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Thooyavan G
Department of Zoology,
Presidency College,
(Autonomous), Chennai, Tamil Nadu, India

Karthikeyan J
Department of Zoology,
Presidency College,
(Autonomous), Chennai, Tamil Nadu, India

Correspondence
Thooyavan G
Department of Zoology,
Presidency College,
(Autonomous), Chennai, Tamil Nadu, India

Phytochemical profiling and GC-MS analysis of *Butea monosperma* seed methanol extract

Thooyavan G and Karthikeyan J

Abstract
Ethnomedicinal plant *Butea monosperma* seeds were analyzed for the presence of biologically active constituents. The preliminary phytochemical studies and quantitative analysis of alkaloids, phenol and flavonids were performed. The observations of results shows maximum number of phytoconstituents were present in methanol seed extract. These extract was further subjected to TLC (Thin layer chromatography), GC-MS (Gas chromatography and mass spectrum) analysis. About 35 different compounds were identified with GCMS analysis. Anthelmintic compounds such as Benzothiazole, Milbimycin B were present along with some of the compounds with varied nature of action like antioxidant, antibacterial and anticancer activities, further studies on *in vitro* and *in vivo* will reveal the exact nature of the compounds.

Keywords: *Butea monosperma*, Phytochemical – GCMS- Benzathizole

1. Introduction
Butea monosperma (Lam.) is commonly known as the Flame of the forest, belongs to the family Fabaceae [1]. It is locally called as palas, palash, mutthuga, bijasneha, dhak, khakara, chichra, bastard teak, bengal Kino, Nourouc and is commonly available throughout India, Burma and Ceylon. In India, palas ranks next to kusum (*Schleichera trijuga*) as a host tree for lac insects [2, 3] and it is a source of constitutive osteogenic agents belonging to isoflavonoid and pterocarpan groups. The genus of Butea also includes *Butea monosperma, Butea parviflora, Butea minor* and *Butea superba* which are widely distributed throughout India [4].
Butea monosperma is extensively used in Ayurveda, Unani and Homeopathic medicine and has become a cynosure of modern medicine. They are commonly used to cure kapha and vata. *Butea monosperma* is used as a tonic, astringent, aphrodisiac, diuretics and inflammations, skin and eye disease, bleeding piles, urinary stones, abdominal discomfort and tumors. The seeds where pounded with lemon juice and applied to the skin, act as a rubefacient [5]. A potential antiviral flavones glycoside has been isolated from the seeds of *Butea monosperma* [6]. It also possess anthelmenthic, appetizer, aphrodisiac activities [7]. Moreover, it is commonly used as an ethno-veterinary medicine for many ailments in various parts of India and South Asia [8, 9]. The present studies are intended to establish various phytoconstituents that exist in the seeds of *Butea monosperma*.

2. Materials and Methods
2.1 Plant material
Butea monosperma seeds were collected in and around Chennai, Tamil Nadu, India. The seeds were identified with the help of Plant Anatomy Research Centre, Chennai.

2.2 Solvent Extraction
The seeds were shade dried and grinded into fine powder and extraction was done using Soxhlet apparatus with different solvent *viz,* hexane, ethyl acetate, chloroform and methanol. The aqueous were prepared using cold maceration method. The extract was vacuum evaporated and stored at 4 ºC until further use.

2.3 Qualitative Phytochemical analysis
Phytochemical analysis were carried out with standard modified protocols for identification of presence and active phytoconstituents [10-12]

2.3.1 Test for Alkaloids
100 mg of extract was dissolved in 5 ml of 1% Hydrochloric acid and filtered. The filtrate was treated with 2 to 3 drops of Dragendorff's reagent, Formation of orange-red precipitate indicates the presence of Alkaloids.

Mayer's test for Alkaloids: To 1 ml of filtrate add few drops of Mayer's reagent appearance of yellowish white precipitate indicates the presence of alkaloids.

2.3.2 Test for Flavonoids
The plant extracts were dissolved in methanol by mild heating, to each of the extract small pieces of magnesium ribbon was added, followed by the addition of a few drops of concentrated hydrochloric acid. The change of colour from orange, pink, red to purple indicates the presence of flavonoids.

2.3.3 Test for Tannins
200 mg of each plant extract was dissolved in about 10 ml of distilled water and then filtered. Two ml of each extract was taken in separate test tube. Few drops of 1% alcoholic ferric chloride solution was added to test tubes and the occurrence of blue green precipitate indicates the presence of tannins.

2.3.4 Test for steroids
100 mg extracts were dissolved in equal volume of acetic acid and chloroform. The sample is cooled at 0 ºC for few minutes and few drops of concentrated sulphuric acid was added, formation of reddish brown or violet-brown ring indicates the presence of a steroid.

2.3.5 Test for Diterpenoids
One ml of each plant extract was treated with a few drops of 1% copper acetate solution, formation of emerald green color indicates the presence of diterpenoids.

2.3.6 Test for Terpenoids
500 mg of extract was dissolved in ethanol equal volume of dissolved extract and acetic anhydride were taken in a clean test tube. Few drops of concentrated sulphuric acid was added to the tubes. The colour change from pink to violet showed the presence terpenoids (or) formation of blue green ring indicates the presence of terpenoids.

2.3.7 Test for Saponins
One gram of each extract was shaken vigorously with 3 to 5 ml of distilled water persistent foam for 10 minutes confirm is the presence of saponins.

2.3.8 Cardiac glycosides (Keller-Killani test).
Three ml of each plant extract was treated with 2 ml of glacial acetic acid and few drops of 1% ferric chloride solution, 1 ml of concentrated sulphuric acid were added. Formation of brown ring at the interface shows the presence of cardiac glycosides.

2.3.9 Carbohydrate Test
Two ml of each plant extract was treated with few drops of Molisch reagent and 1 ml concentrated sulphuric acid. Formation of red coloured ring at the interface indicates the presence of carbohydrates.

2.3.10 Test for Protein
Two ml of each extract was treated with few drops of 1% Ninhydrin solution. Formation of blue colour on mild heating in the water bath confirms the presence of protein.

2.3.11 Test for oil and waxes
Five mg of *Butea monosperma* extracts were placed in between two Whatman No. 1 filter paper when kept undisturbed, the presence of oil stain in the filter paper indicates the presence of oil and waxes.

2.4 Quantitative phytochemical analysis
2.4.1 Estimation of total Phenolic content
Total phenol content was estimated by Folin-Ciocalteu method [13]. The reaction mixture consists of 0.5 ml extract and 0.1 ml of Folin-Ciocalteu reagent (0.5N), it was incubated at 37 ºC for 5 minutes, after incubation 2.5 ml of saturated sodium carbonate was added and it was further incubated for 30 minutes. After incubation the color developed was read at 760nm using spectrophotometer.

2.4.2 Estimation of Flavonoids
In a test tube take 0.5 ml of extract, 0.5 ml of Aluminium chloride and 0.5 ml of Potassium acetate (120mM). The tube was incubated for 30 minutes at room temperature. The color developed was read at 417 nm using spectrophotometer. Distilled water was used as blank and Quercetin was used as standard [14]

2.4.3 Estimation of Alkaloid
5mg of each extracts of *Butea monosperma* was dissolved in dimethyl sulphoxide (DMSO). 1ml of 2N Hydrochloric acid was added and filtered. This solution was transferred to a separating funnel, 5ml of Bromocresol green solution and 0.5 ml of phosphate buffer solution were added. The mixture was shaken with 1, 2, 3 and 4 ml of chloroform by vigorous shaking and collected in a 10 ml volumetric flask and diluted to the volume with chloroform. The absorbance for test and standards were determined against the reagent blank at 470 nm in Spectrophotometer. The total alkaloid content was expressed as mg of AE/g of extract [14].

2.5 Thin Layer Chromatography
A part of the extract was dissolved in acetone and applied on a silica gel pre coated plates with the help of TLC capillary tubes. The plates were placed in the solvent system Methanol: Chloroform: Hexane (2:2:1) to separate the various constituents of the extracts. The developed plates were air dried and observed under visible and UV light (254 and 366 nm). Various separated spots were noted for their *Rf* values.

2.6 GCMS Analysis
GC-MS analysis was performed with Agilent GC 7890A 240MS with Ion Trap gas-chromatograph equipped with (HP5, 30 meters, 0.32mm x 0.25µm) HP5 capillary column (30mx0.32 mm; coating thickness 0.25 µm) interfaced with Agilent 240 MS Ion Trap mass detector. Analytical conditions: Injector and transfer line temperature 220 and 2400C, respectively oven temperature programmed from 800C to 3000C at 40C/min; carrier gas, helium at 1 ml/min; injection 0.2 µl of a n-hexane. The identification of the components was performed for both the columns by comparison of their retention times with those of pure, authentic samples and by mean of their liner retention indices and by computer matching against commercial mass spectra libraries NIST and MS literature data [15]

2.7 Statistical analysis
Estimation of total phenolic compounds, flavonoid and alkaloid contents were conducted in triplicate. The values of each sample was calculated as the Mean ± SD.

3. Results

Different extract of *Butea monosperma* seed, were prepared by Soxhlet extraction. The Yield of crude extract is obtained by measuring its dry weight of concentrated extract. The yield of Hexane (2.8%), Ethyl acetate (1.8%) extracts were when compared to Chloroform (4.1%) Aqueous (9.27) and Methanol (12.23%).

3.1 Qualitative Photochemical

Presence or absence of various phytocontituents were analyzed in five different extract of *Butea monosperma* seeds. The results were presented in Table (1). Alkaloids, flavonoid, diterpenoids and tannins are important secondary metabolites that exist in all the extracts may increase the medicinal value of the tested plant. The maximum yield was observed with reference to methanol extract.

3.2 Quantitative phytochemical analysis

Analysis of the results presented in Table (2) reveals that methanolic extract of *Butea monosperma* seeds. It shows the presence of maximum amount of Phenol (43.03±0.09), Flavanoids (36.87±0.4), Alkaloids (20.76±0.32) when compared to other extracts tested. The analysis confirms the presence of various bioactive compounds present in methanol extracts and they were further subjected to TLC and GCMS analysis the results were presented in Table (3) respectively.

3.3 Thin layer chromatography

Thin layer chromatography studies of Methanol seed extract of *Butea monosperma* shows the presence of different number of bands with respect to band width observed 5, 8 and 12 bands were observed with reference to visible range 254nm and 366 nm respectively.

3.4 GCMS Analysis

GCMS results presented in table 3 with reference to the database. It is observed that the following components were present in the methanolic extract of 0.50% Diphenylamine, 0.36% Benzothiazole, 1.6% Tetracosanoic acid, methyl ester, 0.30% Milbemycin B, 0.30% 2,3,4,6-Tetrahydroisoquinolin, 0.50% 3-Benzyl 2-methyl 1-cyclohexyl-4-hydroxy present in the methanol seed extract.

4. Discussion

Butea monosperma seeds consist of numerous secondary metabolite compounds with various biological functions. The results shows the presence different components in the methanol exctract and it confirms the presence of various classes of metabolites such as flavanoids, alkaloids, tannins and terpenoids. Presence of 43.03 ±0.09 mg/g GAE of total phenlic compound was also evident from the result. About 36.87 mg/g of QE flavonoids and 20.76 mg/g AE of alkaloids were observed in the study. Phenolic compounds possess potential activities such as antioxidant, antidaibetic, hepatic protective, anticancer, antimicrobial activities [16]. The presence of these compounds increase the medicinal value of *Butea monosperma* further when methanol was subjected to GCMS of which 35 volatile chemical compounds belonging to various functional groups such as hydrocarbon, esters, alcohol *etc* were identified.

Different functional groups of phytoconstituents were identified using GC-MS studies [17]. The present findings also reveals the presence of medicinally important compounds in the methanolic seed extract of *Butea monosperma*. The compounds were identified with the reference to the standard databases.

5. Conclusion

In Conclusion methanolic seed extract of *Butea monosperma* is considered as a natural compound for the treatment of various diseases as it possess the compounds with the varied nature of activity. Detail studies on *in vitro* and *in vivo* reveals the nature and mechanism of action of the novel compounds present in the seeds.

Table 1: Qualitative analysis of Phytoconstituents in the seed extracts of *Butea monosperma*

Compounds	Hexane	Ethyl Acetate	Cholroform	Methanol	Water
Alkaloids	+	+	+	++	+
Phenolic acid	+	+	-	+	-
Flavinoids	+	+	+	+++	+
Tannins	+	+	+	+	+
Terpeniods	+	+	+	+	+
Diterpeniods	+	+	+	+++	+
Steroids	-	+	+	+	-
Cardiac Glycosides	+	+	+	+	-
Wax&oil	++	++	++	+++	+
Carbohydrate	+	+	+	++	+
Protein	-	-	+	+	+

Table 2: Total Phenol, Flavonoid, Alkaloid content of methanol seed extract of *Butea monopserma*

Solvents	Total Phenol (mg of GAE/g of extract)	Flavanoid (mg of QE/g of extract)	Alkaloids (mg of AE/g of extract)
Hexane	17.8±0.36	21.9±1.4	14.77±0.5
Ethyl Acetate	14.8±0.3	26.9±0.8	7.5±0.36
Chloroform	13.1±0.2	18.9±1.6	10.43±0.41
Methanol	43.03±0.09	36.87±0.4	20.76±0.61
Aqueous	9.43±0.15	10.93±0.2	9.33±0.32

Mean ± S.D

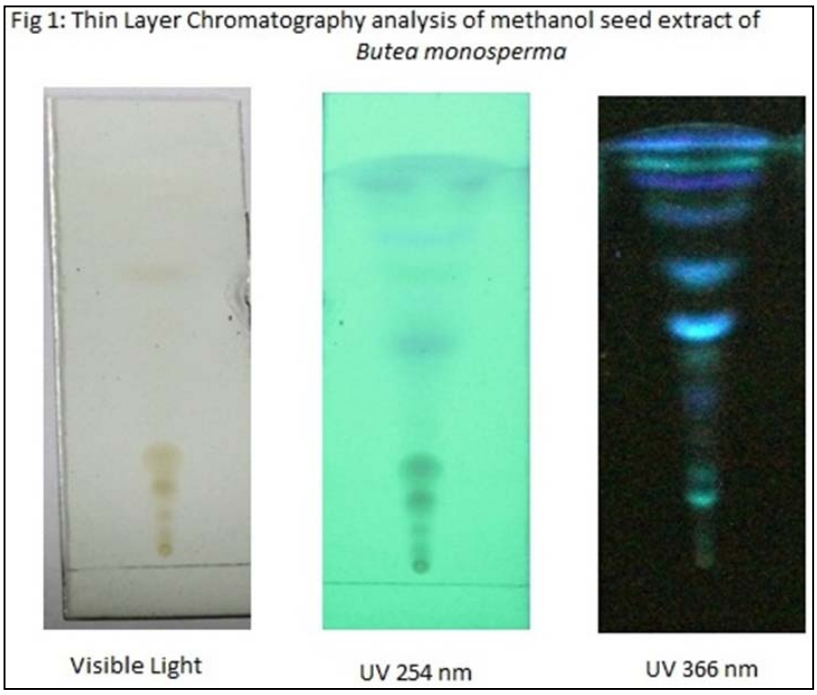

Table 3: List of compounds identified in the GC-MS analysis of Methanol Seed extract of *Butea monosperma*

S. No	RT	Area %	Compound Name	Activity
1	22.22	0.50	Diphenylamine	Antioxidant [18]
2	29.15	0.51	Benzenamine, 4-(1-methylethyl)-N-phenyl-amine	Antioxodant [19]
3	30.04	0.90	Hexadecanoic acid, methyl ester	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Antiandrogenic, flavor, Hemolytic, 5-Alpha reductase inhibitor [20]
4	30.41	0.36	Benzothiazole, 2-(2-hydroxyethylthio)	Anthelmintic activity [21]
5	43.03	0.70	Methyl 20-methyl-heneicosanoate	Insecticidal activity [22]
6	46.19	0.25	Dotriacontyl penta fluoropropionate	
7	46.48	0.42	Estra-1,3,5(10)-trien-17-one, 3,4-bis	
8	46.75	1.6	Tetracosanoic acid, methyl ester	
9	49.41	0.18	Pregn-5-en-20-one, 3,16,17,21-tetrakis	
10	49.61	0.25	Octadecane, 1,1'-[1,3-propanediylbis(oxy) bis	
11	49.82	0.02	psi.,.psi.-Carotene, 3,3',4,4'-tetradehydro-1,1'2,2'tetra hydro1,1''	Antioxidant [23]
12	49.88	0.02	Rhodopin	
13	49.92	0.02	N'-(4-Chlorobenzoyl)-2-(2,4-dichlorophen	
14	50.26	0.05	6,6'-Diacetyl-7,7'-dihydroxy-2,2',4,4',5	
15	50.55	0.10	Acetamide, N-[5-(diethylamino)	
16	51.59	0.06	Phenanthro[3,4-b]phenanthrene, 8-acetox	
17	52.24	0.09	Lycopene	Anticancer [24]
18	52.30	0.02	Normorphine, bis(trimethylsilyl) ether	
19	53.34	0.14	Methanamine, N-(1-[1,1'-biphenyl]-2-ylet	
20	53.56	0.18	9,10-Anthracenedione, 1-(methylamino)	
21	53.63	0.11	Silane, [(3-beta-5-alpha-11-beta - 20S]	
22	53.94	0.23	5-beta-Pregnane-3 alpha 20-beta-diol	
23	54.31	0.10	Lycoxanthin	Anticancer activity[25]
24	54.91	0.30	Milbemycin B	Anthelmintic drug [26]
25	54.95	0.18	2,2-Bis[4-[(4,6-dichloro-1,3,5-triazin-2)	
26	55.03	0.30	2,3,4,6-Tetrahydroisoquinoline-6-one	Antimalarial Drug [27]
27	55.11	0.50	10-(Methoxycarbonyl)-N-acetylcolchinol	
28	55.63	0.27	Perylo[1,12-def]-1,3-dioxepin-5,11-dione	
29	56.39	0.81	[3-Methyl-2-(4-nitro-phenyl)-4-oxo-1,2,3	
30	56.55	0.70	1',1'-Dicarboethoxy-1.beta.,2.beta.-dihydro 3-H-cycloprop [1, 2]- cholesta	
31	56.66	0.50	1 3-Benzyl 2-methyl 1-cyclohexyl-4-hydroxy-5-oxo-2,5-dihydro1H-pyrrole	
32	56.92	0.30	Oxazepam ditms	
33	57.11	0.50	Calconcarboxylic acid	
34	60.48	0.10	3-Phorbinepropanoic acid, 9-ethenyl-14-ethyl-14 formyl-4,8,trimethyl	
35	60.60	0.10	Palladium(0), bis(.eta.-2-butadiene) 1,1,4,5,8,8 hexa(tret-butyl)-1,4	

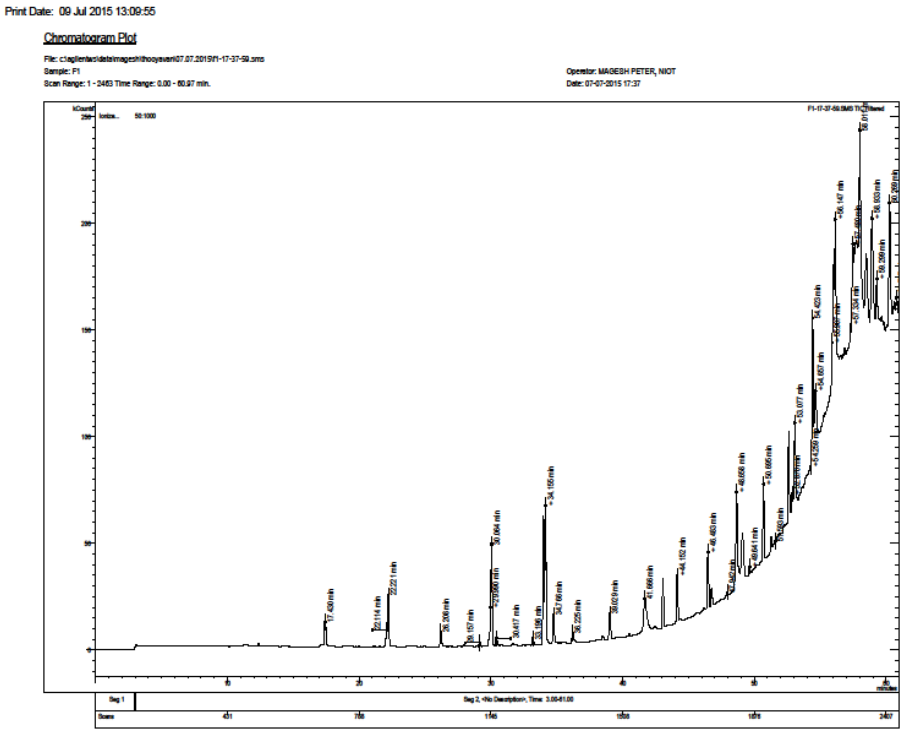

Fig 2: GCMS Chromatogram of methanol seed extract of *Butea monosperma*

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