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ANALYSIS OF JOJOBA OIL EXTRACTED FROM *in vitro* CALLUS AND SEEDS BY GC/MS.

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ABSTRACT

The jojoba oil extracted from various types of callus for *Simmondsia chinensis* and the jojoba seeds; had been analyzed using the combined separation and analytical technique by Gas Chromatography-Mass Spectrometry (GC-MS). Oil of grinded seeds, yellowish compact callus (S1), greenish white semi-compact callus (S2) and green friable callus (S3); cultured on MS media containing different concentrations of growth regulators, and Knop's medium supplemented with TDZ (2mg/l) were used for analysis. It was found that the major fatty acids for S1 sample were gadoleic acid ($C_{20:1}$; 27.01%), oleic acid ($C_{18:1}$; 15.46%) and nervonic acid ($C_{24:1}$; 10.15%), while the most excited for S2 oil were C_{16} dicarboxylic acid (11.27%) and arachidic acid (C_{20}; 10.6%). In case of green friable callus, nervonic acid ($C_{24:1}$) was the most found fatty acid in its oil extract (50.65%). The oil extracted from seeds (control), contained gadoleic acid ($C_{20:1}$; 42.61%), oleic acid ($C_{18:1}$; 15.9%) and erucic acid ($C_{22:1}$; 9.73%). The composition of medium was effective indetermining different fatty acids which varied in their quality and quantity.

***Key words**: Jojoba oil, GC/MS , Fatty acids*

1. INTRODUCTION

Jojoba (*Simmondsia chinensis*) plant is native to Sonoran Desert of Northern Mexico and South Western USA, also known as goat nut, deer nut, pignut, wild hazel, quinine nut, coffeeberry, and gray box bush. It belongs to the Simmondsiaceae family. It is a dioecious plant, with hermaphrodite flowers being extremely rare. It is highly saline-tolerant woody shrub of arid, semiarid or marginal lands (Hogan, 1979).

Jojoba as a commercial crop is cultivated today for its wax. The oil represents 50% of the jojoba seed by weight, is colorless, odorless and does not break down quickly at high temperatures with unique physical and chemical properties. It was used mostly in the cosmetics and lubricants industries because of its superior lubricating ability and uniform viscosity over a wide range of temperatures (Low and Hackett 1981and Wang and Janick 1986). This increased interest in the agricultural production of jojoba but it is a crop that requires further research and development to improve quality and consistency of yields (Lee, 1988).

Jojoba oil is a mixture of wax esters, with 36 to 46 carbon atoms in length. Each molecule consists of a fatty acid and a fatty alcohol joined by an ester bond. The approximate percentages of fatty acids in jojoba oil are as follows: Eicosenoic (66-71%), Docosenoic (14-20%) and Oleic (10-13%).

Gayland *et al.*, (1977) described and compared two analytical procedures for determining composition of jojoba liquid wax esters. First, the more tedious, involves separation of wax ester homologs by high pressure liquid chromatography (HPLC), followed by determination of the acid and alcohol moieties from each homolog. The second allows rapid determination of wax ester composition by gas chromatographic (GC) separation of hydrogenated jojoba wax esters according to chain length, followed immediately by ancillary mass spectrometric identification of the acid and alcohol moieties. Double bonds in the alkyl chains in jojoba liquid waxes were almost exclusively (98%) *w-9,* when examined by gas chromatography/ mass spectrometry (GC/MS) and ozonolysis/GC/MS. Zouari *et al.* (2012) reported that the chemical characterization of *Allium roseum* var. *odoratissimum*, and identified new bioactive natural compounds in its flower essential oil. These compounds were extracted by hydro-distillation and were analyzed by GC and GC/MS,

using an apolar column. Kayani *et al.* (1990) reported that, HPLC and GC-MS were used to analyze jojoba wax ester and fatty acid composition. Fatty alcohols were determined by a different method that assumes a molar material balance.

The objective of this study was to use GC-MS analysis of fatty acids to monitor changes in the composition of jojoba oil extraction from callus grown in media supplemented with different compounds (salts and growth regulators) and were compared with each other in comparison with the control (oil extract from grinded seeds).

2. MATERIALS AND METHODS

The jojoba seeds were obtained from the Natural Oils Unit, National Research Center-Dokki, Egypt. The GC-MS analysis of samples was carried out at the Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.

2.1. Samples type uses

-Seeds (control)

-S1 = callus (yellowish compact callus) grown on MS medium (Murashige and Skoog**,** 1962) supplemented with 1.5 ml\l 2, 4-D + 2 ml/l NAA

-S2 = callus (greenish white semi-compact callus) grown on MS medium supplemented with 5.5 ml/l BA (benzylaminopurine) + 2.5 ml/l NAA (naphthaleneacetic acid)

-S3 = callus (green friable callus) grown on Knops' medium supplemented with 2ml/l TDZ (thidiazuron)

2.2. Extraction of jojoba oil

All samples (callus and grinded seeds) were collected and immediately stored at -20°C before subjecting to fatty acid extraction. The oil content of the samples was determined by complete extraction using Soxhlet extractor. Solvents used for oil extraction were commercial hexane: methanol 1:1 at 60°C for 12h.

2.3. Methylation of fatty acids

Thirty milligrams of oil were placed with 0.5ml reagent (45 g NaOH, 150 ml methanol, 150 ml H_2O) in a capped heat-resistant glass tube. Saponification of fatty acid methyl ester was conducted by heating the tube for 30 min then put in an oven at 100°C for 3h. The tubes were then cooled down quickly to room temperature in a water bath. Finally, the methyl esters of fatty acids were subjected to GC-MS analysis.

2.4. Method of analysis

2.4.1. GC-MS analysis Instrument operating conditions

GC-MS analyses were performed on an Agilent 6890 series II gas chromatograph ,an Agilent 5973 mass spectrometer with electron ionization, mode (EI) generated at 70 eV (ion source at 230 °C and transfer line at 280 °C). The GC was performed using a HP5-MS capillary column (30 m × 0.25 mm, film thickness of 0.25 μm). Operating conditions were as follows: carrier gas, helium with a flow rate of (1 ml min^{-1}). The oven temperature was programmed from 80 °C to 280 °C (at 8 °C min^{-1}), and maintained at 260 °C for 5 min.

All compounds were identified by comparison of both the mass spectra (Wiley and Nist library). The ratio of the compounds specific peaks were indicated by giving an Area% and retention time (Rt).

3. RESULTS AND DISCUSSION

3.1. Jojoba oil

The oil yield from jojoba plant seeds and their respective callus cultures grown on different media composition were compared. Jojoba oil was obtained from seeds (cotyledons) and from all samples (S1, S2 and S3) of callus cultures. Lee and Thomas (1985) obtained cotyledonary structures arising from asexual embryos in jojoba. These cotyledonary structures contained wax bodies and liquid wax identical to that of seeds. Under all experimental conditions, jojoba tissues contained high amount of lipids. Gaber (1993) also obtained i*n vitro* jojoba liquid wax from somatic embryos. GC/MS method could be applied only to fully saturated wax esters (Aasen *et al.* 1971).

Data in Tables (1, 2, 3, and 4) and Figures (1, 2, 3 and 4), showed that C_{17} (margaric acid) and C_{18} (stearic acid) fatty acids were the main saturated fatty acids in all oil extracted in this investigation. It was found that the proportion of both fatty acids differed between the samples. However, the highest percent of C_{17} was obtained in S3 (1.33%), followed by 0.58% obtained from S2, while the lowest proportion was obtained from S1 and the control (0.15 and 0.34%, respectively). Likewise, the C_{18} resides in all samples in descending order, S2, control, S3 and S1 giving 2.35, 2.09, 0.59 and 0.17%, respectively.

Regarding to the control (oil extracted from seeds) as compared to that extracted from callus (S1, S2 and S3) under investigation, it was found that C_{12} ratio for control was 2.78% , increased in S3 to 9.48%, and decreased in S1 to 1.18%, while it was absent in S2.

The C_{14} fatty acid was one of the main fatty

Table (1): Fatty acid constituents quantified in jojoba oil extracted from grinded seeds (control) analysis by GC-MS.

Peak No.	Rt (min)	Components			Area %
		Carbon atoms	Systematic name	Trivial name	
1	4.390	C_{12}	Dodecanoic acid	Lauric acid	2.78
2	5.683	C_{13}	Tridecanoic acid	Tridecylic acid	1.24
3	6.090	-	u.n	u.n	1.65
4	7.080	C_{14}	Tetradecanoic acid	Myristic acid	0.96
5	9.929	C_{16}	Hexadecanoic acid	Palmitic acid	1.24
6	11.308	C_{17}	Heptadecanoic acid	Margaric acid	0.34
7	11.463	-	u.n	u.n	0.78
8	12.636	C_{18}	Octadecanoic acid	Stearic acid	2.09
9	17.179	$C_{18:1}$	Octadecenoic acid	Oleic acid	15.90
10	19.937	C_{20}	Eicosanoic acid	Arachidic acid	7.29
11	20.560	$C_{20:1}$	Eicosenoic acid	Gadoleic acid	42.61
12	21.556	-	u.n	u.n	1.94
13	23.496	$C_{20:2}$	Eicosadienoic acid	-	4.97
14	24.097	$C_{22:1}$	Docosenoic acid	Erucic acid	9.73
15	24.932	-	u.n	u.n	2.38
16	27.610	-	u.n	u.n	1.78
17	31.529	-	u.n	u.n	2.32

Rt = retention time, Non identified peaks= 10.85%, Identified peaks= 89.15%, u.n= unknown.

Table (2): Fatty acid constituents quantified in jojoba oil extracted from *in vitro* yellowish compact callus culture (S1) analysis by GC-MS.

Peak No.	Rt (min)	Components			Area %
		Carbon atoms	Systematic name	Trivial name	
1	4.396	C_{12}	Dodecanoic acid	Lauric acid	1.18
2	5.683	-	u.n	u.n	1.00
3	6.976	$C_{14:1}$	Tetradecenoic acid	Myristoleic acid	0.43
4	7.079	-	u.n	u.n	1.25
5	8.922	-	u.n	u.n	0.43
6	9.837	$C_{16:1}$	Hexadecenoic acid	Palmitoleic acid	9.90
7	10.501	-	u.n	u.n	0.48
8	11.308	C_{17}	Heptadecanoic acid	Margaric acid	0.15
9	11.371	C_{18}	Octadecanoic acid	Stearic acid	0.17
10	11.777	-	u.n	u.n	1.46
11	12.555	$C_{18:1}$	Octadecenoic acid	Oleic acid	15.46
12	14.461	-	u.n	u.n	3.18
13	15.388	$C_{20:1}$	Eicosenoic acid	Gadoleic acid	27.01
14	17.196	$C_{18:1}$trans	Octadecenoic acid	Oleic acid "trans"	0.79
15	17.585	-	u.n	u.n	0.93
16	22.088	$C_{22:1}$	Docosenoic acid	Erucic acid	6.65
17	14.932	-	u.n	u.n	4.41
18	25.596	$C_{23:1}$	Tricosenoic acid	Tricosene	4.49
19	28.417	$C_{24:1}$	Tetracosenoic acid	Nervonic acid	10.15
20	29.160	-	u.n	u.n	5.48
21	29.893	-	u.n	u.n	3.29
22	31.026	-	u.n	u.n	1.71

Rt = retention time, Non identified peaks= 23.62%, Identified peaks= 76.38%, u.n= unknown.

Table (3): Fatty acid constituents quantified in jojoba oil extracted from *in vitro* greenish white semi-compact callus culture (S2) analysis by GC-MS.

Peak No.	Rt (min)	Components			Area %
		Carbon atoms	Systematic name	Trivial name	
1	7.886	-	u.n	u.n	0.07
2	9.625	-	u.n	u.n	1.54
3	14.357	C_{16}	Hexadecanoic acid	Palmitic acid	0.50
4	15.187	-	u.n	u.n	0.17
5	15.768	C_{17}	Heptadecanoic acid	Margaric acid	0.58
6	15.924	C_{16}	Octanoic acid, 2-ethylhexyl ester	C16 ester	3.09
7	16.603	-	u.n	u.n	0.99
8	17.112	C_{18}	Octadecanoic acid	Stearic acid	2.35
9	17.361	$C_{18:1}$	Octadecenoic acid	Oleic acid	0.46
10	17.646	-	u.n	u.n	1.43
11	18.897	$C_{18:2}$	9,12-octadecadienoic acid	Linoleic acid	3.03
12	19.410	-	u.n	u.n	4.61
13	19.608	-	u.n	u.n	4.43
14	20.780	C_{19}	Nonadecane	Nonadecanoic	1.15
15	21.221	C_{20}	Eicosanoic acid	acid	10.60
16	21.574	-	u.n	Arachidic acid	1.79
17	22.124	-	u.n	u.n	2.76
18	23.048	-	u.n	u.n	4.58
19	23.515	-	u.n	u.n	3.14
20	24.781	-	u.n	u.n	8.42
21	25.087	-	u.n	u.n	7.59
22	25.704	C_{16}	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	u.n C16 dicarboxylic acid	11.27
23	26.161	-	u.n		3.01
24	26.565	-	u.n	u.n	8.04
25	27.557	-	u.n	u.n	7.73
26	28.963	-	u.n	u.n	3.96
27	29.590	-	u.n	u.n	1.63
28	29.917	-	u.n	u.n	1.08

Rt = retention time, Non identified peaks= 67.97%, Identified peaks= 33.03%, u.n= unknown.

acids found in jojoba oil, having 0.96% ratio for control, while in S3 it was 2.01% and was absent in S2. S1 contained $C_{14:1}$(myristoleic acid), at the ratio of 0.43%.

The results and chromatograms obtained from GC-MS analysis revealed that palmitic acid (C_{16}) was the dominant saturated fatty acid in all investigated oil standard taken from seeds and callus. It was 1.24% for control as compared with S3 (3.41%), while it was 0.5% for S2 and, for it had two different types of C_{16}, *i.e.* C_{16} ester (3.09%) and C_{16} dicarboxylic acid at high ratio (11.27%) , while the palmitoleic acid ($C_{16:1}$) was found in S1at the ratio of 9.9%.

The results showed also that the gadoleic acid (eicosenoic acid; C20:1) represented the highest percentage of total fatty acids (42.61%), followed by oleic acid ($C_{18:1}$) of 15.9% in oil of control, as compared with S1 (27.01 and 15.46% respectively), while $C_{20:1}$ was not found in S2 but $C_{18:1}$ was found (0.46%). In S3, did not contain any of them.

As for eicosenoic acid, this study agreed with that by Thomas, (1971) where the eicosenoic acid was the major constituent that contributed to the non random wax formation, where as the percentage of the major component, eicosenoic acid in seed samples, remained unchanged at 35% of the total jojoba oil.

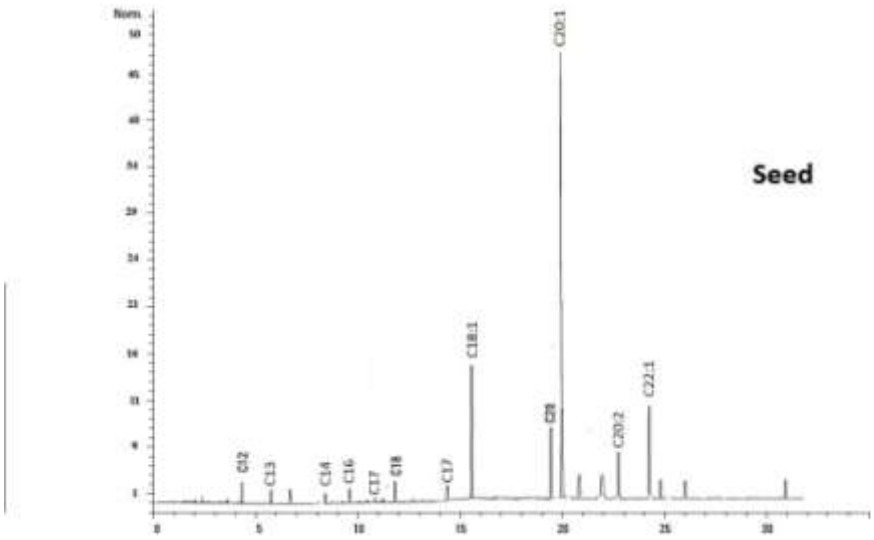
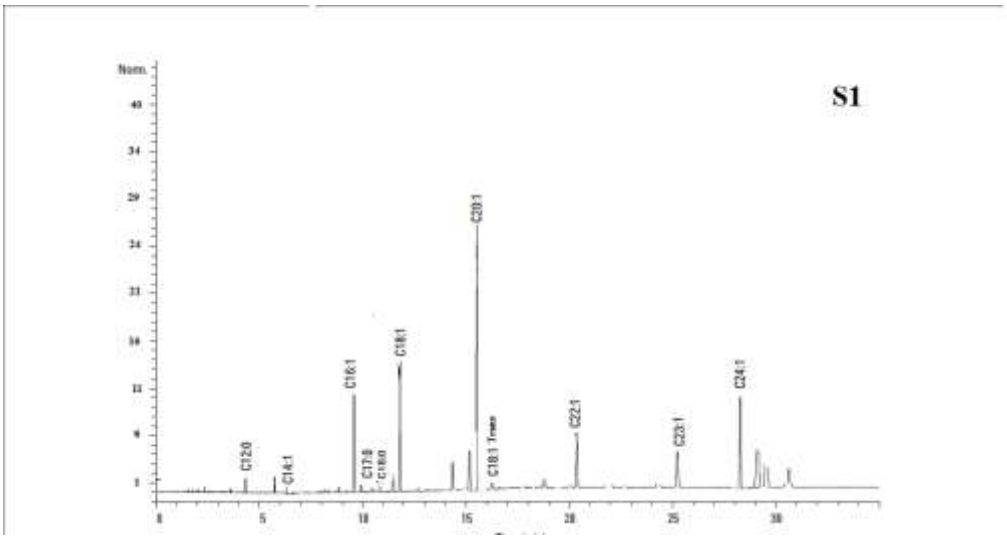


Fig (1): GC-MS chromatogram of fatty acid constituent quantified in jojoba oil extracted from seeds (control).



Fig(2): GC-MS chromatogram of fatty acid constituent quantified in jojoba oil extracted from S1 sample

Concerning to erucic acid ($C_{22:1}$), the results showed that both S2 and S3 did not have this kind, but the control and S1 contained a high proportion of this fatty acid (9.73 and 6.65%, respectively).
The obtained data in Tables 1, 2, 3, and 4 and Figures (1, 2, 3 and 4) gave an explaination where C_{13} and $C_{20:2}$ were absent in all treatments of callus cultures, they were existed in the control.

However, the total components identified for control, S1 and S3 (89.15, 76.38 and 74.72%, respectively) were higher than that of S2 (33.03%).

In this study, jojoba oil was obtained from seeds (cotyledons) and callus cultures on various media types and hormones (treatments). Accordingly, the oil extracted from callus contained types of fatty acids varied in quantity and quality from the oil extracted from seeds. This variation may be due to the effect of components of the media and growth regulators used. There are several reports on the substantial influence of plant growth regulators such as auxin (2, 4-D) and cytokinin (BA) on plant lipid composition and lipid metabolism in terms of fatty acids induction and alteration of their composition (Wennuan *et al*., 1995; Banibrata and Gadgil 1984; Marta, 1993 and Matsuda *et al.*,2001). In another study relating the structure-activity relationship (Roja *et al*., 1987), biosynthesis of secondary products was best reported in callus and suspension cultures and altered metabolism had been also observed during organ differentiation.

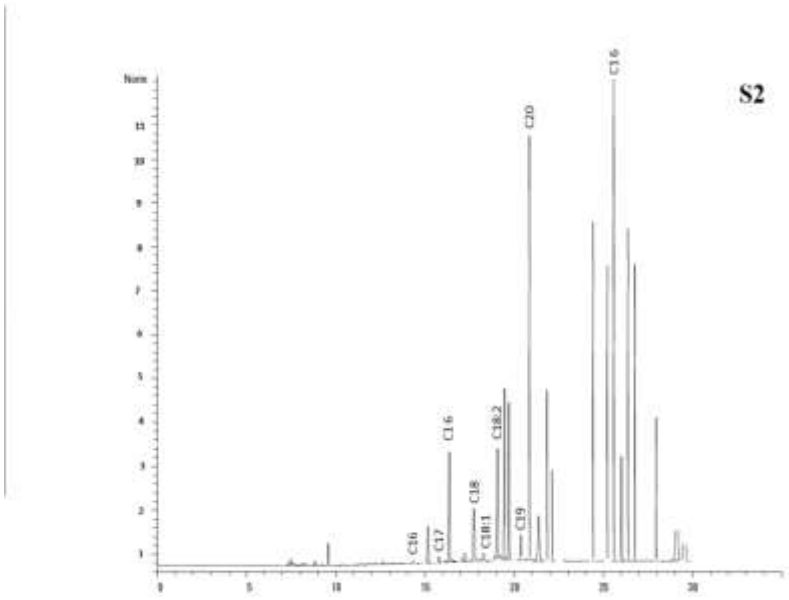
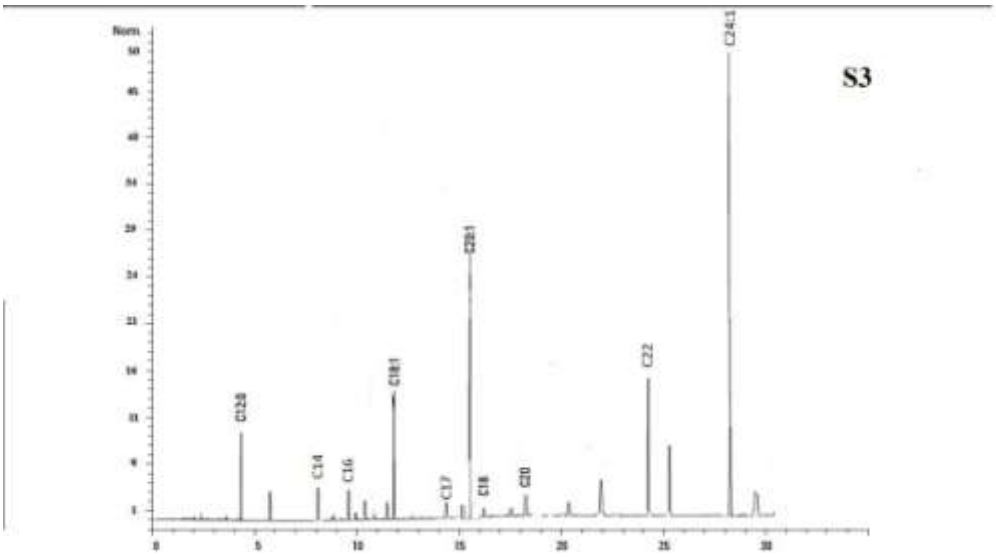


Fig (3): GC-MS chromatogram of fatty acid constituents quantified in jojoba oil extracted from S2 sample.



Fig(4): GC-MS chromatogram of fatty acid constituents quantified in jojoba oil extracted from S3 sample.

Consequently, yield of secondary products may either decrease or even increase further. Reported oil yields from various cultures also vary between different samples and it is not known whether this reflects environmental or genetic differences between cultures (Allan *et al*., 1998).

The recap, in view of increasing commercial demand of jojoba oil, the results from the present investigation foresee further refinement and standardization of plant cell and tissue culture protocols to provide an alternative means for oil recovery.

Table (4): Fatty acids constituents quantified in jojoba oil extracted from *in vitro* green friable callus culture (S3) analysis by GC-MS.

Peak No.	Rt (min)	Components			Area %
		Carbon atoms	Systematic name	Trivial name	
1	4.379	C_{12}	Dodecanoic acid	Lauric acid	9.48
2	6.067	-	u.n	u.n	2.55
3	7.074	C_{14}	Tetradecanoic acid	Myristic acid	2.01
4	8.510	-	u.n	u.n	0.81
5	9.929	C_{16}	Hexadecanoic acid	Palmitic acid	3.41
6	11.462	-	u.n	u.n	2.31
7	14.592	C_{17}	Heptadecanoic acid	Margaric acid	1.33
8	15.491	-	u.n	u.n	1.61
9	17.654	C_{18}	Octadecanoic acid	Stearic acid	0.59
10	18.729	-	u.n	u.n	0.49
11	19.988	C_{20}	Eicosanoic acid	Arachidic acid	1.19
12	20.349	-	u.n	u.n	2.01
13	23.227	-	u.n	u.n	1.07
14	24.720	-	u.n	u.n	4.50
15	24.938	C_{22}	Docosanoic acid	Behnic acid	14.02
16	28.394	$C_{24:1}$	Tetracosenoic acid	Nervonic acid	50.65
17	29.904	-	u.n	u.n	1.97

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تحليل زيت الجوجوبا المستخلص من الكالوس الناتج من مزارع الانسجة النباتية، والزيت المستخرج من البذور بواسطة التحليل الكروماتوجرافي الغازي بمقياس الكتلة GC /MS .

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ملخص

تم تحليل زيت الجوجوبا المستخلص من الكالوس الناتج من مزارع الانسجة النباتية باستخدام تقنية المدمجة بين التحليل و الفصل الكروماتوجراقي الغازي GC /MS. حيث تم تحليل الزيت المستخرج من مجروش البذور (للمقارنة) وكالوس متكتل ومتجمع مصفر (S1) وكالوس شبه متكتل ذو لون ابيض مخضر (S2) وكالوس مفتت اخضر (S3) هذه العينات المزروعة على بيئة النباتية موراشيج وسكوج MS والتي تحتوي على تركيزات مختلفة من الهرمونات النباتية والتي زرعت على بيئة Knop's النباتية المحتوية على 2مجم/لتر من هرمون الTDZ وذلك للحث على تكوين الكالوس بكمية وفيرة.

وجد من نتائج البحث، ان المكون الرئيسي للاحماض الدهنية للعينة S1 هو الحمض جاديوليك ($C_{20:1}$) بنسبة 27.01٪ يليه حمض الاوليك ($C_{18:1}$) بنسبة 15.46 ٪ ثم حمض النيرفونيك ($C_{24:1}$) بنسبة 10.15 ٪ ، بينما كانت اعلى نسبة من الاحماض الدهنية للزيت الناتج من الكالوس S2 هو الحمض الاتي C_{16} dicarboxylic acid بنسبة 11.27 ٪ ويليه (C_{20}) حمض الار اشيدك (10.6 ٪). بالنسبة للزيت المستخرج من الكالوس المفتت الاخضر (S3) كان حمض النيرفونيك ($C_{24:1}$) هو الغالب في نوعية الاحماض الدهنية المكونة للزيت (50.65٪)، اما الزيت المستخرج من البذور المقارن فوجد انه يحتوي على حمض جلاديوليك ($C_{20:1}$) بنسبة 42.61٪ يليه حمض الاوليك ($C_{18:1}$) بنسبة 15.9٪ ثم يليه حمض الايروسيك بنسبة9.73٪ ($C_{22:1}$)، يعتبر تركيب البيئة النباتية ومحتواها من الهرمونات النباتية عاملا مؤثرا على نوع وكمية الاحماض الدهنية المكونة للزيت.

المجلة العلمية لكلية الزراعة – جامعة القاهرة – المجلد (64) العدد الأول (يناير2013):59-66.