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Fungistatic and fungicidal activity of East African medicinal plants

Fungistatische und fungizide Wirksamkeit ostafrikanischer Heilpflanzen

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Schlüsselwörter. Pflanzenextrakte, antimyzetische Aktivität, fungistatische Aktivität, fungizide Aktivität.

Summary. Extracts of the traditionally used medicinal plants *Entada abyssinica* (stem bark), *Terminalia spinosa* (young branches), *Harrisonia abyssinica* (roots), *Ximenia caffra* (roots), *Azadirachta indica* (stem bark), *Zanha africana* (stem bark) and *Spilanthes mauritiana* (roots and flowers) were investigated for fungistatic and fungicidal activity against *Candida* spp. and *Aspergillus* spp. by a microtitre serial dilution technique. *Entada abyssinica*, *T. spinosa*, *X. caffra*, *A. indica*, and *Z. africana* showed activity against various *Candida* species. The minimum inhibitory concentrations (MICs) ranged from 0.006 to >8 mg ml^{-1} and the minimum fungicidal concentrations (MFCs) from 0.06 to >8 mg ml^{-1}. Extracts from *S. mauritiana* (both roots and flowers) exhibited no activity against *Candida* spp., but against *Aspergillus* spp., the MIC and MFC values ranged from 0.13 to 0.25 mg ml^{-1} and from 0.13 to 1 mg ml^{-1} respectively. It is concluded that the extracts contain compounds with high antifungal potency.

Zusammenfassung. Extrakte der traditionell verwendeten Heilpflanzen *Entada abyssinica* (Stammrinde), *Terminalia spinosa* (junge Zweige), *Harrisonia abyssinica* (Wurzeln), *Ximenia caffra* (Wurzeln), *Azadirachta indica* (Stammrinde), *Zanha africana* (Stammrinde) und *Spilanthes mauritiana* (Wurzeln und Blüten) wurden auf fungistatische und fungizide Wirkungen gegen *Candida* spp. und *Aspergillus* spp. mittels einer Mikroreihenverdünnungstechnik untersucht. *Entada abyssinica*, *T. spinosa*, *X. caffra*, *A. indica* und *Z. africana* zeigten Aktivität gegen verschiedene *Candida* species. Die minimale Hemmkonzentration reichte von 0,006 bis >8 mg ml^{-1}, die minimale fungizide Konzentration von 0,06 bis >8 mg ml^{-1}. Extrakte von *S. mauritiana* (sowohl Wurzeln als auch Blüten) zeigten keine Wirkung gegen *Candida* spp., aber gegen *Aspergillus* spp. mit MIC- und MFC-Werten, die von 0,13 bis 0,25 mg ml^{-1} und 0,13–1 mg ml^{-1} reichten. Es wird gefolgert, daß die Extrakte Bestandteile mit hoher antimyzetischer Potenz enthalten.

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Introduction

The accelerating loss of plant species as a result of destruction of their tropical habitat has yielded a revival of interest in plant-derived products [1]. Secondary metabolites from higher plants can serve as defence agents against invading microorganisms [2]. Recent screenings have yielded extracts with antifungal activity [3–6] and active compounds could also be isolated [3, 6]. In the present study six plants that have been used in the traditional medicine of East Africa were selected. The investigated plants had the following traditional uses.

- *Entada abyssinica:* The roots of this plant are crushed and the sap is used as to treat fresh wounds and skin infections [7].
- *Terminalia spinosa:* This has preservative properties and is applied to fresh skins of slaughtered

animals to prevent them from rotting. Some related species, such as *Terminalia glaucescens*, are used to treat circumcision wounds in Masai boys [8].

- *Harrisonia abyssinica:* Patients with sexually transmitted diseases such as gonorrhoea are given a water extract of the roots of this plant [7]. These roots are also mixed with those of *Entada abyssinica* for the treatment of *Mycobacterium tuberculosis* infections [8].
- *Ximenia caffra:* Sap from the roots of this plant is a remedy for infected eyes [7]. A water decoction of leaves is taken for stomachache [8].
- *Azadirachta indica:* This plant contains an insect anti-feeding compound, azadirachtin [9], and is traditionally widely used along the east coast of Africa against several diseases. The Swahili word for this plant is *Marubaini*, meaning a cure for 40 diseases.
- *Spilanthes mauritiana:* This is a small plant that rarely grows longer than 17 cm. Several tribes in Eastern Africa use its various parts for medicinal purposes. The sap from its roots is a cure for mouth and throat infections [7]. Some tribes crush the leaves and use the water decoction to cure thrush. The whole plant is crushed and the juice drunk as a remedy for stomachache and diarrhoea [8].
- *Zanha africana:* This plant is a remedy for various skin diseases in Tanzania [8].

Materials and methods

Plant material

Plant materials collected from various locations in Kenya were authenticated by S. Mathenge of the University of Nairobi Herbarium, where duplicate specimens are held for reference. The material was dried under shade, and ground to a mince using a type 8 Lab mill (Christy and Norris, 49970). The mince was hermetically sealed in polythene bags until extraction if not extracted immediately.

Extraction

A methanol Soxhlet extraction with 40–50 g of minced material was performed for 10 h or until the extract was clear. The solvent was removed *in vacuo* below 50 °C to give a crude extract. A solid or paste of the extract was produced by further drying under vacuum over anhydrous copper sulphate.

Strains

A total of 73 strains included clinical isolates and reference strains. They were 10 *Candida albicans* (including ATCC 90873, ATCC 26790, ATCC 76615, DSM 70006), 10 *C. tropicalis* (including ATCC 90847, DSM 70151), 10 *C. parapsilosis* (including ATCC 90875, DSM 70126), 10 *C. glabrata* (including ATCC 90876, DSM 70614, DSM 70615), 10 *C. guilliermondii* (including ATCC 34134, ATCC 91877, DSM 70051), 10 *C. krusei* (including ATCC 90878, DSM 70075), 11 *Aspergillus fumigatus* (including ATCC 90906, NCPF 2129, NCPF 2140), one *A. flavus*, and one *A. niger*. Before testing, the strains were cultured on Sabouraud glucose agar medium [10] at 35 °C for 24–48 h.

Susceptibility tests

MIC determination was performed by a serial dilution technique using 96-well microtitre plates. Extracts were dissolved in Mueller–Hinton broth (MHB; Unipath, Wesel, Germany) to achieve concentrations of 4 μg ml^{-1} to 8 mg ml^{-1} with a final concentration of 2.5% *N,N*-dimethylformamide (DMF; Merck, Darmstadt, Germany). Growth controls also contained 2.5% DMF. Samples from cultures were suspended in 0.85% NaCl solution to give a turbidity equivalent to a McFarland 1 standard and diluted in MHB to achieve a final inoculum concentration of approximately 1×10^5 CFU ml^{-1} in a final volume of 100 μl per well. The plates were incubated for 24 h at 35 °C. The lowest concentration without visible growth was defined as MIC except that for *C. albicans* and *C. glabrata* a growth of up to 30% of the growth control well was disregarded. The MFC was determined by subcultivation of a 2-μl aliquot with a disposable multipoint inoculator (Dynatch, Denkendorf, Germany) into microtitre plates containing 100 μl of broth per well and further incubation of 24 h at 35 °C. The lowest concentration with no visible growth was defined as the MFC, indicating $\geqslant$99.5% killing of the original inoculum.

Results

Well-demarcated end points could be obtained using the applied microtitre dilution technique, with the exception of *C. albicans* and *C. glabrata*. The MIC and MFC values of extracts active against *Candida* spp. are listed in Tables 1–5. Extracts of *T. spinosa* had low MIC values against all tested *Candida* spp., whereas other extracts showed high MICs against several *Candida* spp.:

Table 1. MIC and MFC of extracts from *Entada abyssinica*

Organism (number tested)	MIC (mg ml^{-1})			MFC (mg ml^{-1})		
	50%	90%	Range	50%	90%	Range
C. albicans ($n=10$)	0.13	0.25	0.13–0.25	8	8	4–8
C. tropicalis ($n=10$)	0.25	0.5	0.13–0.5	>8	>8	>8
C. parapsilosis ($n=10$)	0.25	0.25	0.06–0.5	2	>8	0.25–>8
C. glabrata ($n=10$)	>8	>8	0.13–>8	>8	>8	0.25–>8
C. guilliermondii ($n=10$)	0.13	0.25	0.13–0.25	0.25	8	0.13–8
C. krusei ($n=10$)	0.13	0.13	0.006–0.25	0.25	0.5	0.06–2
Aspergillus spp. ($n=13$)	>8	>8	>8	>8	>8	>8

The 50% and 90% values are the concentrations that inhibit (MIC) or kill (MFC) 50% and 90% of strains respectively.

Table 2. MIC and MFC of extracts from *Terminalia spinosa*

Organism (number tested)	MIC (mg ml^{-1})			MFC (mg ml^{-1})		
	50%	90%	Range	50%	90%	Range
C. albicans ($n=10$)	0.06	0.25	0.03–0.5	4	8	4–8
C. tropicalis ($n=10$)	0.13	0.25	0.13–0.5	>8	>8	>8
C. parapsilosis ($n=10$)	0.25	0.5	0.06–0.5	2	>8	0.5–>8
C. glabrata ($n=10$)	0.25	0.5	0.13–0.5	0.5	4	0.5–4
C. guilliermondii ($n=10$)	0.13	0.5	0.13–1	0.25	4	0.13–4
C. krusei ($n=10$)	0.13	0.25	0.03–0.25	0.5	4	0.03–4
Aspergillus spp. ($n=13$)	4	8	4–8	>8	>8	4–>8

The 50% and 90% values are the concentrations that inhibit (MIC) or kill (MFC) 50% and 90% of strains respectively.

Table 3. MIC and MFC of extracts from *Ximenia caffra*

Organism (number tested)	MIC (mg ml^{-1})			MFC (mg ml^{-1})		
	50%	90%	Range	50%	90%	Range
C. albicans ($n=10$)	0.25	0.5	0.06–0.5	>8	>8	8–>8
C. tropicalis ($n=10$)	>8	>8	0.5–>8	>8	>8	>8
C. parapsilosis ($n=10$)	>8	>8	0.5–>8	>8	>8	4–>8
C. glabrata ($n=10$)	>8	>8	0.5–>8	>8	>8	4–>8
C. guilliermondii ($n=10$)	0.5	0.5	0.25–>8	4	>8	4–>8
C. krusei ($n=10$)	0.25	0.25	0.13–0.25	0.5	8	0.13–>8
Aspergillus spp. ($n=13$)	>8	>8	4–>8	>8	>8	8–>8

The 50% and 90% values are the concentrations that inhibit (MIC) or kill (MFC) 50% and 90% of strains respectively.

E. abyssinica against *C. glabrata*, *X. caffra* against *C. tropicalis*, *C. parapsilosis* and *C. glabrata*, and both *A. indica* and *Z. africana* against *C. tropicalis* and *C. parapsilosis*. On a weight basis the MFC values of *T. spinosa* were the lowest, all the other plants exhibiting very high values. The MIC and MFC values of the extract from *H. abyssinica* were mostly above 8 mg ml^{-1} for all tested fungi. The same was true for *S. mauritiana* when tested against *Candida* spp. However, *S. mauritiana* was active against *Aspergillus* spp. The MIC and MFC values against 50% of *Aspergillus* strains were 0.13 and 0.5 mg ml^{-1} respectively, and against 90% were 0.25 and 1 mg ml^{-1} respectively, with ranges of 0.13–0.25 and 0.13–1 mg ml^{-1}. These values were the same for both roots and flowers.

Discussion

The data indicate that most extracts have considerable *in vitro* activity against *Candida* spp., with MIC values similar to or lower than those achieved by extracts of *Solanum nigrescens* [3]. The MFC values, however, indicate that most extracts have fungistatic action except against some tested

Table 4. MIC and MFC of extracts from *Azadirachta indica*

Organism (number tested)	MIC (mg ml^{-1})			MFC (mg ml^{-1})		
	50%	90%	Range	50%	90%	Range
C. albicans ($n=10$)	0.5	0.5	0.25–0.5	>8	>8	8–>8
C. tropicalis ($n=10$)	1	>8	0.5–>8	>8	>8	>8
C. parapsilosis ($n=10$)	>8	>8	0.25–>8	>8	>8	>8
C. glabrata ($n=10$)	0.5	0.5	0.25–1	>8	>8	8–>8
C. guilliermondii ($n=10$)	0.5	0.5	0.25–0.5	8	>8	4–>8
C. krusei ($n=10$)	0.25	0.25	0.13–0.5	1	4	0.25–4
Aspergillus spp. ($n=13$)	>8	>8	8–>8	>8	>8	>8

The 50% and 90% values are the concentrations that inhibit (MIC) or kill (MFC) 50% and 90% of strains respectively.

Table 5. MIC and MFC of extracts from *Zanha africana*

Organism (number tested)	MIC (mg ml^{-1})			MFC (mg ml^{-1})		
	50%	90%	Range	50%	90%	Range
C. albicans ($n=10$)	1	1	0.25–1	>8	>8	8–>8
C. tropicalis ($n=10$)	>8	>8	2–>8	>8	>8	>8
C. parapsilosis ($n=10$)	>8	>8	0.5–>8	>8	>8	4–>8
C. glabrata ($n=10$)	0.25	0.5	0.13–1	>8	>8	4–>8
C. guilliermondii ($n=10$)	1	1	0.5–1	>8	>8	4–>8
C. krusei ($n=10$)	0.5	0.5	0.25–1	8	>8	1–>8
Aspergillus spp. ($n=13$)	>8	>8	4–>8	>8	>8	4–>8

The 50% and 90% values are the concentrations that inhibit (MIC) or kill (MFC) 50% and 90% of strains respectively.

organisms, especially *C. guilliermondii* and *C. krusei*. An interesting profile of activity was shown by the extracts from *S. mauritiana* (both roots and flowers), which were only active against *Aspergillus* spp. The low MFC/MIC ratio indicates fungicidal activity.

Research into new antifungal substances is being stimulated by the development of resistance to azole derivates [11, 12]. Our results suggest that compounds with high antifungal potency could be isolated from the most active extracts. This underlines the importance of screening tropical medicinal plants for antifungal activity before the definitive loss of their habitat.

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References

1 Anon. (1994) Pharmaceuticals from plants: great potential, few funds (editorial) *Lancet* **343**, 1513–1515.
2 Balandrin, M. F., Klocke, J. A., Wurtele, E. S. & Bollinger, W. H. (1985) Natural plant chemicals: sources of industrial and medicinal material. *Science* **228**, 1154–1160.
3 Xian-guo, H., Mocek, U., Floss, H. G., Caceres, A., Giron, L., Buckley, H., Cooney, G., Manns, J. & Wilson, B. W. (1994) An antifungal compound from *Solanum nigrescens*. *J. Ethnopharm.* **43**, 173–177.
4 Irobi, O. N. & Daramola, S. O. (1993) Antifungal activities of crude extracts of *Mitracarpus villosus* (Rubiaceal). *J. Ethnopharm.* **40**, 137–140.
5 Giron, L. M., Aguilar, G. A., Caceres, A. & Arroyo, G. L. (1988) Anticandidal activity of plants used for the treatment of vaginitis in Guatemala and clinical trial of a *Solaneum nigrescens* preparation. *J. Ethnopharm.* **22**, 307–313.
6 Hafford, C. D., Lia, S. & Clark, A. M. (1988) The antifungal activity of *Trillum grandiflorium* constituents. *J. Nat. Prod.* **51**, 94–98.
7 Watt, P. M. & Brayer-Brandwijk, M. C. (1962) *The Medicinal and Poisonous Plants of Southern and Eastern Africa*, 2nd edn. Edinburgh: E+S Livingstone.
8 Kokwaro, J. O. (1976) *Medicinal Plants of East Africa*. Kampala, Nairobi: East African Literature Bureau.
9 Schmutterer, H., Ascher, K. R. S. & Rembold, H. (eds) (1980) *Natural Pesticides from the Neem Tree (Azadirachta indica* A. Juss). Eschborn, Germany: German Agency for Technical Cooperation.
10 Hülsewede, J. W. & Dermoumi, H. (1994) Comparison of high-performance liquid chromatography and bioassay of amphotericin B in serum. *mycoses* **37**, 17–21.
11 Dermoumi, H. (1992) *In vitro* susceptibility of yeast isolates from the blood to fluconazole and amphotericin B. *Chemotherapy* **38**, 112–117.
12 Dermoumi, H. (1994) *In vitro* susceptibility of fungal isolates of clinically important specimens to itraconazole, fluconazole and amphotericin B. *Chemotherapy* **40**, 92–98.