

# In vitro assessment of the synergism between extracts of *Zanthoxylum zanthoxyloides* and *Zanthoxylum leprieurii* and some standard antibiotics



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## Abstract

**Purpose:** To survive in harsh environments, plants developed functional and metabolic adaptive mechanisms. One of the most relevant defense strategies is the biosynthesis of secondary metabolites, including terpenoids, alkaloids, flavonoids, and phenolics that are accumulated in cellular organelles or secretory structures. Hence, plants are recognized as a valuable source of natural products and for thousands of years very diverse herbal formulations were created to treat several diseases. *Zanthoxylum zanthoxyloides* and *Zanthoxylum leprieurii*, two Rutaceae species native to Guinea-Bissau, are well known for their ethnopharmacological relevance.

**Methods:** In the present study, the *in vitro* antimicrobial activity of these plants against human pathogens was assessed and the phytochemical profile was screened. The extracts of roots and young leaves were obtained by sequential extraction of increasing polarity (*n*-hexane, CH<sub>2</sub>Cl<sub>2</sub>, EtOAc, MeOH and H<sub>2</sub>O) and tested against Gram-positive and Gram-negative bacteria. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined, as well as the evaluation of the synergic potential of the extracts.

**Results:** *Z. leprieurii* leaves extracts, the most apolar ones, had the highest antimicrobial activity, being able to inhibit the growth of *Enterococcus hirae* and all the *Staphylococcus* strains assayed, including the resistant ones. A synergic effect between the *Zanthoxylum* species extracts and standard antibiotics was found, reverting the activity of resistant strains. The phytochemical screening revealed the presence of terpenes, flavonoids, and phenolic compounds, known to have antibacterial properties.

**Conclusions:** The obtained results point to the validation of their use in tradition medicine and emphasize the worthwhile of additional studies of these species to better understand the compounds and mechanisms that may be valuable to restore antibacterial activity.

**Keywords:** *Zanthoxylum zanthoxyloides*; *Zanthoxylum leprieurii*; Antimicrobial activity; antibiotics; Synergic effect; Bioactivity

**Abbreviations:** MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration; LISC: Lisbon University Herbarium

## Introduction

The use of plants as a medicine has been reported since the beginning of civilization. Diverse plant parts and extracts led to innumerable formulations created to treat a wide variety of diseases [1-3]. Nowadays, plants are still part of traditional medicine but also play a greater role on the source of new compounds that can be active by themselves or be used as lead molecules to developed new drugs.

The discovery of penicillin in the early 1940s, led to the development of several classes of compounds with antibacterial activity which allowed the reduction of mortality and morbidity caused by infectious diseases. However, the irrational and uncontrolled use of antibiotics by health professionals, animal industry and agriculture resulted in the emergence of resistant microbial populations. As a result of this strong selective pressure

and increased volume of intercontinental travel, the development and transmission of multiresistant bacteria is turning into a serious public health problem [4,5].

According to [6], the mechanisms of antibiotic resistance can be summarized in four major classes: I. Modifications of the antibiotic molecule; II. Decreased antibiotic penetration and efflux; III. Changes in target sites; IV. Resistance due to global cell adaptations. The combined effects of these mechanisms associated with rapid growth rates and the ability to exchange genes led to the development of methicillin resistant (MRSA), Vancomycin Intermediate *Staphylococcus aureus* (VISA) and Vancomycin Resistant Enterococci (VRE). For instance, in 15 European countries more than 10% *Staphylococcus aureus* infections are caused by methicillin-resistant strains (MRSA), with several countries, namely Portugal presenting resistance rates closer to 50%. Several highly resistant gram-negative pathogens like *Acinetobacter* species, multidrug-resistant, *Pseudomonas aeruginosa*, carbapenem-resistant *Klebsiella* species and *Escherichia coli* are also emerging as significant pathogens, transforming antibiotic resistance in one of the greatest public health threats of the 21st century [7].

The occurrence of various infectious diseases and the increasing prevalence of antibiotic-resistant pathogens have become a serious threat to human health, and it is necessary to find new antimicrobial agents capable of reversing antibiotic resistance. Pharmaceutical companies, in the past few decades, have shifted their development efforts to chronic diseases and antiviral compounds instead of the development of new antibiotics molecules [8]. Several previous studies proved that there is a huge potential of plant-derived compounds as antibacterial and as a resistance-modifying of other antibiotics through synergistic behavior [9]. Therefore, *in vitro* antibacterial assessment of plants extracts, their phytochemical profile screening and the evaluation of the synergic affect when combined with antibiotics reversing the bacteria resistance may lead to a new approach on anti-infective therapy [10].

The selected species for the present study are autochthonous plants from Guinea-Bissau, a small African Portuguese speaking country, where there is a wide range of plants used in herbal medicine to treat endemic diseases as result of fragile health services, easily found in local markets and pharmacies [11]. This country is estimated to have a vascular flora with around 1507 species, 1495 of which are native. Since 1997, to protect biodiversity, a network of protected areas was established in Guinea-Bissau, a collaboration between National Institute for Biodiversity and Protected Areas and International Union for the Conservation of Nature [12]. Traditionally used and present in Guinea-Bissau flora, there are two species of *Zanthoxylum* with medical properties. *Zanthoxylum zanthoxyloides* is used to treat malaria, sick cell anemia, tuberculosis, ulcers, hemorrhoids, injuries, and syphilitic wounds as well as arthritic pain [13]. *Zanthoxylum leprieurii* is traditionally used in the treatment of HIV,

malaria, urinary infections, rheumatic pain and used as antiseptic [14]. The present work aims to evaluate the *in vitro* antimicrobial activity of the two *Zanthoxylum* species, *Z. zanthoxyloides* and *Z. leprieurii*, against a selected panel of microorganisms, to better understand their application in traditional medicine. The potential synergistic effect of plant extracts with standard antibiotics was tested as well as screening of the phytochemical profile.

## Materials and Methods

### Plant materials

Samples of *Z. zanthoxyloides* and *Z. leprieurii* roots and young stems were collected during 2016-2017, in Orango island, Bagos archipelago, Guinea-Bissau and dried at room temperature. Voucher specimens were deposited and identified at the Lisbon University Herbarium (LISC).

### Plant extracts

The plant material was powdered, obtaining 6g of leaves and 30g of roots of *Z. zanthoxyloides* and 13g of leaves and 27g of roots of *Z. leprieurii*. Each powdered material was subjected to extractions with five ascending polarity solvents: *n*-hexane (*n*-hex), dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>), ethyl acetate (AcOEt), methanol (MeOH) and water (H<sub>2</sub>O). Ensuing the usual procedures [15] each solvent extraction took about 24h, at room temperature, with occasional shaking followed by decantation, filtration, drying and storage at -20°C, until use.

### Bacterial strains

To assess the *in vitro* antimicrobial activity of each plant, extract the model proposed by [16] was considered. The microorganisms include a range of Gram-positive and Gram-negative bacteria, all known to be the cause of several human diseases Table 1.

### Antibiotics

The standard antibiotics amoxicillin and oxacillin, purchased from Sigma (Madrid, Spain).

### Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) values were determined by the microplate broth microdilution method according to CLSI (2019). Briefly, on each well of the microplate, 100 µL of the medium plus 100µL of each extract solution to be tested were added, obtaining concentrations ranging between 500-7.5µg/mL. An inoculum of each microorganism was also added (10µL; final concentration 10<sup>4</sup>cfu/mL). Appropriated antibiotics were used as reference for antibacterial activities. After incubation at 37°C for 24 h, the optical density at 630nm was measured in a Biotek ELX 808 plate spectrophotometer to assess the bacterial growth and confirmed by macroscopic evaluation. The samples with MIC value ≤ 100µg/mL were determined to have antibacterial activity. All assays were performed in triplicate.

### Minimum bactericidal concentration (MBC)

The minimum bactericidal concentration (MBC) was evaluated following the CLSI method [17] with some modifications. 10µL of suspension from each well showing no visible growth was spread

out on a Muller-Hinton agar plate. Colony growth was observed after 24h incubation at 37°C. MBC corresponds to the lowest concentration of extract that reduces almost at 100% the viability of the bacteria.

**Table 1:** Selected panel of gram-positive and Gram-Negative Bacteria.

Microorganism	Strain
<b>Gram-positive bacteria</b>	
<i>Bacillus subtilis</i>	ATCC1 6633
<i>Enterococcus hirae</i>	CIP2 5855
<i>Enterococcus faecalis</i>	ATCC 51299
<i>Staphylococcus aureus</i>	ATCC 6538 – MSSA3
	ATCC 43866 – MRSA4
	CIP 106760 – VISA5
<i>Staphylococcus epidermidis</i>	ATCC 12228
<b>Gram-negative bacteria</b>	
<i>Escherichia coli</i>	ATCC 8739
<i>Klebsiella pneumoniae</i>	ATCC 9997
<i>Pseudomonas aeruginosa</i>	ATCC 9027

1ATCC: American Type Culture Collection, Maryland, USA; 2CIP – l'Institut Pasteur Collection, Paris, France; 3MSSA: Methicillin-Sensitive *Staphylococcus aureus*; 4MRSA: Methicillin-Resistant *S. aureus*; 5VISA: Vancomycin-Intermediate *S. aureus*.

### Synergic effect of extracts with standard antibiotics

In order to determine the type of interaction between the extracts and the standard antibiotics (amoxicillin and oxacillin), a checkerboard assay was performed against the MRSA strain ATCC 43866 and VISA strain CIP 106760 according to [18]. Two-fold serial dilutions of antibiotic was prepared on the horizontal rows of microtiter plate and then cross- diluted vertically by two-fold serial dilutions of the extracts. The concentration of each antibiotic ranged from 1 to 1/2048 of the MIC and the concentration of the compounds from 1/2 to 1/64 of the MIC.

The synergic effect was determined based on the fractional inhibitory concentration index (FICI), calculated according to the formula

$$FICI = FIC(A) + FIC(B)$$

where, FIC(A)= MIC (A in the presence of B)/MIC (A alone) and FIC(B)= MIC (B in the presence of A)/MIC (B alone) [18]. When FICI ≤ 0.5 a synergic effect is considered. FICI ranging 0.5-4 are classified as indifference and FICI values > 4 an antagonistic effect is pondered [18].

### Phytochemical screening

A semi-quantitative phytochemical analysis to detect the major chemical groups found in each extract was carried out through thin layer chromatography (TLC) on silica gel plates [19]. Proper mixtures of eluents were used to develop and the spots were revealed with appropriated spray-reagents made according to [20]. being: anisaldehyde-sulfuric acid reagent for terpenoids,

Dragendorff reagent for alkaloids, natural products–polyethylene glycol (NEU) reagent for flavonoids and Fast Blue salt reagent for phenolic compounds. Results were displayed between absent (-) and a strong intensity (+++).

## Results

### Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

A total of 20 extracts of two *Zanthoxylum* species were prepared where the bactericidal and bacteriostatic activity (MBC and MIC) were evaluated against an enlarged panel of Gram positive and Gram negative sensitive and resistant bacteria strains. The results are presented in Table 2.

The MSSA growth was inhibited by *Z. zanthoxyloides* *n*-hex root extract (MIC 60µg/mL) and by both *Z. leprieurii* leaves and roots AcOEt extracts (MIC 60µg/mL). *Z. zanthoxyloides* AcOEt leaves extract and *Z. leprieurii* *n*-hex and CH<sub>2</sub>Cl<sub>2</sub> leaves extracts inhibit the development of the MRSA strains (MIC 60 µg/mL). The VISA strain was inhibited by *Z. leprieurii* CH<sub>2</sub>Cl<sub>2</sub> leaves extract (MIC 60µg/mL). *Z. leprieurii* CH<sub>2</sub>Cl<sub>2</sub> and AcOEt leaves extracts also presented activity against *S. epidermidis* (MIC respectively 15 and 60µg/mL). *Z. zanthoxyloides* leaves *n*- hex extract, *Z. leprieurii* leaves CH<sub>2</sub>Cl<sub>2</sub> and AcOEt extracts and *Z. leprieurii* roots AcOEt extract exhibited inhibitory activity against *E. hirae* (MIC 60µg/mL). The most active plant part was the *Z. leprieurii* leaves and the extract presenting major activity was the CH<sub>2</sub>Cl<sub>2</sub> extract which displayed activity against MRSA, VISA, *S. epidermidis* and *E. hirae* strains.

**Table 2:** Antibacterial activity (MIC and MBC, µg/mL) of *Z. zanthoxyloides* and *Z. lepreurii* extracts.

Plant Extracts	<i>S. Aureus</i>						<i>S. Epidermidis</i>		<i>E. Faecalis</i>		<i>E.Hirae</i>	
	MSSA		MRSA		VISA				VRE			
	ATCC 6538		ATCC 43866		CIP 106760		ATCC 12228		ATCC 51299		CIP 5855	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
<i>Z. zanthoxyloides</i> (leaves) <i>n-hex</i> <i>CH<sub>2</sub>Cl<sub>2</sub></i> <i>AcOEt</i> <i>MeOH</i> <i>H<sub>2</sub>O</i>	250	-	125	-	500	-	500	-	500	-	<b>60</b>	60
	500	-	500	-	500	-	500	-	500	-	500	-
	125	-	<b>60</b>	nd	250	-	250	-	500	-	125	-
	250	-	500	-	500	-	500	-	500	-	500	-
	250	-	500	-	500	-	500	-	500	-	500	-
<i>Z. zanthoxyloides</i> (leaves) <i>n-hex</i> <i>CH<sub>2</sub>Cl<sub>2</sub></i> <i>AcOEt</i> <i>MeOH</i> <i>H<sub>2</sub>O</i>	<b>60</b>	125	500	-	500	-	500	-	500	-	125	-
	250	-	500	-	500	-	500	-	500	-	125	-
	500	-	500	-	500	-	500	-	500	-	250	-
	500	-	500	-	500	-	125	-	500	-	250	-
	125	-	500	-	500	-	500	-	500	-	250	-
<i>Z. lepreurii</i> (leaves) <i>n-hex</i> <i>CH<sub>2</sub>Cl<sub>2</sub></i> <i>AcOEt</i> <i>MeOH</i> <i>H<sub>2</sub>O</i>	125	-	<b>60</b>	60	125	-	500	-	500	-	250	-
	125	-	<b>60</b>	nd	<b>60</b>	125	<b>15</b>	125	250	-	<b>60</b>	125
	<b>60</b>	250	125	-	125	-	<b>60</b>	125	500	-	<b>60</b>	125
	500	-	250	-	500	-	500	-	500	-	125	-
	250	-	250	-	500	-	500	-	500	-	125	-
<i>Z. lepreurii</i> (leaves) <i>n-hex</i> <i>CH<sub>2</sub>Cl<sub>2</sub></i> <i>AcOEt</i> <i>MeOH</i> <i>H<sub>2</sub>O</i>	125	-	250	-	500	-	250	-	500	-	250	-
	125	-	500	-	500	-	250	-	250	-	125	-
	<b>60</b>	125	250	-	250	-	250	-	250	-	<b>60</b>	125
	125	-	250	-	500	-	250	-	500	-	250	-
	125	-	500	-	500	-	500	-	500	-	500	-

### Synergic effect of extracts with standard antibiotics

In order to evaluate the interactions between the extracts and two reference antibiotics used to treat *S. aureus* infections (amoxicillin and oxacillin), the checkerboard assay was performed with two *S. aureus* strains one methicillin-resistant (MRSA, ATCC 43866) and vancomycin-resistant (VISA, CIP 106760). The results

for extracts with fractional inhibitory concentration index (FICI) 1 or lower are displayed in Table 4 and Table 5. FICI values under 0.5 are considered to show a synergic interaction between the antibiotic and the plant extracts [18].

### Phytochemical screening

Results are displayed in Table 3.

**Table 3:** Phytochemical screening of *Zanthoxylum zanthoxyloides* and *Zanthoxylum leprieurii*; - absent; + slight intensity; ++ moderate intensity; and +++ strong intensity.

Species	Part	Extract	Alkaloids	Terpenes	Flavonoids	Phenolics
<i>Zanthoxylum zanthoxyloides</i>	leaves	<i>n</i> -hex CH <sub>2</sub> Cl <sub>2</sub> AcOEt MeOH H <sub>2</sub> O	-	+++	++	-
			-	++	+++	-
			-	++	+++	-
			-	-	+	++
			-	-	+	++
	roots	<i>n</i> -hex CH <sub>2</sub> Cl <sub>2</sub> AcOEt MeOH H <sub>2</sub> O	+	++	-	-
			+	++	-	+
			+	++	-	+
			+	+	+	+++
			+	-	+	+++
<i>Zanthoxylum leprieurii</i>	leaves	<i>n</i> -hex CH <sub>2</sub> Cl <sub>2</sub> AcOEt MeOH H <sub>2</sub> O	-	+++	++	-
			-	++	++	-
			-	++	+++	++
			-	-	+++	++
			-	-	+	+
	roots	<i>n</i> -hex CH <sub>2</sub> Cl <sub>2</sub> AcOEt MeOH H <sub>2</sub> O	+	++	+++	-
			+	++	+++	+
			++	++	+++	+
			++	-	++	++
			+	-	+	+

**Table 4:** Minimum inhibitory concentration (MIC) and fractional inhibitory concentration index (FICI) values of amoxicillin combination with extracts for vancomycin-resistant (CIP 106760) and methicillin-resistant (ATCC43866) *Staphylococcus aureus* strains.

<i>S. Aureus</i> Strains	Plant Extract	MIC (µg/mL)		FIC	FICI	Output
		Alone	Combination			
CIP 106760	<i>Z. zanthoxyloides</i> (roots) MeOH amoxicillin	500	60	0,12	0,18	Synergism
		60	4	0,06		
	<i>Z. leprieurii</i> (roots) AcOEt amoxicillin	250	125	0,50	0,75	Indifference
		60	15	0,25		
ATCC 43866	<i>Z. zanthoxyloides</i> (roots) H <sub>2</sub> O amoxicillin	500	250	0,50	1,00	Indifference
		60	30	0,50		
	<i>Z. leprieurii</i> (leaves) CH <sub>2</sub> Cl <sub>2</sub> amoxicillin	60	15	0,25	0,46	Synergism
		60	4	0,06		
	<i>Z. leprieurii</i> (roots) MeOH amoxicillin	250	60	0,25	0,50	Synergism
		60	30	0,25		
	<i>Z. leprieurii</i> (roots) H <sub>2</sub> O amoxicillin	500	250	0,50	1,00	Indifference
		60	30			



**Table 5:** Minimum inhibitory concentration (MIC) and fractional inhibitory concentration index (FICI) values of oxacillin combination with extracts for vancomycin-resistant (CIP 106760) and Methicillin-Resistant (ATCC 43866) *Staphylococcus aureus* strains.

S. Aureus Strains	Plant Extract	MIC ( $\mu\text{g/mL}$ )		FIC	FICI	Output
		Alone	Combination			
CIP 106760	<i>Z. zanthoxyloides</i> (roots) MeOH oxacillin <i>Z. leprieurii</i> (leaves) AcOEt oxacillin	500	125	0,25	0,31	Synergism
		125	7,5	0,06		
		125	30	0,25	0,31	Synergism
		125	7,5	0,06		
ATCC 43866	<i>Z. zanthoxyloides</i> (roots) H <sub>2</sub> O oxacillin <i>Z. leprieurii</i> (leaves) CH <sub>2</sub> Cl <sub>2</sub> oxacillin <i>Z. leprieurii</i> (leaves) AcOEt oxacillin <i>Z. leprieurii</i> (roots) MeOH oxacillin	500	125	0,25	0,37	Synergism
		125	15	0,12		
		60	30	0,50	1,00	Indifference
		125	60	0,50		
		125	60	0,50	0,75	Indifference
		125		0,25		
		250	60	0,24	0,31	Synergism
125	7,5	0,06				

MSSA: Methicillin-Sensitive *Staphylococcus aureus*; MRSA: Methicillin-Resistant *Staphylococcus aureus*; VISA: Vancomycin-Intermediate *Staphylococcus aureus*; VRE: Vancomycin-Resistant *Enterococcus*; Nd: Non-Defined; (-) not tested.

## Discussion

Analyzing the results displayed in (Table 2), the bioactive extracts are the non-polar ones, mainly CH<sub>2</sub>Cl<sub>2</sub> and AcOEt extracts which are rich in terpenes and flavonoids (Table 3). Terpenoids have antibacterial properties in nature, acting by an unclear mechanism. They seem to disrupt the membrane of bacteria cell [8]. Flavonoid's bioactivity is markedly related with the antioxidant action. Nevertheless, several properties have been reported as anti-inflammatory, antimicrobial, antiviral, antiallergic and antitumor [21-23].

No antibacterial activity was found for Gram-negative bacteria and *B. subtilis*. The presence of a highly hydrophobic outer membrane that works as a permeability barrier in this group, can explain those results [24]. Comparing the MIC / MBC values (Table 2), it is possible to deduce that the extracts that presented antibacterial activity will act as bacteriostatic agents [25]. MIC is considered the standard parameter to determine the susceptibility of bacteria to an external agent like a compound or extract and corresponds to the lowest concentration of compound/extract that inhibits the bacterial grow. MBC is considered a relevant index of the bactericidal activity of antimicrobial agents. MBC corresponds to the lowest concentration of extract that reduces almost at 100% the viability of the bacteria. If the MIC and MBC are in the same range, the extract will probably kill the bacteria.

The antimicrobial activity of *Z. zanthoxyloides* polar extracts (ethanol and water extracts) against resistant isolates of *S. aureus*

was previously reported by [16]. Similarly, the antibacterial activity of *Z. leprieurii* and *Z. zanthoxyloides* essential oils against a MSSA strain was reported by [26,27] published the results of the antibacterial activity of steam bark MeOH/H<sub>2</sub>O extract of *Z. leprieurii* against a MSSA strain. Those results sustained the ethnopharmacological properties described for these *Zanthoxylum* species, once they are mostly used for intestinal disorders and wound care.

*Staphylococcus aureus* strains are one of the major pathogens worldwide related with a large range of clinic manifestations with different levels of severity, triggering lethal infections, by synthesizing a large variety of toxins and enzymes. *S. aureus* strains developed an increasing antibiotic resistance such as a methicillin resistance (MRSA) and a vancomycin-intermediate resistance (VISA) [28]. The emergence of multidrug resistant bacteria strains and the increase of the pathogenicity of the strains are intimately related [29]. One approach to overcoming bacterial resistance mechanisms and restoring antibiotic efficacy is the use of inactive plant extracts when administered individually, in combination with antibiotics. This combinatory strategy may play an important role on the management of challenging infectious diseases [18].

In order to establish the potential interaction between the plants extracts and references antibiotics the checkerboard assay was performed as previously described. The extract that stood out was the *Z. zanthoxyloides* roots MeOH extract, which by itself

presents no relevant antibacterial activity against resistant VISA strain (MIC= 500µg/mL) but was able to restore synergistically the antibacterial activity of the two antibiotics tested, amoxicillin from 60 to 4 µg/mL, (FICI = 0.18) and oxacillin from 125 to 7.5µg/mL (FICI = 0.31), corresponding in both cases to a 16-fold reduction. *Z. lepreurii* leaves AcOEt was also able to interact synergistically with oxacillin against the VISA strain, lowering the antibiotic MIC from 125 mg/mL to 7.5µg/mL (FICI = 0.31), corresponding to a 16-fold reduction.

For the MRSA strain (ATCC 43866) *Z. lepreurii* CH<sub>2</sub>Cl<sub>2</sub> extract was able to revert synergistically the antibacterial activity of amoxicillin decreasing the MIC value from 60 to 4µg/mL (FICI= 0.46) corresponding to a 16-fold reduction. *Z. lepreurii* roots MeOH extract also displayed some synergistic interaction with amoxicillin against this strain, lowering the antibiotic MIC from 60 to 30µg/mL (FICI =0.5). The water extract of *Z. zanthoxyloides* roots when combined with oxacillin, decreased the antibiotic MIC value from 125µg/mL to 15µg/mL against MRSA strain (FICI=0.37).

## Conclusion

Due to the growing problematic of drug-resistant bacteria it is urging to discover new compounds and new pathways to control and revert antibiotic resistance, to regulate the increasing bacterial derived diseases worldwide. The use of natural products as plant extracts reveal to have a multi target mechanism of action leading to a reduced prevalence of bacterial resistance. Therefore, the study of plant compounds and their synergistic interaction is a fundamental step in overcoming community health problems arising from current bacterial infections.

Some of the studied extracts were active against Gram positive strains but the results that stood out were the synergistic activity that some of the extracts presented, being able to restore the activity of the tested antibiotics. Overall, these results emphasize the worthwhile of additional studies of these species to better understand their efficacy and safety in traditional medicine and the mechanisms behind the restore of the antibacterial activity.

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