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Full Length Research Paper

Antimicrobial activity of *Agave sisalana*

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This study was carried out to evaluate the antimicrobial activity of extracts of the leaves and leaf waste discarded in the process of obtaining the hard fibers of *Agave sisalana*. The antimicrobial activity was determined by the paper disk diffusion method using Gram-positive and Gram-negative bacteria (non-resistant and resistant to antibiotics) and a fungus. The hydroalcoholic extract obtained from leaves and from sisal waste showed significant inhibition of *Candida albicans*, on the other hand, it was inactive against three strains of *Staphylococcus aureus*, two strains of *Escherichia coli*, a strain of *Micrococcus luteus, Bacillus cereus, Pseudomonas aeruginosa* and *Salmonella choleraesuis*. The methanol extract of leaves showed weaker reduction in the inhibitory action of *C. albicans* when compared with the above extracts, and it was also inert against the other microorganisms tested.

Key words: *Agave sisalana*, antimicrobial activity, *Candida albicans*.

INTRODUCTION

Infections caused by pathogenic microorganisms are responsible for high rates of morbidity and mortality in Brazil (Coelho et al., 2007; Souza et al., 2007). These infections can occur in invasive form, and are an increasing problem due to the increase of their incidence in hospitals, especially in patients who are undergoing cancer treatment, transplantation or are immunosuppressed for other reasons (Oliveira et al., 2001).

The search for new compounds with antimicrobial activity from plants has been the subject of intense research in recent years (Harvey, 2007; Lee et al., 2007; Hostettmann et al., 2003). This is due mainly to the fact that the plants are widely used in folk medicine to combat various diseases in humans caused by bacteria and fungi (Stefanello et al., 2006; Duarte et al., 2004; Cruz et al., 2007). In this sense, many researchers are aiming to scientifically prove the use of plant extracts as an effective control of infections of the skin (Weckesser et al., 2007), the mouth (More et al., 2008) and other infections caused by a range of Gram-positive and Gram-negative bacteria (Vuuren, 2008; Lee et al., 2007; Chauhan et al., 2007).

Agave sisalana Perrine, popularly known as sisal, belonging to the Agavaceae family and is a monocotyledonous plant from Mexico. It is well adapted to the semi-arid region of Northeast Brazil. Brazil is the world's largest producer of *A. sisalana* for the supply of the sisal fiber, and the sisal culture is one of the main economic activities in the semi-arid Bahia State, which accounts for about 90% of its production (Oashi, 1999). Production of sisal is mainly carried out by small farmers and has an important social function, because these producers would find it difficult to cultivate other crops with satisfactory economic results due to the unfavorable weather conditions in the region (Silva and Beltrão, 1999).

The search for natural products from agro-industrial waste, which may became useful to society, has grown in recent years. Only 5% of the decortications of the leaves of sisal (*A. sisalana*) produce a hard fiber that is used for various purposes; the remaining 95% consists of solid waste (mucilage) and waste liquid (juice of the sisal) that are normally discarded by sisal farms (Oashi, 1999). Thus, sisal waste principally contains plant tissue (lignin

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and cellulose), primary and secondary metabolites, and water, amongst others. In an attempt to utilize this waste, some small local producers have systematically applied this residue to crops in an attempt to obtain improved production, or in the feeding of animals. In this way, researchers have studied this practice and published the use of sisal waste as fertilizer (Lacerda et al., 2006), pesticides (Barreto, 2003) and animal feed (Faria et al., 2008a, b). Pizarro et al. (1999) described the use of sisal waste as being used against larvae of mosquitoes, which transmit tropical diseases.

In this work, we evaluated the antimicrobial activity of *A. sisalana* (leave and sisal waste) in strains that were resistant or non-resistant to antibiotics, which were collected in the Bahia state, Brazil.

MATERIALS AND METHODS

The sisal waste was collected directly using the paraibana-type machine after the decortication process of the leaves of *A. sisalana* in a sisal farm located in the Municipality of Valente, Bahia state, in March 2006. The leaves are also collected in the same local. The sisal residue was subjected to compression and the resultant liquid was filtered and dried under a controlled temperature (60°C) to give the crude extract of the sisal residue (ESR). Leaves of *A. sisalana* (approximately 1.3 kg) was crushed in a semi-industrial blender and divided into two portions. These portions were extracted separately by reflux for 12 h using hydroalcoholic solution (3:7) and methanol to provide the crude hydroalcoholic extract (ESH) and methanol (ESM), respectively.

The microorganisms used in this study were: *Staphylococcus aureus* CCMB 262 (resistant to streptomycin and dihydrostreptomycin), *S. aureus* CCMB 263 (resistant to novobicin), *S. aureus* CCMB 285, *Escherichia coli* CCMB 261 (sensitive to sulphonamide), *E. coli* CCMB 284, *Pseudomonas aeruginosa* CCMB 264, *Micrococcus luteus* CCMB 283, *Salmonella choleraesuis* CCMB 281, *Bacillus cereus* CCMB 282 and a clinical isolate of *Candida albicans* CCMB 266. Evaluations were made by a diffusion test using 6 mm diameter paper discs impregnated with 5 µL of each sample at a concentration of 1 mg per disc. The tests were performed in triplicate. Positive controls were patterns of inhibition of microbial growth discs impregnated with the antibiotic chloramphenicol (30 mg) and the antifungal, nystatin (10 mg). The bacterial inoculum (100 µL of a suspension containing 10^7 CFU/mL) was uniformly spread using sterile cotton swab on Mueller-Hinton agar poured on Petri dish. The discs loaded with natural products were placed onto the surface of the agar. The same procedure was performed to assess the activity of the yeast, but suspension of this microorganism was standardized to a concentration of approximately 5 x 10^5 CFU/mL. The plates were incubated at 37°C for the bacteria and 28°C for the yeast, for 24 and 48 h respectively. The results of the study were obtained with the aid of a millimeter ruler, and the mean and the average standard deviation of the results was calculated. All tests were performed in triplicate.

The MIC for *C. albicans* CCMB 266 was established by testing its susceptibility by microdilution in broth. The tests were performed in Mueller Hinton broth. The ESR and ESH were resuspended in a solution ultrapure autoclaved water and DMSO (1:1). Then, the resuspended extracts were sterilized by filtration through cellulose acetate membrane (0.22 mm). We prepared serial dilutions of 100 to 0.0488 mg/mL of the extracts in sterile microtiter plates of 96 wells. Then, each well received 10 µL of suspension (Scale 3, Mc Farland) of each micro-test. The plates were incubated at 28°C for 48 h. We performed a verification of the purity of the suspension of inoculum by a subculture of the volume used in the test (10 µL) in the Mueller Hinton agar plate in a simultaneous incubation. After the period of incubation, were added 50 µL of triphenyl tetrazolium chloride 2-3-5 (VETEC) in the final concentration of 1250 mg/well for qualitative analysis of microbial growth in the wells in order to determine the antimicrobial activity of each dilution of the samples. Controls were performed to also test for the viability of microorganisms and the sterility of the culture medium. All tests were performed in triplicate.

RESULTS AND DISCUSSION

The extract of the sisal residue (ESR) and foliar polar extracts (ESH) and (ESM) of *A. sisalana* were tested against Gram-positive and Gram-negative bacteria (non-resistant and resistant to antibiotics) and a fungus, by the disk diffusion method (Table 1). It was noted that there was a low efficiency of these extracts against the bacteria tested, however, there was significant inhibition of the growth of *C. albicans* by the ESR and ESH. This fact can be attested by their halos of inhibition, which were similar to those produced following treatment with nystatin. The MIC for *C. albicans* was evaluated for these extracts by microdilution in broth (Table 2).

The classification of Aligiannis et al. (2001) quantifies the biological activity of natural products based on the results obtained in the MIC test: strong inhibitors up to 0.5 mg/mL, moderate inhibitors function between 0.6 and 1.5 mg/mL and weak inhibitors functions above 1.6 mg/mL. The result obtained by MIC of the extracts ESR and ESH (Table 2) shows effective inhibition of *C. albicans*.

The antimicrobial activity of the extracts of *Agave* species are documented in the literature, however, little is known about the liquid residue of *A. sisalana*, which is discarded during the production of hard fibers. Verastegui et al. (1996), in an evaluation of ethanolic extracts of *Agave lechiguilla* against 20 microorganisms (*Clostridium perfringens*, *Listeria monocytogenes*, *Yersinia enterocolitica*, *E. coli, Proteus vulgaris*, *Salmonella thyphimurium*, *Shigella dysenteriae*, *Nocardia asteroids*, *Nocardia brasiliensis, Candida krusei*, *C. albicans*, *Candida rugosa*, *Cryptococcus neoformans*, *Cryptococcus laurenti*, *Cryptococcus albidus*, *Microsporum canis*, *Microsporum gypseum*, *Trichophyton tonsurans*, *Epidermophyton floccosum* and *Sporotrix schenckii*), showed action of this plant against the bacteria *C. perfringens* and *S. dysenteriae* and all fungi tested, except against *C. krusei*. In the other work, Verastegui et al. (2008) examined the biological activity of ethanolic extracts obtained from the leaves of four species of Agave (*A. lecheguilla, Agave picta, Agave scabra and Agave lophanta*), and showed that all the extracts tested are active against the fungus *C. neoformans.* In addition, the ethanolic extract of *A. picta* also showed activity against *E. coli*.

Yang et al. (2006) tested the antimicrobial activity of 28

Table 1. Antimicrobial activity of extracts from *Agave sisalana.*

Microorganism	Inhibition zone (mm)			
	ESR[1]	ESH	ESM	P[2]
Bacteria Gram +				
S. aureus CCMB 285	-	-	-	20
S. aureus CCMB 263[3]	-	-	-	16
S. aureus CCMB 262[4]	-	-	-	16
B. cereus CCMB 282	-	-	-	18
M. luteus CCMB 283	-	-	-	20
Bacteria Gram -				
E. coli CCMB 284	-	-	-	-
E. coli CCMB 261[5]	-	-	-	-
P. aureginosa CCMB 264	-	-	-	-
S. cholereasuis CCMB 281	-	-	-	14
Fungi				
C. albicans CCMB 266	18	19	12	20

[1]The extracts were tested in the concentration of 1 mg/disc.
[2]P: bactericidal chloramphenicol (30 µg/disc) and fungicidal nystatin (10 µg/disc)
[3]Resistent to novobicin.
[4]Resistent to streptomycin and dihydrostreptomicin.
[5]Sensitive to trimethoprim and resistant to sulfonamide.

Table 2. Minimal inhibitory concentration (mg/mL) of liquid sisal residue extract (ESR) and hydroetanolic extract of the leaves (ESH) from *Agave sisalana.*

Microorganism	ESR	ESH	Nystatin
C. albicans CCMB 266	0.39	0.39	0.00195

compounds isolated from monocotyledonous plants. Among these compounds, ten were saponins with steroidal nucleous as aglycone with different units of sugars in their structure, and they showed biological activity that is similar to the positive control of amphotericin against *C. neoformans* and *Aspergillus fumigatus*. Specificically, the saponin of *Agave americana* also showed antifungal activity against the pathogenic microorganisms *C. albicans, Candida glabrata, C. krusei, C. neoformans* and *A. fumigatus*.

Among the antifungal compounds produced by plants, the action of saponins is apparently due to their ability to form complexes with sterols that are present in the membranes of the fungi, which causes the loss of membrane integrity. However, the exact mechanism is not yet fully understood (Osbourne and Morrissey, 1999).

Thus, the hydroethanolic extract obtained of leaves and the foliar sisal residue concentrate of *A. sisalana* demonstrated significant inhibition of *C. albicans*. The methanolic extract of leave showed some action against *C. albicans* when compared with the above extracts. Ujikawa and Purchio (1989) isolated and partially characterized some compounds glycosylated from the leaves of *A. sisalana,* which had a pronounced inhibitory effect against the fungus *C. neoformans*, *Saccharomyces cerevisiae* and *Crebrothecium ashbyi*. These results suggest that the bioactive compound(s) of the extract studied here can contain its glycosilated structural unit (principally saponin). At present, this residue is discarded by the sisal farms, but could constitute a potentially useful raw material, which when stabilized and treated adequately, could be useful in the treatment of diseases caused by *C. albicans*.

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