International Standard Serial Number (ISSN): 2249-6807

International Journal of Institutional Pharmacy and Life Sciences 6(4): July-August 2016

INTERNATIONAL JOURNAL OF INSTITUTIONAL PHARMACY AND LIFE SCIENCES

Life Sciences **Research Article……!!!**

Received: 21-05-2016; Revised: 29-06-2016; Accepted: 01-07-2016

DETERMINATION OF PHYTOCOMPONENTS IN *CORALLOCARPUS EPIGAEUS* RHIZOME USING GC-MS

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Keywords:

Gas chromatography and Mass spectroscopy, *Corallocarpus epigaeus* rhizome, Phytochemistry

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ABSTRACT

The aim of this study was to carry out for identification of bioactive compounds from the whole plant methanolic extract of *Corallocarpus epigaeus* rhizome by Gas chromatography and Mass spectroscopy (GC-MS). GCMS 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 2-Hexadecen-1-ol, 3,7,11,15-tetram, 9-Octadecenoic acid, Octadecanoic acid, methyl esters, l-(+)-Ascorbic acid 2,6-dihexadecanoate, Oleic Acid, 9,12-Octadecadienoic Acid (Z,Z), Octadecanoic Acid, Methyl Ester, Octadecanoic acid, 1,2-Benzenedicarboxylic acid and Stigmast-5-en-3-ol in the methanolic extract of *Corallocarpus epigaeus* rhizome. These findings support the traditional use of *Corallocarpus epigaeus* rhizome in various disorders.

1. INTRODUCTION

Plants have been an important source of medicine with qualities for thousands of years. Plants are used medicinally in different countries, and they are the source of many potent and powerful drugs. Mainly on traditional remedies such as herbs for their history, they have been used as popular folk medicines [1]. It has been shown that *in vitro* screening methods could provide the needed preliminary observations necessary to elect crude plant extracts with potentially useful properties for further chemical and pharmacological investigations [2]. Phytochemistry or plant chemistry has developed in recent years as a distinct discipline, somewhere in between natural product organic chemistry and plant biochemistry and is closely related to both. It is concerned with the enormous variety of organic substances that are elaborated with and accumulated by plants and deals with the chemical structures of these substances, their biosynthesis, turn over and metabolism, their natural distribution and their biological function [3].

Phytochemicals are the chemicals extracted from plants. These organic chemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary constituents include the common sugars, aminoacids, proteins, purines and pyrimidines of nucleic acids, chlrophyll's etc. Secondary constituents are the remaining plant chemicals such as alkaloids (derived from aminoacids), terpenes (a group of lipids) and phenolics (derived from carbohydrates) [4]. Plant produces these chemicals to protect itself but recent research demonstrates that emphasizes the plant source of most of these protective, disease-preventing compounds. A true nutritional role for phytochemicals is becoming more probable every day as research uncovers more of their remarkable benefits [5]. 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 [6].

The chosen medicinal plant namely as *Corallocarpus epigaeus* rhizome L belongs to the Cucurbitaceae family. *Corallocarpus epigaeus* rhizome Gaertn.f. (Cucurbitaceae) is widely distributed in India, Nepal and Bhutan. In India, the species is distributed from Himachal Pradesh to Assam, Tripura, West Bengal, Bihar and Orissa, Eastern districts of Madhya Pradesh extending further to the Eastern Ghats of Andhra Pradesh [7]. The literature survey revealed that no biological activity and phytochemical works has been done so far with the oleoresin of this plant. The biological activity was screened against the micro organisms causing skin allergies, diarrhea and dysentery. A recent study with methanol extract of mature rhizomes reported anti-

inflammatory and antinociceptive activity [8 -13]. The aim of this study is to determine the organic compounds present in the *Corallocarpus epigaeus* rhizome extract with the aid of GC-MS Technique, which may provide an insight in its use in tradition medicine.

2. MATERIAL AND METHODS

2.1 Plant materials:

The fully mature *Corallocarpus epigaeus* rhizomes were collected in April 2013 from Kolli hills, Namakkal District, Tamil Nadu, India from a single herb. The rhizomes were identified and authenticated by Dr.S.John Britto, The Director, the Rapiant 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.

2.2 Preparation of extract:

The collected *Corallocarpus epigaeus* rhizomes were washed several times with distilled water to remove the traces of impurities from the rhizomes. The rhizomes were dried at room temperature and coarsely powdered. The powder was extracted with 70% ethanol 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.

2.3 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.

3. RESULTS AND DISCUSSION

Plants have been an important source of medicine with qualities for thousands of years. Phytochemicals are the chemicals extracted from plants. These organic chemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. GC-MS method used for the analysis of the obtained extract can be an interesting tool for testing the amount of some active principles in herbs used in various industries. Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen substituted derivatives. Most are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total. These substances serve as plant defense mechanisms against, insects and herbivores. Flavonoids exhibit several biological effects such as anti-inflammatory, anti-fungal, anti-hepatotoxic and anti-ulcer actions [14].

3.1 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.

3.2 GC-MS ANALYSIS

Twenty compounds were identified in *Corallocarpus epigaeus* 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 2-Hexadecen-1-ol, 3,7,11,15-tetram, 9-Octadecenoic acid, Octadecanoic acid, methyl esters, l-(+)-Ascorbic acid 2,6-dihexadecanoate, Oleic Acid, 9,12-Octadecadienoic Acid (Z,Z), Octadecanoic Acid, Methyl Ester, Octadecanoic acid, 1,2-Benzenedicarboxylic acid and Stigmast-5-en-3-ol.

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.

ACKNOWLEDGEMENT

The authors are grateful to Dr. S. Velavan, Director, Harman Institute of Science Education and Research (www.harmanresearchcentre.com), Thanjavur, Tamil Nadu for his support and facility.

Figure 1: Chromatogram obtained from the GC/MS with the extract of *Corallocarpus epigaeus* rhizome

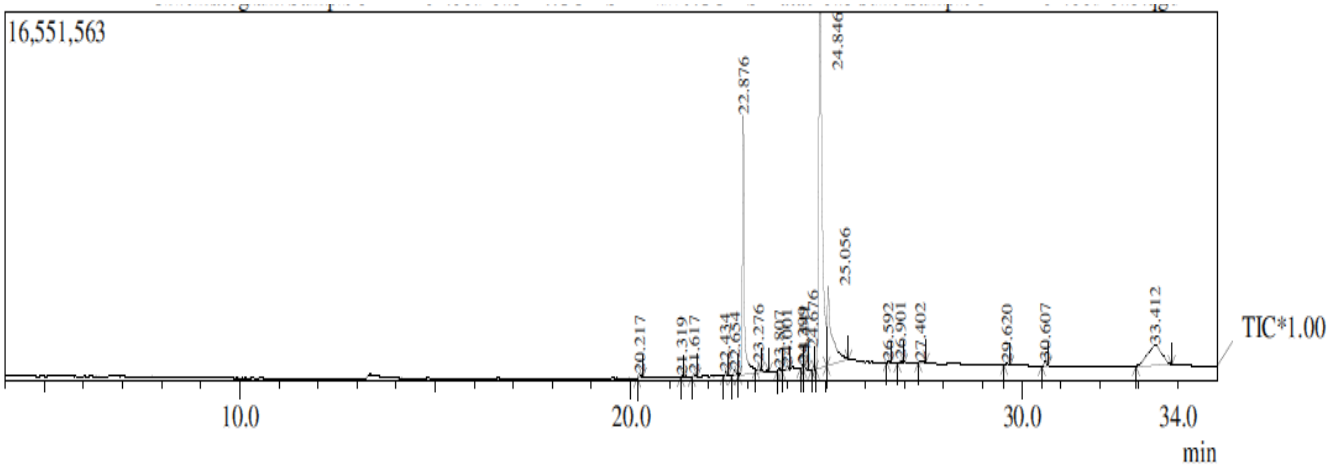

Table 1 Shows the components identified in methanolic extract of *Corallocarpus epigaeus* rhizome (GC MS study)

Peak	R.Time	Area %	Height %	Molecular Formula	Name of the compound
1	20.217	0.35	0.63	$C_{13}H_{26}O_2$	Tridecanoic acid
2	21.319	0.10	0.26	$C_{20}H_{40}O$	2-Hexadecen-1-ol, 3,7,11,15-Tetramethyl
3	21.617	0.26	0.45	$C_{18}H_{34}O_2$	9-Octadecenoic acid
4	22.434	0.20	0.49	$C_{19}H_{38}O_2$	Octadecanoic acid, methyl ester
5	22.654	0.54	0.78	$C_{18}H_{34}O_2$	Octadec-9-enoic acid
6	22.876	20.65	33.50	$C_{38}H_{68}O_8$	l-(+)-Ascorbic acid 2,6-dihexadecanoate
7	23.276	0.36	0.92	$C_{16}H_{26}$	Benzene, p-di-tert-pentyl
8	23.807	0.14	0.21	$C_{18}H_{34}O_2$	Oleic Acid
9	24.001	0.26	0.43	$C_{17}H_{34}O_2$	Heptadecanoic acid
10	24.399	0.25	0.63	$C_{19}H_{34}O_2$	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
11	24.441	0.31	0.74	$C_{19}H_{36}O_2$	9-octadecenoic acid, methyl ester
12	26.676	0.15	0.40	$C_{19}H_{38}O_2$	Octadecanoic acid, methyl ester
13	24.846	49.77	46.04	$C_{22}H_{40}O_2$	cis-13,16-Docasadienoic acid
14	25.056	14.03	10.20	$C_{18}H_{36}O_2$	Octadecanoic acid
15	26.592	0.13	0.21	$C_{16}H_{32}O_2$	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester
16	26.901	0.44	0.42	$C_{18}H_{37}NO_2$	Palmidrol
17	27.402	0.25	0.25	$C_{18}H_{34}O_2$	9-Octadecenoic acid (Z)
18	29.620	0.22	0.27	$C_{21}H_{36}O$	Phenol, 3-pentadecyl- $$
19	30.607	0.42	0.57	$C_{24}H_{38}O_4$	1,2-Benzenedicarboxylic acid
20	33.412	11.18	2.60	$C_{29}H_{50}O$	Stigmast-5-en-3-ol, (3.BETA.)
		100.00	100.00		

Table 2: Activity of phyto-components identified in the methanolic extracts of the *Corallocarpus epigaeus* rhizome by GC-MS.

S.NO.	R.Time	Name of the compound	Biological activity **
	20.217	Tridecanoic acid	No activity reported
1.	21.319	2-Hexadecen-1-ol, 3,7,11,15-tetram	**Cancer-Preventive,** Antimicrobial anti-inflammatory, anti-diuretic Antioxidants
2.	21.61	9-Octadecenoic acid	Antihypertensive, Increase HDL and decrease, LDL Cholesterol.
3.	22.434	Octadecanoic acid, methyl esters	**Anti-tumour**
4.	22.876	l-(+)-Ascorbic acid 2,6-dihexadecanoate	Vitamin c, antioxidant, immunomodulators
5.	23.80	Oleic Acid	5-Alpha-Reductase-Inhibitor, Allergenic, Alpha-Reductase-Inhibitor, Anemiagenic, Antialopecic, Antiandrogenic, Antiinflammatory, Antileukotriene-D4 (Anti-platelet activating factor), **Cancer-Preventive**, Choleretic, Dermatitigenic Flavor, Hypocholesterolemic, Insectifuge Irritant, Percutaneostimulant, Perfumery, Propecic
6.	24.399	9,12-Octadecadienoic Acid (Z,Z)-, Me	Anticoronary, Antialopecic,s
7.	26.676	Octadecanoic Acid, Methyl Ester	**Anti-tumour**
8.	25.056	Octadecanoic acid	Cosmetic, Flavor, Hypocholesterolemic, Lubricant, Perfumery, Prophetic, Suppository
9.	30.607	1,2-Benzenedicarboxylic acid,	Antimicrobial, Antifouling
10.	33.41	Stigmast-5-en-3-ol	Antihepatotoxic, Antiviral, Antioxidant, **Cancer preventive**, Hypocholesterolemic

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

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