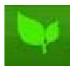
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REGULAR ARTICLE

EVALUATIONS OF PHYTOCHEMICAL CONSTITUENTS AND ANTIMICROBIAL ACTIVITY OF *BUTEA MONOSPERMA* (FABACEAE)

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SUMMARY

The present paper deals with the physico-chemical, quantitative phytochemical determination and antimicrobial activity of *Butea monosperma* (Lamk.) Taub., an important medicinal tree in India. The *in-vitro* antimicrobial activity of ethanol, chloroform and petroleum ether extracts were studied using MIC (Minimum Inhibitory Concentration) by well diffusion method against pathogenic microbes *viz., Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus subtilis.* Among the solvents used, the ethanol extract was found to be more effective against *Bacillus subtilis*, and *Staphylococcus aureus*. The petroleum ether extract did not inhibit *Pseudomonas aeruginosa*, while the *Escherichia coli* did not inhibit any type of extract. Activities of the various extracts were comparable to those of standard antibacterial agent ampicillin as control. The results provided evidence that the studied plant might indeed be potential sources of phytochemical constitution and antimicrobial agents and showing that this plant can be used as a complementary source for traditional medicines.

Key words: *Butea monosperma*, Antimicrobial, Physico-chemicals, Phytochemical

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1. Introduction

Phytochemicals which possess many ecological and physiological roles are widely distributed as plant constituents. Woody plants can synthesize and accumulate in their cells a great variety of phytochemicals including alkaloids, flavonoids, tannins, cyanogenic glycosides, phenolic compounds, saponins and lignins [1]. Over 50% of all modern clinical drugs are of natural product origin [2]. Natural products play on important role in drug development programmes in the pharmaceutical industry [3]. There are a few reports on the use of plants in traditional healing by either tribal people or indigenous community [4-8].

The antimicrobial activity have been screened because of their great medicinal relevance with the recent years, infections have increased to a great extent and resistant against antibiotics, becomes an ever increasing therapeutic problem [9]. Natural products of higher plants may give a new source of antimicrobial agents. There are many research groups that are now engaged in medicinal plants research (10-12). The development of drug resistance in human pathogens against commonly used antibiotics has necessitate the search for new antimicrobial substance from other sources. Screening of medicinal plants for antimicrobial activities and phytochemical is important for finding potential new compounds for therapeutic uses.

2. Materials and Methods

Collection of plant materials, Bacterial strains and Growth conditions

The plant materials (Leaves) *Butea monosperma* (Lamk.) Taub. were collected from the Dr. Babasaheb Ambedkar Marathwada University, Aurangabad (M.S.)

campus. The plant materials were identified using the Flora of Marathwada [13].

Sample preparation

Fully grown leaves and bark of *B. monosperma* weighed (0.5kg). The plant samples were shade dried ground and sieved with 2mm copper sieve to form uniform powder and stored in airtight bottles.

Tested microorganisms

Various cultures of human pathogenic, Gram positive and Gram negative bacteria were used. These are *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Bacillus subtilis.* The cultures were obtained from Department of Microbiology, Government Institute of Science, Aurabgabad, (M.S.) India. The microorganisms were repeatedly subcultured in order to obtain pure isolates. A loop full test organism was inoculated on nutrient broth and incubated for 24 h at 37±1°C and maintained in sterile condition.

Selection of reference antibiotic

Reference antibiotic Amphicilin was obtained from authorized medical shop Auragabad. The purity of the antibiotic is 99.8%

Screening for antibacterial properties

Antibacterial activities of plant extracts were tested by Agar well diffusion method [14]. The culture plates were prepared by pouring 20 ml of sterile nutrient agar.1 ml inoculum suspension was spread uniformly over the agar medium using sterile glass rod to get uniform distribution of bacteria. A sterile cork borer (8 mm) was used to make wells in each plate for extracts. These plates were labeled and 100µl of each plant extracts (at concentration of 50,100 mg/ml) was added aseptically into the well. Then the plates were incubated for 24 h at 37°C during which the activity was evidenced by the presence of zone of inhibition surrounding the well. Each test was repeated three times and the antibacterial activity was expressed as the mean of diameter of the inhibition zones (mm) produced by the plant extracts when compared to the controls.

Extraction of plant materials

The plant materials were dried in shade and powdered in a mechanical grinder. The powder of Leaf and bark were initially defatted with petroleum benzene at (60 - 80˚C) followed by 250 ml of ethanol by using a Soxhlet extractor for 36 hours at a temperature not exceeding the boiling point of the solvent. The extract was filtered using Whattman filter paper (No.1) and then concentrated at 45˚C. The extract were kept in a sterile bottle under refrigerated condition about 2-8˚C for further analysis.

Chemical analysis

Histochemical tests were performed on fresh plant materials [15, 16]. The moisture content was determined by heating the drug at 105oC to a constant weight and calculating the loss of weight. The extracts of drug samples were prepared by using solvents and total acid insoluble and acid soluble ash content obtained [17].

Two grams of each of the plant samples were weighed and taken in a previously weighed vitrosil silica crucible, to which added 2 drops of the mixture of H2SO4: HNO3 (2:1). Then it was heated on the hot plate for about 30 minutes, till the sample was sufficiently charred and turns black. After this, replace the lid of the crucible and keep it in muffle furnace. The temperature allowed to rise up to 600°C and kept it constant for 2 hours. The crucible was removed on cooling and 50 ml of 5 N HCl was added to the ash in crucible. The mixture was heated for 30 minutes in hot water. Then it was allowed to cool and filtered through Whatman filter paper No. 42 and volume was made up to 100 ml with deionized water. This solution was used for mineral analysis. Calcium (Ca) content was determined [18]. Phosphorus (P) content was estimated by colorimetric method [19]. Potassium (K) content was determined on a flame photometer (model Mediflame-127) [20].

Nitrogen (N) content in dry plant material was estimated by micro-Kjeldal method [21]. The amount of Tannins by Folin-Denis Method, lignin, cellulose,

hemicellulose and reducing sugars were estimated [22].

3. Results and Discussion

Indian systems of medicine such as Ayurveda and Siddha uses majority of the crude drugs that are of plant origin. It is necessary that standards have to be laid down to control and check the identity of the plant an ascertain its quality before use. A detailed pharmacognostic evaluation therefore is highly essential prerequisite [23].

Histology

Histological results indicate presence of tannins, lignin, starch grains, Saponins, Phenolic acids and calcium oxalate crystals.

Physio-chemical characters

The physio-chemical characters summarized in Table 1.

Table 1. Physico-chemical evaluation of *Butea monosperma* leaves

Physical evaluation (%)		Chemical evaluation (%)	
	Leaves		Leaves
Extractive values		Lignin	5.90
a) Petroleum Ether	0.50	Tannins	9.74
b) Alcohol	14.30	Cellulose	24.80
c) Methanol	13.10	Hemicellulose	22.15
e) Water	15.60	Reducing sugar	5.00
Ash values		Nitrogen	1.55
a) Total ash	6.40	Calcium	1.20
b) A.I.A.	0.23	Phosphorus	0.060
c) A.S.A	6.17	Potassium	1.40

Table 2. Antibacterial efficacy of different solvent extracts of *Butea monosperma* leaves

Sr. no.	Microorganism	S train +/-	Concentration (mg/ml)	Zone of inhibition (mm)			
				Petroleum ether	Chloroform	Ethanol	Amphicillin (40 µg/ml)
1.	*Escherichia coli*	-ve		00	00	00	
			50	00	00	00	16
2.	Pseudomonas	-ve	100	00	00	05	
	aeruginosa		50	00	02	06	18
3.		+ve	100	03	04	08	
	Staphylococcus		50	05	05	13	27
4.	*aureus*	+ve	100	09	09	09	
			50	13	13	13	20
	Bacillus subtilis		100				

Figures are diameter of zone of inhibition (in triplicates)

Phytochemical evaluation

The preliminary studies revealed presence of various phytochemicals viz Lignin, Tannins, Cellulose, Hemicellulose, Reducing sugar, Nitrogen, Crude protein, Calcium, Phosphorus, Potassium. The value obtained for various phytochemicals in drug sample are presented in Table 1.

The results obtained for the antibacterial tests performed on different solvent extracts of *B. monosperma* are presented (Table 2). Among the solvents used, the ethanol extract was found to be more effective against *Bacillus subtilis*, and *Staphylococcus aureus* (13 mm at 100mg/ml). The petroleum ether extract not inhibit *Pseudomonas aeruginosa*, while the *Escherichia coli* did not inhibited by any type of extract. Activities of the various extracts were comparable to those of standard antibacterial agent ampicillin as control. The differences in the observed activities of the various extracts may be due to varying degree of solubility of the active constituents in the solvents used. It has been documented that different solvents have

diverse solubility capacities for different phytochemical constituents

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