ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF SELECTED MEDICINAL PLANTS AVAILABLE IN KERALA

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Thesis submitted in partial fulfilment of the
requirement for the degree of

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University, Thrissur

2010

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DECLARATION

I hereby declare that the thesis entitled **"ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF SELECTED MEDICINAL PLANTS AVAILABLE IN KERALA"** is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Mannuthy
30/04/2010

SABITHA JOSE

CERTIFICATE

Certified that this thesis, entitled **"ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF SELECTED MEDICINAL PLANTS AVAILABLE IN KERALA"** is a record of research work done independently by **Dr. Sabitha Jose.**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, associateship or fellowship to her.

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ACKNOWLEDGEMENT

With a deep sense of gratitude, I prevail this opportunity to accentuate my thankfulness for my advisor, ***Dr. Usha, P.T.A.,*** *Associate Professor, Department of Pharmacology and Toxicology for the meticulous guidance, personal attention, keen interest, affectionate encouragement, persuasion and help offered to me from the initiation of work to the shipshaping of the manuscript. Without her strong support and cooperation throughout the study, the successful completion of this work would not have been possible.*

I depict my deep sense of indebtedness and utmost gratitude to ***Dr. A.M. Chandrasekharan Nair,*** *Professor and Head, Department of Pharmacology and Toxicology and member of the advisory committee for his expert advice, personal attention, persuasion and help rendered in all possible ways throughout the course of my study.*

I record my sincere gratitude to ***Dr. C.M. Aravindakshan,*** *Associate Professor, Department of Pharmacology and Toxicology and member of the advisory committee for extending incessant help, expert advice, personal attention, valuable guidance and encouragement rendered all the time.*

There are no words to pay my respect and deep sense of gratitude to, ***Dr. Priya, P.M.,*** *Assistant Professor, Department of Veterinary Microbiology, the other member of the advisory committee for her valuable suggestions, personal attention, constructive criticism, keen interest and affectionate encouragement, whenever I needed and providing necessary facilities for carrying out this work.*

I take great pleasure in thanking ***Dr. E. Nanu,*** *Dean, faculty of Veterinary and Animal Sciences for providing me the facilities for my research.*

I am thankful to ***Dr. A.D. Joy,*** *Professor, Department of Pharmacology and Toxicology for his words of inspiration, kindness and professional guidance during the entire period of research.*

I am obliged, thankful and grateful to ***Shri. V.R. Raghunandanan****, Associate Professor and* ***Dr. Nisha A.R,*** *Assistant Professor, Department of Pharmacology and Toxicology, for all the help, inspirations and co- operations rendered from time to time.*

My great indebtedness and gratitude are due to ***Dr. N. Krishnan Nair****, Professor and Head, Department of Veterinary Microbiology,* ***Dr. Usha Narayana Pillai,*** *Associate Professor, Department of Clinical medicine,* ***Dr. B. Sunil,*** *Associate Professor, Department of Veterinary Public Health, for all the help and cooperation rendered from time to time.*

I gratefully acknowledge the timely help by ***Smt. K. A. Mercy,*** *Professor, Department of Statistics for their help and whole hearted suggestions offered in the statistical analysis of data.*

I am indebted to ***Kerala Agricultural University*** *for the fellowship awarded to me for the postgraduate study.*

I gratefully acknowledge the wholehearted help rendered for correction of my thesis by ***Dr. Rani Joy.***

The boisterous support, linear help and sincere friendship rendered by my seniors, ***Drs. Eliza Jose, Priya A.R., Midhun M.V., Bibu John, Suja rani S. and Vineetha*** *whose affectionate friendship, mental support and timely advice helped me in overcoming the hardships faced during the period of study and research work.*

Life would have been dull and monotonous without friends and their uninhibited expression of even the most absurd thoughts that lightened my heavier moments. I cherish the sweet memories of collegue ***Dr. Ranjana Mooken.***

I also warmly acknowledge the timely help and support of my junior colleagues ***Dr. Sreelekha K.P.*** *and* ***Dr. Dhanya V.R.***

I gratefully acknowledge the wholehearted help rendered by my friend ***Dr. Sariprabha P*** *during my research work*

I am grateful to my friend ***Dr. Reeba Paul*** *and my senior* ***Dr. P.K. Senthil Kumar*** *for their help in collecting the literature for the research programme.*

The purity and self less backing of ***Drs. Rani Chacko, Chinchu Jose*** *and all other beloved friends are far more valuable than they might ever regard. I pleasantly acknowledge that this thesis is also a memorabilia of my cherished friendship.*

Words posses no enough power to reflect my thankfulness to my friends ***Drs. Sreeji, Ambili, Remya, Divya rani*** *for their sprit buoyant with abiding courage and pleasure through their help and moral support during my golden days of research work at COVAS, Mannuthy, Thrissur.*

With great fondness, I recall the constant encouragement, love, help and support rendered to me by my juniors, especially ***Akhila, Shruti, Shyma, Mekha, Aswathi*** *and* ***Lakshmi.***

I sincerely acknowledge the staff of our department ***Smt. Victoria, Mr.Wilson and Mr. Ravichandran*** *for their timely help.*

I would like to give special thanks to ***Suresh*** *for the timely help and support provided to me.*

I cannot confine my feelings for my family to a mere gratitude. Without their prayers, love, support and blessings I would not have been able to complete this study successfully.

I thankfully remember all those who directly and indirectly helped me and contributed to finalize the work.

Last but not the least, my sincere solicitude to the ***Almighty God,*** *who helped and gave me mental as well as physical strength to contend any situation during this great endeavour.*

Sabitha Jose

Dedicated to my family

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Introduction

1. INTRODUCTION

For centuries medicinal plants have been used throughout the world as remedies for various diseases. The potential of the medicinal plant as a source for new drugs is still largely unexplored. Now-a-days there is wide spread interest in drugs derived from plants. This interest primarily stems from the belief that green medicine is safe and dependable compared to synthetic drugs. Medicinal plants represent a rich source of antimicrobial agents and scientific experiment on the antimicrobial properties of plant components were first documented in the late nineteenth century. Natural antimicrobials can be derived from plants, animals and microorganism. Random screening as a tool in discovering new biologically active molecule has been most productive in the area of antibiotics.

Increasing failure of antimicrobial agents and drug resistance exhibited by pathogenic microbial infectious agents has led to the need for new agents. Considering the vast potentiality of plants as a source for antimicrobial drugs with reference to antifungal and antibacterial agents, a systematic investigation was undertaken to screen the antibacterial and antifungal activity of *Annona squamosa, Cassia alata, Coleus amboinicus, Myristica fragrans* and *Tectona grandis.*

Annona squamosa Linn. is a plant belonging to the family Annonaceae, also known as custard apple. It is found in all parts of India especially in Southern parts. Roots are employed internally for spinal diseases. Bark is known to be a powerful astringent. In Ayurveda, fruits are considered as good tonic. They enrich blood and are used as expectorant. They increase muscular strength, cooling, lessens burning sensation. They act as sedative to heart and relieves vomiting. The seeds are said to be abortifacient and good to destroy hair lice. Leaves are used as poultice over burns and ulcers. Seeds are powerful irritant of conjunctiva and produce ulcers in the eye (Patel and Kumar, 2008). *Annona squamosa* is traditionally used for the treatment of epilepsy, dysentery, cardiac problems, worm infestation, constipation, haemorrhage, bacterial infection,

dysurea, fever and ulcer. It also has antifertility, antitumour and abortifacient properties (Kaleem *et al.*, 2008).

Cassia alata Linn., belonging to the class Caesalpiniaceae, from the family Leguminosae and subfamily of Fabeceae, is a pantropical, ornamental shrub, distributed from tropical America to India. Other synonym is *Senna alata.* It is commonly known as ringworm senna. *Cassia alata* grows aggressively in areas where there is high water table. It prefers open areas and sunlight. The leaves and barks are used for medicinal purpose. *Cassia alata* contain phytochemical constituents such as alkaloids, lectins, saponins, cyanogenic glycosides, isoflavones and phytoestrogens (Reezal *et al.*, 2002). The leaf extracts of the plant have been reported to possess medicinal properties and used against ringworm, scabies, ulcers and other skin diseases such as pruritis, eczema and itching (Abubacker *et al.,* 2008). The leaf extracts also have anti-inflammatory, analgesic, laxative and antiplatelet aggregating activity (Moriyama *et al.*, 2003b). The roots are used for treating rheumatism and as a strong laxative. *Cassia alata* is very effective for treating uterine disorders and snake bites. The bioactivity of the plant includes antibacterial, antifungal, diuretic, laxative, analgesic and choleretic.

Coleus amboinicus is a powerful aromatic plant, which belongs to the family Laminaceae. It is commonly called as Indian borage. It is used to treat insect bite, hepatopathy, helminthiasis, headaches, fever, colic, bronchitis, dyspepsia, asthma, renal and vesicular calculi. Leaves are used as aromatic carminative. It has specific action on the bladder and is useful in urinary disease, vaginal discharge etc. It has been recommended for chronic cough, epilepsy and convulsive seizures, allergies, hay fever and sinusitis. *Coleus amboinicus* reported to have antioxidant, anti-inflammatory and antimicrobial properties (Kumar *et al.*, 2008). The leaves contain flavonoids like quercetin, apigenin, luteolin, salvigenin, genkwanin, and volatile oils (Kaliappan and Viswanathan, 2008).

Myristica fragrans belongs to the family Myristicaceae and is commonly known as nutmeg. It has been claimed to possess psychomimetic, antidiarrheal, sedative, hepatoprotective, analgesic, anti-inflammatory and memory enhancing activities. *Myristica fragrans* have been reported to possess hypolipidaemic, antimicrobial, acaricidal and anxiogenic activities (Narasimhan and Dhake, 2006).

Tectona grandis belongs to Verbenaceae family. It is commonly known as teak. Traditionally, *Tectona grandis* is used in the treatment of diabetes, lipid disorders, inflammation, ulcer, and bronchitis (Warrier, 1994). *Tectona grandis* is reported to have antiulcer (Pandey *et al.*, 1982), antimicrobial (Sumthong *et al.*, 2006) and anticancer activity (Khan and Miungwana, 1999). *Tectona grandis* can be used to treat anemia (Diallo *et al.*, 2008). It possesses anthelmintic and expectorant properties. *Tectona grandis* leaf extract are widely used in the folklore for the treatment of various kinds of wound, especially burn wounds, piles, leucoderma and dysentery (Shalini and Srivastava, 2009). *Tectona grandis* contains several naphthoquinones, lapachol, trichione, 5-hydroxy lapachol. Lapachol is reported to have anti-ulcer and nitric oxide scavenging activity (Majumdar *et al.*, 2007).

The present study was conducted to evaluate antimicrobial activity of the above mentioned plants, so that they can be used in the antimicrobial therapy effectively.

Review of Literature

2. REVIEW OF LITERATURE

2.1 Annona squamosa

2.1.1 ANTIMICROBIAL ACTIVITY STUDIES ON *Annona squamosa*

Thaker and Anjaria (1985) studied the antimicrobial and infected wound healing response of some traditional drugs like *Ocimum sanctum, Azadirachta indica, Annona squamosa* and *Bergia odorata.* The result of the experiment indicated that the plant extracts have significant inhibitory effect on *Staphylococcus aureus, Streptococcus pyogenes* and *Escherichia coli* about 75 to 99 per cent except that of *Corynebacterium* spp. and *Pseudomonas aeruginosa* by *Annona squamosa, Azadirachta indica* respectively.

Ethanol extract of *Annona squamosa* fruit showed significant inhibitory activity against *Staphylococcus aureus* and *Streptococcus pneumoniae* giving mean diameters of inhibition zones of 14 mm and 13 mm respectively with disc concentration 5 mg/disc. Gram negative bacteria as well as *Enterobacter faecalis* and *C. albicans* were not inhibited by the plant extract (Au *et al.*, 2003).

Rahman *et al.* (2005) reported the presence of Annotemoyin-1, Annotemoyin-2, squamocin and cholesteryl glucopyranoside from the seeds of *Annona squamosa.* These compounds and crude extracts showed remarkable antimicrobial activities and cytotoxic activities.

Patel *et al.* (2007) studied the antibacterial activity of extracts of *Acacia nicotica, Annona squamosa, Azadirachta indica* and *Ocimum sanctum* against *Staphylococcus aureus* and *Pseudomonas aeruginosa.* The investigation revealed that methanol extract turns out to be better extract than acetone and petroleum ether in inhibiting *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

The petroleum ether, chloroform, methanol and aqueous extracts of *Acacia catechu, Acacia nilotica, Aegle marmelos, Azadirachta indica, Annona*

squamosa, *Trachyspermum ammi*, *Holarrhena antidysenterica* and *Ocimum basilicum* were tested against enteropathogenic *Escherichia coli* (Patel *et al.*, 2008). The result showed that *Annona squamosa* inhibited microbial growth at a concentration of 500 µg/ml.

Patel and Kumar (2008) did antimicrobial screening of four different solvents extracts of *Annona squamosa* using agar diffusion method and phytochemical analysis by HTPLC. Methanol extract showed highest antimicrobial activity against all tested microorganisms. Phytochemical analysis revealed the presence of Linalool, Borneol, Eugenol, Farnesol and Geraniol which may be responsible for antimicrobial activity.

2.1.2 OTHER PHARMACOLOGICAL EFFECTS OF *Annona squamosa*

Chopra *et al.* (1956) reported that *Annona squamosa* seeds have insecticidal and abortifacient properties.

Seetharaman (1986) reported the presence of flavonoids in the leaves of *Annona squamosa* and *Polyalthia longifolia.*

Pharmacological screening of an ethanol extract of defatted seeds of *Annona squamosa* using experimental models was done by Saluja and Santani (1994). Ethanol extract at an oral dose of 250 mg/kg in rats, produced a depressant effect on the central nervous system; potentiated the hypnotic effect of pentobarbitone; showed anticonvulsant activity against electrically-induced convulsions and raised the pain threshold when tested by analgesiometer. Phytochemical analysis revealed the presence of alkaloids.

Chao-Ming *et al.* (1997) reported that *Annona squamosa* seeds contain octacyclopeptide named Annosquamosine A.

The chemical characterization of the fruit pulp of *Annona squamosa* was done by Andrade *et al.* (2001). It revealed the presence of sugars (58 per cent of dry mass), triglyceride, diterpenoid compound Kaur-16-en-18-oic acid (0.25 per

cent of dry mass), pinene (25.3 per cent), sabinene (22.7 per cent) and limonene (10.1 per cent).

Araya *et al.* (2002) isolated two bistetrahydrofuran acetogenins, Squamosine O1 and Squamosine O2 from metanolic extracts of *Annona squamosa* seeds. Their structures were also determined by spectral method.

Free radical scavenging activity of *Annona squamosa* leaf extracts was reported by Shirwaikar *et al.* (2004a).

Shirwaikar *et al.* (2004b) investigated the anti-diabetic activity of alcohol extract of *Annona squamosa* leaf in Type-2 diabetes mellitus induced with standardised doses of streptozotocin and nicotinamide. The findings showed the significant anti-diabetic potential of the extract in ameliorating the diabetic conditions in diabetic rats.

The study conducted by Gupta *et al.* (2005) revealed that *Annona squamosa* has anti-diabetic activity. According to this study the water extract of *Annona squamosa* was found to be useful in controlling elevated blood glucose levels in diabetes induced by alloxan and streptozotocin in rabbits and rats respectively.

Pardhasaradhi *et al.* (2005) evaluated the differential cytotoxic effects of *Annona squamosa* seed extracts on human tumour cell lines and found that *A. squamosa* seeds possess potent proapoptotic characteristics for several human tumour cells.

Suresh *et al.* (2006) investigated chemopreventive and anti-lipid peroxidative efficacy of *Annona squamosa* bark extract in 7,12-dimethylbenz(a) anthracene (DMBA) induced hamster buccal pouch carcinogenesis. Oral administration of aqueous and ethanolic bark extracts of *Annona squamosa* at a dose of 500 mg/kg and 300 mg/kg respectively, prevented the tumor formation. It also found to decrease the levels of lipid peroxidation and enhanced the

antioxidants defense mechanism in DMBA painted hamsters. The effect of ethanolic bark extract is more potent than aqueous extract of *Annona squamosa* barks.

Panda and Kar (2007) evaluated the possible ameliorative effect of *Annona squamosa* seed extract in the regulation of hyperthyroidism in mouse model. Simultaneous administration of the Annona seed extract (200 mg/kg) or quercetin (10 mg/kg) for 10 days reversed all effects of T4-induced hyperthyroidism. Phytochemical analysis revealed the presence of quercetin in the seed extract and its role in the mediation of antithyroidal activity of *Annona squamosa* seed extract.

Suresh *et al.* (2007) studied the modifying effect of *Annona squamosa* leaf extracts in 7, 12 dimethylbenz(a)anthracene induced hamster buccal pouch carcinogenesis. It was found that *A. squamosa* leaf extract have potent chemopreventive activity and can modify the abnormalities in cell surface glycoconjugates during neoplastic transformation.

Kaleem *et al.* (2008) demonstrated the anti-diabetic effect of *Annona squamosa* in streptozotocin induced diabetes mellitus in rats. *Annona squamosa* leaves extract lowered blood glucose with simultaneous increase in the plasma insulin levels.

Saleem *et al.* (2008) demonstrated the hepato-protective effect of alcoholic and water extract of *Annona squamosa* (custard apple) in isoniazid and rifampicin induced hepatotoxicity in wistar rats. There was a significant decrease in total bilirubin accompanied by significant increase in the level of total protein and also significant decrease in ALP, AST, ALT and γ-GT in treatment group as compared to the hepatotoxic group.

Bagavan *et al.* (2009) studied the adulticidal and larvicidal efficacy of some medicinal plant extract against tick, fluke and mosquitoes. All plant extracts showed moderate toxic effect on parasites after 24 hours of exposure;

however, the highest mortality was found in hexane extract of *Annona squamosa* leaf, acetone and methanol extracts of *Gloriosa superba,* methanol extracts of *Pergularia daemia* and *Phyllanthus emblica* against *Haemaphysalis bispinosa.*

Raj *et al.* (2009) studied the hepato-protective effect of alcoholic extract of *Annona squamosa* leaves on Diethyl nitroso amine induced liver injury in swiss albino mice. They found that *A. squamosa* possesses hepato-protective effect at a dose rate of 5 g/kg bodyweight for 30 days.

Chavan *et al.* (2010) studied the analgesic and anti-inflammatory activity of Caryophyllene oxide from *Annona squamosa* bark. They found that Caryophyllene oxide at the doses of 12.5 and 25 mg/kg body weight and unsaponified petroleum ether extract at a dose of 50 mg/kg body weight showed significant central as well as peripheral analgesic, along with anti-inflammatory activity.

2.2 Cassia alata

2.2.1 ANTIMICROBIAL ACTIVITY STUDIES ON *Cassia alata*

Ikenebomeh and Metitiri (1988) have reported the antimicrobial activity of *Cassia alata* against *Escherichia coli, Aspergillus niger, Penicillium expansum* and *Trichophyton tonsurans.*

Palanichamy and Nagarajan (1990) tested *Cassia alata* leaf extract for its antifungal activity against *Candida albicans, Aspergillus fumigatus, Aspergillus flavus, Mucor* spp., *Rhizopus* spp. and few species under dermatophytes; *Trichophyton mentagrophytes, T. rubrum* and *M. gypseum.* They reported that 20 per cent w/v of crude extract did not show any significant activity against the contaminant fungi, whereas 2.5 and 3 per cent crude extract completely inhibited the growth of dermatophytes.

Crockette *et al.* (1992) found that water extract of *Cassia alata* could inhibit the growth of *Escherichia coli* and *Candida albicans.* MIC and minimum

bactericidal concentration (MBC) against *E. coli* were 1.6 mg/ml and 60 mg/ ml, respectively. MIC and minimum fungicidal concentration (MFC) against *C. albicans* were 0.39 mg/ml and 60 mg/ ml, respectively.

Ibrahim and Osman (1995) reported that ethanolic extract of the *Cassia alata* at 500 mg/ml concentration showed high activity against dermophytic fungi: *Trichophyton mentagrophytes var interdigitale, T. mentagrophytes var. mentographytes, T. rubrum, Microsporium gypseum* (MIC: 125 mg/ml) and *Microsporium canis* (MIC: 25 mg/ml). It showed low activity against non dermophytic fungi. Bacterial and yeast species showed resistance against *in vitro* treatment with the extract.

The *in vitro* antimicrobial activity of *Cassia alata* extracts has been investigated against *Staphylococcus aureus, Bacillus subtilis, B. cereus, B. stearothermophillus, Escherichia coli, Vibrio cholerae, Salmonella typhi, S. dysenteriae* and *Klebsiella pneumoniae* by Sakharkar and Pati (1998). The acetone and ethanol (95 per cent) extracts of *Cassia alata* showed high activity against nearly all test microorganisms. The inhibitory effects of extracts are very close and identical in magnitude and are comparable with that of standard antibiotics used.

Ranganathan and Balajee (2000) reported that the combination of extract of *Ocimum sanctum* and *Cassia alata* inhibited growth of the *Cryptococcus* at a concentration ranging from 62.5–125 mg/ml and fungicidal at a concentration of 125 mg/ml. The activity of combination of the extracts was heat-stable and worked at acidic pH. The MIC value of ethanolic extract of *C. alata* ranged from 500 - 1000 mg/ml at acidic pH.

Vaijayanthimala *et al.* (2000) have reported the inhibitory activity of water extract from *Cassia alata* leaves against *Candida albicans.*

Khan *et al.* (2001) reported that methanol extracts of *Cassia alata* leaves, flowers, barks and roots at 4 mg/ml concentration inhibited many types of

bacteria including *Escherichia coli* and *Staphylococcus aureus*, but not moulds (*Candida albicans, Aspergillus niger* and *Trichophyton mentagrophytes*). This antibacterial activity was increased on fractionation (petrol, dichloromethane, ethyl acetate), the dichloromethane fraction of the flower being the most effective.

Crude ethanol and water extract of leaves from *Cassia alata* were tested *in vitro* against *Staphylococcus aureus* and *Escherichia coli* and *in vivo* to evaluate the effect of both extracts in liver cells of mice. Both extracts showed activity against *Staphylococcus aureus.* The water extract exhibited higher antibacterial activity than the ethanol extract from leaves (inhibition zone of 11 to 14 mm and 9 to 11 mm respectively). *Escherichia coli* showed resistance to both extract. Histological examination on the liver cells suggests that both extracts induced mild hepatocyte degeneration and the lesion was dose-dependent. The ethanol extract exhibited more lesions compared to the water extract of the plant (Elysha-Nur *et al.*, 2002)

Crude ethanol and aqueous extract of leaves and bark from *Cassia alata* were tested for antifungal activity against *Aspergillus fumigatus, Microsporum canis* and *Candida albicans* using the disc diffusion method. *Candida albicans* showed dose dependent sensitivity towards ethanol and aqueous extract of bark, but resistant for both ethanol and aqueous extract of leaves. The inhibitory zones of the ethanol bark extract for *Candida albicans* ranged from 10 mm to 14 mm and as for the aqueous bark extract the inhibition zones ranged from 12 mm to 16 mm. *Aspergillus fumigatus* and *Microsporum canis* appeared to be resistant towards both ethanol and aqueous extracts of leaf and bark of *Cassia alata* (Reezal *et al.*, 2002).

Somchit *et al.* (2003) screened ethanol and water extracts of leaf and bark from *Cassia alata* against fungi (*Aspergillus fumigatus* and *Microsporum canis*), yeast (*Candida albicans*) and bacteria (*Staphylococcus aureus* and *Escherichia coli*). *C. albicans* showed concentration dependent susceptibility towards both

the ethanol and water extracts from the bark, but resistant towards the extracts of leaves. The growth of *Aspergillus fumigatus* and *Microsporum canis* were not affected by all types of the plant extracts. The antibacterial activity of *C. alata* extracts on *S. aureus* was detected only with the water and ethanol extracts of leaves. The water extract exhibited higher antibacterial activity than the ethanol extract from bark and leaves. *E. coli* showed resistance to all types of extracts.

Phongpaichit *et al.* (2004) investigated crude methanol extracts from leaves of *Cassia alata, Cassia fistula* and *Cassia tora* for their antifungal activities on three pathogenic fungi *Microsporum gypseum, Trichophyton rubrum* and *Penicillium marneffei. C. alata* was the most effective leaf extract against *T. rubrum* and *M. gypseum* with the 50 per cent inhibition of hyphal growth at 0.5 and 0.8 mg/ml, respectively, where as the extract of *C. fistula* was the most potent inhibitor of *P. marneffei*. In addition, it was found that all three *Cassia* leaf extracts affected *M. gypseum* conidial germination.

Owoyale *et al.* (2005) screened methanolic, ethanolic and petroleum ether extracts of *Cassia alata* leaves for phytochemicals, antibacterial and antifungal activities. The result indicated that crude extract of *Senna alata* leaves showed both antibacterial and antifungal activities. The methanolic extract was the most active of the three crude extracts tested against bacterial and fungal organisms. The phytochemical screening revealed that most active chromatographic component is a flavonoid glycoside. Minimum inhibitory concentration of the active component on the moulds and yeasts are 70 µg/ml and 860 µg/ml respectively, while the MIC of the active component on the bacteria is 860 µg/ml.

Idu *et al.* (2007) conducted studies on phytochemistry and antimicrobial activity of *Cassia alata* flowers. Extracts showed *in vitro* antimicrobial activities against clinical isolates of *Staphylococcus aureus, Candida albicans, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa* and *Bacillus subtilis* at a final concentration of 500 µg/ml. The zones of inhibitions produced

by the extracts in agar diffusion assay ranged from 4 to 10 mm while the gentamicin antibiotic control, produced zones that measured 5 mm. Preliminary phytochemical analysis of the plant extracts revealed the presence of phenols, tannins, anthraquinones, saponins and flavonoids.

The antibacterial and antifungal activity of the aqueous and methanol extracts of *Cassia alata* leaves has been evaluated by Makinde *et al.* (2007). They found that the extracts exhibited more antifungal activity than antibacterial properties. The methanolic extracts of *C. alata* exhibited very strong activity against bacteria and fungi with maximum activity in the fractions containing alkaloid salts and alkaloid base.

Abubacker *et al.* (2008) investigated aqueous flower extract *Cassia alata* for antifungal activity by agar diffusion method against three distinct groups of fungi, viz. aflatoxin producing fungi, *Aspergillus flavus* and *A. parasiticus*; plant pathogenic fungi, *Fusarium oxysporum* and *Helminthosporium oryzae* and human pathogenic fungi *Candida albicans* and *Microsporum audouinni*. Total inhibition of growth was seen at 10 and 15 mg/ml concentration for aflatoxin producing fungal strains, whereas total inhibition for human pathogenic fungi and plant pathogenic fungi was at 15 mg/ml concentration. The MIC values of the extract ranging from 5.75 to 8.00 mg/ml.

El-Mahmood and Doughari (2008) screened leaves and roots of *Cassia alata* for phytochemical components and antibacterial activity. Phytochemical screening revealed the presence of alkaloids, carbohydrates, tannins, saponins, phenols, flavonoids, anthraquinones and cardiac glycosides. Result of antibacterial screening indicated that *Staphylococcus aureus, Streptococcus pyogenes* and *Proteus mirabilis* were more susceptible, while *Escherichia coli* and *Pseudomonas aeruginosa* were less sensitive against water, methanol and chloroform extracts. The effectiveness of the crude extracts were enhanced at elevated temperatures and at near neutrality pH values, which attests to its use in traditional medicine to treat skin, urinary tract and gastrointestinal infections.

The methanolic extract of the leaves of *Cassia alata* was sequentially partitioned in increasing polarity to afford the hexane, chloroform, butanol and residual extract. Crude extracts were evaluated against MRSA (Methicillin-Resistant *Staphylococcus aureus*) using the agar well diffusion assay. The butanol and chloroform extracts exhibited inhibition against MRSA with inhibition indexes of 1.03 ± 0.16 and 0.78 ± 0.07 at the concentration of 50 mg/ml (Hazni *et al.*, 2008).

In vitro screening of two flavonoid compounds isolated from *Cassia alata* leaves for fungicidal activities was done by Rahman *et al.* (2008). The compound 2,5,7,4′-tetrahydroxy isoflavone (100 µg/disc) was found active against *Trichophyton longifurus* and *Pseudallescheria boydii* but showed no activities against *Epidermophyton floccosum.* Compound 3,5,7,4′- tetrahydroxy flavone (100 µg/disc) also showed inhibition against most of the fungi excepting the *Epidermophyton floccosum.*

Alam *et al.* (2009) evaluated methanol, ethanol, ethyl acetate and chloroform extracts of *Achyranthes aspera* and *Cassia alata* for their antibacterial activities against *Escherichia coli*, *Bacillus subtilis*, *Vibrio cholerae*, *Salmonella typhi* and *Staphylococcus aureus.* The methanolic extract of both stem and leaves of *Cassia alata* exhibited activity against *Bacillus subtilis* and *Salmonella typhi.* The corresponding MIC values of the leaf extracts were estimated as 1.25 and 1.5 mg/ml respectively. The ethanolic extracts of both the stem and leaf parts were found equally effective only to *S. aureus.*

35 per cent ethanolic extract from *Cassia alata* leaves showed antifungal activity against *Trichophyton mentagrophyte, Trichophyton rubrum* and *Microsporum gypsum.* The activity was determined by agar diffusion method. Minimum inhibitory concentration (MIC) of methanolic extract against *T. mentagrophyte was* found to be 15 mg/ml. It was also found that 35 per cent ethanolic extract showed less activity against *Staphylococcus aureus* at 10 per cent W/V concentration (Nantachit, 2009).

Nebedum *et al.* (2009) studied the ethanolic extracts of *Cassia alata*, *Juglan nigra*, *Ocimum basilicum* and *Aloe vera* for their *in vitro* antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* using agar diffusion method. All the extracts showed antimicrobial activity against tested microorganism. *Juglan nigra* has the highest activity against all tested organisms while the least activity against tested organism was shown by *Ocimum basilicum*. Preliminary phytochemical screening showed the presence of tannin, saponins, glycosides, fats and oil in the ethanolic extracts of all tested plants.

2.2.2 OTHER PHARMACOLOGIC EFFECTS OF *Cassia alata*

Leeuwenberg (1987) reported that *Cassia alata* contains many diverse constituents such as alkaloids, lectins, saponins, cyanogenic glycosides, isoflavones and phytoestrogens.

Palanichamy *et al.* (1988) studied the oral effectiveness of *Cassia alata* leaf extract on streptozotocin- induced hyperglycemia in rats and the results compared with glibenclamide. The extract showed no effect on glucose levels in normoglycemic animals, but it reduced the blood sugar value in streptozotocin-induced hyperglycemic animals.

Studies conducted by Palanichamy *et al.* (1991) revealed significant mast cell stabilizing effect of solvent-free extract of *Cassia alata* leaf. Pre-treatment with *Cassia alata* extract (10, 100, 1000 µg/ml) significantly reduced the percentage of degranulation of mast cells induced by carbachol. A dose dependent inhibition has been observed for *C. alata* extract against mast cell degranulation.

Cassia alata is reported to have laxative properties (Ogunti and Elujoba, 1993).

Damodaran and Venkataraman (1994) reported the therapeutic efficacy of *Cassia alata* leaf extract against Pityriais versicolor for the first time involving humans. They found that the leaf extract has no side effects.

Samappito *et al.* (2002) had done the molecular characterization of root-specific chalcone synthases from *Cassia alata*. They found that roots of *Cassia* also accumulate anthraquinones, chrysophanol, emodin and rhein.

Villasen *et al.* (2002) tested hexane, chloroform and ethyl acetate extracts of the leaves of *Cassia alata* for their anti-mutagenic, antifungal, analgesic, anti-inflammatory and hypoglycemic activities. The hexane extract was analgesic. Both the hexane and ethyl acetate extracts exhibited anti-inflammatory, antifungal and hypoglycemic activity. The chloroform extract was anti-mutagenic and possessed antifungal activity.

Kaempferol-3-*O*-gentiobioside, the major flavonoid glycoside in *Cassia alata* was quantified in various parts of the plant. The mature leaf was found to contain the highest content of this metabolite. The contents ranged from 2.0 to 5.0 per cent and 1.0 to 4.0 per cent in mature and juvenile leaves, respectively. The other parts studied were flower (sepal and petal), rachis, stem and seed. The highest kaempferol-3-*O*-gentiobioside content was detected in the leaflets while very little or none was found in the flower, stem and seed (Moriyama *et al.*, 2003a).

Moriyama *et al.* (2003b) isolated Adenine from the leaves of *Cassia alata* by HPLC using a triacontylsilyl silica (C30) column. Adenine was found to be an inhibitor of platelet aggregation induced by collagen, although its inhibitory potency is weaker than Adenosine. Adenine had little inhibitory effect on the platelet aggregation induced by ADP.

Moriyama *et al.* (2003c) studied the anti-inflammatory activity of heat-treated *Cassia alata* leaf extract and its flavonoid glycoside. They observed that the extracts of heat treated and sun-dried *C. alata* leaves have strong inhibitory

effects on concanavalin A-induced histamine release, COX-1 and COX-2, and 5-lipoxygenase activities, whereas K3G as a major flavonoid glycoside in *C. alata* leaf showed weak or little inhibitory effects. From these observations they assumed the heat-treated extract may contain other constituents responsible for the stronger inhibitory effects.

Panichayupakaranant and Intaraksa (2003) analysed the content of hydroxyanthracene derivatives from the leaves, flowers and pods of *Cassia alata* and found that highest amount is in the leaves.

Panichayupakaranant and Kaewsuwan (2004) investigated the antioxidant activity of crude methanol extracts from the leaves, flowers and pods of *Cassia. alata* using DPPH radical scavenging assay. They found that the extract from leaves exhibited a stronger antioxidant activity than the extracts from the flowers and pods.

The protective activity of *Cassia alata* against carbon tetrachloride (CCl_4) induced hepatotoxicity in rats was assessed by Wegwu *et al.* (2005). They observed significant reduction in the level of Serum aspartate aminotransferase and alanine aminotransferase in rats pretreated with alcoholic extracts of petals of the plant, 18 hours after CCl_4 administration.

Pieme *et al.* (2006) studied the acute and subacute toxicities of hydro-ethanolic extract of leaves of *Cassia alata* in swiss mice and wistar albino rats. The results showed that the hydro-ethanolic extract of *Cassia alata* is nontoxic.

Oladunmoye (2007) reported that the ethanolic extract of *Cassia alata* leaf has immunostimulatory activity on swiss albino rats infected with *Staphylococcus aureus.*

Fernand *et al.* (2008) investigated the presence of pharmacologically active compounds, rhein, kaempferol, aloe-emodin, emodin, chrysophanol and physcion in root extracts of *Cassia alata* by HPLC-UV detection method.

Hepatoprotective activity of the alcoholic extract of the dried leaves of *Cassia alata* was studied against paracetamol induced hepatic injury in albino rats. Pretreatment of the *Cassia alata* extract reduced the biochemical markers of hepatic injury like serum glutamate pyruvate transaminase (SGPT), serum oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), total bilirubin and gamma glutamate transpeptidase (GGTP). Histopathological observations also revealed that pretreatment with *Cassia alata* extract protected the animals from paracetamol induced liver damage (Anandan *et al.*, 2009).

2.3 Coleus amboinicus

2.3.1 ANTIMICROBIAL ACTIVITY STUDIES ON *Coleus amboinicus*

Studies conducted by Rianti and Yogyarti (2006) revealed the antimicrobial effects of *Coleus amboinicus*, *Lourfolium infusum* against *Candida albicans* and *Streptococcus mutans.* They used *Coleus Amboinicus, Lourfolium infusum* with 12.5, 15, 17.5, 20 and 22.5 per cent concentrations. Degree of inhibition of growth increases with increasing of *Coleus amboinicus, Lourfolium infusum* concentration. The most effective concentration to decrease *C. albicans* and *S. mutans* colonies was found to be 22.5 per cent.

Kumar *et al.* (2008) observed that aqueous extract of *Coleus amboinicus* leaves showed selective antimicrobial activity towards *Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Proteus vulgaris, Klebsiella pneumonia* and *Pseudomonas aeruginosa.* The MIC results indicated that *Proteus vulgaris, Bacillus subtilis* and *Staphylococcus aureus* were least susceptible. *Escherichia coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa* were not susceptible to aqueous extract of *Coleus amboinicus.*

Muthuvelan and Raja (2008) reported that *Coleus amboinicus* possessed antimicrobial and antioxidant activities. Among the ten medicinal plants tested, *Coleus amboinicus* is found to have high antioxidant activity (91.64 per cent)

Gurgel *et al.* (2009) studied the antibacterial effects of *Coleus amboinicus* against Methicillin Resistant *Staphylococcus aureus* (MRSA). They observed that the hydroalcoholic extracts of leaves of *C. amboinicus* have shown a promising activity against MRSA strains. The minimum inhibitory concentration ranged from 9.3 to 18.7 mg/ml.

Murthy *et al.* (2009) investigated *Coleus amboinicus* (Indian borage) for antifungal activity through agar well diffusion assay. Indian borage oil (IBO) was found to be effective against various fungi tested, as it inhibited the radial growth of mycelia and exhibited broad fungitoxic properties against *Aspergillus flavus, Aspergillus niger, Aspergillus ochraceus, Aspergillus oryzae, Candida versatilis, Fusarium* spp., *Penicillium* spp. and *Saccharomyces cerevisiae.*

2.3.2 OTHER PHARMACOLOGICAL EFFECTS OF *Coleus amboinicus*

Carvacrol and p-cymene were found to compose about 74 per cent of the essential oil of *Coleus amboinicus* leaves (Valera *et al.*, 2003).

Jose *et al.* (2005) studied the modulatory effect of *Coleus amboinicus* leaves on ethylene glycol induced nephrolithiasis in rats. They observed that *C. amboinicus* have significant anti-lithotic activity.

Study conducted by Kumar *et al.* (2007) evaluated the mast cell stabilization property of the *Coleus aromaticus* leaf extract in rat mesentery. They found that concentrations of 10 and 100 µg/ml of both the aqueous and hydroalcoholic extracts of *C. aromaticus* produced dose-dependent and significant mast cell stabilization property.

Activity-guided isolation of radical-scavenging antioxidant compounds from *Coleus aromaticus* resulted in the identification of three compounds, namely, rosmarinic acid, chlorogenic acid and caffeic acid. The antioxidant activity of these isolated compounds were assessed by measuring their capacity to scavenge the DPPH (1,1-diphenyl-2-picrylhydrazyl). The result indicated that

they were potent antioxidants and rosmarinic acid is having highest activity (Kumaran and Karunakaran, 2007).

Kaliappan and Viswanathan (2008) reported the presence of flavonoids, terpenoids, saponins, steroids, tannins, proteins, carbohydrates and volatile oil in the leaves of *Coleus amboinicus.*

2.4 Myristica fragrans

2.4.1 ANTIMICROBIAL ACTIVITY STUDIES ON *Myristica fragrans*

Orabi *et al.* (1991) isolated two antimicrobial resorcinols, malabaricone B and malabaricone C from the dried seed covers *of Myristica fragrans.* Both compounds exhibited strong antifungal and antibacterial activities against a variety of microorganisms, including *Staphylococcus aureus* and *Candida albicans.* Structure modifications by methylation or reduction resulted in diminished activity.

De *et al.* (1999) screened some Indian spices for their antimicrobial activity. The results revealed that *Myristica fragrans* (nutmeg) have antibacterial activity.

Dorman and Deans (2000) assessed the volatile oils of black pepper (*Piper nigrum*), clove (*Syzygium aromaticum*), geranium (*Pelargonium graveolens*), nutmeg (*Myristica fragrans*), oregano (*Origanum vulgare*) and thyme (*Thymus vulgaris*) for antibacterial activity against 25 different genera of bacteria. The volatile oils exhibited considerable inhibitory effects against all the tested organisms while their major components demonstrated various degrees of growth inhibition.

Takikawa *et al.* (2002) studied the antimicrobial activity of nutmeg against *Escherichia coli* O-157. They found that *Escherichia coli* O-157 were susceptible to the extract, but non pathogenic strains were not susceptible.

Rani and Khullar (2004) evaluated some medicinal plants for their anti-enteric potential against multi-drug resistant *Salmonella typhi*. Fifty four plant extracts (methanol and aqueous) were assayed for their activity against multi-drug resistant *Salmonella typhi*. Strong antibacterial activity was shown by the methanol extracts of *Aegle marmelos*, *Salmalia malabarica*, *Punica granatum*, *Myristica fragrans*, *Holarrhena antidysenterica*, *Terminalia arjuna* and Triphal (mixture of *Emblica officinalis*, *Terminalia chebula* and *Terminalia belerica*).

Singh *et al.* (2005) studied the antifungal, antibacterial, and antioxidant potentials of essential oil and acetone extract of *Myristica fragrans*. They found that both the essential oil and acetone extract of mace exhibited broad spectrum of antimicrobial activity against the tested microorganisms and it possess high antioxidant activity when compared with synthetic antioxidants.

Indu *et al.* (2006) evaluated antibacterial activity of extracts of *Allium sativum* (garlic), *Myristica fragrans* (nutmeg), *Zingiber officinale* (ginger), *Allium cepa* (onion) and *Piper nigrum* (pepper) against 20 different serogroups of *Escherichia coli*, 8 serotypes of *Salmonella*, *Listeria monocytogenes* and *Aeromonas hydrophila*. Nutmeg showed good anti-listerial activity, although activity against *E. coli* and *Salmonella* were serotype dependent. Both garlic and nutmeg extracts were effective against *A. hydrophila*.

Antibacterial principles from *Myristica fragrans* seeds were studied by Narasimhan and Dhake (2006). The antibacterial constituents of *M. fragrans* include trimyristin, myristic acid and myristicin. All the constituents isolated from *Myristica fragrans* exhibited good antibacterial activity against selected gram positive and gram negative organisms.

2.4.2 OTHER PHARMACOLOGICAL EFFECTS OF *Myristica fragrans*

Ozaki *et al.* (1989) investigated the anti-inflammatory effect of methanol extract of *Myristica fragrans* and its active principles by carrageenin-induced edema in rats and acetic acid-induced vascular permeability in mice. They

suggested that the anti-inflammatory effect of the extract is due to the presence of myristicin.

Ethanolic extract of *Myristica fragrans* was studied in albino rabbits for its effects on experimentally induced hyperlipidaemia. A sharper and much more significant decrease in serum total and LDL cholesterol and triglyceride levels as compared with the control group. There is no significant difference in HDL cholesterol levels. The extract of *Myristica fragrans* also possesses anti-platelet activity and there is an increased bleeding time (Ram *et al.*, 1996).

Olajide *et al.* (1999) observed that the chloroform extract of *Myristica fragrans* has anti-inflammatory, analgesic and antithrombotic activities in rodents. The extract inhibited the carrageenin-induced rat paw oedema, produced a reduction in writhings induced by acetic acid in mice and offered protection against thrombosis induced by ADP/adrenaline mixture in mice.

Nutmeg (*Myristica fragrans*) has been reported to have analgesic and anti-inflammatory activity in rats, mice and rabbits (Thabrewa *et al.*, 2003).

According to Sonavane *et al.* (2001) the acetone soluble part of n-hexane extract of *Myristica fragrans* possesses anxiogenic, sedative and analgesic activity.

Sonavane *et al.* (2002a) reported that the n-hexane extract of *Myristica fragrans* seeds, acetone-insoluble part of the n-hexane extract of *Myristica fragrans* (AIMF) and trimyristin (TM) possess anxiogenic activity.

Sonvane *et al.* (2002b) studied anticonvulsant and behavioural actions of *Myristica fragrans* seeds. They observed that *Myristica fragrans* have complex actions in the central nervous system and possess anticonvulsant activity against seizures induced by maximum electroshock, pentylenetetrazol and lithium sulphate- pilocarpine nitrate.

Study conducted by Morita *et al.* (2003) revealed the hepato-protective effect of myristicin from nutmeg (*Myristica fragrans*) on lipopolysaccharide/D-galactosamine- induced liver injury in rats.

Tajuddin *et al.* (2003) reported that the systemic use of 50 per cent ethanolic extracts of nutmeg and clove has sexual behavior enhancing effect in male mice.

Parle *et al.* (2004) studied the effect of *Myristica fragrans* seeds on learning and memory in mice. The learning and memory parameters were assessed using elevated plus-maze and passive-avoidance apparatus. They observed that *Myristica fragrans* extract enhanced learning and retention capacities of both young and aged mice. This extract also reversed scopolamine- and diazepam-induced impairment in learning and memory of young mice.

Tajuddin *et al.* (2005) showed that 50 per cent ethanol extract of nutmeg administered orally resulted in sexual function improving activity of normal male rats, increasing aphrodisiac activity, libido and potency.

Study conducted by Dhingra *et al.* (2006) estimated the acetylcholine esterase inhibiting activity of extracts of *Glycyrrhiza glabra, Myristica fragrans* seeds and ascorbic acid and compare these values with a standard acetylcholine esterase inhibiting drug, metrifonate. They found that *G. glabra, M. fragrans,* ascorbic acid and metrifonate significantly decreased acetylcholinesterase activity as compared with their respective vehicle-treated control groups.

Dhingra and Sharma (2006) investigated the effect of an *n*-hexane extract of *Myristica fragrans* seeds on depression in mice by using the forced swim test (FST) and the tail suspension test (TST). They found that the extract of *M. fragrans* at a dose of 10 mg/kg elicited a significant antidepressant-like effect in mice. The antidepressant-like effect of the extract seems to be more efficacious than fluoxetine and imipramine.

Olaleye *et al.* (2006) investigated aqueous extract of the seed of *Myristica fragrans* (nutmeg) for its phytochemical constituents, anti-nutrients and antioxidant properties. Phytochemical screening of extracts revealed the presence of alkaloids, flavonoids and anthraquinones. The extract exhibited strong antioxidant properties. Behavioural changes and weight gain also noticed in treated animals.

Chatterjee *et al.* (2007) evaluated antioxidant potential of phenolic compounds from green pepper (*Piper nigrum*) and lignans from fresh mace (*Myristica fragrans*) for their ability to scavenge 1,10-diphenyl-2-picrylhydrazyl (DPPH) radical, inhibit lipid peroxidation and protect plasmid DNA damage upon exposure to gamma radiation. They found that green pepper and fresh mace phenolics are powerful antioxidants.

Chirathaworn *et al.* (2007) investigated the effect of *Myristica fragrans* methanol extract as anti-cancer agent using the Jurkat Leukemia T cell line. They further investigated the effect of this plant extract on SIRT1 (silent information regulator two ortholog 1) gene expression. The result indicated that methanol extract of *Myristica fragrans* induced apoptosis of Jurkat leukemia T cell line in a mechanisms involving SIRT1 mRNA down regulation.

Checker *et al.* (2008) described the immunomodulatory and radiomodifying properties of lignans present in the aqueous extract of fresh nutmeg mace in mammalian splenocytes. These mace lignans inhibited the proliferation of splenocytes in response to polyclonal T cell mitogen concanavalin A (Con A). Mace ligans protected splenocytes against radiation-induced intracellular ROS (reactive oxygen species) production in a dose dependent manner.

Somani and Singhai (2008) investigated the hypoglycaemic activity of seeds of *Myristica fragrans* in normoglycaemic and alloxan- induced diabetic rats. The results obtained showed that the petroleum ether extract of *M. fragrans*

decreased blood glucose levels in normal, glucose fed and alloxan- induced diabetic rats, compared with their respective control groups.

Tarmizi *et al.* (2008) determined the effects of essential oil from *Myristica fragrans* on GABA (A) receptor. The essential oil from *Myristica fragrans* enhanced the maximum chloride current of $\alpha_1\beta_2\gamma_2$ receptors.

2.5 Tectona grandis

2.5.1 ANTIMICROBIAL ACTIVITY STUDIES ON *Tectona grandis*

Srinivasan *et al.* (2001) reported that aqueous extract of *Tectona grandis* showed broad spectra of antimicrobial activity against *Chromobacterium* spp., *Enterobacter faecalis*, *Candida albicans*, *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella paratyphi* and *S.typhi*.

Takahashi *et al.* (2004) reported the leishmanicidal activity of *Tectona grandis* against amastigotes of *Leishmania donovani* in mouse peritoneal macrophages.

Study conducted by Neamatallah *et al.* (2005) revealed that the methanol extract from teak *(Tectona grandis)* bark inhibited *Listeria monocytogenes* and methicillin resistant *Staphylococcus aureus.*

Shalini and Srivastava (2009) investigated the antifungal activity of methanolic crude extract of *Tectona grandis, Shilajit, Valeriana wallachi* was against *Alternaria cajani*, *Curvularia lunata*, *Fusarium* spp., *Bipolaris* spp. and *Helminthosporium* spp. HPLC analysis of the crude extract of medicinal plants showed four different Phenolic acids (Tannic acid, Gallic acid, Ferulic acid and Caffeic acid). The extract of *Tectona grandis* showed 90 per cent and 86.84 per cent inhibition growth against *Alternaria cajani* and *Helminthosporium.*

2.5.2 OTHER PHARMACOLOGICAL EFFECTS OF *Tectona grandis*

Lapachol, a naphthaquinone isolated from the roots of *Tectona grandis* given at a dose of 5 mg/kg was found to have an anti-ulcerogenic effect on subsequently induced experimental gastric and duodenal ulcers in rats and guinea-pigs. Its action appears to be associated with an effect on the protein content of gastric juice, and it reversed aspirin-induced changes in peptic activity, protein and sialic acid (Goel *et al.*, 1987).

Pathak *et al.* (1988) isolated Betulin aldehyde together with lupeol, betulin and betulinic acid from the stem bark of *Tectona grandis* and characterised by spectral analysis.

Khan and Miungwana (1999) isolated a new compound, 5-hydroxylapachol (1) along with the known constituents lapachol, dehydro-a-lapachone, methylquinizarin and squalene from *Tectona grandis*. Both hydroxylapachol and lapachol were found to be cytotoxic to *Artemia salina* (brine shrimp) with an LC50 of 5 ppm.

The study conducted by Majumdar *et al.* (2007) evaluated the effect of hydrochloric extract of *Tectona grandis* on experimentally induced wounds in rats and compare the effects with a known wound healing agent, *Aloe vera.* It was observed that *Tectona grandis* leaf extract applied topically (5 per cent and 10 per cent gel formulation) or administered orally (250 mg and 500 mg/kg body weight) possesses wound healing activity.

Diallo *et al.* (2008) studied the effect of *Tectona grandis* on phenylhydrazine induced anaemia in rats. The extract of *Tectona grandis* leaves increased the concentration of haemoglobin, red blood cells number, haematocrit and reticulocytes rate. Moreover, the extract of *T. grandis* enhanced the osmotic resistance of the red blood cells that confirm the important presence of young red blood cells.

Ghaisas *et al.* (2009) investigated the effect of ethanolic extract of *Tectona grandis* bark in dexamethasone induced insulin resistance in mice. The results indicated that *Tectona grandis* may prove to be effective in the treatment of Type-II Diabetes mellitus owing to its ability to decrease insulin resistance. The levels of antioxidant enzymes GSH, SOD and catalase were significantly increased and there was significant decrease in level of lipid peroxidation.

Nayeem and Karvekar (2010) reported that methanolic extract of the leaves of *Tectona grandis* has shown significant analgesic and anti-inflammatory activity. The phytochemical analysis has revealed the presence of flavonoids, steroids, glycosides, anthroquinones, saponins, tannins, carbohydrates and proteins.

2.6 OTHER AGENTS HAVING ANTIMICROBIAL ACTIVITY

Naqvi *et al.* (1994) studied the antibacterial activity of 50 per cent ethanolic extracts of leaves, stem and flowers of *Nerium indicum* and *Hibiscus rosasinensis.* The crude extracts showed activity against gram positive and gram negative bacteria, both pathogenic and non-pathogenic strains.

Khan and Omoloso (2002) reported that the crude methanolic extract of *Barringtonia asiatica* (leaf, fruits, seeds, stem and root bark) and the fractions (petrol, dichloromethane, ethyl acetate, butanol) exhibited a very good level of broad spectrum antibacterial activity.

Singh *et al.* (2002) evaluated *Curcuma longa* rhizome extracts for antibacterial activity against pathogenic strains of gram positive (*Staphylococcus aureus* and *Staphylococcus epidermidis*) and gram negative (*Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*) bacteria. Essential oil was found to be most active and its activity was compared to standard antibiotics gentamicin, ampicillin, doxycycline and erythromycin in these strains. They found that essential oil fraction is more effective against gram positive strains compared to gram negative strains.

Clinical isolates of dermatophytes (*Trichophyton rubrum, Trichophyton mentagrophytes* and *Microsporum nanum*) were treated with extracts of leaves and seeds of the plant *Azadirachta indica* (neem) for antifungal activity by *in vitro* tube dilution technique. The MIC of neem seed extracts was 31 µg/ml for all the dermatophytes tested. The neem seed extract at 15 µg/ml concentration was observed to be sufficient for distorting the growth pattern of the organisms tested (Natarajan *et al.*, 2003).

Lloyd *et al.* (2005) studied the antifungal activity of 10 different extracts of seed kernels of *Azadirachta indica* on *Candida* spp. isolated from immuno-compromised patients. The ethanol extract of commercial neem seed oil, ethanol extract of neem seed kernels and the hexane extract showed best results. All strains were resistant to methanol, water and chloroform extracts.

Ghahfarokhi *et al.* (2006) evaluated the antifungal activity of aqueous extracts prepared from *Allium cepa* and *Allium sativum* against various strains of *Malassezia furfur*, *Candida albicans*, other *Candida* spp. as well as various dermatophyte species and compared with the activity of a known antifungal drug, ketoconazole by using an agar dilution assay. All the extracts and ketoconazole were found to be able to inhibit growth of all fungi tested in a dose-dependent manner.

Hassawi and Kharma (2006) studied the antimicrobial activity of twelve medicinal plant species that belong to six genera (*Achillea, Salvia, Convolvulus, Plantago, Anthemis* and *Artemisia)* against *Candida albicans*. The antimicrobial activity was carried out by using the hole-plate diffusion method. The effect of plant species, extract amounts and their interaction were highly significant. *Achillea santolina, Salvia dominica* and *Salvia officinalis* inhibited the growth of *Candida albicans* at all concentrations of extract (200, 150, 100 and 50 mg/ml). The extracts of *Salvia spinosa*, *Convolvulus althaeoides* and *Plantago lanceolata* showed no activity against *Candida albicans*.

Various parts of *Derris elliptica, Derris indica* and *Derris trifoliata* on fractionation with a number of solvents (petrol, dichloromethane, ethyl acetate, butanol and methanol) gave fractions which demonstrated a varied level of broad spectrum antibacterial activity. None of the plants showed antifungal activity (Khan *et al.*, 2006).

Ali *et al.* (2007) determined the antibacterial and antifungal activities of various solvent extracts of *Piper longum* against a wide variety of pathogenic bacteria and fungi. Crude extracts of *Piper longum* showed mild to moderate activities against most of the tested bacteria. Petroleum ether extracts of *Piper longum* were found to be inactive against most of the tested organisms. Ethyl acetate extracts showed relatively better antimicrobial effect against most of the tested organisms. Chloroform extract of leaves showed higher antifungal activity as compared to the other extracts of the plant.

Nikitina *et al.* (2007) reported that polyphenolic compounds isolated from plants of Geraniaceae and Rosaceae families have antibacterial activity of against gram positive and gram negative bacteria of the genera, *Azotobacter*, *Bacillus* and *Pseudomonas*.

Jalalpure *et al.* (2008) evaluated antimicrobial activity of petroleum ether, chloroform, acetone, methanol and aqueous extracts of leaves of *Alternanthera sessilis* by cup plate method and turbidimetric method. Chloroform and acetone extracts showed maximum zone of inhibition against almost all organisms and chloroform extract showed significant MIC values.

Mahesh and Satish (2008) observed that methanol leaf extracts of *Acacia nilotica, Sida cordifolia, Tinospora cordifolia, Withania somnifera* and *Ziziphus mauritiana* showed significant antibacterial activity against *Bacillus subtilis, Escherichia coli, Pseudomonas fluorescens, Staphylococcus aureus* and *Xanthomonas axonopodis* and antifungal activity against *Aspergillus flavus, Dreschlera turcica* and *Fusarium verticillioides* when compared to extracts of root or bark.

The crude methanol and flavonoid (free and bound) extracts of *Marchantia polymorpha* L. (Marchantiaceae) were screened against *Escherichia coli, Proteus mirabilis, Staphylococcus aureus, Aspergillus flavus, A. niger, Candida albicans* and *Trychophyton mentagrophytes*. Disc diffusion and microbroth dilution techniques were performed for evaluation of antimicrobial activity of the extracts. All the microorganisms were found to be sensitive against the extracts tested. Total activity for *P. mirabilis* and *S. aureus* for methanol extract was found to be the same (Mewari and Kumar, 2008).

Rath *et al.* (2008) assessed the antibacterial activity of Jasmine (*Jasminum sambac*) flower essential oil, synthetic blends and six major individual components against *Escherichia coli* (MTCC-443) strain. The activity was bactericidal. Minimum inhibitory concentration was determined by tube dilution technique and the minimum inhibitory concentration ranged between 1.9-31.25 µl/ml.

The antibacterial and antifungal activities of the ethanol and petroleum ether extracts of *Anacardium occidentale* were tested against *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Candida albicans* and *Aspergillus niger* by cup plate method. Minimum inhibitory concentration values of each active extract were determined. They observed that petroleum ether extract and ethanolic extract of *Anacardium occidentale* leaves exhibited significant antimicrobial and antifungal activity (Dahake *et al.*, 2009).

Dulger (2009) studied antifungal activities of the ethanol extracts obtained from the leaves, rootstock, and the combined formulation of endemic *Lamium tenuiflorum* against *Candida* and *Cryptococcus* species by the visual broth macrodilution method. The MIC values ranged from 3.12 to 25 mg/ml. All the extracts exhibited a strong antifungal effect against the yeast cultures. The extracts exhibited greater antifungal effect against *Candida* species than *Cryptococcus* species.

Study conducted by Hasan *et al.* (2009) determined the antibacterial and antifungal activities of chloroform extract of *Polygonum hydropiper* root against bacteria and fungi using the disc diffusion method. The extract showed significant antibacterial activities against four gram positive (*Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus* and *Enterobacter aerogenes*) and four gram negative (*Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi* and *Shigella sonnei*) bacteria. MIC values against these bacteria ranged from 16 to 64 µg/ml. The antifungal activities were found to be strong against *Aspergillus fumigatus, Aspergillus niger, Aspergillus flavus, Candida albicans, Rizopus oryzae* and *Tricophyton rubrum.*

An experiment was carried out by Jagtap *et al.* (2009) to study the antimicrobial activity of petroleum ether, ethanol and water extract of *Centella asiatica* using agar diffusion method against *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Propionibacterium vulgaris, Aspergillus niger, Aspergillus flavus* and *Candida albicans.* Zone of inhibition produced by petroleum ether, ethanol and water extract in different doses was measured and compared with standard antibiotics ciprofloxacin (10 µg/ml). The results demonstrated that the ethanolic extract of *Centella asiatica* has higher antimicrobial activity than petroleum ether and water extract.

Uma *et al.* (2009) investigated the *in vitro* anticandidal activity of *Asparagus racemosus* root and tubers extract against *Candida albicans, Candida tropicalis, Candida krusei, Candida guillermondii, Candida parapsilosis* and *Candida stellatoida,* which are isolated from vaginal thrush patients. The extract of *Asparagus racemosus* showed high degree of activity against all the *Candida* strains. The inhibitory effect of the extract against all the *Candida* spp. tested was found comparable with that of standard antibiotics.

Materials and Methods

3. MATERIALS AND METHODS

3.1 PLANT MATERIALS

The leaves of the plants *Annona squamosa* (Aatha), *Cassia alata* (Anathakara), *Coleus amboinicus* (Panicoorka), *Myristica fragrans* (Nutmeg) and *Tectona grandis* (Teak) were collected from the campus of College of Veterinary & Animal Sciences, Mannuthy, Thrissur district, Kerala and were identified by Department of Botany, Calicut University, Calicut, Kerala, India (Fig.1-5).

3.1.1 PREPARATION OF EXTRACTS OF PLANT MATERIALS

The leaves of the plants were dried under shade and ground into fine powder using electric blender. 100 g of dried powder was soaked in 1 Litre ethanol for 48 hours with intermittent shaking. The plant extracts were filtered through Whatman No.1 filter paper. (Duraipandiyan *et al.*, 2006). The extracts were concentrated to near dryness using rotatory vacuum flash evaporator under reduced pressure at 30°C and kept in the refrigerator in airtight containers. The extracts were further diluted with Dimethylsulphoxide (DMSO) for experimentation (Ahmad *et al.*, 1998; Patel *et al.*, 2008).

3.1.2 PHYTOCHEMICAL SCREENING

The ethanolic extract and fresh juice of leaves of plants were tested for the presence of various active chemical constituents namely steroids, alkaloids, phenolic compounds, flavonoids, glycosides, diterpenes, triterpenes and saponins by the method described by Harborne (1991). Tests for nitrate (Householder *et al.*, 1966) and cyanide (Bark and Higson, 1963) were also conducted.

Fig 1. ***Annona squamosa***

Fig 2. ***Cassia alata***

Fig 3. ***Coleus amboinicus***

Fig 4. ***Myristica fragrans***

Fig 5. *Tectona grandis*

3.1.2.1 Tests for Detection of Steroids

Salkowski test

About 5 mg of the extract was dissolved in 3 ml of chloroform and then shaken with about 3 ml concentrated sulphuric acid. If a red colour develops, it indicates the presence of steroids.

Liberman Burchardt test

About 5 mg of the extract was dissolved in 3 ml of chloroform in a test tube. Then five drops of acetic anhydride and 1 ml of concentrated sulphuric acid were added to it through the sides of the test tube. A reddish ring at the junction of two layers indicates the presence of steroids.

3.1.2.2 Tests for Detection of Alkaloids

About 0.5 g of the extract was mixed with 5 ml of ammonia and then extracted with equal volume of chloroform. To this, 5 ml diluted hydrochloric acid was added. The acid layer obtained was used for the following chemical tests for alkaloids

Mayer's test (potassium mercuric iodide)

To 1 ml of acid layer, a few drops of Mayer's reagent (1.358 g of mercuric chloride dissolved in 60 ml of water and poured into a solution of 5 g of potassium iodide in 10 ml of water and then make up the volume to 100 ml with distilled water) was added. Development of a creamy white precipitate indicates the presence of alkaloids.

Wagner's test

Few drops of Wagner's reagent (2 g of iodine and 6 g of potassium iodide dissolved in 100 ml of water) were added to 1 ml of the acid layer. If

there is presence of reddish brown coloured precipitate, the presence of alkaloids is indicated.

Hager's test (saturated solution of picric acid)

To 1 ml of the acid extract, few drops of Hager's reagent (1 g of picric acid dissolved in 100 ml of water) were mixed. A yellow precipitate is formed, if alkaloids are present.

Dragendroff's test

Few drops of Dragendroff's reagent (Stock solution (1) 0.6 g bismuth sub nitrate was dissolved in 2 ml of concentrated hydrochloric acid and 10 ml of water was added. Stock solution (2) 6 g potassium iodide was dissolved in 10 ml of water. Then both the stock solutions (1) and (2) were mixed together and then it was mixed with 7 ml of concentrated hydrochloric acid and 15 ml of water. Sufficient amount of distilled water was added to the mixture to make up the volume to 400 ml) was mixed with 1 ml of acid layer. Development of a reddish brown precipitate indicates the presence of alkaloids.

3.1.2.3 Test for Detection of Phenolic compounds

About 5 mg of the extract was dissolved in 1 ml of water and five drops of 10 per cent ferric chloride was added to it. Development of dark brown colour indicates the presence of phenolic compounds.

3.1.2.4 Tests for Detection of Tannins

Ferric chloride test

Two milligram of the extract was mixed with 3 ml of one per cent ferric chloride solution. If blue, green or brownish green colour is obtained, it indicates the presence of tannins.

Gelatin test

About 0.5 g of the extract was mixed with few drops of one per cent solution of gelatin containing 10 per cent sodium chloride. Development of a white precipitate indicates the presence of tannins.

3.1.2.5 Tests for Detection of Flavonoids

Ferric chloride test

To 2 ml of alcoholic solution of the extract (0.5 g extract in 10 ml methanol), a few drops of neutral ferric chloride solution was mixed. Development of green colour indicates the presence of flavonoids.

Lead acetate test

To 2 ml of alcoholic solution of the extract (0.5 g extract in 10 ml methanol), a few drops of neutral ten per cent lead acetate was mixed. If yellow precipitate appears, the presence of flavonoids is indicated.

3.1.2.6 Tests for Detection of Glycosides

Sodium hydroxide test

A small amount of the extract (about 5 mg) was mixed with 1 ml water and 5-6 drops of 10 per cent sodium hydroxide solution were added. Development of yellow colour indicates the presence of glycosides.

Benedict's test

To about 1 ml of the extract (0.5 g extract in 1 ml of water), 5 ml of Benedict's reagent was added. The mixture was boiled for two minutes and cooled. If development of brown to red colour occurs, it indicates the presence of glycosides.

3.1.2.7 Test for Detection of Diterpenes

About 5 mg of the extract was mixed with 3 ml of copper acetate solution (5 per cent). Development of green colour indicates the presence of diterpenes.

3.1.2.8 Tests for Detection of Triterpenes

Salkowski test

About 3 mg of the extract was dissolved in 3 ml of chloroform and then it was shaken with 3 ml of concentrated sulphuric acid. If lower layer turn to yellow on standing, it is an indication of the presence of triterpenes.

Lieberman Burchardt test

Few drops of acetic acid and 1 ml concentrated sulphuric acid were added to 3 ml chloroform solution of the extract (about 3 mg extract in 3 ml chloroform). Deep red ring at the junction of two layers indicates the presence of Triterpenes.

3.1.2.9 Test for Detection of Saponins

Foam test

A small amount of the extract (about 5 mg) was shaken with 3 ml of water. Development of the foam that persists for ten minutes indicates the presence of saponins.

3.1.2.10 Test for the detection of nitrates

The plant material was tested with diphenylamine reagent (Diphenylamine stock solution was prepared by dissolving 0.5 g of diphenylamine in 20 ml of water and concentrated sulphuric acid was added to bring the volume to 100 ml. Equal part of this stock solution was mixed with 80 per cent sulphuric acid.) The test was conducted by adding few drops of reagent

:o the crushed plant material. A bright blue colour indicates presence of nitrate (Householder *et al.*, 1966).

3.1.2.11 Test for the detection of cyanides

Finely chopped fresh plant materials (5 g) were added to 100 ml boiling tube. 4-12 drops of chloroform was added. To the stoppered lid, a picrate paper was anchored in such a way that this picrate paper was hanging away from the bottom of the liquid. The test tube was kept on a water bath at 30-37^0 C for about 3 hours. If the test is positive, yellow picrate paper will turn to red (Bark and Higson, 1963).

3.2 MICROORGANISM

The organisms used in this study consisted of five bacterial and three fungal strains. These include *Staphylococcus aureus subsp. aureus* (MTCC 96), *Salmonella enteritidis* (MTCC 3219), *Escherichia coli* (MTCC 723), *Pasteurella multocida subsp. multocida* (MTCC 1161), *Pseudomonas aeruginosa* (MTCC 741), *Aspergillus fumigatus* (MTCC 870), *Candida albicans* (MTCC 227) and *Cryptococcus neoformans var neoformans* (MTCC 4404). The organisms were obtained as freeze dried pure cultures from the Microbial Type Culture Collection, Institute of Microbial Technology (IMTECH), Chandigarh, India and were maintained at 4°C on nutrient agar slants.

3.3 CHEMICALS AND REAGENTS

Mueller Hinton agar, Mueller Hinton broth, Nutrient agar, Sabouraud dextrose broth, Sabouraud dextrose agar, Czapek yeast extract agar, yeast malt agar, Brain heart infusion agar, sterile discs and standard discs were purchased from Hi- Media, Mumbai.

Standard disc: Penicillin G (10 units/disc)

Furazolidone 50 µg

Ketoconazole 10 µg

Clotrimazole 10 µg

Dimethylsulphoxide (DMSO) from Merck India Ltd, Mumbai and MTT (3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl-2H-tetrazoliumbromide) from Fischer Scientific World wide Company, Hong Kong.

3.4 SCREENING FOR ANTIMICROBIAL ACTIVITY

3.4.1 PREPARATION OF EXTRACT

Various concentrations of the extracts of *Annona squamosa* (Aatha), *Cassia alata (*Anathakara), *Coleus amboinicus (*Panicoorka), *Myristica fragrans* (Nutmeg) and *Tectona grandis* (Teak) were prepared using dimethylsulphoxide (DMSO). The extracts were sterilised using sterile Whatman syringe filter (0.45 µm).

3.4.2 PREPARATION OF INOCULUM

Stock cultures were maintained at 4°C on slopes of nutrient agar. Pure cultures used as inoculums. Three to four similar colonies were selected and transferred to 5 ml of Mueller-Hinton broth (MHB). The broth was incubated at 37°C for 2-8 hrs till light moderate turbidity developed. Turbidity was adjusted to match that of 0.5 McFarland standards. If the turbidity in the broth was sufficient, further incubation was not necessary.

Inoculum for fungi: Five distinct colonies of approximately 1 mm from 24 hours old Sabouraud dextrose agar were suspended in 5 ml sterile 0.85 per cent saline and incubated at 35 ± 2°C. The turbidity is adjusted to yield 1×10^6 - 5×10^6 cells/ml (0.5 McFarland standard).

0.5 McFarland standard – 0.5 ml of 1 per cent barium chloride + 99.5 ml of 1 per cent sulphuric acid (0.36 N).

3.4.3 ANTIMICROBIAL SUSCEPTIBILITY TEST

The disc diffusion method (Bauer *et al.*, 1966) was used to screen the antimicrobial activity. *In vitro* antimicrobial activity was screened by using Mueller Hinton Agar (MHA) obtained from Himedia, Mumbai.

The MHA plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 minutes. The plates were incubated at 37°C for 24 hrs for checking sterility.

Dip sterile non-toxic cotton swab on a wooden applicator in to the standardized inoculum (turbidity so adjusted, as to obtain semi confluent growth on the petriplate) and rotated the soaked swab firmly against upper inside wall of the tube to express excess fluid. The broth suspension of the organism was streaked evenly on the agar surface three times with swab, turning the plate 60° angle between each streaking. The inoculum was allowed to dry for 5 minutes.

The different concentrations of extracts (1.25, 2.5, 5, 10 and 15 mg/disc) were loaded on 6 mm sterile disc. The loaded disc was placed on the surface of medium and the compound was allowed to diffuse for 5 minutes and then the plates were kept for incubation at 37°C for 24 hrs for bacteria and 30°C for 48 hrs for fungi. The reference drug disc was also placed on the surface of the agar. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. These studies were performed in triplicate (Duraipandiyan *et al.*, 2006).

The standard drug used for gram positive organisms was penicillin G and that for gram negative organism was furazolidone. Clotrimazole and ketoconazole were the standard antifungal agents used.

3.4.4 MICROTITRE PLATE DILUTION METHOD

Various concentrations (200 µg-1 mg/ml) of extracts were prepared and 0.02 ml of diluted extract was mixed with 0.05 ml bacterial culture (inoculums of 50 per cent transmission at 530 nm in normal saline) and nutrient broth in a total volume of 0.18 ml in a 96 well microtitre plate. The plate was incubated for a period of 24h at 37°C. After the incubation, 0.02 ml of MTT (3-(4,5-dimethylthiazol-2-yl) 2,5-diphenyl-2H-tetrazoliumbromide) 5 mg/ml was added in to each well and incubated for 30 min at 37°C. After the incubation period, the inhibition of growth was detected as colorless wells. The lowest concentration of extract that completely inhibited bacterial growth was assumed as the minimum inhibitory concentration. Pencillin and furazolidone were used as reference drug. All the experiments were repeated thrice (Sheena *et al.*, 2003).

The bacterial suspensions were used as positive control and extracts in broth were used as negative control. The Minimum bactericidal concentration (MBC) was determined by subculture of the well showing no apparent growth in a sterile agar plate. The least concentration showing no visible growth on agar subculture was taken as MBC value (Quinn *et al.*, 1994; Basri and Fan, 2005).

3.4.5 BROTH DILUTION METHOD FOR FUNGI

An amount of 2 ml of the diluent solvent was added to each vial containing extract and from this stock solution various volumes were drawn for the MIC assay such that in each volume the concentration of extract was 1, 0.5, 0.25, 0.125 and 0.0625 mg. Mueller Hinton broth was used in this assay. The highest dilution of extract, at which inhibition of test organism was observed, was recorded as the MIC. Aliquots from each of the tubes were subcultured ~~onto~~ on Saboraud's dextrose agar (SDA) and incubated overnight. Minimum fungicidal concentration (MFC) of the extract was read as the highest dilution of extract that showed no growth on SDA. Ketoconazole was used as antifungal control (Quinn *et al.*, 1994; Charmaine *et al.*, 2005).

3.5 STATISTICAL ANALYSIS

Values are expressed as mean ± standard error. The data obtained were analyzed by analysis of variance (ANOVA) followed by Duncan's multiple range test and paired t-test (Snedecor and Cochran, 1985) to determine the level of significance. The value of $P<0.05$ was considered statistically significant.

Results

4. RESULTS

The present study was undertaken to evaluate the antibacterial and antifungal activities of the leaves of the plants *Annona squamosa* (Aatha), *Cassia alata* (Anathakara), *Coleus amboinicus* (Panicoorka), *Myristica fragrans* (Nutmeg) and *Tectona grandis* (Teak). Fresh juice and cold ethanolic extracts of the leaves were used for the study.

4.1 YIELD OF FRESH JUICE AND COLD ETHANOLIC EXTRACT OF THE LEAVES

The yields of cold ethanolic extract of *Annona squamosa, Cassia alata, Coleus amboinicus, Myristica fragrans* and *Tectona grandis* were 18.07, 13.96, 10.28, 7.12 and 11.82 per cent respectively.

The yields of fresh juice of *Annona squamosa, Cassia alata, Coleus amboinicus, Myristica fragrans, Tectona grandis* were 3.5, 4, 8 ml, 2, 3.5 ml respectively from 10 g of the fresh tender leaves.

The results obtained are shown in Table 1.

4.2 SCREENING OF *Annona squamosa* FOR ACTIVE PRINCIPLES

4.2.1 Steroids

A red colour and red ring were obtained in the Salkowski test and Lieberman Burchadt test respectively. That indicated the presence of steroids in the fresh juice and ethanolic extract of *Annona squamosa* leaves.

4.2.2 Alkaloids

A creamy white precipitate in Mayer's test and a characteristic yellow coloured precipitate with Hager's test were obtained with the extract. With Wagner's reagent, characteristic reddish brown precipitate was obtained. Dragendroffs test gave a characteristic reddish brown precipitate. So the

Table 1. YIELD OF FRESH JUICE AND COLD ETHANOLIC EXTRACT OF THE LEAVES

Plant	Cold alcoholic extract (%)	Fresh juice (ml/10g)
Annona squamosa	18.07	3.5
Cassia alata	13.96	4
Coleus amboinicus	10.28	8
Myristica fragrans	7.12	2
Tectona grandis	11.82	3.5

presence of alkaloids was confirmed in the fresh juice and cold ethanolic extract of *Annona squamosa* leaves.

4.2.3 Tannins

Brownish green colour was obtained in ferric chloride test and a white precipitate was obtained in gelatin test. These results indicated the presence of tannins in the cold ethanolic extract and fresh juice of *Annona squamosa* leaves.

4.2.4 Flavonoids

A green colour in the ferric chloride test and a yellow coloured precipitate in lead acetate test indicated the presence of flavonoids in the cold ethanolic extract and fresh juice of *Annona squamosa* leaves.

4.2.5 Glycosides

As per Benedict's test, brown colour was developed indicating the presence of glycosides in the sample. A yellow colour was obtained by mixing the extracts with sodium hydroxide reagent, which also indicated the presence of glycosides in the cold ethanolic extract and fresh juice of *Annona squamosa* leaves.

4.2.6 Phenolic compounds

The extract mixed with 10 per cent ferric chloride produced a characteristic dark blue colour, indicating the presence of phenolic compounds in the cold ethanolic extract and fresh juice of *Annona squamosa* leaves.

4.2.7 Diterpenes

Diterpenes were detected in the cold ethanolic extract and fresh juice of *Annona squamosa* leaves as indicated by the green colour when mixed with copper acetate solution.

4.2.8 Triterpenes

For ethanolic extract and fresh juice of *Annona squamosa* leaves, lower layer turned to yellow on standing as per Salkowski test, and by Leiberman

Burchadt's test, a deep ring appeared at the junction of the two layers. These results indicated the presence of triterpenes in the extract.

4.2.9 Saponins

In the foam test, foam persisted for 10 minutes, which indicated the presence of saponins in the extract and fresh juice.

4.2.10 Test for the detection of nitrates

Absence of bright blue colour with diphenylamine reagent indicated that nitrate was not present.

4.2.11 Test for the detection of cyanides

There was no change in color of yellow picrate paper which indicated the absence of cyanide.

4.3 SCREENING OF *Cassia alata* FOR ACTIVE PRINCIPLES

4.3.1 Steroids

As per Salkowski test, red colour was obtained and Lieberman Burchadt test gave a reddish ring at the junction. Thus it could be concluded that detectable level of steroids were present in the fresh juice and ethanolic extract of *Cassia alata* leaves.

4.3.2 Alkaloids

A creamy white precipitate as per Mayer's test and a reddish brown coloured precipitate as per Wagner's test were obtained. Characteristic yellow coloured precipitate with Hager's test and reddish brown precipitate with Dragendroffs test were also obtained with the fresh juice and the extract. Thus the tests revealed the presence of detectable level of alkaloids in the fresh juice and cold ethanolic extract of *Cassia alata* leaves.

4.3.3 Tannins

Characteristic brownish green colour in ferric chloride test and white precipitate in gelatin test indicated the presence of tannins in the cold ethanolic extract and fresh juice of *Cassia alata* leaves.

4.3.4 Flavonoids

Green colour in the ferric chloride test and characteristic yellow coloured precipitate in lead acetate test indicated the presence of flavonoids in the cold ethanolic extract and fresh juice of *Cassia alata* leaves.

4.3.5 Glycosides

A red colour obtained in the Benedict's test indicated the presence of glycosides in the sample. A yellow colour was obtained by mixing the extracts with sodium hydroxide reagent, which also indicated the presence of glycosides in the cold ethanolic extract and fresh juice of *Cassia alata* leaves.

4.3.6 Phenolic compounds

A characteristic dark blue colour was produced with 10 per cent ferric chloride which indicated the presence of phenolic compounds in the cold ethanolic extract and fresh juice of *Cassia alata* leaves.

4.3.7 Diterpenes

Diterpenes were detected in the cold ethanolic extract and fresh juice of *Cassia alata* leaves as indicated by the green colour when it was mixed with copper acetate solution.

4.3.8 Triterpenes

As per Salkowski test, lower layer turned to yellow on standing and by Leiberman Burchadt test, a deep ring appeared at the junction of the two layers. These results indicated the presence of triterpenes in the ethanolic extract and fresh juice of *Cassia alata* leaves.

4.3.9 Saponins

In the foam test, foam persisted for 10 minutes, which indicated the presence of saponins.

4.3.10 Test for the detection of nitrates

Absence of bright blue colour with diphenylamine reagent indicated that detectable amount of nitrates were not present in the extract and fresh juice.

4.3.11 Test for the detection of cyanides

No change in color of yellow picrate paper indicated the absence of cyanide in the fresh juice and alcoholic extract of *Cassia alata* leaves.

4.4 SCREENING OF *Coleus amboinicus* FOR ACTIVE PRINCIPLES

4.4.1 Steroids

A red colour as per Salkowski test and red ring as per Lieberman Burchadt test were obtained. That indicated the presence of steroids in the fresh juice and ethanolic extract of *Coleus amboinicus* leaves.

4.4.2 Alkaloids

A creamy white precipitate in Mayer's test and a reddish brown coloured precipitate with Wagner's test were obtained. Characteristic yellow coloured precipitate with Hager's test and reddish brown precipitate with Dragendroffs test were also obtained. These tests revealed the presence of detectable level of alkaloids in the fresh juice and cold ethanolic extract of *Coleus amboinicus* leaves.

4.4.3 Tannins

There were characteristic brownish green colour in ferric chloride test and white precipitate in gelatin test. It indicated the presence of tannins in the cold ethanolic extract and fresh juice of *Coleus amboinicus* leaves.

4.4.4 Flavonoids

The characteristic green colour obtained in the ferric chloride test and the yellow coloured precipitate in lead acetate test indicated the presence of flavonoids in the cold ethanolic extract and fresh juice of *Coleus amboinicus* leaves.

4.4.5 Glycosides

A red colour with Benedict's test and a yellow colour with sodium hydroxide reagent indicated the presence of glycosides in the cold ethanolic extract and fresh juice of *Coleus amboinicus* leaves.

4.4.6 Phenolic compounds

The extract and fresh juice mixed with 10 per cent ferric chloride did not produce a characteristic dark blue colour, which indicated the absence of phenolic compounds in the cold ethanolic extract and fresh juice of *Coleus amboinicus* leaves.

4.4.7 Diterpenes

Diterpenes were absent in the ethanolic extract and fresh juice of *Coleus amboinicus* leaves since there was no green colour when mixed with copper acetate solution.

4.4.8 Triterpenes

For ethanolic extract and fresh juice of *Coleus amboinicus* leaves, the lower layer did not turn to yellow on standing as per Salkowski test, and by Leiberman Burchadt's test, a deep ring did not appear at the junction of the two layers. These results indicated the absence of triterpenes in the extract.

4.4.9 Saponins

In the foam test, foam persisted for 10 minutes, which indicated the presence of saponins.

4.4.10 Test for the detection of nitrates

A bright blue colour was not observed with diphenylamine which indicated the absence of nitrate in the extract and fresh juice.

4.4.11 Test for the detection of cyanides

Color of yellow picrate paper was not changed to red which indicated the absence of cyanide in the fresh juice and alcoholic extract of *Coleus amboinicus* leaves.

4.5 SCREENING OF *Myristica fragrans* FOR ACTIVE PRINCIPLES

4.5.1 Steroids

Red colour and red ring were not observed in the Salkowski test and Lieberman Burchadt test respectively. Thus steroids were not detected in the fresh juice and ethanolic extract of *Myristica fragrans* leaves.

4.5.2 Alkaloids

Both Mayer's test and Wagner's test were found to be negative. Dragendroffs test did not yield characteristic reddish brown precipitate. Characteristic yellow coloured precipitate was not obtained with Hager's test. This revealed the absence of detectable level of alkaloids in the fresh juice and cold ethanolic extract of *Myristica fragrans* leaves.

4.5.3 Tannins

There were no brownish green colour with ferric chloride test and white precipitate in gelatin test which indicated the absence of tannins in the cold ethanolic extract and fresh juice of *Myristica fragrans* leaves.

4.5.4 Flavonoids

Green colour in the ferric chloride test and characteristic yellow coloured precipitate in lead acetate test indicated the presence of flavonoids in the cold ethanolic extract and fresh juice of *Myristica fragrans* leaves.

4.5.5 Glycosides

A red colour obtained in the Benedict's test indicated the presence of glycosides in the sample. A yellow colour was obtained by mixing the extracts with sodium hydroxide reagent. These tests indicated the presence of glycosides in the cold ethanolic extract and fresh juice of *Myristica fragrans* leaves.

4.5.6 Phenolic compounds

The presence of phenolic compounds in the ethanolic extract and fresh juice of *Myristica fragrans* leaves was indicated by the development of a characteristic dark blue colour with 10 per cent ferric chloride.

4.5.7 Diterpenes

Characteristic green colour was not developed with copper acetate solution. This indicated the absence of diterpenes in the cold ethanolic extract and fresh juice of *Myristica fragrans* leaves.

4.5.8 Triterpenes

As per Salkowski test, the lower layer did not turn to yellow on standing, and by Leiberman Burchadt's test, no deep ring appeared at the junction of the two layers. These results indicated the absence of triterpenes.

4.5.9 Saponins

In the foam test, foam persisted for 10 minutes, which indicated the presence of saponins in the extract and fresh juice.

4.5.10 Test for the detection of nitrates

With diphenylamine reagent no bright blue colour was developed. That indicated the absence of nitrate in the extract and fresh juice.

4.5.11 Test for the detection of cyanides

There was no change in color of yellow picrate paper to red, which indicated the absence of cyanide in the fresh juice and alcoholic extract of *Myristica fragrans* leaves.

4.6 SCREENING OF *Tectona grandis* FOR ACTIVE PRINCIPLES

4.6.1 Steroids

As per Salkowski test, red colour was not obtained and Lieberman Burchadt test did not give a reddish ring at the junction. Thus it could be concluded that no detectable level of steroids was present in the fresh juice and ethanolic extract of *Tectona grandis* leaves.

4.6.2 Alkaloids

No creamy white precipitate in Mayer's test and no characteristic yellow coloured precipitate with Hager's test were obtained. There was no characteristic reddish brown precipitate with Wagner's reagent. Dragendroffs test also gave a negative result. All these tests indicated that no detectable levels of alkaloids were present in the fresh juice and cold ethanolic extract of *Tectona grandis* leaves.

4.6.3 Tannins

A characteristic brownish green colour was obtained in ferric chloride test and white precipitate in gelatin test. These observations indicated the presence of tannins in the ethanolic extract and fresh juice of *Tectona grandis* leaves.

4.6.4 Flavonoids

A green colour in the ferric chloride test and a characteristic yellow coloured precipitate in lead acetate test indicated the presence of flavonoids in the cold ethanolic extract and fresh juice of *Tectona grandis* leaves.

4.6.5 Glycosides

A red colour obtained in the Benedict's test indicated the presence of glycosides in the sample. A yellow colour was obtained by mixing the extracts with sodium hydroxide reagent, which also indicated the presence of glycosides in the cold ethanolic extract and fresh juice of *Tectona grandis* leaves.

4.6.6 Phenolic compounds

The extract and fresh juice mixed with 10 percent ferric chloride produced a characteristic dark blue colour, which indicated the presence of phenolic compounds in the ethanolic extract and fresh juice of *Tectona grandis* leaves.

4.6.7 Diterpenes

When extract and fresh juice were mixed with copper acetate solution, the green colour was not developed. Thus it indicated the absence of detectable amount of diterpenes in the ethanolic extract and fresh juice of *Tectona grandis* leaves.

4.6.8 Triterpenes

For ethanolic extract and fresh juice of *Tectona grandis* leaves, Salkowski test and Leiberman Burchadt's test were found to be negative. These indicated the absence of triterpenes in the extract.

4.6.9 Saponins

In the foam test, foam persisted for 10 minutes, which indicated the presence of saponins in the extract and fresh juice.

4.6.10 Test for the detection of nitrates

The plant material was tested with diphenylamine reagent by adding a few drops of reagent to the crushed plant material. The absence of bright blue colour indicated that no nitrate was present in the extract and fresh juice.

4.6.11 Test for the detection of cyanides

Yellow picrate paper was not turned to red which indicated the absence of cyanide in the fresh juice and alcoholic extract of *Tectona grandis* leaves.

The results obtained in the phytochemical study are furnished in table 2.

TABLE 2. PHYTOCHEMICAL SCREENING OF MEDHNAL PLANTS

Phytochemical constituents	*Annona squamosa*		*Cassia alata*		*Coleus amboinicus*		*Myristica fragrans*		*Tectona grandis*	
	Ethanolic extract	Fresh juice	Ethanolic extract	Fresh juice	Ethanolic extract	Fresh juice	Ethanolic extract	Fresh juice	Ethanolic extract	Fresh juice
Steroids	Present	Present	Present	Present	Present	Present	Absent	Absent	Absent	Absent
Alkaloids	Present	Present	Present	Present	Present	Present	Absent	Absent	Absent	Absent
Tannins	Present	Present	Present	Present	Present	Present	Absent	Absent	Present	Present
Flavonoids	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present
Glycosides	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present
Phenolic compounds	Present	Present	Present	Present	Absent	Absent	Present	Present	Present	Present
Diterpenes	Present	Present	Present	Present	Absent	Absent	Absent	Absent	Absent	Absent
Triterpenes	Present	Present	Present	Present	Absent	Absent	Absent	Absent	Absent	Absent
Saponins	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present
Nitrates	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent
Cyanides	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent	Absent

4.7 ANTIMICROBIAL SUSCEPTIBLITY TESTNG BY DISC DIFFUSION METHOD

The antimicrobial susceptibility test was performed using various concentrations (1.25, 2.5, 5, 10 and 15 mg) of the ethanolic extract and fresh juice (50 µl) of *Annona squamosa, Cassia alata, Coleus amboinicus, Myristica fragrans* and *Tectona grandis.* The diluent used was 5 per cent Dimethyl sulphoxide (DMSO). DMSO was selected as vehicle by disc diffusion method because it did not show inhibitory action against microorganisms.

The diameter of inhibitory zone obtained for Penicillin was 31.20 ± 0.21 mm against *Staphylococcus aureus.* The diameter of inhibitory zone obtained for Furazolidone against *Escherichia coli, Pasteurella multocida, Pseudomonas aeruginosa* and *Salmonella enteritidis* were 23.50 ± 0.31 mm, 18.57 ± 0.17 mm, 20.17 ± 0.17 mm and 21.83 ± 0.21 mm respectively. The inhibitory zone diameter of Ketoconazole against *Aspergillus fumigatus, Candida albicans* and *Cryptococcus neoformans* were 16.67 ± 0.21 mm, 25.50 ± 0.22 mm and 16.33 ± 0.21 mm respectively. The diameter of inhibitory zone for Clotrimazole against *Aspergillus fumigatus, Candida albicans* and *Cryptococcus neoformans* were 20.83 ± 0.21 mm, 20.33 ± 0.21 mm and 20.17 ± 0.17 mm respectively.

4.7.1 ANTIMICROBIAL ACTIVITY OF *Annona squamosa*

Ethanolic extract of *Annona squamosa* showed marked activity against *Escherichia coli, Staphylococcus aureus, Pasteurella multocida, Pseudomonas aeruginosa, Aspergillus fumigatus* and *Cryptococcus neoformans. Salmonella enteritidis* and *Candida albicans* were found to be resistant to all tested concentrations of alcoholic extract of *Annona squamosa* leaf. The highest activity was observed against *Staphylococcus aureus.* A dose dependant increase in the diameter of inhibition zone was observed. The diameter of zones of inhibition varied significantly at each concentration ($p<0.01$). Ethanolic extract of *Annona squamosa* was found to be effective against *Escherichia coli* at concentrations of 5, 10 and 15 mg/disc.

The diameter of inhibitory zone obtained at concentrations (1.25 mg, 2.5 mg, 5 mg, 10 mg and 15 mg) of *Annona squamosa* leaf extract are summarized in the table 3 and presented in figure 6.

Fresh juice of *Annona squamosa* leaves was found to be effective against *Staphylococcus aureus*. The diameter of inhibition zone obtained was 8.67 ± 0.21 mm.

The diameter of inhibition zones for fresh juice of *Annona squamosa* leaves are presented in table 8 and presented in figure 11.

4.7.2 ANTIMICROBIAL ACTIVITY OF *Cassia alata*

Staphylococcus aureus, Pasteurella multocida, Pseudomonas aeruginosa, Aspergillus fumigatus, Candida albicans and *Cryptococcus neoformans* were susceptible to all concentrations of *Cassia alata* leaf extracts. The growth of *Salmonella enteritidis* and *Escherichia coli* were not affected by ethanolic extract of *Cassia alata* leaf. *Pseudomonas aeruginosa* was found to be more sensitive to ethanolic extract of *Cassia alata* leaf. Dose dependant increase in the diameter of inhibition zone was observed. Diameter of inhibition zone differs significantly ($p<0.01$) at each concentration against all susceptible organism except *Aspergillus fumigatus* ($p<0.05$). For *Aspergillus fumigatus* the diameter of inhibition zone at 1.25 mg/disc does not vary significantly to that obtained at 2.5 mg/disc concentration. There is no significant difference observed for diameter of inhibition zone at concentrations of 2.5, 5, 10 and 15 mg/disc for *Aspergillus fumigatus*.

The diameter of inhibitory zone obtained at concentrations (1.25 mg, 2.5 mg, 5 mg, 10 mg and 15 mg) of *Cassia alata* leaf extract is given in table 4 and presented in figure 7.

Candida albicans was the only organism sensitive to fresh juice of *Cassia alata* leaves. The diameter of inhibition zone obtained was 10.33 ± 0.21 mm.

TABLE 4. ANTIMICROBIAL ACTIVITY OF *Cassia alata*

Organisms	Diameter of zone of inhibition in mm								
	Ethanolic extract of leaves (mg/disc)					P (10units)	FR (0.05mg)	KT (0.01mg)	CC (0.01mg)
	1.25	2.5	5	10	15				
S. aureus	9.67^{a} ±0.21	10.67^{b} ±0.21	12.50^{c} ±0.34	14.33^{d} ±0.33	16.17^{e} ±0.31	31.20 ±0.21	–	–	–
E. coli	–	–	–	–	–	–	23.50 ±0.31	–	–
S. enteritidis	–	–	–	–	–	–	21.83 ±0.21	–	–
P. multocida	9.83^{a} ±0.17	11.17^{b} ±0.17	12.33^{c} ±0.21	13.67^{d} ±0.21	15.67^{e} ±0.49	–	18.57 ±0.17	–	–
P. aeruginosa	13.33^{a} ±0.21	15.33^{b} ±0.21	17.33^{c} ±0.21	19.17^{d} ±0.17	20.83^{e} ±0.31	–	20.17 ±0.17	–	–
C. albicans	8.17^{a} ±0.17	9.67^{b} ±0.21	11.50^{c} ±0.22	12.83^{d} ±0.17	14.67^{e} ±0.21	–	–	25.50 ±0.22	20.33 ±0.21
C. neoformans	8.83^{a} ±0.17	10.33^{b} ±0.21	11.83^{c} ±0.17	13.50^{d} ±0.22	15.67^{e} ±0.21	–	–	16.33 ±0.21	20.17 ±0.17
A. fumigatus	8.17^{a} ±0.17	9.83^{ab} ±0.17	10.83^{b} ±0.17	11.67^{b} ±0.21	12.17^{b} ±0.17	–	–	16.67 ±0.21	20.83 ±0.21

(Values of zone of growth inhibition are presented as mean ± SE; (-) indicates no inhibition; within a row, means bearing same superscript do not differ significantly. P- Penicillin; FR- furazolidone; KT – Ketoconazole; CC - Clotrimazole)

Fig. 6 ANTIMICROBIAL ACTIVITY OF *Annona squamosa*

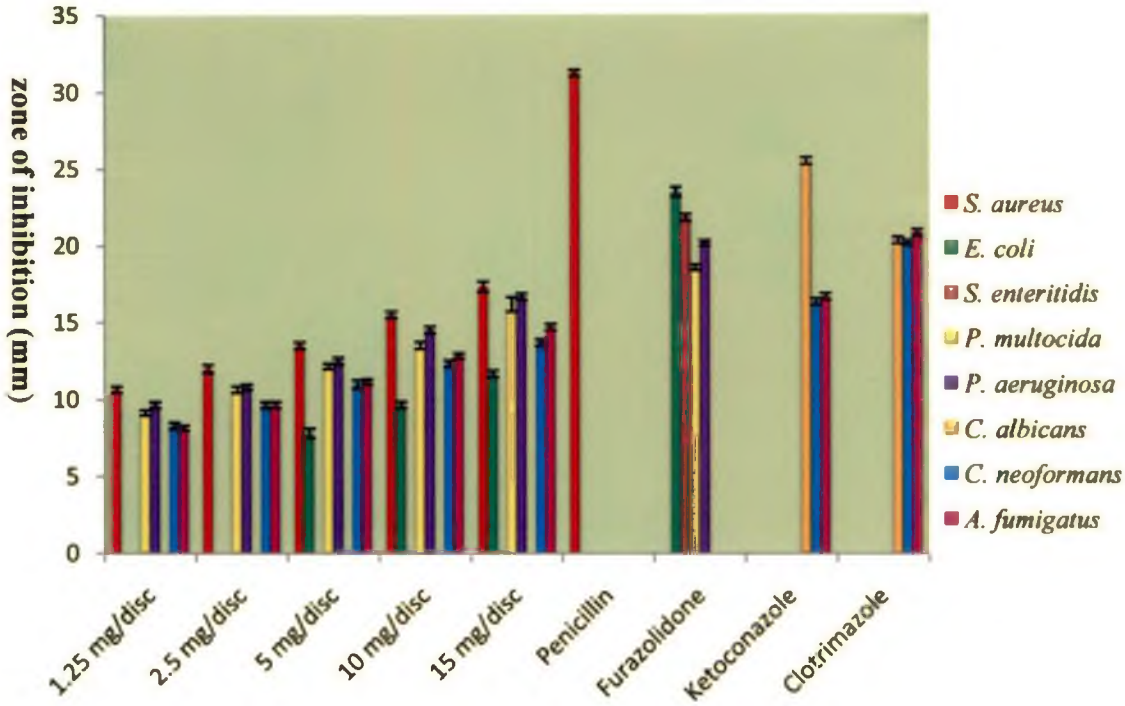
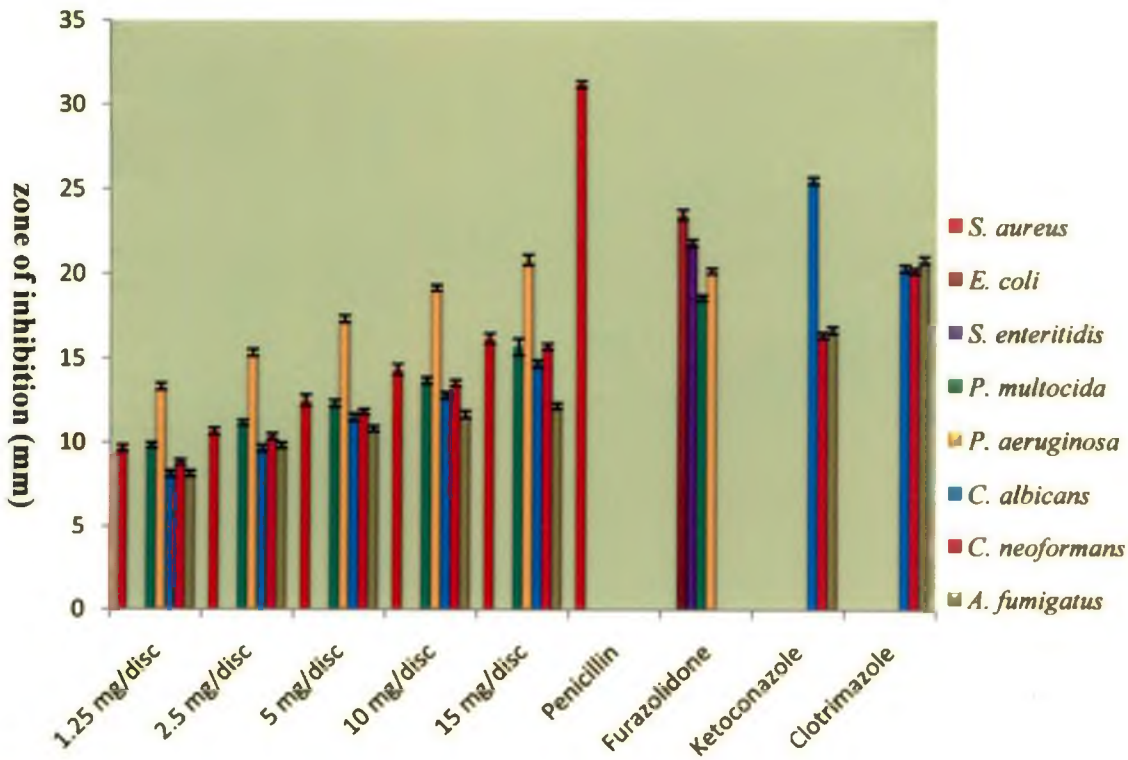


Fig. 7 ANTIMICROBIAL ACTIVITY OF *Cassia alata*



The diameter of inhibition zone obtained for fresh juice of *Cassia alata* leaves is given in table 8 and presented in figure 11.

4.7.3 ANTIMICROBIAL ACTIVITY OF *Coleus amboinicus*

Escherichia coli, Staphylococcus aureus, Pasteurella multocida, Pseudomonas aeruginosa, Salmonella enteritidis, Aspergillus fumigatus and *Candida albicans* were found to be sensitive and *Cryptococcus neoformans* was found to be resistant to ethanolic extract of *Coleus amboinicus* leaves. Most sensitive organism was *Candida albicans*. Dose dependant increase in the diameter of inhibition zone was observed. Significant difference ($p<0.01$) has been observed at each concentration of the extract.

The diameter of inhibitory zone obtained at concentrations (1.25 mg, 2.5 mg, 5 mg, 10 mg and 15 mg) of alcoholic extract of *Coleus amboinicus* leaf is shown in table 5 and presented in figure 8.

Staphylococcus aureus and *Escherichia coli* were found to be sensitive to fresh juice of *Coleus amboinicus* with inhibition zones of 10.17 ± 0.17 mm and 9.67 ± 0.21 mm diameters respectively.

The diameter of inhibition zone obtained for fresh juice of *Coleus amboinicus* leaves is shown in table 8 and presented in figure 11.

4.7.4 ANTIMICROBIAL ACTIVITY OF *Myristica fragrans*

Growth of *Staphylococcus aureus, Pasteurella multocida, Pseudomonas aeruginosa, Salmonella enteritidis* and *Candida albicans* was inhibited by alcoholic extract of *Myristica fragrans* leaves. *Escherichia coli, Cryptococcus neoformans* and *Aspergillus fumigatus* were resistant to *Myristica fragrans* leaf extract. *Staphylococcus aureus* was the most sensitive organism. Dose dependant increase in the diameter of inhibition zone was observed. Diameter of inhibition zone differs significantly ($p<0.01$) at each concentration. Ethanolic extract of *Myristica fragrans* was found to be effective against *Candida albicans* at concentrations of 5, 10 and 15 mg/disc.

TABLE 5. ANTIMICROBIAL ACTIVITY OF *Coleus amboinicus*

Organisms	Diameter of zone of inhibition in mm								
	Ethanolic extract of leaves (mg/disc)					P (10units)	FR (0.05mg)	KT (0.01mg)	CC (0.01mg)
	1.25	2.5	5	10	15				
S. aureus	10.50^{a} ±0.62	13.17^{b} ±0.31	15.17^{c} ±0.31	16.50^{d} ±0.22	18.67^{e} ±0.21	31.20 ±0.21	–	–	–
E. coli	8.83^{a} ±0.17	10.67^{b} ±0.21	12.50^{c} ±0.22	14.50^{d} ±0.22	16.83^{e} ±0.17	–	23.50 ±0.31	–	–
S. enteritidis	8.50^{a} ±0.22	10.17^{b} ±0.21	11.67^{c} ±0.21	13.67^{d} ±0.21	15.50^{e} ±0.22	–	21.83 ±0.21	–	–
P. multocida	9.83^{a} ±0.17	11.67^{b} ±0.21	12.67^{c} ±0.21	14.17^{d} ±0.31	16.00^{e} ±0.26	–	18.57 ±0.17	–	–
P. aeruginosa	9.83^{a} ±0.17	11.67^{b} ±0.21	13.50^{c} ±0.22	16.00^{d} ±0.21	18.83^{e} ±0.31	–	20.17 ±0.17	–	–
C. albicans	14.67^{a} ±0.21	16.50^{b} ±0.22	18.67^{c} ±0.21	21.50^{d} ±0.34	23.83^{e} ±0.17	–	–	25.50 ±0.22	20.33 ±0.21
C. neoformans	–	–	–	–	–	–	–	16.33 ±0.21	20.17 ±0.17
A. fumigatus	15.83^{a} ±0.17	17.33^{b} ±0.21	18.83^{c} ±0.17	20.67^{d} ±0.21	22.83^{e} ±0.17	–	–	16.67 ±0.21	20.83 ±0.21

(Values of zone of growth inhibition are presented as mean ± SE; (-) indicates no inhibition; within a row, means bearing same superscript do not differ significantly. P- Penicillin; FR- furazolidone; KT - Ketoconazole; CC - Clotrimazole)

The diameter of inhibitory zone obtained at concentrations (1.25 mg, 2.5 mg, 5 mg, 10 mg and 15 mg) of ethanolic extract of *Myristica fragrans* leaf is summarized in table 6 and presented in figure 9.

Pseudomonas aeruginosa and *Staphylococcus aureus* were sensitive to fresh juice of *Myristica fragrans* leaves. The diameter of zone of inhibition was 12.50 ± 0.22 mm for *Staphylococcus aureus* and 10.83 ± 0.17 mm for *Pseudomonas aeruginosa.* None of the fungal strains tested were sensitive to fresh juice of *Myristica fragrans* leaves.

The diameter of inhibition zone obtained for fresh juice of *Myristica fragrans* leaves is given in table 8 and presented in figure 11.

4.7.5 ANTIMICROBIAL ACTIVITY OF *Tectona grandis*

Staphylococcus aureus, Escherichia coli, Pasteurella multocida, Pseudomonas aeruginosa, Salmonella enteritidis and *Candida albicans* were inhibited by alcoholic extract of *Tectona grandis* leaves. *Cryptococcus neoformans* and *Aspergillus fumigatus* were resistant to *Tectona grandis* leaf extract. *Staphylococcus aureus* was found to be the most sensitive. Dose dependant increase in the diameter of inhibition zone was observed. Diameter of inhibition zone differs significantly ($p<0.01$) at each concentration. Ethanolic extract of *Tectona grandis* was found to be effective against *Salmonella enteritidis* and *Candida albicans* at concentrations of 5, 10 and 15 mg/disc.

The diameter of inhibitory zone obtained at concentrations (1.25 mg, 2.5 mg, 5 mg, 10mg and 15 mg) of ethanolic extract of *Tectona grandis* leaf is summarized in table 7 and presented in figure 10.

Staphylococcus aureus was the only organism sensitive to fresh juice of *Tectona grandis* leaves. The inhibitory zone diameter observed was 10.67 ± 0.21 mm.

The diameter of inhibition zones for fresh juice of *Tectona grandis leaves* are presented in table 8 and presented in figure 11.

TABLE 6. ANTIMICROBIAL ACTIVITY OF *Myristica fragrans*

Organisms	Diameter of zone of inhibition in mm								
	Ethanolic extract of leaves (mg/disc)					P (10units)	FR (0.05mg)	KT (0.01mg)	CC (0.01mg)
	1.25	2.5	5	10	15				
S. aureus	12.50^{a} ±0.34	14.17^{b} ±0.17	15.33^{c} ±0.21	16.67^{d} ±0.21	18.33^{e} ±0.33	31.20 ±0.21	–	–	–
E. coli	–	–	–	–	–	–	23.50 ±0.31	–	–
S. enteritidis	8.17^{a} ±0.17	9.33^{b} ±0.21	10.67^{c} ±0.21	11.83^{d} ±0.17	13.50^{e} ±0.22	–	21.83 ±0.21	–	–
P. multocida	9.33^{a} ±0.21	11.33^{b} ±0.21	12.50^{c} ±0.22	13.67^{d} ±0.21	15.83^{e} ±0.17	–	18.57 ±0.17	–	–
P. aeruginosa	8.67^{a} ±0.21	10.33^{b} ±0.21	12.67^{c} ±0.21	14.67^{d} ±0.21	16.67^{e} ±0.21	–	20.17 ±0.17	–	–
C. albicans	–	–	8.50^{a} ±0.22	10.33^{b} ±0.21	12.17^{e} ±0.17	–	–	25.50 ±0.22	20.33 ±0.21
C. neoformans	–	–	–	–	–	–	–	16.33 ±0.21	20.17 ±0.17
A. fumigatus	–	–	–	–	–	–	–	16.67 ±0.21	20.83 ±0.21

(Values of zone of growth inhibition are presented as mean ± SE; (-) indicates no inhibition; within a row, means bearing same superscript do not differ significantly. P- Penicillin; FR- furazolidone; KT - Ketoconazole; CC - Clotrimazole)

Fig. 8 ANTIMICROBIAL ACTIVITY OF *Coleus amboinicus*

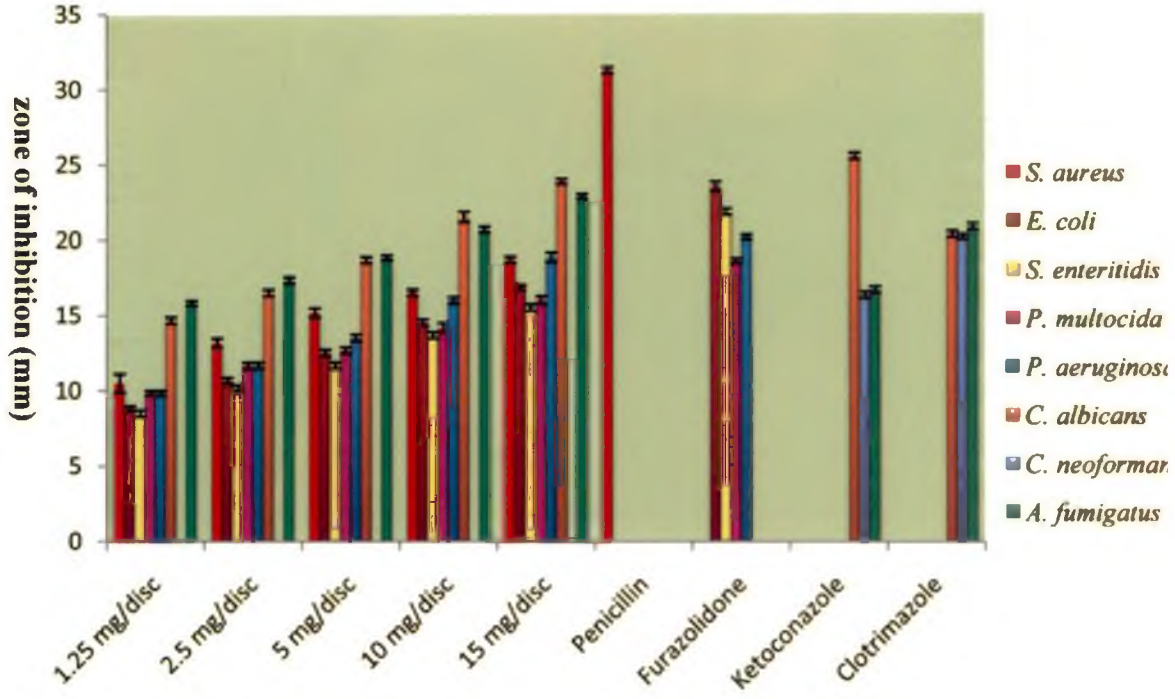


Fig. 9 ANTIMICROBIAL ACTIVITY OF *Myristica fragrans*

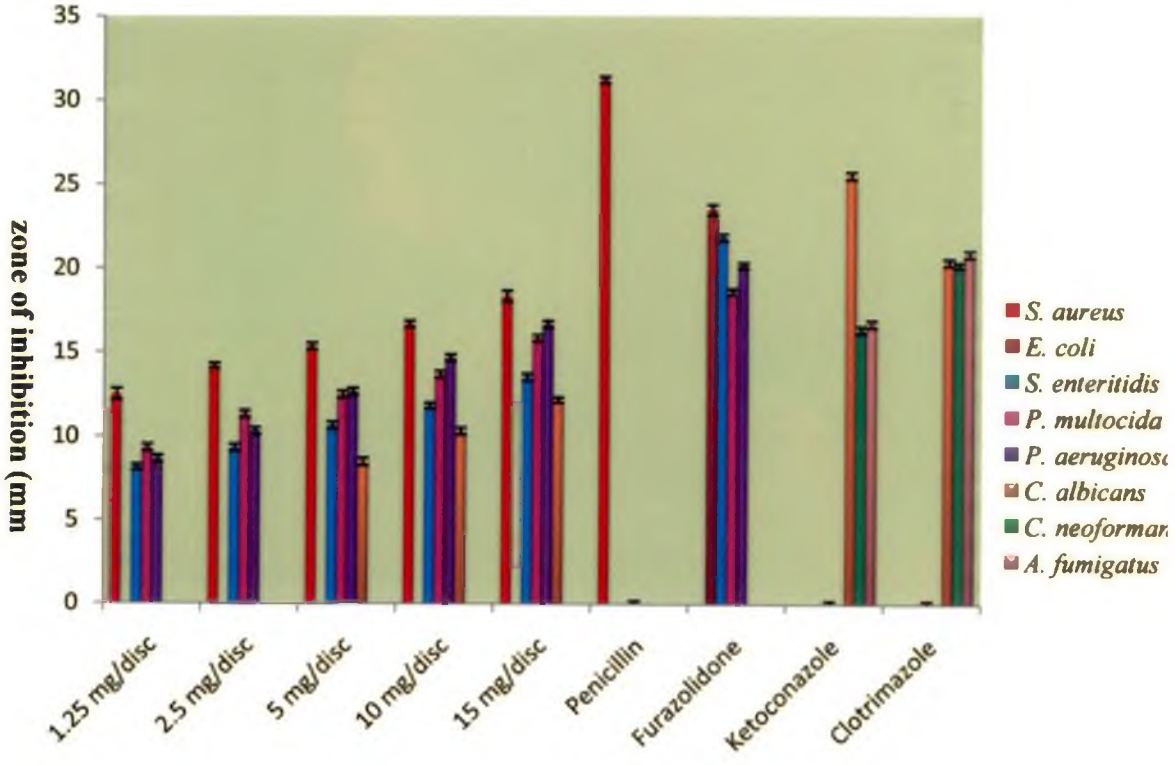


TABLE 7. ANTIMICROBIAL ACTIVITY OF *Tectona grandis*

Organisms	Diameter of zone of inhibition in mm								
	Ethanolic extract of leaves (mg/disc)					P (10units)	FR (0.05mg)	KT (0.01mg)	CC (0.01mg)
	1.25	2.5	5	10	15				
S. aureus	14.17^{a} ±0.17	16.00^{b} ±0.26	17.50^{c} ±0.22	18.83^{d} ±0.17	22.50^{e} ±0.34	31.20 ±0.21	–	–	–
E. coli	8.17^{a} ±0.17	9.67^{b} ±0.21	11.00^{c} ±0.22	12.50^{d} ±0.22	14.33^{e} ±0.21	–	23.50 ±0.31	–	–
S. enteritidis	–	–	8.67^{a} ±0.21	10.33 ± 0.21^{b}	11.67^{e} ±0.21	–	21.83 ±0.21	–	–
P. multocida	8.67^{a} ±0.21	10.67^{b} ±0.21	13.17^{c} ±0.31	15.50 ± 0.34^{d}	18.33^{e} ±0.21	–	18.57 ±0.17	–	–
P. aeruginosa	10.33^{a} ±0.21	12.00^{b} ±0.26	14.50^{c} ±0.22	17.17 ± 0.31^{d}	19.50^{e} ±0.34	–	20.17 ±0.17	–	–
C. albicans	–	–	9.17^{a} ±0.17	10.17^{b} ±0.17	11.83^{c} ±0.17	–	–	25.50 ±0.22	20.33 ±0.21
C. neoformans	–	–	–	–	–	–	–	16.33 ±0.21	20.17 ±0.17
A. fumigatus	–	–	–	–	–	–	–	16.67 ±0.21	20.83 ±0.21

(Values of zone of growth inhibition are presented as mean ± SE; (-) indicates no inhibition; within a row, means bearing same superscript do not differ significantly. P- Penicillin; FR- furazolidone; KT - Ketoconazole; CC – Clotrimazole)

TABLE 8. DIAMETER OF INHIBITION ZONE OBTAINED FOR 50 µl FRESH JUICE OF LEAVES (mm)

Organisms	*Annona squamosa*	*Cassia alata*	*Coleus amboinicus*	*Myristica fragrans*	*Tectona grandis*	P (10 units)	FR (0.05mg)	KT (0.01mg)	CC (0.01mg)
S. aureus	8.67 ±0.21	–	10.17 ±0.17	12.50 ±0.22	10.67 ±0.21	31.20 ±0.21	–	–	–
E. coli	–	–	9.67 ±0.21	–	–	–	23.50 ±0.31	–	–
S. enteritidis	–	–	–	–	–	–	21.83 ±0.21	–	–
P. multocida	–	–	–	–	–	–	18.57 ±0.17	–	–
P. aeruginosa	–	–	–	10.83 ±0.17	–	–	20.17 ±0.17	–	–
C. albicans	–	10.33 ±0.21	–	–	–	–	–	25.50 ±0.22	20.33 ±0.21
C. neoformans	–	–	–	–	–	–	–	20.17 ±0.17	16.33 ±0.21
A. fumigatus	–	–	–	–	–	–	–	20.83 ±0.21	16.67 ±0.21

(Values of zone of growth inhibition are presented as mean ± SE and (-) indicates no inhibition
P- Penicillin; FR- furazolidone; KT - Ketoconazole; CC - Clotrimazole)

Fig 10. ANTIMICROBIAL ACTIVITY OF *Tectona grandis*

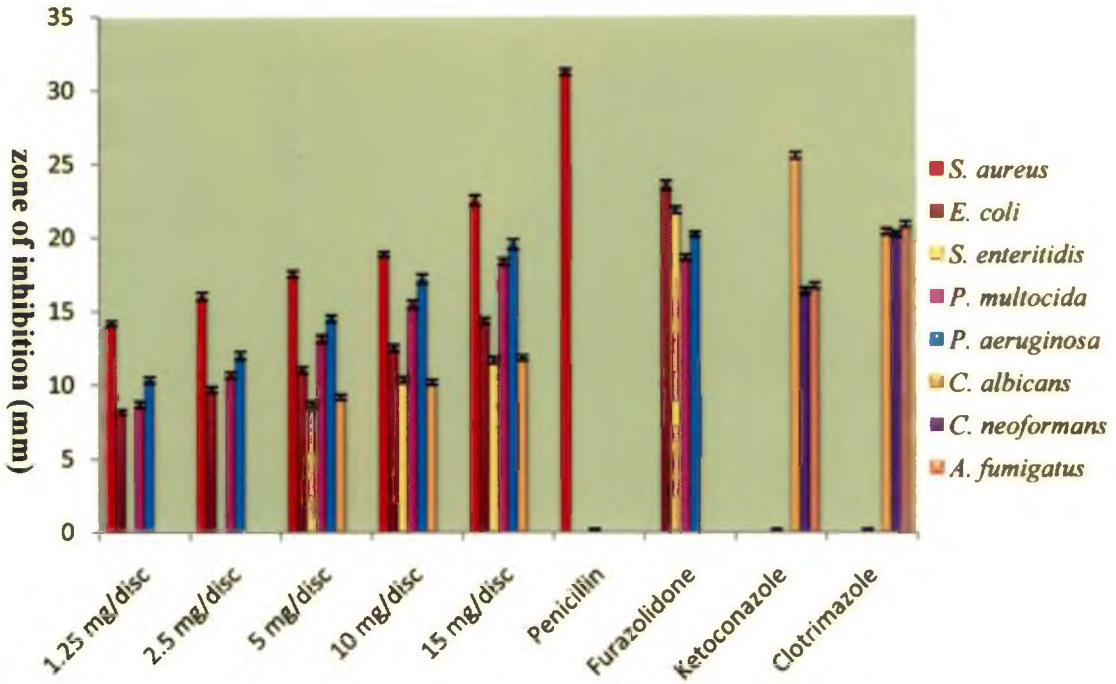
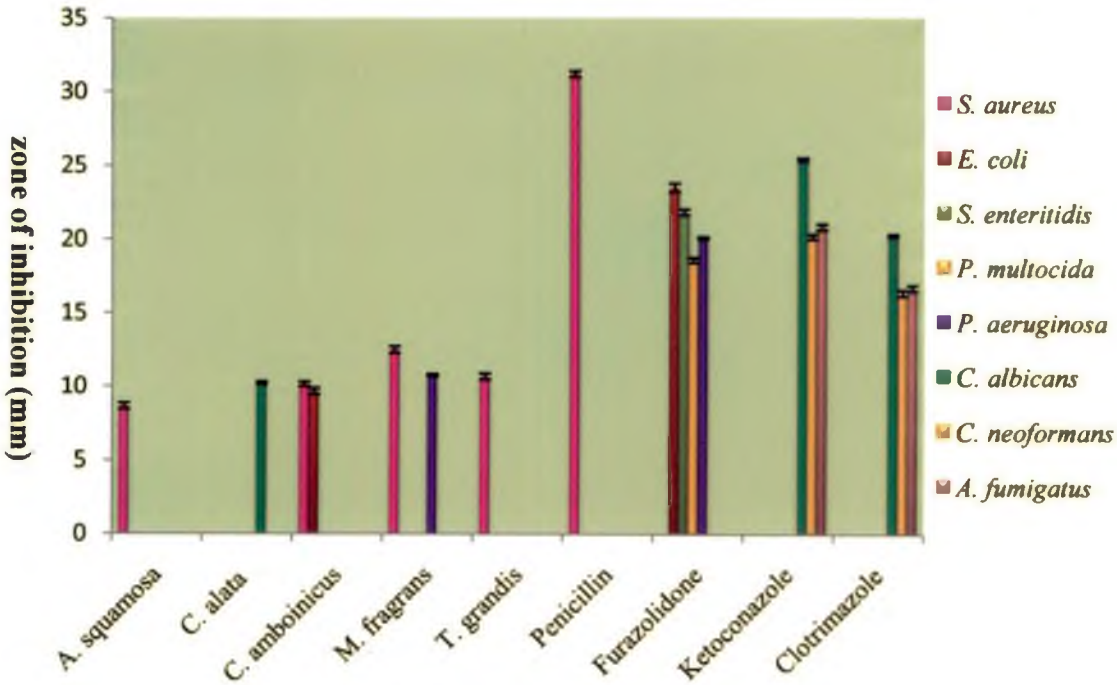


Fig. 11 ANTIMICROBIAL ACTIVITY OF FRESH JUICE OF LEAVES



4.7.6 ANTIBACTERIAL ACTIVITY AGAINST *Staphylococcus aureus*

Annona squamosa, Cassia alata, Coleus amboinicus, Myristica fragrans and *Tectona grandis* were found to be effective against *Staphylococcus aureus.* The activity found to be increased with increase in concentration. *Tectona grandis* is having highest activity against *Staphylococcus aureus.* Fresh juice of *Annona squamosa, Coleus amboinicus, Myristica fragrans* and *Tectona grandis* leaves also inhibited the growth of *Staphylococcus aureus. Staphylococcus aureus* is sensitive to Penicillin (10 units) with inhibitory zone of 31.20 ± 0.21 mm diameter.

The diameter of zones of inhibition for *Staphylococcus aureus* are shown in table 9 and presented in figure 12.

4.7.7 ANTIBACTERIAL ACTIVITY AGAINST *Escherichia coli*

Annona squamosa, Coleus amboinicus and *Tectona grandis* were effective against *Escherichia coli.* Ethanolic extract of *Annona squamosa* was found to be effective only at concentrations of 5, 10 and 15 mg/disc. At different concentrations the diameter of inhibition zone produced by the above plants varied significantly ($p<0.01$). Extract of *Coleus amboinicus* produced maximum inhibition against *Escherichia coli* at all tested concentrations. Fresh juice of *Coleus amboinicus* leaves also inhibited the growth of *E. coli. E. coli* was sensitive to furazolidone (50 µg disc) with inhibitory zone of 23.50 ± 0.31 mm diameter.

The diameter of zones of inhibition for *Escherichia coli* are summarized in table 10 and presented in figure 13.

TABLE 9. DIAMETER OF ZONE OF INHIBITION OBTAINED FOR *Staphylococcus aureus* (mm)

PLANT	Ethanolic extract of leaves (mg/disc)					PENCILLIN (10units)
	1.25	2.5	5	10	15	
Annona squamosa	10.67^{a} ±0.21	12.00^{b} ±0.26	13.50^{b} ±0.22	15.50^{b} ±0.22	17.33^{b} ±0.33	31.20 ±0.21
Cassia alata	9.67^{a} ±0.21	10.67^{a} ±0.21	12.50^{a} ±0.34	14.33^{a} ±0.33	16.17^{a} ±0.31	31.20 ±0.21
Coleus amboinicus	10.50^{a} ±0.62	13.17^{c} ±0.31	15.17^{c} ±0.31	16.50^{c} ±0.22	18.67^{c} ±0.21	31.20 ±0.21
Myristica fragrans	12.50^{b} ±0.34	14.17^{c} ±0.17	15.33^{c} ±0.21	16.67^{c} ±0.21	18.33^{c} ±0.33	31.20 ±0.21
Tectona grandis	14.17^{c} ±0.17	16.00^{d} ±0.26	17.50^{d} ±0.22	18.83^{d} ±0.17	22.50^{d} ±0.34	31.20 ±0.21

(Values of zone of growth inhibition are presented as mean ± SE; (-) indicates no inhibition; within a column, means bearing same superscript do not differ significantly.)

TABLE 10. DIAMETER OF ZONE OF INHIBITION OBTAINED FOR *Escherichia coli* (mm)

PLANT	Ethanolic extract of leaves (mg/disc)					Furazolidone (0.05 mg)
	1.25	2.5	5	10	15	
Annona squamosa	–	–	7.83^{a} ±0.31	9.67^{a} ±0.21	11.67^{a} ±0.21	23.50 ±0.31
Cassia alata	–	–	–	–	–	23.50 ±0.31
Coleus amboinicus	8.83 ±0.17	10.67 ±0.21	12.50^{c} ±0.22	14.50^{c} ±0.22	16.83^{c} ±0.17	23.50 ±0.31
Myristica fragrans	–	–	–	–	–	23.50 ±0.31
Tectona grandis	8.17 ±0.17	9.67 ±0.21	11.00^{b} ±0.22	12.50^{b} ±0.22	14.33^{b} ±0.21	23.50 ±0.31

(Values of zone of growth inhibition are presented as mean ± SE; (-) indicates no inhibition; within a column, means bearing same superscript do not differ significantly.)

Fig. 12 ANTIBACTERIAL ACTIVITY AGAINST
Staphylococcus aureus

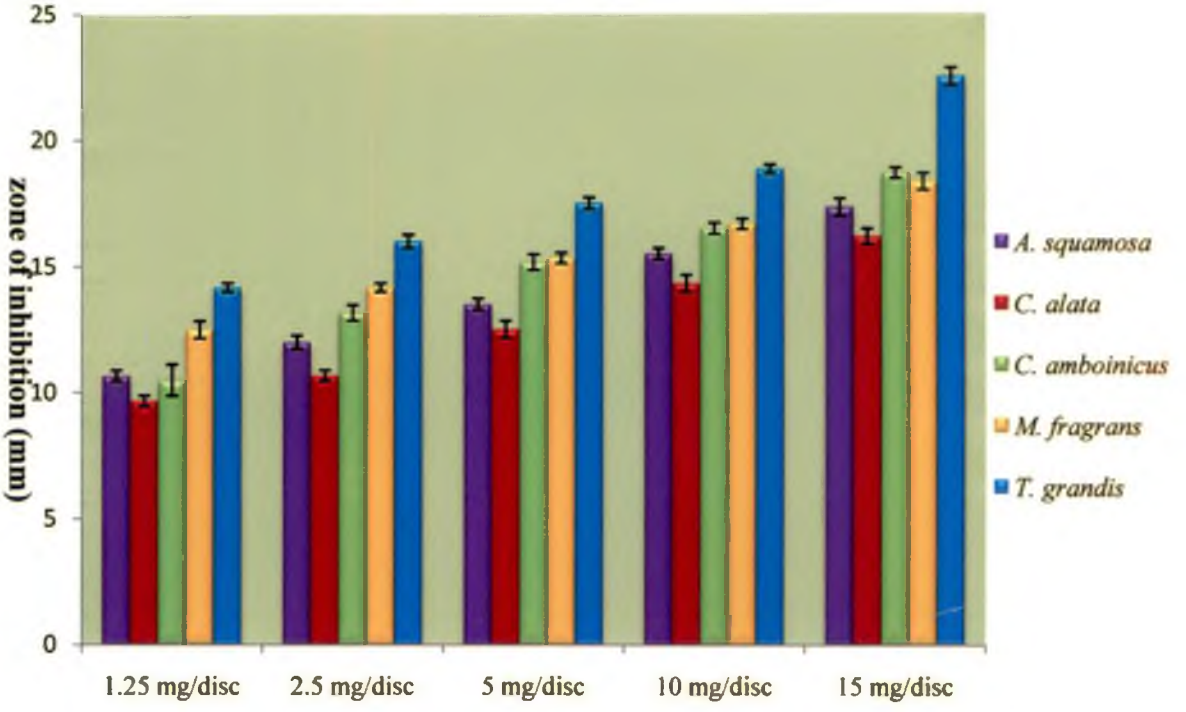
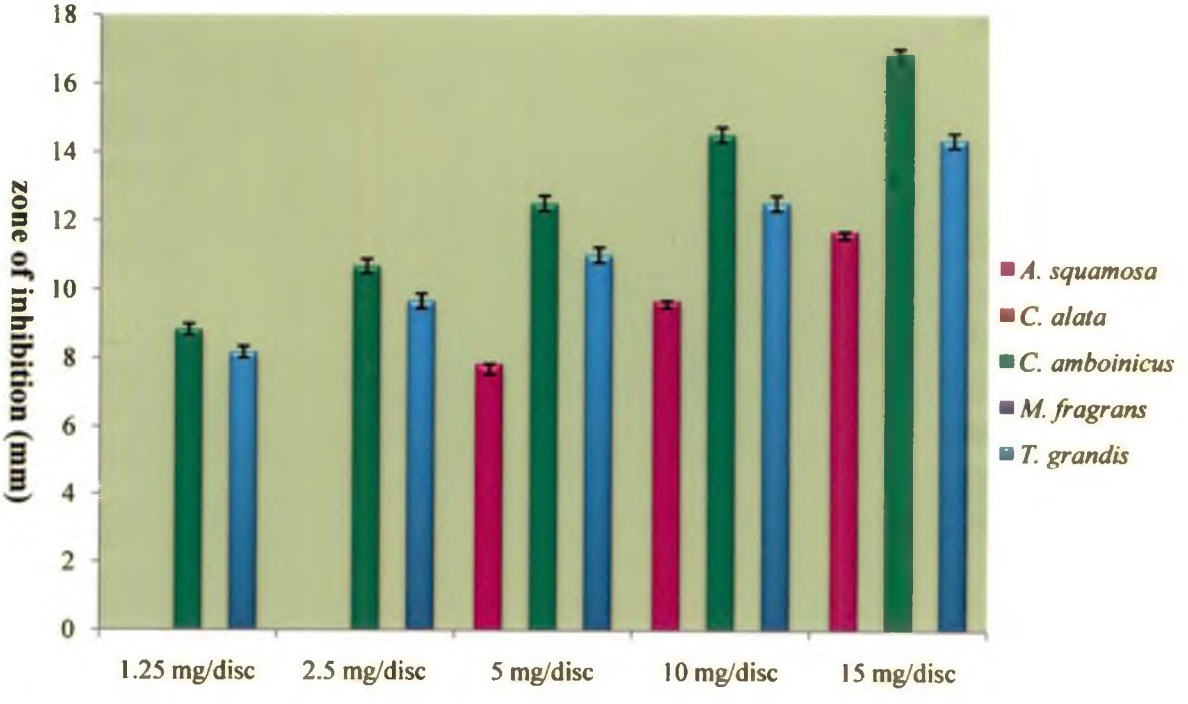


Fig. 13 ANTIBACTERIAL ACTIVITY AGAINST
Escherichia coli



4.7.8 ANTIBACTERIAL ACTIVITY AGAINST *Pasteurella multocida*

Pasteurella multocida was found to be sensitive to *Annona squamosa, Cassia alata, Coleus amboinicus, Myristica fragrans* and *Tectona grandis*. Standard drug furazolidone (50 µg disc) produced inhibitory zone of 18.57 ± 0.17 mm against *Pasteurella multocida.* The highest inhibition was observed in ethanolic extract of *Tectona grandis.* Activity of other plants did not vary significantly. The activity was found to be increased with concentration.

The diameter of zones of inhibition obtained for *Pasteurella multocida* are given in table 11 and presented in figure 14.

4.7.9 ANTIBACTERIAL ACTIVITY AGAINST *Salmonella enteritidis*

Salmonella enteritidis was susceptible to *Coleus amboinicus, Myristica fragrans* and *Tectona grandis* being the most effective. The antimicrobial activities of the plants differed significantly in all tested concentrations ($p<0.01$). *Salmonella enteritidis* was sensitive to standard drug furazolidone (50 µg disc) with inhibitory zone diameter of 21.83±0.21 mm. Susceptibility to the plant increased with increase in concentration of the extract. At concentrations of 1.25 and 2.5 mg/disc the organism was not sensitive to *Tectona grandis.*

The diameter of zone of inhibition obtained for *Salmonella enteritidis* is given in table 12 and presented in figure 15.

4.7.10 ANTIBACTERIAL ACTIVITY AGAINST *Pseudomonas aeruginosa*

Pseudomonas aeruginosa was susceptible to all the tested concentrations of *Annona squamosa, Cassia alata, Coleus amboinicus, Myristica fragrans* and *Tectona grandis*. *Cassia alata* was more effective in inhibiting *Pseudomonas aeruginosa.* At different concentrations the activity of the plants varied significantly ($p<0.01$). Fresh juice of *Myristica fragrans* leaves has also shown

TABLE 11. DIAMETER OF ZONE OF INHIBITION OBTAINED FOR *Pasteurella multocida* (mm)

PLANT	Ethanolic extract of leaves (mg/disc)					Furazolidone (0.05 mg)
	1.25	2.5	5	10	15	
Annona squamosa	9.17^{ab} ±0.17	10.67^{a} ±0.21	12.17^{a} ±0.17	13.50^{a} ±0.22	16.17^{a} ±0.48	18.57 ±0.17
Cassia alata	9.83^{c} ±0.17	11.17^{a} b ±0.17	12.33^{a} ±0.21	13.67^{a} ±0.21	15.67^{a} ±0.49	18.57 ±0.17
Coleus amboinicus	9.83^{c} ±0.17	11.67^{b} ±0.21	12.67^{ab} ±0.21	14.17^{a} ±0.31	16.00^{a} ±0.26	18.57 ±0.17
Myristica fragrans	9.33^{bc} ±0.21	11.33^{b} ±0.21	12.50^{ab} ±0.22	13.67^{a} ±0.21	15.83^{a} ±0.17	18.57 ±0.17
Tectona grandis	8.67^{a} ±0.21	10.67^{a} ±0.21	13.17^{b} ±0.31	15.50^{b} ±0.34	18.33^{b} ±0.21	18.57 ±0.17

(Values of zone of growth inhibition are presented as mean ± SE; (-) indicates no inhibition; within a column, means bearing same superscript do not differ significantly.)

TABLE 12. DIAMETER OF ZONE OF INHIBITION OBTAINED FOR *Salmonella enteritidis* (mm)

PLANT	Ethanolic extract of leaves (mg/disc)					Furazolidone (0.05 mg)
	1.25	2.5	5	10	15	
Annona squamosa	–	–	–	–	–	21.83 ±0.21
Cassia alata	–	–	–	–	–	21.83 ±0.21
Coleus amboinicus	8.50 ±0.22	10.17 ±0.21	11.67^{c} ±0.21	13.67^{c} ±0.21	15.50^{c} ±0.22	21.83 ±0.21
Myristica fragrans	8.17 ±0.17	9.33 ±0.21	10.67^{b} ±0.21	11.83^{b} ±0.17	13.50^{b} ±0.22	21.83 ±0.21
Tectona grandis	–	–	8.67^{a} ±0.21	10.33^{a} ±0.21	11.67^{a} ±0.21	21.83 ±0.21

(Values of zone of growth inhibition are presented as mean ± SE; (-) indicates no inhibition; within a column, means bearing same superscript do not differ significantly.)

Fig. 14 ANTIBACTERIAL ACTIVITY AGAINST
Pasteurella multocida

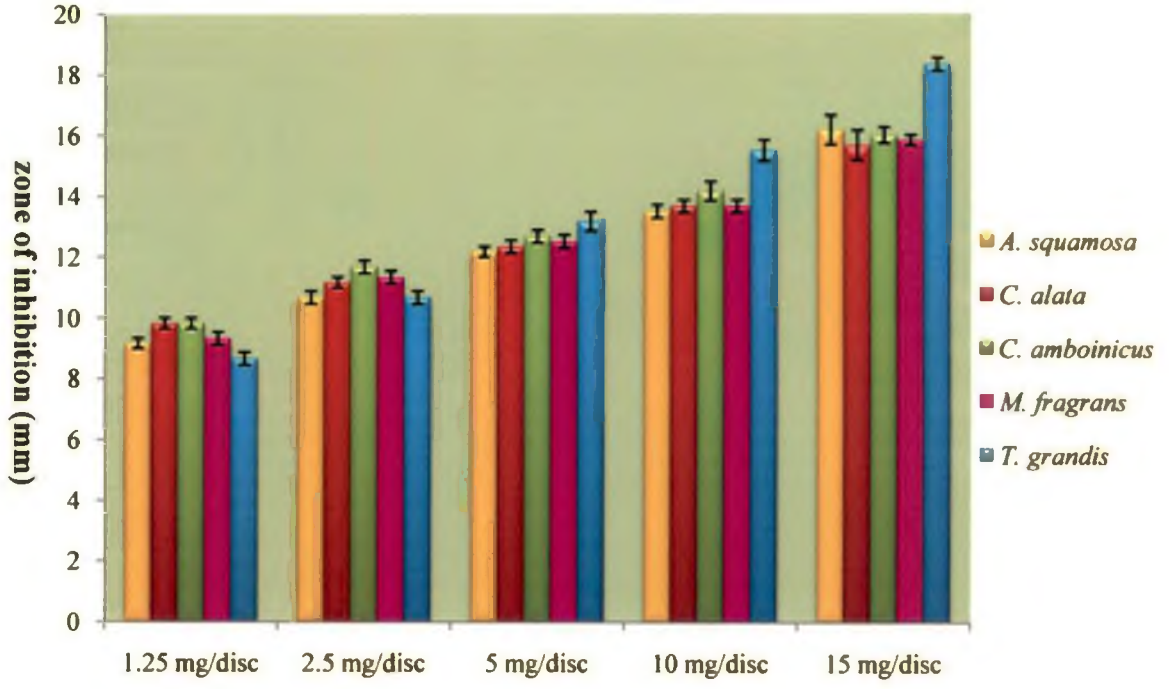
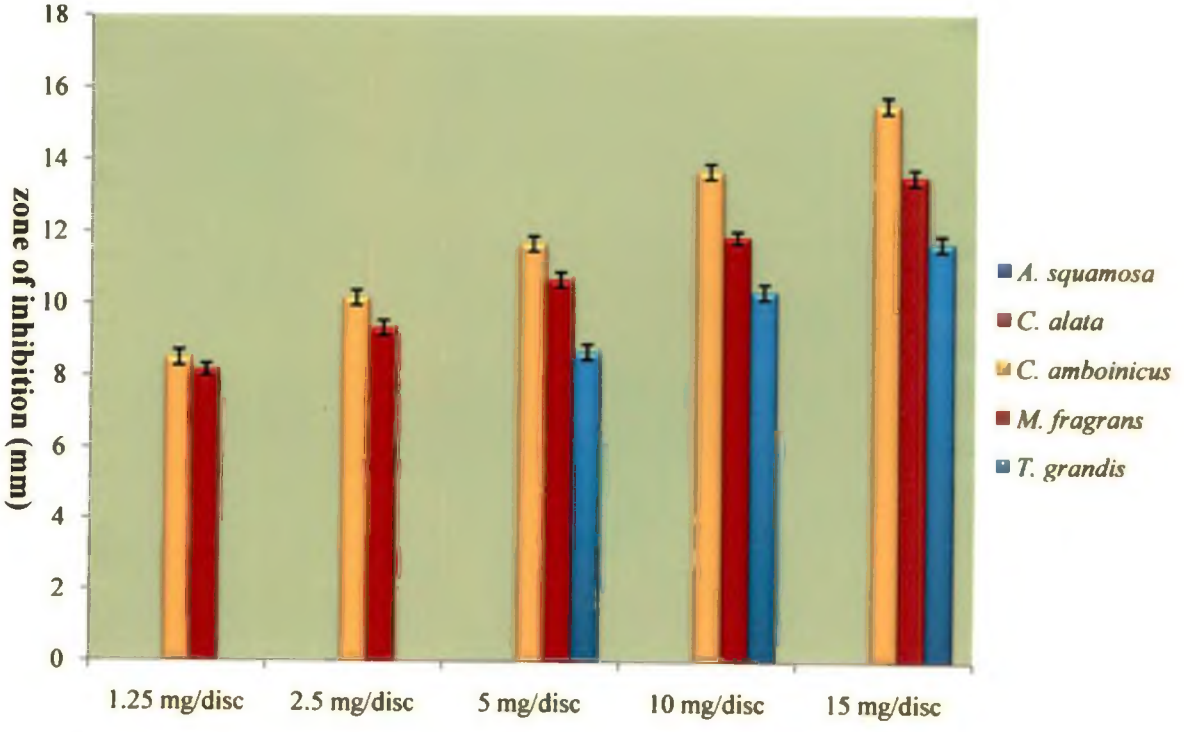


Fig. 15 ANTIBACTERIAL ACTIVITY AGAINST
Salmonella enteritidis



activity against *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* was sensitive to standard drug furazolidone (50 µg disc) with inhibitory zone diameter of 20.17 ± 0.17 mm.

The diameter of zone of inhibition obtained for *Pseudomonas aeruginosa* is given in table 13 and presented in figure 16.

4.7.11 ANTIFUNGAL ACTIVITY AGAINST *Aspergillus fumigatus*

Annona squamosa, *Cassia alata* and *Coleus amboinicus* were found to be effective against *Aspergillus fumigatus*. Ethanolic extract of *Coleus amboinicus* leaves was more effective. There was no significant difference in the activity of *Annona squamosa* and *Cassia alata* against *Aspergillus fumigatus* at all tested concentrations ($p<0.01$). There was a dose dependant increase in the activity noticed. The inhibitory zone obtained for standard drugs ketoconazole and clotrimazole were 16.67 ± 0.21 mm and 20.83 ± 0.21 mm respectively.

The diameter of zones of inhibition obtained for *Aspergillus fumigatus* are shown in table 14 and presented in figure 17.

4.7.12 ANTIFUNGAL ACTIVITY AGAINST *Candida albicans*

Candida albicans was susceptible to *Cassia alata*, *Coleus amboinicus*, *Myristica fragrans* and *Tectona grandis*. *Cassia alata* and *Coleus amboinicus* showed inhibitory activity at all tested concentrations and the activity varied significantly ($p<0.01$). *Coleus amboinicus* was the most effective among tested plants. The organism is sensitive to fresh juice of *Cassia alata* leaves. The antifungal activity of *Myristica fragrans* and *Tectona grandis* against *Candida albicans* did not vary significantly. The diameter of inhibitory zones obtained for standard drugs ketoconazole and clotrimazole were 25.50 ± 0.22 mm and 20.33 ± 0.21 mm respectively.

The diameter of zones of inhibition obtained for *Candida albicans* are reported in table 15 and presented in figure 18.

TABLE 13. DIAMETER OF ZONE OF INHIBITION OBTAINED FOR *Pseudomonas aeruginosa* (mm)

PLANT	Ethanolic extract of leaves (mg/disc)					Furazolidone (0.05 mg)
	1.25	2.5	5	10	15	
Annona squamosa	9.67^{b} ±0.21	10.83^{a} ±0.17	12.50^{a} ±0.22	14.50^{a} ±0.22	16.67^{a} ±0.21	20.17 ±0.17
Cassia alata	13.33^{d} ±0.21	15.33^{c} ±0.21	17.33^{d} ±0.21	19.17^{d} ±0.17	20.83^{c} ±0.31	20.17 ±0.17
Coleus amboinicus	9.83^{bc} ±0.17	11.67^{b} ±0.21	13.50^{b} ±0.22	16.00^{b} ±0.21	18.83^{b} ±0.31	20.17 ±0.17
Myristica fragrans	8.67^{a} ±0.21	10.33^{a} ±0.21	12.67^{a} ±0.21	14.67^{a} ±0.21	16.67^{a} ±0.21	20.17 ±0.17
Tectona grandis	10.33^{c} ±0.21	12.00^{b} ±0.26	14.50^{c} ±0.22	17.17^{c} ±0.31	19.50^{b} ±0.34	20.17 ±0.17

(Values of zone of growth inhibition are presented as mean ± SE; (-) indicates no inhibition; within a column, means bearing same superscript do not differ significantly.)

TABLE 14. DIAMETER OF ZONE OF INHIBITION OBTAINED FOR *Aspergillus fumigatus* (mm)

PLANT	Ethanolic extract of leaves (mg/disc)					CC (0.01mg)	KT (0.01mg)
	1.25	2.5	5	10	15		
A. squamosa	8.17^{a} ±0.17	9.67^{a} ±0.21	11.17^{a} ±0.17	12.83^{a} ±0.17	14.67^{a} ±0.21	20.83 ±0.21	16.67 ±0.21
C. alata	8.17^{a} ±0.17	9.83^{a} ±0.17	10.83^{a} ±0.17	11.67^{a} ±0.21	12.17^{a} ±0.17	20.83 ±0.21	16.67 ±0.21
C. amboinicus	15.83^{b} ±0.17	17.33^{b} ±0.21	18.83^{b} ±0.17	20.67^{b} ±0.21	22.83^{b} ±0.17	20.83 ±0.21	16.67 ±0.21
M. fragrans	–	–	–	–	–	20.83 ±0.21	16.67 ±0.21
T. grandis	–	–	–	–	–	20.83 ±0.21	16.67 ±0.21

(Values of zone of growth inhibition are presented as mean ±SE; (-) indicates no inhibition; within a column, means bearing same superscript do not differ significantly. KT - Ketoconazole; CC - Clotrimazole)

Fig. 16 ANTIBACTERIAL ACTIVITY AGAINST
Pseudomonas aeruginosa

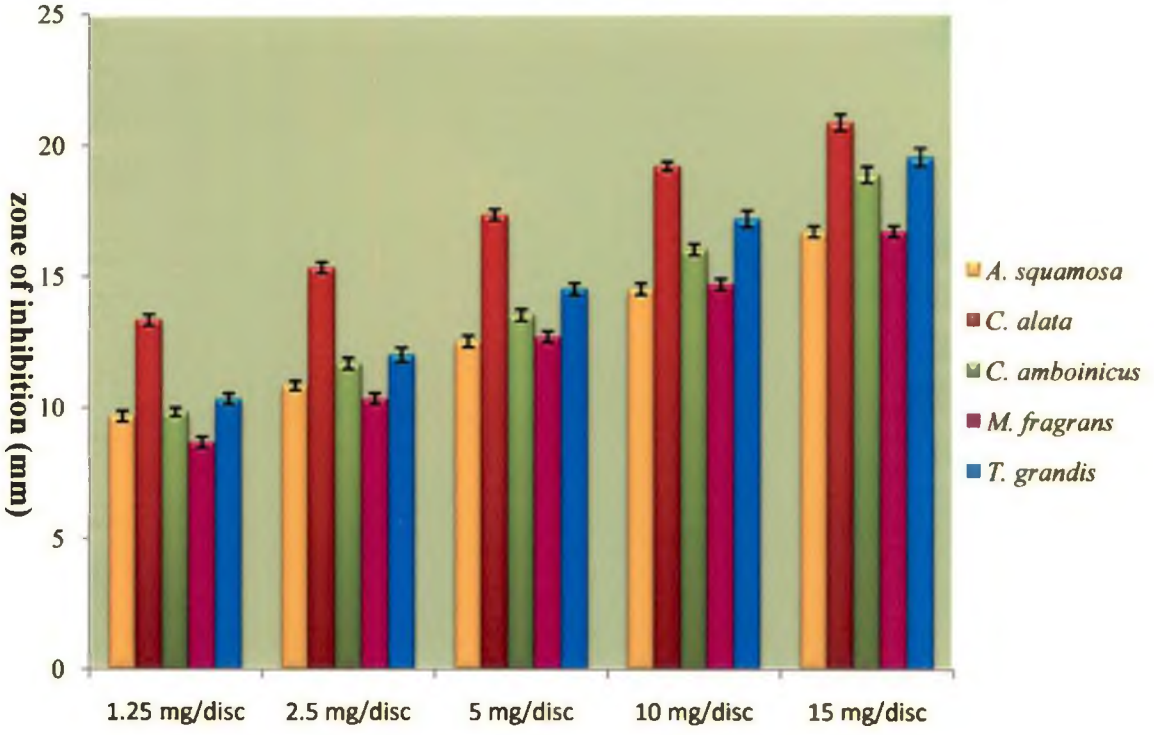
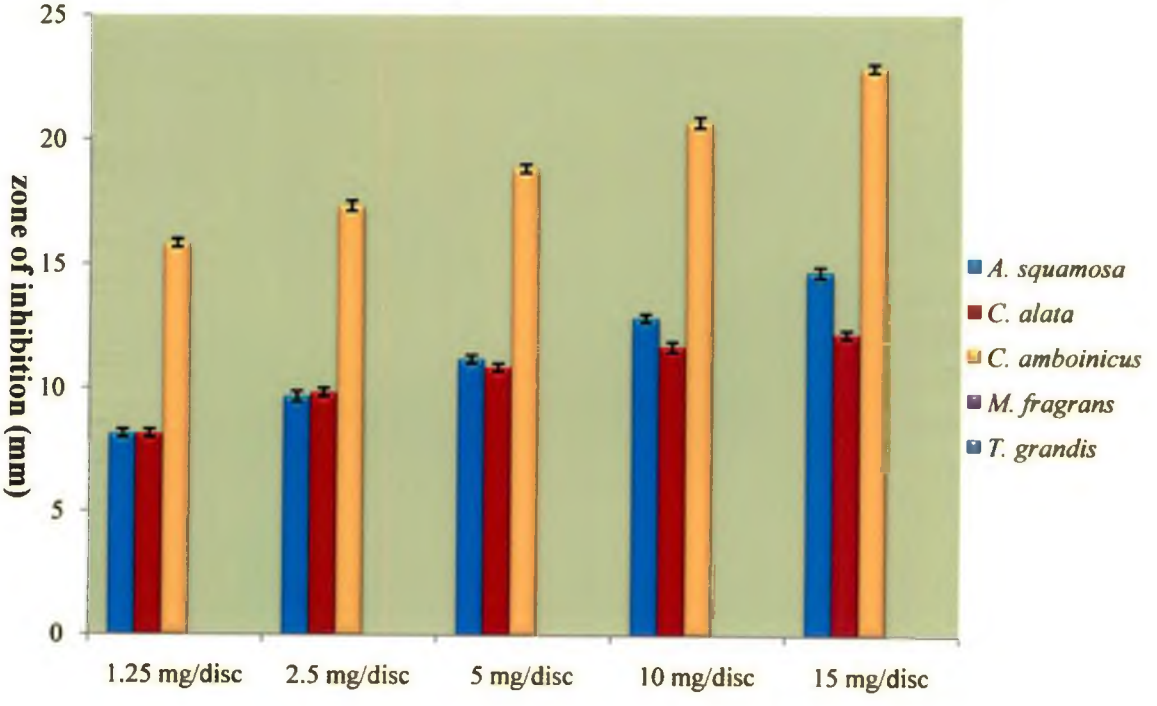


Fig. 17 ANTIFUNGAL ACTIVITY AGAINST
Aspergillus fumigatus



4.7.13 ANTIFUNGAL ACTIVITY AGAINST *Cryptococcus neoformans*

Annona squamosa and *Cassia alata* were found to be effective against *Cryptococcus neoformans. Cryptococcus neoformans* was resistant to *Coleus amboinicus, Myristica fragrans* and *Tectona grandis. Cassia alata* is more effective in inhibiting *Cryptococcus neoformans* than *Annona squamosa.* At concentration 1.25 mg/disc no significant difference was noticed in the activity of *Annona squamosa* and *Cassia alata.* The diameter of inhibitory zone obtained for standard drugs ketoconazole and clotrimazole were 16.33 ± 0.21 mm and 20.17 ± 0.17 mm respectively.

The diameter of zones of inhibition obtained for *Cryptococcus neoformans* are given in table 16 and presented in figure 19.

4.8 ANTIBACTERIAL ACTIVITY OF THE EXTRACTS BY MICROTITRE PLATE DILUTION METHOD

4.8.1 MIC AND MBC FOR *Annona squamosa*

MIC and MBC values of *Annona squamosa* against *Staphylococcus aureus* was 500 µg/ml and 1000 µg/ml; *Pasteurella multocida* was 800 µg/ml and 1000 µg/ml; *Pseudomonas aeruginosa* was 500 µg/ml and 800 µg/ml. The MIC value against *Escherichia coli* was 1000 µg/ml.

4.8.2 MIC AND MBC FOR *Cassia alata*

MIC and MBC values of *Cassia alata* against *Staphylococcus aureus* was 500 µg/ml and 800 µg/ml; *Pasteurella multocida* was 500 µg/ml and 800 µg/ml; *Pseudomonas aeruginosa* was 800 µg/ml and 1000 µg/ml.

4.8.3 MIC AND MBC FOR *Coleus amboinicus*

MIC and MBC values of *Coleus amboinicus* against *Staphylococcus aureus* was 400 µg/ml and 800 µg/ml; *Pasteurella multocida* was 800 µg/ml and 1000 µg/ml; *Pseudomonas aeruginosa* was 500 µg/ml and 800 µg/ml;

TABLE 15. DIAMETER OF ZONE OF INHIBITION OBTAINED FOR *Candida albicans* (mm)

PLANT	Ethanolic extract of leaves (mg/disc)					CC (0.01mg)	KT (0.01mg)
	1.25	2.5	5	10	15		
A. squamosa	–	–	–	–	–	20.33 ±0.21	25.50 ±0.22
C. alata	8.17 ±0.17	9.67 ±0.21	11.50^{b} ±0.22	12.83^{b} ±0.17	14.67^{b} ±0.21	20.33 ±0.21	25.50 ±0.22
C. amboinicus	14.67 ±0.21	16.50± 0.22	18.67^{c} ±0.21	21.50^{c} ±0.34	23.83^{c} ±0.17	20.33 ±0.21	25.50 ±0.22
M. fragrans	–	–	8.50^{a} ±0.22	10.33^{a} ±0.21	12.17^{a} ±0.17	20.33 ±0.21	25.50 ±0.22
T. grandis	–	–	09.17^{a} ±0.17	10.17^{a} ±0.17	11.83^{a} ±0.17	20.33 ±0.21	25.50 ±0.22

(Values of zone of growth inhibition are presented as mean ± SE; (-) indicates no inhibition; within a column, means bearing same superscript do not differ significantly. KT - Ketoconazole; CC - Clotrimazole)

TABLE 16. DIAMETER OF ZONE OF INHIBITION OBTAINED FOR *Cryptococcus neoformans* (mm)

PLANT	Ethanolic extract of leaves (mg/disc)					CC (0.01mg)	KT (0.01mg)
	1.25	2.5	5	10	15		
Annona squamosa	8.33^{NS} ±0.21	9.67* ±0.21	11.00* ±0.26	12.33** ±0.21	13.67** ±0.21	20.17 ±0.17	16.33 ±0.21
Cassia alata	8.83^{NS} ±0.17	10.33* ±0.21	11.83* ±0.01	13.50** ±0.22	15.67** ±0.21	20.17 ±0.17	16.33 ±0.21
Coleus amboinicus	–	–	–	–	–	20.17 ±0.17	16.33 ±0.21
Myristica fragrans	–	–	–	–	–	20.17 ±0.17	16.33 ±0.21
Tectona grandis	–	–	–	–	–	20.17 ±0.17	16.33 ±0.21

(Values of zone of growth inhibition are presented as mean ± SE; (-) indicates no inhibition; within a column, means bearing same superscript do not differ significantly; NS- no significant variation; *P<0.05, significant at 5 per cent level. **P<0.01, significant at 1 per cent level. KT - Ketoconazole; CC - Clotrimazole)

Fig. 18 ANTIFUNGAL ACTIVITY AGAINST
Candida albicans

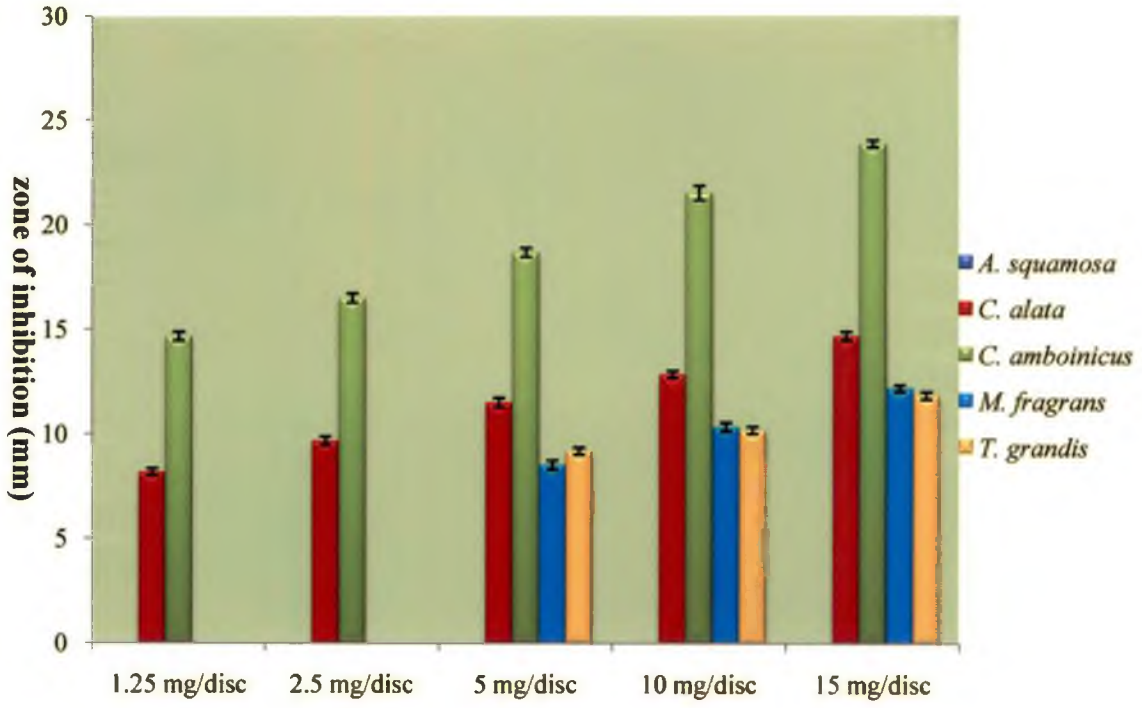
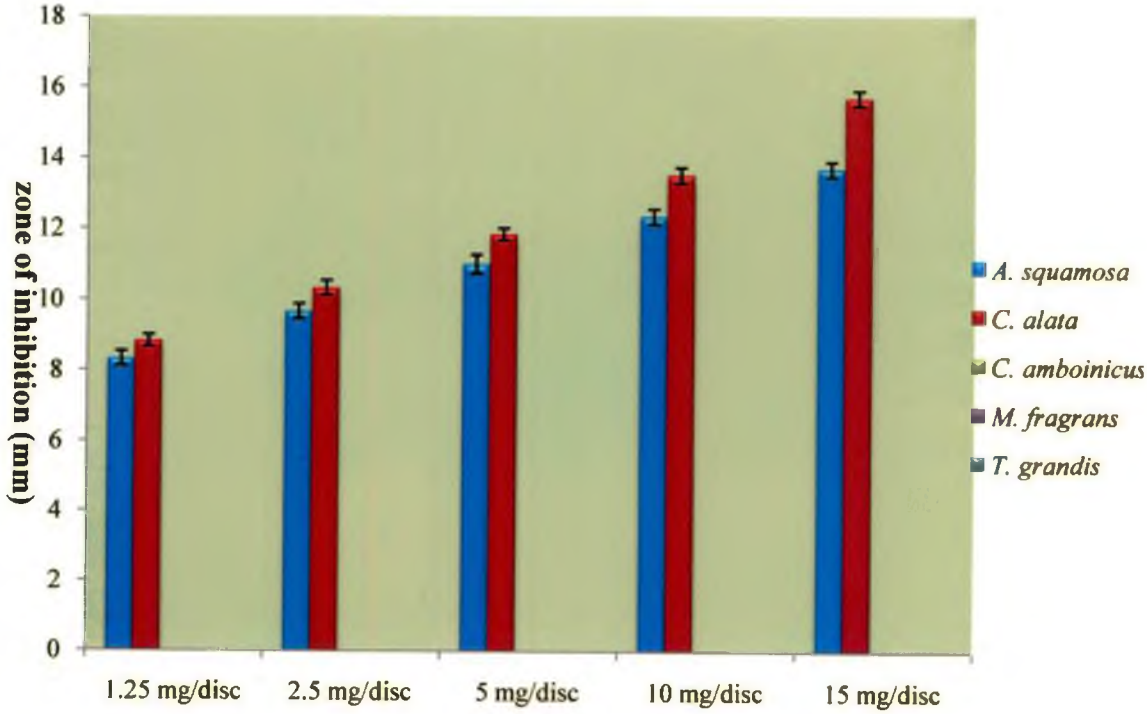


Fig. 19 ANTIFUNGAL ACTIVITY AGAINST
Cryptococcus neoformans



Salmonella enteritidis was 400 µg/ml and 800 µg/ml; *Escherichia coli* was 400 µg/ml and 800 µg/ml.

4.8.4 MIC AND MBC FOR *Myristica fragrans*

MIC and MBC values of *Myristica fragrans* against *Staphylococcus aureus* was 500 µg/ml and 800 µg/ml; *Pasteurella multocida* was 500 µg/ml and 1000 µg/ml; *Pseudomonas aeruginosa* was 800 µg/ml and 1000 µg/ml; *Salmonella enteritidis* was 500 µg/ml and 1000 µg/ml.

4.8.5 MIC AND MBC FOR *Tectona grandis*

MIC and MBC values of *Tectona grandis* against *Staphylococcus aureus* was 400 µg/ml and 800 µg/ml; *Pasteurella multocida* was 500 µg/ml and 800 µg/ml; *Pseudomonas aeruginosa* was 200 µg/ml and 500 µg/ml; *Salmonella enteritidis* was 500 µg/ml and 800 µg/ml; *Escherichia coli* was 400 µg/ml and 800 µg/ml.

The Minimum inhibitory concentration (figure 20) and minimum bactericidal concentration of ethanolic extract of leaves are summarized in table 17 and presented in figure 21.

4.9 BROTH DILUTION METHOD FOR FUNGI

4.9.1 MIC AND MFC FOR *Annona squamosa*

MIC and MFC values obtained for ethanolic extract of *Annona squamosa* leaves against *Aspergillus fumigatus* were 500 µg/ml and 1000 µg/ml. MIC value obtained for *Cryptococcus neoformans* was 1000 µg/ml.

4.9.2 MIC AND MFC FOR *Cassia alata*

MIC and MFC values obtained for ethanolic extract of *Cassia alata* leaves against *Cryptococcus neoformans* were 500 µg/ml and 1000 µg/ml. The MIC values for *Aspergillus fumigatus* and *Candida albicans* were 1000 µg/ml.

4.9.3 MIC AND MFC FOR *Coleus amboinicus*

MIC and MFC values observed for ethanolic extract of *Coleus amboinicus* leaves against *Candida albicans* and *Aspergillus fumigatus* were 250 µg/ml and 500 µg/ml.

4.9.4 MIC AND MFC FOR *Myristica fragrans*

MIC and MFC value obtained for ethanolic extract of *Myristica fragrans* leaves against *Candida albicans* were 500 µg/ml and 1000 µg/ml.

4.9.5 MIC AND MFC FOR *Tectona grandis*

MIC and MFC values obtained for ethanolic extract of *Tectona grandis* leaves against *Candida albicans* were 500 µg/ml and 1000 µg/ml

Minimum inhibitory concentration (figure 20) and minimum fungicidal concentration of ethanolic extract of leaves are furnished in table 18 and presented in figure 22.

TABLE 17. MINIMUM INHIBITORY CONCENTRATION AND MINIMUM BACTERICIDAL CONCENTRATION OF ETHANOLIC EXTRACT OF LEAVES (µg/ml)

PLANT	*S. aureus*		*E.coli*		*S.enteritidis*		*P.multocida*		*P.aeruginosa*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Annona squamosa	500	1000	1000	-	-	-	800	1000	500	800
Cassia alata	500	800	-	-	-	-	500	800	800	1000
Coleus amboinicus	400	800	400	800	400	800	800	1000	500	800
Myristica fragrans	500	800	-	-	500	1000	500	1000	800	1000
Tectona grandis	400	800	400	800	500	800	500	800	200	500

TABLE 18. MINIMUM INHIBITORY CONCENTRATION AND MINIMUM FUNGICIDAL CONCENTRATION OF ETHANOLIC EXTRACT OF LEAVES (µg/ml)

PLANT	*C.albicans*		*C.neoformans*		*A.fumigatus*	
	MIC	MFC	MIC	MFC	MIC	MFC
Annona squamosa	-	-	1000	-	500	1000
Cassia alata	1000	-	500	1000	1000	-
Coleus amboinicus	250	500	-	-	250	500
Myristica fragrans	500	1000	-	-	-	-
Tectona grandis	500	1000	-	-	-	-

Fig. 20 MINIMUM INHIBITORY CONCENTRATION OF MEDICINAL PLANTS

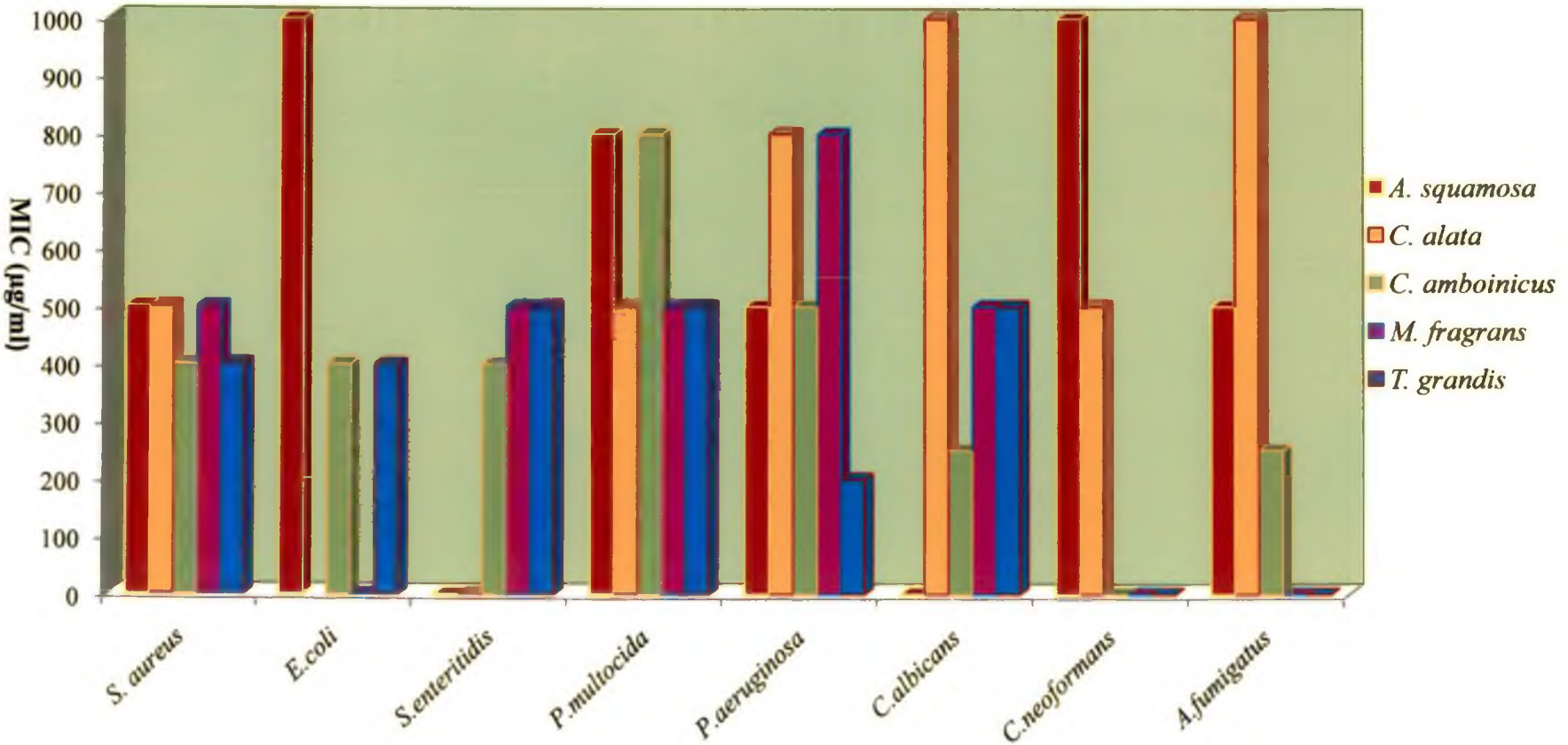


Fig. 21 MINIMUM BACTERICIDAL CONCENTRATION OF MEDICINAL PLANTS

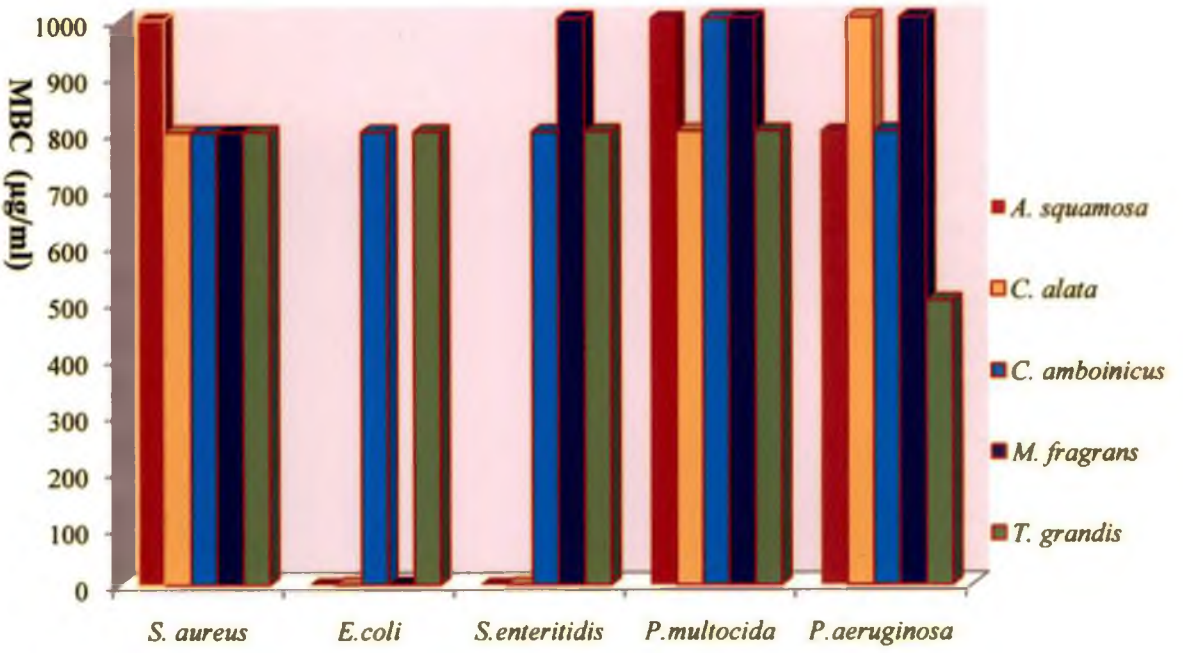
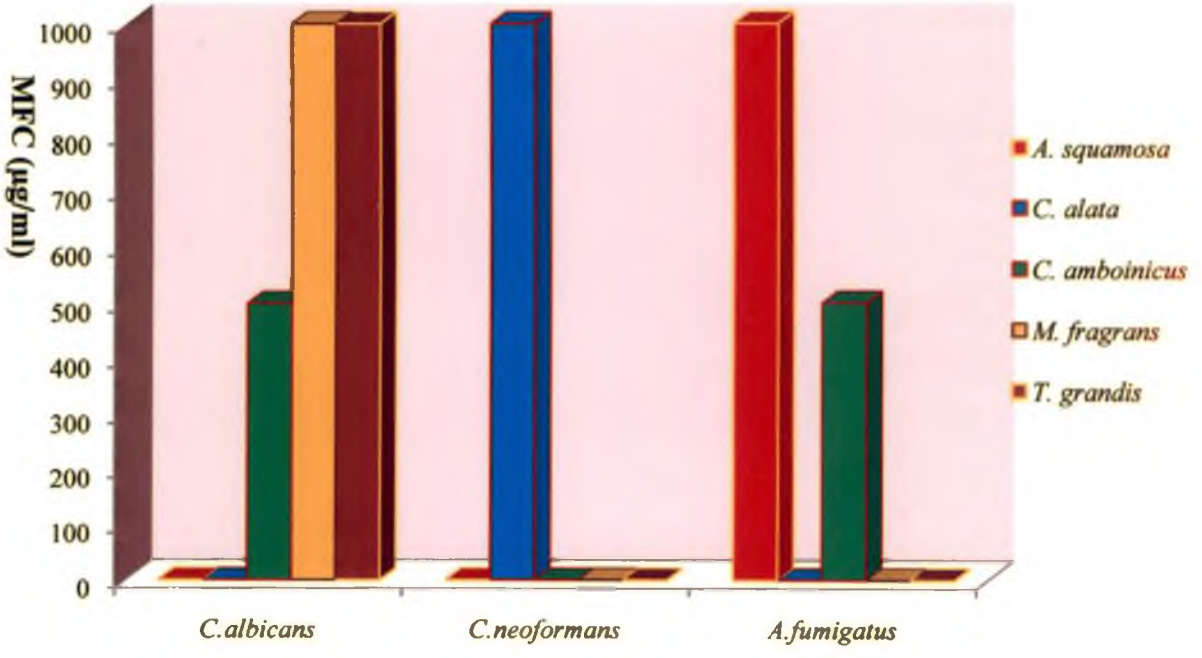


Fig. 22 MINIMUM FUNGICIDAL CONCENTRATION OF MEDICINAL PLANTS



Discussion

5. DISCUSSION

Medicinal plants constitute the base of healthcare systems in many societies. Globally about 85 per cent of traditional medicine used for primary healthcare is derived from plants. In recent times, focus on plant research has increased all over the world and a large number of evidence has been collected to show immense potential of medicinal plants used in various traditional systems. This increasing interest is due to a tremendous historical legacy in the use of plants as medicine and their easy availability, cost of effectiveness and presumed safety. Increasing prevalence of antibiotic resistance among pathogenic bacteria and undesirable side effects of some synthetic antibiotics, add urgency to the search for new infection fighting strategies. Researchers consider medicinal plants as a good choice against broad spectrum of antibiotic resistant bacteria. Medicinal plant based antimicrobials represent a vast untapped source for medicine. In many parts of the world the extracts of medicinal plants are used for their antibacterial, antifungal and antiviral properties (Hassawi and Kharma, 2006). Plant based antimicrobials have enormous therapeutical potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials.

In the present study, the ethanol extracts of leaves of *Annona squamosa, Cassia alata, Coleus amboinicus, Myristica fragrans* and *Tectona grandis* showed antimicrobial activity against *Escherichia coli, Pasteurella multocida, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella enteritidis, Aspergillus fumigatus, Candida albicans* and *Cryptococcus neoformans.* The phytochemical screening of these plants revealed the presence of various active principles. The results support the traditional use of these plants in the treatment of bacterial and fungal infections.

The yields of ethanolic extract from 100 g of the dried leaves were determined in this study and the maximum yield was obtained for *Annona squamosa* (18.07 per cent). Leaves of *Coleus amboinicus* produced the

maximum amount of fresh juice (8 ml from 10 g of fresh juice) among the five plants.

5.1 PHYTOCHEMICAL SCREENING

The active principles of plants are secondary metabolites (Kubmarawa *et al.*, 2007). Therefore, basic phytochemical investigation of these extracts for their major phytoconstituents is also vital.

The various active principles detected in *Annona squamosa* leaf extract by different qualitative tests included steroids, alkaloids, tannins, flavonoids, glycosides, phenolic compounds, diterpenes, triterpenes and saponins. Phytochemical study on ethanolic extract and fresh juice of *Annona squamosa* revealed that no detectable level of nitrates and cyanides is present in them. The presence of aporphine alkaloids anonaine, roemerine, norcorydine, corydine, norisocorydine, isocorydine and glaucine from *Annona squamosa* has been reported (Bhakuni *et al.*, 1972). Bhaumik *et al.* (1979) have tried to isolate alkaloids from the leaves of *Annona squamosa* and they isolated alkaloids named xylopine, O-methylarmepavine and lauginosine for the first time from this source. Seetharaman (1986) reported the presence of flavonoids in the leaves of *Annona squamosa*. The alkaloids and saponins have been reported to have powerful antifungal effects (Zehavi and Polacheck, 1996; Agarwal *et al.*, 2008). Flavonoids isolated from aqueous extract of *A. squamosa* have showed antimicrobial activity (Kotkar *et al.*, 2001). Study conducted by Shirwaikar *et al.* (2004b) reported that *Annona squamosa* leaf contains steroids, alkaloids, saponins, terpenes, tannins, phenolic substances, carbohydrates, volatile oil and mucilage. Volatile compound from leaves of *A. squamosa* was found to possess antibacterial activity (Chavan *et al.*, 2006). Patel and Kumar (2008) found that *Annona squamosa* leaves contain flavonoids. These findings are in agreement with the present study. The antifungal and antibacterial activity may be due to the presence of alkaloids, saponins and flavonoids in *Annona squamosa* leaves.

Phytochemical screening of fresh juice and ethanolic extract of *Cassia alata* leaves revealed the presence of steroids, alkaloids, tannins, flavonoids, glycosides, phenolic compounds, saponins, diterpenes and triterpenes. Nitrates and cyanide were absent in fresh juice and ethanolic extract of *Cassia alata* leaves. Anthraquinone, flavonoids, sterols, tannins, triterpenoids have been isolated from *Cassia alata* leaves (Khan *et al.*, 2001). Reezal *et al.* (2002) isolated diverse constituents like alkaloids, lectin, glycoside, isoflavonones and phytoestrogens from *Cassia alata*. Makinde *et al.* (2007) reported that alkaloids, phenolics and terpenoids were present in the aqueous and methanolic extract of *Cassia alata* leaves and they observed that the fractions containing alkaloids have exhibited strong antimicrobial activity. Phytochemical screening of *Cassia alata* leaves conducted by Owoyale *et al.* (2005) revealed that the most active component was flavonoid glycoside and it has got antimicrobial properties. Idu *et al.* (2007) reported the presence of phenols, tannins, anthraquinones, saponins and flavonoids in the extract of *Cassia alata* flowers. El-Mahmood and Doughari (2008) observed the presence of alkaloids, carbohydrates, tannins, saponins, phenols, flavonoids, anthraquinones and cardiac glycosides from leaves and roots of *Cassia alata*. Rahman *et al.* (2008) have isolated two flavonoids from *Cassia alata* leaves and they possessed antifungal activity. Nebedum *et al.* (2009) recently reported that ethanolic extract of *Cassia alata* leaves contain tannins, fat or oils, saponins and glycosides. These results support the present study and antimicrobial activity of *Cassia alata* may be due to the presence of alkaloids and flavonoids.

The preliminary phytochemical investigation carried out showed that some secondary metabolites such as steroids, alkaloids, tannins, flavonoids, glycosides and saponins in the fresh juice and ethanolic extract of *Coleus amboinicus* leaves. Phenolic compounds, diterpenes, triterpenes, cyanide and nitrates were absent in the ethanolic extract and fresh juice of *Coleus amboinicus* leaves. This is in accordance with the study conducted by Kaliappan and Viswanathan (2008), they observed that *Coleus amboinicus* leaves contain

flavonoids, saponins, steroids, tannins, proteins, carbohydrates and volatile oils. Rianti and Yogyarti (2006) reported that alkaloids were present in *Coleus amboinicus* and it is effective as antimicrobial agent. The present study also revealed the presence of alkaloids.

The phytochemical components present in fresh juice and ethanolic extract of *Myristica fragrans* were glycosides, flavonoids, saponins and phenolic compounds. Alkaloids, steroids, tannins, diterpenes, triterpenes, cyanide and nitrates were absent in the fresh juice and ethanolic extract of *Myristica fragrans* leaves. Dorman and Deans (2000) reported that the components with phenolic structures, such as carvacrol, eugenol and thymol, were highly active against the microorganisms. Singh *et al.* (2005) stated that phenolic compounds constitute considerable percentage of *Myristica fragrans* extract and hence the antimicrobial activity could be due to the presence of these phenolic compounds. Olaleye *et al.* (2006) reported that alkaloids, flavonoids, saponins, glycoside and anthraquinones were present in *Myristica fragrans* seeds.

Qualitative chemical tests revealed the presence of tannins, flavonoids, glycosides, phenolic compounds and saponins in fresh juice and ethanolic extract of *Tectona grandis* leaves. No detectable levels of alkaloids, steroids, diterpenes, triterpenes, cyanide and nitrates were present in ethanolic extract and fresh juice of *Tectona grandis* leaves. Majumdar *et al.* (2007) reported that *Tectona grandis* leaves contain tannins, which have got anti-inflammatory activity. Shalini and Srivastava (2009) isolated four different phenolic acids (tannic acid, gallic acid, ferulic acid and caffeic acid) from *Tectona grandis* and they suggested that antifungal activity may be due to the presence of phytochemical constituents. Basri and Fan (2005) suggested that the antimicrobial activity of medicinal plants seemed to depend on the presence of tannin in the extracts.

The results obtained from phytochemical screening of *Annona squamosa, Cassia alata, Coleus amboinicus, Myristica fragrans* and *Tectona grandis* indicated the existence of active principles with antimicrobial activity in the

crude ethanolic extracts of leaves and some showed a good correlation between the reported uses of these plants in traditional medicine against infectious diseases.

5.2 ANTIMICROBIAL ACTIVITY OF MEDICINAL PLANTS

5.2.1 DISC DIFFUSION METHOD

5.2.1.1 *Annona squamosa*

Ethanol extract of *Annona squamosa* leaves exhibited a higher degree of antimicrobial activity than fresh juice of leaves. From the present study it is evident that ethanolic extract of *Annona squamosa* leaves could significantly inhibit the growth of *Escherichia coli, Staphylococcus aureus, Pasteurella multocida, Pseudomonas aeruginosa, Aspergillus fumigatus* and *Cryptococcus neoformans*. *Candida albicans* and *Salmonella enteritidis* appeared to be resistant. The activity against *Escherichia coli* was observed at concentrations higher than 5 mg/disc. Fresh juice of *Annona squamosa* leaves was found to be effective against *Staphylococcus aureus*. The growth of other tested microorganisms was not affected by fresh juice of *Annona squamosa* leaves.

This agrees with the findings of Thaker and Anjaria (1985) who reported that chloroform extract of *Annona squamosa* leaves inhibited the growth of *Staphylococcus aureus, Escherichia coli, Streptococcus pyogenes,* Corynebacterium sp. and *Pseudomonas aeruginosa.* Petroleum ether, chloroform and ethanol extract of *Annona squamosa* showed antimicrobial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Aspergillus flavus, A. niger* and *A. fumigatus.* As in the present study the growth of *Candida albicans* was not affected by extracts of *Annona squamosa.* (Au *et al.*, 2003; Rahman *et al.*, 2005). These results correlated with the present study. In the present study ethanolic extract of *Annona squamosa* leaves was found to be most active against *Staphylococcus aureus.* Chariandy *et al.* (1999) concluded that petroleum ether extract of *Annona squamosa* leaves was highly effective against

S. aureus, P. aeruginosa and *E. coli.* Patel *et al.* (2008) reported that methanolic extract of *Annona squamosa* was able to inhibit the growth of *Escherichia coli.* Patel and Kumar (2008) reported that petroleum ether, chloroform and methanol extract of *Annona squamosa* leaves had inhibitory action against *E. coli, P. aeruginosa* and *S. aureus.* These observations are consistent with the present study.

5.2.1.2 *Cassia alata*

The results indicated that ethanolic extract of *Cassia alata* leaves appeared to be effective in inhibiting the growth of *Staphylococcus aureus, Pasteurella multocida, Pseudomonas aeruginosa, Aspergillus fumigatus, Candida albicans* and *Cryptococcus neoformans. Escherichia coli* and *Salmonella enteritidis* were found to be resistant. The fresh juice of *Cassia alata* leaves exhibited activity against *Candida albicans* only.

Ikenebomeh and Metitiri (1988) reported the antimicrobial activity of *Cassia alata* against *E. coli.* Crockette *et al.* (1992) found that water extract of *Cassia alata* could inhibit the growth of *E. coli* and *C. albicans.* But in the present study growth of *E. coli* was not affected by *Cassia alata* leaves. This may be due to variation in strain of *E. coli* and extract used. In a similar study, Khan *et al.* (2001) observed that methanol extracts of leaves, flowers, root and stem of *Cassia alata* inhibited many types of bacteria including *E. coli, S. aureus, P. aeruginosa,* but not moulds (*C. albicans, Aspergillus niger* and *Trichophyton mentagrophytes*). The study conducted by Elysha-Nur *et al.* (2002) revealed that *E. coli* was resistant and *S. aureus* was sensitive to ethanolic and water extract of *Cassia alata* leaves. Similar study conducted by Reezal *et al.* (2002) showed that aqueous and ethanol extracts of *cassia alata* leaves could not inhibit the growth of *Candida albicans* and *Aspergillus fumigatus.* Somchit *et al.* (2003) reported that *C. albicans* was resistant and *S. aureus* and *A. fumigatus* were sensitive to ethanol and water extracts of *Cassia alata* leaves. These findings support the present study. But ethanol and water extract of *Cassia alata*

bark inhibited the growth of *C. albicans.* Our findings about susceptibility of *C. albicans* to ethanol extract of *Cassia alata* leaves were supported by Owoyale *et al.* (2005). They reported that *C. albicans* was sensitive to crude ethanolic, methanolic, petroleum ether extracts of *Cassia alata* leaves. Similar observations have been reported by Makinde *et al.* (2007). Duraipandiyan *et al.* (2006) reported that hexane and methanol extracts of *Cassia alata* leaves showed slight activity against *S. aureus.* They also reported that *E. coli, P. aeruginosa and C. albicans* were found to be resistant to the extracts. Extracts of *Cassia alata* flowers were found to be effective against *S. aureus, C. albicans, E. coli* and *P. aeruginosa* (Idu *et al.,* 2007). Alam *et al.* (2009) recently reported that ethanolic extract of *Cassia alata* leaves inhibited the growth of *S. aureus, E. coli,* and *Salmonella typhi.* Nebedum *et al.* (2009) also reported that crude ethanolic extracts of *Cassia alata* could inhibit the growth of *C. albicans, E. coli* and *S. aureus*. These reports tie in with the present study.

Ibrahim and Osaman (1995) reported that ethanol extract of *C. alata* leaves have no activity against *C. albicans* and *C. neoformans,* but the growth of Aspergillus sp. were inhibited. In the present study ethanol extract of *C. alata* leaves showed inhibitory activity against *C. albicans* and *C. neoformans.* This variation in observation may be due to difference in the concentration of the extract, strain of organism, extraction procedure, physiological and morphologic state of the plant (Goyal *et al.*, 2008). Ranganathan and Balajee (2000) reported the anti-cryptococcus activity of combination of ethanolic extracts of leaves of *C. alata* and *Ocimum sanctum.* Vaijayanthimala *et al.* (2000) reported the antimicrobial activity of water extract from *C. alata* leaves against *C. albicans.*

5.2.1.3 *Coleus amboinicus*

From the present results, it is evident that the alcoholic extract of *Coleus amboinicus* leaves showed antibacterial activity against *Escherichia coli, Staphylococcus aureus, Pasteurella multocida, Pseudomonas aeruginosa, Salmonella enteritidis, Aspergillus fumigatus* and *Candida albicans.* There was

no inhibition observed in the case of *Cryptococcus neoformans*. The most sensitive organism was *Candida albicans*. The degree of inhibition increased with increase in concentration. *Staphylococcus aureus* and *Escherichia coli* were found to be sensitive to fresh juice of *Coleus amboinicus*. Rianti and Yogyarti (2006) reported that *Coleus amboinicus* leaves showed inhibitory activity against *Candida albicans* and *Streptococcus mutans*. Kumar *et al.* (2008) reported that aqueous extract of *Coleus amboinicus* leaves showed antimicrobial activity against *Staphylococcus aureus, Bacillus subtilis* and *Proteus vulgaris*. *Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumonia* were found to be resistant. Gurgel *et al.* (2009) observed that the hydroalcoholic extracts of *C. amboinicus* leaves showed a promising activity against MRSA. In the present study *Staphylococcus aureus* has shown susceptibility to both fresh juice and ethanolic extract of *C. amboinicus* leaves. Murthy *et al.* (2009) reported that *Coleus amboinicus* effectively inhibited the growth of *Aspergillus* spp., *Candida* spp., *Penicillium* spp., *Saccharomyces cereviciae* and *Fusarium* spp.

5.2.1.4 *Myristica fragrans*

In the present investigation growth of *Staphylococcus aureus, Pasteurella multocida, Pseudomonas aeruginosa, Salmonella enteritidis* and *Candida albicans* were inhibited by alcoholic extract of *Myristica fragrans* leaves. *Escherichia coli, Cryptococcus neoformans* and *Aspergillus fumigatus* were found to be resistant to extract of *Myristica fragrans* leaves. *Staphylococcus aureus* was the most sensitive organism. Dose dependant increase in the diameter of inhibition zone was observed. *Pseudomonas aeruginosa* and *Staphylococcus aureus* were sensitive to fresh juice of *Myristica fragrans* leaves. Orabi *et al.* (1991) reported that resorcinol isolated from *Myristica fragrans* seeds shown antimicrobial activity against *Staphylococcus aureus* and *Candida albicans*. This observation is in accordance with the present study. Dorman and Deans (2000) reported the antibacterial activity of volatile oil from *Myristica fragrans* against *Escherichia coli* and *Staphylococcus aureus*. Study conducted by Singh *et al.*

(2005) revealed that essential oil and acetone extract of *Myristica fragrans* showed antibacterial activity against *Staphylococcus aureus* and *Bacillus cereus.* Both essential oil and extract were ineffective against *Pseudomonas aeruginosa* and *E. coli.* The results of the present study disagree with Indu *et al.* (2006), who reported that inhibitory activity of *Myristica fragrans* against *E. coli* and *Salmonella* was serotype dependent. There was no inhibition observed for the growth of *Salmonella enteritidis.*

5.2.1.5 *Tectona grandis*

Results of the present study indicated that *Staphylococcus aureus, Escherichia coli, Pasteurella multocida, Pseudomonas aeruginosa, Salmonella enteritidis* and *Candida albicans* were inhibited by alcoholic extract of *Tectona grandis* leaves. *Cryptococcus neoformans* and *Aspergillus fumigatus* were resistant to *Tectona grandis* leaf extract. *Staphylococcus aureus* was found to be the most sensitive. *Staphylococcus aureus* was the only organism sensitive to fresh juice of *Tectona grandis* leaves. Present study showed that ethanol extract of *Tectona grandis* inhibited bacteria better than fungi. The results of the present study was supported by Srinivasan *et al.* (2001) who reported that aqueous extract of *Tectona grandis* leaf inhibited the growth of *Staphylococcus aureus, Salmonella* spp., *Pasteurella multocida, Pseudomonas aeruginosa* and *Candida albicans.* There was no inhibition observed for *Escherichia coli* and *Aspergillus fumigatus.* Neamatallah *et al.* (2005) reported that methanol extract of *Tectona grandis* bark was inhibitory to MRSA.

Various studies revealed that polarity of antibacterial compounds is crucial for their activity (Goyal *et al.*, 2008). Therefore it is obvious that extracts prepared using organic solvents were more active against bacterial species. Similar findings have been reported by Thongson *et al.* (2004). The pattern of inhibition largely depends upon extraction procedure, plant part, physiological and morphological state of plant, extraction solvent and microorganism tested. It has been demonstrated that extracts prepared using dried plant material is much

more effective than the fresh plant materials (Goyal *et al.*, 2008; Ramya *et al.*, 2008). In the present study also ethanol extracts have been found to be more effective than fresh juice of the leaves

5.2.2 MICROTITRE PLATE DILUTION METHOD

The minimum inhibitory concentration of the extracts revealed a decline in activity as the concentrations decreased which implies the extracts are more active at high concentrations than at low concentrations. MIC determination is important in giving a guideline to the choice of an appropriate and effective concentration of antimicrobial agents.

5.2.2.1 *Annona squamosa*

Results of the present study indicated that ethanolic extract of *Annona squamosa* leaves significantly inhibited the growth of *Staphylococcus aureus, Pasteurella multocida, Pseudomonas aeruginosa* and *Escherichia coli. Pseudomonas aeruginosa* was the most sensitive organism among the tested bacteria. These results agree with observation of Thaker and Anjaria (1985). They have reported that chloroform extract of *Annona squamosa* leaves inhibited the growth of *Staphylococcus aureus, Escherichia coli, Streptococcus pyogenes, Corynebacterium* spp. and *Pseudomonas aeruginosa.* Chariandy *et al.* (1999) also reported the activity of *Annona squamosa* leaves against *E. coli, P. aeruginosa* and *S. aureus.* Patel *et al.* (2007) showed the antibacterial activity of water, methanol, acetone and petroleum ether extracts of *Annona squamosa* leaves against *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

5.2.2.2 *Cassia alata*

In the present study *Cassia alata* leaves completely inhibited the growth of *Staphylococcus aureus, Pasteurella multocida* and *Pseudomonas aeruginosa. Staphylococcus aureus* and *Pasteurella multocida* appeared to be equally sensitive. Study conducted by Sakharkar and Pati (1998) revealed the

susceptibility of *Staphylococcus aureus* to ethanol and acetone extract of *Cassia alata.* Elysha-Nur *et al.* (2002) reported that *S. aureus* was sensitive to ethanolic and water extract of *Cassia alata* leaves. Khan *et al.* (2001) have described the antimicrobial activity of methanol extracts of leaves, flowers, root and stem against *S. aureus* and *P. aeruginosa.* Duraipandiyan *et al.* (2006) have observed that hexane and methanol extracts of *Cassia alata* leaves showed slight activity against *S. aureus,* whereas *Pseudomonas aeruginosa* was found to be resistant. Alam *et al.* (2009) and Nebedum *et al.* (2009) reported that ethanolic extract of leaves of *Cassia alata* inhibited the growth of *S. aureus.*

5.2.2.3 *Coleus amboinicus*

Ethanol extract of *Coleus amboinicus* inhibited the growth of *Staphylococcus aureus, Pasteurella multocida, Pseudomonas aeruginosa, Salmonella enteritidis* and *Escherichia coli. Staphylococcus aureus, Salmonella enteritidis* and *Escherichia coli* were found to be more susceptible to ethanolic extract of *Coleus amboinicus* leaves. Kumar *et al.* (2008) reported that aqueous extract of *Coleus amboinicus* leaves inhibited the growth of *Staphylococcus aureus* and no inhibition was observed against *Escherichia coli* and *Pseudomonas aeruginosa.* Gurgel *et al.* (2009) have reported the antibacterial activity of hydroalcoholic extracts of *C. amboinicus* leaves against MRSA.

5.2.2.4 *Myristica fragrans*

Ethanol extract of *Myristica fragrans* leaves was found to inhibit the growth of *Staphylococcus aureus, Pasteurella multocida, Pseudomonas aeruginosa* and *Salmonella enteritidis.* Study conducted by Orabi *et al.* (1991) reported that resorcinol isolated from *Myristica fragrans* seeds possessed antimicrobial activity against *Staphylococcus aureus.* Dorman and Deans (2000) reported the antibacterial activity of volatile oil from *Myristica fragrans* against *Escherichia coli* and *Staphylococcus aureus.* But there was no inhibition observed for *Pseudomonas aeruginosa.* Study conducted by Singh *et al.* (2005) revealed the antibacterial activity of essential oil and acetone extract of *Myristica*

fragrans against *Staphylococcus aureus.* Both essential oil and extract were ineffective against *Pseudomonas aeruginosa* and *E. coli.* Indu *et al.* (2006) reported the antimicrobial activity of *Myristica fragrans* against *E. coli* and Salmonella sp. In the present study, *Pseudomonas aeruginosa* was found to be sensitive and *E. coli* was found to be resistant to extract of *Myristica fragrans.* This variation may be due to difference in the type of extract, extraction procedure or the parts of the plant used.

5.2.2.5 *Tectona grandis*

Growth of *Staphylococcus aureus, Pasteurella multocida, Pseudomonas aeruginosa, Salmonella enteritidis* and *Escherichia coli* was inhibited by ethanol extract of *Tectona grandis* leaves. *Pseudomonas aeruginosa* was found to be more sensitive compared with other organisms. Srinivasan *et al.* (2001) reported that aqueous extract of *Tectona grandis* leaf inhibited the growth of *Staphylococcus aureus, Salmonella* spp., *Pasteurella multocida* and *Pseudomonas aeruginosa. Escherichia coli* was resistant to the extracts of *Tectona grandis* leaves. In the present study *Escherichia coli* was found to be susceptible to *Tectona grandis* leaves. This difference in observation may be due to type of extraction, strain of microorganism, differences in antimicrobial screening methods and phytochemical constituents in the extract (Goyal *et al.*, 2008). In another study Neamatallah *et al.* (2005) reported that methanol extract of *Tectona grandis* bark was inhibitory to MRSA.

5.2.3 BROTH DILUTION METHOD FOR FUNGI

5.2.3.1 *Annona squamosa*

Ethanolic extract of *Annona squamosa* leaves showed inhibitory activity against *Aspergillus fumigatus* and *Cryptococcus neoformans. Annona squamosa* leaves appeared to be more potent in inhibiting *Aspergillus fumigatus* than *Cryptococcus neoformans.* The extract showed satisfactory inhibition at concentrations at or below 1 mg/ml. Study conducted by Rahman *et al.* (2005)

showed that petroleum ether, chloroform and ethanol extract of *Annona squamosa* inhibited the growth of *Aspergillus fumigatus.* But there is no report on anti-cryptococcal activity of *Annona squamosa.*

5.2.3.2 *Cassia alata*

In the present study it was observed that ethanolic extract of *Cassia alata* leaves inhibited growth of *Cryptococcus neoformans, Aspergillus fumigatus* and *Candida albicans. Cryptococcus neoformans* is found to be more susceptible than *Aspergillus fumigatus* and *Candida albicans.* Study conducted by Ibrahim and Osaman (1995) reported that ethanol extract of *C. alata* leaves has no inhibitory activity against *C. albicans* and *C. neoformans.* They also reported the susceptibility of *Aspergillus* spp. to *Cassia alata* leaves. But the present observation proved that ethanol extract of *Cassia alata* leaves got activity against *C. albicans* and *C. neoformans.* Ranganathan and Balajee (2000) reported anti-cryptococcus activity of ethanolic extracts of leaves of *Cassia alata* and *Ocimum sanctum.* Study conducted by Vaijayanthimala *et al.* (2000) described the antimicrobial activity of water extract from *Cassia alata* leaves against *C. albicans.* Nebedum *et al.* (2009) have reported the activity of crude ethanolic extracts of *Cassia alata* against *C. albicans.* These reports are consistent with the present study.

5.2.3.3 *Coleus amboinicus*

Ethanolic extract of *Coleus amboinicus* leaves was found to be effective against *Candida albicans* and *Aspergillus fumigatus.* Study conducted by Rianti and Yogyarti (2006) revealed the susceptibility of *Candida albicans* to leaves of *Coleus amboinicus.* The result of the present study was supported by Murthy *et al.* (2009), who reported that *Coleus amboinicus* effectively inhibited the growth of *Aspergillus* spp. and *Candida* spp.

5.2.3.4 *Myristica fragrans*

Ethanolic extract of *Myristica fragrans* leaves inhibited the growth of *Candida albicans*. This result ties in with the findings of Orabi *et al.* (1991), who reported that resorcinol isolated from *Myristica fragrans* seeds has shown antimicrobial activity against *Candida albicans.*

5.2.3.5 *Tectona grandis*

The growth of *Candida albicans* was inhibited by ethanolic extract of *Tectona grandis* leaves. This result is in accordance with the observation of Srinivasan *et al.* (2001). They reported the antimicrobial effect of aqueous extract of *Tectona grandis* leaf against *Candida albicans.*

Comparison with pertinent results data from literatures indicate that, according to the methodology adopted in studies on antimicrobial activity, the most diverse results can be obtained.

All the medicinal plants used in this work showed promising antimicrobial properties indicating the potential for discovery of new antimicrobial principles. Further phytochemical studies are required to determine all types of compounds responsible for the antimicrobial effects of these plants. The results also indicated that scientific studies carried out on medicinal plants having traditional claims of effectiveness might warrant fruitful results. Further study also required for the *in vivo* efficacy of these plants against the tested pathogens.

Summary

6. SUMMARY

This study was undertaken to assess the *in vitro* antibacterial and antifungal activity of selected medicinal plants available in Kerala. *Annona squamosa* (Aatha), *Cassia alata* (Anathakara), *Coleus amboinicus* (Panicoorka), *Myristica fragrans* (Nutmeg) and *Tectona grandis* (Teak) are the plants used in this study. Ethanolic extract and fresh juice of the leaves from these plants were tested *in vitro* for their antimicrobial activity. The organisms used in this study consisted of five bacterial and three fungal strains. These include *Staphylococcus aureus subsp.aureus* (MTCC 96), *Salmonella enteritidis* (MTCC 3219), *Escherichia coli* (MTCC 723), *Pasteurella multocida subsp.multocida* (MTCC 1161), *Pseudomonas aeruginosa* (MTCC 741), *Aspergillus fumigatus* (MTCC 870), *Candida albicans* (MTCC 227) and *Cryptococcus neoformans var neoformans* (MTCC 4404). The standard drugs used were penicillin (10 units) for gram positive organisms, furazolidone (50 µg) for gram negative organisms and Ketoconazole (10 µg) and clotrimazole (10 µg) for fungal strains.

Yield of ethanolic extract and fresh juice of the leaves, diameter of inhibitory zone at various concentrations of the extract, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were determined to evaluate the antimicrobial activity of the above mentioned plants. Disc diffusion method, microtitre plate dilution technique and broth dilution technique were used to assess the antimicrobial activity of medicinal plants. The alcoholic extract and fresh juice of leaves were tested qualitatively for the presence of various active chemical constituents.

The yields of ethanolic extract from 100 g of the dried leaves were determined in this study and the maximum yield was obtained from *Annona squamosa* leaves. Leaves of *Coleus amboinicus* produced the maximum amount of fresh juice among the five plants.

Phytochemical screening revealed the presence of steroids, alkaloids, tannins, flavonoids, glycosides, phenolic compounds, diterpenes, triterpenes and saponins in the fresh juice and ethanolic extract of *Annona squamosa* and *Cassia alata.* Alkaloids, tannins, flavonoids, glycosides, steroids and saponins were present in the fresh juice and ethanolic extract of *Coleus amboinicus* leaves. Glycosides, flavonoids, saponins and phenolic compounds were present in the fresh juice and ethanolic extract of *Myristica fragrans* leaves. Qualitative chemical tests revealed the presence of tannins, flavonoids, glycosides, phenolic compounds and saponins in fresh juice and ethanolic extract of *Tectona grandis* leaves. Phytochemical analysis revealed the presence of various metabolites in the leaves, thus providing knowledge of the metabolites responsible for its therapeutic efficacy.

The antibacterial screening by disc diffusion method revealed the susceptibility of *Escherichia coli* to ethanolic extract of *Annona squamosa, Coleus amboinicus* and *Tectona grandis. Coleus amboinicus* leaves appeared to be more effective and *Annona squamosa* leaves were the least active. The growth of *Escherichia coli* was not affected by ethanolic extract of *Cassia alata* and *Myristica fragrans* leaves. The growth of *Staphylococcus aureus, Pasteurella multocida* and *Pseudomonas aeruginosa* was inhibited by all the five plants. *Tectona grandis* was more effective in inhibiting the growth of *Staphylococcus aureus* and *Pasteurella multocida* and the least activity was observed for *Cassia alata. Cassia alata* was found to be more effective against *Pseudomonas aeruginosa. Annona squamosa* and *Myristica fragrans* were having less inhibitory action on the growth of *Pseudomonas aeruginosa. Salmonella enteritidis* was inhibited by *Tectona grandis, Myristica fragrans* and *Coleus amboinicus. Coleus amboinicus* produced the highest inhibitory action on the growth of *Salmonella enteritidis.*

In vitro antifungal assay revealed that *Candida albicans* was inhibited by ethanolic extract of *Cassia alata, Coleus amboinicus, Myristica fragrans* and *Tectona grandis. Coleus amboinicus* was found to be more effective in inhibiting

the growth of *Candida albicans* and *Tectona grandis* was less effective. *Annona squamosa* and *Cassia alata* were inhibited the growth of *Cryptococcus neoformans.* The highest inhibitory activity against *C. neoformans* was shown by *Cassia alata. A. fumigatus* was found to be susceptible to *Annona squamosa, Cassia alata* and *Coleus amboinicus. Coleus amboinicus* showed the highest activity against *A. fumigatus* and *Cassia alata* was found to be less effective.

Fresh juice of the leaves was tested *in vitro* for their antimicrobial activity. The results indicated that fresh juice of *Annona squamosa, Coleus amboinicus, Myristica fragrans* and *Tectona grandis* leaves was found to inhibit the growth of *Staphylococcus aureus*. Fresh juice of *Coleus amboinicus* has got inhibitory action on the growth of *Escherichia coli. Pseudomonas aeruginosa* was found to be sensitive to fresh juice of *Myristica fragrans.* The growth of *Candida albicans* was inhibited by fresh juice of *Cassia alata* leaves.

Results of Broth dilution test revealed that *Staphylococcus aureus, Pseudomonas aeruginosa* and *Pasteurella multocida* were susceptible to all the tested medicinal plants. *Escherichia coli* was found to be resistant to *Cassia alata* but sensitive to others. The growth of *Salmonella enteritidis* was inhibited by *Coleus amboinicus, Myristica fragrans* and *Tectona grandis. Candida albicans* appeared to be sensitive to all plants except *Annona squamosa. Cassia alata* and *Annona squamosa* inhibited the growth of *Cryptococcus neoformans* and *Aspergillus fumigatus. Coleus amboinicus* was found to have high inhibitory action on the growth of *Aspergillus fumigatus.* MIC determination is important in giving a guideline to the choice of an appropriate and effective concentration of antimicrobial agents.

Results of the present study indicated that the five medicinal plants used in this work showed promising antimicrobial properties indicating the potential for discovery of antimicrobial principles. It could be concluded that these plants could be effectively used in the treatment of various fungal and bacterial infections.

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ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF SELECTED MEDICINAL PLANTS AVAILABLE IN KERALA

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Abstract of the thesis submitted in partial fulfilment of the requirement for the degree of

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2010

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ABSTRACT

In the present study cold ethanolic extract and fresh juice of five medicinal plants were screened for their *in vitro* antibacterial and antifungal activities. The plants were *Annona squamosa* (Aatha), *Cassia alata* (Anathakara), *Coleus amboinicus* (Panicoorka), *Myristica fragrans* (Nutmeg) and *Tectona grandis* (Teak). Antimicrobial activity was tested against *Staphylococcus aureus subsp.aureus* (MTCC 96), *Salmonella enteritidis* (MTCC 3219), *Escherichia coli* (MTCC 723), *Pasteurella multocida subsp.multocida* (MTCC 1161), *Pseudomonas aeruginosa* (MTCC 741), *Aspergillus fumigatus* (MTCC 870), *Candida albicans* (MTCC 227) and *Cryptococcus neoformans var neoformans* (MTCC 4404). Phytochemical analysis was conducted for the presence of routine secondary plant metabolites.

The diameter of inhibitory zone at various concentrations of the extract minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were used to evaluate the *in vitro* antimicrobial activity of the above mentioned plants. Disc diffusion method, microtitre plate dilution technique and broth dilution technique were used. The reference drugs used in this study were penicillin G, furazolidone ketoconazole and clotrimazole.

The maximum yield was obtained from ethanolic extract of *A. squamosa* leaves (18.07 per cent). Leaves of *C. amboinicus* produced the maximum amount of fresh juice among the five plants (8 ml from 10 g of the fresh tender leaves).

Phytochemical analysis reported the presence of steroids, alkaloids tannins, flavonoids, glycosides, phenolic compounds, diterpenes, triterpenes and saponins in the leaves of *A. squamosa* and *C. alata*. Alkaloids, tannins flavonoids, glycosides, steroids and saponins were present in the leaves of *C. amboinicus*. *M. fragrans* leaves contain glycosides, flavonoids, saponins and phenolic compounds. Qualitative chemical tests revealed the presence of tannins,

flavonoids, glycosides, phenolic compounds and saponins in the leaves of *T. grandis*.

All the extracts showed varying degrees of antimicrobial activity on the microorganisms tested. The antimicrobial screening revealed the susceptibility of *E. coli* to *A. squamosa, C. amboinicus* and *T. grandis*. The growth of *S. aureus, P. multocida* and *P. aeruginosa* was inhibited by all the five plants. *S. enteritidis* was found to be susceptible to *T. grandis, M. fragrans* and *C. amboinicus*.

MIC values ranged from 200-1000 µg/ml and MBC values ranged from 500-1000 µg/ml. In case of *A. squamosa,* MBC value against *E. coli* was more than 1000 µg/ml.

The antifungal screening revealed that the growth of *C. albicans* was inhibited by *C. alata, C. amboinicus, M. fragrans* and *T. grandis. A. squamosa* and *C. alata* inhibited the growth of *C. neoformans. A. fumigatus* appeared to be susceptible to *A. squamosa, C. alata* and *C. amboinicus.*

MIC values ranged from 250-1000 µg/ml for the fungal strains. MFC values ranged between 500-1000 µg/ml except for *A. squamosa* and *C. alata.* MFC of *C. alata* against *A. fumigatus* and *C. albicans* was more than 1000 µg/ml. MFC of *A. squamosa* against *C. neoformans* was found to be more than 1000 µg/ml.

The growth of *S. aureus* was inhibited by fresh juice of *A. squamosa, C. amboinicus, M. fragrans* and *T. grandis* leaves. *E. coli* was susceptible to fresh juice of *C. amboinicus* leaves. Fresh juice of *C. alata* was found to be effective against *C. albicans. P. aeruginosa* was inhibited by fresh juice of *M. fragrans* leaves.

All the plants under the study were found to possess antimicrobial properties, thereby justifying their popular use in the treatment of infectious diseases caused by resistant microorganisms. Further study is required to assess the in vivo efficacy of these plants for the said action.