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Full Length Research Paper

In vitro evaluation of antioxidant, antimicrobial and toxicity properties of extracts of *Schinopsis brasiliensis* Engl. (Anacardiaceae)

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This study aimed to investigate the phenolic content, antioxidant capacity, the antibacterial and toxicological profile of the methanolic extract of *Schinopsis brasiliensis* Engl. (MEx*Sb*). The phenolic content was determined by Folin-Ciocalteu methodology and the flavonoids content by complexation with chloride aluminum. The antioxidant activity was evaluated by DPPH method. The antimicrobial activity was tested by agar diffusion method and the minimum inhibitory concentration (MIC) was determined. The toxicological profile was obtained using tests with larvae of *Artemia salina* Leach. High levels of phenolic compounds (825.65 ± 40.99 tannic acid equivalents in mg/g material) were found in MEx*Sb*, where 55% and 1.8% (in g/100 g dry weight of extract) of these corresponded to tannins and flavonoids, respectively. The MEx*Sb* showed high antioxidant capacity (EC_{50} 8.80 ± 0.94 µg.mL^{-1}). Also a high antimicrobial activity was observed, particularly against strains of *S. aureus* and *P. aeruginosa*, with MIC of 125 and 62.5 µg.mL^{-1}, respectively. Finally, the MEx*Sb* showed moderate toxicity against *A. salina*. These findings allow concluding that the MExSb is a valuable source of molecules with antioxidant and antimicrobial capacity. Other studies, such as identification and quantification of major active components of MExSb are running and will evaluate the potential of the isolated compounds.

Key words: *Artemia salina*, "baraúna", brine shrimp assay, DPPH, MIC, multidrug resistance, phenolic compounds.

INTRODUCTION

Plants have been used for therapeutic purposes by different cultures around the world for centuries. The need for bioactive compounds with medicinal properties presents a tremendous challenge and has encouraged scientists to explore, in detail, plants that are potential sources of promising compounds. However, it is only in the last century that the various medicinal properties of plants have been studied scientifically (Holetz et al., 2002; Novais et al., 2003).

Among the species of the Anacardiaceae family is a tree endemic to Brazil called *Schinopsis brasiliensis* Engl. It is popularly known as baraúna, braúna, quebracho and chamacoco (Braga, 1960; Prado et al., 1995; Cardoso et al., 2005). Different parts of *S. brasiliensis*, including the leaves, bark, stem and fruit have been used in folk medicine as anti-inflammatory agents for various illnesses, such as influenza, fever, cough, diarrhea, impotence and osteoporosis (Almeida et al., 2005; Albuquerque, 2006; Albuquerque et al., 2007). *S. brasiliensis* has also been used as a natural antiseptic to treat wounds and superficial mycoses (Saraiva, 2007), as well as for the treatment of veterinary zoonoses (Cardoso, 2001).

Current studies indicate that many of these folk uses

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may have high levels of antioxidant activity (Dreifuss et al., 2010; Hevesi et al., 2009). Antioxidants inhibit the formation of damaging reactive oxygen species in the body (Velioglu et al., 1998). Antioxidants can also inhibit the peroxidation of biological molecules by chelating transition metals that generate hydroxyl radicals through the Haber-Weiss and Fenton reactions (Chew et al., 2009). Phenolic compounds, represented mainly by tannins and flavonoids, stand out as the major group of natural antioxidants. They act as efficient scavengers of free radicals and, due to their ability to act as hydrogen donors; they interrupt oxidative chain reactions (Delazar et al., 2006; Higdon and Frei, 2003).

Ethnopharmacological studies guide the worldwide search for new antimicrobial drugs from medicinal plants used by traditional communities (Sartoratto et al., 2004). Bacterial diseases have a profound economic impact on public health, especially in tropical regions and in immunodeficient or immunosuppressed patients (Saraiva, 2007). Despite the existence of powerful antibiotics, newly emerged multidrug-resistant strains of bacteria cause infections with high mortality, particularly in hospitals (Nascimento et al., 2000; Stapleton et al., 2004). Therefore, there is a need for further research devoted to the understanding of the genetic mechanisms of resistance in bacteria and to identify new drugs that have different mechanisms of action from the current antibiotics (Silver and Bostian, 1993; Alves et al., 2000).

The purpose of this study was to evaluate the antioxidant and antimicrobial potential of the methanolic extract from *S. brasiliensis* leaves, because there is a correlation between the antimicrobial potential of a compound and its antioxidant capacity. The toxicological profile of the extract, as well as its total phenolic and flavonoid content, was also determined.

MATERIALS AND METHODS

Plant material

Plant material (leaf of *S. brasiliensis*) was collected from Cacimba Nova farm, located in the municipality of Mirandiba in the state of Pernambuco in Brazil (08°07'13"S × 38°43'46"W, altitude 450 m), in March 2008. The voucher specimen (70.007) was identified by the curator A. Bocage and deposited in the Instituto Agronômico de Pernambuco (IPA) Herbarium. The sample was placed in an oven for three days at 45 ± 5°C and powdered to 16 mesh.

Chemicals

Acetic acid (99.8%) and aluminum chloride hexahydrate was purchased from Merck (Darmstadt, Germany). Anhydrous sodium carbonate, casein, dimethylsulfoxide (99.5%), ferrous sulfate heptahydrate (99%), methanol (99.8%), n-hexane (99%) and pyridine (99%) were obtained from Vetec (Rio de Janeiro, Brazil). 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,3,5-triphenyltetrazolium chloride, 3-(2-pyridyl)-5,6-di(2-furyl)-1,2,4-triazine-4′,4″-disulfonic acid disodium salt (Ferrozine), Folin-Ciocalteu phenol reagent, tetracycline, gentamycin and oxacillin were purchased from Sigma-Aldrich (Munich, Germany).

Preparation of crude extract

The extracts were obtained by macerating the sample, which was then extracted with n-hexane followed by methanol. The hexane fraction was discarded. The methanolic leaf extract of *S. brasiliensis* (MEx*Sb*) was filtered and the solvent removed by rotary evaporation under pressure (Marconi MA 120) at temperature of 45°C, obtaining yield of 26.27%. The use of methanol for the extraction of phenolic compounds from plant tissue is recommended due to its ability to inhibit the oxidation of polyphenols, a process which can alter antioxidant activity (Yao et al., 2004).

Determination of phenolic content

The total phenolic content (TPC) was measured by the Folin-Ciocalteu method. The total tannin content (TTC) is the difference between the total phenolic content and the residual phenolic content. The latter was determined by casein precipitation followed by the Folin-Ciocalteau procedure (Amorim et al., 2008). Briefly, TPC was determined by adding 1 mL of MEx*Sb* (10 mg.ml^{-1}, w/v) to 5 mL of aqueous solution of Folin-Ciocalteu reagent (10%, v/v), 10 mL of aqueous solution of sodium carbonate (75 mg/L, w/v) and 84 mL of distilled water. The reaction was incubated for 30 minutes at ambient temperature and the absorbance was measured at 760 nm. To determine the residual phenolic content, 15 mL of MEx*Sb* were combined with 1 g of casein and the mixture was agitated for three hours. The sample was filtered and distilled water was added to a final volume of 25 ml. The residual phenol was determined in 5 mL of the filtrate using the Folin-Ciocalteu method. The calibration equation for tannic acid was $y = 0.074x + 0.0044$ ($R^2 = 0.9993$). The total phenolic content and tannins were expressed as milligrams of tannic acid equivalent per gram of extract (mg TAE/g of MEx*Sb*). All analyses were performed in sextuplicate.

The total flavonoid content (TFC) was determined by the formation of the flavonoid-aluminum complex (Peixoto Sobrinho et al., 2008). One milliliter of MEx*Sb* (10 mg.ml^{-1}, w/v) was mixed with 0.6 mL of acetic acid, 10 mL of methanol solution of pyridine (20% v/v), 2.5 ml of methanol solution of aluminum chloride (50 mg/L, w/v) and 10.9 ml of distilled water. The reaction was incubated for 30 minutes at ambient temperature and the absorbance was measured at 420 nm. The results were expressed as milligrams of rutin equivalent per gram of extract (mg RE/g of MEx*Sb*). The rutin calibration equation was $y = 0.0251x + 0.0139$ ($R^2 = 0.9994$). All analyses were performed in sextuplicate.

Evaluation of antioxidant activity

DPPH radical scavenging assay

The free radical scavenging activity of MEx*Sb* was evaluated by the method of Sousa et al. (2007). Briefly, 0.5 ml of different concentrations (10-100 µg.ml^{-1}) of MEx*Sb* or ascorbic acid was added to 3 mL of a methanol solution of 0.1 mM DPPH (ABS_{sample}). The sample was incubated for 30 minutes and the absorbance was measured at 517 nm. The results were compared to a control, which consisted of a methanol solution of 0.1 mM DPPH ($ABS_{control}$). How blank were used 0.5 ml of methanol (ABS_{blank}). The antioxidant activity was calculated from the regression obtained by plotting concentrations of MEx*Sb* or ascorbic acid *versus* percentages of the radical scavenging activity (RSA) and expressed as efficient concentration value (EC_{50}; in µg.ml^{-1}), that is, the sample concentration required to reduce the absorbance of the

control by 50%. The EC_{50} of ascorbic acid (positive control) was 20.04 ± 1.37 μg.ml⁻¹.

$$RSA\ (\%) = \frac{ABS_{control} - (ABS_{sample} - ABS_{blank})}{ABS_{control}} \times 100$$

Chelating activity

The activity of the ferrous ion chelating (FIC) was evaluated using the method described by Chew et al. (2009). One milliliter of methanol solution of ferrous sulfate (0.1 mM, w/v) and 1 ml of methanol solution Ferrozine (0.25 mM, w/v) were mixed with 1 ml of Exm*Sb* (1 to 7 mg/ml) or EDTA (10 to 100 mg/ml) (ABS_{sample}). The solution was incubated in the dark for 10 min and the absorbance was measured at 562 nm. The measurements were compared with a control consisting of 1 ml of methanol, 1 ml of methanol solution of ferrous sulfate and 1 ml of methanol solution of Ferrozine ($ABS_{control}$). How blank were used dilutions of the samples with 2 ml of methanol (ABS_{blank}). The ability of the sample to chelate ferrous ions was calculated from a calibration curve obtained by the percentages of chelating activity (CA) versus sample concentrations and expressed as efficient concentration value (EC_{50}; in $mg.ml^{-1}$), that is, the concentration of sample needed to reduce the absorbance of control by 50%. The FIC of EDTA (positive control) was of 9.73 ± 0.11 $mg.ml^{-1}$.

$$CA\ (\%) = \frac{ABS_{control} - (ABS_{sample} - ABS_{blank})}{BSA_{control}} \times 100$$

Antimicrobial activity

Preparation of the extracts

MEx*Sb* was dissolved in aqueous solution of dimethylsulfoxide (20%, w/v) (Sakagami et al., 2005) at various concentrations ranging from 12.5 to 100 $mg.ml^{-1}$ (Leite et al., 2006). Known antimicrobial agents that were assayed alongside MEx*Sb* included tetracycline (0.030 mg/well), gentamycin (0.010 mg/well) and ketoconazole (0.050 mg/well). To determine the MIC, a range of dilutions of MEx*Sb* (from 3.9 to 2000 $\mu g.ml^{-1}$) and the antibiotics (gentamycin, tetracycline and oxacillin, the latter used only for strains of *Staphylococcus aureus*) ranging from 1 to 64 $\mu g.ml^{-1}$ were prepared.

Microbial strains

All microorganisms were obtained from the collection of the Microbiological Analysis Laboratory, Department of Pharmaceutical Sciences, Federal University of Pernambuco, Brazil (the identification codes begin with the letters "AM"). The microorganisms used in this study were:

Strains of *S.* aureus: MRSA (Brazilian Epidemic Clones AM793, AM858, AM895 and AM902; Pediatric Clones AM642 and AM922; Sporadic Clone AM594), MSSA (AM632, AM672 and AM532) and Standard AM103 (same as ATCC 6538) and AM106 (same as ATCC 6538P);
Strains of other species of *Staphylococcus*: coagulase-negative strains (AM789 and AM1001), *S. saprophyticus* (AM245), *S. epidermidis* (AM235) and *Staphylococcus sp*. AM109 (same as ATCC 23235);
Strains of *Enterococcus faecalis*: AM126, AM202, AM997, AM1058 (same as ATCC 29212) and AM1056 (same as ATCC 51299);
Strains of *Pseudomonas* aeruginosa: AM428, AM460, AM462, AM470 and AM206 (same as ATCC 14502);
Strains of *Escherichia coli*: AM31 (same as ATCC 9723), AM167 (same as ATCC 10536), AM177, AM215, AM273 and AM1050 (same as ATCC 35218);
Strains of *Klebsiella pneumoniae*: AM327, AM343, AM379, AM410 and AM1047 (same as ATCC 700603);
Strains of *Salmonella typhimurium*: AM1046 and AM1052 (same as ATCC 14028);
Strains of *Candida albicans* (AM1140, AM1141 and AM1155);
Strains of *Candida krusei* (AM1138, AM1139 and AM1157).

Agar diffusion method (well)

Of the various techniques used to assess antimicrobial activity, the agar well diffusion test, in spite of the larger volumes used (Caetano et al., 2002), has the advantage of permitting the use of surfactants, which help to improve the solubility of sample constituents. The enhanced solubility permits greater radial and surface diffusion of the components of the sample and results in larger zones of inhibition, and better performance of the antimicrobial agents, with inhibition of a greater number of bacteria (Alves et al., 2008).

Sterile swabs were used to inoculate 20 × 100 mm sterile Petri dishes containing 20 ml of Mueller-Hinton agar (for bacteria) (CLSI, 2003) or Sabouraud agar (for fungi) (CLSI, 2004). The plates had 6 mm-diameter wells that were filled with 100 μl of MEx*Sb* extracts of different concentrations (Sakagami et al., 2005). As a positive control, the wells contained 100 μl of the antibiotics and as a negative control, 100 μl of dimethylsulfoxide (20%, v/v). The plates were pre-incubated at room temperature for 3 hours to allow for complete diffusion of the extracts (Möller, 1966), after which they were incubated aerobically at 37 ± 1℃ (for bacteria) and 30 ± 1℃ (for fungi) for 24 h. Antibacterial activity was assessed by measuring the diameter of the inhibition zones.

Minimum Inhibitory Concentration (MIC)

The MICs were determined by the agar dilution method proposed by the Clinical Laboratory Standards Institute (CLSI, 2003), with the microorganism that showed inhibition zone greater than 13 mm. Dilutions of MEx*Sb* (3.9 to 2000 $\mu g.ml^{-1}$) or antibiotics (1 to 64 $\mu g.ml^{-1}$) were incorporated into the culture medium, whether Muller-Hinton agar (for bacteria) or Sabouraud agar (for fungi). 100 μl of the inoculum were distributed aseptically into the holes of a multi-inoculator (Stears) and then applied to the surface of the medium. After inoculation with bacteria, the plates were incubated at 37 ± 1℃. The positive and negative controls were performed in duplicate at the beginning and end of the process (Sakagami et al., 2005).

Selective toxicity profile

The toxicity assay employs the larvae of the brine shrimp *Artemia salina* Leach. and is based on the method of Meyer et al. (1982). Saline water was prepared with artificial sea salt (30 g/L of salt in distilled water; pH between 7 and 8). The cysts of *A. salina* (20 mg) were incubated in a vat containing non-toxic artificial saline water and subjected to artificial light for 48 hours to enable the larvae to hatch. Ten larvae of *A. salina* were transferred to test tubes containing 5 ml of different concentrations of MEx*Sb* dissolved in saline water (50 to 1000 $\mu g.ml^{-1}$) or a negative control (artificial saline water only). Dead larvae were collected after 24 h and Probit analysis was used to calculate the lethal concentration value (LC_{50}) defined as the sample concentration that causes the death of 50%

Table 1. Results of the agar diffusion method and minimum inhibitory concentration (MIC) of the methanolic extract of *Schinopsis brasiliensis* Engl. (MExSb) against Gram-positive bacteria.

Assay	Sample		***S. aureus*[1]**												***Staphylococcus*[1]**					***E. faecalis*[1]**				
			103	**106**	**532**	**672**	**632**	**594**	**793**	**642**	**858**	**895**	**902**	**922**	**1001**	**789**	**235**	**245**	**109**	**126**	**202**	**997**	**1056**	**1058**
Agar diffusion method[2]	MEx*Sb*[3]	10.0	28	28	26	31	26	32	29	28	26	28	26	26	26	27	27	27	28	24	23	25	25	22
		5.0	27	26	25	29	24	29	27	26	24	25	24	24	25	25	25	25	25	21	20	23	22	21
		2.5	24	23	23	26	22	26	24	24	22	23	22	21	22	22	23	22	24	15	18	18	20	19
		1.25	19	19	20	24	20	23	21	22	20	19	19	19	20	18	21	19	21	14	16	17	17	18
	Tetracycline	0.03	34	36	34	38	27	14	15	29	23	18	-	37	-	-	-	-	-	-	-	-	-	-
	Gentamycin	0.01	-	-	-	-	-	-	-	-	-	-	-	-	-	35	23	35	28	26	26	18	-	23
MIC[4]	MEx*Sb*		250	125	250	250	125	125	125	125	125	250	125	250	500	500	500	500	500	2	2000	1000	1000	1000
	Tetracycline		1	1	<1	<1	<1	64	64	4	64	64	32	1	-	-	-	-	-	-	-	-	-	-
	Oxacillin		2	2	<1	2	2	>64	32	32	64	32	32	32	-	-	-	-	-	-	-	-	-	-
	Gentamycin		-	-	-	-	-	-	-	-	-	-	-	-	16	1	<1	<1	1	1	1	8	>64	1

[1]The strain numbers below each species are strain identification codes beginning with the letters AM and are described in detail in section *Microbial strains*. [2]Inhibition zones in millimeter; [3]Concentrations for well in milligram; [4]Concentrations in µg.mL⁻¹.

of the larvae.

RESULTS

Determination of phenolic content and antioxidant activity

The extract of *S. brasiliensis* contained high levels of phenolic compounds (825.65 ± 40.99 mg TAE/g) of which about 455.81 ± 50.41 mg TAE/g were tannins and 11.29 ± 0.94 mg RE/g were flavonoids. The MEx*Sb* showed high antioxidant activity (EC_{50} 8.80 ± 0.94 µg.ml^{-1}), however, the FEC showed low activity (1440.62 ± 180.67 mg.ml^{-1}).

Antimicrobial activity

The inhibition zones results for *S. aureus* AM672 of 31 mm was observed with an MEx*Sb* concentration of 10 mg/well; for *S. saprophyticus* AM245, MEx*Sb* produced a zone of inhibition of 25 mm at a concentration of 5 mg/well; for *E. faecalis* AM1056, a zone of inhibition of 20 mm was obtained with MEx*Sb* at a concentration of 2.5 mg/well; and a zone of inhibition of 21 mm was seen with 1.25 mg/well MEx*Sb* for *S. aureus* AM793, a multidrug-resistant MRSA strain of *S. aureus* (Table 1). MEx*Sb* was active against multiple strains of *S. aureus* (MRSA, MSSA and Standard) (MICs between 125 and 250 µg.ml^{-1}) and against strains of *S.* coagulase-negative (MICs of 500 µg.ml^{-1}). On the other hand, it displayed low activity against *E. faecalis* strains (MICs of 2000 µg.ml^{-1}) (Table 1). The antibiotics (gentamycin, oxacillin and tetracycline) were used to confirm the antibiotic sensitivity (or resistance) of the microorganisms tested.

Growth inhibition zones of 32, 31, 28 and 26 mm were observed for P. aeruginosa at MEx*Sb* concentrations of 10, 5, 2.5 and 1.25 mg/well, respectively. The two highest concentrations of MEx*Sb* used in the assay were active or very active against species of the Enterobacteria. A zone of inhibition of 28 mm (for *E. coli* AM251) and 26 mm (for *K. pneumoniae*) using a 10 and 5 mg/well concentration of MEx*Sb*, respectively. Zones of inhibition of 19 mm (for *E. coli*) and 21 mm (for *K. pneumoniae*) were observed with MEx*Sb* concentrations of 2.5 and 1.25 mg/well, respectively. The MIC data are consistent with the results obtained from the agar wells diffusion method. MIC values of 31.25 and 250 µg.ml^{-1} were obtained for wild strains of *P. aeruginosa* and *P. aeruginosa* standard (AM206) (Table 2).

In a study of antifungal activity, MEx*Sb* at a concentration of 5 and 10 mg/well produced growth inhibition zones of 21 mm (for *C. albicans*) and 16 mm (for *C. krusei*), respectively, in the agar wells diffusion assay.

When the individual values of the zones of inhibition at each MEx*Sb* concentration are compared between species or between organisms, it is clear that the antimicrobial activity of MEx*Sb* has no correlation with the antibiotic susceptibility

Table 2. Results of the agar diffusion method and minimum inhibitory concentration (MIC) of the methanolic extract of *Schinopsis brasiliensis* Engl. (MEx*Sb*) against Gram-negative bacteria.

Assay	Sample		*P. aeruginosa*[1]					*E. coli*[1]						*K. pneumoniae*[1]					*Salmonella*[1]	
			428	**460**	**462**	**470**	**206**	**177**	**215**	**273**	**167**	**31**	**1050**	**327**	**343**	**379**	**410**	**1047**	**1046**	**1052**
Agar diffusion method[2]	MEx*Sb*[3]	10.0	32	29	32	29	28	19	28	23	22	19	21	25	22	17	20	28	22	21
		5.0	31	26	29	27	26	15	26	20	19	14	18	21	19	14	16	26	17	17
		2.50	28	25	27	25	24	13	17	19	14	-	-	14	14	-	-	24	13	14
		1.25	26	22	24	23	22	10	-	17	-	-	-	-	-	-	-	21	-	-
	Gentamycin	0.01	-	-	-	-	19	-	29	-	-	24	24	24	24	24	24	27	24	24
MIC[4]	MEx*Sb*		31.25	31.25	62.5	31.25	250	250	250	1000	1000	500	1000	2000	1000	2000	2000	2000	2000	2000
	Gentamycin		>64	>64	>64	32	4	8	4	8	8	4	4	<1	4	<1	<1	<1	2	2

[1]The strain numbers below each species are strain identification codes beginning with the letters AM and are described in detail in section *Microbial strains*. [2]Inhibition zones in millimeter; [3]Concentrations for well in milligram; [4]Concentrations in µg.ml^{-1}.

or resistance of any strain or species, including that of *S. aureus* and *P. aeruginosa*. Some strains of *S. aureus* (AM594, AM793, AM858, AM902) were sensitive to one of the eight antibiotics tested, whereas some *P. aeruginosa* strains (AM460, AM462, AM470) were not sensitive to any of the fourteen antibiotics *Pseudomonas* strains were tested against (Figure 1). For strains of *Staphylococcus* and *P. aeruginosa*, the average zone of inhibition readings generated by MEx*Sb* at a concentration of 1.25 mg/well was 20 mm and 22 mm, respectively.

Toxicity profile

Meyer et al. (1982) established a lethal concentration (LC_{50}) based on the toxicity of substances to the larvae of *A. salina*. According to the scale, LC_{50} values of < 500 µg.ml^{-1} indicate toxicity, LC_{50} values of 500 to 1000 µg.ml^{-1} denote moderate toxicity while LC_{50} values of > 1000 µg.ml^{-1} suggest a lack of toxicity. The LC_{50} value of MEx*Sb* was 705.54 ± 60.46 µg.ml^{-1}, which implies that the extract is moderately toxic. A small decrease in the movement of the larvae at an MEx*Sb* concentration of 750 µg.ml^{-1} was also observed when compared to the negative control. This result demonstrates that high concentrations of the extract can negatively impact the metabolism of *A. salina*. This can be explained by the high concentration of polyphenols (tannin, flavonoids and other phenolics compounds) which is well known for their toxicity against *A. salina* (Santos et al., 2010). Despite showing moderate toxicity, some phenolic compounds that may be responsible for the antimicrobial activity may not show toxicity.

DISCUSSION

Melo et al. (2010) classified primary antioxidant activity into three categories based on the activity of the putative antioxidant relative to a positive control. Compounds in Group I possess good antioxidant activity ($EC_{50} < 60$ µg.ml^{-1}; up to three fold greater than the EC_{50} of the positive control). Group II compounds show moderate antioxidant activity (60 µg.ml^{-1} $< EC_{50} <$ 140 µg.ml^{-1}; between three and seven fold greater than the EC_{50} of the positive control) while those in group III have low activity ($EC_{50} > 140$ µg.ml^{-1}; more than seven fold greater than the EC_{50} of the positive control). Based on this system of classification, MEx*Sb* showed high antioxidant activity (EC_{50} = 8.80 ± 0.94 g.ml^{-1}) when compared to the ascorbic acid (EC_{50} = 20.04 ± 1.37 µg.ml^{-1}). However, the same extract showed low chelating activity (FIC 1440.62 ± 180.67 mg.ml^{-1}) in comparison with control EDTA (FIC of 9.73 ± 0.11 mg.ml^{-1}). The results suggest that the compounds from MExSb have with higher primary antioxidant potential and low chelating activity of metal ions. This is an important factor for the development of a phytomedicine due to the need of adding a chelating agent to inhibit the catalytic action of the chelates.

The antimicrobial activity of a substance is determined by measuring the diameter of the growth inhibition zone between the putative antimicrobial agent and the microorganism being

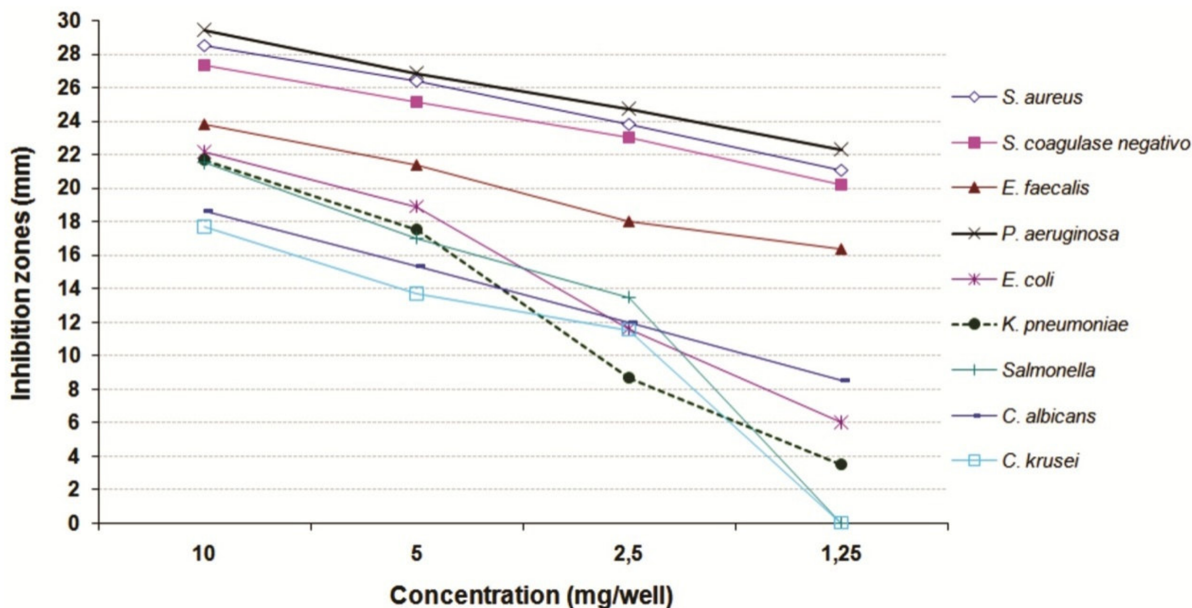

Figure 1. Average of the inhibition zones of the strains in relation the concentrations to the methanolic extract of *Schinopsis brasiliensis* Engl. (MExSb).

Table 3. Results of the agar diffusion method of the methanolic extract of *Schinopsis brasiliensis* Engl. (MEx*Sb*) against yeast obtained.

Assay	Sample		*C. krusei*[1]			*C. albicans*[2]		
			1138	1139	1157	1140	1154	1155
Agar diffusion method[2]	*MExSb*[3]	10.0	17	20	19	15	21	17
		5.0	15	15	16	11	16	14
		2.5	12	12	12	-	11	12
		1.25	8	9	-	-	-	-
	Ketoconazole	0.05	17	30	31	35	23	21

[1]The strain numbers below each species are strain identification codes beginning with the letters AM and are described in detail in section *Microbial strains*. [2]Inhibition zones in millimeter; [3]Concentrations for well in milligram.

tested (Lenette et al., 1987). The four decreasing concentrations (10, 5, 2.5 and 1.25 mg / well) (Leite et al., 2006) was used for the technique of agar wells diffusion to investigate the decline of the antimicrobial activity of the extract against microorganism. These results are presented in Figure 1. The MIC of an extract is defined as the lowest concentration that is capable of inhibiting the growth of a particular microorganism. The strength of antimicrobial activity were classified according to parameters established by Alves et al. (2000), in which a zone of inhibition of < 9 mm indicates lack of activity, a zone of inhibition of 9 to 12 mm denotes low activity, and zones of 13 to 18 mm and > 18 mm are indicative of activity and high activity. Plant extracts with MIC values of < 100 µg.mL^{-1} are considered to be highly active antimicrobial agents; those with MICs of 100 to 500 µg.mL^{-1} are defined as active; those with MICs of 500 to 1000 µg.ml^{-1} are defined as moderately active; those with MICs of 1000 to 2000 µg.mL^{-1} are considered to have low activity; and those with MICs of > 2000 µg.ml^{-1} are defined as inactive.

Our analysis showed that MEx*Sb* was active or very active in inhibiting the growth of all the Gram-positive bacteria and *P. aeruginosa* strains tested (MIC lower than 500 µg.ml^{-1}) by agar wells diffusion and MIC, except this latter method that showed moderately and low activity of MEx*Sb* against *E. faecalis* strains (MIC 1000 and 2000 µg.mL^{-1}). Moreover, the MEx*Sb* was also moderately active against strains of *E. coli* (MIC of 1000 µg.ml^{-1}) and it displayed low activity against strains of *K. pneumoniae* and *Salmonella* (MICs of 2000 µg.ml^{-1}). The antibiotic, gentamycin, was used to confirm the antibiotic sensitivity (or resistance) of the microorganisms tested.

As noted in Figure 1, the average of inhibition zones of

the antimicrobial activity of MExSb no showed great variation in the different multiresistant microorganisms and of lesser or greater sensitivity to groups of antibiotics used in clinical. This indicates that the mechanism by which the constituents of MEx*Sb* act upon micro-organisms is distinct from the resistance mechanisms acquired by these bacteria. The mean inhibition zones against the *Enterobacteriaceae* (*E. coli*, *K. pneumoniae* and *Salmonella*) and yeast (*C. albicans* and *C. krusei*) are only indicative of substantial antimicrobial activity at the two highest concentrations of MEx*Sb* (10 and 5 mg/well). These data indicate that the antimicrobial potential of MEx*Sb* is lower against these micro-organisms than against the Gram-positive strains (*Staphylococcus* and *E. faecalis*) and *P. aeruginosa*.

A close relationship between the antioxidant and antimicrobial activity of tannins has been reported in the literature (Nijeveldt et al., 2001; Vattem and Shetty, 2005), by association of the mechanism of action of phenolic compounds with power their inhibition of free radicals in Gram-positive and Gram-negative bacterias (Nijeveldt et al., 2001; Zaidi-Yahiaoui et al., 2008). Still, contributes to antimicrobial and antioxidant activities of MEx*Sb* the presence of gallic acid, methyl gallate, quercetin, megastigmona, ellagic acid and 5,6,7,8,3',4'-hexahidroxi-flavonol, substances already isolated and identified in the leaves of *Schinopsis brasiliensis* (Souza, 1990; Moreira, 2009).

Conclusion

We conclude that the antimicrobial activity of MEx*Sb* is directly related to the concentration of phenolic compounds in the extract, particularly the tannins, which account for 55% of the total phenolic content. Their abundance may explain the high antioxidant and antimicrobial activity and the toxicity of moderate of the MEx*Sb*. In particular the MEx*Sb* displayed high antimicrobial activity, especially against the pathogenic strains, *S. aureus* and *P. aeruginosa*, which are among the most commonly isolated bacteria from nosocomial infections. This result has stimulated to further studies to isolate and characterize the antimicrobials constituents of the extracts from the leaves of *S. brasiliensis* Engl.

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Abbreviations: MIC, Minimum inhibitory concentration; **IPA,** Instituto Agronômico de Pernambuco; **DPPH,** 2,2-diphenyl-1-picrylhydrazyl; **MExSb,** methanolic leaf extract of S. brasiliensis; **TPC,** total phenolic content; **TTC,** total tannin content; **TFC,** total flavonoid content; **RSA,** radical scavenging activity; **FIC,** ferrous ion chelating; **CA,** chelating activity; **LC_{50},** lethal concentration.

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