Antimicrobial and Antioxidant Activity of *Nerium oleander* Flower Extract (Apocynaceae)

SARANYA.S

(15PZO008)

Thesis submitted to

Avinashilingam Institute for Home Science and Higher Education

for Women, Coimbatore – 641 043

In Partial Fulfilment of the Requirements for the Degree of

Master of Science in Zoology

APRIL 2017

10/4/2017

Signature of the
Head of the Department

10.4.17

Signature of the
Supervisor

I. INTRODUCTION

From the beginning of human civilization, plant and plant products are usually used to treat different diseases (Joshi *et al.,* 2009). As plants have substances of medicinal values, therefore, they are used to treat number of diseases since long time. Use of plants had minimal or less side effect on human beings (Doughari, 2006). With the passage of time the usage of plants is increasing in pharmaceutical industries, suggested that if a plant is used as remedy of disease then it would have some important ingredients (Nostro *et al.,* 2000).

Researchers have great interest in those substances which are derived from plants because they are versatile in their applications. Various phytochemicals can be obtained from plants which are very beneficial for mankind and medicinal plants have become the richest biological resource of such chemicals which are used in manufacturing of traditional drugs as well as in modern nutraceuticals, food supplements, medicines, folk medicines, raw material and pharmaceutical intermediates for synthetic drugs (Tumwine, 2011).

Nerium oleander (Family: Apocyanacea) is a beautiful free flower especially suited to sunny and dry localities (Lokesh *et al*., 2010). Flowers are the most attractive part of the plant. These are rich in color and sweet fragrance. Not only humans, but the animals and the insects all gets attracted to it. Honey bee sucks nectar from the flowers; by which natural honey is prepared. Flowers are used to show different emotions; happiness, grief, sadness, lost, celebrations and many more. Red flowers are mostly used to denote love and affection; whereas yellow, white and pale pink is used to express friendship, sadness etc.

Most of the flowers occupy the potency as they are used in different medicinal fields. In *Chinese therapies, Ayurveda,* Naturopathy etc flowers plays a

major role for the treatment of many diseases. Here, we will be talking about one of the most beautiful flower which is also Sweet scented. Well, it is commonly called Oleander.

Leaves, roots, roots and bark is also used to treat various ailments. Charka prescribed the leaves of white flowered variety externally in chronic and obstinate skin diseases of serious nature including leprosy. Root powdered with water was applied to alleviate venereal diseases. The powder of leaves was used as a snuff for treating epilepsy.

The oleander tree is bushy one and reaches up to 10-12 ft in height. It has small stem with multiple branches and each branch has three pair of 6-8 inch long leaves. Those leaves are completely green, smooth, and shiny even rough. Oleander flowers are found in different shades like pale pink, dark pink, red, white and yellow even. The trees are grown in temples, gardens, and parks and also used as the ornamental plant. It bears flowers throughout the year; while the yellow oleander gives the illusion of having spring and rainy season at the same time.

A wide range of plants express complex mixtures of secondary metabolites within each species (Arguedas *et al.,* 2005). Phenols, terpenoids, flavonoids, glycosides, tannins, alkaloids, steroids, saponins and resins are some important photochemicals found in plants (Tiwari *et al.,* 2011). An estimated 74% of pharmacologically active plant derived components were discovered after following up on ethnomedicinal use. Thus, medicinal plants can be regarded as the richest bio-resource of drugs of modern medicine, folk medicine and chemical entities or templates for synthetic drugs (Joshual & Takudzwa, 2013).

Antimicrobial compounds are a group of chemical compounds which are synthetically or biosynthetically produced which either destroy or usefully suppress the growth and metabolism of variety of microorganisms (Lavanya & Brahmaprakash, 2011).

Pharmacologically important plants present a large source of antimicrobial agents and serve as a drug in many countries (Mahesh & Satish, 2008). This antimicrobial activity of plant is due to their constituents of oils and extracts which are used in pharmaceutical and other related fields (Hammer *et al.,* 1999).

Globally, researchers are using extracts of plants for their antiviral, antibacterial, and antifungal activities. The characteristics of the plants that retard the growth of micro-organisms have been investigated in different laboratories around the world since 1926 (Bakht *et al.,* 2012). Hence, in the present investigation, same efforts are continued in the progression of searching novel therapeutics against antibiotic activity.

It has been established that oxidative stress is among the major causative factors in induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immune suppression, neurodegenerative diseases and others (Young and Woodside, 2001). A great number of aromatic, medicinal, spice and other plants contain chemical compounds exhibiting antioxidant properties.

Oxidative process is one of the most important routes for producing free radicals in foods, drugs and even in living systems (Halliwell, 1994). The most effective path to eliminate and diminish the action of free radicals which cause the oxidative stress is anti oxidative defense mechanisms. Antioxidants are those substances which possess free radical chain reaction breaking properties.

Recently there has been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing oxidative stress-induced tissue injury (Pourmorad *et al.,* 2006). Among the numerous naturally occurring antioxidants; ascorbic acid, carotenoids and phenolic compounds are more effective (Duh *et al*., 1999). They are known to inhibit lipid peroxidation (by

inactivating lipoxygenase), to scavenge free radicals and active oxygen species by propagating a reaction cycle and to chelate heavy metal ions.

The studies carried out on medicinal plants and vegetables strongly support the idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems. In the present investigation, we studied ethanolic flower extracts for the search of antioxidant activity of *Nerium oleander*. The free radical scavenging activity against DPPH and reducing power assay were evaluated during the course of work. The standard, ascorbic acid with antioxidant activity was also determined. The assessment of such properties remains an interesting and useful task, particularly for finding new sources for natural antioxidants.

Plants depend upon radiant energy for the energy necessary to carry on photosynthesis and other physiological processes. The green plant has been called the converter of solar energy. In the presence of sunlight it synthesizes complex organic compounds such as sugars, fats, proteins, etc., from simple inorganic compounds such as water, carbon dioxide, minerals, salts, etc. The interaction of plants with radiant energy is of interest to the botanist, forester, geographer, biophysicist, biochemist, ecologist, hydrologist and agronomist (Gates *et al*., 1965).

Therefore, the analysis of bioactive constituents in plants using spectral studies would help in determining various biological activities of plants. The determination of phytoconstituents is largely performed by relatively expensive and often laborious techniques such as gas and liquid chromatography combined with specific detection schemes (Uzer *et al.,* 2005 & Eisenhauer *et al*., 2009).

In the present study, simple, cost-effective and rapid tests for detecting phytocomponents are used. Spectroscopic ultraviolet-visible, Fourier transform

infrared (UV-Vis, FT-IR) methods together or separate can be used in this sense as well as conventional methods (Hazra *et al*., 2007 & Eberhardt *et al*., 2007).

UV-Vis spectrophotometry related to the spectroscopy of photons in the UV-visible region. UV-Vis spectroscopy uses light in the visible ranges or its adjacent ranges. The color of the chemicals involved directly affects the absorption in the visible ranges. The FT- IR has proven to be a valuable tool for characterization and identification of compounds or functional groups (chemical bonds) present in an unknown mixture of plants extract (Aysal *et al.,* 2007 & Ibrahim et al 2008). In addition, FT-IR spectra of pure compounds are usually so unique that they are like a molecular fingerprint.

The therapeutic effect of medicinal plants for the treatment of various diseases was based on the chemical compounds of these plants. The major components are organic compounds, some of which have biological activity, but none act independently and cannot replace the functions of the medicinal plant as a whole. Analysis has revealed that medicinal plants are rich in many trace elements, and it is suggested that this is an important factor in the curative effect of these plants (Olabanji *et al.*, 1997; Singh *et al.*, 2012; Pereira and Felcman, 1998).

The chemical states in which trace elements are found are originally bound, completed and free; besides, different states have different functions, toxicity, and absorption rates by the body. Trace elements co-exist with numerous organic compounds (many of which are complex agents) in the infusions of medicinal plants (Remington, 1995), and most will be bound to organic compounds, therefore, the concentration of the free trace elements can be very low.

The continuing interest in trace elements has stimulated the development of several powerful analytical techniques for their detection and quantitative

determination. One of these is X-ray diffraction (XRD), where a source of X-ray photons is used to study the elemental composition of materials. Absorption of these photons by the photoelectric effect produces vacancies in the inner electron shells of the atoms of the material, followed by the emission of characteristic X-rays of the elements present. The peaks in X-ray spectrum indicate what kind of atoms are present, while the number of counts (the area under the peak) in relates to the number of atoms in the sample, alluring the quantitative measurements to be made (Burham, 1984).

Moreover, the method gives information about the elements present in the sample irrespective of their state of chemical combination or the phases in which they exist (Piorek, 1978). The chemical constituents of most of the plants used by traditional healers are still unknown. It is, therefore, of paramount importance to know the chemical composition of every reported plant and to make permanent records of the knowledge from natural sources before they all pass away. Hence in the present study, an attempt was made to create a permanent record of the chemical constituents of the flowers of *Nerium oleander* by evaluating the antimicrobial, antioxidant and spectral studies.

With this background, we have formulated the following objectives

1. To analyze the major phytochemical components present in the flower extracts of *Nerium oleander*
2. To evaluate the antimicrobial activity of flower extracts of *Nerium oleander* in different solvent systems
3. To estimate the antioxidant activity of flower extracts of *Nerium oleander* using free radical systems
4. To identify the major bioactive components in the flower extract of *Nerium oleander* using different spectral studies

II. REVIEW OF LITERATURE

2.1. Herbal medicine

Traditional use of herbal medicines implies substantial historical use, and this is certainly true for many products that are available as traditional herbal medicines. In many developing countries, a large proportion of the population relies on traditional practitioners and their armamentarium of medicinal plants in order to meet health care needs. Although modern medicine may exist side-by-side with such traditional practice, herbal medicines have often maintained their popularity for historical and cultural reasons. Such products have become more widely available commercially, especially in developed countries.

In this modern setting, ingredients are sometimes marketed for uses that were never contemplated in the traditional healing systems from which they emerged. An example is the use of ephedra (Ma huang) for weight loss or athletic performance enhancement (Shaw, 1998). While in some countries, herbal medicines are subject to rigorous manufacturing standards, this is not so everywhere. In Germany, for example, where herbal products are sold as ‘phytomedicines’, they are subject to the same criteria for efficacy, safety and quality as are other drug products. In the USA, by contrast, most herbal products in the market place are marketed and regulated as dietary supplements, a product category that does not require pre-approval of products on the basis of any of these criteria.

The pharmacological treatment of disease began long ago with the use of herbs (Schulz *et al.,* 2001). Methods of folk healing throughout the world commonly used herbs as part of their tradition. Some of these traditions are briefly described below, providing some examples of the array of important healing practices around the world that used herbs for this purpose

2.1.1. **Traditional Chinese medicine**

Traditional Chinese medicine has been used by Chinese people from ancient times. Although animal and mineral materials have been used, the primary source of remedies is botanical. Of the more than 12000 items used by traditional healers, about 500 are in common use (Li, 2000). Botanical products are used only after some kind of processing, which may include, for example, stir-frying or soaking in vinegar or wine.

In clinical practice, traditional diagnosis may be followed by the prescription of a complex and often individualized remedy. More than half the population regularly uses traditional remedies, with the highest prevalence of use in rural areas. About 5000 traditional remedies are available in China; they account for approximately one fifth of the entire Chinese pharmaceutical market (Li, 2000).

2.1.2. **Japanese traditional medicine**

Many herbal remedies found their way from China into the Japanese systems of traditional healing. Herbs native to Japan were classified in the first pharmacopoeia of Japanese traditional medicine in the ninth century (Saito, 2000).

2.1.3. **Indian traditional medicine**

Ayurveda is a medical system primarily practiced in India that has been known for nearly 5000 years. It includes diet and herbal remedies, while emphasizing the body, mind and spirit in disease prevention and treatment (Morgan, 2002).

2.2. **An overview of *Nerium oleander***

Classification of *Nerium oleander*

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Gentianales
Family	:	Apocynaceae
Genus	:	Nerium
Species	:	oleander

Nerium oleander is an evergreen shrub comes under the dogbane family or Apocynaceae, a family of flowering plants in the Gentianales order that includes trees, shrubs, herbs, and lianas. The shrub is characterized by dark green, lanceolate leaves, flowers with a deeply 5 - lobed corolla clustered at the end of the branches, and fruit in the form of a long, narrow capsule with numerous compose seeds. Oleander is native to a broad area from Morocco and Portugal eastward through the Mediterranean region and southern Asia to Yunnan in southern parts of China (Huxley *et al.,* 1992). It typically occurs around dry stream beds.

Common name is rosebay and oleander. Other common names for *N. oleander* include adelfa, alheli extranjero, baladre, espirradeira, flor de Sao Jose, laurel de jardín, laurel,Rosa, Laurier rose, Flourier rose, olean, aiwa, rosa Francesca, rosa laurel, and rose-bay or rose bay (Laborde 1989). In Chinese it is known as *jia zhu tao*. The ancient city of Volubilis in Morocco took its name from the old Latin name for the flower.

Oleander grows to 2 to 6 meters (6.5 feet to 19.7 feet) tall, with spreading to erect branches. The leaves are in pairs or whorls of three, thick and leathery, dark green, narrow lanceolate, 5 to 21 centimeters (2 - 8 inches) long and 1 to 3.5 centimeters (0.4 - 1.4 inches) broad, and with an entire margin. The flowers grow in clusters at the end of each branch; they commonly are white, pink, red, yellow or purple, 2.5 to 5 centimeters (1-2 inches) in diameter, with a deeply 5 - lobed corolla with a fringe round the central corolla tube. They are often, but not always, sweetly scented. The fruit is a long narrow capsule 5 to 23 centimeters (2-9 inches) long, which splits open at maturity to release numerous downy seeds.

Nerium oleander is the only species currently classified in the genus Nerium. Oleander offers important ecological and aesthetic values. Ecologically, various animals can use it for food, such as the oleander caterpillar that feeds only on oleanders. For humans, the showy and often sweetly scented oleander

flowers, which come in a variety of colors (white, red, pink, yellow, purple), are used for aesthetic purposes. The plants are used for ornamental purposes in parks, along roadsides, and in some states as a decorative freeway median.

2.3. **Chemical constituents**

The most well known effects of oleander are due to two glycosides, neriin and, and an alkaloid, oleandrin which have a cardiostimulatory action (Jayabalan *et al*., 1995), and to the glycosides gentiobiosyl - oleandrin, gentiobiosyl-nerigoside and gentiobiosyl-beaumontoside extracted from the leaves (Mallet *et al.,*1994).

In addition, its lymph is rich of minerals (Bai *et al,* 2007) and α-tocopherol, an important antioxidant (Hussain *et al.,*2004). Adyregenin is a compound with no cardiac effect. There are also weakly active cardenolides (heterosides of uzarigenine) and inactive cardenolides (heteroside of adynergenine, of digitalose), triterpenoids, a resin, tannins, glucose, a paraffin, ursolic acid, vitamin C and an essential oil. The seeds contain glucosides (oleandrine, odorosides, adigoside). The bark also contains glucosides (rosaginoside,nerioside,corteneroside). The roots contain steroids.

2.4. Habitat range and ecology

N. oleander is either native or naturalized to a broad area from Mauritania, morocco and Portugal eastward through the Mediterranean region and Sahara (where it is only found sporadically), to the Arabian Peninsula Southern Asia, and as Far East as Yunnan in southern parts of china (Pankhurst 2009). It typically occurs around stream beds in river valleys, where it can alternatively tolerate long seasons of drought and inundation from winter rains.

Nerium oleander is planted in many subtropical and tropical areas of the world. On the East Coast of the US, it grows as far north as Virginia Beach, Virginia, while in California and Texas it is naturalized as a median strip planting.

Because of its durability, Oleander was planted prolifically on Galveston Island in Texas after the disastrous Hurricane of 1900.

They are so prolific that Galveston is known as the Oleander City; an annual Oleander festival is hosted every spring. Oleander can be grown successfully outdoors in southern England, particularly in London and mild coastal regions of Dorset and Cornwall.

Caterpillars of the polka-dot wasp moth (*Syntomeida epilais*) feed specifically on oleanders and survive by eating only the pulp surrounding the leaf-veins, avoiding the fibers. Larvae of the common crow butterfly (*Euploea core*) also feed on oleanders, and they retain or modify toxins, making them unpalatable to would-be predators such as birds, but not to other invertebrates such as spiders and wasps.

The flowers require insect visits to set seed, and seem to be pollinated through a deception mechanism. The showy corolla acts as a potent advertisement to attract pollinators from a distance, but the flowers are nectar less and offer no reward to their visitors. They therefore receive very few visits, as typical of much reward less flower species (Herrera J, 1991).

2.5 Ornamental gardening

Oleander is a vigorous grower in warm subtropical regions, where it is extensively used as an ornamental plant in parks, along roadsides, and as a wind break. It will tolerate occasional light frost down to −10 °C (Huxley, *et al*., 1992) though the leaves may be damaged. The plant is tolerant of poor soils, salt spray, and sustained drought, although it will flower and grow more vigorously with regular water.

Nerium oleander also responds well to heavy pruning, which should be done in the autumn or early spring to keep plants from becoming unruly. In cold-winter climates Oleander can be grown in greenhouses and conservatories, or as

potted indoor plants that can be kept outside in the summer. Oleander flowers are showy, profuse, and often fragrant, which makes them very attractive in many contexts.

Over 400 cultivars cultivar have been named, with several additional flower colors not found in wild plants having been selected, including red, pink, yellow, and salmon; white and a variety of pinks are the most common. Double flowered cultivars like Mrs. Isadore Dyer or Mont Blanc are enjoyed for their large, rose-like blooms and strong fragrance. Many dwarf cultivars have also been developed, which grow to only about 10 at maturity. In most Mediterranean climates they can be expected to bloom from April through October, with their heaviest bloom usually in May or June.

2.6 Cultivation and uses

Oleander grows well in warm subtropical regions. It is drought tolerant and will tolerate occasional light frost down to -10°C (Huxley *et al.,* 1992). It is tolerant of a variety of poor soils. Oleander also can be grown in cooler climates in green houses and conservatories, or as indoor plants that can be kept outside in the summer. Young plants grow best in spaces where they do not have to compete with other plants for nutrients.

Oleander flowers are showy and fragrant and are grown for ornamental purposes. Oleander is extensively used in warmer climates as an ornamental plant in landscapes, parks, and along roadsides. It is commonly used as a decorative freeway median in many states.

Over 400 cultivars have been named, with several additional flower colors not found in wild plants having been selected, including red, purple, pink, and orange; white and a variety of pinks are the most common. Many cultivars also have double flowers.

2.7 Therapeutic efficacy

Tincture of flowers exhibited cardio tonic, root CNS - active and spasmolytic activity. Externally, root exhibited healing properties for haemorrhoids and ulcers. Oil of root bark gave good results in leprosy. In Homoeopathy, tincture of *Nerium oleander* (red laurel) leaves is used in diseases of nervous system, hemiplegia and paralytic conditions under strict medical supervision (Khare, 2004).

Various medicinal properties viz. cardiotonic, analgesic, Anti diabetic, Anti-inflammatory, Antibacterial, Anticancer / Antineoplastic, Antifungal, Depressant, Anti mitotic, Insecticidal, Larvicidal are attributed to this plant. Oleander is also diuretic and lentive on dermatosis and contusion (Erdemoglu *et al.*, 2003).

Other properties attribute are inhibition of Nuclear factor- kappa B (NF-/kB) activation, Muscle stimulation, effective against Asthma, Seizures, Cancer, Menstrual pain, Skin problems, Warts, epilepsy, leprosy, malaria, ringworm, indigestion, and venereal disease and also cause abortions. The present investigation was undertaken to find out the antibacterial potential of crude extract of different parts of *N. oleander* against some Gram positive and Gram negative bacteria.

Nerium oleander, showed the highest inhibitory zone against *Klebsiella pneumoniae Proteus vulgaris, Salmonella typhi and Escherichiacoli* (Jude, 2013). The ethanolic flower extracts of *N. oleander* showed anti-fungal activity *in vitro* against four important plant pathogenic fungi *viz*; *Fusarium oxysporum, Alternaria alternata, Fusarium solani* and *Rizoctonia solani* using agar dilution bioassay. Oleander exhibited the best inhibition against *F. oxysporum* and *F. solani* (Hadizadeh *et al.,* 2009). Essential oil is obtained from the flowers of *N. oleander* and showed *in vitro* antibacterial activity against *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*. It also showed a very effective bactericidal activity with minimum inhibitory concentrations (MIC) ranging from 1.45 to 5.10 $mg.ml^{-1}$ (Derwich *et al.,* 2010).

The flowers of *Nerium oleander* possess insecticidal and anti-feedant property against *Plutella xylostella* (Jacobson 1975). This plant has been also reported for larvicidal activity against *Aedes aegypti* by (Komala misra *et al.,* 2005). Insect growth regulatory activity against *Anopheles stephensi* and *Culex quinquefasciatus* of this plant also recorded. The aqueous leaf extract of *Nerium oleander* were exhibited ovicidal and larvicidal properties (Kumar *et al.,* 2012) and for ovicidal and adulticidal activity of this plant against *Anopheles stephensi* was also recorded. It was revealed that flower extract with hexane possessed high larvicidal activity when compared to aqueous extracts.

Single dose of ethanolic extract of *Nerium indicum* has significant anti diabetic activity which reduced the blood glucose level. The chloroform extract showed significant reduction of blood glucose after one hour of treatment while the ethanolic extract showed significant reduction after three hours. However, the water extract of this plant did not able to reduce glucose level at sub-acute level, The activity of *Nerium oleander* observed in chloroform extract showed that the diabetic rats had lower body weights, high blood glucose level as compared to normal rats. Beside this orally administered *Nerium oleander* chloroform extract and ethanolic extract significantly increased the body weight and decreased blood glucose level in diabetic rats (Lahsissene *et al.,* 2009).

Nerium oleander showed antitumor activity on the cell lines, Ehrlich Ascites Carcinoma (Ali et al., 2010). In another study, different amounts of Anvirzel (1.0 ng.ml-1 to 500 microgram.ml-1) or Oleandrin (0.01 ng.ml-1 to 50 microgram.ml-1) in both continuously treated and pulse-treated/recovery Cell cultures. Both Oleandrin and Anvirzel were able to induce cell killing in human cancer cells, but not in murine cancer cells (Patel *et al.,* 2010).

In an ethnopharmacological screening, plants used in Nepalese traditional medicine including *Nerium oleander* were evaluated for antiviral activity. Methanolic and methanolic-aqueous extracts derived out of 23 species were

assayed in two *in vitro* viral systems, influenza virus/MDCK cells and herpes simplex virus/Vero cells. Two species, *Bergenia ligulata* and *Nerium indicum* showed the highest antiinfluenza viral activity with 50% inhibitory dose of 10 microg/ml. *Holoptelia integrifolia* and *N. indicum* exhibited considerable antiviral activity against herpes simplex virus. None of these extracts showed cytotoxic effects (*Rajbhandari M, et al., 2001).*

The methanolic extract of the plant Nerium is used for the treatment of cell proliferation disease in animals and humans Methanolic flowers extract of *Nerium indicum* was evaluated for hepatoprotective in rats. The rats treated with methanolic extract of *Nerium indicum* at a dose of 500 and 1000 mg/kg prevented carbon tetrachloride induced reduction in ascorbic acid (Patel Govind 2010).

2.8 Health benefits of Oleander flower

There are many properties of Oleander which are listed below-

- It has been discovered that the root, skin and seeds contains contain glycosides.
- It regulate *kapha* and *vata*; hence promotes digestion.
- This very plant is cool in nature.
- It acts as a blood purifier and is antipyretic in nature.

Some of the prominent Health benefits of Oleander is listed below-

1. For Heart pain

- Give 100-200 mg of bark of its root after small meals.
- It causes heavy urination; which cures the heart pain.
- It even cures other disorders related to heart.

2. As a toothbrush

- In India, many like to use the twigs and thin branches of some plants as their toothbrush.
- The branch of white oleander can be used to brush teeth.
- It strengthens even the loose teeth.

3. For Headache

- Grind the flowers of oleander with Indian gooseberry in kanji (a fermented drink prepared using beets, carrots, mustard).
- Apply this paste on forehead. It gives immense relief.

4. For Piles

- Grind the root of oleander with cold water and apply on the boils.
- Must apply those on the boils while easing.
- It cures the boils.

5. As a face pack

- Grind the flowers of white oleander and apply on the face.
- It improves texture and complexion of the skin.

6. For Joint pain

- Grind the leaves of Oleander and apply on the painful joints.
- One can even use the decoction of white oleander to rinse the ulcers and wounds.

7. For snake bite

- In case of snake bite, give 125-250 mg of bark of its root or 1-2 leaves repeatedly after a short interval.

- It causes the patient to vomit which then actually releases the poison from the body.

8. For eczema

- Prepare oil from the root bark of white oleander and apply on the affected parts.
- It cures all types of eczema, itching and dermatoses.

9. For Contagious diseases

- In case of any kind of monsoonal/contagious disease, massage with oil of its leaves.
- It prevents growth of micro-organisms that causes such diseases.

10. For Itching

- Apply the medicated oil prepared from oleander leaves.
- It cures itching within an hour.
- Paste of oleander leaves or flowers with olive oil can be applied in any kind of itching.
- It is beneficial in any type of itching.

2.9 Spectral characterization

The active principles of many drugs was found in plants or produced from one of the plants secondary metabolites. The remarkable contribution of plants to the drug industries was possible because of the large number of the phytochemical studies all over the world. These studies established methods and techniques for extraction, separation, chemical identification and biological testing of plants constituents.

Many reagents were developed as diagnostic for different chemical principles (Evans, 2002) and this progress resulted in isolation of many active compounds used as a good remedy for many diseases. The biosynthesis of

secondary metabolites although genetically controlled, was affected by environmental influences. The soil, the season and the gathering time are some of the important variable factors with plants and can hardly be expected that the amount of constituents would be constant under all conditions.

III. MATERIALS AND METHODS

The materials used and methods adopted in the present study entitled "**Antimicrobial and Antioxidant activities of *Nerium oleander* flower extract**" is presented in the following headings

3.1. Collection and authentication of the plant

Nerium oleander flowers (Fig.1) were collected in December 2016 from the campus of Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore. The whole plant was identified (BSI/SRC/5/23/2017/Tech/3265) and their authenticity was confirmed by Dr. C. Murugan, Scientist D, Botanical survey of India, T.N.A.U Campus, Coimbatore.

Fig.1. A view of *Nerium oleander* flower

3.2. Preparation of the Nerium oleander flower

The flowers were cleaned thoroughly and dried at room temperature for 5-7 days in the shade. The dried samples were powdered using an electrical grinder. The powdered samples were stored in screw cap bottles until further analysis (Fig.2).

Fig.2. Nerium oleander flower powder

3.3. Preparation of extract

Five hundred grams of powder from the whole dried *Nerium oleander* flower was taken, to which 50 ml of different solvents (ethanol, chloroform and water) were added, mixed, and kept for four days. The contents were periodically shaken using an electric shaker. After four days, the contents were filtered through a Buchner funnel in a conical flask and it was further concentrated by evaporation by keeping the filtrate in a round-bottomed flask, till the solvent completely evaporated and the extract settled down to the bottom.

3.4. Preliminary phytochemical screening

Preliminary screening of the extracts and identification was done by color tests adapting standard methods by Raman (2006).

3.4.1 Test for Alkaloids

- **Mayer's test**

  A fraction of extract was treated with Mayer's test reagent (1.36 g of mercuric chloride and 5 g of potassium iodide in 100 ml of water) and observed for the formation of cream colour precipitate.

- **Wagner's test**

  A fraction of extract was treated with Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 ml water) and observed for the formation of reddish brown colour precipitate.

- **Dragendroff's test**

  1ml of the extract was added to 1ml of Dragendroff's reagent. Appearance of orange colour precipitation indicates the presence of alkaloids.

3.4.2 Test for Flavonoids

- **Alkaline reagent test**

  1 ml of the extract was treated with aqueous NaOH and HCl. The formation of yellow orange colour indicates the presence of flavonoids.

- **Lead acetate test**

  Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.

3.4.3 Test for Sterols

- **Liebermann-Burchard test**

  Extract (1ml) was treated with chloroform, acetic anhydride and drops of H_2SO_4 was added and observed for the formation of dark pink or red colour.

- **Salkowski**

  When concentrated sulphuric acid was added to a chloroform solution to the extracts (10 mg of extract in 1 ml of chloroform), a reddish blue colour was produced in the chloroform layer and green fluorescence in acid layer, suggesting the presence of steroids.

3.4.4 Test for Phenols

- **Ferric chloride test**

The fraction of extract was treated with 5% ferric chloride and observed for the formation of deep blue or black colour

3.4.5 Test for saponins

- **Foam test**

To the extract, 20ml of distilled water was added and agitated on a graduated cylinder for 15 minutes. The formation of about 1 cm layer of foam indicates the presence of saponins.

3.4.6 Test for Tannins

- **Gelatin test**

To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

- **3.4.7 Test for Quinones**

A small amount of extract was treated with concentrated HCl and observed for the formation of yellow color precipitate.

3.4.8 Test for Proteins

- **Ninhydrin test**

A small quantity of extract solution was boiled with 0.2% solution of Ninhydrin. Blue colour indicates the presence of amino acids.

- **Biuret test**

The extract was treated with equal volume of 40% Sodium hydroxide and two drops of 1% copper sulphate solution. Pink or purple colour indicates the presence of proteins.

3.4.9 Test for Carbohydrates

- **Molisch's test**

To small quantities of solvent free methanolic extract, few drops of 1% α-naphthol in ethanol were added. Conc.H_2SO_4 was then added to sides of the

test tubes. A brown purple ring formed at the junction of the two liquids indicates the presence of sugars.

- **Fehling's test**

Small quantities of solvent free methanolic extract were separately dissolved in minimum amount of distilled water and filtered. To the filtrates equal volume of Fehling's solution were mixed in a test tube separately and heated for few minutes. Formation of brick red precipitate confirmed the presence of sugars.

3.5. Antimicrobial activity

3.5.1 Test organisms

Two Gram-positive bacteria (*Bacillus subtilis, Staphylococcus aureus*) and three Gram-negative bacteria (*Escherichia coli, Salmonella typhi, Pseudomonas*) were used for antibacterial activity. Four fungi (*Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus, Aspergillus rhizopus*) were used for antifungal activity.

3.5.2. Methodology

Antibacterial and antifungal activity studies were carried out by agar diffusion method (Barry *et al.,* 1976). Standard antibiotic disc of Chloramphenicol (K-30 µg/disc) was used as the standard reference drug for antibacterial assay, Nystatin (50 µg/disc) was used for antifungal activity study (sarkar *et al*.,1998).

The pure cultures of different pathogens were grown overnight in sterile nutrient broth and incubated at 37°C for 24 hours. The 0.1ml of the culture was seeded on 25 ml of solidified nutrient agar and rose bengal plates for bacterial and fungal cultures, respectively. The wells were bored with 8mm borer in seeded agar, and then the particular concentrations (20µl) of the extracts were added in each well. Soon after the plates were then kept at 10°C for 30min. After it normalized to room temperature plates were incubated at 37°C for 24hrs. After incubation period is completed, the zone of inhibition was measured and recorded.

3.5.3. Determination of minimum inhibitory concentration (MIC) using Microbroth dilution test

The antimicrobial effectiveness of a compound is often described in terms of its minimum inhibitory concentration (MIC), the lowest concentration of the compound capable of inhibiting the growth of the challenging organism (Mann and Markham, 1998). In the present study, the effectives of *Nerium oleander* flower extract were selected to find out the MIC. Different doses were tested (5, 2.5, 1.25, .625, 0. 3125, 0.15 mg) to find out the MIC values.

The minimum inhibitory concentration (MIC) was determined by micro dilution method using serially diluted plant extracts in microtitre plate (Fig.3) according to the NCCLS protocol (NCCLS, 2000). Plant extracts was diluted to get a series of concentration from 5µl to .312mg/µl in sterile nutrient broth 1µl and potato dextrose broth (1µl). The microbial suspension of 100µl was added to the extracts and broths respectively. These were incubated for 24 hours at 37ºC (bacteria) and at room temperature for 5 days (fungi). MIC of the extract was taken as the lowest concentration that did not given any visible microbial growth.

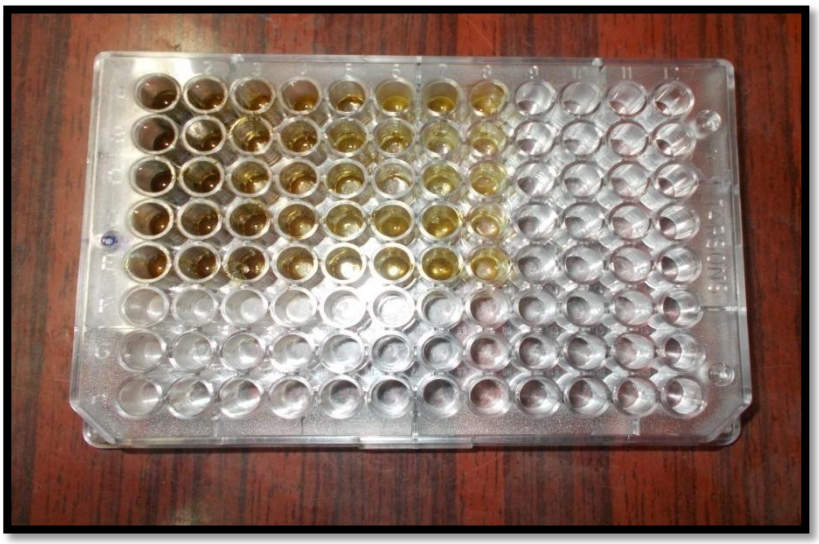


Fig.3. MIC assay

3.6. Antioxidant studies

3.6.1. DPPH free radical scavenging activity (Mensor *et al*., 2001)

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma Aldrich Co., St. Louis, USA. The diluted working solutions of the test extracts were prepared in ethanol. Ascorbic acid was used as standard in 5-30µg/ml solution. 0.002% of DPPH was prepared in ethanol and 1 ml of this solution was mixed with 1 ml of sample solution and standard solution separately.

These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using UV spectrophotometer. Ethanol (1 ml) with DPPH solution (0.002%, 1 ml) was used as blank. The optical density was recorded and % inhibition was calculated using the formula given below:

Percent (%) inhibition of DPPH activity = 100- (A-B/A) x100

Where A = optical density of the blank and B = optical density of the sample.

3.6.2. Reducing power assay (Oyaizu, 1986)

Reaction mixtures were prepared by adding 2.5 ml of phosphate buffer (0.2 M, pH 6.6), 2.5 ml potassium ferricyanide (1%) and varying concentrations of extracts (5-30µg/ml). After, the reaction mixtures were incubated at 50°C in water bath for 30 min, allowed to cool at room temperature (28°C), and 2.5 ml of 10% TCA (Trichloro acetic acid) were added to each reaction mixture, and then centrifuged at 2000 rpm for 10 min. The supernatant (2.5 ml) was separated in the test tube and added with 2.5 ml of distilled water and 0.5 ml $FeCl_3$ (1.0%), and allowed to react for 10 min at room temperature and the absorbance was measured at 700 nm. Ascorbic acid solution was used as standard.

3.7. Spectral Characterization of the flower extract

3.7.1. UV- Visible analysis

To detect the UV-VIS spectrum profile of the *Nerium oleander* flower, the extract were scanned in the wavelength ranging from 100 - 700nm by using UV spectrophotometer (Shimadzu, Japan) and the characteristic peaks were detected. The peaks values of the UV-VIS were recorded.

3.7.2. FT-IR analysis

FTIR measurements were performed on Spectroscope (Shimadzu, Japan) using 500-4000 nm. To prepare sample for FT-IR test a small amount (about one gram) of potassium bromide KBr 3 was mixed with the sample and compressed in order to make a suitable capsule for FT-IR device.

3. 7. 3. XRD analysis

The sample was made powder using mortar in order to perform XRD. The XRD analysis was carried out to confirm the purity of the synthesize materials using Shimadzu 6000 X-ray diffractometer with Cu-Kα radiation of a wavelength of λ= 1.5406 Å source.

IV. RESULTS

The results pertaining to the study "**Antimicrobial and Antioxidant activities of *Nerium oleander* flower extract**" are presented in the following headings

4.1. Preliminary phytochemical screening *Nerium oleander* flower

The phytochemical constituents serve as therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicine. Investigations on secondary plant constituents have made phenomenal advance during the past few decades. Based on the above concept few analysis were done with the extracts used in the present study which are described below (Table I).

Table I: Preliminary phytochemical analysis of flower extracts of *Nerium oleander*

S. No.	Constituents	Test for constituents	Solvents		
			(Ethanol)	(Chloroform)	(Aqueous)
1	Alkaloids	Mayer's	+	+	+
		Wagner's	+	-	+
		Dragendroff's	+	+	_
2	Flavonoids	Alkaline reagent	+	+	_
		Lead acetate test	+	-	_
3	Sterols	Libermann Burchard	-	-	_
		Salkowski	+	-	_
4	Phenols	Ferric chloride	-	+	+
		Lead acetate	+	-	_
5	Saponins	Foam test	+	+	+
6	Tannins	Gelatin test	+	+	+
7	Quinones	Alcoholic KOH	_	-	_
8	Proteins	Ninhydrin	+	-	_
		Biuret test	+	-	+
9	Carbohydrates	Molisch's test	+	+	_
		Fehling's test	+	+	+

+ Present - Absent

4.2. Antimicrobial activity

Antimicrobial resistant bacteria are the causes of numerous clinical problems worldwide. Infectious disease caused by resistant microorganisms is responsible for increased health costs as well as high morbidity and mortality, particularly in developing countries. The increase in the prevalence of multiple drug resistance has slowed down the development of new synthetic antimicrobial drugs and has necessitated the search for new antimicrobials from natural sources.

4.2.1. Antibacterial activity of flower extract *of Nerium oleander*

The flower extracts were evaluated for its antibacterial activity against five clinical bacterial isolates namely *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Salmonella* and *Pseudomonas aeruginosa*. Table.2 described the antibacterial activity of ethanol, chloroform and water extracts of the *Nerium oleander* against selected bacterial isolates. Plate I shows the zone of inhibition of *Nerium oleander* against bacterial isolates.

From the Table 2 it was observed that the zone of inhibition was found to be maximum in the ethanol extract and was found to be more active against *Pseudomonas aeruginosa* (28mm) *Salmonella* (25mm) *Staphylococcus aureus* (21mm), *Escherchia coli* (20mm) followed by *Bacillus subtilis* (17mm). The chloroform extract exhibited the zone of inhibition of 18mm, 15mm, 12mm, 10mm, 9mm against *Pseudomonas aeruginosa, Salmonella typhi*, *Staphylococcus aureus, Escherchia coli* and *Bacillus subtilis* respectively.

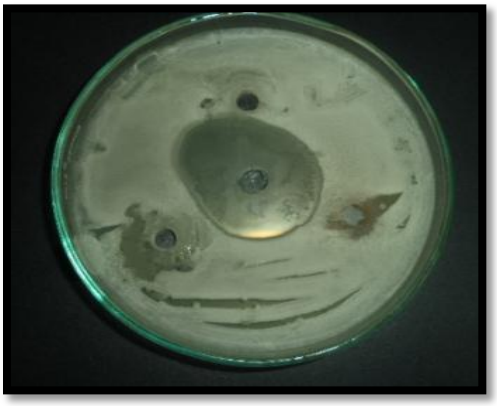
TABLE 2: Antibacterial activity of the flower extract of *Nerium olenander* against the selected bacterial isolates

Bacterial Isolates	Zone of inhibition in diameter (mm)			
	ETA	CHL	AQE	Control*
Bacillus subtilis	17	9	15	25
Escherichia coli	20	10	22	30
Pseudomonas aeruginosa	28	18	25	36
Staphylococcus aureus	21	12	10	35
Salmonella typii	25	15	20	30

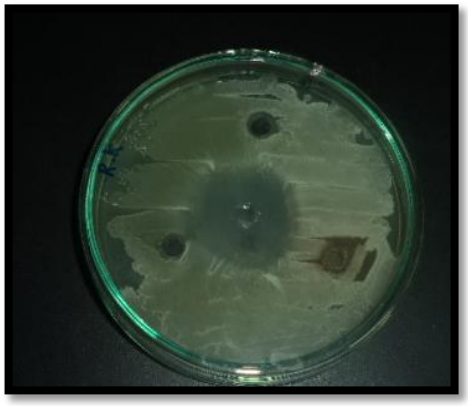
ETA - Ethanol extract **CHL – Chloroform extract**

AQE - Aqueous extract ***Control - Chloramphenicol**

PLATE I

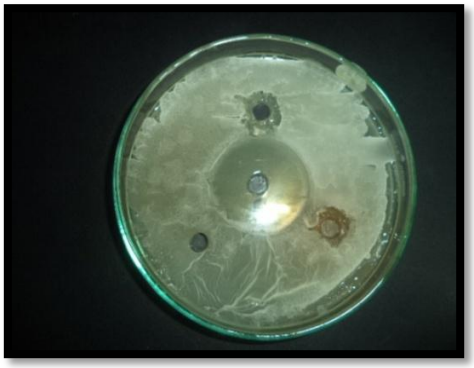


Bacillus subtilis



Escherichia coli

Pseudomonas aeruginosa



Staphylococcus aureus

Salmonella

PLATE I: ANTIBACTERIAL ACTIVITY *OF NERIUM OLEANDER* FLOWER EXTRACTS

4.2.2. Antifungal activity of flower extracts of *Nerium oleander*

The antifungal activity of the *Nerium oleander* flower extract was determined against the fungal isolates namely *Aspergillus niger*, *Aspergillus flavus, Aspergillus fumigates and Rhizopus* species. Table.3 depicts the antifungal activity of *Nerium oleander* flower extracts.

The ethanol extract of the *Nerium oleander* showed the maximum activity against *Aspergillus flavus* (18mm) Rhizopus (18mm) whereas the minimum activity was reported against *Aspergillus fumigates* (17mm) and *Aspergillus niger (13)*.

The Chloroform extract of *Nerium oleander* showed the maximum activity against *Aspergillus flavus* (17mm) and the minimum activity against Rhizopus(17mm), *Aspergillus fumigates* (16mm) followed by *Aspergillus niger* (15mm).

The aqueous extract exhibited the zone of inhibition of 18mm, 14mm, 13mm, and 10mm against *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigates* and rhizopus respectively.

The inhibition zone for the tested fungi ranged from 10 - 19mm indicating a remarkable antifungal effect when compared with that of Nystatin, the positive control, which ranged from 21 - 25mm.

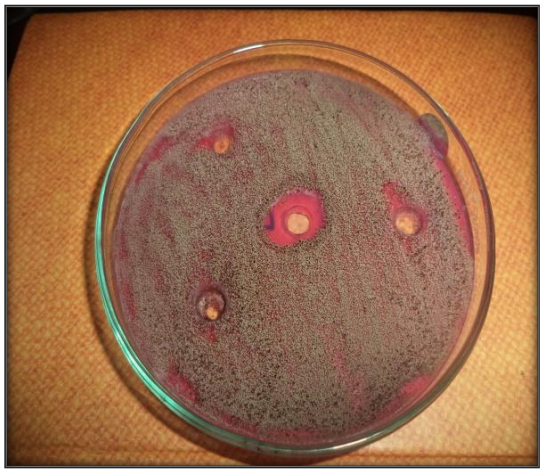
Table 3. Antifungal activity of the flower extracts *of Nerium oleander* against the fungal isolates

Fungal Isolates	Zone of inhibition in diameter (mm)			
	ETA	CHL	AQE	Control NYN
Aspergillus niger	13	15	14	15
Rhizopus	18	17	10	17
Aspergillus flavus	18	17	18	13
Aspergillus fumigatus	17	16	13	15

ETA - Ethanol extract **CHL – Chloroform extract**

AQE – Aqueous extract **NYN- Nystatin**

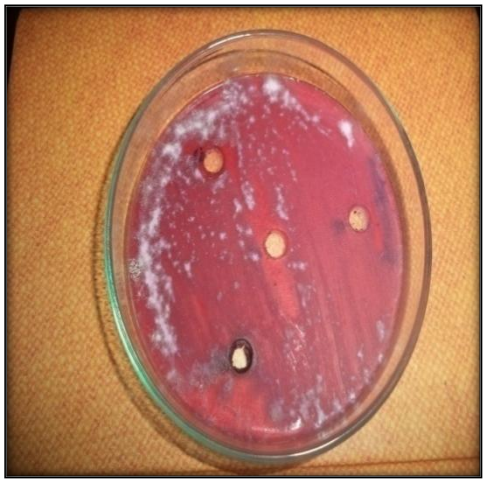
PLATE II



Aspergillus niger

Aspergillus flavus

Aspergillus flavus



Rhizopus

PLATE II: ANTIFUNGAL ACTIVITY OF *NERIUM OLEANDER* FLOWER EXTRACTS

4.2.3. Determination of minimum inhibitory concentration (MIC) using microbroth dilution test

The antimicrobial effectiveness of a compound is described in terms of its Minimum Inhibitory Concentration (MIC), the lowest concentration of the compound capable of inhibiting the growth of the challenging organism is described in terms of MIC values. In the present study *Bacillus subtilis* inhibited at a concentration of 0.161 mg/ml, *Pseudomonas* aeruginosa at 0.624mg/ml, *Salmonella typhi* at 0.423mg/ml, *Staphylococcus aureus* at 0.368mg/ml and *Escherichia coli* at 0.478mg/ml respectively (Table 4).

Table 4: Minimum inhibitory concentration of bacterial isolates

Bacterial isolates	Concentration(mg/ml)
Bacillus subtilis	0.161
Pseudomonas aeruginosa	0.624
Salmonella typhi	0.423
Staphylococcus aureus	0.368
Escherichia coli	0.478

Similarly fungal isolates such as *Aspergillus niger* inhibited the pathogens at a concentration of 0.240mg/ml, *Aspergillus flavus* at 0.070 mg/ml, *Aspergillus fumigates* at 0.614 mg/ml and *rhizopus* at 0.043 mg/ml (Table. 5).

Table 5: Minimum inhibitory concentration of fungal isolates

Fungal isolates	Concentration (mg/ml)
Aspergillus niger	0.240
Aspergillus flavus	0.070
Aspergillus fumigatus	0.614
Rhizopus	0.043

4.3. Antioxidant activity

4.3.1. DPPH radical scavenging activity

The results of the assay are expressed in scavenging activity of DPPH free radical expressed in percentage. The DPPH assay of ethanol extract of *Nerium oleander* and the reference compound ascorbic acid is given in Table. 6. The results showed that the radical scavenging activity of *Nerium oleander* flower extract increases with increasing concentration (Table.6).

Table 6: DPPH radical scavenging activity of *Nerium oleander* flower.

S. No.	Concentration (µg/ml)	Scavenging activity (%)	
		Ascorbic acid	ethanol extract
1	5	50	42
2	10	52	48
3	15	52	50
4	20	65	56
5	25	67	67
6	30	69	70

The ethanol extract of flowers of *Nerium oleander* showed better antioxidant potential when compare to standard ascorbic acid by DPPH scavenging assay method. It means alcoholic extract of plant at higher concentration captured more free radicals formed by DPPH resulting into decrease in absorbance (Fig.4).

The half maximal inhibitory concentration obtained as 5µg/ml and 15µg/ml for ascorbic acid and ethanol extract respectively.

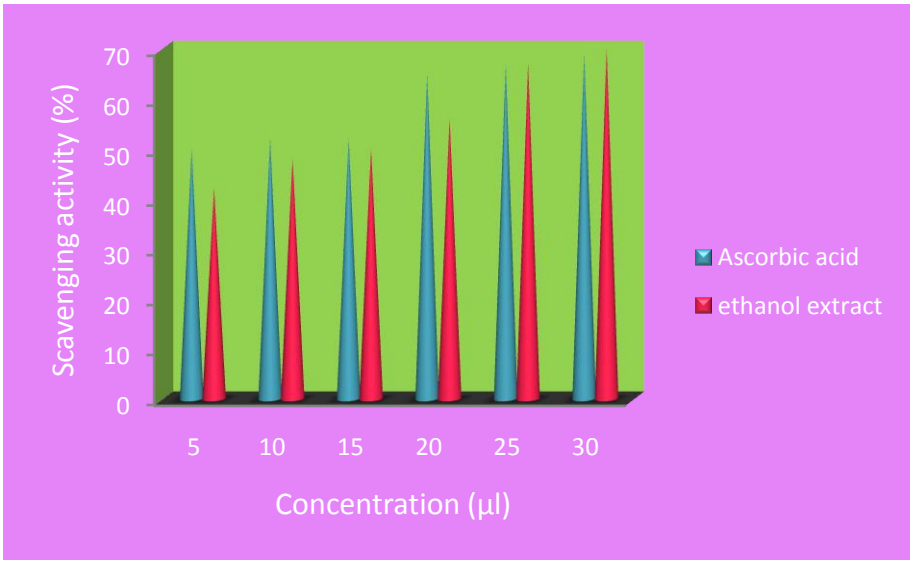


Fig.4. DPPH radical scavenging activity of *Nerium oleander* flower

4.3.2. Reducing power ability of *Nerium oleander* flower extracts

The reducing power of different concentration of *Nerium oleander* was found to be remarkable and the absorbance of each concentration was found to rise as the concentration gradually increases. The reducing power follows in the order of 30 µg/ml > 25 µg/ml > 20 µg/ml > 15 µg/ml >10 µg/ml > 5 µg/ml as shown in the Table. 7.

Table 7. Reducing power assay of *Nerium oleander* flower

S. No.	Concentration (µg/ml)	Absorbance
		Ethanol extract
1	5	0.43
2	10	0.55
3	15	0.65
4	20	1.02
5	25	1.09
6	30	1.50

Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of free radical reactions, so that they can act as primary and secondary antioxidants. From the graph (Fig. 5) it is clear that as the absorbance of the extracts increased, the reducing power ability also increased suggesting the presence of electron donors in the extract which act as intermediates for radical scavenging reactions.

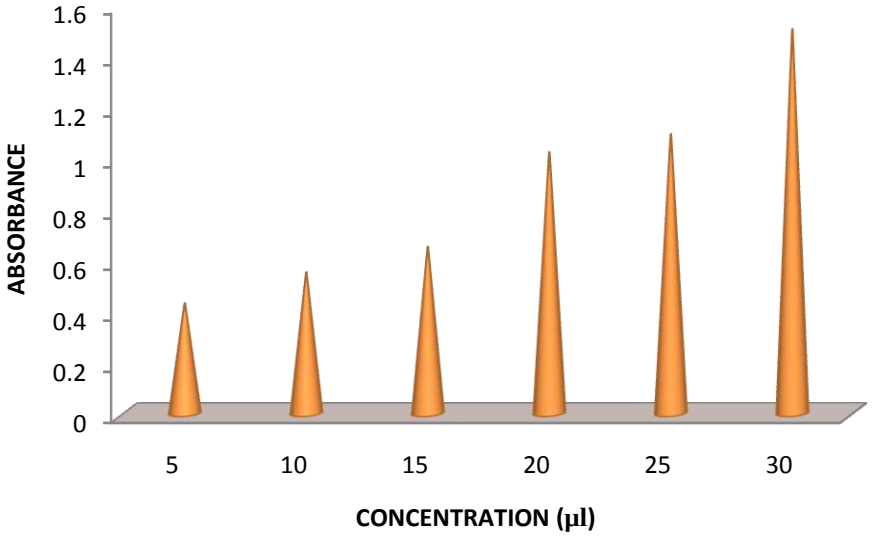


Fig . 5. Reducing power assay of *Nerium oleander* flower

Also the antioxidants present in the flower extracts of *Nerium oleander* reduces Fe^{+3}/ ferricyanide complex to the ferrous form (Fe^{+2}) resulting in formation of Perl's Prussian blue color which was read at 700 nm (Fig. 6)

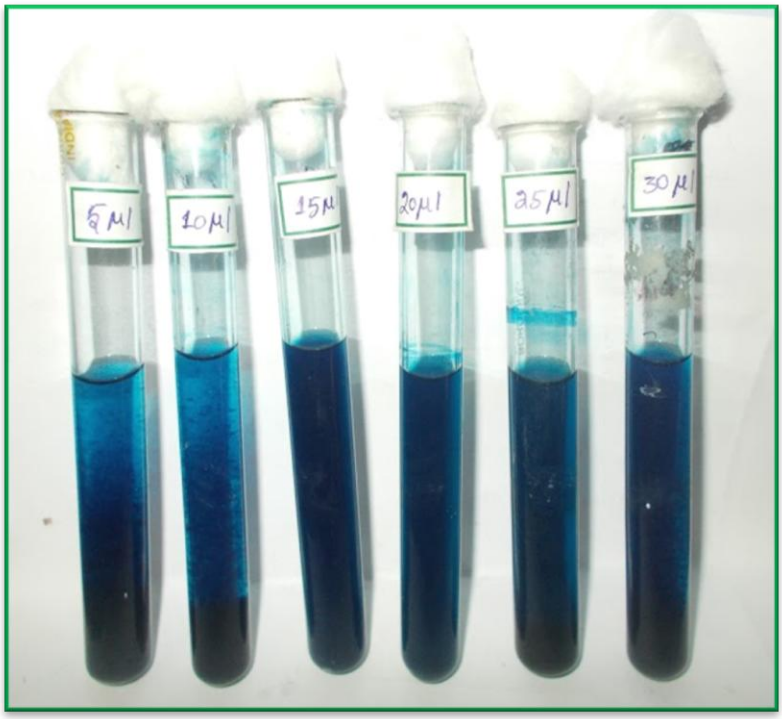


Fig.6. Reducing power ability of flower extracts of *Nerium oleander*

4.4. Spectral characterization

4.4.1. UV- Visible spectral analysis

The UV-VIS profile (Fig. 7) of *Nerium oleander* was studied at a wavelength range of 100 to 700 nm. One major band was recorded at 420 nm with absorbance value of 2.00. The spectra for terpenoid compounds typically lie in the range of 400-420 nm. Thus the result of UV-VIS spectroscopic analysis confirms the presence of terpenoids in the ethanolic extract of *N.oleander*.

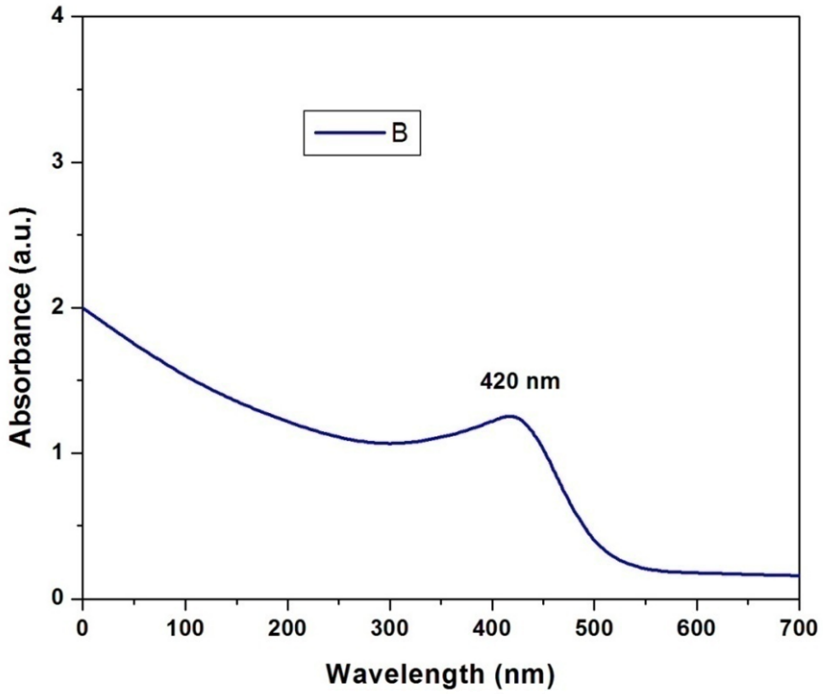


Fig.7. UV-Visible spectrum profile of *Nerium oleander*

4.4.2. FT- IR spectral analysis

The FT- IR spectrum was performed to identify the functional groups present in ethanol extract of *Nerium oleander* based on the peak values in the region of infrared radiation. The major bands were observed at 3446.7 cm^{-1}, 1631.7 cm^{-1}, 1350.1 cm^{-1} and 767.7cm^{-1} (Fig.8). The peak at 3446.7cm^{-1} indicates the O-H stretch, the peak at 1631.7 cm^{-1} indicates C=C stretch, the peak at

1350.1 cm $^{-1}$ indicates C-H bonding and the band at 767.7cm^{-1} corresponds to aromatic ring. In addition, some weak absorption bands were also recorded in the spectra.

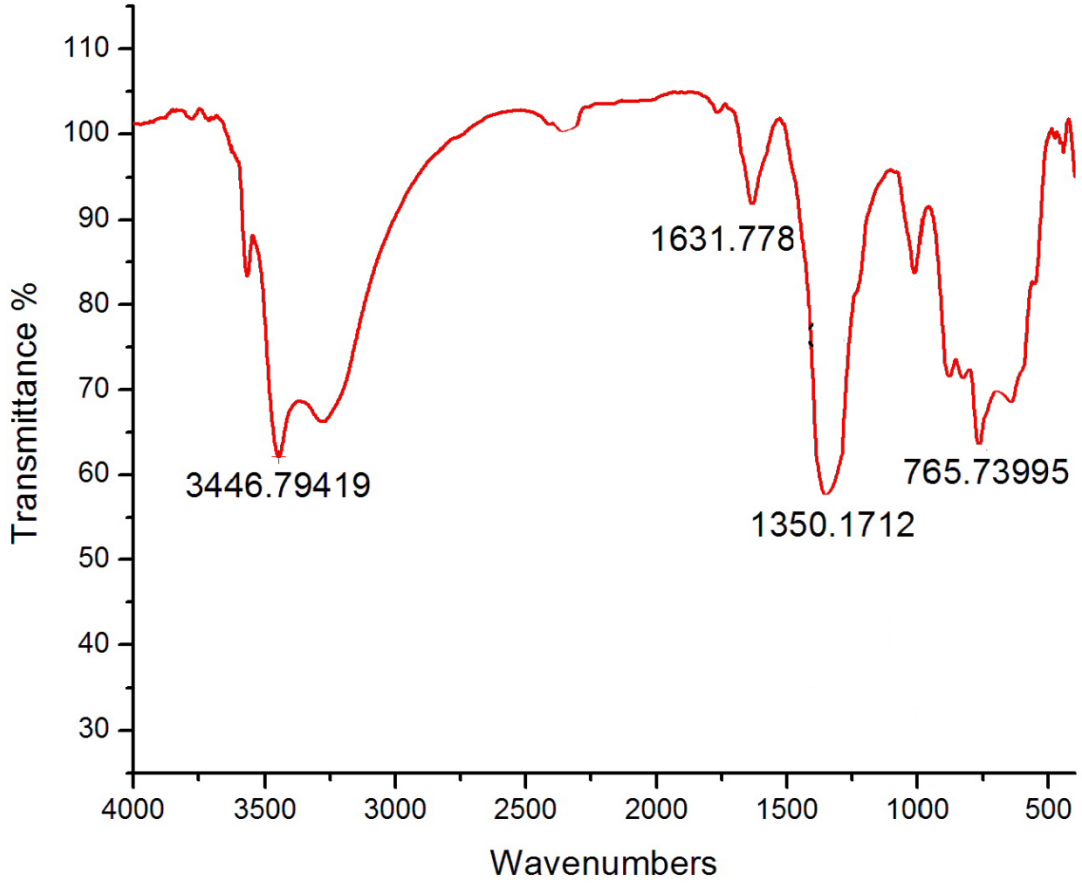


Fig.8. FT-IR spectrum profile of *Nerium oleander* flower extracts

4.4.3. XRD analysis

XRD analysis of the flower extract of *Nerium oleander* exhibited three major peaks and four minor peaks. The extracted flower samples showed three main peaks at 2θ = 4 a.u$_s$, 3.5 a.u$_s$ and 2.5 a.u$_s$ representing amorphous and crystalline peaks.

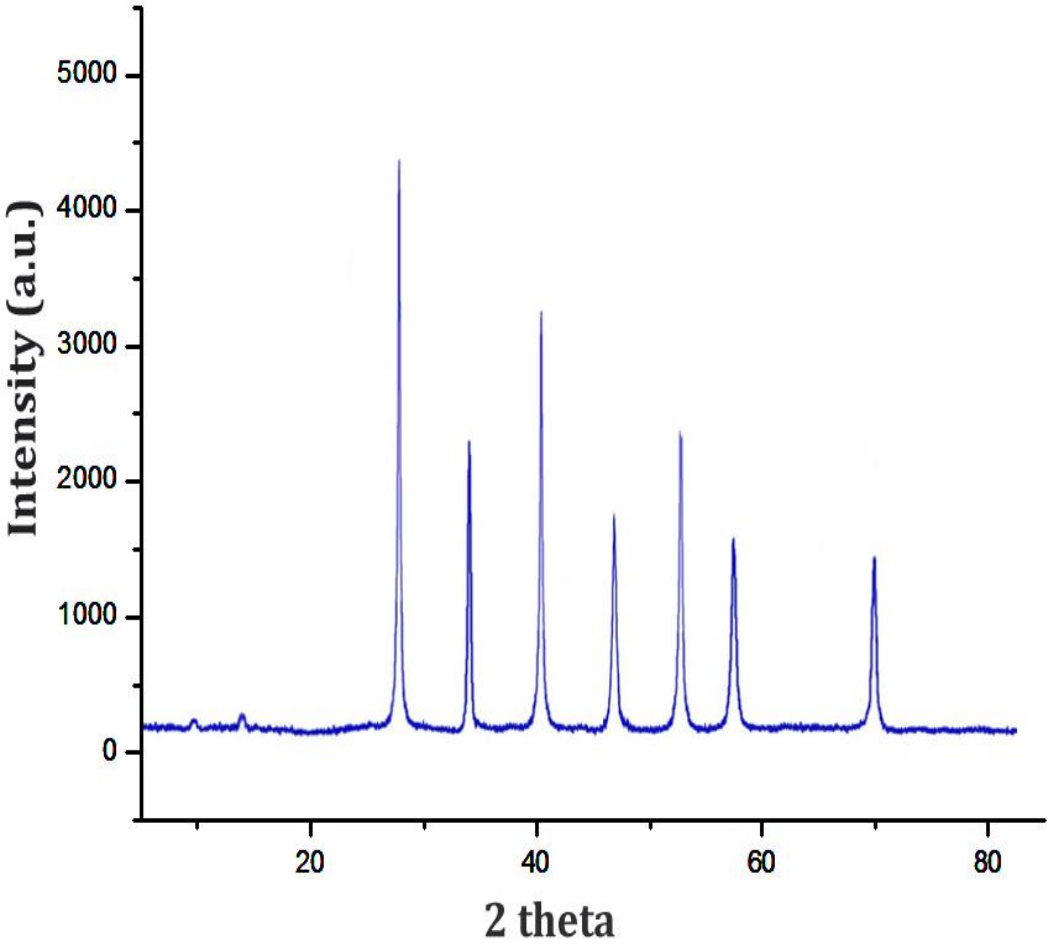


Fig.9. XRD analysis of *Nerium oleander* flower extracts

V. DISCUSSION

Use of flowers as a source of medicine has been inherited and is an important component of the health care system in India. Flower extracts are given singly of as concoctions for various ailments. Many investigations have demonstrated to elucidate the chemical compounds of flower origin. In the present study, phytochemical, antimicrobial and antioxidant activities of the flower extracts was carried out to find out the major bioactives present in the flower extracts.

The phytochemical screening and qualitative estimation of the plant studies showed that the flowers were rich in alkaloids, flavanoids, phenols and triterpenoids in all the extracts. Some extract showed presence of carbohydrates and sterols too. Saponins and tannins were found to be present in almost all the extracts of the flower. It should be noted that steroidal compounds are of importance and of interest in pharmacy due to their relationship with such sex hormones.

Flavonoids are polyphenolic compounds and consist of flavones, flavonols, flavanols, flavanone and flavanonols. These compounds represent the majority of plant secondary metabolites and have shown to possess remarkable health promontory effects such as anti-inflammatory, antioxidant, antimicrobial, anticancer properties. Interception of free radicals or other reactive species is mainly by radical scavenging and is caused by various antioxidants like vitamin C and E, glutathione, other thiol compounds, carotenoids, flavonoids, etc.

All the plant extracts used in this study were primarily screened against the test microorganisms by different methods like agar well diffusion and disc diffusion and the determination of minimum inhibitory concentrations. According to the World Health Organization (2012) the evolving public health threat of antimicrobial resistance is driven by both appropriate and inappropriate use of anti-infective medicines. The development of bacterial resistance to presently available antibiotics has necessitated the need to search for new antibacterial

agents. Different antibiotics exercise their inhibitory activity on different pathogenic organisms (Chanda and Rakholiya, 2011).

In the present investigation, different solvents of flower extract showed varying activity against gram positive and gram negative bacteria. Gram positive bacteria such as *Staphylococcus aureus* is mainly responsible for post operative wound infections, toxic shock syndrome, endocarditis, osteomyelitis and food poisoning (Benayache *et al*., 2001). Gram negative bacterium such as *Escherichia coli* is present in human intestine and causes lower urinary tract infection, coleocystis or septicaemia (Benhassaini *et al.,* 2003; Benjilali *et al.*, 1986).

Multiple drug resistance in human pathogenic microorganisms has been developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. The development of antibiotic resistance is multifactorial, including the specific nature of the relationship of bacteria to antibiotics, the usage of antibacterial agent, host characteristics and environmental factors. This situation has forced scientists to search for new antimicrobial substances from various sources as novel antimicrobial chemotherapeutic agents, but the cost production of synthetic drugs is high and they produce adverse effects compared to plant derived drugs (Abirama sundari *et al*., 2011).

Kumaraswamy *et al*.,(2008) stated that the biochemical molecules of plant origin appear to be a suitable alternative to overcome the problems caused by antibiotic resistant pathogens. As per the observation of Jeyachandran *et al.,* (2010) the ethanolic extract of *Nerium oleander* showed maximum zone of inhibition (28 mm) against *S. typhi*. Salie *et al.,* (1996) showed that *B. subtilis* was found to be more sensitive than gram negative bacteria.

The result obtained from the study points out that the active component present in Ethanol extract can prove to be a great remedy for treating diseases. The mean inhibition zone for the tested bacteria ranged from (9mm-28mm)

indicating a remarkable antibacterial effect when compared with Chloramphenicol the positive control, which ranged from 25mm - 36mm.

The infections caused by *Pseudomonas aeruginosa* can be treated with ethanol extract of *Nerium oleander* exhibited the more inhibitory activity of the extract against the pathogen. Hence, it can be stated that *Pseudomonas aeruginosa* and *Escherichia coli* were susceptible to aqueous and chloroform extract, whereas the remaining bacterial isolates were resistant and moderately susceptible to aqueous and chloroform extracts.

Similar results were reported by Umamaheswari *et al.,* 2010, in which the role of phytoconstituents towards the action of pseudomonas resistance. Disruption of adhesion of the pathogenic organism to host might be an effective way of preventing the disease manifestation. Fungal cells have very high Emgor pressure and even a minor chink in the cell wall structure can lead to bursting and death. The cell wall synthesis is alternative target site for developing new antibiotics. The effect of extracts on cell wall is determined using the plate bioassay method (Vidula and pragna, 2000).

The efficacy of ethanol extract of flowers of *Nerium oleander* demonstrated the presence of cell wall active antifungal agents which could lead to the discovery and development of novel antifungal treatment therapies. Similarly such results were documented by Aplomb and Semple (2011) who reported that thick murine layer in the outer membrane prevent the entry of inhibitory substance inside the cell.

Reddy *et al.,* (2001) reported that the possible mechanism of antimicrobial activity of some phenolics containing extracts is by creating an acidic environment that causes the disruption of bacterial cell membrane disruption. Goyal *et al*., (2008) also reported that the polarity of antibacterial compounds crucial for their activity.

Since antiquity, natural products, especially those of plant origin have always been an important source of therapeutic agents. Recent data from the pharmaceutical industry show that natural products represent a valuable source for the production of new chemical entities. Indeed, Reactive Oxygen Species (ROS) released by the human body are eliminated by molecules with antioxidant properties.

Antioxidants are found in all plants (Muanda *et al*., 2011). The soluble free radical DPPH is well known as a good hydrogen abstractor yielding DPPH-H as by product. Thus, the scavenging of DPPH radicals by phenols are most of the time very effective. The results obtained from the present investigation, highlight the very important antioxidant activity of phenolic fractions in the flower extracts.

The DPPH radical has been extensively used to evaluate the reducing substances and is a useful reagent for investigating the free radical scavenging activities of compounds. The radical scavenging activity of the ethanol extract from *Nerium oleander* flowers and ascorbic acid at five concentrations was tested by DPPH method and the results showed relatively high DPPH scavenging activity comparing with those extracts from other parts of *Nerium oleander*.

Different studies have indicated that the reducing capacity of bioactive compounds is associated with its antioxidant activity (Siddhuraju *et al*., 2002). In this study, the reducing power of *Nerium oleander* extracts was determined. The extracts showed some degree of electron donation capacity in a concentration-dependent manner, but the capacities were lower than that of ascorbic acid.

Analysis of the flower extract of *Nerium oleander* under UV-VIS and FT-IR spectroscopic techniques showed the presence of terpenoid compounds which can be isolated and further screened for different kind of biological activities depending their therapeutic uses. Further research will be needed to find out the structural analysis of terpenoid compound by use of different analytical methods such as NMR and Mass spectrophotometer.

XRD analysis of the flower extract of *Nerium oleander* exhibited three major peaks and four minor peaks. The extracted flower samples showed three main peaks at 2θ = 4 $a.u_s$, 3.5 $a.u_s$ and 2.5 $a.u_s$ representing amorphous and crystalline peaks.

Similar results were reported by Chaturbhuj *et al.*, 2016, who measured the crystalline behavior of cellulose fibers by XRD analysis. The X-ray diffractogram of raw fiber, pulp and bleached pulp from *Gigantochloa scortechinii* contains partially crystalline and amorphous structure resulting from the placement of glycosidic chain, which is held closely by mutual H-bonding in the crystalline region and absence of such organized H-bonding in amorphous region.

Abraham *et al*.,2016 find out that cellulose exhibits crystalline and amorphous peaks in an X-ray diffractrogram. The amorphous peak occurs around 2θ = 15° and the crystallographic plane representing crystallinity occurs at 2θ = 22. The raw and extracted microfibres of hemp showed three main peaks at 2θ = 15.4°, 20.8° and 22.4° representing amorphous and crystalline peaks.

In another study Hamed *et al*., 2016 carried out the physicochemical analysis of cellulose from microalgae confirmed the hypothesis that the variable of NaOH concentration of 2 to 4% shows an amorphous structure, which becomes increasingly abundant to a maximum concentration of 10% sodium hypochlorite

VI. SUMMARY AND CONCLUSION

The therapeutic effects of medicinal plants for the treatment of various diseases are based on the chemical compounds of these plants. The major components are organic compounds, some of which have biological activity, but none act independently and cannot replace the functions of the medicinal plant as a whole.

Phytochemical analyses revealed that ethanolic extracts of *Nerium oleander* contain terpenoids, alkaloids and flavonoids which have been reported to be responsible for the antimicrobial properties and, it could serve as antimicrobial agents for the treatment of microbial infections. Pure isolated alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic, antispasmodic and bactericidal effects. They exhibit marked physiological activity when administered to animals.

Flavonoids on the other hand are potent water soluble antioxidants and free radical scavengers, which prevent oxidative cell damage, have strong anticancer activity. Flavonoids in intestinal tract lower the risk of heart disease. As antioxidants, flavonoids from candidate plants provide anti-inflammatory activity. This may be the reason *Nerium oleander* would be suggested for an alternative in herbal medicine.

The development of antibiotic resistance is multi factorial, including the specific nature of the relationship of bacteria to antibiotics, the usage of antibacterial agent, host characteristics and environmental factors. This situation has forced scientists to search for new antimicrobial substances from various sources as novel antimicrobial chemotherapeutic agents, but the cost production of synthetic drugs is high and they produce adverse effects compared to plant derived drugs.

Generally microbes have the genetic ability to transmit and acquire resistance to drugs used as therapeutic agents. One way to prevent antibiotic

resistance is by using new compounds which are not based on the existing synthetic antimicrobial agents. These antimicrobial substances are of natural origin, and it is thought that their influences on the environment are few and can be used as biological control agents.

However, some medicinal herbs for some reasons have not found wider application and sometimes are referred as ‘forgotten plants’ such as Nerium oleander. Taking this into account it is reasonable to revise the ‘forgotten plants’ by assessing their applicability and benefits using modern scientific analysis methods.

The result obtained from the study points out that the active component present in ethanol extract of *Nerium oleander* can prove to be a great remedy for treating diseases. The zone of inhibition for the tested microorganism, such as bacteria and fungi indicating a remarkable antibacterial effect when compared with chloramphenicol, the positive control.

The antioxidant activity of various medicinal plants can be determined precisely, conveniently, and quickly using DPPH testing. The development in antioxidant activity obtained by using the DPPH method is comparable to trends found using other methods reported in the literature. This method can be used effectively for compact samples without prior extraction procedure and concentration problems, which saves time.

The radical scavenging activity of the ethanol extract from *Nerium oleander* flowers and ascorbic acid at five concentrations was tested by DPPH method and the results showed relatively high DPPH scavenging activity comparing with those extracts from other parts of *Nerium oleander*.

Different studies have indicated that the reducing capacity of bioactive compounds is associated with its antioxidant activity. In this study, the reducing power of *Nerium oleander* extracts was determined. The extracts showed some degree of electron donation capacity in a concentration-dependent manner, but the capacities were lower than that of ascorbic acid.

Spectral analysis has revealed that medicinal plants are rich in many trace elements, and it is suggested that this is an important factor in the curative effect of these plants. The chemical states in which trace elements were found are organically bound, complex and free. Besides, different states have different functions, toxicity, and absorption rates by the body.

Analysis of the flower extract of *Nerium oleander* under UV-VIS and FT-IR and XRD spectroscopic techniques showed the presence of terpenoid compounds which can be isolated and further screened for different kind of biological activities depending their therapeutic uses. Further research will be needed to find out the structural analysis of terpenoid compound by use of different analytical methods such as NMR and Mass spectrophotometer.

VI. REFERENCES

Abdel Rahman. S., Abd-Ellatif, S., Deraz, S. and Khalil, A. (2011). Antibacterial activity of some wild medicinal plants collected from western Mediterranean coast, Egypt: Natural alternatives for infectious disease treatment. *African Journal of Biotechnology*, 10(52): 10733-10743.

Abiramasundari, P., Priya ,V., Jeyanthi,G.P. and GayathriDevi,S. (2011). Evaluation of the antibacterial activity of *Cocculus hirsutus . Hygeia - Journal for Drugs and Medicines*, l3(2), 26-31.

Abraham., Reinu E., Cynthia ,S., Wong and Munish Puri . (2016). Enrichment of cellulosic waste hemp (Cannabis sativa) hurd into non-toxic microfibres. *Materials*, 9: 562.

Ali, D., Hussein, S.M.S., Malik, A. and Ahmed, Z. (2003). Chemical constituents of the genus Launaea. *Journal of Chemical Society of Pakistan*, 25: 341-347.

Ali, H.F.M., El-Ella, F.M.A., and Nasr.N.F (2010). Screening of chemical analysis, antioxidant antimicrobial and antitumor activities of essential oil of oleander (*Nerium oleander*) flower. *International journal of biological Chemistry* .4(4): 190–202.

Arguedas .C, Coley ,D. (2005). Phenotypic variation and spatial structure of secondary chemistry in a natural population of a tropical tree species, *OIKOS* 108: 410-420.

Aysal,P., Ambrus,A.D., Lehotay,S.J. and Cannavan,A. (2007). Validation of an efficient method for the determination of pesticide residues in fruits and

vegetables using ethyl acetate for extraction, *Journal of Environmental Science and Health.* 42: 481-490.

Bai, L., Wang, L., Zhao, M., Toki. A., Hasegawa, T., Ogura, H.,Kataoka,T., Hirose, K., Sakai, J., Bai, J.and Ando, M. (2007). *J Nat Prod*., 70(1): 14- 28.

Bakht, J., Azra.G., and Shafi. M.(2012). Antimicrobial activity of *Nicotiana tabacum* using different solvents extracts. *Pak. J.Bot*., 44(1): 459-463.

Barry, A. L.(1976). Principle and Practice of Microbiology Lea & Fabager. Philadelphia.

Bayati, F. and Sulaiman, K.(2008). In vitro antimicrobial of Salvadora persica L.extracts against some isolated oral pathogen in Iraq*. J.Agric*.*Food chem*., 47:397-492.

Benayache,S., Benayache,F.and Benyahia,S. (2001). Leaf oils of some eucalyptus species growing in Algeria. *J.Essent.Oil., Res*.,13: 210-213.

Benhassaini,H.,Benabderrahmane,M. and Chikhi,K. (2003). Contribution à l'évaluation de l'activité antiseptique de l'oléorésine et des huiles essentielles du pistachier de l'Atlas sur certaines sources microbiennes : Candida albicans (ATC 20027), Candida albicans (ATCC 20032) et *Saccharomyces cerevisiae*. *Ethnopharmacologie.fév*., (30): 38-46.

Benjilali,B.,Tantaoui-Elaraki,A., Ismaïli-Alaoui,M. and Ayadi, A .(1986).Méthode d'études des propriétés antiseptiques des huiles essntielles par contact direct en milieu gélosé. *Plant Médicinal Phytotherapy*, 20: 155-167.

Burham.B.O. (1984). Chemical Constituents of Selected Sudanese Medicinal and Aromatic Plants.

Cao.G., Sofic, E.R. and Prior, R.L. (1996). Antioxidant capacity of tea and common vegetables. *J. Agric*. Food Chem., 44: 3426-3431.

Chanda,S. and Rakholiya, K. (2011). Combination therapy: Synergism between natural plant extracts and antibiotics against infectious diseases. Science against microbial pathogens: communicating current research and technological advances A. Méndez-Vilas (Ed.).

Chaturbhuj, K., Saurab., Mustapha, A., Masri, M., Owolabi, A.F., Syakir, M. I., Rudi Dungani, M. T. , Paridah, M., Jawaid. and Abdul Khalil, H. P. S. (2016.) Isolation and characterization of cellulose nanofibers from gigantochloa scortechinii as a reinforcement material. *Journal of Nanomaterials*.1-8

Darwish, R. and Aburja, A., T (2010). Effect of ethnomedicinal plants used in folklore medicine in Jordan as antibiotic resistant inhibitors on *Escherichia coli. BMC Complementary and Alternative Medicine*, 1-12.

Doughari, J.H. (2006). Antimicrobial activity of *Tamarindus indica Linn*. *Tropical J. Pharma.* Res., 5(2): 597-603.

Duh, P.D., Tu. Y.Y. and Yen, G.C. (1999). Antioxidants activity of aqueous extract of Harnjyur (*Chrysanthemum morifolium* Ramat). *Lebensmwiss Technol*. 32: 269-277.

Eberhardt, T.L., Li,X,. Shupe,T.F. and Hse,C.Y.(2007). Chinese Tallow tree (*Sapium sebiferum*) utilization: Characterization of extractives and cell-wall chemistry. *Wood Fiber Sci*., 39: 319-324.

Egwaikidi, P.A., Okeniyi,S.O.and Gimba, C.E.(2009). Screening for antimicrobial activity and phytochemical constituents, of some Nigerianmedicinal plants. *J. Med*. *Plant. Res*., 3: 1088-1091.

Eisenhauer,N.,Klier.M.and Partsch,A. (2009) No interactive effects of pesticides and plant diversity on soil microbial biomass and respiration, *Applied Soil Ecology***,** 42: 31-36.

Erdemoglu, N., Küpeli, E.and Yeşilada E. (2003). Biological screening of some Turkish medicinal plant extracts for antimicrobial and toxicity activities. *J. Ethnopharmacol*, 89(1) 129.

Evans, W.C. (2002). Trease and Evans Pharmacognosy. WB. Saunders. London. Fifteenth Edition.

Gates,D.M. and David, M., Harry,J.(1965). A review of electron spin spectroscopy and its application to the study of paramagnetic defects in crystalline quartz. *Phys Chem Minerals* 10: 149.

Gopal reddy,P., Prajakta tathe,K. and Salunke,K.J. (2011). In vitro evaluation of antibacterial activity of leaf extracts. *Plant Sci,* 7 (1) : 122-125.

Gupta. P.D.,and Thorsteinson.A.J. (1960). Food plant relationships of the diamondback moth (*Plutella maculipennis* (Curt. gustation and olfaction in relation to botanical specificity of the larva. *Entomologia Experimentalis et Applicata*, 3: 241–250.

Hadizadeh, L., Peivastegan, B. and Kolahi. M., (2009). Antifungal activity of nettle *(Urtica dioica L*.), colocynth (*Citrullus colocynthis L. Schrad*), oleander (*Nerium oleander* L.) and konar *(Ziziphus spina-christi* L.)

extracts on plants pathogenic fungi. *Pakistan Journal of Biological Sciences* ,12(1): 58–63.

Halliwell, B. (1994). Free radicals, antioxidants, and human disease. *Lancet,* 344: 721-724.

Hamed, B., Mohamed, B.H., schéhérazade , K. and Abiayad sidi–mohammed . 2016 Physicochemical analysis of cellulose from microalgae *Nannochloropsis gaditana. African Journal of Biotechnology*, 15(24): 1201-1207.

Hammer, K.A., Carson, C.F. and Riley, T.V. (1999). Antimicrobial activity of essential oils and other plant extract. *J. app. Microbiol*., 86: 985-990.

Hazra,K.M., Roy,R.N., Sen,S.K.and Laska,S. (2007) Isolation of antibacterial pentahydroxy flavones from the seeds of *Mimusops elengiLinn*, *African Journal of Biotechnology*, 6(12):1446-1449.

Henary, H.A., Kurzrock, R.and Falchook, G.S. (2011). Final Results of a First-in-Human Phase 1 Trial of PBI-05204, and Inhibitor of AKT, FGF-2, NF-Kb and P70S6K in advanced cancer patients. *J. Clin Oncol*. 29 (supplement; abstract 3023).

Herrera, J. (1991). The reproductive biology of a riparian Mediterranean shrub, *Nerium oleander L.* (Apocynaceae). *Botanical journal of the Linnean Society*, 106(2):147-172.

Hussain,M.A,. Gorsi, M.S., (2004.) Antimicrobial Activity of Nerium oleander Linn. *Asian Journal of plant sciences,* 3(2): 177-180.

Huxley, A., Griffiths, M. and Levy,M. (eds.). (1992). The New RHS Dictionary of Gardening. Macmillan. ISBN 0333474945.

Ibrahim,M., Hameed,A.J.and Jalbout,A. (2008) Molecular spectroscopic study of river Nile sediment in the greater Cairo region, *Applied Spectroscopy,* 62(3):306-311

Jacobson.M. (1975) Insecticides from plants a review of the literature, 1954–1971. Agriculture Handbook 461, USDA, Washington, DC, 138 p.

Jayabalan, M., K., Rjaranthinam, G.D. Augustus, P.S., Sekar, T., and Veerasamy. S., (1995). Preliminary studies on anti-infl ammaory mactivity of Zingiber moffi cinale Rosc., *Vitex negundo Linn*. and Tinospora cordifolia (Willid) Miers in albino. *Journal of Ecotoxicology and Environmental Monitoring,* 5(1):pp. 45-49.

Jeyachandran, R., Baskaran ,X. and Cindrella,L. (2010).Screening of phytochemical and antibacterial Potential of Four indianmedicinal plants. *Libyan Agriculture Research Center Journal Internation*, 1(5): 301-306.

Joshi, B., Sunil,B. and Anuja,S.(2009). Antibacterial property of different medicinal plants: *Ocimum sanctum, Cinnamomum zeylanicum, Xanthoxylum armatum and Origanum majorana. Kathmandu University Journal of Science, Engineering and Technology*, 5(1): 143-150.

Joshua.,M. and Takudzwa .M. (2013). Antibacterial properties of mangifera indica on

Jude, C.A. (2013). Extraction, characterization and industrial applications of tobacco seed oil (*Nicotiana tabacum*). *Chemistry and Materials Research,* 3(2): 19-21.

Khare CP. (2004). Encyclopedia of Indian Medicinal Plants, *Springer-Verlag-Heidelberg,* 328-330.

Komalamisra, N., Trongtokit,Y., Rongsriyam ,Y.and Apiwathnasorn, C. (2005) .Screening for larvicidal activity in some Thai plants against four mosquito vector species. *Southeast Asian Journal of Tropical Medicine and Public Health,* 36(6): 1412–1422.

Kumar,G., Karthik, L., Rao. K.V.B., Kirthi. A.V.and Rahuman.A.A. (2012) Phytochemical composition and mosquito controlling property of *Nerium oleander* leaves (Apocynaceae) against *Culex tritaeniorhynchus and Culex gelidus* (Diptera: Culicidae). *Asian Pacific Journal of Tropical Biomedicine* ,2: 1–6.

Kumaraswamy, M. V., Kavitha, H. U.and Satish, S. (2008). Antibacterial evaluation and phytochemical analysis of *Betula utilis* D. Don against some human pathogenic bacteria. *World Journal of Agricultural Sciences,* 4(5), 661-664.

Laborde, A. (1989). *Nerium oleander* L. Poisons Information Monograph 366. International Programme on Chemical Safety (IPCS) Inchem.

Lahsissene. H., Kahouadji. A .and Hseini, S. (2009). Catalog of medicinal plants used in the region of Zaer (Morocco Occidental). *Lejeunia*, 186: 0457–4184.

Lavanya, G, and Brahmaprakash. G.P, (2011). Phytochemical screening and antimicrobial activity of compounds from selected medicinal and aromatic plants. *Int. J. Sci & Nature*, 2(2): 287-291.

Li, L. (2000) [Opportunity and challenge of traditional Chinese medicine in face of the entrance to WTO (World Trade Organization]. *Chin. Inform. trad. Chin. Med.,* 7, 7–8

Lokesh, R., Leonard Barnaba, E., Madhuri,P., Saurav,K.and Sundar.K.,(2010)Larvicidal activity of *Trigonella foenum and Nerium oleander* leaves against mosquito larvae found in vellre city, *India current Research Journal of Biologycal Science,* 3:154-160.

Mahesh, B. and Satish. S. (2008). Antimicrobial activity of some important medicinal plant against plant and human pathogens. *World J. Agri. Sci*., 4(S): 839-843.

Mallet, J.F., Cerrati, C., Ucciani, E., Gamisans, J. and Gruber M. (1994). Norditerpene furan glycosides from Tinospora cordifo *Food chemistry* 49(1): pp. 61-65.

Mann,and markhan, J.L (1988). A new method for determining the minimum inhibitory concentration of essential oils*. Journal of applied microbiology*, 84:538-544.

Mensor, L.L., Menezes, F.S., Leitao, G.G., Reis, A.S., Santos, T.S. and Coube, C.S. (2001).Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytother. Res*., 15: 127–130.

Morgan, K.(2002) Medicine of the Gods: Basic Principles of Ayurvedic Medicine [http://www. compulink.co.uk/~mandrake/ayurveda.htm]

Muanda.S., François, N., Rachid Soulimani, S. and Amadou Dicko,G. (2011) Study on biological activities and chemical composition of extracts from

Desmodium adscendens leaves.. 4(2011): 100-1-107 ISSN 0974 – 5211.

Neha Sahu.N.and Jyoti Saxena.J. (2013) Phytochemical analysis of *Bougainvillea glabra* Choisy by FTIR and UV-VIS Spectroscopic Analysis. 33, 196-198.

Nostro, A., Germano,M.P, Angelo,V.D., Marino,A.,and Cannatelli,M.A. (2000). Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity*. Letters in Applied Microbiology*, 30: 379-384.

Olabanji, S. O., Makanji, O. V., Ceccato, D., Buoso, M. C., Haqu, A. M., Cherubini, R., and Moschini, G. (1997). PIGE-PIXE analysis of medicinal plants and vegetables of pharmacological importance*. Trace Elem.* Res., 58 (3): 223-236.

Omer Burham,B. (1984). Chemical constituents of selected substance in medicinal and aromatic in plants.

Oyaizu, M. (1986). Studies on product of browning reaction prepared from glucose amine. *J. Nutr*., 44: 307-15.

Pankhurst, R., Editor (2009*). Nerium oleander* L. Flora Europaea.Royal Botanic Garden Edinburgh.

Patel ,G., Nayak, S.,& Shrivastava, S ., (2010.) Antiulcer Activity of Methanolic Leaves of Nerium Indicum Mill. *International Journal of Biomedical Research* 1(2): 55–61.

Patel .G. 2010. Antiulcer activity of *Nerium indicum* in rats. *Research journal of pharmacology* , 4(3): 66-68.

Pereira. C. E. and Felcman. J. (1998). Correlation between five minerals and the healing effect of Brazilian medicinal plants. Biol. *Trace Elem. Res*., 65 (3). 251-259.

Piorek. S.(1978). Radioisotope Excited X-ray Analysis of Environment sample, Nucleons, 23: 267-290.

Pourmorad, F., Hosseinimehr, S.J.and Shahabimajd, N. (2006). Antioxidant activity, phenols, flavanoid contents of selected Iranian medicinal plants. *S. Afr. J. Biotechnol*., 5: 1142-1145.

Rajbhandari.M., Wegner. U., Juilich, M., Schopke.T.and Mentel .R.S .(2001). Screening of Nepalese medicinal plants for antiviral activity, *Journal of Ethnopharmacology,* 74(3), 251- 255.

Rajendran, R. (2011). Isolation, characterization, pharmacological and corrosion inhibition studies of flavonoids obtained from *Nerium oleander* andtecoma stans. *Int. J. Pharm. Tech. Res*., 2: 1005-1013.

Raman, N.(2006). Phytochemical Technique. New Indian Publishing Agencies: New Delhi,pp.19.

Reddy, P. S., Jamil.K. and Madhusudhan,P.(2001). Antibacterial activity of isolates from *Piper longum* and *Taxus baccata. Pharmaceutical Biol*. 39: 236-238.

Remington. J.P. (1995). The Science and Practice of Pharmacy, Vol. II, (19th edition), pp. 1495-1523. Mack Publishing Company, Easton. Pennsylvania, USA.

Saito, H. (2000) Regulation of herbal medicines in Japan. *Pharmacol. Regul*., 41, 515–519.

Salie,F., Eagles, P.F.K. and Leng, H.M.J. (1996). Preliminary antimicrobial screening of four South African Asteraceae species. *J. Ethnopharmacol,* 52: 27-33.

Sarkar, M. K., Ergil, D.,Tamer, A. U. and Sahin, N. (1998). Antiamoebic and Spasmolytic activities of extracts from some antidiarrhoeal traditional preparations used in Kinshasa Fitoterapia, 69: 457.

Schulz, V., Hänsel, R.and Tyler, V.E, (2001) Rational Phytotherapy. A Physician's Guide to Herbal Medicine, 4th Ed., Berlin, Springer-Verlag.

Shaw, D. (1998). Risks or remedies ? Safety aspects of herbal remedies. J. Roy. Soc. Med., 91,294–296

Siddhuraju, P.P., Mohan, S. and Becker, K. (2002) Studies on the antioxidant activity of Indian laburnum (*Cassia fistula* L.): a preliminary assessment of crude extracts from stem bark, leaves, flowers and fruit pulp. *Food Chem*. 79: 61-67.

Singh G., Kumar P. (2012) Antibacterial potential of alkaloids of *Withania somnifera* L. and Euphorbia hirta L. *Int J Pharm and Pharm Sci*.4(1): 78-81.

Tiwari.P, Kumar,B**.** (2001). Phytochemical screening and Extraction: A Review. *internationale pharmaceuticasciencia*,98.

Tumwine, W. (2011). Implementation of the framework convention on tobacco control in Africa: Current Status of Legislation. *Int. J. Environ.,Res. and Public Health*, 8: 4312-4331.

Umamaheswari,N., Rhama,S., Kannahi,M. and Madhavan,S. (2010). Antifungal activities of Clitoria ternatea leaf extract, Advances in plant sciences, 23(2): 377-378.

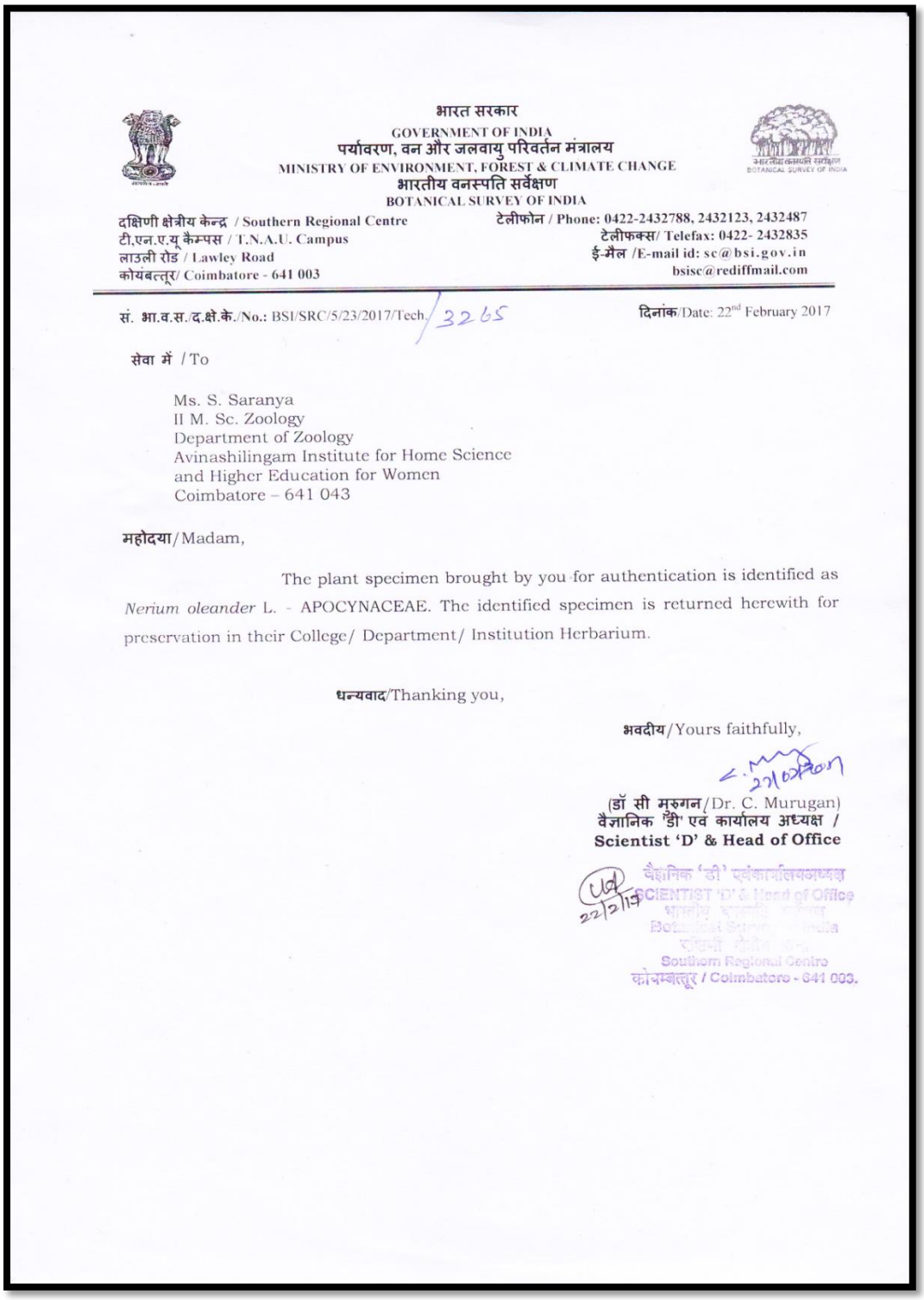
Uzer,A, E. Ercag,E.and R. Apak,.R. (2005) Selective spectrophotometric determination of TNT in soil and water with dicyclohexylamine extraction, *Analytica Chimica Acta,* 534**:** 307-317

Vidula, R.D. and Pragna, D.D. (2000), An agar planted assay to detect cell wall active antifungal agent, *current science*. 78(5):615-617.

Young. I.S., Woodside .J.V. (2001). Antioxidants in health and disease*. J.Clin*. Pathol., 54: 176-186.

APPENDICES

PLANT VOUCHER CERTIFICATE



भारत सरकार
GOVERNMENT OF INDIA
पर्यावरण, वन और जलवायु परिवर्तन मंत्रालय
MINISTRY OF ENVIRONMENT, FOREST & CLIMATE CHANGE
भारतीय वनस्पति सर्वेक्षण
BOTANICAL SURVEY OF INDIA

दक्षिणी क्षेत्रीय केन्द्र / Southern Regional Centre
टी.एन.ए.यू. कैम्पस / T.N.A.U. Campus
लाउली रोड / Lawley Road
कोयंबत्तूर/ Coimbatore - 641 003

टेलीफोन / Phone: 0422-2432788, 2432123, 2432487
टेलीफक्स/ Telefax: 0422- 2432835
ई-मैल /E-mail id: sc@bsi.gov.in
bsisc@rediffmail.com

सं. भा.व.स./द.क्षे.के./No.: BSI/SRC/5/23/2017/Tech./3265

दिनांक/Date: 22nd February 2017

सेवा में / To

Ms. S. Saranya
II M. Sc. Zoology
Department of Zoology
Avinashilingam Institute for Home Science
and Higher Education for Women
Coimbatore – 641 043

महोदया/Madam,

The plant specimen brought by you for authentication is identified as *Nerium oleander* L. - APOCYNACEAE. The identified specimen is returned herewith for preservation in their College/ Department/ Institution Herbarium.

धन्यवाद/Thanking you,

भवदीय/Yours faithfully,

22/02/2017

(डॉ सी मुरुगन/Dr. C. Murugan)
वैज्ञानिक 'डी' एवं कार्यालय अध्यक्ष /
Scientist 'D' & Head of Office

22/2/17

वैज्ञानिक 'डी' एवंकार्यालयअध्यक्ष
SCIENTIST 'D' & Head of Office
भारतीय वनस्पति सर्वेक्षण
Botanical Survey of India
दक्षिणी क्षेत्रीय केन्द्र
Southern Regional Centre
कोयम्बत्तूर / Coimbatore - 641 003.