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Divya S
Department of Zoology, Thiruvalluvar University, Vellore, Tamil Nadu, India

Arivoli S
Department of Zoology, Thiruvalluvar University, Vellore, Tamil Nadu, India

Samuel T
Department of Zoology, Madras Christian College, Chennai, Tamil Nadu, India

Raveen R
Department of Zoology, Madras Christian College, Chennai, Tamil Nadu, India

Jayakumar M
Department of Zoology, University of Madras, Chennai, Tamil Nadu, India

Correspondence
Dr. Arivoli S
Department of Zoology, Thiruvalluvar University, Vellore, Tamil Nadu, India

Gas chromatography-mass spectrometry analysis of *Cardiospermum halicacabum* Linnaeus (Sapindaceae) and *Chenopodium album* Linnaeus (Chenopodiaceae) leaves

Divya S, Arivoli S, Samuel T, Raveen R and Jayakumar M

Abstract
Plants produce enormous varieties of chemicals which are believed to be important in mediating the interaction between them and their environment. Modern chemistry has discovered the structures of many of these biologically active compounds and systematic studies of natural products for plant protection which became recognized within the field of chemistry. Secondary metabolites have been studied using the approach of classical phytochemistry, focused on knowledge of the chemical components of a plant. In the present study the Gas Chromatography-Mass Spectrometry of *Cardiospermum halicacabum* and *Chenopodium album* leaf extracts were analyzed.

Keywords: *Cardiospermum halicacabum*, *Chenopodium album*, leaf extracts, GC-MS analysis, phytochemical compounds

1. Introduction
Secondary metabolites have been studied using the approach of classical phytochemistry, focused on knowledge of the chemical components of a plant. Often, plant secondary metabolites may be referred to as plant natural products, in which case they elicit effects on other organisms [1]. Plant families generally make use of chemical structures for defensive functions [2]. Secondary metabolites can be classified according to their chemical structure, composition, solubility in different solvents, or on the basis of their synthesis pathway. A simple classification, based on chemical structure, includes three main groups: terpenes-composed almost entirely of carbon and hydrogen and including plant volatiles, cardiac glycosides, carotenoids and sterols, phenolics-with the common feature of having one or more phenol rings and including phenolic acids, coumarins, flavonoids, tannins and lignins, and nitrogen-containing compounds which are extremely diverse, including alkaloids and glucosinolates [3]. In the present study, *Cardiospermum halicacabum* and *Chenopodium album* leaf extracts were analyzed using Gas Chromatography-Mass Spectrometry for their phytocompounds.

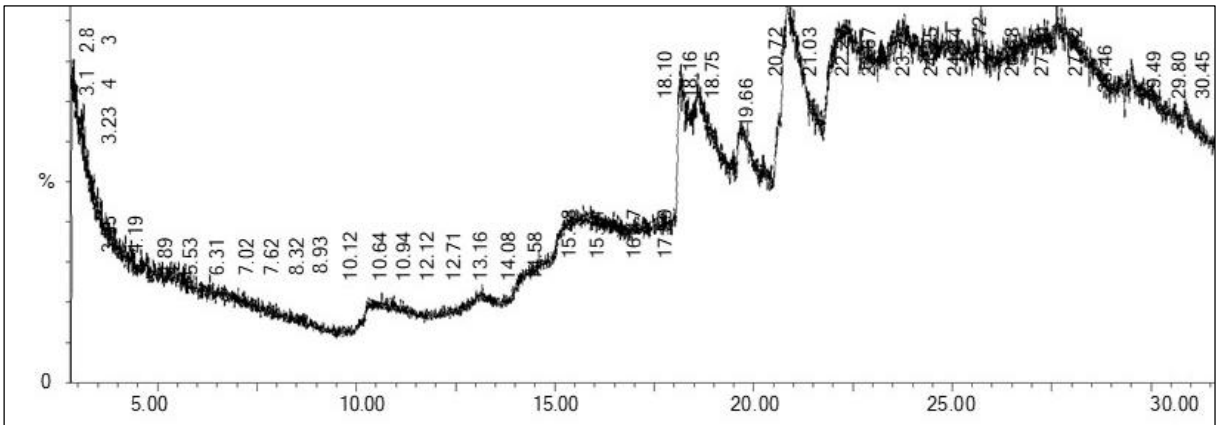
2. Materials and Methods
2.1 Preparation and screening of phytoextracts
Fresh healthy leaves of *Cardiospermum halicacabum* and *Chenopodium album* were collected in and around Walajahpet (12.9250ºN, 79.3669ºE) and Ponnai (13.1241ºN, 79.2536ºE), Ranipet, Vellore district, Tamil Nadu, India. *Cardiospermum halicacabum* leaves were washed under running tap water to remove all traces of soil particles and other dirt and shade dried for 10-15 days. The leaves were then powdered using an electric blender and sieved to obtain a fine powder. The powdered leaves were used for the extraction process. The plant material (1Kg) was soxhleted subsequently with 3L of solvents *viz*., chloroform and methanol each [4]. The leaf extract was concentrated by evaporation. Likewise, the same methodology was adopted to obtain the chloroform and methanolic extract of *Chenopodium album* leaves.

2.2 Gas chromatography-mass spectrometry (GC-MS)
GC-MS is a technique; consisting of two analytical procedures in sequence, namely a Gas Chromatography (GC) separation followed by Mass Spectroscopy (MS) detection. The purpose of the GC step is to separate multiple compounds in a sample so that they reach the MS detector one at a time. GC uses a high-resolution fused silica capillary column housed in a temperature-controlled oven.

The capillary column contains a stationary phase; a fine solid support coated with a non-volatile liquid. The solid remains to be the stationary phase. GC plays a fundamental role in determining how many components and in what proportion they exist in a mixture. However, the ability to establish the nature and chemical structure of these separated and quantified compounds is ambiguous and reduced, and requires a spectroscopic detection system. The most used, is the Mass Spectrometric Detector (MSD), which allows obtaining the "fingerprint" of the molecule, i.e. its mass spectrum. Mass spectra provide information on the molecular weight, elemental composition, if a high resolution mass spectrometer is used, functional groups present, and, in some cases, the geometry and spatial isomerism of the molecule.

3. Results

The GC-MS of the chloroform and methanolic leaf extract of *Cardiospermum halicacabum* showed presence of methylene chloride, 11-hexadecen-1-ol, (Z)-, 3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol, n-hexadecanoic acid, 1-hexyl-2-nitrocyclohexane, naproxen (Figure 1; Table 1) and β-D-glucopyranoside, methyl decanoate, n-hexadecanoic acid, 11-hexadecen-1-ol, (Z)-, 11-eicosenoic acid, (Z)-, e-15-heptadecenal (Figure 2; Table 2) respectively. On the other hand the phytochemicals for *Chenopodium album* leaf extracts were phytol, n-hexadecanoic acid, 1-hexyl-2-nitrocyclohexane, di-n-octyl phthalate, ergosta-5, 24 (28)-dien-3-ol, (3β)-, hexatriacontane (Figure 3; Table 3) and phytol, n-hexadecanoic acid, 2H-pyran, 2-(7-heptadecynyloxy) tetrahydro, di-n-octyl phthalate, squalene, heptacosane, tetratetracontane, vitamin E, heptacosane,1-chloro, betulin, 3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol (Figure 4; Table 4) respectively.



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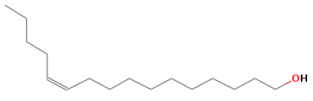
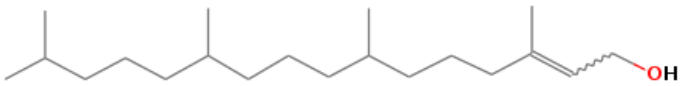
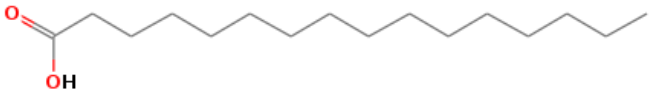
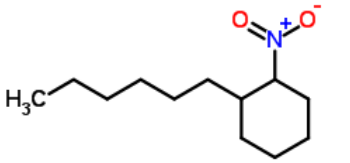
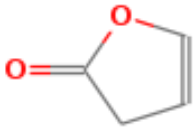
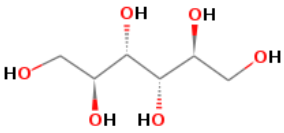
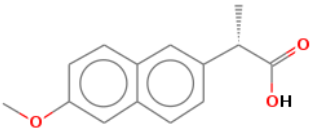
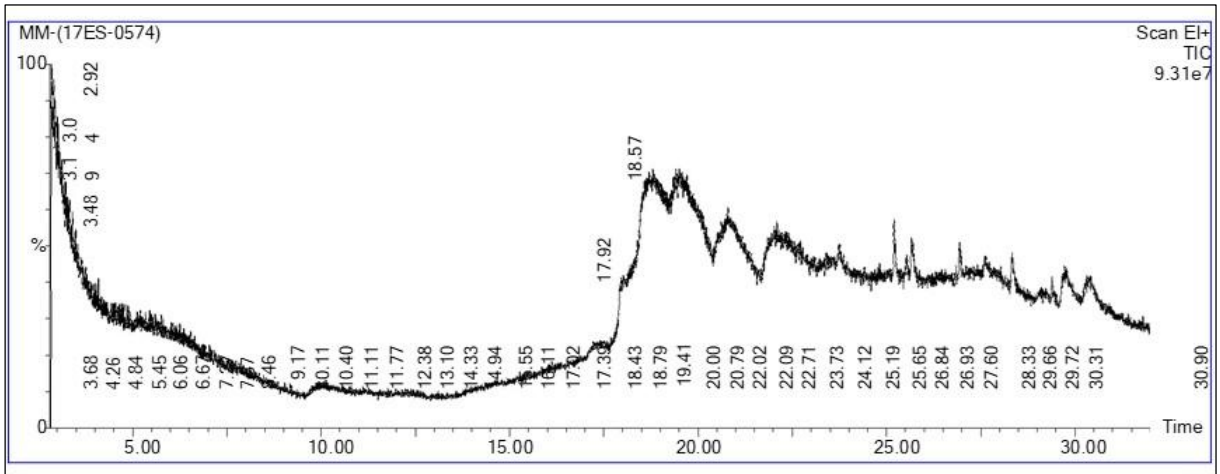
3	18.209	3081	11,698,990	1,033,477.6	2.877	5.74
4	18.595	3158	8,480,202	1,414,054.1	3.937	7.86
5	20.831	3605	20,878,678	7,679,990.5	21.382	42.68
6	22.266	3892	8,456,726	3,455,200.0	9.619	19.20
7	22.406	3920	7,165,270	865,882.1	2.411	4.81
8	25.717	4582	10,803,607	930,864.1	2.592	5.17
9	27.618	4962	6,951,706	1,235,797.4	3.441	6.87

Fig 1: GC-MS analysis of *Cardiospermum halicacabum* chloroform leaf extract

Table 1: Phytochemical constituents of GC-MS analysis of *Cardiospermum halicacabum* chloroform leaf extract

Phytochemical constituent	Molecular formula (Molecular weight)	Structure
Methylene chloride	CH_2Cl_2 (84.933)	
11-Hexadecen-1-ol, (Z)-	$C_{16}H_{32}O$ (240.424)	

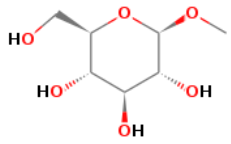
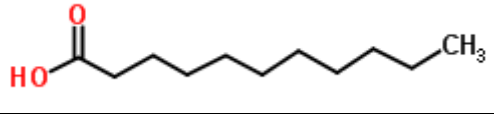
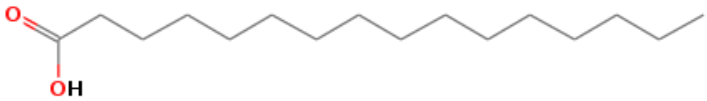
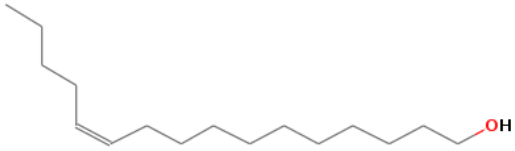
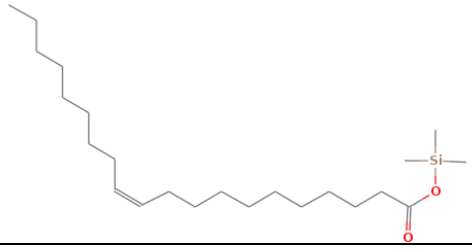
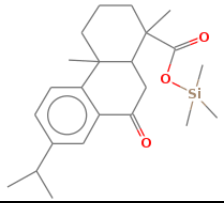
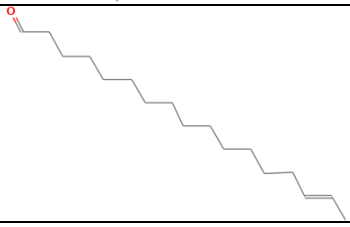
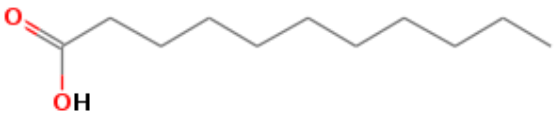
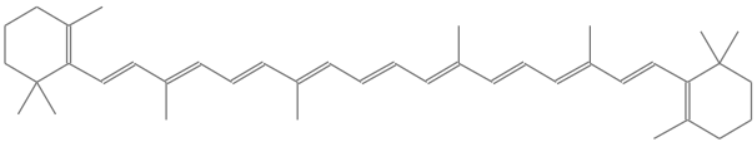
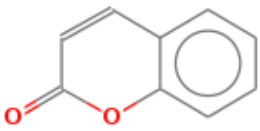
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$ (296.531)	
N-Hexadecanoic acid	$C_{16}H_{32}O_2$ (256.424)	
1-Hexyl-2-nitrocyclohexane	$C_{12}H_{23}O_2N$ (213.316)	
2(3H)-Furanone	$C_4H_4O_2$ (84.073)	
D-Mannitol	$C_6H_{14}O_6$ (182.171)	
Hexadecanal	$C_{16}H_{32}O$ (240.424)	
Naproxen	$C_{14}H_{14}O_3$ (230.259)	

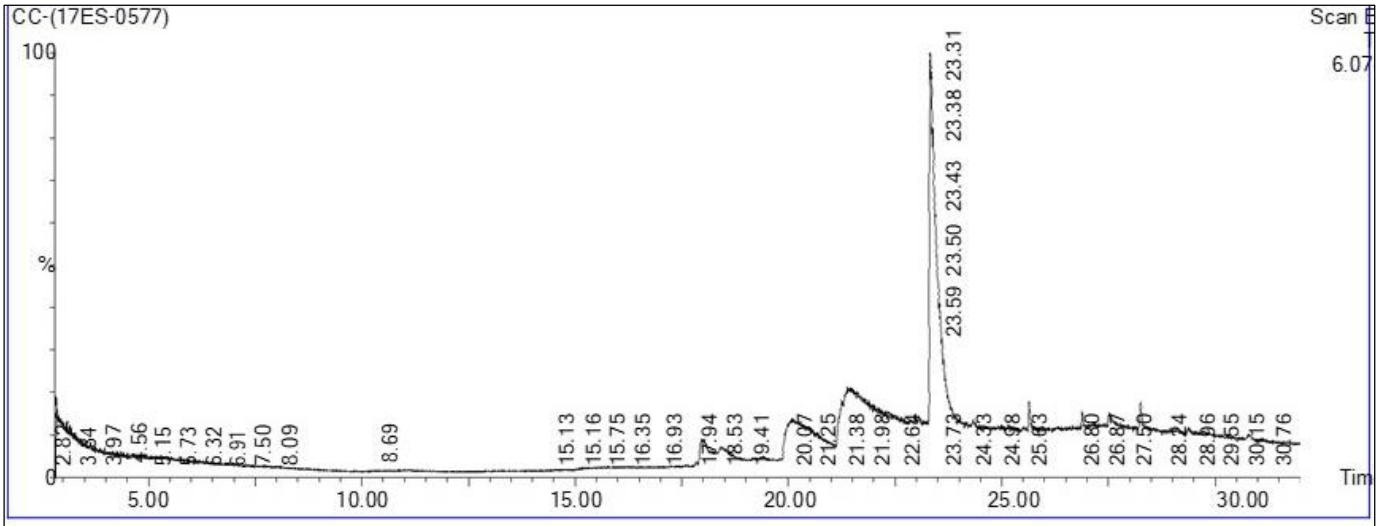


#	RT	Scan	Height	Area	Area %	Norm %
1	18.790	3197	40,886,848	35,937,600.0	29.966	100.00
2	19.495	3338	39,323,192	34,211,548.0	28.527	95.20
3	20.786	3596	26,395,164	20,989,914.0	17.502	58.41
4	22.086	3856	19,576,104	13,610,571.0	11.349	37.87
5	22.706	3980	13,521,020	3,785,983.0	3.157	10.53
6	23.407	4120	9,037,938	3,101,695.0	2.586	8.63
7	23.732	4185	10,392,479	2,192,044.2	1.828	6.10
8	24.817	4402	6,589,922	1,203,363.2	1.003	3.35
9	25.192	4477	16,897,254	1,752,782.1	1.462	4.88
10	25.653	4569	11,296,981	1,189,653.2	0.992	3.31
11	30.315	5501	7,382,792	1,950,796.8	1.627	5.43

Fig 2: GC-MS analysis of *Cardiospermum halicacabum* methanolic leaf extract

Table 2: Phytochemical constituents of GC-MS analysis *Cardiospermum halicacabum* methanolic leaf extract

Phytochemical constituent	Molecular formula (Molecular weight)	Structure
β-D-Glucopyranoside, methyl	$C_7H_{14}O_6$ (194.182)	
Methyl decanoate	$C_{11}H_{22}O_2$ (186.295)	
N-Hexadecanoic acid	$C_{16}H_{32}O_2$ (256.424)	
11-Hexadecen-1-ol, (Z)-	$C_{16}H_{32}O$ (240.424)	
11-Eicosenoic acid, (Z)-	$C_{23}H_{46}O_2Si$ (382.695)	
7-Oxodehydroabietic acid, trimethylsilyl ester	$C_{23}H_{34}O_3Si$ (386.599)	
E-15-Heptadecenal	$C_{17}H_{32}O$ (252.435)	
Undecanoic acid	$C_{11}H_{22}O_2$ (186.291)	
β Carotene	$C_{40}H_{56}$ (536.872)	
Coumarin	$C_9H_6O_2$ (146.142)	
Methylene chloride	CH_2Cl_2 (84.933)	



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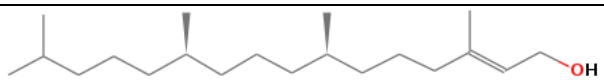
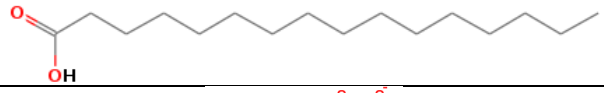
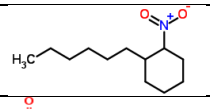
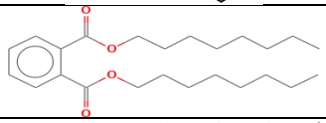
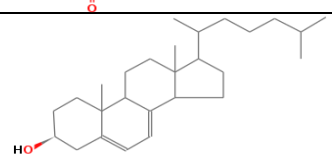
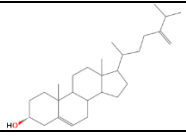
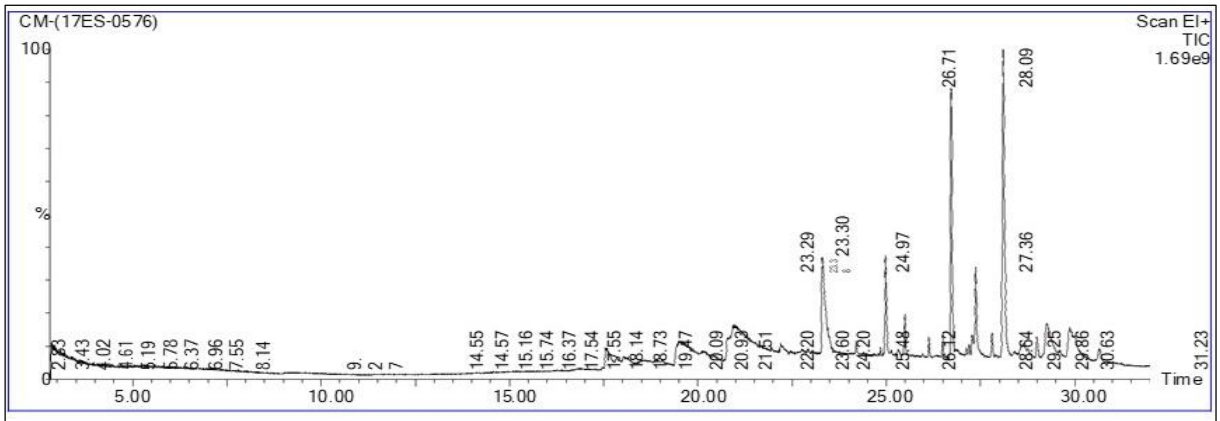
3	21.376	3714	79,045,664	58,736,536.0	27.243	60.65
4	22.326	3904	27,622,966	6,591,722.0	3.057	6.81
5	23.306	4100	530,873,216	96,837,120.0	44.915	100.00
6	27.503	4939	21,649,996	5,477,938.5	2.541	5.66
7	28.243	5087	42,273,304	6,321,021.0	2.932	6.53

Fig 3: GC-MS analysis of *Chenopodium album* chloroform leaf extract

Table 3: Phytochemical constituents of GC-MS analysis of *Chenopodium album* chloroform leaf extract

Phytochemical constituent	Molecular formula (Molecular weight)	Structure
Phytol	$C_{20}H_{40}O$ (296.531)	
N-Hexadecanoic acid	$C_{16}H_{32}O_2$ (256.424)	
1-Hexyl-2-nitrocyclohexane	$C_{12}H_{23}O_2N$ (213.316)	
Di-n-octyl phthalate	$C_{24}H_{38}O_4$ (390.556)	
Cholesta-5,7-dien-3-ol, (3β)-	$C_{27}H_{44}O$ (384.637)	
Ergosta-5,24(28)-dien-3-ol, (3β)-	$C_{28}H_{46}O$ (398.664)	
Hexatriacontane	$C_{36}H_{74}$ (506.972)	



#	RT	Scan	Height	Area	Area %	Norm %
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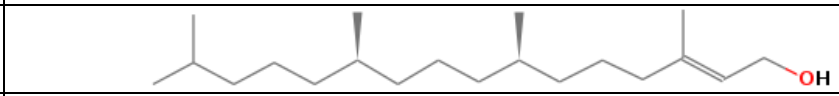
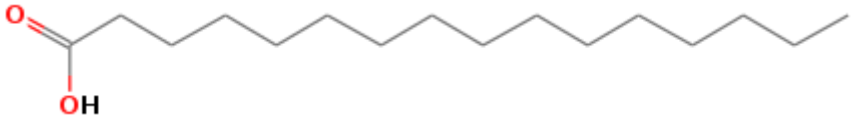
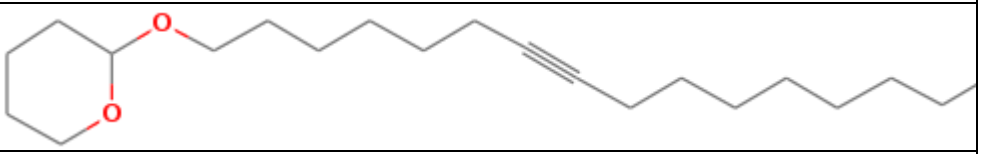
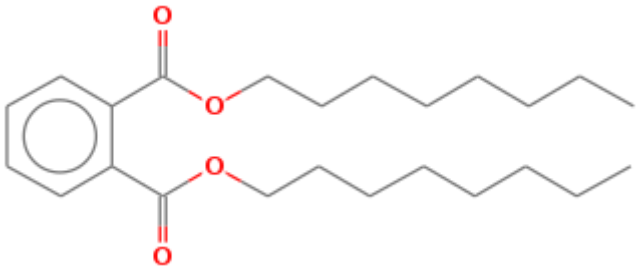
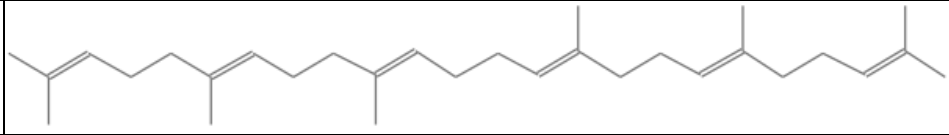
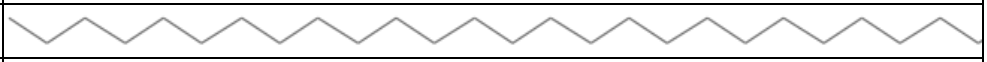
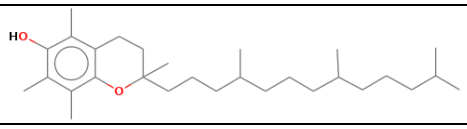
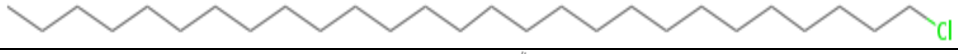
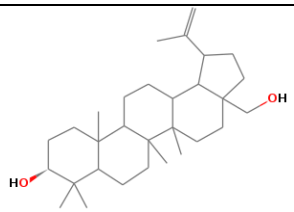
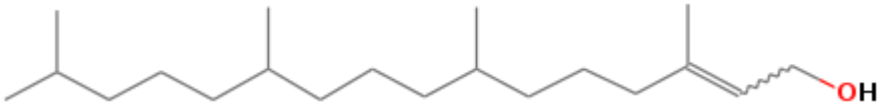
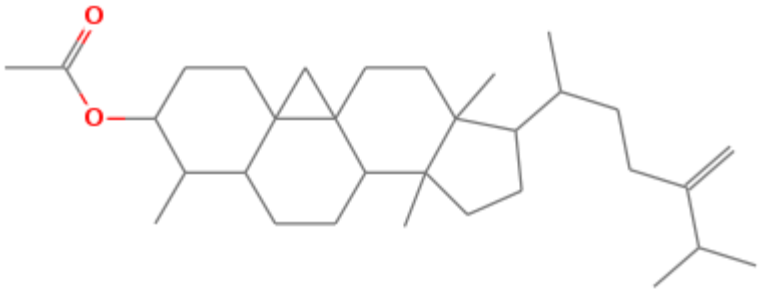
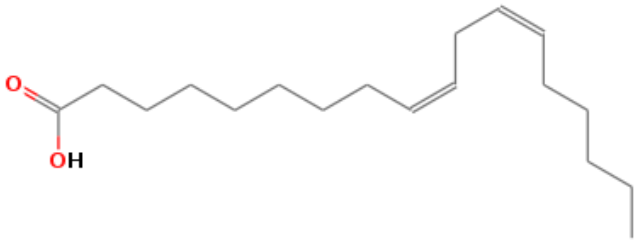
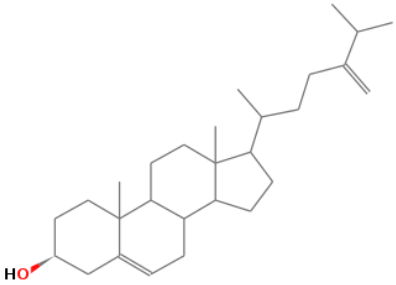
2	19.510	3341	102,232,072	29,026,608.0	6.294	30.61
3	20.956	3630	163,332,272	76,503,424.0	16.589	80.67
4	23.296	4098	484,513,056	55,856,596.0	12.112	58.90
5	24.967	4432	493,935,104	21,199,700.0	4.597	22.35
6	25.482	4535	210,409,888	8,029,864.0	1.741	8.47
7	26.708	4780	1,363,319,424	58,311,296.0	12.644	61.48
8	27.268	4892	101,084,800	5,438,716.5	1.179	5.73
9	27.358	4910	441,333,792	20,219,694.0	4.384	21.32
10	27.788	4996	125,053,576	5,346,526.0	1.159	5.64
11	28.088	5056	1,552,890,240	94,839,528.0	20.565	100.00
12	28.989	5236	109,215,008	6,123,342.5	1.328	6.46
13	29.259	5290	166,264,272	28,719,072.0	6.227	30.28
14	29.864	5411	149,767,648	32,815,950.0	7.116	34.60
15	30.649	5568	62,309,872	5,420,792.0	1.175	5.72

Fig 4: GC-MS analysis of *Chenopodium album* methanolic leaf extract

Table 4: Phytochemical constituents of GC-MS analysis of *Chenopodium album* methanolic leaf extract

Phytochemical constituent	Molecular formula (Molecular weight)	Structure
Phytol	$C_{20}H_{40}O$ (296.531)	
N-Hexadecanoic acid	$C_{16}H_{32}O_2$ (256.424)	
2H-Pyran, 2-(7-heptadecynyloxy) tetrahydro-	$C_{22}H_{40}O_2$ (336.551)	
Di-n-octyl phthalate	$C_{24}H_{38}O_4$ (390.556)	
Squalene	$C_{30}H_{50}$ (410.718)	
Heptacosane	$C_{27}H_{56}$ (380.733)	
Tetratetracontane	$C_{44}H_{90}$ (619.185)	

Vitamin E	$C_{29}H_{50}O_2$ (430.706)	
Heptacosane, 1-chloro-	$C_{27}H_{55}Cl$ (415.179)	
Betulin	$C_{30}H_{50}O_2$ (442.716)	
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$ (296.531)	
9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acetate, (3β,4α,5α)-	$C_{32}H_{52}O_2$ (468.754)	
9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$ (280.445)	
Hexatriacontane	$C_{36}H_{74}$ (506.972)	
Ergosta-5,24(28)-dien-3-ol, (3β)-	$C_{28}H_{46}O$ (398.664)	

4. Discussion

The plant kingdom represents a huge reservoir of new molecules to be discovered; the plants produce enormous varieties of chemicals which are believed to be important in mediating the interaction between plants and their environment. There is a plethora of scientific and ethnobotanical literature listing plants with known pest control properties [5]. The botanical extracts are obtained from plant fractionation by various processes and their composition varies depending on the botanical sample, the experimental conditions, and the physicochemical properties of the compounds [6]. The complexity of the plant metabolism results in a large number of molecules, and the extracts from the same plant are not only complex, moreover their molecular composition also varies from one extraction to another [7]. Modern chemistry has discovered the structures of many of these biologically active compounds, and systematic studies of natural products for plant protection became recognized within the field of chemistry [8]. The approaches employed when studying secondary metabolites, to achieve applied significance, must combine three readily available technologies: (i) separation techniques (extraction, partitioning, and chromatography), (ii) structural elucidation methods (spectrometry, chemical conversions, and X-ray crystallography) and (iii) bioassays [9].

GC-MS analysis of chloroform and methanolic leaf extract of *Cardiospermum halicacabum* revealed presence of various phytochemical components. The Sapindaceae (soapberry family) is a family of flowering plants with about 2000 species occurring from temperate to tropical regions throughout the world. Phytochemical studies on Sapindaceae species are abundant and various kinds of natural products have been isolated and elucidated like flavonoids, triterpenes, xanthenes and cathequines. Several extracts, fractions or pure

compounds of Sapindacceae species have been tested against diverse species of lepidopterans, dipterans and coleopterans of major importance in agriculture [10, 11]. Species of Sapindaceae family seem to be characterized by the occurrence of triterpenes [12-14]. Earlier reports revealed methoxylated flavones, apigenin 7-methyl ether and apigenin 7,4'-dimethyl ether from *Cardiospermum halicacabum* [12], and chrysoeriol 7-O-glucuronide and acacetin only in *Cardiospermum halicacabum* [12, 15] and additionally, a dimethyl ether flavonol was found in *Cardiospermum halicacabum*, kaempferol 30, 40-dimethyl ether [12]. On the other hand, alkaloids, steroids, flavonoids, phenols, and saponins found in Chenopodiaceae plants has been identified and have been deemed to be responsible for various biological effects on insects [16, 17]. *Chenopodium album* reduces larval growth, pupal weight and kills the larvae of the noctuids [18-20]. Phenolic and flavonoid contents are reported to produce several effects in insects. Flavonoids can act as potential grain protectants through contact, oviposition deterrent and ovicidal action. Flavonoids are found to alter moulting in insects, causing death. Most of the studied flavonoids either act as anti-estrogens or inhibit cytochrome P450 isozyme expression and activity [21].

Plants and plant derived products are rich in natural phytochemicals, which make them effective against different microbes and pests. Phytochemicals derived from plant sources act as insect larvicides, insect growth regulators, repellents, ovipositor attractants and have different activities. The secondary metabolites produced by plants have a wide spectrum of activity; they affect insects at the cellular, tissue and organismal level. In general, their action disturbs the cellular and physiological processes responsible for maintaining homeostasis, and they can provoke sublethal changes within various tissues and organs, which can ultimately lead to death. However, secondary metabolites also have sublethal implications, such as reduced fecundity, reduced viability or deformities in parental and filial generations. In addition, these compounds reduce the number of individuals in populations both directly (as a result of death) but also, or even primarily, indirectly. Secondary plant metabolites can disturb development, lead to malformations or malfunctions, extend the duration of developmental stages or act as repellents [22]. Further, in a similar study, Arivoli *et al*. [23] suggested that the phytochemical constituents obtained from the leaves and flowers of *Jasminum fluminense* may be used in the ecofriendly management of mosquitoes. In conclusion, it may be reported that based on literature, the phytocompounds identified from the leaf extracts of *Cardiospermum halicacabum* and *Chenopodium album* can be used as an additional tool in the ecofriendly management of insect pests.

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