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ORIGINAL ARTICLE

Rhamnus pallasii subsp. *sintenisii* fruit, leaf, bark and root: Phytochemical profiles and biological activities

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Abstract The genus *Rhamnus* has received a lot of interest as a source of phenolic chemicals. There have been no reports on the phytochemicals and biological activities of *R. pallasii* subsp. *sintenisii* various morphological components (fruit, leaf, bark, and root) in Iran to yet. Two crude ether petroleum (EP) and hydro-methanolic (HM) extracts were obtained from the separate parts. The antioxidant and antibacterial capabilities of the extracts, as well as their phytochemical screening (total phenolic, flavonoid, phenolic acid, and anthocyanin concentrations), were measured. Furthermore, the phytochemical profiles of EP and HM extracts were determined using GC–MS and LC-ESI–MS, respectively. LC-ESI-MS detected 59 chemicals in HM extracts, including flavonoids (62.71 %), phenolic acids (10.16 %), and anthraquinones (16.94 %). Furthermore, the predominant group components in EP extracts examined by GC–MS were fatty acids (58.82%), phenolic compounds (49.28%), and hydrocarbons (35.15 to 59.45 %). In terms of biological testing (DPPH radical scavenging and anti-bacterial activity), all examined extracts, particularly the fruit, had the highest activities in both assays (IC_{50}: 7.52 to 22.39 μg/ml and MIC: 0.39 to 3.12 mg/ml), owing to their high phenolic content. As a result, individual morphological elements of the species might be thought of as natural antioxidant and antibacterial agents.

1. Introduction

Rhamnus is a genus known as buckthorns in the *Rhamnaceae* family, with over 150 recognised species of small trees or shrubs. Species (deciduous and evergreen) range in height from 1 to 10 m and are endemic to East Asia and North America. Leaves with serrate margins that are 3–15 cm long and grouped in opposing pairs or subopposite. The branches terminate in a woody spine. Fruits are berry-like, red or black, 2–4 stoned, and globose in shape. Male and female yellowish green flowers are on distinct plants. The seeds are oblong in shape and have a long, narrow furrow. *Rhamnus pallasii* subsp. *sintenisii* (Rech. f.) Browicz &. J. Zielinski is a spiny shrub native to Iran and

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Turkey that can grow to a height of 3 m. The leaves are simple, alternately arranged in opposite pairs, and smaller than those of other *Rhamnus* species (Akkemik et al., 2014).

Plants of the *Rhamnus* genus have been used in traditional medicine as antioxidants, radical scavengers, anti-inflammatory agents, and for the treatment of liver disorders, constipation, and laxatives (Zeouk and Bekhti, 2020; Nigussie et al., 2021; Nekkaa et al., 2021). Furthermore, various chemicals have been found in plants related to the *Rhamnus* genus, including quercetin, rhamnetin, kaempferol, kaempferide, rhamnazin, anthrones, isorhamnetin, rhamnocitrin, and naphthaline derivatives (Cuoco et al., 2014; Nigussie et al., 2021; Rocchetti et al., 2019). Sakushima *et al.* extracted a dihydroflavonol, 2,3-dihydromyricetin-4′-*O*-methyl ether, as well as seven recognized flavonoids from the bark of Turkish *R. pallasii* in 1983: kaempferol, quercetin, isorhamnetin, mearnsetin, aromadendrin, eriodictyol, and taxifolin (Sakushima et al., 1983). Coşkun *et al.* isolated an anthraquinone glycoside known as physcion-8-*O*-β-primeveroside and a naphthalide known as α-sorinin from the bark of the same sources later in 1984 (Coşkun et al., 1984). There have been no reports on the chemical profile or biological effects of *R. pallasii* to date. The objectives of this study were to characterize the phytochemical profile of *R. pallasii* extracts prepared with EP and HM (80%) solvents using gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–electrospray ionization mass spectrometry (LC-ESI-MS), as well as to determine the antioxidant and anti-bacterial activities of these extracts, which had never been done before. In herbal medicine, LC-MS and GC–MS are sensitive technologies for identifying and profiling multi-components.

2. Material and methods

2.1. Plant material

R. pallasii subsp. *sintenisii* fruits, leaves, barks, and roots were collected in August 2020 from the Chakhmaqlu mountains of North Khorasan province, Iran (37°29′34″N 56°56′52″E) (Fig. 1). A voucher specimen (803893) has been deposited in the Gonbad Kavous University herbarium. The individual portions were dried at 30 °C in a well-ventilated room and stored in the dark until use.

2.2. Chemicals and reagents

Caffeic acid, gallic acid, quercetin, cyanidin-3-glucoside, butylated hydroxytoluene (BHT), sodium hydroxide, hydrochloric acid, sodium molybdate, sodium carbonate, sodium acetate, aluminum chloride, potassium acetate, potassium chloride, Folin-Ciocalteu reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), ether petroleum, methanol and formic acid were purchased from Sigma Aldrich (USA). The other compounds that were employed were of analytical grade. Aqueous solutions were also prepared using deionized water. Microorganism cultures were obtained from Iranian microbial collections, Pasteur Institute of Iran. The cultures of Gram-positive *Staphylococcus aureus* (ATCC 9144) and Gram-negative *Escherichia coli* (ATCC 25922) were used for the study.

2.3. Preparation of the extracts

The dried powder of species' fruits, leaves, barks, and roots (2 g) were extracted separately with 20 ml of EP at room temperature for 24 h (three times), and residues were extracted with water-methanol under the same conditions (80%). All of these extracts were filtered using a vacuum pump, and the organic solvents were extracted using a rotary evaporator at 40 °C under decreased pressure. Finally, concentrated extracts were lyophilized to dryness in a freeze dryer and stored in darkness at +4 °C for further analysis. The extraction yields (w/w) for EP extracts ranged from 1.2% to 3.5% and 2.9% to 6.3% for HM extracts.

2.4. Quantification of total phenolic content

The total phenolic content (TPC) of HM extracts from species' fruits, leaves, bark, and roots was evaluated using the Folin-Ciocalteu spectrophotometric method described by Singleton et al. (1999), with minor modifications (Singleton et al., 1999). In brief, 200 µL of diluted extracts were combined with

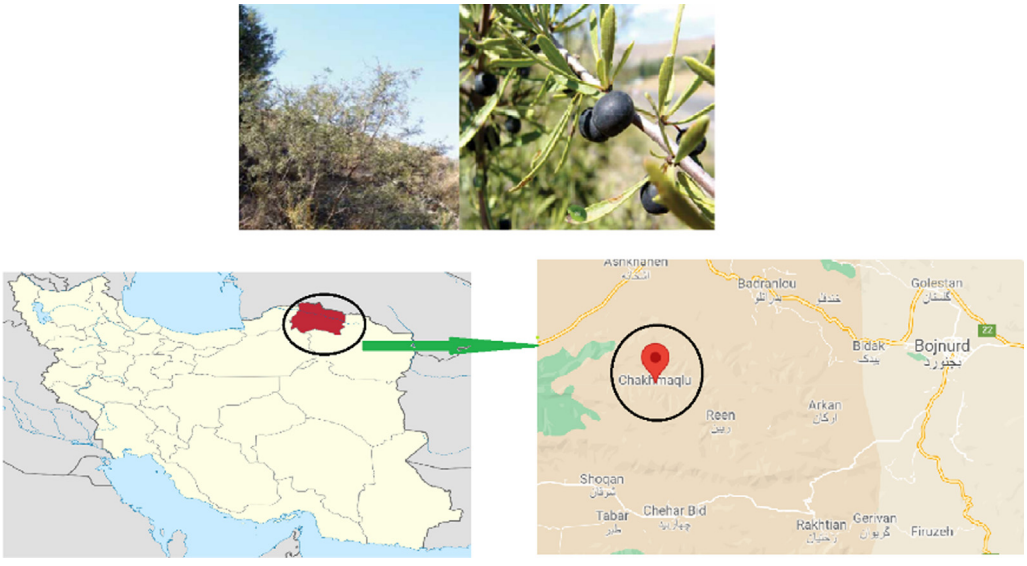

Fig. 1 *Rhamnus pallasii* subsp. *sintenisii* and map showing the location of the sampling (Chakhmaqlu altitudes, North Khorasan, Iran).

0.25 M Folin–Ciocalteu reagent, 600 μL of H_2O, and 1000 μL of 1.0 M Na_2CO_3. The absorbance of the solutions was measured at 760 nm after 1 h of incubation at room temperature in the dark. The findings were reported in milligrams of gallic acid equivalents (GAE) per gram of dried extract (mg GAE/g DE).

2.5. Quantification of total flavonoid content

The total flavonoid content (TFC) of all HM extracts was measured using the aluminum chloride colorimetric method described previously (Zhishen et al., 1999), with quercetin standard. In brief, diluted extracts or quercetin standard solutions were combined with 720 μL of distilled water, 90 μL of 5% $NaNO_2$, 600 μL of NaOH, and 90 μL of $AlCl_3$. The absorbance of reaction mixtures was measured at 510 nm after incubation at room temperature, and the TFC was reported as milligram of quercetin equivalents per gram of dry extract (mg QE/g E).

2.6. Quantification of total phenolic acid content

The total phenolic acid content (including hydroxycinnamic acid derivatives) was assessed using the Matkowski et al. (2008) method for determining the interaction of phenolic acids with sodium nitrite-sodium molybdate. Each extract (1 ml) was combined with 2 ml HCl (0.5 M), 2 ml Arnow reagent (10 g sodium molybdate and 10 g sodium nitrite diluted to 100 ml with deionized water), 2 ml NaOH (8.5 % w/v), and 3 ml water. The solutions were compared to a control mixture that did not contain Arnow reagent. The absorbance at 490 nm was measured, and the total hydroxycinnamic acid concentration was estimated using a caffeic acid calibration curve and represented as mg caffeic acid equivalent (CAE) per gram of dried extract (mg CAE/g DE).

2.7. Quantification of anthocyanin content

The anthocyanin content of all HM extracts was measured using differential pH methods (Camelo-Méndez et al., 2013), two diluted solutions were prepared, one in 0.4 M sodium acetate buffer with a pH of 4.5; and the other in 0.025 M potassium chloride buffer with a pH of 1.0. At 510 and 700 nm, the absorbance was determined using a spectrophotometer. The absorbance was calculated as follows:

$$A = (A_{510} - A_{700})\,pH_1 - (A_{510} - A_{700})\,pH_{4.5}.$$

The content of anthocyanins was determined using the absorbance of (A) and the molar absorptivity of cyanidin 3-glucoside (29,600). The TAC values were calculated as mg cyanidin-3-glucoside per gram dry extract.

$$TAC = (A/e \times L) \times (449.2) \times D/G \times V \times 100.$$

Where A is absorbance; e (26,900) is the molar extinction coefficient, of cyanidin 3-glucoside (Giusti and Jing, 2008); L (1 cm) is the cell length; 449.2 is anthocyanins molecular weight; D is dilution factor; V (ml) is final volume and G (mg) is the dry weight (dw) of samples.

2.8. DPPH radical-scavenging activity

The DPPH radical scavenging activities of HM extracts was monitored according to the method of Cavin et al. (1998). Five different concentrations of each extract were added to 915 μL methanol, then 200 μL DPPH solution in methanol (0.022%) were added. After 30 min incubation at room temperature in the dark, the reaction mixture's absorbance was measured at 517 nm. The absorbance of extracts was compared to that of methanol without DPPH as a blank.

DPPH radical-scavenging activity was determined by:

$$\%\ \text{Inhibition rate} = (A_{\text{control}} - A_{\text{sample}})/A_{\text{control}} \times 100.$$

The effective concentration necessary to inhibit 50% of the DPPH radicals was expressed by IC_{50} value (half maximal inhibitory concentration).

2.9. Antibacterial activity

The antibacterial activity of *R. pallasii* extracts against Gram-positive *Staphylococcus aureus* (ATCC 9144) and Gram-negative *Escherichia coli* (ATCC 25922) was tested using the microdilution broth technique as described by Suffredini et al. (2006) with certain modifications. Strains were obtained from Iranian microbial collections, Pasteur Institute of Iran. Two-fold serial dilutions of the extracts of fruit, leaf, bark and root were prepared in Mueller Hinton Broth ranging from 25 to 0.195 mg/ml in 96-wells plates. Additionally, gentamicin discs and each fraction's solvents were employed as positive and negative controls, respectively. After 24 h of incubation, the plates inhibitory effect on bacteria growth was determined visually by examining the growth in each well. After incubation, the MIC value was determined as the lowest concentration of plates at which microorganisms displayed no observable growth. Additionally, the MBC value was calculated using the lowest concentration of plates that exhibited no bacterial growth. Each microorganism was subjected to three independent analyses.

2.10. HPLC-ESI-MS analysis

The HPLC-ESI-MS analysis was implemented by using the Waters Alliance 2695 HPLC system, which was connected with micro mass quattro micro API mass spectrometer with electrospray ion source (ESI). A standard solution containing uracil, 4-hydroxymethyl benzoate, 4-hydroxy ethyl benzoate and benzophenone was injected into the device in order to validate the reliability of the system.

2.10.1. HPLC analysis

All crude HM extracts (1 mg) were diluted in 1 ml methanol and filtered through a 0.45 m Millipore filter before being injected into the HPLC. HPLC separations were carried out on a Zorbax SB-C18 column (3, 2.1100 mm) using a gradient mobile phase composed of acetonitrile + 0.1 % formic acid (solvent A) and H_2O + 0.1 % formic acid (solvent B). Gradient elution was performed as follows: 0–2 min, 10% A; 2–10 min, 10–50 % A; 10–16 min, 50% A; 16–20 min, 50–90% A; 20–24 min, 90% A; 24–26 min, 90–10 % A; 26–30 min,

10% A. The injection volume was set to 10 L and the column temperature to 35 °C. 0.2 ml/min flow rate was used.

2.10.2. ESI-MS analysis

Electrospray ionization (ESI, negative mode) was used to generate the ions, with nitrogen serving as the cone and desolvation gas. The following parameters were used in the spray chamber: capillary voltage, 3.5 kV; cone voltage, 25 V; extractor voltage, 2 V; collision energy, 30 eV; source temperature, 120C; desolvation temperature, 300C; gas flow, 200 L/h; and nebulizer pressure, 15 psi. Acquisitions of full scans were made in the 150–2000 m/z range. The extracted ion chromatograms (EIC) from total ion chromatograms were used to examine the samples (TIC). The MZmine analysis software program, version 2.3, was used to process the data.

2.11. GC–MS analysis

The GC–MS analyses were performed using an Agilent 6890 gas chromatograph linked to a 5973 MSD mass spectrometer and an HP-5 ms column with a 30 m 0.25 mm i.d. and 0.25 m film thickness. On the basis of Wiley 7n.L and NIST libraries, the chemical profiles of fruit, leaf, bark, and root were identified. Separation of the compounds occurred at a rate of 3°Celsius per minute along a temperature gradient extending from 50 to 280 °C. The instrumentation used a 250 °C analyzer and ion source, a split ratio of 1:20, a 1 μL injection volume, a 70 eV ionization potential, a helium carrier gas flowing at 1.0 ml/min, and a mass range of 50–550 m/z. The components were identified by comparing their mass spectra to those in the Wiley 7.0 mass spectral library and the literature (Adams, 2007). This procedure is similar to that described by Faizi et al., but with minor variations (Faizi et al., 2014).

2.12. Statistical analysis

Each test was conducted in triplicate. Results were expressed as a mean ± standard deviation. SPSS statistics version 20 software and ANOVA procedures were used for statistical analysis. A significance level of 0.05 was considered.

3. Result and discussion

The phytochemical composition of *Rhamnus* species varies greatly. It is well established that genetic (species, organ, and developmental stage) and environmental diversity contribute significantly to the nutritional quality and phytochemical content of plants. To our knowledge, no investigations on the phytochemical screening, chemical profile by LC-MS, GC–MS, or biological activity of *R. pallasii* have been published.

3.1. Total phenolics (TPC), total flavonoids (TFC), total phenolic acids (TFAC) and total anthocyanin contents (TAC)

Fig. 2a illustrates the phytochemical analysis of *R. pallasii* fruit, leaf, bark, and root extracts. The extracts contained a total of 69.8 1.4 to 232.8 2.5 mg GAE/g DE. The fruit extract had the greatest TPC concentration (232.8 1.5 mg GAE/g), followed by the leaf (208.6 2.3 mg GAE/g), the bark (124.3 1.8 mg GAE/g), and the root (69.8 1.4 mg GAE/g) extracts, respectively. The TPC of a methanolic extract of *R. alaternus* examined in the literature (Moussi et al., 2015). The leaves contained 77.8 mg GAE/g TPC, which was lower than the value observed in our investigation. Another study determined the TPC of a 60% ethanol extract of *R. prinoides* stems to be 228.21 ± 13 mg of GAE/g (Chen et al., 2020). Additionally, the TPC of *R. lycioides* leaves was 259.33 ± 4.95 mg of GAE/g, which was greater than the value found in our study (Benamar et al., 2019). A similar result was achieved when species total flavonoids content was determined. However, the fruit extract (187.03 ± 2.09 mg QE/g DE) had a higher TFC value than the leaf (98.6 ± 2.5 mg QE/g), bark (83.3 ± 1.2 mg QE/g), and root (46.8 ± 2.4 mg QE/g) extracts. The TFC values calculated in this study for the leaves extract were higher than those previously reported for *R. alaternus* collected in Algeria (30.11 ± 5.76 mg QE/g) (Moussi et al., 2015), but were lower than those previously reported for *R. alaternus* collected in Tunisia (283 ± 11 mg QE/g) (Ammar et al., 2007). In previous research, the TFC of methanol extracts of *R. kurdica* and *R. lycioides* leaves was determined to be 86.32 ± 2.98 mg catechin equivalent per mg plant and 74.08 ± 2.10 mg catechin equivalent per g dry extract, respectively (Gholivand and Piryaei, 2014; Benamar et al., 2019). Additionally, we determined the total anthocyanin concentration of fruit, leaf, bark, and root extracts (Fig. 2a). The fruit (75.14 ± 0.03 mg cyanidin 3-glucoside/g DE) and bark (51.76 ± 0.02 mg/g) extracts had the highest TAC values, followed by the leaf and root extracts at 42.21 ± 1.02 and 12.41 ± 2.3 mg/g, respectively. Gholivand discovered that *R. kurdica* flowers and leaves have a significant concentration of anthocyanin (21.53 ± 0.57 and 12.36 ± 0.84 g/100 mg fw, respectively) (Gholivand and Piryaei, 2014). The anthocyanin content of extracts varied considerably. These distinctions are related to the diversity of chemicals that make up plant pigments. Finally, the extracts total phenolic acid content (TPAC) was determined (25.01 ± 1.3 to 63.14 ± 2.2 mg CAE/g DE). The fruit extract (63.14 ± 2.2 mg/g) had the highest TPAC value, followed by the leaf (52.4 ± 1.5 mg/g), bark (41.3 ± 2.2 mg/g), and root (25.01 ± 1.3 mg/g) extracts, respectively.

3.2. Antioxidant activity

For the first time, the antioxidant activities of several parts of *R. pallasii* were determined using the DPPH radical scavenging assay, and the results were reported as IC_{50} values (Fig. 2b). Among the *R. pallasii* extracts, the fruit and leaf extracts displayed the highest scavenging activity, with IC_{50} values of 7.52 ± 2.1 and 11.81 ± 1.06 g/ml, respectively, which are significantly more active than the positive control butylated hydroxytoluene (BHT) (IC_{50} = 19.3 ± 1.06 g/ml). The bark and root extracts had the lowest IC_{50} values, at 20.01 ± 2.5 and 22.39 ± 0.10 g/ml, respectively. The extracts free radical scavenging activity may be a result of their high TPC and TFC content, which have hydrogen-donating capabilities (Rice-Evans et al., 1997). Only a few publications in the literature have discussed the DPPH assay in relation to other species. Our values are lower than those previously reported for other species, including methanolic leaf extract of *R. kurdica* (IC_{50} of 21.04 ± 1.35 g/ml), 60 % ethanolic stem extract of *R. prinoides* (IC_{50} of 51.21% 0.046 g/ml), and methanolic leaf

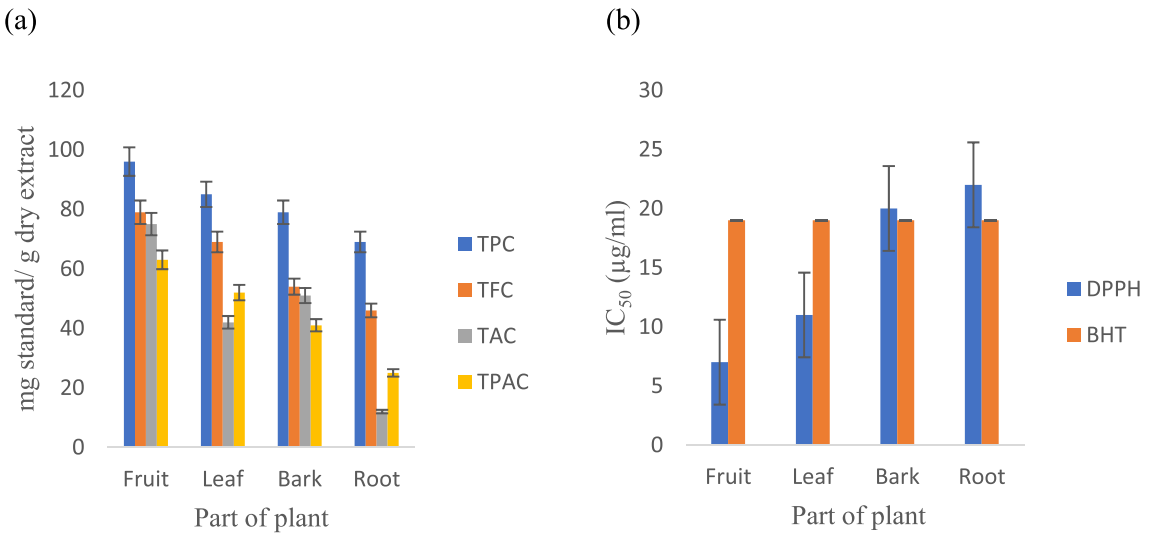

Fig. 2 Total phenolics, total flavonoids, total anthocyanins and total phenolic acids contents (a) and antioxidant activities (b) of the HM extracts of *R. pallasii*.

extract of *R. lycioides* (IC_{50} of 29.69 ± 0.33 g/ml) (Gholivand and Piryaei, 2014; Chen et al., 2020; Benamar et al., 2019).

3.3. Antibacterial activity

Table 1 summarizes the antimicrobial activity of EP and HM extracts of *R. pallasii* fruit, leaf, bark, and root. The extracts were antimicrobial against both microorganisms that cause food poisoning (*Staphylococcus aureus* and *Escherichia coli*). The results indicated that the extracts had significantly more antibacterial action against *S. aureus* than against *E. coli*, owing to the bacterial strains different cell wall structures. Gram-negative bacteria have an outer membrane composed of lipids and a polysaccharide component that serves as a barrier to antimicrobial drug penetration (Lambert, 2002). When compared to gentamicin, the antibacterial effects of the HM extract of fruit and EP extract of root were much stronger than those of other extracts (positive control). The extracts had MIC and MBC values of 0.39 to 3.12 and 0.78 to 6.25 mg/ml respectively. No investigations on the antibacterial activity of *R. pallasii* have been conducted to date, however various studies have reported on the antimicrobial activity of other species. Molla demonstrated that methanol and chloroform leaf extracts of *R. prinoides* were bactericidal against four bacterial strains with MIC values ranging from 8.13 to 32.5 mg/ml (Molla et al., 2016). EP and methanolic extracts of *R. alaternus* had no detectable inhibitory action on gram-negative and gram-positive bacteria until 6 mg/ml (Ben Ammar et al., 2007). Another study found that the MIC and MBC values of *R. prinoides* fruit and leaf extracts against *S. aureus* and *E. coli* were 1.3 to 5.23 and 2.08 to 8.33 g/ml, respectively (Kibret, 2019). Carranza et al. discovered that methanolic extracts of *R. californica* leaves had MIC values of 5.0 to 6.0 mg/ml against six bacterial species (Carranza et al., 2015). The results of this study suggest that the significant antibacterial activity of the EP and HM extracts of *R. pallasii* may be attributed to the presence of terpenes, phenols, and flavonoids, which act as antimicrobial agents via a variety of different mechanisms (Guimarães et al., 2019).

3.4. GC–MS analysis

GC–MS analysis was used to determine the chemical composition of an EP extract of *R. pallasii* (fruit, leaf, bark, and root). The extract yields were 3.5 % (fruit), 1.2 % (leaf), 3.1 % (bark), and 2.8 % (stem) based on the plant's dry weight. The chemicals that have been identified are listed in Table 2. A total of 68 chemicals were isolated from EP extracts of various plant sections. Our findings indicate that fruit extract contains 26 compounds that account for 93.62 % of its

Table 1 MIC and MBC (mean ± SD) (mg/ml) of *R. pallasii* EP and HM extracts against pathogenic bacteria.

Plant part	*S. aureus*				*E.coli*			
	MIC		MBC		MIC		MBC	
	EP	HM	EP	HM	EP	HM	EP	HM
Fruit	3.12 ± 2.13	0.39 ± 1.32	6.25 ± 2.14	0.78 ± 3.16	3.12 ± 1.42	0.39 ± 2.94	3.12 ± 2.92	1.56 ± 3.12
Leaf	0.78 ± 1.09	1.56 ± 1.45	0.78 ± 1.65	3.12 ± 3.52	0.78 ± 1.24	0.78 ± 2.31	1.56 ± 2.81	0.78 ± 2.34
Bark	3.12 ± 3.12	1.56 ± 4.12	3.12 ± 2.34	3.12 ± 2.17	1.56 ± 2.41	0.78 ± 1.49	1.56 ± 1.56	3.12 ± 1.93
Root	0.39 ± 1.21	3.12 ± 3.16	0.78 ± 1.56	3.12 ± 2.41	0.78 ± 1.56	3.12 ± 1.11	1.56 ± 2.86	6.25 ± 3.32
Gentamicin	2.32 ± 1.49 (µg/ml)		32.12 ± 2.12 (µg/ml)		16.31 ± 1.29 (µg/ml)		128.21 ± 3.12 (µg/ml)	

Table 2 Chemical composition (%) of the EP extract from fruit, leaf, bark and stem of *R. pallasii*.

Compounds	Molecular Formula	Classification	RT (min)				Percentage %			
			Fruit	Leaf	Bark	Root	Fruit	Leaf	Bark	Root
2-Methylheptan	C_8H_{18}	Aliphatic hydrocarbon	–	3.553	–	–	–	0.321%	–	–
3-Methylheptan	C_8H_{18}	Aliphatic hydrocarbon	–	3.696	–	–	–	0.328%	–	–
Octane	C_8H_{18}	Aliphatic hydrocarbon	–	4.208	–	–	–	0.206%	–	–
cis-1,2-Dimethyl cyclohexane	C_8H_{16}	Cycloalkane	–	4.871	–	–	–	0.084%	–	–
2-Ethylhexanol	$C_8H_{18}O$	Fatty Alcohols	–	4.999	–	–	–	0.086%	–	–
4-Methyloctane	C_9H_{20}	Aliphatic hydrocarbon	–	5.744	–	–	–	0.066%	–	–
3-Methyloctane	C_9H_{20}	Aliphatic hydrocarbon	–	5.947	–	–	–	0.058%	–	–
Nonane	C_9H_{20}	Aliphatic hydrocarbon	–	6.722	6.723	–	–	0.133%	0.167%	–
2,6,7-Trimethyldecane	$C_{13}H_{28}$	Aliphatic hydrocarbon	–	–	7.739	–	–	–	0.199%	–
3-Ethyl-2-methylheptane	$C_{10}H_{22}$	Aliphatic hydrocarbon	–	–	7.942	–	–	–	0.170%	–
1,1,2,3-Tetramethylcyclohexane	$C_{10}H_{20}$	Cycloalkane	–	–	8.364	–	–	–	0.196%	–
4-Methylnonane	$C_{10}H_{22}$	Aliphatic hydrocarbon	–	–	8.567	–	–	–	0.305%	–
2-Methylnonane	$C_{10}H_{22}$	Aliphatic hydrocarbon	–	–	8.650	–	–	–	0.221%	–
2,6-Dimethyloctane	$C_{10}H_{22}$	Aliphatic hydrocarbon	–	–	8.846	–	–	–	0.428%	–
1-Methyl-2-propylcyclohexane	$C_{10}H_{20}$	Cycloalkane	–	–	9.290	–	–	–	0.302%	–
Decane	$C_{10}H_{22}$	Aliphatic hydrocarbon	9.812	9.756	9.749	9.794	0.904%	0.441%	1.630%	0.220%
4-Methyldecane	$C_{11}H_{24}$	Aliphatic hydrocarbon	10.504	10.449	–	–	0.384%	0.192%	–	–
2-Methyldecane	$C_{11}H_{24}$	Aliphatic hydrocarbon	–	–	–	11.781	–	–	–	0.194%
Undecane	$C_{11}H_{24}$	Aliphatic hydrocarbon	12.928	12.835	12.858	12.888	1.621%	0.627%	3.282%	1.043%
Cycloundecene,1-methyl	$C_{12}H_{22}$	Cycloalken	–	–	–	15.778	–	–	–	0.679%
Naphthalene,decahydro-1,6-dimethyl	$C_{12}H_{22}$	Polycyclic hydrocarbon	15.857	–	15.944	16.305	1.096%	–	1.783%	2.621%
2,6-Dimethyldecalin	$C_{12}H_{22}$	Polycyclic hydrocarbon	16.007	–	–	16.004	0.748%	–	–	1.202%
Naphtalene,decahydro-2,3-dimethyl	$C_{12}H_{22}$	Polycyclic hydrocarbon	16.203	16.531	15.824	16.591	1.036%	0.684%	2.600%	3.108%
Cycloheptanon,2-(-2-methyl propylidene	$C_{11}H_{18}O$	Cyclic ketone	16.293	–	–	–	0.438%	–	–	–
4,8-decadien-3-ol,5,9dimethyl	$C_{12}H_{22}O$	Alcoholic compound	16.519	–	–	–	1.788%	–	–	–
Decahydro-1,2-dimethylnaphthalene	$C_{12}H_{22}$	Polycyclic hydrocarbon	–	–	16.562	–	–	–	3.799%	–
Naphtalene,decahydro-1,5-dimethyl	$C_{12}H_{22}$	Polycyclic hydrocarbon	16.760	16.689	16.712	16.749	2.656%	1.159%	1.630%	5.099%
Tridecane	$C_{13}H_{28}$	Aliphatic hydrocarbon	18.702	18.624	16.639	18.699	0.609%	0.299%	**6.439%**	2.861%
2-Methyltridecane	$C_{14}H_{30}$	Aliphatic hydrocarbon	–	–	–	20.393	–	–	–	0.825%
2,6,10-Trimethyltetradecane	$C_{17}H_{36}$	Aliphatic hydrocarbon	–	–	–	20.732	–	–	–	0.902%
farnesane	$C_{15}H_{32}$	Sesquiterpene	–	–	20.657	20.739	–	–	1.016%	1.469%

Table 2 (*continued*)

Compounds	Molecular Formula	Classification	RT (min)				Percentage %			
			Fruit	Leaf	Bark	Root	Fruit	Leaf	Bark	Root
Tetradecane	$C_{14}H_{30}$	Aliphatic hydrocarbon	21.344	21.281	21.312	21.379	1.078%	0.572%	2.975%	2.006%
Pentadecane	$C_{15}H_{32}$	Aliphatic hydrocarbon	23.844	–	23.781	23.856	0.268%	–	0.778%	7.068%
Hexadecane	$C_{16}H_{34}$	Aliphatic hydrocarbon	26.230	–	26.144	26.242	0.381%	–	2.244%	1.948%
Neoisolongifolene,8-bromo	$C_{15}H_{23}Br$	Sesquiterpene	–	–	27.018	–	–	–	0.875%	–
Banzoic acid,4heptyl-4-cyanophenyl ester	$C_{21}H_{23}NO_2$	Benzoic acid derivatives	27.103	–	–	27.108	0.298%	–	–	3.445%
Banzan,1,3,5tris(1-methyl propyl)	$C_{18}H_{30}$	Benzene derivatives	27.540	–	27.492	27.559	0.350%	–	1.190%	2.013%
Paullinic acid	$C_{20}H_{38}O_2$	Fatty acid	–	–	28.538	28.591	–	–	1.596%	2.648%
Octadecane	$C_{18}H_{38}$	Aliphatic hydrocarbon	30.603	–	30.571	30.638	0.418%	–	3.026%	2.289%
Phytol	$C_{20}H_{40}O$	Diterpene	–	36.720	31.354	–	–	2.122%	0.934%	4.247%
Methyl palmitate	$C_{17}H_{34}O_2$	Fatty acid aster	33.216	–	–	–	1.213%	–	–	–
palmitic acid	$C_{16}H_{32}O_2$	Fatty acid	34.367	34.093	34.011	–	2.756%	2.921%	2.611%	–
Eicosane	$C_{20}H_{42}$	Aliphatic hydrocarbon	–	–	34.560	34.628	–	–	3.847%	4.730%
2-Methyl-1-hexadecanol	$C_{17}H_{36}O$	Alcoholic compound	–	–	36.141	–	–	–	1.386%	–
Methyl oleate	$C_{19}H_{36}O_2$	Fatty acid ester	36.588	–	–	–	3.373%	–	–	–
Ethyl linoleate	$C_{20}H_{36}O_2$	Fatty acid ester	–	–	37.278	–	–	–	4.054%	–
Oleic acid	$C_{18}H_{34}O_2$	Fatty acid	38.252	37.466	–	–	**45.934%**	4.394%	–	–
Docosane	$C_{22}H_4$	Aliphatic hydrocarbon	–	–	38.204	–	–	–	3.752%	–
Acetyl tributyl citrate	$C_{20}H_{34}O_8$	Fatty acid aster	–	39.423	39.416	–	–	1.323%	1.968%	–
Bis(2-ethylhexyl) adipate	$C_{22}H_{42}O_4$	Fatty acid aster	–	41.591	–	–	–	1.637%	–	–
Octadecane,3-ethyl-5-(2-ethyl butyl)	$C_{26}H_{54}$	Aliphatic hydrocarbon	43.265	–	47.576	–	1.804%	–	3.611%	–
17-pentatriacontene	$C_{35}H_{70}$	Aliphatic hydrocarbon	–	–	48.118	41.629	–	–	1.664%	**8.621%**
Tribehenin	$C_{69}H_{134}O_6$	Fatty acid aster	–	–	–	44.730	–	–	–	5.098%
Heptacosane	$C_{27}H_{56}$	Aliphatic hydrocarbon	–	46.108	41.591	38.294	–	1.143%	5.901%	4.461%
Erucamide	$C_{22}H_{43}NO$	Fatty amid	47.533	–	–	47.455	3.549%	–	–	**7.418%**
Squalene	$C_{30}H_{50}$	Triterpene	–	47.997	–	–	–	2.072%	–	–
Nonacosane	$C_{29}H_{60}$	Aliphatic hydrocarbon	49.084	48.976	44.678	–	6.516%	4.725%	4.192%	–
d-Allo-dec-2-enonic acid,5,8-anhydro2,3,4,9-tetradeoxy-8-c-(hydroxymethyl)3-methyl-7,8-O-(1-methylethylid)	$C_{18}H_{28}O_8$	Carbohydrate derivatives	–	49.721	–	–	–	3.506%	–	–
Tetratetracontane	$C_{44}H_{90}$	Aliphatic hydrocarbon	–	–	50.406	–	–	–	3.125%	–
Hentriacontane	$C_{31}H_{64}$	Aliphatic hydrocarbon	–	52.205	–	–	–	**8.934%**	–	–
Stigmast-5-en-3-Ol,oleate	$C_{47}H_{82}O_2$	Triterpenoids	–	–	–	52.295	–	–	–	7.356%
Stigmastan-3,5-diene	$C_{29}H_{48}$	Sterols	52.351	–	–	–	**9.764%**	–	–	
α-Tocopherol (vitaminE)	$C_{29}H_{50}O_2$	Tocopherol (Phenolics)	53.179	53.146	52.958	53.071	**6.602%**	**44.282%**	**11.897%**	**10.084%**
7,8-Epoxylanostan-11-ol, 3-acetoxy-	$C_{32}H_{54}O_4$	Triterpenoid	55.437	–	51.099	–	1.416%	–	1.217%	–

(*continued on next page*)

Table 2 (*continued*)

Compounds	Molecular Formula	Classification	RT (min)				Percentage %			
			Fruit	Leaf	Bark	Root	Fruit	Leaf	Bark	Root
Tritetracontane	$C_{43}H_{88}$	Aliphatic hydrocarbon	–	56.609	–	–	–	1.540%	–	–
Clionasterol	$C_{29}H_{50}O$	Sterol (Phytosterol)	–	57.264	57.302	–	–	**7.561%**	**12.990%**	–
β-sitosterol	$C_{29}H_{50}O$	Sterol (Phytosterol)	–	–	–	57.399	–	–	–	6.342%
α -Amyrin	$C_{30}H_{50}O$	Triterpenoid	–	59.371	–	–	–	3.583%	–	–

Major Grouped Compounds	Fruits	Leaves	Barks	Roots
Terpenes	1.416%	7.777%	4.042%	13.072%
Phenolics	6.602%	**44.282%**	11.897%	10.084%
Fatty acids,Fatty acid asters and fatty amides	**54.825%**	10.275%	9.229%	14.164%
Steroids	9.764%	7.561%	12.990%	6.342%
Hydrocarbons	18.51%	19.5%	**57.45%**	**42.87%**
Miscellaneous	2.524%	3.592%	1.386%	3.445%
Total Identified%	93.62%	92.97%	96.97%	89.92%

composition; leaf extract contains 29 compounds that account for 92.97 % of its composition; bark extract contains 38 compounds that account for 96.97 % of its composition; and root extract contains 28 compounds that account for 89.92 % of its composition. Each extract contained the following major compounds (in percentages): fruit (oleic acid 45.93 %, stigmastan-3,5-diene 9.76 %, -tocopherol 6.60 %); leaf (-tocopherol 44.28 %, hentriacontane 8.93 %, clionasterol 7.56 %); bark (clionasterol 12.99 %, -tocopherol 11.89 %, tridecane 6.43 %); and root (-tocop Generally, the extracts are a good source of biological components. Terpenes, phenolics, fatty acids, fatty esters, steroids, and hydrocarbons are all significant types of chemicals found in *R. pallasii* extracts (Table 2). A review of the literature indicated that no data on GC–MS studies of *R. pallasii* extracts were given, while data on the volatile components of other species were reported (Chouitah et al., 2012; Mekala et al., 2017).

3.5. LC-ESI-MS analysis

The profile of bioactive chemicals in HM extracts of *R. pallasii* fruits, leaves, barks, and roots was published in this work for the first time using an LC-ESI-MS method in the negative ion mode. All extracts included 59 chemicals, including 24 flavonols, 6 flavones, 4 flavanones, 3 flavanonols, 6 phenolic acids, 10 anthraquinones, 3 naphthaenic lactone derivatives, 2 naphthalene derivatives, and 1 coumarin derivative (Table 3). Fig. 3-A-H illustrates the total ion chromatogram (TIC) of extracts and instances of extracted ion chromatograms (EIC). Peaks were identified using molecular weights, retention times (Rt), complete ESI-MS, and matching mass adducts ($[M-H]^-$, [2 M], [2 M−H], [M−2H], and [M−2H + Na]), as well as comparisons to published data. Only ten of the 59 compounds had been identified previously in *R. pallasii*, and they were all kaempferol, quercetin, isorhamnetin, mearnsetin, aromadendrin, taxifolin, eriodictyol, pallasiin, -sorinin, and physclon-8-*O*- β-primeveroside from the barks of Turkish species and leaves of Georgian species (Sakushima et al., 1983; Coşkun et al., 1984). There are no data on the phytochemical profiles of other components of this plant to our knowledge.

3.5.1. Flavonoids

The most abundant class of chemicals discovered were flavonoids. They are potent antioxidants composed of two phenyl rings and a heterocyclic ring. Plants include a variety of flavonoid classes, including flavones, flavanones, flavonols, and anthocyanins. The flavonoids found in *Rhamnus* species tested in this study were classified into four classes: flavonols, flavones, flavanones, and flavanonols, as shown in Table 3. Only eight of the 38 flavonoids discovered in *R. pallasii* have been previously detected: quercetin, kaempferol, isorhamnetin, mearnsetin, aromadendrin, taxifolin, eriodictyol, and pallasiin (Sakushima et al., 1983; Coşkun et al., 1984).

3.5.1.1. Characterization of flavonols. Flavonols were identified as quercetin, kaempferol, isorhamnetin, mearnsetin, rhamnazin, and rhamnocitrin aglycones and their derivatives at C-7 and/or C-3 locations. Compounds 1–6 with $[M-H]^-$ ions at *m*/*z* 301, 463, 609, 755, and 477 were identified as quercetin, quercetin-3-*O*-glucoside (isoquercitrin), quercetin-7-*O*-glucoside, quercetin-3-*O*-rhamnoside, quercetin-3-*O*-robinobioside, quercetin-3-rhamnino quercetin-3-methyl ether-7-*O*-glucoside, respectively, based on the comparison of data obtained with literature findings (Sakushima et al., 1983; Chen et al., 2016; Ammar et al., 2009; Moussi et al., 2015; Marzouk et al., 1999). Additionally, derivatives of kaempferol have been found in *R. pallasii* and other *Rhamnus* species. Compounds 7–10 were identified as kaempferol, kaempferol-7-*O*-glucoside, kaempferol-3-*O*-acetyl-rhamninoside, and kaempferol-3-*O*-robinoside, respectively, using $[M-H]^-$ ions at *m*/*z* 285, 447, 781, and 593. These chemicals have been isolated and identified from the fruit, leaf, and bark of more *Rhamnus* species, including *R. davurica*, *R. saxatilis*, *R. disperma*, and *R. libanoticus* (Chen et al., 2016; Ammar et al., 2009; Moussi et al., 2015; Sakushima et al., 1983; Marzouk et al., 1999). The ions found at *m*/*z* 739 were

Table 3 Characterization of phenolic compounds in *R. pallasii* fruits, leaves, barks and roots by LC-ESI-MS in the negative ion mode.

No.	Compounds	Formula	[M−H]− (*m*/*z*)	Rt/ Intensity (E^n)				Parts reported in literature[1]	Ref.
				Fruit	Root	Bark	Leaf		
1	**Quercetin**	$C_{15}H_{10}O_7$	301	19.1/ $9.9E^4$	18.9/ $3.8E^5$	–	**18.5/ $8.7E^5$**	F, L*, B*	(Sakushima et al., 1983; Chen et al., 2016; Ammar et al., 2009; Moussi et al., 2015)
2	Quercetin-3-*O*-glucoside (isoquercitrin)	$C_{21}H_{20}O_{12}$	463	16.1/ $1.5E^5$	16.2/ $1.4E^5$	16.1/ $1.8E^5$	16.5/ $6.2E^5$	B	(Chen et al., 2016)
3	Quercetin-7-*O*-glucoside	$C_{21}H_{20}O_{12}$	463	–	16.7/ $6.8E^4$	–	16.5/ $4.7E^5$	AP	(Marzouk et al., 1999)
4	Quercetin-3-*O*-robinobioside	$C_{27}H_{30}O_{16}$	609	9.1/ $1.1E^5$	8.7/ $9.6E^4$	9.2/ $1.8E^5$	8.3/ $5.5E^5$	F	(Marzouk et al., 1999)
5	**Quercetin-3-rhamninoside**	$C_{33}H_{40}O_{20}$	755	3.5/ $5.7E^4$	**3.9/ $6.9E^6$**	3.6/ $1.1E^5$	3.1/ $6.4E^5$	F	(Marzouk et al., 1999)
6	Quercetin-3-methyl ether-7-*O*-glucoside	$C_{22}H_{22}O_{12}$	477	10.8/ $9.2E^4$	10.7/ $1E^5$	11/ $2.1E^5$	–	AP	(Marzouk et al., 1999)
7	Kaempferol	$C_{15}H_{10}O_6$	285	20.9/ $2.1E^5$	21.3/ $4.4E^5$	21.5/ $4.7E^5$	21.2/ $5.4E^5$	F, L*, B*, AP	(Chen et al., 2016; Ammar et al., 2009; Moussi et al., 2015)
8	Kaempferol-7-*O*-glucoside	$C_{21}H_{20}O_{11}$	447	17.5/ $1.4E^5$	17.7/ $1.9E^5$	17.7/ $1.7E^5$	17.5/ $1.4E^5$	B	(Chen et al., 2016)
9	Kaempferol-3-*O*-acetyl-rhamninoside	$C_{35}H_{42}O_{20}$	781	8.5/ $1.8E^5$	8.6/ $1.1E^5$	8.6/ $1.3E^5$	8.4/ $4.6E^4$	F	(Cuoco, Mathe, and Vieillescazes, 2014)
10	Kaempferol-3-*O*-robinoside	$C_{27}H_{30}O_{15}$	593	10.2/ $8.2E^4$	10.5/ $2.5E^5$	10/ $1.6E^5$	10.7/ $5.9E^4$	F	(Marzouk et al., 1999)
11	Kaempferol-3-*O*-rhamninoside	$C_{33}H_{40}O_{19}$	739	7.5/ $2E^5$	–	6.9/ $7.5E^4$	7.3/ $6.8E^5$	F, AP	(Marzouk et al., 1999)
12	Kaempferol-4′-*O*-rhamninoside	$C_{33}H_{40}O_{19}$	739	7.9/ $9.1E^4$	–	8.2/ $1.4E^5$	8.4/ $6.4E^5$	F	(Ammar et al., 2009)
13	Rhamnetin	$C_{16}H_{12}O_7$	315	19.8/ $5.7E^4$	20.1/ $1.6E^5$	–	19.4/ $4.8E^5$	F, L, AP	(Cuoco, Mathe, and Vieillescazes, 2014; Marzouk et al., 1999; Ammar et al., 2009)
14	Isorhamnetin	$C_{16}H_{12}O_7$	315	20.3/ $8.9E^4$	–	–	19.8/ $3.7E^5$	F, L*, B*, AP	(Cuoco, Mathe, and Vieillescazes, 2014; Sakushima et al., 1983; Marzouk et al., 1999)
15	**Isorhamnetin-3-*O*-rhamninoside**	$C_{33}H_{40}O_{19}$	769	**4.5/ $2.7E^5$**	4.5/ $5.3E^5$	–	5.1/ $8.3E^5$	F	(Marzouk et al., 1999)
16	**Rhamnetin-3-*O*-rhamninoside**	$C_{34}H_{42}O_{20}$	769	4.3/ $1.3E^5$	4.1/ $1.3E^5$	–	**4.6/ $1.3E^6$**	F, L, AP	(Marzouk et al., 1999; Benamar et al., 2019)
17	Rhamnazin	$C_{17}H_{14}O_7$	329	21.5/ $1.1E^5$	20.9/ $9E^4$	21.3/ $3.2E^5$	20.6/ $8.5E^4$	F, L, AP	(Cuoco, Mathe, and Vieillescazes, 2014; Ammar et al., 2009)
18	**Rhamnazin-3-*O*-acetyl- rhamninosid**	$C_{37}H_{46}O_{21}$	825	**5.6/ $2.2E^5$**	5.5/ $1.4E^5$	6.2/ $7.3E^4$	6.1/ $1.1E^5$	F	(Cuoco, Mathe, and Vieillescazes, 2014)
19	Rhamnazin-3-*O*-robinoside	$C_{29}H_{34}O_{16}$	637	10.1/ $1.8E^5$	9.6/ $9.8E^4$	9.8/ $6.3E^4$	9.6/ $6.5E^4$	AP	(Marzouk et al., 1999)
20	Rhamnocitrin	$C_{16}H_{12}O_6$	299	21.8/ $1.9E^5$	22.1/ $5.3E^5$	–	22.5/ $3.6E^5$	F, L, B, AP	(Nindi et al., 1999)
21	Rhamnocitrin-3-*O*-acetyl-rhamninoside	$C_{36}H_{44}O_{20}$	795	5.6/ $7.7E^4$	4.9/ $9E^4$	–	5.2/ $5.4E^4$	F	(Cuoco, Mathe, and Vieillescazes, 2014)
22	Rhamnocitrin-4′-*O*-rhamninoside	$C_{34}H_{42}O_{19}$	753	–	–	–	5.3/ $1.8E^5$	F	(Ammar et al., 2009)
23	Rhamnocitrin-3-*O*-rhamninoside	$C_{34}H_{42}O_{19}$	753	5.7/ $7.8E^4$	5.2/ $1E^5$	5.2/ $1.1E^5$	5.1/ $5E^5$	F, AP	(Ammar et al., 2009)
24	Mearnsetin	$C_{16}H_{12}O_8$	331	18.1/ $1.5E^5$	18.3/ $1.8E^5$	18/ $1.9E^5$	17.8/ $1.8E^5$	B*	(Sakushima et al., 1983)
25	Luteolin	$C_{15}H_{10}O_6$	285	–	21.3/ $3.8E^5$	22.1/ $3.6E^5$	21.9/ $5.3E^5$	L, B	(Chen et al., 2016; Moussi et al., 2015; Benamar et al., 2019)
26	**Apigenin**	$C_{15}H_{10}O_5$	269	23.9/ $1.4E^5$	**24.1/ $1.1E^6$**	**24.5/ $1.5E^6$**	**24.1/ $3.3E^6$**	L, B	(Chen et al., 2016; Ammar et al., 2009; Moussi et al., 2015)
27	**Orientin**	$C_{21}H_{20}O_{11}$	447	**15.5/ $2.2E^5$**	15.7/ $7.6E^4$	–	15.3/ $5.8E^4$	B	(Chen et al., 2016)
28	Isoorientin	$C_{21}H_{20}O_{11}$	447	15.6/	16/	–	–	B	(Chen et al., 2016)

(*continued on next page*)

Table 3 (*continued*)

No.	Compounds	Formula	[M−H]− (*m*/*z*)	Rt/ Intensity (E^n)				Parts reported in literature[1]	Ref.
				Fruit	Root	Bark	Leaf		
				1.9E^5	1.3E^5				
29	Vitexin	$C_{21}H_{20}O_{10}$	431	17.2/ 8.8E^4	17.3/ 1.1E^5	17.5/ 1.3E^5	–	B	(Chen et al., 2016)
30	Diosmetin-7-*O*-glucoside	$C_{22}H_{22}O_{11}$	461	18/ 6.8E^4	18.1/ 2.7E^5	17.9/ 1.4E^5	18.3/ 7.8E^4	B	(Chen et al., 2016)
31	Eriodictyol	$C_{15}H_{12}O_6$	287	22.8/ 1.1E^5	23.1/ 1.9E^5	23.5/ 4.4E^5	23.1/ 7.1E^4	L, B*, AP	(Sakushima et al., 1983; Marzouk et al., 1999; Benamar et al., 2019)
32	**Naringenin**	$C_{15}H_{12}O_5$	271	24.1/ 9.8E^4	–	**24.3/ 5.1E^5**	24.5/ 2.8E^5	B	(Chen et al., 2016)
33	Sakuranetin	$C_{16}H_{14}O_5$	285	–	25.2/ 2.2E^5	25.3/ 2.7E^5	25.5/ 5.1E^5	B	(Chen et al., 2016)
34	Sakuranetin dimer	$C_{32}H_{26}O_{10}$	551	24.9/ 5.7E^4	24.8/ 6.5E^4	25.3/ 3.2E^5	25.2/ 3.0E^4	B	(Chen et al., 2016)
35	Aromadendrin	$C_{15}H_{12}O_6$	287	–	23.3/ 1.7E^5	–	23.5/ 5.9E^4	B	(Sakushima et al., 1983; Chen et al., 2016)
36	Taxifolin	$C_{15}H_{12}O_7$	303	19.3/ 1.4E^5	–	19.0/ 1.0E^5	–	L, B*, AP	(Sakushima et al., 1983; Chen et al., 2016; Benamar et al., 2019)
37	Pallasiin	$C_{16}H_{14}O_8$	333	18.7/ 1.1E^5	19/ 1.1E^5	–	18.6/ 9.8E^4	B*	(Sakushima et al., 1983)
38	Protocatechuic acid	$C_7H_6O_4$	153	1.8/ 1.5E^5	1.7/ 1.9E^5	1.5/ 1.6E^5	1.6/ 1.1E^5	F, AP	(Marzouk et al., 1999; SATAKE et al., 1993)
39	p-hydroxybenzoic acid	$C_7H_6O_3$	137	2.1/ 2E^5	2.6/ 2E^5	2.4/ 3.2E^5	–	F	(SATAKE et al., 1993)
40	2–5-dihydroxybenzoic acid	$C_7H_6O_4$	153	–	–	2.1/ 2.6E^5	2.2/ 7.4E^4	AP	(Marzouk et al., 1999)
41	Gallic acid	$C_7H_6O_5$	169	1.8/ 1.6E^5	1.6/ 2.3E^5	1.5/ 3.1E^5	–	L	(Ammar et al., 2009; Moussi et al., 2015)
42	ferulic acid	$C_{10}H_{10}O_4$	193	–	2.1/ 1.3E^5	2.4/ 2.5E^5	–	L	(Ammar et al., 2009; Moussi et al., 2015)
43	p-Coumaric acid	$C_9H_8O_3$	163	3.4/ 1.4E^5	3.5/ 1.5E^5	–	–	L	(Ammar et al., 2009; Moussi et al., 2015)
44	Physclon-8-*O*-β-primeveroside	$C_{27}H_{30}O_{14}$	697	8.4/ 9.8E^4	8.5/ 5.3E^4	8.2/ 9.1E^4	8.1/ 1.2E^5	B*	(Coşkun et al., 1984)
45	**Emodin**	$C_{15}H_{10}O_5$	269	20.1/ 1.1E^5	20.6/ 7.2E^4	**20.4/ 1.3E^6**	**20.9/ 2.5E^6**	F, L, B	(Benamar et al., 2019; Nindi et al., 1999; SATAKE et al., 1993)
46	Chrysophanol	$C_{15}H_{10}O_4$	253	28.1/ 1E^5	–	28.3/ 1.6E^5	–	L	(Benamar et al., 2019; Nindi et al., 1999)
47	Physcion	$C_{16}H_{12}O_5$	283	28.2/ 8.1E^4	–	–	–	F, L, B	(Benamar et al., 2019; Nindi et al., 1999)
48	Physcion-8-*O*-glucoside	$C_{22}H_{22}O_{10}$	445	16.7/ 1.2E^5	16.8/ 1.2E^5	16.3/ 9.2E^4	16.1/ 6.6E^5	B	(Chen et al., 2016)
49	Physcion-8-*O*-rutinoside	$C_{22}H_{22}O_{10}$	591	13.1/ 1.1E^5	–	–	12.8/ 6.9E^4	B	(Chen et al., 2016)
50	Emodin-1-glucoside	$C_{21}H_{20}O_{10}$	431	–	–	13.8/ 2.2E^5	13.1/ 5.3E^4	F, B	(SATAKE et al., 1993)
51	Emodin anthrone	$C_{15}H_{12}O_4$	255	26.3/ 7.9E^4	–	26.1/ 1.6E^5	26.2/ 9.3E^4	F, L	(Benamar et al., 2019)
52	Emodin bianthrone	$C_{30}H_{22}O_8$	509	–	26.2/ 1.4E^5	25.8/ 1.8E^5	25.6/ 4E^5	F	(Bezabih and Abegaz, 1998)
53	Prinoidin	$C_{25}H_{26}O_{10}$	485	14/ 7.8E^4	13.8/ 2.4E^5	14.1/ 6.5E^4	13.9/ 5.6E^4	F	
54	**Sorigenin**	$C_{12}H_8O_4$	215	–	**27.9/ 3.5E^6**	28.1/ 8.9E^4	–	L, B*	(Nindi et al., 1999)
55	α-sorinin	$C_{24}H_{28}O_{14}$	539	12.5/ 8.3E^4	12.8/ 9.2E^4	12.3/ 2.6E^5	–	B	(Coşkun et al., 1984)
56	**Geshoidin**	$C_{18}H_{18}O_{10}$	377	28.6/ 8.2E^4	29.1/ 9.4E^5	–	**28.7/ 9.5E^5**	L	(Nindi et al., 1999)
57	**Isofraxetin**	$C_{10}H_8O_5$	207	28.2/ 1.2E^5	28.1/ 2.5E^5	**27.9/ 1.1E^6**	27.4/ 1.3E^5	AP	(Marzouk et al., 1999)
58	**Isotorachrysone**	$C_{14}H_{14}O_4$	245	27.9/	**27.5/**	–	–	B	(Hsiao et al., 1996)

Table 3 (*continued*)

No.	Compounds	Formula	[M−H]− (*m*/*z*)	Rt/ Intensity (E^n)				Parts reported in literature[1]	Ref.
				Fruit	Root	Bark	Leaf		
59	**Musizin**	$C_{13}H_{12}O_3$	215	$8.3E^4$ –	**$1.7E^6$ 26.3/ $2.5E^6$**	–	–	L	(Nindi et al., 1999)

[1] Plant parts from other *Rhamnus* species previously reported in literature; Fruit (F), Leaf (L), Flower (Fl), Bark (B), Seed (S), Root (R) and Aerial part (AP).* Plant parts reported from *R. pallasii* in the literature.

identified as kaempferol-*O*-rhamninoside isomers (kaempferol-3-*O*-rhamninoside and kaempferol-4′-*O*-rhamninoside) (Ammar et al., 2009; Marzouk et al., 1999). Two molecules, 13 and 14, were identified as rhamnetin and isorhamnetin at *m*/*z* 315. (Sakushima et al., 1983; Cuoco et al., 2014; Marzouk et al., 1999). Additionally, compounds 15 and 16 were identified as isorhamnetin-3-*O*-rhamninoside and rhamnetin-3-*O*-rhamninoside, respectively, due to their deprotonated molecules at *m*/*z* 769. Previously, these chemicals were discovered in the fruit and leaves of *R.catharticus* and *R.disperma* (Marzouk et al., 1999; Benamar et al., 2019). Rhamnazin 17 (*m*/*z* 329) and two rhamnazin-*O*-glycosides 18 (*m*/*z* 825) and 19 (*m*/*z* 637) were identified as rhamnazin-3-*O*-acetylrhamninosid and rhamnazin 3-*O*-D-robinoside, respectively. Compounds from other *rhamnus* species, including R. saxatilis, R.prinoides, R. alaternus, and R. disperma, were also given (Ammar et al., 2009; Cuoco et al., 2014; Marzouk et al., 1999). A $[M-H]^-$ at *m*/*z* 299 and 795 was used to identify rhamnocitrin 20 and its derivative, rhamnocitrin-3-O-acetyl-rhamninoside 21. For rhamnocitrin-4′-*O*-rhamninoside 22 and rhamnocitrin-3-*O*-rhamninoside 23, an identical pseudomolecular ion peak at *m*/*z* 753 was also found (Nindi et al., 1999; Cuoco et al., 2014; Ammar et al., 2009). Similarly, compound 24 was identified as mearnsetin, as previously described for *R. pallasii* (Sakushima et al., 1983).

3.5.1.2. Characterization of flavones. Eight compounds isolated from various sections of *R. pallasii* demonstrated flavone structural features. Five of these (25–29) were identified as the flavone aglycones luteolin, apigenin, orientin, isoorientin, and vitexin, respectively, using [M−H] ions at *m*/*z* 285, 269, 447, and 431. On the basis of comparisons to published data, one flavone glycoside (30) was identified from extracts as diosmetin-7-*O*-glucoside (*m*/*z* 461). Earlier this year, compounds with a similar pattern were found in *R. davurica*, *R. alaternus*, and *R. lycioides* (Moussi et al., 2015; Chen et al., 2016; Benamar et al., 2019; Ammar et al., 2009).

3.5.1.3. Characterization of flavanones. Four flavanone derivatives (31–34) were identified in the plant extracts with *m*/*z* values of 287, 271, 285 and 551 and were identified as eriodictyol, naringenin, sakuranetin, and sakuranetin dimer, respectively. For *R. disperma*, *R. lycioides*, and *R. davurica*, these chemicals were already mentioned in the literature (Sakushima et al., 1983; Marzouk et al., 1999; Benamar et al., 2019; Chen et al., 2016).

3.5.1.4. Characterization of flavanonols. Three flavanonol derivatives were found as previously published by *R. davurica*, *R.disperma*, and *R.lycioides*: aromadendrin 35 (*m*/*z* 287), taxifolin 36 (*m*/*z* 303), and pallasiin 37 (*m*/*z* 333). (Sakushima et al., 1983; Chen et al., 2016; Benamar et al., 2019).

3.5.2. Phenolic acids

Phenolic acids are another class of phenolic chemicals that are utilized to prevent heart disease. Additionally, they have an effect on the bitter and sour flavors of food plants (Rashmi and Negi, 2020). Essentially, hydroxybenzoic and hydroxycinnamic acids are two distinct subclasses of phenolic acids. Procatechuic acid 38 (*m*/*z* 153), p-hydroxybenzoic acid 39 (*m*/*z* 137), 2–5-dihydroxybenzoic acid 40 (*m*/*z* 153), and gallic acid 41 (*m*/*z* 169. 231). Ferulic acid 42 (*m*/*z* 193) and p-coumaric acid 43 (*m*/*z* 163) are hydroxycinnamic acid derivatives. These derivatives of *R. thymifolius*, *R. disperma*, and *R. alaternus* have already been reported in the literature (Marzouk et al., 1999; satake et al., 1993; Ammar et al., 2009; Moussi et al., 2015).

3.5.3. Anthraquinones

Natural pigment derivatives known as anthraquinones or anthracenedione are generated from anthracenes and include two keto groups on the central ring. They exhibit a broad range of biological properties, including antioxidant, antifungal, anticancer, and antibacterial properties (Malik and Müller, 2016). Compounds 44–46 were identified as physclon-8-*O*-β-primeveroside (*m*/*z* 697), aloe-emodin (*m*/*z* 269), and chrysophanol (*m*/*z* 253), respectively, based on the identical patterns stated previously. Previous works on *R.prinoides*, *R.thymifolius*, *R.lycioides*, and *R.libanoticus* have discussed the proposed structures (Benamar et al., 2019; Nindi et al., 1999; Satake et al., 1993; Coşkun et al., 1984). A molecule with *m*/*z* 283 was identified as physcion 47. This chemical was recently discovered in *Rhododendron davurica*, *R. lycioides*, *R. prinoides*, and *R. nakaharai* (Benamar et al., 2019; Nindi et al., 1999; Chen et al., 2016). Additionally, two anthraquinone glycosides, physcion 8-*O*-glucoside 48 (*m*/*z* 445) and physcion 8-*O*-rutinoside 49 (*m*/*z* 591), were discovered. Compound 50 was identified as emodin-1-glucoside at *m*/*z* 431. Our findings corroborated earlier research (Chen et al., 2016; Satake et al., 1993). Along with the anthraquinones, three anthrone derivatives were found, notably emodin athrone 51 (*m*/*z* 255), emodin bianthrone 52 (*m*/*z* 509), and prinoidin 53 (*m*/*z* 485). To our knowledge, these chemicals have been iso-

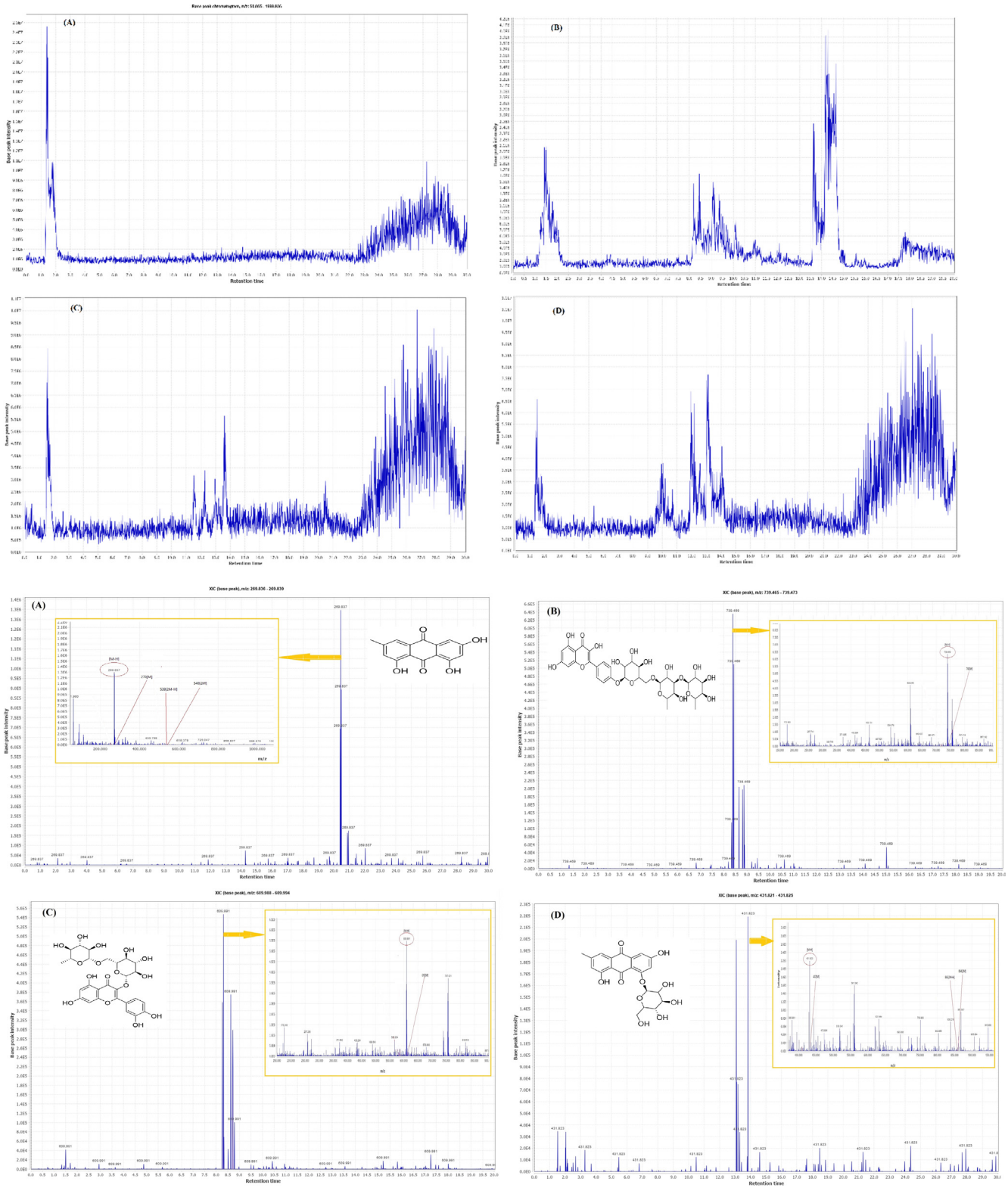

Fig. 3 Chromatograms and corresponding mass adducts in the HM extracts of *R. pallasii*. (A) Total ion chromatogram (TIC) of fruit; (B) TIC of leaf; (C) TIC of bark; (D) TIC of root; (E) Emodin chromatogram (XIC) and mass adducts, *m*/*z* 269.837; (F) Kaempferol-4′-*O*-rhamninoside XIC and mass adducts, *m*/*z* 739.469; (G) Quercetin-3-*O*-robinobios XIC and mass adducts, *m*/*z* 609.991; (H) Emodin-1-glucoside XIC and mass adducts, *m*/*z* 431.823.

lated from *R. prinoides*, *R. lycioides*, and *R. nepalensis* (Mai et al., 2001; Bezabih and Abegaz, 1998).

3.5.4. Other compounds

Three naphthaenic lactone compounds were identified in *R. pallasii* extracts. Sorigenin 54 possesses a deprotonated molecular ion peak at *m*/*z* 215 and was earlier found by *R.prinoides* (Nindi et al., 1999). In any case, chemicals 55 and 56 have been tentatively identified as α-sorinin (*m*/*z* 539) and geshoidin (*m*/*z* 377, respectively. These naphthalene glycosides are well-studied in *R.prinoides* (Coşkun et al., 1984; Nindi et al., 1999). A coumarin derivative was tentatively attributed to one of the compounds 57 with *m*/*z* 207. Isofraxetin was the name given to this chemical in the literature (Marzouk et al., 1999). Additionally, two naphthalene derivatives from *R. pallasii* were identified: isotorachrysone 58 (*m*/*z* 245.145) and musizin 59 (*m*/*z* 215.145). These chemicals were identified using previously published data on *R. davurica*, *R. nakaharai*, and *R.prinoides* (Chen et al., 2016; Hsiao et al., 1996; Nindi et al., 1999).

3.5.5. Comparison between phenolic compounds in different parts of R. Pallasii

The profile and relative intensity (E^n) of detected compounds varied according to the plant's morphology, as 49, 45, 41, and 48 compounds with varying intensities were discovered in fruit, leaf, bark, and root extracts, respectively. Flavonols (44.89 % of total phenols, $5.7E^4$ to $2.7E^5$) were found in the fruits, followed by anthraquinones (16.32 %, $4.7E^4$ to $1.2E^5$), flavones (10.20 %, $6.8E^4$-$2.2E^5$), phenolic acids (8.16 %, $1.1E^5$ to 2.0 E^5), flavanones (6.12 %, $5.7E^4$ to $1.1E^5$), flavanono (8.16 %, $8.2E^4$ to $1.2E^5$). In turn, flavonols (53.33 %, $4.6E^4$ to $1.3E^6$) were more abundant in leaves than anthraquinones (17.77 %, $4.0E^4$ to $2.5E^6$), flavanones (8.88 %, $3.0E^4$ to $5.1E^5$), flavones (6.66 %, $7.8E^4$-$3.3E^6$), flavanonols (4.44 %, $5.9E^4$ to $9.8E^4$), phenolic acids (4.44 %, $1.3E^5$ to $9.5E^5$). However, flavonols were the most abundant class in the barks (39.02 %, $6.3E^4$ to $3.6E^5$), followed by anthraquinones (19.51 %, $6.5E^4$ to $1.3E^6$), phenolic acids (12.9 %, $1.6E^5$ to $3.2E^5$), flavanones (9.75 %, $2.7E^5$ to $5.1E^5$), flavones (9.75 %, $1.3E^5$-$1.5E^6$), flavanon (12.5 %, $9.2E^4$ to $3.5E^6$). Among the phenolic chemicals found in plant components, the most abundant were quercetin-3-rhamninoside (flavonol, $6.9E^6$), sorigenin (naphthaenic lactone, $3.5E^6$), apigenin (flavone, $3.3E^6$), emodin (anthraquinone, $2.5E^6$), musizin and isotorachrysone (naphthalene derivatives, 1.7–$2.5E^6$) and rhamnetin-3-*O*-rhamninoside (flavonol, $1.3E^6$). Overall, all samples, particularly the fruit extract, exhibited a high concentration of flavonoid and phenolic acid components, indicating that they were excellent natural sources of antioxidant and antibacterial agents.

4. Conclusion

This is the only study that we are aware of that examines the phytochemical profile, antioxidant activity, and antibacterial activity of several sections of *R. pallasii* subsp. *sintenisii*. LC-ESI-MS and GC–MS analyses were performed on individual morphological parts of species (fruit, leaf, bark, and root) in order to identify the chemicals responsible for their biological activity. The HM extracts of all samples, particularly the fruit and leaf, were high in polyphenols, including flavonols, flavones, flavanones, flavanonols, phenolic acids, and anthraquinones, and shown substantial antioxidant and antibacterial activity. The EP extract of the root, on the other hand, was a rich source of terpenes and had substantial antibacterial activity. These results may be explained by components of quercetin-3-rhamninoside, apigenin, emodin, quercetin, isorhamnetin-3-*O*-rhamninoside, and orientin discovered in this work. Additionally, the polar and nonpolar extracts of this species may provide valuable natural chemicals for the creation of novel medications. Additional research on the morphological characteristics of species is required to unravel the mechanism of antioxidant and antibacterial activity and to isolate bioactive components from extracts with higher therapeutic effects.

CRediT authorship contribution statement

Soghra Mahmoodi: Investigation, Methodology. **Akram Taleghani:** Conceptualization, Funding acquisition, Writing – original draft, Software. **Reza Akbari:** Conceptualization, Investigation, Formal analysis. **Majid Mokaber-Esfahani:** Investigation, Methodology.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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