

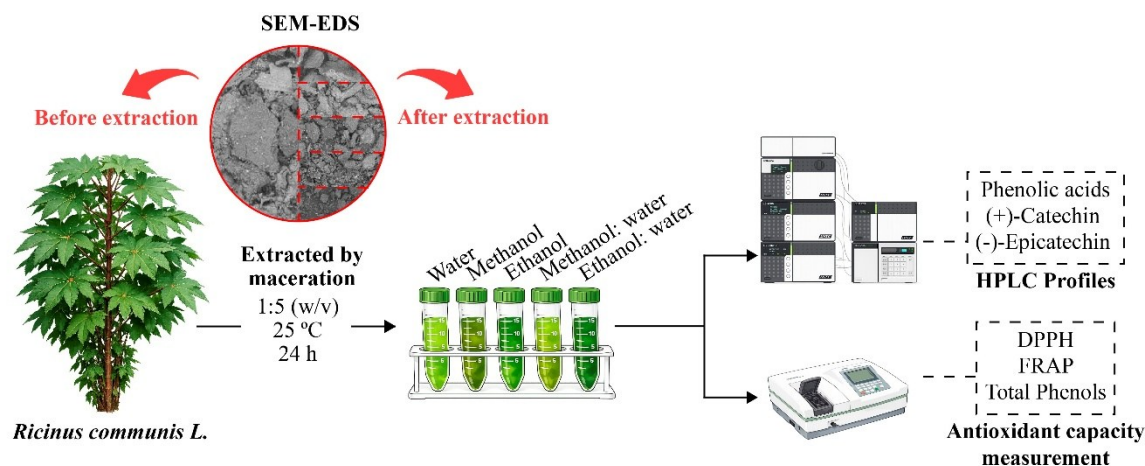
Antioxidant Properties of *Ricinus communis* Leaf Extracts: Evaluation of Solvents and Their Mixtures

Carlos A. Sagaste-Bernal ^{a,b,§} Conrado Garcia-Gonzalez ^{c,*,§} Marcos A. Coronado-Ortega ^{c,*} José R. Ayala-Bautista ^c José L. Arcos-Vega ^c Fernando A. Solis-Dominguez ^b Andrea Urbano-Nila ^c Samuel Lepe-de-Alba ^c Mario A. Curiel-Álvarez ^c Benjamín A. Rojano ^d and Mónica Carrillo-Beltrán ^c

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DOI: DOI: 10.15376/biores.21.3.6105-6122

GRAPHICAL ABSTRACT



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The shrub *Ricinus communis*, originally from Asia, has adapted to different regions of Mexico. The main value of this crop lies in the production of non-edible vegetable oil. In this work, extracts from the leaves of *R. communis* were obtained using various solvents: methanol, ethanol, and water; as well as 80:20 mixtures of methanol:water and ethanol:water. The extraction yield ranged between 1.00 and 2.10 g of extract per 100 g of plant material. The aqueous extract showed the highest yield, while the methanol: water mixture had the lowest. A SEM-EDS analysis was applied to the leaves before and after the extraction. The extracts were tested as antioxidants through free radical scavenging assays, ferric reducing capacity, and total phenolic content. The extract that exhibited the highest IC₅₀ value was the water, followed by ethanol, with values of 38.94 and 331.01, respectively. The extract from the leaves of *R. communis*, demonstrated antioxidant properties. This suggests that this biomass can serve as a source of natural antioxidants, thus utilizing this by product of the crop. The SEM-EDS results indicate that the leaves were not contaminated with metals or other sources, supporting the use of these extracts in applications such as biodiesel.

DOI: 10.15376/biores.21.3.6105-6122

Keywords: *Ricinus communis*; Antioxidants; Biomass valorization; Plant extracts; Green chemistry

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INTRODUCTION

The *Ricinus* genus belongs to the Euphorbiaceae family and consists of herbs with one known species, *Ricinus communis* L., which is commonly referred to as the castor oil plant. Its morphology can vary greatly depending on the region where it grows, ranging from dwarf shrubs to trees up to 7 meters in height (Kumar and Verma 2025). Similarly, its biological cycles depend on environmental conditions. Although it is native to tropical regions of Eurasia and Africa, it is also well adapted to Mediterranean climates and areas prone to droughts. The selection of specimens has led to most cultivated varieties having annual cycles and better resistance to water stress than their wild counterparts (Abomuhaid

et al. 2024). The shrubs typically have abundant foliage, with alternately arranged leaves. Each leaf is composed of 5 to 11 acuminate lobes. The coloration depends on the level of maturation, typically being a deep green with reddish or purple veins (Nour *et al.* 2023), as shown in Fig. 1.

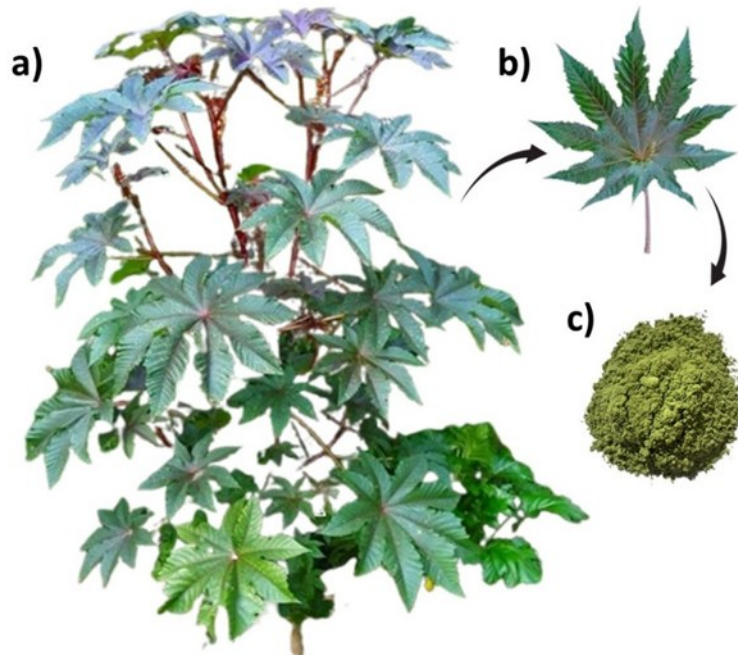


Fig. 1. Specimen of *R. communis*: a) entire shrub; b) leaf detail; c) powder resulting from grinding dry leaves (Author's own creation)

Mexico is ranked 17th among the world's top producers of *R. communis*. The cultivation of *R. communis* has acclimatized well to certain regions of the country (Pari *et al.* 2020), achieving yields of 2.6 t/ha in annual cycles (Valencia *et al.* 2019). The map in Fig. 2 shows the data of countries with the highest castor bean production in 2022, led by India, Mozambique, Brazil, and Myanmar, which averaged over 1.2 million tons of castor beans between 2014 and 2022 (FAOSAT 2024). Mexico's production between 2014 and 2022 averaged 2,961 t/year (SIAP 2022), which is a fraction of the amounts reported by India. The cultivation of *R. communis* has led to its expansion into other regions, where it has gained significant economic importance. The primary use of *R. communis* is the extraction of oil from its seeds, which is a source of hydroxylated fatty acids such as ricinoleic acid, and contains significant amounts of oleic and linoleic acids (Nitbani *et al.* 2022). In a cultivation plantation of *R. communis*, pruning the leaves constitutes a crucial maintenance practice that facilitates optimal plant development. This procedure enhances air circulation and significantly reduces the prevalence of pests and diseases within the crop. Moreover, the removal of senescent or damaged foliage promotes the emergence of new foliar structures, thereby augmenting the photosynthetic efficiency of the plant (Karmakar *et al.* 2024). Proper management techniques not only maximize the productivity of oilseed crops but also ensure the durability and resilience of *R. communis* under adverse environmental conditions.

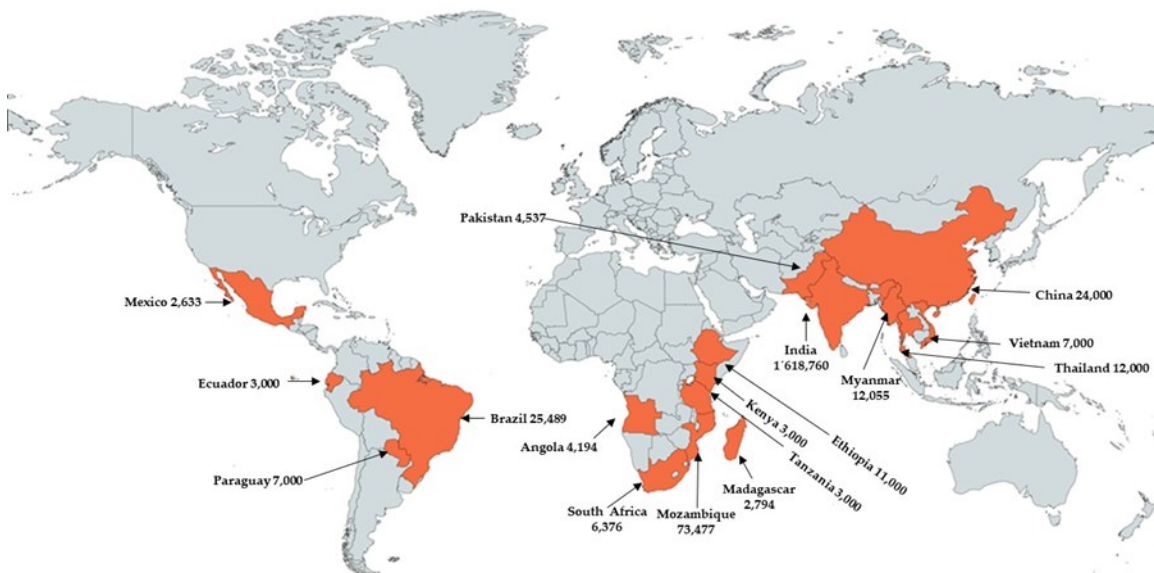


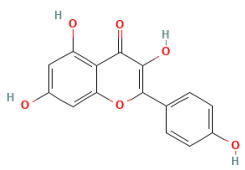
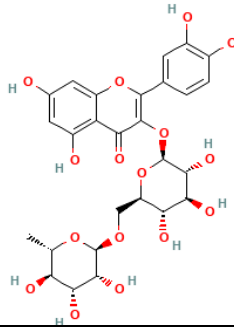
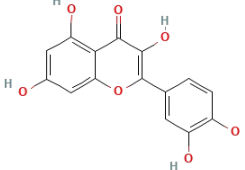
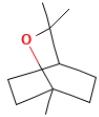
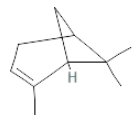
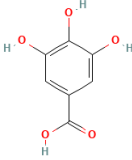
Fig. 2. Main producers of *R. communis* in 2022. Quantities expressed in tons (t), adapted with information from FAOSAT (FAOSAT 2024)

The leaves obtained from the pruning of the *R. communis* shrub are a rich source of secondary metabolites with antioxidant properties, which are often underutilized (Kesdek *et al.* 2025). These advantages highlight the considerable interest in investigating safe, natural antioxidants (Outaki *et al.* 2023; Luzardo-Ocampo *et al.* 2024). It is essential to analyze antioxidant properties of *R. communis* leaf extracts content for each country to provide valuable scientific insights. These findings could significantly benefit emerging industries such as biofuel conservation (Mintiwab and Jeyaramraja 2021). This revelation would be an excellent addition to the scientific literature. In addition to the value and importance of the oil from the seeds of *R. communis*, other authors have explored the properties of its leaves and stems.

Table 1 shows some of the most representative molecules that have been identified in *R. communis* leaf extracts by other researchers. The three functional groups listed in the table are commonly cited as responsible for the bioactivity of extracts and essential oils, conferring antimicrobial, fungicidal, repellent, and antioxidant properties (Ambika and Chauhan 2009; Kim and Cheng 2023; Abomuhaid *et al.* 2024).

Although castor plant residues, such as leaves and seeds, contain compounds that are toxic to humans and animals (Abomuhaid *et al.* 2024), the novelty of this work lies in revalorizing castor leaves from northwestern Mexico through an antioxidant application that utilizes the phytochemical compounds still present in these residues for applications that are not for human or animal consumption. Therefore, the objective of this study was to evaluate the antioxidant activity and to identify key phytochemicals in extracts obtained from castor leaves. Extracts were prepared using water, methanol, ethanol, as well as mixtures of methanol-water and ethanol-water. The antioxidant techniques employed included total phenols, ferric reducing antioxidant power (FRAP), and free radical scavenging (DPPH). Each of the extracts was analyzed by High Performance Liquid Chromatography (HPLC) to identify key phytochemicals. The castor leaf waste after extraction was analyzed using scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM-EDS) to study the destruction effect of the solvent extraction.

Table 1. Bioactive Compounds in *R. communis* Leaf Extracts (PubChem 2025)

Group	Compound	PubChem CID	Formula	MW (g/mol)	Structure
Flavonoids	Kaempferol	5280863	C ₁₅ H ₁₀ O ₆	286.24	
	Quercetin-3-o-rutinoside	52808025	C ₂₇ H ₃₀ O ₁₆	610.50	
	Quercetin	5280343	C ₁₅ H ₁₀ O ₇	302.23	
Monoterpenes	1,8-Cineole	2758	C ₁₀ H ₁₈ O	154.25	
	alpha-pinene	6654	C ₁₀ H ₁₆	136.23	
Phenol	Gallic Acid	370	C ₇ H ₆ O ₅	172.12	

EXPERIMENTAL

Reagents

2,4,6-tripyridyl-s-triazine (TPTZ), 2,2-diphenyl-2-picrylhydrazyl free radical (DPPH), gallic acid, and ascorbic acid, were purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol, Folin-Ciocalteu reagent, sodium carbonate, sodium phosphate dibasic, sodium phosphate monobasic, sodium chloride, dimethyl sulfoxide, hydrochloric acid and ferric chloride hexahydrate were purchased from Merck (Darmstadt, Germany). Distilled water was obtained from a local supplier.

Plant Material

A certified batch of *R. communis* seeds was donated to the Autonomous University of Baja California as part of a collaboration agreement with the National Institute of Forestry, Agricultural, and Livestock Research (INIFAP), a Mexican organization highly specialized in this field. These seeds were planted in a specially designated section within the gardens of the Vice-Rectorate at the Mexicali campus of the university. The shrubs, approximately four years old, were pruned to collect the leaves, which served as the raw material for the antioxidant analyses performed.

The University is located at 32.62°N and 115.45°W, at an altitude of 8 meters above sea level. The city lies in the Sonoran Desert, spanning northwestern Mexico and the southeastern United States, and has a BWh climate on the Köppen scale, indicating a hot desert climate. The collected material was taken from the shrub at a height of 1.5 m from the ground using steel scissors. The plant material was immediately transported to the laboratory and kept at room temperature for 24 h.

Extracts

The plant material was rinsed with running water to remove dust, impurities, and insects, then rinsed with deionized water. Once rinsed and dry to the touch, the material was weighed and left to dry at room temperature (25 °C) for an additional 48 h. After this period, the material was weighed again. Part of the material was reserved for SEM analysis, while the rest proceeded to the extraction process.

Leaves of *R. communis* were detached from the branches and subjected to drying at room temperature, following the procedure described by Nemudzivhadi and Masoko (2015). In the event that the dried plant material did not exhibit a significant increase in mass, this result could suggest minimal or negligible interactions under the evaluated experimental conditions, after which the material was subsequently ground into a powder using a cast iron hand mill. A portion of the ground material was reserved for elemental analysis, and the remainder continued with the extraction process. The powdered material was sieved using a 190-mesh (0.08 mm) screen and subjected to extraction with five different solvents: ethanol, methanol, and water as pure solvents, as well as 80:20 mixtures of methanol: water and ethanol: water.

The extraction process was performed one time and involved maceration at a controlled room temperature of 25 °C, similar to the method described by Jeyaseelan and Jashothan (2012), using 20 g of plant material per 100 mL of solvent and allowing it to rest in an Erlenmeyer flask for 24 h. The extract was then filtered through Whatman No. 1 filter paper and concentrated in a DLAB RE 100-PRO rotary evaporator (DLAB Scientific Instrument Inc., Ontario, CA, USA) under reduced pressure. The operating conditions were 45 °C at 120 rpm, with a vacuum of 500 mmHg for 40 min. After concentration, the extract was weighed to determine the mass yield, and stored at 4 °C. This procedure was repeated for all solvents. The residues of the plant matrix were recovered for SEM-EDS analysis.

Microstructure Analysis by SEM-EDS

Leaf samples of *R. communis* were analyzed using SEM-EDS to observe microstructural modifications before and after the extraction process. For extract preparation, a portion of the leaf batch was air-dried at room temperature and subsequently used in the extraction procedures. Independently, another portion of the leaf batch was specifically prepared for SEM analysis: six samples were dehydrated under controlled conditions required for electron microscopy, mounted on conductive supports with carbon

tape, and analyzed without metallic coating. A JEOL JSM-6010LA analytical scanning electron microscope (Tokyo, Japan) was used, operating at a working distance of 10 mm, an acceleration voltage of 15 kV in high vacuum, and a backscattered electron detector for imaging. SEM images were obtained at 80x magnification.

Elemental Analysis

Elemental analysis of the dried plant material was performed using a Thermo Flash 2000 elemental analyzer with the copper reduction method. This provided the percentages of C, H, N, and S, as well as the O content by difference. The operating conditions included a reactor temperature of 950 °C and an oven temperature of 65 °C. The column length was 2 m with a helium flow rate of 140 mL/min as the carrier gas.

High Performance Liquid Chromatography Analysis

Phenolic acids were quantified by high performance liquid chromatography (HPLC) using a UFLC Shimadzu LC-20AD system (Shimadzu Scientific Instruments, Kyoto, Japan), equipped with a SIL-20AHT autosampler, a CBM-20A system controller, and an SPD-M20A photodiode array detector set at 320 nm. Separation was achieved on an RP-18 Restek® column (4.6 × 250 mm, 5 µm particle size; Restek Corporation, Bellefonte, PA, USA).

The analyzed compounds included gallic, cinnamic, p-coumaric, caffeic, ferulic, and chlorogenic acids, as well as (+)-catechin and (–)-epicatechin. Quantification was performed using external calibration curves prepared for each standard compound.

The mobile phase consisted of methanol and acidified water (pH 2.78), delivered at a flow rate of 0.6 mL/min. The analysis was conducted on a single sample, and the results are expressed as milligrams of compound per 100 g of sample (mg/100 g).

Antioxidant Activity

DPPH free radical scavenging

To prepare the sample for the test, each extract was diluted to concentrations of 10%, 1%, and 0.1% in methanol, with only one dilution selected for analysis according to a calibration curve with ascorbic acid. The free radical scavenging activity was measured using Brand-Williams' technique (Brand-Williams *et al.* 1995), with modifications (Sagaste *et al.* 2019). The test involved reacting 1,980 µL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) methanol solution at 6 µM with 20 µL of the working sample. After stirring, the mixture was left in a dark room for 30 minutes. Absorbance was measured at 517 nm using a VWR UV-3100PC (VWR International bv, Leuven, Belgium), and it was performed in triplicate. The antioxidant effect of the extract was represented by calculating the percentage reduction in absorbance compared to the blank (%inh), as in Eq. 1,

$$\%Inh = (1 - (A_s - A_b) / A_r) * 100 \quad (1)$$

where %inh is the inhibition percentage of the DPPH radical, and A_s , A_b , and A_r are the 517 nm absorbance of the working sample, blank, and reference, respectively. The antioxidant capacity was measured as micromole equivalents of ascorbic acid per gram of extract (mAAE/g) using a calibration curve. The entire assay was performed in triplicate. Based on the obtained data, the concentration at which 50% of DPPH radicals are inhibited, known as IC₅₀, was determined.

Ferric reducing antioxidant power (FRAP) assay

In this determination, each extract was diluted to concentrations of 10%, 1%, and 0.1% in their corresponding solvent or mixture of solvents. A dilution within the range of the ascorbic acid calibration curve was selected. For the FRAP method, a working solution was prepared by adding 1 mL of TPTZ solution at 10 mmol/L, 1 mL of FeCl₃ at 20 mmol/L, and 10 mL of an acetate buffer at pH 3.4. Each sample was prepared by adding an aliquot of 100 µL combined with 1,900 µL of the working solution. The mixture was incubated for 30 min and the absorbance was measured at 593 nm using a VWR UV-3100PC (VWR International bv, Leuven, Belgium). The antioxidant capacity was measured as micromole equivalents of ascorbic acid per gram of extract (mAAE/g). All samples were prepared in triplicate (Benzie and Strain 1996).

Total phenols determination

For this test, each extract was diluted to concentrations of 10%, 1%, and 0.1% of their corresponding solvent or mixture of solvent, to select the working solution for the method. Only one dilution was selected for the analysis according to a calibration curve with gallic acid. Total phenols in the extract were determined using the Singleton and Rossi technique on a 2,000 µL cell (Singleton and Rossi 1965). This technique consists of reacting 250 µL of Folin Ciocalteu reagent with 100 µL of the working sample and 850 µL of water inside a sample cell. After 5 min, the sample cell was brought to a volume of 2,000 µL using a 7.1% w/v solution of Na₂CO₃ and allowed to react for 1 h. Absorbance was measured at 760 nm using a VWR UV-3100PC (VWR International by, Leuven, Belgium). Results were expressed as µmol of gallic acid equivalent per gram of extract (GAE/g), and the test was performed by triplicate.

Statistical analysis

All data assays were repeated at least three times. The research study was a mixed methods approach with a descriptive and comparative experimental design. The representativeness of these data was presented by the mean ± standard deviation. Statistical analyses were conducted using the Pearson correlation technique and were performed using Excel software and MiniTab14, considering a significance level, α , of 0.05.

RESULTS AND DISCUSSION

Extracts

The extraction yield ranged between 1.00 and 2.10 g of extract per 100 g of plant material, as shown in Table 2.

Table 2. Extraction Yields of the Different Solvent Mixtures Used

Solvent	Yield (g of extract/100 g of plant material)
Methanol	1.2 ± 0.07
Methanol:Water	1.0 ± 0.10
Water	2.1 ± 0.09
Ethanol	1.0 ± 0.11
Ethanol:Water	1.9 ± 0.13

This metric has been previously analyzed by Ghaffar and Perveen (2024), who concluded that the differences in the mass yield of extracts from the Malvaceae family are due to the varying polarity of the solvents and their affinity for different bioactive compounds. The aqueous extract showed the highest yield, while the methanol: water mixture had the lowest. The extracts exhibited a green-brown appearance and low viscosity.

SEM-EDS Results

The SEM images in 80x magnification were arranged according to the reduction in the size of the castor leaf, as shown in Fig. 4. In Fig. 4a, the sample before any extraction is shown, where large complete sections and some small particles are visible. Figure 4b shows the castor leaf after the preparation of the extract with water, where large and complete sections can be observed with a slightly cleaner image compared to the previous one. In Fig. 4c, smaller fragments of the *R. communis* leaf are observed; the solvent used in this extract was a methanol-water mixture, suggesting that the presence of alcohol more easily reduced the leaf's morphology.

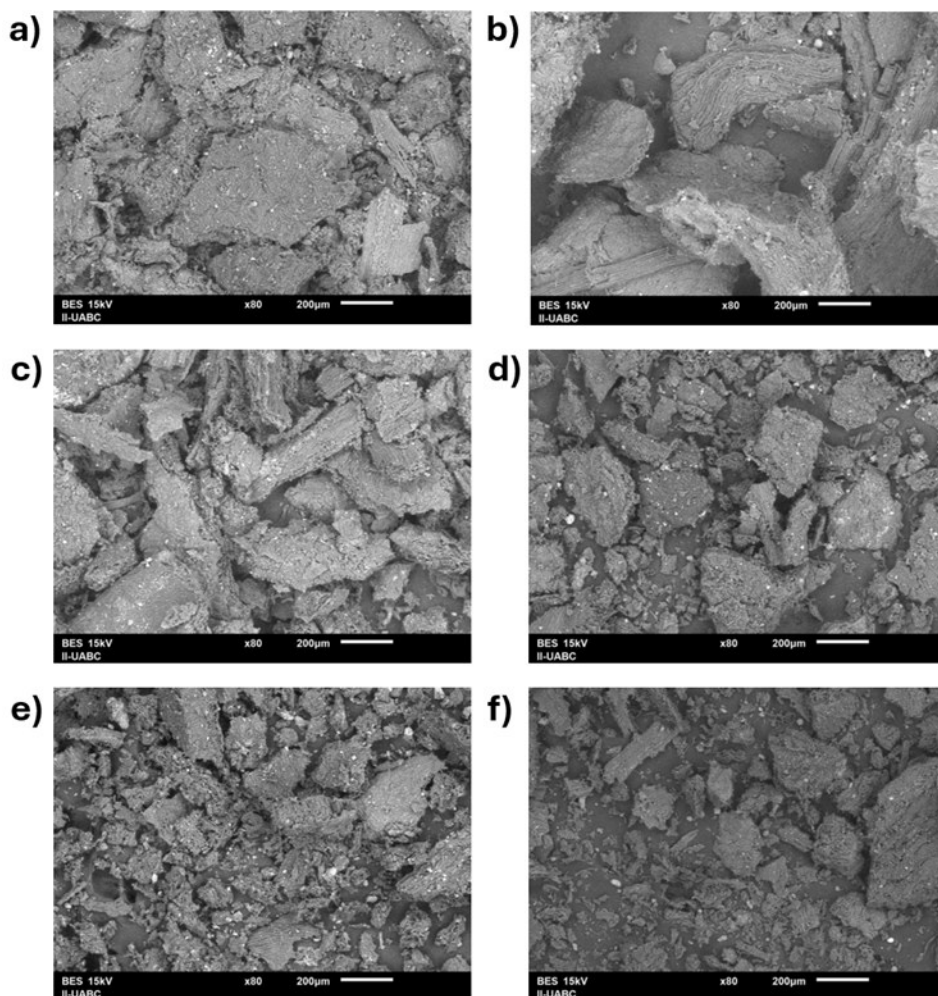


Fig. 4. SEM images of plant material a) before extraction or after extraction with b) water, c) methanol: water, d) methanol, e) ethanol: water, or f) ethanol. Scale bar = 200 μm .

The solvent used in Fig. 4d was pure methanol, and the trend in particle size reduction continues with the increased methanol concentration. Figures 4e and 4f used an ethanol-water mixture and pure ethanol, respectively, showing the maximum leaf degradation in the images from Fig. 4, suggesting that increasing the carbon chain in the alcohol enhanced the leaf destruction effect.

The identification of certain chemical elements by SEM-EDS may correspond to their solubility in different solvents, as shown in Table 3. The materials extracted with water, ethanol:water, and methanol:water mixtures contained a higher elemental mass percentage of Ca and K compared to the original sample or those extracted with pure organic solvents.

Irrigation water is a fundamental factor in the transport of essential nutrients from the soil to the plant's roots. However, the salinity of this water can result in excessive concentrations of sodium and chlorine, which may induce toxic effects that compromise the physiological functions and growth of *R. communis*. The development of this species is profoundly influenced by variables related to the management and quality of irrigation water. Fluctuations in the quality, volume, and frequency of water supply can have a direct impact on the elemental content and chemical composition of the plant, thereby affecting its overall productivity and ability to adapt to different environmental conditions.

Table 3. Detected Elements by SEM-EDS in Plant Material Before and After Extraction

Element	Before	Methanol	Methanol: Water	Water	Ethanol	Ethanol: Water
C	57.83 ± 0.11	55.03 ± 0.10	52.49 ± 0.14	60.12 ± 0.11	52.49 ± 0.14	55.06 ± 0.12
O	36.38 ± 0.33	37.55 ± 0.30	34.8 ± 0.44	34.27 ± 0.33	34.80 ± 0.44	35.95 ± 0.35
Mg	0.30 ± 0.06	1.00 ± 0.06	0.30 ± 0.09	0.20 ± 0.06	0.30 ± 0.09	0.79 ± 0.07
Al	0.21 ± 0.06	0.29 ± *	-	0.19 ± 0.06	-	-
Si	0.29 ± 0.06	0.59 ± 0.06	0.80 ± 0.10	-	0.80 ± 0.10	-
P	0.23 ± *	-	-	0.20 ± *	0.19 ± 0.08	-
S	0.47 ± *	1.09 ± 0.06	0.19 ± 0.08	0.77 ± 0.06	0.97 ± 0.08	1.03 ± 0.07
Cl	0.52 ± *	-	0.97 ± 0.07	-	-	0.77 ± 0.06
K	1.36 ± 0.10	1.84 ± 0.10	2.96 ± 0.18	1.83 ± 0.11	2.96 ± 0.18	3.54 ± 0.15
Ca	2.41 ± 0.83	2.60 ± 0.12	7.51 ± 0.28	2.40 ± 0.14	7.51 ± 0.28	2.86 ± 0.16

(-): not detected. (*): negligible.

Elemental Analysis Results

The results of the elemental analysis show that the plant material is composed of 40.13% C, 5.09% H, 3.64% N, 0.22% H, and the remaining 50.92% O. These results are similar to those reported by Maldonado and Morales (2022), who determined 38.96% C, 4.35% N, and 5.30% H, with no clear metal contamination.

HPLC Results

The detection of the eight target phenolic compounds in the analyzed samples contributes to a mechanistic interpretation of the antioxidant activity exhibited by *R. communis* extracts. The results of the determination of active compounds by HPLC are summarized in Table 4.

Table 4. Results of the Determination of Active Compounds by HPLC

Antioxidants content (mg/100 g)	Methanol	Methanol/Water (80:20)	Water	Ethanol	Ethanol/Water (80:20)
Gallic acid	11.2	18.4	14.2	17.8	16.1
Cinnamic acid	78.2	69.4	41.8	56.1	72.0
<i>p</i> -Coumaric acid	3.4	6.2	5.4	3.8	4.4
Caffeic acid	1.7	5.7	3.3	2.1	5.1
Ferulic acid	1.3	6.3	3.6	5.7	7.2
Chlorogenic acid	9.1	8.6	6.0	8.3	7.6
(+)-Catechin	81.8	88.5	70.6	76.8	91.8
(-)-Epicatechin	110.6	116.9	103.2	107.3	122.9
Total	297.3	320	248.1	277.9	327.1

The phenolic acids and flavan-3-ols such as catechin and epicatechin are widely recognized for their capacity to donate hydrogen atoms or electrons, operating through hydrogen atom transfer (HAT) and single electron transfer (SET) mechanisms. This behavior explains their effectiveness in scavenging stable free radicals such as DPPH•. In contrast, the comparatively lower response observed in the FRAP assay suggests that the antioxidant action of the extracts is more closely associated with radical neutralization than with ferric ion reduction, highlighting the relevance of the underlying reaction mechanism in interpreting assay outcomes.

A clear relationship between phenolic concentration and antiradical performance was observed. The aqueous extract, which contained the lowest levels of the identified phenolic compounds, exhibited the weakest DPPH radical-scavenging activity. This trend supports the contribution of these compounds to the overall antioxidant capacity and suggests that solvent polarity plays a critical role in the selective extraction of bioactive constituents.

While phenolic acids are often considered primary contributors to antioxidant activity, the complexity of plant matrices indicates that synergistic interactions among multiple phytochemical classes may enhance the observed effects. In this regard, flavonoids and other secondary metabolites potentially present in *R. communis* extracts could act additively or synergistically, thereby broadening the antioxidant response beyond that explained solely by the quantified phenolic acids. Future studies aimed at a more comprehensive phytochemical characterization will be essential to fully elucidate these interactions and their impact on antioxidant mechanisms.

Determination of Antioxidant Capacity

Table 5 summarizes the antioxidant properties of the extracts, measured in IC₅₀, free radical scavenging capacity, reducing activity, and phenolic content. Regarding the first parameter, considering the scale proposed by Phoingpaichit *et al.* (2007) based on IC₅₀, all extracts fell into the strong antiradical classification, as this parameter yielded values below 50 µg/mL (Ambika and Chauhan 2014). Similarly, using the IC₅₀ and the values obtained at different concentrations, the equivalent in micromoles of ascorbic acid per gram of extract (mAAE/g) was calculated.

Table 5. Results of LSD Analysis for the Solvent Factor *versus* IC₅₀, DPPH, FRAP and Total Phenols

Sample	IC ₅₀ (µg/mL)				DPPH (mAAE/g) *				FRAP (mAAE/g) *				Total Phenols (GAE/g) **			
	LSD	0.03			LSD	4.71			LSD	7.92			LSD	4.83		
	Mean	Group			Mean	Group			Mean	Group			Mean	Group		
Ethanol	31.01		B		120.58			D	158.10		B		64.48		B	
Ethanol:water	16.83			C	222.19		C		171.05		B		87.14	A		
Methanol	9.25				404.15	A			172.51	A	B		92.82	A		
Methanol:water	13.18			D	283.65		B		189.14	A			83.17	A		
Water	38.94	A			96.01			E	123.27			C	47.56			C

*mAAE/g: ascorbic acid antioxidant equivalent, micromole or ascorbic acid per gram of extract.

**GAE/g: gallic acid equivalent in micromole of gallic acid per gram of extract.

Different letters mean statistical differences.

Statistical analysis indicated significant differences among the mean values obtained with the five antioxidant activity assays. Post hoc comparisons showed that, for IC₅₀ and DPPH, all means differed significantly. In contrast, for the remaining two methods, the means clustered into statistically similar groups, as shown in Table 5. Notably, the aqueous extract was not statistically similar to any other solvent in any assay and consistently exhibited the lowest performance.

The DPPH results can also be expressed as the percentage of inhibition, as shown in Eq. 1. The values obtained were 83.7% for the methanolic extract, 83.4% for the methanol:water extract, 76.3% for the aqueous extract, 66.0% for the ethanolic extract, and 73.4% for the ethanol:water extract. In the literature, DPPH inhibition values range from 44.4% to 79.8% (Mintiwab and Jeyaramraja 2021; Lee *et al.* 2022; Outaki *et al.* 2023; Luzardo-Ocampo *et al.* 2024), with the most favorable results typically observed with methanolic extracts. This comparison indicates that the results obtained in this study fell within the range reported by other authors, with a slight tendency toward the higher end of inhibition range.

The total phenolic content of *R. communis* leaf extracts ranged from 21.0 to 142.7 mg GAE/g, depending on the solvent used (Mintiwab and Jeyaramraja 2021; Surco-Laos *et al.* 2022). The results obtained in this study showed some variation compared to those reported in the literature, as the ethanolic extract contained a higher amount of phenolic compounds than the ethanol-water extract. This trend is the opposite of what has been observed by other authors (Surco-Laos *et al.* 2022). Regarding FRAP, no information was found in the literature for this type of extract.

Some authors have identified the compound rutin (quercetin-3-O-rutinoside) in *R. communis* leaf extracts through HPLC (Lee *et al.* 2022; Outaki *et al.* 2023), a flavonoid commonly found in plant leaves. Its composition has been detected up to 25.6% in the extract mixture (Luzardo-Ocampo *et al.* 2024). Given its chemical characteristics, this compound can be attributed with the antioxidant effect of *R. communis* extract. Additionally, its concentration increases in methanolic extracts, which explains the increase in the TPC values obtained (Outaki *et al.* 2023). Because there is no information in the literature regarding FRAP tests on *R. communis* leaf extracts, it is useful to correlate these findings with data from the DPPH method, as there is a direct correlation between them, allowing for the inference of result trends.

The FRAP assay yielded results complementary to those obtained by the DPPH method. The trend in DPPH and FRAP results was proportional, meaning that the extract with the highest antiradical activity also exhibited the greatest reducing capacity. This correlation could be explained if the compounds responsible for the antiradical action are more soluble in one solvent, while those responsible for the reducing activity are less so. However, when calculating the equation for the methanolic extract, the correlation coefficient between antioxidant activity by DPPH and FRAP was 0.83, as shown in Fig. 5. Further analysis of the extracts is necessary to explain this trend, such as the measurement of total flavonoids and phenolic acids.

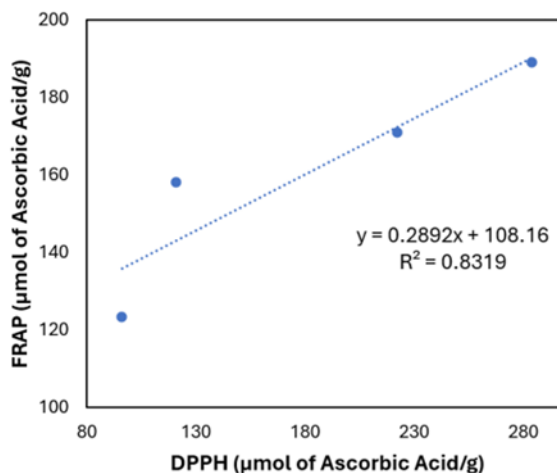


Fig. 5. Correlation between antiradical activity (horizontal axis) and reducing power (vertical axis) excluding the methanolic extract

Similarly, the determination of total phenols showed a correlation with antiradical activity, with higher phenol content resulting in greater activity by the DPPH method. This correlation is illustrated in Fig. 6.

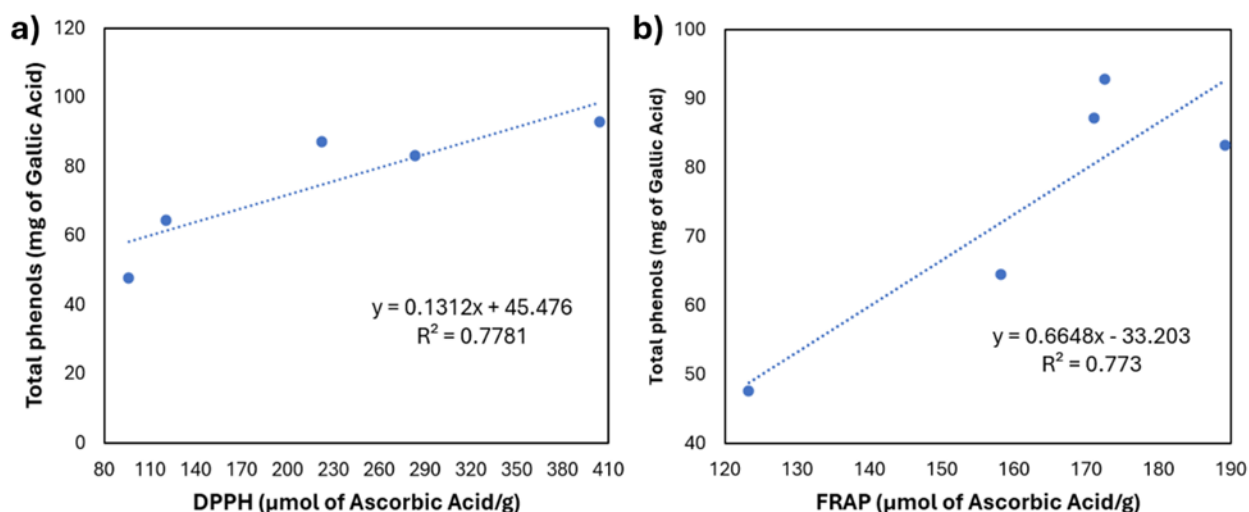


Fig. 6. Correlation between antiradical activity (horizontal axis) and a) antioxidant power or b) total phenolic content

The relationship between phenols measured by the Folin-Ciocalteu reagent method and antioxidant activity by DPPH radical scavenging has been reported by other authors, such as Aryal *et al.* (2019), who evaluated these properties in native plants of Nepal, finding an $R^2=0.75$ when comparing both values. The author also reported a correlation of $R^2=0.84$ for total flavonoid content using the DPPH radical scavenging method.

In turn, the correlation between reducing power and phenolic content followed a more moderate correlation ($R^2=0.773$). This aligns with findings reported by Morales *et al.* (2017), who found correlations ranging from 0.84 to 0.87 between phenolic content and antioxidant power using the ORAC and FRAP methods, respectively, when studying extracts from various varieties of *Mangifera indica*.

If, instead of graphing reducing activity and phenols against antiradical activity, one graphs them with respect to IC_{50} , a much clearer linearity is observed, with R^2 coefficients reaching 0.95, as shown in Fig. 7. As noted previously, the methanolic extract deviates from this trend. This can again be attributed to solubility, as methanol is less selective than ethanol in dissolving organic compounds, potentially leading to “saturation” with highly polar compounds that have significant antioxidant power, but they are not soluble in water or ethanol.

The effect of solvents on extract properties has been studied by other authors. In analyzing various extraction techniques and their bioactivity, Felix-Sagaste *et al.* (2023) argues that ethanol and water are usually good solvents for phenolic compounds. However, the data obtained in this work show the presence of methanol-soluble compounds with antioxidant activity in the leaves of *R. communis*. This aligns with the finding that phenolic content is higher in pure methanol, with the methanol: water mixture being the second-best performing sample, both showing the best antioxidant performance as measured by both techniques. Further chemical analysis of the extracts will be necessary to identify the individual compounds contributing to the antioxidant performance.

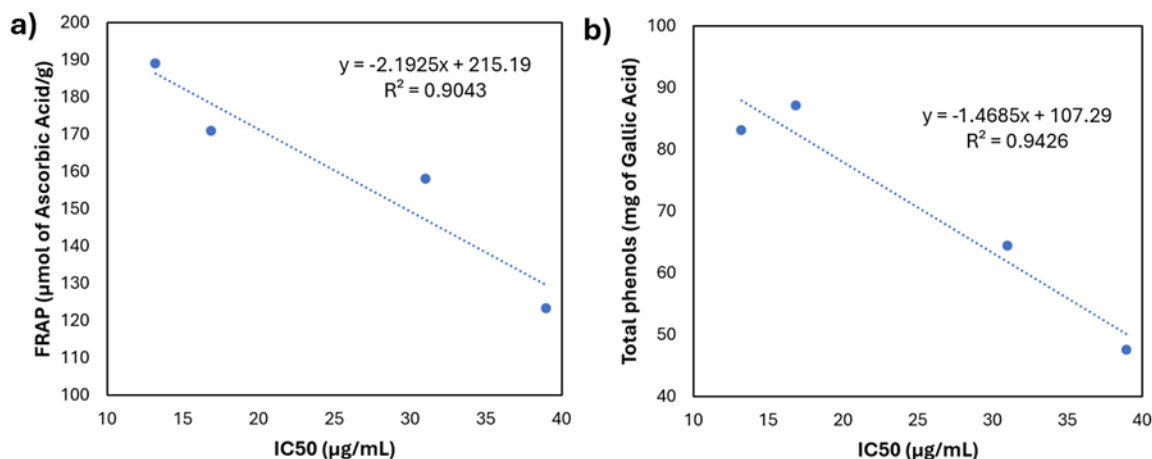


Fig. 7. Correlation between IC_{50} (horizontal axis) and a) antioxidant power or b) total phenolic content

Regarding the methanol: water mixture, the authors suggest exploring its use, which, although it has lower antioxidant power, would result in lower costs and be safer to handle compared to pure alcohol. This cost reduction could offset its modest properties.

Diem Do *et al.* (2014) analyzed extracts of *Limnophila aromatica* using various solvents: water, ethanol, methanol, and acetone, as well as their 1:1 and 1:3 mixtures with

water. The results showed that the correlation of total phenols against IC₅₀ by the DPPH method was $R^2 = 0.72$, provided the pure water extract is excluded, as its inclusion breaks the correlation between the two properties. The aqueous extract followed the general trend that lower phenolic content corresponds to higher IC₅₀, and thus lower antiradical capacity by the DPPH method. These results are similar to those presented in the present work, where the aqueous extract showed low antiradical activity and low phenolic content. These findings can also be attributed to the different solubilities of the bioactive compounds in the plant matrix studied. Other authors attribute the antioxidant activity observed in any of the methods applied in this work to the presence of flavonoids, phenols, and some terpenes such as 1,8-cineole and α -pinene, compounds known for their antioxidant activity (Ambika and Chauhan 2009; Ahmed *et al.* 2015; Jia *et al.* 2019). In this work, the characterization of the extract did not include the identification of terpenes; however, some have been reported in extracts of *R. communis* (Abomuhaid *et al.* 2024).

The results obtained in this work can complement one of the potential applications of *R. communis* cultivation: the production of biofuels. The oil from this plant, being non-edible, has been explored as a raw material for biofuel. This energy source has the disadvantage of being prone to oxidation, requiring antioxidants to improve its stability. With antioxidant properties, the extracts evaluated in this work can be part of the integrated process, from cultivating the plant to converting the oil into biofuel.

The use of extracts to enhance oxidative stability in biodiesel has already been explored by other authors (Park *et al.* 2008; Knothe and Razon 2017). Similarly, the authors of this study improved the oxidative stability of canola biodiesel almost fourfold by adding extracts, as measured by induction time (Sagaste *et al.* 2019). This enhancement can be complemented by reports on the antimicrobial and reducing activity of *R. communis* extracts (Linima *et al.* 2023).

Extracts were obtained using various solvents and solvent mixtures from the aerial parts of *R. communis*. The extraction yield ranged between 1 and 2.1 g of extract per 100 g of plant material, the aqueous extract showed the highest yield, while the methanol: water mixture had the lowest. The plant material showed an elemental composition consistent with other biomass matrices. The extracts demonstrated significant strong antioxidant activity, as they were able to scavenge DPPH radicals and exhibited good reducing capacity at low concentrations. The results obtained provided valuable data on the antioxidant capacity of various *R. communis* leaf extracts, measured using the FRAP method. These findings revealed a proportional relationship with the antioxidant activity determined through the DPPH technique, underscoring the consistency and reliability of both methods in evaluating the antioxidant potential of the extracts.

The extract that performed best was the one obtained using pure methanol as the solvent. It can be established that *R. communis* extracts constitute a valuable product as a natural antioxidant. The leaf extract from *R. communis* cultivated in Mexico represents a valuable resource as an antioxidant source. This presents an opportunity to utilize a plant that currently lacks applications and is even considered an invasive species. Further analysis of the extract obtained in this work is necessary, with the determination of including determining total flavonoid content and the identification of the compounds responsible for the strong antioxidant activity. It would be useful to include further studies specifically assessing oxidative stability in fuel systems are required to determine whether this activity translates into measurable improvements in oxidative stability index.

CONCLUSIONS

1. The performance of the castor leaves extracts demonstrates that there are still phytochemicals having antioxidant capacity, which be applied to applications not related to human or animal consumption.
2. Their potential to enhance stability and prevent oxidative degradation positions these extracts as an effective and sustainable solution as an antioxidant. The antioxidant activity of *R. communis* extracts increases the agronomic and economic value of this crop, particularly in arid and low-precipitation regions where it is well adapted.
3. The influence of the extraction solvent is evident from the results, which show significant differences in antioxidant activity among the solvents evaluated, with water being the least suitable. This behavior may be attributed to the greater solubility of bioactive compounds in alcohol-based solvents.
4. In addition to its established use for non-edible oil production, the recovery of antioxidant compounds may be integrated into a value-added production chain, similar to biorefinery approaches proposed for *Moringa oleifera*.

ACKNOWLEDGMENTS

The authors thank the Universidad Autónoma de Baja California for its support in the development of this work.

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Article submitted: August 8, 2025; Peer review completed: February 13, 2026; Revised version received: April 13, 2026; Accepted: May 6, 2026; Published: May 19, 2026.
DOI: 10.15376/biores.21.3.6105-6122