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Research Article

DETERMINATION OF BIOACTIVE COMPONENTS OF *CARALLUMA UMBELLATA* HAW. (APOCYNACEAE) BY GAS CHROMATOGRAPHY AND MASS SPECTROSCOPY ANALYSIS

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ABSTRACT

Objective: The aim of this study was to carry out Gas Chromatography and Mass Spectroscopy (GC–MS) studies and to determine the bioactive compounds of *Caralluma umbellata* whole plant extract.

Methods: Whole plant of *C. umbellata* was cleaned, shade dried, and pulverized to powder in a mechanical grinder. Required quantity of powder sample was weighed and transferred to stoppered flask separately and treated with ethanol until the powder is fully immersed. The flask was shaken every hour for the first 6 h and then it was kept aside and again shaken after 24 h. This process was repeated for 3 days and then the extracts were filtered. The extracts were collected and evaporated to dryness using vacuum distillation unit.

Results: A total of 29 components of *C. umbellata* whole plant were identified. The prevailing components in the ethanol extract of whole plant were betulin (16.11%), campesterol (11.69%), 5α-Ergost-8(14)-ene (10.16%), 5-androsten-17α-ethynyl-3β, 17β-diol (9.06%), β-sitosterol (8.17%), cholestane-3,7,12,25-tetrol, tetraacetate (3α,5β,7α,12α) (7.94%), β-amyrin (7.02%), stigmasterol (6.78%), 13,16-octadecadiynoic acid, methyl ester (3.53%), lanosterol (3.51%), trans-geranylgeraniol (3.25%), Vitamin E (2.90%) and retinol, acetate (2.81%).

Conclusion: The application of *C. umbellata* for various ailments is confirmed by the presence of various bioactive compounds by traditional practitioners.

Keywords: *Caralluma*, Bioactive compound, Betulin, β-sitosterol, β-amyrin.

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INTRODUCTION

The nutritional incidence in the phytochemical compounds and their role in health and disease increased the interest in researching about the compounds [1,2]. Studying about the organic compounds from plants and their activities has increased rapidly in recent years. Using phytochemical analysis of plants in folklore resulted in the production of number of compounds with various pharmacological activities. Plants with rich metabolites such as tannins, terpenoids, alkaloids, and flavonoids have been found to process various biological properties. In recent years, there is an increase in the usage and search for drugs and dietary supplement which are derived from plants [3,4]. There is a requirement in the regularity of plant materials. Various pharmacopoeia holds the monographs of the plant materials which illustrates only its physicochemical parameter. The herbals and its formulations can be properly regulated using the modern methods of identification and quantification of active constituents in the plant material. Furthermore, the WHO has emphasized the need of ensuring the quality of production of medicinal plants using modern controlled techniques and applying suitable standards [5-7]. Gas Chromatography and Mass Spectroscopy (GC–MS) is the best approach to label the bioactive constituents of long-chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino acid and nitro compounds, etc. [8].

Caralluma R. Br. (family Apocynaceae) is a xerophytic genus of 120 species. Various medicinal uses of *Caralluma* species which is documented in the Arabic and Indian traditional medicine includes the treatment of diabetes, cancer, tuberculosis, snake and scorpion bites, skin rashes, scabies, fever, and inflammation [9-11]. Various species of the genus *Caralluma* are rich in pregnane glycosides, flavones, and megastigmane glycosides and several esters which accredit its medicinal importance [12]. Antioxidant, anticancer, antidiabetic, anti-inflammatory, antimicrobial, antieczemic, antimalarial, and antifungal properties of various *Caralluma* extracts exposed its pharmacological importance [13]. The medicinal importance of this plant was taken into consideration, and the ethanol extract of the whole plant of *Caralluma umbellata* was examined for the 1st time using GC–MS. Research in literature reveals that there is a lack in the information on the GC–MS analysis of *C. umbellata*. Hence, the objective of the present study is to identify the phytochemical constituents with the help of GC–MS techniques.

METHODS

Collection of plant sample

The whole plant of *C. umbellata* was collected from Parvathipuram, Kanyakumari District, Western Ghats, Tamil Nadu. The plant was identified with the help of local flora and the same is authenticated in Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu. The voucher specimens were preserved in the Ethnopharmacology Unit, Research Department of Botany, V.O. Chidambaram College, Tuticorin, Tamil Nadu.

Preparation of plant extract

Whole plant of *C. umbellata* was cleaned, shade dried, and pulverized to powder in a mechanical grinder. Required quantity of powder samples was weighed and transferred to a stoppered flask separately. This is treated with ethanol until the powder is fully immersed. The flask was shaken every hour for the first 6 h and then it was kept aside and again shaken after 24 h. This process was repeated for 3 days and then the extracts were filtered. The extracts were collected and evaporated to dryness using vacuum distillation unit. The final residue thus obtained was then subjected to GC–MS analysis.

GC–MS analysis

GC–MS analysis of ethanol extract was performed with GC Clarus 500 Perkin Elmer system and GC interfaced to a MS equipped with an

Elite-1 fused silica capillary column (30 mm × 0.25 mm 1D × 1 um df, composed of 100% dimethylpolysiloxane). For GC–MS detection, electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1 ml/min and an injection volume of 2 µl was employed (Split ratio of 10:1); 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 0.5 s and fragments from 45 to 450 Da. Total GC running time was 36 min. The relative % 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 Turbomass. Interpretation on mass spectrum of GC–MS was done using the database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight (MW), and structure of the components of the test materials were ascertained.

RESULTS

The compounds present in the ethanol extract of whole plant of *C. umbellata* were identified by GC–MS analysis (Fig. 1).

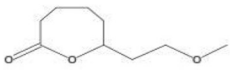
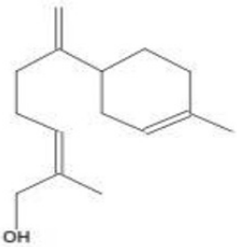
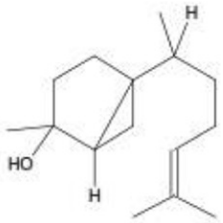
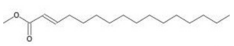
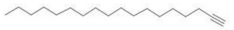
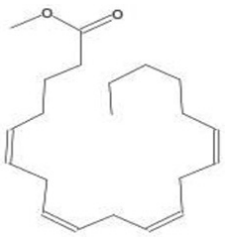
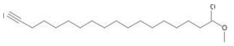
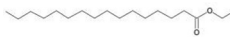
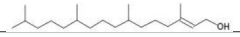
The active principles with their retention time (RT), molecular formula, MW, molecular structure, and concentration % in the ethanol extract of whole plant of *C. umbellata* are presented in Table 1.

The prevailing compounds in ethanol extract of whole plant were betulin (16.11%), campesterol (11.69%), 5α-ergost-8(14)-ene (10.16%), 5-androsten-17α-ethynyl-3β, 17β-diol (9.06%), β-sitosterol (8.17%), cholestane-3,7,12,25-tetrol, tetraacetate (3α,5β,7α,12α) (7.94%), β-amyrin (7.02%), stigmasterol (6.78%), 13,16-octadecadiynoic acid, methyl ester (3.53%), lanosterol (3.51%), trans-geranylgeraniol (3.25%), Vitamin E (2.90%) and retinol, and acetate (2.81%). Table 2 summarizes the major phytocomponents and its biological activities obtained through GC–MS study of *C. umbellata.*

DISCUSSION

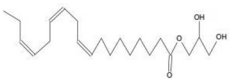
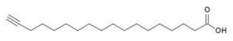
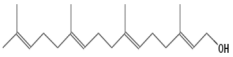
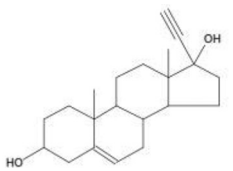
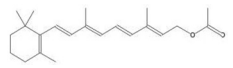
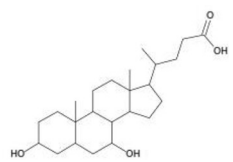
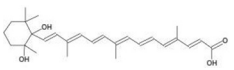
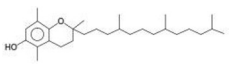
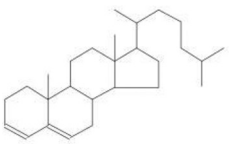
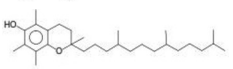
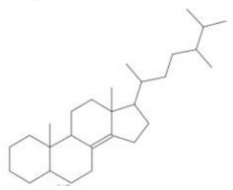
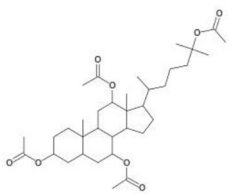
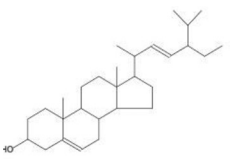
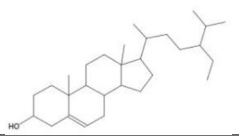
Authentication of medicinal plants as genetic and chemical level is a critical step in the use of their botanical materials for both research purposes and commercial preparations. For any living organism, identity is very important in order to distinguish itself from other organisms within the population and other populations. During this molecular stage, in plant taxonomy, the morphological characters which played a key role in plant systematic study are used as an implement for the categorization of a taxon. In the current era, anatomical, cytological,

Table 1: Phytocomponents detected in whole plant of *C. umbellata*

RT	Name of the compound	Molecular formulae	Molecular weight	Peak area %	Molecular structure
7.20	Octanoic acid, 6-hydroxy-8-methoxy-, ε-lactone	$C_9H_{16}O_3$	172	0.03	
11.27	Lanceol, cis	$C_{15}H_{24}O$	220	0.51	
11.42	7-epi-trans-sesquisabinene hydrate	$C_{15}H_{26}O$	222	0.47	
13.36	2-Hexadecenoic acid, methyl ester, (E)-	$C_{17}H_{32}O_2$	268	0.30	
13.41	1-Octadecyne	$C_{18}H_{34}$	250	0.44	
14.39	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	$C_{21}H_{34}O_2$	318	0.10	
14.66	17-Octadecynoic acid, methyl ester	$C_{19}H_{34}O_2$	294	0.53	
15.38	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	0.32	
16.99	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	0.56	

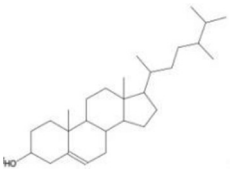
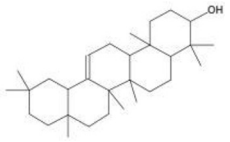
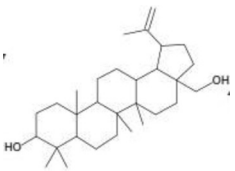
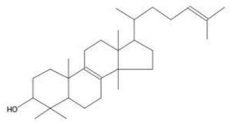
(Contd...)

Table 1: ***(Continued)***

RT	Name of the compound	Molecular formulae	Molecular weight	Peak area %	Molecular structure
17.68	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z, Z, Z)-	$C_{21}H_{36}O_4$	352	0.07	
21.62	17-Octadecynoic acid	$C_{18}H_{32}O_2$	280	0.36	
23.11	9-Eicosyne	$C_{20}H_{38}$	278	0.50	
26.38	13,16-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	290	3.53	
27.30	trans-Geranylgeraniol	$C_{20}H_{34}O$	290	3.25	
27.44	5-Androsten-17α-ethynyl-3β,17β-diol	$C_{21}H_{30}O_2$	314	9.06	
28.23	Retinol, acetate	$C_{22}H_{32}O_2$	328	2.81	
28.45	Ursodeoxycholic acid	$C_{24}H_{40}O_4$	392	0.48	
28.60	Azafrin	$C_{27}H_{38}O_4$	426	0.58	
30.08	β-Tocopherol	$C_{28}H_{48}O_2$	416	0.87	
31.44	Cholesta-3,5-diene	$C_{27}H_{44}$	368	0.92	
31.73	Vitamin E	$C_{29}H_{50}O_2$	430	2.90	
33.06	5α-Ergost-8 (14)-ene	$C_{28}H_{48}$	384	10.16	
33.26	Cholestane-3,7,12,25-tetrol, tetraacetate, (3α,5β,7α,12α)-	$C_{35}H_{56}O_8$	604	7.94	
33.60	Campesterol	$C_{28}H_{48}O$	400	11.69	
34.31	Stigmasterol	$C_{29}H_{48}O$	412	6.78	

(Contd...)

Table 1: ***(Continued)***

RT	Name of the compound	Molecular formulae	Molecular weight	Peak area %	Molecular structure
35.71	β-Sitosterol	$C_{29}H_{50}O$	414	8.17	
36.61	β-Amyrin	$C_{30}H_{50}O$	426	7.02	
37.96	Betulin	$C_{30}H_{50}O_2$	442	16.11	
39.49	Lanosterol	$C_{30}H_{50}O$	426	3.51	

C. umbellata: Caralluma umbellata

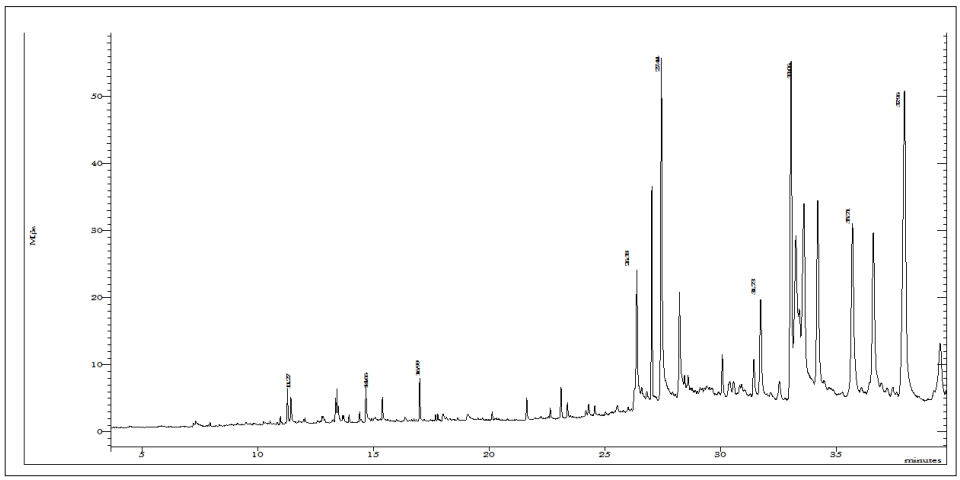


Fig. 1: Gas chromatography and mass spectroscopy chromatogram of the ethanol extract of whole plant of *Caralluma umbellata*

and biochemical parameters are also utilized along with morphological markers to sort out the organisms [14]. GC–MS is an esteemed tool for gaining reliable identification of phytocompounds [15].

Research has indicated that certain cancers, namely, ovarian, prostate, breast, and colon cancers can be obviated using stigmasterol. Studies also reveal that increasing phytosterols in diet will restrict the cholesterol absorption and reduce the serum cholesterol levels by attempting the intestinal absorption. When laboratory animals are fed with stigmasterol over a 6-week period, it is found that both cholesterol and sitosterol absorption lowered to 23% and 30%, respectively. It was exposed that it prevents several proinflammatory and matrix degradation mediators which were typically involved in osteoarthritis-induced cartilage degradation [16]. It also acquires potent antioxidant, hypoglycemic, and thyroid-inhibiting properties [17].

Vitamin E has various biological functions where the antioxidant function is the most important and well known [18]. Vitamin E also plays a key role in neurological functions [19]. Vitamin E also protects lipids and averts the oxidation of polyunsaturated fatty acids [20]. Alpha-tocopherol has been used by the researchers in the human supplementation studies regarding Vitamin E. Alpha-tocopherol usage affects the levels of other forms of Vitamin E by reducing the serum gamma and delta-tocopherol concentrations. The 2007 clinical study about alpha-tocopherol pointed out that the risk of major cardiovascular disorder happenings to middle aged and older men will not be reduced by supplementation [21].

β-amyrin has been found to exhibit antifungal and antimicrobial activity against some microbes. A recent research on the leaves of *Siraitia grosvenorii* explains that β-amyrin and other bioactive compounds were attained, and their activities against the growth of oral bacterial species *Streptococcus mutans*, *Actinobacillus actinomycetemcomitans*, and *Fusobacterium nucleatum* and the yeast *Candida albicans* were figured out *in vitro*. A slight inhibition of *S. mutans* and *F. nucleatum* was exhibited by β-amyrin [22].

Table 2: Activity of phytocomponents identified in the ethanol extract of whole plant of *C. umbellata*

Name of the compound	Peak area %	Compound nature	**Activity
Octanoic acid, 6-hydroxy-8-methoxy-, ε-lactone	0.03	Lactone compound	Antibacterial
Lanceol, cis	0.51	Sesquiterpene oxide	Antitumor, analgesic, antibacterial, anti-inflammatory, sedative, fungicide
7-epi-trans-sesquisabinene hydrate	0.47	Sesquiterpene alcohol	Antitumor, analgesic, antibacterial, anti-inflammatory, sedative, fungicide
5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	0.10	Unsaturated fatty acid ester	Cardioprotective, hypocholesterolemic
Hexadecanoic acid, ethyl ester	0.32	Palmitic acid ester	Antioxidant, hypocholesterolemic nematicide, pesticide, lubricant, antiandrogenic, flavor, hemolytic 5-alpha-reductase inhibitor
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.56	Diterpene alcohol	Antimicrobial, anti-inflammatory
9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (Z, Z, Z)-	0.07	Linolenic acid ester	Hypocholesterolemic nematicide, antiarthritic, hepatoprotective, antiandrogenic, hypocholesterolemic, 5-alpha-reductase inhibitor, antihistaminic, anticoronary, insectifuge, antieczemic, antiacne
trans-Geranylgeraniol	3.25	Alcoholic compound	Fragrance, source, antimicrobial
5-Androsten-17α-ethynyl-3β,17β-diol	9.06	Steroid	Antimicrobial, anti-inflammatory, anticancer, antiasthma, hepatoprotective, diuretic
Retinol, acetate	2.81	Vitamin A precursor	Vitamin A producer, antimicrobial, anti-inflammatory
Ursodeoxycholic acid	0.48	Acidic compound	Antimicrobial
Azafrin	0.58	Phenolic compound	Antimicrobial, anti-inflammatory, analgesic, antioxidant
β-Tocopherol	0.87	Vitamin E compound	Antiaging, analgesic, antidiabetic, anti-inflammatory, antioxidant, antidermatitic, antileukemic, antitumor, anticancer, vasodilator, antispasmodic, antibronchitic, anticoronary
Cholesta-3,5-diene	0.92	Steroid	Antimicrobial, anti-inflammatory, anticancer, antiasthma, hepatoprotective, diuretic
Vitamin E	2.90	Vitamin E	Antiaging, analgesic, antidiabetic, anti-inflammatory, antioxidant, antidermatitic, antileukemic, antitumor, anticancer, hepatoprotective, hypocholesterolemic, antiulcerogenic, vasodilator, antispasmodic, antibronchitic, anticoronary
5α-Ergost-8 (14)-ene	10.16	Steroid	Antimicrobial, anti-inflammatory, anticancer, antiasthma, hepatoprotective, diuretic
Cholestane-3,7,12,25-tetrol, tetraacetate, (3α,5β,7α,12α)-	7.94	Steroid	Antimicrobial, anti-inflammatory, anticancer, antiasthma, hepatoprotective, diuretic
Campesterol	11.69	Steroid	Antimicrobial, anti-inflammatory, anticancer, antiasthma, hepatoprotective, diuretic
Stigmasterol	6.78	Steroid	Antioxidant, anti-inflammatory, sedative, cancer-preventive, antiviral, ovulant, hypocholesterolemic, estrogenic, artemicide
β-Sitosterol	8.17	Steroid	Antimicrobial, anti-inflammatory, anticancer, antiasthma, hepatoprotective, diuretic
β-Amyrin	7.02	Triterpene	Antibacterial, antioxidant, antitumor, cancer-preventive, immunostimulant, chemopreventive, lipoxygenase-inhibitor, pesticide
Betulin	16.11	Triterpene	Antibacterial, antioxidant, antitumor, cancer-preventive, immunostimulant, chemopreventive, lipoxygenase-inhibitor, pesticide
Lanosterol	3.51	Triterpene	Antibacterial, antioxidant, antitumor, cancer-preventive, immunostimulant, chemopreventive, lipoxygenase-inhibitor, pesticide

**Source: Dr. Duke's phytochemical and ethnobotanical databases, *C. umbellate: Caralluma umbellata*

n-hexadecanoicacid,hexadecanoicacid,phytol,9,12,15-octadecatrienoic acid, and 13,16- octadecadienoic acid were identified in the ethanol extract of *C. umbellata* whole plant. Similarly, the research undergone by Arunkumar and Muthuselvam [23] and Kumar *et al*. [24] observed the presence of the above said compounds in the leaf extracts of *Aloe vera* and *Vitex negundo,* respectively. 9,12-octadecadienoic acid presents in the potential antioxidant and anticancer activities of the *Croton tiglium* seed was found in these compounds. Studies reported that the alcohol extract of the leaves of *Kigelia pinnata* [25] and *Melissa officinalis* [26] contains an element named hexadecanoic acid.

The first step in analyzing this GC–MS type is understanding the nature of active principle in the medicinal plants which result in further studies. However, productive results can be derived by isolating an individual phytochemical constituent and then instructing it to the biological probation. It could be concluded that the whole plant of *C. umbellata* contains several bioactive compounds. Hence, it is recommended as a plant of pharmaceutical importance. However, further, research is needed to attempt its bioactivity and toxicity profile.

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