Acta Scientific MICROBIOLOGY (ISSN: 2581-3226)

Volume 6 Issue 10 October 2023

Research Article

Comparative Antimicrobial Activity of Different Extracts of Different Parts of *Butea monosperma*

Abhishek Pandey* and Salma Khanam

Al-Ameen College of Pharmacy, Bangalore, India

***Corresponding Author:** Abhishek Pandey, Al-Ameen College of Pharmacy, Bangalore, India.

DOI: 10.31080/ASMI.2023.06.1304

Received: August 07, 2023

Published: September 21, 2023

Abstract

Extracts of leaves, flower and bark of *Butea monosperma* belonging to the family: *Fabaceae* were prepared and subjected to preliminary antibacterial evaluation. The methanolic extracts obtained by soxhlation method were found to possess better activity compared to the aqueous extracts prepared by refluxation method. The extracts were found to contain significant amounts of phenols, flavonoids and tannins, which are known for their antibacterial properties.

The antibacterial activity of extracts was determined against *Staphylococcus aureus, Bacillus cereus, Escherichia coli* and *Salmonella typhi* using agar diffusion and MIC determination methods. The extracts were found to be active at a concentration of 1mg/ml. The results indicated that extracts of *Butea monosperma* possess good antibacterial property.

Keywords: *Butea monosperma*; Anti-bacterial; Soxhlation; Refluxation; *Staphylococcus aureus; Bacillus cereus; Escherichia coli* and *Salmonella typhi*; MIC

Abbreviation

Sl. No.: Serial Number; +ve: Positive; -ve: Negative; w/w: Weight/Weight; V/V: Volume/volume; W/V: Weight/Volume; Hrs.: Hours; **µg**/mcg: Microgram; %: Percentage; MIC: Minimum Inhibitory Concentration; IP: Indian Pharmacopoeia; Temp: Temperature; MTCC: Microbial Type Culture Collection; DMSO: Dimethyl Sulphoxide; TLC: Thin Layer Chromatography; DSC: Differential Scanning Calorimetry; MBC: Minimum Bactericidal Concentrations

Introduction

Butea monosperma (Family: Fabaceae). This is a moderate sized deciduous tree which is widely distributed throughout India, Burma and Ceylon, popularly known as 'dhak' or 'palas', commonly known as 'Flame of forest' [1] *Butea monosperma* is a plant which is commonly found in the drier parts of the India [2] The plant is traditionally reported to possess astringent, bitter, alterative, aphrodiasiac, anthelmintic [3], antibacterial and anti-asthmatic properties [4] Flowers yields a brilliant yellow coloring matter due to presence of chalcones.

The flowers are widely used in treatment of hepatic disorders, viral hepatitis, diarrhea, depurative and tonic [5] The flowers are also good source of flavonoids [6] *Butea monosperma* flowers are known to contain flavonoids and glucosides Butin, isobutrin and butein are main phytoconstituents of flowers [7] Roots contain glucose, glucosides, glycine, and aromatic compounds. Bark contains various tannins like Kino-tannic acid, pyrocatechin. It also contains gallic acid, butolic acid, palasitrin, butrin, alanind, allophanic acid, cyanidin, histidine, lupenone, lupeol, miroestrol, medicarpin, shellolic acid and palasimide. Leaves contain glucoside, linoleic acid, palmitic lignoceric acid, 3-alphahydroxyeuph- 25-enylheptacosanoate and 3,9-dimethoxypterocapan (Mishra et al., 2000). Gum contains tannins, pyrocatechin and mucilaginous

material [8].

Plants and other natural sources can provide a huge range of complex and structurally diverse compounds. Recently, many researchers have focused on the investigation of plant and microbial extracts, essential oils, pure secondary metabolites and new synthesized molecules as potential antimicrobial agents [9]. Antibacterial activity of methanolic extracts of Leave and flower of *Butea monosperma* has been reported against *S. aureus, B. cereus* and *B. subtitles* [10]. Ethanol extract of *Butea Monosperma* was able to produce sensitivity in *S. typhi*, *E. coli, Pseudomonas* and *S. paratyphi. Shigella flexneri* was strongly inhibited by the same extract. Moderate sensitivity was observed in *S. aureus, E. aerogenes, S. typhimurium, Pr. vulgaris* and *Klebsiella* sp reported [11].

Materials and Methods

- Collection: Different parts of *Butea monosperma* Family-Fabaceae including leaves, flowers and bark were collected from Garhwa, Jharkhand.
- Authenticated by Dr. T.N. Shivananda principal Scientist and scientist in charge, IIHR Bangalore. The sample of leaves, bark and flower were subjected to drying at room temperature. The bark sample dried at 55°C for 72 hours and all the sample were powder mesh size #16.

Preparation of extracts

Extraction by soxhelet method

Procedure: 50 gm of powdered bark, flower, and leaves were subjected to Soxhelet extraction with methanol for 18 hrs. All the extracts were concentrated by using Rotatory vacuum evaporator. They were then air dried.

Extraction by refluxation method

Procedure: 50 gm of powdered bark, flower, and leave parts were subjected to Refluxation extraction with water for 18 hours. All the extracts were filtered and concentrated by using Rotatory vacuum evaporator. They were then air dried.

Phytochemical screening

The leaf, flower and bark extracts of *Butea Monosperma* showing good activity were subjected to phytochemical screening tests to identify the presence of tannins, carbohydrates, sterols, Saponins, alkaloids and aglycones. The results are tabulated in Table 03 and 04.

Preliminary Anti-microbial activity

Six extracts prepare by two different methods of extraction were subjected to anti-microbial screening using Agar diffusion method against *E. Coli* and *B. subtilis* at concertation of 10 mg/ml. The extract prepared by soxhlation were found to be possess better activity. Hence the extract prepare by soxhlation were subjected to anti-microbial studies.

Microorganism used

Standard cultures of bacteria - *Bacillus cereus* (MTCC 1272), *Staphylococcus aureus* (MTCC 96), *E. coli* (MTCC 433) and *Salmonella typhi* (MTCC 735). Were obtained from Eureka Analytical PVT LTD, Bangalore.

Materials

- Standard antibacterial: Tetracycline
- Medium: Nutrient agar medium (for bacteria)
- 0.9% NaCl solution.

Preparation of standard inoculum

McFarland Nephelometer standards

A chemically induced precipitation reaction can be used to approximate the turbidity of a bacterial suspension which is produced by the interaction of barium chloride with sulfuric acid. A series of tubes with varying turbidity's using this reaction has been standardized.

Procedure

Ten test tubes of equal size were set up. A 1% chemically pure Sulphuric acid solution was prepared. A 1.175 % aqueous solution of barium chloride ($BaCl_2$) was prepared. Slowly with constant agitation the designated amount of the two solutions were added to the tubes as given in the table to make a total of 10 ml per tube. The tubes were sealed and the suspended barium sulfate precipitate corresponds approximately to homogenous cells densities. The McFarland standard tubes were stored in the dark at room temperatures, as they are stable for six months.

The preparation of different McFarland standards and their cell density equivalents are shown in Table 1.

Preparation of inoculum

A 24 Hours old culture was used for the preparation of bacterial

In ml/ Tube No.	0.5	1	2	3	4	5	6	7	8	9	10
BaCl2	0.05	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
Approx. Cell Density (1x108)	1.5	3	6	9	12	15	18	21	24	27	30

Table 1: McFarland standards.

suspension. A suspension of bacteria was made in a 5ml sterile isotonic solution of sodium chloride, the turbidity was adjusted with sterile saline to approximately a turbidity of # 0.5, l and 2 McFarland standards respectively.

Culture medium

Nutrient agar medium was used for study of antibacterial activity. The medium was prepared by dissolving the different ingredients in water and sterilized Composition of nutrient agar is shown in Table 2.

Standard preparation

Sl. No.	Ingredients	Weight
1.	Beef Extract	4.0
2.	Peptone	5.0
3.	Agar	20.0
4.	Distilled Water	q.s 100 ml
5.	PH	5.4

Table 2: Composition of nutrient agar medium.

A stock solution of standard Tetracycline 30 µg/ml in sterile distilled water was prepared.

Agar diffusion method

Standardization of method of anti-bacterial activity was done using Tetracycline. Sterile nutrient agar was inoculated with standardized inoculum of Bacillus cereus, Staphylococcus aureus, E-coli, and Salmonella typhi each adjusted to # 0.5,1, 2 by spread plate method. 0.2ml of inoculum was used. One bore each was made in each of the plate using a sterile cork borer. 0.1 ml of 30 µg/ml solution of Tetracycline was added and kept in refrigerator for 2 hours to allow diffusion. The Petri dishes were incubated at 37± 1°C for 24 hours and observed for the zones of inhibition measured using a scale. The 0.5 McFarland concentration was selected for further studies.

Anti-bacterial activity of the extract

Total six of extracts of Flower, Bark and Leaves of *Butea Monosperma* obtained by Refluxation and soxhlation were subjected to screening for anti-microbial activity.

Sample preparation

1gm, 2gm and 5gm of extracts was dissolved in 10ml of DMSO concentration of sample was 100, 200 and 500 mg/ml solution.

Procedure

About 30 ml of sterile Nutrient agar medium was allowed to solidify in sterile Petri dishes. After solidification 0.2 ml bacterial suspension adjusted to # 0.5 McFarland standard was added on solidified medium and spread using a glass spreader. Bores were made in each plate, each of the extracts were tested on separate plates. 0.1 ml of the extracts were added into the cups. 0.1 ml of a 30 µg/ml solution of a standard tetracycline was added into the third cup. While the fourth cup contained DMSO as blank. The petri dishes were kept two hours for diffusion in refrigerator and after diffusion the petri dishes were incubated at 37± 1°C for 24 hours and after 24 hours the zone of inhibition were observed and measured using a scale. This method has been done with all bacteria in similar manner. The results are tabulated in Table no. - And the Photograph shown in Photo.

Extracts obtained by soxhelet method were found to be active hence they were selected for further studies.

Determination of minimum inhibitory concentration (MIC)

MIC determination of the extracts was carried out by serial dilution method. Media used was Muller Hinton broth for bacteria. Organisms used were *E coli*, and *B. subtilis*. McFarland constant selected was 0.5 which contains an approximate cell density of 1x108 cells/ml. The test tubes were serially diluted and different concentrations of extracts were added along with the inoculum. The tube with inoculum without extract served as positive control whereas the tube without inoculum served as negative control. The results are tabulated in Table 05, 06 and 07.

Results and Discussion

Phytochemical screening

Extracts were subjected to qualitative evaluation in order to find out the chemical constituents present in methanolic and aqueous extract. The results are shown in Table 03 and 04.

The methanolic extract obtained by soxhlation of bark was found to contain Alkaloids, Glycosides, Phenols, Tannins and Flavonoids. The methanolic extract obtain soxhlation of leaves was found to contain Alkaloids, Phytosterols, flavonoids and Carbohydrates. The methanolic extract obtain soxhlation of flower was found to contain Alkaloids, Phenols, Tannins and Flavonoids. The aqueous extracts obtained by Refluxation of bark was found to contain Alkaloids, Carbohydrates and Flavonoids the aqueous extracts obtained by Refluxation of leaves was found to contain Carbohydrates and Diterpenes. The aqueous extracts obtain Refluxation of flower was found to contain Alkaloids, Phenols, Carbohydrates and Flavonoids. These results are similar to the reports published in the literature. All the extracts were found to be contain tannins and flavonoids which are known to be antibacterial.

Chemical Constituents	Tests	Flower	Bark	Leaves
Alkaloids	1. Mayer's test	+ve	+ve	+ve
	2. Dragendroff's	+ve	-ve	+ve
	3. Wagner's test	-ve	+ve	-ve
	4. Hager's test	-ve	-ve	+ve
Carbohydrates	1. Molishc's test	-ve	-ve	+ve
	2. Benedict's test	-ve	-ve	+ve
	3. Fehling's test	+ve	+ve	+ve
Glycosides	1. Modified Bontrager's test	-ve	+ve	-ve
	2. Legal test	-ve	-ve	-ve
Saponins	1. Foam test	-ve	-ve	-ve
Phytosterols	1. Salkowski test	-ve	-ve	+ve
	2. Libermann Burchard	-ve	-ve	-ve
	3. Tschugajew test	-ve	-ve	-ve
Fats & Oil	1. Stain test	-ve	-ve	+ve
Resins	1. Acetone water test	-ve	-ve	-ve
Phenols	1. Ferric chloride test	+ve	+ve	-ve
Tannins	1. Alkaline reagent	+ve	+ve	-ve
Flavonoids	1. Gelatin test	+ve	+ve	-ve
	2. Lead acetate test	-ve	+ve	-ve
	3. Shinoda test	+ve	+ve	+ve
Proteins	1. Xanthoproteic test	-ve	-ve	-ve
	2. Ninhydrin test	-ve	-ve	-ve
	3. Biuret test	-ve	-ve	-ve
Diterpenes	1. Copper acetate test	-ve	-ve	-ve

Table 3: Results of Qualitative Chemical Tests of Extracts of *Butea monosperma* by Soxhelet Method with methanol.

Chemical Constituents	Tests	Flower	Bark	Leaves
Alkaloids	1. Mayer's test	+ve	+ve	-ve
	2. Dragendroff's	+ve	-ve	+ve
	3. Wagner's test	-ve	+ve	-ve
	4. Hager's test	-ve	-ve	+ve
Carbohydrates	1. Molishc's test	-ve	-ve	+ve
	2. Benedict's test	-ve	-ve	+ve
	3. Fehling's test	+ve	+ve	+ve
Glycosides	1. Modified Bontrager's test	-ve	+ve	-ve
	2. Legal test	-ve	-ve	+ve
Saponins	1. Foam test	-ve	-ve	-ve
Phytosterols	1. Salkowski test	-ve	-ve	+ve
	2. Libermann Burchard	-ve	-ve	-ve
	3. Tschugajew test	-ve	-ve	-ve
Fats & Oil	1. Stain test	-ve	-ve	+ve
Resins	1. Acetone water test	-ve	+ve	-ve
Phenols	1. Ferric chloride test	+ve	-ve	-ve
Tannins	1. Alkaline reagent	-ve	-ve	-ve
Flavonoids	1. Gelatin test	-ve	-ve	-ve
	2. Lead acetate test	-ve	-ve	-ve
	3. Shinoda test	+ve	+ve	-ve
Proteins	1. Xanthoproteic test	-ve	-ve	-ve
	2. Ninhydrin test	-ve	-ve	-ve
	3. Biuret test	-ve	-ve	-ve
Diterpenes	1. Copper acetate test	-ve	-ve	+ve

Table 4: Results of Qualitative Chemical Tests of Extracts of *Butea monosperma* by Refluxation Method with water.

Preliminary anti-bacterial activity

Standardization of anti-bacterial procedure

Agar diffusion method described by Parmer et al was used for anti-bacterial studies. The method was standardized using tetracycline at the concentration of 10 µg/ml. Four bacteria viz: - *Bacillus cereus (MTCC 1272), Staphylococcus aureus (MTCC 96), E. coli (MTCC 433)* and *Salmonella typhi (MTCC 735)* were used. The inoculum prepared by using 0.5 McFarland showed uniform growth with clear zone of inhibition was observed after 24-hour incubation at 37±1°C. Hence this was used for further studies.

The various extracts obtained by different methods of extraction were subjected to antibacterial susceptibility testing by agar diffusion method. The zones of inhibition of different extracts are expressed in mm. The results are tabulated are in Table 5 and 6. All the extracts were found to be active at a concentration of 10 mg/ml. The methanolic soxhlet extracts were found to possess better activity in terms of Zones of inhibition.

	Zones of inhibition (mm)									
	Bark extract mg/ml			Leaf extract mg/ml			Flower extract mg/ml			Tetracycline mg/ml
	10	20	50	10	20	50	10	20	50	0.1 mg
B. cereus	20	25	28	18	22	28	21	28	29	32
S. aureus	16	18	20	12	12	18	15	18	20	25
E. coli	-	-	18	-	-	20	-	-	22	40
S. typhi	15	20	23	12	15	22	17	21	25	30

Table 5: Data showing Zones of inhibition of extracts obtained by Refluxation.

	Zones of inhibition (mm)									
	Bark extract mg/ml			Leaf extract mg/ml			Flower extract mg/ml			Tetracycline mg/ml
	10	20	50	10	20	50	10	20	50	0.1 mg
B. cereus	29	30	32	22	27	30	24	28	30	32
S. aureus	17	20	22	12	15	20	15	18	25	25
E. coli	-	-	18	-	-	22	-	-	26	40
S. typhi	18	22	25	12	18	25	17	21	24	30

Table 6: Data showing Zones of inhibition of extracts obtained by Soxhlation.

Hence, they were selected for further anti-bacterial study using LB medium at a lower concentration.

Anti-Bacterial screening of extracts using LB medium

Luria Bertani medium was used to confirm the antibacterial activity. LB medium is used for further and uniform growth of bacteria

Determination of zones of inhibition of selected methanolic extracts

The methanolic extracts were subjected to anti-bacterial screening at a concentration of 1 mg/ml and 2 mg/ml using LB medium. The zones of inhibition are shown in Table and photographs are shown in Photo 4-15 The comparative activity depicted in Figure 1 and 2.

All the extracts were found to be active at 1 mg and 2 mg against all the organism except *E. Coli.* Bark extracts exhibited maximum zones of inhibition at 1 mg concentration compare to other extracts.

Antibacterial activity of bark extract against pathogens

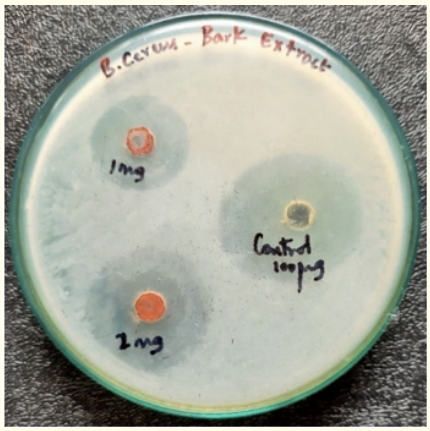

Photo 1: Bark extract against *B. Cereus*

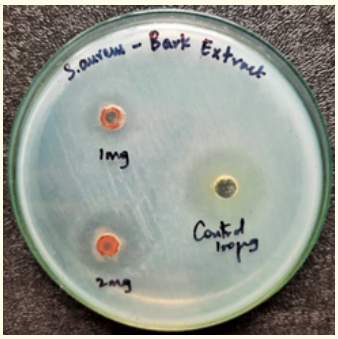

Photo 2: Bark extract against *S. aureus.*

Photo 3: Bark extract against *E. Coli.*

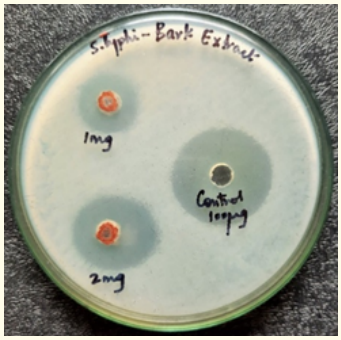

Photo 4: Bark extract against *S. typhi.*

Antibacterial activity of leaves extract against pathogen

Photo 5: Leaf extract against *B. Cereus.*

Photo 6: Leaf extract against *S. aureus.*

Photo 7: Leaf extract against *E. Coli.*

Photo 8: Leaf extract against *S. Typhi.*

Antibacterial activity of flower extract against pathogen

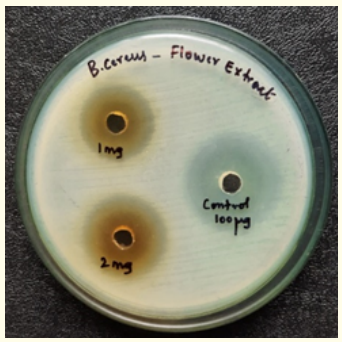

Photo 9: Flower extract against *B. Cereus.*

Photo 10: Flower extract against *S. aureus.*

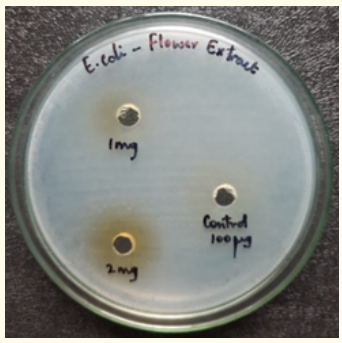

Photo 11: Flower extract against *E. Coli.*

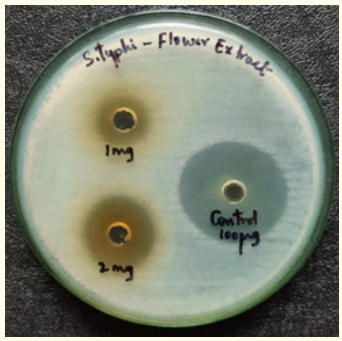

Photo 12: Flower extract against *S. typhi.*

MIC of methanolic extract of flower was found to be 1000 µg against *S aureus*, 1000 µg against *Bacillus cereus*, 2000 µg against *E-coli* and 31.25 µg against *S. typhi*.

MIC of methanolic extract of bark were found to be 100 0 µg against *Bacillus cereus*, 1000 µg/ml against *Staphylococcus aureus*, 2000 µg against *E-coli* and 62.5 µg against *Salmonella typhi* shown in photograph 13, 14 and 15.

MIC of methanolic extract of leaves were found to be 500 µg against *Bacillus cereus,* 500 µg against *Staphylococcus aureus*, 2000 µg against *E-coli* and 125 µg against *Salmonella typhi*. The comparative results are depicted in Figure 3.

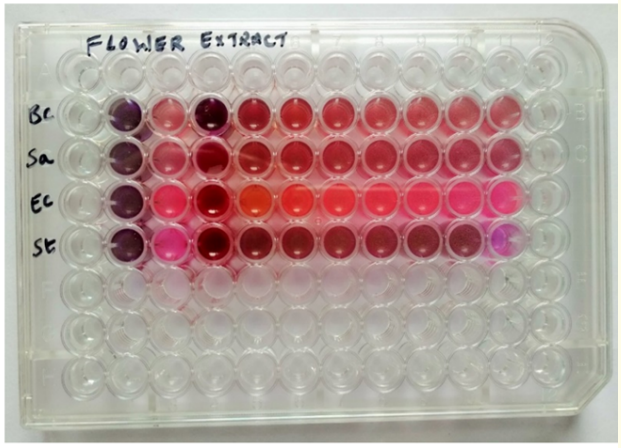

Photo 13: Flower extract micro titer plate.

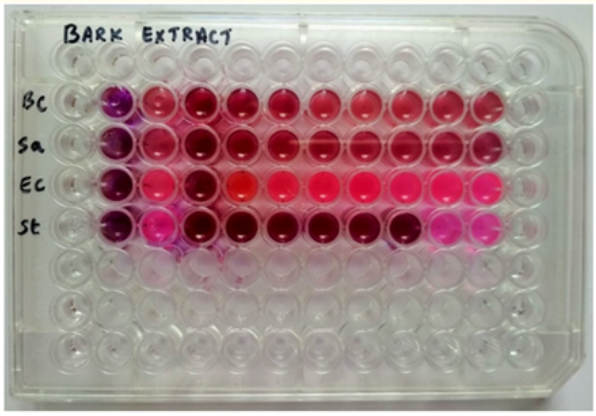

Photo 14: Bark extract micro titer plate.

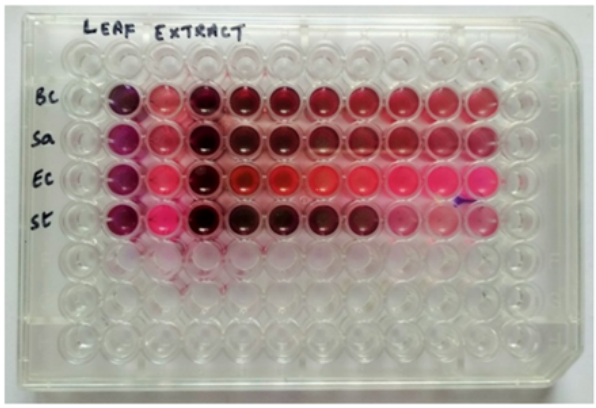

Photo 15: Leaf extract micro titer plate.

Organisms	Inhibition zone of Sample extract in millimeter						
	Bark extract		Leaf extract		Flower extract		Tetracycline
	1 mg	2 mg	1 mg	2 mg	1 mg	2 mg	0.1 mg
B. cereus	29	30	22	27	24	28	32
S. aureus	17	20	12	15	15	18	25
E. coli	-	-	-	-	-	-	40
S. typhi	18	22	12	18	17	21	30

Table 7: Data Showing Zones of Inhibition of Extracts Using LB Medium.

Sample	Concentration (µg/ml)	*B. cereus*	*S. aureus*	*E. coli*	*S. typhi*
Flower	31.25	+	+	+	--
	62.5	+	+	+	--
	125	+	+	+	--
	250	+	+	+	--
	500	+	+	+	--
	1000	--	--	+	--
	2000	--	--	--	--
Leaf	31.25	+	+	+	+
	62.5	+	+	+	+
	125	+	+	+	--
	250	+	+	+	--
	500	--	--	+	--
	1000	--	--	+	--
	2000	--	--	--	--
Bark	31.25	+	+	+	+
	62.5	+	+	+	--
	125	+	+	+	--
	250	+	+	+	--
	500	+	+	+	--
	1000	--	--	+	--
	2000	--	--	--	--

Table 8: Results of determination MIC of Extracts.

- Inhibit, + Growth

Conclusion

- The aqueous and alcoholic extracts of leaves, flowers and bark of Butea monosperma (Lam.) Taub, were, found to possess significant antibacterial activity.
- The methanolic extract prepared using soxhelet method was better than the aqueous one.
- The antibacterial activity was, attributed to presence of significant amount of phenolic compound in the extract.

Bibliography

1. Altemimi A., *et al*. "Phytochemicals: Extraction, isolation, and identification of bioactive compounds from *Butea Monosperma* extracts". *Plants* 6 (2017).
2. Gupta A., *et al*. "a Review on Pharmacognostic Study of *Butea Monosperma*". *International Journal of Research in Ayurveda and Pharmacy* 8.2 (2017): 196-199.
3. Borkar VS., *et al*. "Evaluation of in vitro antihelmintic activity of leaves of *Butea Monosperma*". *International Journal of Phytomedicine* 2.1 (2010): 31-35.
4. Tiwari P., *et al*. "*Butea Monosperma*: Phytochemistry and Pharmacology". *Acta Scientific Pharmaceutical Sciences* (2019): 344-348.
5. Babu PJ., *et al*. "Review of *Butea Monosperma"*. *Frontiers of Materials Science* 5.4 (2011): 379-387.
6. Wagner H., *et al*. "Isobutrin and Butrin, the Antihepatotoxic Principles of *Butea Monosperma* Flowers". *Planta Medica* 2 (1986): 77-79.
7. Iqbal D., *et al*. "Physico-chemical Standardization of *Butea Monosperma* (Lam.) Kuntze (Palasha): An Ayurvedic Drug". *International Journal of Pharmaceutical Quality Assurance* 2.1 (2010): 4951.
8. Kinsalin VA., *et al*. "Antimicrobial activity of methanol extracts of some traditional medicinal plants from Tamil Nadu, India". *Asian Journal of Pharmaceutical and Clinical Research* 7.2 (2014): 38-42.
9. Jarald E., *et al*. "Determination of Rutin Content and Antioxidant Activity of Extracts of *Butea Monosperma* Flowers Extracted Using Various Extraction Methods". *Pharmacognosy Journal* (2009): 344-348.
10. Dave K., *et al*. "Antimicrobial Activity and Phytochemical Study of Plant Parts of *Butea Monosperma*". *Journal of Drug Delivery and Therapeutics* 9.4-A (2019): 344-348.