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ANALYSIS OF BIOACTIVE COMPOUNDS IN METHANOL EXTRACT OF *CISSUS VITIGINEA* LEAF USING GC-MS TECHNIQUE

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ABSTRACT

The aim of this study was to investigate the bioactive compounds from methanolic extract of *Cissus vitiginea* leaves by Gas chromatography and Mass spectroscopy (GC-MS). GC-MS analysis of methanolic extract was done by standard protocol using the equipment Perkin-Elmer Gas Chromatography–Mass Spectrometry, while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. The GC-MS analysis revealed the presence of various compounds like Tetradecanoic acid (19.658), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (20.921), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (21.144), Hexadecanoic acid, methyl ester (21.636), Oleic Acid (21.865), l-(+)-Ascorbic acid 2,6-dihexadecanoate(22.057), 9-Octadecenoic acid (22.712), Andrographolide (22.947), Heptadecanoic acid (23.106), Octadecanoic Acid, methyl ester (23.817), 9,12-Octadecadienoic acid (24.552) and 22-Tricosenoic acid (26.836) in the methanolic extract of *Cissus vitiginea*. These findings support the traditional use of *Cissus vitiginea* in various disorders.

Keyword: Gas chromatography and Mass spectroscopy, *Cissus vitiginea,* Phytochemistry

INTRODUCTION

Plants have basic nutritional importance by their content of protein, carbohydrate, fats and oils minerals, vitamins and water responsible for growth and development in man and animals. Phytochemical simply means plant chemicals. “Phyto” is the Greek word for plant. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. According to OPS[1] a *medicinal plant* [1] is any plant used in order to relieve, prevent or cure a disease or to alter physiological and pathological process[2], or any plant employed as a source of drugs or their precursors. A *phytopharmaceutical preparation* or *herbal medicine* is any manufactured medicine obtained exclusively from plants (aerial and non-aerial parts, juices, resins and oil), either in the crude state or as a pharmaceutical formulation[2]. Current research in drug discovery from medicinal plants involves a multifaceted approach combining botanical, phytochemical, biological, and molecular techniques. In order to understand the biological activity of a plant, be it medicinal, poisonous, or nutritive, it is necessary to know its chemical constituents. Thus, they are plant secondary and primary metabolites (e.g. alkaloids, terpenoids, phenolics, gums, mucilages, carbohydrates, amino acids, proteins, fatty acids, glycolipids, etc.) that organize medicinal plants[3]. Knowledge of plant bioactivity has been accumulated by experimentation over centuries by people living in intimate association with their environment. Therefore, phytochemical research is very useful in drug discovery and development[4].

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 structural determination of phytochemicals. Gas Chromatography Mass Spectroscopy, a hyphenated system which is a very compatible technique and the most commonly used technique for the identification and quantification purpose. The unknown organic compounds in a complex mixture can be determined by interpretation and

also by matching the spectra with reference spectra[5]. The aim of this study is to determine the bioactive compounds present in *Cissus vitiginea* (Family: Vitaceae) leaf extract with the aid of GC-MS Technique, which may provide an insight in its use in tradition medicine.

EXPERIMENTAL

Plant materials

The *Cissus vitiginea* leaves were collected in January 2015 from Tamil University, Thanjavur District, Tamil Nadu, India from a single herb. The leaves were identified and authenticated by Dr. S. John Britto, The Director, the Rabiant Herbarium and centre for molecular systematics, St. Joseph's college Trichy-Tamil Nadu. India. A Voucher specimen has been deposited at the Rabinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil nadu, India.

Preparation of extracts:

The collected *Cissus vitiginea* leaves were washed several times with distilled water to remove the traces of impurities from the leaves. The plant was dried at room temperature and coarsely powdered. The powder was extracted with 70% methanol for 48 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in desiccator until used. The extract contained both polar and non-polar phytocomponents of the plant material used.

GC –MS analysis

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column Elite-1 fused silica capillary column (30 x 0.25mm ID x 1µMdf, composed of 100% Dimethyl polydiloxane), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1 ml /min and an injection volume of 0.5 µI was employed (split ratio of 10:1) injector temperature 250 ºC; ion-source temperature 280 ºC. The oven temperature was programmed from 110 ºC (isothermal for 2 min), with an increase of 10 ºC/min, to 200ºC, then 5ºC/min to 280ºC, ending with a 9min isothermal at 280ºC. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 36min. min. The relative percentage 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 Ver 5.2.0[6].

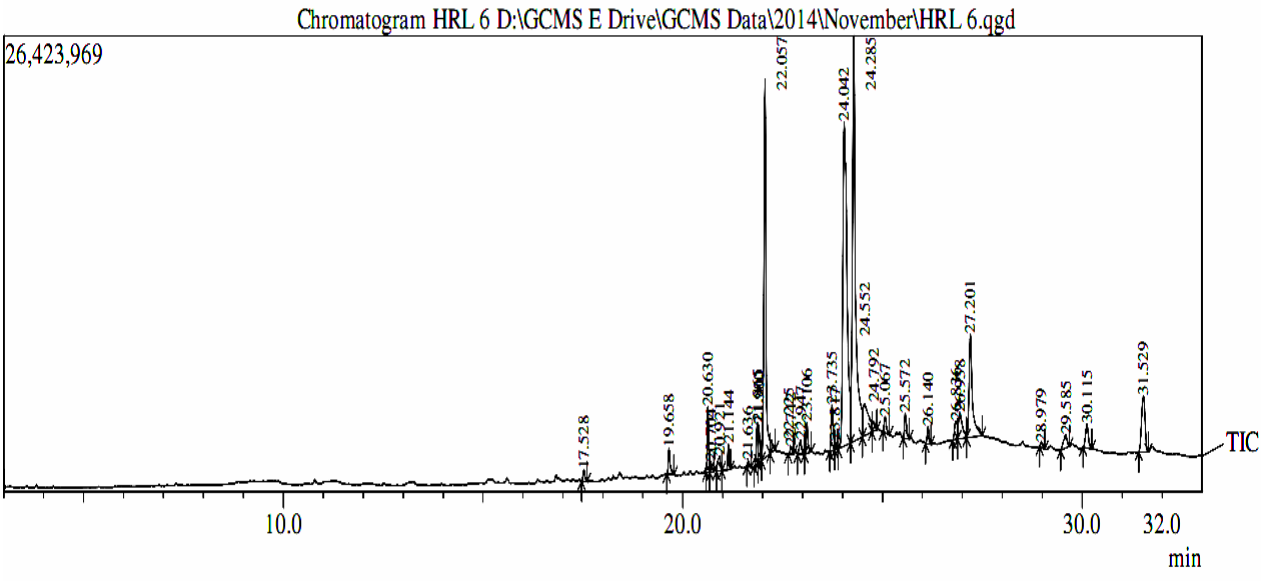

Fig.-1: GC MS analysis of *Cissus vitiginea* leaf extract

RESULTS AND DISCUSSION

Gas chromatography–mass spectrometry (GC-MS) is an analytical method that combines the features of gas-chromatography and mass spectrometry to identify different substances within a test sample. Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation, inorganic, biochemistry and identification of unknown samples. Additionally, it can identify trace in materials that were previously thought to have disintegrated beyond identification. GC-MS has been widely heralded as a "gold standard" for forensic substance identification because it is used to perform a specific test. GC-MS instruments have been used for identification of hundreds of components that are present in natural and biological system[7].

Table-1: GC MS analysis of *Cissus vitiginea* leaf extract

Peak	R.TIME	AREA%	NAME OF COMPOUND	MOLECULAR FORMULA
1	17.528	0.42	Diethyl Phthalate	$C_{12}H_{14}O_4$
2	19.658	0.87	Tetradecanoic acid	$C_{14}H_{28}O_2$
3	20.630	1.74	2,6,10-Trimethyl,14-ethylene-14-pentadecne	$C_{20}H_{38}$
4	20.704	0.33	2-Hexadecene, 3,7,11,15-tetramethyl	$C_{20}H_{40}$
5	20.921	0.74	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}$ O
6	21.144	0.67	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{38}$
7	21.636	0.16	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$
8	21.865	1.39	Oleic Acid	$C_{18}H_{34}O_2$
9	21.900	1.15	Nonanedioic acid, dibutyl ester	$C_{17}H_{32}O_4$
10	22.057	15.02	l-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$
11	22.225	0.50	Dibutyl phthalate	$C_{16}H_{22}O_4$
12	22.712	0.29	9-Octadecenoic acid (Z)-	$C_{18}H_{34}O_2$
13	22.947	0.65	Andrographolide	$C_{20}H_{30}O_5$
14	23.106	1.02	Heptadecanoic acid	$C_{17}H_{34}O_2$
15	23.735	1.77	2-hexadecen-1-OL, 3,7,11,15-tetramethyl	$C_{20}H_{40}O$
16	23.817	0.20	Octadecanoic acid, Methyl ester	$C_{19}H_{38}O_2$
17	24.042	26.18	9-octadecenoic acid	$C_{18}H_{34}O_2$
18	24.285	25.02	Octadecanoic acid $$ Stearic	$C_{18}H_{36}O_2$
19	24.552	3.40	9,12-Octadecadienoic acid	$C_{17}H_{32}O_2$
20	24.792	0.38	1H-Cyclopropa[A]naphthalene, 1A,2,4,5,6,7,7A,7B-OCTAHY	$C_{15}H_{24}$
21	25.067	0.64	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$
22	25.572	0.95	Nonadecanoic acid	$C_{19}H_{38}O_2$
23	26.140	0.63	Hexadecanoic acid, 2-hydroxy-1,3-Propanediyl ESTER	$C_{35}H_{68}O_5$
24	26.836	0.90	22-Tricosenoic acid	$C_{23}H_{44}O_2$
25	26.938	2.06	22-Tricosenoic acid	$C_{23}H_{44}O_2$
26	27.201	6.81	Icosanoic acid	$C_{20}H_{40}O_2$
27	28.979	0.19	Octadecanoic acid, 2,3-dihydroxypropyl ester	$C_{21}H_{42}O_4$
28	29.585	0.90	9-Octadecenoic acid, 1,2,3-propanetriyl ester,	$C_{57}H_{10}O_6$
29	30.115	1.36	Glycidol stearate	$C_{21}H_{40}O_3$
30	31.529	3.65	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$

Identification of components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute 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. The biological activities

listed (Table-2) are based on Dr.Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA[8].

Table-2 : Biological activity of *Cissus vitiginea* leaf extract

S.No	R.TIME	NAME OF COMPOUND	COMPOUND NATURE	BIOLOGICAL ACTIVITY** **Source: Dr.Duke's phytochemical and ethnobotanical databases [Online database].
1	19.658	Tetradecanoic acid	Myristic acid	Antioxidant, Lubricant, Hyper-cholesterolemic, cancer-preventive, cosmetic.
2	20.921	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Terpene alcohol	Antimicrobial.
3	21.144	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	Phytol	Cancer Preventive.
4	21.636	Hexadecanoic acid, methyl ester	Palmittic acid	Antioxidant, Flavor, Anti fibrinolytic, Hypocholesterolemic, Antiandrogenic, Lubricant, Hemolytic, 5-Alpha reductase inhibitor, Nematicide, Antialopecic.
5	21.865	Oleic Acid		5-Alpha reductase, Allergenic, Anemiagenic, Antialopecic, Antiandrogenic, Anti inflammatory, Antileukotriene-D4, Cancer Preventive, Choleretic, Dermatitigenic Flavor, Hypocholesterolemic, Insectifuge Irritant, Percutaneostimulant, Perfumery, propecic.
6	22.057	l-(+)-Ascorbic acid 2,6-dihexadecanoate	Ester	Vitamin C, antioxidant, Immunomodulator.
7	22.712	9-Octadecenoic acid	Oleic acid	Antihypertensive, Increase HDL and decrease LDL Cholesterol.
8	22.947	Andrographolide	Diterpenoid	Cell signalling, Immunomodulator, used in stroke.
9	23.106	Heptadecanoic acid		
10	23.817	Octadecanoic Acid, methyl ester	Stearic acid	No active reported.
11	24.552	9,12-Octadecadienoic acid	Linoleic acid	Anti inflammatory, Nematicide, Insectifuge, Hypocholesterolemic, cancer preventive, Heptaoprotective, Antistaminic, Antiacne, Anti arthritic, Antieczemic,5-Alpha reductase inhibitor, Antiandrogenic, Anticoronary. Anti coronary, Anti cancer,
12	26.836	22-Tricosenoic acid	Saturated Fatty acid	Act as lipid anchor in bio membranes, Anti xiokytic.

GC-MS Analysis

Thirty compounds were identified in *Cissus vitiginea* by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in (Table-1 and Fig.-1). The prevailing compounds were The GC-MS analysis revealed the presence of various compounds like Tetradecanoic acid (19.658), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (20.921), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (21.144), Hexadecanoic acid, methyl ester (21.636), Oleic Acid (21.865), l-(+)-Ascorbic acid 2,6-dihexadecanoate(22.057), 9-Octadecenoic acid (22.712), Andrographolide (22.947), Heptadecanoic acid (23.106), Octadecanoic Acid, methyl ester (23.817), 9,12-Octadecadienoic acid (24.552) and 22-Tricosenoic acid (26.836). This study explores the goodness of the

leaf of the plant *Cissus vitiginea* which has a commendable sense of purpose and can be advised as a plant of phytopharmaceutical importance.

Karpagasundari and Kulothungan[9] screened the bioactive components of *Physalis minima* leaves have been evaluated using GCMS. GC/MS analysis of extract of *Physalis minima* leaves revealed the existence of Heneicosanoic acid (25.22), Bicyclo [4.1.0] Hepta-2, 4-dien (27.41) Octadecanoic acid (CAS), Stearic acid (31.19) and Octadeca-9, 12-dienoic acid (32.02). This study supports our finding compounds.

Prabhadevi *et al* [10] explored the phytochemical constituents present in *Allamanda cathartica (A. cathartica)* L. using GC-MS. The GC-MS analyses determined the presence of 28 different phytochemical compounds in the ethanolic leaf extract of *A. cathartica*. The major phytoconstituents were 9,12,15-octadecatrienoic acid (Z,Z,Z)- (16.39%), n-hexadecanoic acid (14.08%), 3-O-methyl-d-glucose (11.03%) and 9,12,15-octadecatrienoic acid ethyl ester (Z,Z,Z)- (10.58%). The ethanolic stem extract of *A. cathartica* showed the presence of 26 different bioactive compounds. The major ones are 3-O-methyl-d-glucose (29.86%), 2-furancarboxaldehyde 5-(hydroxymethyl)- (14.87%), n-hexadecanoic acid (9.13%) and 9,12,15-octadecatrienoic acid (Z,Z,Z)- (7.34%). Similar types of compounds were agreement with our study.

The investigation concluded that the stronger extraction capacity of methanol could have been produced number of active constituents responsible for many biological activities. So that those might be utilized for the development of traditional medicines and further investigation needs to elute novel active compounds from the medicinal plants which may be created a new way to treat many incurable diseases including cancer.

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