

## GC-MS analysis of *Caralluma diffusa* (Wight) N.E.Br

**K. Kalimuthu\*, K. Kalaiyarasi, R. Prabakaran, V. Preetha and T. Sasikala\*\***

*Plant Tissue Culture Division, PG and Research Department of Botany, Government Arts  
College (Autonomous), Coimbatore, India*

*\*\*Government Arts College (Autonomous), Salem, India*

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### ABSTRACT

*Caralluma diffusa* is an indigenous succulent plant belonging to the sub family Asclepiadoideae. The present study was carried out to identify the phytochemicals present in the ethanolic extract of *C. diffusa* by GC-MS analysis to ascertain its usage by the local community as 'a plant possessing medicinal properties'. The ethanolic extracts result shows twenty one compounds were identified. The major constituents are 2-(3',4'-Dimethoxybenzoylmethyl)benzoic acid, [(E)-1-butyl-2-hex-2-enylidene] cyanamide, 6-[(N-Cyclohexylimino-N'-cyclohexylamino)methyl] benzimidazo [1,2-a] benzimidazole and some of the bio active components are Neophytadiene, 2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R\*,R\*-(E)]]- Hexadecanoic acid, Methyl ester (CAS). The whole plants of *C. diffusa* are used as appetite suppressant and weight loss promoter. The present research was design to investigate ethonolic extract of *C. diffusa* showed the existence of various pharmaceutically important chemical compounds with different chemical structure. The presence of various bioactive compounds confirms the application of this plant for various ailments by traditional practitioners.

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### INTRODUCTION

Plant have been used since ancient times as medicines for the treatment of a range of diseases. Medicinal plants form the backbone of traditional medicine in the last few decades with intense pharmacological studies. They are regarded as potential sources of new compounds of therapeutic value and as sources of lead compounds in drug development. Medicinal plants have been used all over the world for the treatment and prevention of various ailments, particularly in developing countries where infectious disease are endemic and modern health facilities and services are inadequate [1]. In developing countries, it is estimated that about 80% of the population rely on traditional medicine for their primary healthcare. There arises a need to screen medicinal plants for bioactive compounds as a basis for further pharmacological studies [2]. Plants are rich sources of secondary metabolites with interesting biological activities. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties [3]. Screening of active compounds from plant has lead to the invention of new medicinal drugs which have efficient protection and treatment roles against various diseases, including cancer [4] and Alzheimer's disease [5].

The genus *Caralluma* belongs to the family Asclepiadaceae, which comprise 200 genera and 2500 species, which are distributed throughout the world. It is a perennial herb with thick, erect, leafless, branching, and succulent. The phytochemistry of genus *Caralluma* is characterized by many pregnane glycosides, and recently megastimane glycosides also have been isolated. *Caralluma* extracts have also been found to be appetite suppressant a property which is well known to Indian tribal and hunters. India folk lore records its use as a potent appetite suppressant and weight loss promoter [6]. The pregnane glycosides of *Caralluma* have been shown to possess antitumor and anticancer activities and in some studies *Caralluma* is reported to protect gastric mucosa and have antiulcer properties [7,8].

*Caralluma diffusa* (Wight) N.E.Br [syn.*Boucerosia diffusa* (Wight) Family: Apocynaceae] is a succulent medicinal plant. In India, the genus is represented by thirteen species and five varieties [9]. The Flora of Tamil Nadu states that *C. diffusa* distribution is only from Coimbatore district in Tamil Nadu and ecological status is mentioned as rare and threatened species [10].

Gas chromatography – mass spectrometry (GC-MS) is a method that combines the features of gas chromatography and mass spectrometry to identify different substances present within a test sample. Application of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation and identification of unknown samples. GC-MS can also be used in airport security to detect substances in luggage or on human beings, additionally it can identify trace elements in materials that were previously thought to have disintegrated beyond identification. The use of mass spectrometer as the detector in gas chromatography was developed of affordable and miniaturized computers has helped in the simplification of the use of this instrument as well as allowed great improvements in the amount of time it takes to analyze a sample.

## MATERIALS AND METHODS

### Plant material:

The present investigation was carried out in the plant *Caralluma diffusa* that were collected from the natural habitat of Madukarai hills, Coimbatore district in Tamil Nadu.

### Plant parts used in traditional medicine:

Aerial plant parts were used.

### Plant sample extraction:

The fresh aerial plant were carefully washed with tap water to remove soil particles and adhered debris, rinsed with distilled water, and air dried for 1 hour. Then it was cut in to small pieces and dried in room temperature for weeks. Then they were ground in to powder with the help of Hand mill and stored in room temperature. Twenty grams of powdered plant aerial parts of shoot is soaked in 50ml of ethanol overnight and then filters through Whatmann filter paper No.41 along with 2gms sodium sulphate to remove the sediments and traces of water in the filtrate. Before filtering the filter paper along with sodium sulphate is wetted with ethanol. The filtrate is then concentrated by bubbling nitrogen gas in to solution and reduces the volume to one ml. The extract contain both polar and non polar phytochemicals of the plant material and plant extract is injected in the Gas chromatography-Mass spectrometer.

One micro litre of the filtrate was injected in to the Gas chromatography column. The sample gets evaporated and carried away by gas helium. It gets segregated in to individual components. The sample fraction coming out of the column was led in to mass detector and the mass spectrum of unknown components were identified.

The GC – MS analysis was carried out using a Clarus 500 Perkin Elmer (Auto system XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold – Perkin Elmer Turbomass 5.2 spectrometer with an Elite – 5MS (5% Diphenyl / 95% Dimethyl poly siloxane), 30m x 0.25 µm DF of capillary column. The instrument was set to an initial temperature of 70°C, and maintained at this temperature for 2 min. At the end of this period the oven temperature was rose up to 250°C, at the rate of an increase of 10°C /min, and maintained for 9 min. Injection port temperature was ensured as 200 °C and Helium flow rate as one ml/min. The ionization voltage was 70eV. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 45-450 (m/z). Using computer searches on a NIST Version –Year 2011 were used MS data library and comparing the spectrum obtained through GC – MS compounds present in the plants sample were identified.

## RESULTS AND DISCUSSION

The GC-MS study of *C. diffusa* has shown many phytochemicals which contribute to the biological activity of the plant. The active principles, with their retention time (RT), molecular formula, molecular weight (MW) and the concentration (peak area %) are presented in table 1. The GC- MS Chromatogram of the number of peaks of the compounds detected was shown in figure.1. The total number of compounds identified in ethanol extract was 21. Among the identified phytochemicals, hexadecanoic acid has the property of antioxidant activity [11]. Palmitic acid is reported to be an antioxidant [12, and also a nematocide [13]. The presence of Hexadecanoic acid may be used as potential antifeedant agents against insects [14]. Neophytadiene is reported to be an antibacterial compound [15]. suitable for treatment of headache, rheumatism and some skin diseases [16]. 2-hexadecan-1-ol, 3,7,11,15-tetramethyl-, [R-[R\*,R\*-(E)]]-(CAS) is a terpene Alcohol. It act as Antimicrobial and Anti-inflammatory [17].

Table-1 GC MS Analysis of Ethanol extracts of *Caralluma diffusa* (Wight) N.E.Br.

S.No	Retention time (min)	Name of the Compound	Peak Area %	Molecular Weight	Molecular Formula
1	6.14	Heptacosane (CAS)	6.68	380	C27H56
2	19.80	Neophytadiene	2.71	278	C20H38
3	22.82	5-methyl-4-hexen-2-one	4.64	112	C7H12O
4	26.08	Nonanoic acid, methyl ester (CAS)	2.71	172	C10H20O2
5	26.33	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]-(CAS)	2.50	296	C20H40O
6	26.69	Hexadecanoic acid, methyl ester (CAS)	3.62	270	C17H34O2
7	29.61	1,2,3-tris(4' Methylbenzoyl) indolizine	1.31	471	C32H25NO3
8	30.21	Hexanoic acid, 3-hydroxy-2-methylene-3-propyl-, methyl ester (CAS)	2.58	200	C11H20O3
9	30.96	4-Phenylenebis[5-( $\zeta$ -pyridyl)-2'-(2',5'bipyrimidine)]	6.90	544	C32H20N10
10	31.41	1,2-Pentanediol, 4,4-dimethyl-, (+-)- (CAS)	1.54	132	C7H16O2
11	31.86	2-(3',4'-Dimethoxybenzoylmethyl) benzoic acid	11.42*	300	C17H16O5
12	32.49	Octacosanoic acid, methyl ester (CAS)	3.66	438	C29H58O2
13	33.42	3,5-O-benzylidene-1-deoxy-d-ribitol	2.94	224	C12H16O
14	33.97	2-Isopropyl-2-phenyl-1,3-dioxolane	1.96	192	C12H16O2
15	34.42	2-Amino-1,3,4-trihydroxy-8-octadecene	4.65	315	C18H37NO3
16	34.93	2-Chloro-1-(1-methyl-1-phenylethyl)-4-phenylpyrrole	2.94	295	C19H18ClN
17	35.69	[(E)-1-butyl-2-hex-2-enylidene]cyanamide	11.30*	178	C11H18N2
18	38.19	decabenzylstannocen	8.63	1150	C80H70Sn
19	39.03	(2-Deuteriomethylphenyl) methanol	4.09	122	C8H9DO
20	39.80	2,3-Diphenyl-2-butenal	4.10	250	C16H14N2O
21	40.15	6-[(N-Cyclohexylimino-N'-cyclohexylamino)methyl] benzimidazole [1,2-a] benzimidazole	9.10*	413	C26H31N5

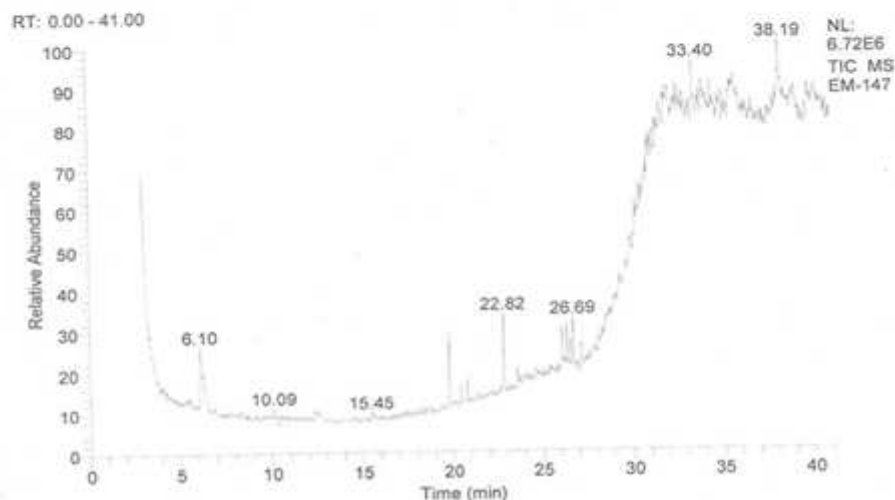


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COIMBATORE - 641 014

Sample ID: EM-147      Low Mass(m/z): 50      Sample Name: PLANT POWDER K  
Operator: RD      High Mass(m/z): 650      Comments:  
Run Time(min): 38.00      Instrument Name: DSQ      Acquisition Date: 05/24/13 02:09:08 PM

EQUIPMENT : THERMO GC - TRACE ULTRA VARI: 5.0,  
THERMO MS DSQ II  
COLUMN : DB 35 - MS CAPILLARY STANDARD NON - POLAR COLUMN  
DIMENSION : 30 MTS, ID : 0.25 MM, FILM : 0.25  $\mu$ M  
CARRIER GAS : HE, FLOW : 1.0 ML/MIN  
TEMP PROG : OVEN TEMP 70 C RAISED TO 250 C AT 10 C/MIN

INJECTION  
VOLUME : 1 MICRO LITER



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**CONCLUSION**

The presence of many phytochemicals in *C.diffusa* lends credence to its use by the local community as a plant with 'medicinal properties' and also holds promise for the production of novel pharmaceuticals as well as a nutraceutical. It would be worthwhile to further isolate the compounds and determine their specific activity and also to understand the synergistic effect of compounds for therapeutic roles.

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