wei, C., Essome, M. and Graham, I. A. 2018. Detailed phytochemical analysis of high-and low artemisinin-producing chemotypes of *Artemisia annua*. *Front. Plant. Sci.*, 9:1-14.

D'Addabbo, T., Carbonara, T., Argentieri, M.P., Radicci, V., Leonetti, P., Villanova, L. and Avato, P., 2013. Nematicidal potential of *Artemisia annua* and its main metabolites. *Eur. J. Plant Pathol.*, 137(2): 295-304.

Da Silva, J. K. R., Pinto, L. C., Burbano, R. M. R., Montenegro, R. C., Guimaraes, E. F., Andrade, E. H. A., and Maia, J. G. S. 2014. Essential oils of Amazon Piper species and their cytotoxic, antifungal, antioxidant and anti-cholinesterase activities. *Ind. Crops. Prod.*, 58: 55-60.

Dar, R. A., Shahnawaz, M. and Qazi, P. H. 2017. General overview of medicinal plants: A review.*Int. J. Phytopharm.*, 6(6): 349-351.

Das, S. 2012. *Artemisia annua* (Qinghao): a pharmacological review. *Int. J. Pharm. Sci. Res.*, 3(12): 4573-4577.

Den Ouden, H., 1958. A new method for culturing plants enabling the observation of nematodes on growing roots. *Tijdschrift Over Plantenziekten.*, 64(3): 269-272.

Dev, S. 1999. Ancient-modern concordance in Ayurvedic plants : Some examples. *Environ. Health Perspect.*, 107(10): 783-789.

Dhaliwal GS and Koul O. 2011. . Biopesticides and pest management . Kalyani pulishers, India. 342 p.

Dhingra, V., Rao, K.V. and Narasu, M.L., 1999. Current status of artemisinin and its derivatives as antimalarial drugs. *Life. Sci***.**, 66(4): 279-300.

Dilshad, E., Zafar, S., Ismail, H., Waheed, M. T., Cusido, R. M., Palazon, J. and Mirza, B. 2016. Effect of rol genes on polyphenols biosynthesis in Artemisia annua and their effect on antioxidant and cytotoxic potential of the plant**.** *Appl. Biochem. Biotechnol.*, 179(8):1456-1468.

E Sa, R. C. D. S., and Andrade, L. N. 2013. A review on anti-inflammatory activity of monoterpenes. *Mol.* (Basel, Switzerland), 18(1): 1227-1254.

Echeverrigaray, S., Zacaria, J. and Beltrão, R., 2010. Nematicidal activity of monoterpenoids against the root-knot nematode *Meloidogyne incognita*. *Phytopathology*., 100(2):199-203.

Efferth, T., Herrmann, F., Tahrani, A. and Wink, M. 2011. Cytotoxic activity of secondary metabolites derived from *Artemisia annua* L. towards cancer cells in comparison to its designated active constituent artemisinin. *Phyto. Med.*, 18(11): 959-969.

Ehrnhöfer-Ressler, M.M., Fricke, K., Pignitter, M., Walker, J.M., Walker, J., Rychlik, M. and Somoza, V., 2013. Identification of 1, 8-cineole, borneol, camphor, and thujone as anti-inflammatory compounds in a *Salvia officinalis* L. infusion using human gingival fibroblasts. *J. Agric. Food Chem*., 61(14): 3451-3459.

Eisenback, J.D., 1985. Detailed morphology and anatomy of second-stage juveniles, males, and females of the genus *Meloidogyne* (root-knot nematodes). *An advance. Treat. on Meloidogyne.*, 1:47-77.

Espinoza, J., Urzúa, A., Bardehle, L., Quiroz, A., Echeverría, J., and González-Teuber, M. 2018. Antifeedant effects of essential oil, extracts, and isolated sesquiterpenes from *Pilgero dendronuviferum* (D. Don) florin heartwood on red clover borer *Hylastinus obscurus* (Coleoptera: Curculionidae). *Mol*., 23(6): 1282-1292.

Falleh, H., Jemaa, M. B., Saada, M., and Ksouri, R. 2020. Essential oils: A promising eco-friendly food preservative. *F. chem.*, 330: 127268.

Feng, X., Cao, S., Qiu, F. and Zhang, B. 2020. Traditional application and modern pharmacological research of *Artemisia annua* L. *Pharma. & Thera.*, 10(7): 6-50.

Fokou, J. B. H., Dongmo, P. M. J. and Boyom, F. F. 2020. Essential Oil's Chemical Composition and Pharmacological Properties. *Oils. of Nat*., 4(8): 1-217.

Francomano, F., Caruso, A., Barbarossa, A., Fazio, A., La Torre, C., Ceramella, J., Mallamaci, R., Saturnino, C., Iacopetta, D. and Sinicropi, M.S., 2019. β-Caryophyllene: a sesquiterpene with countless biological properties. *Appl. Sci.*, 9(24): 5420.

Fu, C., Yu, P., Wang, M. and Qiu, F. 2020. Phytochemical analysis and geographic assessment of flavonoids, coumarins and sesquiterpenes in *Artemisia annua* L. based on HPLC-DAD quantification and LC-ESI-QTOF-MS/MS confirmation. *F. Chem.*, 312: 126070.

Gholami BA, Faravani M and Kashki MT. 2011. Allelopathic effects of aqueous extract from *Artemisia kopetdaghensis* and *Satureja hortensis* on growth and seed germination of weeds. *J. Appl. Environ. Biol. Sci.* 1(4): 283-290.

Habibi, Z., Ghanian, S., Ghasemi, S. and Yousefi, M. 2013. Chemical composition and antibacterial activity of the volatile oil from seeds of *Artemisia annua* L. from Iran. *Nat. Pro. Res.*, 27(2): 198-200.

Haghighian, F., Jalali Sendi, J., Aliakbar, A. and Javaherdashti, M. 2008. The growth regulatory, deterrency and ovicidal activity of worm wood [*Artemisia annua* L.] on *Tribolium confusum* Duv. and identification of its chemical constituents by GC-MS. *Pest. Chem.*, 1(2):51-59.

Haider, F., Kumar, N., Banerjee, S. and Naqvi, A.A. 2009. Effect of altitude on the essential oil constituents of *Artemisia roxburghiana* Besser var. purpurascens (Jacq.) Hook. *J. Essent. Oil Res.*, 21:303–304.

Haider, S.Z., Mohan, M., and Andola, H.C. 2014.Constituents of *Artemisia indica* Willd. from Uttarakhand Himalaya: A source of davanone. *Pharmacogn. Res.*, 8: 257–259.

Haider, S.Z., Andola, H.C. and Mohan, M. 2012. Constituents of *Artemisia gmelinii* Weber ex Stechm. from Uttarakhand Himalaya: A source of artemisia ketone. *Indian J. Pharm. Sci*., 74:265–267.

Haider, S.Z., Lohani, H., Naik, G., Chauhan, D. and Chauhan, N., 2019. Chromatographic Fingerprinting of *Artemisia vulgaris* L. Grown in Various Locations of Uttarakhand Himalaya, India. *J. Essent. Oil Bear. Plants.*, 22(6): 1509-1518.

Harris, E.M. 1995. Infloroscence and floral ontogeny in Asteraceae: a synthesis of historical and current concepts. *Bot. Rev.*, 61:93-278.

Hayat, M. Q., Khan, M. A., Ashraf, M. and Jabeen, S. 2009. Ethnobotany of the genus *Artemisia* L. (Asteraceae) in Pakistan. *J. Pharm. Sci*., 8:32-45.

Helal, E.G., Abou-Aouf, N., Khattab, A.M. and Zoair, M.A., 2014. Anti-diabetic effect of *Artemisia annua* (kaysom) in alloxan-induced diabetic rats. *Egypt. J. Hosp. Med.*, 57:422-430.

Ho, W. E., Peh, H. Y., Chan, T. K. and Wong, W. S. F. 2014. Artemisinins: Pharmacological actions beyond anti-malarial. *Pharma. and Thera.*, 142:126–139.

http://www.theplantlist.org/

Huang, J., Ma, L.Q., Yang, Y., Wen, N., Zhou, W., Cai, C., Liu, Q.H. and Shen, J. 2017. Chloroform extract of *Artemisia annua* L. relaxes mouse airway smooth muscle. *Evid. Comp. Alt. Med.*, 20:17-36.

Hussy, R.S and Barker, K.R. 1973. A comparison of method of collecting inocula of *Meloidogyne* spp. Including a new technique. *Plant Dis*., 57: 1925-1928.

Iqbal, T., Hussain, A.I., Chatha, S.A.S., Naqvi, S.A.R. and Bokhari, T.H., 2013. Antioxidant activity and volatile and phenolic profiles of essential oil and different extracts of wild mint (*Mentha longifolia*) from the Pakistani Flora. *J. Anal. Method. Chem.*, 4(8): 1-7.

Islamuddin, M., Chouhan, G., Want, M. Y., Tyagi, M., Abdin, M. Z., Sahal, D. and Afrin, F. 2014. Leishmanicidal activities of *Artemisia annua* leaf essential oil against Visceral Leishmaniasis. *Front. Micro.*, 5:626.

Ivanescu, B., Vlase, L., Corciova, A. and Lazar, M. I. 2010. HPLC-DAD-MS study of polyphenols from *Artemisia absinthium, A. annua,* and *A. vulgaris. Chem. nat. comp.*, 46(3): 468-470.

Javier, A.M.V., Ocampo, V.R., Ceballo, F.A. and Javier, P.A., 2017. Insecticidal activity of selected essential oil extracts against common cutworm, *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae). *Philipp. J Sci.*, 146(3) : 247-256.

Jia, J. W., Crock, J., Lu, S., Croteau, R. and Chen, X. Y. 1999. (3R)-Linalool synthase from *Artemisia annua* L.: cDNA isolation, characterization, and wound induction. *Arc. Biochem. Biophy.,* 372(1): 143-149.

Joshi, R. K. 2013. Antimicrobial activity of volatile oil of *Artemisia capillaris* growing wild in Uttarakhand Himalaya. *J. Pharma. Phyto. chem.,* 1(6):56-75.

Joshi, R. K. 2020. Chemical Constituents of *Artemisia nilagirica* (Clarke) from Western Himalaya of Uttarakhand, India. *Asian J. Pharma. An.,* 10(4):324-354.

Joshi, R.K. 2015. Volatile oil composition of *Artemisia japonica* from western himalaya of Uttarakhand. *J. Pharmacogn. Phytochem*., 3:96–97.

Juteau F., Masotti V., Bessie‘re J. M., Dherbomez M. and Viano J. 2002. Antibacterial and antioxidant activities of *Artemisia annua* essential oil. *Fit. Chem.,* 73(6): 532–535.

Kabera, J. N., Semana, E., Mussa, A. R., and He, X. 2014. Plant secondary metabolites: biosynthesis, classification, function and pharmacological properties. *J. Pharm. Pharmacol*., *2*(7): 377-392.

Kar, B., Kumar, R. S., Karmakar, I., Dola, N., Bala, A., Mazumder, U. K., and Hadar, P. K. 2012. Antioxidant and in vitro anti-inflammatory activities of *Mimusopselengi* leaves.*Asian. Pac. J. Trop. Biomed*., 2(2): 976-980.

Karthikeyan, S., Sanjappa, M. and Moorthy, S. 2009. *Flowering Plants of India Dicotyledons (Acanthaceae – Avicenniaceae). Bot. Sur. Ind*., 1: 365.

Kataev, V. E., Strobykina, I. Yu., Andreeva, O. V., Garifullin, B. F., Sharipova, R. R., Mironov, V. F., and Chestnova, R.V. 2011. "Synthesis and Anti tuberculosis Activity of Derivatives of *Stevia rebaudiana* Glycoside Steviol bioside and Diterpenoid Isosteviol Containing Hydrazone, Hydrazide, and Pyridinoyl Moieties." *Russ. J. Bioorganic Chem.,* 37 (4): 483-91.

Keskes, H., Mnafgui, K., Hamden, K., Damak, M., El Feki, A. and Allouche, N., 2014. *In vitro* anti-diabetic, anti-obesity and antioxidant proprieties of *Juniperus phoenicea* L. leaves from Tunisia. *Asian Pac. J. Trop. Biomed.*, 4: 649-655.

Khaleel, C., Tabanca, N and Buchbauer, G. 2018. α-Terpineol, a natural monoterpene: A review of its biological properties. *Open. Chem. J.*, 16(1): 349-361.

Khatoon, N., Sharma, Y., Sardar, M. and Manzoor, N. 2019. Mode of action and anti-Candida activity of *Artemisia annua* mediated-synthesized silver nanoparticles. *J. Mycol Med,* 29(3): 201-209.

Khosravi, R., Sendi, J.J. and Ghadamyari, M., 2010. Effect of *Artemisia annua* L. on deterrence and nutritional efficiency of lesser mulberry pyralid (*Glyphodes pylolais* Walker) (Lepidoptera: Pyralidae). *J. Plant Prot. Res*., 50(4): 424-427.

Kim, W. S., Choi, W. J., Lee, S., Kim, W. J., Lee, D. C., Sohn, U. D. and Kim, W. 2015. Anti-inflammatory, antioxidant and antimicrobial effects of Artemisinin extracts from *Artemisia annua* L. *J. Physiol. Pharma.*, 19(1): 21-27.

Klayman, D.L.; Lin, A.J.; Acton, N.; Scovili, J.P.; Hoch, J.M.; Milhous, W.K. andTheoharides, A.D. **1984**. Isolation of artemisinin (Qinghaosu) from *Artemisia annua* growing in the US. *J. Nat. Prod*., 47: 715-717.

Kumar, A. and Singh, A.K., 2021. *Trachyspermum ammi* (AJWAIN): A COMPREHENSIVE REVIEW. *World J. Pharm. Res.* 10(6): 724-736.

Kumar, R., Kumar, R., Prakash, O., Srivastava, R.M. and Pant, A.K., 2019. Chemical composition, in vitro antioxidant, anti-inflammatory and antifeedant properties in the essential oil of Asian marsh weed *Limnophila indica*L.(Druce). *J. pharmacogn. phytochem*., *8*(1): 1689-1694.

Kunwar, G., Prakash, O., Chandra, M. and Pant, A.K., 2013. Chemical composition, antifungal and antioxidant activities of *Perilla frutescens* (L.) syn. *P. ocimoides* L. collected from different regions of Indian Himalaya. *Asian J. Trad. Med*. 8: 88-98.

Lawrence, G.H.M. 1973. Taxonomy of Vascular Plants. Oxford and IBM Publishing Co. *Rakesh Press, New Delhi,* India. 823 p.

Lee, J., Kim, M. H., Lee, J. H., Jung, E., Yoo, E. S. andPark, D. **2012**. Artemisinic acid is a regulator of adipocyte differentiation and C/EBP delta expression. *J. Cell. Biochem.*, 113:2488–2499.

Leyton, M., Mellado, M., Jara, C., Montenegro, I., González, S. and Madrid, A. 2015. Free radical-scavenging activity of sequential leaf extracts of *Embothrium coccineum. Open. Life Sci*., 10(1): 260-268.

Libbey, L.M. and Sturtz, G., 1989. Unusual Essential Oils Grown in Oregon II. *Artemisia annua* L. *J. Essent. Oil Res*., 1(5): 201-202.

Lohani, H., Gwari, G., Bhandari, U., Haider, S. Z., Andola, H., and Chauhan, N 2016. Variability in the essential oils from aerial parts of *Artemisia vulgaris* L. grown in Uttarakhand (India). *J. Essent. Oil-Bear. Plants.*, 19(1): 103-107.

Lopez-Lutz, D., Alviano, D.S., Alviano, C.S., Kolodziejczyk, P.P., 2008. Screening of chemical composition, antimicrobial and antioxidant activities of *Artemisia* essential oils. *J. Phytochem*., 69: 1732–1738.

Lubbe, A., Seibert, I., Klimkait, T. and Van der Kooy, F. 2012. Ethnopharmacology in overdrive: the remarkable anti-HIV activity of *Artemisia annua*. *J. Ethnopharmacol.*, 141(3):854-859.

Ludwiczuk, A., Skalicka-Wozniak, K., and Georgiev, M. I. 2017. Terpenoids. *Pharmacogn.*, Academic Press. pp. 233-266.

Mabberley, D.J.2017. Mobberley's Plant-Book: A portable dictionary of plants, their classification and uses. *Cambridge University Press*. 1102 p.

Mahboubi, M., 2014. *Artemisia sieberi* in traditional and modern medicine. *J. Biol. Act. Prod. Nat.*, 4(2): 149-157.

Malebo, H. M., Tanja, W., Cal, M., Swaleh, S. A., Omolo, M. O., Hassanali, A. and Ndiege, I.2009. Antiplasmodial, anti-trypanosome, anti-leishmanial and cytotoxicity activity of selected Tanzanian medicinal plants *Tanzan. J. Health Res*., 11:226–234.

Malviya N, Jain S and Malviya S. 2010. Antidiabetic potential of medicinal plants. *Acta. Pol. Pharm*., 67(2): 113-118.

Mandal, D. I. P. A. K., Bhowmik, P. A. R. A. M. I. T. A., and Baral, K. A. N. C. H. A. N. 2013. Evaluation of insecticides for the management of Bihar hairy caterpillar, *Spilosoma oblique* walk.(Lepidoptera: Arctiidae) in black gram (*Vignamungo* L.). *The Bioscan*., 8(2), 429-431.

Manns, D. and Hartmann, R. 1992. Annuadiepoxide, a new polyacetylene from the aerial parts *of Artemisia annua*. *J. Nat. Prod*., 55:29-32.

Marinas, I.C.; Oprea, E.; Chifiriuc, M.C.; Badea, I.A.; Buleandra and M.; Lazar, V.2015. Chemical composition and antipathogenic activity of *Artemisia annua* essential oil from Romania. *Chem. Biodivers*., 12:1554-1564.

Maritim, A. C., Sanders, A., and Watkins Iii, J. B. 2003. Diabetes, oxidative stress, and antioxidants: a review. *J. Biochem. Mol. Toxicol.*, 17(1), 24-38.

Martini, M.C., Zhang, T., Williams, J. T., Abramovitch, R. B., Weathers, P. J. and Shell, S.2020. *Artemisia annua* and *Artemisia afra* extracts exhibit strong bactericidal activity against *Mycobacterium tuberculosis*. *J. Ethnopharmacol*., 262:113-191.

Martins, F. T., Doriguetto, A. C., de Souza, T. C., de Souza, K. R., dos Santos, M. H., Moreira, M. E., and Barbosa, L. C. 2008. Composition, and anti-inflammatory and antioxidant activities of the volatile oil from the fruit peel of *Garcinia brasiliensis*.*Chem. Biodivers*., 5(2): 251-258.

Masotti, V., Juteau, F., Bessière, J.M. and Viano, J., 2003. Seasonal and phenological variations of the essential oil from the narrow endemic species *Artemisia molinieri* and its biological activities. *J. Agric. Food Chem*., 51(24): 7115-7121.

Massiha, A., Khoshkholgh-Pahlaviani, M. M., Issazadeh, K., Bidarigh, S., and Zarrabi, S. 2013. Antibacterial activity of essential oils and plant extracts of Artemisia (*Artemisia annua L*.) *in vitro*. Zahedan . *J. Res. Med. Sci*., *15*(6):14.

Mata AT, Proenaca C, Ferreira AR, Serralheiro ML, Nogueira JM and Araujo ME. 2007. Antioxidant and anti acetylcholinesterase activities of five plants used as Portuguese food spices. *Food Chem*., 103:778–86.

Máthé, Á., Hassan, F., and Kader, A. A. 2015. Medicinal and aromatic plants of the world. *Med Aromat. Plants. World*., 4(6): 287-345.

Mathela, C.S., Kharkwal, H.and Shah, G.C. 1994. Essential oil composition of some Himalayan *Artemisia* species. *J. Essent. Oil. Res.*, 6: 345–348.

Mercke, P., Bengtsson, M., Bouwmeester, H. J., Posthumus, M. A. and Brodelius, P. E. 2000. Molecular cloning, expression, and characterization of amorpha 4, 11-diene synthase, a key enzyme of artemisinin biosynthesis in *Artemisia annua L.Arch. Biochem. Biophy*., 381:173-180.

Mesa, L. E., Vasquez, D., Lutgen, P., Vélez, I. D., Restrepo, A. M., Ortiz, I. and Robledo, S. M. 2017. *In vitro* and *in vivo* antileishmanial activity of *Artemisia annua* L. leaf powder and its potential usefulness in the treatment of uncomplicated cutaneous leishmaniasis in humans. *Rev. Soc. Bras. Med. Tr*., 50(1):52-60.

Metcalfe, C. R. and Chalk, L. 1950, Anatomy of the Dicotyledons: Leaves, Stem, and Wood in Relation to Taxonomy with Notes on Economic Uses. *Claredon Press, Oxford*. 2 p.

Miguel, M. G. 2010. Antioxidant and anti-inflammatory activities of essential oils: a short review. *Mol*., 15(12): 9252-9287.

Miraj, S. and Alesaeidi, S. 2016. A systematic review of anti-malarial properties, immunosuppressive properties, anti-inflammatory properties, and anti-cancer properties of *Artemisia annua*. *Elect. Phys.*, 8(10): 31-50.

Mishra, K., Ojha, H., and Chaudhury, N. K. 2012. Estimation of antiradical properties of antioxidants using DPPH assay: A critical review and results. *Food. Chem.,* 130(4): 1036-1043.

Mohammadreza, V.R., 2008. Variation in the essential oil composition of *Artemisia annua* L. of different growth stages cultivated in Iran. *Afr. J. Plant Sci*., 2(2): 016-018.

Mojarab-Mahboubkar, M., Sendi, J.J. and Aliakbar, A., 2015. Effect of *Artemisia annua* L. essential oil on toxicity, enzyme activities, and energy reserves of cotton bollworm *Helicoverp aarmigera* (Hübner) (Lepidoptera: Noctuidae). *J. Plant Prot. Res*, 55(4): 371-377.

Montenegro, I. J., del Corral, S., Diaz Napal, G. N., Carpinella, M. C., Mellado, M., Madrid, A. M., and Cuellar, M. A. 2018. Antifeedant effect of polygodial and drimenol derivatives against *Spodoptera frugiperda* and *Epilachna paenulata* and quantitative structure activity analysis. *Pest Manag. Sci.,* 74(7): 1623-1629.

MR, Verdian-rizi 2009. Chemical composition and antimicrobial activity of the essential oil of *Artemisia annua* L. from Iran. *Pharmacogn. Res.*, 1(1): 21-24.

Muller, M., and Buchbauer, G. 2011. Essential oil components as pheromones. A review. *Flavou. Fragr. J.*, 26(6): 357-377.

Naik, P. M., and Al-Khayri, J. M. 2016. Abiotic and biotic elicitors–role in secondary metabolites production through in vitro culture of medicinal plants. *Abiotic and Biotic Stress in Plants, Recent Advances Future Perspectives. Intech.Open* 2(8): 247-277.

Nazir, N., Zahoor, M., Uddin, F. and Nisar, M. 2021. Chemical composition, in vitro antioxidant, anticholinesterase, and antidiabetic potential of essential oil of *Elaeagnus umbellate* Thunb. *BMC. Complement. Altern. Med.*, 21(1): 1-13.

Nekoei, M., Mohammadhosseini, M. and Akhlaghi, H. 2012. Chemical composition of the volatile oils from the aerial parts of *Artemisia annua L.* (Asteraceae) by using head space solid phase microextraction and hydro distillation methods prior to gas chromatographic-mass spectrometric determination: A comparative investigation. *J. Essent. Oil-Bear. Plants*., 15(6):926-933.

Nerio, L. S., Olivero-Verbel, J., and Stashenko, E. 2010. Repellent activity of essential oils: a review. *Bioresour. Technol*., 101(1): 372-378.

Nessler CR. 1994. Metabolic engineering of plant secondary products. *Transgenic. Res.,* 3:109–115.

Nofal SM, Mahmoud SS, Ramadan A, Soliman GA andFawzy R. 2**009**. Anti diabetic effect of *Artemisia judaica* extracts. *Res. J. Med. Sci*., 4(1):42–48.

Ntalli, N.G., Ferrari, F., Giannakou, I. and Menkissoglu Spiroudi, U., 2011. Synergistic and antagonistic interactions of terpenes against *Meloidogyne incognita* and the nematicidal activity of essential oils from seven plants indigenous to Greece. *Pest Manag., Sci*., 67(3): 341-351.

Ogbole E. A., Ogbole, Y., Peter, J. Y., Builders, M. I. and Aguiyi, J. C. 2014. Phytochemical screening and *in vivo* antiplasmodial sensitivity study of locally cultivated *Artemisia annua* leaf extract against *Plasmodium berghei*. *Am. J. Ethnomed,* 1(1):42-49.

Olsson, M. E., Olofsson, L. M., Lindahl, A. L., Lundgren, A., Brodelius, M. and Brodelius, P. E.2009. Localization of enzymes of artemisinin biosynthesis to the apical cells of glandular secretory trichomes of *Artemisia annua L*. *Phytochem.*, 70:1123–1128.

Omer, E.A., Abou Hussein, E.A., Hendawy, S.F., Ezz El-din Azza, A. and El Gendy, A.G., 2013. Effect of soil type and seasonal variation on growth, yield, essential oil and artemisinin content of *Artemisa annua* L. *Int. Res. J. Hortic.*, 1(1) :15-27.

Padalia, R.C., Verma, R.S., Chauhan, A., Chanotiya, C.S. and Yadav, A., 2011. Variation in the volatile constituents of *Artemisia annua* var. CIM-Arogya during plant ontogeny. *Nat. Prod.Commun*., 6(2):239-242.

Paddon, C. J., Westfall, P. J., Pitera, D. J., Benjamin, K., Fisher, K. and McPhee, D.2013. High-level semi-synthetic production of the potent antimalarial artemisinin. *Nat.*, 496:528–532.

Panda, S. K., da Silva, L. C. N., Sahal, D., and Leonti, M. 2019. Ethnopharmacological studies for the development of drugs with special reference to Asteraceae. *Front. pharmacol.*, 10: 955.

Pandey, A. K. and Singh, P. 2017. The genus *Artemisia*: A literature review on chemical composition, antimicrobial, insecticidal and antioxidant activities of essential oils. *Med.*, 4(3):68.

Patel DK, Kumar R, Prasad SK, Sairam K and Hemalatha S. 2011. Antidiabetic and in vitro antioxidant potential of *Hybanthusennea spermus* (Linn) F. Muell in streptozotocin-induced diabetic rats. *Asian Pac. J. Trop. Biomed*., 1(4): 316-322.

Ponnusamy S, Ravindran R, Zinjarde S, Bhargava S and Kumar AR. 2011. Evaluation of traditional Indian antidiabetic medicinal plants for human pancreatic amylase inhibitory effect in vitro. *Evid. Based Complement .Alternat. Med*., 2011: 515647.

POWO, 2021. "Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. Published on the Internet; http://www.plantsoftheworld online.org/Retrieved 13 August 2021."

Prakash, O., Gondwal, M., and Pant, A. K. 2011. Essential oils composition and antioxidant activity of water extract from seeds and fruit pulp of *Skimmia anquetilia* NP Taylor & Airy Shaw. *Indian. J. Nat.Prod. Resour*., 2(4): 435-441.

R. S. Bhakuni, D. C. Jain, R. P. Sharma, and S. Kumar. 2001. Secondary metabolites of *Artemisia annua* and their biological activity. *Curr. Sci.*, 80(1): 35–48.

Radulović, N.S., Randjelović, P.J., Stojanović, N.M., Blagojević, P.D., Stojanović-Radić, Z.Z., Ilić, I.R. and Djordjević, V.B., 2013. Toxic essential oils. Part II: Chemical, toxicological, pharmacological and microbiological profiles of *Artemisia annua* L. volatiles. *Food Chem. Toxicol.*, 58: 37-49.

Rahimi, A. R., Mousavizadeh, S. J., Mohammadi, H., Rokhzadi, A., Majidi, M., and Amini, S. 2013. Allelopathic effect of some essential oils on seed germination of *Lathyrus annuus* and *Vicia villosa*. *J Biodivers Environ Sci.*, 3(4): 67-73.

Rana, V.S.; Juyal, J.P.; Blazquez, M.A. and Bodakhe, S.H. 2003. Essential oil composition of *Artemisia parviflora* aerial parts. *Flavour. Fragr. J*., 18:342–344.

Rao, M. R., Palada, M. C., and Becker, B. N. 2004. Medicinal and aromatic plants in agroforestry systems. *New. Vist. in agroforest*, pp. 107-122.

Rashid, A. N. M.M., Hossain, M. S., Hassan, N., Dash, B. K., Sapon, M. A. and Sen, M. K. 2014. A review on medicinal plants with antidiabetic activity. *Int. J. Pharmacogn. Phytochem.*, 3(4):149-159.

Rashid, S.; Rather, M.A.; Shah, W.A. and Bhat, B.A.2013. Chemical composition, antimicrobial, cytotoxic and antioxidant activities of the essential oil of *Artemisia indica*. *Food.Chem*., 138:693–700.

Rezelman D. and Goris H.2008. The role of herbal products containing *Artemisia annua* in malaria treatment, a proposal for further research. *Tradit. Complement. Med.*, 4(1): 121.

Rolta, R., Sharma, A., Sourirajan, A., Mallikarjunan, P. K., and Dev, K.2021. Combination between antibacterial and antifungal antibiotics with phytocompounds of *Artemisia annua* L: a strategy to control drug resistance pathogens. *J. Ethnopharmacol.*, 266:113420.

Roy S, Mukhopadhyay A. 2010. . Field efficacy of a biopesticide prepared from *Clerodendrumviscosum* Vent. (Verbenaceae) against two major tea pests in the sub-Himalayan tea plantation of North Bengal, India . *Pestic. Sci.,* 83 : 371 – 77.

Rungqu, P., Oyedeji, O., Nkeh-Chungag, B., Songca, S., Oluwafemi, O. and Oyedeji, A., 2016. Anti-inflammatory activity of the essential oils of *Cymbopogon validus* (Stapf) Stapf ex Burtt Davy from Eastern Cape, South Africa. *Asian Pac. J. Trop. Med*., 9(5): 426-431.

Ryu, J. H., Lee, S. J., Kim, M. J., Shin, J. H., Kang, S. K., Cho, K. M. and Sung, N. J. 2011. Antioxidant and anticancer activities of *Artemisia annua* L. and determination of functional compounds. *J. Korean. Soc. Food. Sci. Nutri.*, 40(4):509-516.

Sadiq, A., Hayat, M. Q., and Ashraf, M. 2014. Ethnopharmacology of *Artemisia annua* L.: A review. *Artemisia annua-Pharm. Biotechnol*., 2(5): 9-25.

Sah, S.; Lohani, H., Narayan, O., Bartwal, S. and Chauhan, N.K. 2010, .Volatile constituents of *Artemisia maritima* Linn. grown in Garhwal Himalaya. *J. Essent. Oil Bear. Plants*., 13: 603–606

Sánchez-Moreno, C. 2002. Methods used to evaluate the free radical scavenging activity in foods and biological systems. *Food Sci. Technol*., 8(3): 121-137.

Sangwan NS, Farooqi AHA, Shabih F and Sangwan RS 2001. Regulation of essential oil production in plants. *Plant. Growth. Regul.,* 34(1):3-21.

Santomauro, F., Donato, R., Sacco, C., Pini, G., Flamini, G. andBilia, A. R. 2016. Vapour and liquid-phase *Artemisia annua* essential oil activities against several clinical strains of *Candida. Planta Med*., 82:1016–1020.

Sati, S.C.; Sati, N.; Ahluwalia, V.; Walia, S. and Sati, O.P. 2013. Chemical composition and antifungal activity of *Artemisia nilagirica* essential oil growing in northern hilly areas of India. *Nat. Prod. Res*., 27: 45–48.

Savithramma, N., Rao, M. L., and Suhrulatha, D. 2011. Screening of medicinal plants for secondary metabolites. *Middle East J. Sci. Res.*, 8(3): 579-584.

Semwal, R.B.; Semwal, D.K.; Mishra, S.P. and Semwal, R. 2015.Chemical composition and antibacterial potential of essential oils from *Artemisia cappillaris, Artemisia nilagirica,* Citrus limon, *Cymbopogon flexuosus, Hedychium spicatum* and *Ocimum tenuiflorum*. *Nat. Prod. J*., 5: 199–205.

Shah GC. 2010. Terpenoid diversity in some Artemisia species of Uttarakhand Himalaya. *Indian. Perfumer*. 54:17-9.

Shakil, N.A., Prasad, D., Saxena, D.B. and Gupta, A.K., 2004. Nematicidal activity of essential oils of *Artemisia annua* against root-knot and reniform nematodes. *Ann. Plant Sci.*, 12(2): 397-402.

Sharma, J. R., Mugdhal, V. and Hajra, P. K. 1997. Floristic diversity and conservation strategies in India (Eds Mugdhal, V. and Hajrap.k.). Botanical Survey of India, Ministry of Environment and Forests.5p.

Sharma, N., 2021. Folkare and Modern Pharmacology of Camphor (*Cinnamomum camphora* Nees and Eberm): Review. *Sch. Int. J. Tradit Complement. Med*., 4(7): 128-135.

Singh, A., Palariya, D., Dhami, A., Prakash, O., Kumar, R., Rawat, D. S. and Pant, A. K. 2020. Biological activities and Phytochemical analysis of *Zanthoxylum armatum* DC. leaves and bark extracts collected from Kumaun region, Uttarakhand, India. *J. Ethnobiol. Ethnomed*., 6: 1-10.

Soetaert, S. S., Van Neste, C. M., Vandewoestyne, M. L., Head, S. R., Goossens, A. and Van Nieuwerburgh, F. C.2013. Differential transcriptome analysis of glandular and filamentous trichomes in *Artemisia annua*. *BMC Plant Biol*., 13:220.

Squires JM and Ferreira JF. 2009. Anti-Diabetic effect of *Artemisia judaica* extracts. *Res. J. Med. Sci.*, 4(1):87-92.

Tahir, H. U., Sarfraz, R. A., Ashraf, A., and Adil, S. 2016. Chemical composition and antidiabetic activity of essential oils obtained from two spices (*Syzygium aromaticum* and *Cuminum cyminum*).*Int. J. Food Prop*., 19(10): 2156-2164.

Tan, C. H., Rasool, S., and Johnston G. A. 2014. "Contact Dermatitis: Allergic and Irritant." *Clin. Dermatol*., 32 (1): 116-24.

Terryn N, Van Montagu M, Inze´ D and Goosens A. 2006. Functional genomic approaches to study and engineer secondary metabolites in plant cell cultures. In: Bogers LJ, Craker LE, Jange D (eds) Medicinal and aromatic plants. Springer, The Netherland: 291-300.

Tewan, K.; Tewari, G.; Pande, C. and Kunwar, G. 2015. Volatile constituents of *Artemisia parviflora* Buch.-Ham. ex Roxb. from Kumaun Himalaya region, *India. J. Essent. Oil Bear. Plants.*, 18:195–198.

Thomas, J., Joy, P. P., Mathew, S., and Skaria, B. P. 1999. Development of Medicinal and Aromatic plants-Outlook for the next millennium. *Indian.J. Arecanu. Species. Med. Pl.*, 1(2): 38-41.

Tripathi, A.K., Prajapati, V., Aggarwal, K.K. and Kumar, S., 2001. Toxicity, feeding deterrence, and effect of activity of 1, 8-cineole from *Artemisia annua* on progeny production of *Tribolium castanaeum* (Coleoptera: Tenebrionidae). *J. Econ. Entomol.*, 94(4): 979-983.

Uniyal, B.P., Sharma, J.R., Choudhery, U. andSingh, D.K. 2007. Flowering Plants of Uttarakhand (A Checklist) Bishen Singh Mahendra Pal Singh, Dehra Dun. 404.

V. Dhingra, K. Vishweshwar Rao, and M. Lakshmi Narasu. 1**999**. Current status of artemisinin and its derivatives as antimalarial drugs *Life. Sci.*, 66(4): 279–300.

V. Mohammadreza. 2008. **Variation in the essential oil composition of** *Artemisia annua* ***L*****. of different growth stages cultivated in Iran.** *Afr. J. Plant Sci*., 2(2):16-18

Verma RS, Rahman L, Verma RK, Chanotiya CS, Chauhan A, Yadav A, Yadav AK, Singh A. 2010 Changes in the essential oil content and composition of *Origanum vulgare* L. during annual growth from Kumaon Himalaya. *urr. Sci***.**, 98: 1010-1012.

Verma, M.K., Sharma, S. and Kumar, S., 2020. A review on pharmacological properties of *Artemisia annua*. *J. pharmacogn. Phytochem*., 9(6): 2179-2183.

Wang, H. F., Wang, Y. K., and Yih, K. H. 2008. DPPH free-radical scavenging ability, total phenolic content, and chemical composition analysis of forty-five kinds of essential oils. *J. Cosmet Sci*., 59(6): 509–522.

Weathers, P. J., Elkholy, S., and Wobbe, K. K. 2006. Artemisinin: the biosynthetic pathway and Its regulation in *Artemisia annua*, a terpenoid-rich species. *In Vitro Cell Dev. Biol. Plant*., 42:309–317.

Weisshaar, B. and Jenkines, G. 1998. Phenylpropanoid biosynthesis and its regulation. *Curr. Opin. Plant Biol.*, 1(3): 251-257.

Wen, W., and Yu, R. 2011. Artemisinin biosynthesis and its regulatory enzymes: progress and perspective. *Pharmacogn. Rev.* 5:189–194.

Woerdenbag, H. J; Bos, R ;Salomons, M. C; Hendriks, H.; Pras, N. and Malingre, T. M. 1993.Volatile constituents of *Artemisia annua* L. (Asteraceae). *Flavour. Frag, J.*, 8:131-137.

Xu, Q., Zhang, L., Yu, S., Xia, G., Zhu, J. and Zang, H., 2021. Chemical composition and biological activities of an essential oil from the aerial parts of *Artemisia gmelinii* weber ex Stechm. *Nat. Prod. Res.*, 35(2): .346-349.

Xu, Z. and Chang, L. 2017. Asteraceae. In: Identification and Control of Common Weeds. 3.pp. 441-721.

Zandi-Sohani N., Hojjati M., Angel A., and Barrachina C., 2012. Bioactivity of *Lantana camara* L. essential oil against *Callosobruchus maculatus* (Fabricius). *Chil. J. Agric. Res*., 72(4): 502.

Zarrelli, A., Pollio, A., Aceto, S., Romanucci, V., Carella, F., Stefani, P. and De Vico, G. 2019. Optimisation of artemisinin and scopoletin extraction from *Artemisia annua* with a new modern pressurised cyclic solid–liquid (PCSL) extraction technique. *Phytochem. Anal.*, 30(5):564-571.

Zhang, Y., Teoh, K. H., Reed, D. W., Maes, L., Goossens, A. and Olson, D. J. 2008. The molecular cloning of artemisinic aldehyde Delta 11(13) reductase and its role in glandular trichome-dependent biosynthesis of artemisinin in *Artemisia annua*. *J. Biol. Chem.*, 283:21501-21508.

Zhigzhitzhapova, S.V., Dylenova, E.P., Gulyaev, S.M., Randalova, T.E., Taraskin, V.V., Tykheev, Z.A. and Radnaeva, L.D., 2020. Composition and antioxidant activity of the essential oil of *Artemisia annua* L. *Nat. Prod. Res.*, 34(18): 2668-2671.

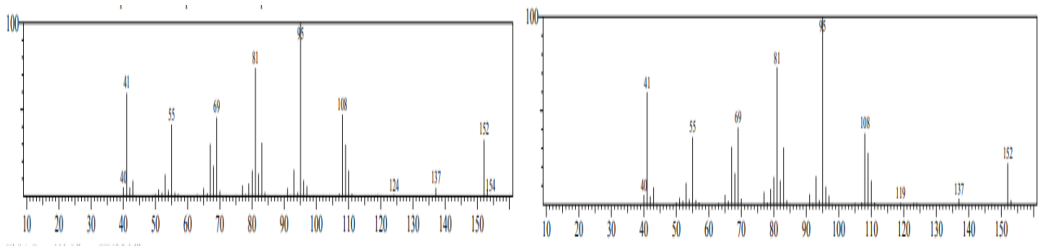
Appendices

Appendix-1

Mass spectra of identified constituents in the essential oils of *Artemisia annua* rainy essential oil (AAREO*)* and *Artemisia annua* winter essential oil *(*AAWEO*)*

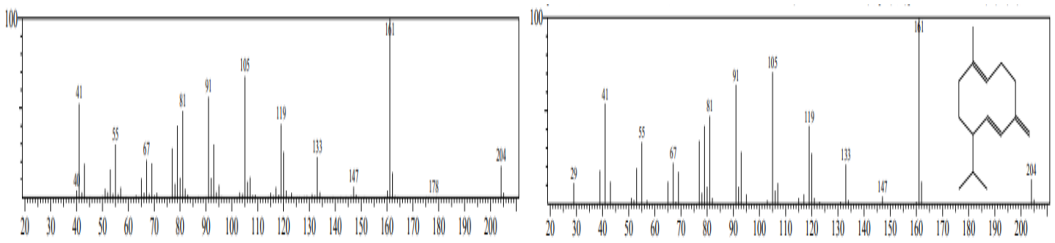
1) Constituent name: camphor, Formula: $C_{10}H_{16}O$, Molecular weight: 152

Target **Constituent identified: camphor**



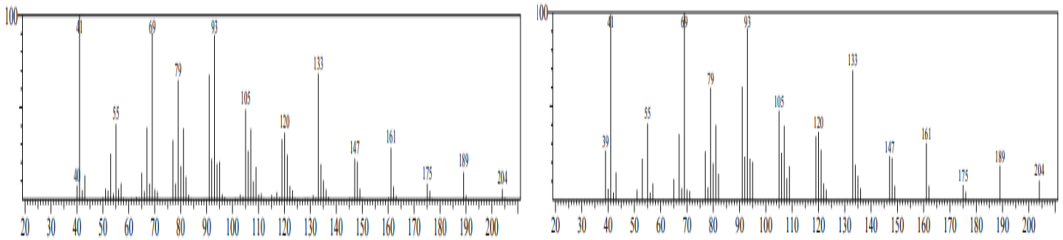
2) Constituent name: germacrene-D, Formula: $C_{15}H_{24,}$ Molecular weight: 204

Target **Constituent identified: germacrene-D**



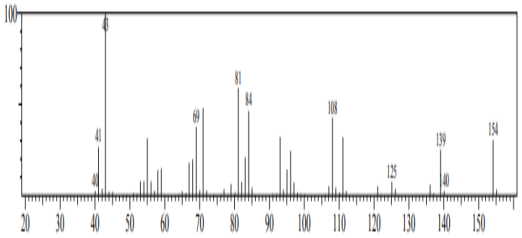
3) Constituent name: *trans*-β-caryophyllene, Formula: $C_{15}H_{24,}$ Molecular weight: 204

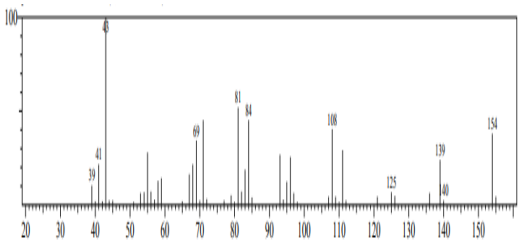
Target **Constituent identified: *trans*-β-caryophyllene**



4) Constituent name: eucalyptol, Formula: $C_{10}H_{18}O$, Molecular weight: 154

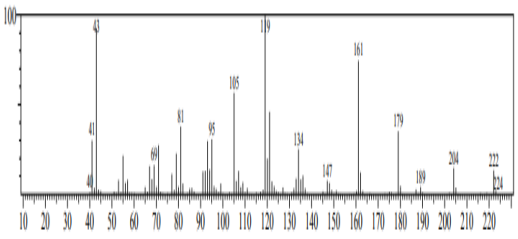
Target **Constituent identified: eucalyptol**

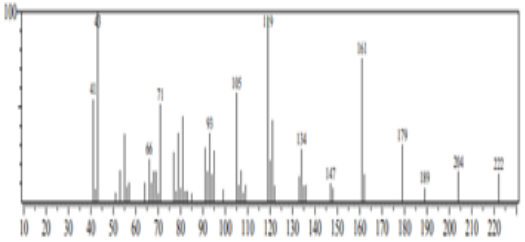




5) Constituent name: *cis*-cadin-4en-7-ol, Formula: $C_{15}H_{26}O$, Molecular weight: 222

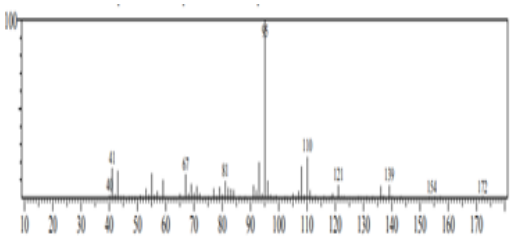
Target **Constituent identified: *cis*-cadin-4en-7-ol**

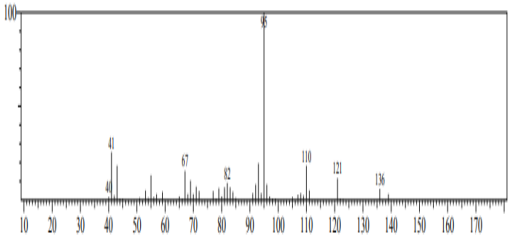




6) Constituent name: isoborneol, Formula: $C_{10}H_{18}O$, Molecular weight: 154

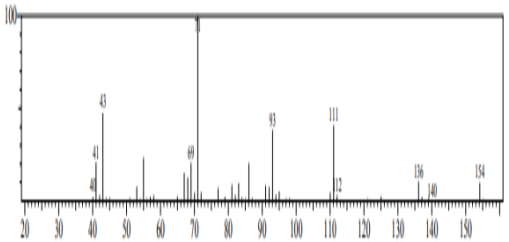
Target **Constituent identified: isoborneol**

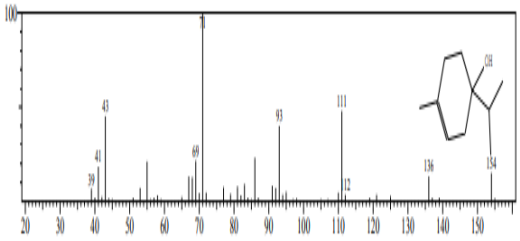




7) Constituent name: terpinene-4-ol, Formula: $C_{10}H_{18}O$, Molecular weight: 154

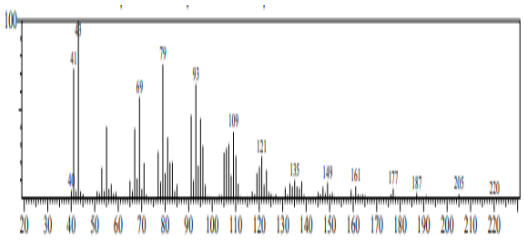
Target **Constituent identified: terpinene-4-ol**

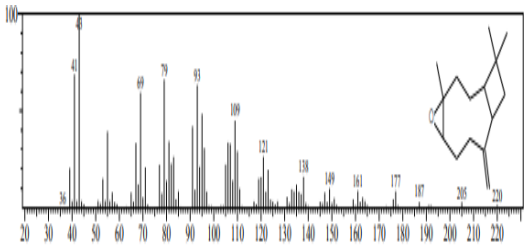




8) Constituent name: caryophyllene oxide, Formula: $C_{15}H_{24}O$, Molecular weight: 220

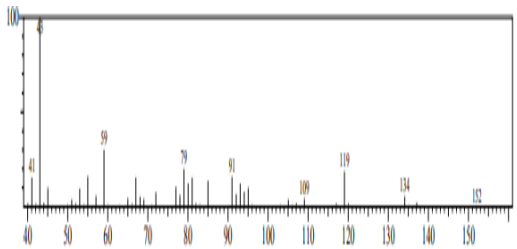
Target **Constituent identified: caryophyllene oxide**

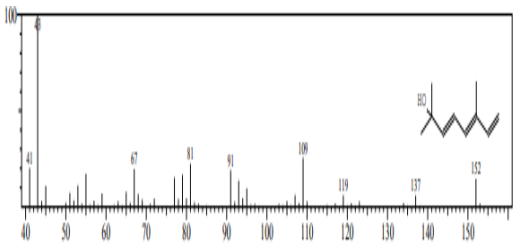




9) Constituent name: (E,E)-2,6-dimethyl-3,5,7-octatrien-2-ol, Formula: $C_{10}H_{16}O$, Molecular weight: 152

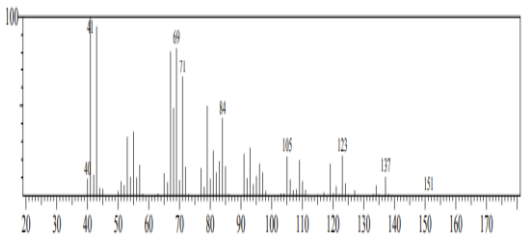
Target **Constituent identified: (E,E)-2,6-dimethyl-3,5,7-octatrien-2-ol**

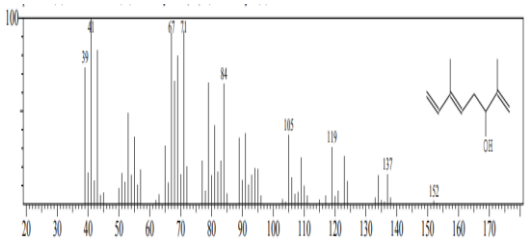




10. Constituent name: (5E)-2,6-dimethyl-1,5,7-octatrien-3-ol, Formula: $C_{10}H_{16}O$, Molecular weight: 152

Target **Constituent identified: (5E)-2,6-dimethyl-1,5,7-octatrien-3-ol**





CURRICULUM VITAE

Name	**:**	Ms. Akanksha Malhotra	**Phone Number**	**:**	9084558989
Mailing Address	**:**	H. No.- 22/6, Premnangar, Dehradun, Uttarakhand	**Permanent Address**	**:**	H. No.- 22/6, Premnangar, Dehradun, Uttarakhand
E-mail	**:**	Ashimalhotra764@gmail.com			
Career Objective	**:**	To expand my learnings, knowledge and skills.			

Educational Qualification :

S. N.	Examination Passed	Institution	Year	Percentage/CGPA
1.	M.Sc.	GBPUA&T, Pantnagar	2021	Pursuing
2.	B.Sc.	D.A.V. P.G. college, Dehradun	2019	6.98
3.	Intermediate	Shri guru nanak public senior secondary school	2015	80.2%
4.	High School	Shri guru nanak public senior secondary school	2013	8.6

Specialization: Major: Chemistry Minor: Nil

Publication: Nil

Conference/Seminars/Workshops/Training Attended: 2

List of papers presented in conference/seminar during degree programme: Nil.

Software Skills: MS Word, MS Excel, MS Power Point.

Professional Skills: Critical thinking and problem solving.

Professional Affiliations (Membership, etc.,): Subject matter expert at chegg india

Awards/Honours/Achievements: Nil.

(Akanksha Malhotra)

Place: Pantnagar
Date:

Name	:	Akanksha Malhotra	**Id. No.**	:	55503
Sem. and year of admission	:	1st Sem., 2019-20	**Degree**	:	M.Sc.
			Department	:	Chemistry
Major	:	Chemistry			
Thesis title	:	**"Seasonal Variation in Chemical Composition and Biological Activities of Essential Oils from *Artemisia annua* L."**			
No. of Pages	:	126	**Advisor**	:	**Dr. Om Prakash**

ABSTRACT

The objectives of the present research study were to investigate the seasonal variation in phytochemical analysis and biological activites of *Artemisia annua* essential oils. The plants were collected from Tarai region of Uttarakhand, Pantnagar in the month of September, 2020 and January 2021 respectively .The essential oils of fresh aerial part of the collected plant materials were extracted by hydrodistillation using Clevenger apparatus and were studied by GC/MS for its phytochemical study and various biological activities. The essential oils isolated in rainy and winter season were designated as AAREO & AAWEO respectively.

The phytochemical analysis of AAREO revealed the identification of 75 constituents which contributed 91.7% of the total essential oil. The major components of AAREO were camphor (14.1%) followed by germacrene –D (9.0%), *trans*-β-caryophyllene (8.7%), eucalyptol (4.2%), *cis*-cadin-4en-7-ol (3.9%), isoborneol (3.2%), p-cymene (2.9%) etc. Similarly, in AAWEO 59 constituents were identified which contributed 94.1% of the total essential oil. The major components of AAWEO were camphor (17.9%) followed by eucalyptol (14.6%), (*E*,*E*)-2,6-dimethyl-3,5,7-octatrien-2-ol (6.3%), (*5E*)-2,6-dimethyl-1,5,7-octatrien-3-ol (5.2%), artemisia alcohol (3.5%), isoborneol (3.2%), caryophyllene oxide (3.1%), artemisia ketone (2.5%) etc besides other minor and trace constituents. Both AAREO & AAWEO showed qualitative and quantitative differences in their phytochemical makeup.

Both AAREO and AAWEO exhibited dose dependent *in-vitro* anti-oxidant activity, evaluated by , DPPH scavenging, metal chelating assay and H_2O_2 radical scavenging as indicated by different IC_{50} values. IC_{50} =77.87±0.528 to IC_{50}=45.588±0.334 µl/mL for DPPH radical scavenging activity similarly IC_{50} =51.40±0.158 to IC_{50}=49.50±0.234 for metal chelating activity and IC_{50} = 44.83±1.304 to IC_{50} =34.79±0.419 for H_2O_2 radical scavenging activity with respect to various standards like, BHT and Na_2EDTA respectively. With respect to diclofenac sodium, the standard anti-inflammatory drug both AAREO and AAWEO exhibited anti-inflammatory activity with IC_{50}= AAWEO (IC_{50}=66.90±0.040) > AAREO (IC_{50}=76.55±0.218) µl/mL respectively. Similarly, significant anti-diabetic activity was observed in both AAREO and AAWEO with IC_{50}= 74.26±3.706µl/mL to IC_{50}= 47.45±0.195µl/mL, respectively compared to standard acarbose (IC_{50}= 38.21±0.03µl/mL). Both AAREO and AAWEO were observed to exhibit strong anti-feedant activity respectively against *Spodoptera litura* (90.85% & 87.92%) .Like wise AAREO & AAWEO were subjected to nematicidal activity against root knot nematode, *Meloidogyne incognita*. It was observed that AAREO exhibited more mortality over AAWEO at different concentration (1-5 µl/mL) , where as in the case of egg inhibition process AAWEO was found to be more effective (5.38% egg hatching) than AAREO (11.41% egg hatching)at a concentration of 10 µl/mL respectively.

Based on observations of above mentioned study, it can be inferred that *Artemisia annua* can be a good natural resource of camphor and 1,8 cineole and the essential oils can be good source of antioxidant, anti-inflammatory, anti-diabetic, anti-feedant and nematicidal activities after proper clinical trials.

(Om Prakash)
Advisor

(Akanksha Malhotra)
Authoress

नम	:	अकांक्षा मल्होत्रा	**परिचयांक**	:	55503
षट्मास एवं प्रवेष वर्ष	:	प्रथम, 2019-2020	**डिग्री**	:	एम0एस0सी0
प्रमुख विषय	:	रासायनिक विज्ञान	**विभाग**	:	रासायनिक विज्ञान
शोध का शीर्षक	:	आर्टेमिसिया एनुआ के सुगंधित तेलों का मौसमी रासायनिक विश्लेषण तथा जैविक गतिविधियां			
पृष्ठ संख्या	:	126	**सलाहकार**	:	डॉ0 ओम प्रकाश

सारांश

वर्तमान शोध का उद्देश्य आर्टेमिसिया एनुआ के सुगंधित तेलों की मौसमी संरचना पर प्रभाव तथा विभिन्न जैविक गतिविधियों का अध्ययन था। शोध हेतु पौधों को पंतनगर के तराई क्षेत्र से क्रमशः सितंबर 2020 तथा जनवरी 2021 के महीने में एकत्रित किया गया। सुगन्धित तेलों को हाइड्रोडिस्टीलेशन विधि द्वारा क्लेवेन्जर उपकरण के माध्यम से निकाला गया। सुगन्धित तेलों को गैस वर्ण लेखन तथा मास स्पेक्ट्रोमेट्री विधि द्वारा विश्लेषण किया गया। विभिन्न मौसमो में एकत्रित सुगन्धित तेलों को AAREO (बरसाती मौसम) तथा AAWEO (सर्दी) नाम से नामित किया गया।

AAREO में 75 अवयव पाए गए जो कुल सुगन्धित तेल का 91.7% प्रतिशत थे। AAREO के प्रमुख घटक कैम्फर (14.1%), डी-जर्मैक्रिन (9.0%), ट्रांस-β-कैरीओफ़ाइलीन (8.7%), सीस-कैडिन-4-एन-7-ओल (3.9%), युकालिपटोल (4.2%), आइसोबोर्नियोल (3.2%), पी-सायमीन (2.9%) आदि थे। इसी प्रकार, AAWEO में 59 अवयव पहचाने गए जिनकी मात्रा 94.1% पायी गयी। AAWEO के प्रमुख घटक कैम्फर (17.9%), युकालिपटोल (14.6%), 2इ, 6इ-डाइमिथाइल-3,5,7-ऑक्टेट्रियन-2-ओल (6.3%), 2इ,6इ -डाइमिथाइल-1,5,7-ऑक्टेट्रियन-3-ओल (5.2%), आर्टेमिसिया अल्कोहल (3.5%), आइसोबोर्नोल (3.2%), कैरियोफिलीन ऑक्साइड (3.1%), आर्टेमिसिया कीटोन (2.5%) आदि थे। AAREO तथा AAWEO दोनों में गुणात्मक और मात्रात्मक अंतर पाया गया।

AAREO तथा AAWEO के द्वारा इन विट्रो प्रति आक्षिकारक प्रतिक्रिया दर्शायी गयी, जिनका अध्ययन डी०पी०पी०एच मूलक उपमार्जक, धातु चिलेटिंग तथा हाइड्रोजन पेरोक्साइड मूलक उपमार्जक विधि से किया गया। प्रतिआक्षिकारक गतिविधि के रूप में डी०पी०पी०एच हेतु IC_{50} = 77.87 ± 0.528 से IC_{50} = 45.588 ± 0.334 माइक्रो ली०प्रति मी०ली० , धातु चिलेटिंग हेतु IC_{50} = 51.40 ± 0.158 से IC_{50} = 49.50 ± 0.234 तथा हाइड्रोजन पेरोक्साइड मूलक उपमार्जक विधि हेतु IC_{50} = 44.83 ± 1.304 से IC_{50} = 34.79 ± 0.419 माइक्रो ली०प्रति मी०ली० पायी गयी। जिनका प्रभाव मानक क्रमश : BHT तथा Na_2EDTA के सापेक्ष देखा गया। डाइक्लोफेनाक सोडियम के सापेक्ष AAREO तथा AAWEO द्वारा एंटी इंफ्लेमेटरी गति विधि पायी गयी जिनका IC_{50} मान क्रमश: AAWEO (IC_{50} = 66.90 ± 0.040)> AAREO (IC_{50} = 76.55 ± 0.218) माइक्रो ली०टी० था। इसी तरह IC_{50}= 74.26±3.076 से IC_{50} =47.45±0.195 एकरबोस (IC_{50} = 38.21 ± 0.03) माइक्रो ली०प्रति मी०ली० के सापेक्ष दोनों सुगन्धित तेलों द्वारा प्रति मधुमेह प्रतिक्रिया दर्शायी गयी। स्पोडोप्टेरा लिटुरा के प्रति AAREO तथा AAWEO द्वारा क्रमश: (90.85% तथा 87.92%) की अच्छी एंटी फीडेंट प्रतिक्रिया पायी गयी। इसी तरह AAREO तथा AAWEO द्वारा मेलोडोगाइनी इन्कॉगनीटा के प्रति निमेटोसाइडल प्रतिक्रिया दर्शायी गयी। AAREO द्वारा 1-5 माइक्रो ली० प्रति मी० ली० की सांद्रता में AAWEO की तुलना में अधिक निमेटोसाइडल नश्वरता पायी गयी, जबकि 10 माइक्रो ली० प्रति मी० ली० की सांद्रता AAWEO (5.38%) में AAREO (11.41%) की तुलना में ज्यादा अंडा निषेधक प्रतिक्रिया पायी गयी।

उपर्युक्त अध्ययन की टिप्पणियों के आधार पर, यह कहा जा सकता है कि आर्टेमिसिया एनुआ, कैम्फर और 1,8 सिनेओल का एक अच्छा प्राकृतिक संसाधन हो सकता है और उचित क्लीनिकल परीक्षणों के बाद आवश्यक तेल एंटी-ऑक्सिडेंट, एंटी-डायबिटीज , एंटी-फीडेंट और नेमाटीसाइडल का अच्छा स्रोत हो सकता है।

(ओम प्रकाश)
सलाहकार

(अकांक्षा मल्होत्रा)
लेखिका