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Determination of chemical composition, antioxidant and antifungal properties of pomegranate (*Punica granatum L.*) and Parsley (*Petroselinum crispum*) seed oil produced in industrial scale

Endüstriyel ölçekte üretilen Nar (Punica granatum L.) ve Maydanoz (Petroselinum crispum) tohumu yağının kimyasal bileşimi, antioksidan ve antifungal özelliklerinin belirlenmesi

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Abstract

In this study, essential oil and oil acid content, antioxidant and antifungal properties of oils obtained from pomegranate (*Punica granatum L.)* and parsley seeds (*Petroselinium crispum*) produced on an industrial scale were investigated. Pomegranate seed oil was obtained cold pressed in an industrial scale, while parsley seed oil was obtained industrial steam distillation. As a result of pomegranate seed oil GC-MS analysis, fifteen components were determined. Parsley seed oil was twelve compounds was identified. Punicic acid (61.19 %) was found as the dominant compound in pomegranate seed oil while apiole (14.21 %) was determined as the dominant compound in parsley seed oil. When the antioxidant capacity of the oils were examined, it was determined that the oils obtained from pomegranate seeds have a moderate antioxidant activity, the oils obtained from parsley seeds have high antioxidant activity. Antifungal activity of pomegranate (*Punica granatum* L.) and parsley (*Petroselinium crispum*) seed oil against five different plant pathogens, *Fusarium. oxysporum f. sp. lycopersici, Botrytis cinerea, Sclerotinia sclerotiorum, Alternaria. solani* and *Rhizoctonia solani* were also determined.

Özet

Bu çalışmada endüstriyel ölçekte üretilmiş olan nar (*Punica granatum* L.) çekirdeği ve maydanoz (*Petroselinium crispum*) tohumundan elde edilen uçucu yağların ve yağ asitlerinin, antioksidan ve antifungal özellikleri incelenmiştir. Nar çekirdeği yağı endüstriyel soğuk press yöntemi ile üretilmiş iken, maydanoz tohumu yağı da endüstriyel buhar destilasyonu yöntemi ile elde edilmiştir. Nar çekirdeği yağında GC-MS analizi sonucunda 15 bileşen tespit edilmiştir. Maydanoz tohumu yağı içinde 12 bileşen tespit edilmiştir. Nar çekirdeği yağında pukinik asit (% 61.19) ana bileşen olarak bulunmuş iken, maydanoz çekirdeği yağında apiyol (% 14.21) ana bileşen olarak tespit edilmiştir. Yağların antioksidan aktivitesi incelendiğinde nar çekirdeğinden elde edilen yağlarda orta miktarda, maydanoz tohumlarından elde edilen yağlarda ise yüksek miktarda antioksidan aktiviteye sahip oldukları tespit edilmiştir. Ayrıca nar (*Punica granatum* L.) ve maydanoz (*Petroselinium crispum*) tohumu yağının beş farklı bitki patojenine *Fusarium. oxysporum f. sp. lycopersici, Botrytis cinerea, Sclerotinia. sclerotiorum, Alternaria. solani* ve *Rhizoctonia solani* antifungal aktivitesi olduğu tespit edilmiştir.

INTRODUCTION

Edible oils are important industrial food ingredients and may contain significant levels of monounsaturated fatty acid, tocopherols, carotenoids, and antioxidative phenolic compound (Parker et al. 2006, Parry et al. 2006). Although more than 100 varieties of plants, which is known oil bearing seeds, commercial seed oil have been very limited (Özcan 2002). Several crops such as corn, safflower, sunflower and soybean have been grown extensively for the oil produced in their seeds from past to present (Parry et al. 2005). Parsley (*Petroselinum crispum*) and pomegranate (*Punica granatum* L.) seed are generally a rich source of oil. However, this resource is only used on a limited scale. On the other hand, vegetable oils (edible or non-edible) are used extensively in cosmetics and other industries, thus

oil obtained from pomegranate and parsley seed can find potential uses in other branches of industry.

Although the humans have begun to cultivate the plant as a food source and additive in Turkey (Can et al. 2017), pomegranete (*Punica granatum* L.) was cultivated and naturalized all over the Mediterranean region since ancient times (Ahangari and Sargolzaei 2012). It is a member of the *punicacea* family (Fadavi et al. 2006). Productions of pomegranate have been rapidly increased for many years in Mediterranean region. In addition, Turkey is one of the main European pomegranate producers (Calişkan and Bayazit 2012). Pomegranate fruits contain seeds ranging between 40 and 100 g kg^{-1} of fruit weight depending of species (Fadavi et al. 2006). Pomegranate seed oil, which is by product pomegranate juice industry, have a wide range of nutritional values such as vitamin E, sterols and punicic acid (Tian et al. 2013).

Parsley (*Petroselinum crispum*) is cultivated widely as an annual. Also, it is a biennial plant. (Petropulos et al. 2004). Parsley belongs to *Umbellifera* plant family (Bakhiet et al. 2010). it was appreciated for medicinal properties long before it became accepted as a food or spice in ancient Greeks (Petropulos et al. 2004). Todays, its extraction becomes of profound industrial interest (Louli et al. 2004). Therefore, oil extraction methods are very important in the industry.

The cold pressing involves neither heat nor chemical treatment. The consumers prefer cold pressed oils as they are natural and safe food products because of these properties. Cold-pressed plant oil have better antioxidant properties and nutritive value, so it has increased to preferability by consumers recently. Also, cold press method has several advantages such as ecological, do not cost much investment, simple and not requiring much energy, as well (Juhaimi and Özcan 2017).

Considering studies in the literature on the pomegranate seed oil, it is seen that there are significant levels of punicic acid, natural antioxidant and polyphenols (Schubert et al. 1999, Sadeghi et al. 2009, Vroegrijk et al. 2011). According to researches the seeds are rich source of lipids. Oil content of pomegranate seed comprises from 12 to 20 % of the seed on a dry-weight basis (Meerts et al. 2009, Mohaghegi et al. 2011). The lipid compositions of pomegranate seed have also gained great attention for their high content of polyunsaturated fatty acid. According to Meerts, the oil is qualified by conjugated linolenic acid, followed by linoleic acid, steraic acid and palmitic acid (Meerts et al. 2009). Also, pomegranate seed oil consist of 75-90% conjugated fatty acid, the most important of which is punicic acid (Elfalleh et al. 2011, Ahangari and Sargolzaei 2012, Jing et al. 2012). Punicic acid is generally use as therapeutic for human health (Yücel 2005). It is known that pomegranate seed oil have high potential of antimicrobial and antioxidant properties (Liu et al. 2009, Tehranifar et al. 2011, Liu et al. 2012). The chemical properties of pomegranate responsible for these protective actions are well-documented (Ismail et al. 2012).

Limited studies of literature were conducted to assess the antioxidant, antifungal and chemical composition in parsley seed oil (Elgayyar et al. 2001, Kurowska and Galazka 2006, Parry et al. 2006). In this regard, the chemical composition of parsley seed oil in native and fermented seed with non-disntigrated and disintegrated were examined. It was found that there were different amount of α-pinene, β-pinene, sabinene, myrtenal and apiole (Stankovic et al. 2005). According to some researcher the main components of parsley seed essential oil are apiole, myristicin, safrole and 2.3.4.5-tetramethoxy-1-allylbenzene (Stankovic et al. 2005), as well as α-thujene, camphene, β-pinene, α-phellandrene, β-phellandrene, limonene, γ-caryophylene (Ahsan et al. 1982), α-pinene (Hol et al. 1990) and terpinolene (Orav et al. 2003).

Although some researchers studied about chemical composition, antifungal and antioxidant properties of pomegranate and parsley seed oil on producing laboratory scale, in the literature, there is few reports about detailed analyses of pomegranate and parsley seed oil produced in industrial scale. Therefore, goal of this study is investigated the essential oil and oil acid profile, antifungal and antioxidant properties of the

pomegranate and parsley seed oil produced in industrial scale. This study can provide important information about pomegranate and parsley seed oil as chemical composition, antioxidant and antifungal properties for industrial production

MATERIAL AND METHODS

Materials

Ripened pomegranate seeds (*Punica granatum*), concerning as a residue, were obtained from juice companies located in Hatay which is Mediterranean region city of Turkey. Parsley (*Petroselinum crispum*) seed were collected from Hatay in Turkey.

Methods

Extraction of Seed Oil

Before the extraction pomegranate (*Punica granatum*) and parsley (*Petroselinum crispum*) seeds were washed with deionized water and dried in room temperature. A commercial cold press was used to obtain the pomegranate seed oil, while commercial steam distillation process was used to obtain the parsley seed oil.

GC-MS Analysis of Pomegranate and Parsley Seed Oil

Before the GC-MS analyses, seed oil (25 µL) was diluted with methanol (1000 µL) and silylated according to Ekman and Holmbom (1989). Identification and quantification of seed oil were carried out by GC-MS on a Shimadzu QP2010 GC-MS system with helium as a carrier gas with a constant linear velocity. The interface and ion source and temperatures were 250 °C, 200 °C and 250 °C respectively, operating at 70 eV ionization energy. The column used was TRB-5MS capillary column (30 m x 0.25mm ID x 0.25 µm film thickness). Temperature was programmed from 60 °C (hold 2 min) 2 °C/min to 200 °C (2 °C/min) hold 10 min. Then to 300 °C (5 min). The injection volume was 1.0 µL the split ratio 1:9 and the injector temperature was 250 °C. The components were identified by comparison of their mass spectra with characteristic features obtained with the NITS and Wiley library spectral data bank. For quantification, normalized peak area was used without any correction factor.

Antioxidant Activity

DPPH Free Radical Scavenging Activity

The capacity of pomegranate and parsley seed oil to scavenge the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity was measured according to the method described by Molyneux. Each extract solution dissolved in methanol. The mixture was shaken vigorously and left to stand at room temperature for 50 min in the dark. The absorbance was read at 517 nm against a control using a spectrophotometer. The values were shown as IC_{50} µg /mL sample representing the concentration of each sample that resulted in 50% scavenging of DPPH radicals. The percentage of DPPH discoloration was calculated using equation: (Equation 1).

Percentage inhibition= $[(Abs_{0control} - Abs_{sample})/ Abs_{0control}]$ x100

β-Carotene-Linoleic Acid Activity

The determination of antioxidant activity was determined by the ability of the compounds to inhibit the bleaching of the β-carotene by linoleic acid. The β-carotene-linoleic acid inhibition activity of the extract was determined using a previously reported method (Huang et al. 2005). Briefly, β-Carotene (0.5 mg) was dissolved in 1 mL chloroform was pipetted into a small round-bottom flask. Then Tween 40 (200 mg) and linoleic acid (25 µL) was added to this solution. Once the chloroform was completely removed using a rotary evaporator under reduced pressure at low temperature, distilled H_2O (100 ml) saturated with oxygen (30 min at a flow rate of 100 mL/min) was added and the resulting mixture was stirred with Vortex for 30 min. Reaction mixture (350 µL) were mixed with seed oil in ethanol (350 µL) in test tubes and measured at 490 nm in a spectrophotometer, before and after the 48h incubation at 24 °C. The same procedure was repeated with poisitive control BHT (butylated hydroxytoluene) an a

blank. The data presented as a percentage of an antioxidant activity % (AA %), using [Equation 2].

$AA\% = [1-(Abs_{0sample} - Abs_{sample})/(Abs_{0control} - Abs_{control})] \times 100$.

Antifungal Activity

In order to determine the effects of pomegranate and parsley seed oil against *B. cinerea, S. sclerotiorum*, *A. solani, R. solani* and *F. oxysporum f. sp. lycopersici* this assay was used. The fungi were obtained from the culture collection of Gümüşhane Vocational School of Higher Education, Department of Organic Farming at Gümüşhane University, Turkey. The in vitro antifungal activity was assayed using a contact that produces mycelial growth inhibition (Kordali et al. 2009). *Botrytis cinerea, Sclerotinia sclerotiorum, Alternaria solani, Fusarium oxysporum f. sp. lycopersici* and *Rhizoctonia solani* were instored on sterile potato dextrose agar (PDA) slants prior to use. First the mycelium of the test strain growths were evaluated in 60 mm Petri dishes filled with PDA solid medium amended with 100, 250, 500 and 1000 ppm extracts of pomegranate and parsley seed oil. The oils were dissolved in ethanol (1/1, v/v). Next, five mm disc of 7-day-old culture of the *B. cinerea, S. sclerotiorum, A. solani, F. oxysporum f. sp. lycopersici* and *R. solani* fungi were placed at the center of the petriplates. Then all inoculated dishes were incubated at 25 °C for 7 days. PDA plates containing 1000µl ethanol (10 mL plate^{-1}), without sample solutions, were used as negative control. In addition, synthetic Maneb fungicide (0.2 g/100 mL PDA) was used as a positive control. After that, colony diameter was measured and recorded after seven days. Three replicate plates were used for each treatment. Finally, percentage inhibition of mycelia growth was calculated by using the following formula [Equation 3].

% inhibition = (dc-dt)/dc x 100,

dc: The average increase in mycelia growth in control

dt: The mean of three replicates of mycelial growth (mm) in treated.

RESULT AND DISCUSSION

Essential oil and oil acid profile of pomegranate and parsley seed oil

Essential oil and oil acid content of pomegranate and parsley seed oils with individual percentages of each component are given in Table 1. Fifteen compounds were identified in pomegranate seed oil, while twelve compounds were identified in parsley seed oil. The pomegranate seed oil was found to be rich unsaturated fatty acid (72.89 %). Punicic, oleic, linoleic, gadoleic acids were among the highest unsaturated fatty acid with the following order: Punicic> Oleic> Linoleic> Gadoleic. Especially punicic acid was found to be the dominant compound for pomegranate seed oil. Punicic acid (61.19%) was determined to be predominant polyunsaturated fatty acid, which was in compatible with results of previous study (Kỳralan et al. 2009, Hernandez et al. 2011, Ahangari et al. 2012). The punicic acids, which is a conjugated linolenic acid, have double bonds in the 9, 11 and 13 positions and it was identified at difference retention times, indicating the existence of different geometric isomers because of the invariable double bonds. However all identified isomers in the present study were considered punicic acids. Our results are in agreement with the literature (Fadavi et al. 2006, Elfalleh et al. 2011, Hernandez et al. 2011). Qualitatively, the punicic acid is similar to previous findings (Liu et al. 2009, Eikani et al. 2012, Liu et al. 2012). Oleic acid (9 and 10) was determined to be second most abundant unsaturated fatty acid followed by linoleic acid. Also, oleic acid was only monosaturated fatty acid (MUFA) detected in pomegranate seed oil and it was found 6.83% of total fatty acid in present study (Table-1). The oleic acid content (6.83%) was similar to Spanish cultivar (4.70-6.49%) (Hernandez et al, 2012) and Georgia cultivar (Pande and Akoh 2009). Although oleic and linoleic acid was reported in most of the studies, it was higher content than our studies (Melgarejo et al. 2000, Fadavi et al. 2006). These quantitative differences on the fatty acid of pomegranate seed could be related to agronomic, environmental, production condition and different genotype. Gadoleic acid (C20:1) was found lesser extent in this study. Gadoleic acids usually were not determined

Table-1 Essential oil and oil acid content of pomegranate seed and parsley seed oil.

Compound	R.I	Parsley seed (GC-MS Peak Area %)	Pomegranate seed (GC-MS Peak Area %)
α-pinene	7.138	12.06	-
Ethylamine	7.633	-	0.72
Norvaline	7.927	-	2.02
β-pinene	8.976	12.48	-
Myrcrene	9.347	0.39	-
β-phellandrene	11.173	12.24	-
Myrtenal	20.826	0.83	-
Blown Oil (2.4-nonadienal)	22.107	-	2.19
Myristcin	42.372	12.69	-
Cis-aserone	44.305	7.86	-
Azulen-3a-ol	46.207	0.95	-
Cis-6-octadecenoic acid	51.325	10.99	-
β-sitesiterol	53.208	0.47	-
Apiole	61.712	14.21	-
Myristic acid (C14:0)	48.45	-	0.21
Palmitic acid (C16:0)	70.639	-	4.07
Linoleic acid (C18:2)	79.654	3.96	4.3
Oleic acid (C18:1)	80.522	-	6.83
Steraic acid (C18:0)	82.138	-	2.18
Punicic acid (C18:3)	92.025	-	61.19
Gadoleic acid (C20:1)	93.048	-	0.57
Arachidic acid (C20:0)	94.585	-	0.63
Behenic acid (C22:0)	104.120	-	0.18
Squalane	111.534	-	2.62
Lignoceric acid (C24:0)	112.198	-	0.07
γ-tocopherol	118.202	-	4.08

in most of studies, but Elfalleh et al. (2011) identified gadoleic acid in pomegranate seed oil (0.50-9.94). The major saturated fatty acid for this study was palmitic acid, which is founded 4.07 %. This palmitic acid content was comparable with literature (Hernandez et al. 2011, Eikani et al. 2012, Liu et al. 2012). On the contrary, the amounts of palmitic acid were lower than those obtained by Melgarejo and Artes et al. (2000), Fadavi et al. (2006), Elfalleh et al. (2011) with palmitic percantages of 2.58-14.9 %, 2.8-16.7 % and 3.13-11.82, respectively. The following saturated fatty acid was determined stearic acid (2.18%). Stearic acid content was similar to previous reported by Melgarejo et al. (2000), Hernandez et al. (2011), Meerts et al. (2009). Arachidic (0.63%), Behenic (0.18%) and Lignoceric acid (0.07%) were found lesser content in present study. These acids were undetected some researches such as Meerts et al. (2009) and Hernandez et al. (2011). The quantity of arachidic acid was found similar to Elfalleh et al. (2011) and Eikani (2012) with arachidic percentage 0.56-1.70 and 0.62 respectively. Likewise, Behenic acid percentages were similar to those obtained by Fadavi et al. (2006), Ahangari and Sargolzaei (2012) and Eikani et al. (2012), 0.03-0.2%, 0.1-0.2% and 0.14% respectively. Lignoceric acid was determined by Ahangari and Sargolzaei (2012), Elfalleh et al. (2011) and Eikani et al. (2012). The saturated/unsaturated acid ratio is important quality criteria in vegetable oil. If unsaturated fatty oil taken 2 to 1, saturated oil could be exhausted by unsaturated oil (Mindel 2009). Squalene is very important industrial and organic compounds. The main source of squalene is fish and thus it is an expensive terpenoid. According Caligiani

et al. (2010) indicated that squalene plays an important role in preventing tumors. Concentrations of squalene was founded considerably higher in pomegranate seed oil (2.62 %) for this study. Squalene was detected in pomegranate seed oil by Caligiani et al. (2010). Considering to other important commercial oils, it seen that they have quite low squalene content, such as 0.03% in maize, peanut, and rapeseed, 0.002% in coconut, 0.01 % in sunflower and cotton, 0.4 % in olive oil and 0.3% in rice bran (He et al. 2002). Stigmasterol is one of a group of phytosterols (plant sterols), that includes β-Sitosterol with chemical structures similar to that of cholesterol (Rosenblat et al. 2013). In this study, stigmasterol and β-Sitosterol were detected 5.59% and 0.2%, This was detected in accordance with previous report on stigmasterol and β-Sitosterol in pomegranate seed oils (commercial), as 30.4±5 and 8.069±135 (Caligiani et al. 2010). Also, these compounds are also known to have cancer chemopreventive properties, including promotion of apoptosis. Stigmasterol and β-Sitosterol in pomegranate seed oil are reported by Kim et al. (2002). γ-tocopherol is one of the chemical compounds that is considered vitamin E, which is detected 4.08% in pomegranate seed oil. They are lipid soluble and show potentials to reduce free radicals (antioxidants), and clinical trials showed the significant role of these antioxidants to scavenge free radicals, such activity contribute to the use of these agents in prevention against stroke and cancer (Elfalleh et al. 2011). Many researches are found γ-Tocopherol in pomegranate seed oil (Caligiani et al. 2010, Elfalleh et al. 2011, Liu et al. 2012).

When the chemical composition of parsley seed oil were examined (Table-1), apiole and myristcin (14.21% and 12.69 %) was found to be the dominant compound. Also, α-pinene (12.06 %), β-pinene (12.48 %) and cis-6-octadecenoic acid (10.99 %) were found significant amounts. According to Young and Tisserand (2014), indicated that apiole is toxic to humans, the lowest fatal daily dose is 770 mg, which was taken 14 days, the lowest single fatal dose is 8g. At least 19 g has been survived. Louli et al. (2004), reported as a result of the qualitative analysis they performed on parsley seed oil that myristicin (36 % and 42%) is the most dominant compound. Authors also found different amount of α-pinene (2.7 % and 1.2 %), β-pinene (2 % and 0.5 %) and apiole (26.7% and 34.6 %) (Louli et al. 2004). Another study examined the quantitative and qualitative compound of the essential oil from parsley seeds (native and fermented). The researchers determined that myristicin was the most dominant compound among the composition of essential oil in parsley with 42.7 % for fermented seeds and 36.7 % for native seeds. Furthermore, α-pinene (20.22 %), β-pinene (16.7 %) and apiole (5.4 %) were determined to be the other most dominant compound (Stankovic et al. 2005). Vokk et al. (2011) conducted in Estonia examined the GC-MS and composition of parsley growing in summer and wintertime that β-phellandrene (35.88% for winter and 21.83 % for summer) and myristicin (30.67 % for winter and 42.65 % for summer) is the most dominant compound. When the components of parsley oil were examined qualitatively, it was found that the literature is largely in parallel with this study. However, if it was examined quantitatively composition of essential oil from parsley seed, it was not compatible with literature. According to Okan et al. (2018) the reason for this is that same plant variety demonstrate differences due to the genetic factor, environmental condition and climate etc. in the synthesis of the essential oil (Okan et al. 2018).

Antioxidant Activity

Molecules known as antioxidants prevent oxidation in living organism by decreasing free radicals or by eliminating these. There are many methods of measuring the antioxidant capacity in natural products (Okan et al. 2019). Antioxidant activity of pomegranate seed and parsley seed oil were tested using DPPH and β-Carotene-linoleic acid assays (Figure-1).

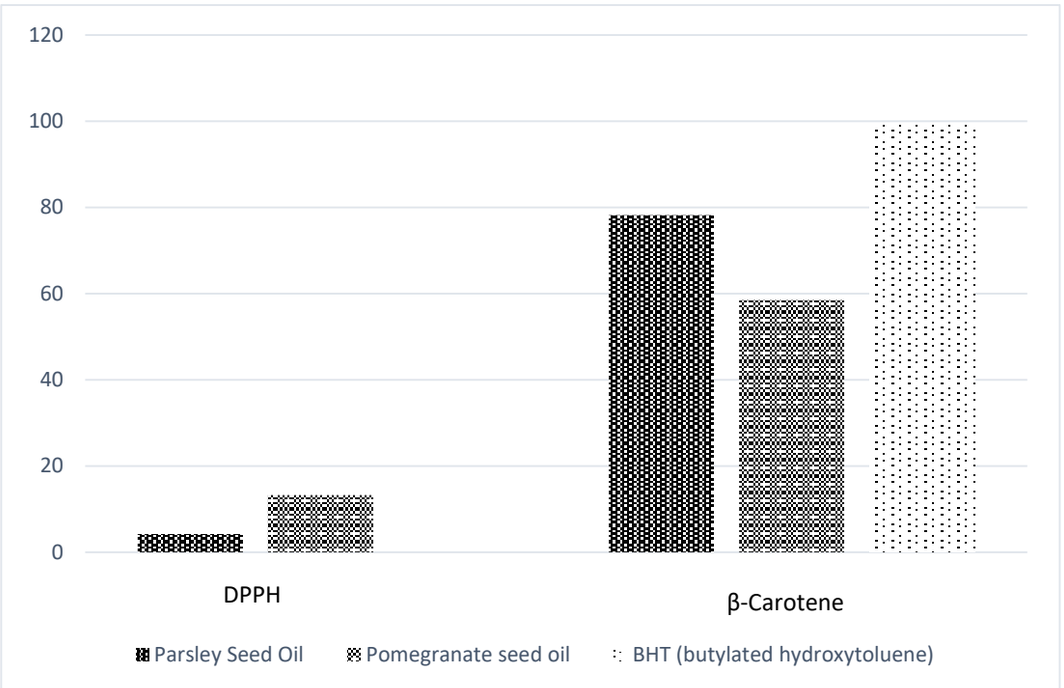

Figure 1. DPPH and β-Carotene-linoleic acid oxidation activities of parsley and pomegranate seed oil

In DPPH assay, free radical scavenging activity of pomegranate and parsley seed oil were determined (IC_{50}: 13.05 µg/ml and IC_{50}: 4.21 µg/ml). According to β-Carotene-linoleic acid antioxidant assay, antioxidant capacity of pomegranate was determined RAA%: 58.51 and compared with positive control BHT. Parsley was determined RAA%: 78.21. Considering to these results, antioxidant activity of pomegranate oil was determined as moderately, but antioxidant activity of parsley oil was determined as strongly. In a different study the antioxidant capacities of the parsley oil was examine using DPPH methods. The result showed that DPPH values was determined quenched 87-91% of the radicals in the reaction mixture in 10 min (Parry et al. 2006). The DPPH antioxidant activity was determined high antioxidant capacity for parsley seed oil (Wei and Shibamoto 2007). According to researchers, myristicin, α -pinene, β-pinene and apiole which is found high amounts parsley oil, are effective in increasing antioxidant capacity. The DPPH activity of pomegranate seed oil was similar to 0.8-5.2 2 µg/ml grown in the China (Liu et al. 2012), 1.8-4.8 µg/ml grown in India (Waghulde et al. 2011) and 1.324-2.577 µg/ml grown in Thailand (Manasathien et al. 2012). These differences may be due to the fact that seconder metabolite synthesis is influenced by environmental biotic and abiotic factors in addition to genotype or different growth conditions (Okan et al. 2019). β-Carotene assay is one of the most used methods for lipophilic antioxidants (Chan and İsmail, 2009). Accordance with previous reports on β-Carotene activities of pomegranate seed oil, as 39, 22, 57% with 100 ppm concentration EtOAc, MeOH and water extract (Sing et al. 2002). β-Carotene activities of pomegranate seed oil was lesser than that in some other edible seed oils including grape seed oil (63-78%) (Jayaprakasha et al. 2001) and buckwheat oil (67%) (Sun and Ho 2005).

Antifungal Activity

The comparative results regarding the antifungal activity assays in parsley (*Petroselinum crispum)* and pomegranete (*Punica granatumun)* seeds are shown in Table 2. The oils exhibited considerable antifungal activity against a broad spectrum of tested fungi. Parsley seed oil is more effective against five fungus than pomegranate seeds oil (Table 2). The oil of parsley seed oil all concentration completely inhibited growth of *B. cinerea* (100%), *S. sclerotiorum*

Table 2. The inhibitory effects of Parsley and Pomegranate seed oil on plant pathogen fungi.

Plant Pathogens		Concentration (ppm)	Mycelial growth inhibition (%)	Mycelial growth (mm)
Botrytis cinerea	Parsley	0 (N- Control)[a]	0.00[d]	60,00[c]
		100	81.70	10.98
		250	100	0,00
		500	100	0,00
		1000	100	0,00
		P-Control (Maneb)[b]	100	0,00
	Pomegranate	0 (N- Control)[a]	0.00	60,00
		100	14.90	51.06
		250	15.78	50.53
		500	17.55	49.47
		1000	20.82	47.51
		P-Control (Maneb)[b]	100	0,00
Sclerotinia sclerotiorum	Parsley	0 (N- Control)[a]	0.00	60.00
		100	22.23	46.66
		250	29.23	42.46
		500	32.33	40.60
		1000	35.50	38.70
		P-Control (Maneb)[b]	100	0.00
	Pomegranate	0 (N- Control)[a]	0.00	60.00
		100	15.62	50.63
		250	16.60	50.04
		500	16.70	49.98
		1000	19.46	48.32
		P-Control (Maneb)[b]	100	0,00
Alternaria solani	Parsley	0 (N- Control)[a]	0.00	48.18
		100	43.21	27.36
		250	55.46	21.46
		500	60.71	18.93
		1000	69.70	14.60
		P-Control (Maneb)[b]	100	0.00
	Pomegranate	0 (N- Control)[a]	0.00	48.18
		100	0.37	48,00
		250	1.04	47.68
		500	2.43	47.01
		1000	3.86	46.32
		P-Control (Maneb)[b]	100	0.00
Fusarium oxysporum f. sp. lycopersici	Parsley	0 (N- Control)[a]	0,00	49.26
		100	42.75	28.20
		250	54.20	22.56
		500	59.83	19.79
		1000	100	0.00
		P-Control (Maneb)[b]	100	0.00
	Pomegranate	0 (N- Control)[a]	0.00	49.26
		100	7.69	45.47
		250	9.16	44.75
		500	13.42	42.65
		1000	21.38	38.73
		P-Control (Maneb)[b]	100	0,00
Rhizoctonia solani	Parsley	0 (N- Control)[a]	0.00	60.00
		100	100	0.00
		250	100	0.00
		500	100	0.00
		1000	100	0.00
		P-Control (Maneb)[b]	100	0.00
	Pomegranate	0 (N- Control)[a]	0,00	60.00
		100	15.75	50.55
		250	15.68	50.59
		500	15.37	50.78
		1000	14.95	51.03
		P-Control (Maneb)[b]	100	0.00

[a]Negative control, [b]Positive control, [c]Radial growth after 7 days (mm) , [d]Percentage (%) growth inhibition was calculated with comparison with the growth of the control (0%).

(35.50%), *A. solani* (69.70%), *F. oxysporum f. sp. Lycopersici* (100%) and *R. solani* (100%). In many cases, antifungal activity of the oil was also found similar or lower than positive control, manep (Table 2). Additionally, *B. cinerea*, *S. sclerotiorum, A. solani*, *F. oxysporum f. sp. lycopersici* and *R. solani*, Pomegranate seeds oils, reduced the mycelial growth of plant pathogens with respect to control. But this inhibition increased depending on rising in concentration and the highest in concentration (1000 ppm) inhibited the mycelial growth of *F. oxysporum f. sp. lycopersici* (21.38%) (Table 2).

Recent reports showed that essential oils, which are possess strong inhibitory effects on the mycelium growth of plant pathogenic fungi (Kordali et al. 2009, Kotan et al. 2008, Lee et al. 2007, Pattnaik et al. 1997). Albayrak et al. (2011) studied the antimicrobial activities of infusion, decoction, hydrosol and methanol extract of parsley seed again six different microbial species and they found that the antimicrobial activity of methanol extract was effective *Bacillus cereus, Morganella morganii* and *Pseudomonas aeruginosa.* Tehranifar et al (2011) found that the seed methanolic and aqueous extract of *Pomegranate* can prevent against *Penicillium italicum*, *Botryris cinereal* and *Rhizopus stolonifera*. A large amount of previous reports about pomegranate and parsley seed oil illustrates that it can confirm the inhibitory effects of seed oil on growth and development of fungi.

CONCLUSIONS

From the present study, it can be concluded that the seed, the by-product of pomegranate fruits was apparently value added. Pomegranate seed oil very rich punicic acid. It was shown to be protective against some cancer type. Squalene and phytosterols was significantly found in this study. Thus, the high content of squalene in pomegranate seed oil may represent a viable new source of squalene. Pomegranate seed oil is rich sources of tocopherols and many antioxidants. Therefore, pomegranate seed oil was exhibited a high antioxidant potential. Parsley seed oil showed powerful antioxidant activity because of apiole, myristicin, α-pinene and β-pinene. Also, it was found to be dominant compound apiole and myristicin. Especially Apiole is known hepatotoxic and nephrotoxic. The results of antifungal activity indicate that pomegranate and parsley seed oil showed strong inhibitory effects on the mycelium growth of plant pathogenic fungi. Thus, they would probably be an important potential candidate plants for the improvement of new generation fungicides. These oils may use for potential medical purposes, because of the fact that pomegranate seed oil have lower saturated fats and parsley seed have high antioxidant capacity as well.

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