Chemical profiling and compound Isolation of different extracts of *Boucerosia pauciflora* Wight using GCMS Analysis

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Abstract: *Boucerosia* is a genus of xerophytic succulent herb included in the family Asclepiadaceae. Out of 13 species reported from India, 11 species occur in peninsular India, of these 5 species and 5 varieties are endemic. Plants belonging to this genus are rich in esterified poly hydroxy pregnane glycosides. The main objectives of the study is to identify the phytochemicals of rare endemic species *Boucerosia pauciflora* and also generate data on active principles with their retention time (RT, molecular formula, molecular weight (MW) and peak area in percentage. GCMS analysis was performed using JEOL GC MATE II GC-MS with data system. It is a high resolution double focusing instrument. For GC MS detection, an electron ionization system operated in electron impact mode with ionization energy of 70 ev. Interpretation of mass spectrum was conducted using data base of national institute standard and technology (NIST) & Wiley spectra libraries. The molecular weight, molecular formula and the number of hits was used to identify the name of the compound. Analytical studies on the Diethyl ether and Ethanol extract revealed the presence of 30 compounds of different types like carbohydrates, glycosides, phenols, steroids and flavonoids. Major compounds separated out were 2 hydroxy-3-cyano pyridine, propane dinitrile, ethylidene, Diethyl phthalate and trans-3-aethoxy-b-methyl-b-nitros etc. The identification of these compounds will help in the production of novel drugs for curing psoriasis, cancer, diabetes etc. Also this study justifies the use of whole plant for treating various ailments usually practiced by traditional healers.

Keywords: *Boucerosia pauciflora*, Retention time, JEOL GC MATE II GC-MS, NIST, Wiley Spectra, 2-Hydroxy-3-cyano pyridine.

INTRODUCTION

Compounds isolated from medicinal plants are nowadays tested for new drugs and pharmacological action. Protective effects of plants are mainly due to the presence of poly phenolic compounds and flavonoids (Mojab et al., 2006). Some toxic products in herbal supplement may cause hepatotoxicity and nephrotoxicity. So caution should be exercised before it is administered directly. The number of phytochemicals isolated so far, nature must still have many more in store. With the advances in synthetic methodology and the development of more sophisticated isolation and analytical techniques, many more of these phytochemicals should be identified.

The plants belongs to the Asclepiadaceae comprises of about 180 genera and 2200 species which are distributed mainly in the tropical and subtropical regions of the world. Asclepiadaceae plants are abundant in esterified polyhydroxy pregnane glycosides which is the main source of antitumor agents (Rizwani, 1991). Mostly the species of caralluma are edible and it is prescribed for the treatment of diabetes. It is nowadays used as an appetite suppressant, treat fever and stimulate central nervous system (Rajendran and Ramaswamy, 2004). Considering the case of Boucerosia of the total 120 species, 15 species are reported in India, 5 species and 5 varieties Endemic. It is a xerophytic diffuse fleshy herb with slender stem. Leaves are minute recurved, inflorescence is umbels reduced to single flower, Calyx-5,lobes narrow, glabrous and Corolla with fringed purple hairs, minutely banded purple concentric lines within. Staminal column short arise from the base of corolla and Ovary-is bicarpellary.

Solvents used for the extraction of biomolecules from plants are chosen based on the polarity of the solute of interest. A solvent of similar polarity to the solute will properly dissolve the solute. Multiple solvents can be used sequentially in order to limit the amount of analogous compounds in the desired yield. The polarity, from least polar to most polar, of a few common solvents used here are as follows: Diethyl ether < Acetone < Ethanol < Methanol < Water (Altemimi et al., 2007).

Gas chromatography mass spectrometry combines the features of Gas liquid chromatography and mass spectrometry to identify different substances within a test sample (Skoog et al., 2007). It separates different compounds in the sample into pulses of pure chemicals based on their volatility Oregon State University, 2012) by flowing an inert gas (mobile phase), which carries the sample, through a stationary phase fixed in the column (Skoog et al., 2007). Spectra of compounds are collected as they exit a chromatographic column by the mass spectrometer, which identifies and quantifies the chemicals according their mass-to-charge

ratio (m/z). These spectra can then be stored on the computer and analyzed (Oregon State University, 2012). The aim of the present study is to screen the phytochemicals present in the plant extracts of Boucerosia pauciflora and list out the bioactive compounds present in it by GC-MS analysis.

MATERIALS AND METHODS

Collection and identification of plant material

Plants were collected from Marunthuvazhmalai hill located in Kanyakumari district situated at the south most tip of the Indian peninsula, it is surrounded by the Bay of Bengal in the east, the Indian Ocean in the south and the Arabian Sea on the west. It stretches for more than a Km, reaching a height of 800 feet at the highest point. It is about 11 Km from Nagercoil. Plant specimens were identified by Dr. Solomon Jeeva, Associate Professor Scott Christian College, Nagercoil and the voucher specimens were stored in Department of Botany, Nesamony Memorial Christian College, Marthandam, Tamilnadu and the details were noted in the field notebook according to field and herbarium techniques.

Preparation of extract for GC-MS Analysis

The aerial stem of Boucerosia pauciflora is washed and homogenized. The shade dried plant parts were powdered and subjected to soxhlet extraction (70±10ºC temp.) with different solvent such as diethyl ether, acetone, methanol and water in 1:4 (w/v) ratios for 18 hrs. The extracts were concentrated using rotary vacuum evaporator (40°C; pressure 70±5 psi), the thick brown masses obtained were kept in vacuum desiccators for complete drying. The dried extracts were stored in air tight container and kept in refrigerator (<10°C). The extracts were suspended in distilled water and used for further pharmacological experiments. The dried plant powder is directly examined using ethanol as a solvent.

Preliminary phytochemical screening of the crude extract

The extract was subjected to preliminary phytochemical screening for the detection of various plant constituents present. The term qualitative analysis refers to the establishing and providing the identity of a substance. The pharmacological actions of crude drugs are determined by the nature of their constituents. The phyto constituents are responsible for the desired therapeutic properties. To obtain these pharmacological effects, the plant materials itself or extract in a suitable solvent or isolated active constituent may be used. The various extracts were subjected to the following chemical tests separately for the identification of various active constituents (Kokate, 1999). Of the five solvent taken only ethanol and diethyl ether extracts are taken for further GC-MS analysis.

Preparation of extracts for GC-MS analysis

The extract of ethanol and diethyl ether was dissolved in corresponding solvent and then filtered through Whatmann filter paper No.41 along with 2gm sodium sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulphate is wetted with chloroform. The filtrate is then concentrated by bubbling nitrogen gas into the solution. The extract contains both polar and non-polar phyto components of the plant material used. 2 µl sample of these solutions is employed for GC/MS analysis.

The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethyl polysiloxane, 30 m × 0.25 mm ID × 250µm df) and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The injector temperature was set at 260°C during the chromatographic run. The 1µL of extract sample injected into the instrument the oven temperature was as follows: 60 °C (2 min); followed by 300 °C at the rate of 10 °C min−1; and 300 °C, where it was held for 6 min. The mass detector conditions were: transfer line temperature 240 °C; ion source temperature 240 °C; and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments from 40 to 600 Da. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS NIST (2008) library.

Identification of Components

Interpretation on mass spectrum of GC-MS is conducted using the database of National Institute Standard and Technology (NIST-12, 62) having more than 62,000 patterns. The spectrum of the unknown component is 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 are ascertained.

RESULT

Preliminary phytochemical analysis of ethanol extract reveals the presence of carbohydrates, glycosides, amino acid, steroids and flavonoids. Alkaloids, protein, terpenoids, phenols, tannins and saponins are absent (Table 1). It was carried out directly with the help of powdered plant part together with ethanol as solvent. Sohxlet extraction of diethyl ether, acetone, methanol and water extract showed the presence of carbohydrates, alkaloids, glycosides and protein in different proportions ranging from 9-100mg/g

(Table 2, 3). Quantitative phytochemical analysis of Diethyl ether extract alone showed the presence of saponin, steroids, phenol and tannin. Considering the overall criteria diethyl ether and ethanol extracts are taken further for GC- MS analysis.

Table - 1: Phytochemical evaluation of Ethanol extract

S.No.	Test	Plant powder
1.	Alkaloids	-
2.	Carbohydrates	+
3.	Glycosides	+
4.	Terpenoids	-
5.	Proteins	-
6.	Amino acids	+
7.	Steroids	+
8.	Flavonoids	+
9.	Phenols	-
10.	Tannins	-
11.	Quinones	-
12	Anthraquinones	-
13	Saponins	-

QUANTITATIVE RESULT- TABLE 2

S.No	Test	TEST SAMPLE	mg/g
1	CARBOHYDRATE	Methanol	31
		Aqueous	32
		Acetone	9
		Diethyl ether	28
2	ALKALOID	Diethyl ether	20
3	GLYCOSIDE	Acetone	2.4
		Diethyl ether	3.6
4	PROTEIN	Methanol	100
		Aqueous	40
		Acetone	30
		Diethyl ether	75

Table -3: Photochemical Evaluation of DIETHYL ETHER Extract

	Methanol	Aqueous	Acetone	Diethyl Ether
Carbohydrate	Present	Present	Present	Present
Protein	Present	Present	Present	Present
Aminoacid	Present	Present	Present	Present
Alkaloid	Absent	Absent	Absent	Present
Flavanoid	Absent	Absent	Absent	Absent
Glycoside	Absent	Absent	Present	Present
Phenol	Absent	Absent	Absent	Absent
Glycoside	Absent	Absent	Present	Present

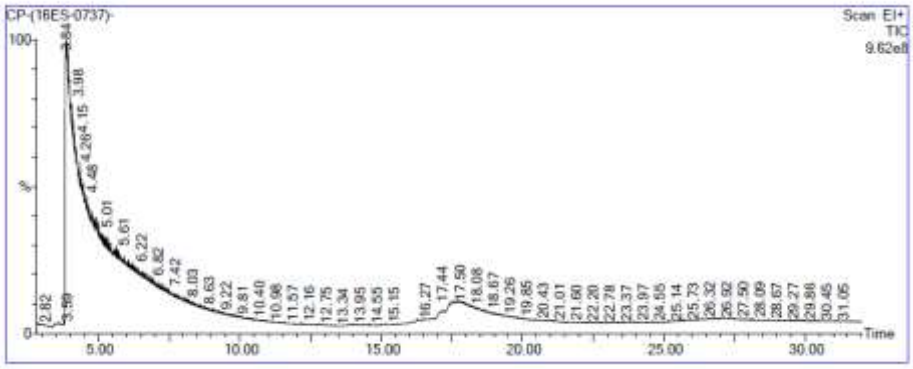
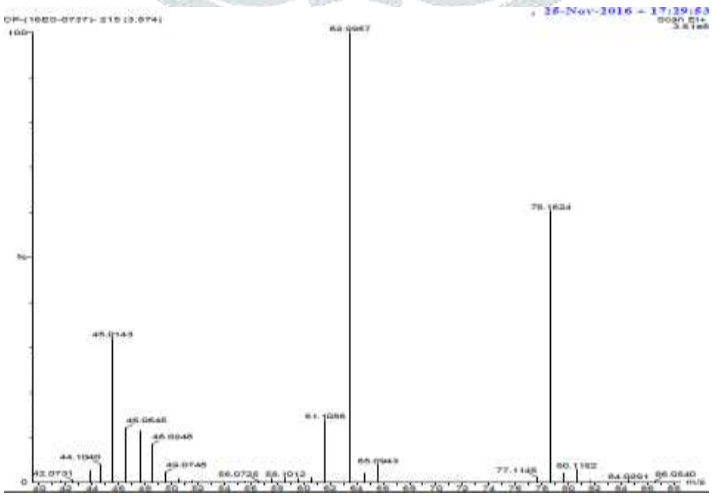


Figure: 1 GC-MS chromatogram of ethanol extract (CPE) of Caraluma pauciflora carried out using GC clarus 680 Perkin Elmer systems.

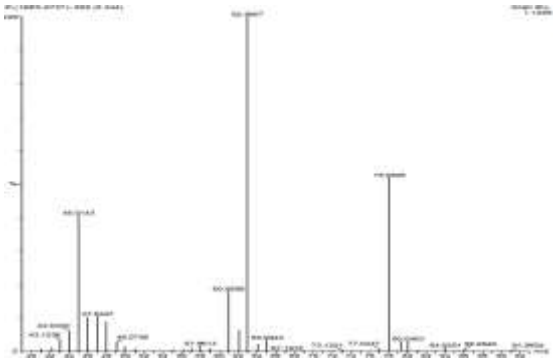
This extracts contributes to varying medicinal activities in proportion to the chemical nature and percentages of compounds present in it. The name of the compounds with their retention time (RT), molecular formula, Molecular Weight (MW), peak area (%) and the compound nature are given in following Table-4. The following figures show the NIST library profile and mass spectrum of compounds.

Table 4 - Details of compounds from GCMS by ethanol extract

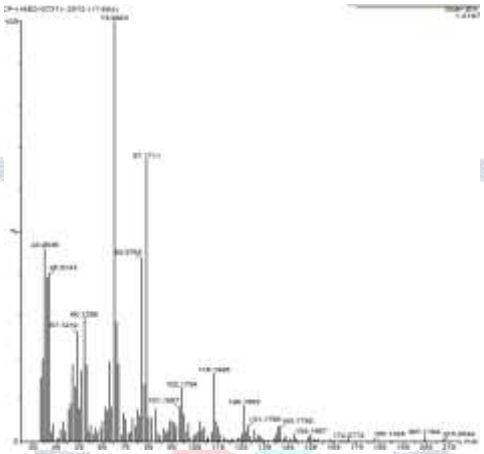
No	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1.	3.87	Dimethyl Sulfoxide	C_2H_6OS	78	59.921
2.	3.98	Butane, 1,1-diethoxy-2-methyl-	$C_9H_{20}O_2$	160	0.20
3.	5.344	Dimethylsulfoxonium formylmethylide	$C_4H_8O_2S$	120	33.387
4.	12.16	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1aà,4aà,7á,7aá,7bà)]- (Synonym: Spathulenol)	$C_{15}H_{24}O$	220	1.08
5.	12.75	1H-3a,7-Methanoazulene, octahydro-1,4,9,9-tetramethyl- (Synonyms: Patchoulane)	$C_{15}H_{26}$	206	0.61
6.	15.15	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$	296	3.87
7.	17.44	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	14.65
8.	17.664	Beta D mannofuranoside, Methyl	$C_7H_{14}O_6$	194	6.692
9.	19.85	Phytol	$C_{20}H_{40}O$	296	2.42
10.	20.43	9,12-Octadecadienoyl chloride, (Z,Z)-	$C_{18}H_{13}ClO$	298	11.08
11.	24.55	Heptacosane	$C_{27}H_{56}$	380	27.58



Mass spectrum of compound I

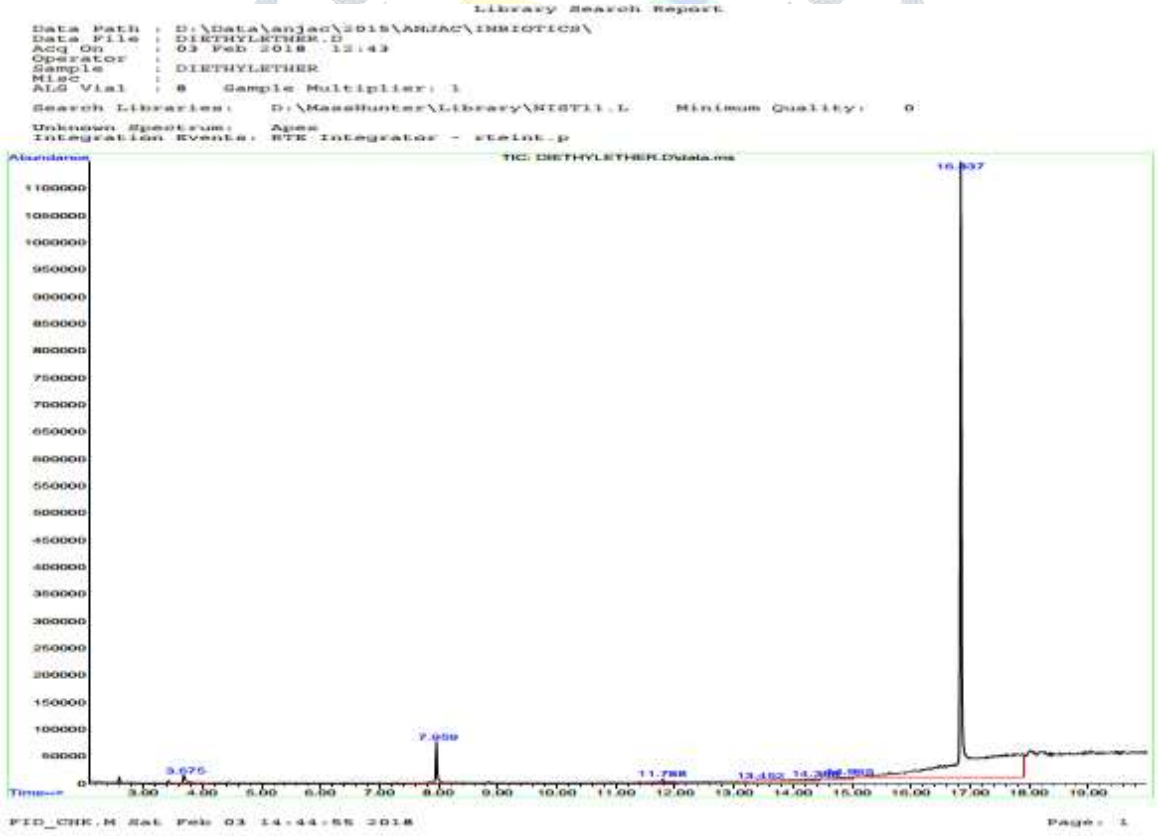


Mass spectrum of compound II



Mass spectrum of compound III

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of Diethyl ether extract



DISCUSSIONS

The curative properties of medicinal plants are due to the presence of various classes of secondary metabolites such as alkaloids,

flavonoids, glycosides, phenols, saponins, sterols etc (Liu, 1991; Yokoi, 1985). The phytochemical analysis conducted on Caralluma nilagiriana extract revealed the presence of different phytoconstituents including steroidal, triterpenoidal, phenolic and flavonoidal compounds (Akerale, 1991).The phytochemical analysis caralluma europaea revealed the presence of flavonoids, alkaloids and phenolic compounds with methanol and aqueous extract. Ethyl acetate extract only reveal the presence of tannin (Hadd hajj et al., 2016). Similar result was observed in the case of diethyl ether.

Sampath kumar et al 2014 mentioned the presence of terpenoids in hydroalcoholic extract of caralluma umbellate. Polyphenolic compounds such as gallic acid, vanillic acid, epicatechin, P- coumaric acid, ferulic acid, quercetin-3β-D glucose and rutin were identified ethanolic extract of caralluma arabica by Chevidenkandy et al., 2014. Using different polar solvent caralluma tuberculata extract provide high rate of phenolic and flavonoids (Rehman et al., 2014).

The GC-MS results of Caralluma trunco coronata revealed that the presence of Thunbergol (68.05%), Vitamin E (9.45%), Squalene and Lupeol (5.67%), β-Tocopherol (4.91%), Acetic acid, 5(dimethyle-6-oxocyclohexylidene) -3-methyl-pent-3-enyl lupeol has a complex pharmacology in humans, displaying antiprotozoal, antimicrobial, anti-inflammatory, antitumor and chemopreventive properties ester (3.78%), β-Sitosterol(1.89%) and Furan, 2-butyltetrahydro- (0.57%) (Margareth, et al., 2009). Among the identified phytochemicals 2 Hydroxy-3-cyanopyridine is having anticancer, antimicrobial, anticonvulsant and antipsoriasis properties.

CONCLUSION

The present study has revealed that ethanol and diethyl ether extract collected from Marunthuvazhmalai hills is found to have better level of chemical profiling. GC-MS analysis revealed the presence of 30 bioactive compounds. Analysing the active principles in Boucerosia pauciflora it was concluded that detailed study will provide valuable information regarding drug isolation. So it is recommended as a plant of phytopharmaceutical importance.

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