Mycoses / Volume 52, Issue 6 / p. 511-517

Free Access

Evaluation of the antifungal potential of Brazilian *Cerrado* medicinal plants

Fernanda Melo E Silva, José Elias De Paula, Laila Salmen Espindola,

First published: 05 October 2009
https://doi.org/10.1111/j.1439-0507.2008.01647.x
Citations: 32

Laila Salmen Espíndola, Laboratório de Farmacognosia, BC 216, Campus Universitário Darcy Ribeiro, Universidade de Brasília, 70910-900 Brasília, DF, Brazil.
Tel.: +55 61 3307 3046. Fax: +55 61 3273 0105.
E-mail: darvenne@unb.br

Summary

Therapeutic limitations, development of fungal drug resistance, drug-related toxicity, drug interactions and insufficient bioavailability of the currently available antifungal drugs have made the development of drugs necessary that would be able to treat the emerging fungal infections. The *Cerrado* is the second greater biome of Brazil and it was identified as one of the most distinguished biomes of South America, becoming an important source of innovative vegetal molecules to treat several conditions. Thus, the objective of this study was to evaluate the antifungal potential of *Cerrado* plants, mainly those used to treat infections and wounds. A total of 57 extracts were screened by the agar-well diffusion technique against *Candida albican*s and *Trichophyton rubrum*. The most promising extracts were tested in smaller concentrations and their minimal

inhibitory concentrations (MIC) were determined by microdilution method. Results were analysed statistically by ANOVA tests. Extracts of *Kielmeyera coriacea*, *Renealmia alpinia*, *Stryphnodendron adstringens* and *Tabebuia caraiba* were very active against *T. rubrum*, presented geometric means of the MIC values between 170.39 and 23.23 μg ml^{-1}. Extracts of *Cerrado* plants are of particular interest as source of new agents for the treatment of dermatophytic infections.

Introduction

Major advances in health care over the past few years have led to an unwelcome increase in the size of a population at risk for life-threatening infections because of pathogenic and opportunistic fungi, especially by increasing the number of patients with impaired immunological defences.[1-3] These infections are associated with significant morbidity and mortality rates; they are difficult to prevent, diagnose, treat and the trend appears to be that the number of people at risk of fungal infections will continue to grow.[4, 5]

Despite the existence of potent antifungal agents, there is an urgent need for developing newer antifungal drugs that would belong to a diverse class compared with the existing ones and present a different mode of action. The recommendation is a consequence of the therapeutic limitations, the development of fungal drug resistances, drug-related toxicity, significant drug interactions, or insufficient bioavailability of most commonly used antifungal drugs.[4, 6-9]

Terrestrial plants and their secondary metabolites play an important role as the source of innovative therapeutic agents for various conditions, including infectious diseases.[10-12] Brazil is one of the countries with the highest plant diversity distributed in different biomes. Brazil's *Cerrado* is the second most important biome, representing 23% of the area of the country, and more than 6000 vegetal species have been catalogued. However, the biome is being rapidly converted into area for agriculture and cattle pastures. For this reason, the *Cerrado* was identified as one of the richest and threatened biomes of the world.[13, 14]

The use of medicinal plants from *Cerrado* for the treatment of many diseases is widespread, mainly in rural areas.[15] Studies based on traditional uses of the *Cerrado* plants have already identified extracts and isolated compounds with potential biological activity,[16-21] including antifungal activity.[22-40] While the vast amount of the *Cerrado* plant species have not yet been chemically or biologically evaluated, the aim of this study was to screen for medicinal plant extracts that could be useful for the development of new tools for the control of infectious diseases. While pursuing this goal, we initiated a systematic evaluation of extracts from the *Cerrado* plant species against the pathogenic fungi *Candida albicans* and *Trychophyton rubrum.*

Materials and methods

Plant material

Botanical materials of 11 native species of *Cerrado* traditionally used in folk medicine by people living in the *Cerrado* area, mainly for the treatment of wounds and infections,[41-49] were collected in Brasília, the Federal District of Brazil, in 2005/2006. Botanical identification was performed by Professor José Elias de Paula of the Vegetal Anatomy Laboratory, Institute of Biology, University of Brasília (UnB). The sample botanic specimens were deposited at the Herbarium (UB) of that institution.

Extracts production

Different parts of the mature plants were air dried in the shade and ground into powdered material using an appropriate mill. The powdered material was macerated with different solvents: hexane, dichloromethane, ethanol or hydroalcoholic solution 90% (4 × 2l). The solution was filtered and the solvents were evaporated at reduced pressure at temperature below 40 °C. After removal of residual solvent, the extracts were kept at −20 °C.

Screening for antifungal activities

Fungal strains.

The microorganisms used for the biological evaluation were provided by the Medical Mycology Specialized Centre (CEMM, Federal University of Ceará, Brazil), where they were maintained in saline (0.9% NaCl) at 28 °C. At the time of the analysis, an aliquot of each suspension was taken and inoculated onto potato dextrose agar (PDA; Difco, Detroit, MI, USA), and then incubated at 28 °C for 2–10 days. The strains used in this study were: *T. rubrum* (eight clinical isolates), *C. albicans* (ATCC 14053) and, for quality control, *Candida parapsilosis* (ATCC 22019) as recommended by the guideline CLSI M27-A2, 2002.

Agar-well diffusion susceptibility tests.

The method used for antifungal susceptibility test was previously described by Fontenelle *et al.* [33] One strain of *C. albicans* (ATCC 14053) and one clinical isolate of *T. rubrum* (CEMM 01-4-021) were subcultured on PDA for 2 and 10 days, respectively, and incubated at 28 °C. For the preparation of the inocula, a suspension of conidia with blastoconidia of *C. albicans* and other of the hyphal fragments of *T. rubrum* were prepared with saline (0.9% NaCl), transferred to a sterile tube and the turbidity were

corrected by adding sterile saline until a McFarland turbidity standard of 0.5 (10^6 colony forming units, CFU ml^{-1}). Petri plates were prepared with PDA, wells of 6 mm in diameter were then made in the medium using a sterile cork borer and 50 µl of the extracts diluted in dimethyl sulphoxide (DMSO; Sigma Chemical Co., St Louis, MO, USA), to obtain the test concentrations of 20, 10 and 5 mg ml^{-1}, were placed into the wells. Each fungal suspension was inoculated onto the surface of the agar. After incubation, 2 days for *C. albicans* and 10 days for *T. rubrum*, at 28 °C, all plates were examined for zones of growth inhibition and the diameters of these zones were measured in millimetres. Itraconazole (10 µg ml^{-1}; Sigma Chemical Co.) was dissolved in DMSO and served as positive control and DMSO 100% as negative control. Each experiment was performed in duplicate and repeated at least twice.

Broth microdilution method.

The minimal inhibitory concentration (MIC) of each extract was determined for eight strains of *T. rubrum* (see Table 3) by using broth microdilution techniques as described by the National Committee for Clinical Laboratory Standards guidelines (M27-A2 and M38-A).[49, 50] MIC values were determined in flat-bottomed 96-well plastic tissue-cultured plates, using RPMI 1640 medium (Sigma Chemical Co.) buffered to a pH 7.0 with 0.165 mol l^{-1} MOPS (Sigma Chemical Co.). For the broth microdilution method, the standardised inocula for *T. rubrum* and *C. parapsilosis* for quality control were also prepared by turbidimetry, as mentioned above. The suspensions were diluted to 1 : 500 for *T. rubrum* and 1 : 2000 for *C. parapsilosis* both with RPMI 1640 medium to obtain inocula of 2.5–5 × 10^3 and 5 × 10^4 CFU ml^{-1} respectively. Extracts stock solutions were two fold diluted from 1000 to 0.98 µg ml^{-1} and a final DMSO concentration ≤5%. The microplates were incubated at 37 °C and read visually after 5 days for *T. rubrum* and 2 days for *C. parapsilosis*. The same tests were performed simultaneously for growth control (RPMI + fungi) and sterility control (RPMI + extract). Itraconazole (Sigma Chemical Co.) was used as reference compound against *T. rubrum* and Anfotericin B (Sigma Chemical Co.) for quality control against *C. parapsilosis.* The MIC was calculated as the highest dilution showing 100% inhibition of tested strain. Only for Itraconazole, MIC was calculated based on 80% inhibition (National Committee for Clinical Laboratory Standards guideline M38-A, 2002). Only extracts that showed important antifungal activity from agar-well diffusion were tested for MIC. All isolates were run in duplicate and repeated at least twice.

Table 3. Minimal inhibitory concentration (MIC) of 5 active extracts against clinical isolates of *Trichophyton rubrum* by the microdilution method

Clinical isolates *T. rubrum*	MIC (μg ml^{-1})					
	Extracts					
	KC SW	KC RW	RA	SA	TC	Itraconazole
CEMM[a] 01-1-010	156.25	78.12	19.53	78.12	78.12	0.25
CEMM 02-6-066	156.25	78.12	19.53	78.12	78.12	0.25
CEMM 01-1-085	156.25	78.12	19.53	78.12	78.12	0.125
CEMM 01-5-020	78.12	39.06	19.53	78.12	78.12	0.125
CEMM 01-1-016	156.25	78.12	19.53	78.12	156.25	0.25
CEMM 02-6-032	156.25	156.25	19.53	78.12	78.12	0.125
CEMM 01-5-030	312.5	156.25	39.06	156.25	156.25	0.031
CEMM 01-2-101	312.5	78.12	39.06	156.25	156.25	0.50
Geometric mean	170.39	85.19	23.23	92.90	101.31	0.16
Modal MIC	156.25	78.12	19.53	78.12	78.12	0.25 and 0.125

KC SW, *Kielmeyera coriacea* SW, d; KC RW, *Kielmeyera coriacea* RW, d; RA, *Renealmia alpinia* L, h; SA, *Stryphnodendron adstringens* L, h; TC, *Tabebuia caraiba* SW, d (SW, stem wood; RW, root wood; L, leaf; h, hexane; d, dichloromethane).

[a]CEMM, clinical isolates of Medical Mycology Specialized Centre.

Statistical analysis

For comparing the different concentrations of each extract and each concentration with the positive control, ANOVA nonparametric (*Kruskal–Wallis H* test) was used, once the preassumptions of ANOVA were not considered (*Shapiro Wilk's* test for normality with $P < 0.05$). The *post hoc* test chosen was the *Dunn* test. After these comparisons, it was interesting to analyse each concentration of one extract against all the experimental groups (multiples comparisons). In this case, a bivariate ANOVA was used and the confidence interval of 95% was observed for the comparisons. Analysis was performed using the SPSS version 14.0.0.

Results

Of 57 extracts tested, fungitoxic activity was found in 18 extracts (20 mg ml^{-1}) of eight medicinal plants from brazilian *Cerrado*, belonging to seven families and the results are presented in Table 1. The zones of inhibition ranged from 0 mm to total inhibition and DMSO 100%, used as negative control, showed no activity. Of all extracts, four prevented growth of *C. albicans* and 17 of *T. rubrum*, while only three extracts were active for both. *Cybistax antisyphilitica, Himatanthus obovatus* and *Serjania lethalis* were the plants whose extracts were completely inactive for both fungi.

Table 1. Plants extracts presenting antifungal activity in the concentration of 20 mg ml^{-1}

Plant species and controls	Extracts (20 mg ml^{-1})	Diameter of inhibition zones (mm)	
		Candida albicans	*Trichophyton rubrum*
Byrsonima crassa	SB,[a] hs[b]	–[d]	18
Calophyllum brasiliense	RB, hs	–	10
	SW, hs	–	11
	R, hs	10	17
Casearia sylvestris var. *lingua*	L, h	–	14
Kielmeyera coriacea	SB, hs	–	16

Plant species and controls	Extracts (20 mg ml^{-1})	Diameter of inhibition zones (mm)	
		Candida albicans	*Trichophyton rubrum*
	SW, d	–	23
	RW, d	10	32
Renealmia alpinia	L, h	9	TI[c]
	Rz, h	–	17
	Rz, d	–	16

[a]SB, stem bark; RB, root bark; SW, stem wood; R, root (wood + bark); L, leaf; RW, root wood; Rz, rhizome.

[b]h, hexane; d, dichloromethane; e, ethanol; hs, hydroalcoholic solution 90%.

[c]TI, total inhibition.

[d]–, no inhibition (0 mm).

Regarding the 17 extracts that were able to prevent growth of *T. rubrum,* some of *Kielmeyera coriacea, Renealmia alpinia, Stryphnodendron adstringens* and *Tabebuia caraiba* showed promising activity, presenting zones of inhibition close to or even larger than that of Itraconazole (25 mm). Besides, the hexanic extract of the leaves of *R. alpinia* presented total inhibition on the growth of the dermatophyte. Therefore, these extracts were tested in three decreasing concentrations against *T. rubrum*. Once the activity against *C. albicans* was weak, further tests were not performed.

Agar serial dilution was carried out in three different concentrations (20, 10 and 5 mg ml^{-1}) against the clinical isolate of *T. rubrum* (CEMM 01-4-021) for the five extracts with promising activity and results are presented in Table 2. In all evaluated concentrations, all the five extracts prevented the growth of the dermatophyte. The higher the concentration, the greater the diameter of the inhibition zone.

Table 2. Serial dilution of the most active extracts against *Trichophyton rubrum* (CEMM 01-4-021) by the agar-well diffusion method

Extracts	Diameter of inhibition zones (mm) ± SD[e]		
	Concentration of the extracts (mg ml^{-1})		
	5	10	20
Kielmeyera coriacea SW[a], d[b]	7.25 ± 0.5A[d]	18 ± 0.82A	23.25 ± 0.5A
Kielmeyera coriacea RW, d	15.25 ± 1.26B	25.75 ± 0.5B	33 ± 2.45B
Renealmia alpinia L, h	27.25 ± 1.26C	TI[c] C	TI C
Stryphnodendron adstringens L, h	8.75 ± 0.5A	23.25 ± 1.26B	30.5 ± 0.58B
Tabebuia caraiba SW, d	21 ± 1.41D	27.75 ± 2.06B	32.25 ± 2.87B

[a]SW, stem wood; RW, root wood; L, leaf.

[b]h, hexane; d, dichloromethane.

[c]TI, total inhibition.

[d]Capital letters A, B, C and D means statistical difference between the extracts in each concentration tested ($P < 0.05$).

[e]Inhibition zone of Itraconazole 10 μg ml^{-1} against *T. rubrum*: 25 mm.

Statistical analyses comparing all the concentrations tested with Itraconazole show that only dichloromethanic extract of the stem wood of *K. coriacea*, even the highest concentration tested, did not present inhibition zones larger than Itraconazole (25 mm), unlike the extract of *R. alpinia* that presented inhibition zones higher than the control when tested with 5 mg ml^{-1} (27.25 mm). In the concentration of 10 mg ml^{-1}, the diameter of the inhibition zone recorded for the dichloromethanic extracts of

stem wood of *T. caraiba* (27.75 mm) and root wood of *K. coriacea* (25.75 mm) were still larger than that of Itraconazole.

Statistical analyses comparing each extract (Table 2) show that the extracts of *R. alpinia* were the most active. Considering the concentration of 20 and 10 mg ml^{-1}, the extracts of *T. caraiba, S. adstringens* and root wood of *K. coriacea* presented the same antifungal potential. When tested with 5 mg ml^{-1}, the diameter of inhibition zones of these extracts presented statistical differences with the extract of *T. caraiba* being the most active and that of *S. adstringens,* the least.

As all the five extracts presented a potent activity, their MIC were determined against eight clinical isolates of *T. rubrum* (Table 3). The quality control of the microdilution test was performed with *C. parapsilosis* ATCC strain using Amphotericin B as susceptibility agent. The value of MIC found was 0.5 µg ml^{-1}, corroborating the recommendation of the guideline CLSI M27-A2 (range 1–0.25 µg ml^{-1}). Geometric mean of the extracts ranged from 170.39 to 23.23 µg ml^{-1} and modal MIC from 156.25 to 19.53 µg ml^{-1}.

Discussion

Additional antifungal agents must be developed to successfully control the new and emerging human fungal pathogens resistant to available antifungals. Once secondary metabolites of terrestrial plants are involved in the relationship of the organism with the environment, including the defence against invading microorganisms and parasites, these biomolecules are strong candidates for development as antimicrobial agents. In this study, a survey of plant species that are important in traditional medicines was conducted to identify species with antifungal activity. While none of the extracts from these plant species greatly inhibited growth of *C. albicans*, several extracts, especially those from *K. coriacea, R. alpinia, S. adstringens* and *T. caraiba,* were identified as having pronounced antifungal activities toward several strains of the most frequently isolated dermatophyte, *T. rubrum.*

It is interesting to note that some of the plant extracts that showed high antifungal activity against *T. rubrum*, were inactive against *C. albicans*. Fontenelle *et al.* [33] has tested an essential oil against *C. albicans* and the dermatophyte *Microsporum canis* and also found a different activity between the species. It is relatively common and indicates differences in the mode of action of antidermatophytes to anti-*candida* compounds.[51]

The plant *K. coriacea* is well-known in Brazilian traditional medicine for the treatment of infectious disease.[52] In this study, extracts of *K. coriacea* presented important antifungal activity, specially the dichloromethanic of the root wood. The present results are consistent with those of Cortez *et al.* [53] who isolated xanthones and one biphenil from the extract mentioned active

against *Cladosporium cucumerinum* and *C. albicans.*

Members of the genus *Tabebuia* have already been tested towards bacteria and fungi. Anthraquinones, naphthoquinones and analogues from *Tabebuia impetiginosa* showed activity against bacteria such as *Helicobacter pylori* and *Staphylococcus aureus*.[54, 55] Furthermore, Barbosa-Filho *et al.* [56] isolated several compounds active against Gram-negative and -positive bacteria. Among them, ethyl *p*-hydroxycinnamate showed activity against *C. albicans.* In Alves *et al.* [24] work, *T. caraiba* did not show antifungal activity. However, their work does not mention which extract was used and test conditions were different as the test performed was bioautographic assay with *C. sphaerospermum*.

Previous findings on *S. adstringens* and *Schinus terebinthifolius* reported potent activity of extracts of these plants against fungi, especially *Candida* species.[22, 39, 57] Although our results showed lower activities of these extracts, this variation may be because of the dose of extracts used in the different studies, the solvent used for extraction, the method of bioassay employed, the age of the plant and the parts used, even the plant source and the period of collection. Passos *et al.* [25] evaluated the antifungal potential of the foliar epicuticular waxes of *Caryocar brasiliensis* and found that the plant collected in March presented different results from that collected in October. Besides, the weak activities demonstrated by these extracts *in vitro*, does not necessarily imply that they would demonstrate weak activities *in vivo*,[58] mostly because they are widely use in the traditional medicine of *Cerrado* for this purpose.

Members of the Zingiberaceae family are important tools in traditional medicine to treat fungal infection. Plants such as *Alpinia galanga*, *Curcuma zedoaria* and *Zingiber purpureum* have been evaluated regarding its antifungal potency and ethanolic extracts showed important activity towards several species of fungi, including resistant ones.[59] The hexanic extract of the leaves of *R. alpinia* was the most promising one, presenting a lower MIC. Interestingly, the best results found in this study are attributable to apolar extracts as well as other studies that reported potent antifungal compounds isolated from apolar extracts of plants used in traditional medicine.[60, 61] We do not narrow the research to a specific polarity of chemical substances of the plant, but we exploit the great variety of secondary metabolites that the plant can offer.

The fact that current dermatophytosis therapy can cause considerable adverse effects in some patients renders these plant-derived compounds the subject of meaningful future research. The plants may provide novel or lead molecules, which could become starting materials for the synthesis of new drugs. Besides, results with plants from Brazilian *Cerrado*, specially *R. alpinia,* open perspectives to find more effective vegetal drugs, less toxic and available to the lowest socioeconomic strata of the

population in the treatment of these diseases. Bioassay-guided fractionations are being performed to determine the active constituents of the plant extracts.

Acknowledgments

We are thankful for CAPES, Brazilian governmental agency, for the financial support in the form of grants and fellowship awards. In particular to Dr José Júlio Costa Sidrim from the Medical Mycology Specialized Centre, Federal University of Ceará, Brazil, for the fungal strain.

References

1 Galván B, Mariscal F. Epidemiología de la candidemia en UCI. *Rev Iberoam Micol* 2006; **23**: 12– 15.
Crossref | PubMed | Google Scholar

2 Venkatesan P, Perfect JR, Myers S. Evaluation and management of fungal infections in immunocompromised patients. *Dermatol Ther* 2005; **18**: 44– 57.
Wiley Online Library | CAS | PubMed | Google Scholar

3 Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective Nationwide Surveillance Study. *Clin Infect Dis* 2004; **39**: 309– 17.
Crossref | PubMed | Web of Science® | Google Scholar

4 Bustamante CI. Treatment of *Candida* infection: a view from the trenches! *Curr Opin Infect Dis* 2005; **18**: 490– 5.
Crossref | PubMed | Web of Science® | Google Scholar

5 Richardson MD. Changing patterns and trends in systemic fungal infections. *J Antimicrob Chemother* 2005; **56**: 5– 11.
Crossref | CAS | PubMed | Web of Science® | Google Scholar

6 Matta DA, Almeida LP, Machado AM *et al.* Antifungal susceptibility of 1000 *Candida* bloodstream isolates to 5 antifungal drugs: results of

a multicenter study conducted in São Paulo, Brazil, 1995–2003. *Diagn Microbiol Infect Dis* 2007; **57**: 399– 404.
Crossref | CAS | PubMed | Web of Science® | Google Scholar

7 Baixench M-T, Aoun N, Desnos-Ollivier M *et al.* Acquired resistance to echinocandins in *Candida albicans*: case report and review. *J Antimicrob Chemother* 2007; **59**: 1076– 83.
Crossref | CAS | PubMed | Web of Science® | Google Scholar

8 Kauffman CA. Clinical efficacy of new antifungal agents. *Curr Opin Microbiol* 2006; **9**: 483– 8.
Crossref | CAS | PubMed | Web of Science® | Google Scholar

9 Pauli A. Anticandidal low molecular compounds from higher plants with special reference to compounds from essential oils. *Med Res Rev* 2006; **26**: 223– 68.
Wiley Online Library | CAS | PubMed | Web of Science® | Google Scholar

10 Cos P, Vlietinck AJ, Berghe DV, Maesa L. Anti-infective potential of natural products: how to develop a stronger in vitro 'proof-of-concept'. *J Ethnopharmacol* 2006; **106**: 290– 302.
Crossref | CAS | PubMed | Web of Science® | Google Scholar

11 Koehn FE, Carter GT. The evolving role of natural products in drug discovery. *Nat Rev Drug Discov* 2005; **4**: 206– 20.
Crossref | CAS | PubMed | Web of Science® | Google Scholar

12 Paterson I, Anderson EA. The renaissance of natural products as drug candidates. *Science* 2005; **310**: 451– 3.
Crossref | PubMed | Web of Science® | Google Scholar

13 Myers N, Mittermeier RA, Mittermeier CG, Fonseca GAB, Kent J. Biodiversity hotspots for conservation priorities. *Nature* 2000; **403**: 853– 8.
Crossref | CAS | PubMed | Web of Science® | Google Scholar

14 Ratter JA, Ribeiro JF, Bridgewater S. The brazilian *Cerrado* vegetation and threats to its biodiversity. *Ann Bot* 1997; **80**: 223– 30.
Crossref | Web of Science® | Google Scholar

15 Silva Júnior MC. *100 Árvores do Cerrado: Guia de Campo*. Brasília: Rede de Sementes do Cerrado, 2005, 278 p.
Web of Science® | Google Scholar

16 Coelho AAM, De Paula JE, Espindola LS. Insecticidal activity of *Cerrado* plant extracts on *Rhodnius milesi* Carcavallo, Rocha, Galvão & Jurberg (Hemiptera: Reduviidae), under laboratory conditions. *Neotrop Entomol* 2006; **35**: 133– 8.
Crossref | PubMed | Web of Science® | Google Scholar

17 Napolitano DR, Mineo JR, De Souza MA, De Paula JE, Espindola LS, Espindola FS. Down-modulation of nitric oxide production in murine macrophages treated with crude plant extracts from the Brazilian *Cerrado*. *J Ethnopharmacol* 2005; **99**: 37– 41.
Crossref | CAS | PubMed | Web of Science® | Google Scholar

18 Mesquita ML, Desrivot J, Bories C *et al.* Antileishmanial and trypanocidal activity of Brazilian *Cerrado* plants. *Mem Inst Oswaldo Cruz* 2005; **100**: 783– 7.
Crossref | PubMed | Web of Science® | Google Scholar

19 Mesquita ML, Grellier P, Blond A *et al.* New ether diglycosides from *Matayba guianensis* with antiplasmodial activity. *Bioorg Med Chem* 2005; **13**: 4499– 506.
Crossref | CAS | PubMed | Web of Science® | Google Scholar

20 Mesquita ML, Grellier P, Mambu L, De Paula JE, Espindola LS. *In vitro* antiplasmodial activity of Brazilian *Cerrado* plants used as traditional remedies. *J Ethnopharmacol* 2007; **110**: 165– 70.
Crossref | PubMed | Web of Science® | Google Scholar

21 Rodrigues AMS, De Paula JE, Degallier N, Molez J-F, Espíndola LS. Larvicidal activity of some *Cerrado* plant extracts against *Aedes aegypti*. *J Am Mosq Control Assoc* 2005; **22**: 314– 7.
Crossref | Web of Science® | Google Scholar

22 Braga FG, Bouzada MLM, Fabri RL *et al.* Antileishmanial and antifungal activity of plants used in traditional medicine in Brazil. *J Ethnopharmacol* 2007; **111**: 396– 402.
Crossref | PubMed | Web of Science® | Google Scholar

23 Alcerito T, Barbo FE, Negri FG *et al.* Foliar epicuticular wax of *Arrabidaea brachypoda*: flavonoids and antifungal activity. *Biochem Syst Ecol* 2002; **30**: 677– 83.
Crossref | CAS | Web of Science® | Google Scholar

24 Alves TMA, Silva AF, Brandão M *et al.* Biological screening of brazilian medicinal plants. *Mem Inst Oswaldo Cruz* 2000; **95**: 367– 73.
Crossref | CAS | PubMed | Web of Science® | Google Scholar

25 Passos XS, Santos SC, Ferri PH *et al.* Antifungal activity of *Caryocar brasiliensis* (Caryocaraceae) against *Cryptococcus neoformans*. *Rev Soc Bras Med Trop* 2002; **35**: 623– 7.
Crossref | PubMed | Google Scholar

26 Gurgel LA, Sidrim JJC, Martins DT, Cechinel Filho V, Rao VS. In vitro antifungal activity of dragon's blood from *Croton urucurana* against dermatophytes. *J Ethnopharmacol* 2005; **9**: 409– 12.
Crossref | Web of Science® | Google Scholar

27 Malheiros A, Cechinel Filho V, Schmitt CB *et al.* Antifungal activity of drimane sesquiterpenes from *Drimys brasiliensis* using bioassay-guided fractionation. *J Pharm Pharmaceut Sci* 2005; **8**: 335– 9.
CAS | PubMed | Web of Science® | Google Scholar

28 Holetz FB, Pessini GL, Sanches NR, Cortez DAG, Nakamura CV, Dias Filho BP. Screening of some plants used in the brazilian folk medicine for the treatment of infectious diseases. *Mem Inst Oswaldo Cruz* 2002; **97**: 1027– 31.
Crossref | PubMed | Web of Science® | Google Scholar

29 Costa TR, Fernandes OFL, Santos SC *et al.* Antifungal activity of volatile constituents of *Eugenia dysenterica* leaf oil. *J Ethnopharmacol* 2000; **72**: 111– 7.
Crossref | CAS | PubMed | Web of Science® | Google Scholar

30 Souza LKH, Oliveira CMA, Ferri PH *et al.* Antifungal properties of brazilian *Cerrado* plants. *Braz J Microbiol* 2002; **33**: 247– 9.
Crossref | Web of Science® | Google Scholar

31 Souza LKH, Oliveira CMA, Ferri PH *et al.* Antimicrobial activity of *Hyptis ovalifolia* towards dermatophytes. *Mem Inst Oswaldo Cruz* 2003; **98**: 963– 5.
Crossref | PubMed | Web of Science® | Google Scholar

32 Leite SP, Vieira JRC, Medeiros PL. Antimicrobial activity of *Indigofera suffruticosa*. *Evid Based Complement Alternat Med* 2006; **3**: 261– 5.
Crossref | PubMed | Web of Science® | Google Scholar

33 Fontenelle ROS, Morais SM, Brito EHS *et al.* Chemical composition, toxicological aspects and antifungal activity of essential oil from *Lippia sidoides* Cham. *J Antimicrob Chemother* 2007; **59**: 934– 40.
Crossref | CAS | PubMed | Web of Science® | Google Scholar

34 Duarte MCT, Figueira GM, Sartoratto A, Rehder VLG, Delarmelina C. Anti-*Candida* activity of Brazilian medicinal plants. *J Ethnopharmacol* 2005; **97**: 305– 11.
Crossref | PubMed | Web of Science® | Google Scholar

35 Irobi ON, Daramola SO. Antifungal activities of crude extracts of *Mitracarpus villosus* (Rubiaceae). *J Ethnopharmacol* 1993; **40**: 137– 40.
Crossref | CAS | PubMed | Web of Science® | Google Scholar

36 Lemos JA, Passos XS, Fernandes OFL *et al.* Antifungal activity from *Ocimum gratissimum* L. towards *Cryptococcus neoformans*. *Mem Inst Oswaldo Cruz* 2005; **100**: 55– 58.
Crossref | CAS | PubMed | Web of Science® | Google Scholar

37 Silva MRR, Oliveira Junior JG, Fernandes OFL *et al.* Antifungal activity of *Ocimum gratissimum* towards dermatophytes. *Mycoses* 2005; **48**: 172– 5.
Wiley Online Library | CAS | PubMed | Web of Science® | Google Scholar

38 Navickiene HMD, Morandim AA, Alécio AC *et al.* Composition and antifungal activity of essential oils from *Piper aduncum*, *Piper arboreum* and *Piper tuberculatum*. *Quim Nova* 2006; **29**: 467– 70.
Crossref | CAS | Web of Science® | Google Scholar

39 Ishida K, Mello JCP, Cortez DAG, Dias Filho BP, Ueda-Nakamura T, Nakamura CV. Influence of tannins from *Stryphnodendron adstringens* on growth and virulence factors of *Candida albicans*. *J Antimicrob Chemother* 2006; **58**: 942– 9.
Crossref | CAS | PubMed | Web of Science® | Google Scholar

40 Sanches ACC, Lopes GC, Nakamura CV, Dias Filho BP, Mello JCP. Antioxidant and antifungal activities of extracts and condensed tannins from *Stryphnodendron obovatum* Benth. *Braz J Pharm Sci* 2005; **41**: 101– 7.
CAS | Google Scholar

41 Lorenzi H, Matos FJA. *Plantas Medicinais no Brasil: Nativas e Exóticas Cultivadas*. Nova Odessa, SP: Instituto Plantarum, 2002, 530 p.
Google Scholar

42 Fenner R, Betti AH, Mentz LA, Rates SMK. Plantas utilizadas na medicina popular brasileira com potencial atividade antifúngica. *Braz J Pharm Sci* 2006; **42**: 369– 94.
Google Scholar

43 Almeida SP, Proença CEB, Sano SM, Ribeiro JF. *Cerrado: Espécies Vegetais Úteis*. Planaltina-DF: EMBRAPA-CPAC, 1998, 464 p.
Google Scholar

44 Silva SR, Silva AP, Munhoz CB, Silva MC Jr, Medeiros MB (eds). *Guia de Plantas do Cerrado Utilizadas na Chapada dos Veadeiros*. WWF, Brasil: Prática Gráfica e Editora Ltda, 2001, 132 p.
Google Scholar

45 Hashimoto G, Nishimoto Y. *Illustrated Cyclopedia of Brazilian Medicinal Plants*. Kamakura city: ABOC-SHA, 1996, 576– 7 pp.
Web of Science® | Google Scholar

46 Alzugaray D, Alzugaray C. *Plantas que Curam. A Natureza a Serviço da Saúde*. São Paulo: Ed. Três livros e fascículos, 1983, 175 p.
Google Scholar

47 Corrêa MP. *Dicionário das Plantas Úteis do Brasil e das Exóticas Cultivadas*. Rio de Janeiro: Imprensa Nacional, 1984, 687 p.
Google Scholar

48 Zhou B-N, Baj NJ, Glass TE *et al.* Bioactive labdane diterpenoids from *Renealmia alpinia* collected in the Suriname rainforest. *J Nat Prod* 1997; **60**: 1287– 93.
Crossref | CAS | PubMed | Web of Science® | Google Scholar

49 National Committee for Clinical Laboratory Standards. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts-Second Edition: Approved Standard M27-A2*. Villanova, PA: Clinical and Laboratory Standards Institute, NCCLS, 2002.
Google Scholar

50 National Committee for Clinical Laboratory Standards. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi: Approved Standard M38-A*. Villanova, PA: Clinical and Laboratory Standards Institute, NCCLS, 2002.
Google Scholar

51 Ali-Shtayeh MS, Abu Ghdeib SI. Antifungal activity of plant extracts against dermatophytes. *Mycoses* 1999; **42**: 665– 72.
Wiley Online Library | CAS | PubMed | Web of Science® | Google Scholar

52 Audi EA, Otobone F, Martins JVC, Cortez DAG. Preliminary evaluation of *Kielmeyera coriacea* leaves extract on the central nervous system. *Fitoterapia* 2002; **73**: 517– 9.
Crossref | CAS | PubMed | Web of Science® | Google Scholar

53 Cortez DAG, Marston A, Hostettmann K. Separation of xanthones and a biphenyl from *Kielmeyera coriacea by* centrifugal partition chromatography. *Chromatographia* 1999; **50**: 7– 10.
Crossref | CAS | Web of Science® | Google Scholar

54 Park B-S, Lee H-K, Lee S-U *et al.* Antibacterial activity of *Tabebuia impetiginosa* Martius ex DC (Taheebo) against *Helicobacter pylori*. *J Ethnopharmacol* 2006; **105**: 255– 62.
Crossref | CAS | PubMed | Web of Science® | Google Scholar

55 Machado TB, Pinto AV, Pinto MCFR *et al.* In vitro activity of Brazilian medicinal plants, naturally occurring naphthoquinones and their analogues, against methicillin-resistant *Staphylococcus aureus*. *Int J Antimicrob Agents* 2003; **21**: 279– 84.
Crossref | CAS | PubMed | Web of Science® | Google Scholar

56 Barbosa-Filho JM, Lima CSA, Amorim ELC *et al.* Botanical study, phytochemistry and antimicrobial activity of *Tabebuia aurea*. *Phyton* 2004; **53**: 221– 8.
Google Scholar

57 Moustafa AMY. Chemical composition and antifungal activity of the essential oil of *Schinus terebenthifolius* of Egyptian origin. *Int J Essent Oil Ther* 2007; **1**: 91– 95.
CAS | Google Scholar

58 Ndip RN, Tarkang AEM, Mbullah SM *et al. In vitro* anti-*Helicobacter pylori* activity of extracts of selected medicinal plants from North West Cameroon. *J Ethnopharmacol* 2007; **114**: 452– 7.
Crossref | PubMed | Web of Science® | Google Scholar

59 Ficker CE, Smith ML, Susiarti S, Leamanb DJ, Irawati Ç, Arnason JT. Inhibition of human pathogenic fungi by members of Zingiberaceae used by the Kenyah (Indonesian Borneo). *J Ethnopharmacol* 2003; **85**: 289– 93.
Crossref | PubMed | Web of Science® | Google Scholar

60 Rahman MM, Gray AI. A benzoisofuranone derivative and carbazole alkaloids from *Murraya koenigii* and their antimicrobial activity. *Phytochemistry* 2005; **66**: 1601– 6.
Crossref | CAS | PubMed | Web of Science® | Google Scholar

61 Thouvenel C, Gantier J-C, Duret P *et al.* Antifungal compounds from *Zanthoxylum chiloperone* var. *angustifolium*. *Phytother Res* 2003; **17**: 678– 80.
Wiley Online Library | CAS | PubMed | Web of Science® | Google Scholar

Citing Literature