STUDY OF JORDANIAN *JOJOBA* (*SIMMONDSIA CHINENSIS*) LIQUID WAX BY GC AND GC/MS

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Abstract

Context: The oil of *Simmondsia chinensis* (Link) C.K. Schneid (Simmondsiaceae) has been traditionally used in ethno-medicine because of its unique chemical constituents.

Objective: This study determined the Jordanian *Jojoba* oil components. Fatty acids and sterols profiles were determined by using gas chromatography (GC), while tocopherols profile was determined by using gas chromatography-mass spectrometry (GC/MS).

Materials and methods: *Jojoba* seeds were subjected for hot extraction by solvent (hexane) in addition to cold pressing extraction.

Results: The fatty acid α-linolenic acid was in higher percentages within the Jordanian oil than those of *Jojoba* oil found in other parts of the world. On the other hand, the fatty acids palmitic acid, oleic acid, vaccenic acid, and gondoic acid were in lower percentages within the Jordanian oil. The avenasterol was in higher percentages within the Jordanian oil and the Jordanian *Jojoba* oil does not contain campesterol or campestanol. In addition, this study found that the major sterols and stanols constituents of the Jordanian *Jojoba* oil are: sitosterol, campesterol, stigmasterol, cholesterol, avenasterol, and brassicasterol. It was also found that *Jojoba* oil (hexane extract) contains stigmastadienol while *Jojoba* oil (cold pressing extract) does not. *Jojoba* oil (cold pressing extract) contains stigmastenol while *Jojoba* oil (hexane extract) does not. This study showed that the Jordanian *Jojoba* oil contains α-tocopherol similar to those of *Jojoba* oil from around the world.

Discussion and conclusion: This is a pioneer results regarding *Jojoba* which is cultivated in Jordan under different environmental conditions.

Keywords: Jojoba oil, GC, GC/MS, fatty acid, sterol, tocopherol

Introduction

Simmondsia chinensis (Link) C.K. Schneid is the only member in its family Simmondsiaceae and it is known as *Jojoba*, which originated from the deserts of USA. It is presently distributed over many deserts. In Jordan, it is cultivated in the Jordan University of Science & Technology farms since 1986 and recently in Al-Ghoor area (AI-Zoubi, 1996).

S. chinensis is unique in many ways (Gentry, 1958). It was the Native Americans who discovered the importance of *Jojoba* oil. Moreover *Jojoba* seeds produce 45-75% by weight of a colorless, odorless oil or liquid wax. This oil has a unique chemical structure in the plant world (McKelvie et al., 1994). The wax is composed of straight chain monoesters of C-20 and C-22 acids and alcohols with two double bonds in about 97% of its content. The acids have been identified as a mixture of cis-11-eicosenoic (C-20) and cis-13-docosenoic (C-22, erucic) acids. The alcohols have been identified as mixtures of cis-11-eicosenol, cis-13-docosenol, and cis-15-tetracosenol (C-24). In addition to quantities of sterols and stanols (around 0.5% total), mainly: campesterol, stigmasterol, sitosterol, cholesterol and avenasterol (Léon et al., 2004; Tada et al., 2005; El-Mallah et al., 2009) along with a trace amounts of triglyceride (Van Boven et al., 2000 b) and different tocopherols, mainly: α-tocopherol (El-Mallah, 2009).

Furthermore, groups of nitrile glycosides known as simmondsin's compounds (10- 20%) have been identified (Van Boven et al., 2000 a; Laszlo et al., 2006). The simmondsins are considered as unusual nitrile glycosides and cannot be compared to most other nitrile glycosides found in plants (Allen, 1997). Also, it contains phospholipids (Léon et al., 2004) and oligosaccharides (Hantus et al., 1997).

Of more than 250,000 identified plant species, *Jojoba* is the only one which produces significant quantities of liquid wax esters similar to the natural restorative esters produced by human sebaceous glands (sebum) and so, it can be used as natural emollient for all skin types and hair. The wax is stable and resistant to both oxidation and rancidity (Mishra et al., 2011).

Therefore, *Jojoba* oil is used as a carrier substance for oxidation sensitive substances such as vitamin A (Gruenwald et al., 1998). Also, *Jojoba* liquid wax is useful in the stabilization of penicillin products. In fact, it has been shown to be the best liquid wax for this purpose (Daugherty et al., 2012). In addition, *Jojoba* oil is used as carrier oil for essential oils in aromatherapy and massage therapy (Buckle, 2007). Moreover, both

quantitative and qualitative analyses of the *Jojoba* wax have been carried out by many authors (El-Mallah, 2009).

The purpose of this work is to study the chemical constituents of *Jojoba* wax of the Jordanian cultivated *S. chinensis*. Since *Jojoba* is successfully cultivated in Jordan, it is worthy to mention that this is the first work in Jordan regarding investigating similar parameters concerning *Jojoba*.

Materials and methods

The plant samples of *S. chinensis* cultivated in Jordan were collected from the farms of Jordan University of Science and Technology (JUST)-Irbid, during the months of October, November and December (2011), with the assistance of Agricultural Services Department in JUST. The seeds were dried at room temperature (25°C) in the shade for about 15 days and then milled and weighed.

Plant specimens were identified with the help of the plant taxonomist Professor Dawud Mohammad Hasan Al-Eisawi, and checked with a herbarium collection at the Department of Biological Sciences, Faculty of Science in the University of Jordan.

Gas chromatography analysis (GC) (Shimadzu 2010 series-USA) was carried out in the food quality lab, chemical labs testing division at the Royal Scientific Society (RSC), Jordan.

Gas chromatography analysis-Mass spectrometry analysis (GC-MS) [(GC; HP Hewlett Packard 5890 series II-USA, MS; Mass selective detector HP Hewlett Packard 5972 series-USA) and Wiley Registry of Mass Spectral Data (Chemical data library) (1995)-Wiley-UK] was carried out in the environmental instrument lab and environmental labs testing division at the RSC.

Extraction methods

Jojoba seeds were soaked in *n*-hexane (95%) in different patches and refluxed for 12 h using Soxhlet apparatus, with optimum control of temperature and pH. The extracts were evaporated under reduced pressure using rotatory evaporator. The liquid wax extracts were kept in amber glass containers to avoid any photo-oxidation.

Cold pressing technique was used for extracting the liquid wax and other related compounds, after complete removal of testa from the seeds. This method is based on a hydraulic presser machine designed specifically for this purpose in the *Jojoba's* cold-pressing unit, which is a part of the Agricultural Services Department in JUST (AI-Zoubi, 1996). All the various extracts were dried, cooled and subjected to identification process.

Gas chromatography (GC) for fatty acids

For determination of fatty acids in *Jojoba* oil, which was obtained from the seeds by hot reflux and cold pressing extraction methods, a reported method was used (International Olive Council, 2001). The method describes a procedure for determining the individual and total fatty acids content of *Jojoba* oils by capillary column gas chromatography.

Through preparation of the fatty acids methyl esters from *Jojoba* oil by trans-esterification with cold methanol solution of potassium hydroxide as an intermediate stage before saponification takes place (IUPAC, 1987).

GC- Method

The column was DB-23 (Agilent Technologies, USA), 60 m long, 0.15 µm film thickness, and 0.25 mm inner diameter with a maximum temperature up to 250°C. In addition, the injection volume was 0.8 µl, and the injection was in a split mode at a ratio of 100 with injector temperature of 230°C. Moreover, the carrier gas was helium, with total flow of 124.2 ml/min, and a column flow at 1.2 ml/min with linear velocity of 26.2 cm/min.

Furthermore, the column oven temperature was held for 8 min at 165°C, then increased by 1 °C/min, and held for 1 min at 185°C. After that, it increased by 5 °C/min, and finally held for 9 min at 220°C in a total run time of 45 min. In addition, the detection was done by a flame ionization detector (FID) at a temperature of 240°C, and the hydrogen flow rate was 40 ml/min along with air flow rate of 400 ml/min

Gas chromatography for sterols and stanols

For determination of sterols, stanols and triterpene dialcohols contents in *Jojoba* oil obtained from the seeds by hot reflux and cold pressing extraction methods were carried out using capillary gas chromatography (International Olive Council, 2011). The method describes a procedure for determining the individual and total sterols and triterpene dialcohols content of *Jojoba* oils by capillary column GC.

The oils, with added α-cholestanol as an internal standard, are saponified with potassium hydroxide in ethanol solution and the unsaponifiable matter is then extracted with ethyl ether. The sterols and triterpene dialcohols fraction are separated from the unsaponifiable matter by preparative thin-layer chromatography on a basic silica gel plate. The fractions recovered from the silica gel are transformed into trimethylsilyl ethers and then analyzed by capillary column gas chromatography, with flame ionization detector (FID).

GC- Method

The column was CP-SI 1 fused silica 8CB (Varian, USA), 30 m long, 0.25 µm film thickness, and 0.25 mm inner diameter. In addition, the injection volume was 0.8 µl, and the injection was in a split mode at a ratio of 60.0 with injector temperature of 280°C. Moreover, the carrier gas was helium, with total flow of 70.9 ml/min, and column flow of 1.11 ml/min at linear velocity of 35 cm/min, under a pressure of 168.6 kpa. In addition, the purge flow was 3 ml/min, and the makeup flow was 30 ml/min.

Furthermore, the column oven temperature was held for 55 min at 255°C, in a total run time of 55 min. In addition, the detection was done by a flame ionization detector (FID) at a temperature of 290°C, and the hydrogen flow rate was 40 ml/min along with air flow rate of 400 ml/min.

Gas chromatography-mass spectrometry (GC/MS) for toccopherols

GC/MS was used for separation of the tocopherols in the *Jojoba* oil obtained from the seeds by hot and cold pressing extraction methods. The column was HP-1 (HP Hewlett Packard, USA), 30 m long and 0.32 mm thick.

The procedure was modified from reported method of Elnimiri & Nimir, 2011. The injection volume was 2 µl with a split ratio of 1:12. The injector temperature was held constant at 280°C. Helium was used as the carrier gas with an inlet pressure of 21.0 kpa, corresponding to a flow rate of 30 ml/min. The column oven temperature was set at 45°C (held for 1 min). Then, the temperature was constantly raised at a rate of 10°C/min until 175°C was reached, followed by raising the temperate at a rate of 25°C/min to 275°C, and finally held at 275°C for 10 min. The detector was mass spectrometer, and it was operated in the electron impact (EI) mode with ionization energy of 70 eV. The transfer line was set at 280°C and mass detector temperature was 300°C.

The chemical constituents of the analyte were identified by comparing the MS fragmentation patterns with those of Wiley registry of mass spectral database library (Wiley, UK, 1995) of the GC/MS system. Sample dilution was 1-100.

Results and discussion

Gas chromatography (GC) for fatty acids

The procedure was done in triplicate. The results were shown in Table 1 and the GC- chromatogram of fatty acids content of Jordanian *Jojoba* oil, as in Figures 1 and 2, respectively. The results, based on GC analyses, of the Jordanian *Jojoba* oil indicated that the oil contains fatty acids of carbon atoms from C 14 to C 24 (saturated and unsaturated).

Furthermore, the main fatty acids constituents of *Jojoba* wax are octadec-9-enoic acid (18:1) (oleic acid) and eicos-11-enoic acid (20:1) (gondoic acid). This is in agreement with reported data. Among these results it was noticed that the fatty acid 9, 12, 15-octadecatrienoic acid (18:3) (α-linolenic acid) was in higher percentages within the Jordanian oil than those of *Jojoba* oil from around the world. In contrast, the fatty acids hexadecanoic acid (16:0) (palmitic acid), octadec-9-enoic acid (18:1) (oleic acid), octadec-11-enoic acid (18:1) (vaccenic acid) and eicos-11-enoic acid (20:1) (gondoic acid) were in lower percentages within the Jordanian oil than those of *Jojoba* oil from around the world. This became clear after comparing Jordanian *Jojoba* oil results with that from the literature data regarding *Jojoba* oil fatty acids constituents (Tada et al., 2005; El-Mallah, 2009).

Gas chromatography for sterols and stanols

The sterols fractions were separated from the unsaponifiable matter by preparative thin-layer chromatography on a basic silica gel plate made of glass (Machery-Nagel-Germany), 20 × 20 cm, thickness 1 mm with fluorescent indicator 254 nm and 365 nm. The fractions recovered from the silica gel are transformed into trimethylsilyl ethers and then analyzed. The procedure was done in triplicate. The results were shown in Table 2 and the GC- chromatogram of sterols-stanols content of Jordanian *Jojoba* oil in Figures 3 and 4, respectively.

The results of the Jordanian *Jojoba* oil indicated that the oil contains sterols and stanols of similar percentages to those of *Jojoba* oil from around the world, and this is in agreement with reported data. Among these results it was noticed that the 5-avenasterol was in higher percentage in the Jordanian oil than those of *Jojoba* oil from around the world. This became clear after comparing Jordanian *Jojoba* oil results with that from the literature data regarding *Jojoba* oil sterols and stanols constituents (El-Mallah, 2009). Also, the results showed that the Jordanian *Jojoba* oil does not contain 7-campesterol and campestanol. The major sterols and stanols constituents of the Jordanian *Jojoba* oil are: sitosterol, campesterol, stigmasterol, cholesterol, avenasterol and brassicasterol.

Moreover, regarding the differences between the yield from the two extraction methods (hot and cold extraction), the findings showed that; *Jojoba* oil (hexane extract) contains 5-23-stigmastadienol while *Jojoba* oil (cold pressing extract) does not. On the other hand, *Jojoba* oil (cold pressing extract) contains 7-stigmastenol while *Jojoba* oil (hexane extract) does not. These differences may be due to the effects of temperature and chemical solvent involved.

Gas chromatography-mass spectrometry (GC/MS) for toccopherols

The chemical constituents of the analyte were identified by comparing the MS fragmentation patterns with those of chemical standards and those of Wiley registry of mass spectral database library (Wiley, UK, 1995) of the GC/MS system. The GC- chromatograms and MS-spectral data of tocopherols content of Jordanian *Jojoba* oil results are shown in Figures 5 and 6, respectively.

These spectral data (Mass spectrometry) for both oil samples were consistent with the structure of α-tocopherol which is the most dominant tocopherol presented in nature (Bhat et al., 2005). The results of the Jordanian *Jojoba* oil showed that the oil contains α-tocopherol similar to those of *Jojoba* oil from around the world.

Among these results it was noticed that; the common tocopherols major peak on GC- chromatogram was consisted of other minor peaks. This was clearer within *Jojoba* oil (hexane extract) than that of *Jojoba* oil (cold pressing extract). This may indicate the presence of other tocopherols such as β, δ and γ, which were reported in literature (El-Mallah, 2009).

The presence of α-tocopherol, which is the pharmacological active form (Bhat et al., 2005) in the Jordanian *Jojoba* oil may explain the biological activities mainly the antioxidant activity, because of the well known antioxidant activity of vitamin E (Kumar et al., 2012). In addition, it may also be related to the action of *Jojoba* oil in wound healing (Ranzatoa et al., 2011) and the great role in treatment of skin disorders (Skidmore-Roth, 2006; Price & Price, 2007). Therefore, the presence of vitamin E may also explain why *Jojoba* oil is preferred to be used as a carrier vehicle for oxidation sensitive substances like vitamin A (Gruenwald et al., 1998).

Conclusion

This study found that; the fatty acid 9, 12, 15-octadecatrienoic acid (18:3) (α-linolenic acid) was in higher % within the Jordanian oil than those of *Jojoba* oil from around the world, while the fatty acids hexadecanoic acid (16:0) (palmitic acid), octadec-9-enoic acid (18:1) (oleic acid), octadec-11-enoic acid (18:1) (vaccenic acid) and eicos-11-enoic acid (20:1) (gondoic acid) were in lower %.

It was observed that the avenasterol was in higher % within the Jordanian oil than those of *Jojoba* oil from around the world and the Jordanian *Jojoba* oil did not contain campesterol and campestanol. In addition, it was found that the major sterols and stanols constituents of the Jordanian *Jojoba* oil are: sitosterol, campesterol, stigmasterol, cholesterol, avenasterol and brassicasterol. Moreover, *Jojoba* oil (hexane extract) was found to contain stigmastadienol while it was not present in the *Jojoba* oil

(cold pressing extract). On the other hand, *Jojoba* oil (cold pressing extract) contained stigmastenol unlike the *Jojoba* oil (hexane extract).

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Tables and Figures

Table 1. Fatty acids content of Jordanian Jojoba oil.

Fatty acids	**Average (%)**	
	Jojoba Oil (hexane extract)	*Jojoba* Oil (cold pressing extract)
C14:0	0.02	0.01
C16:0	0.65	0.57
C16:1	0.09	0.09
C16:1	0.03	0.03
C17:0	0.06	0.06
C17:1	0.04	0.03
C18:0	0.03	0.02
C18:1	5.43	4.84
C18:1	0.37	0.37
C18:2	0.05	0.04
C18:3	0.15	0.12
C20:0	0.05	0.05
C20:1	37.71	37.61
Others	21.52	21.68
C22:0	0.12	0.11
Others	7.33	7.10
Others	0.45	0.46
Others	20.91	21.54
Others	0.63	0.65
C24:0	0.02	0.02
Others	0.77	0.75
Others	3.57	3.85
sum 16:1	0.13	0.12
sum 18:1	5.80	5.21

Table 2. Sterols / Stanols content of Jordanian Jojoba oil.

No.*	Sterols / Stanols	Average (%)	
		Jojoba Oil (hexane extract)	*Jojoba* Oil (cold pressing extract)
2	Cholesterol	3.3941	2.5332
3	Brassicasterol	1.2983	2.2796
4	24-methylene-cholesterol	1.1240	0.6530
5	Campesterol	16.4319	17.4638
6	Campestanol	0.0000	0.0000
7	Stigmasterol	6.3706	6.2640
8	7-campesterol	0.0000	0.0000
9	5-23-stigmastadienol	0.3575	0.0000
10	Clerosterol	1.3738	0.9554
11	Sitosterol	65.2015	65.1473
12	Sitostanol	0.4658	0.3791
13	5-avenasterol	2.4693	3.5998
14	5-24-stigmastadienol	0.2236	0.3402
15	7-stigmastenol	0.0000	0.2756
16	7-avenasterol	1.2895	0.1093
		100.00	100.00
	wt. of sample (g)	5.02	5.03
	Total Sterols	2256.61	3148.08

*****The numbering sequences represent the same sequence of the Retention Time on the GC- chromatograms in Figures 3 and 4. [No.1 on the GC- chromatograms represents the internal standard]

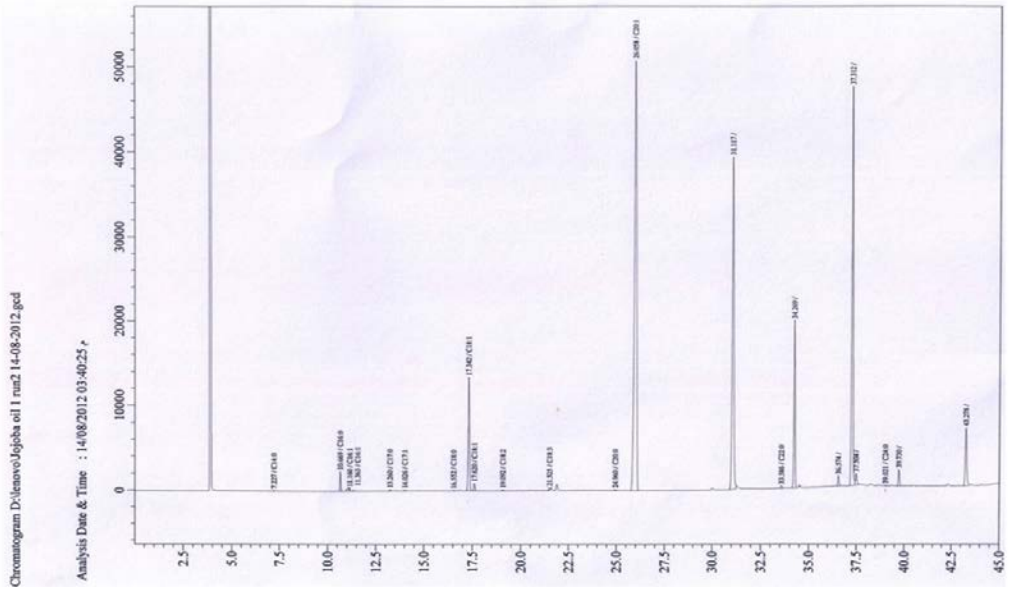

Figure 1. GC- chromatogram of fatty acids content of Jordanian Jojoba oil (hexane extract)

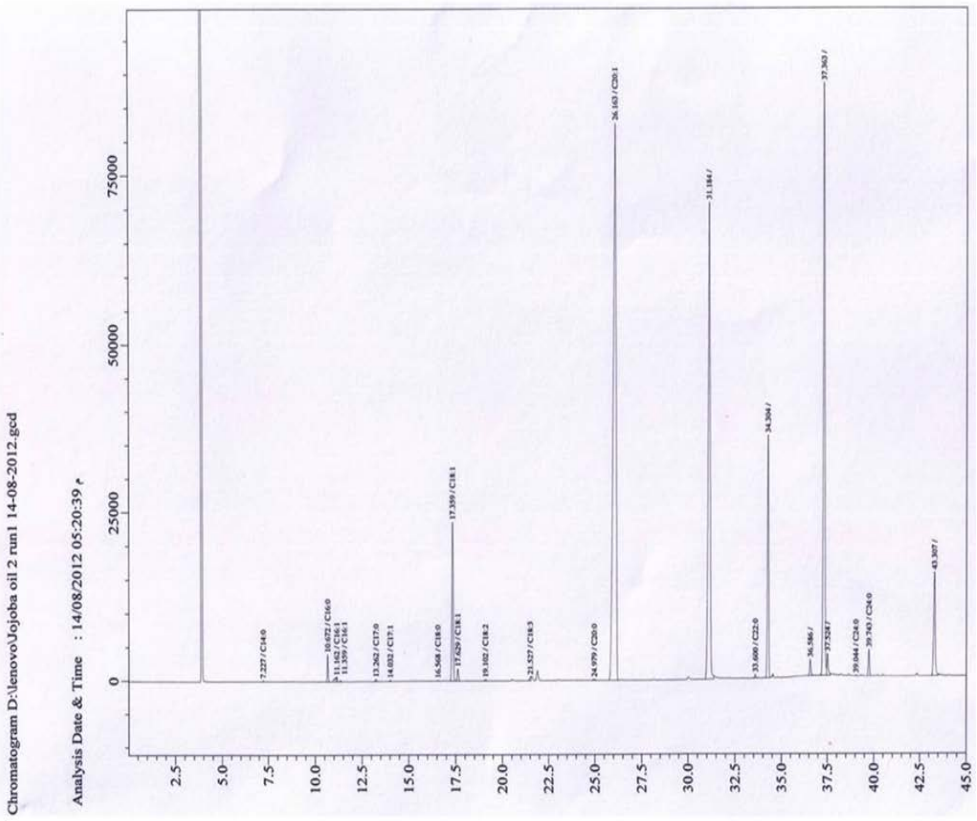

Figure 2. GC- chromatogram of fatty acids content of Jordanian Jojoba oil (cold pressing extract)

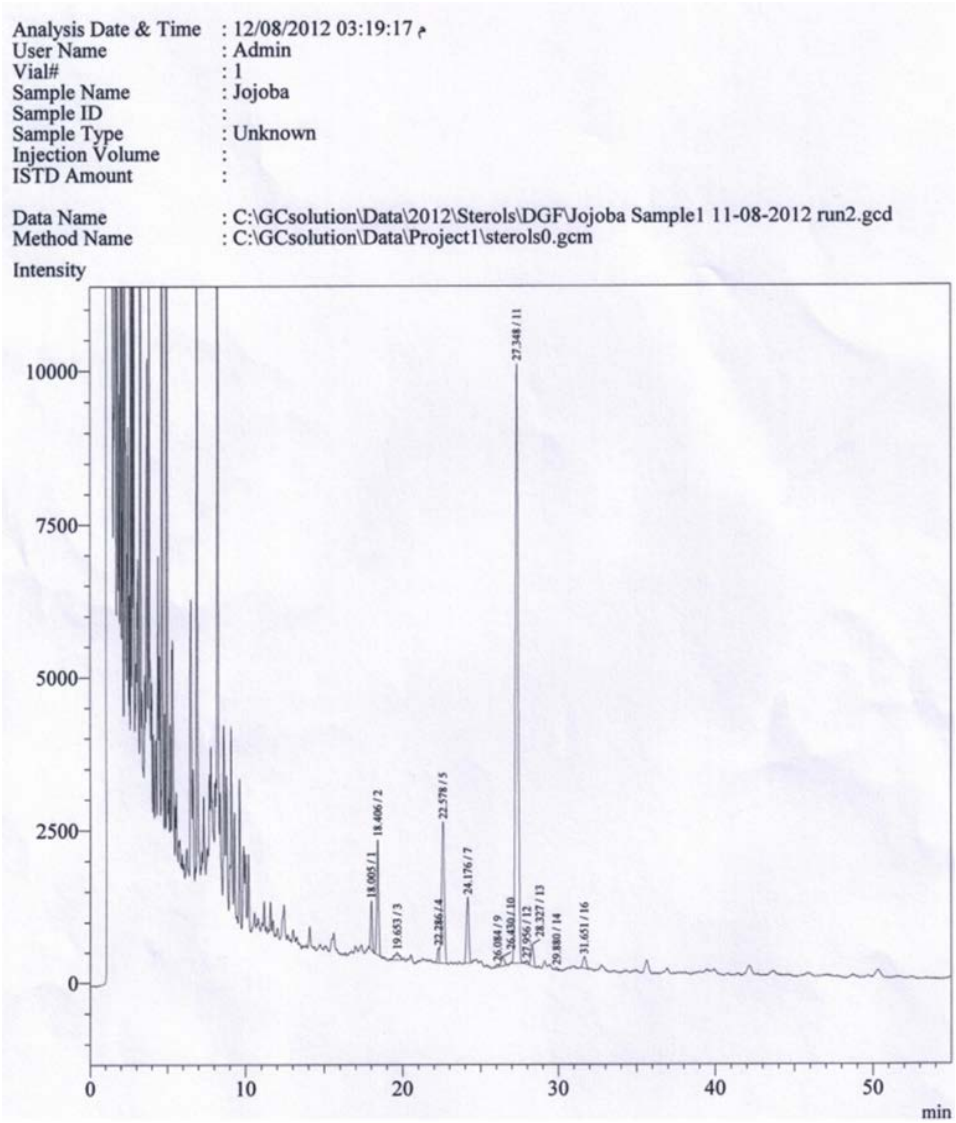

Figure 3. GC- chromatogram of Sterols / Stanols content of Jordanian Jojoba oil (hexane extract)

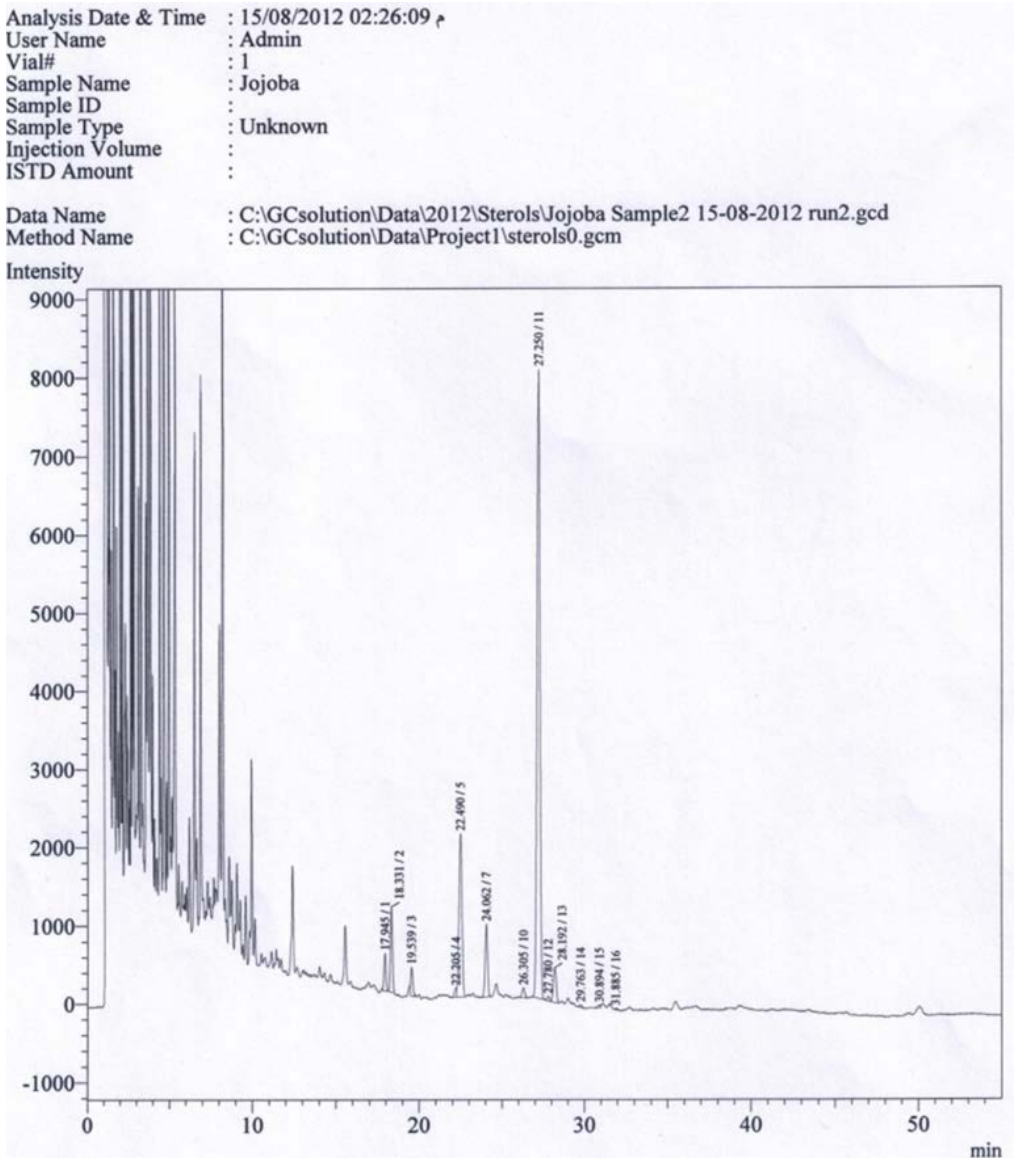

Figure 4. GC- chromatogram of Sterols / Stanols content of Jordanian Jojoba oil (cold pressing extract)

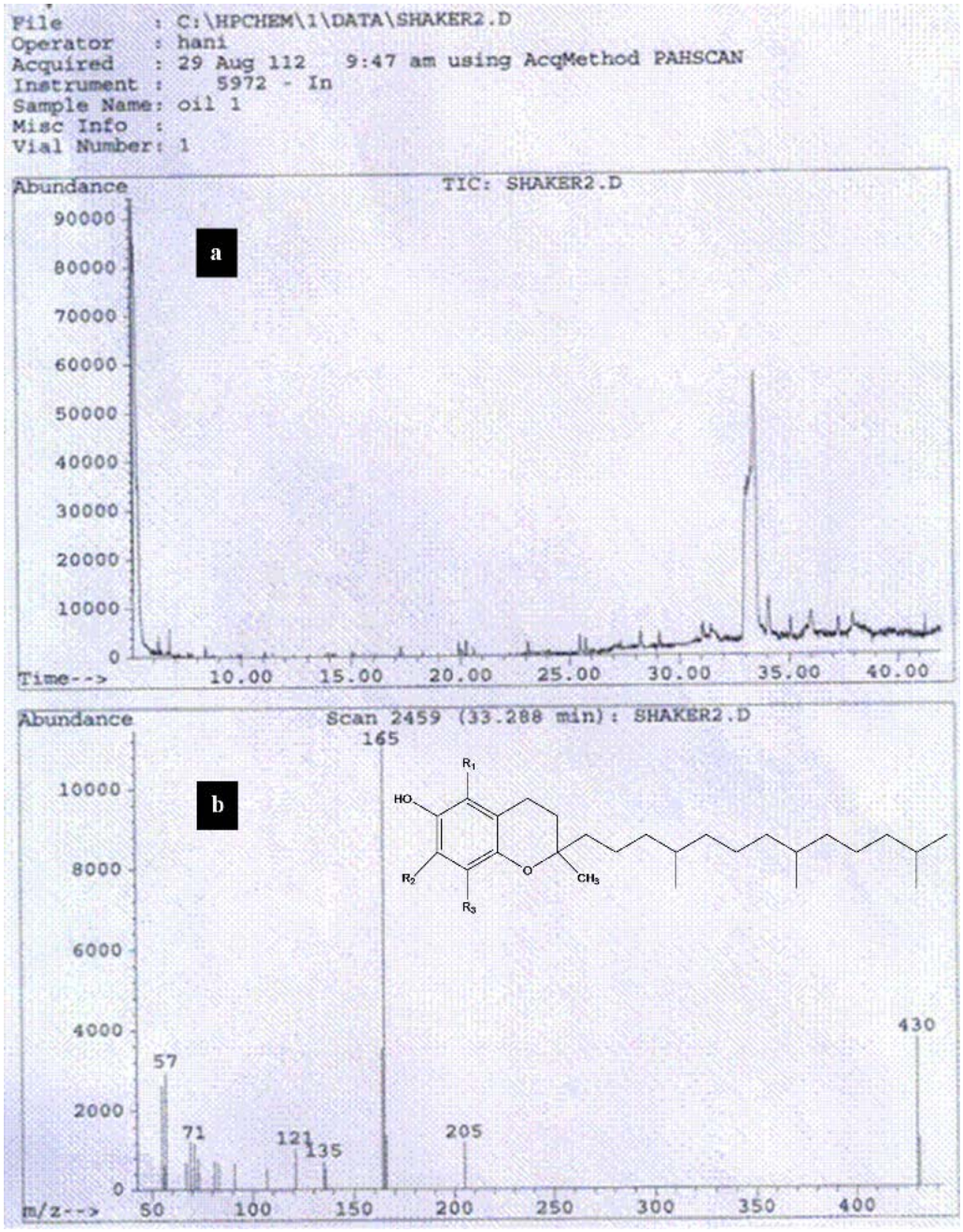

Figure 5. GC- chromatogram (a.) and MS-spectral data (b.) of toccopherols content of Jordanian Jojoba oil (hexane extract)

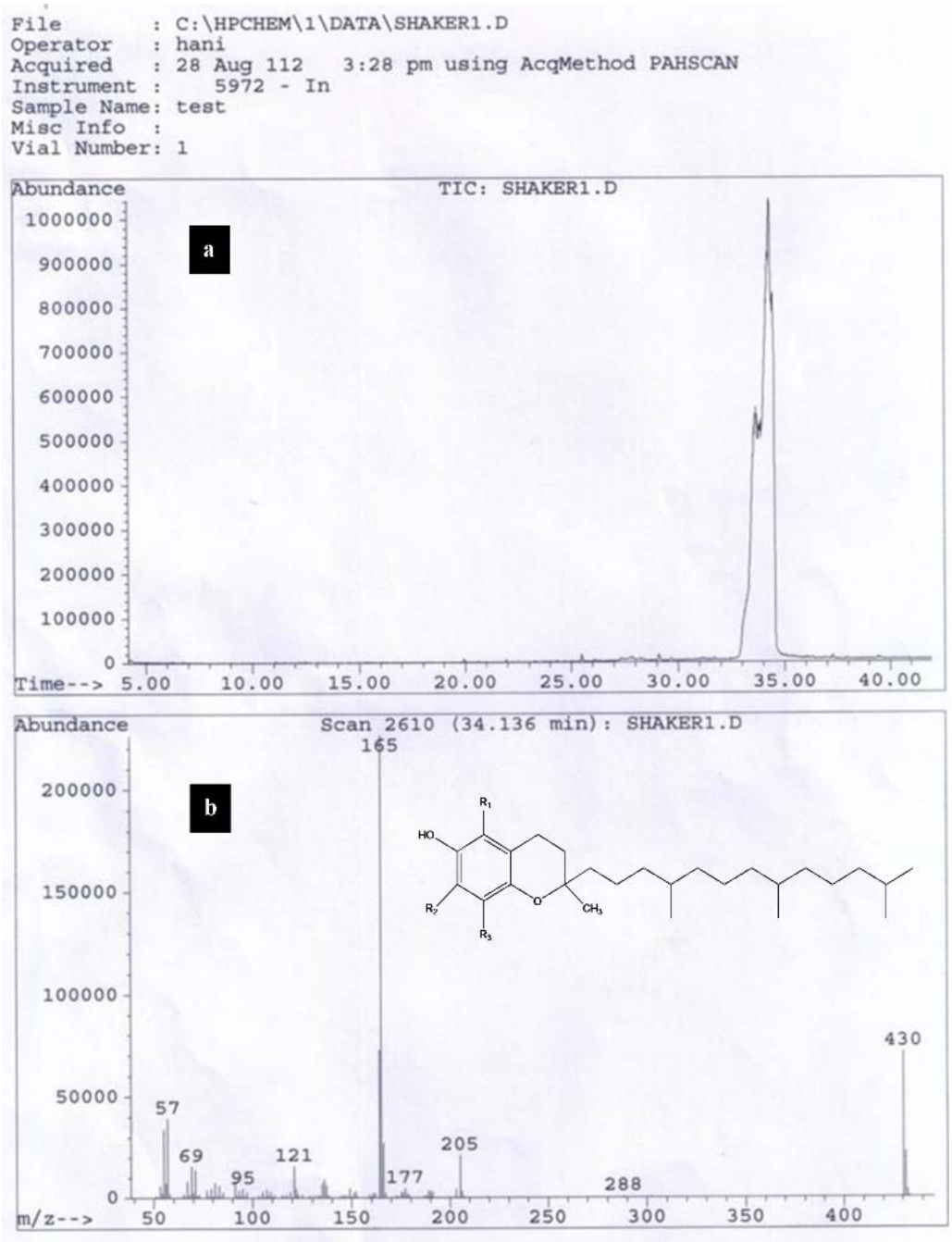

Figure 6. GC- chromatogram (a.) and MS-spectral data (b.) of toccopherols content of Jordanian Jojoba oil (cold pressing extract)

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