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PHYSICO-CHEMICAL CHARACTERIZATION AND BIOACTIVITY OF CULTIVATED AND WILD POMEGRANATE (*Punica granatum* L.) SEED OILS

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The aims of this study were to determine and compare the basic physico-chemical quality parameters of cultivated and wild pomegranate seed oils (CPSO and WPSO, respectively), along with their antioxidant, antihyperglycemic, antiproliferative, and antimicrobial activities. Both cultivated and wild pomegranate seed oils displayed a similar physico-chemical composition, with eicosapentaenoic acid being the predominant fatty acid, accounting for approximately 80 % in both oils. The carotenoid content was higher in cultivated pomegranate seed oil (7.53 mg β-carotene/kg), whereas wild pomegranate seed oil had a greater total chlorophyll content (0.21 mg pheophytin-a/kg). The total phenol contents (0.47 and 0.50 mg GAE/g), flavonoids (0.03 mg Qc/g for both), flavonols (0.55 mg Qc/g for both), and flavan-3-ols (20.03 and 25.00 mg CAT/g) in CPSO and WPSO, respectively, were comparable between the two oil samples. Both oils exhibited strong antioxidant activity against DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) free radicals and demonstrated good antihyperglycemic activity. However, neither oil showed antimicrobial activity against *Salmonella enteritidis* and *Listeria monocytogenes*, or antifungal activity on yeast *Candida albicans*. Antiproliferative activities were observed for both CPSO and WPSO on HeLa (epithelioid carcinoma of the cervix), MCF-7 (breast adenocarcinoma), and MRC-5 (normal fetal lung fibroblasts) cell lines, with IC_{50} values below 200 μg/ml. For the HT-29 cell line, the IC_{50} values were 652.04 μg/ml for CPSO and 414.88 μg/ml for WPSO, indicating stronger antiproliferative activity of WPSO. The inhibitory activities (IC_{50}) correlate with the ability of the sample to inhibit cell proliferation by 50 %, with lower IC_{50} values indicating a stronger antiproliferative effect.

Keywords: pomegranate; seed oil; physico-chemical characteristics; phenolic compounds; bioactivity

КАРАКТЕРИЗАЦИЈА НА ФИЗИЧКО-ХЕМИСКИТЕ СВОЈСТВА И БИОАКТИВНОСТА НА МАСЛО ОД СЕМКИ НА КУЛТИВИРАНИ И ДИВОТРАСТЕЧКИ КАЛИНКИ (*Punica granatum* L.)

Целта на оваа студија е да се определат и споредат основните физичко-хемиски параметри за квалитет на масло добиено од семки на култивирани и диворастечки калинки (CPSO и WPSO), како и нивните антиоксидациски, антихипергликемиски, антипролиферативни и антимикробни активности. Најдено е дека обете масла од семки, и од култивирани и од диворастечки калинки, имаат слични физичко-хемиски својства и еикозапентаенова киселина е најзастапената масна киселина со масен удел од околу 80 % во двете масла. Содржината на каротеноиди беше поголема во маслото од семки на култивираните калинки (7,53 mg β-каротен/kg), додека маслото од семки на диворастечките калинки имаше поголема содржина на вкупен хлорофил (0,21 mg феофитин-a/kg). Вкупната содржина на феноли (0,47 и 0,50 mg GAE/g), флавоноиди (0,03 mg Qc/g за двете масла), флавоноли (0,55 mg Qc/g за двете масла), и флаван-3-оли (20,03 и 25,00 mg CAT/g), соодветно во CPSO и WPSO, беше споредлива во двете масла. Обете масла покажаа силна

антиоксидациска активност испитувана со слободни радикали од DPPH (2,2-дифенил-1-пикрилхидразил) и ABTS (2,2'-азино-bis(3-етилбензотиазолин-6-сулфонска киселина), како и добра антихипергликемиска активност. Освен тоа, ниту едно масло не покажа антимикробна активност против *Salmonella enteritidis* и *Listeria monocytogenes*, ниту пак антифунгална активност спрема *Candida albicans*. Антипролиферативни активности со вредности за IC_{50} пониски од 200 μg/ml беа забележани кај двете масла спрема клеточните линии HeLa (епителоиден карцином на цервикс), MCF-7 (аденокарцином на дојка) и MRC-5 (нормални фетални фибробласти на белите дробови). За клеточните линии HT-29, вредностите за IC_{50} беа 652,04 μg/ml за CPSO и 414,88 μg/ml за WPSO, укажувајќи на посилна антипролиферативна активност на WPSO. Инхибиторната активност (IC_{50}) корелира со способноста на примерокот да ја инхибира пролиферацијата на клетките за 50 % и пониската IC_{50} вредност укажува на посилен антипролиферативен ефект.

Клучни зборови: калинка; масло од семки; физичко-хемиски својства; фенолни соединенија; биоактивност

1. INTRODUCTION

Pomegranate (*Punica granatum* L.) is considered one of the oldest edible fruits, belonging to the *Punicaceae* family. This plant species originates from Asia, specifically the region from Iran to northern India. Wild pomegranate refers to a variety that grows naturally in the wild. Wild pomegranate fruits are smaller, whereas cultivated pomegranate fruits are larger, heavier, and possess bright red seeds (arils) measuring 8 – 12 mm.

Approximately 50 % of the total mass of the pomegranate fruit consists of the pericarp, which is rich in bioactive compounds such as flavonoids, ellagitannins, and proanthocyanidins. The remaining 50 % is made up of the arils, the edible portion of the fruit, which are further divided into the outer fleshy red part (78 %) and the inner seeds (22 %).[1,2] The fruit is divided into several carpels (cells) by carpellar membranes, which are packed with rounded, succulent arils.[3]

Research by Guo et al.[4] has reported that wild pomegranate contains higher phenolic content than cultivated pomegranate varieties. Additionally, wild pomegranates generally have higher vitamin C levels than cultivated ones.[5] The enzyme α-glucosidase (EC 3.2.1.20), found in the small intestine of the digestive tract of humans, plays a critical role in carbohydrate digestion. By inhibiting this enzyme, the breakdown of carbohydrates is slowed, resulting in reduced post-meal glucose spikes.[6] Various scientific studies have investigated the effects of pomegranate fractions on fasting blood glucose – a key variable in type 2 diabetes.[7–9]

Pomegranate seeds are rich in punicic acid, punicalagin, ellagic acid, gallic acid, oleanolic acid and ursolic acid, which exhibit anti-diabetic properties. Notably, studies indicate significant decreases in fasting blood glucose levels due to punicic acid,[10] methanolic seed extract,[11] and pomegranate peel extract.[12]

Pomegranate seed oil is highly nutritious and versatile, with applications in both cosmetics and medicine. It is rich in fatty acids, particularly conjugated linolenic acid – a metabolite of punicic acid (PA) belonging to the omega-5 (ω-5) family – known for potent bioactivity.[13] Phenolic and flavonoid contents in pomegranate seed oil have been recorded at 39.06 and 12.4 mg/g, respectively. Additionally, substantial levels of carotenoids and sterols are present.[14] Tocopherols and sterols form another important class of lipophilic compounds found in bioactive oil, with γ-tocopherol (0.136 mg/ml) being the dominant tocopherol and β-sitosterol (2.68 mg/ml) being the major sterol.[15] The oil possesses estrogenic, anti-inflammatory, and antimicrobial properties.[16]

Pomegranate seed oil can be extracted using various techniques, including microwave-assisted extraction, ultrasound-assisted extraction, cold pressing techniques, and Soxhlet's extraction with different organic solvents.

This study aimed to determine and compare the physical characteristics of pomegranate seeds and the physico-chemical and biological characteristics of cultivated and wild pomegranate seed oils. In addition to chemical analyses, the study investigated fatty acid profiles, mineral content, phenolic compound concentrations, as well as the antioxidant, antimicrobial, antihyperglycemic, and antiproliferative activities of the oils.

2. EXPERIMENTAL

2.1. *Origin of pomegranate samples and seed characterization*

Wild pomegranate fruits were harvested in November 2019 from Stolac, Bosnia and Herzegovina, (43° 05′ N, 17° 58′ E). Cultivated pomegranate samples, originating from Turkey, were purchased at a local market in Banja Luka, Bosnia

and Herzegovina, during November 2019. The mass of 1000 seeds, seed moisture content, volume mass, and specific mass were determined as described by Grubačić and Vasilišin.[17] After extracting the juice, the pomegranate seeds were thoroughly washed and air-dried at room temperature.

2.2. *Oil extraction*

Before oil extraction, the seeds were ground using a laboratory mill (E-1350 blender; Ema, Istanbul, Turkey). Cultivated (CPSO) and wild (WPSO) pomegranate seed oils were obtained by extraction with hexane using a Soxhlet's apparatus for 10 hours. After evaporation of the hexane, the oils were stored at +4 °C in a refrigerator until analysis.

2.3. *Oil chemical analysis*

Oil density, refractive index, acid number, peroxide number, saponification number, Hanush iodine number, and ash content were determined according to standard AOAC methods.[18] Heavy metal contents were determined using an Agilent Technologies atomic absorption spectrophotometer model 240FS/240Z AA Duo (Agilent Technologies, Santa Clara, California). The metal contents were determined using the flame technique, except for arsenic, which was determined using the hydride technique.

The fatty acid composition was determined using gas chromatography with an flame ionization detector (FID) BAS EN ISO 12966-4 (Agilent Technologies, Santa Clara, California). Carotenoid content in oils, expressed as β-carotene, was determined following the method described by Munasinghe et al.[19] The chlorophyll content, expressed as pheophytin-a, was determined according to the method detailed by Pokorny et al.[20]

2.4. *Oil bioactivity analysis*

Total phenols were determined by the modified Folin-Ciocalteu method,[21] with gallic acid used to construct the calibration curve. The total flavonoid content was determined using the method of Ordoñez et al.,[22] and results were expressed as quercetin equivalents. Total flavonol content was determined according to the method of Kumaran and Karunakaran,[23] with results expressed as quercetin-equivalent flavonols. The total flavan-3-ols were determined following the method of Revilla et al.,[24] using catechin to create the calibration curve.

Antioxidant activity was evaluated using the DPPH$^{\bullet}$ (2,2 diphenyl-1-picryl-hydrazyl) assay, following the method of Liyana-Pathirana and Shahidi.[25] Similarly, antioxidant activity of the oils was assessed using the ABTS$^{\bullet+}$ (2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonyl)-diammoniumsalt) test as per the method described by Re et al.[26]

The α-glucosidase inhibitory potential was assessed according to the method of Tumbas Šaponjac et al.[27] Antiproliferative effects were determined on four human tumor cell lines: HeLa (epithelioid carcinoma of the cervix), MCF-7 (breast adenocarcinoma), HT-29 (colon adenocarcinoma), and MRC-5 (normal fetal lung fibroblasts). Cell growth inhibition was assessed using the colorimetric sulforhodamine-B (SRB) assay, following the method described by Cetojevic-Simin et al.,[28] with effects expressed as a percentage relative to the control.

2.5. *Antimicrobial activity analysis*

The antibacterial and antifungal activity of the oils were assessed using the well diffusion method, as described by Balouiri et al.,[29] Valgas et al.,[30] and Magaldi et al.[31] For the antibacterial tests, two bacterial species were selected: *Salmonella enteritidis* ATCC 13076 (Gram-negative) and *Listeria monocytogenes* ATCC 13932 (Gram-positive). Antifungal activity was tested against *Candida albicans* ATCC 10231 (yeast). All microorganisms used in this study were obtained from the Microbiologics reference strain collection (St. Cloud, USA) in the form of KWIK-STIK units, which include lyophilized microorganism pellets, a hydrating fluid ampoule, and an inoculating swab. These KWIK-STIKs were stored at 2–8 °C until use.

To prepare the cultures, a swab was streaked on Tryptic Soy Agar (TSA; Merck KGaA, Darmstadt, Germany) for the bacterial species and Sabouraud Dextrose Agar (SDA; Merck KGaA, Darmstadt, Germany) for yeast. The plates were incubated at 37 °C for 24 hours to obtain isolated colonies. Afterward, bacterial cultures were transferred to Mueller-Hinton Broth (MHB), and yeast cultures were transferred to a saline solution. The microbial density of both bacterial and yeast cultures was adjusted to 10^6 CFU/ml, as determined by comparison with the 0.5 McFarland standard.[32]

For the assay, the prepared bacterial and yeast suspensions were inoculated onto Petri dishes (9 cm in diameter) containing Mueller-Hinton Agar (MHA) and Sabouraud Dextrose Agar (SDA), respectively, using sterile cotton swabs. Wells (6 mm in diameter) were created in the inoculated agar plates using a sterile borer, and 50 μl of the oils (50 mg/ml in 80 % ethanol) were intro-

duced into the wells. The plates were incubated upright at 37 °C for 24 hours. After incubation, the diameters of the inhibition zones, including the well width (6 mm), were measured.

For comparison, several antibiotics served as positive controls: nalidixic acid (30 µg), cephalothin (30 µg), cefpodoxime (10 mg), vancomycin (30 µg), and novobiocin (500 µg) for bacterial species, and natamycin (30 µg) for yeast. An 80 % ethanol (v/v) solution was used as a negative control.

2.6. *Statistical analysis*

The experiments were conducted with at least three repetitions. Results were expressed as mean value standard deviation (SD). A one-factor analysis of variance (ANOVA) was performed on all data, and the Duncan test was performed to determine statistically significant differences between mean values at a significance level of $p < 0.05$. Statistical analyses were carried out using the following software: Excel (Microsoft Office 2010), Origin 5.0 (OriginLab, USA), and Statistica 12.0 (StatSoft, Inc.).

3. RESULTS AND DISCUSSION

The physical characteristics of cultivated and wild pomegranate seed oils (CPSO and WPSO, respectively) are shown in Table 1. The moisture content of the seeds was almost identical for both cultivated and wild pomegranate, measuring 7.76 % and 7.59 %, respectively. These values were lower than those reported by Juhaimi et al.,[33] where the moisture content ranged from 10.44 to 12.64 %.

T a b l e 1

Physical characteristics and physico-chemical characteristics and mineral composition of cultivated and wild pomegranate seed oils

Parameter	**CPS**	**WPS**	**Literature data**
Moisture content (%)	7.76 ± 0.03[a]	7.59 ± 0.07[b]	–
Mass of 1000 seeds (g)	24.64 ± 0.58[a]	28.10 ± 0.14[b]	–
Hectolitre mass (kg)	49.8 ± 0.4[a]	48.9 ± 0.1[b]	–
Specific gravity (kg/m^3)	935.58 ± 0.79[a]	885.00 ± 1.06[b]	–
	CPSO	**WPSO**	
Oil density at 20 °C (g/ml)	0.935 ± 0.001[a]	0.926 ± 0.003[b]	0.893–0.931 g/ml [34]
Refraction index at 20 °C	1.5162 ± 0.0003[a]	1.5097 ± 0.0006[b]	1.499[35]
Iodine number (g I_2/100g)	171.05 ± 4.07[a]	165.09 ± 4.69[a]	206.9–221.64 [33,34,36]
Saponification number (mg KOH/g)	190.51 ± 0.72[a]	188.36 ± 0.22[b]	156–199.61 [34]
Acidic number (mg NaOH/g)	1.99 ± 0.16[a]	2.25 ± 0.16[a]	4.21–7.51[33] 0.63–8.36[34]
Peroxide number (mmol O_2/kg)	0.47 ± 0.00[a]	0.83 ± 0.00[b]	5.44–8.47[33] 0.39–5.96[34] 21.1–23.8[35]
Ash content (%)	0.06 ± 0.01[a]	0.09 ± 0.01[b]	1.59–1.887[36]
Minerals content (mg/kg)			
Zn	0.61 ± 0.03[a]	0.62 ± 0.04[a]	33.71–52.36[33] 29.17–70.96[36]
Cu	1.21 ± 0.10[a]	1.21 ± 0.03[a]	2.11–6.81[33] 5.49–9.81[36]
Fe	4.32 ± 0.10[a]	4.20 ± 0.26[a]	31.84–61.44[33] 16.28–31.23[36]
Mn	ND	ND	8.21–15.81[33] 7.54–12.44[36]
Co	ND	ND	–
Cr	ND	ND	–
Ni	ND	ND	–
As	ND	ND	–

*Results are presented as mean ± standard deviation SD (n = 3). ND – not determined. Mean values with different superscript letters in the same row are statistically different ($p < 0.05$).

The yields of both types of pomegranate oils were approximately similar, at 13.60 % for CPSO and 13.34 % for WPSO. These results for the cultivated pomegranate oil align with the literature values, which range from 3.57 to 24.13 %.[37,38] Variations in yield may be attributed to factors such as the method of extraction, pomegranate variety, climatic factors, and environmental conditions.[16,39]

The physico-chemical characteristics of cultivated and wild pomegranate seed oil are shown in Table 1. The density of wild pomegranate seed oil was 0.926 g/ml, slightly lower than that of cultivated pomegranate oil. Published values for oil density ranged from 0.893 to 0.931 g/ml.[34] The refractive index of a substance, which depends on the wavelength of incident light and experimental temperature, serves as a characteristic and identifying feature.[40] For wild pomegranate oil, the refractive index was 1.5097, while for cultivated oil, it was 1.5162. Hajib et al.[35] reported a refractive index of 1.499 for pomegranate seed oil extracted with hexane.

The iodine number of wild pomegranate oil was 165.09, lower than reported literature values ranging from 206.9 to 221.64.[33,34,36] The saponification number of the wild pomegranate oil was 188.36, slightly lower than that of the cultivated pomegranate oil and consistent with reported literature values ranging from 156 to 199.61.[34] The saponification value depends on the length of the fatty acid chains in the triacylglycerol molecule; fats with shorter chains exhibit higher values. Oils with higher saponification numbers are richer in fatty acids of lower molecular weight.[40]

The acid value of wild pomegranate seed oil was 2.25 mg NaOH/g, higher than that of cultivated pomegranate oil. This acid value aligns with the results by Paul and Radhakrishnan[34] and is lower than the results published by Juhaimi et al.,[33] which ranged from 4.21 to 7.51. The acid value represents free acidity and indicates the degree of hydrolytic decomposition of fatty acids. For cultivated pomegranate seed oil, the acid value was low. Pomegranate seed oil extracted with n-hexane typically has an acid value between 3.78 and 8.36 ml/g, while oil obtained via cold pressing technology exhibits a lower acid value, around 0.63 ml/g.[41]

The peroxide value of wild pomegranates was 0.83 mmol O_2/kg, almost double that of cultivated pomegranate oil. This value aligns with published data[34] but is much lower than the results reported by Juhaimi et al.[33] In contrast, the peroxide value for cultivated pomegranate oil was substantially lower compared to values reported by Demir and Demir.[35] Generally, a lower peroxide value indicates better oil quality, as primary oxidation process, measured by the peroxide value, is negligible in the fresh oil.[41]

The mineral composition of both oils was approximately similar (Table 1). Heavy metals such as cobalt, nickel, chromium, arsenic, and manganese were not observed in any of the oils. However, zinc, copper, and iron contents were lower in the oils compared to their levels in the seeds of cultivated pomegranate.[33,36]

Fatty acid composition of both oils is shown in Table 2, with chromatograms of the two samples and a standard mixture presented in Figure 1. Fatty acids with fewer than 15 carbon atoms were not observed in any of the tested oils. The palmitic acid (C16:0) content of wild pomegranate seed oil was slightly lower than that of cultivated oil. These values align with findings from Paul and Radhakrishnan,[34] where C16:0 content ranged from 2.89 to 22.08 %.

Fatty acids C16:1, C17:0, and C17:1 were not detectable (less than 0.05 %), consistent with results obtained by Dadashi et al.[36] and Paul and Radhakrishnan.[34] The contents of steric acid (C18:0) and trans-linoleic acid (C18:2) were consistent with literature values for cultivated pomegranate oil, which ranged from 1.60 to 3.19 % and 0.30 to 5.13 %, respectively.[36,42,43] The C18:0 content of wild pomegranate oil also matched the findings of Paul and Radhakrishnan.[34] Arachidic acid (C20:0) levels were approximately equal in both oils, with slightly higher levels observed in cultivated pomegranate compared to the study by Jing et al.,[43] while wild oil values were consistent with Paul and Radhakrishnan.[34]

The C22:0 content of both oils was consistent with published data.[34,42] The most abundant fatty acid in both oils was eicosapentaenoic acid (C20:5, cis 5, 8, 11, 14, 17), constituting 80.64 % in wild pomegranate and 77.59 % in cultivated pomegranate. However, this finding contrasts with the literature, where the most abundant fatty acid was identified as punicic acid (C18:3).[34,36,42,43] The lignoceric acid (C24:0) content in both oils was consistent with the published results.[34,39,42]

The total carotenoid content, determined spectrophotometrically, was approximately 3 times higher in CPSO compared to WPSO (Table 3). The carotenoid content in pomegranate oil exceeded the values reported by Yoshime et al.[44] (0.9 mg/kg). The chlorophyll content was slightly higher in wild pomegranate seed oil, amounting to 0.213 mg pheophytin-a/kg. Costa et al.[45] did not detect chlorophylls in pomegranate oil using the HPLC method. However, in the study by Loukhmas et al.,[46] pheophytin levels ranged from 0.39 to 3.87 mg/kg, depending on the variety of pomegranate.

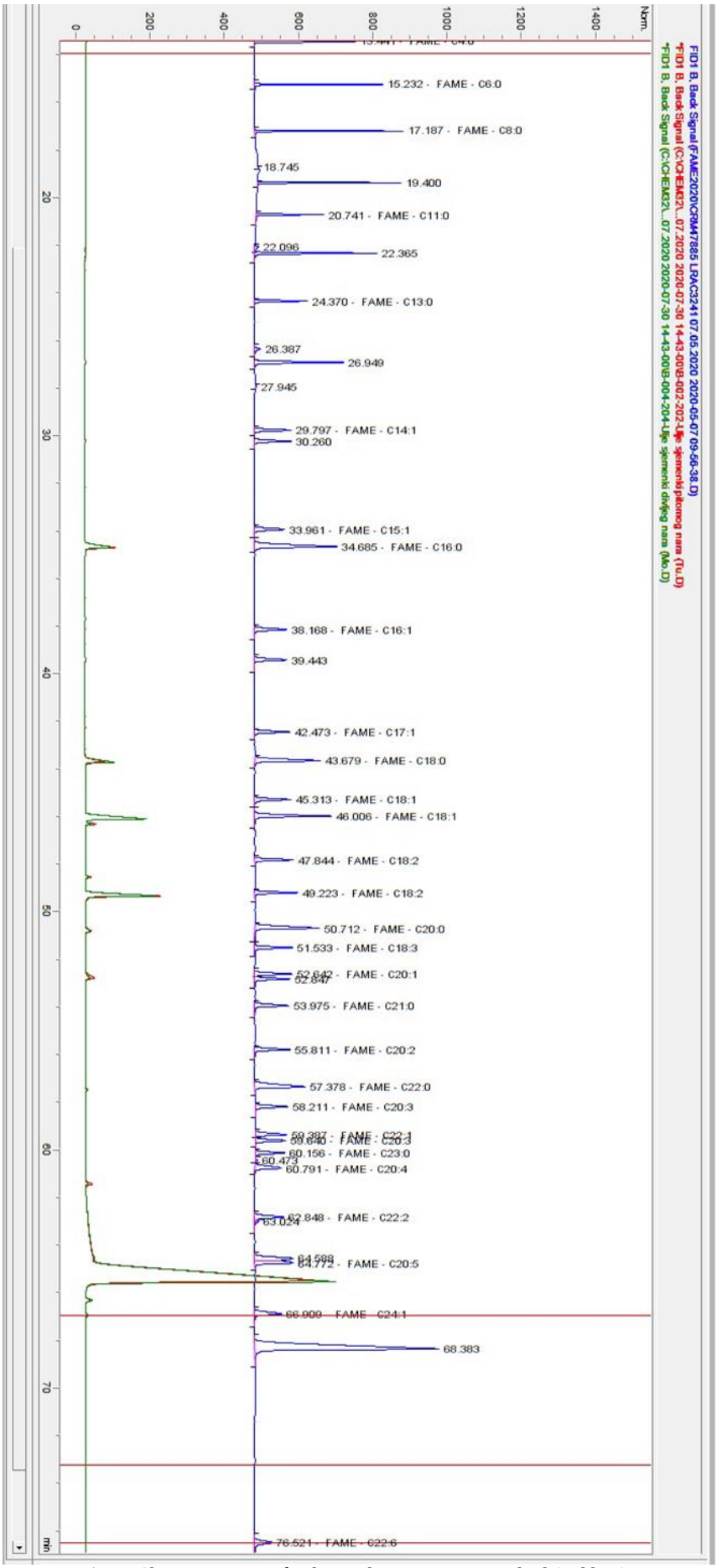

Fig. 1. Chromatograms of cultivated pomegranate seed oil (red line), wild pomegranate seed oil (green line), and standards (blue line)

T a b l e 2

Fatty acid composition in pomegranate seed oils (g per 100 g of sample)

Fatty acid	CPSO	WPSO	Literature data
C16:0	3.11	2.47	2.89–22.08[34]
C16:1 (cis 9)	ND	ND	0.06–0.09[36] 0.02–1.88[34]
C17:0	ND	ND	0.01[36] 0.05–0.54[34]
C17:1 (cis 10)	ND	ND	0.01–0.02[36] 0.83[34]
C18:0	1.73	2.62	1.60–3.19[36,42,43] 1.44–8.94[34]
C18:1 cis 9	5.92	5.62	
C18:1 (trans 9)	0.77	0.42	
C18:2 (all trans 9, 12)	0.38	0.24	0.30–5.13[36,42,43]
C18:2 (all cis 9, 12)	6.88	5.7	
C20:0	0.57	0.53	0.25–0.35[43] 0.38–0.91[34]
C18:3 (cis 6, 9, 12)	ND	ND	74.43–84.11[34] 72.07–73.31[36] 69.79–81.69[42] 73.45–78.80[43]
C18:3 (cis 9, 12, 15)	1.14	0.56	
C22:0	0.2	0.12	0.11–1.25[34] 0.12–0.27[42]
C23:0	0.56	0.12	
C24:0	ND	ND	0.05–0.58[34] 0.00–0.12[42] 0–8.1[39]
C20:5 (cis 5, 8, 11, 14, 17)	77.59	80.64	
C24:1 (cis 15)	0.14	0.21	

*ND – not determined, defined as ≤ 0.05 %

The total phenol content in CPSO was 0.47 mg GAE/g, slightly lower than that in WPSO, which measured 0.50 mg GAE/g. These values were significantly lower than those reported by Juhaimi et al.[33] (8.71 – 19.17 mg GAE/g). The total flavonoid and flavonol contents were identical in both oils, while the concentration of flavan-3-ol was slightly higher in WPSO (Table 3).

The antioxidant activities of pomegranate seed oils are shown in Table 4. Wild pomegranate oil demonstrated better antioxidant activity in the ABTS test, with activity approximately 1.13 times higher than that of the cultivated pomegranate oil. Conversely, cultivated pomegranate oil showed greater antioxidant activity in the DPPH test, about 1.3 times higher than wild pomegranate oil. Jing et al.[43] reported IC_{50} values for the DPPH test in the range of 11.2 – 19.8 mg/ml for the methanolic extract of pomegranate seeds, while Loukhmas et al.[46] found values ranging from 0.69 to 1.76 mg/ml. The ABTS test results for cultivated pomegranate oil in this study were lower than those reported by Amri et al.[47] (3.00 mg/ml), indicating stronger antioxidant capacity.

Wild pomegranate seed oil showed a slightly lower antihyperglycemic effect compared to cultivated pomegranate oil (Table 4). Başyiğit et al.[48] reported an IC_{50} value of 5.16 mg/ml for pomegranate seed oil.

The IC_{50} values for the antiproliferative effect are also shown in Table 4. For MCF-7 tumor cells, both oils had IC_{50} values below 200 μg/ml, though these were higher than previously published values for methanolic extracts of pomegranate seed samples, which reported IC_{50} values below 5 μg/ml.[49] Hydrophilic fractions of pomegranate seed oil have been shown to suppress the viability of MDA-MB-231 and MCF-7 cells after 24 h of incubation, without inducing significant apoptosis. Interestingly, inedible parts of the pomegranate fruit and tree, such as roots, bark, and fruit, have been reported to inhibit the proliferation of MCF-7 cells by up to 94 %. In animal studies, the addition of 0.01 % and 0.1 % of pomegranate seed oil to diets suppressed the occurrence and number of colon adenocarcinomas, significantly reducing their incidence.[50]

According to research by Đurđević et al.,[51] the tested oil showed no cytotoxic effects against normal MRC-5 (IC_{50} > 200 μg⁄ml) but demonstrated moderate cytotoxic effect against malignant HeLa cells (IC_{50} = 49.51 μg⁄ml).

The oils from both cultivated and wild pomegranate seeds demonstrated no antimicrobial activity against the tested bacterial strains (Table 5). Similarly, Başyiğit et al.[48] reported no observable effects of pomegranate oil on *Candida albicans*

Table 3

Total carotenoids (TCr), chlorophylls (TChl), phenols (TP), flavonoids (TF), flavonols (TFl), and flavan-3-ols (TFl3ol) in pomegranate seed oils

	CPSO	WPSO	Literature data
Total carotenoids content (mg β-caroten/kg)	7.53 ± 0.17[a]	2.49 ± 0.15[b]	0.9 mg/kg[44]
Total chlorophylls content (mg pheophytina/kg)	0.161 ± 0.010[a]	0.213 ± 0.010[b]	n.d. [45] 0.39–3.87 mg/kg [46]
TP (mgGAE/g)	0.47 ± 0.00[a]	0.50 ± 0.0[b]	8.71–19.17[33]
TF (mgQc/g)	0.03 ± 0.00[a]	0.03 ± 0.00[a]	–
TFl (mgQc/g)	0.55 ± 0.02[a]	0.55 ± 0.02[a]	–
TFl3ol (mgCAT/g)	20.03 ± 0.36[a]	25.00 ± 0.72[b]	–

*Results are presented as mean ± standard deviation SD (n = 3). GAE – gallic acid, Qc – quercetin hydrate, CAT – catechin. Mean values with different superscript letters in the same row are statistically different ($p < 0.05$).

Table 4

Antioxidant, antihyperglycemic, and antiproliferative activities of pomegranate seed oils

	Antioxidant activity			
	DPPH test	ABTS test		
Samples	**IC_{50} (μg/ml)**	**IC_{50} (μg/ml)**		
CPSO	3031.42 ± 77.19[a]	1167.93 ± 69.62[a]		
WPSO	4028.42 ± 170.54[b]	1031.10 ± 8.75[b]		
Trolox	12.56 ± 0.72	2.97 ± 0.19		
	Antihyperglycemic activity (at final concentration 1.25 mg/ml for oil samples and 0.0005 mg/mL for standard)			
CPSO	34.85 ± 5.33[a]			
WPSO	36.82 ± 4.04[a]			
Acarbose	39.51 ± 1.42			
	Antiproliferative activity, IC_{50} (μg/ml)			
	HeLa	**MCF7**	**HT-29**	**MRC-5**
CPSO	< 200[a]	< 200[a]	652.04 ± 95.92[a]	< 200[a]
WPSO	< 200[a]	< 200[a]	414.88 ± 71.79[b]	< 200[a]

*Results are presented as mean ± standard deviation SD (n = 3). Mean values with different superscript letters in the same column are statistically different ($p < 0.05$).

Table 5

Antimicrobial activities of pomegranate seed oils and controls

Samples	*Salmonella enteritidis*	*Listeria monocytogenes*	*Candida albicans*
	ATCC 13076	**ATCC 13932**	**ATCC 10231**
CPSO	0[e]	0[e]	0[a]
WPSO	0[e]	0[e]	0[a]
Nalidixic acid 0.03 mg	27.34 ± 1.38[b]	6.91 ± 0.45[d]	–
Cephalothin 0.03 mg	22.65 ± 1.07[c]	29.22 ± 1.56[b]	–
Cefpodoxime 10 mg	29.90 ± 0.34[a]	16.73 ± 0.54[c]	–
Novobiocin 0.5 mg	19.84 ± 0.78[d]	44.85 ± 1.48[a]	–
Vancomycin 0.05 mg	–	29.9 ± 0.79[b]	–
Natamycin 0.05 mg	–	–	15.63 ± 0.45[b]

*Results are presented as mean ± standard deviation SD (n = 3). Mean values with different superscript letters in the same column are statistically different ($p < 0.05$).

4. CONCLUSIONS

The oils obtained from the cultivated and wild pomegranate seeds showed similar levels of total phenols, flavonoids, flavonols, and flavan-3-ols. However, total carotenoid content was higher in cultivated pomegranate seed oil, while total chlorophyll content was higher in wild pomegranate seed oil. The predominant fatty acid in both oils was eicosapentaenoic acid. Wild pomegranate oil showed superior antioxidant activity in the ABTS test (approximately 1.13 times higher), whereas cultivated pomegranate oil showed better antioxidant activity in the DPPH test (approximately about 1.3 times higher).

Neither oil showed antimicrobial activity against the tested bacteria, but they demonstrated good antihyperglycemic effects and notable antiproliferative activity against HeLa, MRC-5, and MCF-7 tumor cells. To the best of our knowledge, no prior research has been conducted of the bioactivities on wild pomegranate seed oil. Given their richness in health-beneficial compounds and bioactivity, pomegranate seed oils should be incorporated into daily diets and used as components of functional foods. Future studies on these oils should focus on *in vivo* investigations.

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