

## ***In vitro* antifungal activity and phytochemical characterization of *Eugenia uniflora*, *Libidibia ferrea* and *Psidium guajava***

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The aim of this study was evaluate the *in vitro* antifungal activity of crude extracts from *Eugenia uniflora*, *Libidibia ferrea* and *Psidium guajava*. The extracts were obtained by turbo-extraction using water (AQ) or acetone-water (AC-W) (7:3, v/v) as solvents and lyophilized to obtain the crude extracts (CE). The CE were characterized by UV-Vis, TLC and HPLC. The activity of CEs was investigated against clinical isolates of *Candida* spp. and the Minimum Inhibitory Concentration (MIC), MIC<sub>50</sub> and MIC<sub>90</sub> were determined. The analysis by TLC showed that all CEs presented polyphenols (flavonoids and tannins). The CEs from *E. uniflora* showed higher amount of polyphenols (30.35 ± 2.15%, AC-W) and the HPLC analysis revealed the tannins in all extracts. The CEs of *E. uniflora* showed MIC range from 1.9 to 500.0 µg/mL, and lower values of MIC<sub>50</sub> and MIC<sub>90</sub> against non-*albicans* *Candida* isolates. Regarding *L. ferrea* and *P. guajava*, the results showing MIC from 3.9 to 1000.0 µg/mL (CE-AQ) against *C. albicans*. The results demonstrate antifungal performance from CE against various species of *Candida* spp., suggesting that the herbal species may be use as new potential antifungal agents. Additionally, the polyphenol content can play a pivotal role in the antifungal properties of CE.

**Keywords:** Medicinal plants. Herbal extracts. Polyphenols. *Candida* isolates. Oral candidiasis.

### **INTRODUCTION**

Oral candidiasis is an opportunistic infection, which begins by proliferation of endogenous commensal yeasts that inhabit the oral cavity. The oral tissue is the main way to various microorganisms, including *Candida* spp.. In healthy individuals, the immune defenses limit the

proliferation of pathogenic microorganisms, reducing the connection with these tissues (Williams, Lewis, 2011). However, under certain conditions, these fungi may take the pathogenic form, especially in a group specific of patients, such as patients with transplant recipients, with Acquired Immunodeficiency Syndrome (AIDS) and resistant to conventional antifungal (Chaves *et al.*, 2013).

Treatment with immunosuppressive depresses the host defense response, meaning a risk to oral infection, besides the association with other complications. In

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patients taking this type of treatment, such as transplant patients, the pathogens most likely to oral are local destruction and opportunistic infections appear due to the inability of the immune system to suppress and destroy such pathogens (Rojas *et al.*, 2012).

The presence of lesions in the oral cavity can be considered as a risk factor for the development of oral infections, such as candidiasis (Sankari *et al.*, 2015). In these cases, candidiasis can become invasive, being an important cause of morbidity and mortality caused by fungal infections that are particularly frequent in the first 6 months after the transplant (Shoham, Marr, 2012).

*Candida albicans* is the principal microorganism associated with this type of infection, although other species such as *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, *C. guilliermondii*, *C. lusitaniae* and *C. krusei* also are considered pathogenic (Sardi *et al.*, 2013), in addition, the species *C. dubliniensis* has been associated (Chaves *et al.*, 2013; Sardi *et al.*, 2013).

The increasing number of fungal infections has created a need for new antifungal agents. Several drugs such as *polyenes* (amphotericin B) are toxic; so becomes the appearance of *azoles* (fluconazole, ketoconazole, voriconazole), these are inexpensive, but *Candida albicans* and non-*albicans Candida* species have become resistance; *echinocandins* (*caspofungin*, *micalofungin* and *anidulafungin*) have low toxicity and few drug interactions; and, *fluoropyrimidines* and others, may be interfere with some of the virulence factors of *Candida* species (Dimopoulos *et al.*, 2013). Furthermore, many of these drugs have limitations such as narrow spectrum of action, side effects and toxicity. Therefore, the excessive and indiscriminate use of antifungal conventional agents increases the development of resistant, complicating the treatment of infections (Wille *et al.*, 2013).

In this context, natural products arise as an important and promising source for the discovery of novel antifungal agents (Ferreira *et al.*, 2013). Since these products have traditionally been used in controlling various diseases, as they are sources of multiple active compounds possessing therapeutic properties and are part of different synthesis of various natural products (Liu *et al.*, 2012). Furthermore, the use of native plant species can be an economical alternative to the use of sustained region.

The aim of the present study was to characterize and evaluate the *in vitro* antifungal activity against clinical isolates from the oral cavity of kidney transplant

recipients of crude extracts of *Eugenia uniflora*, *Libidibia ferrea* and *Psidium guajava*.

## MATERIAL AND METHODS

### Plant material

Samples of *E. uniflora* (leaves), *L. ferrea* (stem bark), and *P. guajava* (leaves) were collected in the states of Pernambuco and Rio Grande do Norte. Voucher specimens were deposited at the Agronomy Institute of Pernambuco (IPA) and the Department of Botany at the Federal University of Rio Grande do Norte (UFRN), under numbers 11763, 88145 and 8214, respectively.

### Preparation of crude extracts

The extracts were obtained by turbo-extraction with four extractive cycles of 1 min interspersed with 4 min of pause [10% (w/v) of dried and ground raw material], using water or acetone: water (7:3, v/v) as solvents. The extracts were filtered and concentrated under reduced pressure in an evaporator (RV10 Basic, IKA®) for complete elimination of the organic solvent. The residue was frozen (-80 °C, 3 days) and then lyophilized (Model L101, Liotop®) to yield a crude extracts (CEs).

### Phytochemical analysis: Thin Layer Chromatography (TLC)

Pre-coated TLC silica gel 60-F254 aluminum plates (10 cm × 10 cm; 250 µm thickness; Macherey-Nagel®) were used. The plates were developed using ethyl acetate: formic acid: water (90:5:5, v/v/v) as the mobile phase. The chamber was saturated with the mobile phase at room temperature (25 ± 2 °C) for 15 min. After development, the plates were dried and the compounds were visualized after derivatization by spraying the NEU+PEG reagent for flavonoids and hydrolysable tannins, and vanillin chloride for condensed tannins. Were used as standards: quercetin, gallic acid and catechin, respectively, for flavonoids, hydrolysable tannins and condensed tannins (Bezerra *et al.*, 2018).

### Determination of Total Polyphenol Content

The methods described by the Brazilian Pharmacopeia (Brazil, 2010) for the determination

of Total Polyphenol Content (TPC) were used as guidelines for analyses of crude extracts and standard (pyrogallol, 98%; Sigma-Aldrich®). Solutions of pyrogallol (1000.0 µg/mL) and crude extracts (1000.0 µg/mL) were prepared in distilled water. Each solution (at the appropriate dilution) was transferred to a 25 mL volumetric flask containing distilled water (10 mL) and the Folin–Ciocalteu (Cinética®) reagent (1 mL); the volume was completed with 29% anhydrous sodium carbonate (w/v). The samples were measured at 760 nm in an ultraviolet/visible spectrophotometer (Micronal®) after 30 min the addition of the last reagent. Distilled water was used as the blank. The experiments were carried out in triplicate.

### High-Performance Liquid Chromatography (HPLC) analysis

The crude extracts were analyzed by HPLC using a system Ultimate 3000 (Thermo Scientific®) equipped with a binary pump (HPG3x00RS, Thermo Fisher Scientific®), autosampler (ACC3000, Thermo Fisher Scientific®) and diode array detector (DAD; Thermo Fisher Scientific®). Chromeleon 6.0 (Dionex®) software was used for data acquisition and processing. The chromatographic separation was performed using a C18 column (250 mm × 4 mm i.d., 5 µm; Dionex®), equipped with a pre-column with the same material (4 mm × 3.9 µm, Phenomenex®). The mobile phase consisted by solvent A (purified water, Purelab Classic UV, Elga®) and solvent B (methanol, HPLC grade, Tedia®), both acidified with 0.05% trifluoroacetic acid (Vetec®) with flow rate at 0.8 mL/min, according to the following gradient program: 20–22.5% B (10 min), 22.5–40% B (10 min), 40–75% B (5 min), 75–20% B (3 min) and 20% B (4 min).

The content of substances found in all crude extracts was determined from the calibration curves of gallic acid (96% of purity), catechin (98% of purity), ellagic acid (95% of purity, from tree bark), myricitrin (99% of purity) and quercetin (98% of purity), all standards purchased from Sigma® (USA). The determination of contents was carried out in triplicate.

The crude extracts of *E. uniflora*, *L. ferrea* and *P. guajava* as well as the standards were dissolved in water and filtered through a 0.45 µm membrane (Macherey-Nagel®) prior to sample injection (20 µL). The peaks of substances in the crude extract were initially identified by comparing the retention times and UV spectra. After

that, the peaks were confirmed by spiking the sample with a small amount of the standards.

### *In vitro* studies: Antifungal Assay

The isolates from oral colonization, were obtained of the 88 patients from two different geographic regions of Brazil (69 from Natal - Rio Grande do Norte and 19 from Maringá - Paraná). Only patients who agreed to take part on a surveillance confidential study, in accordance to the Local Research Ethics committee from The Onofre Lopes University Hospital, approved under the number 152/07, were enrolled in this study. The species were identified in accordance with studies of (da Silva-Rocha *et al.*, 2014).

### Preparation of the yeast suspension

To prepare the inoculum, yeast cells were suspended in sterile saline solution (SSS, 0.9% of NaCl) vortexing and the concentration was adjusted at 90% transmittance in 530 nm spectrophotometer (Bausch & Lomb®). The turbidity was adjusted according to 0.5 tube of the McFarland scale, compatible with 1.0-5.0 × 10<sup>6</sup> colony-forming units per mL (CFU/mL). From this suspension, two new dilutions were performed: the first of 1:50 in sterile saline solution and a second dilution of 1:20 in RPMI-1640 medium (Gibco®).

### Preparation of the culture medium

The RPMI-1640 medium (Gibco®) was prepared together with a buffer solution of morpholino propanesulfonic acid-MOPS (Sigma-Aldrich®) and supplemented with 2% Glucose (Sigma-Aldrich®), then stabilizing the pH of this solution to 7.0. Then, the medium was sterilized by membrane filtration porosity of 0.22 µm (Milipore®). After filtration, an aliquot was separated for the sterility at 37 °C for 24 hours. Subsequently, the medium was stored at temperature between 2-5 °C until use.

### Preparation of samples for analysis

To prepare the samples, the crude extracts were weighed into eppendorfs and diluted with 950 µL of water and 50 µL of dimethylsulfoxide (DMSO, Synth®) to yield the final concentration of 2000 µg/mL, which is the stock solution. The working range

used for the MIC determination was from 1.9 to 1000.0 µg/mL.

### The antifungal activity of crude extracts

The susceptibility tests were carried out through the method of broth microdilution for fluconazole (Pfizer®) according to the Clinical and Laboratory Standards Institute standard M27-A3 (CLSI, 2008) with modifications for natural products.

The tests were made in sterile plastic microplates (Nunclon®) with 96 wells arranged in eight series from A to H, each one with 12 wells numbered 1 until 12. Each line corresponded to a strain of yeast and received 100 µL of the corresponding inoculum. The extracts were diluted in the medium used, on the proportion 1:2.

In wells of columns 2 to 11 was added aliquots of medium RPMI-1640 (100 µL) and 200 µL in column 12. The extracts, 100 µL, was added in the columns 1 and 2 of the microplates. With the multichannel pipette, a serial dilution was performed starting from the second column, homogenizing and removing 100 µL of the mixture (medium and extracts) and adding to the well of the next column. The procedure was repeated the same way, to up the column 10, discarding the remaining 100 µL. In this way, the concentrations of crude extracts ranged from 1.9 to 1000.0 µg/mL.

For each isolate, the following controls were included: negative (only RPMI-1640); positive (RPMI-1640 plus inoculum, without antifungal addition) and diluent (DMSO and inoculum). In each microplate a strain of *Candida parapsilosis* (ATCC 22019) was included as reference yeast. The microplates mounted were incubated in stove at 35 °C/48-72 h with daily monitoring. After 48 h the reading of the fluconazole test was performed in microplate reader (Asys Hitech GmbH®), and after 72 h that of the plant extracts was made through visual comparison, by mirror reflex (Shinobu-Mesquita *et al.*, 2011; Ferreira *et al.*, 2013).

### Minimal Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) was considered as the smallest concentration of the extracts (*E. uniflora*, *L. ferrea* and *P. guajava*) or of fluconazole that inhibited 100% of the growth of each yeast, with reference to its respective positive control (n = 3). The MIC50 and MIC90 were defined as the MIC

that inhibited 50% and 90% of the isolates, respectively (Shinobu-Mesquita *et al.*, 2011).

## RESULTS

### Phytochemical Analyses of Crude Extracts

The qualitative phytochemical profile by TLC of crude extracts (aqueous and acetone-water) from *E. uniflora*, *L. ferrea* and *P. guajava* showed the presence of polyphenols (hydrolysable tannins and flavonoids) in all extracts. The results of the assay of Folin-Ciocalteu for total phenolic content (TPC) are shown in Table I. A comparison of the average of TPC showed that the acetone-water extracts exhibited the highest content of polyphenols.

The chromatographic fingerprints of the crude extracts are shown in Figure 1. The peak correspondent to gallic acid was identified in all chromatograms, eluting at a retention time (Rt) of 9.5 min with maximum of absorbance at 270 nm. In chromatographic profile obtained for CE of *E. uniflora* (Figure 1A), was possible identified the presence of a flavonoid myricitrin at 21.5 min. In the CE of *L. ferrea* (Figure 1B), was identified peak correspondent to catechin (Rt = 17.5 min), and others majorities peaks of derivative catechins, with maximum of absorbance at 280 nm. And, in the CE of *P. guajava* (Figure 1C) was possible identified the presence of flavonoid quercetin at Rt = 26.5 min and ellagic acid at Rt = 28.5 min, with maximum's absorptions at 355 nm and 254 nm, respectively. The results found by HPLC corroborated with results by UV-Vis that showed the acetone-water extracts presented highest content of each compound analyzed.

In this study, three plant species (native from Northeastern of Brazil) widely used as medicinal by the population were screened against various yeasts from *Candida* species, such as *C. albicans*, *C. krusei*, *C. dubliniensis*, *C. glabrata* and others. All crude extracts exhibit an inhibitory potential with broad spectrum, as they inhibited all yeasts species tested.

Overall, the CE from *E. uniflora* species exhibited a significant antifungal activity against *Candida* spp. isolates from the oral cavity of kidney transplant patients. Concentrations between 3.9 and 1000.0 µg/mL of aqueous crude extract prevented the growth of *C. albicans* isolated from the Natal-RN; and between 1.9 and 500.0 µg/mL as the isolates of *C. albicans* from Maringá-PR. Since the ranges of MIC were lower for

**TABLE I -** Chemical content of marker by HPLC and Total polyphenol (TPC) by UV-Vis of crude extracts

Compound	<i>E. uniflora</i> (%)		<i>L. ferrea</i> (%)		<i>P. guajava</i> (%)	
	AQ	AC-W (7:3, v/v)	AQ	AC-W (7:3, v/v)	AQ	AC-W (7:3, v/v)
Gallic acid	0.16 (0.29)	0.17 (0.95)	0.12 (0.07)	0.13 (0.82)	0.31 (1.34)	0.35 (0.50)
Myricitrin	1.64 (0.37)	1.77 (0.06)	-	-	-	-
Catechin	-	-	0.95 (1.25)	1.09 (1.36)	-	-
Quercetin	-	-	-	-	0.93 (0.12)	1.15 (0.08)
Ellagic acid	-	-	-	-	0.23 (0.22)	0.24 (0.41)
TPC*	25.13 (0.83)	30.37 (2.15)	22.79 (1.12)	25.69 (0.39)	20.55 (0.16)	22.23 (0.69)

All data represented as mean (relative standard deviation) of three independent measurements. Values are expressed as percentages ( $\mu\text{g/g}$ ) of each compound in the crude extracts. AQ: Aqueous; AC-W: Acetone:water. \*Values expressed as pyrogallol equivalent (PE) per gram of crude extracts.

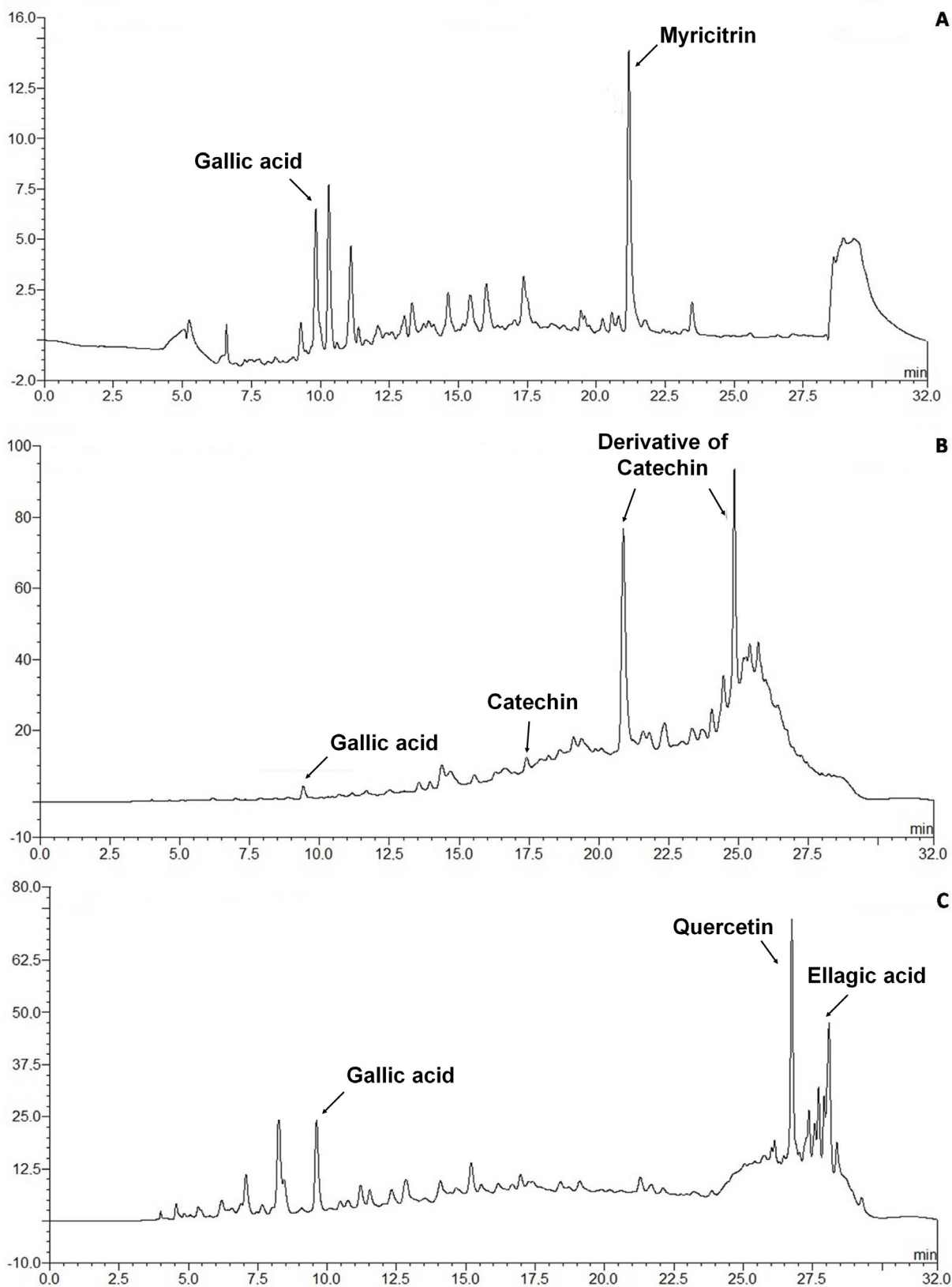
crude extract of type acetone: water (7:3, v/v), remaining in the range of 1.9 to 1000.0  $\mu\text{g/mL}$  in Natal-RN and 1.9 to 500.0  $\mu\text{g/mL}$  in Maringá-PR. Regarding the MIC50, showed value of 62.5  $\mu\text{g/mL}$  for *C. albicans*, those isolated from patients in the region of Natal; already isolates of the Maringá, the crude extract obtained with acetone: water (7:3) showed the lowest MIC50 (31.2  $\mu\text{g/mL}$ ) compared to the aqueous extract. MIC90 values showed values for the crude extracts acetone:water (7:3) equal to 500.0  $\mu\text{g/mL}$ . In relation to the species of non-*C. albicans* *Candida* (non-*C. albicans*), the MIC50 was lowest and equal to 31.2  $\mu\text{g/mL}$  for the crude extract aqueous and 15.6  $\mu\text{g/mL}$  for crude extract acetone: water, suggesting that the species may be new potential antifungal agent (Table II).

Regarding to *L. ferrea*, the results were similar in the regions where the isolates were collected and the type of extract, with the same range of MIC values (between 3.9 to 1000.0  $\mu\text{g/mL}$  and 3.9 to 500.0  $\mu\text{g/mL}$ , respectively, for *C. albicans* and non-*C. albicans*, both as acetonic and aqueous extracts). The MIC50 and MIC90 values were also compared the type of extracts

analyzed (MIC50 = 125.0  $\mu\text{g/mL}$  and MIC90 = 500.0  $\mu\text{g/mL}$  for the *C. albicans*), and in relation to isolates of non-*C. albicans* the values of MIC50 (15.6  $\mu\text{g/mL}$ ) and MIC90 (125.0  $\mu\text{g/mL}$ ) were less for the aqueous extract.

For *P. guajava*, the results were similar to aqueous extracts of *E. uniflora* and *L. ferrea* in relation to the range of minimum inhibitory concentration, when observed across species *C. albicans* (3.9 - 1000.0  $\mu\text{g/mL}$ ). The extracts obtained with acetone: water (7:3) had lower range of MIC values in the isolates of non-*C. albicans* (3.9-250.0  $\mu\text{g/mL}$ ). The MIC50 and MIC90 for clinical isolates of *C. albicans* showed higher values as respectively 250.0 and 500.0  $\mu\text{g/mL}$  (extract aqueous) and 125.0 to 250.0  $\mu\text{g/mL}$  (Table II).

In relation the non-*C. albicans* species, variability was found in the MIC values against the same plant species. Moreover, the crude extracts obtained with acetone: water (7:3) showed better results when compared with *C. albicans*. The MIC values remained between 1.9 and 500.0  $\mu\text{g/mL}$  to as extracts of *E. uniflora*; between 3.9 and 500.0  $\mu\text{g/mL}$  for of *L. ferrea* and 3.9 and 250.0  $\mu\text{g/mL}$  for *P. guajava* (Table II).



**FIGURE 1** - Chromatographic fingerprints obtained by HPLC of crude extracts (acetone-water) from *E. uniflora* (A), *L. ferrea* (B) and *P. guajava* (C).

**TABLE II** - Antifungal activity of crude extracts against *Candida albicans* and non-*C. albicans* isolated from the oral cavity of kidney transplant recipients

Crude Extract	Microorganisms	n	<i>E. uniflora</i>			<i>L. ferrea</i>			<i>P. guajava</i>		
			Range of MIC	MIC <sub>50</sub>	MIC <sub>90</sub>	Range of MIC	MIC <sub>50</sub>	MIC <sub>90</sub>	Range of MIC	MIC <sub>50</sub>	MIC <sub>90</sub>
			µg/mL			µg/mL			µg/mL		
AQ	<i>C. albicans</i>	74	3.9-1000.0	62.5	125.0	3.9-1000.0	125.0	500.0	3.9-1000.0	250.0	500.0
	non- <i>C. albicans</i>	14	1.9-500.0	31.2	500.0	3.9-500.0	15.6	125.0	3.9-500.0	31.2	250.0
AC-W (7:3)	<i>C. albicans</i>	74	1.9-1000.0	31.2	500.0	3.9-1000.0	125.0	500.0	7.8-1000.0	125.0	250.0
	non- <i>C. albicans</i>	14	1.9-500.0	15.6	125.0	3.9-500.0	31.2	250.0	3.9-250.0	31.2	250.0

N: Number of isolates; MIC: Minimum Inhibitory Concentration; lowest concentration of each drug able to inhibit 100% of the growth of each yeast. MIC<sub>50</sub> and MIC<sub>90</sub>: MIC of each extract able to inhibit 50% and 90% respectively of the number of isolates. The results presented are the mean obtained from three tests carried out.

## DISCUSSION

The plant species natives from region Northeastern of Brazil are used in folk medicine or as commercial herbal. However, few ethnobotanic and ethnopharmacological studies have been described about the species related in this study (Biasi-Garbin *et al.*, 2016; Ferreira *et al.*, 2013). Besides, some few reports describing the antifungal activity of extracts or substances derived from plant on *Candida* species (Santos *et al.*, 2013).

All the crude extracts studied had considerable amounts of phenolic compounds. The antifungal activity sometimes it's related with a presence of polyphenols (Shahzad *et al.*, 2014), and polyphenols are an abundant source of structures containing phenolic hydroxyl that can be quantified by Folin-Ciocalteu method, like described in this study and others studies. Comparison of the average of TPC in crude extracts showed that the TPC from the aqueous extracts were all less than the TPC from the acetone-water extracts, corroborating with data from the literature that emphasize the maximization of polyphenols extraction when using the mixture acetone: water as a solvent (de Araújo *et al.*, 2014; Martins *et al.*, 2015).

The presence of gallic acid corroborates with results previously described in crude extracts from *L. ferrea* and *P. guajava* (de Araújo *et al.*, 2014), but the presence in crude extract AC-W of *E. uniflora* was described at the first time. Moreover, the presence of derivative flavonoidics were described in others studies in with leaves of *E. uniflora* (Bezerra *et al.*, 2018).

The literature regarding the antifungal activity against clinical isolates from the oral cavity of kidney transplant recipients of the species *E. uniflora*, *L. ferrea* and *P. guajava* is scarce. This study associated with ethnopharmacological uses of these species, may contribute to investigations as antifungal agents. Some studies related the activity of ethanolic extract from leaves of *E. uniflora* against dermatophytes (Biasi-Garbin *et al.*, 2016); thus, the association of the extract of *E. uniflora* to metronidazole showed a potential antifungal activity against *C. tropicalis* (Santos *et al.*, 2013). Crude extracts from leaves of *E. uniflora* and *P. guajava* and stem barks of *L. ferrea* (water; ethanol: water, 1:1; acetone: water, 1:1) showed significant activity against four standard species of *C. albicans*, *C. dubliniensis*, *C. glabrata* and *C. krusei* (Ferreira *et al.*, 2013).

In relation to the *Candida* spp. clinical isolates (da Silva-Rocha *et al.*, 2014) classified from the 88 yeasts obtained, like 77.6% of *C. albicans*, that was the most commonly isolated species on the oral cavity, as observed in studies where the frequency, up to 70% of isolates. Among the non-*C. albicans* species considered pathogenic such as *C. tropicalis*, *C. glabrata*, *C. dubliniensis*, *C. orthopsilosis* and *C. metapsilosis* has been detected in cases of oral candidiasis, especially those related to immunosuppression (Chaves *et al.*, 2013). The use of clinical isolates from patients who are colonized or from fungal infections has been an important strategy to investigate potential of new antifungal agents.

The degree of susceptibility of yeasts obtained was different in relation to the species (*C. albicans* and non-*C. albicans*) and the type of extract studied. As the isolates of *C. albicans* from Natal were more resistant to *E. uniflora*, in both types of extracts evaluated (aqueous or acetone-water); however when evaluated non-*C. albicans* isolates, the crude extracts obtained with acetone-water were most effective, with average MIC equal to 56 µg/mL (range 1.9 to 250 µg/mL).

For the species *L. ferrea* and *P. guajava*, both types of extract, for *C. albicans* were less resistant to those presented in the region of Maringá, highlighting the average MICs 86 µg/mL for the aqueous extract of *L. ferrea* (with range 3.9 to 500.0 µg/mL for aqueous and acetone-water extract) and 74 µg/mL for extract acetone-water of *P. guajava* (with range 3.9 to 500.0 µg/mL for aqueous extract and 3.9 to 250 µg/mL for acetone: water extracts). In accordance with the values of MIC and the MIC50 and MIC90 values, it's possible affirms that the crude extract from *E. uniflora*, *L. ferrea* and *P. guajava* demonstrated strong antifungal activity (Shinobu-Mesquita *et al.*, 2011; Ferreira *et al.*, 2013).

Based on these findings, the crude extracts of *E. uniflora*, *L. ferrea* and *P. guajava* are optimistic since there was antifungal activity with low concentrations of crude extract. It is possible that after purification steps to achieve the required concentration antifungal activity is less than the one obtained in this work. Thus, further studies are needed to purify fractions or components, followed by new susceptibility testing in order to search new alternatives of antifungal drugs available to the population.

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## CONFLICTS OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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