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PHYTOCHEMICAL STUDIES AND GC-MS ANALYSIS OF *CARALLUMA FIMBRIATA* WALL.

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ABSTRACT

The investigation was carried out to determine the possible phytochemical components from the aqueous, ethyl acetate, ethanolic & methanolic extracts of *Caralluma fimbriata* Wall. Among the phytochemical screening of these extracts, methanolic extract showed that the whole plant was rich in alkaloids, flavonoids, glycosides, phenolic compounds, saponins and quinones. This study was extended by analyzing the potent bioactive compounds in the methanolic extract of *Caralluma fimbriata* Wall. using GC-MS. The analysis revealed that *Caralluma fimbriata* Wall. contains mainly n-Hexadecanoic acid (44.23%) and oleic acid (21.08%). Medicinal potential of these compounds needs further research on toxicological aspects to develop safe drug.

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INTRODUCTION

Caralluma fimbriata Wall. an edible succulent cactus is a perennial herb growing in dry parts of Tamil Nadu, India. It belongs to the family Apocynaceae is also a well known as Famine Food, Appetite Suppressant & thirst quencher among tribal population. Genus *Caralluma* comprises about 200 genera & 2500 species[1]. It grows wild all over India & is also planted as a roadside shrub & boundary marker in gardens. Several members of the genus *Caralluma* have found medicinal uses in the treatment of Rheumatism, Diabetes, Leprosy, Antiseptics & Disinfectants[2].

The species of *Caralluma* found in India are edible and form part of the traditional medicine system of the country. *Caralluma fimbriata* is listed in The Wealth of India (1992) as medicinal plant used as an appetite suppressant and has also been used to treat diabetic, pain, fever, and inflammation. Native Indian diets over many centuries have included these edible wild succulent cacti, with claims in folklore about its Appetite Suppressant Activity. An investigation was carried out to find out the effect of *Caralluma fimbriata* extract on appetite, food intake and anthrometry in adult Indian men and women[3]. The extract of *Caralluma fimbriata* in the form of capsules, has been released under the trade name GENASLIM for body weight control.

Phytochemicals in fruits, vegetable, spices and traditional herbal medicinal plants have been found to play protective role against many human chronic diseases including cancer and cardio vascular disease (CVD). Phytocomponents including phenolics, flavonoids, tannins proanthocyanidins and various plants or herbal extracts have been reported to be Radical Scavengers and inhibitors of Lipid Peroxidation[4]. When Phytochemicals compounds react with a free radical, it is the delocalization of the gained electron over the phenolic antioxidant and the aromatic nucleus, that prevents the continuation of the free radical chain reaction. This is often called “Radical Scavenging”. But polyphenolic compounds inhibit oxidation through a variety of mechanisms[5].

Due to uniqueness of curing different ailments this whole plant was selected for the study. Hence the present investigation was carried out to determine the possible phytochemical components from *Caralluma fimbriata* Wall. and to analyze the potent bioactive compound by GC-MS.

MATERIALS AND METHODS

The apocynaceae family members are mainly distributed in the Himalayan, southern and western parts of India. The plants chiefly inhabit arid soil. The plants were collected from Medicinal garden, J.J College of Arts and Science, Pudukkottai (District), Tamil Nadu, India and authenticated in Botanical Survey of India, Coimbatore. MS media, sucrose and all the chemicals for this study were purchased from HiMedia, Mumbai, India. Glasswares were purchased from Borosil, India.

Preparation for extracts

The plant was collected, washed and dried. Then it was ground in a grinding machine to fine powder and passed through a 24-mesh sieve and the extract is weighted and stored at room temperature.

Extraction of plant material

The powdered sample (20g) of *Caralluma fibriata* was successively extracted with 200ml of solvent (ethanol, ethyl acetate and methanol) using magnetic stirrer and stirred for 3hrs. Then it was filtered using whatmann filter paper. Again the residue was dissolved with 200ml solvent and stirred for 2hrs. The solvent containing the extract is dried under reduced pressure. The aqueous extract was prepared with 10g of powder in 100ml of distilled water & stirred for 3 hrs. The supernatant was boiled up to minimum volume.

Phytochemical Studies [6, 7]

The freshly prepared crude extract was qualitatively tested for the presence of biochemical constituents.

Test for alkaloids

5ml of the extract was added to 2ml of HCl. To this acidic medium, 1ml of Wagners reagent was added. A reddish precipitate brown produced immediately indicates the presence of alkaloids.

Test for glycosides

To a small amount of extract, 1ml of Fehling`s solution was added and heated, orange precipitate indicates the presence of glycosides.

Test for flavonoids

To 1ml of the extract, a few drops of dilute sodium hydroxide was added. An intense yellow colour was produced in the plant extract, which become colourless on addition of a few drops of dilute acid indicates the presence of flavonoids.

Test for saponins

The Extract was diluted with 20 ml of distilled water and it was agitated in a graduated cylinder for 15 min. The formation of 1cm layer of foam showed the presence of saponins.

Test for phenolic compounds

Small amount of various extracts were taken separately in water and tested for the presence of phenolic compounds with dilute ferric chloride solution. Violet color indicates the presence of phenolic components.

Test for quinines

To a small amount of extract, concentration of sulphuric acid is added. Appearance of red color indicates the presence of quinones.

Test for reducing sugar

To few drops of the test solution, 2ml of Fehlings reagent & 3ml of water is added. Appearance of Red orange indicates the presence of Reducing sugar.

GC-MS analysis

GC-MS analysis on the methanolic extract of *Caralluma fimbriata* was carried out in the Indian Institute of Crop Processing Technology, Thanjavur. GC Clarus 500 Perkin Elmer system comprising a AOC-20i auto sampler and gas chromatograph interfaced to a masss pectrometer (GC-MS) instrument was used, employing the following conditions: Column Elite-1 fused silica capillary column (30mm×0.25mm ID ×1 µ M df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%)was used as carrier gas at a constant flow of 1ml/minand an injection volume of 0.5 µ l was employed (split ratio of10:1) injector temperature 250 °C; ion-source temperature 280 °C. The oven temperature was programmed from 110 °C (isothermal for 2 min), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5seconds and fragments from 45 to 450 Da.Total GC running time is 36 mins. The plant extract was dissolved in methanol and filtered with polymeric solid phase extraction (SPE) column and analyzed in GC-MS for different components.

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.

RESULTS

Phytochemical studies

Qualitative analysis of phytochemicals were carried out by Harborne & Kokate[6,7] method. Maximum extraction of phytochemicals were found to be present in Methanolic extract when compared with Ethanolic, Ethyl acetate (EA) and aqueous extract of *Caralluma fimbriata* (Table 1). The phytochemical screening of methanolic extract showed that the whole plant was rich in alkaloids, flavonoids, glycosides, phenolic compounds, saponins and quinones. These compounds may be responsible for several medicinal activity of *Caralluma fimbriata.*

Table 1: Phytochemical Analysis

S.No	Phytochemicals	Ethanol Extract	Ethyl acetate Extract	Methanol Extract	Aqueous Extract
1	Alkaloids	++	+	+++	++
2	Glycosides	+	+	++	+
3	Flavonoids	++	+	++	+
4	Saponins	+++	++	+++	+++
5	Phenolic Compounds	++	+	++	+
6	Quinone	+	+	++	+
7	Reducing Sugar	_	_	_	_

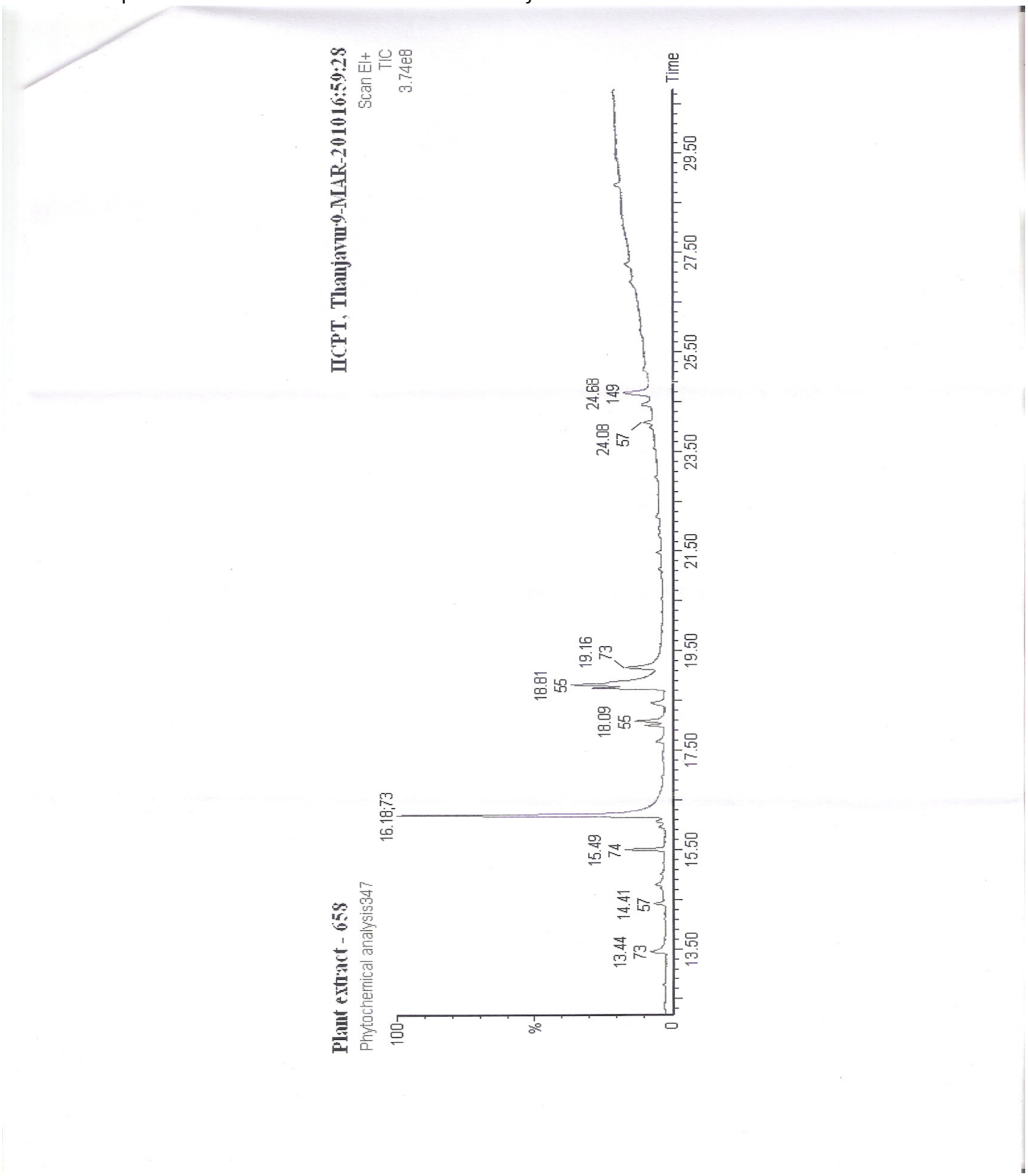
GC-MS: Phytocomponents in methanolic extract of *Caralluma fimbriata* by GC-MS Report

The presence of phytochemicals in methanolic extracts of *Caralluma fimbriata* is tabulated and represented by graphical method. Fourteen compounds were identified in *Caralluma fimbriata* by GC-MS analysis. The active principles with their retention time(RT), molecular formula, molecular weight (MW) and concentration (%) are found. The prevailing compound was n-Hexadecanoic acid (44.23%) and oleic acid (21.08%). The chemical compounds shown in (Table 2) and the corresponding chemical shift peaks of the spectrum were shown in Fig. 1.

Table 2: Phytocomponents in methanolic extract of *Caralluma fimbriata* by GC-MS

S.No	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1	13.44	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	3.19
2	14.41	3,7,11,15 – Tetramethyl – 2 - hexadecen – 1 – ol	$C_{20}H_{40}O$	296	1.05
3	15.49	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	3.56
4	16.18	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	44.23
5	17.67	Heptadecanoic acid	$C_{17}H_{34}O_2$	270	1.29
6	18.00	9,15 - Octadecadienoic acid, methyl ester, (Z,Z)-	$C_{19}H_{34}O_2$	294	1.38
7	18.09	9 – Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	296	2.62
8	18.44	Heneicosanoic acid, methyl ester	$C_{22}H_{44}O_2$	340	2.27
9	18.75	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	6.74
10	18.81	Oleic acid	$C_{18}H_{34}O_2$	282	21.08
11	19.16	Octadecanoic acid	$C_{18}H_{36}O_2$	284	5.36
12	21.47	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	326	0.58
13	24.68	1,2 – Benzenedicarboxylic acid, diIsooctyl ester	$C_{24}H_{38}O_4$	390	5.34
14	28.86	Squalene	$C_{30}H_{50}$	410	1.32

Figure 1: GC-MS spectrum of methanolic extract of *Caralluma fimbriata*



DISCUSSION

Plant products including phenols, flavonoids, tannins proanthocyanidins in the plants extracts have been reported to be radical scavengers and inhibitors of Lipid Peroxidation[4,8]. The antioxidant properties of phenolic acids and flavonoids are due to their redox properties, ability to chelate metals and quenching of singlet oxygen[9]. When Phytochemicals compounds react with a free radical, it is the delocalization of the gained electron over the phenolic antioxidant and the aromatic nucleus that prevents the continuation of the free radical chain reaction. This is often called "Radical Scavenging". But polyphenolic compounds inhibit oxidation through a variety of mechanisms[5]. The compounds identified by GC-MS in methanolic extract are medicinally valuable and possess various pharmaceutical applications. The identified phytocomponents needs further research on toxicological aspects to develop safe drug.

CONCLUSION

From the present study, it is concluded that the maximum extraction of phytochemicals was obsereved in methanolic extract than ethanolic, ethyl acetate and aqueous extract which reveals that *Caralluma fimbriata* is highly valuable in medicinal usage for the treatment of various human aliments.

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