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Phytochemical analysis, antibacterial activity of stem and leaf extracts of *Butea monosperma* (Lam.) Taub

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Abstract
The aim of the present study was to evaluate the phytochemical analysis of different solvent extracts of stem and leaves of *Butea monosperma*. Phytochemical screening was carried out for six solvent extract revealed the presence of various bioactive compounds include tannin, flavonoid, terpinoids, lipids, acedic compounds, reducing sugar and carbohydrates. The antibacterial activity of *Butea monosperma* stem extract, was studied by agar well diffusion method against the test organisms, *Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumoniae.* The extract of stem was used to determine the antibacterial activity of tested organisms and the zone on inhibition were measured. From these analyses of *Butea monosperma* leaf extracts had the maximum inhibition activity against *Pseudomonas aeruginosa* (12mm) followed by *Escherichia coli* (11mm) and *Klebsiella pneumoniae* (07 mm). The stem extracts of *Butea monosperma* had the maximum inhibition activity against *Pseudomonas aeruginosa* (09 mm) followed by *Escherichia coli* (07 mm) and *Klebsiella pneumoniae* (04 mm).

Keywords: antibacterial activity, *Butea monosperma*, leaves, stem, phytochemical analysis

Introduction

The practice of medicinal plants for the treatment of a wide range of illnesses has been recorded since ancient times, and they have become increasingly relevant in healthcare. While the use of phytomedicines was previously focused on empiricism, empirical evidence about their chemical composition and related medicinal properties is now becoming more prevalent (Petrovska, 2012) [16]. Many medicinal plants with identical morphology and the same folk name among species within the same genus may be misidentified and deliberately or unintentionally substituted in commercial goods during the manufacturing phase, resulting in a loss of effectiveness and protection. Biological species authentication is typically conducted by professional taxonomists; however, morphological recognition may be limited by the lack of various phenotypic characteristics. Furthermore, herbal products are found in refined ways such as spices, herbal content (i.e. essential oils), herbal preparations (i.e. extracts), and finished herbal products (i.e. capsules and tablets), making morphological recognition difficult (Zhao, S. Y. *et al.*, 2017; Ahmad, M. *et al.*, 2009) [19, 1]. As a result, phytochemical studies can provide a valuable tool for the identification and differentiation of related plant species.

The phytochemical characterization of plant material is important because it is related to therapeutic behaviour. It is perhaps self-evident that different plant species have different chemical constituents. These variations, however, may extend to different varieties or even the same variety grown in different locations or harvested at different times. Different active constituents can be found in different areas of the plant, including the leaves, bark, seeds, stems, berries, and pods. The phytochemical evaluation of plants is needed for the analysis of pharmacological activities. It can be accomplished by qualitative chemical analysis with particular reagents for specific constituents, followed by validation with various chromatographic techniques such as TLC, HPTLC, and HPLC. HPTLC is a significant development of the TLC concept, involving less time and higher resolution. As a result, before continuing with pharmacological and toxicological trials, a herbal drug's plant authentication and phytochemical assessment are critical requirements (Srivastava 2011) [13].

Plants possess a wide range of biologically active compounds. Many of them have been shown to have antimicrobial effects (Cowan, 1999) [6]. Plant-derived drugs have been used in most parts of the world for thousands of years, and there is growing interest in plants as sources of agents to combat microbial diseases (Chariandy *et al.*, 1999) [4]. Numerous plant species have been used as traditional medicine by Australia's indigenous native people (Ahamed *et al.*, 1998). However, only a handful of these have been studied for antimicrobial and antiviral effects. Many plants have been used as insecticides, molluscicides, and rodenticides (Anwar *et al.*, 1992) [3].

Herbs and spices are one of the world's oldest disciplines. Just recently has scientific research begun to pay attention to the properties of spices (Chaudhry *et al.*, 2006) [5]. The use of natural ingredients as an alternative to traditional therapy in curing and treatment of multiple diseases has increased in the last few decades due to the side effects of conventional medicine. Since medicinal plants contain a plethora of biologically active chemical constituents, they have antibacterial activity. Plant preparations used as food snuff, insecticides, CNS active, cardio active, antitumor, and antimicrobial agents are only a few examples of the vast chemical richness in plants that mankind has discovered. Natural plant items, such as compresses, cataplasms, gargles, and ointments, can provide a new source of antibacterial agents for external usage. Anila and Vijayalakshmi (2000) [2], In human health treatment, an antimicrobial agent is commonly used. For the antimicrobial action, various medicinal plant extracts were used. In the active stage, a significant number of medicinal plants are used. It is expected that screening and scientific assessment of plant extracts for antimicrobial activity will yield novel antimicrobial substances from Indian medicinal plants.

Butea monosperma (Lam.) Taub. (Bastard Teak, Flame of the Forest; Synonyms: Butea frondosa Roxb; Butea frondosa Willd; *Butea monosperma* (Lam.) Kuntze and Butea braamania DC.) Belongs to the fabaceae family and is native to tropical southeastern Asia. It is a common ornamental tree grown all over the world. It is a deciduous tree that can reach a height of 12–15 m and has a crooked trunk diameter of up to 20-40 cm in mature trees. The leaves are pinnate, with a petiole of 8-16 cm and three leaflets of 10-20 cm each. The flowers are 2.5 cm tall, light orange in colour, and appear in racemes up to 15 cm long in spring. The fruit is a pod that grows to be between 15-20 cm long and 4-5 cm wide before turning brown (Kirtikar and Basu 1935; Kapoor 2005; Kala 2004; Suguna *et al.*, 2005) [11, 10, 9, 18].

In various areas of the world, the herb is used to treat a variety of diseases such as stomatitis, sores and skin problems, constipation, ringworm, insomnia, dysentery, muscle pains, digestive disorders, ulcer, tumour, fever, gonorrhoea, diabetes, arthritis, fungal infection, masses, urinary disease, asthma, and leucorrhoea and is the source of a various form of chemical constituents including fatty acids, amino acids, terpenoids, phenolics, flavonoids, alkaloids, steroids, glycosides, tannins and many others (Sharma Ajay Kumar and Deshwal Neetu 2011; Firdaus Rana and Mazumder Avijit 2012; Das Manas Kumar *et al.*, 2011) [17, 8, 7].

Based on the above, we conducted a preliminary phytochemical screening of *Butea monosperma* leaf and stem extracts, along with antibacterial activities against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* using the Agar Well Diffusion method.

Materials and Methods

Plant Material

The stem and leaves of *Butea monosperma (Lam) Taub*. were collected and prepared the samples for this study.

Phytochemical Analysis

Phytochemical studies of plant stem and leaf extract were carried out to investigate the qualitative phytochemical analysis.

Sample Preparation

The plant parts of *Butea monosperma* were properly washed in running tap water and then in RO water. The washed stem and leaves were allowed to shade dry for 2 – 3 Weeks. After that the shade dried leaves were pulverize using a sterile electric blender, to obtain a powder form. The powdered forms of plant parts were stored in airtight glass containers, protected from sunlight until required for analysis.

Preparation of extracts

The powdered plant parts were extracted with Acetone, Benzene, Chloroform, Diethyl ether, Ethyl acetate and Ethanol. The each extract of plant was prepared by soaking 5g in 100 ml of corresponding solvents. The extract was incubated at 48 hours and the extract was then filtered through normal or what man No.1 filter paper. The extracts were further used for phytochemical analysis.

Qualitative Phytochemical Tests

Test for Tannins

About 0.5g of powdered sample of plant are boiled in 20ml of different solvents in a test tube and then filtered. The filtration method used normally, which includes a conical flask and filter paper. 0.1% ferric chloride (FeCl3) solution was added to the filtered samples and observed for brownish green or a blue black coloration, which shows the presence of tannins.

Test for Phlobatannins

To the 10ml of filtered extract of each solvent are boiled with 1ml of 1%HCl acid in a test tube or conical flask. If the plant sample carries phlobatannins, a deposition of a red precipitate will occur and indicates the presence phlobatannins.

Test for Flavanoids

Few drops of 1% NH3 (ammonia) solution is added to all the filtered extracts in a test tube. A yellow coloration is observed if flavonoids compounds are present.

Test for Terpenoids

To 5ml of each filtered extracts of plant sample are mixed with 2ml of CHCl3 (chloroform) in a test tube. 3ml of concentrated H2SO4is carefully added to the mixture to form a layer. An interface with reddish brown coloration is formed if terpenoids constituents are present.

Test for Cardiac Glycosides

One ml of concentrated H_2SO_4 is prepared in separate test tubes. 5ml of filtered extracts from each solvent are mixed with 2ml of glacial CH3CO2H (acetic acid) containing 1drop of FeCl3. The above mixture is carefully added to the 1ml of concentrated H_2SO_4. The concentrated H_2SO_4 underneath the mixture. If cardiac glycoside is present in the sample, a brown ring will appear, indicating the presence of the cardiac glycoside constituents.

Test for Phenol

To the 2ml of each filtered extracts are mixed with 2ml of $FeCl_3$. The black precipitation indicates the presence of phenol in the plant sample.

Test for Steroids

To the 2ml of acetic anhydride is added to the each 2ml of various filtrates. The few drops of concentrated H_2SO_4 are carefully added to the side of the test tube. The blue or green ring indicates the presence of steroids.

Test for Lipids

One ml of filtrate is added to the 2ml 1N NaOH. The irritating odour will appear, if the lipids present in the plant sample.

Test for Cholesterol (LibermannBurchard test)

To the 2ml of powdered sample is added with 2ml of each solvent. Then 10 drops of acetic anhydride solution is added and mixed well. 2 drops of concentrated H_2SO_4 is carefully added to the side of the test tube. Deep green color formed if cholesterol present in the plant sample.

Test for Protein (Ninhydrine test)
To the 2ml of all filtrates, 5 drops of 1% Ninhydrine is added boil for few minutes. After incubation violet or purple color formed to indicates the presence of proteins.

Test for Acidic Compounds
The small amount of evaporated extract is taken. Sodium bicarbonate solution is added to the various extracts. Then observe for production of effervescence, which indicates the presence of acidic compounds.

Test for Reducing Sugar (Fehling's test)
Fehling's A and B solution equally added to the diluted evaporated extracts heated for 30 minutes. Then observed for the formation of brick red precipitation, this indicates the presence of reducing sugar in the plant sample.

Test for Anthraquinone
The each filtered extracts are mixed with equal volume of 10% NH3 (ammonia solution). Pink or red color observed in the ammonial layer if anthraquinone present.

Test for Carbohydrates (Molish's test)
Two drops of molish reagent (0.5g of α-napthol in 10 ml of ethanol) is added to the 2ml of various filtrates. The concentrated H_2SO_4 is carefully added to the side of the test tube. The red or violet color ring will appear in the interface of the solution, which indicates the presence of carbohydrates in the plant sample.

Antibacterial Activity
Media Used
Muller Hinton Agar Medium and nutrient broth were used for culturing of microorganisms.

Test Organisms
Three bacterial cultures *viz, Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumoniae* were used for this study and the culture were obtained from Microbial Type Culture Collection (MTCC).

Plant Extracts Preparation
The plant material were collected and washed with distilled water to remove the dust and dried in shade. Dried plant materials were finely ground with mixer grinder. These powdered materials were used for further studies.
The powdered plant material was immersed with ethanol and distilled water (1:1) for 24 hrs. Then the extracts were separated by filtration and the residual plant material was further extracted with respective solvents. The solvent was evaporated under reduced pressure and semi solid gummy material was obtained.

Agar well Diffusion Method
Agar well diffusion method was adopted for evaluation of antibacterial activity of *Butea monosperma* leaf and stem extracts. Muller Hinton Agar Medium was poured into sterile Petri dishes to a uniform depth of 4mm and then allowed to solidify at room temperature. After solidification, the test organisms *Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumoniae* were inoculated with the help of a sterile swab soaked in bacterial culture or suspension. Thus provided the uniform surface growth of bacterium and is used for antibacterial sensitivity studies. Well were punched in agar media using a stainless steel bores. Each well was filled with *Butea monosperma* stem and leaf extracts (Approximately 0.1ml). The plants were incubated at 37˚c for 24 hrs, after which they were observed for the zone of inhibition. Diameter of inhibition zone were calculated and expressed in mm.

Results and Discussion
Qualitative Phytochemical Analysis
The qualitative study of *Butea monosperma* reveals that the presence of number of secondary metabolites which has therapeutic values and that is considered to be good constituents which leads to further detailed isolation, purification studies to elucidate the value based secondary metabolite component which is indulged in *Butea monosperma*. All the plant parts (Stem and leaves) of *Butea monosperma* are said to possess lipids in all the six solvent extracts of Acetone, Benzene, Chloroform, Diethyl Ether, Ethyl Acetate and ethanol (Table-1). Terpenoids are found to be seen in all plant parts (Stem and leaves) of *Butea monosperma* except chloroform extract of leaves and Ethyl Acetate extract of leaves. Reducing Sugar are found to be seen in all plant parts (Stem and leaves) of *Butea monosperma* except acetone, benzene extracts of stem and chloroform extract of leaves.
Whereas, the plant parts (Stem and leaves) of *Butea monosperma* does not possess phenol, protein and anthroquinone. Phlobatannin are found to be absent in all plant parts (Stem and leaves) of *Butea monosperma* except acetone leaves extract. Cardiac glycosides are found to be absent in all plant parts (Stem and leaves) of *Butea monosperma* except chloroform and ethanol stem extracts. Steroids are found to be absent in all plant parts (Stem and leaves) of *Butea monosperma* except benzene leaf extract and stem extract of diethyl ether. Cholesterol are found to be absent in all plant parts (Stem and leaves) of *Butea monosperma* except leaf extracts of acetone, diethyl ether and ethyl acetate. Similar to our results the flower extract of *Butea monosperma* showed the presence of the following phytochemicals like cardiac glycosides, flavonoids, phenols, carbohydrates, saponins, tannins, alkaloids and terpenoids in methanol solvent extract (Milan Hait *et al.*, 2019) [14].

Antibacterial Activity
The antibacterial effect of *Butea monosperma*, was studied by agar well diffusion method against the test organisms, *Escherichia coli, Pseudomonas aeruginosa and Klebsiella pneumoniae.* The extract of stem was used to determine the antibacterial activity of tested organisms and the zone on inhibition were measured (Table - 2).
From these interpretations of *Butea monosperma* leaf extracts had the maximum inhibition activity against *Pseudomonas aeruginosa* (12mm) followed by *Escherichia coli* (11mm) and *Klebsiella pneumoniae*(07 mm).The stem extracts of *Butea monosperma* had the maximum inhibition activity against *Pseudomonas aeruginosa* (09 mm) followed by *Escherichia coli* (07 mm) and *Klebsiella pneumoniae* (04 mm). Similar to our results Pattari Lohitha *et al.*, (2011) [15] tested the antimicrobial activity of aqueous and ethanol extracts of *Butea monosperma* plant parts against different microbial pathogens. Among the different pathogens tested, *E. coli* and *Candida albicans* showed maximum zone of inhibition in both the solvent extracts. Mahesh Chandra Sahu and Rabindra Nath Padhy (2011) [12] analysed the

antimicrobial activity of aqueous as well as ethanolic extracts of the root, stem and leaf of *Butea monosperma* was tested against different pathogens. In their studies the root extracts showed maximum inhibition zone effect compare with stem and leaves of *Butea monosperma.* From our studies also the antibacterial activity of ethanol extract of *Butea monosperma* leaves and stem showed maximum inhibition activity against the tested two pathogens.

Table 1: Phytochemical Analysis of different solvent extracts of *Butea monosperma*

S.No	Test	Acetone		Benzene		Chloroform		Diethyl Ether		Ethyl Acetate		Ethanol	
		Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf	Stem	Leaf
1.	Tannins	-	-	+	+	+	-	+	-	-	+	+	+
2.	Phylobatannins	-	+	-	-	-	-	-	-	-	-	-	-
3.	Flavonoids	+	-	+	-	+	+	-	-	+	-	+	-
4.	Terpenoids	+	+	+	+	+	-	+	+	+	-	+	+
5.	Cardiac Glycosides	-	-	-	-	+	-	-	-	-	-	+	-
6.	Phenol	-	-	-	-	-	-	-	-	-	-	-	-
7.	Steroids	-	-	-	+	-	-	+	-	-	-	-	-
8.	Lipids	+	+	+	+	+	+	+	+	+	+	+	+
9.	Cholesterol	-	+	-	-	-	-	-	+	-	+	-	-
10.	Protein	-	-	-	-	-	-	-	-	-	-	-	-
11.	Acedic Compounds	+	+	+	+	+	+	-	-	+	-	-	-
12.	Reducing Sugar	-	+	-	+	+	-	+	+	+	+	+	+
13.	Anthraquinone	-	-	-	-	-	-	-	-	-	-	-	-
14.	Carbohydrates	+	+	+	-	+	-	+	-	-	-	+	-

"+" indicates Presence, "-" indicates Absence

Table 2: Antibacterial activity extracts of *Butea monosperma*

Microorganisms	Zone of Inhibition (mm)	
	Stem	leaf
Escherichia coli	07	11
Pseudomonas aeruginosa	09	12
Klebsiella pneumoniae	04	07

Conclusion

The results indicate that the stem and leaves of *Butea monosperma* are a potentially beneficial source of beneficial pharmaceuticals owing to the presence of phytochemicals and may be used in the treatment of different diseases as well as suitable for use in the pharmaceutical and cosmetic industries. However, further research is required to separate the active principle from the crude extract for appropriate medication development.

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