GC-MS BASED PHYTOCHEMICAL SCREENING IN ETHYL ACETATE ROOT EXTRACT OF *BLEPHARISPERMUM SUBSESSILE* DC

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ABSTRACT

Historically Plants have been the exemplary source of medicine for thousands of years. *Blepharspermum subsessile* DC has various medicinal properties used in folklore medicine in Odisha. Phytochemical screening using GC-MS of root extracts of *B. subsessile* DC was performed in methanol and ethyl acetate. All the spectral analysis was matched with NIST (National Institute of Standards and Technology) library. GC-MS analysis revealed the presence of 10 different bioactive compounds in ethyl acetate root extract of *B. subsessile* DC. The compounds were identified by comparing their retention time and peak area with that of literature and by interpretation of mass spectra. Many of these are widely used in industry for various applications like antioxidant, anti-inflammatory, anti-malarial, anti-septic, anesthetic, antimicrobial, anti-nematocidal, herbiside, molluscaides, flavor, pesticide and cancer preventive. This study will help to predict the structure of biomolecules which can be used as drugs for candidate.

KEYWORDS: *Blepharispermum subsessile* DC, GC-MS, Phytochemical constituents, Ethyl acetate root extract, NIST.

1. INTRODUCTION

Medicinal plants have been the mainstay of traditional herbal medicine amongst rural dwellers worldwide since antiquity to date. From Sumerian and Akkadan civilizations plants have been used therapeutically (Douhari, 2012). The use of medicinal plants and herbs are known as the first medicines is a universal phenomenon. Every culture on earth, through written or oral tradition, has relied on the vast variety of natural chemistry found in healing plants for their therapeutic properties (Serrentino, 1991). For a longer period of time till now, plant based medicinal drugs have been in practice (Ruikar et al., 2009). More than three quarters of the world's population are still depends on traditional medicinal plant system for primary health care (Akin-Osanaiye et al., 2012). Around the globe, plant based traditional medicine, plays a pivotal role in human health maintenance. Plants are rich source of secondary metabolites with interesting biological activities. Secondary metabolites are major source with wide range of structural arrangements and properties (De-Fatima, et al., 2006).

Blepharispermum subsessile DC, is a rare medicinal shrub found in India, belonging to the family Compositae or Asteraceae, used for treatment of various health ailments by local medical practioners (Agarwal et al., 1999). It grows in the dry deciduous forest of Madhya Pradesh, Maharashtra, Karnataka, Odisha and Tamil nadu. The root of *B.subsessile DC* has anti-arthritic activity and leaf juice is used as "*eye drop*" in various ophthalmic diseases (Khare, 2008). It is also used for the treatment of diarrhea (Dash, 2006), skin diseases (Arunachalam, 1996), irregular menstruation (Prusti, 2007) and anti-inflammatory diseases. The rhizome of *B.subsessile DC* has an aromatic odour and is used in havan samagri (Agarwal, 1999).

2. MATERIALS AND METHODS

2.1. Plant material: The roots of *Blepharispermum subsessile* DC was collected from Gurudangar Reserve forest (Odisha) in October, 2011. The plant was identified by the Botanical Survey of India, (BSI) Coimbatore, Tamilnadu, India and the voucher specimen was kept in the institutional herbarium for future reference (BSI/SRC/5/23/2011-12/Tech.1250).

2.2. Sample collection: The root of *Blepharispermum subsessile* DC was collected from Gurudanger Reserve Forest, Odisha, India. The roots were washed thoroughly in running tap water to remove soil particles and other debris of the plant washed with sterile distilled water. The roots were shade dried separately and ground into fine powder using mortar and pestle. The powdered materials were stored in air tight polythene bags until use.

2.3. Preparation of crude extracts: The whole plant was dried under shade. Then shade dried plant were chopped and cut into small pieces and then grinded to the powered form. The powered material (1kg) of the plant was immersed in methanol for 5 days. The extract was separated by filtration and evaporated to dryness by using rotary evaporator. Under reduced pressure, this afforded 80 gm of ethyl acetate extract.

2.3. GC-MS analysis: The Varian 450 GC used in the analysis employed a fused capilary column packed with Elite-1 (50% phenyl, 95% methyl siloxane, 30 nm X 0.25 nm ID 1 µm df) and the components were separated using Helium as carrier gas at a constant flow of 1 mL/min. The 2 µl sample extract injected into the instrument was detected by the Turbo gold mass detector (Varian) with the aid of the Turbo mass 6.8 software. During the 50 th minute GC extraction process, the oven was maintained at a temperature of 80 °C with 2 minutes holding. The injector temperature was set at 280 °C (mass analyser). The different parameters involved in the operation of the Varian 450 MS, were also standardized (Inlet line temperature: 200 °C; Source

temperature: 200 °C). Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da (Prabhadevi et al., 2012)

2.4. Identification of components: Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technique, WILEY8 and FAME having more than 65,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST, WILEY8 and FAME library. The name, molecular weight, molecular formula and structure of the component of the test material was ascertained (Thanga et al., 2012). The relative percentage 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 GC MS solution ver. 6.

3. RESULTS

The results of GC-MS analysis leads to the identification of number of compounds from fractions of the ethyl acetate extracts of root. These compounds were identified through mass spectrometre attached with GC. The various components present in the root of *B. subsessile* DC that were detected by the GC-MS are shown in Table 1. The main phytoconstituents isolated from ethyl acetate root extracts were Betulin, Dronabinol, Benzoic acid,4-heptyl-, 4-cyanophenyl ester, Benzamide, 4-chloro,o-(2,6-dimethoxybenzoyl) oxime, 9,12-Octadecadienoic acid (Z,Z)-, n-Propyl 9, 12-octadecadienoate, n-Propyl 9, 12-octadecadienoate, 2-Amino-4-(2-cyclohexyl-ethyl)-7-methyl-5-oxo-4H,5H-Pyranol(4,3-b)pyran-3-carbonitrile, Carbamult and Carvacrol.

The GC-MS spectrum confirmed the presence of various components with different retention times as listed in Table 1. The biological activities listed are based on Dr. Duke's phytochemical and ethnobotanical databases by Dr. Jim Duke' of the Agricultural Research Service/USDA.

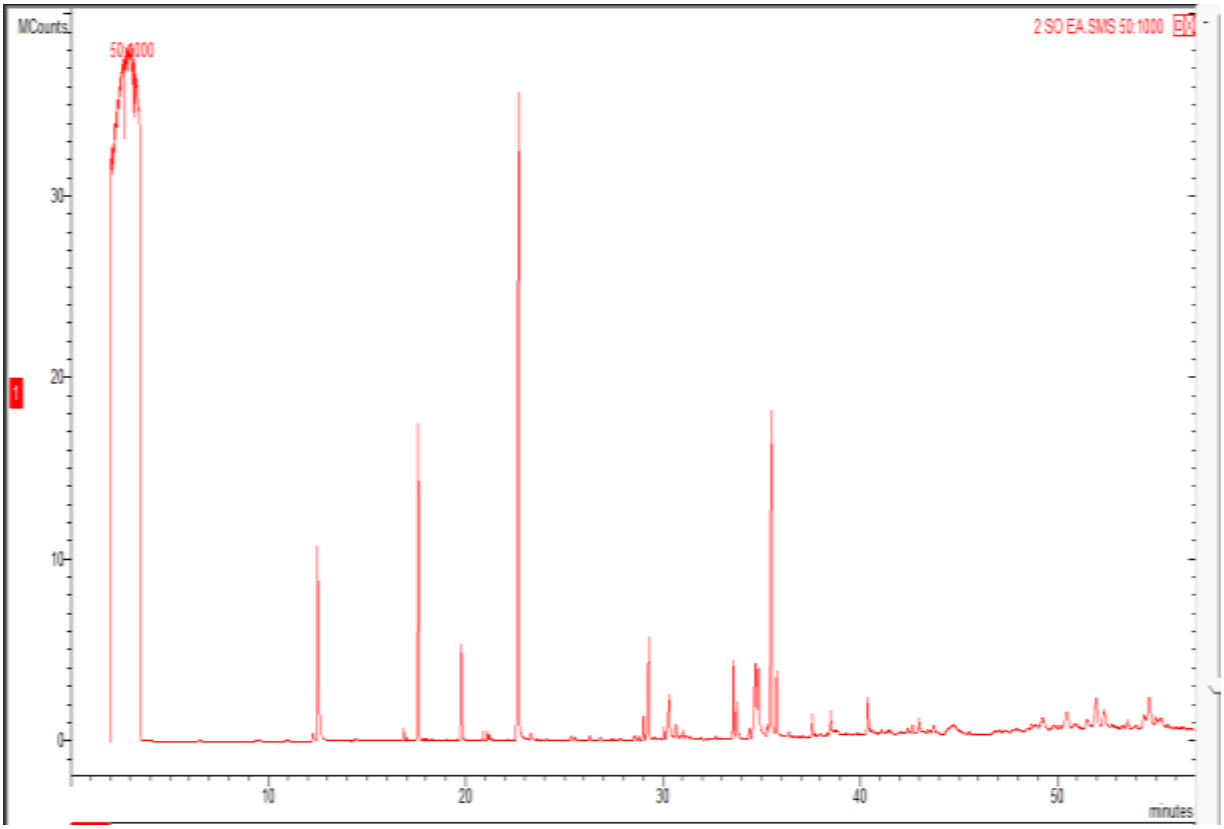

Fig 1: Components detected in ethayl acetate root extract of *Blepharispermum subsessile*

Medicinal plants are consumed worldwide for treatment of disease and are important raw material for pharmaceutical industry production of phytopharmaceuticals. The significant increase in the use of herbal medicine is related to popular knowledge and the costs of synthetic drugs, especially in developing countries. Like other pharmaceuticals, however, herbal drugs interact with the organism and can have both side-effects and beneficial effects (Rodrigues et al., 2012). They have contributed many ingredients to fight against various diseases and illness. The analysis and extraction of plant material play an important role in the development, modernization and quality control of herbal formulations (Gomathi et al., 2013). The prediction of the biological activities by applying the Duke's databases was confirmed with previous observations and supported

the traditional usage of the *Blepharispermum subsessile*. By interpreting these compounds, it is found that it possess various therapeutic applications.

Table 1: Components detected in ethayl acetate root extract of *Blepharispermum subsessile*

No	RT	Name of the compound	Molecular formula	Molecular Weight	Peak Area %	Activity
1.	12.507	Carvacrol	$C_{10}H_{14}O$	150.217	4.88	Nematocide
2.	17.609	Carbamult	$C_{12}H_{17}NO_2$	207.13	3.54	Pesticide
3.	22.71	2-Amino-4-(2-cyclohexyl-ethyl)-7-methyl-5-oxo-4H,5H-Pyranol(4,3-b)pyran-3-carbonitrile	**	**	2.00	**
4.	29.029	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.424	18.34	Anti-inflammatory
5.	29.302	n-Propyl 9, 12-octadecadienoate	$C_{10}H_{38}O_2$	322.525	1.48	**
6.	30.349	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_32O_2$	280.445	10.68	Anti-inflammatory, acne reductive, antioxidant
7.	35.775	Benzamide, 4-chlo, o-(2,6-dimethylbenzoyl) oxime	**	**	0.96	**
8.	35.775	Benzoic acid,4-heptyl-, 4-cyanophenyl ester	$C_{14}H_{12}O_3$	228.243	0.88	**
9.	51.969	Dronabinol	$C_{21}H_{28}O_4$	344.445	8.66	Analgesic, anti-inflammatory
10.	54.667	Betulin	$C_{30}H_{50}O_2$	442.72	6.44	Tumor disorders, skin irritations, insect bites and tuberculosis

The gas chromatogram shows the relative concentrations of various compounds getting eluted as a function of retention time. The heights of the peak indicate the relative concentrations of the components present in *Blepharispermum subsessile*. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. These mass spectra are fingerprint of that compound which can be identified from the data library. The presence of phytochemicals have been shown to possess anti-inflammatory, analgesic, pesticide, tumor disorders, skin irritations, insect bites and nematocide activities. Hence, the results of the GC-MS profile can be used as pharmacognostical tool for the identification of *B. subsessile*.. The present study helps to predict the formula and structure of biomolecules which can be used as drugs. This also enhances the traditional usage of *B. subsessile*. Which possess several known and unknown bioactive compounds. Further investigation may lead to the development of drug.

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