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GC/MS Analysis of Chemical Compounds Soluble in n-Hexane and Petroleum Ether From *Trigonella foenum-graecum* Seeds

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ABSTRACT

Background and Objective: *Trigonella foenum-graecum* (fenugreek), belonging to the Fabaceae family, is an ancient medicinal herb with various pharmacological effects, including hypoglycemic properties. This study aims to analyze the chemical compositions of non-polar extracts from fenugreek seeds sold in Omdurman, Sudan and Al-Madina, Saudi Arabia. **Materials and Methods:** Fenugreek seeds were obtained in April 2022 from markets in Omdurman, Sudan and Al-Madina, Saudi Arabia. The seeds were macerated separately for 48 hrs in n-hexane and petroleum ether solvents. The chemical compositions were analyzed using GC/MS on a QP-2010 Shimadzu spectrometer, with identification based on retention times and mass spectra from the Wiley Library. **Results:** The n-hexane extract, Sudan sample of *Trigonella foenum-graecum* contains oleic acid (9.09%) and butylphosphonic acid, di(4-octyl) ester (8.72%) as major components, while petroleum ether extract contains O-cymene (27.69%) and Gamma-Terpinene (23.92%) as major components. Saudi Arabia samples, n-hexane extract contains 9-octadecanamide (Z)- (17.14%) and stearic acid (13.51%), while petroleum ether extract contains D-Limonene (36.46%) and Gamma-sitosterol (10.95%) as major constituents. **Conclusion:** The petroleum ether extract of fenugreek seeds from Sudan is rich in Gamma-terpinene and O-cymene, whereas the extract from Saudi Arabia is rich in D-limonene and Gamma-sitosterol.

KEYWORDS

GC/MS analysis, chemical composition, *Trigonella foenum-graecum*, n-hexane, petroleum ether, therapeutic potential, spectroscopic analysis

INTRODUCTION

Fenugreek, scientifically known as *Trigonella foenum-graecum*, is a legume renowned worldwide for its contribution to enhancing the sensory appeal of culinary dishes. Beyond its culinary significance, fenugreek is celebrated for its array of medicinal properties, encompassing antidiabetic, antifertility, anticarcinogenic, hypocholesterolemic, antimicrobial, antiparasitic, lactation stimulant, antioxidant and immunological effects[1,2].

Fenugreek harbors an extensive array of chemical constituents, including steroidal sapogenins and the notable component diosgenin found in seeds. The seeds boast significant mucilage content, alongside

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volatile oil, alkaloids like trigonelline and choline, proteins and a yellow coloring substance. With its rich nutritional profile comprising proteins, fats, carbohydrates and dietary fiber, fenugreek emerges as a noteworthy source of essential nutrients, including iron. Fenugreek seeds also contain minute quantities of volatile and fixed oils[3].

The GC/MS and FTIR analysis of fenugreek seed oil revealed that the oil is rich in essential omega-6 fatty acids (linoleic acid), which are highly effective for the prevention of coronary heart diseases, inflammation and cancer. The main constituents of the oil namely, linoleic acid, palmitic acid, pinene and other components with lower portions were found to be very useful in reducing free radicals due to their natural antioxidant properties[4].

Fenugreek seeds have a rich history of use as both a spice and a medicinal herb, owing to their complex chemical makeup. These seeds contain fragrant small molecules, amino acid derivatives like 4-hydroxyisoleucine, alkaloids such as trigonelline, galactomannan-rich polysaccharides, phenolic compounds including flavonoids and aromatic acids and complex saponins. Trigonelline, a key bioactive component in *Trigonella foenum-graecum*, has antidiabetic properties, acts as a CNS stimulant and may benefit individuals with Alzheimer's disease.

The essential oils extracted from Bulgarian and Indian fenugreek seeds are noted for their distinctive, pungent aroma. Analysis of these oils shows that both contain cubenol, gamma-n-amylbutylrolactone and palmitic acid as primary constituents. Moreover, oxygenated sesquiterpenes are the dominant fraction in both types of oils.

Fenugreek oil is highly unsaturated, with only 15-19% saturated fatty acids, mainly palmitic acid. It also contains 18-27% monounsaturated acids, such as oleic and erucic acids. Fenugreek exhibits a wide range of pharmacological activities, including antidiabetic, antiulcer, anticancer, anti-inflammatory, antipyretic and CNS stimulant effects. The seeds also aid in wound healing, act as an expectorant, stomachic and laxative, boost immunity and enhance mental and reproductive health in women. Placebo studies have demonstrated its safety, with no significant side effects reported[5-11].

The objective of this research work was to explore the compounds present in the n-hexane and petroleum ether extracts of *Trigonella foenum-graecum* seeds obtained from two different origins (Sudan and Saudi Arabia) by implicating Gas Chromatography-Mass Spectroscopy (GC/MS) technique.

MATERIALS AND METHODS

Study area: *Trigonella foenum-graecum* seeds were sourced from Omdurman, Sudan, in July, 2022 and from Al-Madina, Saudi Arabia, in October, 2022. The experimental procedures were performed at the Department of Chemistry, Faculty of Science and Technology, Omdurman Islamic University and at the Central Laboratory, National Centre for Research, Khartoum, Sudan.

Plant material: The plant under investigation (samples from two sources Omdurman and AL-Madina Local Markets) were authenticated at the Department of Botany by one author, Prof. Hatil, Hashim ELKamali, Omdurman Islamic University.

Preparation of crude plant extracts: The dried plant material was ground into a coarse powder using mortar and pestle. One hundred and fifty grams of the powder were soaked in n-hexane and petroleum ether, separately for three days and filtered and then stored until used.

Gas Chromatography-Mass Spectrometry (GC/MS) analysis[12]: The qualitative and quantitative analysis of the sample was carried out by using GC/MS technique model (GC/MSQP2010-Ultra) from Japan's Shimadzu Company, with serial number 020525101565SA and capillary column

(Rt×-5 ms-30 m×0.25 mm×0.25 um). The sample was injected using split mode, with helium as the carrier gas flowing at a rate of 1.61 mL/min. The temperature program started at 60°C, increasing at a rate of 10°C per minute until it reached a final temperature of 300°C, which was held for 5 min. The injection port temperature was maintained at 300°C, the ion source temperature was set at 200°C and the interface temperature was 250°C. The sample was analyzed in scan mode, with a mass-to-charge ratio (m/z) range of 40-500. Identification of the sample components was achieved by computer searches in a commercial library, the National Institute of Standards and Technology (NIST).

RESULTS AND DISCUSSION

GC/MS analysis of *Trigonella foenum-graecum* (n-hexane extract, Sudan sample): Table 1 shows 34 compounds were identified from *Trigonella foenum-graecum* seeds n-hexane extract, Sudan sample. Butylphosphonic acid, di (4-octyl) ester (8.72%), 9-octadecenoic acid 1,2,3-propanetriyl ester (8.55%), 9-octadecenamide, (Z)- (8.00%), stigmastan-3,5-diene (5.59%), and 4-fluorobenzyle alcohol (4.44%) were found as the major chemical components.

Table 1: Chemical constituents of *Trigonella foenum-graecum* seeds (n-hexane extract, Sudan sample)

No.	Compound name	Other name	RT	Percentage
1	Benzaldehyde, 4-fluoro-	Benzaldehyde,p-fluro	3.433	4.23
2	4-Fluorobenzyl alcohol	p-Flurobenzyl alcohol	4.770	4.44
3	Butylphosphonic acid, di(4-octyl) ester	-	12.804	8.72
4	Cyclohexanone, 5-butyl-3,3,5-trimethyl-	3-Butyl-3,5,5-trimethylcyclohexanone	13.053	1.31
5	Phytol, acetate	-	14.146	0.70
6	Hexadecanoic acid, methyl ester	Palmitic acid, methyl ester	15.056	1.38
7	n-Hexadecanoic acid	Hexadecanoic acid; Palimatic acid	15.551	3.58
8	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Linoleic acid, methyl ester	16.706	2.68
9	11-Octadecenoic acid, methyl ester, (Z)-	Cis-11-Octadecenoic acid, methyl ester	16.750	3.69
10	Methyl stearate	Stearic acid, methyl ester	16.972	0.76
11	Oleic Acid	9-Octadecenoic acid (Z)-	17.288	9.09
12	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	Palmitin-2-mono-	18.121	1.72
13	Tributyl acetylcitrate	1,2,3-propanetricarboxylic acid,2-(acetyloxy)-, tributyl ester	18.234	1.03
14	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethaned i1y8l .ester	Palmitin,1,2-di-	18.510	1.87
15	Oleoyl chloride	Oleic acid chloride	19.615	3.04
16	Unidentified	$C_{21}H_{34}O_2$	19.670	0.81
17	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E, E1)9-	2,3-Bis[(9E)-9-Octadecenoyloxy]propyl	19.993	8.55
18	Methyl (Z)-5,11,14,17-eicosatetraenoate	-	20.050	2.04
19	cis, 6-Octadecenoic acid, trimethylsilyl ester	Trimethylsilyl(6E)-6-Octadecenoate	20.117	3.77
20	Diisooctyl phthalate	Bis(6-methylheptyl)phthalate	20.582	1.26
21	Unidentified	$C_{32}H_{54}O_3$	20.993	
22	E-11-Hexadecenal	-	21.192	0.76
23	13-Octadecenal, (Z)-	Cis-13-Octadecenal	21.561	1.19
24	18.alpha.-Olean-3.beta.-ol, acetate	Oleanan-3-yl acetate	22.263	1.68
25	9-Octadecenamide, (Z)-	Adigen 73; Oleamide; Oleic acid amide	22.338	8.00
26	Olean-12-ene-3,28-diol, (3.beta.)-	Erythrodiol	22.430	1.31
27	Oxirane, 2,2-dimethyl-3-(3,7,12,16,20-pentamethyl-233,7.4,1112, 15,19-h e1n6e2i4c 6o3sa0pentaen 0y.l9)-9, (all-E)-	-	23.412	0.12
28	Unidentified	$C_{29}H_{48}$	24.639	1.34
29	Vitamin E	-	24.822	2.26
30	Cholest-5-en-3-ol, 4,4-dimethyl-, (3.beta.)-	Cholest-5-en-3beta-ol,4,4-dimethyl-	25.970	1.10
31	Gamma-Sitosterol	Stigmast-5-en-3-ol,(3beta,24S)-	26.601	3.63
32	Cycloartanol	pollinastanol	26.932	2.64
33	9,19-Cyclolanost-24-en-3-ol, (3.beta.)-	-	27.425	4.08
34	Stigmastan-3,5-diene	Stigmasta-3,5-diene	27.972	5.59

Table 2: Chemical constituents of *Trigonella foenum-graecum* seeds (petroleum ether extract, Sudan sample)

No.	Compound name	Other name	RT	Percentage
1	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	3-thujene; alpha-thujene	3.090	0.11
2	.beta.-Pinene	2(10)-pinene	3.689	4.83
3	o-Cymene	Benzene,1-methyl-2(1-methylethyl)-	4.277	27.69
4	Gamma-Terpinene	1,4-cyclohexadiene	4.721	23.92
5	Thymol	Phenol,5-methyl-2-(1- methylethyl)-	8.238	6.29
6	Phytol, acetate	-	14.811	0.73
7	Unidentified	$C_{22}H_{42}O_2$	15.285	0.42
8	Hexadecanoic acid, methyl ester	Palimatic acid, methyl ester	15.783	1.25
9	Methyl 10-trans,12-cis-octadecadienoate	-	17.505	4.75
10	9-Octadecenoic acid (Z)-, methyl ester	Oleic acid, methyl ester	17.551	7.15
11	Cyclooctasiloxane, hexadecamethyl-	Hexadecamethyl-cyclooctasioxane	17.630	0.51
12	Methyl stearate	Stearic acid, methyl ester	17.784	1.16
13	1-Propene-1,2,3-tricarboxylic acid, tributyl ester	Tributyl aconitate	18.166	0.79
14	Decanedioic acid, dibutyl ester	Sebacic acid, dibutyl ester	18.239	0.81
15	Tributyl acetylcitrate	1,2,3-propanetricarboxylic acid, 2-(acetyloxy)-, tributyl ester	19.091	3.08
16	Unidentified	$C_{18}H_{54}O_9Si_9$	21.356	0.71
17	Unidentified	$C_{18}H_{54}O_9Si_9$	22.444	0.73
18	Heptadecane, 8-methyl-	methylheptadecane	22.627	0.40
19	Unidentified	$C_{24}H_{42}O_4$	22.809	0.89
20	1,2-Cyclohexanedicarboxylic acid, cyclohexylmethyl ester	-	22.993	1.91
21	Unidentified	$C_{26}H_{48}O_4$	23.162	1.20
22	Unidentified	$C_{26}H_{48}O_4$	23.225	1.10
23	Unidentified	$C_{26}H_{48}O_4$	23.344	1.47
24	Cyclononasiloxane, octadecamethyl-	Octadecamethyl-cyclononasiloxane	23.449	1.98
25	1,2-Cyclohexanedicarboxylic acid, dinonyl ester	-	23.540	1.68
26	Unidentified	$C_{26}H_{48}O_4$	23.738	0.59
27	dl-.alpha.-Tocopherol	(+/-)-alpha-Tocopherol	26.431	0.99
28	.gamma.-Sitosterol	Stigmast-5-en-3-ol,(3beta,24S)-	28.903	2.87

GC/MS analysis of *Trigonella foenum-graecum* L. (petroleum ether extract, Sudan sample): Table 2 shows 28 compounds were identified from *Trigonella foenum-graecum* seeds petroleum ether extract, Sudan sample. The compounds: O-cymene (27.69%), Gamma-terpinene (23.92 %), 9-octadecenoic acid (Z)-, methyl ester (Oleic acid) 7.15%), thymol (6.29%), beta-pinene (4.83%) and methyl 10-trans, 12-cis-octadecadienoate (4.75%) were found as the major phytochemical constituents.

GC/MS analysis of *Trigonella foenum-graecum* (n-hexane extract, Saudi sample): Table 3 shows 39 compounds were identified from *Trigonella foenum-graecum* seeds n-hexane extract, Saudi sample. The compounds: 9-Octadecanamide, (Z)- (17.14%), octadecanoic acid (stearic acid) (13.51%), Gamma-sitosterol (9.71%), Gamma-terpinene (4.51%), o-cymene (4.37%) and butyl phosphonic acid, di (4-octyl) ester (4.06%) were found as the major phytochemical components.

GC/MS analysis of *Trigonella foenum-graecum* (petroleum ether extract, Saudi sample): Table 4 shows twenty six compounds were identified from *Trigonella foenum-graecum* seeds n-petroleum ether extract, Saudi sample. The compounds: D-Limonene (36.46%), Gamma-sitosterol (10.95%), 13-docosenamide (Z)- (10.80%), 9-octadecenoic acid, 1,2,3-propanetriyl ester (5.69%), thymoquinone (3.59%) and floxuridine (2.73 as the major phytochemical constituents.

Table 5 showed the major compounds in the *Trigonella foenum-graecum* obtained from two different origins extracted by two non-polar solvents and analyzed by GC/MS.

Table 3: Chemical constituents of *Trigonella foenum-graecum* seeds (n-hexane extract, Saudi sample)

No.	Compound name	Other name	RT	Percentage
1	Benzaldehyde, 4-fluoro-	Benzaldehyde,p-fluro	3.442	1.73
2	Bicyclo[3.1.1]heptane, 6,6-dimethyl-2-methylene-	2(10)-pinene (1S,5S)-(-)-	3.576	1.82
3	O-Cymene	Benzene,1-methyl-2(1-methylethyl)-	4.135	4.37
4	.gamma.-Terpinene	1,4-cyclohexadiene, 1-methyl-4-(1-methylethyl)-	4.556	4.51
5	Hexadecane	n-Hexadecane	6.942	1.36
6	Cyclohexasiloxane, dodecamethyl-	Dodecamethylcyclohexasiloxane	7.703	0.61
7	Diethyl Phthalate	Phthalic acid, diethyl ester	11.599	2.82
8	Butylphosphonic acid, di(4-octyl) ester	-	12.788	4.06
9	Phytol, acetate	-	14.124	1.64
10	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	2-Hexadecen-1-ol,3,7,11,15-tetramethyl	14.571	0.81
11	n-Hexadecanoic acid	Hexadecanoic acid ; Palmitic acid	15.498	2.92
12	cis-9-Hexadecenal	9-Hexadecenal,(Z)-	17.220	3.24
13	5.alpha.-Pregn-16-en-20-one, 12.beta.-hydroxy-, ac e1t7at.	20-Oxopregn-16-en-12-yl acetate #	17.290	0.71
14	Octadecanoic acid	Stearic acid	17.387	13.51
15	Hexadecane	n-Hexadecane; n-Cetane	17.552	17.55
16	Hexadecanamide	Palmitamide	17.608	2.56
17	Octadecanoic acid, (2-phenyl-1,3-dioxolan-4-yl)me t1h7y.l6 e9s2t er, cis-	-	17.692	2.22
18	9-Octadecenamide, (Z)-	Adogen 73; Oleamide; Oleic acid amide	19.136	17.14
19	Octadecanamide	Stearamide	19.367	0.75
20	Octadecanoic acid, 2,3-dihydroxypropyl ester	Stearin,1-mono-	19.782	0.74
21	1,3-Dipalmitin trimethylsilyl ether	-	19.947	1.19
22	Decane, 1,9-bis[(trimethylsilyl)oxy]-	-	20.110	0.90
23	Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester	Stearin,1,3-di-	20.155	1.07
24	Diisooctyl phthalate	Bis(6-methylheptyl)phthalate	20.552	0.64
25	Octacosanol	nooctracosanol	20.790	0.70
26	13-Docosenamide, (Z)-	Erucylamide	22.326	1.70
27	Unidentified	$C_{32}H_{54}O_3$	22.392	
28	Squalene	2,6,10,14,18,22-Tetracosahexaene,2,6,10,15,19,23- hexamethyl-,(all-E)-	22.546	0.89
29	.beta.-Tocopherol	-	24.190	0.76
30	Lup-20(29)-en-3-ol, acetate, (3.beta.)-	Lupenyl acetate	24.491	0.90
31	Stigmastan-3,5-diene	-	24.600	2.10
32	dl-.alpha.-Tocopherol	(+/-)-alpha-Tocopherol	24.777	3.40
33	5-Cholestene-3-ol, 24-methyl-	Ergost-5-en-3-ol	25.716	1.24
34	Gamma.-Sitosterol	Stigmast-5-en-3-ol,(3beta,24S)-	26.543	9.71
35	Fucosterol	Stigmasta -5,24(28)-dien-3-ol, (3beta,24E)-	26.725	1.93
36	Unidentified	$C_{30}H_{50}O$	27.354	0.72
37	Lanostan-3.beta.-ol, 11.beta.,18-epoxy-19-iodo-, ac e2t7a.t8e	-	27.891	0.58
38	9,19-Cyclolanostan-3-ol, 24-methylene-, (3.beta.)-	9,19-Cyclo-9.beta-lanost-24-en-3beta.-ol	28.047	0.97
39	Phytol, acetate	-	28.801	0.74

Table 6 shows the major chemical classes of *Trigonella foenum-graecum* seeds n-hexane and petroleum Ether extracts, in Sudan and Saudi samples.

n-Hexane extract, Sudan sample: Eight fatty acid compounds were identified in the n-hexane extract, Sudan sample (27.07 %), the result revealed that oleic acid (9.09 %) was found as a major component followed by 11-octadecenoic acid, methyl ester (Z)- (3.69 %) and palmitic acid (n-hexadecanoic acid) (3.58%).

Table 4: Chemical constituents of *Trigonella foenum-graecum* seeds (petroleum ether extract, Saudi sample)

No.	Compound name	Other name	RT	Percentage
1	O-Cymene	Benzene,1-methyl-2(1-methylethyl)-	4.292	1.25
2	D-Limonene	Cyclohexene, 1-methyl-4-(1-methyl ethenyl)-,(R)-	4.339	36.46
3	.gamma.-Terpinene	1,4-cyclohexadiene, 1-methyl-4-(1-methylethyl)-	4.734	1.56
4	Thymoquinone	2,5-cyclohexadiene-1,4-dione,2-methyl-5-(1-methylethyl)-	7.565	3.59
5	Thymol	Phenol,5-methyl-2-(1-methylethyl)-	8.309	1.95
6	Diethyl Phthalate	1,2-Benezenedicarboxylic acid, diethyl ester	12.208	2.00
7	Phytol, acetate	-	15.328	0.85
8	Phthalic acid, butyl undecyl ester	-	17.459	2.79
9	Oleic Acid	9-Octadecenoic acid (Z)-	20.421	1.11
10	Oleoyl chloride	Oleic acid chloride	24.878	1.47
11	9-Octadecenoic acid, 1,2,3 -propanetriyl ester, (E,E, E2)5-	2,3-Bis(9E)-9-Octadecenoyloxy]propyl	25.618	5.69
12	Floxuridine	Uridine ,2-deoxy-5-fluro	25.881	2.73
13	13-Docosenamide, (Z)-	Erucyamide	30.325	10.80
14	Squalene	2,6,10,14,18,22-tetracosahexaene, 2,6,10,15,19, 23- hexamethyl-, (all-E)-	30.818	1.70
15	Oxirane, 2,2-dimethyl-3-(3,7, 12,16,20-pentamethyl-332,7.5, 1017, 15,19-he n4e1i0c3o5sa1p entaen 0y.l9)-9, (all-E)-	-	32.507	0.32
16	Unidentified	$C_{54}H_{108}Br_2$	33.526	0.95
17	erythro-9,10-Dibromopentacosane	9,10-Dibromopentacosane	34.169	1.61
18	dl-.alpha.-Tocopherol	(+/-)-,alpha-Tocopherol	35.227	2.41
19	Tetrapentacontane, 1,54-dibromo-	1,54-Dibromotetrapentacontane	36.873	1.14
20	Tetracosamethyl-cyclododecasiloxane	-	37.551	1.07
21	Gamma-Sitosterol	Stigmast-5-en-3-ol,(3beta,24S)-	37.924	10.95
22	Fucosterol	Stigmasta-5,24(28)-dien-3-ol,(3beta,24E)-	38.202	1.57
23	1,16-Hexadecanediol	-	38.350	1.13
24	Hexacontane	-	38.395	1.95
25	9,19-Cyclolanost-24-en-3-ol, (3.beta.)-	9,19-Cyclo-9.beta-lanost-24-en-3beta.-ol	39.154	0.95
26	Silane, diethylheptyloxyoctadecyloxy-	-	39.275	1.32

Table 5: Major compounds in the *Trigonella foenum-graecum* obtained from two different origins extracted by two non-polar solvents and analyzed by GC/MS

	n-Hexane (%)		Petroleum ether (%)	
Compound name	Sudan sample	Saudi sample	Sudan sample	Saudi sample
Butylphosphonic acid, di(4-octyl) ester	8.72	4.06	-	-
Oleic acid	9.09	-	-	1.11
9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E, E1)-	8.55	-	-	5.69
9-Octadecenamide, (Z)-	8.00	17.14	-	-
.gamma.-Sitosterol	3.63	9.71	2.87	10.95
O-cymene	-	4.37	27.69	1.25
D-limonene	-	-	-	36.46
Gamma-terpinene	-	4.51	23.92	1.56
Octadecanoic acid	-	13.51	-	-
dl-alpha-tocopherol	-	3.40	0.99	2.41
Beta-pinene	-	-	4.83	-
Thymol	-	-	6.29	1.95
13-docosenamide (Z)-	-	1.70	-	10.80
Oleic acid, methyl ester (9-Octadecenoic acid (Z)- methyl ester)	-	-	7.15	-

Seven ester compounds were identified in this extract (25.89%) and the result revealed that butyl phosphonic acid, di (4-octyl) ester (8.72 %) was found as a major component followed by 9-octadecenoic acid -1,2,3-propane-triyl ester (E,E,E 1)- (8.55%). Whereas three phytosterol compounds were identified in extract (10.56 %), the result revealed that stigmastan-3,5-diene (5.59 %) was found as a major component followed by Gamma-sitosterol (3.63%).

Table 6: Statistics of major chemical class of *Trigonella foenum-graecum* seeds n-hexane and petroleum ether extracts, Sudan and Saudi samples

	Concentration (%)		Concentration (%)	
Chemical class	n-Hexane extract, Sudan sample	Petroleum ether extract, Sudan sample	n-Hexane extract, Saudi sample	Petroleum ether extract, Saudi sample
Terpenes	8.87	63.88	13.85	43.02
Fatty acids	27.07	15.12	16.43	8.27
Alkanes	-	4.33	22.24	8.04
Phytosterols	10.56	2.87	13.74	12.52
Amides	8.00	-	22.15	10.80
Ketones	1.31	-	0.71	3.59
Esters	25.89	9.63	12.74	4.79
Vitamin E	2.26	0.99	4.16	2.41
Aldehydes	6.18	3.08	4.97	-
Alcohols	7.08	-	2.71	-

Petroleum ether extract, Sudan sample: Six terpenes compounds were identified in petroleum ether extract, Sudan sample (63.88 %) and the result revealed that O-cymene (27.69 %) was formed as a major component followed by Gamma-terpinene (23.92 %). Five fatty acids compounds were identified in this extract oleic acid (15.12%) and methyl ester (7.15%) were found as major components followed by methyl 10 trans-12, cis-octadecadienoate (4.75%).

Eight ester compounds were identified in this extract (9.63%), 1,2-cyclohexanedicarboxylic acid, cyclohexyl methyl ester (1.91%) was found as a major component followed by 1,2- cyclohexane dicarboxylic acid, dinonyl ester (1.68%).

n-Hexane extract, Saudi sample: Five alkane compounds were identified in n-hexane extract, Saudi sample (22.24%) and the result revealed that hexadecane (17.5 %) was found as a major component. Four amide compounds were identified in this extract (22.15%) and the result revealed that 9-octadecenamide, (Z)- (17.14%) was found as a major component. Two fatty acid compounds were identified (16.43%) and the result revealed that stearic acid (13.51%) was found as a major component followed by palmitic acid (2.92%).

Petroleum ether extract, Saudi sample: Six terpene compounds were identified in the petroleum ether extract, Saudi sample (43.02%) and the result revealed that D-Limonene (36.46%) was found as a major component. Two phytosterol compounds were identified in this extract (12.52%) and the result revealed that Gamma-sitosterol (10.95%) was formed as a major component, followed by fucosterol (1.57%). Whereas three one-amide compounds were identified in this extract (10.80%), 13-Docosenamide, (Z)- (10.80%) was found as a major component.

Gamma-sitosterol (Clionsterol; beta-dihydrofucosterol) compound was found in all studied samples, but high content was found in both n-hexane and petroleum ether extracts of Saudi sample (9.71% and 10.95%, respectively). Gamma sitosterol was reported to possess antihyperglycemic activity by increasing insulin secretion in response to glucose[13].

The Gamma-terpinene is a monoterpene that has multiple pharmacological properties and has structural similarity to antinociceptive monoterpenes, such as limonene[14]. The 9,12-octadecadienoic acid (Z,Z), methyl ester (linoleic acid) it serves as anti-inflammatory, antiarthritic, antieczemic, antifibrinolytic, antihistaminic, antimenorrhagic, antiprostatic, cancer- preventive, carcinogenic and nematicide[15].

Tributyl citrate is an effective plasticizer for biodegradable polymers. It serves as an ideal additive for the paints, coatings and industrial chemicals industries, catering to companies aiming to formulate with

natural and environmentally friendly ingredients[16]. It is a perfect additive in the paints, coatings and industrial chemicals industries, for companies looking to formulate with natural, environmentally-friendly additives[16].

Campesterol (24a-methyl-5-cholesten-3b-ol) it possess a wide range of biological functions such as protection against pathogens[17]. Thymoquinone is used as cytoprotective, against acetaminophen-induced hepatotoxicity, as an anti-inflammatory, antioxidant, anticancer and antitumor[18]. Floxuridine is a pyrimidine analogue used as an antineoplastic agent, usually as a continuous hepatic arterial infusion to treat hepatic metastases from colon cancer[19]. The 9-octadecanamide (Z)- Oleamide) compound was found in both n-hexane extract, the Sudan sample and Saudi sample (8.00 and 17.14%, respectively). The oleamide, a polymer lubricant and a bioactive compound, from various plastic, marketed containers for food/beverages and medicines into polymer contact liquid[20]. Oleamide is a potent hyperlipidemic agent as regarding its effects on decreasing serum TG, TC, LDL-C and hepatic TG[21].

CONCLUSION

Analysis of n-hexane and petroleum ether extracts of *Trigonella foenum-graecum* seeds obtained from two different sources (Sudan and Saudi Arabia) in this study is an attempt to gain a better understanding of the primary and secondary metabolites profile which would be beneficial in the evaluation of the therapeutic potential of this valuable medicinal and nutritive plant and to develop phytochemical standards for the quality control purpose. The present study revealed that the terpenes, fatty acids, esters, amides and alkane derivatives compounds in studied plants may be explored for manufacturing industrial products.

SIGNIFICANCE STATEMENT

This research aims to detect and identify primary and secondary metabolites in two different non-polar seed extracts of *Trigonella foenum-graecum* using GC/MS. Currently, there are no published reports on the GC/MS analyses of n-hexane and petroleum ether extracts of *Trigonella foenum-graecum* grown in Sudan and Saudi Arabia. This study provides novel information and data valuable to the scientific community and supports further research. Various bioactive compounds have been discovered, which can be utilized based on their biological activities. The findings are intriguing and highlight the significance of this research for the development of herbal medicines.

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