International Journal of Pharmacology

ISSN 1811-7775

science alert

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OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2024.936.941

Research Article
Extraction of Omega 5 (ω-5) a Pharmacologically Active Ingredient from the Waste Parts of Pomegranate Seeds

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Abstract

Background and Objective: Pomegranate seed oil (PSO) is important because of its positive effects on human health due to its antidiabetic, hypolipidemic, anti-inflammatory and anti-cancer activity properties. Pomegranate seeds are a rich source of conjugated linolenic acid as a metabolite of punicic acid (PA), derived from the omega 5 family (ω-5). Therefore, the extraction and amount of pomegranate seed oil were evaluated in this study. **Materials and Methods:** For this purpose, the total oil yield and ω-5 amounts in the seeds were determined by different drying methods applied to the beans obtained from three different phenotypes, namely Hicaz, Nuz and Devedişi. Solid-liquid extraction method was used to determine ω-5 amounts and total fat ratios. **Results:** When the total oil yields were compared, the highest yield was obtained in the natural drying method for all three species. The highest total oil yield was obtained from naturally dried Nuz pomegranate seeds with a value of 9.08%. Considering the total oil yields of other species, it was determined that the efficiency was 8.09% in natural dried seeds of Hicaz pomegranate and 4.36% in natural dried seeds of Devedişi. Oil yield was determined as 4.08% in Hicaz pomegranate, from which oil was extracted after drying in the oven and 3.97% in Devedişi pomegranate. **Conclusion:** Considering the amount of ω-5 detected by the gas chromatography device, the highest value was observed at 65.973% in the oil obtained from Devedişi pomegranate dried in the shade. As a result, the presence of pharmacologically effective ω-5 in different pomegranate phenotypes was detected in this study.

Key words: Pomegranate seed oil, omega 5, anti-cancer activity, anti-diabetic, anti-inflammatory

Citation: Oğuzkan, S.B. and M. Özaslan, 2024. Extraction of omega 5 (ω-5) a pharmacologically active ingredient from the waste parts of pomegranate seeds. Int. J. Pharmacol., 20: 936-941.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The pomegranate is a perennial fruit belonging to the *Punica* genus of the Punicaceae family and the most important species is *Punica granatum* L.[1]. In addition to the use of pomegranate fruit as food, its seed and peel are also used in various industrial areas. In addition to these, pomegranate is a fruit that has been considered sacred in many beliefs since ancient times[2]. It is thought to be one of the oldest cultivated fruits[3]. The consumable part of this fruit, which is very important in terms of nutritional value, is 52%. As 78% of this rate is fruit and 22% is seeds[4].

In pomegranate consumption, it is generally preferred in the form of pomegranate juice. However, according to recent studies, the presence of very valuable nutrients in pomegranate seeds has been detected. In particular, it was determined that the oil of the pomegranate seed was extracted and contained very important fatty acids at high rates[5].

Studies have shown that pomegranate seed oil is a source of important unsaturated fatty acids. When the fatty acids in the pomegranate seeds are examined, it contains high levels of punicic acid, oleic acid, palmitic acid, stearic acid, arachidic acid and important fatty acids[6].

Pomegranate seed oil (PSO) is punicic acid, also known as ω-5, as the major molecule. Many studies have been conducted to examine the effects of punicic acid on human health. These studies cover certain topics such as its antidiabetic properties, hypolipidemic properties, anti-inflammatory properties, anticancer activity, antinephrotoxic activity and antioxidant capacity[7].

The pharmacological efficacy of PSO against malignancies such as prostate cancer has been determined[8]. In recent years, protective effects of PSO on bone have been observed in ovariectomized animals[9].

There are many methods used today for oil extraction from plants. Among the features sought in these methods are parameters such as obtaining the oil without reducing the yield and quality, choosing harmless and effective solvents if solvents are to be used and paying attention not to spoil the composition of the oil if heat treatment is to be applied.

In the oil extraction process, there are extraction methods such as obtaining oil with the help of a soxhlet mechanism, solvent extraction, maceration and distillation. In addition to these, methods such as cold pressing and hot pressing can also be tried[10]. The most commonly used laboratory type trial type in oil extraction is mostly soxhlet and maceration.

The most preferred solvent is hexane, which can best extract non-polar materials[11,12].

In a study conducted for the comparison of pomegranate seed oil extraction, solvent extraction, soxhlet extraction and accelerated solvent extraction were studied comparatively at room temperature and the highest efficiency was observed in solvent extraction, while the working efficiencies of soxhlet and accelerated extraction were subsequently improved[13].

In the present study, the total oil yield and omega 5 amounts of different drying conditions applied to the seeds obtained from three different phenotypes of pomegranate species from Gaziantep Region: Hicaz pomegranate, Nuz pomegranate and Deve Dişi pomegranate were determined. Thus, the seeds remaining as waste during consumption and processing during fruit juice production can be converted into value-added products by evaluating the data in this study.

The omega 5 is a little-known but important component of unsaturated fatty acids with pharmacological effect. In this study, determining the omega 5 amounts obtained from pomegranates of different phenotypes is important both for the literature and for the purpose of determining the source of a compound with pharmacological activity.

MATERIALS AND METHODS

Supply of pomegranate seeds: In this study, pomegranate seeds of 3 different phenotypes (the local name of Hicaz, Nuz and Devedişi), which are widely grown in Oğuzeli District of Gaziantep Province, were collected at the harvest time (October to December, 2022) and after being identified by the Agricultural Engineer at Gaziantep District Directorate of Agriculture, they belonged to all three phenotypes.

Drying of pomegranate seeds: Information on the drying of pomegranate seeds obtained from different pomegranate species in the study was given in Table 1.

Table 1: Drying processes applied to pomegranate seeds

	Natural drying		Oven drying	
Pomegranate type	Drying temperature (°C)	Drying time(hr)	Kurutma temperature (°C)	Drying time (hr)
Nuz	25	48	65	10
Devedişi	25	48	65	10
Hicaz	25	48	65	10

Table 2: GC-MS column furnace temperature program

	Rate (°C/min)	Value (°C)	Hold time (min)
		80	0
Ramp 1	20	130	1
Ramp 2	10	170	0
Ramp 3	5	215	12
Ramp 4	40	230	3

Obtaining oil from pomegranate seeds: The dried beans were ground into powder in a plant blender and extracted with hexane solvent for 72 hrs. At the end of this period, the hexane phase was separated from the mixture by filtration and then evaporated in a rotary evaporator to obtain oil.

Devices used: Solvent evaporation was carried out with Heidolph Hei-Vap Value HL/G1 brand Rotary Evaporator. Gas chromatography was performed on an Agilent 7890A GC System coupled to an Agilent 5975C inert MSD with Triple Axis Detector brand gas chromatography mass spectroscopy instrument. Agilent HP5-MS (30 m×0.25 mm×0.25 µm) column was used as GC column.

Determination of omega 5 quantities: The GC-MS device was used to determine the amount of ω-5 fatty acids in the obtained oils. For the oils to be read in GC-MS, the process of converting the oils to methyl esters was carried out. For this process, 0.1 g of oil sample was dissolved with 2 mL of hexane, then 0.2 mL of 2 M methanolic KOH solution was added to provide an esterification reaction.

The clear solvent was injected into the column. The GC column furnace temperature program is as in Table 2. High purity helium was used as the carrier gas and the total duration of the GC column furnace temperature program was 31,875 min. Injection port temperature was set to 200°C and a splitless model inlet was used. The injection volume was determined to be 1.0 µL. Wiley and NIST (Wiley Registry of Mass Spectral Data, 7th Edition, NIST 98 Library) was used as the library. All chemicals used were of analytical purity and were commercially available from Sıgma-Aldrıch.

RESULTS

Pomegranate seed oil is a special, highly unsaturated oil. The determination of the amount of fatty acids in the pomegranate seed was made based on relative percentage. More than 73% of the total weight of pomegranate seed oil contains 9,11,13-octadic-trienoic acid (18:3) and the identification was made by comparison with previously published data. The results of ω-5 and total oil amounts obtained after drying processes and solid liquid extraction applied to different types of pomegranate seeds were shown in Table 3. When the results were evaluated, it was determined that the pomegranate seed type/type with the highest oil yield was the naturally dried Nuz pomegranate with a value of 9.08%. Among all pomegranate species/species, the highest ω-5 content with a value of 65.973% was found in the oil content obtained from naturally dried ostrich pomegranate seeds. When drying processes were compared, it was determined that the total oil and omega 5 amounts of naturally dried pomegranate seeds were higher than those of oven-dried pomegranate seeds. When the GC-MS chromatogram results in Fig. 1 were examined, the presence of omega 5 was detected when the blue color chromatogram obtained from the device's library and the peaks of the oil belonging to the Hicaz pomegranate seed were compared. The other phenotypes GC-MS chromatogram are reported in supporting info files.

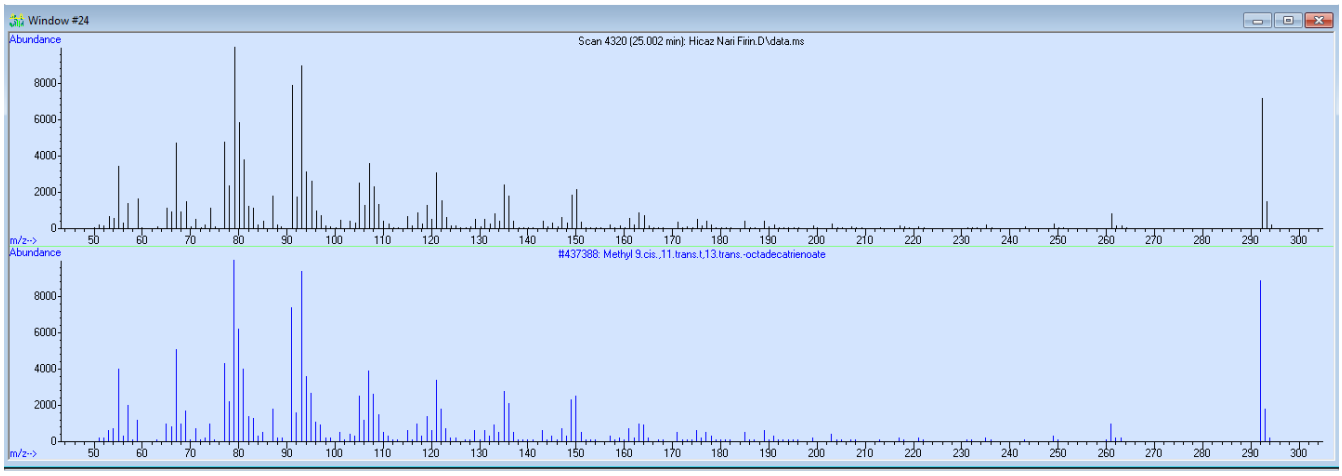

Fig. 1: GC-MS chromatogram results

Table 3: Total oil and ω-5 amounts of pomegranate seeds

Sample	Total oil yield (%)	ω -5 (punicic acid) (%)
Natural dried hicaz pomegranate	8.09	51.757
Oven dried hicaz pomegranate	4.08	46.344
Natural dried Nuz pomegranate	9.08	46.941
Oven dried Nuz pomegranate	5.06	38.822
Natural dried Devedişi pomegranate	4.36	65.973
Oven dried Devedişi pomegranate	3.97	51.889

DISCUSSION

Pomegranate is a type of fruit from the henna family, consisting of hundreds of particles that form the fruit body with small seeds, with a slightly sour and sweet taste depending on the type, grown in temperate climates. Pomegranate consists of 4 main parts.

These parts are consist of shell, kernel, grains and white membrane[14]. The edible part of the pomegranate fruit constitutes approximately 52% of the whole fruit by weight and the active bioactive components are found at a very high rate in the peel and seed. It has been reported that the higher antioxidant activity of commercially sold pomegranate juices is due to the polyphenols transferred from the peel to the pomegranate juice as a result of pressing the fruit as a whole during production[15]. The seeds of the pomegranate fruit vary between 40 and 100 g/kg fruit weight, depending on the variety. The seeds contain 6.63-19.3% lipid on dry matter[16]. They determined that the fatty acid composition of pomegranate seeds obtained from 7 regions of Spain consists of 4.16-26.5% saturated and 73.4-95.8% unsaturated fatty acids. The fatty acid composition of the seed oils of a total of 25 varieties of pomegranate, 8 of which are sweet, 3 of which are mayhos and 14 of which are minus species, grown in Iran were investigated.

The fatty acid composition of sweet variety pomegranate seeds consists of 6.8-13.3% saturated and 86.7-93.2% unsaturated, 6.4-12.1% saturated and 87.9-93.6% unsaturated types of sour varieties and 4.8-26.8% saturated and 73.2-94.4% unsaturated fatty acids in minus species were determined[17]. The pomegranate seed oil has gained importance in recent years, as it is a promising phytochemical and its novelty is cheap by consumers. Pomegranate seed oil is preferred as a nutraceutical compound due to its novelty, good consumer acceptance, cheap availability and promising phytochemical composition[18,19]. The pomegranate seed oil contains 10-20% of the total seed weight and has an ideal high unsaturated fatty acid content consisting mainly of punicic acid, the rare conjugated linolenic acid isomer, whose activities against metabolic syndrome and inflammation have been confirmed by various *in vivo* experiments[20,21]. Pomegranate seed oil also increases serum Insulin-like Growth Factor Type 1 (IGF-1) levels and activates liver secretion by increasing histone acetylation in the IGF-1 gene in bone, thereby increasing bone mass[22,23]. For this reason, the high efficiency of omega 5, which is effective on health, in pomegranate seed oil was examined in this study. In the past years, many researchers have only determined the type and amount of fatty acids and have not conducted any studies on the physical and chemical properties of pomegranate seed oil. For example, El-Shaarawy and Nahapetian[24] have shown that 8% of the fatty acids in pomegranate seed oil are saturated, 10% mono, 10% saturated and about 70% conjugated, most likely punicic acid. El-Nemr *et al.*[25] showed that 83.6% of the fatty acid is saturated and 16.3% is unsaturated. In another study, Kýralan *et al.*[26] reported the fatty acid composition of 15 commercial pomegranate cultivars grown in Turkey. The oil content and dominant unsaturated fatty acid of the examined cultivars were found to be 24.13 to 13.95% and punicic acid (70.42-76.17%), respectively. In current study, it was determined that an average of 73% of pomegranate seeds in 3 different phenotypes in Oguzeli was punicic acid. In addition, in present study, the effect of drying on the production of punicic acid was examined by comparing the methods of drying pomegranate seeds in an oven (40°C) and drying in the shade naturally. In this study, it was determined how different drying conditions had an effect on the amount of this active ingredient and in accordance with the results obtained, the omega 5 amounts in the oils obtained from naturally dried pomegranate seeds were found to be much more efficient than the oven drying process.

CONCLUSION

Fats, which constitute a part of human basic nutrients, have led to the need to focus on vegetable oil production. The import and export of oil obtained from plant seeds has an important place in the world. The uses of oils are not limited to edible oil but also have an important place in the pharmaceutical and cosmetic industry. Although there is no data on the use of pomegranate seed oil in the edible oil class, it is known to be used in the pharmaceutical and cosmetic industries. This study, which examines the presence of valuable conjugated unsaturated fatty acids in pomegranate seed oil, is extremely important in terms of developing products with high added value from waste materials.

SIGNIFICANCE STATEMENT

Since the benefits and source of omega 5, which is the least known among the unsaturated fatty acids, are not fully known. This study was important in terms of detecting it in the waste parts of pomegranate. In addition, determining the amount of pomegranate seeds in different phenotypes is important in terms of agricultural biotechnology.

ACKNOWLEDGMENTS

The author thanks the head of the department Prof. Dr. Halil İbrahim Uğraş, Dr. Elif Sine Düvenci and Dr. Şeref Karadeniz for the use facilities of Central Laboratory of Düzce University.

REFERENCES

1. Jurenka, J., 2008. Therapeutic applications of pomegranate (*Punica granatum* L.): A review. Altern. Med. Rev., 13: 128-144.
2. Hamdi, A.S., D.M.M. Bakhsh and M. Al-Sayyad, 2017. Indications of total hip arthroplasty at a tertiary hospital in Jeddah. Saudi Surg. J., 5: 106-109.
3. Martinez, J.J., F. Hernandez, H. Abdelmajid, P. Legua, R. Martinez, A. El Amine and P. Melgarejo, 2012. Physico-chemical characterization of six pomegranate cultivars from Morocco: Processing and fresh market aptitudes. Sci. Hortic., 140: 100-106.
4. Prakash, C.V.S. and I. Prakash, 2011. Bioactive chemical constituents from pomegranate (*Punica granatum*) juice, seed and peel-A review. Int. J. Res. Chem. Environ., 1: 1-18.
5. Zielińska, A., K. Wójcicki, D. Klensporf-Pawlik, M. Marzec and M. Lucarini *et al*., 2022. Cold-pressed pomegranate seed oil: Study of punicic acid properties by coupling of GC/FID and FTIR. Molecules, Vol. 27. 10.3390/molecules27185863.
6. Okumuş, G., E. Yildiz and A. Akpinar-Bayizit, 2015. Natural antioxidant compounds: Potential use of pomegranate by-products as antioxidants [In Turkish]. J. Agric. Fac. Uludag Univ., 29: 203-214.
7. Cheng, J., J. Li, R.G. Xiong, S.X. Wu and S.Y. Huang *et al*., 2023. Bioactive compounds and health benefits of pomegranate: An updated narrative review. Food Biosci., Vol. 53. 10.1016/j.fbio.2023.102629.
8. Jeune, M.A.L., J. Kumi-Diaka and J. Brown, 2005. Anticancer activities of pomegranate extracts and genistein in human breast cancer cells. J. Med. Food, 8: 469-475.
9. Lansky, E.P., G. Harrison, P. Froom and W.G. Jiang, 2005. Pomegranate (*Punica granatum*) pure chemicals show possible synergistic inhibition of human PC-3 prostate cancer cell invasion across Matrigel™. Invest. New Drugs, 23: 121-122.
10. Saravani, M., H.K. Mehrjerdi, A. Mirshahi and A.A. Goli, 2014. Protective effects of pomegranate seed oil on ovariectomized rats as a model of postmenopausal osteoporosis: A multi-detector computed tomography evaluation. Vet. Res. Forum, 5: 263-267.
11. Jing, P., T. Ye, H. Shi, Y. Sheng and M. Slavin *et al*., 2012. Antioxidant properties and phytochemical composition of China-grown pomegranate seeds. Food Chem., 132: 1457-1464.
12. Paul, A. and M. Radhakrishnan, 2020. Pomegranate seed oil in food industry: Extraction, characterization, and applications. Trends Food Sci. Technol., 105: 273-283.
13. Masci, A., A. Coccia, E. Lendaro, L. Mosca, P. Paolicelli and S. Cesa, 2016. Evaluation of different extraction methods from pomegranate whole fruit or peels and the antioxidant and antiproliferative activity of the polyphenolic fraction. Food Chem., 202: 59-69.
14. Bryś, J., M. Obranović, M. Repajić, K. Kraljić and D. Škevin *et al*., 2021. Comparison of different methods of extraction for pomegranate seeds. Proceedings, Vol. 70. 10.3390/foods_2020-07657.
15. Martinez, J.J., P. Melgarejo, F. Hernandez, D.M. Salazar and R. Martinez, 2006. Seed characterisation of five new pomegranate (*Punica granatum* L.) varieties. Sci. Hortic., 110: 241-246.
16. Gil, M.I., F.A. Tomas-Barberan, B. Hess-Pierce, D.M. Holcroft and A.A. Kader, 2000. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. J. Agric. Food Chem., 48: 4581-4589.
17. Kawaii, S. and E.P. Lansky, 2004. Differentiation-promoting activity of pomegranate (*Punica granatum*) fruit extracts in HL-60 human promyelocytic leukemia cells. J. Med. Food, 7: 13-18.
18. Fadavi, A., M. Barzegar and M.H. Azizi, 2006. Determination of fatty acids and total lipid content in oilseed of 25 pomegranates varieties grown in Iran. J. Food Compos. Anal., 19: 676-680.
19. Fadavi, A., M. Barzegar, M.H. Azizi and M. Bayat, 2005. Physicochemical composition of ten pomegranate cultivars (*Punica granatum* L.) grown in Iran. Food Sci. Technol. Int., 11: 113-119.
20. Dalimov, D.N., G.N. Dalimova and M. Bhatt, 2003. Chemical composition and lignins of tomato and pomegranate seeds. Chem. Nat. Compd., 39: 37-40.
21. Melgarejo, P. and F. Artés, 2000. Total lipid content and fatty acid composition of oilseed from lesser known sweet pomegranate clones. J. Sci. Food Agric., 80: 1452-1454.
22. Sassano, G., P. Sanderson, J. Franx, P. Groot, J. van Straalen and J. Bassaganya-Riera, 2009. Analysis of pomegranate seed oil for the presence of jacaric acid. J. Sci. Food Agric., 89: 1046-1052.

23. Bachagol, D., G.S. Joseph, G. Ellur, K. Patel and P. Aruna *et al*., 2018. Stimulation of liver IGF-1 expression promotes peak bone mass achievement in growing rats: A study with pomegranate seed oil. J. Nutr. Biochem., 52: 18-26.
24. El-Shaarawy, M.I. and A. Nahapetian, 1983. Studies on pomegranate seed oil. Fette Seifen Anstrichmittel, 85: 123-126.
25. El-Nemr, S.E., I.A. Ismail and M. Ragab, 1990. Chemical composition of juice and seeds of pomegranate fruit. Mol. Nutr. Food Res., 34: 601-606.
26. Kýralan, M., M. Gölükcü and H. Tokgöz, 2009. Oil and conjugated linolenic acid contents of seeds from important pomegranate cultivars (*Punica granatum* L.) grown in Turkey. J. Am. Oil Chem. Soc., 86: 985-990.