International Journal of Phytopharmacy
Vol. 2 (3), pp.84-90, May-Jun 2012

Research Article

PHYTOCHEMICAL AND GC-MS STUDIES *ONSTYLOSANTHES FRUTICOSA* LINN

M. Paul John Peter and J. Yesu Raj

Department of Chemistry, St. Joseph's College (Autonomous), Tiruchirappalli, India

Corresponding Author: m.peter1989@gmail.com

Abstract
Medicinal plants, herbs, spices and herbal remedies are integral components of alternative system of medicine since times immemorial. *Stylosanthes fruticosa* is a potential folklore medicinal plant (Fabacea) used for Aurveda and Siddha systems of medicine. In this study Alkaloid, Carbohydrate and Glycoside, Saponin, Protein & Amino acid, Phenolic compounds & Flavonoids and Phytosterols were identified as the major phytochemical constituents in the ethanol fractions of *Stylosanthes fruticosa* leaf extract. Their structures were elucidated, on the basis of GC-MS data. (2R, 3R)-4-methyl-2,3-epoxypentan-1-ol(9.26%),1-Cyclohexyl-2,2-difluoro-4-(1,3-dioxolan-2-yl)-4-iodobutanone (9.26%), 9-(Tetrahydropyran-2''-yl)-6-[2'-phenyl-',4',5',6''-tetrapropylphenyl]-9H-purine(9.26%) these different active phytochemicals have been found to possess a wide range of activities. In conclusion *Stylosanthes fruticosa* contains biologically active compounds that may serve as candidate for the discovery of new drugs in the treatment of antimicrobial activities.
Keywords: GC-MS, Phytochemicals, *Stylosanthes fruticosa*, antimicrobial activities

1. Introduction

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value ofthese plants lies in some chemical substances thatproduce a definite physiological action on the humanbody. The most important of these bioactive constituentsof plants are alkaloids, tannins, flavonoids, and phenoliccompounds [1]. Many of these indigenousmedicinal plants are used as spices and food plants.They are also sometimes added to foods meant forpregnant and nursing mothers for medicinal purposes[2]. 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 diseases[3-5]. Knowledge of the chemical constituents of plant is helpful in the discovery oftherapeutic agent as well as new sources of economic materials like oil and gums. The mostimportant bioactive constituents of the plants are alkaloids, tannins, flavonoids and phenoliccompounds. In India large number of plant species had been screened for their pharmacologicalproperties but still a vast wealth of endangered species are unexplored. Medicinal plants are atinterest to the field of biotechnology, as most of the drug industries depend in part on plants forthe production of pharmaceutical compounds.[6]

1.1 Morphological Description: *Stylosanthes fruticosa* (Family *Fabacea*) commonly known as *Wild Lucerne.* 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 subtropics.

1.2 Distributions: Native to the South Sahelian and North Sudanian eco zones from Senegal to Rep. of Sudan (Kordofan) and to East and South Africa. Found in the Sudan, Nigeria, Kenya, Uganda, Tanzania, Zambia, Mozambique, Zimbabwe, South Africa and south India.[7-9]

Stylosanthes fruticosa is much sought after by all kinds of livestock and is grazed heavily by stock in the Sudan and Tanzania (Skerman, 1970). This stylo is suitable for the rehabilitation of fallow land.[10] Analgesic activity has been reported.[11]The present investigation deals with extraction of essential biological active compounds. This study will help to design the new drugs for many incurable drugs.

2. Materials and Methods

2.1 Collection of plant material: The leaves of *Stylosanthes fruticosa*werecollected from the Bharadhidasan university herbarium, Thiruchirappalli, Tamil Nadu, India. They were identified and authenticated by the St.Joseph's College herbarium, Thiruchirappalli, Tamil Nadu, India.

2.2 Preparation of powder and extract: Leaves of *Stylosanthes fruticosa* (500g) was shade dried, powdered and extracted with ethanol for 6-8 hours using soxhlet apparatus. The extract was then filtered through Whatmann filter paper No.41 along with 2g sodium sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulphate is wetted with absolute alcohol. The filtrate is then concentrated by bubbling nitrogen gas into the solution and reduce the volume to 1ml.The extract contains both polar and non-polar phyto components.

2.3 GC-MS Analysis: The GC-MS analysis of *Stylosanthes fruticosa* powder leaves extract with in absolute alcohol, was performed using a Clarus 500 Perkin Elmer gas chromatography equipped with a Elite-5 capillary column (5% phenyl 95% dimethyl polysiloxane) (30nm X 0.25mm ID X 0.25µmdf) and mass detector turbomass gold of the company which was operated in EI mode. Helium was the carriers gas at a flow rate of 1ml/min. and the injector was operated at 290ºC and the oven temperature was programmed as follows; 50ºC at 8ºC/min to 200ºC (5min) at 7ºC/min to 290ºC(10min).

2.4 Identification of components: Interpretation on mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST), WILEY8, FAME having more than 62,000 patterns. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the (NIST) ,WILEY8, FAME library. The name, molecular weight and structure of the components of the test materials were ascertained.[12-13]

3. Results

GC-MS chromatogram of the ethanolic leaf extract of *Stylosanthes fruticosa* (Fig-2) showed 33 peaks indicating the presence of thirty three compounds. The chemical compounds identified in the ethanolic extract of the leaf of *Stylosanthes fruticosa* presented in Table 1. GC-MS analysis revealed that the presence of1-(p-Methyl phenyl)-1-(phenylthio)-2,2-diphenylethene, Ethyl 2-methyl-4-(2-thienyl)-6-trifluoromethylpyridine-3-carboxylate is showed as minimum percent. The phenolic type compounds are recorded predominantly. trans-5-Hexyl-1,4-dioxane-2-carboxylic acid(9.26%), (2R,3R)-4-methyl-2,3-epoxypentan-1-ol(9.26%), (2R,3R)-4-methyl-2,3-epoxypentan-1-ol(9.26%), Dodecanoic acid, methyl ester(6.58%), Nonanoic acid(6.58%), methyl ester(6.58%), 5-methyl-10-(3,5-dinitrobenzyl)-5,10-dihydrophenazine(6.58%). Carbohydrates like allose and sucrose are considered amount is present. The GC-MS analyses revealed that the alcoholic extract is mainly composed of oxygenated hydrocarbons and predominantly phenolic hydrocarbons.

4. Discussions

These phytochemicals are responsible for various pharmacological actions like antimicrobial activity. This study is only a preliminary study of the occurrence of certainproperties of *Stylosanthes fruticosa* bark extract an in-depth study will provide a good concrete basefor all the biochemical and phytochemical functions mentioned above. New scientific strategiesfor the evaluation of natural products with specific biological activities require theimplementation of large screening process.

Stylosanthes fruticosais a potential folklore medicinal plant used for many diseases and infections. Phytochemical analysis by GC-MS revealed presence of fatty acid esters, fatty acid amide, terpenoids, diterpene alcohols and phytol as major compound groups in the methanol fractions. Compositional variation in quantities, qualities and structural features may influence compounds behavior on GC-MS, as well as bioactivities of their precursor fractions.

Fig 1: Plant of *Stylosanthes fruticosa*

Fig 2.GC-MS Profile of leaves extract of *Stylosanthes fruticosa*

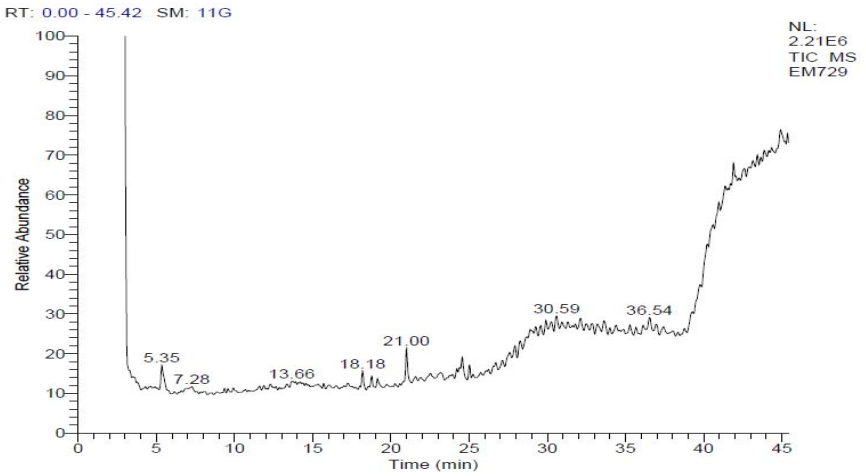
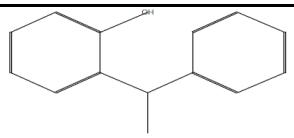
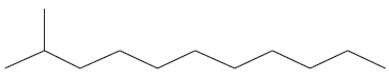
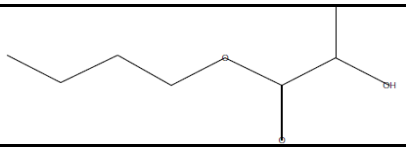
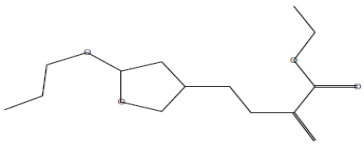
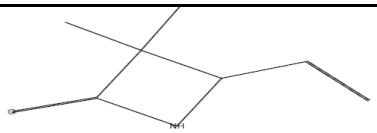
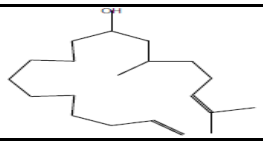
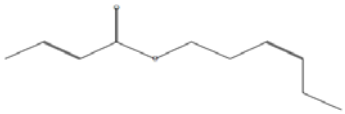
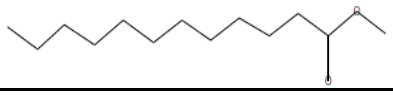
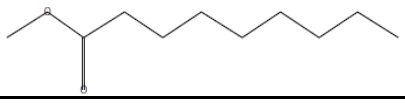
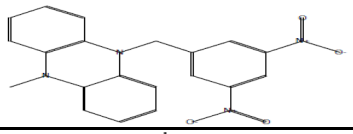
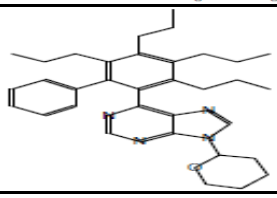
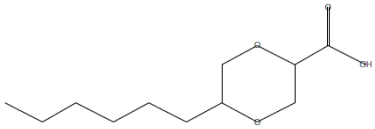
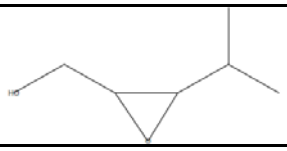
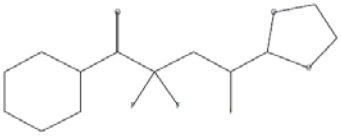
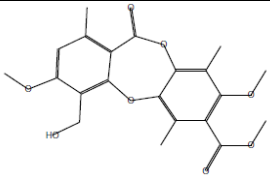
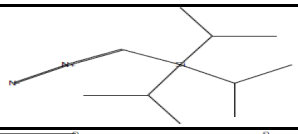
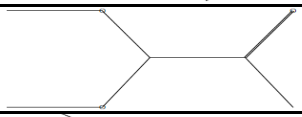
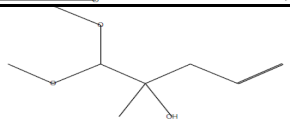
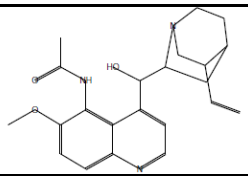
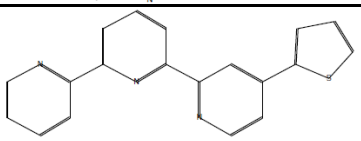
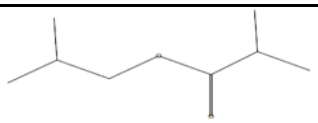
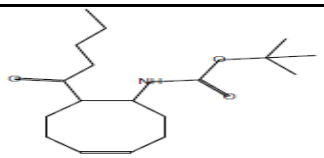
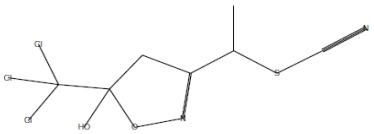
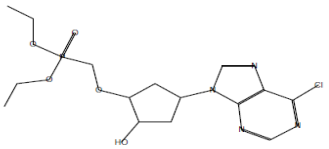
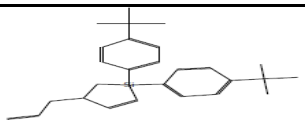
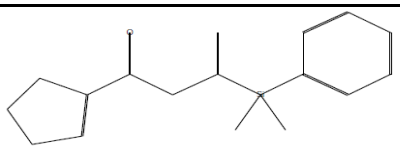
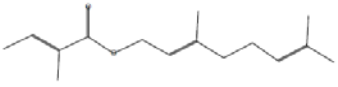


Table1.Compounds present in the leaves extract of *Stylosanthes fruticosa*using GC-MS analysis

S.No	Phytochemical compound	RSI	% Peak area	Structure
1	Phenol, 2-(1-phenylethyl)- Formula: $C_{14}H_{14}O$ MW:198	978	5.11	
2	Undecane Formula: $C_{16}H_{26}$ MW:170	978	5.11	
3	Propanoic acid, 2-hydroxy-, butyl ester Formula: $C_7H_{14}O_3$ MW:146	976	5.11	
4	n-Pentadecane Formula :$C_{15}H_{32}$ MW:212	974	5.11	

5	trans-4-(3-Carbethoxy-3-butenyl)-2-propoxytetrahydrofuran Formula $C_{14}H_{24}O_4$ MW:110	945	3.16	
6	3,3-dimethyl-4-vinyl-2-azetidinone Formula: $C_7H_{11}NO$ MW:125	923	3.18	
7	(6R)-2,6-Dimethyl-2,17-octadecadien-8-ol Formula: $C_{20}H_{38}O$ MW:294	889	3.18	
8	(Z)-3-hexenyl butenoate Formula: $C_{10}H_{16}O_2$ MW:168	880	3.18	
9	Dodecanoic acid, methyl ester Formula: $C_{13}H_{26}O_2$ MW:214	999	6.58	
10	Nonanoic acid, methyl ester Formula: $C_{10}H_{20}O_2$ MW:172	993	6.58	
11	5-methyl-10-(3,5-dinitrobenzyl)-5,10-dihydrophenazine Formula:$C_{20}H_{16}N_4O_4$ MW :376	912	6.58	
12	9-(Tetrahydropyran-2"-yl)-6-[2'-phenyl-',4',5',6"-tetrapropylphenyl]-9H-purine Formula:$C_{13}H_{44}N_4O$ MW:524	902	2.23	
13	trans-5-Hexyl-1,4-dioxane-2-carboxylic acid Formula: $C_{11}H_{20}O_4$ MW:216	921	9.26	
14	(2R,3R)-4-methyl-2,3-epoxypentan-1-ol Formula: $C_6H_{12}O_2$ MW:116	717	9.26	
15	1-Cyclohexyl-2,2-difluoro-4-(1,3-dioxolan-2-yl)-4-iodobutanone Formula: $C_{13}H_{19}F_2IO_3$, MW: 388,	710	9.26	
16	Methyl 4-hydroxymethyl-3,8-dimethoxy-1,6,9-trimethyl-11-oxo-11H-dibenzo[b,e][1,4]dioxepin-11-one Formula: $C_{21}H_{22}O_8$ MW:402	746	2.38	

17	Triisopropylsilyldiazomethane Formula: $C_{10}H_{22}N_2Si$, MW: 198	797	3.06	
18	2-Propanone,1,1-dimethoxy- Formula: $C_5H_{10}O_3$, MW: 118,	858	2.91	
19	1,1-Dimethoxy-2-methyl-4-penten-2-ol Formula: $C_8H_{16}O_3$, MW: 160,	816	2.91	
20	Acetamide, N-[(8à,9R)-9-hydroxy-6'-methoxycinchonan-5'-yl]- Formula: $C_{22}H_{27}N_3O_3$, MW: 381,	803	2.91	
21	4-(2'-Thienyl)-2,2',6',2''-terpyridine Formula: $C_{19}H_{13}N_3S$, MW: 315,	704	2.91	
22	Propanoic acid, 2-methyl-, 2-methylpropyl ester (CAS) Formula: $C_8H_{16}O_2$, MW: 144,	982	2.79	
23	cis-5-Valeryl-6-([tert-butoxycarbonyl]amino)cyclooctene Formula: $C_{18}H_{31}NO_3$, MW: 309,	972	3.33	
24	5-Trichloromethyl-3-[1-(cyanothio)ethyl]-4,5-dihydroisoxazol-5-ol Formula: $C_7H_7Cl_3N_2O_2S$, MW: 288,	934	3.33	
25	9-[(1'à,3'à,4'á)-4'-(Diethylphosphono)methoxy-3'-hydroxycyclopentyl]-6-chloropurine Formula: $C_{15}H_{22}ClN_4O_5P$, MW: 404	899	3.33	
26	1,1-bis(4-tert-butylphenyl)-4-(2-propenyl)-1-silacyclo-2-pentene Formula: $C_{27}H_{36}Si$, MW: 388,	895	2.40	
27	1-Cyclopentenyl 2-(Dimethylphenylsilyl)-2-propenyl Ketone Formula: $C_{17}H_{22}OSi$, MW: 270,	855	2.40	
28	Geranyltiglate Formula: $C_{15}H_{24}O_2$, MW: 236,	758	2.38	

5. Phytochemical Studies

5.1 Preliminary Phytochemical Analysis: Qualitative phytochemical studies of different extracts of leaves of stylosanthes fruticosa linn were performed on its alcoholic and water extracts to identify its Alkaloid, Carbohydrate and Glycoside, Saponin, Protein & Amino acid, Phenolic compounds & Flavonoids and Phytosterols by using suitable chemicals and reagents (Table 2).Alkaloid test results of leaf showed slightly positive in all four tested reagents. Qualitative phytochemical studies of Carbohydrate & Glycoside showed a good characteristic colour and precipitate in all five tested reagent. Slight presence of Saponin was confirmed by foam test in leaf in all extracted solvents. Protein and amino acid was found absent in all tests. However in Millon's test alcoholic extract showed slight presence of protein. Phenolic compounds and Flavonoids were abundantly present in all the extracts. However alkaline test showed the moderate result in comparison to other two tests [14-18]. Libermann-Burchards test showed slight presence of phytosterol in all the extracts. The above qualitative phytochemical screening showed that the whole plant is a rich source of Glycosides, Phenols & Flavonoids. However, presence of protein and alkaloids is limited in leaves.[19-20]

Table 2: Qualitative Phytochemical Screening of leaves of *Stylosanthes Fruticosa Linn*

	Cold Maceration		**Sohxalation**
Phytochemical test	**Alcoholic Extract**	**Alcoholic Extract**	**Ethanolic Extract by Sohxalation**
1. Alkaloids			
Mayer's test	+	-	+
Wagner's test	+	-	+
Hager's test	+	+	-
Dragendorff's test	+	+	+
2. Carbohydrates & Glycosides			
Molish's test	+++	+++	+++
Fehling's test	+++	+++	+++
Barfoed's test	+++	+++	+++
Benedict's test	+++	+++	+++
Borntrager's test	+++	+++	+++
3.Saponins			
Foam test	+	+	+
4. Proteins & amino acid			
Millon's test	+	-	-
Biuret's test	-	-	-
Ninhydrin test	-	-	-
5. Phenolic compounds & flavonoids			
Ferric chloride test	+++	+++	+++
Lead acetate test	+++	+++	+++
Alkaline test	++	++	++
6. Phytosterol			
Libermann-Burchards test	+	+	+
-,Negative; +, Slight; ++, Moderate;+++,Frequent;			

Acknowledgement

We sincerely thank to S.Maria arul kulanthai raj St.Joseph's College, Thiruchirappalli, for doing this valuable work. I warmly thank Mr.Arockiam, for his valuable advice and friendly help. His extensive discussions around my work and interesting explorations in operations have been very helpful for this study.

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