DETERMINATION OF ANTIMICROBIAL ACTIVITY OF *Melia azedarach* L. AGAINST CLINICAL MICROBES

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ABSTRACT

Melia azedarach L., a deciduous tree of moderate size, grown throughout warm countries possesses various uses in our life. The extract of *leaves* in different solvent systems (aqueous and methanol) when applied to mosquito repellent. *Melia azedarach* L., a close relative of neem from the family Meliaceae which occurs in India and other tropical and subtropical countries. It has been reported to possess antimicrobial, insecticidal and nematicidal properties. The study of medicinal properties of *Melia azedarach* is used as home medicine in India and it may contains more bioactive compounds to treat various diseases such as Diabetes, Skin diseases, etc., The effect of different concentration of neem leaf extract against four bacterial and four fungal pathogens (bacterial pathogens: *E.coli, K.pneumoniae, Entrococcus* sp, and *S. aureus.* fungal pathogens: *Aspergillus niger, A.terreus, Penicillium* sp, and *Fusarium* sp). The aqueous and methanol extract of *Melia azedarach* exhibited broad spectral activity against both bacterial and fungal pathogens. The antibacterial properties of *Melia azedarach* leaf with different concentration of 25, 50, 75 and 100µl and two solvents like aqueous and methanol was individually detected. The maximum antibacterial activity of *M. azedarach* with 100µl leaf extract concentration was excellent performance then the lower concentration whereas the minimum activity at 25µl concentration was recorded with respective plant leaf extract. The effect of antifungal activity was tested. The higher concentration 100µl of *Melia azedarach* leaf extract showed extraordinary inhibition recorded against clinical fungi. Whereas minimum concentration of 25µl has moderate activity was observed. Therefore the crude extracts of Mountain neem exhibited antibacterial and antifungal activities. The active phytocompounds isolation and identification is necessary to find out the pharmacological important compounds to treat various diseases.

KEYWORDS: *Melia azedarach*, microbes, antimicrobial activity.

INTRODUCTION

Ayurveda- an ancient medical science is even more recognized for its worth and efficacy. Indian cultured heritage is also amply enriched with the presence of Unani and Siddha systems of medicine. In the present time when allopathy has become so advance, the world has been attracted towards traditional Indian systems of medicine. India has several traditional medical systems such as Ayurveda and Unani which has survived through more than 3000 years mainly using medicinal plant-based drugs. Medicinal plants are containing inherent active ingredients used to cure disease or relieve pain (Okigbo *et al*., 2008). Micro-organisms such as virus, bacteria and fungi cause disease and lead to decrease the quality of human life. Identification of effective and natural drug can help in protecting from diseases. Medicinal plants are the natural resources which are used to treat several diseases caused by bacteria, fungi and viruses because medicinal plants have secondary metabolites such as alkaloids, carbohydrates, flavonoids, glycosides, sterols, saponins, terpenoids, coumarins, quinones and tannins (Cowan, 1999). Usage of natural drugs are more efficient than chemical drugs and no side effects. There is an increasing need to search for new compounds with antibacterial and antifungal activity to microbial infections.

MATERIALS AND METHODS

Collection of plant materials

Healthy plants of *Melia azedarach* collected from Eswari nagar, Thanjavur, Tamilnadu, India. The leaf materials were cleaned and free from dirt particles and shade dried.

Preparation of plant extracts (Harbone, 1957)

Soxhlet method used for extraction of crude compounds. Twenty gram of powder leaves blended with 50 ml of different solvents separately (aqueous and methanol) for different periods (48 h) with agitation at room temperature. After the plant extracts were allowed to filtration by using a 0.45 Millipore filter paper. Then, the plant extracts concentrated using a rotary evaporator at 40°C under reduced pressure. Finally, the extracts were allowed to weigh and store at -20°C till their usage in the different tests.

ANTIMICROBIAL ACTIVITY (Nya-Agha *et al*., 1987)

Agar well – diffusion method

Agar well – diffusion method was followed for determination of antimicrobial activity. Nutrient agar (NA) and Potato Dextrose Agar (PDA) plates were swabbed (sterile cotton swabs) with 24 hours culture and 48 hours old broth culture of respective bacteria and fungi were used. Agar wells (5mm diameter) were made in each of these plates using sterile cork borer. The microbes of bacteria like *E.coli, K.pnemoniae, Entrococcus* sp and *S.aureus* and fungi like *Aspergillus niger, A.terreus, Penicillum* sp and *Fusarium* sp were selected antimicrobial activity by the *Melia azedarach* leaf extract. About 25, 50, 75 and 100µl of different solvents of plant leaf extracts of *Melia azedarach* added using sterilized dropping pipettes into the wells and

plates were left for 1 hour to allow a period of pre-incubation diffusion in order to minimize the effects of variation in time between the applications of different solutions in the plates were incubated in an upright position at 37± 2°C for 24 hrs for bacteria and 28± 2°C for 48 hrs for fungi. The organic solvents (aqueous and methanol) were acted as a negative control. Results were recorded, as the presence or absence of inhibition zone. The inhibitory zone around the well indicated the absence of tested organism and it was reported as positive and absence of zone is negative. The diameters of the zones were measured using diameter measurement scale. Triplicates were maintained and the average values were recorded for antimicrobial activity.

Table 1: Antibacterial activity of *Melia azedarach* in different extract against bacteria

	Zone of inhibition (mm)							
	Aqueous extract				Methanol extract			
Name of the bacteria	25µl	50µl	75µl	100µl	25µl	50µl	75µl	100µl
E.coli	12.4±0.22	23.3±0.76	26.0±0.66	27.3±9.11	16.6±5.53	22.6±7.53	18.3±6.12	24.6±8.22
K.pneumoniae	21.0±0.23	24.6±0.22	24.0±0.21	30.2±1.02	11.6±3.86	14.3±4.76	20.6±6.86	22.6±7.53
Entrococcus sp	13.3±0.43	24.0±0.21	23.6±0.86	24.6±8.22	10.6±3.53	17.2±5.66	20.6±6.86	22.6±7.53
S.aureus	21.6±0.20	19.0±0.33	23.2±0.66	23.3±7.76	11.6±3.86	16.0±5.23	27.0±8.23	27.6±7.20

Standard division ± error

Table 2: Antifungal activity of *Melia azedarach* in different extract against fungi

	Zone of inhibition (mm)							
	Aqueous extract				Methanol extract			
Name of the fungi	25µl	50µl	75µl	100µl	25µl	50µl	75µl	100µl
A.niger	5.55±1.83	10.3±3.43	11.6±3.86	13.0±4.33	21.3±7.11	14.6±4.86	23.6±7.86	24.2±8.02
A.terreus	6.33±2.00	7.60±2.53	9.62±2.53	12.0±4.11	16.6±5.53	20.3±6.76	17.6±5.86	22.3±1.15
Penicillium sp	5.54±1.66	7.22±2.33	8.66±2.86	9.22±3.22	13.3±4.43	13.3±4.43	22.0±7.33	18.6±6.20
Fusarium sp	3.56±1.16	4.50±1.52	7.66±2.53	8.00±2.66	20.0±6.66	21.6±7.21	16.6±5.53	19.2±6.33

Standard division ± error

RESULT AND DISCUSSION

Antimicrobial activity of herbal preparation is not an absolute characteristic and should be seen in comparative terms specifically its toxicity to the target pathogen and in target host. In recent past several contrasting reports on effectiveness of herbal antimicrobials are quite confusing; one group claims that microbes may not easily develop resistance to antimicrobial activity of herbal drugs while other group claims herbal drug resistance as a common phenomenon (Vadhana *et al*, 2016). The revealed *invitro* antimicrobial activity against all tested bacteria. Most susceptible organisms were *Staphylococcus aureus* and *Pseudomonas aeruginosa* which is of interest in the treatment of infections caused by these organisms. The effect of extracts of all plants for antibacterial and antifungal activities against selected microbes *invitro* was undertaken. It was clear that, most of the plants possessed antimicrobial activity with few exceptions. However, there was a slight variation in the activity of the plant extracts. It was clear from this screening that *Emblica officinalis*, *Curcuma longa*, *Cyperus rotundus* and *Melia azedarach* extracts exhibited maximum antimicrobial activity against selected test bacterium and fungi. (Saheb and Shinde, 2018). Screening work, no extracts of *Pedalium murex* were found to be inactive against any organism, such as Gram positive, Gram negative and fungal strains were resistant to all the extracts of *Pedalium murex* From the above results the activities of ethanolic extract of *Pedalium murex* shows significant antibacterial and antifungal activity. The present study when we compare zone of inhibition of various extracts treated strain maximum zone of inhibition was observed for ethanolic extract treated strain for antibacterial and antifungal strains. The results claimed uses of leaves in the traditional system of medicine to treat various infectious disease caused by the microbes. Therefore, it may be concluded from the above results, that the crude extracts obtained from the leaves of *Pedalium murex* used enough as drug to treat disease caused by those bacteria, which are sensitive to the above mentioned samples. But before use in human being isolation of pure toxicological study, and clinical trial in animal model should observed may be compound, be carried out thereafter. However, further studies are needed to better evaluate the potential effectiveness of the crude extracts as the antimicrobial agents (Sule *et al*., 2017).

In the present investigation recognized that the medicinal plant *Melia azedarach* with different concentration of 25, 50, 75 and 100µl treated for antibacterial activity two solvents like aqueous and methanol against *E.coli, K.pnemoniae, Entrococcus* sp and *S.aureus* were performed. The maximum antibacterial properties of *Melia azedarach* leaf aqueous extracts was 27.3±9.11, 30.2±1.02, 24.6±8.22 and 23.3±7.76mm zone of inhibition exhibited with *E.coli, K.pnemoniae, Entrococcus* sp and *S.aureus* recorded at 100µl concentration of respective plant leaf extract whereas minimum in 25µl concentration against *E.coli, K.pnemonia, Entrococcus* sp and *S.aureus* was 12.4±0.22, 21.0±0.23, 13.3±0.43 and 21.6±0.20mm zone of inhibition were observed respectively.

The effect of antibacterial activities of *Melia azedarach* with different concentration of 25, 50, 75 and 100µl treated against *E.coli, K.pnemonia, Entrococcus* sp and *S.aureus*. It was 24.6±8.22, 22.6±7.53, 22.6±7.53 and 27.6±7.20mm zone of inhibition recorded at 100µl plant leaf extract has maximum antibacterial effect when compared with low concentration whereas minimum effect of 25µl concentration was 16.6±5.53, 11.6±3.86, 10.6±3.53 and 11.6±3.86 mm zone of inhibition observed respectively. The plant phytochemicals which responsible for antibacterial properties because the presence of potential source of compounds. The efficacy of antifungal properties of *Melia azedarach* plant leaf extract of various concentrations of 25, 50, 75 and 100µl treated. The fungi like *Aspergillus niger, A.terreus, Penicillium* sp and *Trichoderma viride* was 13.0±4.33, 12.0±4.11, 9.22±3.22 and 8.00±2.66 mm zone of inhibition at maximum level in the higher concentration of 100µl excellent biological assay whereas minimum at low concentration of 25µl was 5.55±1.83, 6.33±2.00, 5.54±1.66, 3.56±1.16 mm zone of inhibition recorded respectively. In the methanolic solvent extract of *Melia azedarach* leaf extract of 100µl concentration was 24.2±8.02, 22.3±1.15, 18.6±6.20 and 19.2±6.33mm inhibition of zone observed against the fungi whereas minimum was 21.3±7.11, 16.6±5.53, 13.3±4.43 and 17.0±6.66 mm inhibition represented with respective bacteria. The some of the phytochemicals compounds has maximum antifungal properties against fungi proved by this investigation. It was concluded that the uses of traditional medicine hold a great promise an easily available source as effective medicinal plants with the wide range of ailments for human life. The scientific evaluation of ethno medicinally important plants with various aspects in pharmacognostic properties of medicinal plants.

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