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Comparing the antimicrobial properties of medicinal plants

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I. INTRODUCTION

Herbal medicines have been known to man for centuries. Therapeutic efficacy of many indigenous plants for several disorders has been described by practitioners of traditional medicine (Natrajan *et al*., 2003).

Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The world health organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population (Ahmed Adiguzel *et al*., 2005).

India has about 2000 species of medicinal plants and a vast geographical area with high production potential and varied agro-climatic conditions. For a long period of time, plants have been a valuable source of natural products for maintaining human health, last decade, with more intensive studies for natural therapies. The use of plant compounds for pharmaceutical purpose has gradually increased. According to the world health organization, medicinal plants would be the best source to obtain a variety of drugs (Chomnawang *et al*., 2005).

Cultivation of medicinal plants offers to considerable scope for rural employment and export for foreign exchange earnings. India is already a major exporter of medicinal plants. Infectious diseases are one of the important health hazard all over the world, both in developing and developed countries. Several synthetic antibiotics are employed in the treatment of infections and communicable diseases (Sereiti *et al*., 1999).

The harmful microorganisms can be controlled with drugs and this result in the emergence of multiple drug resistant bacteria and it has created alarming clinical situations in the treatment of infections. The pharmacological industries have produced a number of new antibiotics, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to synthetic drugs which are utilized as therapeutic agents.

Therefore, actions must be taken to reduce this problem, such as to minimize the use of antibiotics, develop research to better understand the genetic mechanism of resistance, among microorganism and to continue studies to develop new drugs, either synthetic or natural to control pathogenic microorganism. Certain natural products such as plants, and different parts of plants – leaves, roots, flowers, fruits, barks, seeds and oils are used for curing some chronic and acute diseases.

The vernacular name of ***Azadirachta indica*** is vepillai. Neem extracts have been reported to possess anti-diabetic, anti-bacterial and anti-viral properties and young fruits are reported to possess astringent, tonic ant anti-periodic properties. The plant is beneficial in malarial fever and useful in cutaneous diseases. Bark, leaf, root, flower and fruit together cure blood morbidity, biliary afflictions, itching, skin ulcers and burning senations, Neem have been extensively used in ayurveda, unani and homeopathic medicines. Neem oil and the bark and leaf extracts have been therapeutically used as folk medicine to control leprosy, intestinal helminthiasis, epistaxis, anorexia, respiratory disorders, constipation and also a general health promoter. These plants forms the main base for the manufacture of drugs of Indian systems of medicine – Allopathy, Ayurveda, Unani, Siddha and Homeopathy.

The vernacular name of ***Phyllanthus amarus*** is keezhanelli. It is useful for the treatment of hepatitis B (both acute and chronics), hepatitis C (both acute and chronic) and other related viral infections of the liver with antihepatotoxic and liver cell regenerating potentials and immunodulating properties. It shown to be effective drugs in the treatment of jaundice, ingastropathy, dropsy, diarrhoea, fever, opthalmopathy, scabies, ulcers and wounds. The decoction of the plant is a remedy for intermittent with infracts of the spleen and liver. The plant is bitter, astringent, stomachic, febrifuge, antiseptic and also used for menorrhagia, genital affections. The juice of leaf is applied to treat pimples. Also as a good tonic and diuretic. It is mainly used for ayurvedic medicine.

The vernacular name of ***Solanum nigrum*** is manathakalli. The plant is diaphoretic, anodyne, narcotic purgative. Herb has antiseptic and anti dysenteric properties. Infusion of plant is used as an enema for infants having abdominal upsets. It is a house holds remedy for anthrax pustule and applied locally. Root bark is laxative, useful in disease of ears, eyes and nose, good for ulcers, on neck, burning of throat, inflammation of

liver, chronic fever. The berries are poisonous. It has been used in early ayurveda along with other ingredients in the treatment of heart disease.

The vernacular name of ***Cynodon dactylon*** is Arugampul. It is highly nutritious fodder, especially for horses. Durva is reputed as a remedy in epitaxis, haematuria and scabies. Plant is used in inflammed tumors, whitlows and fleshy excrescences. Juice of plant is astringent, used as application to fresh cuts and wounds. Decoction of root is diuretic and used in dropsy. Infusion of root is used for stop bleeding from piles. It is used to treat asthma, diabetes, urinary tract infection, chronic diarrhea, and any type of infections. The juice of the leaf is applied to ring worm. Ayurvedic medicines are produced by several thousand companies in India. The key suppliers in Ayurveda are dabur, Baidyanath, zandu, himalaya herbal health care, charka, vicco, aimil and emami groups.

The objective of this research was to evaluate the potential of plant extracts and standard bacterial strains. Therefore the present study was carried out with the following objectives;

1. Selection of plants for the medicinal properties.
2. Preparation of plant extracts using cold water, hot water and methanol.
3. Studies on the antimicrobial activities of medicinal herb extracts against ***Staphylococcus aureus***, ***Pseudomonas aeruginosa*** and ***Escherichia coli*** by the following methods:

a) Agar well diffusion method
b) Paper disc assay method
c) Tube dilution method

II. REVIEW OF LITERATURE

It is well known that infectious diseases account for high proportion of health problems, especially in the developing countries. Microorganisms have developed resistance to many antibiotics and this has created immense clinical problem in the treatment of infectious diseases (Davis, 1994). This resistance has increased due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This situation forced scientists to search for new antimicrobial substances from various sources, such as medicinal plants (Karaman *et al*., 2003).

The microorganisms used for testing antimicrobial activity of plant extracts were chosen for the following reasons:

The bacterium ***Staphylococcus aureus*** was used due to its clinical relevance as a major cause of hospital acquired infections of surgical wounds and infections associated with indwelling medical devices. Besides, ***Staphylococcus aureus*** rapidly develops resistance to many antimicrobial agents. (Esquenazi *et al*., 2002).

The bacterium ***Pseudomonas aeruginosa*** is one of the most trouble some agents causing nosocomial infections. The most common infection caused by ***Pseudomonas aeruginosa*** is suppurative otitis. The pre-eminent role of ***Pseudomonas aeruginosa*** in hospital infection is due to its resistance to common antibiotic and antiseptics, and its ability to establish itself widely in hospitals. (Morrison *et al*., 1984).

The bacterium ***Escherichia coli*** is the best-known member of the normal microbiota of the human intestine and a versatile gastrointestinal pathogen. The varieties of ***Escherichia coli*** that cause diarrhoea are classified into named pathotypes, including enterotoxigenic, enteroinvasive, enteropathogenic, and enterohemorrhagic ***Escherichia coli***. Individual strains of each pathotype possess a distinct set of virulence-associated characteristics that determine the clinical, pathological and epidemiological features of the diseases they cause (Robins-Browne and Hartland 2002).

The literature pertaining in the present studies are briefly reviewed here under the following topics.

2.1 Anti bacterial properties of medicinal herbs
2.1.1 Medicinal properties of ***Azadirachta indica.***
2.1.2 Biological activity of ***Azadirachta indica.***
2.1.3 Medicinal properties of ***Phyllanthus amarus.***
2.1.4 Biological activity of ***Phyllanthus amarus.***
2.1.5 Medicinal properties of ***Solanunm nigrum.***
2.1.6 Biological activity of ***Solanunm nigrum.***
2.1.7 Medicinal properties of ***Cynodon dactylon.***
2.1.8 Biological activity of ***Cynodon dactylon.***

2.1 ANTI BACTERIAL PROPERTIES OF MEDICINAL HERBS:

Naque *et al.* (1991) found that fruits of ***Termindia bellerica*** was active against ***Staphylococcus aureus*** and ***Shigella sonnei.*** Wright *et al*. (1993) reported that Eucalptus contains essential oils which showed activity against ***Escherichia coil*** and ***Staphylococcus aureus.*** The antibacterial sensitivity pattern for the isolated bacteria was studied by the disc diffusion method (Kirby *et al*., 1996)

Encarnacion dimayuga *et al.* (1998) reported the use of traditional, medicine of Baja california antimicrobial activity against S***taphylococcus aureus, Bacillus Subtilis, Streptococcus taecalis, Escherichia coli*** and ***Candida albicans.***

Krishnakumar *et al*. (1997) screened twelve medicinal plants for their antibacterial activity of ethanol extracts of all plants showed activity against ***Staphylococcus aureus*** and ***Escherichia coli***. The antibacterial activity was maximum in ***Termindia cocciea*** followed by ***Termindia acuminata***. Dey and choudhuri (1984) studies the antibacterial properties of essential oil from ***O***cimum sanctum against ***Staphylococcus aureus***. Sinha and gulati (1990) studied the antibacterial properties of essential oil from ***Ocimum*** species viz ***Ocimum sanctum, Ocimum Barilieum*** against ***Staphylococcus aureus*** and ***Bacillus Subtilis.***

The antibacterial and antifungal activities of certain medicinal plants such as ***Allium sativum, Azadirachata indica*** and ***Ocimum Sanctum*** were studied by many researchers (Anesini and Derez 1993: Belachow desta 1993. Youvaraj *et al.,* 1995: David 1997; Saxena 1997; Ahmed *et al*. 1998; Nimri *et al*., 1999)

Gislene *et al*. (2000) evaluated antimicrobial activity of plant extracts from ***Caryophyllus aromatics*** and ***Syzygyum joabobnum***. and phytochemical with antibiotic susceptible and resistant microorganisms. The highest antimicrobial potential was observed for the extracts ***Caryophyllus aromaticus.***
Staphylococcus must be assayed for antibiotic sensitivity as soon as possible, even though therapy usually should commence before such results are available. Once theraphy has begun, it should be intensive to kill all organisms before mutation to a slightly higher level for drug resistance can occur (Volk *et al*., 1995).

Medicinal plants exhibited antibacterial activity. Since the contain innumerable biologically active chemical constituents. The use of plant preparations as food stuffs, insecticide, cardioactive antitumous and antimicrobial agents were some examples of immense chemical diversity in plants (sereiti *et al*., 1999).

Different medicinal plants extracts were used for the antimicrobial activity. Antimicrobial agents are currently used in human health care. A large number of medicinal plants are used in active stage. Screening and scientific evaluation of plant extract for their antimicrobial activity may provide new antimicrobial substances. Considerable work on the anticandial activity of medicinal plants were reported. (Arora,1993).

Natural products of higher plants may offer a new source of antibacterial agents for external use eg. Compressor cataplasms, gargles,and ointments (Brantner and Edith Grien, 1994).
Valsaraj *et al*. (1997) prepared different concentration of 80% ethanol extract of 78 traditionally used medicinal plants and tested against four bacterial pathogens *viz.*, ***Bacilus Subtilis***, ***Staphylococcus aureus Escherichia .coli*** and ***Pseudomonas aeruginosa***. They found that 90% of plant extracts showed antibacterial activity at a concentration of 25 mg/ml.

Iqbal Ahmed and Beg (2001) studied the antimicrobial activity of ethanolic extracts of 45 medicinal plants against certain resistant bacteria. Of these, 40 plant extract showed varied levels of antimicrobial activity against one or more test bacteria. Overall broad spectrum antimicrobial activity was observed in medicinal plants *viz*., ***Eucalyptus sp***. and ***Ocimum Sanctum.***

El Astal *et al*. (2003) evaluated the antimicrobial activity of aqueous, ethanolic, methanolic and phenolic extracts from three Palestinian folk medicinal plants in addition to their commercial oils against ten pathogenic microorganisms. ***Phyllanthus amarus*** which is useful for the treatment of hepatitis Band hepatitis C (both acute and chronic) and others related viral infections of the liver with antihepatotoxic and liver cell regenerating potential and immumomodulating properties (Thyagarajan, 2003).

Ezcifeka *et al.* (2004) evaluated the antimicrobial effect of the ethanol and aqueous extracts of locally available plants, ***Cajanuscajan Gascina kola*** and ***Xylopia aethiopia*** on ***Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli*** and ***Candida albicans***. The agar gel diffusion and paper disc assay methods were used to determine the inhibitory effects of both the leaves and seeds extract of plants on the test microorganisms.

The ethanol extracts of the test plants were more effective in producing inhibition zones against the microorganisms than water extract. It is expected that they could be used to treat infections and diseases caused by these organisms.

Omoregbe *et al* .(2005) studied the antimicrobial activity of three plant extracts ***Momordica charantia, Alstonia boonie,*** and ***Salmonella paratyphi*** and ***Shigella dysentriae*** was examined.

Onacha *et al.*(2005) reported antimicrobial properties of extracts of ***Combretum racemosum (Combretaceae)*** a straggling shrub wide spread across Africa is traditionally reputed to be antihelmintic and antimicrobial for genitourinary and gastrointestinal. The bacterial strain were sensitive to both extracts methanol and ethyl acetate sensitive of ***Salmonella typhi, Escherichia coli*** and ***Pseudomonas aeroginosa.***

Osadabe po *et al*. (2004) conducted a comparative study of the Phytochemical and antimicrobial properties leaves of ***Azadirachta indica, Irviniga gabonesis, Persea americana*** using standard methods. The results showed marked variations. The extracts from ***Persea americana*** and ***Azadirachata indica*** showed significant activity against ***Staphylococcus aureus.***

The medical plants are widely used by the traditional medical practitioners for curing various disease in day to day practice (prakash and gupta, 2005).

Tadeg *et al*. (2005) determined the treatment of skin disorder by traditional ethopian medicinal plants the results indicated the potential of there herbal drugs in treating microbial infections of the skin. For many diseased conditions a variety of *in vitro* test can now be employed as the biochemical mechanisms underlaying disease and healing processes were under stood (Houghton *et al*., 2005).

Klock *et al*. (2005) tested nine ethanol extracts of some Peruvian medicinal plants. Among the plants tested ***Phyllanthus amarus*** and ***Terminalia Catappa*** showed the most promising antibacterial properties inhibiting all of the strain tested with minimum inhibitory concentrations.

Swati Bipte and mussaddig (2005) conducted ***in vitro*** studies on antimicrobial activities of ***Azudirachta indica*** against ***Pseudomonas, Xanthomonas staphylococcus, Fusarium, Rhizoctonia spp*** using certain aqueous extracts of green leaves, tender twigs and dry leaves of a neem bark. Among the aqueous extracts, the green leaves was found to be more microbiotoxic followed by tender twigs, neem seeds and dry leaves.

2.1.1 **MEDICINAL PROPERTIES OF *Azadirachta indica:***

Natarajan *et al*. (2003) determined the minimum inhibitory concentration and minimum fungicidal concentration for the extracts of the leaves and seeds of the plant ***Azadirachta indica*** against various dermatophytes. The medicinal properties of the plant ***Azadirachta indica*** were studied by several workers. The antipyretic effect, antidiabetic effect, antifertility effect on the central nervous system and cardiovascular effect.

Biswas *et al*. (2002) have recently reviewed the biological activities of some of the neem compounds, pharmacological actions of the neem extracts. Clinical study and plausible medicinal applications of neem along with their safety evaluation. More than 135 compounds have been isolated from different pants of neem and several reviews have also been published on the chemistry and structural diversity of their compounds. The compounds have been divided in to two major classes iso prenoids (like diterpenoids and triterpenoids containing protomeliacins, limonoids, azadirone and its derivatives gedunin and its derivatives vilasinin type of compounds and C- secome liquins such as nimbin salonin and axadirachtin) and non iso prenoids such as proteins (aminoacid) and carbolydrates (polysaccharides) sulphurous compounds, poly phenolics such as flavonoids and aliphatic compounds etc.

2.1.2 BIOLOGICAL ACTIVITY OF *Azadirachta indica*

Immunostimulant activity:

The aqueous extract of neem bark and leaf also posseses anticomplement and immunostimulant activity. Neem oil has been shown to possess activity by selectively activating the cell-mediated immune mechanisms to elicit and enhanced response to subsequent mitogenic or antigenic challenge.

Hypoglycaemic activity:

Aqueous extract of neem leaves significantly decreases blood sugar level and prevents adrenaline as well as glucose-induced hyperglycaemia. Recently, hypoglycaemic effect was observed with leaf extract and seed oil, in normal as well as alloxan-induced diabetic rabbits.

Antiulcer effect:
Neem leaf and bark aqueous extracts produce highly potent antiacid secretory and antiulcer activity.

Antifertility effect:
Intra-vaginal application of neem oil, prior to coitus, can prevent pregnancy. It could be a novel method of contraception.

Antimalarial activity:
Neem seed and leaf extracts are effective against both choroquin-resistant and sensitive strain malarial parasites.

Antifungal activity:
Extracts of neem leaf, neem oil seed kernels are effective against certain fungi including ***Trichophyton, Epidermophyton, Microspor Trichosporon, Geotricum*** and ***Candida.***

Antibacterial activity:
Oil from the leaves, seet and bark posseses a wide spectrum of antibacterial action against Gram-negative and Gram-positive microorganisms, including ***Mycobacterium tuberculosis*** and streptomycin resistant strains. *In vitro*, it inhibits ***Vibrio Cholerae Kelebsiella Pneumoniae, Mycobacterium tuberculosis and Mycobacterium pyogenes***. Antimicrobial effects of neem extract have been demonstrated against ***Streptococus mutans*** and ***Streptococcus faecalis.***

Antiviral activity:
Aqueous leaf extract offers antiviral activity against Vaccinia virus, Chikungemya and measles virus.

Anticancer activity:
Neem leaf aqueous extract effectively suppresses oral squamous cell carcinoma induced by 7, 12-dimethylbenz (a) anthracene (DMBA), as revealed by reduced incidence of neoplasm. Neem may exert its chemopreventive effect in the oral mucosa by modulation of glutathione and its metabolizing enzymes.

Antioxidant activity:
The antioxidant activity of neem seed extract has been demonstrated *in vivo* during horse-grain germination.

Effect on central nervous system:
Varying degrees of central nervous system (CNS) depressant activity in mice was observed with the leaf extract. Fractions of acetone extract of leaf showed significant CNS depressant activity. Various plants of the neem tree have been used as traditional Ayurvedic medicine in India. Medicinal used to expel worms from the body, burning sensation near the heart, fever, cough, ulcers, inflammations and leprosy used as an insecticidal for eye problems, inflammation of the liver and skin diseases, urinary problems, tumors, piles and toothache.

Every past of the tree, oils from seeds, juice, leaves and basks are used in medicine. Bark yields a clear amber coloured gum used in medicines. Neem is a hardly quick growing, evergreen showy tree. The green fruits turn bright yellow when ripe and is one seeded. Flowers are small, fresh leaves and flowers, new leaves are purple in colour (Supriya kumar, 2000).

2.1.3 MEDICINAL PROPERTIES OF *Phyllanthus amarus*
Phyllanthin (bitter constituent) ang hypophyllanthin (non-bitter compounds) were isolanted from parts phyllanthine (4-methoxy-securinine) and 4-methoxy-non secusinine were identitied. Lintetralin was also islated from the plant unusal hydrolysable tannin, Phyllanthuslin D was also isolated from the plant (Clark, 1996).

2.1.4 BIOLOGICAL ACTIVITY OF *Phyllanthus amarus*
The alcoholic extract of the whole plant has anti-cancer activity against Freund virus leukemia, antispasmodic activity on isolated guinea pig ileum. Aqueous extract of whole plant has hydroxyl and alloxan-diabetic rabbits and leaves to be higher than that of tolbutamide.

Hindu physicians considered the plant deobstruent, diuretic, astringent and cooling. They prescribed the dry powder or fresh juice for jaundice. The plant was also used in skin disease, scabby affections, offensive sores and bruises. In western pants of India it was used as a diabetes, gonorrhoea and acidity of the urine. The root with rice water was a remedy for menorrhagia and chronicdysentry (Cousins, 1995).

2.1.5 MEDICINAL PROPERTIES OF *Solanum nigrum*

The plant is used in the treatment of cough. It is sedative, diuretic and digestive. Leaves are used as hemostatic. The fumes of burning seeds are inhaled for tooth ache. The juice of the fruit decoction is used to gonorrhoea. It is an expectorant and is employed in cough, asthma and pains in chest. The plant is accredited with diuretic properties and is used to cure dropsy. The juice of the leaves mixed with black pepper is prescribed in rheumatism (Gamble, 1979).

2.1.6 BIOLOGICAL ACTIVITY OF *Solanum nigrum*

In Guam," in waste ground and newly cultivated soil; weedy but not especially trouble some" (Stone, 1970). In niue," a very common weed in plantations and other areas of disturbed ground" (Sykes, 1970). In new Guinea,' a weed of cultivation and waste land in pasture about yards and trees. From low altitudes to 3000 m" (Henry and Pritchard 1975). In tonga " frequent … as plantation weed" (Yuncker,1959).

The leaves strongly promote perspiration and purge the bowels the next day. The juice of the fresh herb is sometimes used for fever and as a pain reliever. Externally, leaf juice preparations are used for eye disease, fevers and rabies externally, the juice or an ointment prepared from the leaves can be used for skin problems and tumors. (Whistler, 1983).

Solanum nigrum is most effective for liver disorders such as chronic enlargement of the liver and associated symptoms eg. Haemoptesis (blood from the mouth, mucoid stools, and other skin manifestation). In inflammatory conditions the plant is taken internally as a vegetable and applied externally as a paste. Alternatively the hot leaves can be applied to the swellings. Decoctions of leaves are diuretic and laxative, decoction of berries and flowers are prescribed in cough and cold (Swasbrick, 1997).

2.1.7 MEDICINAL PROPERTICES OF *Cynodon dactylon*

Cynodon dactylon is mainly used for the diabetic patients. It was used worldwide for the treatment of diabetic illness (Mohan, 2000). The cold infusion of the root often stops bleeding from piles. The roots crushed and mixed with crude are a Deccan remedy for chronic feet. The expressed juice of the plant is an ayurveda specific for hysteria, epilepsy and insanity. The expressed juice of the plant, however is a popular stringent commonly used as an application to fresh cuts and wounds and given internally in cases of chronic diarrhoea and dysentery. It is highly nutritious fodder, especially for horses. The juice of which is used as chronic diarrhoea and dysentery (Hubbard, 1959).

In malta ***Cynodon dactylon*** has also been regarded to be a medicinal plant plants are widely known for their medicinal folk preparation. ***Cynodon dactylon*** was used to treat urinary tract infection, prostatitis and various other condition. Other local uses collected by personal communication include treatment of shock, asthma and any type of infections (Lanfranco *et al.*, 1975).

2.1.8 BIOLOGICAL ACTIVITY OF *Cynodon dactylon*

Most of the commercial drug is imported from the continent and frequently consist of the rhizome of ***Cynodon dactylon***. The grass is employed as a diuretic in certain affections of the bladder. The decoction of the plant is useful in dropsy and anasarca oral administration of the juice mixed with honey 2 days was found effective in menorrhagia (Calus, 1986).

III. MATERIALS AND METHODS

GENERAL METHODS:

3.1.1 CLEANING OF GLASS WARES:

All the glasswares were first soaked in cleaning solution (100g of potassium dichromate was added to 100 ml to distilled water followed by addition of 50 ml of concentrated sulphuric acid) for about 12 hrs and washed in tap water. Then they were boiled in soap water and washed in tap water. Finally, they were cleaned in distilled water, dried and used for the study.

3.1.2 STERILIZATION:
All the media were sterilized in autoclave at 15lb pressure for 20 minutes. The glasswares were sterilized at 180^0C for 3 hours in hot air oven.

3.1.3 CHEMICALS:
All the chemicals used in the experiments were of analytical reagents (AR) grade and glass distilled water used throughout the present study.

3.2.1 PLANT MATERIALS USED:

Scientific name	**Family**	**Vernacular name**
Azadirachta indica	**Meliaceae**	**Vepillai**
Phyllanthus amarus	**Euphorbiaceae**	**Keezhanelli**
Solanum nigrum	**Solanaceae**	**Manathakalli**
Cynodon dactylon	**Cypraceae**	**Arugampul**

3.2.2 CULTURES USED FOR PRESENT STUDY:
The three bacterial cultures ***viz***, ***Staphylococcus aureus*** (ATCC 25923), ***Pseudomonas aeruginasa*** (ATCC 278753) and ***Escerichia coli*** (ATCC 25922) used for the study were obtained from the Division of Medical Microbiology, Christian Medical College and Hospital, Vellore.

Table 3.1
CHARACTERISTICS OF ISOLATED ORGANISMS

Organisms Charecteristics	***Staphylococcus aureus***	***Pseudomonas aeruginosa***	***Escherichia Coli***
CULTURE			
Nutrient agar	**Colorless Colonies**	**Green pigment**	**Convex Colonies**
Blood agar	**Yellow to cream**	**Hemolytic**	**Mucoid**
Macconkey agar	**NLF**	**NLF**	**LF**
MORPHOLOGY	**Gram positive Cocci in clusters**	**Gram negative Rods**	**Gram negative short rods**
BIOCHEMICAL CHARACTERS			
Lactose	+	-	+
Mannnitol	+	-	+
Glucose	+	+	+
Sucrose	+	-	+
Indole	+	-	+
Oxidase	-	+	-
Urea	+	+	-
Citrate	-	+	-
Coagulase	+	-	-
TSI slant **Butt** **H2s** **Gas**	**Yellow** **Yellow** - -	**Red** **Red** - -	**Yellow** **Yellow** - +
MR/VP	-/-	-/-	+/-

3.2.3 MEDIA USED:

1) Muller-Hinton Agar:

Beef infusion	:	300	g
Casein acid hydrolysate	:	17.5	g
Starch	:	1.5	g
Agar	:	17.0	g
Distilled water	:	1000 ml	
P^H	:	7.3	

The constituents were dissolved in distilled water and autoclaved before use.

2. Nutrient Agar:

Peptone	:	5 g		
Beef-extract			:	3 g
Nacl			:	5 g
Agar			:	15 g
Distilled water		:	1000 ml	
P^H			:	7.2

The constituents were dissolved in distilled water and autoclaved before use

3. Nutrient Broth:

Peptone	:	5 g		
Beef-extract			:	3 g
Nacl			:	5 g
Distilled water		:	1000 ml	
P^H			:	7.2

The constituents were dissolved in distilled water and autoclaved before use.

3.3 EXPERIMENTAL DESIGN AND DETAILS:

3.3.1 PLANT MATERIALS USED:

1)Azadirachta indica:

Azadirachta indica belongs to the family meliaceae. Its local name is neem. The English name of the plant is Indian lilac, margosa tree and the Sanskrit name of the tree is aristha meaning 'reliever of sickness' and hence is considered as sarbaroganibarini. Plate.1.

Habit:

A medium to large size tree.

Habitat:

It is commonly found throughout the greater part of India and often cultivated.

Morphology:

A large, evergreen tree, with long, spreading branches forming a broad crown. The bask is grey and rough,' the leaves are alternate, the leaflets 8-19, glossy and bluntly serrate,' the flowers are white or pale yellow, small, scented, numerous and found in long, axillary panicles. The drupes are yellow on ripening, aromatic, oblong and smooth, with a exalbuminous seed.

Chemical Constituents

More than 135 compounds have been isolated from different part of neem. The compounds have been divided in to two major classes. Isoprenoids (like diter penodis and triterpenoids containing protomeliacins, limonoids, azadirone and its derivatives, gedunin and its derivatives, vilasinin type of compounds and C- secomeliacins such as nimbin, salanin and azadirachtin) and non-isoprenoids, which are proteins (amino acids) and carbohydrates (polysaccharides).

Part uses: Whole tree

Product range:

Pliex (veincare), pure hands, Purim (Hemocare), Anti-Dandruff Hairs oil, Blood purifier capsule, cream, revitalizing Hairs oil, Erinal plus.

2. Phyllanthus amarus:

Phyllanthus amarus belongs to the family Euphorbiaceae. Its local name is 'Bhuiamala'. The Sanskrit name of the plant is Bhumyaamalaki and the hindi name of the plant is Jar-Ar.plate.2.

Habit:

It is an annual, glabrous herb grows up to 15-60cm high

Habitat:

It is found mainly in waste places, agricultural and riverbanks. In waste lands it glows abundantly during the season.

Morphology:
It has an erect stem, naked below and leaf branches above leaves are numerous, sub sessile, pale green, often distichously imbrielliptic to oblong, obtuse and stipules subulate. Flowers arise in leaf axis very numerous. Sepals of male orbicular and obovate to oblong in females. Seeds are 3-gonous, rounded and with longitudinal regular parallel ribes on the back.

Chemical Constituents:
Twenty two compounds were identified, including coumarin, 1-octadecne and diterpenic, cupresenic and kaurenoic acid.
Part uses: leaves, seeds
Product range:
Diabecon (Glucocare). Chyavanprasha

3. Solanum nigrum:
Solanum nigrum belongs to the family solanceae. Its local name is kachmachu. Common name of the plant is black-berry night shade, garden night shade, poison berry, other names such as piludu (gujarti), Gurskamai (Hindi), kakisopu (kannada), Buddakasa (Telugu), Gudkamai (Bengali), morelle noire (French), lierbamora (Spanish) Sanskrit (kakahva).plate.3.
Habit:
Average height 3 feet
Habitat:
Found all over the plains of India and warm hilly places.
Morphology:
A herbaceous or suffructescent weed. Erect, delicate, branched, annual herb. It has several arching, leafy branches and ting, star like, white flower. The stem and braches are smooth and soft. It has ovate, dark green leaves with small and shiny, almost black berries as small as pepper, growing in clusters. Berries can sometimes be red or yellow.
Chemical Constituents:
Solasamine and solamargine in leaver and glucoalkaloids from immature fruits.

Part used: Leaves, berries.
Product range:
Evecare (Menstricare), Geriforte (Gericare / stresscare)

4. Cynodon dactylon:
Cynodon dactylon belongs to the family Cypraceae. Its local name is wakha. The English name of the plant is Dhub Grass, Bermuda (or) Bahama Grass. The sanskrit name of the plant is Durva, Haritali. It is commonly known as 'nigem' in maltese. It is also know as 'week' in south Africa, 'Doob' in India; 'couch' in australia' 'Bermudagrass' in the united states, and as 'wire grass', 'Dogs tooth couch grass', and 'creeping finger grass' in the Biritish Isles.plate.4.

Habit:
A perrennial herb

Habitat:
It is usually found growing on uncultivated ground, dry places and roadsides.

Morphology:
It is a long-jointed creeping grass with very long, branched, rhizomes bearing many erect branches. It forms a dense tuft on the surface of the soil and is sometimes described as being mat-forming grass. The slender stem may end bearing a group of several, narrow finger- like spreading spikes which are purple or pale green in colour.
Part uses: whole plant
Chemical Constituents:
An alkaloid from the plant caused a slowing of blood flow in mesenteric capillaries of rats and mice. A glycoside from the plant caused hypotension in cat.
Product range:
Herbolax (Laxacare), chyavanaprasha

3.4 PREPATATION OF PLANT EXTRACT:

The leaves of medicinal plants were collected washed with distilled water, drained the water, shade dried and used for the preparation of cold water, hot water and methanol extracts.

3.4.1 COLD WATER EXTRACT:

Five grams of shade dried medicinal plant leavers were homogenized with pestle and mortar in 5ml of sterile distilled water (1:1 w/v) and filtered through a double layered cheese cloth. Filtrate was collected and evaporated under room temperature. 5mg of collected residue was dissolved in 5ml of fives per cent dimethyl sulfoxide (DMSO) and considered as standard cold water medicinal plant extract (1000µg ml^{-1}).

3.4.2 HOT WATER EXTRACT:

After shade drying, 5gm, of powdered medicinal leavers was plunged in 5ml of water (1:1 w/v), taken in beaker and heated over a water bath at 80^0C for 10 minutes. Then the materials were filtered through a double layered cheese cloth and filtrate was collected. It was evaporated under room temperature and residues were obtained. 5mg residue was dissolved in 5ml of five percent dimethyl sulfoxide (DMSO) which formed standard hot water extract (1000 µg ml^{-1}).

3.4.3. METHANOL EXTRACT:

Five gram of shade dried medicinal plant leaves was macerated using pestle and mortar with five ml of methanol (1:1 w/v). After maceration, it was filtered through double layered cheese cloth. Then the filtrate was evaporated under room temperature and residues were collected. 5mg of residue was dissolved in 5ml of 5% dimethylsulfoxde (DMSO) and used as a standard methanol plant leaves extract (1000 µg ml^{-1}).

3.5 METHODS:

ANTIMICROBIAL PROPETIES OF MEDICINAL PLANT EXTRACTS WERE TESTED BY THE FOLLOWING METHODS.

Agar well diffusion method
Paper disc assay method
Tube dilution method

3.5.1 AGAR WELL DIFFUSION METHOD:

Agar well diffusion method was adopted for evaluation of antibacterial activity of four medicinal plant extracts. Muller hinton agar medium was prepared and autoclaved at 15 lb pressure for 20 min and cooled to 45^0C. Cell suspension (10^6 cells|ml) of the test organism *viz.*, ***Staphylococcus aureus , Pseudomonas aeruginosa*** and ***Escherichia coli*** was added separately to muller hinton agar medium at 1ml|100ml of medium to prepare seeded medium. Seeded medium was poured in to sterile petriplates and allowed for solidification wells were punched in agar media using a stainless steel bores. Each well was filled with the cold water , hot water and methanol extracts of the four medicinal plants at different concentration *viz.*, 1000,500,250 µg/ml. The plates were incubated at 37^0C for 24hrs, after which they were observed for the zone of inhibition. Diameter of inhibition zone were calculated and expressed in mm.

3.5.2 PAPER DISC ASSAY METHOS:

Paper disc assay method was adopted for evaluation of antibacterial activity of four medicinal plant extracts. Nutrient agar medium was prepared and autoclaved at 15lb pressure for 20 minutes and cooled to 45^0C. cell suspension (10^6 cells/ ml) of the test organism *viz.*, ***Staphylococcus aureus. Pseudoman aeruginosa*** and ***Escherichia coli*** was added separately to nutrient agar medium at 1ml / 100 ml of medium to prepare seeded medium. Seeded medium was poured in to sterile petriplates and allowed for solidification and filled with the cold water, hot water and methanol extracts of four medicinal plants at different concentration *viz*., 1000,500,250µg/ml prepared and impregnated with whatman No. 1 sterile paper discs (6mm diameter). Sterile paper disc dipped in 5%. DMSO served as control. Three replications were maintained. The plants were incubated for 24hrs at 37^0C. After incubation period, the diameter of the zone formed around the paper discs were measured and expressed in mm. Diameter of inhibition zone were calculated and expressed in mm.

3.5.3 TUBE DILUTION METHOD:

Minimum inhibitory concentration method was adopted for evaluation of antibacterial activity of four medicinal plant extracts. Nutrient broth was prepared and autoclaved at 15lb pressure for 20min. cell suspension (10^6 cells/ml) of the test Organism *viz.*, ***Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli*** was added separately to nutrient broth at 1ml /100ml of medium.

Nutrient broth was poured in to sterile test tubes and cold water, hot water and methanol extracts of four medicinal plants were added at different concentration *viz*., 125,250,500,1000µg/ml. The tubes were incubated at 37^0C for 24hrs after which they were observed for the presence or absence of growth. The concentration at which no growth was recorded is considered as Minimum inhibitory concentration of the organism.

IV. EXPERIMENTAL RESULTS:

ANTIMCROBIAL ACTIVITY OF FOUR MEDICINAL PLANT EXTRACTS:

The antimicrobial effect of cold water, hot water and methanol extracts of the four medicinal plants *viz*., ***Azadirachta indica, Phyllanthus amarus, Solanum nigrum*** and ***Cynodon dactylon*** at different concentration *viz*., 250µg/ml, 500 µg/ml and 1000 µg/ml, was studied by three methods against the test organisms *viz*., ***Staphylococcus aureus, Pseudomonas aeruginosa*** and ***Escherichia coli*** and the results of present investigation are given below.

4.1.1 Inhibitory effect of cold water, hot water and methanol extracts of the four medicinal plants against S*taphylococcus aureus* by agar well diffusion method.

The results on the Inhibitory effect of cold water, hot water and methanol extracts of the four medicinal plants against ***Staphylococcus aureus*** are presented in Table 1. Plate.5.

Table 4.1
Inhibitory effect of medicinal plant extracts against *Staphylococcus aureus* by agar well diffusion method

Leaf extract of the plant	Diameter of inhibition zone (mm)											
	Cold water extract				Hot water extract				Methanol extract			
	Control	250 µg /ml	500 µg /ml	1000 µg /ml	Control	250 µg /ml	500 µg /ml	1000 µg /ml	Control	250 µg /ml	500 µg /ml	1000 µg /ml
Azadirachta indica	-	1.2	2.6	4.5	-	4.0	6.5	9.0	-	8.5	11.0	15.0
Phyllanthus amarus	-	0.8	3.0	4.0	-	2.0	5.1	7.0	-	3.0	7.6	9.0
Solanum nigrum	-	1.1	2.0	3.8	-	1.3	4.0	5.5	-	2.0	4.0	6.8
Cynodon dactylon	-	-	0.7	2.9	-	0.2	2.4	4.8	-	1.0	2.2	6.0

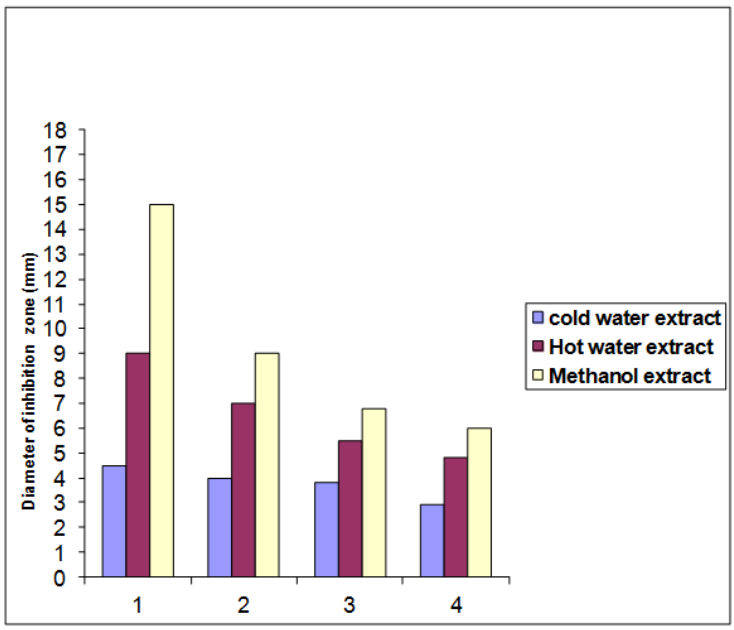


Fig. 4.1
Inhibitory effect of medicinal plant extracts against S*Staphylococcus aureus* by agar well diffusion method

The four medicinal plants *viz.,* ***Azadirachta infica, Phyllanthus amarus, Solanum nigrum*** and ***Cynodon dactylon*** recorded inhibitory activity against ***Staphylococus aureus*** in different concentration was compared with the control. The inhibitory effect increased with increase in the concentration of the extracts. Methanol extracts recorded more inhibitory effect than hot water and cold water extract.

The cold water extract at 1000µg/ml of ***Azadirachta indica*** recorded the diameter of inhibition zone (4.5 mm), followed by ***Phyllanthus amarus*** (4.0 mm), S***olanum nigrum*** (3.8 mm) and C***ynodon dactylon*** (2.9 mm) against the test pathogens of ***Staphylococcus aureus.***

The hot water extract at 1000µg/ml of ***Azadirachta indica*** recorded the diameter of inhibition zone (9.0 mm), followed by ***Phyllanthus amarus*** (7.0 mm), ***Solanum nigrum*** (5.5 mm) and ***Cynodon dactylon*** (4.8 mm) against the test pathogens of ***Staphylococcus aureus.***

The methanol water extract at 1000µg/ml of ***Azadirachta indica*** recorded the diameter of inhibition zone (15.0 mm), followed by ***Phyllanthus amarus*** (9.0 mm), ***Solanum nigrum*** (6.8 mm) and ***Cynodon dactylon*** (6.0 mm) against the test pathogens of ***Staphylococcus aureus.***

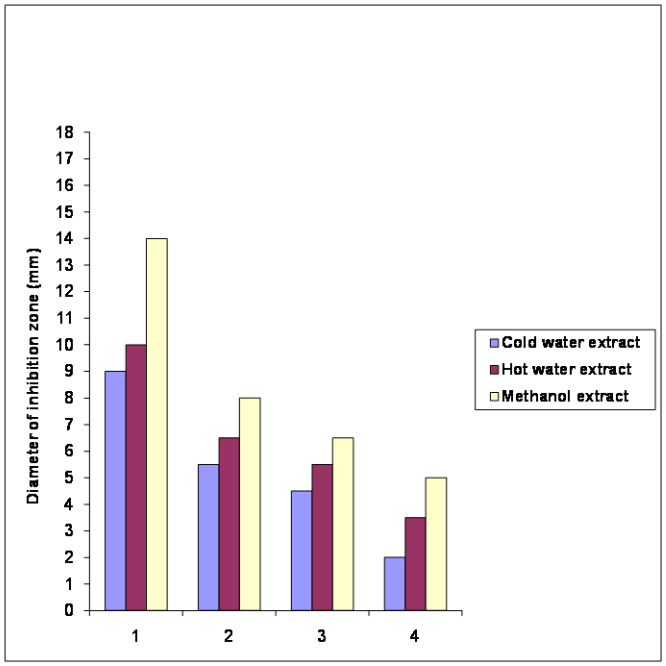
4.1.2 Inhibitory effect of cold water, hot water and methanol extracts of the four medicinal plants against *Pseudomonas aeruginosa* by agar well diffusion method.

The results on the inhibitory effect of cold water, hot water and methanol extracts of the four medicinal plants against ***Pseudomonas aeruginosa*** are presented in Table 2. Plate 6.

The four medicinal plants recorded inhibitory activity against ***Pseudomonas aeruginosa*** in different concentration was compared with the control. The inhibitory effect increased with increase in the concentration of the extracts. Methanol extracts recorded more inhibitory effect than hot water and cold water extracts.

Table 4.2
Inhibitory effect of medicinal plant extracts against *Pseudomonas aeruginosa* by agar well diffusion method

Leaf extract of the plant	Diameter of inhibition zone (mm)											
	Cold water extract				Hot water extract				Methanol extract			
	Control	250 µg /ml	500 µg /ml	1000 µg /ml	Control	250 µg /ml	500 µg /ml	1000 µg /ml	Control	250 µg /ml	500 µg /ml	1000 µg /ml
Azadirachta indica	-	-	3.5	9.0	-	3.0	6.0	10.0	-	3.9	7.1	14.0
Phyllanthus amarus	-	-	-	5.5	-	-	2.1	6.5	-	2.3	5.2	8.0
Solanum nigrum	-	-	-	4.5	-	-	-	5.5	-	2.0	4.1	6.5
Cynodon dactylon	-	-	-	2.0	-	-	-	3.5	-	-	1.5	5.0



A.indica ***P.amarus*** ***S.nigrun*** ***C.dactylon***

Inhibitory effect of medicinal plant extracts against *Pseudomonas aeruginosa* by agar well diffusion method

The cold water extract at 1000µg/ml of ***Azadirachta indica*** recorded the diameter of inhibition zone (9.0 mm), followed by ***Phyllanthus amarus*** (5.5 mm), ***Solanum nigrum*** (4.5 mm) and ***Cynodon dactylon*** (2.0 mm) against the test pathogens of ***Pseudomonas aeruginosa.***

The hot water extract at 1000µg/ml of ***Azadirachta indica*** recorded the diameter of inhibition zone (10.0 mm), followed by ***Phyllanthus amarus*** (6.5 mm), ***Solanum nigrum*** (5.5 mm) and **C*ynodon dactylon*** (3.5 mm) against the test pathogens of ***Pseudomonas aeruginosa.***

The methanol water extract at 1000µg/ml of ***Azadirachta indica*** recorded the diameter of inhibition zone (14.0 mm), followed by ***Phyllanthus amarus*** (8.0 mm), ***Solanum nigrum*** (6.5 mm) and ***Cynodon dactylon*** (5.0 mm) against the test pathogens of ***Pseudomonas aeruginosa.***

4.1.3 Inhibitory effect of cold water, hot water and methanol extracts of the four medicinal plants against *Escherichia coli* by agar well diffusion method.

The results on the inhibitory effect of cold water, hot water and methanol extracts of the four medicinal plants against ***Escherichia coli*** are presented in Table 3.

Table 4.3
Inhibitory effect of medicinal plant extracts against *Escherichia coil* by agar well diffusion method

Leaf extract of the plant	Diameter of inhibition zone (mm)											
	Cold water extract				Hot water extract				Methanol extract			
	A. indica	250 µg /ml	500 µg /ml	1000 µg /ml	Control	250 µg /ml	500 µg /ml	1000 µg /ml	Control	250 µg /ml	500 µg /ml	1000 µg /ml
Azadirachta indica	-	3.5	7.5	10.5	-	4.0	8.2	12.0	-	7	10.5	11.5
Phyllanthus amarus	-	-	1.8	5.5	-	-	2.2	7.0	-	-	3.4	7.5
Solanum nigrum	-	-	1.2	3.8	-	-	2.5	5.5	-	-	2.2	6.0
Cynodon dactylon	-	-	-	3.5	-	-	-	4.5	-	-	-	4.0

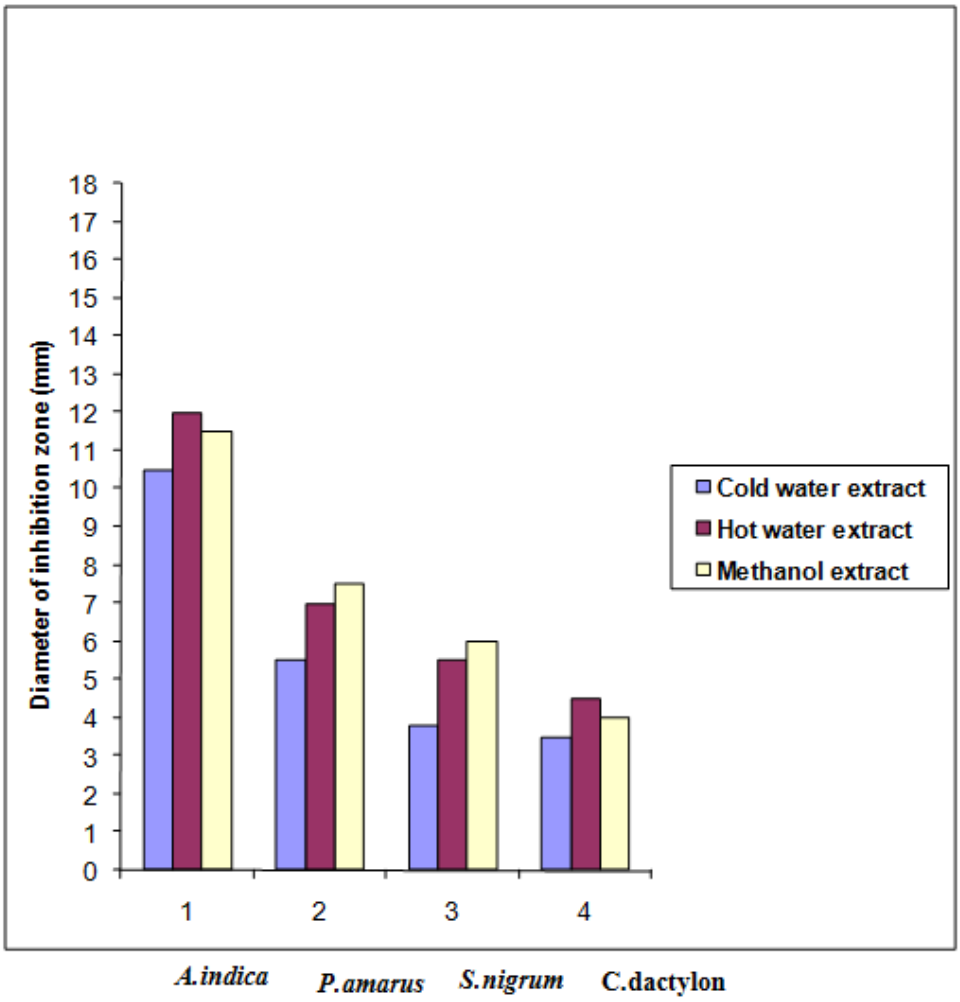


Fig.4.3
Inhibitory effect of medicinal plant extracts against *Escherichia coil* by agar well diffusion method

The four medicinal plants recorded inhibitory activity against ***Escherichia coli*** in different concentration was compared with the control. The inhibitory effect increased with increase in the concentration of the extracts. Methanol extracts recorded more inhibitory effect than hot water and cold water extracts.

The cold water extract at 1000µg/ml of ***Azadirachta indica*** recorded the diameter of inhibition zone (10.5 mm), followed by ***Phyllanthus amarus*** (5.5 mm), ***Solanum nigrum*** (3.8 mm) and ***Cynodon dactylon*** (3.5 mm) against the test pathogens of ***Escherichia coli.***

The hot water extract at 1000µg/ml of ***Azadirachta indica*** recorded the diameter of inhibition zone (12.0 mm), followed by ***Phyllanthus amarus*** (7.0 mm), ***Solanum nigrum*** (5.5 mm) and ***Cynodon dactylon*** (4.5 mm) against the test pathogens of ***Escherichia coli.***

The methanol water extract at 1000µg/ml of ***Azadirachta indica*** recorded the diameter of inhibition zone (11.5 mm), followed by ***Phyllanthus amarus*** (7.5 mm), ***Solanum nigrum*** (6.0 mm) and ***Cynodon dactylon*** (4.0 mm) against the test pathogens of ***Escherichia coli.***

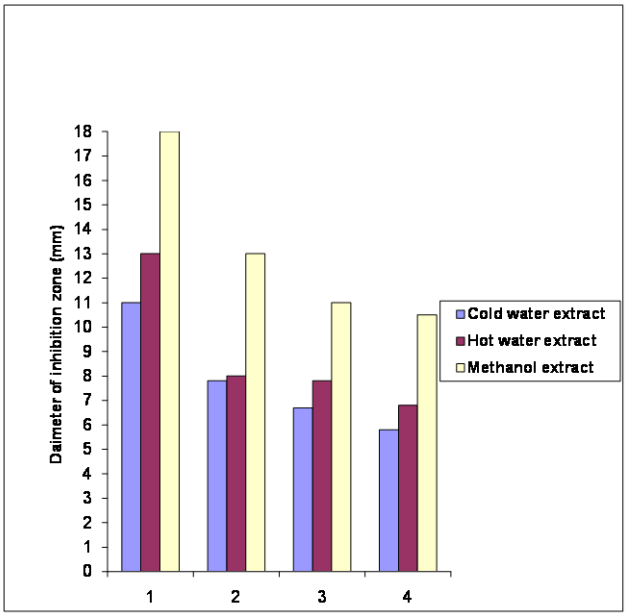
4.2.1 Inhibitory effect of cold water, hot water and methanol extracts of the four medicinal plants against *Staphylococcus aureus* by paper disc assay method.

The results of the inhibitory effect of cold water, hot water and methanol extracts of the four medicinal plants against ***Staphylococcus aureus*** are present in Table 4. plate. 7.

The four medicinal plants *viz.*, ***Azadirachta indica, Phyllanthus amarus, Solanum nigrum*** and ***Cynodon dactylon*** recorded inhibitory activity against ***Staphylococcus aureus*** in different concentration was compared with the control. The inhibitory effect increased with increase in the concentration of the extracts. Methanol extracts recorded more inhibitory effect than hot water and cold water extracts.

Table 4.4
Inhibitory effect of medicinal plant extracts against *staphylococcus aureus* by paper disc assay method

Leaf extract of the plant	Diameter of inhibition zone (mm)											
	Cold water extract				Hot water extract				Methanol extract			
	Control	250 µg/ml	500 µg/ml	1000 µg/ml	Control	250 µg/ml	500 µg/ml	1000 µg/ml	Control	250 µg/ml	500 µg/ml	1000 µg/ml
Azadirachta indica	-	4.9	9.8	11.0	-	6.3	10.8	13.0	-	9.0	13.2	18.0
Phyllanthus amarus	-	-	5.9	7.8	-	4.1	5.8	8.0	-	7.0	9.8	13.0
Solanum nigrum	-	2.1	4.9	6.7	-	4.0	5.2	7.8	-	6.8	8.5	11.0
Cynodon dactylon	-	2.0	3.8	5.8	-	2.1	4.2	6.8	-	6.0	8.0	10.5



A.indica ***P.amarus*** ***S.nigrum*** ***C.dactylon***

Fig. 4.4

Inhibitory effect of medicinal plant extracts against *staphylococcus aureus* by paper disc assay method

The cold water extract at 1000µg/ml of ***Azadirachta indica*** recorded the diameter of inhibition zone (11.0 mm), followed by ***Phyllanthus amarus*** (7.8 mm), ***Solanum nigrum*** (6.7 mm) and ***Cynodon dactylon*** (5.8 mm) against the test pathogens of ***Staphylococcus aureus.***

The hot water extract at 1000µg/ml of ***Azadirachta indica*** recorded the diameter of inhibition zone (13.0 mm), followed by ***Phyllanthus amarus*** (8.0 mm), ***Solanum nigrum*** (7.8 mm) and ***Cynodon dactylon*** (6.8 mm) against the test pathogens of ***Staphylococcus aureus.***

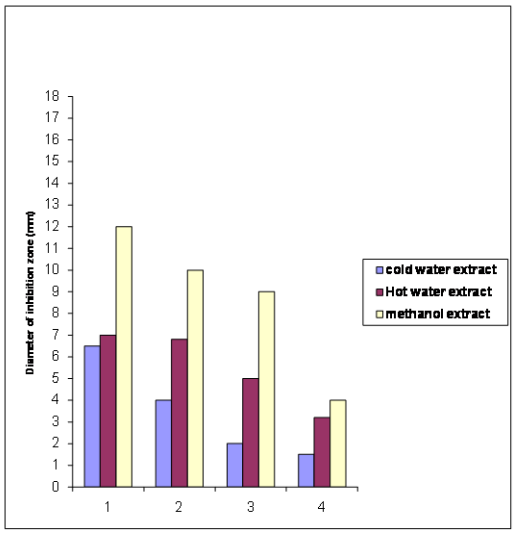
The methanol water extract at 1000µg/ml of ***Azadirachta indica*** recorded the diameter of inhibition zone (18.0 mm), followed by ***Phyllanthus amarus*** (13.0 mm), ***Solanum nigrum*** (11.0 mm) and ***Cynodon dactylon*** (10.5mm) against the test pathogens of ***Staphylococcus aureus.***

4.2.2 Inhibitory effect of cold water, hot water and methanol extracts of the four medicinal plants against *Pseudomonas aerugionsa* by paper disc assay method.

The results of the inhibitory effect of cold water, hot water and methanol extracts of the four medicinal plants against ***Pseudomonas aerugionsa*** are present in Table 5. plate.8.

Table 4.5
Inhibitory effect of medicinal plant extracts against *Pseudomonas aeruginosa* by paper disc assay method

Leaf extract of the plant	Diameter of inhibition zone (mm)											
	Cold water extract				Hot water extract				Methanol extract			
	Control	250 µg/ml	500 µg/ml	1000 µg/ml	Control	250 µg/ml	500 µg/ml	1000 µg/ml	Control	250 µg/ml	500 µg/ml	1000 µg/ml
Azadirachta indica	-	1.7	2.8	6.5	-	1.5	4.0	7.0	-	6.1	9.0	12.0
Phyllanthus amarus	-	-	2.0	4.0	-	1.0	3.2	6.8	-	4.3	7.0	10.0
Solanum nigrum	-	-	0.7	2.0	-	-	1.8	5.0	-	3.0	6.4	9.0
Cynodon dactylon	-	-	0.2	1.5	-	-	1.1	3.2	-	-	2.3	4.0



A.indica ***P.amarus*** ***S.nigrum*** ***C.dactylon***

Fig. 4.5

Inhibitory effect of medicinal plant extracts against *Pseudomonas aeruginosa* by paper disc assay method
S
The four medicinal plants *viz.*, ***Azadirachta indica***, ***Phyllanthus amarus,*** Solanum nigrum and ***Cynodon dactylon*** recorded inhibitory activity against ***Pseudomonas aerugionsa*** in different concentration was compared with the control. The inhibitory effect increased with increase in the concentration of the extracts. Methanol extracts recorded more inhibitory effect than hot water and cold water extracts.

The cold water extract at 1000µg/ml of ***Azadirachta indica*** recorded the diameter of inhibition zone (6.5 mm), followed by ***Phyllanthus amarus*** (4.0 mm), ***Solanum nigrum*** (2.0 mm) and ***Cynodon dactylon*** (1.5 mm) against the test pathogens of ***Pseudomonas aerugionsa.***

The hot water extract at 1000µg/ml of ***Azadirachta indica*** recorded the diameter of inhibition zone (7.0 mm), followed by ***Phyllanthus amarus*** (6.8 mm), ***Solanum nigrum*** (5.0 mm) and ***Cynodon dactylon*** (3.2 mm) against the test pathogens of ***Pseudomonas aerugionsa.***

The methanol water extract at 1000µg/ml of ***Azadirachta indica*** recorded the diameter of inhibition zone (12.0 mm), followed by ***Phyllanthus amarus*** (10.0 mm), ***Solanum nigrum*** (9.0 mm) and ***Cynodon dactylon*** (4.0 mm) against the test pathogens of ***Pseudomonas aerugionsa.***

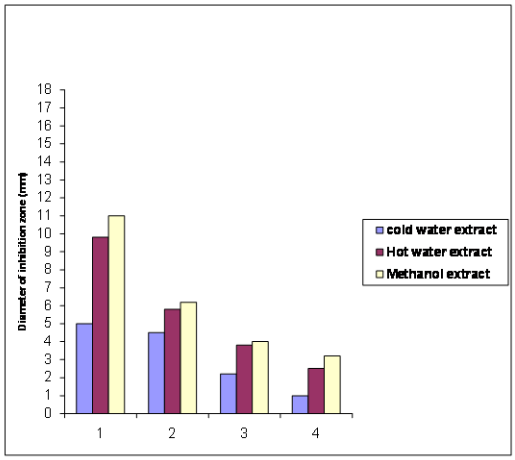
4.2.3 Inhibitory effect of cold water, hot water and methanol extracts of the four medicinal plants against *Escherichia coli* by paper disc assay method.

The results of the inhibitory effect of cold water, hot water and methanol extracts of the four medicinal plants against ***Escherichia coli*** are present in Table 6.plate.9.

The four medicinal plants *viz.*, ***Azadirachta indica, Phyllanthus amarus, Solanum nigrum*** and ***Cynodon dactylon*** recorded inhibitory activity against ***Escherichia coli*** in different concentration was compared with the control. The inhibitory effect increased with increase in the concentration of the extracts. Methanol extracts recorded more inhibitory effect than hot water and cold water extracts.

Table 4.6
Inhibitory effect of medicinal plant extracts against *Escherichia coli* by paper disc assay method

Leaf extract of the plant	Diameter of inhibition zone (mm)											
	Cold water extract				Hot water extract				Methanol extract			
	Control	250 µg/ml	500 µg/ml	1000 µg/ml	Control	250 µg/ml	500 µg/ml	1000 µg/ml	Control	250 µg/ml	500 µg/ml	1000 µg/ml
Azadirachta indica	-	0.5	1.2	5.0	-	1.8	4.0	9.8	-	4.0	9.0	11.0
Phyllanthus amarus	-	-	2.0	4.5	-	-	2.6	5.8	-	2.0	3.8	6.2
Solanum nigrum	-	-	-	2.2	-	-	-	3.8	-	-	0.2	4.0
Cynodon dactylon	-	-	-	1.0	-	-	-	2.5	-	-	-	3.2



A.indica ***P.amarus*** ***S.nigrum*** ***C.dactylon***

Fig. 4.6
Inhibitory effect of medicinal plant extracts against *Escherichia coli* by paper disc assay method

The cold water extract at 1000µg/ml of ***Azadirachta indica*** recorded the diameter of inhibition zone (5.0 mm), followed by ***Phyllanthus amarus*** (4.5 mm), ***Solanum nigrum*** (2.2 mm) and ***Cynodon dactylon*** (1.0 mm) against the test pathogens of ***Escherichia coli.***
The hot water extract at 1000µg/ml of ***Azadirachta indica*** recorded the diameter of inhibition zone (9.8 mm), followed by ***Phyllanthus amarus*** (5.8 mm), ***Solanum nigrum*** (3.8 mm) and ***Cynodon dactylon*** (2.5 mm) against the test pathogens of ***Escherichia coli.***

The methanol water extract at 1000µg/ml of ***Azadirachta indica*** recorded the diameter of inhibition zone (11.0 mm), followed by ***Phyllanthus amarus*** (6.2 mm), ***Solanum nigrum*** (4.0 mm) and ***Cynodon dactylon*** (3.2 mm) against the test pathogens of ***Escherichia coli.***

4.3.1 Inhibitory effect of cold water, hot water and methanol extracts of the four medicinal plants against *Staphylococcus aureus* by tube dilution method.
The results of the inhibitory effect of cold water, hot water and methanol extracts of four medicinal plant against ***Staphylococcus aureus*** are presented in Table 7.

Table 4.7
Inhibitory effect of medicinal plant extracts against *Staphylococcus aureus* by tube dilution method

Leaf extract of the plant	Minimal inhibitory concentration														
	Cold water extract					Hot water extract					Methanol extract				
	Control	125 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml	Control	125 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml	Control	125 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml
Azadirachta indica	+	+	+	-	-	+	+	-	-	-	+	-	-	-	-
Phyllanthus amarus	+	+	+	+	-	+	+	+	-	-	+	+	-	-	-
Solanum nigrum	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-
Cynodon dactylon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-

Growth +
No Growth -

The four medicinal plants *viz.*, ***Azadirachta indica, phyllanthus amarus, Solanum nigrum*** and ***Cynodon dactylon*** recorded inhibitory activity against ***Staphylococcus aureus*** in different concentration was compared with the control.

The minimal inhibitory concentration ***Azadirachta indica*** for ***Staphylococcus aureus*** was 500µg/ml for cold water extract, 250 µg/ml for hot water extract and 125µg/ml for methanol extract.

The minimal inhibitory concentration ***Phyllanthus amarus*** for ***Staphylococcus aureus*** was 1000µg/ml for cold water extract, 500 µg/ml for hot water extract and 250µg/ml for methanol extract.

The ***Solanum nigurm*** plant extract could not inhibit ***Staphylococcus aureus*** in cold water extract at all concentration tested (125-1000 µg/ml). The minimal inhibitory concentration of ***Solanum nigrum*** for ***Staphylococcus aureus*** was 1000 µg/ml for hot water extract, 500 µg/ml for methanol extract.

The ***Cynodon dactylon*** plant extract could not inhibit ***Staphylococus aureus in*** cold water and hot water extracts al all concentration tested
(125-1000 µg/ml). The minimal inhibitory concentration of ***Cynodon dactylon*** for ***Staphylococus aureus*** was 1000 µg/ml for methanol extract.

4.3.2 Inhibitory effect of cold water, hot water and methanol extracts of the four medicinal plants against *Pseudomonas aeruginosa* by tube dilution method.

The results of the inhibitory effect of cold water, hot water and methanol extracts of four medicinal plant against ***Pseudomonas aeruginosa*** are presented in Table 8.

Table 4.8
Inhibitory effect of medicinal plant extracts against *Pseudomonas aeruginosa* by tube dilution method

Leaf extract of the plant	Minimal inhibitory concentration														
	Cold water extract					Hot water extract					Methanol extract				
	Control	125 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml	Control	125 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml	Control	125 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml
Azadirachta indica	+	+	+	+	-	+	+	+	-	-	+	+	-	-	-
Phyllanthus amarus	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-
Solanum nigrum	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-
Cynodon dactylon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

The four medicinal plants *viz*., ***Azadirachta indica, Phyllanthus amarus, Solanum nigrum*** and ***Cynodon dactylon*** recorded inhibitory activity against ***Pseudomonas aeruginosa*** in different concentration was compared with the control.

The minimal inhibitory concentration ***Azadirachta indica*** for ***Pseudomonas aeruginosa*** was 1000µg/ml for cold water extract, 500 µg/ml for hot water extract and 250µg/ml for methanol extract.

The phyllanthus amarus plant extract could not inhibit ***Peseudomonas aeruginosa*** in cold water extract at all concentration tested (125-1000 µg/ml). The minimal inhibitory concentration of ***Phyllanthus amarus*** for ***Peseudomonas aeruginosa*** was 1000µg/ml hot water extract and 500 µg/ml for methanol extract.

The ***Solanum nigrum*** plant extract could not inhibit ***Peseudomonas aeruginosa*** in cold water extract and hot water extracts at all concentration tested (125-1000 µg/ml). The minimal inhibitory concentration of ***Solanum nigrum*** for ***Peseudomonas aeruginosa*** was 1000 µg/ml for methanol extract.

The ***Cynodon dactylon*** plnat extract could not inhibit ***Peseudomonas aeruginosa*** in cold water, hot water and methanol extracts at all concentration tested (125-1000 µg/ml).

4.3.3 Inhibitory effect of cold water, hot water and methanol extracts of the four medicinal plants against *Escherichia coli* by Tube dilution method.
The results of the inhibitory effect of cold water, hot water and methanol extracts of four medicinal plant against ***Escherichia coli*** are presented in Table 9.

The four medicinal plants *viz*., ***Azadirachta infica, Phyllanthus amarus,*** Solanum nigrum and ***Cynodon dactylon*** recorded inhibitory activity against ***Escherichia coli*** in different concentration was compared with the control.

Table 4.9
Inhibitory effect of medicinal plant extracts against *Escherichia coli* by tube dilution method

Leaf extract of the plant	Minimal inhibitory concentration														
	Cold water extract					Hot water extract					Methanol extract				
	Control	125 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml	Control	125 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml	Control	125 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml
Azadirachta indica	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-
Phyllanthus amarus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Solanum nigrum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cynodon dactylon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

The ***Azadirachta indica*** plant extract could not inhibit ***Escherichia coli*** in cold water extract at all concentration tested (125-1000 µg/ml). The minimal inhibitory concentration of ***Azadirachta indica*** for ***Escherichia coli*** was 1000µg/ml hot water extract and 500 µg/ml for methanol extract.

The ***Phyllanthus amarus*** plant extract could not inhibit ***Escherichia coli*** in cold water extract and hot water extract at all concentration tested (125-1000 µg/ml). The minimal inhibitory concentration of ***phyllanthus amarus*** for ***Escherichia coli*** was 1000 µg/ml for methanol extract.

The ***Solanum nigrum*** plant extract could not inhibit ***Escherichia coli*** in cold water, hot water and methanol extract at all concentration tested (125-1000 µg/ml).

The ***Cynodon dactylon*** plant extract could not inhibit ***Escherichia coli*** in cold water, hot water and methanol extracts at all concentration tested (125-1000 µg/ml).

V. DISCUSSION

Plant based drug have been in use against various diseases since time immemorial. The primitive man used herbs as therapeutic agents and medicament, which they were able to produce easily. The nature has provided abundant plant wealth for all living creatures, which possess medicinal virtues. The essential values of some plants have long been published but a large number of them remain unexplored as yet. So there is a necessity to explore their uses and to conduct pharmacognostic and pharmacological studies to ascertain their therapeutic properties (Baquar, 1989).

Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The world health organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the worlds population. Turkish people have a tradition of using a number of plant species for the treatment of infections diseases and various ailments.

The antimicrobial activities of ***Azadirachta indica, Phyllanthus amarus, Solanum nigrum, Cynodondactylon*** extracts against the microorgaenisme were examined in the present study. Osadabe po *et al.* (2004) conducted a comparative study of the phytochemical and antimicrobial properties of the leaves of ***Azadirachta indica, Irvingia gabonesis and Persea americana.*** The results showed marked variations. The extracts from ***Persea americana*** and ***Azadirachta indica*** showed significant activity against ***Staphylococcus aureus.***

The extraction of antimicrobial substance from medicinal plants was carried out by solvents, such as cold water, hot water and methanol. This conflict can be explained that the better extraction of an antimicrobial compounds from various medicinal plants may require different solvents. Since the extracts of the plant produced good inhibition zone against the test organisms. Ezcifeka *et al.* (2004) evaluated the antimicrobial effect of the ethanol and aqueous extracts of locally available plants, ***Cajanuscajan Gascina kola*** and ***Xylopia aethiopia*** on ***Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli*** and ***Candida albicans***. The ethanol extracts of the test plants were more effective in producing inhibition zones against the microorganisms than water extract. In the present study, methanol extract of all the plants exhibited higher activity than hot water and could water extract. This may be due to the ability of methanol solvent to extract, the antimicrobial active components effectively than hot water and cold water extract.

In the present study ***Azadirachta indica*** recorded the highest diameter of inhibition zone for ***Staphycococcus aureus*** (15 mm), followed by ***Pseudomonas aeruginosa*** (14.0 mm) and ***Escherichia coli*** (11.5 mm) in agar well diffusion method.

The inhibition zone formed by ***Phyllanthus amarus*** for ***Staphylococcus aureus*** (9.0 mm), followed by ***Pseudomonas aeruginosa*** (8.0 mm) and ***Escherichia coli*** (7.5 mm) in agar well diffusion method.

The inhibition zone formed by ***Solanum nigrum*** for ***Staphylococcus aureus*** (6.8 mm), followed by ***Pseudomonas aeruginosa*** (6.5 mm) and ***Escherichia coli*** (6.0 mm) in agar well diffusion method.

The inhibition zone formed by ***Cynodon dactylon*** was the least among the four plants tested (4.0 – 6.0 mm). Survey and screening at different medicinal herbs for antibacterial properties were carried out by various workers. Krishnakumar *et al.* (1997) screened twelve medicinal plants for their antibacterial activity of ethanol extracts of all plants showed activity against ***Staphylococcus aureus*** and ***Escherichia coli***.

In the present study ***Azadirachta indica*** recorded the highest diameter of inhibition zone for ***Staphylococcus aureus*** (18.0 mm), followed by ***Pseudomonas aeruginosa*** (12.0 mm) and ***Escherichia coli*** (11.0 mm) in paper disc assay method.

The inhibition zone formed by ***Phyllanthus amarus*** for ***Staphylococcus aureus*** (13.0 mm), followed by ***Pseudomonas aeruginosa*** (10.0 mm) and ***Escherichia coli*** (6.2 mm) in paper disc assay method.

The inhibition zone formed by ***Solanum nigrum*** for ***Staphylococcus aureus*** (11.0 mm), followed by ***Pseudomonas aeruginosa*** (9.0 mm) and ***Escherichia coli*** (4.0 mm) in paper disc assay method.

The inhibition zone formed by ***Cynodon dactylon*** was the least among the four plants tested (3.2 – 10.5 mm). Valsaraj *et al.* (1997) reported that different concentration of 80% ethanol extract of 78 plants recorded antimicrobial activity at higher concentrations. In the lowest test concentration of 1.6 mg ml^{-1} , only 8 plants were active.

In the present study minimal inhibitory concentration of ***Azadirachta indica*** for ***Staphylococcus aureus*** was 125µg/ml followed by ***Pseudomonas aeruginosa*** (250 µg/ml) and ***Escherichia coli*** (500 µg/ml) in tube dilution method.

The inhibitory concentration of ***Phyllanthus amarus*** for ***Staphylococcus aureus*** was 250µg/ml followed by ***Pseudomonas aeruginosa*** (500 µg/ml) and ***Escherichia coli*** (1000 µg/ml) in tube dilution method.

The inhibitory concentration of ***Solanum nigrum*** for ***Staphylococcus aureus*** was 500µg/ml followed by ***Pseudomonas aeruginosa*** (1000 µg/ml). The ***Solanum nigrum*** plant extracts could not inhibit ***Escherichia coli*** at all concentration tested (125-1000µg/ml) in tube dilution method.

The ***Cynodon dactylon*** plant extacts could not inhibit ***Pseudomonas aeruginosa*** and ***Escherichia coli*** at all concentration tested (125-1000µg/ml) in tube dilution method. Klock *et al*. (2005) tested nine ethanol extracts of some Peruvian medicinal plants. Among the plants tested ***Phyllanthus amarus*** and ***Terminalia Catappa*** showed the most promising antibacterial properties inhibiting all of the strain tested with minimum inhibitory concentrations.

VI. SUMMARY

The present investigation on the antimicrobial activity in medicnde plants *viz*., ***Azadirachta indica, Phyllanthus amarus, Solanum nigrum, Cynodon dactylon*** against common bacteria is summarized here under.

Bacterial cultures of ***Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli*** were obtained from Christian Medical College, Vellore for testing them against the above medicinal plant extracts.

1. Three methods were employed to find out the antimicrobial activity of ***Azadirachta indica, Phyllanthus amarus, Solanum nigrum, Cynodon dactylon*** plant extracts against common bacteria:

a) Agar well diffusion method
b) Paper disc assay method
c) Tube dilution method

2. In vitro study on the antimicrobial activity of the four medicinal plants against pathogenic bacteria was tested. Methanol extract of the medicinal plants possessed more antibacterial activity than hot water extract and cold water extract. The plant extracts of the four plants showed increase in the diameter of inhibition with the increased in the concentration of extract from 250 µg/ml to 1000 µg/ml for all the three pathogenic bacteria tested. The highest inhibition zone was recorded in methanol extract of ***Azadirachta indica*** to ***Staphycococcus aureus*** (15 mm), followed by ***Pseudomonas aeruginosa*** (14.0 mm) and ***Escherichia coli*** (11.5 mm) in agar well diffusion method.

3. The diameter of inhibition with the increased in the concentration of plant extract from 250 µg/ml to 1000 µg/ml for all the three pathogenic bacteria tested. The highest inhibition zone was recorded in methanol extract of ***Azadirachta indica*** to ***Staphycococcus aureus*** (18.0 mm), followed by ***Pseudomonas aeruginosa*** (12.0 mm) and ***Escherichia coli*** (11.0 mm) in paper disc assay method.

4. The inhibition of growth of bacteria increased with the increase in the concentration of plant extract from 125 µg/ml to 1000 µg/ml for the three pathogenic bacteria tested. The minimal inhibitory concentration of ***Azadirachta indica*** for ***Staphylococcus aureus*** was 125µg/ml followed by ***Pseudomonas aeruginosa*** (250 µg/ml) and ***Escherichia coli*** (500 µg/ml) in tube dilution method.

5. In addition, almost all species of plants were found to have activity on at least against one bacterial strain. The antimicrobial activity profile also showed that ***Staphylococcus aureus*** and ***Escherichia coli*** were the most susceptible bacterial strains.

6. The result indicate the potential of these herbal drugs in treating microbial infection of the skin. Thus, justifying their claimed uses in the treatment of various skin disorders. The majority of which are of infectious origin. Based on these results, the methanol extract has a stronger and broader spectrum of antimicrobial activities compared with other extract.

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