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Evaluation of Antibacterial Potential of Butea Monosperma Leaf Extract

E. Shravya puri¹, A. Sabitha Rani^{2*}, G. Sulakshana³

²Department of Botany, University College of Science, Osmania University, Hyderabad-500007, India ^{1,3}Department of Botany, Osmania University College for Women, Koti, Hyderabad-500095, India

*Corresponding Author: sabitaammana@yahoo.com, mobile no.9290609610

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Abstract: Butea monosperma leaf extract was evaluated *in vitro* for its antibacterial activity against various bacterial species i.e. Bacillus subtilis, staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa using Agar well diffusion technique. Different concentrations of leaf (500- 2000μ g), extracted with petroleum ether was evaluated. All the concentrations of leaf extract shoed antibacterial activity. The antibacterial potential of extract increased with the increase in concentration of test solution. Higher activity was reported at 2000μ g of leaf extract in all four bacterial genera tested. Among all, high inhibition zone were observed against *P.aeruginosa* (1.9 cm) followed by *S.aureus* (1.8 cm), *B.subtilis* (1.7 cm) and *E.coli* (1.6 cm).

Keywords: Antibacterial activity, Butea monosperma, leaf extract, zone of inhibition, Bacillus subtilis, staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa

I. INTRODUCTION

Higher plants are the rich source of bioactive chemical constituents, which have various pharmacological and antimicrobial properties. From ancient times, these plant products have been used in the treatment of various diseases. The antimicrobial potential of plant products have been used in the development of many potent drugs in defence against insects, bacteria and fungi. The plants and their products can be exploited for production of natural protection agents for control of plant diseases. Extracts from various parts of plant like root, stem, leaf, flowers contains many secondary metabolites, which possess various antibacterial, antifungal and insecticidal properties [1]. Hence, the use of plant based pesticides is one of the best ways to reduce damage caused by synthetic chemicals.

Butea monosperma (Lam.) belongs to the family Fabaceae and commonly known as "Flame of the Forest". It is a medium sized tree, distributed in tropical and sub-tropical regions of Indian Subcontinent. B. monosperma is traditionally used as antitumor, anti-inflammatory, antidiabetic, antimicrobial, diuretic, anthelmintic, astringent carminative. Stem of B.monosperma is used in bleeding haemorrhoid disorder, dysmenorrhoeal, liver disorders, gonorrhoea, wound, worm infections, scorpion sting, cough and cold [2,3]. Seed of B. monosperma is used in the treatment of inflammation, urinary stones, abdominal troubles, intestinal worms and tumour. Gum is used in stomatitis, ring worm, leucorrhoea, sore throat [4]. The leaves of B. monosperma contain glucoside, kino-oil containing oleic, linoleic acid, palmitic and lignoceric acid [5]. The leaf extract possess many medicinal properties

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and employed in preparation of traditional medicinal formulations. The leaves are also utilized as a packing material. The orange dye obtained from flowers is used in preparation **Natural Holi colour** and also dyeing the fabric. In the present work, leaf extract of *Butea monosperma* was evaluated *in vitro* for its antibacterial activity using Agar cup bioassay.

II. RELATED WORK

In vitro evaluation of plant products and extracts for their antimicrobial activity is the primary step in the development of plant based antimicrobial agents. A vast number of medicinal plants have been recognized as valuable resources of plant products with antimicrobial properties. Majority of these are secondary metabolites which are defensive in nature and produced as a result of reciprocal interactions between plants and microbes. Hence, screening the plant extracts for their biological activity has an immense significance in development of natural antimicrobial agents. Hence, the study was designed to explore *in vitro* antibacterial potential of *Butea monosperma*, a widely distributed perennial tree.

III. MATERIALS AND METHOD

Collection of plant material

Fresh and healthy leaves were collected from the wildly occurring plants of *B. monosperma*. The leaves were washed thoroughly under running tap water, shade dried and powdered coarsely and used for the preparation of extract.

Dried leaf powder of 50 gm. was extracted with 500 ml of Petroleum ether ($60-80^{\circ}$ C) for 8-12 hrs using Soxhlet apparatus. The plant extract was concentrated to dryness with the help of rotary evaporator under reduced pressure. Different concentrations (0.5 mg to 2.0 mg) was prepared by dissolving the leaf extract in DMSO (Dimethyl Sulphoxide) and used for *in* vitro evaluation of antibacterial activity.

Bacterial cultures used for antibacterial activity:

Four different bacterial species were evaluated *in vitro* for antibacterial activity. Pure cultures of bacteria were obtained from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh, India. The test organisms were maintained on Nutrient Agar Media slants and stored in the refrigerator. The following are the list of bacterial cultures used for evaluation of antibacterial activity.

- 1. Pseudomonas aeruginosa (MTCC 25619)
- 2. *Staphylococcu saureus*(MTCC 9144)
- 3. Bacillus subtilis(MTCC 633)
- 4. Escherichia coli (MTCC 25922)

Preparation of Nutrient Agar Media and maintenance of bacterial cultures:

The test organisms were maintained on nutrient agar media slants and stored in the refrigerator at 4°C. To prepare the Nutrient agar media, 23.0 grams of nutrient agar media (Hi-media) was weighed and dissolved in 90ml of distilled water, stirred well and made up to one litre with the addition of distilled water. The media was autoclaved at 121°C at 15 psi for 20 minutes. The medium was poured into Petri dishes under aseptic conditions in a laminar flow chamber and left to solidify for evaluation of antibacterial activity. The cultures of the test organisms were maintained on nutrient agar slants.

In vitro evaluation of plant extract for antibacterial activity:

The screening for antibacterial activity of leaf extract of *Butea monosperma* was done by agar well diffusion technique [6]. The Petri dishes were inoculated with 0.5 ml of 24 hours old culture of bacteria. After inoculation, wells were cut out with 10mm sterile cork borer and the lids of the dishes were replaced. To each well different concentrations of plant extracts (500µg, 1000µg, 1500µg and 2000µg) were added. Controls were maintained with DMSO and standard antibacterial agent, Streptomycin and Penicillin G. Three replicates were maintained for each treatment. Results were noted after 24 hrs of incubation period by measuring the diameter of zone of inhibition in centimetres (cm).

IV. RESULT AND DISCUSSION

The *Butea monosperma* leaf extract exhibited antibacterial activity against the four test bacteria. The zone of inhibition increased with the increase in concentration of the test solution (Fig.1). Higher activity was observed at

 2000μ g in all the organisms tested (Fig.2). The antibacterial potential of various parts of medicinal plants have been reported in many medicinal plants [7,8,9,10].

In the present study, high inhibition zone were observed against *P.aeruginosa* (1.9 cm) followed by *S.aureus* (1.8 cm). Slightly lesser activity was observed in *E.coli* (1.6) and *B.subtilis* (1.7) (**Table.1**). Similarly, Rajput et al [11] reported significant antibacterial activity of leaf extract of *Butea monosperma*. In another study, antimicrobial efficiency of seed oil of *B. monosperma* was tested in *vitro* by filter paper disk method, against several human pathogenic bacteria and fungi [12]. The oil obtained from the bark of *Butea* showed a significant fungicidal and bactericidal activity [13].

V. CONCLUSION

In the present study, the leaf extract of Rutea monosperma exhibited antibacterial potential against four different bacterial genera. B. monosperma is medium sized deciduous tree, growing in all types of soils. The plants have abundant number of leaves and are the major source of bioactive properties. Hence, leaf extract of Butea monosperma can be utilized for production of cost effective and ecofriendly field formulations for control of many bacterial and fungal diseases of plants. The medicinal properties of leaves also can be exploited for development of clinically effective plant based therapeutics for control of major human and animal diseases.

Table.1. Antibacterial activity of *Butea monosperma* leaf extract against four different bacteria

Conc. of	Zones of inhibition (cm)			
extract	E.coli	P.aeruginosa	B.subtilis	S.aureus
(µg)		0		
500	0.7	0.8	0.5	0.5
1000	1.5	1.1	0.9	0.9
1500	1.6	1.5	1.5	1.2
2000	1.6	1.9	1.7	1.8



Fig.1. Antibacterial activity of Butea monosperma leaf extract

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Fig.2.Antibacterial activity of *Butea monosperma* leaf extract *E. coli* B-*P.aeruginosa* C-*B.subtilis* D-*S.aureus*

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AUTHOR PROFILES

Dr. Shravya Puri, presently working as a Lectures (PTL)I in Dept. of Botany Lecturer in University College for Women, Koti, Hyderabad (TS). She Completed M.Sc Botany with a specialization of Cytogenetics from Osmania University. She was awarded Ph.D. from Osmania



University on the topic of Evaluation of different plant extracts for the control of Wilt and Anthracnose diseases of *Capsicum annum* L. She had presented 8 papers in National and International Seminars and published more than eight research papers. She had attended five workshops and actively involved in various activities related to research and academics.

Dr. A.Sabitha Rani is presently working as Associate Professor, Dept. of Botany, Osmania University, Hyderabad. She completed M.Sc. Botany from Kakatiya University, Warangal and Ph.D. from CPMB, Dept. of Genetics , Osmania University. She worked as **Post**-



Doctoral Fellow at CSIR-IICT, Hyderabad under **DST Young Scientist & Women Scientist Program**. She has 30 years of research and 16 years of teaching experience. She published 12 book chapters,50 research papers in reputed journals and participated in 60 National and International conferences. She completed many **r**esearch projects and many student awarded Ph.D. under her guidance. Her research interests are Plant Biotechnology, Bio-evaluation and Plant secondary metabolite production through tissue culture

Dr.G.Sulakshana is presently working as Assistant Professor (C) in Botany Department, Osmania University College for Women, Hyderabad. She completed M.Sc. Botany with specialization 'Field Pathology and Plant Protection' from O.U.C.W, Koti, Hyderabad. She was awarded



Ph.D in Botany from Osmania University in Plant tissue culture. She had presented 13 papers in National and International Seminars, published more than fifteen research papers. She had attended six workshops and actively involved in research and academic activities.