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ANTIFUNGAL ACTIVITY OF BRAZILIAN AMAZON PLANTS EXTRACTS AGAINST SOME SPECIES OF *CANDIDA* SPP.

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ABSTRACT

The increase in both opportunistic mycoses and antimicrobial resistance of pathogenic microorganisms has determined the need to develop new chemotherapies agents. Amazon Forest Plants has been used as natural drug by local people in the treatment of various tropical diseases. The objective of the study is to evaluate the antifungal activity of twenty-eight species of plants extracts belonging to twenty botanical families from the Amazon forest. The minimum inhibitory concentration of the one hundred fourteen crude extracts of dichloromethane, methanol and water were evaluated against three *Candida* species: *Candida albicans*, *Candida glabrata* and *Candida parapsilosis*. Seventy-four extracts showed activity, with minimum inhibitory concentration between 0.06 and 1mg/mL, against the three species evaluated. The results observed in this study, mainly about the families Arecaceae, Apocynaceae, Salicaceae and Urticaceae, showed that these extracts are promising for the development of new drugs that can be used in the treatment against opportunistic fungal infections.

Key words: plants, extracts, Amazon forest, mycoses, antifungal activity, *Candida* spp.

INTRODUCTION

Medicinal plants have been used in developing countries as alternative medical treatments and extracts and essential oils isolated from plants have been shown biological activity *in vitro* and *in vivo* (Hofling *et al*., 2010; Portillo *et al*., 2001; Webster *et al*., 2008). Some countries, like Brazil, have been highlighted due to its great diversity of flora and utilization of medicinal plants in the treatment of bacterial and fungal infections (Ahmad and Beg 2001; Rios and Recio 2005; Svetaz *et al*. 2010).

Brazil is the country with the greatest plant genetic diversity of the world, with more than 55,000 species cataloged of a total estimated of 350,000 to 550,000. However only 8% of plant species of the Brazilian flora were studied regard its bioactive while only 1,100 species have been assessed in their medicinal properties (Simões *et al*., 2003). The vast Amazonian biodiversity together with the traditional knowledge of the forest people could represent a potential source for the discovery of new therapeutic agents (Brandao *et al*., 2008; Duarte *et al*., 2005; Svetaz *et al*., 2010).

Duarte *et al*. (2007) have reported that in the last 10 years there has been an increase in the research of natural products active against *Candida* spp, including approximately 258 plant species, from 94 families. Brazilian medicinal plants have been used as natural medicines by local population due their therapeutic effect in various diseases, including fungal and bacterial infections (Alves *et al*., 2000; Newman and Cragg, 2012).

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Yeasts are considered opportunistic pathogens that can determine infectious processes from clinical asymptomatic to severe and fatal disease (Erkose and Erturan, 2007; Hofling *et al*., 2010; Repentigny *et al*., 2004). In recent decades, due to acquired immunodeficiency syndrome (AIDS) and increase of immunocompromised patients related to new therapies for cancer as well as advances in surgical techniques of organ transplantation, the mycoses has acquired an important role in public health world (Duraipandiyan and Ignacimuthu 2011; Fostel and Lartey 2000; Svetaz *et al*.,,,,, 2010). There are about 200 described species of *Candida* and approximately 10% of these cause infections disease. Fungal diseases by *Candida* spp. are 78.3% of cases nosocomial infection. Although the yeast *C. albicans* remains more frequently involved in pathological processes, the emergence of other species as *C.tropicalis, C. parapsilosis, C. krusei, C. lusitaniae, C. glabrata* has been observed (Erkose *et al*., 2007; Hofling *et al*., 2010; Sardi *et al*., 2010).

The treatment of fungal infections is a challenge of medical practice, and have few drug, the treatment is long, has high toxicity and is not always efficient (Duraipandiyan *et al*., 2011; Portillo *et al*., 2001; Spampinato and Leonardi, 2013). This study evaluated the antifungal activity of Brazilian Amazon plant extracts against three species of *Candida* considered opportunistic pathogens.

MATERIAL AND METHODS

Plants

The plants were collected in several parts of the Amazon region and identified at the Herbarium of the Botanical Research Coordination of the National Research Institute of Amazonia (INPA) (Table 1).

Extraction methods

Plant material was dried, powdered and then extracted with dichloromethane using ultrasound bath for 20 min and then filtered. The extraction procedure was repeated thrice. The plant material was then dried and extracted with methanol and finally with water, by using the same ultrasound procedure.

In order to use less environment aggressive solvents, we change dichloromethane by hexane, and performed the extractions in the same way. Organic extracts were concentrated on rotaevaporator and aqueous extracts by lyophilisation.

Strains prepration

Candida albicans (ATCC 11006), *Candida glabrata* (ATCC 2001*)* and *Candida parapsilosis* (ATCC 22019) were cultivated on 4% Sabouraud Dextrose Agar (SDA) for 24h at 25˚C. The suspension inoculum was carried out in sterile saline 0.85% from a colony alone. This suspension, after shaking in vortex by 15s, was adjusted to 0.5 of McFarland scale, resulting in a concentration of $1x10^6$ cells/mL, diluted, 1:50 in Sabouraud broth for bioassays.

Evaluation of the antifungical activity of the extracts

The search for antifungal potential of 114 extracts was performed using the technique of microdilution in broth, as the NCCLS-CLSI (2003). For susceptibility testing, 75 µL of Sabouraud broth was distributed in whole plate, except in the wells periphery, which was added sterile water. Also were added 75 µL of the plant extract in serial dilution starting from 2 mg/mL. The plates were incubated at 25 ˚C for 48 h. The results were determined by optical density through the Elisa reader 630 nm. The Minimal Inhibitory Concentration (MIC) was determined for those extracts presenting antifungical activity in the screening assay. Amphotericin B was used as positive control.

RESULTS AND DISCUSSION

For many years, medicinal plants have been responsible in traditional therapeutics worldwide, including Brazil, moreover, many of the currently available drugs are derived from plants, which has stimulated the search for new antimicrobial drugs with antifungal activity in plant extracts (Ahmad *et al*., 2001; Cowan, 1999; Duarte *et al*., 2007).

In the present study, it was determinate the antifungal activity of dichloromethane, methanol and aqueous extracts from different parts of 28 species of plants belonging to families Arecaceae, Apocynaceae, Capparaceae, Fabaceae, Hypericaceae, Lamiaceae, Lecythidaceae, Melastomataceae, Moraceae, Myristicaceae, Olacaceae, Picrodendraceae, Rubiaceae, Rutaceae, Salicaceae, Sapotaceae, Smilacaceae, Urticaceae, Verbenaceae, Zingiberaceae. These crude extracts were evaluated and the MIC results are shown in Table 1.

Among 114 extracts evaluated, 74 were active against *Candida albicans*, 82 against *C. glabrata* and 85 against *C. parapsilosis*, with MIC between 0.06 and 1 mg/mL. The three species of *Candida* were susceptible to 73 plant extracts belonging to almost all families tested, except Hypericaceae. In general, there is no agreement on the level of acceptance for plant activity when compared with standards; therefore, some authors consider only activity comparable to antibiotics, while others consider even higher values (Aligiannis *et al*. 2001; Duarte *et al*. 2005).

Therefore, we have established 2.0 mg/mL as the highest concentration, so that only the extracts presenting a MIC below 2.0 mg/mL were considered as having potential antimicrobial activity and in our work proposed a classification for plant materials, based on MIC results

as follows: strong inhibitors – MIC until 1 mg/mL; weak inhibitors – MIC between 1.1 and 2.0 mg/mL (Table 2).

The extracts of *Ferdinandusa goudotiana, F. hirsuta* and *Palicourea guianensis* (Rubiaceae) were active against three strains of *Candida* with MIC between 0.12 and 1.0 mg/mL. The activity of these extracts may be associated with the possible presence of indol alkaloids, triterpenoids, since these substances are present in these species, which are commonly found in Rubiaceae family species, whose antimicrobial activity has been demonstrated previously (Figueiredo *et al*., 2009; Nunez *et al*., 2009; Nunez *et al*., 2012).

The extract from *Miconia argyrophylla* (Melastomataceae), *Ficus cf. trigonata* (Moraceae), *Minquartia guianensis* (Olacaceae), *Zanthoxylum* sp. (Rutaceae), *Salix martiana* (Salicaceae) and *Manilkara huberi* (Sapotaceae) shown strong activity against the three species of *Candida*, with MIC 0.12 and 1 mg/mL. The families Olacaceae, Sapotaceae, Lilliaceae, Rutaceae, Euphorbiaceae, Moraceae, Asclepidaceae, have been studied regard their bioactive potential (Ahmad *et al*., 2001; Cursino *et al*., 2011; Fernandes *et al*., 2009; Ramos *et al*., 2008). It is possible that antimicrobial activity is related with flavonoids, saponins and alkaloids present in species of this family (Bukola *et al*., 2008).

Regarding *Miconia argyrophilla*, the aqueous and dichloromethane extract of leaves were inactive, while the branches presented high activity. These can be due to an uneven distribution of the plant constituents. More studies should be conducted in order to determine why this species produces antifungic compounds in the branches which are not transported to the leaves. Several substances as tannins, gallic acid, some catechins, flavonoids, and terpenoids can show antimicrobial activity, compounds already reported in members of this species (Yazdani *et al*., 2011).

Only the dichloromethane extract of barks of *Zanthoxylum* sp. shown to be active against all species of *Candida* sp., where the leaves were bioactive against the *C. albicans* and *C. parapsilosis.* The family Rutaceae has aroused interest because it presents a significant of different secondary metabolites, generating many biological and pharmaceutical, such as alkaloids and flavonoids that showed antibacterial activity in gram-positive and gram-negative bacteria, such as *Cryptococcus neoformans* and *C. albicans* (Harborne and Williams, 2000; Nissanka *et al*., 2001).

In the fractionation of extracts of plants using apolar solvents such as diclomethane and hexane allows the extraction of steroid groups (stigmasterol, β-sistosterol), coumarin, oleic acid esters, lactones, terpenoids, flavonoids, benzofuran, xantona, quinone and phenolic compounds. Some studies have shown that the alcohol was found to be a better solvent for extraction of antimicrobially active substances compared to water and hexane (Ahmad *et al*., 2001; Cowan, 1999).

Recent studies isolated from *Salix martiana* acetylsalicylic acid an agent with antibacterial activity (Fernandes *et al.*, 2009) and *S. martiana* and other species of this genus showed antioxidant properties and has an inhibitory action on tumor cells, since this substance induces apoptosis of these cells (El-Shemy *et al*., 2007).

The Arecaceae, Apocynaceae, Capparaceae, Picrodendraceae and Lecythidaceae families, represented in this study by *Orbignya phalerata, Asclepias curassavica, Crateva benthami, Piranhea trifoliata and Eschweilera pedicellata*, respectively, showed activity against *C. albicans* and *C. parapsilosis* with MIC between 0.06 – 1 mg/mL and *C. glabrata* with MIC between 0.12 – 1 mg/mL. The sensitivity of *C. parapsilosis* in this study was similar to *C. glabrata*, as regards the plant species and solvents used, about 80 plant extracts were active and 70 showed strong activity.

Both *C. albicans* and *C. parapsilosis* presents weak inhibition to all extracts of *Zingiber zerumbet* (Zingeberaceae), as well as the methanol extract of seeds of *Campsiandra comosa* (Fabaceae) showed weak activity against *C. glabrata* and *C. parapsilosis*, but the aqueous extract of this species extract was shown to be inactive. In others studies, some species of the Fabaceae showed the antimicrobial activity (Lima *et al*., 2011; Ramos *et al.*, 2008; Svetaz *et al*., 2010).

The aqueous extract of pod *Campsiandra laurifolia* (Fabaceae) against *C. albicans* and *C. glabrata*, already had an antimicrobial activity described in other studies (Alves *et al*., 2000; Chokchaisiri *et al*., 2009; Ramos *et al.*, 2008), the dichloromethane extract of the root of *Smilax brasilienses* (Smilacaceae) against *C. albicans* and *C. parapsilosis* and three extracts of Urticaceae family (*Pourouma* sp. and *P. guianensis*) against *C. glabrata* and *C. parapsilosis*, was strong inhibition to with MIC of ≤ 0.6 mg/mL.

So to assess the activity of the family Verbenaceae can be observed that just the dicloromethane (Jantan *et al*. 2003) extract of the leaves of *Lippia macrophyla* were not showed activity against the species of *Candida* studies (Aguiar *et al*., 2008). From Lamiaceae family, only the dichloromethane extract of flowers of *Vitex cymosa* was active against the three species of *Candida*. This fact is probably related to various bioactive compounds in often attributed to volatile components from their essential oil, such as monoterpenes, terpenoids, sesquiterpenes and diterpenes has already isolated from species of the family Lamiaceae, which may be acting synergistically against microorganisms evaluated (Abedini *et al*., 2013; Duarte *et al*., 2005; Gazim *et al*., 2010; Gazim *et al*., 2013).

Table 1. Evaluation of antifungal activity of the families examined against of the species of *Candida* sp. (mg/mL).

Species	Family	Plant Part	Extract	*Candida albicans*	*Candida glabrata*	*Candida parapsilosis*
Orbignya phalerata Mart.	Arecaceae	mesocarps	DCM	0.50	0.12	2.0
Orbignya phalerata Mart.	Arecaceae	mesocarps	MeOH	0.50	0.12	0.50
Orbignya phalerata Mart.	Arecaceae	mesocarps	H_2O	1.0	0.12	0.50
Asclepias curassavica L.	Apocynaceae	leaves	DCM	1.0	1.0	0.50
Asclepias curassavica L.	Apocynaceae	leaves	MeOH	0.50	0.50	0.25
Asclepias curassavica L.	Apocynaceae	leaves	H_2O	0.50	0.50	0.25
Asclepias curassavica L.	Apocynaceae	roots	DCM	2.0	1.0	1.0
Asclepias curassavica L.	Apocynaceae	roots	MeOH	Inative	0.12	2.0
Asclepias curassavica L.	Apocynaceae	roots	H_2O	Inative	0.12	2.0
Crateva benthami Eichler	Capparaceae	leaves	DCM	0.25	0.50	0.50
Crateva benthami Eichler	Capparaceae	leaves	MeOH	0.25	0.50	0.50
Crateva benthami Eichler	Capparaceae	leaves	H_2O	0.50	1.0	0.50
Crateva benthami Eichler	Capparaceae	barks	H_2O	0.50	0.50	0.50
Campsiandra comosa Benth.	Fabaceae	bark	MeOH	1.0	2.0	0.50
Campsiandra comosa Benth.	Fabaceae	branch	MeOH	0.12	0.50	0.12
Campsiandra comosa Benth.	Fabaceae	seed	MeOH	1.0	2.0	2.0
Campsiandra comosa Benth.	Fabaceae	seed	H_2O	Inative	Inative	Inative
Campsiandra comosa Benth.	Fabaceae	leaves	MeOH	0.50	0.50	1.0
Campsiandra laurifolia Benth.	Fabaceae	branch	DCM	0.25	0.50	1.0
Campsiandra laurifolia Benth.	Fabaceae	branch	MeOH	Inative	Inative	Inative
Campsiandra laurifolia Benth.	Fabaceae	branch	H_2O	0.25	0.50	0.50
Campsiandra laurifolia Benth.	Fabaceae	fruit	H_2O	0.12	2.0	0.12
Campsiandra laurifolia Benth.	Fabaceae	fruit	MeOH	Inative	0.06	2.0
Campsiandra laurifolia Benth.	Fabaceae	pod	MeOH	1.0	2.0	0.12
Campsiandra laurifolia Benth.	Fabaceae	pod	H_2O	≤0.06	0.06	1.0
Vismia sp	Hypericaceae	bark	MeOH	Inative	Inative	Inative
Vitex cymosa Bertero ex Spreng.	Lamiaceae	branches	H_2O	Inative	Inative	Inative
Vitex cymosa Bertero ex Spreng.	Lamiaceae	roots	H_2O	Inative	Inative	Inative
Vitex cymosa Bertero ex Spreng.	Lamiaceae	flowers	H_2O	Inative	Inative	Inative
Vitex cymosa Bertero ex Spreng.	Lamiaceae	flowers	DCM	0.12	0.50	1.0
Eschweilera pedicellata (Rich.) S.A. Mori	Lecythidaceae	leaves	H_2O	0.50	1.0	0.12
Eschweilera pedicellata (Rich.) S.A. Mori	Lecythidaceae	branch	H_2O	Inative	Inative	Inative
Miconia argyrophylla DC.	Melastomataceae	branches	DCM	1.0	1.0	0.50
Miconia argyrophylla DC.	Melastomataceae	branches	MeOH	0.12	0.25	0.50
Miconia argyrophylla DC.	Melastomataceae	branches	H_2O	1.0	0.50	0.50
Miconia argyrophylla DC.	Melastomataceae	leaves	DCM	Inative	Inative	Inative
Miconia argyrophylla DC.	Melastomataceae	leaves	H_2O	Inative	0.50	1.0
Ficus cf. trigonata L.	Moraceae	barks	DCM	Inative	Inative	Inative
Ficus cf. trigonata L.	Moraceae	barks	MeOH	1.0	0.50	1.0
Ficus cf. trigonata L.	Moraceae	barks	H_2O	1.0	0.12	1.0
Ficus cf. trigonata L.	Moraceae	branches	DCM	Inative	0.50	0.12
Ficus cf. trigonata L.	Moraceae	branches	MeOH	0.12	0.50	0.12
Ficus cf. trigonata L.	Moraceae	branches	H_2O	2.0	1.0	0.50
Virola calophylla (Spruce) Warb.	Myristicaceae	leaves	MeOH	Inative	Inative	2.0
Minquartia guianensis Aubl.	Olacaceae	leaves	DCM	Inative	Inative	Inative
Minquartia guianensis Aubl.	Olacaceae	leaves	MeOH	Inative	Inative	Inative
Minquartia guianensis Aubl.	Olacaceae	leaves	H_2O	0.25	2.0	1.0
Minquartia guianensis Aubl.	Olacaceae	branches	DCM	0.25	2.0	1.0

Minquartia guianensis Aubl.	Olacaceae	branches	MeOH	0.50	0.50	0.25
Minquartia guianensis Aubl.	Olacaceae	branches	H_2O	1.0	1.0	1.0
Piranhea trifoliata Baill.	Picrodendraceae	leaves	DCM	Inative	Inative	2.0
Piranhea trifoliata Baill.	Picrodendraceae	leaves	MeOH	Inative	Inative	Inative
Piranhea trifoliata Baill.	Picrodendraceae	branches	DCM	0.25	2.0	0.50
Piranhea trifoliata Baill.	Picrodendraceae	branches	MeOH	0.25	0.25	0.06
Ferdinandusa paraensis Ducke	Rubiaceae	leaves	H_2O	Inative	Inative	Inative
Ferdinandusa paraensis Ducke	Rubiaceae	leaves	DCM	1.0	0.50	0.50
Ferdinandusa paraensis Ducke	Rubiaceae	leaves	MeOH	1.0	0.50	1.0
Ferdinandusa paraensis Ducke	Rubiaceae	branches	H_2O	2.0	1.0	1.0
Ferdinandusa hirsuta Standl.	Rubiaceae	leaves	H_2O	Inative	Inative	Inative
Ferdinandusa hirsuta Standl.	Rubiaceae	leaves	DCM	Inative	Inative	Inative
Ferdinandusa hirsuta Standl.	Rubiaceae	leaves	MeOH	1.0	0.25	1.0
Ferdinandusa hirsuta Standl.	Rubiaceae	branches	DCM	0.25	0.50	1.0
Ferdinandusa hirsuta Standl.	Rubiaceae	branches	MeOH	Inative	Inative	Inative
Ferdinandusa hirsuta Standl.	Rubiaceae	leaves	MeOH	Inative	Inative	Inative
Ferdinandusa hirsuta Standl.	Rubiaceae	leaves	H_2O	1.0	1.0	1.0
Ferdinandusa goudotiana K. Schum.	Rubiaceae	leaves	DCM	1.0	0.25	1.0
Ferdinandusa goudotiana K. Schum.	Rubiaceae	leaves	MeOH	0.12	1.0	0.50
Palicourea corymbifera (Müll. Arg.) Standl.	Rubiaceae	leaves	H_2O	1.0	0.25	0.50
Palicourea corymbifera (Müll. Arg.) Standl.	Rubiaceae	leaves	DCM	Inative	Inative	Inative
Palicourea corymbifera (Müll. Arg.) Standl.	Rubiaceae	leaves	MeOH	2.0	0.50	1.0
Palicourea guianensis Aubl.	Rubiaceae	leaves	H_2O	0.50	0.50	1.0
Palicourea guianensis Aubl.	Rubiaceae	leaves	DCM	Inative	Inative	Inative
Palicourea guianensis Aubl.	Rubiaceae	leaves	MeOH	2.0	0.50	0.50
Zanthoxylum sp.	Rutaceae	roots	H_2O	Inative	Inative	Inative
Zanthoxylum sp.	Rutaceae	stalks	MeOH	Inative	Inative	Inative
Zanthoxylum sp.	Rutaceae	barks	DCM	0.12	0.12	0.12
Zanthoxylum sp.	Rutaceae	leaves	DCM	0.12	Inative	0.50
Zanthoxylum sp.	Rutaceae	leaves	H_2O	Inative	0.12	0.50
Salix martiana Leyb.	Salicaceae	leaves	DCM	1.0	1.0	0.50
Salix martiana Leyb.	Salicaceae	branches	DCM	0.25	0.12	0.12
Salix martiana Leyb.	Salicaceae	branches	MeOH	0.12	0.25	1.0
Salix martiana Leyb.	Salicaceae	branches	H_2O	1.0	1.0	0.50
Manilkara huberi (Ducke) A. Chev.	Sapotaceae	barks	DCM	0.12	0.50	0.12
Manilkara huberi (Ducke) A. Chev.	Sapotaceae	barks	MeOH	Inative	Inative	Inative
Manilkara huberi (Ducke) A. Chev.	Sapotaceae	barks	H_2O	Inative	Inative	Inative
Smilax brasiliensis Spreng.	Smilacaceae	leaves	DCM	0.50	0.50	0.12
Smilax brasiliensis Spreng.	Smilacaceae	leaves	MeOH	0.50	0.25	2.0
Smilax brasiliensis Spreng.	Smilacaceae	leaves	H_2O	0.50	0.25	2.0
Smilax brasiliensis Spreng.	Smilacaceae	branches	DCM	Inative	Inative	Inative
Smilax brasiliensis Spreng.	Smilacaceae	branches	MeOH	1.0	2.0	0.50
Smilax brasiliensis Spreng.	Smilacaceae	branches	H_2O	0,50	0.50	0.12
Smilax brasiliensis Spreng.	Smilacaceae	roots	DCM	0.06	0.25	0.06
Smilax brasiliensis Spreng.	Smilacaceae	roots	MeOH	0.50	0.25	0.12

Smilax brasiliensis Spreng.	Smilacaceae	roots	H_2O	1.0	0.25	0.12
Cecropia cf. hololeuca Miq	Urticaceae	branches	DCM	0.12	1.0	0.12
Cecropia cf. hololeuca Miq	Urticaceae	branches	MeOH	Inative	1.0	1.0
Cecropia cf. hololeuca Miq	Urticaceae	branches	H_2O	Inative	Inative	Inative
Coussapoa magnifolia Trécul	Urticaceae	fruits	DCM	Inative	Inative	Inative
Coussapoa magnifolia Trécul	Urticaceae	fruits	MeOH	Inative	1.0	1.0
Coussapoa magnifolia Trécul	Urticaceae	fruits	H_2O	0.06	0.06	0.06
Pourouma sp	Urticaceae	leaves	DCM	Inative	Inative	Inative
Pourouma sp	Urticaceae	leaves	MeOH	0.12	0.06	0.06
Pourouma sp	Urticaceae	leaves	H_2O	1.0	0.06	0.06
Pourouma guianensis Aubl.	Urticaceae	leaves	DCM	0.12	0.12	0.06
Pourouma guianensis Aubl.	Urticaceae	leaves	MeOH	Inative	Inative	Inative
Pourouma guianensis Aubl.	Urticaceae	leaves	H_2O	Inative	0.06	0.06
Lippia microphylla Cham.	Verbenaceae	leaves	MeOH	0.12	0.12	1.0
Lippia microphylla Cham.	Verbenaceae	leaves	H_2O	0.12	0.50	2.0
Lippia microphylla Cham.	Verbenaceae	leaves	DCM	Inative	Inative	Inative
Zingiber zerumbet (L.) Roscoe ex Sm.	Zingiberaceae	leaves	MeOH	2.0	0.50	2.0
Zingiber zerumbet (L.) Roscoe ex Sm.	Zingiberaceae	leaves	H_2O	2.0	0.50	2.0
Zingiber zerumbet (L.) Roscoe ex Sm.	Zingiberaceae	leaves	DCM	2.0	0.06	2.0
Zingiber zerumbet (L.) Roscoe ex Sm.	Zingiberaceae	roots	MeOH	2.0	0.12	2.0
Zingiber zerumbet (L.) Roscoe ex Sm.	Zingiberaceae	roots	DCM	2.0	0.50	2.0

H_2O= water; DCM= dichloromethane; Hex= hexane; MeOH= methanol

The extracts of *Zingiber zerumbet* in Zingiberaceae family was active against all species of *Candida*, but the MIC against *C. albicans* and *C. parapsilosis* from 2 mg/ mL, while the MIC for *C. glabrata* was from 0.06 to 0.5 mg/mL. Members of the Zingiberaceae family are commonly used in traditional medicine. Some plants from this family show a high activity against dermatophytes, *Candida albicans* and *Cryptococcus neoformans* (Jantan *et al*., 2003; Yob *et al*., 2011). Flavonoids are the main bioactive constituents found in the roots of these plants, and soluble flavonoids were reported to be more active than glycosidic forms naturally present in plants (Rauha *et al*., 2000). Species of yeast were more resistant when compared with fungi (Parekh and Chanda, 2008).

About the activity of the family Urticaceae can be observed that eight of the twelve extracts tested were active against at least one species of *Candida* evaluated. The aqueous extract of the fruits of *Coussapoa magnifolia* been active for three species of *Candida* (MIC of the 0.06 mg/mL), this fact is probably related to aqueous solvent extract the bioactive substances such as phenolics compounds and flavonoids, that appears to be directly related to the antimicrobial activity (Modarresi *et al*., 2010).

The use of natural products, with therapeutic properties, is as ancient as human civilization and, for a long time, minerals, plants and animal products were the unique sources of drugs (Duraipandiyan *et al*., 2011; Van Vuuren, 2008). This behavior has been drawing the attention of the scientific community to investigate the effectiveness of natural products as well as to promote its safe use. Several studies have been conducted to assess the biodiversity of various plants, which show antibacterial, antifungal and antiviral activity and antioxidant and immnumodulater properties in others works (Rios *et al.*, 2005; Van Vuuren, 2008).

It is expected that the traditional knowledge about medicinal plants indicates the presence of biologically active substance. The collection of plants for biological testing from its traditional use can be a great advantage, or a shortcut, increasing the chances of discovering new drugs (Cowan, 1999; Oliveira *et al*., 2006; Soejarto, 1996; Van Vuuren, 2008).

Added to this the fact, Brazil has a rich biodiversity, which offers a wide range of source to products of economic importance, especially plant sources. However, the extracts may contain active substance that can antagonized or potentiated in presence of other ones (Newman *et al.*, 2012). Moreover, the molecular diversity of plants that confer a variety of

Table 2. Extracts activity against *C. albicans, C. glabrata, C. parapsilosis*.

	Number of extracts active by inhibition level		
	Strong (until 1.0 mg/mL)	**Weak (1.1 to 2.0 mg/mL)**	**Inactive***
C. albicans	64	10	40
C. glabrata	74	8	32
C. parapsilosis	70	15	29

*Higher than 2 mg/mL

structures with biological potential is an advantage of the natural products when compared to synthetic, as this generally complicates the process of synthesis (Arunotayanun and Gibbons, 2012; Calixto, 2005). The results obtained showed the expressive contribution of the some plants extracts of Brazilian flora with anti-Candida activity.

CONCLUSION

Among one hundred and fourteen extracts tested against all species of *Candida*, seventy-four were active against the *C. albicans*, eighty-two against the *C. glabrata* and eighty-five against *C. parapsilosis*. The results of this study indicate that families of the Amazon plants have considered promising antifungal activity mainly the Arecaceae, Apocynaceae, Salicaceae and Urticaceae, which extracts all show up before the active isolates for the development of new drugs that can be used in therapy against opportunistic infections caused by these microorganisms.

The active phytocompounds of these plants against these *Candida* species showed that products of plant from Brazilian Amazon are an important source of bioactive substances and it reinforces the need for studies to identify the potential of these products of plant origin, as well as the active ingredient responsible for its activities.

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