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GC-MS Analysis of bioactive components on the Leaves extract of *Stylosanthes fruticosa*- A potential folklore medicinal plant

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ABSTRACT

The present study of phytochemical analysis in the leaf powder extract with absolute alcohol,the phytochemical compound screened by GC-MS method. In this GC-MS analysis, 33 bioactivephytochemical compounds were identified in leaf powder of Stylosanthes fruticosa .The 33 compounds predominantly Phenolic compounds and Flavonoids derivatives are present included , Carbohydrateand Glycoside, Saponin,, and Phytosterols compounds.protein and alkaloids is limited in the leaf extract. These different active phytochemicals have been found to possess a wide range of activities, which may help in the protection against incurable diseases.

Key words: GC-MS, Phytochemicals, *Stylosanthes fruticosa.*

INTRODUCTION

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[1-3]. 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 phenolic compounds. In India large number of plant species had been screened for their pharmacological properties but still a vast wealth of endangered species are unexplored. Medicinal plants are at interest to the field of biotechnology, as most of the drug industries depend in part on plants for the production of pharmaceutical compounds.[4]

***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.

Distributions: Native to the South Sahelian and North Sudanian ecozones 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. [5-7]

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.[8] The present investigation deals with extraction of essential biological active compounds. This study will help to design the new drugs for many incurable drugs.

MATERIALS AND METHODS

Collection of plant material

The leaves of *Stylosanthes fruticosa*werecollected from the Bharadhidasan university herbarium, Trichirappalli, Tamil Nadu, India. They were identified and authenticated by the Bharadhidasan university herbarium, Trichirappalli, Tamil Nadu, India.

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 phytocomponents.

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).

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.[9-10]

RESULTS AND DISCUSSION

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-Methylphenyl)-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. 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*

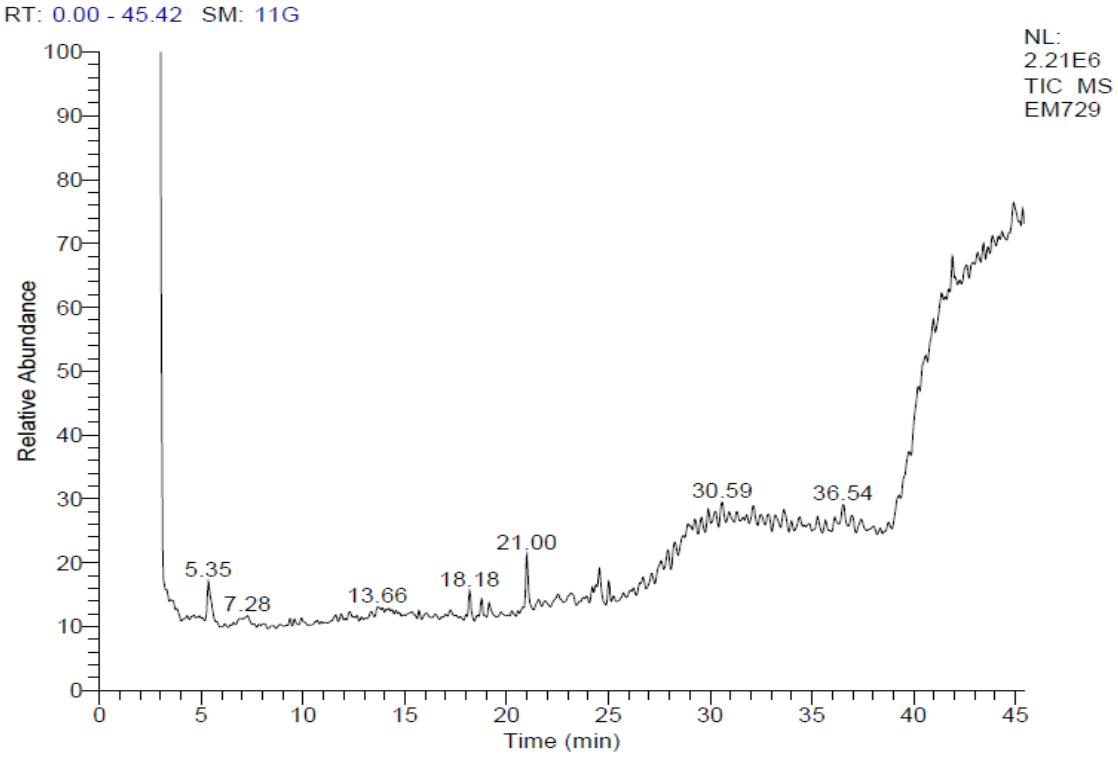
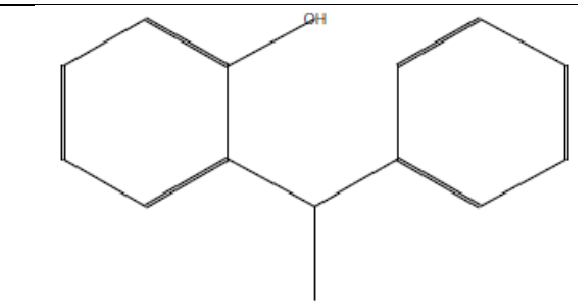
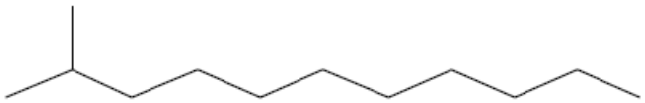
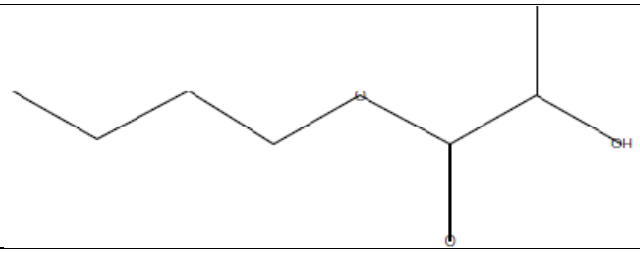
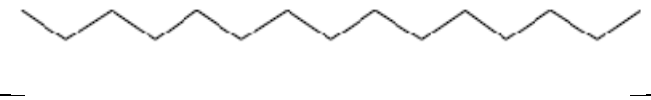
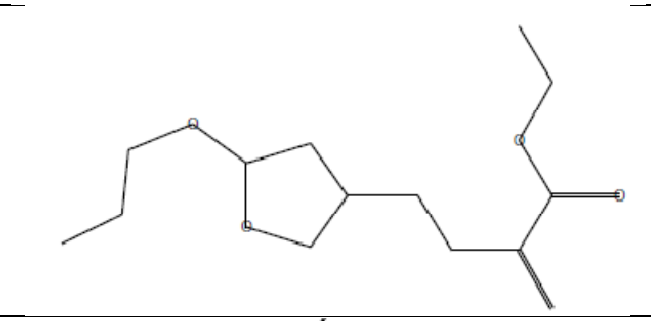
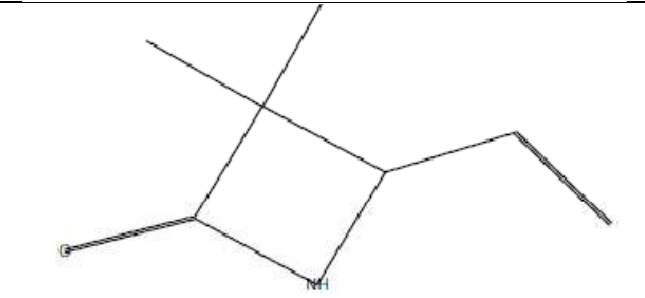
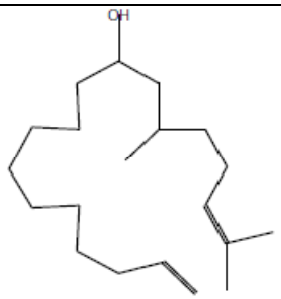
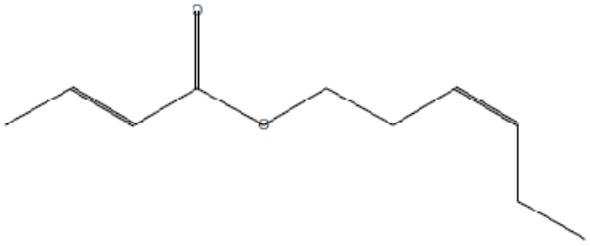
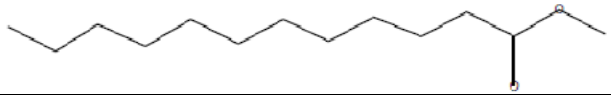
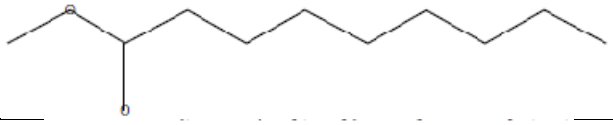
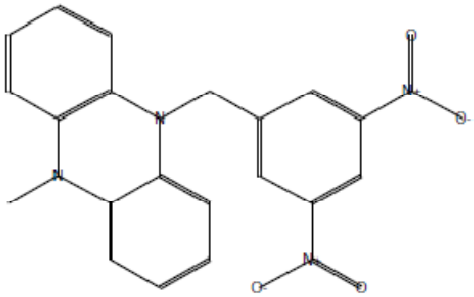
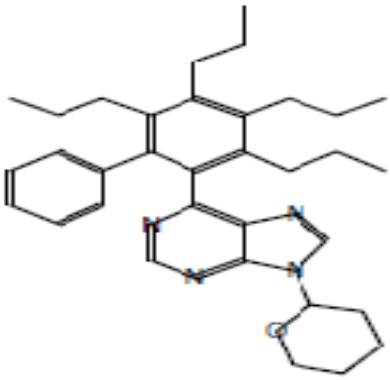
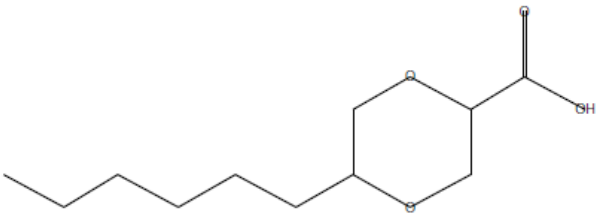
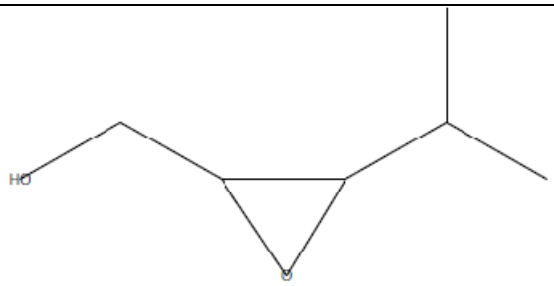
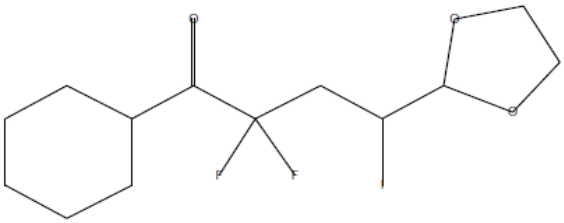
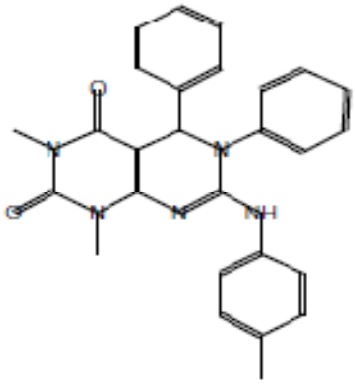
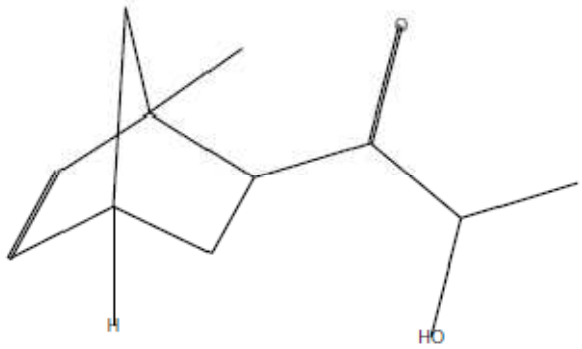
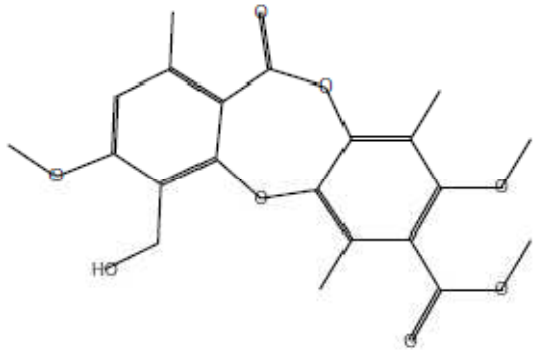
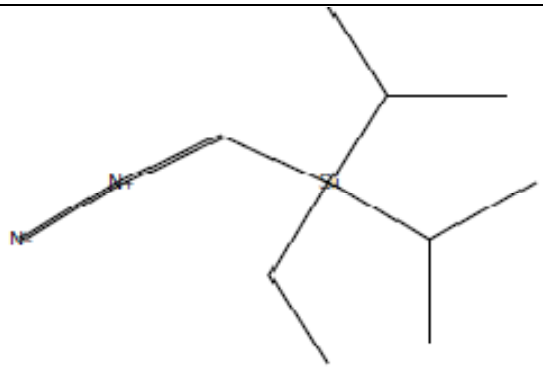
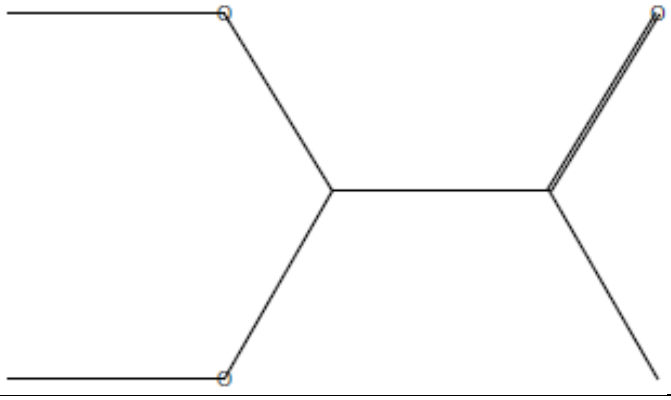


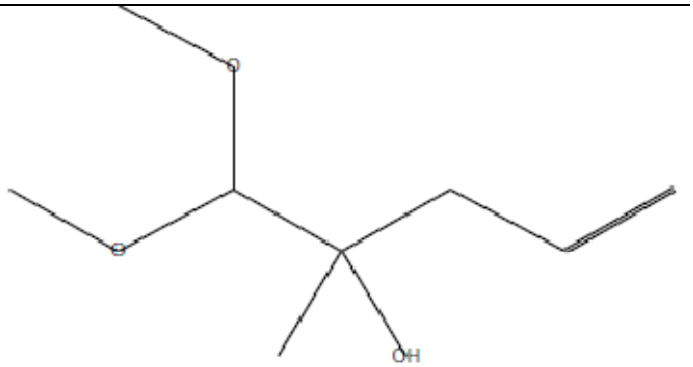
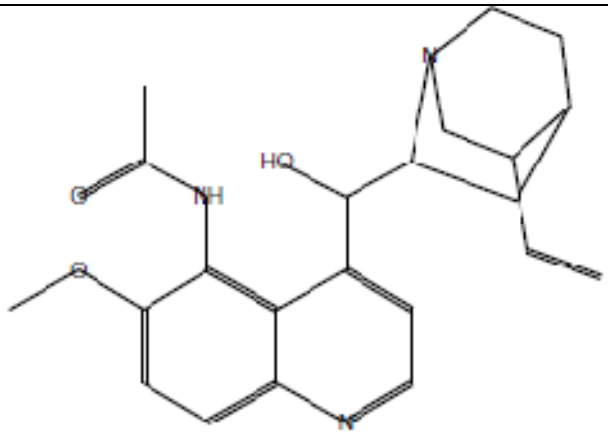
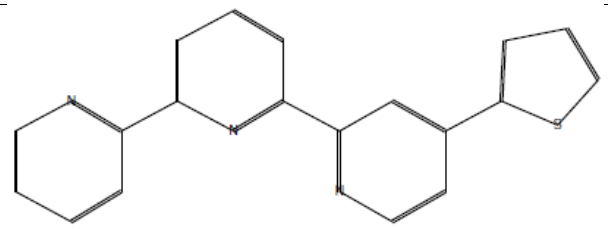
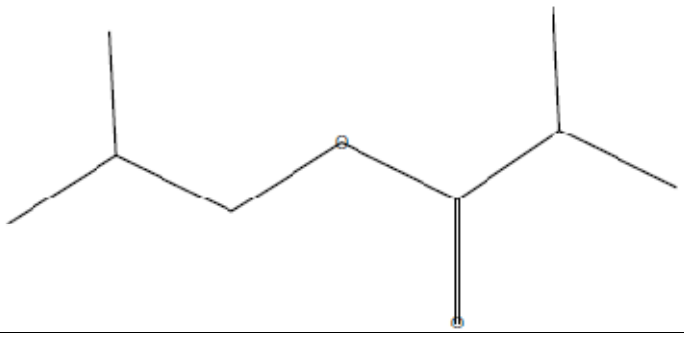
Table1.Compounds present in the leaves extract of *Stylosanthes fruticosa* using GC-MS analysis

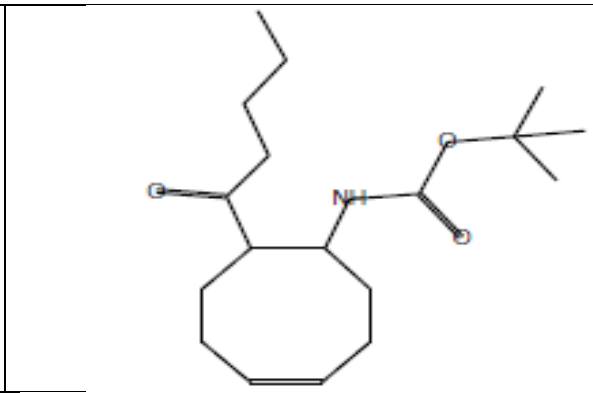
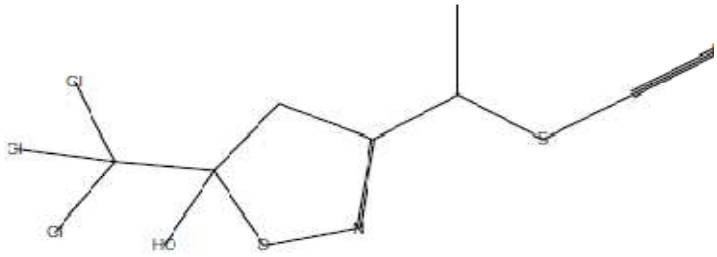
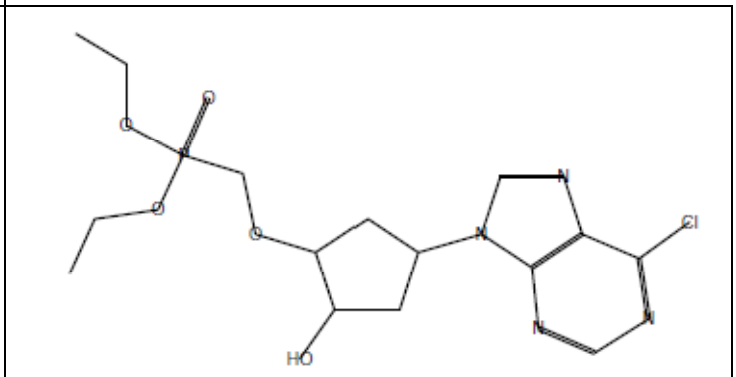
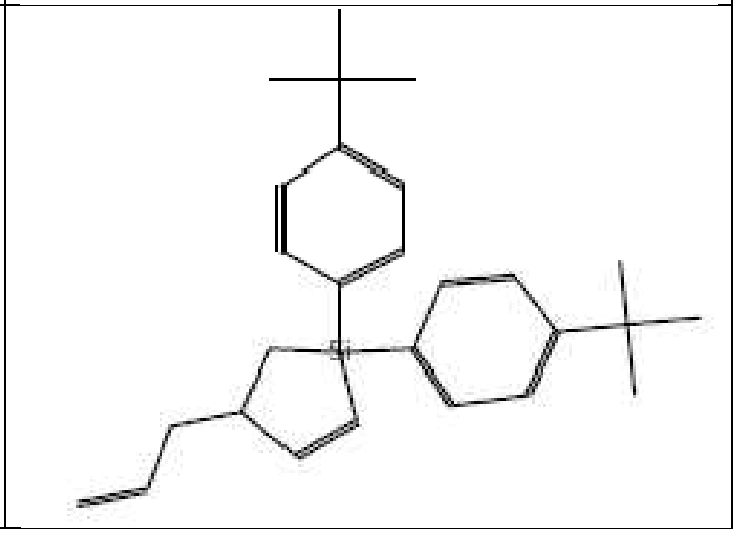
Serial No	Phytochemical compound	RSI	% Peak area	Structure
1	Phenol, 2-(1-phenylethyl)- Formula: C14H14O MW:198	978	5.11	
2	Undecane Formula: C16H26 MW:170	978	5.11	
3	Propanoic acid, 2-hydroxy-, butyl ester Formula: C7H14O3 MW:146	976	5.11	
4	n-Pentadecane Formula :C15H32 MW:212	974	5.11	
5	trans-4-(3-Carbethoxy-3-butenyl)-2-propoxytetrahydrofuran Formula C14H24O4 MW:110	945	3.16	
6	3,3-dimethyl-4-vinyl-2-azetidinone Formula: C7H11NO MW:125	923	3.18	

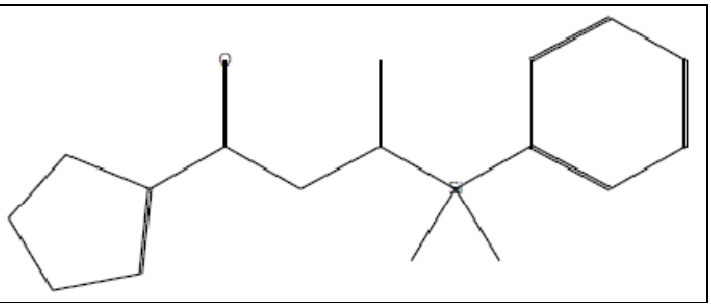
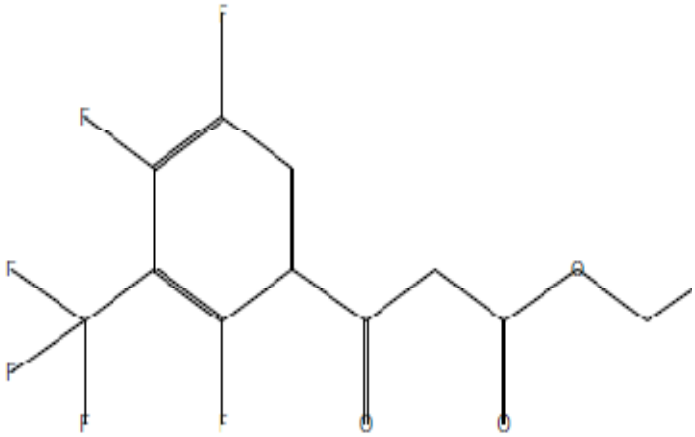
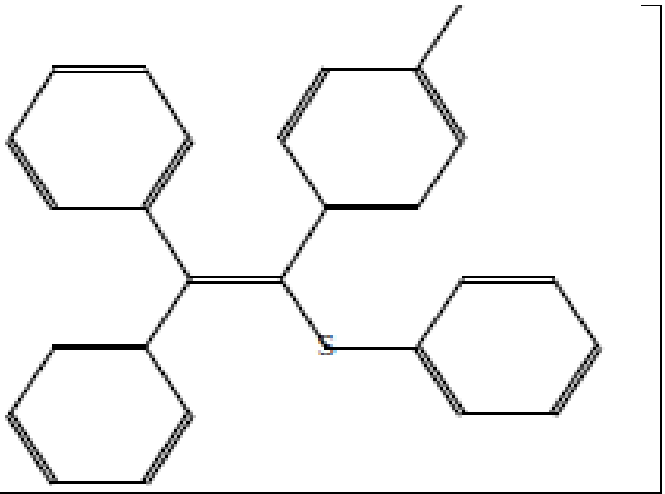
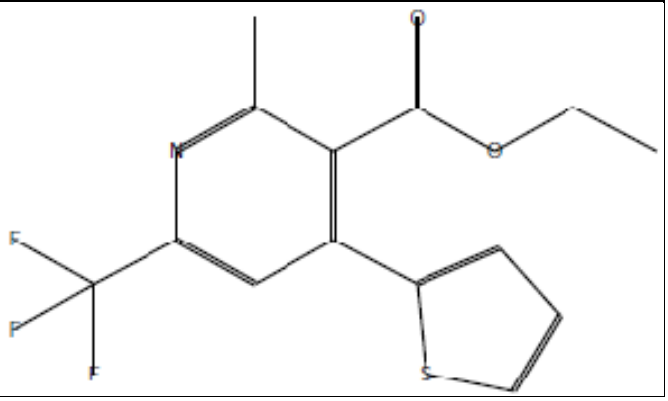
7	(6R)-2,6-Dimethyl-2,17-octadecadien-8-ol Formula: C20H38O MW:294	889	3.18	
8	(Z)-3-hexenyl butenoate Formula: C10H16O2 MW:168	880	3.18	
9	Dodecanoic acid, methyl ester Formula: C13H26O2 MW:214	999	6.58	
10	Nonanoic acid, methyl ester Formula: C10H20O2 MW:172	993	6.58	
11	5-methyl-10-(3,5-dinitrobenzyl)-5,10-dihydrophenazine Formula:C20H16N4O4 MW :376	912	6.58	
12	9-(Tetrahydropyran-2"-yl)-6-[2'-phenyl-',4',5',6"-tetrapropylphenyl]-9H-purine Formula:C13H44N4O MW:524	902	2.23	

13	trans-5-Hexyl-1,4-dioxane-2-carboxylic acid Formula: C11H20O4 MW:216	921	9.26	
14	(2R,3R)-4-methyl-2,3-epoxypentan-1-ol Formula: C6H12O2 MW:116	717	9.26	
15	1-Cyclohexyl-2,2-difluoro-4-(1,3-dioxolan-2-yl)-4-iodobutanone Formula: C13H19F2IO3, MW: 388,	710	9.26	
16	1,3-Dimethyl-7-(4-methylphenyamino)-5,6-dihydro-5,6-diphenylpyrimido[4,5-d]pyrimidine-2,4(1H,3H)-dione Formula: C27H25N5O2 MW: 451	861	2.38	
17	Geranyl tiglate Formula: C15H24O2, MW: 236,	758	2.38	

18	(2S)-2-Hydroxy-1-((1R,2R,4R)-1-(methyl1bicyclo[2.2.1]hept-5-en-2-yl)propan-1-one Formula: C11H16O2, MW: 180,	757	2.38	
19	Methyl 4-hydroxymethyl-3,8-dimethoxy-1,6,9-trimethyl-11-oxo-11H-dibenzo[b,e][1,4]dioxepin-11-one Formula: C21H22O8 MW:402	746	2.38	
20	Triisopropylsilyldiazomethane Formula: C10H22N2Si, MW: 198	797	3.06	
21	2-Propanone,1,1-dimethoxy-(CAS) Formula: C5H10O3, MW: 118,	858	2.91	

22	1,1-Dimethoxy-2-methyl-4-penten-2-ol Formula: C8H16O3, MW: 160,	816	2.91	
23	Acetamide, N-[(8à,9R)-9-hydroxy-6'-methoxycinchonan-5'-yl]-(CAS) Formula: C22H27N3O3, MW: 381,	803	2.91	
24	4-(2'-Thienyl)-2,2',6',2''-terpyridine Formula: C19H13N3S, MW: 315,	704	2.91	
25	Propanoic acid, 2-methyl-, 2-methylpropyl ester (CAS) Formula: C8H16O2, MW: 144,	982	2.79	

26	cis-5-Valeryl-6-([tert-butoxycarbonyl]amino)cyclooctene Formula: C18H31NO3, MW: 309,	972	3.33	
27	5-Trichloromethyl-3-[1-(cyanothio)ethyl]-4,5-dihydroisoxazol-5-ol Formula: C7H7Cl3N2O2S, MW: 288,	934	3.33	
28	9-[(1'à,3'à,4'á)-4'-(Diethylphosphono)methoxy-3'-hydroxycyclopentyl]-6-chloropurine Formula: C15H22ClN4O5P, MW: 404	899	3.33	
29	1,1-bis(4-tert-butylphenyl)-4-(2-propenyl)-1-silacyclo-2-pentene Formula: C27H36Si, MW: 388,	895	2.40	

30	1-Cyclopentenyl 2-(Dimethylphenylsilyl)-2-propenyl Ketone Formula: C17H22OSi, MW: 270,	855	2.40	
31	Ethyl2,4,5-Trifluoro-á-oxo-3-(trifluoromethyl)benzenepropanoate Formula: C12H8F6O3, MW: 314,	975	2.34	
32	1-(p-Methylphenyl)-1-(phenylthio)-2,2-diphenylethene Formula: C27H22S, MW: 378,	948	2.34	
33	Ethyl 2-methyl-4-(2-thienyl)-6-trifluoromethylpyridine-3-carboxylate Formula: C14H12F3NO2S, MW: 315,	387	2.34	

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