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RESEARCH ARTICLE

Screening of traditionally used Tanzanian medicinal plants for antifungal activity

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Abstract

Fungal infections represent a significant cause of morbidity and mortality especially in immunocompromised patients in the world today. Dichloromethane (DM) and aqueous (W) extracts of nine plants used traditionally for the treatment of fungal infections in Bukoba rural district in Tanzania were screened for antifungal activity against *Candida albicans*, *Cryptococcus neoformans*, and *Aspergillus niger* using agar well and disk diffusion methods. Dichloromethane extracts of *Capparis erythrocarpos* [CE] Isert (Capparaceae), *Cussonia arborea* [CA] Hochst. Ex A. Rich (Araliaceae), *Dracaena steudneri* [DS] Engl. (Dracaenaceae), *Lannea schimperi* [LS] (A. Rich) Engl. (Anacardiaceae), *Rauvolfia vomitoria* [RV] Afz (Apocynaceae), and *Sapium ellipticum* [SE] (Krauss) Pax (Euphorbiaceae) showed activity against all three fungi. Extracts of *Rumex usambarensis* [RU] (Dammer) Dammer (Polygonaceae) and *Zehneria scabra* [ZS] (L.f.) Sond. (Cucurbitaceae) had an activity limited to only one or two of the test organisms. *Rhoicissus tridentata* [RT] (L.f.) Wild & Drum (Vitaceae) was the only plant without activity. Fractions of the active extracts CE, CA, DS, LS, and SE exhibited higher antifungal activity against one or more of the three fungi. Four compounds isolated from *S. ellipticum* also exhibited antifungal activity against one or more of the three fungi. The minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs), determined using the microplate assay method, ranged between 0.4 and 50.0 µg/mL for crude extracts, 1.6 and 50.0 µg/mL for semi-purified fractions, and 0.12 and 1.0 µg/mL for pure compounds, as compared to 0.016–1.5 µg/mL for fluconazole. We confirm the potential of traditionally used plants as a source of new drugs for treatment of fungal infections.

Keywords: *Antifungal activity;* in vitro *evaluation; traditional medicines*

Introduction

For centuries, medicinal plants have been used throughout the world for the treatment and prevention of various ailments, particularly in developing countries where infectious diseases are endemic and modern health facilities and services are inadequate (Samie et al., 2005). In Africa, the use of medicinal plants in traditional medicine has been practiced since antiquity, and more than 70% of the population depend on it for their primary health care (Kamanzy et al., 2002). In Tanzania, traditional medicine is well recognized, and more than 60% of the population use medicinal plants to treat various diseases (Ministry of Health and Social Welfare, 2002).

In the last two decades, invasive fungal infections have been recognized as a major cause of morbidity and mortality in the world (Hossain & Ghannoum,

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2000). In sub-Saharan Africa, fungal infections, especially *Candida* infections, are very common and cause significant morbidity among patients (Feldmesser, 2003; Moshi et al., 2007). Fungal infections tend to be chronic, often require prolonged chemotherapy, and present particular risks for immunocompromised individuals (Martin & Ernst, 2004). Most fungal infections are caused by *Candida albicans*, *Aspergillus* spp., and *Cryptococcus neoformans*, but the emergence of other fungal infections is changing the spectrum of the disease (Maertens et al., 2001; Lemos et al., 2005). Most common fungal infections are frequently difficult to eradicate with topical preparations and may require long-term use of systemic drugs. Virtually all synthetic antifungals are associated with serious adverse effects; for some fungal infections, there is still no effective cure (Ernst, 2001).

Effective management of fungal infections has thus been impaired by the emergence of resistant fungal strains and side effects associated with most conventional antifungal drugs such as polyenes (amphotericin B) and the azoles (itraconazole and fluconazole) (Hossain & Ghannoum, 2000; Lemos et al., 2005). In some cases, especially for *Candida*, there is a tendency of pathogen shift from *C. albicans* to less sensitive species such as *C. glabrata* and *C. krusei*, thus reducing the number of available, effective antifungal agents (Bastert et al., 2001). The high cost of antifungal agents also renders them unaffordable by the majority of the population in developing countries including Tanzania, where resources are limited (Moshi et al., 2007).

The difficulties associated with the management of fungal infections necessitate the development of new antifungal remedies. For the majority of the rural population in Tanzania, the only option for them is to utilize locally available plant remedies to manage the infections. Plants that are widely used in Tanzanian traditional medicine could offer a potentially useful resource for new and safe drugs for the treatment of fungal infections. The aim of the present study was therefore to determine the antifungal activity of some plants used in traditional medicine to manage fungal infections in Tanzania. Very little or no work has so far been done on the antifungal activities of some of the plant species analyzed in this study, particularly for *Capparis erythrocarpos* Isert (Capparaceae), *Cussonia arborea* Hochst. Ex A. Rich (Araliaceae), *Dracaena steudneri* Engl. (Dracaenaceae), *Lannea schimperi* (A. Rich) Engl. (Anacardiaceae), and *Sapium ellipticum* (Krauss) Pax (Euphorbiaceae). There are currently no chemical data known for *S. ellipticum*, and there is therefore the need to investigate the antifungal activity of possible bioactive compounds from the plant. The study has confirmed the potential of traditionally used plants as a source of new drugs for the treatment of fungal infections.

Materials and methods

Ethnobotanical survey

Ethnobotanical surveys were carried out between September and October 2006 in Bukoba rural district, Kagera region in Northern Tanzania. Having obtained a prior informed consent (PIC), information on plants used in traditional medicine to treat fungal infections was obtained from 30 authentic herbal practitioners (21 women and nine men). With the assistance of local administrative officers, the herbal practitioners were identified through a Participatory Rural Appraisal (PRA) approach according to Martin (1995). They were then interviewed using semi-structured open-ended questionnaires. Interviews were conducted in the local *Kihaya* language except for a few cases where the respondents could understand *Kiswahili*. The respondents were requested to give the local names of the plants, parts used, preparation, administration, and the fungal conditions treated with the plants. Since most of the respondents were not aware of the clinical manifestations of various fungal infections, the symptoms of the infections were described to the healers to enable them to give the appropriate plant species they usually use to manage the infections.

Collection and identification of plant materials

Within the same period of ethnobotanical surveys, the practitioners were used as guides in field excursions to collect plant material. Plant materials were collected on the basis of ethnopharmacological surveys. Voucher specimens were identified by Mr. Suleiman Haji and Mr. Frank Mbago of the Department of Botany, University of Dar es Salaam. The voucher specimens were then assigned collection numbers and deposited at the department's herbarium. The plant species collected for analysis were *Capparis erythrocarpos* Isert (Capparaceae) [roots] (Collection No. DK028/06), *Cussonia arborea* Hochst. Ex A. Rich (Araliaceae) [bark] (Collection No. DK022/06), *Dracaena steudneri* Engl. (Dracaenaceae) [bark] (Collection No. DK014/06), *Lannea schimperi* (A. Rich) Engl. (Anacardiaceae) [bark] (Collection No. DK047/06), *Rauvolfia vomitoria* Afz. (Apocynaceae) [bark] (Collection No. DK030/06), *Rhoicissus tridentata* (L.f.) Wild & Drum. (Vitaceae) [roots] (Collection No. DK072/06), *Rumex usambarensis* (Dammer) Dammer (Polygonaceae) [leaves] (Collection No. DK060/06), *Sapium ellipticum* (Hochst.) Pax (Euphorbiaceae) [bark] (Collection No. DK019/06), and *Zehneria scabra* (L.f.) Sond. (Cucurbitaceae) [leaves] (Collection No. DK017/06).

Preparation and extraction of the plant material

Plant materials were cut into small pieces and then air dried under shade for a period of 10 days at ambient temperature. The dried plant materials were then pulverized using a grinder. About 600 g of each of the pulverized materials was partitioned into two. One part of the material (about 300 g) was extracted by maceration in dichloromethane while the other part was extracted using 100% water. The organic crude extracts were concentrated *in vacuo* using a rotary evaporator at a temperature not exceeding 40°C. The aqueous extracts were lyophilized using a freeze dryer according to Monks et al. (2002) and Banjaw and Schmidt (2004). Both the organic and the aqueous extracts were dissolved in dimethylsulfoxide (DMSO) and kept in a refrigerator before use. The yield of extraction for organic crude extracts ranged between 7.8 and 16.51 g, while that of aqueous extracts ranged between 2.6 and 9.3 g.

Fractionation of crude extracts and isolation of pure compounds

Fractionation of crude extracts of five plant species, *C. erythrocarpos* (CE), *C. arborea* (CA), *D. steudneri* (DS), *L. schimperi* (LS), and *S. ellipticum* (SE), which have scanty literature information, was done using vacuum liquid chromatography (VLC). Column chromatography (CC) was used to isolate compounds from the SE 3 fraction (5.59 g), one of the six fractions obtained from *S. ellipticum* (SE) crude extracts. The isolated compounds were identified using standard spectroscopic methods ^{1}H nuclear magnetic resonance (NMR), ^{13}C NMR, distortionless enhancement by polarization transfer (DEPT), H,H-correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple bond coherence (HMBC), electron ionization, and high resolution mass spectrometry) and by comparison of their data to those reported in the literature.

Test microorganisms and preparation of the inoculum

Three fungal species, *Candida albicans* (ATCC 90028), *Cryptococcus neoformans* (ATCC 90112), and *Aspergillus niger* (AZN 8240), were used for the *in vitro* antifungal assays. The microbes were obtained from the Department of Molecular Biology and Biotechnology (MBB), University of Dar es Salaam and the Department of Microbiology and Immunology, Muhimbili University of Health and Allied Sciences (MUHAS). Malt extract broth (MEB) was prepared according to the manufacturer's instructions and dispensed (10 mL) in capped test tubes, autoclaved, and left overnight. Pure isolates of subcultured fungal colonies were then asceptically transferred into the broth and adjusted to a turbidity equivalent to 0.5 McFarland standard with approximate cell concentration of 1×10^6 CFU/mL. The inoculated broth was transferred to a shaking incubator at 150 rpm orbital for an overnight period.

Media preparation and antifungal activity tests

Kirby–Bauer disk and agar well diffusion methods were used to determine the *in vitro* antifungal activity of crude extracts according to Bauer et al. (1966) and Perez et al. (1990), respectively. Semi-purified fractions and pure compounds were tested using the agar well diffusion method only. Malt extract agar was prepared according to the manufacturer's instructions. The molten medium was inoculated with 0.2 mL of the broth culture, which was spread evenly on the agar surface using a sterile Drigalski spatula. For the agar well diffusion method, equidistant wells were prepared in the plates with the help of a sterile cork-borer. About 75 µL of the plant extracts, semi-purified fractions, or pure compounds and their controls were introduced into the wells (Rojas et al., 2006; Parekh & Chanda, 2007). For the disk diffusion method, sterile 5 mm Whatman No. 1 filter paper disks were saturated with 75 µL of the test crude extracts and controls, allowed to dry, and then introduced on the upper layer of the seeded agar plates. In both cases, the treated plates were pre-incubated in a refrigerator at 4°C for 4–6 h to allow diffusion of the extracts and controls into the agar while arresting the growth of the test microbes. They were then transferred to an incubator for 24 h at 30°C. The tests were carried out in duplicate.

Determination of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC)

A broth microdilution method (Murray & Hospenthal, 2004; Clinical and Laboratory Standards Institute, 2006) was used to determine the minimum inhibitory concentration (MIC) of crude extracts, semi-purified fractions, and pure compounds. The test was performed in Sero-Wel® sterile flat-bottomed 96-well microplates. Solutions of extracts and compounds were made in DMSO in the concentration range of 50–0.4 µg/mL for crude extracts and semi-purified fractions, and 0.5–0.0078 µg/mL for pure compounds and the positive control fluconazole. Serial dilutions of 100 µL of the test extracts and compounds were dispensed in duplicate into columns 1–10, while fluconazole was dispensed into columns 11 and 12. The serial dilutions were made vertically from rows A to G. The negative controls of inoculated and uninoculated broth were made in row H. An inoculum of approximately10^6 CFU/mL (100 µL) was added into the wells to make a volume of 200 µL for each well with the test drugs, followed by the addition of 40 µL of 0.2% iodonitrotetrazolium

chloride (Sigma-Aldrich, St. Louis, MO, USA) and incubation of the plates at 30 ± 2°C for 24 h. Fungal growth in the plates was observed when the indicator turned from colorless to purple, thus indicating the absence of extract or compound activity. The MIC end-point was recorded when the color of the indicator remained clear, indicating activity of the extracts or compounds. The contents of the microplate wells that showed no visible fungal growth were cultured into malt extract agar plates to determine the fungicidal effects of the extracts. The plates were incubated for a further 24–48 h. The minimum fungicidal concentration was recorded as the lowest concentration of the extract that did not show any fungal growth on the solid agar medium (Mann et al., 2008).

Results

During the ethnobotanical surveys, the nine plant species analyzed in this study were found to treat one or more of the following fungal infections: skin rashes, oral candidiasis, ringworm, aspergillosis, cryptococcal meningitis, general skin eruptions, and skin lesions. Extracts of eight out of nine plants showed antifungal activity against one or more of the three fungal species tested (Table 1). Generally, activity was detected in the dichloromethane extracts except for *C. erythrocarpos*, *C. arborea*, *D. steudneri*, *R. vomitoria*, and *Z. scabra*, whose activity was also in the aqueous extract. Dichloromethane extracts of *C. erythrocarpos*, *C. arborea*, *D. steudneri*, *L. schimperi*, *R. vomitoria*, and *S. ellipticum* showed activity against all three fungi, while extracts of *R. tridentata* were inactive against all three fungal species. Out of a total of 33 active crude extracts in both agar well and disk diffusion assays, only six of them were aqueous.

Fractionation of dichloromethane extracts yielded 23 fractions with relatively higher antifungal activity than their initial crude extracts, as indicated by bigger growth inhibition zones (Table 2). Only dichloromethane crude extracts were fractionated, as they exhibited better activities than water extracts during the preliminary screening. Three fractions, CE 1, CE 2, and CE 3, were obtained from *C. erythrocarpos*, where CE 3 was the most active against all three fungi. Five fractions CA 1–5 were obtained from *C. arborea* extract, of which CA 5 was the most active, inhibiting the growth of the three fungal species. The *D. steudneri* dichloromethane extract yielded five fractions, DS 1–5, and DS 3 was the

Table 1. Antifungal activity of crude extracts observed in the agar well and disk diffusion methods.

				Inhibition zone diameter (mm)					
				Agar well method			Disk diffusion method		
Plant name	Part extracted	Extract	Conc. (mg/mL)	*C. albicans*	*C. neoformans*	*A. niger*	*C. albicans*	*C. neoformans*	*A. niger*
Capparis erythrocarpos (CE)	Roots	DM	250	6.50 ± 1.41	14.00 ± 0.00	20.50 ± 3.05	6.50 ± 1.41	—	—
		W	250	12.00 ± 0.00	—	—	—	12.00 ± 0.00	—
Cussonia arborea (CA)	Bark	DM	100	—	17.00 ± 1.53	16.00 ± 0.00	6.00 ± 0.00	—	—
		W	100	—	—	16.00 ± 5.67	—	—	—
Dracaena steudneri (DS)	Bark	DM	200	12.50 ± 0.71	—	—	11.00 ± 2.83	13.50 ± 2.12	10.50 ± 0.71 AI: 0.39
		W	250	15.00 ± 0.00	12.00 ± 0.00	—	—	5.00 ± 1.41	—
Lannea schimperi (LS)	Bark	DM	100	—	16.5 ± 1.53	9.00 ± 0.00	7.00 ± 0.00	8.00 ± 0.00	—
		W	100	—	—	—	—	—	—
Rauvolfia vomitoria (RV)	Bark	DM	250	—	—	19.00 ± 5.67	5.00 ± 0.71	6.5 ± 0.71	—
		W	250	—	—	—	—	11.00 ± 2.83	—
Rhoicissus tridentata (RT)	Roots	DM	250	—	—	—	—	—	—
		W	170	—	—	—	—	—	—
Rumex usambarensis (RU)	Leaves	DM	130	—	—	17.00 ± 0.00	—	—	12.00 ± 0.71 AI: 0.44
		W	165	—	—	—	—	—	—
Sapium ellipticum (SE)	Bark	DM	250	—	18.00 ± 0.00	24.00 ± 7.19	8.00 ± 1.41	—	10.00 ± 0.71 AI: 0.37
		W	200	—	—	—	—	—	—
Zehneria scabra (ZS)	Leaves	DM	200	—	—	—	4.5 ± 0.71	—	—
		W	250	—	10.00 ± 0.00	—	—	—	—
Fluconazole			2 µg/mL	35.00 ± 7.07	29.00 ± 1.41	32.50 ± 3.53	29.00 ± 1.41	34.00 ± 0.00	27.00 ± 0.71

Results are presented as mean ± SD of the inhibition zone diameter (mm).

AI, the ratio between the zone of inhibition of an extract compared to that of the standard drug; DM, dichloromethane extract; W, aqueous extract.

most active against the three fungi, with excellent activity against *A. niger*. LS 1–4 fractions of *L. schimperi* also showed higher activity than the crude extract, but none of them was active against all three fungi. The *S. ellipticum* dichloromethane extract produced six fractions, SE 1–6, all with higher activity than the original extract, with SE 3 being the most active against all three fungal species. Only one fraction, CA 3 from the dichloromethane extract of *C. arborea*, was inactive. Overall, the fractions that were active against all three fungal species were CE 3, CA 5, DS 3, and SE 3.

Column chromatography of extracts of the SE 3 fraction of *S. ellipticum* yielded four compounds identified as lup-20(29)-en-3-one (**1**), a triterpene; β-sitosterol (**2**), a steroid; α-amyrin (**3**), a triterpene; and acetyl aleuritolic acid (**4**), a triterpene (Figure 1). Lup-20(29)-en-3-one (**1**) was active against *C. albicans* and *A. niger*, β-sitosterol (**2**) and α-amyrin (**3**) were active against *A. niger* only, while acetyl aleuritolic acid (**4**) was active against *C. albicans*. None of the compounds was active against *C. neoformans* (Table 3).

The MIC values ranged between 12.5 and 0.4 µg/mL for crude extracts, 25 and 1.6 µg/mL for fractions, 0.5 and 0.12 µg/mL for pure compounds, and 0.5 and 0.016 µg/mL for fluconazole (Tables 4 and 5). For the crude extracts, the lowest MIC value (0.4 µg/mL) was recorded for *R. vomitoria* against *A. niger*. Fraction DS 3 had the lowest MIC value of 1.6 µg/mL against *C. albicans*, while compound α-amyrin (**3**) had the lowest MIC value of 0.12 µg/mL against *A. niger*. Crude extract of *R. vomitoria*, fraction DS 3, and compound α-amyrin (**3**) were also the most fungicidal, with MFCs of 1.2, 3.2, and 0.36 µg/mL, respectively (Tables 4 and 5).

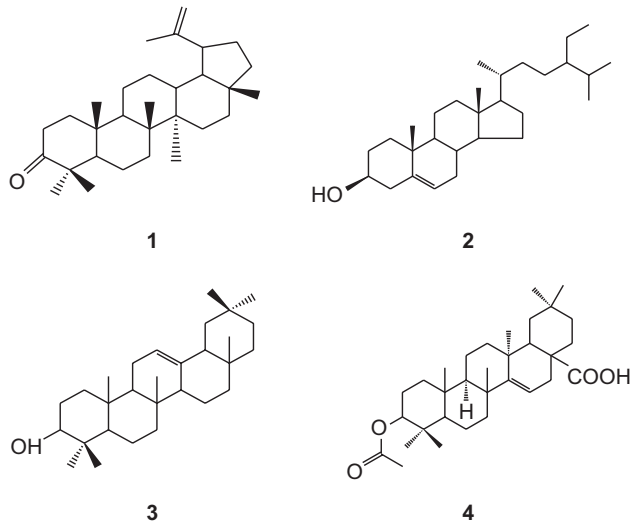

Figure 1. Chemical structures of compounds isolated from SE 3 fraction of *S. ellipticum* stem bark extract.

Table 2. Growth inhibition zone means ± SD (mm) of semi-purified fractions observed in agar well diffusion assay.

		Inhibition zone diameter (mm)		
Fraction code	Conc. (mg/mL)	*C. albicans*	*C. neoformans*	*A. niger*
CE 1	100	9.00 ± 0.00	9.00 ± 0.00	—
CE 2	100	—	10.00 ± 0.00	—
CE 3	100	10.00 ± 0.00	9.50 ± 0.00	15.00 ± 0.00
CA 1	100	—	—	15.00 ± 0.00
CA 2	100	13.00 ± 0.00	—	14.00 ± 0.00
CA 3	100	—	—	—
CA 4	100	15.00 ± 0.00	—	13.50 ± 2.12
CA 5	100	15.00 ± 0.00	15.00 ± 5.66	15.00 ± 0.00
DS 1	100	—	—	9.00 ± 6.36
DS 2	100	—	20.00 ± 0.00	14.65 ± 5.03
DS 3	100	11.50 ± 0.71	10.00 ± 0.00	21.00 ± 1.41
DS 4	100	—	—	11.00 ± 0.00
DS 5	100	—	10.00 ± 0.00	19.50 ± 0.71
LS 1	100	—	—	12.00 ± 1.41
LS 2	100	—	—	13.00 ± 1.41
LS 3	100	15.00 ± 0.00	12.00 ± 8.48	—
LS 4	100	11.00 ± 0.00	—	—
SE 1	100	14.00 ± 0.00	—	—
SE 2	100	20.00 ± 0.00	12.50 ± 0.00	—
SE 3	100	19.00 ± 0.00	16.00 ± 0.00	16.50 ± 0.00
SE 4	100	12.00 ± 0.00	—	—
SE 5	100	15.50 ± 0.00	—	17.00 ± 0.00
SE 6	100	10.00 ± 0.00	14.00 ± 0.00	—
Fluconazole	2 µg/mL	22.00 ± 0.00	25.00 ± 0.00	22.00 ± 1.41

Table 3. Antifungal activity of pure compounds by agar well diffusion method.

Compound	Conc. (mg/mL)	Inhibition zone diameter (mm)		
		C. albicans	*C. neoformans*	*A. niger*
Lup-20(29)-en-3-one (**1**)	1.0	15.00 ± 0.71	—	15.00 ± 2.12
β-Sitosterol (**2**)	1.0	—	—	15.00 ± 0.71
α-Amyrin (**3**)	1.0	—	—	17.00 ± 1.41
Acetyl aleuritolic acid (**4**)	1.0	10.00 ± 1.41	—	—
Fluconazole (μg/mL)	1.0	25.00 ± 2.83	9.00 ± 0.00	18.00 ± 1.41

Results are presented as mean ± SD of the inhibition zone diameter (mm).

Table 4. Minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs) of dichloromethane crude extracts and semi-purified fractions.

Crude extract[a]/ fraction[b]	*C. albicans*		*C. neoformans*		*A. niger*	
	MIC (μg/mL)	MFC (μg/mL)	MIC (μg/mL)	MFC (μg/mL)	MIC (μg/mL)	MFC (μg/mL)
Capparis erythrocarpos	12.5	25.0	—	—	6.3	18.9
CE1	25.0	50.0	25.0	50.0	—	—
CE 2	—	—	25.0	50	—	—
CE 3	12.5	25.0	12.5	50.0	—	—
Cussonia arborea	12.5	50.0	6.3	12.6	—	—
CA 1	—	—	—	—	25.0	50.0
CA 2	6.3	12.6	—	—	12.5	25.0
CA 4	6.3	12.6	—	—	12.5	25.0
CA 5	25.0	25.0	12.5	50.0	25.0	50.0
Dracaena steudneri	3.1	6.2	3.1	9.3	12.5	25.0
DS 1	—	—	—	—	6.3	12.6
DS 2	—		6.3	12.6	3.1	9.3
DS 3	1.6	3.2	6.3	12.6	3.1	6.2
DS 4	—	—	—	—	12.5	50.0
DS 5	—	—	12.5	50.0	12.5	50.0
Lannea schimperi	12.5	12.5	12.5	25.0	—	—
LS 1	—		—		12.5	50.0
LS 2	—		—		25.0	50.0
LS 3	25.0	50.0	12.5	25.0	—	—
LS 4	12.5	25.0	—	—	—	—
Rauvolfia vomitoria	12.5	25.0	6.3	12.6	0.4	1.2
Rumex usambarensis	—	—	—	—	12.5	50.0
Sapium ellipticum	12.5	25.0	—	—	6.3	12.6
SE 1	12.5	25.0	—	—	—	—
SE 2	12.5	50.0	12.5	12.5	—	—
SE 3	6.3	12.6	6.3	18.9	12.5	25.0
SE 4, 5, and 6	—	—	—	—	—	—
Zehneria scabra	12.5	25.0	—	—	—	—
Fluconazole	0.5	1.5	0.25	0.5	0.016	0.048

[a]Full plant species names represent crude extracts.
[b]Code names represent semi-purified fractions.

Table 5. Minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MFCs) of pure compounds.

Compound	*C. albicans*		*C. neoformans*		*A.niger*	
	MIC (μg/mL)	MFC (μg/mL)	MIC (μg/mL)	MFC (μg/mL)	MIC (μg/mL)	MFC (μg/mL)
Lup-20(29)-en-3-one (**1**)	0.25	0.5	—	—	0.25	0.25
β-Sitosterol (**2**)	—	—	—	—	0.5	1.0
α-Amyrin (**3**)	—	—	—	—	0.12	0.36
Acetyl aleuritolic acid (**4**)	0.25	0.5	—	—	—	—
Fluconazole	0.5	1.5	0.25	0.5	0.016	0.048

Discussion

The present study indicates that extracts of the plants studied have potential for treating fungal infections. This is confirmed by the fact that the majority of the extracts were active against one or more of the three fungal species tested. There was basically no activity for most water extracts, probably because the active constituents are soluble in organic solvents and not in water, as similarly observed by Nair and Chanda (2006). Semi-purified fractions and pure compounds were tested using the agar well diffusion method only, as it was superior to the disk assay method in the screening tests.

Although it is not recommended to compare the biological activity of standard drugs such as fluconazole with the activity of a complex mixture of substances (Suffredini et al., 2006), the zones of inhibition of crude extracts for *C. erythrocarpos* and *S. ellipticum* and fractions DS 3 and DS 5 against *A. niger* were relatively comparable to that of fluconazole. The MIC and MFC values for these extracts were, however, not comparable to the standard except for fraction DS 3, which was relatively comparable against *C. albicans*. The MIC and MFC values of active compounds were also quite favorably comparable with those of fluconazole. Compounds lup-20(29)-en-3-one (**1**) and acetyl aleuritolic acid (**4**) were more active than fluconazole against *C. albicans*, while compound α-amyrin (**3**) was also favorably comparable against *A. niger* (Table 5), indicating a strong potency of these natural compounds. Since no synergistic action can be associated with pure compounds, it can be argued that the isolated compounds were relatively very active against the respective fungal species. Therefore, probably the compounds were responsible for the strong activity observed in the dichloromethane crude extract of *S. ellipticum*, especially against *A. niger.*

In closely related studies, Sama and Ajaiyeoba (2006) have reported inhibitory activities of the leaf and stem extracts of *Capparis thonningii* and *C. tomentosa* against *C. albicans* and *A. flavus*, while Mahasneh (2002) reported antifungal activity of aerial parts of *C. spinosa* against the same fungal strains. These findings augment the antifungal activity of *C. erythrocarpos* observed in the present study. Okunji et al. (1996) reported fungistatic and fungicidal activity of spiroconazole A, a saponin compound from *Dracaena mannii* and *D. arborea*. In a related study by Diallo et al. (2001), extracts from *Cussonia barteri* and *Lannea velutina* gave a positive response against *C. albicans*, thus corroborating the anticandida activity of *C. arborea* and *L. shimperi* observed in the present study. *R. vomitoria* has also been reported to possess antifungal activities (ENVIS (Environmental Information System) Center, 2007). This work also confirms the report by Desta (1993) on the anticandida activity of the leaf extract of *Z. scabra*, though with very mild sensitivity. Nevertheless, methanolic leaf extracts of this plant were non-inhibitory against *C. albicans*, *A. niger*, and *Trichophyton mentagrophytes*, as reported in a different study by Messele et al. (2004). A probable reason for this discrepancy could be that the anticandida compounds were less polar and therefore extractable by dichloromethane used in the present study rather than by methanol used in the previous study.

The present study confirms reports by Ghosal et al. (1978) and Tangmouo et al. (2006) on the anticandida and antiaspergillus activity of compound lup-20(29)-en-3-one (**1**). There is, however, no information on the anticryptococcal activity of this compound. In a different study, the antifungal activity of compound β-sitosterol (**2**) showed a percentage inhibition of spore germination and germ-tube elongation of *A. niger* (Aderiye et al., 1989). An anticandida activity of compound α-amyrin (**3**) and its derivatives has been reported by Johann et al. (2007). However, information on its antifungal potential against *C. neoformans* and *A. niger* is not yet known. So far, there is no report on the antifungal activity of compound acetyl aleuritolic acid (**4**) against the three fungal species tested.

The antifungal activities of the extracts tested confirm many of their traditional applications in the treatment of fungal infections caused by the studied pathogens. For example, *C. erythrocarpos*, *D. steudneri*, *R. vomitoria*, *R. usambarensis*, *S. ellipticum*, and *Z. scabra* were all used traditionally in the treatment of oral candidiasis. The present *in vitro* anticandida activity for these extracts confirmed these claims, except for *R. usambarensis* extract, which did not inhibit the growth of *C. albicans*. The antifungal assays also confirm the use of *C. erythrocarpos*, *D. steudneri*, *R. vomitoria*, *S. ellipticum*, *Z. scabra*, and *C. arborea* in the treatment of cryptococcal meningitis by traditional healers. An exception is for *R. tridentata*, whose claim is not confirmed. Only *C. erythrocarpos* and *S. ellipticum* were associated with the treatment of aspergillosis, and their antiaspergillus activity is confirmed in this study.

Conclusion

This study authenticates that the information obtained from traditional healers could lead to the discovery of therapeutically useful agents. The detection of antifungal activity in nearly all the studied plant species gives a significant credibility to the claims by traditional healers in their treatment of fungal infections. Crude extracts of *C. erythrocarpos*, *C. arborea*, *D. steudneri*, *L. schimperi*, and *S. ellipticum* have been screened for the first time

in this study against one or more of the fungal species studied, where they have portrayed various significant activities. The study supports the importance of ethnopharmacological leads in identifying bioactive molecules in drug discovery. Based on the results, extracts of the studied plants could effectively be used to manage the fungal infections associated with the test organisms. There would be a need to scale up the antifungal investigations of the bioactive compounds isolated to *in vivo* levels as an onset for preclinical trials. Alternatively, herbal formulations of the active crude extracts could be developed based on standardized dosages, for cheaper accessibility by the majority of the population in Tanzania who cannot afford the usually expensive conventional medicines.

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