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Antimicrobial Activity of the Crude Ethanol Extract and Fractions from *Eugenia uniflora* Leaves Against *Pseudomonas aeruginosa*

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SUMMARY. This study evaluated the antimicrobial activity of the crude ethanol extract and fractions from *Eugenia uniflora* L. leaves against *Pseudomonas aeruginosa*. A total of 72 *P. aeruginosa* isolated from patients of three hospitals in Goiânia and 8 standard strains were selected to test antimicrobial activity. The bacteria susceptibility profile against 15 antimicrobial agents was determined using the disc diffusion method. The minimum inhibitory concentration of the crude ethanol extract and the ethyl acetate, chloroform and hexane fractions was determined. Most of the *P. aeruginosa* analyzed proved resistant to a number of antimicrobial substances and their sensitivity varied from 81.9% sensitivity to meropenem to 0.0% sensitivity to cefotaxim. All the *P. aeruginosa* were inhibited by the crude extract and by the ethyl acetate fraction. Only 11.25% of the bacteria analyzed were inhibited by the chloroform fraction (8.75 mg/mL MIC). The hexane fraction presented no activity.

INTRODUCTION

P. aeruginosa is an aerobic, non-fermenting ubiquitous Gram-negative bacteria usually found in humid environments [1]. Within the hospital environment this bacteria is especially relevant to feeble patients who present alterations in their physical barriers and immunosupression [2]. The spectrum of infections caused by this agent ranges from skin infections to fulminant septicemias. *P. aeruginosa* can cause acute infection by producing a number of toxins as well as chronic infections by forming a biofilm rich in alginate such as in cystic fibrosis [3].

Infections caused by *P. aeruginosa* are relevant due to the intrinsic resistance of this microorganism to multiple classes of antimicrobial agents and due to their ability to acquire induced resistance during therapy [4]. The bacteria are resistant to several β-lactams, some quinolones, chloramphenicol, tetracyclins, macrolides, trimethoprim-sulfamethoxazole and rinfampicin [1].

There are a limited number of antimicrobial agents active against *P. aeruginosa,* including penicillins and anti-pseudomona cephalosporins, carbapenens and fluorquinolones, particularly ciprofloxacin [5]. Marques *et al.* [6] studied 84 *P. aeruginosa* isolates in people attending an outpatient center in Belém-Pará, Brazil, detected isolates resistant to cotrimazol (95 %), ampicillin/sulbactam (91.3%) and ticarcillin (81.6%) but sensible to piperacillin/tazobactam/piocianin (80.9%) and to colistin (100 %). Figueiredo *et al.* [7] noted that *P. aeruginosa* isolates in two hospitals in Recife – Pernambuco, Brazil, presented a cross resistance rate between 22.9 and 38.1% between the β-lactams (carbapenens and piperacillin/tazobactam) and the aminoglycosides and quinolones (agents normally used as adjuvants in the treatment of serious *Pseudomonas* infections).

The problem of microbial resistance is increasing and the prognosis regarding the use of antimicrobial agents in the future is still uncertain, therefore strategies must be devised to reduce microbial multiresistance, such as control measures for the use of antimicrobial agents, detection and comprehension of genetic resistance mechanisms and the quest for new synthetic or

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natural substances that have antimicrobial activity. The main objective is to offer efficient and appropriate antimicrobial drugs to patients with infections, especially when caused by multiresistant microorganisms [8].

Plants have always been a valuable source of natural health products. Due to more intensive studies over the last years, their components have been described and characterized [8]. The antibacterial and antifungal activity of products obtained from medicinal plants has been verified in studies on phytoalexins, fixed and essential oils, cumarins, terpens, flavonoids, isoflavonoids, amides, imides, alkaloids and so on [9].

The Myrtaceae family comprises around 140 genera. The chemical profile of this family is characterized by the presence of tannins, flavonoids, mono and sesquiterpenes, triterpenes, phloroglucynol derivatives, chromenes, stilbenzoids and others. The Myrtaceae species are usually used for treating diarrhea, hemorrhages, fever, cystitis, urethritis, rheumatism, and hyperglycemia [9]. *Eugenia uniflora* L. (pitangueira) is one of the species described. It is a well known and highly appreciated plant in Brazil that produces edible fruit. The tea made from its leaves is used in popular medicine especially as a hypotensor, antigout, stomachic, hypoglycemiant [10] and antibiotic agent [11].

In vitro research has reported the antimicrobial activity of the essential oils from *E. uniflora* leaves and fresh fruit against *Pseudomonas aeruginosa* [12], *Staphylococcus aureus, Escherichia coli* [13], *Bacillus subtilis, Micrococcus luteus* [11] and against the following fungi: *Trichophyton mentagrophytes* [12], *Candida krusei* [14], *C. parapsilosis* e *C. tropicalis* [13], *Microsporum canis, M. gypseum, T. rubrum* and *T. mentagrophytes* [15].

The purpose of this study was to test the antimicrobial activity of the crude ethanol extract and ethyl acetate, chloroform and hexane fractions of *E. uniflora* leaves against 8 standard strains and 72 *P. aeruginosa* isolates as well as comparing the susceptibility profile of *P. aeruginosa* against several antimicrobial agents.

MATERIALS AND METHODS

Botanical Material

The botanical material consisting of leaves from the *E. uniflora* species was collected in the municipality of Goiânia, Goiás (16º 36´15.1"S and 49º 16´ 0.70" W, at an altitude of 778 m) from February through April 2005, identified by Dr. José Realino de Paula, professor at the Federal University of Goiás. Voucher specimens were deposited in the herbarium of that institute under registration number UFG/29859. The leaves were oven dried with air circulation at 40 °C and then pulverized by blade mill.

Preparation of the crude ethanol extract, fractions and determination of the TLC profile

The *E. uniflora* leaf powder was macerated in ethanol at 95% (W/W) P.A. in a 1:3 proportion at room temperature, with occasional shaking for 72 h, followed by filtration. The extract obtained was concentrated in a rotavapor at 40 °C and the vegetable residue was extracted twice again analogously, thereby obtaining the crude ethanol extract. The crude ethanol extract was solubilized in MeOH/H_2O 7:3 to obtain the fractions. The resulting solution was extracted successively with hexane, chloroform, and ethyl acetate; these fractions were concentrated in a rotavapor at 40 °C [16].

For TLC the fractions were re-dissolved in ethanol 95% (W/W) and applied to TLC plate containing silicagel 60 F254 (MERCK). The TLC analyses were performed with the following solvent mixtures for mobile phase: cetone/toluene/formic acid (3:3:1), ethyl acetate/formic acid/glacial acetic acid/water (100:11:11:27), ethyl acetate/methanol/water (100:13.5:10), chloroform/glacial acetic acid/methanol/water (64:32:12:8), and ethyl acetate/formic acid/water (90:5:5). For the detection of the main characteristic compounds were used: $FeCl_3$/HCl (condensed and hydrolysable tannins and flavonoids), vaniline/H_2SO_4 (terpenes, condensed and hydrolysable tannins, flavonoids and saponins), KOH at 10% (anthraquinones, anthrones, anthrol and cumarins), NP 1% (diphenyl boric acid 1% in methanol)/PEG (polyethyleneglycol) 4000 at 5% in ethanol (flavonoids, aloína), UV (lignans and chlorophyll). The compounds on TLC plates were detected by visible light and UV (365 nm), and analyzed according to Wagner & Bladt [17].

Total phenol, tannin and flavonoid dosages

The total phenol and tannin dosages were performed in triplicate according to the methodology described by Hagerman and Butler [18] and the total flavonoids in triplicate according to the methodology described for *E. uniflora* in Brazilian Pharmacopoeia [19].

Antimicrobial activity evaluation

Microorganisms

The antimicrobial activity evaluation was performed with 72 *Pseudomonas aeruginosa* clinical specimen isolates from patients that attended three hospitals in Goiânia, Goiás – Brazil, 4 standard strains of *P. aeruginosa* (ATCC 2919, ATCC 2724, ATCC 2443, ATCC 9027) and 4 production of metallo-β-lactamase (IMP-1, IMP-2, VIM-2, SPM-1). The *P. aeruginosa* investigated were isolated from 41.7% of the patients hospitalized in the clinical ward, 38.9% in the intensive care unit (ICU) and 19.4% in the surgical ward. Of the 72 *P. aeruginosa* analyzed, 22 were isolated from urine (30.5%), 15 from blood (20.8%), 1 from ascitic liquid (1.4%), 2 from bronchic liquid (2.8%), 1 from a subcutaneous abscess (1.4%), 2 from spittle (2.8 %), 2 from catheter tips (2.8%) and from different secretions: 5 postoperative wounds (6.9%), 1 abdominal (1.4%), 1 breast (1.4%), 5 tracheal (6.9%), 1 plantar ulcer (1.4%), 1 leg lesion (1.4%), 1 right foot lesion (1.4%), 1 gastrotomy (1.4%), 2 from oropharynxes (2.8%), 1 from a drain (1.4%), 1 from a bone fracture (1.4%), 1 from a venous dissection (1.4%), and 6 from scabs (8.3 %) (Table 1).

The microorganisms used are part of a collaborative project between the Medical Bacteriology Laboratory of the Microbiology Depart-

P. aeruginosa	Hospital sectors	Local collection	Hospital	*P. aeruginosa*	Hospital sectors	Local collection	Hospital
6	ICU	urine	AJ	307	clinical ward	gastrotomy secretion	HC
9	clinical ward	blood	AJ	309	clinical ward	urine	AJ
18	surgical ward	ascitic liquid	AJ	313	ICU	blood	AJ
21	ICU	blood	AJ	318	clinical ward	oropharynxes secretion	HC
24	surgical ward	bronchic liquid	AJ	321	clinical ward	catheter tips	HC
46	ICU	urine	AJ	325	clinical ward	urine	HC
56	surgical ward	postoperative wounds	HC	326	clinical ward	catheter tips	HC
61	ICU	urine	AJ	329	clinical ward	spittle	HC
71	ICU	blood	HC	330	clinical ward	oropharynxes secretion	HC
73	surgical ward	urine	HC	345	clinical ward	blood	HC
96	surgical ward	abdominal secretion	HC	346	ICU	drain secretion	SC
100	clinical ward	blood	HC	356	clinical ward	scabs secretion	HC
120	surgical ward	blood	AJ	370	surgical ward	blood	AJ
124	surgical ward	bronchic liquid	AJ	379	clinical ward	urine	HC
134	ICU	subcutaneous abscess	SC	380	ICU	blood	HC
140	surgical ward	breast secretion	HC	407	ICU	tracheal secretion	HC
170	ICU	tracheal secretion	HC	415	ICU	urine	HC
171	ICU	postoperative wounds	HC	427	ICU	tracheal secretion	HC
187	clinical ward	spittle	HC	433	clinical ward	scabs secretion	HC
201	ICU	tracheal secretion	AJ	436	clinical ward	bone fracture secretion	HC
202	clinical ward	urine	SC	439	ICU	blood	AJ
223	surgical ward	postoperative wounds	HC	446	clinical ward	tracheal secretion	HC
237	clinical ward	urine	HC	449	ICU	urine	AJ
238	clinical ward	blood	AJ	453	surgical ward	blood	SC
240	clinical ward	urine	AJ	477	surgical ward	postoperative wounds	HC
251	clinical ward	plantar ulcer secretion	HC	495	ICU	venous dissection secretion	HC
253	ICU	postoperative wounds	HC	496	clinical ward	scabs secretion	HC
254	clinical ward	urine	HC	499	clinical ward	urine	HC
256	surgical ward	blood	AJ	515	clinical ward	scabs secretion	HC
269	ICU	leg lesion secretion	HC	516	ICU	scabs secretion	HC
270	clinical ward	right foot lesion secretion	HC	522	clinical ward	scabs secretion	HC
272	ICU	blood	AJ	570	ICU	urine	AJ
278	ICU	urine	HC	574	ICU	urine	AJ
279	clinical ward	urine	HC	584	ICU	urine	AJ
280	clinical ward	urine	HC	585	ICU	urine	AJ
304	surgical ward	blood	HC	586	ICU	urine	AJ

Table 1. Origin of 72 *Pseudomonas aerugionosa* clinical specimen isolates from patients that attended three hospitals in Goiânia, Goiás – Brazil. **AJ** = Hospital Araújo Jorge; **HC** = Hospital das Clínicas; **SC** = Santa Casa; **ICU** = Intensive Care Unit.

ment in the Tropical Pathology and Public Health Institute (IPTSP) of the Federal University of Goiás and the Catholic University of Goiás, and were stored in inclined simple agar (ISA) at 4 °C.

Disc diffusion test and phenotypical detection of carbapenemic resistance

The disc diffusion test was performed according to the CLSI [20]. *P. aeruginosa* were cultivated in triple soy agar for 24 h at 37 °C. The bacterial inocule was prepared with a turbidity equivalent to half the 1.0 McFarland scale and spread on Muller-Hinton agar plates with a sterile swab. The discs of antimicrobial aminoglycosides (amikacin, gentamycin, tobramycin), betalactams (aztreonam), cephalosporins (cefepime, cefotaxim, ceftazidim, ceftriaxone), quinolones (ciprofloxacin, levofloxacin), carbapenemics (imipenem, meropenem), polymyxin B penicillins (piperacillin, piperacillin/tazobactam) and carboxipenicillin (ticarcillin) (Oxoid, Basingstoke, England) were placed on the agar surface. The inhibition halo readings were performed according to CLSI (2005) criteria after incubation. The interpretation of the polymyxin B results was based on the work published by Gales *et al.* [21]. The isolates with intermediary inhibition halos were considered resistant.

P aeruginosa with reduced sensitivity to carbopenemics and ceftazidim and sensitive to aztreonam underwent phenotypic testing using the disc approximation method with ethylenediaminetetraacetic acid (ETDA) and 2-methoxypropionic acid (2-MPA), as suggested by Arakawa *et al.* [22]. In the metallo-β-lactamase producing isolates a distortion was noted in the inhibition halo between the ceftazidim disc and the inhibitor disc. For positive controls the following were used: metallo-β-lactamase producing strains: *P. aeruginosa* carrying the gene bla_{IMP1}, bla_{VIM1}, bla_{VIM2} and bla_{SPM1} and *Acinetobacter baumanni* bla_{IMP2}. For negative control *P. aeruginosa* ATCC 27853 was used.

Metallo-β-lactamase production by *P. aeruginosa* was confirmed by the Etest® (AB Biodisk, Stockholm, Sweden). The MBL Etest® strip contains imipenem (4-256 µg/mL) on one side and imipenem (1-64 µg/ml) associated with EDTA (320 µg/ml) on the opposite side (Walsh *et al.,* 2002). This test was used as a phenotypic test to confirm the production of metallo-β-lactamase for the positive isolates in the disc approximation test. A bacterial suspension with turbidity equal to half the McFarland 1 scale was uniformly spread on the Müeller-Hinton agar plate and 15 min later the MBL Etest strips were placed on the inoculated plate.

The plates were incubated at 37 °C, over a period of 18 to 24 h. The isolate that presented a reduction ≥ 3 dilutions (log_2) in the MIC of imipenem associated with ETDA was considered the metallo-β-lactamase producer [23]. For positive control Acinetobacter baumanni blaIMP2 was used as the metallo-β-lactamase producing strain. *P. aeruginosa* ATCC 27853 was used for negative control.

Determination of the minimum inhibitory concentration (MIC) of E. uniflora

To determine the MIC of the crude ethanol extract and fractions of *E. uniflora* against *P. aeruginosa,* 700 mg of crude ethanol extract and 350 mg of ethyl acetate, chloroform and hexane fractions of *E. uniflora* were diluted in 2 mL of DMSO and underwent a 1:2 serried dilution. Next 19 mL of Müeller Hinton agar liquefied at 50 °C was added to each tube, homogenized and poured onto sterile Petri dishes. After dilution the crude ethanol extract concentrations varied from 17.50 to 0.55 mg/mL and the concentration of the fractions varied from 8.75 to 0.27 mg/mL. Plates containing 1.0 mL of DMSO were prepared for negative control. The plates were incubated at 37 °C for 24 h to test sterility.

Bacterial suspensions with turbidity equal to half the McFarland 1.0 scale were prepared, transferred to the Steers inoculator [24] and placed on the Müller Hinton agar plates containing the different concentrations of *E. uniflora* crude ethanol extract and fractions. The plates were incubated at 37 °C for 24 h. The lowest concentration capable of inhibiting the microbial development was considered MIC.

RESULTS

Preparation of the crude ethanol extract and fractions and determination of the TLC profile

The crude ethanol extract from *Eugenia uniflora* powder yielded 25.78% and the crude extract fractions yielded 13.8% for the hexane fraction, 11.7% for the chloroform fraction and 9% for the ethyl acetate fraction.

The TLC analyses indicated the presence of flavonoids in the ethyl acetate and chloroform fractions, tannins in the ethyl acetate fraction and terpens in the hexane and chloroform fractions.

Dosage of total phenols, tannins and flavonoids

In the *E. uniflora* sample the total phenol content was 9.22%, tannins were 5.08% and total flavonoids were 0.53 %.

Antimicrobials	*P. aeruginosa* (n = 72)	
	Sensitive %	Resistant %
Amikacyn	65.3	34.7
Gentamicin	56.9	43.1
Tobramicin	70.9	29.1
Aztreonam	69.4	30.6
Cefepime	72.2	27.8
Cefotaxim	0.0	100
Ceftazidim	66.7	33.3
Ceftriaxone	8.3	91.7
Ciprofloxacin	66.7	33.3
Levofloxacin	63.9	36.1
Imipenem	77.8	22.2
Meropenem	81.9	18.1
Piperacillin	62.5	37.5
Piperacillin/Tazobactan	65.3	34.7
Ticarcillin	40.3	59.7
***E. uniflora* (MIC)**		
Crude ethanol extract	100	0.0
Ethyl acetate fraction	100	0.0
Chloroform fraction	11.3	88.7
Hexane fraction	0.0	100

Table 2. Susceptibility profile of *P. aeruginosa* to antimicrobials and to the *E. uniflora* crude extract and fractions.

Antimicrobial activity test

The disc diffusion test showed that among the 72 *P. aeruginosa* isolates sensitivity varied from 81.9% to meropenem to 0.0% to cefotaxim. The antimicrobials that presented the highest sensitivity to *P. aeruginosa* were meropenem (81.9%), imipenem (77.8%), cefepime (72.2%). Cefotaxim, cephtriaxone and ticarcyllin presented lower sensitivity rates: 0.0, 8.3 and 40.3 % respectively (Tables 2 and 3). In general the carbapenemic compounds presented the highest percentages of sensitivity while the cephalosporins presented the highest resistance rates.

The crude ethanol extract from *E. uniflora* leaves inhibited the development of all the *P. aeruginosa* (Table 2), namely 47.5% in the 4.37 mg/mL concentration, 27.5% in the 8.75 mg/mL concentration, 17.5% in the 2.187 mg/mL concentration, 5% in the 17.5 mg/mL concentration and 2.5% in the 1.093 mg/mL concentration (Table 4).

The ethyl acetate fraction also inhibited 100% of the *P. aeruginosa* analyzed (Table 2), with a MIC inferior to that of the crude extract. Half the isolates were inhibited with a MIC of 1.09 mg/mL, 25% with a MIC of 2.19 mg/mL, 21.25% with a MIC of 4.37 mg/mL and 3.75 % with a MIC of 8.75 mg/mL (Table 3). Only 11.3% of the bacteria were inhibited by the chloroform fraction (Table 2), (MIC of 8.75 mg/mL). The hex-

P. aeruginosa	*E. uniflora* leaves		Antimicrobials														
	MIC (mg/ml)		Disk diffusion test														
	Crude ethanol extract	Ethyl acetate fraction	A	B	C	D	E	F	G	H	I	J	L	M	N	O	P
96	4.37	1.09	S	R	R	R	R	R	S	R	R	S	S	R	R	R	S
134	2.18	1.09	S	S	S	R	S	S	R	S	S	S	S	S	S	S	S
171	4.37	1.09	S	R	R	R	R	R	R	S	R	R	R	R	R	R	S
202	8.75	2.18	R	R	S	R	R	R	R	R	R	R	R	R	S	R	R
254	8.75	2.18	R	S	R	R	R	R	R	R	S	R	S	R	R	R	R
278	4.37	2.18	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R
279	4.37	1.09	R	R	R	R	R	R	R	R	S	R	S	R	R	R	R
304	8.75	4.37	R	R	R	R	R	R	R	R	S	R	S	S	R	R	R
321	4.37	1.09	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R
325	8.75	4.37	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R
415	4.37	4.37	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R
495	4.37	1.09	S	S	S	R	S	R	S	S	S	S	S	S	S	S	S
516	8.75	2.18	R	S	R	R	R	R	R	R	R	R	R	R	S	R	R
584	17.5	2.18	S	S	S	R	S	R	S	S	S	S	S	S	S	S	S

Table 3. Susceptibility profiles of 14 metallo-β-lactamase producing *P. aeruginosa* to the *E. uniflora* crude ethanol extract, ethyl acetate fraction and antimicrobials. A = amikacin; B = aztreonam; C = cefepime; D = Cefotaxim; E = ceftazidim; F = ceftriaxone; G = ciprofloxacin; H = gentamicin; I = imipenem; J = levofloxacin; L = meropenem; M = piperacillin; Piperacillin/TAZO; O = ticarcillin; P = Tobramicin; R = resistant; S = sensitive.

Minimum inhibitory concentration (mg/L)	***E. uniflora* leaves**			
	***Pseudomonas aeruginosa* (n = 80)**			
	Crude ethanol extract	**Ethyl acetate fraction**	**Chloroform fraction**	**Hexane fraction**
1.09	2	40	0	0
2.19	14	20	0	0
4.38	38	17	0	0
8.75	22	3	9	0
17.5	4	0	0	0
% total	100 %	100 %	11.25 %	0 %

Table 4. Minimum inhibitory concentration of crude extract and fractions from *E. uniflora* leaves *against P. aeruginosa*.

ane fraction in the concentrations analyzed did not inhibit the development of the *P. aeruginosa* tested (Tables 2 and 4). Of the 72 *P. Aeruginosa* analyzed, 14 (19.4%) were metallo-beta-lactamase producers and were inhibited by the crude ethanol extract and the ethyl acetate fraction (Table 3).

DISCUSSION

P. aeruginosa isolates from patients, with resistance profiles and/or metallo-β-lactamase production, presented 100% sensitivity to different concentrations of the crude extract and ethyl acetate fraction of *E. uniflora*. These bacteria proved resistant to the antimicrobial agents tested with resistance percentages varying from 100% to cefotaxim to 18.1% to meropenem. *P. aeruginosa* resistance to multiple antimicrobial agents has been reported by a number of authors. Paviani *et al.* [2] noted that *P. aeruginosa* isolates in a hospital in the municipality of Porto Alegre, Rio Grande do Sul – Brazil, presented higher sensitivity to ceftazidim, cefepime and imipenem with the following percentages: 77%, 78% and 67% respectively and lower sensitivity rates to amikacyn, gentamicyn and ciprofloxacin with 47%, 38% and 42% respectively. Torres *et al.* [25] showed that 7.71% of *P. aeruginosa* isolates from various clinical samples from the General Hospital in Fortaleza, Ceará – Brazil produced MBL, with 82% sensitivity to piperacillin/tazobactam, 73.63% to piperacillin, 62.70 % to imipenem, 62.05% to ticarcillin-clavulanic acid and 44.37% to ceftazidim. Babay [26] when analyzing *P. aeruginosa* isolated in a university hospital in Riyadh, Saudi Arabia from 2001 through 2005, noted a gradual increase in *P. aeruginosa* resistance to most antimicrobial agents, particularly to aztreonam, ceftazidim, piperacillin/tazobactam and imipenem. These reports on the emergence of multi-resistant *Pseudomonas* reinforce the importance of controlling the use of antimicrobials as well as searching for new substances with antimicrobial activity. In this respect, *E. uniflora* can be a promising alternative since it inhibited the development of this bacterium.

In this study, 19.4% of the isolated *P. aeruginosa* were MBL producers. According to Bush [27], MBL belong to the group 3 extended spectrum β-lactamases, part of a common functional class of metalloenzymes classified according to their ability to hydrolyze imipenem at a measurable level, as well as their susceptibility to the available β-lactamase inhibitors.

MBL production widens the bacterial resistance arsenal since it causes the hydrolysis of penicillins, cephalosporins and carbapenems [2]. The acquisition of new antimicrobial resistance mechanisms highlights the increasing need to search for new natural drugs. In this regard, the ethanol extract and ethyl acetate fraction of *E. uniflora* come as molecule forerunners to control infections caused by *P. aeruginosa,* including MBL producers.

The TLC analysis indicated the presence of tannins and flavonoids in the ethyl acetate fraction, flavonoids in the chloroform fraction and chlorophyll and terpens in the hexane and chloroform fractions. Tannins can act as antiseptic and antimicrobial agents, present anti-hemorrhagic and anti-diarheal activity and have cicatrizing effect [28]. Scalbert [29] noted that several tannin rich substracts inhibited bacteria belonging to a number of genera, including the *Pseudomonas* genus, while many flavonoids presented antibacterial and anti-inflammatory activity [30]. The antimicrobial activity detected in this study

against *Pseudomonas* may be linked to the presence of tannins and flavonoids detected in the ethyl acetate fraction, as this fraction presented the best inhibition results for the *P. aeruginosa* tested.

CONCLUSIONS

The antimicrobial activity of the crude extract and ethyl acetate fraction from *E. uniflora* leaves against *P. aeruginosa* resistant to multiple antimicrobials shows hope for future treatment of multiresistant hospital infections caused by this pathogen. This activity justifies the popular use of this plant as an antibiotic and may be related to the presence of tannins and flavonoids in its chemical constitution.

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