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Studies on Antimicrobial Activities ofMedicinal Plant Mesua Ferrea

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Abstract –

The plant is used in inflammation and septic conditions. The plant is used for its antiseptic, purgative, blood purifier, worm control, tonic properties. it is used to treat fever, cold, asthma and as carminative, expectorant, cardiotonic, diuretic and antipyretic agent. An attempt was made to analyse antibacterial activity of medicinally important plant Mesua Ferrea against human bacterial pathogens. Antibacterial study was carried out by disc diffusion method against the pathogens by using the methanol and aqueous extracts. The antifungal activity of medicinal plant Mesua Ferrea was tested against plant pathogenic fungi by agar well – diffusion method. The plant leaves was extracted with various solvents like chloroform, ethanol and aqueous. The aim of the study was to evaluate the antimicrobial activity of extracts of plant species used in traditional herbal medicine. The results obtained in the present study suggest that they can be used in treating diseases caused by the test organism. Therefore the Plant Mesua Ferrea can be considered as a source of antimicrobial agents.

Key words –

Human pathogens, medicinal plant, disc diffusion method, antibacterial, antifungal activity, agar well –diffusion method.

Introduction

Traditional medicines for human diseases have been widely used in many parts of the world. Herbal plants are usually the primary source of medicine in many developing countries. Natural product compounds from plants provide biologically active compounds. The number of multi-

drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. In addition, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulterations

And side effects. Therefore, there is need to search new infection-fighting strategies to control microbial infections. The aim of this study was to evaluate the antimicrobial activity of some medicinal plant used in Ayurveda and traditional medicinal system for treatment of manifestations caused by microorganisms. Therefore, extracts of the Mesua Ferrea plant was tested for their potential activity against microbial pathogens.

Mesua ferrea Linn. belonging to the family Clusiaceae (Guttiferea) is known in Hindi as 'Nagkeshar' and in English as Ceylon Ironwood. It is a medium to large evergreen tree with short trunk, often buttressed at the base, found in the Himalayas from Nepal eastward, in north-eastern India it occurs in dry hilly forests of Rajasthan, Madhya Pradesh, Gujarat, Bihar, Assam, Orissa, Deccan Peninsula and the Andaman Islands, ascending to an altitude of 1.500m. The different parts of the plant contain glycosides, coumarins, flavanoids, xanthones, triglycerides and resins. Specifically it contains α -copaene and germacrene D , ve β-amyrin, β-sitosterol, and a new cyclohexadione compound named as mesuaferrol (I), mesuanic acid(13), triterpenoids and resins, reducing sugars, and tannins , saponins, Mesuaferrone B, mesuol etc. The plant has shown various pharmacological activities including antibacterial, immunomodulatory etc. Antibacterial activity of plant Mesua Ferrea was carried out by disc diffusion method. And Antifungal activity of plant Mesua Ferrea was carried out by agar well diffusion method. The aim of this present study was to evaluate the activity of different extracts against fungus. Phytochemical investigations find active constituents which help in drug development. The aim of this study was to evaluate the antimicrobial activity of some medicinal plant used in Ayurveda and traditional medicinal system for treatment of manifestations caused by microorganisms. The solvent like methanol was used to extract the bioactive components from plant and screened for their antibacterial activity. Therefore, extracts of the Mesua Ferrea plant was tested for their potential activity against microbial pathogens. In disease of bacterial origin, the plants function as a result of antibacterial activity against the causative agents. This is in pursuance of the attempts to search for drugs from plants and verification of the scientist basis of some known practices in conventional medicines.

The raising high members of bacteria, that are expanding resistance to traditional antibiotics drive, much of the current interest on plant antimicrobial molecules in hope that may give useful leads into anti-infective drugs. This is due to its toxicities in human beings. Mesua Ferrea phytocomplex has not yet clearly defined and therefore this study will improve this knowledge. In literature, there is no survey that reports the antimicrobial activity of Mesua Ferrea. As a result,

the main objective of this study is the evaluation of the antimicrobial activities of the extracts will carried out.

Plant Material

Mesua Ferrea is selected for antimicrobial activities. This plant is collected from Pachmarhi district Hoshangabad Madhya Pradesh. It is authenticated by Dr. J. Anuradha (Department of Botany), NIET, NIMS University, Jaipur, Rajasthan.

Solvent Extraction

The leaves of plant was dried and made 250g of powdered material. Powder was mix with 600ml methanol as a solvent and heated in Soxhlet apparatus near about 10 hrs. The extract was concentrated under vacuum and dried in desiccators. The powdered material mixed with 500ml of distilled water and kept near about 10 days

at room temperature. The extract obtained from water and filtered. Residue evaporated at 15°C.

Materials used

The total eight microorganisms were used for antibacterial properties. The antimicrobial study was done in the department of microbiology and the microorganisms obtained from microbial collection centre, in Nims Institute of Allied Medical Science & Technology, NIET, NIMS University, Jaipur, Rajasthan.

Preparation of standard solution and nutrient broth

Standard drug Streptomycin Sulphate was used. The concentration was 10mg/ml. 4gm Nutrient broth component were dissolved in 100ml water and pH was adjusted to 7.3. This solution was sterilized by autoclaving at 10lbs/110°C for 15min.

Preparation of nutrient agar (medium)

- Nutrient agar 2 gm
- Distilled water 100ml

The above nutrient agar was dissolved in 100ml water and pH was adjusted to 7.5. It was sterilized by autoclaving at 10lbs/110°C for 15min.

Preparation of Inoculums

The microorganisms were pre-cultured in nutrient broth overnight in a rotary shaker at 40° C; centrifuged at 10000 rpm for 5min. pellet was suspended in double distilled water.

Preparation of antimicrobial disc

For the present study commercially available sterile discs were used. Sometimes antimicrobial discs were also prepared in the laboratory. The sterile discs were refrigerated at the temperature instructed by the manufacturer. About one hour before use, the working stock of discs was allowed to warm at room temperature.

1. Discs of 5-6 mm in diameter were punched out from whatman no. 1 filter paper.
2. Then, they were placed in petridishes allowing a distance of 2-4 mm. between each disc.Petridishes were then sterilized in a hot air over at 160°c for 1 hour.
3. After allowing the discs to cool, 0.02ml of the plant extract was pipette out to each disc.
4. The disc were dried at 35-37° C in incubator for 1 hour and then used for antimicrobial susceptibility testing.

Disc diffusion method

1. Nutrient agar plate was labeled with the name of the organisms.
2. Turbidity of nutrient broth was matched with a defined standard.
3. The surface on the nutrient agar plate was inoculated with the help of sterile cotton swab after expressing excess culture from the swab by pressing or rotating the tube above the culture level. The surface of nutrient agar plate was covered evenly by swabbing in three directions. A final sweep was made on the agar rim with the swab.

4. Three to five minutes were allowed for the agar surface to dry.

5. Each extract soaked disc is picked up by sterilized forceps and was put on nutrient agar plate at least 15mm. from the edge of the plate.

6. Each nutrient agar plate was inverted and incubated at 40° C for 30 hours.

7. After incubation period the antimicrobial activity of the plant extract was measured in terms of inhibition zones appearing around the disc.

Antibacterial Activity

The antibacterial test was performed by disc diffusion method. Different concentration of extracts (50ml) was prepared with methanol. The test microorganisms spread on the plate. Cultures bacteria grow in nutrient. It is solidify and filter paper disc of 6mm in diameter impregnated with extract were test on microorganisms. 10 mg/ml streptomycin sulphate used as positive control and methanol solvent (100mg/ml) used as negative control. Antibacterial assay plates were incubated at 40° C near about 30hrs and diameter of inhibition zones were measured in millimeters.

Table- 1

Antibacterial activity of methanol and aqueous extracts (100mg/ml) of plant by discdiffusion assay

an Pathogenic Bacteria	Zone of inhibition (in mm)		Streptomycin Sulphate (10mg/ml)
	Methanol	Aqueous	
K. Pneumoniae	15	11	22
S. auerus	12	09	23
P. vulgaris	21	6	20
E. coli	10	8	17
Lactobacillus	13	11	16
P. aeruginosa	14	12	18
S. typhi	11	7	19
B. subtilis	16	14	24

Antifungal activity of plant Mesua Ferrea was carried out by agar well diffusion method. The aim of this present study was to evaluate the activity of different extracts against fungus.

Sterilization of plant material

The disease free and fresh plant was taken. About 3g of fresh and healthy leaves were taken for each solvent extraction. They were washed with distilled water for three times. Then surface sterilized with 1% mercuric chloride for 25 sec. Again, the leaves were washed thoroughly with distilled water. (Three times)

Preparation of plant extracts

Five grams of sterilized plant leaves were kept in the 15ml organic solvents such as methanol, ethanol and aqueous. Then they were ground well with the help of mortar and pestle. The plant materials were subjected to centrifugation, for 15-20 min (at 10000 rpm) again it was filtered through whatman No.1 Filter paper. The supernatant was collected and made to known volume by adding sterile aqueous, methanol and ethanol stored for further antimicrobial screening purpose.

Activation of Fungi

The plant extracts were assayed for antifungal activity against the fungal strain C.falcatum**,** Aspergillus flavus, Aspergillus niger, Candida albicans**.** The antifungal study was done in the department of microbiology and the microorganisms obtained from microbial collection centre, in Nims Institute of Allied Medical Science & Technology, NIET, NIMS University, Jaipur, Rajasthan. These fungi was grown on PDA plate at 28°C and maintained with periodic sub – culturing at 4°C.

Preparation of potato dextrose agar medium (PDA agar medium)

The potato tubers were peeled off and weighed for about 200g tubers were chopped in to small pieces in to the sterile conical flask. After boiling the supernatant were collected and dextrose (12g) with agar (15g) to dissolve the ingredients. The medium was mentioned and adjusted to 6.6pH. Finally the medium was sterilized in pressure cooker for 15min.

Preparation of inoculums

Two days prior to these testing, inoculations of the above fungal cultures were made in the PDA medium and then incubated at 28° C to produce fungal spore. This spore can produced in liquid medium.

Antifungal Activity

Antifungal activity was screened by agar well diffusion method. The methanol, ethanol and aqueous extracts of plant were tested against plant pathogens C.falcatum, Aspergillus flavus, Aspergillus niger, Candida albicans. The PDA medium was poured in to the sterile petriplates and allowed to solidify. The test fungal culture was evenly spread over the media by sterile cotton swabs. Then wells (6 mm) were made in the medium using sterile cork borer, (7 mm diameter holes cut in the agar gel, 20 mm apart from one another). 200µl of each extracts were transferred in to the separate wells. The plates were incubated at 27°C for 48-72 hrs. After the incubation the plates were observed for formation of clear incubation zone around the well indicated the presence of antifungal activity. The zone of inhibition was calculated. Antifungal activity of medicinal plant extract was assayed by agar well diffusion method.

Table-2

Inhibition Spectrum of the Medicinal Plant Mesua Ferrea		**Zone of inhibition (mm)**	
Fungi	**Aqueous**	**Ethanol**	**Chloroform**
Colletotrichum falcatum	-	25	18
Aspergillus flavus	-	16	22
Aspergillus niger	-	24	19
Candida albicans	-	23	17

Result and discussion

Antibacterial activities of the aqueous and methonolic extracts of leaves of plant Mesua Ferrea was investigated against Gram positive and Gram negative bacteria by disc diffusion method. To find out the growth of microorganism, we had used inhabitants like antibiotics Streptomycin

Sulphate and methanolic and aqueous extracts of the medicinal plant Mesua Ferrea, their comparative results for antibacterial activity was discussed as per available in recorded data.

Results obtained in the present study revealed that tested medicinal plant possess antibacterial activity against K. Pneumoniae, S. Auerus, P. Vulgaris, E. coli, Lactobacillus, P. aeruginosa, S. typhi, and B. subtilis. When tested by the disc diffusion method, the methanol leaf extracts of the plant showed significant activity against all the tested microorganisms when compared to aqueous extracts. It shows the presence of broad spectrum antimicrobial compounds. The highest antibacterial activity recorded in P. Vulgaris (21mm), B. subtilis (16mm) followed by K. Pneumoniae (15mm) P. aeruginosa (14mm), Lactobacillus (13mm), S. Auerus (12mm), and S. typhi (11mm), E. coli (10mm) compared to aqueous extract.

The present study shows that the methanolic and aqueous extract of leaves of Mesua Ferrea have the all phytochemical constituent studied with negligible variation. In recent times there has been great attention in the use of plant material as another method to control pathogenic microorganism and many components of plant products have been shown to be specially targeted against resistant pathogenic bacteria. The emergence of multidrug resistant strain of many pathogens is a serious threat and makes chemotherapy more difficult. Moreover, the current cost of most of the chemotherapeutic agents is unbearable to the public especially in developing countries like India. Thus, attempts must be directed towards the development of effective natural, nontoxic drug for treatment.

Antifungal activity of plant Mesua Ferrea extracts was assayed by agar well diffusion method. The result revealed that the extract of medicinal plant showed significant reduction in growth of fungi. The ethanol extract of the plant (24mm) showed maximum antifungal activity compared with chloroform extract (19mm) against Colletotrichum falcatum. The ethanol and chloroform extracts of plant showed moderate activity against Aspergillus niger (23 and 18mm) and Candida albicans (22 and 16mm). The chloroform and ethanol extracts of plant exhibited least activity against Aspergillus flavus (21 and 15mm). The result of antifungal effect of aqueous extract of theplant showed no activity against these fungi.

The plant Mesua Ferrea was used for antifungal activity against these fungi. The plant material was extracted with water ethanol and chloroform. The ethanol extract of plant is more efficient as compared to the chloroform.

To develop environmental friendly alternatives to synthetic fungicides for control of fungal plant diseases the interest on plant extracts has been increased. This study provides new scientific information on fungal activity of plant against these fungi. The extracts should be tested against the diseases under the diseases field conditions. Our climate is rich support for availability of variety of herbs which have medicinal value and can be used to obtain active drugs having their significant role in treatment of various diseases and disorders. In this study the Mesua Ferrea plant was selected for investigation of bioactive compounds.

Conclusion

The present work was a pioneer to explore the antimicrobial property of Mesua Ferrea. Thus, the significant activity against P. vulgaris and B. subtilis may be due to their Phytochemical or secondary metabolites. The present studies support the use of plant against several diseases. Various phytocompounds are present in plant as secondary metabolites for conferment of antibacterial activities. Antimicrobial activity of this plant was also studied to check antibacterial activity against gram positive and gram negative bacteria. The results

obtained confirm the zoneof inhibition against tested bacteria.

It is concluded that this study would lead to the establishment of some valuable compound that has to be used to formulate new, different and more potent antimicrobial drugs of natural origin. They have advantage that while using own human body they have minimum side effects or sometimes no side effect irrespective of synthetic english drugs.

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