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ANTIMICROBIAL ACTIVITIES OF *AVICENNIA MARINA*, *CAESALPINIA PULCHERRIMA* AND *MELASTOMA MALABATHRICUM* AGAINST CLINICAL PATHOGENS ISOLATED FROM UTI.

A SHEELA DEVI [1]*, JOHANNA RAJKUMAR [2], M RAJATHI D MODILAL [1], R.ILAYARAJA[3]

[1]Department of Biotechnology, Karpaga Vinayaga College of Engineering and Technology G.S.T. Road, Chinna Kolambakkam, Palayanoor Post, Madurantagam Taluk, Kanchipuram-603308, Tamilnadu.
[2]Department of Biotechnology, Rajalakshmi Engineering College, Thandalam, Chennai, Tamilnadu.
[3]Department of Microbiology, Sree Renga Hospital, No:12, Varadha reddy street, Chengalpattu, Kancheepuram dist-603001, Tamilnadu.

ABSTRACT

The ethnobotanical efficacy of Indian Medicinal plants leaves of *Avicennia marina, Caesalpinia Pulcherrima* and *Melastoma Malabathricum* was evaluated against Gram positive (*Staphylococcus aureus,*) and Gram-negative (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa)* bacteria. Leaves were extracted using different solvents such as hexane, chloroform, ethyl acetate and methanol by soxhlet extraction method. The antibacterial activity was evaluated using agar well diffusion method. The plant extracts showed antibacterial activity against the four bacterial species taken in the present investigation. Among treatments, maximum invitro inhibition was scored in the extract of the plant *Avicennia marina* followed by *Caesalpinia Pulcherrima* and *Melastoma Malabathricum*. All plant extracts showed significant zone of inhibition for *Staphylococcus aureus* than for other bacterial species taken for the study. Among the four solvents tested the methanol and ethyl acetate extracts were more potent in their antibacterial activity. Preliminary phytochemical screening was also done for the leaves extract of *Avicennia marina, Caesalpinia Pulcherrima* and *Melastoma Malabathricum.* The results obtained in the present study shows that *Avicennia marina*, *Caesalpinia pulcherrima* and *Melastoma Malabathricum* can be used in treating diseases caused by the test organisms.

KEY WORDS : **Medicinal plants, antibacterial activity, phytochemical screening, leaf extracts, *Avicennia marina, Caesalpinia Pulcherrima* and *Melastoma Malabathricum***

A SHEELA DEVI
Department of Biotechnology, Karpaga Vinayaga College of Engineering and Technology G.S.T. Road, Chinna Kolambakkam, Palayanoor Post, Madurantagam Taluk, Kanchipuram-603308. Tamilnadu.

*Corresponding author

INTRODUCTION

Plants produce a diverse range of bioactive molecules, making them rich sources of different types of medicine. Most of the drugs today are obtained from natural sources or semi synthetic derivatives of natural products and used in the traditional systems of medicine. Thus it is a logical approach in drug discovery to screen traditional natural products. Approximately 20% of the plants found in the world have been submitted to pharmaceutical or biological test and a sustainable number of new antibiotics introduced on the market are obtained from natural or semi synthetic resources (Shariff N 2006).

Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world (Saxena and Sharma, 1999). It has been reported that the higher plants have shown to be a potential source for the new antimicrobial agents (Mitscher *et al*., 1987). Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries (Sokmen *et al*., 1999). Studies on antibacterial and antifungal activity of the plants and their prospects for use in different systems require scientific experimentation. In recent years, drug resistance to pathogenic microorganisms has been commonly and widely reported in literature (Mulligen *et al*., 1993), therefore antimicrobials may have a significant clinical value in treatment of resistant microbial strains. Because of the side effects and the resistance that pathogenic microorganisms build against antibiotics, many scientists have recently paid attention to extracts and biologically active compounds isolated from plant species used in herbal medicines (Essawi and Srour, 2000).

A. marina (Forssk.) is commonly known as gray mangrove tree classified in the plant family *Avicenniaceae*, and is commonly used for treatment of ulcers (Subashree *et al*., 2010), rheumatism, small pox and other ailments (Bandaranayake *et al*., 2002). Some studies were done about the antiparasitic, antifungal and antibacterial activity of *Avicennia marina* (Abeysinghe *et al*., 2006; Khafagi *et al*., 2003; Premananthan *et a*l., 1999). They provide a rich source of steroids, triterpenes, saponins, flavonoids, alkaloids and tannins. *Caesalpinia Pulcherrima is* a species of flowering plant in the pea family Fabaceae and is commonly known as peacock flower. In traditional Indian Medicine *Caesalpinia Pulcherrima* is used in the treatment of tridosha, fever, ulcer, abortifacient, emmenagogue, asthma, tumors, vata and skin diseases; it also possesses antibacterial properties (Maheshwara *et al*., 2006; Nasimul Islam *et al*., 2003) and prominent antifungal properties (Nasimul Islam *et al*., 2003). *Melastoma malabathricum* L.(Melastomataceae) is a very well known medicinal plant used by peoples of different community all over the world. The leaves of *Melastoma malabathricum* L. is used traditionally to treat diarrohea, dysentery, leucorrhoea, hemorrhage infection during confinement, toothache, flatulence, sore legs and thrush. The ethanolic extract of Melastoma malabathricum L. was found to have antinoceptive and antibacterial effect (Sulaiman *et al*., 2004). Therefore, in the present investigation the above mentioned three plants were screened against multi-drug resistant bacteria including *Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus* for their antibacterial activity.

MATERIALS AND METHODS

Preparation of Plant Extract

The leaves of *Avicennia marina; Caesalpinia pulcherrima* and *Melastoma malabathricum* were collected from Vadakara taluk, Calicut district, Kerala, India. They were carefully identified and authenticated at Siddha Central Research Institute Arumbakkam, Chennai. Fresh leaves were washed thoroughly 2 – 3 times with running tap water and then with sterile water and subsequently shade-dried, powdered and used for extraction. The

powder obtained was subjected to successive soxhlet extraction with organic solvents i.e. methanol, ethyl acetate, hexane and chloroform respectively.

Bacterial Strains

The human pathogenic bacteria such as one Gram-positive (*Staphylococcus aureus*) and three Gram-negative (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*) were collected from Sree Renga Hospitals Chengalpattu, Tamilnadu, used for this study. The organisms were sub-cultured on Nutrient agar medium incubated at 37°C for 24 h and maintain stock culture with 20% glycerol.

Preparation of aqueous plant extracts

25g of shade dried, powder of plant materials were macerated separately with 50 ml of sterile distilled water using pestle and mortar. The macerate was first filtered through three layers of musin cloth and then the filtrate was centrifuged at 10000 rpm for 15 mins at room temperature. Supernatant was filtered through What man No.1 filter paper and heat sterilised at 120^0C for 30 mins. The extract was preserved aseptically in a bottle at 4^0C until further use.

Preparation of Solvent Extractions

25g of shade dried, powdered of plant material were filled separately in the thimble and extracted successively with 150 ml of each of methanol, ethyl acetate, hexane and chloroform using a Soxhlet extractor for 48 hrs. All the extracts were concentrated using rotary flash evaporator. After complete solvent evaporation, each of these solvent extracts was weighed and preserved at 4^0C in airtight bottles until further use. 1g of each solvent residues dissolved in 10 ml of respective solvents were used as the test extracts for antimicrobial activity assay.

Determination of antibacterial activity

The crude methanol, ethyl acetate, hexane and chloroform extracts of the *Avicennia marina, Caesalpinia Pulcherrima* and *Melastoma Malabathricum* were subjected to antibacterial activity and was confirmed by using the agar well diffusion method as described by (Kirby *et al*., 1966). 5mm-dia. wells were made on pathogen spread MHA agar plates and then 50µl of each solvent were added to the wells. After drying, the plates were kept for 2 hrs in a refrigerator to facilitate diffusion of solvents in agar. The inoculated plates were then incubated for 24 hrs at 37^oC and the diameter of the zone of inhibition in millimeters was measured and recorded and the experiment was repeated in three replicates.

PHYTOCHEMICAL SCREENING

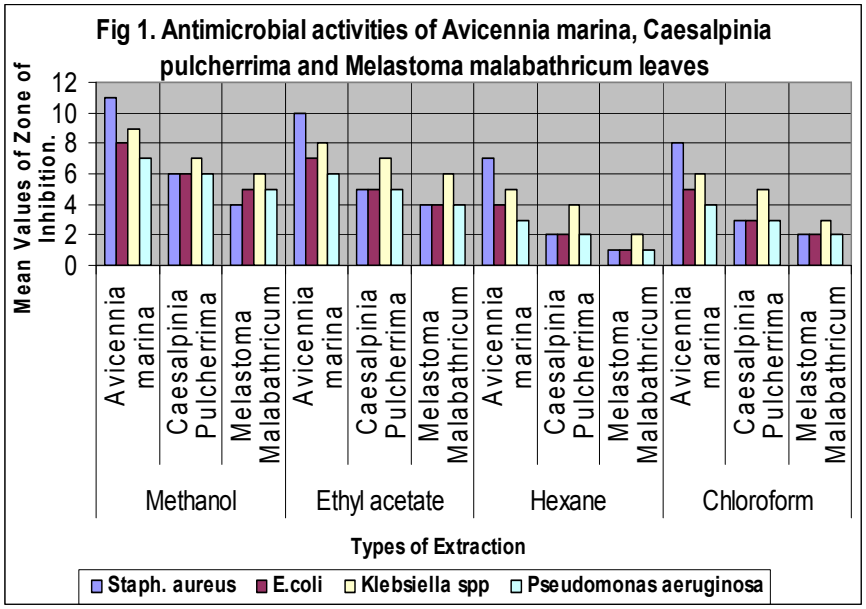
The method of Martinez *et al*., 2003 was implemented to identify the general phytochemical groups of compounds in the crude methanol, ethyl acetate, hexane and chloroform extracts of *Avicennia marina; Caesalpinia pulcherrima* and *Melastoma malabathricum.* Draggendorff's; Mayor's and Wagner's test for alkaloids, Keller-Killiani test for glycosides, Benedict's, Fehling's and Molisch's tests for carbohydrates, test for phytosterols, oils and fats, Liebermann - Burchard test for steroids, shimoda's test for flavanoids, froth test for saponins, ferric chloride test for phenols, gelatine or ferric solution test for tannins, Biuret and ninhydrin test for protein and aminoacids, Salkowski test for terpenoids.

RESULT AND DISCUSSION

India is a home to a variety of traditional medicine systems that relay or spread to a very large extent on native plant species for their raw drug materials (Santhi *et al*., 2006). The plant products over synthetic compounds in the treatment of diseases are needed, because it does not have a deleterious effect in higher plants and animals including man. The urge in research on new drugs from natural sources is now moving out of the herbalists shop, away from the core texts into the drug research laboratories (Chitravadivu, 2009). Therefore, now there is a need to look back towards the traditional medicine which can serve as novel therapeutic agent.

The ethnobotanical efficacy of various solvents of four different extracts (hexane,

chloroform, ethyl acetate and methanol) of *Avicennia marina, Caesalpinia Pulcherrima* and *Melastoma Malabathricum* leaves showed significant zone of inhibition against "Gram-positive" bacteria, *Staphylococcus aureus* and three "Gram-negative" bacterium *Pseudomonas aeruginosa, Klebsiella pneumonias* and *Escherichia coli* (Figure 1).



All plant extracts exhibited more inhibition for *Staphylococcus aureus* than for other bacterial species taken for the study. Our data show that, in general, the plant antibacterial extracts substances appear to be more inhibitory to Gram-positive organism than to the Gram-negative types. Unlike Gram-positive bacteria, the lipopolysaccharide layer along with proteins and phospholipids are the major components in the outer layer of Gram-negative bacteria. So the outer lipopolysaccharide layer may hinder access of antibacterial compounds to the peptidoglycan layer of the cell wall (Abeysinghe, 2011). The highest antibacterial activity for the four bacterial strains was shown by the extract of the plant *Avicennia marina* followed by *Caesalpinia Pulcherrima* and *Melastoma Malabathricum*. Extracts from different mangrove plants are reported to possess diverse medicinal properties. Mangroves and mangrove associates possess novel compounds of medicinal value and biologically active compounds. Extracts from different mangrove plants and mangrove associates are active against human and plant pathogens (Ravikumar *et al*., 2010). *Caesalpinia Pulcherrima* exhibited a broad spectrum of antimicrobial activity, particularly against *Escherichia coli* (enteropathogen), *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Parekh and Chanda, 2007). Several literature reflect the efficiency of plants of Melastomataceae family to have potential antimicrobial ability. Various species of *Miconia* belonging to Melastomataceae family showed significant antimicrobial activity against different Gram (+ve) and Gram (-Ve) bacteria (Choudhury *et al*., 2011).

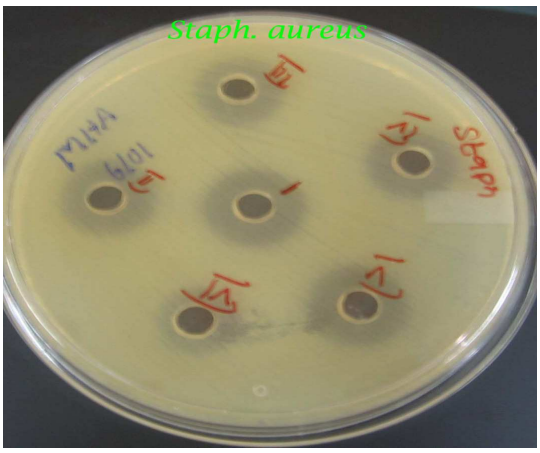


Figure 2
Zone of Inhibition in mm against Staphylococcus aureus

Among the four solvents tested methanol was more potent in its antibacterial activity. In accordance with the present study, in an earlier report methanol was found to be a better solvent for extraction of antimicrobially active substances compared to other solvents (Ahmad *et al*., 1998). Preliminary phytochemical screening of the extracts of *Avicennia marina, Caesalpinia Pulcherrima* and *Melastoma Malabathricum* leaves were carried out (Table 1, 2 and 3) .

Table 1
Phytochemical screening of different solvent extracts of Avicennia marina leaves

S.No	Plant constituents	Petroleum ether extract	Chloroform Extract	Ethyl acetate extract	Methanol extract
1	Test for alkaloids	++	++	++	++
2	Test for glycosides	++	++	-	-
3	Test for Carbohydrates	- +	- +	- +	- +
4	Test for phytosterols	-	-	-	-
5	Test for steroids	-+	-+	-+	-+
6	Test for flavanoids	++	++	++	++
7	Test for saponins	++	++	++	++
8	Test for phenols	++	++	-+	-+
9	Test for tannins	++	-+	-+	-+
10	Test for protein and aminoacids	-+	-+	-+	++
11	Test for terpenoids	++	++	++	++
12	Test for oils and fats	-	-	-	-

Table 2
Phytochemical screening of different solvent extracts of Caesalpinia pulcherrima leaves

S.No	Plant constituents	Petroleum ether extract	Chloroform Extract	Ethyl acetate extract	Methanol extract
1	Test for alkaloids	++	-	- +	++
2	Test for glycosides	++	- +	++	- +
3	Test for Carbohydrates	++	++	++	++
4	Test for phytosterols	++	++	++	++
5	Test for steroids	- +	- +	-	- +
6	Test for flavanoids	++	-	++	-
7	Test for saponins	-	- +	- +	-
8	Test for phenols	- +	- +	- +	-
9	Test for tannins	-	- +	- +	++
10	Test for protein and aminoacids	-	++	-	-
11	Test for terpenoids	++	++	++	-
12	Test for oils and fats	-	-	-	-

Table 3
Phytochemical screening of different solvent extracts of Melastoma malabathricum leaves

S.No	Plant constituents	Petroleum ether extract	Chloroform Extract	Ethyl acetate extract	Methanol extract
1	Test for alkaloids	- +	- +	- +	- +
2	Test for glycosides	++	++	++	++
3	Test for Carbohydrates	++	++	++	++
4	Test for phytosterols	-	-	-	-
5	Test for steroids	++	++	++	++
6	Test for flavanoids	++	++	++	++
7	Test for saponins	++	- +	- +	- +
8	Test for phenols	- +	- +	- +	- +
9	Test for tannins	- +	- +	- +	- +
10	Test for protein and aminoacids	- +	- +	- +	- +
11	Test for terpenoids	++	++	++	++
12	Test for oils and fats	-	-	-	-

The antimicrobial activity of *Avicennia marina may* be due to the presence of alkaloids, flavonoids, saponins, glycosides, phenol, sugars, tannins, protein and amino acids in the plant extracts.The antimicrobial activity of the *Caesalpinia Pulcherrima* may be due to

the presence of phytochemical like terpenoids, flavonoids, tannins, phytosterol, sugars, protein and amino acids in the plant extracts. The antimicrobial activity of the *Melastoma Malabathricum* may be due to the presence of phytochemical like terpenoids, flavonoids, steroids, saponin and alkaloids in the plant extracts. Some of the phytochemical compounds e.g. glycoside, saponin, tannin, flavonoids, terpenoid, alkaloids, have previously been reported to have antimicrobial activity (Okeke *et al*., 2001; Ebi and Ofoefule *et al*., 1997). This possibly means that the compound responsible for the antimicrobial activity is present in three different plants. It is promising that the tested plant species could be used to synthesise novel antibiotics for bacterial infections, especially for antibiotic resistant bacterial infections. Further research is necessary for successful separation, purification and characterization of biologically active compounds using chromatographic methods and spectroscopic techniques.

Future Perspective

Continued further exploration of these plants-derived antimicrobial is needed today. Further research is necessary to determine the identification of the antibacterial compounds from with in these plants and also to determine their full spectrum of efficacy. However the present study of in vitro antibacterial evaluation of some plants forms a primary platform for further pharmacological studies to discover new antibiotic drugs.

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