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Preliminary phytochemical and antibacterial studies of seed oil of *Butea Monosperma* Lam

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Abstract
The objective of present studies deals with the Preliminary Phytochemical and antimicrobial studies oil of seed of *Butea monosperma* Lam. Seed oil exhibited antimicrobial activity against all five microorganisms the paper disc diffusion method was employed. From zone of inhibition oil showed prominent antibacterial activity. Seed oil of *Butea monosperma* Lam. was more active against *B. subtilis* and fungus *C. albicans* (zone of inhibition 13.66 ± 2.08 mm, 13.66 ± 0.5 mm respectively). Oil of seed of *Butea monosperma* Lam. was also active against gram positive bacteria *S. aureus* (zone of inhibition 11.33 ± 0.57 mm) while it is less effective against gram negative bacteria *P. aeruginosa* and *E. coli* (zone of inhibition 6.4 ± 0.30 mm, 7.3 ± 0.26 mm). It can also be seen that the MIC and MBC values of the oil on *S. aureus* was same that is 156.25µg/ml.
The present study on preliminary phytochemical and antibacterial studies of Seed oil of Butea monosperma Lam. might be useful to supplement information in regard to its identification parameters assumed significantly in the way of acceptability of herbal drugs in present scenario lacking regulatory laws to control quality of herbal drugs.

Keywords: *Butea monosperma*, seeds, pharmacognosy, phytochemical

Introduction
Butea monosperma (Lam.) Taub (Syn. Butea frondosa Willd. Family Faboideae), a deciduous tree, is found chiefly in the mixed or dry deciduous forests of Central and Western India. This plant is popularly known as dhak or palas, palash, mutthuga, bijasneha, khakara, chichara and commonly known as 'Flame of the forest'. This tree grows to 50 ft high, with stunning flower clusters. Tree is almost leafless during spring season forming an orange red hue of flowers on the upper portion, giving the appearance of flame from a distance [1, 2].
Butea monosperma is extensively used in Ayurveda, Unani, Homeopathy and Traditional systems of medicine. Flowers of B. monosperma are used as anticonvulsant, antioxidant, antistress, antigout, diuretic, antileprotic, anti-inflammatory, antiulcer, astringent, antiestrogenic activity, antihepatotoxic, eye disorder [3, 4], diarrhea, depurative, tonic, leprosy, skin diseases and thirst [5].
Phytochemical studies of flower extract have shown chemical constituents like triterpene, flavonoids and glycosides like butein, butin, isobutrin, coreopsin, isocoreopsin, sulphurein, monospermoside, isomonospermoside, chalcones, aurones and steroids [6-8]. Each plant drug possesses unique properties in terms of its botany, chemical constituents and therapeutic potency. So it is important to study pharmacognostic characters of each medicinal plant to differentiate the genuine plant sample. Isolation and pharmacological studies have been extensively made on all parts of *B. monospema* but, very less is known about pharmacognosy.
The Present work is to frame a standard Preliminary Phytochemical and antibacterial studies for the seeds of *Butea monosperma* useful in authentification and standardization of the drug, which give the quality and purity of the drug Figure 1.

Material and Method
Plant material
The plant specimens for the proposed study were collected from Chopda Tehsil (Adawad) MS, India in the month of April 2017 care was taken to select healthy plants and for normal organs. The plant was authenticated by Botanical Survey of India (BSI), Pune, Maharashtra, India. A voucher specimen (No. SSS 01) was deposited at B.S.I., Pune, India [11].

Preliminary phytochemical parameters
Preliminary phytochemical test of seeds of *Butea monosperma* Lam. were performed and the chemical constituents were detected Table 1 [9, 15].

Antibacterial Activity [16, 22]
The paper disc diffusion method was employed for antibacterial activity.
Microbial strains used: The test organisms was gram-positive bacteria *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538), gram negative *Escherichia coli* (ATCC 10538), *Pseudomonas aeruginosa* (ATCC 27853) and fungus *Candida albicans* (ATCC 10239) were obtained from the microbiology department, R. C. Patel Art Science and Commerce college, Shirpur (NMU University), Maharashtra, India. The Nutrient agar medium composition are shown in Table 2
Preparation of test solution: Stock solution of was prepared in dimethyl sulfoxide (DMSO) at a concentration 5000 µg/ml. Accurately weighed 2 gm of the each extract dissolved in 400 ml of DMSO solution. Stored the solutions in the refrigerator at 4ºC.
Preparation bacterial stock culture: Stock cultures were maintained at 4°C on slopes of nutrient agar in test tubes. Active cultures for experiments were prepared by selecting

At least three to five well-isolated colonies of the same morphological type from the stock cultures. The top of each colony is touched with a loop and the growth is transferred into a test tube containing 4 to 5 ml of a nutrient agar media for bacteria and incubated without agitation for 24 hrs at 37°C. The experiment was performed under strict aseptic conditions.

Preparation fungal stock culture: Stock cultures were maintained at 4°C on slopes of potato dextrose agar medium in test tubes. Active cultures for experiments were prepared by selecting at least three to five well-isolated colonies of the same morphological type from the stock cultures. The top of each colony is touched with a loop, and the growth is transferred into a test tube containing 4 to 5 ml of a potato dextrose agar medium for fungi and incubated without agitation for 3-5 days at 30°C.

Preparation of sterile of bacterial suspension (Inoculum): The loop used for delivering the culture which made of 20 gauge wire and has a diameter of 2 mm. Transferred loopful culture in test tube containing 3 to 4 ml saline preparation and shaken the tube for proper mixing.

Inoculation of microbial suspension on the test plates: The agar plates inoculated with the test microbial suspension. The microbial suspensions were spreaded over the surface of the agar media with the help of sterile spreader to ensure uniform inoculation and confluent growth.

Application of discs to inoculated agar plates: Sterile 6 mm disc filter paper disc were impregnated with 100 µL of the plant extracts. Discs should be placed on the agar with forceps which is sterilized by pass the forceps through a Bunsen burner flame. Allowed them to cool. Made sure that your red hot loop is cool enough prior to touch the microbes and used them to pick up the paper discs. Each disc must be pressed down to ensure complete contact with the agar surface.

They distributed evenly so that they are no closer than 24 mm from center to center. A disc was not be relocated once it has come into contact with the agar surface. The plates are placed in freezing condition for 5 minutes. After that plates are inverted and placed in an incubator set to 35°C.

Incubation of disc loaded plates: The bacterial plates were incubated at 37±0.1 °C for 24 hr. while yeast plates were incubated at 28±0.1 °C for 48 hr. in incubator.

Reading plates and interpreting results: After incubation each plate is examined. The diameters of the zones of complete inhibition are measured, including the diameter of the disc with the help of ruler which is held on the back of the inverted petri plate. The diameter of these zones was measured in millimetres. The petri plate is held a few inches above a black, nonreflecting background.

All tests were performed under sterile conditions in duplicate and repeated three times. The results of antimicrobial activity of oil was shown in Table 3 and the photo documentation was shown in Figure 2 and 3.

Standard drug used for activity: Antimicrobial poly discs are rings containing multiple antibiotics. The disc is produced using cotton-based paper of high-quality, which has fewer ionic sites for the binding of antibiotics. The antibiotic tip is also printed with a 3-letter code based on the Internationally agreed name of the antibiotic and is colour-coded with an inert dye.

Adding the dye to the drug gives assurance that none of the antimicrobic has contaminated another part of the disc and that the tip does in fact contain the drug.

Poly discs with different combinations of antibiotics for gram positive, gram negative. Combination for each type is taken with common and effective antibiotic used for the particular organisms / infection. Poly discs applied to agar plates by use of a sterilized forceps which was used as positive controls and the solvent such as DMSO served as negative control. The list of drug, abbreviation for drug and concentration per each disc were shown in Table 4 and antimicrobial activity of standard drug was given in Table 5.

Minimum Inhibitory Concentration (MIC)

MIC is defined as the lowest concentration where no visible turbidity is observed in the test tube (bacteriostatic concentration). The result of MIC was mentioned in Table 6.

Minimum Bacterial Concentration (MBC)

Minimum bacterial concentration (MBC) is defined as the lowest concentration where no bacterial growth is observed (bacteriocidal concentration). This was determined by the sub culturing of MIC tubes in antimicrobial free agar plates. In this technique the contents of the test tubes resulting from MIC was streaked using a sterile wire loop on agar plate free of bacteria and incubated at 37ºC for 18 hours. The lowest concentration of the extract which showed no bacterial growth was noted and recorded as the MBC. The result of MBC of extract was shown in Table 7.

Results and Discussion

Macroscopy of Seed

Colour, shape and appearance: Flat 25 to 40 mm long, 15 to 25 mm wide and 1.5 to 2 mm thick. The seed-coat is reddish-brown in colour, wrinkled and two large yellowish cotyledons.

Odour and taste: Odourless, characteristics Figure 1.

Fig 1: Seeds of *Butea monosperma* lam.

Preliminary Phytochemical Studies

Ethanolic extract of seed of *Butea monosperma* Lam. showed the presence of various Phytoconstituents such as Phytosterols, saponins, triterpenoids, tannins and flavanoids Table 1.

Table 1: Preliminary phytochemical screening of seed oil *Butea monosperma* Lam.

Sr. No.	Test	Expressed seed oil
1.	Test for Phytosterols	
	Salkowaski test	+
	Lieberman-Burchard test	+
	Sulphur test	-
2.	Test for Alkaloids	
	Mayer's reagent test	-
	Dragendorff's reagent test	-
	Hager's test	-
	Wagners test	-
3.	Test for glycoside	
	Legal test	-
4.	Test for Terpenoids	+
5.	Test for Anthraquinones	-
6.	Test for Flavonoids	
	Shinoda Test	+
7.	Test for Phenols	+
8.	Test for Tannins	+
9.	Test for Saponins	
	Foam Test	+
10.	Test for Carbohydrates	
	Molish test	-
	Fehling's Test	-
11.	Test for Proteins	
	Millions test	-
12.	Test for fats and oil	
	Solubility test	+
	Filter paper test	+

Remarks: + (Present) **;** – (Absent)

Antibacterial activity Results

Table 2: Composition nutrient agar medium

Sr. No.	Ingredients	Gm/batch
1	Beef extract	10.0 gm
2	Peptone	10.0 gm
3	Agar	17.0 gm
4	Glycerine	10.0 gm
5	Sodium chloride	3.0 gm
6	Distilled water	1000 mL
	Final P^H	6.9-7.1

Table 3: Antimicrobial activity of oil against test microbes

Sr. No.	Category	Strain	Conc. (mg/mL)	Mean ± SD of diameter for zone of inhibition (mm)
				Butea monosperma seed oil
				Petroleum ether extract
1	Gram positive	*B. subtilis*	5.0	13.66 ± 2.08
2	Gram positive	*S. aureus*	5.0	11.33 ± 0.57
3	Gram negative	*P. aeruginosa*	5.0	6.4 ± 0.30
4	Gram negative	*E. coli*	5.0	7.3 ± 0.26
5	Fungi	*C. albicans*	5.0	13.66 ± 0.5

Table 4: Composition and abbreviation of each drug in Master Multidisc

Sr. No.	Drug	Abbreviation	Concentration (µg)
1	Amoxycillin	AN	10.0
2	Augmentin	AU	30.0
3	Cephotaxime	CX	30.0
4	Ceftriaxone	CA	30.0
5	Cefuroxime	CO	30.0
6	Ciprofloxacin	CP	5.0
7	Clindamycin	CY	2.0
8	Erythromycin	ER	15.0
9	Gentamicin	G	10.0
10	Lincomycin	L	2.0
11	Ofloxacin	OF	5.0
12	Pefloxacin	PF	5.0

Table 5: Antimicrobial activity of standard drug against test microbes

Sr. No.	Control	*B. subtilis* (mm)	*P. aeruginosa* (mm)	*S. aureus* (mm)	*E. coli* (mm)	*C. albicans* (mm)
1	AN	21	14	11	-	-
2	AU	15	09	15	10	-
3	CX	16	-	-	-	-
4	CA	24	12	22	13	-
5	CO	16	14	-	-	-
6	CP	-	11	-	-	-
7	CY	23	-	12	16	-
8	ER	-	-	13	-	-
9	G	15	19	-	-	-
10	L	-	21	-	17	-
11	OF	-	15	-	-	-
12	PF	20	-	-	-	-
13	Fluconazole	-	-	-	-	15
14	DMSO	00	00	00	00	00

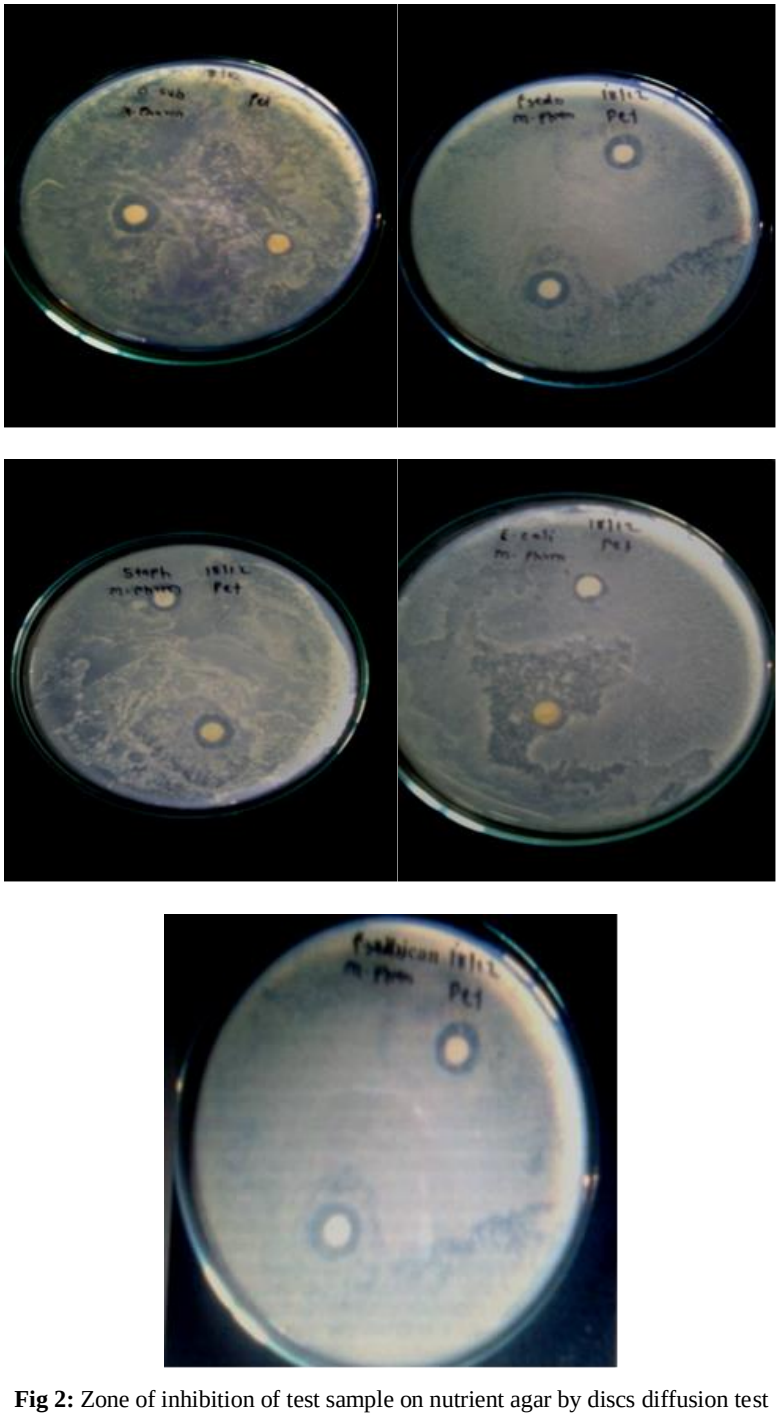

Fig 2: Zone of inhibition of test sample on nutrient agar by discs diffusion test

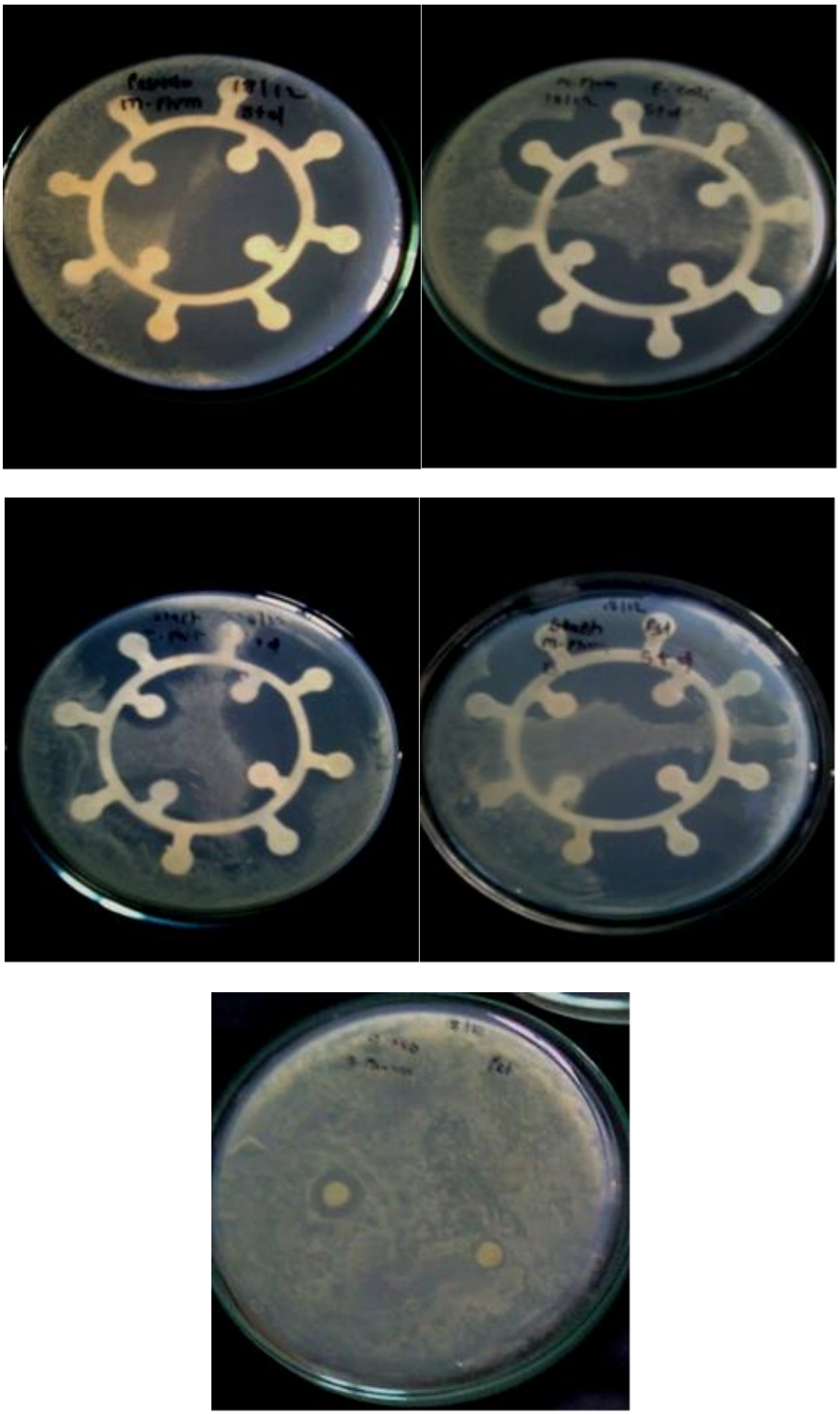

Fig 3: Zone of inhibition of standard drug on nutrient agar by disc diffusion test

Table 6: Minimum inhibitory concentration (MIC) values for bacterial strain against oil of seed of *Butea monosperma*

Sr. No.	Conc. (µg /ml)	*B. subtilis*	*P. aeruginosa*	*S. aureus*	*E. coli*	*C. albicans*
1	5000	+	+	+	+	+
2	2500	+	+	+	+	+
3	1250	+	+	+	+	+
4	625	+	+	+	β	+
5	312.5	+	+	+	-	+
6	156.25	+	+	β	-	+
7	78.125	β	+	-	-	+
8	39.062	-	+	-	-	β
9	19.331	-	β	-	-	-
10	9.765	-	-	-	-	-

11	4.882	-	-	-	-	-
12	2.441	-	-	-	-	-
13	1.2205	-	-	-	-	-
14	0.610	-	-	-	-	-
15	0.305	-	-	-	-	-

Remarks: - Resistance (growth of bacteria/fungi or turbidity).
+ Concentrations show no turbidity (inhibition of bacterial growth).
β Least concentration showing no turbidity (MIC).

Table 7: Minimum bacterial concentration (MBC) Values for bacterial strain against oil of seed of *Butea monosperma*

Sr. No.	Conc. (µg/ml)	*B. subtilis*	*P. aeruginosa*	*S. aureus*	*E. coli*	*C. albicans*
1	5000	+	+	+	+	+
2	2500	+	+	+	+	+
3	1250	+	+	+	+	+
4	625	+	+	+	+	+
5	312.5	+	+	+	β	+
6	156.25	+	+	β	-	+
7	78.125	+	+	-	-	+
8	39.062	β	+	-	-	+
9	19.331	-	+	-	-	+
10	9.765	-	+	-	-	β
11	4.882	-	β	-	-	-
12	2.441	-	-	-	-	-
13	1.2205	-	-	-	-	-
14	0.610	-	-	-	-	-
15	0.305	-	-	-	-	-

Remarks: Resistance (growth of bacteria/fungi or turbidity).
+ Concentrations show no turbidity (inhibition of bacterial growth).
β Least concentration showing no turbidity (MBC).

Conclusion

The Present work is to frame a standard Preliminary Phytochemical and antibacterial studies for the seeds of *Butea monosperma* useful in authentification and standardization of the drug, which give the quality and purity of the drug. With most bactericidal antimicrobial the MIC and MBC are often near or equal in value as has typically been observed in this study. It was seen that the MIC value for *B. subtilis, P. aeruginosa, E. coli and C. albicans* was 78.125 µg/ml, 19.331 µg/ml, 625 µg/ml & 39.062 µg/ml respectively while MBC was 39.062 µg/ml, 4.882 µg/ml, 312.5 µg/ml & 9.765 µg/ml respectively. From above results it was seen that the seed oil have antibacterial activity against both gram positive, negative and fungal strain under study. The results of this investigation are indicative of possible pure active principle of natural origin from the extract with possible high potency which could serve as chemotherapeutic agents.

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Conflict of Interest

There is no conflict of interest with this research.

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