

FATTY ACID COMPOSITION AND ANTIOXIDANT CAPACITY OF DEFATTED, NON-DEFATTED AND OILS EXTRACTS OF *QUERCUS ILEX* FRUIT FROM ALGERIA

Hadjira Guenane¹, Boulanouar Bakchiche¹, Ramazan Erenler², Ilyas Yildiz², Omar M. Khalaf³, Amal M. Saad⁴, Mosad A. Ghareeb⁴

¹Laboratory of Biological and Agricultural Sciences (LBAS), Amar Telidji University, Laghouat 03000, Algeria

²Department of Chemistry, Faculty of Arts and Sciences, Tokat, Gaziosmanpasa University, 60240 Tokat, Turkey

³Chemistry of Natural Product Department, Ministry of Education, Anbar Education Directorate, Iraq

⁴Medicinal Chemistry Department, Theodor Bilharz Research Institute, Kornaish El Nile, Warrak El-Hadar, Imbaba (P.O. 30), Giza 12411, Egypt

ABSTRACT

Background. The nutritional value and health-promoting properties cause the fruits (acorns) of *Quercus ilex* to have great potential for use in the food industry as functional ingredients and antioxidants source.

Objective. In this study, the amount of total phenolic compounds, flavonoids in different extracts (defatted, non-defatted) and composition of fatty acids in the fruits oils of *Quercus ilex* were investigated. Besides, antioxidant activity was determined.

Material and Methods. Fatty acids were extracted with n-hexane and determined by gas chromatography with mass spectrometry detection (GC-MS). Total phenolic and flavonoids contents in the extracts were measured spectrophotometrically and the antioxidant activities were tested by the DPPH (2,2-diphenyl-1-picrylhydrazyl), free radical scavenging assay, free radical-scavenging ABTS and total antioxidant capacity.

Results. The amount of total phenolic and flavonoid compounds in the defatted *Q. ilex* were 634.36±27.41 mg GAE/g DW and 96.85±2.13 mg RE/g DW, respectively. Unsaturated fatty acids were detected in higher amounts than saturated fatty acids. The primary unsaturated fatty acids of the *Quercus ilex* oil were oleic acid (65.38%), 9,12-octadecadienoic acid (16.64%) and palmitic acid (12.81%). Besides, defatted *Q. ilex* extract showed remarkable DPPH and ABTS radical scavenging activity with IC₅₀ values of 0.008±0.0008, 0.005±0.001 mg/ml respectively, while high total antioxidant capacity of the non-defatted extract with VCEAC value 0.13±0.006.

Conclusions. *Q. ilex* oil contained high amounts of polyphenols, high essential fatty acids and antioxidant potential for producing specific health promoting antioxidants in food and pharmaceutical industry.

Key words: *Quercus ilex*, total phenolics, total flavonoids, fatty acids, GC-MS, antioxidants

INTRODUCTION

The Algerian ecosystem is rich in important medicinal plants and natural sources that are widely used in traditional medicine to treat various pathological phenomena. It is worth noting that Algerian medicinal plants have promising biological activities due to their unique secondary metabolites [1-9].

Oak acorns, one of the species of *Quercus* genus, are of vital importance for both humans and animals. They have been widely used as food for many thousands of years in many regions worldwide

[10]. According to Bainbridge et al. (2006) acorns were a staple food throughout Europe, the Mideast, North Africa, Asia, and North America [11]. Acorns are nutritional dense functional food with health properties. Some of the health benefits are attributed to the high level of phenolic compounds found in acorns. These phenolic compounds provide acorn fruit with high levels of antioxidants, which could have potential health benefits [10, 12]. The green oak (*Quercus ilex*) is a tree of *Fagaceae* family, it is mainly found in the western part of the Mediterranean basin. The fruits are achenes called acorns, they are a rich source of

Corresponding author: Prof. Boulanouar Bakchiche, Laboratory of Biological and Agricultural Sciences (LBAS), Amar Telidji University, Laghouat 03000, Algeria; e-mail: b.bakchiche@lagh-univ.dz

This article is available in Open Access model and licensed under a Creative Commons Attribution-Non Commercial 4.0 International License (CC BY-NC) (<https://creativecommons.org/licenses/by-nc/4.0/>)

Publisher: National Institute of Public Health NIH - National Research Institute

carbohydrates, amino acids, proteins, lipids and sterols [13].

In Algeria the acorns of *Q. ilex* are widely used in traditional nutrition, but without knowing its biological properties attributed to their bioactive molecules such as polyphenol. There are reports on the antioxidative action of some acorn components like the skin and the endosperm that can contribute to the antioxidant ability [13]. The potential use of acorn oil appears to be promising, as indicated by a chemical composition that is rich in phytochemicals, especially sterols, tocopherols, and terpenic alcohols, suggesting possible applications in the pharmaceutical industry. α -tocopherol, a chain-breaking antioxidant that traps peroxy free radicals, is the principal and most potent lipid-soluble antioxidant in plasma and low-density lipoprotein [14] and is frequently present in *Quercus* species [13]. The potential of acorn oil as antioxidant supplement could add value to an underutilized agricultural product. For long, different parts of the *Quercus* tree have been used as natural remedy for the treatment of gastrointestinal inflammations and disorders. Extracts from leaves, bark and wood have shown antioxidant, antimicrobial, anti-inflammatory, antitumor and gastroprotective properties, attributed to their high content of phenolic compounds [15]. Also, the intake of polyphenols is associated with their beneficial bioactivity related to cardiovascular protection, inhibiting the oxidation of low-density lipoprotein cholesterol, and to glucose metabolism, promoting absorption and helping to prevent hyperglycemia [16-18].

In recent times, acorns oil has attracted the attention of the scientific community [19, 20]. The three primary fatty acids found in acorn oil are oleic (C18:1), linoleic (C18:2), and palmitic (C16:0) acids [21]. Tocopherols and phytosterols are present in high amounts in acorns oils [19, 22, 23].

Acorns oil has been used in the dietary industry since the nineteen sixties [21]. The oil is also used in cosmetic preparations and combined with other ingredients like avocado oil and beeswax to treat skin irritation and eczema [19]. Studies have shown that acorns oil possess similar nutritional quality and physicochemical properties as olive oil [19, 24, 25, 26].

The object of this research was to measure the total polyphenol and flavonoid of (defatted, non-defatted) extracts, the fatty acid composition of the oil extract of *Quercus ilex* fruit from Algeria, and to examine the antioxidant activity using DPPH, ABTS and phosphomolybdenum tests.

MATERIAL AND METHODS

Plant collection

Q. ilex fruits were directly gathered from two or three individual trees from Laghouat of Algeria. The

mature acorns were taken and identified by the Process Engineering Laboratory, University of Laghouat, Algeria. Collected fruits (acorns) were sorted and cleared of all impurities, separated from the shell, the oak acorns were subsequently in the shade and at room temperature then powdered using electric grinder and passed through a 425 μ m sieve, then conserved for future use.

Oil extraction

Q. ilex fruits oils were extracted using Soxhlet method. Acorn powder (10 g) was weighed into a cellulose extraction cartridge and the Soxhlet apparatus (JOAN Lab Glassware; China) containing the cartridge was fitted to a distillation flask containing 100 mL *n*-hexane. After 6 hours of extraction, the extract was filtered and dehydrated with anhydrous sodium sulphate and the solvent was evaporated under vacuum at 50°C [27]. The defatted of *Q. ilex* was left in a fume hood overnight to evaporate the remaining solvent and then stored in sealed containers at -18 to -16°C until use.

Preparation of phenolic extracts

Q. ilex fruits were thoroughly rinsed and air dried. They were ground to fine powder and 50 g of fruits was defatted with *n*-hexane and then extracted with ethanol for 72 hours. Another 50 g of fruits (non-defatted) was soaked in ethanol for 72 hours. All the filtrates of each extract were concentrated using rotary evaporator at 40°C. The crude extracts were weighed and stored at 4°C till further use.

Total phenolic content

Total phenolic content of the extract was determined by spectroscopic method using Folin-Ciocalteu's reagent [28]. A volume of 100 μ L of (defatted, non-defatted) extracts were added to 200 μ L of 1:10 diluted Folin-Ciocalteu's reagent and 2 mL of water. After 3 min, 1 mL of saturated sodium carbonate solution was added. After 2 hours of incubation at room temperature, the absorbance was measured at 765 nm using a UV-Vis-spectrometer (Specord 200 Plus, Analytik Jena, Jena, Germany). Simultaneously, a standard curve was prepared using various concentrations of gallic acid (ranging from 0.25 to 1 mg/mL) and subjected to the same reaction conditions. The results were expressed as milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g).

Measurement of flavonoid content

Aluminum-chloride colorimetric assay was used to determine the total flavonoid contents in the extract as previously reported by Shengwei et al. (2019) [29]. Briefly, 1 mL of (defatted, non-defatted) extracts

were mixed with the same volume of 2% aluminum trichloride (AlCl_3) solution and allowed to stand for 15 min. The absorbance of the mixture was then determined at 430 nm with a UV-Vis spectrometer (Specord 200 Plus, Analytik Jena, Jena, Germany). Simultaneously, a standard curve was prepared using various concentrations of rutin (ranging from 0.01 to 0.1 mg/mL) and subjected to the same reaction conditions. The data were expressed as milligrams of rutin equivalents per gram of dry extract (mg RE/g).

Antioxidant Activity

Determination of antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical

The antioxidant activity was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. Experiments were carried out according to the method of Apriliyanti et al. (2020) [30]. The reduction of the radical is followed by a decrease in the absorbance at 517 nm. A volume of 0.2 mL of extract was put into test tubes and 1.8 mL of DPPH solution was added. The tubes were covered with parafilm and kept in the dark for 1 h. Absorbance at 517 nm was measured with a UV-Vis spectrophotometer (Jasco V-530). Each assay was carried out in triplicate.

The inhibition activity (I(%)) was calculated as follows:

$$I(\%) = (A_0 - A_1) / A_0 \times 100$$

Where A_0 is the absorbance of the control, A_1 is the absorbance of the extract/standard. The extract concentration providing 50% of radical scavenging activity (IC_{50}) was calculated from the graph of inhibition percentage against extract concentration. Ascorbic acid, BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) were used as standards.

Determination of ABTS radical-scavenging

ABTS free radical activity was performed according to the method of Li et al. (2018) with slight modification [31]. ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) cation was produced by reacting ABTS solution (7 mM) with potassium persulfate (2.45 mM) and then the mixture to stand in dark (12-16 hours, at the room temperature). The mixture was diluted with ethanol to give an absorbance of 0.700 ± 0.02 at 734 nm for the study. The extract (15 μL) and ABTS solution (1.485 mL) were mixed. The mixture absorbance was read at 734 nm after the incubation period (15 min, at room temperature). The results were expressed as IC_{50} values.

Determination of total antioxidant capacity

Total antioxidant capacity (TAC) of extract was determined by the method reported by Kandasamy et al. (2021) [32]. The assay is based on the reduction of Mo (VI) to Mo (V) by samples and formation of green colored phosphate Mo (V) complex at acidic pH. 0.3 mL of extract was mixed with 3 mL of reaction mixture containing 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate into the test tubes. The test tubes were incubated at 95°C for 10 min to complete the reaction. After cooling at room temperature, extract absorbance was measured at 695 nm using a spectrophotometer against a blank solution.

Fatty acids composition

The fatty acid profile was determined by derivatization, followed by gas chromatography, coupled with mass spectrometry detection (GC-MS). The methyl esters were prepared by the following procedure: the fatty acids in the oils were esterified into methyl esters by saponification with 0.5 N methanolic NaOH and transesterified with 14% BF_3 (v/v) in methanol [33], then analysed by GC-MS.

GC-MS analysis

The analysis of the samples was carried out in the Technical Platform of Physico-Chemical Analysis (PTAPC-CRAPC)-Laghouat-Algeria, using a SHIMADZU GCMS-QP2020 Instruments, equipped with a fused Rxi®-5ms capillary column (Phase: Crossbond® 5% diphenyl/95% dimethyl polysiloxane). Its dimensions are: 30 m \times 0.25 mm and 0.25 μm film thickness. This column has similar phase to the following columns: HP-1ms, HP-1msUI, DB-1ms, DB-5ms, DB-1msUI, Ultra-1, VF-1ms, ZB-1, ZB-1ms and considered also equivalent to USP G1, G2, G38 phases. A volume of 0.5 μL of sample was injected in split mode (1:10). Injector and detector temperatures were maintained at 250°C and 300°C, respectively the column temperature was programmed at: 80°C fixed for 4 min then increased to 200°C with an increase increment of 3°C/min then fixed for 5 min, after that raised to 300°C with an increase increment of 10°C/min, and maintained at that temperature for 5 min. The carrier gas used was helium (99.995% purity) with a flow rate of 1 mL/min. The mass spectrometer conditions were as follow: ionization voltage 70 eV, ion source temperature 200°C, and electron ionization mass spectra were acquired over the mass range of 45-600 m/z [34].

Statistical analysis

All experiments were performed in triplicate. The values of different parameters were expressed as the mean \pm standard deviation (Mean \pm SD).

RESULTS

Total phenolic and flavonoid contents

As shown in Table 1, total phenolic contents (TPC) are given as gallic acid equivalents by reference to standard curve. TPC of *Q. ilex* fruits showed highly significant differences ($p \leq 0.05$) depending on extracts. Defatted *Q. ilex* extract showed a high phenolic content (634.36 ± 27.41 mg GAE/g of DW), which was higher than that found in non-defatted *Q. ilex* (469.92 ± 26.00 mg GAE/g of DW).

The results of the determination of flavonoids by the aluminum chloride method are presented in Table 1. TFC were determined as rutin equivalents (RE). Flavonoids content of defatted *Q. ilex* (96.85 ± 2.13 mg RE/g of DW) was higher ($p < 0.05$) than that determined in non-defatted *Q. ilex* extract (84.98 ± 9.15 μ g RE/mg dry extract).

Antioxidant capacity

DPPH radical scavenging activity

Substances which are able to perform reduction by either hydrogen or electron-donation can be considered as radical scavengers and therefore antioxidants. The color change degree of DPPH radicals from violet to yellow upon reduction indicates the radical scavenging potential of the antioxidant. Results showed that both extracts exerted considerable dose-dependent scavenging activity on DPPH radical (Table 2).

In this research, *Q. ilex* extract was being assessed its antioxidant activity as a free-radical scavenger (DPPH test) and expressed in IC_{50} value. An IC_{50} value was defined as extract concentration to show radical scavenging activity (RSA) of 50%. The highest antioxidant activity indicated by the lowest IC_{50} , ascorbic acid, BHA (butylated hydroxyanisole)

and (BHT) butylated hydroxytoluene were employed as positive controls.

However, defatted *Q. ilex* extract with $IC_{50} = 0.0080.0008$ mg/mL, was significantly more potent ($p < 0.05$) than non-defatted *Q. ilex* that gave an IC_{50} value of 0.13 ± 0.04 mg/mL, while the lowest ($IC_{50} = 0.70 \pm 1.07$ mg/mL) were observed in *Q. ilex* oil were found to be lower to ascorbic acid, BHA and BHT (0.0022 ± 0.0003 , 0.0033 ± 0.0005 and 0.0042 ± 0.0002 mg/mL).

ABTS radical scavenging activity

In this research, the conversions for the ABTS⁺ radical cation inhibition or hunting capacities of each *Q. ilex* samples in various extracts were investigated in comparison with the standard Trolox. The trial data for ABTS radical scavenging potential of each plant extract is shown in Table 2.

For the activity against ABTS radical the IC_{50} values showed the defatted *Q. ilex* as the most active with an estimated value of $IC_{50} = 0.005 \pm 0.001$ mg/mL, followed by the non-defatted *Q. ilex* extract (0.006 ± 0.001 mg/mL). The *Q. ilex* oil exhibit the lowest IC_{50} with a value of 0.016 ± 0.007 mg/mL. However, these values classified as slightly lower when compared with the reference substance (Trolox 0.004 ± 0.0002 mg/mL).

Thus, the ABTS scavenging effect increased in the order of *Q. ilex* oil < non-defatted *Q. ilex* < defatted *Q. ilex* < Trolox.

Total antioxidant capacity

PM assay is based on the reduction of phosphate Mo (VI) to phosphate Mo (V) by the sample and subsequent formation of a bluish green colored phosphate Mo (V) complex at acid pH. The phosphomolybdenum

Table 1. Total phenolic and flavonoids contents in *Q. ilex* extracts

<i>Q. ilex</i> extracts	Total phenolic content (mg GAE/g DW)	Total flavonoids content (mg RE/g DW)
Non defatted <i>Q. ilex</i>	469.92 ± 26.00	84.98 ± 9.15
Defatted <i>Q. ilex</i>	634.36 ± 27.41	96.85 ± 2.13

Table 2. Antioxidant activity of extracts for *Q. ilex*, expressed in IC_{50} (mg/mL) for DPPH and ABTS assay, VCEAC (vitamin C equivalents mmol of vitamin C/g dry weight) for phosphomolybdate assay

<i>Q. ilex</i> extracts	$IC_{50/DPPH}$ (mg/mL)	$IC_{50/ABTS}$ (mg/mL)	Phosphomolybdate assay VCEAC
Non defatted <i>Q. ilex</i>	0.13 ± 0.04	0.006 ± 0.001	0.13 ± 0.006
Defatted <i>Q. ilex</i>	0.008 ± 0.0008	0.005 ± 0.001	0.017 ± 0.004
<i>Q. ilex</i> oil	0.70 ± 1.07	0.016 ± 0.007	0.0007 ± 0.0003
Ascorbic acid	0.0022 ± 0.0003	ND	ND
BHT	0.0042 ± 0.0002	ND	ND
BHA	0.0033 ± 0.0005	ND	ND
Trolox	ND	0.004 ± 0.0002	ND

method is routinely applied in the laboratory to evaluate the total antioxidant capacity of plant extracts [35]. The total antioxidant capacity of the extracts was determined by the Phosphomolybdate method. The different extracts of *Q. ilex* (defatted, non-defatted and oil) showed different degrees of total antioxidant capacity (Table 2). Non defatted *Q. ilex* extract showed the highest total antioxidant capacity (VCEAC=0.13±0.006) and followed by defatted *Q. ilex* (VCEAC=0.017±0.004). While, the *Q. ilex* oil indicated the lowest total antioxidant capacity (VCEAC=0.0007±0.0003). This capacity might be associated to the existence of antioxidant compounds in *Q. ilex*.

Fatty acid compositions

The fatty acid composition of acorn oil was presented in Table 3. As shown, the fatty acids in acorn oil were saturated fatty acids (SFA) and unsaturated (UFA). The total contents of SFA, monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) were 16.28%, 66.80% and 16.64%, respectively. The most abundant fatty acids determined in the acorn oil samples were oleic acid (C18:1) (65.38%), 9,12-octadecadienoic acid (C18:2) (16.64%) and palmitic acid (C16:0) (12.81%). Furthermore, minor quantities of 11-eicosenoic acid (C20:1), methylnonadecanoic acid (C20:0), cis-13-octadecenoic acid, (C18:1) and 20-methyl-heneicosanoic acid (C22:0) were detected (below 0.5%).

DISCUSSION

The results generally of phenolic confirmed that the ethanol solvent (non-defatted) extract provide satisfactory phenolic content and defatted extract

Table 3. The fatty acid compositions (%) of *Q. ilex*

Peak no.	Compound name	Area %
1	Palmitic acid (C16:0)	12.81
2	9,12-octadecadienoic acid (C18:2)	16.64
3	Oleic acid (C18:1)	65.38
4	Vaccenic acid (C18:1)	1.00
5	Stearic acid (C18:0)	3.05
6	11-eicosenoic acid (C20:1)	0.32
7	Methylnonadecanoic acid (C20:0)	0.32
8	Cis-13-octadecenoic acid (C18:1)	0.10
9	20-methyl-heneicosanoic acid (C22:0)	0.10
	∑ SFA	16.28
	∑ MUFA	66.80
	∑ PUFA	16.64

SAFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids

were found to be more effective for isolation and determination of phenolic compounds from different plant materials; and this statement is a confirmation for our findings reported in the present study. The higher phenolic content of the defatted compared with the non-defatted extracts showed that defatting the plant before extraction could enhance the availability of phenols in the plant (Table 1). The higher content of phenols in the defatted extracts of all the plants compared to the non-defatted extracts could be as a result of the removal of fatty substances from the extracts making the phenolic compounds more polar.

Noteworthy, results observed in the present work were higher than those previously reported by Zarroug et al. (2021) [36]. TPC and TFC of Tunisian *Q. ilex* flour were 23.56 mg GAE/g DW and 7.08 mg CE/g DW. The study of Custódio et al. (2013) [37] on acorns collected from Portugal, revealed lower contents of TPC and TCT, but higher levels of TFC. However, the content of TPC was in accordance with that (33.11%) registered by Masmoudi et al. (2020) on *Q. suber* on acorns collected from Portugal, revealed lower contents of TPC and TCT, but higher levels of TFC. However, the content of TPC was in accordance with that (33.11%) registered by Masmoudi et al (2020) on *Q. suber* [38].

Taib et al. (2024) reported that the total polyphenol contents were found to be 98.3 and 212.5 mg GAE/g of dry extract for *Q. faginea* and *Q. rotundifolia*, respectively. While, the total flavonoid contents were 6.2 and 10.3 mg/g of dry extract for *Q. faginea* and *Q. rotundifolia*, respectively [39].

Recent research reported that the flavonoid contents were ranged between 212.26 and 279.82 mg CE/kg dry weight and 122.99 and 131.6 mg CE/kg of oil for *Q. flour* and *Quercus* oil, respectively. The significant higher amount was obtained in *Q. ilex* species for the two different extracts [39]. These results are close to that found by Sousa et al. (2021) who showed that the bark ethanol extracts *Q. rotundifolia* bark had a high proportion of phenolic compounds (572.8 mg GAE/g extract; 3.7 g GAE/g dry bark), in which flavonoids constituted the major classes (247.6 mg CE/g extract. While, the bark water extracts contained a much lower amount of phenolic compounds; total phenolics 219.5 mg GAE/g of extract, flavonoids 162.5 mg CE/g of extract) [40].

DPPH is routinely used to evaluate the free radical scavenging capacity of chemical compounds. This assay enlightens the effect of the antioxidant molecules toward a stable free radical through a known mechanism involving a hydrogen atom transfer coupled to a fast electron transfer. Phenolic compounds scavenge DPPH by their ability to form o-quinone intermediates upon free radical H-atom abstraction and its subsequent disproportionation [41].

Santos et al. (2010) tested the scavenging activity of different extracts prepared from cork of *Q. suber* using DPPH radicals and reported an IC_{50} values ranging from 2.79 to 5.84 $\mu\text{g}/\text{mL}$ [42]. According to studies conducted by Makhoulouf et al. (2019) the flour extract of *Q. ilex* species exhibited the strongest scavenging capacity on DPPH (52.62 g TE/g dry weight) [27]. The obtained data on the antioxidant activity shows that the methanolic extract of acorn can be used to reduce the stable DPPH radical with IC_{50} values of 318 $\mu\text{g}/\text{ml}$ [36]. *Quercus* extract exhibited the strongest scavenging capacity against DPPH radicals. These findings were directly related to the higher content of phenolic and flavonoid compounds.

ABTS scavenging activity is also one of the most commonly used method to evaluate the antioxidant properties in fruits and plants. This activity varied from 1.27 to 3.23 mg TE/g for acorn oil and from 36.19 to 44.50 mg TE/g for acorn flour as reported by Makhoulouf et al. (2019) [27].

The significant antioxidant activity of *Quercus* fruit is due to the inductive effect of the natural antioxidants present in the fruit such as phenolic compounds and flavonoids which reduce and discolor free radicals (ABTS⁺) because of their ability to yield hydrogen [43, 44].

Phosphomolybdate is another important *in vitro* antioxidant assay to access the total antioxidant capacity of the plant extract. The assay principal follows the conversion of Mo (VI) to Mo (V) by extract or the compound which possess antioxidant potential resulting in green phosphate Mo (V). The electron/hydrogen donating pattern of antioxidants depends upon its structure and series of redox reactions occurring in the activity [45]. Our findings showed that non defatted *Q. ilex* extract has good antioxidant potential due to presence of flavonoid and phenolic contents. Phosphomolybdenum assay showed significant correlation with total flavonoid contents as well as total phenolic contents. Jan et al., also reported the best phosphomolybdenum activity of aqueous extract and a significant correlation with TPC and TFC [46].

The saturated and unsaturated fatty acids are influenced by environmental conditions, such as temperature, rainfall, and genotypes [47]. The amount of fatty acids, on the genotypic, is one of the most influential factors [48, 49]. Some authors have observed, that fatty acid contents were significantly influenced by years, various physiological, geographical, ecological and cultural factors [48,50]. Indeed, the level, of this fatty acid, has been shown to be greatly influenced by the impact of abiotic conditions, such as water availability [51].

Since the acorn oil was rich in both oleic and linoleic acids, it might be considered healthier for the

human diet [52]. It has long been acknowledged that plant oils containing relatively low concentrations of omega-6, and higher levels of MUFA (mainly oleic acid) may contribute to the lower rate of CHD and a nutritional perspective [53].

The minor difference in fatty acid levels noticed within the same species could be explained by various factors, including oak acorn maturity, differences in oil processing, environmental conditions, or different harvest dates [52, 22]. As previously stated, the main MUFA found in acorn oils is oleic acid. Numerous studies have clearly demonstrated the health benefits of this fatty acid, particularly in reducing cardiovascular disease risk and preventing type 2 diabetes mellitus, besides contributing to improving hemostasis, glucose metabolism, and endothelial dysfunction [54]. Zarroug et al. (2021) also determined the MUFA and PUFA were 67.94% and 17.35%, respectively. The most abundant fatty acids were oleic acid (67.2%), linoleic acid (16.46%) and palmitic acid (11.51%) [36].

Karabas (2013) showed myristic, arachidonic, palmitoleic and gadoleic acids were minor fatty acids constituting 0.09%, 0.37%, 0.08% and 0.61% [55]. Furthermore, the main fatty acids in acorn oils of *Quercus* were oleic (54.77%, 49.88), linoleic (23.04%, 28.25%), and palmitic acid (18.23%, 17.57%) in *Q. ilex* and *Q. coccifera* species, respectively [56]. However, Santos et al. (2003) reported the concentration of oleic acid was >63% of total fatty acids in all cases, followed by palmitic and linoleic acids at similar concentrations (12-20%) [13]. The most abundant fatty acids of *Q. aegilops* (QA), *Q. infectoria* (QI), and *Q. calliprinus* (QC) were oleic (53.3-56.1%), linoleic 21.3-23.4%, palmitic 17.8-18.7%, linolenic 1.5-1.6 %, and stearic acid 1.02-1.60% [19].

CONCLUSION

According to the current study, mature fruits of *Q. ilex* are revealed to be an interesting source for the production of oil. Furthermore, acorns possessed rich sources of potentially functional components (such as flavonoids and phenolics) and showed higher antioxidant capacity. Fatty acid composition showed the richness of oil with more than 67% of unsaturated fatty acids playing the potential preventative role in reducing the coronary heart disease and cancer. Results showed that *Q. ilex* oils were generally characterized as high methyl oleate. All these good qualities make acorn oil a suitable ingredient for many industrial applications in food, pharmaceutical, nutraceutical and cosmetic domains. Highlighting the good chemical composition, oxidative stability and the richness in natural antioxidants of acorn oil.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

The authors are grateful to the DGRSDT and the Ministry of Higher Education and Scientific Research of Algeria for PRFU Project (A16N01UN030120230001), and the team of Medicinal Chemistry Department, Theodor Bilharz Research Institute, Egypt.

REFERENCES

- Bakchiche B, Gherib A, Bronze MR, Ghareeb MA. Identification, quantification, and antioxidant activity of hydroalcoholic extract of *Artemisia campestris* from Algeria. *Turk J Pharm Sci.* 2019;16(2):234-239. doi: 10.4274/tjps.galenos.2018.99267.
- Cheraif K, Bakchiche B, Gherib A, Bardaweel SK, Ayvaz MC, Flamini G, et al. Chemical composition, antioxidant, anti-tyrosinase, anti-cholinesterase and cytotoxic activities of essential oils of six Algerian plants. *Molecules.* 2020;25(7):1710. doi:10.3390/molecules25071710.
- Bakchiche B, Temizer İK, Güder A, Çelemlı ÖG, Yegin SÇ, Bardaweel SK, et al. Ghareeb MA. Chemical composition and biological activities of honeybee products from Algeria. *J Appl Biotechnol Rep.* 2020;7(2):93-103. doi: 10.30491/JABR.2020.109498.
- Bakchiche B, Guenane H, Sahin B, Öztürk M, Ghareeb MA, Miguel MG. Fatty acid, mineral content and antioxidant activities of Algerian fat bee pollen. *Nova Biotechnol Chim.* 2020;19(2):208-215. doi: 10.36547/nbc.v19i2.738.
- Bireche M, Bakchiche B, Gherib A, Gil-Izquierdo A, Domínguez-Perles R, Ghareeb MA. Fatty acid and amino acid composition of *Citrullus colocynthis* seeds growing in Algeria. *Egypt J Chem.* 2021;64(8):4727-4737. doi: 10.21608/EJCHEM.2021.73414.3627.
- Bakchiche B, Gören AC, Aydoğmuş Z, Mataraci-Kara E, Ghareeb MA. *Artemisia campestris* and *Artemisia herba-alba*: LC-HRESI-MS profile alongside their antioxidant and antimicrobial evaluation. *Acta Pharm Sci.* 2022;60(2):131-152. doi: 10.23893/1307-2080.APS.6010.
- Kemal ME, Bakchiche B, Kemal M, Cheraif K, Kara Y, Bardaweel SK, et al. Six Algerian plants: Phenolic profile, antioxidant, antimicrobial activities associated with different simulated gastrointestinal digestion phases and antiproliferative properties. *J Herb Med.* 2023;38:100636. doi: 10.1016/j.hermed.2023.100636.
- Teggar N, Bakchiche B, Abdel-Aziz ME, Bardaweel SK, Ghareeb MA. Chemical composition and biological evaluation of Algerian propolis from six different regions. *Jordan J Pharm Sci.* 2023;16(2):184-197. doi: 10.35516/jjps.v16i2.1319.
- Mohamed-Benkada M, Achira DY, Ghareeb MA. Assessment of antimicrobial activity and mycelium insights of *Terfezia clavaryi* truffle harvested from Tindouf desert. *J Appl Pharm Sci.* 2024;14(02):161-173. doi: 10.7324/JAPS.2024.169988.
- Hoeche U, Kelly A, Noci F. Acorn: Staple food from the past or novel food for the future? An investigation into the desirability and acceptability of acorn flour products. *Dublin Gastronomy Symposium.* Dublin; 2014. p. 1-14.
- Bainbridge DA. Acorns as food: History, use, recipes, and bibliography [Internet]. *Scotts Valley, CA: Sierra Nature Prints;* 2006. Available from: https://www.academia.edu/3829415/Acorns_as_Food_Text_and_Bibliography.
- Rakić S, Petrović D, Tešević V, Simić M, Maletić R. Oak acorn, polyphenols and antioxidant activity in functional food. *J Food Eng.* 2006;74(3):416-423. doi: 10.1016/j.jfoodeng.2005.03.057.
- Cantos E, Espin J, Carlos-Lopez BC, Delahoz L, Ordonez JA, TomasBarberan FA. Phenolic compounds and fatty acids from acorns (*Quercus* spp.), the main dietary constituent of free ranged Iberian pigs. *J Agric Food Chem.* 2003;51:6248-6255. doi: 10.1021/jf030216v.
- Kaul N, Devaraj S, Jialal I. α -Tocopherol and atherosclerosis. *Evid Based Med.* 2001;226:5-12. doi: 10.1177/153537020122600102.
- Karioti A, Bilia AR, Gabbiani C, Messori L, Skaltsa H. Proanthocyanidin glycosides from the leaves of *Quercus ilex* L. (Fagaceae). *Tetrahedron Lett.* 2009;50(16):1771-1776. doi: 10.1016/j.tetlet.2009.01.158.
- Ghareeb MA, Mohamed T, Saad AM, Refahy LA, Sobeh M, Wink M. HPLC-DAD-ESI-MS/MS analysis of fruits from *Firmiana simplex* (L.) and evaluation of their antioxidant and antigenotoxic properties. *J Pharm Pharmacol.* 2018;70:133-142. doi: 10.1111/jphp.12843.
- Ghareeb MA, Sobeh M, Rezaq S, El-Shazly AM, Mahmoud MF, Wink M. HPLC-ESI-MS/MS profiling of polyphenolics of a leaf extract from *Alpinia zerumbet* (Zingiberaceae) and its anti-inflammatory, anti-nociceptive, and antipyretic activities *in vivo*. *Molecules.* 2018;23:3238. doi: 10.3390/molecules23123238.
- Sobeh M, Mahmoud MF, Hasan RA, Abdelfattah MAO, Sabry OM, Ghareeb MA, et al. Tannin-rich extracts from *Lannea stuhlmannii* and *Lannea humilis* (Anacardiaceae) exhibit hepatoprotective activities *in vivo* via enhancement of the anti-apoptotic protein Bcl-2. *Sci Rep.* 2018;8:9343. doi: 10.1038/s41598-018-27452-8.
- Al-Rousan WM, Ajo RY, Al-Ismail KM, Attlee A, Shaker RR, Osaili TM. Characterisation of acorn fruit oils extracted from selected Mediterranean *Quercus* species. *Fats Oils.* 2013;64(5):554-560. doi: 10.3989/gya.023313.
- Makhlouf FZ, Squeo G, Barkat M, Trani A, Caponio F. Antioxidant activity, tocopherols and polyphenols of acorn oil obtained from *Quercus* species grown in Algeria. *Food Res Int.* 2018;114:208-213. doi: 10.1016/j.foodres.2018.08.010.
- Lopes IM, Bernardo-Gil MG. Characterisation of acorn oils extracted by hexane and by supercritical carbon dioxide. *Eur J Lipid Sci Technol.* 2005;107(1):12-19. doi: 10.1002/ejlt.200401039.

22. Górnas P. Oak *Quercus rubra* L. and *Quercus robur* L. acorns as an unconventional source of gamma- and beta-tocopherol. *Eur Food Res Technol.* 2019;245(1):257-261. doi: 10.1007/s00217-018-3150-0.
23. Rabhi F, Narváez-Rivas M, Tlili N, Boukhchina S, León-Camacho M. Sterol, aliphatic alcohol and tocopherol contents of *Quercus ilex* and *Quercus suber* from different regions. *Ind Crops Prod.* 2016;83:781-786. doi: 10.1016/j.indcrop.2015.11.020.
24. Taib M, Bouyazza L, Lyoussi B. Acorn Oil: Chemistry and Functionality. *J Food Qual.* 2020;2020:8898370. doi: 10.1155/2020/8898370.
25. Makhlouf FZ, Squeo G, Difonzo G, Faccia M, Pasqualone A, Summo C, et al. Effects of storage on the oxidative stability of acorn oils extracted from three different *Quercus* species. *J Sci Food Agric.* 2021;101(1):131-138. doi: 10.1002/jsfa.10623.
26. Charef M, Yousfi M, Saidi M, Stocker P. Determination of the fatty acid composition of acorn (*Quercus*), *Pistacia lentiscus* seeds growing in Algeria. *J Am Oil Chem Soc.* 2008;85(10):921-924. doi: 10.1007/s11746-008-1283-1.
27. Makhlouf FZ, Squeo G, Barkat M, Pasqualone A, Caponio F. Comparative study of total phenolic content and antioxidant properties of *Quercus* fruit: flour and oil. *Nor Afr J Food Nutr Res.* 2019;03(05):148-155. doi: 10.51745/najfnr.3.5.148-155.
28. Kuhkheil A, Naghdi BH, Mehrafarin A, Abdossi, V. Phytochemical and morpho-physiological variations in sea buckthorn (*Hippophae rhamnoides* L.) populations of Taleghan region in Iran. *J Med Plants.* 2020;19(76):21-35. doi: 10.29252/jmp.19.76.21.
29. Shengwei S, Jian H, Meijuan L, Guangling Y, Xuguang Z. A great concern regarding the authenticity identification and quality control of Chinese propolis and Brazilian green propolis. *J Food Nutr Res.* 2019;7(10):725-735. doi: 10.12691/jfnr-7-10-6.
30. Apriliyanti MW, Ardiyansyah M, Febrianti W, Arum P, Jayus SA. Effect of time and temperature on vitamin C contents and antioxidant activity in the hot water extraction of melinjo peels. *In vitro biological evaluation and phytochemical contents of three *Centaurea* L. species growing from Eastern Anatolia in Turkey.* *J Agric Nat.* 2020;23(1):148-156. doi: 10.1088/1755-1315/980/1/012045.
31. Li Y, Jianyan C, Lili C, Liang L, Fang W, Zhengping L, et al. Characterization of a novel polysaccharide isolated from *Phyllanthus emblica* L. and analysis of its antioxidant activities. *J Food Sci Technol.* 2018;55(7):2758-2764. doi: 10.1007/s13197-018-3199-6.
32. Kandasamy S, Cengiz S, Saliha SS, Rabia BS, Wang M-H. Phytochemical composition, antioxidant, and enzyme inhibition activities of methanolic extracts of two endemic *Onosma* species. *Plants.* 2021;10:1373. doi: 10.3390/plants10071373.
33. Berber A, Zengin G, Aktumsek A, Aydin Sanda M, Uysal T. Antioxidant capacity and fatty acid composition of different parts of *Adenocarpus complicatus* (Fabaceae) from Turkey. *Rev Biol Trop.* 2014;62(1):337-46. doi: 10.15517/rbt.v62i1.7887.
34. Bouafia M, Benarfa A, Gourine N, Yousfi M. Seasonal variation of fatty acid composition, tocopherol content and lipid antioxidant activity of extracts from *Centaurea* sp. *Food Bioscience.* 2020;37:100728. doi: 10.1016/j.fbio.2020.100728.
35. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Anal Biochem.* 1999;269:337-341. doi: 10.1006/ABIO.1999.4019.
36. Zarroug Y, Sriti J, Boulares M, Mejri J, Sfayhi D, Hamdaoui G, et al. Chemical composition and sterol profile of Tunisian *Quercus ilex* oil. *La Rivista Italiana delle Sostanze Grasse.* 2021;98:145-153.
37. Custódio L, Patarra J, Neng NR, Nogueira JMF, Romano A. Extracts from *Quercus* sp. acorns acts neuroprotective *in vitro* by inhibiting cholinesterase and protecting SHSY5Y cells from hydrogen mediated cytotoxicity. *Ind Crops Prod.* 2013;45:114. doi: 10.1016/j.indcrop.2012.12.011.
38. Masmoudi M, Besbes S, Bouaziz MA, Khelifi M, Yahyaoui D, Attia H. Optimization of acorn (*Quercus suber* L.) muffin formulations: Effect of using hydrocolloids by a mixture design approach. *Food Chem.* 2020;328:127082. doi: 10.1016/j.foodchem.2020.127082.
39. Taib M, Damiri F, Rezzak Y, Berrada M, Bouyazza L. Chemical composition, nutritional, and antioxidant activity of two *Quercus* species acorns growing in Morocco. *Lett Appl Nano BioSci.* 2024;13(1):15. doi: 10.33263/LIANBS131.020.
40. Sousa V, Ferreira JPA, Miranda I, Quilhó T, Pereira H. *Quercus rotundifolia* bark as a source of polar extracts: Structural and chemical characterization. *Forests.* 2021;12:1160. doi: 10.3390/f12091160.
41. Berraouan A, Ziyat A, Mekhfi H, Sindic M, Fauconnier M-L, Legssyer A, et al. Chemical Composition of Cactus Pear Seed Oil: phenolics identification and antioxidant activity. *J Pharmacopuncture.* 2022;25(2):121-129. doi: 10.3831/KPI.2022.25.2.121.
42. Santos S, Pinto PR, Silvestre AJD, Neto CP. Chemical composition and antioxidant activity of phenolic extracts of cork from *Quercus suber* L. *Ind Crops Prod.* 2010;31(3):521-526. doi: 10.1016/j.indcrop.2010.02.001.
43. Servili M, Esposto S, Fabiani R, Urbani S, Taticchi A, Mariucci F, et al. Phenolic compounds in olive oil: antioxidant, health and Organoleptic activities according to their chemical structure. *Inflammopharmacology.* 2009;17(2):76-84. doi: 10.1007/s10787-008-8014-y.
44. Turkmen N, Velioglu YS, Sari F, Polat G. Effect of extraction conditions on measured total polyphenol contents and antioxidant and antibacterial activities of black tea. *Molecules.* 2007;12(3):484-96. doi: 10.3390/12030484.
45. Gupta K, Maurya S, Agarwal S, Kushwaha A, Kumar R. Antioxidant assessment of extracts obtained through hot extraction process. *Cell Mol Biol.* 2016;62:129. doi: 10.4172/1165-158X.1000129.

46. Jan S, Khan MR, Rashid U, Bokhari J. Assessment of antioxidant potential, total phenolics and flavonoids of different solvent fractions of *Monotheca buxifolia* fruit. *Osong Public Health Res Perspect.* 2013; 4:246-254. doi: 10.1016/j.phrp.2013.09.003.
47. Sadeghi H, Talaii AR. Impact of environmental conditions on fatty acids combination of olive oil in Iranian olive CV, Zard. *Acta Hort.* 2000;586:579-581. doi: 10.17660/ActaHortic.2002.586.121.
48. Uzun B, Ulger S, Cagirgan IM. Comparison of determinate and indeterminate types of sesame for oil content and fatty acid composition. *Turk J Agric For.* 2002;26:269-274.
49. Tulukcu E. A comparative study on fatty acid composition of black cumin obtained from different regions of Turkey, Iran and Syria. *Afr J Agric Res.* 2011;6(4):892-895. doi: 10.5897/AJAR10.286.
50. Al-Rousan WM, Ajo RY, Al-Ismaïl KM, Attlee A, Shaker RR, Osaili TM. Characterization of acorn fruit oils extracted from selected Mediterranean *Quercus* species. *Grasas y Aceites.* 2013;64(5):554-560. doi: 10.3989/gya.023313.
51. Msaada K, Hosni K, Ben Taarit M, Chahed T, Hammami M, Marzouk B. Changes in fatty acid composition of coriander (*Coriandrum sativum* L.) fruit during maturation. *Ind Crops Prod.* 2009;29(2-3):269-274. doi: 10.1016/j.indcrop.2008.05.011.
52. Laribi B, Kouki K, Sahli A, Mougou A, Marzouk B. Essential oil and fatty acid composition of a Tunisian caraway (*Carum carvi* L.) seed ecotypes cultivated under water deficit. *Adv Environ Biol.* 2011;5(2):257-264.
53. Ryan E, Galvin K, O'Connor TP, Maguire AR. Phytosterol, squalene, tocopherol content and fatty acid profile of selected seeds, grains, and legumes. *Plant Food Hum Nutr.* 2007;62(3):85-91. doi: 10.1007/s11130-007-0046-8.
54. Riley T, Petersen K, Kris-Etherton P. Health aspects of high-oleic oils. *High Oleic Oils.* 2022;201-243. doi: 10.1016/B978-0-12-822912-5.00002-2.
55. Karabas H. Biodiesel production from crude acorn (*Quercus frainetto* L.) kernel oil: An optimisation process using the Taguchi method. *Renew Energy.* 2013;53:384-388. doi: 10.1016/j.renene.2012.12.002 .
56. Lassoued R, Abderrabba M, Mejri J. Comparative chemical composition of two *Quercus* species seeds growing in Tunisia. *S Afr J Bot.* 2021;146(10):71-76. doi: 10.1016/j.sajb.2021.10.003.

Received: 02.07.2024

Revised: 19.08.2024

Accepted: 21.08.2024

Published online first: 13.09.20243