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Research Article

PHYTOCHEMICAL CONSTITUENTS AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS OF *CARALLUMA DIFFUSA* (WIGHT) N. E. BR. AERIAL PART

*KARTHIK PRABU M, SAMYDURAI P, SUBBAIYAN B AND V THANGAPANDIAN

PG and Research Department of Botany, Kongunadu Arts and Science College (Autonomous), Coimbatore-641 029, Tamil Nadu, India. Email: karthikprabu23@gmail.com

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ABSTRACT

Objective: The present study was aimed to investigate the phytochemical constituents and Gas Chromatography-Mass Spectrometry (GC-MS) of aerial part extract of *Caralluma diffusa.*

Methods: Powdered sample (125 g) was extracted with 500 ml of solvent (petroleum ether, acetone and methanol) using soxhlet apparatus. The aqueous extract was prepared with 10 g of powder in 100 ml of distilled water and stirred for 12 h. Then it was filtered and dried under reduced pressure. All these extracts subjected to qualitative phytochemical screening and methanol extract was taken for GC-MS analysis.

Results: Among these four extracts, the methanolic extract showed positive results for alkaloid, carbohydrate, saponin, flavonoid, glycoside, steroids, phenols, terpenoids and cardiac glycoside. As these major bioactive compounds were present in methanolic extracts, further screening by GC-MS analysis revealed the presence of 18 bioactive compounds. The results of GC-MS revealed that *Caralluma diffusa* contains mainly 2-Furancarboxaldehyde 5-(hydroxymethyl)-(28.67 %), 1, 5-Anhydro-6-Deoxyhexo-2, 3-Diulose (17.19%) and 1, 3-Propanediol, 2-ethyl-2-(hydroxymethyl)- (11.89 %) were identified based on percentage of peak area.

Conclusions: The results of the present study enhance the traditional usage of *C.diffusa* which possesses several bioactive compounds. By isolation, identification, and develop the structure for biomolecules which can be used as drugs and further investigation may lead to the development of drug formation. On the basis of the medicinal potential of these bioactive compounds further research may be done on toxicological aspects to develop safe drugs.

Keywords: *Caralluma diffusa*, Methanol extract, Bioactive compounds, GC-MS analysis.

INTRODUCTION

Nature has provided a complete store house of remedies to cure ailments of mankind. The knowledge of drugs has accumulated over thousands of years as results of man's inquisitive nature so that we possess many effective means of ensuring health care [1]. Medicinal plants remain an endless source of new drugs, new drug leads and new chemical entities [2]. In developing countries, medicinal plants have been the most accessible source of medicaments and in rural areas, traditional medicine is part of the first line treatment for common pathologies [3]. Plants are good sources for new safe, biodegeradable and renewable drugs. The medicinal plant used as therapeutic agent in addition to being used as food in many countries [4]. Presently there is an increasing interest worldwide in herbal medicines accompanied by increased laboratory investigations into the pharmacological properties of the bioactive ingredients and their ability to treat various diseases. Various drugs have entered into the international market through exploration of ethnopharmacology and traditional medicine. Although scientific studies are carried out on a large number of plants but smaller number of marketable drugs or phytochemical entities has entered the evidence based therapeutics [5]. This has led to intensified efforts on the documentation of medicinal plants [6].

The genus *Caralluma* (Asclepiadaceae) which are comprises about 200 genera and 2500 species. The member of the genus is small plant, erect, fleshy. *Caralluma diffusa* is rare and an endemic succulent medicinal plant of Southern Western Ghats of Tamil Nadu [7]. The species of *Caralluma* found in India are edible and form the part of the traditional medicine system of the country [8]. The species of this family possess significant anti-inflammatory, antitumor, anticancer, antiulcer activity and cytoprotective, analgesic, antioxidant, hypolipidemic, antihyperglycemic, antidiabetic, treating paralysis and joint pain and antipyretic properties [9]. The major constituents of *Caralluma* species is pregnane glycosides, saponin and flavonoids [10]. Hence, the present investigation was carried out identify bioactive constituents of *Caralluma diffusa* by preliminary phytochemical screening and Gas chromatography-mass spectrometry (GC-MS) analytical method.

MATERIALS AND METHODS

The aerial part of *Caralluma diffusa* (Wight). N.E.Br was collected from the Western Ghats of Coimbatore, Tamil Nadu, India. The aerial plant parts were washed thoroughly for four times in running tap water to remove soil particles and finally with sterile distilled water. The aerial portions (fleshy stem) were cut, shade dried, ground into fine powder and stored in air tight plastic container until use.

Extraction of the plant material

About 125 g of the powdered plant material was subjected into successive solvent extraction in soxhlet extractor using petroleum ether, acetone and methanol. The aqueous extract was prepared by soaking 10 g of powdered sample in 100 ml distilled water followed by rotary shaker for 12 h. The water extract is filtered by using Whatman No. 1 filter paper. All the extracts were concentrated using a rotary evaporator under reduced pressure and dried in a vaccum oven for further usage.

Phytochemical screening

The petroleum ether, acetone, methanol and aqueous extracts were subjected into phytochemical screening for the presence of alkaloid, carbohydrates, tannins, saponin, flavonoid, steroids, terpenoid, glycosides, cardiac glycosides, phenol, fixed oils and fats. Phytochemical analyses of the extracts of plant were carried out the bioactive compounds were determined by the standard methods [11,12,13].

Gas Chromatography – Mass spectrum analysis

GC-MS analysis on the methanol extract of *Caralluma diffusa* was carried out at Sargam Laboratory, Chennai. GC-MS analysis of this extract was performed using a GC Claurus 500 Perkin Elmer system comprising a AOC-20i auto sampler and gas chromatography interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: Column Elite-1

fused silica capillary column (30 mm x 0.25 mm ID x 1µM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70eV; helium (99.999%) was used as the carrier gas at a constant flow rate of 1ml/min and an injection volume of 2 µl was employed (split ratio of 10:1) injector temperature 240°C; Ion-source temperature 200°C. The oven temperature was programmed from 110°C (isothermal for 2 minutes), with an increase of 10°C/minutes, to 200°C, then 5°C/minutes to 280°C, ending with 9 minutes isothermal at 280°C. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC running time was 20 minutes. 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 bioactive compounds

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 1,000,000 patterns. The mass spectrum of the unknown components was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the bioactive compounds of the test materials were ascertained.

RESULTS

The results obtained from the preliminary phytochemical screening of *Caralluma diffusa* revealed that the presence of terpenoids, alkaloids, carbohydrates, steroid, glycoside, cardiac glycoside, saponin, phenol, flavonoid, fatty acid and fixed oil. Methanol extract were showed positive results for maximum amount of phytoconstituents when compared with acetone, petroleum ether and aqueous extract. At the same time phytochemical constituents like tannin were absent in these four extract (Table 1). The compounds present in the methanol extract of *Caralluma diffusa* were identified by GC-MS analysis. The GC-MS analysis of *Caralluma diffusa* revealed that the presence of eighteen compounds that could contribute the medicinal quality of the plant (Table 2). The active principles with their retention time (RT), molecular weight (MW), molecular formula (MF) and peak area in percentage are presented in Table 2 and Figure 1. It was found that main phytoconstituents of aerial parts are 2-Furancarboxaldehyde, 5-(hydroxymethyl) (28.67%), 1, 5-Anhydro-6-Deoxyhexo-2,3-Diulose (17.19%), 1,3-Propanediol, 2-ethyl-2-(hydroxymethyl) (11.89%), n-Hexadecanoic acid (8.32%), Oleic Acid (6.21%) (Figure 2-6). Our results showed the presence of flavonoids, fatty acid, glycoside and phenol derivatives in the aerial part of *Caralluma diffusa*.

Table 2: Phytochemical components identified in the methanol extract of aerial part of *C. diffusa* by using GC-MS showing their RT, peak area percentage, molecular weight, molecular formula and their biological activity.

S. No.	RT	% of Peak area (%)	Name of the compound	MW	MF	Compound nature	Biological activity
1	5.294	2.69	3-Acetoxydodecane	228	$C_{14}H_{28}O_2$	Fatty acid	No activity
2	5.941	4.48	Cyclopentane, 1-acetyl-1,2-epoxy-	126	$C_7H_{10}O_2$	Steroids	Precursor for cyclopentane monoterpenoid synthesis
3	7.015	17.19	1,5-Anhydro-6-Deoxyhexo-2,3-Diulose	144	C_6H8O_4	Glycoside	Preservative
4	7.558	2.47	Benzoic acid	122	$C_7H_6O_2$	Phenolic acid	Antimicrobial, antimutagenic, keratolytic, antioxidant, algicidal
5	8.477	28.67	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	126	$C_6H_6O_3$	Aldehyde	Antimicrobial, preservative
6	8.691	3.90	1,2,3-Propanetriol, Diacteate	176	$C_7H_{12}O_5$	Lipids	Antifungal, cosmetics, food additives, generate NAD^+ in brain and skeletal muscle cells of mammals
7	9.424	3.37	Heptanoic acid, 6-oxo-	144	$C_7H_{12}O_3$	Fatty acid	Antiviral
8	9.692	1.59	CIS-Dimethyl Morpholine	115	$C_6H_{13}NO$	Alkaloid	Fungicide, antibiotics, antitumor agent
9	10.615	0.46	Propane, 2-isocyanato-2-methyl-	99	C_5H_9NO	Terpenes	They are used in the synthesis for the target molecules such as pharmaceuticals, pesticides, textile softener, lubricants and industrial disinfectants.
10	10.896	1.13	2-Furanmethanol, tetrahydro	102	$C_5H_{10}O_2$	Furfuryl alcohol	Coupling solvents for pesticides and textile auxiliaries
11	12.176	11.89	1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)-	134	$C_6H_{14}O_3$	Polyphenol	Additive for perfumes, skin and hair care products
12	12.872	1.30	3',5'-Dimethoxyacetophenone	180	$C_{10}H_{12}O_3$	Phenol	Antioxidant, analgesic, antipyretic and anti-inflammatory.
13	15.056	2.08	Tetradecanoic acid	228	$C_{14}H_{28}O_2$	Myristic acid	Hypocholesterolemic, nematicide, antioxidant, lubricant, cancer preventive
14	16.688	0.67	Heptadecanoic acid, methyl ester	284	$C_{18}H_{36}O_2$	Saturated fatty acid (Margaric acid)	Antimicrobial, antioxidant
15	16.930	1.29	9-Hexadecenoic acid	254	$C_{16}H_{30}O_2$	Palmitic acid	Increasing insulin secretion
16	17.132	8.32	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	Palmitic acid	Lubricant, anti-androgenic flavor, hypocholesterolemic, flavor, hemolytic, antioxidant, nematicide, pesticide, 5-alpha reductase inhibitor
17	18.801	6.21	Oleic Acid	282	$C_{18}H_{34}O_2$	Mono unsaturated fatty acid	Anti-inflammatory, anti-androgenic flavor, cancer preventive, dermatitigenic hypocholesterolemic, 5-alpha reductase inhibitor, anemiagenic, insectifuge
18	19.000	2.29	Octadecanoic acid	284	$C_{18}H_{36}O_2$	Stearic acid	Cosmetics, lubricant, used to produce dietary supplements and anti-inflammatory.

Table 1: Preliminary phytochemical analysis of aerial part extracts of *Caralluma diffusa.*

Bioactive compounds	Phytochemical test	Petroleum ether	Acetone	Methanol	Aqueous
Alkaloids	Dragendorff test	-	+	+	-
	Mayer's test	-	+	+	-
	Wagner's test	-	+	+	-
Carbohydrates	Molisch's test	-	++	+++	-
	Benedict's test	-	++	+++	-
	Fehlig's test	-	++	+++	-
Flavonoids	Ferric chloride test	-	+	+++	++
	Lead acetate test	-	+	+++	++
	Shinoda test	-	+	+++	++
Phenol	Ferric chloride test	+	+	++	+
	Ellagic acid test	+	+	++	+
Terpenoids	Salkowski test	+	++	++	-
	Liberman–Burchard's test	+	++	++	-
Tannins	Ferric chloride test	-	-	-	-
	Lead acetate test	-	-	-	-
Glycosides	Keller – Killiani test	-	-	+	+
Steroids	Salkowski test	-	-	+++	+
	Liberman – Burchard's test	-	-	+++	+
Cardiac Glycosides	Keller – Killiani test	-	-	+	+
Fixed oils and Fats	Spot test	+++	-	-	-
Saponin	Frothing test	-	-	++	++

+ indicates weakly present, ++ indicates moderately present, +++ indicates strongly present, -indicates absent

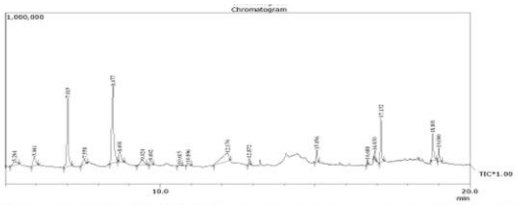

Fig. 1: GC-MS Chromatogram of methanolic extract of *Caralluma diffusa*

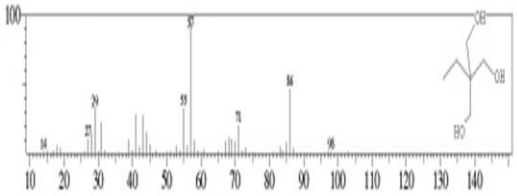

Fig. 4: 1,3-Propanediol, 2-ethyl-2-(hydroxmethyl)-

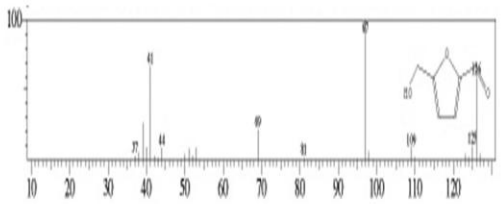

Fig. 2: 2-Furancarboxaldehyde, 5-(hydroxymethyl)-

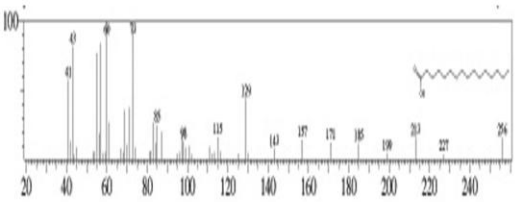

Fig. 5: n-Hexadecanoic acid

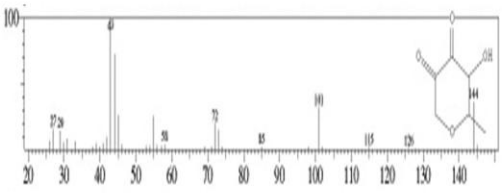

Fig. 3: 1,5-Anhydro-6-deoxyhexo-2,3-diulose

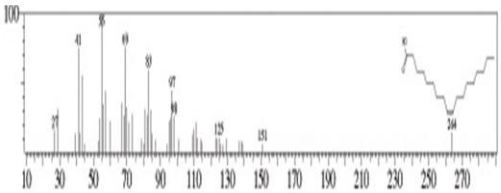

Fig. 6: Oleic acid

DISCUSSION

In the present study, the qualitative phytochemical screening of petroleum ether, acetone, methanol and aqueous extracts of *C.diffusa* revealed that the presence of alkaloid, terpenoids, steroid, glycoside, cardiac glycoside, saponin, phenol, flavonoid, fatty acid and fixed oil. GC-MS chromatogram of the methanol extract showed 18 peaks indicating the presence of eighteen phytochemical constituents. The fragmentation pattern of the major compound are 2-Furancarboxaldehyde, 5-(hydroxymethyl) retention time is 8.477 and peak area percentage is 28.67 have mainly antimicrobial activity. Fatty acid like Heptanoic acid, 6-Oxo has antiviral activity. Tetradecanoic acid is an antioxidant, cancer preventive, hypocholesterolemic, nematicide, lubricant properties and 9-Hexadecenoic acid is an increasing insulin secretion. The n-Hexadecanoic acid and Oleic acid possesses anti-androgenic flavor, hypocholesterolemic, hemolytic, nematicide, pesticide, 5-alpha reductase inhibitor, antioxidant, anti-inflammatory, anti-androgenic, cancer preventive, dermatitigenic, anemiagenic and insectifuge properties of this plant.

The methanol extract of *Catharanthus* species exhibited more phytochemical constituents were identified and responsible for antibacterial activities [14,15]. Oleic acid and n-Hexadecanoic acid were major phytochemical compounds of methanol extract of *Caralluma fimbriata* [16] and *Cissus quadrangularis* [17], ethanol extract of *Tylophora indica* [18]. GC-MS analysis of methanol extracts of *Gmelina arborea*, *Garcinia indica* [19] and ethanol extract of *Vitex altissima* revealed that the presence of n - hexadecanoic acid, octadecanoic acid, tetradecanoic acid [20]. The n-hexadecanoic acid, methyl/ethyl ester of Hexadecanoic acids are considered as fatty acids and these play crucial role in biological process [21]. Presence

of high levels of unsaturated fatty acids in tribal pulses are nutritionally desirable and also are comparable with some edible legumes like Goa bean and Soybean [22]. 2-Furancarboxaldehyde, 5-(hydroxymethyl) has the property of antimicrobial and preservative as reported by earlier workers [17, 23, 24].

CONCLUSION

From the present study, it is concluded that the threatened plant *Caralluma diffusa* are highly valuable in medicinal usage for the treatment of various human diseases along with bioactive compounds present in this plant. So it is recommended as a plant of phytopharmaceutical importance in drug industries. However, further studies will need to be undertaken to ascertain fully its bioactivity, toxicity profile, drug development.

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