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In vitro biological potential of Guanxuma-of-Horn [*Sebastiania corniculata* (Vahl) Mull. Arg.] in infection control

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ABSTRACT

Medicinal plants with antimicrobial properties built into the problem of infection have been investigated, guide the discovery of efficient against the emerging pathogens herbal related to bacterial and fungal infection. This study aimed to enlarge the possibilities of knowledge about antibacterial and antifungal activities of this plant, in the treatment of infections. The results of antimicrobial activity and cytotoxicity of two fractions in MeOH/H_2O from ethanol and acetone extracts of guanxuma-of-Horn [Sebastiania corniculata (Vahl) Mull. Arg.] (Euphorbiaceae) evaluated the extracts by disk diffusion test (DD) and the method of the Minimum Inhibitory Concentration (MIC). The extracts were active for Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus pneumoniae, Pseudomonas aeruginosa, Acinectobacter calcoaceticus and Proteus mirabilis in the DD test. However, the best results for these fractions were against P. aeruginosa with an MIC from 250 to 125 µg/mL, respectively. In the evaluation of cytotoxic for cell viability by MTT, only a dose of 100 µg/mL exhibited moderate cytotoxicity with $p < 0.0001$. In this perspective, the other extracts showed significant cytotoxicity, which suggests further research to evaluate the anti-tumor activity and consolidate the evidence obtained in the research present.

Key words: *Euphorbiaceae*, Microbial Sensitivity Tests, Cell Viability.

INTRODUCTION

The use of plants with medicinal purposes, either in the treatment, cure or prevention of disease, is one of the oldest forms of medical practice of humanity [1-2]. Bio-monitoring studies are developed and improved, since plants with proven efficacy for a particular biological activity are possible raw materials for natural or synthetic drugs [3].

The search for a natural product that is able to contain the fungal and bacterial infection, when installed, and reduce antibiotic resistance and its adverse effects [4], and consequently enhance the control of the infection, is valid for the prospect of a new herbal medicine that promote this action. The incentive of the World Health Organization (WHO) to the use of alternative treatments for health has stimulated research and the use of therapeutic practices considered by many health professionals as popular or non-scientific practices, and including it has provided the reintroduction of medicinal herbs as an alternative or add-on therapy [5-6].

The cost with the infected wound treatment is costly and thus there is great interest among nurses in the expansion of their knowledge in the area of treatment of infections in wounds, being more than a nursing care, a specialty that requires multi-professional interventions, including the development of new technologies to meet the needs of the individual with wounds [7]. In this context, it is highlighted the nurses and the nursing process applied which involves skills since data collection until the evaluation of the final results of its interventions. Another aspect to highlight is the orientation toward health promotion and self-care involving wounds and infection control [8].

In addition to providing healthcare, the use of all the benefits that the technology through basic search makes it possible to offer, can relate to knowledge from basic research in the context of clinical practice. Therefore, the findings that the technology makes it possible contribute to the use of what nature has to offer [9]. In this way, it is important the participation of health professionals in this area, aimed at an integration of conventional knowledge used by popular health system, because the alternative therapies may enable the individual relative autonomy in relation to health care [10].

It is common to use plants in experimental research especially in *in vitro* test and the evidence of results of tests with emphasis on antimicrobial activity of several plant species, like *Symphytum officinale* L. (Confrey) and *Copernicia prunifera* [11-12] used successfully in biological processes, in order to provide improvements in wound healing, including present antimicrobial activity.

The promising results with the use of these plant species strengthen the importance of investigations to assess the biological potential of Brazilian flora, through experimental tests so that their results will be used in clinical practice [13]. Several plant species of the *Euphorbiaceae* family are popularly used against viral etiology diseases, antimicrobial, antinflammatory, antiulcerogenic, analgesic, anti-hypertensive, muscle relaxer [14-15].

The object of study is the species is the *Sebastiania corniculata* (Vahl) Mull. Arg., belonging to the genus *Sebastiania*, Family Euphorbiaceae, order Malpighiales, Magnoliophyta class, Magnoliopsida Division and the Kingdom Plantae, popularly known as false-guanxuma and/or guanxuma-of-Horn [16]. The genus *Sebastiani*a includes approximately 158 species distributed mainly in tropical regions, where the largest centers of dispersal are in South America and Africa [17].

Some species *Sebastiania* are used popularly as antidiarrheal drug, antibacterial and for the elimination of kidney stones. Among other activities reported for species of the genus it is highlighted the antifungal, antibacterial, analgesic, antispasmodic, antiviral and Antinociceptive [18]. Phytochemical studies conducted with species of the genus *Sebastiania* describe the occurrence of compounds with great structural diversity, such as acetophenone xantoxilina, steroids, triterpenes, coumarins, flavonoids, phenolic alkaloids and derivatives that corroborate the biological activities, popularly cited [18-20].

It is searched with the study monitoring species *Sebastiania corniculata* enlarging the possibilities of knowledge about antibacterial and antifungal activities of this plant, in the treatment of infections.

EXPERIMENTAL SECTION

In vitro experimental study, conducted in the Laboratory of Research in Treatment of Wounds (LpTF) of the School of Nursing and Pharmacy at the Federal University of Alagoas, using plant extracts of guanxuma-of-horn [*Sebastiania corniculata* (Vahl) Mev. Arg].

Vegetal Material

The species *Sebastiana corniculata* (MAC nº 10763) collected from different localities of Alagoas, was ceded by the Institute on the Environment of the State of Alagoas, where the plant specimens are deposited. The whole plant was used in the test.

Preparation of extracts

The preparation of extracts and fractions was performed at the Laboratory of Research in Chemistry of Natural Products (IQB/UFAL). The vegetal material (1,360 g) after drying at room temperature and trituration was submitted to maceration with acetone and ethanol 90%. The crude extracts in acetone (38.3 g) and ethanol (21.5 g), were obtained in a rotary evaporator. The extracts were suspended later in MeOH/H_2O solution (6:4) and successively extracted with C_6H_{14} and in CH_2Cl_2. The fractions in C_6H_{14} were treated with NaOH to 2%. In the present study, it was used the ethanolic extract/fraction MeOH/H_2O (16.2 g) and the acetone extract/fraction MeOH/H_2O (13.0 g).

Micro-organisms and conditions for cultivation

The 16 patterned lines, distributed by CEFAR Diagnostics Ltda., São Paulo, SP, Brazil, 10 bacteria: *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* CCCD S011, *Streptococcus pneumoniae* CCCD, *Shigella flexneri* CCCD S006, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* CCCD P001, *Klebsiella pneumoniae* CCCD K001, *Enterobacter aerogenes* CCCD E001, *Acinectobacter calcoaceticus* CCCD A001 *enteric subspecies Salmonella* CCCD S004, *Enterobacter cloacae* CCCD E010, grown on the AMH 35º C, and 05 fungi: *Candida albicans* ATCC 10231, *Candida tropicalis* ATCC13803, *Candida parapsihoris* ATCC 22019, *Aspergillus brasiliensis* ATCC 16404 and *Saccharomyces cerevisiae* ATCC 9763, grown in ADS to 28 ºC.

Antimicrobial Testing

Disk diffusion

Evaluation of the *in vitro* antimicrobial potential of vegetal species occurred by paper disk technique by Kirby-Bauer methodology cited by Santos et al. [21], with adaptations, in which diluted suspensions of microorganisms were inserted in conveniently plates with solid state culture medium. The dilution factors were adjusted to cloudiness, in accordance with the scale of 0.5 McFarland (10^8 CFU mL-1) for use in antimicrobial activity test [22]. In the end, the paper disks received 20 µL of extract solutions, resulting in a concentration of 50 mg/mL. Subsequently, these plates were placed in an bacteriological oven for 24 hours to 35° C in the case of bacteria, and to 28° C for 48 hours, of fungus [23] in an inverted position.

After the incubation period, visual readings were performed, observing the halos of inhibition of bacterial growth and quantified in millimeters with the aid of a caliper. It was adopted the following criteria for classification as microbial growth inhibition [24]: a) inhibition zone greater than 75%, the extract was considered active; b) inhibition zone of more than 25% but less than 75%, the extract was considered moderately active; c) zone of inhibition less than 25%, the extract was considered inactive. Bioassays were performed in triplicate.

For each micro-organism, standard antibiotic was used as positive control, which was chosen from the antimicrobial susceptibility test, being for Gram-positive bacteria the Ceftriaxona 30 µg and Gram-negative ciprofloxacin 5 µg. To fungus, *S. cerevisiae* and *C. parapsilosis*, Tioconazole 25 µg for *A. brasiliensis* and *C. albicans* Miconazole nitrate 50 µg and *C.* tropicalis Nystatin 100 IU. As negative control, it was used ethanol (EtOH) absolute PA. Extracts considered active or moderately active were again evaluated for determination of minimum inhibitory concentration (MIC). The tests were conducted in triplicate

Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) was obtained through the method of Microdilution in stock, held in sterile microplates 96 wells, flat bottom, as described previously in the literature [12], with adaptations. For the realization of the test it was prepared a stock solution of the sample of 2000 µg/mL. Microbial Inoculants on concentration of 0.5 McFarland (10^8 CFU/mL) were rediluted 1:10 (v/v) for obtaining the standard concentration used (10^4 CFU/mL). A 200 µL volume of the stock solution at a concentration of 2000 µg/mL of vegetable samples tested was inoculated in triplicate, on the columns of the row 9 to 1.

The remaining holes from the line B were filled with 100 µL BHI stock doubly concentrated [25]. Then a rate of 100 µg/mL of the contents of every orifice of the line A was transferred to the holes of line B and, after homogenization, the same volume was transferred to the line C, repeating the procedure until the line H, obtaining the concentrations in µg/mL of 1000; 500; 250; 125; 62.5; 31.2 and 15.6. Subsequently, in each hole were added 5 µL of microbial inoculum.

For the positive control bacterial viability, BHI stock were used and the microbial inoculum (5 µL); the negative control was evaluated through the inhibitory activity of the solvent DMSO; and for the control of sterility, it was used only the stock [8]. Each concentration sample was tested at tripling. The microplates were placed in incubator at 35 ºC (bacteria) for 18 hours and 28 ºC for 48 hours (fungus).

After this time interval, to each of the 20 µL holes were added of an aqueous solution of TTC (v/v) to 0.5% and the microplates again re-incubated for another three hours. The presence of a red color in the holes was interpreted as negative evidence of inhibitory effect of extract, while the absence of red coloring is considered proof positive of the inhibitory action of the extract. The degree of the activity was determined according to the criteria: MIC < 100 µg/mL: Active; 100 µg/mL < MIC < 500 µg/mL: moderately active; 500 µg/mL < MIC µg/mL ≤ 1000: low activity; and MIC ≥ 1000 µg/mL: inactive [12].

Cytotoxicity test

For this test, we used the method of the MTT colorimetric, based on mitochondrial activity of cells by MTT reduction, through the cleavage of tetrazolium salt, as described previously in the literature [26]. The optical density resulting from the MTT test was determined by spectrophotometer. Line macrophages J774were used, in the density of 2 x 10^5 cells per well, cultured in DMEM and supplemented with 10% fetal bovine serum, sown in plates of 96 wells. The results were analyzed in the program *Graphpad prism*® 5.0. The data were presented as mean ± standard error of the mean (SEM), compared by ANOVA complemented by Tukey test with a significance level of 5% ($p \leq 0.05$) and expressed in graphics.

RESULTS AND DISCUSSION

The Table 1 presents the results of antimicrobial activity of guanxuma-of-Horn for seven of the 16 microorganisms tested against the Ethanol extracts/MeOH/H_2O fraction and fraction of acetone/MeOH/H_2O.

Extracts subjected to antibacterial testing against the gram-negative strains of *Klebsiella pneumoniae, Salmonella enterica* subspecies, *Enterobacter cloacae enteric, Enterobacter aerogenes*, were considered inactive, since it did not show halo of inhibition. Likewise, in the face of strains of *Candida albicans, Candida tropicalis, Candida parapsilosis, Saccharomyces cerevisiae, Aspergillus brasiliensis*, being also considered inactive.

Table 1: Antimicrobial activity to disk/diffusion of vegetal species *S. corniculata*. Maceió/AL, Brazil, 2014

Microorganism	Concentration (mg/disk)	Inhibition Halo diameter (mm)/Inhibition				C+	C-
		Ethanol/fraction MeOH/H_2O		Acetone/fraction MeOH/H_2O			
		DHI	ICM (%)	DHI	ICM (%)		
S. aureus	100	13	51	12	47	25	-
P. aeruginosa	100	10	29	16	47	34	-
S. epidermidis	100	11	33	14	40	34	-
S. pneumoniae	100	11	31	14	39	35	-
A. calcoaceticus	100	-	-	7	24	30	-
P. mirabilis	100	10	25	21	52	41	-
S. flexneri	100	12	29	19	46	41	-

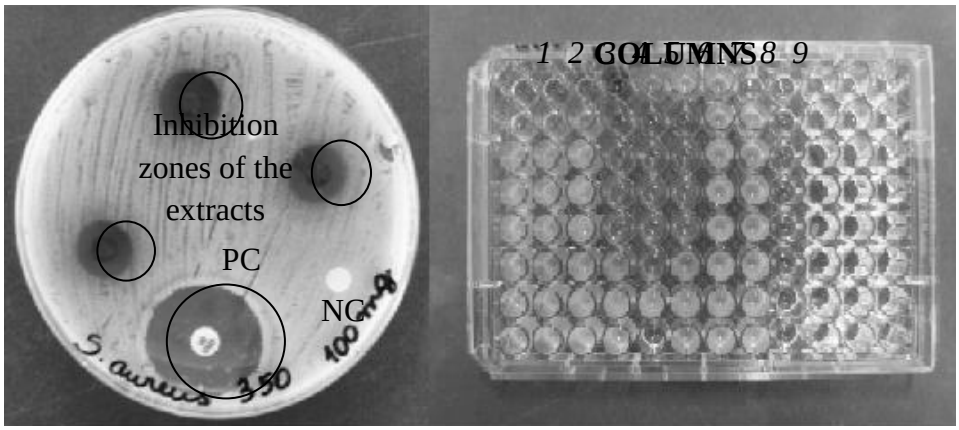
Key - DHI: Average of halo diameter of inhibition in triplicate; ICM: microbial growth inhibition; (-): Absence of inhibition; C +: Standardized (Ceftriaxone 30 µg 5 µg and ciprofloxacin); C-: 20 µL/EtOH absolute PA disk.

From the results achieved outside the disk diffusion test, it was found that the tested extracts of the species *Sebastiania corniculata* presented best inhibitory activities classified as moderately active, to the species *S. aureus* and *P. aeruginosa* (Figure 1). In other studies, plant species of the family *Euphorbiaceae*, among them *palidullus Croton, Croton ericoides, Phyllanthus acidus* inhibited the growth of *S. aureus* [27-28]. *Croton heterocalyx and Euphorbia hirta*, plant species of the family *Euphorbiaceae*, corroborate results found for *P. aeruginosa* [29].

The moderate inhibitory potential of *S. epidermidis* in extracts tested is important, since this is the primary microorganism causing fast progressing bacteremia may present and sepsis associated with implanted devices that produce a polysaccharidic layer connecting the catheters and shunts, protecting them from antibiotics and inflammatory cells [30]. Absence or reduction of the bactericidal action of vegetable samples against Gram-negative bacteria have been observed by other researchers, thus corroborating the obtained results [12]. While the absence of fungicidal activity may be due to the vegetable samples tested, not compromising the synthesis of ergosterol, not acting on the fungal membrane and cellular permeability [31].

The fraction MeOH/H_2O from the ethanolic extract of *S. corniculata* presented to Gram-positive bacteria (*S. aureus, S. epidermidis, S. pneumoniae*-MIC of 250 µg/mL), while for the Gram-negative species (*P. aeruginosa* and *P. mirabilis*-MC of 250 µg/mL for *A. calcoaceticus* of 1000 µg/mL and *S. flexneri* CIM greater than 1000 µg/mL), as shown in Table 2.

Figure 1: Halos of growth inhibition of *S. aureus* (A); Minimum Inhibitory Concentration of extracts against *P. aeruginosa* (B). Maceió/AL, Brazil, 2014



A **B**

Halos of inhibition of the growth of strain against S. aureus (A). Micro-dilution test in triplicate (B), with the ethanolic extracts (column 1 to 3) and acetone (column 4 to 6), growth control (column 7), negative control (column 8) and sterility control (column 9) of S. corniculata against P. aeruginosa

Table 2. Minimum Inhibitory Concentration (MIC) of plant extracts of *S. corniculata.* Maceió/AL, Brazil, 2014

Microorganism	Vegetal extract (µg/mL)	
	Ethanol/Fraction MeOH/H_2O	Acetone/Fraction MeOH/H_2O
S. aureus	250	250
P. aeruginosa	250	125
S. epidermidis	250	500
S. pneumoniae	250	1000
A. calcoaceticus	1000	500
P. mirabilis	250	1000
S. flexneri	>1000	1000

In relation to the evaluation of the Minimum Inhibitory Concentration (MIC), the plant extracts of *S. corniculata* tested is performed with great prospects for the continuation of the tests for further developing a phytotherapeutic.

From the results of the MIC, *S. aureus, S epidermidis and P. aeruginosa* proved with inhibitory potential moderately active, results that corroborate previous studies that presented inhibitory potential of moderate to high front to this bacteria [27-29].

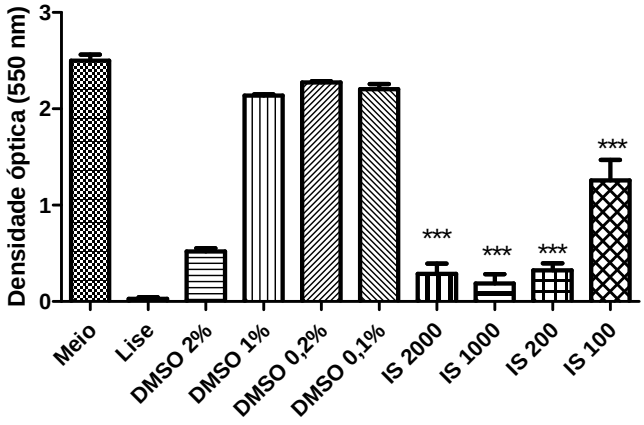
The cytotoxicity test assesses cell viability of MeOH/H_2O fraction from the extract in acetone, in concentrations of 100, 200, 1000 and 2000 µg/mL. In the control group, the feasibility was 100%, while in the samples tested at concentrations of 100, 200, 1,000 and 2000 µg/mL were obtained the percentages of 50.3%, 13.0%, 7.5% 11.5%, respectively. Only the concentration of 100 µg/mL showed moderate cell viability when compared with the control group (middle). The comparative analysis of the data showed that the viability of crops treated with the specimen tested in different concentrations for 48 hours was significantly toxic when compared to the culture medium.

For the absorbencies were found the values of 2.502 ± 0.05998 for the control group, 0.02752 ± 0.01901 for Lise, 0.5217 ± 0.03219, 2.139 ± 0.01233, 2.274 ± 0.01090 and 2.204 ± 0.05523 - for the 2% DMSO, 1%, 2%, 1%, 0,2% e 0,1%; respectively. As for the samples IS 2000, IS 1,000, IS 200, IS 100 of the 350 samples, the absorbencies found were 0.2887± 0.1040, 0.1880 ± 0.09580, 0.3267 ± 0.07083 and 1.259 ± 0.2109, respectively. In this study the absorbance values found were lower than control cells (Middle – 100%) indication of reduction in the rate of cell proliferation.

Other studies corroborate with statistically significant cytotoxicity in the family *Euphorbiaceae* species are cytotoxic to cancer cells, including *Euphorbia milii, Euphorbia tirucalli, Jatropha curcas, Ricinus communis, Manihot utilissima, Euphorbia Kansui* [15, 26].

The lowest values found for the samples IS 2000, IS 1000 and IS 200 show that, after 48 hours of the test, there was a low of viable cells. So, from the evaluation of cell viability, it can be said that the fraction MeOH/H_2O from the acetone extract in their concentrations induced a cellular toxicity in line J774 macrophages. This result contributes to a study that describes the MTT cytotoxicity increased significantly, in samples from in acetone extracts [32].

Figure 2: Cell viability of acetone extract/MeOH/H_2O. Maceió/AL, Brazil, 2014



*Effects of MeOH/H_2O fraction of the entire plant (IS) in concentrations 2000, 1000, 200 and 100 µg/mL * ($p < 0.05$) ** ($p < 0.01$) and * ** ($p < 0.0001$)*

The results showed that the best antimicrobial activity of both fractions in MeOH/H_2O of *S. corniculata* against the *P. aeruginosa*, which ranging from 250 to 125 µg/mL, respectively. The comparative analysis of the data showed that the viability of crops treated with the specimen tested in different concentrations for 48 hours was significantly less than the half at the same time experimental. The comparative analysis of the data showed that the viability of the cultures treated with the specimen tested at different concentrations for 48 hours was significantly lower compared to the culture medium revealed a trend of toxicity for these extracts.

CONCLUSION

This research clearly demonstrates the antibacterial activity of fractions in ethanol and acetone, the extract derived MeOH/H_2O against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Acinectobacter calcoaceticus*. However, the best results for these fractions were against *P. aeruginosa* with an MIC from 1250 to 125 µg/mL, respectively.

The comparative analysis showed that the viability of the cultures treated with the specimen tested at different concentrations for 48 hours was significantly lower compared to the culture medium revealed a trend of toxicity for these extracts. The results of the cell viability test point further studies to evaluate the anti-tumor potential of said extract.

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