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Anti-microbial Evaluation of twigs of *Butea monosperma* Lam.

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Abstract :
Extracts of twigs of *Butea monosperma* were evaluated for antimicrobial activity against pathogenic strains of Gram-positive (Staphylococcus aureus, Bacillus subtitis and Bacillus cereus) and Gram-negative (Escherichia coli, Pseudomonas aeruginosa, S.albony) bacteria and fungal stain (Candida albicans) Methanolic extract was found to be more active, and its activity was compared to standard antibiotics gentamycin, ciproflaxacin, chloremphenicol, erythromycin and fluconazol in these strains. Only methanolic extrat of the bark of the plant showed significant activity against these strains. The use of methanolic extracts of twigs of Butea monosperma as a potential antiseptic in prevention and treatment of microbial infections has been suggested.
Key words: Butea monosperma, Anti-microbial activity, disc diffusion method, methanolic extract**.**

INTRODUCTION

World Health Organization (WHO) noted that majority of the world's population depends on traditional medicine for primary healthcare. Plant extracts have been used for many thousands of years [1] in food preservation, pharmaceuticals, alternative medicine and natural therapies [2, 3]. The purpose of this work was therefore to evaluate antimicrobial activity of *Butea monosperma* barks on different microbial strains.

Butea monosperma (Lam.) is commonly known as Flame of forest, belongs to the family Fabaceae [4].*Butea monosperma* (Lam.) kuntze is one among four species belonging to the genus *Butea* Koenig, three species of which occur in India [5]. It holds an important place because of its medicinal and other miscellaneous uses of economic value. Bark fibers are obtained from stem for making cordage [6].Stem bark powder is used to stupefy fishes. Young roots are used for making ropes [5]. Green leaves are good fodder for domestic animals. Leaves are used for making platters, cups, bowls and beedi wrappers [5,7] Leaves are also used for making Ghongda to protect from rains and are eaten by buffaloes and elephants. Tribals use flowers and young fruits as vegetables. Flowers are boiled in water to obtain a dye [4]**.** Orange or red dye is used for colouring garments and for making skin antiseptic ointments [8]. Fresh twigs are tied on horns of bullocks, on occasion of 'pola' and dry twigs are used to feed the sacred fire [4]. In addition wood of the plant is mainly

used for well-curbs and water scoop. It is also employed as a cheap board wood and for structural work; wood pulp is suitable for newsprint manufacturing [7]

Flower: Triterpene [9], butein, butin, isobutrin, coreopsin, isocoreopsin (butin 7-glucoside), sulphurein, monospermoside (butein 3-e-D-glucoside) and isomonospermoside, chalcones, aurones, flavonoids (palasitrin, prunetin) and steroids [12, 13].Gum: Tannins, mucilaginous material, pyrocatechin [7]. Seed: Oil (yellow, tasteless), proteolytic and lypolytic enzymes, plant proteinase and polypeptidase. (Similar to yeast tripsin). A nitrogenous acidic compound, along with palasonin is present in seeds [13]. It also contains monospermoside (butein 3-e-D-glucoside) and somonospermoside. From seed coat allophanic acid has been isolated and identified [12, 13]. Resin: Jalaric esters I, II and laccijalaric esters III, IV. Z- amyrin, e-sitosterone its glucoside and sucrose; lactone-nheneicosanoic acid-{-lactone [13,14). Sap: Chalcones, butein, butin, colourless isomeric flavanone and its glucosides, butrin [5].

Leaves: Glucoside, Kino-oil containing oleic and linoleic acid, palmitic and lignoceric acid [15]. Bark: Kino-tannic acid, Gallic acid, pyrocatechin. The plant also contains palasitrin, and major glycosides as butrin, alanind, allophanic acid, butolic acid, cyanidin, histidine, lupenone, lupeol, (-)-medicarpin, miroestrol, palasimide and shellolic **acid** [15, 16, 17, 18, 19, 20, 21, 22]. Stem: 3-Z-hydroxyeuph-25-ene and 2,14-dihydroxy-11,12- dimethyl-8-oxo-octadec-11-enylcyclohexane[21] Stigmasterol-e-D-glucopyranoside and nonacosanoic acid [24] Stem: 3-Z-hydroxyeuph-25-ene and 2,14-dihydroxy-11,12- dimethyl-8-oxo-octadec-11-enylcyclohexane [23]. Stigmasterol-e-D-glucopyranoside and nonacosanoic acid [24]

In the literature, *B. monosperma* is ascribed to have many medicinal properties. It has been used as tonic, astringent, aphrodisiac and diuretic. Its flowers are widely used in the treatment of hepatic disorders and viral hepatitis, diarrhoea and possess anti-implantation activity [25] Roots of *B. monosperma* are reported to be useful in the treatment of filariasis, night blindness, helminthiasis, piles, ulcers and tumors. Pippali rasayana, an Indian Ayurvedic drug, employs *B. monosperma* and is used in the management of giardiasis [26]. The bark is reported to possess antitumor and antiulcer activities. The root bark is used as an aphrodisiac, analgesic and antihelmintic whereas the leaves possess antimicrobial property[27]. *B. monosperma* flowers are well-known antihepatotoxic principles of *B.* monosperma [29] . Gum is useful as astringent, depurative and useful in diarrhoea, haemorrhoids, haepoptysis, haematemesis, leprosy, skin diseases [30, 31] Therefore, the plants have long since been deemed a valuable source of natural products for maintaining human health. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance to therapeutic treatments. Therefore, such plants should be investigated to better understand their properties, safety profiles and levels of efficiency against pathogenic microbes. The development of bacterial resistance to presently available antibiotics has necessitated the search for new antibacterial agents [32]

MATERIAL AND METHODS

Plant Material

Fresh twigs *of Butea monosperma* were collected from forests of area adjoining to Bhopal in the month of April and authentication of plant material (specimen voucher no. 864/22) was done by

taxonomist Dr. Manjusa Saxena at department of botany, Govt. Maharaja College, Chhatarpur (M.P.).

Preparation of Extracts

Twigs *of Butea monosperma* (200g) were ground finely in a mortar and pestle by adding little water and were subjected to steam distillation. Oily fraction (fraction-I, 0.742 g) was collected and residue in water was filtered. The filtrate was evaporated under vacuum to give water extract (fraction-IV,1.8 g). The residue wad air dried and left over night in chloroform extract from(200 ml), filtered and re- extracted twice with chloroform (2 X 100 ml). All the chloroform extracts were combined and solvent was evaporated to give chloroform extract (Fraction-II, 1.2 g). The residue left after chloroform extract, was extracted with methanol (2 X 100 ml) to give the methanol extract (fraction-3, 4.2 g) Activity screened against *Escherichia Coli*. (NCTC-9002), *S. albony* (NCTC-6017), *Staphylococcus Aureus* (ATCC-6538), *Psedomonas auragenosa* (ATCC-9027) and *Candida albicans* (ATCC-10231), *Bacillus subtitis* MTCC 441, *Bacillus* and *cereus* MTCC 430, Procured from All India Institute of Medical Sciences, New Delhi.

Evaluation of antimicrobial activity (Disc diffusion method)

Bacterial strains were grown at 37°C on nutrient broth (Hi Media, Mumbai)for 12-14 h, and were maintained on nutrient agar slant (Hi Media, Mumbai) at 4^{o}C,whereas fungal strains were grown at 30°C in Sabouraud dextrose agar at pH 7.4 for 48 hrs followed by frequent sub culturing to fresh medium and were used as test micro-organisms. The extracts were dissolved in ethylene glycol; membrane filter (0.47 µl) sterilized and tested for antimicrobial activity using disc diffusion method. A concentration of 2000 µg / ml/ disc was chosen based on available literature. Sterile 6-mm diameter filter paper discs were impregnated with 2000 µg of the sterile test material, and placed on to nutrient agar surface spread with 0.1 ml of bacterial culture (ca. 3x 10^8 cells/ml using McFarland's 1 as Standard). The plates were incubated at 37 °C for 12-14 h. The experiments were carried out in triplicate. The results (mean value n = 3) were recorded by measuring the zone of growth inhibition around the discs. The statistical analysis was carried out using student's t test. [15, 35] The antimicrobial spectra showing zone of inhibition in mili metres and percentage calculated by taking gentamicin as positive control for bacterial strains and fluconazol for fungal strain with 100% inhibition. Control discs contain ethylene glycol only. For comparison, standard antibiotics gentamycin and cloremphenicol inhibiting bacterial cell wall biosynthesis and erythromycin inhibiting bacterial protein synthesis were included in the assy.

MIC Assay

The agar dilution method recommended by the National Committee for Clinical Laboratory Standards [14, 34] was used. A series of two fold micro dilution of methanolic extract with saline at a final concentration ranging from 100µg /ml to 10µg /ml was prepared nutrient agar at 48°C. Plates were dried at room temperature for 30 min prior to spot inoculation. Inoculated plates were incubated at 37°C for 18 h and the MIC was determined. Experiments were carried out in triplicate. Inhibition of bacterial growth in the plates containing test extract was judged by comparison with growth in blank control plates. The MIC values were taken as the lowest

concentration of the extracts in the wells of the microtiter plate that showed no turbidity after 24 hours of incubation at 37° C.

RESULTS AND DISCUSSION

Screening of Antimicrobial activity of methanolic extract of *Butea monosperma*

The screening of anti microbial activity was performed with the help of disc diffusion method. Following tables shows anti microbial activity of methenolic extract of *Butea monosperma* at different concentration against gram negative bacteria, gram positive bacteria and fungi (*Candida albicans*) given in table no. 1.

Table 1 . Zone of inhibition of various extracts from *twigs of Butea monosperma* compared to reference drugs: activity against Gram-positive bacteria.

Micro-Organism	***A.actinomycetemcomitans***		***P. gingivalis***		***B. forsythus***	
Name of drug	**In mm Mean**	**As %**	**In mm Mean**	**As %**	**In mm Mean**	**As %**
Gentamycin (10 mg/ml)	17.67±1.47	100	16.88±0.87	100	19.34±0.68	100
Chloromphenicol (20 mg/ml)	16.33±0.33	92	10.45±0.87	61	11.56±0.63	59
MeOH Extract of *Butea monosperma* (mg/ml) 15 30 45 60 90	 6.00± 00 6.00± 00 6.00± 00 8.00± 00 9.36± 0.47*	 00 00 00 45 52	 6.00± 00 6.00± 00 8..44± 00 9. 34±0.85* 9. 49±0.46*	 00 00 50 55 56	 6.00± 00 6.00± 00 7.42±0.54 8.20±0.52 9.89±0.38*	 00 00 38 42 51
Control	6.00±00	00	6.00±00	00	6.00±00	00

Mean, Mean value of diameter of inhibition zone with standard error.
As the diameter of paper disc used was 6 mm, 6 mm diameter included in the table is indicative of no activity. Percent was calculated after subtracting disc diameter (6mm) from all observations.
* indicates significant activity at $p<0.05$

Screening of Methanolic extract of *Butea monosperma*

Screening of Methanolic extract of *Butea monosperma* have shown the significant activity against *A.actinomycetemcomitans* on the concentration 90 mg/ml, against *P. gingivalis* on the concentration 60 and 90 mg/ml and against *B.forsythus* it has shown significant activity on 90 mg/ml concentration. On the concentration of 15, 30 mg/ml no zone of inhibition was observed and shown in table no. 2

Table 2. Zone of inhibition of various extracts from *twigs of Butea monosperma* compared to reference drugs: activity against Gram-negative bacteria**.**

Micro-Organism **Name of drug**	***Streptococcus mutans***		***S.mitis***		***S. sanguis***	
	In mm Mean	**As %**	**In mm Mean**	**As %**	**In mm Mean**	**As %**
Gentamycin (10 mg/ml)	18.00±0. 68	100	16.00±0. 68	100	17.00±0. 68	100
Chloromphenicol (20 mg/ml)	15.84 ±0.34	87	15.84 ±0.34	85	14.84 ±0.34	86
MeOH Extract of *Butea monosperma* (mg/ml) 15 30 45 60 90	6.00±00 6.30±0.32 8.00±0.85 9.54±0.64* 10.68±0.24*	00 35 44 52 59	6.00±00 6.30±0.32 8.00±0.22 9.44±0.34* 10.66±0.20*	00 35 43 51 59	6.00±00 6.00±0.30 8.40±0.85 8.54±0.12* 10.00±0.38*	00 34 42 42 58
Control	6.00±00	00	6.00±00	00	6.00±00	00

Mean, Mean value of diameter of inhibition zone with standard error.
As the diameter of paper disc used was 6 mm, 6 mm diameter included in the table is indicative of no activity. Percent was calculated after subtracting disc diameter (6mm) from all observations.
* indicates significant activity at $p< 0.05$

Methanolic extract of *Butea monosperma* have shown zone of inhibition 9.54 ± 0.64 mm on 60 mg/ml and 10.68 ± 0.24 mm on 90 mg/ml and have shown significant activity against *Streptococcus Mutans* on the concentration 60 and 90 mg/ml. On the concentration of 15 mg/ml no zone of inhibition was observed. And inhibition was 52 % and 59% as compared to the standard drugs.

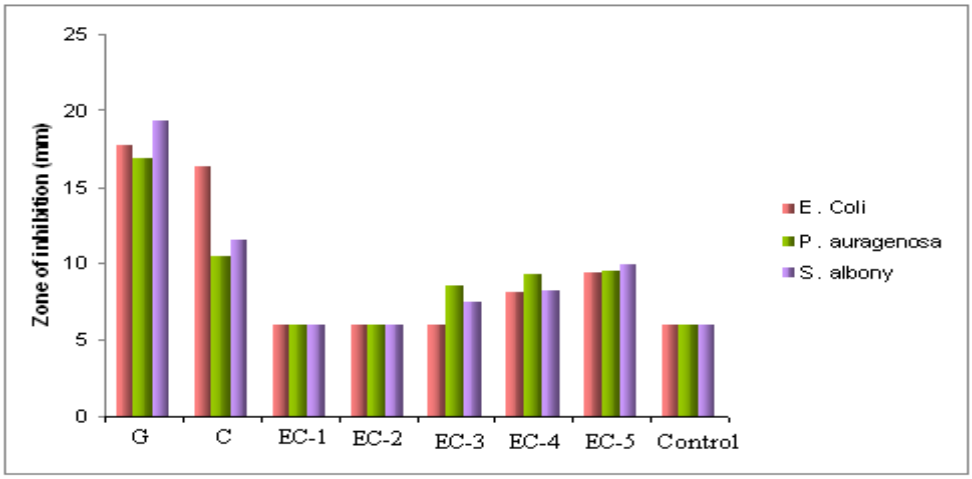

Fig. 1: Zone of inhibition for various concentrations of *Butea monosperma* compared to reference drugs: activity against gram negative bacteria. G = Gentamicin, C = Chorempenicol, EC-1= extract on the concentration of 15 mg/ml, EC-2 = extract on the concentration of 30 mg/ml, EC-3 = extract on the concentration of 45 mg/ml, EC-4 = extract on the con. On 60 mg/ml, EC- 5 = extract on the concentration of 90 mg/ml.

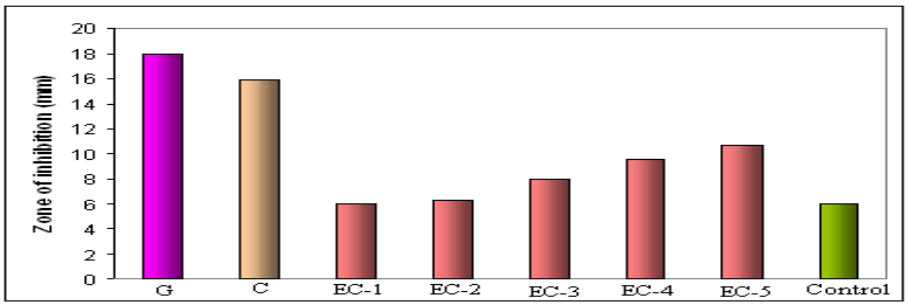

Fig 2: Zone of inhibition for various concentrations of *Butea monosperma* compared to reference drugs: activity against gram positive bacteria.

Table 3. Zone of inhibition of various extracts from *twigs of Butea monosperma* compared to reference drugs: activity against fungal strain **.**

Micro- Organism **Name of drug**	***C. albicans***	
	In mm **Mean**	**as** **%**
Fluconazol (10 mg/ml)	14.00±0.34	100
MeOH Extract of *Butea monosperma* (mg/ml) 15 30 45 60 90	 6.00±00 6.00±00 6.80±00 8.46±0.48* 9.28±0.37*	 00 00 48 60 66
Control	6.00±00	00

Mean, Mean value of diameter of inhibition zone with standard error.
As the diameter of paper disc used was 6 mm, 6 mm diameter included in the table is indicative of no activity. Percent was calculated after subtracting disc diameter (6mm) from all observations.
* indicates significant activity at $p< 0.05$

Methanolic extract of *Butea monosperma* have shown the significant activity on the concentration 60 and 90 mg/ml and zone of inhibition 8.46±0.48 mm (60% inhibition) and 9.28 ± 0.37 mm (66 % inhibition) respectively against C. albicans as compared to the standard drug fluconazol. On the concentration of 15and 30 mg/ml no zone of inhibition was observed.

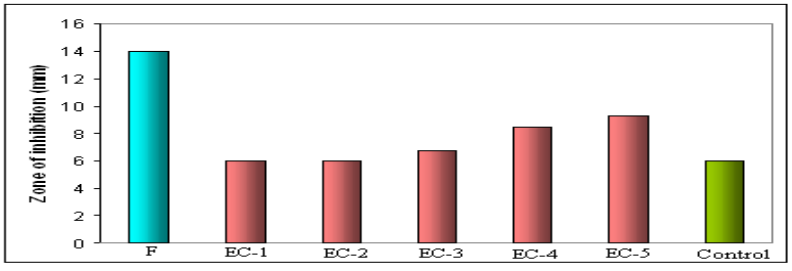

Fig 3: Zone of inhibition for various concentrations of *Butea monosperma* compared to reference drugs: activity against *Candida albicans*.

F = fluconazole as standard drug. EC-1= extract on the concentration of 15 mg/ml, EC-2 = extract on the concentration of 30 mg/ml, EC-3 = extract on the concentration of 45 mg/ml, EC-4 = extract on the con. On 60 mg/ml, EC- 5 = extract on the concentration of 90 mg/ml.

Table 4. Minimum inhibitory concentration of fraction-III (Methanolic) on Gram positive bacteria with gentamycin as standard reference.

Micro-Organism	***A.actinomycetemcomitans* Zone of inhibition**		***P. gingivalis* Zoneof inhibition**		***B.forsythus* Zone of inhibition**	
Name of drug	In mm Mean	As %	In mm Mean	As %	In mm Mean	As %
Gentamycin (10 ug/ml)	24.67±1.47	100	25.88±0.87	100	21.00±0.68	100
Chloromphenicol (10ug/ml)	22.33±0.33	95	9.45±0.87	38	8.56±0.63	31
Ciprofloxacin (10 ug/ml)	23.66±1.88	98	6.00±0.00	00	6.00±0.00	00
Erythromicin (10ug/ml)	16.00±0.44	62	6.42±0.64	00	6.00±0.52	00
Fraction -I	6.00± 00	00	7.00± 00	27	6.90± 00	20
Fraction-II	7.00± 00	33	7..00± 08	30	7.00±0.00	33
Fraction-III	14.84.± 0.62	61*	13.34±0.85	59*	10.66±0.52	51*
Fraction-IV	7.00 ± 00	20	7. 42±0.46	15	7.89±0.38	14
Control	6.00±00	00	6.00±00	00	6.00±00	00

Diameters of paper disc = 6mm , indicative of no activity, percentage was calculated after subtracting disc diameter (6 mm).

Mean values shown in the table are mean of diameters of zone of inhibition taken with standard error.

Zone of inhibition of various fractions from *Butea monosperma* compared to reference drugs: activity against fungal strain.

Micro- Organism **Name of drug**	***C. albicans*** **Zone of inhibition**	
	In mm (Mean)	**As %**
Fluconazol (10 ug/ml)	27.38±1.22	100
Fraction –I	6.00± 00	00
Fraction-II	7.00± 00	25
Fraction-III	15.66± 00	67*
Fraction-IV	9.26±0.47	31
Control	6.00±00	00

Only acetone fraction (fraction-III) shown significant activity against gram positive, negative and fungal strains so only this fraction was considered for further study**.** Various extracts such as steam distilled (Fraction-I essential oil), chloroform (Fraction-II), methanol (Fraction III) and water (Fraction-IV) were tested for antimicrobial activity in *vitro system.*

CONCLUSION

Methanolic extracts of the bark of the plant produced good inhibition zones against the test organisms. So it is expected that they could be used to treat infections and diseases caused by these organisms and if the active ingredients of the extracts are isolated and possibly crystallized, therapeutic antibiotics could be produced from these compounds.

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