

PHYTOCHEMICAL SCREENING, CHARACTERIZATION AND *IN VITRO* ANTIMICROBIAL ACTIVITY OF *BUTEA MONOSPERMA* FLOWER, LEAVES AND GUM: METHANOLIC AND AQUEOUS EXTRACT

M. O. MALPANI*¹, P. R. RAJPUT², V. D. MANE¹, A. R. DESHPANDE³

¹Department of Chemistry, S. K. College, Akola, ²Department of Chemistry, V. B. M. V. Amravati, ³Department of Microbiology, S. K. College, Akola. Email: manojkumar732@rediffmail .com

Received: 17 Aug 2011, Revised and Accepted: 19 Oct 2011

ABSTRACT

The present paper deals with the Characterization, Phytochemical determination and antimicrobial activity of *Butea monosperma*, an important medicinal tree in India. The *in-vitro* antimicrobial activity of water and methanol extracts were studied by cup plate agar well diffusion method against pathogenic bacteria viz., *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*. Among the solvents used, the water extract was found to be more effective against all pathogenic bacteria. The methanol extract does not inhibit *Escherichia coli* and *Pseudomonas aeruginosa*. After characterization acid contents were separate out this found to be more effective against all the bacteria used. Non Acid content of Gum inhibit the activity of *Bacillus subtilis* and *Pseudomonas aeruginosa*. Activities of the various extracts were comparable to those of standard antibacterial agent Ampicillin and Vancomycin as control. The results provided evidence that the studied plant might indeed be potential source of phytochemical constitution, antimicrobial agent and showing that this plant can be used as a complementary source for traditional medicines.

Keywords: *Butea monosperma*, Antimicrobial, Characterization, Phytochemical screening.

INTRODUCTION

It is evident that without nature human being life is impossible. There are three basic necessity of humans is food, clothes and shelter and now the fourth one is good health, which provided by plant kingdom. Nature stands a golden mark and provided the storehouse of remedies to cure all ailments of mankind. Plant kingdom represents a rich house of organic compounds, many of which have been used for medicinal purposes and could serve as lead for the development of novel agents having good efficacy in various pathological disorders in the coming years. Herbs have always been the principal form of medicine in India and presently they are becoming popular throughout the world, as people strive to stay healthy in the face of chronic stress and pollution, and to treat illness with medicines that work in count with the body's own defence. There is a widespread belief that green medicines are healthier and more harmless or safer than synthetic ones (1).

In traditional medicine, there are many natural crude drugs that have the potential to treat many disease and disorders one of them is *Butea monosperma* popularly known as 'dhak' or 'palas', commonly known as 'Flame of forest'. It has 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-inflammatory activity (2).

Roots of *B. monosperma* are reported to be useful in the treatment of filariasis, night blindness, helminthiasis, piles, ulcers and tumours. Pippali rasayana, an Indian Ayurvedic drug, employs *B. monosperma* and is used in the management of giardiasis (3).

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 (4). *B. monosperma* flowers contain butin, butein, butrin, isobutrin, palasitrin, corepsin, isocorepsin, chalcones, and aurones (5). Gum is useful as astringent, depurative and useful in diarrhoea, haemorrhoids, haemoptysis, haematemesis, leprosy, skin diseases (6-7). In some tribes (Banjara) from Maharashtra (India), gum of *B. monosperma* is used to treat microbial and fungal infections (8). To substantiate this claim, the present study was undertaken to evaluate the antimicrobial potential of leaves, flower and gum extract of *B. monosperma* by disc diffusion assay and broth dilution assay.

MATERIALS AND METHODS

Plant material

The leaves, flower, and Gum of *Butea monosperma* plant were collected from the Melghat region of Amravati, District of Maharashtra, India, in the month of December – February and it was authenticated by the taxonomists Dr. S. P. Rothe from the Department of botany, Shri Shivaji College Akola. A voucher specimen (ML-101) was deposited in the herbarium of Department of Botany, Shri Shivaji College, Akola.

Extraction and isolation

The leaves, flower, and Gum of *Butea monosperma* plant were shade dried at room temperature, ground in a manual mill to get coarse powder. The powder were kept in the air tight polythene bags and stored at dry place. The powder was extracted with solvent water and methanol by using soxhlet apparatus. The extracts were concentrated for further studies at 40 °C in water bath. Test extracts were then dried, crushed and stored in air tight bottle for further study. The water extracts of flower were screened for different phytochemical constituents. All the extracts were characterized for different constituents and screened for antimicrobial tests.

Phytochemical Screening

The chemical tests were performed for testing different chemical groups present in water extract of flower (9).

- 1) **Test for Sugar**
 - a) Molisch's Test: - Positive
 - b) Iodine Test: - Negative
- 2) **Test for Flavonoids**
 - a) Shinoda test: - Positive
 - b) H₂SO₄ test: - Negative
- 3) **Test for Sterols**
 - a) Salkowaski test: - Positive
 - b) Vanillin test: - Positive
- 4) **Test for Alkaloids**
 - a) Wagner's reagent test: - Negative
 - b) Mayer's reagent test: - Negative
- 5) **Test for Tannin**
 - a) FeCl₃ test: - Positive
 - b) Lead acetate test: - Negative
- 6) **Test for Protein and Amino acid**
 - a) Biuret test: - Negative

b) Xanthoprotein test: - Negative

7) **Test for Resin**

a) NaOH test:- Positive

Chemicals

Mueller Hinton agar, SDA (Himedia Lab); Methanol (Ranbaxy laboratories Ltd.); Standard Discs of Ampicilin, Vancomycin (Himedia Lab); Nutrient Broth, PDA (Himedia Lab)

Microorganisms Used

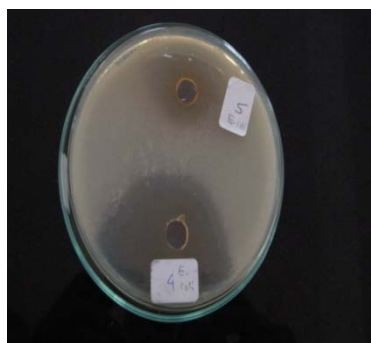
The microorganisms which are used for the antibacterial activity were brought from National Chemical Laboratory Pune. These are as follows.

Bacteria :- *Escherichia coli* NCIM No. 2931, ATCC No. 25922, *Staphylococcus aureus* NCIM No. 5021, ATCC No. 25923, *Bacillus subtilis* NCIM No. 2063, ATCC No. 6633, *Pseudomonas aeruginosa* NCIM No.2036, ATCC No.19429.

Antibacterial Assay

The methanol and water extracts were examined for their antibacterial potency by Cup plate agar method (10) against four bacterial species viz., *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*. Petri plates were prepared with 25ml sterile Mueller Hinton Agar. A sterile cork borer (8 mm) was used to make wells in each plate. 1 ml inoculum suspension was swabbed uniformly over the agar medium to get uniform distribution of bacteria. These plates were labelled and 100µl of each plant extracts (at concentration of 200 mg/ml) was added aseptically into the well. The Petri plates were then incubated at 37°C for 24 hrs during which the activity was evidenced by the presence of zone of inhibition surrounding the well. The negative control was prepared using respective solvent. Ampicilin disc (10 mcg/disc) and Vancomycin disc (30 mcg/disc) were used as positive control. The zone of inhibition was recorded in millimetres by using Himedia Zone Reader Scale.

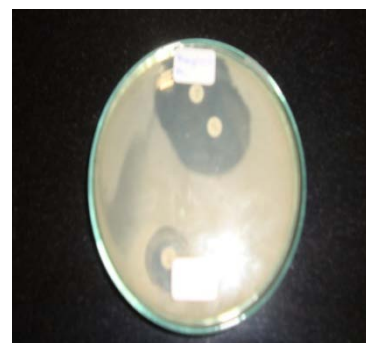
Antibacterial activity against *E.coli*



Activity against *E. Coli* for extracts 4 & 5

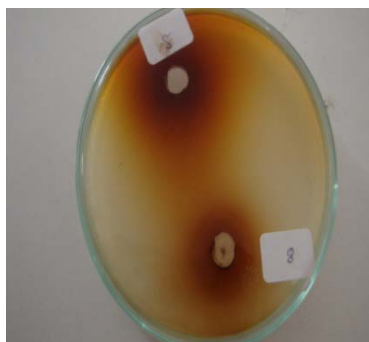


Activity against *E. Coli* for extracts 7 & 8



Control 11 & 12 against *E. Coli*

Antibacterial activity against *S.aureus*

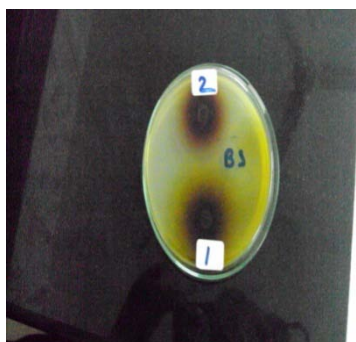


Activity against *S. aureus* for extracts 5 & 8

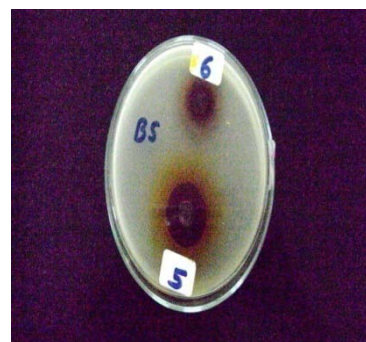


Control 11 & 12 against *S. aureus*

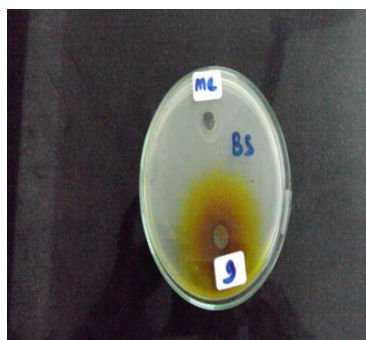
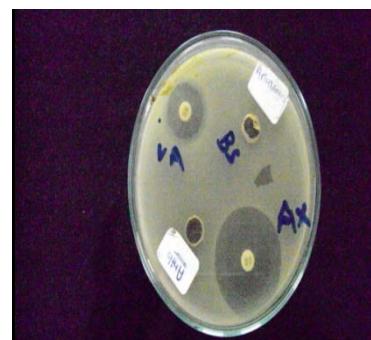
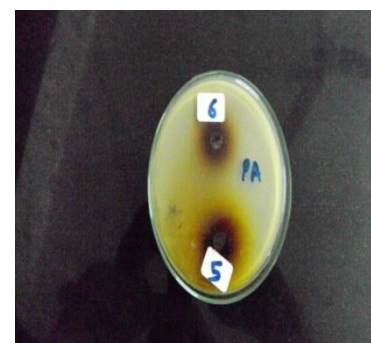
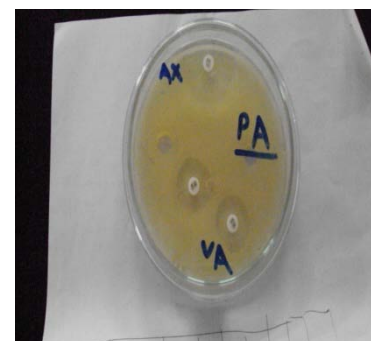
Antibacterial activity against *B.subtilis*



Activity against *B.subtilis* for Extracts 1 & 2



Activity against *B.subtilis* for extracts 5 & 6

Activity against *B.subtilis* for extracts 3&4Activity against *B.subtilis* for extracts 9 & control methanolActivity against *B.subtilis* for control 11 & 12Antibacterial activity against *P.aeruginosa*Activity against *P.aeruginosa* for extracts 3 & 4Activity against *P.aeruginosa* for extracts 1 & 2Activity against *P.aeruginosa* for extracts 5 & 6Activity against *P.aeruginosa* for extracts 7 & 8Activity against *P.aeruginosa* for extracts 9 & control methanolActivity against *P.aeruginosa* for control 11 & 12Scheme No. 1: Photos showing the Antibacterial activity of different extracts of the plant *Butea monosperma*.Table 1: Antibacterial activity of different extracts of the plant *Butea monosperma*

Extracts	Concentration	Inhibitory zones in mm			
		<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Pseudomonas aeruginosa</i>
Water extract of leaves	200 mg/ml	16mm	12mm	38mm	16mm
Water extract of Gum	200 mg/ml	--	--	36mm	---
Water extract of Flower	200 mg/ml	--	14mm	21mm	15mm
Acid content of Gum (H ₂ O Extract)	200 mg/ml	36mm	18mm	26mm	26mm
Acid content of Flower (H ₂ O Extract)	200 mg/ml	18mm	24mm	26mm	19mm
Non Acid content of Gum (H ₂ O Extract)	200 mg/ml	---	---	25mm	22mm
Methanol extract of leaves	200 mg/ml	---	15mm	---	---
Methanol extract of Gum	200 mg/ml	---	19mm	---	---
Methanol extract of Flower	200 mg/ml	---	---	15mm	---
Methanol (Control)	----	---	---	18mm	14mm
Ampicilin disc (Control)	----	30mm	19mm	25mm	29mm
Vancomycin disc (Control)	----	20mm	13mm	20mm	19mm

RESULT AND DISCUSSION

The results obtained for the antibacterial tests performed on different solvent extracts of *B. monosperma* are presented in Table 1. Among the solvents used, the water extract was found to be more effective against all pathogenic bacteria. The methanol extract does not inhibit the growth of *Escherichia coli* and *Pseudomonas aeruginosa*. Acid contents separated from gum and flower, found to be more effective against all the pathogens under examination. Non Acid content of Gum inhibit the activity of *Bacillus subtilis* and *Pseudomonas aeruginosa* to a remarkable extent. The result obtain were assessed on their comparison with the activities of standard antibacterial agent like Ampicilin and Vancomycin as control. The differences in the observed activities of the various extracts may be due to varying degree of solubility of the active constituents in the solvents. It has been documented that different solvents have different solubility capacities for different phytochemical constituents (9).

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 systems of medicines. It may provide a natural blueprint for the development of new drugs. In the present study four different bacterial were used to screen the antimicrobial activity of *B. monosperma* extracts. A result clearly indicates that extracts showed significant antibacterial activity. So it is expected that they could be used to treat infections and diseases caused by these organisms.

ACKNOWLEDGEMENT

The author is grateful to Dr. P. R. Rajput, Associate Professor, Department of Chemistry, Vidyabharati Mahavidyalaya, Amravati for his gracious guidance. He acknowledge to the Dr. S. P. Rothe,

Department of Botany, Shri Shivaji College, Akola for the authentication of *B. monosperma* flower, leaves and gum.

REFERENCES

1. Sindhia VR, Bairwa R. Plant review: *Butea monosperma*. International Journal of Pharmaceutical and Clinical Research 2010; 2(2): 90-94.
2. Agarwal AK, Tripathi DM, Sahai R, Gupta N, Saxena RP, Puri A, et al. Management of giardiasis by a herbal drug 'Pippali Rasayana': A clinical study. J Ethnopharmacol 1997; 56: 233-236.
3. Kasture VS, Chopde CT, Deshmukh VK. Anticonvulsive activity of *Albizzia lebbek*, *Hibiscus rosa sinesis* and *Butea monosperma* in experimental animals. J Ethnopharmacol 2000; 71: 65-75.
4. Gupta SR, Ravindranath B, Seshadri TR. Glucosides of *Butea monosperma*. Phytochemistry 1970; 9: 2231-2235.
5. Vaidyaratnam P. Indian Medicinal Plants, Vol 1. Madras: Orient Longman Publisher; 1995. pp. 314.
6. Kirtikar K, Basu B. Indian Medicinal Plants, Vol 1. Periodical experts; 1975. pp. 785.
7. 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.
8. Lohitha et al, Phytochemical screening and in vitro antimicrobial activity of *Butea monosperma* (L) bark ethanolic and aqueous extract. IJPSR 2010; Vol. 1 (10): 150-155.
9. Dhale DA, Chamle DR, Panchal VH. Evaluations of Phytochemical constituents and antimicrobial activity of *Butea monosperma* (Fabaceae). Journal of Phytology 2010; 2(12): 17-21.
10. Kavanagh F. Analytical microbiology, Part II. Academic Press; New York: 1972. pp. 126.