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Phytochemical and chromatographic analysis of flavonoid fraction isolated from methanolic extract of *Pterocarpus marsupium*

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ABSTRACT

Medicinal plants are considered as rich sources of active components which can be used in drug development. *Pterocarpus marsupium* is a medicinal plant known for therapeutic efficacy against Diabetes mellitus and inflammatory conditions. The proximate analysis, phytochemical screening, GC-MS analysis and the HPTLC analysis of flavonoid fraction isolated from methanolic extract of *Pterocarpus marsupium* were performed in the present study. The total ash, crude protein, crude fat, crude fibre, acid insoluble ash, carbohydrate content, calcium and phosphorus content were all measured. Standard techniques were used to examine the methanolic leaf extract for various phytochemical components. The presence of alkaloid, saponins, phytosterols, phenolic compounds, flavanoids, terpenoids and cardiac glycosides was revealed from the methanolic extract of *P. marsupium*. TLC analysis of crude methanolic extract of *P. marsupium* was conducted to study the separation pattern with different solvent systems and solvent combination with effective separation of components. The HPTLC finger print profiling of flavonoid fraction of methanolic extract of leaf of *P. marsupium* were also conducted. The GC-MS analysis revealed the composition of 13-Docosenamide and 1-Bromo-4-bromomethyldecane.

Keywords: *Pterocarpus marsupium*, Methanolic extract, Flavonoids, Phytochemical, Proximate content, GC- MS analysis.

INTRODUCTION

Herbal medicine has long been recognised for its important role in sustaining human health and increasing the quality of life. Secondary metabolites such as alkaloids, steroids, tannins and phenol compounds, which are generated and deposited in specific plant sections, are the most common chemicals with pharmacological properties in plants [1]. They may function by mimicking or antagonising endogenous metabolites, hormones, signal transduction molecules, or neurotransmitters and so have favourable medical effects on humans/animals due to similar target sites [2]. *Pterocarpus* is a Fabaceae family genus of pantropical trees. There are roughly 35 species in this genus, with *P. marsupium* being one of the more well-known [3]. These plants have been claimed to offer therapeutic potential for the treatment of illnesses like diabetes, inflammation and bleeding for a long time. It was also used to treat chest and body pain as an astringent, anti-inflammatory, haemostatic and anthelmintic [4]. The previous studies show that the genus *Pterocarpus* to be a rich source of polyphenolic compounds [5]. The extract contains stilbene, pterostilbene, catechin, epicatechin, marsupol, isoflavonoid glycol, etc. An isoflavone c-glucoside marcrocarposide isolated from heartwood of *P. marsupium* has been characterized as 5, 7, 2, 4-tetrahydroxy isoflavonone 6-6 glucoside [6]. The purpose of this study was to determine the proximate contents, phytochemical ingredients and GC-MS analysis of the methanolic extract of *P. marsupium* leaves, as well as the isolation of a flavonoid fraction from the methanolic extract of *P. marsupium* leaves.

MATERIALS AND METHODS

Plant Extraction

Collection and identification of plant materials

The *Pterocarpus marsupium* plant leaves utilised in this study were obtained from Sultan battery Wayanad Kerala India and were taxonomically recognised by a botanist from Department of Botany Calicut University An herbarium for morphological investigations was prepared and a voucher specimen was placed at the Calicut university herbarium Kozhikode Kerala.

Preparation of plant materials

The plant materials were collected cleaned and dried in the shade to remove water The powdered leaves were sieved to remove the coarse particles after being powdered in an electrically operated mortar The fine powder was collected and stored in an airtight container until it was used.

Preparation of crude methanolic extract

The powdered plant material of *P. marsupium* was utilised for extraction using methanol in a Soxhlet extraction device attached to a rotating vacuum evaporator (Buchi, Switzerland) according to the modified method of [7]. The powdered plant material (100g) was kept in filter paper thimbles (Whatman filter paper No.1) and extracted using methanol for eight refluxes in extraction chamber of Soxhlet apparatus. Solvents were extracted using a rotary vacuum evaporator at temperatures ranging from 40 to 45° C. The dried extract's weight was recorded and the extractive yield was computed as follows,

$$\text{Extractive value} = \frac{\text{Weight of the extract} \times 100}{\text{Weight of powdered plant sample taken}}$$

Phytochemical examination of crude extracts was performed by dissolving the extract in methanol.

Analysis of Plant Material

Analysis of crude powder of leaf of P. marsupium for proximate principles

Standard proximate analysis techniques were used to determine the ash crude fibre crude protein carbohydrate crude fat dry matter and moisture content of the individual plants [7].

Phytochemical Analysis

The methanolic leaf extract was subjected to phytochemical analysis according to the standard protocol [8,9]. Qualitative tests used for identification of phytoconstituents is described in (Table 3).

Chromatography

Thin layer chromatography (TLC)

The chromo plate for TLC is prepared by spotting the methanolic extract of the plant sample on a silica gel pre-coated aluminium paper plates with thickness 0.25mm (Merck TLC plate F245) [10]. It is placed in a slanting manner in a glass tank containing fixed composition of the solvent system such that the sample spots are just above the solvent level. The tank is closed with a lid. The solvent which constitutes the mobile phase ascends by capillary action carrying the various components at different rates due to their differences in attraction to the stationary phase. This results in separation of the components and can be analyzed. Mobile phases of various compositions were tested and the solvent system that provided the most efficient subcomponent separation was chosen as the solvent system for column chromatographic separation. For column chromatography, a 78: 20: 2 ratio of hexane, ethyl acetate and acetic acid was utilised.

Flash Chromatography

The flash chromatographic system (M/s Buchi, Switzerland) equipped with C-601binary gradient pump, C-620 control unit, C-660 fraction collector, Buchi UV Photometer C-640 detector and sepacore control 1.2 software for data analysis were used for the analysis [11]. Silica gel (230-400mesh, 200g) was pre-heated 100ºC for 1hr and packed in 36/460-044041 columns. Column was initially stabilized with least polar solvent of the solvent system. Approximately 5g of methanolic extract of *P. marsupium* dissolved in minimum quantity of hexane was loaded by manual injection. Flavonoid fractions eluted from the column were collected in separate glass tubes from the fraction collector.

Column size: glass column 460mm long, 36mm diameter

Stationary phase: 200g of silica gel (230-400mesh, Merck)

Sample: 5g methanolic extract of *P. marsupium*

Mobile phase: Solvent 1: Petroleum Benzene, Solvent 2: Ethyl acetate

The flavonoid fraction was concentrated by removing the solvents in rotary vacuum evaporator and solvent completely removed by evaporation. Then the analysis of flavonoid fraction was done by HPTLC. The solvent used is a mixture of hexane and ethyl acetate in the ratio 9:1 and 8:2. Scanning was done with a Camag TLC scanner in fluorescence mode at 254 nm and 366 nm, utilising win CATS software (version 1.4.1). Plates were examined in visible light, UV 254 nm and UV 366 nm.

High Performance Thin Layer Chromatography (HPTLC) Analysis of Isolated Flavonoid Fraction

HPTLC analysis of the flavonoid fraction isolated from the methanolic extract of leaf of *P. marsupium* was carried out on a Camag HPTLC (M/s Camag, Switzerland) system [12]. Chromatographic separation was performed on Merck TLC plates (20 cm×10 cm with 200 µm thickness) pre coated with silica gel from E. Merck, Germany. The methanolic extract of different concentrations was applied on to the plates as a band width 6 mm using Camag Linomat 5 applicator (M/s Camag, Switzerland). Linear ascending development was carried out in a twin trough glass chamber containing hexane and ethyl acetate in the ratio 90: 10. Scanning was done with a Camag TLC scanner in fluorescence mode at 254 nm and 366 nm, utilising win CATS software (version 1.4.1). Plates were examined in visible light, UV 254 nm and UV 366 nm.

Gas chromatography-Mass spectroscopy (GCMS) analysis

The crude methanolic extract of *P. marsupium* was analyzed by GC-MS using a GC-MS QP 2010 gas chromatograph (M/s Shimadzu Corporation, Kyoto, Japan) [13]. The column used for this analysis was a 30 m × 0.25 µm × 0.25 mm internal diameter, RX1 SILMS (Restek, USA). One milligram of the extract was dissolved in one millilitre of hexane and 1 microlitre of this was injected into the split mode of the GC-MS instrument. With a flow rate of 1 ml/minute, helium was used as the carrier gas. For the first 5 minutes, the column temperature was kept at 60°C. Then it was set to 220°C at a ramp rate of 5°C per minute, with a final temperature of 280°C kept for 5 minutes. The injector and detector temperatures were set to 250 and 290ºC, respectively. The MS operating parameters as follows as:

Ionization energy = 70 eV
Ion source temperature = 250°C
Solvent delay = 5.0 minutes
Scan range = 40 to 700 u

The components were identified on the basis of matching of their fragmentation spectra available with mass spectral library (M/s Wiley 08, NIST 11).

RESULTS AND DISCUSSION

Analysis of crude powder of leaf of *P. marsupium* for proximate principles

The methanolic extracts of *P. marsupium* leaf had extractive values of 9.29 per cent. The per cent values for crude protein, crude fat, dry

matter, moisture, crude fibre, ash and carbohydrate contents derived from proximate analysis of crude powder of *P. marsupium* leaf are shown in table 1 and figure 2. Table 2 shows the per cent values obtained for inorganic content of crude powder from *P. marsupium* leaf. Using standard proximate analytical procedures, the dry matter, moisture, crude fibre, crude protein, ash carbohydrate and mineral contents of *P. marsupium* leaf were determined. Dry matter content was 91.13 per cent, total ash, crude protein, crude fat, crude fibre, acid insoluble ash and carbohydrate content were 7.66, 20.93, 1.77, 26.97, 0.85, and 60.76 per cent, respectively, according to the results of the analysis. Calcium and phosphorus percentages were 3.62 per cent and 0.63 per cent, respectively. The proximal principles of *P. marsupium* were studied by [14]. They found that the total ash value, acid insoluble ash, and water-soluble ash were 2 per cent, 0.35 per cent, and 0.30 per cent, respectively. Other proximate concepts were not mentioned in any other reports.

Table 1: Proximate principles in the crude powder of leaf of *P. marsupium*

Plant	Biochemical contents (%)						
	Dry Matter	Moisture	Crude fiber	Crude protein	Crude fat	Ash	Carbohydrate
Pterocarpus marsupium	91.13	8.86	26.97	20.93	31.77	7.66	60.76

Figure 1: Fresh leaves of *P. marsupium* used for the experiment

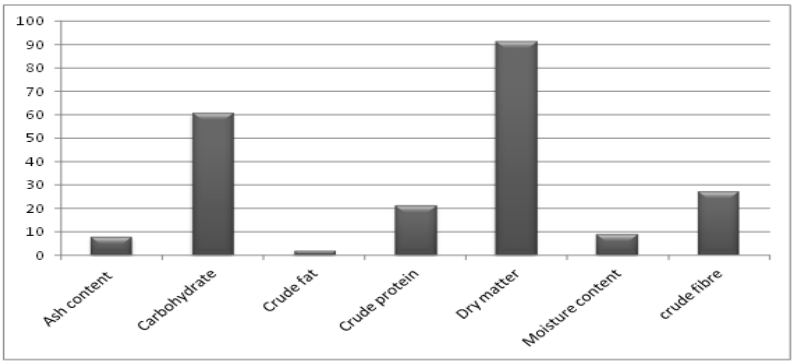

Figure 2: Figure depicting the proximate principles in the crude powder of leaf of *P. marsupium*

Table 2: Inorganic contents in the crude powder of leaf of *P. marsupium*

Plant	Biochemical contents (%)			
	Total Ash	Acid insoluble ash	Calcium	Phosphorus
Pterocarpus marsupium	7.66	0.85	3.62	0.63

Phytochemical analysis

The phytochemical properties of the *P. marsupium* methanolic extract studied were summarised in Table 3. Alkaloids, saponins, phytosterols, phenolic chemicals, flavonoids and terpenoids were found in the methanolic extract of the leaf, however carbohydrates, reducing sugars, gums and mucilages were not found in the extract. [15] reported that *P. marsupium* is used as a medicine for the treatment of diabetes and the stem bark contain phenols, tannin, flavons, flavonoids, alkaloids, terpenoids and cardiac glycosides. Badkhane Y et al [16] reported that pterostilbene, pigalin methyl ester, pigalin, metlaline are isolated from the methanol extract of *P. marsupium* heartwood. Pterostilbene has anti-oxidant, antidiabetic and anticancer activity. Abouelela M et al [17] studied the structure of marsupol. This is the first reported naturally occurring hydrobenzoin. Mohan P et al [18] isolated three isoflavone glycosoides from the heart wood of *P. marsupium*. They are retusin 7-glucoside (1), irisolidone 7-rhamnoside (2) and 5, 7-dihydroxy-6-methoxyisoflavone 7-rhamnoside (3). Phytosterols are helpful to human health, as they can lower plasma cholesterol levels and have anti-inflammatory, anti-diabetic and anticancer properties.

TLC analysis of crude methanolic extract of *P. marsupium*

Polarity of solvents was increased by adding highly polar substance in less amount and studied the separation pattern with different solvent

systems. Effective separations of components were observed in solvent combination, Hexane: Ethyl acetate in 8:2 ratio. That is, an increasing polarity of solvent system resulted better separation of components. Therefore, polarity is further increased by adding small amount of acetic acid. In Hexane: Ethyl acetate: Acetic acid combinations, better separation of components was observed in 7.8:2:0.2 ratio.

Table 3: Qualitative phytochemical analysis of methanolic extract of leaf of *P. marsupium*

No	Phytochemicals	Tests	Inference
1	Alkaloids	Mayer's test	-
		Dandruff's test	-
		Hager's test	++
		Wagner's test	-
2	Carbohydrate	Molisches test	-
3	Reducing sugar	Fehling's test	-
		Benedict's test	-
4	Saponin	Foam test	+
		Froth test	+
5	Phytosterols	Salkowski's test	++
		Liberman Bouchard's test	+
6	Phenolic compounds	Ferric chloride test	+
		Lead acetate test	+
7	Tannins	Ferric chloride	-
8	Flavonoids	Lead acetate test	+
		Alkaline reagent test	+
9	Cardiac glycosides	Keller-kill ani test	+
10	Proteins and amino acids	Millions test	-
		Biuret test	-
		Ninhydrin test	-
11	Terpenoids	Salkowski's test	+
12	Fixed oils and fats	Spot test	-
		Saponification test	-
13	Gums and mucilage's	ruthenium red solution	-

Note : ++ Higher + Lowe - Absent

Table 4: Combinations of solvents used in TLC plates

9:1 H:E	8:2 H:E	7:3 H:E	6:4 H:E	9:1 H:C	8:2 H:C
8.8:2:0.2 H:E:A	7.8:2:0.2 H:E:A	6.8:2:0.2 H:E:A	5.8:2:0.2 H:E:A	7:3 H:C	6:4 H:C

HE-Hexane :Ethyl acetate, HEA- Hexane :Ethyl acetate: Acetic acid, HC-Hexane :Chloroform

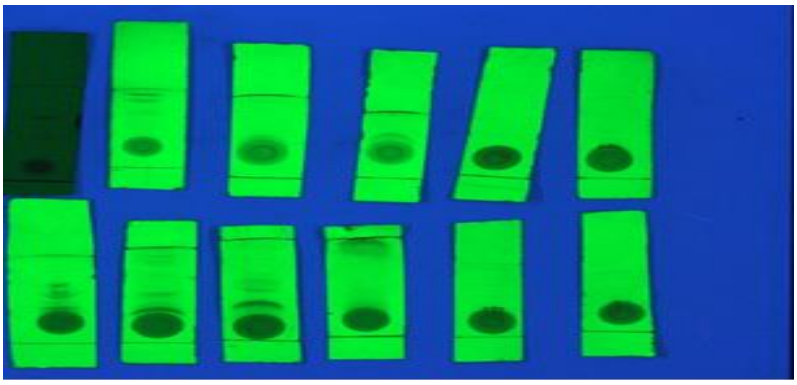

Figure 3: TLC chromatogram of methanolic extract leaf of *P. marsupium*- 250nm

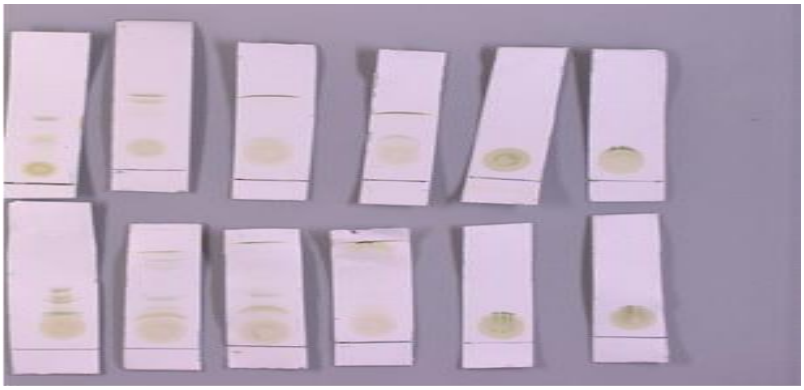

Figure 4: TLC chromatogram of methanolic extract leaf of *P. marsupium* in visible light

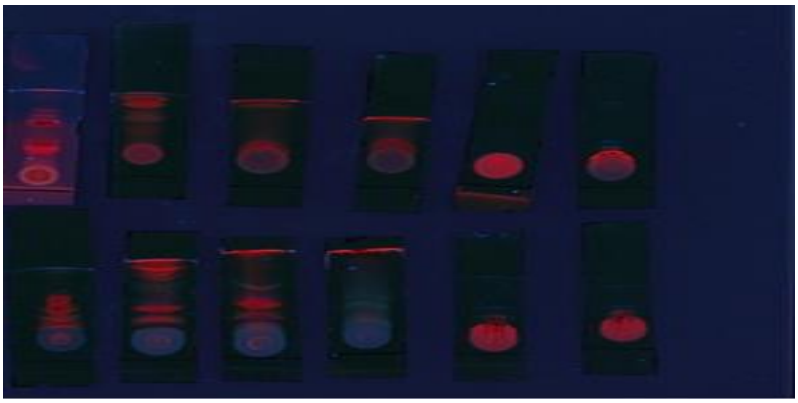

Figure 5: TLC chromatogram of methanolic extract leaf of *P. marsupium*- 366nm

HPTLC analysis of flavonoid fraction isolated from *P. marsupium*

The HPTLC finger print profiling of flavonoid fraction of methanolic extract of leaf of *P. marsupium* are presented in figure 6.7.8 and Table 5. The peak numbered 3 (R_f 0.23) in 254 nm and 3 (R_f 0.23) in 490 nm showed major peak area percent. [15] isolated five new flavonoid compounds from the aqueous extract of *P. marsupium* heartwood. They are 6-hydroxy-2-(4-hydroxybenzyl)-benzofuran-7-C-b-d-glucopyranoside (1), 3-(a-methoxy-4-hydroxybenzylidene)-6-hydroxybenzo-2(3H)-furanone-7-C-b-d-glucopyranoside (2), 2-hydroxy-2-p-hydroxybenzyl-3(2H)-6-hydroxybenzofuranone-7-C-b-d-glucopyranoside (3), 8-(C-b-d-glucopyranosyl)-7, 30, 40-trihydroxyflavone (4) and 1, 2-bis (2, 4-dihydroxy, 3-Cglucopyranosyl)-ethanedione (5). [16] reported that after the oral administration of Ethyl acetate extract of *P. marsupium* heartwood and its flavonoid constituent like marsupin, pterosupin and liquiritigenin the serum lipid levels like serum triglyseride and total cholesterol in rats with hyperlipidemia were reduced. Liquiritigennin and pterosupin cause reduction in serum cholesterol, LDL-cholesterol and atherogenic index. In addition to this pterosupin shows reduction in serum triglyceride.

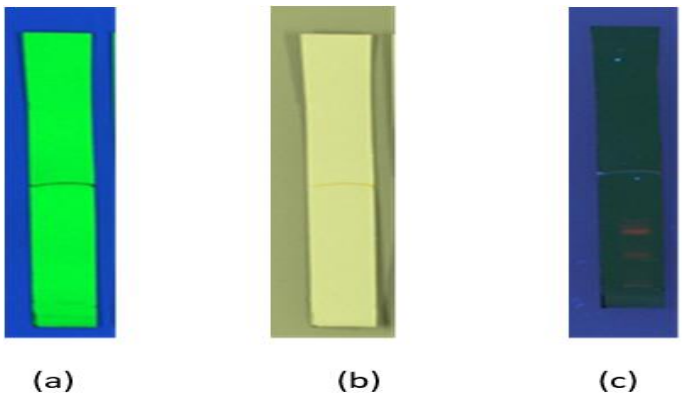

Figure 6: HPTLC chromatogram of flavonoid fraction of methanolic extract of leaf of *P. marsupium* visualized in various lights: (a), 254nm, (b), Visible and (c), (366nm), Solvent system: Hexane: Ethyl acetate (9:1)

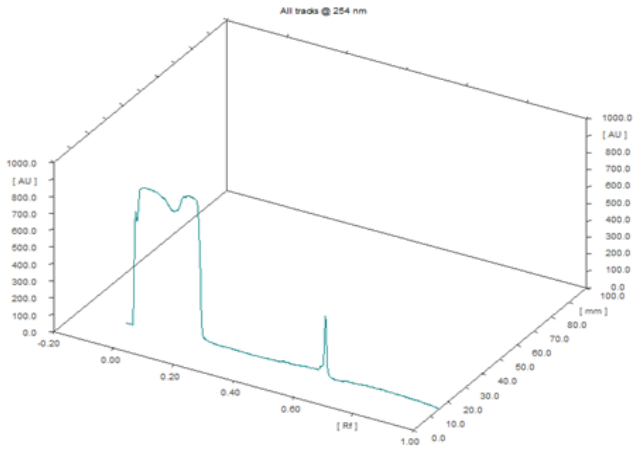

Figure 7: The HPTLC, finger print profiling of flavonoid fraction of methanolic extract of leaf of *P. marsupium* (254nm)

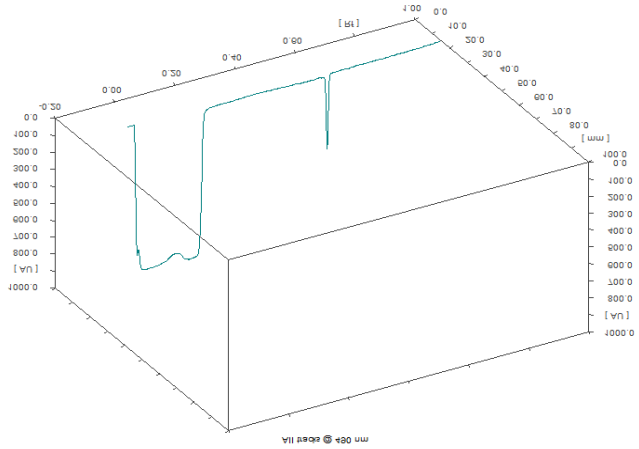

Figure 8: The HPTLC finger print profiling of flavonoid fraction of methanolic extract of leaf of *P. marsupium* (490nm)

Table 5: HPTLC analysis of flavonoid fraction isolated from *P. marsupium*

Sample	Peak No.	R_f Value	Area%
Flavonoid fraction of methanolic extract (254nm)	1	0.03	21.32
	2	0.11	35.28
	3	0.23	40.76
	4	0.64	2.45
	5	0.70	0.19
Flavonoid fraction of methanolic extract (490nm)	1	0.03	21.47
	2	0.11	37.70
	3	0.23	38.53
	4	0.65	2.30

GC-MS analysis of crude methanolic extract of *P. marsupium*

Analysis of methanolic extract of *P. marsupium* by Gas Chromatography and Mass Spectrometer (GC/MS) revealed the presence of nearly a total 100 compounds (Table 6). The GC-MS chromatogram of *P. marsupium* is shown in the figure 5. The predominant compounds were 13-Docosenamide, (Z)- (23.46%),1-Bromo-4-bromomethyldecane (8.37%), d-Ribose, 2-deoxy-bis(thioheptyl)-dithioacetal (6.85%), Diisooctyl phthalate (6.02%),1,3,5-Trisilacyclohexane (5.14%) n-Hexadecanoic acid (2.85%), Eicosanoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester (1.85%), Heneicosane (1.26%), 6-epi-shyobunol (1.99%) (Fig.9). Structures of important compounds present in the methanolic extract of leaf of *Pterocarpus marsupium* is shown in Figure 10 (a,b,c,d). Hugar AL et al [19] reported the presence of 3-O-methyl –d-glucose, n-hexadecanoic acid, 1,2-benzenedicarboxylic acid, tetra decanoic acid etc by GC-MS analysis of ethanolic extract of *P. marsupium* wood and bark.

Table 6: Chemical composition of methanolic extract of *P. marsupium* leaf

Area	Area %	Compounds
75817	0.31	Tetradecane
184033	0.74	Tridecane
46278	0.19	N-(Trifluoroacetyl)-N,O,O',O''-tetrakis(trimethylsilyl)norepinephrine
67528	0.27	Nonadecane
52628	0.21	Heptane, 2,4,6-trimethyl-
56135	0.23	1-Heptanol, 2-propyl-
106500	0.43	Sulfurous acid, pentadactyl 2-propyl ester
73343	0.3	2-Bromo dodecane
47989	0.19	Cyclohexasiloxane, dodecamethyl-
274346	1.11	Tetradecane
276850	1.12	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
73358	0.3	2-Undecanone, 6,10-dimethyl-
133275	0.54	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
122723	0.5	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
34890	0.14	Decani, 1-iodo-
165582	0.67	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
42130	0.17	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione
160834	0.65	Dibutyl phthalate
28775	0.12	Oxalic acid, allyl tetradecyl ester
58886	0.24	1,2-Benzenedicarboxylic acid, dipentyl ester
39944	0.16	Hexadecenoic acid, 15-methyl-, methyl ester
35984	0.15	3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy)tetrasiloxane
110612	0.45	Sulfurous acid, 2-ethylhexyl isohel ester
200193	0.81	Dibutyl phthalate
698257	2.82	n-Hexadecenoic acid
266180	1.08	Dibutyl phthalate
127076	0.51	2-Bromo dodecane
31355	0.13	Citronellol
128455	0.52	Octadecanoic acid, ethyl ester
240153	0.97	Heptadecane
41343	0.17	Pentosane, 13-phenyl-
152160	0.62	1,2-Benzenedicarboxylic acid, decyl octyl ester
67452	0.27	1,1,1,3,5,7,7,7-Octamethyl-3,5-bis(trimethylsilyl) tetra siloxane
36710	0.15	9-Hexadecenoic acid, methyl ester, (Z)-
69099	0.28	2-methyloctacosane
481844	1.95	Phytol
83996	0.34	cis-13,16-Docasadienoic acid
262399	1.06	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-
35766	0.14	Sulfurous acid, hexyl pentadactyl ester
74387	0.3	Z,E-2,13-Octadecadien-1-ol
179018	0.72	Decanoic acid, 1,2,3-propanetriyl ester
57339	0.23	Octadecanoic acid, ethyl ester
171507	0.69	Octacaine
86098	0.35	2-methyltetracosane
140424	0.57	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsilyl) tetra siloxane
36462	0.15	1-Bromo-4-bromomethyldecane

30717	0.12	Malonic acid, 2-chloropropyl tridecyl ester
45057	0.18	Triacontane, 1,30-dibromo-
69547	0.28	Dotriacontylheptfluorobutyrate
56755	0.23	Cyclohexanone, 2-methyl-5-(1-methylethenyl)-, trans-
137274	0.56	Hexadecenoic acid, 1-[(2-aminoethoxy) hydroxyphosphinyl] oxy]methyl]-1,2-ethanediyl ester
53236	0.22	3,6-Dioxa-2,4,5,7-tetrasilaoctane, 2,2,4,4,5,5,7,7-octamethyl-
47780	0.19	Tetrapentacontane, 1,54-dibromo-
86849	0.35	10-Methylnonadecane
115109	0.47	Nonadecane, 9-methyl-
110756	0.45	4,8,12,16-Tetramethylheptadecan-4-olide
197314	0.8	Metasilicate, hexadecamethyl-
28538	0.12	2-Isopropyl-5-methyl-1-heptanol
39443	0.16	2,6,10,14-Hexadecatetraen-1-ol, 3,7,11,15-tetramethyl-, acetate, (E,E,E)-
60748	0.25	11-Dodecenoic acid, 2,4,6-trimethyl-, methyl ester, (2S,4R,6R)-(+)-
30032	0.12	Cyclohexanone, 2-(3-oxobutyl)-
77486	0.31	Nonadecane, 9-methyl-
103915	0.42	2-methylhexacosane
63106	0.26	Tetrapentacontane, 1,54-dibromo-
136329	0.55	Z-2-Octadecen-1-ol
156314	0.63	Hen eicosane, 10-methyl-
198484	0.8	7-Methylxanthine, bis(trimethylsilyl) derivative
53965	0.22	D,L-3-Camphorcarboxylic acid
116782	0.47	Tetrapentacontane, 1,54-dibromo-
491308	1.99	6-epi-shyobunol
312290	1.26	Scleral (sclareolidelactol)
150137	0.61	2-Butyloxycarbonyloxy-1,1,10-trimethyl-6,9-epidioxydecalin
1271735	5.14	,
104503	0.42	1,1'-Bicyclohexyl, 4-propoxy-4'-propyl-
422940	1.71	Metasilicate, hexadecamethyl-
52876	0.21	Butyl phosphonic acid, di(but-1-yn-3-yl) ester
1692702	6.85	d-Ribose, 2-deoxy-bis(thioethyl)-dithioacetal
79306	0.32	1-Bromo-11-iodoundecane
153971	0.62	16-Hentriacontanone
311814	1.26	Hen eicosane
322509	1.3	1-Decanol, 2-hexyl-
50169	0.2	(7S,8S)-cis-syn-trans-Tricyclon[7.3.0.0(2,6)]dodecane-7,8-diol
100888	0.41	3-Methyl-Z,Z-4,6-hexadecadiene
119592	0.48	2-Butanone, 3,3-dimethyl-1-[5-(1-methylethyl)tetrahydrofuran-2-yl]-
1488516	6.02	Diisooctyl phthalate
31814	0.13	Butanol, 1-[2,2,3,3-tetramethyl-1-(3-methyl-1-penynyl)cyclopropyl]-
49631	0.2	1,1'-Bicyclohexyl, 4-methoxy-4'-pentyl-
212461	0.86	Octadecanoic acid, ethyl ester
2070214	8.37	1-Bromo-4-bromomethyldecane
189799	0.77	Cyclononasiloxane, octadecamethyl-
36655	0.15	2,6-Dimethyltridecanenitrile
44732	0.18	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol
456456	1.85	Eicosanoid acid, 2,3-bis[(trimethylsilyl)oxy] propyl ester
29010	0.12	Triacontane, 1-bromo-
219565	0.89	Glutaric acid, isohels 3-phenylprop-2-enyl ester

244274	0.99	Tetra tetracontane
42701	0.17	1,16-Dibromohexadecane
37571	0.15	9-Undecen-2-one, 6,10-dimethyl-
180100	0.73	Cyclononasiloxane, octadecamethyl-
5798353	23.46	13-Docosenamide, (Z)-

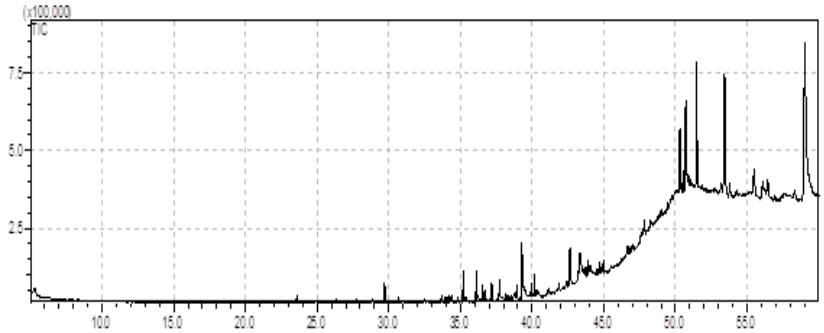

Figure 9: The GC-MS chromatogram of methanolic extract of leaves of *P. marsupium*

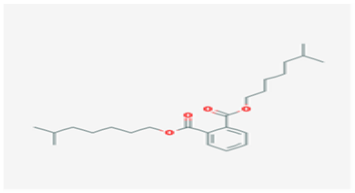

Fig 10(a). Diisooctyl phthalate

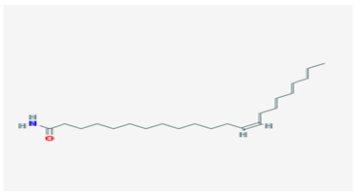

Fig 10(b). Docosenamide

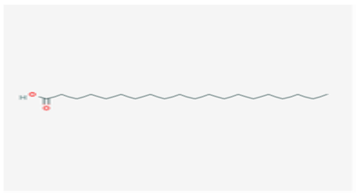

Fig 10 (c). Eicosanoic acid

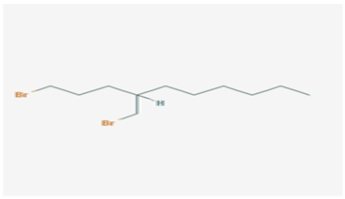

Fig 10 (d). 1-bromo-4-(bromomethyl

Figure 10: (a, b, c, d), Structures of important compounds present in the methanolic extract of leaf of *P. marsupium*

CONCLUSION

Methanolic extract of the stem of *Pterocarpus marsupium was screened* for the presence of active compounds by GC-MS analysis and Chromatographic separations. Standard techniques were used to examine the methanolic leaf extract for various phytochemical constituents, which revealed the presence of alkaloids, saponins, phytosterols, phenolic compounds, flavonoids and terpenoids. TLC analysis of crude methanolic extract of *P. marsupium* was conducted to study the separation pattern with different solvent systems and solvent combination with effective separation of components were analysed. The HPTLC finger print profiling of flavonoid fraction of methanolic extract of leaf of *P. marsupium* were analysed. The GC-MS analysis revealed the presence of compounds which can be pharmacologically active. Further research is being done to determine the vast range of pharmacological activities of the plant. This research could help with the structural elucidation and quantification of bioactive compounds of the plant in the future.

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Conflict of Interest

None declared.

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