



Phytochemical Analysis of *Stylosanthes fruticosa* using UV-VIS, FTIR and GC-MS

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Available online at: www.isca.in, www.isca.me

Received 17th August 2012, revised 3rd October 2012, accepted 24th August 2013

Abstract

The present study was carried out to characterize the bioactive constituents present in different leaf extracts of *Stylosanthes fruticosa* using UV-VIS, FTIR and GC-MS. The crude extracts were scanned in the wavelength ranging from 200-1100nm by using Perkin Elmer spectrophotometer and the characteristic peaks were detected. For GC-MS analysis, 10 g sample is extracted with 30 ml ethanol, filtered in ash less filter paper with 2 g sodium sulphate and the extract is concentrated to 1 ml by bubbling nitrogen into the solution. The compound detection employed the NIST Ver.2.0 Year 2005 library. The biological activities are based on Dr. Duke's phytochemical and ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA. The UV-VIS profile showed different peaks ranging from 400-700 nm with different absorption respectively. The FTIR spectrum confirmed the presence of secondary alcohols, phenols, alkanes, alkenes, carboxylic acids, aromatics, nitro compounds and amines in different extracts. The results of the GC-MS analysis provide different peaks determining the presence of 21 phytochemical compounds with different therapeutic activities. The major phyto constituents were Trans-5-Hexyl-1,4-dioxane-2-carboxylic acid (9.26%), dodecadienoic acid, Methyl-ester(6.58%) and Nonanoic acid Methyl ester (6.58%). Hence, this study offers base of using as herbal alternative for the synthesis of antimicrobial agents.

Keywords: Phytochemical, *Stylosanthes fruticosa* ., GC-MS, antimicrobial, ethnobotanical

Introduction

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds¹. Many of these indigenous medicinal plants are used as spices and food plants. They are also sometimes added to foods meant for pregnant and nursing mothers for medicinal purposes². Plants have great potential uses, especially as traditional medicine and pharmacopoeial drugs. A large proportion of the world population depends on traditional medicine because of the scarcity and high costs of orthodox medicine. Medicinal plants have provided the modern medicine with numerous plant-derived therapeutic agents. Many plants contain a variety of phytopharmaceuticals, which have found very important applications in the fields of agriculture, human and veterinary medicine. Natural products play a dominant role in the development of novel drug leads for the treatment and prevention of diseases³⁻⁵.

A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials⁶. In order to promote the use of medicinal plants as potential sources of antimicrobial compounds, it is important to thoroughly investigate their composition and activity and thus validate their

use⁷. Some phytochemicals produced by plants have antimicrobial activity and used for the development of new antimicrobial drugs⁸. It has been shown that *in-vitro* screening methods could provide the needed preliminary observations to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations⁹. The determination of phyto constituents is largely performed by relatively expensive and often laborious techniques such as gas (GC) and liquid (LC) chromatography combined with specific detection schemes¹⁰⁻¹¹. Analysis of small amounts of chemicals has become easier and more cost-effective owing to the development of hyphenated chromatographic techniques such as GC or LC-MS. GC-MS analysis can identify pure compounds present at less than 1 μ m¹². However, simple, cost-effective and rapid tests for detecting phyto components are necessary. Spectroscopic (UV-Vis, FTIR) methods together or separate can be used in this sense as well as conventional methods¹³⁻¹⁵.

Copiously branching woody herb, ascending shrub or under shrub, reaching 50 cm in height. Branches densely clothed with short yellowish pubescence. Leaflets oblanceolate narrowed to both ends, long mucronate at the apex, 9 to 18 mm long, prominently nerved, and both surfaces nearly glabrous, Flowers in dense oblong terminal heads. Pod with two articulations, about 6 mm long, both faces and remains of style densely silky (Andrews, 1952). Beaks 1.5 to 3 mm long and the plant have evenly pubescent stems. It is a perennial which may behave as an annual in the subtropics¹⁶⁻¹⁷. In the last few years,

spectroscopic methods have become firmly established as a key technological platform for secondary metabolite profiling in both plant and non plant species¹⁸⁻¹⁹. Therefore, the present research was conducted to investigate the phytochemical constituents of *Stylosanthes fruticosa* using UV-VIS, FTIR and GC-MS.

Material and Methods

Collection and processing of plant material: The leaves of the plant *Stylosanthes fruticosa* were collected from the natural habitats of Thiruchirappalli district, Tamil Nadu, India. The samples were washed with sterile distilled water. The leaves were cut, shade dried, ground into fine powder and stored in air tight polythene bags until use.

Plant sample extraction: 2 g of air dried powder of leaf sample was extracted with 50 ml of solvents such as ethanol and acetone with gentle stirring for 72 h. The sample was kept in dark for 72 h with intermittent shaking. After incubation the solution was filtered through Whatmann No. 1 filter paper and the filtrate was collected (crude extracts). It was then transferred to glass vials and kept at 4°C before use.

UV-VIS and FTIR Spectroscopic analysis: The extracts were examined under visible and UV light for proximate analysis. For UV-VIS and FTIR spectrophotometer analysis, the extracts were centrifuged at 3000 rpm for 10 min and filtered through Whatmann No. 1 filter paper by using high pressure vacuum pump. The sample is diluted to 1:10 with the same solvent. The extracts were scanned in the wavelength ranging from 200-1100 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected. FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks and their functional groups. The peak values of the UV-VIS and FTIR were recorded. Each and every analysis was repeated twice for the spectrum confirmation.

GC-MS analysis: 10 g of powdered leaf sample is soaked with 30 ml ethanol overnight and filtered through ash less filter paper with sodium sulphate (2 g). The extract is concentrated to 1 ml by bubbling nitrogen into the solution. The extract contained both polar and non-polar phyto components. 2µl of the ethanolic extract of *Stylosanthes fruticosa* was employed for GC-MS analysis²⁰. The Clarus 500 GC used in the analysis employed a fused silica column packed with Elite-1 [100% dimethyl polysiloxane, 30 nm × 0.25 mm ID × 1µm df] and the components were separated using Helium as carrier gas at a constant flow of 1 ml/min. The 2µl sample extract injected into the instrument was detected by the Turbo gold mass detector (Perkin Elmer) with the aid of the Turbo mass 5.1 software. During the 36th minute GC extraction process, the oven was maintained at a temperature of 110°C with 2 minutes holding. The injector temperature was set at 250°C (mass analyser). The different parameters involved in the operation of the Clarus 500 MS, were also standardized (Inlet line temperature: 200°C; Source temperature: 200°C). Mass spectra were taken at 70 eV; a scan

interval of 0.5 s and fragments from 45 to 450 Da. The MS detection was completed in 36 minutes.

Identification of components: The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The detection employed the NIST (National Institute of Standards and Technology) Ver.2.0-Year 2005 library. The compound prediction is based on Dr. Duke's Phytochemical and Ethnobotanical Databases by Dr. Jim Duke of the Agricultural Research Service/USDA. Interpretation of GC-MS was conducted using the database of 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.

Results and Discussion

The qualitative UV-Vis spectrum profile of *Stylosanthes fruticosa*, acetone extract was selected from 390 to 1100 nm due to sharpness of peaks and proper baseline. The profile showed the peaks at 390 to 1100 nm and the profile showed the peaks 405, 534, 605 and 661 nm with absorption 1.8206, 0.6211, 0.5741, 1.0093 respectively. The UV-Vis spectrum of *S. fruticosa*, the ethanol extract was taken at the 0.5741 and 1.0093 respectively.

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The results of FTIR peak values and functional groups were represented in table 2. Performing the next advanced phytochemical analysis technique of FTIR the presence of various functional groups of different compounds was found. The solvent had its respective functional group like amines, cycloalkanes, carboxylic acids, amino acid, halogen compounds, ethers, non-conjugated dienes etc. Hence, the crude extracts subjected to UV-VIS and FTIR analysis is used for the identification of chemical constituents present in *Stylosanthes fruticosa*. In addition, UV-VIS and FTIR spectroscopy is proved to be a reliable and sensitive method for detection of bio molecular composition²¹. The results pertaining to GC-MS analysis leads to the identification of number of compounds from the GC fractions of the ethanolic extract of *Stylosanthes fruticosa*. These compounds were identified through mass spectrometry attached with GC. These observations may be due to the nature of biological active components and the stronger extraction capacity of ethanol could have been produced number of active constituents responsible for antibacterial activity.

The biological activities based on Dr. Duke's Phytochemical and Ethnobotanical Databases were tabulated in table 3. The results revealed the presence of 12 different compounds namely (SE)-3-Acetoxy-6,10-methyl (1,59 andecadien-2-one (2.34%), Ethyl 2,4,5- Trifluoro - a- oxo - 3 - (trifluoromethyl) benzenepropanate(2.34%), Hazaleamidemonoepitoxide(2.55%),

Propanoic acid 2-methyl-2-Methylpropyl ester (2.79%), Trans-4-(3-carbethoxy-3-butenyl) -2-propyrtetrahydrofuran (3.18%), Azidodiphenyl-borane(3.68%), Phenol, 2-(phenylethyl) (5.11%), Undecane, 2-methyl-(5.11%), Propanic acid 2-hydroxy-butyl ester (5.11%), Dodecanoic acid, Methy-ester (6.58%), Nonanoic acid Methyl ester (6.58%), Trans-5-Hexyl-1,4-dioxane-2-carboxylic acid (9.26%).

The phytochemical screening chemical constituents of the plants studied showed that the leaves were rich in sugar, amino acids and secondary metabolites like steroids, alkaloids, flavonoids, saponins, tannin etc. (table-3). They were known to show medicinal activity as well as exhibiting physiological activity²³. The presence of saponin in *Stylosanthes fruticosa* in the present study is in corroborate with the opinion of Gill²³ who noted that saponin is one of the active constituents. Also, the presence of saponin contradicts the observation of Taylor-Smith R.²⁴ who reported that saponin was absent in this taxon.

GC-MS analysis was done using the organic solvent ethanol and it shows the presence of twelve different chemical compounds present in the plant sample. The sample was extracted with ethanol because of the effect of antibacterial activity in this

solvent. GC-MS analysis also provides the spectrum for the ethanolic extract. The spectrum profile of GC-MS confirmed the presence of 21 major components with the retention time 5.35,5.35, 5.35, 18.18, 21.00, 21.00, 24.56, 27.92, 29.90, 30.56, 32.88,36.97 respectively.

This 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 *Stylosanthes fruticosa*. The individual fragmentation patterns of the components were illustrated.

Conclusion

These mass spectra are fingerprint of the compound which can be identified from the NIST data library. Hence, the identified phyto components using GC-MS can be used as a pharmacognostical tool for the identification of adulterants. It paves the way for the development of several treatment regimens based on this extract. In addition, further research is necessary to identify and purify the active compounds responsible for therapeutic activity.

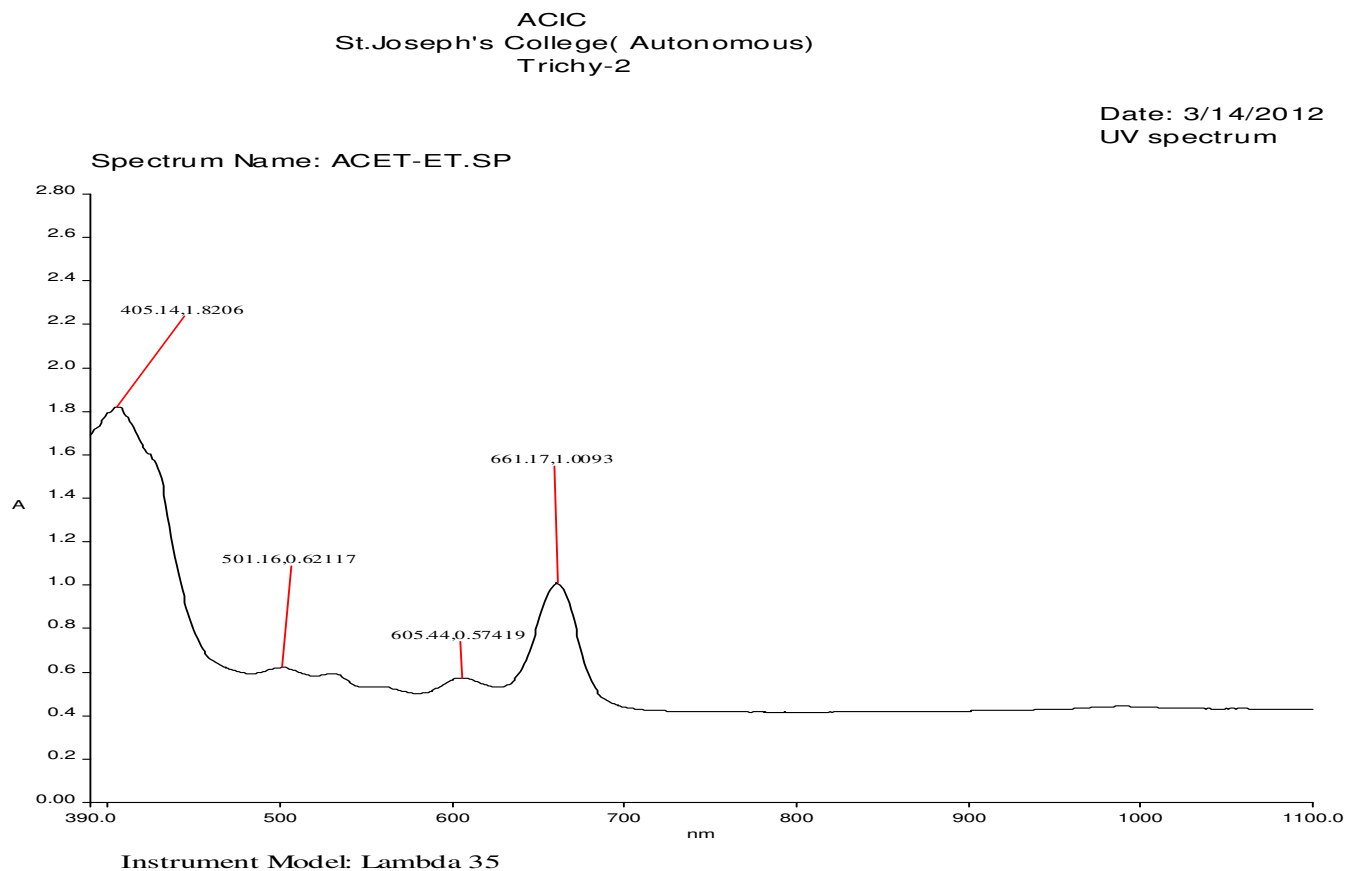


Figure-1
UV-Visible spectrum of Acetone extract of *Stylosanthes fruticosa*

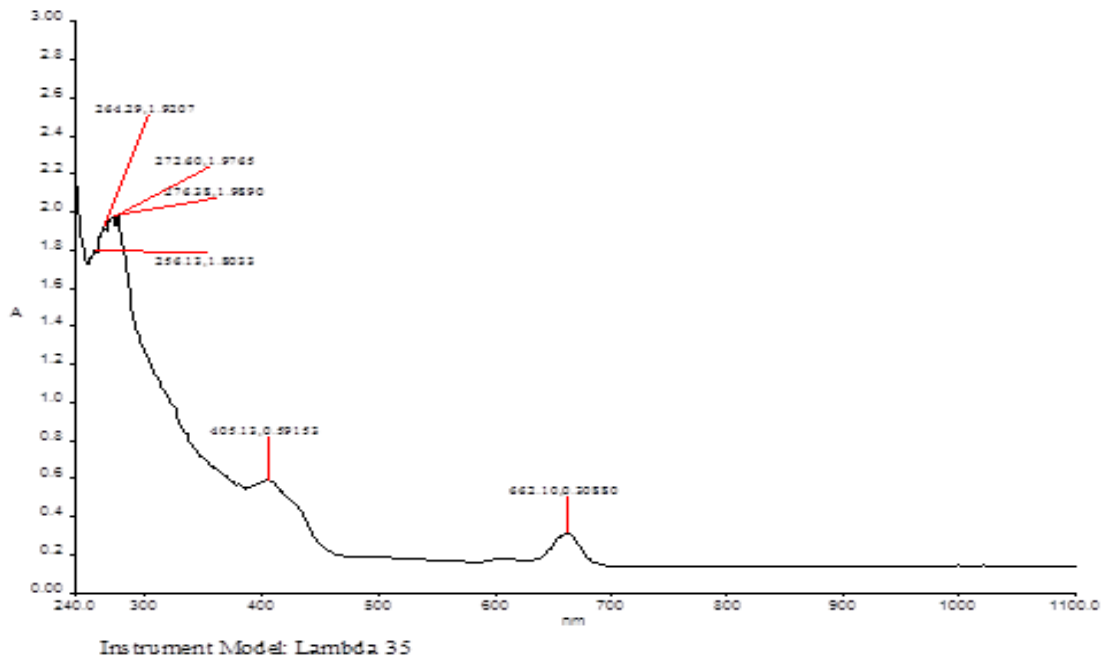


Figure-2
 UV-Visible spectrum of Ethanol extract of *Stylosanthes fruticosa*

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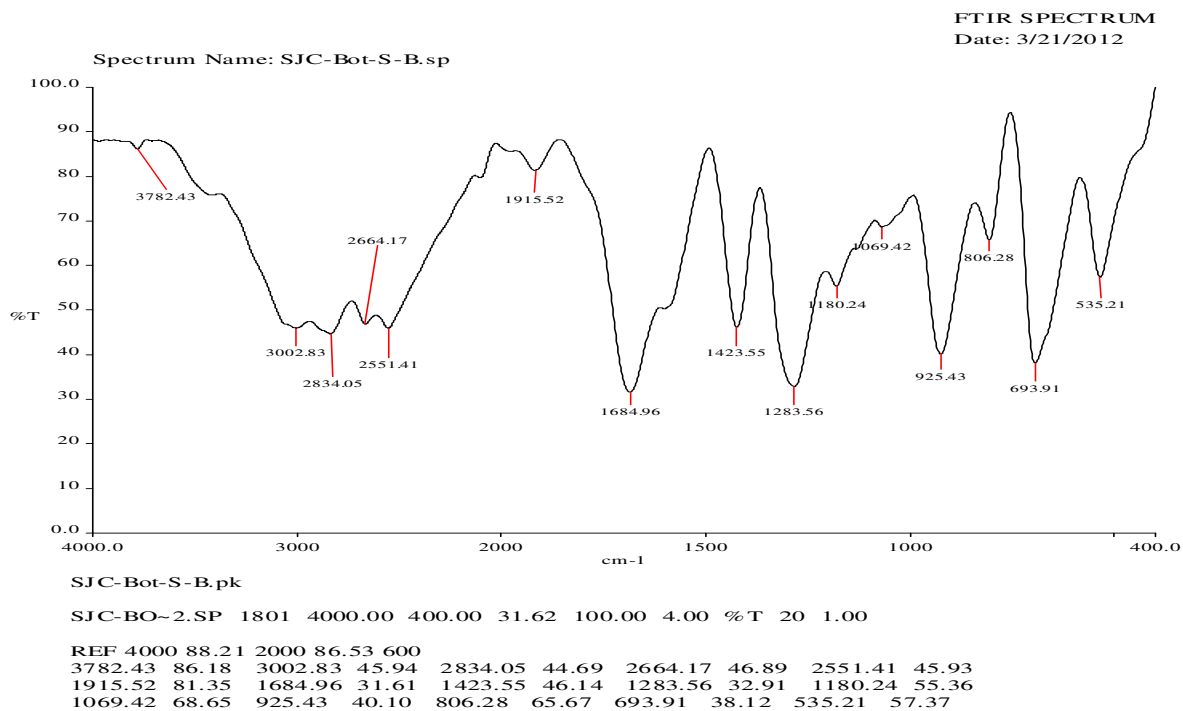


Figure-3
 FTIR analysis of Ethanolic Extracts of *Stylosanthes fruticosa*

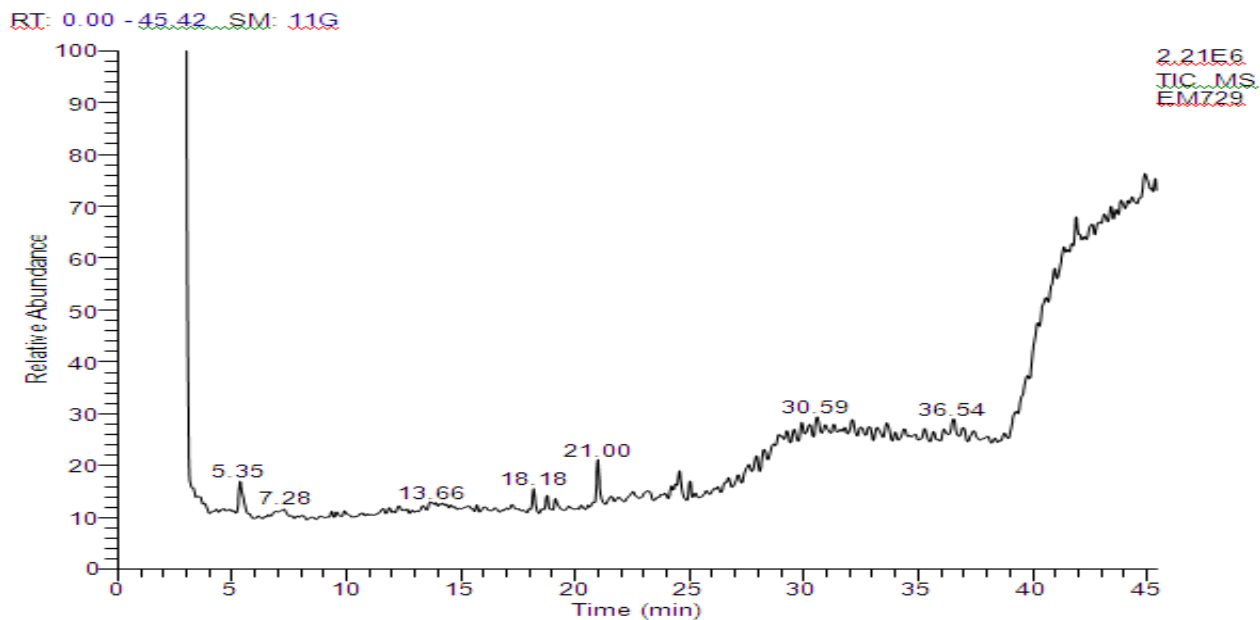


Figure-4

Gas chromatography and mass spectroscopy (GC-MS) analysis of ethanolic sample of *Stylosanthes fruticosa*(Retz.) Alston

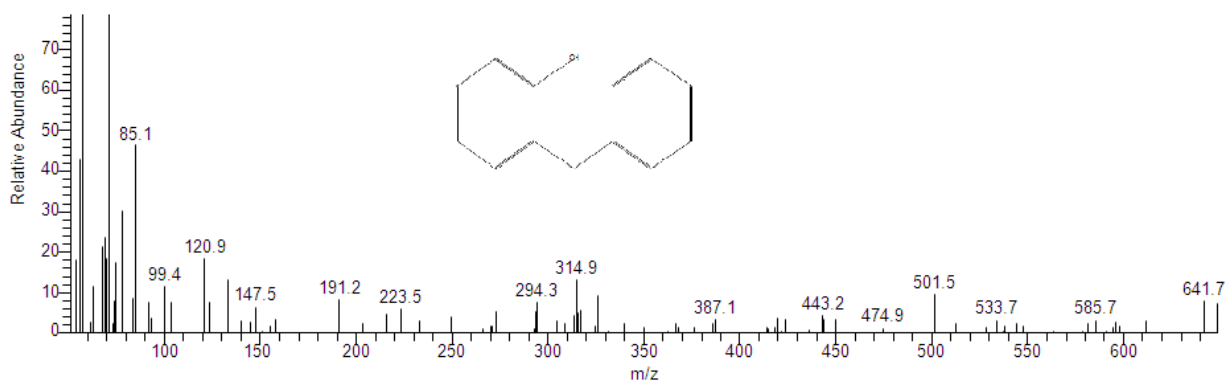


Figure-5

Mass spectrum of phenol, 2-(phenylethyl)

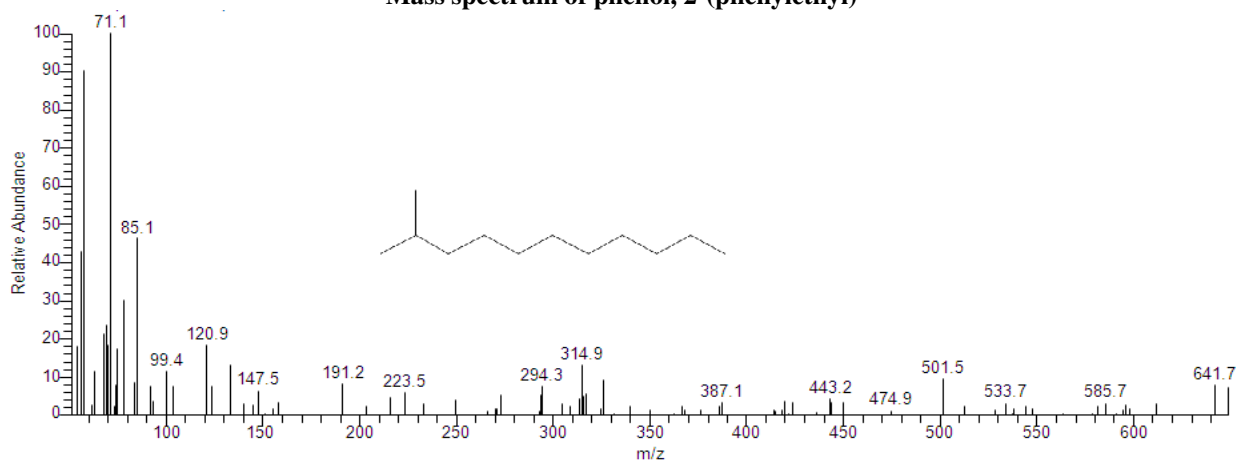


Figure-6

Mass Spectrum of Undecane, 2-methyl-(CAS)

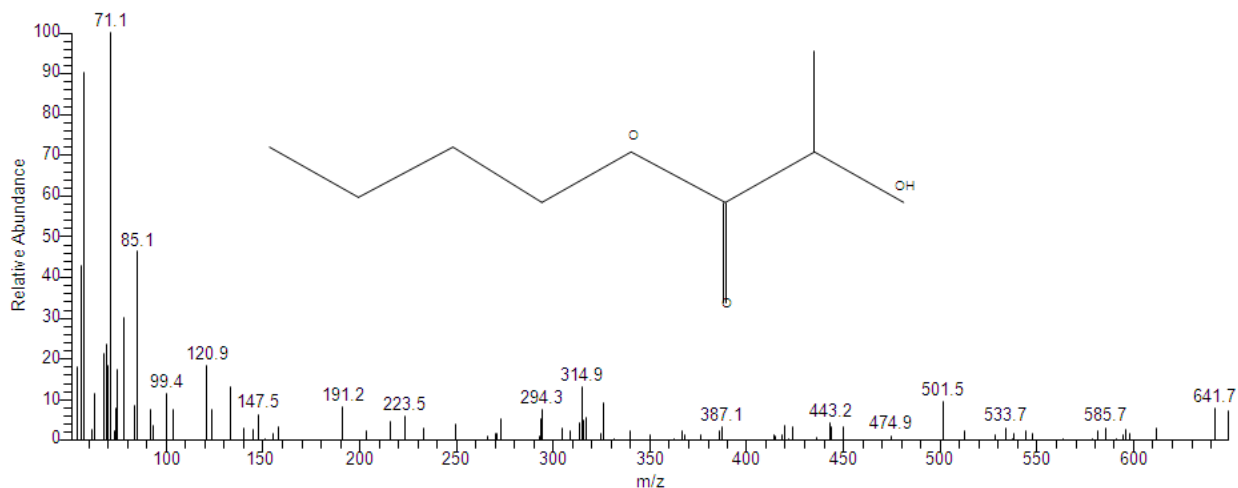


Figure-7
Mass spectrum of Propanoic acid 2-hydroxy-butyl ester

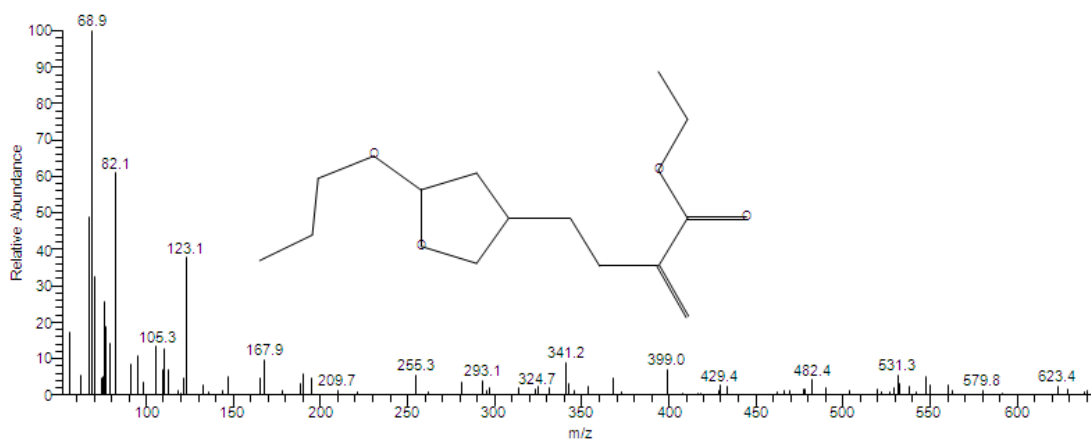


Figure-8
Mass spectrum of Trans-4-(3-carbethoxy-3-butenyl)-2-propyltetrahydrofuran

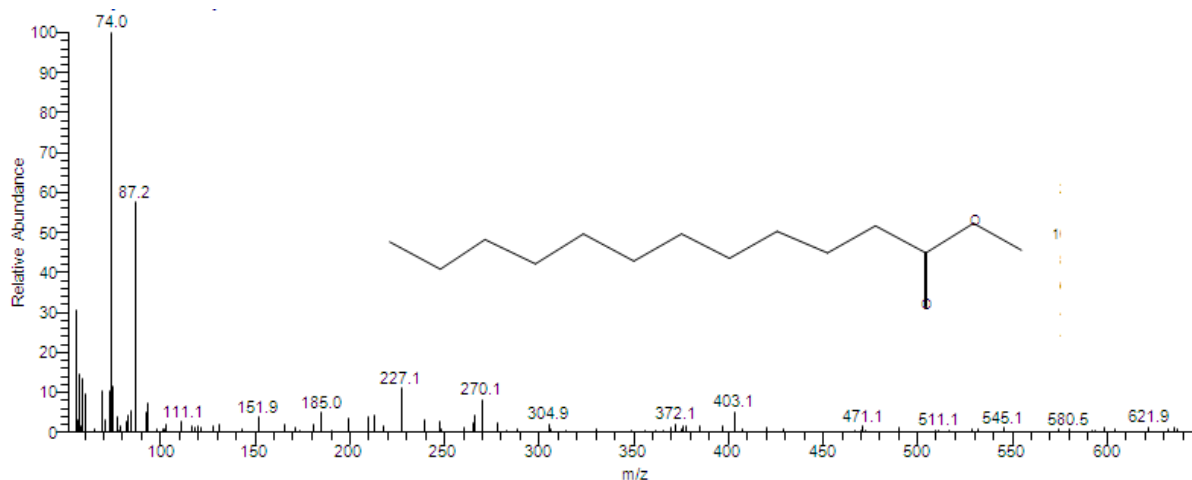


Figure-9
Mass spectrum of Dodecanoic acid, Methyl-ester

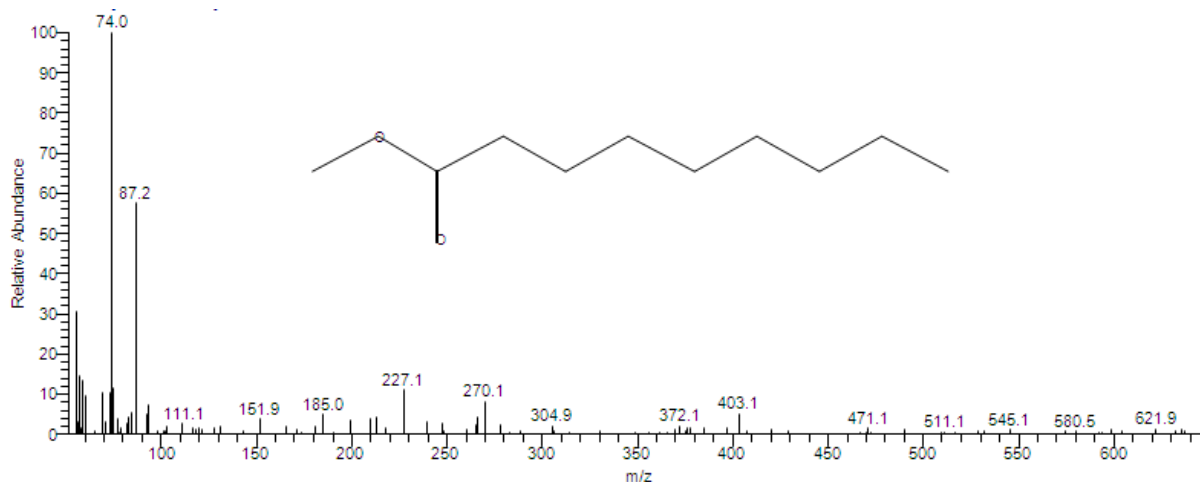


Figure-10
Mass spectrum of Nonanoic acid Methyl ester

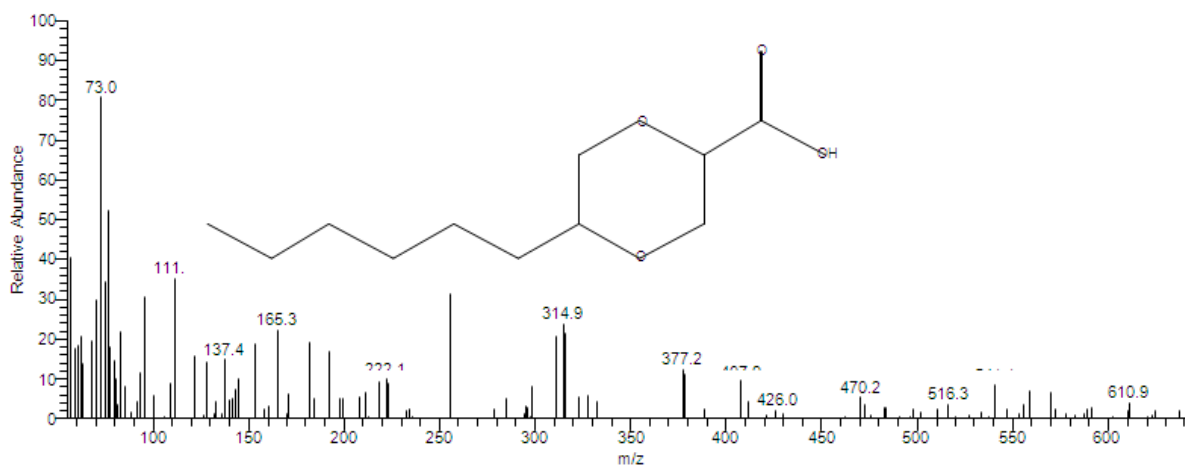


Figure-11
Mass spectrum of Trans-5-Hexyl-1,4-dioxane-2-carboxylic acid

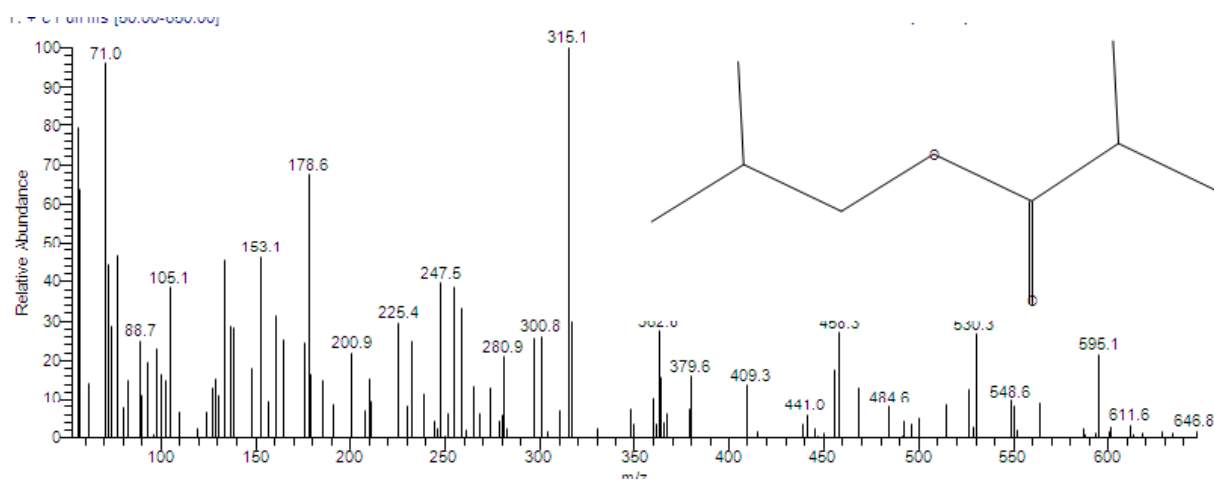
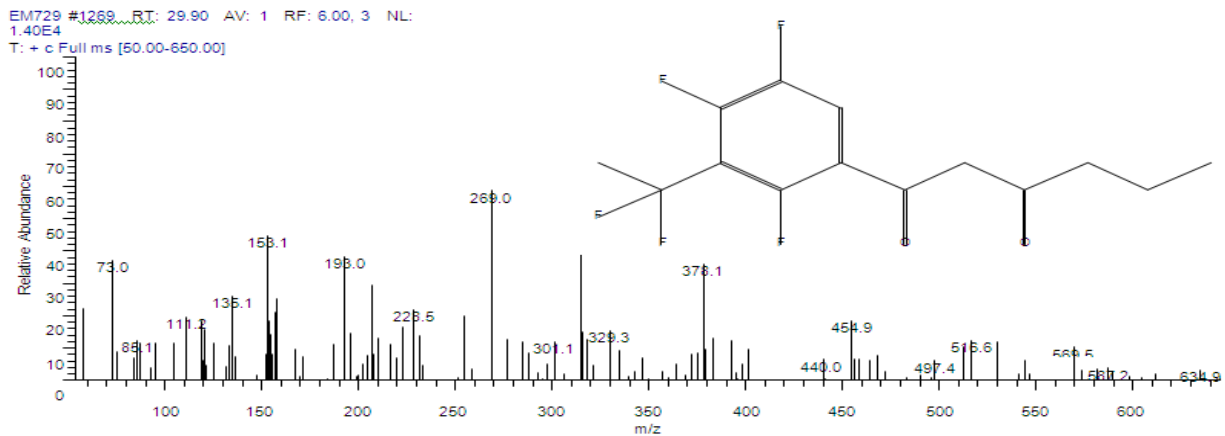
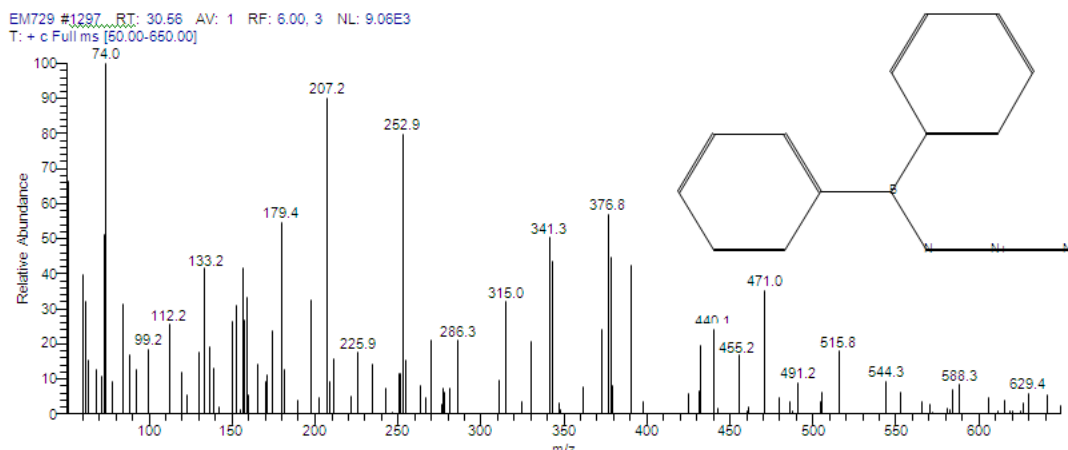


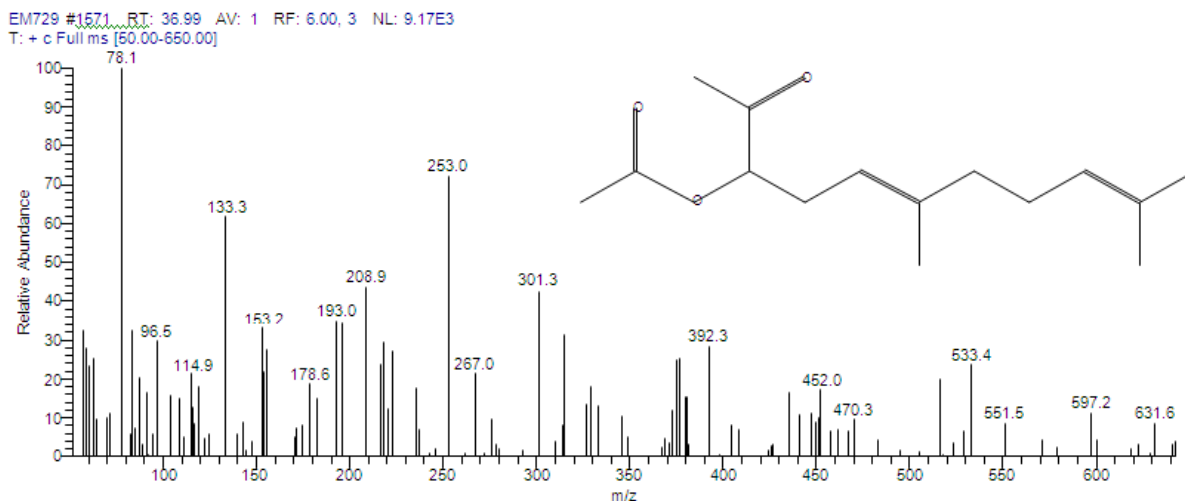
Figure-12
Mass spectrum of Propanoic acid 2-methyl-Methylpropyl ester



Mass spectrum of 2,4,5-Trifluoro-a-oxo-3-(trifluoromethyl) benzenepropanate



Mass spectrum of Azidodiphenyl-borane



Mass spectrum of (SE)-3-Acetoxy-6,10-methyl (1,59 andecadien-2-one

Table-1
UV-VIS peak values of different extracts of Stylosanthes fruticosa

S. No.	Acetone		Ethanol	
	Nanometer	Absorption value	Nanometer	Absorption value
1.	405	1.820	264	1.920
2.	501	0.621	272	1.976
3.	605	0.574	276	1.9860
4.	661	1.009	405	0.591

Table-2
FTIR peak values and functional groups of different extracts of Stylosanthes fruticosa L.

S. No.	Peak Value	Functional Group
1.	3782.43	Phenol
2.	3002.83	Alkene
3.	2834.05	Alkane
4.	2664.17	Ester
5.	2551.41	Para Benzene
6.	1915.52	Alkene
7.	1684.96	Alkene
8.	1423.55	Nitrogen
9.	1283.56	Secondary Alcohol
10.	1180.24	Nitro compounds

Table-3
Phytochemicals identified in ethanolic leaf extract of Stylosanthes fruticosa L. using GC-MS

S. No.	RT	Name of Compound	Molecular formula	MW	Peak Area %	Nature of Compound	Biological Activity
1.	5.35	Phenol, 2-(phenylethyl)	C ₁₄ H ₁₄ O	198	5.11	Alkane	No activity
2.	5.35	Undecane, 2-methyl-(CAS)	C ₁₂ H ₂₆	170	5.11	Aromatic ether	Analgesic
3.	5.35	Propanoic acid 2-hydroxy-butyl ester (CAS)	C ₇ H ₁₄ O ₃	146	5.11	Palmitic acid	Antipyretic
4.	18.18	Trans-4-(3-carbethoxy-3-butenyl)-2-propyltetrahydrofuran	C ₁₄ H ₂₄ O ₄	256	3.18	Steroid	Anaesthetic
5.	21.00	Dodecanoic acid, Methyl-ester (CAS)	C ₁₃ H ₂₆ O ₂	214	6.58	Biterpene	Anticancerous
6.	21.00	Nonanoic acid Methyl ester (CAS)	C ₁₀ H ₂₀ O ₂	172	6.58	α-linolenic acid	Anticancerous
7.	24.56	Trans-5-Hexyl-1,4-dioxane-2-carboxylic acid	C ₁₁ H ₂₀ O ₄	216	9.26	Aromatic alcohol	Antiinflammatory
8.	27.92	Propanoic acid 2-methyl-2-Methylpropyl ester (CAS)	C ₈ H ₁₆ O ₂	144	2.79	Alkene compound	Anticancerous activity
9.	29.90	Ethyl 2,4,5-Trifluoro-oxo-3-(trifluoromethyl) benzenepropanate	C ₁₂ H ₈ F ₆ O ₃	314	2.34	α-sitosterol	Anti-hypercholesterolemia
10.	30.56	Azidodiphenyl-borane	C ₁₂ H ₁₀ BN ₃	207	3.68	Extra Compound	Fungicide
11.	32.88	Hazaleamidemonoepitoxide	C ₁₈ H ₂₉ NO ₂	291	2.55	Amino group	Antiseptic, Antipyretic
12.	36.97	(SE)-3-Acetoxy-6,10-methyl (1,59 undecadien-2-one	C ₁₅ H ₂₄ O ₃	252	2.34	Hexo-Pentane	Anti-androgenic, Hemolytic, Nematicide

Acknowledgement

The authors are grateful to Mr. Arockiam St. Joseph's College (Autonomous), Tiruchirappalli for providing the valuable instructions to carry out the whole experiment.

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