Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6 suppl 2, 2014

Research Article

GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS OF BIOACTIVE CONSTITUENTS IN THE ETHANOLIC EXTRACT OF *SACCHARUM SPONTANEUM* LINN.

J. AMUTHA ISWARYA DEVI*, A. KOTTAI MUTHU

Department of Pharmacy, Annamalai University, Annamalai Nagar 608002, Tamil Nadu, India. Email: shreeenkay@yahoo.com

Received: 24 Dec 2013, Revised and Accepted: 14 Jan 2014

ABSTRACT

Objective: To isolate and analyze the chemical composition in Ethanolic extract from the whole plant of *Saccharum spontaneum* Linn. by gas chromatography-mass spectrometry(GC-MS).

Methods: The shade dried whole plant powder was extracted with ethanol by using Soxhlet extractor and crude ethanolic extract was obtained.

Results: Qualitative analyses of ethanolic crude extract of *S. spontaneum* by using GC-MS showed that they were different types of high and low molecular weight compounds. Most of the isolated and identified compounds by GC-MS in the crude extracts are basically biologically important. Further, the *Saccharum spontaneum* possessed certain characteristics that can be ascribed to cultivation on a domestic plantation. The crude extract was prepared from the whole plant powder of *S. spontaneum* for respective compounds can be chosen on the basis of above GC-MS analysis.

Conclusion: The bioactive compounds in the ethanolic extract of *S. spontaneum* have been screened using this analysis, Isolation of individual components would however, help to find new drugs for various ailments by traditional practitioners.

Keywords: GC-MS analysis, Phytocomponents, Ethanolic extract, *Saccharum spontaneum*

INTRODUCTION

In the recent past, there has been growing interest in exploiting the biological activities of different ayurvedic medicinal herbs, owing to their natural origin, cost effectiveness and lesser side effects[1]. An estimate of the World Health Organization (WHO) states that around 85-90% of the world's population consumes traditional herbal medicines[2]. Herbal medicines are safer than synthetic medicines because the phytochemicals in the plant extract target the biochemical pathway. Medicinal plants have been used all over the world for the treatment and prevention of various ailments, particularly in developing countries where infectious diseases are endemic and modern health facilities and services are inadequate[3]. There is growing awareness in correlating the phytochemical constituents of a medicinal plant with its pharmacological activity[4-10].

Saccharum spontaneum Linn.; Synonyms, Ahlek, loa, wild cane, wild sugar cane. Family: Poaceae. This occurs throughout India and it is widely distributed in Andhra Pradesh, Vellore district in Tamilnadu. It grows as waste land weed and it is popular folk medicine. It is considered as valuable medicinal herb in traditional systems of medicine in India.

The whole plant used to diseases of vomiting, mental diseases, abdominal disorders, dyspnoea, anaemia, and obesity. The roots are used as sweet, astringent, emollient, refrigerant, diuretic, lithontriptic, purgative, tonic, aphrodisiac and useful in treatment of dyspepsia, burning sensation, piles, sexual weakness, gynaecological troubles, respiratory troubles etc.

The fresh juice of the stem of *Saccharum spontaneum* plant to the treatment of mental illness and mental disturbances by the vaidhiyars[11] and also used for renal and vesicol calculi dyspepsia, haemorrhoids, menorrhagia dysentery, agalactia phthisis and general debility.

Leaves are employed as cathartic and diuretics. And nobody has isolated crude ethanolic extract and analyzed this extract by GC-MS. This work is the first time report for the preparation of crude extract and analysis by GC-MS. For this reason, the aim of this work was to isolate, investigate and characterize the bioactive chemical constituents in the ethanolic crude extract by using GC-MS from *S. spontaneum*.

MATERIALS AND METHODS

Collection and preparation of plant material

The fresh whole plants of *S. spontaneum* were collected from the natural habitats of Tirunelveli district, Tamil Nadu, India. The samples were washed thoroughly in running tap water to remove soil particles and adhered debris and finally washed with sterile distilled water. The whole plants were shade dried and ground into fine powder. The powdered materials were stored in air tight polythene bags until use.

Plant sample extraction

The powder samples of S. spontaneum were extracted with ethanol at temperature between 60-65ºC by using soxhlet extractor. The solvent was evaporated by rotavapor to obtained viscous semi solid masses. The semi dry ethanol crude extract from the whole plant of S. spontaneum analysed by GC-MS, it has led to the identification and characterization of 22 different organic compounds.

GC-MS Analysis

GC-MS analysis of ethanolic extract were performed using a Perkin-Elmer GC clauses 500 system and Gas Chromatograph interfaced to a mass spectrometer (GC-MS) employed a fused silica capillary column packed with Elite-1 (100% dimethyl poly siloxane, 30 nm × 0.25 nm ID × 1μm df) For GC/MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1 ml/min and an injection volume of 2 μl was employed (Split ratio of 10:1) injector temperature was set at 250°C; ion-source temperature was set at 280°C.

The oven temperature was programmed from 110°C(isothermal for 2 min) with an increase of 10°C/min to 200°C, then5°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 seconds and fragments from 45 to 450 Da.

Total GC detection time was completed in 36 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass Spectra and chromatogram was a Turbo mass.

Identification of Components

Interpretation on mass spectrum of GC-MS was conducted using the database of National Institute of 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 was ascertained.

RESULTS

The results pertaining to GC-MS analysis led to the identification of number of compounds from the GC fractions of the ethanolic extract of *S. spontaneum*. These compounds were identified through mass spectrometry attached with GC. The results of the present study were tabulated in Table 1. The compound prediction is based on Dr.Duke's Phytochemical and Ethnobotanical Databases. The results revealed that the presence of Propane, 1,1,3-triethoxy-(3.65%), 2-Propyl-tetrahydropyran-3-ol (3.87%), 2-Furancarboxaldehyde,5-(hydroxymethyl)- (22.63%), 2,4-Octadienoic acid, 7-hydroxy-6-methyl-, [r-r*,s*-(E,E)]]- (4.06%), 9-Acetoxynonanal (7.42%), d-Mannose (14.52%), Vitamin d3 (3.32%), D-Glucose,4-O-à-D-glucopyranosyl- (1.17%), trans-2-Undecen-1-ol (2.60%), 8-Methyl-6-nonenoic acid (2.00%), Tetradecanoic acid (4.79%), Octadecanoic acid, ethyl ester (3.40%), Phytol (1.45%), 9,12-Octadecadienoic acid, methyl ester, (E,E)- (4.91%), 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- (2.87%), Docosanoic acid, ethyl ester (0.66%), 1,2-Benzenedicarboxylic acid, diisooctyl ester (3.43%), 9-Octadecenamide (Z)- (Oleamide) (1.15%), Z,Z,Z-1,4,6,9-Nonadecatetraene (1.37%), 2H-Pyran, 2-(7-heptadecynyloxy)tetrahydro- (4.00%), Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3á,17á)- (3.51%), cis-Z-à-Bisabolene epoxide (3.23%). The spectrum profile of GC-MS confirmed the presence of 22 compounds with retention time 2.52, 3.59, 4.85, 5.38, 6.05, 8.61, 10.20, 10.52, 11.05, 11.50, 12.65, 12.79, 14.24, 14.85, 14.95, 15.27, 19.96, 23.54, 26.93, 27.70, 29.72 and 30.80 respectively. The individual fragmentation of the components is illustrated in Figures 2A-2Q.

Table 1: Chemical composition of ethanolic extract of *S. spontaneum* Linn.

S. No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1.	2.52	Propane, 1,1,3-triethoxy-	$C_9H_{20}O_3$	176	3.65
2.	3.59	2-Propyl-tetrahydropyran-3-ol	$C_8H_{16}O_2$	144	3.87
3.	4.85	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	126	22.63
4.	5.38	2,4-Octadienoic acid, 7-hydroxy-6-methyl-, [r-r*,s*-(E,E)]]-	$C_9H_{14}O_3$	170	4.06
5.	6.05	9-Acetoxynonanal	$C_{11}H_{20}O_3$	200	7.42
6.	8.61	d-Mannose	$C_6H_{12}O_6$	180	14.52
7.	10.20	Vitamin d3	$C_{27}H_{44}O$	384	3.32
8.	10.52	D-Glucose,4-O-à-D-glucopyranosyl-	$C_{12}H_{22}O_{11}$	342	1.17
9.	11.05	trans-2-Undecen-1-ol	$C_{11}H_{22}O$	170	2.60
10.	11.50	8-Methyl-6-nonenoic acid	$C_{10}H_{18}O_2$	170	2.00
11.	12.65	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	4.79
12.	12.79	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312	3.40
13.	14.24	Phytol	$C_{20}H_{40}O$	296	1.45
14.	14.85	9,12-Octadecadienoic acid, methyl ester, (E,E)-	$C_{19}H_{34}O_2$	294	4.91
15.	14.95	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292	2.87
16.	15.27	Docosanoic acid, ethyl ester	$C_{24}H_{48}O_2$	368	0.66
17.	19.96	1,2-Benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	3.43
18.	23.54	9-Octadecenamide, (Z)- (Oleamide)	$C_{18}H_{35}NO$	281	1.15
19.	26.93	Z,Z,Z-1,4,6,9-Nonadecatetraene	$C_{19}H_{32}$	260	1.37
20.	27.70	2H-Pyran,2-(7-hepta decynyloxy)tetrahydro-	$C_{22}H_{40}O_2$	336	4.00
21.	29.72	Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one, 3-hydroxy-, (3á,17á)-	$C_{22}H_{32}O_2$	328	3.51
22.	30.80	cis-Z-à-Bisabolene epoxide	$C_{15}H_{24}O$	220	3.23

Table 2: Activity of phytocomponents identified in *S. spontaneum* Linn. by GC-MS

RT	Name of the compound	Nature of compound	Activity
2.52	Propane 1,1,3-triethoxy-	Ether	No activity reported
3.59	2-Propyl-tetrahydropyran-3-ol	Alcohol	No activity reported
4.85	2-Furancarboxaldehyde,5-(hydroxymethyl)-	Aldehyde	Antimicrobial Preservative
5.38	2,4-Octadienoic acid, 7-hydroxy-6-methyl-,[r-[r*,s*-(E,E)]]-	Allenic fatty acid	No activity reported
6.05	9-Acetoxynonanal	Aldehyde	Antimicrobial
8.61	d-Mannose	Sugar	No activity reported
10.20	Vitamin d3	Secosteroids	osteoporosis, hypovitaminosis D, vitamin D deficiency
10.52	D-Glucose,4-O-á-D-glucopyranosyl-	Sugar moiety	No activity reported
11.05	Trans-2-Undecen-1-ol	Alcohol	No activity reported
11.50	8-Methyl-6-nonenoic acid	Fatty acid	No activity reported
12.65	Tetradecanoic acid	Fatty acid	Antioxidant Cancer preventive Cosmetic Hypercholesterolemic Nematicide

			Lubricant
12.79	Octadecanoic acid, ethyl ester	Fatty ester	No activity reported
14.24	Phytol	Diterpene	Antimicrobial, antiinflammatory, anticancer diuretic
14.85	9,12- Octadecadienoic acid, methyl ester, (E,E)-	Polyenoic fatty acid	Hepatoprotective, antihistaminic, hypocholesterolemic, antieczemic
14.95	9,12,15- Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	Polyenoic fatty acid	Antiinflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge Antihistaminic, Antiarthritic, Anticoronary, Antieczemic, Antiacne, 5-Alpha reductase inhibitor Antiandrogenic
15.27	Docosanoic acid, ethyl ester	Fatty ester	No activity reported
19.96	1,2- Benzenedicarboxylic acid, diisooctyl ester	Plasticizer compound	Antimicrobial Antifouling
23.54	9- Octadecenamide, (Z)- (Oleamide)	Amide	Antimicrobial
26.93	Z,Z,Z- 1,4,6,9- Nonadecatetraene	Alkene	No activity reported
27.70	2H-Pyran, 2- (7-heptadecynyloxy) tetrahydro-	Flavonoid fraction	Antimicrobial Anti-inflammatory Antioxidant
29.72	Spiro [androst-5 - ene- 17, 1' - cyclobutan] - 2' - one, 3- hydroxy-, (3á, 17á)-	Steroid	No activity reported
30.80	Cis- Z- á- Bisabolene epoxide	Pheromone compound	To increase sex hormone Activity

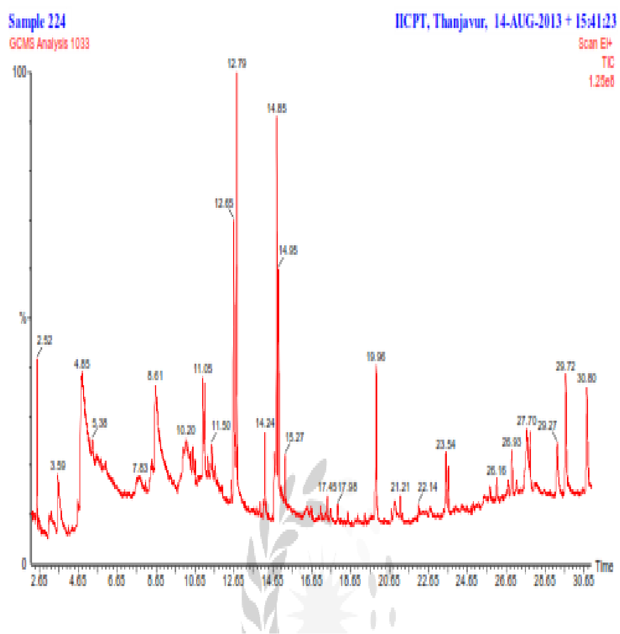

Fig.1: GC-MS Chromatogram of ethanolic extract of *Saccharum spontaneum* Linn.

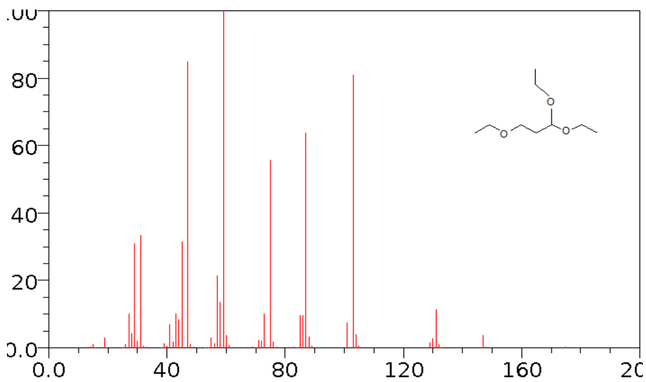

Fig. 2A: Propane,1, 1, 3-triethoxy-

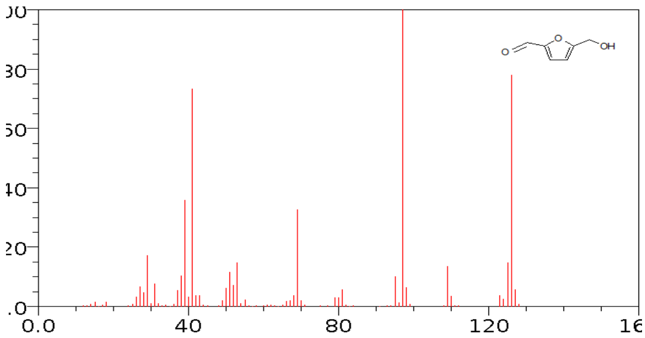

Fig. 2B: 2-Furancarboxaldehyde,5-(hydroxymethyl)

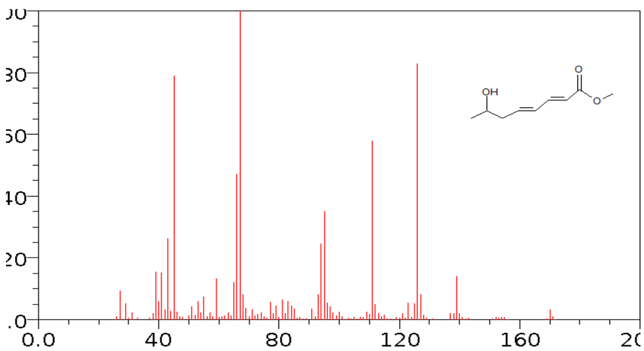

Fig. 2C: 2,4-Octadienoic acid, 7-hydroxy-6-methyl-, [r-r*,s*-(E,E)]]

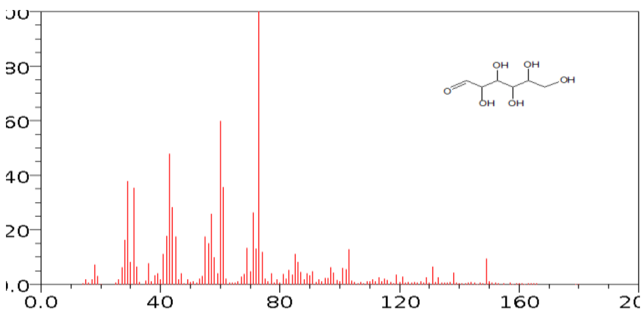

Fig. 2D: d-mannose

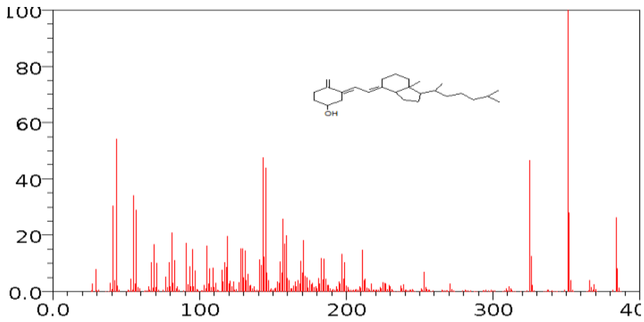

Fig. 2E: Vitamin d3

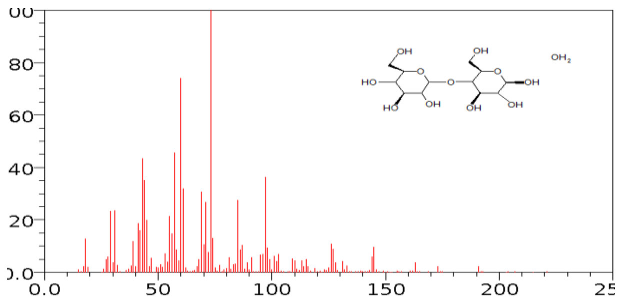

Fig. 2F: D-Glucose,4-O-á-D-glucopyranosyl-

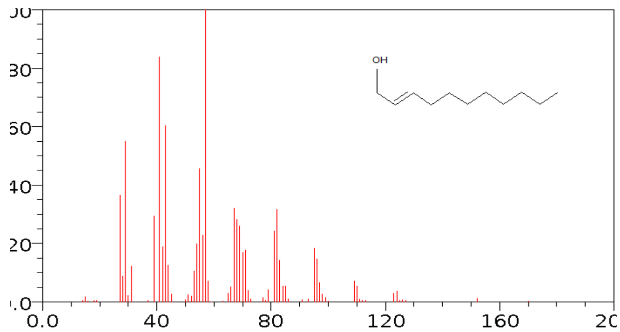

Fig. 2G: trans-2-Undecen-1-ol

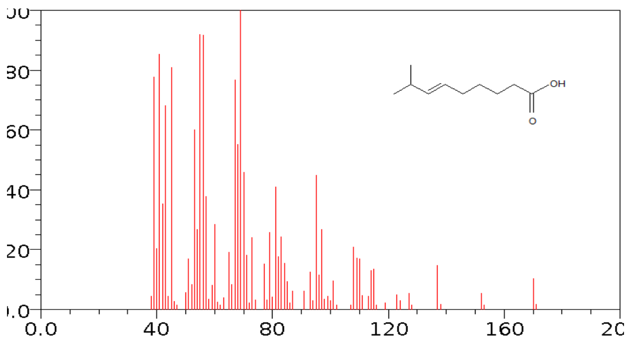

Fig. 2H: 8-Methyl-6-nonenoic acid

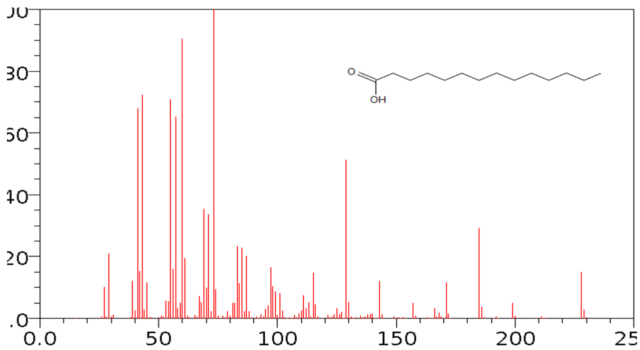

Fig. 2I: Tetradecanoic acid

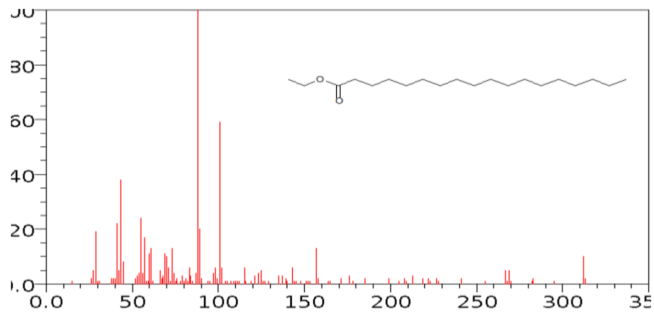

Fig. 2J: Octadecanoic acid

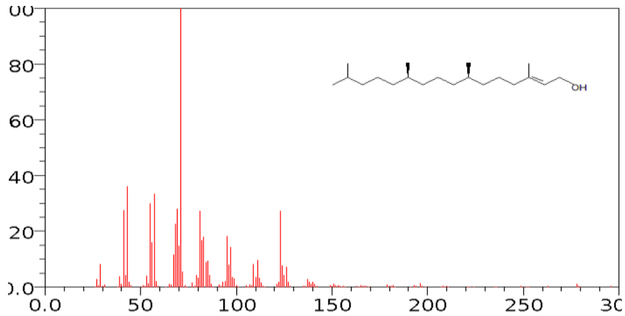

Fig. 2K: Phytol

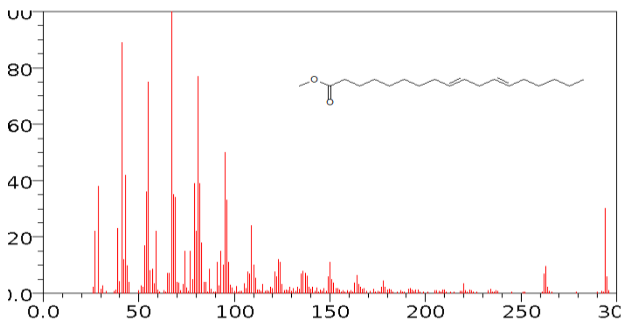

Fig. 2 L: 9,12- Octadecadienoic acid, methyl ester, (E,E)-

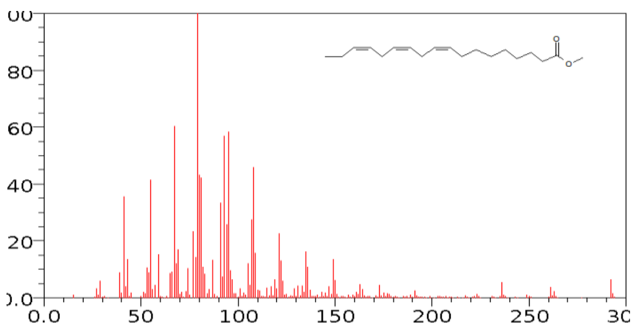

Fig. 2M: 9, 12, 15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-

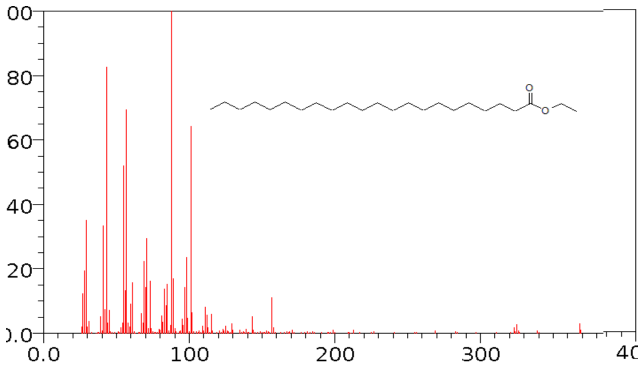

Fig. 2N: Docosanoic acid, ethyl ester

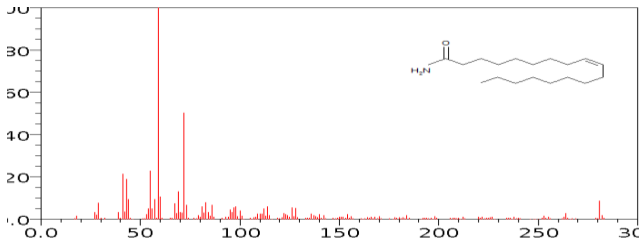

Fig. 2O: 9-Ocadecenamide,(Z)-

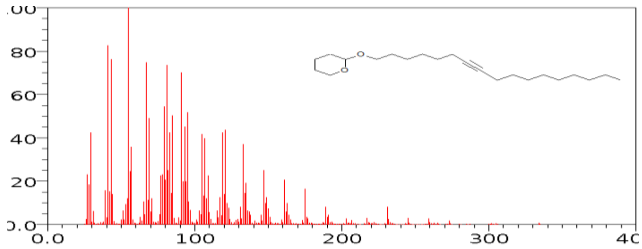

Fig. 2P: 2H-Pyran, 2-(7-heptadecynyloxy)tetrahydro-

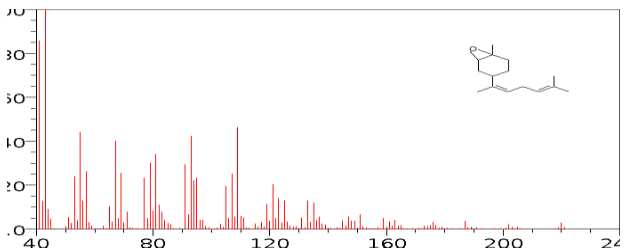

Fig. 2Q: Cis- Z- á- Bisabolene epoxide

DISCUSSION

2-Furancarboxaldehyde,5-(hydroxymethyl)- to be an aldehyde compound and it may be acts as an antimicrobial and preservative. 9-Acetoxynonanal is suggested to be an aldehyde and 9-Octadecenamide, (Z)- (Oleamide) to be an amide compound both may be employed as an antimicrobial. Vitamin d3 is suggested to be a secosteroid compound and it may be acts as an osteoporosis, hypovitaminosis D, vitamin D deficiency. Tetradecanoic acid is a fatty acid compound it may act as an antioxidant, cancer preventive, cosmetic, hypercholesterolemic, nematicide and lubricant. Phytol is a diterpene compound and it may be act as an antimicrobial, anti inflammatory, anti cancer and diuretic. 9,12- Octadecadienoic acid, methyl ester, (E,E)-to be a polyenoic fatty acid compound and it may be acts as an antihistaminic, hepatoprotective, hypocholesterolemic and antieczemic. 9,12,15- Octadecatrienoic acid, methyl ester, (Z,Z,Z)- is a polyenoic fatty acid compound and it may be acts as an anti-inflammatory, hypocholesterolemic, cancer preventive, hepatoprotective, nematicide, insectifuge, anti histaminic, anti arthritic, anti coronary, anti eczemic, anti acne, 5-alpha reductase inhibitor and anti androgenic. 1,2- Benzenedicarboxylic acid, diisooctyl ester to be a plasticizer compound, it may be acts as an antimicrobial and antifouling. 2H-Pyran, 2- (7-heptadecynyloxy) tetrahydro- to be a flavonoid fraction and it may be acts as an antimicrobial, anti-inflammatory and antioxidant. Cis- Z- á-Bisabolene epoxide is a pheromone compound,it may be acts as to increase sex hormone Activity. The Activity of phytocomponents identified in *S. spontaneum* were tabulated in Table 2. Several phytochemical screening studies have been carried out in different parts of the world using GC-MS[12-14]. In the present study we characterized the chemical profile of S. spontaneum using GC-MS. The gas chromatogram shows the relative concentrations of various compounds getting eluted as a function of retention time. The heights of the peak indicate the relative concentrations of the components present in the plant. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to appearance of peak at different m/z ratios. These mass spectra are fingerprint of that compound which can be identified from the data library. This report is the first of its kind to analyze the chemical constituents of *S. spontaneum* using GC-MS. In addition to this, the results of the GC-MS profile can be used as pharmacognostical tool for the identification of the plant. The result of the present study supported and supplemented the previous observations[12-15].

GC-MS analysis showed the existence of various compounds with different chemical structures. The presence of various bioactive compounds confirms the application of *S. spontaneum* for various ailments by traditional practitioners. However, isolation of individual phytochemical constituents may proceed to find a novel drug.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. S. Kumaravel, Quality Manager, Food Testing Laboratory and The Director, Indian Institute of Crop Processing Technology(IICPT), Thanjavur, for providing the laboratory facilities (GC-MS) and support to carry out the work.

REFERENCES

1. Naik GH, Priyadarsini KI, Satav JG, Banavalikar MM, Sohani DP, Bivani MK. Comparative antioxidant activity of individual herbal components used in Ayurvedic medicine. Phytochemistry. 2003; 63: 97-104.
2. WHO Report, World Health Organization, Geneva, WHO/EDM/TRM/ 2002, 21, 19.
3. Zaidan MRS, Rain NA, Badrul AR. *In vitro* screening of five local medicinal plants for antibacterial activity using disc diffusion method. Trop Biomed. 2005; 22: 165-170.
4. Prachayasittikul S, Buraparuangsang P, Worachartcheewan A, Isarankura-Na-Ayudhya C, Ruchirawat S, Prachayasittikul V. Antimicrobial and antioxidant activity of bioreactive constituents from *Hydnophytum formicarum* Jack. Molecules. 2008; 13: 904-921.
5. Nogueira JC, Diniz Mde F, Lima EO. *In vitro* antimicrobial activity of plants of acute *Otitis externa. Braz.* J Otorhinolaryngol. 2008; 74: 118-124.
6. Costa ES, Hiruma-LimaC A, Limo EO, Sucupira GC, Bertolin AO, Lolis SF. Antimicrobial activity of some medicinal plants of cerrado. Brazil Phytother Res. 2008; 22: 705-707.
7. Al-Bayati FA, Al-Mola HF. Antibacterial and antifungal activity of different parts of *Trubulus terrestris* L. growing in Iraq. J Zhejiang Univ Sci. 2008; B9: 154-159.
8. Chen IN, Chang CC, Wang CY, Shyu YT, Chang TL. Antioxidant and antimicrobial activity of *Zingiberaceae* plants in Taiwan. Plant Foods Hum Nutr. 2008; 63: 15-20.
9. Pesewu GA, Cutler RR, Humber DP. Antibacterial activity of plants used in traditional medicine in Ghana, with particular reference to MRSA. J Ethnopharmacol. 2008; 116: 102-111.
10. Turker AU, Usta C, Biological screening of some Turlish medicinal plants for antimicrobial and toxicity studies. Nat Prod. 2008;22:136-146.
11. C.A.Suresh kumar, R.Varadharajan, P.Muthumani, R. Meera, P.Devi, B.Kameswari. Pharmacognostic and Preliminary Phytochemical Investigations on the stem of *Saccharum spontaneum.* J. Pharm. Sci. & Res. 2009;1(3): 129-136.
12. Wu L, Gao H, Wang X, Ye J, Lu J, Liang Y. Analysis of chemical composition of *Chrysanthemum indicum* flowers by GC/MS and HPTLC. J Med Plants Res. 2010; 4(5): 421-426.
13. Vohra A, Kaur H. Chemical investigation of medicinal plant *Ajuga bracteosa.* J Nat Prod Plant Resour. 2011;1(1): 37-45.
14. Sangeetha J, Vijayalakshmi K. Determination of bioactive components of ethyl acetate fraction of *Punica granatum* Rind extract. Int J Pharm Sci & Drug Res. 2011; 3(2): 116-122.
15. Roy S, Rao K, Bhuvaneswari Ch, Giri A, Mangamoori LN. Phytochemical analysis of *Andrographis paniculata* extract and its antimicrobial activity. World J Microbiol Biotechnol. 2010; 26: 85-91.