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A Study of Phytochemical Constituents in *Caralluma Umbellata* By Gc-Ms Anaylsis.

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ABSTRACT: *Caralluma umbellata is known as a valuable medicinal plant. It has varied medicinal properties that can be exploited for the treatment of many diseases. The phytochemical analysis showed the presence of potent phytochemicals like flavanoids, terpenoids, tannius, glycosides sterols, phenols and saponins. These compounds correspond to varied medicinal properties that can be exploited for the treatment of many diseases.*

Keywords: *Phytochemical studies, Caralluma umbellata, GC – MS anaylsis.*

I. INTRODUCTION

Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. The research into plants with alleged folkloric use as pain relievers, anti-inflammatory agents, should therefore be viewed as a fruitful and logical research strategy in the search for new analgesic and anti-inflammatory drugs. *C. umbellata* is a perennial herb that is found in the wilds of Sri Lanka and in Nepal. In India, occasional in hilly regions of Orissa, Andhra Pradesh, Tamilnadu and in Karnataka.

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, succulent, perennial herb. Flower during January and June.

Plant derived drugs have a market of about and 20 billion annually in the United State alone. It is also estimated that only 5-15% of potential useful plants have so for been systematically explored for useful chemicals. There fore, there is great potential for using plant cultures for the production many highly valuable chemicals (Balasubramanian *et al.*, 1996).

Gas Chromatogarphy – Mass Spectrometry (GC – MS) is a method that combines the features of gas liquid chromatography and mass spectrometry to identify different substances with in a test sample. Applications 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 a 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. In 1996, the top of the line high speed GC-MS units completed analysis of the fire accelerants in less than 90 seconds, whereas first generation GC-MS would have required at least 16 minutes. The GC-MS is composed of two major building blocks; the gas chromatograph and the mass spectrometer. The gas chromatograph utilizes a capillary column which depends on the columns dimensions (length, diameter, film thickness) as well as the phase properties (e.g. 5% (phenyl) Poly siloxane).

The GC-MS has been widely heralded as a "gold standard" for forensic substance identification because it is used to perform a specific test. A specific test positively identifies the actual presence of a particular substance in a given sample. A non-specific test merely indicates that a substance falls into a category of substances although non-specific statistically suggests the identity of the substances, this could lead to false positive identification.

1. REVIEW OF LITERATURE

Phytochemical studies

The aim of the present study is to identify the bio-active phytocomponents of the plant by through GC-MS analysis of the bark extract of *Caralluma umbellate.* The most important bioactive constituents of the plants are alkaloids ,tannis,flavonoids and phenolic compounds. In India large number of plant species had been screened for their pharmacological properties but still a vast wealth of endangered species are unexplored. Medicinal plants are at interest to the field of biotechnology, as most of the drug industries depent in part on plants for the production of pharmaceutical compounds (Velmurgan *et al*., 2010).

(Ertuk et al., 2006) Recent studies are involved in the identification and isolation of new therapeutic compounds of medicinal importance from higher plants for specific diseases. The chemical composition of essential oil of Ocimun basilicum L.C.V. purple and Ocimun basilicum L.C.V. green, cultivated in Iran were investigated through GC – MS by (Seyed *et al.*, 2006), (Castello et al., 2002). Natural products derived from plants are being tested for presence of new drugs with new modes of pharmacological action. A special feature of higher plants is their capacity to produce a large number of secondary metabolites.

Mukherjee, 2002 Fresh stem were collected and air –dried at room temperature. The dried material was then homogenized to obtain coarse powder and stored in air-light bottles for further analysis. The shade dried, powdered stem were extracted with ethanol solvent by hot extraction using soxhlet apparatus collected and stored in a vial for further analysis. A fast and simple GC/MS method for lignan profiling in *Anthraces sylvestris* was done by albert Koulman *et al.,* (2001).

The combination of Gas Chromatograph and Mass Spectrometry (GC – MS) is a versatile technique for analyzing natural products in the early days of GC MS analysis of natural products. The technique was used for the analysis of volatile species such a flavours and essences (Mellon, 2000). However using appropriate derivatization procedures less volatile molecules can also be analyzed now. The essential oil of *Chrysanthemum maximum* was isolated and constituents were analyzed by GC/MS (Jozefcy *et al.,* 1999).

(Lafferly et al., 1989) The identify of the components in the extracts was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literature. NISTO8s.GC – MS analysis for the first time underivatized alkaloid mixture from *Narcissus pseudonarcissus* encouraged by the excellent results of (Kreh *etal.,* 1985) prior toils application in the plant genera. GC – MS has had a fairly long history in metabolic profiling (Horning and Horning, 1971) through the detailed study of metabolic disorders in humans. Analysis present in wet or freeze dried plants tissues are normally extracted with methanol – water prior to derivatization. Lipophitc components may be peartitiones using chloroform (Roessner *et al.*, 2000). The essential oil which were obtained from the leaves, flowers and stems of *Ocimum gratissimum* L were examinated by GC – MS fifteen constituents in the essential oil were identified with geraid, as the major constituent. Other major components included gamma muurolens beta caryophyllene, neural and limonene (Charles and Simon, 1992).

II. MATERIALS AND METHODS

1.1. Plant Material

The present investigation was carried out in plant *Caralluma Umbellata* (Figure - 1) that were collected from Alagirirattyapatty, Tiruchirappalli, District in Tamil Nadu.

1.2. Plant parts used in traditional medicine:

Aerial Parts of Shoot

1.3. Plant Sample Extraction

Twenty grams of powdered plant aerial parts of shoot is soaked in 50 ml of absolute alcohol overnight and then filteres through Whatman filter paper No.41 along with 2gms sodium sulphate to remove the sediments and traces of water in the filtrate. Before filing the filter paper along with sodium sulphate is wetted with absolute alcohol. The filtrate is then concentrated by bubbling nitrogen gas into the solution and reduces the volume to one ml. The extract contains both polar and non-polar phytocomponents of the plant material and the plant extract is injected in the Gas Chromatography – Mass Spectrometer.

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

1.4. Principle and application of GC-Ms Detector in Phytochemical analysis

GC-MS plays a key role in the analysis of unknown components of plant origin. GC-MS ionizes compounds and measures their mass numbers. Ionization is typically, the C.I (Chemical ionization) and E.I. (Electron ionization) The E.I. method provides good results for Quantitative analysis as well of the compounds and it is a highly selective method for interfering components. Gas Chromatography technique involves the

separation of volatile components in a test sample using suitable capillary column coated with polar and non-polar or intermediate polar, chemicals.

Elite-1 column (100% Dimethyl poly siloxane) is a non-polar column used for analysis of phytocomponents in medicinal plants and pesticide residues. Elite-5 column (5% phenyl and 95% methyl polysiloxane) is an intermediate column used for the estimation of pesticide residues in soft drinks and food grains. Elite wax (polyethylene glycol) is a polar column used in the estimation of fragrances in rice, alcohol, flower and fatty acid profile of edible oils. An inert gas such as hydrogen or nitrogen or helium is used as a carrier gas.

The components of test sample is evaporated in the injection port of the GC equipment and segregated in the column by adsorption and adsorption technique with suitable temperature programme of the oven controlled by software. Different components are eluted from the column based on the boiling point of the individual components. The GC column is heated in the oven between 60 to 270ºC. The time at which each component eluted from the GC column is termed as retention time (RT).

The eluted component is detected in the mass detector. The spectrum of the unknown component is compared with the spectrum of the known components stored of the NIST library and ascertains the name, molecular weight and fragrances, floral fragrances, pesticide residues. Therpenes, steroids, alkaloids and fatty acids are some of the useful components analyzed in the GC-MS study.

III. RESULT AND DISCUSSION

a. GC-MS Analysis

The GC-MS analysis revealed the presence of thirty two compounds in the leaves of Caralluma umbellate by comparing their retention times and by interpretation of their mass spectra. The compounds identified such as: Propane, 3 Hexadecyne, a-Sitosterol, Betulin, Ethyl iso-allochlate, Lupeol, Phytol, 2H-1-Benzopyran-2-one,3-(3[1,1-bipherny1]-4-y1-1,2,3,4-tetrahydro-1-naphthleny1)–4–hydroxyl, Psi.,.Psi, carotene 1,1',2,2' -tetrahydro-1, 1' -dimethoxy- [Table 1].

These compounds correspond to hypoglycemic, antimicrobial antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, antiandrogenic, flavor, hemolytic 5-Alpha reductase inhibitor, anti-inflammatory, anticancer, antieczemic, antiacane, antiandrogenic, antiarthritic, anticoronary, insectifuge properties. The result clearly indicates that *caralluma umbellata* has varied medicinal properties that can be exploited for the treatment of many diseases [Table 2].

Arunkumar and Muthuselvam (2009) reported in the GC-MS analysis 26bioactive phytochemical compounds were identified in the ethanolic extract of Aloe Vera. The identification of phytochemical compounds is based on the peak area, molecular weight and molecular formula. J. Sitosterol ($C_{29}H_{50}O$) with RT 38.78 had peak area 13.19%, Oleic acid ($C_{18}H_{34}O_2$) with RT (21.85) and 9,12,15-Octadecatrienoic acid, methyl ester (Z,Z,Z) ($C_{19}H_{33}O_2$) with RT 22.6 ranks net having peak area 11.74% and 11.36% respectively.

Ivanka Kostova *et al*. (2002) investigated fourteen aromatic and 24 aliphatic acids were determined by GC-MS analysis of acidic fractions obtained from Paronia peregrine and Paeonia tenuifolia roots. Benzoic acid and its monohydroxy-dihydroxy-and tri-hydroxy derivatives are the main acid compounds of both Paronia species. Some fractions could serve as a source of benzoic, 4-hydroxy benzoic, vanillic and gallic acids as well as of ethyl gallate.

Ahmed Al-harrasi and Salim Al-Saidi (2008) reported phytochemically centrified oleogum resin of Boswellia acra essential oil revealed the presence of 34 monterpense and 16 sequterpenes.

b. Analysis of Samples

The given sample was extracted with ethanol and analyzed in GC-MS for identification of different phytocomponents.

1. GC Programme

Column: Elite-5MS (5% Diphenyl /95% Dimethyl poly siloxane), 30 x 0.25µm df Equipment: GC Clarus 500 Perkin Elmer
Carrier gas: 1 ml per min, Split: 10:1
Detector: Mass detector Turbo mass gold-Perkin Elmer
Software: Turbomass 5.2
Sample injected: 3µl

Oven temperature Programme-

110o C-2 min hold
Up to 200º C at the rate of 10º C/min-No hold
Up to 280º C at the rate of 5 º C/min-9 min hold
Injected temperature 250 º C
Total GC running time 36 min

2. MS Programme

Library used NIST Version – Year 2005
Inlet line temperature 200 º C
Source temperature 200 º C
Electron energy: 70 eV
Mass scan (m/z): 45-450
Solvent Delay: 0-2 min
Total MS running time: 36 min

IV. CONCLUSION

This phytochemical analysis has shown the presence of potent phytochemicals like flavanoids, terpenoids, tannius, glycosides sterols, phenols and saponins. These compounds correspond to hypoglycemic, antimicrobial antioxidant, hypocholesterolemic, nematicide, pesticide, lubricant, antiandrogenic, flavor, hemolytic 5-Alpha reducatse inhibitor, anti-inflammatory, anticancer, antieczemic, antiacne, antiandrigenic, antiarthritic, anticoronary, insectifuge properties. The result clearly indicates that *Caralluma umbellata* has varied medicinal properties that can be exploited for the treatment of many diseases.

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FIGURE – 1

TABLE – 1

Components identified in *Caralluma umbellata*

[GC MS]

No.	RT	Name of the compound	Molecular formula	MW	Peak Area %
1.	11.62	3-Hexadecyne	$C_{16}H_{30}$	222	2.25
2.	14.96	Phytol	$C_{20}H_{40}O$	296	4.49
3.	27.64	Difenakum	$C_{31}H_{24}O_3$	444	4.82
4.	29.73	Betulin	$C_{30}H_{50}O_2$	442	4.33
5.	30.64	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	8.03
6.	31.15	Tetrahydrospirilloxanthin	$C_{42}H_{64}O_2$	600	8.99
7.	32.42	á-Sitosterol	$C_{29}H_{50}O$	414	14.77
8.	34.37	Lupeol	$C_{30}H_{50}O$	426	52.33

TABLE – 2

Activity of Phyto Components identified in *Caralluma umbellata*
[GC MS]

01.	11.62	3-Hexadecyne	$C_{16}H_{30}$	222	2.25	Unsaturated Hydrocarbon	No activity reported
02.	14.96	Phytol	$C_{20}H_{40}O$	296	4.49	Diterpene	Antimicrobial Antiinflammatory Anticancer Diuretic
03.	27.64	Difenakum	$C_{31}H_{24}O_3$	444	4.82	Coumarin compound	Antimicrobial Antiinflammatory Anticancer Antioxidant
04.	29.73	Betulin	$C_{30}H_{50}O_2$	442	4.33	Triterpene	Aniinflammatory Antimicrobial Anticancer Diuretic Chemo preventive
05.	30.64	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	8.03	Steroid	Aniinflammatory Antimicrobial Anticancer Antiasthma Diuretic Antiarthritic Antioxidant
06.	31.15	Tetrahydrospirilloxanthin	$C_{42}H_{64}O_2$	600	8.99	Carotene compound	Skin conditioner Nutrient
07.	32.42	á-Sitosterol	$C_{29}H_{50}O$	414	14.77	Steroid	Aniinflammatory Antimicrobial Anticancer Antiasthma Diuretic Antiarthritic Antioxidant
08.	34.37	Lupeol	$C_{30}H_{50}O$	426	52.33	Triterpene	Aniinflammatory Antimicrobial Anticancer Diuretic Chemo preventive