Original article

Comparative Study on Secondary Metabolites, Physicochemical Properties and Fatty Acids Composition of Seed Oils Extracts of *Gossypium hirsutum* L.'Hamid' and *Gossypium barbadense* L.'Barakat 90'

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Abstract

Cotton seeds oils have their potential biological activity and specific physicochemical properties, due to which they are playing vital role in human nutritional diet for health benefits. The aim of this study was to compare secondary metabolites, physicochemical properties and fatty acids composition of seeds oil extracts of *Gossypium hirsutum* L. and *Gossypium barbadense* L. Cotton seeds used in this study were obtained from the Agricultural Research and Technology Corporation (ARTC), Wad Medani, Sudan. Seed oils methanolic extracts of *Gossypium hirsutum* and *Gossypium barbadense* were screened for their accumulated secondary compounds. Results indicated that *G. hirsutum* seed oil has more alkaloid than *G. barbadense*, whereas no variation in the amount of saponins, tannins, phenolic compounds, flavonoids and steroids & terpenoids in two mentioned species. Physicochemical properties analyses were carried out using standard analytical methods. Results of physical properties showed that the specific gravity and refractive index@25C0 were 6.73 and 1.470 for *G. hirsutum* compared to 6.87 and 1.475 for *G. barbadense* respectively. Density (g/c3) of seed oils in order were 0.935 and 0.939 whereas pH was 6.76 and 5.76. The chemical properties of oils of two studied species recorded acid values (mg KOH/ g), % of free fatty acids, peroxide values (meq/Kg fat), saponification value (mg KOH/g) and iodine value (I/g oil) were in order 7.1and 20.3; 3.3and 10.3; 0.00 and 0.00; 188.0 and 195.0; 113.6 and 110.4 for *G. hirsutum* and *G. barbadense* respectively. Fatty acids composition of seed oils of two species were revealed using the gas chromatography-mass spectrometry (GC-MS) technique. The dominant unsaturated fatty acids of *G. hirsutum* seed oils were oleic acid 13.11% and linoleic acid 44.25%, while the saturated fatty acids were palmitic acid 29.16 %, methyl stearate acid (stearic acid) 5.06%, methyl tetradecanoate (methyl myristate) 1.48% and arachidic acid 0.67%. Comparatively, the unsaturated fatty acids of G. barbadense were oleic acid 22.63 % and linoleic acid 9.65 %, whereas the saturated fatty acids were palmitic acid 40.12%, methyl stearate (stearic acid) 7.18%, methyl tetradecanote (methyl myristate) 1,71% and arachidic acid 1.51%. In conclusion, the study revealed that oils extracted from *G. hirsutum* and *G. barbadense*, grown in Sudan contain the main saturated and unsaturated fatty acids. Physicochemical properties showed standard values in most parameters especially for *G. hirsutum*. Secondary metabolites obtained indicated the presence of biological activities in the oils of these species.

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Introduction

Gossypium is a cotton genus belongs to family Malvaceae, it comprises of about 50 species and few new species continue to be discovered. The species of this genus are generally shrubs or shrub-like plants which are extraordinarily diverse in morphology and adaptation (Wendel *et al*., 2009).

Cotton is one of the most important crops produced in Sudan. More than three hundred thousand families in the Sudan are still depending on cotton for earning their livelihood. Several other

thousands are engaged in cotton related activities.

Today *G. hirsutum* and *G. barbadense* are the major cultivated species with *G. hirsutum* accounting for 90% of world cotton production (Jerkins, 2003), while *G. barbadense* represents approximately 5% (Wu *et al*., 2008). *Gossypium hirsutum* is called long-staple cotton, and the characteristic length of its fibers is 22-36 mm. *Gossypium barbadense*, extra-long-staple cotton, has fibers usually over 35 mm in length; it is cultivated mainly in Egypt, Peru, Sudan, USA and some Central Asian countries.

Sudan is characterized with a variety of climates zones, from the desert in the North to tropical zone in the South, this gives it favorable environments for all agricultural activities as well as integrated investment in industries (Bushara, 2016).

The seeds, even though extensively and intensively used worldwide as well, tend to be regarded as a secondary product or byproduct. The seeds are used to obtain edible oil, which is considered to be of very good quality within the range of vegetable oils (O'Brien *et al*., 2005); as chaff for livestock feed; and as high-protein cake and flour, which are used mainly for livestock feed. Cottonseed oil is of interest as a lubricant and a biofuel (Karaosmano *et al*., 1999). Cotton seed oil is used in foods as a coating agent, emulsifying agent, formulation aid, and texturizer (National Academy of Science, 1996a). Cottonseed oil and hydrogenated cottonseed oil have been used in margarines, shortenings, and cooking oils (Applewhite, 1985). In addition to their economic importance, the leaf, root, bark and seeds of Gossypium species have been widely explored for their medicinal values.

Vegetable oils have their specific physicochemical properties due to which they are playing vital role

in human nutritional diet for health benefits. Phytochemicals analysis of differential solvent extracts of the leaves of *Gossypium hirsutum* showed that they contained alkaloids, phenolic compounds, terpenoids, tannins, saponins, flavonoids, cardiac glycosides and protein of the dried flower bud (Chan and Lukefahr , 1978).

Phenolic acids are intermediates of phenylpropanoid metabolism (Cvikrová *et al*., 1996) and precursors of lignin (Lewis and Yamamato, 1990) and phenyl propanoid phytoalexins (Kessmann *et al*., 1990).

Muhammad (2014) was evaluated the phytochemistry composition of *G. barbadense* cotton leaves. The phytochemical analysis of methanolic extract of leaves revealed the presence of alkaloid (3.82±0.17), flavonoid (2.80±0.18), total phenols (5.94±0.41), cyanogenic glycosides (18.07±0.54) and saponins (7.28±0.19) in mg/100g, anthraquinones and terpenoids were absent. The phytochemical screening of *Gossypium hirsutum* leaves revealed the presence of alkaloids, saponins, flavonoids, tannins and cardiac glycosides whereas, terpenoids and steroids were absent, subsequent quantification analysis revealed that *Gossypium hirsutum* contained 12.20% alkaloids, 2.63% saponins, 11.90% flavonoids, 2.73mg/100g tannins and 1.62mg/100g total phenol (Ayeni, 2015).

After soybean, cotton plant is considered as one the best source of protein and also it is the fifth major oilseed crop after soybean, palm, canola and sunflower (Sawan *et al*., 2006). The main three fatty acids of cotton seed oils are palmitic, oleic and linoleic with an average percentage 22, 20 and 54 respectively (Sekhar and Rao, 2011). Cotton crop is also well-known for their dual-use purpose, one for its fiber producing nature and secondly it contributes about 4% of vegetable oil production in the world (Ashraf,2002).

Fatty acids composition of various cultivated species of *Gossypium hirsutum* were about 45% linoleic, 30% oleic, 21% palmitic, 2% myristic, and smaller amounts of stearic and arachidic acids. It has a specific gravity at $25C^0$ of 0.915 to 0.921, a saponification value of 189 to 198, and an iodine value of 99 to 113. It has a maximum acid value of 2.0 (Nikitakis and McEwen 1990b).

The proximate composition, physicochemical analysis and characterization of *Gossypium hirsutum* seed and it's oil was carried out in the characterization of fatty acid present in the oil with GC-MS Spectroscopy system, the major unsaturated fatty

acid values were 14.53% for oleic acid and 55.38% for linoleic acid while the palmitic acid and stearic acid values which were saturated acid are 27.39% and 2.23% respectively. The percentage values of the rest of the fatty acid present in the oil were very low. The parameters determined were within the international and Nigerian industrial standard for vegetable oil (Okonkwo *et al*., 2016).

Three cotton genotypes of species *Gossypium hirsutum* L., Cukurova 1518, PAUM 15 and BA 119 were investigated for their certain physicochemical properties of oils such as free fatty acids, peroxide value, iodine value, unsaponifiable matter, total carotenoid and tocopherol contents and fatty acids composition in Cukurova region in Turkey. Seed oil content ranged 17.2-19.6% and PAUM 15 was found to be genotype with the highest oil content. The range of other physicochemical properties and their values are as follows; free fatty acids 1.7-2.8%, peroxide value 5.3-6.0 meq O 2 kg-1, unsaponifiable matters 2.1-2.3%, iodine value 102-110, total carotenoid content 119-140 mg kg-1, total tocopherol content 887-920 mg kg-1, linoleic acid 52.00-55.82%, palmitic acid 24.85-25.63%, oleic acid 14.06-17.00%, stearic acid 3.01-3.13% in the cottonseed oils. PAUM 15 was determined to be more suitable for food consumption as edible oil due to its highest oil content and quality characteristics than the other genotypes. (Dilsat, 2017).

To the best of our knowledge, there is no research was conducted on secondary metabolites of seeds, physical and chemical properties and fatty acids composition of seed oils extract from *Gossypium hirsutum* and *Gossypium barbadense* grown in Sudan, although there are some researches were conducted on *Gossypium hirsutum* in other countries. Vegetable oils have their specific physicochemical properties due to which they are playing vital role in human nutritional diet for health benefits, thus the present research aimed to investigate the secondary metabolites of seeds, physicochemical properties, and fatty acids composition of seed oils extract from *Gossypium hirsutum* L. and *Gossypium barbadense* L. grown in Sudan. It is obvious that the seeds of different variety of cotton vary as grown in diverse agroclimatic conditions with respect to oil, and other content.

Materials and Methods

Plant Materials

Healthy seeds of *Gossypium hirsutum* L. and *Gossypium barbadense* L were obtained from the Agricultural Research and Technology Corporation (ARTC), Wad Medani, Sudan.

Preparation of Plant Crude Extracts

Twenty-five gm of dry seeds of *Gossypium hirsutum* L. and *Gossypium barbadense* L. were cleaned and ground using blender. The extracts were prepared by the method described by Akbar *et al* (2009) followed with slight modification. The extraction was carried out by soxhlet apparatus, using petroleum ether (200 ml) in 60-80 ° C. The process was continued for 3 hrs. The extracted plant material was air dried and repacked again then extracted under the same conditions by methanol (200ml). The obtained extracts were filtered with a filter paper and were evaporated at room temperature to get a dried solid. The products were stored in dried bottles at 4 °C. Each extraction was run in three replicates and %yield was determined.

Qualitative Analysis of Secondary Metabolites

Phytochemical examinations were carried out for all the extracts to evaluate the presence of secondary metabolites such as alkaloids, saponins, phenolic compound, tannins, flavonoids and steroids & terpenoid by using different standard methods described by Evans (1997), Kokate (1999), (Wolfe *et al*.,2003), Kokate (1994), Peach and Tracey (1956) and Harborne (1992) for steroids & terpenoid, respectively for the mentioned secondary metabolites.

Physicochemical Analysis of Seed Oils

The specific gravity of *Gossypium hirsutum* L. and *Gossypium barbadense* L seed oils were determined using specific gravity bottle method as described by (Pearson, 1976). The refractive indices were read at 25° C by Abbe Refractometer as (Singhal

and Sekiya, 2003) The method of density determination was adopted by Akpan *et.al.*, (2005). pH of oils was determined by the method of AOAC (2000), using digital pH meter.

Iodine value determined by Hanus iodine method (AOAC,1975), while saponification values, acid values and peroxide values were determined according to (AOAC,1990). Amount of free fatty acid (FFA) was calculated as being equivalent to half the value of acid. All the analyses were done in triplicate and reagents used were of analytical.

Gas Chromatography/Mass Spectrometry (GC/MS) Conditions Analysis of Fatty Acid

Methyl ester were prepared from total lipids by the method (AOAC,1990). These fatty acids methyl esters were analyzed by gas chromatography, Shimadzu series (GC.MS.QP.2010), equipped with mass spectrometer detector and Rtx.50 column having internal diameter 0.25mm and length 30cm. Fatty acids profile obtained through this gas chromatography with relevant standards.

Statistical Analysis

All data were presented by means ± S.D. Statistical analysis for all the assays results was done using Microsoft program (2010).

Results and Discussion

Screening of secondary metabolites of methanol extracts from *Gossypium hirsutum* L. and *Gossypium barbadense* L were revealed by positive reaction with respective test reagent. Results in table (1) was represented secondary constituents of alkaloids, saponins, tannins, phenolic compounds, flavonoids and steroids &terpenoids in *Gossypium hirsutum* L. and *Gossypium barbadense* L respectively. These results were consistent with that reported by Ayeni (2015) and Muhammad (2014). High amounts of flavonoids, steroids and terpenoid agree with that of Kannan et al. (2009), who reported that *G. hirsutum* fruits contained complex antibiotic compounds which cured various diseases like cancer, cardiovascular and digestive diseases.

Table (1) Screening for secondary metabolites of cotton seeds methanolic extracts of *Gossypium hirsutum* L. 'Hamid' and *Gossypium barbadense* L .'Barakat 90'

Test	Reagent	*Gossypium hirsutum L.*' Hamid'	*Gossypium barbadens L* .'Barakat 90'	Observation
Alkaloids	Mayers	++	+	White creamy precipitate
	Wagner,s	++	+	Reddish-brown precipitate
	Dragendorff,s	++	+	Yellow precipitate
Saponins	H_2O	++	++	Persistent foam
Tannins	Gelatin	++	++	White precipitate
	Fecl3	++	++	Bluish black colour
Flavonoids	NaOH	++	++	Yellow colour
	Lead acetate	++	++	Yellow precipitate
Phenolic compounds	$FeCl_3$	+++	+++	Bluish black colour
	Folin-Ciocalteu	+++	+++	Blue color
Steroids and Terpenoids	Salkowski,s test	++	++	Reddish brown colour ring

+ =compound is detected. + = low ++ = medium +++ = high

Physicochemical characteristics of cottonseed oil derived from these two species were shown in figures (1&2). Results obtained were agree with that of Dilsat (2017) who determined the level of free fatty acids 1.7-2.8%, peroxide

value 5.3-6.0 meq, iodine value 102-110 in *Gossypium hirsutum* L., Cukurova 1518, PAUM 15 and BA 119.To the best of our knowledge, there is no documented study on seed oil extracted from *Gossypium barbadense* L.'Barakat 90' ,although the results obtained to some extent were similar to that of *Gossypium hirsutum* L.'Hamid' (figures 1&2).

The dominant unsaturated fatty acids of *G. hirsutum* 'Hamid' seed oils were oleic acid 13.11% and linoleic acid 44.25%, while the saturated fatty acids were palmitic acid 29.16 %, methyl stearate acid (stearic acid) 5.06%, methyl tetradecanoate (methyl myristate) 1.48% and arachidic acid 0.67% (table 2 &figure 3). These finding was consistent to that of (Okonkwo *et al*.,2016). The unsaturated fatty acids of *G. barbadense* L. 'Barakat 90' were oleic acid 22.63 % and linoleic acid 9.65 %, whereas the saturated fatty acids were palmitic acid 40.12%, methyl stearate (stearic acid) 7.18%, methyl tetradecanote (methyl myristate) 1,71% and arachidic acid 1.51%(table 3 &figure 4).These results were inconsistent with that reported by Jones and King,(1996).

Various factors affecting variation in cottonseed oil mainly includes soil, fertilizer genotype and environmental conditions (Cherry, 1983 Jones and King, 1996).

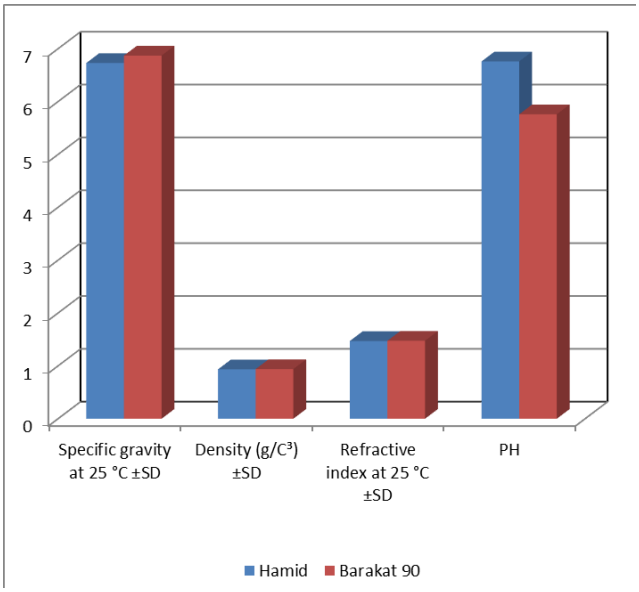

Figure (1) Physical properties of seeds oil of *Gossypium hirsutum* L. 'Hamid' and *Gossypium barbadens* L. 'Barakat 90'

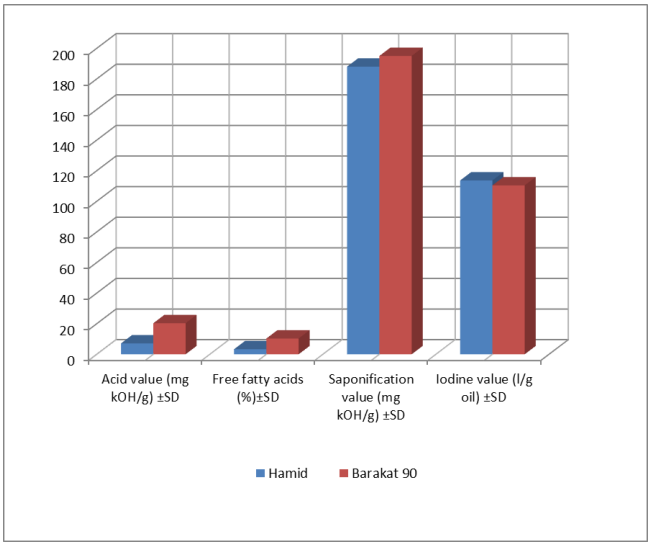

Figure (2). Chemical properties of seeds oil of *Gossypium hirsutum* L. 'Hamid' and *Gossypium barbadens* L.'Barakat 90'

Table (2) MS chromatogram of petroleum ether of *Gossypium hirsutum* L. 'Hamid'

NO	RT	Compound name	Class of compound	Area %	Formula	MW
1	13.182	Methyl tetradecanoate	Fatty acid ester	1.48	$C_{15}H_{30}O_2$	242
2	14.260	Pentadecanoic acid, methyl ester	Fatty acid ester	0.03	$C_{16}H_{32}O_2$	256
3	15.084	methyl palmitoleinate	Monounsaturated	1.07	$C_{17}H_{32}O_2$	268
4	15.310	palmitic acid methyl ester	(saturated)	29.16	$C_{17}H_{34}O_2$	270
5	16.045	cis-10-Heptadecenoic acid, methyl ester	Fatty acid ester	0.18	$C_{18}H_{34}O_2$	282
6	16.256	Heptadecanoic acid, methyl ester	Fatty acid ester	0.16	$C_{18}H_{36}O_2$	284
7	16.981	linoleic acid ,methyl ester	Essential fatty acid unsaturated	44.25	$C_{19}H_{34}O_2$	294
8	17.019	oleic acid ,methyl ester	Fatty acid ester unsaturated	13.11	$C_{19}H_{36}O_2$	296
9	17.045	Elaidic acid, methyl ester	Fatty acid ester	1.31	$C_{19}H_{36}O_2$	296
10	17.195	Methyl stearate	Fatty acid ester	5.06	$C_{19}H_{38}O_2$	298
11	17.960	10-Nonadecenoic acid, methyl ester	Fatty acid ester	0.49	$C_{20}H_{38}O_2$	310
12	18.547	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester	Fatty acid ester	1.12	$C_{18}H_{31}ClO$	298
13	18.716	9-Octadecenoic acid, 12-hydroxy-, methyl ester, [R-(Z)]-	Fatty acid ester	0.39	$C_{19}H_{36}O_2$	312
14	18.940	Eicosanoic acid, methyl ester	Fatty acid ester	0.67	$C_{21}H_{42}O_2$	326
15	19.038	PGH1, methyl ester	Fatty acid ester	0.28	$C_{22}H_{38}O_4$	366
16	20.558	Docosanoic acid, methyl ester	Fatty acid	0.34	$C_{23}H_{46}O_2$	354
17	21.323	Tricosanoic acid, methyl ester	Fatty acid ester	0.07	$C_{24}H_{48}O_2$	368
18	22.061	Tetracosanoic acid, methyl ester	Fatty acid ester	0.26	$C_{25}H_{50}O_2$	382
19	22.777	Pentacosanoic acid, methyl ester	Fatty acid ester	0.06	$C_{26}H_{52}O_2$	396
20	23.462	Hexacosanoic acid, methyl ester	Fatty acid ester	0.05	$C_{27}H_{54}O_2$	410
21	26.983	.gamma.-Sitosterol	Stigmastanes or derivatives	0.46	$C_{29}H_{50}O$	414
				100.00		

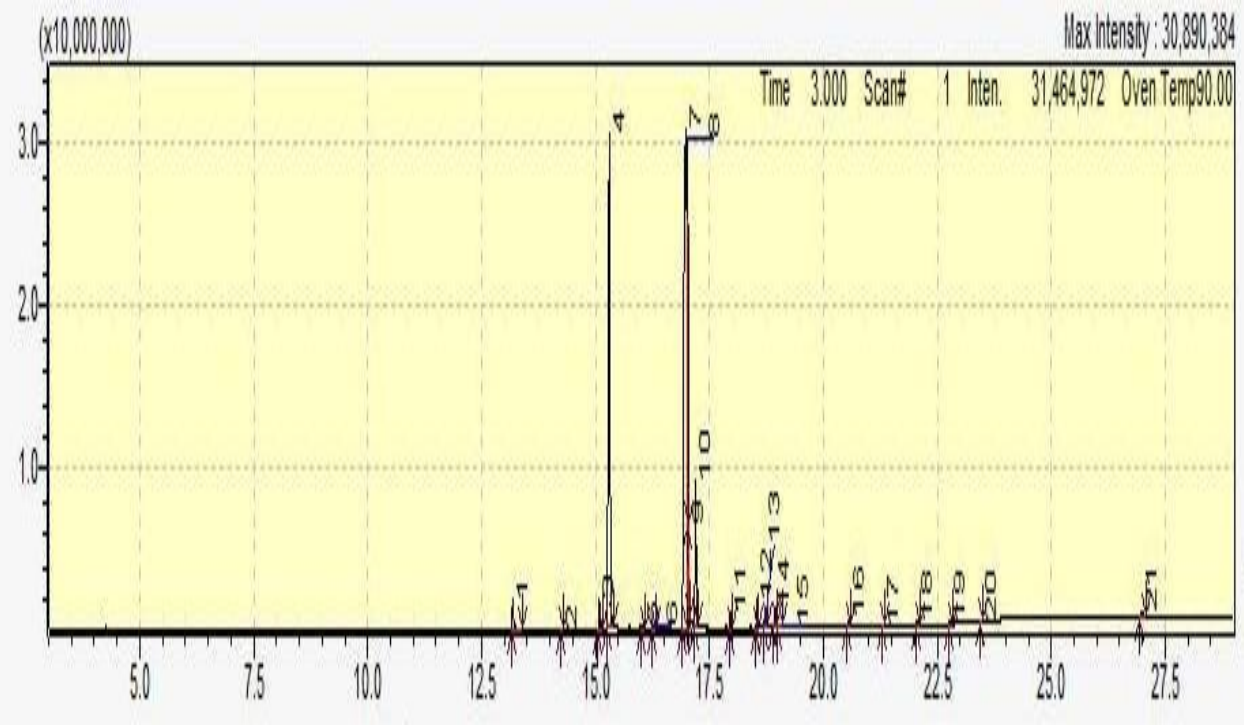

Fig (3) GC-MS chromatogram of petroleum ether of *Gossypium hirsutum* L. ' Hamid '

Table (3) GC-MS chromatogram of petroleum ether of *Gossypium barbadens* . 'Barakat 90'

NO	RT	Compound name	Class of compound	Area%	Formula	MW
1	3.105	Hexanoic acid, methyl ester	Fatty acid ester	0.47	$C_7H_{14}O_2$	130
2	5.595	Octanoic acid, methyl ester	Fatty acid ester	0.22	$C_9H_{18}O_2$	158
3	6.971	Nonanoic acid, methyl ester	Fatty acid ester	0.05	$C_{10}H_{20}O_2$	172
4	9.021	2-Octenal, 2-butyl-	Aldehydes	0.14	$C_{12}H_{22}O$	182
5	9.844	Nonanoic acid, 9-oxo-, methyl ester	Fatty acid ester	0.19	$C_{10}H_{18}O_3$	186
6	10.871	Dodecanoic acid, methyl ester	Fatty acid ester	0.04	$C_{13}H_{26}O_2$	214
7	11.208	Nonanedioic acid, dimethyl ester	Fatty acid ester	0.22	$C_{11}H_{20}O_4$	216
8	13.178	Methyl tetradecanoate	Fatty acid ester	1.71	$C_{15}H_{30}O_2$	242
9	15.081	methyl palmitoleinate	Fatty acid ester monounsaturated	1.15	$C_{17}H_{32}O_2$	268
10	15.305	palmitic acid methyl ester	(saturated)	40.12	$C_{17}H_{34}O_2$	270
11	15.735	n-Hexadecanoic acid	Saturated Fatty acid	3.47	$C_{16}H_{32}O_2$	256
12	16.027	Cis-10-Heptadecenoic acid, methyl ester	Fatty acid ester	0.23	$C_{18}H_{34}O_2$	282
13	16.249	Heptadecanoic acid, methyl ester	Fatty acid ester	0.32	$C_{18}H_{36}O_2$	284

14	16.929	linoleic acid methyl ester	(Essential fatty acid unsaturated)	9.65	$C_{19}H_{34}O_2$	294
15	16.988	oleic acid ,methyl ester	Fatty acid ester	22.63	$C_{19}H_{36}O_2$	296
16	17.024	Elaidic acid, methyl ester	Fatty acid ester	1.01	$C_{19}H_{36}O_2$	296
17	17.189	Methyl stearate	Fatty acid ester	7.18	$C_{19}H_{36}O_2$	298
18	17.395	Oleic Acid	unsaturated Fatty acid	0.59	$C_{18}H_{34}O_2$	282
19	17.956	10-Nonadecenoic acid, methyl ester	Fatty acid ester	0.36	$C_{20}H_{38}O_2$	310
20	18.401	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl]cyclopropyl]methyl]-, methyl ester	Fatty acid ester	0.46	$C_{22}H_{38}O_2$	334
21	18.471	11,14-Eicosadienoic acid, methyl ester	Fatty acid ester	0.46	$C_{21}H_{38}O_2$	322
22	18.548	linoleic acid	unsaturated Fatty acid	3.19	$C_{18}H_{31}ClO$	298
23	18.709	Oxiraneoctanoic acid, 3-octyl-, methyl ester, cis-	Fatty acid ester	1.51	$C_{19}H_{36}O3$	312
24	18.800	17-Octadecynoic acid, methyl ester	Fatty acid ester	1.59	C19H34O2	294
25	18.939	Eicosanoic acid, methyl ester	Fatty acid ester	1.19	C21H42O2	326
26	19.480	Tricyclo[20.8.0.0(7,16)]triacontane, 1(22),7(16)-diepoxy-		1.02	C30H52O2	444
27	20.556	Docosanoic acid, methyl ester	Fatty acid ester	0.55	C23H46O2	354
28	22.057	Tetracosanoic acid, methyl ester	Fatty acid ester	0.28	C25H50O2	382
				100.00		

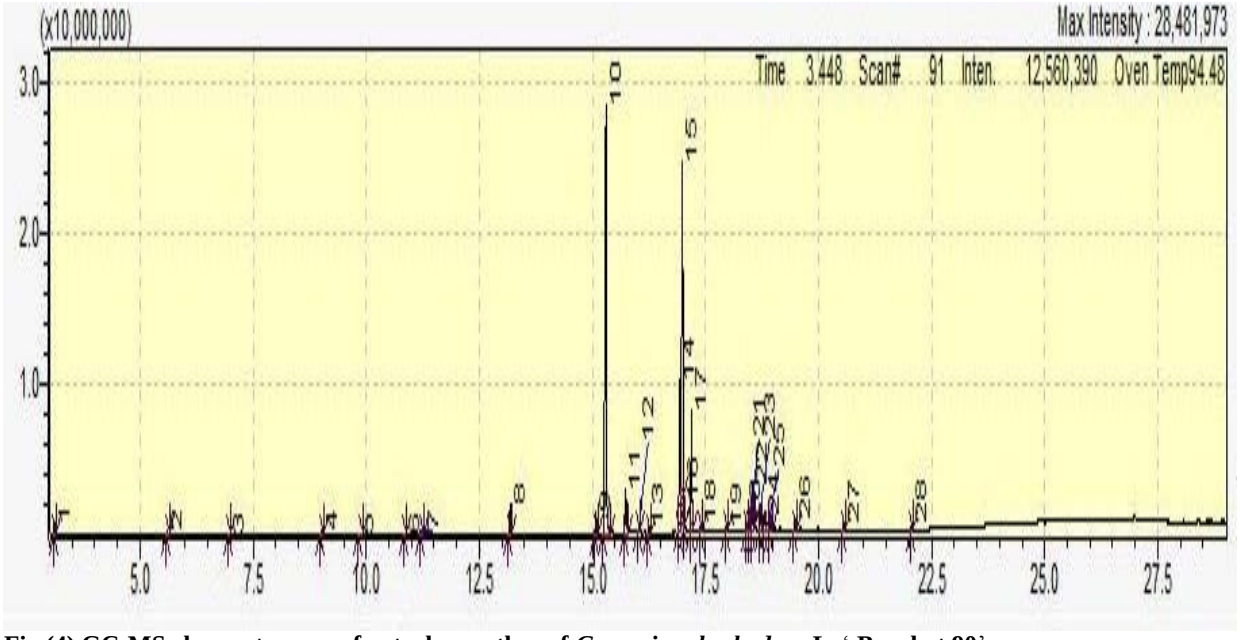

Fig (4) GC-MS chromatogram of petroleum ether of *Gossypium barbadens* L. ' Barakat 90'

Conclusion

The research revealed that oils extracted from *G. hirsutum* and *G. barbadense*, grown in Sudan contain the main saturated and unsaturated fatty acids. *G. hirsutum* contain high amount of poly unsaturated fatty acids linoleic acid 44.25%. The results suggest that *G. hirsutum* seed oil have great potential for future industrial oil seeds crops, whereas *G. barbadense* contain high amount of saturated fatty acids palmitic acid 40.12%. Physicochemical properties showed standard values in most prameters especially for *G. hirsutum*. Comparable secondary metabolites compounds were obtained from the two species under study. The amount of secondary metabolites support the use of oils of both species in herbal cure remedies.

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