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Phytochemical studies on the seeds, pseudofruits, and roots of *Rosa pimpinellifolia*

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ABSTRACT: The roots and pseudofruits of *Rosa pimpinellifolia* (Rosaceae) are used in traditional medicine as hemorrhoids, various infections, abdominal pain complaints, heart disease, flu, cold, and anemia treatment in Turkey. In this study, the phytochemical composition of the *R. pimpinellifolia* was revealed by using chromatographic and spectroscopic techniques. Two condensed tannin precursor, (-)-catechin **(1)**, the mixture of catechin and epicatechin **(2)** and three triterpenoid glycosides, kaji-ichigoside F1 **(3)**, 2*α*,3*β*,19*α* trihydroxyurs-12-en-28-*O*-*β*-D-glucopyranoside **(4)**, 2*α*,3*β*,19*α*,23*β*-tetrahydroxyurs-12-en-28-O-*β*-D-glucopyranoside **(5)** were isolated from the roots of *R. pimpinellifolia*. The isolation studies were performed by column chromatography. The chemical structures of the isolated compounds were elucidated with 1H and 13C NMR spectroscopy. Total phenolic compound contents were determined as 31.1937 mg/g dry extract in the pseudofruit; 9.8909 mg/g dry extract in the root via HPLC. The seeds of *R. pimpinellifolia* were extracted with diethyl ether in Soxhlet apparatus. The methyl esters of fatty acid were obtained from fixed oil and analyzed by GC/MS. The total ratio of unsaturated fatty acids in the seeds was 93.4%; containing 51.40% ratio of linoleic acid and 41.20% ratio of homo-γ-linolenic acid.

KEYWORDS: *Rosa pimpinellifolia;* Triterpenoid glycosides; phenolic compounds; fatty acid.

1. INTRODUCTION

Rosa (Rosaceae) genus, known as "Gül, Kuşburnu" in Turkey, has 24 species [1, 2]. In Turkey, *R. pimpinellifolia* L. is known as "karakuşburnu, koyungözü" and its synonym is also used as *R. spinossima* L. in the literature. *R. pimpinellifolia* is used in traditional medicine as tea alone or in a mixture with other herbs for hemorrhoids, various infections, abdominal pain complaints, heart disease, flu, cold, and anemia treatment [3-6].

Rosehip known as vitamin C source is used for preparing baby food, fruit juice, marmalade, tea, wine, and also as an additive ingredient in the pastry and confectionery industry [7]. It is usually added in medicinal preparations as a vitamin C source [8]. Ascorbic acid has a cytotoxic effect on cancer cells and cannot cause damage because they are resistant to healthy cells [9]. There is a good correlation between antioxidant activity and vitamin C as well as phenolic compounds [9-11].

Rosehip oil is rich in essential fatty acids, carotenoids, and vitamin A (retinol). Due to its rich chemical content, the cosmetic industry uses the oil in moisturizing and anti-aging as well as skin problems such as dermatitis, eczema, acne, burns [12]. "Essential Fatty Acids" are fatty acids having more than two bonds, cannot be produced in the human body, and should be taken by diet, [13, 14]. Essential fatty acids have four basic tasks in the body: (i) modulation of membrane structure, (ii) the formation of eicosanoids (prostaglandins, leukotrienes, and thromboxanes), (iii) control of possible permeability of other membranes such as the digestive tract and blood-brain barrier, (iv) regulation of cholesterol transport and cholesterol synthesis [15-17].

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Flavonoid derivatives [18], anthocyanidins [19], aurons [20], phenolic acids [21], phenylethanoids and glycosides [22], triterpenic compounds [23], steroidal compounds [24], sesquiterpenes [25], carotenoids [26], tannins [18], fatty acids [27], and essential oil [28] are found in the contents of pseudofruits, seeds, leaves, and flowers of *Rosa* species. These findings are in line with the outcomes of the previous studies conducted by the roots of *R. heckeliana* [21], *R. davurica* [29, 30], *R. taiwanensis*[31], *R. multiflora* [32-34], *R. laevigata* [35, 36], *R. odorata* var. *gigantean* [37], and *R. rugosa* [38].

Some biological activity studies conducted on the roots of *Rosa* species reported that root extracts including condensed tannins and triterpenes have anti-inflammatory and hepatoprotective activites [30, 39-41]. Çoruh et al. reported that catechin and epicatechin, isolated from the roots of *R. heckeliana*, have anticancer activity[39]. An et al. reported that kaji-ichygoside F1, rosamultin, euchaphic acid, and tormentic acid isolated from the roots of *R. rugosa*, also have antinociceptive and anti-inflammatory activity [38]. These results made us suggest that *R. pimpinellifolia* roots may be a rich source for important biological activities.

Although there hasn't been much research on *R. pimpinellifolia*, Porter et al. reported that the systematic characters of *Rosa* genus are with flavonol glycosides acylated with 3-hydroxy-3-methylglutaric acid. In their study performed on *R. pimpinellifolia*'s leaves, 3-*O*-*a*-L-rhamnopyranosyl-(1→2)-[6-*O*-(3-hydroxy-3-methylglutaryl)-*β*-D-galactopyranosides] of kaempferol (3,5,7,4' -tetrahydroxyflavone) and quercetin (3,5,7, 3',4' –pentahydroxyflavone) were reported [42].

Biological activity studies conducted on *R. pimpinellifolia* pseudofruits report remarkable results. One of these conducted by Mavi et al. showed that there are high antioxidant activities of *R. pimpinellifolia* pseudofruits via peroxidation inhibitory and DPPH activity mechanisms [43].

Although there are some studies in the literature reporting various biological activities and chemical composition of *R. pimpinellifolia* pseudofruit; there is no available study conducted on the chemical composition of *R. pimpinellifolia* roots. Our study has an original value of investigating the chemical composition of *R. pimpinellifolia* roots.

This study aims to determine the chemical composition of *R. pimpinellifolia* plant used in traditional medicine. Thus, it can be a pioneer for investigating the different pharmacological activities of *Rosa* species from roots to fruit.

2. RESULTS

2.1. Isolation and structure identification

At the beginning of the study, the roots of *R. pimpinellifolia* were extracted with MeOH and its chemical contents were obtained by column chromatography. ^{1}H NMR and ^{13}C NMR data of the known compounds were in line with data given in the literature for (-) catechin [44], epicatechin [45], Kaji-ichigoside F1 [46], 2*a*, 3*β*, 19*a* trihydroxyurs-12-en-28-*O*-*β*-D-glucopyranoside [47-49], 2*a*, 3*β*, 19*a*, 23*β*-tetrahydroxyurs-12-en-28-*O*-*β*-D-glucopyranoside [50, 51] (Figure 1).

2.1.1. Compound 1 ((-) catechin)

^{1}H NMR (400 MHz, CD_3OD) δ 6.82 (*d*, 1H, H-2', *J* = 1.8 Hz), 6.75 (*d*, 1H, H-5' *J* = 8.0 Hz), 6.70 *dd*, 1H, H-6', *J* = 1.8, 8.0 Hz), 5.92 (*d*, 1H, H-8 *J* = 2.2), 5.84 (*d*, 1H, H-6, *J* = 2.2), 4.55 (*d*, 1H, H-2, *J* =7.7), 3.96 (*ddd*, 1H, H-3, *J* =(7.7, 5.5, 8.3), 2.49 (*dd*, 1H, H-4a, *J* =16.1, 8.3), 2.83 (*dd*, 1H, H-4b *J* =16.1, 5.5). ^{13}C-NMR (100 MHz, CD_3OD) δ 156.4 (C-7), 156.7 (C-5), 155.7 (C-9), 145.1 (C-3',C-4'), 131.0 (C-1'), 118.8 (C-6'), 114.9 (C-2'), 114.1 (C-5'), 99.6 (C-10), 95.1 (C-6), 94.3 (C-8), 81.7 (C-2), 67.6 (C-3), 27.3 (C-4). ^{1}H NMR and ^{13}C NMR data were in agreement with data given in the literature for (-) catechin [44] (Figure S1, S2).

*2.1.2. Compounds 2 (*The mixture of catechin and epicatechin*)*

Catechin. ^{1}H NMR and ^{13}C NMR data of catechin in the mixture were in agreement with the data given above. *Epicatechin*. ^{1}H NMR (400 MHz, CD_3OD) *δ* 6.95 (*d*, 1H, H-2', *J* = 2.0 Hz), 6.79 (*dd*, 1H, H-6', *J* = 2.0, 8.2 Hz), 6.75 (*d*, 1H, H-5' *J* = 8.2 Hz), 5.93 (*d*, 1H, H-6, *J* = 2.2), 5.90 (*d*, 1H, H-8 *J* = 2.2), 4.2 (bs, H-2, 1H), 3.82 (m, 1H, H-3), 2.85 (*dd*, 1H, H-4b, *J* =16.1, 5.8), 2.73 (*dd*, 1H, H-4a, *J* =16.1, 3.0). ^{13}C-NMR (100 MHz, CD_3OD) *δ* 156.4 (C-7), 156.5 (C-5), 155.7 (C-9), 145.1 (C-3',C-4'), 131.0 (C-1'), 118.8 (C-6'), 114.9 (C-2'), 114.1 (C-5'), 99.6 (C-10), 95.1 (C-6), 94.3 (C-8), 81.7 (C-2), 67.6 (C-3), 27.3 (C-4). ^{1}H NMR and ^{13}C NMR data were in agreement with data given in the literature for epicatechin [45] (Figure S3, S4).

2.1.3. Compound 3 (Kaji-ichigoside F_1)

^{1}H NMR (400 MHz, CD_3OD) δ 5.30 (*d*, 1H, H-1′, *J* = 8.0), 5.30 (*s*, 1H, H-12), 3.9 (*m*, 1H, H-2), 3.3 (*m*, 1H, H-3), 3.79 (*dd*, 2H, H-6′, *J* = 12.09, 1.83), 3.67 (*dd*, 2H, H-6′, 2H *J* = 11.73, 4.4), 3.3-3.64 (*m*, 1H, sugar protons, H-2′, H-3′, H-4′, H-5′), 2.59 (*ddd*, 2H, H-16, *J* = 4.0, 9.2), 2.5 (*s*, 1H, H-18), 1.99 (*m*, 2H, H-11), 1.81 (*m*, 2H, H-15), 1.8 (*m*, 1H, H-9), 1.78 (*m*, 2H, H-22), 1.57 (*m,* 2H, H-7), 1.55 (*m*, 2H, H-1), 1.4 (*m*, 2H, H-6), 1.32 (*s*, H-27, CH_3), 1.18 (*m*, 2H, H-21), 1.18 (*s*, H-29, CH_3), 0.97 (*s*, H-25, CH_3), 0.96 (*s*, H-23, CH_3), 0.92 (*d*, H-30, CH_3, *J* = 6.6), 1.26 (*m*, 1H, H-20), 1.22 (*m*, 1H, H-5), 0.84 (*s*, H-24, CH_3), 0.74 (*s*, H-26, CH_3). ^{13}C-NMR (100 MHz, CD_3OD) δ177.4 (C-28), 138.2 (C-13), 128.2 (C-12), 94.5 (C-1'), 78.8 (C-3), 77.2 (C-3'), 76.9 (C-5'), 72.5 (C-2'), 72.3 (C-19), 69.8 (C-4'), 65.8 (C-2), 61.1 (C-6'), 53.6 (C-18), 47.9 (C-5), 47.0 (C-9), 46.8 (C-17), 41.4 (C-1), 41.4 (C-20), 41.2 (C-14), 40.1 (C-8), 38.1 (C-10), 36.9 (C-22), 38 (C-4), 32.7 (C-7), 28.3 (C-15), 27.9 (C-23), 25.8 (C-21), 25.7 (C-29), 25.1 (C-16), 23.4 (C-11), 23.4 (C-27), 21.1 (C-24), 17.9 (C-6), 16.4 (C-26), 15.7 (C-25), 15.2 (C-30). ^{1}H NMR and ^{13}C NMR data were in agreement with data given in the literature for Kaji-ichigoside F_1 [46] (Figure S5-S13).

2.1.4. Compound 4 (2*a*, 3*β*, 19*a* trihydroxyurs-12-en-28-*O*-*β*-D-glucopyranoside)

^{1}H NMR (400 MHz, CD_3OD) δ 5.30 (*d*, 1H, H-1′, *J* = 8.1), 5.2 (*brs*, 1H, H-12), 3.55 (*m*, 1H, H-2), 2.83 (*d*, 1H, H-3, *J* = 9.7), 3.70, 3.61 (*m,* 2H, H-6′), 3.35-3.2 (*m*, 1H, sugar protons, H-2′, H-3′, H-4′, H-5′), 2.5 (*m*, 2H, H-16), 2.43 (*s*, 1H, H-18), 1.9 (*m*, 2H, H-11), 1.89 (*m*, 1H, H-9), 1.81 (*s*, 2H, H-15), 1.72, 1.55 (*m*, 2H, H-22), 1.48 (*m,* 2H, H-7), 1.7 (*m*, 2H, H-21), 1.45 (*d*, 2H, H-6, *J* = 10.2), 1.25 (*s*, H-27, CH_3), 1.25 (*m*, 1H, H-20), 1.1 (*s*, H-29, CH_3), 0.90 (*s*, H-25, CH_3), 0.90 (*s,* H-23, CH_3), 0.86 (*d*, H-30, CH_3, *J* = 6.5), 0.8 (*m*, 2H, H-1), 0.78 (*m*, 1H, H-5), 0.73 (*s*, H-24, CH_3), 0.69 (*s*, H-26, CH_3). ^{13}C-NMR (100 MHz, CD_3OD) δ177.1 (C-28), 138.3 (C-13), 128.1 (C-12), 94.4 (C-1'), 83.1 (C-3), 77.2 (C-3'), 76.9 (C-5'), 72.4 (C-2'), 72.2 (C-19), 69.7 (C-4'), 68.1 (C-2), 61 (C-6'), 53.5 (C-18), 55 (C-5), 47.2 (C-9), 47.8 (C-17), 46.9 (C-1), 41.5 (C-20), 41.3 (C-14), 39.9 (C-8), 37.8 (C-10), 36.9 (C-22), 39.1 (C-4), 32.7 (C-7), 28.2 (C-15), 27.9 (C-23), 25.8 (C-21), 25.8 (C-29), 25.1 (C-16), 23.4 (C-11), 23.3 (C-27), 16.2 (C-24), 18.3 (C-6), 16.0 (C-26), 15.7 (C-25), 15.2 (C-30). ^{1}H NMR and ^{13}C NMR data were in agreement with data given in the literature for 2*a*, 3*β*, 19*a* trihydroxyurs-12-en-28-*O*-*β*-D-glucopyranoside [47-49] (Figure S14-S24).

2.1.5. Compound 5 (2*a*, 3*β*, 19*a,* 23*β*-tetrahydroxyurs-12-en-28-*O*-*β*-D-glucopyranoside)

^{1}H NMR (400 MHz, CD_3OD) δ 5.23 (*d*, 1H, H-1′, *J* = 8.1), 5.23 (*s*, 1H, H-12), 3.75, 3.62 (*m,* 2H, H-6′), 3.62 (*dm*, 1H, H-2 *J* = 8.5), 3.2, 3.42 (*d,* 2H, H-23, *J* = 11.1), 3.3-3.25 (*m*, 1H, sugar protons, H-2′, H-3′, H-4′, H-5′), 3.28 (over loaded sugar protons 1H, H-3), 2.5 (*ddd*, 2H, H-16, *J* = 4.24, 13.2), 2.43 (*s*, 1H, H-18), 1.94 (*m*, 2H, H-11), 1.76, 1.55 (*m*, 2H, H-22), 1.75 (*m*, 2H, H-15), 1.7 (*m*, 1H, H-9), 1.7 (*m*, 1H, H-5), 1.7 (*m*, 2H, H-21), 1.59 (*m,* 2H, H-7), 1.35 (m, 2H, H-6), 1.29 (*m*, 1H, H-20), 1.26 (*s*, H-27, CH_3), 1.21, 0.81 (*m*, 2H, H-1), 1.12 (*s*, H-29, CH_3), 0.95 (*s*, H-25, CH_3), 0.86 (*d*, H-30, CH_3, *J* = 4.4), 0.69 (*s*, H-26, CH_3), 0.61 (*s*, H-24, CH_3). ^{13}C-NMR (100 MHz, CD_3OD) δ 177.1 (C-28), 138.3 (C-13), 128.1 (C-12), 94.4 (C-1'), 77.3 (C-3'), 77.3 (C-5'), 76.9 (C-3), 72.4 (C-2'), 72.2 (C-19), 69.7 (C-4'), 68.3 (C-2), 65 (C-23), 61 (C-6'), 53.6 (C-18), 46.8 (C-5), 47.8 (C-17), 47.1 (C-9), 46.5 (C-1), 42.7 (C-4), 41.5 (C-20), 41.4 (C-14), 39.9 (C-8), 37.6 (C-10), 36.9 (C-22), 32.1 (C-7), 28.2 (C-15), 25.8 (C-21), 25.7 (C-29), 25.1 (C-16), 23.4 (C-11), 23.3 (C-27), 17.8 (C-6), 16.3 (C-26), 16.2 (C-25), 15.2 (C-30), 12.5 (C-24). ^{1}H NMR and ^{13}C NMR data were in agreement with data given in the literature for 2*a*, 3*β*, 19*a,* 23*β*-tetrahydroxyurs-12-en-28-*O*-*β*-D-glucopyranoside [50, 51] (Figure S25-S40).

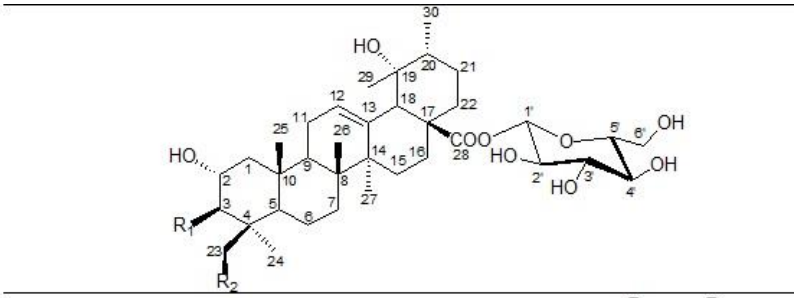
2.2. HPLC analysis in the pseudofruits and roots of *R. pimpinellifolia*

2.2.1. Fingerprint analysis

In this study, fingerprint analysis of the root extracts of *R. pimpinellifolia* has been carried out for the first time via a method that is described in the literature [52]. The correlation coefficient, regression equation, LOD, and LOQ were determined as shown in Table 1. (The amount of the phenolic content of the plant was expressed as mg/g.)

The first three major compounds in the pseudofruit were found to be benzoic acid (21.9115 mg/g dry extract), caffeic acid (12.5583 mg/g dry extract), chlorogenic acid (4.8085 mg/g dry extract) respectively. The amount of total phenolic compounds determined in the dry extract of the pseudofruit was 31.1937 mg/g (Table 2, Figure 2).

The first three major compounds in the root extract were found to be benzoic acid (11.3593 mg/g dry extract), protocatechuic aldehyde (4.1719 mg/g dry extract), and vanillic acid (1.5754 mg/g dry extract) respectively. The amount of total phenolic compounds determined in the dry extract of the pseudofruit was 9.8909 mg/g (Table 3; Figure 3).



	R_1	R_2
Kaji-ichigoside F_1 (3)	α-OH	H
2α, 3β, 19α trihydroxyurs-12-en-28-*O*-β-D-glucopyranoside (4)	β-OH	H
2α, 3β, 19α, 23β-tetrahydroxyurs-12-en-28-*O*-β-D-glucopyranoside (5)	β-OH	β-OH

Figure 1. Isolated triterpene compounds from the roots of *Rosa pimpinellifolia*.

Table 1. Method validation data for thirteen compounds determined via HPLC.

Compounds	LOD (µg/mL)	LOQ (µg/mL)	Correlation coefficient (r)	Regression equation
1- Gallic acid	0.03	0.12	0.9935	Y=46685X-33209
2- Protocatechuic acid	0.04	0.11	0.9984	Y=39214X-27944
3- Protocatechuic aldehyde	0.03	0.09	0.9968	Y=46438X+6147.1
4- *p*-Hydroxybenzoic acid	0.02	0.07	0.9974	Y=39080X-10794
5- Chlorogenic acid	0.12	0.35	0.9930	Y=9208.8X-399.4
6- Vanillic acid	0.02	0.08	0.9980	Y=49369X-63110
7- Caffeic acid	0.15	0.15	0.9973	Y=27764X-9126.7
8- Vanillin	0.02	0.07	0.9976	Y=38753X-1671.6
9- Syringaldehyde	0.11	0.34	0.9982	Y=13655X-2503.5
10- *p*-Coumaric acid	0.03	0.11	0.9955	Y=53747X-39534
11- Ferulic acid	0.06	0.19	0.9975	Y=81674X-55584
12- Sinapic acid	0.12	0.38	0.9997	Y=63367X-44638
13- Benzoic acid	0.28	0.86	0.9967	Y=6422X-11532

Table 2. The concentration of compounds in *R. pimpinellifolia* pseudofruit extract.

Compounds	Concentration (mg/g dry extract)
1-Gallic acid	*
2-Protocatechuic acid	*
3-Protocatechuic aldehyde	1.0800
4-*p*-Hydroxybenzoic acid	0.4937
5-Chlorogenic Acid	4.8085
6-Vanillic Acid	0.6592
7-Caffeic Acid	12.5583
8-Vanillin	0.8957
9-Syringaldehyde	4.2989
10-*p*-Coumaric Acid	0.7297
11-Ferulic Acid	1.0225
12-Sinapic Acid	4.6472
13-Benzoic Acid	21.9115

*Not detected

2.3. Determination of free fatty acids

As a result of Soxhlet extraction, we determined that 4.74% of seeds of *R. pimpinellifolia* is fixed oil. After hydrolysis of the oil and esterification of the fatty acids with MeOH, we detected fatty acid composition by using GC and GC-MS. Thus, the composition of *R. pimpinellifolia* seed oil contains mainly fatty acids (98.5%) in which unsaturated fatty acids are found as 93.40%. The major unsaturated fatty acids were linoleic acid ($C_{18:2}$) (51.4%) and homo-γ-linolenic acid ($C_{20:3}$) (41.2%) (Table 4).

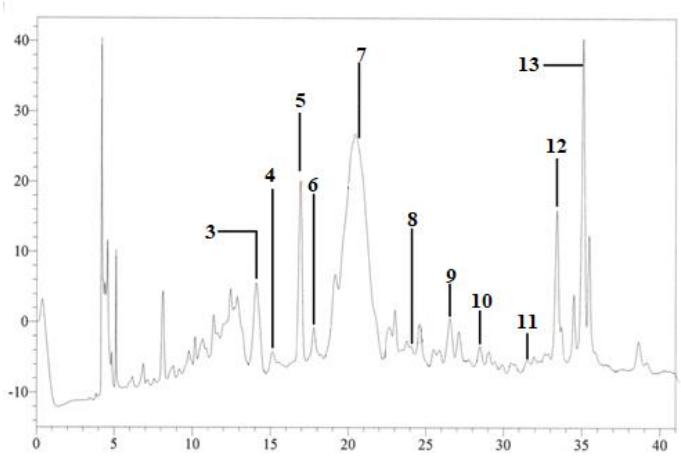


Figure 2. Compounds in *R. pimpinellifolia* pseudofruit extract.

Table 3. The concentration of compounds in the root extract of *R. Pimpinellifolia.*

Compounds	Concentration (mg/g dry extract)
1-Gallic acid	0.1114
2-Protocatechuic acid	0.1060
3-Protocatechuic aldehyde	4.1719
4-*p*-Hydroxybenzoic acid	0.0953
5-Chlorogenic Acid	0.2590
6-Vanillic Acid	1.5754
7-Caffeic Acid	0.5791
8-Vanillin	0.1199
9-Syringaldehyde	0.6990
10-*p*-Coumaric Acid	0.4658
11-Ferulic Acid	0.1880
12-Sinapic Acid	1.5201
13-Benzoic Acid	11.3593

3. DISCUSSION

In this study, we determined the chemical composition and the possible health-beneficial compounds of *R. pimpinellifolia* pseudofruits, seeds, and roots. We determined the fatty acids composition of *R. pimpinellifolia*'s seed oil and 11 compounds from pseudofruits. Additionally, We determined 13 compounds from the roots of *R. pimpinellifolia* for the first time.

In the NMR studies of compound **5,** we determined $J_{2,3}$=8.5 Hz which shows *trans* structure of H-2 and H-3 according to each other. CH_2OH attached to C-4 gave an AB system ($J_{23a,23b}$=11.1 Hz) at d=3.42 and 3.18 ppm as doublets which exactly confirms attached CH_2OH and CH_3 to C-4. In 1984, Seto et al. [49] reported a very similar compound to compound **5** in which all functionalities are the same but only CH_2OH attached to C-4 is structured as alfa. Therefore, to elucidate the structure of CH_2OH we needed further 2D NMR studies.

The intensity of the methyl signal of $H_3C(24)$ (0.61 ppm) increased when the signal of H-3 (3.28 ppm) was irradiated in the NOE experiment. However, the intensity of the signal of H-2 (3.62 ppm) did not increase as expected. These show that H-3 and $H_3C(24)$ were in the same directions and H-2 and H-3 were in different directions (Figure S38- S40).

Shameh et al. reported the phenolic contents of pseudofruits of 5 different *Rosa* species (*R. canina, R. moschata, R. damascene, R. webbiana, R. hemisphaerica*) and stated that chlorogenic acid and gallic acid were the main components [53].

In the study by Okan et al., it is reported that *R. pimpinellifolia* pseudofruits have a high total phenolic and flavonoid content and contain phenolic compounds such as gallic acid, protocatechuic acid, catechin, and quercetin. Besides, some enzyme inhibitory effects (acetylcholinesterase, xanthin oxidase, and urease) were examined and their potential activities were shown. In our study, gallic acid and protocatechuic acid were not detected. While caffeic acid was the major component, it was not detected in their study [54]. These distinct results obtained from the same plants can arise from various factors including location, altitude, and climate [53].

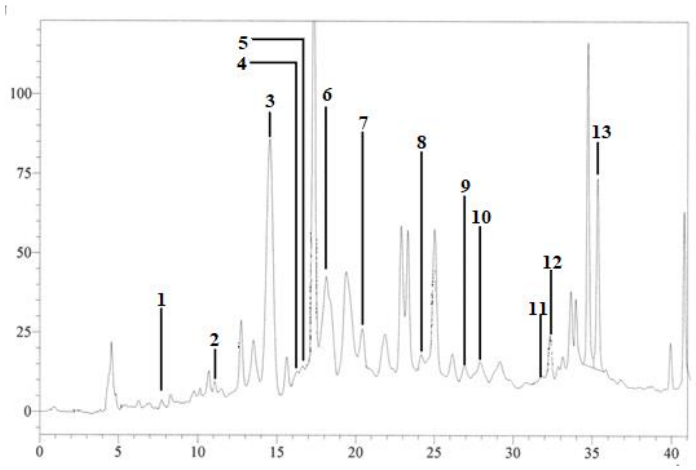


Figure 3. Compounds in *R. pimpinellifolia* root extract.

Table 4. The fatty acids composition of *R. pimpinellifolia*'s seed oil (%) [a, b].

Fatty acid	Symbol	Serial	% Area	Retention time	Retention indices
Lauric acid	C 12:0	-	0.1	37.22	1525
Tetradecanoic acid	C 14:0	-	0.1	44.29	1725
Pentadecanoic acid	C 15:0	-	0.1	47.59	1826
Palmitic acid	C 16:0	-	0.2	50.14	1906
Heksadecanoic acid	C 16:0	-	3.0	50.81	1928
Heksadecadienoic acid	C 16:2	ω-12	0.1	52.89	1997
8,11,14-Dokosatrienoic acid	C 22:3	ω-8	0.1	53.10	2004
Heptadecanoic acid	C 17:0	-	0.1	53.79	2027
Linoleic acid	C 18:2	ω-6	**51.4**	56.21	2111
Homo-γ-linolenic acid	C 20:3	ω-6	**41.2**	56.40	2118
Stearic acid	C 18:0	-	1.5	56.78	2131
11,14,17-Eikozatrienoic acid	C 20:3	ω-3	0.1	57.32	2150
11Z-Eikozenoic acid	C 20:1	ω-9	0.6	61.52	2304
Σ	98.50				
Σsaturated	5.10				
Σunsaturated	93.40				

[a] RI calculated from retention times relative to that of FAMEs (C_4-C_{24}) on the non-polar HP-5 column.
[b] Percentages obtained by FID peak-area normalization.

Phenolic contents of the root of *R. pimpinellifolia* were also measured for the first time in our study. The amount of phenolic substance of the roots was less than that of the pseudofruit. The total amount of phenolic acids were detected as 31.24 mg/g extract in pseudofruit and 9.795 mg/g dry extract in the root.

In our study, we detected linoleic acid, homo-γ-linolenic acid, and stearic acid content as 51.4%, 41.2%, 1.5%, respectively. Thus, for the first time, we herein report that the presence of homo-γ-linolenic acid as fatty acid in the seeds of *R. pimpinellifolia* has not been reported in the other *Rosa* species. We suppose that these differences are thought to be due to the location, climate, and stress conditions of the collected plant material. Our comment is also supported by Çakır et al 2004 who suggest that the chemical contents of the same species are changeable according to location [55]. The ratio of homo-γ-linolenic acid was found to be very high in *R. pimpinellifolia* seeds. Therefore, it may be thought that *R. pimpinellifolia* seed oil can be used in medical materials or dietary supplements. In this context, further biological and chemical studies are required.

In the study conducted by Murathan et al., the fatty acids consist of four *Rosa* species, one of which was *R. pimpinellifolia*. Researchers reported that *R. pimpinellifolia* had the highest amount of linoleic acid (41.21%) and stearic acid (19.2%) [56].

In a study investigating the oil content and fatty acid composition of different *Rosa* species, Ercisli et al. reported the oil content changes between 4.60-5.37% in which the ratio of unsaturated fatty acids is between 77.80-91.85% [57].

3. CONCLUSION

From the results of the present research, it can be concluded that the traditional use of *R. pimpinellifolia* roots is due to the rich triterpenes and phenolic compounds in the plant's content. Also, the pharmacological and biological activities of seed oils, and the isolated active ingredients should be investigated.

4. MATERIALS AND METHODS

4.1. General experimental procedures

^{1}H NMR and ^{13}C NMR spectra were recorded with a Varian Mercury plus spectrometer at 400 MHz and 100 MHz. Sephadex LH-20 (Sigma-Aldrich) and silica gel (Kieselgel 60, 0.063-0.2 mm Merck 7734 and 0.040-0.063 mm Merck 9385 and LiChroprep RP-18, 25-40 µm, Merck 9303) for column chromatography (CC); silica gel 60 F254 (Merck, 05554) for TLC were used. TLC spots were detected with a UV lamp and spraying 1% Vanillin/H_2SO_4 and heating at 120 °C for 1-2 min. Solvents used in isolation and solvent system are ethyl acetate, formic acid, methanol, H_2SO_4, H_2O, HCl, hexane, chloroform (Merck), vanillin (Fluka). HPLC analysis was practiced using a Shimadzu liquid chromatography (Shimadzu Corporation, LC 20 AT, Kyoto, Japan) and C18 column (Zorbax, 4.6 mm x 150 mm, 5 µm particle size). GC analysis was carried out using a Shimadzu 2010 Plus gas chromatography coupled to a Shimadzu QP2010 Ultra mass selective detector.

4.2. Plant material

R. pimpinellifolia roots were collected in Köşk village, Erzurum, Turkey (altitude 1900 m) in September 2013 and were authenticated by Prof. Dr. Ufuk Özgen. A voucher specimen (ATA 9876) has been deposited in Atatürk University Science Faculty.

4.3. Extraction and isolation

Schematic isolation steps were shown in Figure 4.

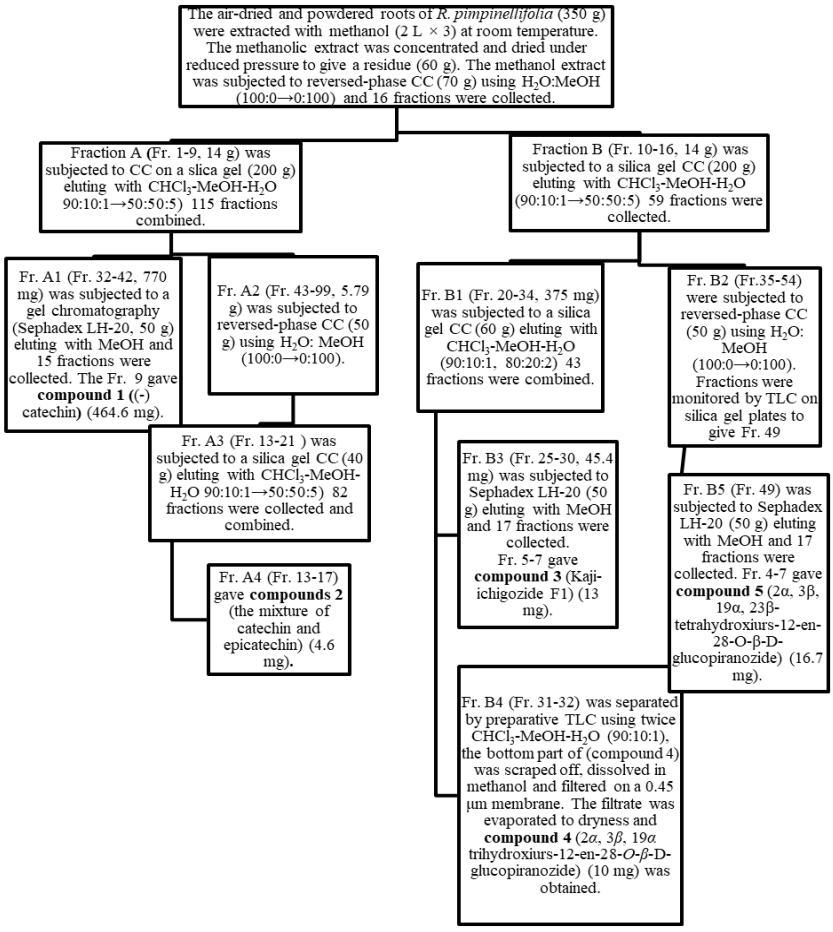


Figure 4. The purification pathway of pure compounds from the roots of *R. Pimpinellifolia.*

4.4. HPLC analysis in pseudofruits and roots of *Rosa pimpinellifolia*

4.4.1. Preparation of the extracts

The dried and powdered roots and pseudofruits of the plant (1 g) were extracted with 20 mL methanol for 12 h at 25 °C in the mechanical stirrer. The solvent was removed under vacuum. The dried methanol extracts were dissolved in HPLC grade methanol (10 mg/mL) and filtered through 0.45 µm membranes.

4.4.2. Fingerprint analysis

The modified method was run for 41 minutes to determine compounds. For gradient elution, the mobile phase consists of 0.5% (v/v) acetic acid in HPLC-grade water/acetonitrile (50:50) (Solvent A) and 2% (v/v adjust to pH 2.85) acetic acid in HPLC-grade water (Solvent B) as mobile phase. The flow rate was 1.2 mL/min. The injection volume was 20 µL for this method. The method was studied with a diode array detector at 270 and 340 nm at 25 °C [52].

4.5. Determination of free fatty acids

4.5.1. Oil extraction

The dried and powdered seeds of *R. pimpinellifolia* (50 g) were extracted with diethyl ether in a Soxhlet apparatus for 2 hours at 75 °C. Then, the solvent was evaporated under reduced pressure to obtain a crude fixed oil (yields:2.37 g, 4.74%)

4.5.2. Gas chromatography-mass spectrometry (GC-MS) analysis

Fatty Acid Methyl Esters (FAMEs) in 1 µL HPLC grade hexane were injected and analyzed by GC-MS under the following conditions. GC-MS analyses were made as described previously [58]. The GC-MS conditions are set out in the following (Table 5).

Table 5. The GC-MS conditions.

Column temperature	: The initial temperature was 60 °C for 2 min, which was increased to 240 °C at 3 °C/min, the final temperature of 250 °C was held for 4 min
Injector temperature	: 250 °C
Detector temperature	: 240 °C
Carrier gas	: Helium (99.999 %)
Flow rate	: 1 mL / min
Injection volume	: 1 µL
Split ratio	: 1/20
Analyse time	: 60 min
MS scanning range (m / z)	: (40-450 m / z)
Electron impaction ionization	: 70 eV

The Wiley and Nist libraries were used to identify the peaks in GC-MS chromatograms obtained from the analyzes. Relative percentages of peaks of fatty acids were calculated using Agilent Software. All samples were injected 2 times and the results expressed as the average of the values obtained after 2 injections.

4.5.3. Identification of constituents

Constituents of the FAMEs were identified by comparing their indices that were determined by the Kovats method using FAMEs (C_4-C_{24}) as standards. Mass spectra of FAMEs were determined with FFNSC1.2, W9N11 mass spectral libraries, and with published data [56-60] (Figure S41).

4.5.4. Esterification of the fixed oil

The obtained lipid samples were warmed with 2 N KOH in MeOH at 80 °C for 2 hours. The solution was cooled. Then 2 N HCl was added to neutralize the mixture. A neutralized mixture was extracted with *n*-hexane. The part of n-hexane was separated and mixed with 5 mL water to wash, dried with anhydrous Na_2SO_4, and filtered. 2 mL methanol was added to the 40 mg sample to dissolved in a test tube and the solutions were cooled in an ice bath; then BBr_3 was added, dropwise. The test tube was waited in a boiling water bath at 100 °C for 6 hours and cooled. Then 3 mL water was added and extracted with 2 mL *n*-hexane

two times. 4 mL, 2% $KHCO_3$ was added to the hexane layers, dried over anhydrous Na_2SO_4, and filtered. The organic solvent was evaporated to yield FAMEs. Fatty acid methyl esters were obtained from the lipid sample as described in the literature with minor modifications [60].

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Appendix A. Supplementary Material

Supplementary material related to this article can be accessed at https://dx.doi.org/10.29228/jrp.6 .

REFERENCES

[1] Davis PH. Nilsson Ö. *Rosa* L. In Flora of Turkey and the East Aegean Islands vol 4. Edinburgh University Press, UK, 1972, pp 106-128.

[2] Baytop T, Türkiye'de Bitkiler ile Tedavi. Istanbul Üniversitesi, Istanbul, Türkiye 1999.

[3] Özgen U, Kaya Y, Houghton P. Folk medicines in the villages of Ilıca District (Erzurum, Turkey). Turk J Biol. 2012; 36(1): 93-106. [CrossRef]

[4] Tetik F, Civelek S, Cakilcioglu U. Traditional uses of some medicinal plants in Malatya (Turkey). J Ethnopharmacol. 2013; 146 (1): 331-346. [CrossRef]

[5] Gürhan G, Ezer N. Halk arasında hemoroit tedavisinde kullanılan bitkiler-I. Hacettepe Univ Eczacı Fak Derg. 2004; 24 (1): 37-55.

[6] Altundag E, Ozturk M. Ethnomedicinal studies on the plant resources of East Anatolia, Turkey. Procedia Soc Behav Sci. 2011; 19: 756-777. [CrossRef]

[7] Coşkun M, Kurucu S, Kartal M. HPLC analysis of ascorbic acid in the fruits of some Turkish *Rosa* species. Sci Pharm. 1997; 65: 169-174.

[8] Vertuani S, Bosco E, Testoni M, Ascanelli S, Azzena G, Manfredini S. Antioxidant herbal supplements for hemorrhoids. Developing a new formula. Nutrafoods. 2004; 3: 19-26.

[9] Ullah M, Khan H, Zubair H, Shamim U, Hadi S. The antioxidant ascorbic acid mobilizes nuclear copper leading to a prooxidant breakage of cellular DNA: implications for chemotherapeutic action against cancer. Cancer Chemother Pharmacol. 2011; 67 (1): 103-110. [CrossRef]

[10] Roman I, Stanila A, Stanila S. Bioactive compounds and antioxidant activity of *Rosa canina* L. biotypes from spontaneous flora of Transylvania. Chem Cent J. 2013; 7(1): 73. [CrossRef]

[11] Shin I-C, Jeong K-J, Shim T-H, Oh H-S, Park S-K, Cheung E-H, Kim S-N, Kim G-G, Choi D-S, Kwon Y-S. Catechin content and antioxidative effect from *Rosa davurica* Pall. Korean J Physiol Pharmacol. 2002; 33 (3): 177-181.

[12] Ahmad N, Anwar F. Rose hip (*Rosa canina* L.) oils. In: Essential oils in food preservation, flavor and safety. Elsevier. 2016; 667-675.

[13] Bruneton J. Pharmacognosy, phytochemistry, medicinal plants. vol Ed. 2. Technique et Documentation Lavoisier, Paris 1993.

[14] Cunnane S, Anderson M. Pure linoleate deficiency in the rat: influence on growth, accumulation of n-6 polyunsaturates, and [1-14C] linoleate oxidation. J Lipid Res. 1997; 38 (4): 805-812.

[15] Schmitz G, Ecker J. The opposing effects of n− 3 and n− 6 fatty acids. Prog Lipid Res. 2008; 47 (2): 147-155. [CrossRef]

[16] Horrobin D. Nutritional and medical importance of gamma-linolenic acid. Prog Lipid Res. 1992; 31 (2): 163-194. [CrossRef]

[17] Feller SE, Gawrisch K. Properties of docosahexaenoic-acid-containing lipids and their influence on the function of rhodopsin. Curr Opin Struct Biol. 2005; 15 (4): 416-422. [CrossRef]

[18] Kumar N, Bhandari P, Singh B, Gupta AP, Kaul VK. Reversed phase-HPLC for rapid determination of polyphenols in flowers of *Rosa* species. J Sep Sci. 2008; 31 (2): 262-267. [CrossRef]

[19] Mikanagi Y, Yokoi M, Ueda Y, Saito N. Flower flavonol and anthocyanin distribution in subgenus *Rosa*. Biochem Syst Ecol. 1995; 23 (2): 183-200. [CrossRef]

[20] Gao X, Yang L, Shu L, Shen Y, Zhang Y, Hu Q. Aurone constituents from the flowers of *Rosa rugosa* and their biological activities. Heterocycles. 2012; 85 (8): 1925-1931. [CrossRef]

[21] Çoruh N, Özdoğan N. Identification and quantification of phenolic components of *Rosa heckeliana* Tratt roots. J Liq Chromatogr Relat. 2015; 38 (5): 569-578. [CrossRef]

[22] Gao XM, Shu LD, Yang LY, Shen YQ, Zhang YJ, Hu QF. Phenylethanoids from the flowers of *Rosa rugosa* and their biological activities. Bull Korean Chem Soc. 2013; 34 (1): 246-248. [CrossRef]

[23] Liu D, Zhu S, Ma R, Zhang J, Li H, Chen H. Triterpenoids from the roots of *Rose odorata* var. *gigantean*. Chin J Nat Med. 2010; 8 (1): 12-15. [CrossRef]

[24] Stoyanova-Ivanova B, Iochkova I, Mladenova K, Daskalov R. Content and composition of neutral components in wax from *Rosa canina* flowers. Dokl Bolg Akad Nauk. 1979; 32 (11): 1503-1506.

[25] Hashidoko Y. The phytochemistry of *Rosa rugosa*. Phytochemistry. 1996; 43 (3): 535-549. [CrossRef]

[26] Novruzov EN. Pigments of species in the genus *Rosa* and their chemotaxonomic value. Acta Horticulturae 690. (Proceedings of the 1st International Rose Hip Conference, 2004). 2005; 225-230.

[27] Ghazghazi H, Miguel MG, Hasnaoui B, Sebei H, Ksontini M, Figueiredo AC, Pedro LG, Barroso JG. Phenols, essential oils and carotenoids of *Rosa canina* from Tunisia and their antioxidant activities. Afr J Biotechnol. 2010; 9 (18): 2709-2716.

[28] Al-Rehaily AJ, Al-Howiriny TA, Bizzo HR Essential oil of *Rosa abyssinica* R. Br. from Saudi Arabia. J Essent Oil Res. 2003; 15 (5): 344-345. [CrossRef]

[29] Yoshida T, Jin Z, Okuda T. Tannins of rosaceous medicinal plants. Part 10. Hydrolyzable tannin oligomers from *Rosa davurica*. Phytochemistry. 1991; 30(8): 2747-2752. [CrossRef]

[30] Park JC, Hwang YH, Choi DR, Jung DY, Park JG, Hur JM, Kim SJ, Kim SN, Kim MS. A triterpenoid glucoside and phenolic compounds from *Rosa davurica*. Nat Prod Sci. 2003; 9(1): 31-33.

[31] Yang SC, Fang JM, Cheng YS. Chemical constituents from the root and aerial parts of *Rosa taiwanensis*. J Chın Chem Soc. 1995; 42(3): 573-577. [CrossRef]

[32] Li Y, Hu L, Lou F. Studies on the constituents of *Rosa multiflora* Thunb. Zhongguo Yaoke Daxue Xuebao. 2002; 33(3): 184-187.

[33] Park KH, Kim SK, Choi SE, Kwon JH, Oh MH, Lee MW. Three new stereoisomers of condensed tannins from the roots of *Rosa multiflora*. Chem Pharm Bull. 2010; 58(9): 1227-1231. [CrossRef]

[34] Yeo H, Chin YW, Park SY, Kim J. Lignans of *Rosa multiflora* roots. Arch Pharm Res. 2004; 27(3): 287-290. [CrossRef]

[35] Li S, Zhai X, Wang T, Ma W, Hu J, Wang S, Li N, Wang K. Flavonoids and triterpenoids from the roots of *Rosa laevigata*. J Mex Chem Soc. 2014; 58(4): 374-377.

[36] He RR, Yao XS, Yao N, Wang M, Dai Y, Gao H, Yu Y, Kurihara H. Protective effects of radix *Rosa laevigata* against *Propionibacterium acnes* and lipopolysaccharide-induced liver injury. Biosci Biotechnol Biochem. 2009; 73(5): 1129-1136. [CrossRef]

[37] Liu DL, Zhu S, Ma R, Zhang JZ, Li HY, Chen H. Triterpenoids from the roots of *Rose odorata* var. *gigantean*. Chin J Nat Med. 2010; 8(1): 12-15. [CrossRef]

[38] An HJ, Kim IT, Park HJ, Kim HM, Choi JH, Lee KT. Tormentic acid, a triterpenoid saponin, isolated from *Rosa rugosa*, inhibited LPS-induced iNOS, COX-2, and TNF-α expression through inactivation of the nuclear factor-κb pathway in RAW 264.7 macrophages. Int. Immunopharmacol. 2011;11(4): 504-510. [CrossRef]

[39] Coruh N, Ozdogan N. Wild-growing *Rosa heckeliana* Tratt.: phenolic constituents with cytotoxic and antioxidative properties. Turk J Biol. 2017; 41: 195-212. [CrossRef]

[40] Park KH, Jeong MS, Park KJ, Choi YW, Seo SJ, Lee MW. Topical application of *Rosa multiflora* root extract improves atopic dermatitis-like skin lesions induced by mite antigen in NC/Nga mice. Biol Pharm Bull. 2014; 37(1): 178-183. [CrossRef]

[41] Kim IT, Ryu S, Shin JS, Choi JH, Park HJ, Lee KT. Euscaphic acid isolated from roots of *Rosa rugosa* inhibits LPS-induced inflammatory responses via TLR4-mediated NF-κB inactivation in RAW 264.7 macrophages. J Cell Biochem. 2012; 113(6): 1936-1946. [CrossRef]

[42] Porter EA., Van den Bos AA, Kite GC, Veitch NC, Simmonds MS. Flavonol glycosides acylated with 3-hydroxy-3-methylglutaric acid as systematic characters in *Rosa*. Phytochemistry. 2012; 81: 90-96. [CrossRef]

[43] Mavi A, Terzi Z, Özgen U, Yildirim A, Coşkun M. Antioxidant properties of some medicinal plants: *Prangos ferulacea* (Apiaceae), *Sedum sempervivoides* (Crassulaceae), M*alva neglecta* (Malvaceae), *Cruciata taurica* (Rubiaceae), *Rosa pimpinellifolia* (Rosaceae), *Galium verum* subsp. *verum* (Rubiaceae), *Urtica dioica* (Urticaceae). Biol Pharm Bull. 2004; 27(5): 702-705. [CrossRef]

[44] Seto R, Nakamura H, Nanjo F, Hara Y. Preparation of epimers of tea catechins by heat treatment. Biosci Biotechnol Biochem. 1997; 61 (9): 1434-1439. [CrossRef]

[45] Davis AL, Cai Y, Davies AP, Lewis J. 1H and 13C NMR assignments of some green tea polyphenols. Magn Reson Chem. 1996; 34 (11): 887-890. [CrossRef]

[46] Guang-yi L. The isolation and structure of-kaji-ichigoside f1 and rosamultin. Acta Bot Sin. 1988; 4: 409-413.

[47] Wang Q, Ju P, Wang Y-F, Luo S-D. Triterpenoids from *Saurauia napaulensis* (Saurauiaceae). Acta Bot Yunnan. 2008; 30 (1): 121-124.

[48] Liu DL, Zhu S, Ma R, Zhang JZ, Li HY, Chen H. Triterpenoids from the roots of *Rose odorata* var. *gigantean*. Chin J Nat Med. 2010; 8 (1): 12-15. [CrossRef]

[49] Seto T, Tanaka T, Tanaka O, Naruhashi N. β-Glucosyl esters of 19α-hydroxyursolic acid derivatives in leaves of *Rubus* species. Phytochemistry. 1984; 23 (12): 2829-2834. [CrossRef]

[50] De Leo M, De Tommasi N, Sanogo R, D'Angelo V, Germanò MP, Bisignano G, Braca A. Triterpenoid saponins from *Pteleopsis suberosa* stem bark. Phytochemistry. 2006. **67**(24): p. 2623-2629. [CrossRef]

[51] Ponou BK, Teponno RB, Ricciutelli M, Quassinti L, Bramucci M, Lupidi G, Barboni L, Tapondjou LA. Dimeric antioxidant and cytotoxic triterpenoid saponins from *Terminalia ivorensis* A. Chev. Phytochemistry. 2010. 71(17-18): p. 2108-2115. [CrossRef]

[52] Aliyazicioglu R, Demir S, Badem M, Sener SO, Korkmaz N, Demi EA, Ozgen U, Karaoglu SA, Aliyazicioglu Y. Antioxidant, Antigenotoxic, Antimicrobial Activities and Phytochemical Analysis of *Dianthus carmelitarum*. Rec Nat Prod. 2017; 11 (3).

[53] Shameh S, Alirezalu A, Hosseini B, Maleki R. Fruits phytochemical composition and color parameters of twenty-one accessions of five *Rosa* species grown in North West Iran. J Sci Food Agric. 2019; 99(13): 5740-5751. [CrossRef]

[54] Okan O, Serencam H, Baltas N, Can Z. Some edible forest fruits their in vitro antioxidant activities, phenolic compounds and some enzyme inhibition effects. Fresenius Environ. Bull. 2019; 28: 6090-6098.

[55] Cakir A. Essential oil and fatty acid composition of the fruits of *Hippophae rhamnoides* L. (Sea Buckthorn) and *Myrtus communis* L. from Turkey. Biochem Syst Ecol. 2004; 32 (9): 809-816. [CrossRef]

[56] Murathan ZT, Zarifikhosroshahi M, Kafkas NE. Determination of fatty acids and volatile compounds in fruits of rosehip (*Rosa* L.) species by HS-SPME/GC-MS and Im-SPME/GC-MS techniques. Turk J Agric For. 2016; 40 (2): 269-279. [CrossRef]

[57] Ercisli S, Orhan E, Esitken A. Fatty acid composition of *Rosa* species seeds in Turkey. Chem Nat Compd. 2007; 43 (5): 605-606.

[58] Renda G, Celik G, Korkmaz B, Karaoglu SA, Yayli N Antimicrobial Activity and Analyses of Six *Geranium* L. Species with Headspace SPME and Hydrodistillation. J Essent Oil Bear Pl. 2016; 19 (8): 2003-2016. [CrossRef]

[59] Adams RP. Identification of volatile oil components by gas chromatography/mass spectroscopy. Allured Publishing Corporation, Carol Stream, IL, USA 2004.

[60] Yaylı N, Tosun G, Yayh B, Gündoğan Z, Coşkunçelebi K, Karaoğlu Ş. Altitude Variation in the Composition of Essential Oils, Fatty Acid Methyl Esters, and Antimicrobial Activities of Two Subspecies of *Primula vulgaris* Grown in Turkey. Nat Prod Commun. 2016; 11 (10): 1505-1510.