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Research Article **Biochemistry**

DETERMINATION OF BIOACTIVE COMPOUNDS IN *Aplotaxis auriculata* DC. RHIZOME EXTRACT USING GC-MS TECHNIQUE

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ABSTRACT

The aim of this study was to investigate the bioactive compounds from rhizome extract of *Aplotaxis auriculata* rhizome by Gas chromatography and Mass spectroscopy (GC-MS). GC-MS analysis of rhizome 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 Ascorbic acid, Hexadecanoic acid methyl ester, Phytol, Andrographolide, Octadecanoic acid - Stearic acid, Tetradecanoic acid - Myristic acid in the rhizome extract of Aplotaxis auriculata. These findings support the traditional use of Aplotaxis auriculata in various disorders. .

Keywords: Gas chromatography and Mass spectroscopy, *Aplotaxis auriculata* rhizome, Phytochemistry

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INTRODUCTION

Herbal medicines have recently attracted much attention as alternative medicines useful for treating or preventing life style related disorders and relatively very little knowledge is available about their mode of action. There has been a growing interest in the analysis of plant products which has stimulated intense research on their potential health benefits. 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 a medicinal plant is [1] any plant used in order to relieve, prevent or cure a disease or to alter physiological and pathological process, or [2] 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 [3]. 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 systemwhich is a very compatible technique and the most commonly usedtechnique for the identification and quantification purpose. Theunknown organic compounds in a complex mixture can bedetermined by interpretation and also by matching the spectra withreference spectra [5].The aim of this study is to determine the bioactive compounds present in *Aplotaxis auriculata* (Tamil : Kostam) (Family : Asteraceae) rhizome extract with the aid of GC-MS technique, which may provide an insight in its use in tradition medicine.

MATERIAL AND METHODS

Plant materials:

The *Aplotaxis auriculata* rhizomes were collected in January 2015 from Kolli hills, Nammakal District, Tamil Nadu, India from a single herb. The rhizomes 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 *Aplotaxis auriculata* rhizomes were washed several times with distilled water to remove the traces of impurities from the rhizome. 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 IDx 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 TurboMassVer 5.2.0 [6].

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].

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].

GC-MS Analysis

In the present study twenty five chemical constituents have been identified from the extract of Leaf by Gas Chromatogram- Mass spectrometry (GC-MS) analysis. The prevailing compounds wereTetradecanoic acid Hexadecanoic acid, methyl

ester cis-10-Nonadecenoic acid l-(+)-Ascorbic acid, Phytol, 9-Octadecenoic Acid Andrographolide, Octadecanoic acid (Table 1 and Fig 1). The presence of various bioactive compounds justifies the use of plant extract for various ailments by traditional practitioners. The biological activity of rhizome extract represented in the table 2.

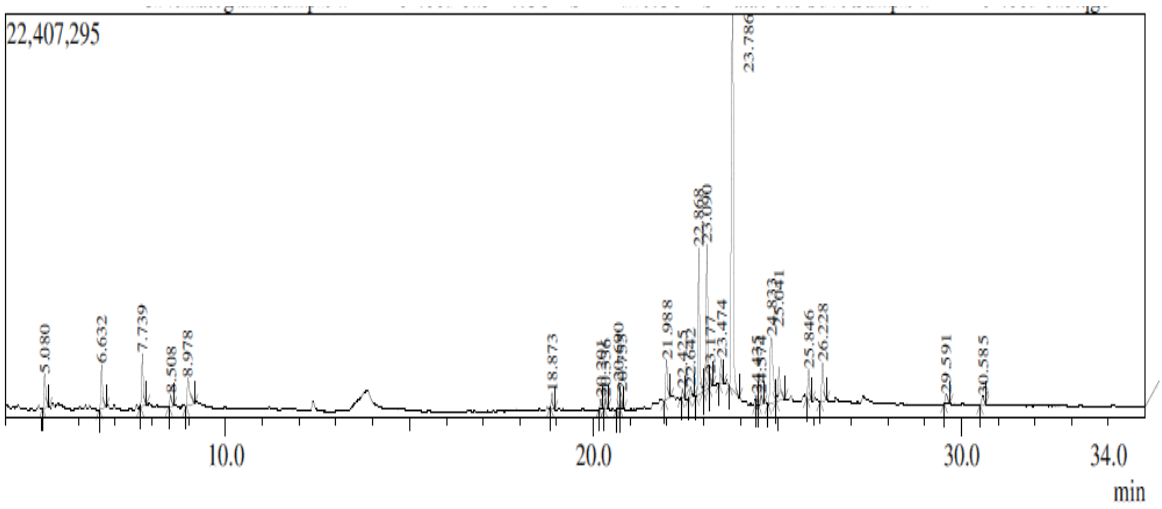

Fig 1: GC-MS analysis of bioactive components from rhizome extract of *Aplotaxisauriculata*

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 ofactive 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 totreat many incurable diseases including cancer.

Table 4 Analysis of bioactive compounds in *Aplotaxis auriculata* rhizomeby GC MS

S. No	R. Time	Area%	Molecular formula	Name of the compounds
1.	5.080	2.64	$C_6H_6O_4S$	Benzenesulfonic Acid, 4-Hydroxy
2.	6.632	3.15	$C_7H_{10}O_2$	Cyclopentane, 1-acetyl-1,2-epoxy-
3.	7.739	3.64	$C_6H_8O_4$	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl
4.	8.508	0.86	$C_6H_7O_6$	**Ascorbic acid**
5.	8.978	3.13	$C_6H_6O_3$	5-Hydroxymethylfurfural

6.	18.873	0.96	$C_{21}H_{34}O_2$	Androstan-17-one, 3-ethyl-3-hydroxy-, (5.alpha
7.	20.201	0.58	$C_{14}H_28O_2$	**Tetradecanoic acid**
8.	20.336	0.61	$C_{15}H_{24}$	Cyclohexane, 1-Ethenyl-1-Methyl-2,4
9.	20.690	1.76	$C_{15}H_{24}$	4,11,11-Trimethyl-8-Methylenebicyclo[7.2.0]Undec-3-Ene
10.	20.755	1.08	$C_{15}H_{24}O$	2-(4a,8-Dimethyl-1,2,3,4,4a,5,6,7-octahydro-na
11.	21.988	2.96	$C_{15}H_{24}$	1H-Cycloprop[E]Azulene, 1A,2,3,5,6,7,7A
12.	22.425	0.56	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester
13.	22.642	0.79	$C_{19}H_{36}O_2$	cis-10-Nonadecenoic acid
14.	22.868	9.57	$C_{38}H_{68}O_8$	**l-(+)-Ascorbic acid 2,6-dihexadecanoate**
15.	23.090	10.51	$C_{22}H_{32}O_2$	Doconexent
16.	23.177	0.84	$C_{15}H_{20}O_2$	Eudesma-5,11(13)-dien-8,12-olide
17.	23.474	1.55	$C_{15}H_{24}$	“KW3 AusEpiglobuloL”
18.	23.786	34.79	$C_{15}H_{18}O_2$	Azuleno[4,5-b]furan-2(3H)-one, 3a,4,6a,7,8,9,9a
19.	24.435	0.42	$C_{19}H_{36}O_2$	9-Octadecenoic Acid, Methyl ester
20.	24.607	0.63	$C_{20}H_{40}O$	**Phytol**
21.	24.833	8.64	$C_{16}H_{30}O$	Cis-9-Hexadecenal $$ 9-Hexadecenal, (Z)-
22.	25.041	3.08	$C_{18}H_{36}O_2$	Octadecanoic acid
23.	25.846	2.17		**Andrographolide**
24.	26.228	3.26	$C_{30}H_{44}$	Dibenzo[a,h]cyclotetradecene, 2,3,11,12-tetraethenyl
25.	29.591	0.94	$C_{21}H_{36}O$	3-Pentadecylphenol $$ 3-N-Pentadecylphenol
26.	30.585	0.89	$C_{24}H_{38}O_4$	Bis(2-ethylhexyl) phthalate $

Table 2 Biological activity of compounds in *Aplotaxis auriculata* rhizome extract

S.NO.	R.Time	Name of the compound	Biological activity **
1	8.508	**Ascorbic acid**	Vitamin c, **Antioxidant, Anticancer**, immunomodulator
2	20.20	**Tetradecanoic acid**	**Antioxidant**, Lubricant, Hypercholesterolemic, **Cancer preventive**, Cosmetic
3	22.42	Hexadecanoic acid, methyl ester	**Antioxidant**, hypocholesterolemic , Anti androgenic , hemolytic, Alpha reductase inhibitor
4	22.64	cis-10-Nonadecenoic acid	**Antitumor**
5	24.43	9-Octadecenoic Acid	ntihypertensive,Increase HDL and decrease LDL Cholesterol.
6	24.60	**Phytol**	Antimicrobial, **Anticancer, Anti-oxidant,** Anti-Diuretic, Immunostimulatory and Anti-Diabetic
7	25.84	Andrographolide	Cell signaling, immunomodulator, used in stroke.
8	25..04	Octadecanoic acid	Lower LDL Cholesterol level

**Source: Dr.Duke's phytochemical and ethnobotanical databases [Online database].

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