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Short Communication

PHYTOCHEMICAL CHARACTERIZATION OF *CARALLUMA INDICA* STEM EXTRACT USING GC MS TECHNIQUE

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ABSTRACT

The senative property of medicinal plants is due to the presence of certain bioactive compounds. The aim of the study was to carry out identification of bioactive compounds from the ethanolic extract of stem of *Caralluma indica* by GC-MS. The compounds were identified by comparing the peak area and their retention time with that of literature and by interpretation of Mass Spectra of GC-MS using database of National Institute Standard and Technology (NIST) library. Nearly 20 phyto-constituents were identified. The superior compounds were hexadecanoic acid, hexadecanoic acid ethyl ester, isobutyl pthylate, 9-octadecanoic acid. Many of these compounds are already in use like antioxidant, hypocholesterolemic, pesticide etc. From this we can subject further research to evaluate therapeutic potential of stem of *Caralluma indica*.

Keywords: GC-MS, Phytochemicals, *Caralluma indica*

1. INTRODUCTION

Main stream medicine focuses much on the traditional practices for the discovery of novel drugs and their efficacy. If a plant is supposed to have a certain therapeutic property, even if the plant has not been studied extensively and if sufficient evidence exists for its use in traditional medicine, the bioactive component in the plant can be characterized and analyzed for therapeutic value. According to the world health organization (WHO) in 2008, more than 80% of world's population relies on traditional medicine for the primary health needs [1]. A knowledge of the chemical constituents of plants are desirable not only for the discovery of therapeutic agents but also because, such information may be of great value in disclosing new resources of economic phytocompounds for the synthesis of complex chemical substances and for discovering the actual significance of folkloric remedies [2]. In recent years GC-MS studies have been applied for the analysis of medicinal plants as the technique has been proved to be a valuable method for the analysis of non polar compounds [3] and alkaloids [4].

Gas chromatography-Mass spectrometry (GC-MS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample [5]. GC can separate volatile and semi volatile compounds with great resolution, but it cannot identify them, MS can provide detailed structural information on most compounds such that they can be exactly identified. But it cannot readily separate them. GCMS can quantitatively estimate the materials present in a sample even at very low concentration. The present study was done to characterize the phytocomponents of the ethanolic extract of the stem of *Caralluma indica* by GC-MS analysis. This work will be beneficial for the therapeutic medicinal value by the identification of compounds in the extract.

2. MATERIAL AND METHODS

2.1. Plant Materials

Plant materials were collected from Kathattipatti (Palaiyapatti North), Sengipatti Village at Thanjavur District in the month of Nov-2017. They were taken as whole plant. The whole plant was identified and authenticated by Dr. S. John Britto, Director, Rabiant Herbarium and Centre for molecular Systematic, St. Joshphe's College, Trichy, Tamil Nadu, India. A voucher specimen (RSV01) has been deposited at the Rapinat Herbarium St. Joshph's college, Trichy, Tamil Nadu, India.

2.2. Preparation of the plant extract

The stem of *Caralluma indica* was first washed several times with distilled water and traces of impurities were

removed from stem. Then old, infected and fungus damaged portion of the stems were removed. Healthy stems was spread out in a plain paper and dried in shade at room temperature for about 10 days. The collected stem was cut into small pieces and fine powder was made using grinder then the powder was extracted with ethanol. After 24 hours, the supernatant was transferred into china dish over water bath at 45°C. The extract was then concentrated until the solvent was completely removed a semi solid extract was obtained after complete elimination of solvent, the obtained residue was used for GC-MS analysis.

2.3. GC-MS Analysis

GC-MS analysis was carried out on Shimadzu 2010 plus comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column RTX 5Ms (Column diameter is 0.32mm, column length is 30m, column thickness 0.50µm), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1.73 ml /min and an injection volume of 0.5 µI was employed (split ratio of 10:1) injector temperature 270°C; ion-source temperature 200°C. The oven temperature was programmed from 40°C (isothermal for 2 min), with an increase of 8°C/min, to 150°C, then 8°C/min to 250°C, ending with a 20min isothermal at 280°C. 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 51.25min. 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 Turbo Mass Ver 5.2.0 [6].

2.4. Identification of components

Interpretation on GCMS 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 [7].

3. RESULTS AND DISCUSSION

3.1. Identification of compound

In the present study 20 chemical constituents have been identified from the ethanolic extract of the stem of *Caralluma indica* by gas chromatogram-mass spectrophotometry (GC-MS) analysis. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The active principles with their retention time (RT), name of the compound, molecular formulae, molecular weight (MW) and concentration are presented in (Table 1). The prevailing compounds were hexadecanoic acid, decanoic acid ethyl ester, dodecanoic acid, 1,2-benzene dicarboxylic acid bis methyl propyl ester.

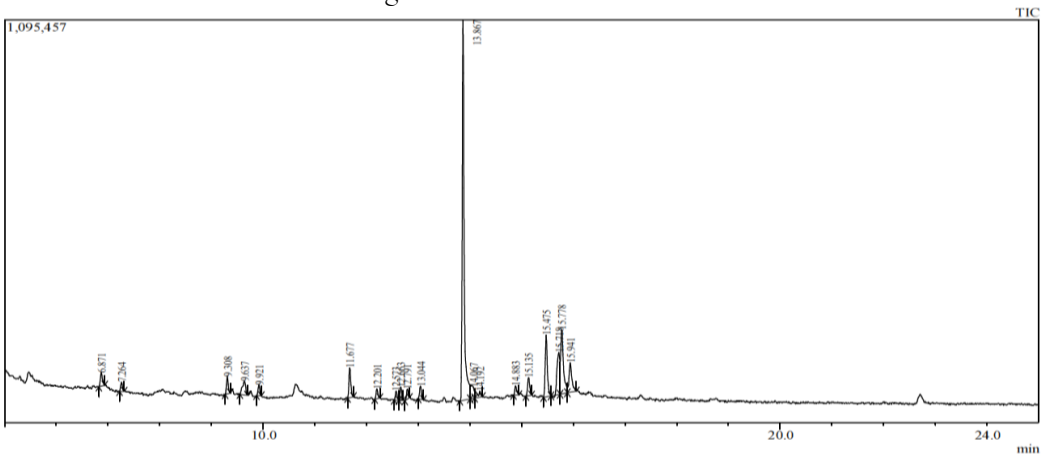

Fig. 1: GC-MS Chromatogram of the ethanolic extract of *Caralluma indica*

The knowledge of the phytocomponents in a medicinal plant is very essential to analyze their therapeutic value. Such precise qualitative analysis can be obtained by Gas Chromatography coupled with Mass Spectrometry (GC-MS) [8]. This study has revealed the presence of 20 compounds that possess various biological properties. The biological activity of the stem of *Caralluma indica* extract is represented in Table 2 [7]. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in Table 1 and Fig 1.

Table 1: Identification of bioactive compounds in ethanolic extract of *Caralluma indica* stem using GC-MS

S.No.	R.Time	Area %	Molecular formulae	Molecular weights	Name of the compounds
1	6.871	1.70	C_4H_4S	84	Thiophene
2	7.264	0.96	$C_{12}H_{26}$	170	Decane 3,7-dimethyl
3	9.308	1.81	$C_{12}H_{24}O_2$	200	Dodecanoic acid
4	9.637	2.65	$C_{15}H_{12}O_4$	256	Benzyl oxy carbonyl benzoic acid
5	9.921	1.11	$C_7H_5NO_3$	151	Benzoldehyde 2-nitro
6	11.677	3.34	$C_{16}H_{32}O_2$	256	Hexadecanoic acid
7	12.201	1.09	C_9H_{18}	126	4,4 dimethyl 1-heptene
8	12.573	1.00	$C_{12}H_{26}O$	186	Delta -4 do decanol
9	12.663	1.28	$C_{12}H_{18}O$	178	6-dodecanone
10	12.791	1.02	$C_{16}H_{32}O_2$	256	Hexadecanoic acid
11	13.044	1.44	$C_{17}H_{36}O$	256	Heptadecanol
12	13.867	44.82	$C_{16}H_3O_2$	256	Hexadecanoic acid
13	14.067	2.51	$C_{16}H_{32}O_2$	256	1,2 benzene di carboxylic acid bis (2-methyl propyl ester
14	14.192	1.39	$C_{12}H_{24}O_2$	200	Decanoic acid ethyl ester
15	14.883	1.08	$C_{16}H_{32}O_2$	256	Hexa decanoic acid
16	15.135	2.30	$C_{16}H_{34}O$	242	1-hexadecanol
17	15.475	7.91	$C_{20}H_{40}O$	296	2-hexa decan 1-ol,37,11,15
18	15.718	6.92	$C_{10}H_{22}O_2$	174	1,10 decanediol
19	15.778	10.67	$C_{19}H_{32}O_2$	292	9,12,15 octa decatrienoic acid methyl ester
20	15.941	4.98	$C_{18}H_{34}0_2$	282	9-octadecanoic acid

Table 2: Biological activity of phytocomponents identified in the ethanolic extract of *Caralluma indica* stem by GC MS

S.No	Name of the Compounds	Nature of compound	Biological activity**
1	Hexadecanoic acid	Palmitic acid	Antioxidant, Hypocholesterolemic, Nemaicide Anti-Androgenic Flavor, Hemolytic, 5-Alpha Reductase Inhibitor
2	Hexadecanoic acid ethyl ester	Fatty acid ester	Antioxidant, Hypocholesterolemic, Anti-Androgenic flavor, Hemolytic, Insectiside
3	Benzoic acid	Benzene	Arachidonic acid inhibitor, Increase aromatic amino acid Decarboxylase Activity and Inhibit production of Uric acid
4	Octadecanoic acid	Fatty alcohol	Anaphylactic, Antitumor, Decrease Nor Epinephrine Production, Increase Natural Killer (NK) Cell Activity, Increase HDL And Decrease LDL Cholesterol, Antihypertensive
5	1-hexadecanol	Fatty alcohol	Antiviral, Diuretic, Antianemic, Insecticide
6	Thiophene	Thiofuran	Antiarrhythmic, Antiatherosclerotic Agents, Anti-microbial, Anti-inflammatory, Analgesic Activity
7	1,2 benzene dicarboxlic acid bis methyl propyl ester	Diethyl pthlate (Plasticizer compound)	Antimicrobial, Antifouling
8	9,12,15 octadecatrienic acid methyl ester	Fatty acid ester	Hypocholesterolemic, 5-Alpha Reductase Inhibitor, Antihistaminic, Insectifuge, Antieczemic, Antiacne
9	9-octadecanoic acid	Oleic acid	Antihypertensive, Increase HDL and decrease LDL Cholesterol.

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

The prevailing compounds were thiophene, dodecanoic acid, hexa decanoic acid, 1-hepta decanol, 1,2-benzene di carboxlic acid bis methyl propyl ester, octadecanoic acid, 9,12,15-octadecatrienoic acid methyl ester. Among the identified N-hexadecanoic acid, palmitic acid is suggested as an antioxidant, hypo cholestrolemic hemolytic properties [9, 10]. The biological activities of prevailing compounds are represented in table 2.

Hexadecanoc acid has been reported as a component in the alcohol extract of *Kigelia africana* fruit [11] and *Melissa offcinalis* [12]. Similar results were also seen in ethanol extract of *Caesalpania sappan* which is found to be effective against acetaminophen induced nephrotoxicity and oxidative stress in male albino rats [13]. Major bioactive compounds methyl ester of hexa decanoic acid was isolated from the leaves of *Annona muricata* and it was proved to have antifungal potentials [14].

Another biochemical compound hexa decanol revealed that it has anti viral diuretic anti anemic properties. 1,2-benzene dicarboxlic acid di ethyl ester is a plasticizer compound act as antimicrobial and antifouling agent [15]. Benzoic acid was found in the ethanolic extract of *Caralluma indica* which may be employed as Arachidonic acid inhibitor, increases aromatic amino acid decarboxylase activity and inhibits production of uric acid. In whitfield's ointment benzoic acid is one of the constituent for the treatment of diseases like Tinea, analgesic and antiseptic in the early 20th century [16]. Meanwhile Octa decanoic acid, identified in *Caralluma indica* is an anaphylactic, decreases LDL cholesterol and increases HDL cholesterol. This study explores the goodness of the stem of *Caralluma indica* which has various bioactive compounds and justifies the use of plant extract for various ailments by traditional practitioners.

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

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