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GC-MS Analysis of Phytocomponents in *Pleiospermium alatum* (Wall. ex Wight & Arn.) Swingle, (Rutaceae)

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Abstract
Pleiospermium alatum (Wall. ex Wight & Arn.) Swingle is one of the medicinally important plants belonging to the family Rutaceae, commonly known as Malai Naarthai. The present study deals with the GC-MS analysis of ethanol extract of the above mentioned plant. Thirteen phytochemical constituents from leaf and eleven phytochemical constituents from bark have been identified by comparing the chromatogram, peak value of the unknown compound with entries in NIST database. The prevalent compounds viz., Squalene (13.57%), Lupeol **(**8.81%), 9,12-Octadecadienoic acid (Z,Z)- (8.36%), n-Hexadecanoic acid (6.11%), ethyl ester, Phytol (5.13%) and Hexadecanoic acid (0.93%) are found in leaf. In bark the prevalent phytochemical constituents are 9,12-Octadecadienoic acid (Z,Z)- (18.81%), All-trans-Squalene (17.55%), n-Hexadecanoic acid (12.61%),Oleic acid (5.88%) and 9-Hexadecanoic acid (2.15%) in bark. These results indicate the ethanol extract of leaf and stem bark of *P. alatum* possess potent antioxidant, anti-inflammatory, anticancer, antitumour, antiarthritic, cancer preventive, antibacterial effects so that it can be recommended as a plant of phytopharmaceutical importance.

Keywords: *P. alatum* - GC-MS- Phytochemical compounds - Antioxidant –Anti-inflammatory- Antiarthritic activity

1. Introduction
Approximately 85-90% of the world's population consumes traditional herbal medicines for during the last few decades there has been an increasing interest in the study of medicinal plants and their traditional use in different parts of the world. Medicinal plants contain a wide range of substances that can be used to treat chronic as well as infectious diseases. Reports available on green plants represent a reservoir of effective chemotherapeutants; these are non-phytotoxic, more systemic and early biodegradable [1, 2].
Within a decade, there were a number of dramatic advances in analytical techniques including TLC, UV, NMR and GC-MS that were powerful tools for separation, identification and structure determination of phytochemicals [3]. GC-MS is a technique used for screening/identification/quantification of many susceptible compounds in plant extracts. Gas chromatography (GC) is used to separate drugs that might be present in the sample. The retention time (RT) is an identifying characteristic of a drug. The detector for the GC is the mass spectrometry (MS). The fragmentation pattern for a drug is unique and therefore is an identifying characteristic of a drug. The identification of a drug by its retention time and fragmentation pattern, along with sample specific information afforded to make GC-MS the foremost confirmation method for analyzing herbal extract. In recent years GC-MS studies have been increasingly applied for the analysis of medicinal plants. This technique has proved to be a valuable method for the analysis of non polar components and volatile essential oil, fatty acid, lipids and alkaloids [4].
P. alatum (Wall. ex Wight & Arn.) Swingle belongs to the family Rutaceae. It is widely grown in India and commonly known as malainaarthai. The juice extracted from hundred grams of fresh leaves of *P. alatum* and hundred grams of fresh leaves of lemon grass (*Cymbopogon citratus* Stapf) is boiled in one litre of neem oil in a low flame for twenty minutes. This oil is applied on the joints, shoulders and the other affected parts. Hot water is sprinkled to get relief from rheumatic complaints by the *Kanikkar* tribals of Kalakad - Mundanthurai Tiger Reserve, Western Ghats, Tamil Nadu [5]. However, perusal of literature reveals that GC-MS analysis of *P. alatum* is totally lacking and hence the present investigation was undertaken. The main objective of the present study is to analyse the various phytochemical constituents found in leaf and stem bark of *P. alatum* which may provide an insight in its use in traditional medicine.

2. Materials and Methods

2.1 Collection of Plant material

The fresh plant materials of leaf and bark of *P. alatum* (Wall. ex Wight & Arn.) Swingle were collected from Anaikatti, Coimbatore district, Western Ghats, Tamil Nadu. The plants were identified with the help of local flora and authenticated in Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu, India. The leaf and bark of *P. alatum* were cut into small fragments and shade dried until the fracture is uniform and smooth. The dried plant materials were granulated or powdered by using a blender, and sieved to get uniform particles by using sieve No. 60. The final uniform powder was used for the extraction of active constituents of the plant materials.

2.2 Gas Chromatography –Mass Spectroscopy analysis

GC-MS analysis of the extract was performed using a Perkin – Elmer GC Clarus 500 system and Gas Chromatograph interfaced to a mass spectrometer (GC-MS) equipped with a Elite-1, fused silica capillary column (30 mm x 0.25 mm 1DX 1 µMdf, composed of 100% Dimethyl poly siloxane). For GC-MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1ml/ min and an injection volume of 2 µl was employed (Split ratio of 10:1); injector temperature 250ºC; Ion – source temperature 280ºC. The oven temperature was programmed from 110ºC (isothermal for 2 min.), with an increase of 10ºC/ min, to 200ºC, then 5ºC / min to 280ºC, ending with a 9 min isothermal at 280ºC. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 36 minutes. The relative % amount of each component was calculated by comparing its average peak area, to the total areas, software adopted to handle mass spectra and chromatograms was a Turbomass [6].

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained [7]

3. Results

GC-MS chromatogram of ethanol extract of *P. alatum* leaf and bark along with their retention Time (RT) are shown in the Fig 1 and 2. Major phyto components present in the leaf of *P. alatum* along with molecular formula, molecular weight, and peak area were presented in Table 1. The GC-MS chromatogram of ethanol extract of *P. alatum* leaf showed the presence of several active principle compounds.

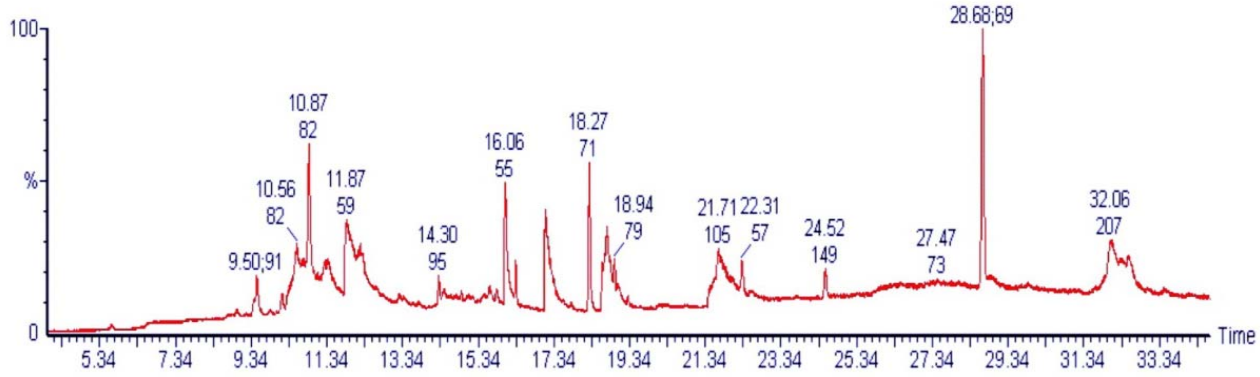

Fig 1: GC-MS Chromatogram of the ethanol extract of *Pleiospermium alatum* leaf

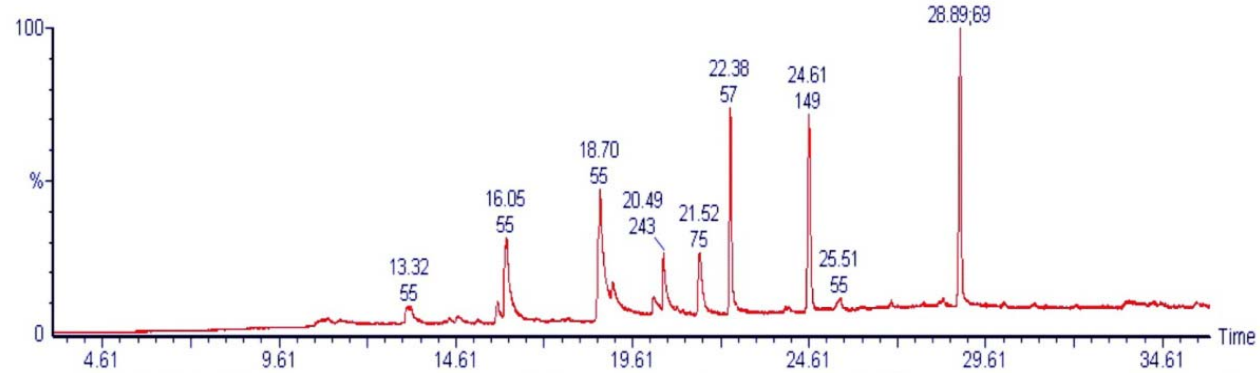

Fig 2: GC-MS Chromatogram of the ethanol extract of *Pleiospermium alatum* bark

Thirteen compounds were identified in ethanolic extracts of P. alatum. The prevailing compounds were E-2-Hexenylbenzoate (13.84%) 2,6,10,14,18,22- Tetracosahexane, 2,6,10,15,19,23-hexamethyl- (all-E) – [All-trans-squalene] (13.57%), 2H,8H-Benzo [1,2-b:5,4-b'] dipyran-2-one, 8,8-dimethyl- (11.12%), Cyclohexanemethanol, 4-ethenyl-qá,á,4-trimethyl-3-(1-methylethenyl)- [1R-91á,3á,4á)–[Elemol] (10.96%), 2-Hydroxymethyl-5- (1-hydroxy-1-isopropyl)-2-Cyclohexen-1-one (10.35%), Lupeol (8.81%), 9,12-Octadecadienoic acid (Z,Z)- (8.36%), Acetyl turicine (8.15%), n-Hexadecanoic acid (6.11%), Phytol (5.13%),2,5-Octadecadienoic acid, methyl ester (1.83%), Hexadecanoic acid ethyl ester (0.93) and á-Cubebene (0.85) (Table 1).

Table 1: Compounds detected in *Pleiospermium alatum* leaf extract

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1.	9.50	2,5-Octadecadienoic acid, methyl ester	C19H30O2	290	1.83
2.	10.14	á-Cubebene	C15H24	204	0.85
3.	10.56	Acetyl turicine	C9H15NO4	201	8.15
4.	10.87	Cyclohexanemethanol, 4-ethenyl-à,à,4-trimethyl-3-(1-methylethenyl)-, [1R-(1à,3à,4á)]-[Elemol]	C15H26O	222	10.96
5.	11.87	2-Hydroxymethyl-5-(1-hydroxy-1-isopropyl)-2-cyclohexen-1-one	C10H16O3	184	10.35
6.	16.06	n-Hexadecanoic acid	C16H32O2	256	6.11
7.	16.33	Hexadecanoic acid, ethyl ester	C18H36O2	284	0.93
8.	17.12	2H,8H-Benzo[1,2-b:5,4-b']dipyran-2-one, 8,8-dimethyl-	C14H12O3	228	11.12
9.	18.27	Phytol	C20H40O	296	5.13
10.	18.94	9,12-Octadecadienoic acid (Z,Z)-	C18H32O2	280	8.36
11.	21.71	E-2-Hexenyl benzoate	C13H16O2	204	13.84
12.	28.68	2,6,10,14,18,22-Tetracosahexane, 2,6,10,15,19,23-hexamethyl-, (all-E)-[All-trans-Squalene]	C30H50	410	13.57
13	32.06	Lupeol	C30H50O	426	8.81

Figure 3 shows the mass spectrum of n-Hexadecanoic acid, á-Cubebene and Elemol detected from the leaf of *P. alatum*. The major phytocomponents and its biological activities obtained through the GC-MS study of the leaf of *P. alatum* are presented in Table 3.

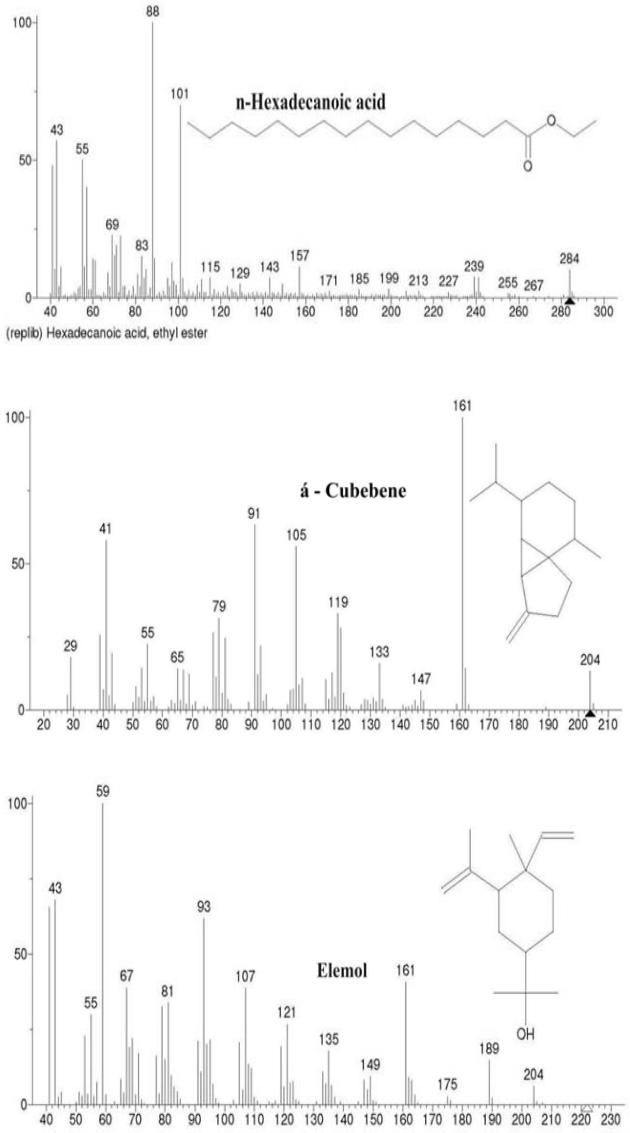

Fig 3: GC-MS spectrum of some compounds present in the ethanol extract of *Pleiospermium alatum* leaf

Table 3: Activity of compounds identified in the GCMS study of *Pleiospermium alatum* leaf extract

No	RT	Name of the compound	Molecular formula	MW	Peak Area %	Compound Nature	**Activity
1.	9.50	2,5-Octadecadienoic acid, methyl ester	C19H30O2	290	1.83	Unsaturated fatty acid ester	No activity reported
2.	10.14	á-Cubebene	C15H24	204	0.85	Sesquiterpene	Anti-tumor, Analgesic, Antibacterial, Anti-inflammatory, Sedative, Fungicide.
3.	10.56	Acetyl turicine	C9H15NO4	201	8.15	Nitrogen compound	No activity reported
4.	10.87	Cyclohexanemethanol, 4-ethenyl-à,à,4-trimethyl-3-(1-methylethenyl)-, [1R-(1à,3à,4á)]-[Elemol]	C15H26O	222	10.96	Sesquiterpene alcohol	Antimicrobial Anti-inflammatory
5.	11.87	2-Hydroxymethyl-5-(1-hydroxy-1-isopropyl)-2-cyclohexen-1-one	C10H16O3	184	10.35	Ketone compound	No activity reported
6.	16.06	n-Hexadecanoic acid	C16H32O2	256	6.11	Palmitic acid	Antioxidant Hypocholesterolemic Nematicide Pesticide Lubricant Antiandrogenic

No	RT	Name of the compound	Molecular formula	MW	Peak Area %	Compound Nature	**Activity
							Flavor Hemolytic 5-Alpha reductase inhibitor
7.	16.33	Hexadecanoic acid, ethyl ester	C18H36O2	284	0.93	Palmitic acid ester	Antioxidant Hypocholesterolemic Nematicide Pesticide Lubricant Antiandrogenic Flavor Hemolytic 5-Alpha reductase inhibitor
8.	17.12	2H,8H-Benzo[1,2-b:5,4-b']dipyran-2-one, 8,8-dimethyl-	C14H12O3	228	11.12	Ketone compound	No activity reported
9.	18.27	Phytol	C20H40O	296	5.13	Diterpene	Antimicrobial Anti-inflammatory Anticancer Diuretic
10.	18.94	9,12-Octadecadienoic acid (Z,Z)-	C18H32O2	280	8.36	Linoleic acid ester	Anti-inflammatory, Hypocholesterolemic Cancer preventive, Hepatoprotective, Nematicide Insectifuge, Antihistaminic Antieczemic, Antiacne, 5-Alpha reductase inhibitor Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge
11.	21.71	E-2-Hexenyl benzoate	C13H16O2	204	13.84	Aromatic compound	No activity reported
12.	28.68	2,6,10,14,18,22-Tetracosahexane, 2,6,10,15,19,23-hexamethyl-, (all-E)-[All-trans-Squalene]	C30H50	410	13.57	Triterpene	Cancer preventive Antimicrobial Sunscreen Chemo preventive Antitumor Immunostimulant Perfumery Pesticide Antioxidant
13	32.06	Lupeol	C30H50O	426	8.81	Triterpene compound	Antimalarial Antiflu Antiviral Antioxidant Anti-inflammatory Antiperoxidant Antitumor Pesticide Antimalarial

****Source: -** Dr. Duke's Phytochemical and Ethnobotanical Databases

Eleven compounds were identified from the ethanolic extract of stem bark of *P. alatum* (Table 4). The results showed the presence of 9, 12-Octadecadienoic acid (Z, Z)- (18.81%), All-trans-Squalene (17.55%), 1, 2-Benzenedicarboxylic acid, diisooctyl ester (13.38%), n-Hexadecanoic acid (12.61%), Diisooctyl adipate (11.80%), Tris(1,3-dichloroisopropyl)phosphate (6.66%), Oleic Acid (5.88%), 3a-(3,4-Methylenedioxy)-hexahydroindole (5.86%), Tetradecanoic acid (3.88%), 9-Hexadecanoic acid (2.15%) and 1-Monolinoleoylglycerol trimethylsilyl ether (1.40%) (Table 4). Mass spectrum of Diisooctyl adipate, Oleic acid and Tetradecanoic acid detected from the stem bark of P. alatum (Fig 4). Table 4 listed the major phytocompounds and its biological activities obtained through the GC-MS study of the stem bark of *P. alatum.*

Table 4: Compounds detected in *Pleiospermium alatum* bark extract

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1.	13.32	Tetradecanoic acid	C14H28O2	228	3.88
2.	15.80	9-Hexadecanoic acid	C16H30O2	254	2.15
3.	16.05	n-Hexadecanoic acid	C16H32O2	256	12.61
4.	18.70	9,12-Octadecadienoic acid (Z,Z)-	C18H32O2	280	18.81
5.	19.05	Oleic Acid	C18H34O2	282	5.88
6.	20.49	3a-(3,4-Methylenedioxy)-hexahydroindole	C15H17NO2	243	5.86
7.	21.52	Tris(1,3-dichloroisopropyl)phosphate	C9H15Cl6O4P	428	6.66
8.	22.38	Diisooctyl adipate	C22H42O4	370	11.80
9.	24.61	1,2-Benzenedicarboxylic acid, diisooctyl ester	C24H38O4	390	13.38
10.	25.51	1-Monolinoleoylglycerol trimethylsilyl ether	C27H54O4Si2	498	1.40
11.	28.89	All-trans-Squalene	C30H50	410	17.55

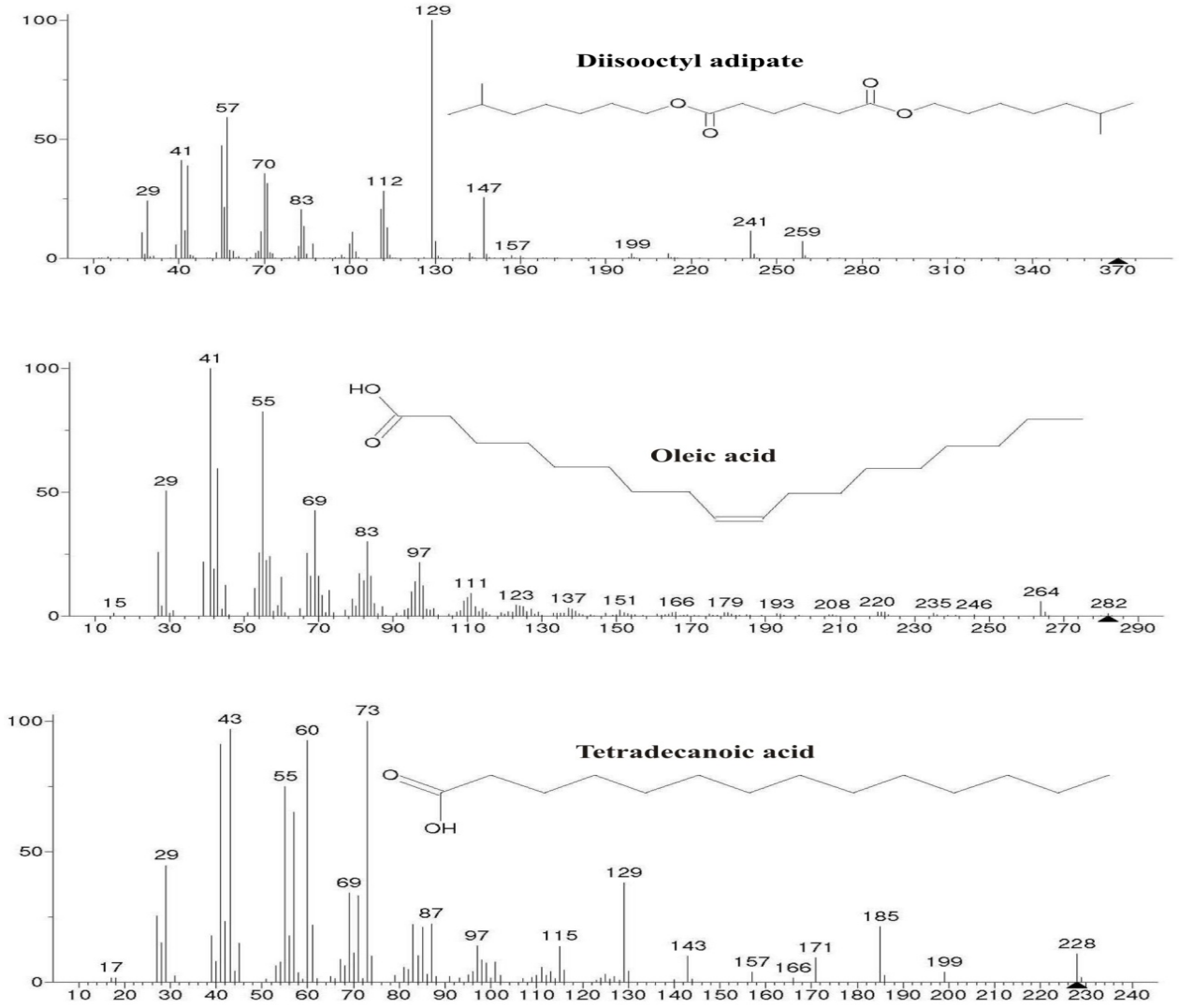

Fig 4: GC-MS spectrum of some compounds present in the ethanol extract of *Pleiospermium alatum* bark

Table 4: Activity of compounds identified in the GCMS study of *Pleiospermium alatum bark* extract

No	RT	Name of the compound	Molecular formula	MW	Peak Area %	Compound Nature	**Activity
1.	13.32	Tetradecanoic acid	C14H28O2	228	3.88		Antioxidant, Cancer preventive Nematicide, Lubricant, Hypocholesterolemic
2.	15.80	9-Hexadecanoic acid	C16H30O2	254	2.15		No report
3	16.05	n-Hexadecanoic acid	C16H32O2	256	12.61	Palmitic acid	Antioxidant Hypocholesterolemic Nematicide Pesticide Lubricant Antiandrogenic Flavor Hemolytic 5-Alpha reductase inhibitor
4	18.70	9,12-Octadecadienoic acid (Z,Z)-	C18H32O2	280	18.81	Linoleic acid ester	Anti-inflammatory, Hypocholesterolemic Cancer preventive, Hepatoprotective, Nematicide Insectifuge, Antihistaminic Antieczemic, Antiacne, 5-Alpha reductase inhibitor Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge
5	19.05	Oleic Acid	C18H34O2	282	5.88	fatty acid	ant antitumor, anti-inflammatory

6	20.49	3a-(3,4-Methylenedioxy)-hexahydroindole	C15H17NO2	243	5.86		
7	21.52	Tris(1,3-dichloroisopropyl)phosphate	C9H15Cl6O4P	428	6.66		
8	22.38	Diisooctyl adipate	C22H42O4	370	11.80		
9	24.61	1,2-Benzenedicarboxylic acid, diisooctyl ester	C24H38O4	390	13.38		Antifouling Antimicrobial
10	25.51	1-Monolinoleoylglycerol trimethylsilyl ether	C27H54O4Si2	498	1.40	Steroid	Antimicrobial Antioxidant Antiinflammatory Antiarthritic Antiasthma, Diuretic
11	28.89	All-trans-Squalene	C30H50	410	17.55	lipids	Antioxidant, Antitumor

4. Discussion

The GC-MS analysis of P. *alatum* leaves revealed the presence of 13 compounds. The identified compounds possess many biological properties. Among the identified phytochemicals, Squalene has the property of antioxidant activity [8, 9] and anticancer activity [10]. Recently it has been found that squalene possess chemopreventive activity against colon carcinogenesis [11, 12]. Squalene has been reported in *Aloe vera* [13], and *Vitex negundo* [14]. Squalene is used in cosmetics as a natural moisturizer [15].

Among the identified phytochemicals, n-Hexadecanoic acid (6.11%), hexadecanoic acid, ethyl ester (0.93%)- Palmitic acid have the property of antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant activities and hemolytic 5-alpha is a reductase inhibitors [16, 17]. n-hexadecanoic acid as the major compound in the leaves of *Cleistanthus collinus* [18]. GC-MS analysis of ethyl acetate extract of *Goniothalamus umbrosus* revealed the presence of n-Hexadecanoic acid [19]. 9,12-Octadecadienoic acid (Z,Z)- is one among the phytocompounds in leaf (8.36%) and stem bark (18.81%) of *P. alatum* was found to have potential cancer preventive, anti-inflammatory and antiarthritic activities. Similar report was made in *Croton tiglium* seed and found to have potential antioxidant and anticancer activity [20] reported that *Euphorbia longan* leaves mainly contained n-hexadecanoic acid and 9, 12- Octadecadienoic acid. These reports are in accordance with the result of this study [21].

Phytol is detected in *P. alatum* leaf which was also found to be effective in different stages of arthritis. Similar results were also observed in the leaves of *Lantana camera* [22], *Mimosa pudica* [23] and aerial parts of *Flueggea leucopyrus* [24]. Phytol was found to give good as well as preventive and therapeutic results against arthritis. The results show that reactive oxygen species promoting substances such as Phytol constitute a promising novel class of pharmaceuticals for the treatment of rheumatoid arthritis and possibly other chronic inflammatory diseases [25].

Lupeol is detected in *P. alatum* leaf part have the properties of antioxidant, anti-inflammatory, antimalarial and antitumour activity [26]. Oleic acid is another compound present in stem bark of *P. alatum* which can act as antioxidant and anti-inflammatory property. Oleic acid may hinder the progression of adrenoleukodystrophy (ALD), a fatal disease that affects the brain and adrenal glands. Oleic acid may be responsible for the hypotensive (blood pressure reducing) effects of olive oil [27]. Several other compounds were also detected through GC/MS chromatogram having notable medicinal property.

The above said compounds found in the ethanol extract of *P. alatum* leaf and stem bark are being used for the pharmacological work. Thus this type of GC-MS analysis is the first step towards understanding the nature of active principles in the medicinal plants and this type of study will be helpful for further detailed study. However, isolation of individual phytochemical constituent and subjecting it to biological activity will definitely give fruitful results. It could be concluded that, *P. alatum* contains various bioactive compounds. So it is recommended as plant of pharmaceutical importance. However, further studies are needed to undertake its bioactivity and toxicity profile.

5. Conclusion

The result of the present investigation reveals that the ethonolic extract of leaves and bark of *P. alatum* possessed significant anti-inflammatory, anticancer, antioxidant, antitumor, immunostimulant and antimicrobial properties. The GC-MS analysis of the ethonolic extract of *P. alatum* reveals the presence of phytoconstituents belonging to the type acids, esters, alcohols, ethers, etc. Thus, the medicinal plant *P. alatum* is found to possess significant phytoconstituents such as Squalene, Lupeol**,** 9,12-Octadecadienoic acid (Z,Z)-, n-Hexadecanoic acid, ethyl ester, Phytol and Hexadecanoic acid are found in leaf. In bark the prevalent phytochemical constituents are 9,12-Octadecadienoic acid (Z,Z)-, All-trans-Squalene, n-Hexadecanoic acid Oleic acid and 9-Hexadecanoic acid in bark. The importance of the study is due to the biological activity of some of these compounds. The present study, which reveals the presence of components in *P. alatum* leaf and stem bark suggest that the contribution of these compounds on the pharmacological activity should be evaluated.

6. Acknowledgement

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