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GC-MS Profile of Methanolic Leaf Extract of *Baliospermum montanum* (Wild.) Muell. Arg

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ABSTRACT: *Baliospermum montanum (Wild.) Muell. Arg.*,is the member of euphorbiaceae. Ethanomedicine has evident the medicinal properties of different parts of this plant. Understanding the role of phytochemicals, current research is been focussed on separation of phytoconstituents by GC-MS technique using Perkin-Elmer Gas Chromatography– Mass Spectrometry. The mass spectra of the compounds found in the extract were matched with the National Institute of Standards and Technology (NIST) library. The compounds identified by GC-MS in methanolic leaf extract may be medicinally valuable and possess various pharmaceutical applications. The identified phytocomponents needs further research on toxicological aspects to develop safe drug.

KEYWORDS: *Baliospermum montanum*, Euphorbiaceae, phytochemicals, .GC-MS, medicinal properties

I. INTRODUCTION

Baliospermum montanum (Wild.) Muell. Arg., (Hindi: danti, Kannada: kaadu haralu, Malayalam: katalavanakku, Marathi: buktumbo Sanskrit: kakubha, Tamil: appaiccevakam, Telugu: ettadundiga) belongs to euphorbiaceae. *B. montanum* is a stout undershrub with numerous branches. Plant is 10 cm to 8 m in height with herbaceous branches from the roots. Leaves are simple, toothed with undulations. Upper leaves are small, lower ones are large, sometimes, 3-30 cm long, 1.5-15 cm broad. Male and female flowers are separated, seen in the same flowering branch, about 3 mm across, greenish yellow, arranged in axillary and terminal racemes, spikes or fascicles. Capsules are distinctly 3-lobed, obovoid, stony, 8-13 mm diameter, minutely densely pubescent. Seeds are egg-shaped. *B. montanum* is distributed throughout the sub-Himalayan region from Khasi Hills to Kashmir. It is common in Bihar, West Bengal, and Peninsular and Central India. Ethanobotany study has assured the medicinal properties of *B.montanum*. The demonstrated medicinal properties of *B. montanum* are listed in table: 1. The table depicts detailed information about the plant parts used and its known medicinal properties with the references.

Sl.No.	Plant part	Medicinal properties	references
1	seeds	rheumatism, gout and in gastric complaints	[1]
2	seeds	purgative, stimulant, rubefacient	[2, 3, 4, 5]
3	seeds	laxative	[6]
4	seeds	snake bite	[7]
5	seeds	constipation	[8]
6	leaves	Asthma & headache	[9, 4, 10]
7	stem	toothache	[11, 12]
8	roots	laxative	[12, 13, 14]
9	roots	dropsy, jaundice, anasarca	[3, 4]
10	roots	rheumatism, anemia	[15]
11	roots	jaundice, skin diseases, helminthic infections, leucoderma and	[5]

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		piles.	
12	leaf	Anti-inflammatory	[16]
13	-	Effectively used in loss of appetite, indigestion, liver disorders, intestinal gas and intestinal worms.	[17]
14		Asthma and bronchitis	[17]
15	Seed	purgative	[18]
16	Leaves	Purgative, antiasthmatic	[18]
17	Latex	body ache and pain of joints.	[18]
18	Root and seed oil	cathartic, antidropsical	[18]
19	leaves	Snake bite	[19]
20	roots	Anthelmintic, carminative, rubefacient and anodyne, used for abdominal pain, constipation, calculus, general anasarca, piles, helminthic, infestation, scabies, skin disorders, swelling and piles	[19]
21	root	heapatoprotective and analgesic activity	[20]
22	root	jaundice, leucoderma, skin diseases, wounds, and as an anthelmintic	[21]
23	Leaves	asthma, bronchitis	[22]
24	leaves	in treating abdominal tumor	[23]
25	seeds	Purgative and its oil as powerful hydragogue cathartic and applied externally in rheumatism.	[24]

Many evidences have shown that phytochemicals grounds the medicinal properties of medicinal plant and hence researchers are focussing on determining and separating the phytochemicals. A knowledge of the chemical constituents of plants is desirable not only for the discovery of therapeutic agents, but also because such information may be of great value in disclosing new sources of economic phytocompounds for the synthesis of complex chemical substances and for discovering the actual significance of folkloric remedies [25]. Chromatography is wildly employed for separating the components of mixture.

Chromatography is the term used to describe a separation technique in which a mobile phase carrying a mixture of components interacts selectively with the stationary phase. It also plays a fundamental role as an analytical technique for quality control and standardization of phyto therapeuticals [26].Gas chromatography-mass spectrometry (GC-MS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. Applications of GC-MS includes identification of phytochemicals and unknown samples, drug detection, fire investigation, environmental analysis, explosives investigation, and. GC-MS can also be used in airport security to detect substances in luggage or on human beings [27]. In the last few years GC-MS has become firmly established as a key technological platform for secondary metabolite profiling in both plant and non plant species [28, 29, 30].

II. MATERIAL AND METHODS

Collection of Plant material

B. montanum was collected from Sirsi, Western Ghats of Karnataka and now plant is being maintained in the Department of Molecular Biology, Bangalore University, Bangalore.

Preparation of plant extract

Leaves were collected from *B. montanum*. They were dried for one week at room temperature (in shade). Dried leaves were grinded in a blender to fine particles. Crude plant's leaf extract was prepared by soxhlet extraction method. 20 gm of dried fine grinded powder of leaves was uniformly packed into thimble and phytochemicals were extracted with 250

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mL of methanol. The extraction was carried out for 24 hours. Later extract was concentrated by keeping it on hot plate at 30 to 40°C and stored at 4°C for further research.

Preparation of stock solution: The soxhlet extracted methanolic leaf solution was filtered and concentrated in a rotary evaporator under reduced pressure (rotary vacuum flash evaporator). Methanolic extracts (1 µl) were injected for GC-MS analysis.

GC-MS analysis of the methanol extract of *B. montanum* (sample name- SB3), was performed using a Perkin–Elmer GC Clarus 500 system comprising an AOC-20i auto-sampler and a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with a Restek RtxR (5% diphenyl/95% dimethyl poly siloxane) fused a capillary column (30 × 0.25 µm ID × 0.25 µm). For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 1 µl was employed. The injector temperature was maintained at 280°C, the ion-source temperature was 200°C, the oven temperature was programmed for40°C (isothermal for 5 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. The solvent delay was 0 to 2 min, and the total GC/MS running time was 60 min. The mass-detector used in this analysis was Turbo-Mass Gold-Perkin-Elmer, and the software adopted to handle mass spectra and chromatograms was a Turbo-Mass ver-5.2.

III. RESULTS

Data Interpretation:

The sample (SB3) was subjected to GC-MS and the total separated peaks are shown in Figure 1. The figure showed major 4 peaks. Extracted ion chromatograms were obtained from all the major peaks. The mass of the compounds and fragments recorded were matched with NIST database for identification of probable compounds present in the sample.

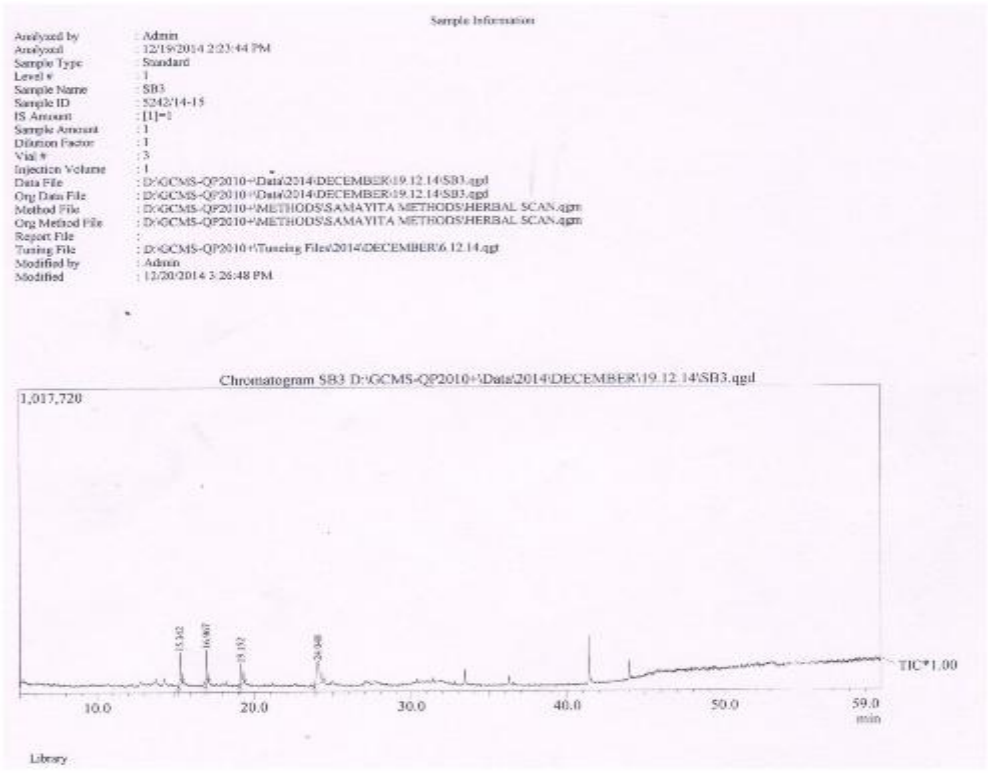

Fig.1: GC-MS analysis of leaf methanolic extract of *Baliospermum montanum*

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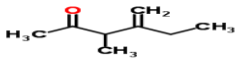
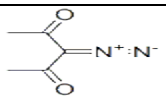
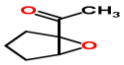
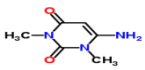
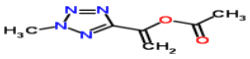
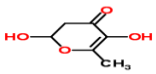
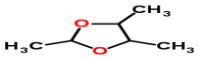
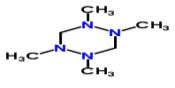
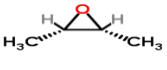
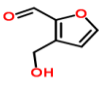
Identification of components

Interpretation on mass spectrum of GC-MS for *Baliospermum montanum* (SB3 sample) was done using the database of National Institute 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 chemical structures of major compounds matched with NIST Library are shown in table 2. This table demos compound name, its molecular formula, structure and its retention time

Table 2: Compounds present in the methanolic extract of *Baliospermum montanum*

SL. No.	Retention time	Compound name	Molecular formula	structure	Molecular weight
1	15.342	3-Methyl-4-methylene-2-hexanone	$C_8H_{14}O$		126 Da.
2	15.342	2,4-Pentanedione,3-diazo-	$C_5H_6N_2O2$		126 Da
3	15.342	Cyclopentane, 1-acetyl-1,2-epoxy-	$C_9H_{14}O_2$		154.206 Da
4	15.342	6-Amino-1,3-dimethyl-2,4(1H,3H)-pyrimidinedione	$C_6H_9N_3O_2$		155.155 Da
5	15.342	1-(2-Methyl-2H-tetrazol-5-yl)vinyl acetate	$C_6H_8N_4O_2$		168.153 Da
6	16.697	2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one	$C_6H_8O_4$		144.125 Da
7	16.697	2,4,5-Trimethyl-1,3-dioxolane	$C_6H_{12}O_2$		116.158 Da
8	16.697	1,2,4,5-Tetramethyl-1,2,4,5-tetrazinane	$C_6H_{16}N_4$		144.137497 Da
9	16.697	(2R,3S)-2,3-Dimethyloxirane	C_4H_8O		72.106 Da
10	19.150	Hydroxymethylfurfural	$C_6H_6O_3$		126.110 Da

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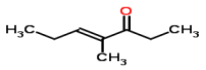
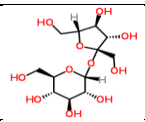
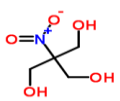
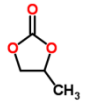
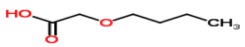
11	19.150	4E-Methyl-4-hepten-3-one	$C_8H_{14}O$		126.196 Da
12	24.050	Sucrose	$C_{12}H_{22}O_{11}$		342.297 Da
13	24.050	2-(Hydroxymethyl)-2-nitropropan-1,3-diol	$C_4H_9NO_5$		151.118 Da
14	24.050	Propylene carbonate	$C_4H_6O_3$		102.089 Da
15	24.050	Butoxyacetic acid	$C_6H_{12}O_3$		132.158 Da

Gas Chromatography-Mass Spectrometry Analysis

The methanol leaf extract of *B. montanum* (sample name- SB3 was analyzed by GC-MS and the chromatogram is shown in Figure 1. The relative retention times (Rt) and mass spectra of the extract components were compared with those of authentic samples and with mass spectra from the NIST library.GC-MS with NIST library analysis of *B. montanum* (sample name- SB3) resulted in the identification of fifteen major compounds viz. 3-methyl-4-methylidenehexan-2-one, 2,4-Pentanedione,3-diazo-, 1-(2,3-Dimethyl-6-oxabicyclo [3.1.0]hex-1-yl)ethanone, 6-Amino-1,3-dimethyl-2,4(1H,3H)-pyrimidinedione, 1-(2-Methyl-2H-tetrazol-5-yl)vinyl acetate, 2,3-dihydro-2,5-dihydroxy-6-methyl-4H-pyran-4-one, 2,4,5-Trimethyl-1,3-dioxolane, 1,2,4,5-Tetramethyl-1,2,4,5-tetrazinane, (2R,3S)-2,3-Dimethyloxirane, Hydroxymethylfurfural, -Methyl-4-hepten-3-one, Sucrose, 2-(Hydroxymethyl)-2-nitropropan-1,3-diol, Propylene carbonate and Butoxyacetic acid, Till this date no one has studied the GC-MS profile of *B. montanum*. We are the first one to report the GC-MS profile of this medicinal plant. GC-MS analysis of a related species of *Drypetes roxborghii* viz. *Pinus roxburghii* is been reported. The essential oil constituents was analysed using GC-MS by two different and independent authors. Wajahat A. Shah & Mahahpara Qadir (2014) reported seven compounds and seventeen compounds were found by Prabodh Satyal et al (2013). This type of GC-MS analysis is the first step towards understanding the nature of active principles of medicinal plants. Such studies assist to uncover enigma of nature and facilitate it to employ those principle for human and other species welfare.

IV. CONCLUSION

The methanolic leaf extract of *B. montanum* was cast for GC-MS analysis. GC-MS with NIST library study showed the presence of fifteen compounds. The identified compounds may have medicinally value and possess various pharmaceutical applications. The identified phytocomponents needs further research on toxicological aspects to develop safe drug.

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