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ANTIMICROBIAL ACTIVITY AND BIOAUTOGRAPHIC STUDY OF ANTISTAPHYLOCOCCAL COMPONENTS FROM *Schinopsis brasiliensis Engl*.

Estudo da atividade antimicrobiana e bioautográfica dos componentes antiestafilocóccicos de *Schinopsis brasiliensis Engl.*

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ABSTRACT

The growth of bacterial resistance to antibiotics is a threat to the world population, and plants are an inexhaustible source of new molecules. *Schinopsis brasiliensis* (baraúna) is a tree found in the Northeastern region of Brazil. The objective of this research was determine the antimicrobial activity of extracts from leaves, flowers, Root Peel, Stem bark and fruit (endocarp, exocarp and seeds) from *S. brasiliensis* against clinical isolates strains of multidrug-resistant *Staphylococcus aureus* MRSA. Antimicrobial activity was evaluated using the agar well diffusion method and Minimum Inhibitory Concentration (MIC) by agar dilution, bioautography and phytochemical study. Results: The methanolic extracts from leaves, flowers, root peel and stem bark showed inhibition halos of 23-27 mm (10 mg.well^{-1}) and 18-25 mm (5 mg.well^{-1}), and MIC of 0.125- 1.0 mg.ml^{-1}. The main groups of secondary metabolites found were phenolic acids, flavonoids, terpenes and steroids. Conclusion: The extracts from leaves, stem bark and root peels and flowers of *S. brasiliensis* were classified as active againsts multidrug-resistant *S. aureus strains*, which justifies further studies aimed at the identification of these compounds.

Keywords: Phytochemicals, Multidrug-resistant *Staphylococcus aureus*, Brazilian epidemic clone, Pediatric epidemic clone, Sporadic clone.

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RESUMO

O crescimento da resistência bacteriana aos antibióticos é uma ameaça à população mundial, sendo as plantas uma fonte inesgotável de novas moléculas. *Schinopsis brasiliensis* (baraúna) é uma árvore encontrado na região Nordeste do Brasil. O objetivo desta pesquisa foi determinar a atividade antimicrobiana dos extratos das folhas, flores, cascas da raiz e do caule, fruto (endocarpo, exocarpo e sementes) de *S. brasiliensis* frente a isolados clínicos de *Staphylococcus aureus* MRSA multirresistentes. A atividade antimicrobiana foi avaliada pela técnica de poços difusão em ágar e Concentração Mínima Inibitória (CMI) por diluição em agar, bioautografia e estudo fitoquímico. Os extratos metanólicos das folhas, flores, casca da raiz e do caule apresentaram halos de inibição da ordem de 23-27 mm (10 mg.poço^{-1}) e 18-25 mm (5 mg.poço^{-1}), e CMI de 0,125-1,0 mg.mL^{-1}. Os principais grupos de metabólitos secundários evidenciados foram ácidos fenólicos, flavonóides, terpenos e esteróides. Os extratos das folhas, casca do caule e raiz e flores de *S. brasiliensis* foram classificados como ativos frente às cepas de *S. aureus* multirresistentes, o que justifica mais estudos que objetive a identificação destes compostos.

Palavras-chave: Fitoquímicos, *Staphylococcus aureus* multirresistente, Clone epidêmico brasileiro, Clone epidêmico pediátrico, Clone esporádico.

INTRODUCTION

The importance of nosocomial infection caused by *Staphylococcus aureus*, especially by methicillin resistant *S. aureus* (MRSA) is well known because its frequency, morbidity, mortality and principally for its treatment difficulty (DUKIC et al., 2013; Rodrigues et al, 2020). The strains of MRSA are resistant to all β-lactamic, macrolides, tetracycline, aminoglycosides, while the two glycopeptides (vancomycin and teicoplamin) and one oxazolidinone (linezolid) are the alternatives of clinical treatment against infections of MRSA multidrug-resistant *S. aureus* (TEIXEIRA et al., 2012; CONG et al., 2020). However, vancomycin intermediate *S. aureus* (VISA) or glycopeptides intermediate *S. aureus* (GISA) strains have recently been identified in different countries. Following it,

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vancomycin-resistant in *S. aureus* (VRSA) strains have also emerged, having a mechanism different from those VISA strains (DEZFULIAN et al., 2012, CONG et al., 2020).

Studies in Brazil have described a clone of methicillin resistant *Staphylococcus aureus* (Brazilian Epidemic Clone-BEC, ST247-SCC*mec*IIIA) that is disseminated and predominated in hospitals along the country. It was also observed that this clone has expanded to other countries in South America (Argentine, Chile, Colombia, Peru, Ecuador, Uruguay), besides Europe (Portugal, Italy and Chech Republic) and Asia (MIRANDA et al*.,* 2007; RODRÍGUEZ-NORIEGA et al*.,* 2010; PEREIRA-FRANCHI et al, 2019). In light of these evidences, it is clear that the identification of new antimicrobial agents is needed.

Among the species of Anacardiaceae family is a tree endemic to Brazil called *Schinopsis brasiliensis* Engl. It is popularly known as baraúna, braúna, quebracho and chamacoco (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 (ALBUQUERQUE et al. 2007). *Schinopsis brasiliensis* has also been used as a natural antiseptic to treat wounds and superficial mycoses (SARAIVA et al., 2011), as well as for the treatment of zoonosis (CARDOSO, 2001).

In literature, the isolation of some substances had been reported which were isolated and identified from hexane and chloroform extracts from stem and methanolic extracts from leaves of *S. brasiliensis*, like: 5α, 8α-epidioxyergosta-6,22-dien-3-beta-ol, stigmast-4en-3-one, stigmast-4-en-one-6-β-ol, methyl 6-eicosanyl-2-hydroxy-4-methoxybenzoate, methyl gallate and gallic acid, 5,6,7,8,3',4'-hexahidroxiflavonol, 5,7,4',5'-tetrahidroxiflavon-3'-o-β-D-glucopyranosede, megastigmane, gallic acid 4-O-β-D-glucopyranoside, gallic acid 4-O-β-D-(6'-O-galloyl)-glucopyranoside, ethyl-O-β-D-(6'-O-galloyl)-glucopyranoside, 2-hydroxy-4-methoxyphenol 1-O-β-D-(6'-O-syringoyl) – glucopyranoside, (2R*,3R*,2''R*,3''R*)-7-Hydroxy-4'-methoxy-flavanone- (3→3'')-3''',7''-dihydroxy-4'''-methoxy-flavanone, (6R,9R)-megastigma-4- en-3-one 9-O-β-glucopyranoside, quercetin-3-O-β-D xylopyranoside, tricetin-3'-O-β-D-glucopyranoside,

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4,2',4'-trihydroxychalcone-(3→O→4")-2"',4"'-dihydroxychalcone, β-1,2,3,4,6-Pentagalloyl-D-glucose (CARDOSO, 2001; CARDOSO et al., 2005; SOUZA, 1990; MOREIRA, 2009; SANTOS et al., 2017; SARAIVA et al. 2020).

The purpose of this study was to evaluate the antimicrobial activity of the extracts from *S. brasiliensis* (leaves, stem bark, root peel, exocarp and endocarp of the fruit, seeds and flower) against *Staphylococcus aureus* strains of different sensibility/resistance clones (Brazilian Epidemic Clone, Pediatric Epidemic Clone and Sporadic Clone) and to correlate between the microbial antibiotics resistance and extent of inhibition halos and minimal inhibitory concentration of the extracts, as well as the inhibition zones in bioautography plate, that would correspond to active metabolites present in these extracts.

MATERIAL AND METHOD

Plant material

The plant was collected in the town of Carnaubeira da Penha, hinterland of Pernambuco (State), latitude 08°19'09", longitude 38º44'41" and altitude of 446 meters (BRASIL, 2005), between the months of March and June, 2004, had been identified by the curator of the Herbarium of Empresa Pernambucana de Pesquisa Agropecuária (IPA), Dra. Rita de Cássia Pereira, with the Voucher nº 70.007. The sample was placed in an oven for three days at 45 ± 5 °C and powdered to 16 mesh.

Preparation of extracts and standard antimicrobial agent

The extracts of parts of *S. brasiliensis* were obtained separately by macerating the samples, which were then extracted with n-hexane followed by ethyl acetate and last methanol. The hexanic, ethyl acetate and methanolic extracts (leaf, stem bark, root peel and flower, except seed, exocarp and endocarp of the fruit, only extracted in methanol) of *S. brasiliensis* were filtered and the solvent was removed by rotary evaporation under

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pressure (Marconi MA 120) at temperature of 45°C, subsequently weighted and its output calculated.

The methanolic extracts were dissolved in aqueous solution of Dimethylsulfoxide (20%, w/v) (SAKAGAMI et al., 2005) and the hexanic and ethyl acetate extracts in aqueous solution of tween 80 (4%, v/v) at two concentrations ranging from 50 $mg.mL^{-1}$ and 100 $mg.mL^{-1}$ (Saraiva et al. 2012). Standard antimicrobial agent (tetracycline, 0.30 $mg.mL^{-1}$) was assayed along side *Schinopsis brasiliensis* extracts. To determine the Minimum Inhibitory Concentration (MIC), a range of dilutions of *Schinopsis brasiliensis* extracts (from 0.3125 to 20 $mg.mL^{-1}$) and the antibiotics, tetracycline and oxacillin, ranging from 0.010 to 0.64 $mg.mL^{-1}$ and 0.010 to 0.256 $mg.mL^{-1}$, respectively, were prepared.

Bacteria strains

A total of twenty-two *Staphylococcus aureus* strains, comprising twenty clinical isolates and two standard strains, were used in the study (Table 1). These isolates were: ten multidrug-resistant *S. aureus* (MRSA) Brazilian Epidemic Clone (BEC), five isolates of Pediatric Epidemic Clone, three isolates correspond to Sporadic Clones and two MSSA strains. All bacteria are from the collection of the Microbiological Analysis Laboratory from Department of Pharmaceutical Sciences, Federal University of Pernambuco, Recife, Brazil (CORDEIRO, 2004; MIRANDA et al., 2007).

Table 1. List of *Staphylococcus aureus* strains assayed

Strains	Profile	Clone	Origin
AM594	MRSA	A_{13}*	Catheter point
AM723	MRSA	A_1	Catheter point
AM791	MRSA	A_8	Secretion of Tenckhoff Outlet
AM793	MRSA	A_1	Tracheal secretion
AM799	MRSA	A_5	Vesical probe point
AM837	MRSA	A_7	Catheter point

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AM858	MRSA	A_{13}	Catheter point
AM895	MRSA	A_6	Hemoculture
AM902	MRSA	A_2	Orifice secretion
AM948	MRSA	A_9	Catheter point
AM599	MRSA	B_3	Ulcer Secretion
AM642	MRSA	B_5	Secretion
AM771	MRSA	B_2	Tracheal secretion
AM922	MRSA	B_6	Left leg wound
AM942	MRSA	B_4	Hemocultura
AM872	MRSA	G	Urine
AM875	MRSA	D	Hemoculture
AM876	MRSA	I	Hemoculture
AM632	MSSA	-	Operation wound
AM672	MSSA	-	Tracheal secretion
AM103	Standard	-	ATCC 6538
AM106	Standard	-	ATCC 6538P

AM: Collection of Microbiological Analysis Laboratory from Department of Pharmaceutical Sciences - UFPE; **ATTC**: American Type Culture Collection; **Clone A:** Brazilian Epidemic Clone (CEB); *AM594 classified like Clone F (CORDEIRO, 2004) and reclassified like Clone A_{13} (MIRANDA et al., 2007); **Clone I, D, G, F:** Sporadic Clone; **Clone B:** Pediatric clone; **MRSA:** *Staphylococcus aureus* Methicillin Resistant; **MSSA:** *Staphylococcus aureus* Methicillin Sensitive

Preparation of inoculates

The inoculates were prepared starting from 24h colonies culture of *S. aureus* in Mueller-Hinton agar and suspended in sterile physiological solute, comparing the turbidity with 0.5 McFarland scale (10^8 UFC.mL^{-1}) (CLSI, 2003).

Agar well diffusion method

The inoculum were applied in surface of Mueller-Hinton agar and after perforated the wells (diameter 6 mm) and added 100 µL in each well of extracts (concentrations 100

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and 50 mg.mL^{-1}), of standard antibiotic (0.3 mg.mL^{-1}) and of the control solution (DMSO at 20% (v/v) or tween 80 at 4% (v/v)) . After incubation at 37 ºC ± 1 for 24 hours, the diameter of the inhibition halos were measured and its results evaluated according to the following scale: inhibition halos of 9 mm - inactive; 9-12 mm - somewhat active; 13-18 mm - active; 18mm - very active (ALVES et al., 2000).

Agar dilution method – Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration for the extracts (basic solution of 20 mg.mL^{-1}), dilutions in water with concentrations of 0.3125 to 20 mg.mL^{-1} had been prepared and incorporated to Mueller-Hinton agar (1:9), thus final concentrations of 0.0315 to 2 mg.mL^{-1} of the extracts remain. The standard antibiotics used were tetracycline and oxacillin (Sigma), with concentrations as indicated by the CLSI (2003).

The inoculates were distributed aseptically in the Stears' multiinoculator, and then being deposited on the surface of the culture medium, and incubated at 36 ºC ± for 24 hours.

Duplicate controls were performed in the beginning and the end of the inoculation process control. The control of the diluents also was done (tween 80 at 4% and DMSO at 20%).

The results were read comparatively to the strain controls and determined by the first plate, the concentration of which inhibits grow.

Plant extracts with MIC values of < 0.10 mg.mL^{-1} are considered to be highly active antimicrobial agents; those with MICs of 0.10 to 0.50 mg.mL^{-1} are defined as active; those with MICs of 0.50 to 1 mg.mL^{-1} are defined as moderately active; those with MICs of 1 to 2 mg.mL^{-1} are considered to have low activity; and those with MICs of > 2 mg.mL^{-1} are defined as inactive (SARAIVA et al., 2011).

Phytochemical analysis

Thin layer chromatography (TLC) of silica-gel were GF° 254 (Merck) was used in phytochemical study. The methanol or ethyl acetate extracts from the leaf, stem bark and

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flower from *S. brasiliensis* at the concentration of 20 $mg.mL^{-1}$ were applied using an analytic capillary (15 µL). A mobile phase for the methanol extracts was the mixture of solvents (1): AcOEt/HCOOH/AcOH/H_2O (100:11:11:26), and for the corresponding ethyl acetate the system (2): AcOEt/HCOOH/AcOH/H_2O (100:2:2:2 and 100:3:3:3). The TLC was performed in duplicate, one being used as a chromatographic reference and the other for bioautography (SARAIVA et al., 2012).

In the phytochemical study, we used standards of gallic acid, ellagic acid, quercetin, kaempferol, catechin, ursolic acid, β-sitosterol, β-amyrin, pilocarpine, iridoids, and glucose.

The visualization of the TLC was realized under UV light (254 and 366 nm), as revealed according to Table 2.

Table 2. Solvent system used in thin layer chromatographic studies of *Schinopsis brasiliensis* Engl.

Metabolites	Elution System	Detection	Reference
Alkaloids	A	Dragendorff	WAGNER et al., 1984
Triterpenoids and steroids	B	Liebermann-Burchard	HARBORNE, 1998
Iridoids	A	Sulfuric Vanilin	WAGNER et al., 1984
Saponins	A	Anisaldehyde	WAGNER & BLADT, 1996
Sugars	C	TTC	METZ, 1961
Coumarins	D	U.V	WAGNER & BLADT, 1996
Cinnamic Derivatives	A	NEU	WAGNER & BLADT, 1996
Flavonoid	A	NEU	WAGNER ET AL., 1984 MARKHAM, 1982
Phenylpropane glycosides	A	NEU	WAGNER & BLADT, 1996
Condensed Proanthocyanidine and Leucoanthocyanidines	A	Vanillin-HCl	ROBERTS et al., 1957

A: EtOAc-HCOOH-AcOH-H2O (100:11:11:26 v/v); **B:** EtOAc-HCOOH-AcOH-H2O (100:0.5:0.5:0.5 v/v); **C:** n-BuOH-Me_2CO- Buffer Phosphate pH = 5.0 (40:50:10 v/v); **D:** Et_2O-toluene-AcOH 10% (50:50:50 v/v); **NEU:** 2-Anino-ethyl-diphenyl borinate, **TTC:** Triphenyl tetrazolium Chlorid.

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Bioautographic technique

The developed chromatogram according to system (1) was subjected to aseptic current air for 8h and another developed by system (2) for 6h, respectively. On the TLC plate melted Mueller-Hinton agar (MH), inoculated with a saline suspension of *S. aureus* ATCC 6538 (bacterial suspension at 10^8 UFC/mL) was added and homogeneously distributed over the plate. After solidification of the MH inoculated, it left for 30 minutes at surrounding 25ºC, for the prediffusion of the active components. Subsequently it was incubated for 24 to 36 hours ±1ºC. After this period the bioautography was revealed with a 2,3,5-triphenyl tetrazolium chloride (TTC) solution at 2.5 $mg.mL^{-1}$ and again incubated for 4 hours.

The presence of inhibition zone indicates the existence of active components (SARAIVA et al. 2012).

RESULTS AND DISCUSSION

Antimicrobial activity

Among the two techniques used for the determination of antimicrobial activity, the agar well diffusion method, although it uses larger volumes (100 µL) (SARAIVA et al., 2012) in relation to those used in disks (10 µL) (VORAVUTHIKUNCHAI & KITPPIPIT, 2005), has the advantage to allow the use of adjuvant to improve the solubility of the extract constituents and to permit a radial as well as a superficial diffusion, which are conditions resulting in better inhibition halos.

With regard to the five *Staphylococcus aureus* strains chosen to determinate the inhibition zones, they represent the three types of multidrug-resistant MRSA clones together with the standard strain (Table 3). The methanolic extract of the leaves (LM) showed the largest inhibition halo for the five tested strains, with values in the order of 23 mm in concentration of 5 $mg.Well^{-1}$, which correspond to the very active classification (Alves et al., 2000). Likewise, the extracts from the leaf, stem bark, root peel and flower

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extracted by ethyl acetate, presented zone of inhibition in the order of 22 mm for the leaf and of 16 mm for the rest of the extracts. The hexane extracts did not present any antimicrobial activity. The diameters of the inhibition halos expressed in millimeters are represented in the form of Table 3 for each extract and for each bacterium. The inhibition halos produced by the tetracycline antibiotic confirmed the phenotype resistance of the assayed clones, in other words, *Staphylococcus aureus* MRSA (AM594, AM723 and AM942) resistant to tetracycline showed inhibition zones in the order of 13 mm or less and the strains of *S. aureus* MRSA AM922 and *S. aureus* ATCC 6538 sensitive to tetracycline presented inhibition zones in the order of 26 mm (MIRANDA et al., 2007; CORDEIRO, 2004).

Table 3. Antimicrobial activity of *Schinopsis brasiliensis* Engl. against *S. aureus*.

Extracts	**mg. Well**	***S. aureus* (AM)**				
		594	**723**	**922**	**942**	**103**
LM	**10**	25*	24	25	25	27
	5	23	23	22	23	25
BM	**10**	22	22	21	22	24
	5	20	20	20	20	22
RM	**10**	21	20	20	21	23
	5	19	18	19	20	21
FM	**10**	21	21	21	21	23
	5	20	19	19	20	22
ExM	**10**	20	18	18	18	20
	5	18	15	14	15	17
EnM	**10**	18	15	16	19	20
	5	16	13	14	15	18
SM	**10**	19	18	18	18	20
	5	17	15	16	16	18
LA	**10**	21	21	22	21	23
	5	21	21	22	20	22
BA	**10**	16	15	15	16	18
	5	14	13	12	12	14
RA	**10**	13	13	16	15	18
	5	-	-	13	13	16

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FA	**10**	14	14	14	13	15
	5	14	12	12	14	14
Tet	**0.03**	11	12	27	12	28

***:** inhibition halo in millimeter; **M**: Methanol; **A**: Ethyl Acetate; **L**: Leaves; **B**: Stem Bark; **R**: Root peel; **FL**: Flowers; **Ex**: Exocarp of the Fruit; **En**: Endocarp of the Fruit; **S**: Seeds, **Tet:** Tetracycline. **AM**: Collection of Microbiological Analysis Laboratory – Department of Pharmaceutical Sciences - UFPE

The MIC values of the assayed extracts are being presented separately, according to the type of multidrug-resistant *S. aureus* MRSA clone. With reference to analyzing the results, the MIC were classified like active, with MIC > 0.1 and ≤ 0.5 $mg.mL^{-1}$, one observes in the case of *Staphylococcus aureus* MRSA Brazilian Epidemic Clone, that some methanolic extracts (RM, FM) present values of 0.125 and 0.5 $mg.mL^{-1}$. The extract of the stem bark presented similar MIC values, in the order of 0.5 $mg.mL^{-1}$. The MIC values for the five Pediatric Clones were 0.125 to 0.25 $mg.mL^{-1}$ for the flower extract obtained from methanol (FM) while the extract obtained from extraction of ethyl acetate indicated lower activity. In Table 4 the MIC of the flower extract from *S. brasiliensis* extracted in methanol (FM) against four Sporadic Clones presented good activity with concentrations of 0.25 to 0.5 $mg.mL^{-1}$. The MIC values of the antibiotics oxacillin and tetracycline confirmed for the first the MRSA or MSSA character of the *Staphylococcus aureus* strains and in the case of tetracycline the phenotype resistance (Table 4). In relation to the DMSO diluents at 50% and Tween 80 to 4%, they do not present any inhibition, with the twenty-two strains growing, which confirmed data already indicated for DMSO by SAKAGAMI (2005). The presented data were the average of two determinations.

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Table 4. Minimum inhibitory Concentration (MIC) of extracts from *Schinopsis brasiliensis* against *Staphylococcus aureus* strains

	Minimal Inhibitory Concentration (MIC - mg.mL^{-1})													
	Extracts from *Schinopsis brasiliensis* Engl.											**Antibiotics**		
Strains	**LM**	**BM**	**RM**	**FM**	**EnM**	**ExM**	**SM**	**LA**	**BA**	**RA**	**FA**	**Tet**	**Oxa**	**Sensitive**	**Resistant**
AM594	1.0	0.5	1.0	0.5	>2.0	>2.0	>2.0	2.0	0.25	>2.0	>2.0	0.032	0.256	1	2, 3, 4, 5, 6, 7, 8
AM723	1.0	1.0	1.0	0.25	1.0	NT	NT	>2.0	NT	>2.0	2.0	0.032	0.064	1	2, 3, 4, 5, 6, 7, 8
AM791	0.5	0.5	0.5	0.25	>2.0	2.0	1.0	1.0	0.5	>2.0	2.0	0.032	0.064	1	2, 3, 4, 5, 6, 7, 8
AM793	0.5	0.5	0.5	NT	>2.0	2.0	>2.0	2.0	0.5	>2.0	2.0	>0.064	0.256	1	2, 3, 4, 5, 6, 7, 8
AM799	0.5	0.5	0.125	0.5	>2.0	2.0	>2.0	2.0	0.5	1.0	2.0	0.064	0.256	1	2, 3, 4, 5, 6, 7, 8
AM837	1.0	1.0	0.5	NT	>2.0	>2.0	>2.0	NT	0.5	NT	NT	0.032	0.256	1	2, 3, 4, 5, 6, 7, 8
AM858	0.5	0.25	NT	0.25	1.0	2.0	2.0	NT	0.5	NT	NT	0.032	0.256	1	2, 3, 4, 5, 6, 7, 8
AM895	0.5	0.5	0.125	0.125	NT	>2.0	>2.0	2.0	0.25	>2.0	2.0	0.064	0.064	1	2, 3, 4, 5, 6, 7, 8
AM902	1.0	1.0	NT	>2.0	>2.0	>2.0	>2.0	NT	0.5	NT	NT	>0.064	0.032	1	2, 3, 4, 5, 6, 7, 8
AM948	0.5	0.5	0.5	NT	NT	1.0	>2.0	2.0	0.5	>2.0	>2.0	0.064	0.256	1	2, 3, 4, 5, 6, 7, 8
AM599	1.0	0.5	1.0	0.25	>2.0	>2.0	>2.0	2.0	0.5	>2.0	>2.0	0.001	0.016	1, 2, 4, 5, 7	3, 6, 8
AM642	1.0	1.0	1.0	0.5	>2.0	NT	NT	>2.0	NT	>2.0	>2.0	<0.001	0.032	1, 4, 5, 7	2, 3, 6, 8
AM771	0.5	0.5	0.5	0.25	2.0	>2.0	0.5	2.0	0.5	>2.0	2.0	<0.001	0.032	1, 2, 4, 5, 7	3, 6, 8
AM922	1.0	1.0	1.0	NT	>2.0	>2.0	2.0	2.0	1.0	>2.0	>2.0	0.002	0.064	1, 2, 3, 4, 7	5, 6, 8
AM942	0.5	1.0	0.5	0.125	0.5	NT	NT	>2.0	NT	>2.0	>2.0	0.032	0.016	1, 2, 3, 5, 7	4, 6, 8
AM872	1.0	1.0	1.0	0.25	>2.0	>2.0	>2.0	>2.0	1.0	>2.0	2.0	0.016	0.128	1, 2, 3, 5, 7	4, 6, 8
AM875	1.0	1.0	0.5	NT	>2.0	2.0	>2.0	2.0	1.0	>2.0	>2.0	<0.001	0.016	1, 2, 4, 5, 7	3, 6, 8
AM876	1.0	0.5	0.5	0.5	1.0	>2.0	>2.0	2.0	0.5	>2.0	2.0	0.002	0.032	1, 2, 3, 4, 5, 7	6, 8
AM632	1.0	1.0	1.0	0.5	2.0	>2.0	>2.0	>2.0	0.5	>2.0	>2.0	0.001	<0.001	1, 2, 4, 5, 6, 7	8, 3
AM672	NT	NT	NT	0.5	2.0	>2.0	>2.0	2.0	0.5	>2.0	2.0	0.001	<0.001	1, 2, 3, 4, 6, 7	5, 8

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AM103	0.5	1.0	0.5	0.5	2.0	1.0	>2.0	2.0	0.25	>2.0	>2.0	<0.001	<0.001	ATCC 6538 standard
AM106	0.125	1.0	0.5	0.25	2.0	NT	>2.0	2.0	0.5	>2.0	>2.0	<0.001	<0.001	ATCC 6538P standard

NT: Not tested, **L** (Leaf); **F** (Flower); **B** (Stem Bark); **R** (Root peel); **Ex** (Exocarp of the Fruit); **En** (Endocarp of the Fruit); **S** (Seed); **M** (Methanol); **A** (Ethyl Acetate); **1:** Vancomycin; **2:** Ciprofloxacin; **3:** Erythromycin; **4:** Tetracycline (Tet); **5:** Gentamycin; **6:** Oxacillin (Oxa); **7:** Trimetropim/Sulphametoxazole; **8:** Penicillin, **AM:** Collection of Microbiological Analysis Laboratory – Department of Pharmaceutical Sciences – UFPE.

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Bioautography

The most expressive zone inhibitory (inhibition halo-diameter > 20 mm) in either concentrations of 10 or 5 mg per well, were the extracts from the leaf methanol (LM), the explanation may be justified by the presence of three polyphenols visualized in the bioautography Rf 0.58 – gallic acid, Rf 0.84 – methyl gallate and another in Rf 0.34, which were characterized presumptively by TLC and according to standards revealed with 2-aminoethyldiphenyl borate. All other parts of the plant also indicated the presence of gallic acid, but not visualized the polyphenol (Rf 0.34). Other metabolites with antimicrobial activity in Rf 0.27 and 0.44 are present in three FM, BM and BA extracts. The polyphenol - Rf 0.62, visualized in stem bark extracted in ethyl acetate (BA), while still present in the flower and root peel of *Schinopsis brasiliensis* (Table 5).

Table 5. Rfs Values related to inhibition zones of the extracts from *Schinopsis brasiliensis* obtained of bioautographies in TLC against *S. aureus* ATCC 6538

Chromatograph System	**Extracts**	**R*f***				
	LM	0.34	0.58	0.84		
AcOEt/HCOOH/AcOH/H_2O	BM	0.18	0.27	0.44	0.62	
(100:11:11:26)	FM	0.12	0.27	0.44		
	BA	0.27	0.44	0.62	0.78	0.90
AcOEt/HCOOH/AcOH/H_2O	FA	0.58	0.80	0.90		
(100:2:2:2 e 100:3:3:3)	LA	0.76	0.86	0.96		

Rf: Readings; **L** (Leaves); **B** (Stem Bark); **F** (Flower); **M** (Methanol); **A** (ethyl Acetate)

Phytochemical study

The secondary metabolites found in the extracts from the parts studied from *S. brasiliensis* with well-known antimicrobial activity are presented in Table 6.

Table 6. Secondary metabolites of *Schinopsis brasiliensis*

Secondary Metabolites	***Schinopsis brasiliensis* Engl.**						
	LM	**BM**	**RM**	**EnM**	**ExM**	**SM**	**FM**
Triterpene/Steroid	+	+	+	+	+	+	+
Cynamic Derivate	+	+	+	+	+	+	+

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Flavonoid	+	+	+	+	+	+	+
Saponin	(-)	(-)	(-)	+	(-)	(-)	(-)
Leucoanthocyanidin	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Proanthocyanidin	(-)	+	+	+	(-)	(-)	(-)
Catechin	(-)	(-)	(-)	+	(-)	(-)	(-)
Iridoid	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Sugar	+	+	+	+	+	+	+
Alkaloid	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Phenolic Acid	+	+	+	+	+	+	+
Catechic Tannin	(-)	+	+	+	(-)	(-)	(-)

+: Metabolite Present; **(-)**: Metabolite Absent; **L** (Leaves); **B** (Stem Bark); **R** (Root peel); **S** (Seeds); **En** (Endocarp of the fruit); **Ex** (Exocarp of the fruit); **F** (Flower); **M** (Methanol).

The genus *Schinopsis* (quebracho) is recognized to be rich in polyphenols, particularly in tannins (SARAIVA *et al*, 2011) and flavonoids, also being observed in species *Schinopsis brasiliensis* Engl., and these groups of secondary metabolites are closely relationship with antimicrobial activity (SARAIVA et al., 2011; BYLKA et al., 2004). Already isolated and identified from *S. brasiliensis* some antimicrobial potential compounds, like: gallic acid, quercetin, methyl gallate (MOREIRA, 2009; SOUZA, 1990; AKIYAMA et al., 2001), sitosterol (CARDOSO, 2001; VIRTUOSO et al., 2005) and ellagic acid (SOUZA, 1990; VATTEM & SHETTY, 2005) that may be related to the antimicrobial activity exhibited by extracts from *S. brasiliensis* Engl.

CONCLUSION

The extracts from *S. brasiliensis* showed excellent antimicrobial activity against the multidrug-resistant *S. aureus* strains. Further studies are needed to isolate and structurally characterize these constituents from leaves and flowers, with at least three molecules, and the stem bark, with at least five molecules with potential antistaphylococcal, observed by bioautography technique, which can be used to guide isolation the antimicrobial potential compounds. This is important, since the discovery of new antistaphylococcal components is necessary, either because the rapid acquisition of antimicrobial resistance possibility of *Staphylococcus aureus* strains. Also, it is necessary to increase the variety of

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antistaphylococcal agents with high potential therapeutic, low toxicity and that to be most effective against the infection produced by multidrug-resistant *S. aureus* MRSA.

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