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Research Article

Phytochemical Screening of *Sphagneticola calendulacea* Leaf Extracts Using Polar and Non-Polar Solvent

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ABSTRACT

Plant products possess therapeutic potential and are duly utilized for the production of traditional as well as contemporary medicines worldwide. It is apparent that the chemical composition of a plant and promising synergy of its constituents have been components of phytomedicine *Sphagneticola calendulacea* are known for its medicinal values in alternative systems of holistic health and herbal medicine. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. The present study was undertaken to investigate the qualitative presence of phytochemicals using polar and non-polar solvent in *S. calendulacea*. Leaves were pulverized into fine powder and extracted sequentially by methanol and chloroform later subjected to GC-MS (Gas Chromatography Mass Spectrum) analysis for the identification of bioactive compounds. Results revealed that the presence of eight bio active compounds in both the solvents. Among the polar and non-polar solvent presence of biologically effective compounds are higher in methanol with maximum peak identified as Tridecanol, 2-ethyl-2- methyl (28.12%). Extracts and metabolites from this plant have been known to possess pharmacological properties. In India, scrutiny on phytochemicals of ethno medicinal origin is extremely needed for the development of affordable antibiotics and hence the present study finds the necessity.

Keywords: *Sphagneticola calendulacea*, GC-MS, Phytochemical analysis, Polar, Non-Polar**.**

INTRODUCTION

In recent years the use of plants in the management and treatment of diseases has gained considerable importance. Plants and fruits are considered as one of the main sources of biologically active compounds. An estimate of the World Health Organization (WHO) states that around 85 - 90% of the world's population consumes traditional herbal medicines[1]. In India around 20,000 medicinal plants have been recorded recently, but more than 500 traditional communities use about 800 plant species for curing different diseases[2]. Medicinal plants and their derived material, have been widely employed in all cultures, throughout history, for the prevention and treatment of diseases. The significant increase in the use of herbal medicines in recent decades may be attributed to popular wisdom, the cost of synthetic drugs and the resurgence of interest in the development of new drugs and the re-establishment of old ones from plant sources[3]. It has been shown that *in vitro* screening methods could provide the needed preliminary observations necessary to elect crude plant extracts with potentially useful properties for further chemical and pharmacological investigations[4].

Phytochemicals are responsible for medicinal activity of plants. These are non-nutritive chemicals that have protected human from various diseases. Phytochemicals are basically divided into two groups that are primary and secondary metabolites based on the function in plant metabolism. Primary metabolites are comprise common carbohydrates, amino acids, proteins and chlorophylls while secondary metabolites consist of alkaloids, saponins, steroids, flavonoids, tannins and so on[5]. Plant produces these chemicals to protect itself but recent research demonstrates that emphasizes the plant source of most of these protective, disease-preventing compounds. A true nutritional role for phytochemicals is becoming more probable every day as research uncovers more of their remarkable benefits[6].

Sphagneticola calendulacea, (L.) Pruski belonging to the family Asteraceae whichis a procumbent, perennial herb found in wet places in Uttar Pradesh, Assam, Arunachal Pradesh and all along the coastal areas[7]. The leaves are used in dyeing grey hair and in promoting the growth of hair. They are considered tonic, alternative, and useful in coughs, cephalalgia, skin diseases, and alopecia. The seeds and flowers, as well as the leaves, are used in decoction, in the quantity of half of teacupful twice daily, as deobstruent. In decoction, the plant is used in uterine haemorrhage and menorrhagia[8].

Gas Chromatography Mass Spectroscopy, a hyphenated system which is a very compatible technique and the most commonly used technique for the identification and quantification purpose[9]. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra.

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Hence, the objective of the present study is to identify the phytochemical constituents using polar and non-polar solvent with the aid of GC-MS technique.

MATERIALS AND METHODS

Collection of Plant Material

The leaves of *Sphagneticola calendulacea* were collected from the natural habitats of Thiruvalluvar district, Tamil nadu, India. The plant material was identified and authenticated by Botanical Survey of India (BSI) with ref no.BSI/SRC/5/23/2015/Tech/2626. The leaves were washed thoroughly for three times in running tap water to remove soil particles and adhered debris and finally with sterile distilled water. The leaves were cut, shade dried and powder of the leaves obtained by grinding them mechanically and stored in air tight bags.

Preparation of Plant Extracts

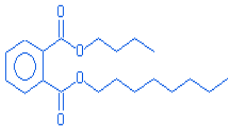
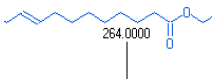
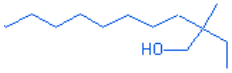
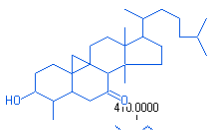
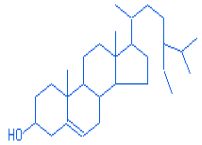
Dry powder of plant sample was extracted with methanol and chloroform using soxhlet apparatus at 70˚C and then concentrated using rotary evaporator[10]. The extract contains both polar and non-polar components of the plant material and 2 µl of the sample of the solutions was employed in GC-MS for analysis of different compounds.

GC-MS Analysis

GC-MS analysis of the polar and non-polar solvent extract of *Sphagneticola calendulacea* was performed in Sophisticated Analytical Instrument Facility Indian Institute of Technology, Madras IIT, Madras 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 an Elite-5MS (5% diphenyl/95% dimethyl poly siloxane) fused a capillary column (30 × 0.25 µm ID × 0.25 µmdf). For GC-MS detection, an electron ionization system was operated in electron impact mode with an 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 2 µl was employed (a split ratio of 10:1). The injector temperature was maintained at 250 °C, the ion-source temperature was 200 °C, the oven temperature was programmed from 110 °C (isothermal for 2 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 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. 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.

Identification of compounds

Table 1: Phytocomponents identified in the methanolic leaf extract of *Sphagneticola calendulacea* by GC-MS

S.No	Retention time	Name of the compound	Molecular formula	Structure
1	15.33	Dodecanoic acid,10 methyl, methyl ester	$C_{14}H_{28}O_2$	
2	15.93	1,2 Benzenedicarboxylic acid, butyl octyl ester	$C_{20}H_{30}O_4$	
3	17.07	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	
4	21.98	Oleic acid, eicosyl ester	$C_{38}H_{74}O_2$	
5	25.05	1-Heptatriacotanol	$C_{37}H_{76}O$	
6	28.12	Tridecanol, 2-ethyl-2- methyl	$C_{16}H_{34}O$	
7	20.3	9,19- Cyclocholestan-3-ol- 7-one,4adimethly-[20R]	$C_{29}H_{48}O_2$	
8	24.2	c-Sitosterol	$C_{29}H_{50}O$	

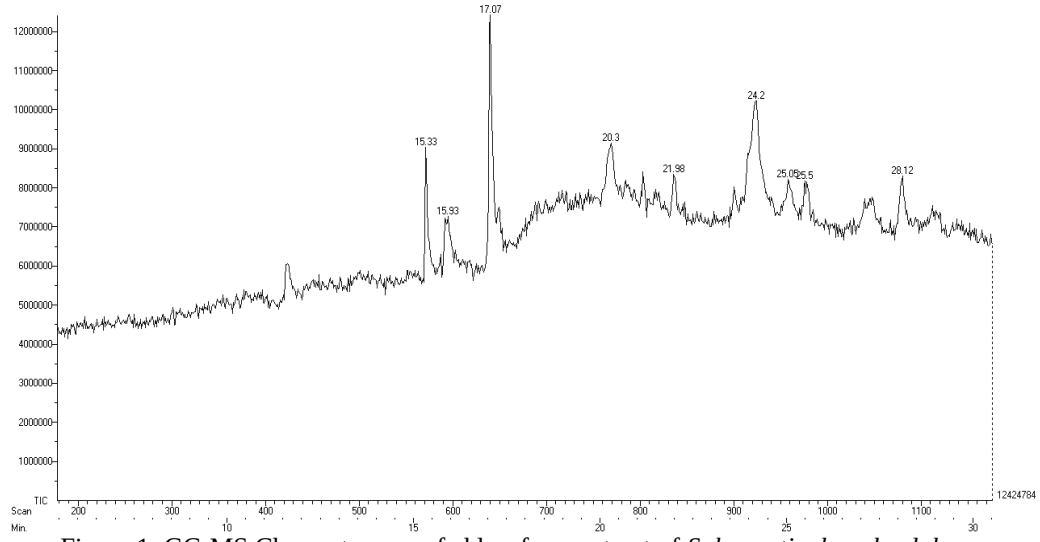


Figure 1: GC-MS Chromatogram of chloroform extract of *Sphagneticola calendulacea*

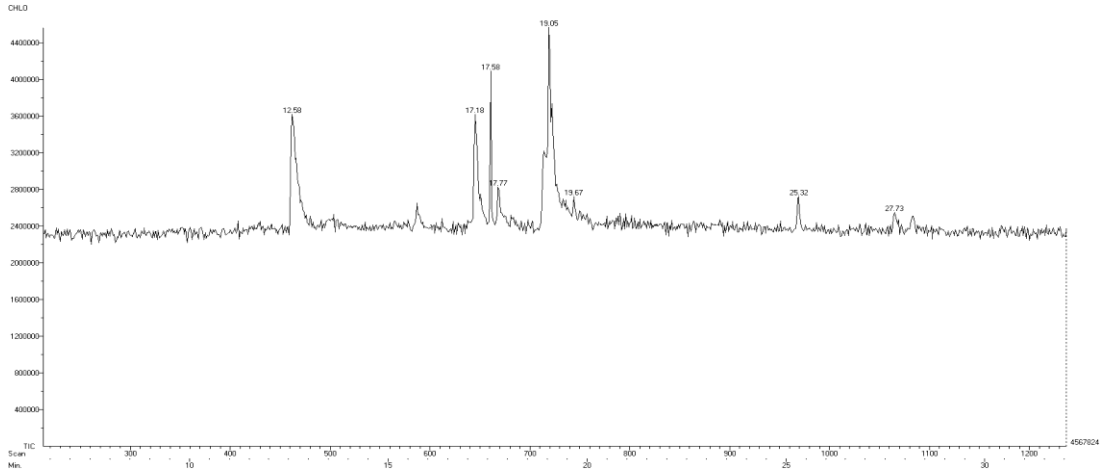


Figure 2: GC-MS Chromatogram of chloroform extract of *Sphagneticola calendulacea*

Interpretation of mass spectrum of GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the known component was 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 were ascertained.

RESULTS AND DISCUSSION

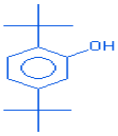
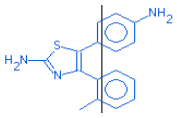
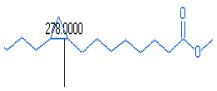
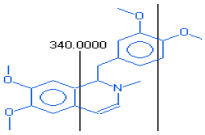
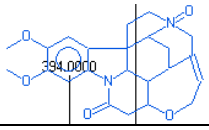
GC-MS methods proved to be very effective and sensitive for the separation and detection of complex mixtures of phytochemicals. The identification of phytochemical compounds is based on their retention time (RT), molecular formula, molecular weight (MW), and concentration (peak area %). Activities of Components with retention time identified in methanol extracts are Tridecanol, 2-ethyl-2-methyl (28.12), 1-Heptatriacotanol (25.05), c-Sitosterol (24.2) Oleic acid, eicosyl ester (21.98), 9,19- Cyclocholestan-3-ol-7-one,4a-dimethly-[20R] (20.3), 10-Octadecenoic acid, methyl ester (17.07), 1,2 Benzenedicarboxylic acid, butyl octy ester (15.93), Dodecanoic acid,10 methyl, methyl ester whereas, in chloroform the compounds with retention time are as follows Strychnidn-10-one,2,3-dimethoxy-, 19-oxide (27.73), 1,2 Dihydro-2-methylpapaverine (25.32), Cyclopropaneoctanoic acid, 2-octyl-,methylester,cis-(19.67), Heptadecanoic acid, 9-methyl-, methyl ester (19.12), Oleic acid (17.77), 5-[p-Aminophenyl]-4-[O-tolyl]-2-thiazolamine (17.58), Phenol 2,5-bis[1,1 dimethylethyl] (12.58), Pentadecanoic acid 14-methyl, methyl ester (17.18).

A knowledge of the chemical constituents of plants is desirable not only for the discovery of therapeutic agents, but also obtaining such information will 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[11].

In the last few years Gas-Chromatography Mass-Spectrometry has become firmly established as a key technological platform for metabolite profiling in both plant and non-plant species[12].

Earlier authors reported that the phytochemical studies of *S. calendulaceae*using methanol solvent yielded eleven bio active compounds[13] which are N-(3,4,4-Trimethyl-1,2-Dioxethane-3-yl-MethoxyCarbonyl)Glycine, Silane, Acetamide, 1H-Pyrimido[4,5,6-IJ][2,7]Naphthyridine-6-

Table 2: Phytocomponents identified in the chloroform leaf extract of *Sphagneticola calendulacea* by GC-MS

S.No	Retention time	Name of the compound	Molecular formula	Structure
1	17.18	Pentadecanoic acid14-methyl, methyl ester	$C_{17}H_{34}O_2$	
2.	12.58	Phenol 2,5-bis[1,1 dimethylethyl]	$C_{14}H_{22}O$	
3	17.58	5-[p-Aminophenyl]-4-[O-tolyl]-2-thiazolamine	$C_{16}H_{15}N_3S$	
4	17.77	Oleic acid	$C_{18}H_{34}O_2$	
5	19.12	Heptadecanoic acid, 9-methyl-, methyl ester	$C_{19}H_{38}O_2$	
6	19.67	Cyclopropaneoctanoic acid, 2-octyl-,methylester,cis-	$C_{20}H_{38}O_2$	
7	25.32	1,2 Dihydro-2-methylpapaverine	$C_{21}H_{25}2NO_4$	
8	27.53	Strychnidn-10-one,2,3-dimethoxy-, 19-oxide	$C_{23}H_{26}N_2O_5$	

Carbonitrile,2-Ethyl-5,8-Dimethoxy-, Acetonitrile-D3, 3-Methoxy-5-(Methoxymethoxy)-7-Methyl-6-(3-(Trimethylsilyl)Propargyl)-1,4-Naphthoquinone, L-Alanine, Ethylester-, Formamide,N-[(dibutylamino)methyl]-N-methyl-, Trans-2-((phenylthio)methyl)-1-(2-propenyl)-1,2,3,4-tetrahydronaphthalene, 2-Acetonyl-3-cyano-2,3-dimethylcyclobutane-1-carboxylic acid, 5,5'-dicarboxy-3'-(2-chloroethyl)-4-(2-acetoxyethyl)-3,4'-dimethylpyrromethane,whereas eight divergent compounds were observed in the current study namely Tridecanol , 2-ethyl-2-methyl, 1-Heptatriacotanol , c-Sitosterol,Oleic acid, eicosyl ester, 9,19- Cyclocholestan-3-ol-7-one,4a-dimethly-[20R], 10-Octadecenoic acid, methyl ester, 1,2 Benzenedicarboxylic acid, butyl octy ester,Dodecanoic acid,10 methyl, methyl ester. Hence, the differences of plant components might arise from several environmental (climatical, seasonal, and geographical) and genetic differences, which were the important factors influencing the quality of medicinal herbs.

In Present study the methanolic leaf extracts of *S. calendulacea* exhibited eight bioactive compounds whereas, chloroform extracts revealed eight disparate compounds.

10 Octadecanoic acid methyl ester, found in the methanolic leaf extract is also known as stearic acid stated as a model compound of unsaturated fatty acids, which selectively inhibits Fab I enzyme in *Staphylococcus aureus* and *Escherichia coli*, catalyzing the final and rate limiting the step of chain elongation process of the type II fatty acid synthesis (FAS- II) in bacteria. The mechanisms of

antimicrobial action of fatty acids are non-specific modes of action[14].

Oleic acid, eicosyl ester proclaimed to encompass anti-inflammatory, cancer preventive, Dermatitigenic hypocholesterolemic and anemiagenic, insectifuge[15]. 1- Heptatriacotanol is an alcoholic compound which showed antimicrobial activity[16]. Sitosterol is an imperative phytosterol that is said to reduce cholesterol levels. 1,2 Benzene dicarboxylic acid slated to possess anti-inflammatory and anti-bacterial activty[17].

oleic acid could be reported as an anti-inflammatory fatty acid playing a role in the activation of different pathways of immune competent cells[18]. Antioxidant and antimicrobial are shown by pentadecanoic acid-,14-methyl-, methyl ester.Phenol 2,5-bis[1,1 dimethylethyl] act as a chemical intermediate for the synthesis of UV stabilizers or antioxidants. Heptadecanoic acid are also possess the property of antioxidant and antimicrobial and second most abundant fatty acids[19].

The results suggest that the phytochemical properties for curing various ailments and possess potential anti-inflammatory, antimicrobial and antioxidant and leads to the isolation of new and novel compounds.

In conclusion, the presence of diverse bioactive compounds substantiate the use of the leaf of *Sphagneticola calendulacea* for numerous ailments by traditional practitioners. Therefore, it is endorsed as a plant of phytopharmaceutical importance. Further, investigation of the plant can increase the isolation of the newer molecules which will be helpful for the study of the pharmacological activities.

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