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Ximenia americana L. enhances the antibiotic activity and inhibit the development of kinetoplastid parasites

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ABSTRACT

Objective: The objective of this work was evaluate the cytotoxic, leishmanicidal and tripanocidal activity, as well as to evaluate its antimicrobial and modulatory activity in association with different antibiotics of the hydroethanolic extract of the *Ximenia Americana* stem bark (EHXA).
Method: *In vitro* tests against *Trypanosoma cruzi*, *Leishmania* sp. and citotoxicity were performed. The evaluation of the antibacterial and bacterial resistance modulatory effect was given by the microdilution method.
Results: The chemical profile show different classes of compounds with significant presence of quercetrin and caffeic acid. The EHXA demonstrated activity only in the concentration of 1000 μg/mL against the *L. infantum* and *L. brasiliensis* promastigotes, causing mortality percentage of 40.66 and 27.62%, respectively. The extract presented a significant toxicity only in the concentration of 1000 μg/mL, causing a mortality of 55.42% of fibroblasts. The antibacterial activity of the EHXA demonstrated a MIC value ≥1024 μg/mL against all the tested bacteria. However, in the modulation assay with EHXA in association with different antibiotics the extract had a synergistic effect against *S. aureus* strains when associated with norfloxacin.
Conclusion: The results of this investigation demonstrate for the first time the chemical composition of the hydroethanolic extract of the *Ximenia Americana* stem bark, your potential antiparasitic and modulatory effect. The low cytotoxic and biological potential against *S. aureus* open therapeutic perspectives against leishmaniosis and bacterial infections.

1. Introduction

"Neglected diseases" is a term used to refer to a group of diseases affecting mainly poor and developing countries, favoring the maintenance of inequality and social exclusion. For the public health, this group of diseases is a big problem, as they can cause physical and cognitive deficits in the individual; as well as impacting the epidemiological profile. In Brazil, we can cite as examples of neglected diseases, in special, leishmaniosis and Chagas' disease [1].

Leishmaniosis is an infectious disease, which is considered zoonotic, caused by protozoan of the genus *Leishmania* and is transmitted to the definitive host by the bite of an infected female sand-fly; it possesses a wide distribution around the world, from Asia to America. According to Palatnik-de-Sousa and Day (2011), leishmaniosis is an endemic disease in 88 countries around the world, of which 60 per cent of disease foci are in well-defined areas of Bangladesh, India and Nepal, with an annual register of 1 million to 1.5 million cases [2]. From 1980 to 2005, Brazil recorded 59,129 cases of visceral leishmaniosis, 82.5%, it has a

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high detection coefficient and is capable of producing deformities [3].

Chagas' disease, caused by flagellated *Trypanosoma cruzi* protozoans, is an endemic disease in 21 countries of the Americas which can be transmitted to humans through the feces of infected triatomine insects, contaminated foods, blood transfusions and organ transplantations through donors infected with the parasite and through trans placental infections. It is estimated that around 8 million people are chronically infected with *T. cruzi*, however, in Brazil the prevalence ranged from 4.4% (95% CI: 2.3–8.3) with highest estimated regional prevalence North and Northeast regions [4].

Another public health problem is pathogenic bacterial resistance to antimicrobials due to the reduction of drugs available for the treatment of diseases caused by such resistant microorganisms this is considered a serious problem [5]. Those which present resistance to one or more classes of antibiotics are considered resistant bacteria [6]. Within the classes of microorganisms which cause the most worry to the public health; Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* are the bacteria which provoke the most deaths in the world and which are resistant to multiple drugs [7]. In this context, research with the brute extracts of medicinal plants have stood out in the search for new substances with therapeutic potential against diseases caused by microorganisms or treatment of leishmaniosis and Chagas' disease [8,9].

Ximenia americana L., a vegetable species belonging to the family Olacaceae and of the genus *Ximenia*, is popularly known by the names of plum, brave-plum, bay-plum or plum-of-thorn and can be easily found in South America and Africa. Several studies have demonstrated that this species have anticancer, antimicrobial activity, anti-inflammatory and anti-neoplssastic properties, analgesic activity, antipyretic activity and anti-trypanosomal activity [10–16].

In Brazil, this species occurs in the Caatinga and is popularly used and commercialized for combating pain, obesity, inflammation, injury healing, diabetes, cough, hoarseness, constipation, venereal diseases, osteoporosis, treatments for inflamed skin, gynecological inflammation, internal organ inflammation, throat, gall bladder, wounds, prostate problems, back pain, kidney pains, injury and fatigue [17–20]

Given the above and given the vast pharmacological potential of the species *X. americana*, this work reports the chemical profile and for the first time the leishmanicidal and tripanocidal activity from the stem bark of the *X. americana* hydroethanolic extract (EHXA), as well as to evaluate its cytotoxic, antimicrobial and bacterial resistance modulatory activity in association with different antibiotics.

2. Materials and methods

2.1. Botanic material

The vegetable material (*X. americana* stem bark) was collected from the Lambedor Farm (coordinate 06° 57′ 26.3″ S and 39° 32′ 17.4″ W), in the Farias Brito city, Ceará state, Brazil. The botanical identification was performed by Prof. Dr. Maria Arlene Pessoa da Silva and a sample was deposited in Herbarium Caririense Dárdano de Andrade-Lima localized in Regional University of Cariri, City of Crato, under the number 10.976.

2.2. Preparation of the extract

X. americana stem bark (2590 g) were previously cleaned, crushed and subjected to maceration for 72 h using a binary mixture of water/ethanol (1:1; v/v) according to methodology adopted by Matos (2007). Following this period, the extracted solution was filtered, and the organic solvent was distilled in a rotary evaporator and lyophilizate. The resulting brown colored solid material was conditioned in an amber glass and stored in the freezer for further analysis [21].

2.3. Chemical analysis of the extract

2.3.1. Chemical prospecting

The qualitative chemical prospecting of the extract and its fractions was performed by determination of secondary metabolites present in the EHXA, following the methodology by Matos (2007) [21], with the existence of the principle classes evaluated, such as: Phenols, Tannins, Anthocyanins, Anthocyanidins, Flavonoids, Leucoanthocyanidins, Catechins, Flavones, Alkaloids, Steroids, Triterpenoids and Saponins. This methodology addresses visual observations of colors and formations of precipitates after the exposure to specific chemical reagents.

2.3.2. Quantification of phenolic compounds by HPLC-DAD

Chemical products acquired from Merck (Darmstadt, Germany) such asacetonitrile, acetic acid, gallic acid, chlorogenic acid, caffeic acid and ellagic acid, of analytic grade were used. The catechin, quercetin, quercitrin, rutin and kaempferol were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

The technical use of the High Performance Liquid Chromatography (HPLC) occurred through the use of chromatograph (Shimadzu, Kyoto, Japan), Auto Sampler (SIL-20 A), equipped with a Shimadzu LC-20AT with alternating pumps connected to the degasser DGU 20A5 with a CBM 20ª integrator, UV-VIS detector with DAD (diode) SPD-M20 A and the Software LC solution 1.22 SP1. We specifically used phosphoric acid, acetonitrile and bi-distilled water, purified by the Milli-Q system, for analyses.

Chromatography analysis of the *X. americana* extract was performed using the reverse phase column Phenomenex C18 (4.6 mm x 250 mm) with particle size of 5 µm in diameter. The mobile phase was water with 2% phosphoric acid (v/v) (solvent A) and acetonitrile (solvent B) at a flow rate of 0.7 mL/min and an injection volume of 50 µL. The gradient composition was: 2% of solvent B, reaching 15% at 10 min; 30% of solvent B in 25 min, 65% of solvent B in 40 min and 98% of solvent B in 45 min, followed by 50 min of isocratic elution until 55 min. After 60 min of the gradient reaching the initial concentrations again, the method described by Boligon et al (2012) [22] was followed, with light variations. The sample and mobile phase were filtered through a membrane filter of 0.45 µm (Millipore), and following this, degasified by ultrasound bath. The standard reference stock solutions were prepared in the mobile phase of the method in a range of concentrations from 0.025 – 0.300. Quantifications were performed through integration of the peaks using the standard external method, at 270 nm for gallic and ellagic acid; 280 nm for catechin, 326 nm for chlorogenic and caffeic acid; and 366 nm for quercetin, quercitrin, rutin and kaempferol.

The chromatographic peaks were confirmed by comparison of the retention time with the standard references and by DAD spectra (200 to 500 nm). All chromatographic operations were performed at room temperature and in triplicates. The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the responses and gradient by means of three curves from independent analyses, as defined by Colpo et al. [23]. LOD and LOQ were calculated as 3.3 and 10 σ / S, respectively, where σ is the standard deviation of the response and S is the slope of the calibration curve.

2.4. Evaluation of leishmanicidal, tripanocidal and cytotoxic activity

2.4.1. Reagents

Sodium Resazurin was obtained from Sigma–Aldrich (St Louis, MO) and stocked at 4 °C under light, and prepared with a 1% phosphate buffer, pH 7 and sterilized by filtration before utilization. Chlorophenol red-β-D-galactopyranoside (CPRG; Roche, Indianapolis, IN) was dissolved in a Triton X-100 0.9% (pH 7,4) solution. Penicillin G (Ern, S.A., Barcelona, Spain), streptomycin (Reig Jofré S.A., Barcelona, Spain) and Dimethyl sulfoxide (DMSO) were also used.

2.4.2. Cellular strains used

For the *T. cruzi in vitro* tests, the CL-B5 clone was used. The stable form transfected parasites with the gene for the *Escherichia coli* (*lacZ*) β-galactosidase were supplied by Dr. F. Buckner, through the Commemorative Gorgas Institute (Panama). The epimastigotes were cultured at 28 °C in *Liver Infusion Tryptose Broth* (Difco, Detroit, MI) culture medium, supplied with 10% Fetal Bovine Serum (FBS) (Gibco, Carlsbad, CA), penicillin (Ern, S.A., Barcelona, Spain) and streptomycin (Reig Jofré S.A., Barcelona, Spain), as described by Le Senne et al [24]. The cells were collected for the tests in the exponential phase of its growth.

Leishmania spp. cultures were obtained from the Research Institute for Health Sciences, Asuncion, Paraguay – IICS and identified by isoenzymatic analysis. Maintenance of the strains, cultivation and isolation methods of the *Leishmania* spp. promastigotes and cytotoxic assays used the fibroblast NCTC929 were followed the procedures described by Roldos et al. [25]. The inhibition assays of the promastigotes were performed using the *L. brasiliensis* (MHOM/CO/88/UA301) and *L. infantum* (MHOM/ES/92/BCN83) strains, cultured at 22 °C in Schneider's Drosophila media supplemented with 20% FBS.

The cytotoxic assays used the fibroblast NCTC929 strain, cultured in Minimal Essential Medium (Sigma). The culture medium was supplied with heat inactivated 10% FBS, penicillin G (100 U/mL) and streptomycin (100 mg/mL). The cultures were maintained at 37 °C in humid atmosphere with 5% CO_2. The viability of the strains was evaluated through the use of Resazurin with a colorimetric method.

2.4.3. Antiepimastigote activity test

The test was performed in a 96-well microplate, with cultures in the exponential phase. The epimastigotes were inoculated in a concentration of 1×10^5 mL^{-1} in 200 µL of liver tryptose broth. The plates were then incubated with the drugs in the concentrations of 100 and 500 µg/mL at 28 °C for 72 h. After this time, 50 µL of CGRP were added to the solution reaching a concentration of 200 µM. The plates were incubated for an additional time of 6 h at 37 °C and were subjected to visualization under 595 nm. Each experiment was performed twice in an independent manner, with each tested concentration in triplicates for each experiment. The efficiency of each compound was estimated through calculation of the percentage of antiepimastigote activity (% EA).

2.4.4. Antipromastigote activity test

L. brasiliensis and *L. infantum* promastigote cultures were cultivated at a concentration of 10^6 cell/mL and then transferred for testing. The compounds were dissolved in DMSO until the concentrations to be tested were reached and were then transferred to the microplates. Each assay was performed in triplicates. The activity of the compounds was evaluated 72 h later by direct counting of the cells after serial dilutions and compared to an untreated control.

2.4.5. Cytotoxicity test

NCTC929 fibroblasts were plated in microdilution plates with 96 wells at a final concentration of 3×10^4 cells/well. The cells were cultivated at 37 °C in 5% CO_2 atmosphere. Following this, the culture medium was removed and the compounds were added at 200 µL, with a new culture being performed for 24 h. Following this incubation, 20 µL of 2 mM Resazurin solution was added to each well. The plates were incubated for 3 h and the reduction of Resazurin was determined through a double absorbance in the wavelengths of 490 and 595 nm. The control (white) value was subtracted.

2.5. Antibacterial and modulatory activity evaluation

2.5.1. Drugs

The *Brain Heart Infusion - BHI* culture medium was provided by Difco Laboratories Ltda. The antibiotics used were: oxacillin, imipenem, gentamicin and norfloxacin (SIGMA Chemicals, St Louis, EUA) prepared in accordance with the manufacturer's instructions.

2.5.2. Bacterial material

The bacterial strains used were obtained through the Microbiology and Molecular Biology Laboratory (LMBM) of the Regional University of Cariri (URCA). The standard bacterial strains used were *Escherichia coli* ATCC 25922; *Staphylococcus aureus* ATCC 25923; *Pseudomonas aeruginosa* ATCC 9027 and the multiresistant strains were the *Escherichia coli* 06, *Staphylococcus aureus* 10 and *Pseudomonas aeruginosa* 24. Before the assays, the strains were cultivated at 35 °C for 24 h in *Brain Heart Infusion Broth* – BHI (Difco Laboratories Ltd.).

2.5.3. Antimicrobial and modulatory activity test

The Minimum Inhibitory Concentration (MIC) was determined by a broth microdilution assay (CLSI, 2012) using an inoculum of 100 µL of each strain, suspended in BHI broth with a concentration of 10^5 CFU/mL in 96-well microdilution plates, with ½ serial dilutions. 100 µL of the extract solution was added in each well. The final concentration of the extracts varied between 512 – 8 µg/mL. The standard antibiotics were used as controls whose final concentrations varied between 512 – 8.0 µg/mL. For the modulation test the extracts were mixed in 10% BHI broth in sub inhibitory concentrations (MIC/8) as report in MIC procedure [26]. An indicator solution of sodium Resazurin (Sigma) was prepared in sterile distilled water in the concentration of 0.01% (w/v) for visualization. Following incubation, 20 µL of the indicator solution was added to each well and the plates spent an incubation period of 1 h at room temperature. The color change from blue to pink, due to the reduction of Resazurin, indicates bacterial growth, aiding the visualization of the MIC, defined as the lowest concentration capable of inhibiting microbial growth, evidenced by an unaltered blue color [27].

2.6. Statistical analysis

Data from the antiparasitic activity were expressed in media and standard deviation was calculated and expressed as concentration dependent percentage mortality. Regarding the antimicrobial activity, the data was expressed in geometric form and statistical significance was evaluated using a Two-way ANOVA test followed by *Bonferroni's post hoc* test (where $p < 0.001$ was considered significant).

3. Results

The extract yield in relation to the dry material was of 6%. The qualitative analysis of the chemical classes presents in the *X. americana* bark (EHXA) highlighted the presence of diverse secondary metabolites classes, as shown in Table 1. The HPLC-DAD analysis present in Table 2 show phenolic derivate as, gallic acid, chlorogenic acid, caffeic acid and ellagic acid, and flavonoids catechin, rutin, quercitrin, quercetin and kaempferol were quantified.

In Table 3 the results referring to the antiparasitic and cytotoxic

Table 1
Qualitative chemical profile of bark hydroethanolic extract of *X. americana*.

Secundary metabolies class	(+) Presence (-) Absence	Secundary metabolies class	(+) Presença (-) Ausência
Phenols	+	**Aurones**	+
Pyrogallol Tannin	–	Flavononols	–
Condensed Tannin	–	**Leucoanthocyanidin**	+
Anthocyanins	+	**Catequins**	+
Anthocyanidins	+	Flavonones	–
Flavones	–	Alkaloids	–
Flavonols	+	**Steroids**	+
Xanthones	–	Pentacyclic Triterpenoids	–
Chalcones	+	Saponine	–

Table 2
Quantitative chemical profiles of Polyphenols present in bark hydroethanolic extract of *X. americana*.

Compound	*X. americana* mg/g	LOD µg/mL	LOQ µg/mL
Gallic Acid	0.19 ± 0.01 [a]	0.009	0.029
Chlorogenic Acidclorogênico	0.23 ± 0.01 [a]	0.011	0.037
Caffeic Acid	1.85 ± 0.03 [c]	0.032	0.106
Elagic Acid	0.79 ± 0.02 [d]	0.017	0.056
Catequine	0.57 ± 0.02 [b]	0.015	0.048
Rutine	0.53 ± 0.01 [b]	0.024	0.079
Quercitrine	1.89 ± 0.01 [c]	0.008	0.027
Quercetin	0.93 ± 0.02 [e]	0.021	0.071
Kaempferol	0.76 ± 0.03 [d]	0.019	0.062

Results are expressed as mean ± standard deviations (SD) of three determinations. Averages followed by different letters differ by Tukey test at $p < 0.05$.

activity of the *X. americana* stem bark hydroethanolic extract are shown. The extract did not obtain a significant result against *T. cruzi* epimastigotes, however, against the *L. infantum* and *L. brasiliensis* promastigotes the EHXA, at the concentration of 1000 µg/mL, causing a mortality percentage of 40.66 and 27.62%, respectively. Nifurtimox (50 µg/mL), which was used as standard drug against the epimastigotes of *T. cruzi* with 93% of kill in epimatigote form. However, pentamidine standard drug used against Leishmania infections killed 94% of the promastigotes form.

Regarding the cytotoxic activity, the extract presented a low toxicity in the concentrations of 250 and 500 µg/mL promoting a fibroblast mortality of 12.75% and 42.74%, respectively. At the highest concentration, 1000 µg/mL, the EHXA possessed a cytotoxic activity above 55% (Table 2).

In the antibacterial activity evaluation assay the EHXA exhibited a MIC ≥ 1024 µg/mL against all the bacterial strains used. However, in the modulation tests, present in Fig. 1, in association with different antibiotic classes, the extract had a synergistic or antagonist effect. The synergic effect was observed against *S. aureus* strains only when associated with norfloxacin, promoting a reduction in MIC of 50%. Still regarding the modulatory activity of the EHXA against *S. aureus 10* strains, the extract promoted an antagonistic effect when associated with oxacillin and imipenem. The EHXA show modulatory effect against *E. coli 06*and *P. aeruginosa 24*, respectively, the results demonstrate that the extract did not have any significant modulatory effect against any of the tested bacterial species when associated with oxacillin; however, when associated with the remaining antibiotics (imipenem, gentamicin and norfloxacin) the EHXA exhibited an antagonistic effect against these microorganisms.

4. Discussion

Qualitative as well as the quantitative chemical analysis of the EHXA the presence of classes belonging to the phenolic acids and flavonoids were verified. However, you can also observe other secondary metabolite classes such as steroids, anthocyanins, anthocyanidins, leucoanthocyanidins, aurones and chalcones and absence of alkaloids. Despite the scarce literature evaluating the chemical composition of this species present similar qualitative composition [16,28]. In study of Le et al (2012) was corroborated with quantitative chemical profile present in this work, identifying the presence of gallic acid, quercetin, quercitrin and kaempferol in aqueous ethanol extract [29]. Reports in the literature identified various secondary metabolites found in *Ximenia americana*, these are the saponins, the flavonoids, glycosides, tannins, phenolic acids, terpenoids and quinones [11].

Natural products are potential sources for the discovery of new substances with biological activity for different target including, antiparasitary activity against leishmaniasis, Chagas disease's and antimicrobial agents [8,9]. In the literature, various studies which prove the potential biological action of *X. americana*, such as: antimicrobial activity [12], analgesic activity [30], antipyretic activity [13] and antitrypanosomal activity [15]. However, this is the first study which evaluated the modulatory effect on bacterial resistance to antibiotics and the leishmanicidal activity of extracts obtained from *X. americana*.

According to results obtained in this study, a tripanocidal action against *T. cruzi* was not evidenced in any of the tested concentrations. However, it was observed that the EHXA demonstrated itself as a possible agent with leishmanicidal action; especially against *L. infantum* promastigotes. This antileishmania effect observed can be associated to chemical constituents present in the extract. The results present in this work shows that clinical standard drug (pentamidine and Nifurtimox) are more effective than EHXA as antiparasitary activity.

In the study by Silva et al. (2012), quercetin, quercitrin and isoquercitrin was tested against *Leishmania* parasites and presented a relevant leishmanicidal activity with IC_{50} values were estimated to be 3.8, 10 and 4.3 µM, respectively [31]. Vila-Nova et al (2011) show that flavonoid quercetin and rutin show toxicity against promastigote and amastigote forms of *Leishmania infantum chagasi* with EC_{50} of 26 and

Table 3
Antiparasitary activity of bark hydroethanolic extract of *X. americana*.

Compounds	Concentraction (µg/ml)	%AP ± DS (*L. infantum*)	%AP ± DS (*L. brasiliensis)*	%AE ± DS (*T. cruzi*)	%CTF ± DS
EHXA	1000	40.66 ± 0.61	27.62 ± 0.57	0.00 ± 1.63	55.42 ± 0.66
	500	0.00 ± 0.64	0.00 ± 0.45	0.00 ± 0.42	42.74 ± 0.49
	250	0.00 ± 0.85	0.00 ± 0.06	0.00 ± 0.46	12.57 ± 0.48
EC_{50}	–	1014*	1041*	> 1000	885.71
Nifurtimox	100	–	–	100.00 ± 0.46	–
	50	–	–	93.00 ± 0.66	–
	10	–	–	84.00 ± 0.62	–
	1	–	–	43.00 ± 0.93	–
	0.5	–	–	13.00 ± 2.50	–
	0.1	–	–	0.00 ± 1.54	–
EC_{50}	–	–	–	3.02	–
Pentamidine	100	93.9 ± 0.30	93.9 ± 0.30	–	–
	50	93.9 ± 0.10	93.9 ± 0.10	–	–
	25	89.2 ± 0.60	89.2 ± 0.60	–	–
	12.5	80.6 ± 0.20	80.6 ± 0.20	–	–
	6.25	54.2 ± 0.30	54.2 ± 0.30	–	–
	3.125	15.5 ± 1.10	15.5 ± 1.10	–	–
EC_{50}		5.69	5.69	–	–

%AP - percentual of antipromastigote activity %AE – percentual of antiepimastigote activity; %SD – standard deviation; %CTF –cytotoxic percentual in fibroblast; EC_{50} – concentration that present 50% of effect.

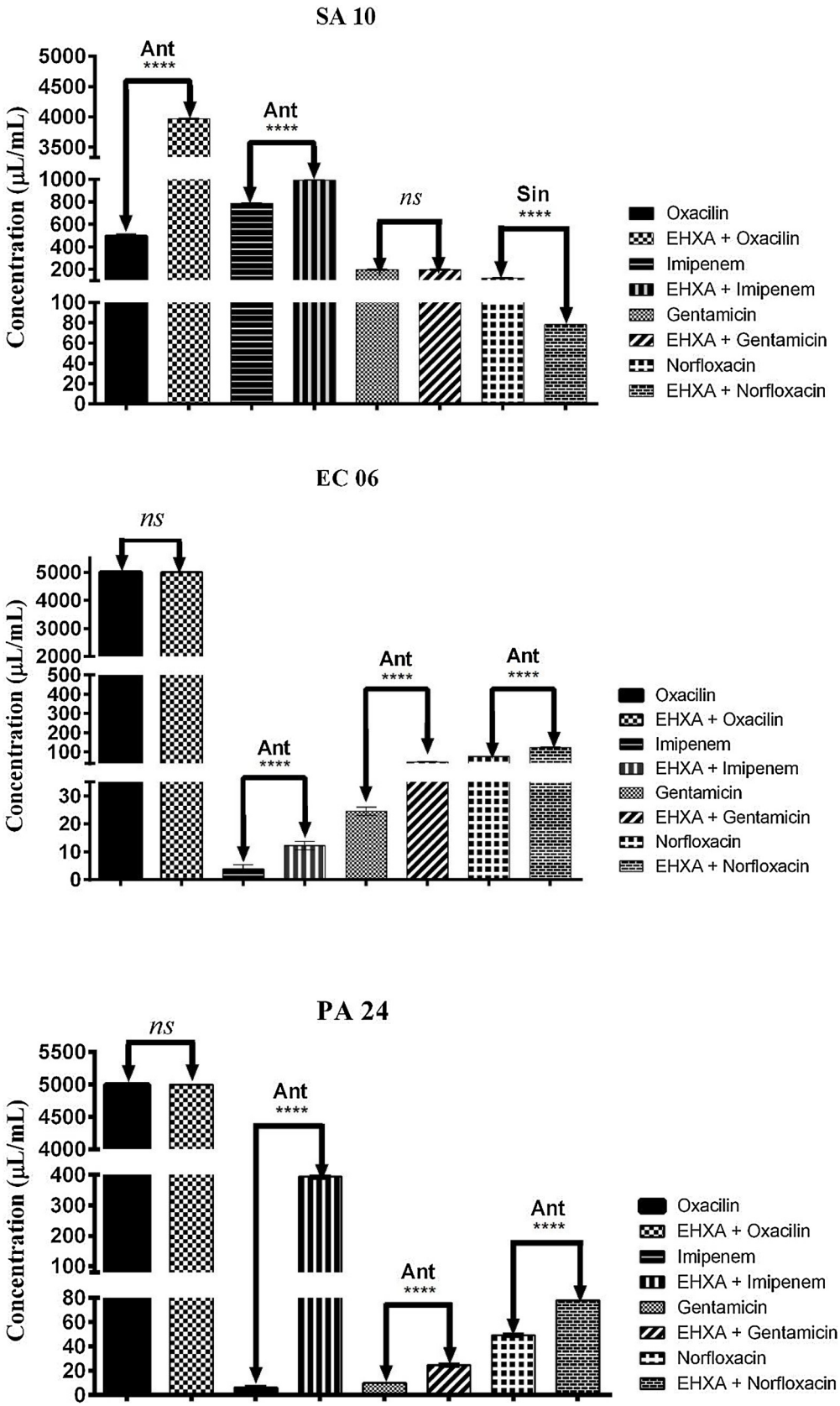

Fig. 1. Minimum Inhibitory Concentration (µg/mL) of antibiotics in the absence and presence of EHXA. SA 10 - *Staphylococcus aureus* 10, EC 06 - *Escherichia coli* 06, PA 24 – *Pseudomonas aeruginosas* 24, EHXA - *Ximenia americana* stem bark hydroethanolic acid. *** Statistically significant value $p < 0.001$ Ant = Antagonism and Syn = Sinergism.

30.3 μg/mL, respectively [32]. These flavonoids presented comparable results to the positive control drug, amphotericin B, against the amastigote forms with EC_{50} for quercetin and rutin of 10.6 and 43.3 μg/mL, respectively. The possible mechanisms of action through which quercetin acts promoting the death of these parasites may be due to its capacity to chelate iron and, consequently, deprive the intracellular forms of these parasites of essential nutrients for their survival [33]. Other way was showed by Fonseca-Silva et al (2013) that also affirmed that quercetin has the capacity to cause the destruction of parasites of the *Leishmania* genus due to its pro-oxidant activity, that is, for its effect of generating reactive oxygen species (ROS) enzymes and, consequently, causing mitochondrial membrane dysfunction of these parasites [34]. In this manner, we can suggest that these can also be possible mechanisms of action through which *X.americana* acts, promoting a leishmanicidal effect, once Monte et al. (2012) affirmed that quercetin is one of the compounds present in this plant [11].

Studies with extracts obtained from *X. americana* reveal that this species has an inhibitory capacity over bacterial growth. Maikai, Maikai e Kobo (2009) affirmed that the extracts obtained from *X. americana* stem bark was efficacious at inhibiting bacterial growth of the *E. coli*, *S. aureus* and *P. aeruginosa* strains [35]. James et al. (2007) evaluated the antibacterial activity of aqueous and methanolic extracts obtained from the *X. americana* roots, stem bark and leaves against *S. aureus*, *Klebsiella pneumoniae* and *Shigella flexneri* strains and concluded that these extracts were efficacious at inhibiting bacterial growth, with this effect being associated with carbohydrates, glycosides, flavonoids and tannins present in this extract [36].

In the study performed by Silva et al. (2015) the ethanolic extract of *X. americana* leaves, when associated with erythromycin, exhibited a synergistic modulatory effect against *Staphylococcus aureus* resistant to erythromycin (SA01, SA 03 and SA 04); the same affirmed that this modulatory effect may be related to the presence of phenolic compounds, which interfere with the membrane permeability favoring a better bactericidal activity [37].

The mechanisms of action of the antibacterial and modulatory effects can be obtained through interactions of secondary metabolites of extracts of one or more vegetables with drugs or antibiotics, causing a synergistic modulatory effect [38]. These mechanisms are varied, as constituents can act as intermediate regulators of bacterial metabolism, activating or blocking enzymatic reactions as, the activity showed by quercetin, has been at least partially attributed to inhibition of DNA gyrase, directly affecting enzyme synthesis at a nuclear or ribosomal level, or changing the membrane structure of these organisms [39–41]. In the same way, the antagonistic activities observed are due to these probable interactions, as, flavonoids and tannins are capable of forming complexes with antibiotics reducing its availability and hindering its penetration. However, new studies should be performed to elucidate which interactions and, thus, possibly, explain the antagonism over pharmaceuticals.

In conclusion, the result obtained point to a new perspective of an alternative therapeutic potential for the treatment of leishmaniosis, cause by the *L. infantum* protozoan species and bacterial infections caused by *S. aureus*. The extract showed a potential safety for use, as it only demonstrated toxic action at the more elevated concentration. It was possible to evidence the modulatory potential of bacterial resistance through antagonistic and synergistic effects when the EHXA was associated to different classes of antibiotics. However, new *in vitro* test must be performed to improve the comprehension of the therapeutic potential and its possible mechanism of action, enabling, thus, future *in vivo* assays.

Conflict of interests

The authors have not any conflict of interest to disclose.

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