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Antibacterial and Antifungal Activity of *Mitragyna parvifolia* Leaf Extracts in Experimental Rats

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ABSTRACT

The aim of this study was to determine the antibacterial and antifungal potency of ethanolic and aqueous extracts of *Mitragyna parvifolia* leaves as an alternative for synthetic medication in the treatment of bacterial and fungal infections caused by human pathogenic microbial strains. The fresh leaves were harvested from a nearby field and then dried and extracted. *Staphylococcus aureus, Pseudomonas aeruginosa, Escherechia coli, Candida albicans, Microsporum gypseum,* and *Aspergillus niger* were used as bacterial and fungal strains, respectively. Normal antibacterial and antifungal agents were chloramphenicol and griseofulvin. The antibacterial and antifungal properties of the extract were verified by the agar diffusion results. *In vivo* studies of this plant extract are needed to further understand its protection, effectiveness, and properties. The antimicrobial potential of the extract may lead to the discovery of new antimicrobial agent, according to the findings of the present study.

Keywords: *Mitragyna parvifolia*, antibacterial activity, antifungal activity, agar well diffusion, minimum inhibition concentration.

1. INTRODUCTION

Antimicrobial chemotherapy has been an effective medicinal practice used worldwide in human medicine, food, agriculture, poultry, and household items since Ehrlich's early research of antibacterial dyes in the twentieth century.[1] By the late 1940s, however, antimicrobial resistance had become a major concern in healthcare facilities such as hospitals and care facilities.[2] Increased use of domestic antimicrobials and industrial antimicrobials promotes resistance to medications designed for human treatment, which may have serious effects for children and the elderly. Methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci, as well as gram-negative rods such as Enterobacteriaceae and *Pseudomonas aeruginosa*, are the most widespread resistant bacteria in hospitals.[3]

Bacterial resistance forces researchers to devise ways to change the mechanisms of antimicrobial compounds in order to prevent their inactivation, but structural changes alone are insufficient to prevent bacterial resistance.[4] The quest for new anti-infection agents has dominated several ethnopharmacology research groups over the last few decades. Several herbal medicines are thought to be potent antimicrobial crude drugs as well as a source of novel antimicrobial compounds with potentially novel modes of action.

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Previous research has discovered a broad variety of conditions for the detection of antimicrobial compounds in plants. Many researchers are interested in evaluating the antimicrobial function of plant extracts, or extracted substances such as essential oils, alkaloids, flavonoids, sesquiterpene lactones, diterpenes, triterpenes, or naphthoquinones. Most of these compounds were extracted or collected by bioassay-guided isolation after antimicrobial activity in the plant extract was detected. It has also previously been established that certain naturally occurring plant compounds can destroy antibiotic resistance of bacteria such as *Bacillus cereus, Escherichia coli, Micrococcus luteus,* and *Staphylococcus aureus*.[5,6]

Mitragyna parvifolia belongs to the Rubiaceae family referred to as Kaim. It is a medium to large tree and can be found emerging wild in the drier areas of India, Pakistan, and Sri Lanka.[7] Various indolic (tetrahydroalstonine, akkuamigine, hirsuteine, etc.) and oxindolic (mitraphylline, isomitraphylline, pteropodine, isopteropodine, etc.) alkaloids can be found in the tree's aerial components, stem bark, and roots. Natives and indigenous peoples have used various parts of the tree, such as fruit pulp, leaves, and stem bark, in herbal medicine since time immemorial.[7,8] Analgesic, antipyretic, anti-inflammatory, antiarthritic, anti-ulcer, anthelmentic, antioxidant, and other medicinal and therapeutic effects of *Mitragyna parvifolia* have been scientifically validated. Its leaves are applied to wounds and ulcers to relieve discomfort, swelling, and promote faster healing.[9]

The current study assessed the potential role of ethanolic and aqueous extracts of *Mitragyna parvifolia* leaves for antibacterial and antifungal activities, taking into account conventional claims and confirmed therapeutic activities.

2. MATERIAL AND METHODS

2.1 Selection of Microorganisms

Three bacterial strains i.e., *Staphylococcus aureus* (Gram positive; MTCC 737), *Pseudomonas aeruginosa* (Gram negative; MTCC 1688) & *Escherichia coli* (Gram negative; MTCC 443) and, three fungal strains i.e., *Candida albicans* (MTCC 183), *Microsporum gypseum* (MTCC 2829) & *Aspergillus niger* (MTCC 282) were procured from MTCC, IMTECH, Chandigarh and maintained at 4°C in Mueller-Hinton agar medium containing beef extract (2 g); casein hydrolysate (17.5 g); starch (1.5 g); agar (17 g), distilled water (1 L); pH-25°C. This was then sterilized in an autoclave at 15 lbs pressure and 121°C for fifteen minutes. The sterilized medium (20 mL) was poured in sterilized petri dishes allowing them to solidify under aseptic condition.[10]

2.2 Preparation of Microbial Inoculum

The density of microbial strains was adjusted equal to that of the 0.5 McFarland standard (1.5 x 108 CFU/ml) by adding sterile distilled water. McFarland standards are used as a reference to correct the turbidity of bacterial suspensions so that the number of bacteria will be within a given range to standardize microbial testing. For the preparation of the 0.5 McFarland standard, 0.05 mL of 1% barium chloride ($BaCl_2$) was added to 9.95 mL of 1% H_2SO_4 (w/v) with constant stirring. The McFarland standard tube was tightly sealed to prevent loss by evaporation and stored for up to 6 months. To aid comparison the test and standard were compared against a white background with a contrasting black line.[11]

2.3 Collection and Extraction of Plant

Fresh leaves of *Mitragyna parvifolia* were taken from different locations in Ghatkesar, Telangana, India. The leaves were then washed thoroughly 2-3 times with running tap water and then with sterile water followed by shade dried, powdered and used for extraction.

Solvents, ethanol (95%) and distilled water were used for the phytochemical extraction of plant parts. For extraction with solvent, 50 g of powdered plant material was dissolved in the solvent to make 100 ml of each extract (25% w/v). The mixture was kept undisturbed at room temperature for 24 hrs in a sterile flask covered with aluminum foil to avoid evaporation and subjected to filtration through sterilized Whatman

no. 1 filter paper. After filtration, the extract was evaporated in water bath. The ethanol and aqueous extracts thus obtained were immediately evaluated for their antibacterial and antifungal activity using agar well diffusion method.[12]

2.4 Antimicrobial Assay

2.4.1 Agar Well Diffusion Method

The antimicrobial activities of 2 crude extracts (ethanolic & aqueous) of the leaves of *Mitragyna parvifolia* against bacterial and fungal strains were evaluated by using agar well diffusion method. For bacteria and fungi, Mueller-Hinton agar growth medium plates were poured with 100 µL of standardized inoculum (1.5 x 108 CFU/mL) of each microorganism and spread with sterile swabs. Wells or cups of 6 mm size were made with sterile borer into agar plates containing the bacterial inoculums. 100 µL of the leaves extract was poured into the well of inoculated plates. Sterilized distilled water and ethanol were used as a negative control. Chloramphenicol and griseofulvin were used as positive control. The plates were placed in an incubator at 37° C for 30 minutes for bacteria and 22℃ for fungal diffusion into agar medium. After 48 hrs incubation for bacteria and 7 days for fungal, the diameter of zone (zone of inhibition) was measured and recorded in mm.[13]

2.4.2 Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration of the plant extracts were tested by the two-fold serial dilution method. The test extract was dissolved in 5% DMSO to obtain 1000 µg/mL stock solutions. 0.5 mL of stock solution was incorporated into 0.5 mL of Mueller Hinton Agar for bacteria, Yeast Nitrogen Base for yeasts and Sabouraud Dextrose Broth for mycelial fungi to get a concentration of 500 µg/mL and serially diluted by double technique to achieve 250, 125, 62.5 and 31.25 µg/mL, respectively. 50 µL of standardized suspension of the test organism was transferred on to each tube. The control tube contained only organisms and not the plant extract. The culture tubes were incubated in BOD incubators at 37°C for 24 h (bacteria) and 28°C for 72-96 h (fungi). The lowest concentrations, which did not show any growth of tested organism after macroscopic evaluation was determined as MIC.[14]

3. RESULTS

Ethanolic extract of *Mitragyna parvifolia* was effective against all four tested bacterial and fungal strains. Ethanolic extract of *Mitragyna parvifolia* recorded significant inhibition zone of 25 mm against *Staphylococcus aureus,* 22 mm against *Pseudomonas aeruginosa*, 20 mm against *Escherechia coli*, 15 mm against *Candida albicans*, 20 mm against *Microsporum gypseum* and 13 mm against *Aspergillus niger*. The aqueous extracts did not show any significant inhibitory activity against any of the test bacterial and fungal strains. The negative control (distilled water & ethanol) did not inhibit any of the microorganisms tested.

Table 1: Antibacterial and antifungal effect of *Mitragyna parvifolia* leaves

S. No.	Organisms	Mean Zone of Inhibition (mm)			MIC (µg/ml)	
		Ethanol	Aqueous	CAP	Ethanol	Aqueous
	Bacteria					
1	*Staphylococcus aureus*	25	10	31	125	250
2	*Pseudomonas aeruginosa*	22	7	28	125	500
3	*Escherechia coli*	20	8	25	125	500
	Fungi					
1	*Candida albicans*	15	9	19	125	125
2	*Microsporum gypseum*	20	11	25	125	250
3	*Aspergillus niger*	13	9	18	125	250

MIC= Minimum Inhibitory Concentration; CAP: Capriomycin

According to Table 1, the ethanolic extract of leaves of *Mitragyna parvifolia* showed MIC of 62.5 µg/mL against *Staphylococcus aureus* whereas MIC of 125 µg/mL against remaining bacteria (*Pseudomonas aeruginosa* & *Escherechia coli*). The MIC of 125 µg/ml against all fungal strains (*Candida albicans*, *Microsporum gypseum* & *Aspergillus niger*) were observed.

4. DISCUSSION

The present study revealed that the ethanolic extract of leaves of *Mitragyna parvifolia* was very effective against *Escherechia coli, Pseudomonas aeruginosa*, *Staphylococcus aureus, Candida albicans, Microsporum gypseum* and *Aspergillus niger* when compared to aqueous extract. In the present study, gram positive bacteria such as *Staphylococcus aureus* were more susceptible than gram negative bacteria such as *Escherichia coli* and *Pseudomonos aeruginosa*.

Among the two extracts used for the present study, the ethanol extract showed a higher antibacterial and antifungal activity. This may be due to the solvent used to extract the different constituents having antimicrobial activity. The crude aqueous extracts of the leaves of *Mitragyna parvifolia* showed limited antibacterial and antifungal activity. Ethanol was the most effective solvent for extracting antibacterial compounds from the selected plant leaves.

Bacterial strains in the current research work were found to be more susceptible to the extract than the fungal strains. These antimicrobial effects were due to the fact that the leaves of the plant *Mitragyna parvifolia* have potential phyto-chemicals such as 16,17-dihydro-17beta-hydroxy isomitraphylline, 16, 17-dihydro-17beta-hydroxy mitraphylline, isomitraphylline and mitraphylline.[15] The higher zone of inhibition for antibacterial activity was recorded at 62.5-125 µg/mL concentration whereas 125 µg/mL was recorded for antifungal activity.

The fact that the ethanolic extract of leaves of *Mitragyna parvifolia* exhibited inhibitory activities against some of the microorganisms implicated in the pathogenesis of bacterial and skin diseases (*Escherechia coli, Pseudomonas aeruginosa*, *Staphylococcus aureus, Candida albicans, Microsporum gypseum* and *Aspergillus niger*) provides some scientific basis for the utilization of this plant in traditional Indian systems of medicine and as alternative to commercially available high cost medicines for the treatment of bacterial and skin diseases.[16] Based on the test data, further chemical and pharmacological investigations may be recommended for *Mitragyna parvifolia*.

5. CONCLUSION

From the present study it can be concluded that the ethanolic extract of leaves of *Mitragyna parvifolia* were highly effective against all the bacterial and fungal strains. The present study is *in vitro* antimicrobial evaluation of the selected plant which forms a primary platform for further phytochemical and pharmacological studies to discover new antibiotic drugs. Further research is necessary to determine the identity of the antimicrobial compounds within this plant and its effectiveness in human.

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