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ORIGINAL ARTICLE

Systematic Screening of Antibacterial and Antifungal Activity of Different Leaves Extracts of Pterocarpus Marsupium

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ABSTRACT
The indigenous herbal medicinal plants are always been the great resource for many bioactive constituents, it contains the capacity to explore antimicrobial, anti-fungal, antihistamine, and antibacterial agents, etc to fight against numerous infectious diseases. These herbal medicinal drugs are safe, eco-friendly, possess minimum side effects, and are available at a low cost in comparison to the chemically formulated drugs, which are harmful with high toxicity . So, this investigation was focused on analysing the antimicrobial activity of a leaf extract of medicinal plants Pterocarpus Marsupium Roxb belongs to the family Fabaceae (commonly known as Malabar Kino, Indian Kino). The antifungal and antibacterial activity against various pathogens was calculated by measuring the minimum inhibitory count (MIC) and zone of inhibition.Minimum bactericidal and fungicidal count ((MBC,MFC) values were also calculated. Methanol extract showed the maximum zone of inhibition and MIC value. Ethanol and water showed a minimum zone of inhibition and a minimum inhibitory concentration (MIC) value. Therefore, various solvent extracts of the leaves of Pterocarpus Marsupium Roxb. have been found to possess antimicrobial activity and could be very effective agents for the isolation of better drugs to cure several antimicrobial diseases. The methanol extracts can be explored in future to develop and discover new drugs with extreme potential to cure the infectious diseases.
Keywords: *Bacterial strain, fungal strains, Pterocarpus Marsupium, MIC, MBC, MFC, and Zone of inhibition.*

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INTRODUCTION

Traditional medicinal plants play a very vital and important role in the development of new drugs and in their discoveries.[3] The best booster and gift given by nature are natural resources that include natural herbal medicinal plants. The indigenous herbal medicinal plants have always been a great resource for many bioactive constituents that can be explored as antimicrobial, antifungal, antihistamine, antibacterial, etc. agents to fight numerous infectious diseases. These herbal, natural plants play a great role in curing diseases as they contain bioactive substances of great therapeutic value. These herbal medicinal drugs are safe, ecofriendly, have minimal side effects, and are available at a low cost in comparison to the chemically formulated drugs, which are harmful with high toxicity and lots of side effects. The use of natural medicinal drugs increased tremendously worldwide as these drugs had a high potential to cure infectious diseases. These drugs can be explored as the best alternative source of drug to fight infectious disease in place of chemical-based drugs. The environment is the reservoir of natural plants; many of them are studied through Ayurveda and show lots of therapeutic values, but still, a lot of herbal plants are not explored for their phytochemical and pharmacological properties. These unknown plants are the source of new developments and discoveries of better drugs for more effective treatments against several diseases. Natural plant resources proved to be the life support system of human beings as the large amount of the world's population depends upon these resources for their survival in the form of food, medicine, etc. An antimicrobial activity from leaf extracts of medicinal plants Pterocarpus marsupium Roxb belongs to the family Fabaceae (commonly known as Malabar Kino, Indian Kino, also known as

Honne in Kannad, Venga or Venna in Malayalam, and Vengai in Tamil). This medicinal plant is a deciduous tree that grows about 31mm in height. The bark part of the plant can be used as an anti-diabetic drug. The medicinal plant is found in the region of the western ghats in Karnataka, Kerala, Nepal, and Sri Lanka. The antimicrobial activity against various pathogens was calculated by measuring the minimum inhibitory count (MIC) and zone of inhibition.[3] Minimum bactericidal count (MBC) and minimum fungicidal count (MFC) values were also determined. Every extract showed activity against various microorganisms, but the petroleum ether extract showed the maximum zone of inhibition and MIC value. Ethanol and water showed a minimum zone of inhibition and a minimum inhibitory concentration (MIC) value. Therefore, various solvent extracts of the leaves of ***Pterocarpus marsupium*** have been found to possess antimicrobial activity and could be very effective agents for the isolation of better drugs to cure several antimicrobial diseases. The petroleum ether sample can be used to discover new drugs of very high potency.

MATERIAL AND METHOD

1. **Preparation of plant extraction**: - ***Pterocarpus marsupium***, leaves were collected , washed, shade-dried, and powdered at room-temperature . Powered sample was blended with different organic solvents (like ethanol, methanol, petroleum ether, and water) with respect to their increasing polarity continuously for 12 hours in the soxhlet apparatus. [4] After the extraction, the excess liquid that is left overnight is removed by the distillation process. The solid, compact substance obtained was dried in an incubator overnight at 400°C. After drying in vacuum inside desiccators, the distillate product was dissolved in 50% DMSO (dimethyl sulfoxide), which was further preserved in cool storage at 40°C in sterile glass tubes.

2. Test Microorganism : The different bacterial and fungal Pathogen taken for antimicrobial activity estimation from the leave extract of pterocarpus Marsupium Roxb was

S.No	Bacterial Strains	*Fungal Strains*
1	Bacillus Cereus	*Aspergillus niger*
2	S.aureus	*Aspergillus flavus*
3	Escherichia Coli	*Rizopusstoionifer*
4.	Pseudomonas aeruginosa	*Fusarium oxisporim*

3. Microbial culture growth Media Composition and its preservation: - The antimicrobial properties (antibacterial and antifungal activities) were analysed on solid (Agar-Agar) media in Petri plates for the bacterial assay. Nutrient Agar (NA) (40 g/l) and fungus PDA (39 g/l) were used for developing surface colony growth. The suspension culture, for bacterial cell growth, was performed with 2 %(w/v) Lauria Broth, and for fungus cell growth, 2.4% (w/v) PDB (potato dextrose broth) was used. [5]

4. Agar Well diffusion method: - This method is widely used to examine antimicrobial activity. The 8–10-hour-old cultured broth plates were smeared for bacteria and fungi, respectively, with Nutrient Agar (NA) and Potato Dextrose Agar (PDA) media. The well was dug in all the 10mm-diameter (and about 2-cm-thick) plates with the help of a sterile corn borer. [6] 80-100ul of solution of various dilutions from various extracts was poured with a decontaminated syringe into the well and maintained at 37 °C for 2-3 hours. Further, all the plates were incubated at 37 °C for 18 to 24 h for bacterial pathogens and at 28 °C for 46–48 h for fungal pathogens. The result was recorded nearby for all the wells as measured by the diameter of the zone of inhibition (mm).

5. Evaluation of antibacterial / antifungal activity: - To analyse the antibacterial / antifungal activities of different leaf extracts, the micro dilution method uses for bacterial pathogens and agar plates method for fungal strain. 0.85% saline containing 0.1% Tween 80 (v/v). The bacterial suspension was regulated with sterile saline to a concentration of 107 CFU/ml. Various inoculums were made and were preserved at 4°C for further use. [7,8]

6. **Determination of MBC/MFC**: - The MBC/MFC is defined as the minimum bactericidal and fungicidal concentration.[11] To determine the MBC/MFC data, serial sub-cultivation of 2 l in micro titer plates with 100 ul of broth per well was used. After the formation of the plates, they were incubated at 28°C for 72 hours. [9,10] The plate that shows no visible growth and has the least concentration towards growth was considered to have an MBC/MFC value. These values suggested that about 99% of the bacterial and fungal infection in the original inoculums was killed. To compare the results, streptomycin was taken as a standard drug for various bacteria strains and greisofluvin for fungal strain (1-3000 ug/ml), which is the standard drug used as positive controls. [12,13]

RESULTS AND DISCUSSION

Table:1 zone of inhibition(,mm)of all leave extracts of plant against microbial strains

Microorganism bacteria	Ethanol	Methanol	Petroleum ether	Aqueous(water)	Standard(streptomycin)
B.cereus	2.6	20.9	18.7	ND	22..6mm
S.aureus	9.4	17.7	13.5	13.6	19.3mm.
E.coli	10.7	15.5	11.5	14.2	21.7mm
P.aeruginosa	ND	16.4	12.4	9.8	19.5mm
fungi	**Ethanol**	**Methanol**	**Petroleum ether**	**Aqueous(water)**	**Standard (griesoflvin)**
A.niger	10.5	16.1	9.3	12.6	18.25mm
A.flavus	8.5	22.5	10.5	11.3	13.88mm
R.stolonifer	ND	19.3	10.6	ND	17.13mm
F.oxisporum	6.5	20.2	8.2	11.1	19.43mm

ND: - not detected the growth of microorganism

In the above-mentioned research, the antimicrobial activities (antibacterial and antifungal) of various leaf extracts (methanol, ethanol, petroleum ether, and water) of Ptercarpus marsupium were calculated and compared to those of microbial strains. The microbial activities were quantitatively evaluated on the basis of the diameter of the zone of inhibition (mm) along with minimum inhibitory concentration (MIC) values. The statistical data was evaluated with the activity of standard streptomycin (1 mg/disc) for bacterial strains and griseofluvin (1 mg/disc) for fungal strains.These extracts can be explored as a powerful and strongly effective antimicrobial source of drug development for all microorganism strains used in the research.[14]

Table 2: **MIC(µg/ml) and MBC of the different leaves sample of plant Pterocarpus Marcarpium against bacterial pathogen**

Microorganism bacteria	Ethanol		Methanol		Petroleum Ether		Aqueous (water)		Standard	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
B.cereus	20.4	34.6	22.4	42.5	29.3	48.4	17.8	52.6	35.7	58.1
S.aureus	14.4	44.8	34.6	61.6	21.4	40.8	15.2	48.7	23.4	63.6
E.coli	13.8	23.7	24.7	42.1	14.9	31.2	26.1	32.6	36.8	64.5
P.aeruginosa	12.2	39.6	18.2	38.2	17.4	49.8	19.2	50.3	26.3	74.6

Table 3 : MIC(µg/ml) and MFC of all leaves extracts of plant Mesa indica against fungal pathogens

Microorganism fungi	Ethanol		Methanol		Petroleum Ether		Aqueous (water)		Standard	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
A.niger	16.3	38.5	18.6	38.7	17.7	33.3	18.5	32.4	21.7	50.2
A.flavus	22.5	35.3	15.5	55.6	14.3	46.7	21.3	38.6	25.5	45.5
R.stolonifer	15.4	29.9	23.4	43.2	18.8	24.0	16.8	17.2	17.6	38.5
F.oxisporum	18.6	30.6	13.9	38.8	20.3	27.3	23.4	32.6	23.5	42.6

The mention of experimental research suggests that different ethanols, methanols, petroleum ethers, and aqueous extracts of the leaves of Pterocarpus marsupium show activity against the bacterial and fungal strains. MIC data analysis shows that all the extracts have variations in the values in various samples. The methanol leaf extract of the plant reflects the significant antibacterial and antifungal activity against several bacterial and fungal strains. These entire samples reveal maximum activity against S. *cereus* and *B. cereus* in bacterial strains *A. flavus* and *F. oxisporum* for fungal strains in the methanol sample. The data also suggest that the petroleum ether and water extracts have average activity towards various bacterial and fungal strains, while the ethanol extract has the lowest activity against some strains. The present research focused on different leaf extracts of the plant Pterocarpus marsupium. The analysis interprets the presence of bioactive compounds that possess high antimicrobial activities against different bacterial and fungal strains. The bioactive substances that are present in these samples may be responsible for their antibacterial and antifungal nature. The study indicates that different leaf extracts of this plant may be explored in the future as rich and effective sources of antimicrobial agents with high potency to cure

infectious disease. These drugs can be explored as high-potential antibacterial and antifungal agents at a cheaper cost in the future.

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