

Comparison and Evaluation of Oil Content, Composition and Antioxidant Properties of *Pistacia atlantica* and *Pistacia khinjuk* Grown as Wild

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Abstract

The growing demands of consumers for edible seed oils containing high unsaturated fatty acid and antioxidant content have resulted in considerable efforts to investigate plants as possible sources of oils and nuts. In this research, the amount of fatty acid compositions, total flavonoid, phenol and antioxidant properties of *Pistacia atlantica* and *Pistacia khinjuk* were evaluated. The kernel oil content of *P. atlantica* and *P. khinjuk* were $24.33 \pm 0.333\%$ and $31.00 \pm 0.577\%$, respectively. Palmitic acid, Stearic acid, Oleic acid, Linoleic acid, and Palmitoleic acid were the main components in the oil of the two *Pistacia* species. The results showed that unsaturated fatty acids accounted for approximately 77.65% and 74.87% of total fatty acids in *P. atlantica* and *P. khinjuk*, respectively. The two *Pistacia* species were rich in phenolic compounds (130.77 ± 3.11 and 126.91 ± 4.41 mg quercetin/100 g oil) and had high antioxidant properties (4.545 ± 0.655 and 15.733 ± 0.689 mg/g oil) in *P. atlantica* and *P. khinjuk*, respectively. Oil content and Oleic acid of the two species of *Pistacia* are shown/known to be higher than some other edible oils. This research showed that the kernel oil of the two species of *Pistacia* have the same value in terms of quality, taste and natural antioxidant qualities with other edible oils.

Keywords: DPPH, Fatty acids, Flavonoid, Phenolic content, *Pistacia*.



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Introduction

The genus *Pistacia* belongs to the Anacardiaceae family, which contains about 15 species. Among them, *P. atlantica*, *P. vera* and *P. khinjuk* are grown as wild in Iran

(Mozaffarian, 2005). *P. atlantica* and *P. khinjuk*, are also known in Persian as "Baneh" and "Kolkhoung", respectively, which are widely distributed in the Zagrossian region of Iran (Bozorgi et al., 2013; Ezatpour et al., 2015). Different parts of these plants, including the fruit, leaf, resin,

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bark and aerial parts, have been largely used as folk medicines for the treatment of various health problems related to cutaneous, respiratory, gastrointestinal, infectious and renal diseases (Soleiman-Beigi and Arzehgar, 2013; Ezatpour et al., 2015). Their fruits and oil are eaten as nuts, and are used for the treatment of heart, stomach and respiratory system disorders, wound healing and gastrointestinal disorders (Hatamnia et al., 2016 a and b).

Previous studies have shown antioxidant, anti-inflammatory and antimicrobial properties of *P. atlantica* and *P. khinjuk* fruit, as well as revealed that they are rich in fatty acids such as Linoleic acid and Oleic acid (Dorehgirae and Pourabdollah, 2015; Hacıbekiroğlu et al., 2015).

These species of *Pistacio* grow to 5-15 m tall. The leaves are alternate and pinnate, and can be either evergreen or deciduous, depending on species. The mentioned species of *Pistacia* are known as the origin of Pistachio. Fruits of *P. atlantica* and *P. khinjuk* used as edible wild nuts. From ancient times, the fruits of these plants have been used as snacks and in cooking (Ghasemi Pirbalouti and Aghae 2011; Bozorgi et al., 2013; Hacıbekiroğlu et al., 2015).

Vegetable oils are normally extracted from plant fruits and have high nutritional value. In addition to widespread uses in human diet and also in industry, the oils have many applications in the pharmaceutical industry due to the numerous therapeutic effects of their natural ingredients. Lack of essential fatty acids in the human diet can be responsible for the development of disorders such as cardiovascular disease, viral infections, certain types of cancers, eczema, diabetes mellitus, rheumatoid arthritis and autoimmune diseases (Moazzami et al., 2015).

The content of bioactive compounds in the seed oils is affected by climatic conditions, species and variety of the *Pistacia* genus (Asadollahzadeh and Shamspur, 2013; Hatamnia et al., 2016a;

Hatamnia et al., 2016b; Tavakoli et al., 2016;).

The most fascinating oleaginous compounds in different species of the *Pistacia* fruit oil include unsaturated fatty acids, phenolic compounds, tocopherols, and phytosterols (Tavakoli and Pazhouhanmehr 2010). Among the *Pistacia* seed oil fatty acids, Oleic acid level was found to be higher than most of the other frequently used edible oils (Salvador et al., 2019).

Pistacia nuts are considered as a great source of biologically active compounds, due to their high essential unsaturated fatty acid content, as well as phenolic compounds (Ghasemynasabparizi et al., 2015; Martínez et al., 2016). Plant oil antioxidants play an important role in protecting health. Among seed oils, *Pistacia* spp exhibit interesting nutritional properties because they contain cardio-protective constituents, such as high Oleic acid, phytosterols, phenolics, tannins, flavonoid and tocopherols, make them as high antioxidant food product (Amarowicz et al., 2004; Matthäus and Özcan, 2006; Ramadan, 2019).

In light of the above mentioned facts, antioxidant activities and phenolic compounds in genus *Pistacia* have been the subject of various research studies (Farhoosh et al., 2011; Gourine et al., 2010; Hatamnia et al., 2014; Hatamnia et al., 2016a; Hatamnia et al., 2016b; Hossein et al., 2005; Tomaino et al., 2010).

Regarding the fact that *P. khinjuk* and *P. atlantica* nuts and oils are widely used, as well as by their potential nutritional properties, resulting in novel gourmet and healthy oils, with added value for the consumers as compared to the most common industrial refined vegetable oils, the present study was conducted to provide information on the content and composition of the oil in two *Pistacia* species, and secondly to evaluate the total phenol and flavonoid content, also their antioxidant activity as a source of edible or non-edible oils.

Materials and Methods

Plant material

To determine the amount of oils, fatty acid compositions and antioxidant properties of two *Pistacia* species in Iran including *P. atlantica* and *P. khinjuk* were investigated. Fruits were collected in September of 2017 from natural forests in the mountains surrounding Kazerun city (Fars Province; Latitude 30° 2' 42.21" N and longitude 52° 16' 34.44" E, at an elevation of 860 meter). The sampling was done by randomized collection from 10–15 trees in an area of about 5000 m². Fruits were isolated manually from the panicle in the laboratory to obtain a weight of 1000–1200 g for each sample.

Oil extraction

The fruits were milled using a grinder to obtain a fine powder. 30 g of a powdered sample from each species of plant fruits were extracted with n-hexane, using a Soxhlet extractor for 8 h. After extraction, the solvent was evaporated under reduced pressure at 60 °C to compute oxidizability (Cox), values for the oil samples were computed according to the formula proposed by Fatemi and Hammond (1980): $Cox = [1(18 : 1\%) + 10.3(18 : 2\%) + 21.6(18 : 3\%)]/100$

Fatty acid profile

Fatty acid composition was transformed into the corresponding methyl esters (FAME) using the method proposed by Metcalf et al. (1966), and analyzed using a gas chromatography system (Unicam 4600) equipped with a FID detector. A fused silica capillary column BPX70 [(30 m×0.22 mm i.d, 0.25 µm film thickness (SGE))] was used as the stationary phase and 0.2 µL of the FAME sample was injected into the chromatograph using a micro-syringe. Helium was the carrier gas with a head pressure of 18 psi and injector and detector temperatures were adjusted at 250 and 300 °C, respectively. Oven temperature was set to 160 °C for 5 min

and subsequently increased by 2.0 °C/min to 200 °C and held at that level for 40 min. The FAME samples were identified through comparison of standards Sigma-Aldrich and the closest match to the retention time data. Fatty acid patterns were calculated and evaluated from the identified fatty acids.

Total phenol content

Total phenolic compounds were determined using Folin-Ciocalteu reagent (Liu and Yao, 2007). Briefly, 0.5 mL diluted extract solution was shaken for 1 min in a reaction mixture contained 250 µL of Folin–Ciocalteu reagent, and 3 mL of distilled water. After 3 min, 2 mL of 20% Na₂CO₃ was added and the mixture was shaken once again for 0.5 min. Absorbance was determined at 750 nm after 1.5 h of reaction at ambient temperature. After which total phenolic content was expressed as mg Gallic acid equivalent per gram of the oil using the standard curve.

Total flavonoid content

Total flavonoid content was determined by utilizing aluminum chloride (AlCl₃) and using quercetin as a standard (Ordóñez et al., 2006) The plant extract (400 µL) was added to 0.3 mL distilled water followed by 5% NaNO₂ (0.03 mL). After 5 min rest at 25 °C, AlCl₃ (0.03 mL, 10%) was added. After a further 5 min rest, the reaction mixture was treated with 0.2 mL of 1 mM NaOH. Finally, the reaction mixture was diluted to 1 mL with water and the absorbance was measured at 510 nm. The results were expressed as mg quercetin QE/g of extract.

DPPH (2, 2'-diphenyl-1-picrylhydrazyl) radical scavenging assay

Antioxidant activity was measured through use of the DPPH free radical scavenging method (Akroum et al., 2010). The stock solution of the oil was prepared at 2 mg/mL of ethanol. Then a serial dilution of 50% was made to obtain 8 concentration ranges (1000–7.8 µg/mL).

After that, 550 μL of the DPPH solution (1 mg/10 mL) was mixed with 50 μL of the oil solution. After 30 min of incubation in the dark, at room temperature, the absorbance of the mixture was read at 517 nm using a spectrophotometer. Radical scavenging activity was gauged based on the following equation:

$$\text{Percentage of radical scavenging activity} = (\text{Abs}_{\text{blank}} - \text{Abs}_{\text{sample}}) / \text{Abs}_{\text{blank}} \times 100$$

where A_{blank} is the absorbance of the control reaction, and A_{sample} is the absorbance of the test compound. The extract IC50 values were calculated from the graph plotting inhibition percentage against extract concentration.

β -carotene-linoleic acid assay

The antioxidant activity of the extract was determined using β -carotene Linoleic acid model system (Jayaprakasha et al., 2001). The carotene solution was prepared by dissolving 0.2 mg mL^{-1} in chloroform. All the samples were assayed in triplicate. The antioxidant activity (AA) was calculated in terms of the percentage inhibition relative to the control, using the following equation:

$$\text{AA} = (\text{R}_{\text{control}} - \text{R}_{\text{sample}}) / \text{R}_{\text{control}} \times 100.$$

where R_{Control} and R_{Sample} are average bleaching rates of the negative control and the antioxidant (plant extracts or BHT), respectively.

Statistical analysis

Statistical analysis of data was performed using a T-test by SPSS version 16. (SPSS Inc., Chicago, USA). The analysis was performed in triplicate and the mean values and standard error were reported.

Results

Oil content

The results of statistical analysis showed

significant differences in the oil content between the two species of *Pistacia*. The Soxhlet extraction gave higher oil yield for *Pistacia khinjuk* ($31.00 \pm 0.577\%$) when compared to *Pistacia atlantica* ($24.33 \pm 0.333\%$) (Table 1).

Table 1. The amount of oil extracted from the kernel samples

Sample	Oil Content (%)
<i>P. atlantica</i>	24.33 ± 0.333
<i>P. khinjuk</i>	31.00 ± 0.577

Fatty acid composition

The fatty acid compositions (% weight of FAME) of the two investigated *Pistacia* species are summarized in Table 2. Results indicated that kernels of *Pistacia* species are identifiable by the presence of Palmitic, Oleic, Stearic, Linoleic and Palmitoleic acids. Gas chromatography analysis indicated that oils of both investigated species contained high amounts of monounsaturated fatty acids (MUFA) followed by saturated fatty acids (SFA), while polyunsaturated fatty acids (PUFA) were presented in lower contents. Unsaturated fatty acids comprised around 80% of the fatty acids in both of the assayed oils (Fig. 1).

The most dominant fatty acids in *P. atlantica* and *P. khinjuk* were Oleic acid (53.15% and 51.49%), Linoleic acid (20.41% and 16.43%) and Palmitic acid (20.20% and 23.32%), respectively. Saturated fatty acid (SFA) contents of *P. atlantica* and *P. khinjuk* were 22.59% and 25.22%, respectively.

The results showed that *P. atlantica* and *P. khinjuk* are rich in these types of fatty acids with amounts of 21.12% and 17.32%, respectively. USFA/SFA ratio is considered as a normative of the tendency of oils to self-oxidative reactions. These amounts in *P. atlantica* and *P. khinjuk* were 3.43 and 2.97, respectively (Table 2).

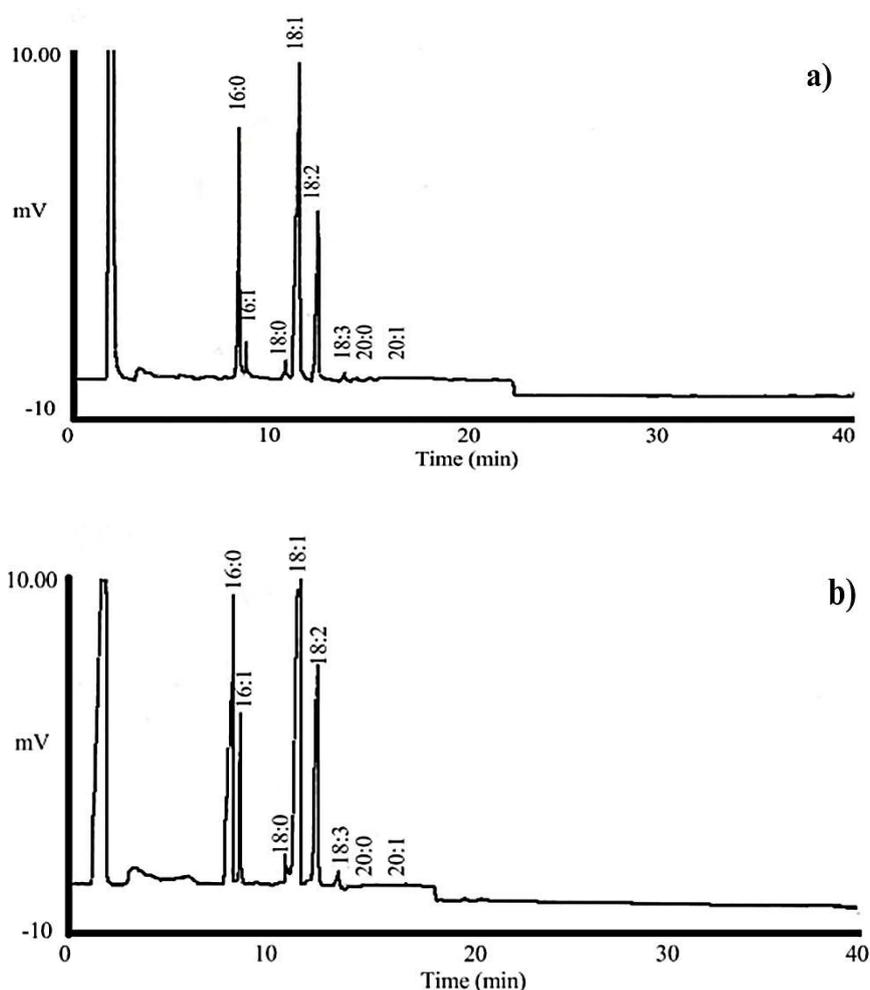


Fig. 1. Chromatograms of fatty acids composition of oil extracted from kernels of *Pistacia atlantica* (a) and *Pistacia khinjuk* (b) fruits.

Table 2. Fatty acid composition of oils obtained from kernels of two *Pistacia* species

Fatty acid	Symbol	Content (%)	
		<i>P. atlantica</i>	<i>P. khinjuk</i>
Palmitic acid	C16:0	20.20± 0.035	23.32± 0.026
Palmitoleic acid	C16:01	2.97± 0.041	5.83± 0.023
Heptadecanoic acid	C17:00	0.06± 0.017	0.03± 0.002
<i>cis</i> -10-heptadecenoic acid	C17:01	---	0.09± 0.002
Stearic acid	C18:00	2.02± 0.088	1.84± 0.049
Oleic acid	C18:01	53.15± 0.195	51.49± 0.041
Linoleic acid	C18:02	20.41± 0.168	16.43± 0.039
α -linolenic acid	C18:03	0.71± 0.036	0.89± 0.011
Arachidic acid	C20:00	0.15± 0.027	0.03± 0.019
<i>cis</i> -11-Eicosenoic acid	C20:01	0.32± 0.058	0.11± 0.003
Σ SFA	/	22.59	25.22
Σ MUFA	/	56.44	57.52
Σ PUFA	/	21.12	17.32
USFA/SFA ratio	/	3.43	2.97
Oxidizability (Cox) value	/	2.79	2.40

Note: *SFA*, saturated fatty acids; *MUFA*, monounsaturated fatty acids; *PUFA*, polyunsaturated fatty acids; *Cox*, calculated oxidizability.

Total phenol and flavonoid

According to the obtained results, total phenol and flavonoid contents showed significant differences between the two species of *Pistacia* (Table 3). Total phenol content of *P. atlantica* and *P. khinjuk* were 130.77 ± 3.11 mg gallic acid/100 g oil and 126.91 ± 4.41 mg quercetin/100 g oil, respectively. Total flavonoid content of *P. atlantica* and *P. khinjuk* were 302.64 ± 21.87 mg gallic acid/100 g oil and 255.29 ± 40.41 mg quercetin/ 100 g oil, respectively (Table 3).

DPPH radical scavenging assay

DPPH radical scavenging assay showed significant differences between the two species of *Pistacia*. The concentration of *P. atlantica* and *P. khinjuk* extracts, which

resulted in 50% of radical inhibition, is shown in Table 3. The range of antioxidant potential revealed in the research showed *P. khinjuk* extract with 15.733 ± 0.689 mg/g having higher inhibitory power than *P. atlantica* extract, with a value of 4.545 ± 0.655 mg/g.

β -carotene-linoleic acid assay

The result is the loss of conjugation and, therefore, a decrease in absorbance at 470 nm. Antioxidants present in the solution can prevent the destruction of β -carotene reaction with the linoleate free radical or any other radical formed in the solution. The inhibition ratios of the oxidation of Linoleic acid by *P. atlantica* and *P. khinjuk* were $38 \pm 0.57\%$ and $62 \pm 1.20\%$, respectively (Table 3).

Table 3. Comparison of total phenol, total flavonoid and antioxidant activity of kernels belonging to two *Pistacio* species

Sample	β -Carotene linoleic acid Inhibition (%)	DPPH assay IC50 (mg/g)	Phenol content (mg Gallic acid/100 g oil)	Total Flavonoids (mg Quercetin/ 100 g oil)
<i>P. atlantica</i>	38.00 ± 0.57	4.545 ± 0.655	130.77 ± 3.11	126.91 ± 4.41
<i>P. khinjuk</i>	62.00 ± 1.20	15.733 ± 0.689	302.64 ± 21.87	255.29 ± 40.41

Discussion

The main reasons for increased interest in *Pistacia* nuts are due to their high oil content and healthy fatty acid profile. According to the obtained results, the two species of *Pistacia* can be considered as good sources of vegetable oils. Although the amount of oil is lower when compared to yields from *Pistacia vera*, it generally contains 50-62 % of oil (Satil et al., 2003; Catalán et al., 2017; Ojeda-Amador et al., 2018). The same results were reported by other studies on *P. khinjuk*, *P. vera* and *P. atlantica* (Mortazavi et al., 2015; Dini et al., 2016; Mohammadi et al., 2019; Labdelli et al., 2019). The high amounts of oil in the kernels of these plants, especially when compared to other oil seed crops; highlight them as possible commercial sources of plant oil.

Numerous studies have reported that the

fatty acid composition of *Pistacia* oils shows a high Oleic acid content, from 51% to 81% (Arena et al., 2007; Sena-Moreno et al., 2015; Esteki et al., 2019), which is higher than many other seed oils (FAO-WHO Codex Stan 210-1999). An investigation into the fatty acid composition of three *Pistacia* species revealed that the main fatty acid component found in *P. khinjuk*, *P. vera* and *P. atlantica* oils is Oleic acid, while Linoleic acid is a secondary most abundant fatty acid in *P. vera* and *P. atlantica* oils, whereas in *P. khinjuk* oil there was no significant difference between Linoleic acid and Palmitic acid contents (Tavakoli and Pazhouhanmehr, 2010). Among nuts, *Pistacia* (*Pistacia* spp.) exhibit interesting nutritional properties because they contain cardioprotective constituents, such as a high Oleic acid content, phytosterols, phenolics

and tocopherols, making it potentially as a high antioxidant and anti-inflammatory food product (Tavakoli and Pazhouhanmehr 2010).

Oleic acid is an essential fatty acid that plays a vital and effective role in the maintenance of the human health. Its high oxidative stability is due to low PUFA contents. The important role of this essential fatty acid in the human body means that natural materials containing this fatty acid may be of strategic importance. Essential fatty acids are only absorbed by the human body from food stuffs; the human body is not capable of synthesizing them.

One of the most important fatty acids for lowering blood cholesterol is α -linolenic acid (ω -3 fatty acid). Foods containing ω -3 are recommended for health, especially for the prevention of cardiovascular disease. Due to the existence of α -linolenic acid, *P. atlantica* and *P. khinjuk* can also be identified as a good plant source of ω -3. The fatty acid composition of *Pistacia* oil is important for edible source seed oils.

Fatty acids that contain several unsaturated bunches, which play vital and effective roles in lowering blood plasma cholesterol levels (Mattson and Grundy, 1985). *Pistacia* is rich in unsaturated fatty acid. The high unsaturated/saturated fatty acid ratio found in *P. atlantica* (3.43) and *P. khinjuk* (2.97) make them to be a worthwhile source of unsaturated fatty acids. Regarding the well-documented harmful effects of SFA and food values of PUFA, the optimum oil in terms of food value is reported to be *P. atlantica*, based on the obtained result in the present study.

The oxidizing index indicates the sensitivity of the oil to oxidation (Parker et al., 2003). According to the above index, both species *P. atlantica* (2.79) and *P. khinjuk* (2.40) can be considered as sources of stable oils. A similar result has been reported in *P. khinjuk* (Tavakoli et al., 2016). There is usually a reverse relationship between the PUFA/SFA ratio

and the oxidizability value from edible oils and their oxidative stability (Tavakoli et al., 2016). According to the obtained results, it is expected that *P. atlantica* contains oil with appropriate oxidative stability, when compared to *P. khinjuk*.

There is a positive correlation between phenols and antioxidant activity for different nuts (Akbari et al., 2012; Hatamnia et al., 2014; Hatamnia et al., 2016b). High amounts of polyunsaturated fatty acids result in poor oxidative durability and short shelf life of oils (Bodoira et al., 2017). Previous studies have shown that high levels of phenolic compounds are a major cause of the antioxidant activity of seed oils. Phenolic compounds are natural antioxidants and key factors for estimating the quality of edible seed oils. According to this study, *P. atlantica* and *P. khinjuk* fruits are good sources of phenolic compounds, which are well known for their antioxidant attributes. Higher antioxidant activity of *P. atlantica* seed oil, compared to the *P. khinjuk* kernel oil, could be because of its higher amounts of phenolic content.

DPPH radical scavenging activity is enhanced by the total phenolic content. Obtained results are in agreement with that reported by Won et al. (2013) and Dong et al. (2015), who reported a positive relationship between antioxidant activity and phenol contents.

β -carotene-linoleic acid assay is based on the change in the color of β -carotene in reaction with Linoleic acid free radical. This radical is formed at high temperature by removing the hydrogen atom between two double bonds of Linoleic acid (Amarowicz et al., 2004). The antioxidant activity of *P. khinjuk* was higher than that of *P. atlantica*. β -carotene/linoleic acid model system are in consistent with the data obtained from DPPH assay.

Conclusion

P. atlantica and *P. khinjuk* kernels are good sources of food oil because of their

proper oil content, essential fatty acids including Oleic acid, Palmitic acid, Linoleic acid, α -Linolenic acid (ω -3) and polyunsaturated fatty acids as well as their high antioxidant properties. Fatty acid composition and high levels of antioxidants are the most important factors for oxidative stability of the *Pistacia* oil and the cause of longer shelf-life of the oil of wild *Pistacia* types, when compared to other commercial varieties. Nowadays, the consumption of foods containing ω -3 is recommended for health, especially for the prevention of cardiovascular diseases. It is also possible to use *P. atlantica* and *P. khinjuk* as a seed oil source containing ω -3. According to the obtained data and previous reports, it can be concluded that the use of *P. atlantica* and *P. khinjuk* oils is characterized as good plant oil in respect to their quality and taste when compared to other edible oils and their oils can be considered as good oils with multiple nutritional values. Therefore, due to high oil content in *P. atlantica* and *P. khinjuk*, which characterized with high nutritional value (high levels of unsaturated and essential fatty acids), cultivation and development of these plants as new oil sources for industrial use and as a substitute for the other oils can be recommended.

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Conflict of interest

The authors declare that they have no conflict of interest.

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