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GC-MS and FT-IR Profiling of leaves methanol extract from the *Pleiospermium alatum* (Wall. ex Wt. & Arn) Swingle Rutaceae family

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ABSTRACT

The present study was aimed to analysis of bioactive constituents of leaves from the *Pleiospermium alatum* (Rutaceae). The methanol extract of the leaves were subjected to Fourier transform infrared spectroscopy (FT-IR) and Gas chromatography- mass spectroscopic (GC-MS) analysis. GC-MS analysis of plant extract was performed using a Perkin-Elmer GC Clarus 500 system and Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) and IR spectrum was recorded in spectrophotometer (Thermo Scientific NICOLET-iS5). FT-IR analysis of peak values with various functional compounds such as amizone, alcohol, phenol, alkanes, protein, enzyme, alkanes, isopropyl. GC-MS analysis of compounds with totally, thirty compounds major chemical compounds were identified, such as 5-Thio-D-glucose, 5-Allylsulfanyl-1-(4-methoxy-phenyl)-1H-tetrazole, E)-10-Heptadecen-8-ynoicacid methyl ester and Z-11-Hexadecenoic acid. The present results concluded that the phytochemicals was observed in methanol extract which revealed that the *P. alatum* is potential use in different fields namely medical and pharmaceuticals and highly valuable in medicinal usage for the treatment of various human aliments.

Keywords: *Pleiospermium alatum*, Phytochemical profile, FT-IR, GC-MS.

INTRODUCTION

Pleiospermium alatum (Wall. ex Wt. & Arn) Swingle (Rutaceae) a medicinal plant commonly called "Kurunthumul thazhai", distributed in India (mainly in the states of Punjab, Bihar, Orissa, Assam, Madhya Pradesh, Bombay, Mysore, and Tamil Nadu), Burma, Thailand, South Western China, Indochina and Ceylon [1]. The leaves and bark are used in the treatment of inflammation and pain management. The folklore claim suggests that the leaf is showing wound healing property [2].

The juice extracted from fresh leaves of *P. alatum* and fresh leaves of lemon grass (*Cymbopogon citratus* Stapf) is boiled in neem oil in a low flame and this oil is applied on the joints, shoulders and the other affected parts for the pain relief. Hot water is sprinkled to get relief from rheumatic complaints by the *Kanikkar* tribals of Kalakad - Mundanthurai Tiger Reserve, Western Ghats, Tamil Nadu[3]. The leaves and bark are used for the fomentation of rheumatic pain; the dried fruit is useful in malignant and pestilent fevers and is used as and for poisons[4]. The stem bark of *P. alatum* along with that of *Azadirachta indica* are boiled in water and the decoction is given orally for post-natal complaints[5].

Leaves and bark of *P. alatum* are used as fomentations in rheumatic pain, Juice as a *Nasya* in *Peenasa* (chronic rhinitis) and it has been described in Telugu verses of Basavarajeeyam are considered to be an elite contribution to the Indian system of medicine [6] .

Plant materials remain as an important resource to combat serious diseases in the world. The traditional medicinal methods, especially the use of medicinal plants are still play a vital role to cover the basic health needs in the developing countries. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds [7].

GC-MS and FT-IR has played an important role in pharmaceutical analysis in recent years [8, 9], recently, spectroscopy has emerged as one of the major tools for biomedical applications and has made significant progress in the field of clinical evaluation. Research has been carried out on a number of natural tissues using spectroscopic techniques, including FT-IR spectroscopy. GC-MS analysis is a breakthrough in analysis of phytoconstituents and structure elucidation of these compounds as they have a sensitivity of detecting compounds as low as 1ng [10]. Higher plants are sources of bioactive compounds continue to play a dominant role in the maintenance of human health. Reports available on the green plants to represent a reservoir of effective chemotherapeutics, which are non-phytotoxic, more systemic and easily biodegradable [11, 12]. Plants are rich source of secondary metabolites with interesting biological activities. In general these secondary metabolites are important source with a variety of structural arrangements and properties [13].

The main objective of the present study is to analyse the various phytochemical constituents using FT-IR and GC-MS found in leaf methanol extract of *Pleiospermium* which may provide an insight in its use in traditional medicine.

MATERIAL AND METHOD

Collection of plant material

The healthy fresh leaves of *Pleiospermium alatum* (Wall ex. Wt. & Arn.) Swingle (Rutaceae) was collected from Silambur (Lat, 11.35 ºN; Long, 79.31ºE), Ariyalur District, Tamil Nadu, India during March to April 2014. Voucher specimens deposited in the Department of Botany, Annamalai University (AUBOT#262). Collected leaves were washed with water, then surface sterilized with 10% sodium hypochlorite solution rinsed with sterile distilled water and shade dried under room temperature. The samples were ground in to a coarse powder.

Preparation of extraction

One hundred grams of powdered materials of leaf samples were extracted in a Soxhlet apparatus for 8 hours with methanol. The extracts were filtered, pooled and the solvents were evaporated with the help of rotary evaporator (Heidolph, Germany) under reduced pressure at 40 ºC and the crude extracts were kept at 4 ºC in refrigerator for further analysis.

GC-MS analysis

Gas chromatography (GC) analysis was carried out using Agilent 6890 N gas chromatography equipped with mass selective detector coupled to front injector type 1079. The chromatograph was fitted with DB 5 MS capillary column (30 m x 0.25 mm i.d., film thickness 0.25 µm). The injector temperature was set at 280 °C and the oven temperature was initially at 45 °C then programmed to 300 °C at the rate of 10 °C/min and finally held at 200 °C for 5 min. Helium was used as a carrier gas with the flow rate of 1.0 mL/min. One microlitre of the sample (diluted with acetone 1:10) was injected in the split mode in the ratio of 1:100. The percentage of sample was calculated by the GC peak area.

GC-mass spectrometry (GC-MS) analysis of sample was performed using Agilent gas chromatography equipped with JEOL GC MATE-II HR Mass Spectrometer. GC conditions were the same as reported for GC analysis and the same column was used. The mass spectrometer was operated in the electron impact mode at 70 eV. Ion source and transfer line temperature was kept at 250 °C. The mass spectra were obtained by centroid scan of the mass range from 40 to 1000 amu. The extract was identified based on the comparison of their retention indices (RI), Retention time (RT), mass spectra of WILEY, NIST library data of the GC-MS system and literature data[14].

Fourier transforms infra-red spectra

IR spectrum was recorded in spectrophotometer (Thermo Scientific NICOLET-iS5). The active principle was mixed with KBr and pellet technique was adopted to record the spectra.

RESULT AND DISCUSSION

Phytochemicals are bioactive chemicals of plant origin. They are regarded as secondary metabolites and they are naturally produced in all parts of the plant body, bark, leaves, stem, root, flower, fruits, seeds etc. i.e. any part of the plant body contain active components [15]. The medicinal value of a plant lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants include alkaloids, tannins, carbohydrates, terpenoids, steroids and flavanoids [7].

The GC-MS study revealed that that presence of same chemical compounds were identified, such as 2-Hydroxymethyl-5-(1-hydroxy-1-isopropyl)-2-cyclohexen-1-one, Benzamide, N-(1,3-dihydro-2- oxo-4-isobenzofuryl, n-Hexadecanoic acid, (Z)6,(Z)9-Pentadecadien-1-ol and 2-Methoxy-4-vinylphenol in the leaves of *P. alatum* Table 1 & Figure 1. The compounds may be acted as highly responsible agent for their antimicrobial and antioxidant activity.

Chatterjee *et al.* [16] have isolated two alkamides from the leaves of *Pleiospermium alatum*. On the basis of spectral and chemical evidence the new alkamide, designated alatamide, has been identified i.e N-(E)-(p-methoxystyryl) benzamide and N-benzoyltyramine methyl ether. Bandara *et al.* [17] studied alkaloids, coumarins, lupeol and stigmasterol. One of the acridones, 1,5,6-trihydroxy-2,3-dimethoxy-10-methyl-9-acridone, was a new compound while *another, 1-hydroxy-2,3,5,6-tetramethoxy-10-methyl-9-acridone* in the leaves of *P. alatum.* Eleven compounds were reported in the ethanolic extract of P. alatum bark, the major compounds include 9, 12-Octadecadienoic acid (Z, Z)-(18.81%), All-trans-Squalene (17.55%) and 1,2-Benzenedicarboxylic acid.

Sethuraman *et al.* [5] have reported thirty-one components comprising 97.3% of the leaf oil, twenty-nine components comprising 96.1% of the stem oil and twenty-three components comprising 95.0% of the root oil were identified. The major components in leaf oil of *P. alatum*. The major components in the leaf oil were elemol (12.5%) followed by pregeijerene (10.5%), α-cadinol (8.5%) and geijerene (8.5%). Elemol (12.6%), α-cadinol (11.3%) and epi-α-muurolol (9.6%). Twenty-four components comprising 95.0% of the oil were identified of which α-Cadinol (27.9%), α-bisabolol (11.5%), α-santalene (11.0%) and elemol (9.8%) were identified as the major components of the root oil.

FT-IR analysis of ethyl acetate extract of leaves of *P. alatum* was carried out the compounds indicated shows that the band at 2956, 2918, 2853, 2849, 2673, 1705, 1464, 1431, 1401, 1307, 1294, 1249, 1227, 1207, 1188, 1098, 937, 720, 687, 541 cm^{-1} (Figure 2). The broad band at 3408 cm-1 amizone to OH stretching in alcohol and phenol group, 2924 cm-1 to 2853 cm-1 attributed to C-H stretching vibration in alkanes group, the peaks around 1662 to 1627 cm-1 are due to the amide I and II region that are characteristic of protein and enzyme, Small bands at 1734 cm^{-1} are represented C=O stretching vibrations of carboxylic acid. 1384 cm^{-1} C–H stretching alkanes group, The weak band at 1038 cm^{-1} can be attributed to the glycoside/C–OH bonds in the polysaccharide/protein structure and 518 to 483 cm^{-1} C-H out of plane bending alkenes group. FT-IR analysis was used to identify the functional group of active components based on peak values in the region of infrared radiation[18]. Ragavendran *et al.*[19] screened the functional groups of carboxylic acids, amines, amides, sulphur derivatives, polysaccharides, organic hydrocarbons, halogens that are responsible for various medicinal properties of *Aerva lanata*. Thangarajan Starlin

et al. [20], while analyzing the ethanolic extracts of *Ichnocarpus frutescens*, by FT-IR, revealed functional group components of amino acids, amides, amines, carboxylic acid, carbonyl compounds, organic hydrocarbons and halogens.

Table 1: GC-MS analysis of methanol extract of leaves of *Pleiospermium alatum*

S. No.	Phytochemical Constituents[a,b]	Retention Time (min)	Peak Area	% Peak area
1.	1H-Pyrrole, 1-methyl	2.58	8867692	3.471.9
2.	2,4-Dihydroxy-2,5-dimethyl- 3(2H)-furan-3-one	5.93	245605	0.0962
3.	Tetrahydrocyclopenta[1,3]dioxin-4-one	6.91	858023	0.3359
4.	4-(4-Methyl-piperazin-1-yl)-1,5,-dihydro-imidazol-2-one	8.94	6048319	2.3681
5.	Propanoic acid, 2-hydroxy-, pentyl ester	9.75	4312243	1.6883
6.	Benzenecarboxylic acid	11.41	13722854	5.3728
7.	Benzofuran, 2,3-dihydro	12.42	10150876	3.9743
8.	Benzene, 1-methoxy-4-propyl-	13.43	454563	0.1780
9.	2-Methoxy-4-vinylphenol	13.86	13817267	5.4098
10.	Phenol, 2,6-dimethoxy-	14.67	752279	0.2945
11.	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)	15.93	12574719	4.9233
12.	à-Caryophyllene	16.84	2283575	0.8941
13.	2-Propenoic acid, 3-phenyl	17.54	12462561	4.8794
14.	Cyclohexene, 4-ethenyl-4- methyl-3-(1-methylethenyl)-1-(1- methylethyl)-, (3R-trans)	18.69	1562939	0.6119
15.	Cyclohexanemethanol, 4- ethenyl-à,à,4-trimethyl-3-(1- methylethenyl)-, [1R-(1à,3à,4á)]	19.70	760359	0.2977
16.	Ethanone, 1-(3,4- dimethoxyphenyl)	20.43	4482929	1.7552
17.	Cyclohexane-1-methanol, 3,3- dimethyl-2-(3-methyl-1,3-butadienyl)	20.59	4412587	1.7276
18.	10,10-Dimethyl-2,6 dimethylenebicyclo[7.2.]undecan-5áol	22.28	4438971	1.6988
19.	2-Hydroxymethyl-5-(1-hydroxy-1-isopropyl)-2-cyclohexen-1-one	23.32	53541632	20.6927
20.	3-Hydroxy-4-methoxybenzoic acid	23.76	3544804	1.3879
21.	3,7,11,15-Tetramethyl-2- hexadecen-1-ol	27.15	5063354	1.9824
22.	2-Propenoic acid, 3-(4-hydroxy- 3-methoxyphenyl)-, methyl ester	28.83	1180415	0.4622
23.	Acetamide, N-(3-methyl-4- phenyl-5 pyrazolyl)-	29.82	1370673	0.5366
24.	n-Hexadecanoic acid	31.18	23028354	9.0161
25.	3,7,11,15-Tetramethyl-2- hexadecen-1-ol	34.17	3836192	1.5020
26.	(Z)6,(Z)9-Pentadecadien-1-ol	35.55	19010318	7.4430
27.	Octadecanoic acid	35.99	1889662	0.7398
28.	5-Ethoxy-2-phenyl-4,5- dihydrooxazol	37.92	1976885	0.7740
29.	Benzamide, N-(1,3-dihydro-2- oxo-4-isobenzofuryl)	40.59	31331836	12.2671
30.	3-Phenyl-1,3-pentanediol	42.65	7530871	2.9485

a Compounds listed in order of elution from DB 35-MS Capillary Standard non-polar column.
b Components identified based on computer matching of the mass peaks with WILEY and NIST Library.

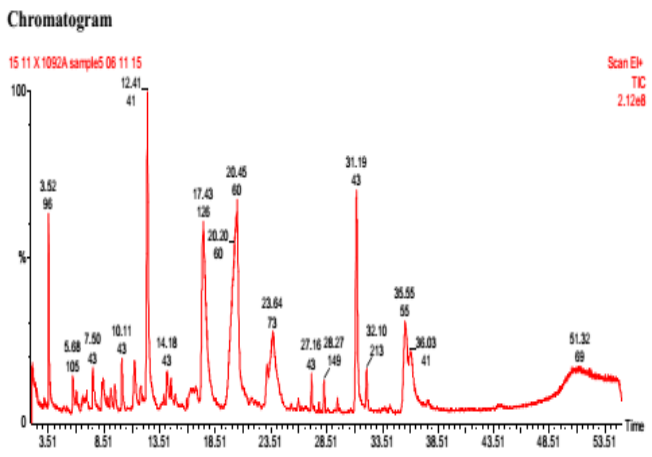

Figure 1: Phytocompounds identified from methanol extract of *Pleiospermium alatum* leaves by GC-MS analysis

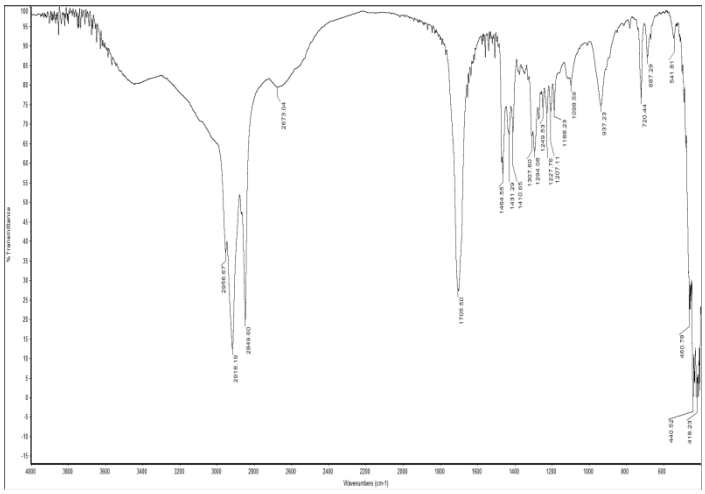

Figure 2: FT-IR spectrum of ethyl acetate extract of leaves of *Pleiospermium alatum*

CONCLUSION

The results of the present work above said compounds found in the methanol extract of *P. alatum* leaf 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. Finally it can be concluded that, *P. alatum* contains various phytocompounds of, 5-Thio-D-glucose, 5-Allylsulfanyl-1-(4-methoxy-phenyl)-1H-tetrazole, E)-10-Heptadecen-8-ynoicacid methyl ester and Z-11-Hexadecenoic acid and its recommended as plant of pharmaceutical importance. However, an isolation and identification and validation of each compound present in the plant species.

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