Chemo profiling of *Nerium oleander* (L.) and *Cascabela thevetia* (L.) leaf extracts and their biological activities

Thesis

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By

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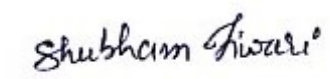

December, 2020
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CERTIFICATE

This is to certify that the thesis entitled **"Chemo Profiling of *Nerium oleander* (L.) and *Cascabela thevetia* (L.) leaf extracts and their biological activities"** submitted in partial fulfilment of the requirements for the degree of **Masters of Science** with major in **Chemistry** of the College of Post Graduate Studies, G.B. Pant University of Agriculture & Technology, Pantnagar, is a record of *bona fide* research carried out by **Mr. Shubham Tiwari, Id. No. 53995**, under my supervision and no part of the thesis has been submitted for any other degree or diploma.

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

Pantnagar
December, 2020

(Shishir Tandon)
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We, the undersigned, members of the Advisory Committee of **Mr. Shubham Tiwari, Id. No. 53995**, a candidate for the degree of **Master of Science** with major in **Chemistry**, agree that the thesis entitled **"Chemo Profiling of *Nerium oleander* (L.) and *Cascabela thevetia* (L.) leaf extracts and their biological activities"** may be submitted in partial fulfilment of the requirements for the degree.

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(Virendra Kasana)
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ABBREVIATIONS AND ACRONYMS

Abbreviations	Description
%	Percentage
^{0}C	Degree Celsius
CTHE	*Cascabela thevetia* hexane extract
CTAE	*Cascabela thevetia* acetone extract
CTME	*Cascabela thevetia* methanol extract
DPPH	Diphenyl picryl hydrazyl
EDTA	Ethylene diamine tetra acetic acid
GC-MS	Gas chromatography Mass Spectrometry
IC_{50}	The half maximum inhibitory concentration
mg	Milli gram
mL	Milli liter
MS	Mass spectrometer
NOHE	*Nerium oleander* hexane extract
NOAE	*Nerium oleander* acetone extract
NOME	*Nerium oleander* methanol extract
pH	Power of hydrogen
ppm	Parts per million
ROS	Reactive oxygen species
SD	Standard deviation
v/v	Volume by volume
w/w	Weight by weight
µg	Microgram
µL	Microliter

Introduction

Biodiversity refers to the variety of organisms found in given place and time. Plant biodiversity of earth includes roughly 350,000 plant species representing an estimated 80-90% of the global total **(Vellend *et al.*, 2017).** India is among those countries which are richest in terms of plant biodiversity. Apart from the abundant flora there is also extraordinary ecosystem diversity. Among this large plant diversity about 7500 species of plants constitute the medicinal plant biodiversity **(Rasbid and Anand, 2008).** According to **Aerts *et al.*, (2018),** biodiversity not only include the variety of species of plants, animals and other microorganisms but also the variety of genes present in those species and the different ecosystems to which they belongs to. A diverse range of bioactive products are produced by plants to either cure or prevent various diseases **(Jamal *et al.*, 2012).** Uttarakhand state is the medicinal plant hub of India due to its richness in plant biodiversity **(Joshi and Joshi, 2014).**

The idea that plants possess healing potential towards many diseases had been known long before mankind discovered the existence of microbes. Since the time immemorial medicines based on plants are used to treat various infectious diseases and some of these traditional medicines are still used (**Rios and Reico, 2005).**

Medicinal plants are found to have immense role in the treatment of various diseases and are used all over the world. According to **Owolabi *et al.*, (2007)** about 80% of the World's population rely mainly on herbal medicines. Drugs can be easily obtained from plants in a less expensive ways and can serve as a base material for the development of new drugs **(Ahmad and Rajagopal, 2013).** A strategic plan for the development and promotion of the use of medicinal plants had been suggested by the world health organization (WHO). In some European countries some herbal products are licensed as medicines with efficacy proven by clinical trials, while in some other countries these are considered as dietary supplements **(Rafieian and Kopaei, 2012).**

According to **Ahmed and Bhardwaj, (2018)** plant-based antimicrobials are a vast untapped source of medicines and they are found to be effective for the treatment of various infectious diseases and also provides benefit against the side effects of

synthetic antimicrobials. In developing countries infections are common and may take the form of epidemics due to overpopulated living conditions, improper sanitation and majorly illiteracy **(Alirol *et al*., 2011).** Apart from the health care plants and plant products can be used for many other purposes like in agricultural field for plant pest protection, in nanotechnology field to prepare nanoparticles and in several other fields. A study shows that nearly 2.5 million tons of synthetic pesticides are used on crops each year and the damage caused by these synthetic pesticides reaches $100 billion annually **(Koul, 2008)**. Hence natural products are an excellent alternative to the synthetic ones which ultimately also reduces the negative impact caused by the latter ones. Some of the bioactive components are also traded in markets as a raw material in many herbal industries **(Renisheya *et al*., 2011).** Thus, study of plants is very useful for developing countries like India.

Since the prehistoric Neanderthal men, historical documents of ancient civilization and also the modern clinical studies have represented the curative potential of medicinal plants **(Pan *et al*., 2014; Petrovska, 2012).** In India medicinal plants are used extensively either as folk remedies or in different indigenous systems of medicine. According to national health experts, in India alone, about 2000 different plants are used for preparation of medicine **(Srinivasan *et al*., 2000)**. According to a survey conducted by UNCTAD 33 percent of total drugs produced by the industrialized nations are derived from plants. Indian scriptures also mention about the medicinal properties of plants like, in Rigveda 67 plants, in Yajurveda 81 plants and in Atharvaveda 290 plants are listed to have therapeutic effects **(Srinivasan *et al*., 2000)**.

Medicinal and aromatic plants are of utmost importance as they are the source of medicine and drugs, we use in our day-to-day life. The uses of medicinal and aromatic plants are convenient, productive and undemanding. Because of these advantages of medicinal and aromatic plants they have been widely used to serve the purpose of folk medicines **(Bilal *et al.*, 2012).** These plants are present in almost all the regions of the world having a large treasure of potential bioactive compounds concealed within them **(Doughari *et al.*, 2012).** Medicinal plants are the primary source of secondary metabolites and vital oils which are of therapeutics importance **(Ahmed *et al*., 2002).**

Plants are also considered as the oldest sources of pharmacologically active compounds with several medicinally important biological compounds for centuries **(Kumari *et al*., 2016).** Plants synthesize a variety of phytochemicals as a part of their normal metabolic activities and these phytochemicals are responsible for the various activities shown by any plant species. The products derived from different parts of the plants like stem bark, leaves, fruits, roots and seeds have been a part of phytomedicine that can produce a definite physiological function in human body **(Phuse and Khan, 2018).** Phytochemicals are chemical compounds produced by plants either through primary or through secondary metabolic processes. They generally play a role in plant growth or defense against pathogens or predators. The major secondary metabolites include alkaloids, flavonoids, coumarins, glycosides, phenols, tannins, terpenes and triterpenoids **(Okwu, 2004)**. The alkaloids and their synthetic derivatives have been reported to show antimicrobial activities **(Ragasa *et al*, 2005; Cuhnie and Lamb, 2006; Soetan *et al.*, 2006)** and these activities shows their efficacy by affecting integrity of the cell membrane of the microorganisms **(Seetharaman *et al.*, 2017).** The extracts and oils obtained from plants have their use in the treatment of various infectious diseases. The amount of extract obtained differ from species to species and also from location to location. The solvent and extraction system may also modify the final results **(Rios *et al*., 2005).** Considering the beneficial effects of the products obtained from the plants there is an increasing interest of scientists to obtain natural antioxidants, antimicrobial and various other compounds that can be beneficial to humans as the products obtained does not have any adverse effect.

Antioxidant are the molecules which scavenge free radicals and decrease the damage caused by oxidative stress. Free radicals are chemical species that possesses unpaired electrons and the presence of these unpaired electrons makes them very unstable and reactive species. Oxidative stress caused by free radicals is believed to be a major cause of a number of human cardiovascular, neurological and other disorders. It has been estimated that about the 5% of the oxygen inhaled by humans gets converted into reactive oxygen species by various physiological processes occurring inside our body.

Free radicals can be produced by the homolytic cleavage of chemical bonds present inside the body by various agents such as, low wavelength electromagnetic radiation (e.g., gamma rays) can split water molecules into hydroxyl radical. Similarly,

UV radiation, however does not have sufficient energy to dissociate water, but can dissociate hydrogen peroxide (H_2O_2). The radicals thus formed are highly reactive than nonradicals and their reactivities varies from radical to radical **(Okezie, 1998).** According to **Narasimhan *et al*., (1996)** oxygen, which is a vital component for the human survival, can transform into water, a superoxide radical, a hydroxyl radical and hydrogen peroxide inside the body.

The imbalances between reactive oxygen species and antioxidants could lead to oxidative stress. Inadequate intake of antioxidant derived diet can be a factor for one such possibility. Since our endogenous antioxidant system itself is not 100 percent efficient, it is reasonable that dietary antioxidant can help us to protect from such damage **(Halliwell, 1996).** In this regard the exogenous consumption of antioxidants obtained from natural resources have proved beneficial for human health **(Sen and Chakraborty, 2011).**

Family Apocynaceae also known as dogbane family is one of the ten largest families of flowering plants. For practical reasons Robert Brown separated family Asclepediaceae from family Apocynaceae and due to the conflict between these two families there is a variation in exactly how many species or genera belong to family Apocynaceae. A revised classification for family Apocynaceae was reported according to this classification family Apocynaceae consists of five subfamilies namely Rauvolfoideae, Apocynoideae, Periplocoideae, Secamonoideae and Asdepiadoideae **(Endress *et al*., 2014).** About 424 genera with more than 46,00 species are distributed among these subfamilies **(Bhadane *et al*., 2018).** In India, this family includes about 89 species **(Singh and Sangwan, 2011; Motwani *et al*., 2012).**

The plants belonging to family Apocynaceae are native throughout India, Bangladesh, China, Pakistan, and Sri Lanka **(Mahmood *et al*., 2011).** Few representative plants, widely used as ornamentals, such as oleander (*Nerium* L.) and the periwinkles (*Vinca* L.) are well known. A characteristic feature of this family is that almost all species belonging to this family produce milky sap**.** Many species belonging to Apocynaceae possess medicinal properties and are used to treat diseases like fever, malaria, pain, diabetes and gastrointestinal ailments. Some non-medicinal plants are used for food, fodder, wood, dye, perfume and ornamentals. Some other species have

proved excellent sources of therapeutic values like anticonvulsant activity, anticancer activity, antimalarial activity, cardiotonic activity, coughing, throat and mouth problems, fever, measles, wound healing and antiviral activity **(Barbon *et al*., 2012; Belayneh and Bussa, 2014; Botelho *et al*., 2017**; **Dai *et al*., 2011; Haldar *et al*., 2015; Joselin *et al*., 2012; Kumar *et al*., 2015; Rahman *et al*., 2017; Ravikumar *et al*., 2012; Singh *et al*., 2013; Wong *et al*., 2014;).** The most studied group of secondary metabolites in this family are indole alkaloids, which are present only in the part of family having less specialized flowers **(Simões *et al*., 2016).**

Two common species belonging to family Apocynaceae are *Cascabela thevetia* and *Nerium oleander* which are cultivated as an ornamental shrub in different parts of the world. However, in some part of the world they are considered to be noxious weeds **(Bandara *et al*., 2010).** In spite of their toxicity both *Cascabela* and *Nerium* have been used as therapeutics for heart failure, leprosy, malaria, ringworm and indigestion **(Osterloh *et al*., 1982).** However due to its adverse gastrointestinal side effects like diuresis and slow ventricular response in cardiac patients it is no longer used as a therapeutic **(Middleton and Chen, 1936).** Others researchers have described antibacterial, insecticidal, molluscidal and rhodenticidal activity of these plants **(Osterloh *et al*., 1982; Obasi and Igboechi, 1991; Oji and Okafor, 2000).**

Considering all the above facts the current research has focused on the study of *Cascabela thevetia* and *Nerium oleander* plants with following objectives:

Objectives:

1. Collection of *Nerium oleander* and *Cascabela thevetia* leaves (family Apocynaceae) from Tarai region of Uttarakhand
2. Extraction of *N. oleander* and *C. thevetia* leaves successively by different solvents using Soxhlet apparatus.
3. Chemical analysis of leaf extracts using GC-MS analysis for identification of their bioactive constituents.
4. Determination of biological activities viz., anti-oxidant, anti-diabetic and anti-inflammatory activities of leaf extracts.

Review of Literature

Both *Cascabela thevetia* and *Nerium oleander* belongs to family Apocynaceae. Both of these genera share a close morphological relationship which are easily deflected in their recognition **(Alvardo-Cárdenas and Ochoterena, 2007).** Both *Cascabela* and *Nerium* are cultivated as ornamental shrubs in different part of world however, both these plants are well known for their toxicity. The whole plant of *Nerium* is toxic and contain cardiac glycosides with the highest concentration in the kernel of seeds, followed by leaves, fruit and sap. Oleander, because of the presence of oleandrin and nerine toxins **s**how toxic properties and hold its toxicity even after drying **(Inchem, 2005). Shaw and Pearn, (1979)** also reported that oleander contains a mixture of extremely toxic cardiac glycosides. Poisoning caused by oleander had been reported from different parts of the world such as India, Sri Lanka, East Asia, Australia, Europe, Southern Africa and United States **(Langford and Boor, 1996; Eddleston and Warrell, 1999).**

2.1 *Cascabela thevetia* L.

C. thevetia (syn. *Thevetia peruviana*) is an ornamental plant and an evergreen shrub which belongs to the family Apocynaceae. This plant gets its name after French monk, Andre Thevet. All parts of this plant are poisonous hence, it is also known as "suicide tree" **(Shivaraja *et al.*, 2018).** Seeds are most toxic among all parts producing cardiac glycosides followed by leaves, fruit and sap **(Kyerematen *et al.*, 1985; Saravanapavananthan and Ganeshamoorthy, 1988).** Some common names of *C. thevetia* are Yellow oleander, Mexican oleander, Be-still tree, Dicky plant, Captain cook tree, Lucky nut, Foreigner's tree, Still tree, Halodhiya karabi, Currant-tree etc. *C. thevetia* is a native plant of Mexico and Central America and is a close relative to *N. oleander* (**Soloman *et al.*, 2016).** It is a widely cultivated ornamental species originally introduced from tropical America and is also distributed in Asia, Malaysia and Pacific islands **(Sowjanya *et al.*, 2013).**

2.1.1 Botanical description

C. thevetia L. commonly known as Indian oleander is an evergreen tropical shrub having willow like leaves in appearance. The leaves are covered with waxy coating to reduce water loss **(Phuse and Khan, 2018).** *Cascabela* and *Thevetia* had some convoluted history with each other. On one hand phylogenic analysis and taxonomic treatments regard both genera as distinct entities, while others treat *Cascabela* as synonym of *Thevetia* **(Alvarado-Cardenas and Soto-Núñez, 2014).**

2.1.2 Chemistry and phytochemistry

The aqueous extract of *C. thevetia* showed the presence of carbohydrates, saponins, alkaloids and terpenoids and chloroform extract showed presence of carbohydrates, saponins and methanol extract showed presence of alkaloids, carbohydrates, cardiac glycosides, phenols, tannins, saponins, and steroids. Because of these secondary metabolites the extract showed a significant number of biological activities such as antidiabetic, antioxidant, antimicrobial, anti-inflammatory, anticarcinogenic activities etc. **(Seetharaman *et al*., 2017).** All parts of this plant are found to be toxic to most vertebrates as they contain cardiac glycosides. The main compounds responsible for its toxicity are thevetin A and thevetin B, others are peruvoside, neriifolin, thevetoxin, and ruvoside **(Seetharaman *et al*., 2017). Ahmed and Bhardwaj, (2018)** described that the leaves of *C. thevetia* contain flavanone and flavanol glycosides which showed inhibitory activities against enzymes HIV-1 reverse transcriptase and HIV-1 integrase and thus can be useful for the treatment of AIDS. **Obasi *et al.*, 2010, Tiwari *et al.*, (2011),** also reported the presence of various other phytochemicals like alkaloids, flavonoids, tannins, saponins, terpenoids, triterpenoids, phenols, cyanins, cardiac glycosides, proteins and carbohydrates in whole plant extract.

Eddleston *et al*., (2000) also reported the presence of cardenolides called thevetin A and thevetin B as the main toxins some others are peruvoside, neriifolin, thevetoxin and ruvoside. These phytochemicals are responsible for various activities like antidiabetic, antioxidant, antimicrobial, anticarcinogenic and anti-inflammatory. **Gogoi and Bhuyan, (2019)** studied the bark extract of *C. thevetia* for primary phytochemical screening and revealed the presence of various plant constituents such

as alkaloids, flavonoids, glycosides, steroids, phenolic compounds, tannins, saponins, carbohydrates, triterpenoids, anthraquinones and fatty acids.

Phuse and Khan, (2018) reported that the extract of *Cascabela* contain phenolic and flavonoids and the amount of phenolics and flavonoids obtained was 0.164mg/ml, and 0.185mg/ml respectively. The methanolic extract of *Cascabela* was found to contain nine cardiac glycosides, six were obtained from methanol extract and three were obtained from hydrolyzed methanol extract catalyzed by own seeds. The compounds present in methanol extract are thevetin A, thevetin B, thevetin C, acetyl thevetin A, acetyl thevetin B, and acetyl thevetin C. Additionally, three monoglycosides thevetosides were identified after enzymatic hydrolysis are neriifolin, acetylnerifolin and acetylpervoside **(Balderas-López *et al*., 2019).**

Shivaraja *at al.*, (2018) presented a case report showing the poisoning effects of *Cascabela thevetia*. He concluded that this plant contains phytotoxins like cardenolides, peruvoside, neriifolin and thevetoxin. He further explained that the cardenolides present are thermally stable and thus retain their toxic effects even after drying or heating.

2.1.3 Biological activities

2.1.3.1 Antioxidant activity

According to **Krinsky, (1992)** antioxidants are chemical substances present in low concentration compared with those of an oxidizable substrate, which significantly delays and prevents the substrate oxidation. The oxidizable substrate may include any biomolecule found in living organisms including proteins, lipids, DNA and carbohydrates. In biological systems antioxidants have multiple functions such as protection against oxidative damage and in major signaling pathways of cells. The major function is to protect cells against the oxidative damage caused by reactive oxygen species such as superoxide radical, hydroxyl radical, peroxide radical and nitric acid radical. DPPH assay is one of the most extensively used method for the determination of antioxidant activity because of its advantage to screen a large number of samples in relatively short time interval **(Coyle *et al*., 1993).** When a DPPH radical interacts with a proton donating substance like an antioxidant species, it gets scavenged, leading to decrease in the absorbance which can be measured easily by a

spectrophotometer **(Seetharaman *et al*., 2017)**. The flower extract and leaf extract of *C. thevetia* possess higher efficiency to neutralize free radicals than ascorbic acid **(Phuse and Khan, 2018).** Some studies showed that the roots of *C. thevetia* also possess antioxidant activity. Antioxidant activity of chloroform extract of plant roots tested between 100 and 200µg/mL range using ABTS [2-2' azinobis (3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging method showed the percentage scavenging activity from 29.66 to 58.66% (IC_{50} =162.5± 5.77) **(Kanthal *et al*., 2018).**

Phuse and Khan, (2018) tested the antioxidant activity of acetone and ethyl acetate extracts of flowers of *Cascabela*. Antioxidant activity was performed by DPPH radical assay, Nitric oxide radical assay and Superoxide radical assay using UV-VIS spectrophotometer technique. The IC_{50} values for DPPH radical, nitric oxide radical and superoxide radical showed higher potential for ethyl acetate extract than for acetone extract. Comparative IC_{50} values of standard ascorbic acid for DPPH assay, nitric oxide assay and superoxide assay were found to be 8.03, 13.29 and 9.93µg/ml respectively. Various other activities were also reported by other researchers like antispermatogenic **(Gupta *et al*., 2011)**, piscicidal **(Singh *et al*., 2010)**, antitermite **(Kareru *et al*., 2010)**, antifungal **(Ambang *et al*., 2010; Gata-Gonclaves *et al*., 2003)**, antidiarrheal and cytotoxic activities **(Hassan *et al*., 2011).**

2.1.3.2 Antimicrobial activity

Antimicrobials obtained from plants have less side effects than the synthetic ones. They have been studied widely as an important source of medicinal agents and also have extraordinary therapeutic potential. Phytochemicals having sufficient antimicrobial activity can also be used to treat many microbial infections. It was found that methanol leaf extract of *C. thevetia* showed highest activity against *S. aureus, P. aeruginosa, E. faecalis,* and fungal pathogen *C. albicans.* Highest activity against *E. coli and P. aeruginosa* was shown by chloroform leaf extract. Moderate activity was shown by hexane extract against respected pathogens **(Sowjanya *et al*., 2013).** The flower extract of *C. thevetia* had also shown activity against different microorganisms like *S. typhi, E. coli, E. faecalis* and *B. cereus* **(Solomon *et al*., 2016).**

Hassan *et al.*, (2011) studied the antibacterial activity of ethanol-extracted leaves of *C. thevetia* against some bacterial species namely *B. polymyxa*, *B. megaterium, B. cereus*, *B. subtilis*, *S. aureus*, *S. typhi, S. sonnei* and *S. flexineri*, using tetracycline as a standard drug. They reported poor activity of extracts against both gram-positive and gram-negative bacteria.

Ahmed and Bhardwaj, (2018) tested the antibacterial activity of the leaf extracts of *C. thevetia* (hexane, acetone and methanol extract) against three bacterial strains namely, *M. luteus, P. aeruginosa* and *S. flexneri* using agar well diffusion method. It was found that hexane extract showed moderate activity at 100mg/ml against *M. luteus* (inhibition zone 12mm), at 25mg/ml against *P. aeruginosa* (inhibition zone 8mm) and results found were poor for *Shigella.* Similarly, methanol extract at 100mg/ml against *P. aeruginosa* (inhibition zone 26mm), 100mg/ml against *S. flexneri* (inhibition zone 8mm) and the activity found was poor against *M. luteus.* In case of acetone extract concentration of 100mg/ml against *M. luteus* (inhibition zone 12mm), whereas 200mg/ml against *S. flexneri* (inhibition zone 12mm) and 200mg/ml against *P. aeruginosa* (inhibition zone 10mm).

Seetharaman *et al.*, (2017) evaluated the antibacterial activity of the whole plant extracts (methanol, chloroform and water) of *C. thevetia*. The bacterial strains used were *K. pneumonia, P. aeruginosa* and *P. vulgaris.* The results had shown that each group possesses antimicrobial activity. However, maximum zone of inhibition was observed for methanolic extract compared to others.

2.1.3.3 Pharmacological activity

The leaf twigs of *Cascabela* were also found to possess immunomodulator suppressant, anticancer and anti-inflammatory activity **(Save *et al.*, 2015).** *Thevetia peruviana* is also used in ethnomedicine for the treatment of external wounds, infected area, ring worms, tumors etc. Grinded leaves of this plant are also used in ethnoveterinary medicine for healing wounds **(Mishra, 2013).** The oral administration of fruit extracts (aqueous, alcohol, ether) of *C. thevetia* (L.) showed abortifacient activity at the doses of 100 and 200mg/kg. Antifertility activity shown by extract may be because of the presence of flavonoids or other constituent present in it, as flavonoids and saponins are reported to possess antifertility activity **(Deshmukh and Zade, 2014).**

Gogoi and Bhuyan, (2017) studied the *in vivo* anti-diabetic activity of the bark of *C. thevetia* in streptozotocin induced diabetic rats. Their study includes the investigation of the fasting blood glucose level, lipid biochemical parameters and histopathological study of the pancreatic tissues. The effects of different doses having concentrations 100mg/kg, 200mg/kg and 400mg/kg of the methanolic extract of *Cascabela* were investigated in the diabetic rats using metformin hydrochloride as standard drug, as a hypoglycemic agent (10mg/kg). Results showed increased levels of triglycerides, cholesterol, and LDL levels and decreased level HDL level in the diabetic animals. Similarly, the serum biomarkers and total protein content were increased significantly in case of the diabetic rats which decreased significantly after the use of standard drug and the test extract and reached to their normal values. The histopathology of the pancreatic tissues in the test group showed partial necrosis and ballooning in 100mg/kg group, while in case of 200mg/kg group cellular integrity was almost normal and in 400mg/kg group cellular integrity was normal to great extent. Thus, it can be concluded that the antidiabetic activity of the extract may sensitize the insulin receptors from the pancreatic cells and hence supports the *in vivo* antidiabetic activity.

Kanthal *et al*., (2018) evaluated the cytotoxic and anthelmintic activity of the chloroform root extract of *C. thevetia. In vitro* cytotoxic activity of the extract was measured at various concentration levels against two different cancer cell lines namely human mammary gland adenocarcinoma and human cervical carcinoma. Anthelmintic activity was tested against earthworm (*Pheritima posthuman)* and antidiabetic by percentage of scavenging. The anticancer activity was evaluated by percent growth inhibition and the concentration range selected was 62.5 to 1000μg/mL. The results showed that both the percent inhibition activity and cytotoxic effect was higher for mammary gland adenocarcinoma than that for human cervical carcinoma. Similarly, the death time and paralysis time was found to be very significant when compared to standard drug albendazole. Thus, this study showed that the plant extract of *C. thevetia* possess anthelmintic and cytotoxic activity.

In-vitro anthelmintic activity of the chloroform extract of *Cascabela thevetia* was also investigated against *P. posthuma* and paralysis time and death time was

compared with reference to standard drug Albendazole. Studies showed paralysis time for earthworm ranged from 87.3 minutes to 125 minutes in case of *Cascabela* extract and 83.6 minutes and 114 minutes in case of standard drug Albendazole **(Kanthal *et al.*, 2018).**

Ramos-Silva *et al.*, (2017) discussed the cytotoxic activity of methanolic extract of *Thevetia (Cascabela)* fruit on breast cancer, lung cancer and prostate cancer cell lines in humans. They showed that the methanolic fruit extract induced strong cytotoxic effect in all four lines (conc. <20µg/mL). They proposed that the fruit extract induces fragmentation of DNA in all four types of human cancer cells. The IC values for the cell lines examined are 12.04µg/mL for lung cells, 1.91µg/mL for prostate cells, 5.78µg/mL for breast cells and 6.30µg/mL for colorectal cell lines. Symptoms of oleander poisoning appears after 4 hours of ingestion. Fatal period is usually 6 days. **Bandara *et al.*, (2010)** reported that oleander ingestion results in nausea, vomiting, abdominal pain, diarrhea, dysrhythmias, and hyperkalemia. Patients with acute intoxication were treated with digoxin-specific fab antibody as a therapeutic agent. **Rahman *et al.*, (2017)** established that the leaves and fruit extract of *Thevetia* possess anti-inflammatory based wound healing properties along with the presence of antioxidants, which signifies the beneficial use of this plant for future use in pharmaceutical industry to develop wound healing drugs. The leaves of *Thevetia peruviana* also found to possess flavanone and flavanol glycosides which showed inhibitory activities against HIV-1 reverse transcriptase and HIV-1 integrase **(Tewtrakul *et al.*, 2002).**

2.1.3.4 Other Uses

Gogoi and Bhuyan, (2019) studied the preliminary phytochemical screening and pharmacognostic study of the bark of *C. thevetia* bark. The pharmacognostic studies include macroscopic and microscopic investigation, physicochemical parameters and fluorescence analysis. Bark powder was successively extracted by solvents like petroleum ether, chloroform, ethyl acetate, and methanol using a Soxhlet apparatus. The presence of cork cells, starch granules, oxalate crystals, parenchyma cells and vascular bundles were shown by transverse microscopy. Similarly, presence of cork cells, calcium oxalate and fiber were shown by powder microscopy.

Due to rapid rise in the price and decrease supply of crude oil researchers are showing interest in the development of biofuels. Biodiesel is one the most prominent renewable energy resource **(Meng *et al.*, 2009).** Biodiesel is an alternative fuel for diesel engines produced by the reaction between a vegetable oil with an alcohol **(Gerpen, 2005).** Biodiesel is a biodegradable, renewable, nontoxic and environment friendly energy source. In 2003, the total world biodiesel production was estimated to be around 1.8 billion liters **(Huang *et al.*, 2010).** However, it is not economically feasible to use food grade vegetable oil therefore, non-edible feedstocks are gaining importance. *C. thevetia* is one of the promising non-edible biodiesel feedstocks having 60-65% oil content. Experiments showed that an engine fueled with 20% *Thevetia* biodiesel and 80% pure diesel shown good efficiency **(Basumatary, 2014). Sut *et al.*, (2016)** also showed that *C. thevetia* is a potential feedstock for the production of biofuel using chemical and thermochemical processes.

2.2 *Nerium oleander* L.

N. oleander, commonly known as pink oleander is an evergreen shrub having a height of 2-5 m. It is a drought-tolerant ornamental plant cultivated throughout the world, particularly in temperate regions (**Zibbu and Batra, 2010).** It is also known as oleander because it possesses its superficial resemblance with an unrelated plant namely, *Olive olea. N. oleander* is indigenous to India-Pakistan subcontinent, distributed in the Himalayas from Nepal to Kashmir up to 1950m, extending to Afghanistan, Baluchistan and found throughout India **(Chaudhary and Prasad, 2014).** This plant can be propagated though its seed and showed great variability in seedling production **(Sinha and Biswas, 2016).** Some common names of *Nerium* are Soland, Kaner, Lorier bol Karabi, Rosebary, Gundari, Arali, Kanher etc.

2.2.1 Botanical description

N. oleander is an evergreen shrub or small tree typically growing to 2-6 meters in height, leaves are green, leathery and are in pair of three or whorled. Leaves are having a prominent midrib and are leathery in texture. Oleander has flexible branches with green, smooth bark eventually turning to dark grey when the plant gains maturity. Flowers are grown in terminal branches in clusters and show various colours from pink to red **(Benson *et al.*, 2015).** Oleander can be propagated through seeds but being

heterozygous shows a great variability in seedling production (**Zibbu and Batra, 2010).**

2.2.2 Chemistry and Phytochemistry

N. oleander is entirely different from *C. thevetia* or yellow oleander as the cardiac glycosides produced by both of them are entirely different. The former one contains cardiac glycosides such as oleandrin, while the latter one contains thevetin **(Benson *et al.*, 2015).** The seeds of *Nerium* contains glucosides oleandrine, odorosides, adigodise while the bark contain glucosides rosaginoside, nerioside, cortenoside, while the roots contain steroids (**Zibbu and Batra, 2010).** *N. oleander* has been used to treat skin diseases, cancer, diabetes, inflammation, CNS depressant and also some microbial infections **(Shankar *et al.*, 2016). Nasua *et al.*, (2002); Newman *et al.*, (2007)** studied the ability of *N. oleander* extract as a chemotherapeutic agent. **Siddiqui *et al.*, (2012**) reported the presence of a pentacyclic triterpene, oleanderocioic acid, two flavonoidal glycosides, quercetin-5-O-[a-L-rhamnopyranosyl-(1→6)]-b-D-glucopyranoside and kaempferol-5-O-[a-L-rhamnopyranosyl-(1→6)-b-D-glucopyranoside, and a cardenolide, oleandigoside in the leaf extract of *N. oleander.* Oleander is one of the most toxic plant in nature and contains numerous toxic compounds (**Zibbu and Batra, 2010).** A new diterpene, oleanderoic acid and a new triterpene, oleanderin had been isolated from the fresh, undried and uncrushed leaves of *N. oleander.* The fresh, undried, winter leaves of *N. oleander* were also found to possess two new cardiac glycosides namely, kaneroside and neriumoside **(Gupta and Mittal, 2010).**

Huq *et al.*, (1998) reported the presence of a new cardenolide, 3β-hydroxy-5β-carda-8,14,16,20(22)-tetraenolide in the roots of *N. oleander*. They also described the isolation, characterization and antibacterial screening of another novel cardenolide, named as 12β-hydroxy-5β-carda-8,14,16,20(22)-tetraenolide. It was concluded by them that latter compound showed antibacterial activity against *B. subtilis, B. cereus, E. coli* and *P. aeruginosa*. The same compound also showed strong cardio-stimulating effect on intact toad heart at a single dose of 30μg.

Hydro distillation of *N. oleander* leaves resulted in the identification of 34 volatile compounds, showing 93.21% of total oil content and yield obtained was 1.76%. The components reported were nerine (22.56%), digitoxigenine (11.25%), amorphane

(8.11%), cineole (6.58%), α-pinine (5.54%), calarene (5.12%), limonene (5.015%), β-phellandrene (4.84%), terpinene-4-ol (3.98%), sabinene (3.22%), isoledene (2.94%), 3-carene (2.56%), humulene (2.29%), β-pinene (2.01%) and cymen-8-ol (1.67%) **(Derwich *et al*., 2010).**

Gupta and Mittal, (2010) reported that water extraction of crushed *N. oleander* leaves yielded 2.3% of a crude polysaccharide. The main fraction (67%) represents a pectic polysaccharide mainly composed of galacturonic acid besides rhamnose, arabinose and galactose. Three new pregnanes, 21- hydroxypregna- 4, 6-diene- 3, 12, 20- trione, 20R-hydroxypregna-4,6-diene- 3, 12- dione, and 16beta, 17beta-epoxy-12beta- hydroxypregna- 4, 6-diene- 3, 20- dione, were also found in *N. oleander*, along with two known compounds, 12beta- hydroxypregna- 4, 6, 16- triene-3,20-dione (neridienone A) and 20S, 21- dihydroxypregna- 4, 6- diene-3, 12- dione (neridienone B). **Sharma *et al*., (2009)** also reported the presence of a number of secondary metabolites, triterpenoids, cardiac glycosides and bioactive pregnanes. **Hameed *et al*., (2015)** investigated the chemical composition of ethanolic leaf extract extract of *N. oleander* by gas chromatography-mass spectroscopy. β-d-allopyranoside, 5-hydroxy methyl furfural, methyl 6-dioxy 2-o-methyl, tetradecamethyl, cycloheptasiloxane, hexadecamethyl, cyclononasiloxane, cyclooctasiloxane, octadecamethyl, eicosamethyl, cyclodecasiloxane, 2,3bis(trimethylsilyl)oxy propyl ester, 2-cyclopenten-1-one, 2-hydroxy-3-methyl, 9,12,15-octadecatrienoic acid 3-eicosene, 1,1,3,3,5,5,7,7,9,9-decamethyl-9-(2-methyl propoxy)penta siloxane, 3-ethyl-5-(2-ethylbutyl), 1-monolinoleoylglycerol trimethylsilyl ether, 2-cyclohexen-1-one, 4 (hydroxy butyl)-3.5.5-trimethyl, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl octasiloxane and octadecane were found in the ethanolic leaf extract by GC-MS.

2.2.3 Biological activities

2.2.3.1 Antioxidant activity

Antioxidant are the molecules which protect the living cells against the oxidative damage caused by various chemicals produced inside the body. Reactive oxygen and nitrogen species are the chemicals produced inside the body in the form of superoxide anions, hydroxyl radicals and hydrogen peroxide from the autooxidation of lipids. These ROS and RNS are formed by UV radiation, smoking and drug

metabolisms and excess of these are likely to damage several cellular components such as lipids, proteins, nucleic acids, and DNAs through the oxidation or nitration process. Further these reactive species damage biomolecules including lipids, proteins and nucleic acids leading to various degenerative diseases such as atherosclerosis, arthritis, cancer, neuro-degenerative disorders etc. **(Naithani *et al.*, 2011).** All aerobic organisms, including human beings have defense against these antioxidants. However, these naturally produced antioxidants can be inefficient if the reactive species are produced in high quantity, in such cases some artificial antioxidants such as butylated hydroxy anisole (BHA) and butylated hydroxytoluene (BHT) are commonly used in processed foods but they have been reported to cause some side effects. Therefore, researches are taking place to find naturally originated antioxidants. *N. oleander* had showed effective antioxidant activity, which include free radical scavenging and reducing power. Polar solvents like methanol had proved to be the most efficient solvent for the extraction of antioxidants form this plant **(Mohadjerani., 2012). Vinayagam and Sudha *et al.*, (2011)** stated that the secondary metabolites like phenolics and flavonoids obtained from the plants show considerable free radical scavenging activity. They also reported that free radical scavenging activity of methanolic extract of the flowers of *Nerium* was greater than leaves. Also, it was found that the total phenolic content is higher in crude flower extract (449 mg/100g), as compared to leaves extract (199mg/100mg).

Benson *et al.*, (2015) compared the antioxidant activity of Aloe vera based extract of *N. oleander* with aqueous extract of *Nerium* and it was concluded that the antioxidant capacity of aloe vera based extract was greater than aqueous extract. **Subbaiya, *et al.*, (2014)** suggested that the extract of *N. oleander* is capable of producing silver nanoparticles without using additives. The hydroxyl ions along with other alkaloids present in *N. oleander* may be responsible for the synthesis of silver nanorods and these were found to have antioxidant activity.

2.2.3.2 Antimicrobial property

Antimicrobial compounds are a group of chemical compounds produced synthetically or biosynthetically and are used either to destroy or to usefully suppress the growth and metabolic activities of microorganisms **(Larvanya *et al.*, 2013).** The

inspiration for the production of new drugs has been provided by plants and thus have made a significant contribution towards human health. The potential for developing antimicrobial from plants lead to development of phytomedicine. Antimicrobial based on plants are very useful as they can be used without any side effect that are associated with the use of synthetic antimicrobials. The antimicrobial activity of plants is due to the presence of bioactive compounds in oils and extract of the plant **(Malik *et al*., 2015).** The extract obtained from *N. oleander* has shown significant antimicrobial property. The methanol, ethanol and chloroform extracts showed considerable antimicrobial activity against *B. pumilus, B. subtilis, S. aureus* and *E. coli* **(Hussain and Gorsi, 2004).**

Hasson, (2016) studied the antioxidant and antimicrobial activities of *N. oleander*. The test organisms used in their studies were *S. aurous, E. coli. P. aeruginosa and K. pneumonia*. They also synthesized silver nanoparticles by green synthesis method and characterized by UV-Vis spectroscopy showing an apparent broad peak at 450nm. The results obtained from antimicrobial assay revealed that the ethanolic extract of *N. oleander* showed remarkable effects on gram-positive bacteria as well as on gram-negative bacteria. However, results showed that gram- positive bacteria were more susceptible than gram-negative bacteria against the ethanolic extract. **Wong *et al*., (2013)** described that the extract obtained from the leaves and root extract of *N. oleander* displayed antibacterial activity against *B. pumilus, B. subtilis, S. aureus* and *E. coli.*

Goktas *et al*., (2007) revealed that beech wood impregnated by *N. oleander extract* at concentration ranging from 0.25-3.00% is effective in suppressing the attack of certain fungal species however, at high concentration shows nutritive properties towards fungi. **Derwich *et al*., (2010)** reported antibacterial activity of the essential oil from *N. oleander* flower against *E. coli, P. aeruginosa* and *S. aureus.* The disc diffusion assay revealed that the oil is active against all the tested bacteria. The MIC concentrations reported were 1.45mg/mL against *E. coli,* 2.87mg/mL against *P. aeruginosa* and 5.10mg/mL against *S. aureus.* Silver nanoparticles synthesized using *N. oleander* were also found to possess antibacterial activity **(Subbaiya *et al*., 2014).**

Antimicrobial activity of *N. oleander* and *O. basilicum were screened against* micro-organisms (*Rhizobium solani, Phytophora sp., Pythium sp., Fusarium sp.* and Sclerotinia) and it was found that ethanol extract of *Nerium* showed highest activity against the tested bacteria. The results showed the presence of various phytochemicals like triterpenoids, flavonoids, saponins, tannins, alkaloids and phenolic compounds in the plant extract **(Bidarigh *et al*., 2012)**. **Bhuvaneshwari *et al*., (2007)** also found that *N. oleander* is effective against *S. aureus, P. aeruginosa* and *S. typhimurium.*

2.2.3.3 Insecticidal activity

The diseases transmitted by insects remain a major cause of illness and death all over the world **(Pavela, 2009).** Mosquitoes are the vectors for the transmission of various dangerous diseases such as malaria, dengue, chikungunya, filariasis and Japanese encephalitis**.** *Culex quinquefasciatus* is one of the dominant house-resting mosquitos found in many tropical countries breeding in polluted water responsible for the transmission of *Wuchereria bancrofti*, which is the major cause of lymphatic filariasis **(Holder, 1999).** Recent studies have suggested the use of natural products obtained from plants as a source of insect controlling agents because of their selective and eco-friendly nature. It has been found that the roots, bark, stem, leaves and flowers of *N. oleander* possess insecticidal and antifeedant activity against insects. Also, studies have shown that the aqueous and hexane extract of *N. oleander* possess larvicidal activity against filarial mosquito vector, *C. quinquefasciatus.* It was found that the hexane flower extract of *N. oleander* possesses high larvicidal activity as compared to aqueous extract with LC_{50} value of 102.54 and 61.11ppm after 24 and 48 hours of experimentation **(Raveen *et al*., 2014).**

2.2.4.4 Pharmacological activity

Triterpenoids isolated from leaves, and cardenolides isolated from stems were cytotoxic to certain type of human cancer cells. Ethanolic extract of leaves, stems and roots have cytotoxic effects on leukemia cell lines. Antiproliferative activity was also shown by the methanolic leaf extract of *N. oleander.* It also produces some secondary metabolites which shows different pharmacological activities like anti-inflammatory, antibacterial, antinociceptive, anticancer and CNS depressant activity **(Gupta and Mittal, 2010).** The roots, stem, bark, leaves and flowers of *N. oleander* have been

reported to possess antibacterial, anti-inflammatory, antinociceptive, and antitumor activity. The extract also possesses insecticidal, antifeedant and larvicidal activity. The aqueous extract obtained from leaves were tested for the ovicidal, larvicidal and repellent activity against some mosquito species **(Raveen *et al*., 2014).** *N. oleander* had also been used for the phytoremediation of fluoride as fluoride is considered both essential at low concentration and pollutant at high concentrations that can cause a number of disorders. It was found that *N. oleander* remove fluoride up to 92% from a solution of NaF at the concentration of 10mg/mL within a time period of 15 days **(Khandare *et al*., 2017).**

Oleandrine is an anti-inflammatory and antitumoral compound obtained from *N. oleander*. The aqueous and alcoholic extract obtained from *Nerium* is supposed to possess antinociceptive and cardiotonic properties **(Gupta and Mittal, 2010).** The plant also possesses some anti-inflammatory activity **(Erdenoglu *et al*., 2003).** Leaves of *Nerium* also reported to possess diaphoretic, diuretic, antibacterial and antiplatelet aggregation activity. Other parts of the plant are also used as therapeutic agents for treating swelling, leprosy, eye and skin diseases **(Tannu *et al*., 2011).**

Ahmed *et al*., (2006) discussed about the analgesic activity of the methanol extract of *N. indicum* in Swiss Albino mice of either sex between 4-5 weeks. The inhibition of writhing in mice by the plant extract was compared against a standard analgesic, aminopyrine. The acetic acid writhing model having *Nerium* flower extract showed 89.14% inhibition at oral doses of 250mg/kg body weight of mice. Similarly, the root extract showed 59.18% and 95.92% writhing inhibition at oral doses of 125 mg/kg and 250 mg/kg body weight of mice respectively. The stem extract however showed only 6.78% and 27.89% inhibition of writhing response at oral doses of 125 mg/kg and 250 mg/kg body weight of mice, respectively.

Govind *et al*., (2010) showed that the methanol extract of *N. indicum* possess antiulcer activity against plus pylorus ligation induced gastric ulcer and indomethacin induced ulcer in rats. A significant reduction in gastric volume, free acidity and ulcer was observed in pylorus ligation model and also the extract showed 69.6% ulcer inhibition in case of indomethacin induced ulcer. **Pan *et al*., (2014)** investigated the antitumor activity of a supercritical CO_2 extract of *N. oleander* in a human pancreatic

cancer Panc-1 orthotopic model. The result of their study shown that only 2 of 8 mice treated for 6 weeks with the supercritical extract showed dissectible tumor at the end of treatment period considered. Thus, they concluded that the cardiac glycoside oleandrin possess strong anti-proliferative activity against various human malignancies.

N. oleander is used for the treatment of diabetes in different parts of the world like in Algeria **(Rachid *et al*., 2002)**, Pakistan **(Hussain *et al*., 2013)**, Morocco **(Jouad *et al*., 2001, Bnouham *et al*. 2002)** and the use of *Nerium* as an antidiabetic is also mentioned in Ayurveda **(Sudha *et al*., 2011)**. **Bas *et al*., (2012)** studied the effect of shoot distillate of *N. oleander* streptozotocin induced diabetes showing enhanced insulin sensitivity. Results showed a considerable beneficial effect by using *N. oleander* distillate on glucose metabolism, insulin resistance, insulinotropic activity, leptin, and liver enzymes which point out the insulin like effect of *N. oleander.* **Dey *et al*., (2015)** also studied antidiabetic activity *N. oleander* through alloxan induced diabetes in mice and found that the leaf extract of *Nerium* possess potent antihyperglycemic activity by reducing blood glucose level to 73.79% after 20 days of treatment.

Calderon-Montano *et al*., (2013) reported that the hydroalcoholic extract of *N. oleander* leaves containing cardiac glycosides possess selective cytotoxic activity towards lung cancer cells which, may be due to the marked inhibition of glycolysis. DNA damage induced by *N. oleander* extract and ROS formation participate in its cytotoxicity was also shown. *N. oleander* is known to possess cardiac glycosides and these cardiac glycosides are found to inhibit Na^+/K^+-ATPase pump. The inhibition of Na^+/K^+-ATPase pump may restrict the activity of SGLTs (sodium glucose linked transporters) and thus interfere with the glucose intake by some type of cells. Also, Inhibition of Na^+/K^+-ATPase pump would decrease glycolysis by inhibiting the enzyme phosphofructokinase of glycolytic cycle and glycolysis inhibition is well known to produce cytotoxicity **(Lopez-Lararo, 2007). Erdenoglu *et al*., (2003)** studied the aqueous and ethanolic extract of *Nerium* flowers and showed that the extract possesses significant antinociceptive activity against p-benzoquinone-induced abdominal contractions in mice also the extract showed potent anti-inflammatory activity against carrageenan-induced hind paw edema model in mice without causing any gastric damage.

Materials and Methods

The study was performed in the Department of Chemistry, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India.

3.1 Materials

3.1.1 Source of plant materials

C. thevetia and *N. oleander* plants were collected from G.B.P.U.A.&T. Pantnagar, India.

3.1.2 Chemicals and Glass wares

The analytical grade chemicals and solvents used were of AR or HPLC grade and were obtained from E. Merck and Hi-Media. Glass wares used during the experiment were of Borosil, JSW and Corning.

3.1.3 Instruments

Following instruments were used during experiment-

- Electronic weighing balance (Alzel)
- Fridge (L.G.)
- Oven (NSW India)
- GC-MS (gas chromatograph HP 6890 and Agilent technologies, USA)
- Grinder (Crompton)
- Hot plate (Gamson Pvt. Ltd., India)
- Micropipette
- pH meter
- Soxhlet apparatus
- UV-VIS spectrophotometer
- Water bath

3.1.4 Preparation of leaf extract

The leaves of *Cascabela thevetia* and *Nerium oleander* were washed thoroughly under tap water, then dried under shade at room temperature (20^0C) for about 20 days. The dried leaves were grinded into powdered form. Grinded powder of leaves (150gm) was subjected to undergo extraction by Soxhlet apparatus using hexane, acetone and methanol successively. The extracts obtained were then dried to evaporate the solvents and solid leaf extracts obtained were used for further studies.

3.2 GC-MS analysis of leaf extract

GC-MS analysis of the leaf extract of both *C. thevetia* and *N. oleander* were performed at Advanced Instrumentation Research Facility, Jawaharlal Nehru University, New Delhi. The analysis was performed by using gas chromatograph HP 6899 having mass selective detector MS 5978 fitted with a DB-6 column, with split-spitless injector and electronic pressure controlling unit. Experimental conditions were as follows:

GC-MS parameters	**Extract**
Column temperature	50 ^{0}C
Injection temperature	260 ^{0}C
Injection mode	Split
Flow control mode	Linear velocity
Pressure	69.0 kPa
Total flow	16.3 mL/min
Column flow	1.21 mL/min
Linear velocity	39.9 cm/sec
Split ratio	10.0
High pressure injection	Off
Carrier gas injection	Off
Splitter hold	Off

Constituents of the extract were identified by matching their mass spectra with those present in NIST-MS, Wiley library and Adams (1995).

3.3. Preparation of test solution

Stock solution of 10,000 ppm was prepared by dissolving 1gm of sample extract in 100mL methanol. Dilution of sample was done by using distilled water to obtain desirable concentration for the experimental needs. All the standard solutions used during entire study were also of same concentrations as that of plant extracts (i.e., 100, 200, 300, 400 and 500µg/mL).

3.4 Antioxidant assays

Antioxidant activity of leaf extracts were done by following methods:

3.4.1 DPPH free radical scavenging activity

The DPPH method was developed by Blois in 1958 to determine the antioxidant activity by using a stable free radical α, α-diphenyl-β-picrylhydrazyl (DPPH). DPPH assay is used extensively for the determination antioxidant activity as it can screen a large number of samples in relatively short time interval and is sensitive enough to detect low concentrations of active species (**Coyle *et al.*, 1993).** The test is based on the measurement of the radical scavenging capacity of antioxidants towards it. The odd electron present on nitrogen atom in the radical gets reduced by receiving a hydrogen from the antioxidant molecule to the corresponding hydrazine **(Contreras and Strong, 1982).** Due to extensive delocalization DPPH radical absorbs strongly at 517 nm and appears violet in colour. The violet colour disappears irreversibly as the radical accept an electron or a hydrogen radical. The disappearance of violet colour also takes place even with very weak antioxidants, if sufficient time is provided to the reaction. The alcoholic solution of concentration nearly 0.5mM are sufficiently coloured and obeys Beer-Lambert law over this range.

3.4.1.1 Reagents required

DPPH, methanol, butylated hydroxy toluene (BHT), ascorbic acid, gallic acid.

3.4.1.2 Procedure

A fresh DPPH solution (0.4mM) was prepared and placed in dark as the radical is light sensitive. Standard stock solutions of ascorbic acid, gallic acid and butylated hydroxy toluene were prepared in ethanol. Stock solution (10,000 ppm) of sample is

diluted to lower concentration of 100, 200, 300, 400 and 500µg/mL by adding distilled water to it. Take 1mL of test solution and add to it 5 mL of DPPH solution. The reaction mixture was then left in dark for 30 minutes for incubation. The absorbance of reaction mixture was then recorded at 517 nm. Same procedure was also performed for control and standard solutions. The radical scavenging activity was calculated by the formula:

$$\textbf{\textit{Inhibition percentage}} = \frac{\textbf{\textit{Ac} - \textit{As}}}{\textbf{\textit{Ac}}} \textbf{X 100}$$

Where, Ac = absorbance of control and As = absorbance of sample

3.4.2 Metal chelating method

This method is based on the principle of Fe^{2+} chelating ability of the antioxidant and measuring the absorbance of the ferrous ion-ferrozine complex formed at 560 nm **(Ocsoy *et al.*, 2013).**

3.4.2.1 Reagents required

EDTA, citric acid, $FeCl_2.6H_2O$, ferrozine, methanol.

3.4.2.2 Procedure

Ferrozine solution (2 mM), $FeCl_2.6H_2O$ (5 mM) and standard solution of EDTA and citric acid were prepared. 1mL of leaf extract of different concentration (100-500µg/mL) was taken in different test tubes. To it 0.2mL $FeCl_2.6H_2O$ and 0.2mL of ferrozine was added and solution was made up to 5mL with methanol. Solutions were stirred properly for 10 minutes and absorbance was measured at 562 nm. EDTA and citric acid were used as standard. Chelating activity was calculated by following equation:

$$\textbf{Inhibition percentage} = \frac{\textbf{Ac} - \textbf{As}}{\textbf{Ac}} \textbf{X 100}$$

Where, Ac = absorbance of control and As = absorbance of sample

3.4.3 Hydroxyl radical scavenging activity

This activity was performed by the method proposed by **Ruch *et al.*, (1989).**

3.4.3.1 Reagents required

Ferrous sulfate ($FeSO_4.7H_2O$), 1,10-phenanthroline, sodium phosphate buffer, hydrogen peroxide.

3.4.3.2 Procedure

Ferrous sulfate solution (0.1mM), 1,10- phenanthroline solution (1mM), phosphate buffer solution (0.2M) and hydrogen peroxide solution (0.7M) was prepared. 60µL of ferrous sulphate solution was mixed with 90µL of aqueous 1,10-phenanthrolin solution. In this solution 2.4mL phosphate buffer solution was added followed by the addition of 150µL hydrogen peroxide (0.7M). Then in this solution, 1mL of sample leaf extracts at different concentration (100-500µg/mL) was added and absorbance was measured at 560nm. Standard used for this activity was ascorbic acid. The scavenging activity was calculated by following equation:

$$\textbf{Inhibition percentage} = \frac{\textbf{Ac} - \textbf{As}}{\textbf{Ac}} \textbf{X 100}$$

Where, Ac = absorbance of control and As = absorbance of sample

3.5 Anti-diabetic activity

Antidiabetic activity of *C. thevetia* and *N. oleander* was determined using alpha-amylase inhibitory activity and alpha-glucosidase activity.

3.5.1 Alpha-amylase inhibitory activity

The assay was carried out according to the standard method explained by **Dey *at el.*, (2015).**

Reagents required: Alpha- amylase enzyme**,** sodium phosphate buffer (pH 6.9), starch, DNS reagent, acarbose.

Procedure: Sodium phosphate buffer solution (0.02M), starch solution (1% in 0.02M of sodium phosphate buffer) and DNS solution (40mM) were prepared. Sample leaf extracts having different concentration (100-500µg/mL) were taken in test tubes and sodium phosphate buffer containing alpha-amylase was added to it and the mixture was preincubated for 10 minutes at 25^{o}C. After preincubation 1% of starch solution was added to it and incubated for 10 minutes at 25^{o}C. Then 2mL of DNS reagent was added

to it for the termination of reaction. Again, the solution was incubated for 5 minutes on water bath at 40^0C and then cooled to ambient temperature. Absorbance was recorded at 540 nm. Standard used was Acarbose. Alpha-amylase inhibition was calculated by the following equation:

$$\textbf{Inhibition percentage} = \frac{\textbf{Ac} - \textbf{As}}{\textbf{Ac}} \textbf{X 100}$$

Where, Ac = absorbance of control and As = absorbance of sample

3.5.2 Alpha-glucosidase inhibitory activity

The assay was carried out according to the standard method explained by **Kim *et al.*, (2005).**

Reagents required: Alpha-glucosidase enzyme, DNS reagent, maltose substrate with tris buffer (pH 8.0).

Procedure: Initially, maltose substrate with tris buffer was incubated with sample leaf extracts of different concentrations (100-500µg/mL) for 30 minutes, at 35^{o}C followed by addition of alpha-glucosidase enzyme 2.1U/mL. Finally, DNS reagent was added to the reaction mixture to stop the reaction and absorbance was measured at 540 nm. Alpha-glucosidase inhibition was calculated by the following equation:

$$\textbf{Inhibition percentage} = \frac{\textbf{Ac} - \textbf{As}}{\textbf{Ac}} \textbf{X 100}$$

Where, Ac = absorbance of control and As = absorbance of sample

3.5 Anti-inflammatory assay

Anti-inflammatory activity of the plant leaf extracts were studied by described method.

3.5.1 *In*-vitro anti-inflammatory activity

The assay was carried out according to the standard method explained by **Zhen *et al.*, (2016).**

Reagents required: Phosphate buffer (pH 6.4), sodium diclofenac and egg albumin.

Procedure: To 0.2mL of egg albumin, 2.8mL of phosphate buffer and 2mL of sample leaf extracts of different concentration (100-500µg/mL) was added and the reaction

mixture was incubated for 15 minutes at 37^{o}C in BOD. After incubation the reaction mixture was heated for 5 minutes at 70^{o}C and then cooled again. Absorbance of the solution was recorded at 660 nm against blank reagent. Diclofenac was used as standard. Protein inhibition was calculated by following equation:

$$\textbf{Inhibition percentage} = \frac{\textbf{Ac} - \textbf{As}}{\textbf{Ac}} \textbf{X 100}$$

Where, Ac = absorbance of control and As = absorbance of sample

3.6 Statistical analysis

The experimental results obtained were means ± standard deviation of all the measurements. Mean values and standard deviation were calculated statistically using SPSS software version 2006.

Results and Discussion

4.1 Percentage yield of plant extract

The extract obtained from the plants were weighed and % yield was calculated (Table 4.1) using the formula:

$$\% \text{ Yield} = \frac{\text{Weight of the extract obtained}}{\text{Weight of the dry sample}} \times 100$$

Table no. 4.1: % yield of extracts obtained from plant leaves

Name of plant	**%yield (in hexane)**	**%yield (in acetone)**	**%yield (in methanol)**
Cascabela thevetia	4.67	5.44	12.01
Nerium oleander	5.03	9.94	15.48

4.2 Chemical analysis of leaf extract

Identification of plant constituents present in the hexane, acetone and methanol leaf extracts was performed using GC-MS.

4.2.1. GC-MS analysis of *Cascabela thevetia* hexane leaf extract

GC-MS analysis of leaf extract showed the presence of 14 compounds. The major compound was mome inositol (89.99%). Other major constituents were 1,6-anhydro-beta -D-Glucopyranose (4.27%), (3 beta)-Lup-20(29)-en-3-ol, acetate (1.11%), n-hexadecanoic acid (1.03%) and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one (0.80). Remaining compounds are described in table 4.2. GC-MS analysis of *C. thevetia* hexane leaf extract is illustrated in figure 4.1.

Table 4.2: GC-MS analysis of CTHE

Sr. No.	Name of compound	Area %	Molecular Formula	Molecular Weight
1	Isosorbide Dinitrate	0.47	$C_6H_8N_2O_8$	236
2	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one	0.80	$C_6H_8O_4$	144
3	6,10,14-trimethyl-2-Pentadecanol	0.27	$C_{18}H_{38}O$	270
4	1,6-anhydro-beta -D-Glucopyranose	4.27	$C_6H_{10}O_5$	162
5	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, methyl ester	0.49	$C_{21}H_{38}O_2$	332
6	Neophytadiene	0.11	$C_{20}H_{38}$	278
7	n-Hexadecanoic acid	1.03	$C_{16}H_{32}O_2$	256
8	(Z, Z, Z)-9,12,15-Octadecatrienoic acid, methyl ester	0.45	$C_{19}H_{32}O_2$	292
9	Mome inositol	89.99	$C_7H_{14}O_6$	194
10	2-hydroxy-1-(hydroxymethyl)ethyl ester Hexadecanoic acid	0.15	$C_{19}H_{38}O_4$	330
11	(3-beta)-Lup-20(29)-en-3-ol, acetate	1.11	$C_{32}H_5O_2$	468
12	2,6,10-Trimethyl,14-ethylene-14-pentadecne	0.33	$C_{20}H_{38}$	278
13	14-methyl-Pregn-4-ene-3,20-dione	0.34	$C_{22}H_{32}O_2$	328
14	(3 beta24S)-Stigmast-5-en-3-ol	0.19	$C_{29}H_{50}O$	414
	Total	100.00		

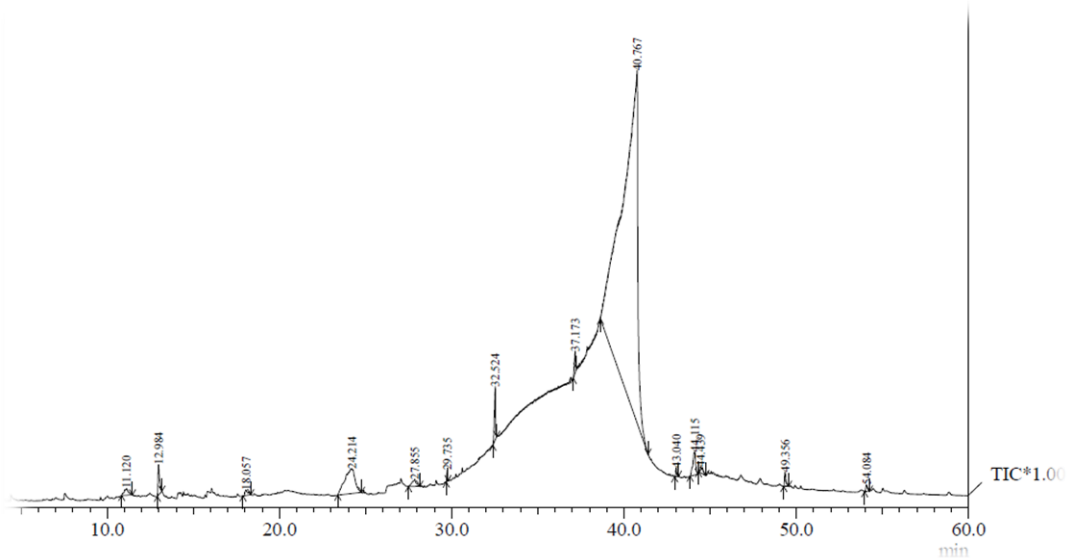

Fig. 4.1: GC-MS of *C. thevetia* hexane extract

4.2.2. GC-MS analysis of *Cascabela thevetia* acetone leaf extract

GC-MS analysis of leaf extract showed the presence of 42 compounds. The major compounds were Neophytadiene (18.18%), n-hexadecanoic acid (17.11%), gamma-Sitosterol (6.63%), 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R- [R*, R*-(E)-3,7,11,15-tetramethyl-2-hexadecene (5.61%), (Z, Z, Z)-9,12,15-Octadecatrienoic acid (5.22%) and 14-Methyl-pregn-4-ene-3,20-dione (5.03%). Other compounds are given in table 4.3. GC-MS analysis of *C. thevetia* acetone leaf extract is illustrated in figure 4.2.

Table 4.3: GC-MS analysis of CTAE

Sr. No.	Name of compound	Area %	Molecular Formula	Molecular Weight
1	1-Ethylpentyl acetate	2.26	$C_9H_{18}O_2$	158
2	2,4-bis(1,1-dimethylethyl)-Phenol	1.15	$C_{14}H_{22}O$	206
3	Tetradecanal	0.24	$C_{14}H_{28}O$	212
4	Tetradecanoic acid	0.64	$C_{14}H_{28}O_2$	228
5	5,6,7,7A-tetrahydro-6-2(4H)-Benzofuranone	1.10	$C_{11}H_{16}O_3$	196
6	(S,E)-4-Hydroxy-3,5,5-trimethyl-4-(3-oxobut-1-en-1-yl)cyclohex-2-enone	1.60	$C_{13}H_{18}O_3$	222
7	3,7,11,15-tetramethyl- [R-[R*,R*-(E)]]-2-Hexadecene	0.45	$C_{20}H_{40}$	280
8	Neophytadiene	18.18	$C_{20}H_{38}$	278
9	3,7,11,15-tetramethyl- [R-[R*,R*-(E)]]-2-Hexadecene	0.93	$C_{20}H_{40}$	280
10	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	3.42	$C_{20}H_{40}O$	296
11	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)-3,7,11,15-tetramethyl-2-hexadecene	5.61	$C_{20}H_{40}O$	296
12	n-Hexadecanoic acid	17.11	$C_{16}H_{32}O_2$	256
13	Phytol	4.15	$C_{20}H_{40}O$	296
14	Linoelaidic acid	0.91	$C_{18}H_{32}O_2$	280
15	(Z,Z,Z)-9,12,15-Octadecatrienoic acid	5.22	$C_{18}H_{30}O_2$	278
16	Octadecanoic acid	1.26	$C_{18}H_{36}O_2$	284

17	S-[2-[N,N-Dimethylamino]ethyl]N,N dimethylcarbamoyl thiocarbohydroximate	0.31	$C_8H_{17}N_3O_2S$	219
18	(6Z)-6-Pentadecen-1-ol	0.24	$C_{15}H_{30}O$	226
19	(Z)-9-Octadecenal	1.03	$C_{18}H_{34}O$	266
20	Tetracosanal	0.39	$C_{24}H_{48}O$	352
21	(3-methylphenyl)-Cyclohexane	0.17	$C_{12}H_{24}$	168
22	Hexadecanal	0.16	$C_{16}H_{32}O$	240
23	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	0.26	$C_{12}H_{23}NO_2$	213
24	Octadecanal	0.63	$C_{18}H_{36}O$	268
25	Docosanal	0.17	$C_{22}H_{44}O$	324
26	2,6,10,15,19,23-Hexamethyl-2,6,10,14,18,22-tetracosahexane	0.60	$C_{30}H_{50}$	410
27	alpha-Tocospiro B	0.14	$C_{29}H_{50}O_4$	462
28	alpha-Tocospiro B	0.23	$C_{29}H_{50}O_4$	462
29	Deoxy-celidoniol	0.49	$C_{29}H_{60}$	408
30	Androstan-1-on-2-en-17-ol acetate	0.47	$C_{21}H_{30}O_3$	330
31	gamma-Tocopherol	0.51	$C_{28}H_{48}O_2$	416
32	14-Methyl-pregn-4-ene-3,20-dione	5.03	$C_{22}H_{32}O_2$	328
33	Deoxy-celidoniol	2.02	$C_{29}H_{60}$	408
34	Vitamin E	3.84	$C_{29}H_{50}O_2$	430
35	(3 beta)-Ergost-5-en-3-ol	1.07	$C_{28}H_{48}O$	400
36	Stigmasterol	0.79	$C_{29}H_{48}O$	412
37	Hexatriacontane	1.79	$C_{36}H_{74}$	506
38	gamma-Sitosterol	6.63	$C_{29}H_{50}O$	414
39	4,4,6A,6B,8A,11,11,14B-Octamethyl-1,4,4A,5,6,6A,6B,7,8,8A,9,10,11,12,12A,14,14A 14B-octadecahydro-2H-picen-3-one	2.25	$C_{30}H_{48}O$	424
40	24-Norursa-3,12-diene	2.11	$C_{29}H_{46}$	394
41	(3 beta)-Olean-12-en-3-ol, acetate	0.56	$C_{32}H_{52}O_2$	426
42	24-Norursa-3,12-diene	3.67	$C_{29}H_{46}$	394
	Total	100		

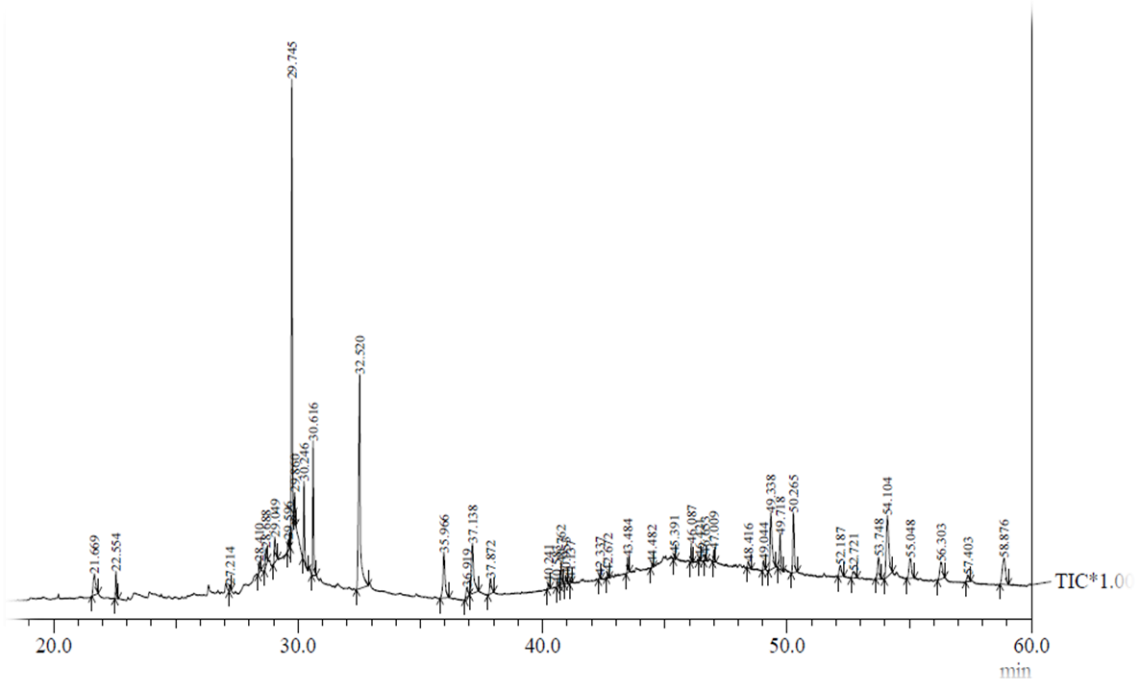

Fig. 4.2: GC-MS of *C. thevetia* acetone extract

4.2.3. GC-MS analysis of *Cascabela thevetia* methanol leaf extract

GC-MS analysis of leaf extract showed the presence of 41 compounds. The major compounds identified were Lup-20(29)-en-3-yl acetate (40.61%), (3 beta)-Olean-12-en-3-ol, acetate (7.20%), n-Hexadecanoic acid (5.98%), (3. beta.)-9,19-Cyclolanost-24-en-3-ol (4.97%), gamma sitosterol (4.85%) and 4,4,6a,6b,8a,11,11,14b-Octamethyl-1, 4, 4a, 5, 6, 6a, 6b, 7, 8, 8a, 9, 10, 11, 12, 12a, 14, 14a, 14b-octadecahydro-2H-picen-3-one (4.36%). Remaining compounds are given in table 4.4. GC-MS analysis of *C. thevetia* methanol leaf extract is illustrated in figure 4.3.

Sathish *et al.*, (2015), analyzed the methanolic lead extract of *Thevetia peruviana* and reported the presence of eight phytochemicals. The compounds reported were 8-Hydroxy-6, 7-epoxydendrolasin, Citronellyl tiglate, Methyl 16-hydroxy-3-3-Dimethylhepatadecanoate, 1-xAllyloxy-1-ethynyl-5-methylcyclohexane, 2-(Methoxycarbonyl)-2-propargyl-1-Cyclopentanol, 1, 2-Benzenedicarboxylic acid, dicyclohexyl ester, 1-(-R)-endo-Methylbornyl E-butenoate, 1, 3, 4, 5-Tetramethylbicyclo [3.2.0] hex-3-ene-2-one.

Sangodare *et al.*, (2012), reported the GC-MS analysis of *Thevetia peruviana* methanolic extract of seeds. The major compounds found were 1,3, dihydroxy-2-propanone (20.98%), n-hexadecanoic acid (36.62%), 2-propanone, 1,3- dihydroxy-, 9-octadecanoic acid (Z), methyl ester.

Table 4.4: GC-MS analysis of CTME

Sr. No.	Name of compound	Area %	Molecular Formula	Molecular Weight
1	5,6,7,7a-tetrahydro-4,4,7a-trimethyl-(R)-2(4H)-Benzofuranone	0.12	$C_{11}H_{16}O_2$	180
2	Octadecanal	0.23	$C_{18}H_{36}O$	268
3	5,6,7,7A-Tetrahydra-6-hydroxy-4,4,7A-trimethyl-(6S-cis)-2(4H)-Benzofuranone	0.27	$C_{11}H_{16}O_3$	196
4	Neophytadiene	0.21	$C_{20}H_{38}$	278
5	6,10,14-trimethyl-2-Pentadecanone	0.08	$C_{18}H_{36}O$	268
6	Pentadecanoic acid	0.17	$C_{15}H_{30}O_2$	242
7	3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-2-hexadecene-1-ol	0.05	$C_{20}H_{40}O$	296
8	(Z, Z, Z)-9,12,15-Octadecatrien-1-ol	0.11	$C_{18}H_{32}O$	264
9	n-Hexadecanoic acid	5.98	$C_{16}H_{32}O_2$	256
10	Palmitic Acid, TMS derivative	0.08	$C_{19}H_{40}O_2Si$	328
11	Phytol	0.19	$C_{20}H_{40}O$	296
12	Cis, cis, cis-7,10,13-Hexadecatrienal	3.14	$C_{16}H_{26}O$	234
13	Octadecanoic acid	0.35	$C_{18}H_{36}O_2$	284
14	4,8,12,16-Tetramethylheptadecan-4-olide	0.07	$C_{21}H_{40}O_2$	324
15	Glycine, N-[N-(2-hydroxybenzoyl)-beta-alanyl]-methyl ester	0.06	$C_{13}H_{16}N_2O_5$	280
16	6-(acetyloxy)-4-methyl-, (E)-4-Heptenal	0.05	$C_{10}H_{16}O_3$	184
17	4-[[(2-methoxy-4-octadecenyl) oxy] methyl]-1,3-Dioxolane	0.04	$C_{25}H_{48}O_4$	412
18	Octacosane	0.11	$C_{28}H_{58}$	394
19	2,6,10,14,18,22-Tetracosahexane, 2,6,10,15,19,23-hexamethyl-	1.70	$C_{30}H_{50}$	410

20	alpha -Tocospiro A	0.12	$C_{29}H_{50}O_4$	462
21	alpha -Tocospiro B	0.13	$C_{29}H_{50}O_4$	462
22	Deoxy-celidoniol	0.04	$C_{29}H_{60}$	408
23	Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-3,7,11,15,19-heneicosapentaenyl)-(all-E)-	0.09	$C_{30}H_{50}O$	426
24	3,7,11,16-tetramethyl-Hexadeca-2,6,10,14-tetraen-1-ol	0.04	$C_{20}H_{34}O$	290
25	3-ethyl-3,4-dimethyl-1-[10-methyldecalin-1,4-dien-3-one-9-yl]-Hexan-1,5-dione	0.21	$C_{21}H_{34}O_3$	334
26	gamma -Tocopherol	0.25	$C_{28}H_{48}O_2$	416
27	14-Methyl-pregn-4-ene-3,20-dione	1.64	$C_{22}H_{32}O_2$	328
28	Vitamin E	2.09	$C_{29}H_{50}O_2$	430
29	(E)-2-Tridecyl-2-heptadecenal	0.21	$C_{30}H_{58}O$	434
30	(3 beta 24Z)-Stigmasta-5,24(28)-dien-3-ol	0.15	$C_{29}H_{48}O$	412
31	(3 beta)-Ergost-5-en-3-ol	0.77	$C_{28}H_{48}O$	400
32	Stigmasterol	0.55	$C_{29}H_{48}O$	412
33	unidentified	0.13	$C_{32}H_{52}O_3$	484
34	gamma -Sitosterol	4.85	$C_{29}H_{50}O$	414
36	(3 beta)-Cholest-5-en-3-ol, 24-propylidene	1.35	$C_{30}H_{50}O$	426
37	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one	4.36	$C_{30}H_{48}O$	424
38	24-Norursa-3,12-diene	7.20	$C_{29}H_{46}$	394
39	(3 beta)-9,19-Cyclolanost-24-en-3-ol	4.97	$C_{30}H_{50}O$	426
40	Lup-20(29)-en-3-yl acetate	40.61	$C_{32}H_{52}O_2$	468
41	(3 beta)-Olean-12-en-3-ol, acetate	17.22	$C_{32}H_{52}O_2$	468
	Total	100.00		

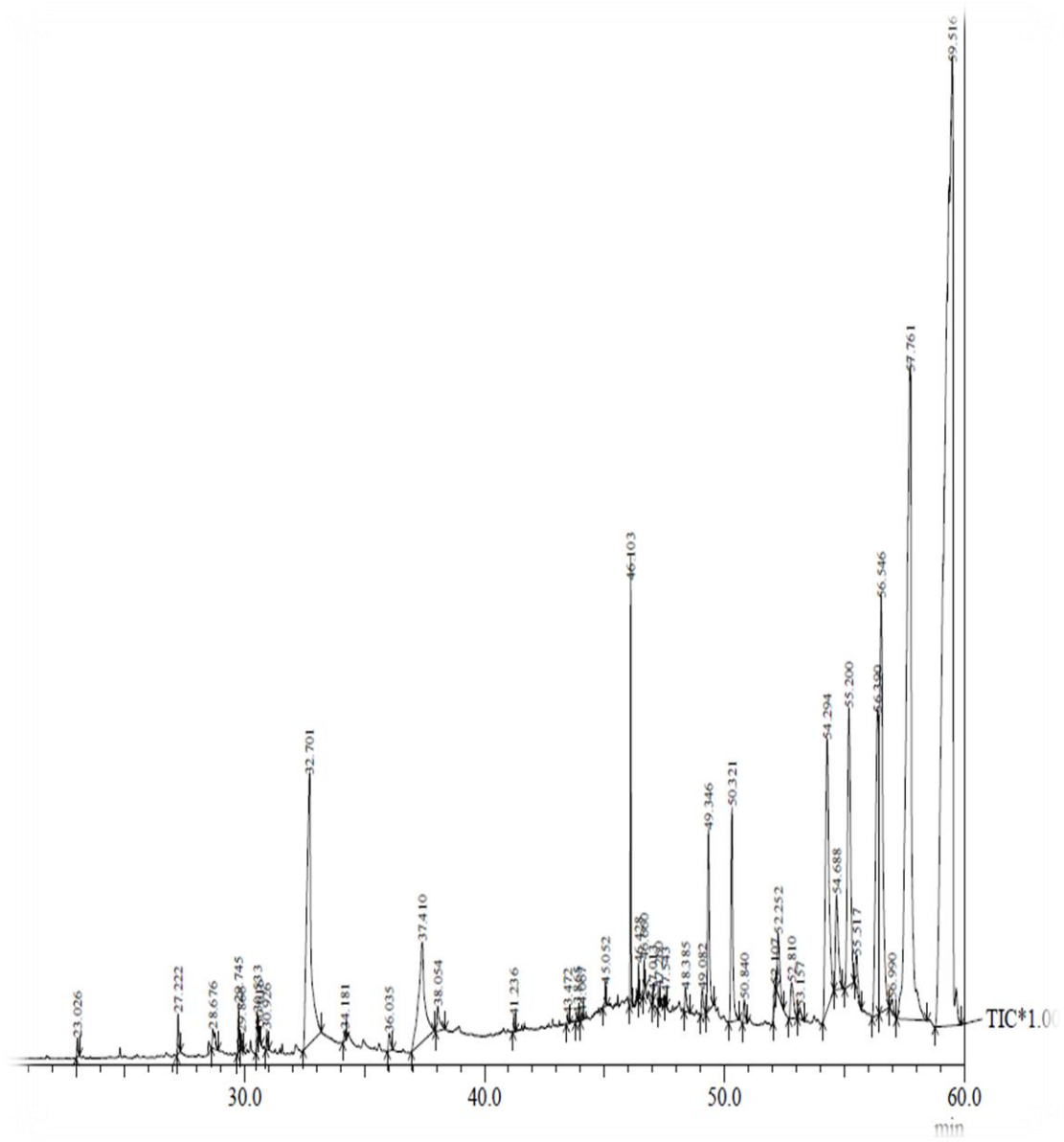

Fig. 4.3: GC-MS of *C. thevetia* methanol extract

4.2.4. GC-MS analysis of *Nerium oleander* hexane leaf extract

GC-MS analysis of leaf extract showed the presence of 32 compounds. The major compounds identified were mome inositol (57.72%), 5-Hydroxymethylfurfural (22.87%), 5,5'-[Oxybis(methylene)]bis-2-furancarbocyaldehyde (4.71%) and n-Hexadecanoic acid (2.10%). The compounds are shown in table 4.5. GC-MS analysis of *N. oleander* hexane leaf extract is illustrated in figure 4.4.

Table 4.5: GC-MS analysis of NOHE

Sr. No.	Name of compound	Area %	Molecular Formula	Molecular weight
1	6,6-Didiuteroundecane	0.12	$C_{11}H_{22}D_2$	158
2	1,3-Dioxane	0.10	$C_4H_8O_2$	88
3	2,3-Dihydro-5-hydroxy-6-methyl-4H-pyran-4-one	0.32	$C_6H_8O_3$	128
4	2,3-dihydro-3,5-dihydroxy-6-methyl-4H-Pyran-4-one	2.04	$C_6H_8O_4$	144
5	5-Hydroxymethylfurfural	22.87	$C_6H_6O_3$	126
6	2-Methoxy-4-vinylphenol	0.10	$C_9H_{10}O_2$	150
7	3,5-bis(1,1-dimethylethyl)- Phenol	0.05	$C_{14}H_{22}O$	206
8	N-(4-methyl-3-nitrophenyl)-Arabinosamine	1.39	$C_{12}H_{16}N_2O_6$	284
9	Mome inositol	57.72	$C_7H_{14}O_6$	194
10	Neophytadiene	0.43	$C_{20}H_{38}$	278
11	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.13	$C_{20}H_{40}O$	296
12	3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-2-Hexadecen-1-ol	0.20	$C_{20}H_{40}O$	296
13	n-Hexadecanoic acid	2.10	$C_{16}H_{32}O_2$	256
14	5,5'-[Oxybis(methylene)]bis-2-furancarbocyaldehyde	4.71	$C_{12}H_{10}O_5$	234
15	9,12-Octadecadienoic acid, methyl ester	0.11	$C_{19}H_{35}O_2$	294
16	(Z,Z,Z)-9,12,15-Octadecatrienoic acid, methyl ester	0.26	$C_{19}H_{32}O_2$	292
17	Oxacycloheptadec-8-en-2-one	0.33	$C_{16}H_{28}O_2$	252
18	(Z,Z,Z)-9,12,15-Octadecatrienoic acid	1.93	$C_{18}H_{30}O_2$	278
19	Octadecanoic acid	0.21	$C_{18}H_{36}O_2$	284
20	1-(1,5-dimethyl-4-hexenyl)-4-methyl Benzene	0.34	$C_{15}H_{22}$	202
21	tetrahydro-2,2,6-trimethyl-6-(4-methyl-3-cyclohexen-1-yl)-, [3S-[3alpha,6alpha(R*)]]-2H-Pyran-3-ol	0.23	$C_{15}H_{26}O_2$	238

22	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	0.23	$C_{19}H_{38}O_4$	330
23	(3 beta)-Olean-12-ene-3,28-diol	0.50	$C_{30}H_{50}O_2$	442
24	Cholestan-22(26)-epoxy-3,16-dione	0.11	$C_{27}H_{42}O_3$	414
25	3-hydroxy- (3beta)-Pregen-5-en-20-one	0.14	$C_{21}H_{32}O_2$	316
26	Ursolic aldehyde	1.69	$C_{30}H_{48}O_2$	440
27	1,2,4a,5,6,7,8,8a-octahydro-4a-methyl-2-Naphthalenamine	0.10	$C_{11}H_{19}N$	165
28	18,22,22-Trimethyl-17,27,29,30-tetranor-c-homoolean-14-ene-3beta,21alpha-diol	0.53	$C_{30}H_{50}O_2$	442
29	Acetyl betulinaldehyde	0.17	$C_{32}H_{50}O_3$	482
30	3,14-dihydroxy- (3 beta,5 alpha)-Card-20(22)-enolide	0.17	$C_{23}H_{34}O_4$	374
31	24-Norursa-3,12-diene	0.20	$C_{29}H_{46}$	394
32	alpha -Amyrin	0.27	$C_{30}H_{50}O$	426
	Total	100.00		

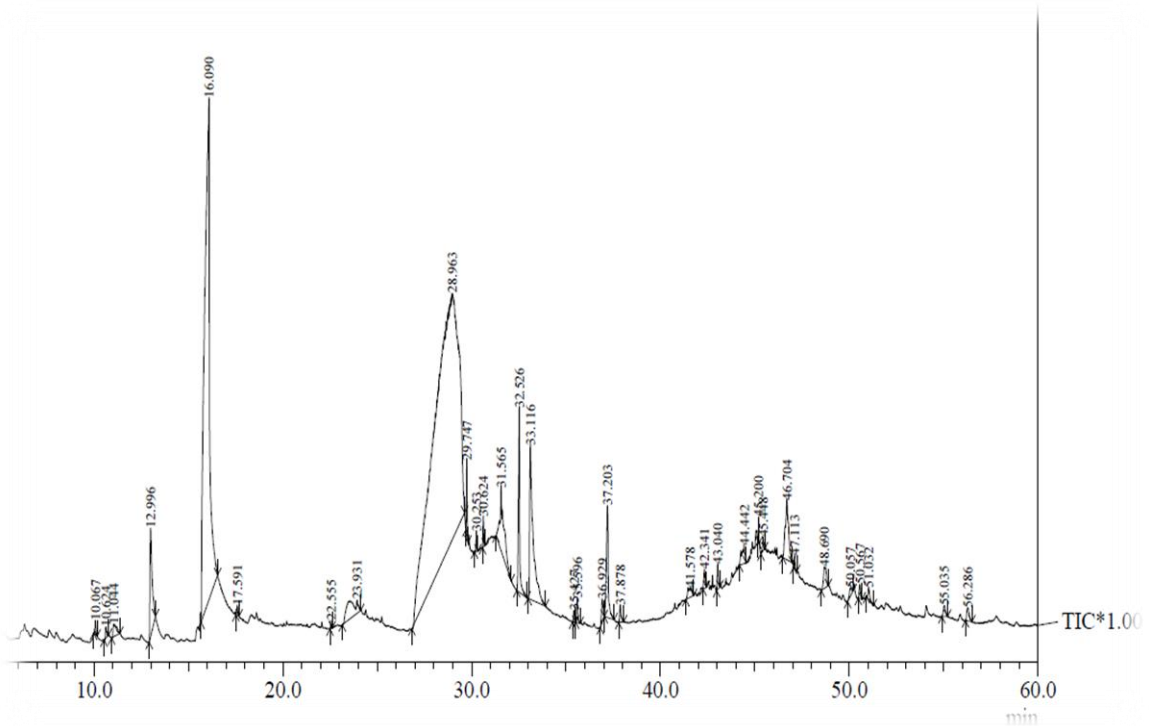

Fig. 4.4: GC-MS of *N. oleander* hexane extract

4.2.5. GC-MS analysis of *Nerium oleander* acetone leaf extract

GC-MS analysis of leaf extract revealed the presence of 17 compounds. The major compound present were mome inositiol (84.29%), neopytadiene (4.93%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (1.58%), phytol (1.23%), 2,3-dihydro-benzofuran (1.22%) and n-hexadecanoic acid (1.14%). Remaining compounds are given in table 4.6. GC-MS analysis of *N. oleander* acetone leaf extract is illustrated in figure 4.5.

According to **Hase *et al.*, (2017)**, GC-MS analysis of *Nerium oleander* L. root extract showed fifty-eight phytochemical compounds. The major phytoconstituents present were 2-methoxy-5-methyl phenol and isomers (2.92%), 2, 6-dimethoxyphenyl ester (4.47%), trans-Isogenol (4.89%),5-ter-butylpyrogallol(2.25%),2-hydroxy-4-isopropyl-7 methoxytropone (3.72%),l-(+)-ascorbic-acid-2,6-dihexadecanoate, (4.47%), trans-Isogenol(4.89%),2,3,5-trimetho oxyamphetamine (2.25%), 2-hydroxy-4-isopropyl-7-methoxytropone (3.72 %), Oleic acid(1.09 %), 2-(9,12- octadecadienyloxy)-,(Z,Z)-ethanol (9.48 %), 9-hexadecenoic acid (2.24%), 9-octadecenamide (3.13%), α-amyrin (10.85 %).

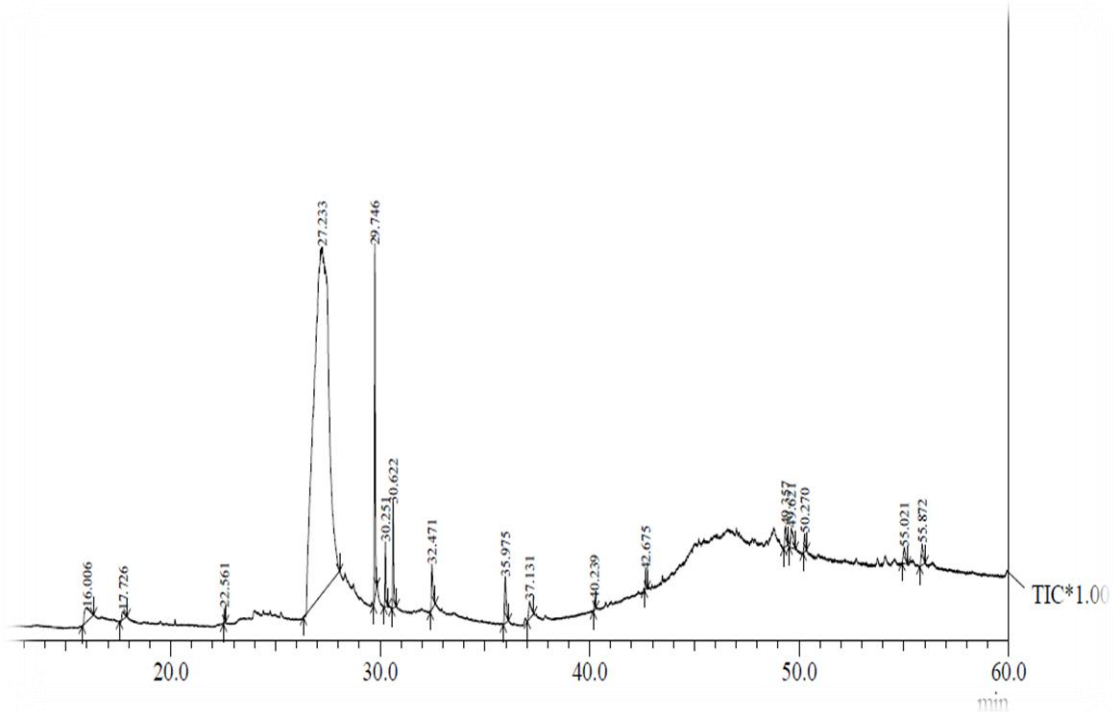

Fig. 4.5: GC-MS of *N. oleander* acetone extract

Table 4.6: GC-MS analysis of NOAE

Sr. No.	Name of compound	Area%	Molecular formula	Molecular weight
1	2,3-Dihydro-benzofuran	1.22	C_8H_8O	120
2	2-Methoxy-4-vinylphenol	0.28	$C_9H_{10}O_2$	150
3	3,5-bis(1,1-dimethylethyl)-Phenol	0.20	$C_{14}H_{22}O$	206
4	Mome inositol	84.29	$C_7H_{14}O_6$	194
5	Neophytadiene	4.93	$C_{20}H_{38}$	278
6	3,7,11,15-tetramethyl-, [R- [R*, R*-(E)]]-2-Hexadecen-1-ol	0.97	$C_{20}H_{40}O$	296
7	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	1.58	$C_{20}H_{40}O$	296
8	n-Hexadecanoic acid	1.14	$C_{16}H_{32}O_2$	256
9	Phytol	1.23	$C_{20}H_{40}O$	296
10	cis, cis, cis-7,10,13-Hexadecatrienal	0.70	$C_{16}H_{26}O$	234
11	Carbonic acid, 2-dimethylaminoethyl neopentyl ester	0.15	$C_{10}H_{21}NO_3$	203
12	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	0.26	$C_{12}H_{23}NO_2$	213
13	22,23-Bisnor-5-cholenic acid, 3beta-hydroxy, acetate(ester)	0.59	$C_{24}H_{36}O_4$	388
14	2-Oxo-2-[3-oxoandrosta -4,9(11),16-trien-16-yl]	0.80	$C_{23}H_{28}O_4$	368
15	Vitamin E	0.38	$C_{29}H_{50}O_2$	430
16	3-(1,5-Dimethyl-hexyl)-3a,10,10,12b-tetramethyl-1,2,3,3a,4,6,8,9,10,10a,11,12,12a,12b-tetradecahydro- benzo[4,5]cyclohepta[1,2-E]indene	0.58	$C_{30}H_{50}$	410
17	4-ethenyl-6-(2- hydroxyacetoxy)-2,4,7,14-tetramethyl-tricyclo [5.4.3.0(1,8)] tetradecan-3-ol-9-one	0.69	$C_{22}H_{34}O_5$	378
	Total	100		

4.2.6. GC-MS analysis of *Nerium oleander* methanol leaf extract

GC-MS analysis of leaf extract revealed the presence of 52 compounds. The major compounds present were n-hexadecanoic acid (11.25%), alpha-amyrin (10.02%), vitamin E (9.77%), gamma-sitosterol (7.50%), Pregna-1,4,7,16-tetraene-3,20-dione (7.25%), stigmasterol (6.79%), 24-norusa-3,12-diene (5.33%) and 16,17-epoxy- (16-alpha.)-Pregn-4-ene-3,20-dione (5.20%). Remaining compounds are described in table 4.7. GC-MS analysis of *N. oleander* methanol leaf extract is illustrated in figure 4.6.

According to **Hameed *et al.*, (2015)**, GC-MS analysis of *N. oleander* ethanol leaf extract showed the presence of 2-hydroxy-3-methyl, 9.12.15-octadecatrienoic acid, tetradecamethyle, β-d-allopyranoside, methyle 6-dioxy-2-o methyl, 5-hydrooxy methylfurfural, cycloheptasiloxane, cyclooctasiloxane, hexadecamethyl, cyclononasiloxane, octadecamethyl, cyclodecasiloxane, eicosamethyl, 2-cyclopenten-1-one, 2,3bis[trimethylsilyl]oxy] propyl ester, octadecane, 1.1.3.3.5.5.7.7.9.9-decamethyl-9-(2-methyl propoxy)pentasiloxane,3-ethyl-5-(2-ethylbutyl), 1-monolinoleoylglycerol trimethylsilyl ether, 1.1.3.3.5.5.7.7.9.9.11.11.13.13.15.15-hexadecamethyl 2-cyclohexen-1-one, 4-(hydroxy butyl)-3.5.5-trimethyl, octasiloxane and 3-eicosene.

Dey *et al.*, (2015), also reported the presence of similar type of compounds in his studies e.g., tetradecanoic acid, n-hexadecanoic acid, phytol, pentadecanoic decanoic methyl ester etc. While we analyzed the leaves and the results obtained are as follows:

Table 4.7: GC-MS analysis of NOME

Sr. No.	Name of compound	Area%	Molecular formula	Molecular weight
1	5,6,7,7a-tetrahydro-4,4,7a-trimethyl 2(4H)-Benzofuranone	1.14	$C_{11}H_{16}O_2$	180
2	4-(1,5-Dihydroxy-2,6,6-trimethylcyclohex-2-enyl) but-3-en-2-one	0.27	$C_{13}H_{20}O_3$	224
3	Tetradecanal	0.12	$C_{14}H_{28}O$	212
4	5,6,7,7A-tetrahydro-6-hydroxy-4,4,7A-trimethyl- (6S-cis)-42(4H)-benzofuranone	0.89	$C_{11}H_{16}O_3$	196
5	Neophytadiene	1.32	$C_{20}H_{38}$	278

6	6,10,14-trimethyl-2-Pentadecanone	0.63	$C_{18}H_{36}O$	268
7	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.48	$C_{20}H_{40}O$	296
8	(Z)- 2-Hexen-1-ol, TMS derivative	0.10	$C_{11}H_{22}O_2Si$	214
9	3,7,11,15-Tetramethyl- [R-[R*,R*-(E)]]-2-hexadecen-1-ol	0.69	$C_{20}H_{40}O$	296
10	n-Hexadecanoic acid	11.25	$C_{16}H_{32}O_2$	256
11	Palmitic Acid, TMS derivative	0.14	$C_{19}H_{40}O_2Si$	328
12	Phytol	4.25	$C_{20}H_{40}O$	296
13	Linoelaidic acid	1.11	$C_{18}H_{32}O_2$	280
14	cis,cis,cis-7,10,13-Hexadecatrienal	1.66	$C_{16}H_{26}O$	234
15	Eicosanoic acid	0.69	$C_{20}H_{40}O_2$	312
16	1,1'-[3-(2-cyclopentylethylidene)-1,5-pentanediyl]bis-Cyclopentane	0.10	$C_{22}H_{38}$	302
17	4,8,12,16-Tetramethylheptadecan-4-olide	0.54	$C_{21}H_{40}O_2$	324
18	3-oxo- octadecanoic acid, ethyl ester	0.86	$C_{20}H_{38}O_3$	326
19	Octacosane	0.08	$C_{28}H_{58}$	394
20	Di-n-octyl phthalate	0.15	$C_{24}H_{38}O_4$	390
21	1,1-dichloro-2,2,3,3-tetramethylcyclopropane	0.20	$C_7H_{12}Cl_2$	166
22	(Z,Z,Z)-9,12,15-Octadecatrienoic acid, phenylmethyl ester	0.33	$C_{25}H_{36}O_2$	368
23	2-Ethylbutyric acid, eicosyl ester	0.16	$C_{26}H_{52}O_2$	396
24	5,6-difluoro-3-hydroxy-, (3.beta.)-Pregnan-20-one	0.22	$C_{21}H_{32}F_2O_2$	354
25	3-hydroxy-, (3.beta.)-Pregnan-20-one	0.20	$C_{21}H_{34}O_2$	318
26	2,5-Dimethyl-1-(trimethylsilyl)-1H-indole	0.19	$C_{13}H_{19}NSi$	217
27	2,6,10,15,19,23-Hexamethyl 2,6,10,14,18,22-tetracosahexane	0.73	$C_{30}H_{50}$	410
28	(2E,6E,10E)-3,7,11,15-Tetramethylhexadeca-2,6,10,14-tetraen-1-yl formate	0.07	$C_{21}H_{34}O_2$	318

29	alpha-Tocospiro A	0.64	$C_{29}H_{50}O_4$	462
30	alpha-Tocospiro B	1.16	$C_{29}H_{50}O_4$	462
31	14-hydroxy- Pregn-4-ene-3,20-dione	0.73	$C_{21}H_{30}O_3$	330
32	Effusanin E	0.12	$C_{20}H_{28}O_6$	364
33	unidentified	3.97	$C_{14}H_{20}O_3$	236
34	3-ethyl-3,4-dimethyl-1-[10-methyldecalin-1,4-dien-3-one-9-yl]-Hexan-1,5-dione	3.02	$C_{21}H_{34}O_3$	334
35	1,5,9-trimethyl-12-(1-methylethyl)-4,8,13-Cyclotetradecatriene-1,3-diol	0.85	$C_{20}H_{34}O_2$	306
36	(R)-6-Methoxy-2,8-dimethyl-2-((4R,8R)-4,8,12-trimethyltridecyl)chroman	1.13	$C_{28}H_{48}O_2$	416
37	gamma-Tocopherol	0.28	$C_{28}H_{48}O_2$	118
38	16,17-epoxy- (16 alpha)-Pregn-4-ene-3,20-dione	5.20	$C_{21}H_{28}O_3$	328
39	Pregna-1,4,7,16-tetraene-3,20-dione	7.25	$C_{21}H_{24}O_2$	308
40	Vitamin E	9.77	$C_{29}H_{50}O_2$	430
41	Desmosterol	1.79	$C_{27}H_{44}O$	384
42	(3 beta)-Ergost-5-en-3-ol	0.86	$C_{28}H_{48}O$	400
43	Stigmasterol	6.79	$C_{29}H_{48}O$	412
44	(3beta,5alpha,6beta,12-beta)-Ergost-25-ene-3,5,6,12-tetrol	0.47	$C_{28}H_{48}O_4$	448
45	gamma -Sitosterol	7.50	$C_{29}H_{50}O$	414
46	(+)-Cedrylacetate	1.29	$C_{17}H_{28}O_2$	264
47	24-Norursa-3,12-diene	5.33	$C_{29}H_{46}$	394
48	Lupeol	0.43	$C_{30}H_{50}O$	426
49	1-Cyclohexene-1-acrylic acid, 2,6,6-trimethyl-3-oxo-methyl ester	1.33	$C_{13}H_{18}O_3$	222
50	alpha -Amyrin	10.02	$C_{30}H_{50}O$	426
51	Stigmast-4-en-3-one	0.50	$C_{29}H_{48}O$	412
52	Lupeol	0.96	$C_{30}H_{50}O$	426
	Total	100		

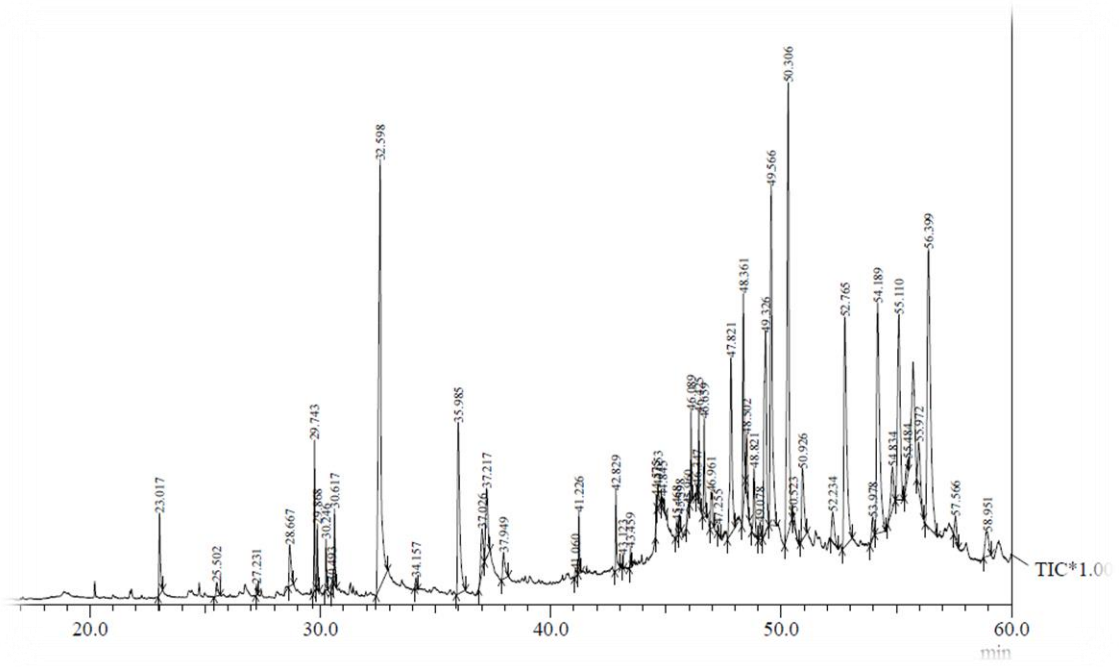

Fig. 4.6: GC-MS of *N. oleander* methanol extract

4.2 Antioxidant activity

The antioxidant activity was assayed using DPPH, ferrous chelating activity and hydroxyl radical scavenging activity. Results are obtained in triplicate manner along with standard deviation.

4.2.1. DPPH free radical scavenging activity

The result of this antioxidant activity had shown that the best scavenging was shown by CTHE (IC_{50} = 106.56µg/mL) which was less than standard ascorbic acid (IC_{50} = 100.15µg/mL). Detailed scavenging activities of all the extracts along with IC_{50} values is given in table 4.8 and related graphs are shown in figure 4.7. The increasing order of antioxidant activity is as follows:

Ascorbic acid > Gallic acid > CTHE > CTAE > CTME

Phuse and Khan, (2018), tested the antioxidant activity of *C. thevetia* flower extract by DPPH, nitric oxide and superoxide assay and solvent used was ethyl acetate and acetone. IC_{50} values obtained from these assays were 90.52µg/mL, 63.45µg/mL and 60.41µg/mL respectively. IC_{50} values from Acetone extract for flower were 59.60µg/mL, 45.10µg/mL and 36.98µg/mL respectively.

Table 4.8: % Scavenging activity of *C. thevetia* extracts with standards

Sr. no.	Sample name		% Scavenging activity of different concentrations (in µg/mL)					
			100	**200**	**300**	**400**	**500**	**IC_{50}**
1.	**Gallic acid**	**R1**	49.40	58.29	67.23	80.77	88.07	112.18
		R2	49.28	58.14	67.11	80.85	88.60	114.56
		R3	49.25	58.26	67.11	80.68	88.02	113.29
		Mean	49.31 ±0.08	58.23 ±0.08	67.15 ±0.07	80.77 ±0.09	88.23 ±0.32	113.35 ±1.19
2.	**Ascorbic acid**	**R1**	50.25	60.10	67.79	78.25	88.86	100.25
		R2	50.37	60.10	67.61	78.40	88.92	100.04
		R3	50.13	60.27	67.90	78.19	88.92	100.15
		Mean	50.25 ±0.12	60.16 ±0.10	67.77 ±0.15	78.28 ±0.11	88.90 ±0.03	100.15 ±0.10
3.	**CTHE**	**R1**	48.82	57.47	66.44	73.19	79.71	104.89
		R2	48.76	57.00	66.56	73.11	79.63	107.24
		R3	48.61	57.18	66.38	73.08	79.51	107.55
		Mean	48.73 ±0.11	57.22 ±0.24	66.46 ±0.09	73.13 ±0.06	79.62 ±0.10	106.56 ±1.46
4.	**CTAE**	**R1**	49.14	55.83	64.95	74.71	83.31	121.32
		R2	49.31	56.45	64.86	74.63	83.43	117.91
		R3	49.11	56.30	64.89	74.54	83.25	119.53
		Mean	49.19 ±0.11	56.19 ±0.32	64.90 ±0.04	74.63 ±0.09	83.33 ±0.09	119.58 ±1.71
5.	**CTME**	**R1**	41.30	48.73	59.92	67.41	75.48	201.60
		R2	41.16	48.64	60.01	67.61	75.50	201.98
		R3	41.07	48.47	60.16	67.49	75.50	202.86
		Mean	41.18 ±0.12	48.61 ±0.13	60.03 ±0.12	67.50 ±0.10	75.49 ±0.02	202.15 ±0.64

	Concentration	**Treatment**	**Conc. X Treat.**
S.Em±	0.319	0.251	0.080
CD at 5%	0.897	0.709	0.636

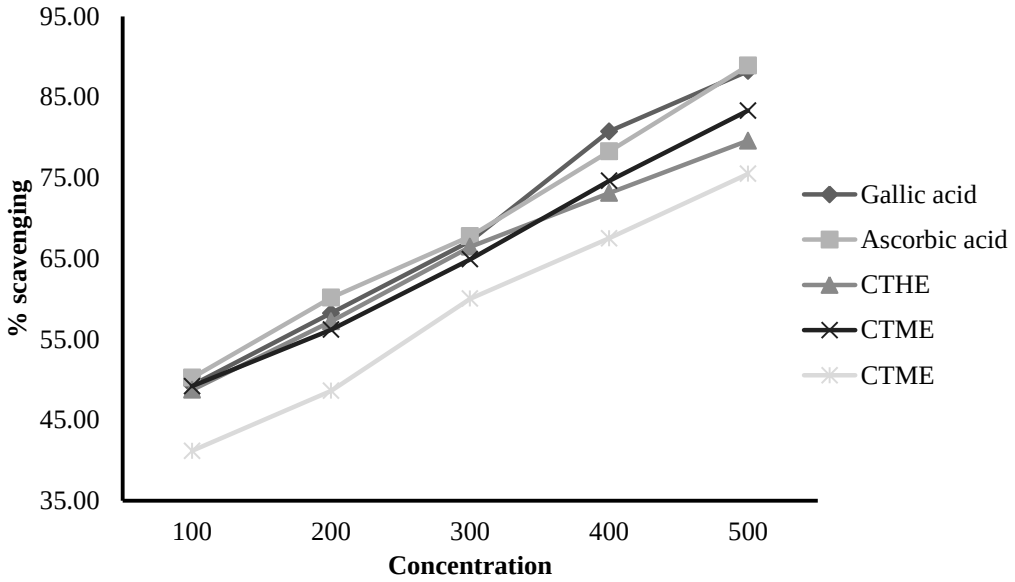

Fig. 4.7: % scavenging of *C. thevetia* extracts at different concentrations

In case of *N. oleander* best inhibition was shown by NOHE (IC_{50} = 109.56µg/mL) which was almost similar to the standard ascorbic acid (IC_{50} = 100.15µg/mL). Detailed % scavenging activity and IC_{50} values are given in table 4.9 and graphs are given in figure 4.8. The increasing order of antioxidant activity is as follows:

Ascorbic acid > Gallic acid > NOHE > NOAE > NOME

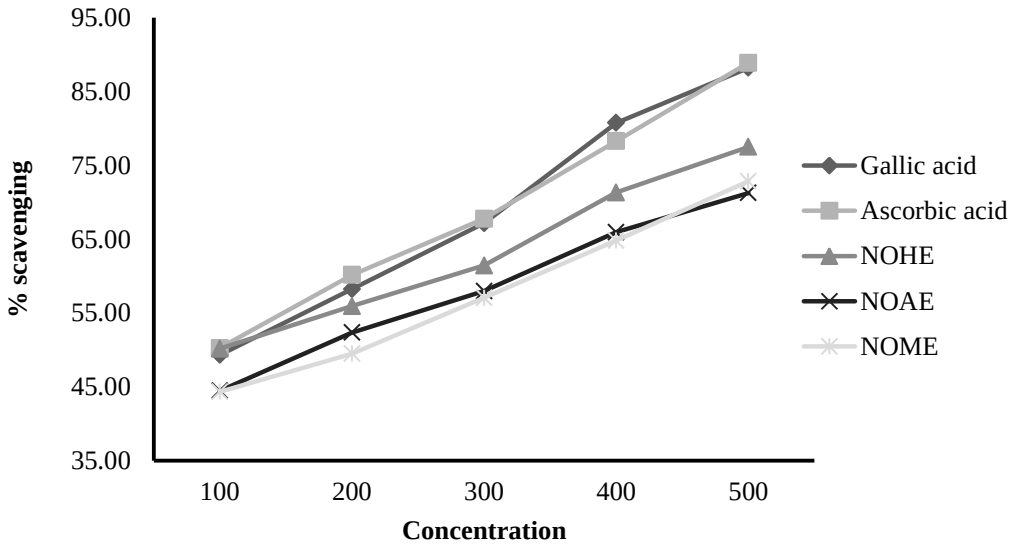

Fig. 4.8: % scavenging of *N. oleander* extracts at different concentrations

Table 4.9: % Scavenging activity of *N. oleander* extracts with standards

Sr. no.	Sample name		% Scavenging activity of different concentrations (in µg/mL)					
			100	200	300	400	500	IC_{50}
1.	Gallic acid	R1	49.40	58.29	67.23	80.77	88.07	112.18
		R2	49.28	58.14	67.11	80.85	88.60	114.56
		R3	49.25	58.26	67.11	80.68	88.02	113.29
		Mean	49.31 ±0.08	58.23 ±0.08	67.15 ±0.07	80.77 ±0.09	88.23 ±0.32	113.35 ±1.19
2.	Ascorbic acid	R1	50.25	60.10	67.79	78.25	88.86	100.25
		R2	50.37	60.10	67.61	78.40	88.92	100.04
		R3	50.13	60.27	67.90	78.19	88.92	100.15
		Mean	50.25 ±0.12	60.16 ±0.10	67.77 ±0.15	78.28 ±0.11	88.90 ±0.03	100.15 ±0.10
3.	NOHE	R1	50.16	55.86	61.39	71.27	77.55	108.49
		R2	50.04	55.92	61.47	71.38	77.46	111.44
		R3	50.31	56.04	61.44	71.35	77.49	108.75
		Mean	50.17 ±0.13	55.94 ±0.09	61.43 ±0.04	71.33 ±0.06	77.50 ±0.04	109.56 ±1.63
4.	NOAE	R1	44.55	52.32	57.91	65.89	71.15	174.64
		R2	44.40	52.35	58.02	65.97	71.29	175.25
		R3	44.43	52.41	57.97	65.97	71.32	175.08
		Mean	44.46 ±0.08	52.36 ±0.04	57.97 ±0.06	65.95 ±0.05	71.26 ±0.09	174.99 ±0.32
5.	NOME	R1	44.43	49.52	57.09	64.83	72.90	192.57
		R2	44.29	49.52	57.00	64.75	72.81	193.82
		R3	44.29	49.43	57.06	64.89	72.84	193.83
		Mean	44.33 ±0.08	49.49 ±0.05	57.05 ±0.04	64.83 ±0.07	72.85 ±0.04	193.41 ±0.72

	Concentration	Treatment	Conc. X Treat.
S.Em±	0.319	0.712	0.227
CD at 5%	0.897	0.201	0.180

Comparison of antioxidant activity between *C. thevetia* and *N. oleander* showed following results (Fig. 4.9):

Ascorbic acid > Gallic acid > CTHE > NOHE > CTAE > NOAE > NOME >CTME

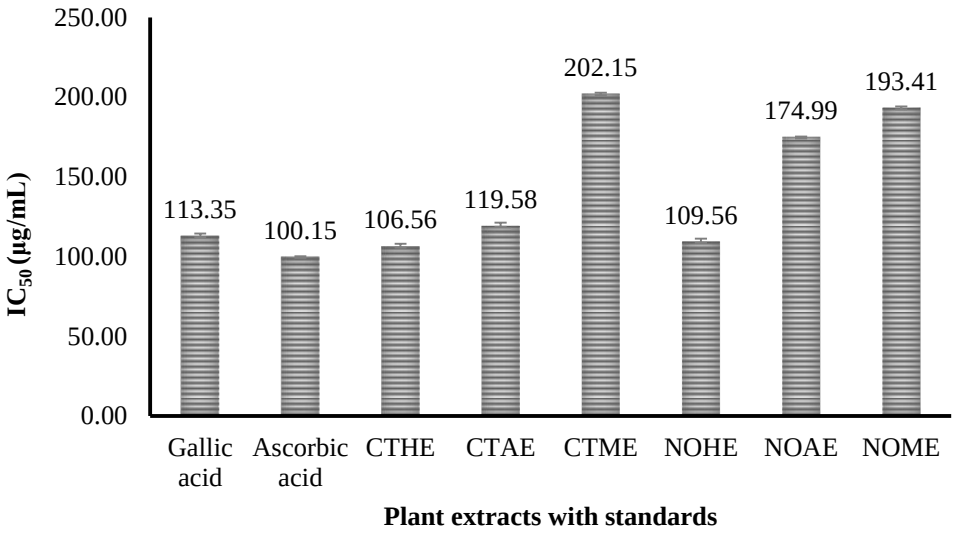

Fig. 4.9: IC_{50} values of all extracts along with their standards

4.2.2 Metal chelating activity

The present investigation of different leaf extracts showed that the plant extract exhibited less chelating power than standards. Among the plant extracts CTHE showed highest chelating power i.e., IC_{50} = 254.37µg/mL while standard showed IC_{50} = 174.02µg/mL. % chelating activity and IC_{50} values are given in table 4.10 and related graphs are shown in figure 4.10. The increasing chelating power of different extracts was in order:

Citric acid > EDTA > CTHE > CTAE > CTME

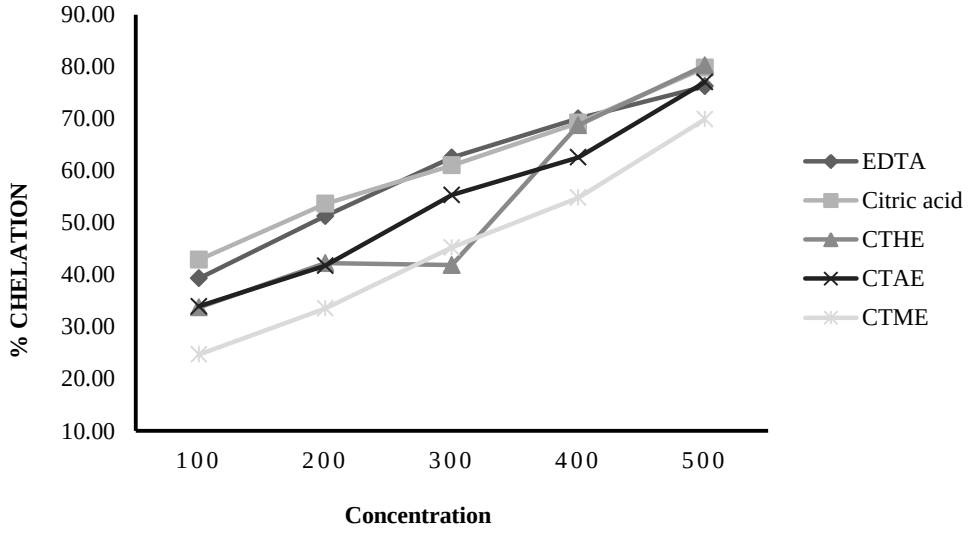

Fig. 4.10: % chelating activity of *C. thevetia* extracts at different concentrations

Table 4.10: % chelating activity of *C. thevetia* extracts with standards

Sr. no.	Sample name		% chelating activity of different concentrations (in µg/mL)					
			100	200	300	400	500	IC_{50}
1.	EDTA	R1	39.39	51.19	62.47	70.03	76.13	193.43
		R2	39.31	51.39	62.67	70.11	76.64	192.64
		R3	39.19	51.27	62.47	69.99	75.93	194.03
		Mean	39.30 ±0.10	51.28 ±0.10	62.54 ±0.11	70.04 ±0.06	76.23 ±0.37	193.37 ±0.70
2.	Citric acid	R1	42.95	53.72	60.85	69.12	79.89	173.29
		R2	42.79	53.56	61.01	69.36	79.73	174.02
		R3	42.87	53.60	61.16	69.16	79.77	173.28
		Mean	42.87 ±0.08	53.63 ±0.08	61.01 ±0.16	69.21 ±0.13	79.80 ±0.08	173.53 ±0.43
3.	CTHE	R1	33.77	42.24	52.65	68.73	80.25	253.81
		R2	33.65	42.12	52.26	68.80	80.13	255.01
		R3	33.69	42.28	52.34	68.69	80.32	254.29
		Mean	33.70 ±0.06	42.21 ±0.08	52.41 ±0.21	68.74 ±0.06	80.23 ±0.10	254.37 ±0.60
4.	CTAE	R1	33.93	41.84	55.27	62.71	76.92	261.44
		R2	33.81	41.77	55.34	62.55	77.04	261.87
		R3	33.97	41.57	55.34	62.39	77.12	262.00
		Mean	33.90 ±0.98	41.73 ±0.14	55.32 ±0.05	62.55 ±0.16	77.03 ±0.10	261.77 ± 0.29
5.	CTME	R1	24.58	33.49	45.25	54.75	69.95	339.22
		R2	24.54	33.53	45.09	54.71	69.83	339.76
		R3	24.86	33.53	45.33	54.91	69.87	338.60
		Mean	24.66 ±0.17	33.52 ±0.02	45.22 ±0.12	54.79 ±0.10	69.89 ±0.06	339.19 ±0.58

	Concentration	Treatment	Conc. X Treat.
S.Em±	0.320	0.253	0.081
CD at 5%	0.901	0.712	0.642

In case of *N. oleander* among the different extracts NOAE showed highest chelating power (IC_{50} = 274.04µg/mL) followed by methanol and hexane extract while standard showed IC_{50} of 173.53 and 193.37µg/mL for citric acid and EDTA respectively. Detailed values are given in table 4.11 and related graphs are shown in figure 4.11. The increasing chelating power of different extracts is in order:

Citric acid > EDTA > NOAE > NOME > NOHE

Table 4.11: % chelating activity of *N. oleander* extracts with standards

Sr. no.	Sample name		% chelating activity of different concentrations (in µg/mL)					
			100	200	300	400	500	IC_{50}
1.	EDTA	R1	39.39	51.19	62.47	70.03	76.13	193.43
		R2	39.31	51.39	62.67	70.11	76.64	192.64
		R3	39.19	51.27	62.47	69.99	75.93	194.03
		Mean	39.30 ±0.10	51.28 ±0.10	62.54 ±0.11	70.04 ±0.06	76.23 ±0.37	193.37 ±0.70
2.	Citric acid	R1	42.95	53.72	60.85	69.12	79.89	173.29
		R2	42.79	53.56	61.01	69.36	79.73	174.02
		R3	42.87	53.60	61.16	69.16	79.77	173.28
		Mean	42.87 ±0.08	53.63 ±0.08	61.01 ±0.16	69.21 ±0.13	79.80 ±0.08	173.53 ±0.43
3.	NOHE	R1	22.57	31.08	41.77	51.23	66.07	369.48
		R2	22.49	31.12	41.61	50.99	66.27	370.01
		R3	22.37	31.20	41.84	51.11	65.99	369.85
		Mean	22.47 ±0.10	31.13 ±0.06	41.74 ±0.12	51.11 ±0.12	66.11 ±0.14	369.78 ±0.27
4.	NOAE	R1	31.59	42.16	50.48	62.59	77.83	274.07
		R2	31.87	41.88	50.36	62.67	77.95	274.03
		R3	31.71	42.08	50.55	62.47	77.75	274.02
		Mean	31.72 ±0.14	42.04 ±0.14	50.46 ±0.10	62.58 ±0.10	77.84 ±0.10	274.04 ±0.02
5.	NOME	R1	23.67	35.35	44.26	53.48	69.60	342.91
		R2	23.52	35.35	44.06	53.56	69.48	343.74
		R3	23.63	35.19	44.18	53.37	69.40	344.17
		Mean	23.61 ±0.08	35.30 ±0.09	44.17 ±0.10	53.47 ±0.10	69.49 ±0.10	343.61 ±0.64

	Concentration	Treatment	Conc. X Treat.
S.Em±	0.320	0.716	0.229
CD at 5%	0.901	0.201	0.181

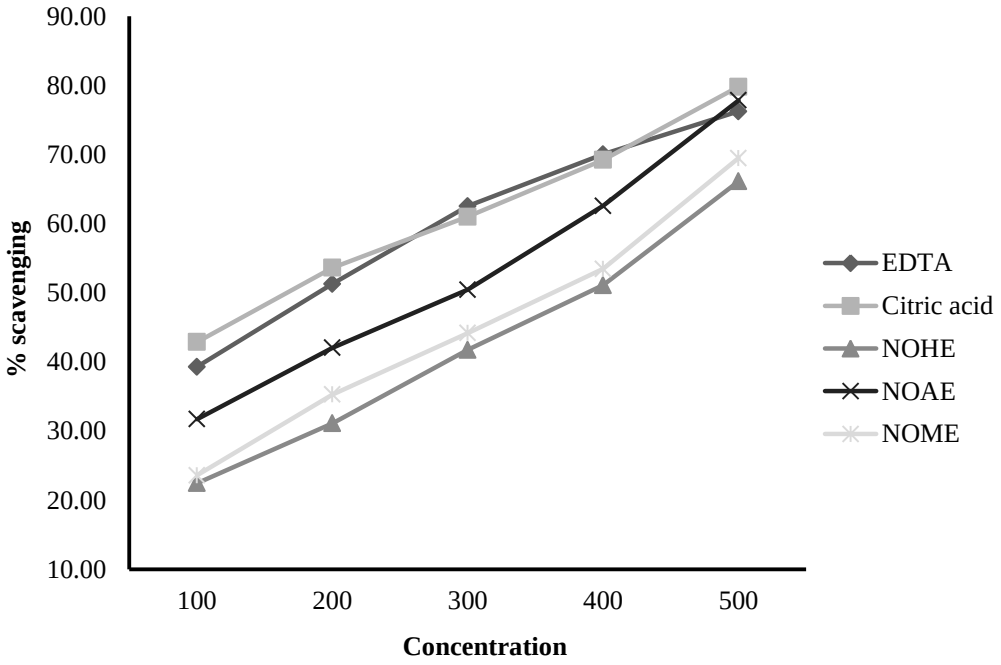

Fig. 4.11: % chelating activity of *N. oleander* extracts at different concentrations

Comparison of chelating power of *C. thevetia* and *N. oleander* (Fig. 4.12):

Citric acid > EDTA > CTHE > CTAE > NOAE > CTME > NOME > NOHE

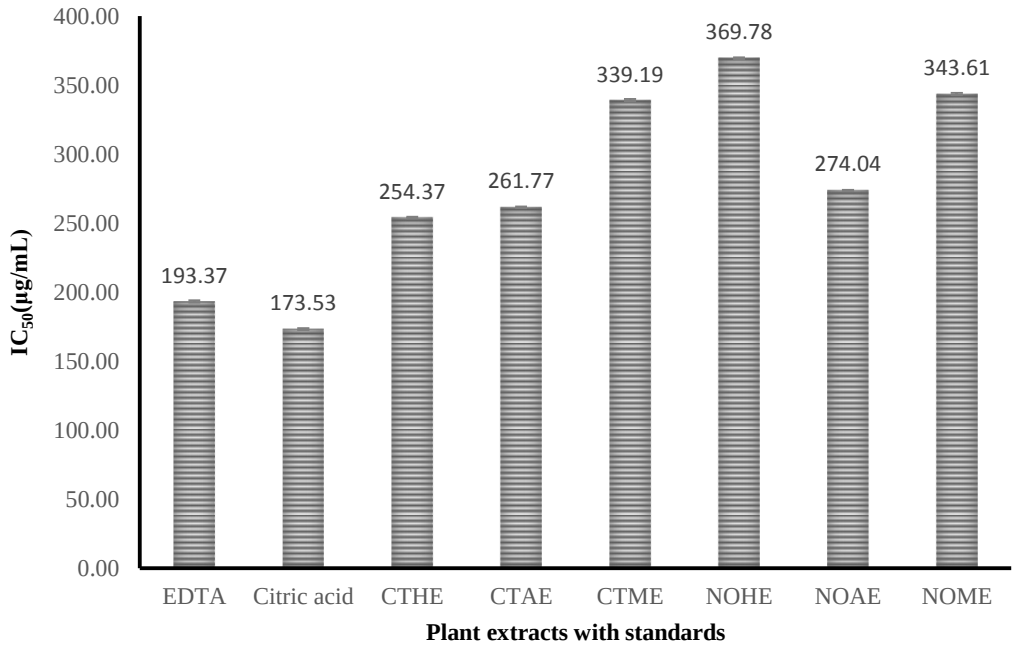

Fig. 4.12: IC_{50} values of all extracts along with their standards

4.2.3. Hydroxyl radical scavenging activity

Among the plant extracts CTME showed highest scavenging power (IC_{50} = 55.79µg/mL) followed by acetone and hexane extract while standard showed IC_{50} = 51.34µg/mL. Values are given in table 4.12 and related graphs are shown in figure 4.13. The increasing chelating power of different extracts was in order:

Ascorbic acid > CTME > CTAE > CTHE

Table 4.12: % Scavenging activity of *C. thevetia* extracts with standards

Sr. no.	Sample name		% Scavenging activity of different concentrations (in µg/mL)					
			100	200	300	400	500	IC_{50}
1.	Ascorbic acid	R1	54.12	61.64	73.53	80.48	88.21	51.82
		R2	54.19	61.80	73.69	80.41	88.30	50.31
		R3	54.03	61.69	73.57	80.29	88.14	51.88
		Mean	54.11 ±0.08	61.71 ±0.08	73.60 ±0.08	80.39 ±0.09	88.22 ±0.08	51.34 ±0.89
2.	CTHE	R1	32.84	42.47	53.96	69.74	82.90	249.88
		R2	32.79	42.40	54.03	69.74	82.73	250.22
		R3	32.89	42.35	53.93	69.70	82.83	250.22
		Mean	32.84 ±0.05	42.41 ±0.06	53.97 ±0.05	69.73 ±0.03	82.82 ±0.08	250.11 ±0.20
3.	CTAE	R1	42.40	50.46	57.29	72.56	84.05	192.30
		R2	42.45	50.25	57.29	72.63	83.96	221.11
		R3	42.38	50.36	57.25	72.73	83.91	192.63
		Mean	42.41 ±0.04	50.36 ±0.11	57.28 ±0.03	72.64 ±0.08	83.97 ±0.07	202.01 ±16.54
4.	CTME	R1	52.78	62.98	75.05	83.25	89.03	56.14
		R2	52.74	62.96	74.98	83.27	89.08	56.94
		R3	53.09	62.91	74.96	83.16	89.01	54.30
		Mean	52.87 ±0.19	62.95 ±0.04	75.00 ±0.05	83.23 ±0.06	89.04 ±0.04	55.79 ±1.35

	Concentration	Treatment	Conc. X Treat.
S.Em±	0.216	0.182	0.039
CD at 5%	0.609	0.515	0.314

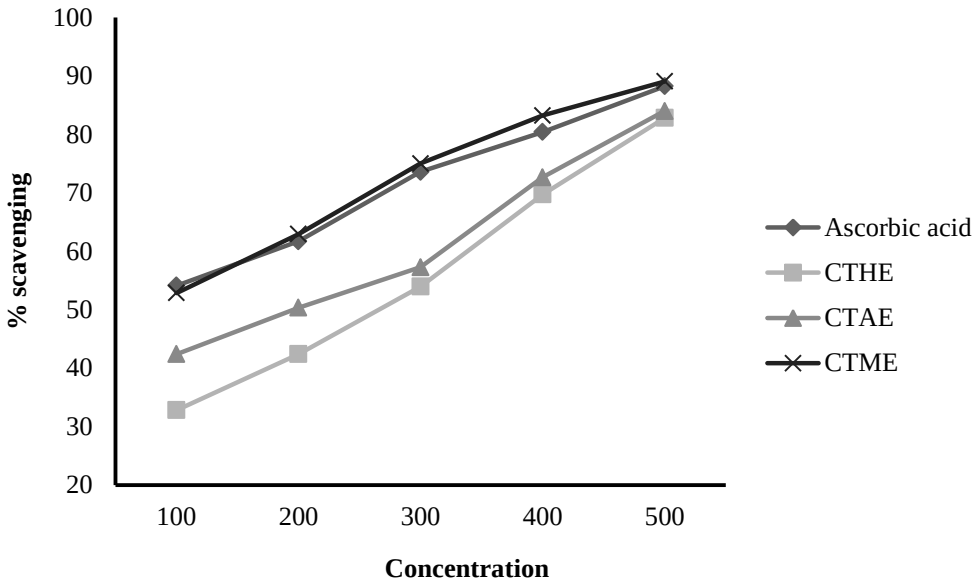

Fig. 4.13: % scavenging of *C. thevetia* extracts at different concentrations

In case of *N. oleander* best inhibition was shown by NOHE (IC_{50} = 54.59µg/mL) which was almost similar to the standard ascorbic acid (IC_{50} = 51.34µg/mL). Detailed results are shown in table 4.13 and related graphs are shown in figure 4.14. The increasing order of antioxidant activity is as follows:

Ascorbic acid > NOHE > NOAE > NOME

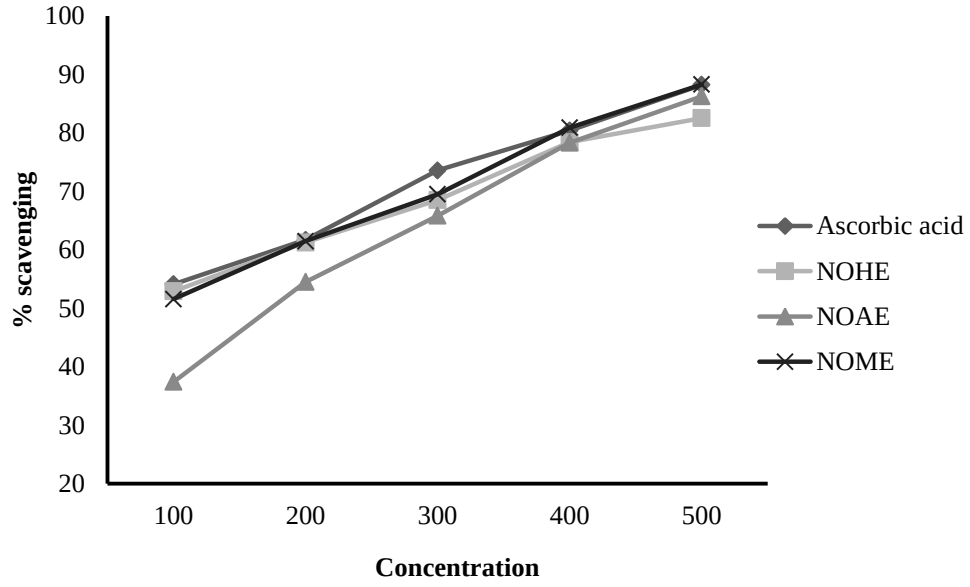

Fig. 4.14: % scavenging of *N. oleander* extracts at different concentrations

Table 4.13: % Scavenging activity of *N. oleander* extracts with standards

Sr. no.	Sample name		% Scavenging activity of different concentrations (in µg/mL)					
			100	200	300	400	500	IC_{50}
1.	Ascorbic acid	R1	54.12	61.64	73.53	80.48	88.21	51.82
		R2	54.19	61.80	73.69	80.41	88.30	50.31
		R3	54.03	61.69	73.57	80.29	88.14	51.88
		Mean	54.11 ±0.08	61.71 ±0.08	73.60 ±0.08	80.39 ±0.09	88.22 ±0.08	51.34 ±0.89
2.	NOHE	R1	52.90	61.31	68.48	78.37	82.45	54.41
		R2	53.07	61.24	68.59	78.46	82.62	53.75
		R3	52.81	61.29	68.52	78.37	82.55	55.63
		Mean	52.92 ±0.13	61.28 ±0.04	68.53 ±0.06	78.40 ±0.05	82.54 ±0.08	54.59 ±0.95
3.	NOAE	R1	37.44	54.55	65.87	78.37	86.26	180.71
		R2	37.37	54.52	65.75	78.34	86.33	181.22
		R3	37.49	54.47	65.91	78.25	86.21	180.66
		Mean	37.44 ±0.06	54.51 ±0.04	65.84 ±0.08	78.32 ±0.06	86.27 ±0.06	180.86 ±0.31
4.	NOME	R1	51.59	61.52	69.42	80.90	88.23	80.58
		R2	51.52	61.50	69.60	80.83	88.42	81.30
		R3	51.63	61.40	69.53	80.95	88.18	80.50
		Mean	51.58 ±0.06	61.48 ±0.06	69.52 ±0.09	80.89 ±0.06	88.28 ±0.12	80.79 ±0.44

	Concentration	Treatment	Conc. X Treat.
S.Em±	0.216	0.483	0.104
CD at 5%	0.609	0.136	0.083

Comparison of scavenging activity (Fig. 4.15) :

Ascorbic acid > NOHE > CTME > NOAE > NOME > CTAE > CTHE

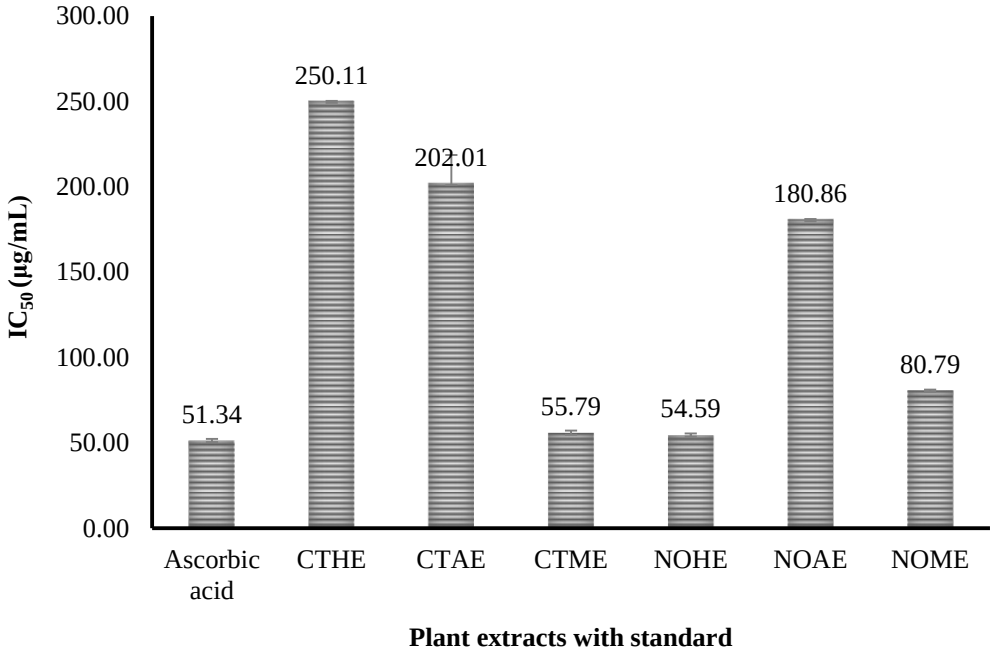

Fig. 4.15: IC_{50} values of all extracts along with their standards

4.3 Antidiabetic assay

Antidiabetic activity of both plants was determined by alpha amylase and alpha glucosidase inhibitory activity. The results obtained are as follows:

4.3.1 Alpha amylase inhibition activity

The results obtained from this study has shown that all the extracts has shown different inhibition activities. The IC_{50} values for hexane, acetone and methanol extracts are 373.08, 147.26 and 135.17µg/mL respectively. Standard acarbose has shown IC_{50} value of 138.61µg/mL. % Inhibition activity along with IC_{50} values is shown in table 4.14 and related graphs in figure 4.16.

Table 4.14: % Inhibition activity of *C. thevetia* extracts with standards

Sr. no.	Sample name		% Inhibition activity of different concentrations (in µg/mL)					
			100	200	300	400	500	IC_{50}
1.	Acarbose	R1	46.86	55.76	68.85	78.53	87.43	131.77
		R2	45.03	54.97	68.06	79.32	88.22	145.43
		R3	45.81	55.67	68.24	78.53	87.61	138.63
		Mean	45.90 ±0.92	55.47 ±0.43	68.38 ±0.41	78.80 ±0.45	87.75 ±0.41	138.61 ±6.83
2.	CTHE	R1	29.06	35.08	39.01	52.62	65.45	363.82
		R2	28.01	34.29	39.53	52.88	63.87	369.62
		R3	27.75	34.82	40.05	51.83	63.35	373.08
		Mean	28.27 ±0.69	34.73 ±0.40	39.53 ±0.52	52.44 ±0.54	64.22 ±1.09	368.84 ±4.68
3.	CTAE	R1	47.12	54.45	58.64	64.40	74.35	147.97
		R2	46.34	53.66	58.38	64.92	73.30	156.99
		R3	48.17	54.71	57.59	65.71	72.51	136.82
		Mean	47.21 ±0.92	54.28 ±0.54	58.20 ±0.54	65.01 ±0.66	73.39 ±0.92	147.26 ±10.11
4.	CTME	R1	48.04	54.42	62.93	73.56	80.89	135.42
		R2	47.96	54.48	62.93	74.87	80.10	133.86
		R3	47.85	54.50	63.01	73.04	80.89	136.22
		Mean	47.95 ±0.09	54.47 ±0.04	62.96 ±0.05	73.82 ±0.94	80.63 ±0.45	135.17 ±1.20

	Concentration	Treatment	Conc. X Treat.
S.Em±	0.171	0.145	0.025
CD at 5%	0.483	0.408	0.197

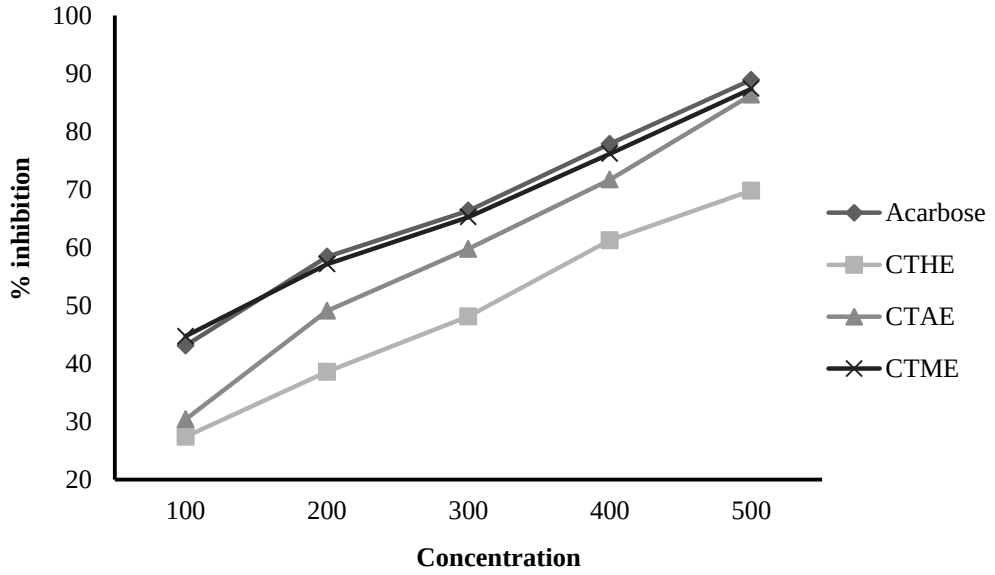

Fig. 4.16: % inhibition of *C. thevetia* extracts at different concentrations

In case if *N. oleander* highest activity was shown by acetone extract (IC_{50}= 133.07µg/mL), followed by methanol extract (IC_{50}= 146.13µg/mL) and then hexane extract (IC_{50}= 320.57µg/mL) shown in Table 4.15 and Fig. 4.17. **Dey *et al.*, (2015),** studied the anti-diabetic activity of *N. oleander* through alloxan induced diabetes in mice. The alpha-amylase inhibitory activity of NOLE showed an IC_{50} value of 703.01 ± 56.47µg/mL. In our study we tested in-vitro activity and results are as follows:

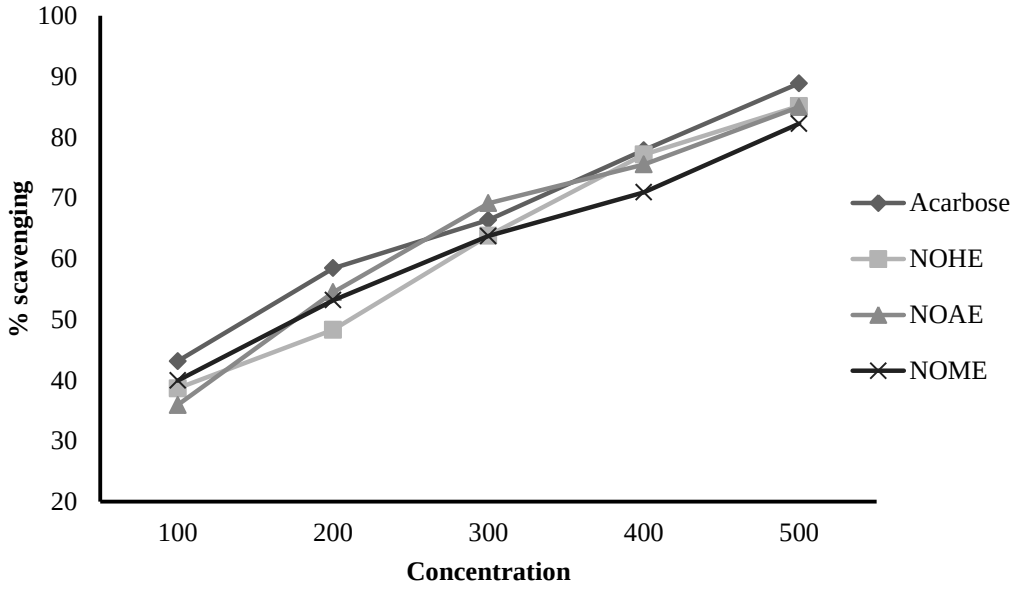

Fig. 4.17: % inhibition of *N. oleander* extracts at different concentrations

Table 4.15: % Inhibition activity of *N. oleander* extracts with standards

Sr. no.	Sample name		% Inhibition activity of different concentrations (in µg/mL)					
			100	200	300	400	500	IC_{50}
1.	Acarbose	R1	46.86	55.76	68.85	78.53	87.43	131.77
		R2	45.03	54.97	68.06	79.32	88.22	145.43
		R3	45.81	55.67	68.24	78.53	87.61	138.63
		Mean	45.90 ±0.92	55.47 ±0.43	68.38 ±0.41	78.80 ±0.45	87.75 ±0.41	138.61 ±6.83
2.	NOHE	R1	32.46	38.74	43.72	57.33	68.32	320.92
		R2	32.46	39.53	44.76	57.33	68.59	316.12
		R3	33.25	37.70	43.19	56.02	68.85	324.67
		Mean	32.72 ±0.45	38.66 ±0.92	43.89 ±0.80	56.89 ±0.76	68.59 ±0.26	320.57 ±4.29
3.	NOAE	R1	49.21	52.09	59.69	68.32	74.61	139.09
		R2	50.79	53.14	58.64	69.11	74.08	121.69
		R3	49.74	51.31	58.90	68.85	73.04	138.44
		Mean	49.91 ±0.80	52.18 ±0.92	59.08 ±0.54	68.76 ±0.40	73.91 ±0.80	133.07 ±9.86
4.	NOME	R1	44.76	56.02	68.06	76.44	85.86	141.86
		R2	43.98	54.71	66.49	76.70	84.82	151.97
		R3	45.03	55.24	67.28	76.96	85.86	144.56
		Mean	44.59 ±0.54	55.32 ±0.66	67.28 ±0.79	76.70 ±0.26	85.51 ±0.60	146.13 ±5.23

	Concentration	Treatment	Conc. X Treat.
S.Em±	0.171	0.383	0.065
CD at 5%	0.483	1.080	0.522

Order of % inhibition (Fig. 4.18):

NOAE > CTME > Acarbose > NOME > CTAE > NOHE > CTHE

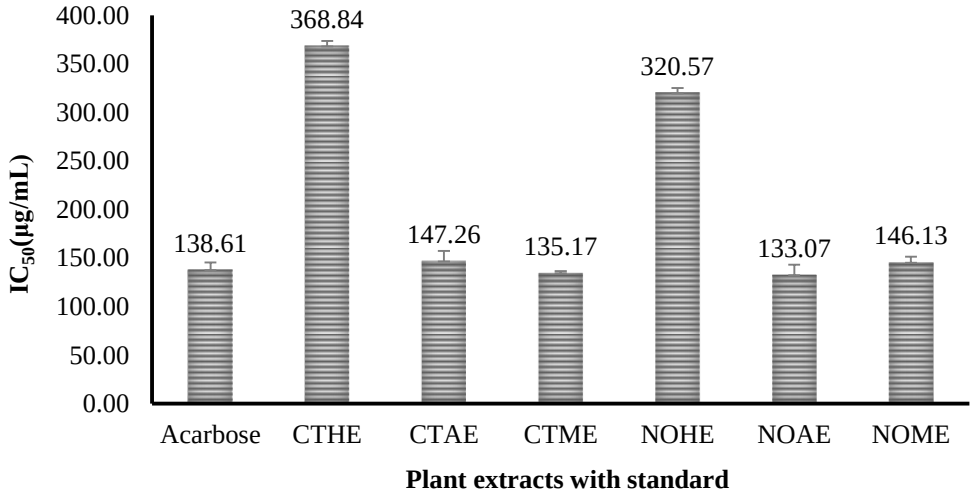

Fig. 4.18: IC_{50} values of all extracts along with their standards

4.3.2 Alpha glucosidase inhibition activity

Among the plant extracts CTME showed highest scavenging power (IC_{50} = 73.47µg/mL) better than standard acarbose (IC_{50} = 99.74µg/mL)**.** Hexane and methanol extracts showed IC_{50} values of 337.55 and 107.25µg/mL respectively. Detailed values are shown in table 4.16 and graphs are shown in figure 4.19. The increasing chelating power of different extracts was in order:

CTME > Acarbose > CTAE > CTHE

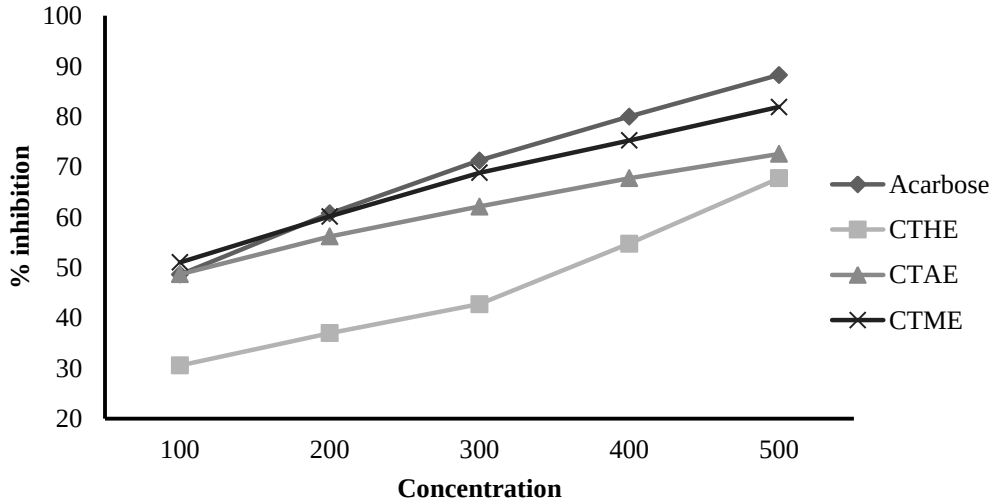

Fig. 4.19: % inhibition of *C. thevetia* extracts at different concentrations

Table 4.16: % Inhibition activity of *C. thevetia* extracts with standards

Sr. no.	Sample name		% Inhibition activity of different concentrations (in µg/mL)					
			100	**200**	**300**	**400**	**500**	**IC_{50}**
1.	**Acarbose**	**R1**	48.48	60.25	70.59	80.57	88.24	103.42
		R2	49.38	60.78	71.48	79.86	88.59	94.71
		R3	47.95	61.14	71.66	79.50	87.88	100.20
		Mean	48.60 ±0.72	60.72 ±0.45	71.24 ±0.57	79.98 ±0.54	88.24 ±0.36	99.44 ±4.40
2.	**CTHE**	**R1**	30.66	37.43	42.07	55.26	67.38	337.57
		R2	30.12	36.90	42.78	54.19	68.09	338.53
		R3	30.84	36.54	43.32	54.72	67.74	336.55
		Mean	30.54 ±0.37	36.96 ±0.45	42.72 ±0.63	54.72 ±0.54	67.74 ±0.38	337.55 ±0.99
3.	**CTAE**	**R1**	48.48	55.61	62.75	68.09	72.73	110.75
		R2	49.55	55.79	61.14	67.38	73.08	105.85
		R3	47.95	57.04	62.39	67.74	71.84	105.13
		Mean	48.66 ±0.82	56.15 ±0.78	62.09 ±0.36	67.74 ±0.84	72.55 ±0.64	107.25 ±3.06
4.	**CTME**	**R1**	50.98	59.89	69.34	75.22	81.64	72.83
		R2	51.00	60.78	68.63	75.22	82.00	70.75
		R3	51.03	59.71	68.45	75.22	82.00	76.83
		Mean	51.00 ±0.03	60.13 ±0.57	68.81 ±0.47	75.22 ±0.00	81.88 ±0.21	73.47 ±3.09

	Concentration	**Treatment**	**Conc. X Treat.**
S.Em±	0.152	0.128	0.019
CD at 5%	0.429	0.362	0.155

In case of *N. oleander* best inhibition was shown by NOAE (IC_{50} = 149.57µg/mL) which was greater than standard acarbose (IC_{50} = 99.44µg/mL). Detailed values are shown in table 4.17 and graphs are shown in figure 4.20. The increasing order of inhibition is as follows:

Acarbose > NOAE > NOME > NOHE

Table 4.17: % Inhibition activity of *N. oleander* extracts with standards

Sr. no.	Sample name		% Inhibition activity of different concentrations (in µg/mL)					
			100	200	300	400	500	IC_{50}
1.	Acarbose	R1	48.48	60.25	70.59	80.57	88.24	103.42
		R2	49.38	60.78	71.48	79.86	88.59	94.71
		R3	47.95	61.14	71.66	79.50	87.88	100.20
		Mean	48.60 ±0.72	60.72 ±0.45	71.24 ±0.57	79.98 ±0.54	88.24 ±0.36	99.44 ±4.40
2.	NOHE	R1	33.51	41.18	51.87	60.96	72.01	280.27
		R2	34.05	40.82	51.87	62.39	71.66	277.70
		R3	32.80	39.57	50.80	60.78	70.77	290.42
		Mean	33.45 ±0.63	40.52 ±0.84	51.52 ±0.62	61.38 ±0.88	71.48 ±0.64	282.80 ±6.73
3.	NOAE	R1	47.77	52.76	60.61	66.13	73.44	143.26
		R2	47.77	52.58	59.36	67.20	74.15	148.37
		R3	45.81	53.48	59.71	66.49	73.80	157.07
		Mean	47.12 ±1.13	52.94 ±0.47	59.89 ±0.64	66.61 ±0.54	73.80 ±0.36	149.57 ±6.98
4.	NOME	R1	39.22	49.91	62.39	74.33	81.64	194.72
		R2	39.93	48.66	61.85	73.44	81.64	197.37
		R3	39.75	49.20	60.96	74.15	81.11	197.46
		Mean	39.63 ±0.37	49.26 ±0.63	61.73 ±0.72	73.98 ±0.47	81.46 ±0.31	196.51 ±1.55

	Concentration	Treatment	Conc. X Treat.
S.Em±	0.152	0.340	0.052
CD at 5%	0.429	0.959	0.411

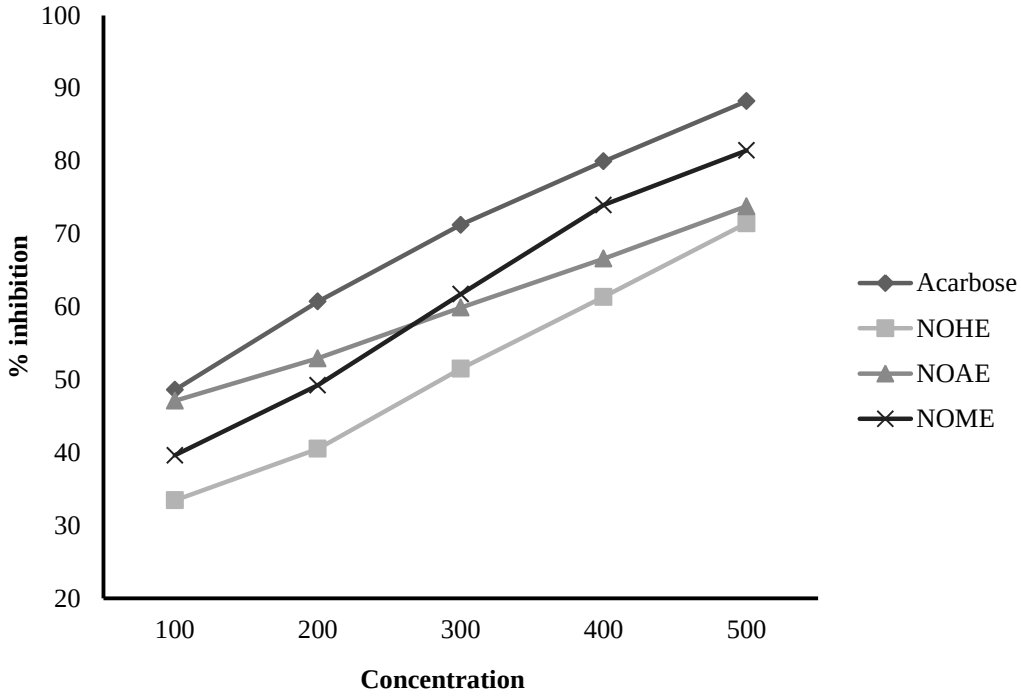

Fig. 4.20: % inhibition of *N. oleander* extracts at different concentration

Order of % inhibition (Fig. 4.21):

CTME > Acarbose > CTAE> NOAE > NOME > NOHE > CTHE

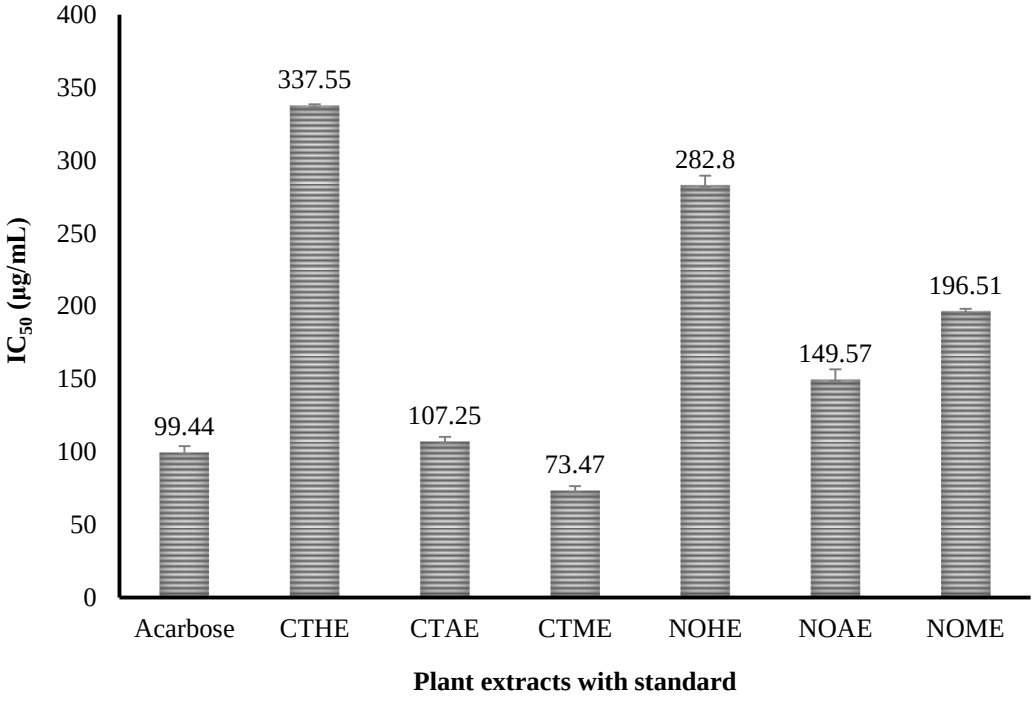

Fig. 4.21: IC_{50} values of all extracts along with their standards

4.4 Anti-inflammatory assay

Anti-inflammatory activity of both plants was determined using sodium diclofenac as standard. CTME showed best scavenging activity with IC_{50} value of 145.40µg/mL, which was slightly better than standard sodium diclofenac with IC_{50} of 147.57µg/mL. Hexane and acetone extract have not shown very good activity. All the values are shown in table 4.18 and related graphs are shown in figure 4.22. The results obtained are as follows:

CTME > Sodium Diclofenac > CTAE > CTHE

Table 4.18: % Scavenging activity of *C. thevetia* extracts with standards

Sr. no.	Sample name		% Scavenging activity of different concentrations (in µg/mL)					
			100	200	300	400	500	IC_{50}
1.	Sodium diclofenac	R1	43.29	58.43	66.27	78.09	89.24	146.99
		R2	43.16	58.17	66.67	77.56	88.98	147.76
		R3	42.90	58.70	66.14	77.95	88.45	147.97
		Mean	43.12 ±0.20	58.43 ±0.26	66.36 ±0.28	77.87 ±0.27	88.89 ±0.40	147.57 ±0.517
2.	CTHE	R1	27.49	38.38	48.07	61.09	69.72	309.71
		R2	26.83	38.38	48.47	61.35	69.59	309.89
		R3	27.89	39.04	47.81	61.35	70.12	307.01
		Mean	27.40 ±0.54	38.60 ±0.38	48.12 ±0.33	61.27 ±0.15	69.81 ±0.28	308.87 ±1.612
3.	CTAE	R1	30.68	49.40	59.50	71.71	86.45	228.58
		R2	30.54	49.00	60.03	71.98	86.19	228.86
		R3	30.01	48.87	59.76	71.45	86.32	231.35
		Mean	30.41 ±0.35	49.09 ±0.28	59.76 ±0.27	71.71 ±0.27	86.32 ±0.13	229.60 ±1.522
4.	CTME	R1	44.36	57.64	65.07	76.23	87.12	145.52
		R2	44.62	57.10	64.94	76.10	87.52	146.79
		R3	45.15	56.71	65.74	76.23	87.52	143.89
		Mean	44.71 ±0.40	57.15 ±0.48	65.25 ±0.43	76.18 ±0.08	87.38 ±0.23	145.40 ±1.454

	Concentration	Treatment	Conc. X Treat.
S.Em±	0.776	0.656	0.509
CD at 5%	0.219	0.185	0.041

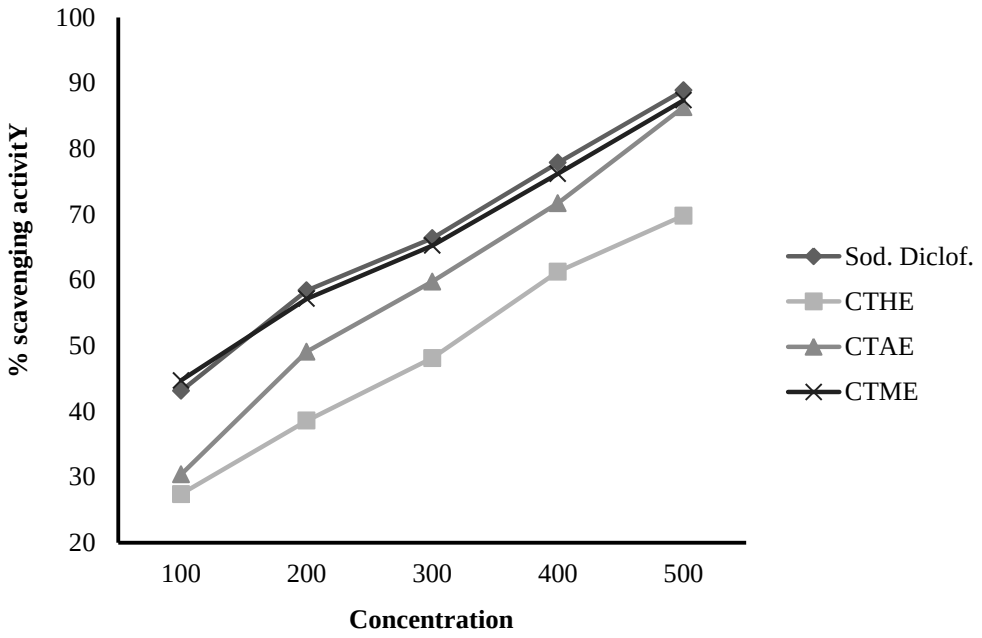

Fig. 4.22: % scavenging of *C. thevetia* extracts at different concentrations

In case of *N. oleander* hexane, acetone and methanol extracts have shown the IC_{50} values of 196.47, 182.42 and 182.75µg/mL respectively. The standard has shown better scavenging with IC_{50} value of 147.57µg/mL. Detailed values are shown in table 4.19 and related graphs are shown in figure 4.23.

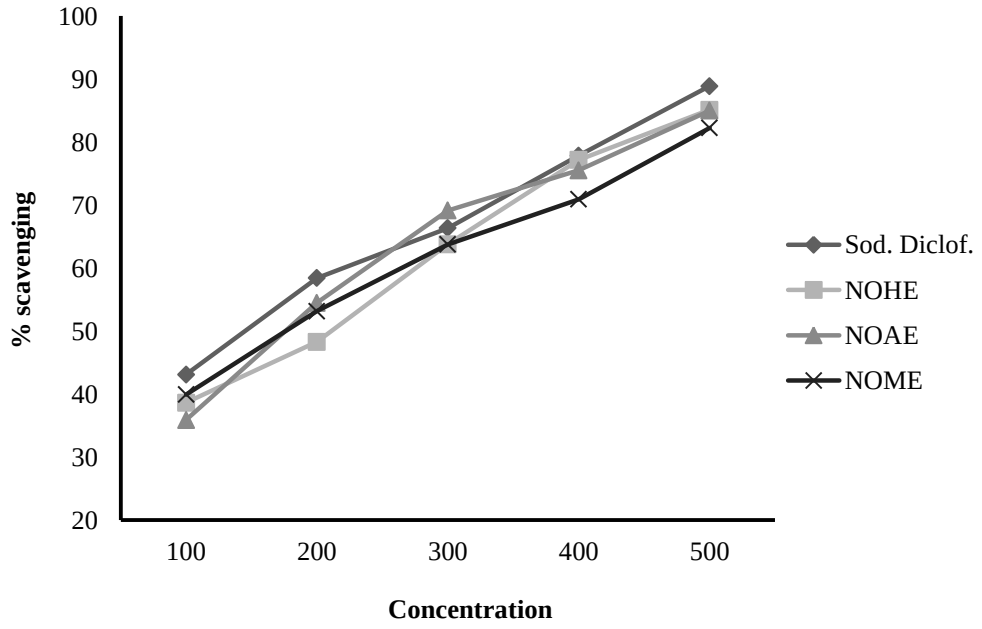

Fig. 4.23: % scavenging of *N. oleander* extracts at different concentration

Table 4.19: % Scavenging activity of *N. oleander* extracts with standards

Sr. no.	Sample name		% Scavenging activity of different concentrations (in µg/mL)					
			100	200	300	400	500	IC_{50}
1.	Sodium diclofenac	R1	43.29	58.43	66.27	78.09	89.24	146.99
		R2	43.16	58.17	66.67	77.56	88.98	147.76
		R3	42.90	58.70	66.14	77.95	88.45	147.97
		Mean	43.12 ±0.20	58.43 ±0.26	66.36 ±0.28	77.87 ±0.27	88.89 ±0.40	147.57 ±0.517
2.	NOHE	R1	38.91	48.07	63.48	77.42	84.86	196.43
		R2	38.65	48.21	64.01	77.29	85.39	196.23
		R3	38.38	48.61	63.88	76.76	85.13	196.74
		Mean	38.65 ±0.26	48.30 ±0.28	63.79 ±0.28	77.16 ±0.35	85.13 ±0.26	196.47 ±0.255
3.	NOAE	R1	35.86	54.45	69.06	75.70	85.13	182.78
		R2	35.99	54.32	69.32	75.56	85.26	182.34
		R3	35.86	54.71	69.06	75.30	84.59	182.14
		Mean	35.90 ±0.08	54.49 ±0.20	69.15 ±0.15	75.52 ±0.21	84.99 ±0.36	182.42 ±0.327
4.	NOME	R1	40.11	53.39	63.75	70.92	81.81	181.20
		R2	39.71	53.39	64.01	70.78	82.60	182.68
		R3	39.97	52.72	63.48	71.05	82.34	184.37
		Mean	39.93 ±0.20	53.17 ±0.39	63.75 ±0.26	70.92 ±0.14	82.25 ±0.40	182.75 ±1.589

	Concentration	Treatment	Conc. X Treat.
S.Em±	0.776	0.174	0.135
CD at 5%	0.219	0.489	0.107

Order of % inhibition (Fig. 4.24):

CTME > Sodium Diclofenac > NOAE >NOME > NOHE> CTAE > CTHE

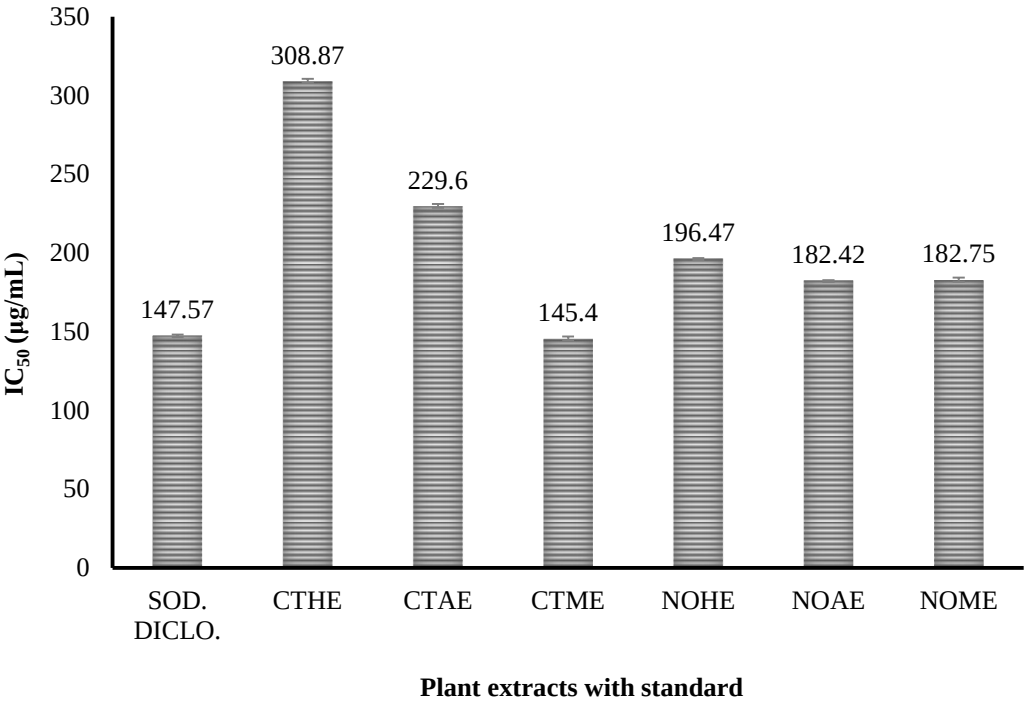

Fig. 4.24: IC_{50} values of all extracts along with their standards

Summary and Conclusions

Chapter 5 SUMMARY AND CONCLUSION

The present study has taken into consideration the study of the phytochemical analysis and biological activities of two plant species *Cascabela thevetia* and *Nerium oleander* belonging to family Apocynaceae. Both the species were collected from Tarai region of Pantnagar. Collection of plant material (leaves) was done in the month of March, 2019 from Pantnagar. The plant leaves had undergone successive extraction using Soxhlet apparatus. The solvents used were hexane, acetone and methanol. The yield of extract obtained was 4.67% for CTHE, 5.44% for CTAE, 12.01% for CTME, 5.03% for NOHE, 9.94% for NOAE and 15.48% for NOME.

The study of chemical composition of the plant extracts was carried out using GC-MS technique. GC-MS analysis of hexane, acetone and methanol extracts of *C. thevetia* revealed the presence of 14, 42 and 41 constituents respectively. Similarly, GC-MS analysis of hexane, acetone and methanol extracts of *N. oleander* showed the presence of 32, 17 and 52 components respectively.

Further the extract was also analyzed against some important biological activities like anti-oxidant activity, anti-diabetic and anti-inflammatory activities. Anti-oxidant activity was performed by different methods like DPPH free radical scavenging activity, metal chelating activity and hydroxyl radical scavenging activity. Anti-diabetic activity was determined by using alpha-amylase inhibitory activity and alpha-glucosidase inhibitory activity. The study was concluded by determining anti-inflammatory activity of the plant extract using egg albumin.

DPPH radical scavenging activities of plant extract was tested against standard antioxidants such Ascorbic acid, BHT and Gallic acid. The results showed the IC values of different extracts were in order:

Ascorbic acid > Gallic acid > CTHE > NOHE > CTAE > NOAE > NOME >CTME

Metal chelating method of the plant extract was studied using EDTA and citric acid as standard. The results showed the IC values in order-

Citric acid > EDTA > CTHE > CTAE > NOAE > CTME > NOME > NOHE

Hydroxyl radical scavenging was studied using ascorbic acid as standard and the IC_{50} values in order-

Ascorbic acid > NOHE > CTME > NOAE > NOME > CTAE > CTHE

Antidiabetic activity using alpha amylase showed IC_{50} values in the order:

NOAE > CTME > Acarbose > NOME > CTAE > NOHE > CTHE

While in case of alpha glucosidase IC_{50} values showed the following order:

CTME > Acarbose > CTAE> NOAE > NOME > NOHE > CTHE

In-vitro anti-inflammatory activities of the plant extract was assessed by protein denaturation technique by using the albumin of hen's egg. The results were compared against standard drug diclofenac sodium. The extract showed significant anti-inflammatory activity with IC_{50} in order:

CTME > Sod. Diclof. > NOAE >NOME > NOHE> CTAE > CTHE

Finally, it is concluded that both the oleander species can be a good source of natural antioxidants and antidiabetic agents. Further they can also be used as an anti-inflammatory agent. Detailed study of these plants is required to exploit their potential biological functions.

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VITA

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ABSTRACT

In this thesis work phytochemical and biological properties of *Cascabela thevetia* and *Nerium oleander* leaf extracts were studied. Leaves of *C. thevetia* and *N. oleander* were collected form Tarai region of Pantnagar. Leaves were washed, air dried in shade and grinded finely. Leaf powder was successively extracted using hexane, acetone and methanol solvents by Soxhlet apparatus. The chemical composition of the extracts was studied by GC-MS. Mome inositol (89.99%), neophtadiene (18.18%) and Lup-20(29)-en-3-yl acetate (40.61%) compounds were in majority in hexane, acetone and methanol extract, respectively in *C. thevetia.* In *N. oleander* hexane, acetone and methanol leaf extract, Mome inositol (57.72%), Mome inositol (84.29%) and n-hexadecanoic acid (11.25%) were major components, respectively. All the six extracts were screened for different biological activities viz., antioxidant, antidiabetic and anti-inflammatory activity. All the extracts exhibited good to moderate antioxidant activity. In DPPH assay hexane and acetone extracts of *C. thevetia* showed good antioxidant activity while methanol extract showed moderate activity. In case of *N. oleander* hexane extract showed good activity while, acetone and methanol extract showed moderate activity. Metal chelating activity of all the extracts was comparable. Methanol extract of *C. thevetia* and hexane extract of *N. oleander* showed good activity in hydroxyl radical scavenging assay while other extracts showed moderate activity. Acetone and Methanol extracts of both the plants showed good *in-vitro* antidiabetic activity while, the activity was moderate in case of hexane extract. *In-vitro* anti-inflammatory activity of the plant leaf extract revealed that methanol extract of *C. thevetia* is most efficient while, other extracts showed moderate efficiency. Based on the results obtained it can be concluded that both *C. thevetia* and *N. oleander* plants are rich source of phytochemicals and can also be used as antioxidants, antidiabetic and anti-inflammatory agent.

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शोध शीर्षक : **नेरियम ओलियंडर (एल.) और कैस्केबेला थिवेशिया (एल.) की पत्ती के अर्क की जैविक गतिविधियों** की **कीमो प्रोफाइलिंग**

सलाहकार : डॉ0 शिशिर टंडन

सारांश

इस शोध में कैस्केबेला थिवेशिया और नेरियम ओलियंडर के जैवरसायानिक गुणों का अध्ययन किया गया। इस अध्ययन के लिए सी. थिवेशिया और एन. ओलियंडर के पत्तों को पंतनगर के तराई क्षेत्र से एकत्र किया गया। धुलाई के बाद एकत्रित पत्तियों को छाया में सुखाया गया और बारीक पीस लिया गया। इसके बाद, पत्तियों के पाउडर का सक्सेलेट तंत्र के द्वारा क्रमबद्ध तरीके से हेक्सेन, एसीटोन और मेथनॉल का उपयोग करके निष्कर्षण किया गया। जीसी-एमएस द्वारा निष्कर्ष की रासायनिक संरचना का अध्ययन किया गया। मोम इनोसिटोल (89.99%), नियोफेटाडिन (18.18%) और ल्यूप -20 (29) -इन-3-आइल-एसीटेट (40.61%) क्रमशः सी. थेवेसिया में हेक्सेन, एसीटोन और मेथनॉल एक्सट्रैक्ट में अधिक मात्रा में पाए गए। मोम इनोसिटोल (57.72%), मोम इनोसिटोल (84.29%) n- हेक्साडिकैनोईक अम्ल (11.25%) क्रमशः एन. ओलियंडर के हेक्सेन, एसीटोन और मेथनॉल निष्कर्ष में प्रमुख घटक पाए गए। सभी निष्कर्षों की विभिन्न जैविक गतिविधियों (एंटीऑक्सिडेंट, एंटीडायबिटिक और एंटी इन्फ्लेमेटरी) का भी परीक्षण किया गया। सभी छह निष्कर्षों में उच्च से मध्यम एंटीऑक्सीडेंट गतिविधिया पाई गई। डी.पी.पी.एच. परख में सी. ठेवेशिया के हेक्सेन और एन. ओलियंडर के एसीटोन निष्कर्ष में अच्छी एंटीऑक्सिडेंट गतिविधि पाई गई, जबकि मेथनॉल अर्क में मध्यम एंटीऑक्सिडेंट गतिविधि पाई गई। सभी निष्कर्षो की मेटल चिलेटिंग गतिविधिया लगभग समान थी। सी. थेवेसिआ के मेथेनॉल निष्कर्ष और एन. ओलियंडर के हेक्सेन एक्सट्रैक्ट ने हाइड्रॉक्सिल रेडिकल स्केवेंजिंग परख में अच्छी गतिविधि मिली जबकि अन्य अर्क ने मध्यम गतिविधियां मिली। दोनों पौधों के एसीटोन और मेथनॉल निष्कर्ष में अच्छी एंटी डायबिटिक गतिविधि मिली, जबकि हेक्सेन निष्कर्ष में गतिविधि मध्यम थी। पत्तियों के निष्कर्ष की एंटी इन्फ्लेमेटरी गतिविधि से पाया गया कि सी. ठेवेसिया का मेथनॉल अर्क सर्वश्रेष्ठ है जबकि अन्य अर्क में मध्यम दक्षता पाई गई। प्राप्त परिणामों के आधार पर यह निष्कर्ष निकाला जा सकता है कि सी. थिवेशिया और एन. ओलियंडर पौंधे एंटीऑक्सिडेंट के समृद्ध स्रोत हैं और इसका उपयोग एंटीडायबिटिक तथा एंटी इन्फ्लेमेटरी एजेंट के रूप में भी किया जा सकता है।

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