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Chemical composition, antibacterial potential, and toxicity of the extracts from the stem bark of *Hancornia speciosa* Gomes (Apocynaceae)

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ABSTRACT

Ethnopharmacological relevance: Hancornia speciosa is a medicinal plant popularly used to treat different medical issues, including infectious diseases. Exploring the therapeutic potentialities of the extracts from medicinal plants combined with conventional antibiotic drugs is a promising horizon, especially considering the rising microbial resistance.

Aim of the study: This study aimed to characterize the chemical composition of the ethereal (EEHS) and methanolic (MEHS) extracts of the stem bark of *H. speciosa*, and also evaluate their antibacterial and drug-modifying activity, and toxicity.

Materials and methods: The extracts were characterized by gas chromatography coupled to mass spectrometry (GC–MS). Additionally, total phenol and flavonoid contents were determined. The antibacterial and antibiotic-modifying activity was evaluated against strains of *Escherichia coli*, *Staphylococcus aureus,* and *Pseudomonas aeruginosa* using the serial microdilution method, obtaining the minimum inhibitory concentration (MIC). The toxicity assay was carried out using the *Drosophila melanogaster* model.

Results: Thirty compounds were identified in the extracts of the stem bark of *H. speciosa*, with triterpenoids being predominant in both extracts. Additionally, fatty alcohols, carbohydrates, fatty acids, phenolic acids, and phytosterols were identified in both extracts. EEHS and MEHS extracts had considerable phenol contents (346.4 and 340.0 mg GAE/g, respectively). Flavonoids were detected in a lower proportion (7.6 and 6.9 mg QE/g, respectively). *H. speciosa* extracts did not display intrinsic antibacterial activity against the bacterial strains evaluated, however, they were capable of modifying the activity of gentamicin, erythromycin, and norfloxacin. EEHS increased the efficacy of norfloxacin against *E. coli* and *S. aureus*, reducing MIC values by 50%. MEHS potentiated the action of gentamicin against all bacterial strains, especially against *E. coli*. The extracts did not display toxicity at clinically relevant concentrations against *D. melanogaster*.

Conclusion: The stem bark of *H. speciosa* was considered a rich source of bioactive compounds. Our findings evidenced the therapeutic potential of *H. speciosa* extracts for the development of new pharmaceutical therapeutics against bacteria. Although the extracts did not exhibit intrinsic antibacterial activity, they enhanced the efficacy of commercial antibiotic drugs and were non-toxic at clinically relevant concentrations. Future studies are needed to elucidate the mechanisms of action of these extracts, ensuring their safety and efficacy.

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1. Introduction

Antibiotics were discovered at the beginning of the 20th century, and since then, they have been widely used for treating and preventing different types of infectious diseases (Hutchings et al., 2019; Ding et al., 2020). However, the intensive and inappropriate use of these drugs has contributed to selecting resistant microorganisms. The appearance of resistant strains is currently outpacing the development of new antimicrobial drugs (Dadgostar, 2019; Udaondo and Matilla, 2020). Microbial resistance reduces the effectiveness of antibiotics, and it is considered a serious threat to the medical systems. Consequently, treating infectious diseases becomes more difficult and costly, affecting the quality of life of the patients under treatment, and in many cases leading to death (Dadgostar, 2019; Morel et al., 2020). According to the World Health Organization (WHO - World Health Organization, 2019), 700,000 deaths are caused by pathogenic microorganisms annually, and the majority of these cases are in underdeveloped and developing countries. Furthermore, it is estimated that by 2050 the world could reach 10 million deaths per year associated with microbial resistance if effective actions are not taken in time. Among these actions, the discovery of new compounds capable of intensifying the activity of commercial antimicrobial drugs has a prominent space (Tagliabue and Rappuoli, 2018; Trotter et al., 2019).

Nosocomial microorganisms such as pathogenic bacteria are known for their capacity to develop microbial resistance. These bacteria are associated with infections acquired in hospital environments, affecting around 10% of the patients and resulting in extra costs for the public health system (Khan et al., 2015; Edwardson and Cairns, 2019). Among the main nosocomial bacteria, *Escherichia coli*, *Staphylococcus aureus,* and *Pseudomonas aeruginosa* stand out globally. These bacterial species are known to cause opportunistic infections, due to their strong resistance, pathogenicity, and virulence. Additionally, these bacteria increase the morbidity and mortality of infected hosts (Hoang et al., 2018; Horn et al., 2018; Khalil et al., 2018). The development of new alternative treatments, including the discovery of new antimicrobial substances has become an urgent need, bringing attention to the use of compounds from medicinal plants (Ueda et al., 2023).

Throughout history, medicinal plants have demonstrated important roles in therapeutics, providing evidence of their effectiveness, which has been passed from generation to generation. The accessibility, low cost, and cultural acceptability make medicinal plants a viable option for treating several infectious diseases, especially in poor and non-developed countries (Hamid et al., 2023). Several medicinal plant species showed great potential against multidrug-resistant bacterial strains (Araújo et al., 2022; Pontes et al., 2022; Almeida-Bezerra et al., 2023). The use of phenolic compounds from plants with strong antimicrobial capacity has been reported as an alternative to the development of new antibacterial agents (Ueda et al., 2023).

Hancornia speciosa Gomes (Apocynaceae) (Fig. 1), commonly known as "mangabeira," is a recognized medicinal species. Ethnopharmacologically, the stem bark of this species is used to treat a variety of medical conditions, including stomach ulcers, stomach pain, diarrhea, dysentery, gastritis, hernia, wound healing, cancer, urinary tract infections, female reproductive system diseases, inflammation and general infections (Ribeiro et al., 2014; Albuquerque and Meiado, 2015; Vieira et al., 2015; Penido et al., 2016; Ribeiro et al., 2017). The therapeutic and healing properties of *H. speciosa* stem bark can be attributed to various compounds found in the species, such as flavonoids, catechins, proanthocyanidins, and tannins (Moraes et al., 2008; D'Abadia et al., 2020; Almeida et al., 2022; Silva et al., 2024). Considering the therapeutic potential of *H. speciosa* stem bark described in ethnomedicinal studies, especially in the treatment of infectious diseases, and due to the increasing resistance of pathogenic bacteria to commercial antimicrobial drugs, there is a need for new compounds with antimicrobial properties. Therefore, it is highly relevant to explore the antimicrobial potential of *H. speciosa* against these types of pathogenic bacterial strains, including those associated with nosocomial infections.

This study hypothesizes that the extracts of the stem bark of *H. speciosa* have antimicrobial effects against bacteria causing infections. The objective of this study was to characterize the chemical composition of sulfuric ether and methanolic extracts from the stem bark of *H. speciosa* and verify their antibacterial and antibiotic-modifying activity against multi-resistant bacterial strains (*E. coli*, *P. aeruginosa,* and *S. aureus*). Additionally, the toxicity of these extracts was tested on *Drosophila melanogaster*.

Fig. 1. Characteristics of *Hancornia speciosa* Gomes (Apocynaceae). (A) = Leaves; (B) = Fruit; (C) = Stem and latex; (D) = Latex collection.

2. Materials and methods

2.1. Collection of botanic material

The samples of stem barks of *H. speciosa* were collected in the municipality of Jardim, state of Ceará, Brazil, in the Environmental Protection Area of Chapada do Araripe, at 920 m altitude, under the coordinates 7°29′02.4″S and 39°16′51.9″W (Fig. 2). The exsiccate of the species was deposited in the Herbarium UFP – Geraldo Mariz under registration number #88,947.

2.2. Preparation of extracts

A quantity of 950 g of dehydrated *H. speciosa* stem bark was crushed and subjected to exhaustive extraction using *n*-hexane for 72 h at room temperature to remove low-polarity substances. After the removal of the residues, two sequential extractions were performed using sulfuric ether and methanolic solvents, for 72 h each. After each step, the samples were filtered and the solvents were removed using a rotary evaporator (Silva et al., 2024). At the end of this process, the sulfuric ether and methanolic extracts of *H. speciosa* (EEHS and MEHS, respectively) were stored in an amber flask at room temperature. These extracts were kept stored until the chemical analysis and biological activity tests.

2.3. Phytochemical analysis

2.3.1. Gas chromatography coupled to mass spectrometry (GC–MS)

Before the analysis, the extracts were derivatized using 50 μL of pyridine, and 50 μL of N, O–Bis(trimethylsilyl)trifluoroacetamide (BSTFA), for 1 h at 70 °C. After derivatization, 1 μL of each sample was injected into a gas chromatography system (GC 6850 Network, Agilent) coupled to a mass spectrometer (MSD VL 5975C, Agilent), equipped with an Agilent HP5-MS column (30 m, 0.25 mm, 0.25 μm). Helium was used as attraction gas under a flow of 1 mL min^{-1}. The temperatures of the injector, quadrupole, and ion source were set to 300 °C, 150 °C, and 230 °C, respectively. Ionization was performed by electron impact at 70 eV, with a recorded mass range from 50 to 600 m/z at a rate of 2.66 $scans^{-1}$. The column temperature was set up to the initial temperature of 100 °C maintained for 5 min, followed by an increase of 5 °C per minute until reaching 320 °C (final temperature). The total analysis period took 49 min.

The spectral data of each extract were processed (peak alignment, deconvolution, and calculation of the Linear Retention Index (LRI)), along with compound identification using the Global Natural Product Social Molecular Networking (GNPS), which generated an information table containing retention time, peak area, molecular ion and possible identification suggestion for each compound. A minimum cosine index of 0.30 and an LRI window of 30 were established for compound identification. Compounds with a cosine index above the minimum but without LRI matching were grouped into the same class (according to GNPS) (Sala-Carvalho et al., 2022).

2.3.2. Total phenols and flavonoids

The Folin-Ciocalteu method was used to determine the total phenol contents (Singleton et al., 1999), with some modifications. Initially, an ethanolic solution (1 mg/mL) of *H. speciosa* extracts was added to a volumetric flask, together with 250 μL of Folin-Ciocalteu reagent and 3 mL of distilled water. After stirring for 30 s, 1 mL of 15% sodium carbonate (Na_2CO_3) was added. After 2 h, the absorbance was measured on a spectrophotometer at 760 nm (SmartSpec Plus, Bio-Rad, USA). The results obtained expressed the total phenolic content in μg of gallic acid equivalents per mg of *H. speciosa* extract (GAE/mg).

To quantify total flavonoids it was used the method described by Woisky and Salatino (1998), with some adaptations. The *H. speciosa*

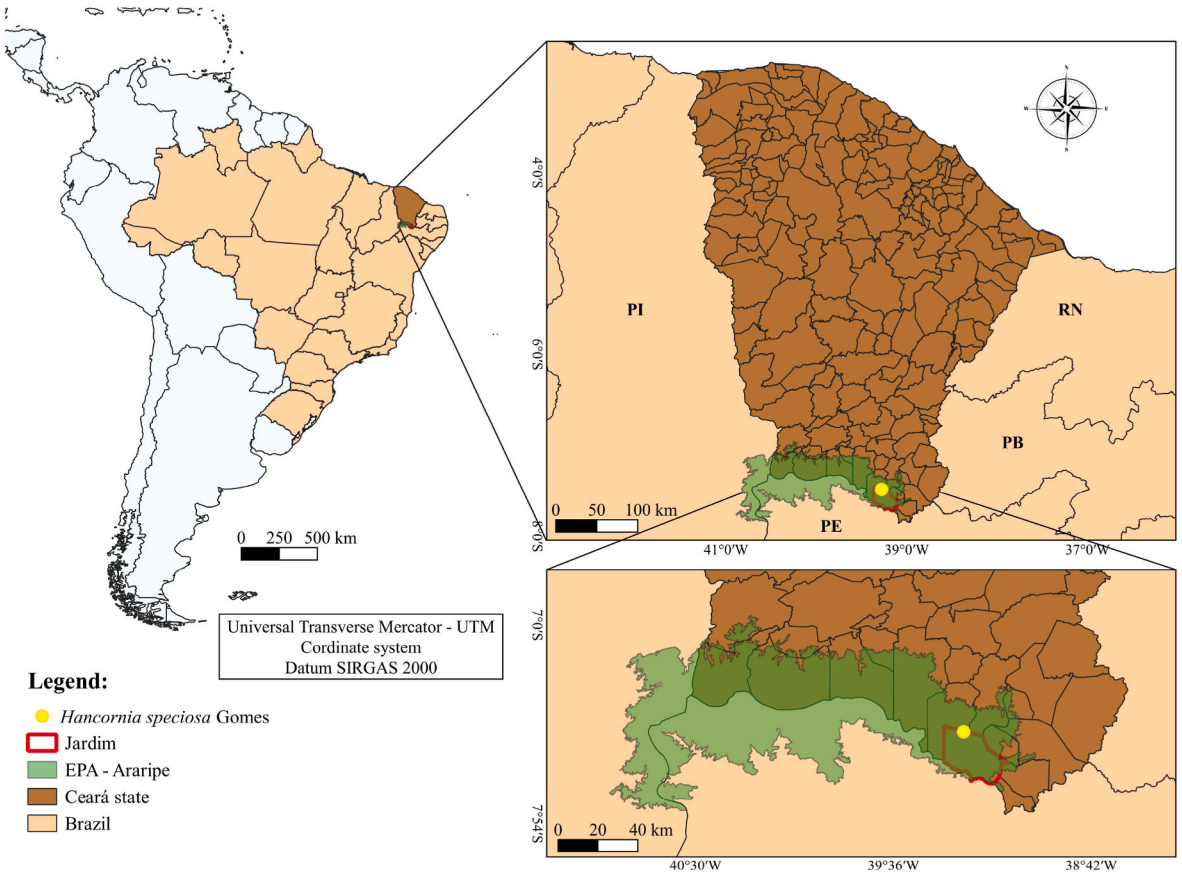

Fig. 2. Map of the sampling site where were collected the stem barks of *Hancornia speciosa* Gomes, Jardim city, Ceará, Brazil.

extracts were diluted in distilled water until they reached a concentration of 1 mg/mL. Then, 3 mL of methanol and 1 mL of aluminum chloride (5% $AlCl_3$) were added to each of the extracts. After standing for 30 min, the absorbance was measured at 425 nm using a spectrophotometer (SmartSpec Plus, Bio-Rad, USA). It was compared to a blank sample solely containing methanol. Total flavonoid content was determined using a quercetin standard curve (Sigma-Aldrich®). The results obtained express the total flavonoid content in μg of quercetin equivalents per mg of *H. speciosa* extract (QE/mg).

2.4. Antibacterial activity

2.4.1. Strains, culture medium, drugs, reagents, and preparation of solutions

Standard bacteria (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 25853, and *Staphylococcus aureus* ATCC 22923), and multidrug-resistant strains (*Escherichia coli* 06, *Pseudomonas aeruginosa* 24, and *Staphylococcus aureus* 10) were used in the tests. The resistance profile of multi-drug resistant bacteria to antibacterial drugs is described in the antibiogram (Table 1). The bacterial strains were inoculated in Petri dishes containing Heart Infusion Agar (HIA), and incubated for 24 h in a microbiological incubator at a constant temperature of 37 °C. After the bacterial growth, they were collected and diluted in sterile saline solution (0.9%) until reaching a turbidity value of 0.5 at the McFarland scale (1×10^8 CFU/mL). From this solution, 150 μL of the inoculum was removed and added to a Brain Heart Infusion (BHI) solution (10%), for use in the antibacterial and drug-modifying assays.

The extracts of *H. speciosa* were diluted in dimethylsulfoxide (DMSO, Merck, Darmstadt, Germany) to reach an initial concentration of 20 mg/mL. Following, the extracts were diluted in sterile distilled water to reach an initial concentration of 1024 μg/mL. The antibacterial drugs norfloxacin, gentamicin, and erythromycin (Sigma-Aldrich, St. Louis, Missouri, USA) were directly diluted in sterile distilled water reaching a concentration of 1024 μg/mL.

2.4.2. Minimum inhibitory concentration – MIC

To determine the minimum inhibitory concentration, 96–well flat-bottom plates (KASVI®, São José dos Pinhais, PR, Brazil) were initially filled with 100 μL of BHI solution + 10% of the inoculum each well. Subsequently, serial dilution (1:1 v/v) was performed with the extracts or standard drugs at decreasing concentrations (512–0.5 μg/mL). After dilution, the plates were kept in a microbial incubator at 37 °C. After 24 h, a 20 μL solution of 0.01% resazurin (Sigma–Aldrich, St. Louis, Missouri, USA) was added to each well to allow redox reactions. A color change indicated bacterial growth. The MIC was considered the well with the lowest concentration that inhibited bacterial growth (Fernandes et al., 2022).

Table 1
Resistance profile of multi-drug resistant bacteria to antibiotic drugs. Source: Laboratory of Microbiology and Molecular Biology – LMBM, Universidade Regional do Cariri – URCA (Ceará, Brazil).

Bacteria	Origin	Drug resistance
Escherichia coli 06	Urine culture	Cefalothin, cephalexin, cefadroxil, ceftriaxone, cefepime, ampicillin-sulbactam, amikacin, imipenem, ciprofloxacin, levofloxacin, piperacillin-tazobactam, ceftazidime, meropenem, cefepime.
Pseudomonas aeruginosa 24	Uroculture	Amikacin, imipenem, ciprofloxacin, levofloxacin, piperacillin-tazobactam, ceftazidime, meropenem, cefepime.
Staphylococcus aureus 10	Rectal smear	Cefadroxil, cefalexin, cephalothin, oxacillin, penicillin, ampicillin, amoxicillin, moxifloxacin, ciprofloxacin, levofloxacin, ampicillin-sulbactam, amoxicillin/clavulanic acid, erythromycin, clarithromycin, azithromycin, clindamycin.

2.4.3. Drug-enhancing activity

After determining the MIC, the drug–enhancing experiment was carried out based on the methodology described by Coutinho et al. (2008). Initially, the extracts were evaluated at sub-inhibitory concentrations (MIC/8). A BHI solution containing 10% of the inoculum and the sub-inhibitory concentration of the extracts was prepared. An aliquot of 100 μL was distributed into the wells of the plates in a uniform pattern. Subsequently, a serial dilution (1:1 v/v) was performed using standard antibacterial drugs at decreasing concentrations (512–0.5 μg/mL). These plates were incubated in a microbial incubator at 37 °C. At the end of the growth period, the plates were read by adding 20 μL of an aqueous solution of resazurin.

2.5. Toxicity analysis

2.5.1. Toxicity assay on Drosophila melanogaster

For toxicity assessment, it was used the ingestion method with *Drosophila melanogaster* as the standard organism (Costa et al., 2020), with some modifications. Adult *D. melanogaster* flies (males and females) aged 6 days were transferred to 300 mL glass flasks (20 flies per flask) (Supplementary Fig. 1). At the bottom of the flasks were placed the basal diet of the flies with the addition of *H. speciosa* extracts at different concentrations (1, 10, and 100 mg/g) for the treated groups, and distilled water for the control group. The choice for these concentrations followed established practices in the literature, which employ logarithmic intervals for efficient coverage of the concentrations (Rand, 2010; Rajan and Perrimon, 2011; Cunha et al., 2015). They were kept under 12-h light cycle (light/dark), at 25 °C ± 1 °C, and 60% relative humidity, for 7 days. The number of dead individuals was counted daily until the end of the experiment (7 days).

2.6. Statistical analysis

All experiments were performed in triplicates, and the means with their respective standard errors (±SEM) were specific to each test. A one-way analysis of variance (ANOVA) was performed, followed by Tukey's test at a 95% confidence level. Significance values were categorized as $p < 0.0001$ (**** = extremely significant), 0.0001 to 0.001 (*** = extremely significant), 0.001 to 0.01 (** = very significant), 0 .01 to 0.05 (* = significant) and $p > 0.05$ (ns = not significant). The statistical analysis was performed using GraphPad Prism 6.0 software (GraphPad Software, San Diego, CA, United States).

3. Results

3.1. Chemical composition

The GC-MS analysis revealed the presence of 30 compounds in the extracts of *H. speciosa* (Table 2 and Fig. 3). Among these compounds, 19 were identified in both extracts (EEHS and MEHS), while nine were found only in the EEHS extract, and the other two exclusively in the MEHS extract. In the EEHS extract, triterpenoids were the predominant compounds (42.07%), followed by fatty acids (6.29%), and carbohydrates (5.17%). The fractions of phytosteroids, alcohols, phenolic acids, and other compounds were also detected, with 2.49%, 0.58%, 0.39%, and 4.38%, respectively. In the MEHS extract, triterpenoids were also identified, but found in smaller quantities (8.70%), followed by fatty acids (4.29%), phytosteroids (2.65%), and alcohols (2.43%). Carbohydrates, phenolic acids, and other compounds were found in relatively small amounts in the total extract composition (1.23%, 0.75%, and 0.98%, respectively).

The total phenolic and flavonoid contents found in the stem bark extracts (EEHS and MEHS) of *H. speciosa* can be observed in Table 3. For EEHS and MEHS, total phenolic contents ranged from 340.0 to 346.4 mg GAE/g per extract, respectively, and did not differ statistically ($p > 0.05$). The total flavonoids in the extracts were comparable ($p > 0.05$)

Table 2
Chemical composition of the sulfuric ether (EEHS) and methanolic (MEHS) extracts of *Hancornia speciosa* analyzed via gas chromatography coupled to mass spectrometry.

Class/Compound	Retention time (min)	Area (%)	Molecular formula	Molecular weight (g/mol)	Biological properties	Extract
Fatty alcohol						
n–Octacosanol **(1)**	44.977/44.967	0.30/ 0.95	$C_{28}H_{58}O$	410.80	**Antibacterial** (Sengupta et al., 2018); **Larvicidal/Insecticidal** (Zavala-Sánchez et al., 2020); **Antifungal** Shehata et al. (2024)	EEHS/ MEHS
n–Tetradecanol **(2)**	23.979/23.975	0.28/ 1.19	$C_{14}H_{30}O$	214.39	**Antibacterial** (Verde et al., 2017)	EEHS/ MEHS
n–Triacontanol **(3)**	47.393	0.29	$C_{30}H_{62}O$	438.80	**Antioxidant/Antibacterial** (Ali et al., 2022)	MEHS
Carbohydrate						
D–Psicofuranose **(4)**	24.924	0.53	$C_6H_{12}O_6$	180.16	–	EEHS
Fructofuranose **(5)**	24.777/24.768	0.51/ 0.36	$C_6H_{12}O_6$	180.16	–	EEHS/ MEHS
Galactopyranose **(6)**	26.558	0.48	$C_6H_{12}O_6$	180.16	–	EEHS
Galactose **(7)**	24.578	0,29	$C_6H_{12}O_6$	180.16	–	EEHS
Glucopyranose **(8)**	26.381	2.35	$C_6H_{12}O_6$	180.16	–	EEHS
Glucose **(9)**	28.085	1.64	$C_6H_{12}O_6$	180.16	–	EEHS
Maltose **(10)**	39.608/39.595	0.37/ 0.87	$C_{12}H_{22}O_{11}$	342.30	–	EEHS/ MEHS
Fatty acid						
Elaidic acid (18:1–*trans*) **(11)**	30.626	0.32	$C_{20}H_{38}O_2$	310.5	**Larvicidal** (Perumalsamy et al., 2015); **Cytotoxic activity** Zha et al. (2021)	EEHS
Hexacosanoic acid (C26:0) **(12)**	43.650/43.639	0.53/ 0.66	$C_{26}H_{52}O_2$	396.7	**Antifungal** (Singh and Singh, 2003); **Antibacterial** (Rehan et al., 2020)	EEHS/ MEHS
Lignoceric acid (C24:0) **(13)**	41.010/40.997	1.13/ 0.55	$C_{24}H_{48}O_2$	368.6	–	EEHS/ MEHS
Triacontanoic acid (C30:0) **(14)**	48.499	0.91	$C_{30}H_{60}O_2$	452.8	–	EEHS
Palmitoleic acid (C16:1) **(15)**	28.554/28.545	1.08/ 1.32	$C_{16}H_{30}O_2$	254.41	**Cytotoxic** (Yamasaki et al., 2003) **Antibacterial** (Watanabe et al., 2021; Wang et al., 2022)	EEHS/ MEHS
Oleic acid (C18:1–*cis*) **(16)**	31.521/31.511	1.43/ 1.01	$C_{18}H_{34}O_2$	282.5	**Antibacterial** (Dilika et al., 2000); **Larvicidal** (Rahuman et al., 2008); **Cytotoxic** (Permyakov et al., 2012); **Antifungal** (Walters et al., 2004; Verma et al., 2014)	EEHS/ MEHS
Linolenic acid (C18:3) **(17)**	31.419/31.414	0.47/ 0.40	$C_{18}H_{30}O_2$	278.4	**Cytotoxic** (Vartak et al., 2000); **Antifungal** (Walters et al., 2004); **Antibacterial** (Obonyo et al., 2012; Jung et al., 2015)	EEHS/ MEHS
Behenic acid (C22:0) **(18)**	38.184/38.174	0.42/ 0.35	$C_{22}H_{44}O_2$	340.6	**Larvicidal** (Wuillda et al., 2019); **Antibacterial** (Ravi et al., 2024)	EEHS/ MEHS
Phenolic acid						
Gallic acid **(19)**	27.364/27.345	0.18/ 0.40	$C_7H_6O_5$	170.12	**Cytotoxic** (Alves et al., 2016; Jiang et al., 2022); **Larvicidal** (Punia et al., 2021); **Antifungal** (Liberato et al., 2022); **Antibacterial** (Keyvani-Ghamsari et al., 2023)	EEHS/ MEHS
Protocatechoic acid **(20)**	24.699/24.649	0.21/ 0.35	$C_7H_6O_4$	154.12	**Cytotoxic** (Babich et al., 2002); **Larvicidal** (Daniel et al., 2020); **Antibacterial** (Liu et al., 2005; Fifere et al., 2022)	EEHS/ MEHS
Phytosterol						
Campesterol **(21)**	46.533/46.532	1.99/ 1.50	$C_{28}H_{48}O$	400.7	**Cytotoxic** (O'Callaghan et al., 2013); **Antifungal** (Choi et al., 2017); **Antibacterial** (Silva et al., 2023)	EEHS/ MEHS
y–Sitosterol **(22)**	47.456/47.562	0.50/ 1.15	$C_{29}H_{50}O$	414.7	**Antifungal** (Mbambo et al., 2012); **Cytotoxic** (Sirikhansaeng et al., 2017); **Larvicidal** (Mishra et al., 2020); **Antibacterial** (Luhata and Usuki, 2021)	EEHS/ MEHS
Triterpenoid						
α–Amyrin **(23)**	47.749	1.93	$C_{30}H_{50}O$	426.7	**Antifungal** (Johann et al., 2007); **Larvicidal** Kuppusamy et al., 2009); **Antibacterial** (Díaz-Ruiz et al., 2012)	MEHS
α-Amyrone **(24)**	47.661/47.667	8.94/ 0.73	$C_{30}H_{48}O$	424.7	–	EEHS/ MEHS
Friedelan–3–one **(25)**	49.728/49.667	3.51/ 2.58	$C_{30}H_{50}O$	426.7	**Antibacterial/Antifungal** (Ichiko et al., 2016; Okafor et al., 2022); **Cytotoxic** (Radi et al., 2023)	EEHS/ MEHS
Lupeol **(26)**	48.357	25.52	$C_{30}H_{50}O$	426.7	**Cytotoxic** (Chaturvedi et al., 2008; Akwu et al., 2020); **Larvicidal** (Nobsathian et al., 2018); **Antibacterial** (Rosandy et al., 2021); **Antifungal** (Javed et al., 2021)	EEHS

(*continued on next page*)

Table 2 (*continued*)

Class/Compound	Retention time (min)	Area (%)	Molecular formula	Molecular weight (g/mol)	Biological properties	Extract
Ursolic acid **(27)**	50.765/50.731	4.10/ 3.46	$C_{30}H_{48}O_3$	456.7	**Cytotoxic** (Ma et al., 2005); **Antifungal** (Shaik et al., 2016); **Antibacterial** (Nascimento et al., 2014; Sycz et al., 2022); **Larvicidal** (Kamatchi et al., 2023)	EEHS/ MEHS
Others						
Glucuronic acid **(28)**	28.284	2.07	$C_6H_{10}O_7$	194.14	**Antibacterial** (Ansari et al., 2019)	EEHS
Shikimic acid **(29)**	25.802/25.814	0.64/ 0.41	$C_7H_{10}O_5$	174.15	**Antibacterial** (Bai et al., 2015); **Antifungal** (Batory and Rotsztejn, 2022); **Cytotoxic** (Meghdadi et al., 2024)	EEHS/ MEHS
Trans–5–*O*–Caffeoyl–D–quinic acid **(30)**	45.422/45.403	1.67/ 0.57	$C_{16}H_{18}O_9$	354.31	**Antibacterial** (Aires et al., 2017)	EEHS/ MEHS

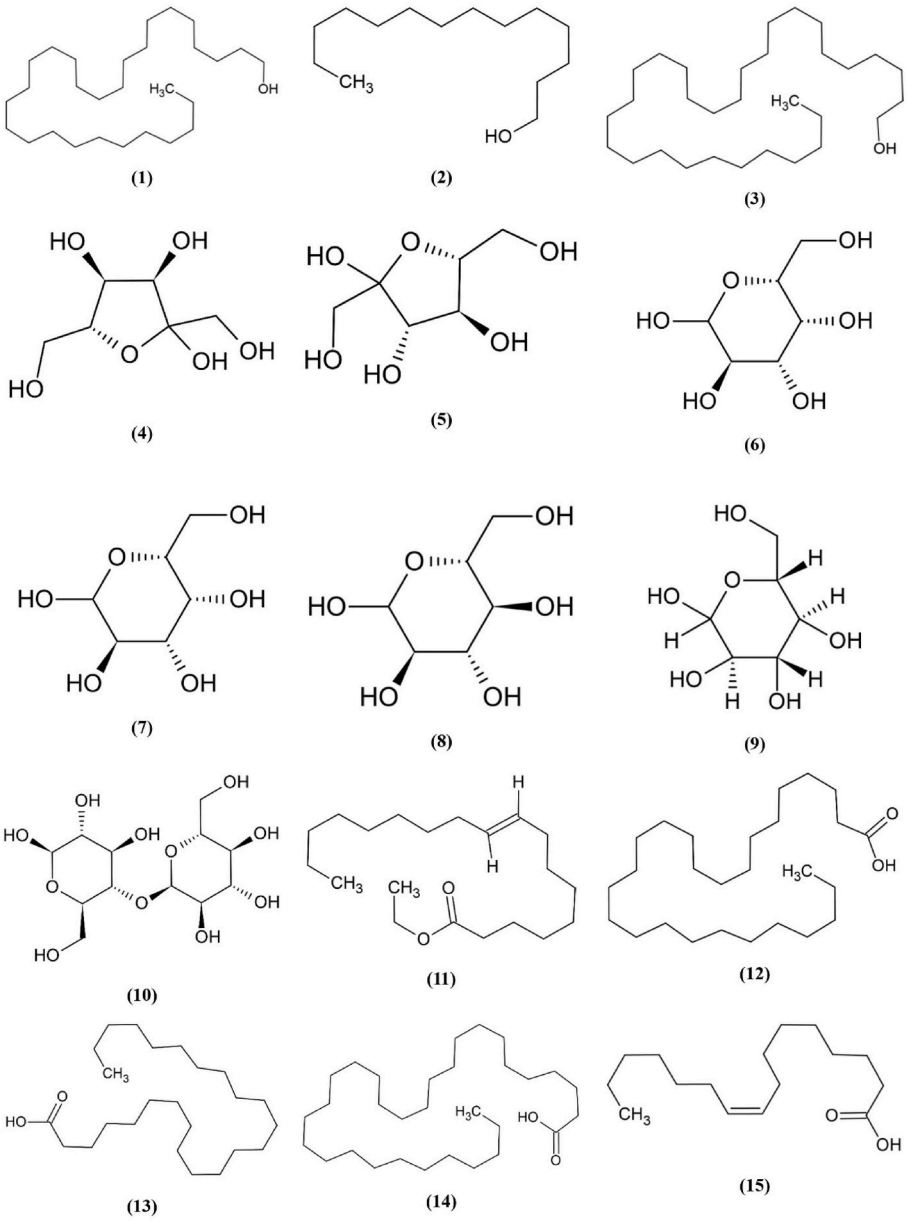

Fig. 3. Chemical structures of the compounds identified in the extracts of the stem bark of *Hancornia speciosa* by gas chromatography-mass spectrometry.

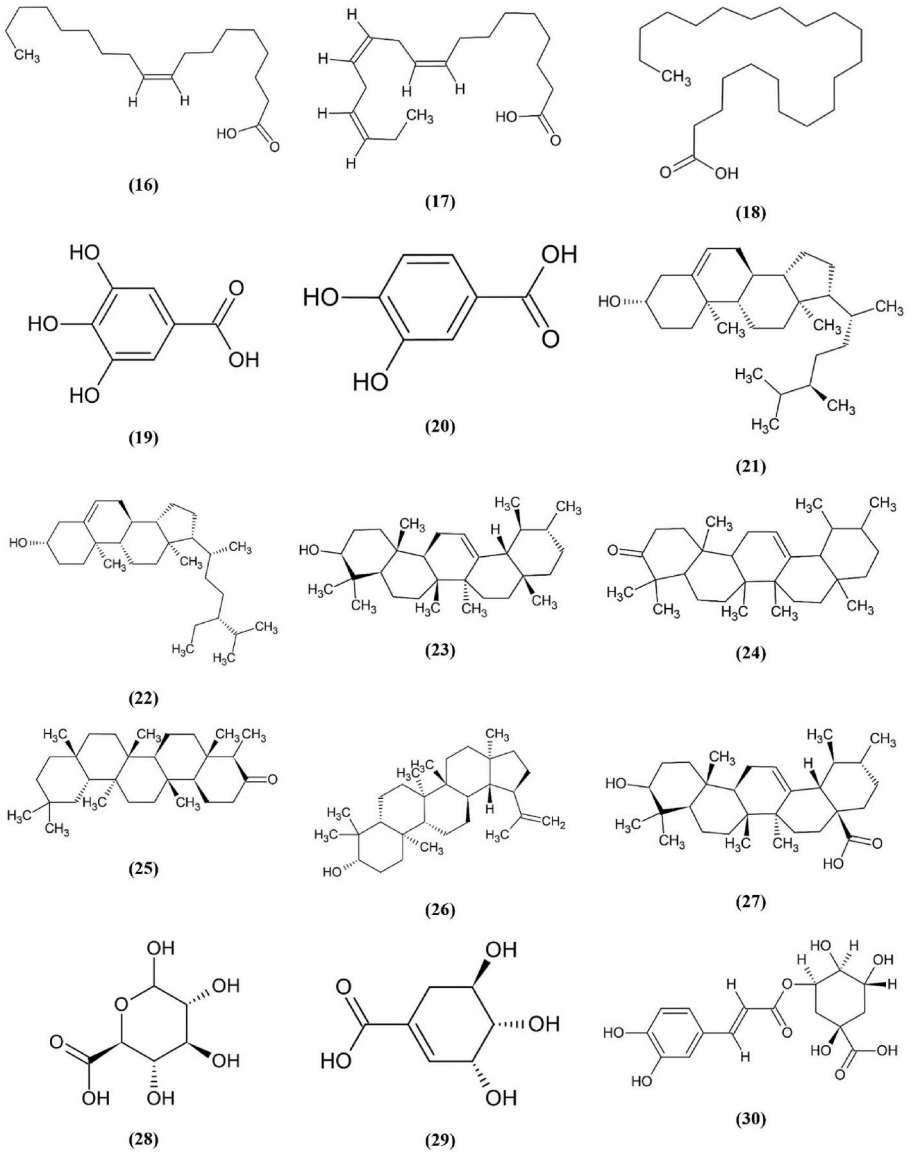

Fig. 3. (*continued*).

Table 3
Total phenolic and flavonoid content in sulfuric ether (EEHS) and methanolic (MEHS) extracts of the stem bark of *Hancornia speciosa*.

Extract	Total phenolics (mg GAE/g)	Total flavonoids (mg QE/g)
EEHS	346.4 ± 17.8	7.6 ± 0.3
MEHS	340.0 ± 45.4	6.9 ± 1.0

± Standard error (n = 3). GAE = gallic acid equivalent, QE = quercetin equivalent.

(Table 3).

3.2. Antibacterial and drug-modifying activity

Although *H. speciosa* is used in herbal medicine for treating infections, its extracts did not show intrinsic antibacterial effects against standard and multi-resistant strains of *E. coli*, *P. aeruginosa,* and *S. aureus* (MIC >512 μg/mL). On the other hand, *H. speciosa* extracts were able to intensify the antibacterial activity of gentamicin and erythromycin against multi-resistant strains of *P. aeruginosa* and *E. coli* ($p < 0.0001$). The EEHS produced a negative effect on the action of two drugs, reducing their effects against *S. aureus*. In this specific case, EEHS significantly ($p < 0.0001$) increased MIC values from 25.39 μg/mL to 101.59 μg/mL for gentamicin; and from 2 μg/mL to 406.37 μg/mL for erythromycin. Regarding the drug norfloxacin, the EEHS extract increased its action against *E. coli* and *S. aureus*, reducing their MIC values by 50% (Fig. 4a).

The MEHS extract of *H. speciosa* potentialized the effect of all multi-resistant strains, notably against *E. coli*, where the MIC value was reduced by more than 50% ($p < 0.001$). When combined with erythromycin, MEHS significantly ($p < 0.0001$) reduced the MIC value of this drug from 128 μg/mL to 25.39 μg/mL against *P. aeruginosa*. On the other hand, this combination increased the MIC value from 2 μg/mL to 80 μg/mL against *S. aureus*. The combination of MEHS extract with norfloxacin made *E. coli* and *S. aureus* strains more susceptible to the drug ($p < 0.0001$), however, it displayed an antagonistic effect against *P. aeruginosa* (Fig. 4b).

3.3. Toxicity

3.3.1. In vivo toxicity assay against Drosophila melanogaster

In the toxicity test against *D. melanogaster*, EEHS extract did not show

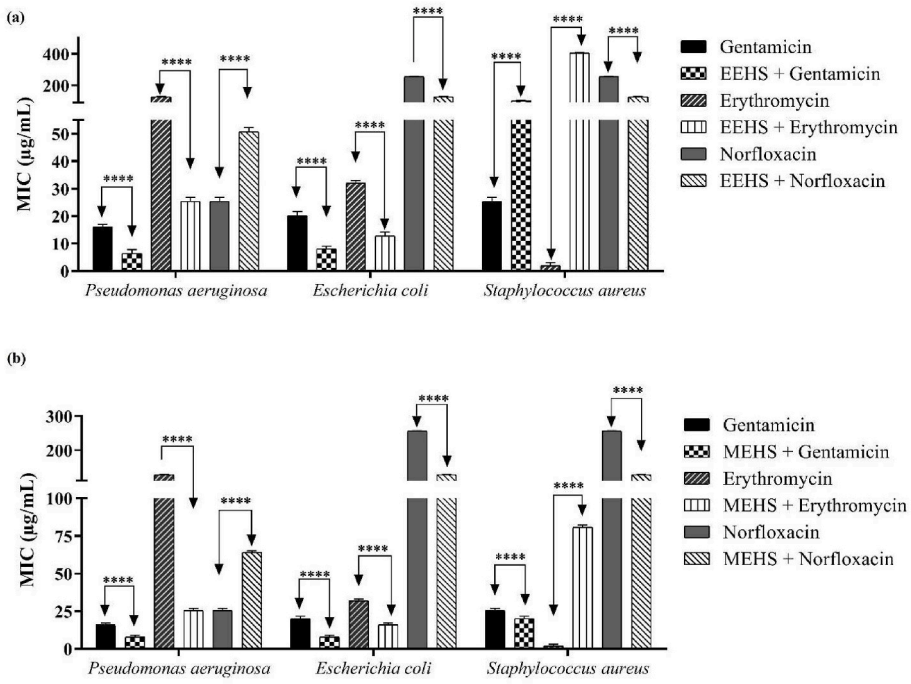

Fig. 4. Minimum Inhibitory Concentration (MIC) of antibiotics combined with sulfuric ether (EEHS) (a) and methanolic (MEHS) (b) extracts of the stem bark of *Hancornia speciosa* against multi-resistant bacterial strains. MIC values are displayed as geometric mean. The bars represent the standard error of the mean (n = 3). **** = $p < 0.0001$.

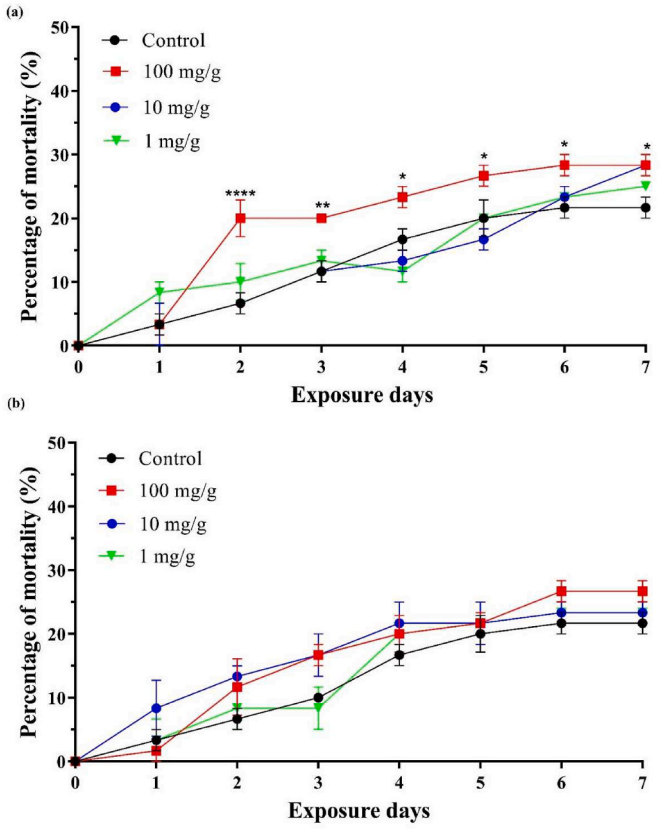

Fig. 5. Toxicity of sulfuric ether (EEHS) (a) and methanolic (MEHS) (b) extracts of the stem bark of *Hancornia speciosa* at different concentrations against *Drosophila melanogaster*. * = $p < 0.05$, **** = $p < 0.0001$. Bars represent the standard error of the mean (n = 3).

toxic effects at clinically relevant concentrations, indicating its safety up to 10 mg/g. However, the extract showed toxicity at a dosage of 100 mg/g from the second day of exposure, leading to a mortality rate of 20%, and reaching 28.3% at the end of the 7-day toxicity assay (Fig. 5a). On the other hand, MEHS extract did not show toxicity in any concentration tested over 7 days period, when compared with the control group ($p > 0.05$) (Fig. 5b).

4. Discussion

The ethnopharmacological knowledge has been a valuable source for the discovery of promising candidates to be used in the development of new antimicrobial agents, as well as for the identification of natural compounds that can enhance the activity of conventional antibiotic drugs (Calixto, 2019; Chaachouay and Zidane, 2024). In the present study, we investigated two extracts from the stem bark *H. speciosa*, a plant commonly used in Brazilian herbal medicine for treating various infectious diseases. According to the literature, chemical constituents of high and medium polarity from *H. speciosa* exhibit remarkable bioactivity (Barbosa et al., 2019; Almeida et al., 2022). Additionally, methanol has been shown in other studies to be effective in extracting a wide range of phytochemical compounds with different polarities (Anoor et al., 2022; Zarrinmehr et al., 2022; Riyadi et al., 2023). Based on this evidence, our study focused on two highly polar extracts: sulfuric ether and methanolic.

In a recent publication by our research group, we used liquid chromatography (UPLC–QTOF–MS/MS) to identify the chemical composition of the ethereal and methanolic extracts of the stem bark of *H. speciosa* (Silva et al., 2024). In this analysis, we mainly identified catechin, chlorogenic acid, epicatechin, procyanidin B dimer, procyanidin B trimer, vanillic acid, quinic acid, and phloretin, cinchonain IIb, lariciresinol hexoside isomers, cinchonain Ib isomers, and gluconic acid. In the current study, using GC–MS we identified fatty alcohols, carbohydrates, fatty acids, phenolic acids, phytosterols, and triterpenoids. These diverse findings highlight the importance of using different methods to investigate the compounds present in plant extracts.

Our current findings corroborate previous studies that also identified phytocompounds such as carbohydrates, fatty acids, phenolic acids, and phytosterols in *H. speciosa* (Santos et al., 2018; Silva and Jorge, 2020; Silva et al., 2024). It is worth noting that some of the metabolites identified in this study, such as hexacosanoic acid, lignoceric acid, triacontanoic acid, and α-amyrone, were identified for the first time in *H. speciosa*. Furthermore, the literature demonstrates that several compounds found in the extracts, such as linolenic acid, behenic acid, gallic acid, lupeol, and ursolic acid, have antibacterial activity (Jung et al., 2015; Sycz et al., 2022; Dwivedi et al., 2024).

Our study also evaluated the total phenolic and flavonoid content in the extracts. These constituents can be an indicator of the pharmacological and biological potential of plant extracts (Angeloni et al., 2021). According to the literature, phenolic and flavonoid compounds exhibit a wide range of activities, such as anti-inflammatory, antioxidant, antiulcer, anticarcinogenic, and antibacterial (Goławska et al., 2023; Sun and Shahrajabian, 2023). Our results showed that EEHS and MEHS extracts had considerable total phenol contents, higher than the findings reported by Panontin et al. (2022) which investigated the leaves of the same species, using a different solvent and extraction method. It is important to emphasize that the contents of phenols and flavonoids can vary depending on soil and climate conditions, site and time of collection, part of the plant extracted, the solvent and method used for extraction (Martins et al., 2016; Adhikari et al., 2020; Valencia et al., 2023).

Regarding antibacterial activity, despite ethnopharmacological records that mention the use of *H. speciosa* in the treatment of infections (Ribeiro et al., 2017; Cruz et al., 2021), our findings showed that the extracts (EEHS and MEHS) from the stem bark did not show an intrinsic antibacterial effect, at clinically relevant concentrations, displaying a MIC >512 μg/mL (Houghton et al., 2007). On the other hand, Santos et al. (2016) reported that the ethanolic extract of *H. speciosa* leaves displayed antibacterial activity against different bacterial strains, including *S. aureus*.

Our results demonstrated that the extracts from the stem bark of *H. speciosa* enhanced the activity of three commercial antibiotic drugs, a promising alternative for improving antibacterial therapy. The evaluation of the enhancing activity of antibiotics in microbiological assays aims to determine the ability of other compounds to increase or intensify the therapeutic effects of the drugs. The identification of compounds that enhance the activity of antibiotics is crucial to optimizing treatments, enabling the reduction of the antibiotic dose and minimizing potential adverse effects (Coutinho et al., 2008; Confessor et al., 2024). These assays generally involve biological models or *in vitro* tests, in which the compound or product is combined with the antibiotic in different concentrations. Synergistic or additive effects of these drug-enhancing compounds can be compared with the results obtained based on the isolated effect of the antibiotic drugs (Carneiro et al., 2019; Confessor et al., 2024).

Multi-resistant bacteria can develop mechanisms to modify the structure of the antibiotics (e.g., aminoglycoside class), reducing or nullifying their action (Garneau-Tsodikova and Labby, 2016). In our study, extracts from the stem bark of *H. speciosa* enhanced the activity of gentamicin, an aminoglycoside antibiotic. Although we cannot assure that the flavonoids found in the extracts are responsible for inhibiting enzymes related to bacterial resistance, Górniak et al. (2019) suggested that these compounds can interact with acetyltransferases, nucleotidyltransferases, and phosphotransferases, inhibiting their action.

It is worth mentioning that the combinations of the stem bark extracts of *H. speciosa* with different antibiotics intensified the action of all drugs against the *E. coli* strain. This bacteria species is one of the main causes of infection in the urinary tract and bloodstream in humans on a global scale (Čurová et al., 2020). *Escherichia coli* is commonly found in the intestinal tract and is normally harmless, being considered a commensal bacterium (Pakbin et al., 2021). However, these microorganisms can acquire resistance genes, resulting in failures in the treatment of intestinal and extraintestinal infections that can lead to health risks including morbidity and mortality (Pokharel et al., 2023). This reinforces the importance of implementing strategies to solve the problem of antimicrobial resistance (Eisinger et al., 2023).

The intensification of the antibacterial effect against *E. coli* observed in our study may be related to the presence of phenolic compounds in the extracts of the stem bark of *H. speciosa*. Different findings corroborated the antibacterial activity of these compounds against *E. coli* (Miklasińska-Majdanik et al., 2018; Ecevit et al., 2022; Lobiuc et al., 2023). Among the mechanisms of action include the inhibition of biofilm formation (Bernal-Mercado et al., 2018; Tian et al., 2022), increase in membrane permeability, and rupture of the bacteria (Hao et al., 2021; Tian et al., 2022). According to recent findings (Bernal-Mercado et al., 2018; Hao et al., 2021; Tian et al., 2022), gallic acid and protocatechuic acid, phenolic compounds identified in our extracts, demonstrated these effects. It is believed that the synergy between the compounds present in the extracts and the antibiotics contributed to increasing antibacterial activity against *E. coli*, fighting the bacteria more effectively.

According to the literature, the combination of conventional antibiotic drugs and natural substances such as plant extracts has shown significant antibacterial activities (Araújo et al., 2022; Pontes et al., 2022; Almeida-Bezerra et al., 2023). Some phytochemicals present in the plant extracts can act through different mechanisms of action against bacteria, which include inhibition of the biosynthesis of nucleic acid and proteins, damage to the bacterial membrane and cell wall, inactivation of the efflux pump, and inhibition of virulence mechanisms of the bacteria (e.g., ability to form biofilms) (Fig. 6) (Ayaz et al., 2019; Khameneh et al., 2019; Górniak et al., 2019; Dassanayake et al., 2021; Mahamud et al., 2022).

The compounds present in the stem bark extracts of *H. speciosa* can operate through diverse and complex mechanisms to produce an antibacterial effect, impacting various aspects of the bacterial cell. Fatty acids, for example, can destabilize cell membranes, interrupt the electron transport chain, uncouple oxidative phosphorylation, inhibit enzymatic activities of the membranes, and reduce nutrient absorption (Yoon et al., 2018). Flavonoids, on the other hand, can interact with the bacterial plasma membrane, increasing its permeability to the drug and inhibiting bacterial growth even at low concentrations (Cushnie and Lamb, 2011; Górniak et al., 2019; Farhadi et al., 2018).

Triterpenoids have lipophilic properties, which facilitate interaction with the bacterial cell wall. These compounds can interfere with the biosynthesis of the cell wall and inhibit virulence mechanisms, such as the formation of biofilms. Furthermore, triterpenoids can penetrate the bacterial cell directly impacting protein synthesis, DNA replication and repair (Ibrahim et al., 2019). The lipophilicity is also crucial to the antibacterial potential of phenolic acids. Their lipophilic nature allows them to cross the bacterial cell membrane, resulting in acidification of the cytoplasm and disruption of the membrane structure. This can induce protein denaturation and increase membrane permeability, allowing ions such as potassium (K^+) to outflow from the cell (Lobiuc et al., 2023).

According to Miklasińska-Majdanik et al. (2018) and Kauffmann and Castro (2023), phenolic compounds can negatively affect bacteria through different mechanisms, such as reducing biofilm formation, disturbing membrane integrity and inhibiting virulence factors. Phenolic compounds present in *H. speciosa* extracts may be associated with the intensification of the observed antibiotic activity. An example of this is eriodictyol, identified in previous studies with these extracts (Silva et al., 2024). This compound has been recognized as one of the most powerful antimicrobials of plant origin (Khameneh et al., 2019). According to Wang et al. (2021), eriodictyol acts by inhibiting different virulence mechanisms of the Sortase A enzyme, including the formation of biofilms of methicillin-resistant *S. aureus*. Furthermore, studies indicate that phloretin, also present in *H. speciosa* extracts (Silva et al., 2024), inhibits the Sortase B enzyme, essential for *S. aureus* infection (Wang et al., 2018), and inhibits the expression of toxin-related genes

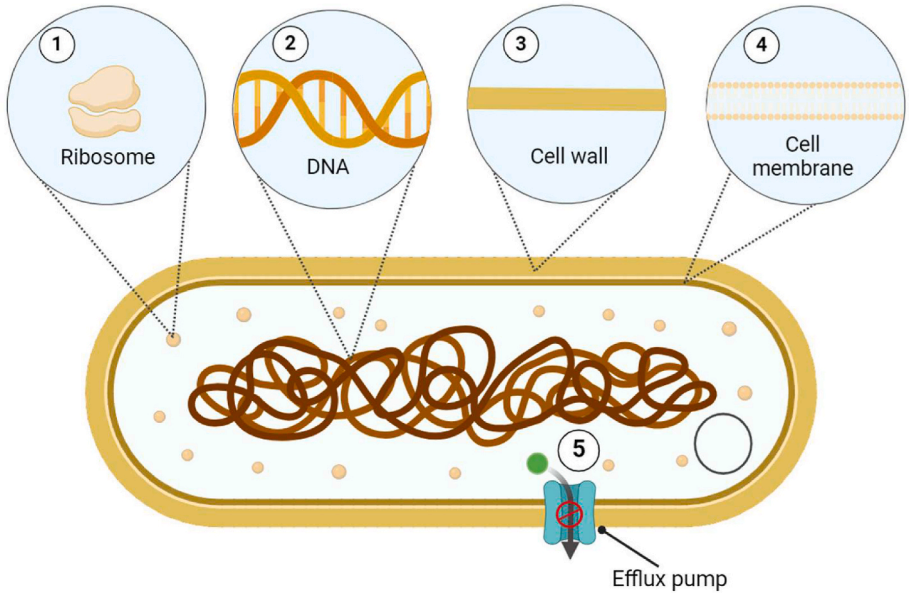

Fig. 6. Target sites of phytochemicals, and their mechanisms of action against bacteria. 1: Inhibition of protein synthesis; 2: Inhibition of nucleic acid synthesis; 3: Damage to the cell wall; 4: Damage and rupture of the cell membrane and 5: Inhibition of the efflux pump.

and signaling systems in *E. coli* (Lee et al., 2011).

The intensification of antibacterial activity observed in this study was probably the result of the synergistic action of the antibiotic with different secondary metabolites present in the extracts. The diversity of mechanisms of action of these compounds may have contributed to the intensification of antibacterial activity by acting on multiple targets and different bacterial resistance mechanisms (Ayaz et al., 2019).

The possible antagonistic effect observed by the association of stem bark extracts of *H. speciosa* with norfloxacin against *P. aeruginosa*, and with gentamicin and erythromycin against *S. aureus* can be attributed to the interference on the sites of action of these antibiotics, as well as the possible chelation with these drugs (Bezerra et al., 2017). Direct interference on sites of action reduces antibiotic effectiveness, while chelation forms stable complexes, decreasing the availability and effectiveness of the antibiotics. These mechanisms can compromise the action of antibiotics, contributing to the occurrence of antagonistic effects of the plant extracts (Oliveira et al., 2017).

In our study, in addition to the evaluation of the antibacterial activity, we also investigated the toxicity of *H. speciosa* extracts against *D. melanogaster*, a viable model organism commonly used to assess the toxic activities of natural products (Supplementary Table 1). *Drosophila melanogaster* is genetically significant because it has about 60% homologous genes with humans, with fewer redundant genes. Additionally, around 75% of human disease-associated genes have counterpart homologs in flies (Ugur et al., 2016). Furthermore, it is a low-cost and highly sensitive model, offering an efficient alternative to traditional animal models in toxicological assays, thus circumventing associated ethical challenges (Cunha et al., 2015; Coutinho et al., 2018; Costa et al., 2020). In our study, the stem bark extracts of *H. speciosa* did not cause *in vivo* toxicity in *D. melanogaster* at clinically relevant concentrations ($\leq$10 mg/g). The absence of toxicity against *Allium cepa* and *Artemia salina* was also verified by Panontin et al. (2021) using the hydroethanol extract from the stem of *H. speciosa*.

The drug-modifying action of the extracts *H. speciosa* and the absence of toxicity effects observed in the present study, qualify this plant for future research on antibiotic development and therapy. However, it is necessary to carry out additional pre-clinical studies to evaluate the mechanisms involved in the pharmacokinetics and pharmacodynamics of *H. speciosa* extracts, which is essential for understanding their efficacy and safety before considering their introduction as a new pharmaceutical product.

5. Conclusion

The findings of this study partially support the ethnopharmacological practices associated with the use of *H. speciosa* in the treatment of infections. Although *H. speciosa* extracts did not show intrinsic antibacterial activity at clinically relevant concentrations against the bacterial strains (*E. coli*, *P. aeruginosa,* and *S. aureus*), they demonstrated the ability to enhance the antibacterial effect of commercial antibiotic drugs (gentamicin, erythromycin, and norfloxacin). Furthermore, the extracts did not show toxicity at clinically relevant concentrations in tests with *D. melanogaster*.

These results evidenced the potentialities of stem bark *H. speciosa* as a source of bioactive compounds for the development of new therapeutic agents. The ability of these extracts to intensify the action of antibiotics brings new strategies for the development of therapeutics against bacterial infections, especially, under the current increasing of bacterial resistance to antibiotics. However, it is important to mention that additional research is needed to better understand the therapeutic potential and mechanisms underlying the properties of *H. speciosa* extracts. The identification and characterization of the bioactive compounds found in the extracts, the investigation of their activity against other pathogens, and the evaluation of their safety and efficacy in more complex animal models, are crucial steps for the development of new antibiotics based on *H. speciosa*.

Ethical standards

This study was approved by the Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SisGen-Brazil) under the registration number A535238, and by the Sistema de Autorização e Informação em Biodiversidade (SISBio-Brazil) under the registration number 80293-1.

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Data statement

Data will be made available on request.

CRediT authorship contribution statement

Viviane Bezerra da Silva: Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **José Weverton Almeida-Bezerra:** Investigation, Formal analysis. **Raimundo Luiz Silva Pereira:** Investigation. **Bruno Melo de Alcântara:** Software. **Cláudia Maria Furlan:** Software. **Janerson José Coelho:** Formal analysis. **Henrique Douglas Melo Coutinho:** Project administration. **Maria Flaviana Bezerra Morais-Braga:** Supervision. **Antonio Fernando Morais de Oliveira:** Writing – review & editing, Writing – original draft, Supervision, 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.

Data availability

Data will be made available on request.

Abbreviations

BHI	Brain Heart Infusion
CFU/mL	Colony Forming Units per Milliliter
DMSO	Dimethylsulfoxide
EEHS	Sulfuric Ether Extract of *Hancornia speciosa*
GAE	Gallic Acid Equivalents
GC–MS	Gas Chromatography coupled to Mass Spectrometry
HIA	Heart Infusion Agar
MEHS	Methanolic Extract of *Hancornia speciosa*
MIC	Minimum Inhibitory Concentration
ns	Not Significant
QE	Quercetin Equivalents
SEM	Standard Error of the Mean
UPLC–QTOF–MS/MS	Ultra-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jep.2024.118631.

References

Adhikari, L., Kotiyal, R., Pandey, M., Bharkatiya, M., Sematy, A., Semalty, M., 2020. Effect of geographical location and type of extract on total phenol/flavon contents and antioxidant activity of different fruits extracts of *Withania somnifera*. Curr. Drug Discov. Technol. 17, 92–99. https://doi.org/10.2174/1570163815666180807100456.

Aires, A., Dias, C., Carvalho, R., Saavedra, M.J., 2017. Analysis of glycosylated flavonoids extracted from sweet-cherry stems, as antibacterial agents against pathogenic *Escherichia coli* isolates. Acta Biochim. Pol. 64, 265–271. https://doi.org/10.18388/ABP.2016_1374.

Akwu, N., Naidoo, Y., Singh, M., Thimmegowda, S.C., Nundkumar, N., Lin, J., 2020. Isolation of lupeol from grewia lasiocarpa stem bark: antibacterial, antioxidant, and cytotoxicity activities. Biodiversitas 21, 5684–5690. https://doi.org/10.13057/biodiv/d211213.

Albuquerque, U.P. de, Meiado, M.V., 2015. In: Sociobiodiversidade Na Chapada Do Araripe, first ed. NUPEEA, Recife.

Ali, J.S., Riaz, N., Mannan, A., Tabassum, S., Zia, M., 2022. Antioxidative-, antimicrobial-, enzyme inhibition-, and cytotoxicity-based fractionation and isolation of active components from *Monotheca buxifolia* (Falc.) A. DC. Stem extracts. ACS Omega 7, 3407–3423. https://doi.org/10.1021/acsomega.1c05647.

Almeida, F.L.C., Oliveira, E.N.A., Almeida, E.C., Souza, W.F.C., Silva, M.O., Melo, A.M., Castro, M.P.J., Bullo, G.T., Luna, L.C., Prata, A.S., Forte, M.B.S., 2022. *Hancornia speciosa*: an overview focused on phytochemical properties, recent achievements, applications, and future perspectives. Int. J. Gastron. Food Sci. 29, 100561 https://doi.org/10.1016/j.ijgfs.2022.100561.

Almeida-Bezerra, J.W., Cruz, R.P., Pereira, R.L.S., Silva, V.B., Sousa, D.O.B., Neto, J.X.S., Souza, L.A.L., Araújo, N.M.S., Silva, R.G.G., Lucetti, D.L., Coutinho, H.D.M., Morais-Braga, M.F.B., Oliveira, A.F.M., 2023. *Caryocar coriaceum* fruits as a potential alternative to combat fungal and bacterial infections: in vitro evaluation of methanolic extracts. Microb. Pathog. 181, 106203 https://doi.org/10.1016/j.micpath.2023.106203.

Alves, A.D.C.S., Mainardes, R.M., Khalil, N.M., 2016. Nanoencapsulation of gallic acid and evaluation of its cytotoxicity and antioxidant activity. Mater. Sci. Eng. C 60, 126–134. https://doi.org/10.1016/j.msec.2015.11.014.

Angeloni, S., Spinozzi, E., Maggi, F., Sagratini, G., Caprioli, G., Borsetta, G., Ak, G., Sinan, K.I., Zengin, G., Arpini, S., Mombelli, G., Ricciutelli, M., 2021. Phytochemical profile and biological activities of crude and purified *Leonurus cardiaca* extracts. Plants 10, 195. https://doi.org/10.3390/plants10020195.

Anoor, P.K., Yadav, A.N., Rajkumar, K., Kande, R., Tripura, C., Naik, K.S., 2022. Methanol extraction revealed anticancer compounds quinic acid, 2(5h)-furanone and phytol in *Andrographis paniculata*. Mol. Clin. Oncol. 17 https://doi.org/10.3892/mco.2022.2584.

Ansari, F., Pourjafar, H., Kangari, A., Homayouni, A., 2019. Evaluation of the glucuronic acid production and antibacterial properties of kombucha black tea. Curr. Pharmaceut. Biotechnol. 20, 985–990. https://doi.org/10.2174/1389201020666190717100958.

Araújo, N.J.S., Silva, A.R.P., Costa, M.S., Freitas, T.S., Barbosa Filho, J.M., Matos, Y.M.L.S., Morais-Braga, M.F.B., Pereira Junior, F.N., Silva, C.A.P., Souza, E.O., Ribeiro, P.R.V., Lacerda, B.C.V.G., Andrade, E.M., Coutinho, H.D.M., Andrade-Pinheiro, J.C., 2022. Chemical characterization UPLC-ESI-QToF-MSE, antibacterial and antibiofilm potential of *Sarcomphalus joazeiro* (MART.) Hauenschild. Food Biosci. 50, 102066 https://doi.org/10.1016/j.fbio.2022.102066.

Ayaz, M., Ullah, F., Sadiq, A., Ullah, F., Ovais, M., Ahmed, J., Devkota, H.P., 2019. Synergistic interactions of phytochemicals with antimicrobial agents: potential strategy to counteract drug resistance. Chem. Biol. Interact. 308, 294–303. https://doi.org/10.1016/j.cbi.2019.05.050.

Babich, H., Sedletcaia, A., Kenigsberg, B., 2002. In vitro cytotoxicity of protocatechuic acid to cultured human cells from oral tissue: involvement in oxidative stress. Pharmacol. Toxicol. 91, 245–253. https://doi.org/10.1034/j.1600-0773.2002.910505.x.

Bai, J., Wu, Y., Liu, X., Zhong, K., Huang, Y., Gao, H., 2015. Antibacterial activity of shikimic acid from pine needles of *Cedrus deodara* against *Staphylococcus aureus* through damage to cell membrane. Int. J. Mol. Sci. 16, 27145–27155. https://doi.org/10.3390/ijms161126015.

Barbosa, A.M., Santos, K.S., Borges, G.R., Muniz, A.V.C.S., Mendonça, F.M.R., Pinheiro, M.S., Franceschi, E., Dariva, C., Padilha, F.F., 2019. Separation of antibacterial biocompounds from *Hancornia speciosa* leaves by a sequential process of pressurized liquid extraction. Sep. Purif. Technol. 222, 390–395. https://doi.org/10.1016/j.seppur.2019.04.022.

Batory, M., Rotsztejn, H., 2022. Shikimic acid in the light of current knowledge. J. Cosmet. Dermatol. 21, 501–505. https://doi.org/10.1111/jocd.14136.

Bernal-Mercado, A.T., Vazquez-Armenta, F.J., Tapia-Rodriguez, M.R., Islas-Osuna, M.A., Mata-Haro, V., Gonzalez-Aguilar, G.A., Lopez-Zavala, A.A., Ayala-Zavala, J.F., 2018. Comparison of single and combined use of catechin, protocatechuic, and vanillic acids as antioxidant and antibacterial agents against Uropathogenic *Escherichia coli* at planktonic and biofilm levels. Molecules 23, 2803–2813. https://doi.org/10.3390/molecules23112813.

Bezerra, C.F., Camilo, C.J., Silva, M.K.N., Freitas, T.S., Ribeiro-Filho, J., Coutinho, H.D.M., 2017. Vanillin selectively modulates the action of antibiotics against resistant bacteria. Microb. Pathog. 113, 265–268. https://doi.org/10.1016/j.micpath.2017.10.052.

Calixto, J.B., 2019. The role of natural products in modern drug discovery. An. Acad. Bras. Cienc. 91, 1–7. https://doi.org/10.1590/0001-3765201920190105.

Carneiro, J.N.P., da Cruz, R.P., Silva, J.P.C., Rocha, J.E., Freitas, T.S., Sales, D.L., Bezerra, C.F., Almeida Martins, W.O., Costa, J.G.M., Silva, L.E., Amaral, W., Rebelo, R.A., Begnini, I.M., Coutinho, H.D.M., Morais-Braga, M.F.B., 2019. *Piper diospyrifolium* Kunth.: chemical analysis and antimicrobial (intrinsic and combined) activities. Microb. Pathog. 136, 103700 https://doi.org/10.1016/j.micpath.2019.103700.

Chaachouay, N., Zidane, L., 2024. Plant-derived natural products: a source for drug discovery and development. drugs and drug candidates 3, 184–207. https://doi.org/10.3390/ddc3010011.

Chaturvedi, P.K., Bhui, K., Shukla, Y., 2008. Lupeol: connotations for chemoprevention. Cancer Lett. 263, 1–13. https://doi.org/10.1016/j.canlet.2008.01.047.

Choi, N.H., Jang, J.Y., Choi, G.J., Choi, Y.H., Jang, K.S., Nguyen, V.T., Min, B.S., Le Dang, Q., Kim, J.C., 2017. Antifungal activity of sterols and dipsacus saponins isolated from *Dipsacus asper* roots against phytopathogenic fungi. Pestic. Biochem. Physiol. 141, 103–108. https://doi.org/10.1016/j.pestbp.2016.12.006.

Confessor, M.V.A., Agreles, M.A.A., Campos, L.A.A., Neto, A.F.S., Borges, J.C., Martins, R.M., Scavuzzi, A.M.L., Lopes, A.C.S., Kretzschmar, E.A.M., Cavalcanti, I.M.F., 2024. Olive oil nanoemulsion containing curcumin: antimicrobial agent against multidrug-resistant bacteria. Appl. Microbiol. Biotechnol. 108 https://doi.org/10.1007/s00253-024-13057-x.

Costa, A.R., Silva, J.R.L., Oliveira, T.J.S., Silva, T.G., Pereira, P.S., Borba, E.F.O., Brito, E.S., Ribeiro, P.R.V., Almeida-Bezerra, J.W., Júnior, J.T.C., Menezes, I.R.A., Kamdem, J.P., Duarte, A.E., Barros, L.M., 2020. Phytochemical profile of *Anacardium occidentale* L. (cashew tree) and the cytotoxic and toxicological evaluation of its bark

and leaf extracts. South Afr. J. Bot. 135, 355–364. https://doi.org/10.1016/j.sajb.2020.09.017.
Coutinho, H.D.M., Costa, J.G.M., Lima, E.O., Falcão-Silva, V.S., Siqueira-Júnior, J.P., 2008. Enhancement of the antibiotic activity against a multiresistant *Escherichia coli* by *Mentha arvensis* L. And chlorpromazine. Chemotherapy 54, 328–330. https://doi.org/10.1159/000151267.
Coutinho, H.D.M., Oliveira-Tintino, C.D. de M., Tintino, S.R., Pereira, L.S.P., Freitas, T.S. de, Silva, M.A.P. da, Franco, J.L., Cunha, F.A.B., Costa, J.G.M., Menezes, I.R.A.de, Boligon, A.A., Rocha, J.B.T. da, Rocha, M.I., Santos, J.F.S., 2018. Toxicity against *Drosophila melanogaster* and antiedematogenic and antimicrobial activities of *Alternanthera brasiliana* (L.) Kuntze (Amaranthaceae). Environ. Sci. Pollut. Res. Int. 25, 10353–10361. https://doi.org/10.1007/s11356-017-9366-x.
Cruz, R.R., Almeida-Bezerra, J.W., Menezes, S.A., Silva, V.B., Santos, L.T., Morais-Braga, M.F.B., de Moraes, J.L., 2021. Ethnopharmacology of the angiosperms of Chapada of Araripe located in northeast of Brazil. JEAP 6, 326–351. https://doi.org/10.24221/jeap.6.4.2021.4272.326-351.
Cunha, F.A.B., Wallau, G.L., Pinho, A.I., Nunes, M.E.M., Leite, N.F., Tintino, S.R., Costa, G.M., Athayde, M.L., Boligon, A.A., Coutinho, H.D.M., Pereira, A.B., Posser, T., Franco, J.L., 2015. *Eugenia uniflora* leaves essential oil induces toxicity in *Drosophila melanogaster*: involvement of oxidative stress mechanisms. Toxicol. Res. 4, 634–644. https://doi.org/10.1039/c4tx00162a.
Čurová, K., Slebodníková, R., Kmeťová, M., Hrabovský, V., Maruniak, M., Liptáková, E., Siegfried, L., 2020. Virulence, phylogenetic background and antimicrobial resistance in *Escherichia coli* associated with extraintestinal infections. J Infect Public Heal 13, 1537–1543. https://doi.org/10.1016/j.jiph.2020.06.032.
Cushnie, T.P.T., Lamb, A.J., 2011. Recent advances in understanding the antibacterial properties of flavonoids. Int. J. Antimicrob. Agents 38, 99–107. https://doi.org/10.1016/j.ijantimicag.2011.02.014.
D'Abadia, P.L., Bailão, E.F.L.C., Júnior, R.S.L., Oliveira, M.G., Silva, V.B., Oliveira, L.A. R., Conceição, E.C., Melo-Reis, P.R., Borges, L.L., Gonçalves, P.J., Almeida, L.M., 2020. *Hancornia speciosa* serum fraction latex stimulates the angiogenesis and extracellular matrix remodeling processes. An. Acad. Bras. Cienc. 92 https://doi.org/10.1590/0001-3765202020190107.
Dadgostar, P., 2019. Antimicrobial resistance: implications and costs. Infect. Drug Resist. 12, 3903–3910. https://doi.org/10.2147/idr.s234610.
Daniel, I.J., Innocent, E., Sempombe, J., Mugoyela, V., Fossen, T., 2020. Isolation and characterization of larvicidal phenolic acids from *Kotschya thymodora* leaves. J. Appl. Sci. Environ. Manag. 24, 1483–1488. https://doi.org/10.4314/jasem.v24i8.26.
Dassanayake, M.K., Khoo, T.-J., An, J., 2021. Antibiotic resistance modifying ability of phytoextracts in anthrax biological agent *Bacillus anthracis* and emerging superbugs: a review of synergistic mechanisms. Ann. Clin. Microbiol. Antimicrob. 20 https://doi.org/10.1186/s12941-021-00485-0.
Díaz-Ruiz, G., Hernández-Vázquez, L., Luna, H., Del Carmen Wacher-Rodarte, M., Navarro-Ocaña, A., 2012. Growth inhibition of streptococcus from the oral cavity by α-amyrin esters. Molecules 17, 12603–12611. https://doi.org/10.3390/molecules171112603.
Dilika, F., Bremner, P.D., Meyer, J.J.M., 2000. Antibacterial activity of linoleic and oleic acids isolated from *Helichrysum pedunculatum*: a plant used during circumcision rites. Fitoterapia 71, 450–452. https://doi.org/10.1016/S0367-326X(00)00150-7.
Ding, Y., Li, Z., Xu, C., Qin, W., Wu, Q., Wang, X., Cheng, X., Li, L., Huang, W., 2020. Fluorogenic probes/inhibitors of β-lactamase and their applications in drug-resistant bacteria. Angew. Chem. 60, 24–40. https://doi.org/10.1002/anie.202006635.
Dwivedi, G.R., Pathak, N., Tiwari, N., Negi, A.S., Kumar, A., Pal, A., Sharma, A., Darokar, M.P., 2024. Synergistic antibacterial activity of gallic acid based chalcone indl 2 by inhibiting efflux pump transporters. Chem. Biodiversity 21. https://doi.org/10.1002/cbdv.202301820.
Ecevit, K., Barros, A.A., Silva, J.M., Reis, R.L., 2022. Preventing microbial infections with natural phenolic compounds. Future Pharmacol 2, 460–498. https://doi.org/10.3390/futurepharmacol2040030.
Edwardson, S., Cairns, C., 2019. Nosocomial infections in the ICU. Anaesth. Intensive Care 20, 14–18. https://doi.org/10.1016/j.mpaic.2018.11.004.
Eisinger, R.W., Williams, M.P., Choe, S.H., Krofah, E., 2023. A call to action-stopping antimicrobial resistance. JAC-Antimicrob. Resist. 5 https://doi.org/10.1093/jacamr/dlac142.
Farhadi, F., Khameneh, B., Iranshahi, M., Iranshahy, M., 2018. Antibacterial activity of flavonoids and their structure–activity relationship: an update review. Phytother Res. 33, 13–40. https://doi.org/10.1002/ptr.6208.
Fernandes, P.A.S., Pereira, R.L.S., dos Santos, A.T.L., Coutinho, H.D.M., Morais-Braga, M. F.B., da Silva, V.B., Costa, A.R., Generino, M.E.M., de Oliveira, M.G., de Menezes, S. A., dos Santos, L.T., Siyadatpanah, A., Wilairatana, P., Portela, T.M.A., Gonçalo, M. A.B.F., Almeida-Bezerra, J.W., 2022. Phytochemical analysis, antibacterial activity and modulating effect of essential Oil from *Syzygium cumini* (L.) Skeels. Molecules 27, 3281. https://doi.org/10.3390/molecules27103281.
Fifere, A., Turin-Moleavin, I.A., Rosca, I., 2022. Does protocatechuic acid affect the activity of commonly used antibiotics and antifungals? Life 12, 1010. https://doi.org/10.3390/life12071010.
Garneau-Tsodikova, S., Labby, K.J., 2016. Mechanisms of resistance to aminoglycoside antibiotics: overview and perspectives. MedChemComm 7, 11–27. https://doi.org/10.1039/c5md00344j.
Goławska, S., Łukasik, I., Chojnacki, A.A., Chrzanowski, G., 2023. Flavonoids and phenolic acids content in cultivation and wild collection of european cranberry bush *Viburnum opulus* L. Molecules 28, 2285. https://doi.org/10.3390/molecules28052285.
Górniak, I., Bartoszewski, R., Króliczewski, J., 2019. Comprehensive review of antimicrobial activities of plant flavonoids. Phytochemistry Rev. 18, 241–272. https://doi.org/10.1007/s11101-018-9591-z.
Hamid, L., Alsayari, A., Tak, H., Mir, S.A., Ali, M., Wahab, S., Bader, G.N., 2023. An Insight into the global problem of gastrointestinal helminth infections amongst livestock: does nanotechnology provide an alternative? Agriculture 13, 1359. https://doi.org/10.3390/agriculture13071359.
Hao, J., Lei, Y., Gan, Z., Zhao, W., Shi, J., Jia, C., Sun, A., 2021. Synergetic inactivation mechanism of protocatechuic acid and high hydrostatic pressure against *Escherichia coli* O157. Foods 10. https://doi.org/10.3390/foods10123053, 3053–3053.
Hoang, S., Georget, A., Asselineau, J., Venier, A.G., Leroyer, C., Rogues, A.M., Thiébaut, R., 2018. Risk factors for colonization and infection by *Pseudomonas aeruginosa* in patients hospitalized in intensive care units in France. PLoS One 13, e0193300. https://doi.org/10.1371/journal.pone.0193300.
Horn, J., Stelzner, K., Rudel, T., Fraunholz, M., 2018. Inside job: *Staphylococcus aureus* host-pathogen interactions. Int J. Med. Microbiol. 308, 607–624. https://doi.org/10.1016/j.ijmm.2017.11.009.
Houghton, P.J., Howes, M.-J., Lee, C.C., Steventon, G., 2007. Uses and abuses of in vitro tests in ethnopharmacology: visualizing an elephant. J. Ethnopharmacol. 110, 391–400. https://doi.org/10.1016/j.jep.2007.01.032.
Hutchings, M.I., Truman, A.W., Wilkinson, B., 2019. Antibiotics: past, present and future. Curr. Opin. Microbiol. 51, 72–80. https://doi.org/10.1016/j.mib.2019.10.008.
Ibrahim, H.A., Elgindi, M.R., Ibrahim, R.R., El-Hosari, D.G., 2019. Antibacterial activities of triterpenoidal compounds isolated from *Calothamnus quadrifidus* leaves. BMC Compl. Alternative Med. 19 https://doi.org/10.1186/s12906-019-2512-x.
Ichiko, C.O., Terrumun, A.T.A., John, O.I., John, V.A., 2016. In vitro antimicrobial properties of friedelan-3-one from *Pterocarpus santalinoides* LHerit, ex Dc. Afr. J. Biotechnol. 15, 531–538. https://doi.org/10.5897/ajb2015.15091.
Javed, S., Mahmood, Z., Khan, K.M., Sarker, S.D., Javaid, A., Khan, I.H., Shoaib, A., 2021. Lupeol acetate as a potent antifungal compound against opportunistic human and phytopathogenic mold *Macrophomina phaseolina*. Sci. Rep. 11, 8417. https://doi.org/10.1038/s41598-021-87725-7.
Jiang, Y., Pei, J., Zheng, Y., Miao, Y., Duan, B., Huang, L., 2022. Gallic acid: a potential anti-cancer agent. Chin. J. Integr. Med. 28, 661–671.
Johann, S., Soldi, C., Lyon, J.P., Pizzolatti, M.G., Resende, M.A., 2007. Antifungal activity of the amyrin derivatives and in vitro inhibition of *Candida albic*ans adhesion to human epithelial cells. Lett. Appl. Microbiol. 45, 148–153. https://doi.org/10.1111/j.1472-765X.2007.02162.x.
Jung, S.W., Thamphiwatana, S., Zhang, L., Obonyo, M., 2015. Mechanism of antibacterial activity of liposomal linolenic acid against *Helicobacter pylori*. PLoS One 10, e0116519. https://doi.org/10.1371/journal.pone.0116519.
Kamatchi, P.A.C., Maheswaran, R., Sivanandhan, S., Ignacimuthu, S., Balakrishna, K., Reegan, A.D., Arivoli, S., 2023. Bioefficacy of ursolic acid and its derivatives isolated from *Catharanthus roseus* (L) G. Don leaf against *Aedes aegypti, Culex quinquefasciatus*, and *Anopheles stephensi* larvae. Environ. Sci. Pollut. Res. 30, 69321–69329. https://doi.org/10.1007/s11356-023-27253-1.
Kauffmann, A.C., Castro, V.S., 2023. Phenolic compounds in bacterial inactivation: a perspective from Brazil. Antibiotics 12. https://doi.org/10.3390/antibiotics12040645, 645–645.
Keyvani-Ghamsari, S., Rahimi, M., Khorsandi, K., 2023. An update on the potential mechanism of gallic acid as an antibacterial and anticancer agent. Food Sci. Nutr. 11, 5856–5872. https://doi.org/10.1002/fsn3.3615.
Khalil, I.A., Troeger, C., Blacker, B.F., Rao, P.C., Brown, A., Atherly, D.E., Brewer, T.G., Engmann, C.M., Houpt, E.R., Kang, G., Kotloff, K.L., Levine, M.M., Luby, S.P., MacLennan, C.A., Pan, W.K., Pavlinac, P.B., Platts-Mills, J.A., Qadri, F., Riddle, M.S., Ryan, E.T., Shoultz, D.A., Steele, D., Walson, J.L., Sanders, J.W., Mokdad, A.H., Murray, C.J.L., Hay, S.I., Reiner, R.C., 2018. Morbidity and mortality due to shigella and enterotoxigenic *Escherichia coli* diarrhoea: the global burden of disease study 1990–2016. Lancet Infect. Dis. 18, 1229–1240. https://doi.org/10.1016/s1473-3099(18)30475-4.
Khameneh, B., Iranshahy, M., Soheili, V., Bazzaz, B.S.F., 2019. Review on plant antimicrobials: a mechanistic viewpoint. Antimicrob. Resist. Infect. Control 8, 1–28. https://doi.org/10.1186/s13756-019-0559-6.
Khan, H.A., Ahmad, A., Mehboob, R., 2015. Nosocomial infections and their control strategies. Asian Pac. J. Trop. Biomed. 5, 509–514. https://doi.org/10.1016/j.apjtb.2015.05.001.
Kuppusamy, C., Murugan, K., Arul, N., Yasodha, P., 2009. Larvicidal and insect growth regulator effect of α-amyrin acetate from *Catharanthus roseus* Linn against the malaria vector *Anopheles stephensi* Liston (Diptera: Culicidae). Entomol. Res. 39, 78–83. https://doi.org/10.1111/j.1748-5967.2009.00196.x.
Lee, J.-H., Regmi, S.C., Kim, J.-A., Cho, M.H., Yun, H., Lee, C.-S., Lee, J., 2011. Apple flavonoid phloretin inhibits *Escherichia coli* O157:H7 biofilm formation and ameliorates colon inflammation in rats. Infect. Immun. 79, 4819–4827. https://doi.org/10.1128/iai.05580-11.
Liberato, I., Lino, L.A., Souza, J.K.D., Neto, J.B.A., Sá, L.G.A.V., Cabral, V.P.F., Silva, C. R., Cavalcanti, B.C., Moraes, M.O., Freire, V.N., Júnior, H.V.N., Andrade, C.R., 2022. Gallic acid leads to cell death of *Candida albicans* by the apoptosis mechanism. Future Microbiol. 17, 599–606. https://doi.org/10.2217/fmb-2021-0139.
Liu, K. Sen, Tsao, S.M., Yin, M.C., 2005. *In vitro* antibacterial activity of roselle calyx and protocatechuic acid. Phyther. Res. 19, 942–945. https://doi.org/10.1002/ptr.1760.
Lobiuc, A., Pavăl, N.-E., Mangalagiu, I.I., Gheorghiță, R., Teliban, G.-C., Mantu, D.A., Stoleru, V., 2023. Future antimicrobials: natural and functionalized phenolics. Molecules 28. https://doi.org/10.3390/molecules28031114, 1114–1114.
Luhata, L.P., Usuki, T., 2021. Antibacterial activity of β-sitosterol isolated from the leaves of *Odontonema strictum* (Acanthaceae). Bioorg. Med. Chem. Lett. 48, 128248.
Ma, C.M., Cai, S.Q., Cui, J.R., Wang, R.Q., Tu, P.F., Hattori, M., Daneshtalab, M., 2005. The cytotoxic activity of ursolic acid derivatives. Eur. J. Med. Chem. 40, 582–589. https://doi.org/10.1016/j.ejmech.2005.01.001.

Mahamud, A.G.M.S.U., Nahar, S., Ashrafudoulla, M.A., Park, S.H., Ha, S.-D., 2022. Insights into antibiofilm mechanisms of phytochemicals: prospects in the food industry. Crit. Rev. Food Sci. Nutr. 64, 1–28. https://doi.org/10.1080/10408398.2022.2119201.

Martins, N., Barros, L., Ferreira, I.C.F.R., 2016. In vivo antioxidant activity of phenolic compounds: facts and gaps. Trends Food Sci. Technol. 48, 1–12. https://doi.org/10.1016/j.tifs.2015.11.008.

Mbambo, B., Odhav, B., Mohanlall, V., 2012. Antifungal activity of stigmasterol, sitosterol and ergosterol from *Bulbine natalensis* Baker (Asphodelaceae). J. Med. Plants Res. 6, 5135–5141. https://doi.org/10.5897/jmpr12.151.

Meghdadi, P., Bamoharram, F.F., Karimi, E., Ghasemi, E., 2024. Shikimic acid nanoformulations: a comprehensive inquiry into anticancer potential and apoptotic induction on A2058 skin cancer cells. Bionanoscience 1–8. https://doi.org/10.1007/s12668-024-01490-1.

Miklasińska-Majdanik, M., Kępa, M., Wojtyczka, R.D., Idzik, D., Wąsik, T.J., 2018. Phenolic compounds diminish antibiotic resistance of *Staphylococcus aureus* clinical strains. Int. J. Environ. Res. Publ. Health 15. https://doi.org/10.3390/ijerph15102321, 2321–2321.

Mishra, M., Sharma, A., Dagar, V.S., Kumar, S., 2020. Effects of B-sitosterol on growth, development and midgut enzymes of *Helicoverpa armigera* Hübner. Arch. Biol. Sci. 72, 271–278. https://doi.org/10.2298/ABS200308021M.

Moraes, T. de M., Rodrigues, C.M., Kushima, H., Bauab, T.M., Villegas, W., Pellizzon, C. H., Brito, A.R.M.S., Hiruma-Lima, C.A., 2008. *Hancornia speciosa*: indications of gastroprotective, healing and anti-*Helicobacter pylori* actions. J. Ethnopharmacol. 120, 161–168. https://doi.org/10.1016/j.jep.2008.08.001.

Morel, C.M., Alm, R.A., Årdal, C., Bandera, A., Bruno, G.M., Carrara, E., Colombo, G.L., de Kraker, M.E.A., Essack, S., Frost, I., Gonzalez-Zorn, B., Goossens, H., Guardabassi, L., Harbarth, S., Jørgensen, P.S., Kanj, S.S., Kostyanev, T., Laxminarayan, R., Leonard, F., Hara, G.L., Mendelson, M., Mikulska, M., Mutters, N. T., Outterson, K., Rodriguez Baño, J., Tacconelli, E., Scudeller, L., 2020. A one health framework to estimate the cost of antimicrobial resistance. Antimicrob. Resist. Infect. Control 9, 1–14. https://doi.org/10.1186/s13756-020-00822-6.

Nascimento, P.G.G., Lemos, T.L.G., Bizerra, A.M.C., Arriaga, A.M.C., Ferreira, D.A., Santiago, G.M.P., Braz-Filho, R., Costa, J.G.M., 2014. Antibacterial and antioxidant activities of ursolic acid and derivatives. Molecules 19, 1317–1327. https://doi.org/10.3390/molecules19011317.

Nobsathian, S., Bullangpoti, V., Kumrungsee, N., Wongsa, N., Ruttanakum, D., 2018. Larvicidal effect of compounds isolated from *Maerua siamensis* (Capparidaceae) against *Aedes aegypti* (Diptera: Culicidae) larvae. Chem. Biol. Technol. Agric. 5, 1–7. https://doi.org/10.1186/s40538-018-0120-5.

O'Callaghan, Y., Kenny, O., O'Connell, N.M., Maguire, A.R., McCarthy, F.O., O'Brien, N. M., 2013. Synthesis and assessment of the relative toxicity of the oxidised derivatives of campesterol and dihydrobrassicasterol in U937 and HepG2 cells. Biochimie 95, 496–503. https://doi.org/10.1016/j.biochi.2012.04.019.

Obonyo, M., Zhang, Li, Thamphiwatana, S., Pornpattananangkul, D., Fu, V., Zhang, Liangfang, 2012. Antibacterial activities of liposomal linolenic acids against antibiotic-resistant *Helicobacter pylori*. Mol. Pharm. 9, 2677–2685. https://doi.org/10.1021/mp300243w.

Okafor, G.C.O., Oyewale, A.O., Habila, J.D., Akpemi, M.A., 2022. Isolation, characterization, and assessment of the *in vitro* antibacterial and antifungal properties of methanol extracts and friedelan-3-one from *Uapaca ambanjensis* (Leandri). J. Appl. Sci. Environ. Manag. 26, 1479–1486. https://doi.org/10.4314/jasem.v26i9.4.

Oliveira, F.S., Freitas, T.S., da Cruz, R.P., Costa, M.S., Pereira, R.L.S., Quintans-Júnior, L. J., Andrade, T.A., Menezes, P.D.P., de Sousa, B.M.H., Nunes, P.S., Serafini, M.R., de Menezes, I.R.A., Araújo, A.A.S., Coutinho, H.D.M., 2017. Evaluation of the antibacterial and modulatory potential of α-bisabolol, β-cyclodextrin and α-bisabolol/β-cyclodextrin complex. Biomed. Pharmacother. 92, 1111–1118. https://doi.org/10.1016/j.biopha.2017.06.020.

Pakbin, B., Brück, W.M., Rossen, J.W.A., 2021. Virulence factors of enteric pathogenic *Escherichia coli*: a review. Int. J. Mol. Sci. 22, 9922. https://doi.org/10.3390/ijms22189922.

Panontin, J.F., Barbosa, R.D.S., Isaac, V., Seibert, C.S., Scapin, E., 2022. Chemical composition, antioxidant activity and development of a facial serum formulation from the extract of *Hancornia speciosa*. Nat. Prod. Res. 36, 6121–6125. https://doi.org/10.1080/14786419.2022.2053968.

Panontin, J.F., Neres, R.P., Fernandes, R.M.N., Scapin, E., Seibert, C.S., 2021. Chemical characterization and toxicological analyses of hydroalcoholic extracts from the stem and leaves of mangabeira (*Hancornia speciosa* Gomes) as a guide for the development of green cosmetics. J. Med. Plants Res. 15, 366–379. https://doi.org/10.5897/JMPR2021.7099.

Penido, A.B., Morais, S.M., Ribeiro, A.B., Silva, A.Z., 2016. Ethnobotanical study of medicinal plants in imperatriz, state of maranhão, northeastern Brazil. Acta Amazonica 46, 345–354. https://doi.org/10.1590/1809-4392201600584.

Permyakov, S.E., Knyazeva, E.L., Khasanova, L.M., Fadeev, R.S., Zhadan, A.P., Roche-Hakansson, H., Håkansson, A.P., Akatov, V.S., Permyakov, E.A., 2012. Oleic acid is a key cytotoxic component of HAMLET-like complexes. Biol. Chem. 393, 85–92. https://doi.org/10.1515/BC-2011-230.

Perumalsamy, H., Jang, M.J., Kim, J.R., Kadarkarai, M., Ahn, Y.J., 2015. Larvicidal activity and possible mode of action of four flavonoids and two fatty acids identified in *Millettia pinnata* seed toward three mosquito species. Parasit. vectors 8. https://doi.org/10.1186/s13071-015-0848-8.

Pokharel, P., Dhakal, S., Dozois, C.M., 2023. The diversity of *Escherichia coli* pathotypes and vaccination strategies against this versatile bacterial pathogen. Microorganisms 11. https://doi.org/10.3390/microorganisms11020344, 344–344.

Pontes, M.C., Cavalcante, N.B., Leal, A.E.B.P., de Oliveira, A.P., Coutinho, H.D.M., de Menezes, I.R.A., Delange, D.M., Turatti, I.C.C., de Oliveira, G.G., Neto, F.C., Tomaz, J.C., Lopes, N.P., Almeida, J.R.G.S., 2022. Chemical constituents and antibacterial activity of *Bromelia laciniosa* (Bromeliaceae): identification and structural characterization. Phytomed. Plus. 2, 100215 https://doi.org/10.1016/j.phyplu.2022.100215.

Punia, A., Chauhan, N.S., Singh, D., Kesavan, A.K., Kaur, S., Sohal, S.K., 2021. Effect of gallic acid on the larvae of *Spodoptera litura* and its parasitoid Bracon hebetor. Sci. Rep. 11, 531. https://doi.org/10.1038/s41598-020-80232-1.

Radi, M.H., El-Shiekh, R.A., El-Halawany, A.M., Abdel-Sattar, E., 2023. Friedelin and 3β-friedelinol: pharmacological activities. Rev. Bras. Farmacogn. 33, 886–900.

Rahuman, A.A., Venkatesan, P., Gopalakrishnan, G., 2008. Mosquito larvicidal activity of oleic and linoleic acids isolated from *Citrullus colocynthis* (Linn.) Schrad. Parasitol. Res. 103, 1383–1390. https://doi.org/10.1007/s00436-008-1146-6.

Rajan, A., Perrimon, N., 2011. *Drosophila* as a model for interorgan communication: lessons from studies on energy homeostasis. Dev. Cell 21, 29–31. https://doi.org/10.1016/j.devcel.2011.06.034.

Rand, M.D., 2010. Drosophotoxicology: the growing potential for *Drosophila* in neurotoxicology. Neurotoxicol. Teratol. 32, 74–83. https://doi.org/10.1016/j.ntt.2009.06.004.

Ravi, L., Ajith Kumar, K., Shree Kumari, G.R., Mathew, J., Harshitha, S., Panda, M., Shivani, S., Paul, A., Chandana, T.S., Anil, A., Megha, J.K., Mukherjee, T., Bhattacharjee, S., Raveendran Nair, M., Subhanjan, V., Mohanasrinivasan, V., Jain, P., 2024. Behenic Acid as a multi-target inhibiting antibacterial phytochemical against *Vibrio parahaemolyticus* and *Aeromonas hydrophila* for effective management of aquaculture infections: an *in-silico, in-vitro* & *in-vivo* experimentation. J. Biomol. Struct. Dyn. 1–16. https://doi.org/10.1080/07391102.2024.2317988.

Rehan, M.S., Ansari, F.A., Singh, O., 2020. Isolation, identification, antibacterial activity and docking of fatty acid and fatty alcohol from *Rumex dentatus* leaf Extract. Int. J. Pharmaceut. Sci. Rev. Res. 64, 7–11. https://doi.org/10.47583/ijpsrr.2020.v64i01.002.

Ribeiro, D.A., de Oliveira, L.G.S., de Macêdo, D.G., de Menezes, I.R.A., da Costa, J.G.M., da Silva, M.A.P., Lacerda, S.R., de, A., Souza, M.M., 2014. Promising medicinal plants for bioprospection in a Cerrado area of Chapada do Araripe, Northeastern Brazil. J. Ethnopharmacol. 155, 1522–1533. https://doi.org/10.1016/j.jep.2014.07.042.

Ribeiro, R.V., Bieski, I.G.C., Balogun, S.O., de, O., Martins, D.T., 2017. Ethnobotanical study of medicinal plants used by ribeirinhos in the north araguaia microregion, mato grosso, Brazil. J. Ethnopharmacol. 205, 69–102. https://doi.org/10.1016/j.jep.2017.04.023.

Riyadi, P.H., Susanto, E., Anggo, A.D., Arifin, M.H., Rizki, L., 2023. Effect of methanol solvent concentration on the extraction of bioactive compounds using ultrasonic-assisted extraction (UAE) from Spirulina platensis. Food Res. 7, 59–66. https://doi.org/10.26656/fr.2017.7(s3).9.

Rosandy, A.R., Ishak, S.S.O., Sabri, N.A., Ahmad, W.Y.W., Al Muqarrabun, L.M.R., 2021. Antibacterial activity of lupeol from the bark of *Dehaasia cuneate* (Lauraceae). Curr. Res. Biosci. Biotechnol. 2, 145–148. https://doi.org/10.5614/crbb.2021.2.2/bofy6724.

Sala-Carvalho, W.R., Montessi-Amaral, F.P., Esposito, M.P., Campestrini, R., Rossi, M., Peralta, D.F., Furlan, C.M., 2022. Metabolome of *Ceratodon purpureus* (Hedw.) Brid., a cosmopolitan moss: the influence of seasonality. Planta 255, 50–77. https://doi.org/10.1007/s00425-022-03857-8.

Santos, U.P. dos, Tolentino, G.S., Morais, J.S., Souza, K.P., Estevinho, L.M., Dos Santos, E. L., 2018. Physicochemical characterization, microbiological quality and safety, and pharmacological potential of *Hancornia speciosa* Gomes. Oxid. Med. Cell. Longev. 1–17. https://doi.org/10.1155/2018/2976985.

Santos, U.P., Campos, J.F., Torquato, H.F.V., Paredes-Gamero, E.J., Carollo, C.A., Estevinho, L.M., Souza, K.P., Santos, E.L., 2016. Antioxidant, antimicrobial and cytotoxic properties as well as the phenolic content of the extract from *Hancornia speciosa* Gomes. PLoS One 11, e0167531. https://doi.org/10.1371/journal.pone.0167531.

Sengupta, S., Nandi, I., Bhattacharyya, D.K., Ghosh, M., 2018. Anti-oxidant and Anti-bacterial properties of 1-octacosanol isolated from rice bran wax. J Plant Biochem. Physiol. 6, 206. https://doi.org/10.4172/2329-9029.1000206.

Shaik, A.B., Ahil, S.B., Govardhanam, R., Senthi, M., Khan, R., Sojitra, R., Kumar, S., Srinivas, A., 2016. Antifungal effect and protective role of ursolic acid and three phenolic derivatives in the management of sorghum grain mold under field conditions. Chem. Biodivers. 13, 1158–1164. https://doi.org/10.1002/cbdv.201500515.

Shehata, A.I., Rasheed, M., Rafiq, H., Khalid, N., Rafique, A., Alhoshy, M., Habib, Y.J., El Basuini, M.F., 2024. Multi-functional application of octacosanol as a feed additive in animal and aquaculture: a review. J. Anim. Physiol. Anim. Nutr. https://doi.org/10.1111/jpn.14002.

Silva, A.C., Jorge, N., 2020. Potential of mangaba (*Hancornia speciosa*), mango (*Mangifera indica* L.), and papaya (*Carica papaya* L.) seeds as sources of bioactive compounds. Rev. Ceres 67, 439–447. https://doi.org/10.1590/0034-737x202067060003.

Silva, F.E.F., das Chagas Lima Pinto, F., Loiola Pessoa, O.D., Marques da Fonseca, A., Martins da Costa, J.G., Pinheiro Santiago, G.M., 2023. Campesterol semi-synthetic derivatives as potential antibacterial: in vitro and *in silico* Evaluation. Chem. Biodivers. 20, e202300536 https://doi.org/10.1002/cbdv.202300536.

Silva, V.B., Almeida-Bezerra, J.W., Novais, M.H.G., Farias, N.S., Coelho, J.J., Ribeiro, P. R.V., Canuto, K.M., Coutinho, H.D.M., Morais-Braga, M.F.B., de Oliveira, A.F.M., 2024. Chemical composition, antifungal, and anti-virulence action of the stem bark of *Hancornia speciosa* Gomes (Apocynaceae) against *Candida* spp. J. Ethnopharmacol. 321, 117506 https://doi.org/10.1016/j.jep.2023.117506.

Singh, B., Singh, S., 2003. Antimicrobial activity of terpenoids from *Trichodesma amplexicaule* Roth. Phyther. Res. 17, 814–816. https://doi.org/10.1002/ptr.1202.
Singleton, V.L., Orthofer, R., Lamuela-Raventós, R., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 152–178. Academic Press.
Sirikhansaeng, P., Tanee, T., Sudmoon, R., Chaveerach, A., 2017. Major phytochemical as γ -sitosterol disclosing and toxicity testing in Lagerstroemia species. Evid. base Compl. Alternative Med. 2017, 7209851 https://doi.org/10.1155/2017/7209851.
Sun, W., Shahrajabian, M.H., 2023. Therapeutic potential of phenolic compounds in medicinal plants—natural health products for human health. Molecules 28, 1845. https://doi.org/10.3390/molecules28041845.
Sycz, Z., Wojnicz, D., Tichaczek-Goska, D., 2022. Does secondary plant metabolite ursolic acid exhibit antibacterial activity against Uropathogenic *Escherichia coli* living in single- and multispecies biofilms? Pharmaceutics 14, 1691. https://doi.org/10.3390/pharmaceutics14081691.
Tagliabue, A., Rappuoli, R., 2018. Changing priorities in vaccinology: antibiotic resistance moving to the top. Front. Immunol. 9, 1068. https://doi.org/10.3389/fimmu.2018.01068.
Tian, Q., Wei, S., Zheng, S., Xu, S., Liu, M., Bo, R., Li, J., 2022. Bactericidal activity of gallic acid against multi-drug resistance *Escherichia coli*. Microb. Pathog. 173, 105824 https://doi.org/10.1016/j.micpath.2022.105824.
Trotter, A.J., Aydin, A., Strinden, M.J., O'Grady, J., 2019. Recent and emerging technologies for the rapid diagnosis of infection and antimicrobial resistance. Curr. Opin. Microbiol. 51, 39–45. https://doi.org/10.1016/j.mib.2019.03.001.
Udaondo, Z., Matilla, M.A., 2020. Mining for novel antibiotics in the age of antimicrobial resistance. Microb. Biotechnol. 13, 1702–1704. https://doi.org/10.1111/1751-7915.13662.
Ueda, J.M., Milho, C., Heleno, S.A., Soria-Lopez, A., Carpena, M., Alves, M.J., Pires, T., Prieto, M.A., Simal-Gandara, J., Calhelha, R.C., Ferreira, I.C.F.R., Barros, L., 2023. Emerging strategies to combat methicillin-resistant *Staphylococcus aureus* (MRSA): natural agents with high potential. Curr. Pharmaceut. Des. 29, 837–851. https://doi.org/10.2174/1381612829666230410095155.
Ugur, B., Chen, K., Bellen, H.J., 2016. *Drosophila* tools and assays for the study of human diseases. Dis. Model. Mech. 9, 235–244. https://doi.org/10.1242/dmm.023762.
Valencia, D., Aguilar-González, D.I., Ortega-García, J., Godoy-Hernández, G., Leyva-Peralta, M.A., Moo-Huchín, V.M., Aarland, R.C., Quintero-Vargas, J., Mendoza-Espinoza, J.A., Zarza-García, A.L., 2023. Phytochemical Profile, Antioxidant and antiproliferative activity from leaves and seeds of *Bixa orellana* L. from the Yucatán peninsula, Mexico. Phcog. Mag. 19, 482–490. https://doi.org/10.1177/09731296231158492.
Vartak, S., Robbins, M.E.C., Spector, A.A., 2000. The selective cytotoxicity of γ-linolenic acid (GLA) is associated with increased oxidative stress. Adv. Exp. Med. Biol. 469, 493–498. https://doi.org/10.1007/978-1-4615-4793-8_72.
Verde, K., Eun, J., Kim, M.J., Kang, M.-J., Jang, A.-R., Kim, J.R., Kim, S., Chang, K.-T., Hong, J.J., Parque, J.H., 2017. Inhibitory effect of 1-tetradecanol on *Helicobacter pylori*-induced production of interleukin-8 and vascular endothelial growth factor in gastric epithelial cells. Mol. Med. Rep. 16, 9573–9578. https://doi.org/10.3892/mmr.2017.7793.
Verma, S., Bhardwaj, A., Vij, M., Bajpai, P., Goutam, N., Kumar, L., 2014. Oleic acid vesicles: a new approach for topical delivery of antifungal agent. Artif. Cells, Nanomed. Biotechnol. 42, 95–101. https://doi.org/10.3109/21691401.2013.794351.
Vieira, L.S., Sousa, R.S., Lemos, J.R., 2015. Plantas medicinais conhecidas por especialistas locais de uma comunidade rural maranhense. Rev. Bras. Plantas Med. 17, 1061–1068. https://doi.org/10.1590/1983-084x/15_009.
Walters, D., Raynor, L., Mitchell, A., Walker, R., Walker, K., 2004. Antifungal activities of four fatty acids against plant pathogenic fungi. Mycopathologia 157, 87–90.
Wang, F., Guo, Y., Cao, Y., Zhang, C., 2022. In vitro antibacterial activity of palmitoleic acid isolated from filamentous microalga *Tribonema minus* against fish pathogen *Streptococcus agalactiae*. J. Ocean Univ. China 21, 1615–1621. https://doi.org/10.1007/s11802-022-5047-6.
Wang, G., Gao, Y., Wang, H., Wang, J., Niu, X., 2018. Phloretin reduces cell injury and inflammation mediated by *Staphylococcus aureus* via targeting sortase B and the molecular mechanism. Appl. Microbiol. Biotechnol. 102, 10665–10674. https://doi.org/10.1007/s00253-018-9376-8.
Wang, L., Li, Q., Li, J., Jing, S., Jin, Y., Yang, L., Yu, H., Wang, D., Wang, T., Wang, L., 2021. Eriodictyol as a potential candidate inhibitor of Sortase A protects mice from methicillin-resistant *Staphylococcus aureus*-induced pneumonia. Front. Microbiol. 12 https://doi.org/10.3389/fmicb.2021.635710.
Watanabe, T., Yano, S., Kawai, T., Jinbo, Y., Nonomura, Y., 2021. Selective antibacterial activity of palmitoleic acid in emulsions and other formulations. J. Surfactants Deterg. 24, 973–979. https://doi.org/10.1002/jsde.12529.
WHO - World Health Organization, 2019. New Report Calls for Urgent Action to Avert Antimicrobial Resistance Crisis. https://www.who.int/news/item/29-04-2019-new-report-calls-for-urgent-action-to-avert-antimicrobial-resistance-crisis. (Accessed 3 May 2024).
Woisky, R.G., Salatino, A., 1998. Analysis of propolis: some parameters and procedures for chemical quality control. J. Apicult. Res. 37, 99–105. https://doi.org/10.1080/00218839.1998.11100961.
Wuillda, A.D.S.J., Martins, R.C.C., Costa, F.D.N., 2019. Larvicidal activity of secondary plant metabolites in aedes aegypti control: an overview of the previous 6 years. Nat. Prod. Commun. 14, 1934578X19862893 https://doi.org/10.1177/1934578X19862893.
Yamasaki, M., Chujo, H., Nou, S., Tachibana, H., Yamada, K., 2003. Alleviation of the cytotoxic activity induced by trans10, cis12-conjugated linoleic acid in rat hepatoma dRLh-84 cells by oleic or palmitoleic acid. Cancer Lett. 196, 187–196. https://doi.org/10.1016/S0304-3835(03)00215-5.
Yoon, B.K., Jackman, J.A., Valle-González, E.R., Cho, N.-J., 2018. Antibacterial free fatty acids and monoglycerides: biological activities, experimental testing, and therapeutic applications. Int. J. Mol. Sci. 19, 1114. https://doi.org/10.3390/ijms19041114.
Zarrinmehr, M.J., Daneshvar, E., Nigam, S., Gopinath, K.P., Biswas, J.K., Kwon, E.E., Wang, H., Farhadian, O., Bhatnagar, A., 2022. The effect of solvents polarity and extraction conditions on the microalgal lipids yield, fatty acids profile, and biodiesel properties. Bioresour. Technol. 344 https://doi.org/10.1016/j.biortech.2021.126303, 126303–126303.
Zavala-Sánchez, M.A., Rodríguez-Chávez, J.L., Figueroa-Brito, R., Quintana-López, C.M., Bah, M.M., Campos-Guillén, J., Bustos-Martínez, J.A., Zamora-Avella, D., Ramos-López, M.A., 2020. Bioactivity of 1-octacosanol from *Senna crotalarioides* (Fabaceae: Caesalpinioideae) to control *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Fla. Entomol. 102 https://doi.org/10.1653/024.102.0410, 731–731.
Zha, S., Ueno, M., Liang, Y., Okada, S., Oda, T., Ishibashi, F., 2021. Induction of apoptotic cell death in human leukemia u937 cells by c18 hydroxy unsaturated fatty acid isolated from red alga *Tricleocarpa jejuensis*. Mar. Drugs 19. https://doi.org/10.3390/md19030138, 138–138.