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Phytochemical analysis of *Caralluma nilagiriana* using GC – MS

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ABSTRACT
The aim of the study was to investigate and identify the constituents of the rare endemic species *Caralluma nilagiriana*. The phytochemical analysis was carried out by using Gas Chromatography-Mass Spectroscopy instrument for the presence of active constituents by qualitative method. The results showed the active ingredients were Alkaloids, Terpenoids, Flavonoids, Steroids, Tannins, and Phenolic compounds in ethanolic extract of the *Caralluma nilagiriana*. The compounds were identified and confirmed by comparing their mass spectrum with the original spectrum obtained from the inbuilt libraries namely WILEY and NIST. These libraries have more than five million compounds.

Keywords: *Caralluma nilagiriana, ethanolic extract, phytochemical compounds, Gas Chromatography, Spectral library.*

1. Introduction
The traditional knowledge of medicinal plants in India has been conveyed orally of various ethnic communities for centuries. It slowly lost its impact due to improper transformation or improper understanding between generations and the modern technologies also played a major role in shadowing the traditional methods. Traditional medicine is an important source of potentially useful compounds of chemotherapeutic agents. A wide range of medicinal plant parts are used for extraction of raw drugs and they possess varied medicinal properties. *Caralluma* is a xerophytic genus which includes about 120 taxa, with a wide African, Asian, South African and Southeast European distribution [1]. *C. nilagiriana* is a succulent medicinal plant depleted due to overexploitation, lack of organized cultivation and completely eaten by sheep and goats, the wild population has become restricted species and has now been added to the list of Indian endemic plants [2]. The phytochemistry of genus *Caralluma* is characterized by the many pregnane glycosides. While recently megastimane glycosides also have been isolated [3], *Caralluma* extracts have also been found to be appetite suppressant, a property which is well known to Indian tribals and hunters. Indian folklore records its use as a potent appetite suppressant and weight loss promoter [4]. Several members of the genus *Caralluma* have found medicinal uses in the treatment of Rheumatism, Diabetes, Leprosy, antiseptics, antimicrobial [5] and disinfectants [6].

Due to the uniqueness of curing different ailments, this whole plant was selected for the study. Hence, the present investigation was carried out to determine the phytochemical components of *Caralluma nilagiriana* and to analyze the potent bioactive compound using GC – MS.

2. Materials and Methods
2.1 Plant Collection
The species, *C. nilagiriana was* collected from foot hills of Ooty, Nilgiris District, Tamil Nadu and confirmed the binomial with the voucher specimen deposit available in the Department of Botany, Government Arts College (Autonomous), Coimbatore. The leaves were washed thoroughly two times with water and then with distilled water. The leaves were shed dried and powdered. The powdered sample was kept in polythene bags.

2.2 Plant Extraction
About 1 g of powdered material was then subjected to extraction using a Soxhlet apparatus in AR grade Methanol, ethanol, chloroform, ethyl acetate, acetone and petroleum ether and were treated for 6 hours. All the extracts were filtered and concentrated in a rotary evaporator under reduced pressure (Vacuum 175mbar for b.p at 40 ^{o}C) to get thick green crude extracts [7].

2.3 Phytochemical Screening

Chemical test was carried out only on the ethanol extract using standardized procedures to identify the constituents as described by sofowara [8], Trease [9] and Evans [10] and Harbone [11].

2.4 GC – MS Analysis

Gc-Ms Instrumentation

The Trace GC Ultra and DSQII model MS from Thermo Fisher Scientific Limited, were engaged for analysis.

The instrument was set as follows, Injector port temperature set to 250 °C, Interface temperature set as 250 °C, source kept at 200 °C. The oven temperature programmed as a variable, 70 °C for 2 mins, 150 °C @ 8 °C/min, up to 260 °C @ 10 °C/min. Split ratio set as 1:50 and the injector used was splitless mode. The DB-35 MS Non-polar column was used whose dimensions were 0.25 mm OD x 0.25 µm ID x 30 metres length procured from Agilent Co., USA. Helium was used as the carrier gas at 1 ml/min. The MS was set to scan from 50 to 650 Da. The source was maintained at 200 °C and <40 mtorr vacuum pressure. The ionization energy was -70eV. The MS was also having inbuilt pre-filter which reduced the neutral particles.

The data system has two inbuilt libraries for searching and matching the spectrum. NIST4 and WILEY9 each contain more than five million references. Only those compounds with spectral fit values equal to or greater than 700 were considered positive identification.

2.5 Identification of compounds

Interpretation of mass spectrum of GC – MS was done using the database of National Institute Standard and Technology (NIST4) and WILEY9 [12]. The spectrum of the known component was compared with the spectrum of the known components stored in the inbuilt library.

3. Results and Discussion

Some compounds were identified in an ethanolic fraction of *Caralluma nilagiriana* extract by GC-MS analysis. Qualitative analyses of phytochemicals were carried out by Harborne & Kokate method [13, 14 & 15]. The chromatograms obtained by an ethanol fraction of *Caralluma nilagiriana* are shown in fig 1, 2 and 3. The active principle, area of the peak, Retention time (RT), molecular formula and molecular weight of the Phytocomponents identified in the ethanolic extract of *C. nilagiriana* are presented in Table 1. Table 2 lists the major phytocomponents and their biological activities

Table 1: Phytocomponents in ethanolic extract of *Caralluma nilagiriana* by GC-MS

S. No.	RT (minutes)	Name of the compound	Formula	M.WT	AREA%
1	3.29	Styrene	C_8H_8	104	0.38
2	4.74	1-(1'-Azetidinyl)-2,6-dimethyl-1-cyclohexene	$C_{11}H_{19}N$	165	0.43
3	5.13	2(1H)-Pyridinone, 1-ethenyl	C_7H_7NO	121	0.36
4	8.07	2-Propenoic acid, 2-ethylhexyl ester	$C_{11}H_{20}O_2$	184	1.39
5	9.63	Acenaphthen-1,2-imine	$C_{12}H_9N$	167	0.21
6	10.10	á-L-mannofuranose, 6-deoxy-, cyclic 1,2:3,5-bis(ethylboronate)	$C_{10}H_{18}B_2O_5$	240	0.77
7	10.41	Cyclotetradecane	$C_{14}H_{28}$	196	3.28
8	10.70	Diphenyl ether	$C_{12}H_{10}O$	170	1.81
9	15.16	1,1'-Biphenyl, 4-(1-methylethyl)-	$C_{15}H_{16}$	196	0.59
10	16.86	5,6-dihydro-5,6-dimethylbenzo[c]cinnoline	$C_{14}H_{14}N_2$	210	1.46
11	18.02	Benzene, (1-ethyldecyl)- (CAS)	$C_{18}H_{30}$	246	0.69
12	20.12	Clovanemagnolol	$C_{33}H_{42}O_3$	486	1.66
13	20.93	1H-Pyrrolizine, hexahydro- (CAS)	$C_7H_{13}N$	111	0.77
14	21.82	Phthalic acid, butyl dodecyl ester	$C_{24}H_{38}O_4$	332	0.70
15	24.65	Phytol isomer	$C_{20}H_{40}O$	296	4.32
16	26.12	Behenic alcohol	$C_{22}H_{46}O$	326	2.31
17	28.11	4-Bromo-1,3-dimethyl-1H-indole	$C_{10}H_{10}BrN$	223	0.69
18	29.39	3à,5-Cyclo-5à-ergosta-6,8(14).22t-triene	$C_{28}H_{42}$	378	0.49
19	30.61	Colchifoleine	$C_{21}H_{23}NO_7$	610	1.76
20	31.06	Di-(2-ethylhexyl)phthalate	$C_{24}H_{38}O_4$	390	0.22
21	34.19	4-Ethoxy-5-octyl-2,6-bis(methylsulfonyl)pyrimidine	$C_{16}H_{28}N_2O_5S_2$	392	0.20
22	34.67	2,6-bis(Dibromomethyl)-3,5-diphenyl-4H-pyran-4-one	$C_{19}H_{12}Br_4O_2$	588	0.18
23	35.17	Stigmasta-5,22-dien-3-ol, (3á,22E)- (CAS)	$C_{29}H_{48}O$	412	3.80
24	36.80	Pirenzepine, 5-isobutyloxycarbonyl-	$C_{24}H_{29}N_5O_4$	451	0.53
25	38.08	2,3-Dichloro-1,4-bis(phenylethynyl)naphthalene	$C_{26}H_{14}C_{l2}$	396	6.64

Table 2: Therapeutic activity of phytocompounds identified from the ethanolic extract of *Caralluma nilagiriana*

S. NO.	RT (minutes)	Compound Name	Nature	Activity
1	3.29	Styrene	Benzoic	Anti ibacterial
2	4.74	1-(1'-Azetidinyl)-2,6-dimethyl-1-cyclohexene	Alkenes	Anti-inflammatory
3	5.13	2(1H)-Pyridinone, 1-ethenyl	Ketone	Anti-Inflammatory
4	8.07	2-Propenoic acid, 2-ethylhexyl ester	Carboxylic Acid	Hepatoprotective
5	10.70	Diphenyl ether	Phenol	Antiseptic
6	15.16	1,1'-Biphenyl, 4-(1-methylethyl)-	Phenolic	Antiseptic
7	16.86	5,6-dihydro-5,6-dimethylbenzo[c]cinnoline	Benzoic	Antimicrobial
8	18.02	Benzene, (1-ethyldecyl)- (CAS)	Benzoic	Antimicrobial
9	21.82	Phthalic acid, butyl dodecyl ester	Plasticizer	antifouling
10	24.65	Phytol isomer	Diterpene	Antimicrobial, Anti-inflammatory
11	26.12	Behenic alcohol	Alcohol	Antimicrobial
12	28.11	4-Bromo-1,3-dimethyl-1H-indole	Alkaloid	Antioxidant
13	29.39	3à,5-Cyclo-5à-ergosta-6,8(14).22t-triene	Alkenes	Anti-inflammatory
14	30.61	Colchifoleine	Flavonoid	Anti-oxidant
15	31.06	Di-(2-ethylhexyl)phthalate	Plasticizer	Antifouling
16	34.67	2,6-bis(Dibromomethyl)-3,5-diphenyl-4H-pyran-4-one	Ketone	Anti-inflammatory
17	35.17	Stigmasta-5,22-dien-3-ol, (3á,22E)- (CAS)	Steroid	Antiasthma, Antiarthritic
18	38.08	2,3-Dichloro-1,4-bis(phenylethynyl)naphthalene	Benzoic	Antibacterial

* Source: Dr. Duke's phyto chemical ethno botanical database [online database]

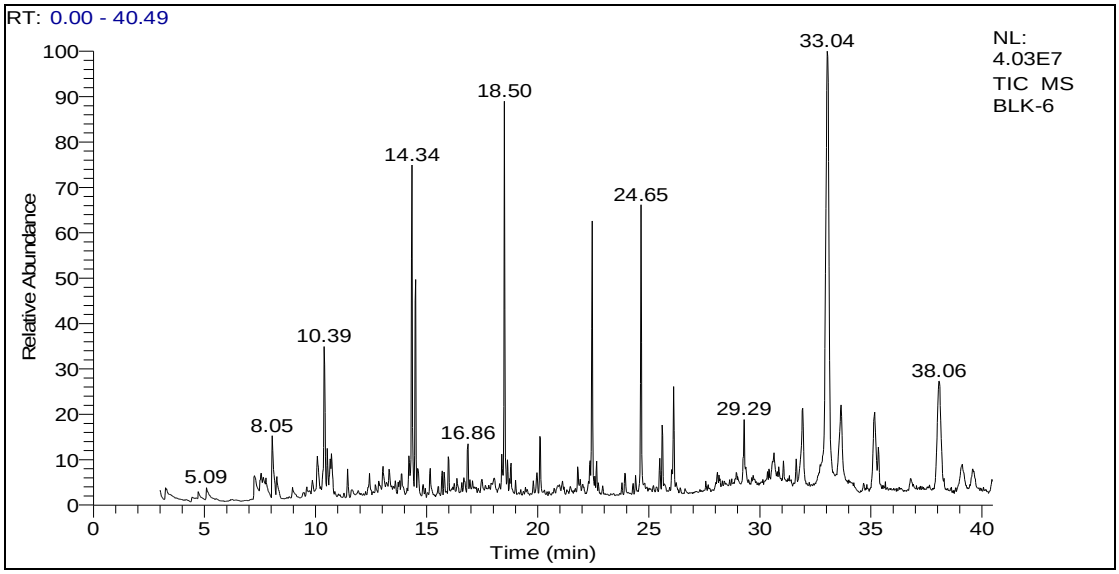

Fig 1: Chromatogram of ethanolic extract of *Caralluma nilagiriana* by GC-MS

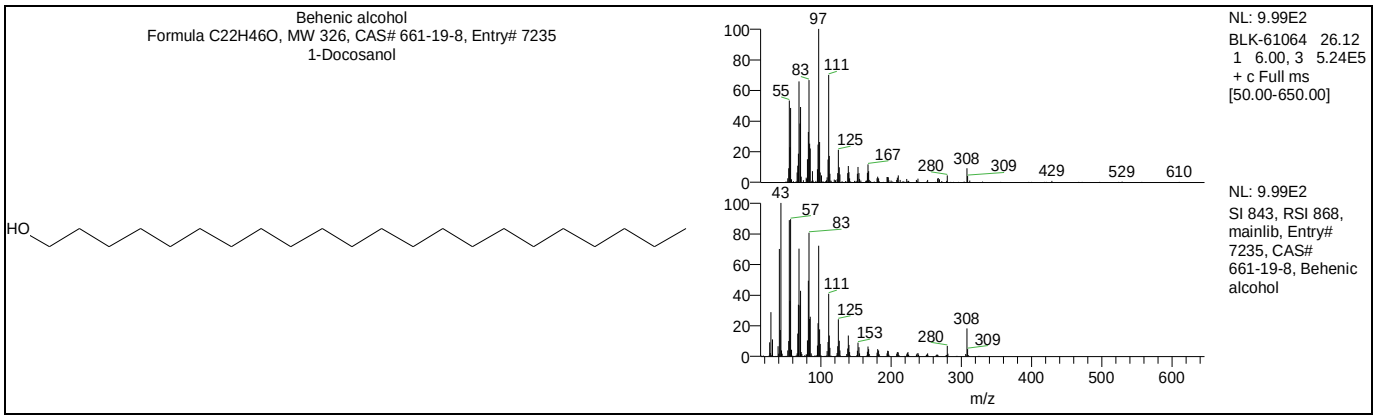

Fig 2: Structure and spectrum comparison of Behenic alcohol

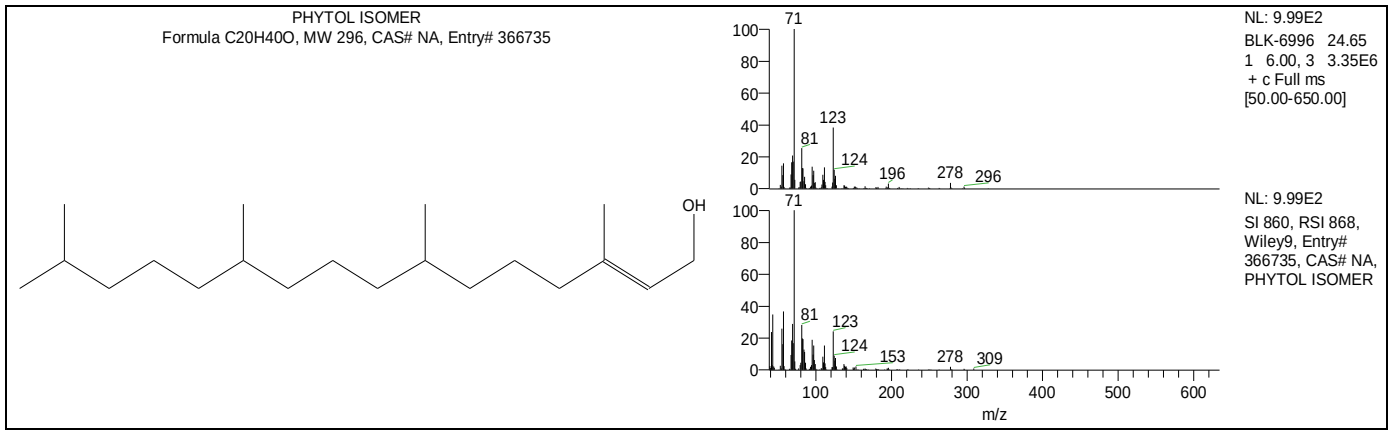

Fig 3: Structure and spectrum comparison of Phytol isomer

The fragmentation pattern of Phytol isomer is given in the above figure whose retention time is 24.65 minutes and the peak area percentage is 4.32%. This compound is known to possess antimicrobial and antioxidant activity [13]. The alcoholic compound namely Behenic alcohol, with a retention time of 26.12 minutes and a peak area percentage is 2.31%, which is reported to having antimicrobial property was also found [14].

3.1 Discussion

The chromatogram which was taken for analysis is subtracted from a blank run chromatogram so the results were refined from unwanted solvent and column eluted peaks. The results from refined chromatogram shows, most of the compounds were extracted and eluted by Ethanol. The eluted compounds were classified into alcoholics, terpenoids, plasticizers, alkenes and steroids.

Terpenoids are the most abundant compound in the plant kingdom. The present study shows the presence of Terpenoids and the presence of such active ingredients in the whole plant justifies its use for various ailments. These compounds of medicinal interest contribute to the fact that the plant *C. nilagiriana* being used traditionally for medicines to provide anti-microbial [6], anti-inflammatory, anticancer, antifouling and anti-arthritic properties.

4. Conclusion

From the present study, it is concluded that the maximum number of phytochemicals were observed in the ethanolic extract than methanolic, ethyl acetate and acetone extract [15]. The presence of various bioactive compounds justifies the use of this plant for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents and subjecting it to pharmacological activity will definitely give more fruitful results.

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