International Journal of Drug Development & Research
| October-December 2011 | Vol. 3 | Issue 4 | ISSN 0975-9344 |
Available online http://www.ijddr.in
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Antimicrobial activity of *Mitragyna parvifolia* barks and *Butea monosperma* leaves extracts against human pathogenic microbial strains

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Abstract

The present study was designed to evaluate the antimicrobial efficacy of *Mitragyna parvifolia* (barks) and *Butea monosperma* (leaves) against human pathogenic microbial strains such as two Gram positive (*Staphylococcus epidermidis, Bacillus subtilis*), two Gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*) and two yeasts (*Saccharomyces cereviseae, Candida albicans*) assayed by using agar well diffusion assay. Three different extracts (ethanol, methanol and water) of each plant were used during the study. *M. parvifolia* extracts showed better activity than the *B. monosperma* extracts. The zone of inhibition in *M. parvifolia* extracts (ethanolic and methanolic) was in the range of 14mm to 25mm and 10mm to 14mm in case of *B. monosperma* extracts. The aqueous extracts did not show any inhibitory activity against any of the test bacterial strains. No antifungal activity was observed against the test yeast strains. The MIC values of methanol extract of *Mitragyna parvifolia* for different bacterial strains ranged from 6.25mg/ml to 12.5mg/ml. On the basis of this finding, the extracts demonstrating antimicrobial efficacy could result in the discovery of novel antimicrobial agents.

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Key words:
Agar well diffusion, antimicrobial activity, bacteria, *Butea monosperma,* fungi, *Mitragyna parvifolia*

***How to Cite this Paper*:**
Pundir Ram Kumar* and Bishnoi Shreya, "Antimicrobial activity of *Mitragyna parvifolia* barks and *Butea monosperma* leaves extracts against human pathogenic microbial strains", Int. J. Drug Dev. & Res., Oct-Dec 2011, 3(4): 141-147

Article History:----------
Date of Submission: 15-06-2011
Date of Acceptance: 09-08-2011
Conflict of Interest: NIL
Source of Support: NONE

INTRODUCTION

Medicinal plants represent a rich source of antimicrobial agents. The development and spread of

resistance to the existing antibiotics by microorganisms are due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of various diseases. Although a number of plants with antimicrobial activities have been identified, great number still remains unidentified[12].

Mitragyna parvifolia belongs to family *Rubiaceae* is commonly known as Kaim[9]. It is credited with innumerable medicinal properties and is widely used by tribal people and other ayurvedic practitioners. The barks and roots are used to treat fever, colic, muscular pain, burning sensation, poisoning, gynecological disorders, cough, edema and as aphrodisiac. The fruit juice augments the quantities of breast milk in lactating mothers and also work as lactodepurant. Wounds and ulcers are dressed with its leaves to alleviate pain, swelling and for better healing[4].

Butea monosperma belongs to family *Fabaceae,* also known as flame of the forest, is wild, medium sized tree found throughout the deciduous forests and also in open areas. It is traditionally used in the treatment of diabetes, leprosy, gout, skin diseases, eye diseases, piles, aphrodisiac, laxative and antihelminitic. It has antistress, antioxidant and anti-inflammatory activity[3].

Taking into consideration the traditional claims and reported activities, possible role of these plants were evaluated for antimicrobial activity. The investigation was undertaken to evaluate the two plants *Mitragyna parvifolia* and *Butea monosperma* for their antibacterial and antifungal activity.

MATERIALS AND METHODS

Procurement and maintenance of test microorganisms

A total of 4 bacteria such as two Gram negatives *Escherichia coli* MTCC 483*, Pseudomonas aeruginosa* MTCC 741, two Gram positives *Staphylococcus epidermidis* MTCC 433*, Bacillus subtilis* MTCC 441, and 2 fungi *Saccharomyces cereviseae* MTCC 170 and *Candida albicans* MTCC 183 were procured from MTCC, IMTECH, Chandigarh and maintained at 4° C in growth medium 3 (components-beef extract-1.0g; yeast extract-2.0 g; peptone-5g; NaCl-5g; agar-15g dist.water-1000ml) agar slants for bacteria and growth medium 5 (yeast extract-3g; peptone-10g; dextrose-20g; agar-15 g; distilled water-1000ml) agar slants for fungi for further uses.

Preparation of microbial inoculum

The density of microbial strains was adjusted equal to that of the 0.5 McFarland standard (1.5 x 10^8 CFU/ml) by adding sterile distilled water. McFarland standards are used as a reference to adjust the turbidity of microbial suspension so that the number of microorganisms will be within a given range. For the preparation of the 0.5 McFarland standard, 0.05ml of barium chloride ($BaCl_2$) (1.17% w/v $BaCl_2.2H_2O$) was added to 9.95 ml of 0.18M H_2SO_4 (1.0% 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[1].

EVALUATION OF PLANT EXTRACTS FOR THEIR ANTIMICROBIAL ACTIVITY

Collection of plants

Fresh leaves of *Butea monosperma* and barks of *Mitragyna parvifolia* were taken from different locations in Kurukshetra, Haryana, India. The respective plant parts were washed thoroughly 2-3 times with running tap water and then with sterile water followed by shade dried, powdered and used for extraction[4].

Preparation of plant extracts

An extract is a mixture of phytochemicals from any plant which is obtained by extraction of specific parts of the plant[6]. Solvent, ethanol (95%), methanol (95%) and distilled water were used for the phytochemical extraction of plant parts. For extraction with solvent, 25 g of powdered plant material was dissolved in the solvent to make 100ml 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 until 20 ml extract was left in the container. The ethanol, methanol and aqueous extracts thus obtained were immediately evaluated for their antibacterial and antifungal activity using modified agar well diffusion method[10].

Antimicrobial assay

Agar well diffusion method

The antimicrobial activity of 6 crude extracts (aqueous, ethanolic and methanolic) of the plant parts against all bacterial and fungal strain were evaluated by using agar well diffusion method[10].For bacteria Growth medium 3 and for fungi Growth medium 5 agar plates were poured with 100µl of standardized inoculum (1.5x10^8 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. 100µl volume of the plant extract was poured into the well of inoculated plates. Sterilized distilled water or solvent (ethanol/methanol) was used as a negative control. Gentamycin and ketaconazole were used as positive control. The plates thus prepared were left at room temperature for ten minutes allowing the diffusion of the extract into the agar. After incubation for 24 hrs at 37˚C, the plates were observed for zone of inhibition surrounding the well containing the plant extract. The zone of inhibition was measured and expressed in millimeters.

Determination of minimum inhibitory concentration (MIC) of *Mitragyna parvifolia* methanolic extract against bacterial strains

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of the antimicrobial agent that will inhibit the visible growth of a microorganism after overnight incubation[1]. MIC of methanol extract of *Mitragyna parvifolia* barks was determined by macrodilution agar method. The MIC was determined following the methodology of Pundir and Jain[10].

Macrodilution agar method

In the macrodilution agar method, a two-fold serial dilution of the extract was prepared in sterile distilled water to achieve a decreasing concentration ranging from 200mg/ml to 1.56mg/ml in eight sterile tubes labeled 1 to 8. Sterile cork borer of 6mm diameter was used to bore well in the presolidified G3 agar medium plates and 100µl volume of each dilution was added aseptically into the wells made in agar plates in triplicate that had bacteria seeded with the standardized inoculum (1.5 X 10^8 CFU/ml). 100µl methanol introduced into the well in place of plant extract was used as negative control. All the test plates were incubated at 37°C and were observed for the growth after 24 hrs. The lowest concentration of an extract showing a clear zone of inhibition was considered as the MIC.

RESULTS AND DISCUSSION

Plant based antimicrobial compounds have enormous therapeutic potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. In the present study, three solvents namely water, ethanol and methanol were selected for the plant extraction. In the present study The *Mitragyna parvifolia* and

the *Butea monosperma* extracts exhibited antibacterial activity in ethanol and methanol solvents (Table. 1 and Fig. 1 and 2). Among treatments, according to Table 1 maximum in vitro inhibition of tested bacteria *E. coli, S. epidermidis, P. aeruginosa* and *B. subtilis* was scored in methanol extract of *Mitragyna parvifolia* which offered inhibition zone of 14mm, 25mm, 15mm and 21mm respectively.

Ethanolic extract of *Mitragyna parvifolia* was effective against all four tested bacteria which recorded significant inhibition zone of 21mm against *S. epidermidis,* 14mm (*P. aeruginosa*) and 19 mm (*B. subtilis*). No activity of ethanolic extract of *Mitragyna parvifolia* was observed against *E. coli*. The antibacterial activity can be due to the presence of major alkaloids like mitraphylline, isomitraphylline rotundifoline, rhynchophyline, isorotundifoline, rhynchociline, mitragynine and speciociliatine[8]. Various indolic and oxindolic alkaloids have also been reported from this species are of significant biological importance[11].

The methanol extract of *Butea monosperma* recorded inhibition zone of 12 mm against *E .coli*, 10 mm against *S. epidermidis,* 13mm against *P. aeruginosa* and 13mm against *B. subtilis* whereas the ethanol extract of *Butea monosperma* offered inhibition zone of 13 mm against *E. coli*, 14mm against *B. subtilis* and 12mm against *P. aeruginosa*. The activity can be due to the presence of some of the phytochemical components like saponins, tannins and phenolic compounds[7] .The activity against *S. epidermidis* was not observed. It might be due to resistance developed by *Staphylococci*[5].

The aqueous extracts did not show any inhibitory activity against any of the test bacterial strains. Since nearly all of the identified components from plants active against microorganisms are aromatic or saturated organic compounds, they are most often obtained through initial ethanol or methanol extraction[2].

No antifungal activity of the plants was observed during the study. Therefore maximum inhibition was shown by *Mitragna parvifolia* methanol extract against Gram positive bacteria than the Gram negative bacteria. This may be due to the presence of outer membrane which acts as effective barrier in Gram negative bacteria. It is evident from table 1 that methanol and ethanol extracts of *Mitragyna parvifolia* showed significant activity against Gram positive *S. epidermidis* which was comparable to the inhibition zone observed for control gentamycin (10µg/ml).

According to Table. 2 and Fig. 3, the methanol extract of *Mitragyna parvifolia* showed MIC of 6.25 mg/ml against *S. epidermidis* whereas MIC of 12.5 mg/ml against all the three bacteria *(E. coli, B. subtilis and P. aeruginosa*). According to Zongo *et al.,*[13] the lowest MIC (0.625mg/ml) of *Mitragyna inermis* was recorded against various human pathogens such as *Proteus mirabilis*, *Staphylococcus aureus* and *S. carmosum*. This MIC value was ten times lower the MIC value reported in the present study. No more literature on the MIC of *Mitragyna parvifolia* barks has been reported till now.

Fig.1: Antibacterial activity of *Mitragyna parvifolia* (A) and *Butea monosperma* methanolic extracts (B) and methanol as negative control (C) against *Bacillus subtilis.*

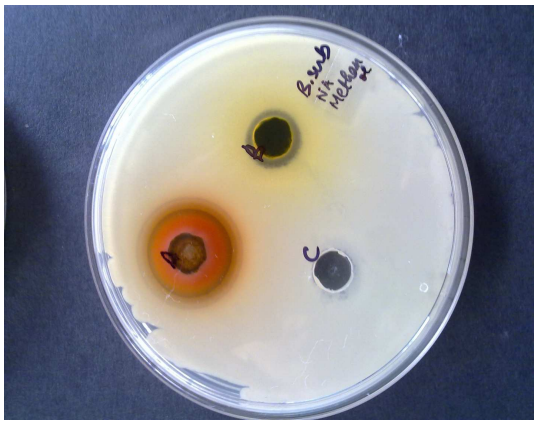


Table 1: Antimicrobial activity of extracts of *Mitragyna parvifolia* barks and *Butea monosperma* leaves against bacterial and fungal strains

	extract	Diameter of zone of inhibition in mm.					
		Staphylococcus epidermidis	***Bacillus subtilis***	***Pseudomonas aeruginosa***	***Escherichia coli***	***Saccharomyces cereviseae***	***Candida albicans***
Mitragyna parvifolia	Methanol	25	21	15	15	**NA**	**NA**
	Ethanol	21	19	14	**NA**	**NA**	**NA**
	Aqueous	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
Butea monosperma	Methanol	10	13	13	12	**NA**	**NA**
	Ethanol	NA	14	12	13	**NA**	**NA**
	Aqueous	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**
Positive control for bacteria	Gentamycin(10µg/ml)	20	31	27	26	**NA**	**NA**
Positive control for fungi	Ketoconazole (10 µg/ml)	**NA**	**NA**	**NA**	**NA**	26	28

NA: No Activity

Fig.2: Antibacterial activity of *Mitragyna parvifolia* and *Butea monosperma* methanolic extracts against bacterial and fungal strains.

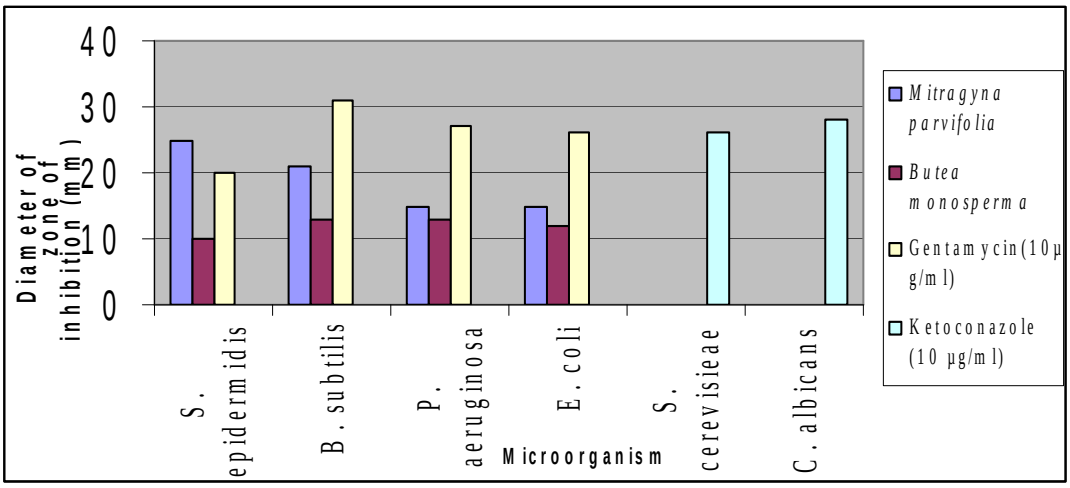
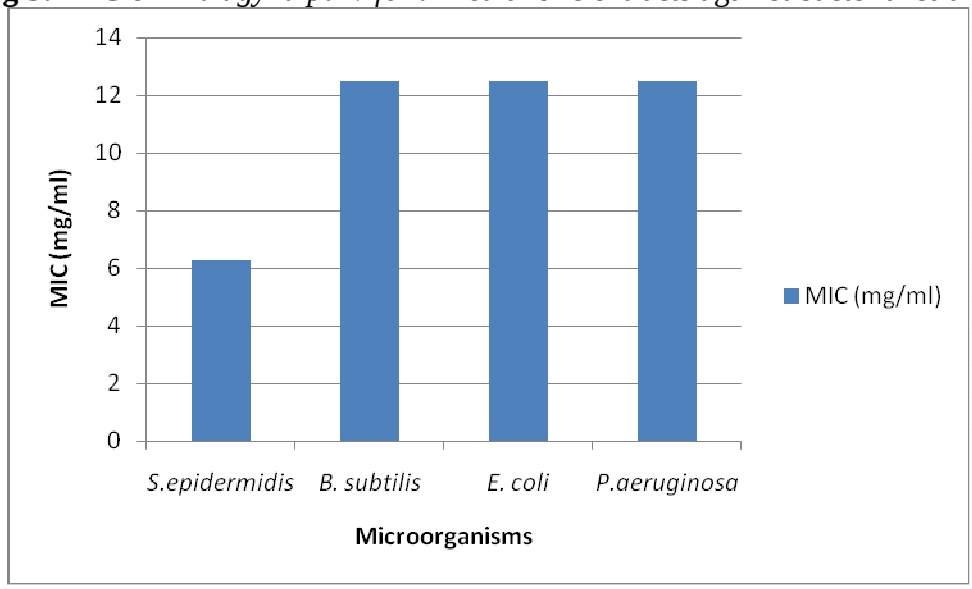


Table 2: Minimum Inhibitory Concentration of *Mitragyna parvifolia* methanolic extract against test bacterial strains

Bacteria	Concentration of *Mitragyna parvifolia* Barks methanolic extract (mg/ml)								
	1.56	**3.13**	**6.25**	**12.5**	**25.0**	**50.0**	**100.0**	**200.0**	**MIC**
Staphylococcus epidermidis	+	+	NA	NA	NA	NA	NA	NA	6.25
Bacillus subtilis	+	+	+	NA	NA	NA	NA	NA	12.5
Escherichia coli	+	+	+	NA	NA	NA	NA	NA	12.5
P. aeruginosa	+	+	+	NA	NA	NA	NA	NA	12.5

NA: No Activity

Fig 3: MIC of *Mitragyna parvifolia* methanolic extracts against bacterial strains



CONCLUSION

From the present study it can be concluded that the methanol extracts of both the plants were highly effective against all the four bacteria. Further research is necessary to determine the identity of the antibacterial compounds within these plants. However the present study of in vitro antibacterial evaluation of some plants forms a primary platform for further phytochemical and pharmacological studies to discover new antibiotic drugs.

ACKNOWLEDGEMENTS

Authors are grateful to Dr. J. K. Sharma (Director), Dr. R. K. Jethi (HOD Biotechnology Engineering Department) and non-teaching staff, ACE, Devsthali, Near Mithapur, Ambala, Haryana for their continuous support while carrying out this research work.

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