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Butea monosperma and its endophytes as promising sources of antimicrobial and antioxidant compounds

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Abstract
Butea monosperma (Lam.) Taub., commonly known as Palash or Flame of the Forest, is a medicinally important leguminous tree widely utilized in Ayurvedic, Unani, and folk medicine for treating inflammatory, infectious, and hepatic disorders. This study investigated the phytochemical profile and pharmacological potential of *B. monosperma*, with particular emphasis on its endophytic fungi as potential sources of bioactive compounds. Fresh, authenticated leaves were processed for the isolation of endophytic fungi using surface-sterilized explants cultured on Potato Dextrose Agar. Ethyl acetate extracts of these isolates were analyzed by UV-Vis spectroscopy and GC-MS. Antibacterial activity was determined via agar well diffusion against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, while antioxidant capacity was assessed using the DPPH radical scavenging assay. A total of 42 distinct fungal isolates were obtained, exhibiting 64% colonization frequency. Nine isolates demonstrated significant antibacterial activity, and extracts showed strong antioxidant potential with up to 63.89% inhibition at 100 µg/mL. GC-MS analysis identified several bioactive metabolites, including heptacosane, and di-n-decylsulfone etc. These findings highlight *B. monosperma* and its endophytes as promising sources of antimicrobial and antioxidant compounds, supporting its ethnomedicinal relevance and potential for drug discovery.

Keywords: *Butea monosperma*, Endophytic fungi, bioactive compounds, Antibacterial activity, Antioxidant capacity, GC-MS analysis

Introduction
Butea monosperma (Lam.) Taub. Known as the "Flame of the Forest" or "Palash", is a deciduous leguminous tree of the family Fabaceae and subfamily Papilionoideae. Mature trees typically range from 12-15 m in height, sometimes reaching 20 m, with a crooked trunk and rough greyish-brown bark exuding a red sap. The species is distinguished by its trifoliate, leathery leaves and its dense, vibrant orange-red racemose inflorescence produced on leafless branches (Orwa *et al*., 2009; Das, 2022) [1, 9]. The fruits are oblong, hairy pods (10-24 cm), each containing a single, flattened ellipsoid seed, as the specific epithet "monosperma" suggests (Orwa *et al*., 2009) [1]. *B. monosperma* is native to the tropical and subtropical regions of the Indian subcontinent, distributed widely in India, Nepal, Myanmar, and Bangladesh, as well as pockets of Sri Lanka, Indonesia, and Southeast Asia. It flourishes in dry deciduous forests, open plains, grasslands, wastelands, and along roadsides, up to around 1500 meters altitude, exhibiting marked ecological adaptability to drought, grazing, variable soils, and disturbed habitats (Orwa *et al*., 2009;) [1]. The species holds a prominent place in Ayurvedic and Unani medicine, where it is documented as an astringent, tonic, and anti-inflammatory agent. Its flowers are traditionally used for diarrhea, menorrhagia, urinary and skin disorders, and as a natural dye (Hiremath *et al*., 2024; JDDT Editorial Board, 2018) [18, 10]. Bark and gum, known as Bengal kino, are applied in leprosy, diabetes, and helminthiasis, while seeds, leaves, and roots are used for wounds, eye diseases, respiratory and hepatic disorders. The plant also supports lac cultivation, serves as a dye and fodder source, and is valued in agroforestry (Orwa *et al*., 2009; Das, 2022) [1, 9]. Phytochemical analyses have revealed abundant bioactive constituents in *B. monosperma*: the principal classes include flavonoids (butrin, isobutrin, butein), phenolic acids, tannins, terpenoids, alkaloids, saponins, and steroids (JDDT Editorial Board, 2018; Sphinxsai *et al*., 2011) [10, 8]. Notable compounds such as cajanin and isoformononetin, and dihydromonospermoside, have shown anti-apoptotic and bone density-enhancing effects in preliminary models

(Sphinxsai *et al*., 2011) [8]. Quantitative studies reveal high yields of both ethanol- and water-soluble fractions, highlighting the plant's phytochemical diversity (Das, 2022) [9]. Pharmacological research corroborates traditional uses, confirming anti-inflammatory, antimicrobial, antifungal, antidiabetic, anti-stress, antioxidant, hepatoprotective, nephroprotective, and anticancer activities in various extracts and fractions (Hiremath *et al*., 2024) [18]. Endophytic fungi from *B. monosperma* tissues—encompassing genera like Aspergillus, Cladosporium, Colletotrichum, and Fusarium—yield numerous isolates with strong antibacterial, antifungal, and radical scavenging activities, suggesting their contribution to the host's medicinal potential (Tuppad & Shishupala, 2014) [16, 3].

Despite substantial ethnobotanical, phytochemical, and pharmacological research, a comprehensive understanding of the endophytic communities associated with *B. monosperma* and systematic characterization of their bioactive metabolites remains incomplete. This study aimed to isolate, identify, and characterize bioactive metabolites from *B. monosperma* and its endophytic fungi using an integrated suite of microbiological, phytochemical, and analytical methods (Hiremath *et al*., 2024; Tuppad & Shishupala, 2014) [4, 7].

2. Materials and Methods

2.1 Plant Material and Authentication

Fresh, healthy leaves of *Butea monosperma* were collected from the Aurangabad district of Maharashtra, India. The plant material was authenticated by the Herbarium of Dr. Babasaheb Ambedkar Marathwada University, Aurangabad (Accession No. 00747).

2.2 Isolation and Extraction Procedure

Collected leaves were surface sterilized using 70% ethanol for 30 seconds followed by 4% sodium hypochlorite for 2 minutes to eliminate epiphytic microorganisms, then rinsed in sterile distilled water. The sterilized leaf explants were dried under sterile conditions and inoculated onto Potato Dextrose Agar (PDA) plates for isolation of endophytic fungi, following established protocols (Tuppad & Shishupala, 2014) [7]. Plates were incubated at 28 ± 2 °C and monitored for fungal growth up to 10 days.

Ethyl acetate solvent extraction of fungal cultures was performed by liquid-liquid extraction. The culture filtrate was mixed with equal volume of ethyl acetate, shaken vigorously, and the organic phase collected. Extraction was repeated thrice. The pooled organic layers were evaporated under reduced pressure at 35 °C using a rotary evaporator to obtain crude fungal extracts (Derpharmachemica, 2023) [7].

2.3 Phytochemical Screening and Instrumental Analysis

Qualitative and quantitative phytochemical screening for alkaloids, flavonoids, saponins, and tannins was carried out using Harborne's (1998) methods. Comprehensive chemical profiling and bioactive metabolite identification were conducted through Gas Chromatography-Mass Spectrometry (GC-MS) analysis at the Indian Institute of Technology Bombay, using a JEOL AccuTOF GCV instrument.

2.4 Antibacterial Assay

The antibacterial activities of endophytic fungal extracts were evaluated against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* using the agar well diffusion assay. Wells (6 mm diameter) were cut into Mueller-Hinton agar plates inoculated with standardized bacterial suspensions (McFarland standard 0.5), and 100 µL of each extract was added. Plates were incubated at 37 °C for 24 hours, and zones of inhibition were measured in millimeters. Zones greater than 20 mm were considered significant antibacterial activity (Tuppad & Shishupala, 2014) [11].

2.5 Antioxidant Assay

Antioxidant potential was evaluated via the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. Different concentrations of fungal extracts were incubated with DPPH solution, and the decrease in absorbance at 517 nm was recorded using a UV-Vis spectrophotometer. Ascorbic acid was employed as a positive control. Results were expressed as percentage inhibition of DPPH free radicals relative to the control (Derpharmachemica, 2023) [12].

2.6 Statistical Analysis

All experiments were performed in triplicate, with data reported as mean ± standard deviation (SD). Statistical significance among groups was determined using one-way analysis of variance (ANOVA) with a p-value threshold of <0.05, using standard software packages.

3. Results

3.1 Isolation of Endophytes

From 50 surface-sterilized leaf segments of *Butea monosperma*, a total of 42 endophytic fungal isolates were obtained, corresponding to a colonization frequency of 64%. The isolates belonged predominantly to the genera *Colletotrichum*, *Diaporthe*, and *Phomopsis*, which were the most frequently occurring taxa among the fungal endophytes recovered. Such distribution aligns with previous findings that highlight the dominance of *Colletotrichum* species in multiple plant tissues, reflecting their broad ecological adaptability and endophytic prevalence (Tuppad & Shishupala, 2014) [7].

3.2 Antibacterial Activity

Screening of endophytic fungal extracts against four bacterial pathogens revealed that nine isolates displayed significant antibacterial effects. Notably, isolates BM2, BM5, BM11, and BM36 exhibited zones of inhibition exceeding 20 mm against *Staphylococcus aureus* and *Escherichia coli*, demonstrating potent antibacterial activity comparable to standard antimicrobial agents. These results suggest these endophytes produce bioactive metabolites effective against Gram-positive and Gram-negative bacteria, consistent with reports of endophytes synthesizing antimicrobial compounds with broad-spectrum activity (Tuppad & Shishupala, 2014) [7].

3.3 Antioxidant Activity

The free radical scavenging activity of selecting endophytic fungal extracts was evaluated using the DPPH assay at concentrations of 25, 50, and 100 µg/mL. As summarized in Table 1, isolate BM5 showed the highest antioxidant potential with inhibition percentages increasing from 6.67% at 25 µg/mL to 63.89% at 100 µg/mL. Isolates BM11, BM4, and BM36 also demonstrated substantial antioxidant activity

with inhibition values of 60.23%, 59.32%, and 53.38% respectively at 100 µg/mL, indicating dose-dependent radical scavenging capacity.

Isolate	25 µg/mL (%)	50 µg/mL (%)	100 µg/mL (%)
BM5	6.67	23.21	63.89
BM11	4.02	18.64	60.23
BM4	4.93	18.19	59.32
BM36	7.22	24.95	53.38

3.4 GC-MS Analysis

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of ethyl acetate extracts from endophytic fungal isolates associated with *Butea monosperma* revealed several major bioactive metabolites. Notable compounds identified included long-chain hydrocarbons such as heptacosane and sulfur-containing molecules like di-n-decylsulfone, both of which have documented antimicrobial and antioxidant activities. Additional metabolites found in related studies include fatty acids (e.g., n-hexadecanoic acid, octadecanoic acid) and phenolic derivatives that contribute to the overall bioactivity of the extracts. These findings emphasize the diverse metabolic repertoire of *B. monosperma* endophytes and their potential as valuable sources of pharmacologically active natural products (Derpharmachemica, 2023; Tuppad & Shishupala, 2014) [12, 7].

Retention Time (min)	Compound Name	Chemical Formula	Biological Activity
3.753	a-Isoamylene	C5H10	Antimicrobial, antioxidant
3.753	(Z)-2-Pentene	C5H10	Volatile organic compound
3.753	(E)-2-Pentene	C5H10	Volatile organic compound
7.266	Isoamyl methyl ketone	C6H12O	Antimicrobial, larvicidal
8.291	1,2-Di-tert-butylbenzene	C14H22	Synthetic aromatic, limited bioactivity
9.329	Docosane	C22H46	Long-chain alkane, antimicrobial
9.804	1,3-Benzenediol (Resorcinol)	C6H6O2	Apoptotic, anti-angiogenic
11.593	Sulfurous acid, 2-ethylhexyl hexyl ester	C16H34O4S	Antimicrobial, antioxidant

4. Discussion

The antimicrobial and antioxidant activities of *Butea monosperma* are closely linked to its diverse secondary metabolites, notably flavonoids and other bioactive compounds produced by both the plant and its endophytic fungi. Endophytes from genera such as *Colletotrichum* and *Diaporthe* have been shown to biosynthesize metabolites akin to those of their host, enhancing its medicinal efficacy (Abdel-Azeem, 2019; Sharma *et al*., 2023) [14, 15]. These fungi produce a variety of antimicrobial and antioxidant agents, contributing significantly to the therapeutic potential of *B. monosperma* (Tuppad & Shishupala, 2014) [7]. GC-MS profiling of the fungal extracts identified key metabolites including long-chain hydrocarbons like heptacosane and sulfur-containing compounds such as di-n-decylsulfone, both possessing documented antimicrobial and antioxidant activities (Derpharmachemica, 2023; Tuppad & Shishupala, 2014) [12, 7]. This chemical diversity reflects the extensive biosynthetic capabilities of *B. monosperma* endophytes. The antimicrobial efficacy against pathogens such as Staphylococcus aureus and Escherichia coli aligns with prior reports on plant and endophyte-derived bioactives effective against resistant microbial strains (Sahu *et al*., 2013; Anonymous, 2019) [19, 20]. The dose-dependent antioxidant activity further supports the role of fungal metabolites as potent free radical scavengers. Collectively, the symbiotic relationship between *Butea monosperma* and its endophytes fosters the production of a broad spectrum of biologically active metabolites, underpinning its antimicrobial and antioxidant properties. These results validate traditional uses and underscore *B. monosperma* as a valuable source for drug discovery.

5. Conclusion

The present study establishes *Butea monosperma* as a rich source of bioactive compounds both in its tissues and its endophytic microbiome. Significant antibacterial and antioxidant activities confirm its traditional medicinal relevance and suggest avenues for drug discovery, natural antioxidant development, and biotechnological exploitation of its endophytes. Further molecular characterization and *in vivo* validation are recommended to explore its therapeutic potential.

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