Acta Botanica Brasilica - 34(2): 442-448. April-June 2020.
doi: 10.1590/0102-33062020abb0003

Short communication

Antifungal activity of selected plant extracts based on an ethnodirected study

Flávia dos Santos Silva[1, 2], Melissa Fontes Landell[3], Gustavo Vasconcelos Bastos Paulino[3], Henrique Douglas Melo Coutinho[4] and Ulysses Paulino Albuquerque[1, 2*]

Received: January 3, 2020
Accepted: April 3, 2020

ABSTRACT

Plants have been reported as used by local populations to treat various infections for a long time, which has directed several pharmacological studies. The main aim of this work was to evaluate three plant selection criteria with better predictive power to detect extracts with antifungal action: (1) medicinal plants that are not used for indications of infection and inflammation; (2) plants with direct citations for inflammation, except for infection; (3) plants with direct citations for inflammation and infection selected quantitatively by Syndromic Importance Value (SIV). We tested the action of 23 hydroethanolic extracts of plants against the fungi *Candida albicans*, *Cryptococcus neoformans,* and *Cryptococcus gattii* and found no differences in the number of active extracts among the different strategies used, but activity quality varied. The extract of *Anacardium occidentale* presented fungicidal activity against the three analyzed fungi. At least five species — *A. occidentale, Myracrodruon urundeuva, Poincianella pyramidalis, Anadenanthera colubrina var. cebil,* and *Mimosa oftalmocentra* — presented fungistatic and fungicidal effects against all strains. Our findings indicate that selecting plants based on popular indications and quantitative prioritization techniques increases the chance of detecting potential antifungal candidates, and that the plants selected by these criteria were more effective against *C. neoformans*.

Keywords: antifungal activity, ethnobotany, ethnopharmacology, natural products, local medical systems

Introduction

One of the great current challenges in the treatment of fungal diseases has been the resistance they have acquired to certain compounds. This required the use of new drugs for the treatment of infectious diseases caused by these microorganisms (Bastos *et al.* 2011; Newman & Cragg 2016). The use of natural products has been an important source in the discovery of new drugs in this area (Newman & Cragg 2016; Biasi-Garbin *et al.* 2016). The use of plants by local populations in the treatment of infectious diseases, such as those caused by fungi have been recorded by several studies

1 Programa de Pós-graduação em Etnobiologia e Conservação da Natureza, Departamento de Biologia, Universidade Federal Rural de Pernambuco, 52171-900, Recife, PE, Brazil
2 Laboratório de Ecologia e Evolução de Sistemas Socioecológicos. Departamento de Botânica, Universidade Federal de Pernambuco, 50670-901, Recife, PE, Brazil
3 Instituto de Ciências Biológicas e da Saúde, Universidade Federal de Alagoas, 57072-900, Maceió, AL, Brazil
4 Departamento de Química Biológica, Universidade Regional do Cariri, 63100-000, Crato, CE, Brazil

* Corresponding author: upa677@hotmail.com

(Maregesi *et al.* 2008; Svetaz *et al.* 2010; Bastos *et al.* 2011; Violante *et al.* 2012) and has been used to direct research.

The set of approaches based on local knowledge (popular, folk, etc.) has been termed ethnodirected and has guided many studies (Braga *et al.* 2007; Silva *et al.* 2013). One of the great challenges in the area, in spite of people's knowledge about medicinal plants, is to find good criteria for prioritizing plants for studies. For example, some studies have evaluated the antimicrobial activity of crude plant extracts popularly used for infections (Maregesi *et al.* 2008; Violante *et al.* 2012), injuries (Mølgaard *et al.* 2011), and inflammations (Braga *et al.* 2007). Testing the *in vitro* activity of plants for indications related to infectious diseases and inflammations may be an interesting criterion in the search of plants with antifungal action, since some studies suggest that the information obtained locally are not always clear regarding diseases caused by microorganisms (Ferreira-Júnior *et al.* 2011).

This study aimed to verify the antifungal activity of medicinal plants collected in the area of Caatinga (dry seasonal forest) that were selected based on different criteria within the ethnodirected approach. We used as reference the Minimum Inhibitory Concentration (MIC) of plant extracts against *Candida albicans*, *Cryptococcus neoformans,* and *Cryptococcus gattii* to test the best selection criteria. These fungi were selected as a model due to their clinical relevance. The species *C. albicans* is the most common agent of candidiasis. This disease has been shown to be very resistant in HIV-positive (Rex *et al.* 2000; Colombo *et al.* 2013) or immunocompromised patients. Infections caused by *C. neoformans* are generally associated with immunosuppressed individuals (Lin 2009; Ahmed *et al.* 2014) and *C. gattii* is very common in immunocompetent individuals (Kwon-Chung *et al.* 2014).

Materials and methods

Data treatment and plant selection

Plants were selected from an ethnobotanical survey executed in a rural community located in the municipality of Altinho, Pernambuco, in northeastern Brazil (Silva *et al.* 2011) and constitute a database of the Laboratório de Ecologia e Evolução de Sistemas Socioecológicos da Universidade Federal de Pernambuco. We selected the plants based on three groups: 1st Group: medicinal plants that are not used for indications of infections and inflammations; 2nd Group: plants with direct citations for inflammation, but not infections; 3rd Group: plants with direct citations on inflammations and infections. We randomly selected 10 plant species for the first two groups using BioEstat 5.3 software (Ayres *et al.* 2007). We reviewed the scientific names of all the medicinal plants using the following databases: The Plant List (TPL) (http://www.theplantlist.org/2013), The International Plant Names Index (PNI) (www.ipni.org/ipni/authorsearchpage.do), and the list of species of Flora do Brasil 2020 online (floradobrasil.jbrj.gov.br).

The plants of the 3rd group were selected based on the Syndromic Importance Value (SIV). The SIV considers the diversity of symptoms cited for each plant, the number of citations attributed by different sources, and the relative importance of each symptom for which the plant was cited (Leduc *et al.* 2006; Araújo *et al.* 2008). The calculation of the SIV is given by the following formula:

$$SIV = \frac{(\sum pxs) + \frac{(\sum pxf)}{F}}{2}$$

Where *p* refers to the weight of each indication; *f* = number of citations for the referred species; *F* = total number of informants; and *s* is the total of local symptoms for each species.

The weight of the indications was attributed based on the degree of association of the indication with the mentioned activities. For this, a literature search was performed on the signs and symptoms associated with microbial infections. The weights of the indications ranged from 0.25 to 1.0, where 1 was given for a highly associated indication; 0.75 for those that are moderately associated; 0.5 poorly associated, and 0.25 weakly associated (Tab. 1). The classification of the symptoms for the plants with direct citations, such as anti-inflammatory, was made based on information obtained from the works of Ferreira-Júnior *et al.* (2011) and Araújo *et al.* (2008).

Table 1. Weighted (p) values attributed to each anti-microbial and anti-inflammatory indication attributed to the plants cited in the free list performed in a rural community located in an area of Caatinga, Pernambuco, Brazil.

Citation	Therapeutic indication (s)	Weight (p)
Direct (inflammation and infection) - moderately associated	Gastritis, ulcer, burn, inflammation, inflammation of the bladder, inflammation of the tooth, "woman's inflammation/woman's remedy", inflammation of the feet, sore throat, ovarian inflammation, renal inflammation, urethritis, inflammation of the bladder, urinary inflammation, vaginal inflammation, inflamed wound, inflammation in the uterus, tuberculosis, hepatitis, urinary tract infection, anti-inflammatory, bronchitis, infection.	0.75
Indirect (inflammation and infection) - poorly associated	Swollen leg, swollen foot, toothache, uterine conditions, rheumatism, thrush, swelling, mouth ulcer, "woman problems", vaginal discharge.	0.5
Indirect (inflammation and infection) - weakly associated	Pain, cut, healing, urinary incontinence, injury, burning, problems in the urethra, hematoma, diarrhea, renal conditions, itchy rectum, leg pain, expectorant.	0.25

Preparation of extracts

The plant material was collected in an area of Caatinga, located in the municipality of Altinho (Pernambuco, NE Brazil). The exsiccates of the collected plants were identified by experts and deposited in the herbaria of the Instituto Agronômico de Pernambuco (IPA).

The plant material (parts of plants used medicinally, as indicated in the database) was collected from at least three individuals of each species and shade dried at room temperature. The extracts were obtained from 30 g of the material that was macerated in hydroalcoholic solvent (70 % ethanol) at room temperature and protected from light. Successive extractions were performed until complete extraction of the plant material. The first one was performed after 48 hours and the others at 24-hour intervals. After this period, the solvent was removed using the rotary evaporator at a temperature of 40 °C. The obtained extract was placed in a desiccator.

Minimum inhibitory concentration test (MIC)

The extracts were tested against *C. albicans* (ATCC 90028), *C. neoformans* (ATCC 40283), and *C. gattii* (ATCC 56990) obtained from the Laboratório de Diversidade Molecular da Universidade Federal de Alagoas (UFAL).

In vitro susceptibility of yeast isolates was performed using broth microdilutions according to the methodology recommended by the Clinical and Laboratory Standards Institute – CLSI in M27-A3 protocol (2008). The strains were previously cultured on YEPD agar medium (2 % glucose, 0.5 % yeasts extract, 0.5 % peptone, 2 % agar) at 35 °C for 48 h to be metabolically active for the tests. An inoculum was prepared by suspension of colonies in saline solution (0.85 %), and the cell density was spectrophotometrically fixed according to an absorbance turbidity equivalent to that of a 0.5 McFarland (~1 x 10^6 a 5 x 10^6 cells/mL). The crude extracts were resuspended in dimethyl sulfoxide (DMSO) in a ratio of 1:1. The concentration tested ranged from 20 to 0.0391 µg/µL, but when necessary, the range was increased from 0.0003 to 20 µg/µl. The microdilution plates containing RPMI-1640 (RPMI tissue culture medium supplemented with glutamine) buffered to pH 7.0 using 0.156 M 3-N-morpholinopropane-sulphonic acid (MOPS) with different concentrations of extracts, were inoculated with 100 µL of diluted culture, resulting in 0.5 x 10^3 to 2.5 x 10^3 cells/mL in each well (total of 200 µL), as recommended by the CLSI broth microdilution method. Following this, the plates were incubated at 35 °C for 24-48 h. The positive control was composed of culture medium and yeast, and the negative control contained DMSO in the concentration used to dilute the extracts. As antifungal control, we used two agents of different classes: Amphotericin B and fluconazole, with the concentrations tested ranging from 16 to 0.0313 µg/mL and 64 to 0.125 µg/µL, respectively, according to Khyriem *et al.* (2006) and the CLSI manual.

The minimal inhibitory concentrations were determined as the minimal compound concentration at which no visible growth (100 % of inhibition) was observed when compared to the control (wells without any antifungal agents). For determining whether the extracts used present fungicidal or fungistatic activity, a small volume (5 µL) of each of the wells with no apparent yeast growth were inoculated in YEPD agar medium and incubated at 35 °C for 48 hours. To avoid antifungal carryover, aliquots were deposited as a spot onto the agar plate and allowed to soak. The result was obtained according to the formation, or not, of colonies at the inoculated site. These were included as control strains in each set of experiments.

Data analysis

Through the demonstrated activities of the plants, the three selection criteria were compared for the number of active extracts using the G test (considering $p < 0.05$).

Results and discussion

Among the 30 plants selected for the three groups of criteria mentioned, only 23 were tested due to difficulties in availability, since the Caatinga environment presents a strong seasonality, which limits the temporal supply of plant material to few months of the year (Tab. 2).

According to the value of the SIV, eight species were indicated as priority (see Tab. 3). The species that were calculated to possess the highest weight were *M. urundeuva* and *A. colubrina*. Both had a higher frequency of citation and weight of the symptoms compared to those of the other species.

There were no significant differences ($G = 2.9503$; $p = 0.566$) among the selection criteria in relation to the number of active extracts for each evaluated strain, indicating that the amount of active extracts does not seem to depend on the technique of selection used. Although the number of active extracts did not differ with respect to the selection criteria, it was possible to observe divergence among them with respect to the degree of inhibitory activity and the number of strains susceptible to the extracts. For example, plants cited as anti-inflammatory and selected by SIV were seen to be more effective against *C. neoformans* alone.

The proportion of active plants has demonstrated the relevance of the ethnodirected approaches to test the *in vitro* activity of crude vegetal extracts against fungi. Studies have confirmed that plants which are reported to be used by local populations have higher antimicrobial potential than those which are selected by other approaches, such as random selection. For example, Svetaz *et al.* (2010) found that the probability of finding plants with anti-fungal properties was higher in those with ethnomedical uses related to fungal infections compared to those that were randomly selected. Besides that, strong activity (MIC≤1000 µg/mL) against dermatophytes was found in the group of plants selected through the ethnodirected approach.

Table 2. Plant species selected based on different selection criteria for antifungal evaluation.

Family / Species / Voucher	Plant	Part	Indication
1st Group: Randomly Selected-Plants without indications for inflammation and infection			
Apocynaceae/ *Catharanthus roseus* (L.) Don/ IPA 91036	boa noite	flower, leaf	insomnia, malaria
Asclepiadaceae/*Calotropis procera* (Aiton) W.T.Aiton/ IPA 91035	algodão de seda	leaf	headache
Lamiaceae/ *Ocimum campechianum* Mill.	alfavaca	Entire plant	analgesic
Malpighiaceae/ *Stigmaphyllon auriculatum* (Cav.) A.Juss./ IPA 91037	louco	leaf	allergy, wart
Myrtaceae/ *Eugenia punicifolia* (Kunth) DC./ IPA 91051	pirim	bark	worms
Myrtaceae/ *Eugenia pyriformis* Cambess.	ubaia	bark	stomachache
Solanaceae/ *Nicotiana glauca* Graham/ IPA 91033	pára raio	leaf	headache
2nd Group: Randomly selected - Plants with indications for inflammation			
Capparaceae/*Tarenaya spinosa* (Jacq.) Raf./ IPA 91045	mussambê	flower	bronchitis
Lythraceae/ *Punica granatum* L./ IPA 91046	romã	peel of the fruit	inflamed tooth
Anacardiaceae/*Schinopsis brasiliensis* Engl./ IPA 91044	braúna	bark	inflammation, tooth inflammation
Capparaceae/*Crateva tapia* L./ IPA 91056	trapiá	bark	inflammation of the urethra
Leguminosae-Caesalpinioideae/*Bauhinia cheilantha* Steud./ IPA 91040	mororó	leaf	gastritis
Leguminosae-Caesalpinioideae/*Libidibia ferrea* (Mart. ex Tul.) L.P.Queiroz/ IPA 91057	jucá	bark	gastritis
Meliaceae/*Cedrela odorata* L.	cedro	bark	inflammation and sinusitis
Rubiaceae/*Coutarea hexandra* (Jacq.) K. Schum.	quina- quina	bark	inflammation and sinusitis
3rd Group: SIV- Plants with indications for inflammation and infection			
Anacardiaceae/*Anacardium occidentale* L./ IPA 90995	cajú roxo	bark	kidney conditions, inflamed tooth, infection, inflammation, "woman's inflammation", sore throat
Anacardiaceae/*Myracrodruon urundeuva* Allemão/ IPA 91068	aroeira	bark	kidney conditions, uterine conditions, wound, dental pain, leg pain, gastritis, infections, urinary infection, inflammation, tooth inflammation, "woman's inflammation", sore throat, uterine inflammation, swollen foot, "women problems", burn,
Capparaceae/*Cynophalla hastata* (Jacq.) J.Presl/ IPA 91063	pau-d'arco roxo	bark	uterine conditions, wound, inflammation, uterine inflammation, tuberculosis
Celastraceae/*Maytenus rigida* Mart./ PEUFR 46182	bom nome	bark	kidney conditions, vaginal discharge, wound, gastritis, hematoma, infection, kidney inflammation, urethritis
Leguminosae-Caesalpinioideae/*Poincianella pyramidalis* (Tul.) L.P.Queiroz/ IPA 91043	catingueira	bark	gastritis, hepatitis, swelling, urinary infection, diarrhea, rheumatism
Leguminosae-Mimosoideae/*Anadenanthera colubrina* var. *cebil* (Griseb.) Altschul/ IPA 91059	angico	bark	kidney conditions, uterine conditions, bronchitis, inflammation, "woman's remedy", tuberculosis, wound
Leguminosae-Mimosoideae/*Mimosa oftalmocentra* Mart. ex Benth./ IPA 91054	jurema preta	bark	uterine conditions, wound, gastritis, infection, inflammation, foot inflammation
Rhamnaceae/*Ziziphus joazeiro* Mart. / IPA 91058	juá	bark	expectorant, flu, tuberculosis

The plants used for indications of infections and inflammation showed interesting results against the analyzed fungi. We found that studies have previously selected plants based on these indications and have observed anti-microbial or anti-fungal properties in these plants. A study implemented in Chile has verified the antifungal action of plants which were used for injuries and associated infections against *Penicillium expansum* and *C. albicans*. Among the 40 evaluated species, 30 presented interesting antimicrobial activities, corroborating with their traditional uses (Silva *et al.* 2013). Braga *et al.* (2007) selected plants traditionally used in infectious diseases and inflammation and evaluated their activity against fungi. Among the 24 methanolic extracts obtained from 20 plants, only those of *Schinus terebintifolius*, *O. gratissimum, Cajanus cajan*, and *Piper aduncum*, with MIC of 1.25 mg/ml, presented activity against *C. albicans*. In contrast, the species *Bixa Orellana, O. gratissimum* and *Syzygium cumini* with MIC of 0.078 mg/ml, presented better activity against *C. neoformans*. The proportion of species with interesting activities has been lower than that observed in our studies. However, the definition of the criteria has been important in the attempt to reduce efforts and costs with *in vitro* tests.

From the total number of extracts evaluated (23), ten extracts showed activity against *C. albicans*, 21 (91 %) against the fungus *C. neoformans*, which was the most sensitive to the evaluated extracts, and 14 (60 %) showed activity against *C. gattii*. The inhibitory activity of the extracts against *C. gattii* was verified if the extracts exhibited activity against *C. albicans* and *C. neoformans*. Among all the extracts tested, 48 % presented weak fungicidal and fungistatic activity against at least one strain, with MIC varying between 0.039 and 20 µg/µl. Of the plants prioritized by the SIV, only five (*A. occidentale, M. urundeuva, P. pyramidalis, A. colubrina var. cebil,* and *M. oftalmocentra*) presented antifungal effects against all three strains (*C. albicans, C. neoformans,* and *C. gattii*), with MIC ranging from 0.0049 to 20 µg/µl (Tab. 4). The extracts that showed strong inhibitory activity were *A. occidentale* bark extract for *C. neoformans*, compared to fluconazole, and extracts of *M. urundeuva* and *P. pyramidalis*, compared to amphotericin B, against the same strain (Tab. 4).

Among the eight randomly selected plants with citations for use in inflammation, extracts of *L. ferrea*, *S. brasiliensis,* and *P. granatum* showed fungicidal action against all strains, with MIC between 0.0049 and 1.25 µg/µl. The extract from the bark of *S. brasiliensis* showed strong fungicidal activity for *C. neoformans* (MIC 0.0049 µg/µl) compared to fluconazole. Among the seven medicinal plants randomly selected (used in cases without indications of inflammation and infection), only *E. pyriformis* extract showed fungistatic activity against *C. albicans* (MIC 5 µg/µl) and fungicidal activity against *C. gattii* (MIC 20 µg/µl) and *C. neoformans* (MIC of 0.009 µg/µl) with a good inhibitory effect. The hydroalcoholic extracts from *B. cheilantha* and *C. tapia* were the only ones considered inactive against the three strains (Tab. 4). However, for this same selection category, most of the extracts reported were inactive against only *C. albicans* (Tab. 4).

A previous study (Cruz *et al.* 2007) evaluated the activity of *Z. joazeiro, Caesalpinia pyramidalis* (valid name: *Poincianella pyramidalis), Bumelia sartorum* (valid name*: Sideroxylon obtusifolium*), and *Hymenaea courbaril*, which are plants popularly known for their treatment of mycoses, against *C. albicans, C. guilliermondii, C. neoformans,* and *Trichophyton rubrum*. Of these, only the aqueous extracts obtained from the leaves of *C. pyramidalis* and from the bark of *Z. joazeiro* were effective (MIC of 6.5 µg/mL) against the fungi *C. guilliermondii* and *T. rubrum*. Similar to our results, *Z. joazeiro* showed substantial activity against *C. neoformans*. However, no activity was reported against *C. albicans* and *Z. joazeiro* presented the best activity in the study carried out by Cruz *et al.* (2007). Finding plants with antifungal potential has not been easy (Souza 2010), because even when such activity is observed, many other substances present a high level of toxicity. Due to this complexity concerning the bioprospecting of plants with antifungal activity, our data show that the use of direct citations for infections and inflammations may be a good tool in the search of potential antifungal candidates, since medicinal plants without these indications did not present better activity.

Data availability

The data used to support the findings of this study are included within the article and can be solicited by request to the authors.

Table 3. Syndromic Importance Value (SIV) of plants used in cases of inflammation and infections in the area of Caatinga, Northeast Brazil.

Species	s	*f*	p	SIV
Myracrodruon urundeuva Allemão	20	77	11.25	117
Anadenanthera colubrina var. *cebil* (Griseb.) Altschul	19	24	11.25	108
Maytenus rigida Mart.	9	38	5	23
Mimosa oftalmocentra Mart. ex Benth.	7	23	3.75	14
Anacardium occidentale L.	5	34	3	8
Cynophalla hastata (Jacq.) J.Presl	5	10	3	7.6
Ziziphus joazeiro Mart.	3	43	1.25	4.3
Poincianella pyramidalis (Tul.) L.P. Queiroz	2	27	1.5	1.7

Note: s: total local symptoms for each species; *f*: number of citations for the cited species; p: weight of each indication; SIV: Syndromic Importance Value.

Table 4. Determination of Minimum Inhibitory Concentration (μg/μl) of the selected plant extracts by different criteria through the ethnodirected approach.

Species	Part	Microorganisms		
		Ca	Cn	Cg
3rd Group: SIV - Plants with indications for inflammation and infections				
Anacardiaceae/*Anacardium occidentale* L.	B	0.312*	0.0049*	0.039*
Anacardiaceae/ *Myracrodruon urundeuva* Allemão	B	0.156**	0.0024*	0.019*
Capparaceae/*Cynophalla hastata* (Jacq.) J.Presl	B	N/A	1.25**	N/T
Celastraceae/ *Maytenus rigida* Mart.	B	N/A	1.25**	N/T
Leguminosae-Caesalpinioideae/ *Poincianella pyramidalis* (Tul.) L.P.Queiroz	B	5**	0.0024*	0.019*
Leguminosae-Mimosoideae/*Anadenanthera colubrina* var. *cebil* (Griseb.) Altschul	B	20*	0.078*	5**
Leguminosae-Mimosoideae/ *Mimosa oftalmocentra* Mart. ex Benth./ IPA 91054	B	0.625**	0.009**	0.019**
Rhamnaceae/*Ziziphus joazeiro* Mart.	B	N/A	20**	N/T
2nd Group: Randomly selected - Plants with no indications of inflammation and infection				
Apocynaceae/*Catharanthus roseus* (L.) Don	Fl, L	N/A	5**	5*
Asclepiadaceae/*Calotropis procera* (Aiton) W.T.Aiton	L	N/A	10**	N/T
Lamiaceae/ *Ocimum campechianum* Mill.	EP	N/A	0.039*	2.5*
Malpighiaceae/*Stigmaphyllon auriculatum* (Cav.) A. Juss.	L	N/A	0.156**	N/T
Myrtaceae/*Eugenia punicifolia* (Kunth) DC.	B	N/A	0.039**	0.312*
Solanaceae/*Nicotiana glauca* Graham	L	N/A	0.312**	20**
Myrtaceae/*Eugenia pyriformis* Cambess.	B	5**	0.009*	20
1st Group: Randomly selected - Plants with indications for inflammation				
Capparaceae/*Crateva tapia* L.	B	N/A	N/A	N/T
Lythraceae/ *Punica granatum* L.	P	0.625**	0.078**	0.078*
Meliaceae/*Cedrela odorata* L.	B	N/A	0.078**	N/A
Capparaceae/*Tarenaya spinosa* (Jacq.) Raf.	Fl	N/A	5**	20*
Leguminosae-Caesalpinioideae/*Libidibia ferrea* (Mart. ex Tul.) L.P.Queiroz	B	1.25**	0.15*	0.07*
Leguminosae-Caesalpinioideae/*Bauhinia cheilantha* Steud.	L	N/A	N/A	N/T
Rubiaceae/*Coutarea hexandra* (Jacq.) K. Schum.	B	0.039**	2.5*	N/A
Anacardiaceae/*Schinopsis brasiliensis* Engl.	B	0.039**	0.0049*	0.019*

Microrganisms: Ca=*Candida albicans* ATCC 90028; Cn=*Cryptococcus neoformans* ATCC 40283; Cg=*Cryptococcus gattii* ATCC 56990. Parts of plants: B=bark; L=leaf; Fl=Flower; EP=entire plant; P=peel. N/A = no activity; N/T = Not tested. * fungicidal; ** fungistatic.

Cryptococcus sp. = Amphotericin B < 0.002 μg/μL and fluconazole< 0.008 μg/μL. *C. albicans* ATCC 90028 = 0.00025 – 0.001 μg/μL for fluconazole and 0.0005 – 0.002μg/μL for Amphotericin B.

Acknowledgements

To the members of Laboratório de Ecologia e Evolução de Sistemas Socioecológicos da Universidade Federal de Pernambuco and to the Laboratório de Microbiologia e Biologia Molecular da Universidade Regional do Cariri (URCA) for their support. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES), Finance Code 001, with contributions from the INCT Ethnobiology, Bioprospecting, and Nature Conservation certified by CNPq, and financial support from FACEPE (Grant number: APQ-0562-2.01/17).

References

Ahmed AS, McGaw LJ, Elgorashi EE, Naidoo V, Eloff JN. 2014. Polarity of extracts and fractions of four *Combretum* (Combretaceae) species used to treat infections and gastrointestinal disorders in southern African traditional medicine has a major effect on different relevant *in vitro* activities. Journal of Ethnopharmacology 154: 339-350.

Araújo TAS, Alencar NL, Amorim ELC, Albuquerque UP. 2008. A new approach to study medicinal plants with tannins and flavonoids contents from the local knowledge. Journal of Ethnopharmacology 120: 72-80.

Ayres M, Ayres MJ, Ayres DL, Santos SA. 2007. BioEstat 5.3: aplicações estatísticas nas áreas das ciências biológicas e médicas. Belém, Sociedade Civil de Mamirauá.

Bastos GM, Nogueira NAP, Soares CL, Martins MR, Rocha LQ, Teixeira AB. 2011. *In vitro* determination of the antimicrobial potential of homemade preparations based on medicinal plants used to treat infectious diseases. Revista de Ciências Farmacêuticas Básica e Aplicada 32: 113-120.

Biasi-Garbin RP, Demitto FO, Amaral RCR, *et al.* 2016. Antifungal potential of plant species from Brazilian caatinga against dermatophytes. Revista do Instituto de Medicina Tropical de São Paulo 58: 18. doi: 10.1590/S1678-9946201658018

Braga FG, Bouzada MLM, Fabri RL, *et al.* 2007. Antileishmanial and antifungal activity of plants used in traditional medicine in Brazil. Journal of Ethnopharmacology 111: 396-402.

Clinical and Laboratory Standards Institute – CLSI. 2008. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard / 3rd ed. CLSI document M27-A3. Philadelphia, Wayne.

Colombo AL, Guimarães T, Camargo LFA, *et al.* 2013. Brazilian guidelines for the management of candidiasis - a joint meeting report of three medical societies: Sociedade Brasileira de Infectologia, Sociedade Paulista de Infectologia and Sociedade Brasileira de Medicina Tropical. The Brazilian Journal of Infectious Diseases 17: 283-312.

Cruz MCS, Santos PO, Barbosa AM, *et al.* 2007. Antifungal activity of Brazilian medicinal plants involved in popular treatment of mycoses. Journal of Ethnopharmacology 111: 409-412.

Ferreira-Júnior WS, Ladio AH, Albuquerque UP. 2011. Resilience and adaptation in the use of medicinal plants with suspected anti-inflammatory activity in the Brazilian Northeast. Journal of Ethnopharmacology 138: 238-252.

Khyriem AB, Sujatha S, Parija SC. 2006. Antifungal susceptibility of *Cryptococcus neoformans* to amphotericin B and fluconazole. Indian Journal of Pathology & Microbiology 49: 307-308.

Kwon-Chung KJ, Fraser JA, Doering TL, *et al.* 2014. *Cryptococcus neoformans* and *Cryptococcus gattii*, the etiologic agents of cryptococcosis. Cold Spring Harbor Perspectives Medicine 4: a019760. doi: 10.1101 / cshperspect.a019760

Leduc C, Coonishish J, Haddad P, Cuerrier A. 2006. Plants used by the Cree Nation of Eeyou Istchee (Quebec, Canada) for the treatment of diabetes: A novel approach in quantitative ethnobotany. Journal of Ethnopharmacology 105: 55-63.

Lin X. 2009. *Cryptococcus neoformans*: morphogenesis, infection, and evolution. Infection, Genetics and Evolution 9: 401-416.

Maregesi SM, Pieters L, Ngasspa OD, *et al.* 2008. Screening of some Tanzanian medicinal plants from Bunda district for antibacterial, antifungal and antiviral activities. Journal of Ethnopharmacology 119: 58-66.

Mølgaard P, Holler JG, Asar B, *et al.* 2011. Antimicrobial evaluation of Huilliche plant medicine used to treat wounds. Journal of Ethnopharmacology 138: 219-227.

Newman DJ, Cragg GM. 2016. Natural products as sources of new drugs from 1981 to 2014. Journal of Natural Products 79: 629-661.

Rex JH, Walsh TJ, Sobel JD, Filler SG, Pappas PG, Dismukes WE, *et al.* 2000. Practice guidelines for the treatment of candidiasis. Clinical Infectious Diseases 30: 662-678.

Silva ACO, Santana EF, Saraiva AM, *et al.* 2013. Which approach is more effective in the selection of plants with antimicrobial activity? Evidence-Based Complementary and Alternative Medicine 2013: 308980. doi:10.1155/2013/308980

Silva FS, Ramos MA, Hanazaki N, Albuquerque UP. 2011. Dynamics of traditional knowledge of medicinal plants in a rural community in the Brazilian semi-arid region. Revista Brasileira de Farmacognosia 21: 382-391.

Souza NAB. 2010. Possíveis mecanismos de atividade antifúngica de óleos essenciais contra fungos patogênicos. PhD Thesis. Universidade Federal da Paraíba, João Pessoa.

Svetaz L, Zuljan F, Derita M, *et al.* 2010. Value of the ethnomedical information for the discovery of plants with antifungal properties. A survey among seven Latin American countries. Journal of Ethnopharmacology 127: 137-158.

Violante IMP, Hamerski L, Garcez WS, *et al.* 2012. Antimicrobial activity of some medicinal plants from the Cerrado of the central- western region of Brazil. Brazilian Journal of Microbiology 43: 1302-1308.