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Antimicrobial potential evaluation of hydroethanolic extracts of the species *Anacardium occidentale* Linn.

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The species *Staphylococcus aureus* and *Escherichia coli* have shown increased antimicrobial resistance, which can be overcome with plant extracts, in view of their molecular complexity, for example extracts of *Anacardium occidentale* Linn (cashew). The objective was to evaluate the *in vitro* antimicrobial activity of cashew stem bark extract. Samples were collected in Santa Cruz / RN / Brazil, and after dried and crushed, the extract was prepared by maceration with ethanol: water solution (70:30 v / v) at a ratio (1:10 w / v) for 7 days. The filtrate was reduced by rotary evaporator and the resulting frozen. Extracts were characterized by Thin Layer Chromatography (TLC) and tested for antimicrobial activity in various concentrations (200 to 6.25 mg / ml) by diffusion disk. TLC testing indicated that the plant extract has phenolics, tannins mainly responsible for the pharmacological properties. The minimum inhibitory concentration was determined at 12.5 mg / ml only for *S. aureus*.

Key words: Cashew, antimicrobial activity, *S. aureus*.

INTRODUCTION

Humans are often affected by infections caused by microorganisms in the environment, especially bacterial species *Staphylococcus aureus* and *Escherichia coli*, which are showing increasing resistance to antibiotics (Tortora et al., 2005; Davis et al., 2005; Teixeira et al., 2008; Gomes, 2008; Howden et al., 2011; Sakunpak and Panichayupakranant, 2012).

An alternative to mitigate this problem refers to the use of medicinal plants whose products have higher molecular diversity than that derived from synthetic products (Davis et al., 2005; Coutinho et al., 2008; Santos, 2011).

Plant species were always very important to humanity, as a food or for the treatment of diseases. Since ancient times there are reports of the use of medicinal plants to treat infections caused by microorganisms (Leite, 2009; Ansarullah et al., 2009; Van der kooy et al., 2009; Chandrasekaran et al., 2010; Brasil, 2012). Thus, there is the importance of scientifically analyze the antimicrobial activity of plant species, because these studies can contribute to a better understanding of its safety and
therapeutic efficacy. In Rio Grande do Norte / Brazil several plants are used for medicinal purposes (Rodrigues and Casali, 2002).

An example of this is the stem bark of Anacardium occidentale Linn, popularly known as cashew (Trevisan et al., 2006). A. occidentale Linn, belonging to the family Anacardiaceae, has the decoction extract of the stem bark used in traditional medicine, both for the oral or topical treatment for diarrhea and wound antisepsis (Lorenzi and Matos, 2002).

The literature reported several pharmacological activities (anti-inflammatory and antimicrobial) that have been proven from this plant (Trevisan et al., 2006; Konan and Bacchi, 2007; Kubo et al., 2011; Santos, 2011; Arekemase, 2011; de Abreu et al., 2013).

Therefore, this study aimed to evaluate the in vitro antimicrobial activity of the stem bark extract of the species A. occidentale Linn (cashew) against the bacteria S. aureus and E. coli.

METHODOLOGY

Sample collection and processing plant

The bark of the stem of the species A. occidentale Linn (cashew) has been previously cleaned with water before the procedures. The identification of plant species was based on the collection of the Herbarium of the Federal University of Rio Grande do Norte.

The samples were dried in an oven (BioPar -S150SD) at 33°C for 7 days. Later there was a size reduction of cashew samples by hand to then be grinded in a mill (Botini - F001643) previously sanitized. After grounding, the samples were stored in a desiccator for further realization of extraction (Gonçalves et al., 2005; Silva et al., 2007; Bertuccil et al., 2009; Palmeira et al., 2010).

Hydroethanolic extraction

The extraction was performed by soaking in ethanol:water (70:30 v/v), 1:10 w/v, for 7 days under periodic agitation. The extract was filtered and the solution was reduced on a rotary evaporator (IKA - Control Model RV 10 CV) at 40°C. The crude extracts were obtained for each sample, which were stored in a freezer maintained at approximately -10°C.

Phytochemical characterization of plant samples

The qualitative phytochemical analysis of hydroethanolic extract was performed by Thin Layer Chromatography (TLC), as fixed phase silica gel F254 plates (MACHEREY-NAGEL) and as a mobile phase, which was used twice, with polar and nonpolar solvents. The first mobile phase used was ethyl acetate: acetic acid: formic acid: distilled water (10: 1:1.1: 2.6 v/v/v/v). The second mobile phase used was tolouene: ethyl acetate:formic acid (5: 5: 0.5 v/v/v), Figure 1. The chromatographic developed users were the sulfuric vanillin (universal developer), ferric chloride (developer phenolic compounds), natural reagent A (specific reagent for the class of flavonoids) and the Dragendorff reagent (revealing specific to the class of alkaloids) (Alves et al., 2011).

Bacteria

To achieve the standard in vitro tests we used strains of S. aureus and E. coli acquired in the Biosciences Center of the Federal University of Rio Grande do Norte - UFRN.

Determination of minimum inhibitory concentration (MIC)

The Hydroethanolic extracts were diluted in sterile distilled water and used in decreasing concentrations (200 to 6.25 mg/mL). The antimicrobial activity on plates was performed by antimicrobial susceptibility testing by disk diffusion, and used filter paper discs (Qualy) having 6 mm diameter, previously sterilized before, being impregnated with the extract of samples to be tested. As the culture medium, we used the Mueller Hinton Agar (DIFCO), being prepared according to the manufacturer's specifications (NCCLS, 2003). Strains of the species S. aureus and E. coli were grown in nutrient broth BHI (Brain Heart Infusion) and incubated at 37°C for 24 h. Then, it was diluted (1 mL of bacterial culture / 10 mL of sterile saline - 0.85% NaCl), and this solution was then used to seed the surface of plates containing culture medium (Catão et al., 2006; Silva et al., 2007).

After the seeding was introduced to the plates disks containing extracts to be tested, it then was incubated for 24 h at 37°C for measurement of the diameter of the inhibition zones (Nccls, 2003). As a positive control we used disks containing the antibiotic gentamicin – 10 μg/disc (Poly Sensidisc DME 15 Gram Positive) for S. aureus and ciprofloxacin - 10 μg/disc (Poly Sensidisc DME 15 Gram negative) to E. coli, as a negative control we used on filter paper discs (Qualy) impregnated with sterile distilled sterile water.
Figure 2. Antimicrobial activity of the hydroalcoholic extract of cashew against S. aureus: A: 200 to 125 mg/mL; B: 12.5 mg/mL.

RESULTS AND DISCUSSION

Phytochemical characterization

Qualitative tests such as Thin Layer Chromatography (TLC) are able to demonstrate the presence or absence of chemical constituents (Costa, 2011). The plates eluted at more polar solvent system, showed the major components of the extract with the formation of well-defined bands. After processing with sulfuric vanillin, universal reagent, it was possible to observe the pink and red color bands. This staining is characteristic for phenolic compounds of the tannin class. These bands were also observed after the revelation of ferric chloride, with the development of blue color (presence of phenolic compounds). There was no formation of flavonoid characteristic bands after the revelation with natural reagent A. The revelation with Dragendorff reagent has not determined the observation characteristics of alkaloids bands.

Several studies have reported that the shell and leaves of the cashew have lots of phenolic compounds, mainly tannins, which are primarily responsible for the pharmacological properties (Hislan, 1966; Mota, 1982; Melo et al., 1997; Konan Ebacchi, 2007; Kubo et al., 2011). The tannins present in cashew shell are seen as responsible for their antimicrobial activity (Castillo-Juárez et al., 2007; Cui et al., 2008).

Minimum inhibitory concentration (MIC)

Microbiological studies showed that the hydroethanolic extract (cashew) was able to inhibit the in vitro growth of S. aureus, with 12.5 mg/mL as a minimum inhibitory concentration (MIC) (Figure 2).

The disks containing 200 mg/ml hydroethanolic extract showed the greatest inhibition zone against S. aureus, while discs containing 12.5 mg/ml showed smaller inhibition zones. However, no inhibition of E. coli species. The negative controls (disks with sterile distilled water) showed no inhibition against bacterial species. Already in the positive controls were observed inhibition zones: ciprofloxacin (10 mg/disc) - 30 mm front of E. coli and gentamicin (10 mg / disc) -32 mm against S. aureus.

The results are similar to those presented by Silva et al. (2007), confirming its potential use as a medicinal plant in infectious processes. In the study of Dahake et al. (2009), the antibacterial activities of hydroethanolic extract and petroleum ether extract of cashew stem bark showed significant variations, and among the extracts tested the hydroethanol showed the highest antimicrobial activity. The better antimicrobial activity was against S. aureus and Bacillus subtilis.

The results observed in a study by Akinjogunlaa et al. (2012) showed that the hydroethanolic extract of cashew stem also has inhibitory effect against Streptococcus mutans. In other studies it was shown that the extract also presents antibacterial effect opposite to the species of Streptococcus (Melo et al., 2006). In addition, inhibitory actions against gram-negative bacteria are reported as Proteus morgani, Pseudomonas aeruginosa, E. coli and Salmonella typhi, with high concentrations of the hydroethanolic extract (Laurens et al., 1992). Therefore, it is appropriate to popularly use for the treatment of infection and inflammation of the gums, mouth and bronchitis for example (Silva et al., 2007).

Among the phenolic compounds, tannins are one of those which have received more attention due to its antimicrobial activity compared to other phenolic
compounds, and the fact that most of them are able to inhibit microorganisms and virulence factors. Furthermore, tannins may also exhibit synergism with antibiotics (Rodriguez Vaquero et al., 2010; Jayaraman et al., 2010; Saavedra et al., 2010). Several mechanisms may explain the effect of tannins in inhibiting bacterial growth, such as destabilization of the plasma membrane, the inhibition of enzyme activity, actions that occur directly on the microbial metabolism and the deprivation of the substrate required for microbial growth, especially essential minerals such as iron and zinc (Dixon et al., 2005; Heinonen, 2007).

**Conclusion**

The hydroethanolic extract of the species *A. occidentale* (cashew) has antimicrobial activity on the species *Staphylococcus aureus*, and its antimicrobial potential is similar to that observed in the literature. The fact of the same species have not shown inhibitory effect on *E. coli* species and positive results presented in other studies may have been due to the extraction method used. However, despite this study along with other show the antimicrobial potential of the extract to *S. aureus*, other studies are needed to better characterize the potential use, including toxicological risks.

**Conflict of Interests**

The authors have not declared any conflict of interests.

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