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Comparative characterization of Fatty acids composition of wild and cultivated varieties *Olea europaea* seed oil by gas chromatography mass spectrometry

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ABSTRACT
Olea europaea is medicinally valuable plant producing a variety of bioactive molecules having important medicinal value in folk medicine. To determine components ofwild and cultivated varieties, Olea europaea seed oil, methyl ester derivatives of fatty acids were analyzed. Hyphenated technique of Gas chromatography-mass spectrometry (GC-MS) was utilized for this analysis. Results showed the presence of15different saturated and unsaturated fatty acids in both varieties of Olea europaea seed oil. Fatty acid methyl esters (FAMEs) Quantifications was done by using triplicate point calibration curves compared with $R^2 > 0.99$) in all cases. Oleic acid (C18:1c) was found at the highest amount of (9.87%) in wild variety and (8.86%) in cultivated variety which is essential for the normal growth and active inhibition activity of the cyclooxiginase-2catalyzed reaction leading to the biosynthesis of prostaglandin. Amongst the additional Fatty acids whose concentrations were found above 1% included: Palmitic acid (3.76%) in wild variety and (3.11%) in cultivated variety and Linoleic acid (2.06%) in wild variety. Remaining fatty acids were having concentrations less than 1%.
Key words: *Olea europaea; Fatty acids, Methylation, GC-MS, FAMEs, EOs.*

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INTRODUCTION

Olive fruit extract possesses anti-inflammatory and anti-nociceptive activities **[**1].Fourier Transmission Infra-red Spectroscopy (FTIR) and GC-MS of olive confirmed different phytochemicals and oil fatty acids [2]. Olive oil analysis showed the presence of saturated fatty acids (SFAs), polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids (MUFAs) of great importance. Phenolic compounds of olive oils in humans reduce cardiac risks and oxidative complexity [3,4]. Various analytical techniques like titrimetry, chromatography, spectroscopy, electrophoresis, and electrochemical conforming procedures

are used in pharmacological research [5]. FT-IR spectroscopy evaluates degree of oxidation, nutritional, physicochemical and sensual characteristics of the edible oil [6]. Gas chromatography(GC) can sensitively characterize different sample mixtures. Liquid Chromatography-Mass Spectrometry (LC-MS) is effective for samples identification based on MS libraries and available resources to help GC-MS metabolite profiling and experimentations[7].The essential oil volatile constituents were analyzed via GC and GC-MS techniques[8].The presence of MUFAs and bioactive components like phospholipids, carotenoids, polyphenolic compounds and tocopherols in olive oils have numerous biological activities and major health benefits. PUFAs less consumptions may adversely affect long chains n-3 PUFAs integration to membranes of infants and fetus brain as well as on the mental and vision growth in early development [9-12].

The essential oils (EOs) and aromatic phytocomponents have been widely used for their biological activities as well as their numerous industrial applications[13-15]. Recently, scientists are attracted to the therapeutic uses of EOs compared to less effective synthetic antibiotics or alternate food preservatives [15].Olive fruits are sources of plant polyphenols having an inhibitor, antimicrobial, antiviral properties. Olive oil markets are too important in the olive industries as about 90% produced olives go for oil processing annually [16].

The present study aims to analyze essential oil composition of wild and cultivated varieties of *O. europaea* to find out the essential oils in olive fruits to get an access to their therapeutic as well as medicinal values. We have compared and characterized two varieties of *O. europaea* FAMEs are using GC-MS technique.

MATERIAL AND METHODS

Materials

The reagents and chemicals were of analytical grade and procured from standard suppliers. Boron trifluoridein 10% alcoholic solution was bought from the Swiss company named Fluka Chemie(Buchs). Merck **(**Darmstadt, Germany**),** provided0.5 N methanolic sodium hydroxide solution and common salt on payment. HPLC grade alcohol and n-hexane purchased from Fischer **(**Leicestershire, United Kingdom**).**Pak gas **(**United Arab Emirates**)** provided chemical element gas **(**99.99%**)** on the purchase. The FAMEs and Tridecanoic acid methyl ester components were bought **(**Accu Standard USA**)**. Deionized water was used throughout the experiment.

Instruments

The following instruments and equipments were used during the present work. Mass spectrometer model QP 2010+(Japan) hyphenated to Gas Chromatograph (Shimadzu) was used which is set with an Auto-injector model AOC20-i and an Autosampler model AOC20S.Helium was used as carrier gas. Capillary column (TRB-FFAP; Technokroma) was used for the entire Chromatographic separation procedures. Column already treated with *Polyethylene glycol* specifications were: Thickness; 0.250 µm, Length-30 m,I.D- 0.35 mm. Additional GC-MS conditions were: Ionic Source Temperature (250°C), 240°C Interface Temperature, 1.8 min solvent cut time-period and Pressure (100 KPa).

Preparation of standard

By mixing tridecanoic acid methyl ester(13.7 mg)withn-hexane (1mL)internal standards were being prepared. External standard was prepared by dissolving 37 components FAMEs mix standards(10 mg)with 10 mL of dichloromethane. More working standards were madefrom this solution.

Preparation of FAMEs and extraction of oil

Using Soxhlet extraction apparatus, approximately 100 g fine powder seed materials were isolated with 250 mL of n-hexane intended for six hours [17]. Using rotary evaporator, the solvent was recovered by the extract concentration. Fatty acids have non-volatile and polar nature, but Gas chromatographic analysis needs volatile sample. To make oil fatty acids volatile, derivation is done for GC-MS analysis. For converting fatty acids into FAMEs, researcher prefer derivatizing reagent, boron trifluoride-methanol as the most acceptable in their scientific procedures[18].Generally, non-volatile fatty acids are changed to volatile FAMEs by means of methylation[19]. Using standard reference method, derivatization was done (AOAC 2000). About25 mg fat equivalent quantity sample was added 0.1 mL internal standard 1.37 mg weighing. Then 1.5mL methanolic caustic soda solution having 0.5 normality was mixed, covered and boiled in heating water bath for about 5 minutes. The mixture was chilled followed by the addition of 2.5 mL of 10% methanol-boron trifluoride made solution. The solution was again sealed and boiled in heating water bath for half an hour time followed by cooling. 5mL brine was added to the esterified mixture and then extracted two times using 1mL of n-hexane as suitable solvent. This extract was filtered using 0.45 µm thick filter paper. After this, extract (1µL)was introduced to GCMS via sample injector[20].

FAMEs Chromatographic separation

Chromatographic separations were done using capillary column (TRB-FFAP; Technokroma)with provisions; Length: 30 m, I.D: 0.35 mm, Thickness: 0.25 µm, polyethylene glycol treated. More GC-MS settings are: interface temperature; 240 µC, temperature of ion source (EI); 250 µC, pressure conditions; 100 KPa, 1.8 minutes cut off time for solvent. About 1 µL amount of sample and standard were introduced to GC column. Auto-injector was run in a fractionate mode of1:50 splitting ratio. Auto-Injector's temperature at 240°C was fixed. The temperature of column system at the beginning was 50°C for 1st minute and after this, increased till 150 °C by the speed equal to 15 °C/min. Then temperature was enhanced up to 175°C for 5 minutes by the constant rate of 2.5°C/min. After this, the temperature was raised to 220 °C at the rate of 2.5 °C/min maintained for 3 minutes. Entire elution's period was about 43 minutes duration followed by MS scanning between nm/z85-380. The supplier provided GC-MS solutions software used for controlling the system for acquiring data. Compounds identification was performed by comparing the mass spectrogram [spectra] obtained to standardized mass spectrogram (spectra) from the NIST 05(National Institute of Standards and Technology)and Adams database[21].

RESULTS AND DISCUSSION

Tables 1 and2 demonstrate the GC-MS analysis acquired results from the wild and cultivated varieties which show the comparative concentrations of separate esterificated Fatty acids. These results are obtained because of the external standardized methods as well as the values among triplicate results of standard deviation in every instance. Three times repeated analysis specific values and concentrations have been reported in Tables 1 and 2,are the average of triplicate measured results. FAMEs Quantifications was done by using triplicate point calibration curves compared with R^2 values > 0.99 in all cases. The *Olive* fruit oils of both (wild and cultivated) GC-MS chromatograms along with correctly assigned detected analytes signals are attached as Figs.1 and 2. The analyzed sample confirmed the presence of saturated and unsaturated fatty acids. Among the fatty acids, Oleic acids(C18:1c) were found in the highest amount (9.87%) in wild variety and (8.861%) in cultivated variety. Amongst the additional Fatty acids whose concentrations were found above 1% included: Palmitic acid (3.72%) in wild variety and (3.11%) in cultivated variety and Linoleic acid (2.06%) in wild variety. Rests of the fatty acids were having concentration amounts <1%.Oleic acid is vitalto normalize growth possessing active inhibition activity of the cyclooxiginase-2 catalyzed reaction leading to the biosynthesis of prostaglandin[22-23]. Obtained results showed that *O.europaea* seeds and fruitspossess various bioactive compounds like saturated and unsaturated fatty acids contain different pharmacological products. The present method is a reliable analyzing method for many fatty acids in a singlyrun simultaneously.

Table-1 Wild variety Olive seed Fatty Acids GC-MS quantitative results

Name of fatty acid methyl ester	Retention time (min)	Area	Concentration (%)
C6:O; Hexanoic acid	2.85	528	0.01
C8:O; Caprylic acid	4.68	8283	0.01
C10:O; Capric acid	6.43	3099	0.01
C12:0; Lauric acid	8.03	14456	0.01
C14:0; Myristic acid	10.09	23278	0.06
C15:0; Pentadecenoic acid	11.51	7121	0.01
C16:0; Palmitic acid	13.30	9030343	3.76
C16:1c; Palmitoleic acid	13.73	200859	0.54
C17:0; Magaric acid	15.31	35595	0.02
C18:2t; Heptadecenoic acid	15.74	7875	0.02
C18:0; Stearic acid	17.69	1918054	0.86
C18:1c; Oleic acid	18.21	8241869	9.87
C18:1n9t; Elaidic acid	18.30	347820	0.85
C18:2c; Linoleic acid	19.31	1705732	2.06
C18:2t; Octadecadienoic acid	19.43	7060	0.01
C18:3n3; Linolenic acid	21.34	140860	0.30
C20:0; Arachidic acid	24.26	256410	0.17
C20:1c; 11-Eicosenoic acid	24.82	73634	0.17
C22:0; Behenic acid	31.46	62692	0.04
C24:0; Lignoceric acid	37.74	38646	0.03
Total			18.71

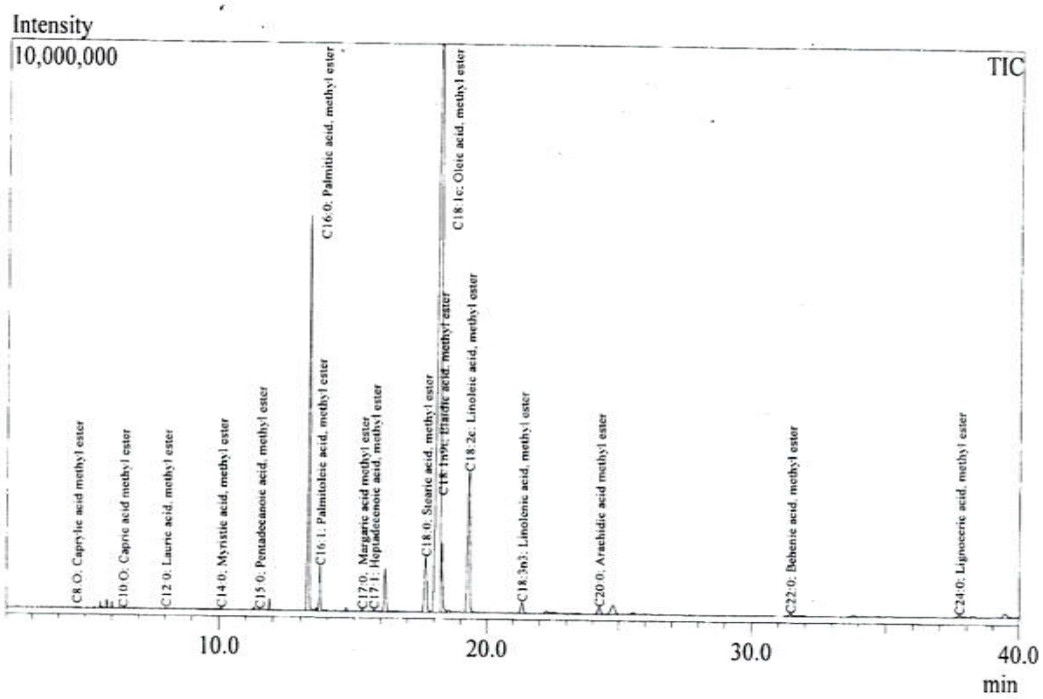

Fig.-1. Chromatogram showing wild variety of *O. europaea* seed fatty acids composition

Table-2 Cultivated variety Olive seed Fatty Acids GC-MS quantitative results

Name of fatty acid methyl ester	Retention time (min)	Area	Concentration (%)
C6:O; Hexanoic acid	2.89	48984	0.13
C8:O; Caprylic acid	4.69	25164	0.04
C10:O; Capric acid	6.43	9988	0.01
C11:0; Undecanoic acid	7.33	1594	0.01
C12:0; Lauric acid	8.03	5425	0.01
C13:0; Tridecanoic acid	9.12	5484	0.01
C14:0; Myristic acid	10.09	10477	0.01
C15:0; Pentadecenoic acid	11.51	5056	0.01
C15:1; Pentadecenoic acid	11.65	1105	0.01
C16:0; Palmitic acid	13.28	6181963	3.11
C16:1; Palmitoleic acid	13.72	76460	0.25
C17:0; Magaric acid	15.31	14797	0.01
C17:1; Heptadecenoic acid	15.74	2748	0.01
C18:0; Stearic acid	17.66	1268972	0.69
C18:1c; Oleic acid	18.16	7049728	8.86
C18:1n9t; Elaidic acid	18.27	308288	0.92
C18:2c; Linolenic acid	19.27	350556	0.52
C18:2t; Octadecadienoic acid	19.39	2181	0.01
C20:0; Arachidic acid	24.27	163679	0.13
C20:1c; 11-Eicosenoic acid	24.80	124354	0.35
C22:0; Behenic acid	31.48	41098	0.03
Total			14.84

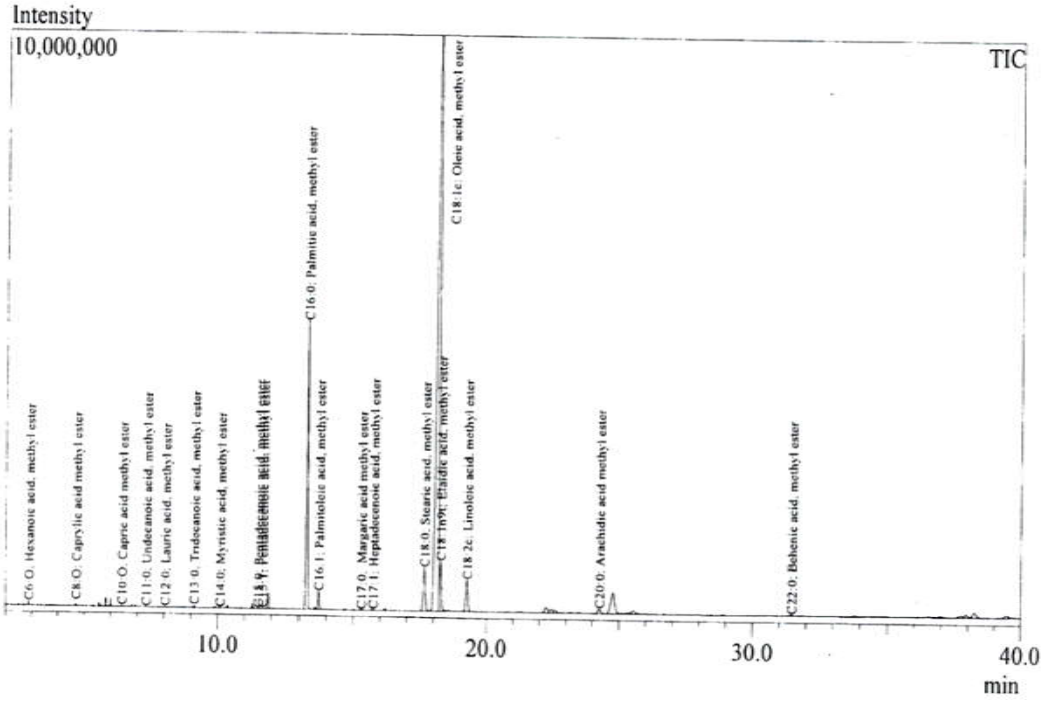

Fig-2. Chromatogram showing cultivated variety of *O. europaea* seed fatty acids composition

The percentage of FAMEs calculated GC-MS quantitative results

From the percent concentration of GC-MS quantitative results tables, the percentages of fatty acid methyl ester were calculated which confirmed that C18:1c; Oleic acid had the highest percentage (53 for wild and 59 for cultivated). Similarly, C10:O; Capric acid had the lowest percentage (0.02) for wild variety fruit and C11:0; Undecanoic acid has the lowest percentage (0.01) in the case of cultivated variety fruits. The details of calculated percentages are formulated in the Tables 3 and 4. From the percent concentration of GC-MS quantitative results tables, the percentages of fatty acid methyl ester were calculated using formula given below:

$$\text{Percentage of fatty acids} = \frac{\text{Concentration (\%)}}{\text{Total Components}} \times 100$$

Table-3. The percentage of wild variety FAMEs calculated from GC-MS quantitative results

Name of fatty acid methyl esters	Percentage
C6:O; Hexanoic acid	0.06
C8:O; Caprylic acid	0.05
C10:O; Capric acid	0.02
C12:0; Lauric acid	0.06
C14:0; Myristic acid	0.08
C15:0; Pentadecenoic acid	0.03
C16:0; Palmitic acid	19.86
C16:1c; Palmitoleic acid	2.89
C17:0; Magaric acid	0.11
C18:2t; Heptadecenoic acid	0.11
C18:0; Stearic acid	4.57
C18:1c; Oleic acid	52.77
C18:1n9t; Elaidic acid	4.55
C18:2c; Linoleic acid	10.99
C18:2t; Octadecadienoic acid	0.07
C18:3n3; Linolenic acid	1.61
C20:0; Arachidic acid	0.88
C20:1c; 11-Eicosenoic acid	0.90
C22:0; Behenic acid	0.22
C24:0; Lignoceric acid	0.14
Total	18.71

Table-4. The percentage of wild variety FAMEs calculated from GC-MS quantitative results

Name of fatty acid methyl esters	Percentage
C6:O; Hexanoic acid	0.87
C8:O; Caprylic acid	0.25
C10:O; Capric acid	0.07
C11:0; Undecanoic acid	0.01
C12:0; Lauric acid	0.03
C13:0; Tridecanoic acid	0.02
C14:0; Myristic acid	0.05
C15:0; Pentadecenoic acid	0.03
C15:1; Pentadecenoic acid	0.03
C16:0; Palmitic acid	20.59
C16:1; Palmitoleic acid	1.66
C17:0; Magaric acid	0.07
C17:1; Heptadecenoic acid	0.05
C18:0; Stearic acid	4.98
C18:1c; Oleic acid	58.68
C18:1n9t; Elaidic acid	6.12
C18:2c; Linolenic acid	3.42
C18:2t; Octadecadienoic acid	0.03
C20:0; Arachidic acid	0.85
C20:1c; 11-Eicosenoic acid	2.31
C22:0; Behenic acid	0.23
Total	15.1

CONCLUSION

The analyzed sample proved the presence of saturated and unsaturated fatty acids, methyl ester. Oleic acid was found in highest amount in wild and cultivated varieties of *O. europaea*. Among the additional fatty acids, Capric acid, Pentadecanoicacid, and Pentadecanoic acid were found in the least amounts in both varieties. From this study, it is concluded that Oleic acid was found in highest amount in wild variety than the cultivated variety as confirmed by the GC-MS analysis.

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