International Journal of Secondary Metabolite
2024, Vol. 11, No. 2, 235–243
https://doi.org/10.21448/ijsm.1391683
journal homepage: https://dergipark.org.tr/en/pub/ijsm
Research Article

Content of saponin, tannin, and flavonoid in the leaves and fruits of Iranian populations from *Rhamnus persica* Boiss. (Rhamnaceae)

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ARTICLE HISTORY

Received: Nov. 16, 2023
Accepted: Feb. 16, 2024

KEYWORDS

Rhamnus persica,
Phytochemical diversity,
Saponin,
Tannin,
Flavonoid
Population.

Abstract: To study the phytochemical properties in the leave and fruit of Iranian populations of *Rhamnus persica* Boiss, eighteen different populations from six geographical regions of Kermanshah province were studied. The results showed that the highest and lowest leaf saponin was present in Aliabad (6.40 mg/g) in Eslamabad-e Gharb and Chaharzebar in Mahidasht (3.29 mg/g), respectively. In fruit saponin, the highest content was in the population of Arkavazi in Eslamabad-e Gharb (4.33 mg/g) and the lowest being in Mahidasht population (Chaharzebar) and Sarpol-e Zahab (Galin) each with 2.20 mg/g. About the leaf tannin, the highest and lowest values were available in Barf-Abad population in Eslamabad-e Gharb (4.57 mg/g) and Chaharzebar in Mahidasht (1.83 mg/g), respectively. In addition, in the case of fruit tannin, the highest amount in Meleh-Sorkh population belonged to Homeil (2.74 mg/g) and the lowest being in of Changizeh population (Sonqor) in 2.28 mg/g. Moreover, the highest and lowest leaf flavonoids were obtained in two populations including Gordi in Eslamabad-e Gharb (6.14 mg/g) and Changizeh from Sonqor (4.09 mg/g), respectively. Regarding fruit flavonoids, the highest and lowest amounts were observed in populations of Meleh-Sorkh from Homeil (3.45 mg/g) and Banganjab in Mahidasht (2.13 mg/g). These findings confirmed the effect of geographical location on changing the phytochemical characteristics of *Rhamnus* populations in Kermanshah province from Iran.

1. INTRODUCTION

Study of different medicinal plants in each country is the first step to enter the global trade market of these important plants. Various biochemical properties such as saponin levels can be studied in various medicinal plants and even used as a marker to identify, differentiate, and classify of these plants (Paterson, 2019). Saponins are steroidal and triterpenoid glycosides that exhibit various biological activities. Extensive presence in plants and potential for medicinal usages has led to the extraction and identification of them in various plant species. Although,

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e-ISSN: 2148-6905

these efforts are important for expanding knowledge about saponin structures that are naturally produced by plants, biosynthesis, and their distribution in have recently been considered (Ahmed *et al*., 2008; Faizal & Geelen, 2013).

Tannins are different compounds that are usually hard and astringent, as well as the ability to adhere. They contain proteins and cause their deposition. Due to this property, they have been used for centuries to turn animal skins into leather (in tanning). Tannins are divided into several general categories, which include hydrolyzable (gallotanes and agitates), dense, and false tannins (complex) (Omidbeygi, 2008). Flavonoids include anthocyanin pigments and isoflavonoids. The main skeleton for the flavonoid group is flavan, flavonos, flavonols, and anthocyanins that are the most important groups of them. The most prominent flavonoids are also anthocyanins and anthocyanins (Lila, 2004).

Rhamnus persica Boiss. (Figure 1) is one of the valuable medicinal plants in the flora of Iran and due to the presence of some bioflavonoids, vitamins (especially vitamin C), valuable minerals, and organic acids are very important in the pharmaceutical industry around the world (Sattarian *et al.*, 2017). The Family Rhamnaceae includes about 100 to 125 species that are distributed in tropical and subtropical regions of the northern hemisphere and *Rhamnus* is the largest genus in this family (Kurylo *et al.*, 2007). The fruits in this genus are round with four-leafed ovaries and the seeds have yellow grooves (Mubberley, 1987; Soufiyan & Dinarvand, 2007). Eight terrestrial species of genus *Rhamnus* are known in Iran and are widely found in the Zagros, Iran-Turanian, and Caspian regions (Soufiyan & Dinarvand, 2007).

The vast plateau of Iran, while being a special geographical unit on the planet, has a variety of climates and environments. Therefore, various plant species are distributed in the country so that the rich flora of Iran includes more than 7500 plant species. The effect of climatic factors on different plants is variable and the role of these factors on the growth and development and effective compounds in medicinal plants should always be investigated with appropriate research. Although, the quantity of secondary metabolites is affected by the set of plant gene bank; but, their content, concentration, and accumulation are significantly affected by the environmental conditions (Omidbeygi, 2008).

So far, there is no study on the phytochemical properties of *R. persica* in Iran including the measurement of its saponin, tannins, and flavonoids. The present study was performed by using eighteen ecotypes of this medicinal plant and its purpose was to determine the content of different compounds in the leaves and the fruits of this valuable medicinal plant for finding a superior population for its later usages. The authors of this article hope to introduce suitable populations of *R. persica* with higher chemical compounds for their usage in Iran and in trade of medicinal plants. This study is very important; because, it is new and can be considered by researchers from different aspects.

Figure 1. *Rhamnus persica*.

2. MATERIAL and METHODS

2.1. Collecting of Samples

In this study, eighteen different populations (ecotypes) of *R. persica* were studied in Kermanshah province from west of Iran which location of them is shown in Table 1. The leaves and fruits of the plant were collected from its natural habitats and samples were identified according to the identification key in the herbarium of Razi University, Kermanshah. Then, were maintained in the refrigerator under a temperature between 4°C to 8 °C.

Table 1. Geographical characteristics of plant collection areas from *R. persica*.

Name	Latitude	Longitude	Height from the see level (m)
Gordi (Homeil)	33 50 21	49 51 22	1353
Meleh-Sorkh (Homeil)	33 57 20	46 55 05	1538
Kandahar (Homeil)	34 53 23	46 52 00	1601
Barf-Abad (Eslamabad-e Gharb)	34 04 41	46 31 18	1409
Ali-Abad (Eslamabad-e Gharb)	34 07 20	46 26 21	1376
Arkavazi (Eslamabad-e Gharb)	34 07 49	46 28 19	1402
Navdar (Guilan-e Gharb)	34 03 22	46 59 03	1026
Anari (Guilan-e Gharb)	34 07 57	46 53 02	895
Vizhenan (Guilan-e Gharb)	34 05 34	45 43 32	946
Nasar (Sarpol-e Zahab)	34 19 01	45 51 36	992
Galin (Sarpol-e Zahab)	34 15 49	45 56 36	796
Sorkheh-Dizeh (Sarpol-e Zahab)	34 24 57	46 01 59	1128
Ali-Yar (Mahidasht)	34 10 42	46 49 36	1511
Chaharzebar (Mahidasht)	34 14 00	46 41 29	1561
Banganjab (Mahidasht)	34 17 48	46 40 34	1450
Parsineh (Sonqor)	34 41 50	47 26 43	1819
Soltan Taher (Sonqor)	34 40 22	47 42 45	1804
Cahngizeh (Sonqor)	34 50 16	47 05 05	2023

2.2. Preparation of Methanolic Extract from *R. persica*

For extraction, mature leaves and fruits of *R. persica* were dried in the shade condition at room temperature and then pulverized. Extraction was performed for 48h by percolation method with methanol 80%. At the end, the extract was concentrated by a rotary apparatus and dried by oven at 60 °C. Finally, the purified extract was stored at 4 °C until its usage (Pormorad *et al.*,2016).

2.3. Saponin Assay

To extract the saponin, purified extract of alcoholic extract was used by diffusion method. The extract from the solvent was separated by vacuum distillation to identify the saponin and determine its amount, pour 1 g of methanolic extract into a test tube, some distilled water were added, and then shaken violently for 5 min. After standing for 30 min, the amount of saponin was measured from the height of the foam created in it (Salehi *et al.*, 1992).

2.4. Flavonoid Assay

To prepare flavonoid extract, method of soaking in methanol 70% with a ratio of 3 to 1 (3 part of methanol and 1 from plant powder) for 24h was used. After this time, the extract was filtered and spilled in a 100 mL jug with a methanol 70%. Then, 2 mL of methanolic extract of the plant with 1.5 mL of methanol, 0.1 mL of aluminum chloride (methanol10%), 0.1 mL of potassium acetate (1M), and 2.8 mL of distilled water was combined. Finally, the solutions were placed under the room temperature for 30 min and absorbance of each reaction compound was measured at 415 nm by spectrophotometer (Ranjith, 2009). The standard curve was prepared

with methanolic solutions at concentrations of 250 to 1000 µg/mL and the flavonoid concentration of the extract was calculated from the following formula.

$P = (C \times 100/1000 \times M) \times 100$

P = Percentage of flavonoids concentration

C = Flavonoid concentration calculated based on standard curve (mg/mL)

M = Sample weight (g)

2.5. Tannin Assay

In order to measure tannin, 1 mL of methanol containing 20 mg of polyvinyl pyrrolidine was added to 1 mL of methanolic extract, put in a vortex for 10 sec and then maintained in ice for 30 min. The samples were centrifuged for 6 min at 6000 rpm. Then, 1.5 mL of the centrifuged solution was poured into microtubes and 10 mg of polyvinyl pyrrolidine was added to it and the extract was re-extracted according to the above method. After centrifugation, 1 mL of the extract was separated and the tannins were measured according to the phenol measurement method. According to this method, tannin compounds were precipitated with polyvinyl pyrrolidine and their amount was calculated from the difference of adsorption rate (Ranjith, 2009).

2.6. Statistical Analysis

Statistical analysis of the data was done in the form of randomized complete block design (RCBD) with three replications and comparison of means was performed by Duncan's multiple range test ($p \leq 0.01$) by using SPSS software, version 22.

3. RESULTS

The results of ANOVA from saponin, tannin, and flavonoids content in the leaves and fruits of *R. persica* are shown in Table 2. Accordingly, different populations have these important compounds in their structure which showed a significant difference at the level of 1%. Also, the results of the mean comparison from saponin, tannin, and flavonoids in the leaves and fruits are also shown in Table 3. Accordingly, in the case of leaf saponin, the highest value was in Ali-Abad population (6.40 mg/g) in Eslamabad-e Gharb and the lowest being Chaharzebar (3.29 mg/g) belong to Mahidasht. By comparing different populations, it was found that the differences between the populations of Sonqor (Soltan Taher) and, Sarpol-e Zahab (Nesar), difference between the populations of Guilan-e Gharb (Anari and Vizhenan), difference between the populations of Eslamabad-e Gharb (Barf-Abad) and Guilan-e Gharb (Navdar), difference among the populations of Sarpol-e Zahab (Galin and Sorkheh-Dizeh) and Mahidasht (Ali-Yar) and finally the difference among the populations of Homeil (Gordi), Mahidasht (Banganjab) and Sonqor (Parsineh and Cahngizeh) were not significant. Regarding the fruit saponin, the highest content was determined in the population of Eslamabad-e Gharb (Arkavazi) at 4.33 mg/g and the lowest being in the population of Mahidasht (Chaharzebar) and Sarpol-e Zahab (Galin) at 2.20 mg/g. Also, differences among the populations of Homeil (Gordi), Sarpol-e Zahab (Galin), Mahidasht (Banganjab), and Sonqor (Changizeh), differences among the populations of Homeil (Meleh-Sorkh), Eslamabad-e Gharb (Arkavazi), Guilan-e Gharb (Navdar), and Sonqor (Parsineh), difference between the populations of Sarpol-e Zahab (Nesar) and Mahidasht (Chaharzebar), and finally the differences among the populations of Guilan-e Gharb (Anari and Vizhenan) and Mahidasht (Ali-Yar) were not significant.

About the leaf tannins, the highest and lowest amounts were presented in Meleh-Sorkh (4.57 mg/g) populations in Eslamabad-e Gharb and Chaharzebar (1.83 mg/g) populations in Mahidasht, respectively. By comparing of different populations, it was found that the differences between the populations of Guilan-e Gharb (Navdar) and Sonqor (Soltan Taher), the difference between the populations of Sarpol-e Zahab (Nesar) and Sonqor (Parsineh), and

finally the difference between the populations of Galin and Sorkheh-Dizeh in Sarpol-e Zahab were not significant. In the case of fruit tannin, the highest amount in Meleh-Sorkh population (2.74 mg/g) belonged to Homeil and the lowest being in the population of Sonqor (Changizeh) was determined to be 2.28 mg/g. In this case, the differences between the populations of Sonqor (Soltan Taher) and Sarpol-e Zahab (Nesar) and finally the differences between the populations of Guilan-e Gharb (Navdar) and Mahidasht (Chaharzebr) were not significant.

Table 2. ANOVA of phytochemical properties from *R. persica*.

Sources of changes	Leaf Saponin	Fruit Saponin	Leaf Tannin	Fruit Tannin	Leaf Flavonoid
Treatment (populations)	23.834	0.846	1.483	0.069	0.285
Block (repeat)	0.117	0.141	0.117	0.0001	0.005
Error	0.216	0.1	0.002	0.002	0.0002

Values of treatment, block, and error.

The highest and lowest leaf flavonoids were obtained in Gordi population (6.14 mg/g) in Homeil and Changizeh population (4.09 mg/g) belonging to Sonqor, respectively. In this case, the difference between the populations of Navdar and Anari in Guilan-e Gharb, the difference between the populations of Guilan-e Gharb (Vizhenan) and Mahidasht (Banganjab), the difference among the populations of Homeil (Meleh-Sorkh), Sarpol Zahab (Galin), and Sonqor (Changizeh) were not significant. Regarding fruit flavonoids, the highest and lowest values were determined in the population of Meleh-Sorkh (3.45 mg/g) belonging to Homeil and Bangangab (2.13 mg/g), respectively. In this case, the difference between the populations of Gordi and Meleh-Sorkh in Homeil, the difference among the populations of Sarpol Zahab (Sorkheh-Dizeh), Guilan-e Gharb (Navdar) and Mahidasht (Ali-Yar and Chaharzebar), the difference among the populations of Guilan-e Gharb (Viznan) and Sarpol-e-Zahab (Nesar and Galin) and Mahidast (Ali-Yar), and finally the differences between the populations of Homeil (Kandahar) and Sonqor (Changizeh) were not significant.

Table 3. Mean comparison from leaf and fruit saponin, tannin, and flavonoids from *R. persica*.

Population	Leaf Saponin (mg/g)	Fruit Saponin (mg/g)	Leaf Tannin (mg/g)	Fruit Tannin (mg/g)	Leaf Flavonoid (mg/g)	Fruit Flavonoid (mg/g)
Gordi (Homeil)	3.38 ^{ji}	2.37 ^{f}	2.67 ^{fg}	2.68 ^{b}	6.14 ^{b}	3.44 ^{a}
Meleh-Sorkh (Homeil)	3.50 ^{lhi}	2.43 ^{ef}	3.63 ^{cd}	2.74 ^{a}	6.08 ^{c}	3.45 ^{a}
Kandahar (Homeil)	3.82 ^{fg}	2.73 ^{cdef}	3.67 ^{C}	2.63 ^{e}	6.04 ^{d}	3.34 ^{bc}
Barf-Abad (Eslamabad-e Gharb)	4.30 ^{e}	3.30 ^{bc}	4.57 ^{a}	2.65 ^{c}	4.40 ^{j}	3.12 ^{fg}
Ali-Abad (Eslamabad-e Gharb)	6.40 ^{a}	4.23 ^{a}	2.52 ^{h}	2.46 ^{k}	4.95 ^{e}	3.24 ^{de}
Arkavazi (Eslamabad-e Gharb)	3.93 ^{f}	4.33 ^{ef}	2.72 ^{f}	2.34 ^{n}	4.87 ^{f}	2.58 ^{j}
Navdar (Guilan-e Gharb)	4.33 ^{e}	2.50 ^{ef}	2.92 ^{e}	2.59 ^{f}	5.34 ^{a}	2.91 ^{h}
Anari (Guilan-e Gharb)	5.13 ^{d}	3.00 ^{bcde}	2.63 ^{g}	2.64 ^{d}	5.35 ^{a}	3.06 ^{g}
Vizhenan (Guilan-e Gharb)	5.07 ^{d}	3.00 ^{bcde}	2.32 ^{ji}	2.50 ^{j}	4.73 ^{g}	2.75 ^{i}
Nasar (Sarpol-e Zahab)	5.40 ^{c}	3.23 ^{bcd}	2.17 ^{k}	2.51 ^{i}	4.39 ^{j}	2.74 ^{i}
Galin (Sarpol-e Zahab)	3.77 ^{fgh}	2.20 ^{f}	2.25 ^{jk}	2.53 ^{h}	5.08 ^{c}	2.70 ^{i}
Sorkheh-Dizeh (Sarpol-e Zahab)	3.33 ^{fgh}	2.67 ^{def}	2.24 ^{jk}	2.54 ^{g}	4.85 ^{f}	2.89 ^{h}
Ali-Yar (Mahidasht)	3.70 ^{fgh}	3.03 ^{bcde}	2.39 ^{i}	2.63 ^{e}	5.35 ^{a}	2.88 ^{h}
Chaharzebar (Mahidasht)	3.29 ^{j}	2.20 ^{bcd}	1.83 ^{l}	2.59 ^{f}	4.51 ^{i}	2.92 ^{h}
Banganjab (Mahidasht)	3.38 ^{ij}	2.30 ^{f}	2.60 ^{g}	2.43 ^{l}	4.74 ^{g}	2.13 ^{fg}
Parsineh (Sonqor)	3.37 ^{ij}	2.53 ^{ef}	2.22 ^{k}	2.34 ^{m}	4.65 ^{h}	3.24 ^{de}
Soltan Taher (Sonqor)	5.60 ^{c}	3.40 ^{b}	2.85 ^{e}	2.52 ^{i}	4.88 ^{f}	3.16 ^{ef}
Cahngizeh (Sonqor)	3.38 ^{ij}	2.30 ^{f}	3.56 ^{d}	2.28 ^{o}	4.09 ^{c}	3.32 ^{bc}

Different letters indicate a significant difference in Duncan's multiple range test ($p \leq 0.01$).

4. DISCUSSION and CONCLUSION

Saponins have many medicinal properties; but, unfortunately few studies have been done on them in various plants, especially in *R. persica* and other plants in the family Rhamnaceae, some of which are discussed below. Generally, saponins were defined as a group of naturally occurring plant glycosides characterized by formation of strong foam in aqueous solutions. To date, saponins have been reported in more than 100 plant families, among which at least 150 types of them have significant anti-cancer properties. There are more than eleven premium classes of saponins including dammaranes, tirucallanes, lupanes, hopanes, oleananes, taraxasteranes, ursanes, cycloartanes, lanostanes, cucurbitanes and steroids. Due to their great structural diversity, saponins always show good antitumor effects through a variety of antitumor pathways. In addition, there is a large amount of saponin that has not yet been identified or studied by chemists (Ando *et al.,* 2008; Acharya *et al.,* 2010).

Certain saponins with strong antitumor effects have also been identified. Ginsenosides, which belong to dammaranes, prevent tumor cells from invading and metastasizing by inhibiting it angiogenesis and suppressing in vascular endothelial cells and then preventing their adhesion. Dioscinis are other steroid saponins whose aglycone diosgenin has been extensively studied for its antitumor effect by stopping cell cycle and apoptosis. Other important molecules discussed include oleanane saponins such as avicins, platycodons, saikosaponins, and soysaponins along with tubeimosides (Man *et al.*,2010).

Kimura *et al.,* (1981) in their studies on the medicinal plant *Hovenia dulcis* Thunb obtained three new saponins including C2(1a), D(2a), and G(3a) from its leaves, which are very valuable and further studies are needed to determine the effects of these newly identified compounds. Tadesse *et al.,* (2012) in their studies on the analysis of phytochemical properties in the root extract of the medicinal plant *Helinus mystacinus* (Aiton) E.Mey. ex Steud. from the family Rhamnaceae stated that the essential oil extract of this plant contains saponins, terpenoids, and glycosides and its methanolic extract also contained alkaloids, saponins, tannins, terpenoids, and glucosides. On the other hand, the oil extract of the plant did not contain any of these tested compounds. Based on phytochemical screening experiments, chloroform extract was subjected to column chromatography and showed two compounds called Betulinic Acid-1 and Benzoic Acid-2 in its structure. In the present study, the leaf and fruit extracts of *R. persica* were studied and only methanolic extraction was performed, which was one of the important reasons for the differences in the results of two studies. However, plant species were also different in them.

Seri Seriab *et al.,* (2020) in their studies on the medicinal plant *Ziziphus mauritiana* Lam from the family Rhamnaceae which is traditionally used in treatment of various diseases found that the main constituents of the methanolic extract from the leaves in growing plant was O-malonyl-ziziphus saponin compound with nine known compounds consisted of two saponins, six flavonoids, and a calcon derivative. The structure of these compounds was determined by analyzing D1, D2-NMR2 spectroscopy data as well as mass spectrometry. Part of *Zizyphus* saponin I (2) along with the other compounds was isolated from the leaves of this plant for the first time. Although in the present study, chemical separation and identification of saponins was not performed; but, this field of research can open many windows of studies on different genera of this family.

There are no direct studies on the study of phytochemical properties, such as the measurement of tannins and flavonoids in *R. persica*; but, there are studies on related species, some of which are mentioned. Pawlowska *et al.*,(2009) investigated flavonoids in *Zizyphus jujuba* and *Zizyphus spina-christi* fruit and concluded that the six major compounds were purified by Sephadex LH-20 column chromatography followed by using HPLC spectroscopy and NMRs. A glycoside C, 5'-di-C-β-d-glucosylphloretin was also detected in *Z. spina-christi*. Quantitative analysis of all compounds reported higher flavonoids content in *Z. jujuba*. Ahmed

et al., (2019) in their study on evaluation of antioxidant activity and phytochemical screening from leaves, skin, stem, and fruit of *Alphitonia philippinensis* Braid from Brunei Darussalam found that most of antioxidant activity in leaf extract can be higher total phenolic content and total flavonoids and flavonols are correlated. According to antioxidant content and results of the assay, the leaf extract had the highest antioxidant properties followed by fruit, shell, and stem. Also, Kohansal-Vajargah *et al.*, (2019) in their study on evaluation and comparison of morphological and phytochemical characteristics from 14 ecotypes of *Z. jujuba* found that flavonoid function was positively and significantly correlated with fruit length and width with length to width ratio. Phenol yield also had a positive and significant relationship with fruit length and width and tannin yield showed a positive and significant correlation with phenol. The results ofmorphological traits including leaf length and width and length to width ratio, fruit length, and width and length to width ratio and 1000-seed weight showed germination type of this medicinal plant in Iran. In terms of morphological traits, ecotype had the maximum effective substance and no statistically significant difference was observed in chemicals. Based on cluster analysis, 14 ecotypes were divided into two separate groups and evaluation of morphological and phytochemical traits showed high diversity among ecotypes. In another study, Murtala *et al.*, (2019) examined the phytochemical screening and physicochemical properties of *Ziziphus abyssinica* when found that phytochemical screening for presence of alkaloids, flavonoids, saponins, phenols, tannins, carbohydrates, glycosides, glycosides, showed ions and triterpenes in aqueous and methanolic extracts. However, steroids were not available. Quantitative chemical analysis showed that flavonoids (0.134 mg/g) were the highest chemical in the leaves while the lowest were saponins (0.6 mg/g).

Characteristics of the studied geographical areas and their effect on the amount of saponin, tannin, and flavonoids in the leaves and fruits of *R. persica* cannot be said with certainty. Based on means comparison, it can be said that similar areas in terms of the climate has almost the same amount of these valuable compounds; but, this position needs further research. Another subject that need more work is to accurately identify saponins types in leaves and fruits and even comparison of them to each other. Unfortunately, as mentioned earlier, studies on this valuable plant are very limited. The authors of this article hope that future researchers will take further steps in this area and explore more characteristics of this valuable plant.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Peymen Asadi: Investigation and Resources. **Masoumeh Farasat**: Visualization and Software. **Mehrnoush Tadayoni**: Formal Analysis and Writing original draft. **Sina Attar Roshan**: Methodology. **Neda Hassanzadeh**: Supervision and Validation

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