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PRELIMINARY PHYTOCHEMICAL SCREENING AND PHARMACOLOGICAL EVALUATION OF THE LEAVES OF *BUTEA MONOSPERMA*

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Antibacterial,
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ABSTRACT: Various parts of the plant *Butea monosperma* have been used traditionally for many of the diseases like anti-inflammatory, antimicrobial, anthelmintic, antidiabetic, diuretic, analgesic, antitumor, anticancer, astringent activities. Among the various parts of the *Butea monosperma* first choice is selection of leaves in our current research work because only scanty work has been done on leaves. So it is an attempt to carryout phytochemical evaluation of various leaf extracts and to isolate active constituents and study the characterization of isolated compounds by FTIR, NMR and Mass spectroscopy. The isolated components evaluated for the pharmacological activities along with antimicrobial studies. The pharmacological studies of Petroleum ether, hexane, Chloroform, Ethyl acetate and Ethanol extracts of *Butea Monosperma* leaves shows the anti-inflammatory activity and anthelmintic activity. The petroleum ether and chloroform extract of *Butea Monosperma* leaves shows antioxidant activity. The Petroleum ether, Hexane, Chloroform, Ethyl acetate and Ethanol extracts of *Butea Monosperma* leaves have no anti bacterial and antifungal activity.

INTRODUCTION: Mankind has a long history in the use of herbal medicines. Rigveda and Ayurveda (4500-1600 BC) reveal that ancient Indians had a rich knowledge of the use of medicinal plants. This is all the more striking when we consider the fact that approximately 80% of the people living in less developed countries rely exclusively on traditional medicine¹ for their health care needs. Records of the Assyrians, ancient Hebrews, Greeks and Chinese have all shown the extensive uses of plants with medicinal properties.

Furthermore, the demand for medicinal plants is increasing due to recognition of natural products being non-narcotic with no side effects, easy availability, cost effectiveness and sometimes being the only source of health care for the poor. So by review of history and previous documentation, it is an attempt to detect the compound responsible for particular pharmacological activity. To isolate, characterize the compound and monitor the activity of prime importance.

Butea monosperma (Lam) Synonym Bengal kino tree, Bastard teak. Family *Fabaceae* collected from Utkal University, Bhubaneswar, Odisha and authenticated at Department of Botany; commonly known as 'palas'. Leaves are used to cure boils, pimples, haemorrhage, it is astringent, diuretic, anti-diabetic^{2, 3} and arrest bleeding. Flowers are

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aphrodisiac and tonic properties. Flowers have anti-inflammatory^{4, 5} and anti-cancer activity^{6, 7}. Seed are used for anti-implantation and anti-ovulatory properties. Bark is having anti-microbial properties⁸, anti-fungal activity⁹ and used in tumors¹⁰, bleeding piles, ulcers. Its medicinal properties are enshrined in ancient Indian scriptures with almost each part of the plant namely roots, stem, bark, leaves, flowers, fruits, seeds and gum are used in Ayurvedic and Unani medicine¹¹.

In recent years, pharmaceutical companies have spent a lot of time and money in developing natural products, extracted from plants to produce more cost effective remedies that are affordable to the population¹². The rising incidence in multi-drug resistance amongst pathogenic microbes has further necessitated the need to search for newer antibiotic sources.

Chemical constituents of *Butea monosperma*:

Leaves contains alkaloids (Euphane trieterpenoid and pterocran), Flowers having (butrin, butein, butin, isobutin, coreoopsin, monospermoside and their isoderivatives and sulphurein, palastrin). Seed contains palasonin, d- mecantharidin proteolytic

and lipolytic enzymes, α -amyrin, β -sitosterol and alkaloid monospermine glycerides of stearic, palmitic, linoceric, oleic and linoleic Acids. Bark contains tannins and gum (Butea gum), leucocyanidin, it tetramer, procyanidin, allic acid and mucilaginous material¹³.

MATERIAL AND METHODS: *Test microorganism* - In the present study strains for antibacterial activity were *Staphylococcus aureus*, *Enterococci faecalis*, *Pseudomonas aeruginosa*, *Kliebsiella aerogenes* and for antifungal activity *Candida albicans*. Disc diffusion method is used for both activities. For both antibacterial and antifungal activity Mueller – Hinton agar medium were used. A loop full test organism was inoculated on nutrient broth and incubated for 24 h at 37±1°C and maintained in sterile condition. Ciprofloxacin (5mcg/ml) for antibacterial and Ketoconazole (10mcg/ml) for antifungal activity.

Anti-inflammatory activity:

In-vitro anti-inflammatory activity was done by HRBC membrane stabilization method (**Table 1, Fig.1**).

TABLE 1: IN-VITRO ANTI-INFLAMMATORY ACTIVITY BY HRBC MEMBRANE STABILIZATION METHOD

S. No	Treatment	Dose mg/ml	Percentage stabilization (Mean ± SEM)
1	Diclofenac sodium	1 mg/ml	59.78 ± 0.235**
2	Petroleum ether extract	1 mg/ml	53.81 ± 0.2235**
3	Hexane extract	1 mg/ml	42.40 ± 0.2236**
4	Chloroform extract	1 mg/ml	57.61 ± 0.2581*
5	Ethyl acetate extract	1 mg/ml	40.42 ± 0.2236**
6	Ethanol extract	1 mg/ml	44.03 ± 0.2236**

P < 0.001 vs Control, ** Highly Significant, * Significant

Samples were prepared with various extracts at a dose of 1mg/ml – 2% acacia solution. Diclofenac sodium was used as standard.

Preparation of Standard - 1ml of std drug + 2 ml of hypotonic saline + 1 ml phosphate buffer + 0.5 ml 10%. Human red blood cell (HRBC) Suspension . Incubate at 37° C., centrifuge with a speed of 3000 RPM for 15-20 minutes. Absorbance of the supernatant was measured at 560 nm¹⁴.

Preparation of Sample : 1ml of extract + 2 ml hypotonic saline + 1ml of phosphate buffer + 0.5 ml of 10% HRBC suspn.

Preparation of Product Control: 1ml isotonic saline + 2 ml hypotonic saline + 1ml phosphate buffer + 0.5 ml of distilled water. Preparation of Test Control: 1ml isotonic saline + 2ml hypotonic saline + 1 ml of phosphate buffer + 0.5 ml 10% HRBC suspension.

$$\text{Percentage stabilization} = \frac{100 - (\text{OD of sample} - \text{OD of product control})}{\text{OD of test control}} \times 100$$

OD of test control

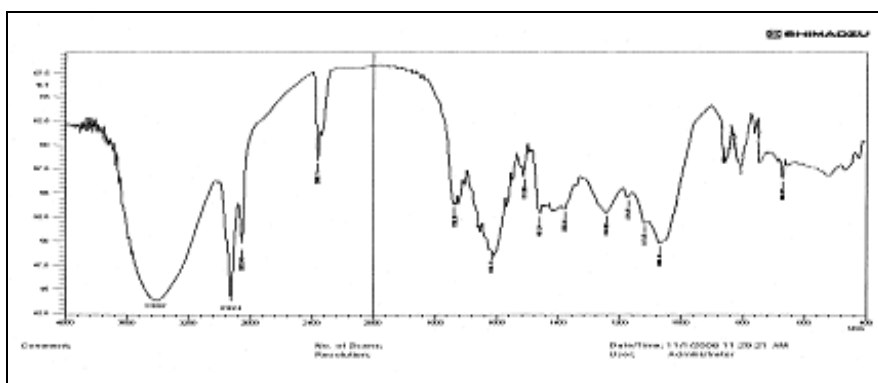


FIG.1: FTIR STUDY OF BUTEA MONOSPERMA

In-vitro antioxidant activity: ¹⁵

Samples was prepared by using various extracts at a dose of 1mg/ml – 2% acacia soln. Standard was

used Ascorbic Acid (1 mg/ml), Absorbance measured at 546 nm (Table 2, Fig. 2).

TABLE 2: NITRIC OXIDE FREE RADICAL SCAVENGING ACTIVITY ^{16, 17} OF EXTRACTS OF BUTEA MONOSPERMA LEAVES

S.N	Tested Extracts	Concentration 1mg/ml	% Inhibition
1	Petroleum ether	1mg	63.71
2	Chloroform	1mg	65.58
3	Ascorbic Acid Standard	1mg	83.81

Sulphanilamide solution: - (1% Sulphanilamide in 5% phosphoric Acid) 1gm sulphanilamide dissolved in 100ml 5% phosphoric acid.

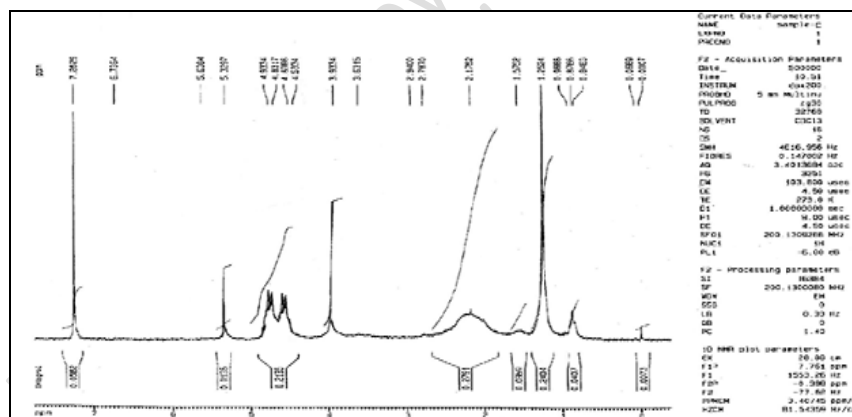


FIG.2: NMR SPECTROSCOPY OF BUTEA MONOSPERMA

NED Solution:

(0.1% N-1 naphthylethyl ethylenediamine di - hydrochloride in water) 100mg NED dissolved in 100 ml water. Nitrite Standard:- (0.1 M sodium nitrite in water) 6.90 gm sodium nitrite dissolved in 1000ml water.

Griess Reagent:

50 ml Sulphanilamide solution + 50 ml NED soln + 1 ml Nitrite STD Standard phosphate Buffer solution (p^H 7.5):- 50 ml 0.2M potassium di - hydrogen phosphate + 39.1 ml of 0.2 M NaOH +

water up to 200ml. 0.2 M potassium di-hydrogen phosphatae:- 27.21 gm of potassium di-hydrogen phosphate dissolved in 1000 ml water. 0.2 M

NaOH solution:

8 gm of NaOH dissolved in 1000 ml water. Sodium nitroprusside solution (5micro M)

$$\text{Inhibition percent} = \frac{\text{Absorbance of control} - (\text{Absorbance of test})}{(\text{Absorbance of Control})} \times 100$$

Anthelmintic activity:

Sample prepared with various Extracts (0.5% and 1%), Standard used was Albendazole (0.5% and 1%), *Pheretima posthuma* was used as test organism, Indian earthworm (Table 3, Fig. 3).

TABLE 3: EVALUATION OF VARIOUS EXTRACTS OF BUTEA MONOSPERMA, LEAVES FOR ANTHELMINTIC ACTIVITY

S.no	Extracts	Concentration %			
		Time for paralysis (min)		Time for death (min)	
		0.5	1.0	0.5	1.0
1	Petroleum ether	75	41	180	160
2	Hexane	71	42	170	155
3	Chloroform	70	41	168	150
4	Ethyl acetate	68	40	155	142
5	Ethanol	68	41	154	143
6	Albendazole	48	40	68	65

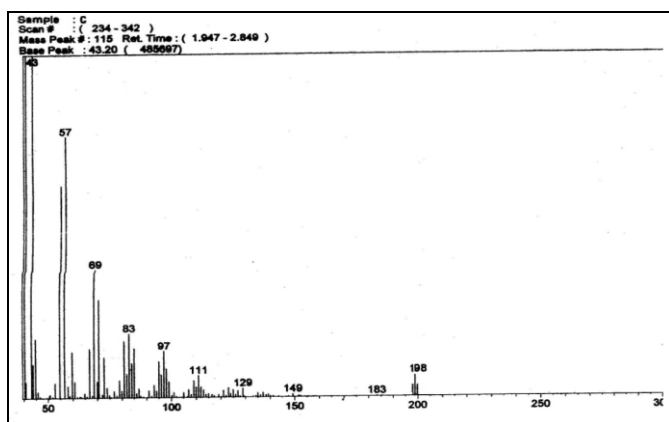


FIG.3: MASS SPECTROSCOPY OF BUTEA MONOSPERMA

Phytochemical screening:

The preliminary phytochemical analysis of *Butea Monosperma* leaves shows the presence of alkaloids in hexane and chloroform extracts. Carbohydrates in ethanol and ethyl acetate extracts, Phytosterols in Petroleum Ether, hexane and Chloroform extract, flavones in petroleum ether and chloroform extract. The compound has melting point 152°C. The compound is white, odorless powder soluble in ethanol, di-methyl sulfoxide and di-methyl formamide. The chemical test shows the presence of ester¹⁸.

Preparation of extracts:

Air dried plant leaves (15 kg) were pulverized with a grinder. Approximately 250g of pulverized plant part was extracted with ethanol by using continuous hot percolation for 72 h at temperature

60 – 65°C. Extract were obtained as dark green color residue¹⁰. Polarity increased by fractionation with petroleum ether, hexane, chloroform and ethyl acetate. Color Change done by Day light and UV light.

RESULTS AND DISCUSSION: The preliminary phytochemical analysis of *Butea Monosperma* leaves shows the presence of alkaloids in hexane and chloroform extracts. Carbohydrates in ethanol and ethyl acetate extracts, Phytosterols in Pet. Ether, hexane and Chloroform extract, flavones in petroleum ether and chloroform extract. The compound has melting point 152°C. The compound is white, odourless powder soluble in ethanol, di-methyl sulfoxide and di-methyl formamide. The chemical test shows the presence of ester. The FTIR spectrum reveals the presence of OH and COO- which are the functional groups of Gallic Acid ester; further the compound was confirmed by the NMR and MASS Spectroscopy.

Petroleum ether and chloroform extract shows highly significant anti-inflammatory activity while hexane, ethyl acetate and ethanol shows moderate anti-inflammatory activity. The petroleum ether and chloroform extracts at a concentration of 1mg/ml exhibits moderate antioxidant activity comparable to that of Ascorbic Acid, studied by Nitric oxide free radical scavenging activity. The petroleum ether, hexane, chloroform, ethyl acetate and ethanol extracts exhibits mild anthelmintic activity at a concentration of 1mg/ml, comparable to that of Albendazole. The petroleum ether, hexane, chloroform, ethyl acetate and ethanol extracts of *Butea monosperma* at a concentration of 1mg/ml have no antibacterial and antifungal activity compared to the standard drug ciprofloxacin and ketoconazole at a concentration of 5 mcg /disc and 10 mcg/disc respectively.

CONCLUSION: The phytochemical screening of various extracts of *Butea Monosperma* leaves reveals the presence of Alkaloids, Carbohydrates, Flavones and Phytosterols. In the current work the chloroform extract was subjected to column chromatography for isolation and one compound was isolated. The chemical test reveals the presence of ester, further the structure of the compound was confirmed by spectral methods.

The pharmacological studies of Petroleum ether, hexane, Chloroform, Ethyl acetate and Ethanol extracts of *Butea Monosperma* leaves shows the anti-inflammatory activity and anthelmintic activity. The petroleum ether and chloroform extract of *Butea Monosperma* leaves shows antioxidant activity. The Petroleum ether, Hexane, Chloroform, Ethyl acetate and Ethanol extracts of *Butea Monosperma* leaves have no anti bacterial and antifungal activity. The different extracts of the plant *Butea Monosperma* leaves contain some other constituents responsible for the above activity. So further study can be made on the isolation and characterization of the compounds in future.

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