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Determination of bioactive compounds in ethanolic extract of *Caralluma indica* using GC-MS technique

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Abstract
The aim of this study is to carry out for identification of bioactive compounds from the plant stem ethanolic extract of *Caralluma indica* Gas chromatography and Mass spectroscopy (GC-MS) have been used for this study. GCMS analysis of ethanolic extract has been done by standard protocol. Mass spectra of the compounds found in the extract. They are matched with the National Institute of Standards and Technology (NIST) library. The GC-MS analysis reveals the presence of various compounds like 1,2-Benzenedicarboxylic acid, diethyl ester (CAS) Ethyl phthalate, 1,2-Benzenedicarboxylic acid, dihexyl ester (CAS) di-n-Hexyl phthalate, 3-Octadecene, 1-Octadecanol, 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (CAS) Methyl linolenate, 9-Octadecenoic acid (Z)- (CAS) Oleic acid in the ethanolic extract of *Caralluma indica*. These findings support the traditional use of *Caralluma indica* in various disorders.

Keywords: Gas chromatography and mass spectroscopy, *Caralluma indica*, ethanolic extract

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. Traditional remedies such as herbs have been used as popular folk medicines Sathyaprabha *et al.* (2010) [13]. 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 Mathekaga and Meyer (1998) [10].

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 Harborne (1986) [6].

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) Liu (2004) [9]. 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 Hamburger and Hostettmann (1991) [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 Roberts and Xia (1995) [11].

Gas Chromatography Mass Spectroscopy (GC-MS) a hyphenated system which is a very compatible technique and the most commonly used technique for the identification and quantification of biochemical components of medicinal plants Ronald Hites (1997) [12]. The chosen medicinal plant namely as *Caralluma indica* stem belongs to Apocynaceae Family. *Caralluma indica* is widely distributed in Tamil Nadu, Andhra Pradesh and Karnataka. The aim of this study is to determine the organic compounds present in the *Caralluma indica* stem extract with the aid of GC-MS Technique.

Material and Methods

Plant materials: The whole plant of *Caralluma indica* Stem were collected in January 2017 from Kathattipatti (Palaiyapatti North) Thanjavur, Tamil Nadu, India from a herb. The plant were identified and authenticated by Dr. S. John Britto, The Director, the Rapinat Herbarium and center 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 *Caralluma indica* stems were washed several times with distilled water to remove the traces of impurities from the plant. The stem was cut into small pieces and and ground in to fine powder using mechanical grinder. The powder was extracted with ethanol for 24 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The *Caralluma indica* stem extract was stored in refrigerator until 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 200 ºC, then 5 ºC/min to 280 ºC, ending with a 9min isothermal at 280 ºC. 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 36 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 Turbo Mass Ver 5.2.0

Results and Discussion

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 Kell *et al.* (2005) [8]. In the last few years, GC-MS has become firmly established as a key technological platform for secondary metabolite profiling in both plant and non-plant species Fernie *et al*. (2004) [4]. 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 De-Fatima *et al*. (2006) [2]. 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 is 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 are ascertained.

Twenty compounds have been identified in *Caralluma indica* stem by GC-MS analysis. Retention time (RT), molecular formula, molecular weight (MW) and concentration (%) of the compounds are presented in (Table 1 and Fig 1). The prevailing compounds were 1,2-Benzenedicarboxylic acid, diethyl ester (CAS) Ethyl phthalate, 1,2-Benzenedicarboxylic acid, dihexyl ester (CAS) di-n-Hexyl phthalate, 3-Octadecene, 1-Octadecanol, 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (CAS) Methyl linolenate and 9-Octadecenoic acid (Z)- (CAS) Oleic acid. The biological activities of identified compounds are listed in (Table 2). They are based on Dr.Duke's Phytochemical and Ethnobotanical Databases of the Agricultural Research Service/USDA.

Among the identified phytochemicals 1-Octadecanol is suggested to be a fatty acid ester and it may employed as Antibacterial, antifungal, anti-larva activities Bodoprost and Rosemeyer (2007) [1], Falodun *et al*. (2009) [3]. 1, 2-benzenedicarboxylic acid, di isooctyl ester is a plasticizer compound and acts as antimicrobial and antifouling agent Heinonen *et al*. (1998) [7].

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.

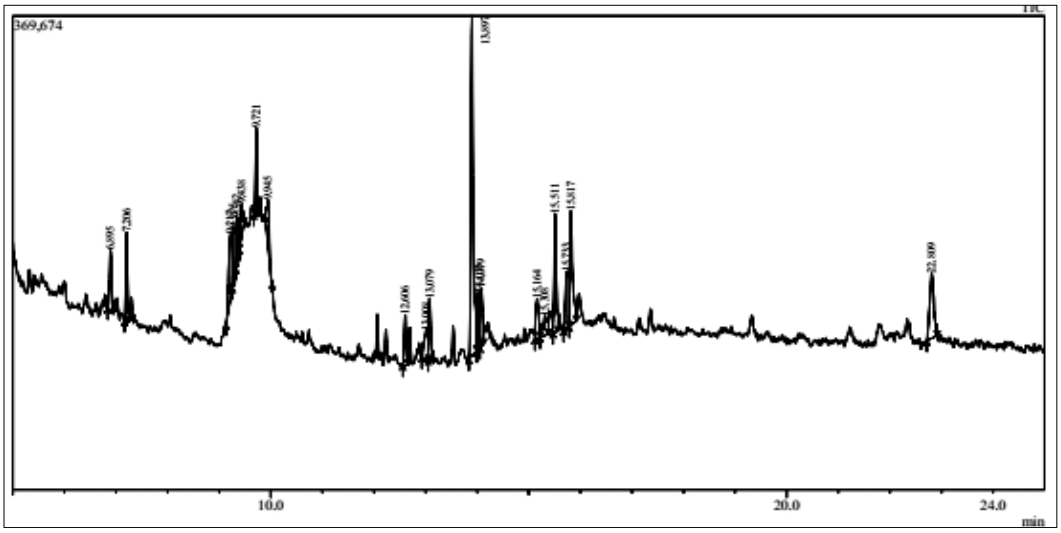

Fig 1: GC-MS Chromatogram of *Caralluma indica* stems ethanolic extract

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

Peak#	R. Time	Area %	Molecular formula	Molecular weight	Molecular name
1	6.895	3.60	C_4H_4S	84	Thiophene (CAS) Thiofuran
2	7.206	4.19	$C_{13}H_{26}$	182	1-Tridecene (CAS) n-Tridec-1-ene
3	9.217	6.35	$C_{20}H_{26}O_4$	330	1,2-Benzoldicarbonsaeure, Di-(Hex-1-En-5-Yl-Ester)
4	9.275	3.85	$C_8H_{10}N_2O$	150	N-Ethyl-4-pyridinecarboxamide
5	9.342	3.06	$C_{12}H_{14}O_4$	222	1,2-Benzenedicarboxylic acid, diethyl ester (CAS) Ethyl phthalate
6	9.438	2.30	$C_8H_{14}O$	126	3-Octyn-2-ol (CAS)
7	9.721	3.74	$C_{18}H_{36}$	252	3-Octadecene, (E)- (CAS)
8	9.945	2.69	$C_{20}H_{26}O_4$	330	1,2-Benzoldicarbonsaeure, Di-(Hex-1-En-5-Yl-Ester)
9	12.606	2.59	$C_{16}H_{32}O$	240	Oxirane, tetradecyl-
10	13.008	2.91	$C_{10}H_{22}$	142	Hexane, 2,2,3,3-tetramethyl- (CAS) 2,2,3,3-Tetramethylhexane
11	13.079	3.55	$C_9H_{18}O$	142	2-Nonen-1-ol, (E)- (CAS) trans-2-Nonenol
12	13.897	20.83	$C_{18}H_{34}O_2$	282	9-Octadecenoic acid (Z)- (CAS) Oleic acid
13	14.033	3.90	$C_{20}H_{30}O_4$	334	1,2-Benzenedicarboxylic acid, dihexyl ester (CAS) di-n-Hexyl phthalate
14	14.079	3.35	$C_{20}H_{30}O_4$	334	1,2-Benzenedicarboxylic acid, dihexyl ester (CAS) di-n-Hexyl phthalate
15	15.164	3.07	$C_{18}H_{38}O$	270	1-Octadecanol (CAS) Stenol
16	15.308	2.61	C_8H_{14}	110	Cyclooctene (CAS) (Z)-Cyclooctene
17	15.511	6.65	$C_{10}H_{20}O$	156	dl-Citronellol $$ 6-Octen-1-ol, 3,7-dimethyl-, (.+-.)- (CAS) Dihydrogeraniol
18	15.733	5.20	$C_9H_{18}O$	142	cis-6-Nonenol
19	15.817	8.46	$C_{19}H_{32}O_2$	292	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (CAS) Methyl linolenate
20	22.809	7.09	$C_9H_{20}O$	144	1-Octanol, 2-Methyl-

Table 2: Biological activity of phytochemicals identified in the ethanol stem extract of *Caralluma indica*

S. No	Compound Name	Biological activity**
1	1,2-Benzenedicarboxylic acid, diethyl ester (CAS) Ethyl phthalate	Plasticizers
2	1,2-Benzenedicarboxylic acid, dihexyl ester (CAS) di-n-Hexyl phthalate	Antimicrobial, Antifouling
3	3-Octadecene	Antibacterial, antioxidant, anticancer
4	1-Octadecanol	Antibacterial, antifungal, anti-larva
5	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (CAS) Methyl linolenate	Flavour, Fungicide, pesticide, perfumery Anti -inflammatory
6	9-Octadecenoic acid (Z)- (CAS) Oleic acid	Antihypertensive, Increase HDL and decrease LDL Cholesterol.

**Duke's. Phytochemical and Ethnobotanical Databases, www.ars-gov/cgi-bin/duke/, 2013.

Conclusion

In this study, GC-MS analysis reveals the presence of phytochemicals in the stem of *Caralluma indica* and justifies the medicinal usage of this plant in Ayurveda medicine. Based on the literature survey we believe that this is the first report of GC-MS analysis of stem extract of this plant. Hence, further studies are insisted to identify the compounds and evaluate its bioactivity through *in vitro* models.

Reference

1. Bodoprost J, Rosemeyer H. Analysis of phenacylester derivatives of fatty acids from human skin surface by reversed-phase HPTLC: Chromatography mobility as a function of physicochemical properties. International Journal of Molecular Sciences. 2007; 8:1111-1124.
2. De-Fatima A, Modolo LV, Conegero LS, Pilli RA, Ferreira CV, Kohn LK *et al*. Lactones and their derivatives: biological activities, mechanisms of action and potential leads for drug design. Curr. Med. Chem. 2006; 13:3371-3384.
3. Falodun A, Siraj R, Choudary MI. GC- MS analysis of insecticidal stem essential oil of *Pyrenacantha staudtii* Hutch and Dalz (Icacinaceae). Tropical Journal of Pharmaceutical Research. 2009; 8:139-143.
4. Fernie AR, Trethewey RN, Krotzky AJ, Willmitzer L. Metabolite profiling: From diagnostics to systems biology. Nat Rev Mol Cell Biol. 2004; 5:763-9.
5. Hamburger M, Hostettmann, K. Bioactivity in plants: the link between phytochemistry and medicine. Phytochemistry. 1991; 30:3864-74.
6. Harborne JB. Plant flavonoids in biology and medicine: Biochemical pharmacological, and structure–activity relationships. NY, USA: Alan R. Liss, 1986, 15-24.
7. Heinonen OP, Alnanes D, Virtamo T. Prostate cancer and supplementation with alpha- tocopherol and beta-carotene: incidence and mortality in a controlled trial. Journal of the National Cancer Institute. 1998; 90(6):440-446.
8. Kell DB, Brown M, Davey HM, Dunn WB, Spasic I, Oliver SG. Metabolic foot printing and systems biology: The medium is the message. Nat Rev Microbiol. 2005; 3:557-65.
9. Liu RH. Potential synergy of phytochemicals in cancer prevention: Mechanism of action. Journal of Nutrition. 2004; 134(12):3479-3485.
10. Mathekaga AD, Meyer JJM. Antibacterial activity of South African *Helichrysum* species. South Afr J Bot. 1998; 64:293-5.
11. Roberts JKM, Xia JH. High-resolution NMR methods for study of higher plants, Methods Cell Biol. 1995; 49:245-258.
12. Ronald Hites A. Gas Chromatography Mass Spectroscopy: Handbook of Instrumental Techniques for Analytical Chemistry, 1997, 609-611.
13. Sathyaprabha G, Kumaravel S, Ruffina D, Praveenkumar PA. comparative study on antioxidant, proximate analysis, antimicrobial activity and phytochemical analysis of *Aloe vera* and *Cissus quadrangularis* by GC-MS. J Pharma Res. 2010; 3:2970-3.