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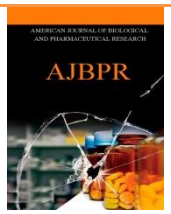

PHYTOCHEMICAL PROFILING AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS) ANALYSIS OF PETROLEUM ETHER ROOT EXTRACT OF *DESMODIUM GANGETICUM* (LINN.)

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ABSTRACT

Plant products are considered to be the most important components of diet for a good health. The present work was designed to investigate the Preliminary Phytochemical screening and GC-MS analysis of Petroleum ether Root extract of *Desmodium gangeticum*. Phytochemical screening of root extract revealed the presence of Alkaloids, Flavonoids, Glycosides and Steroid compounds. In GCMS analysis 17 biologically active constituents were identified. The analysis of *D.gangeticum* root revealed the existence of Elemicin, Spathulenol, Cubenol, Azulol, Asarone, Caryophyllene oxide, Lanceol, cis, and à-Curcumene.Findings from current study support the presence of some of these constituents in the plant extract provides the scientific evidences for the antibacterial, antioxidant, immunomodulatory and anti-tumor properties of the plant.

INTRODUCTION

Medicinal plants are nature's gift to human beings and animals for disease free healthy life [1]. Medicinal plants and their several parts or their extracts are used for the treatment of various diseases in India. World Health Organization (WHO) reported that traditional medicine is estimated to be used by 80% of the population of most developing countries [2]. These plant-based medicines are used for primary health care needs. Although plants are unique in their activities, it has also been found that a particular plant may be used by different tribes or countries for different ailments. This shows that plants possess a wide range of healing powers which are attributed to their chemical composition.

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Despite the wealth of human experience and folklore concerning the medicinal uses of plants, proper scientific investigation has only been applied to a small fraction of the world's plants. Phytochemical are very important in medicine and constitute most of the valuable drugs [3].

A knowledge of the chemical constituents of plants are desirable not only for the discovery of therapeutic agents, but also it 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 [4]. Hence a thorough validation of the herbal drugs has emerged as a new branch of science emphasizing and prioritizing the standardization of the natural drugs due to the several phytochemicals complementary and overlapping mechanism. Mass spectrometry, coupled with chromatographic separations such as Gas chromatography (GC/MS) is normally used for

direct analysis of components existing in medicinal plants. In recent years GC-MS studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the analysis of non polar components and volatile essential oil, fatty acids, lipids [5] and alkaloids [6].

Desmodium gangeticum (L.) is used widely in the Indian system of medicine particularly in Ayurveda. It is an important and well explored species of genus *Desmodium*. The National Medicinal Plant Board of India (NMPB) has identified this plant as "high trade sourced medicinal plant species from tropical forests" and also included in the list of vulnerable group of species that needs immediate management attention [7]. It is a slender shrub. Root are alternate, unifoliate, petioles long, striate at the base. Traditionally, it is of great therapeutic value in treating diseases such as typhoid, piles, asthma, and bronchitis [8]. It has high medicinal value and is used as bitter tonic, febrifuge, digestive, anti catarrhal, anti emetic, in inflammatory conditions of the chest and in various other inflammatory conditions [9,10]. Hence the present study focused on phytochemical profiling and gas chromatography-mass spectrometry (gc-ms) analysis of petroleum ether root extract of *Desmodium gangeticum*

MATERIALS AND METHODS

Plant material collection

The plant *Desmodium gangeticum* (L.) was collected from S.B. college Botanical garden Chenganacherry, Kottayam, Kerala, India. It was taxonomically identified and authenticated by Rev. Dr. S. John Britto SJ, Director, The Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College (Autonomous), Tiruchirappalli, Tamil Nadu, India. The voucher specimen was deposited at the Rapinat herbarium and the voucher number is MS001.

Plant sample extraction

The powdered sample was successively extracted with petroleum ether by hot continuous percolation method in Soxhlet apparatus for 24 hrs. The extract was concentrated by using a rotary evaporator and subjected to freeze drying in a Lyophilizer till dry powder was obtained. The dried powder was subjected in to GC-MS analysis.

Gas Chromatography-Mass spectrometry (GC-MS) analysis

The GC-MS analysis was carried out using a Clarus 500 Perkin- Elmer (Auto System XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold – Perking Elmer Turbomas 5.2 spectrometer with an Elite-1 (100% Dimethyl ply siloxane), 300 m x 0.25 mm x 1 µm df capillary column. The instrument was set to an initial temperature of 110°C, and maintained at this temperature for 2 min.

At the end of this period, the oven temperature was raised upto 280°C, at the rate of an increase of 5°C/min, and maintained for 9 min. Injection port temperature was ensured as 250°C and Helium flow rate as 1 ml/min. The ionization voltage was 70 eV. The samples were injected in split mode as 10:1. Mass Spectral scan range was set at 45-450 (mhz). The chemical constituents were identified by GC-MS. The fragmentation patterns of mass spectra were compared with those stored in the spectrometer database using National Institute of Standards and Technology Mass Spectral database (NIST-MS). The percentage of each component was calculated from relative peak area of each component in the chromatogram.

RESULTS AND DISCUSSION

Herbal medicine represents one of the most important fields of traditional system for preventive as well as the therapeutic aid for various ailments. Plants are rich source of secondary metabolites with interesting biological activities [11]. Plants have been used as medicine for thousands of years and also a hallmark in the search of new medicine [12]. Many plant species have been used in traditional medicine to treat many health problems. Even today compounds from plants continue to play a major role in primary health care as therapeutic remedies in many developing countries. The application of modern instrument and technique for standardization and formulation is the need of the hour. A lot of physico and chemico data are available. But there are no advanced and modern methods to describe the identification and quantification of active constituents in the plant materials [13].The preliminary phytochemical screening revealed the presence alkaloids, flavonoids, glycosides and steroids. Totally 17 compounds have been identified from petroleum ether extract of the plant root of *D.gangeticum* by GC-MS (Table2, Figure1). They are Cyclohexanol,1-methyl 4(1methylethyl), àCurcumene,2Butanone, 4(4methoxyphenyl), à-Farnesene, Elemicin, à-Bisabolol, 2-Octenoic acid, 4-isopropylidene-7-methyl-6-methylene-, methyl ester, Spathulenol, Caryophyllene oxide, Asarone, trans-Z-à-Bisabolene epoxide, ë-Cadinol, (-)-,Lanceol, cis, Azulol, 2H-Pyran, 2-(7-heptadecynyloxy) tetrahydro-, 2,4,5,5,8a-Pentamethyl-6,7,8,8a-tetrahydro-5H-chromene and Cubenol .

Omolo *et al.*, demonstrated the repellent effect of caryophyllene oxide against *Anopheles gambiae* [14]. A previous study showed that β-caryophyllene exhibited antiproliferative activity in human renal adenocarcinoma and amelanotic melanoma cells [15]. Futhermore, β-caryophyllene has also been reported to increase the anticancer activity of α-humulene, isocaryophyllene and paclitaxel against tumour cell lines [16]. In the present study β-caryophyllene oxide was more in *D.gangeticum* root. Hence the root possesses good biological activities.

Cadinol or 10α-hydroxy-4-cadinene is an organic compound, a sesquiterpenoid alcohol. It act as anti-fungal

and hepatoprotective and was proposed as a possible remedy for drug-resistant tuberculosis. Asarone is a volatile fragrance oil. Lee demonstrated that β-asarone is used in traditional medicine to treat diabetes [17]. A study by Geng *et al.*, has shown β-asarone as a potential candidate for a therapeutic agent to manage cognitive impairment associated with conditions such as Alzheimer's disease [18]. In a number of in vitro experiments, β-asarone was found to have anticholinergic activity [19].

Bisabolol also known as levomenol. It is a natural monocyclic sesquiterpene alcohol. Bisabolol has a weak sweet floral aroma and is used in various fragrances. It has also been used for hundreds of years in cosmetics because of its perceived skin healing properties. Bisabolol is known to possess anti-irritant, anti-inflammatory and anti-microbial properties. Bisabolol is also demonstrated to enhance the percutaneous absorption of certain molecules [20].

Elemicin is a phenylpropene, a natural organic compound. Phenylpropanoids are therapeutically beneficial and generally not toxic. Most phenylpropanoids are analgesic, anti-inflammatory and hypotensive. Cyclohexanol is an organic compound and an important feedstock in the polymer industry, firstly as a precursor to nylons, but also to various plasticizers. Small amounts are used as a solvent. Cyclohexanols were found to exhibit antitumor, cytotoxic and anti-echinococcal activities [21, 22].

Table 1. Phytochemical Analysis of *Desmodium gangeticum* (L.)

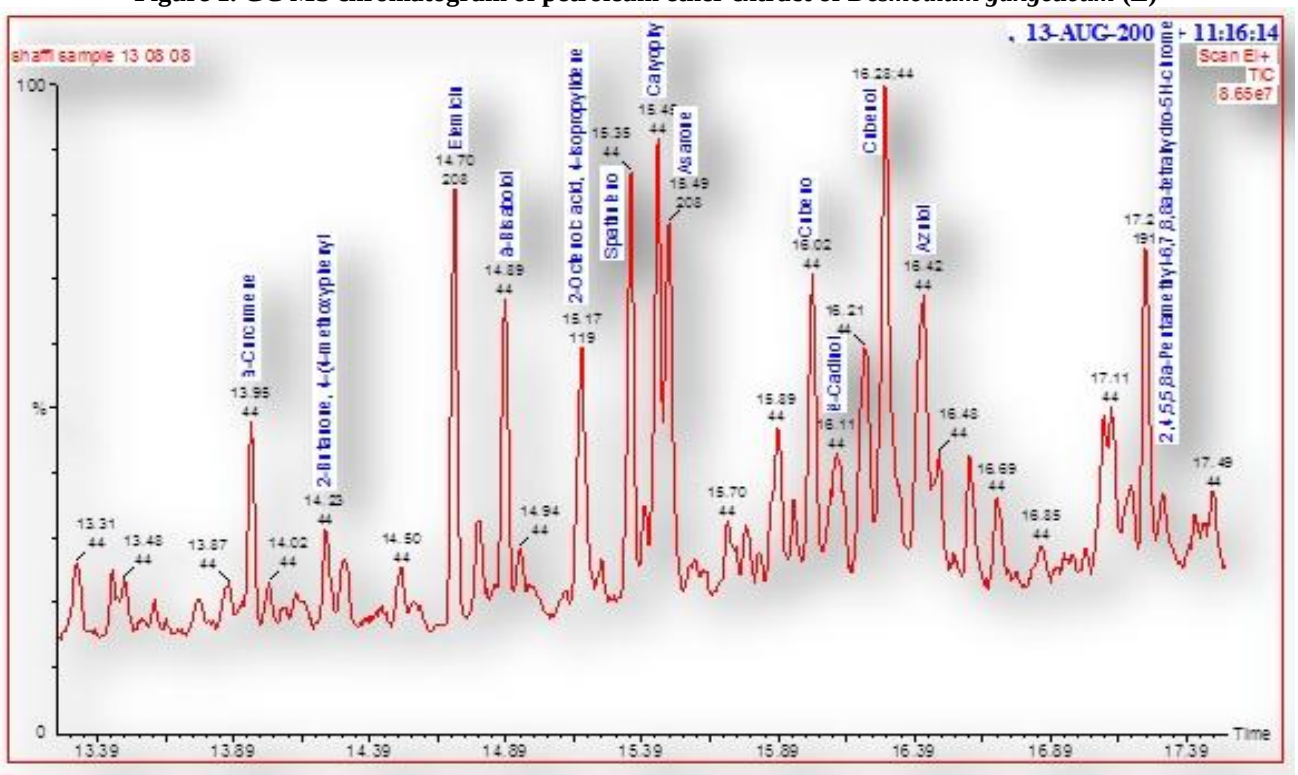
S.No	Phytochemicals	Petroleum ether
1	Alkaloid	+
2	Carbohydrate	-
3	Phytosterol	-
4	Protein	-
5	Glycoside	+
6	Flavonoid	+
7	Saponin	-
8	Steroid	+
9	Amino acid	-

Table 2. GC-MS Analysis of *Desmodium gangeticum* (L.)

S.No	Peak name	Retention time	Peak Area	%Peak Area
1	Cyclohexanol, 1-methyl-4-(1-methylethyl)- Formula: $C_{10}H_{20}O$ MW: 156	9.81	4507830	0.9510
2	à-Curcumene Formula: $C_{15}H_{22}$ MW: 202	13.95	16443750	3.4691
3	2-Butanone, 4-(4-methoxyphenyl)- Formula: $C_{11}H_{14}O_2$MW: 178	14.23	9415494	1.9864
4	à-Farnesene Formula: $C_{15}H_{24}$ MW: 204	14.30	7496679	1.5816
5	Elemicin Formula: $C_{12}H_{16}O_3$ MW: 208	14.70	36796032	7.7628
6	à-Bisabolol Formula: $C_{15}H_{26}O$ MW: 222	14.89	29593496	6.2433
7	2-Octenoic acid, 4-isopropylidene-7-methyl-6-methylene-, methyl ester Formula: $C_{14}H_{22}O_2$ MW: 222	15.17	26203186	5.5281
8	Spathulenol Formula: $C_{15}H_{24}O$ MW: 220	15.35	35884780	7.5706
9	Caryophyllene oxide Formula: $C_{15}H_{24}O$ MW: 220	15.45	23192261	4.8929
10	Asarone Formula: $C_{12}H_{16}O_3$ MW: 208	15.49	15491507	3.2682
11	trans-Z-à-Bisabolene epoxide Formula: $C_{15}H_{24}O$	15.95	37302979	7.8698

	MW: 220			
12	ë-Cadinol, (-)- Formula: $C_{15}H_{26}O$ MW: 222	16.11	11110190	2.3439
13	Lanceol, cis Formula: $C_{15}H_{24}O$ MW: 220	16.21	23200868	4.8947
14	Azulol Formula: $C_{15}H_{18}$ MW: 198	16.42	28705992	6.0561
15	2H-Pyran, 2-(7-heptadecynyloxy)tetrahydro- Formula: $C_{22}H_{40}O_2$ MW: 336	17.19	9933273	2.0956
16	2,4,5,5,8a-Pentamethyl-6,7,8,8a-tetrahydro-5H-chromene Formula: $C_{14}H_{22}O$ MW: 206	17.24	30392960	6.4120
17	Cubenol Formula: $C_{15}H_{26}O$ MW: 222	17.84	56662972	11.9542

Figure 1. GC MS chromatogram of petroleum ether extract of *Desmodium gangeticum* (L)



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CONFLICT OF INTEREST:
The authors declare that they have no conflict of interest.

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