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Gc-Ms Analysis of Bio-Active Compounds in Ethanolic Extract of Sarcostemma Acidum Stem

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Abstract
The present investigation has been carried out to characterize the possible bioactive compounds present in stem extract of *Sarcostemma acidum* using Gas Chromatography-Mass Spectrum (GC-MS). GC-MS method used for the analysis of the obtained extracts, can be an interesting tool for testing the amount of some active constituents in herbs used in various industries. Eighteen compounds has been identified in stem extract of *Sarcostemma acidum*. The prevailing compounds in the ethanolic extract of stem of *Sarcostemma acidum* were 1,2-benzenedicarboxylic acid, diethyl ester, n-hexadecanoic acid, stigmasterol, phytol, 9,12-octadecadienoic acid, 9,12,15-octadecatrienoic acid and methyl ester.
Keyword: *Gas chromatography, Mass spectroscopy, Sarcostemma acidum, Phytocompounds.*

INTRODUCTION

Plants are used medicinally in different countries, and they are the source of many potent and powerful drugs. Plants have been an important source of medicines for thousands of years. Mainly, they have been used as popular folk medicines (Sathyaprabha *et al.,* 2010). 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).

Phytochemistry or plant chemistry has been developed in recent years as a distinct discipline, somewhere in between natural product organic chemistry and plant biochemistry and is closely related to both. It is concerned with the enormous variety of organic substances that are accumulated in plants and deals with the chemical structures of these substances, their biosynthesis, turn over and metabolism, natural distribution and biological function (Harborne 1986).

Phytochemicals are the chemicals extracted from plants. These organic chemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. Primary constituents include the common sugars, aminoacids, proteins, purines and pyrimidines of nucleic acids, chlrophylls etc. Secondary constituents are the remaining plant chemicals such as alkaloids (derived from aminoacids), terpenes (a group of lipids) and phenolics (derived from carbohydrates) (Liu, 2004). Plant produces these chemicals to protect itself, but recent research demonstrates that these are the protective, disease-preventing compounds. A true nutritional role for phytochemicals is becoming more probable every day, as research uncovers more of their remarkable benefits (Hamburger and Hostettmann, 1991).

In recent years, the variety of analytically advanced instruments including UV, NMR, FTIR and GC-MS are used as important tools for identification, separation and structural analysis of bioactive compounds (Roberts and Xia, 1995). GC-MS may be a very compatible technique and therefore the most typically used method for the quantification and identification purpose. The unidentified organic compounds in a mixture are often analysed by interpretation and also by matching with reference standard spectra (Ronald Hites et al., 1997). The chosen medicinal plant namely as *Sarcostemma acidum* (Roxb.) Voigt (Tamil: Kodikkalli) belongs to *Asclepiadaceae* Family. *Sarcostemma acidum* is native to India and distributed in many parts of Asia, including south Taiwan and in North Australia (Liu *et al*., 2010). The purpose of the study is to determine the organic chemicals present in the *Sarcostemma acidum* stem extract with the aid of GC-MS technique.

MATERIAL AND METHODS

Plant materials

The stem of *Sarcostemma acidum* were collected from Thanjavur District, Tamil Nadu, India. The *Sarcostemma acidum* were first washed well and dust was removed from the stems. Then the stems were dried at room temperature and coarsely powdered.

Preparation of extracts

The *Sarcostemma acidum* were first washed well and dust was removed from the stem. Then the stem was dried at room temperature and coarsely powdered. The powder was extracted with ethanol 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 Shimadzu 2010 plus comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column RTX 5Ms (Column diameter is 0..32mm, column length is 30m, column thickness is 0.50µm), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1.73 ml /min and an injection volume of 0.5 µI was employed (split ratio of 10:1) injector temperature 270 ºC; ion-source temperature 200 ºC. The oven temperature was programmed from 40 ºC (isothermal for 2 min), with an increase of 8 ºC/min to 150ºC/min, then 8ºC/min to 250ºC, ending with a 20 min isothermal at 280ºC. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds. Total GC running time is 51.25min. 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 Turbo Mass Ver 5.2.0 (Srinivasan *et al*., 2013).

Identification of components

Interpretation on GCMS 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 (Dr. Dukes, 2013).

RESULTS AND DISCUSSION

Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen substituted derivatives. Most of them are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total. These substances serve as plant defense mechanisms against, insects and herbivores. Flavonoids exhibit several biological effects such as anti-inflammatory, anti-fungal, anti-hepatotoxic and anti-ulcer actions (Dsse-Fatima *et al*., 2006).

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.

GC-MS ANALYSIS

Eighteen compounds were identified in *Sarcostemma acidum* stem by GC-MS analysis. The active constituents with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in (Table 1 and Fig 1). The prevailing compounds were 1,2-benzenedicarboxylic acid, diethyl ester, n-hexadecanoic acid, stigmasterol, phytol, 9,12-octadecadienoic acid and 9,12,15-octadecatrienoic acid, methyl ester.

Among the identified phytochemicals, hexadecanoic acid is suggested to be a fatty acid ester and it may be employed as antioxidant, antimicrobial, flavor, hypocholesterolemic agent and has larvicidal activities (Bodoprost and Rosemeyer, 2007; Falodun *et al*., 2009).

Compounds like n-hexadecanoic acid, 12-octadecanoic acid, dodecanoic acid, tetradecanoic acid, 1, 2-benzene dicarboxylic acid, butyl octyl ester, hexadecanoic acid, ethyl ester and 9,12-octadecadienoic acid (Z,Z) were identified in the ethanolic leaf extract of *Vitex altissima* (Sathish *et al*., 2012). Likewise, hexadecane, dodecanoic acid, nonadecane, eicosane, tetradecanoic acid, oleic acid, heptacosane, 9,12-octadecenoic acid, ethyl ester; n-hexadecanoic acid; 1,2-benzenedicarboxylic acid and 9-octadecenoic acid (Z)-ethyl ester were reported in *Clerodendrum inerme* and *C. phlomidis* leaves (Anandhi and Ushadevi, 2013; Balaji and Kilimozhi, 2014).

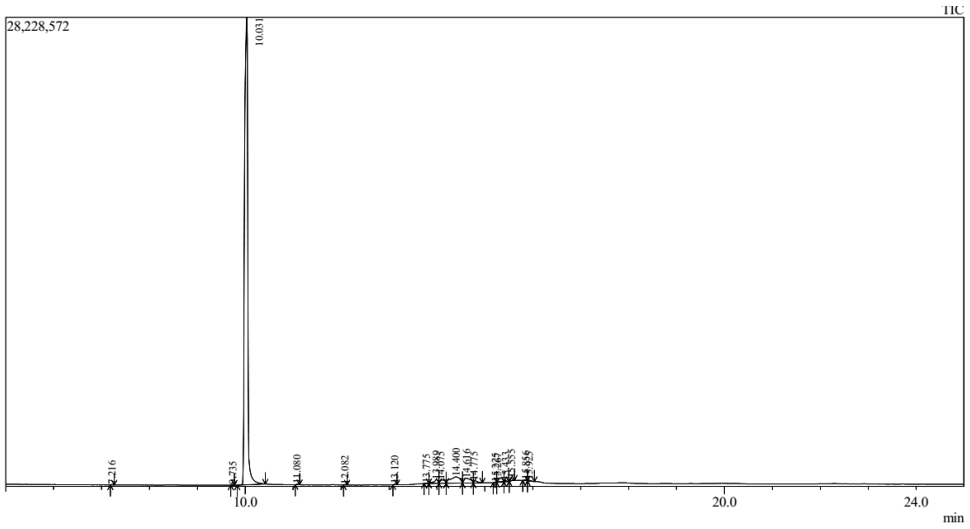

Figure 1: Chromatogram obtained from the GC/MS with the extract of *Sarcostemma acidum*

Table 1: Shows the components identified in ethanolic extract of *Sarcostemma acidum* (GC MS study)

Peak #	R. Time	Area %	Height %	M. weight (g/mol)	Molecular formula	Compound Name
1	7.216	0.05	0.11	130	$C_8H_{18}O$	1-Heptanol 6-methyl
2	9.735	0.04	0.10	172	$C_{11}H_{24}O$	1-Undecanol
3	10.031	89.78	93.92	222	$C_{12}H_{14}O_4$	1,2-Benzenedicarboxylic acid diethyl ester
4	11.080	0.08	0.15	330	$C_{20}H_{26}O_4$	1,2-Benzoldicarbonsaeure di-(hex-1-EN-5-yl-ester)
5	12.082	0.04	0.10	587	$C_{36}H_{75}O_3P$	Phosphonic acid dioctadecyl ester
6	13.120	0.06	0.11	390	$C_{24}H_{38}O_4$	1,2-Benzenedicarboxylic acid dioctyl ester
7	13.775	0.08	0.07	220	$C_{15}H_{24}O$	Aromadendrenoxid-(2)
8	13.989	1.07	0.76	256	$C_{16}H_{32}O_2$	n-Hexadecanoic acid
9	14.075	1.15	0.62	414	$C_{29}H_{50}O$	Stigmast-8(14)-en-3.beta.-ol
10	14.400	3.99	1.27	414	$C_2H_{50}O$	Chondrillastenol
11	14.616	2.12	0.98	412	$C_{29}H_{48}O$	Stigmasterol
12	14.775	0.49	0.46	180	$C_{12}H_{20}O$	2-Propen-1-ol, 3-(2,6,6-trimethyl-2-cyclohexen-1-yl)-
13	15.225	0.06	0.09	154	$C_{10}H_{18}O$	Z-4-decenal
14	15.267	0.20	0.12	218	$C_{15}H_{22}O$	2,6,6,11-Tetramethyltricyclo[5.4.0.0~2,8~] undec-10-en-9-one
15	15.433	0.16	0.22	651	$C_{45}H_{78}O_2$	Cholest-5-en-3-ol (3.beta.)- 9-octadecenoate
16	15.555	0.31	0.52	296	$C_{20}H_{40}O$	Phytol
17	15.856	0.18	0.22	280	$C_{18}H_{32}O_2$	9,12-Octadecadienoic acid
18	15.925	0.15	0.17	292	$C_{19}H_{32}O_2$	9,12,15-Octadecatrienoic acid methyl ester

Table 2: Activity of phyto-components identified in the ethanolic extracts of the *Sarcostemma acidum* by GC-MS.

R. Time	Name of the compounds	Biological activity**
10.031	1,2-Benzenedicarboxylic acid, diethyl ester	Anti-fouling, **Antimicrobial**.
13.989	n-Hexadecanoic acid	Antioxidant, hypocholesterolemic nematicide, pesticide, anti-androgenic flavor, hemolytic, 5- Alpha reductase inhibitor.
14.616	Stigmasterol	Antihepatotoxic, Antiviral, **Antioxidant**, **Cancer preventive**, Hypocholesterolemic.
15.555	Phytol	**Antimicrobial**, **Anticancer**, Diuretic, Antiinflammatory
15.856	9,12-Octadecadienoic acid (Z,Z)- (CAS) Linoleic acid	Antiinflammatory, Nematicide, Insectifuge, Hypocholesterolemic, **Cancer preventive**, Hepatoprotective, Antihistaminic, Antiacne, Antiarthritic, Antieczemic.
15.925	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)	Antiinflammatory, Hypocholesterolemic, **Cancer preventive**, Hepatoprotective, Nematicide, Insectifuge, Antihistaminic, Antiarthritic, Anticoronary, Antieczemic Antiacne, 5-Alpha reductase inhibitor Antiandrogenic.

**Source: Dr.Duke's phytochemical and ethnobotanical databases [Online database].

Similar results were also observed in the leaves of *Gmelina asiatica* which showed Pregnane – 3,11, 12,14,20 – pentol, 3,12, 20, triacetate 11 (hydroxyacetate), (3a, 11a, 12a, 14a), Tridecanoic acid, methyl ester, 10-Octadecanoic acid, methyl ester, 16-Octadecanoic acid, methyl ester, 2,7- Diphenyl-1,6-dioxopyridazino (4,5:2,3) pyrrolo (4,5,-d) pyridazine, spiro (androstane-3,2- thiazolidine). The compounds have anthelminthic, anti- Inflammatory and anti-microbial activities and anti-cancerous activity (Azhagumurugan and Rajan, 2014).

Similarly Uraku (2015) identified the Chemical Compositions of *Cymbopogon citrates l*eaves 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.

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.

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

CONCLUSION

The present study characterized the phytochemical profile of the *Sarcostemma acidum* stem extract using GC-MS. The chromatogram shows the comparative concentration of different components getting eluted with respect to retention time. The heights of the different peaks indicates the relative concentration of the compounds existing in the ethanolic extract of *Sarcostemma acidum* stem. The mass spectrometer had been used to analyse the compounds which were eluted at different time intervals and the nature and structure of the compounds were determined. The investigation established that ethanol has strong extraction ability and hence number of bioactive components have been extracted for various biological properties.

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