Palynological and comparative Antimicrobial studies of selected Medicinal Plants

BY

ZULQARNAIN

Department of Plant Sciences
Quaid-i-Azam University, Islamabad
Pakistan
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BY

ZULQARNAIN

Department of Plant Sciences
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بسم الله الرحمن الرحيم

DECLARATION

The whole of the experimental work including described in this thesis was carried out by me in the Plant Taxonomy Laboratory, Department of Plant Sciences, Quaid-i-Azam University, Islamabad. The findings & conclusion are of my investigation with discussion of my supervisor Associate Prof. Dr. Rizwana Aleem Qureshi. No part of this work has been presented for any other degree.

ZULQARNAIN
(Signature of the student)

I confirm that the above statement is correct. I have found that this thesis is completed and satisfactory in all respects and that any/all revisions required by the final examining committee have been made.

Associate Prof. Dr. Rizwana Aleem Qureshi
(Signature of Supervisor)

APPROVAL CERTIFICATE

This thesis submitted by, **Mr. Zulqarnain** is accepted in its present form by the Department of Plant Sciences, Quaid-i-Azam University, Islamabad as satisfying the thesis requirement for the degree of Master of Philosophy in the Plant Taxonomy.

Supervisor : _____
Associate Prof. Dr. Rizwana Aleem Qureshi

External Examiner : _____
Prof. Dr. Rehana Asghar

Chairperson : _____
Prof. Dr. Mir Ajab Khan

Dated: 15-07-2008

DEDICATION

DEDICATED TO BELOVED PARENTS, BROTHERS AND SISTERS.

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Abstract

Eleven crude extracts in methanol with four dilution of each medicinal plants, used in ethnomedicine to treat common infectious were screened out for Antimicrobial activities against two Gram positive (*Staphylococcus aureus, Micrococcus luteus*) and Gram negative (*Escherichia coli, Pseudomonas auregenosa*). Most of the plants showed antibacterial activities against Gram positive bacteria but have no effect on Gram negative bacteria except few plants that displayed activity against *Pseudomonas auregenosa.* Extract from *Phyllanthus emblica* L. leaves have been the most active among the 11 plants. Comparing the results with the standard Ampicillin and Chloramphinicol, both the standard have greater zone of inhibition against *Escherichia coli* and *Staphylococcus aureus* than the plant extracts.

In Palynological studies all the pollen grains were monad, psilate and tri-colpate except in *Adhatoda vasica* Nees where bicolpate pollen was observed and *Lantana camara* L. in which the pollen was tetra-colpate. In *Adhatoda vasica* Nees shape in polar view was circular as well as tubular while in *Lantana camara* L. shape in polar view was rectangular. Nearly all the pollens were subulate to prolate to sub-prolate in equatorial view. The largest polar diameter 54 µm was observed in *Bauhenia variegata* L. and smallest 16.25 µm in *Debregeasia salicifolia* (D.Don) Rendle. The highest percentage fertility 96 was observed in *Olea ferruginea* Royle and lowest 80 in *Caryopteris grata* Benth. *Pinus roxburghii* Sargent has a bisaccate (or vesiculate) pollen grain consisting of a body with two latarally-placed bladders (sacca, vesicles), which is the characteristic of the Gymnosperms.

CHAPTER 01

INTRODUCTION

INTRODUCTION

1.1 MEDICINAL PLANTS

Since a long time, mankind has been developing throughout the world a traditional medicine based on the knowledge of medicinal plants. This knowledge got enriched over numerous generations due to experimentation but also through observation of animal behaviors. It represents for the local population possibility of simple and cheap treatment. In addition it is a source of potentially important new pharmaceutical substances.(Diehl *et al.*, 2004)

It is estimated that there are 250, 000 to 500,500 species of plants on Earth (Borris, 1996). A relatively small percentage (1 to 10%) of these is used as food by both humans and other animal species. It is possible that even more are used for medicinal purposes. Hippocrates (in the late fifth century B.C) mentioned 300 to 400 medicinal plants (Schultes, 1978).

Mainstream medicine is increasingly receptive to the use of antimicrobial and other drugs derived from plants, as traditional antibiotics (products of microorganisms or their synthesized derivatives) become ineffective and as new, particularly viral, diseases remain intractable to this type of drug. Another driving factor for the renewed interest in plant antimicrobials in the past 20 years has been rapid rate of plant species extinction (Lewis, 1995). There is a feeling among natural products chemists and microbiologists alike that the multitude of potentially useful phytochemical structures which could be synthesized chemically is at risk of being lost irretrievably (Borris, 1996). There is a scientific discipline known as ethnobotany (or ethnopharmacology), whose goal is to utilize the impressive array of knowledge assembled by indigenous people about the plant and animal products they have used to maintain health (Vaden Berghe *et al.*, 1986).

Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives (Geissman, 1963). Most are secondary metabolites, of which at least 12000 have been isolated. These plant

substances serve as plant defense mechanism against predation by microorganisms, insects and herbivores.

About one quarter of the prescription drugs dispensed by community pharmacies in the United States contain at least, one active ingredient derived from plant material (Fransworth 1976). The Flavonols, phenolic acids are particularly attractive as they are known to exhibit various beneficial pharmacological properties such as vasoprotective, anticarcinogeics antiviral, anti-inflammatory, antinepplastics as well as antiallergic and antiroliferative activity on tumor cells. (Carr *et al.*, 2000).

Bacteria have been the worst human diseases causing organism (Ritter *et al.*, 1996). Generally, the most prevalent human pathogens are bacteria, viruses and some fungi, protozoan or parasitic worms (Ritter *et al.*, 1996). Many plant extract and essential oil isolated from plants have been shown to exert biological activity in vitro and vivo, which justified that research on traditional medicine, was focused on characterization of antimicrobial activities of these plants (Marinez *et al.*, 1996). While a number of synthetic and natural antibacterial agents are available for controlling bacterial infections increased resistance calls for new antibacterial drugs, one source of which are traditional medicinal plants (Greenwood. 1982).

1.2 PALYNOLOGY

The term *palynology* was introduced by Hyde and Williams in 1944, following correspondence with the Swedish geologist Antevs, in the pages of the Pollen Analysis Circular (one of the first journals devoted to pollen analysis, produced by Paul Sears in North America). Hyde and Williams chose *palynology* on the basis of the Greek words *paluno* meaning 'to sprinkle' and *pale* meaning 'dust' (and thus similar to the Latin word *pollen*). (Hyde & Williams, 1944).

Pollen is the characteristic of seed plants, which include the Gymnosperms and the Angiosperms. They are the vehicles in which the male gamete carried genetic code to the female gamete. Pollen grains appear as yellow powder of the flower with naked eye. However light microscope and scanning Electron microscope exploit various types of sculpturing on its outer resistant coat (Exine) like spines, spores, grooves, reticulates etc and such variations provide a means of identifying a pollen grain of particular taxa. Thus Palynology can also helps in solving taxonomic problems concerned with the hybrid plants. Using light microscope the identification of pollen

grain is some times limited to family or generic level, but there are occasions when identification to species is possible. Palynological characters are useful in solving complicated problems of inter-relationship between various Taxa and assessment of their status in the classification, particularly with reference to the families, sub-families, tribes, genera, species and sub-species. As Palynological characters are usually specific for a particular Taxon, keys have been constructed for identification based purely on pollen characters (Jaffery, 1964).

1.2.1 History of Palynology

The earliest reported observations of pollen under a microscope are likely to have been in the 1640s by the English botanist Nehemiah Grew who described pollen, the stamen and successfully predicted that pollen was required for successful reproduction in plants. As microscopes began to improve further studies included work by Robert Kidston and P. Reinsch examined the presence of spores in coal and compared them to modern spores. (Jansonius, 1996). The early pioneers also included Christian Gottfried Ehrenberg (radiolarians and diatoms), Gideon Mantell (desmids) and Henry Hopley White (dinoflagellates). The earliest quantitative analysis of pollen was published by Lennart von Post who laid out the foundations of modern pollen analysis in his Kristiania lecture of 1916. Pollen analysis was initially confined to Nordic countries because many early publications were in Nordic languages. This isolation ended with the publication of Gunnar Erdtman's thesis of 1921 when pollen analysis became widespread throughout Europe and North America for use in studies of Quaternary vegetation and climate change. (Faegri, 1973).

1.3 DISCRIPTION OF THE PLANTS SPECIES

The description of the plants studied for the antibacterial assay and Palynology on the basis of ethnobotany is given below:

1.3.1 *Adhatoda vasica* Nees

Family: Acanthaceae

Local Name: Arusa, Adulsa, Bhekar

The plant is a small ever green,sub herbaceous bush which grows commonly in open plains and foot hills of Himalayans range upto 4000 ft. (Hakim.1956). *Adhatoda vasica* Nees. grows about 1.2-2.4 m high.the leaves are of

dark green colour above and pale yellow below, opposite, entire, lanceolate and shortly petiolate, tapering towards apex and base. The flowers are typical white or purple arranged in pedunculated spike with four seeded fruit and is small capsule.

The plant part used are leaves, root, stem bark and flowers. The flowers and fruit are bitter, aromatic and antispasmodic. Fresh leaves, root and bark are applied on wounds, relative bronchial spasm and considered as remedy in asthma especially in combination with *belladonna* (Nadkarni, 1976). The leaves are useful in gonorrhea. Commonly used for bleeding due to idiopathic thrombocytopenic purpura, local bleeding due to peptic ulcer, piles and menorrhagia (Doshi, 1983).

The leaves contain an essential oil and alkaloids vasicine 45-95 %(the mucolytic drug bromhexine was developed from this alkaloid), N-oxides of vasicine, vasicinone, deoxyvasicine, oxyvasicine and maintone (http://www.globalherbalsupplies.com/herb-informaton/adhatoda-vasica.htm).

1.3.2 *Bauhenia variegata* Linn.

Family: Ceasalpinaceae

Local Name: Kachnar

Wild in Forrest of foothills Himalayans up to 1000 m. Locally found in Shakerparian, Gokina, Rawal lake. A medium sized tree with dark brown nearly smooth bark; young shoots pubescent. Leaves petiolate. Inflorescence few flowered pubescent raceme Pods hard, flat, dehiscent 10-15 seeded; stipe glabrous. (Ali and Nasir, 1973).

Bauhenia variegate L. is a medicinal plant (Warrier *et al.* 1993) traditionally used for varied purposes. The bark of this plant is described as astringent, alliterative, tonic and useful in scrofula, skin disease and ulcers. The root and bark are acrid, cooling, constipating, depurative, anthelminthic, anti-inflammatory and styptic. They are useful in diarrhea, dysentery, skin disease, leprosy, intestinal worms, wound, ulcers, tumors, coughs and diabetes (Warrier *et al.* 1993).

Genus *Bauhenia* contains terpenoids, alkaloids, steroids, triterpenes, tannin, quinines, bibenzyls and more frequently flavonoids (Silva and Cechinel Filho, 2002;

Apisantiyakom *et al.*, 2004; Athikomkulchai *et al.*, 2005; Maheswara *et al.*, 2005; Yadav and Bladoria, 2005; Zhao *et al.*, 2005).

The main chemical compounds isolated from the bark of *B. variegate* L.. are quercitroside, isoquercitroside and rutoside, myrecitol glycoside and kamferol glycoside (Duret & Pairs 1977). Gupta *et al.* (1979) isolated 5, 7-dihydroxyflavoanone 4-O-a-l rhamnopyranosyl-b-Dglucopyranoside (I) from the stem. The stem contains b-sitosterol, lupeol, kempferol-3-glucoside and 5, 7-dimethyl ether 4 rhamnoglucoside (Gupta *et al.* 1980). The stem barks of *B. variegata* L. yields four substances viz. hentriacontane, octacosanol, b-sitosterol and stigmasterol (Anandaprakash, 1978). Phytochemical studies on the stems (Gupta *et al.*, 1979, 1980, 1984), flowers (Rahman and Begum, 1966; Wahab *et al.*, 1987), and seeds (Yadava and Reddy, 2001) of this species have led to the isolation of several flavonoids.

1.3.3 *Bombax ceiba* Linn.

Family: Bombacaceae

Local Name: Simbal

Wild in sub Himalayans areas and in lower valleys up to 1400 m. Locally found in Shakerparian, Rumli, Shahdara, Rawal lake. Large deciduous tree with symmetrical branching pattern leaves palmate with 5-7, stalked leaflets. Flowers large, showy, clusterd towards the end of bare horizontally spreading branches. Fruit capsule, woody. Seeds embedded in white cottony hairs. (Nasir and Rafiq 1995, Sheikh 1993, Qaiser 1978).

Root powder is used as a tonic for impotency, for which 10 g powder is advised daily with a glass of milk. Powder of stem prickles is used to treat asthma; about 10 g (one spoonful) powder is taken with a glass of cow's milk/fresh water in the morning for 3/4 months. Seed paste with water is applied on small-pox boils. (Singh *et al.*, 2002) Half cup extract of bark and flower is given for 3 days to both men as well as women in sexual diseases like hydrocele, leucorrhoea, gonorrhea (Jain *et al.*, 2003).

Shamimin a new flavonol C-glycoside has been isolated as a pale yellow powder from the ethanolic extract of fresh, undried leaves of *Bombax ceiba* (Faizi S *et al.*, 1999).

Mangiferin, 2-β-D-glucopyranosyl-1,3,6,7-tetrahydroxy-9H-xanthen-9-one, obtained directly from methanolic extracts of *Bombax ceiba* leaves in substantial amounts demonstrated strong antioxidant activity (EC50 5.8±0.96 μg/ml or 13.74 μM) using DPPH assay comparable to rutin, commonly used as antioxidant for medical purposes (Dar *et al.*, 2005).

1.3.4 *Carissa opaca* Stapf ex Haines

Family: Apocynaceae

Local Name: Garanda

Foothills of Himalayans and adjacent plains up to 1400 m. Locally found through out. Ever green thorny shrub up to 3 m or more tall. Young shoots with milky juice. Spines arising between the petiole. Leaves opposite, glabrous, shinnig.The sweet scented white flowers are borne in terminal cymes. Fruit berry. (Nasir and Rafiq 1995, Nazimuddin and Qaiser 1983).

Root is grounded and put in worm infested sores of animals. Fruit and leaves are known as cardiac stimulants. Leaf decoction is used in asthma. Tannin is the major constituent in leaves and bark. The major constituent of bark and leaves is tannin. (Shinwari and Khan, 1998, Baquar 1989, CSIR 2003).

1.3.5 *Caryopteris grata* Benth.

Family: Verbenaceae

Distributed in outer and sub-Himalayan tracts. A straggling or rambling shrub, often purplish or brownish in colour.Leaves lanceolate or elliptic, Cymes short, axillary, 1.5-2 (-2.5) cm long. Flowers small, 5-6 mm across, white or purplish;. Capsules red when ripe. (Jafri and Ghafoor, 1974).

Caryopteris incana (Thumb.) Miq. one of the parents of the studied hybrid, has been used in China as a folk medicine for the relief of colds, coughs and rheumatic pains (Gao, 1997). The decoction of root and whole plant of *Caryopteris paniculata* are used for Diarrhea, skin itch, diminish inflammation, acesodyne (Long *et al.*, 2003).

1.3.6 *Debregeasia salicifolia* (D.Don) Rendle

Family: Urticaceae

Local Name: Puruni, Siaru, Siharu

In Pakistan, it is found in Swat, Salt range, Murree Hills, usually near springs and water courses. (Akbar *et al.*, 2000). Common in moist places in the Northern Himalayas and Salt range, up to 2000 m. A dioecious, evergreen tall shrub or small tree. Stem with dark brown fibrous bark scabrous, young shoots whitish tomentose. Leaves densely tomentose petiole; lamina oblong – lanceolate, deciduous. Male flower clusters larger than female flower heads. Achenes fleshy, yellow. (Stewart, 1972).

A new triterpene, 3_-19_-dihydroxy-30-norurs-12-ene (1), has been isolated from the methanolic extract of Debregeasia salicifolia stems. Besides, lupeol, sitosterol, stigmasterol and oleanolic acid are also reported for the first time from this species. (Akbar *et al.*, 2000).

1.3.7 *Lantana camara* Linn.

Family: Verbenaceae

Local Name: Panch Phuli

Weed of waste places, roadsides etc. in Pakistan, It is naturalized in Sindh, Punjab and Azad Kashmir. Locally found as invasive in Shakarparian, Rawal lake, Siadpur and Nurpur etc. Aromatic rambling or struggling, evergreen, shrub upto 1-3cm high with rough stems and branches. Stem with short hooked spines, 4-angled. Leaves simple opposite, ovate, usually crenate or toothed. Flowers small borne on a permanently capitates heads, yellow or orange. Fruit drupe. (Nasir and Rafiq 1995, Jafri and Ghafoor 1974).

The leaves of the plant are boiled like tea and the decoction is a remedy against cough (Watt *et al.*, 1962). A decoction of the plant is given as treatment for tetanus, rheumatism, and malaria. Pounded leaves are applied to cuts, ulcers and swellings; a decoction of the leaves is used as a lotion for wound. Anti-feedant, larval mortality/repellency, anti-fungal and antibacterial activities of extracts of *Lantana* leaves have been reported (Pandey *et al.*, 1977). Powdered roots with milk are given orally (5_/10 g per day) to children suffering from colic pain and stomachache.

Infusion of whole plant is used orally (20_/50 ml twice a day) for the treatment of bronchitis. Leaf decoction is used orally (50 ml at bed time) in treating constipation. (Singh *et al.*, 2002).

Eight triterpenoids, betulonic acid (3), betulinic acid (4), oleanolic acid (5), lantadene A (6),lantadene B (7), icterogenin (8), lantanilic acid (9), and ursolic acid (10), three flavonoids,hispidulin (11), pectolinarigenin (12), and pectolinarin (13), as well as β-sitosteryl-3-O-β-Dglucoside(2) and a mixture of campesterol (1a), stigmasterol (1b), and β-sitosterol (1c) were isolated from the leaves of the yellow flowering taxa of *Lantana camara* L.(Juang *et al.*, 2005).

1.3.8 *Melia azedarach* Linn.

Family: Meliaceae

Local Name: Drek

Wild in Himalayas up to 1700 m and distributed throughout the country. Locally found in Rawal Lake, Gokins and Saidpur. Deciduous tree up to 12 m tall with tomentose young shoots. Leaves pinnate, Leaflet opposite, acuminate. Flowers lilac, sweet-scented, Fruit drupe globose, yellow when ripe. (Abdulla 1972 b, Sheikh 1993).

Aqueous extract is useful in asthma, leaves and barks are used internally and externally in leprosy, leaf juice is used internally as diuretic, anthelmentic. Bark is used as cathartic and emetic. flowers are used as resolvent. Seeds are used in rheumatism. Gum used as remedy for spleenic enlargement. ripe fruit is used against diabetes. Bark of the root and stem is used for various skin diseases in the form of a powder mixed with mustard oil.Fried flowers are used as tonic and stomachic. Ripe fruits are used as purgative, insecticide and antiseptic.(Shinwari and Khan 1998, CSIR 2003). Tender leaves and shoots are eaten for blood purification. A decoction of the fresh leaves is a bitter tonic and an alternative in chronic malarial fever. Oil of seeds is used as an astringent, applied to cure leprosy and piles. Oil is also used to remove lice and pests from hairs (Singh *et al.*, 2002). *Melia azedarach* L. is used against intestinal worms, in skin diseases, stomach ache, intestinal disorders, uterine illnesses and cystitis, as diuretic and febrifuge. (Khan *et al.*, 2000).

The Chemical investigation on the products of the neem tree was extensively undertaken in the middle of the twentieth century. Since the early report by Siddiqui in 1942 on the isolation of nimbin, the first bitter compound isolated from neem oil, more than 135 compounds have been isolated from different parts of neem and several reviews have also been published on the chemistry and structural diversity of these compounds. (Koul *et al.*, 1990, Govindachari, 1992). The compounds have been divided into two major classes isoprenoids and others18. The isoprenoids include diterpenoids and triterpenoids containing protomeliacins, limonoids, azadirone and its derivatives, gedunin and its derivatives, vilasinin type of compounds and Csecomeliacins such as nimbin, salanin and azadirachtin.

The nonisoprenoids include proteins (amino acids) and carbohydrates (polysaccharides), sulphurous compounds, polyphenolics such as flavonoids and their glycosides,dihydrochalcone, coumarin and tannins, aliphatic compounds etc. The details of the chemistry of various compounds falling under these groups have already been reviewed (Kraus *et al.*, 1995, Devakumar *et al.*,1996). Only a few compounds whose bioactivity has been studied are presented here. As the pesticidal and antifeedant activities of azadirachtin and the related compounds have been reviewed earlier (Govindachari *et al.*, 1992). A new triterpenoid, 1a,7a diacetoxyapotirucall-14-ene-3a, 21, 22, 24, 25-pentaol -1 and the two known compounds odoratone -2. and 2b, 3b, 4b-trihydroxypregnan-16-one -3 were isolated from a methanolic extract of the seed kernels of Azadirachta indica.(Luo *et al.*, 2000). Two new degraded limonoids, azedararide and 12-acetoxy fraxinellone, were isolated together with two known degraded limonoids, fraxinellone and fraxinellonone, as ichthyotoxic substances from the root bark of *Melia azedarach* L. Their structures were elucidated by spectroscopic and chemical means. (Nakatani *et al.*, 2001).

1.3.9 *Olea ferruginea* Royle

Family: Oleaceae

Local Name: Kahu/Kao

Warmer foothills tracts from 450-2000 m forming climax with *Acacia modesta*. Locally found in Saidpur, Suban, Rawal lake, Nurpur. Medium sized ever green tree, leaves opposite, simple smaller, oblong-lanceolate, entire. Flower whitish

bisexual,panicle about as long as leaves. Fruit drupe. (Grohman 1974, Sheikh 1993, Nasir and Rafiq 1995).

The leaves and bark are bitter, astringent, antiseptic, antiperiodic and diuretic.Oil obtained from fruit is used as rubefacient. Fruit is used as antidiabetic. Root is useful for asthma.The major constituent of pulp and seeds is oil. (Baquar 1989, Shinwari and Khan 1998, CSIR 2003).

The distribution and biosynthesis of iridoid glucosides in the Oleaceae is reviewed and five distinct biosynthetic pathways to iridoids have been identified in the family, deoxyloganic acid apparently being a common intermediate. Likewise, the distributions of caffeoyl phenylethanoid glycosides (CPGs), i.e. verbascoside and its analogues, as well as cornoside are listed. Iridoid glucoside data exist for 17 genera of Oleaceae and the occurrence of iridoids from the different biosynthetic pathways correlate extremely well with the phylogenetic classification inferred from recent chloroplast DNA sequence data.The two tribes Jasmineae and Oleeae (the remaining genera) both contain iridoids from the oleoside pathway.(Jensen *et al.*, 2002).

1.3.10 ***Phyllanthus emblica*** Linn.

Syn: ***Emblica officinalis*** Gaertn.

Family: Euphorbiaceae

Local Name: Amla

Wild in sub Himalayan tracts from 900-1000 m and cultivated in plains distributed in Nurpur, Saidpur, Subban. Deciduous tree up to 15 m tall with pendant branches. Leaves simple, alternate, leaf blade linear oblong. Flower unisexual small. Male flower yellowish green.Female flower with stouter pedicels. Fruit succulent, subglobose, pale green. (Sheikh 1993, Nasir and Rafiq 1995).

Root and bark is astringent, leaves and bark are used as poultice in cutaneous diseases.Flowers are used as refrigerant. Powder obtained from the fruit is used as purgative and certain parasitic skin diseases, also employed in hemorrhage, diarrhea, dysentery, jaundice, dyspepsia, anemia and asthma. (Baquar 1989, Shinwari and Khan 1998, NIH 2003). Bark decoction, 20 ml twice daily for 2/3 days, is used for treating diarrhoea, dysentery and cholera. Bark powder mixed with water is applied on sores

and pimples. Fruits are used in the Ayurvedic medicine 'triphala' as one of the ingredients (Singh *et al.*, 2002). Traditionally used in the treatment of asthma, sore throat, vomiting, cough, diarrhea, bleeding piles, gout and heart and bladder diseases (Kirtikar, 1935).

Glutamate is to be considered a nociceptive neurotransmitter and glutamatergic antagonists present antinoceptive activity. In this study we investigated the effects of the naturally occurring antinociceptive compounds rutin, geraniin and quercetine extracted from *Phyllanthus* (Martini *et al.*, 1999). Its fruit is reputed to probably have the highest content of vitamin C compared with any other naturally occurring substances in nature.
(Pornpimon *et al.*, 2007). Flavonoids from *Emblica officinalis* and *Mangifera indica* effectively reduce lipid levels in serum and tissues of rats induced hyperlipidemia.(Anila *et al.*, 2001). Various chemical compounds have been extracted from *P.emblica* such as flavonoids, alkaloids, triterpenes (Subramanian *et al,*. 1971).

1.3.11 *Pinus roxburghii* Sargent

Family: Pinaceae

Local Name: Chir

Wild in sub Himalayan tracts from 600-1800 m forming pure stands.Locally found in Rawal lake, Pirsohawa, Margalla top and Shakarparian. Tree up to 30 m tall with a soft flaky bark. Leaves in clusters. Male cone long yellowish in dense tetrminal clusters. Female cone solitary at the tip of branches. Mature ones woody, bracts and scale distinct, seeds winged. (Nasir and Rafiq 1995, Sheikh 1993).
Wood is used as refrigerant and expectorant. Resin is used in snake bite and scorpion bite.Oil from resin acts as expectorant and is useful in chronic bronchitis and gangrene of the lungs. Male cones is used in antidibetic formulation.

Crude extracts of water and solvent extractable tannin fractions from pine needles were found to contain tannin concentrations of 10.15% and 13.15% tannic acid equivalents respectively. Thin Layer Chromatography revealed the presence of four distinct phenolic compounds,amongst which two were tannic acid like compounds. Both the extracts were found to be inhibitory to several microbes of agricultural importance.(Selvakumar *et al.*, 2007).

CHAPTER 02

REVIEW OF LITERATURE

REVIEW OF LITERATURE

2.1 MEDICINAL PLANTS

Due to adverse effects of chemical substances as antimicrobial agents, the medicinal plants are now considered to be effective alternative antibiotic and have gained importance, especially to combat disease problems both in human beings and plants. A classical example is Neem (*Azadirachta indica*). Every part of the neem plant bark, root bark, young fruit, nuts or seeds, flowers , leaves, gum and sap, all possess medicinal properties (Akilandesusari *et al*., 2003).

Brantner and Grein (1994) studied 28 plant families selected on the basis of medicinal folklore reports and literature data, for their antibacterial activity. When used externally, the results indicated that about 60 % of the plant extracts tested exhibited some level of antimicrobial action.

Sakanaka *et al*., (2000) studied inhibitory action of green tea poly phenol towards the development and growth of bacteria. Tea polyphenol showed antibacterial effects towards *Bacillus stearothermophilus*, which are thermophilic bacteria.

Coe (1994) reported antimicrobial potential of Chile peppers used as a food item found nearly ubiquitously. Besides this, the Chile peppers contain Vitamin C, provitamin A and E and several B vitamins are also found in Chile pepper.

Cichewiez and Thrope (1996) found that capsaicin a turpenoid found in Chile peppers might enhance growth of *Candida albicans* but it clearly inhibits various bacteria growth to certain extant.

Oliver-Bever (1986) worked on Papaya (*Carica papaya*), which yields a milky sap, often called latex, which is a complex mixture of chemicals. Chief among them is papain, which is a proteolytic enzyme. Osato *et al*., (1993) found that latex of Papaya is bacteriostatic to *Bacillus subtilis, Enterobacter cloacae, Escherichia coli, Staphylococcus aureus and Proteus vulgaris.*

Thomson (1978) reported that any part of the plant may contain active components e.g. roots of Ginseng contains active saponins and essential oils, leaves of Eucalyptus contain essential oils and tannins. Some trees yield useful substances in their bark, leaves and shoots.

Zhang and Lewis (1997) were of the view that initial screening of the plants for antimicrobial activities begun by using crude aqueous or alcoholic extraction and can be followed by various organic extraction methods. Since nearly all of the identified components from plants, active against the microorganisms are aromatic or saturated organic compounds. Therefore they are most often obtained through initial methanolic or ethanoloic extraction. The exceptional water soluble compounds such as polysaccharides, starch or polypeptides are commonly more effective as inhibitors of pathogen adsorption, usually effective against viruses.

According to Zhang and Lewis (1997) aqueous extraction is recommended for anthocyanins, starches, tannins, saponins, polypeptides and lectins. Ethanolic extraction is best for extraction of tannins, polyphenols, polysaccharides, flavonol, terpenoids, saponins, tannins, xanthophylls, totarol, quassinoids, lactones, flavones, phenones and polyphenols.

Meral and Karabay (2002) extracted plant materials for *in vitro* antimicrobial activity of three Hypericum species from West Anatolia. Methanol at 80 C was used for soxhlation for extraction of powdered plant material. *In vitro* antibacterial studies were carried out by disc diffusion method, three out of four Hypericum species were found to be antibacterial against both gram positive and gram negative organisms.

Batistata *et al.*, (1994) were of the view that in vitro antimicrobial screening of plant materials may be performed with pure substances or crude extracts. The two most commonly used screens to determine antimicrobial susceptibility is broth dilution assay (Ayafor, 1994) and tha disc or agar well diffusion assay (Narvarro, 1996). After initial screening of phytochemicals, more detailed studies of their antibiotic effects should be conducted. In such cases more specific media can be

used and MICs can be effectively compared to those of a wider range of currently used antibiotics (Tsuchiya *et al.*, 1996).

Loganathan *et al.*, (2001) investigated phytochemical and antimicrobial activities of extracts of plant root of *Achyranthes aspera* Linn. They used two solvents i.e., chloroform and ethanol 95 % for extraction of active principles from dried powdered plant roots. The phytochemical tests of the extracts revealed the presence of carbohydrates, alkaloids, phytosterols and glycosides (saponins). Antimicrobial activity of these extracts was studied against four gram positive (*Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus albus and Bacillus subtillus*) and four gram-negative (*Escherichia coli, Klabsiella aerogenes, Proteus vulgaris and Pseudomonas aeruginosa*). The zones of inhibition were compared with standard antibiotic (Gentamcycin 10 gm) under similar conditions. Both the extracts exhibited marked effectiveness against Gram-positive and gram negative test organisms.

Ogundipe and Oladipo (2001) studied phytochemical and antimicrobial potential of *Persea americana* Mill. Using its bark and leaf as source materials. The phytochemical screening confirmed that the leaves and bark contained tannins, saponins, falvanoids and anthocyanin. The antimicrobial assay of the leaf Ethanolic and aqueous extracts showed inhibitory effects on *Staphylococcus aureus, Pseudomonas aeruginosa and Shigella flexneri.* Whereas there was no inhibitory effects obsereved on *Escherichia coli* and *Bacillus subtillus.* The antimicrobial or otherwise effects exhibited against these organisms are attributed the organic compounds in these plant materials.

Water extract of garlic and clove possesses antimicrobial activity. Some bacteria showing resistance to certain antibiotics were sensitive to extract of both garlic and clove. On the other hand, water extract of Miswak (*Salvedora persica*) roots and stem contain potential antimicrobial anionic components such as chloride, sulphate, thyocyanate, and nitrate. (Darout *et al.*, 2000).

The decoction of the six of the studied Jordanian medicinal herbs (*Teucrium polium, marjoram vulgare, Anabasis syriaca, Cloeme droserifolia* and *Calendula*

officinalis) displayed good antibacterial activity against pseudomonas aureginosa (Khuzaie *et al.*, 1999).

A comparison of the inhibitory effect of he twelve plant extracts (*Calendula officinalis, Commiphora malmol, Hamamelis virgiana, Allium sativum, Berberis vulgaris, Hydrasis canadensis, Alove vera, Populus candicans, Tymus vulgaris, Rosmarinus officinalis, Eucalyptus smithii,* and *Melaleuca alternifolia*) on *Candida albicans* were carried out. *Thymus vulgaris* and *Populus candicans* essential oil, which are not usually considered to be particularly antifungal, were found to be highly inhibitory at normal therapeutic concentration and much higher dilutions (Mcfadden, 1995).

(Li, 1998) reported that the insecticidal activities of limonoids from Meliaceous plants which possess ingredients causing repellence, antifeeding, poisoning and growth inhibition against many important agricultural insects. Limonoids from the genera *Melia* and *Azadirachta* of family Meliaceae show admirable effectiveness in bioactiviry. Some species of genera Aglaia, Trichilia and Tmna contain very effective limonoids against insects as well.

2.2 PALYNOLOGICAL STUDIES

Research on Palynology in Pakistan started from sixties by Butta and his colleagues on Paleobotany and Taxonomy. From 1960-1970 pollen research was confined to fossil pollen and research on living pollen started after 1970.

Atlas or pollen flora of Pakistan has not yet completed and nearly all this is done on simple microscope.

Erdtman (1952) divided *Papaveraceae* into three subfamilies i.e.,. *Fumaroideae*, *Papaveroideae* and *Hypecoideae.* According to him all these subfamilies are quite heterogeneous palynologically, especially *Fumaroideae* palynology also supports its exclusion from *Papaveraceae.*

The pollen morphology of 353 species from Karachi, belonging to 67 families of angiosperms, distributed in 58 dicots and 9 monocots were investigated by scanning

electron microscope (S.E.M.) and light microscope (L.M.). Examination of these families revealed great pollen diversity in their qualitative and quantitative characters. However, from a phylogenetic and evolutionary point of view, polarity, symmetry, apertural types and exine sculpturing are the most important characters. In general, dicot pollen is relatively more specialized than that of monocots. Dicotyledons are generally characterized by radially symmetrical, isopolar, colporate, colpate and porate pollen, whereas monocotyledon are usually heteropolar, bilaterally symmetric, boat–shaped, monocolpate and monoporate pollen. With few problematic exceptions, the pollen data supports the general classification. The relatively most primitive pollen type i.e. monosulcate (monocolpate), heteropolar, bilaterally symmetric pollen are restricted to the most primitive subclass of dicots i.e., Magnoliidae and to the monocots. In contrast to this, the highly advanced subclass Asteridae exhibits the greatest array of specialized pollen types, especially the family compositae.(Perveen. A, 1998).

The pollen morphology of 11 species representing 5 genera of the subfamily Caesalpinioideae from Pakistan was examined by light and scanning electron microscope. Caesalpinioideae is ± a eurypalynous subfamily. The pollen grains are generallly radially symmetrical, isopolar, tricolporate and triangular-trilobed. The tectum is commonly reticulate-rugulate or fossulate-foveolate, and often striate. The pollen morphology of the subfamily is significantly useful at the generic and specific levels. On the basis of apocolpium, mesocolpium and tectum features, three pollen types were recongnized, namely, *Bauhinia variegata* - type, *Caesalpinia pulcherrima* - type and *Senna holosericea* - type. (Perveen and Qaiser, 1997).

Gambel (1933) divided the family *Convolvulaceae* into two groups on the basis of spinulose and non-spinolose pollen, with further division on the basis of apertural types.

Pollen morphology of 40 species representing six genera viz., *Andrachne, Chrozophora, Dalechmpia, Euphorbia, Mallotus and Phyllanthus* of the family Euphorbiaceae from Pakistan has been examined by light and scanning electron microscope. Euphorbiaceae is eurypalynous family. Pollen grains usually radially symmetrical, isopolar, prolate-spheroidal to sub-prolate or prolate often oblate-

spheroidal, colporate(tri rarely 6-7), colpi generally with costae, colpal membrane psilate to sparsely or densely granulated, ora la-longate, sexine as thick as nexine or slightly thicker or thinner than nexine.Tectal surface commonly reticulate or regulate-reticulate rarely striate or verrucate. On the basis of exine pattern 5 distinct pollen types viz., *Andrachne-aspera* – type, *Chrozophora oblongifolia*-type, *Euphorbia hirta* – type, *Mallotus phlilippensis*-type and *Phyllanthus urinaria*- type are recognized. (Perveen and Qaiser, 2005).

Khan (2005) presented pollen morphology of 8 taxa belonging to 3 genera of the family *Brassicaceae* by light microcscope.

Pollen morphology of 3 species of the genus *Oxalis* L., (Oxalidaceae) from Pakistan has been examined by light and scanning electron microscope.Pollen grains usually radially symmetrical, isopolar or apolar, prolate-subprolate, rarely oblate-spheroidal, colpate.sexine thinner or thicker than nexine.Tectum reticulate.On the basis of pollen shape, 2 distinct pollen types viz., *Oxalis carniculata*- type and *Oxalis pescaprae*-type are recognized. (Perveen and Qaiser, 2003).

Weber (1998) reported a systematic approach to categorize pollen types that enable the reader to recognize pollen characteristics of the most common botanical source and determine the relevant contributers top the aeroallergen burden

The pollen morphology of 25 species belonging to 23 genera distributed in 13 families have been examined by light microscopy and scanning electron microscope. Generally pollen grains occur singly and rarely in polyads. Pollen grains generally 3-colporate in families viz., Bignoniaceae, Myrtaceae, Mimosaceae, Anacardiaceae, Apocynaceae, Caesalpiniaceae, Caricaceae, Moringaceae, Rhamnaceae and Sapotaceae. However, in Bignoniaceae and Myrtaceae both colporate and colpate types of pollen grains are found. In the family Malvaceae pantoporate pollen grains are found. The shapes of pollen grains are also different. Generally pollen grains are sub-prolate, prolate and prolate-spheroidal, rarely oblate and spheroidal. Tectum generally reticulate or rugulate in addition to this scabrate and echinate.(Aftab and Perveen, 2006).

Several recent papers have discussed pollen morphological variation in relation to the systematics of Acanthaceae. Scotland (1991) presented a cladistic analysis of 25 genera, but that study was incomplete as alternative character codings of pollen morphological variation were not explored and the full extent of pollen variation was not fully described and documented. The aims of this paper are to describe, illustrate and analyse pollen morphological variation of 36 Acanthaceae genera with contorted corollas, and to explore the ramifications of alternative character codings. (Scotland, 1992).

CHAPTER 03

MATERIALS AND METHODS

MATERIALS AND METHODS

3.1 ANTIBACTERIAL STUDIES OF SELECTED MEDICINAL PLANTS

The present research work was carried out in the Department of Plant sciences, Quaid-i-Azam University, Islamabad. Brief account of materials as well as procedure used in it, are described below.

3.1.1 Plant materials

Plant material was collected from the Himalayan foothills of Islamabad, Pakistan on 26th Oct 2007. The plants were identified by Dr.Rizwana Aleem Qureshi (Taxonomist), Department of Plant Sciences, Quaid-i-Azam University Islamabad.

3.1.2 Extraction

Fresh aerial plant material was taken, rinsed with distilled water and kept under shade till drying. Leaves of the plants were weighed.

3.1.3 Extraction from leaves

Extraction from the leaves was carried out by simple maceration process. The leaves were taken and grounded in methanol using kitchen blender. The poorly homogenized mixture was kept for 4 weeks at room temperature (25°C ± 2°C) in extraction bottle. After 4 weeks, maximum amount of methanol was separated from the mixture. Filterate was filtered twice, first using ordinary filter paper and then Whatman-41 filter paper. Methanol was then completely evaporated by rotary evaporator to obtain the extract.

3.1.4 Requirements

Test samples, nutrient agar (20g/l).McFarland Barium sulphate turbidity standard of 0.5, cultures of bacterial strains, sterile normal saline solution (0.9%NaCl w/v), Bacterial Slants, Sterile cork borer, micropipette, Petri dishes, organic solvent (DMSO), incubator, standard antibiotics (Ampicillin and chloramphinicol), spirit lamp.

3.1.5 Preparation of sample

Sample prepared for antibacterial assay was

- Methanolic extract of leaves

One hundred and Twenty milligrams of the extract was dissolved in 1 ml of DMSO. This stock solution was used for further dilution with DMSO. Solution of Ampicillin and Chloramphinicol, 2mg/ml in DMSO, were prepared for positive control. Pure DMSO was used as negative control.

3.1.6 Media for bacteria

Nutrient broth medium (Merck) was used to grow bacteria for inoculums preparation. Its composition was:

a) Peptone from meat =5gm/l

b) Meat extract =3gm/l

Nutrient agar medium (Merck) was used to perform antibacterial assay. Its composition was:

a) Peptone from meat =5gm/l

b) Meat extract =3gm/l

c) Agar-agar =12gm/l

3.1.7 Preparation of media for bacteria

Nutrient broth medium was prepared by dissolving 0.13gm/ 10 ml nutrient broth in distilled water; pH was adjusted at 7.0 and was autoclaved.

Nutrient agar medium was prepared by dissolving 20gm/l of nutrient agar in distilled water; pH was adjusted to 7.0 and was autoclaved.

3.1.8 McFarland 0.5 $BaSO_4$ turbidity standard

The standard was prepared by adding 0.5 ml of 0.048M $BaCl_2$ to 99.5 ml 0.36N H_2SO_4. Barium Sulphate turbidity standard (4-6 ml) was taken in screw capped test tube and was used to compare the turbidity (Koneman, 1988).

3.1.9 Microorganisms used

Four strains of bacteria were used. Two were Gram Positive, which were *Staphylococcus aureus* (ATCC 6538), *Micrococcus luteus* (ATCC 10240) and two were Gram Negative, which were *Escherichia coli* (ATCC 25922), *Pseudomonas aureginosa* (ATCC 7221). The organisms were maintained on nutrient agar medium at 4°C.

3.1.10 Preparation of inocula

Centrifuged pallets of bacteria from twenty-four hours old culture in nutrient broth(SIGMA) of selected bacterial strains was mixed with physiological saline until a McFarland 0.5 $BaSO_4$ turbidity standard [10^8 colony forming unit (CFU)per ml]. Then this inocula is used for seeding the nutrient agar.

3.1.11 Preparation of seeded agar plates

Nutrient agar medium was prepared by suspending nutrient agar (MERCK) 20gm in 1 liter distilled water; pH was adjusted at 7.0 and was autoclaved. It was allowed to cool up to 45°C.Then it was seeded with 10 ml of prepared inocula to have 10^6 CFU per ml.Petri plates were prepared by pouring 75 ml of seeded nutrient agar and allowed to solidify. Four wells per plate were made with sterile cork borer (5mm).

3.1.12 Pouring of test solution; incubation and measurement of zone of inhibition

Using Micropipette, 100 µl of test solutions was poured in respective wells. Four concentrations of extracts, positive control (Ampicillin and chloramphinicol) were applied to Petri plates. These plates were incubated at 37°C. After 24 hours of incubation the diameter of the clear zone, showing no bacterial growth, around each well was measured. Antibacterial activity of all dilutions of extract was determined against four bacterial strains.

3.5 PALYNOLOGICAL STUDIES

Palynological study was confined to pollen morphology and fertility estimation.

3.5.1 Preparation of glycerin jelly

Glycerin jelly was prepared according to modified method of Ahmad *et al* (2008) who followed Erdtman (1952). A 50 ml of distilled water was taken in a beaker and heated it on a hot plate (model UELP scientifica, Germany). 35 gm of gelatin was added when temperature reaches to 70-80°C. After increase in temperature it become a viscous liquid. The whole situation was kept on heating for one hour. 35 gm of glycerol was mixed in it with few crystals of phenol. Then 0.1% safranine was added by 1/8 th volume with glycerin jelly. It was stirred till uniform pink colour appeared. Jelly was stabilized at room temperature.

3.5.2 Method of pollen study

Mature floral buds were taken from fresh plant material for pollen study. Fresh polleniferous material was used according to special technique known as woodhouse technique (Ronald 2000) with little modifications. Anthers were dissected from flower under binocular light microscope and placed in the center of clean glass slide with 1-2 drops of acetic acid for one minute. Anthers were then crushed to release pollen. The anther wall material was discarded and waited for the evaporation of excess acetic acid. Then added a small drop of melted glycerin jelly and stirred with needle to secure even distribution of pollen. Cover slip was placed on the prepared pollen glycerin jelly mixture. When cooled, the glass slide was labeled and edges of the cover slip were sealed with transparent nail polish.

3.5.3 Palynomorph features

Qualitative Characters includes Type of pollen, shape in polar view, shape in equatorial view, sculpturing where as Quantitative Character include Polar diameter, equatorial diameter, P/E ratio, length and width of colpi, exine thickness, fertile pollen and sterile pollen. Each value was taken ten times to ensure the accurate data.

3.5.4 Pollen fertility

The prepared slides were observed carefully. The percentage of full, well stained grains in a total of at least 200 grains was calculated by the following formula

Fertile pollen / Total pollen x 100

CHAPTER 04

RESULTS

RESULTS

4.1 ANTIBACTERIAL STUDIES OF SELECTED MEDICINAL PLANTS

Methanolic extracts of eleven different plants with four different concentrations were used against four strains of bacteria. Two strains were gram positive i-e *Staphylococcus aureus* (ATCC 6538), *Micrococcus luteus* (ATCC 10240), and two were gram negative i-e *Escherichia coli* (ATCC 25922) and *Pseudomonas auregenosa* (ATCC 7221). Zone of inhibition were measured in millimeter. All dilutions of the extracts were made in Dimethyl sulphoxide (DMSO). This solvent has no inhibitory effect on the growth of bacteria. Four concentrations (120 mg/ml, 90 mg/ml, 60 mg/ml and 30mg/ml) were used for the test against four strains of bacteria.

When the methanolic extracts of the leaves of *Adhatoda vasica* Nees was tested against the tested microorganisms, it was found to be inactive against *Escherichia coli* and active against *Staphylococcus aureus, Pseudomonas auregenosa* and *Micrococcus luteus.* The extract at the concentration of 120 mg/ml displayed 08mm, 10mm, 13mm zone of inhibition against *Pseudomonas auregenosa, Staphylococcus aureus* and *Micrococcus luteus.* The extract at the concentration of 90 mg/ml displayed 08mm, 10mm, 10mm zone of inhibition against *Pseudomonas auregenosa, Staphylococcus aureus* and *Micrococcus luteus.* The extract at the concentration of 60 mg/ml showed no effect on *Pseudomonas auregenosa* and *Staphylococcus aureus* and 10mm against *Micrococcus luteus.* While the extract at the concentration of 30mg/ml showed no effect on *Pseudomonas auregenosa* and *Staphylococcus aureus* and 10mm zone of inhibition against *Micrococcus luteus.*

When the methanolic extracts of the leaves of *Bauhenia variegata* L. was tested against the tested microorganisms, it was found to be inactive against *Escherichia coli* and *Pseudomonas auregenosa* and active against *Staphylococcus aureus,* and *Micrococcus luteus.* The extract at the concentration of 120 mg/ml displayed 12mm and 15mm zone of inhibition against *Staphylococcus aureus* and *Micrococcus luteus.* The extract at the concentration of 90 mg/ml displayed 10mm, 12mm zone of inhibition against *Staphylococcus aureus* and *Micrococcus luteus.* The extract at the

concentration of 60 mg/ml showed 10mm, 10mm zone of inhibition against *Staphylococcus aureus* and *Micrococcus luteus*. While the extract at the concentration of 30mg/ml showed 08mm, 09mm zone of inhibition against *Staphylococcus aureus* and *Micrococcus luteus*.

When the methanolic extracts of the leaves of *Bombax ceiba* L. was tested against the tested microorganisms, it was found to be inactive against *Escherichia coli* and *Pseudomonas auregenosa* and active against *Staphylococcus aureus*, and *Micrococcus luteus*. The extract at the concentration of 120 mg/ml displayed 12mm and 15mm zone of inhibition against *Staphylococcus aureus* and *Micrococcus luteus*. The extract at the concentration of 90 mg/ml displayed 12mm, 15mm zone of inhibition against *Staphylococcus aureus* and *Micrococcus luteus*. The extract at the concentration of 60 mg/ml showed 12mm, 13mm zone of inhibition against *Staphylococcus aureus* and *Micrococcus luteus*. While the extract at the concentration of 30mg/ml showed 08mm, 2mm zone of inhibition against *Staphylococcus aureus* and *Micrococcus luteus*.

When the methanolic extracts of the leaves of *Carissa opaca* Stapf ex Haines was tested against the tested microorganisms, it was found to be inactive against *Escherichia coli* and active against *Staphylococcus aureus, Pseudomonas auregenosa* and *Micrococcus luteus*. The extract at the concentration of 120 mg/ml displayed 11mm, 12mm, 11mm zone of inhibition against *Pseudomonas auregenosa, Staphylococcus aureus, Micrococcus luteus*. The extract at the concentration of 90 mg/ml displayed 10mm, 11mm, 10mm zone of inhibition against *Pseudomonas auregenosa, Staphylococcus aureus, Micrococcus luteus*. The extract at the concentration of 60 mg/ml showed 09mm, 11mm, 10mm zone of inhibition against *Pseudomonas auregenosa, Staphylococcus aureus, Micrococcus luteus*. While the extract at the concentration of 30mg/ml showed 08mm, 11mm, 08mm zone of inhibition against *Pseudomonas auregenosa, Staphylococcus aureus* and *Micrococcus luteus*.

When the methanolic extracts of the leaves of *Caryopteris grata* Benth. was tested against the tested microorganisms, it was found to be inactive against *Escherichia coli* and active against *Staphylococcus aureus, Pseudomonas auregenosa* and

Micrococcus luteus. The extract at the concentration of 120 mg/ml displayed 13mm, 17mm, 13mm zone of inhibition against *Pseudomonas auregenosa, Staphylococcus aureus, Microzoccus luteus*. The extract at the concentration of 90 mg/ml displayed 12mm, 13mm, 13mm zone of inhibition against *Pseudomonas auregenosa, Staphylococcus aureus, Micrococcus luteus*. The extract at the concentration of 60 mg/ml showed 06mm, 13mm, 13mm zone of inhibition against *Pseudomonas auregenosa, Staphylococcus aureus, Micrococcus luteus*. While the extract at the concentration of 30mg/ml showed no effect on *Pseudomonas auregenosa* and 11mm, 12mm zone of inhibition against *Staphylococcus aureus* and *Micrococcus luteus*.

When the methanolic extracts of the leaves of *Debregeasia salicifolia* (D.Don) Rendle was tested against the tested microorganisms, it was found to be inactive against *Escherichia coli* and active against *Staphylococcus aureus, Pseudomonas auregenosa* and *Micrococcus luteus*. The extract at the concentration of 120 mg/ml displayed 10mm, 11mm, 14mm zone of inhibition against *Pseudomonas auregenosa, Staphylococcus aureus, Micrococcus luteus*. The extract at the concentration of 90 mg/ml displayed 10mm, 11mm, 10mm zone of inhibition against *Pseudomonas auregenosa, Staphylococcus aureus, Micrococcus luteus*. The extract at the concentration of 60 mg/ml showed 10mm, 09mm, 08mm zone of inhibition against *Pseudomonas auregenosa, Staphylococcus aureus, Micrococcus luteus*. While the extract at the concentration of 30mg/ml showed 11mm, 09mm, 08mm zone of inhibition against *Pseudomonas auregenosa, Staphylococcus aureus* and *Micrococcus luteus*.

When the methanolic extracts of the leaves of *Lantana camara* L. was tested against the tested microorganisms, it was found to be inactive against *Escherichia coli* and active against *Staphylococcus aureus, Pseudomonas auregenosa* and *Micrococcus luteus*. The extract at the concentration of 120 mg/ml displayed 10mm, 11mm, 15mm zone of inhibition against *Pseudomonas auregenosa, Staphylococcus aureus, Micrococcus luteus*. The extract at the concentration of 90 mg/ml displayed 10mm, 11mm, 14mm zone of inhibition against *Pseudomonas auregenosa, Staphylococcus aureus, Micrococcus luteus*. The extract at the concentration of 60 mg/ml showed 10mm, 11mm, 13mm zone of inhibition against *Pseudomonas auregenosa, Staphylococcus aureus, Micrococcus luteus*. While the extract at the concentration of

30mg/ml showed 10mm, 11mm, 13smm zone of inhibition against *Pseudomonas auregenosa, Staphylococcus aureus* and *Micrococcus luteus.*

When the methanolic extracts of the leaves of *Melia azedarach* L. was tested against the tested microorganisms, it was found to be inactive against *Escherichia coli* and *Pseudomonas auregenosa* and active against *Staphylococcus aureus* and *Micrococcus luteus.* The extract at the concentration of 120 mg/ml displayed 10mm and 11mm zone of inhibition against *Staphylococcus aureus* and *Micrococcus luteus.* The extract at the concentration of 90 mg/ml displayed 10mm, 11mm zone of inhibition against *Staphylococcus aureus* and *Micrococcus luteus.* The extract at the concentration of 60 mg/ml showed 10mm, 11mm zone of inhibition against *Staphylococcus aureus* and *Micrococcus luteus.* While the extract at the concentration of 30mg/ml showed 08mm, 09mm zone of inhibition against *Staphylococcus aureus* and *Micrococcus luteus.*

When the methanolic extracts of the leaves of *Olea ferruginea* Royle was tested against the tested microorganisms, it was found to be inactive against *Escherichia coli, Pseudomonas auregenosa* and *Staphylococcus aureus* and active against *Micrococcus luteus.* The extract at the concentration of 120 mg/ml displayed 30mm zone of inhibition against *Micrococcus luteus.* The extract at the concentration of 90 mg/ml displayed 28mm, 15mm zone of inhibition against *Micrococcus luteus.* The extract at the concentration of 60 mg/ml showed 28mm zone of inhibition against *Micrococcus luteus.* While the extract at the concentration of 30mg/ml showed 22mm zone of inhibition against *Micrococcus luteus*

When the methanolic extracts of the leaves of *Phyllanthus emblica* L. was tested against the tested microorganisms, it was found to be inactive against *Escherichia coli* and active against *Staphylococcus aureus, Pseudomonas aureginosa* and *Micrococcus luteus.* The extract at the concentration of 120 mg/ml displayed 25mm, 17mm, 23mm zone of inhibition against *Pseudomonas auregenosa, Staphylococcus aureus, Micrococcus luteus.* The extract at the concentration of 90 mg/ml displayed 22mm, 16mm, 22mm zone of inhibition against *Pseudomonas auregenosa, Staphylococcus aureus, Micrococcus luteus.* The extract at the concentration of 60 mg/ml showed 22mm, 15mm, 19mm zone of inhibition against *Pseudomonas auregenosa,*

Staphylococcus aureus and *Micrococcus luteus*. While the extract at the concentration of 30mg/ml showed 15mm, 12mm, 16mm zone of inhibition against *Pseudomonas auregenosa, Staphylococcus aureus* and *Micrococcus luteus.*

When the methanolic extracts of the leaves of *Pinus rouxberghii* Sargent was tested against the tested microorganisms, it was found to be inactive against *Escherichia coli* and active against *Staphylococcus aureus, Pseudomonas auregenosa* and *Micrococcus luteus.* The extract at the concentration of 120 mg/ml displayed 18mm, 13mm, 16mm zone of inhibition against *Pseudomonas auregenosa, Staphylococcus aureus, Micrococcus luteus.* The extract at the concentration of 90 mg/ml displayed 16mm, 13mm, 15mm zone of inhibition against *Pseudomonas auregenosa, Staphylococcus aureus* and *Micrococcus luteus.* The extract at the concentration of 60 mg/ml showed 14mm, 11mm, 15mm zone of inhibition against *Pseudomonas auregenosa, Staphylococcus aureus, Micrococcus luteus*. While the extract at the concentration of 30mg/ml showed 14mm, 11mm, 14mm zone of inhibition against *Pseudomonas auregenosa, Staphylococcus aureus* and *Micrococcus luteus.*

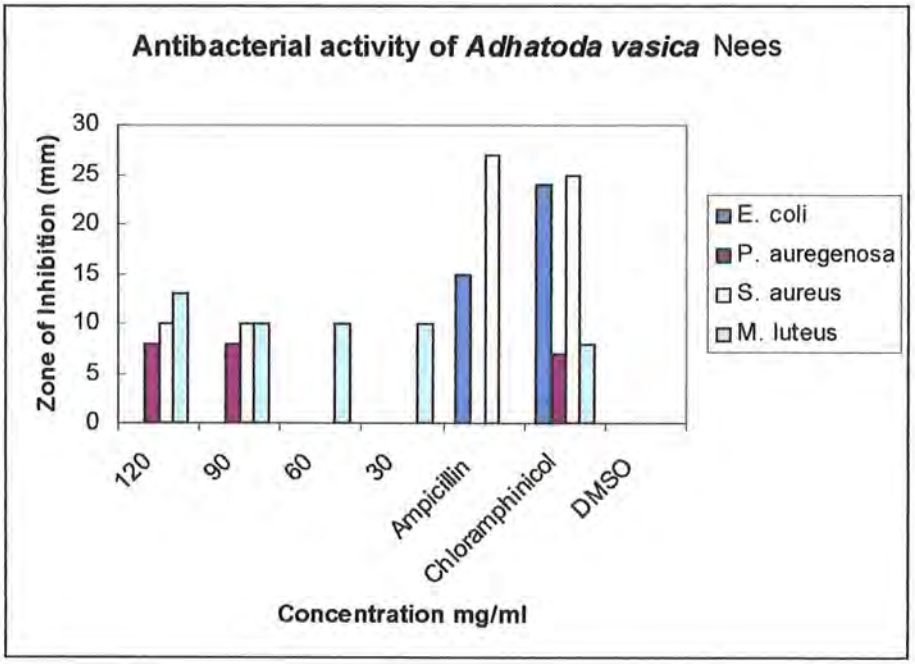
Results displayed that all the plants have Antimicrobial activity against Gram Positive bacteria (*Staphylococcus aureus* and *Micrococcus luteus*). Few Plants have Antimicrobial activity against *Pseudomonas aerugenosa* (Gram Negative) and all the Plants have no inhibitory effect on *Escherichia coli* (Gram Negative). Comparing the result with standard antibiotics i-e Ampicillin and Chloramphinicol, the inhibitory effect of Ampicillin against *Stapylococcus aureus* was about 27mm, 15mm against *Escherichia coli* have no inhibitory effect on *Pseudomonas auregenosa* and *Micrococcus luteus.*

The inhibitory effect of Chloramphinicol was about 25mm agaist the *Stphylococcus aureus,* 24mm against *E.coli,* 7mm against *Pseudomonas aureginosa* and 8mm against *Micrococcus luteus.*

All the inhibitory zones of the standards were found to be smaller in size against *Micrococcus luteus* and *Pseudomonas auregenosa* when compared with the inhibitory zones of the plant extracts. While the inhibitory effect of the standards were found to be larger against *Staphylococcus aureus* and *Escherichia coli.*

Table 1: Antibacterial activity of *Adhatoda vasica* Nees

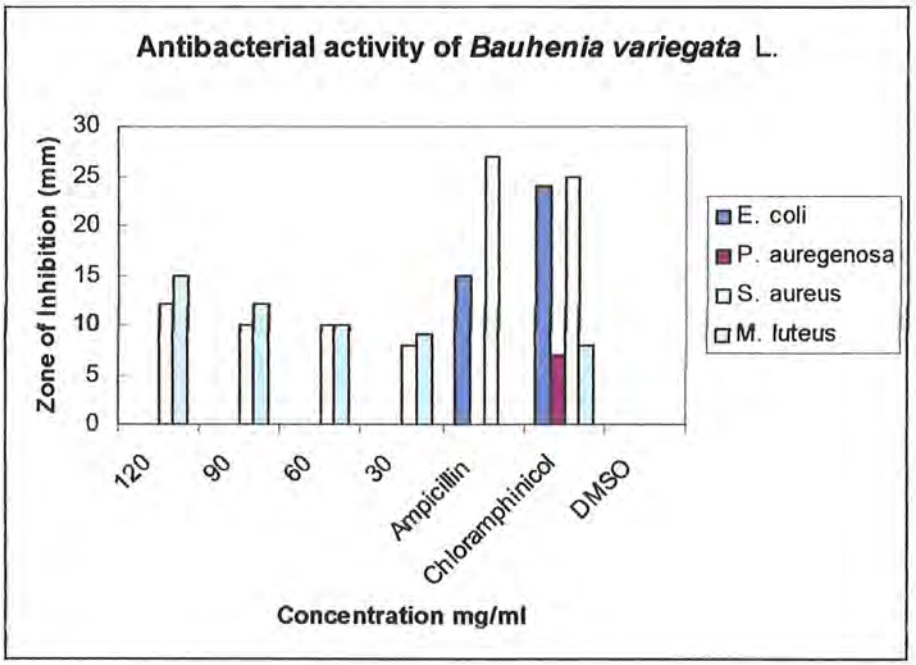
		Zone of Inhibition (mm) after 24 hrs			
S/No	**Conc mg/ml**	***E.coli***	***P.auregenosa***	***S.aureus***	***M.luteus***
1	**120**	—	**8**	**10**	**13**
2	**90**	—	**8**	**10**	**10**
3	**60**	—	—	—	**10**
4	**30**	—	—	—	**10**
5	**Ampicillin**	**15**	—	**27**	—
6	**Chloramphinicol**	**24**	**7**	**25**	**8**
7	**DMSO**	—	—	—	—



E. coli: *Escherichia coli*
P. auregenosa: *Pseudomonas auregenosa*
mm : Millimeter
S. aureus: *Staphylococcus aureus*
M. luteus: *Micrococcus luteus*
DMSO : Dimethyl sulfoxide

Table 2: Antibacterial activity of *Bauhenia variegata* L.

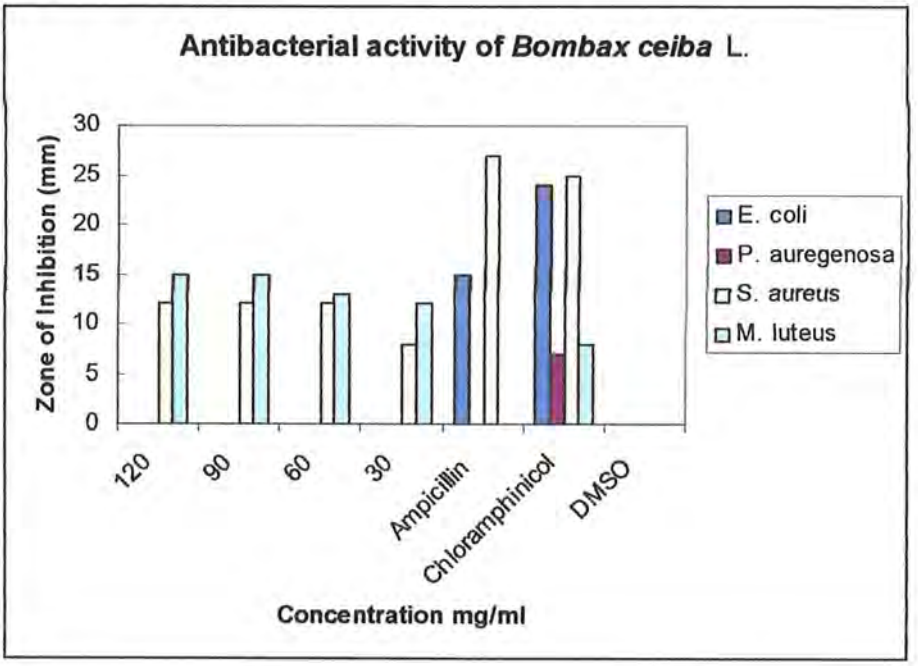
		Zone of Inhibition (mm) after 24 hrs			
S/No	**Conc mg/ml**	***E.coli***	***P.auregenosa***	***S.aureus***	***M.luteus***
1	**120**	—	—	**12**	**15**
2	**90**	—	—	**10**	**12**
3	**60**	—	—	**10**	**10**
4	**30**	—	—	**8**	**9**
5	**Ampicillin**	**15**	—	**27**	—
6	**Chloramphinicol**	**24**	**7**	**25**	**8**
7	**DMSO**	—	—	—	—



E. coli : *Escherichia coli* *S. aureus: Staphylococcus aureus*
P. auregenosa: Pseudomonas auregenosa *M. luteus: Micrococcus luteus*
mm : Millimeter DMSO : Dimethyl sulfoxide

Table 3: Antibacterial activity of *Bombax ceiba* L.

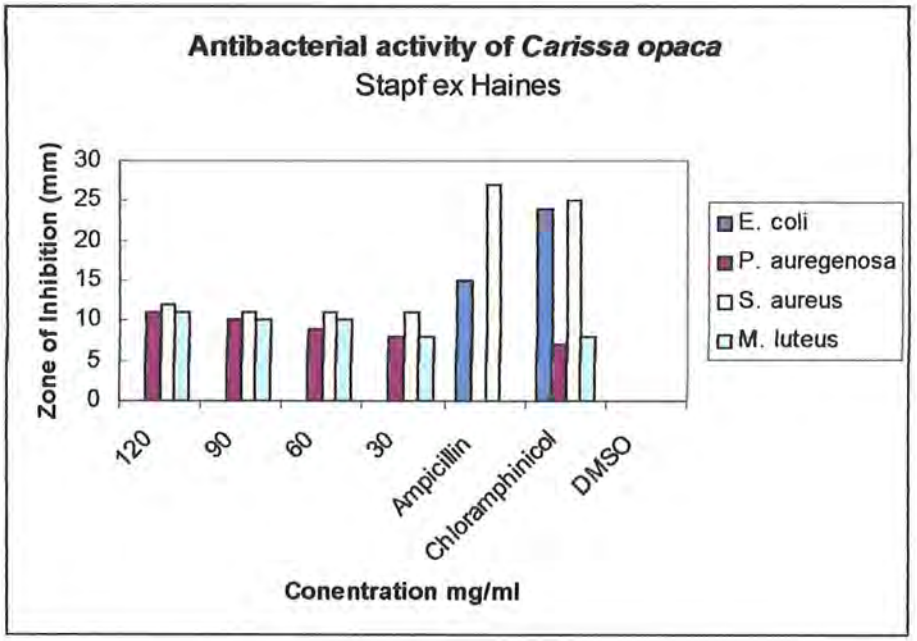
		Zone of Inhibition (mm) after 24 hrs			
S/No	**Conc mg/ml**	***E.coli***	***P.auregenosa***	***S.aureus***	***M.luteus***
1	**120**	—	—	**12**	**15**
2	**90**	—	—	**12**	**15**
3	**60**	—	—	**12**	**13**
4	**30**	—	—	**8**	**12**
5	**Ampicillin**	**15**	—	**27**	—
6	**Chloramphinicol**	**24**	**7**	**25**	**8**
7	**DMSO**	—	—	—	—



E. coli : *Escherichia coli*
P. auregenosa: *Pseudomonas auregenosa*
mm : Millimeter
S. aureus: *Staphylococcus aureus*
M. luteus: *Micrococcus luteus*
DMSO : Dimethyl sulfoxide

Table 4: Antibacterial activity of *Carissa opaca* Stapf ex Haines

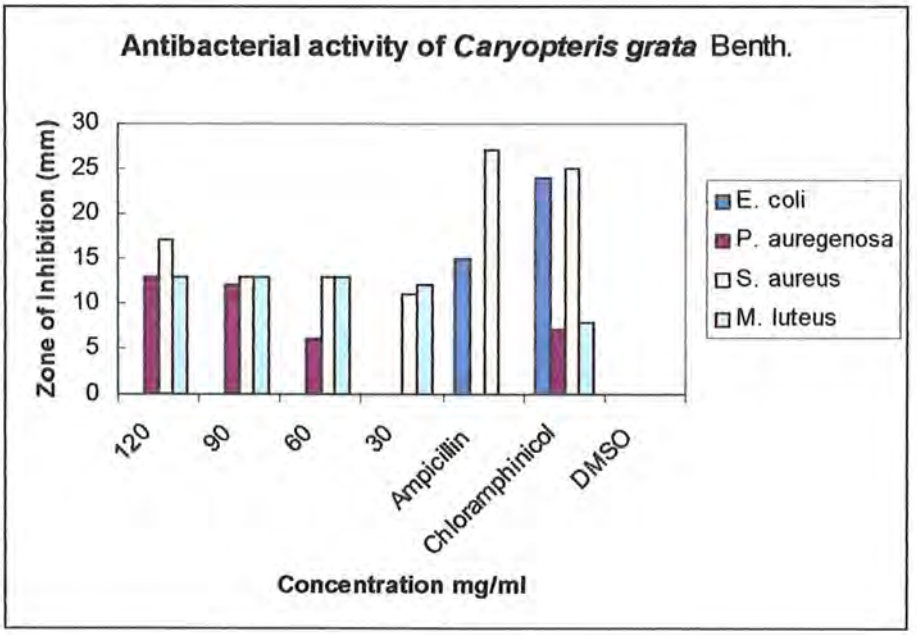
		Zone of Inhibition (mm) after 24 hrs			
S/No	**Conc mg/ml**	***E.coli***	***P.auregenosa***	***S.aureus***	***M.luteus***
1	**120**	—	**11**	**12**	**11**
2	**90**	—	**10**	**11**	**10**
3	**60**	—	**9**	**11**	**10**
4	**30**	—	**8**	**11**	**8**
5	**Ampicillin**	**15**	—	**27**	—
6	**Chloramphinicol**	**24**	**7**	**25**	**8**
7	**DMSO**	—	—	—	—



E. coli : *Escherichia coli*
P. auregenosa: *Pseudomonas auregenosa*
mm : Millimeter
S. aureus: *Staphylococcus aureus*
M. luteus: *Micrococcus luteus*
DMSO : Dimethyl sulfoxide

Table 5: Antibacterial activity of *Caryopteris grata* Benth.

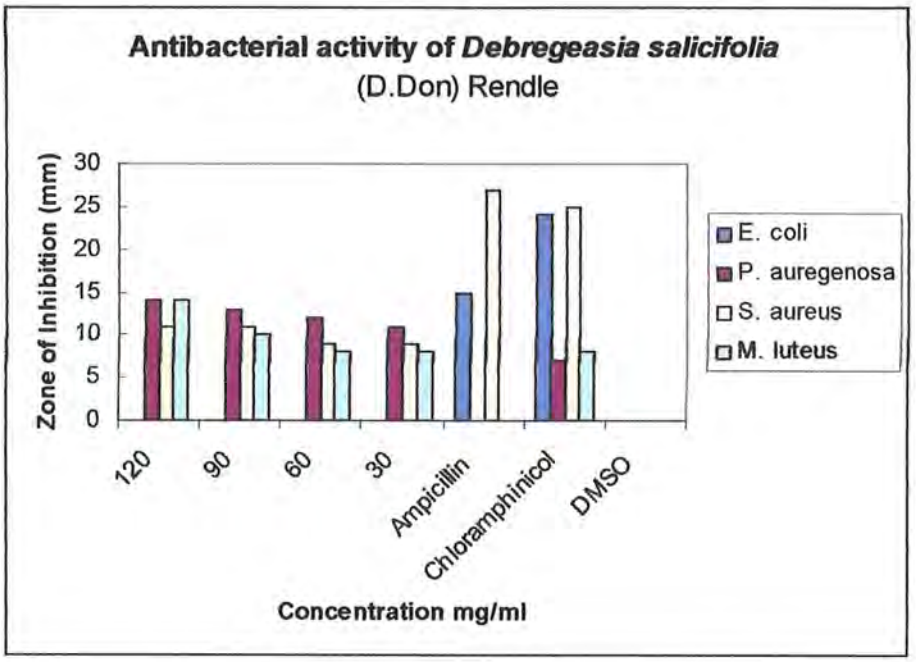
	Zone of Inhibition (mm) after 24 hrs				
S/No	**Conc mg/ml**	***E.coli***	***P.auregenosa***	***S.aureus***	***M.luteus***
1	**120**	—	**13**	**17**	**13**
2	**90**	—	**12**	**13**	**13**
3	**60**	—	**6**	**13**	**13**
4	**30**	—	—	**11**	**12**
5	**Ampicillin**	**15**	—	**27**	—
6	**Chloramphinicol**	**24**	**7**	**25**	**8**
7	**DMSO**	—	—	—	—



E. coli: *Escherichia coli* *S. aureus: Staphylococcus aureus*
P. auregenosa: Pseudomonas auregenosa *M. luteus: Micrococcus luteus*
mm : Millimeter DMSO : Dimethyl sulfoxide

Table 6: Antibacterial activity of *Debregeasia salicifolia* (D.Don) Rendle

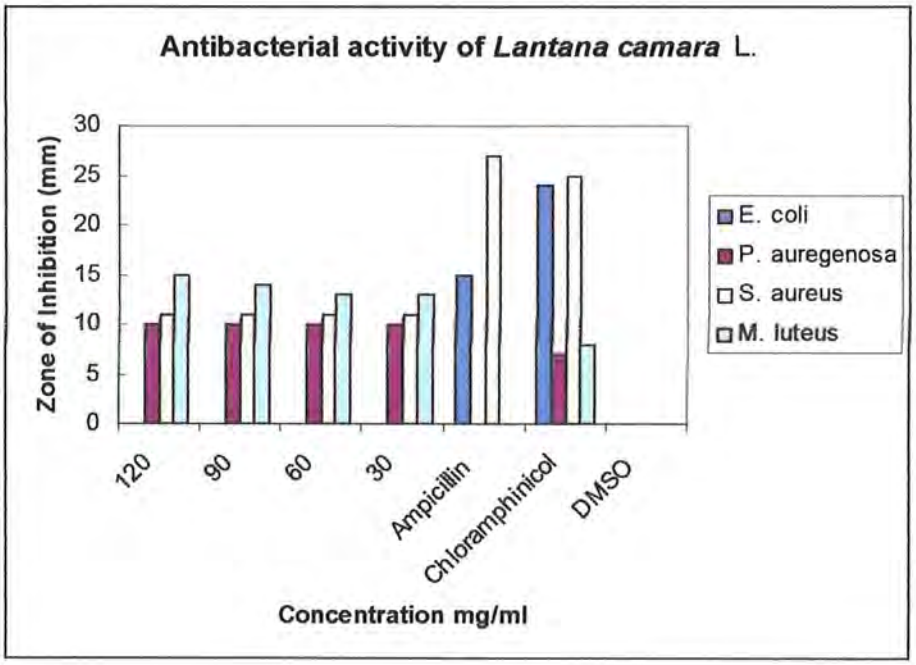
		Zone of Inhibition (mm) after 24 hrs			
S/No	**Conc mg/ml**	***E.coli***	***P.auregenosa***	***S.aureus***	***M.luteus***
1	**120**	—	**14**	**11**	**14**
2	**90**	—	**13**	**11**	**10**
3	**60**	—	**12**	**9**	**8**
4	**30**	—	**11**	**9**	**8**
5	**Ampicillin**	**15**	—	**27**	—
6	**Chloramphinicol**	**24**	**7**	**25**	**8**
7	**DMSO**	—	—	—	—



E. coli: *Escherichia coli* *S. aureus: Staphylococcus aureus*
P. auregenosa: Pseudomonas auregenosa *M. luteus: Micrococcus luteus*
mm : Millimeter DMSO : Dimethyl sulfoxide

Table 7: Antibacterial activity of *Lantana camara* L.

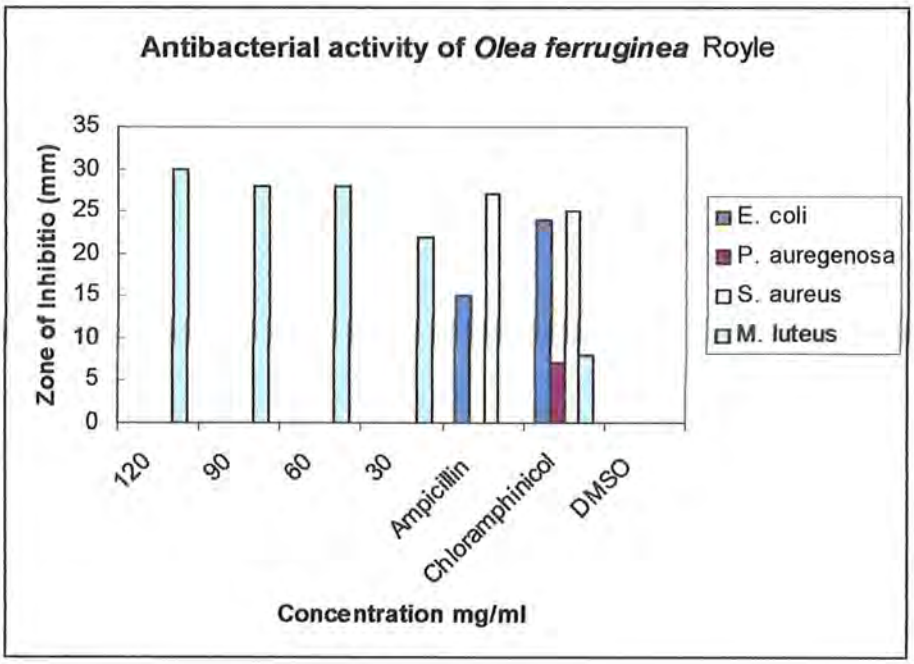
		Zone of Inhibition (mm) after 24 hrs			
S/No	**Conc mg/ml**	***E.coli***	***P.auregenosa***	***S.aureus***	***M.luteus***
1	**120**	—	**10**	**11**	**15**
2	**90**	—	**10**	**11**	**14**
3	**60**	—	**10**	**11**	**13**
4	**30**	—	**10**	**11**	**13**
5	**Ampicillin**	**15**	—	**27**	—
6	**Chloramphinicol**	**24**	**7**	**25**	**8**
7	**DMSO**	—	—	—	—



E. coli: *Escherichia coli*
P. auregenosa: *Pseudomonas auregenosa*
mm : Millimeter
S. aureus: *Staphylococcus aureus*
M. luteus: *Micrococcus luteus*
DMSO : Dimethyl sulfoxide

Table 9: Antibacterial activity of *Olea ferruginea* Royle

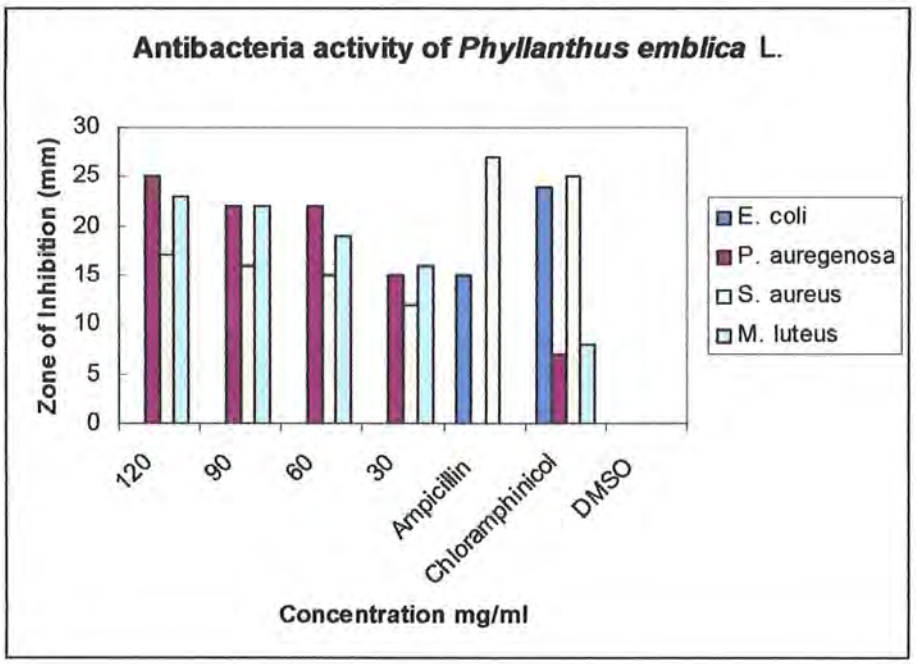
		Zone of Inhibition (mm) after 24 hrs			
S/No	**Conc mg/ml**	***E.coli***	***P.auregenosa***	***S.aureus***	***M.luteus***
1	**120**	—	—	—	**30**
2	**90**	—	—	—	**28**
3	**60**	—	—	—	**28**
4	**30**	—	—	—	**22**
5	**Ampicillin**	**15**	—	**27**	—
6	**Chloramphinicol**	**24**	**7**	**25**	**8**
7	**DMSO**	—	—	—	—



E. coli: *Escherichia coli* *S. aureus: Staphylococcus aureus*
P. auregenosa: Pseudomonas auregenosa *M. luteus: Micrococcus luteus*
mm : Millimeter DMSO : Dimethyl sulfoxide

Table 10: Antibacterial activity of *Phyllanthus emblica* L.

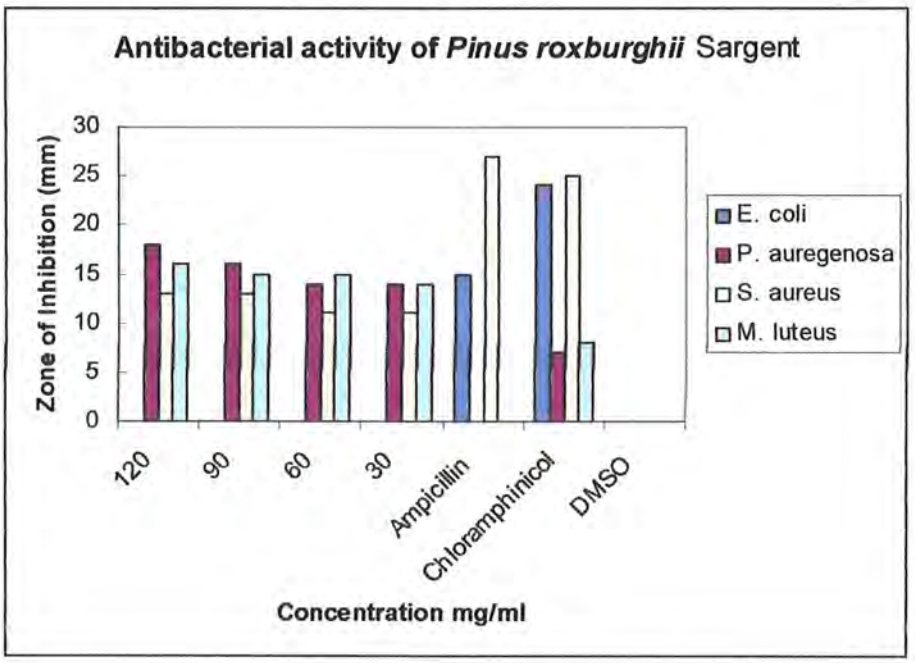
	Zone of Inhibition (mm) after 24 hrs				
S/No	**Conc mg/ml**	***E.coli***	***P.auregenosa***	***S.aureus***	***M.luteus***
1	**120**	—	**25**	**17**	**23**
2	**90**	—	**22**	**16**	**22**
3	**60**	—	**22**	**15**	**19**
4	**30**	—	**15**	**12**	**16**
5	**Ampicillin**	**15**	—	**27**	—
6	**Chloramphinicol**	**24**	**7**	**25**	**8**
7	**DMSO**	—	—	—	—



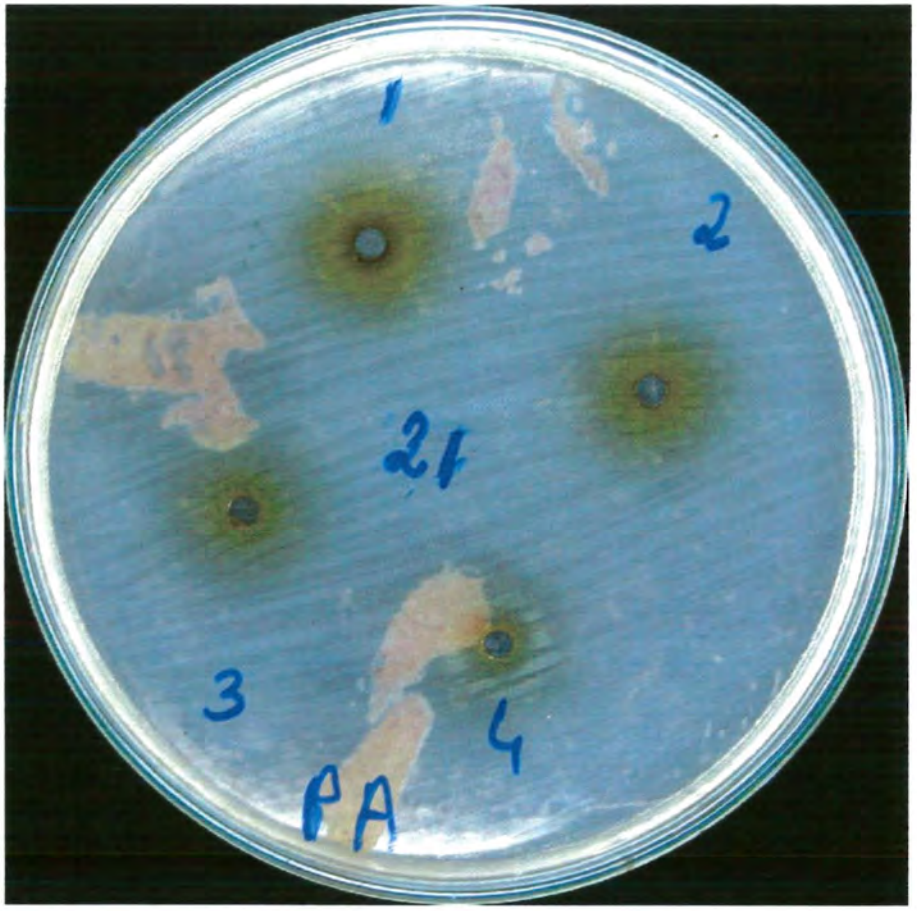
E. coli: *Escherichia coli* *S. aureus: Staphylococcus aureus*
P. auregenosa: Pseudomonas auregenosa *M. luteus: Micrococcus luteus*
mm : Millimeter DMSO : Dimethyl sulfoxide

Table 11: Antibacterial activity of *Pinus roxburghii* Sargent

		Zone of Inhibition (mm) after 24 hrs			
S/No	**Conc mg/ml**	***E.coli***	***P.auregenosa***	***S.aureus***	***M.luteus***
1	**120**	**—**	**18**	**13**	**16**
2	**90**	**—**	**16**	**13**	**15**
3	**60**	**—**	**14**	**11**	**15**
4	**30**	**—**	**14**	**11**	**14**
5	**Ampicillin**	**15**	**—**	**27**	**—**
6	**Chloramphinicol**	**24**	**7**	**25**	**8**
7	**DMSO**	**—**	**—**	**—**	**—**

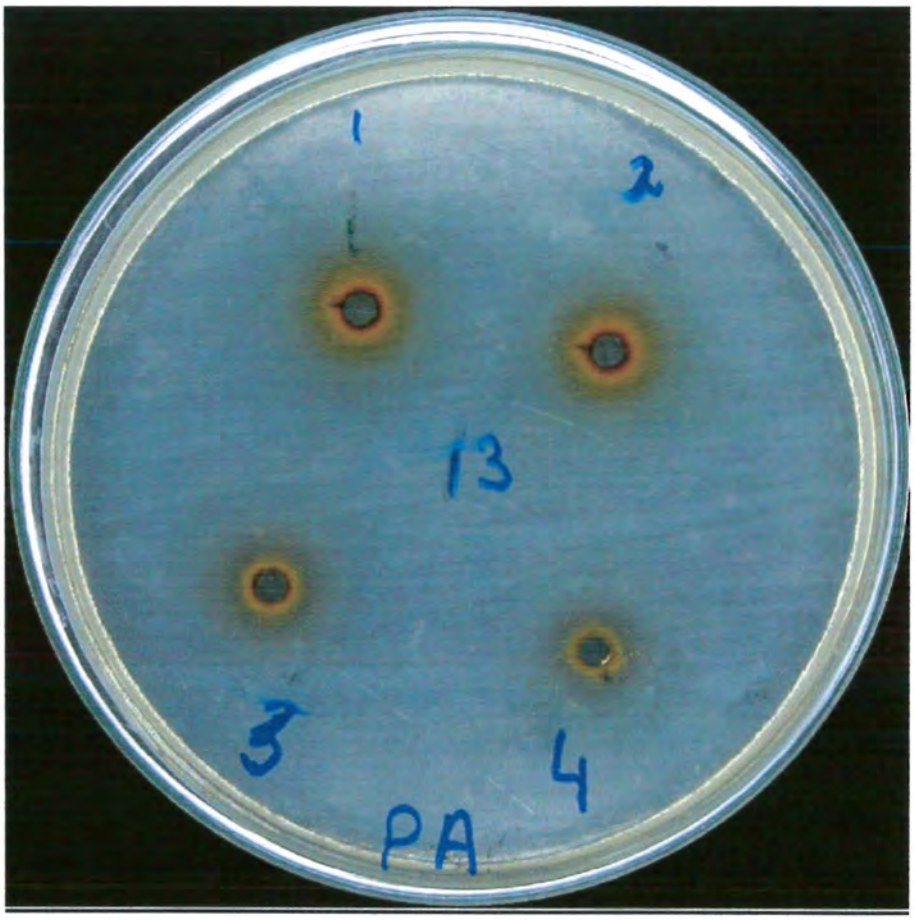


E. coli: *Escherichia coli* *S. aureus: Staphylococcus aureus*
P. auregenosa: Pseudomonas auregenosa *M. luteus: Micrococcus luteus*
mm : Millimeter DMSO : Dimethyl sulfoxide



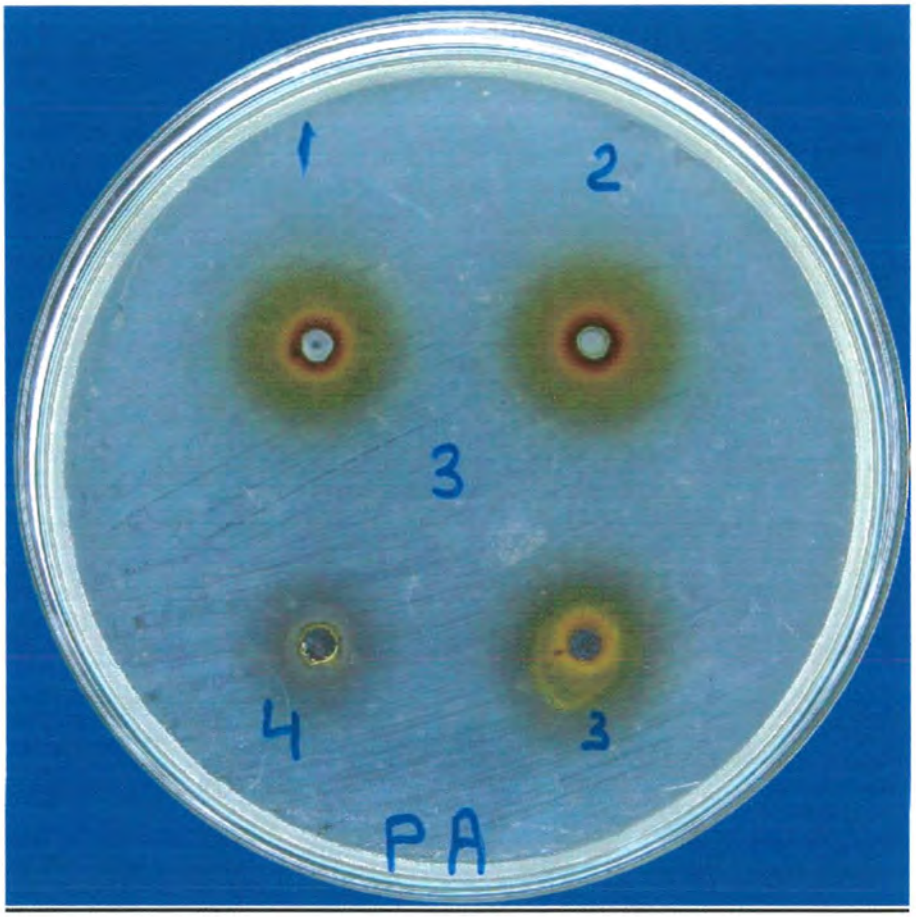
1 : 120 mg/ml **2 : 90 mg/ml**
3 : 60 mg/ml **4 : 30 mg/ml**

Plate I: Antibacterial activity of *Lantana camara* L. against *Pseudomonas auregenosa*



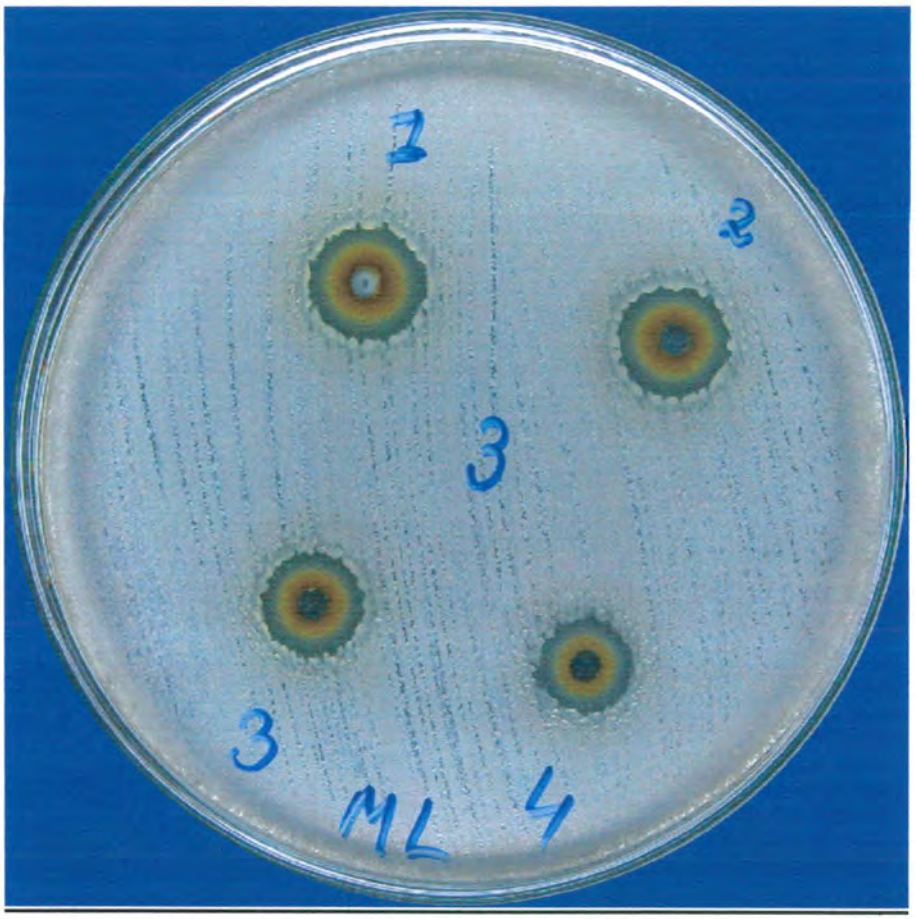
1 : 120 mg/ml **2 : 90 mg/ml**
3 : 60 mg/ml **4 : 30 mg/ml**

Plate II: Antibacterial activity of *Debregeasia salicifolia* (D.Don) Rendle **against *Pseudomonas auregenosa***



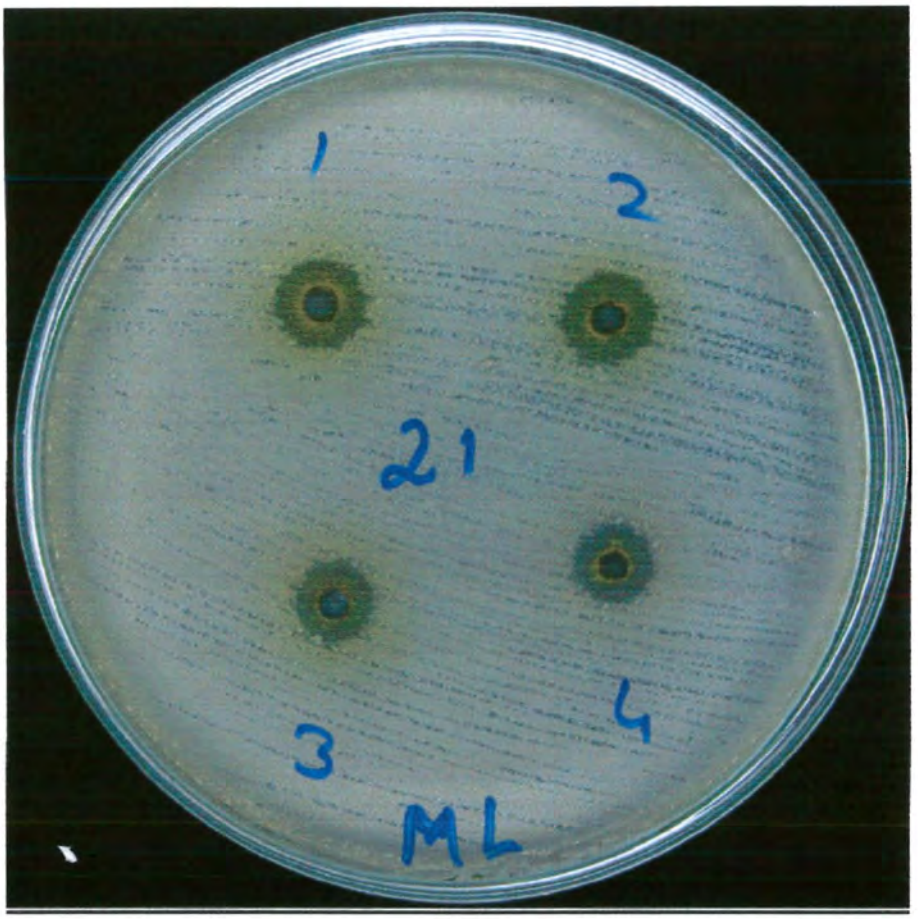
1 : 120 mg/ml **2 : 90 mg/ml**
3 : 60 mg/ml **4 : 30 mg/ml**

Plate III: Antibacterial activity of *Phyllanthus emblica* L. against *Pseudomonas auregenosa*



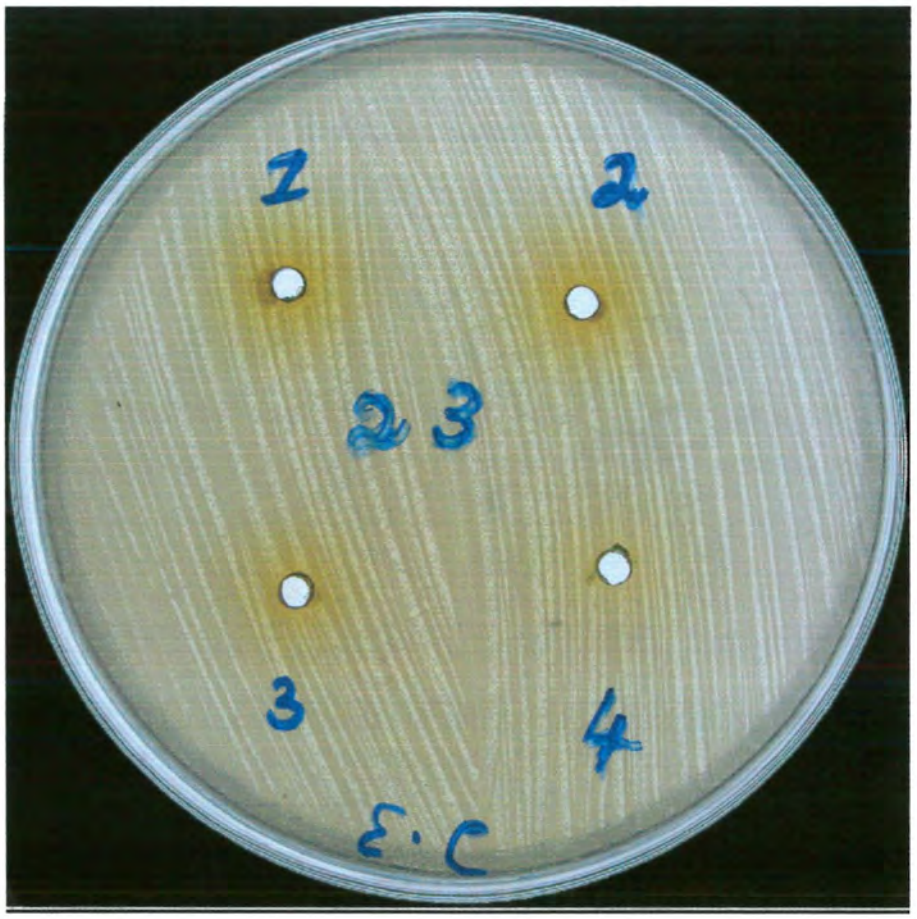
1 : 120 mg/ml **2 : 90 mg/ml**
3 : 60 mg/ml **4 : 30 mg/ml**

Plate IV: Antibacterial activity of *Phyllanthus emblica* L. against *Micrococcus luteus*



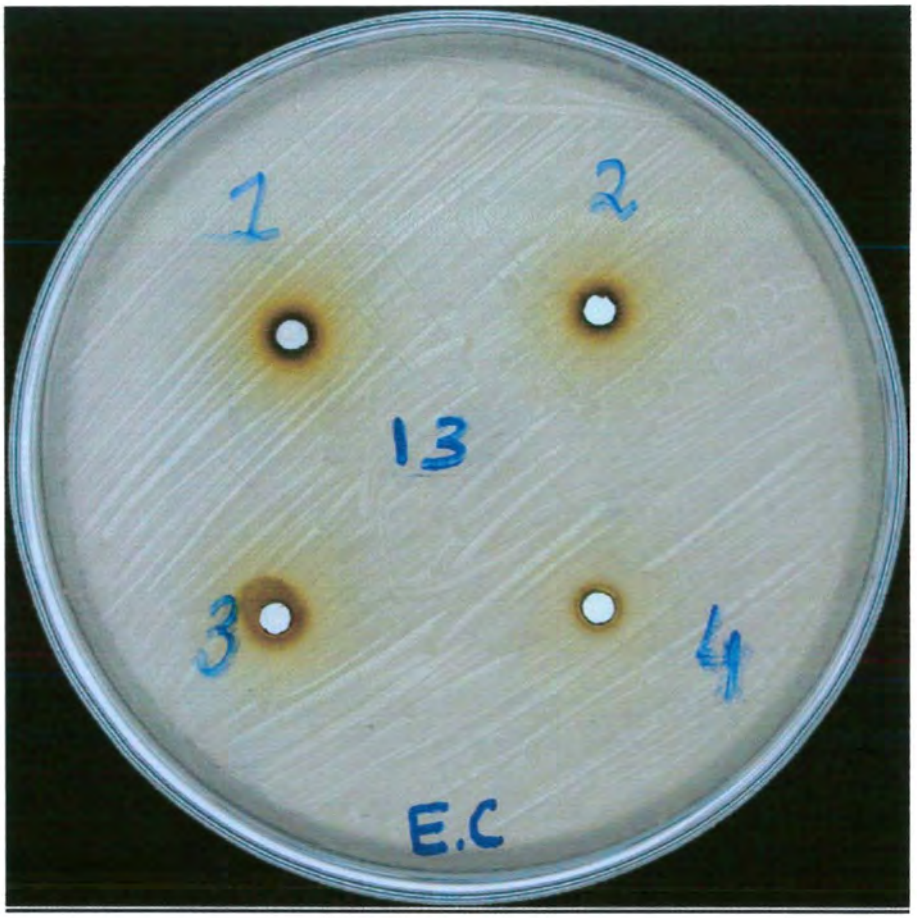
1 : 120 mg/ml **2 : 90 mg/ml**
3 : 60 mg/ml **4 : 30 mg/ml**

Plate V: Antibacterial activity of *Lantana camara* L. against *Micrococcus luteus*



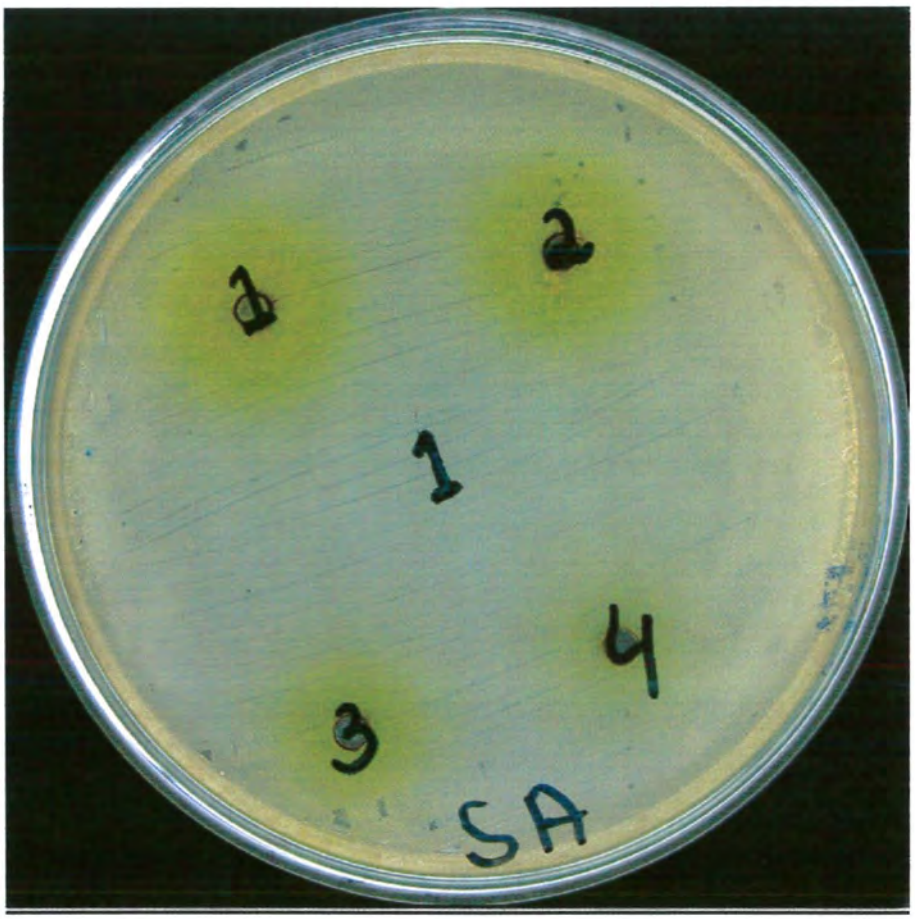
1 : 120 mg/ml **2 : 90 mg/ml**
3 : 60 mg/ml **4 : 30 mg/ml**

Plate VI: Antibacterial activity of *Caryopteris grata* Benth. against *Escherichia coli*



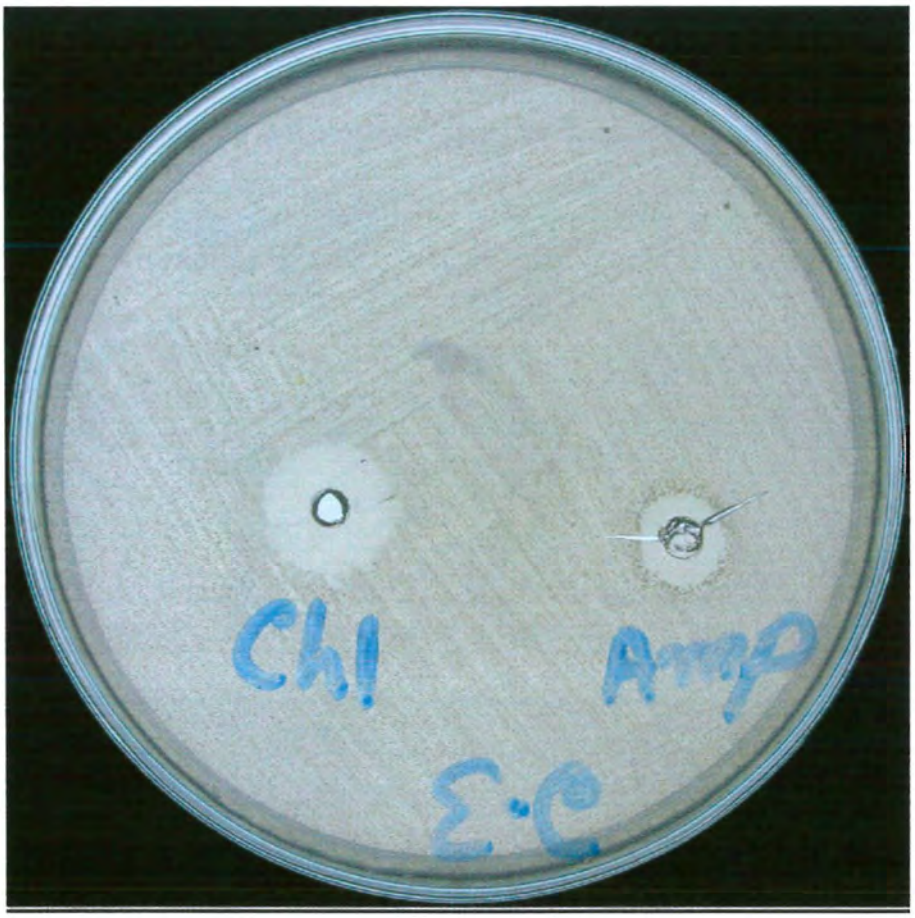
1 : 120 mg/ml **2 : 90 mg/ml**
3 : 60 mg/ml **4 : 30 mg/ml**

Plate VII: Antibacterial activity of *Debregeasia salicifolia* (D.Don) Rendle against *Escherichia coli*



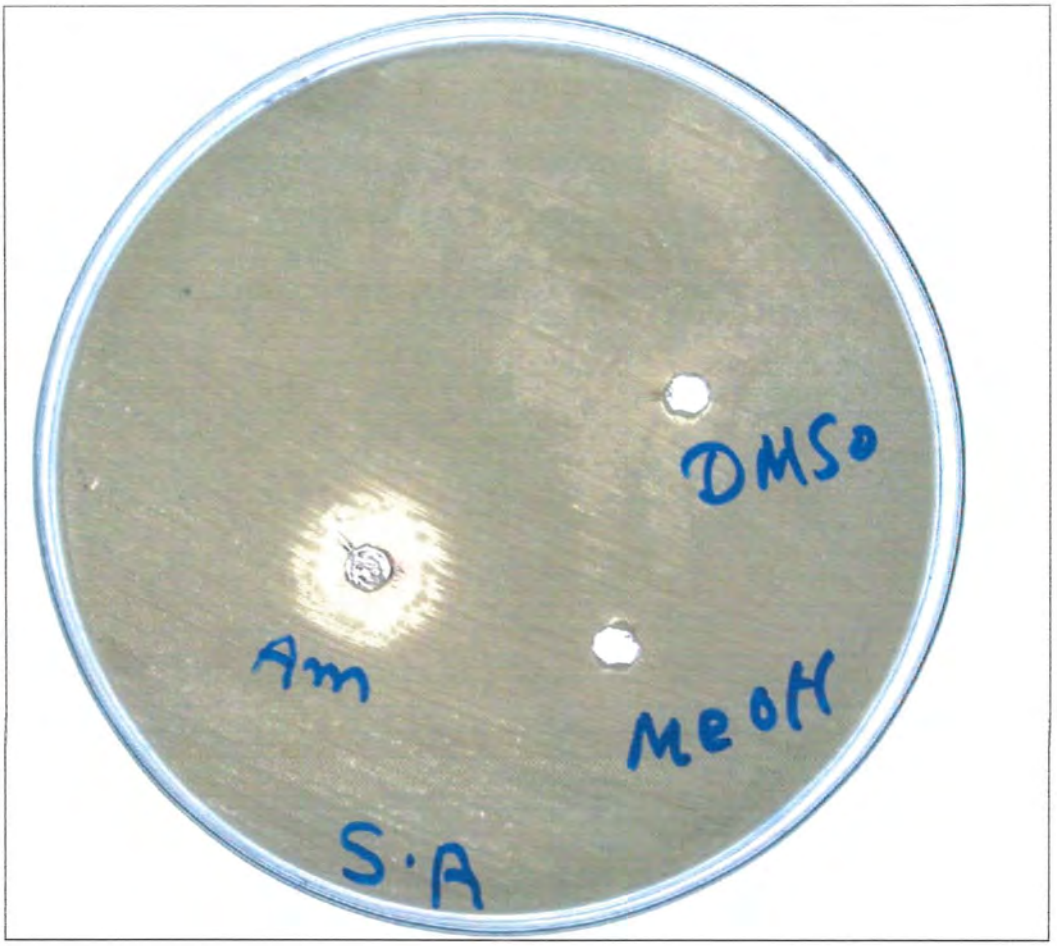
1 : 120 mg/ml **2 : 90 mg/ml**
3 : 60 mg/ml **4 : 30 mg/ml**

Plate VIII: Antibacterial activity of *Adhatoda vasica* Nees against *Staphylococcus aureus*



Chl : Chloramphinicol **Amp : Ampicillin**

Plate IX: Activity shown by Ampicillin and Chloramphinicol against *Escherichia coli*



Am : Ampicillin **MeOH : Methyl alcohol**
DMSO : Dimethyl sulphoxide

Plate X: Activity shown by Ampicillin, DMSO, MeOH against *Staphylococcus aureus*

4.2 PALYNOLOGICAL STUDIES OF SELECTED MEDICINAL PLANTS

4.2.1 Pollen grains of *Adhatoda vasica* Nees

Pollen type: Monad, Psilate, Bicolpate sometimes tricolpate.

Shape of Pollen grains:

In Polar view: Tubular and Circular

In equatorial view: Prolate.

Dimensions:

Equatorial diameter: 25 µm

Polar diameter: 25 µm

P/E ratio: 01 µm

Exine Thickness: 2.5 µm

Colpi Length: 2.5 µm

Colpi Width: 2.5 µm

Fertility: 92%

4.2.2 Pollen grains of *Bauhenia variegata* L.

Pollen type: Monad, Psilate, Bicolpate sometimes tricolpate.

Shape of Pollen grains:

In Polar view: Circular

In equatorial view: Subulate to Prolate to Perprolate.

Dimensions:

Equatorial diameter: 54 µm (50-60 µm).

Polar diameter: 47.85 µm (32.5-75 µm)

P/E ratio: 1.12 µm

Exine Thickness: 2.5 µm

Colpi Length: 5 µm

Colpi Width: 2.5 µm

Fertility: 94%

4.2.3 Pollen grains of *Bombax ceiba* L.

Pollen type: Monad, Psilate, Bicolpate sometimes tricolpate.

Shape of Pollen grains:

In Polar view: Circular

In equatorial view: Subprolate to prolate.

Dimensions:

Equatorial diameter: 38.5 µm (35 µm -40 µm).

Polar diameter: 49.107 µm (46.25 µm -50 µm)

P/E ratio: 1.27 µm

Exine Thickness: 2.5 µm

Colpi Length: 2.5 µm

Colpi Width: 5 µm

Fertility: 92%

4.2.4 Pollen grains of *Carissa opaca* Stapf ex Haines

Pollen type: Monad, Psilate and Tricolpate

Shape of Pollen grains:

In Polar view: Circular

In equatorial view: Suboblate to Subprolate

Dimensions:

Equatorial diameter: 27.5 µm (22.5 µm -30 µm)

Polar diameter: 27.5 µm (20 µm -30 µm)

P/E ratio: 01 µm

Exine Thickness: 2.5 µm

Colpi Length: 3.5 µm

Colpi Width: 3 µm

Fertility: 90%

4.2.5 Pollen grains of *Caryopteris grata* Benth.

Pollen type: Monad, Psilate and Tricolpate

Shape of Pollen grains:

In Polar view: Circular

In equatorial view: Spheroidal to Perprolate

Dimensions:

Equatorial diameter: 16.25 µm -27.5 µm).

Polar diameter: 27.5 µm (20 µm -35 µm)

P/E ratio: 1.25 µm

Exine Thickness: 1.25 µm

Colpi Length: 1.25 µm

Colpi Width: 1.25 µm

Fertility: 80%

4.2.6 Pollen grains of *Debregeasia salicifolia* (D.Don) Rendle

Pollen type: Monad, Psilate and Tricolpate

Shape of Pollen grains:

In Polar view: Circular

In equatorial view: Spheroidal to Perprolate

Dimensions:

Equatorial diameter: 14 µm (10 µm -15 µm).

Polar diameter: 16.25 µm (15 µm -17.5 µm)

P/E ratio: 1.16 µm

Exine Thickness: 1.25 µm

Colpi Length: 2.5 µm

Colpi Width: 2.5 µm

Fertility: 92%

4.2.7 Pollen grains of *Lantana camara* L.

Pollen type: Monad, Psilate and Tricolpate some times Tetracolpate

Shape of Pollen grains:

In Polar view: Circular to Semiangular to Rectangular

In equatorial view: Subprolate to Prolate-Spheroidal to Prolate.

Dimensions:

Equatorial diameter: 32.5 µm (27.5 µm -42.5 µm).

Polar diameter: 33 µm (27.5 µm -40 µm)

P/E ratio: 1.01 µm

Exine Thickness: 2.5 µm

Colpi Length: 2.5 µm

Colpi Width: 2.5 µm

Fertility: 80 %

4.2.8 Pollen grains of *Melia azedarach* L.

Pollen type: Monad, Psilate and Tricolpate

Shape of Pollen grains:

In Polar view: Circular to Semiangular

In equatorial view: Irregular

Dimensions:

Equatorial diameter: 30.2 µm (22 µm -34 µm)

Polar diameter: 32.2 µm (30 µm -40 µm)

P/E ratio: 1.07 µm

Exine Thickness: 2.5 µm

Colpi Length: 2.5 µm

Colpi Width: 2.5 µm

Fertility: 84 %

4.2.9 Pollen grains of *Olea ferruginea* Royle

Pollen type: Monad, Psilate and Tricolpate

Shape of Pollen grains:

In Polar view: Circular

In equatorial view: Spheroidal to prolate.

Dimensions:

Equatorial diameter: 15.62 µm (12.5 µm -17.5 µm)

Polar diameter: 19.16 µm (17.5 µm -20 µm)

P/E ratio: 1.22 µm

Exine Thickness: 2.5 µm

Colpi Length: 2.5 µm

Colpi Width: 2.5 µm

Fertility: 95%

4.2.10 Pollen grains of *Pinus roxburghii* Sargent

Pollen grains consist of Two Sacs and Pollen body (Corpus).

Exine Thickness: 2 µm

Corpus Diameter: 46- 49 µm

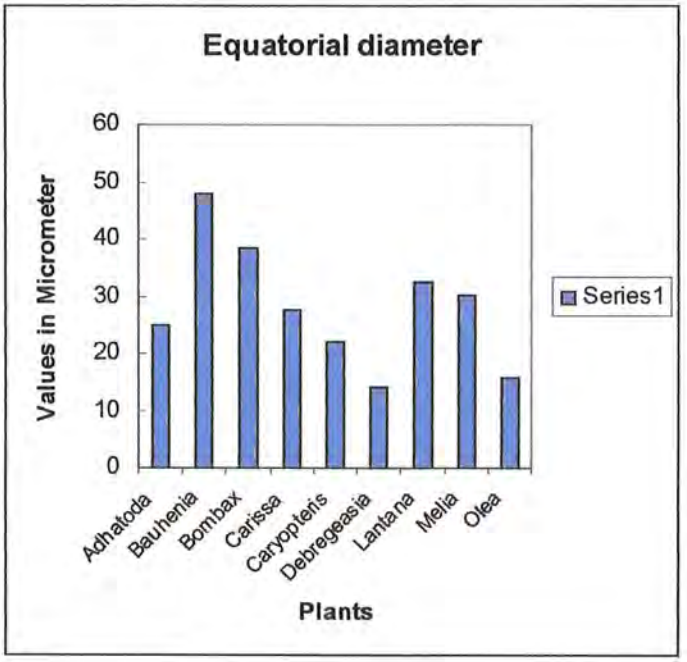
Height of Saccus: 16 -20 µm

Width of Saccus: 23 -27 µm

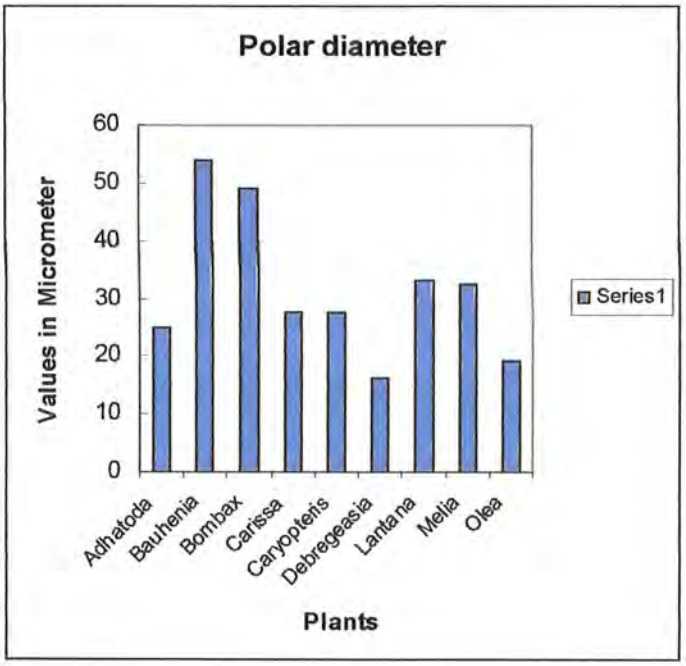
Table: Qualitative and Quantitative data on pollen features

S.No	Taxa	Polar diameter (µm)	Equatorial diameter (µm)	P\E Ratio (µm)	Exine thickness (µm)	Shape in polar view	Shape in equatorial view	Type	Colpi length (µm)	Colpi Width (µm)	Sculpturing	%age fertility
1.	*Adhatoda vesica* Nees	25	25	01	2.5	near to tubular, Circular	Prolate	Bicolpate, tricolpate	2.5	2.5	psilate	92.30
2.	*Bauhenia variegata* L.	54(50-60)	47.85 (32.5-75)	1.12	2.5	Circular	Subulate to Prolate to Perprolate	tricolpate	5	2.5	psilate	94
3.	*Bombax ceiba* L.	49.10(46.25-50)	38.5(35-40)	1.27	1.25	Circular	Subprolate to Prolate	tricolpate	2.5	5	psilate	92
4.	*Carissa opaca* Stapf ex Haines	27.5(20-30)	27.5 (22.5-30)	1	1.25	Circular	Subulate to Subprolate	tricolpate	1.75	1.56	psilate	90
5.	*Caryopteris grata* Benth.	27.5(20-35)	21.87 (16.25-27.5)	1.25	1.25	Circular	Spheroidal to perprolate	tricolpate	1.25	1.25	psilate	80
6.	*Debregeasia salicifolia* (D.Don) Rendle	16.25(15-17.5)	14(10-15)	1.16	1.25	Circular	Spheroidal to Perprolate	tricolpate	2-5	2-5	psilate	96
7.	*Lantana camara* L.	33(27.5-40)	32.5 (27.5-42.5)	1.01	2.5	Circular, Semiangular to Rectangular	Subprolate, Prolate-Spheroidal	Tricolpate, Tetra-colpate	2.5	2.5	psilate	85
8.	*Melia azedarach* L.	32.5(30-40)	30.2(22-34)	1.07	2	Circular to Semiangular	irregular	tricolpate	2.5	2.5	psilate	84
9.	*Olea ferruginea* Royle	19.16(17.5-20)	15.62 (12.5-17.5)	1.22	1.25	semi-angular	spheroidal to prolate	tricolpate	2.5	2.5	Psilate	98

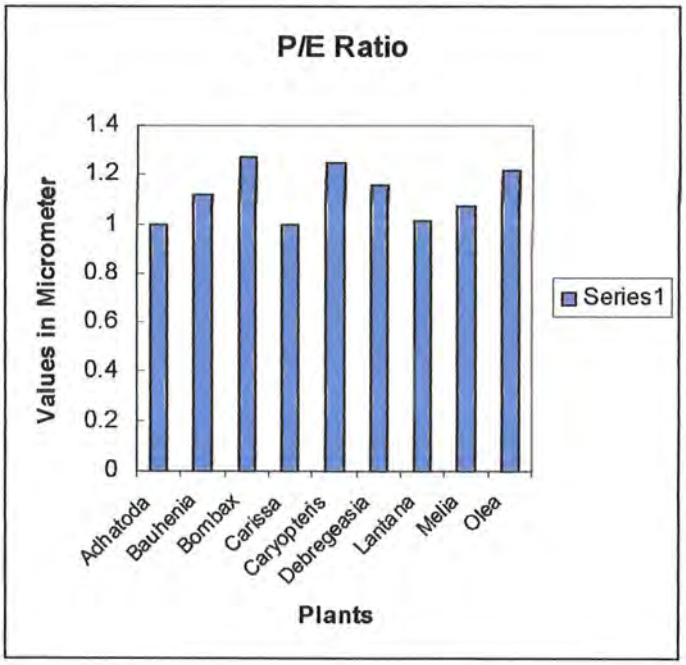
Graph Representing Equatorial Diameter of the Studied Plants



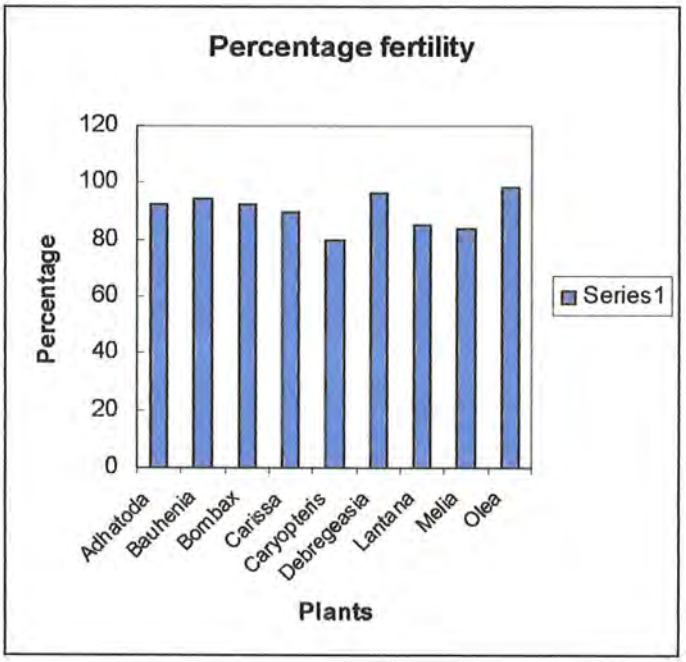
Graph Representing Polar Diameter of the Studied Plants

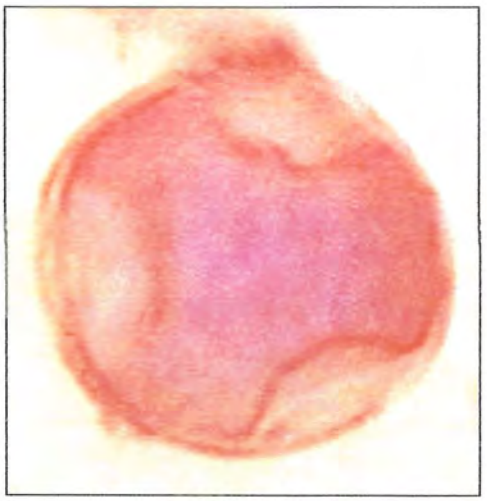


Graph Representing P/E ratio of the Studied Plants

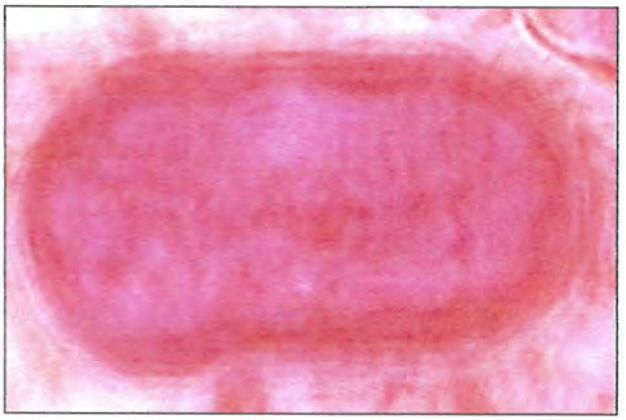


Graph Representing Percentage Fertility of the Studied Plants



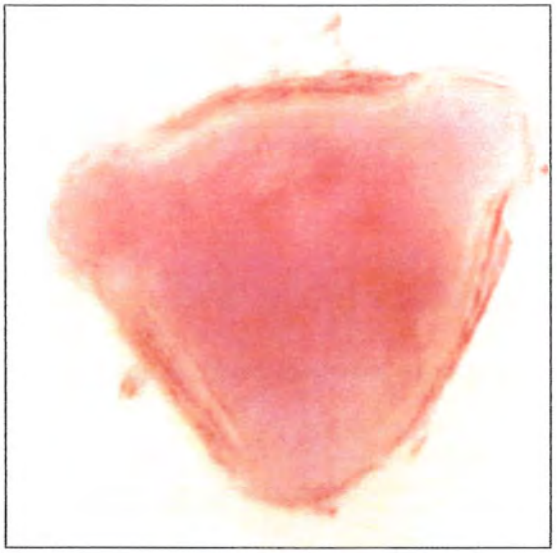


A **Polar view**

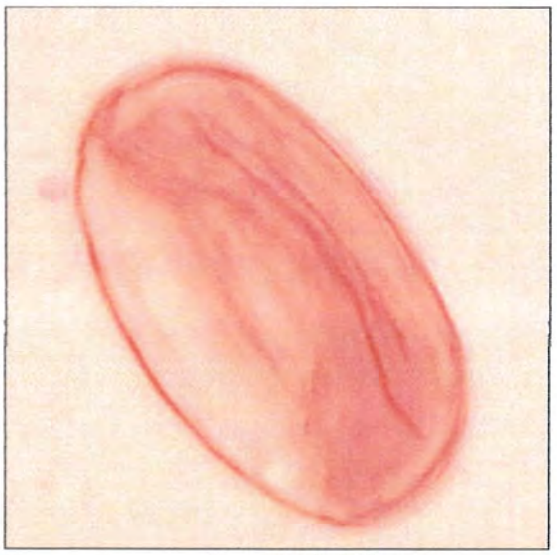


B **Equatorial view**

Plate I: Pollen light Micrograph of *Adhatoda vasica* Nees

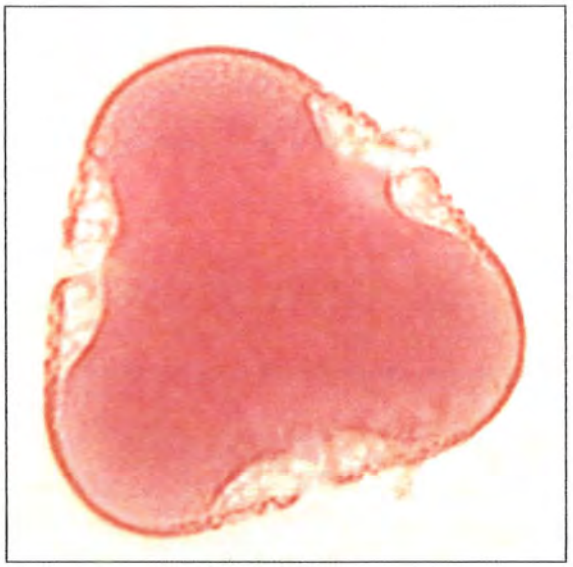


A **Polar view**



B **Equatorial view**

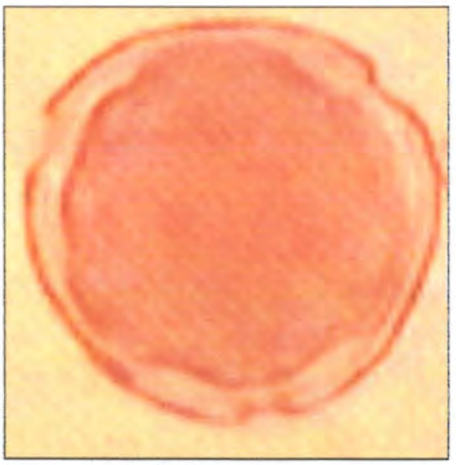
Plate II: Pollen light Micrograph of *Bauhenia variegata* L.



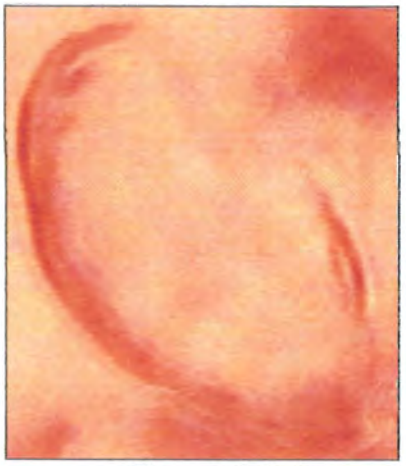
A **Polar view**

B **Equatorial view**

Plate III: Pollen light Micrograph of *Bombax ceiba* L.

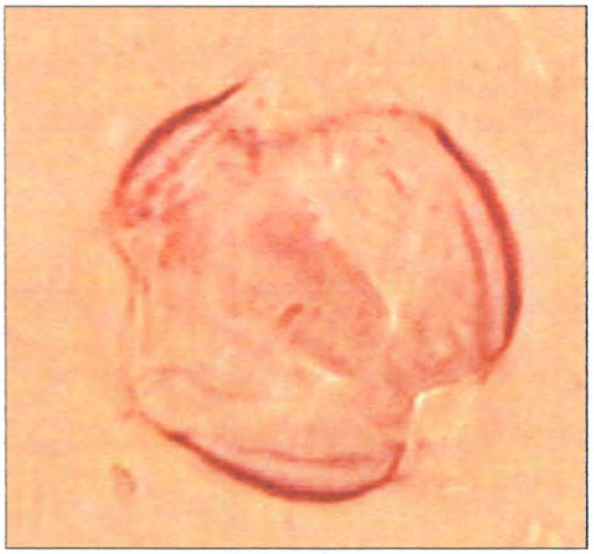


A **Polar view**

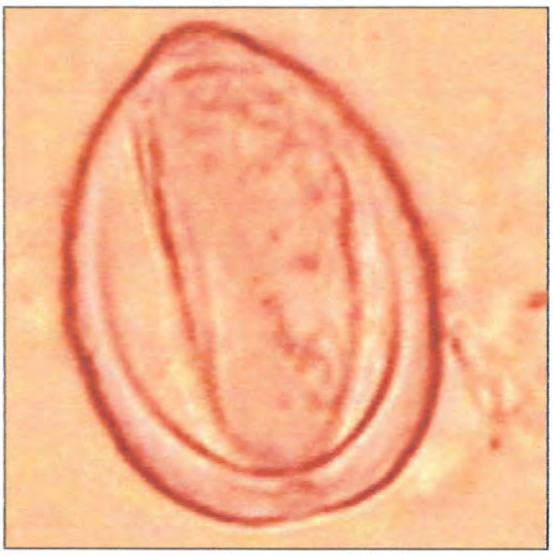


B **Equatorial view**

Plate IV: Pollen light Micrograph of *Carissa opaca* Stapf ex Haines

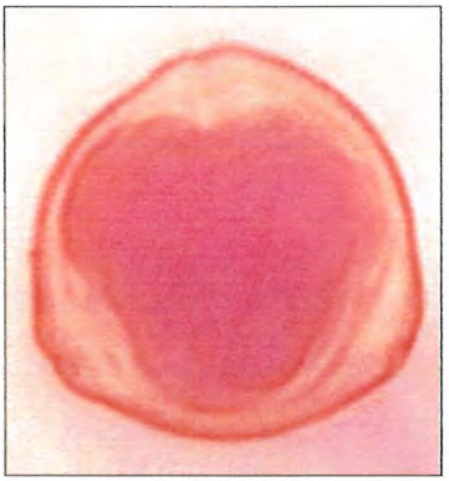


A **Polar view**

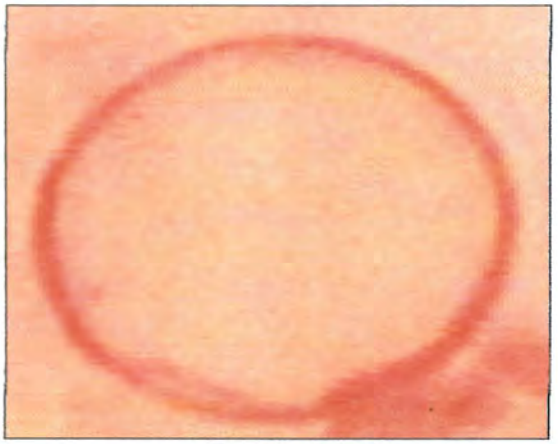


B **Equatorial view**

Plate V: Pollen light Micrograph of *Caryopteris grata* Benth.

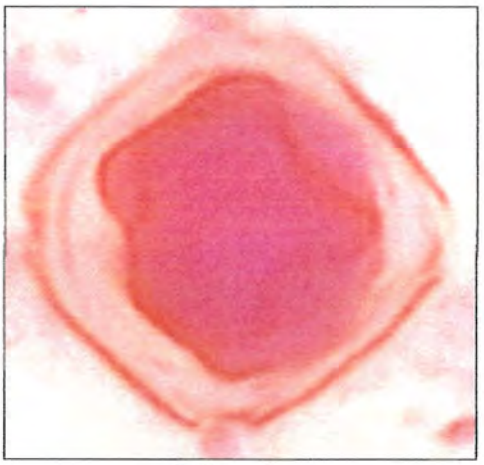


A **Polar view**

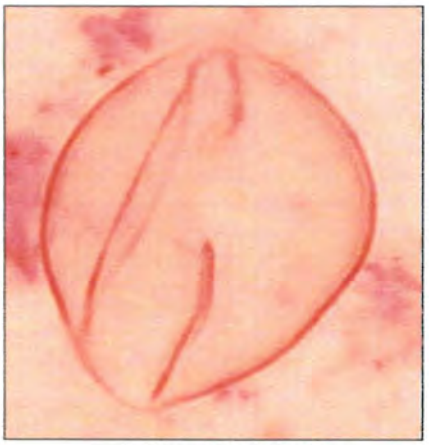


B **Equatorial view**

Plate VI: Pollen light Micrograph of *Debregeasia salicifolia* (D.Don) Rendle

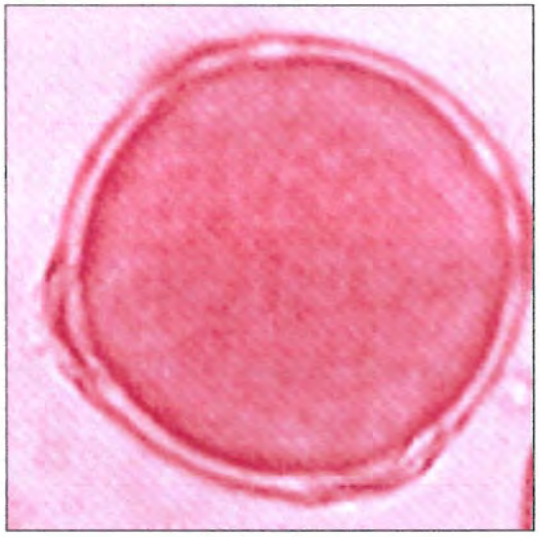


A **Polar view**

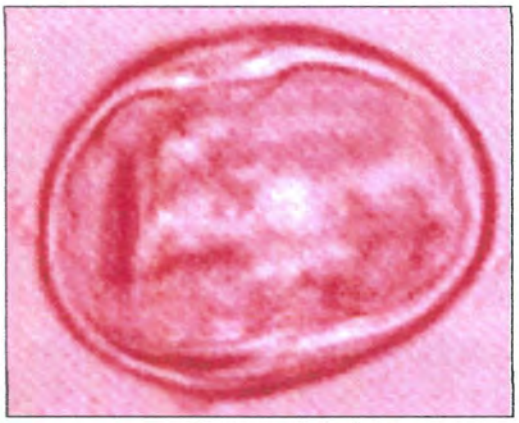


B **Equatorial view**

Plate VII: Pollen light Micrograph of *Lantana camara* L.

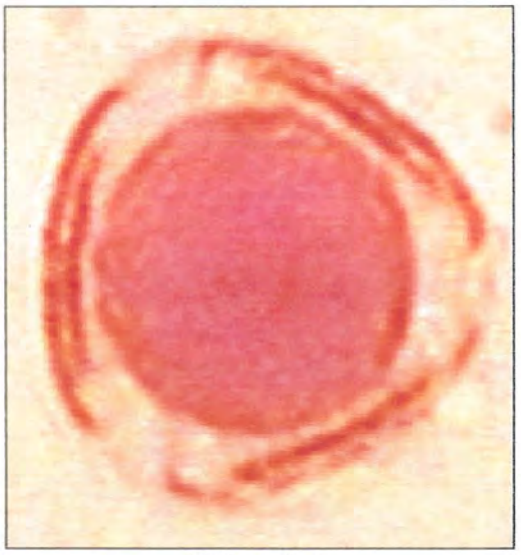


A **Polar view**

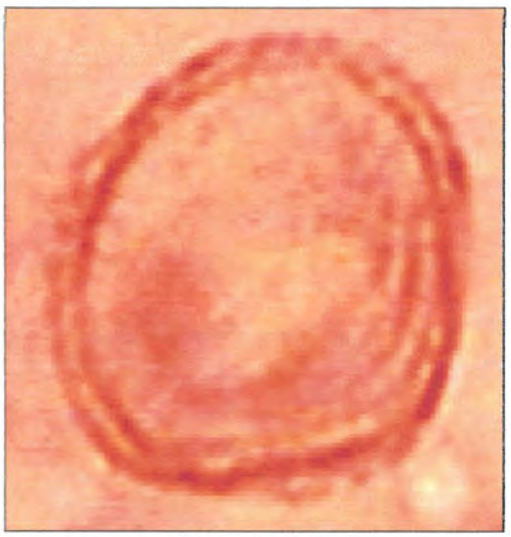


B **Equatorial view**

Plate IX: Pollen light Micrograph of *Melia azedarach* L.

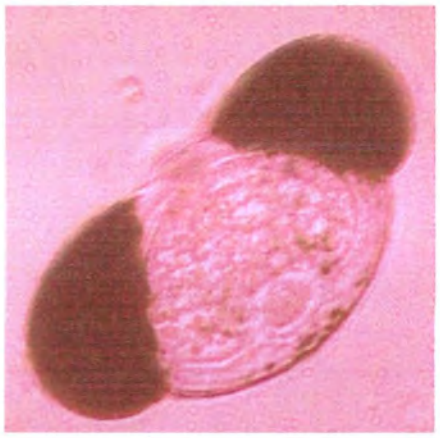


A **Polar view**

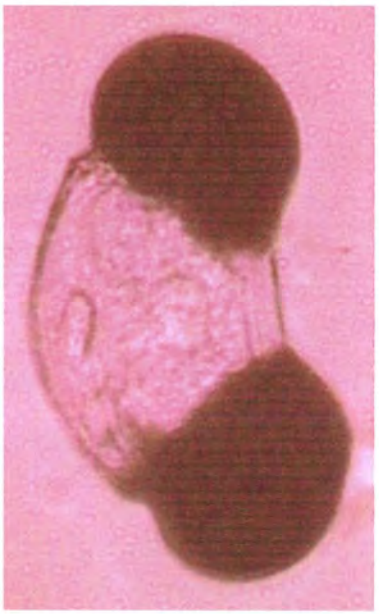


B **Equatorial view**

Plate X: Pollen light Micrograph of *Olea ferruginea* Royle



A



B

Plate X: Pollen light Micrograph of *Pinus roxburghii* Sargent

CHAPTER 05

DISCUSSION

DISCUSSION

5.1 ANTIBACTERIAL STUDIES OF SELECTED MEDICINAL PLANTS

Pakistan is a rich source of medicinal plants and people are willing to practice traditional medicine. Since Unani system of medicine, is now revived in recognition of unparallel contribution of Arabs and Greeks, closely associated with Muslims. This system of medicine is gaining revival in Pakistan and many Muslim countries, in the name of Tibe-Islami.

Among large number of plants, *Adhatoda vasica* Nees, *Bauhenia variegata* L., *Bombax ceiba* L., *Carrisa opaca* Stapf ex Haines, *Caryopteris grata* Benth., *Debregeasia salicifolia* (D.Don) Rendle, *Lantana camara* L., *Melia azedarach* L., *Olea ferruginea* Royle, *Phyllanthus emblica* L., *Pinus roxburghii* Sargent appear to have potential for testing as a plant of high medicinal values for various microbial activities as well other medicinal activities. These plants are abundantly found in Pakistan. Therefore easily accessible.

Agar well diffusion and Disc diffusion methods are very commonly used for the screening of antimicrobial activities. In these studies we used Agar well diffusion method for determining antimicrobial activity of the methanolic extracts of the selected medicinal plants. Similar technique has been used by some other workers like Navarro in 1996.

The culture medium used in the current study was Nutrient Agar, which extensively used by microbiologist for culturing routine pathogens. This medium proved to be efficient enough for supporting the growth of all pathogens used in this study i-e *Staphylococcus aureus, Escherichia coli, Pseudomonas auregenosa, Micrcoccus luteus.*

Methanol is used as solvent for the extraction of the crude extract. Methanol is the better solvent for the extraction of plant parts. Almost all the chemical compounds which have better antimicrobial activity are soluble in the methanol. (Chandrasekaran

and Venkatesalu, 2004). Study revealed that *Phyllanthus emblica* L. has the greater antimicrobial activity than rest of the Plants.

Pokhrel *et al.*, (2001) reported the antimicrobial activity of the *Bauhinia variegata* L. against *Bacillus subtilis, Pseudomonas aeruginosa, Salmonella typhi, Shigella dysenteriae, Staphylococcus aureus and Vibrio cholerae*. The largest zone of inhibition (18 mm) was found to be exhibited against *Bacillus subtilis*. Rajkapoor *et al.*, (2005) reported that Oral administration of ethanol extract of *Bauhinia variegata* L. (250 mg/kg) effectively suppressed liver tumor induced by DEN as revealed by decrease in DEN induced elevated levels of serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), total bilirubin, gamma glutamate transpeptidase (GGTP), lipid peroxidase (LPO), glutathione peroxidase (GPx) and glutathione S-transferase (GST). The extract produced an increase in enzymatic antioxidant (superoxide dismutase and catalase) levels and total proteins when compared to those in liver tumor bearing rats.

Dar *et al.*, (2005) reported Mangiferin, 2-β-D-glucopyranosyl-1,3,6,7-tetrahydroxy-9H-xanthen-9-one, obtained directly from methanolic extracts of *Bombax ceiba* L. leaves in substantial amounts demonstrated strong antioxidant activity (EC50 5.8±0.96 µg/ml or 13.74 µM) using DPPH assay comparable to rutin, commonly used as antioxidant for medical purposes. Faizi S and Ali M.(1999) reported Shamimin a new flavonol C-glycoside has been isolated as a pale yellow powder from the ethanolic extract of fresh, undried leaves of *Bombax ceiba* L. Its structure has been elucidated as 2-(2, 4, 5-trihydroxyphenyl)-3, 5, 7-trihydroxy-6-C- glucopyranosyloxy-4H-1-benzopyran-4-one through extensive spectroscopic methods (IR, mass, 1H- and 13C-NMR), and 2D-NMR experiments. Shamimin showed antimicrobial activity against a few bacteria and fungi.
Chun-lin Long *et al.*, (2003) reported that the decoction of root and whole plant of caryopteris paniculata are used for Diarrhea, skin itch, diminish inflammation.

Akbar *et al.*, (2000) reported a new triterpene, 3_-19_dihydroxy-30-norurs-12-ene 1, has been isolated from the methanolic extract of *Debregeasia salicifolia* (D.Don) Rendle stem.

Kumar *et al.*, (2006) reported that *Lantana camara* L. exhibit significant antimicrobial activity and properties that support folkloric use in the treatment of some diseases as broad-spectrum antimicrobial agents. This probably explains the use of these plants by the indigenous people against a number of infections. Oliveira *et al.*, (2006) screened 26 plants collected in the Brazilian southeast region, to identify plant extracts with antibacterial properties against *Aeromonas hydrophila, Bacillus subtilis, Pseudomonas aeruginosa and Staphylococcus aureus*. Initially, the agar diffusion method was employed. Then, those extracts presenting activity were submitted to a broth microdilution assay to determine the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). It was observed that 13 of the tested extracts showed antibacterial activity. The best results were obtained with those from *Lantana lilacina* and *Phyllanthus tenellus*.

Markin *et al.*, (2002) reported that olive leaves exhibit 'in vitro' antimicrobial activity against bacteria and fungi.

Ghosh *et al.*, (2007) reported the antibacterial activity of *Phyllanthus emblica* L. against five pathogenic bacteria *Staphylococcus aureus, Bacillus subtilis, Escherichia coli Proteus vulgaris and Enterobacter aerogenes*. Active extracts of *P. emblica* have been shown to possess several pharmacological properties, e.g., analgesic, anti-inflammatory, antioxidant and chemoprotective activities.

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All the plants we have studied display antibacterial activity against Gram Positive bacteria except few Plants showed activity against *Pseudomonas aerugenosa*, all the plants have no activity against Gram Negative bacteria. Chandrasekaran and Venkatesalu (2004) also reported more antibacterial activity of *Syzygium jambolanum* seeds against Gram Positive strains than Gram Negative strains.

5.2 PALYNOLOGICAL STUDIES OF SELECTED MEDICINAL PLANTS

Most of the taxonomist used morphological characters for the identification of plant species. Recently Palynology also proved to be the best tool for the identification of plant species. (Diaz and lifante, 1991).

Over all view of the present palynological study was that the largest polar diameter 54µm was observed in *Bauhenia variegata* L. and smallest 16.25 µm in *Debregeasia salicifolia* (D.Don) Rendle. In all the species the pollens were tricolpate except *Lantana camara* L. where tetracolpate is also observed. *Lantana camara* L. and *Caryopteris grata* Benth. belongs to Verbenaceae but displayed variation in Pollen Morphology. Pollen grain of *Caryopteris grata* Benth. is circular in polar view and spheroidal in equatorial view and the Pollen grain of *Lantana camra* L. is circular to semi-angular in pollar view and subprolate to prolate–spheroidal in equatorial view (Zafar et al., 2006). The results displayed rectangular shape in *Lantana camara* L. in pollar view and subprolate to prolate–spheroidal in equatorial view. In the same way polar and equatorial diameter of *Lantana camara* L. is about 33 µm and 32.5 µm of *Caryopteris grata* Benth. is 27 µm and 21 µm. So it shows clear difference between species of the same genera.

Pollen grains of the *Bruvaisiu berlandieriunu* (Nees) of family Acanthaceae is bicolpate polar view obtusely rectangular (Robert W. Scotland 1992). *Adhatoda vasica* Nees (Acanthaceae) has also such pollen characters. Pollen grains of *Melia azedarach* L. is circular and semiangular in polar view and usually irregular in equatorial view. Aftab and Perveen (2006) reported Prolate to spheroidal shape of equatorial view in *Melia azedirahta*. Pollen grains of *Carissa opaca* Stapf ex Haines (Apocynaceae) are tricolpate and subulate to subprolate in equatorial view. Aftab and Perveen (2006) reported tricolporate and prolate type of pollen in equatorial view of *Plumeria acutifolia* (Apocynaceae). Pollen grain of *Bauhenia variegata* L. are subulate to prolate shape in equatorial view and nearly circular in polar view. Perveen and Qaiser (1997) reported oblate-spheroidal shape in Polar view and sub-prolate in an equatorial view.

Pinus roxburghii Sargent has a bisaccate (or vesiculate) pollen grain consisting of a body with two latarally-placed bladders (sacca, vesicles), which is the characteristic

of the Gymnosperms. The vesicles helps in the distribution of the pollens to far distant places and to reach the Female cones.

Pollen fertility indicates an important tool in taxonomy used to distinguish the putative hybrid from the parent plants and is useful to determine the degree of fertility(stain ability)in plants grown under unfavorable conditions (Lawrence, 1969).

Pollen fertility range from 80-96%, which indicates the establishment of all the species of Medicinal plants. The highest percentage fertility 96 was observed in *Olea ferruginea* Royle and lowest 80 in *Caryopteris grata* Benth.

5.3 CONCLUSION

The result of the Antibacterial assay confirm the great potential of medicinal plants for the production of bioactive compounds and are useful for rationalizing the use of medicinal plants in primary health care. The phytochemical characterization of the extracts, the identification of responsible bioactive compound and quality standard are necessary.

Palynology also proved to be the best tool for the identification of plant species at lower taxa to solve the Taxonomic problems. Pollen fertility indicates an important tool in taxonomy used to distinguish the putative hybrid from the parent plants.

CHAPTER 06

REFERENCES

REFERENCES

- Abdulla, P. 1972b. In: Nasir, E. and S. I. Ali (Eds.): Flora of Pakistan, "Meliaceae" No. 17.
- Aftab, R., Perveen, A. 2006. A palynological Study of Some Cultivated Trees from Karachi. Pak. J. Bot., 38(1): 15-28.
- Akbar Erum, Riaz Muhammad, Malik Abdul. 2000. Ursene Type Nortriterpene from Debregeasia salicifolia. Fitoterapia 72Ž2001.382_385.
- Akilandeswari, S., Ganapathy, S., Kumarasundari, S.K. And Valarmathi, R. 2003. Neem (*Azadirachta Indica*) Extract as Antimicrobial Agents Against Bacteria *Proteus Vulgaris* and Fungi *Candida Albicans*. Hamdard Medicus. XL VI(1): 21 – 22. Ali, M. and Singh, S. 2003. Biological of Some Indian Medicinal Plants. Phytochemistry and Pharmacology. 239-245.
- Ali, S. I. 1973. In: Nasir, E. And S. I. Ali (Eds.): Flora Of Pakistan, "Caesalpinaceae" No. 54.
- Anandaprakash, R.L. 1978. Natural Constituents of *Bauhinia Variegata*. Journal of Research In Indian Medicine, Yoga And Homeopathy 13, 96–97.
- Anila. L, Vijayalakshmi N.R. 2001. Flavonoids From *Emblica Officinalis* and *Mangifera indica* effectiveness for dyslipidemia. Journal of Ethnopharmacology 79 (2002) 81–87.
- Ayafor, J.F., M.H.K. Tchuendem and B. Nyasse. 1994. Novet Bioactive Diterpenoids from Aframonium Aulacocarpos. J. Nat Prod. 57: 917-923.
- B. Rajkapoor, B. Jayakar, N. Murugesh, D. Sakthisekaran. 2005. Chemoprevention and Cytotoxic Effect of *Bauhinia Variegata* against N-Nitrosodiethylamine Induced Liver Tumors And Human Cancer Cell Lines. Journal Of Ethnopharmacology 104 (2006) 407–409.
- Baquar, S. R. H. 1989. Medicinal and Poisnous Plants Of Pakistan. Printas Karachi, Pakistan, P.506.

- Batista, O., A. Duarte, J. Nascimento, and M.F. Simones. 1994. Structure and Antimicrobial Activity Of Diterpense from The Roots Of *Plectanthus hererocensis*. J. Nat. Prod. 57: 858-861.
- Borris, R.P. 1996. Natural Products Research: Perspectives from a major Pharmaceutical company. J. Ethnopharmacol. 51: 29-38.
- Brantner, A., and E. Grein. 1994. Antibacterial Activity of Plant Extracts Used Externally in Traditional Medicine. J. Ethnopharmacol. 44: 35-40. Burdick, E.M. 1971.
- Carr, A.C, Zhu, B.Z & Frei, B. (2000), Potential Antiathero-Genic –Echanis-of Ascorbate (Vitamin C) and Alpha Tocopherol (Vitamin E). Circulation Research , 87,349-354.
- Chandrasekaran, M. And Venkatesalu, V. (2004). Antibacterial and Antifungal Activity Of *Syzgium Jambulanum* Seeds. 91: 105-108.
- Cichewiez., R.H., And P.A. Thrope. 1996. The Antimicrobial Properties of Chile Pepers (*Capsium* Species) and their uses in Mayan Medicine. J. Ethnopharmacol. 52: 61-70.
- Coe, S.D. 1994. America's First Cuisines. University Of Taxas Press, Austin. Del Camp, J., Amoit, M.J.And Nguyen, C. 2000. Antimicrobial Effect of Rosemary Extracts. Journal of Food Protection. 63(10): 1359-1368. Dorman, H.J.D. and Deans, S.G. 2000.
- CSIR 2003. The Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products. Raw Materials Vol. I-XI Revised Edition. Publication and Information Directorate, Council Of Scientific and Industrial Research (CSIR), New Delhi India.
- Dar Alısana, Faizi Shaheen, Naqvi Sabira, Roome Talat, Zikr-Ur-Rehman Sadia, Ali Muhammad, Firdous Sadiqa, S. T. Moin, 2005. Analgesic and Antioxidant Activity Of Mangiferin and its derivatives: The Structure Activity Relationship. : Biological & Amp; Pharmaceutical Bulletin, 2005 (Vol. 28) (No. 4) 596-600.

- Darout, I., Cristy, A., Skaug, N. And Egeberge, P. 2000. Identification and Quantification of Some Potentially Antimicrobial Anionic Components in Miswak Extract. Ind. J. Pharm. 32: 11-4.
- Devakumar, C. And Sukhdev, In Neem (Eds Randhawa And Parmar, B. S.), 1996, 2nd Edn, Pp. 77–110.
- Diaz, Z.I. And Lifante, Z.D. 1991. *Asphodelus Cirerae* A Forgotton Species Of *Asphodelus* Sec, Verina (Liliaceae), Morphological, Palynological, Karyological and Ecologial Characterization, Flora Mediterranea, 1, 87-109.71.
- Diehl, M.S., Kamanzi Atindehou, K., Tere, H., Betschart, B., 2004. Prospect for anthelminthic plants in the Ivory Coast using ethnobotanical criteria. Journal of Ethno pharmacology. 95, 277-284.
- Doshi, J. J. 1983. Int. J. Crude Drug Res., 21: 173 (www.Sirismpex.Com/Adhatoda.Htm).
- Duret, S. & Paris, R.R. 1977 Plants Of Nepal. V. The Flavonoids of Various *Bauhinia, B. Valhii, B. Variegata, B. Malabarica*; Liguminosa Caesalpina. Planta Medica Phytologica Et Therapeutica 11, 213–221.
- Erdtman, G. 1952 Pollen Morphology & Plant Taxonomy. Angiosperms. Chronica Botanica Co., Waltham, Massachuselts.
- Erdtman, G.1952. Pollen Morphology and Plant Taxonomy. Angiosperms. Almqvist and Wiksell, Stockholm.
- Faegri, Knut (1973). "*In Memoriam O. Gunnar E. Erdtman*". *Pollen Et Spores* 15: 5-12.
- Faizi S, Ali M. 1999. Shamimin: A New Flavonol C-Glycoside from Leaves of *Bombax Ceiba.* : Planta Med. 1999 May; 65(4):383-5.
- Fransworth, N. R, and R.W, Morris. 1976. Higher Plants: The Sleeping Giant of Drug Development. Am. J. Pharm. March / April -46.).
- Gamble, J.S. 1933. Flora of The Presidency Of Madras V.Pp. 769-962.
- Gao. J, Han. G, Photochemistry 44 (1997) 759.

- Geissman, T.A. 1963. Flavonoid Compounds, Tannins, Lignins and Related Compounds, P.265. In M. Florkin and E.H. Stotz (Ed), Pyrrole Plgments, Isoprenoid Compounds And Phenolic Plant Consitituents, Vol. 9. Elsevier, New York, N.Y Hayat, M.M., Ansar, S.H., Ali M. And Wani, F.A. 2002. Antimicrobial Activity of *Betula Pendula*. Hamdard Medicos. XLV(1): 41-43. Karpagam, S.K.S., Valarmathi, R., Akilandeswari, S. And Reckmani, K. 2001.
- Ghosh Anupam, Das Bidus Kanti, Roy Arup, Mandal Biplab, Chandra Goutam,. 2007. Antibacterial Activity of Some Medicinal Plant Extracts. J Nat Med (2008) 62:259–262.
- Govindachari, T. R., Curr. Sci., 1992, 63, 117–122.
- Green Wood .D. (1982) Antimicrobial Chemotherapy . OMP. London. HIRTE, M. (2002). Benefits of Mango For The Human Health. Http://Www.Preda.Org/Archives/2002/R02111802.Html (Last Accessed 2nd January.2007).
- Grohman, F. 1974. In: Nasir, E. and S. I. Ali (Eds.); Flora Of Pakistan, "Oleaceae" No. 59.
- Gupta, A.K., Chauhan, J.S., 1984. Constituents from the stem of *Bauhinia Variegata.* National Academy Of Science Letters 7, 15–16.
- Gupta, A.K., Vidyapati, T.S. & Chauhan, J.S. 1979. 5-7 Dihydroxy Flavonone-4-O-A-L-Rhamnopyranosyl B-D-Glucopyranoside of *Bauhinia Variegata.* Indian Journal of Chemistry 18, 85–86.
- Gupta, A.K., Vidyapati, T.S. & Chauhan, J.S. 1980. Chemical Examination of the stem of *Bauhinia Variegata.* Planta Medica 38, 174–176.
- Hakim, M.H.A. 1956. Kitab Al Mufaradat. 2nd Ed. Sheikh Ghulam Ali & Sons Lahore.
- Http://Www.Globalherbalsupplies.Com/Herb-Informaton/Adhatoda-Vasica.Htm.
- Hyde, H.A.; D.A. Williams (1944). "The Right Word.". Pollen Analysis Circular 8: 6.

- Indica—Effectiveness for Dyslipidemia. Journal Of Ethnopharmacology 79 (2002) 81–87
- Jaffery, C 1964. A note on Pollen Morphology is Cucurbitaceae. Kew Bull. 17:473-477.
- Jafri, S. M. H. And A. Ghafoor. 1974. In: Nasir, E. and S. I. Ali (Eds.): Flora of Pakistan, "Verbenaceae" No. 77.
- Jain Anita, Katewa S.S., Chaudhary B.L., Galav Praveen. 2003. Folk Herbal Medicines Used In Birth Control and Sexual Diseases by Tribals of Southern Rajasthan, India. Journal of Ethnopharmacology 90 (2004) 171–177.
- Jansonius, J; D.C. Mcgregor (1996). "Introduction, Palynology: Principles and Applications". *AASP Foundation* 1: 1-10.
- Jensen Soren Rosendal, Franzyka Henrik, Wallander Eva. 2002. Chemotaxonomy of the Oleaceae: Iridoids as Taxonomic Markers. Phytochemistry 60 (2002) 213–231.
- Juang Fu-Chiang, Chen Yu-Fang Lin, Fuh-Mei and Huang Keh-Feng. 2005. Constituents from The Leaves Of *Lantana Camara*. J Chin Med 16(2-3): 149-155, 2005.
- Khan M.R, Kihara. M, Omoloso A.D. 2000. Antimicrobial Activity of *Horsfieldia Helwigii* and *Melia Azedarach.*
- Khan, R. 2005. Studies On The Pollen Morphology Of The Genus Sisymbrium and Monotypic Genera *Atelanthera* And *Acryosperma* (Brassicaceae) From Pakistan. Pak. J. Bot., 37 (1):15-22.
- Khuzaie, R., Rashan, L. And Al-Douri N. 1999. Invitro Antimicrobial Activity of Crude Aqueous Extracts of some Local Medicinal Plants Growing In Jordan. Jordanian J. Appl, Sci, 1: 46-52.
- Kirtikar KR, Basu BD (1935) *Terminalia Chebula.* In: Kirtikar KR, Basu BD (Eds) Indian Medicinal Plants, Vol 1, 2nd Edn. Lalit Mohan Basu Publications, Allahabad, Pp 1020–1023.

- Koneman, E. W., Allen, S. D., Dowell, V. R., Janda, W. M., Soomers, H. M And Winn, W. C. (1988). Color Atlas and Text Book Of Diagnostic Microbiology, (3rd Ed). Pp 479-481.
- Koul, O., Isman, M. B. And Ketkar, C. M., Can. J. Bot., 1990, 68,1–11.
- Kraus, W., In the Neem Tree: Source Of Unique Natural Products For Integrated Pest Management, Medicine, Industry And Other Purposes (Ed. Schmutterer, H.), 1995, Pp 35–88.
- Kumar V. Prashanth, Chauhan Neelam. S, Padh Harish, Rajani. M. 2006. Search for Antibacterial and Antifungal Agents from Selected Indian Medicinal Plants. Journal of Ethnopharmacology 107 (2006) 182–188.
- Lawrence, H. M. 1969. Taxonomy of Vascular Plants. Macmillan, New York.
- Lewis, W.H., and M.P. Elyin-Lewis. 1995. Medicinal Plants as Sources Of New Therapeutics. Ann. Mo. Bio. Gard. 82: 16-24.
- Li Xiaodong. 1998. Recent Studies On Insecticidal Activities Of Limonoids From Meliaceous Plants.
- Loganathan, V., Manimaram, S., Akilandeswari, S. and Rajasekaran, A. 2001. Antimicrobial Activity Of Root Extracts Of *Achyranthes Aspera, Pteronia Incana* and *Rosmarinus Officinalis* on Selected Bacteria and Yeast Strains. Letters in Applied Microbiology. 28: 291-296
- Long Chun-Lin, Li Rong. 2003. Ethnobotanical Studies on Medicinal Plants Used By The Red-Headed Yao People In Jinping, Yunnan Province, China. Journal of Ethnopharmacology 90 (2004) 389–395.
- Luo Xiao-Dong, Wu Hua-Shao, Ma Bao-Yun, Wu Gang-Da. 2000. A New Triterpenoid from *Azadirachta Indica*. Fitoterapia 71_2000.668-672.
- Marinez, M.J , Betancourt, J., Alonso –Gonzalez. N., Jaurengui, A., 1996. Screening of some Cuban Medicinal Plants for Antimicrobial .Journal Of Pharmacoglogy 52, 171-174.
- Markin. D, Duek. L, and Berdicevsky. I. 2002. In Vitro Antimicrobial Activity Of Olive Leaves. Mycoses, 46, 132–136.

- Martin: Lucia H., R. Souza Carla, B. Marques Patricia, Calixto João B, Yunes Rosendo A., and Souza Diogo O.1999. Compounds Extracted From *Phyllantus* and *Jatropha Elliptica* Inhibit The Binding Of [3H] Glutamate And [3H] GMP-PNP In Rat Cerebral Cortex Membrane. Neurochemical Research. Vol. 25, No. 2, 2000, Pp. 211–215.
- Mcfadden, R. 1995. Comparison of the Inhibitory effects of various Plants Extracts on the Growth of *C. Albicans* Invitro. Eur, J. Herbal Medicine. (3): 26-31.
- Meral, G.E. And Karabay, N.U. 2002. In Vitro Anitacterical Activities of Three Hypericum Species From West Anatolia. Turkish Electonic Journal of Biotechnology. Special Issue: 6-10.
- Naba Raj Pokhrel1, R.P. Adhikari and M.P. Baral. 2001. In-Vitro Evaluation of the Antimicrobial Activity of *Bauhinia Variegata*, Locally Known As Koiralo. World Journal Of Microbiology & Biotechnology 18: 69–71.
- Nadkarni, K. M. 1976. Indian Materia Medica. Popular Parakashan Bombay, India. 1: 1252-1253.
- Nakatania Munchiro, Chun Huangb Ruo, Okamuraa Hiroaki, Iwagawaa Tetsuo and Tadera Kenjiro. 2001. Degraded Limonoids from *Melia Azedarach.*
- Narvarro, V., M.L., Villarreal, G. Rojas, and X. Lozoya. 1996. Antimicrobial Evaluation of some Plants used in Mexican Traditional Medicine for the Treatment of Infectious Diseases. J. Ethnopharmacol. 53: 143-147.
- Nasir, Y. J. and Rafiq, R. 1995. Wild Flowers of Pakistan. Oxford University Press, Pakistan.
- Nazimuddin, S. and M.Qaiser. 1983. In: Nasir, E. and S. I. Ali (Eds.): Flora of Pakistan, "Apocynaceae" No.148.
- Ogundipe, O.T. and Oladipo, O.B, 2001. The Phytochemical and Antimicrobial Studies of *Persea Americana* Mill. Hamdard. XLIV(4): 44-50.
- Oliveira Denilson.F, Pereira Aline. C, Figueiredo Henrique C.P, Carvalho Douglas. A, Silva Greiciele, Nunes Alexandro. S, Alves Dejane. S, Carvalho

Hudson W.P. 2006. Antibacterial Activity of Plant Extracts from Brazilian Southeast Region. Fitoterapia 78 (2007) 142–145.

- Oliver-Bever, B. 1986. Medicinal Plants in Tropical West Africa. Cambridge University Press, New York, N.Y.
- Pandey ND, Singh M, Tewari GC. Indian J Ent 1977; 39: 60.
- Perveen Anjum and Qaiser Mohammad. 2003. Pollen Flora of Pakistan-XXII. Oxalidaceae. Pak . J. Bot ., (1):3-6, (2003).
- Perveen Anjum and Qaiser Muhammad. 1997. Pollen Flora of Pakistan -X- Leguminosae (Sub Family: Caesalpinioideae). Turkish Journal of Botany 22 (1998) 145-150.
- Perveen Anjum. 1998. Pollen Characters and Their Evolutionary Significance with Special Reference to the Flora of Karachi. Turkish. Journal of Botany. 22 (1998) 145-150.
- Pornpimon Mayachiew, Sakamon Devahastin. 2007. Antimicrobial and Antioxidant Activities of Indian Gooseberry and Galangal Extracts. Swiss Society of Food Science and Technology. Published By Elsevier Ltd.
- Qaiser, M.1978. In: Nasir, E. and S. I. Ali (Eds.): Flora of Pakistan, "Bombacaceae" No.119.
- R. R. Stewart, Ann. Cat. Vasc. Pl. W. Pak & Kash. 195.1972.
- Ritter , B., Coombs, R.F, Drysdale, R.B., Gardner, G.A. & Lunn, D.T(1996) Biology , Nelson Canada, British Columbia Edition , Ontario , Canada.
- Ronald, O. K. 2000. Pollen and Spores. 2nd Ed. American Association of Stratographic Palynologist. 13-21.
- Sakanata, S., Junega, L.R. And Taniguchi, M. 2000. Antimicrobial Effects of Green Tea Polyphenois on Thermophilic Spore Forming Bacteria. Journal of Biosciences and Bioengineering. 90(1): 81-85.
- Schultes, R.E. 1978. The Kingdom Of Plants, P.208. In W.A.R. Thomson (Ed)., Medicines From The Earth Mcgraw-Hill Book Co., New York N.Y. Singh, N.B. 1998. Current Concepts of Multidiscipline approach to the Medicinal Plants.

- Scotland Robert W. 1992. Pollen Morphology of Contortae (Acanthaceae) Botanical Journal of The Linnean Socieb (1993), 111: 471-504
- Selvakumar. G, Saha Supradip, Kundu. S. 2007. Inhibitory Activity of Pine Needle Tannin Extracts on Some Agriculturally Resourceful Microbes. Indian J. Microbiol. (September 2007) 47:267–270.
- Sheikh, M. I. 1993. Trees of Pakistan, Pictorial Printers (Pvt.) Ltd., Islamabad, Pakistan.
- Shinwari, M. I. And M. A. Khan. 1998. Ethnobotany of Margalla Hills National Park of Islamabad. Department of Biological Sciences, Quaid-I-Azam University, Islamabad-Pakistan. PASTIC Printing Press:1-100.
- Singh. A. K, Raghubanshi A.S, Singh J. S. 2002. Medical Ethnobotany of the Tribals of Sonaghati of Sonbhadra District, Uttar Pradesh, India. Journal of Ethnopharmacology 81 (2002) 31- 41.
- Subramanian, S.S., Nagarajan, S. And Sulochana, N. (1971): Euphorbiaceae Flavonoids of some Euphorbiaceous Plants. Phytochemistry 10: 2548–2549.
- Thomson, W.A.R. (Ed) 1978. Medicines from the Earth. Mcgraw-Hill Book Co., Maidenhead, United Kingdom.
- Tsuchiya, H., M. Sato T. Miyazaki, S. Fujiwara, S. Tanigaki, M. Ohyama, T. Tanaka, and M. Ilinum. 1996. Comparative Study on the Antibacterial Activity of Photochemical Flavanones against Methicillin-Resistant *Staphylocous aureus*. J. Ethnopharmacol. 50: 27-34.
- Vanden Berghe, D.A., A., A.J. Vlietinck and Hoof. 1986. Plant Products as Potential Antiviral Agents. Bull. Inst. Pasteur 84: 101-147.
- Wahab, S.M.A.E., Wassel, G.M., Ammar, N.M., Hanna, T., 1987. Flavonoid Constituents in the Different Organs of Selected *Bauhinia* Species and their effect n Blood Glucose. Herba Hungeria 26, 27–39.
- Warrier, P.K., Knambiar, V.P. & Amankutty, C.R. 1993 Indian Medicinal Plants: A Compendium of 500 Species, Vol. I. Madras, India: Orientlongman Limited. ISBN 0-86311464-4.Ology 95, 277–284.

- Watt JM, Breyer-Brandwijk MG. The Medicinal and Poisonous Plants of Southern and Eastern Africa. E & S Livington Ltd; 1962. P. 1947.
- Weber, R. W. 1998. Pollen Identification. Ann-Allery-Asthma-Immunol. 80(2):141-147.
- Yadav, S., Bladoria, B.K., 2005. Indian J. Chem. 44b, 2604.
- Yadava, R.N., Reddy, V.M.S., 2001. A New Flavone Glycoside, 5-Hydroxy-7, 30, 40, 50-Tetramethoxyflavone 5-O-D-Xylopyranosyl-L-Rhamnopyranoside from *Bauhinia Variegata.* Journal of Asian Natural Products Research 3, 341–346.
- Zafar. M, Ahmad Mushtaq and Khan Mir Ajab. 2006. Palynological Studies Of Verbenaceae From Margalla Hills, Islamabad, Pakistan. Pak. J. Pl. Sci., 12(1):21-25, 2006.
- Zhang, Y., And K. Lewis. 1997. Fabatins: New Antimicrobial Plant Peptides. FEMS Microbial. Lett. 149: 59-64.
- Zhao, Y.Y., Cui, C.B., Cai, B., Han, B., Sun, Q.S., 2005. J. Asian. Nat. Prod. Res. 7, 835.