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A N I N T E R N A T I O N A L J O U R N A L

Antimicrobial Activity of *Syagrus coronata* (Martius) Beccari

Alice Ferreira da Silva Hughes[1,4], Fabíola Guedes de Lima[1], Angélica Maria Lucchese[2,4], Aristóteles Góes Neto[1,4] and Ana Paula Trovatti Uetanabaro[3,4*]

[1]Laboratório de Pesquisa em Microbiologia; Departamento de Ciências Biológicas; Universidade Estadual de Feira de Santana; 44036-900; Feira de Santana - BA - Brasil. [2]Laboratório de Química de Produtos Naturais e Bioativos; Departamento de Ciências Exatas; Universidade Estadual de Feira de Santana; 44036-900; Feira de Santana - BA - Brasil. [3]Laboratório de Microbiologia da Agroindústria e Departamento de Ciências Biológicas; Universidade Estadual de Santa Cruz; 45662-900, Ilhéus - BA - Brasil.[4]Programa de Pós-graduação em Biotecnologia; Universidade Estadual de Feira de Santana; 44036-900; Feira de Santana - BA - Brasil

ABSTRACT

This work aimed to evaluate the antimicrobial activity of aqueous and methanol extracts of leaves, inflorescences, nut-shell, liquid and solid endosperm nuts of Syagrus coronata *against pathogenic bacteria and yeast. Screening was initially performed using the agar dilution method. The extracts regarded as bioactive underwent liquid-liquid partition for determination of their minimum inhibitory concentration and minimum bactericide concentration (MIC and MBC) and those of their respective fractions against the microorganisms inhibited in preliminary tests. Antimicrobial activity was observed only in inflorescences. The corresponding aqueous extract was effective against* B. cereus *and the three strains of* S. aureus*, and the corresponding MIC and MCB values were lower than those of dichloromethane, ethyl acetate and butanol fractions of the same extract. The methanol extract was effective against* B. cereus*, and the corresponding MIC and MBC values were higher than those of ethyl acetate and butanol fractions of the same extract.*

Key words: *Syagrus coronata***,** Arecaceae, antimicrobial activity

INTRODUCTION

The biological activity of medicinal plants has been the subject of intense scientific investigation. Higher aromatic plants are widely used in folk medicine for their broad spectrum of activities and their known inhibitory properties against the pathogenic bacteria and fungi (Hulin et al. 1998). There are many antimicrobial activity studies with the members of the Arecaceae family (Venkataraman et al. 1980, Esquenazi et al. 2002) and the *Syagrus* genus (Silveira et al. 2005), which are popularly used for the treatment of several diseases due to their tonic, carminative, stomachic, vermifuge, antithermic and cicatrizant properties (Hadi and Bremner 2001, Silveira et al. 2005). The Arecaceae family has been investigated for its antimicrobial activity based on the following criteria: (i) medicinal use of the plants and their possible content of antimicrobial compounds (Esquenazi et al. 2002, Silveira et al. 2005, Agra et al. 2008, Rufino et al. 2008); (ii) chemical composition, since alkaloids (sometimes pyrimidine), proanthocyanidines, flavonoids, saponines and triterpene methyl esters may be present (reviewed in Silveira et al. 2005); (iii)

*Author for correspondence: anapaula.uetanabaro@pq.cnpq.br

large geographic distribution area, with species found in many parts of Brazil, especially in the Northeast and Southeast (Lorenzi 2000).

Syagrus coronata (Martius) Beccari belongs to the Arecoideae subfamily (Noblick 1991). This is the largest subfamily of Arecaceae, with 115 genera and 1500 species to-date (Uhl et al. 1995). Among several names by which the species is popularly known in Brazil, licuri is mostly used in the outskirts of the state of Bahia; other names such as ouricuri, aricuri, nicuri, coqueiro dicori, coqueiro-cabeçudo-alicuri, and baba-de-boi are occasionally used (Bondar 1938, Crepaldi et al. 2004).

S. coronata has also been used in folk medicine from some regions in Northeastern Brazil. Some of the parts of the plant used in medicinal applications include the nut-shell for snake bites (Agra et al. 2008), coconut water (liquid endosperm) as drops for eye inflammation, mycoses (*Tinea negra* and *Tinea versicolor*) and wound healing; and licuri root tea, indicated for the treatment of spinal pain (Rufino et al. 2008). Furthermore, *S. coronata* is a source for candle and soap making products and plays an important role in the subsistence economy of the semi-arid parts of the state of Bahia, through the trade of manufactured products made from different parts of the plant (Crepaldi et al. 2004) and artisanal production of feed (Gonçalves et al. 2005).

The purpose of this study was to evaluate the antimicrobial activity of the extract and fractions of *Syagrus coronata* (licuri), for potentially using these products as phytotherapeutic agents in the medicinal and/or cosmetic formulations for human use, as well as provide scientifically-grounded primary data about the potential use of species from semi-arid regions.

MATERIALS AND METHODS

Collection of Materials

S. coronata (Mart.) Becc. was collected from five specimens in the city of Feira de Santana in the state of Bahia, Brazil. In November 2005, inflorescences and leaves were collected, and in February and April 2006, unripe and ripe nuts, respectively, were collected from the same previously marked specimens. The samples were deposited in the Herbarium of Universidade Estadual de Feira de Santana (HUEFS) and registered under the numbers 114796, 114797, 114798, 114799 and 1147800. The materials were identified by comparison with exsiccates identified by the experts and also based on specialized literature (Noblick 1991).

Obtainment of the Crude Extract

Crude Aqueous Extract

Sixty grams of fresh material (leaves, inflorescences, nut-shell and solid endosperm) was macerated with 600 mL of distilled water. The extracts were filtered using filter paper and submitted to shaking at 200 rpm at room temperature for 3 h. Subsequently, the extracts were filtered and the plant residues were re-extracted with 600 mL of distilled water. The liquid endosperm of unripe nuts was collected using a 3.0 ml syringe and then filtered to complete the final volume of 300 mL. All the filtrates were lyophilized and stored at - 80 °C until the trials.

Crude Methanol Extract

The dried leaf, inflorescence, nut-shell and solid endosperm materials (60 g) were crushed in absolute methanol (500 mL) and left for 7 days at room temperature. Subsequently, the extracts were filtered and the plant residues were re-extracted with 500 mL of methanol. The filtrates were concentrated by rotary evaporation under reduced pressure until completely dried (Chechinel Filho and Yunes 1998). Concentrated materials were stored at - 80 °C until the trials.

Obtainment of crude extract fractions

The extracts showing bioactivity on the agar diffusion test were subjected to fractioning. The methanol extract (10 g) was dissolved in 200 mL of absolute methanol and 60 mL of distilled water, while the aqueous extract (10 g) was dissolved in 260 mL of distilled water. The extracts were then subjected to liquid-liquid partition with solvents of increasing polarity, namely hexane, dichloromethane, ethyl acetate and butanol. The concentration and storage of the fractions were performed as described above for methanol extracts.

Antimicrobial Activity

Test Microorganisms

The antimicrobial activity of the extracts was tested against the *Bacillus cereus* CCMB 282, *Escherichia coli* CCMB 284, *E. coli* CCMB 261, sensitive to trimetoprim and resistant to sulphonamide, *Pseudomonas aeruginosa* CCMB

264, *Salmonella choleraesuis* CCMB 281, *Staphylococcus aureus* CCMB 285, *S. aureus* CCMB 262, resistant to streptomycin and dihydrostreptomycin, *S. aureus* CCMB 263, resistant to novobiocin, and against *Candida albicans* CCMB 266, *C. albicans* CCMB 286, resistant to amphotericin-B and fluconazole, and *Malassezia furfur* CCMB 293. These microorganisms were cultured in Müeller-Hinton Agar (MHA) medium, except for *M. furfur* which was cultured in Sabouraud agar added with long-chain fatty acids (2% glucose, 1% peptone, 0.5% yeast extract, 1% olive oil, 3% bovine bile, 5% Tween 80, 2% agar, 0.1% chloramphenicol), modified Sadrim and Rocha (2004) medium, and incubated at 37°C (bacteria) and 28°C (yeasts) for 24 and 48 h, respectively. The test strains were provided by the Microorganism Cultures Collection of the state of Bahia (CCMB/UEFS).

Agar Diffusion Test

Initial screening for antimicrobial activity was performed using the agar diffusion method with slightly modified filter paper disks (Bauer et al. 1966). A suspension of the test organism (0.1 mL of the MacFarland 0.5 scale for bacteria and 0.1 mL of the MacFarland 3 scale for yeasts) was spread onto a Sabouraud agar-covered surface added with long-chain fatty acids as previously mentioned for *M. furfur*, and MHA (approximately 25 mL) for other test microorganisms on a Petri dish (100 mm diameter x 15 mm height). The filter paper disks (6 mm diameter) were impregnated with 5.0 µL of the crude extract samples at initial concentrations of 200, 100 and 50 mg/mL and placed on the plates inoculated with the test microorganisms. Extract-containing disks were air dried at room temperature for solvent evaporation. Nystatin (10 µg/disk) and chloramphenicol (30 µg/disk) samples were selected as standards for the comparison of results. Inoculated plates containing the extract-impregnated disks and controls were incubated as previously described. The diameters of inhibition zones, including the disk diameter, were measured using a millimeter ruler. Tests were performed in triplicate and the average diameter was calculated for each test.

Determination of the Minimum Inhibitory Concentration (MIC)

Extracts showing antimicrobial activity on the agar diffusion test and their respective fractions underwent broth microdilution tests for the determination of the minimum inhibitory concentration (MIC) against the bacteria inhibited (CLSI 2003) in the aforementioned preliminary trial. Tests were performed in Müeller-Hinton broth. The aqueous extract was re-suspended in the water, while the methanol extract and other fractions were re-suspended in 25% of dimethyl-sulfoxide (DMSO) and then sterilized by filtration through an acetate cellulose membrane (0.22 µm). Serial dilutions of 100 mg/mL at 0.0488 mg/mL were prepared from the extracts and fractions on sterile 96-well microtitration plates. Each well then received 10 µL of the test microorganism suspension (1.5 x 10^5 UFC/mL per well). The plates were incubated at 37 °C for 24 h. A purity verification of the suspension was performed by subculture of a corresponding aliquot on MHA plate for simultaneous incubation. After incubation, 50 µL of aqueous TTC (triphenyl tetrazole chloride) solution at 0.5% were added to each of the wells and the microplates were reincubated for three more hours, as reported by Custódio et al. (2010), at 37°C for qualitative assessment of microbial growth. Chloramphenicol dilutions (6 at 0.0488 mg/mL) were used as the controls for data comparison between the independent experiments and as indicators for relative evaluation of the inhibition level of the samples tested. Controls were also prepared for viability assessment of the test microorganisms and sterility assessment of the culture medium and the solvent used for dissolution of extracts and fractions, for verification of any possible effects on microorganisms. All the tests were performed in triplicate.

The activity pattern used for interpretation of minimum inhibitory concentrations was based on the values established by Aligiannis et al. (2001): strong inhibition, MIC lower than 0.5 mg/mL; moderate inhibition, MIC between 0.6 and 1.5 mg/mL; and poor inhibition, MIC higher than 1.5 mg/mL.

Determination of the Minimum Bactericide Concentration (MBC)

For the determination of the minimum bactericide concentration (MBC), the wells showing bacterial development inhibition in the MIC trial were selected. A sample of 20 µL was removed from each of these wells and inoculated onto MHA plates at 37 °C for 24 h. After incubation, the cultures were inspected for visual verification of microbial growth. All the tests were performed in

triplicate. The MBC was defined as the lowest concentration yielding negative subcultures or only one colony (Sanches et al. 2005).

RESULTS

The aqueous inflorescence extract was the only among all the aqueous extracts tested by agar diffusion to show antimicrobial activity, particularly against *B. cereus* and the three strains of *S. aureus* (Table 1). The methanol inflorescence extract was the only to show antibacterial activity against *B. cereus* with halos of 10 and 8 mm for the concentrations of 1000 and 500 μg/disk, respectively. The minimum inhibitory concentration (MIC) and the minimum bactericide concentration (MBC) of the aqueous and methanol licuri inflorescence extracts and fractions against *B. cereus* CCMB 282, *S. aureus* CCMB 262, 263 and 285 were quantitatively analyzed based on the values in Table 2. The yield for the hexane fraction of the aqueous inflorescence extract was null and thus MIC and MBC could not be calculated.

Table 1 - Average diameters (mm) of inhibition zones of aqueous *S. coronata* extracts and controls against test microorganisms as determined by agar diffusion tests.

	Inhibition zones (mm)															
Microorganisms	**Leaves**			**Inflorescences**			**Liquid endosperm**			**Solid endosperm**			**Nut Shell**			**Controls**
	C1	**C2**	**C3**	**C1**	**C2**	**C3**	**C1**	**C2**	**C3**	**C1**	**C2**	**C3**	**C1**	**C2**	**C3**	
B.c CCMB 282	0	0	0	14	12	10	0	0	0	0	0	0	0	0	0	32*
E.c CCMB 284	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9*
E.c CCMB 261	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0*
P.a CCMB 264	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0*
S.a CCMB 262	0	0	0	12	10	8	0	0	0	0	0	0	0	0	0	12*
S.a CCMB 263	0	0	0	12	10	8	0	0	0	0	0	0	0	0	0	12*
S.a CCMB 285	0	0	0	10	8	7	0	0	0	0	0	0	0	0	0	12*
S.c CCMB 281	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10*
C.a CCMB 286	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0**
C.a CCMB 266	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	24**
M.f CCMB 293	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0**

B.c: *Bacillus cereus*; E.c: *Escherichia coli*; P.a: *Pseudomonas aeruginosa;* S.a: *Staphylococcus aureus*; S.c: *Salmonella choleraesuis*; C.a: *Candida albicans*; M.f: *Malassezia furfur*; C1: 1000 μg extract/disc; C2: 500 μg extract/disc; C3: 250 μg extract/disc; Controls: chloramphenicol * 30 μg/disc e Nystatin ** 10 μg/disc.

Table 2 - Minimum inhibitory concentration (MIC) and minimum bactericide concentration (MBC) values in mg/mL for *S. coronata* inflorescence extracts and fractions, chloramphenicol (mg/mL) and DMSO (%).

Microorganisms	**AE**	**DF[a]**	**EAF[a]**	**BF[a]**	**ME**	**HF[m]**	**DF[m]**	**EAF[m]**	**BF[m]**	**CHL**	**DMSO**
MIC											
B.c CCMB 282	0,19	1,56	1,56	1,56	0,78	0,78	0,78	0,19	0,39	0,09	12,5
S.a CCMB 262	0,78	0,78	1,56	3,12	*	*	*	*	*	1,56	12,5
S.a CCMB 263	0,78	1,56	1,56	3,12	*	*	*	*	*	1,56	12,5
S.a CCMB 285	0,78	1,56	1,56	6,25	*	*	*	*	*	1,56	12,5
MBC											
B.c CCMB 282	0,78	1,56	6,25	3,12	0,78	1,56	1,56	0,39	0,39	0,19	12,5
S.a CCMB 262	3,12	6,25	6,25	6,25	*	*	*	*	*	3,12	12,5
S.a CCMB 263	3,12	6,25	6,25	6,25	*	*	*	*	*	3,12	12,5
S.a CCMB 285	3,12	6,25	6,25	6,25	*	*	*	*	*	3,12	12,5

B.c: *Bacillus cereus*; S.a: *Staphylococcus aureus*; AE: crude aqueous inflorescence extract; ME: crude methanol inflorescence extract; HF: hexane fraction; DF: dichloromethane fraction; BF: butanol fraction; EAF: ethyl acetate fraction; a: fractions of the aqueous inflorescence extract; m: fractions of the methanol inflorescence extract; CHL: chloramphenicol; DMSO: dimethyl-sulfoxide; "*": Test not performed due to lack of inhibition of the methanolic crude extract of the inflorescence, using the disk diffusion test.

DISCUSSION

The inflorescence was the only among all the investigated parts of *S. coronata* to show any antimicrobial activity. The aqueous extract was effective against *B. cereus* and the three strains of *S. aureus*, with no distinction between the two antibiotic-resistant strains and the antibiotic-sensitive strain, while the methanol extract was effective only against *B. cereus* (Table 1). According to the criteria of Aligiannis et al. (2001), aqueous extracts from the inflorescence of *S. coronata* showed moderate activity (MIC = 0.78 mg/mL) against *S. aureus* strains and strong activity (MIC = 0.19 mg/mL) against *B. cereus*, while the methanol extract showed moderate activity (MIC = 0.78 mg/mL) against *B. cereus*.

The MBC of the aqueous inflorescence extract of *S. coronata* against the strains *S. aureus* CCMB 262, 285 and 263 and *B. cereus* was four times higher than the respective MIC. The MBC of the methanol inflorescence extract against *B. cereus* was identical to the MIC. The bactericide activity concentration of chloramphenicol was twice the MIC. Evaluation of the minimum bactericide activity pattern is very important, once direct and prolonged contact of the *S. aureus* strains with antibiotics may be related to the development of antimicrobial resistance (Bernardes et al. 2004). Silveira et al. (2005) investigated the antimicrobial activity of *S. oleracea* and observed that the substances in hexane extracts of the epicarp/mesocarp were likely to be primarily responsible for the antimicrobial activity against *S. aureus* when compared to ethanol extracts. These authors suggested that the substances in the hexane extracts were probably the main responsible for the antimicrobial activity of *S. oleracea*.

On the other hand, according to Table 2, the crude aqueous extract of *S. coronata* showed higher antimicrobial activity against *B. cereus* and *S. aureus* strains when compared with the fractions investigates. This could be explained by the molecular synergism in the said extract. The crude methanol inflorescence extract showed low antimicrobial activity against *B. cereus* when compared with the fractions of higher polarity (FAE^m and FB^m), which suggested that the antimicrobial activity of the inflorescence was mainly due to higher polarity compounds.

The antibacterial activity of the aqueous extract and more polar fractions of methanol extract could be related to the presence of phenolic compounds, mostly soluble as esters or heterosides in water and organic solvents (Mello and Santos 2001) that formed complex with extracellular proteins of the bacterial membrane, leading to cell death (Cowan 1999).

No antimicrobial activity was observed in the aqueous and methanol extracts against the Gram-negative strains investigated, unlike the *S. oleracea*, which was effective against *P. aeruginosa* and *E. coli* in the studies conducted by Silveira et al. (2005). Some studies have shown that Gram-negative bacteria are not as susceptible to the plant extracts when compared to Gram-positive (Ojala et al. 2000, Sanches et al. 2005, Mothana et al. 2008). The resistance of Gram-negative bacteria to antibacterial substances has been demonstrated by the presence of liposaccharides on the external membrane (Gao et al. 1999), as well as by the presence of enzymes capable of breaking down the substances introduced in the periplasmic space (Duffy and Power 2001).

None of the study extracts were effective against *C. albicans* and *M. furfur*. This could be due to the fungal cell wall acting as protective barrier against the extracts, or due to the latter not affecting or inhibiting the cell wall formation mechanisms. The results of this study, thus, showed that the use of liquid endosperm in folk medicine for the treatment of mycoses was not scientifically sound. The results of this study also pointed to the potential antimicrobial properties of *S. coronata* inflorescences. New trials are required, with the isolation and identification of bioactive substances, to aim to find out new therapeutic alternatives for the infections caused particularly by antibiotic-resistant *S. aureus* strains.

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