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R Sowbaraniga
Ph.D Research Scholar, PG and Research Department of Botany, Government Arts College (Autonomous), Coimbatore, Tamil Nadu, India

Dr. M Chitra
Assistant Professor, PG and Research Department of Botany, Government Arts College (Autonomous), Coimbatore, Tamil Nadu, India

Correspondence
R Sowbaraniga
Ph.D Research Scholar, PG and Research Department of Botany, Government Arts College (Autonomous), Coimbatore, Tamil Nadu, India

Phytocompounds identification in *Cucumis dipsaceus* Ehrenb ex. Spach fruits by gas chromatography-mass spectrometry (GC-MS) analysis

R Sowbaraniga and Dr. M Chitra

Abstract
Phytochemicals identification is necessary to explore its usefulness towards medicinal value and commercial products preparation. *Cucumis dipsaceus* Ehrenb ex. Spach is a less explored plant in India and the present study focused to find out the phytochemicals present in its fruits by Gas Chromatography –Mass Spectrometry analysis. Fruit extracts were prepared using Petroleum ether, Acetone, Ethanol and Water solvents by successive soxhlet extraction. Crude extracts were subjected to GC-MS analysis and the spectra of the obtained components were compared with the GC-MS NIST (2008) library. The results revealed the presence of bioactive compounds, Octadecanoic acid, Pentadecanoic acid, Eicosanic acid, Oleic acid, Tetradecanal, octadecanal, Pentadecanal, Tridecanoic acid, alkyne and amide compounds. Thus, the present study explored the presence of bioactive compounds in fruits of *Cucumis dipsaceus*.

Keywords: *Cucumis dipsaceus*, GC-MS analysis, phytochemicals, bioactive compounds

Introduction
Identification of phytochemicals in plants is an essential factor to know its potential towards the therapeutic and commercial applications. Commercial products and Drugs derived from plant sources gains importance due to its non toxicity property. Traditional as well as modern medicines, nutraceuticals, food products are obtained from plants which are richest source of phytocompounds [1]. Exploration of each and every compound in plants paves way for invention and development of non toxic commercial products and medicines. Modern techniques and equipments in phytochemical field play a vital role in identification of compounds. Gas Chromatography-Mass Spectrometer is one of such technology helpful in identification of specific compounds and used to determine the amount of active compounds in plants [2].

Cucumis dipsaceus Ehrenb ex. Spach of Cucurbitaceae family is an annual climbing herb. Cucurbitaceae family members (Cucumber, Pumpkin, Zucchini, melon varieties, gourd varieties etc.) are well known as economically as well as medicinally important plants. The genus *Cucumis* mainly includes cucumber varieties and melon varieties, of which many of the species were originated in African countries [3]. *Cucumis dipsaceus* commonly known as Arabian cucumber, Hedgehog cucumber and teasel gourd and its origin is known to be Ethiopia [4]. It is an less explored plant in India and few studies were reported of its phytoconstituents in leaves and fruits. The earlier study on GC-MS analysis of leaves of *Cucumis dipsaceus* revealed the presence of compounds such as fatty acid esters, tetradecanal, tetradeconic acid, squalene and nonadecanoic acid [5]. GC-MS analysis of *Cucumis dipsaceus* fruits was not yet reported previously, hence the present study focused to identify the bioactive compounds present in fruits by GC-MS analysis.

Materials and Methods
Preparation of plant material and Fruit extracts
Fruits of *Cucumis dipsaceus* were collected in Coimbatore district, Tamil nadu during the month of October- November (2016) and authenticated by Botanical Survey of India, Coimbatore. Shade dried cleaned fruits were milled into coarse powder by mortar and pestle which was used to extract phytocompounds. Successive Solvent extraction method was adopted with the help of soxhlet extractor using Petroleum ether, Acetone, Ethanol and Water solvents. Concentrated crude extract was stored in sterile container at room temperature for further analysis.

Gas Chromatography-Mass Spectrometer (GC-MS) analysis

Gas Chromatography- Mass Spectrophotometer (GC-MS) analysis was carried out for fruit extracts (Petroleum ether, Acetone, Ethanol and Water) to identify the extracted phytocompounds. The Gas Chromatogram (Clarus 680) was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethyl polysiloxane, 30 m × 0.25 mm ID × 250µm df) and the components were separated using Helium as carrier gas at a constant flow of 1 mL/min. The injector temperature was set at 260°C during the chromatographic run. The 1µL of extract sample injected into the instrument the oven temperature was as follows: 60 °C (2 min); followed by 300 °C at the rate of 10 °C min−1; and 300 °C, where it was held for 6 min. The mass detector conditions were the transfer line temperature of 240 °C, ion source temperature of 240 °C and ionization mode electron impact at 70 eV, a scan time 0.2 sec and scan interval of 0.1 sec. The fragments were obtained from 40 to 600 Da. The spectra of the obtained components were compared with the database stored in the GC-MS NIST (2008) library.

Results

GC-MS analysis

From the findings of the present study, totally 60 major compounds were identified in four fruit extracts. Petroleum ether extract possess 21 compounds, Acetone extract has 19 compounds, Ethanol extract has 20 compounds and Water extract has 21 compounds. The major compounds were presented in Table-1 with its retention time, compound name, molecular weight, molecular formula, area% and chemical abstract service number (CAS No.).

Petroleum ether Extract

The retention time from 15.44 to 29.39 showed the maximum peak area (Fig. 1). The maximum area% observed was 23.10 at retention time 19.99 for the alkene compound 1,19-eicosadiene followed by area% 14.88 at retention time 29.04 and fatty aldehydes identified wer octadecanal, tetradecanal and pentadecanal. The alkane compounds octacosane and heptacosane was noted with area% of 8.08 at retention time 25.88.

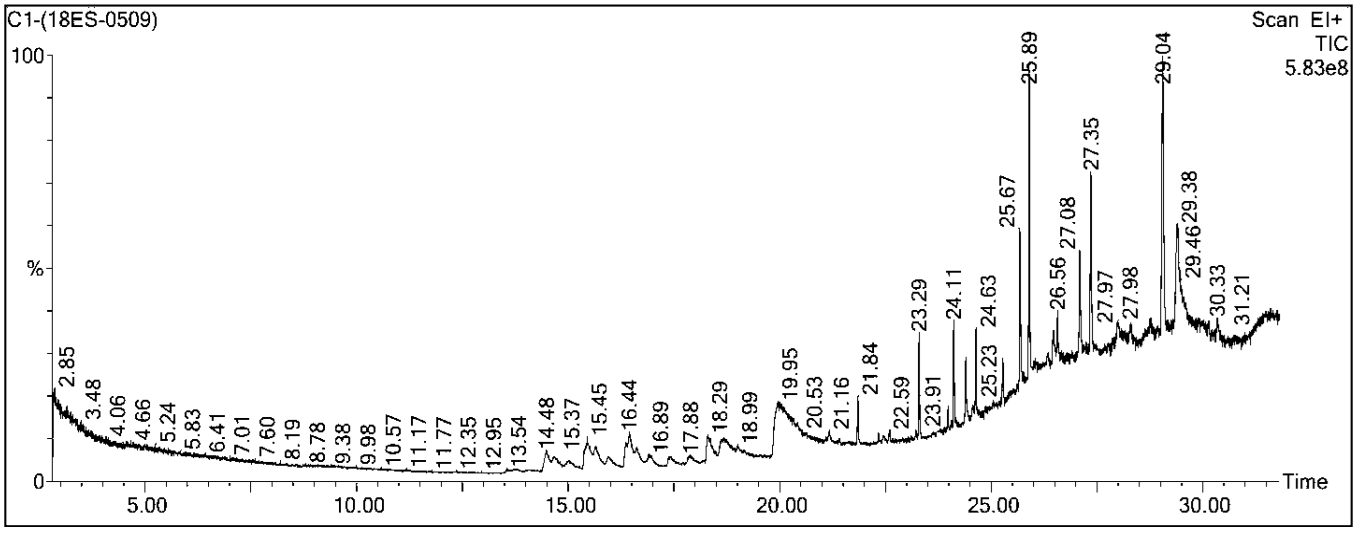

Fig 1: Chromatogram of Petroleum ether extract of *Cucumis dipsaceus* fruits

Acetone Extract

The maximum peak range noted from the retention time of 18.48 to 27.12 (Fig. 2). The compounds z,e-3,13-octadecadien-1-ol and 1,19-eicosadiene were showed the maximum area% of 48.40 at the retention time 19.92. The other major compounds observed were n-hexadecanoic acid, eicosanoic acid, octadecanoic acid, nonadecanoic acid, pentadecanoic acid, undecanoic acid, heptadecanoic acid, tridecanoic acid, n-decanoic acid and nonanoic acid with area% of 14.76 at the retention time 18.50.

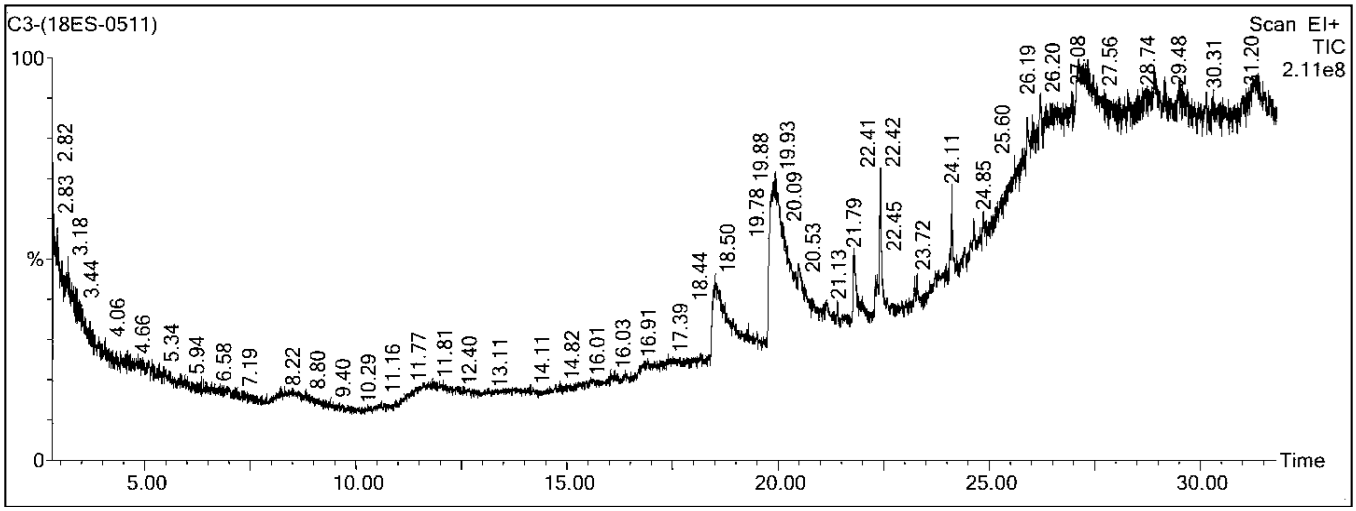

Fig 2: Chromatogram of Acetone extract of *Cucumis dipsaceus* fruits

Ethanol Extract

The retention time from 18.45 to 27.36 showed maximum peak area range (Fig. 3). The maximum area% of 37.44 was noted at retention time 19.89 showed the presence of 1-tridecyne, 1-octadecyne, 1-tetradecyne, 1-hexadecyne and area% of 17.58 at retention time 18.45 denoted the fatty acid compounds, n-hexadecanoic acid, n-decanoic acid, octadecanoic acid, eicosanoic acid, undecanoic acid, pentadecanoic acid, tridecanoic acid, nonadecanoic acid and heptadecanoic acid.

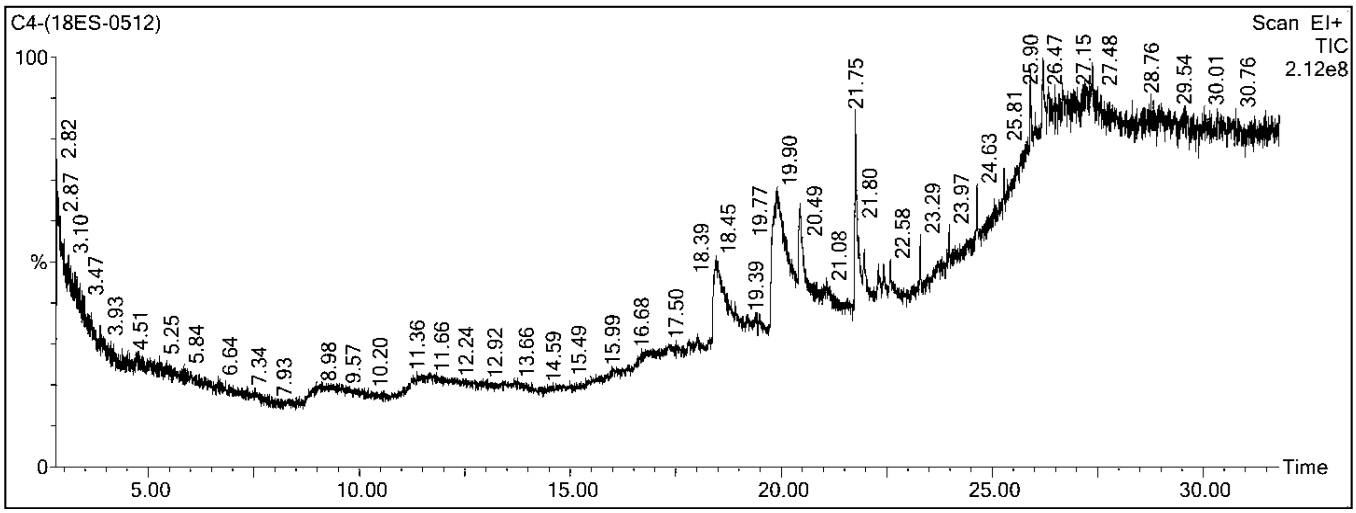

Fig 3: Chromatogram of Ethanol extract of *Cucumis dipsaceus* fruits

Water Extract

The retention time from 7.59 to 33.42 showed peak area range (Fig. 4). The maximum area% of 41.65 at retention time 34.40 was noted which showed presence of amide compounds such as 13-Docosenamide, (Z)-, cis-11-Eicosenamide, trans-11-Icosenamide, 9-Octadecenamide. 9,12,15-Octadecatrienoic acid,2,3-bis[(trimethylsilyl)oxy]propyl ester, (Z,Z,Z)- observed with area% of 2.62 at retention time 31.36 and dotriacontane with area% of 2.58 at retention time 10.46.

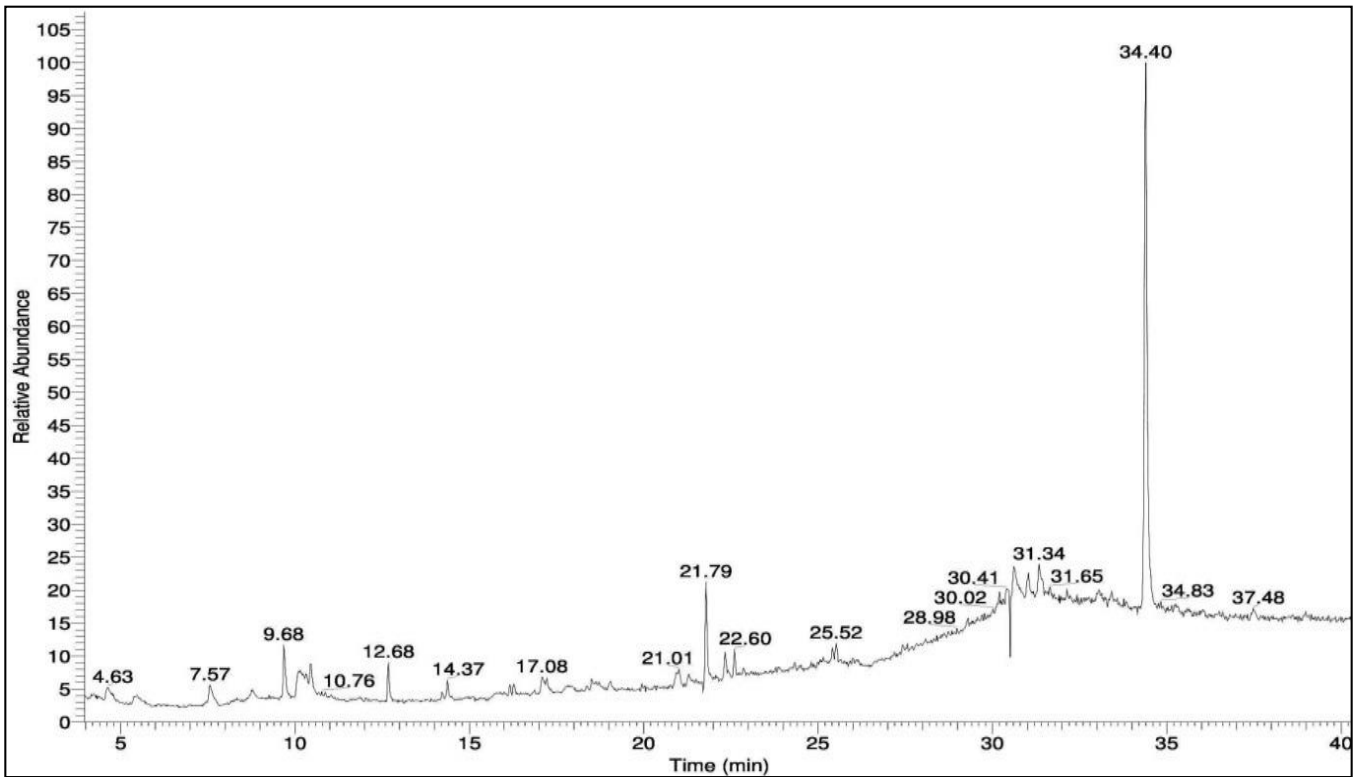

Fig 4: Chromatogram of water extract of *Cucumis dipsaceus* fruits

Table 1: Compounds identified by GC-MS analysis of fruit extracts of *Cucumis dipsaceus*

Compound Name	MW	MF	PE		AE		EE		WE		CAS No.
			RT	Area%	RT	Area%	RT	Area%	RT	Area%	
Esters											
1,2-benzenedicarboxylic acid, butyl octyl ester	334	$C_{20}H_{30}O_4$	18.29	2.98	-	-	-	-	-	-	84-78-6
phthalic acid, isobutyl octadecyl ester	474	$C_{30}H_{50}O_4$	18.29	2.98	-	-	-	-	-	-	900309-06-1
di-n-octyl phthalate	390	$C_{24}H_{38}O_4$	-	-	22.42	5.06	-	-	-	-	117-84-0
fumaric acid, 2-dimethylaminoethyl nonyl ester	313	$C_{17}H_{31}O_4N$	-	-	-	-	21.75	9.06	-	-	900331-64-8
Hexadecanoic acid, ethyl ester	284	$C_{18}H_{36}O_2$	-	-	-	-	-	-	22.34	1.75	628-97-7
Octadecanoic acid, ethyl ester	312	$C_{20}H_{40}O_2$	-	-	-	-	-	-	22.34	1.75	111-61-5
9-Octadecenoic acid (Z)-, ethyl ester	310	$C_{20}H_{38}O_2$	-	-	-	-	-	-	22.34	1.75	6114-18-7
sulfurous acid, pentadecyl 2-propyl ester	334	$C_{18}H_{38}O_3S$	25.88	8.08	-	-	-	-	-	-	900309-12-6
9,12,15-Octadecatrienoic acid,2,3-bis[(trimethylsilyl)oxy]propyl ester, (Z,Z,Z)-	496	$C_{27}H_{52}O_4Si_2$	-	-	-	-	-	-	31.36	2.62	54284-45-6
Alkenes											
1,19-eicosadiene	278	$C_{20}H_{38}$	19.99	23.10	19.92	48.40	-	-	-	-	14811-95-1
Alkanes											
tetratetracontane	618	$C_{44}H_{90}$	23.28	2.97	-	-	-	-	-	-	7098-22-8
hexatriacontane	506	$C_{36}H_{74}$	23.28	2.97	-	-	-	-	-	-	630-06-8
tetratriacontane	478	$C_{34}H_{70}$	23.28	2.97	-	-	-	-	-	-	14167-59-0
octacosane	394	$C_{28}H_{58}$	25.88	8.08	-	-	-	-	-	-	630-02-4
heptacosane	380	$C_{27}H_{56}$	25.88	8.08	-	-	-	-	-	-	593-49-7
Dodecane	170	$C_{12}H_{26}$	-	-	-	-	-	-	7.59	1.92	112-40-3
Docosane	310	$C_{22}H_{46}$	-	-	-	-	-	-	7.59	1.92	629-97-0
Dotriacontane	450	$C_{32}H_{66}$	27.34	6.73	-	-	-	-	10.46	2.58	544-85-4
Alkynes											
1-octadecyne	250	$C_{18}H_{34}$	-	-	20.21	9.77	19.89	37.44	-	-	629-89-0
1-tetradecyne	194	$C_{14}H_{26}$	-	-	20.21	9.77	19.89	37.44	-	-	765-10-6
1-dodecyne	166	$C_{12}H_{22}$	-	-	20.21	9.77	20.16	2.44	-	-	765-03-7
1-tridecyne	180	$C_{13}H_{24}$	-	-	20.21	9.77	19.89	37.44	-	-	26186-02-7
9-eicosyne	278	$C_{20}H_{38}$	-	-	20.21	9.77	-	-	-	-	71899-38-2
1-hexadecyne	222	$C_{16}H_{30}$	-	-	-	-	19.89	37.44	-	-	629-74-3
1-undecyne	152	$C_{11}H_{20}$	-	-	-	-	20.16	2.44	-	-	2243-98-3
Fatty acids											
oleic acid	282	$C_{18}H_{34}O_2$	26.46	1.96	-	-	-	-	-	-	112-80-1
pentadecanoic acid	242	$C_{15}H_{30}O_2$	26.46	1.96	18.50	14.76	18.45	17.58	-	-	1002-84-2
eicosanoic acid	312	$C_{20}H_{40}O_2$	26.46	1.96	18.50	14.76	18.45	17.58	-	-	506-30-9
nonadecanoic acid	298	$C_{19}H_{38}O_2$	26.46	1.96	18.50	14.76	18.45	17.58	-	-	646-30-0
octadecanoic acid	284	$C_{18}H_{36}O_2$	26.46	1.96	18.50	14.76	18.45	17.58	-	-	57-11-4
n-hexadecanoic acid	256	$C_{16}H_{32}O_2$	-	-	18.50	14.76	18.45	17.58	-	-	57-10-3
undecanoic acid	186	$C_{11}H_{22}O_2$	-	-	18.50	14.76	18.45	17.58	-	-	112-37-8
heptadecanoic acid	270	$C_{17}H_{34}O_2$	-	-	18.50	14.76	18.45	17.58	-	-	506-12-7
tridecanoic acid	214	$C_{13}H_{26}O_2$	-	-	18.50	14.76	18.45	17.58	-	-	638-53-9
n-decanoic acid	172	$C_{10}H_{20}O_2$	-	-	18.50	14.76	18.45	17.58	-	-	334-48-5
nonanoic acid	158	$C_9H_{18}O_2$	-	-	18.50	14.76	-	-			112-05-0
6-Octadecenoic acid	282	$C_{18}H_{34}O_2$	-	-	-	-	-	-	25.52	1.70	4712-34-9
trans-13-Octadecenoic acid, cis-13-Octadecenoic acid	282	$C_{18}H_{34}O_2$	-	-	-	-	-	-	25.52	1.70	13126-39-1
Octadec-9-enoic acid	282	$C_{18}H_{34}O_2$	-	-	-	-	-	-	25.52	1.70	2027-47-6
Fatty aldehydes											
hexadecanal	240	$C_{16}H_{32}O$	25.66	5.30	-	-	-	-	-	-	629-80-1
16-heptadecenal	252	$C_{17}H_{32}O$	25.66	5.30	-	-	-	-	-	-	900144-57-9
octadecanal	268	$C_{18}H_{36}O$	29.04	14.88	-	-	21.82	3.43	-	--	638-66-4
tetradecanal	212	$C_{14}H_{28}O$	29.04	14.88	-	-	21.82	3.43	-	-	124-25-4
pentadecanal-	226	$C_{15}H_{30}O$	29.04	14.88	-	-	-	-	-	-	2765-11-9
Fatty alcohols											
z,e-3,13-octadecadien-1-ol	266	$C_{18}H_{34}O$	-	-	19.92	48.40	-	-	-	-	900131-10-4
Falcarinol	244	$C_{17}H_{24}O$	-	-	-	-	-	-	15.78	0.83	21852-80-2
Fatty amides											
13-Docosenamide, (Z)-	337	$C_{22}H_{43}NO$	-	-	-	-	-	-	34.40	41.65	112-84-5
cis-11-Eicosenamide, trans-11-Icosenamide	309	$C_{20}H_{39}NO$	-	-	-	-	-	-	34.40	41.65	-
9-Octadecenamide	281	$C_{18}H_{35}NO$	-	--	-	-	-	-	34.40	41.65	3322-62-1
Triterpenoids											
squalene	410	$C_{30}H_{50}$	24.10	2.67	-	-	-	-	-	-	7683-64-9
Alkaloid											
Pseudojervine	587	$C_{33}H_{49}NO_8$	-	-	-	-	-	-	18.55	1.24	36069-05-3
Flavonols											
Quercetin 7,3',4'-trimethoxy	344	$C_{18}H_{16}O_7$	-	-	-	-	-	-	30.41	2.83	6068-80-0

Amino acids											
Glycyl-D-asparagine	189	$C_6H_{11}N_3O_4$	-	-	-	-	-	-	18.55	1.24	-
L-Aspartic acid, N-glycyl-	190	$C_6H_{10}N_2O_5$	-	-	-	-	-		18.55	1.24	4685-12-5
Other compounds											
oxirane, tetradecyl-	240	$C_{16}H_{32}O$	-	-	20.47	3.82	-	-	-	-	7320-37-8
hexadecanal, 2-methyl-	254	$C_{17}H_{34}O$	-	-	-	-	21.82	3.43	-	-	55019-46-0
undecanal, 2-methyl-	184	$C_{12}H_{24}O$	-	-	-	-	21.82	3.43	-	-	110-41-8
6-Iodoacetoveratrone	306	$C_{10}H_{11}IO_3$	-	-	-	-	-		22.60	1.08	-
D-Mannitol, hexaacetate	434	$C_{18}H_{26}O_{12}$	-	-	-	-	-	-	33.42	1.13	642-00-2
d-Xylitol, pentaacetate	362	$C_{15}H_{22}O_{10}$	-	-	-	-	-	-	33.42	1.13	-

MW=molecular weight; MF= Molecular Formula; CAS no= chemical abstract service number; RT= Retention time; PE= Petroleum ether extract; AE = Acetone extract; EE= Ethanol extract; WE= Water extract

Discussion

The plants possess pharmacologically important bioactive compounds and metabolites involved in various metabolism processes. GC-MS is a very helpful analysis to identify those compounds which are known for its medicinal property and commercial use. The results of present study in the fruit extracts of *Cucumis dipsaceus* Ehrenb ex. Spach revealed the presence of such bioactive compounds with the help of GC-MS analysis. Compounds like Tetratriacontane, Octacosane, Pentadecanoic acid, Eicosanic acid (arachidic acid), Pentadecanal, Tridecanoic acid, Nonanoic acid, Dodecane, Docosane, Hexadecanoic acid ethyl ester and Quercetin 7,3',4'-trimethoxy plays role as a plant metabolite [6]. Tetratetracontane, Hexadecanal (Palmitaldehyde) and Undecanoic acid serves as human metabolite [6]. Squalene is a isoprenoid compound and has a vital role as antioxidant and cancer therapy [7]. Oleic acid is an unsaturated fatty acid which is commercially used in the preparation of oleates and lotions. It also has herbicide, and fungicide properties [8]. N-Hexadecanoic acid (Palmitic acid) is saturated long-chain fatty acid which is major component of oil palm fruits, commercially used to produce soaps and cosmetics and precursor in fatty acid synthesis [8]. Undecanoic acid involved in control of triacylglycerol synthesis and has a role as an antifungal agent [8]. Nonadecanoic acid is a long chain fatty acid which has a property as pheromone used by some of the insects [8]. It is an intermediate product in n-icosane biodegradation and inhibits cancer growth [9]. Pentadecanal is a long chain fatty aldehyde which has a role as an antimicrobial agent, a volatile oil component [10]. n-decanoic acid (capric acid) is a saturated medium chain fatty acid used to make esters for perfumes and fruit flavours [11]. Nonanoic acid (Pelargonic acid) has antifungal properties and also used as herbicide [12]. Di-n-octyl phthalate presence was identified which is an ester of phthallic acid and used as a plasticizer for many resins and elastomers [13]. Falcarinol is a natural pesticide [14]. Docosane, Heptacosane, 1-Octadyne,1, Hexdecyne, octadecanoic acid has antibacterial activity [15-18]. Dodecane has role as antifungal agent [18]. Hexadecanoic acid, ethyl ester and tetratetracontane has antioxidant property [19, 20]. 6-Octadecenoic acid prevents cancer [21]. 13-Docosenamide, (Z)- has antimicrobial activity [22]

Conclusion

The present study was carried out to find out the phytocompounds present in fruit extracts of *Cucumis dipsaceus* Ehrenb. ex spach by GC-MS analysis. Fatty acid esters and its derivatives were the major constituents present in all fruit extracts. Major bioactive compounds identified in petroleum ether extract were fatty acids, fatty aldehydes and alkanes. In acetone and Ethanol extract, alkynes compounds and fatty acids were identified. In Water extract, esters, fatty amides, aminoacids were also identified. Thus, it is concluded that present study revealed the presence of bioactive compounds in fruits of *Cucumis dipsaceus* Ehrenb. ex spach.

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