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Research Article

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Antimicrobial activity of methanolic extracts of *Azadirachta indica*, *Rosmarinus officinalis* and *Lagenaria siceraria* leaves on some important pathogenic organisms

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ABSTRACT

In India, a large number of medicinal plants occur in the wild state. Presences of the phytochemical constituents such as alkaloids, flavonoids, tannin, and phenolics compounds have been reported to be important compounds in many other medicinal plants. These compounds exhibit diverse actions such antibacterial, analgesic, antifungal .antidiabetic and antioxidant etc. The main objective of this work was to study the antibacterial activity of methanolic extracts of Neem (Azadirachta indica), Rosemary (Rosmarinus officinalis) and Bottle guard (Lagenaria siceraria) leaves on some important Pathogenic organisms. The methanolic extracts of leaves of Neem, Rosemary and Bottle guard were evaluated for its antibacterial activity against staphylococcus aureus pseudomonas aeruginosa. , E.coli and Klebsiella using Agar well diffusion technique. The methanolic extracts of leaves of Neem, Rosemary and Bottle guard (MEAIL, MERML and MELSL) showed zones of inhibition on agar plates. The methanol leaf extracts of Neem leaves, Rosemary leaves and Bottle guard fruit showed significant antibacterial activity against Escherichia coli, Pseudomonas, Staphylococcus aureus and Klebsiella. Extensive studies may yield cost effective antibiotics for future .

Key words: Antibacterial, Agar disk, Agar well diffusion, Medicinal plants.

INTRODUCTION

Medicinal plants represent a rich source of antimicrobial agents. A wide variety of natural products are used in the treatment of common infection in traditional medicine in developing countries.[1,2]Herbal medicines are an important part of the culture and traditions of many developing countries and depend on herbal medicines for their health care needs [3]·In India, a large number of medicinal plants available in the wild state. Presences of the phytochemical constituents such as alkaloids, flavonoids, tannin, and phenolic compounds have been reported to be important compounds in many other medicinal plants [4, 5]

Azadirachta indica (Neem) is a tree in the mahogany family, native to India, Burma, Bangladesh, Sri Lanka, Malaysia and Pakistan, growing in tropical and semi-tropical regions. It blossoms in spring with the small white flowers. Several pharmacological activities and medicinal applications of various parts of Neem are well known [6] Biological activity of Neem is reported with the crude extracts and their different fractions from leaf, bark, root, seed and oil [7] Neem oil, the bark and leaf extracts have been therapeutically used as folk medicine to control leprosy, intestinal helminthiasis, respiratory disorders, and constipation and also as a general health promoter[8]Its use for

the treatment of rheumatism, chronic syphilitic sores and indolent ulcer has also been evident. The Neem plant is reported to have antimicrobial action, and is not well studied on leaves [9, 10] so this made us to carry out the present study on Neem leaves. The herb *Rosemary Rosmarinus officinalis* has been used as a food spice and as a medicine since ancient times. Traditional medicinal uses of rosemary leaf preparations taken internally include digestive distress, headaches, and anxiety. The fragrance of rosemary leaf has been said to enhance memory. Rosemary oil was applied to the skin to treat muscle and joint pain and taken internally to promote abortions. Rosemary essential oil, like many essential oils, has antimicrobial properties when it comes in direct contact with bacteria and other microorganisms. [12-16].

Biological evaluation of plant products, based on their use in traditional medicine is the key to ideal development of new drugs from plants. One such plant is *Lagenaria siceraria* (Molina) standley (LS) (Family: Cucurbitaceous). It is a large, softly pubescent, annular, climbing or trailing herb growing throughout the India. Traditionally all parts of the plant are used as folk medicines. The fruits, leaves, oil, and seeds are edible and used by local people as folk medicines in the treatment of jaundice, diabetes, ulcer, piles, colitis, insanity, hypertension, congestive cardiac failure, and skin diseases. The fruit pulp is used as an emetic, sedative, purgative, cooling, diuretic, antibilious, and pectoral. The flowers are an antidote to poison. The stem bark and rind of the fruit are diuretic. The seed is vermifuge. Extracts of the plant have shown antibiotic activity. Leaf juice is widely used for baldness. [17, 18 and 19]. The flavonoids in LS fruits are mainly isovitexin, isoorientin, saponarin, and saponarin 4'-O-glucoside [20]. Leaf extract of *Lagenaria siceraria*) was studied for its antimicrobial action in our study.

EXPERIMENTAL SECTION

Fresh *Azadirachta indica* leaves were collected from a Neem tree within the premises of Mamata Medical College April 2013. Fresh *Lagenaria siceraria* leaves were collected from a vegetable garden near Khammam, Andhra Pradesh. The plant leaves were identified by Botany head of the department Government degree college at Khammam. Packed powder of *Rosmarinus officinalis* leaves was obtained from an authenticated Ayurvedic shop at Khammam.

Extract Preparation

The leaves of *Azadirachta indica* and *Lagenaria siceraria* were washed and air dried in the laboratory for twenty days and fine powder was made. The extraction of *Azadirachta indica*, *Lagenaria siceraria* and *Rosmarinus officinalis* powders were done with 95% ethanol by Soxhelts apparatus in department of Pharmacology. The extracts were dried under vacuum, stored at room temperature and protected from direct sunlight.

Phytochemical Analysis

Preliminary photochemical analysis was made for presence of alkaloid, flavonoids, carbohydrates, glycosides, proteins and amino acids, steroids, vitamin C, fat and fixed oil

Organisms Used

The test organisms included four clinical isolates of staphylococcus aureus pseudomonas aeruginosa, E.coli and Klebsiella and an attempt has been made to test the in vitro antibacterial activity of above leaf extracts against staphylococcus aureus, E.coli, Klebsiella and pseudomonas aeruginosa. Clinical isolates of these organisms isolated during the study period from pus for staphylococcus aureus and from urine for Escherichia coli and Klebsiella and pseudomonas sp. were utilized for this study.

Micro dilution assay

The minimum inhibitory concentration was defined as the lowest concentration of the compound to inhibit the growth of microorganisms [21] .The minimum inhibitory concentration values were determined by broth dilution assay of micro dilution assay. Varying Concentrations of the extracts (500mg/ml, 400mg/ml, 300mg/ml, 200mg/ml, 100mg/ml and 50mg/ml) were prepared. 0.1ml of standardized test organism of Controls was equally set up by using solvents and test organisms without extract.

Antimicrobial Screening

Agar well diffusion technique was used to determine the antibacterial activity. The above reference strains were cultured over night in thioglycolate broth and culture was streaked on a plate of blood agar. Five wells of 5×5 mm measure were made with the help of a template on the surface of the agar plate. *Azadirachta indica* leaf extract

(MEAIL) in 50000 µg in 0.1ml was delivered in to the well using a micropipette. The other two wells were filled with 5 % of Amikacin and 0.9% normal saline as positive and negative controls respectively. They were then incubated at 37°c for 24 hours, and the antibacterial activity was assessed by the diameters of the inhibition zones measured in millimeters.[22] same procedure was repeated with *Rosmarinus officinalis* **(**MERML) and *Rosmarinus officinalis* **(**MELSL) leaf extracts. Amoxicillin/Cloxacillin combination was used for testing staphylococcus aureus sensitivity. All tests were done in triplicate.

RESULTS

In the present study, the ethanol leaf extracts of Neem (MEAIL) showed inhibition zone of 16mm on E.coli, 15mm on Klebsiella, 14mm on pseudomonas aeruginosa and 20mm zone of inhibition on staphylococcus aureus as shown in Table 1.. The antibacterial activity of ethanol leaf extract of Rosemary **(**MERML) showed inhibition zone of 19mm on E.coli, 15mm on Klebsiella, 17mm on pseudomonas aeruginosa and19mm zone of inhibition on staphylococcus aureus as shown in Table 1. The antibacterial activity of ethanol leaf extract of bottle guard **(**MELSL) showed 18 mm of inhibitory zone on E.coli, 17 mm on Klebsiella,17 mm on pseudomonas aeruginosa and 16 mm of inhibitory zone on staphylococcus aureus as shown in Table1. Ethanol leaf extract of Neem exhibited maximum inhibition zone on staphylococcus aureus followed by E.coli, Klebsiella and pseudomonas aeruginosa. Where as Rosy Mary had equal inhibitory zone on staphylococcus aureus and E.coli followed by pseudomonas aeruginosa. Bottle guard showed maximal inhibitory zone on E.coli followed by other organisms

Table 1 Antibacterial activity of some medicinal plant methanol extracts (50mg /ml 1) and antibiotic (20 µg ml 1) against bacterial species tested by Agar well diffusion technique

Plant extract	Negative Control 0.9% Normal saline	Positive Control	*E.coli*	*Klebsiella*	*pseudomonas aeruginosa*	*staphylococcus aureus*
MEAIL	0	27	16	15	14	20
MERML	0	26	19	15	17	19
MELSL	0	27	18	17	17	16

MEAIL- Methanolic extracts of Azadirachta indica, MERML- Methanolic extracts of Rosmarinus officinalis, and MELSL- Methanolic extracts of Lagenaria siceraria leaves, Positive—Control Discs = Amikacin (E. coli, Klebsiella, pseudomonas aeruginosa), Augmentin- (Amoxicillin/Cloxacillin combination) (Staphylococcus aureus)

DISCUSSION

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. Vast research was done and many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants [23, 24, 25, 26, 27, 28, and 29].The active principles responsible for such activities are helpful in the developing of drugs for the therapeutic use in human beings. Synthetic drugs may cause various side effects. Hence, drugs developed from plant sources can be used in developing newer drugs with minimal side effects [30] and Plant based products have been effectively proven for their utilization as source for antimicrobial compounds. *Azadirachta indica* leaves possessed good anti bacterial activity confirms the presence of bioactive compounds and is useful for rationalizing the use of this plant in primary health care [31].The extracts of Neem when used as medicinal plant, could be useful for the growth inhibition of the carcinogenic bacterium, *S. sobrinus*. [32] The phytoconstituents alkaloids, glycosides, flavonoids and saponins are antibiotic principles of plants. These antibiotic principles are actually the defensive mechanism of the plants against different pathogens[33].The result was also supported by one of the studies.[34].In our study *Azadirachta indica* leaf extract showed inhibitory effect on all tested pathogens namely staphylococcus aureus followed by E.coli , Klebsiella and pseudomonas aeruginosa and supports above statement. Rosemary plants are rich sources of phenolic compounds with high antimicrobial activity against both Gram-positive and Gram-negative bacteria. Some studies [35, 36, and 37] reported antimicrobial activity of this plant. High percent of the antimicrobial activity they attributed to carnosic acid and carnosol. It is clear that rosemary extracts have bioactive properties, but their antimicrobial activities have not been deeply characterized. Antimicrobial activities of plant essential oils have been known for centuries, but their strong flavor limited their use in food [38].There is also some evidence that minor components have a critical part in antibacterial activity, possibly by producing a synergistic effect between other components [39]. Antimicrobial activity of this plant leaf is confirmed in our study supports above statements. Extracts of the plant *Lagenaria siceraria* have shown antibiotic activity. Leaf juice is widely used for baldness. [40, 41, 42] *Lagenaria siceraria* (Molina) standley (LS) (Family: Cucurbitaceae).Chemically, LS contains various biologically active phytoconstituents including flavonoids, saponins, triterpenes, and volatile principles. The

flavonoids in LS fruits are mainly isovitexin, isoorientin, saponarin, and saponarin 4'-O-glucoside [20]**.** In Ethiopian traditional medicine, LS is widely used for treatment of skin disorders. Goji evaluated antimicrobial activity of methanolic extracts of the leaves, seeds, and fruit-flesh of L. siceraria (Cucurbitaceae) using the agar-well diffusion method. Results revealed LS extract to show activity against Pseudomonas aeruginosa and Streptococcus pyogenes, but not against clinical isolates of S. aureus and Escherichia coli. Thus LS can be used to treat various skin disorders. [43].In our study also LS showed inhibitory zone not only on Pseudomonas aeruginosa but also on clinical isolates of S. aureus and others. Our study observed significant inhibitory zones on all four microorganisms. The absence of inhibition zone does not necessarily mean that compounds are inactive. For example, non-polar compounds may not diffuse into the culture medium [37].

CONCLUSION

Many plants have been used to screen the antimicrobial property because of their antimicrobial traits, which are due to presence of alkaloids [44, 45]. It was concluded that rosemary extract has antibacterial activity against E.coli, pseudomonas sps and Staphylococcus aureus. The most susceptible bacteria with *Azadirachta indica* was Staphylococcus aureus, E.coli followed by Klebsiella. *Rosmarinus officinalis* had equal inhibitory zone on staphylococcus aureus and E.coli followed by pseudomonas aeruginosa and. *Lagenaria siceraria has* shown maximal inhibitory zone on E.coli followed by other organisms. Extensive research should be carried out on Phytochemicals of these plants for the development of cost effective drugs for future.

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