Antimicrobial, Antioxidant, and Phytochemical Activities of *Rhus coriaria* L. and its Phenolic Compounds and Volatile Component Analyses

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Volatile oil analysis, phenolic constituents, antioxidant capacity, antimicrobial activity, vitamin C, and enzyme activities of the fruits of *Rhus coriaria* L. were studied. The chemical with the highest percentage was sesquiterpene hydrocarbons with 40.4%. The major compound was detected as caryophyllene (36.9%). The main phenolic constituents of fruit samples were gallic acid, syringic acid, protocatechuic acid, and 4-hydroxybenzoic acid. The highest phenolic constituent of fruits was gallic acid. Ferric (III) ion reducing antioxidant power (FRAP) capacity (14.9 mg $FeSO_4$ eq./g), free radical scavenging (ABTS) capacity (68.8 mg AA eq./g), ABTS % inhibition rate (98.0%), free radical scavenging (DPPH) (53.1 mg AA eq./g), and DPPH % inhibition (79.6%) amounts were determined in antioxidant capacities of the samples. The bioactive component contents of the samples were total antioxidant amounts (TAC) (32.8 mg GA/g), total flavonoid substance amounts (TFC) (73.8 mg QE eq./g), and total phenolic substance amounts (TPC) (41.4 mg GA eq./g). The results of the antimicrobial activity analysis of *R. coriaria* fruit samples showed antimicrobial activity against *Staphylococcus aureus* and *Listeria monocytogenes* microorganisms. The amount of vitamin C and enzyme inhibitor activity in the fruits of *R. coriaria* were determined as 35.5 mg/100 g and 0.07 mg/mL, respectively.

DOI: 10.15376/biores.18.4.6842-6861

Keywords: GC-MS/FID; HPLC-DAD; Chemical and phenolic composition; Antioxidant-antimicrobial-enzyme activities; Rhus coriaria L.

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INTRODUCTION

The bioactive constituents of nutrients offer to promote health and support for the human immune system (Najafi *et al.* 2016; Tahvilian *et al.* 2016; Zangeneh *et al.* 2016; Galanakis 2020; Zannou *et al.* 2022). Meanwhile, new challenges facing the world in nutrition, demographics, and health have led to the search for viable and sustainable ways to solve them. For example, bioactives-containing food ingredients are of big relevance as promising solutions for nutrition, health, and cosmetics industries (Gürbüz *et al.* 2019; Baltacı *et al.* 2022; Ji and Ji 2022; Zannou *et al.* 2022). A wide variety of biomolecules, such as phenolics, tocopherols, carotenoids, and sterols, are found in plants (Sarikurkcu and Tlili 2022). Since early times, plants have generally played a crucial role in disease treatment and health care (Fereidoonfar *et al.* 2019). Different plant species have been utilized for the prevention and cure of many diseases, ranging from simple headaches to

main illnesses like cognitive and cancer ailments. This could be attributed to the large biodiversity in plants' bioactive secondary metabolites (Elagbar *et al.* 2020). From this perspective, *R. coriaria* has great potential as a public medicine.

Fig. 1. *R. coriaria* leaves and fruits (Photo: Mehmet Öz, 19.09.2021)

Rhus coriaria is a wild-growing herb of the Anacardiaceae family (Langroodi *et al.* 2019). It is widely known as sumac (Alsamri *et al.* 2021). While the genus *Rhus* contains rougly 250 species (Gök *et al.* 2020) worldwide, there is only *R. coriaria* in Türkiye (Davis 1965). It is distributed over Southern Europe, the Middle East, North Africa, Iran, and Afghanistan (Brunke *et al.* 1993; Kosar *et al.* 2007). The appearance of the fruits and leaves of *R. coriaria* is shown in Fig. 1.

Rhus coriaria has been accepted in herbal medicine as an antiseptic, food flavoring agent, natural antioxidant, and an antimicrobial constituent (Nasar-Abbas and Halkman 2004; Kosar *et al.* 2007; Gharaei *et al.* 2013; Langroodi *et al.* 2019). The many curative impacts of *R. coriaria* could be attributed to its numerous biological properties, for example antioxidant, antibacterial, anti-inflammatory, antipyretic, hypoglycemic, DNA protective, anti-ischemic, hepatoprotective, vasorelaxant, hypolipidemic activities (Beretta *et al.* 2009; Chakraborty *et al.* 2009; Mohammadi *et al.* 2010; Pourahmad *et al.* 2010; Peter 2012; Abu-Reidah *et al.* 2014; Foroughi *et al.* 2016; Zhaleh *et al.* 2018; Sakhr and El Khatib 2020). Consumption of sumac fruits is increasing worldwide and is of great economic importance as a natural source of bioactive compounds (Kizil and Turk 2010; Shabbir 2012; Morshedloo *et al.* 2018).

In folk medicine, *R. coriaria* is recommended to treat the liver, diarrhea, wound healing, for respiratory system illnesses like catarrh and the common cold, ulcers, diabetes, diuresis, stroke, hypertension, indigestion, anorexia, hemorrhagia, kidney stones, gout, hematemesis, dysentery, urinary system issues, dentistry, rash, edema, bruise, atherosclerosis, smallpox, stomach ache, ophthalmia, hyperglycemias, measles, aconuresis, timulate perspiration, headaches, reduce cholesterol, pox incidence in the eye, eye trachoma, uric acid level, and blood sugar (Tabata *et al.* 1994; Honda *et al.* 1996; Mohammadi *et al.* 2010; Polat *et al*. 2013; Tuttolomondo *et al.* 2014; Abu-Reidah *et al.* 2015; Paksoy *et al.* 2016; Giovanelli *et al.* 2017; Farag *et al.* 2018; Mahdavi *et al.* 2018; Morshedloo *et al.* 2018; Fereidoonfar *et al.* 2019; Elagbar *et al.* 2020; Gök *et al.* 2020; Alsamri *et al.* 2021). It has been used as a conventional medicine for the cure of several diseases including cancer (Farag *et al.* 2018; Elagbar *et al.* 2020; Sakhr and El Khatib 2020).

Presently, over 200 phytochemicals have been extracted from *R. coriaria*, and these contain flavonoids, isoflavonoids, terpenoids, phenolic acids, phenolic constituents conjugated with malic acid derivatives, organic acids, anthocyanins, hydrolysable tannins, and other constituents, for instance coumarin, iridoid, and butein derivatives (Tohma *et al.* 2019; Alsamri *et al.* 2021). Former works demonstrated that sumac included essential oil, tannins, anthocyanin, phenolic acids, flavonoids, nitrite, and nitrate contents (Mavlyanov *et al.* 1997; Özcan and Akbulut 2007; Zannou *et al.* 2022). Volatile oils can be extracted from several parts as fruits, leaves, flowers, stems, and roots. During the last years, there has been a rising interest in pharmacological studies on volatile oils, and it appears that the volatile oils have been useful for control and inhibition of human and animal bacterial infections (Zhaleh *et al.* 2018; Radonić *et al.* 2020). *R. coriaria* is rich in *β*-caryophyllene and cembrene with regard to volatile oil constituents, which are potent antibacterial agents (Dahham *et al.* 2015; Zhaleh *et al.* 2018).

As far as the authors' knowledge, in comparison to many other pharmaceutical-industrial plants, there is particularly minimal data about the vitamin C, enzyme inhibition, phenol constituents, and antimicrobial properties of *R. coriaria* volatile oil collected in Gümüşhane province, northeast of Türkiye. Hence, the goal of the current research is to ensure a comprehensive overview of the pharmacological and phytochemical on *R. coriaria* fruits.

EXPERIMENTAL

Plant Material

In this research, *R. coriaria* fruit samples were gathered in Torul-Köstere Village (40°36'20"N, 39°19'17"E, Altitude: 1040 m) located within the borders of Gümüşhane Province, Türkiye. The leaves and fruits of the plant samples are shown in Fig. 1. The fruit (500 g) samples from *R. coriaria* were gathered. The taxonomic diagnosis of plant sample was identified by Assoc. Prof. Mutlu GÜLTEPE (Department of Forestry, Dereli Vocational School, Giresun University, Giresun, Türkiye). The plant sample was listed in the Herbarium of Department of Biology (located in Karadeniz Technical University, Faculty of Science), with the identification number of KTUB Gültepe 719.

Extraction and GC-MS/FID Analysis

The volatile oil obtained by hydrodistillation method (at 100 °C) in the modified Clevenger system, which is cooled inside and outside, was dissolved in hexane, passed through a 0.45-micron filter, and placed in amber colored vials and placed in the autosampler. Components were determined by gas chromatography-flame ionization detection (GC-MS/FID; MS Agilent 5975C, GC-FID Agilent-7890A model, Agilent Technologies Inc, Santa Clara, CA, USA). After the volatile constituents were separated on the gas chromatography column, the mass spectra of each of them were taken individually in the mass spectrophotometer and their structures were elucidated by comparing the mass spectra of each component with the reference constituents of the Willey and NIST libraries. To confirm the detected constituents, the Kovats indices of the constituents were compared with the literature data. The measurement of the volatile oil was made with the GC-FID instrument. For GC, the split ratio was adjusted as 1:5 by injecting 1 µL of volatile oil in hexane into the same column. The GC-MS/FID analyses were performed on Agilent-7890 model device and an HP-5 model apolar capillary column

(30 m x 0.32 mm, film thickness 0.25 µm) was used for analysis. The injector, ion source and quadrupole rod temperatures were 250, 230, and 150 °C, respectively. Injections were applied in split (25:1) mode using helium (>99.999%), as the carrier gas with a flow rate of 1 mL/min. Then, 1 µL of essential oil solution in hexane (GC class) was injected and initially the GC oven temperature program kept at 60 °C for 2 min, increased to 240 °C with a rise of 3 °C/min, and spectra were obtained. Mass spectra were acquired at a scan speed of 2 spectra per second after a solvent delay of 3.8 min, and the mass scan range was set at *m*/*z* 45 to 450. The FID detector temperature was maintained at 250 °C with a hydrogen flow of 35 mL/min and air flow of 350 mL/min.

Extraction with Methanol

The extraction process was performed using an ultrasonic bath (3 L 320 W Bandelin Ultrasonic Bath). After the fruit parts were ground, 10 g were taken, 50 mL of 80% aq. MeOH was added, and then ultrasound-assisted extraction process was applied at 60 min and 40 °C. At the end of 60 min, it was filtered 2 times through Whatman 1 filter and centrifuged at 4000 rpm for 10 min and plant extracts were obtained. At the end of centrifugation, the upper part was taken into a beaker and the extracts were obtained by completely evaporating the methanol at 40 °C (Dranca and Oroian 2016).

Determination of Phenolic Constituents

All specimens were ultrasonically bathed for 20 min and filtered through a syringe filter (0.45 µm) before analysis. Chromatographic analysis of methanol extracts of fruit samples was performed using an Agilent 1260 Infinity high performance liquid chromatography-diode array detector HPLC-DAD system (Agilent Technologies, Waldbronn, Germany) device. Due to its speed, simplicity and convenience, HPLC-DAD is the most widely used among various chromatographic techniques (Irakli *et al.* 2012). Pyrzynska and Biesaga (2009) stated that routine detection in HPLC and CE typically relies on measuring UV absorption, usually using diode array detection (DAD), and that the DAD detector can simultaneously detect chromatograms of different wavelengths. For the analysis, the following were used as standards: gallic acid, sesamol, paracoumaric acid, benzoic acid, protocatechuic acid, catechin, syringic acid, vanillin, syringaldehyde, rutin, protocatechuic aldehyde, vanillic acid, rutin, 4-hydroxybenzoic acid, ferulic acid, coumarin, epicatechin, rosmarinic acid, *t*-cinnamic acid, quercetin, kaempferol, caffeic acid, and chyricin. The analysis method of the phenolic compounds of the samples was studied by modifying the gradient flow of the mobile phase with some changes (Paje *et al.* 2022). Chromatographic isolation of individual constituents was performed using a Hypersil HPLC Column (250 x 4.6 mm^2, 5 µm). Mobile phase solvent A was used as mixture 0.5% acetic acid in water (0.5: 95.5, v/v) and acetonitrile (solvent B). The gradient elution was started with 95% of solvent A and reduced to 75% after 20 min. Solvent A was reduced to 50% at 45 min and to 10% at 55 min. It was then increased to 65% at 65 min and continued for up to 70 min. The flow ratio was 1.0 mL/min and the injection capacity was 10 µL. The wavelength used in the DAD detector were 240, 250, 254, 280 and 324 nm.

Determination of Antioxidant Activity

The antioxidant activities of the attained methanol extract of *R. coriaria* fruits were found according to ferric (III) ion reducing antioxidant power (FRAP) capacity, free radical scavenging (ABTS and DPPH) activities. In addition, some bioactive component amounts

were detected by total antioxidant amounts (TAC), total flavonoid substance amounts (TFC), and total phenolic substance amounts (TPC) studies. The FRAP analysis of methanol extracts was determined using the Ahmed *et al.* (2015) method using FRAP solution. A total of 500 µL of distilled water was utilized as blank. A total of 250 µL of the standards were taken and the same procedures were performed. The FRAP amounts in samples using the correct equation of the calibration graph obtained with the $FeSO_4$ solution, the total iron reducing capacity was determined as mg $FeSO_4$ equivalent/g (Ahmed *et al.* 2015). The ABTS activity analysis (Ahmed *et al.* 2015) was made using ABTS solution according to the method. A total of 150 µL of methanol was utilized as blank. Then, 150 µL of standards (ascorbic acid) were taken and the same procedures were performed. The acquired solution was then read at a spectrophotometer absorbance at 734 nm. The ABTS cation removal activity amounts in the samples were calculated following Ahmed *et al.* (2015), Eq. 1. Results are given as mg AA eq./g, mg Trolox eq./g, and % free radical removal.

Inhibition(%)=(Control Absorbance–Example Absorbance/Control Absorbance)x100 (1)

The DPPH activity of the methanol extracts obtained from the fruit was determined using 2,2-diphenyl-1-picrylhydrazil according to the Sanchez-Moreno method (Sağdıç *et al.* 2011). The method was applied by mixing the methanol extract and DPPH solutions with specific concentrations by vortexing and keeping them at room temperature and in the dark for 30 min. At the end of the period, the absorbance of the specimens at 517 nm was read, and the amount of DPPH remaining in the reaction medium was calculated according to Eq. 2. Results are given as mg AA eq./g, mg Trolox eq./g, and % free radical removal.

Inhibition(%)=(Control Absorbance-Example Absorbance/Control Absorbance)x100 (2)

The analysis of TAC content in methanol extract of fruit was performed using molybdate reagent according to the Kasangana method. A total of 250 µL of pure water was utilized instead of the sample as a blank. The absorbance of the resulting reaction mixtures was measured in a 695 nm spectrophotometer. A total of 500 µL of the standards were taken and the same procedures were performed. The amount of TAC in methanol extract samples was given as mg GA eq./g using the correct equation of the calibration graph obtained with the solution of ascorbic acid (Kasangana *et al.* 2015). The TFC content in methanol extracts of fruit was detected following the Kasangana method. The absorbance of the resulting mixture was read in a spectrophotometer at 506 nm. A total of 500 µL of pure water was utilized as blank. Then, 500 µL of the standards were taken and the same procedures were performed. The amount of TFC in the samples was determined as mg QE eq/g using the correct equation of the calibration graph obtained with Catechin or Quercetin (ethanol was dissolved) solution (Kasangana *et al.* 2015). Analysis of the TPC amount, one of the bioactive components of methanol extracts, was carried out according to the Kasangana method using Folin-Ciocalteu reagent (Kasangana *et al.* 2015). After the prepared mixture was whirlpooled, it was incubated in the dark at room temperature for 120 min. At the end of the incubation period, the absorbance of the mixture at 760 nm was read. The amount of 3.7 mL water, 500 µL methanol + 100 µL Folin-Ciocalteu reagent + 600 µL 10% Na_2CO_3 mixture was used as a blank. The amounts of phenolic substances in the samples were expressed as mg GA eq/g using the correct equation of the calibration graph obtained with the gallic acid solution.

Determination of Antimicrobial Activity

Microorganisms utilized in the research were attained from the laboratories of Gümüşhane University, Department of Food Engineering. The antimicrobial analyses of the methanol extracts were detected by disk-diffusion method against 13 microorganisms, including 10 bacteria and 3 yeast-molds (Matuschek *et al.* 2014). Antimicrobial activity was realized in two phases: preparation of bacteria and yeasts and preparation of examples. Bacteria were used in Nutrient Broth medium after 24 h of first activation at 36 °C and after 18 h of second activation at 36 °C. A total of 1% of the microorganisms to be used in the study were added to the prepared sterile solid media and they were poured into petri dishes and allowed to solidify. Then, 5 mm diameter wells were opened on the solidified media. The incubation process was conducted by adding the solutions of the methanol extract prepared with hexane to the opened wells. Petri dishes including bacteria were incubated for 24 h at 36 °C, and petri dishes including yeast and mold were incubated for 48 h at 27 °C. After the determined period, the outcomes were found by measuring the transparent areas around the discs.

Determination of Enzyme Inhibitory Activities

The α-glucosidase inhibitory activity of the samples was studied by modifying it (Yu *et al.* 2012). In the study, first, 650 µL of phosphate buffer (pH: 6.8 and 0.1 M) was added to the test tubes. Then, 20 µL of sample and 30 µL of α-glucosidase enzyme (*Saccharomyces cerevisiae*, lyophilized powder ≥ 10 units/mg protein) prepared in phosphate buffer were added. After the mixture was incubated at 37 °C for 10 min, 75 µL of substrate (4-nitrophenyl-α-D-glucopyranoside) was added. The mixture was kept at 37 °C for 20 min; then 650 µL of 1 M Na_2CO_3 was added to all tubes and the reaction was stopped. Absorbance ratios were measured at 405 nm in an ultraviolet/visible (UV/VIS) spectrophotometer (UV 1800, Shimadzu, Kyoto, Japan). Different concentrations of acarbose (positive control) were studied as the standard inhibitor. The study was performed in three parallel and reagent-sample blanks. The IC_{50} values of acarbose and samples (sample concentration that halves the enzyme activity present in the environment) were calculated.

Determination of the Analysis of Vitamin C

Vitamin C analyses of the specimens were made using the HPLC-UV device with UV 1000 detector according to HPLC-UV detector method (Thermo Finnigan, San Jose, CA, USA). Analytical column RP C18 (250 x 4,6 mm, 5 µm), mobile phase: methanol: water (5:95, v/v) pH= 3 (H_3PO_4), flow 1 mL/min, injection volume 20 µL, with detection by UV at 254 nm. For the calibration curve, standard solutions of 10, 30, 60, 90, and 120 mg/L concentrations were prepared from L-ascorbic acid. Then, 10 g of *R. coriaria* fruits were taken and divided into pieces in a shredder. A total of 70 mL, a sufficient amount of metaphosphoric acid (15% m/m), was added to the smashed fruits and mixed in the homogenizer. The homogenized samples were completed to 100 mL and filtered through filter paper. After the filtrates were passed through a 0.45-micron filter, they were taken into vials and given to the HPLC device. The amount of analyzed vitamin C in the sample was calculated using the calibration graph method (y = 9498.7 x - 4236) (Öz *et al.* 2018).

RESULTS AND DISCUSSION

The GC-MS/FID analysis and chromatogram results of volatile oils attained from *R. coriaria* fruits are shown in Table 1 and Fig. 2. As a result of the analysis of essential oils by GC-MS/FID processes, the structure of a total of 74 constituents in *R. coriaria* fruits was found, but the structure of 4 constituents could not be defined. Caryophyllene (36.9%), thunbergene (12.95%), and (*E*,*E*)-2,4-decadienal (5.99%) were observed to be the highest constituents in volatile oils isolated from fruits. It is seen that the most common major compound in fruit samples is caryophyllene.

The GC-MS/FID analyses of the volatile oils determined 57 compounds in total. (*E*)-caryophyllene (50.3%), n-nonanal (23.3%), cembrene (21.7%), *α*-pinene (19.7%), and (*2E,4E*)-decadienal (16.5%) were determined as the major compounds of the volatile oils (Morshedloo *et al.* 2018). The results demonstrated that *β*-caryophyllene (34.3%) was the most frequently found constituent in *R. coriaria* (Zhaleh *et al.* 2018). *β*-caryophyllene (30.7%) was the main compound of Iranian sumac volatile oils (Gharaei *et al.* 2013). (*E*)-Caryophyllene, one of the main components of the species examined in this study, has been described as the main component of sumac essential oil in previous studies in Southeastern region of Türkiye (Bahar and Altug 2009), in Türkiye (Brunke *et al.* 1993), in Italy (Giovanelli *et al.* 2017), and in northern Iran (Gharaei *et al.* 2013). Alike, *α*-pinene is often explained as the major component of sumac volatile oils (Brunke *et al.* 1993). Fidyt *et al.* (2016) reported that *β*-caryophyllene and *β*-caryophyllene oxide have the ability to increase the efficacy of classical anticancer drugs such as paclitaxel or doxorubicin, as well as their direct anti-cancer activities. Sain *et al.* (2014) reported that beta caryophyllene and caryophyllene oxide, which they isolated, can act as potent anti-inflammatory agents. These compounds, which were identified as the main components within the scope of the present study, can potentially be used for the stated benefits.

The main components obtained in this study were similar to the main components found in previous studies. However, it was determined that there were differences in the percentages of these components. Different amounts and main components of *R. coriaria* were formerly shown for the volatile oil compound and dissimilar chemical profiles have been reported from dissimilar geographical and environmental conditions of the World (Brunke *et al.* 1993; Akbulut *et al.* 2009; Bahar and Altug 2009; Peter 2012; Giovanelli *et al.* 2017; Morshedloo *et al*. 2018).

Table 1. The Volatile Oil Constituents of Fruits in *R. coriaria*

No.	RT (min)	Area %	Constituents	Compound Classification	RI[a]	RI[b]
1	5.00	0.37	Heptane	Hydrocarbon	699	700
2	5.26	0.02	3-Methyl-3-buten-1-ol	Alcohol	711	716
3	5.48	0.43	Methyl cyclohexane	Hydrocarbon	720	720
4	7.13	0.07	1-Octene	Hydrocarbon	789	789
5	7.41	5.38	Octane	Hydrocarbon	800	800
6	7.61	0.13	(*Z*)-2-Octene	Hydrocarbon	806	806
7	7.87	0.10	(*E*)-3-Octene	Hydrocarbon	812	814
8	8.24	0.05	1,3-Octadiene	Hydrocarbon	822	826
9	9.27	0.06	(*E*)-2-Hexenal	Aldehyde	850	850
10	9.90	0.14	1-Hexanol	Alcohol	867	867
11	10.10	0.17	(*Z*)-4-Heptenal	Aldehyde	896	895

12	11.19	0.18	Heptanal	Aldehyde	901	901
13	12.63	0.16	*α*-Pinene	Monoterpene	932	932
14	13.19	0.16	Camphene	Monoterpene	945	945
15	13.70	4.46	(*E*)-2-Heptenal	Aldehyde	955	955
16	13.90	0.04	Benzaldehyde	Aldehyde	960	960
17	14.26	0.02	(*E*)-2-Hepten-1-ol	Alcohol	968	970
18	14.69	0.23	1-Octen-3-one	Ketone	977	977
19	14.75	0.35	1-Octen-3-ol	Alcohol	979	979
20	15.11	0.31	6-Methyl-5-Hepten-2-one	Ketone	986	986
21	15.31	0.39	2-Pentyl-furan	Other	990	990
22	15.59	0.11	Unidentified		996	
23	15.68	0.09	Decane	Hydrocarbon	999	1000
24	15.86	0.30	Octanal	Aldehyde	1002	1002
25	16.25	0.23	(*E,E*)-2,4-Heptadienal	Aldehyde	1010	1010
26	16.91	0.06	*p*-Cymene	Monoterpene	1024	1024
27	17.11	0.13	Limonene	Monoterpene	1028	1028
28	17.25	0.11	3-Ethyl-2-methyl-1,3-hexadiene	Hydrocarbon	1030	1030
29	17.56	1.19	*cis*-β-Ocimene	Monoterpene	1037	1037
30	17.86	0.09	Benzeneacetaldehyde	Aldehyde	1043	1043
31	18.08	1.98	*trans*-β-Ocimene	Monoterpene	1048	1048
32	18.55	0.97	(*E*)-2-Octenal	Aldehyde	1057	1057
33	19.05	0.15	(*E*)-2-Octen-1-ol	Alcohol	1067	1067
34	19.17	0.20	1-Octanol	Alcohol	1070	1070
35	19.28	0.11	*cis*-Linalool oxide	Monoterpenoid	1072	1072
36	20.04	0.14	*α*-Terpinolen	Monoterpene	1088	1088
37	20.31	0.14	3,5-Octadien-2-one	Ketone	1093	1093
38	20.59	0.07	Linalool	Monoterpenoid	1099	1099
39	20.83	3.08	Nonanal	Aldehyde	1104	1104
40	22.01	0.43	Allo-Ocimene	Monoterpene	1128	1128
41	23.19	0.03	(*E,E*)-2,6-Nonadienal	Aldehyde	1153	1153
42	23.49	0.59	(*E*)-2-Nonenal	Aldehyde	1159	1159
43	23.59	0.09	(*trans*-3-Pinanone) (Pinocamphone)	Monoterpenoid	1161	1161
44	25.13	0.25	*α*-Terpineol	Monoterpenoid	1193	1193
45	25.29	0.32	*cis*-4-Decenal	Aldehyde	1196	1199
46	25.68	0.20	Decanal	Aldehyde	1205	1205
47	26.08	0.54	(*E,E*)-2,4-Nonadienal	Aldehyde	1214	1214
48	27.24	0.08	Pulegone	Monoterpenoid	1239	1239
49	28.03	0.10	Unidentified		1256	
50	28.38	5.58	(*E*)-2-Decenal	Aldehyde	1263	1263
51	29.24	0.16	Vitispirane	Monoterpenoid	1282	1281
52	30.23	0.22	Thymol	Monoterpenoid	1304	1304
53	30.37	0.21	Undecanal	Aldehyde	1307	1307
54	30.87	**5.99**	**(*E,E*)-2,4-Decadienal**	Aldehyde	1319	1319
55	32.88	0.45	3-Dodecenal	Aldehyde	1364	1365
56	34.91	0.21	Isocaryophyllene	Sesquiterpene	1411	1411
57	35.63	**36.9**	**Caryophyllene**	Sesquiterpene	1428	1428
58	35.82	0.17	Unidentified		1433	
59	36.69	0.91	*trans*-Geranylacetone	Monoterpenoid	1453	1453
60	36.91	2.87	Humulene	Sesquiterpene	1459	1459

61	38.24	0.03	*α*-Selinene	Sesquiterpene	1491	1491
62	39.39	0.28	*δ*-Cadinene	Sesquiterpene	1520	1520
63	39.73	0.15	*β*-Bisabolene	Sesquiterpene	1528	1528
64	40.91	0.85	Caryophyllenol	Sesquiterpenoid	1558	1568
65	42.17	2.24	Caryophyllene oxide	Sesquiterpenoid	1590	1590
66	43.16	0.19	Tetradecanal	Aldehyde	1616	1616
67	43.93	0.11	Epicubenol	Sesquiterpenoid	1637	1637
68	44.04	0.26	*γ*-Eudesmol	Sesquiterpenoid	1639	1639
69	44.19	0.84	α-Caryophylladienol	Sesquiterpenoid	1644	1644
70	44.97	1.32	*α*-Eudesmol	Sesquiterpenoid	1664	1664
71	45.31	0.11	Unidentified		1673	
72	45.46	0.71	(*E*)-14-hydroxy-9-epi-caryophyllene	Sesquiterpenoid	1677	1674
73	51.41	0.13	Hexahydrofarnesyl acetone	Sesquiterpenoid	1845	1845
74	53.33	0.03	Nonadecane	Hydrocarbon	1902	1900
75	53.94	0.15	Farnesyl acetone	Sesquiterpenoid	1921	1921
76	54.64	**12.95**	**Thunbergene**	Diterpene	1942	1941
77	54.99	0.27	3E-Cembrene A	Diterpene	1953	1951
78	55.51	0.73	Neocembrene A	Diterpene	1969	1960

RT: Retention time, RI[a]: Retention indices computed against, RI[b]: Literature retention indices supported on NIST, WILLEY, and Adams 2007.

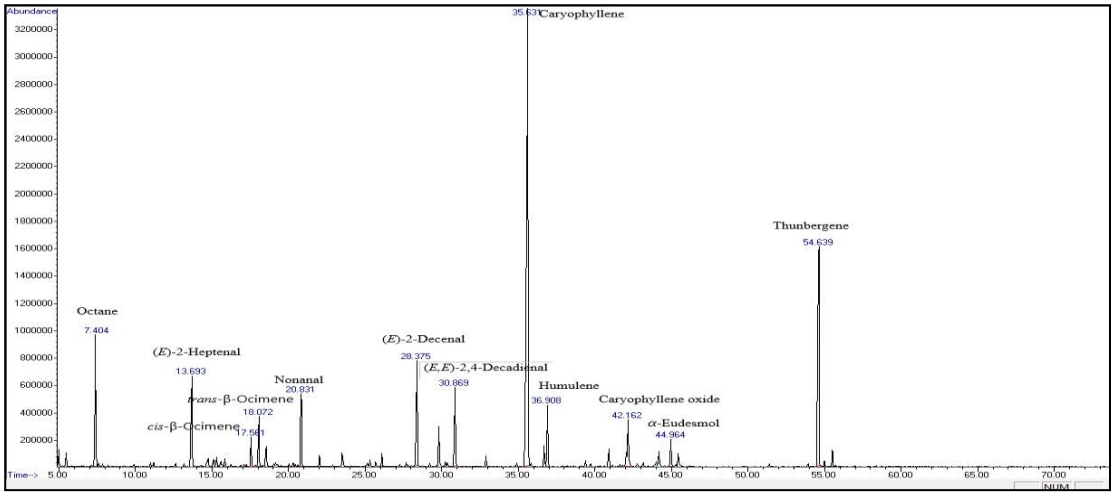

Fig. 2. GC-MS/FID chromatograms of the volatile oils from fruit of *R. coriaria*

In Table 2, 74 constituents, whose structures were clarified regarding the outcomes of the analysis on the fruits of the *R. coriaria* plant, were classified as 10 groups. These groups and numbers of constituent were determined as alcohols 6, aldehydes 20, ketones 3, hydrocarbons 10, monoterpenes 8, monoterpenoids 8, sesquiterpenes 6, sesquiterpenoids 9, diterpenes 3, and others 1. As a result of the analysis of the fruits of the *R. coriaria*, the highest common chemical classes were determined as sesquiterpenes with 40.42% and aldehydes with 23.68%.

Among the monoterpenoids, *α*-pinene (19.7%) was the most plentiful constituent in the studied species. In contrast, sesquiterpenoids consisted primarily of caryophyllene oxide, α-humulene, and (*E*)-caryophyllene. Diterpenes and aliphatic constituents, including fatty acids and aldehydes, were the other major chemical classes of volatile oil constituents (Morshedloo *et al.* 2018).

Table 2. Chemical Classification of Constituents Determined in Fruits Volatile Oil of *R. coriaria*

Compound Classification	Compound Number	Ratio (%)	Main Compound
Alcohols	6	0.88	1-Octen-3-ol
Aldehydes	20	23.68	(*E*,*E*)-2,4-Decadienal
Ketones	3	0.68	6-Methyl-5-Hepten-2-one
Hydrocarbons	10	6.76	Octane
Monoterpene hydrocarbons	8	4.25	*trans*-*β*-Ocimene
Oxygenated monoterpenes	8	1.89	*trans*-Geranylacetone
Sesquiterpene hydrocarbons	6	40.42	Caryophyllene
Oxygenated sesquiterpenes	9	6.61	Caryophyllene oxide
Diterpene hydrocarbons	3	13.95	Thunbergene
Others	1	0.39	2-Pentyl-furan
Unidentified constituents	4	0.49	
Total	**78**	**100**	

The analysis and chromatogram results of phenolic constituents of *R. coriaria* fruits by HPLC-DAD methods are shown in Table 3 and Fig. 3.

Table 3. Phenolic Constituents in Fruits of *R. coriaria*

Number	Constituents	Fruits (mg/kg)
1	Protocatechuic Acid	327.23
2	4-Hydroxybenzoic Acid	362.72
3	Syringic Acid	465.71
4	Gallic Acid	3708.60
5	Catechin	< 0.1 mg/kg*
6	Sesamol	< 0.1 mg/kg
7	Paracoumaric Acid	< 0.1 mg/kg
8	Benzoic Acid	< 0.1 mg/kg
9	Caffeic Acid	< 0.1 mg/kg
10	Rutin	< 0.1 mg/kg
11	Vanillin	< 0.1 mg/kg
12	Protocatechuic Aldehyde	< 0.1 mg/kg
13	Syringaldehyde	< 0.1 mg/kg
14	Vanillic Acid	< 0.1 mg/kg
15	Ferulic Acid	< 0.1 mg/kg
16	Coumarin	< 0.1 mg/kg
17	Epicatechin	< 0.1 mg/kg
18	Rosmarinic Acid	< 0.1 mg/kg
19	t-cinnamic Acid	< 0.1 mg/kg
20	Quercetin	< 0.1 mg/kg
21	Kaempferol	< 0.1 mg/kg
22	Chyrisin	< 0.1 mg/kg

*0.1 mg/kg: LoQ (limit of quantitation) value

The main phenolic constituents of fruit samples were gallic acid (3708.60 mg/kg), syringic acid (465.71 mg/kg), protocatechuic acid (327.23 mg/kg), and 4-hydroxybenzoic acid (362.72 mg/kg). The highest phenolic constituent of fruits is gallic acid. Naturally

occurring gallic acid is highly antioxidant and may play a protective role in healthy individuals by inhibiting apoptosis (Zahrani *et al.* 2007).

Phenolic constituents are among the rich sources of natural antioxidants (Akbulut *et al.* 2009). Gallic acid was determined as the major phenolic compound in both the authors' study and previous study (Kosar *et al.* 2007). The authors' results confirmed the data explained in the former works that noticed the same findings (Kosar *et al.* 2007; Fereidoonfar *et al.* 2019).

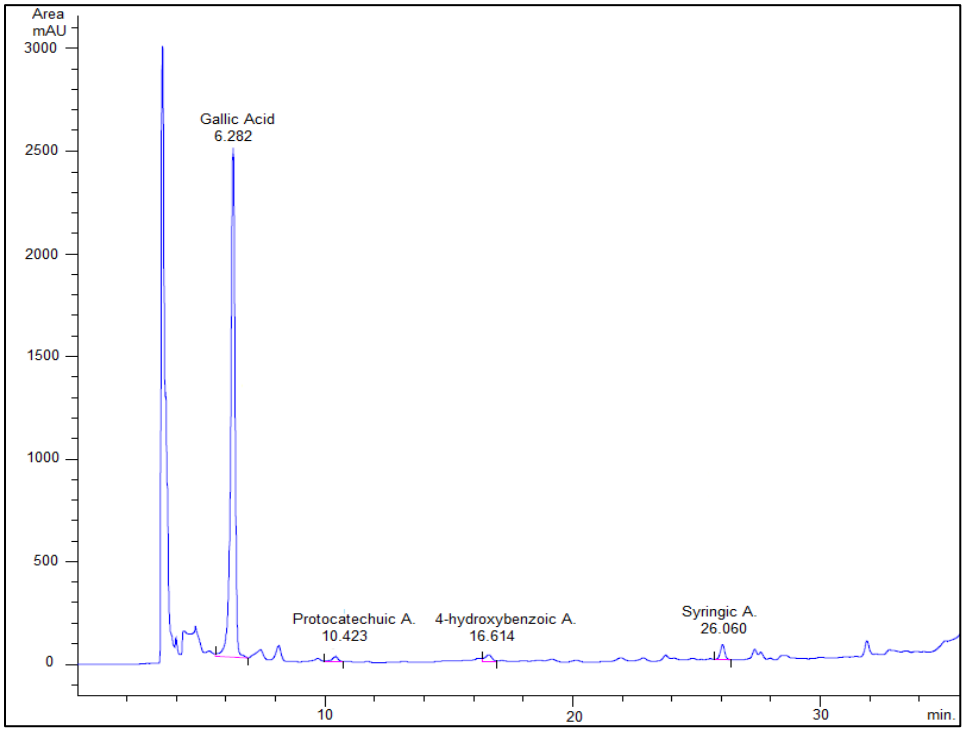

Fig. 3. Chromatogram of phenolic compounds from *R. coriaria*

Antioxidant activity analysis results of methanol extracts obtained from *R. coriaria* fruits are presented in Table 4. In the current work, the content of FRAP in methanol extract of fruit was determined as 14.9 mg $FeSO_4$ eq./g. The ABTS amounts of the samples were determined as 68.8 mg AA eq./g and 100.4 mg Trolox eq./g in fruit methanol extracts. The ABTS % inhibition rate was 98.0 in methanol extract of fruit. While the amount of DPPH was determined by 53.1 mg AA eq./g and 64.1 mg Trolox eq./g in methanol extract of *R. coriaria* fruit, the % inhibition rate of DPPH in the same samples was 79.6% (Table 4). It is seen that the FRAP, ABTS, and DPPH values in methanol extract of fruit samples studied have similar results with the literature.

In this study, the amount TAC, which is one of the bioactive component contents of the samples, was observed as 32.8 mg GA eq./g in methanol extract of fruit. The TFC content in methanol extract of fruit was 73.8 QE eq./g. The TPC content in methanol extract of fruit was determined as 41.4 mg GA eq./g.

The rates of TPC were determined in ranges of 44.5 to 125.0 mg GA eq./g and 36.3 to 114.5 mg GA eq./g for UA eq./g and HA eq./g, respectively. The TFC ranged from 4.95 to 13.9 mg EC eq./g for UA eq. and from 4.08 to 17.6 mg EC eq./g for HA eq./g (Zannou *et al.* 2022). In addition, the average TPC in sumac was 498 mg GA eq./g DW (Unver *et al.* 2009). Further, the average TPC in sumac fruits was determined as 152 mg GA eq./g

DW (Raodah *et al.* 2014). While phenolic amount varied, ascorbic acid ranged from 10.0 to 45.0 mg per g, from 77.5 to 389.3 mg GA eq./g (Fereidoonfar *et al.* 2019). Anthocyanin fraction contained pelargonidin, petunidin, peonidin, cyanidin, and delphinidin glucosides and coumarates, while gallic acid was the major phenolic acid in the extracts. Phenolic quantity ranged from 77.5 to 389.3 mg GA eq./g DW. The determined phenol value of sumac was 172 mg GA eq./g DW (Kosar *et al.* 2007).

If the antioxidant activity results obtained from the authors' plant samples are evaluated, it is seen that they are compatible with the literature. This situation shows parallelism with antimicrobial activities. There are some studies on the antioxidant activity of sumac. Both fruits and leaves have been reported for their antioxidant activities. It was determined that the tannin fractions of these samples had a powerful antioxidant capacity (Zalacain *et al.* 2000, 2002; Kosar *et al.* 2007). Phytochemicals and especially phenolic compounds are expressed as secondary metabolites and are known to have strong antioxidant effects. In recent work, it is stated that the consumption of plant materials with antioxidant activity may reduce the risk of various illnesses (Cory *et al.* 2018). *R. coriaria* may be useful in the correction or cure of various pathological disorders, for instance overweight and obesity (Jamous *et al.* 2018; Alsamri *et al.* 2021), myopathies (Najjar *et al.* 2017), and skin injuries (Nozza *et al.* 2020).

Table 4. Antioxidant Activity Contents and Bioactive Compounds of Methanol Extracts Attained from Fruits of *R. coriaria*

Antioxidant Activity Amounts			
DPPH	mg AA eq./g 53.08* ± 1.52**	mg Trolox eq./g 64.14 ± 1.81	% Inhibition 79.63 ± 2.25
ABTS	mg AA eq./g 68.84 ± 0.71	mg Trolox eq./g 100.39 ± 0.99	% Inhibition 97.98 ± 0.96
FRAP	mg $FeSO_4$ eq./g 14.92 ± 0.40		
Bioactive Components			
TPC	mg GA eq./g 41.39 ± 3.99		
TFC	mg QE eq./g 73.77 ± 8.99		
TAC	mg GA eq./g 32.79 ± 0.89		

*: Means (The average of three parallel studies), ** ±: Standard deviation

At the end of the study, the results of methanol extract examples showing antimicrobial activity are given in Table 5. It was determined that the fruit of *R. coriaria* formed zones of 5.10 mm and 8.02 mm in diameter against *Listeria monocytogenes* and *Staphylococcus aureus*, respectively. Thus, it was shown that the methanol extracts of *R. coriaria* fruit showed antimicrobial activity. In the meantime, it was determined that the fruit of *R. coriaria* has antimicrobial effects against *Listeria monocytogenes* and *Staphylococcus aureus*.

Sumac ethanolic extract demonstrated a strong antimicrobial effect against the investigated bacteria. *Salmonella enteric, Staphylococcus aureus, Escherichia coli,* and *Bacillus cereus* isolates were the most to the least sensitive bacteria shown toward the ethanolic extract, respectively. *E. coli* showed the most resistance toward ethanolic extract among the examined standard strains (Mahdavi *et al.* 2018). There was a similarity in both

the authors' study and a previous study (Mahdavi *et al.* 2018). In both studies, it was determined that they showed antimicrobial effect against *Staphylococcus aureus* bacteria.

Table 5. Antimicrobial Activity of Crude Extract of *R. coriaria* Fruits

Bacteria Species	5000 ppm	1000 ppm	500 ppm	250 ppm	Penicillin G** (10 mg)
Enterococcus faecalis ATCC 29212	-	-	-	-	32 ± 0.01
Aeromonas hydrophila ATCC 35654	-	-	-	-	34 ± 0.01
Shigella flexneri ATCC 12022	-	-	-	-	30 ± 0.01
Listeria monocytogenes ATCC 7644	8.02* ± 0.01	-	-	-	30 ± 0.01
Escherichia coli ATCC 25922	-	-	-	-	34 ± 0.01
Salmonella typhimurium ATCC 23566	-	-	-	-	34 ± 0.01
Staphylococcus aureus ATCC 25923	5.10 ± 0.01	-	-	-	38 ± 0.01
Bacillus cereus ATCC 9634	-	-	-	-	30 ± 0.01
Escherichia coli O157:H7 35150	-	-	-	-	34 ± 0.01
Bacillus subtilis ATCC 6633	-	-	-	-	34 ± 0.01
Yeast-Molds					
Saccharomyces cerevisiae S288C	-	-	-	-	14 ± 0.01
Candida albicans ATCC 10231	-	-	-	-	22 ± 0.01
Aspergillus flavus ATCC 46283	-	-	-	-	25 ± 0.01

*Expressed as inhibition zone in mm, ** Penicillin G (10 mg) was used as the standard for bacteria, yeast, and molds

In the study, the results of the enzyme inhibitor activity in *R. coriaria* fruits are shown in Table 6. The enzyme inhibitor activity in *R. coriaria* fruits was 0.07 mg/mL.

Fruit extract *α*-glucosidase inhibition (IC_{50}) was 56.48 µg/ML (Gök *et al.* 2020). There was a similarity between the authors' study and a previous study. Moreover, the authors' study was slightly higher than their work (Gök *et al.* 2020).

Yu *et al.* (2012) stated in their study that the lower the IC_{50} value of the sample, the more effective it is in enzyme inhibition. The lower the IC_{50} value of the studied sample, the more effective it is in enzyme inhibition. Inhibitory activities on pancreatic lipase, α-amylase, and α-glucosidase were investigated with 80% extracts made from the fruits and leaves of the plant. Against all three enzymes analyzed, the detected IC_{50} ratios of the fruit extracts were higher than the leaf extracts. Their research has also demonstrated that *R. coriaria* fruit and leaf extracts have antidiabetic potentials *in vitro* (Gök *et al.* 2020).

Table 6. Amount of Enzyme Inhibitory Activities in Fruits of *R. coriaria*

	IC_{50} (mg/mL)	R^2
Acarbose*	0.021** ± 0.02***	0.9911
Fruits	0.069 ± 0.04	0.9812

*: For positive control, **: The average of three parallel studies, ***±: Standard deviation

The amounts of vitamin C in the examined fruit samples of *R. coriaria* are given in Table 7. The amount of vitamin C in the fruits of *R. coriaria* was determined as 35.54 mg/100 g.

Table 7. Amount Vitamin C in Fruits of *R. coriaria*

Vitamin C (mg/100 g)	
	Fruits
	35.54*
S.D.	0.27

*: The average of three parallel studies, SD: Standard deviation

CONCLUSIONS

1. In the gas chromatography – mass spectrometry with flame ionization detection (GC-MS/FID) analysis of the obtained volatile oils, 74 constituents were detected as the number of compounds in the fruits. The chemical groups with the most constituents in the volatile oils of the fruits of the *R. coriaria* were aldehydes. Sesquiterpene hydrocarbons were determined with 40.4% in the fruits of the chemical groups with the maximum percentage of constituents in the essential oils of plant parts. The main component found in the essential oils of plant parts was caryophyllene (36.9%) in its fruits.
2. The main phenolic constituents of fruit samples were protocatechuic acid, syringic acid gallic acid, and 4-hydroxybenzoic acid. The highest phenolic constituents of fruits were gallic acid.
3. The ABTS amounts of the samples were 68.8 mg AA eq./g and 100.4 mg Trolox eq./g in fruit methanol extracts. ABTS % inhibition rate was 98.0 in methanol extract of fruit. While the amount of DPPH was 53.1 mg AA eq./g and 64.1 mg Trolox eq./g in methanol extract of *R. coriaria* fruit, the % inhibition rate of DPPH in the same samples was 79.6%. The antioxidant capacity in the methanol extracts of plant parts was 14.9 mg $FeSO_4$ eq./g in FRAP capacity, 68.8 mg AA eq. and 100.4 mg Trolox eq./g in free radical scavenging (ABTS) capacity, 98.0% in ABTS % inhibition rate, 53.1 mg AA eq./g and 64.14 mg Trolox eq./g in free radical scavenging (DPPH), and 79.6% in DPPH % inhibition rate. Among the bioactive components of the samples, TAC amounts (32.8 mg GA/g), TFC amounts (73.8 mg QE eq./g), and TPC amounts (41.4 mg GA eq./g) were determined.
4. Concerning the results of the antimicrobial activity analysis of *R. coriaria* fruit samples, they showed antimicrobial activity against *Staphylococcus aureus* and *Listeria monocytogenes* microorganisms. Enzyme inhibitor activity in *R. coriaria* fruits was 0.069 mg/mL.
5. It is known that the lower the value of the studied sample, the more effective it is in enzyme inhibition. The amount of vitamin C in the fruits of *R. coriaria* was 35.5 mg/100 g.

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Article submitted: May 10, 2023; Peer review completed: June 18, 2023; Revised version received: July 16, 2023; Accepted: July 25, 2023; Published: August 7, 2023.
DOI: 10.15376/biores.18.4.6842-6861