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RESEARCH ARTICLE

Antimicrobial activity of *Butea monosperma* Lam. Gum

SHAILENDRA S. GURAV, VIJAY D. GULKARI, NANDKISHORE J. DURAGKAR and ARUN T. PATIL

For author affiliations, see end of text.

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ABSTRACT

Gum of Butea monosperma is used to treat microbial and fungal infections in folk medicine. To validate this use, the in- vitro antimicrobial activity of petroleum ether and alcoholic extract of Butea monosperma gum was evaluated against various microbial strains such as Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Salmonella typhimurium, Pseudomonas aeuriogenosa, Escherichia coli, Candida albicans and Saccharomyces cerevisiae by using disc diffusion method. Minimum inhibitory concentration (MIC) was determined by agar dilution technique. Both extracts showed significant inhibition against reference gram positive bacteria and fungal strains. MIC value of petroleum ether extract against gram positive and fungal strains was 300 μg / ml and that of alcoholic extract was 200 μg / ml. Neither extract showed inhibitions against gram negative bacteria.

Keywords: *Butea monosperma, Antimicrobial activit, Antifungal activity, Disc diffusion method, Agar dilution method*

The spread of drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases. Down the ages, essential oils and other extracts of plants have evoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases [1]. World Health Organization (WHO) noted that majority of the world's population depends on traditional medicine for primary healthcare. Plant extracts have been used for many thousands of years [2] in food preservation, pharmaceuticals, alternative medicine and natural therapies [3, 4]. It is necessary to scientifically investigate those plants which have been used in traditional medicine to improve the quality of healthcare.

The development and spread of resistance to the existing antibiotics by microorganisms, calls for increased efforts in the development of new antibiotics. Although a number of plants with antimicrobial activities have been identified, great number still remains unidentified. The purpose of this work was therefore to evaluate antimicrobial activity of *Butea monosperma* gum on different microbial strains.

Butea monosperma Lam. (Fabaceae) also known as flame of the forest, is wild, medium sized tree found throughout the deciduous forests and also in open areas. In the literature, *B. monosperma* is ascribed to have many medicinal properties. It has been used as tonic, astringent, aphrodisiac and diuretic. Its flowers are widely used in the treatment of hepatic disorders and viral hepatitis, diarrhoea and possess anti-implantation activity [5]. Roots of *B. monosperma* are reported to be useful in the treatment of filariasis, night blindness, helminthiasis, piles, ulcers and tumors. Pippali rasayana, an Indian Ayurvedic drug, employs *B. monosperma* and is used in the management of giardiasis [6]. The bark is reported to possess antitumor and antiulcer activities. The root bark is used as an aphrodisiac, analgesic and antihelmintic whereas the leaves possess antimicrobial property [7]. *B. monosperma* flowers contain butin, butein, butrin, isobutrin, palasitrin, coreipsin, isocoreipsin, chalcones, and aurones [8]. Butrin (7, 30, 40-trihydroxy.avanone-7, 30-diglucoside) and isobutrin (3, 4, 20, 40-tetra-hydroxy-chalcone-3, 40-diglucoside) are the well-known antihepatotoxic principles of *B. monosperma* [9]. Gum is useful as astringent, depurative and useful in diarrhoea, haemorrhoids, haepoptysis, haematemesis, leprosy, skin diseases [10, 11].

In some tribes (Banjara) from Maharashtra (India), gum of *B. monosperma* is used to treat microbial and fungal infections [12]. To substantiate this claim, the present study was undertaken to evaluate the antimicrobial and antifungal potential of gum of *B. monosperma* by disc diffusion assay and broth dilution assay.

Table 1. Antimicrobial activity of *Butea monosperma* gum extracts

Microorganisms	Zone of Inhibition (mm) Pet. Ether Extract (mg/ml)					MIC µg/ml	Zone of Inhibition (mm) Alcoholic Extract (mg/ml)					MIC µg/ml	References Ciprofloxacin		Amphotericin B	
	2	4	6	8	10		2	4	6	8	10		Zone of inhibition	MIC µg/ml	Zone of inhibition	MIC µg/ml
Staphylococcus aureus ATCC 25923	9	11	12	12	12	400	11	12	12	13	13	300	16	0.5	ND	ND
Bacillus subtilis MTCC 441	11	12	13	14	14	300	12	13	14	15	14	200	17	2.0	ND	ND
Bacillus cereus MTCC 430	11	12	13	14	14	300	12	13	14	14	15	200	15	1	ND	ND
Pseudomonas aeruginosa MTCC 424	n. s.	9	9	10	10	500	-	9	9	10	10	500	14	0.25	ND	ND
Salmonella typhimurium NCTC 74	n. s	n. s	n. s	n. s	n. s	n. s	n. s	n. s	n. s	n. s	n. s	n. s	14	0.50	ND	ND
Escherichia coli MTCC 443	n. s	n. s	n. s	n. s	n. s	n. s	n. s	n. s	n. s	n. s	n. s	n. s	13	0.05	ND	ND
Candida albicans MTCC 227	10	11	12	13	14	300	13	13	14	15	15	200	ND	ND	18	1
Saccharomyces cerevisiae MTCC 170	11	11	10	13	13	300	12	13	14	14	15	200	ND	ND	15	1

Values are means of three replications, MIC- Minimum Inhibitory concentration (µg/ml),
n.s. - Not sensitive at the concentration, - = No zone of inhibition, ND- Not determined.

MATERIALS AND METHODS

Plant Material

Gum of *B. monosperma* plant was collected from local region of Nagpur, District of Maharashtra, India in the month of July 2005. The botanical identity was confirmed by a taxonomist, Dr. Vinayak Naik, Department of Botany; Zandu Pharmaceuticals where voucher specimen (No. 237/4) has been deposited for further reference.

Preparation of Extracts

The gum of *B. monosperma* was subjected to size reduction to get coarse powder. Extraction was performed by using soxhlet apparatus (40 cycles each), carried out first with petroleum ether (60-80 ºC) to defat the material (yield: 1.49% w/w). The defatted material was then extracted with alcohol to get alcoholic extract (yield: 8.15% w/w). The extracts were concentrated for further studies at reduced pressure and temperature in a rotary evaporator and tested for presence of secondary metabolites by different phytochemical tests. Test extracts were then dried crushed to fine powder and dissolved in 10% aqueous dimethylsulfoxide (DMSO) for further study.

Chemicals

Mueller Hinton agar and Sabourand dextrose agar (Himedia Lab); Ethanol (Ranbaxy laboratories Ltd. Punjab); Petroleum ether (60-80) and Tween 80 (S.D. Fine chemicals, Mumbai, India); standard Discs of Ciprofloxacin and Amphotericin B (Himedia Lab).

Microbial Strains

The test extracts were individually tested against a panel of microorganisms including *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* MTCC 441, *Bacillus cereus* MTCC 430, *Pseudomonas aeruginosa* MTCC 424, *Salmonella typhimurium* NCTC 74, *Escherichia coli* MTCC 443, *Candida albicans* MTCC 227, *and Saccharomyces cerevisiae* MTCC 170. These strains were obtained from Institute of Microbiological Technology, Chandigarh and National Chemical Laboratory, Pune. Bacterial strains were grown at 37 °C in Mueller Hinton agar (MHA) whereas fungal strains were at 30 °C in Sabouraud dextrose agar at pH 7.4 for 48 hrs followed by frequent subculturing to fresh medium and were used as test micro-organisms. The inoculum size of each test strain was 10^8 bacteria/ml for disc diffusion assay, which was standardized by adjusting the optical density of the bacterial suspension to a turbidity corresponding to spectrophotometric absorbance = 0.08 (OD_{620} = 0.08) at 620 nm.

Screening for Antimicrobial Activity

Disk diffusion method was used for the determination of antimicrobial activity and the MIC assay was performed by broth microdilution method. The MICs of

ciprofloxacin and amphotericin B were also determined in parallel experiments in order to control the sensitivity of the test microorganisms. All studies were performed in triplicate and mean value was calculated.

Disc Diffusion Method

Screening of extracts for antibacterial activity was done by the disk diffusion method [13]. It was performed using an 18 h culture at 37°C in 10 ml of Mueller Hinton broth. Bacterial inoculums were spread over the plates containing Mueller-Hinton agar using a sterile cotton swab in order to get a uniform microbial growth on both control and test plates. The extracts were dissolved in 10% aqueous dimethylsulfoxide (DMSO) with Tween 80 (0.5% v/v for easy diffusion) and sterilized by filtration through a 0.45 µm membrane filter. Under aseptic conditions, empty sterilized discs (Whatman no. 5, 6 mm dia) were impregnated with 100 µl of each of the extracts of different concentration and left to dry under laminar flow cabinet overnight and then placed on the agar surface. Paper disc moistened with aqueous DMSO was placed on the seeded petriplate as a vehicle control. Standard discs containing ciprofloxacin (0.8 µg/ml) and amphotericin (1.5 µg / ml) were used as reference control. All petridishes were sealed with sterile laboratory parafilm to avoid eventual evaporation of the test samples. The plates were left for 30 min at room temperature to allow the diffusion of test drugs and kept for incubation.

Incubation of Plates

The plates containing the bacterial culture were incubated at 37°C for 18 h (18 h was fixed as the optimum time since there was no change in the inhibition up to 24 h). On the other hand, the plates with fungal suspension were incubated at 25°C for 72 h. After the incubation time, all the plates were examined for the presence of zones of inhibition with a caliper as a property of antimicrobial activity. The antibacterial activity was interpreted from the size of the diameter of zone of inhibition measured to the nearest millimeter (mm) as observed from the clear zones surrounding the discs.

MIC Assay

The agar dilution method recommended by the National Committee for Clinical Laboratory Standards [14] was used. A series of twofold microdilution of each extract with saline at a final concentration ranging from 1000µg /ml to 100µg /ml was prepared in Muellur Hinton agar at 48°C. Plates were dried at room temperature for 30 min prior to spot inoculation with 3 µl aliquots of culture containing approximately 10^5 bacteria/ml of each organism. Inoculated plates were incubated at 37°C for 18 h and the MIC was determined. Experiments were carried out in triplicate. Inhibition of bacterial growth in the plates containing test extract was judged by comparison with growth in blank control plates. The MIC values were taken as the lowest concentration of the extracts in the wells of the microtiter plate that showed no turbidity after 24 hours of incubation at 37° C. The turbidity of the wells in the microtiter plate was interpreted as visible growth of the microorganisms.

RESULTS

Preliminary phytochemical screening of extracts revealed presence of sterols and coumarins in petroleum ether extract whereas showed positive tests for triterpenes and sugar in alcoholic extract.

The petroleum ether and alcoholic extract of the gum were used in the present study to investigate their antimicrobial potential. Both gram-negative and gram positive bacteria and fungi were used as test organisms. Ciprofloxacin and amphotericin were used as positive controls.

The anti-bacterial activity of petroleum ether and alcoholic extract by disc diffusion method is summarized in Table 1. The results revealed that, in disc diffusion assay, both extracts showed antibacterial activity with varying magnitudes against gram positive and fungal strains. The zone of inhibition above 6 mm in diameter was taken as positive result. No extract showed antimicrobial activity upto 10 mg/ml against gram negative strains except *Pseudomonas aeruginosa*. In comparison with petroleum ether extract, alcoholic extract showed slightly wider zone of inhibitions against all test microorganisms. In case of gram positive strains, both extracts showed good antimicrobial activity against *Bacillus subtilis* and *Bacillus cereus* than *Staphylococcus aureus*. Also *Candida albicans* is found to be more sensitive to both extracts than *Saccharomyces cerevisiae*. Activity of alcoholic extract was similar to that of conventional antibiotic amphotericin B in case of *Saccharomyces cerevisiae*. Ciprofloxacin and amphotericin showed prominent zone of inhibitions and MIC against test bacterial and fungal strains respectively.

MIC values of both extracts, by agar dilution method against all bacterial and fungal strains are also shown in Table 1. No extract showed sensitivity against gram negative strains except *Pseudomonas aeruginosa* at 500 µg/ml for both extracts. MIC values of petroleum ether and alcoholic extracts against *Candida albicans* and *Saccharomyces cerevisiae* were 300 µg/ml and 200 µg/ml respectively. The lower MIC values of both the extracts against *Bacillus subtilis* and *Bacillus cereus* in comparison with *Staphylococcus aureus* showed their greater sensitivity towards the extracts of the *B. monosperma* gum.

DISCUSSION

Plants have provided a source of inspiration for novel drug compounds as plant-derived medicines have made significant contribution towards human health. Phytomedicines can be used for the treatment of diseases as is done in case of Unani and Ayurvedic system of medicines or it can be the base for the development of a medicine, a natural blueprint for the development of new drugs.

In the present study six different bacterial and two fungal strains were used to screen possible antimicrobial

activity of *B. monosperma* gum extracts. A result clearly indicates that both extracts of gum showed significant antimicrobial and antifungal activity.

We found that the extracts of the *B. monosperma* gum inhibited the gram-positive bacteria better than the gram-negative. Generally, plant extracts are usually more active against gram positive bacteria than gram-negative bacteria [15]. The range of MIC values for all the test microorganisms correlated well with the results obtained using the disc diffusion method.

The MIC values for both extracts against *Bacillus subtilis* and *Bacillus cereus* are lower when compared with *Staphylococcus aureus*. This shows that these gram-positive bacterial strains are more susceptible to the effect of the extracts of gum of *B.monosperma* with respect to its effect against *Staphylococcus aureus.*

Coumarins [16, 17, 18] and triterpenes [19, 20, 21] in plant extracts were found to be possess antimicrobial activity so it can be said that coumarins from petroleum ether extract and triterpenes from alcoholic extract may be responsible for proposed activity.

CONCLUSION

Both extracts of the plant produced good inhibition zones against the test organisms. So it is expected that they could be used to treat infections and diseases caused by these organisms and if the active ingredients of the extracts are isolated and possibly crystallized, therapeutic antibiotics could be produced from these compounds.

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REFERENCES

1. Tepe B, Daferera D, Sokmen M, Polissiou M, Sokmen A. *In vitro* antimicrobial and antioxidant activities of the essential oils and various extracts of *Thymus eigii* M. Zohary et P.H. Davis. *J Agric Food Chem* 2004; 52:1132-1137.
2. Jones FA. Herbs – useful plants. Their role in history and today. *Euro J Gastroenterol Hepatol* 1996; 8:1227-1231.
3. Reynolds JEF. Martindale – the Extra Pharmacopoeia. 31st edition, London; Royal Pharmaceutical Society of Great Britain; 1996.
4. Lis-Balchin M, Deans SG. Bioactivity of selected plant essential oils against *Listeria monocytogenes*. *J Appl Bacteriol* 1997; 82:759-762.
5. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal plants. Council of Scientific and Industrial Research: New Delhi, 1956.
6. Agarwal AK, Tripathi DM, Sahai R, Gupta N, Saxena RP, Puri A, Singh M, Misra RN, Dubey CB, Saxena KC. Management of giardiasis by a herbal drug 'Pippali Rasayana': a clinical study. *J Ethnopharmacol* 1997; 56: 233–236.
7. Kasture VS, Chopde CT, Deshmukh VK. Anticonvulsive activity of Albizzia lebbeck, *Hibiscus rosa sinesis* and *Butea monosperma* in experimental animals. *J Ethnopharmacol* 2000; 71: 65–75.
8. Gupta SR, Ravindranath B, Seshadri TR. Glucosides of *Butea monosperma*. *Phytochemistry* 1970; 9: 2231–2235.
9. Wagner H, Geyer B, Fiebig M, Kiso Y, Hikino H. Isobutrin and butrin, the antihepatotoxic principles of *Butea monosperma* flowers. *Planta Med* 1986; 2: 77–79.
10. Vaidyaratnam P. Indian Medicinal Plants, Vol 1. Madras: Orient Longman Publisher, 1995. pp. 314.
11. Kirtikar K, Basu B. Indian Medicinal Plants, Vol 1. Periodical experts, 1975. pp. 785.
12. Ethnobiological investigation in Maharashtra (No. 14/17/90-MAB-RE): Project Report 1991-97, Major research project financed by Department of Environment and Forest, Govt. of India, New Delhi.
13. Andrews JM. BSAC standardized disc susceptibility testing method. *J Antimicrob Chemother* 2001; pp. 48-57.
14. NCCLS (National Committee for Clinical Laboratory Standards): Methods for dilution antimicrobial susceptibility tests of bacteria that grow aerobically. In *Approved Standard M100-S12* Wayne. PA, NCCLS; 2002.
15. Lin J, Opaku AR, Geheeb-Keller M, Hutchings AD, Terblanche SE, Jäger AK. Preliminary screening of some traditional Zulu medicinal plants for anti-inflammatory and anti-microbial activities. *J Ethnopharmacol* 1999; 68: 267-74.
16. Hoult JRS, M Paya. Pharmacological and biochemical actions of simple coumarins: natural products with therapeutic potential. *Gen Pharmacol* 1996; 27:713–722.
17. Soine TO. Naturally occurring coumarins and related physiological activities. *J Pharm Sci* 1964; 53:231–264.
18. Scheel LD. The biological action of the coumarins. *Microbiol Toxins* 1972; 8:47–66.
19. Barre JT, Bowden BF, Coll JC, Jesus J, Fuente VE, Janairo GC, Ragasa CY. A bioactive triterpene from *Lantana camara*. *Phytochemistry* 1997; 45: 321–324.
20. Scortichini M, Pia Rossi M. Preliminary *in vitro* evaluation of the antimicrobial activity of terpenes and terpenoids towards *Erwinia amylovora* (Burrill) Winslow *et al*. *J Appl Bacteriol* 1991; 71:109–112.
21. Mendoza L, Wilkens M, Urzua A. Antimicrobial study of the resinous exudates and of diterpenoids and flavonoids isolated from some Chilean *Pseudognaphalium* (Asteraceae). *J Ethnopharmacol* 1997; 58: 85–88

CURRENT AUTHOR ADDRESSES

Shailendra S. Gurav, Department of pharmaceytical Sciences, Campus, Nagpur-33, Maharashtra, India. E-mail: shailugurav@rediffmail.com (Corresponding author)

Vijay D. Gulkari, Department of pharmaceytical Sciences, Campus, Nagpur-33, Maharashtra, India.

Nandkishore J. Duragkar, Department of pharmaceytical Sciences, Campus, Nagpur-33, Maharashtra, India.

Arun T. Patil, Department of pharmaceytical Sciences, Campus, Nagpur-33, Maharashtra, India.