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Investigation effects of extracted compounds from shell and cluster of pistachio nut on the inactivation of free radicals

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ABSTRACT

Essential oils (EOs) are known for uses in various fields such as perfume, cosmetic, pharmaceutical and food industries. Agricultural wastes are among the resources of EOs that produced and disposed of in large quantities annually. Hence, in this study, for the first time, EOs available from pistachio fruit [fruit pistachio shells (FPS) and fruit pistachio cluster (FPC)] were used to the extraction of EOs. The Clevenger device and distilled water were used to extract EOs. The amount of total phenolic compounds (TPC) by Folin-ciocalteu reagent and the radical scavenging ability (RSA%) of FPS and FPC extracted by the soaking method were also measured. The RSA% of EOs and extracts in the presence of DPPH free radicals was evaluated by the IC₅₀ index. Chemical composition of EOs detected by mass spectrometric gas chromatography. Notwithstanding amounts of extraction efficiency by water in the soaking method from FPS and FPC was 4.6% and 3.2% respectively, EOs extraction efficiency from FPC and FPS was 2.10% and 0.13% respectively. TPC in FPS and FPC was 958.38 and 796.25 mgGA/100g dry material respectively. The amount of IC₅₀ of FPS was 3760.69 ppm and near to RSA% of BHT (2354.36 ppm). Statistical difference was observed between the RSA of EOs and positive control antioxidant (P < 0.05). The RSA of antioxidant extracts and TPC showed positively correlated. The major components of FPC were α -thujene and α -pinene, abundance respectively.

1. Introduction

Reactive oxygen species (ROS) are active molecules produced by intracellular enzymatic reactions. In normal physiological conditions, ROSs are produced at very low levels. These compounds are required at this small amount for the general application of cells, and the internal antioxidants of the immune system have the ability to prevent the harmful effects of ROS. Free radicals have an effect on DNA, protein, fat, and other small cellular molecules in the development of pathological processes, including arteriosclerosis, joint rheumatism, and cancers. Therefore, antioxidants are important to protect the body from oxidative stress. The oxidation of lipids by ROSs such as anion superoxide, radical hydroxyl, and hydrogen peroxide, reduce the nutritional value of lipids (Halliwell and Gutteridge, 1984; Chattopadhyay et al., 2010; Emami Bistgani et al., 2017).

One of the most important crops in Iran is pistachio, which is

cultivated on a large scale in different regions of Iran. According to the Food and Agriculture Organization of the United Nations (FAO) statistics, Iran produces 478,600 tons of pistachio per year, the world's largest producer of pistachios (FAO, 2015; Celik and Demirer, 2015). Pistachio fruit is harvested with its green shells after it arrives, and the process of removing this green peel from pistachios, depending on the weather conditions and the variety of pistachios, is carried out in a wet or dry manner. Most of the varieties that are cultivated in Iran have a larger seed than other varieties, and in this condition, the product is susceptible to rapid decomposition, so the removal of green shell from freshly picked pistachio should do immediately (Khodabakhshian and Bayati, 2011). The freshly harvested green pistachio has about 40% green shell; is often discarded and, in a few cases, it is fed to the animal and a very small part of this amount of pistachio hull consumed for production of jam (Behgar et al., 2011; Barreca et al., 2016). Many studies have been performed on the content of phenolic compounds, antioxidant and antimicrobial

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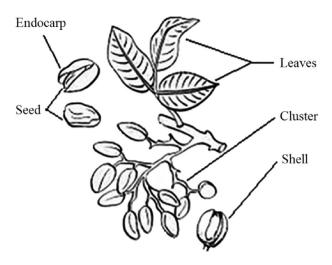


Fig. 1. Morphology of different parts of pistachio seed.

activity of pistachios and its green shell (Barreca et al., 2016; Tomaino et al., 2010; Goli et al., 2005; Abolhasani et al., 2017; Rajaei et al., 2010; Tsantili et al., 2011; Rabadan et al., 2017). Essential oils have antioxidant and antibacterial properties that are widely used in pharmaceutical and food industries (Adrar et al., 2016; Said et al., 2016; Alviano et al., 2005; Kerekes et al., 2013).

The antibacterial activity of essential oils, due to the prolonged shelf life, has led to their widespread use in many foods and beverages. Consumers' tendency to use natural additives has increased over recent years in comparison with their synthetic types (Lou et al., 2017; Araújo et al., 2017). Studies were done on mango waste and citrus peel extracted for determining the antioxidant activity of compounds, total flavonoids and measure the radical scavenging activity of essential oils (Dorta et al., 2012; Bustamante et al., 2016; Wu et al., 2017). Some researchers showed that EOs extracted from Anise, Peppermint, Clove, Cinnamon, Pepper, Citronella, and Camphor (Tu et al., 2018) and *Ocimum tenuiflorum* (Bhavya et al., 2018) had insecticide effects on Tu et al. (2018) showed that EOs have Insecticidal activity.

However, due to the limited studies done on the essential oils in pistachio peel, investigation of the antioxidant and antimicrobial properties of essential oils in pistachio green shell is necessary. Therefore, due to the potential of waste products in agriculture for extraction of effective compounds as well as the creation of added value and finding a method for the optimal use of waste produced during the pistachio harvesting stages, in this study essential oils in pistachio shell were extracted and antioxidant properties, total phenolic compounds and chemical composition of EO and water extracted were studied.

2. Materials and methods

2.1. Raw material

Pistachio green shell variety of "Ohadi" was collected (different parts of pistachio tree and nuts showed in Fig. 1), and taken in the period from the beginning of August till the end of the October 2018, Then were spread on the bed in the shade and dried in the warm summer weather. Two parts of the shell and cluster in the waste were separated from each other and kept in a dark place until the time of consumption.

2.2. Extraction of essential oils

Essential oils in Fruit Pistachio Shell (FPS) and Fruit Pistachio Cluster (FPC) were extracted by the Clevenger system. For this purpose, 150 g of FPS and 50 g of FPC were weighed and immersed separately in a 2,000 mL at distilled water. The extraction time started when the contents of the Clevenger's balloons were boiled for 4 h. At the end of the extraction

process, sodium sulfate was used to remove the moisture content of the samples and were stored at -18 °C (Araújo et al., 2017).

2.3. Extraction of antioxidant extract

For extraction of phenolic compounds from pistachio green shell, using the traditional soaking method, the mixing ratio of 1:20 (sample: solvent) and distilled water was used as a solvent. The extraction process was carried out on a magnetic stirrer at ambient temperature over a period of 18 h. The extract obtained after evaporation under vacuum was stored in a vacuum oven at 40 °C and the extract powdered was kept at -18 °C.

2.4. Measurement of extraction efficiency

After the extraction process was completed, the essential oils in the FPS and FPC were collected in micro-tubes and the extraction efficiency was calculated using the following Eq. (1).

Extraction Efficiency% =
$$\frac{m_1 - m_0}{m_2} \times 100$$
 (1)

where m_0 is the weight of the micro-tube, m_1 , the weight of the microtube with the essential oil, m_2 is the weight of the primary sample that has been extracted (Yen and Lin, 2017). The extraction efficiency of soaking extract measured by Eq. (1) also.

2.5. Determination of the total phenolic compounds (TPC)

The appropriate concentrations of extracted EOs and extracts were prepared (for soaking extract used from water and methanol for EOs as solvent) and 0.5 mL of them with 2.5 mL of the Folin-ciocalteu reagent (10 times diluted in distilled water), transferred to the tubes and finally 2 mL of sodium carbonate 7.5% was added and kept at ambient temperature for 30 min. In the end, the absorbance of the samples was read at 765 nm (UV-Visible Recording spectrophotometer, Shimadzu, Japan, model: UV-160A). For control samples, 0.5 mg distilled water was used. The total phenolic compounds in terms of mg of gallic acid per 100 g of dry pistachio dry shell were calculated (Bajalan et al., 2017).

2.6. Measuring the radical scavenging ability (RSA)

The effect of essential oils extracted to eliminate DPPH free radicals was studied using the recommended method by Luís et al. (2016). Samples were prepared at different concentrations in methanol and 2 mL of each of them was added to 0.5 mL of DPPH 0.2 mM solution. The prepared mixture was stored in a dark place for 30 min and then read the absorbance using a spectrophotometer at 517 nm wavelength. Scavenging Ability of DPPH free radicals by plant extracts and essential oils was calculated using Eq. (2).

$$RSA\% = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$
 (2)

where Abs _{control}, absorbs the control and Abs_{sample}, sample absorption. To compare the radical scavenging power of antioxidant extracts, an IC_{50} factor equal to the effective concentration for the absorption of 50% of free radicals in the environment was used. For a better comparison of DPPH free radical scavenging, BHT (as a synthetic antioxidant) and ascorbic acid (as a natural antioxidant) were used (Luis et al., 2016).

2.7. Identification of chemical composition of essential oils using gas chromatography-mass spectrometer (GC-MS)

Gas chromatography-mass spectroscopy (GC/MS) analysis carried out by Agilent 7890B gas chromatograph connected with a mass detector (model: 5977A, Agilent Technologies, US). The gas chromatograph was

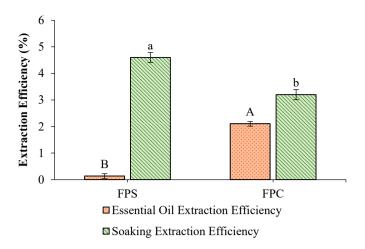


Fig. 2. Soaking Extraction efficiency and essential oil extraction efficiency from FPS and FPC. *- A and B had significant differences. **- a and b had significant differences.

Table 1

TPC in FPS and FPC soaking extracts.

	TPC (mg GA per 100 g DM)		
	Soaking Extract	Essential Oil	
FPS	958.38 ^a	205.68 ^a	
FPC	796.25 ^b	112.54 ^b	

"a" and "b" are statistical signs and have a significant difference (α =0.05).

equipped with an HP-5 MS capillary column (phenyl methyl siloxane, 30 m \times 0.25 mm internal diameter, 0.25 µm, Agilent technologies). The temperature of injector was 270 °C and the oven temperature was programmed from 60 °C (0 min) to 200 °C at 5 °C/min rate. Helium was selected as the carrier gas while the flow rate was adjusted to 1 mL/min and injection volume (1 µL). Also, the mass spectrometer was set in EI mode at 70 eV. The interface temperature was adjusted to 280 °C and the mass range was 35–500 m/z (Abbaszadegan et al., 2015).

2.8. Statistical analysis

The results were analyzed using the ANOVA table in a completely randomized factorial design with a significance level of $\alpha < 0.05$. Duncan's test was also carried out between the averages. SPSS software version 17 (United States) was used for statistical analysis.

3. Results and discussion

3.1. Extraction efficiency

The extraction efficiency for FPS and FPC extracts obtained by soaking was calculated using Eq. (1). The extraction efficiency of essential oils was also calculated from FPS and FPC. The results showed that the extraction efficiency of FPS was higher than FPC, but the extraction efficiency of essential oils was higher than FPC. The presence of a higher amount of essential oils in the FPC than its FPS suggests a lower FPC yield than FPS. Because distilled water was used for soaking, distilled water was not able to extract essential oils due to its polarity, and due to the higher level of low polarization compounds in FPC, including essential oils, the FPC extraction efficiency was lower (Fig. 2).

3.2. Total phenolic compound measurement

The number of phenolic compounds extracted from FPS was higher

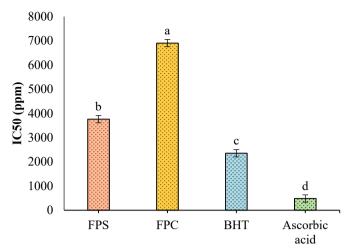


Fig. 3. Radical scavenging ability of essential oils extracted from FPC and FPS compare to BHT and Ascorbic acid antioxidants.

than its value in the FPC and its results are presented in Table 1. Due to the fact that the water was used for the extraction process, and given that the polarization of the phenolic compounds in the FPS was more similar to that of water polarity, higher levels of phenolic compounds extracted from FPS are possible. TPC in FPS essential oil was higher than FPC and there was a significant difference between them (P < 0.05). According to Table 1, the TPC in essential oil was lower against soaking extract. In the extraction condition, the polarity of solvent is one of the important parameters. Due to the polarity of phenolic compounds is very extreme and contain low polar until high polar compounds, the solubility of phenolic compounds is rather in soaking extracts. In some researches pointed that water was the better solvent for extraction of TPC (Difonzo et al., 2017; Chen et al., 2007) even though some researches pointed to other solvents, for example, ethanol, methanol and etc (Al-Dabbas et al., 2006; Spigno et al., 2007).

3.3. Radical scavenging ability measurement

The radical scavenging ability of extracts extracted from FPS and FPC was measured using a spectrophotometric method in the presence of a DPPH colorant and by changing its color from violet to pink. The results showed that the strength of the extract obtained from FPS was greater than that obtained from FPC for the free radical release of DPPH. According to Fig. 3, the IC₅₀ value for extract extracts from FPS was about half the IC₅₀ value of extract extracts from FPC. The IC₅₀ concentration has an inverse relationship with radical scavenging power, it can be stated that the radical scavenging power of the FPS extract is approximate twice the radical scavenging power of the FPC receptor (Fig. 3).

The radical scavenging ability of essential oils extracted from FPS and FPC was compared with two other antioxidants and it was found that the strongest antioxidant among the tested compounds was ascorbic acid, and then the essential oils obtained from FPS had the highest radical scavenging ability. The essential oils obtained from FPC showed the lowest DPPH free radical scavenging power. In general, it can be stated that the free radical scavenging of DPPH by essential oils of FPC was more similar to the BHT antioxidant, and FPS had more similarity in terms of free radicals scavenging power to ascorbic acid. Yen and Lin (2017), who studied the radical scavenging power of Cymbopogon citrus, stated that by increasing the concentration of essential oils in the environment, the scavenging capacity of free radicals increases (Yen and Lin, 2017). Lou et al. (2017) showed that the scavenging capacity of free radicals by essential oils of *Citrus medica* L. var. sarcodactylis in the microscopic state was more than pure radical scavenging ability (Lou

Table 2

Composition of the essential oil from the FPC.

	Compound	Category	Molecular formula	Molecular weight (g/mol)	Retention time (min.)	Area Sum %
1	tricyclene		C10H16	136	4.449	0.63
2	α-thujene	Monoterpenes	C10H16	136	4.51	31.9
3	α-pinene	Monoterpenes	C10H16	136	4.652	15.84
4	camphene	Monoterpenes	C10H16	136	4.943	3.13
5	sabinen	monoterpenes	C10H16	136	5.406	1.39
6	β-pinene	Monoterpenes	C10H16	136	5.495	3.22
7	β-myrcene	monoterpenes	C10H16	136	5.719	2.36
8	3-carene	monoterpenes	C10H16	136	6.187	0.99
9	α-terpinen	Monoterpenes	C10H16	136	6.324	0.80
10	D-limonene	Monoterpenes	C10H16	136	6.606	25.01
11	β-ocimene	Monoterpenes	C10H16	136	7.014	0.52
12	γ-terpinen	Monoterpenes	C10H16	136	7.302	0.89
13	terpinolene	Monoterpenes	C10H16	136	8.034	7.9
14	terpinen-4-ol	Monoterpenes	C10H18O	154	10.343	1.28
15	L-bornyl acetate	*	C12H20O2	196	13.184	3.33
16	thujopsene	Sesquiterpenes	C15H24	204	16.958	0.37
Total		- *				99.54

Table 3

Composition of the essential oil from the FPS.

	Compound	Category	Molecular formula	Molecular weight (g/mol)	Retention time (min.)	Area Sum %
1	α-thujene	Monoterpenes	C10H16	136	4.51	6.48
2	α-pinene	Monoterpenes	C10H16	136	4.653	12.86
3	camphene	Monoterpenes	C10H16	136	4.945	2.1
4	β-pinene	Monoterpenes	C10H16	136	5.495	1.76
5	β-myrcene	monoterpenes	C10H16	136	5.719	1.46
6	3-carene	Monoterpenes	C10H16	136	6.188	2.76
7	4-carene	monoterpenes	C10H16	136	6.326	1.23
8	β-cymene	monoterpenes	C10H16	136	6.507	1.1
9	D-limonene	Monoterpenes	C10H16	136	6.613	50.53
10	β-ocimene	Monoterpenes	C10H16	136	7.016	0.56
11	γ-terpinen	Monoterpenes	C10H16	136	7.304	1.51
12	terpinolene	Monoterpenes	C10H16	136	8.034	9.63
13	L-bornyl acetate	•	C12H20O2	196	13.186	3.96
Total	·					95.94

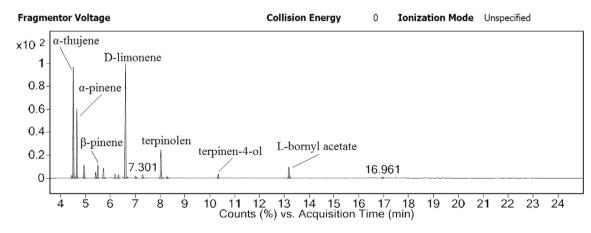


Fig. 4. GC-MS chromatogram of FPC.

et al., 2017).

3.4. Chemical compounds of the shell and cluster of pistachio fruit

Identified compounds from two parts of pistachio fruit (cluster and shell) are presented in Tables 2 and 3, respectively, by the GC-MS. Thirteen compounds were identified in FPS and 16 compounds were identified in FPC. The yield of essential oils in FPC ranged from 0.37 to 31.9 (Table 2) (Fig. 4) and amounts of essential oils in FPS ranged from 0.56 to 50.53 (Table 3) (Fig. 5). A total of eleven compounds were shared between the compounds identified in the FPS and FPC. The unique

compounds related to FPS were 4-carene and β -cymene. The number of unique compounds identified by FPC was higher, including tricyclene, sabinene, α -terpinene, terpinen-4-ol, and thujopsene. The results showed that the highest amount of FPC compound was α -thujene, from the monoterpenes category. The amount of this compound in the FPC was 25.1%, and the highest combination found in FPS, D-limonene, was 53.50%. D-Limonene belongs to the monoterpenes category.

In this study, the pistachio wastes, which are often discarded, were used to extract essential oils. Due to the fact that these wastes have two parts of the shell and cluster, the compounds in these two parts were extracted and identified. The D-limonene contained in the FPS, which is a

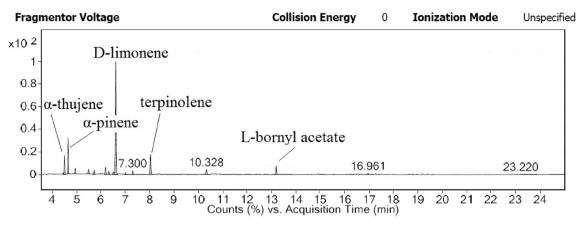


Fig. 5. GC-MS chromatogram of FPS.

major component, is a non-polar compound, soluble in solvents with a polarity of less than 5, and cannot be dissolved in solvents with a polarity of more than 5.5. D-Limonene dissolves in very small amounts in water (13.8 mg/L) and dissolves in organic solvents such as ethanol, acetone, chloroform, tar, and alkenes. α -Thujene combines with a boiling point of 151 °C, which is the dominant essential oil in the Savory (Gruenwald, 2004). It has been extracted from other sources, including cumin, *Thymus vulgaris* and ajowan (Dubey et al., 2017; Mohtashami et al., 2018; Zrig et al., 2016). According to the pharmaceutical toxicity of α -thujene, ecotypes with the minimum amount of this compound are suitable (Mirzahosseini et al., 2017).

4. Conclusion

The results of experiments performed on extracts and essential oils extracted from two parts of pistachio fruit (cluster and shell) showed that the number of phenolic compounds in FPS was higher than FPC and consequently, the radical scavenging power of FPS was higher. The FPS had more water-soluble compounds, but the essential oils in the FPC were higher. The combination of the extracted from FPS was D-limonene and the combination of the index in FPC was α -thujene based on the results of gas chromatography.

Declarations

Author contribution statement

Morteza Mohammadi: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Mohammad Ghorbani: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Adel Baik Babaei: Conceived and designed the experiments; Wrote the paper.

Samira Yeganehzad: Performed the experiments; Contributed reagents, materials, analysis tools or data.

Alireza Sadeghi-Mahoonak: Performed the experiments.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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