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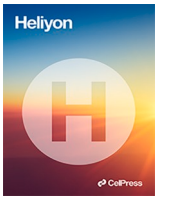

Research article

Physicochemical, nutritional properties, and antioxidant potential of 'limilla' fruit (*Rhus aromatica* var. schmidelioides (Schltdl.) Engl.)

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ABSTRACT

Rhus aromatica inhabits humid oak and oakpine forests in the State of Michoacán (Mexico). The fruit of *R. aromatica* is edible and is traditionally used in the preparation of soft drinks, ice pops, ice creams and 'atole'. The objective of the present investigation was to carry out a physical and chemical characterization and analysis of the antioxidant capacity of fruit. For the physical characterization, the equatorial and longitudinal diameter, weight and percentage of pulp were determined. In the chemical characterization, a proximal analysis was carried out, quantification of polyphenols and flavonoids was performed, and the antioxidant capacity was determined. The results showed that the fruit had a longitudinal diameter of 6.58 ± 1.02 mm, an equatorial diameter of 7.17 ± 0.66, a weight of 55.22 ± 5.47 mg, and a 40 % pulp proportion. The chemical characterization analysis indicated 8.7 % moisture, 30.6 % lipids, 8.7 % proteins, 29.4 % total sugars, 3.8 % ashes and 18.7 % crude fibre, 3.1 °Brix, pH 3.1, 1.92 % acidity total and a caloric intake of 4.27 kcal/g. The polyphenol content was higher in 60 % ethanol extracts with 88.6 ± 50.89 mg EAG/g; for flavonoids from extracts with 100 % acetone, it was 26.52 ± 0.65 mg EQ/g, and the total carotenoid content was 46.37 mg/100 g. The total antioxidant activity was higher in extracts with 80 % acetone, with 87.17 % inhibition of the DPPH radical and 90 % inhibition of ABTS without showing a significant difference with the different solvents used. The lowest IC_{50} values were presented in 100 % ethanol and 60 % methanol extracts for the DPPH radical and for the ABTS radical were the 80 % ethanol and 60 % methanol extracts. The lipid, protein, carotenoid, and polyphenol contents and antioxidant capacity of the fruit of *R. aromatica* were as high as those of other fruits consumed in the human diet.

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1. Introduction

The edible fruits of various plant species have considerable levels of functional compounds that provide health benefits beyond their basic nutritional value [1,2]. Among the active compounds that provide functional attributes, natural antioxidants have drawn attention due to their safety and broad therapeutic effects [3]. The trend to consume little-known wild edible fruits is due to the numerous beneficial health effects and organoleptic properties. Great interest has been shown in wild fruits due to their importance as a supplement in the diet of rural populations [4–6]. However, the consumption of wild fruits has decreased because conventional fruits have been improved genetically and agronomically. In this situation, scientific validation of the beneficial contributions to health and nutritional value would increase the added value of wild fruits [7,8]. The rescue of wild species, sustainable development in rural communities and food security, together with interest in the use of underutilized species, could allow the resumption of the use and consumption of wild fruits [9–11].

Regarding the bioactive properties of wild fruits, most research has focused on demonstrating their antioxidant properties [7]. The evaluation of the antioxidant activity in fruits is important since various studies have shown that the intake of some types of fruits has been related to a lower risk of suffering from cancer and has also been related to the prevention of chronic degenerative diseases [12–14]. Polyphenols, flavonoids and carotenoids are bioactive compounds present in foods of plant origin. The study of these compounds in foods of natural origin has increased in recent years due to their importance in protecting health and improving nutrition and their high antioxidant capacity against the action of free radicals; therefore, they contribute to disease prevention [15]. Fruits contain bioactive phenolic compounds such as flavonoids, phenolic acids, stilbenes, coumarins, tannins, and anthocyanins, among others, which play an important role in the attenuation of free radicals [16–18].

Carrying out studies that focus on compounds present in wild fruits with a beneficial effect on health may help to understand the benefits and promote a greater consumption of these fruits, including their use for the formulation of functional foods, nutraceutical and pharmaceutical products, and increase the variety in diet [19].

Rhus aromatica, commonly called fragrant sumac, also known locally as 'limilla', 'jaripos' or 'agrillos', is a wild fruit produced by a plant that belongs to one of the 250 species of the *Rhus* genus. Species in this genus are widely distributed in temperate and tropical regions around the world. In Mexico, it is possible to find *R. aromatica* fruit in the area of 'El Bajío', which includes the states of Michoacán, Guanajuato and San Luis Potosí. In the municipality of Puruándiro, Michoacán state, the fruit is collected by the inhabitants and is sold during the months of April to June, but its use is limited only to the preparation of soft drinks, ice cream, ice pop and 'atole' [20].

More than 200 chemical components have been identified in fruits of species of the genus *Rhus*. The main chemical components include hydrolysable tannins, phenolic acids, anthocyanins, flavonoids, organic acids, terpenoids and essential oils in the case of *R. coriaria* [21]; antioxidants, phenolics (gallic acid, tannic acid) and flavonoids in *R. chinensis* [22]; and flavonoids, anthocyanins and pyranoanthocyanins in *R. typhina* [23]. The aforementioned components are attributed to different pharmacological properties, such as antileishmanial, antibacterial, antioxidant, anti-inflammatory, antidiabetic, antihyperlipidemic, neuroprotective, and cardioprotective properties, ameliorating hepatic glycolipid metabolism disorder [24–30]. However, for the species *R. aromatica*, basic information such as physicochemical characteristics, proximal composition, and the presence of antioxidant components is limited. Therefore, it is important to explore the chemical composition and biological activity in the fruits of this species.

Some reports have described that fruits of other species of the *Rhus* genus (*R. coriaria*, R. *chinensis*, and *R. typhina*) have bioactive properties due to the presence of flavonoids, isoflavonoids, hydrolysable tannins, anthocyanins, and other phytochemical components, which are compounds responsible for antimicrobial, anticancer, antihyperglycemic, antihyperlipidemic, anti-inflammatory, and antioxidant activities [21–23]. However, for the species *R. aromatica*, basic information such as physicochemical characteristics and proximal composition is limited; therefore, it is important to explore the chemical composition and biological activity of the fruits of this species. Another type of compound with antioxidant potential and an important role in disease prevention is carotenoids, which are important lipophilic secondary metabolites with antioxidant properties [31]. There is no report on the presence of this type of compound in species of the genus *Rhus* because carotenoids are natural pigments of yellow, orange and red colors, and the presence of these compounds in the fruits of species of the genus *Rhus* was assumed. Therefore, the objective of this research was to determine the physical properties and chemical composition of the *R. aromatica* fruit, as well as to quantify the total content of phenolic compounds, flavonoids, carotenoids, and antioxidant capacity.

2. Materials and methods

The fruits of *R. aromatica* were acquired in stores in the municipality of Puruándiro (Michoacán state, Mexico). The fruits were selected and allowed to dry for 24 h at 40 °C in a drying oven (Novatech, HS45AID, Mexico).

2.1. Determination of physical characteristics of the fruit: weight, dimensions and proportion of pulp-seed

The physical characteristics of the fruit were evaluated from 50 fruits with commercial maturity taken at random, to which the weight was determined with the help of an analytical balance (Precisa, Switzerland-Dietikon). The longitudinal and equatorial diameter and thickness of the fruit were measured with a digital Vernier calliper (Luzeren, Mexico). The seed was separated from the pulp, and the mass of both parts was determined to calculate the proportion of the pulp.

2.2. Determination of the physicochemical characteristics: °Brix, titratable acidity, pH, and soluble solids

To determine degrees Brix (°Brix), titratable acidity and pH, 2 g of dehydrated pulp was mixed with 100 ml of distilled water [32]. The pH was measured using a potentiometer (HANNA Instruments, Romania). Soluble solids were measured with a portable digital refractometer (HANNA Instruments, Romania). The titratable acidity was obtained from 100 mL of the aqueous solution of the fruit mixed with 0.3 mL of 1 % phenolphthalein as an indicator, and the titration was carried out with 0.1 N NaOH. The results were expressed as % citric acid (% w/w).

2.3. Proximal composition

2.3.1. Determination of moisture content

The moisture content (*Hbs*) of the fruit was determined by the oven-drying method [33]. Ten grams (*mh*) of fresh fruit was weighed to dehydrate in a drying oven (Novatech, HS35-ED, Mexico) at 80 °C until a constant weight (*ms*) was achieved. The moisture percentage was calculated using the following formula:

$$Hbs\ (\%) = (mh\text{-}ms)/ms \times 100$$

2.3.2. Determination of total lipid content

The total lipid content determination was carried out using the Soxhlet method [34]. Briefly, 3 g of sample (*m*) was placed in a cellulose cartridge for extraction, 200 mL of hexane was added to the system, and the extraction was carried out by recirculating the solvent for 2 h. Then, the solvent was evaporated, and the extract (*m2*) was obtained. The fat content present in the sample was calculated with the following formula:

$$\text{Crude fat}\ (\%) = (m2\text{-}m1)/m \times 100$$

where m is the weight of the sample; *m1* is the weight of the flask alone; and *m2* is the weight of the flask with grease.

2.3.3. Determination of ash content

The ash content was determined by the muffle calcination method [35]. Two grams of dry sample was weighed into a clean porcelain crucible (*W1*) and placed in a muffle (Novatech, HS35-ED, Mexico) for approximately 5 h at a temperature of 500 °C. After 5 h, the crucible was removed and placed in a desiccator until it cooled, and its final weight (*W2*) was recorded. The total ash content was calculated with the following formula:

$$\text{Ash content}\ (\%) = (W2/W1) \times 100$$

2.3.4. Determination of total carbohydrates

The total carbohydrate content was quantified by the phenol-sulfuric method [36]. From an extract prepared with 0.1 g of dried fruit in 5 ml of distilled water. The reaction was carried out by mixing 125 μL of the previously prepared extract with 250 μL of 5 % aqueous phenol and 625 μL of concentrated H_2SO_4 and recording the absorbance at 490 nm. The calculation of total carbohydrates was carried out using the equation obtained from the calibration curve that was constructed from the known glucose concentrations 0.2, 0.6, 1.0, 1.4, 1.8 and 2.0 μg/mL.

2.3.5. Determination of crude protein content

Crude protein was determined according to the micro-Kjeldahl method [37]. Briefly, 1.0 g pulverized dry sample was digested in 10 mL H_2SO_4 at 420 °C using copper sulfate and potassium sulfate as the catalyst mixture. The digested sample was distilled using 40 % NaOH. Ammonia was captured in a 4 % boric acid solution and then titrated with 0.02 N HCl to estimate the total nitrogen content. The crude protein content was evaluated using a factor of 6.25.

2.3.6. Determination of fibre content

The fibre content was determined by the AOAC [38] nonenzymatic gravimetric method with some modifications. Defatted and pulverized 2 g samples were boiled in 0.25 N H_2SO_4 solution for 30 min. Next, the hydrolysed fraction was filtered using a Buchner funnel, and the residue was washed with water. The residue obtained was boiled in a 0.3 N NaOH solution for 30 min, filtered under vacuum and washed with hot water. The residue obtained was washed with a 0.25 N H_2SO_4 solution and then with hot water, followed by three washes with petroleum ether. The final residue was placed in a crucible and dried in an oven at 105 °C for 12 h. After the drying time, the mass of the crucible with residue was recorded, and then it was introduced into a muffle furnace at 550 °C for 3 h. Then, it was cooled, and the mass of the final residue was recorded to carry out the corresponding calculation with the following equation:

$$\text{Fibre content}\ (\%) = (A\text{-}B)/C \times 100$$

where *A* is the weight of the crucible with dry residue (g), *B* is the weight of the crucible with ash (g), and *C* is the weight of the sample (g).

2.3.7. Caloric intake

The caloric intake of the fruit was calculated using the Atwater factors: 9 kJ/g lipids, 4 kJ/g carbohydrates and 4 kJ/g proteins [39].

2.4. Preparation of extracts

The fruit pulp, separated from the seed, was dehydrated at 40 °C in a convective dryer for 24 h. For this study, extracts were prepared in glass bottles from 0.125 g of pulp with 5 ml of the different solvents under agitation for 24 h at room temperature (25 °C) in the dark; the solvents used were absolute methanol, 80 % methanol, 60 % methanol, absolute ethanol, 80 % ethanol, 60 % ethanol, absolute acetone, 80 % acetone and 60 % acetone. The extracts were centrifuged at 1500 rpm to obtain the supernatant, and the quantification of polyphenols, flavonoids and antioxidant capacity was immediately performed [40,41].

2.4.1. Quantification of total polyphenols and flavonoids

The total content of polyphenols was determined by the Folin-Ciocalteu method [42] with some modifications; the calibration curve was made with gallic acid at concentrations of 40, 80, 120, 160, 200, 240, 280, 320, 360, 380, 420 and 500 μg/mL. Fifty microliters of each concentration was taken, 750 μL of distilled water and 50 μL of Folin-Ciocalteu reagent were added, and the mixture was left to settle for 3 min. Then, 150 μL of 15 % sodium carbonate solution was added, and the mixture was incubated for 30 min at 50 °C. After incubation, 250 μL of each of the prepared solutions was used to measure the absorbance in a microplate absorbance spectrophotometer (Bio-Rad, xMarkTM, USA) at a wavelength of 760 nm. For the quantification of total polyphenols in the extracts, 50 μL of the extracts was used. The calculation of total polyphenols was carried out using the equation obtained from the calibration curve, and the result was expressed in equivalent milligrams of gallic acid/g of dry weight (mg EGA/g).

The determination of total flavonoids was carried out by the formation of aluminum complexes [43]. Fifty microliters of extract was added to a tube containing 100 μL of 1 M potassium acetate, 100 μL of 10 % aluminum nitrate and 500 μL of 80 % ethanol, and the mixture was left to rest for 30 min at room temperature. Subsequently, 250 μL of the mixture was taken and placed in a microplate, and the absorbance was read in a spectrophotometer at a wavelength of 415 nm. The calculation of total flavonoids in the extracts was carried out using the equation obtained from the quercetin calibration curve that was built from the concentrations of 60, 100, 140, 180, 220, 240, 280 and 300 μg/mL, and the results were expressed in milligrams quercetin equivalents/g dry weight (mg EQ/g).

2.4.2. Determination of total carotenoid content

The determination of carotenoids was performed by spectrophotometry, with some modifications [44]. Briefly, 2 g of dried fruit was homogenized in 20 mL of a mixture of acetone:ethanol (1:1) and left to settle for 24 h at 4 °C. Subsequently, the solution was filtered and adjusted to 100 mL with an acetone:ethanol (1:1) mixture. Then, the solution was transferred to a separatory funnel, 50 mL of hexane and 25 mL of water were added, and the mixture was stirred for 5 min. The mixture obtained was left to rest for 30 min for phase separation. The organic phase was recovered, and the absorbance was measured at 470 nm in a microplate spectrophotometer (Bio-Rad, xMark ™, USA). The results were expressed as μg β-carotene equivalent/100 g using the following equation:

$$\mu g\ \beta\ \text{carotene equivalent}/100\ g = (A \times V \times 106)\ /A1cm \times 100 \times PMx$$

where *A* is the sample absorbance, *V* is the total volume of the extract (mL), *A1cm* is the β-carotene absorptivity coefficient (2500), and *PMx* is the sample weight (g).

2.5. Antioxidant capacity

Because antioxidant capacity is a complex property, it is recommended to use more than one method to measure it. For this study, the ABTS and DPPH assays were chosen. The first allows the determination of the antioxidant capacity of both lipophilic and hydrophilic compounds, which is an advantage due to the type of solvents used for the preparation of the extracts. The DPPH assay evaluates the antioxidant capacity of hydrophilic compounds that have been extracted with organic solvents in solution.

2.5.1. Antioxidant capacity, DPPH assay

The antioxidant capacity of pulp extracts from *R. aromatica* fruits was evaluated using the DPPH assay [45] with some modifications [46]. The 0.1 mM DPPH radical was adjusted to an absorbance of 0.75 at a wavelength of 515 nm using a spectrophotometer. Next, 230 μL of the radical with 20 μL of extract was placed in a microplate, the mixture was incubated in the dark at room temperature for 30 min, and the absorbance at 515 nm was measured and contrasted against control samples. Antioxidant activity was expressed as the percentage of DPPH radical scavenging activity (% RSA) relative to the control. The dose response was also obtained at different concentrations of methanolic extracts against the radical (0.1, 0.5, 1.0, 2.0, 4.0 and 6 μg/ml). Finally, the effective concentration of antioxidant required to decrease the initial concentration by 50 % (IC_{50}) was determined according to Brand-Williams et al. [47].

2.5.2. Antioxidant capacity, ABTS assay

The antioxidant capacity assay by ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) was performed as described by Re

et al. [48] with some modifications. The stock solution was prepared with 14 mM ABTS mixed with 2.6 mM potassium persulfate and left to rest for 24 h at 4 °C. To carry out the assay, the solution was adjusted to an optical density of 0.75 at 734 nm. A total of 230 μL was placed in a microplate of the ABTS radical and 20 μL of the extracts at the concentrations already described for the DPPH assay, and the absorbance was read. In both DPPH and ABTS assays, the decrease in absorbance of the sample indicates the capacity to eliminate free radicals. For this test, the dose response of the extracts and the IC_{50} value were also determined.

2.6. Statistical analysis

The experimental design was completely random with three replications. The analysis was performed using Statistix software ver 10 (Analytical Software 2105 Miller Landing Rd Tallahassee, FL 32312, USA); mean comparisons were performed by Tukey's test ($p \leq 0.05$) when ANOVA showed significant differences among treatments.

3. Results

3.1. Physical characteristics of the R. aromatica fruit

The fruits of *R. aromatica* weighed an average of 55.22 ± 5.47 mg; the polar and equatorial diameters were 6.60 ± 1.02 mm and 7.15 ± 0.66 mm, respectively. The dimensions of the fruit allowed us to characterize it as a subglobular fruit, and these data allow the design of processes for the selection, cleaning and classification of the fruit. On the other hand, the portion of the pulp (edible) represented 40 % in relation to the total weight of the fruit, which indicates that it is possible to make an integral use including the seed.

3.2. Physicochemical characteristics and proximal analysis of the fruit pulp

The results of proximal composition are presented in Table 1; from this analysis, the high content of total fats stands out, so it is pertinent in future research to carry out a specific characterization of the lipid content of the fruit of *R. aromatica*.

The values represent the mean of three replications.

Table 2 shows the percentage of the requirement pattern of the proximal components [49–51] and the percentage of contribution of the 'limilla' per 100 g of fruit. The results of this work show that 'limilla' fruit is a good source of lipids and is low in carbohydrates.

3.3. Content of polyphenols, flavonoids and carotenoids

Polyphenolic compounds are important since they are nonnutritive components synthesized by the secondary metabolism of plants and play an important role in human health, in addition to presenting various bioactive properties [52,53]. The content of total polyphenols quantified in the pulp of the *R. aromatica* fruit presented significant differences ($p \leq 0.001$) depending on the solvent and the proportion of water used to obtain the extracts (Fig. 1). The content of total polyphenols was higher in the extract obtained with 80 % methanol, followed by the extracts obtained with 60 % ethanol and 60 % acetone (Table 3). With these results, it is confirmed that polar organic solvents mixed with water allow the extraction of phenolic compounds more efficiently, possibly due to the presence of glycosides with external hydrophilic hydroxyls in the chemical structure [54,55].

Flavonoids are the most common type of polyphenols and are mainly divided into six classes based on the degree of oxidation. These classes include flavones, isoflavones, flavanones, flavonols, anthocyanins, and proanthocyanidins [56]. The quantification of the total flavonoid content yielded significant differences ($p \leq 0.001$) depending on the solvent and the proportion of water used to obtain the extracts. The majority of total flavonoids were obtained in the extracts with acetone, ethanol, and methanol, all at 100 %; the highest amount of polyphenols was obtained with 100 % acetone (Table 3).

Carotenoids are natural compounds responsible for the typical colors of some fruits that give them red, orange and yellow tones, depending on their type and content; in this regard, 'limilla' fruit is typically associated with shades of red–orange color. Table 4 shows the content of total carotenoids expressed in mg β-carotene equivalents/100 g of fruit and the contribution with respect to other

Table 1
Proximal composition of *R. aromatica* fruits.

Component	*Rhus aromatica*
Moisture (%)	8.7
Ash (%)	3.8
Fat (%)	30.6
Crude Protein (%)	8.7
Crude Fibre (%)	18.7
Total Carbohydrates (%)	29.4
Caloric Intake (kcal/100 g)	415.8
pH	3.1
°Brix	3.1
Titratable acidity	1.9

Table 2
Proximate composition of *R. aromatica* and percentage contribution with respect to the requirement patterns for different age groups.

Component		Infants		Children								Adults	
		0.5–1 year		1–3 years		4–8 years		9–13 years		14–18 years		>18 years	
	Contribution of *R. aromatica* (g/100 g)	RP	RCP	RP	RCP	RP	RCP	RP	RCP	RP	RCP	RP	RCP
Carbohydrates	29.4	60–95	49–31	130	23	130	23	130	23	130	23	130	23
Fat	30.6	30	100	22	133	22	133	63	133	22	133	22	133
Protein	8.7	9–13	96–64	13	70	19	46	34	26	52	17	56	16
Fibre	18.7	ND	ND	19	98	25	75	31	60	38	49	38	49

RP = Requirement patterns for the different age groups (grams of component). %RPC= Contribution to the percentage requirement. ND= Not determined.

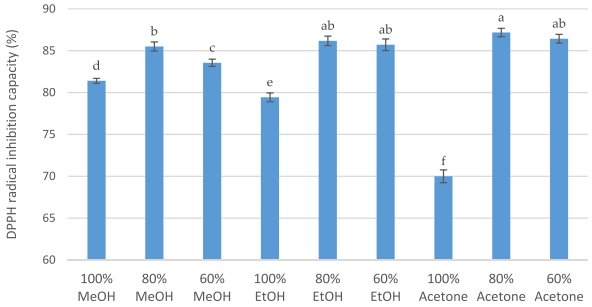

Fig. 1. Free radical scavenging activity of DPPH from *R. aromatica* extracts (mean ± SD, n = 3). Bars with the same letter are not significantly different (Tukey's test, $p \leq 0.05$).

Table 3
Total content of polyphenols and flavonoids in the pulp of *R. aromatica* fruits.

Solvent	Total polyphenols (mg AG/g)	Total flavonoids (mg EQ/g)
100 % MeOH	66.53 ± 0.45 f	17.45 ± 0.33 c
80 % MeOH	80.55 ± 0.62 c	7.30 ± 0.18 g
60 % MeOH	71.71 ± 1.13 e	10.09 ± 0.21 e
100 % EtOH	62.28 ± 0.13 g	19.29 ± 0.69 b
80 % EtOH	72.50 ± 0.90 de	8.38 ± 0.47 fg
60 % EtOH	88.65 ± 0.89 a	9.22 ± 0.15 ef
100 % Acetone	56.98 ± 0.19 h	26.52 ± 0.65 a
80 % Acetone	74.28 ± 0.15 d	10.35 ± 0.58 e
60 % Acetone	85.94 ± 0.05 b	12.78 ± 0.13 d

Values in the same column with a different letter are significantly different (Tukey, $p < 0.05$). The values shown represent the mean ± standard deviation.

Table 4
Total content of carotenoids in *R. aromatica* and other fruits consumed daily.

Source	mg total carotenoids/100 g of fruit	Contribution with respect to *R. aromatica* (%)
R. aromatica	46.37 ± 3.27[a]	100
Mango	7.47 [57]	16
Orange bell pepper	6.99 [58]	15
Carrot	5.47 [59]	11.7
Mammy	4.42 [59]	9.5

[a] The data is the mean value with standard deviation, n = 3.

sources of fruits and vegetables of daily consumption reported by other authors [57–59].

3.4. Antioxidant capacity

The total antioxidant capacity was significantly ($p \leq 0.001$) different depending on the solvent used for the extraction (Fig. 1). The highest values of total antioxidant activity (80–87.17 %) were obtained in extracts with 80 % methanol, 80 % ethanol and 80 % acetone.

In the ABTS assay (Fig. 2), the extracts with different solvents and proportions of water did not show a significant difference in the total antioxidant capacity, and the radical elimination capacity in the different extracts was 90 %. A similar effectiveness was determined in fruits of R. *chinensis* Mill [60].

In Figs. 3–5, the antioxidant capacity in dose response is shown; most of the extracts presented a DPPH free radical scavenging capacity greater than 60 % at concentrations of 4 μg/mL, except for the extracts with pure acetone and 80 % acetone. The best values of radical elimination capacity (greater than 80 %) were obtained with 6 μg/mL from the different 80 % and 60 % solvents, which allows an advantage for the use of more environmentally friendly solvents to obtain compounds with antioxidant capacity.

On the other hand, IC_{50} was also determined with DPPH and ABTS; in both trials, the values obtained were significantly different between the different extracts tested ($p < 0.05$). The lower the IC_{50} value, the better its radical inhibition capacity. The IC_{50} values obtained according to the solvents used at the different concentrations are shown in Table 5.

The best IC_{50} values for the inhibition of DPPH radicals were obtained with the extracts with 60 % methanol and 100 % ethanol, although they were not significantly different from the IC_{50} value obtained with 80 % MeOH. For ABTS, the best IC_{50} values were obtained with 80 % EtOH, 60 % MeOH, 80 % acetone, and 80 % MeOH.

4. Discussion

R. aromatica has physical characteristics similar to fruits of other species of the same genus (*Rhus*) and are described as subglobular fruits. Such is the case for the fruit of *R. coriaria*, which has a thickness of 2.51 mm and polar and equatorial diameters of 4.98 mm and 5.54 mm, respectively [61]. Similar to the percentage of pulp found for the fruit of *R. aromatica* below 50 %, it has been reported for other species of the genus *Rhus* that the majority percentage is seed, such as *Rhus chinensis*, where the seed represents 78 % of the fruit [62]. The value of total soluble solids of *R. aromatica* is similar to that reported in *R. coriaria* [63], so both species lack the possibility of obtaining juice from their fruits. The pH values (2.66–3.90) reported for *R. coriaria* fruits [64] are similar to the pH found in this study for *R. aromatica* (Table 1); the variation between the pH values even when they belong to the same species can be influenced by the amount of organic acids contained in the fruit [65]. The fruits of *R. aromatica* and *R. coriaria* are considered to be highly acidic, according to the classification of foods with pH values similar to those reported in this study.

The titratable acidity value (2.1–7.8 %) reported for *R. coriaria* [64] is similar to that of *R. aromatica* (Table 1). Various investigations have revealed that the acidic taste of *R. coriaria* is due to the presence of malic acid; therefore, it could be that malic acid is the major organic acid of *R. aromatica*.

The moisture value of *R. aromatica* (Table 1) was lower than that reported for *R. coriaria* (9.6 %) [61] and higher than that reported for *R. typhina* (6.64 %) [23]. The difference between the moisture values of these fruits may be influenced by the time the fruit is collected, since once ripe, depending on the climatic conditions, the fruits tend to lose moisture.

The crude protein content of *R. aromatica* (Table 1) was higher than that reported for *R. coriaria* (2.6 %) [61] and for *R. typhina* (4.31 %) [23]. In another investigation in *R. coriaria* [66] and R. *chinensis* [62], crude protein values of 11.56 % and 9.14–10.55 %, respectively, were obtained. Regarding the quality of the protein, only the presence of essential amino acids in low amounts has been reported for *R. typhina* [23], and for *R. coriaria*, only the total amount of free amino acids ranges from 25.01 to 166.38 mg GlyE/g DW [64].

As part of the chemical composition of the fruits that belong to the genus *Rhus*, the lipid content is the most representative. There is precedent for interest in the fruits of this genus as an alternative to oilseeds. In this regard, the total lipid content (30.6 %) of *R. aromatica* can be considered high compared to that reported for *R. coriaria* (7.4 %) [61] and *R. typhina* (11.52 %) [23], so the fruit could be an adequate source of vegetable lipids, particularly an appropriate source of lipophilic pigments given the waxy consistency and colouration of the fruit.

Regarding the total fibre content, *R. aromatica* fruit showed a higher content than *R. coriaria* (14.6 %) [61] and less than *R. typhina* (32.9 %) [23]. In this regard, it is known that fruits that present values greater than or equal to 14.6 % of crude fibre are a potential source of dietary fibre that could be used to favour gastrointestinal disorders.

The fruit of *R. aromatica* adequately contributes to the recommended daily intake requirements for all age groups; a high

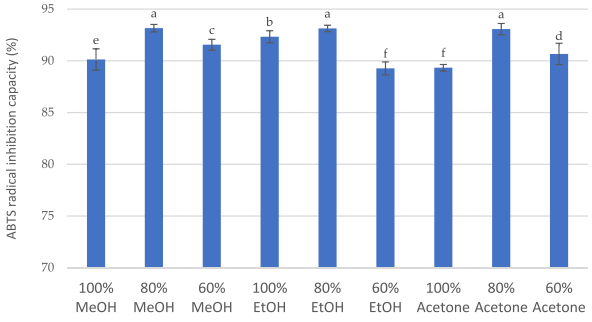

Fig. 2. Free radical scavenging activity of ABTS from *R. aromatica* extracts (mean ± SD, n = 3). Bars with the same letter are not significantly different (Tukey's test $p \leq 0.05$).

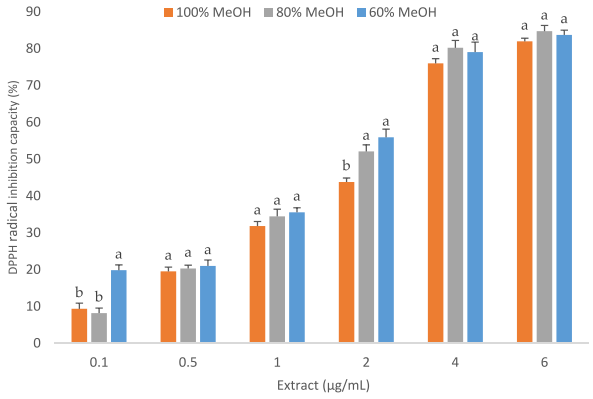

Fig. 3. Dose–response relationship in the elimination of DPPH radicals from extracts with methanol of *R. aromatica* fruits. Values are shown as the mean ± SD (n = 3). Means with different letters at the same concentration are significantly different (Tukey's test, $p < 0.05$).

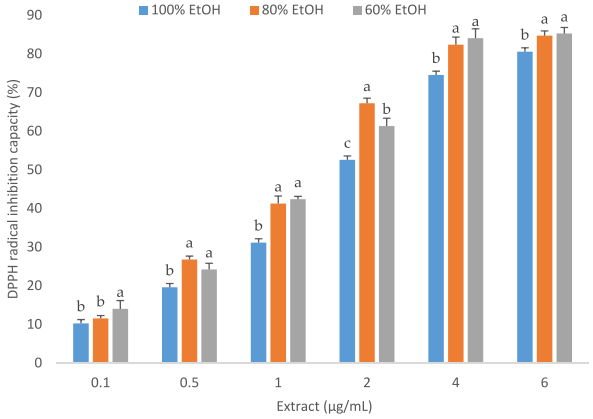

Fig. 4. Dose–response relationship in the elimination of DPPH radicals from extracts with ethanol of *R. aromatica* fruits. Values are shown as the mean ± SD (n = 3). Means with different letters at the same concentration are significantly different (Tukey's test, $p < 0.05$).

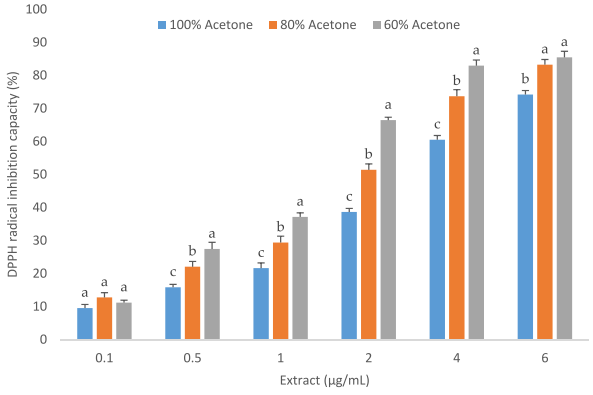

Fig. 5. Dose–response relationship in the elimination of DPPH radicals from extracts with acetone of *R. aromatica* fruits. Values are shown as the mean ± SD (n = 3). Means with different letters at the same concentration are significantly different (Tukey's test, $p < 0.05$).

contribution of fibre, carbohydrates and proteins can be highlighted.

Phenolic compounds, also called polyphenols, are metabolic products widely distributed in plant foods; they have biological and pharmacological activities that could provide protection against chronic diseases [24]. These compounds have a greater antioxidant effect than vitamins and are capable of neutralizing the effects of oxidative free radicals [40]. There are various investigations in which the amount of polyphenols and flavonoids in fruits of species belonging to the *Rhus* genus, mainly *R. coriaria*, *R. typhina*, and R.

Table 5
IC_{50} values (μg/mL) for the inhibition of DPPH and ABTS radicals from different extracts of *R. aromatica* fruit.

Extract	IC_{50} DPPH	Extract	IC_{50} ABTS
100 % EtOH	1.87 ± 0.04^{d}	80 % EtOH	1.32 ± 0.04^{d}
60 % MeOH	1.89 ± 0.03^{d}	60 % MeOH	1.48 ± 0.19^{cd}
80 % MeOH	2.08 ± 0.04^{cd}	80 % Acetone	1.53 ± 0.16^{cd}
80 % Acetone	2.35 ± 0.04^{bc}	80 % MeOH	1.81 ± 1.97^{bcd}
80 % EtOH	2.63 ± 0.17^{b}	100 % EtOH	2.083 ± 0.15^{bc}
60 % Acetone	2.73 ± 0.03^{b}	100 % Acetone	2.25 ± 0.10^{b}
100 % MeOH	3.41 ± 0.08^{a}	100 % MeOH	2.43 ± 0.42^{b}
60 % EtOH	3.54 ± 0.20^{a}	60 % EtOH	2.44 ± 0.06^{b}
100 % Acetone	3.67 ± 0.08^{a}	60 % Acetone	3.23 ± 0.10^{a}

Values are the mean of triplicate determinations (n = 3) ± standard deviation; means with different letters are significantly different (Tukey $p < 0.05$).

tripartite, among others, has been quantified. However, for the species *R. aromatica*, this study is the first to quantify these components. *R. aromatica* showed a higher content of polyphenols compared to *R. coriaria* [67], which is reported from 36.38 to 58.66 mg GAE/g in 15 genotypes from extracts with methanol, and other values reported in this range from different populations of *R. coriaria* where it is mentioned that the polyphenol content in fruit extracts with 80 % methanol is 77.54 mg GAE/g [74]. On the other hand, concentrations similar to those of *R. aromatica* were found in extracts of fruits with 80 % methanol of the species *R. hirta* with a content of 81.6 mg GAE/g [68], and values close to those reported in R. tripartite with a content of 102.06 mg GAE/g [69], also in fruit extracts with methanol. Finally, for *R. typhina* fruits, the polyphenol content exceeds that reported in this study, with values of 151 mg GAE/g in extracts with 20 % ethanol [66], and concentrations similar to those reported in extracts of *R. hirta* with ethanol acidified with 1 % HCl (81.6 mg GAE/g) [68]. In relation to the solvents for the extraction of total polyphenols, the present investigation coincides with that reported by Zhang [60], where the highest content of polyphenols was obtained from extracts with 80 % ethanol. Due to the high content of total polyphenols found in this study for *R. aromatica*, this fruit can be considered an adequate source of polyphenols since it exceeds the value reported for fruits of regular consumption: blueberries 7.07 mg/g [70], blackberries 4.12 mg/g [71], strawberries 2.35 mg/g and raspberries 3.09 mg/g [72].

The flavonoid content of *R. aromatica* compared to other species of the same genus is higher; in extracts with 80 % methanol, *R. coriaria* fruits presented values between 2.19 and 7.54 mg GAE/g [64]. On the other hand, Wu et al. [68] reported 4.97 mg GAE/g in extracts with 80 % ethanol, and another author reported 14.28 mg EAG/g in extracts with methanol in fruits of *R. tripartita* and 11.93 mg EAG/g in *R. pentaphylla* [69].

Carotenoid content is not reported for species other than *R. aromatica* (within the *Rhus* genus). The study of the concentration of total carotenoids present in 'limilla' fruit is relevant since carotenoids are an important part of the human diet. Carotenoids play a vital role in health and nutrition with positive effects in preventing vitamin A deficiency, as well as reducing the incidence of age-related eye diseases, cancer, and cardiovascular diseases [73]. In addition, there are carotenoids that are used as pigments, such as astaxanthin, which is added to fish feed to obtain a desirable pink meat color [74]. The 'limilla' fruit has a higher content of carotenoids than other fruits and vegetables of daily use. This first report on the content of carotenoids in species of the genus *Rhus* opens the possibility for future research that elucidates the profile of carotenoids and the biological properties that they can contribute.

The antioxidant capacity of *R. aromatica* is higher than that reported in fruits of various cultivars of *R. coriaria* [64,65], of which the total antioxidant capacity is reported to range from 73.37 to 79 % in extracts with methanol.

The dose–response obtained for the elimination of DPPH radicals by the fruit extracts of *R. aromatica* is better than those obtained from extracts of *R. chinensis* [60], in which, at a dose of 5 μg/mL of extract, the ability to eliminate free radicals does not exceed 70 %. The dose response for the elimination of ABTS radicals was also more efficient for the extract of *R. aromatica* because with 4 μg/mL of extract, elimination capacities greater than 85 % and 90 % were obtained in extracts obtained with methanol and ethanol, respectively, at 100 %. Likewise, a dose–response is reported for the elimination of ABTS radicals by extracts of *R. chinensis* at 40 % with a concentration of 2.5 μg/mL of extract [60], in comparison with a similar concentration of extract (2 μg/mL) of *R. aromatica*, which eliminates the ABTS radicals in a higher percentage (greater than 60 %), with the exception of the extract with 100 % acetone.

The IC_{50} values, with DPPH, obtained with extracts of *R. aromatica* fruits were lower than those reported in extracts of fruits of other species of the *Rhus* genus. For extracts of *R. coriaria* with ethanol and water, IC_{50} values of 20 ± 2.6 and 54 ± 2.77 were obtained, respectively [21]. In extracts of *R. chinensis* with 80 % methanol, 80 % ethanol and 80 % ketone, IC_{50} values of 3.72, 3.92 and 3.64 were obtained, respectively [60]. In the case of extracts with methanol from fruits of *R. tripartita* and *R. pentaphyla*, IC_{50} values of 22.83–35.47 and 12.90 to 15.09 are reported, respectively [69]. For the ABTS radical, the extracts of *R. tripartitum* obtained with methanol showed IC50 values of 3.81–55.5 [75]; compared to the values of this study, they are on the order of two to forty times higher.

According to the results obtained in this investigation, the content of polyphenols, flavonoids and antioxidant capacity, it is pertinent to investigate further in the identification of specific components of the fruit of *R. aromatica* and start with the first studies that focus on exploring the pharmacological properties of this species.

5. Conclusion

According to the values obtained in this research, the fruit of *Rhus aromatica* has some similarities to the fruits of species belonging to the same genus, such as physical characteristics, yield in the edible part, pH, and protein and fibre content. However, the lipid content for the fruit of *R. aromatica* is higher than that for the other species. On the other hand, due to the values of the total polyphenol content obtained, the fruit of *R. aromatica* can be considered an adequate source of polyphenols since it mostly exceeds the values of other species. Additionally, the results of the antioxidant capacity are higher compared to the other species, so the fruits of *R. aromatica* can be considered in future research for the assessment of pharmacological properties focused on antiobesity, anticancer, antimicrobial, and antihyperlipidemic effects, among others properties. For the first time, the content of total carotenoids in the fruit of one of the species of the genus *Rhus* (*R. aromatica*) is reported, presenting a higher contribution than some fruits and vegetables of daily consumption.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Gonzalo Soria-Melgarejo: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Juan C. Raya-Pérez:** Validation, Data curation. **Juan G. Ramírez-Pimentel:** Visualization. **Jorge Covarrubias-Prieto:** Validation. **Glenda M. Gutiérrez-Benicio:** Validation, Resources. **Isaac Andrade-González:** Data curation. **Cesar L. Aguirre-Mancilla:** Writing – review & editing, Software, Project administration, Methodology, Investigation, Conceptualization.

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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