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Chemical Analysis of Pomegranate Flower Essential Oil in Türkiye

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Abstract: *Punica granatum* L. (Pomegranate) grows naturally in a wide area from northwest Türkiye to western and northern Pakistan and is also cultivated throughout subtropical and tropical regions of the world. Pomegranate is in the form of a spiny shrub or a small tree. The barks, fruit peels, flowers, and seeds of the plant are used in traditional medicine due to their ellagitannin and polyphenol content and are used to treat circulatory system disorders. In this study, essential oil (EO) of *P. granatum* flowers was obtained by hydrodistillation and analyzed by GC-FID and GC-MS. As a result, the chemical composition of the essential oil obtained was elucidated. As a result, nine compounds representing 99.9% of the essential oil of P. granatum flowers were characterized, with hexadecanoic acid (52.4%), linoleic acid (15.2%), heptacosane (10.1%), and pentacosane (10.1%) as the major components. Notably, this research marks the first comprehensive examination of the essential oil of pomegranate flowers in Türkiye.

Keywords: *Punica granatum*, Punicaceae, Essential oil, Hydrodistillation, GC-FID, GC-MS.

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1. INTRODUCTION

The Punicaceae family is represented by a single genus, *Punica* L., is characterized by deciduous shrubs or trees, sometimes with spiny branches. Leaves of the family are simple without stipules. The flowers are located at the tips of the branches. The large fruit has a leathery exocarp and bears many seeds (1). *Punica granatum* L. (pomegranate) is a cosmopolite plant that is distributed widely from northwest Türkiye to western and northern Pakistan and is cultivated in subtropical and tropical regions of the world (1-3).

For centuries, people have used fragrant plants and their processed forms as flavorings. More recently, these plants have become a focus for finding natural antioxidants and antibacterial agents. Pomegranate flowers have a long history of use in traditional Indian medicine systems like Ayurveda and Unani. These flowers possess anti-diabetic and astringent properties, and an infusion made by boiling the flower buds is employed to treat chronic diarrhea, especially in children. As there is growing interest in utilizing plant-derived compounds in food and pharmaceuticals, carefully studying plant extracts for these beneficial properties has become increasingly important. Moreover, the stem bark, fruit peel, flowers, and seeds of pomegranate are used in folk medicine for their anthelmintic and antimicrobial properties. Despite the low aromatic intensity of fresh pomegranates, only a handful of studies have looked at the volatile aroma compounds in pomegranates, and these mainly focused on pomegranate juices. For example, one study could only identify nine compounds in pomegranate juices from Iran. Later, a pomegranate and berry juice survey found just ten volatile compounds in the pomegranate. Additionally, there is a lack of research on the fatty acids in pomegranate flowers, except for a study that characterized the fatty acid methyl esters in five different Iranian pomegranate flower cultivars (4,5). Other phytochemical components of the plant consist of tannins, flavonoids, alkaloids, organic acids, triterpenes, steroids, coumarins, and lignans (6-10). Pomegranate plant is known to be used to treat circulatory system disorders, it also has antimicrobial, antioxidant, anticancer, antidiabetic, and immunomodulatory activities (11-15). The research also reported that extracts from *P. granatum* arils possess strong *in vitro* antibacterial activity against many bacterial strains tested. Most

of those investigations suggested that the presence of phytocompounds in the extracts, including phenols, tannins, and flavonoids, as major active constituents may be responsible for these activities. However, research on the antibacterial activity of pomegranate flower extracts is scarce. Moreover, it is not well known whether volatile components could be involved in the antibacterial effect of pomegranate flower extracts and essential oils (5). Moreover, the flowers from Türkiye possess significant phytochemical and antioxidant properties, making them a valuable source of bioactive compounds. The flower extracts, particularly rich in phenolic compounds such as punicalagin, have shown good antioxidant activity (16).

When we review the literature, we find that essential oils (EOs) obtained from the fruit peels have been studied extensively. However, there are only a few studies on the composition of the EO obtained from the flowers (5). As far as we know, this is the first study examining the essential oil obtained from the flowers of the pomegranate plant growing in Türkiye naturally.

2. EXPERIMENTAL SECTION

2.1. Plant Material

Plant material was collected from Kahramankazan, Ankara (Türkiye), specifically from Güvenç Köyü at an altitude of 1000 meters on 30/06/2021. A voucher specimen was deposited in the Ankara University Faculty of Pharmacy Herbarium with the herbarium number AEF 30716. Collected flowers were dried in the shade.

2.2. Isolation and Analysis of Essential Oil

The hydrodistilled essential oil was analyzed by GC-MS and GC-FID (17-19). Table 1 contains the result. The EO was obtained by hydrodistillation using a Clevenger-type apparatus for three hours. A small amount of EO of *P. granatum* was trapped in n-hexane. The EO was stored at 4°C in an amber vial.

The essential oil compounds were identified by comparing their relative retention indices (RRI) to those of authentic samples using computer matching against commercial data (MassFinder 4.0 Library, Wiley GC-MS Library). The identification was carried out using an in-house "Başer Library of Essential Oil Constituents" compiled from genuine compounds of recognized essential oils and the MS reported in the literature (17, 18).

3. RESULTS AND DISCUSSION

9 compounds representing 99.9% of the essential oil of the flowers were characterized with hexadecenoic acid (52.4%), linoleic acid (15.2%), heptacosane (10.1%), and pentacosane (10.1%) as major components. The composition of the EO is shown in Table 1. The EO of pomegranate flowers contains various compounds, including nonadecane, tricosane, tetracosane, pentacosane, hexacosane, heptacosane, nonacosane, hexadecenoic acid, and linoleic acid. Hexadecanoic acid, also known as palmitic acid. Palmitic and linoleic acids are fatty acids. Palmitic acid (C16:0) is a saturated lipid with a 16-carbon chain. Linoleic acid (C18:2) is a polyunsaturated fatty acid (PUFA) with the molecular name omega-6 or cis-9,12-octadecadienoic acid. It is an essential fatty acid, which means that the human body cannot produce it and must receive it from the diet.

Table 1: Composition of the EO of *Punica granatum* flowers.

RRI	Compound	%
1900	Nonadecane	1.0
2300	Tricosane	2.3
2400	Tetracosane	0.8
2500	Pentacosane	10.1
2600	Hexacosane	1.6
2700	Heptacosane	10.1
2900	Nonacosane	6.4
2931	**Hexadecenoic acid**	**52.4**
3290	**Linoleic acid**	**15.2**
	Total	**99.9**

When we searched the literature, we saw that many studies were performed on fruit juice, particularly focusing on its preservation. And studies conducted on essential oils were performed mostly on the EOs obtained from fruit peels.

In a study by Hadrich et al. (2014), the essential oil of Tunisian pomegranate peels was analyzed, revealing camphor (60.32%), benzaldehyde (20,98%), and borneol (4.75%) as the main components of the EO (20).

In a study, essential oil of the peel was obtained both with hydro-distillation and super fluid extraction methods, and the obtained results were compared. Hydrodistillation yielded 73 compounds; however, superfluid extraction just yielded 46 components. While major compounds of hydrodistillation procedure were found to be (-)-borneol (12.97%), oleic acid (11.65%), and dibutyl phthalate (10.82), the superfluid extraction procedure yielded oleic acid (12.49%), palmitic acid (11.65%), and (-)-borneol (9.5%) as major components (21).

In another study performed on fruit peels by Wahba (2020), linoleic acid was found to be the main component of the essential oil (29.33%), along with *D*-limonene (13.79%), caryophyllene (13.9%), *cis*-vaccenic acid (12.66%), and squalene (9.12%) (22). In the study conducted by Gültepe et al. (2019) on fruit peels, methyl stearate (43.21%), metilox (16.82%), and methyl palmitate (7.90%) were dominant in the essential oil (23).

As it can be found from the results of these studies, the main components are usually different than the results of our study, which is not surprising by the way since the plant parts used to isolate essential oils were different.

In a study conducted in Tunisia, essential oils obtained from pomegranate flowers were compared to reveal the influence of different types of cultivation methods with respect to the essential oil

composition. 4 different types of cultivators (Tounsi, Nabli, Gabsi, and Chelfi) were selected for this purpose, and the essential oils of flowers were examined. Esters, alcohols, terpenes, aldehydes, ketones, and hydrocarbons were identified to be present in the chemical composition of the EOs. The results of these studies are listed in Table 2 (5). When we examine the results of this study, we can recommend that only the composition of the Gabsi cultivator was similar to the findings of our study. *n*-Pentacosane found to be present in this cultivar (13.59%) was also present in the essential oil that we have obtained from the flowers.

Table 2: The major components of the essential oils from the flowers of Tunisian pomegranate cultivars.

Cultivar	Major Compounds	(%)
Tounsi	2,3-Butandiol	18.98
	Eugenol	15.85
	Ethyl hexadecanote	5.96
Nabli	Ethyl hexadecanote Ethyl	35.92
	oleate	18.88
	Nezukol	12.75
Gabsi	Ethyl hexadecanote	19.31
	n-Pentacosane	13.59
	Abietadiene	12.37
Chelfi	2,3-Butanediol	20.65
	Eugenol	18.85
	Abietadiene	10.79

Pomegranate is a fruit of significant commercial importance, and it is cultivated in Southern, Middle East, Asia, and Mediterranean regions. Its seeds are rich in bioactive components, including fatty acids, tocopherols, tocotrienols, phytosterols, and carotenoids. Pomegranate seed fixed oil is a non-traditional oil with health benefits, rich in polyunsaturated fatty acids (PUFAs), namely linoleic acid (omega-6) and linolenic acid (omega-3), as well as an effective conjugated linolenic acid, punicic acid (24).

The other study compared three different methods (microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), and cold pressing) for extracting pomegranate seed oil and found that the ultrasound-assisted extraction technique was the most efficient, with a maximum extraction efficiency of 26.31% under optimized conditions. The cold pressing method yielded the lowest oil extraction efficiency at 5%, but the extracted oil had the highest punicic acid content at 88.33%. The other major fatty acids in the cold-pressed oil were oleic acid (3.68%), linoleic acid (3.22%), palmitic acid (2.19%), and stearic acid (1.56%). The MAE technique using dimethyl succinate as the solvent had an extraction efficiency range of 5.94 to 22.01%. The extracted oils were also dominated by punicic acid, which ranged from 87.65 to 88.15%. Other notable fatty acids included oleic acid (1.58-3.70%), linoleic acid (3.32-3.64%), and palmitic acid (2.20-2.69%). When hexane was used as the solvent for MAE, the extraction efficiency increased to 25.3%, and the punicic acid content was 88.39%. The UAE method demonstrated the highest extraction efficiency, ranging from 6.71 to 26.3%. The extracted oils had a wider variation in fatty acid composition, with punicic acid ranging from 74.4 to 88.0%. Other significant fatty acids included linoleic acid (3.4-7.5%), oleic acid (3.6-7.1%), and palmitic acid (2.3-5.0%). Using hexane as the solvent for UAE further increased the extraction efficiency to 31.2%, and the punicic acid content was 75.26% (25).

The second main compound of flower essential oil is linoleic acid. Although it is close to the fixed oil content in this aspect, it has a different chemical composition in other compounds.

4. CONCLUSION

In this research, we have investigated the composition of essential oil obtained from pomegranate flowers collected from Ankara, Türkiye. To the best of our knowledge, this is the first research examining the essential oil of pomegranate flowers from plants growing naturally in Türkiye, with hexadecenoic acid (52.4%) identified as the major component. And thus, this study sheds light on the chemical composition of *P. granatum* flower essential oil, showcasing the major of specific compounds that contribute to its aromatic and potentially therapeutic properties. The findings provide valuable insights into the potential applications of pomegranate essential oil in various fields, including aromatherapy and cosmetics, for its bioactive constituents.

5. CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

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