Phytochemical Analysis, Antibacterial and Antioxidant Activities of Methanolic Extract of *Butea Monosperma* Leaves

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Abstract: **Most of the medicinal plants possess antimicrobial activity since they are rich in phytochemicals that possess medicinal properties. The current study analyses the phytochemical studies, antibacterial and antioxidant activities of leaves of *Butea monosperma.* Methods: The methanol extract of the leaves of *Butea monosperma* were used for the above mentioned studies. Reducing power assay was used to determine the antioxidant activity. Disc diffusion method was performed to determine the antibacterial activity against *Bacillus subtilis, Staphylococus aureus, and Escherichia coli*. The phytochemical analysis of the methanol extract revealed that the plant has major phytochemicals except Terpenoids and Arthroquinones**.

***Index terms*: Antioxidant activity, *Butea monosperma,* Antibacterial activity, Phytochemicals.**

I. INTRODUCTION

Butea monosperma is a medicinal plant that belongs to the plant family Fabaceae and the order Fabales. The bright orange color of the flower gives it the name "Flame of the forest".[1, 2]It is effective in treating skin problems, diarrhoea, diabetes, sore throat, intestinal worms . It belongs to deciduous family of trees and are being cultivated in drier parts, open grasslands and wastelands.[3,4] This plant possesses antidiabetic,[5,6] antifertility,[7] anticonvulsive,[8] antistress,[9] anthelmintic,[10] anticancerous,[11,12] antiinflammatory,[13-15] antidiarrhoeal,[16] and antibacterial properties[17]. The medicinal parts of the plant include leaves, seeds, flowers, stem and gum. Microbes are becoming resistant to synthetic antibiotics and therefore the search for antimicrobial activity of medicinal plants are carried out all over the world. Because of this specific property, phytochemicals that are present in the plants have been considered as important. Phytochemicals are naturally occurring chemicals that are present in most of the medicinal plants. These phytochemicals have an ability of antimicrobial and antioxidant activities. The present study on the leaves of *Butea monosperma* selected due to the fact that not many studies have been done on the leaves among the various plant parts. Hence, this study aims on the analysis of *Butea monosperma* with respect to medicinal aspects.

II. MATERIALS AND METHODS

Collection of plant material

Butea monosperma leaves were collected from Kuruvambatti, Salem, Tamilnadu, India and authenticated by the Botanist at ABS garden Kuruvambatti, Salem, Tamilnadu, India with the reference id 7136124388.

Extraction of plant material

Butea monosperma leaves were washed with distilled water and made into powder. The powder was mixed with methanol at 10 g/100 ml concentration and maintained at room temperature overnight.

Phytochemical Screening

The following Phytochemical analysis was carried out for the 10 g/100 ml methanol extract of sample as per standard methods described by Brain and Turner 1975 and Evans 1996. The alkaloid test was conducted by Mayer's test and Wagner's test. Flavonoids were detected by Wagner's test and Lead acetate test H_2SO_4 test. Steroids were detected by Liebermann-Burchard test. Terpenoids were detected by Salkowski's test. Anthroquinones were detected by Borntrager's test. Phenols were detected by ferric chloride test and lead acetate test. Saponins were detected by Froth test**.** Tannins were detected by Ferric chloride test. Carbohydrates were detected by Fehling's test. Oils and resins were detected by Spot test.

Antioxidant Activity:

Reducing Power Assay:

The reducing power was determined according Oyaizu's method (1986). The sample with 1.5 ml of 1% Ascorbic acid solution was spiked with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was kept at 50° in a water-bath for 20 m. The resulting solution was cooled rapidly, spiked with 2.5 ml of 10% trichloroacetic acid, and centrifuged at 3000 rpm for 10 m. 5 ml of supernatant was collected and mixed with 5 ml of distilled water and 1 ml of 0.1% ferric chloride. The absorbance was detected at 700 nm using UV visible spectrometer for every 10 m. The higher the absorbance the stronger the reducing power. The reducing power assay was expressed in terms of ascorbic acid equivalent per gram of dry weight basis.

Formula

$$\% IC_{50} = \frac{\text{OD OF SAMPLE} - \text{OD OF CONTROL}}{\text{OD OF CONTROL}} \times 100 \quad (1)$$

Where % IC_{50} - Minimum inhibitory concentration
OD – Optical density in nm

Screening of antibacterial activity

Preparation of inoculum

Inoculum was prepared by transferring a loop full of culture from the stock cultures to test tube of Muller-Hinton broth (MHB) incubated without agitation for 24 h at 37º and 25º respectively. The cultures were diluted with fresh MHB to achieve optical densities corresponding to 2.0 X 10^6 colony forming units (CFU/ml) for bacteria.

Antimicrobial susceptibility test

The disc diffusion method (Bauer *et al.,* 1966) was used to screen the antimicrobial activity and ampicillin was used as a positive control (100 mg/ml). The bacterial cultures used were *Bacillus subtilis* MTCC 441, *Staphylococus aureus* MTCC 737, *and Escherichia coli* MTCC 1687. *In vitro* antimicrobial activity was screened by using Muller Hinton Agar (MHA) obtained from Hi-media (Mumbai). The MHA plates were prepared by pouring 15 ml of molten media into sterile petri plates and inoculums were swabbed uniformly. 40 mg of extracts were loaded on 6 mm sterile disc. The plates were kept for incubation at 37º for 24 h. At the end of incubation, inhibition zones formed and were measured with transparent ruler in millimeter.

III. RESULTS

Phytochemical Analysis of *Butea monosperma*:

Phytochemical screening of *Butea monosperma* were performed to confirm the presence of phytochemicals and it showed that the plant has sufficient phytochemicals needed for drug production (Table 1).

Table 1: Phytochemical screening of methanol extract

Phytochemicals	Observations	Presence/Absence
Alkaloids Mayer's test Wagner's test	Cream colour Reddish brown solution/ precipitate	+ +
Flavonoids Lead acetate test H_2SO_4 test	Yellow orange Reddish brown / Orange colour precipitate	+ +
Steroids Liebermann-Burchard test	Violet to blue or Green colour formation	+
Terpenoids Salkowski test	Reddish brown precipitate	-
Arthroquinone Borntrager's test	Pink colour	-
Phenols Ferric chloride test Lead acetate test	Deep blue to Black colour formation White precipitate	++
Saponin	Stable persistent	+
Tannin	Brownish green / Blue black	+
Carbohydrates	Yellow / brownish / blue / green colour	+
Oils & Resins	Filter paper method	-

Where + represents presence and – represents absence.

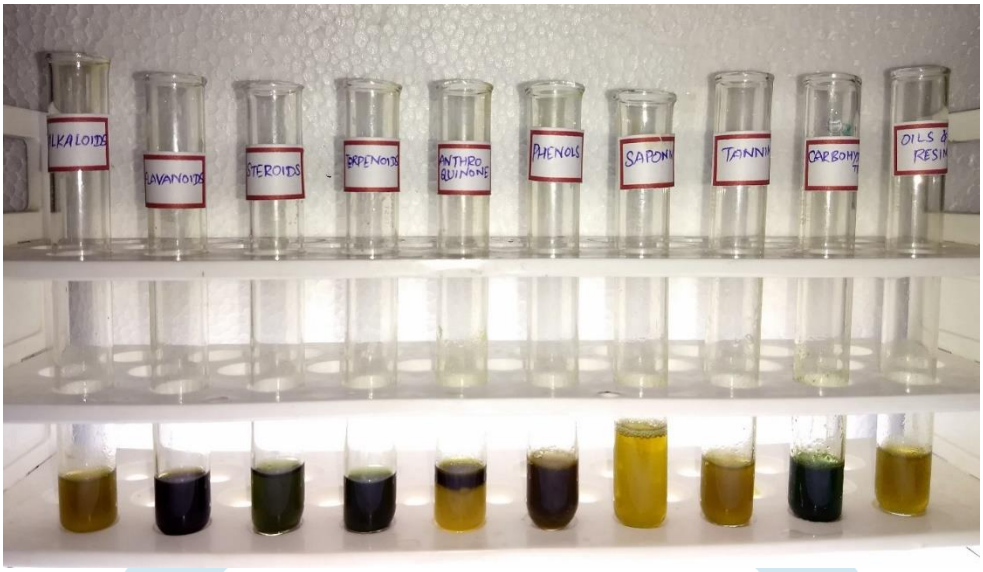


Figure 1: Phytochemical analysis of methanol extract of *Butea monosperma*

The presence of phytochemicals were confirmed by different tests (Figure 1). During this study, it was found that the expected color changes did not occurred for anthroquinones and terpenoids. Therefore, these phytochemicals were considered to be absent.

Antioxidant Activity

Reducing Power Assay

Table 2: Determination of half maximal inhibitory concentration (IC_{50}) of methanol extracts of *Butea monosperma*

S .No	Concentration (µg/ml)	OD (nm)	% IC_{50}	IC_{50}
1.	250	0.199	35.37	591.30
2.	500	0.219	48.97	
3.	750	0.227	54.42	
4.	1000	0.245	66.66	

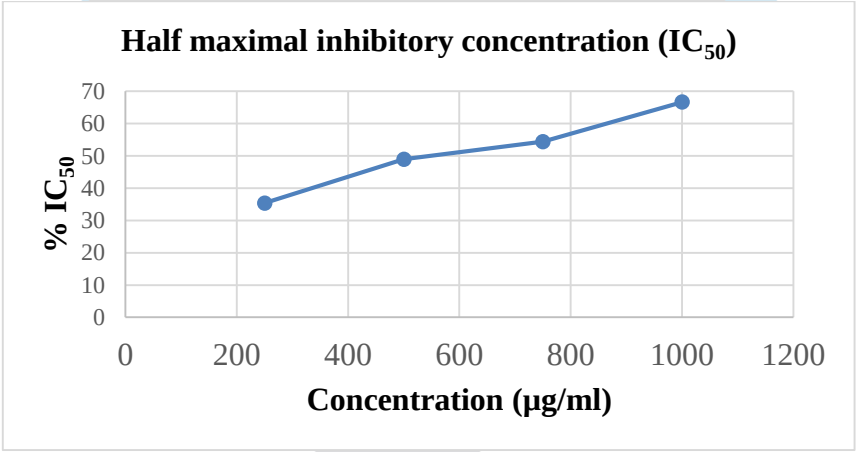


Figure 2: Half maximal inhibitory concentration (IC_{50}) of the sample

The antioxidant activity was performed by reducing power assay method. This activity neutralizes free radicals which is an important property for drug production. The **%IC_{50}** is the lowest concentration of a drug that inhibits the growth of a microorganism. The **%IC_{50}** values at 250, 500, 750 and 1000 (µg/ml) concentration was found to be 35.37, 48.97, 54.42, and 66.66 respectively using the formula (1). The absorbance of control was found to be 0.471 nm. The half maximal inhibitory concentration (IC_{50}) represents the property of drugs to inhibit the activity of microorganisms by 50% (Figure 2). The (IC_{50}) value was found to be 591.30 (Table 2).

Antibacterial Study

The antibacterial activity exhibited by methanol extract is summarized (Table 3). The methanol extract of *Butea monosperma* restricted the activities of bacterial species such as *Bacillus subtilis, Staphylococus aureus, and Escherichia coli* at 40, 50, 60, and

70 mg/ml concentrations. The zone of inhibition occurred between 13 to 20 mm. At 70 mg/ml concentration, the methanol extract exhibited better antibacterial activity.

Table 3: Antibacterial activity of *Butea monosperma*

S .No	Organisms	Control mg/ml	40 mg/ml	50 mg/ml	60 mg/ml	70 mg/ml
1.	*B. subtilis*	28	13	15	17	18
2.	*S. aureus*	25	15	16	18	19
3.	*E. coli*	23	14	16	18	20

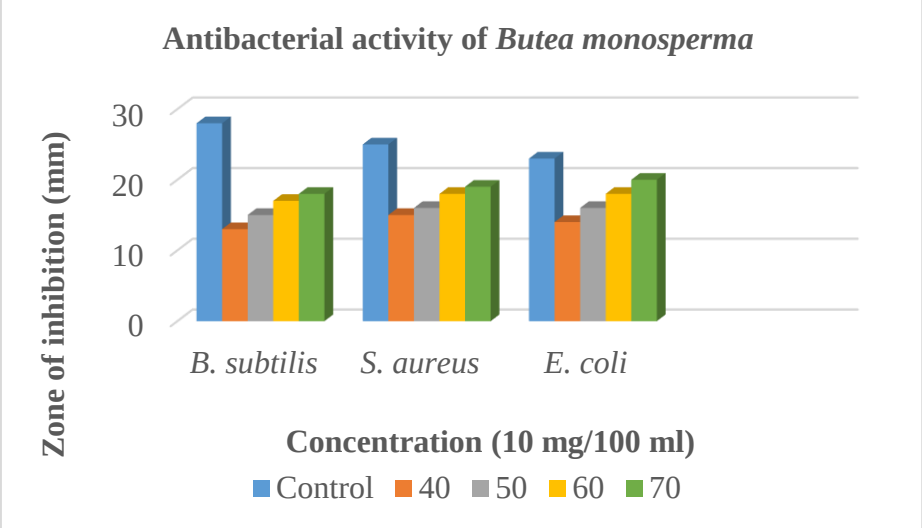
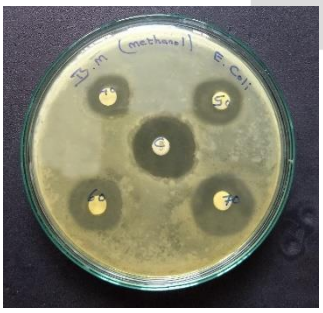
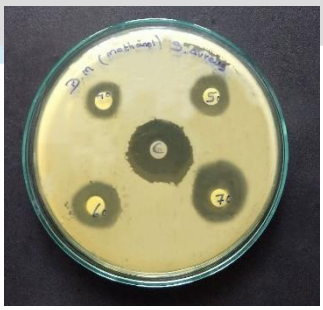


Figure 3: Antibacterial activity of *Butea monosperma*

Figure 4: Disc diffusion method



Zone of inhibition observed on *Escherichia coli*



Zone of inhibition observed on *Staphylococus aureus*

Zone of inhibition observed on *Bacillus subtilis*

IV. DISCUSSION

The antibacterial activity has been evaluated by disc diffusion method. The antibacterial activity increases with the concentration of methanol extract and lower concentrations has less activity on bacterial species. The methanol extract inhibited both gram positive and gram negative bacterial species. Sweta daru *et al* (2016) reported antibacterial activity for *B. subtilis, S. aureus, E.coli* as 18, 11, 14 mm diameter of inhibition zones respectively and present study summarize that the antibacterial activity was found to be 18, 19, 20 mm diameter of inhibition zones for *B. subtilis, S. aureus, E.coli* respectively. The phytochemical screening showed that

plants contains some major phytochemicals. Sweta daru *et al.,* (2016) summarized the phytochemical study in the absence of steroids and glycosides and this study reports that the phytochemicals such as anthroquinones and terpenoids are absent. The %IC50 value increased with the increase in concentration. In their previous study, Sweta daru *et al.,* (2016) mentioned that the minimum inhibitory concentration of the methanol extract shows turbidity activity in between the range of 100 – 6000 µg/ml. The current study reports the $\%IC_{50}$ values between 200 – 1000 µg/ml concentrations and was found to be 35.37, 48.97, 54.42, and 66.66.

V. CONCLUSION

It is evident from this study that the methanol extract restricted several bacterial species and also has antioxidant activities for the neutralization of free radicals. The phytochemical studies carried out in this study showed that the plant is eligible for drug productions. Hence this study indicates that *Butea monosperma* has properties which help in producing drugs. Further characterizations can be can be in this plant for treating diseases.

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