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Original Article

GAS CHROMATOGRAPHY AND MASS SPECTROMETRY ANALYSIS OF BIOACTIVE CONSTITUENTS OF ADIANTUM CAPILLUS-VENERIS L.

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ABSTRACT

Objective: In this present study, to analyze Gas Chromatography and Mass Spectrometry analysis of whole plant methanol extract of *Adiantum capillus-veneris*.

Methods: Gas chromatography and mass spectrometry analysis of whole plant extract carried out with instrument GC-MS-QP 2010 [SHIMADZU].

Results: The methanol extracts of *Adiantum capillus-veneris* to identified thirty seven bioactive compounds which are major compound such as 5-7A-Isoprpenyl-4, 5-Dimethyloctahydro-1h-inden-4yl)-3-methyl-2-penta, (24.49%), n-hexadecanoic acid (18.29%) and gamma-sitosterol (10.61%), cis-vaccenic acid(9.25%), 5-7A-Isopropenyl-4,5-Dimethyl-octahydro-inden-4-yl)-3-methyl-pent-2-EL (2.63%), Tetrdecanoic acid (2.20%) and Phenanthrene, 9-dodecyltetradecahydro (2.15%). The lowest percentage of peak area 0.25% and their compound as 2-methoxy-4-propyl, among these bioactive compounds are including Vitamin E (1.58%) also present.

Conclusion: In this identified compounds are having in antioxidant, antimicrobial, anti-inflammatory, diuretic and analgesic properties also these thirty seven bioactive compounds are biologically significance in many activities.

Keywords: Gas Chromatography-Mass Spectrometry, *Adiantum capillus-veneris*, Bioactive compounds, Vitamin E

INTRODUCTION

Plants are man's friend in survival, giving him food, fuel and medicine from the days beyond dawn of civilization [1]. During the twentieth century, when exploring the natural environment, man has made great discoveries that have enabled him to use a considerable number of natural resources [2]. According to World Health Organization about 80% of the world population depends on the natural product for their health due to minimal side effect and cost effective [3]. In recent period the gas chromatography and mass spectroscopy studies have been increasingly applied for the analysis of most of the or medicinal plant alone medicinal plants as this technique has proved to be a valuable method for the analysis of non polar components and volatile essential oil, fatty acids and lipids [4].

Adiantum capillus-veneris (Family: Adiantaceae) is one of the most common pteridophyte species with potential importance for medicinal and nutritive purpose. Adiantaceae generally occur in the mountainous region of throughout India; in plains they grow on rocks, inhabiting in shady places near swamps and on slopes of lower hills [5]. In traditional herbal medicinal system, *Adiantum capillus-veneris* is used as expectorant, diuretic, febrifuge, as hair tonic, in chest diseases, in catarrhal infection, to treat hard tumours in spleen, antimicrobial and anticancerous [6,7,8,9,10].

As folk medicine, the Pteridophytes which constitute ferns and ferns allies, have been known to man for more than 2000 years, and also been mentioned in ancient literature [11, 12, 13]. India is profusely rich in the history of medicinal plants and its 75% folk population is still using herbal preparations in the form of powder, extracts and decoctions because these are easily available in nature and the natives have stronger faith on traditional knowledge [14]. GC-MS is a powerful technique used for many applications which has very high sensitivity and specificity. Generally its application is oriented towards the specific detection and potential identification of compounds based on the molecular mass in a complex mixture. The combination of a principle separation technique (GC) with the best identification technique (MS) made GC–MS an ideal for qualitative and quantitative analysis for volatile and semi-volatile compounds [15].

MATERIALS AND METHODS

Plant material

The whole plant of *Adiantum capillus-veneris* were collected from field at foothills of Valparai hills Western Ghats of Coimbatore district, of southern India. The samples of plants were identified self and binomially by Botanical Survey of India (Southern part Coimbatore, Tamilnadu, India) and voucher specimens were deposited at the Herbarium Department of Botany, Kongunadu Arts and Science College (Autonomous), Coimbatore, Tamilnadu, India.

Extraction of plant material

Healthy fresh plant of *Adiantum capillus-veneris* was collected from Valparai region of Western Ghats of Coimbatore district. 50g of fine powder was packed with Whatman No.1 filter paper and placed in soxhlet apparatus along with solvent petroleum ether and followed by methanol. The residues were collected and dried at room temperature, 30˚C after which yield was weighed and then performed to activity.

GC-MS analysis

5 ml of methanol extract was evaporated to dryness and reconstituted in to 2 ml methanol. The extracts were then subjected to GC-MS analysis. Chromatographic separation was carried out with instrument GC-MS-QP 2010 [SHIMADZU] instrument with Db 30.0 column (0.25µm diameter × 0.25um thickness). The oven temperature was programmed from 70 °C (isothermal for 5 min), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 35 min isothermal at 280°C. Mass spectra was taken at 70 eV; a scan interval of 0.5 s and Scan range from 40–1000 m/z. Helium was used as carrier gas at 99.999 % pressure with flow 1.0 ml/min and electronic pressure control on. Samples were dissolved in methanol and injected automatically.

Analytical condition

Injection temperature at 240^{0}C, interface temperature at 240^{0}C and ion source temperature at 70^{0}C were determined. Injection was performed in split less mode.

Table 1: Gas chromatography and mass spectrum analysis of the methanol extract of *Adiantum capillus-venerisi* L

S. No.	Chemical constituents	RT	Peak Area %	MW	MF	Compound nature	Biological activities
1	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	7.296	0.67	144	$C_6H_8O_4$	Flavonoids	Antimicrobial, anti-inflammatory
2	Hydroquinone	9.545	1.33	110	$C_6H_6O_2$	Alkaloids	Antioxidant
3	2-methoxy-4-venylphenol	9.712	0.25	150	$C_9H_{10}O_2$	Phenol	Antioxidant, antimicrobial
4	Phenol, 4 propyl	10.734	1.09	136	$C_9H_{12}O$	Phenol	Antioxidant, Antidermatitic, Antileukemic
5	Dodecanoic acid	12.908	0.40	200	$C_{12}H_24O_2$	Carboxylic acids	Un known
6	Tetradecanoic acid	15.184	2.20	228	$C_{14}H_28O_2$	Carboxylic acids	Antioxidant, cancer preventing, nematicide, Hypocholesterolemic
7	2,6,10-trimethyl,14-ethylene-14-pentadecne	15.820	1.26	278	$C_{20}H_{38}$	Alcoholic	Unknown
8	cis-9-Hexadecenoic acid	17.050	1.78	254	$C_{16}H_{30}O_2$	Fatty acid	Flavoring agent
9	n-Hexadecanoic acid	17.349	18.29	256	$C_{16}H_{32}O_2$	Palmitic acid	Antioxidant, Hypocholesterolemic Nematicide, Antiandrogenic, Flavor, Hemolytic
10	Phytol	18.568	1.43	296	$C_{20}H_{40}O$	Triderpene	Antimicrobial, anticancer, anti-inflammatory, diuretic
11	Cis-vaccenic acid	19.015	9.25	282	$C_{18}H_{34}O_2$	Fatty acid	Cosmetics
12	Octadecanic acid	19.164	0.34	284	$C_{18}H_{362}$	Fatty acid	Cosmetics
13	2-hydroxy-3-[(9E)-9 octadecenoyloxy] propyl(9E)-9-octadcenoate	20.067	0.44	620	$C_{39}H_{72}O_5$	Fatty acid	Antioxidant, Antidermatic, Antileukemic
14	15-Hydroxypentadecanic acid	20.233	1.09	258	$C_{15}H_{30}O_3$	Fatty acid	Antidermatic, cosmetic
15	2,5,8,11,-Pentaoxahexadecan-16-ol	20.567	0.51	252	$C_{11}H_{24}O_6$	Ester	Antitumor, antioxidant, Antimicrobial
16	2-hydroxy-3-[(9E)-9-octadecenoyloxy] propyl(9E)-9-octadecenoate	21.708	0.32	620	$C_{39}H_{72}O_5$	Fatty acid	Rheumatoid arthritis, osteoarthritis,
17	1,2-Bensenedicarboxilic acid,mono (2-ethyl)ester	22.162	0.64	278	$C_{16}H_{22}O_4$	Ester	Flavouring agent, perfumes, Ice-cream
18	2-[2-[2[-[2-[2-(2-Methoxyethoxy)ethoxy]ethoxy]ethoxy]ethol	22.749	0.92	296	$C_{13}H_{28}O_7$	Alcoholic	Toxicity
19	9-octadecenamide	24.051	0.64	281	$C_{18}H_{35}NO$	Nitrogen compound	Fertilizer
20	Squalence	24.166	2.13	410	$C_{30}H_{50}$	Triterpene	Antibacterial, Antioxidant, Antitumor, Cancer preventive, Immunostimulant, Chemo preventive, Pesticide
21	Hexaethylene glycol monododecyl ether	24.734	1.60	450	$C_{24}H_{50}O_7$	Ester	Flavouring agent, perfumes
22	Cholesta-3,5-diene	24.893	0.40	368	$C_{27}H_{44}$	Fatty acid	Cosmetics, nutraceutical
23	Olean-12-en-28-al, cyclic 1,2 –ethandiyl mercaptal	26.102	0.84	500	$C_{32}H_{52}S_2$	Triterpene	Antimicrobial, anticancer, anti-inflammatory,
24	3,9B-epoxy-9BH-benz[E]indene, dodecahydro-3,3A,6,6,9A- pentamethyl-	26.156	1.07	262	$C_{18}H_{30}O$	Hydrocarbon	Lubricants, fuel, paraffin wax
25	Vitamin E	26.411	1.58	430	$C_{29}H_{502}$	Vitamin compound	Analgesic, Anti-diabatic Anti-inflammatory, Antioxidant, Antidermatitic, Antileukemic, Antitumor, Anticancer, Hepatoprotective, Antispasmodic
26	2-[2-[2[-[2-[2-[2-(2-Methoxyethoxy)ethoxy]ethoxy]ethoxy]ethoxy] ethoxy]ethoxy]	26.580	0.93	384	$C_{17}H_{369}$	Alcoholic	Toxicity
27	Ergost-5-EN-3-OL, (3, BETA)	27.308	1.44	400	$C_{28}H_{48}O$	Steroids	Liver diseases, Jaundice, atherosclerosis
28	Gamma,-Sistosterol	28.101	10.61	414	$C_{29}H_{50}O$	Steroids	Pain killer and Jaundice
29	2,6,10,15,19,23-hexamethyl	28.357	1.19	332	$C_{15}H_{25}I$	Triterpene	Antibacterial, Antioxidant, Antitumor, Cancer preventive, Immunostimulant,
30	4-(2,6,6-trimethyl-1-cyclohexen-1-YL)-3-penten-2-one	28.101	1.92	206	$C_{14}H_{22}O$	Aldehyde	Melamine, dyes
31	5-(7A-isopropenyl-4,5-dimethyloctahydro-1H-inden-4-yl	28.871	24.49	332	$C_{22}H_{362}$	Alcoholic	Toxicity
32	Lup-20(29)-EN-28-OL	28.931	0.35	426	$C_{30}H_{50}O$	Triterpenoids	Antibacterial, Antioxidant, Antitumor
33	Phenanthrene, 9-dodecyltetradecahydro-	29.349	2.15	360	$C_{26}H_{48}$	Phenol	Antioxidant
34	Stigmast-4-En-3-one	29.446	1.05	412	$C_{29}H_{48}O$	Stearic acid	Hepatoprotective
35	2-Azapentane-1,5-dione, 4-methyl-1,5-diphenyl-3-(p-tolyl)-	29.758	1.74	357	$C_{24}H_{23}NO_2$		Antimicrobial, anticancer, anti-inflammatory,
36	5-(7A-isopropenyl-4,5-dimethyl-octahydro-inden-4-YL)-3methyl-pent-2-EI	30.443	2.63	290	$C_{20}H_{34}O$	Alcoholic	Un known
37	1-Eicosanol	33.201	1.01	298	$C_{20}H_{42}O$	Triterpene	Un known

Identification of compounds (Data analysis)

The mass spectra of compounds in samples were obtained by electron ionization (EI) at 70 eV and the detector operator in scan mode from 40 to 1000 m/z atomic mass units. Identification based on the Molecular weight, Molecular formula, Retention time and peak area %.

Identification of compounds

Identification was based on the active principles with their Retention time (RT), Molecular formula (MF), Molecular weight (MW) and concentration (peak area %). It is done in order to determine whether this plant species contains any individual compound or group of compounds which may substantiate its current commercial and traditional use as herbal medicine, in addition to determine the most appropriate methods of extracting these compounds. These results will consequently be discussed in the light of their putative biological and therapeutic relevance.

RESULTS AND DISCUSSION

Gas Chromatography-Mass Spectroscopy

The present research work deals with the methanolic extracts of whole plant extract of *Adiantum capillus-veneris* to analysis Gas Chromatography-Mass Spectroscopy. The extracts are a complex mixture of many constituents totally thirty seven compounds identified Table-1 and Figure 1. Best of our knowledge and literature survey there is no report of gas chromatography and mass spectrum analysis to identify the chemical compounds from the plant species of *Adiantum capillus-veneris.* GC-MS analysis showed the revealed that existence of the major compound such as 5-7A-Isoprpenyl-4, 5-Dimethyloctahydro-1h-inden-4yl)-3-methyl-2-penta,(24.49%), n-hexadecanoic acid (18.29%) and gamma-sitosterol (10.61%). The bio active compounds like Gamma-sitosterol, Vaccenic acid, Octadecanic acid, Phytol, Squalene, Hexaethylene glycol monododecyl ether, Hydroquinine, 9-Octadecenamide, 15-Hydroxypentadecanic acid, Cis-vaccenic acid, cis-9-Hexadecanic acid and also among these compounds including vitamin E also present. The active principles with their retention time (RT), Molecular formula, Molecular weight, and concentration (peak area %) are present. The GC-MS chromatogram of the seven peaks of the compounds was detected. Chromatogram GC-MS analysis of the methanolic extract of *Adiantum capillus-veneris* showed the presence of 3 major peaks and the components corresponding to the peaks were determined.

Many medicinal plants are rich source of secondary metabolites such as alkaloids, phenol, cardiac glycosides, flavonoids, tannins and terpenoids determined by gas chromatography and mass spectrum [16, 17]. Olagunju et al. [18] revealed that secondary plant metabolites exert a wide range of biological activities on physiological systems. Kumar et al.[19] also reported that the activities of some plant constituents with compound nature of flavonoids, palmitic acid (hexadecanoic acid, ethyl ester and n-hexadecaonoic acid), unsaturated fatty acid and linolenic (docosatetraenoic acid and octadecatrienoic acid) as antimicrobial, anti-inflammatory, antioxidant, hypocholesterolemic, cancer preventive, hepatoprotective, antiarthritic, antihistimic, antieczemic and anticoronary. Identified squalene has also derivatives of hydrocarbon and a triterpene that the property of antioxidant and chemopreventive activity against colon carcinogenesis [20].

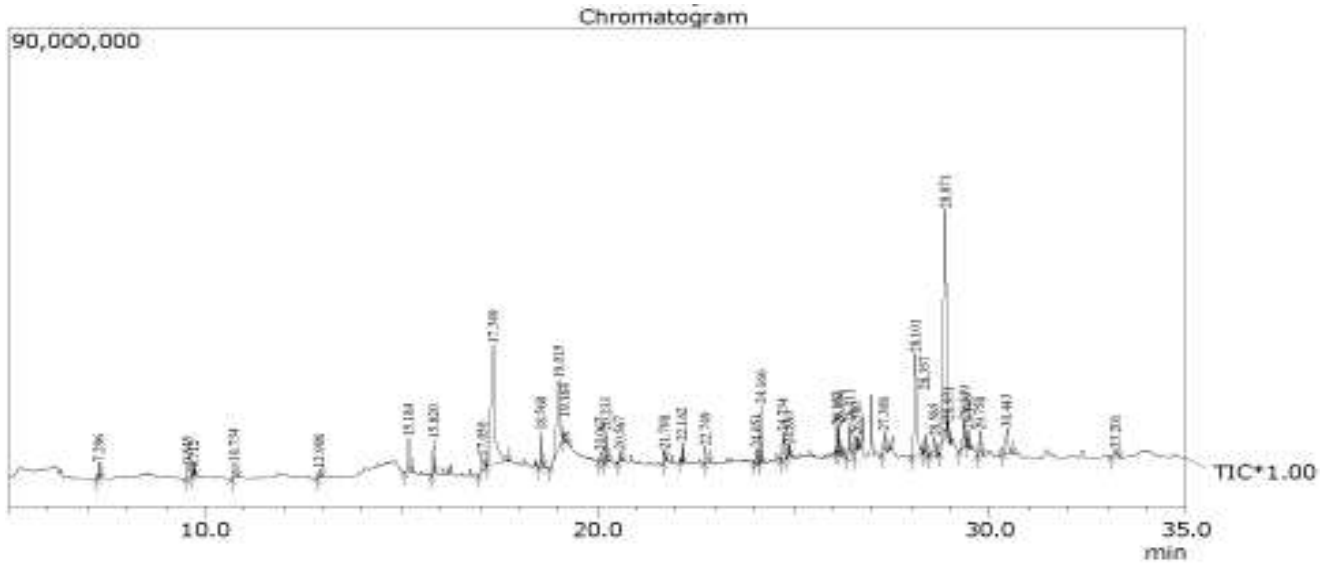

Fig. 1: Showing GC-MS analysis of methanol extract of *A. capillus-veneris*

Squalene also involved in the synthesis of cholesterol, steroid hormones and vitamin D in the human body and it is also able to protect human against cancer [21]. Bharathy et al. [22] analyzed that the Phytol is a diterpene with antimicrobial properties, significantly against many bacterial strains. In recent reports Gas Chromatography-Mass Spectroscopy analysis was carried out on the ethanolic extract of *Canthium parviflorum* and 22 compounds were identified [23]. Among the identified Phytocompounds Dodecanoic acid, Tetrdecanoic acid and n-Hexadecanoic acid have the property of anti oxidant and antimicrobial activities [24]. Sterols are important constituents of all eukaryotes and play vital role in plant cell membranes. Plant sterols possess valuable physiological activities; they are biogenetic precursors of many hormones and oviposition stimulants of some insects [25]. Terpenoids are an important compound of volatiles from plants. Most of them possess different allele chemical functions. *Carthamus lanatus* were identified two sesquiterpenes, α-bisabolol, caryophyllene oxide and α-Bisabolol fucopyranoside are main constituents analysed by gas chromatography and mass spectrum [26]. Balaji et al. [27] reported that the GC-MS analysis of various extracts of *clerodendrum phlomidis* leaf. Grover and Patni [28] also reported that the GC-MS analyses of methanolic extract of woodfordia fruticosa twenty one compounds were identified. Similarly, our present result showed that the GC-MS analyses of methanolic extract of *Adiantum capillus-veneris* thirty seven compounds were recorded. Peak area percentage was more than 10% for gamma,-Sistosterol, n-Hexadecanoic acid, 5-(7A-isopropenyl-4,5-dimethyloctahydro-1H-inden-4-yl and below peak area percentage 10% of Hydroquinone

(1.33%), Phenol 4 propyl (1.09%), Dodecanoic acid (0.40%), Tetrdecanoic acid (2.20%), Phytol (1.43%), Vitamin E (1.58%) were given in Table 1. The present study has been found useful in the identification of several constituents present in the methanolic extract of the plants. The presence of various bioactive compounds such as triterpene, palmitic acid, fatty acid, steroids, carboxylic acid and Vitamin E compounds justifies that the use of the whole plant for various treatments by traditional practitioners.

CONCLUSION

In the present study determined that 37 phytoconstituents were identified from methanol extract of the whole plant of *A. capillus-veneris* by Gas chromatogram and mass spectrometry (GC-MS) analysis. This plant derived bioactive compounds used as source of antibiotic, antioxidant, anti-inflammatory, anticancer properties and pharmaceutical industries used for drug formulation. This plant crude extract showed the phytochemical constituent has great potential for food resource and malnutrition of human health.

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