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SCREENING AND CHARACTERIZATION OF ANTIBACTERIAL AGENTS FROM *BUTEA MONOSPERMA* (LAM.) TAUB SEED EXTRACTS

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ABSTRACT

In vitro antibacterial activity of *Butea monosperma* (Lam.) Taub. root extract was evaluated against five bacterial species. Among the tested organisms, *Proteus vulgaris* exhibited the highest inhibition at 100% concentration, followed by *Erwinia amylovora*, *Pectobacterium*, *Staphylococcus aureus*, and *Pseudomonas syringae*. The inhibition zones ranged from 20.0 mm to 31.0 mm for *E. amylovora* and *Pectobacterium*, while *P. vulgaris* and *S. aureus* showed 24.0 mm and 25.0 mm inhibition, respectively. The methanolic root extract was further subjected to thin-layer chromatography (TLC) to separate its bioactive components, yielding three fractions: Fraction I, Fraction II, and Fraction III. Among these, Fraction II demonstrated notable antibacterial activity against all tested strains, with marked inhibition observed in *E. coli*, *Pectobacterium*, *P. vulgaris*, *P. syringae*, and *S. aureus*. *E. amylovora* exhibited the greatest sensitivity, showing inhibition zones ranging from 31.0 mm to 39.0 mm across different concentrations, followed by *P. vulgaris* and *S. aureus*, which showed inhibition between 23.0 mm and 34.0 mm.

KEYWORDS: *B. monosperma, antibacterial activity, bioactive compound.*

INTRODUCTION

The excessive use of commercial antimicrobial drugs in treating infectious diseases has led to the development of multiple drug resistance in both human and plant pathogens (Jamuna *et al.*, 2011). Medicinal plants, endowed with rich botanical diversity, have been utilized for thousands of years as natural remedies (Artu et al., 2020). With the growing interest in traditional medicine, the use of herbal medicines continues to rise, as many individuals increasingly practice herbal self-medication (Mahamane Idi Issa Abdoulahi *et al.*, 2023; Eisenberg *et al.*, 1993).

The therapeutic value of medicinal plants is primarily attributed to the presence of phytochemicals such as tannins, phenolics, flavonoids, alkaloids, anthocyanins, essential oils, and terpenoids. In India, nearly two-thirds of the plant species used in modern medicine are indigenous (Joshi *et al.*, 2009). Herbal treatments are generally regarded as safe and harmonious with nature, offering an effective alternative for managing various health conditions and diseases (Ali *et al.*, 2008). In the present study, the methanolic extract and bioactive compounds of *Butea monosperma* (Lam.) Taub. L. root, belonging to the family Fabaceae, were evaluated for their antibacterial activity.

MATERIALS AND METHODS

Plant Material: Healthy and disease-free seeds of *Butea monosperma* (Lam.) Taub. were collected from Mysore. The seeds were thoroughly washed two to three times with running tap water, followed by a final rinse with sterile distilled water. The root material was then air-dried under shade on a sterile blotter and subsequently used for extraction.

Solvent extraction: Thoroughly washed seeds of *Butea monosperma* (Lam.) Taub. were shade-dried for five days and then ground into a fine powder using a Waring blender. A quantity of 25 grams of the dried powder was

placed in a thimble and successively extracted with methanol using a Soxhlet apparatus for 48 hours. The obtained solvent extract was concentrated under reduced pressure, and after complete evaporation, 1 gram of the concentrated extract was dissolved in 9 ml of methanol for use in the antibacterial assay (Lalitha *et al*., 2011).

Test pathogens: Five pathogenic bacterial strains — *Escherichia coli*, *Enterobacter aerogenes*, *Proteus vulgaris*, *Bacillus cereus*, and *Staphylococcus aureus* — were obtained from the Research Center, Pooja Bhagavat Memorial Mahajana P.G. Centre, K.R.S. Road, Metagalli, Mysore. The cultures were subcultured on nutrient agar medium and incubated at 37°C for 24 hours. After incubation, the bacterial cultures were aseptically preserved at low temperature for future use.

Preparation of Inoculum

Preparation of standard culture inoculums of test organism: All the test bacterial strains were inoculated into 2 ml of nutrient broth and incubated at 37°C for 24 hours, until the turbidity of the broth matched the 0.5% McFarland standard, as recommended by the WHO (Bole et al., 2010).

Separation of different fractions by Thin Layer Chromatography (TLC)

***Preparation of TLC plates and separation of fractions*:** Five glass plates (20 × 20 cm) were prepared for TLC by coating with silica gel. The plates were thoroughly cleaned with detergent, rinsed with distilled water, wiped with acetone to remove grease, and air-dried. A slurry of 25 g silica gel in 60–70 ml distilled water was prepared and evenly spread on the plates using a TLC spreader set at a 0.25 mm thickness. The coated plates were dried at 110–120°C overnight and cooled in a desiccator before use. The concentrated methanolic extract of *Butea monosperma* was dissolved in 10 µl of methanol and spotted onto the prepared plates. The plates were developed using a methanol:chloroform solvent system (9:1). When the solvent front reached three-fourths of the plate, the plates were removed and observed under visible light and UV light (254 nm and 366 nm). Three distinct fractions were obtained, and the Rf values were calculated using the formula-Rf = Distance moved by compound / Distance moved by solvent front**.** Each fraction was collected and used for antibacterial activity testing (Sharma et al., 2009).

Separation of different fractions: After the separation and identification of the Rf values, each fraction—consisting of three distinct bands—was carefully marked and individually collected. The silica gel portions containing the separated bioactive compounds were gently scraped from the TLC plates using a clean spatula and transferred into sterile beakers. To extract the compounds from the silica gel, each sample was treated with methanol and thoroughly mixed to ensure complete dissolution of the bioactive constituents. The resulting mixture was filtered through Whatman No. 1 filter paper to remove silica residues. The clear filtrate containing the dissolved compounds was then collected and allowed to evaporate at room temperature until complete dryness. The residue obtained after solvent evaporation represented the purified bioactive fractions. The total yield of each fraction was measured and recorded for further antibacterial activity analysis.

Antibacterial activity of different fractions: The dried residues obtained from the different TLC bands were dissolved in 1 µl of methanol to prepare stock solutions. From these stock solutions, various concentrations — 500 ppm, 1000 ppm, 1500 ppm, and 2000 ppm — were prepared for testing antibacterial activity. Nutrient agar medium was prepared and supplemented with the respective concentrations of each fraction. As a control, nutrient agar plates containing equivalent concentrations of methanol (without extract) were also prepared to ensure that the solvent itself did not influence bacterial growth. Each nutrient agar plate was uniformly inoculated with the test bacterial cultures using sterile cotton swabs to achieve a consistent lawn of bacterial growth. After inoculation, the plates were incubated at 37°C for 24 hours. Following incubation, the antibacterial activity of each fraction was evaluated by measuring the zone of inhibition (in millimeters) around the sample spots. The diameter of the inhibition zones indicated the effectiveness of the respective fractions and concentrations against the tested bacterial strains.

Antibacterial assay of Bioactive compound: The same procedure was followed as aqueous extract for Bioactive compound tested at 100 to 1000ppm concentration compared to synthetic antibiotic Chloramphenicol

RESULT

Antibacterial activity of methanol extract: *Proteus vulgaris* exhibited the highest antibacterial activity among the tested bacterial species, showing a maximum inhibition zone of 36.0 mm at 100% concentration, followed by 35.0 mm at 90% concentration and 34.0 mm at concentrations ranging from 40% to 80%. *Erwinia amylovora* and *Pectobacterium* also demonstrated strong antibacterial responses, with inhibition zones ranging from 20.0 mm to 31.0 mm across the concentration gradient of 10% to 100%. Similarly, *Staphylococcus aureus* showed inhibition zones varying between 19.0 mm and 31.0 mm across the same concentration range. The least antibacterial activity was observed in *Pseudomonas syringae*, which recorded inhibition zones between 19.0 mm and 25.0 mm at concentrations from 10% to 100%. When compared to the standard antibiotic tetracycline**,** the methanolic extract of *Butea monosperma* (Lam.) Taub. root exhibited comparable antibacterial efficacy. The inhibition zones recorded for the standard antibiotic were 22.0 mm for *E. amylovora*, 21.0 mm for *Pectobacterium*, 23.0 mm for *P. vulgaris*, 24.0 mm for *S. aureus*, and 25.0 mm for *P. syringae*. These results, summarized in Table 1, indicate that the methanolic extract of *B. monosperma* possesses notable antibacterial properties, particularly against *P. vulgaris*,

suggesting the presence of potent bioactive compounds effective against both Gram-positive and Gram-negative bacteria.

Table 1: Antimicrobial activity of Methanol extract of *B. monosperma (Lam.) Taub.* L.(Root)against important species of bacteria.

Bacteria	Inhibition (mm) Methanol Concentration of the solvent extract										Standard Antibiotic Chloramphenicol 25mg
	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%	
E. coli	20.0 ±0.0	22.0 ±0.0	22.0 ±0.1	23.0 ±0.1	24.0 ±0.0	24.0 ±0.1	26.0 ±0.0	27.0 ±0.1	29.0 ±0.1	31.0 ±0.1	22.0±0.0
Pectobacteria	20.0 ±0.1	21.0 ±0.1	22.0 ±0.0	24.0 ±0.1	24.0 ±0.1	25.0 ±0.0	26.0 ±0.1	27.0 ±0.0	30.0 ±0.1	31.0 ±0.1	21.0±0.0
P. vulgaris	23.0 ±0.1	27.0 ±0.0	31.0 ±0.1	32.0 ±0.1	32.0 ±0.0	33.0 ±0.1	33.0 ±0.0	34.0 ±0.1	35.0 ±0.1	36.0 ±0.0	23.0±0.0
S. aureus	19.0 ±0.0	21.0 ±0.1	21.0 ±0.1	22.0 ±0.1	24.0 ±0.1	26.0 ±0.0	29.0 ±0.1	29.0 ±0.1	30.0 ±0.1	31.0 ±0.1	24.0±0.0
P. syringae	19.0 ±0.0	20.0 ±0.1	21.0 ±0.1	22.0 ±0.1	22.0 ±0.0	23.0 ±0.1	24.0 ±0.1	24.0 ±0.1	25.0 ±0.0	25.0 ±0.0	25.0±0.0

- Values are the mean of five replicates, ±standard error.
- The means followed by the same letter (s) are not significantly different at P 0.05 when subjected to Tukey's HSD.
- Pattern of percentage inhibition increase is not uniform for all the microorganism

Separation of different fractions and determination of R_f value: The methanolic extract of *Butea monosperma* (Lam.) Taub. root was subjected to thin-layer chromatography (TLC) to separate its bioactive components. Upon development of the chromatogram, three distinct bands were clearly observed, each representing a different fraction of the extract based on their movement along the silica gel plate. These were designated as Fraction I**,** Fraction II, and Fraction III. The Rf values (retention factor values) for the separated bands were determined to assess their relative mobility within the solvent system. Fraction I exhibited Rf values ranging from 1.0 to 2.7**,** Fraction II showed Rf values between 2.7 and 5.0, and Fraction III displayed Rf values from 5.0 to 6.7 (Table 2). Each of these fractions was carefully collected and preserved for further bioassay testing. Among them, Fraction II was noted for its distinct coloration and intensity under UV illumination, suggesting a higher concentration of bioactive compounds. These fractions were subsequently evaluated for their antibacterial potential against selected pathogenic bacterial strains.

Table 2: Separation of different fractions of *B. monosperma (Lam.) Taub.* L. (seed) and determination of R_f value.

Methanol extract		
Fraction I	**Fraction II**	**Fraction III**
R_f value		
1.0-2.7	2.7-5.0	5.0 to 6.7

Antibacterial activity of different fractions: Among the three fractions separated from the methanolic extract of *Butea monosperma* (Lam.) Taub. root, each was tested at a concentration of 50 µl against all the selected pathogenic bacterial strains to evaluate their antibacterial efficacy. Of the three, Fraction II exhibited the most pronounced antibacterial activity against all tested organisms. The inhibition zones recorded were 22.0 mm for *Escherichia coli*, 25.0 mm for *Pectobacterium*, 31.0 mm for *Proteus vulgaris*, 22.0 mm for *Pseudomonas syringae*, and 24.0 mm for *Staphylococcus aureus* (Table 3). These results clearly indicate that Fraction II contains potent bioactive compounds responsible for strong antibacterial action. Notably, *Proteus vulgaris* showed the highest sensitivity to this fraction, suggesting that the active constituents in Fraction II may possess broad-spectrum antibacterial potential effective against both Gram-positive and Gram-negative bacteria.

Table 3: Antibacterial activity of different fractions of Methanol extract of *B. monosperma (Lam.) Taub.*L. (Root)

Bacteria	Methanol extract			Standard Antibiotics Chloramphenicol 25mg
	Fraction I	Fraction II	Fraction III	
	Concentration of the plant extract			
E.coli	–	22.0±0.0	–	22.0±0.0
Pectobacteria	–	25.0±0.1	–	21.0±0.0
P.vulgaris	–	31.0±0.0	–	23.0±0.0
S.aureus	–	24.0±0.0	–	24.0±0.0
P.syringae	–	22.0±0.0	–	25.0±0.0

Antibacterial activity of Bioactive compound: The bioactive compound isolated from Fraction II exhibited an Rf value of 2.8, indicating a distinct and well-separated component on the TLC plate. When evaluated for antibacterial activity at varying concentrations (400 ppm, 500 ppm, 600 ppm, 700 ppm, 800 ppm, 900 ppm, and 1000 ppm), Fraction II showed strong inhibitory effects against all tested bacterial strains. The highest activity was observed against *Erwinia amylovora*, which recorded inhibition zones of 31.0 mm**,** 33.0 mm**,** 34.0 mm**,** 36.0 mm**,** 37.0 mm**,** 38.0 mm**,** and 39.0 mm across the respective concentrations. *Proteus vulgaris* followed closely, with inhibition zones of 30.0 mm**,** 30.0 mm**,** 31.0 mm**,** 32.0 mm**,** 32.0 mm**,** 34.0 mm**,** and 36.0 mm at the same concentration range. *Staphylococcus aureus* also exhibited significant antibacterial activity, showing inhibition zones ranging from 23.0 mm to 34.0 mm. Moderate antibacterial activity was observed in *Pseudomonas syringae* and *Pectobacterium*, which recorded inhibition zones of 23.0 mm and 21.0 mm**,** respectively, at the highest concentration (1000 ppm).When compared to the standard antibiotic Chloramphenicol, the inhibition zones recorded were 22.0 mm for *E. amylovora*, 21.0 mm for *Pectobacterium*, 23.0 mm for *P. vulgaris*, 24.0 mm for *S. aureus*, and 25.0 mm for *P. syringae* (Table 4).

Table 4: Antibacterial activity of Bioactive compound of *B. monosperma (Lam.) Taub.* L. (Root) against important species of bacteria.

Bacteria	Concentration of the Bioactive compound										Chloramphenicol 25mg
	100 ppm	200 ppm	300 ppm	400 ppm	500 ppm	600 ppm	700 ppm	800 ppm	900 ppm	1000 ppm	
E. coli	23.0 ±0.0	26.0 ±0.0	29.0 ±0.0	31.0 ±0.0	33.0 ±0.0	34.0 ±0.0	36.0 ±0.0	37.0 ±0.0	38.0 ±0.1	39.0 ±0.0	22.0±0.0
Pectobacteria	10.0 ±0.1	11.0 ±0.1	13.0 ±0.0	14.0 ±0.0	15.0 ±0.0	16.0 ±0.1	17.0 ±0.0	18.0 ±0.0	20.0 ±0.1	21.0 ±0.0	21.0±0.0
P.vulgaris	25.0 ±0.0	27.0 ±0.0	28.0 ±0.0	30.0 ±0.0	30.0 ±0.0	31.0 ±0.1	32.0 ±0.0	32.0 ±0.0	34.0 ±0.1	36.0 ±0.0	23.0±0.0
S.aureus	23.0 ±0.0	25.0 ±0.0	28.0 ±0.1	29.0 ±0.0	29.0 ±0.1	30.0 ±0.1	30.0 ±0.1	31.0 ±0.0	32.0 ±0.0	34.0 ±0.1	24.0±0.0
P.syringae	12.0 ±0.1	14.0 ±0.0	15.0 ±0.0	16.0 ±0.0	16.0 ±0.0	17.0 ±0.0	18.0 ±0.0	19.0 ±0.1	20.0 ±0.0	23.0 ±0.1	25.0±0.0

- Values are the mean of five replicates, ±standard error.
- The means followed by the same letter (s) are not significantly different at P 0.05 when subjected to Tukey's HSD.
- Pattern of percentage inhibition increase is not uniform for all the microorganisms

DISCUSSION

According to the World Health Organization (2003)**,** approximately 80% of the global population relies on traditional medicine to meet their primary healthcare needs. Among the countries rich in medicinal plant diversity, India holds a prominent position as one of the twelve recognized mega biodiversity hotspots in the world. It is estimated that India possesses nearly one-fifth of all known medicinal plant species**,** with around 25,000 plant-based formulations currently being utilized in folk and indigenous medicine systems by rural and tribal communities.

Medicinal plants form an invaluable component of the world's natural wealth, serving as a primary source of healthcare and contributing significantly to the discovery of new therapeutic agents. Their bioactive compounds, including alkaloids, flavonoids, terpenoids, phenolics, and tannins, have been shown to exhibit diverse pharmacological properties such as antimicrobial, anti-inflammatory, antioxidant, and anticancer activities.

However, the growing incidence of infectious diseases and the rapid emergence of antibiotic-resistant pathogens have created an urgent need to identify and develop novel bioactive compounds from natural sources. Plant-based antimicrobials, in particular, represent a vast and largely untapped reservoir of potential therapeutic agents

that could serve as effective alternatives to synthetic antibiotics.

In this context, *Butea monosperma* (Lam.) Taub. (L.)**,** a plant traditionally used in Ayurvedic and folk medicine, has shown promising potential as a potent antibacterial agent**.** Its root, bark, flowers, and seeds are known to contain several phytochemicals with proven medicinal properties. Continued research on the isolation, characterization, and evaluation of these bioactive constituents could lead to the development of new, safe, and effective plant-derived antimicrobial drugs, contributing significantly to global health and sustainable medical practices.

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