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Pharmacological Constituents of *Butea monosperma* Crude Leaves Extracts and Antibacterial Activity against Clinical Isolates

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ABSTRACT

Nature is full with resources for medical plants and medicinal based on herbs, but some of these sources are still hidden because of a scarcity of knowledge and awareness about their therapeutic capabilities. For the treatment and prevention of numerous infections and disorders, herbal medicines have the potential to be a source of therapeutic treatment. *Butea monosperma*, a naturally occurring tree from the tribal area of the Baglan Region of India, has been referred as the Palas (Palash). *Butea monosperma* was assessed for pharmacological constituents and evaluated antibacterial activity against clinical isolates. Ethanolic and acetone leaves extract of *Butea monosperma* detected with phytoconstituents such as amino acids and proteins, carbohydrates, phenols, tannins, flavonoids, glycosides, and saponins.Both extracts i.e., ethanol and acetone exhibited the higher diameter of the inhibition zone, i.e., 16 and 17 mm against *Salmonella paratyphi B*, followed by an ethanol extract *with1*6 mm against *Streptococcus pyogenes*. Both ethanol and acetone extractpotentially exerted antimicrobial activity against clinical isolates.

Key words: *Butea monosperma, Palas, Antimicrobial activity, Phytoconstituents*

Introduction

Since ancient times, medicinal plants have formed the foundation of traditional herbal medicine among rural populations across the world. Plants were employed by human beings for a number of purposes. The majority of medicines are made with natural substances, particularly ones that originate in plants. When it comes to plant biodiversity, India is the richest nation. Since the previous few decades, the rise of drug resistance to widely used antibiotics and the emergence of unfavorable side effects of certain pharmaceuticals have alarmed researchers into looking for intriguing antimicrobial medicinal products, mostly from herbal sources (Balouiri *et al.,* 2016; Jayasree *et al.,* 2015). In the literature, several hundreds of herbal remedies and medicines are known to have the great potential to treat many infections and diseases (Jayasree *et al.,* 2015). Now a days there is vast demand for herbal medicines and drugs of plant origin, because of less or none of side effects of ayurvedic medicines (Ncube *et al.,* 2008; Balouiri *et al.,* 2016). *Butea monosperma* is a member of the sub family *Caesalpinioideae* and *Fabaceae* family (More *et al.,* 2019). The Palash tree is known as flame of the forest because of its vivid orange-yellow blossoms that resemble flames (Saroj and Shah, 2023; Pawar *et al.,* 2019; Yadav *et al.,* 2020). As a deciduous tree that is native to all of India, *Butea monosperma* thrives during the dry season. The three leaflets of

the pinnate, dark green leaves that grow from the stems are grouped in a pinnate configuration (Yadav, 2020). Palash's blooms have striking orange coloring, numerous wide petals, are aligned in a cluster or raceme shape, and are edible despite being very bitter (Vaidya and Pandita, 2017). The plant *Butea monosperma,* previously explained as being proven to beanti-helminthic, anti-convulsant, anti-diabetic, anti-diarrheal, anti-estrogenic, anti-fertility, anti-microbial, anti-fungal, anti-bacterial, anti-stress, chemo-preventive, hepatoprotective, radical scavenging, thyroid inhibitory, antiperoxidative, hypoglycemic, and wound healing activities (More *et al.*,2019; Vaidya and Pandita, 2017; Gunakkunru *et al.*,2005; Sahu and Padhy, 2013; Bhatwadekar *et al.*,1999; Gupta *et al.*,2012; Saroj and Shah, 2023; Pawar *et al.,* 2019; Singh *et al.,* 2020; Tiwari*et al.,* 2019; BandaraRatnayake *et al.*,1989; Panda *et al.*,2009; Sharma*et al.,* 2019).

Materials and Methods

Collection of Plant Material

The Butea monosperma leaves were brought to the lab after being collected from the college campus and the Baglan District Forest area in Nashik, India. Dr. B. R. Pawar, Taxonomist and Head (Ret.), Department of Botany, verified the plant's authenticity. The leaves of the plant were collected and thoroughly cleaned by being rinsed three times in distilled water in order to remove all contaminants and other particulates. In a lab at room temperature, the leaves were shade dried for 15 days. To make a fine powder, the dried leaves were grounded. Samples were kept in tightly closed bottles.
Extraction of plant material
The plant material extracts were prepared as follows

Extraction in Organic solvents

Fine powder of leaves extracted with organic solvents (Ethanol and Acetone). Alcohol is considered to be best solvent for extraction of phytochemicals (Vaidya and Pandita, 2017). 200 ml of each solvent were used to soak 20 g of powdered leaves. Three days of the extraction procedure comprised using a rotary shaker at 120 rpm at 26 °C. Using a rotary vacuum evaporator at 45 °C, the extracts were dried. Until further investigation, the dried extracts were kept in sterile bottles and stored in the refrigerator.

Analysis of phytoconstituents in extracts

For the purpose of identifying significant compounds, the crude extracts performed phytochemical analysis. According to previous investigations, the phytochemical analysis of all crude extracts was conducted to detect the presence of sugars, amino acids, proteins, glycosides, tannins, terpenoids, phenols, alkaloids, and saponins (Trease and Evans 2005; Vasait, 2017; Shaikh and Patil, 2020; Vaidya and Pandita, 2017).

Preparation of plant extracts for antibacterial activity

Stock solutions of plant leaves extracts were prepared by mixing them with sterile distilled water. 100 mg of alcohol and acetone extract was mixed with 100 ml of sterile distilled water and designated as stock solution (Saliem and Abed, 2018). These extracts were used for the evaluation of an antibacterial activity.

Test organisms for antibacterial activity

By using a diffusion assay with Muller-Hinton media, antibacterial activity against clinical isolates, Gram positive bacteria like *Streptococcus pyogenes* and *Staphylococcus aureus*, and Gram-negative bacteria like *Salmonella typhi, Salmonella paratyphi B, E. coli*, and *Proteus mirabilis* was investigated.

Antibacterial diffusion assay

The agar diffusion method has been used for the evaluation of the antibacterial potency of crude extracts. In this test, extracts were impregnated on paper discs of size 6 mm and used for assaying. The disc diffusion assay has performed the testing of the antibacterial potential of each extract (Wayne CLSI, 2012). Mueller Hinton agar containing Beef extract: 2.0 g/l, Acid hydrolysate of casein: 17.5 g/l, Starch: 1.5 g/l, Agar-agar: 17.0 g/l, and pH: 7.3±0.1 was used as a basal and seed medium for the antibacterial assay. Sterile basal agar plates and seed agar butts were prepared. After the pouring and solidification of seed agar, sterile filter paper discs were impregnated with the crude extract dilutions and placed on the surface of the medium. The discs impregnated with sterile distilled water were also placed on an agar surface as a negative control. Standard antibiotic was used in the antimicrobial assay study as a positive control. Then all plates were incubated at 37 °C for 24 hours. After incuba-

tion, the diameters of the zones of inhibition were measured in mm.

Results

Morphological features of *Butea monosperma* (Palas)

As stated above there is striking arrangement of leaves present in their morphology of palas. The leaves trifoliate, large, alternate and stipulate small (Figure 1). Petiole are about 10-15 cm long. Leaflets are obtuse, globrous above (Kavitha *et al.,* 2012) *Butea monosperma* is a medium to large tree of tropical and subtropical climate (Figure 2).

Fig. 1. Leaves of *Butea monosperma*

Fig. 2. Tree of *Butea monosperma*

Phytochemical Analysis of crude extracts

Standard chemical assays were used for phytochemical analysis to look for the compounds that were present in the extracts. Pharmacological components like carbohydrates, amino acids, proteins, glycosides, phenols, flavonoids, saponins, and others were evaluated. The majority of phytoconstituents are reported to be utilized in pharmaceuticals for a variety of acute and chronic infections and disorders and play a significant role as antimicrobials. Table 1 summarizes the phytochemicals as were investigated. It was determined of both extracts, ethanol and acetone, contained tannins, flavonoids, glycosides, saponins, amino acids, proteins, phenols, and carbohydrates. The presence of phytochemicals such as carbohydrates, tannins, flavonoids, starch, glycosides, proteins, and carbohydrates in *Butea monosperma* plant extracts was evaluated in previous studies (Vaidya and Pandita, 2017; Sahu and Padhy, 2013; Lakshmayya *et al.,* 2000; Shah *et al.,* 1992). Due to their secondary metabolites, plants have a significant role in medicine. It is widely understood that plant saponins have antibacterial, anti-inflammatory properties (Tatli and Somuncuoglu, 2021). It was evaluated that terpenoids and flavonoids are thought to have antibacterial and antidiarrheal properties. It has been revealed that plant secondary metabolites have therapeutic benefits and are involved in a variety of processes in herbal medicines.

Table 1. Phytoconstituents detected in extracts of *Butea monosperma*

Phytochemicals	*Butea monosperma* Extracts	
	Ethanol	Acetone
Carbohydrates	+	+
Amino acids and peptides	+	+
Glycosides	+	+
Tannins	+	+
Terpenoids	-	-
Phenols	+	+
Saponins	+	+
Alkaloids	-	-
Flavonoids	+	-

Antibacterial Assay

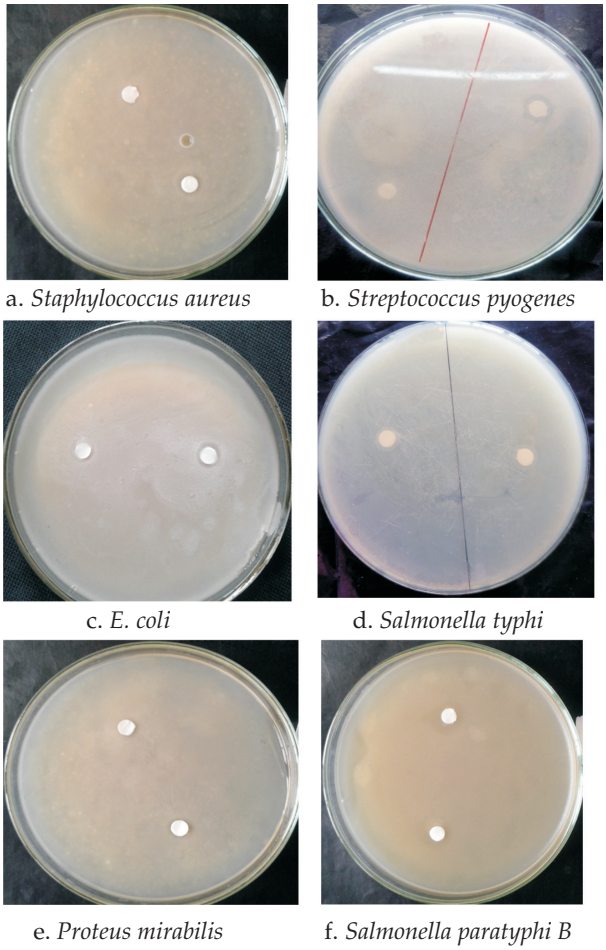
Plants produce secondary metabolites including saponins, flavonoids, and tannins that are thought to be toxic to microbes. Tannins have antibacterial potential due to their basic character, which may allow

them to react with enzymes and proteins which leads in the disruption of cell membrane, thereby being considered as bactericidal by cessation of some metabolic activities (Cano *et al.,* 2020).

Acetone extract exhibited less antibacterial activity as compared to ethanol extract. Both extracts of *Butea monosperma* found inhibitory and showed zone of inhibition against all test clinical isolates (Table 2). Test organisms *Staphylococcus aureus* and *Salmonella paratyphi B* were found more susceptible to both of the extracts of leaves of *Butea monosperma.* All extracts exhibited antibacterial activity against the test organisms in comparison with the control. In the present study, the antibacterial activity of the leaves extracts was evaluated against both Gram-positive and Gram-negative organisms. Both extracts i.e., ethanol and acetone exhibited the higher diameter of the inhibition zone, i.e., 16 and 17 mm respectively against *Salmonella paratyphi B*, followed by an ethanol extract with 16 mm (Figure 3). Test organisms *E. coli* and *Salmonella typhi* were evaluated moderately sensitive to leaves extract of *Butea monosperma*. Previously reported that extracts of *Butea monosperma* found effective against *S. typhimurium*, *P. aeruginosa* and *E. coli* and had higherantibacterial activity against *B. subtilis* (Jayasree *et al.,* 2015). It was reported that leaves and flower methanol extract of *Butea monosperma* exhibited higher zone of inhibition against test organisms *S. aureus* and *B. subtilis* in a range of 7.0-22.0 mm (Nag *et al.,* 2021). It was studied that leaf-extracts prepare in hot water and ethanol have shown significant antibacterial activity against all bacteria (Sahuand Padhy, 2013). Previously reported that *Butea monosperma* has not only antibacterial activity but also antifungal activity (Ratnayake Bandara *et al.,* 1989). *Butea monosperma (Lam.)* evaluated antibacterial and antidiarrheal activityof *B. monosperma* bark extract against *Enterobacter cloacae* (Sharma *et al.,* 2019). According to Lakshmayya *et al.* (2000) *Butea monosperma* leaves extract evaluated to be antibacterial against *Staphylococcus aureus*, *E. coli* and *Pseudomonas aeruginosa*.

Table 2. Antibacterial activity of extracts evaluated against test organisms

Test Organism	Diameter of Zone of Inhibition (mm)	
	Ethanol Extract	Acetone Extract
Staphylococcus aureus	12	6
Streptococcus pyogenes	16	8
E. coli	14	12
Salmonella typhi	12	12
Salmonella paratyphi B	16	17
Proteus mirabilis	8	8



a. *Staphylococcus aureus* b. *Streptococcus pyogenes* c. *E. coli* d. *Salmonella typhi* e. *Proteus mirabilis* f. *Salmonella paratyphi B*

Fig. 3. Antibacterial activity of leaves extracts of *Butea monosperma* against test organisms a. *Staphylococcus aureus,* b. *Streptococcus pyogenes,* c. *E. coli,* d. *Salmonella typhi*, e. *Proteus mirabilis* and f. *Salmonella paratyphi B.*

Conclusion

According to the findings of this study, the leaf extract of *Butea monosperma* from the Baglan District of Nashik has inhibitory activity against the bacteria tested: Gram-negative *E. coli, Proteus mirabilis, Salmonella typhi,* and *Salmonella paratyphi B,* and Gram-positive *S. aureus* and *Streptococcus pyogenes*. Because of the presence of phytoconstituents, the plant is ef-

fective for treating multiple conditions and has the potential to produce useful pharmaceuticals for human use. The majority of the physiologically active phytochemicals detected in *Butea monosperma* ethanolic and acetone extracts were discovered in this study. Because the ethanolic extract exhibited potential antibacterial action against all test species, it can be encouraged for future research.

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Conflict of Interest: None.

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