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Effect of *Pistacia atlantica* (Bane) Essential Oil on Oxidative Stability of Sunflower Oil

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HIGHLIGHTS

- Basic components of *Pistacia atlantica* (Bane) essential oil were monoterpene and sesquiterpene hydrocarbons.
- All concentrations (200, 400,600,800, and 1,000 ppm) of *P. atlantica* essential oil had a stabilizing effect on sunflower oil.
- *P. atlantica* essential oil can be used as a natural antioxidant to stabilize edible oil during storage.

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Acronyms and abbreviations
DPPH=2,2-Diphenyl-1-Picrylhydrazyl
FRAP=Ferric Reducing Antioxidant Power
P-AnV=P-anisidine Value
PV=Peroxide Value
RSA=Radical Scavenging Activity
TBHQ=Tertiary Butyl Hydroquinone

ABSTRACT

Background: The antioxidant activity of Bane (*Pistacia atlantica*) has been proved in different researches. This study evaluated the potential of Bane (*Pistacia atlantica*) essential oil (as a natural antioxidant) on the oxidative stability of sunflower oil.

Methods: The essence of Bane was added to sunflower oil at concentrations of 200, 400,600,800, and 1,000 ppm. Tertiary Butyl Hydroquinone (TBHQ) was applied as synthetic antioxidant. All samples with the control were stored at 65℃ for 20 days. Gas Chromatography-Mass Spectrometry was used for the essence analysis. The 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay, rancimat, p-anisidine value (P-AnVs), and peroxide value (PV) were determined to assess the efficacy of differecnt concentration of essence (200, 400,600,800, and 1,000 ppm). Data were analyzed by Statistical Analysis System (SAS) version 9 Software.

Results: The essential oil yield was 0.1% v/w. The basic components of essence were monoterpene and sesquiterpene hydrocarbons. Synthetic antioxidant had the highest scavenging activity, followed by the mixture sample. PVs were in the range of 19.56-20.73 milliequivalents (meq)/kg for the treated samples after 20 days, while it was 38.74 on the 20th day for the control. For all treatments, PV was increased with increasing storage time. P-AnVs were 8.58-17.14 for stabilized samples and 18.02 for control sample on the 20th day of storage. In all stages, control sample had the highest P-AnV. For all samples, P-AnV increased as a subject of storage time.

Conclusion: *P. atlantica* (Bane) essential oil had a stabilizing effect on sunflower oil and can be used as a natural antioxidant to stabilize edible oil during storage.

Introduction

Fats and oils are the main sources of vital precursors of metabolic processes in the body, energy storage, and important ingredients of cell membranes and other biological conformations. They play considerable roles in

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the absorption of fat-soluble nutrients and act as essential fatty acids, cellular transport components, fat-soluble vitamins, and dietary supplements (Kostik et al., 2013). Apart from biological and nutritional actions, lipids play a key role in the quality, processing, texture, and organoleptic characteristics of food crops (Ying et al., 2018). Quality of fats and oils is changed through destructive reactions, both during heating and long-term storage (Farhoosh and Tavassoli-Kafrani, 2010). The major subversive process is oxidation and destruction of its products, which resulted in loss of sensory quality and nutritional value. Prevention of this process is vital for the food producers and individuals involved in the food milling from the plant to the user (Olmedo et al., 2018). Synthetic antioxidants have been applied for stabilizing oil and fats. But their safety has been problemed (Farhoosh and Tavassoli-Kafrani, 2010). Therefore, there is a tendency among scientists and consumers to replace these compounds with natural materials.

Sunflower oil is among the world's five most important vegetable oil crops. This oil has many applications, including baking, cooking, and frying (Spring, 2021). Sunflower oil is high in mono-unsaturated fatty acids such as oleic acid (14.0-39.4%) and poly-unsaturated fatty acids, linoleic acid (48.3-74.0%; Sousa et al., 2021). Sunflower oil is appreciated for its higher concentration of poly-unsaturated fatty acids, as well as the presence of large levels of phytosterols and vitamin E (Spring, 2021).

The *Pistacia* genus is in the family of *Anacardiaceae*. It contains about 70 genera and over 600 species (Bozorgi et al., 2013). It has eleven or more species that are shrubs or trees. Three species of *Pistacia*, including *P. khinjuk* Stocks, *P. vera* Linnaeus, and *P. atlantica* Desf present in Iran. *P. atlantica* has been distributed from Northwest Africa to Southwest Asia. *P. atlantica* which is named Bane in Iran, consists of four subspecies of *kurdica*, *cabolica*, *mutica*, and *atlantica* (Bozorgi et al., 2013). Bane trees grow in central, eastern, and western parts of Iran. The leaves and fruits of Bane (*P. atlantica*) are applied in folk medicine to cure throat infections and stomach disorders. Local people consume the fruit of this plant after pulverizing and blending it with other constituents as food and use unripe fruit to produce jam. Oleoresin of Bane is used for the making of chewing gum in Iran (Hatamnia et al., 2016). The main components of essential oil among the different species of *Pistacia* are hydrocarbons and oxygenated monoterpenes which α-pinene has been reported as a major monoterpene hydrocarbon. The α-terpinolene, limonene, and ocimene are among other compounds. Sesquiterpenes with a greater amount are germacrene-D and β-caryophyllene. Also, diterpenoids, triterpenoids, phenolic components, fatty acids, and sterols in trace levels have been isolated. These constituents have antioxidant, antimutagenic, antimicrobial, and antiviral, anti-inflammatory, and antinociceptive activity. Also, they were effective on gastrointestinal disorders (Bozorgi et al., 2013).

The antioxidant activity of this plant has been proved in different researches. In the study of Hatamnia et al. (2014), the antioxant activity of leaves from 10 Bane genotypes was considered using Ferric Reducing Antioxidant Power (FRAP) test. The results showed that all genotypes have revealed higher antioxidant activity than that reported for butylated hydroxyanisol. Also, the antioxidant activity of Bane essential oil was assayed using FRAP and 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) tests, and Bane essential oil showed significant antioxidant activity using FRAP test (Rezaie et al., 2015). In another study, Bane (*P. atlantica*) hull oil was more effective on the level of 2% for stabilizing sunflower oil during the frying process relative to sesame oil and rice bran oil (Sharif et al., 2009). So, due to the importance of natural compounds for replacing synthetic antioxidants in edible oils, this study investigated the antioxidant effect of Bane on sunflower oil during storage.

Materials and methods

Materials

Refined sunflower oil was provided from the agro-industry complex and vegetable oil of Golbarg Baharan, Karaj, Iran. The Bane plant was purchased from the Kermanshah market. All chemical materials were of analytical grade and purchased from Merck (Merck Millipore, Darmstadt, Germany). DPPH and Tertiary Butyl Hydroquinone (TBHQ) were obtained from Sigma Chemical Co (St. Louis, MO, USA).

Extraction

The fruit of *P. atlantica* was dried at ambient temperature and was ground, and 100 g of its powder was placed in a flask including 1,200 ml distilled water. It was then steam distilled by clevenger- apparatus for 3 h matching the British method. The essential oil was dried using anhydrous sodium sulfate (Na_2SO_4), and then the essence was stored at 4℃ for further analysis (Sadeghi et al., 2016).

Gas Chromatography (GC) analysis

The essential oil was analyzed by an Agilent HP-6,890 GC containing capillary column HP-5MS (5% phenylmethyl siloxane) (0.25 µm film thickness, 0.25 mm×30 m; Restek, Bellefonte, PA, USA) including an Agilent HP-5,973 mass selective detector in a mode of electron impact. Ionization energy was 70 eV. The

temperature of 50℃ was used in the oven for 5 min, then it was increased to 250℃ with speed of 3℃/min and was made steady at 250℃ for 10 min. N-hexane was used for the dilution of essential oil and sample injection was performed in 0.1 µl volume with a split ratio of 1:50. The carrier gas was helium at 1.1 ml/min. The essential oil ingredients were identified by account of their retention indices under the programmed conditions on the set for essential oil and n-alkanes (C_8-C_{20}) and by the comparison of their retention indices and mass spectra with valid samples and those brought in the literature (Küçükbay et al., 2014).

Sample provision

The essence of Bane was added to sunflower oil at concentrations of 200, 400,600,800, and 1,000 ppm. TBHQ was applied at 120 ppm. Also, mixtures of TBHQ (60 ppm) and essence (60 ppm) were used. All samples with the control were stored at 65℃ for 20 days. Chemical tests were performed in intervals of four days in triplicate.

Antioxidant activity parameters

-Rancimat test

A Metrohm rancimat model 679 (Herisau, Switzerland) was applied to determine the Oil Stability Index (OSI). Assays were performed with 2.5 g of each sample at 110℃ and airflow of 20 L/h (ISO, 2006). In this method constitution of volatile acids is appreciated by altering electrical conductivity when they exit from the oxidizing oils by air.

-DPPH assay

The radical scavenging activity assay state the efficacy of antioxidants to scavenge natural or synthetic radicals in opposition to the antioxidant ability of a standard antioxidant (Zaporozhets et al., 2004). DPPH assay was done according to Gyamfi et al. (1999). Essential oil methanolic solution (50 µl) at different concentrations was added to DPPH solution (0.004% in methanol; 5 ml). The control sample was prepared by adding 50 µl solvent to the DPPH solution. Afterward, the samples were stored in darkness and at ambient temperature for 30 min. The sample's absorbance was read at 517 nm. The Radical Scavenging Activity (RSA) was measured by the following equation:

$$RSA=[(A_{DPPH}-A_S)/A_{DPPH}]\times 100$$

Where A_S and A_{DPPH} are the absorbance of samples and DPPH solution, respectively.

-P-Anisidine Value (P-AnV) and Peroxide Value (PV)

American Oil Chemists' Society (AOCS) cd 18-90 and cd 8-53 methods were used to measure P-AnV and PV, respectively (AOCS, 1990). P-AnV is applied to measure the secondary products of oxidation. Its base is the reaction of p methoxy aniline (anisidine) and aldehydic compounds (Hashemi et al., 2014). It is a reliable method for calculating oxidative rancidity in oils, fats, and fatty foods.

Statistical analysis

Statistical analysis was done in a completely randomized design in three repetitions by Statistical Analysis System (SAS) version 9 Software. ANOVA test was used to analyze the data. Significant differences of (p<0.05) were determined by Duncan's multiple range test.

Results

Chemical components of the essential oil

The essential oil yield was 0.1% v/w. Gas Chromatography-Mass Spectrometry (GC-MS) was served for the detection of essential oil compounds. Fifteen major constituents including 95.98% of the essential oil were detected (Table 1). The basic components of essence were monoterpene and sesquiterpene hydrocarbons.

Antioxidant activity parameters

-Rancimat test

Induction periods for various treatments have presented in Table 2. Obtained results showed that the induction period increased with a rise in antioxidant concentrations, but there was a slight difference among different doses of the essential oil. However, it should be noted the highest induction period belonged to the TBHQ and then the mixture sample. The higher induction period in the mixture sample attributed to the presence of synthetic antioxidant.

-DPPH assay

The percentages of free RSA for different treatments have shown in Table 2. According to these results, synthetic antioxidant had the highest scavenging activity, followed by the mixture sample. Different concentrations of the essential oil showed low RSA.

-PV

PVs were in the range of 19.56-20.73 milliequivalents (meq)/kg for the treated samples after 20 days, while it was 38.74 on the 20th day for the control (Figure 1). For all treatments, PV was increased with increasing storage time. Control sample had the highest PV in all stages, followed by SFO-200, SFO-400, SFO-600, SFO-800, SFO-1,000, SFO-Mixture, and SFO-TBHQ, respectively. In control sample, PV had a tremendous rise on the 4th day and rose to the 20th day. For other samples, it increased gradually to the storage final stage. All concentrations showed significant (p<0.05) stabilizing effect on sunflower oil, and in the final storage period, SFO-Mixture was even better than a synthetic antioxidant. Maximum PVs were 20.73, 20.24, 20.11, 20.09, 19.99, and 19.56 for concentrations 200, 400, 600, 800, 1,000 ppm and mixture sample, respectively.

-P-AnV

P-AnVs were 8.58-17.14 for stabilized samples and 18.02 for control sample on the 20th day of storage (Figure 2). In all stages, control sample had the highest P-AnV. For all samples, P-AnV increased as a subject of storage time. On the 4th day, the maximum value was related to control, followed by SFO-600, SFO-800, SFO-400, SFO-1,000, SFO-200, SFO-Mixture, and SFO-TBHQ, respectively. Finally, on the 20th day, the arrangement of P-AnV was SFO-1,000, SFO-800, SFO-200, SFO-400, SFO-Mixture, SFO-600, and SFO-TBHQ. Totally, in all stages of storage, all of the essential oil concentrations had an inhibitory effect on the formation of secondary products of oxidation, and differences among treats and storage periods were significant (p<0.05).

Table 1: Chemical components of *Pistacia atlantica* essential oil

No	Compound name	% (W/W)	RI [a]
1	Myrcene	2.21	1,109
2	Camphene	4.17	1,186
3	α-Pinene	14.36	1,327
4	β-Pinene	5.29	1,474
5	Phellandrene	49.73	1,609
6	Limonene	5.13	1,632
7	γ-Terpinene	2.02	1,792
8	α-Terpinolene	0.89	1,842
9	Linalool	2.15	2,136
10	Thujopsene	1.07	2,878
11	Caryophyllene oxide	1.86	3,925
12	Hexadecanoic acid	0.94	5,256
13	Octadecanoic acid	3.87	6,023
14	9- Octadecenoic acid	1.03	6,183
15	Ethyl oleate	1.26	6,612
Total		95.98	

RI [a]: Retention Indices relative to C_8-C_{20} n-alkanes on HP-5MS capillary column

Table 2: Antioxidant activity of *Pistacia atlantica* essential oil on different treatments and control groups of sunflower oil

Treatments	Ctrl	SFO-200	SFO-400	SFO-600	SFO-800	SFO-1,000	SFO-Mixture	SFO-TBHQ
Induction period (h)	4.68±0.05 ^{f}	4.70±0.01 ^{f}	4.73±0.04 ^{f}	4.79±0.03 ^{e}	4.9±0.04 ^{d}	5.2±0.05 ^{c}	9.85±0.02 ^{b}	10.97±0.08 ^{a}
RSA (%)		6.08±0.04 ^{g}	8.26±0.04 ^{f}	14.78±0.02 ^{e}	25.21±0.04 ^{d}	37.39±0.06 ^{c}	77.82±0.08 ^{b}	92.17±0.03 ^{a}

Data are means±standard deviation of treats in three replicates. Similar characters mean lack significant difference among treatments (p<0.05).
SFO=Sunflower Oil; RSA=Radical Scavenging Activity; TBHQ=Tertiary Butyl Hydroquinone

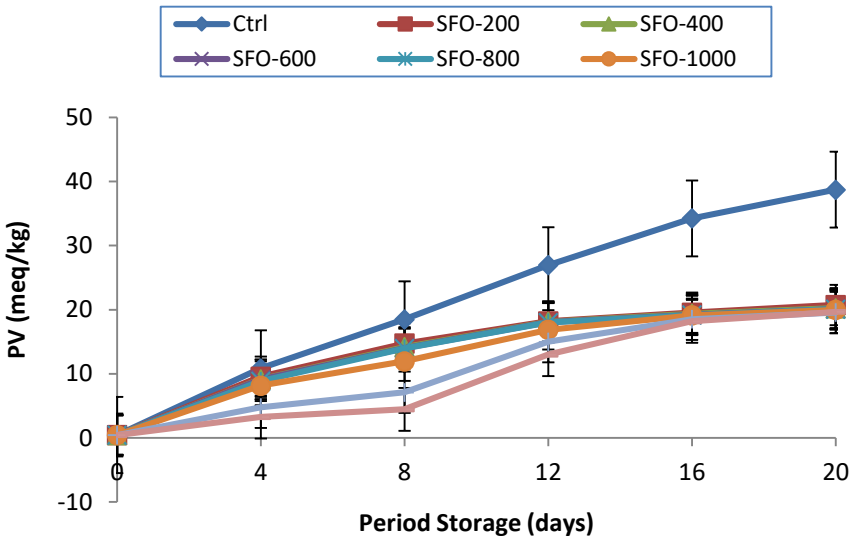

Figure 1: Effect of Bane essential oil on Peroxide Value (PV) of control and treated samples during storage period; SFO=Sunflower Oil; TBHQ=Tertiary Butyl Hydroquinone

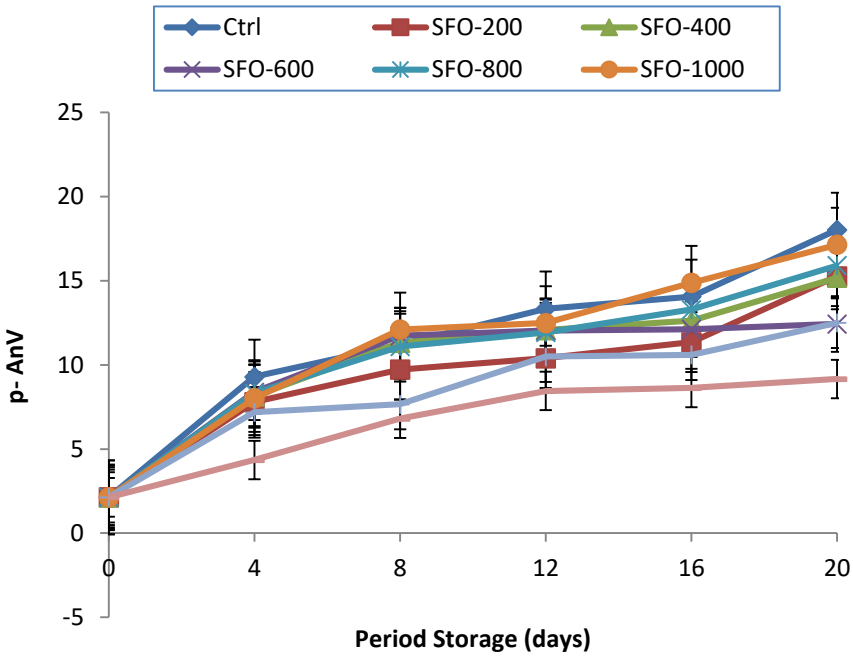

Figure 2: Effect of Bane essential oil on P-anisidine Value (P-AnV) of control and treated samples during storage period; SFO=Sunflower Oil; TBHQ=Tertiary Butyl Hydroquinone

Discussion

According to Table 1, phellandrene and α-pinene were major components of essential oil. In a study, the main compounds in the essential oil of fresh leaves from the female and male plant of *P. atlantica* (Laghouat, Algiers) were a-pinene/a-thujene and δ-3-carene, respectively (Gourine et al., 2010). In a study by Rezaie et al. (2015), α-pinene was the major compound in the essential oil of the Bane fruit hull (Marvdasht, Fars, Iran). Fathollahi et al. (2019) observed α-pinene as the dominant ingredient in the fruit essential oil of *P. atlantica* subsp. *Kurdica* (Kurdistan, Iran). The difference among the ingredients of essential oil in different researches has been reported to be linked to several factors including part and plant species, harvesting time, cultivar sex, climatic condition, and geographical source (Gourine et al., 2010).

According to the obtained results, the sample with 120 ppm TBHQ had the highest induction period. Tavakoli et al. (2017) considered the effect of unsaponifiable materials extracted from *P. khinjuk* fruit oil (Meimand forest, Fars, Iran) on the oxidative stability of olive oil. They also observed the highest oxidative stability index for the sample containing TBHQ (100 ppm). In the study of Sadeghi et al. (2017), the effect of *Ferulago angulata* (Dalahoo Mountains, Kermanshah, Iran) essential oil was considered on the oxidative stability of sunflower oil. The data for the oil stability index also showed the superiority of synthetic antioxidant relative to different concentrations of essential oil. The lower oxidative stability index for essential oils perhaps is due to the volatility of essential oils at high temperatures.

Various concentrations of the essential oil exhibited low radical scavenging activity. Similar results were observed by essential oil of Bane hull (Marvdasht, Fars, Iran) (IC_{50}=23µg/ml) in comparison with Butyl Hydroxyl Toluene (BHT), ascorbic acid, and α-tocopherol (Rezaie et al., 2015). In another study, the antioxidant activity (IC_{50}) of leaves of *P. atlantica* from four regions in Algeria was in the range of 8.8–27.48 mg/ml (Gourine et al., 2010). Also, Hasheminya and Dehghannya (2020) obtained IC_{50}=25.2 mg/ml for hull essential oil of *P. atlantica* subsp. *kurdica* (Ilam, Iran). In another study, the antioxidant activity of the essential oil extracted from 14 Tunisian *P. lentiscus* populations was assayed using the DPPH. The IC_{50} volues were in range of 299-993 µg/g. The IC_{50} value for BHT was 29.4 µg/ml (Aissi et al., 2016). Monoterpenes are not regarded as strong scavengers. Therefore, the low antioxidant efficacy of the essence is probably related to the high amount of these components in the essential oil (Wojtunik et al., 2014). Moreover, essential oils have lower content of active compounds.

Peroxides are initial oxidation products that may decompose in the final stages of oxidation. PVs were in the range of 19.56-20.73 meq/kg for the treated samples after 20 days. These quantities are lower than those of sunflower oils stabilized by guava leaves (Anwar et al., 2006), pomegranate peel extracts (Iqbal et al., 2008), garlic extract (Iqbal and Bhanger, 2007), roselle, roselle seed, and kenaf seed extracts (Nyam et al., 2013), but they are higher than the results obtained for stabilized sunflower oil by essential oil of *F. angulata* (Sadeghi et al., 2017). Levels of PV in a study by Olmedo et al. (2018) on stabilizing effect of *Aloysia triphylla* and *Minthostachys mollis* essential oils in sunflower oil during accelerated storage at 60 ˚C in concentrations of 0.02, 0.1, and 0.2% for 14 days were higher than obtained data in this study. Also, in sunflower oil supplemented with essential oils of oregano, rosemary, and laurel (0.02 and 0.1%) for 28 days at 60 ˚C, greater PV was observed (Olmedo et al., 2015). Mezza et al. (2018) studied the effect of essential oil rosemary and its fractions (by molecular distillation) on sunflower oil at a dose of 0.1/100 g at room temperature for 115 days. The PV declared higher than 20 meq/kg for treated oil at the end of the storage period. SFO-1,000 had the maximum effect on stability of oil and different doses of natural antioxidants were effective for prevention of primary oxidation products. The antioxidant activity in essential oil of Bane is related to monoterpene and sesquiterpene hydrocarbons.

P-AnVs were 8.58-17.14 for stabilized samples and 18.02 for control on the 20th day of storage. In a study by Olmedo et al. (2018) on stabilizing effect of *A. triphylla* and *M. mollis* essential oils on sunflower oil during accelerated storage at 60 ˚C (concentrations of 0.02, 0.1, and 0.2%), P-AnV was lower than 6 after 14 days but in sunflower oil supplemented with essential oils of oregano, rosemary, and laurel (0.02 and 0.1%) for 28 days at 60 ˚C, similar results were obtained by Olmedo et al. (2015). Wang et al. (2018) observed P-AnV 189.4 for supplemented sunflower oil with essential oil of *Coriandrum sativum* L (1,200 ppm) after 24 days store at 65 ˚C. Also, Okhli et al. (2020) studied the oxidative stability of sunflower oil using citron peel (*Citrus medica* L.) essential oil at the concentration of 800 ppm for 5 days at 65 ˚C. They observed P-AnV 64.01 after 5 days. The outcomes of this test were not following PV and DPPH and rancimat outcomes. In other words, changes in secondary products of oxidation were not according to essential oil concentration. However, in all tests, synthetic antioxidants had the strongest activity. Researchers suggest that the high antioxidant efficacy of TBHQ is due to its ability to donate hydrogen owing to the existence of two para-hydroxyl groups in its

construction (Alizadeh et al., 2016; Gourine et al., 2010). Also, a synthetic antioxidant is a pure compound, while the essential oil is not pure. Therefore, its higher antioxidant activity is normal. Moreover, Bane essential oil has low active compounds for scavenging of formed free radicals as a result of peroxides decomposition.

Conclusion

Obtained results from various parameters exhibited that all concentrations of *P. atlantica* (Bane) essential oil had a stabilizing effect on sunflower oil. Also, it was more effective for inhibition of the primary oxidation products ratio of secondary oxidation products. Also, it was a weak scavenger for DPPH radical that it attributed to a low amount of antioxidant compounds in the essential oil. However, it can be used as a natural antioxidant to stabilize edible oil during storage.

Author contributions

E.S. designed the study; M.A., M.K., and M.A.-L. conducted the experimental work; F.K. analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

The authors declare that there is no conflict of interest.

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