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Determination of Phytocompounds in *Spermacose hispida* Leaf Extract Using Gas Chromatography and Mass Spectroscopic Technique

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Abstract: ***The phytocomponents of Spermacose hispida leaves were evaluated by standard protocol using the equipment Perkin-Elmer Gas Chromatography–Mass Spectrometry. The mass spectrum of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. The GC-MS analysis revealed the presence of various compounds like 2-Hexadecen-1-olm, 2-Pentadecanone, 6, 10, 14-trim, 3, 7, 11, 15-Tetramethyl-2-hexade, Hexadecanoic acid, Ethyl ester, n-Nonadecanol-1, Phytol, Heptadecanoic acid ethyl ester, Squalene, 1, 2-Benzenedicarboxylic acid in the methanolic extract of Spermacose hispida. These findings support the traditional use of Spermacose hispida in various disorders.***

Keyword: Gas chromatography and Mass spectroscopy, *Spermacose hispida,* Phytocompounds

1. Introduction

Plants are a rich source of secondary metabolites with interesting biological activities. In general, these secondary metabolites are an important source with a variety of structural arrangements and properties (de-Fátima *et al*., 2006). Different medicinal plants and their medicinal values are widely used for various ailments throughout the world. Various chemical compounds isolated and characterized from Boraginaceous plant species are described. Distinguished examples of these compounds include flavonoids, phenols and phenolic glycosides, saponins and cyanogenic glycosides (Shahidi, 2000 and Shahidi, *et al*., 2008). Natural products from microbial sources have been the primary source of antibiotics, but with the increasing recognition of herbal medicine as an alternative form of health care, the screening of medicinal plants for active compounds has become very significant because these may serve as talented sources of book antibiotic prototypes (Meurer-Grimes *et al*., 1996; Koduru *et al*., 2006). It has been shown that in vitro screening methods could provide the needed preliminary observations necessary to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations (Mathekaga and Meyer, 1998).

Within a decade, there were a number of dramatic advances in analytical techniques including FTIR, UV, NMR and GC- MS that were powerful tools for separation, identification and structural determination of phytochemicals. 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. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra (Ronald Hites, 1997). The aim of this study is to determine the bioactive compounds present in *Spermacose hispida* (Family: Rubiaceae) leaf extract with the aid of GC- MS techniques which may provide an insight in its use in tradition medicine.

2. Material and Methods

Plant Materials

The *Spermacose hispida* leaves were collected in January 2014 from Karur, Karur District, Tamil Nadu from a single herb. The leaves were identified and authenticated by Dr. M. Kandhasamy, Department of Botany, Government Arts College, Karur- 639 005,Tamil Nadu, India.

Preparation of Extracts

The collected *Spermacose hispida* leaves were washed several times with distilled water to remove the traces of impurities from the leaves. The plant was dried at room temperature and coarsely powdered. The powder was extracted with methanol for 24 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in desiccator until used. The extract contained both polar and non-polar phytocomponents of the plant material used.

GC –MS Analysis

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column Elite-1 fused silica capillary column (30 x 0.25mm ID x 1µMdf, composed of 100% Dimethyl polydiloxane), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1 ml /min and an injection volume of 0.5 µI was employed (split ratio of 10:1) injector temperature 250 ºC; ion-

source temperature 280 ºC. The oven temperature was programmed from 110 ºC (isothermal for 2 min), with an increase of 10 ºC/min, to 200ºC, then 5ºC/min to 280ºC, ending with a 9min isothermal at 280ºC. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 36min. min. 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 TurboMass Ver 5.2.0 (Srinivasan et al., 2013).

3. Results and Discussion

Gas chromatography–mass spectrometry (GC-MS) is an analytical method that combines the features of gas-chromatography and mass spectrometry to identify different substances within a test sample. Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation, inorganic, biochemistry and identification of unknown samples. Additionally, it can identify trace in materials that were previously thought to have disintegrated beyond identification. GC-MS has been widely heralded as a "gold standard" for forensic substance identification because it is used to perform a specific test. GC-MS instruments have been used for identification of hundreds of components that are present in natural and biological system (Ronald Hites, 1997).

Identification of Components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown 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. The biological activities listed (Table 2) are based on Dr.Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA (Duke's, 2013). The nature and structure of the compounds were identified at different time intervals using mass spectrometer. The heights of the different peaks indicate the relative concentration of the different components present in the sample. The finger prints of the compound which can be identified from NIST library database.

GC-MS Analysis

Twenty one compounds were identified in *Spermacose hispida* by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in (Table 1and Fig 1). The prevailing compounds were 2-Hexadecen-1-olm, 2-Pentadecanone, 6,10,14-trim, 3,7,11,15-Tetramethyl-2-hexade, Hexadecanoic acid, Ethyl ester, n-Nonadecanol-1, Phytol, Heptadecanoic acid ethyl ester, Squalene, 1, 2-Benzenedicarboxylic acid present in the extract. The pharmacological activity of *Spermacose hispida* represented in table 2. This study explores the goodness of the leaf of the plant *Spermacose hispida* which has a commendable sense of purpose and can be advised as a plant of phytopharmaceutical importance.

The investigation concluded that the stronger extraction capacity of methanol could have been produced number of active constituents responsible for many biological activities. So that those might be utilized for the development of traditional medicines and further investigation needs to elute novel active compounds from the medicinal plants which may be created a new way to treat many incurable diseases including cancer.

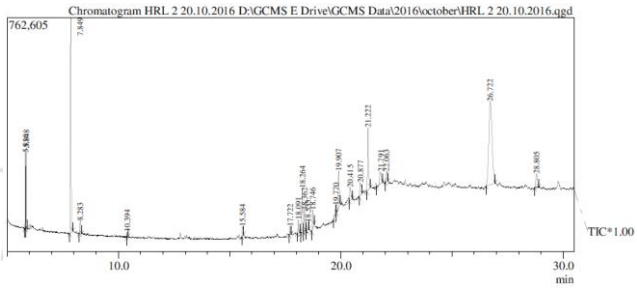

Figure 1: GC MS chromatogram of *Spermacose hispida* leaf extract

Phytol is reported to have antioxidant, antiallergic (Santos *et al*., 2013) antinociceptive and anti-inflammatory activities (Ryu *et al*., 2011). Recent studies have revealed that phytol is an excellent immunostimulant. It is superior to a number of commercial adjuvants in terms of long-term memory induction and activation of both innate and acquired immunity (Lim *et al*., 2006). Phytol has also shown antimicrobial activity against *Mycobacterium tuberculosis* and *Staphylococcus aureus* (Saikia *et al*., 2010). Similarly Maria Jancy Rani *et al.* (2011) observed the presence of phytol in the leaves of *Lantana camara* and Sridharan *et al.* (2011) in *Mimosa pudica* leaves. Similar result was also observed in the leaves of *Lantana camara* (Sathish kumar and Manimegalai, 2008). Phytol was observed to have antibacterial activities against *Staphylococcous aureus* by causing damage to cell membranes as a result there is a leakage of potassium ions from bacterial cells (Inoue *et al.,* 2005). Phytol is a key acyclic diterpene alcohol that is a precursor for vitamins E and K1. It is used along with simple sugar or corn syrup as a hardener in candies.

Hexadecanoic acid, ethyl ester is recommended to be a saturated fatty acid and it might as act as an Antioxidant, hypocholesterolemic, anti androgenic, hemolytic and alpha reductase inhibitor (Sermakkani, 2012). Hexadenoic acid has earlier been reported as a component in alcohol extract of the leaves of *Kigelia pinnata* (Grace *et al.,* 2002) and *Melissa officinalis* (Sharafzadeh *et al.,* 2011). Parasuraman *et al.* (2009) identified 17 compounds with n-Hexadecanoic acid and Octadecanoic acid as the major compounds in the leaves of *Cleistanthus collinus.* GC-MS analysis of ethyl acetate extract of *Goniothalamus umbrosus* revealed the presence of n-Hexadecanoic acid (Siddig Ibrahim *et al.,* 2009). n-hexadecanoic acid, Hexadecanoic acid, Phytol, 9, 12 -

Octadecadienoic acid, 9, 12, 15-Octadecatrienoic cidand Squalene were Identified in the ethanol leaf extract of *Aloe vera* (Arunkumar and Muthuselvam, 2009) and *Vitex negundo* (Praveen kumar *et al.,* 2010). Squalene has earlier been reported as antimicrobial, antioxidant, anticancer , Neutralize different xenobiotics, anti-inflammatory, anti-atherosclerotic and anti-neoplastic, role in skin aging and pathology and Adjuvant activities and cosmetics as a natural moisturizer (Ponnamma and Manjunath, 2012). Devi *et al.* (2009) reported that *Euphorbia longan* leaves mainly contained n-hexadecanoic acid and Octadecadienoic acid. These reports are in accordance with the result of this study.

Uraku (2015) investigated the Chemical Compositions of *Cymbopogon citrates* Leaves by Gas Chromatography-Mass Spectrometry (GC-MS) Method. Six compounds were identified in the methanol leaf extract and they include; hexadecanoic acid (8.11%), hepta-9,10,11-trienoic acid (17.43%), octadecenoic acid (8.41%), 2-ethenyltetradecan-1-ol (13.28%), eicosane aldehyde (37.56%) and 1-ethoxyoctadecane (15.20%) as the major chemical constituents.

Naji Alsultan *et al* (2016) reported that GC-MS Analysis of Mangosteen Leaf Extracts against Plant Pathogenic Bacteria. The GC-MS spectrum range confirmed the vicinity of 314 varied constituents with different retention times beside twelve elements in the high peak section (Caryophyllene, Spinacene, 2-(2-Quinolinyl)-1-naphthol, 2-(2-Quinolinyl)-1-naphthol, Silane, dimethyl (1-phenylpropoxy) tridecyloxy-, 12.beta.-Hydroxy-5.alpha.-pregnane, methoxyacetate, Phenol, 4,4'-(1-methylethylidene) bis-, Chromium, cyclopentadienyl-hexaethylbenzene, Docosane, 3,5-Dimethyldocosane, Cycloartenol, 4,4'-Methylenebis (2,6-di-tert-butylphenol), and Terephthalylidenebis (p-butylaniline)). With regards to the percentage amount, Caryophyllene (3.87%), docosane (5.50%), Cycloartenol (4.16%) and Phenol, 4,4'-Methylenebis (2,6-di-tert-butylphenol) (3.97%) were noticeable in *G. mangostana* which may possibly add to the antimicrobial feature of the plant.

Das and Sudhakar Swamy (2016) determined the bioactive compounds by GC-MS in fruit methanol extracts -a comparative analysis of three *Atalantia* species from south India. Twenty seven compounds were identified from the mass spectra obtained. 1,3,4,5-Tetrahydroxycyclohexanecarboxylic acid was the major compound identified. In *A. racemosa* also 27 compounds were identified and n- Hexadecanoic acid was the major compound.

Uraku (2016) examined the Bioactive Constituents of Methanol Fraction of *Spilanthes uliginosa* (Sw) Leaves. The major phytocompounds identified in the leaf extract are hexadecanoic acid (8.68%), hepta-9, 10, 11-trienoic acid (19.36%), octadecenoic acid (8.14%), 5-hydroxylmethyl heptadecane (14.02%), docosane aldehyde (41.72%) and 1-ethoxyoctadecane (8.08%).

Table 1: GC-MS analysis revealed the presence of Phytochemical component in leaf of *Spermacose hispida*

Peak	R.Time	Area %	Height %	Molecular Formula	Mol. Weight	Name of the compound(s)
1	5.816	6.50	9.95	$C_9H_{20}O_2$	160	Butane 1,1-diethoxy-3-methyl
2	5.848	5.19	10.57	$C_9H_{20}O_2$	160	Pentane, 1,1-diethoxy-
3	7.849	17.02	28.06	$C_9H_{20}O_3$	176	Propane, 1,1,3-triethoxy-
4	8.283	0.94	1.68	$C_{10}H_{22}O_3$	190	1,1,3-Triethoxybutane
5	10.394	0.41	0.68	$C_{16}H_{16}O_2$	240	Ethanone, 2-ethoxy-1,2-diphe
6	15.584	1.05	1.18	$C_{12}H_{14}O_4$	222	1,2-Benzenedicarboxylic acid, diethy
7	17.722	0.80	1.11	$C_{20}H_{28}$	268	Tricyclo[3.3.1.13,7]decane, [[2,2-dimet
8	18.091	1.31	1.71	$C_{17}H_{34}O_2$	207	Isopropyl myristate
9	18.264	4.20	5.81	$C_{20}H_{40}O$	296	2-Hexadecen-1-ol, 3,7,11,15-Tetramethyl
10	18.362	2.46	2.83	$C_{18}H_{36}O$	268	2-Pentadecanone, 6,10,14-trim
11	18.535	0.45	1.02	$C_{20}H_{38}$	278	2,6,10-Trimethyl,14-Ethylene-14-P
12	18.746	1.83	2.37	$C_{20}H_{40}O$	296	3,7,11,15-Tetramethyl-2-hexade
13	19.770	1.36	1.20	$C_{13}H_{16}O_4$	236	2-((3-Methylbutan-2-yloxy)carbonyl)benzoic
14	19.907	4.15	4.81	$C_{18}H_{36}O_2$	284	Hexadecanoic acid, Ethyl ester
15	20.415	2.24	1.75	$C_{19}H_{40}O_2$	328	Hexadecanoic acid, trimethylsilyl ester
16	20.877	2.30	1.69	$C_{19}H_{40}O$	284	n-Nonadecanol-1
17	21.222	8.87	8.15	$C_{20}H_{40}O$	296	Phytol Isomer
18	21.791	2.75	1.54	$C_{16}H_{30}O$	238	Z,Z-8,10-Hexadecadien-1-ol

Table 2: GC-MS analysis revealed the presence of phytochemical component in leaf of *Spermacose hispida* and their pharmacological activities

Peak#	R.Time	Area%	Height %	Name of the compound	Pharmacological Activity**
1.	18.264	4.20	5.81	2-Hexadecen-1-olm	Cancer-preventive
2.	18.362	2.46	2.83	2-Pentadecanone, 6,10,14-trim	Cancer-preventive
3.	18.746	1.83	2.37	3,7,11,15-Tetramethyl-2-hexade	Cancer-preventive
4.	19.907	4.15	4.81	Hexadecanoic acid, Ethyl ester	anti-oxidant nematicide and hypocholesterolemic,Nematicide, Antiandrogenic, Hemolytic 5-Alpha reductase inhibitor
5.	20.877	2.30	1.69	n-Nonadecanol-1	Anti-microbial and cytotoxic properties

6.	21.222	8.87	8.15	Phytol	Antimicrobial, Anticancer, Anti-Inflammatory, Anti-Diuretic, Immunostimulatory and Anti-Diabetic
7.	22.063	1.28	1.28	Heptadecanoic acid, ethyl ester	Antioxidant
8.	26.722	32.00	10.83	Squalene	Antimicrobial, Antioxidant, Anticancer ,Neutralize different xenobiotics, Anti-Inflammatory, Anti-Atherosclerotic and Anti-Neoplastic, Role in Skin aging and Pathology and Adjuvant Activities
9.	28.805	2.88	1.77	1, 2-Benzenedicarboxylic acid	Anti fouling

**Source: Dr. Duke's phytochemical and ethnobotanical database (online database)

4. Conclusion

The present study characterized the phytochemical profile of the *Spermacose hispida* leaf extract using GC-MS. The chromatogram shows the comparative concentration of different components getting eluted as a purpose of retention time. The heights of the different peaks indicates the relative concentration of the compounds exist in the methanolic extract of *Spermacose hispida* leaf. The mass spectrometer analyses the compounds which were eluted at different time intervals to recognize the nature and structure of the compounds. These spectrum are finger print of the compound which can be identified from the NIST library. The identification of various bioactive compounds confirms the therapeutic application of *Spermacose hispida* leaf for a variety of diseases. Further research is in progress for the evaluation of biological activity in *Spermacose hispida* leaf.

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