Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(4): 721-725
Received: 12-05-2017
Accepted: 13-06-2017

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GC/MS Analyses of Avocado and Sesame Fixed Oils

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Abstract
The present study showed GC/MS analyses of the saponifiable matters of Avocado fruit and Sesame seed fixed oils, which obtained from Souss Argane Company. Avocado (*Persa americana*) fruit fixed oil contained saturated, monounsaturated and polyunsaturated fatty acids with percentages 8.87%, 44.61% and 6.40%, respectively. The major fatty acids were oleic acid methyl ester (43.61%). While, Sesame (*Sesamum indicum*) seed fixed oil contained saturated, monounsaturated and polyunsaturated fatty acids with percentages 23.05%, 33.65% and 39.79%, respectively. The major fatty acids were linoleic acid methyl ester (37.40%) followed by oleic acid methyl ester (29.09%).

Keywords: Avocado, Sesame, Fixed oil, Saponification, GC/MS.

1. Introduction
Plant derived natural products have considerable significant attention in recent years due to their various pharmacological properties including anti-inflammatory [1], anti-hyperglycemic [1], gastroprotective [1] and hepatoprotective [2] activities. Avocado and Sesame are considered as very important plants because of their high economic and therapeutic values. The Avocado (*Persea americana* Mill.), known in the past as alligator pear, midshipman's butter, vegetable butter, or sometimes as butter pear and called by Spanish-speaking people (aguacate, cura, cupandra, or palta); in Portuguese, (abacate) and in French, (avocatier) [3]. It is the only important edible fruit of the laurel family, Lauraceae [3]. Oil expressed from the flesh was rich in vitamins A, B, G and E. It had a digestibility coefficient of 93.8% [3]. It had many pharmacological activities *viz*., analgesic [4], anti-inflammatory, Hypotensive [5], antiviral [6], wound healing [7],…..etc. The profile of bioactive compounds in Avocado pulp oil influenced by the drying processes and extraction methods [8]. The oil yield was maximal with lyophilization and soxhlet extraction, but lyophilization and cold pressing produced oils, which had greater concentrations of antioxidants and other bioactive compounds [8]. GC/MS analyses of Avocado oil samples showed α-tocopherol, squalene, cycloartenol acetate, β-sitosterol, campesterol and stigmasterol [8].
The genus Sesamum has 37 species, of which *Sesamum indicum* L. is the main cultivated species. Their distribution is mainly in three regions *viz*., Africa, India and the Far East [9]. The oil has been ranked as 9th among the top thirteen oil seed crops, which make up 90% of the world population of edible oils [10]. The result of the qualitative phytochemical analysis revealed that the constituents of white *S. indicum* (Pedaliaceae) seed oil contained various secondary metabolites as alkaloids, saponins, flavonoids, tannins, steroids, terpenoids, anthraquinone and phenols. While, cardiac glycosides and phlobatannins were absent [11]. On the other hand, HPLC analysis of Sesame oil showed a total lignan content of 9.32% (6.54% sesamin and 2.78% sesamolin) [12]. Sesamin, a major lignan in Sesame seeds, induced neuronal differentiation in PC12 cells through activation of ERK1/2 signaling pathway [13]. Furthermore, GC/MS analyses of hexane extract of two varieties of Sesame seed oil revealed many fatty acids [14]. The first variety (White Sesame seed oil) showed 11-octadecenoic, oleic, decanoic, stearic, arachidic, nonenoic, ricinoleic, tridecanoic, hexadecanoic and linolelaidic fatty acids [14]. While, the second one (Brown Sesame seed oil) exhibited the presence of 11-octadecenoic, oleic, decanoic, stearic, arachidic, 7-nonenoic, ricinoleic, palmitic, erucic, in addition to 10-undecenoic fatty acids [14].
Due to the high economic and therapeutic values of Avocado fruit and Sesame seed fixed oils. This provoked us to standardize both of these fixed oils, which obtained from Souss Argane Company, Morocco, for our future skin healing study.

2. Materials, Equipments and Methods

2.1 Materials

2.1.1 Reagents

The reagents used were: sulphuric acid (10%), concentrated. sulphuric acid, dichloromethane, distilled water, litmus paper, absolute methanol and anhydrous calcium chloride.

2.1.2 Fixed oils

2.1.2.1. Avocado (*Persa americana*) fruit fixed oil (AFFO)

Color: Yellow viscous oil, Boiling point: 270 °C, Solubility: Organic solvents (chloroform and benzene), Refractive index: 1.46, Storage temp: 2-8 °C and Source: Souss Argane company. Morocco.

2.1.2.2 Sesame (*Sesamum indicum*) seed fixed oil (SSFO)

Color: Faint yellow oil, Boiling point: 210 °C, Solubility: Organic solvents (chloroform and benzene), Refractive index: 1.47, Storage temp: 2-8 °C and Source: Souss Argane company. Morocco.

2.2 Equipments

The column used was Rtx-Ms 30 meter in length, 0.25 mm in diameter and 0.25 µm film thickness. The detector FID (Flame Ionization Detector) connected to the Mass Spectrometer. Rotary evaporator (BUCHI R-144, Switzerland), Water distiller (BHANU Basic, India) and Water bath (BUCHI B466, Switzerland).

2.3 Methods

2.3.1 Preparation of the fatty acids

The oil was acidified with 20 ml sulphuric acid (10%). The liberated fatty acids were extracted with successive portions of dichloromethane. The combined dichloromethane extracts were washed with distilled water till washings were neutral to litmus paper. The dichloromethane was distilled off and the residue of the total fatty acids was oily brown matter [15, 16].

2.3.2 Preparation of methyl esters

The residue of the total fatty acids was refluxed with 1.5 ml concentrated sulphuric acid with 50 ml absolute methanol for two hrs on a boiling water bath. Then, the major part of methanol was distilled off. The liquid left was diluted with twice its volume of distilled water. This liquid was extracted with several portions of dichloromethane until exhaustion. The combined dichloromethane extracts were washed with distilled water till washings were neutral to litmus paper. The dichloromethane was distilled off. The and the residue of fatty acid methyl esters was dried over anhydrous calcium chloride [15, 16].

2.3.3 GC/MS analysis

2.3.3.1 GLC of saponifiable matter

This carried out for saponifiable matters of both AFFO and SSFO. Colum temperature was programmed to start from 40 °C and gradually increased to 240 °C through time of (50 min). The temperature of detector was 270 °C and the sample was injected as 1µl at 240 °C. Helium was used as the carrier gas at a flow rate of 1 ml/min. The total run time was 48 min.

2.3.3.2 Mass analysis

Total ion chromatograms and mass spectra were recorded in the electron impact ionization mode at 70 eV, using ACQ Mode of scan from 35 to 500 m/z in 0.3 s.

3. Results

The identification of fatty acids methyl esters was carried out by direct comparison of retention times and pattern of fragmentation of each of the separated compounds with those of reference fatty acids methyl esters analyzed under the same conditions [17]. The quantitative estimation was based on peak area integration.

3.1 Avocado fruit fixed oil (AFFO)

The results of GC/MS analysis of AFFO saponifiable matters are displayed in Figure 1 and presented in Table 1. They revealed the presence of 20 fatty acids from which 6 compounds (59.88%) were identified, whereas 14 fatty acids (40.12%) couldn't be identified. The identified fatty acids were classified as saturated, monounsaturated and polyunsaturated fatty acids in percentages 8.87%, 44.61 and 6.40%, respectively. Oleic acid methyl ester was the major identified monounsaturated fatty acid from the saponifiable matter (43.61%). Its structure was shown in Figure 3.

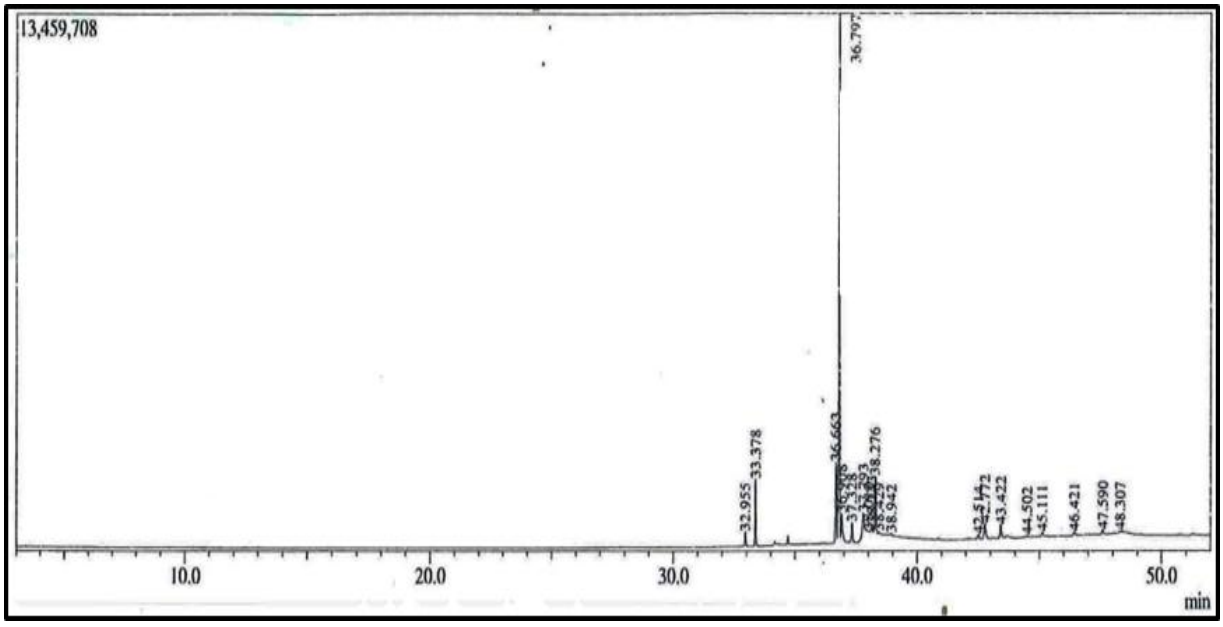

Fig 1: GLC chromatogram of AFFO.

Table 1: Identification of the components of the saponifiable matter of AFFO.

Peak No.	Chemical Name	Molecular Formula	Molecular Weight	Retention Time (min)	RRT	Relative Area (%)
1	9*Z*-Hexadecenoic methyl ester (Palmitoleic acid methyl ester)	$C_{17}H_{32}O_2$	268	32.955	0.896	1.02
2	Hexadecanoic acid methyl ester (Palmitic acid methyl ester)	$C_{17}H_{34}O_2$	270	33.375	0.907	5.00
3	9*Z*,12*Z*-Octadecanoic acid methyl ester (Linoleic acid methyl ester)	$C_{19}H_{34}O_2$	294	36.665	0.996	6.40
4	9*Z*-Octadecenoic acid methyl ester (Oleic acid methyl ester= elaidic acid methyl ester)	$C_{19}H_{36}O_2$	296	36.795	1.000	43.59
5	Unknown	-----	-----	36.910	1.003	3.57
6	*N*-Octadecanoic acid methyl ester (stearic acid methyl ester)	$C_{19}H_{38}O_2$	298	37.330	1.014	2.03
7	Unidentified	-----	-----	37.795	1.027	8.68
8	Unidentified	-----	-----	38.010	1.033	1.21
9	Unidentified	-----	-----	38.145	1.037	3.05
10	Unidentified	-----	-----	38.275	1.040	8.01
11	Unidentified	-----	-----	38.430	1.044	3.17
12	Unidentified	-----	-----	38.940	1.058	1.77
13	Unidentified	-----	-----	42.515	1.155	1.08
14	Unidentified	-----	-----	42.770	1.162	2.77
15	Unidentified	-----	-----	43.420	1.180	2.10
16	Unidentified	-----	-----	44.500	1.203	1.07
17	Unidentified	-----	-----	45.110	1.226	1.03
18	Unidentified	-----	-----	46.420	1.262	1.36
19	Docosanoic acid methyl ester (Behenic acid methyl ester)	$C_{23}H_{46}O_2$	354	47.590	1.293	1.84
20	Unidentified	-----	-----	48.305	1.313	1.25
Percentage of unidentified compounds 40.12%						
Percentage of saturated fatty acids methyl ester 8.87%						
Percentage of monounsaturated fatty acids methyl ester 44.61%						
Percentage of polyunsaturated fatty acids methyl ester 6.40%						

RRT=Relative Retention Time

3.2 Sesame seed fixed oil (SSFO)

The results of GC/MS analysis of the saponifiable matter of SSFO are shown in Figure 2 and listed in Table 2. They revealed the presence of 15 fatty acids.

The identified fatty acids were classified as saturated, monounsaturated and polyunsaturated fatty acids in 23.05%, 33.65% and 39.79%, respectively. Linoleic acid methyl ester (37.40%) was the major identified fatty acid followed by oleic acid methyl ester (29.09%) and palmitic acid methyl ester (11.50%). Their structures were demonstrated in Figure 3.

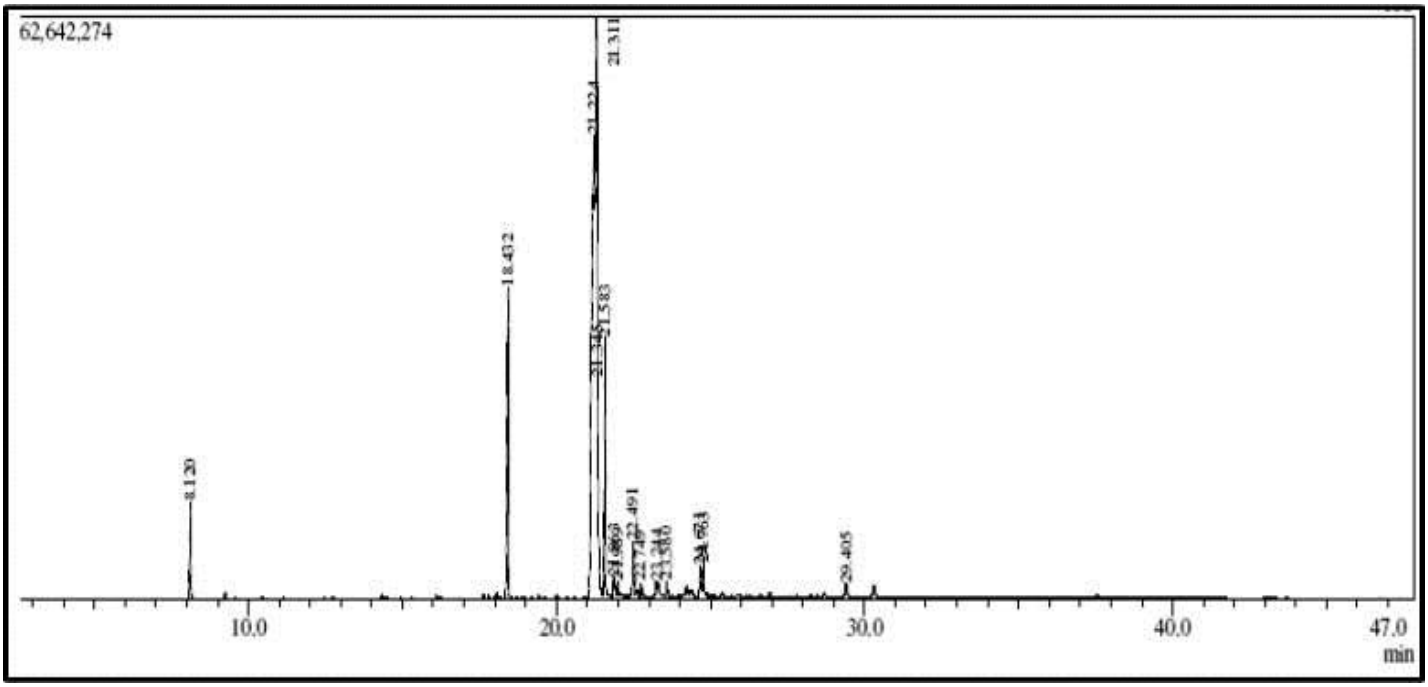

Fig 2: GLC chromatogram of SSFO.

Table 2: Identification of the components of the saponifiable matter of SSFO.

Peak No.	Chemical Name	Molecular Formula	Molecular Weight	Retention Time (min)	RRT	Relative Area (%)
1	Unidentified compound	-----	-----	8.120	0.383	2.67
2	Hexadecanoic acid methyl ester (Palmitic acid methyl ester)	$C_{17}H_{34}O_2$	270	18.430	0.869	11.50
3	9*Z*,12*Z*-Octadecadienoic acid methyl ester (Linoleic acid methyl ester)	$C_{19}H_{34}O_2$	294	21.225	1.000	37.40
4	9*Z*-Octadecenoic acid methyl ester (Oleic acid methyl ester)	$C_{19}H_{36}O_2$	296	21.310	1.004	29.09
5	9*E*-Octadecenoic acid methyl ester	$C_{19}H_{36}O_2$	296	21.345	1.006	4.56
6	Octadecanoic acid methyl ester (Stearic acid methyl ester)	$C_{19}H_{38}O_2$	298	21.585	1.017	8.34
7	6*E*,9*E*-Octadecadienoic acid methyl ester	$C_{19}H_{34}O_2$	294	21.865	1.030	0.53
8	Unidentified compound	-----	-----	21.990	1.036	0.35
9	8*E*,11*E*-Octadecadienoic acid methyl ester	$C_{19}H_{34}O_2$	294	22.490	1.060	1.86
10	2-Furanpentanoic acid, tetrahydro-5-nonyl-, methyl ester	$C_{19}H_{36}O_3$	312	22.750	1.072	0.31
11	Octadecanoic acid, 9,10,12-trimethoxy-methyl ester	$C_{22}H_{44}O_5$	388	23.245	1.095	0.30
12	Unidentified compound	-----	-----	23.580	1.111	0.49
13	Eicosanoic acid methyl ester (Arachidic acid methyl ester)	$C_{21}H_{42}O_2$	326	24.670	1.162	1.05
14	9-hydroxy pentadecanoic- anoic acid methyl ester	$C_{19}H_{38}O_3$	314	24.765	1.167	1.08
15	Docosanoic acid methyl ester (Behenic acid methyl ester)	$C_{23}H_{46}O_2$	354	29.405	1.386	0.47
Percentage of unidentified compounds 3.51%						
Percentage of saturated fatty acids methyl ester 23.05%						
Percentage of monounsaturated fatty acids methyl ester 33.65%						
Percentage of polyunsaturated fatty acids methyl ester 39.79%						

RRT=Relative Retention Time.

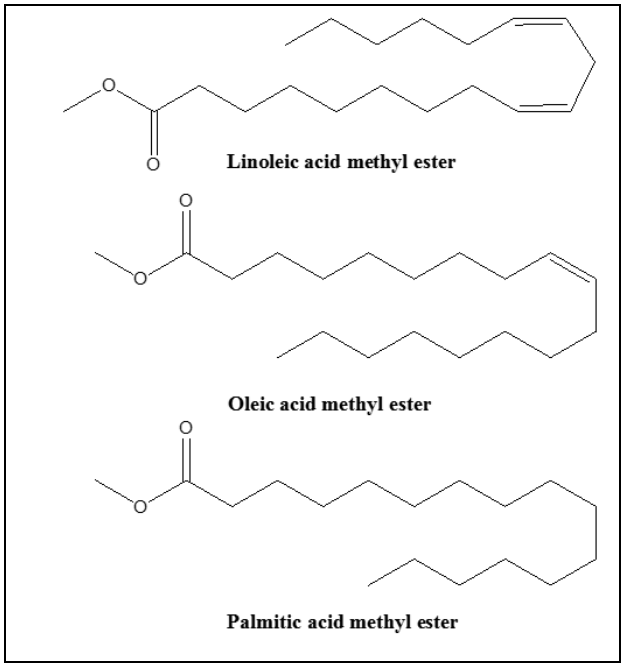

Fig. 3: Major identified compounds in both AFFO and SSFO.

4. Discussion

GC/MS analysis of AFFO showed high content of monounsaturated fatty acids 44.61%, while GC/MS analysis of SSFO contained high content of both mono- and polyunsaturated fatty acids with percentages 33.65% and 39.79%, respectively.

AFFO contained oelic acid (monounsaturated fatty acid) with 43.59%, which is less than the two varieties of Avocado (Hess and Fuerte) [18] as well as Olive oil [19], which reported to have percentages 54.5%, 58.13% and 55.13%, respectively.

Furthermore, AFFO contained 6.40% linoleic acid (polyunsaturated fatty acid). In comparison with the reported percentage of it in the two varieties of Avocado (Hess and Fuerte) [18] and Olive oil [19], it revealed higher percentages with 15.7%, 10.6% and 13.85%, respectively.

On the other hand, SSFO showed lower percentage of oleic acid 29.09% than Brown Sesame 47.85% [20] and Olive oil 55.13% [19].

Furthermore, our GC/MS analysis of the polyunsaturated fatty acid in SSFO in comparison with Brown Sesame and Olive oil showed the presence of linoleic acid 37.40%. SSFO is nearly had the same percentage of linoleic in Brown Sesame 37.63% [20], while Olive oil displayed the least percentage with 13.85% [19].

High monounsaturated fatty acids diets exhibited various benefits on cardiovascular disease risk factors beyond those associated with plasma lipids and lipoproteins. A diet rich in monounsaturated fat had beneficial effects on blood pressure and glucose metabolism [21, 22].

Both oleic and linoleic acids had different mechanisms on the inflammatory phase of wound healing in rats. Therefore, pro-inflammatory effect of these acids may speed up the wound healing process [23].

Moreover, further research on the therapeutic potential of adjuvant conjugated-linoleic acid in the treatment of human papillomavirus (HPV)-induced laryngeal papillomatosis [24].

5. Conclusion

Analyses of Avocado and Sesame fixed oils indicated their high contents of mono- unsaturated fatty acids. Therefore, these oils can be used in many formulations for different diseases such as burns, bed sores,…..etc.

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