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O‘ZBEKISTONDA O‘SADIGAN *Punica granatum L.* o‘simligi “QAYUM” NAVI BARGLARI VA GULLARINING UCHUVCHAN KOMPONENTLARINI O‘RGANISH

STUDY OF VOLATILE COMPONENTS OF LEAVES AND FLOWERS OF *Punica granatum L.,* VARIETY “KAYUM” GROWING IN UZBEKISTAN

ИССЛЕДОВАНИЕ ЛЕТУЧИХ КОМПОНЕНТОВ ЛИСТЬЕВ И ЦВЕТКОВ *Punica granatum L.* СОРТА «КАЮМ», ПРОИЗРАСТАЮЩЕГО В УЗБЕКИСТАНЕ

Saminov Khusniddin Numonjon o‘g‘li[1] , Ibragimov Alijon Aminovich[2], Nazarov Otabek Mamadaliyevich[3]

[1]Saminov Khusniddin Numonjon o‘g‘li[1] — Lecturer, Department of chemistry, Fergana State University

[2]Ibragimov Alijon Aminovich[2] — Doctor of Chemistry, professor, Department of chemistry, Fergana State University

[3]Nazarov Otabek Mamadaliyevich[3] — PhD, associate professor, Department of chemistry, Fergana State University

Annotatsiya

O‘zbekiston Respublikasining Farg‘ona viloyatida o‘suvchi Punica granatum L. o‘simligi Qayum navining ommaviy gullash davrida to‘plangan barglari va gul barglari efir moyi tarkibi, shuningdek, gulkosasining benzol ekstrakti, xromato-mass-spektrometriya usulida o‘rganildi.

Barglarning efir moyida 59 ta komponent aniqlangan, shundan 51 ta komponent (efir moyining 91,36%) aniqlangan, bu yerda (+)-2-bornanon (kamfora) (21,81%), β-tuyon (8,27%), (-)- borneol (7,39%), cis-digidrokarbon (7,22%) va α-tuyon (4,81%). Punica granatum gul barglari efir moyi tarkibida 41 ta komponent topilgan, ulardan 27 ta birikma aniqlangan (efir moyining 96,41%), asosiy komponentlari bis-(2-etilgeksil) ftalat (40,33%), furfurol (16,08%), palmitin (15,15%) va linolein (5,64%) kislotalar, shuningdek, 3,5,6-trixloropiridin-2(1H)-on (4,07%). Gulkosasi benzol ekstraktida 7 ta komponent topilgan, ulardan 4 tasi aniqlangan (ekstraktning 76,18%) va ekstraktda 2-pentadekanol (36,08%) va etil linoleat (22,01%) ustunlik qilgan. Punica granatum barglari, gul barglari va gulkosasining uchuvchi komponentlari tarkibini qiyosiy tahlil qilish har bir namuna uchun dominant komponentlarni aniqlash imkonini berdi.

Аннотация

В состав эфирного масла листьев и лепестков цветков, а также бензольного экстракта околоцветника Punica granatum L. сорта Каюм, собранные в период массового цветения в Ферганской области Республики Узбекистан, исследовали методом хромато-масс-спектрометрии. В эфирном масле листьев обнаружено 59 компонентов, из них идентифицирован 51 компонент (91,36% эфирного масла), где (+)-2-борнанон (камфора) (21,81%), β-туйон (8,27% эфирного масла), (-)-борнеол (7,39%), цис-дигидрокарвон (7,22%) и α-туйон (4,81%). В составе эфирного масла лепестков цветков P. granatum обнаружен 41 компонент, из них идентифицировано 27 соединений (96,41 % эфирного масла), а основными компонентами являются бис-(2-этилгексил)фталат (40,33 %).), фурфурол (16,08%), пальмитиновая (15,15%) и линолевая (5,64%) кислоты, а также 3,5,6-трихлорпиридин-2(1H)-он (4,07%). В бензольном экстракте околоцветников обнаружено 7 компонентов, из которых идентифицировано 4 (76,18 % экстракта), причем в экстракте преобладают 2-пентадеканол (36,08 %) и этиллинолеат (22,01 %). Сравнительный анализ состава летучих компонентов листьев, лепестков цветков и околоцветников P. granatum позволил установить доминирующие компоненты для каждого образца.

Abstract

The composition of the essential oil of leaves and flower petals, as well as the benzene extract of perianths Punica granatum L. of the Kayum variety, collected during the period of mass flowering in the Fergana region of the Republic of Uzbekistan, were studied by chromatography-mass spectrometry. In the essential oil of the leaves, 59 components were found, of which 51 components were identified (91.36% of the essential oil), where (+)-2-bornanone (camphor) (21.81%), β-thujone (8.27%), (-)- borneol (7.39%), cis -dihydrocarvone (7.22%) and α-thujone (4.81%). In the composition of the essential oil of P. granatum flower petals, 41 components were found, of which 27 compounds were identified (96.41% of the essential oil), while the major components were bis-(2-ethylhexyl)phthalate (40.33%), furfural (16.08%), palmitic (15.15%) and linoleic (5.64%) acids, as well as 3,5,6-trichloropyridin-2(1H)-one (4.07%). In the benzene extract of perianths, 7 components were found, of which 4 were identified (76.18% of the extract), and 2-pentadecanol (36.08%) and ethyl linoleate (22.01%) dominated in the extract. A comparative analysis of the composition of the volatile components of the leaves, flower petals, and perianths of P. granatum made it possible to establish the dominant components for each sample.

Kalit so‘zlar: *Punuca. granatum L., gidrodistillash efir moyi benzol ekstrakti xromato-massa spektrometriyasi.*

Ключевые слова: *Пунука. granatum L., гидродистилляция эфирное масло бензольный экстракт хромато-масс-спектрометрия.*

Key words: *Punuca. granatum L., hydrodistillation essential oil benzene extract chromato-mass spectrometry.*

INTRODUCTION

Pomegranate (*Punica granatum* L.) is a fruit-bearing deciduous shrub or tree from the Loosestrife family *(Lythraceae)*, genus Pomegranate (*Punica)* , reaching a height of 5-10 m [1]. The genus *Punica* contains only two species: *P. granatum* L. and *P. protopunica* Balf. f. 1882. The species *P. protopunica* is endemic to Socotra Island (Yemen) [2,3,4] and is considered to be the predecessor of *P. granatum* L.[5].

The pomegranate is widely grown in the Middle East and the Caucasus, northern and tropical Africa, the Indian subcontinent, Central Asia, the drier parts of Southeast Asia and the Mediterranean basin [6].

Punica granatum L. is one of the medicinal plants with remarkable medicinal value, which is often used in folk medicine to treat a number of diseases such as cardiovascular diseases, cancer, diabetes, gastritis, ulcers, diarrhea, etc. Various parts of this plant contain many biologically active compounds (flavonoids, alkaloids, tannins, elagotannins, catechins, organic acids) [7]. Clinical trials have proven their antioxidant [8,9], antimicrobial [10,11,12], antifungal [13,14,15], anticancer [16,17,18,19,20], anti-inflammatory [21], and antidiabetic activities. [22]. Extracts from the plant exhibit hypotensive and antianginal properties [23].

Despite the wide range of compounds isolated from *P. granatum* L., the essential oils of the leaves and flowers are not well understood. Works on the study of the essential oil of the peel of the fruits of the plant are known. Thus, in the essential oil of the peel of *P. granatum* L. growing in Iran, 74 compounds were identified, among which the main ones were (-)-borneol (12.97%), oleic acid (11.65%), dibutyl phthalate (10.82%), palmitic acid (9.86%), butylbenzene (4.88%), cineole (3.14%), and camphor (3.05%) [24]. In the essential oil of the peel of *P. granatum* L. growing in Tunisia, camphor (60.32%), benzaldehyde (20.98%), endoborneol (4.75%) and 4-(1-methylethyl)-1-cyclohexene-1-carboxaldehyde (2.53%) were found as the main components [3]. In the methanol extract obtained from the leaves of *P. granatum* L. of Indian origin, 20 compounds were identified, among which the main ones were 3,7,11,15-tetramethyl-2-hexadecen-1-ol (26.28%), 1-octadecine (9.60%), 1-octadecanol (7.69%), 2-propen-1-one, 1-[2-[(phenylmethoxy)methyl]-1-cyclohexen-1-yl]-3-(trimethylsilyl)-(6.24%) and 10-methyl-3,10-diazabicyclo [4.3.1] decan-4-one(5.71%) [25].

Due to geographical conditions, Uzbekistan is a suitable place for the cultivation of many medicinal plants, which are valuable raw materials for fundamental and applied research in the field of chemistry of natural compounds. Although the country is not among the most important regions of the world for growing pomegranate, the plant is widely cultivated in some regions of the country. The analysis of the literature shows that the chemical composition of non-polar compounds and the essential oil of leaves and flowers of *P. granatum* L. was studied little, so it was advisable to study this plant in more depth to assess its chemical composition. The purpose of this research is to study the essential oil of the leaves, flower petals, as well as the volatile components of the perianths of *P. granatum* L. variety "Kayum" growing in Uzbekistan.

MATERIALS AND METHODS

*Plant materials.*For the study, the leaves and flowers of *P. granatum* L. variety "Kayum" were collected in 2021 during the period of mass flowering in the Fergana region of the Republic of Uzbekistan. The flowers were manually divided into petals and perianths. Leaves, flower petals and perianths were dried in air at room temperature in a dark place, protected from direct sunlight. Air-dry samples were ground in a mill and sieved through sieves with a mesh size of 2 mm.

Isolation of essential oil. Essential oil from leaves and flower petals was isolated from finely ground (see above) plant samples by hydrodistillation in a Clevenger apparatus for 3.5 hours. The resulting oil was separated from the aqueous phase by extraction with *n*- hexane, the extract was dried over anhydrous Na_2SO_4 . The yield of essential oil from the leaves (100 g) was 0.16%, from the flower petals (90 g) 0.39% (from the dry mass of raw materials). The essential oils of two samples were a pale yellow mobile liquid with a specific odor. Prior to GC-MS analysis, essential oils were stored in tightly closed vials in the dark at 4°C in a refrigerator.

Perianth extraction. Perianth extraction was carried out by 3-fold infusion with benzene at room temperature with an interval of 8 hours at a ratio of raw material: extractant 1:6 (weight-volume). The extracts were filtered, combined, concentrated by distillation in vacuo and dried over

anhydrous Na_2SO_4 . The composition of the isolated volatile substances was determined by gas chromatography-mass spectrometry (GC-MS).

GC/MS Analysis of volatile compounds. The qualitative and quantitative composition of volatile compounds of the samples was studied on an Agilent 5975C inert MSD/7890AGC chromato-mass spectrometer with an Agilent HP-INNOWax quartz capillary column (30 m ×250 μm ×0.25 μm) in the temperature regime: 60 °C (2 min) - 4 °C/ min up to 220 °C (10 min) - 1 °C/min up to 240 °C (20 min). The volume of the introduced sample was 1.0 μl, the flow rate of the mobile phase (H_2) was 1.1 ml/min. The temperature of the evaporator is 220 °C, the temperature of the ion source is 230 °C. The ionization of molecules was carried out by the electron impact method (70 eV). EI-MS spectra were obtained in the range *m/z* 45-550 amu. Components were identified based on a comparison of the characteristics of the mass spectra with the data of electronic libraries W9N11.L (Wiley Registry of Mass Spectral Data-9 th Ed., NIST Mass Spectral Library, 2011) and a comparison of the retention indices (RI) of compounds determined with respect to the time retention of a mixture of *n*- alkanes (C_9-C_{34}). The quantitative content of the components of essential oils was calculated from the areas of chromatographic peaks. The results are presented in tables 1-3.

RESULTS AND DISCUSSION

59 components were found in the composition of the essential oil from the leaves of *P. granatum.* Of these, 51 components were identified, making up 91.36% of the essential oil. The main components of the leaf essential oil are bicyclic monoterpene ketones (+)-2-bornanone (21.81%), *β* -thujone (8.27%) and *α* -thujone (4.81%), monocyclic monoterpene alcohol (-)-borneol (7.39%), the monocyclic monoterpene ketone *cis* -dihydrocarvone (7.22%) and the monocyclic sesquiterpene alcohol *α* -bisabolol (3.41%) (Table 2). The identity of the mass spectra of unidentified compounds (from 52 to 59) was less than 75% with those of library data.

Table 1 The composition of the essential oil of the leaves *Punica granatum* L.

№	Component	RI*	RT**	%
1	Isoamyl alcohol	1125	3.738	0.33
2	Eucalyptol	1135	3.910	0.16
3	β-Thujone	1265	8.498	8.27
4	α-Thujone	1274	8.979	4.81
5	(+)-2-Bornanone (camphor)	1417	10.831	21.81
6	β-ocimene	1461	12.119	0.21
7	(1R)-(+)-Nopinone	1468	12.321	0.12
8	Bornylacetate	1482	12.724	0.27
9	6-Methyl-3,5-heptadien-2-one	1494	13.051	0.36
10	γ-terpinene	1506	13.407	1.85
11	Sabinaketone	1521	13.840	0.36
12	5-Ethenyldihydro-5-methyl-2(3H)-furanone	1551	14.695	0.66
13	thuyl alcohol	1563	15.063	0.88
14	α-Terpineol	1571	15.294	0.34
15	Diethylsuccinate	1573	15.378	0.46
16	(-)-Verbenone	1587	15.775	0.18
17	(-)-Borneol	1593	15.959	7.39
18	Camphene	1598	16.084	0.98
19	D-Carvone	1618	16.660	1.28
20	trans-1,2-Dihydroperylaldehyde	1638	17.217	0.33
21	Ethyl 2-phenylacetate	1667	18.042	0.45
22	Tricyclo[4.2.1.1(2,5)]decan-3-ol	1676	18.286	0.27
23	3-tert-Butylphenol	1695	18.832	1.26
24	trans-Carveol	1720	19.514	1.21
25	α-Dimethylstyrone	1731	19.817	0.57
26	cis-2-(3,3-Dimethylcyclohexylidene)-ethanol	1737	19.977	0.20
27	exo-2-hydroxycineol	1739	20.043	0.28

28	Carveol	1747	20.256	0.24
29	Ethyl-3-phenylpropionate	1759	20.577	0.13
30	2α-Hydroxy-1,4-cineol	1764	20.719	0.18
31	Methyleugenol	1840	23.907	2.60
32	Octanoic acid	1861	25.064	0.32
33	4-Isopropylbenzyl alcohol	1873	25.746	1.08
34	3-(2',4'-Dimethylpenta-1',4'-dien-3'-ylidene)-6,6-dimethylcyclohex-1-ene	1887	26.494	0.34
35	3-Allyl-6-methoxyphenol	1898	27.141	0.30
36	Carvacrol	2008	28.411	1.23
37	β-Selinene	2014	29.272	0.16
38	cis-Dihydrocarvone	2016	29.616	7.22
39	4-(4-Methoxyphenyl)-2-butanone	2018	29.812	0.34
40	α-Bisabolol	2019	30.050	3.41
41	caryophyllene oxide	2020	30.198	2.00
42	4,4,7α-trimethyl-5,6,7,7α-tetrahydro-benzofuran-2(4H)-one	2023	30.548	0.16
43	Coumarin	2037	32.715	0.63
44	α-epoxide of bisabolene	2041	33.243	0.21
45	Leden oxide-(II)	2058	35.724	0.34
46	β-humulene	2059	35.967	0.24
47	cis-2,11-Dioxo-3-oxabicyclo - [7.3.0(9.13)]trideca-6-ene	2069	37.344	2.79
48	1-Methoxy-2,6,6-trimethyl-cyclohepta-1,3-diene	2071	37.754	0.67
49	Methylparaben	2103	42.353	0.17
50	Palmitic acid	2104	42.496	0.31
51	3,6,9-Trimethyl-2,8-dioxo-(octahydro)-naphthaleno[1,2-d]furan	2221	59.744	1.12
Σ				91.36

RI*-Kovach Index, RT**-Retention time

41 components were present on the GC-MS chromatogram of the essential oil of flower petals, of which 27 compounds were identified with a total content of 96.41% of the essential oil (Table 2). The major components were bis(2-ethylhexyl) phthalate (40.33%), palmitic acid (15.15%), furfural (16.08%), linoleic acid (5.64%), 3,5,6-trichloropyridin-2(1H) -one (4.07%), *n*-heptacosane (3.35%) and elaidic acid (2.37%).

Table 2 Essential oil composition of flower petals *Punica granatum* L.

№	Component	RI*	RT**	%
1	4-Methyl-3-penten-2-one	1060	2.795	0.11
2	Decamethylcyclopentasiloxane	1114	3.536	0.46
3	2-heptanol	1220	6.178	0.14
4	α-angelicalactone	1265	8.522	0.16
5	Furfural	1281	9.353	16.08
6	(3trans,5trans)-Hepta-3,5-dien-2-one	1404	10.463	0.38
7	cis-6-Nonenal	1447	11.698	0.50
8	5-Methyl-2-furfural	1469	12.339	0.66
9	4-Cyclopentene-1,3-dione	1475	12.505	0.28
10	p-Cyclohexylphenol	1616	16.618	0.22
11	2-(2-butoxyethoxy)-ethanol	1682	18.446	0.41
12	n-Hexanoic acid	1731	19.817	0.28
13	6,10,14-Trimethylpentadecan-2-one	2001	27.325	0.84
14	2-Methoxy-4-vinylphenol	2004	27.830	0.26
15	Versalid	2024	30.708	0.25
16	Galaxolide	2025	30.857	0.22

17	diethyl phthalate	2030	31.634	1.10
18	n-Pentacosane	2060	36.062	0.88
19	3,5,6-Trichloropyridin-2(1H)-one	2085	39.694	4.07
20	n-Heptacosan	2086	39.944	3.35
21	Palmitic acid	2104	42.579	15.15
22	Cyclotetradecane	2106	42.852	0.62
23	7-Isopropyl-4a-methyloctahydro-2(1H)-naphthalenone	2108	43.143	0.73
24	1-hexadecene	2112	43.772	0.92
25	Elaidic acid	2145	48.580	2.37
26	Bis(2-ethylhexyl) phthalate	2151	49.499	40.33
27	Linoleic acid	2157	50.313	5.64
Σ				96.41

RI*-Kovach Index, RT**-Retention time

On the GC-MS chromatogram of the benzene extract of perianths, peaks of 7 components were detected, of which 4 compounds were identified with a total content of 76.18% by weight of the extract. The main components were 2-pentadecanol (36.08%) and ethyl linoleate (22.01%) (Table 3).

Table 3 Volatile components of the benzene extract of perianths *Punica granatum* L

№	Component	RI*	RT**	%
1	Palmitic acid	2104	42.496	9.32
2	Linoleic acid	2156	50.152	8.77
3	Ethyllinoleate	2174	52.823	22.01
4	2-Pentadecanol	2212	67.513	36.08
Σ				76.18

RI*-Kovach Index, RT**-Retention time

The common component for the three samples is palmitic acid. Its content varies from 0.31% to 15.15%. Its predominant amount is contained in the essential oil of flower petals. Linoleic acid is present in the essential oil of the flower petals and in the benzene extract of the perianths. Also, in the essential oil of flower petals, a *trans* - isomer of oleic acid, elaidic acid (2.37%), was found in minor amounts. Saturated and unsaturated fatty acids are found in the essential oils of plants. Examples are the plants *Trigonella foenum graecum*, *Micromeria persica* Boiss., *Avicennia schaueriana* Stapf & Leechm, *Ligustrum quihoui*, *Gypsophila laricina* Schreb and *Lactuca sativa* L., whose essential oils contain palmitic acid [26,27,28,29,30,31,32]. Linoleic acid is found in the essential oil of the stems of *Vinca major* var. *variegate* Loudon [33], rhizomes *of Aspidistra phanluongii* Vislobokov [34], aerial parts of *Micromeria persica* Boiss [27], leaves of garden lettuce *Lactuca sativa* L. [31], its methyl ester was found in the essential oil of aerial parts of *Justicia procumbens* L. [35].

In the essential oil of *P. granatum leaves* contains a significant amount of terpene ketones and alcohols; the predominant component is (+)-2-bornanone (camphor), the major ones are β-thujone, (-)-borneol, *cis* - dihydrocarvone, α-thujone and α-bisabolol. The essential oil of flower petals is dominated by bis(2-ethylhexyl) phthalate, diethyl phthalate (1.1%) is also identified. Diethyl phthalate is of biogenic origin and is present in the essential oils of some plants [36,37]. Bis(2-ethylhexyl) phthalate is referred to as "atypical" phthalates. It can be of biogenic [38,39] or abiogenic [36] origin. Plants are known that contain dibutyl phthalate, bis (2-methylpropyl) phthalate and 1-butyl 2- (8-methylnonyl) phthalate in essential oil [24]. Thus, the discovery of phthalic acid esters in the composition of essential oils of plants is not a rare case. In addition, recent studies show that not only plants, but also fungi are able to produce phthalic esters in significant amounts [40,41,42]. The study of phthalates biosynthesis in fungi showed that diethyl phthalate can be formed via the shikimate pathway. The intermediate product of biosynthesis, protocatechuic acid, then turns into o-phthalic acid, which then forms the target product.

A comparative analysis shows that the essential oil of the leaves and flower petals of *P. granatum* of Uzbek origin contains some components characteristic of the peel of the fruit. foreign

samples of *P. Granatum* [23,24] camphor, (-)-borneol (leaves), phthalic acid esters - dibutyl phthalate (peel), diethyl phthalate and di 2-ethylhexyl phthalate (flower petals).

CONCLUSIONS

Thus, the conducted studies made it possible for the first time to establish the qualitative and quantitative compositions of the essential oil of leaves and flower petals, as well as the volatile components of the benzene extract of the perianth Punica granatum L. of the Kayum variety, cultivated in the Fergana region of the Republic of Uzbekistan, using the GC-MS method, and to identify for each sample dominant components. The common component for the three studied samples is palmitic acid. The leaves of Punica granatum L. can serve as a raw material for obtaining an essential oil enriched with (+)-2-bornanone (camphor), β-thujone, (-)-borneol and cis - dihydrocarvone.

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