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Article

Antimicrobial activity and bioautographic study of antistaphylococcal components from *Caesalpinia pyramidalis* Tull.

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The antimicrobial activity of dry methanol and ethyl acetate extracts for the leaves, bark of the stem, peel of the root, flower, fruit and seed of *Caesalpinia pyramidalis* Tull. (catingueira) was performed against seventeen isolates of *Staphylococcus aureus* MRSA multiresistant strains, which included two isolates of *S. aureus* MSSA and two ATCC strains. The antimicrobial activity was tested by the agar diffusion method and the Minimum Inhibitory Concentration (MIC) was determined. The dry methanol extract of the root showed good antimicrobial activity with a MIC of less than 0.5 $mg.mL^{-1}$. The dry ethyl acetate extracts exhibited lower antimicrobial activity, which might be explained by solubility problems and less diffusion in the agar medium. Results of the bioautographies also confirmed inhibition halos corresponding to the active substances present in the leaves, as well as in the flower of *C. pyramidalis.* The phytochemical study of the leaves, bark of the stem, peel of the root, flower and fruit of extracts from *C. pyramidalis* confirmed the presence of a number of known antimicrobial agents including ursolic acid, quercetin, catechin, ellagic acid, sitosterol, flavonoids, proanthocyanidins and gallic acid.

Uniterms: *Caesalpinia pyramidalis*/antimicrobial activity. *Caesalpinia pyramidalis/*bioautographic study. *Caesalpinia pyramidalis/*antistaphylococcal components. *Staphylococcus aureus/*multiresistant. Brazilian Epidemic Clone.

A determinação da atividade antimicrobiana dos extratos metanólicos e em acetato de etila da folha, casca do caule, casca da raiz, flor, fruto e semente de *Caesalpinia pyramidalis* Tull. foi realizada frente a dezessete isolados de *Staphylococcus aureus* MRSA multirresistentes, dois isolados de *S. aureus* MSSA e duas cepas padrão, pelas técnicas de poço/difusão em ágar e determinação das CMI pelo método de diluição em agar/multiinoculador de Stears. O extrato metanólico de casca da raiz indicou uma boa atividade, com CMI inferior a 0.5 $mg.mL^{-1}$. Os extratos secos por extração em acetato de etila apresentaram menor atividade que se poderia explicar por problemas de solubilidade e menor difusão no meio de cultura em ágar. Resultados das bioautografias confirmaram zonas de inibição correspondente às substâncias ativas presente na folha, como também na flor da *C. pyramidalis*. No estudo fitoquímico das folhas, casca da caule, casca da raiz, flor e fruto dos extratos de *C. pyramidalis* evidenciou-se a presença de vários constituintes com reconhecida atividade antimicrobiana, entre estes o ácido ursólico, quercetina, catequina, ácido elágico, sitosterol, flavonóides, proantocianidinas e ácido gálico. Entre todos os metabólitos citados, somente o ultimo não observamos, por CCD, na casca da raiz de *C. pyramidalis*.

Unitermos: *Caesalpinia pyramidalis/*atividade antimicrobiana. *Caesalpinia pyramidalis/*estudo bioautográfico. *Caesalpinia pyramidalis/*componentes antiestafilocócicos. *Staphylococcus aureus/* multirresistente. Clone Epidêmico Brasileiro.

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INTRODUCTION

The importance of nosocomial infection caused by *Staphylococcus aureus*, especially by methicillin resistant *S. aureus* (MRSA) is well known for its frequency, morbidity, mortality and principally for its difficulty to treat (Nascimento *et al*., 2000). The strains of MRSA are resistant to all β-lactamics, macrolides, tetracycline, aminoglycosides, while the two glycopeptides (vancomycin and teicoplanin) remain the only alternatives for clinical treatment against infections of MRSA multiresistant *S. aureus* (Shibata *et al*., 2005). However, *S. aureus* with intermediate susceptibility to vancomycin (VISA) or *S. aureus* glycopeptides (GISA) has recently been identified in different countries. Subsequently, strains of *S. aureus* resistant to vancomycin (VRSA) have also emerged, possessing a different mechanism to those of VISA strains (Nishi *et al*., 2004).

Studies conducted in Brazil have described a clone of methicillin resistant *Staphylococcus aureus* (Brazilian Epidemic Clone-BEC, ST247-SCC*mec*IIIA) that is disseminated and predominates within hospitals throughout the country. It was also observed that this clone has spread to other countries in South America (Argentine, Chile, Colombia, Peru, Ecuador, Uruguay), besides Europe (Portugal, Italy and the Czech Republic) and Asia (Miranda *et al.,* 2007; Sola *et al*., 2002; Rodríguez-Noriega *et al.,* 2010). In light of this evidence, the need to identify new antimicrobial agents is clear.

Caesalpinia pyramidalis Tull., Leguminous family (Fabaceae) is a tree found in the Northeastern region of Brazil, popularly known as "catingueira", "pau-de-porco" and "mussitaiba". In folk medicine, its flowers, seeds, leaves and bark of the stem are used for the treatment of catarrhal infections, diarrheas and dysentery, besides being endowed with antipyretic and diuretic properties (Mendes *et al*., 2000; Braga, 1960; Rêgo Júnior *et al*., 2011).

A large number of metabolites have been isolated from `catingueira`, such as: phenylpropanoids, lupeol, β-sitosterol, bioflavonoids (agastiflavone, amentoflavone, sequoiaflavone and podocarpusflavone), chalcone, kaempferol, apigenin, lignane, stigamasterol and methyl gallate (Mendes *et al*., 2000; Bahia *et al*., 2010; Novais *et al*., 2003). Crude ethyl acetate from the leaves and roots of *C. pyramidalis* Tull. tested against strains of *S. aureus* and *Escherichia coli* by the agar disc diffusion method showed inhibition halos in the order of 10 mm for the *S. aureus* strains.

MATERIAL AND METHOD

Collection and identification

The plant was collected in the town of Carnaubeira da Penha, in the hinterland of Pernambuco (State), at a latitude of 08°19'09", longitude of 38º44'41" and altitude of 446 meters (MME, 2005), between the months of March and June, 2004. Samples were identified by employees of the Herbarium of the Empresa Pernambucana de Pesquisa Agropecuária (IPA), Dr. Rita de Cássia Pereira, and deposited with voucher nº 70.008.

Preparation of extracts

For determination of the antibacterial activity of *C. pyramidalis*, different fresh material parts of the plant including leaves, bark of the stem, peel of the root, flower, seeds and fruit were triturated, weighed and submitted to three successive extractions by the process of infusion, with medium intervals of 72 hours for each solvent. The order of solvents used was *n*-hexane, followed by ethylic acetate and lastly methanol. The extracts thus obtained were filtered and solvents evaporated at 40 ºC under reduced pressure. These were subsequently weighed and their output calculated.

The dry extracts obtained from the methanol extraction were reintroduced into water/DMSO (1:1 v/v) (Sakagami *et al*., 2005) at a concentration of 100 mg.mL^{-1}. The dry extracts obtained from the ethyl acetate and n-hexane extractions were also reintroduced in tween 80/water (4.8:0.2 v/v) at a concentration of 100 mg mL^{-1}.

Bacteria strains

A total of twenty-one *Staphylococcus aureus* strains, comprising nineteen clinical isolates and two standard strains, were used in the study (Table I). These consisted of eight multiresistant *S. aureus* MRSA of the Brazilian epidemic clone (BEC), five isolates of the pediatric epidemic clone, four isolates of the sporadic clones and two MSSA strains which were only resistant to the antibiotics penicillin, erythromycin and gentamycin. The multiresistant *S. aureus* MRSA and *S. aureus* MSSA were strains from the Microbiological Analysis Laboratory collection, derived from a study on the susceptibility/resistance profile of *S. aureus* in Recife-Pernambuco state (Cordeiro, 2004).

Preparation of inoculates

The inoculates were prepared starting with the 24 h

TABLE I - List of *Staphylococcus aureus* strains assayed

Reg. Nº	Profile	Clone	Origin
AM723	MRSA	A1	Catheter point
AM793	MRSA	A1	Tracheal secretion
AM799	MRSA	A5	Vesical probe point
AM837	MRSA	A7	Catheter point
AM858	MRSA	A13	Catheter point
AM895	MRSA	A6	Hemoculture
AM902	MRSA	A2	Orifice secretion
AM948	MRSA	A9	Catheter point
AM599	MRSA	B3	Ulcer Secretion
AM642	MRSA	B5	Secretion
AM771	MRSA	B2	Tracheal secretion
AM922	MRSA	B6	Left leg wound
AM942	MRSA	B4	Hemoculture
AM594	MRSA	F	Catheter point
AM872	MRSA	G	Urine
AM875	MRSA	D	Hemoculture
AM876	MRSA	I	Hemoculture
AM632	MSSA	-	Operation wound
AM672	MSSA	-	Tracheal secretion
AM103	-	-	ATCC 6538
AM106	-	-	ATCC 6538P

Reg. Nº: Register Number; **AM**: Microbiological analysis laboratory collection – Pharmaceutical Science Department –UFPE; **ATCC:** American type culture collection; **Clone A:** Brazilian epidemic clone (BEC); **Clone I, D, G, F**: Sporadic clone; **Clone B:** Pediatric clone; **MRSA:** Methicillin resistant *Staphylococcus aureus*; **MSSA:** Methicillin sensitive *Staphylococcus aureus*

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

Agar well diffusion method

The inoculum were applied to the surface of the Mueller-Hinton agar and after perforation of the wells (perforator 6 mm in diameter), 100 µL of the extracts (concentrations of 100 mg .mL^{-1} and 50 mg mL^{-1}), standard antibiotic (300 µg mL^{-1}) and of the control solution (DMSO at 50% (v/v) or tween 80 at 4% (v/v)) were added to each well. After incubation at 37 ºC ± 1 for 24 hours, the diameter of the inhibition halos were measured and 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 – Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration for the extracts (basic solution of 20 mg.mL^{-1}), dilutions in water with concentrations of 312.5 µg.mL^{-1} to 20.10^3 µg.mL^{-1}, had been prepared and incorporated to Mueller-Hinton agar (1:9), thus final concentrations of 31.5 µg.mL^{-1} to 2000 µg.mL^{-1} of the extracts remained. The standard antibiotics used were tetracycline and oxacillin (Sigma), with concentrations as indicated by the CLSI (2003) norms.

The twenty-one inoculates were distributed aseptically in the Stears multi-inoculator, and then deposited on the surface of the culture medium, and incubated at 36 ºC ± for 24 hours.

Duplicate controls were performed at the beginning and end of the inoculation control process. Control of the diluents was also done (tween 80 at 4% and DMSO at 50%).

The results were interpreted comparatively to the strain controls and determined by the first plate whose concentration inhibited growth.

Phytochemical analysis

Thin layer chromatography (TLC) with silica-gel GF°254 (Merck) was used. The methanol or ethyl acetate extracts of the leaves and flower of *Caesalpinia pyramidalis* at the concentration of 20 mg mL^{-1} were applied using an analytic capillary (15 µL). The mobile phase for the methanol extracts was the chromatographic system (1): AcOEt/MeOH/H2O (81:11:08), while for the corresponding ethyl acetate the system (2): AcOEt/HCOOH/AcOH/H_2O (100:2:2:2 and 100:3:3:3) was employed. TLC was performed in duplicate, one being used as a chromatographic reference and the other for bioautography.

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

The visualization of TLC was carried out under UV light (254 and 366 nm), as shown in Table II.

Bioautographic technique

The developed TLC according to system (1) was subjected to aseptic current air for 5 minutes, and another developed by system (2) for 6h, respectively. On the TLC

TABLE II - Chromatographic systems used for phytochemical screening of *Caesalpinia pyramidalis* Tull.

Metabolites	Elution System	Revelator	Reference
Alkaloids	A	Dragendorff	Wagner *et al*., 1984
Triterpenoids and Esteroids	B	Liebermann-Burchard	Harbone, 1998
Iridoids	A	Sulfuric Vanilin	Wagner *et al*., 1984
Saponins	A	Anisaldehyde	Wagner and 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	Chloridric Vanillin	Robertson *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-Amino-ethyl-diphenyl borinate, TTC: Triphenyl Tetrazolium Chloride.

surface, melted Mueller-Hinton agar (MH) was added, inoculated with a saline suspension of *S. aureus* ATCC 6538 (bacterial suspension at 10^8 UFC/mL) and homogeneously distributed over the TLC plate. After solidification of inoculated MH, it was left for 30 minutes at the surrounding ambient temperature of 25 ºC for predifusion of the active components. Subsequently, it was incubated for 24 to 36 hours ±1 ºC. After this period the bioautography was revealed with a solution of 2,3,5-triphenyltetrazolium chloride (TTC) at 2.5 mg mL^{-1} and incubated for a further 4 hours.

The presence of an inhibition zone indicated the existence of active components (Pessini *et al*., 2003).

RESULTS AND DISCUSSION

Antimicrobial activity

The results of methanol and ethyl acetate extracts from *C. pyramidalis* against four clinical isolates and one standard strain (AM103) of *S. aureus* are given in Table III. The hexane extracts showed no activity. The diameters of the inhibition halos expressed in millimeters are expressed in the form of a table for each extract and each bacterium.

The MIC values of the assayed extracts are shown separately, according to type of *S. aureus* MRSA multiresistant clone. Table IV summarizes the results of eight Brazilian epidemic clones, five pediatric clones, four sporadic multiresistant clones, two strains of *S. aureus* MSSA, and finally two standard strains.

TABLE III - Antimicrobial activity of *Caesalpinia pyramidalis* against *S. aureus*

Extracts	mg/Well	*S. aureus* (AM) halo diameter in mm				
		594	723	922	942	103
LM	10 mg	18	20	20	21	20
	5 mg	15	15	16	18	16
BM	10 mg	20	18	18	19	21
	5 mg	17	15	16	16	19
RM	10 mg	19	16	17	17	20
	5 mg	19	16	17	17	20
FLM	10 mg	20	22	22	22	23
	5 mg	17	18	18	19	21
FM	10 mg	20	20	19	21	23
	5 mg	18	18	16	19	20
SM	10 mg	17	16	19	17	20
	5 mg	15	14	16	16	18
LA	10 mg	-	-	-	-	12
	5 mg	-	-	-	-	-
BA	10 mg	16	15	15	16	18
	5 mg	14	13	12	12	14
RA	10 mg	18	18	20	19	20
	5 mg	16	17	16	17	18
FA	10 mg	14	13	12	18	18
	5 mg	12	-	-	15	-
TT	30 mcg	11	12	27	12	28

L: (leaves); **FL** (flower); **B** (bark of stem); **R** (peel of root); **F** (fruit); **S** (seeds); **M** (methanol); **A** (ethyl acetate) **TT**: Tetracycline; **AM**: Microbiological analysis laboratory collection – Pharmaceutical Science Department –UFPE

Bioautography

The bioautographic results for the leaves and flower extracted in methanol and ethyl acetate, and peel of the root extracted in ethyl acetate, from *C. pyramidalis* are listed in Table V.

Phytochemical study

The secondary metabolites found in the extracts of the parts studied from *C. pyramidalis* with well-known antimicrobial activity are shown in Table VI.

Among the two techniques used for the determina-

TABLE IV - Minimum Inhibitory Concentration (MIC) of extracts from *Caesalpinia pyramidalis* against *Staphylococcus aureus* strains

STRAINS		MIC of extracts from *Caesalpinia pyramidalis* (mg mL^{-1})									MIC of Antibiotics (mg mL^{-1})		Antibiotics	
		LM	BM	RM	FLM	FM	SM	RA	FA	BA	Tet	Oxa	Sensitive	Resistant
AM	**723**	1	1	1	2	>2	>2	2	>2	2	0.032	0.064	1	2, 3, 4, 5, 6, 7, 8
AM	**793**	1	1	0.5	0.5	1	1	0.25	1	>2	>0.064	0.256	1	2, 3, 4, 5, 6, 7, 8
AM	**799**	1	1	0.5	0.5	2	1	0.25	0.5	>2	0.064	0.256	1	2, 3, 4, 5, 6, 7, 8
AM	**837**	2	2	1	1	NT	NT	NT	NT	2	0.032	0.256	1	2, 3, 4, 5, 6, 7, 8
AM	**858**	1	1	0.25	1	NT	NT	NT	NT	>2	0.032	0.256	1	2, 3, 4, 5, 6, 7, 8
AM	**895**	1	1	0.5	1	2	1	0.25	1	NT	0.064	0.064	1	2, 3, 4, 5, 6, 7, 8
AM	**902**	>2	>2	1	2	NT	NT	NT	NT	2	>0.064	0.032	1	2, 3, 4, 5, 6, 7, 8
AM	**948**	NT	NT	0.5	0.5	2	>2	1	0.5	2	0.064	0.256	1	2, 3, 4, 5, 6, 7, 8
AM	**599**	2	>2	1	1	2	2	NT	1	>2	0.001	0.016	1, 2, 4, 7, 5	3, 6, 8
AM	**642**	2	2	1	1	2	1	>2	2	>2	0.0005	0.032	1, 7, 5, 4	3, 2, 6, 8
AM	**771**	2	1	0.5	0.5	>2	1	NT	0.5	2	0.0005	0.032	1, 2, 4, 7, 5	3, 6, 8
AM	**922**	>2	1	1	1	1	2	2	1	2	0.002	0.064	1, 2, 3, 4, 7	6, 8, 5
AM	**942**	1	0.5	1	1	1	>2	NT	2	2	0.032	0.016	1, 2, 3, 5, 7	4, 6, 8
AM	**594**	0.5	1	1	1	2	2	>2	0.125	1	0.032	0.256	1	3, 4, 6, 2, 7, 5, 8
AM	**872**	>2	1	1	2	>2	>2	NT	0.125	>2	0.016	0.128	1, 2, 3, 5, 7	4, 6, 8
AM	**875**	2	2	0.5	1	2	2	NT	0.5	2	0.0005	0.016	1, 2, 4, 7, 5	3, 6, 8
AM	**876**	2	2	0.5	0.5	2	1	>2	0.5	2	0.002	0.032	1, 2, 3, 4, 7, 5	6, 8
AM	**632**	2	2	1	1	2	1	2	1	>2	0.001	0.00013	1, 2, 6, 4, 7, 5	3, 8
AM	**672**	2	2	NT	NT	>2	1	2	0.5	>2	0.001	0.00013	1, 2, 3, 4, 6, 7	5, 8
AM	**103**	2	2	0.5	0.5	2	1	2	0.25	2	0.00025	0.00006	ATCC 6538	
AM	**106**	2	1	0.5	1	2	0.5	>2	0.25	2	0.00025	0.00013	ATCC 6538P	

NT: Not tested; **L:** (leaves); **FL** (flower); **B** (bark of stem); **R** (peel of root); **F** (fruit); **S** (seeds); **M** (methanol); **A** (ethyl acetate).; (**1**) – vancomycin; (**2**) – ciprofloxacin; (**3**) – erythromycin; (**4**) –tetracycline (**Tet**); (**5**) – gentamicin; (**6**) – oxacillin (**Oxa**); (**7**) – sulfamethoxazole/trimethoprim; (**8**) – penicillin.; **AM**: Microbiological analysis laboratory collection – Pharmaceutical Science Department –UFPE

TABLE V - Retention factor values related to inhibition halos of extracts of *Caesalpinia pyramidalis* obtained by bioautographic technique

Chromatograph Systems	Extracts	Retention Factor (R*fs*)			
AcOEt/MeOH/H_2O (81:11:08)	LM	0.55	0.84	0.94	
	FLM	0.19	0.29	0.48	
AcOEt/HCOOH/AcOH/H_2O (100:2:2:2 and 100:3:3:3)	LA	0.51	0.62	0.80	0.94
	FLA	0.62			
	RA	0.26	0.39	0.58	0.68

L: (leaves); **FL** (Flower); **R** (Peel of Root); **M** (Methanol); **A** (Ethyl Acetate); AcOEt: Ethyl acetate, MeOH: Methanol, HCOOH: Formic Acid, AcOH: Acetic Acid.

TABLE VI - Secondary metabolites from *Caesalpinia pyramidalis* with well-known antimicrobial activity

Secondary Metabolites	*Caesalpinia pyramidalis*				
	Leaves	Stem of Bark	Peel of Root	Fruit	Flower
Ursolic Acid	**(-)**	**(-)**	+	**(-)**	**(-)**
Sitosterol	+	+	+	+	+
Cinnamic Derivatives	+	+	+	+	+
Flavanoids of Aglicones	+	+	+	+	+
Quercetin	**(-)**	**(-)**	+	**(-)**	**(-)**
Condensed Proanthocyanidins	**(-)**	+	+	**(-)**	**(-)**
Catechin	**(-)**	+	+	**(-)**	**(-)**
Gallic Acid	**(-)**	**(-)**	**(-)**	+	+
Ellagic Acid	**(-)**	**(-)**	+	+	+

+: Metabolite Present; (-): Metabolite Absent

tion of antimicrobial activity, the agar well diffusion technique, despite using larger volumes (100 µL) (Caetano *et al*., 2002) compared to disks (10 µL) (Voravuthikunchai and Kitppipit, 2005), has the advantage of allowing the use of adjuvant to improve the solubility of the extract constituents and to permit radial as well as superficial diffusions, conditions resulting in better inhibition halos.

With regard to the five *Staphylococcus aureus* strains studied, four represent the three types of MRSA multiresistant clones and one the standard strain.

In Table III, the extract of the flower methanol (FLM) showed the largest inhibition halos for the five tested strains, with values in the order of 22 mm, at the highest concentration (10 mg/well), corresponding to the very active classification (Alves *et al*., 2000), and halos in the order of 16 mm for concentrations of 5 mg per well , with extracts of leaves, peel of the root, fruit and seed also having good activity.

In general, extracts from retrieval in ethyl acetate showed smaller inhibition halos compared with those extracted from methanol. This fact can be due to the polarity characteristics of its constituents which resulted in lower diffusion in aqueous environs and therefore produced smaller inhibition halos despite having substances with antimicrobial activity (Lenette *et al*., 1987).

Observing Table III of the corresponding extracts (leaves, bark of the stem, peel of the root, fruit and seed) in ethyl acetate, the extract of peel of the root produced inhibition halos in the order of 20 mm, while the extract of the leaves showed no activity except against the standard strain (AM103). Experimentally, this difference may be explained by the fact that the dry extract of peel of the root is in the form of powder and dissolved very well in tween 80/water 4% whereas extract from the leaves had a pasty consistency and was not well dissolved, hindering its solubility.

The inhibition zone produced by the standard antibiotic, tetracycline, confirmed the resistance phenotype of the clone strains, i.e. *Staphylococcus aureus* MRSA AM594, *S. aureus* AM723 and *S. aureus* AM942, resistant to tetracycline had inhibition halos in the order of 13 mm or less while the strains *S. aureus* MRSA AM922 and *S. aureus* AM103 sensitive to tetracycline had inhibition halos in the order of 26 mm.

Finally, the two diluents, DMSO at 50% and tween 80 at 4%, exhibited no inhibition.

The presented data are mean values of two determinations.

On the other hand, the presence of active substances in the leaves as well as in the flower for both types of extracts was evidenced in the results of the bioautographies (Table V).

The bioautography technique permits the detection of inhibition halos that reveal the presence of active substances in the TLC (Pessini *et al*., 2003) against *S. aureus* ATCC 6538 in the methanolic extracts, one in R*f* 0.48 for the flower extract and R*f* 0.55 for the leaves extract. Similarly, for the extracts in ethyl acetate, one showed the presence of inhibition halos in R*f* 0.62 for the flower extract, the R*f* 0.51, R*f* 0.62, R*f* 0.80 and R*f* 0.94 for the leaves extract and the R*f* 0.26, R*f* 0.39, R*f* 0.58, R*f* 0.68, , R*f* 0.77 and R*f* 0.92 for the stem bark extract (Table V).

The phytochemical investigation evidenced the presence of substances such as flavonoids (flavonoid aglycones), sitosterol and cinnamic derivatives for all the extracts of *C. pyramidalis* tested. The presence of gallic acid and ellagic acid was also evidenced in the flower extract in methanol . Other components identified were

polyphenols (phenolic acid) in the fruit, and ursolic acid and quercetin from the bark of the stem and peel of the root (root and bark of the stem) thus confirming data from the literature (Bahia *et al*., 2010; Bahia *et al*., 2005).

With reference to MIC (Table IV), analysis of the results reveals that, in the case of *S. aureus* MRSA Brazilian epidemic clone, that some of the values are less than 0.5 $mg.mL^{-1}$ for the methanol extracts as well as ethyl acetate extracts of the peel of the root.

Similar results were found for the methanol extract of the flower.

The MIC values for the five pediatric clones were again low for the extracts of the peel of the root, being in the order of 0.25 $mg.mL^{-1}$ for both methanol and ethyl acetate extracts.

Also, in Table IV featuring MIC for the four sporadic clones together with the susceptible *S. aureus* MSSA strains and standard strains, the extracts of the peel of the root in ethyl acetate had some values as low as 0.125 mg mL^{-1}, even less than those of the MSSA strains (0.5 mg mL^{-1}) and standard strains (0.25 mg mL^{-1}).

The MIC values of tetracycline and oxacillin confirmed the phenotype resistance and MRSA or MSSA character of isolates of *S. aureus* strains, respectively.

In relation to the DMSO diluents at 50% and tween 80 at 4%, these showed no inhibition against any of the twenty-one strains, confirming earlier data for DMSO by Sakagami *et al*. (2005).

CONCLUSION

The extracts from *C. pyramidalis* showed good antimicrobial activity against the S. aureus multiresistant strains. Also, the bioautography technique allowed visualizing six inhibition halos for the extracts in ethyl acetate from stem bark and three from the leaves, which allows us to conclude that there are at least six plus three active compounds, respectively.

Further experiments aimed at the isolation and structural identification of the active compounds are needed, as well as more pharmacological data to validate the popular use of the extracts from the *C. pyramidalis.* such as data determining its safety and toxicity.

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