CHAPTER – 1

1.1. INTRODUCTION

Plants are the most vital constituents, for every day life on earth. Plants have played an important part in the development of mankind. It provides us with food, medicine and cosmetics. Herbal medicine is the oldest form of medicine known to mankind using plants. Herbs have been used by human being, since antiquity for their extra-ordinary leading abilities and pain relieving properties. Presently man has increasingly started using herbs to overcome various illnesses and suffering. Today approximately 75% of all prescription drugs are delivered from trees, shrubs on herbs.

Medicinal plants have become a major component of human health care as they have no (or) least side effects. Surveys conducted in Australia and America indicates that almost 48.5% and 34% of respondents had used at least one form of unconventional therapy including herbal medicine. The investigations of the efficiency of plant based drugs used in the traditional medicine have been paid great attention because they elicit meager side effects and are cheap. According to WHO, still 80% of the world population rely on plant drugs (Dharmasri et al., 2002).

The development of antimicrobial agents for clinical use has bought unquestionable benefit to individuals and society. Infectious diseases that were formerly often fatal became curable (Shirwaikarkumar et al., 1995). However, mankind is now confronted with new re-emerging infections for which no effective treatments are available. In contrast, to other types of medication, antibiotics ultimately lose their effectiveness as they are used overtime and resistant strains of bacteria develop (Jeannette Day et al., 2002). There is thus an urgent need to identify novel, active chemo types as lead for drug development. Natural products could play a crucial role in meeting this demand of drugs approved between 1983 and 1994 by either the united states Food and Drug Administration (FDA) or comparable entities in other countries, Drugs of natural origin predominated (78%) in the area of antibacterial research (Padmaja et al., 1993). Many plants derived from nature possess antimicrobial and insecticidal activities. The interest in these plants is increasing because of finding safer microbicides in combination with the need of preventing environmental degradation.
The use of different parts of several medicinal plants to cure specific ailments has been in vague from ancient times. The indigenous system of medicine namely Ayurvedic, Siddha and Unani have been in existence for several centuries. Medicinal plants are nature’s gift to cure a number of diseases. Enormous plants are in use as therapeutic agents for thousands of years in treating different diseases. About 80% of the world’s population depends on traditional phyto medicines in numerous treatments and disorders (Farnsworth, 1994). Plants used in traditional medicines contain a wide range of ingredients that can be used to treat chronic as well as infectious diseases. The bioactive compounds like alkaloids, flavonoids, tannins, terpenes and phenolic compounds are the reason for the medicinal value of plants that produce a definite physiological action on the body (Hemashenpagam et. al, 2009).

India is one of the largest producers of medicinal plants in the world (Seth, & Sharma, 2004). The Indian traditional healthcare system, Ayurveda provides relatively organized database and more exhaustive description of botanical materials, many of which have been used as templates for novel drug development (Nordstrom, 1988). Medicinal plants have been used in traditional medicine for many years (Abu Rabia, 2005). India is an exquisite example of biodiversity. It has a very old history of the use of plants in the indigenous systems of medicines dating back to over 5000 years. It has been estimated that over 8000 plants are used in traditional, folk and herbal medicines (Alice and Asha, 2007). Today according to World Health Organization (WHO), as many as 80% of the world’s people depend on traditional phyto medicine for their primary healthcare needs. In the developed countries, 25% of the medicinal drugs are based on plants and their derivatives (Principe, 1991). Traditional medical knowledge of medicinal plants and their use by indigenous cultures are not only useful for conservation of culture and biodiversity but also for community healthcare and drug development in the present and future (Busia, 2005).

Phytochemicals have been used as drugs for millennia. For example, Hippocrates may have prescribed willow tree leaves to abate fever. Salicin, having anti-inflammatory and pain-relieving properties, was originally extracted from the bark of the white willow tree and later synthetically produced became the staple over-the-counter drug called aspirin. There is evidence from laboratory studies that phytochemicals in fruits and vegetables may reduce the risk of cancer, possibly due to
dietary fibers, polyphenol antioxidants and anti-inflammatory effects. Specific phytochemicals, such as fermentable dietary fibers, are allowed limited health claims by the US Food and Drug Administration.

Secondary metabolites are chemicals produced by plants for which no role has yet been found in growth, photosynthesis, reproduction, or other "primary" functions. These chemicals are extremely diverse; thousands have been identified in several major classes. Each plant family, genus, and species produces a characteristic mix of these chemicals, and they can sometimes be used as taxonomic characters in classifying plants. Humans use some of these compounds as medicines, flavorings, or recreational drugs.

Secondary metabolites are important in plant use by humans. Most pharmaceuticals are based on plant chemical structures, and secondary metabolites are widely used for recreation and stimulation (the alkaloids nicotine and cocaine; the terpene cannabino). The study of such plant use is called ethnopharmacology. Psychoactive plant chemicals are central to some religions, and flavors of secondary compounds shape our food product. Which are causes aroma and flavor of such compounds nitrogen-and sulfur-containing chemicals, glucosinolates, which protect these plants from many enemies. The astringency of wine and chocolate derives from tannins. The use of spices and other seasonings developed from their combined uses as preservatives (since they are antibiotics).

Secondary metabolites can be classified on the basis of chemical structure (for example, having rings, containing a sugar), composition (containing nitrogen or not), their solubility in various solvents, or the pathway by which they are synthesized (e.g., phenylpropanoid, which produces tannins). A simple classification includes three main groups: the terpenes (made from mevalonic acid, composed almost entirely of carbon and hydrogen), phenolics (made from simple sugars, containing benzene rings, hydrogen, and oxygen), and nitrogen-containing compounds (extremely diverse, may also contain sulfur).

Recent research is identifying more and more primary roles for these chemicals in plants as antioxidants, and other functions, so "secondary" may not be an accurate description in the future. Consuming some secondary metabolites can have
severe consequences. Alkaloids can block ion channels, inhibit enzymes or interfere with neurotransmission, producing hallucinations, loss of coordination, convulsions, vomiting, and death. Some phenolics interfere with digestion, slow growth, block enzyme activity and cell division, or just taste awful. Most herbivores and plant pathogens possess mechanisms that ameliorate the impacts of plant metabolites, leading to evolutionary associations between particular groups of pests and plants. Some herbivores (for example, the monarch butterfly) can store (sequester) plant toxins and gain protection against their enemies. Secondary metabolites may also inhibit the growth of competitor plants (allophty). Pigments (such as terpenoids carotenes, phenolics, and flavonoids) color flowers and, together with terpene and phenolic odors, attract pollinators.

Developing countries like India and China have been using the traditional system of medicine from centuries for treating various ailments and diseases. Now a day’s plants have been used as a powerful and potential medicine. Medicinal plants are mainly focused as an alternative source against manifestations caused by various pathogenic microorganisms due to the increasing resistance of existing antimicrobial agents. Studies by various researchers have proved that plants are one of the major sources for drug discovery and development (Rates SMK, 2001, Pasquale et al., 1984, Gordon et al., 2005). Plants are reported to have antimicrobial, anticancer, antiinflammatory, antidiabetic, hemolytic, antioxidant, larvicidal properties etc.

Antibiotic resistance has become a global concern in these days especially in developing countries. Bacterial resistance to antibiotics has become a serious problem of public health that concerns almost all antibacterial agents and that manifests in all fields of their application. Novel antimicrobial compounds against new bacterial targets and drug resistance mechanisms are urgently needed. Plant derived antibacterials are always a source of novel therapeutics. As infectious diseases are one of the major causes of mortality in these countries, the screening of higher plants for their natural products has been extensively focused. This could lead to its successful use as a potential drug which confers to its therapeutic use.

Medicinal plants contain physiologically active principles that over the years have been exploited in traditional medicine for the treatment of various ailments (Adebanjo, A et al. 1983) as they contain anti-microbial properties (Sokmen, A et al. 1999).
These medicinal herbs constitute indispensable components of the traditional medicine practiced worldwide due to the low cost, easy access and ancestral experience (Martin-Bettolo, G. B. 1980).

Most of the medicinal plants contain active constituents which needs further screening for its use as antimicrobial agent. Studies suggested that secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids that are present in these plants might confer to its antimicrobial activity (Gayathri Gunalan et. al., 2011). Due to a rapid increase in the rate of antibiotic resistance in microorganisms and side effects of synthetic antibiotics, medicinal plants are gaining popularity over these drugs and these plants are readily available in rural areas at relatively cheaper than modern medicine.

Plants are rich in a wide variety of secondary metabolites belonging to chemical classes (tannins, terpenoids, alkaloids, and polyphenols) represent different biological activities that depend on the diversity and quantity (Geyid A, et al. 2005). For example, Quinine (Cinchona) and berberine (Berberis) are alkaloids obtained from plants which are highly effective against microbes such as S. aureus, E. coli (Bibi Y, et al. 2011) Therefore, the determination of the compounds responsible for any biological activity would facilitate the selection of the plants for future investigation.

1.1.1. Alkaloids

A cyclic organic compound that contains nitrogen in a negative oxidation state and is of limited distribution among living organisms. Over 10,000 alkaloids of many different structural types are known; and no other class of natural products possesses such an enormous variety of structures. Therefore, alkaloids are difficult to differentiate from other types of organic nitrogen-containing compounds. (Schultz et al. 2011).

Simple low-molecular-weight derivatives of ammonia, as well as polyamines and acyclic amides, are not considered alkaloids because they lack a cyclic structure in some part of the molecule. Amines, amine oxides, amides, and quaternary ammonium salts are included in the alkaloid group because their nitrogen is in a negative oxidation state (the oxidation state designates the positive or negative
character of atoms in a molecule). Nitro and nitrous compounds are excluded as alkaloids. The almost-ubiquitous nitrogenous compounds, such as amino acids, amino sugars, peptides, proteins, nucleic acids, nucleotides, prophyrrins, and vitamins, are not alkaloids. However, compounds that are exceptions to the classical-type definition (that is, a compound containing nitrogen, usually a cyclic amine, and occurring as a secondary metabolite), such as neutral alkaloids (colchicine, piperine), the β-phenyl-ethylanines, and the purine bases (caffeine, theophylline, theobromine), are accepted as alkaloids. (Heinstein et al. 2011).

Colchicine, from the corms and seeds of the autumn crocus, is used as a gout suppressant. Caffeine, which occurs in coffee, tea, cocoa, and cola, is a central nervous system stimulant; it is used as a cardiac and respiratory stimulant and as an antidote to barbiturate and morphine poisoning. Emetine, the key alkaloid of ipecac root (*Cephaelis ipecacuanha*), is used in the treatment of amebic dysentery and other protozoal infections. Epinephrine or adrenaline, produced in most animal species by the adrenal medulla, is used as a bronchodilator and cardiac stimulant and to counter allergic reactions, anesthesia, and cardiac arrest (Qian et al., 1984).

While most alkaloids have been isolated from plants, a large number have been isolated from animal sources also. They occur in mammals, anurans (frogs, toads), salamanders, arthropods (ants, millipedes, ladybugs, beetles, and butterflies), marine organisms, mosses, fungi, and certain bacteria. Many alkaloids exhibit marked pharmacological activity, and some find important uses in medicine. Atropine, the optically inactive form of hyoscyamine, is used widely in medicine as an antidote to cholinesterase inhibitors such as physostigmine and insecticides of the organophosphate type; it is also used in drying cough secretions. Morphine and codeine are narcotic analgesics, and codeine is also an antitussive agent, less toxic and less habit-forming than morphine (Kopp et al. 2011).

### 1.1.2. Flavonoids

Phytonutrients of this phenol subclass enhance the effects of ascorbate-vitamin C. Flavonoids were once lumped together as vitamin P, but there are well over 1,500 of them. The biologic activities of flavonoids include action against allergies, inflammation, free radicals, hepatotoxins, platelet aggregation, microbes, ulcers, viruses and tumors. Flavonoids also inhibit specific enzymes. For example,
flavonoids block the angiotensin-converting enzyme (ACE) that raises blood pressure: By blocking the "suicide" enzyme cyclooxygenase that breaks down prostaglandins, they prevent platelet stickiness and hence platelet aggregation. Flavonoids also protect the vascular system and strengthen the tiny capillaries that carry oxygen and essential nutrients to all cells (Kopp et al., 2011). Additionally, flavonoids block the enzymes that produce estrogen, thus reducing the risk of estrogen-induced cancers. One way they do this is by blocking estrogen synthsase, an enzyme that works overtime in binding estrogen to receptors in several organs.

Although their way of doing so is not yet fully understood, flavonoids also appear to retard development of cataracts in individuals with inborn errors in sugar metabolism such as diabetes. Cataracts can be a complication of diabetes because diabetics, unable to metabolize sugar normally, build up damaging levels of "alcohol sugars." These in turn cause clouding of the lens of the eye (cataract). It is suspected flavonoids prevent cataracts by blocking aldose-reductase (a digestive enzyme), which can convert the sugar galactose into the potentially harmful form of galacticol (Crozier et al., 2006).

1.1.3. Terpenoids

The terpenoids, sometimes referred to as isoprenoids, are a large and diverse class of naturally occurring organic chemicals similar to terpenes, derived from five-carbon isoprene units assembled and modified in thousands of ways. Most are multicyclic structures which differ from one another not only in functional groups, but also in their basic carbon skeletons. These lipids can be found in all classes of living things, and are the largest group of natural products.

Plant terpenoids are extensively used for their aromatic qualities. They play a role in traditional herbal remedies and are under investigation for antibacterial, antineoplastic and other pharmaceutical effects. Terpenoids contribute to the scent of eucalyptus, the flavors of cinnamon, cloves and ginger and the color of yellow flowers. Well-known terpenoids include citral, menthol, camphor and the cannabinoids found in the Cannabis plant. The steroids and sterols in animals are biologically produced from terpenoid precursors. Sometimes terpenoids are added to proteins, e.g. to enhance their attachment to the cell membrane; this is known as isoprenylation (Montamat EE et al., 2000).
1.1.4. Terpenes

Terpenes such as those found in green foods, soy products and grains, comprise one of the largest classes of phytonutrients. The most intensely studied terpenes are carotenoids-as evidenced by the many recent studies on beta carotene. The terpenes function as antioxidants, protecting lipids, blood and other body fluids from assault by free radical oxygen species including singlet oxygen, hydroxyl, and peroxide and superoxide radicals. Terpenoids are dispersed widely throughout the plant kingdom, protecting plants from the same reactive oxygen species that attack human cells (Schultz et al., 2011).

1.1.4.1. Classification of Terpenes

Terpenes are hydrocarbons resulting from the combination of several isoprene units. Terpenoids can be thought of as modified terpenes, where methyl groups have been moved or removed, or oxygen atoms added. (Some authors use the term "terpene" more broadly, to include the terpenoids.) Just like terpenes, the terpenoids can be classified according to the number of isoprene units used:

1. Monoterpenoids, 2 isoprene units
2. Sesquiterpenoids, 3 isoprene units
3. Diterpenoids, 4 isoprene units
4. Sesterterpenoids, 5 isoprene units
5. Triterpenoids, 6 isoprene units
6. Tetraterpenoids, 8 isoprene units
7. Polyterpenoids with a larger number of isoprene units

Terpenoids can also be classified according to the number of cyclic structures they contain. Simplified version of the steroid synthesis pathway with the terpenoid intermediates isopentenyl pyrophosphate (IPP), dimethylallyl pyrophosphate (DMAPP), geranyl pyrophosphate (GPP) and squalene shown. Some intermediates are omitted for clarity (Graf E et al., 1990).

1.1.5. Carotenoids

This terpene subclass consists of bright yellow, orange and red plant pigments found in vegetables such as tomatoes, parsley, oranges, pink grapefruit, spinach and red palm oil. We even find carotenoids lending bright colors to animals; flamingos
owe their color to carotenoids, as do shellfish. Egg yolks are yellow because of carotenoids that protect the unsaturated fats in the yolk.

The Carotenoid family actually includes two distinct types of molecules. One type, the carotenes, is chemically classified as 40-carbon tetraterpenes, which do not include specific chemical features like hydroxyl or keto groups. This type of Carotenoid includes the familiar molecule beta carotene. The second type of carotenoids, the xanthophylls, includes the chemical compounds known as the Carotenoid alcohols and keto-carotenoids. In this second category are included the molecules Zeaxanthin, Cryotpxanthin, and Astazanthin (Crozier et al., 2006).

There are more than 600 naturally occurring carotenoids. Most people think of this family of phytonutrients as being precursors to vitamin A, but fewer than 10 percent have vitamin A activity. Among the carotenes, only alpha, beta and epsilon carotene possess vitamin A activity. Of these, beta carotene is the most active. Alpha carotene possesses 50 percent to 54 percent of the antioxidant activity of beta carotene, whereas epsilon carotene has 42 percent to 50 percent of the antioxidant activity (Warren et al., 2011).

The above-mentioned carotenes, along with gamma carotene and the carotenes lycopene and lutein, which do not convert to vitamin A, seem to offer protection against lung, colorectal, breast, uterine and prostate cancers. Carotenes are tissue-specific in their protection. Overall protective effects are therefore greater when all carotenes are taken together. Carotenes also enhance immune response and protect skin cells against UV radiation. Additionally; they "spare" the glutathionine Phase II detoxification enzymes in the liver that we rely on to safely eliminate pollutants and toxins from the body (Urbano et al., 2000).

The xanthophyll type of carotenoids also includes many interesting molecules. One xanthophyll, canthaxantin, was popular as a tanning pill a few years ago. It migrates to the skin and protects it from sunlight. Other important xanthophylls are cryptoxanthin, zeaxanthin and astaxanthin. Xanthophylls are important because they appear to protect vitamin A, vitamin E and other carotenoids from oxidation. Evidence is emerging that xanthophylls are tissue specific. Cryptoxanthin, for example, may be highly protective of vaginal, uterine and cervical tissues (Montamat EE et al., 2000).
1.1.6. Phytosterols

Sterols occur in most plant species. Although green and yellow vegetables contain significant amounts, their seeds concentrate the sterols. Most of the research on these valuable phytonutrients has been done on the seeds of pumpkins, yams, soy, rice and herbs. Phytosterols compete with dietary cholesterol for uptake in the intestines. They have demonstrated the ability to block the uptake of cholesterol (to which they are structurally related) and facilitate its excretion from the body. Cholesterol has long been implicated as a significant risk factor in cardiovascular disease (Fred R et al., 1957).

Investigations have revealed that phytosterols block the development of tumors in colon, breast and prostate glands. The mechanisms by which this occurs are not well understood, but we do know that phytosterols appear to alter cell membrane transfer in tumor growth and reduce inflammation (Hartmann et al., 2011).

1.1.7. Phenols

These phytonutrients comprise a large class that has been the subject of extensive research as a disease preventive. Phenols protect plants from oxidative damage and perform the same function for humans. Blue, blue-red and violet colorations seen in berries, grapes and purple eggplant are due to their phenolic content. Bilberries, for example, are high in phenolic anthocyanidins and are red in color. The outstanding phytonutrient feature of phenols is their ability to block specific enzymes that cause inflammation. They also modify the prostaglandin pathways and thereby protect platelets from clumping (Crozier et al., 2006).

The plants known as having medicinal values are rich in secondary metabolites which include alkaloids, glycosides, amines, steroids, terpenes, flavonoids, polyphenols and relative active metabolites are of great medicinal value and have been extensively used in drug and pharmaceutical industry (Atal and Kapur, 1982). Medicinal plants are believed to be an important source of new substances with potential therapeutic effects. Many plants synthesize substances that are useful to the maintenance of health in humans and other animals. They include aromatic substances, most of which are phenols (or) their oxygen substituted derivatives such as tannins. Herbal therapy is used to treat a large variety of ailments and symptoms, e.g., inflammation, fever and pain; however, there are no adequate experimental evidences about their effectiveness.
Based on importance of Indian medicinal plants in herbal medicines, *Delonix elata* is selected for the present study.

*Delonix elata* is a deciduous tree about 2.5-15 m tall commonly known as white gold mohur (Vadhanarayana in Tamil), with a spreading, rather rounded crown, crooked poor stem form and drooping branches. Bark smooth, shining; sometimes flaking. The genera comprises of 3 tropical species. D. elata is a varied species; two variants are recognized in east Africa. *Delonix elata* (Caesalpiniaceae) is a small sized tree found in Gujarat, Western eninsula and Southern India. Trunk of the tree is smooth, ash coloured, leaves compound, rachis 15-30 cm long, bipinnate, leaflets 10-20 pairs, flowers yellowish white in terminal corymbiform racemes, pods small, 12-18 cm long, seeds 4-8.

*Delonix* is native to Madagaskar, later introduced and naturalized in India, commonly known as “Sandesaro”. The leaves of which are used both internally and for external application in cases of inflammatory joints by applying paste or by taking the expressed juice by local people. Medicated oil prepared from the leaves is marketed under the name of “Vathanarayana”. Leaves are used as a folklore remedy for inflammatory joint disorders. Delonix is from the Greek word “delos”, meaning evident and “onux”. Delonix is a genus of flowering plants in the pea family, Fabaceae, subfamily Caesalpinioideae. By far the best known species is the Royal Poinciana (*D. regia)*.

*Delonix* (Gamble, J.S. 1986), a genus of tribe Eucaesalpiniea consists of two species growing in India (*Delonix elata* and *Delonix regia*). *Delonix elata* Gamble (Gamble, J.S. 1986, Matthew, K.M. 1983, Kirtikar, K.R. and Basu, B.D. 1984) (Syn. Poinciana *eLata* Linn.) is an erect tree, 20-30 feet, high, reported to occur wild in some parts of Kathiwar and South India and frequently planted as an avenue tree. Bark is tolerably smooth and ash-colored. Leaves abruptly 2-pinnate, 10-20 cm. long; main rachis slender; pinnae 4-8 pairs, opposite. Leaflets 10-20 pairs, subsessile, 3 by 8 mm, closely set along the rachis, linear-oblong, rounded and usually apiculate at the apex, glabrous, caducous. Flowers in terminal few flowered corymbiform racemes; pedicels stout, finely pubescent. Calyx 2-2.5 cm. long, coriaceous, silkypubescent outside; segments linear-oblong, acute. Petals suborbicular, yellow, scarcely exerted,
the upper a little smaller and of a deeper color than the others, the margins of all much curled. Filaments often 6.3 cm long. Villous and thickened at the base. Pods 12.5-18 by 2-2.3 cm, attenuated at both ends, reticulately veined, glabrous.

The generic name is derived from the Greek words (delos), meaning "evident," and (onyx), meaning "claw," referring to the petals. The common name, Poinciana, comes from a former genus of the same name in which the members of the current genus Delonix were classified along with plants now placed in the genus Caesalpinia. A claw in allusion to the shape of the petals; the epithet “elata” means lofty or tall. A pychosomatic medicinal use relating to scorpion bite treatment is reported from India. The leaf and bark in the form of paste is used by local people to reduce inflammation and pain.

**Taxonomy**

- **Current name**: Delonix elata
- **Authority**: (L.) Gamble
- **Family**: Fabaceae - Caesalpinioideae

**Synonym(s)**

- Caesalpinia elata (L.) Sw.
- Poinciana elata L.

**Common names**

- **English**: Creamy peacock flower, flamboyant tree, tiger bean, white, gulmohur
- **French**: Flamboyant
- **Gujarati**: Sandesra
- **Swahili**: mseele
- **Tamil**: padenarayan, pandenarayan, vadhanarayana

**Functional uses**

**Fuel**

D. elata is very promising as a firewood source having high density, calorific value and carbon percentage, and low silica and nitrogen.
Timber

The wood weighing 90 kg/cu. ft after seasoning, is yellow, even-grained and easily worked. It is suitable for cabinet work, carvings and utensils.

Gum

The tree yields a dark coloured, mucilaginous gum.

Medicine

The leaf extracts are anti inflammatory, a root decoction is drunk for abdominal pains.

1.2. MATERIALS AND METHODS

1.2.1. Plant materials

The leaves of *Delonix elata* were collected from Kolli hills, Namakkal District.

1.2.2. Preparation of extracts for phytochemical analysis

The plant material was allowed to shadow dry and afterwards pulverized by using mortar and pestle. 10 grams pulverized material were dissolved in 100 ml of solvent (Methanol, ethanol, acetone and water) and kept in a shaker for overnight. The obtained extracts were filtered with Whatmann No.4 filter paper and the filtrate was collected and used for analysis (Kokate, 1994).

1.2.3. Test microorganisms

Fresh cultures of the microorganisms were grown in nutrient broth. The density of microorganisms was adjusted to Mc Farland 0.5 standard. The invitro antimicrobial activity was performed by agar disc diffusion method against bacterial viz. *Staphylococcus aureus, Bacillus subtilus, Klebsiella, E.coli, Proteus sp.* and *Pseudomonas sp.* and fungi viz. *Aspergillus Niger, Pencillium sp., Candida albicans* respectively.

1.2.4. Preliminary Phytochemical analysis

1.2.4.1. Detection of carbohydrates

A minimum amount of the extract was dissolved in 5 ml of distilled water and filtered. The filtrate was subjected to Molisch’s test to detect the presence of carbohydrates.
1.2.4.1.1. Molisch’s test
The minimum amount of extract was treated with 2 to 3 drops of 1 percent alcoholic alpha-naphthol and 2 ml of concentrated sulphuric acid. This was added along the sides of the test tube. Formation of a violet ring at the junction of two layers will indicate the presence of carbohydrates.

1.2.4.1.2. Fehling’s test
The minimum amount of extract was treated with 1 ml of Fehling’s solution and heated. Formation of a reddish orange precipitate will indicate the presence of reducing sugar.

1.2.4.1.3. Benedict’s test
The minimum amount of extract was treated with 1 ml of Benedict’s solution and heated. Formation of a reddish precipitate will indicate the presence of reducing sugar.

1.2.4.1.4. Barford’s test
The minimum amount of extract was treated with 1 ml of Barford’s solution and heated. Formation of a reddish precipitate will indicate the presence of monosaccharide.

1.2.4.2. Detection of alkaloids
A small quantity of the extract was separately treated with few drops of dilute hydrochloric acid and filtered. The filtrate was used for the following tests. The minimum amount of extract was treated with Mayer’s reagent. Cream color precipitates if obtained with the aqueous extracts, will indicate the presence of alkaloids. The minimum amount of extract was treated with dragendorff’s reagent. Reddish brown precipitate, if obtained, will indicate the presence of alkaloids.

1.2.4.3. Detection of phytosterols
A small quantity of the aqueous extract was dissolved in 5 ml of chloroform separately. Then these solutions were subjected to Libermann Buchard test for the detection of phytosterols.
1.2.4.3.1. Libermann burchard’s test

The chloroform solution was treated with few drops of concentrated sulphuric acid followed by 1 ml of acetic anhydride solution. Purple color change was observed. It showed the presence of phytosterols.

1.2.4.4. Detection of gums and mucilage

Add about 10 ml of aqueous extract slowly to 25 ml of absolute alcohol with constant stirring. Filter the precipitate and dry in air. Examine the precipitate for its swelling properties and for the presence of carbohydrates.

1.2.4.5. Detection of Saponins

Dilute 1 ml of alcoholic and aqueous extracts separately with distilled water to 20 ml and shake in a graduated cylinder for 15 minutes. A one centimeter layer of foam indicates the presence of saponins. The saponins content was classified as follows. No froth = Negative; Froth less than 1 cm = weakly positive; Froth 1.2 cm high = Positive and froth greater than 2 cm high = strongly positive (Segelman and Farnsworth, 1969).

1.2.4.6. Detection of proteins and free amino acids

Dissolve small quantities of extracts in a few ml water and subject the solution to Biuret, Ninhydrin and Xanthoproteic tests.

1.2.4.7. Detection of phenolic compounds and tannins

Small quantity of the aqueous extract was dissolved in water and tested for the presence of phenolic compounds and tannins with dilute ferric chloride solution (5%), 1 percent solution of gelatin, containing 10 percent sodium chloride, 10 percent lead acetate and aqueous bromine solution. Formation of a white precipitate will show the presence of phenolic compounds and tannins.

1.2.4.8. Detection of flavonoids

5 ml of dilute ammonia solution were added to the extract of each sample followed by addition of concentrated sulphuric acid. A yellow coloration was observed and it indicates the presence of flavonoids.
1.2.5. Antibacterial assay

The antibacterial activity of the extracts was determined by the disc diffusion method. Briefly, overnight bacterial cultures were diluted in the Mueller-Hinton broth (O.D. 600=0.08) to obtain a bacterial suspension of 10^8 CFU/ml. Petri plates containing 20 ml of Mueller-Hinton agar media were inoculated with 200 μl of diluted cultures by the spread plate technique and were allowed to dry in a sterile chamber. The test samples were applied on sterile paper discs (6 mm diameter) and placed on the inoculated agar surface. A 20 μl of the extracts (100 mg/ml) were loaded on to the filter paper discs and were allowed to dry completely. Standard antibiotic Streptomycin 10 μg/disc was placed as standard. Plates were incubated at 37° C for 24 h. The antibacterial activity was assessed by measuring the inhibition zone. All the tests were performed in triplicate.

1.2.6. Antifungal assay

For the evaluation of antifungal effects, PDA medium was incubated with fungal cells. The plates were incubated for 3 days at 25° C. Further processes were repeated as above mentioned.

1.2.7. GC-MS Analysis of Samples

The methanolic extract of Delonix Elata was analyzed in GC-MS for identification of different phytocomponents.

1.2.7.1. GC Programme

Column: Elite-5MS (5% Diphenyl / 95% Dimethyl poly siloxane), 30 x 0.25mm x 0.25m df
Equipment: GC Clarus 500 Perkin Elmer
Carrier gas: 1ml per min, Split: 10:1
Detector: Mass detector Turbo mass gold-Perkin Elmer
Software: Turbomass 5.2
Sample injected: 3l

Oven temperature Programme

110° C -2 min hold
Up to 200° C at the rate of 10 °C/min-No hold
Up to 280 °C at the rate of 5° C / min-9 min hold
Injector temperature 250° C
Total GC running time 36 min
1.2.7.2. MS Programme

Library used NIST Version-Year 2005
Inlet line temperature 200° C
Source temperature 200 ° C
Electron energy: 70 eV
Mass scan (m/z): 45-450
Solvent Delay: 0-2 min
Total MS running time: 36 min

1.3. RESULTS AND DISCUSSION

Phytochemical analysis revealed that Methanol, ethanol, acetone and aqueous extracts of *Delonix elata* leaves contains alkaloids, flavonoids, phytosterol, saponins, tannins and phenolic compounds (Table 1).

**Table 1: Phytochemical (Qualitative) analysis of Leaf extracts of Delonix elata**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Phytochemical</th>
<th>Test</th>
<th>Organic solvent</th>
<th>Aqueous extract</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Acetone extract</td>
<td>Ethanol extract</td>
</tr>
<tr>
<td>1</td>
<td>Carbohydrates &amp; Glycosides</td>
<td>A. Molish’s</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B. Fehling’s</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C. Benedict’s</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D. Barford’s</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Alkaloids</td>
<td>A. Mayer’s</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B. Hagner’s</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C. Wagner’s</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Phytosterol</td>
<td>Libermann burchard</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Gums &amp; Mucilages</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Saponins</td>
<td>Foam test</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Protein &amp; Amino acids</td>
<td>A. Biuret</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B. Ninhydrin</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C. Xanthoproteic</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Phenolic compounds</td>
<td>Ferric chloride</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Flavonoids</td>
<td>A. Aqueous NaoH</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B. con H2So4</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Carotenoids</td>
<td></td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>
Table 2: Anti-Bacterial activity of Delonix elata leaves in various extracts

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Test micro organism</th>
<th>Diameter of Zone Inhibition (mm)</th>
<th>Standard</th>
<th>Negative control</th>
<th>Methanolic extract</th>
<th>Ethanolic Extract</th>
<th>Acetone extract</th>
<th>Aqueous extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Staphylococcus aureus</td>
<td>20</td>
<td>-</td>
<td>18.5</td>
<td>16</td>
<td>11</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Bacillus subtilis</td>
<td>17</td>
<td>-</td>
<td>15</td>
<td>15</td>
<td>6</td>
<td>5.3</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Klebsiella</td>
<td>15</td>
<td>-</td>
<td>18</td>
<td>20</td>
<td>8</td>
<td>6.8</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>E.coli</td>
<td>15</td>
<td>-</td>
<td>21</td>
<td>36</td>
<td>8.4</td>
<td>7.2</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Proteus</td>
<td>15</td>
<td>-</td>
<td>15</td>
<td>30</td>
<td>11</td>
<td>8.2</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Pseudomonas</td>
<td>16</td>
<td>-</td>
<td>16.5</td>
<td>11</td>
<td>26</td>
<td>7</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean of three replicates

Negative Control: Distilled water

Concentration: 1 mg/ml

Standard: Streptomycin 10 μg/disc

Table 3: Anti-Fungal activity of Delonix elata leaves in various extracts

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Test micro organism</th>
<th>Diameter of Zone Inhibition (mm)</th>
<th>Methanolic extract</th>
<th>Ethanolic Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aspergillus niger</td>
<td>12</td>
<td>10.8</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Pencillium sp.,</td>
<td>11.2</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Candida albicans</td>
<td>14.2</td>
<td>11.2</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean of three replicates

Concentration: 1 mg/ml

Negative Control: Distilled water
Since earlier studies on phytochemicals reported the antibacterial activity of terpenoids, saponins, tannin, alkaloids and flavonoids isolated from plant materials (Mahmoud et al., 1999; Tsuchiya et al., 1996). The presence of phytochemicals in this study might be a factor that is responsible for the antibacterial activity of Delonix elata leaves. In the present study the maximum activity was observed against all the species using Delonix elata leaves. Thus, these plants can be useful, seems to be a potential source for arresting the growth and metabolic activities of various general bacteria and fungi (Table 2 & 3). The exact dosage concentration and the synergistic antimicrobial activity of Delonix elata leaves need to studied further.

Table 4: GC-MS Analysis of methanolic leaf extract of Delonix elata

<table>
<thead>
<tr>
<th>No.</th>
<th>RT</th>
<th>Name of the compound</th>
<th>Molecular formula</th>
<th>MW</th>
<th>Peak Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>4.96</td>
<td>Undecane, 2-methyl-</td>
<td>C12H26</td>
<td>170</td>
<td>12.66</td>
</tr>
<tr>
<td>2.</td>
<td>5.55</td>
<td>Undecane, 3,7-dimethyl-</td>
<td>C13H28</td>
<td>184</td>
<td>12.40</td>
</tr>
<tr>
<td>3.</td>
<td>8.23</td>
<td>Benzoic acid, 4-ethoxy-, ethyl ester</td>
<td>C11H14O3</td>
<td>194</td>
<td>29.02</td>
</tr>
<tr>
<td>4.</td>
<td>11.62</td>
<td>3,7,11,15-Tetramethyl-2-hexadecen-1-ol</td>
<td>C20H40O</td>
<td>296</td>
<td>7.65</td>
</tr>
<tr>
<td>5.</td>
<td>13.09</td>
<td>Phthalic acid, butyl octyl ester</td>
<td>C20H30O4</td>
<td>334</td>
<td>4.22</td>
</tr>
<tr>
<td>6.</td>
<td>14.98</td>
<td>Ethyl iso-allocholate</td>
<td>C26H44O5</td>
<td>436</td>
<td>5.01</td>
</tr>
<tr>
<td>7.</td>
<td>24.39</td>
<td>Rhodopin</td>
<td>C40H58O</td>
<td>554</td>
<td>5.54</td>
</tr>
<tr>
<td>8.</td>
<td>27.21</td>
<td>Astaxanthin</td>
<td>C40H52O4</td>
<td>596</td>
<td>10.03</td>
</tr>
<tr>
<td>9.</td>
<td>31.18</td>
<td>Stigmasterol</td>
<td>C29H48O</td>
<td>412</td>
<td>5.28</td>
</tr>
<tr>
<td>10.</td>
<td>32.45</td>
<td>á-Sitosterol</td>
<td>C29H50O</td>
<td>414</td>
<td>8.18</td>
</tr>
</tbody>
</table>

The GC-MS analysis revealed the presence of Undecane, 2-methyl-, Undecane, 3,7-dimethyl-, Benzoic acid, 4-ethoxy-, ethyl ester, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, Phthalic acid, butyl octyl ester, Ethyl iso-allocholate, Rhodopin, Astaxanthin, Stigmasterol and á-Sitosterol (Table 4). The GC-MS analysis also reveals that substantial amount of Astaxanthin in Delonix elata which is a potent antioxidant agent.
1.4. CONCLUSION

*Delonix elata* is used in the treatment of various diseases by local folks. Since, these plants possess many medicinal properties; the present study was designed to evaluate the phytochemicals and the antimicrobial activity of leaf extract of *Delonix elata*. The invitro antimicrobial activity was performed by agar disc diffusion method against bacterial viz. *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella*, *E.coli*, *Proteus* sp. and *Pseudomonas* sp. and fungi viz. *Aspergillus Niger*, *Pencillium* sp., *Candida albicans*. The organic extracts especially methanolic extract, showed maximum against the micro organism. The presence of broad range of phytochemicals in *Delonix elata* can certainly serve as an effective natural herbal source against inflammatory diseases.

Screenings of plant extract (*Delonix elata*) and plant products for antimicrobial activity have shown that higher plants represent potential sources of new-anti-infective agents. The organic extraction of plants (especially methanolic extract) greater activity than aqueous extract. Hence the study suggests that the organic solvent especially methanolic solvent is suitable to screen for the antibacterial activity. The result of present study reveals that the extract of plant exhibited potential antibacterial activity against the tested pathogens. The study also supports the view that several medicinal plants might be useful as antimicrobial agent. In the present study the notable activity was observed against all tested micro organisms. This shows that the plant can be used for medicinal purposes.