

Phenolic Compounds and Antioxidant Activity of the *Rhus aromatica* var. *schmidelioides* (Schltdl.) Engl. Fruit Extracts

Gabriel Alejandro Reveles-Hernández, Jesús Nicodemo González-Gallegos, Evelia Martínez-Cano*

Departamento de Ciencias de la Tierra y de la Vida, Centro Universitario de los Lagos, Universidad de Guadalajara, Jalisco, México.

*Corresponding author: Evelia Martínez-Cano, email: evelia.martinez@academicos.udg.mx

Received November 15th, 2023; Accepted September 5th, 2024.

DOI: <http://dx.doi.org/10.29356/jmcs.v69i3.2169>

Abstract. The fruit of *Rhus aromatica* var. *schmidelioides* (Schltdl.) Engl. (RHSC) is commonly consumed for the properties attributed to it in traditional medicine. However, to the best of our knowledge, there is no information on the bioactive compounds present in the species. The content of phenolic compounds and the antioxidant activity of the RHSC fruit were evaluated in different extracts: methanol (M), ethanol (E), ethanol-water (MW), and ethanol-water (EW). The values obtained in each extract were compared, and it was found that the highest value for total phenol was EW, for total flavonoids it was E, and for anthocyanin, it was M. While for antioxidant activity the highest value obtained for DPPH was MW, for ABTS it was EW and for FRAP it was M. In general, the antioxidant activity could be verified in all extracts. This study corresponds to the first report on the content of phenolic compounds and antioxidant activity in different extracts, highlighting that the RSS fruit contains a high content of compounds with antioxidant properties.

Keywords: *Rhus aromatica* var. *schmidelioides* (Schltdl.) Engl.; phenolic compounds; antioxidant activity.

Resumen. El fruto de *Rhus aromatica* var. *schmidelioides* (Schltdl.) Engl. (RHSC), es comúnmente consumido por las propiedades que se le atribuyen en la medicina tradicional. Sin embargo, hasta donde sabemos no existe información de los compuestos bioactivos presentes en la especie. El contenido de compuestos fenólicos y la actividad antioxidante del fruto de RHSC fueron evaluadas en diferentes extractos: metanol (M), etanol (ET), metanol-agua (MA) y etanol-agua (EA). Al comparar los valores obtenidos en cada extracto se encontró que el valor más alto para fenoles totales fue en EA, para flavonoides totales fue E y para antocianinas fue M. Mientras que para la actividad antioxidante el mayor valor obtenido para DPPH fue MA, para ABTS fue EA y para FRAP fue M. En general, se pudo comprobar en todos los extractos la actividad antioxidante. Este estudio corresponde al primer informe sobre el contenido de compuestos fenólicos y la actividad antioxidante en diferentes extractos, resaltando, que el fruto de RSS contiene un alto contenido de compuestos con propiedades antioxidantes.

Palabras clave: *Rhus aromatica* var. *shmidelioides* (Schltdl.) Engl.; compuestos fenólicos; actividad antioxidante.

Introduction

Rhus aromatica var. *schmidelioides* (Schltdl.) Engl (RHSC) belonging to Anacardiaceae family, is a wild plant in Los Altos de Jalisco, Mexico. RHSC fruits, commonly known as agrillo are traditionally used for preparing refreshing drinks and liquors. Fruits of RHSC, as well as different species of the genus *Rhus*, are edible and are used in traditional medicine. Extracts of some of these species have shown remarkable anticancer, antiinflammatory, antifungal, antiviral, and antioxidant properties [1-3]. This species is distributed in the north-

central region of México, specifically in the states of Sonora, Chihuahua, Coahuila, Nuevo León, Tamaulipas, Durango, Zacatecas, Querétaro, San Luis Potosí, Guanajuato, Michoacán and Jalisco. Some species of the *Rhus* genus are characterized by their biological properties, including antioxidant activity mainly attributed to phenolic compounds (PC) [2,4-5]. PC are secondary metabolites present in plant species, which play an important role in preventing and treating diseases related to oxidative stress [6,7]. PC has been identified in some species of the genus *Rhus*, for example, epicatechin, rutin, naringenin and hesperidin in *Rhus natalensis* [8]; myricetin-3-O- β -glucoside and taxofolin in *Rhus tripartita* [9,10]; quercetin, butin, fustin, sulphuretin in *Rhus verniciflua* [11-13]; myricetin in *Rhus coriaria* [14]. However, to our knowledge, there are no reports on the phytochemical composition of RHSC. Therefore, this study aimed to assess the phenolic composition and *in vitro* antioxidant activity in different extracts of the RHSC fruit.

Experimental

Plant material

Fresh ripe fruits of RHSC were collected in Jesus Maria region (South of Jalisco, Mexico; coordinates: 20°38'20.9"N 102°13'21.1"W) in April of 2022 (Fig. 1). The plant was authenticated and identified by Emmeth Josafat Rodriguez Perez. The species was deposited in the Herbarium of the Unidad Académica de Ciencias Biológicas of the Universidad Autónoma de Zacatecas, with a replica in the Herbario del Estado de Zacatecas (HZAC), with voucher specimen No. 6778.



Fig. 1. *Rhus aromatica* var. *schmidelioides* (Schltdl.) Engl. species.

Preparation of extracts

The fruits were rinsed with distilled water after being cleaned with running tap water to remove dirt. They were left to shade-dry at room temperature and ground to obtain small particles. Ethanol (E), methanol (M), ethanol-water (EW), and methanol-water (MW) were used as solvents. 10 g of ground fruits and 70 mL of E, M, EW (50:20, v/v), and MW (50:20, v/v) were mixed separately for each extraction and left to macerate for 48 hours at room temperature. The extracts were filtered with filter paper Whatman No. 1, and 20 mL of the crude extract was fractionated by liquid-liquid extraction with petroleum ether (1:1, v/v). The aqueous phase of each extract was poured into a brown glass bottle and kept at 4°C until further use. Three experiments were performed in triplicate for each method used.

Chemicals and reagents

Folin-Ciocalteu reagent (2N), gallic acid, quercetin, 2,2'-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), ascorbic acid, 2,4,6-tripyridil-S-triazine (TPTZ) were obtained from Sigma-Aldrich (St. Louis, MO, USA). All the other reagents and solvents used were of analytical grade.

Phenolic compounds

Total phenol content

Total phenol content in each extract was determined using the Folin-Ciocalteu method, with several modifications [15]. Briefly, 50 μL extract solution was mixed with 200 μL of Folin-Ciocalteu reagent (1 N). After 5 min, 1000 μL of Na_2CO_3 (7 %, w/v) was added. The mixture was homogenized and incubated for 30 minutes in the dark. The absorbance was measured at 765 nm using a spectrophotometer (Perkin-Elmer lambda 365 UV/Vis). Gallic acid was used as a standard (0.1 mg/mL), and the results were expressed as milligrams of gallic acid equivalents (GAE) per gram of fresh sample (FS).

Total flavonoid content

Total flavonoid content in each extract was determined using the aluminum colorimetric method, with some modifications [16]. An aliquot of 20 μL of each extract was mixed with 300 μL of NaNO_2 (5 %, w/v) solution. After 6 minutes, 150 μL of $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ (10 %, w/v) solution was added and the mixture was allowed to stand another 5 minutes. Then, 500 μL of NaOH (1 M) was added and mixed. The absorbance was measured at 510 nm. Quercetin was used as standard (1 mg/mL), and the results were expressed as milligrams of quercetin equivalents (QE) per gram of fresh sample (FS).

Total anthocyanin content

Total anthocyanin was quantified using a pH differential method, with slight modifications [17]. Briefly, 30 μL of each extract was diluted with 2970 μL of two different buffers: KCl (0.025 M, pH 1.0) and $\text{C}_2\text{H}_3\text{Na}_2\text{O}_2$ (0.4 M, pH 4.5), respectively. After 30 min of incubation at room temperature, the absorbance was measured at 520 and 700 nm in each buffer solution. The results were expressed as milligrams of cyanidin-3-glucoside equivalents (CE) per gram of fresh sample (FS). The results were calculated as follows:

$$A = (A_{520 \text{ nm}} - A_{700 \text{ nm}}) \text{ pH } 1.0 - (A_{520 \text{ nm}} - A_{700 \text{ nm}}) \text{ pH } 4.5$$

$$\text{Total anthocyanin content } (\mu\text{g/g}) = \frac{(A) \times (\text{MW}) \times (\text{DF}) \times (10^3)}{(\epsilon) \times (\lambda) \times (m)}$$

Where A is the difference sample absorbance between pH 1.0 and 4.5; MW is a molecular weight; DF is a dilution factor, ϵ is the molar absorption coefficient; λ is a cuvette optical path length (1 cm) and m is the weight of the sample (g).

Antioxidant activity

DPPH radical scavenging activity

Radical scavenging activity was measured using the stable radical DPPH $\cdot$. The procedure was followed according to Brand-Williams, with some modifications [18]. An aliquot of 5 μL of each extract was mixed with 2850 μL of methanol solution of DPPH (150 μM). After the mixture was allowed to stand in the dark at room temperature for 30 minutes. The absorbance was measured at 517 nm. Trolox was used as standard (0.25 mg/mL), and the results were expressed as milligrams of Trolox equivalents (TE) per gram of fresh sample.

ABTS $\cdot^+$ radical scavenging activity

The ABTS $\cdot^+$ assay was adopted based on the method of Morales and Paredes, with some modifications [16]. Briefly, 2 μL of each extract was mixed with 2400 μL of ABTS $\cdot^+$ (100 μM) and incubated at room temperature in the dark for 6 min and thereafter the absorbance was measured at 734 nm. Trolox was used as

standard (0.025 mg/mL), and the results were expressed as milligrams of Trolox equivalents (TE) per gram of fresh sample.

Ferric reducing antioxidant power (FRAP) assay

FRAP assay was performed according to the method described by Benzie and Strain, with slight modifications [19]. The FRAP solution was prepared by mixing acetate buffer (300 mM, pH 3.6), TPTZ (10 mM), and $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (20 mM). An aliquot of 2 μL of each extract was mixed with 2400 μL of FRAP solution. The absorbance was measured at 593nm after the mixture was incubated at room temperature in the dark for 30 min. Ascorbic acid was used as a standard (0.01 mg/mL), and the results were expressed as milligrams of ascorbic acid equivalents (AAE) per gram of fresh sample (FS).

Statistical analysis

All experiments were expressed as mean $\pm$ standard deviations (SD). Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test, with significance set at $p < 0.05$. GraphPad Prism 9.0 (GraphPad, San Diego, CA, USA) statistical program was used for all statistical analysis.

Results and discussion

Total phenol content

The results obtained showed that the total phenolic content (mg GAE/g of FS), determined by the modified Folin-Ciocalteu method, varied among the extraction solvents, as shown in Table 1. The lowest value was determined in methanol, where the average result was 19.30 mg/g of FS, rising in ethanol and ethanol-water extracts. The highest values were obtained for methanol-water extract and were a little more than 3-fold higher than those for methanol extract. The results indicated that ethanol-water and methanol-water were better than ethanol and methanol ($p < 0.0001$) in the extraction of phenolic compounds from the RHSC fruits (Fig. 2(A)). In comparison with the methanolic extract of *Rhus tryphina* and *Rhus verniciflua* fruits (183.42 ± 2.29 mg GAE/g of FS and 68.9 ± 7.7 mg GAE/g of FS) [11,20], our results showed a lower concentration of total phenols. On the other hand, the methanol-water extract of *Rhus vulgaris* fruit presented a higher total phenolic content (83.15 ± 7.6 mg GAE/g FS). Additionally, the extract of *Rhus hirta* revealed values of 81.6 ± 0.3 mg GAE/g FS in the ethanolic extract and 46.3 ± 0.2 mg GAE/g of FS in the aqueous extract [21]. Our results showed that ethanol-water and methanol-water extracts were more successful than ethanol and methanol extracts. PC are secondary metabolites found in a wide range of vegetables and fruits, playing important roles in diverse physiological processes in plants [6]. Several studies have shown that the PC present in many *Rhus* species has health benefits, such as anti-inflammatory, antimutagenic, antiviral, antitumorigenic, antimicrobial, and antioxidant effects [3].

Table 1. Total phenol, flavonoid, and anthocyanin content in various extracts of RHSC fruits.

Extract	Total phenol content (mg GAE/g FS)	Total flavonoid content (mg QE/g FS)	Total anthocyanin content (μg CE/g FS)
E	20.92 ± 1.89^a	107.60 ± 5.96	124.40 ± 2.55
M	19.39 ± 1.66^a	67.23 ± 7.12	202.20 ± 3.20
EW	77.15 ± 3.26	26.80 ± 2.79	145.10 ± 0.81
MW	50.23 ± 5.26	14.47 ± 2.23	85.54 ± 0.41

Data are presented as mean $\pm$ SD (n=3, in triplicate).

Superscript letter (a) within the same column indicates there are no significant differences ($p < 0.05$).

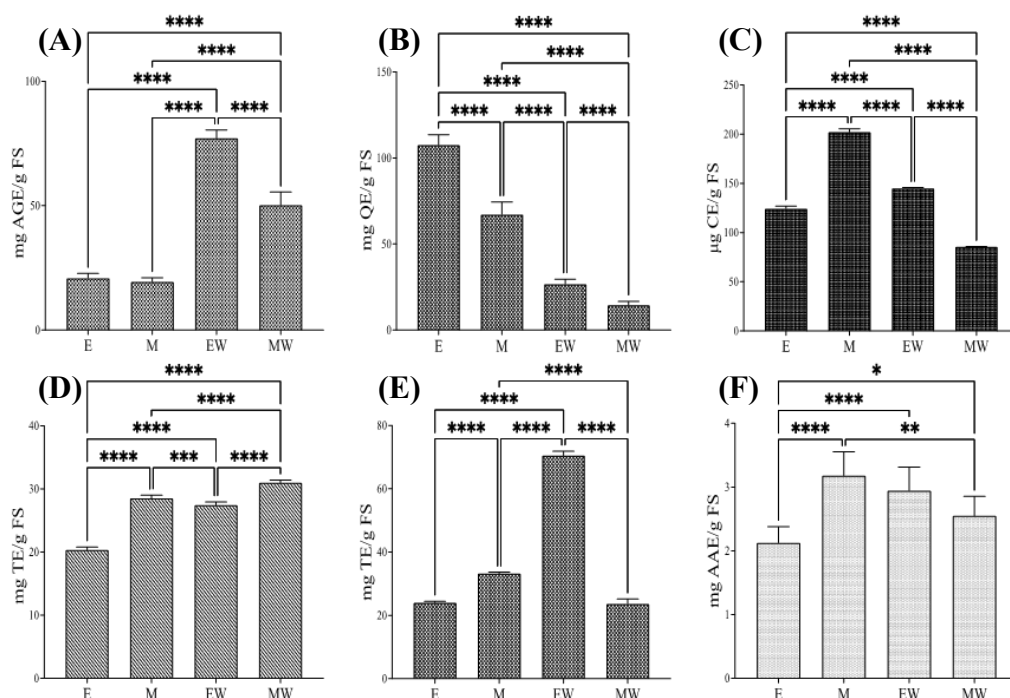


Fig. 2. Total phenol content (A), total flavonoid content (B), total anthocyanin content (C), DPPH radical scavenging activity (D), ABTS radical scavenging activity (E), and FRAP assay (F) in ethanol (E), methanol (M), ethanol-water (EW), and methanol-water (ME) extracts prepared from RHSC fruit. All results are expressed as median $\pm$ SD (n=3, in triplicate). Significance was determined using ANOVA followed by Tukey test for multiple comparisons (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$ ****, $p < 0.0001$).

Total flavonoid content

The total flavonoid content of the extracts ranges from 14.47 mg GAE/g of FS for ethanol-water extract to 107.60 mg GAE/g of FS for ethanolic extract (Table 1) and decrease in the following order: ethanol > methanol > ethanol-water > methanol-water, respectively. It was also found that the total flavonoid content in the extracts decreased in ethanol-water and methanol-water systems. Based on our results, the best extracting solvent was ethanol. Analysis of results revealed differences between all pairs of extracts evaluated ($p < 0.0001$) (Fig. 2(B)). These results differ from those reported in other studies carried out on species of the same family. For example, the total flavonoid content in the methanolic extract of *Rhus typhina* and *Rhus verniciflua* fruits was reported 54.53 ± 1.34 mg EQ/g of sample and 7.7 ± 1.5 mg catechin equivalent/g of sample, respectively [11,20]. On the other hand, for *Rhus hirta* values of 4.97 ± 0.08 mg of catechin equivalent/g of sample were reported in the ethanolic extract and 3.08 ± 0.06 mg catechin equivalent/g of sample in the aqueous extract [21]. In this study, it was observed that the ethanolic extract had the highest concentration of flavonoids, establishing it as the most efficient solvent. Our results differ when compared to those obtained for other *Rhus* species. However, our study demonstrates the relevance of the solvent used in the extraction. Flavonoids are well-known and scientifically proven antioxidants, in both *in vitro* and *in vivo* studies. In general, flavonoids exhibit a broad range of applications, especially anti-inflammatory, anticancer, antiallergic, and antioxidant activities, among other things [6]. The content of flavonoids in the extracts of RHSC fruit supports their therapeutic potential as attributed in traditional medicine.

Total anthocyanin content

The total anthocyanin content is shown in Table 1. Among all solvents in the extraction process, methanol showed the highest anthocyanin content ($202.2 \mu\text{g CE/g of FS}$), followed by ethanol, ethanol-water, and methanol-water. The total anthocyanin content between extracts was significantly different ($p < 0.0001$)

(Fig. 2(C)). It is important to note that no reports are available for total anthocyanin content in RHSC fruits. However, a study on the aqueous extract of *Rhus trilobata* stems reported 9.98 ± 1.5 g EC/g sample, which was higher than the value obtained in this study. This finding underscores the variability in anthocyanin content across different parts of the *Rhus* genus [20-23]. Currently, anthocyanin has various applications in human health, serving as a bioactive component in traditional medicine [1,2]. Therefore, the bioavailability of anthocyanin in RHSC fruits may be a key factor in maintaining good health and preventing various diseases.

Antioxidant activity

DPPH radical scavenging activity

The results of DPPH radical scavenging activity of RHSC fruit extracts are summarized in Table 2. The activity ranges of the extracts range from 20.33 mg TE/g of FS for ethanol extract to 30.97 mg TE/g of FS for methanol-water extract. Methanol-water exhibits the highest DPPH radical scavenging activity with 30.97 mg TE/g of FS, followed by methanol, ethanol-water, and ethanol with 28.1, 27.42, and 20.33 mg TE/g of FS, respectively. When evaluating the results, differences were found between all the extracts evaluated ($p < 0.0001$) (Fig. 2(D)). These results are similar to those reported in the fruit of *Rhus vulgaris* in which an antioxidant capacity of 22.86 ± 3.71 mg ascorbic acid equivalents/g of the sample was found in a methanol-water extract [21]. However, despite the similarity in the results between the *Rhus Vulgaris* fruit and the RHSC fruit, it is important to mention that reference standards are different, which limits the direct comparison.

Table 2. Effect of extracting solvent on the antioxidant activity in the RHSC fruit.

Extract	DPPH (mg TE/g FS)	ABTS (mg TE/g FS)	FRAP (mg AAE/g FS)
E	20.33 ± 0.43	24.00 ± 0.43^a	2.12 ± 0.25
M	28.51 ± 0.47	33.26 ± 0.38	3.18 ± 0.37^a
EW	27.42 ± 0.50	70.52 ± 1.30	$2.94 \pm 0.36^{a,b}$
MW	30.97 ± 0.42	23.70 ± 1.48^a	2.55 ± 0.30^b

Data are expressed as median $\pm$ SD (n=3, in triplicate).

Superscript letter (a) within the same column indicates there are no significant differences ($p < 0.05$).

ABTS radical scavenging activity

There is considerable variation in radical scavenging activity among the different RHSC extracts (Table 2). The ABTS radical scavenging abilities in the extracts revealed that ethanol-water extract (70.52 mg TE/g of FS) had the highest, while methanol, ethanol, and methanol-water were the lowest. Antioxidant activity, measured by the ABTS assay, showed variation among the RSS fruit extracts, with values ranging between 23.7 to 70.52 mg TE/g of FS. The analysis of the results reported differences between all the extracts evaluated ($p < 0.0001$), as seen in Fig. 2(E). Its antioxidant activity has been demonstrated in other species of *Rhus* genus. In a study, it was found that the aqueous extract of *Rhus verniciflua* contained 91.5 ± 4.6 mg of vitamin C equivalents/g of sample [11]. Although it is not possible to directly compare our results with this study, we verified that all extracts of the RHSC fruit can eliminate the ABTS radical. These findings suggest that RHSC fruit is a source of natural antioxidants.

Ferric reducing antioxidant power (FRAP) assay

The results obtained showed that the antioxidant activity evaluated by the FRAP assay ranged between 2.12 to 3.18 mg AAE/g of FS as shown in Table 2. The methanol and methanol-water extracts showed the highest antioxidant activity, with 3.18 and 2.94 mg AAE/g FS, respectively, followed closely by methanol-water with 2.55 mg AAE/g of FS and ethanol with 2.12 mg AAE/g of FS. When analyzing the results, differences were shown between ethanol and methanol extracts ($p < 0.0001$); ethanol and ethanol-

water ($p < 0.0001$; methanol and methanol-water ($p = 0.00015$); and ethanol and methanol-water ($p = 0.0458$) (Fig. 2(F)). The previous results are different from those reported in the fruit of *Rhus hirta*, in which values of 14.087 ± 0.1 in ethanolic extract and 9.789 ± 0.08 mg AAE/g of FS in aqueous extract were reported [20], their values are considerably higher compared of RHSC, these differences may be due to various factors such as the type of species, growing conditions, type of soil, relative humidity, or extraction methods, which underline the importance of carrying out specific studies for the different species and varieties of *Rhus*.

Conclusions

The results of this research show that the fruits of RHSC represent a source of phenolic compounds with antioxidant activity, suggesting that this edible species which grows wild in Los Altos de Jalisco, could be a valuable resource of bioactive compounds potentially useful as natural agents for health promotion. To the best of our knowledge, this is the first study to determine the total phenols, flavonoids, and anthocyanin in different extracts of the RHSC fruits, along with their antioxidant activity. Our results demonstrate that the total phenolic content in the extracts is lower than the values reported for *Rhus hirta* (81.6 mg GAE/g FS), *Rhus trypana* (46.2 mg GAE/g FS), *Rhus verniciflua* (183.42 mg GAE/g FS), and the *Rhus trilobata* (68.9 mg GAE/g FS). In contrast, the extracts exhibited higher flavonoid and anthocyanin content compared to those reported by other researchers. Furthermore, the antioxidant activity observed in our extracts is comparable to that of other fruit species. These findings suggest that, although the contents of phenol, flavonoids, and anthocyanins vary among species, our extracts possess antioxidant capacities similar to those of other fruit species. Of course, further investigations are needed. In the future, isolating and elucidating the structure of phenolic components may be interesting. Additionally, more research is necessary to better understand their mechanisms of action as antioxidants.

Acknowledgments

The authors thank the Programa de Incorporación y Permanencia de Posgrado (PROINPEP) for their support in purchasing of reagents. Gabriel Alejandro Reveles-Hernández thanks the Consejo Nacional de Humanidades, Ciencias y Tecnologías (CONAHCyT) for the doctoral scholarship (779709).

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