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Determination of phytocompounds in *Withania somnifera* and *Smilax china* using GC-MS technique

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Abstract
GC-MS method used for the analysis of the obtained extract can be an interesting tool for testing the amount of some active principles in herbs used in various industries. The aim of this study was to carry out for identification of bioactive compounds from the whole plant methanolic extract of *Withania somnifera* and *Smilax china* by Gas chromatography and Mass spectroscopy (GC-MS). GCMS analysis of methanolic extract was done by standard protocol using the equipment Perkin-Elmer Gas Chromatography–Mass Spectrometry, while the mass spectra 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 Thirteen (13) compounds were identified in *Withania somnifera* whreas sixteen (16) compounds were identified in *Smilax china*. In the ethanolic extract of *Withania somnifera* and *Smilax china* these findings support the traditional use of *Withania somnifera* and *Smilax china* in various disorders.

Keywords: Gas chromatography and Mass spectroscopy, *Withania somnifera*, *Smilax china* and phytochemistry

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 medicine with qualities for thousands of years. Mainly on traditional remedies such as herbs for their history, they have been used as popular folk medicines Sathyaprabha *et al.* (2010) [16]. 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 Mathekaga, and Meyer (1998) [12].

Phytochemistry or plant chemistry has 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 elaborated with and accumulated by plants and deals with the chemical structures of these substances, their biosynthesis, turn over and metabolism, their natural distribution and their biological function Harborne (1986) [8].

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, chlrophyll's 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) [11]. 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 Hamburger and Hostettmann (1991) [7]. Within a decade, there were a number of dramatic advances in analytical techniques including TLC, UV, NMR and GC-MS that were powerful tools for separation, identification and structural determination of phytochemicals Roberts and Xia (1995) [13].

Gas Chromatography Mass Spectroscopy (GC-MS) a hyphenated system which is a very compatible technique and the most commonly used technique for the identification and quantification of biochemical components of medicinal plants Ronald Hites (1997) [14]. The chosen medicinal plant namely as *Withania somnifera* and *Smilax china* belongs. *Withania somnifera* and *Smilax china* is widely distributed in India, Nepal and Bhutan.

The aim of this study is to determine the organic compounds present in the *Withania somnifera* and *Smilax china* extract with the aid of GC-MS Technique.

Material and methods

Plant material and preparation of extracts: The roots of *Withania somnifera* and *Smilax china* barks were purchased from Traditional Medicinal shop, Thanjavur, Tamil Nadu, India. Healthy roots and barks were washed several times with distilled water to remove the traces of impurities from the roots. Shade dried at room temperature for about 10 days and ground in to fine powder using mechanical grinder. The powder was extracted with ethanol. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The roots of *Withania somnifera* and *Smilax china* bark extract was stored in refrigerator until 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 Turbo Mass Ver 5.2.0.

Results and discussion

Gas chromatography – mass spectrometry (GC-MS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample Kell *et al.* (2005) [10]. In the last few years, GC-MS has become firmly established as a key technological platform for secondary metabolite profiling in both plant and non-plant species Fernie *et al.* (2004) [6]. Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen substituted derivatives. Most 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 De-Fatima *et al.* (2006) [4]. 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.

Thirteen (13) compounds were identified in *Withania somnifera* by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in (Table 1 and Fig 1). The prevailing compounds were Hexadecanoic acid, ethyl ester and Ethyl iso-allocholate in table 2. Sixteen (16) compounds were identified in *Smilax china* by GC-MS analysis. The active principles with their retention time (RT), molecular formula, molecular weight (MW) and concentration (%) are presented in (Table 3 and Fig 2) Oleic Acid, 8,11,14-Eicosatrienoic acid, (Z,Z,Z)- and 9,12-Octadecadienoyl chloride, (Z,Z)- in table 4. The biological activities of identified compounds were are based on Dr. Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA.

Among the identified phytochemicals hexadecanoic acid is suggested to be a fatty acid ester and it may employed as antioxidant, antimicrobial, flavor, hypocholesterolemic agent and larvicidal activities Bodoprost and Rosemeyer (2011) [3], Falodun *et al.* (2009) [5]. 1, 2- benzenedicarboxylic acid, diisooctyl ester is a plasticizer compound and acts as antimicrobial and antifouling agent Heinonen *et al.* (1998) [9]. Compounds like n-hexadecanoic acid, 12-octadecanoic acid, dodecanoic acid, tetradecanoic acid, 1,2-Benzenedicarboxylic acid, dibutyl ester, hexadecanoic acid, ethyl ester and 9,12-octadecadienoic acid (Z,Z) were identified in the ethanolic leaf extract of Vitex altissima, a Verbenaceae member Sathish *et al.* (2012) [15]. 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) [1], Balaji and Kilimozhi (2014) [2].

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.

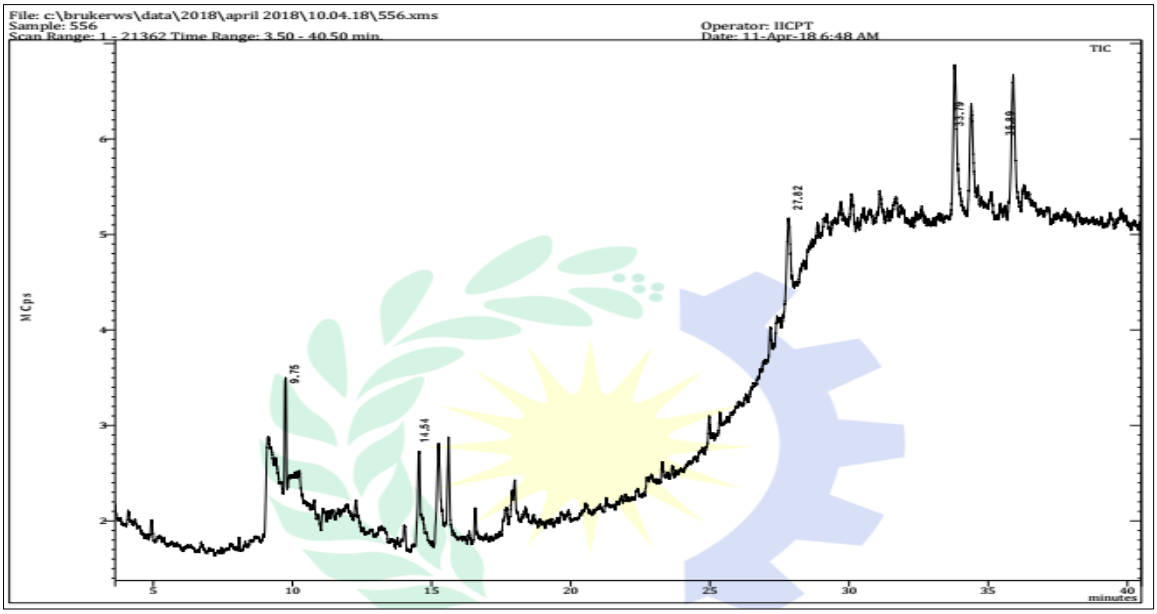

Fig 1: Compounds Identified in the *Withania somnifera*

Table 1: Compounds Identified in the *Withania somnifera*

S. No.	RT	Name of the compound	Molecular Formula	Molecular Weight	Peak Area %
1.	9.13	Dodecanoic acid, 3hydroxy-	$C_{12}H_{24}O_3$	216	9.10
2.	9.75	Phenol, 2,4-bis(1,1 dimethylethyl)	$C_{14}H_{22}O$	206	7.99
3.	12.28	7-Methyl-Z-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	268	1.04
4.	14.01	Z,Z,Z-4,6,9-Nonadecatriene	$C_{19}H_{34}$	262	1.51
5.	14.54	cis-5,8,11,14,17-Eicosapentaenoic acid	$C_{20}H_{30}O_2$	302	6.79
6.	15.25	Estra-1,3,5(10)-trien-17β-ol	$C_{18}H_{24}O$	256	9.41
7.	15.60	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284	6.94
8.	16.57	Methyl abietate isomer	$C_{21}H_{32}O_2$	316	1.98
9.	17.99	cis-13-Octadecenoic acid	$C_{18}H_{34}O_2$	282	2.23
10.	27.82	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan-4-yl)methyl ester, cis	$C_{28}H_{44}O_4$	444	11.05
11.	33.79	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436	16.25
12.	34.39	10,12-Docosadiynedioic acid ditms	$C_{28}H_{50}O_4Si_2$	506	15.12
13.	35.89	Stigmasta-5,22-dien-3-ol, acetate, (3β)-	$C_{31}H_{50}O_2$	454	10.57

Table 2: Biological Activity of Phytocomponents Identified in the Ethanol Extract of *Withania somnifera*

S. No	Compounds name	Biological Active compounds**
1.	Hexadecanoic acid, ethyl ester	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Flavor, Lubricant, Antiandrogenic, Hemolytic 5-Alpha reductase inhibitor.
2.	Ethyl iso-allocholate	Antimicrobial Diuretic Anti-inflammatory Anti-asthma

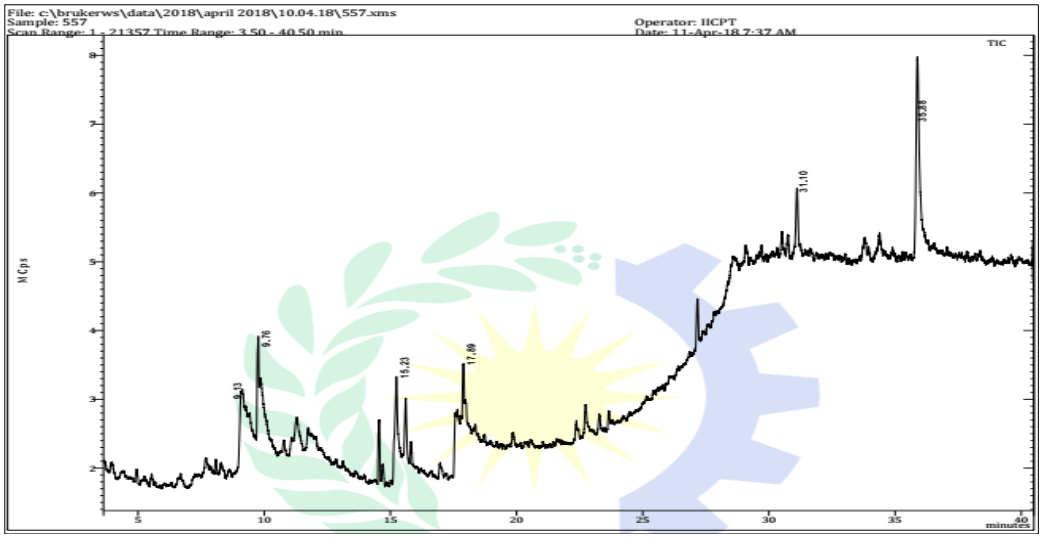

Fig 2: Compounds identified in the *Smilax china*

Table 3: Compounds identified in the *Smilax china*

S. No.	RT	Name of the compound	Molecular Formula	Molecular Weight	Peak Area %
1.	7.70	Pterin-6-carboxylic acid	$C_7H_5N_5O_3$	207	0.20
2.	8.08	2H-Oxecin-2-one, 3,4,7,8,9,10-hexahydro-4-hydroxy-10-methyl-, [4S-(4R*,5E,10S*)]-	$C_{10}H_{16}O_3$	184	0.75
3.	9.13	Maltose	$C_{12}H_{22}O_{11}$	342	4.36
4.	9.76	2H-Indeno[1,2-b]furan-2-one, 3,3a,4,5,6,7,8,8b-octahydro-8,8-dimethyl	$C_{13}H_{18}O_2$	206	9.51
5.	11.29	l-Gala-l-ido-octose	$C_8H_{16}O_8$	240	4.97
6.	11.86	2,2-Dimethyl-6-methylene-1-[3,5- dihydroxy-1-pentenyl]cyclohexan-1-perhydrol	$C_{14}H_{24}O_4$	256	0.18
7.	14.56	7,9-Di-tert-butyl-1-oxaspiro (4, 5)deca-6, 9-diene-2,8-dione	$C_{17}H_{24}O_3$	276	3.82
8.	14.70	12-Methyl-E,E-2,13-octadecadien-1-ol	$C_{19}H_{36}O$	280	1.48
9.	15.23	Dodecanoic acid, 2,3-bis (acetyloxy) propyl ester	$C_{19}H_{34}O_6$	358	11.97
10.	15.61	Oleic Acid	$C_{18}H_{34}O_2$	282	7.86
11.	15.82	10-Heptadecen-8-ynoic acid, methyl ester, (E)-	$C_{18}H_{30}O_2$	278	1.88
12.	17.68	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-	$C_{20}H_{34}O_2$	306	6.33
13.	17.89	9,12-Octadecadienoyl chloride, (Z,Z)-	$C_{18}H_{31}ClO$	298	9.03
14.	22.73	Hexadecenoic acid, Z-11-	$C_{16}H_{30}O_2$	254	1.29
15.	31.10	2H-Pyran, 2-(7-heptadecynyloxy) tetrahydro	$C_{22}H_{40}O_2$	336	4.73
16.	35.88	Digitoxin	$C_{41}H_{64}O_{13}$	764	31.63

Table 4: Biological activity of Phytocomponents identified in the ethanol extract of *Smilax china*

S. No	Compounds name	Biological Active compounds**
1.	Oleic Acid	Antihypertensive, Increase HDL and decrease LDL Cholesterol. Antiinflammatory,
2.	8,11,14-Eicosatrienoic acid, (Z, Z, Z)-	Cardio protective, Hypocholesterolemic Anticoronary, Anticancer
3.	9,12-Octadecadienoyl chloride, (Z,Z)-	Hypocholesterolemic, Nematicide Antiarthritic, Hepatoprotective, Antiandrogenic, Nematicide,5-Alphareductaseinhibitor, Antihistaminic, Anticoronary, Insectifuge, Antieczemic, Anticancer

Conclusion

The investigation concluded that the stronger extraction capacity of ethanol 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.

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