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# A novel approach towards evaluation of primary metabolite and antimicrobial screening in *Butea monosperma* (Lamarck.) Kuntze.

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**ABSTRACT**

The quantification of primary metabolites and antimicrobial activities of medicinal plant *Butea monosperma* (Lam.) kuntze against clinical isolates was evaluated. The present study was aimed to determine the antibacterial and antifungal activities of the chloroform and ethanolic extract of leaf, flower, root and seed of the *Butea monosperma* (Lam.) kuntze. Antimicrobial activity was determined by using agar well diffusion method. Ethanolic extracts of leaves and flowers possessed highest antibacterial activity against *E.coli* and highest antifungal activity against *Trichoderma reesei* and *Fusarium oxysporium.* Chloroform extracts of seeds have the highest antibacterial activity against *Bacillus subtilis* and highest antifungal activity was observed in chloroform extracts against *Fusarium oxysporium* . The present results showed potential of this medicinal plant which can be used as herbal drug as therapeutic ventures in future aspects.



## Introduction

In recent years attention has been devoted to novel molecules which can act as substituent of synthetic drugs. Primary metabolites are responsible for growth and development of plant. They are primarily used as industrial raw materials, food or food additives. Therefore, in the present study primary metabolites from leaves, flower, roots and seeds of *Butea monosperma* have been evaluated. Plants are considered to be the best source for antimicrobial agents; an attempt has been made by us towards the screening of crude extracts of selected plant species. With the development of antimicrobials, microorganisms have adapted and become resistant to previous antimicrobial agents (Bonjar *et al.* 2005). Therefore, the present study has been designed to focus on antimicrobial activity of different solvents like ethanolic and chloroform and against selected microorganisms, *Trichoderma reesei* (ATCC -13631) , *Aspergillus niger* (ATCC-9029), *Fusarium oxysporium* (ATCC-62506) and *Penicillium funicullosum* (ATCC -11797) and bacteria *Escherichia coli*, (MTCC -443) *Streptomyces grisveus* (MTCC-4734), *Bacillus subtilis* (MTCC-10619) and *Staphylococcus aureus* (MTCC -3381) *Butea monosperma* (palas) is a medium-sized deciduous tree belongs to family fabaceae-papilioneae. This tree is also called 'Flame of the Forest' and Bastard Teak (Kirtikar and Basu, 1935). They comprise one of the largest families of flowering plants, numbering 630 genera and 18000 species (The wealth of India.1988). It grows throughout the Indian subcontinent, especially in Indo-gangetic plains (Chopra *et al.*1958). It is said that the tree is a form of Agnidev,'God of Fire'. Flowers are offered in place of blood in sacrifice rituals to goddess Kali (Ambasta and B.P.1994). The plant is reported to possess various bioactivities like anticonvulsive (Kasture *et al.*2002), anti-inflammatory (Mengi *et al.* 1995), antidiabetic (Somani *et al.*2006), antidiarrhoeal (Ramana *et al.* 2000). Free radical scavenging (Schoeller *et al.* 1938) anti helmintic (Iqbal *et al.*2006).

## Material and Methodology

**Plant material:** Healthy plants of *B. monosperma* were collected from Amer, Delhi road, Jaipur and authenticated by the Herbarium (**RUBL.211650),** University of Rajasthan, Jaipur, Rajasthan, India*:*

- **RUBL**-Rajasthan University Botanical Library

**Chemical:** All the chemicals and growth regulators used are analytical grade and purchased from Hi Media Pvt. Ltd., Mumbai, India.

### Quantification of Primary metabolites

The flower, root, seed and leaf parts of *B. monosperma* were evaluated quantitatively to estimate the total levels of soluble sugars, Loomis and Shull(1937), starch, McCready (1950), proteins, Lowery *et al.*,(1951), lipids, Jayaraman (1981) and phenols, Bray and Thorpe(1954) following the established methods for the sugars, starch, lipid, protein and phenol. All experiments were repeated five times for precision and values were expressed in mean ± standard deviation in terms of shade dried material.

### Antimicrobial activity of *B.monosperma*

Antibacterial and Antifungal activities of the plant extracts were tested using Agar well diffusion method of Perez *et al.*,(1990) and Bonzar *et al.*, (2005) respectively. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter.

### Preparation of Extracts:

The flowers, leaves, seeds and roots of *B.monosperma* were dried and powdered for experimentation. The resultant was then subjected to extraction with methanol in Soxhlet apparatus. The extracts were then concentrated in vacuum under plates were incubated at 27°C for 24 hrs and fungal plates at 24°C for 72hrs. The diameter of the minimum zone of inhibition was recorded and calculated in mm

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**Result and Discussion:**

All the plant parts of *Butea monosperma* were evaluated quantitatively for the analysis of total soluble sugars, starch, protein, phenol, lipid and ascorbic acid. Table 1:

**Table.1. Estimation of primary metabolites (mg/gdw) in different parts of *Butea monosperma* (mg/gdw).**

| Experiments Plant Parts | Sugar | Starch | Protein | Phenol | Lipid |
|---|---|---|---|---|---|
| Root | 5 | 7 | 36 | 1.98 | 46 |
| Flower | 14 | 16 | 250 | 7.56 | 12 |
| Leaf | 10.4 | 22 | 290 | 9.8 | 8 |
| Seed | 9.6 | 24 | 100 | 3.41 | 32 |

mg/gdw = milli gram per gram dry weight

Biochemical studies of the individual plant parts is a necessary prerequisite in order to evaluate their importance in the overall metabolism of the plant, as well as the role of specific substances that may be produced as direct or indirect products of metabolism in same physiological processes. Hence, carbohydrates, proteins, amino acids, chlorophyll, vitamins, hormones, phenols etc., are very essential for plant without which the plant life is hampered. In the present investigation among various primary metabolites maximum total soluble sugars were observed in flowers (14mg/gdw) and starch were observed in seeds (24 mg/gdw)), while minimum in root (7mg/gdw). Leaves had maximum protein content (290 mg/gdw) and roots had minimum (36mg/gdw). Maximum lipids were found in roots (46 mg/gdw), while content in leaves was minimum (8mg/gdw). Phenols were maximum in seeds (7.56mg/gdw) and minimum in roots (1.98mg/gdw).

**Antimicrobial activity**

The maximum zone of inhibition in bacteria culture was observed in chloroform extract of seed against *Bacillus subtilis* (24 mm) and minimum was observed in ethanolic extract of *Bacillus subtilis* (10 mm) while in the case of fungal strains the highest zone of inhibition was shown against *Fusarium oxysporium* in chloroform extract (18mm) of leaves whereas minimum in chloroform extract of roots against *Aspergillus niger* (4mm).

**Evaluation of antibacterial activity**

**Table 2.Antibacterial activity of chloroform extracts of *B. monosperma* .**

| S.No. | Bacterial colonies | | | |
|---|---|---|---|---|
| | ***Bacillus Subtilis*** | ***E. coli*** | ***Streptomyces grisveus*** | ***Staphylococcus aureus*** |
| Flowers | NA | NA | NA | NA |
| Leaves | NA | NA | NA | NA |
| Seeds | IZ- 24<br>AI- 0.12 | NA | NA | NA |
| Roots | NA | NA | NA | NA |

**Table 3. Antibacterial activity of Ethanolic Extract of *B. monosperma.***

| S.No. | Bacterial colonies | | | |
|---|---|---|---|---|
| | ***Bacillus Subtilis*** | ***E. coli*** | ***Streptomyces grisveus*** | ***Staphylococcus aureus*** |
| Flowers | IZ- 11<br>AI- 0.55 | IZ- 16<br>AI-0.8 | NA | NA |
| Leaves | NA | IZ- 12<br>AI-0.6 | NA | IZ-18<br>AI- 0.9 |
| Seeds | IZ- 10<br>AI- 0.50 | NA | NA | NA |
| Roots | IZ- 11<br>AI-- 0.55 | NA | NA | NA |

Standard: Ciprofloxacin -20mm IZ= Inhibition zone (in mm) AI- activity index = IZ of test sample / IZ of standard.

**Table 4. Antifungal activity of Chloroform extract of *B.monosperma.***

| S.No. | Fungal colonies | | | |
|---|---|---|---|---|
| | ***Trichodema ressei*** | ***Aspergillus niger*** | ***Penicillium funiculosum*** | ***Fusarium oxysporium*** |
| Flowers | NA | NA | NA | IZ-12<br>AI- 0.55 |
| Leaves | NA | NA | NA | IZ-18<br>AI- 0.82 |
| Seeds | NA | NA | NA | NA |
| Roots | NA | IZ-4<br>AI-0.18 | NA | NA |

**Table 5. Antifungal activity of ethanolic extract *B.monosperma.***

| S.No. | Fungal colonies | | | |
|---|---|---|---|---|
| | ***Trichoderma ressei*** | ***Aspergillus niger*** | ***Penicillium funiculosum*** | ***Fusarium oxysporium*** |
| Flowers | IZ-16<br>AI – 0.73 | NA | NA | IZ-16<br>AI- 0.73 |
| Leaves | IZ-14<br>AI- 0.64 | NA | NA | IZ-12<br>AI- 0.55 |
| Seeds | NA | NA | NA | NA |
| Roots | NA | NA | NA | NA |

Standard: Ketokonazole- 22mm (fungus) IZ= Inhibition zone (in mm) AI- activity index = IZ of test sample / IZ of standard.

**Discussion**

Herbal drugs have gained importance in recent years because of their efficacy and cost effectiveness. The continuous evolution in bacterial resistance to currently available antibiotics has necessitated the search for novel and effective antimicrobial compounds. The first step towards these goals is the *in vitro* antibacterial activity assay Tanwer et al., (2010). Many primary metabolites lie in their impact as pharmacologically active metabolites in pharmaceutical compounds (Sagwan et al., 2011).

Plant synthesizes primary metabolites (lipid, protein, starch, phenol etc for the normal growth and development of itself. Polysaccharides extracted from Chinese medicinal herbs possess immmmunomodulatory and antimicrobial activity (Wong et al., 1994). Bioactivity of carbohydrates derivatives has also been reported by Nobmann (2009).Further work to isolate and characterize the active compounds responsible for this activity in the plant is recommended in future.

**Conclusion**

The results of the present investigation clearly indicate that the study of primary metabolites and antimicrobial activity of this plant could consider it as a natural herbal source. Thus, the study ascertains the value of plants used in Ayurveda, which could be of considerable interest to the development of pharmaceutical drugs.

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