Scientific Paper

Phytochemical composition and antibacterial properties of *Ricinus communis* L.

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Abstract

Objective: To evaluate the phytochemical composition and antibacterial properties of *Ricinus communis* L. leaf extracts.

Materials and Methods: Leaves from adult plants were used for extractions in 90 % ethanol and distilled water. The presence of flavonoids, terpenoids, tannins, saponins, steroids, coumarins, anthraquinones and cardiotonic glycosides was qualitatively determined. The contents of phenols, soluble and fixed to the walls, reducing sugars and total soluble proteins were quantified. The antibacterial activities of the ethanolic extract were evaluated by the well technique on Gram-negative bacteria *Escherichia coli* ATCC 25922, *Proteus* sp. and *Klebsiella pneumonie* and on Gram-positive *Staphylococcus aureus* ATCC 25923.

Results: Abundant presence of flavonoids, terpenoids and tannins was observed in both solvents, and lesser presence of steroids, saponins and coumarins. The total phenol content was 56,5 mg g-1 (fresh mass). The ethanolic extract showed the highest values of reducing sugars and soluble proteins and antibacterial activity against *S. aureus* and *Proteus* sp. The inhibitory effect was lower on Gram-negative bacteria *E. coli* and *Klebsiella* sp.

Conclusions: *R. communis* leaves have bioactive compounds with antibacterial properties. The extracts showed potential to control infectious diseases, so it is of interest to promote further studies to develop their use in traditional medicine.

Keywords: pathogenic bacteria, biochemistry, secondary metabolites

Introduction

One of the problems currently faced by the World Health Organization is the resistance of bacteria to antibiotics. This problem has led to the search for new drugs of botanical origin that, because they have different action mechanisms, constitute a viable alternative to conventional drugs, which, in addition to being expensive, have adverse effects (Shamsudin *et al*., 2022).

Ricinus communis L. (fig tree) is a species of the *Euphorbiaceae* family, which is used in traditional medicine to treat various infectious diseases (Kebede and Shibeshi, 2022). It is considered an unconventional forage resource due to its protein-energy value and high rumen degradability (Ramírez *et al*., 2017; Palma-García, 2018). Recently, Ramírez-Navarro *et al*. (2020) proved that its inclusion in sheep diets did not affect productive indicators, for which they consider it as a nutritional alternative.

Phytochemical studies with extracts from leaves, roots and bark of *R. communis* have shown the presence of secondary metabolites (flavonoids, terpenes, saponins, tannins, glycosides and alkaloids). These compounds have the active principles that form the basis of biological properties useful for the agricultural and medical-pharmaceutical sector (Rashmi *et al*., 2019; Nawaz *et al*., 2022). However, the phytochemical profile of plants depends on numerous factors, including soil and climate conditions, variety and genotype (Rahul *et al*., 2022); thus, it is necessary to conduct studies with the local flora in order to corroborate whether the potential for its use in the treatment of infectious diseases is maintained. Therefore, the objective of this research was to evaluate the phytochemical and antibacterial properties of *R. communis* leaf extracts.

Materials and Methods

Location. The studies were carried out in the laboratories of the Center for Biotechnological Studies (CEBIO) of the School of Agricultural Sciences of the University of Matanzas, Cuba.

Selection, identification and characterization of plant material. *R. communis* plants were obtained from the Botanical Garden of the University of Matanzas, located at coordinates, 23°02′06″N 81°30′36″W / 23.034377-81.507485. Taxonomic iden-

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tification was carried out by specialists of this entity from the herbarium and morphological traits *in situ*. A quantity of 2,5 kg of fresh leaves from 15 adult plants, which were between 2,0 and 2,5 m tall, was selected. The plants showed no mechanical damage, disease symptoms or pest attack. The collection was carried out in February, 2019, between 8:00 and 9:00 am. The average temperature for the month was 21,5 ºC. The soil in the area is classified as typical Ferralitic Red.

Extract preparation. The leaves were washed with distilled water and dried for 48 h in a natural circulation oven at 45 ºC. The dried material was crushed to a particle size of 1 mm, with the aid of an electrical grinder. Extracts were prepared from 100 g of sample and 2 L of solvent. The alcoholic ones with 90 % ethanol and the aqueous ones with distilled water. The mixtures were shaken over an orbital shaker (HDL®APPARATTUS) at 160 rpm for 24 h and then filtered with Whatmann # 40 paper. The supernatants were stored in amber flasks at 4 ºC. Remaining solids were again homogenized in 1 L for another 24 h. Once filtered, the obtained supernatants were added to the previous ones.

The extracts were concentrated under vacuum with the aid of a rotoevaporator (Heidolfph, Germany). The sediments were placed on Petri dishes and dried in an oven at 50 °C (Mohammed *et al*., 2013). The dried solids were stored in amber flasks until use in phytochemical studies and microbiological tests.

Qualitative determination of secondary metabolites. The determination of secondary metabolites was performed according to the procedure described by Chigodi *et al*. (2013). The contents were evaluated qualitatively using the non-parametric system of crosses (MINSAP, 1997): +++ = abundant, ++ = moderate, + = low, - = absence (table 1).

Content of reducing sugars (RS). It was determined by the dinitrosalicylic acid method using D-glucose (Sigma) as a standard (Miller, 1959).

Table 1. Procedures for determining metabolites.

Metabolites	Essay	Reagents/procedure	Positive result
Flavonoids	Sodium hydroxide test	To 100 mg of dry extract 1 ml of NaOH 0,1 mol L^{-1} is added and then equal volume of 0,1 mol L^{-1} is added	Yellow color
Terpenoids	Salkowski's test	100 mg of dry extract are mixed with 1 ml of chloroform and 2 ml of concentrated H_2SO_4.	Reddish-brown color
Tannins	Ferric chloride test	To 100 mg of dry extract 2 ml of distilled water are added. The mixture is heated in bain-Marie and filtered. Two drops of dissolution of 5 % ferric chloride in methanol (1:1) are added to the supernatant.	Dark green or dark blue color
Saponins	Foam test	To 100 mg of dry extract 3 ml of distilled water are added and it is vigorously shaken.	Formation of persistent foam
Steroids	Salkowski's test	100 mg of dry extract are mixed with 3 ml de $CHCl_3$. 2 ml of concentrated H_2SO_4 is added on the sides of the test tube.	Red color in the top layer and green in the H_2SO_4 layer
Coumarins	Sodium hydroxide test	100 mg of dry extract are mixed with 3 ml of distilled water. The mixture is shaken and filtered. To 1 ml of the filtrate 1 ml of 10 % NaOH is added.	Yellow color
Anthraquinones	Ammonium test	200 mg of dry extract are mixed with 3 ml of 10 % HCl. The mixture is heated at 100 ºC during three minutes in bain-Marie and filtered. The supernatant is cooled until reaching room temperature and 3 ml of $CHCl_3$ and a few drops of 10 % ammonium dissolution are added. This mixture is heated again in bain-Marie during three minutes.	Pink color
Cardiotonic glycosides	Keller-Kiliani's test	200 mg of dry extract are mixed with 5 ml of distilled water. The mixture is shaken and filtered. To 3 ml of the supernatant 2 ml are added of glacial acetic acid, which contains a drop of 1 % ferric chloride and 1 ml of concentrated H_2SO_4.	Brown ring in the interphase along with a purple ring below.

The absorbance was measured at a wavelength of 456 nm.

Content of total soluble proteins (TSP). It was determined colorimetrically by the method of Lowry *et al*. (1951) with bovine serum albumin (BSA) as standard. The absorbance values were measured at a wavelength of 750 nm and the concentrations (mg ml^{1}) were determined using a standard curve.

Content of soluble, wall-bound and total phenols. Phenol extraction was performed according to the method described by Quiñones-Galvez *et al*. (2021). For the soluble phenols soluble phenols, 100 mg of sample were mixed in 1 ml of methanol. The suspension was shaken vigorously in a vortex apparatus (IKA® Vortex 3). It was then centrifuged at 15 000 rpm for 5 min to collect the supernatant.

The extraction of the phenols bound to the cell wall was carried out from the precipitate obtained in the previous procedure; 250 ml of sodium hydroxide, 2 mol L^{-1} were added to the remaining solid and homogenized with the aid of a vortex. Subsequently the mixture was neutralized with equal volume of hydrochloric acid (2 mol L^{-1}) and centrifuged again under the same conditions to obtain the supernatant. The concentrations of soluble and cell wall-bound phenols were determined colorimetrically using chlorogenic acid (0,05 mol L^{-1}) as a standard. The absorbance values were obtained in a spectrophotometer, at wavelength 725 nm Ultrospect 2000 (Pharmacia Biotech, Sweden).

Antibacterial activity. The *in vitro* antibacterial activity of the ethanolic extract was evaluated against Gram-positive bacteria *S. aureus* ATCC 25923 and Gram-negative bacteria *E. coli* ATCC 25922, *Proteus* sp. and *K. pneumonie*. The assay was performed through the well diffusion method, according to the procedure described by Kebede and Shibeshi (2022).

The strains were previously rejuvenated in brain-heart broth medium at 37 °C. Subsequently, they were inoculated in Mueller-Hinton broth medium with the aid of a sterile swab and incubated at 37 °C until a turbidity equivalent to 0,5 on the McFarland scale was obtained in the tube. The wells were made with the aid of a sterile 8-mm diameter borer and 100 µL (200 mg ml^{-1}) of extract were added (Arekemase *et al*., 2019). Similar volume was used in the controls. All plates were incubated for 18 h at 37 °C.

As a negative control, hydroalcoholic dilution, with which the ethanolic extract was prepared, was used. The antibiotics cephalexin 30 µg for *S. aureus* (Gram positive) and amikacin 30 µg for Gram negative were taken as positive control. Antibacterial activity was obtained from the diameter of the bacterial growth inhibition zone (Voleti *et al*., 2022).

Statistical analysis. Qualitative determination of secondary metabolites, antibacterial activity and absorbance readings for the quantification of reducing sugars, total soluble proteins and phenols were performed in triplicate. Data were processed with the statistical package Statgraphic plus 5.1 on Windows, after checking that the data conformed to a normal distribution using the Kolmogorov-Smirnov goodness-of-fit test. For variance homogeneity, Bartlett's tests were performed. For comparison between means, a simple rank analysis of variance was performed, and to determine the antimicrobial activity, Tukey's multiple range test was used. The Kruskal-Wallis test was applied to the data referred to total soluble protein, reducing sugars and phenols, which did not meet the aforementioned premises, with a confidence level of 95 %. The Student Newman-Keuls test was used to compare means ($p < 0,05$).

Results and Discussion

Phytochemical study. Aqueous and ethanolic extracts showed in *R. communis* leaves a high presence of terpenoid compounds and tannins (table 2). Flavonoids were notably detected in the ethanolic extract and moderately in the aqueous extract. Although in lower concentrations, coumarins prevailed in the aqueous extract. Steroids and saponins were other metabolites found as traces in both extracts. No anthocyanins, anthraquinones or cardiotonic glycosides were found.

The contents of total soluble proteins and reducing sugars were higher in the ethanolic extract ($p < 0,05$). Variations in the relative metabolite contents were ascribed to differences in their polarities.

These results coincide with studies in which tannins, saponins, steroids, terpenes and flavonoids were also found (Maldonado-Santoyo and Morales-López, 2022; Wadankar *et al*., 2022). In contrast with the results of this research, Nawaz *et al*. (2022) found cardiotonic glycosides and anthocyanins, which may be associated with various factors, such as genotype, soil and climate conditions, physiological age of the plant, time of collection, and techniques used for extraction and detection of secondary metabolites (Rahul *et al*., 2022).

The presence of metabolites in *R. communis* extracts confirms that this plant is a potential source of bioactive substances, with applications in

Table 2. Ratios of secondary metabolites, total soluble proteins and reducing sugars in aqueous and ethanolic extracts of *R. communis* leaves.

Secondary metabolites	Extract	
	Aqueous	Ethanolic
Flavonoids	++	+++
Terpenes	+++	+++
Anthocyanins	-	-
Steroids	+	+
Saponins	+	+
Tannins	+++	+++
Coumarins	++	+
Cardiotonic glycosides	-	-
Anthraquinones	-	-
TSP, mg ml^{-1}	13,39 ± 0,21	14,66 ± 0,23
RS, mg ml^{-1}	4,48 ± 0,09	6,62 ± 0,11

Content: +++ = abundant, ++ = moderate, + = low, - = absent.
Statistical differences between extracts for the same metabolite, according to the *Kruskal-Wallis* test ($p < 0,05$). ± Standard Error
TSP: content of total soluble proteins, RS: content of reducing sugars

the pharmaceutical and technological fields and in human and veterinary medicine. Several scientific studies have shown that polyphenolic compounds, tannins, flavonoids, coumarins and phenolic acids possess antioxidant, antibacterial, antifungal, antiviral and anticancer properties (Kaczmarek, 2020; Kebede and Shibeshi, 2022; Fink, 2023; Nguyen *et al*., 2023).

The contents of cell wall-bound phenols were significantly higher ($p < 0.05$) than soluble phenols (36.54 vs. 19.11 mg ml^{-1}) and total phenols reached high values (Figure 1). The presence of these polyphenolic compounds has also been referenced by Nawaz *et al.* (2022).

In recent years, the mechanisms of action of secondary metabolites on pathogenic microorganisms

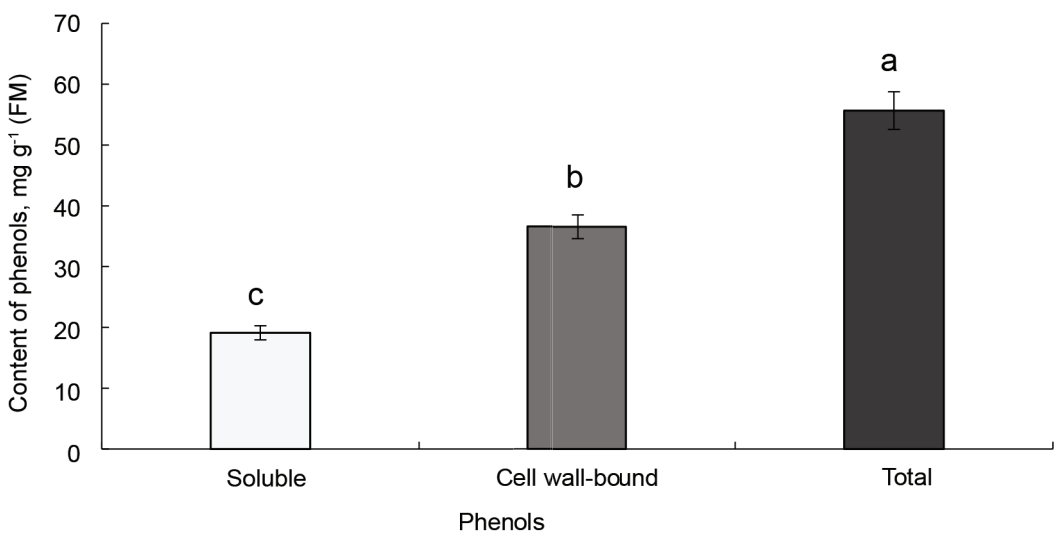

Figure 1. Contents of soluble, cell wall-bound and total phenols in ethanolic extracts of *R. communis* leaves.
FM: fresh mass
Different letters indicate significant differences between extracts, according to *Student Newman-Keuls* test ($p < 0,05$).
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have been studied in depth. This is because, unlike traditional antibiotics, bacteria exposed to these compounds do not develop antimicrobial resistance.

Flavonoids decrease cell viability, since by increasing the permeability of membranes in microorganisms, they promote the release of important cellular components, such as nucleic acids (Thebti *et al*., 2023).

Tannins affect metabolic processes because, in addition to reducing the activities of enzymes and other polypeptides through hydrophobic interactions and hydrogen bonding, they have the ability to chelate an essential cofactor for ATP and NADPH production, iron. This interference in metabolic energy generation leads to the death of microorganisms (Kaczmarek, 2020; Nguyen *et al*., 2023).

Saponins were also proved to have antibacterial activity, associated with the hydrophobic and hydrophilic nature of their chemical structure, which confers an amphipathic character to the molecule. This characteristic allows the metabolite to easily penetrate biological membranes and alter their physical properties, leading to the death of the microorganism (Dong *et al*., 2020).

Terpenoid compounds weaken bacterial membranes and increase their permeability, an action that in addition to affecting ion transfer, functions of membrane proteins and cellular enzymes, inhibits DNA synthesis (Mutlu-Ingok *et al*., 2020).

Antibacterial activity. The evaluation of antibacterial activity was performed with the ethanolic extract, due to the superior effect shown by alcoholic solvents in relation to aqueous solvents (Voleti *et al*., 2022). The results showed a high antibacterial effect against *S. aureus*, with inhibition halos of 15,33 mm, although statistically lower than the positive control (table 3). With regards to Gram-negative bacteria, the highest activity was obtained against *Proteus* sp. with inhibition halos of 8,67 mm, followed by *E. coli*, with average values of 5,67 mm. The extract showed no antibacterial activity against *Klebsiella* sp.

These results are in correspondence with those referred by El-Kahlout *et al*. (2018), who observed that the ethanolic extract of fig leaves (200 mg ml^{-1}) showed lower antibacterial activity against *S. aureus* and higher on *E. coli*. The above-cited authors also report that the extract did not show activity against *Klebsiella* sp. The discrepancies found are ascribed to differences in the resistance of the used strains, although other factors that may affect the final concentrations of the metabolites, such as environmental factors or extraction processes, are not ruled out.

The effectiveness of the extract against the Gram-positive bacteria *S. aureus* was higher than that obtained against the evaluated Gram-negative bacteria. This result coincides with that observed by Byadgi *et al*. (2017), and may be related to the complexity of the cell walls possessed by Gram-negative bacteria. These bacterial groups have, in addition to the peptidoglycan layer, another lipopolysaccharide layer that constitutes a barrier for the entry of water-soluble secondary

Table 3. Antibacterial activity of ethanolic extracts of *R. communis* leaves.

Treatment	*S. aureus*		*E. coli*	
	DIZ, mm	SE ±	DIZ, mm	SE ±
Amikacin (control +)	24,00[a]	0,58	-	-
Cefalexin (control +)	-	-	14,83[a]	0,58
Hydroalcoholic dissolution	1,03[c]	0,33	0,00[c]	0,00
R. communis extract	15,33[b]	0,88	5,67[b]	0,67
Treatment	*Proteus* sp.		*Klebsiella* sp.	
	DIZ, mm	SE ±	DIZ, mm	SE ±
Amikacin (control +)	-	-	-	-
Cefalexin (control +)	12,00[a]	0,33	4,00	0,2
Hydroalcoholic dissolution	0,00[c]	0,00	0,00	0,00
R. communis extract	8,67[b]	0,88	0,00	0,00

DIZ: diameter of the inhibition zone.
Data represent means of three replicas.
Different letters indicate significant difference according to Tukey's multiple range test ($p < 0,05$).

metabolites with antibacterial action (Kaczmarek, 2020). On the other hand, the resistance of Gram-negative bacteria may be associated with the ability of these strains to produce enzymes and/or toxins that degrade the bioactive compounds present in the extract (Arekemase *et al*., 2019).

Recent studies, but with methanolic extracts, confirmed the presence of the bioactive compounds and their antibacterial effects on the same strains evaluated (Kebede and Shibeshi, 2022; Voleti *et al*., 2022). However, they report superior inhibition halos against Gram-negative bacteria, responses that may be ascribed to genotypic differences between strains and/or with the extraction potential of the used solvent.

The antibacterial activity observed in this research is related to the synergistic action of the bioactive compounds found, such as flavonoids, terpenoids, tannins, coumarins, saponins and polyphenols, which are defined as antibacterial agents of different pathogenic microorganisms (Nguyen *et al*., 2023; Thebti *et al*., 2023).

Conclusions

The leaves of *R. communis* contain bioactive compounds with antibacterial properties that have a potential use for the treatment of infectious diseases in animals and humans. It is therefore of interest to promote further studies to support and enhance their use in traditional medicine.

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Conflict of interests

The authors declare that there is no conflict of interest.

Authors' contributions

- Marlene María Martínez-Mora. Design and setting-up of experiments, statistical analysis and data interpretation, manuscript drafting and revision.
- Gladys Sardiña-Alfonso. Design and setting-up of experiments and manuscript drafting.
- Ana Julia Rondón-Castillo. Design of experiments, data analysis and interpretation and manuscript revision.
- Arley Pérez-Rojas. Data analysis and interpretation and manuscript revision.
- Yunel Pérez-Hernández. Design of experiments, statistical analysis and data interpretation, manuscript revision and supervision.

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