



## IN-VITRO ANTI MICROBIAL ACTIVITY OF AQUEOUS EXTRACT OF MEDICINAL PLANTS *BUTEA MONOSPERMA*

Nand Kishor<sup>1</sup>, Dr. Vivekanand Katare\*<sup>1</sup>, Mrs. Abhilasha Delouri<sup>1</sup>, Mr. Prabhat Kumar Jain<sup>2</sup>

<sup>1</sup>Vivekanand College of Pharmacy, Bhopal (M.P.)

<sup>2</sup>Scan Research Laboratories, Bhopal (M.P.)

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**\*Correspondence Info:**

**Dr. Vivekanand Katare,  
Vivekanand College of  
Pharmacy, Bhopal (M.P.)**

*Email:*

[vivekanandkatare@gmail.com](mailto:vivekanandkatare@gmail.com)

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**ABSTRACT**

The aim of this work is carried out the phytochemical analysis and antimicrobial activity of *Butea monosperma* (leaves). The Determination of total flavonoids content was performed by aluminum chloride method and was found to be 0.547 mg/100mg in quercetin equivalents The TLC (Thin Layer Chromatography) has been performed which showed the RF value 0.52 of the extract. Antimicrobial activity was performed against 2 stains of human pathogenic bacteria by well diffusion method. The aqueous extract of *Butea Monosperma* showed good antimicrobial activity against selective bacteria. The results of this study show that the aqueous extract of *Butea Monosperma* can be used as easily accessible source of antimicrobial agent.

**Key words:** *Butea Monosperma*, Phytochemical analysia, TLC, Antimicrobial Studies.

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**INTRODUCTION:**

Medicinal plants are rich sources of antimicrobial agents. Plants are used medicinally in different countries and are the source of potential and powerful drugs (Sivastava *et al.*, 1996). A wide range of medicinal parts are used to get different rasayanas which possess different medicinal properties against, different microbes. Although hundreds of plants species have been tested for antimicrobial properties, the majority of these have not been adequately evaluated

(Balandrin *et al.*, 1985). The antimicrobial compounds produced by plants are active against plant and human pathogenic microorganisms (Lee *et al.*, 1998).

This paper deals with the phytochemical analysis and antimicrobial activity of *Butea monosperma* (leaves). *Butea monosperma* (Palas), is a medium-sized deciduous tree belongs to family Leguminosae-Papilionae. This tree is also called 'Flame of the Forest' and Bastard Teak. It grows throughout the

Indian subcontinent, especially in Indo-Gangetic plains. The trunk becomes twisted and gnarled by the wind, making it a conversation piece. Use it as a specimen, or as a background component of the canopy (Vashist and Jindal, 2012; Murti, 1940).

*Butea Monosperma* has astringent ant diarrheal ant dysenteric febrifuge aphrodisiac purgative antihelmintic properties. It is used for timber, resin, fodder, medicine, and dye. The bark and the flowers and the leaves and the gum and even the seeds are used to prepare herbal remedies. The gum from the tree, called kamarkas in Hindi, is used in certain food dishes. The gum is also known as Bengal Kino, and is considered valuable by druggists because of its astringent qualities, and by leather workers because of its tannin (Saldanha and Nicolson, 1976).

## **MATERIALS AND METHODS**

### **Plant material collection**

The Leaves of *Butea Monosperma* was collected from local area of Bhopal (M.P.) in the month of Jan, 2022.

**Extraction procedure** (Pandey and Tripathy, 2014)

*Butea Monosperma* (leaves) was shade dried at room temperature. The shade dried plant

material was coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defeating of the material had taken place. Dried powdered *Butea Monosperma* (leaves) was extracted with water (Aqueous) using maceration process for 48 hrs, filtered and dried using vacuum evaporator at 40 °C.

### **Phytochemical screening**

The *Butea Monosperma* (leaves) extract obtained was subjected to the preliminary phytochemical analysis following standard methods by Kokate and Harborne (Kokate, 1994; Harborne, 1976). The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavonoids, glycosides, Saponins, alkaloids, fats or fixed oils, protein, amino acid and tannins.

### **Total flavonoid content estimation**

Determination of total flavonoids content was based on aluminum chloride method. 50 mg quercetin was dissolved in 50 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 1gm of dried powder of drug was extracted with 100 ml methanol, filter, and make up the volume up to 100 ml. One ml (1mg/ml) of this extract was for the estimation of flavonoid. 1 ml of 2% AlCl<sub>3</sub> methanolic

solution was added to 1 ml of extract or standard and allowed to stand for 60 min at room temperature; absorbance was measured at 420 nm (Merva *et al.*, 2009).

### **TLC (Thin Layer Chromatography) profile**

For the separation of different phytochemical compounds in the aqueous extract of *Butea Monosperma*, the extract was spotted manually using a capillary tube on pre coated silica gel G TLC plates (15X5 cm with 3 mm thickness). The spotted plates were put into a solvent system to detect the suitable mobile phase as per the method of Wagner *et al.* (Wagner *et al.* 1996; Wagner *et al.* 1984). After the separation of phytochemical constituents, the spraying reagents such as Dragendorff reagent, 10% ethanolic sulphuric acid, 10% sulphuric acid, 5% ferric chloride, Kedde reagent, vanillin phosphoric acid reagent and vanillin sulphuric acid reagent were used to identify the respective compounds. The colour of the spots was noted and Rf values were calculated by using the following formula:

$$\text{Retention time (Rf)} \\ = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

### **Antimicrobial activity Pathogenic antimicrobial used**

The pathogenic bacteria used in the current study were obtained from Microbial Culture Collection, BMHRS, Bhopal, and Madhya Pradesh, India.

### **Antibiogram studies**

Broth cultures of the pure culture isolates of those test microorganisms which are sensitive towards the phytoextracts used in present study were prepared by transferring a loop of culture into sterile nutrient broth and incubated at 37°C for 24-48 hrs. A loop full was taken from these broths and seeded onto sterile nutrient agar plates through sterile cotton swab to develop diffused heavy lawn culture. The well diffusion method was used to determine the antimicrobial activity of the extract prepared from the plant material of *Butea Monosperma*, using standard procedure (Bauer *et al.*, 1966).

There were 3 concentrations used which are 25, 50 and 100 mg/ml for each extracted phytochemical in antibiogram studies. Its essential feature is the placing of wells with the antibiotics on the surfaces of agar immediately after inoculation with the organism tested. Undiluted overnight broth cultures should never be used as an inoculum. The plates were incubated at 37 °C for 24 hrs and then

examined for clear zones of inhibition around the wells impregnated with particular concentration of drug.

## RESULTS AND DISCUSSION

Phytochemical screening of the plant showed the presence of flavonoids and flavonoids. Proteins and amino acids, carbohydrates, Diterpenes, alkaloids and Saponins were found to be absent. Table 1

**Table 1: Result of phytochemical screening of aqueous extract of *Butea Monosperma***

S. No.	Constituents	Aqueous Extract
1	Alkaloids	-
2	Flavonoids	+
3	Diterpenes	-
4	Phenolics	+
5	Amino Acids	-
6	Carbohydrate	-
7	Proteins	-
8	Saponins	-

A number of developing solvent systems were tried, but the satisfactory resolution was obtained in the solvent systems mentioned in table 2.

After development of plates, they were air-dried and numbers of bands were noted & RF values were calculated.

**Table 2: Calculation of Rf. Value**

Compound	Extract	Rf Value
Quercetin	Toluene: Ethyl acetate: Formic acid (5:4:1)	0.521

Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve:

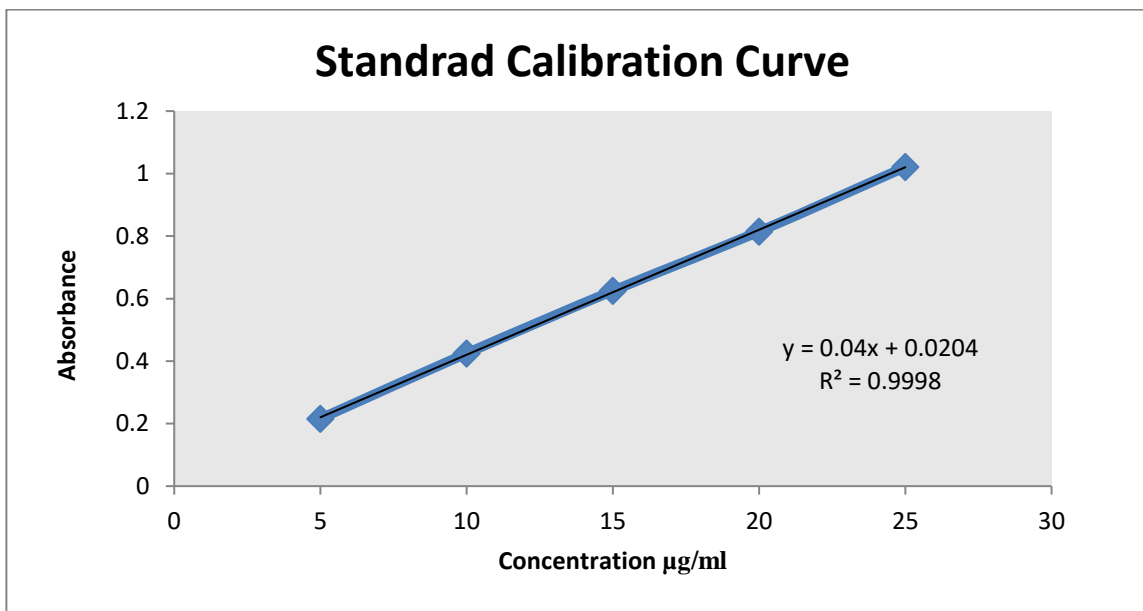
$Y=0.04X + 0.020$ ,  $R^2=0.999$ , where X is the quercetin equivalent (QE) and Y is the absorbance table 3 and fig 1.

**Table 3: Preparation of calibration curve of quercetin**

S. No.	Concentration	Absorbance
0	0	0
1	5	0.216
2	10	0.425
3	15	0.625
4	20	0.815
5	25	1.021

The total flavonoid contents were determined by established methods and were found to be

0.546 mg/100mg in quercetin equivalents table 4.



**Figure 1: Estimation of total flavonoids content**

**Table 4: Estimation of total flavonoids content of *Butea Monosperma***

S. No	Plant material	Total flavonoids Equivalent to Quercetin mg/ 100 mg of extract
1.	<i>Butea Monosperma</i>	0.546

The lawn cultures were prepared with all the microbes used under present study and sensitivity of bacteria towards the various phytochemicals extracts obtained from the *Butea Monosperma* was studied at the concentration of 25-100 mg/ml using well diffusion method. Antimicrobial activity was

performed against 2 stains of human pathogenic bacteria by well diffusion method. The aqueous extract of *Butea Monosperma* showed good antimicrobial activity against selective bacteria table 5.

**Table 5: Antibacterial activity of *Butea Monosperma* on different microbes**

Extract	Name of microbes	Zone of inhibition		
		100mg/ml	50 mg/ml	25mg/ml
Aqueous extract of <i>Butea Monosperma</i>	Escherichia coli	6	10	12
	Staphylococcus aureus	9	11	12

## CONCLUSION

The preliminary phytochemical investigation indicates the presence of flavonoids in the plant material. In addition, the aqueous extract of *Butea Monosperma* found to contain a noticeable amount of flavonoids, which play a major role in controlling microbes. The results

of this study show that the aqueous extract of *Butea Monosperma* can be used as easily accessible source of antimicrobial agent. However, the components responsible for the antimicrobial activity of aqueous extract of *Butea Monosperma* are currently unclear. Therefore, further works have been performed on the isolation and identification of the

antioxidant components present in aqueous extract of *Butea Monosperma*.

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