Extraction, Analysis and Study of Antioxidant Activity and Total Phenolic of Pomegranate (Punica Granatum L.) Seed Oil from four Different Regions of Iran (Yazd, Saveh, Kashan and Varamin)

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ABSTRACT

In this study varieties of pomegranates were collected from four different regions of Iran (Yazd, Saveh, Kashan and Varamin) and the oils were extracted in Soxhlet by using Hexane as a solvent. The fatty acids composition of the seed oil of pomegranate varieties (*Punica granatum L.*) was determined by GC-MS. The results showed 6.8-9.0 % saturated fatty acid, 8.1-34.5% monounsaturated, 0-8.7% diunsaturated and 50.9-78.2% poly unsaturated fatty acids. Antimicrobial properties were studied the disc diffusion method on four microbial samples of *Saccharomyces cerevisae* (ATCC 2365), *Basilus subtilis*, *Staphylococcus aureus* (ATCC 25923), *Esherichiacoli* (ATCC 25922). None of the samples showed antimicrobial effects. The samples were also subjected to screening for their possible antioxidant activity by using DPPH as a stable free radical, Ravand Kashan variety showed the most antioxidant activity of about 90% while the Shirin Saveh variety had the least activity of 50% compared with trolox as a control sample with 94% antioxidant activity. Four oil samples from different regions of Iran were studied for their total phenolic compounds according to Folin-Ciocalteau method. The Ravand Kashan variety had the highest amount of approximately 37.97 µg/mg and the Shirin Saveh variety had the least amount, 22.61 µg/mg.

Key Words

Extraction, analysis, antioxidant activity, pomegranate, *Punica granatum L.*, seed oil.

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1. Introduction

The pomegranate's medicinal qualities have been known for thousands of years.References in the Bible and Roman mythology mention the tree's unique healing powers, and some Middle Eastern, Asian, and South American people stile chew its bark, petals, and peel to treat conditions as diverse as dysentery and diseases of the mouth and gums (Ross, 1999) . Modern research has shown that the pomegranate contains polyphenols and anthocyanidins that are powerful free-radical scavengers and are more effective against disease than are those in green tea (Burton, 2003). It is widely used in traditional medicine to cure inflammation, diabetes, cardiac disease, AIDS, ischemia and cancer. On this basis, the possible anticarcinogenic effects of the pomegranate have been further explored. For example, the application of pomegranate extract to the skin of mice before they were exposed to a carcinogenic agent was shown to inhabit the appearance of erythemas and hyperplasia and the activity of epithelial ornithine decarboxylase (Van Elswijk et al., 2004). The pomegranate has also been shown to induce programmed cell death and to inhibit tumor invasion, proliferation and angiogenesis. It targets several proteins in the cell-signaling pathway.

The unique biochemistry of the pomegranate tree is quiet intriguing. In addition to the high levels of antioxidant-

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rich tannis and flavonids in its juice and peel, the crushed and dry seeds of its fruit produce distinct oil, about 60% of which is a very rare 18-carbon fatty acid, also referred to as punicic acid. This oil contains isoflavone genistein, the phytoestrogen coumestrol, and the sex steroid estrone. In fact, the pomegranate tree is one of the only plants in nature known to contain estrange (Aviram et al., 2001). Its estron-containing nature may in part explain its therapeutic efficacy, given that several lines of evidence suggest a possible role of phytoestrons in preventing a range of diseases, not least of which are the hormonally dependent cancers (Van Elswijk et al., 2004).

The Anthocyanidins are components of pomegranate fruit that contribute to the antioxidant activity. Anthocyanidins (delphinidin, cyaniding, and pelargonidin) have been shown to have free – radical scavenging activities and inhibitory effects on lipid proxidantion. They inhibit hydrogen peroxideinduced lipid peroxidation in the ret brain homogenates (Toi et al., 2003). Evidence suggests that polyphenolic antioxidants contained in pomegranate juice can contribute to the reduction of oxidative stress and atherogenesis through the activation of redox- sensitive genes ELK-1 and p-JUN and increased eNOS expression (Gasmi et al., 2010).

The pomegranate is native to the region of Armenia, Iran and the western Himalayan range, and has been cultivated in Iran, Iraq, Armenia, Afghanistan, Pakistan, India, Russia, and the Mediterranean region for several millennia (Doijode, 2001). *Punica granatum L.* (Figure 1) belongs to the family Punicaceae which includes only one genus and two species, the other one, *protopunica balf*. Pomegranate fruits contain considerable amounts of seeds, ranging between 40 and 100 g/kg of fruit weight depending on cultivar (Parashar et al., 2008).

The main objective of the present study was to identify of the lipid content especially fatty acids and study of antioxidant activity and total phenolic content of pomegranate (*Punica granatum L.*) seed oil on Yazd, Saveh, Kashan and Varamin regions in Iran.

2. Material and Methods

In this research, the seed oil from the varieties of pomegranate in Iran was extracted through solvent extraction technique as described in the method of AOAC (1990); hexane used as solvent was recovered by Rotary Evaporator. The extracted oil was injected into chromatograph equipment with a mass spectra detector (GC- MS). Components were identified by comparison of the retention time and mass spectra of the unknowns with those of authentic samples and also comparative analysis of kovats index & using references of Eight peak.

It should be noted that the extraction and identification was performed separately for each region.

The DPPH Method: A simple method that has been developed to determine the antioxidant activity of foods utilizes the stable 2, 2- diphenyl-1-picrylhydrazyl (DPPH) radical. The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color. The color turns from purple to yellow as the molar absorptive of the DPPH radical at 517 nm reduces from 9660 to 1640 when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolonization is stoichiometric with respect to number of electrons captured. Antioxidant compounds may be water-soluble lipid soluble, insoluble, or bound to cell walls. Hence, extraction efficiency is an important factor in quantification of antioxidant activity of foods. Trolox (as the reference standard) and the sample are reacted with DPPH solution in methanol/water for four hours at 35°C in a vessel mounted on a rotary shaker and the absorbance changes are measured at 517 nm (Miller et al., 2000).

Folin-Ciocalteu Method: The total phenolic contents of the samples were determined in triplicate in Gallic Acid Equivalents (GAE) using the modifications of Folin-Ciocalteu method (Singleton and Rossi, 1965). The procedure consisted of the following: 50 µL of cachaça sample, 3.7 mL of distilled water, 250 µL of Folin-Ciocalteu reagent 2 N and 1 mL of sodium carbonate 20% (w/v) were placed in calibrated test tubes. The test tubes were shaken to homogenize their contents and allowed to rest in the dark for 30 min to stabilize the reaction. The absorbance at 750 nm was determined in a Shimadzu UV mini-1240 spectrophotometer in quartz cells with a 10-mm optical path. A calibration curve was prepared with gallic acid solutions in the concentration range of 0 to 1000 mg L^{-1} and the results were expressed in milligrams as Gallic Acid Equivalents (GAE) per liter of cachaça (mg_{GAE} L^{-1}). The following calibration curve, was used to calculated the concentration of total phenolic (mg_{GAE} L^{-1}): Total phenolic mg_{GAE} L^{-1} = 1068.6x Abs_{750nm} + 26.54, correlation coefficient r = 0.99351.

Disk diffusion test: The agar diffusion test, or the Kirby-Bauer disk-diffusion method, is a means of measuring the effect of an antimicrobial agent against bacteria grown in culture. The bacteria in question are swabbed uniformly across a culture plate. A filter-paper disk,

Fig. 1. *Punica granatum* L.

impregnated with the compound to be tested, is then placed on the surface of the agar. The compound diffuses from the filter paper into the agar. The concentration of the compound will be highest next to the disk, and will decrease as distance from the disk increases. If the compound is effective against bacteria at a certain concentration, no colonies will grow where the concentration in the agar is greater than or equal to the effective concentration. This is the zone of inhibition. Thus, the size of the zone of inhibition is a measure of the compound's effectiveness: the larger the clear area around the filter disk, the more effective the compound (Tepe et al, 2005).we studied the agar diffusion test on four microbial samples of *Saccharomyces cerevisae* (ATCC 2365), *Basilus subtilis*, *Staphylococcus aureus* (ATCC 25923), *Escherichiacoli* (ATCC 25922).

3. Results

In this research, the components identified in GC-MS analysis of pomegranate seed oil samples is shown the below Table 1.

Results of this research indicate that compounds identified are saturated fatty acids Palmitic (2.38-4.37%) acid, Stearic acid (1.59-3.64%) and Ecosanoic acid (0.87-5.32%), monounsaturated fatty acids Oleic acid (7.10-19.86 %) and 11-Ecosenoic acid (1.00-14.62 %), polyunsaturated fatty acids alpha-linoleic acid (50.90-78.24 %) and Linoleic acid (5.30-8.76%).

Also, we researched the antimicrobial activities of pomegranate seed oil samples and none of the samples showed antimicrobial effects.

The antioxidant activities of pomegranate seed oil sample from four different regions of Iran (Yazd, Saveh, Kashan and Varamin) are shown in Table 2.

The total phenolic contents of the samples is indicated in Table 3.

4. Discussions

In conclusion, these results indicate that the most dominant fatty acid was α-Linolenic acid (50.90-78.24 %). Alpha-linolenic acid is an n-3 fatty acid (Burdge & Calder, 2005). Research shows that omega-3fatty acids reduce inflammation (Gil, 2002) and may help lower risk of chronic diseases (Lord & Bralley, 2002; Tran etal., 2010) such as heart disease (Reiffel & McDonald, 2006; Chattipakorn et al, 2009), cancer (Hardman, 2002; Simon et al., 2009; Adhami et al., 2011), and arthritis (Calo et al., 2005; Landmark, 2006; Coursodon-Boyiddle et al., 2012). Omega-3 fatty acids are highly concentrated in the brain (Allen et al, 2001) and appear to be important for brain memory and behavioral function (Bousquet et al., 2008). In similar studies, Malik et al (2005), Gasmi & Sanderson (2010), Vroegrijk et al (2011) and Gozlekçi et al (2011) on pomegranate seed oil found that punicic acid is the main constituent of pomegranate seed oil (70-80%). Punicic acid (c9t11c13-CLNA) is an omega-5 long chain polyunsaturated fatty acid. It is a conjugated linolenic acid and has three conjugated double bonds (Vroegrijk et al., 2011).

Also, the results in research showed that pomegranate seed oil contains polyphenols and anthocyanidins. In addition, pomegranate seed oil on kashan area showed the most antioxidant activity and total phenolic compounds. Antioxidants are vital substances, which possess the ability to protect the body

Table 1. The compound identified in the chloroform phase of pomegranate (*Punica granatum* L.) seed oils from four different regions of Iran (Yazd, Saveh, Kashan and Varamin).

Compound	MF	KI	% of total			
			Aghda Yazd	Shirin Saveh	Ravand Kashan	Shahpar Varamin
Fatty acid	$C_{16}H_{32}O_2$	1740	3.44	4.37	2.38	4.09
Saturated fatty acid						
Palmitic acid	$C_{18}H_{36}O_2$	1904	2.49	3.64	1.59	3.37
Stearic acid	$C_{18}H_{36}O_2$	1904	2.49	3.64	1.59	3.37
Ecosanoic acid	$C_{20}H_{40}O_2$	2040	0.87	1.01	5.32	1.03
Mono-unsaturated fatty Acid						
Oleic acid	$C_{18}H_{34}O_2$	1887	7.10	8.18	19.86	9.46
11-Ecosenoic acid	$C_{20}H_{38}O_2$	2029	1.00	2.35	14.62	2.05
Poly- unsaturated fatty acid						
α-Linolenic acid	$C_{18}H_{30}O_2$	2016	78.24	71.57	50.90	67.93
Linoleic acid	$C_{18}H_{32}O_2$	2040	6.86	8.76	5.30	6.40

MF: Molecular Formula
KI: Kovats Index
%: Percent of the compound

Table 2. IC50 values of extracts by DPPH radical method.

Sample	Percent of inhibiting free radical	IC50 (µg/mg)
Trolox	94.76	503
Aghda Yazd	80.80	550
Shirin Saveh	55.74	572
Ravand Kashan	90.32	514
Shahpar Varamin	85.89	627

Table 3. Rate of total phenolic content in extracts.

Sample	GAE (µg/mg)
Aghda Yazd	24.47
Shirin Saveh	22.61
Ravand Kashan	37.97
Shahpar Varamin	34.65

GAE: Gallic Acid Equivalents

from damages caused by free radical-induced oxidative stress. The polyphenols of pomegranate seed oil have been shown to exert anticancer effects on human (Van Elswijk et al., 2004). In similar studies, Aviram et al (2001), Parashar et al (2008), Gozlekçi et al (2011) and Adhami et al (2011) showed that pomegranate seed oil contains polyphenols and anthocyanidins.

Overall the effectiveness using of pomegranate seed oil is in health and possibly in preventing inflammation (Gil, 2002), brain disorders (Allen et al, 2001), diabetes (Stirban et al., 2010), oxidative stress, hypoxia, hyperlipidemia (possibly decreased low-density lipoprotein (LDL) and increased high-density lipoprotein (HDL) cholesterol), cardiac disease (Aviram et al., 2002; Teres, 2008), AIDS, ischemia and cancer (especially Skin, Colon, Breast, Prostate and lung) (Longtin, 2003; Malik et al., 2005; Seeram et al., 2007; Grossmann et al., 2010) Monounsaturated fat consumption has been associated with cholesterol, and (Teres, 2008).

Results of in this study showed that the muscle tissues of female and male blue swimming crab (*Portunus pelagicus*) are rich in fatty acids especially Omega-3 alpha- Linoleic acid (46.9-47.2%) and Omega-9 Oleic acid(16.0-16.2%), so these crabs are one of the healthiest seafood, and they are also suitable as a raw material in the processing industry.

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