

Inhibition of human pathogenic fungi by ethnobotanically selected plant extracts

Hemmung humanpathogener Pilze durch ethnobotanisch ausgewählte Pflanzenextrakte

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Summary

In this study, 36 extracts derived from 29 plant species selected using an ethnobotanical approach were tested for antifungal activity against a taxonomically diverse group of 13 human pathogenic fungi. We compared the inhibitory characteristics of these plant extracts with those of the commonly used antifungals, amphotericin B and ketoconazole, and the plant-derived antifungal, berberine. Several plant extracts, notably those from *Zingiber officinale* (ginger) and *Juglans cinerea* (butternut), had pronounced antifungal activity against a wide variety of fungi, including strains that were highly resistant to amphotericin B and ketoconazole. Further exploration of *Z. officinale* as an antifungal is warranted as this species is generally regarded as safe for human consumption.

Zusammenfassung

Es wurden 36 Extrakte von 29 Pflanzenarten auf ihre antimyketische Aktivität gegen 13 humanpathogene Pilzarten untersucht. Die Hemmcharakteristik dieser Pflanzenextrakte wurde mit der von Amphotericin B, Ketoconazol und Berberin verglichen. Mehrere Pflanzenextrakte, besonders die von *Zingiber officinale* (Ingwer) und *Juglans cinerea* (Butternuss) zeigten eine deutliche antimyketische Aktivität gegen ein breites Pilzspektrum einschließlich solcher Stämme, die hochresistent gegen Amphotericin B und Ketoconazol waren. Die weitere Untersuchung der antimyketischen Aktivität von *Z. officinale* erscheint vielversprechend, da diese Art allgemein als sicher für menschlichen Konsum angesehen wird.

Key words: antifungal activity, plants, phytomedicine.

Schlüsselwörter: Antimyketische Aktivität, Pflanzen, Phytomedizin.

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Introduction

During the past decade there has been an increase in the number of patients with alterations in immune status associated with human immunodeficiency virus (HIV), cancer chemotherapy and organ and bone marrow transplantations. Coinciding with this rise in the immunocompromised patient population has been an increase in the incidence of human systemic mycoses (reviews in: ^{1–3}). Amphotericin B and the azole group of antifungal agents have been used extensively in the treatment of fungal infections. Unfortunately, the widespread use of these antifungals has led to the emergence of drug resistance in several common pathogenic fungi.^{4,5} In addition, fungi that were previously regarded as saprobes are now occurring on human hosts, with some of these fungal strains having an innate resistance to the presently available antifungal drugs.^{1,6–8}

Due to this emergence of antibiotic resistant human pathogenic fungi, it is of great importance to develop new antifungal agents. The field of ethnobotanical research has expanded greatly in recent years as the value of this type of research in identifying novel therapeutics has come to be more widely recognized.⁹ Using an ethnobotanical approach, this study identifies plant extracts with significant antifungal activities that are appropriate for further study and development as antifungal phytochemicals. The inhibitory characteristics of 36 extracts from 29 plant species used in traditional remedies are reported and compared with those of amphotericin B and ketoconazole. A taxonomically diverse group of human pathogenic fungi were tested to determine their susceptibilities to amphotericin B and ketoconazole, from which 13 strains were selected for use in the plant extract assays. An analysis of published literature using the NAPALERT database (obtained from the University of Illinois) revealed that, although the traditional uses of most of the plant species used in this study are well documented, few have been tested for antifungal activity against a broad range of fungi.

Materials and methods

Plant material and extract preparation

Extracts from 29 plant species (Table 1), representing 16 families, that are used in traditional remedies were obtained from the University of Ottawa Herbarium; 11 were from Togo, West Africa, 12 were from Costa Rica, Central America, and six were from Eastern Ontario, Canada. Plants were collected and preserved in alcohol in the field, then transported to the lab for processing.

The preserved plant material was ground in a blender with ethanol (1 : 3 w/v), then filtered and the solvent was roto-evaporated at 45 °C to near dryness, after which the extract was freeze-dried for 48 h to achieve complete dryness. Two hundred milligrams of the dried extract was reconstituted in 1 mL ethanol for a final concentration of 2 mg/10 µL. More than one plant part of six of the species was extracted, resulting in a total of 36 extracts.

Fungal strains and inoculum preparation

The sources of yeast-like and filamentous fungi used in this study are given in Table 2. The strains are all opportunistic human pathogens. Fungi were cultured on Sabouraud glucose agar medium at 30 °C and are maintained at Carleton University, (Ottawa, ON, Canada). All transfers of fungal strains were carried out under a biological containment hood (Bioclone2, Microzone, Ottawa, ON, Canada). Inocula of yeast-like fungi were prepared in liquid Sabouraud glucose broth and adjusted to an OD₆₀₀ of ~2.0. Inocula of filamentous fungi were prepared by placing a 1 × 2 cm block of agar medium containing hyphae from the colony margin into 10 mL of Sabouraud glucose broth and fragmenting the hyphae for 1.5 min at high speed in a water-cooled Waring blender.

Disk diffusion bioassays

Fungal inoculum (150 µL) was spread onto Sabouraud glucose agar plates with a sterile bent glass rod. Three sterile 3M paper discs, 7.5 mm in diameter, were impregnated with 10 µL (2 mg) of ethanolic plant extract, allowed to air dry and placed face down on the inoculated agar surface. A negative control consisting of 10 µL DMSO and a positive control consisting of 2 mg berberine in 10 µL of ethanol were also prepared.

To test for ultraviolet light effects on antifungal activity of the plant extracts, dark and UV-light treatments were performed. For UV-light treatments, disks impregnated with plant extracts were placed onto freshly inoculated Petri dishes and irradiated with 10 W/m² for 2 h using three 20 W black light blue tubes (320–400 nm range). For dark treatments, plates were wrapped in aluminium foil for 2 h prior to incubation. All treatments were incubated in the dark at 30 °C for 48 h at which time the diameter of the fungal growth inhibition zone was recorded. An extract was classified as having antifungal activity when the diameter of the inhibition zone was ≥8 mm, 0.5 mm larger than the diameter of the paper disk. Fungi

Table 1 Origin of plant species tested and their uses in traditional medicine.

Origin, species	Family	Designation	Traditional medicinal usage ¹
Togo			
<i>Euphorbia hirta</i> L	Euphorbiaceae	UO-18554	Intestinal pain, wounds, antimicrobial
<i>Euphorbia odoratum</i> L	Euphorbiaceae	UO-18557	Malaria, astringent, antispasmodic, antihypertensive, cough, anti-inflammatory
Harungana			
<i>madagascariensis</i> (Lam.) Poir.	Hypericaceae	UO-18555	Emmenagogue, abortive, aphrodisiac, facilitate birth, interrupt menses, antidiarrheal
<i>Mormodica charantia</i> L	Cucurbitaceae	UO-18552	Laxative, diseases of the spleen and liver, gout, rheumatism, diabetes
<i>Phyllanthus amarus</i> L	Euphorbiaceae	UO-18550	Jaundice, stomach ache, skin diseases, colds
<i>Pleiocarpa mutica</i> Benth.	Burseraceae	UO-18558	Fever, malaria
<i>Psidium guajave</i> L	Myrtaceae	UO-18556	Malaria, diarrhea, dysentery
<i>Trema guineensis</i> (Schum. & Thonn.) Ficalho	Ulmaceae	UO-18551	Bronchial congestion, asthma, anthelmintic, antispasmodic, female infertility
<i>Veronia conferta</i> von Schreber	Asteraceae	UO-18560	Increase lactation, aphrodisiac, sleeping sickness, cough, stomach ache, antiparasitic
<i>Ximenia americana</i> L.	Oleaceae	UO-18559	Anthelmintic
<i>Zingiber officinale</i> Roscoe	Zingiberaceae	UO-17722	Cough, tuberculosis, bronchitis, malaria, antimicrobial, contraceptive
Costa Rica			
<i>Cabralea</i> sp. (bark)	Meliaceae	UO-19049	Antimicrobial
<i>Cedrela odorata</i> L	Meliaceae	UO-19089	Wounds, abdominal pain, chills, cough, malaria
<i>Cedrela salvadorensis</i> L	Meliaceae	UO-19025	Gastrointestinal pain, chills, abortive
<i>Piper tuberculatum</i> L	Piperaceae	UO-19061	Regulate menstrual cycle, bronchial infections, cough, female infertility, gonorrhea, syphilis
<i>Protium glabrum</i> (Rose) Engl	Burseraceae	UO-19053	Anti-inflammatory, analgesic
<i>Protium panamensis</i> (Rose) I. M. Johnst	Burseraceae	UO-19054	Anti-inflammatory
<i>Renalmia aromatica</i> (Aubl.) Griseb	Zingiberaceae	UO-19052	Febrifuge
<i>Swietenia mahogoni</i> L	Meliaceae	UO-19046	Malaria, diabetes, postpartum hemorrhage, hypertension, diarrhea
<i>Trichilia americana</i> (fruit) Browne, P	Meliaceae	UO-19013	Antiprotozoal
<i>Trichilia glabra</i> L	Meliaceae	UO-19020	Antiprotozoal
<i>Trichilia pleena</i> (A. Juss.) C. DC.	Meliaceae	UO-19002	Antimalarial
<i>Trichilia triflolia</i> Browne, P	Meliaceae	UO-18464	Purgative, abortive, emmenagogue
Ontario (eastern Canada)			
<i>Acer rubrum</i> L	Aceraceae	UO-18500	Antimicrobial
<i>Acer saccharum</i> Marsh.	Aceraceae	UO-18501	Antimicrobial
<i>Betula papyrifera</i> Marsh.	Betulaceae	UO-18502	Antimicrobial
<i>Juglans cinerea</i> L	Juglandaceae	UO-18507	Antimicrobial
<i>Prunus serotina</i> Ehrh.	Rosaceae	UO-18508	Antimicrobial
<i>Quercus rubra</i> L	Fabaceae	UO-18511	Antimicrobial

¹Sources = NAPALERT database, University of Illinois; 31.

showing growth inhibition when treated with nine (25%) or more of the plant extracts were considered extract susceptible. This value is somewhat arbitrary but serves as a frame of reference against which the antifungal susceptibility of the fungi can be compared.

Amphotericin B and ketoconazole minimum inhibitory concentration (MIC) bioassays

Antifungal susceptibility testing was based on standardized method.¹⁰ Minimum inhibitory concentration (MIC) values were recorded as the lowest tested

concentration of antifungal activity that resulted in a prominent reduction ($\geq 80\%$) of fungal growth. The MIC bioassays were performed in sterile, disposable microtiter plates (Costar, 96 V-shaped well). Fungal inoculum, prepared as described above, was diluted with Sabouraud glucose broth (1 : 500) to obtain $\sim 10^3$ CFU/mL. One hundred microliters of the diluted fungal inoculum was dispensed into each microtiter well within a row. A three-fold dilution was performed from an initial amphotericin B (Sigma, Oakville, ON, Canada) or ketoconazole (Sigma, Oakville, ON, Canada) concentration of 90 $\mu\text{g/mL}$ in the first microtiter well to a final concentration of 0.005 $\mu\text{g/mL}$ in the second to last

microtiter well. The last well in each row served as a drug-free control. Using the three-fold dilution series resulted in a large gap of untested antifungal concentrations from 30 to 90 µg/mL. To define MIC values within this range, bioassays were subsequently repeated using a 0.25 step-wise dilution ranging from 6.8 to 90 µg/mL. Microtiter plates were sealed with parafilm and maintained stationary in the dark at 30 °C for 48 h at which time they were examined for fungal growth. The MIC assays were performed independently at least three times for each fungal strain/antifungal combination.

Results

We examined the susceptibility of 22 fungal strains to amphotericin B and ketoconazole (Table 2). For filamentous fungi, we used hyphal fragments as inoculum rather than conidia for our assays, the rationale being that inhibiting the growth of hyphae is more relevant to pathogenesis than is conidial germination. In addition, not all of the filamentous fungal strains we used readily produced conidia. Fungi with MIC values above ~2 µg/mL for amphotericin B and ~8 µg/mL for ketoconazole were considered antifungal resistant based on

Table 2 Infection types and minimum lethal concentration (MLC) values of fungal strains used in this study.

Fungal species/strain	Source ¹	Infection types ²	MLC values (µg/mL)	
			Amphotericin B	Ketoconazole
Yeast-like				
<i>Candida albicans</i> (Robin) Berkhout		Systemic and subcutaneous infections		
D10	N. D. Lees		90	>90
CN1A	N. D. Lees		90	90
al-1	OGH-308-1329		1.1	0.37
al-2	OGH-311-0628		1.1	0.12
al-3	OGH-312-0305		3.3	30
al-4	OGH-316-0588		1.1	0.13
al-5	OGH-316-0504		1.1	30
al-6	OGH-316-433		3.3	10
al-7	OMH-FR2853			
<i>Candida krusei</i> (Castellani) Berkhout		Systemic and subcutaneous infections		
kr-1	OGH-238-0967		3.3	10
kr-2	OMH-FR2428		1.1	3.3
<i>Candida kefyr</i> (Beijerinck)				
van Uden et Buckley	OGH-301-0788	Systemic and subcutaneous infections	3.3	0.13
<i>Candida glabrata</i> (Anderson)				
Meyer et Yarrow	OGH-299-378	Systemic and subcutaneous infections	3.3	0.13
<i>Cryptococcus neoformans</i> (Sanfelice)				
Vuillemin	OMH-FR2704	Systemic and subcutaneous infections, meningoencephalitis	0.04	0.04
<i>Saccharomyces cerevisiae</i> Hansen	ATCC 48252	Systemic infections in immunocompromised patients	0.37	1.1
<i>Wangiella dermatitidis</i> (Kano) McGinnis	OMH-FR2236	Cutaneous infections and central nervous system	>90	30
Filamentous				
<i>Alternaria alternata</i> (Fries) Keissler	OMH-FR9884	Cutaneous and subcutaneous infections	0.37	0.37
<i>Aspergillus fumigatus</i> Fresenius	OMH-FR2837	Respiratory, subcutaneous infections	1.1	10
<i>Fusarium oxysporum</i> Schlechtendahl	OMH-FR6448	Keratomycosis, subcutaneous infections	90	>90
<i>Microsporium gypseum</i> (Bodin)				
Guiart et Grigorakis	OMH-FR2385	Tinea corporis, tinea pedis	30	30
<i>Pseudallescheria boydii</i> (Shear)				
McGinnis <i>et al.</i>	OMH-FR2625	Sinusitis, central nervous system colonizer	67.5	90
<i>Rhizopus</i> sp.	OMH-FR2874	Invasion of tissue and surrounding vessels	0.01	30
<i>Trichophyton mentagrophytes</i> (Robin)				
Blanchard	OMH-T2379	Tinea corporis, tinea pedis	67.5	67.5

¹N.D. Lees, IUPUI, Indianapolis IN USA; OGH, Ontario General Hospital, Ottawa ON Canada, OMH, Ontario Ministry of Health, Toronto, ON Canada, ATCC, American type culture collection.

²Sources: ^{2,6,8,32,33-35}.

therapeutic concentrations reported in the literature (reviews in: ^{3,4,11}). Most of the yeast-like strains were susceptible to both antifungals at or below these concentrations, with the exception of *C. albicans* strains D10, CN1A, al-3 and al-5, and *W. dermatitidis*, which were resistant to either amphotericin B or ketoconazole, or to both. Notably, all filamentous fungi except *A. alternata* were resistant to at least one of the two antifungals. The amphotericin B and ketoconazole MIC values given in Table 2 are compatible with values reported for the fungal species examined, which tend to vary in a strain-specific manner.^{12–16} From these initial susceptibility assays, we selected 13 fungal strains that showed a wide range of susceptibility to the antifungals, and represented both yeast-like and filamentous growth forms. The strains selected were *C. albicans* strains al-1, D10, and CN1A, *Cr. neoformans*, *S. cerevisiae*, *W. dermatitidis*, *A. alternata*, *A. fumigatus*, *F. oxysporum*, *M. gypseum*, *P. boydii*, *Rhizopus* sp. and *T. mentagrophytes*.

The results of testing the 36 ethanolic plant extracts and berberine against these six yeast-like and seven filamentous fungi are summarized in Table 3. Notably, the plant-derived berberine is a highly effective inhibitor of a wide variety of fungi. Of the 36 crude plant extracts tested, all but six (*E. hirta* leaves, *P. amarus* leaves, *V. conferta* leaves, *Cabralea* spp. bark, *C. odorata* fruit and *T. pleena* bark) were inhibitory to the growth of at least one fungal strain. Photoactivation of plant-derived inhibitors is commonly observed.¹⁷ Significant effects (positive or negative) on antifungal activities of plant extracts by the UV-light treatment were observed in several cases (Table 3). For example, UV-light exposure enhanced the inhibitory effects of *J. cinerea* bark extract on *Cr. neoformans* and *S. cerevisiae*. However, the UV-light treatment of *J. cinerea* wood extract caused a reduction in the zone of inhibition in disk assays with *C. albicans* strain D10.

Figure 1 shows a trend whereby fungal strains that are inhibited by larger numbers of extracts also tend to have larger zones of inhibition by any given extract. Using either criterion, mean inhibition zone diameter or number of plant extracts that inhibit growth (Fig. 2), we found that 11 fungi fell into one of three distinct groups. Group 1 included *C. albicans* strains CN1A and D10, *T. mentagrophytes*, *W. dermatitidis* and *M. gypseum*, all of which were neither susceptible to amphotericin B (MIC ≥ 2 $\mu\text{g}/\text{mL}$) nor to ketoconazole (MIC ≥ 8 $\mu\text{g}/\text{mL}$) but were inhibited by $>25\%$ of plant extracts. Group 2 included *F. oxysporum* and *P. boydii*, which were neither susceptible to antifungal activity nor to $\leq 25\%$ of the plant extracts tested. Finally, group 3 strains, *A. alternata*, *C. albicans* strain al-1, *Cr. neoformans* and

S. cerevisiae, were susceptible to both antifungals and to $\leq 25\%$ of the plant extracts. The strains representing *Rhizopus* sp. and *A. fumigatus* fell into groups 2 and 3 as both were susceptible to amphotericin B but not to ketoconazole. Evidently, several fungi (group 1) that are not susceptible to commonly used antifungals may be inhibited by several plant-derived compounds. The *C. albicans* strains D10 and CN1A that are highly resistant to amphotericin B and ketoconazole, for example, are inhibited by 13 and 12 of the plant extracts, respectively. The *M. gypseum* strain proved to be the most susceptible to the plant-derived growth inhibitors but was not susceptible to amphotericin B or to ketoconazole. The pattern associated with group 2 suggests that the active compounds in the plant extracts have different modes of action from either of the antifungals tested. Both amphotericin B and azole antifungals target ergosterol, the major sterol component of the fungal plasma membrane (reviews in: ^{3,4,11,18,19}). Amphotericin B, a polyene antifungal, is believed to act primarily by binding to ergosterol and forming channels in the membrane through which cellular components, particularly potassium ions, leak, thereby destroying the proton gradient and causing cell death. Ketoconazole and other azoles act primarily by inhibiting lanosterol 14- α demethylase, a cytochrome P450-dependent enzyme involved in the conversion of lanosterol to ergosterol. Azole exposure results in the accumulation of 14 α -methylsterols which induce a variety of functional alterations including membrane leakiness, changes in membrane-bound enzymes and inhibition of growth. The strains CN1A and D10 have mutations that effect the P-450 ergosterol biosynthetic pathway and result in a decreased cell membrane ergosterol content and resistance to ketoconazole and amphotericin B.^{5,20,21} These alterations may increase the permeability of the cell membrane, and thus the sensitivity of the group 1 strains to growth inhibitors in the plant extracts. By extension, the resistance to amphotericin B and ketoconazole displayed by the fungi in group 2, *F. oxysporum* and *P. boydii*, is likely achieved by a different mechanism than alterations in the cell membrane ergosterol content, as these fungi are also quite resistant to inhibition by the plant extracts. Other potential means of resistance, including altered uptake mechanisms and increased activity of efflux pumps. (review in: ⁴), may result in general insensitivity to a wide variety of inhibitors. Because of the overall resistance of the pathogens belonging to group 2, inhibitors of fungi in this group should be actively pursued.

Plant extracts with inhibitory activity against five or more fungal strains included *M. charantia* leaves,

Table 3 Growth inhibition of fungi by plant extracts.

Agent tested ³	Mean diameter ¹ (mm) of growth inhibition zone for fungal strains ²																										
	Cn		Sc		al-1		D10		CN1A		Wd		Aa		Af		Fo		Mg		Pb		Rh		Tm		
	+UV ⁴	-UV ⁴	+UV	-UV	+UV	-UV	+UV	-UV	+UV	-UV	+UV	-UV	+UV	-UV	+UV	-UV	+UV	-UV	+UV	-UV	+UV	-UV	+UV	-UV	+UV	-UV	
Togo																											
<i>E. hirta</i> (l)																											
<i>E. odoratum</i> (l)																											
<i>H. madagascariensis</i> (l)																											
<i>M. charantia</i> (l)																											
<i>P. amarus</i> (l)																											
<i>P. mutica</i> (l)																											
<i>P. guagave</i> (l)																											
<i>T. guineensis</i> (l)																											
<i>V. conferta</i> (l)																											
<i>X. americana</i> (l)																											
<i>Z. officinale</i> (r)																											
Costa Rica																											
<i>Cabralea</i> spp. (b)																											
<i>C. odorata</i> (b)																											
<i>C. odorata</i> (f)																											
<i>C. odorata</i> (l)																											
<i>C. salvadorensis</i> (w)																											
<i>P. tuberculatum</i> (l)																											
<i>P. glabrum</i> (l)																											
<i>P. panamense</i> (l)																											
<i>R. aromatica</i> (l)																											
<i>S. mahogoni</i> (w)																											
<i>T. americana</i> (f)																											
<i>T. americana</i> (l)																											
<i>T. glabra</i> (f)																											
<i>T. pleena</i> (w)																											
<i>T. pleena</i> (b)																											
<i>T. triflora</i> (w)																											
Eastern Canada																											
<i>A. rubrum</i> (b)																											
<i>A. saccharum</i> (b)																											
<i>B. papyrifera</i> (w)																											
<i>J. cinerea</i> (b)																											
<i>J. cinerea</i> (w)																											
<i>P. serotina</i> (b)																											
<i>P. serotina</i> (w)																											

<i>Q. rubra</i> (b)	11.5	23.5*	11.5	10.8	9.5
<i>Q. rubra</i> (w)	8.5	9.5	24.8	18.8	24.8
Berberine	31	25	24.2	24.8	24.2
DMSO	31	25	24.2	24.8	24.2

¹n ≥ 3, minimum diameter possible = 8 mm. Standard error was <15% of mean in all cases. * indicates significant difference between +UV and -UV treatments.

²Cn, *C. neoformans*; Sc, *S. cerevisiae*; al-1, D10; CN1A, *C. albicans*; Wd, *W. dermatitidis*; Aa, *A. alternata*; Af, *A. fumigatus*; Fo, *F. oxysporum*; Mg, *M. gypseum*;

Pb, *P. boydii*; Rh, *Rhizopus* sp.; Tm, *T. mentagrophytes*.

³(b), bark; (f), fruit; (l), leaves; (r), rhizome; (w), wood; positive and negative controls were berberine, a potent antimicrobial agent³⁶ and DMSO, respectively.

⁴+UV and -UV= with and without UV light exposure, respectively.

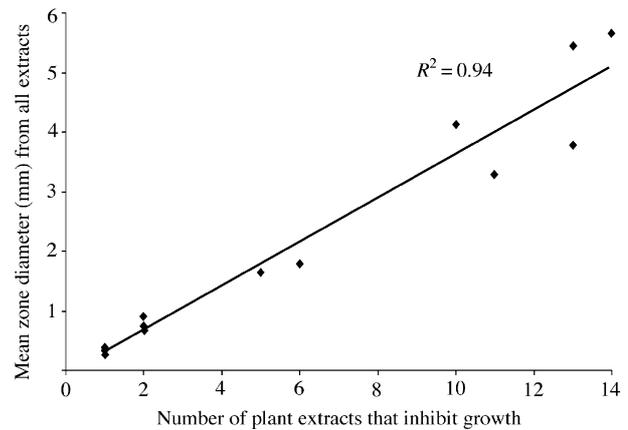


Figure 1 Correlation between mean inhibition zone diameters and number of plant extracts that inhibit fungal strains (from Table 3).

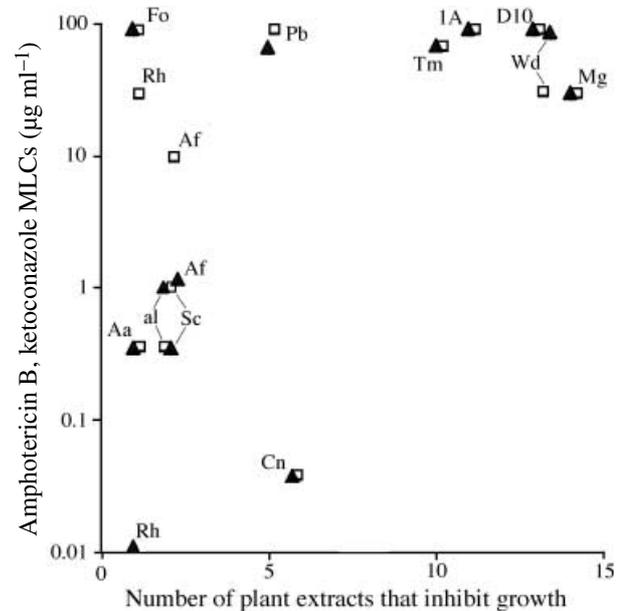


Figure 2 Comparison of inhibition of fungal strains by ketoconazole (open squares) or amphotericin B (closed triangles) and by plant extracts. Susceptibility to ketoconazole and amphotericin B is considered to be about ≥ 2 $\mu\text{g}/\text{mL}$ and ≥ 8 $\mu\text{g}/\text{mL}$, respectively. Fungi that were inhibited by nine (25%) or more of the 36 plant extracts tested were considered extract sensitive. *C. albicans* strains al-1 (al), CN1A (1A) and D10, *T. mentagrophytes* (Tm), *W. dermatitidis* (Wd), *M. gypseum* (Mg), *F. oxysporum* (Fo), *P. boydii* (Pb), *Rhizopus* sp., (Rh) *A. fumigatus* (Af), *A. alternata* (Aa), *C. neoformans* (Cn), *S. cerevisiae* (Sc).

Z. officinale rhizome, *J. cinerea* bark, *Q. rubra* bark and *P. serotina* wood. Of the extracts tested, *J. cinerea* bark and *Z. officinale* rhizome demonstrated the broadest range of antifungal activity, measured as either the number of fungi inhibited or as the average diameter of

the zones of inhibition. Fungi inhibited by *J. cinerea* bark and *Z. officinale* rhizome included those in group 2. Of particular interest, only the *Z. officinale* rhizome extract exhibited activity against the *Rhizopus* sp. strain that was not inhibited by berberine, ketoconazole or any other plant extracts tested. Likewise, *J. cinerea* bark was the only extract inhibiting the growth of the amphotericin B- and ketoconazole-resistant *F. oxysporum* strain.

Discussion

Additional antifungal agents must be developed to successfully control the new and emerging human fungal pathogens resistant to available antifungals. Intense selective pressure on plants by fungal pathogens has resulted in the evolution of a wide range of phytochemicals with antifungal activity. In this study, a survey of plant species important in traditional medicines was conducted to identify species with antifungal activity. Their traditional uses suggest that these plants are favored by low overall toxicity to humans. While none of the extracts from these plant species inhibited growth of all fungal strains, several extracts, especially those from *J. cinerea* bark and *Z. officinale* rhizome, were identified as having pronounced antifungal activities toward a wide range of fungi.

J. cinerea bark was previously found to be a potent antibacterial agent.¹⁷ The antimicrobial activity of *J. cinerea* is thought to be the result of the presence of quinones.²² The potential toxicity of quinones renders these extracts unsafe for use against systemic mycoses but is of potential value as a topical antifungal. Extracts of *Z. officinale* have been reported to possess numerous biological activities^{23–26} and have been used extensively in antinausea clinical trials,^{27–29} including trials conducted in pregnant women.³⁰ As adverse side-effects have not been reported in any of the clinical trials, *Z. officinale* is generally regarded as safe for human use and further investigation into the antifungal activity of this species and its possible use as a phytomedicine is warranted.

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