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Gas chromatography-mass spectrometry analysis and *in vitro* biological studies on fixed oil isolated from the waste pits of two varieties of *Olea europaea* L.

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Abstract – Olive oil isolated from the fruits of the *Olea europaea* L. is an important part of Mediterranean diet. It is known for its diverse biological actions. Furthermore, a little amount of fixed oil and other bioactive components can also be extracted from the olive seeds which are considered as byproduct of olive oil extraction. Therefore, this study was designed to analyze the fatty acid composition and to perform *in vitro* biological studies on fixed oil isolated from olive seeds. The fixed oil was isolated from the olive seeds of Syrian and Greek black olive fruits by using Soxhlet apparatus. The purity was checked by measuring its refractive index. Composition of two isolated oils and a commercially available virgin olive oil was determined by preparing their Methyl esters (FAME) followed by GC-MS analysis. Various *in vitro* assay methods were used to investigate activities such as antioxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH), cytotoxic activity by using Brine shrimps lethality bioassay and antimicrobial activity against two bacterial strains; *Staphylococcus aureus* and *Escherichia coli* by agar well diffusion method. GC-MS analysis revealed that the two isolated oils differ quantitatively in chemical composition with oleic acid identified as the major chemical constituent (62.6% and 73.56%). Both the fixed seed oils showed a concentration dependent DPPH radical scavenging activity ranging from 8 to 76% inhibition. The oils also exhibited excellent cytotoxic activity but no antimicrobial activity was observed. The chemical composition of the isolated fixed olive seed oil is found to be almost similar to the commercially available fruit olive oil. The fixed oil from the seeds of olive fruits possesses useful biological actions. Further studies are needed to isolate and quantify their bioactive constituents.

Keywords: antioxidant / antimicrobial / cytotoxic activity / olive oil / olive seeds

Résumé – Analyse par chromatographie en phase gazeuse couplée à la spectrométrie de masse (GC-MS) et études biologiques *in vitro* d'huiles résiduelles issues des noyaux de deux variétés d'*Olea europaea L.* L'huile d'olive extraite des fruits de l'*Olea europaea L.* constitue une part importante du régime alimentaire méditerranéen. Elle est connue pour ses diverses actions biologiques. En outre, une petite quantité d'huile résiduelle et d'autres composants bioactifs peuvent également être extraits des noyaux d'olive considérés comme des sous-produits de l'extraction de l'huile d'olive. Cette étude a donc été conçue pour analyser la composition en acides gras et réaliser des études biologiques *in vitro* sur une huile résiduelle isolée à partir de noyaux d'olive. L'huile a été isolée des noyaux d'olives syriennes et d'olives noires grecques à l'aide d'un appareil Soxhlet. La pureté a été vérifiée en mesurant son indice de réfraction. La composition des deux huiles résiduelles et d'une huile d'olive vierge disponible dans le commerce a été déterminée en préparant leurs esters méthyliques (FAME) suivies d'une analyse par GC-MS. Diverses méthodes de dosage *in vitro* ont été utilisées pour étudier des activités telles que l'activité antioxydante du 1,1-diphényl-2-picrylhydrazyle (DPPH), l'activité cytotoxique à l'aide du dosage biologique de la létalité des larves de crevettes dans l'eau de mer et l'activité antimicrobienne contre deux souches bactériennes (*Staphylococcus aureus* et *Escherichia coli*) par la méthode de diffusion sur puits d'agar. Une analyse par

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GC-MS a révélé que la composition chimique des deux huiles issues des noyaux d'olive diffère quantitativement, l'acide oléique étant identifié comme le constituant principal (62,6 et 73,56 %). Les deux huiles de noyaux d'olive ont montré une activité de piégeage des radicaux de la DPPH dépendant de la concentration allant de 8 à 76 % d'inhibition. Les huiles présentaient également une excellente activité cytotoxique, mais aucune activité antimicrobienne n'a été observée. La composition chimique de l'huile de graines d'olive résiduelle de noyau est presque identique à celle de l'huile d'olive issue du fruit disponible dans le commerce. L'huile produite à partir des noyaux d'olive possède des actions biologiques utiles. Des études complémentaires sont nécessaires pour isoler et quantifier leurs constituants bioactifs.

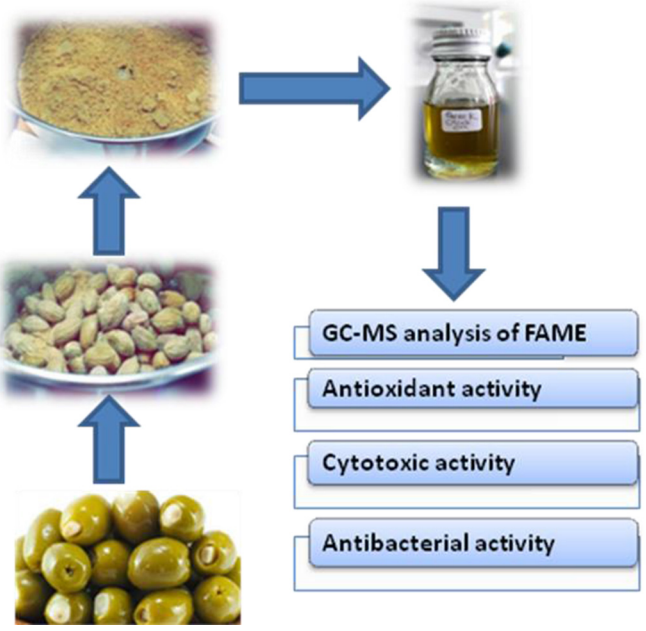
Mots clés : antioxydant / antimicrobien / activité cytotoxique / huile d'olive / noyaux d'olive

1 Introduction

Olea europaea L. tree belonging to the family Oleaceae is one of the oldest known cultivated crops in the world particularly in Mediterranean region. It is known as Olive in English and Zaitoon in Arabic (Farhangi *et al.*, 2014). The Mediterranean climate is optimal for the cultivation of this small, slow growing but long lived evergreen tree of agricultural importance (Abaza *et al.*, 2015). The olive tree is known for its edible fruit, which is the prime source of olive oil. Olive oil is regarded as a healing agent as well as an excellent food since ancient time. It is consumed regularly as a main source of lipid and hence forms an essential and integral part of Mediterranean diet (Schwingshackl *et al.*, 2017). A number of epidemiological studies have reported that consumption of olive oil promotes good health (Gorzynik-Debicka *et al.*, 2018) and reduces risk of morbidity and mortality (Cicerale *et al.*, 2009) by protecting against several neurodegenerative disorders (Fung *et al.*, 2009; Scarmeas *et al.*, 2009), cardiovascular diseases (Estruch *et al.*, 2006; Nocella *et al.*, 2018) and certain types of cancer (Dixon *et al.*, 2007; Boss *et al.*, 2016).

The health benefits of consuming Virgin olive oil have been attributed to its high content of a monounsaturated fatty acid (MUFA) namely oleic acid (70–80% of total fatty acids), and presence of other phenolic bioactive compounds (Caravita *et al.*, 2007; Cicerale *et al.*, 2009; Cioffi *et al.*, 2010). The biological properties of olive oil and its constituents affect health through multiple mechanisms ranging from antioxidant, anti tumour, antimicrobial and modulation of gene functions etc (Rahmani *et al.*, 2014). In traditional system of medicine, olive oil is used to improve digestion, as a laxative, to treat rheumatic pain, colic, to reduce muscle aches, to maintain skin, hair and muscle health (Hashmi *et al.*, 2015). The oil because of its nutritional benefits, stability, delicate flavor, commercial and medicinal value is used in cooking, cosmetics, and pharmaceutical industry (Ghanbari *et al.*, 2012).

The olive seed or pit is obtained as an important by product of olive oil extraction. The olive stone once dumped as a waste material is nowadays used as a source of renewable energy in industrial sector. The main constituents of olive stone are hemicellulose, cellulose and lignin while protein, fat, free sugars are also present in considerable amount (Rodríguez *et al.*, 2008). The olive seed also contains little amount of oil in addition to other bioactive compounds but unfortunately very few studies have been carried out to explore the potential of olive seed oil. We hypothesized that chemical composition of seed oil would be different from the commercial oil extracted from olive pulp and it also possesses useful biological activities. We therefore aimed to (i) analyze and compare the chemical composition of olive seed oil extracted from Greek and Syrian olive fruits variety with that of commercial oil available in market by Gas chromatography coupled with Mass spectrometry (GC-MS) and (ii) investigate the antioxidant, cytotoxic and antimicrobial activity of isolated seeds oil by *in vitro* methods.



2 Materials and methods

2.1 Chemicals

All reagents and solvents used in current study were of analytical grade. A commercial olive oil "Rafael Salgado" (RS) was also purchased from the market for comparison purpose. Brine shrimp eggs of San Francisco Bay Brand, USA were used for cytotoxic activity. *Escherichia coli* (gram negative) and *Staphylococcus aureus* (Gram-positive), two pathogenic bacterial strains were obtained from the Department of Natural Sciences, Oman Medical College, Sultanate of Oman.

2.2 Isolation and extraction of fixed oil from olive pits

Syrian and Greek black olives were purchased from the local market in the month of June 2016. Whole seeds were separated from the pulp with the help of a knife, cleaned under running water and then dried under the sun. Approximately 250 grams of dry seeds were weighed and pulverized using the domestic blending mixer. The fixed oil from the Syrian and Greek olive seed powder (150 g) was extracted with the help of petroleum ether solvent (900 ml) by continuous hot extraction method using Soxhlet apparatus at a temperature of 65 °C for 4 h. The fixed olive seed oil was obtained by removing the excess of solvent from the extract with the help of a rotary vacuum evaporator under reduced temperature and pressure. The oil was transferred into small glass bottles, weighed to calculate percentage yield with reference to dry seed powder and stored at 18 °C until further use.

2.3 Preparation of fatty acid methyl esters (FAME) from olive seed oil and virgin oil by Boron trifluoride-methanol method

The methyl esters of olive oil were prepared as per the reported method with slight modification (Kyriakidis and Dionysopoulos, 1983). Approximately 0.5 g of oil was dissolved in 6 ml of 2% w/v methanolic sodium hydroxide solution. The mixture was heated under reflux for 10 minutes on a water bath for complete hydrolysis. It was followed by the addition of 7 ml of 14% boron-trifluoride (BF_3) solution in methanol to the above mixture and then refluxing for 3 more minutes. After the esterification, 5 ml of heptane was added and the boiling was continued for 2 minutes. The reaction mixture was cooled, diluted with 15 ml of saturated sodium chloride solution and shaken vigorously for few seconds. The supernatant hydrocarbon layer (FAME) was collected and dried over anhydrous sodium sulphate for gas chromatographic (GC) analysis.

2.4 GC-MS analysis of fatty acid methyl esters (FAME) of the isolated olive seed oil

GC-MS analysis was performed on a Shimadzu GC-2010 Plus, fitted with a SP-2560 *Supelco* capillary column (100 m × 0.250 mm i.d. × 0.2 μm film thickness) coupled to GCMS-QP2010 ULTRA MS. Ultra-high purity helium (99.9999%) from air products was used as carrier gas at a constant flow of 1.0 ml/min. The injection port temperature was kept at 250 °C while the transfer line and ion source temperatures were maintained at 240 °C. The ionizing energy was 70 eV. Electron multiplier (EM) voltage was obtained from autotune. All data were obtained by collecting the full-scan mass spectra within the scan range 35–500 atomic mass unit (amu). The injected sample volume was 1 μl with a split ratio of 30:1. The oven temperature program was 50 °C (hold for 5 minutes) and accelerated at a rate of 4 °C/min – 250 °C hold for 5 minutes.

The unknown compounds were identified by comparing the spectra obtained with mass spectrum libraries (NIST 2011 v.2.3 and Wiley, 9th edition)and further confirmed with Supelco 37 component FAME mixture (cat.# 47885-U).

2.5 Determination of physico-chemical properties

The λmax, pH and refractive index of the two isolated seed oils and RS commercial oil were measured at the room temperature by using double beam UV-spectrophotometer, digital pH meter and Abbe- refractometer, respectively.

2.6 *In vitro* antioxidant activity

The *in vitro* antioxidant activity of the fixed oil was determined by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay method as described by Al-Hakmani *et al.* (2013) with slight modification (Al-Hakmani *et al.*, 2013). Briefly, the DPPH solution (0.04 mg/ml) and different concentrations of oil (20, 40, 80 and 160 mg/ml) were prepared in ethyl acetate. In each test tube, 1 ml of the test sample of different concentrations was mixed with 2 ml of DPPH and shaken for two minutes. The test tubes were covered with aluminum foil and kept aside in the dark for 30 min. The color intensity (optical density) of each solution was measured at 517 nm using double beam spectrophotometer. Similarly, a blank solution was prepared by substituting the test sample with 1 ml of ethyl acetate. The percent inhibition of DPPH radical was calculated using the standard formula and reported as mean ± standard deviation of the triplicate reading. The EC_{50} value for each sample was also determined.

2.7 Cytotoxic activity of olive oil by Brine shrimp lethality Bioassay

Brine shrimp (*Artemia salina*) eggs were hatched in sea water filled in a clean plastic container (Hamidi *et al.*, 2014). The container was partially covered and kept at room temperature for 36 h. The hatched and matured *nauplii* were attracted to the wall of the container by placing a lamp above the open side. Cytotoxic activity of the olive oils at three different concentrations (1000, 100 and 10 μg/ml) was evaluated following the standard procedure of Sarah *et al.* (2017). Briefly, the stock solution was prepared by dissolving 20 mg of oil in 2 ml ethyl acetate. An accurately measured quantity of stock solution (500, 50 and 5 μl) was transferred to the cleaned and labeled test tubes containing 10 shrimp larvae and the final volume was made up to 5 ml with sea water to obtain the desired concentrations of 1000, 100 and 10 μg/ml, respectively. The test tubes were incubated for 24 h and the total number of dead larvae was counted to calculate the percentage mortality for each concentration. The experiment was performed in a triplicate.

2.8 Evaluation of antimicrobial activity

Antibacterial activity of the isolated fixed oils was determined by well diffusion method using standard Muller Hinton agar (MHA) media against *S. aureus* and *E. coli* bacterial strains (Al-Aamri *et al.*, 2018). Wells of approximately 5 mm diameter were made in Petri plates using sterile borer followed by the addition of 5 and 10 μl of each neat oils to these wells under aseptic conditions. Ampicillin (25 μg/disc) was used as a positive control. The plates were incubated at 37 °C for 24 h and then the diameter of zone of inhibition

Table 1. Physiochemical properties of fixed oil isolated from olive pits.

Oil type	Refractive index(RI) at		pH	λ_{max}	Color	Percent yield
	24.2 °C	20 °C				w/w
RS Commercial oil	1.4658	1.4677	4.82	270	Yellowish green	–
Greek seed oil	1.4686	1.4705	5.82	268	Light green	2.66
Syrian seed oil	1.4667	1.4686	4.95	267.2	Light green	2.33

around the well was measured in mm. All experiments were performed in triplicate.

3 Results and discussion

3.1 Percentage yield and physicochemical properties of isolated fixed oil

The percentage yield of fixed oils extracted was calculated on the dried powder weight of seeds used for extraction. The Greek and Syrian olive pits yielded 2.66% w/w and 2.33% w/w of fixed oil. The percentage yield was found to be slightly lower than the reported values in the literature (Banat *et al.*, 2013). The percentage yield could have been affected by olive variety, less effective penetration of solvent, duration of extraction, harvesting time, size of pits, etc used for extraction. The isolated oils were of light green color and were observed to be less darker and viscous than the RS commercial virgin oil, hence showed a slight difference in λ_{max} values (Tab. 1). The pH of both the isolated oils and a positive control RS commercial olive oil was found to be acidic (4.82–5.82). However, the commercial oil was more acidic (4.82) suggesting a difference in fatty acids composition and content of oils. The refractive index of all the three oils was computed at 20 °C by using the following formula:

$$\eta^{20^\circ} = \eta^{\text{observed temp}^\circ} + 0.00045\,(\text{Observed temp at } 20\,^\circ\text{C}).$$

The refractive index of the olive oils (1.4677–1.4705) was found to be in agreement with the reported ranges for virgin olive oil (Codex Alimentarius, 2009).

3.2 Chemical composition of fixed oil by GC-MS analysis

GC-MS is widely used to study the fatty acid composition and to detect the adulteration of olive oil and other vegetable oils. The fatty acid methyl esters (FAME) of olive oils were prepared as per the standard method to obtain the sharp peaks in GC chromatogram (Ichihara and Fukubayashi, 2010). The content and composition of the isolated oils and commercial olive oil identified by GC-MS analysis (Figs. 1a–1c) are presented in Table 2. A total of 13 fatty acids methyl esters were identified in the Greek seed oil while the Syrian and commercial olive oils showed presence of 12 and 8 fatty acids respectively. Oleic acid (18:1 Cis 9), a monounsaturated fatty acid was identified as the major component in all the three oils which constituted 61.75% of the commercial oil, 62.61% of the Greek oil and 73.56% of the Syrian oil. The health benefits of olive oil are partially attributed to the high content of this monounsaturated ω-9 fatty acid. The commercial olive oil had the highest amount of saturated palmitic acid (18.81%) as compared to the seed oils (11.60% and 7.70%). The polyunsaturated fatty acids (linoleic acid, 18:2 and linolenic acid, 18:3) content of commercial oil was 8.62% and 3.38% while linoleic acid could not be detected in either of the seed fixed oils. Amongst the oils, only commercial one showed the presence of ω-6 fatty acid but seed oils showed slightly higher level of ω-3 fatty acids. The Syrian variety contained the highest amount of linolenic acid (4.99%) followed by Greek oil (3.96%). The results of our study are in agreement with literature that olive oil contains higher amount of unsaturated fatty acids (UFA) (Poulli *et al.*, 2006) but interestingly the Syrian variety had the highest level of UFA (83.07%) followed by commercial olive oil (76.94%) indicating it to be more nutritious and beneficial for daily use as an edible oil. The olive seed oils fatty acid composition was also found similar to the type I virgin oil *i.e.* high content of olive oil and low concentration of linoleic and palmitic acid (Fig. 2) (Diraman and Dibeklioglu, 2009; Asık and Özkan, 2011). The Syrian seed oil might also be more beneficial in reducing the risk of coronary heart disease due to high proportionate values of C18: C16 and UFA: SFA (Tab. 3) (Grundy, 1986).

3.3 Antioxidant activity using DPPH

Olive oil has been shown to possess good antioxidant activity which is attributed to the presence of different classes of chemical compounds (Kiralan *et al.*, 2009). A simple, popular and reliable *in vitro* DPPH colorimetric assay was employed to evaluate the free radical scavenging activity of isolated oils. The average % inhibition of commercial olive oil used as a reference ranged from 9.44 to 65.55%, while the Syrian and Greek oils showed % inhibition from 14.72 to 76.24% and 8.06 to 43.41%, respectively tested at concentration of 20–160 mg/ml. It was observed that free radical scavenging activity of oils increased with an increase in concentration (Tab. 4). The Syrian oil exhibited better antioxidant activity than the commercial virgin oil at all concentrations. It has been reported that antioxidant capacity of olives is related to their type, location and maturity (Yildiz and Uylaser, 2015).

3.4 Cytotoxic activity of olive oil using Brine shrimps

Olive oil has been reported to have protective role against cancer (Fabiani *et al.*, 2006). The Brine Shrimp lethality (BSL) bioassay is a rapid, inexpensive, simple and comprehensive test for cytotoxicity evaluation for the bioactive compounds. (Asaduzzaman *et al.*, 2015). Hence, BSL bioassay was used to investigate the cytotoxicity of the fixed oils. All oils showed concentration dependent cytotoxic activity. The % mortality

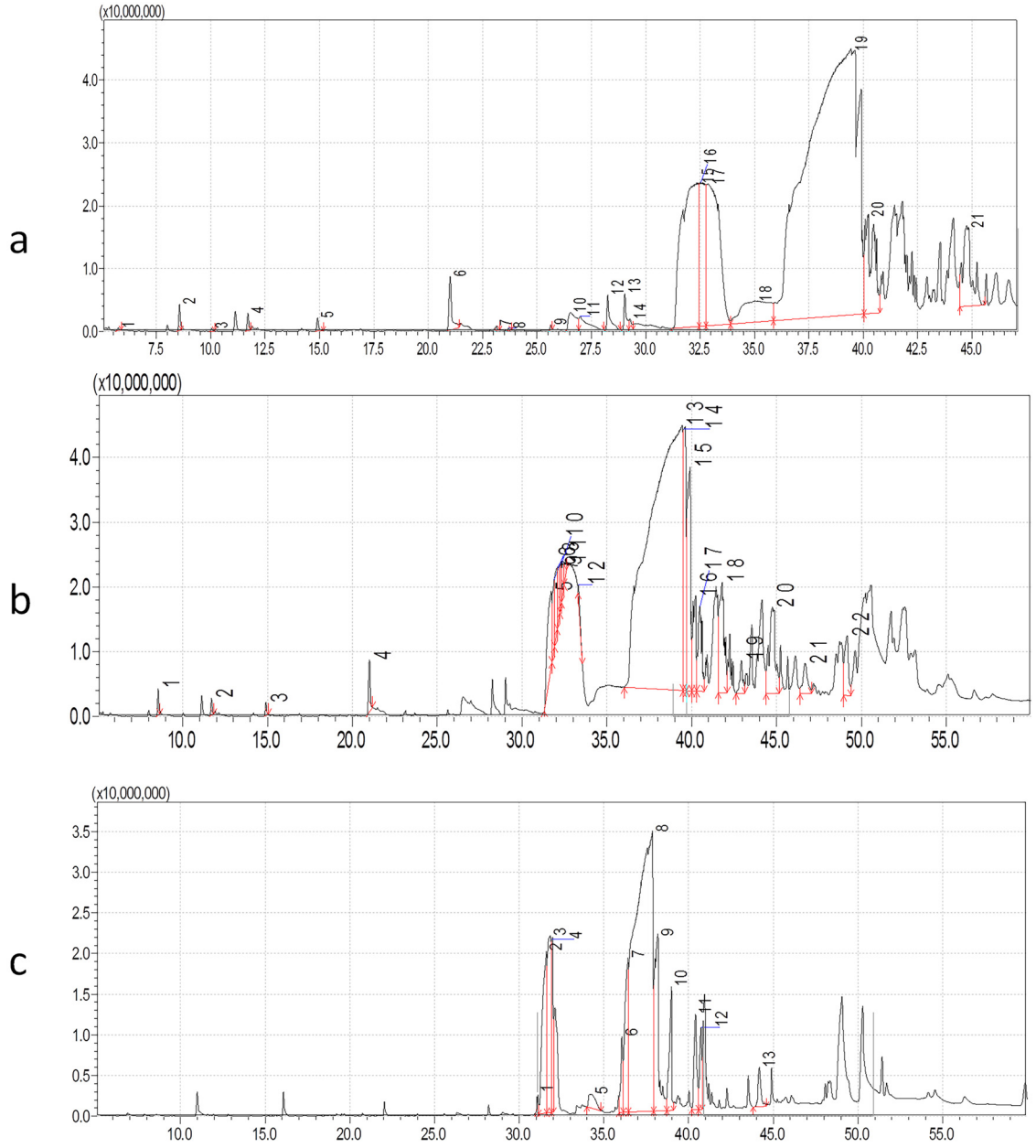


Fig. 1. a: GC chromatogram of Greek olive seed oil; b: GC chromatogram of Syrian olive seed oil; c: GC chromatogram of commercial olive oil.

rate for the oils was observed in the following order; Greek > Commercial RS > Syrian. Greek seed oil caused mortality was 46.7, 50.0 and 80.0% at 1000, 100 and 10 μg/ml. At the same concentrations, the RS oil showed percent mortality of 50.0, 60.0 and 73.3% while Syrian seed oil was the least cytotoxic with % mortality rate of 63.3, 66.7 and 73.3%. The LC_{50} values of oils are given in Table 5.

3.5 Antimicrobial activity by agar well diffusion method

Agar well diffusion method was used to screen the *in vitro* antimicrobial activity of two different concentrations (5 and 10 μl) of the 3 olive oils against two types of bacteria, *E. coli* (gram negative) and *S. aureus* (gram positive). Surprisingly, no antimicrobial activity was observed for the isolated oils against the tested bacterial strains. Though, there are conflicting reports about their antibacterial activity. In a similar study conducted by Charu *et al.* olive oil failed to inhibit the growth of bacteria and no zone of inhibition was observed against the same bacterial strains (Charu *et al.*, 2008).

4 Conclusion

The chemical composition of the isolated fixed olive seed oils is found to be almost identical to that of commercial fruit olive oil but a slight difference in physicochemical properties

Table 2. Fatty acid analysis of isolated oils by GC-MS.

No.	Retention time (Rt)	Area	Area%	Name
		Greek olive seed oil		
1	5.744	2134954	0.019464	Heptanoic acid, methyl ester (C7:0)
2	8.555	20004688	0.182384	Octanoic acid, methyl ester (C8:0)
3	11.704	14842899	0.135323	Nonanoic acid, methyl ester (C9:0)
4	14.909	13200327	0.120348	Decanoic acid, methyl ester (C10:0)
5	21.012	77673751	0.708156	Dodecanoic acid, methyl ester (C12:0)
6	26.532	122307733	0.611483	Myristic acid, methyl ester (C14:0)
7	32.425	658556867	11.19572	Palmitic acid, methyl ester (C16:0)
8	35.076	312962264	2.853293	cis-10-Heptadecenoic acid, methyl ester (C17:1)
9	39.46	6867035057	62.60712	Oleic acid methyl ester (C18:1)
10	40.221	433854979	3.955479	Linolenic acid, methyl ester (C18:3)
11	41.815	89069892	0.812055	cis-11-Eicosenoic acid, methyl ester (C20:1)
12	42.969	61583504	0.56146	Heneicosanoic acid, methyl ester (C21:0)
13	44.82	348162111	3.174212	Docosanoic acid, methyl ester (C22:0)
		Syrian olive seed oil		
1	8.555	19596371	0.245883	Octanoic acid, methyl ester (C8:0)
2	11.704	13905707	0.17448	Nonanoic acid, methyl ester (C9:0)
3	14.909	11325931	0.14211	Decanoic acid, methyl ester (C10:0)
4	21.012	58343623	0.732058	Dodecanoic acid, methyl ester (C12:0)
5	32.245	613780278	7.701318	Palmitic acid, methyl ester (C16:0)
6	39.44	5862428617	73.55797	Oleic acid methyl ester (C18:1)
7	40.23	398151090	4.995743	Linolenic acid, methyl ester (C18:3)
8	41.798	359178983	4.506746	cis-11-Eicosenoic acid, methyl ester (C20:1)
9	42.94	60571693	0.760014	Heneicosanoic acid, methyl ester (C21:0)
10	44.769	339083707	4.254603	Docosanoic acid, methyl ester (C22:0)
11	46.701	88252932	1.107341	Tricosanoic acid, methyl ester (C23:0)
12	49.178	145188910	1.821737	Tetracosanoic acid, methyl ester (C24:0)
		Commercial olive oil (RS)		
1	31.829	833332411	18.81107	Palmitic acid, methyl ester (C 16:0)
2	34.291	48342758	1.091256	cis-10-Heptadecenoic acid, methyl ester (C17:1)
3	36.428	2735648546	61.75264	Oleic acid methyl ester (C18:1)
4	38.175	381831200	8.619194	Linoleic acid, methyl ester (C18:2)
5	39.982	149594785	3.376849	Linolenic acid, methyl ester (C18:3)
6	40.421	131049469	2.958220	Eicosanoic acid, methyl ester (C20:0)
7	40.737	93100242	2.101581	cis-13-Eicosenoic acid, methyl ester (C20:1)
8	44.182	57111070	1.289186	Docosanoic acid, methyl ester (C22:0)

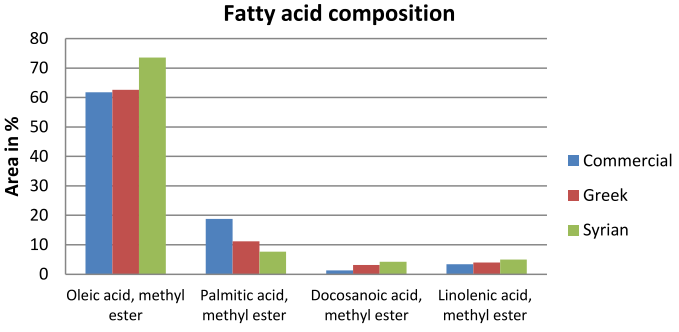


Fig. 2. Comparison of fatty acid composition of commercial and isolated oils from seeds.

was noted. The refractive index of the isolated oil is similar to the reference sample and is in agreement with the reported values in the literature but they showed slightly higher pH and little lower percentage yield and λ_{max} values with respect to the commercial olive oil. These differences are most probably due to the factors such as such as time of harvesting, climate, environmental and soil conditions. However, olive seeds oils were found to contain high content of monounsaturated fatty acids and the Syrian olive seed oil showed promising antioxidant as well cytotoxic potential. Thus, the oil from the waste seeds can be used as an additional economical and alternative source of olive oil. It can also be added to our diet to prevent progression of cardiovascular and neurodegenerative

Table 3. Fatty acid composition of olive oils.

Parameter	RS olive oil	Syrian olive seed oil	Greek olive seed oil
Mono unsaturated fatty acid (MUFA)	64.94%	78.07%	66.27%
Poly unsaturated fatty acid (PUFA)	12.0%	5.0%	3.96%
Saturated fatty acid (SFA)	23.06%	16.93%	29.77%
Unsaturated fatty acid (UFA)	76.94%	83.07%	70.23%
C18:C16 ratio	3.92	10.2	5.94
MUFA: PUFA ratio	5.41	15.61	16.73
UFA:SFA ratio	3.34	4.91	2.36

Table 4. Free radical scavenging activity of olive oil by DPPH method.

Concentration mg/ml	% Inhibition of DPPH radical		
	RS olive oil	Syrian olive seed oil	Greek olive seed oil
20	9.44 ± 2.60	14.72 ± 5.66	8.06 ± 0.24
40	26.77 ± 1.21	49.65 ± 4.27	24.86 ± 7.48
80	32.01 ± 1.82	58.68 ± 7.52	36.81 ± 0.32
160	65.55 ± 2.20	76.24 ± 2.90	43.41 ± 1.07
EC_{50}	119.53	75.48	172.49

Values are mean ± SD ($n = 3$).

Table 5. Mean % mortality of Brine shrimp larvae by olive oil after 24 h incubation.

S. No.	Oil type	Concentration (μg/ml)			LC_{50} (μg/ml)
		10	100	1000	
1.	Greek olive seed oil	46.7 ± 5.8%	50.0 ± 10%	80.0 ± 17.3%	171.97
2.	Syrian olive seed oil	63.3 ± 15.3%	66.7 ± 5.8%	73.3 ± 5.8%	81.33
3.	RS olive oil	50.0 ± 10%	60.0 ± 17.3%	73.3 ± 5.8%	109.8

Values are mean ± SD ($n = 3$).

diseases. Further studies are needed to isolate and quantify the bioactive constituents.

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Conflict of interest. The authors declare that they have no conflicts of interest in relation to this article.

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