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Original Article

GC-MS Analysis of Bioactive Components of *Petiveria alliacea* L. Whole Plant (Phytolaccaceae)

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ABSTRACT

The investigation was carried out to determine the phytocompounds of ethanol extract of *Petiveria alliacea* whole plant. GC-MS analysis of ethanol extract was performed using a Perkin-Elmer GC clarus 500 system and Gas Chromatograph interfaced to a Mass Spectometer(GC-MS) equipped with a Elite-1,fused silica capillary column(30mm×0.25mm 10×1µMdf, composed of 100% Di methyl poly siloxene). Interpretation on mass spectrum GC-MS was conducted using the database of National Institute standard and Technology (NIST). Fourteen compounds were identified. The prevailing compounds were Asarone, 2-propenonic acid, 3-(4-methoxy phenyl), Ester, Phytol, a-D-glucopyranose-4-O-a-D-galactopyranosyl, Heptadecane 2, 6, 10, 14 tetramethyl-, Squalene, Z, Z,-2-5-pentadecadien-1-ol (2.28%), Vitamin E (1.14%).

Keywords: *Petiveria alliacea*, Phytol, Asarone, Squalene, Vitamin E, Anti ageing

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1. INTRODUCTION

Throughout human history, natural products have been used as remedies to cure or treat illness. In some of the world, this tradition has been suppressed by the amazing technological and pharmaceutical developments that have emerged with the promise of easier healing. Humans continue to be affected by several diseases, mainly due to natural forces such as

drug- resistant microbes and insects. Consequently, an imperative need exists to connect the ethno pharmacological information with the newest drug-discovery technologies and scientific efforts, in order to discover new active natural metabolites. Human are continuously learning none about and attaching value to natural products and their therapeutic properties [1].

Petiveria alliacea L. (Phytolaccaceae) is claimed to have several medicinal properties. It is used in folk medicine to enhance memory and in the treatment of the common cold, flu, other viral or bacterial infections, inflammation, diabetes and cancer [2-5]. Previous work on *P. alliacea* revealed the presence of triterpenoids, saponins, polyphenols, coumarins, benzaldehyde, benzoic acid, flavonoids, fredelinol, pinitol and allantonin, varying their concentrations in the root, stems and leaves [6,7]. Taking into consideration of the medicinal importance of the plant the ethanol extract of *P. alliacea* was analyzed for the GC-MS. This work will help to identify the compounds of therapeutic value. GC-MS is one of the technique to identify the bioactive constituents of long chain branched chain hydrocarbons, alcohols, acids, esters, etc

2. MATERIALS AND METHODS

2.1 Collection of plant sample

Whole plant of *Petiveria alliacea* was collected from Kalakad, Southern Western Ghats, Tamil Nadu. With the help of flora, voucher specimen were identified and preserved in the Ethnopharmacology Unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin, Tamil Nadu for further references.

2.2 Plant sample extraction

The whole plants were cleaned, shade dried and pulverized to powder in a mechanical grinder. Required quantity of powder was weighed and transferred to Stoppard flask and treated with ethanol until the powder is fully immersed. The flask was shaken every hour for the first 6 hours and then it was kept aside and again shaken after 24 hours. This process was repeated for 3 days and then the extract was filtered. The extract was collected and evaporated to dryness by using a vacuum distillation unit. The final residue obtained was then subjected to GC-MS analysis.

2.3 GC-MS Analysis

GC-MS analysis of these extracts were performed using a Perkin-Elmer GC Clarus 500 system and Gas Chromatograph interfaced to a Mass Spectometer (GC-MS) equipped with a Elite-1,fused silica capillary column(30mm×0.25mm 10×1µMdf, composed of 100% Di methyl poly siloxene). For GC-MS detection an electron ionization system with ionizing energy of 70ev was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate of 1ml/min and an injection volume of 2µ l was employed (split ratio of 10:1); injector temperature 250^0 C; ion-source temperature 280^0 C. The oven temperature programmed from 110^0 c (isothermal for 2 min) with an increase of 10^0 c/min to 200^0 C, then 5^0 C/min to 280^0 C, ending with a 9 min isothermal at 280^0 C, mass spectra were taken at 70ev; a scan interval of 0.5 seconds and fragments from 45 to 450Da, total GC running time was 36mlnutes. 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.

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute of Standard and Technology (NIST) having more than 62,000 paterns. 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 [8, 9].

3. RESULT AND DISCUSSION

Fourteen compounds were identified in *P. alliacea* whole plant by GC-MS analysis. The compounds present in the ethanol extract of *P. alliacea* whole plant identified by GC-MS analysis are shown in figure 1. The active principles with their retention time (RT) molecular formula, molecular weight (MW), concentration (%) and mass spectrum (Structure) in the ethanol extract of *P. alliacea* whole plant are present in table-1. The prevailing compounds were Asarone (26.64%), 2-propenonic acid, 3-(4-methoxy phenyl), Ester (20.95%), Phytol (17.43%), a-D-glucopyranose-4-O-a-D-galactopyranosyl (14.75%), Heptadecane 2, 6, 10, 14- tetramethyl-, (7.29%), Squalene (4.33%), Z, Z,-2-5-pentadecadien-1-ol (2.28%), Vitamin E (1.14%), 5, 8, 11, 14- Eicosatetraenoic acid ,Phenyl methyl ester (all-Z) (1.09%), 1-Napthalenepropanol, a`-ethyldecahydro-5(hydroxymethyl)- a`, 5, 8a- trimethyl-2- methylene, [1S- [l a` (S*), 4aa` 5a` 8aa]] (10.7%). Table 2 listed the various phyto chemicals constituents which contribute to the biological activity of ethanol extract of *P. alliacea* whole plant.

The results pertaining to GC-MS analysis led to the identification of number of compounds from the GC fraction of the ethanol extract of *P. alliacea* whole plant. These compounds were identified through mass spectrometry attached with GC. Among the identified phyto chemicals, Phytol is one among the compounds of the present study. Phytol was observed to have antibacterial activities against *Staphylococcus aureus* by causing damage to cell membranes as a result there is a leakage of potassium ions from bacterial cells [10]. Phytol is a key acyclic diterpene alcohol that is a precursor for Vitamins E and K_1. It is used along with simple or corn syrup as a hardener in candies. Phytol is detected in whole plant of *P. alliacea* which was also found to be effective at different stages of arthritis. It was found to give good as well as preventive and therapeutic results against arthritis [11].

Vitamin E is the main lipid, soluble antioxidant in the body. As antioxidant, Vitamin E acts in cell membrane where prevents the propagation of free radical reaction, although it has been also shows to have proxidant activity [12]. Vitamin E supplements may help reduce PMS symptoms, including anxiety, craving, and depression. Vitamin E keeps the skin young by reducing the appearance of fine lines and wrinkles. Free radicals are believed to play an important role in the ageing of the skin and also have antiageing, antioxident, antitumor property

Squalene possesses chemo-preventive activity against the colon carcinogenesis [13]. In addition, Squalene is the main component of skin surface. Polyunsaturated lipids show some advantages for the skin as an emollient and antioxidant and for hydration and its antitumor activities [14]. With several pharmacological prospects such as antisteoarthritic, anti hypercholestrolemic, antitumor, hypoglycemic, antimutagenic, antiinflammatory and CNS effects [15, 16]. Many spiro compounds possess very promising biological activity of anticancer, antibacterial, anticonvlsant, antituberculosis anti Alzheimer's, pain relief, anti dermatitis and microbial agents [17]. Recently Spiro compounds have also used as antioxidants [18]. Beta-asarone may be a potential candidate for development as a therapeutic agent to manage cognitive impairment associated with conditions such as Alzheimer's disease [19]. The above said components found in the whole plant of *P. alliacea* are being used for the pharmacological work.

4. CONCLUSION

Gas chromatography and mass spectroscopy analysis showed the existence of various compounds with variable chemical structures. The investigation concluded that the stronger extraction capacity of

ethanol could have produced number of active constituents which are responsible for many biological activities. So that it might be utilized for the development of traditional medicines and further investigation is in need to elute novel active compounds from the medicinal plants which may create a new way to treat many incurable diseases. At end point it can be concluded that the *in vivo* studies on biological systems can open up new way for natural drugs that can also be employed for clinical trials which may generate successful results in future.

Table 1: Phytocomponents detected in *P. alliacea* whole plant

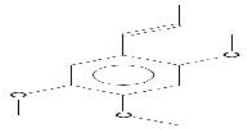
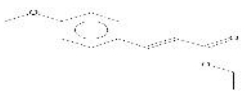
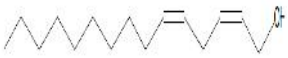
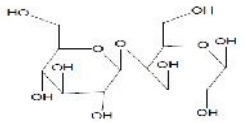
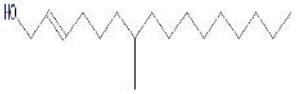
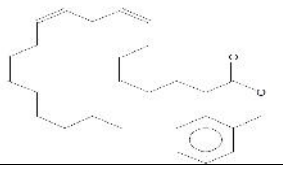
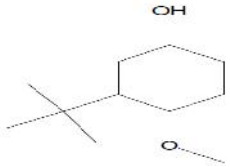
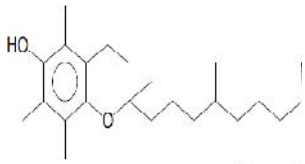
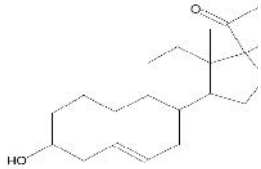
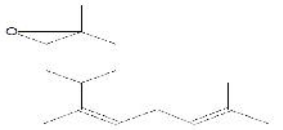
No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %	Structures
1.	7.22	Heptadecane, 2,6,10,14-tetramethyl-	$C_{21}H_{44}$	296	7.29	
2.	8.75	Asarone	$C_{12}H_{16}O_3$	208	26.64	
3.	10.52	2-Propenoic acid, 3-(4-methoxyphenyl)-, ethyl ester	$C_{12}H_{14}O_3$	206	20.95	
4.	10.94	Z,Z-2,5-Pentadecadien-1-ol	C15H28O	224	2.28	
5.	12.21	á-D-Glucopyranose, 4-O-á-D-galactopyranosyl-	C12H22O11	342	14.75	
6.	14.09	Phytol	C20H40O	296	17.43	
7.	14.69	9-Tetradecen-1-ol, acetate, (E)-	C16H30O2	254	0.40	
8.	17.41	5,8,11,14-Eicosatetraenoic acid, phenylmethyl ester, (all-Z)-	C27H38O2	394	1.09	
9.	18.89	3,trsns-(1,1-dimethylethyl)-4,trans-methoxycyclohexanol	C11H22O2	186	0.54	
10.	23.38	Squalene	C30H50	410	4.33	
11.	27.55	Vitamin E	C29H50O2	430	1.14	
12.	30.37	Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3á,17á)-	C22H32O2	328	1.04	
13.	31.88	trans-Z-à-Bisabolene epoxide	C15H24O	220	1.04	
14.	34.48	1-Naphthalenepropanol, à-ethyldecahydro-5-(hydroxymethyl)-à,5,8a-trimethyl-2-methylene-, [1S-[1à(S*),4aá,5à,8aà]]-	C20H36O2	308	1.07	

Table 2: Activity of phytocomponents identified in the ethanol extract of *P. alliacea* whole plant

No.	RT	Name of the compound	Molecular Formula	Compound Nature	**Activity
1.	8.75	Asarone	$C_{12}H_{16}O_3$	Aromatic compound	Tranquilizer, Sedative Pesticide, Fungicide Emetic, Antipyretic Antispasmodic, Cardio depressant Anticonvulsant Myorelaxant Psychoactive
2.	10.52	2-Propenoic acid, 3-(4-methoxyphenyl)-, ethyl ester	$C_{12}H_{14}O_3$	Ester compound	Antibacterial Antioxidant
3	12.21	á-D-Glucopyranose, 4-O-á-D-galactopyranosyl-	$C_{12}H_{22}O_{11}$	Sugar moiety	Preservative
4.	14.09	Phytol	$C_{20}H_{40}O$	Diterpene	Antimicrobial Anticancer Anti-inflammatory Diuretic

5.	**17.41**	**5,8,11,14-Eicosatetraenoic acid, phenylmethyl ester, (all-Z)-**	**$C_{27}H_{38}O_2$**	**Unsaturated fatty acid ester**	**Cardio protective**
6.	**23.38**	**Squalene**	**$C_{30}H_{50}$**	**Triterpene**	**Antibacterial Antioxidant Antitumor Cancer preventive Immunostimulant Chemo preventive Lipoxygenase-inhibitor Pesticide**
7.	**27.55**	**Vitamin E**	**$C_{29}H_{50}O_2$**	**Vitamin compound**	**Antiageing, Analgesic, Antidiabatic Anti-inflammatory, Antioxidant, Antidermatitic, Antileukemic, Antitumor,Anticancer, Hepatoprotective, Hypocholesterolemic, Antiulcerogenic, Vasodilator, Antispasmodic, Antibronchitic, Anticoronary**
8.	**30.37**	**Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3á,17á)-**	**$C_{22}H_{32}O_2$**	**Steroid**	**Antiarthritic Anticancer Hepatoprotective Antimicrobial Antiasthma Diuretic**
9.	**31.88**	**trans-Z-à-Bisabolene epoxide**	**$C_{15}H_{24}O$**	**Steroid**	**Antiarthritic Anticancer Hepatoprotective Antimicrobial Antiasthma Diuretic**

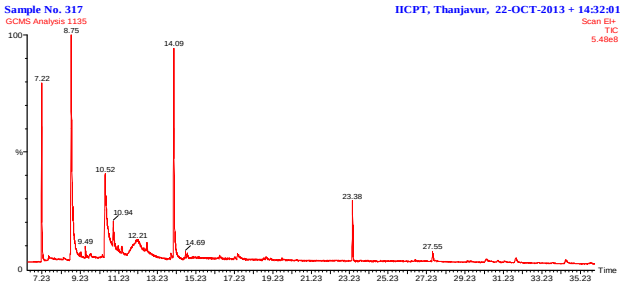


Fig 1: GC-MS chromatogram of the ethanol extract of *P. alliacea* whole plant

5. ACKNOWLEDGEMENT

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