BIOLOGICAL ACTIVITIES AND PHYTOCHEMICAL SCREENING OF LEAF EXTRACTS FROM *Zanthoxylum caribaeum* L. (Rutaceae)

ATIVIDADES BIOLÓGICAS E PROSPECÇÃO FITOQUIMICA DE EXTRATOS VEGETAIS DAS FOLHAS DE Zanthoxylum caribaeum *L. (Rutaceae)*

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ABSTRACT: The Brazilian flora is known for its vast biodiversity; however, many species have been still little studied regarding to their chemical composition and biological potential. Thus, this study aimed to determine the antimicrobial, antioxidant and acaricidal activity of the extracts of leaves of *Zanthoxylum caribaeum* L. In addition, phytochemical screening of these extracts was carried out to determine the main classes of secondary metabolites present in *Z. caribaeum*. Using the *Z. caribaeum* leaves, aqueous and organic extracts were obtained using the following solvents (ethanol, methanol, hexane, acetone, dichloromethane and ethyl acetate). The antimicrobial activity of extracts was determined by broth microdilution method, and to detect antioxidant activity the method of capturing the free radical 2,2-diphenyl-1-picryl hydrazyl (DPPH) was used. The acaricidal activity of the extracts was tested on *Dermanyssus gallinae* (De Geer) (Acari: Dermanissidae). Ethanolic and methanolic extracts presented antimicrobial activity for most of the bacterial strains tested, as well as for yeast *Candida albicans*. The ethanolic extract presented high free radical sequestration potential (71.2%) and antioxidant capacity (the lowest IC_{50} value - 24.39 μg mL^{-1}). The crude extracts obtained with methanol and acetone were the most promising. In general, phytochemical screening indicated the presence of steroids, flavanones, flavones, flavonols, saponins, tannins, triterpenoids and xanthones.

KEYWORDS: Antimicrobial activity. Antioxidant activity. Acaricidal activity. Poultry red mite. *Salmonella enterica*.

INTRODUCTION

Brazil is home to one of the richest floras in the world, with about 10% of the world's total species (MUGGE et al., 2016). Brazilian megadiversity represents an invaluable source of new substances, derived from the secondary metabolism of plants, which may present antimicrobial (COSTA et al., 2010; SILVA et al., 2017), antioxidant (PEREIRA et al., 2016; SANTI et al., 2017) and acaricide activity (FERRAZ et al., 2017). In addition, many products marketed by the pharmaceutical industry, originate from plants, being possible to mention digoxin from *Digitalis* spp. (Plantaginaceae); vincristine and vinblastine from *Catharanthus roseus* (L.) G. Don (Apocynaceae); atropine of *Atropa belladonna* L. (Solanaceae), among others (RATES, 2001).

Secondary plant metabolites may also serve as a model molecule for pesticides' synthesis, such as pyrethroids synthesized using pyrethrins, extracted from chrysanthemum flowers *Tanacetum cinerariifolium* (Trevir.) Sch. Bip (Asteraceae) (PALMQUIST; SALATAS; FAIRBROTHER, 2012) and are widely used as pesticides.

Among the numerous botanical families with recognized biological activities, the Rutaceae family, whose representatives are known for the synthesis of alkaloids, coumarins, lignans, flavonoids and limonoids (SUPABPHOL; TANGJITJAREONKUN, 2014; TUNDIS; LOIZZO; MENICHINI, 2014) are noteworthy. Many substances belonging to these chemical classes present antioxidant properties (Baj et al., 2017) and are toxic to microorganisms (ADAMSKA-SZEWCZYK; GLOWNIAK; BAJ, 2016; ZHANG et al., 2017) and arthropods (GREGER, 2017; LV et al., 2015).

In this context, the species *Zanthoxylum caribaeum* L. (Rutaceae), despite the scarcity of studies in the literature, is notable for the presence of antimicrobial activity of its extracts and its essential oil against *Salmonella* serotypes of poultry origin (SOUZA et al., 2017), as well as for *Staphylococcus aureus* and *Escherichia coli* (ORDAZ PICHARDO et al., 2014). Its toxicity as

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an acaricide for *Rhipicephalus microplus* (Canestrini) (Acari: Ixodidae) (NOGUEIRA et al., 2014a) and as an insecticide for *Rhodnius prolixus* (Stal) (Hemiptera: Reduviidae) (NOGUEIRA et al., 2014b) has also been confirmed.

This specie of the Rutaceae family is widely distributed in the neotropical region, occurring in all Brazilian states (PIRANI, 2015). In addition, *Z. caribaeum* presents antimalarial and antirheumatic properties, and it is also used in the popular medicine in the treatment of several diseases (FERREIRA et al., 2007; SCHNEE, 1984).

Thus, considering the biological activities of plants' secondary metabolites and the scarcity of *Z. caribaeum* information, the present study aimed to evaluate the antimicrobial, antioxidant and acaricide activity of secondary metabolites of *Z. caribaeum* leaf extracts. In addition, the main classes of secondary metabolites presented in *Z. caribaeum* leaves extracts were investigated by phytochemical screening.

MATERIAL AND METHODS

Botanical material

The leaves of *Z. caribaeum* were collected from January to April 2015 in the ecological park Paulo Gorski, Cascavel, PR, Brazil (24º57'51 "S, 53º26'2" W). An exsiccate of the plant was deposited in State University of the West of Paraná Herbarium (UNOP) (voucher number UNOP 1849). The leaves were dried and ground following the assumptions of Weber et al. (2014), originating the vegetal powder used to obtain the extracts.

Aqueous extract

Initially, distilled water (Aq) (100 mL) was added to the dry botanical material (40 g), the mixture was maintained on a rotary incubator (220 rpm, 24 h, 22 °C). After this period, the mixture was filtered using Whatman nº 1 filter paper and centrifuged at 5000 rpm for 15 minutes. The supernatant was collected giving rise to the aqueous extract, it was maintained at a temperature of 4 º C until used in the bioassays.

Organic solvent extracts

Organic extracts were obtained according to the assumptions of Pandini et al. (2015), with modifications. Ethanol (EtOH), methanol (MeOH), hexane (Hex), acetone (AcO), dichloromethane (CH_2Cl_2) and ethyl acetate (EtOAc) were used as solvents. The vegetable powder (40 g) was subjected to extraction with the solvents (100 mL) and followed the same procedure as with the aqueous extract, then the supernatant was collected and the solvent was removed on a rotary evaporator, yielding the crude soluble extracts in EtOH, MeOH, Hex, AcO, CH_2Cl_2 and AcOEt. The extracts were kept in a freezer at 4ºC.

Phytochemical screening

Aqueous and organic solvent extracts of *Z. caribauem* were tested for the presence of alkaloids, anthocyanins, anthocyanins, aurones, chalcones, coumarins, steroids, flavanones, flavones, flavonols, saponins, condensed tannins, triterpenoids and xanthones, according to a methodology described by Matos (1997). The qualitative results were expressed as presence/positive reaction (+) weakly positive or strong positive reaction (++) with higher intensity (more concentrated reactions), and absence /negative reaction (-) of phytochemicals.

Microorganisms used and inoculum preparation

The strains of the collections *American Type Culture Colletion* (ATCC) and "*Cefar Diagnóstica"* (CCCD), were used to evaluate the antimicrobial potential of *Z. caribaeum*, six of the strains were Gram-negative, *Salmonella enterica* subspecies *enterica* sorovar Enteritidis (ATCC 13076), *Salmonella enterica* subspecies *enterica* sorovar Typhimurium (ATCC 14028), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus mirabilis* (ATCC 25933) and *Klebsiella pneumoniae* (ATCC 13883) and four strains were Gram-positive: *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Enterococcus faecalis* (ATCC 19433) and *Bacillus subtilis* subspecies *spizizenii* (CCCD-B005) and the yeast *Candida albicans* (ATCC 10231). In addition, three serotypes of *Salmonella enterica* subspecies *enterica* of the highest occurrence in the western region of Paraná, Brazil (SCUR et al., 2014), were isolated from chicken broilers from the region: Heildelberg, Gallinarum and Newport yielded by Veterinary Laboratory, MercoLab from Cascavel, Paraná, Brazil (www.mercolab.com.br).

The microorganisms were recovered in Brain Heart Infusion (BHI) broth and incubated for 24 hours at 36 ± 0.1 ° C in a B.O.D. After that, the strains were harvested in Muller Hinton Agar (MH) culture media and incubated for 24 h at 36 ± 0.1 ° C in a B.O.D. To standardize the inoculum, the strains were diluted in saline solution (0.85%) resulting in the final concentration of 1×10^5 CFU mL^{-1} for bacteria and 1×10^6 CFU mL^{-1} for yeast *C. albicans* (Mcfarland scale).

Antimicrobial activity

Aqueous and organic solvent extracts were used to determine the Minimum Inhibitory Concentration (MIC) according to the microdilution method of Pandini et al. (2015), with modifications. For the bioassay with the bacteria, the plant extracts were solubilized in methyl alcohol and Mueller-Hinton broth (MH). Whereas for *C. albicans* Roswell Park Memorial Institute broth (RPMI-1640) was used. Concentrations ranging from 200 to 0.09 mg mL^{-1} of each extract were obtained. Then the inocula (10 µL) were added to each well, and the plates were incubated for 18 to 24 h at 36 ± 0.1 ° C. After that time, 0.5% triphenyltetrazolium chloride (TTC) (10 µL) was added and the plates were again incubated for 3 h at 36 ± 0.1 ° C. Presence of red staining was indicative of microorganismal growth. As a positive control, the antibiotics gentamicin (200 mg mL^{-1}) and nystatin (200 mg mL^{-1}) were added to the culture media MH and RPMI, respectively. Microbial wells were reserved for control of broth sterility (MH/RPMI only), bacterial growth (bacterial suspension and MH/RPMI), reference antimicrobial action (bacterial suspension, MH/RPMI and gentamicin 200 mg mL^{-1}) and solvent (bacterial suspension, MH/RPMI and methanol).

According to the method proposed by Weber et al. (2014) with modifications, prior to the addition of TTC in all wells, as described above, aliquots (2 µL) were withdrawn from each well containing culture medium and the microorganisms, which was inoculated onto the surface of MH agar. The plates were incubated for 24 h at 36 ± 0.1 ° C and after this time the Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC) was defined as the lowest of the plant extract capable of causing the inoculum death. The MIC and the MBC of the extracts were classified according to the methodology of (PANDINI et al., 2015), the activity was considered high (<12.5 mg mL^{-1}), moderate (12.5 to 25 mg mL^{-1}), low (50 to 100 mg mL^{-1}) and very low (> 100 mg mL^{-1}).

Antioxidant activity

Aqueous and organic solvent extracts had the antioxidant activity evaluated according to the free radical reduction method 2,2-diphenyl-1-picryl hydrazyl (DPPH) (PANDINI et al., 2015). For that, alíquots of aqueous and organic solvent extracts (0,1 mL) were solubilized in MeOH PA (200 mg mL^{-1}) and added the methanolic solution of DPPH (0,2 mM) (3,9 mL). The absorbance of the samples was read at 515 nm using the FEMTO spectrophotometer, 700 Plus. As negative control methyl alcohol (50%), acetone (70%) and water (2: 2: 1) were used, the synthetic antioxidant Butylhydroxytoluene (BHT) was used as a positive control. The ability to eliminate DPPH (% antioxidant activity) was calculated using the following equation: $I\% = \left[\frac{(A_0 - A_1)}{A_1}\right] \times 100$, where A_0 is the absorbance of the control, A_1 is the absorbance of sample and $I\%$ is the percentage of antioxidant activity. The IC_{50} (amount of antioxidant substance needed to reduce the initial concentration of DPPH by 50%) was calculated based on the equation of the straight line obtained from the calibration curve.

Acaricidal activity

The poultry red mite *Dermanyssus gallinae* (De Geer) (Acari: Dermanyssidae) was collected in a commercial poultry layer house , without reports of the use of sanitary products, located in the city of Matelândia / PR, Brazil (25º26'8.15º S, 54º04'39.5ºW). Engorged females were selected (FLECHTMANN, 1985) and packed in glass tubes closed with *voile* tissue. The mites were maintained for acclimation for 24 h under controlled conditions (25 ± 1 ° C, 70% U.R. and 14 h photophase), the bioassays were also conducted under the same conditions.

The trials were conducted in a completely randomized design, with five replicates per treatment, and the experimental plot consisted of 25 mites. The treatments consisted of plant extracts at the concentrations of 125, 250, 500, 750 and 1000 mg mL^{-1}. Organic solvent extracts were solubilized in MeOH P.A., thus MeOH were employed as negative control. Whereas for the aqueous extract bioassay, distilled water was as negative control.

For the treatment application, engorged females were transferred to Petri dishes with the edges insulated with solid vaseline, to avoid escape. Then, the mites received 2 mL of each extract, by mean of airbrush coupled to an air compressor, under constant pressure of 0.84 Kgf / cm^2 of exit. The volume of the solution for each experimental plot was 2 mL. After spraying, the females were transferred to glass tubes closed with *voile* tissue . The mortality evaluation was performed after 24 h, considering dead mites as those that did not present mobility to the touch with a brush. For the statistical analysis, the data were submitted to Kruskal-Wallis non-parametric test using R® software (R DEVELOPMENT CORE TEAM, 2017).

RESULTS AND DISCUSSION

Phytochemical screening

Steroids, flavonoids, flavones, flavonols, saponins, tannins, triterpenoids and xanthones were detected (Table 1).

Table 1. Phytochemical prospection of aqueous and organic solvent extracts from leaves of *Zanthoxylum caribaeum.*

Chemical compounds	Extracting solvent[1,2]						
	AcO	AcOEt	EtOH	Hex	MeOH	CH_2Cl_2	Aq
Alkaloids	-	-	-	-	-	-	-
Anthocyanidins	-	-	-	-	-	-	-
Anthocyanins	-	-	-	-	-	-	-
Aurones	-	-	-	-	-	-	-
Chalcones	-	-	-	-	-	-	-
Cumarines	-	-	-	-	-	-	-
Steroids	+	+	+	+	+	++	-
Flavanonols	-	-	-	-	-	-	+
Flavones	+	+	+	+	+	+	-
Flavonols	+	+	+	+	+	+	-
Saponins	-	-	-	-	-	-	+
Condensed tannins	-	-	+	-	++	-	+
Triterpenoids	+	+	+	+	+	+	+
Xanthones	+	+	+	+	+	+	+

[1]Acetone (AcO), ethyl acetate (AcOEt), ethanol (EtOH), hexane (Hex), methanol (MeOH), dichloromethane (CH_2Cl_2), and aqueous (Aq); 2 (+) Positive reaction, (++) strong positive reaction and (-) negative reaction.

Except for the aqueous extract, all the others presented positive reaction (+) for the presence of steroids (Table 1). The strong positive reaction (++) found for the soluble extract in CH_2Cl_2 is highlighted, this can be explained by the fact that steroids are apolar compounds. On the other hand, the aqueous extract was the only one that presented flavanonols, which are considered partially polar flavonoids and moderately soluble in polar solvents, such as water (BARAN, 1997 DIXON; PAIVA, 1995).

Flavones and flavonols have been detected in all organic solvents, independent of the polarity of the solvent, because although most flavonoids are moderately soluble in polar solvents, others tend to be more soluble in non-polar solvents such as ethers and chloroform (BARAN, 1997 DIXON; PAIVA, 1995). On the other hand, Saponins were found only in the aqueous extract. It is known that saponins have high polarity (MAJINDA, 2012). Also, because they were phenolic compounds, condensed tannins were detected when more polar solvents were used, such as ethanol, methanol and water (MELLO; SANTOS, 2001). The triterpenoids and xanthones were detected in all the extracts and did not present any difference in relation to the polarity.

The classes of secondary metabolites detected in *Z. caribaeum* leaves extracts, in the present work, are in agreement with the reported in the literature for other species of the same genus. Phytochemical studies with *Zanthoxylum spp.* reported the presence of steroids, flavonoids, saponins, tannins, triterpenoids and xanthones (PAMHIDZAI; ISAAC, 2013; RAVIKUMAR et al.; 2012) which may be related to the biological activities found in the present work.

Antimicrobial activity

With the exception of the aqueous extract, all others promoted growth inhibition of Gram-positive and Gram-negative bacteria, with the most pronounced results being found for soluble extracts in EtOH and MeOH (Table 2). This is because, they showed activity to practically all the microorganisms used in the bioassays.

In relation to gram-negative strains, soluble extracts in MeOH and EtOH showed moderate activity for *E. coli*, *S.* Enteritidis and *S.* Typhimurium, with MIC and MBC of 25 mg mL^{-1}. The strains *P. aeruginosa* and *P. mirabilis* were more sensitive to the extract soluble in MeOH, which presented high activity, with MIC and MBC of only 6.25 mg mL^{-1}. For *K. pneumoniae*, the extract that showed the best activity was the soluble in EtOH, with MIC and MBC of 6.25 mg mL^{-1} (high activity). In relation to the Heidelberg and Newport serotypes, the extract that presented the best values of MIC and MBC was that soluble in AcOEt, whereas the Gallinarum serotype was more sensitive to the AcO extract.

Table 2. Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), Minimum Fungicidal Concentration (MFC) of aqueous and organic solvent extracts on leaves of *Zanthoxylum caribaeum* against different microbial strains.

Microorganisms	MIC / MBC-MFC (mg mL^{-1})[1]					
	Extracting Solvent[2]					
Gram Negatives	AcO	AcOEt	EtOH	Hex	MeOH	CH_2Cl_2
Proteus mirabilis ATCC 25933	50/50	50/50	12.5/12.5	100/100	6.25/6.25	50/50
Klebsiella pneumoniae ATCC 13883	25/25	25/25	6.25/6.25	50/50	6.25/12.5	25/25
Pseudomonas aeruginosa ATCC 27853	50/50	50/50	12.5/12.5	100/100	6.25/6.25	50/50
Escherichia coli ATCC 25922	50/50	50/50	25/25	100/100	25/25	25/50
Salmonella Enteritidis ATCC 13076	50/50	25/50	25/25	100/100	25/25	50/50
Salmonella Typhimurium ATCC 14028	50/50	25/50	25/25	100/100	25/25	50/50
Salmonella Heidelberg*	50/50	25/25	50/50	100/200	25/50	200/200
Salmonella Newport*	50/50	25/50	50/50	50/100	-	200/200
Salmonella Gallinarum*	12.5/50	25/50	50/50	100/100	50/50	100/200
Gram Positives						
Bacillus subtilis CCCD-B005	6.25 /6.25	3.12/3.12	6.25 /6.25	Na	6.25 /6.25	0.78/0.78
Staphylococcus aureus ATCC 25923	25/50	25/25	12.5/12.5	6.25/12.5	6.25/12.5	25/25
Staphylococcus epidermidis ATCC 12228	12.5/25	25/25	12.5/12.5	25/25	6.25/12.5	6.25/25
Enterococcus faecalis ATCC 19433	50/50	25/25	25/50	25/25	25/50	12,5/50
Yeast						
Candida albicans ATCC 10231	50/50	50/50	25/25	50/50	25/25	50/50

[1](-) No activity; [2]Acetone (AcO), ethyl acetate (AcOEt), ethanol (EtOH), hexane (Hex), methanol (MeOH) and dichloromethane (CH_2Cl_2); * *Salmonella enterica* serotypes.

For the group of Gram-positive strains, *B. subtilis* was more susceptible to the CH_2Cl_2 extract, with MIC and MBC of 0.78 mg mL^{-1}, the best value observed for antimicrobial activity among all microorganisms tested (high activity). The extracts soluble in MeOH and Hex were more effective against *S. aureus*, with MIC and MBC of 6.25 and 12.5 mg mL^{-1}, respectively, indicating high activity of both extracts. The same was verified for the strain *S. epidermidis*, referring to the extract MeOH. For the *E. faecalis* bacterium, all extracts presented almost the same efficacy, except for the CH_2Cl_2 extract that obtained the best inhibition, with a MIC value of 12.5 mg mL^{-1}.

The standard yeast *C. albicans* was also sensitive to all extracts; soluble extracts in EtOH and MeOH showed moderate activity with MIC and MFC of 25 mg mL^{-1}, followed by extracts soluble in Hex, AcO, CH_2Cl_2 and AcOEt, with low activity, with MIC and MFC of 50 mg mL^{-1} and the aqueous extract showed no fungicidal activity.

The genus *Zanthoxylum* is a group of plants that presents as one of its characteristics bioactive compounds with antimicrobial activity. It is possible to exemplify the moderate activity of the aqueous extract of the leaves of *Zanthoxylum capense* (Thunb.) Harv. (Rutaceae) against *B. subtilis, E. coli* and *K. pneumoniae*, *S. aureus*, whereas the ethanolic extract presented high activity for *B. subtilis, E. coli, K. pneumoniae* and *S. aureus* (BUWA; STADEN, 2006). In the case of *Z. capense*, the acetone-soluble extract showed excellent activity against *S. aureus, E. coli, E. faecalis* and *P. aeruginosa* (ADAMU; VINNY; ELOF, 2014). Meanwhile, the ethanolic extract of *Zanthoxylum chalybeum* Engl. (Rutaceae) did not

present an inhibitory action against *E. coli, E. faecalis, P. aeruginosa* and *S. aureus*, but demonstrated antifungal potential against *C. albicans* yeast (KUGLEROVA et al., 2011).

In relation to *Z. caribauem*, an antimicrobial action of soluble extracts in methanol, ethanol, acetone, ethyl acetate, dichloromethane, hexane and aqueous was observed against different *Salmonella* serotypes of poultry origin. However, as in our study, the aqueous extract did not obtain satisfactory results, demonstrating inhibition of growth only against the serotype *S. enterica* Mbandaka at the concentration of 200 mg mL^{-1} (SOUZA et al., 2017).

In general, it can be seen that both the MeOH extract and EtOH showed antimicrobial activity against most of the strains tested. This result may be justified by the fact that these extracts exhibit the presence of tannins, which are known to act on cell membranes and may alter permeability or even lead to the destruction of the cell (SCHENKEL et al,; 2001). In bacteria, growth retardation has been associated with the formation of complexes between the tannins and the cell wall of secreted extracellular bacteria or enzymes, causing inhibition of nutrient transport into the cell (MCSWEENEY et al., 2001).

It is important to highlight that the use of solvents with different polarities in obtaining the extracts reflects the composition of the secondary metabolites found, which justify the differences regarding the antimicrobial activity of the extracts (MIGLIATO et al., 2011). With this, it was verified that the extracts with the solvent with greater polarity (EtOH and MeOH) proved to be efficient in the antimicrobial action, in relation to the others.

The absence of the antimicrobial activity as in the aqueous extract of *Z. caribaeum* on different the microorganism may be related to the low concentrations of the potentially active substances, since the phytochemical screening allows us a qualitative and non-quantitative analysis of the secondary metabolites present (AYRES et al., 2008). In addition, since plant extracts are formed from complex mixtures, there may often be interactions between their constituents, which directly influence bioactivity (HEINRICH, 2010; JUNIO et al., 2011).

Antioxidant activity

It was observed that the extract soluble in EtOH obtained from the leaves of *Z. caribaeum* showed the highest percentage of free radical sequestration (DPPH) (71.2%) and the lowest IC_{50} value (24.39 μg mL^{-1}) when compared to the other extracts. Soluble extracts in AcO and MeOH also showed free radical sequestration above 50%. The soluble extract in AcOEt had a free radical scavenger effect of 48.56%, and these last three extracts had a moderate / intermediate percentage of antioxidant activity when compared to the commercial antioxidant BHT, with antioxidant activity of 92.80% and value of IC_{50} = 7.93 μg mL^{-1} (Table 3). While the soluble extracts CH_2Cl_2, Hex and Aq showed less than 10% sequestration, being considered without antioxidant activity.

Table 3. Antioxidant activity of aqueous and organic solvent extracts from the leaves of *Zanthoxylum caribaeum* by the DPPH method.

	Test solution [1]	% capture DPPH[2]	IC_{50} (μg mL^{-1})[3]
Extracting Solvent	EtOH	71,12	24,39
	AcO	66,16	29,67
	MeOH	61,99	27,98
	AcOEt	48,56	29,67
	BHT (positive control)	92,80	7,93

[1]Etanol (EtOH), acetone (AcO), methanol (MeOH), ethyl acetate (AcOEt) and BHT (commercial synthetic antioxidant Butylhydroxytoluene); [2]Percentage of radical sequestration DPPH (2,2-diphenyl-2-picrylhydrazyl); [3]Concentration of *Z. caribauem* leaves extract is necessary to reduce 50% of the DPPH radical.

Although no studies have been found in the literature exploring the antioxidant activity of *Z. caribaeum*, Kuglerova et al. (2011) reported the antioxidant it' activity of *Z. chalybeum* Engl. (Rutaceae), which presented IC_{50} of 22.66 μg mL^{-1}, whereas for the Trolox positive control, the IC_{50} was 3.49 ± 0.19 μg mL^{-1}. Likewise, the methanol and ethyl acetate soluble fractions of methanolic *Z. rhetsa* DC (Rutaceae) seeds exhibited expressive rapture activity compared to the ascorbic acid control with more than 50% free radical sequestration (PRABHASH; AGNEL, 2015).

The antioxidant compounds are very diverse, commonly found in plants, such as vitamins C and E, carotenoids and phenolic compounds (OU et al., 2002). To Pérez-Jiménez and Saura-Calixto (2006) what can determine the extraction of these metabolites with antioxidant capacity is the type of

solvent and its polarity, which are formed by the transfer of electrons from hydrogen atoms that give rise to compounds different antioxidants.

In the research of Xu and Chang (2007) it was verified that different solvents used in the extraction, resulted in different phenolic compositions and consequently differences in their antioxidants. In agreement with the authors above, it was recognized that extracts obtained through high polarity solvents, such as the soluble extract in EtOH, are more effective as free radical scavengers and are considered to be good bacterial inhibitors when compared to those obtained from solvents of less polarity.

Therefore, the highest percentage of DPPH free radical sequestration of the EtOH-soluble extract in the present work is probably due to the antioxidant effect of phenolic compounds such as tannins and flavonoids, which are substances categorized as free radical scavengers, being very efficient in the prevention of autoxidation (SHAHIDI; JANITHA; WANASUNDARA, 1992).

Acaricidal activity

As for the *Z. caribaeum* extracts tested against the *D. gallinae* mite, the extract with the highest acaricidal activity was the soluble in MeOH, with a maximum mortality of 37.6% at the concentration of 1000 mg mL^{-1}, followed by the extract EtOH with 27.2% and AcO with 25.6%. The other plant extracts presented very low toxicity for *D. gallinae* with values lower than 20% (Table 4). The extracts obtained from MeOH and EtOH presented the highest values of mortality in comparison with the others, suggesting that their acaricide action is mainly related to the presence of steroids and condensed tannins, differing them from the other extracts tested.

Although the mortality caused by extracts has not been as pronounced, is possible suppose the existence toxic some metabolites for this mite in *Z. caribauem* (Table 4).

Table 4. Mortality from *Dermanyssus gallinae* treated with aqueous and organic solvent extracts from the leaves of *Zanthoxylum caribaeum* in different concentrations.

Bioassays	Treatments[1]	Concentration (mg mL^{-1})	Mortality (%)
Bioassay 1	Negative control	0	0.8 ± 0.80
	Extract soluble in MeOH	125	8.0 ± 4.19
		250	20.8 ± 5.71
		500	24.0 ± 4.38
		750	32.8 ± 4.63*
		1000	37.6 ± 4.11*
	Extract soluble in AcOEt	125	4.8 ± 2.93
		250	4.0 ± 1.78
		500	3.2 ± 2.33
		750	11.2 ± 4.63
		1000	5.6 ± 0.97
	Extract soluble in EtOH	125	5.6 ±3.70
		250	10.4 ±2.71
		500	24.8 ±5.85
		750	29.6 ±6.14
		1000	27.2 ± 2.65
		$\chi^2 = 54.5822$; df = 15; $p \leq 0.001$	
Bioassay 2	Negative control	0	1.6 ± 0.97
	Extract soluble in Hex	125	0.8 ± 0.80
		250	5.6 ± 2.40
		500	3.2 ± 1.49
		750	0.0 ± 0.00
		1000	6.4 ± 1.60
	Extract soluble in AcO	125	0.8 ± 0.80
		250	4.0 ± 1.26
		500	8.0 ± 3.09
		750	24.8 ± 5.27*
		1000	25.6 ± 2.71*

	Extract soluble in CH_2Cl_2	125	6.4 ±3.70
		250	0.8 ±0.80
		500	2.4± 1.60
		750	1.6 ± 1.60
		1000	1.6±1.60
		χ^2 =45.186; df = 15; p ≤ 0.001	
Bioassay 3	Negative control	0	0.0 ± 0.00
	Aqueous extract	125	3.2 ± 2.33
		250	4.8 ± 2.93
		500	8.0 ± 1.78
		750	4.8 ± 1.49
		1000	8.8 ± 2.33
		χ^2 = 8.4022; df = 5; p = 0.1354	

[1]Methanol (MeOH), ethyl acetate (EtOAc), ethanol (EtOH), hexane (Hex), acetone (AcO), dichloromethane (CH_2Cl_2), and aqueous (Aq). *Means with statistical difference by non-parametric Kruskal-Wallis test (p≤0.05).

The toxic activity of plant extracts of the genus *Zanthoxylum* against arthropods is well known, both to insect (MOUSSAVI et al., 2015) as larvae of the *R. microplus* tick (SANTOS et al., 2013). In relation to the species *Z. caribauem*, a high toxicity of the leaf essential oil on the *R. microplus* tick was reported, as mortality of 97% (NOGUEIRA et al., 2014a), however, this is the first study in which the toxicity of *Z. caribaeum* to the *D. gallinae* mite. So, phytochemical composition study is necessary and recommended.

CONCLUSIONS

The methanolic and ethanolic extracts were the most effective and with a broad spectrum of action against all tested microorganisms, including yeast *C. albicans*.

As for the antioxidant activity, the ethanolic extract proved to be a good free radical scavenger. Soluble extracts in methanol and acetone showed toxicity to the *D. gallinae* mite.

The phytochemical characterization evidenced the presence of steroids, flavanones, flavones, flavonols, saponins, tannins, triterpenoids and xanthones in the aqueous and organic solvent extracts of *Z. caribauem* leaves.

The results found in the present work show the biological potential of *Z. caribaeum* leaves extracts to obtain new bioactive substances.

RESUMO: A flora brasileira é conhecida pela sua vasta biodiversidade, no entanto, muitas espécies ainda são pouco estudadas quanto à composição química e ao potencial biológico. Assim, esse trabalho teve como objetivo determinar a atividade antimicrobiana, antioxidante e acaricida dos extratos vegetais das folhas de *Zanthoxylum caribaeum* L. Adicionalmente, foi realizada triagem fotoquímica desses extratos para determinar as principais classes de metabólitos secundários presentes em *Z. caribaeum.* Empregando-se as folhas de *Z. caribaeum* foram obtidos o extrato aquoso e orgânicos, utilizando os seguintes solventes (etanol, metanol, hexano, acetona, diclorometano e acetato de etila). A atividade antimicrobiana dos extratos foi determinada pelo método de microdiluição em caldo, e para detecção da atividade antioxidante foi empregado o método de captura do radical livre 2,2-difenil-1-picril hidrazil (DPPH). A atividade acaricida dos extratos foi avaliada frente a *Dermanyssus gallinae* (De Geer) (Acari: Dermanissidae). Os extratos brutos etanólico e metanólico apresentaram atividade antimicrobiana para a maioria das cepas bacterianas testadas, e também para a levedura *Candida albicans*. O extrato etanólico apresentou elevado potencial de sequestro de radicais livres (71,2%) e o menor valor de IC_{50} (24,39 μg mL^{-1}), revelando, portanto, sua capacidade antioxidante. No que se refere à atividade acaricida, os extratos obtidos com metanol e acetona foram os mais promissores. De modo geral, a triagem fitoquímica indicou a presença de esteroides, flavanonas, flavonas, flavonóis, saponinas, taninos, triterpenóides e xantonas.

PALAVRAS-CHAVE: Atividade antimicrobiana. Atividade antioxidante. Atividade acaricida. Ácaro vermelho das galinhas. *Salmonella enterica*.

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