Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(1):319-328

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

In vitro evaluation of antimicrobial, antioxidant and larvicidal activities from extracts of *Zeyheria tuberculosa* (Vell) Bur. (Bignoniaceae)

Naélia Monique Moreira Brito Silva[1], Isabelle Souza de Mélo Silva[2], Raíssa Fernanda Evangelista Pires dos Santos[1], Talita Lucio Chaves Vasconcelos[1], Max Denisson Maurício Viana[1], Eliane Aparecida Campesatto[1], Lucia Maria Conserva[3], Eliana Maria Maurício da Rocha[4], Ednaldo Cavalcante de Araújo[5], João Xavier de Araújo-Júnior[1] and Maria Lysete de Assis Bastos[1*]

[1]Escola de Enfermagem e Farmácia. Universidade Federal de Alagoas. Campos A.C. Simões, Av. Lourival Melo Mota, s/n, Tabuleiro dos Martins, CEP: 57072-900: Maceió – Brasil
[2]Universidade Tiradentes/UNIT. Av. Murilo Dantas, 300, CEP: 49032-490: Aracaju, SE – Brasil
[3]Instituto de Química e Biotecnologia. Universidade Federal de Alagoas. Campos A.C. Simões, Av. Lourival Melo Mota, s/n, Tabuleiro dos Martins, CEP: 57072-900: Maceió – Brasil
[4]Universidade Federal de São João Del-Rei. Rua Sebastião Gonçalves Coelho, 400 Chanadour 35501-296 - Divinopolis, MG – Brasil
[5]Av. Prof. Moraes Rego, 1235 - Cidade Universitária, Recife - PE - CEP: 50670-90

ABSTRACT

This study aimed to investigate in vitro antimicrobial properties from extracts of Zeyheria tuberculosa, by antimicrobial susceptibility testing as well as testing their potential larvicide, compared to larvae of the mosquito Aedes aegypti, and investigate possible antioxidant properties of the synthetic method by DPPH (2,2-diphenyl-2-picryl-1-hidrazila). All plant material was extracted with EtOH, and the leaf extract of spent process liquid/liquid extraction and column chromatography (CC), the stem was subjected to the technique of Spray drying and the stem bark was obtained by maceration and drying in rotary evaporators. Samples that showed sensitivity in the Drilling Test in Agar were qualitatively evaluated by determining the Minimum Inhibitory Concentration (MIC). The results showed that the filtration $CHCl_3$ was moderately active against strains of S. aureus, S. epidermidis and P. mirabilis, as it presented a MIC < 125 µg/mL for the three microorganisms. The crude extract of the stem presented active against strains of S. aureus and S. epidermidis with a MIC < 62.5 µg/mL and MIC < 31.2 µg/mL, respectively, and moderately active front P. mirabilis, with a MIC < 125 µg/mL. The samples evaluated against synthetic DPPH (2,2-diphenyl-1-picryl-hidrazila) showed weak activity or were inactive. In addition, the samples evaluated against larvae in 4th stage of Aedes aegypti were considered inactive, with mortality less than 25%.

Keywords: Bignoniaceae; Medicinal Plants; Microbial Sensitivity Tests; *Aedes*; Antioxidants.

INTRODUCTION

For thousands of years, medicinal plants have been used by world population in the treatment of several diseases. It is estimated that 80% of the population of underdeveloped and developing countries are dependent on the folk medicine. The World Health Organization (WHO) confirms these data and further states that, of this total, at least 30% were by medical prescription [1].

In Brazil, the use of medicinal plants occupies a prominent place in popular usage as a result of indigenous knowledge, slaves and immigrants, as well as of the plant genetic diversity, which has over 55.000 species cataloged from a total estimated at between 350.000 and 550.000 copies. Of these, only about 8% were investigated from a chemical perspective and approximately 1.100 taxons regarding medicinal properties [2].

Currently, several factors have contributed for increasing use of plants as medicinal resource, among them, the high cost of manufactured drugs, poor access of the population to health care, as well as the tendency to use products with natural origin [3].

Increasingly experimental researches which aim to evaluate the potential of plant species have been developed. An *in vitro* evaluation showed, for example, the antimicrobial potential of *Sebastiania corniculata* (Vahl) Mull. Arg. against the strains of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Acinectobacter calcoaceticus*. However, best results for this species was against *P. aeruginosa* [4].

Another study showed the antimicrobial potential of *Tabebuia roseo-alba* (White Ipe) that showed moderate antimicrobial activity in three Gram-positive bacterial species: *S. aureus*, *S. epidermidis*, *E. faecalis*; and Gram-negative: *K. pneumoniae* [4]. And it was proven *in vitro* antibacterial activity of the stem bark ethanolic extract of *Capparis flexuosa* Linn. ("brave beans"), showing promising antimicrobial potential against the strains of *S. aureus*, *E. coli*, *E. aerogenes*, *A. calcoaceticus* and *S. flexneri* [5].

Zeyheria tuberculosa belongs to the Bignoniaceae family which comprises 120 genus, many monotypical and 800 species. It has a wide distribution in tropical and subtropical regions, and offbeat in the subtropics. Its flowers are pollinated by bees, wasps, butterflies, moths, birds and bats, and the seeds are dispersed mainly by wind [6].

Bignoniaceae family has many genus of great exuberance during flowering which are used as ornamental, decorating squares, streets and avenues, and its wood shows high economic value as a raw material in the timber industry [7].

Large numbers of species from Bignoniaceae family is used in folk medicine for the treatment of various diseases, and naphthoquinones present in many species of this family can be the compounds responsible for different biological activities reported. Several naphthoquinones derived of lapachol and lapachone show a variety of biological activities, and for a long time have been studied as antitumor, anti-inflammatory, antimicrobial and antiprotozoal [8].

Phytochemical investigations with *Z. tuberculosa* led to the isolation of four flavonoids (4'-hydroxy-5,6,7,8-methoxyflavone; 4',5,6,7-tetraidroxiflavona; 3,5,7,8-tetrametoxiflavona, and 5,6,7,8-tetrametoxi- flavone) [9].

Flavonoids comprise a wide class of natural origin substances that have a number of pharmacological properties which permit them act on biological systems. Stand out among others, the following effects of flavonoids on biological systems: antioxidant capacity, anti-inflammatory activity with vasodilator effect, antiallergic action, activity against tumor development, antiplatelet, as well as antimicrobial and antiviral actions [10,11].

According to biological activities reported by the Bignoniaceae family and genus *Zeyheria*, this study aimed to investigate the antimicrobial properties of the extracts of *Zeyheria tuberculosa* (Vell) Bur. (Bignoniaceae), as well as test the potential larvicide against larvae in 4th stage of *Aedes aegypti*, the main vector of dengue in the world, and also investigate possible antioxidant properties by the DPPH (2,2-diphenyl-1-picryl-hidrazila) synthetic radical method.

EXPERIMENTAL SECTION

Collecting, identification and obtaining plant material

Plant material was collected in the municipality of Coruripe, Alagoas, Brazil, located 85 km away from Maceió (geographical coordinates S 10°08'53" and W 36°10'86"). The identification was done at the Institute of the Environment of the State of Alagoas (IMA / AL), where exsicates were deposited, with identification MAC nº. 23 816.

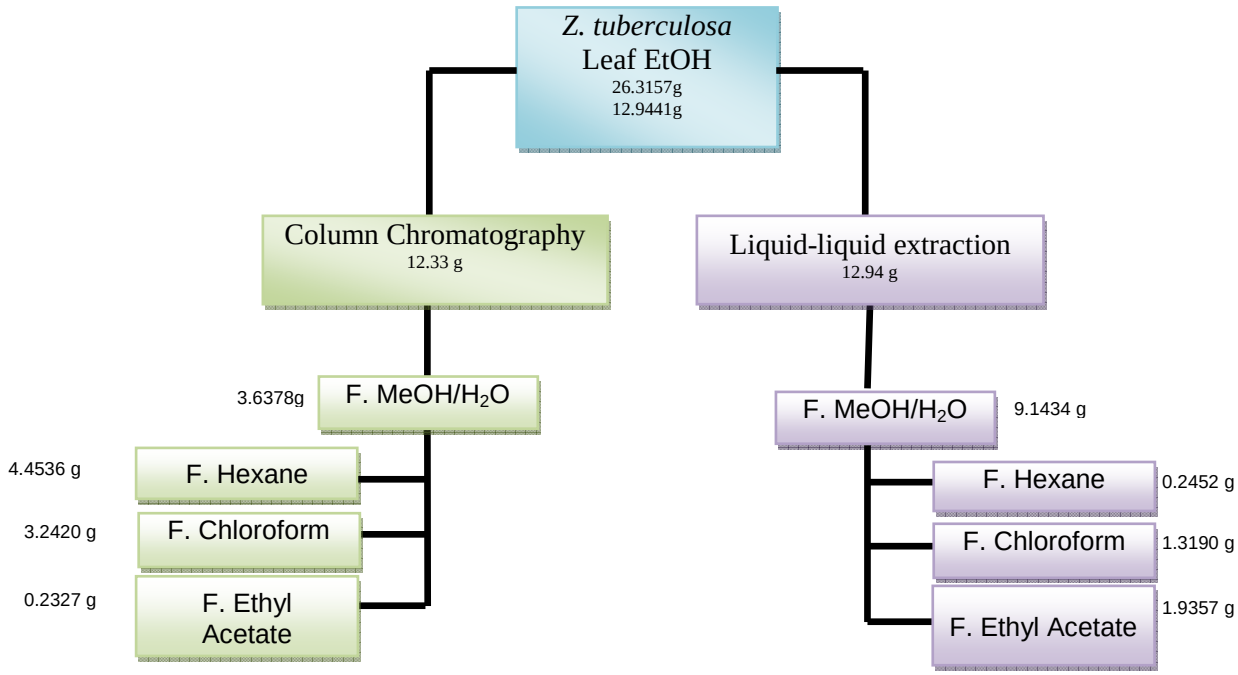
The parts of the plant (leaf, stem and stem bark) were separated and placed to dry at room temperature, then were ground. Crude extracts of the leaves, stem and stem bark of *Z. tuberculousa* were obtained by maceration with the solvent ethanol (EtOH). The extracts were concentrated in rotary evaporator and drying at room temperature. Thereafter, the crude extract from leaf, adequately dried, was subjected to liquid/liquid extraction, also known as solvent extraction or partition, and at a column chromatography (CC), known as filtration, with solvents of increasing polarity.

For liquid/liquid extraction 12.94 g of the crude extract was used, which was then suspended in a MeOH/H_2O solution 2:3 (methanol-water) and extracted exhaustively and successively in n-C_6H_{14} (hexane), $CHCl_3$ (chloroform), and EtOAc (ethyl acetate).

The other part of the extract (12.33 g) was used to perform column chromatography, using solvents of increasing polarity. Several extractions were performed until the exhaustion of soluble substances in the solvents used. The phases obtained after partition and filtration were concentrated in a rotary evaporator and total drying was performed at room temperature (Figure 1).

The ethanolic extract of the stem was subjected to the spray drying technique, in order to evaluate the activity of the extract through this drying method.

Figure 1 - Flowchart of liquid-liquid extraction/partition and Column Chromatography/ filtration. Maceió/AL, 2014



Evaluation of free radical scavenging capacity

A qualitative analysis of synthetic activity against DPPH (2,2-diphenyl-1-picryl-hidrazila) was performed based on the methodology proposed by Soler-Rivaset et al. (2000). Cromatoplacas were prepared containing aliquots of extracts of the stem and the stem bark as well as the fractions of the partition and filtration from leaf which were eluted with mixtures of suitable solvents for each sample tested (Table 1). Then, the positive standard [(+)-catechin, 1 mg/mL in MeOH] was applied and the plate was immersed for 10 seconds in a methanolic solution 0.4 µM of synthetic radical DPPH (1 mg/mL in MeOH).

Table 1 – Extracts and fractions subjected to qualitative assays (DPPH). Maceió/AL, 2014

Parts of Plant	Extracts	Eluents Systems
Leaves	Partition C_6H_{14}	C_6H_{14}-EtOAc 6:4
	Partition $CHCl_3$	$CHCl_3$-MeOH 9:1
	Partition EtOAc	EtOAc 100%
	Partition MeOH	$CHCl_3$-MeOH 7:3
	Filtration C_6H_{14}	C_6H_{14}- EtOAc 9:1
	Filtration $CHCl_3$	$CHCl_3$-MeOH 9:1
	Filtration EtOAc	EtOAc -$CHCl_3$ 8:2
	Filtration MeOH	NT
Stem bark	Crude ethanolic	EtOAc -$CHCl_3$ 7:3
	Crude ethanolic (Spray dryer)	NT

NT = Not tested.

After drying the cromatoplacas at room temperature, a possible antioxidant activity was suggested by the appearance of yellowish spots on a purple background when compared with the positive standard used [12].

The samples which showed positive results in qualitative assays were subjected to quantitative assays as shown in Table 2 [12,13].

Table 2 – Extracts and fractions subjected to quantitative assays (DPPH). Maceió/AL, 2014

Parts of Plant	Extracts	Eluents Systems
	Partition $CHCl_3$	$CHCl_3$-MeOH 9:1
Leaves	Partition EtOAc	EtOAc 100%
	Partition MeOH	$CHCl_3$-MeOH 7:3
Stem bark	Crude ethanolic	EtOAc -$CHCl_3$ 7:3

The consumption of DPPH was monitored by decreasing in absorbance at UV-VIS spectrophotometer, at a wavelength of 515 nm. For construction of the calibration curve was prepared a solution of 50 mL DPPH (solubilized in methanol) in a concentration of 40 mg/mL (100 µmol/L). From this, decreasing dilutions were prepared (40, 35, 30, 25, 20, 15, 10, 5 and 1 mg/mL).

The calibration curve was constructed from the absorbance measurements of UV/VIS radiation at 515 nm, containing 1 mL of each dilution. The experiment was performed in triplicate, with methanol as white and readings were made at intervals of 1 minute. After obtaining the absorbance values, the mathematical equation of the calibration curve: Y = a + bx was determined by linear regression analysis using the *Microcal Origin Pro* 7.0 program, where *y* is the mean or steady absorbance after 60 minutes; *a*, the linear coefficient obtained from the calibration curve; *b*, the slope or gradient of the line obtained from the calibration curve; and *x*, the concentration of DPPH at 60 minutes [13].

For quantitative evaluation, stock solutions of each sample were prepared at a concentration of 200 µg/mL (2 mg of each sample/10 mL methanol) and subsequent dilutions (150, 100, 50 and 25 µg/mL), DPPH solutions (200 µmol – 100, 50, 25, 12.5 µmol), as well as the calibration curve to obtain various measures of dispersion, such as standard deviation (SD) and correlation coefficients (R).

The calculation of the Effective Concentration (IC_{50}) values for the samples and standards used was performed in triplicate, at concentrations listed above and on the same conditions described for the calibration curve. Absorbance measurements of the reaction mixtures were obtained from 0.1 mL aliquots of sample or standard and 0.9 mL of the stock solution of DPPH [40 mg/mL (100 µmol/L)]. These measurements were made at 515 nm, every 15 minutes, totaling 60 minutes. The absorbance of the solutions were recorded against a blank (methanol).

The concentrations and the percentage of remaining DPPH (% $DPPH_{Rem}$) were determined by the relationship between the concentration of DPPH in the middle after reaction with the extract ([DPPH]T = t) and the initial radical concentration in the medium (40 mg/L or 100 µmol/L), from the mathematical equation of the calibration curve (Y = a + bx), and the constant values of absorbance obtained after 60 minutes for each concentration, according to the following equation:

$$\%[\mathbf{DPPH}]_{\mathbf{Rem}} = \frac{[\mathbf{DPPH}]_{\mathbf{T=t}}}{[\mathbf{DPPH}]_{\mathbf{T=0}}} * \mathbf{100}$$

Thus, IC_{50} value of the extract able to decrease 50% of initial concentration of DPPH was determined from the exponential equation obtained by the relative concentration of the samples or standard versus percentage $DPPH_{Rem}$, through the Microcal OriginPro 7.0 program [14,15].

A high potential to scavenge free radicals is expressed through a low rate of IC_{50}, because the lower the concentration of the extract required to inhibit oxidation of the radical at 50%, best antioxidant activity. Ascorbic acid (IC_{50} 37.37 ± 3.18 µg/mL, rapid kinetics) and BHT (IC_{50} 97.86 ± 2.52 µg/mL, slow kinetics) were used as positive standards. Samples with IC_{50} > 200 µg/mL were considered inactive.

Antimicrobial evaluation

The extracts and fractions were tested against the following strains of Gram-positive bacteria: *Staphylococcus aureus* (25923), *Staphylococcus epidermidis* (14990), *Streptococcus pneumoniae* (6303); Gram-negative: *Escherichia coli* (25922), *Pseudomonas aeruginosa* (15442), *Acinetobacter calcoaceticus* (23055), *Enterobacter aerogenes* (13048), *Proteus mirabilis* (7002) and the fungus *Candida albicans* (10231). The microorganisms were standardized and distributed by diagnostic CEFAR Ltda., São Paulo/SP, and American Type Cell Cellection (ATCC), Manassas/VA/USA.

Standardizing, storage and preparation of inoculum
For antimicrobial activity evaluation, bacterial and fungal inoculums were prepared from the suspension of each microorganism in Buffered Saline Solution (BSS). The inoculum was prepared by comparison with the scale MacFarland until to obtain turbidity vial number 0.5, corresponding to 1.5 x 10^8 CFU/mL. This, in turn, was sown on the surface of Petri dishes containing Mueller-Hinton Agar (MHA) for bacteria and Sabouraud Dextrose Agar (SDA) in the case of the fungus as standardized by the Clinical and Laboratory Standards Institute [16].

Drilling Test in Agar
A qualitative evaluation of the antimicrobial activity was determined by Drilling Test in Agar. A superficial base layer was prepared in Petri dishes with medium MHA or SDA. For preparation of wells in sterile medium, five inverted pipette tips with 7 mm in diameter were placed in equidistant points. In sterile test tubes containing 10 mL culture medium were added 100 µL of microbial solution and poured entire contents in the Petri dishes. After hardening of the culture medium, pipette tips were removed, giving rise to five wells.

Samples were prepared with 100 mg of the crude extract, fraction or partition, plus 1 mL of saline solution (0.9%) and 2 drops of Cremophor, thereby obtaining a test sample to 10%. Then, 50 µL of these test samples were added into each well previously made.

The negative control was composed of 1 mL of saline solution (0.9%) with 2 drops of cremophor, used in solubilizing the samples, and the positive control of bacterial viability was selected based on the results of antimicrobial susceptibility test [17]. For Gram-positive bacteria was used Ceftriaxone (30 µg/disk) and for Gram-negative Ciprofloxacin (5 µg/disk). For the fungus *Candida albicans* was used Miconazole (50 µg/disc).

For evaluation of microbial growth, the Petri dishes were incubated at 35°C for 24 h for bacteria and at 28°C for 48 hours for fungus. After this time, the zones of growth inhibition were measured with the aid of a manual caliper [16].

The results were analyzed according to the following criteria: halo of inhibition less than 9 mm characterizes inactive samples; between 9-14 mm, moderately active; greater than 14 and less than 17 mm, active; and greater than 17 mm, very active. Bioassays were performed in triplicate [17].

Determination of Minimum Inhibitory Concentration (MIC)
To determine the Minimum Inhibitory Concentration (MIC), the inoculum of 1.5 x 10^8 CFU/mL was rediluted in a proportion at 1:10 (v/v) to obtain the standard concentration used (1.0 x 10^4 CFU/mL). The MIC was performed in sterile microplates of polystyrene 96-well, with 12 columns (1 through 12) and 8 rows (A-H). A volume of 200 µL of the stock solution at a concentration of 2000 mg/mL of the plant samples was inoculated in triplicate in columns 1 through 9 of the line A. The other wells from line B were filled with 100 µL of BHI doubly concentrated.

Right after, an aliquot of 100 µL of the contents of each well was transferred line A to line B and after homogenization, the same volume was transferred to the C line, repeating this procedure until the line H. Thus, there were obtained decreasing concentrations in µg/mL: 1000; 500; 250; 125; 62.5; 31.25 and 15.62. Subsequently, 5 µL of the bacterial inoculum were added to each well.
For positive control of bacterial viability was used BHI doubly concentrate and microbial inoculum (5 µL); the negative control was assessed by the inhibitory activity of the diluent Dimethylsulfoxide (DMSO); and for the sterility control, only the culture medium was used.

The plates were incubated at 35°C for 18 hours for bacteria, and at 28°C for 36 hours for fungi. After this time, 20 µL of Trifenilte Trazolium Chloride 5% (v/v) were added to each well and the plates were reincubated for 3 hours. The change from colorless to red color meant the presence of microorganisms. The degree of activity was determined, MIC $\leq$ 100 µg/mL (active); 100 < MIC $\leq$ 500 µg/mL (moderately active); 500 < MIC $\leq$ 1000 µg/mL (low activity); and MIC $\geq$ 1000 µg/mL (inactive) [17].

Evaluation of larvicidal activity
The tests against 4th stage larvae (L4) of the mosquito *Aedes aegypti* were performed in the insectary of the Laboratory for Research on the Chemistry of Natural Products IQB/UFAL, according to the recommendations of the World Health Organization, with some modifications [18].

A. aegypti larvae were reared and kept in the Insectarium from Institute of Biological Sciences and Health of the Federal University of Alagoas, at an average temperature of 27 ± 4°C and relative humidity of 80 ± 4%, with photo

period of about 12 hours. The larvae were obtained from eggs deposited on filter papers, which were placed in plastic containers with water for hatching. Then, the larvae were fed with autoclaved chow for developing of pupae. Pupae were separated with aid of pipettes and placed in cages with protected screens for the development of adult mosquitos, whose feed was performed with 10% glucose soaked in cotton balls, exchanged daily. For maturation of eggs, females were fed with blood of pigeons from *Columbia livia* species.

Preliminary experiments were performed in triplicate at a concentration of 250 µg/mL. For this, 75 mg of each plant species sample were dissolved in aqueous solution of Dimethylsulfoxide (DMSO) to 0.33% (Table 3). For negative control, an aqueous solution of DMSO at 0.33% was used; and for positive control, a solution of 1% Temephos reformulated at a concentration of 3 µg/mL.

Table 3 – Extracts and fractions subjected to larvicidal tests. Maceió/AL, 2014

Parts of Plant	Extracts
Leaves	Fractions of the extract in $CHCl_3$ and Filtration in $CHCl_3$ and EtOAc
Stem bark	Ethanolic Extract

Fifteen larvae were used in the 4th stage of *A. aegypti* test with three replicates for each sample. 1/3 of a grain ration was added in each container containing larvae to eliminate any doubt as to whether mortality of larvae by the absence of food. The exposure of the larvae to the samples and positive and negative controls was 48 hours, and the test was read every 24 hours from the start of the test.

The larvicidal activity was evaluated through percentage mortality of larvae sensitive to the samples according to the following criteria: larval mortality greater than 75% characterizes promising activity; mortality between 50 and 75% partially promising; mortality between 25 and 50%, weakly promising; and mortality less than 25%, were considered inactive [19,20].

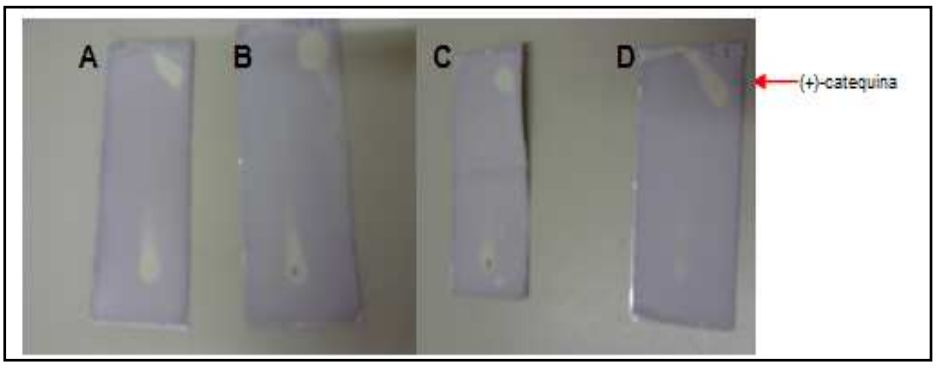
Samples with promising results in preliminary tests at a concentration of 250 µg/mL were tested in lowest concentrations of 200, 150, 100 and 50 µg/mL. Afterwards, the results were analyzed by Probity method for determining the Lethal Concentration (LC_{50}), along with a confidence interval of 95% (IC_{95}) [21].

RESULTS AND DISCUSSION

Evaluation of free radical scavenging capacity

Among the extracts that were subjected to the tests, a possible activity was suggested for the crude extract of the stem bark in EtOH and fractions from the leaf ethanolic partition in EtOAc, $CHCl_3$ and MeOH-H_2O. That is, all of these samples presented yellowish spots on a purple background compared to the positive control [(+)-catechin]. Thereby, only these extracts were quantified to confirm the antioxidant activity (Figure 2).

Figure 2 – Chromatograms after reaction with DPPH. Maceió/AL, 2014



Positive samples: A = partition in EtOAc; B = partition in MeOH-H_2O; C = crude ethanolic extract of the stem bark; and D = partition in $CHCl_3$.

Samples with positive results in qualitative assays were quantitatively evaluated regarding the radical scavenging capacity. In this analysis, it was determined the sample concentration required to reduce the initial concentration of

DPPH by 50% (IC_{50}) which is a measure of efficiency of the samples analyzed. IC_{50} values are inversely proportional to the activity, in other words, the smaller the value, the higher the antioxidant activity.

All samples tested showed IC_{50} higher than 200 µg/mL. These results suggested that these extracts are poor sequestrants free radicals or inactives (Table 4).

Table 4 – Results of the evaluation of activity from crude extract of the stem bark and leaves fractions against radical DPPH. Maceió/AL, 2014

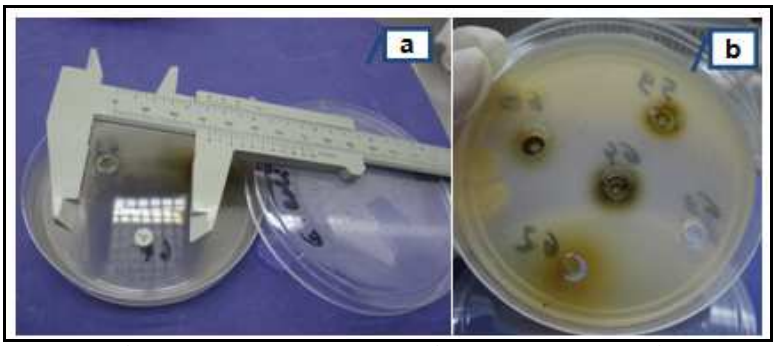
Parts of Plant	Extracts	DPPH (Qualitative)	DPPH CI_{50} ± DP(µg/mL)
Leaves	Partition C_6H_{14}	-	
	Partition $CHCl_3$	+	213.1± 5.2
	Partition EtOAc	+	264.0 ± 1.4
	Partition MeOH	+	1014.0 ± 0.7
	Filtration C_6H_{14}	-	
	Filtration $CHCl_3$	-	
	Filtration EtOAc	NT	
	Filtration MeOH	-	
Stem bark	Crude ethanolic	+	225.0 ± 2.0
Stem	Crude ethanolic (Spray dryer)	NT	

(-) = Negative; (+) = Positive; NT = Not Tested.

Evaluation of antimicrobial activity

The filtration fraction in $CHCl_3$ of the ethanolic leaf was moderately active against strains of *S. aureus* and *S. epidermidis* (inhibition zones of 9-14 mm). The crude ethanolic extract of the stem was active against these same lines (inhibition zones of 14-17 mm) and the leaf partition in acetate was considered moderately active against the strain of *P. mirabilis* (Table 5 and 6). For the other tested bacteria and for fungus, the extracts were inactive, which means that these microorganisms are not sensitive to the action of the analyzed samples (Figure 3).

Figure 3 – Drilling in Agar of fractions and extracts of *Z. tuberculosa* for *E. coli* bacteria. Maceió/AL, 2014



(a) Measurement of the positive control; (b) Example of tests with no inhibition of microbial growth.

Table 5 – Results of the antimicrobial activity of leaves samples in ethanol of *Z. tuberculosa*. Maceió/AL, 2014

Microorganisms	Partition/filtration and size of the halo (mm)					
	Positive Control	Negative Control	C_6H_{14}	$CHCl_3$	Acetate	MeOH
A. clocoaceticus	32.0	-/-	-/-	-	-/-	-/-
C. albicans	30.3	-/-	-/-	-	-/-	-/-
S. aureus	24.3	-/-	-/-	13.0	-/-	-/-
S. epidermidis	29.7	-/-	-/-	12.7	-/-	-/-
S. pneumoniae	35.0	-/-	-/-	-/-	-/-	-/-
E. aerogenes	32.3	-/-	-/-	-/-	-/-	-/-
E. coli	33.0	-/-	-/-	-/-	-/-	-/-
P. aeruginosa	35.0	-/-	-/-	-/-	-/-	-/-
P. mirabilis	36.3	-/-	-/-	-/-	9.7	-/-

(-) No zone of inhibition of bacterial growth.Tests performed in triplicate.

Table 6 – Antimicrobial activity of the crude ethanolic extract of stem bark from *Z. tuberculosa*. Maceió/AL, 2014

Microorganisms	Crude Extract/ Size of the halo (mm)			
	Positive Control	Negative Control	Stem bark/ Crude extract	Stem/ Crude extract
A. clocoaceticus	32.0	-	-	-
C.albicans	30.3	-	-	-
S.aureus	24.3	-	-	14.7
S.epidermidis	29.7	-	-	15.0
S.pneumoniae	35.0	-	-	-
E.aerogenes	32.3	-	-	-
E.coli	33.0	-	-	-
P.aeruginosa	35.0	-	-	-
P.mirabilis	36.3	-	-	-

(-) No zone of inhibition of bacterial growth.Tests performed in triplicate.

These results corroborate the study whose crude extract from leaf of *Z. tuberculosa* showed promising results against *S. aureus* and were inactive against strains of *C. albicans* and other fungi. This extract showed a zone of inhibition of 29 mm, greater than Gentamicin (positive control) and the fraction of filtration/$CHCl_3$ evaluated in these studies showed a zone of inhibition of 13 mm, both for strains of *S. aureus* [22]. When compared with this study, there is a divergence related to the fractions derived from the crude ethanolic extract from leaf, concluding that the crude extract showed better activity.

In studies of antimicrobial activity of crude extracts from plant species, the antimicrobial potential frequently is not due to a single substance, but rather a set of such substances. A plant drug may have multiple active ingredients with the same effect, allowing the phenomenon of synergism, and it can be superior in efficacy compared to isolates compounds [23].

Samples which showed sensitivity in the Drilling Test in Agar were qualitatively evaluated to determine the MIC. The results obtained with the MIC determination demonstrated that the filtration in $CHCl_3$ of leaf was moderately active against strains of *S. aureus*, *S. epidermidis* and *P. mirabilis*, as it showed a MIC < 125 µg/mL (Figure 4). Whereas the crude extract of the stem presented active against strains of *S. aureus* and *S. epidermidis* with a MIC < 62.5 µg/mL and MIC < 31.2 µg/mL, respectively (Figure 5), and moderately active against *P. mirabilis*, with MIC < 125 µg/mL (Table 7).

Tabela 7 - Resultado da CIM das amostras que apresentaram halo de inibição para três bactérias das nove testadas. Maceió/AL, 2014

Microorganisms	Extracts tested/ MIC (µg/mL)							
	Leaves (Filtration in $CHCl_3$)							
	≥ 1000	≤ 500 ≥	< 250	< 125	< 62.5	< 31.2	< 15.6	≤ 7.8
S. aureus	a	a	a	a	cb	cb	cb	cb
S. epidermidis	a	a	a	a	cb cb	cb	cb	cb
P. mirabilis	a	a	a	a	cb	cb	cb	cb
Crude extract of the stem bark								
	≥ 1000	< 500	< 250	< 125	< 62.5	< 31.2	< 15.6	≤ 7.8
S. aureus	a	a	a	a	a	cb	cb	cb
S. epidermidis	a	a	a	a	a	a	cb	cb
P. mirabilis	a	a	a	a	cb	cb	cb	cb

*(a) Activities of extracts; (cb) Inactivity of extracts; *Tests performed in triplicate.*

Evaluation of larvicidal activity

The literature reports several studies involving the activity of plant extracts against *A. aegypti* larvae. However, regarding to *Z. tuberculosa*, no report on the activity of this species was found. No significant results derived from extracts from leaf EtOH and crude extract of the stem bark subjected to preliminary tests (concentration of 250 ppm) against larvae in 4th stage for 24 and 48 hours were observed. Accordingly, all samples were considered inactive, with mortality less than 25%. Larval mortality was not observed in the negative control (solution 0.33% DMSO) and positive control (Temephos solution) the mortality rate was 100%. The results of the test larvicide are presented in Table 8.

Figure 4 – MIC in triplicate of the filtration fraction ($CHCl_3$) from ethanolic leaf (line D, column 1 to 3) and crude extract from stem (line E, column 1 to 3) negative control (column 7), growth control (Column 8) and sterility control of plate (Column 9) from *Z. tuberculosa* against *S. aureus*. Maceió/AL, 2014

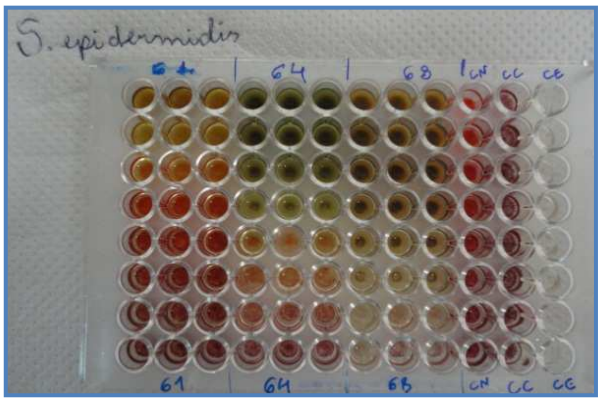


Figure 5 – MIC in triplicate of the filtration fraction ($CHCl_3$) from ethanolic leaf (line D, column 3 to 6) and crude extract from stem (line F, column 7 to 9) negative control (column 10), growth control (Column 11) and sterility control of plate (Column 12) from *Z. tuberculosa* against *S. epidermidis*. Maceió/AL, 2014

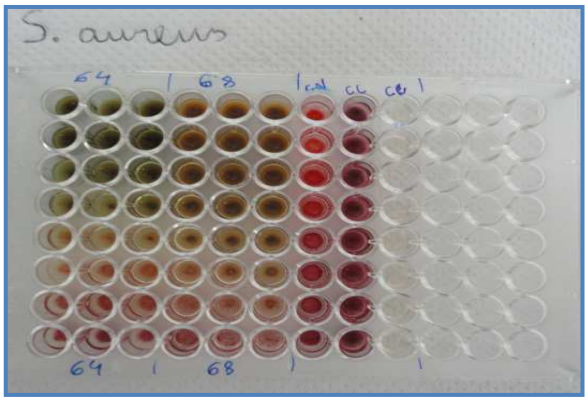


Table 8 – Preliminary results of larvicidal activity (250 µg/mL) with extracts and fractions from leaves and stem. Maceió/AL, 2014

Samples	Mortality 24 hours (%)	Mortality 48 hours (%)	Results
Leaves – Partition ($CHCl_3$)	13.33	4.44	Inactive
Leaves – Filtration ($CHCl_3$)	0.00	0.00	Inactive
Leaves – Filtration (EtOAc)	8.86	17.77	Inactive
Crude ethanolic extract from stem bark (EtOH)	0.00	0.00	Inactive
Temephos reformulated	100.00	100.00	Active

CONCLUSION

The tested samples of *Z. tuberculosa* showed up promising antibacterial activity ranging from moderately active to active against three of the nine bacteria evaluated, they are: *S. aureus*, *S. epidermidis* and *P. mirabilis*. Thus it showed promising antibacterial potential of this species confirming its popular use in treating bacterial infections. It was shown also that this species is a weak free radical scavenging, and shows no larvicidal activity against *Aedes aegipty* larvae.

Studies like this can be useful in order to guide researchers in the origin of new investigations on this plant. More systematic and thorough investigations of this plant species and their chemical components are needed to serve as subsidies for the future development of herbal medicine, promoting the incorporation of new scientific knowledge, as well as the development of unpublished researches with this species.

REFERENCES

[1] GF Oliveira. Avaliação da atividade antimicrobiana, *in vitro* do extrato hidroalcoólico bruto das folhas de *Syzygium cumini* (L.) Skeels (Jambolão). [Dissertação de Mestrado em promoção a Saúde]. Franca: Pós-graduação da Universidade de Franca, **2005,** [acesso em: 2012 jun. 20]. Disponível em: http://www.promocaodesaude.unifran.br/dissertacoes/2005/GUILHERME_FERREIRA_DE_OLIVEIRA.pdf
[2] A de Lima. Caracterização química, avaliação da atividade antioxidante *in vitro* e *in vivo*, e identificação dos compostos fenólicos presentes no Pequi (*Caryocar brasiliense*, Camb.). Tese (Doutorado)- Faculdade de Ciência Farmacêuticas da Universidade de São Paulo. Departamento de Alimentos e Nutrição Experimental. **2008,** São Paulo, 182p.
[3] ARM Iserhard; MLD Budó; ET Neves, MR Badke. Práticas culturais de cuidados de mulheres mães de recém-nascido de risco do Sul do Brasil. *Esc Anna Nery*, **2009,** 13(1), 116-22.
[4] ISM Silva, RFEP Santos, TVC Melo, et al. *J. Chem. Pharm. Res*., **2014,** 6(4), 663-69.
[5] JIF Filho, MGS Araujo; WVS dos Santos; MEL Correia; TLC Vasconcelos; RCSS Veríssimo et al. Atividade antibacteriana *in vitro* do extrato etanólico da *Capparis flexuosa* Linn. *Revista Enfermagem Atual in Derme*, **2014**, 68.
[6] MSM Alves; CM Patrizia; JGP Vieira; E Ozela; WLR Barbosa; JOC Silva Júnior. Análise farmacognóstica das folhas de *Arrabidaea chica* (Humb. &Bonpl.) B. Verlt., Bignoniaceae. *Rev.Bras.Farmacog,* **2010,** 20(2), 215-21.
[7] H Lorenzi. Árvores brasileiras: manual de identificação e cultivo de plantas arbóreas do Brasil. 5st Edition. Nova Odessa: Instituto Plantarum, **2008**.
[8] LN Seito. Caracterização da droga *Zeyheria montana* Mart. (Bignoniaceae) e fracionamento biomonitorado de seus constituintes ativos. [Tese de doutorado]. Botucatu – SP: Universidade Estadual Paulista, Instituto de Biociências de Botucatu, **2008,** 89p.
[9] MLA Bastos; LM Conserva; EMM Rocha; VS Andrade; RPL Lemos. Flavonoides e Atividade Antimicrobiana do caule da *Zeyheria tuberculosa* (Vell) Bur. (Bignoniaceae). ***25º Reunião Anual da Sociedade Brasileira de Química*** *– SBQ*, **2008,** 1-2.
[10] EA Rosa da; BC Silva; FM Silva. Da; CM A Tanaka; RM Peralta; CMA Oliveira de et al . Flavonoides e atividade antioxidante em Palicourea rigida Kunth, Rubiaceae. *Rev. bras. Farmacogn*, **2010,** 20(4), 484-88.
[11] MMM Lopes; RM de Carvalho-Okano; AL de Souza; HN de paiva. Crescimento de Mudas de Cipó-Cravo (*Tynanthus fasciculatus Miers*), uma Liana com Potencial Medicinal. *Revista Árvore*, **2008,** 32(20), 211-16.
[12] W; Brand-Williams; ME Cuvelier; C Berset. *Food Science and Technology*, **1995,** (28), 25-30.
[13] C Sánchez-Moreno; JA Larrauri; F Saura-Calixto. *Journal of the Science of food and Agriculture*, **1998,** (76), 270-76.
[14] CMM Sousa; HR Silva; GM Vieira-Jr.; MCC Ayres; CLS Costa; DS Araújo; LCD Cavalcante; EDS Barros; PBM Araújo; MS Brandão; M Chaves. Fenóis totais e atividade antioxidante de cinco plantas medicinais. *Química Nova*, **2007,** 30(2), 351-55.
[15] SMP Duavy; LJ da Silva; JGM da Costa; FFG Rodrigues. Atividade biológica de extratos de folhas de *Caryocar coriaceum* Wittm.: estudo *in vitro. Cad. Cult. Ciên*c, **2012,** 11(1), 13-19.
[16] CLSI - ClinicalandLaboratory Standards Institute. Performance Standards for AntimicrobialSusceptibility Testing; Twenty-Second. Informational Supplement, **2012,** 32(3), M100-S22.
[17] MCC Ayres; MS Brandão; GM Vieira-Júnior; JCAS Menor; HB Silva; MJS Soares. Atividade antibacteriana de plantas úteis e constituintes químicos da raiz de *Copernicia prunifera*. *Rev Bras. Farmacogn*, **2008,** 18(1), 90-97.
[18] WORLD HEALTH ORGANIZATION (WHO) [a] Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides, **1981,** (807), 1-6.
[19] PV Oliveira; JC Ferreira Junior; FS Moura; GS Lima; FM de Oliveira; PE Oliveira et al. *Parasitol. Res.*, **2010,** 107(2), 403-17.
[20] RB dos Santos; ER Cole; V Lacerda Junior; HR Rezende. Atividade larvicida, inseticida e repelente do óleo essencial dos frutos de *Schinus terebinthifolius* Raddi. *Sociedade Brasileira de Química*. **2008,** 31ª Reunião Anual da Sociedade Brasileira de Química.
[21] DJ Finney. Probit analises. *Cambridge University Press*, **1972**,14(1), 72.
[22] RS Ramos; PA Sarmento; TH Lins; IML Lúcio; LM Conserva; MLA Bastos. Atividade antimicrobiana *in vitro* dos extratos hexânico e etanólico das folhas de *Zeyheria tuberculosa. Rev Rene***. 2012**, 13(5), 1015-24.
[23] JB Müller. Avaliação das atividades antimicrobiana, antioxidante e antinociceptiva das folhas da *Luehea divaricata martius*. **2006.** Programa de pós-graduação em ciências farmacêuticas. Santa Maria, RS, Brasil.