

Screening of Venda medicinal plants for antifungal activity against *Candida albicans*

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Abstract

Crude methanol and water extracts of 32 plant species, used for the treatment of infectious diseases in Venda, were screened for *in vitro* activity against *Candida albicans* standard strain (ATCC 10231) and five clinical isolates. Water extracts of 16 plant species and methanol extracts of 11 plant species inhibited candidiasis growth. Inhibition at < 1 mg/ml, against the *C. albicans* strains tested, was observed for the methanol extracts of *Combretum molle* (root), *Piper capense* (bark), *Solanum aculeastrum* (fruits), *Syzygium cordatum* (bark) and *Zanthoxylum davyi* (bark) as well as the aqueous bark extract of *Afzelia quanzensis* and root extract of *Tabernaemontana elegans*. These results implicate that the extracts contain compounds with therapeutic potential against *C. albicans*.

Opportunistic diseases cause substantial morbidity, necessitate toxic and expensive therapies, result in hospitalisation and shorten the survival of people with HIV infection (Moore and Chaisson, 1996). A decrease in the incidence of fungal infections has been observed in countries where antiretroviral therapy is widely available, however, this is not true for countries where this treatment is not affordable (Ruhnke, 2004). In Africa, opportunistic fungus infections are increasing with the increase in number of immuno-compromised patients in the health care system. In immuno-compromised patients, *Candida albicans* is an important opportunistic fungal pathogen and the major cause of oropharyngeal candidosis (Rex et al., 1995).

In this study we screened 32 plant species used as traditional medicine in Venda, in the treatment of infectious diseases, to determine activity against *C. albicans* standard strain and five recent clinical isolates.

Plants for investigation were selected from the literature (Arnold and Gulumian, 1984 and Mabogo, 1990). Voucher specimens of the plants with collection number NH were collected and identified by Dr. N Hahn and are lodged at the Soutpansbergensis herbarium whereas those with collection number LT are lodged at the herbarium in Department of Toxicology, Onderstepoort Veterinary Institute (Pretoria). Identity of the latter specimens was confirmed by the South African National Biodiversity Institute (Tshwane).

Methanol and water extracts of the plant species were prepared by adding 1 g dried powdered plant material to 10 ml solvent and allowing the mixtures to stand overnight at room temperature, after which the supernatants were filtered. Antifungal activity was assessed using the plate-hole diffusion assay of Ieven et al. (1979) and the macro-broth tube dilution method defined by the National Committee for Clinical Laboratory Standards (NCCLS, 2002). The plate-hole diffusion assay served as a screening method to determine whether the plant extracts possessed antifungal activity or not. Amphotericin B served as positive control (Mast Group, Meyerside, UK) and all assays were performed in triplicate.

In the present study methanol and/or water extracts of 17 of the 32 plant species commonly used as traditional medicine in Venda showed anti-*Candida* activity against the standard and/or clinical isolates (Table 1). The antifungal compound, amphotericin B, inhibited growth of all strains tested (< 10 µg/ml).

MIC: minimal inhibitory concentration representing the mean value of three replicates.

– MIC not determined since the crude plant extract showed no zone of inhibition when using the plate-hole diffusion assay.

In previous studies concentrating on Venda medicinal plants the antibacterial activity was investigated, but not the antifungal activity (Obi et al., 2003 and Tshikalange et al., 2005). However, a few studies investigating a series of plants used by other populations have included single species evaluated in this study. Reports on the inhibition of *C. albicans* growth by methanolic root extracts of *Terminalia sericea* (Fyhrquist et al., 2002 and Moshi and Mbwambo, 2005) and bark extracts of *Burkea africana* (Diallo et al., 2001), support the present findings. Fyhrquist et al. (2002) noted that the methanolic extracts of the leaves of *Combretum molle* inhibited growth of *C. albicans*, a finding similar to the methanolic extracts of the root, found in the present study. Antimicrobial activity for fruit extracts of *Solanum aculeastrum* has been reported, however, activity against *C. albicans* was not determined in both studies (Wanyonyi et al., 2003 and Koduru et al., 2006).

Bioassay-guided isolations have resulted in the identification of the responsible antifungal agents of some of the plants screened. These include tannins and saponins in *Terminalia* species (Baba-Moussa et al., 1999), tannins in *Combretum* species (Kolodziej et al., 1999) and ecodysteroids in the genus *Asparagus* (Dinan et al., 2001). The inhibitory activity of *Brackenridgea zanguebarica* against the fungus, *Cladosporium cucumerinum*, has been attributed to the benzofuran derivatives (Marston et al., 1996).

Water extracts of 16 plant species and methanol extracts of 11 plant species inhibited candidiasis growth. The effectivity of the water extracts is worth noting since traditional medicine in Venda is mainly prepared as decoctions or infusions, taken orally (Arnold and Gulumian, 1984). Although more aqueous extracts inhibited *Candida* growth, the methanol extracts had the lowest MIC values. Inhibition at concentrations < 1 mg/ml, against *C. albicans* strains tested was observed for the methanol extracts of *C. molle* (root), *Piper capense* (bark), *S. aculeastrum* (fruits), *Syzygium cordatum* (bark) and *Zanthoxylum davyi* (bark) as well as the aqueous bark extract of *Afzelia quanzensis* and root extract of *Tabernaemontana elegans*. These antifungal results implicate that the extracts contain compounds with therapeutic potential against *C. albicans*.

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Plant (family)	Voucher number	Plant part	Solvent		MIC (mg/ml) <i>Candida albicans</i>				
					Clinical isolates				
				ATCC	U1	U7	M42	M43	M44
				10231					
<i>Afzelia quanzensis</i> Welw. (Fabaceae)	LT 13	Bark	Methanol	–	–	–	–	–	–
			Water	–	0.92	–	–	–	–
<i>Albizia versicolor</i> Welw. Ex Oliv. (Fabaceae)	LT 1	Bark	Methanol	–	–	–	–	–	–
			Water	–	–	–	–	–	–
<i>Asparagus falcatus</i> Thunb. (Asparagaceae)	LT 5	Root	Methanol	–	–	–	–	–	–
			Water	16.00	–	–	3.66	3.66	3.66
<i>Brackenridgea zanguebarica</i> Oliv. (Ochnaceae)	NH 682	Root	Methanol	–	–	–	–	–	–
			Water	6.50	1.48	–	–	–	–
<i>Bridelia micrantha</i> (Hochst.) Baill. (Euphorbiaceae)	LT 22	Bark	Methanol	8.00	–	–	–	–	–
			Water	10.00	–	–	2.25	–	–
<i>Burkea africana</i> Hook. (Fabaceae)	LT 15	Bark	Methanol	13.50	3.00	3.00	3.00	3.00	1.50
			Water	1.25	2.25	–	2.25	2.35	2.25
<i>Capparis tomentosa</i> Lam. (Capparaceae)	NH 1882	Root	Methanol	–	–	–	–	–	–
			Water	–	–	–	–	–	–
<i>Cassine transvaalensis</i> (Burt. Davy) Codd (Celastraceae)	LT 18	Bark	Methanol	20.20	–	–	–	–	–
			Water	–	–	–	–	–	–
<i>Combretum molle</i> R.Br. ex G. Don. (Combretaceae)	NH 1881	Root	Methanol	1.00	–	–	–	–	–
			Water	6.50	–	–	1.52	–	–
<i>Combretum paniculatum</i> Vent. (Combretaceae)	NH 1919	Root	Methanol	5.55	5.55	5.55	5.55	5.55	5.55
			Water	14.44	–	–	–	14.44	14.44

Plant (family)	Voucher number	Plant part	Solvent		MIC (mg/ml) <i>Candida albicans</i>				
					Clinical isolates				
				ATCC					
				10231	U1	U7	M42	M43	M44
<i>Dalbergia melanoxylon</i> Guill. & Perr. (Fabaceae)	LT 20	Bark	Methanol	–	–	–	–	–	–
			Water	10.00	–	–	–	–	–
<i>Dichrostachys cinerea</i> (L.) Wight & Arn. subsp <i>africana</i> Bren. & Brumm. (Fabaceae)	NH 1871	Bark	Methanol	–	–	–	–	–	–
			Water	–	–	–	–	–	–
<i>Ficus capensis</i> Thunb. (Moraceae)	LT 14	Fruit	Methanol	–	–	–	–	–	–
			Water	–	–	–	–	–	–
<i>Ficus sycomorus</i> L. (Moraceae)	LT 6	Fruit	Methanol	–	–	–	–	–	–
			Water	–	–	–	3.72	3.72	–
<i>Gyrocarpus americanus</i> Jacq. subsp <i>africanus</i> Kubitzki (Hernandiaceae)	LT 21	Root	Methanol	–	–	–	–	–	–
			Water	–	–	–	–	–	–
<i>Hexalobus monopetalus</i> (A. Rich) Engl. and Diels (Annonaceae)	LT 23	Root	Methanol	–	–	–	–	–	–
			Water	–	–	–	–	–	–
<i>Lannea schweinfurthii</i> (Engl.) Engl. (Anacardiaceae)	LT 19	Root–bark	Methanol	–	–	–	–	–	–
			Water	–	–	–	–	–	–
<i>Obetia tenax</i> (N.E.Br.) Friis (Urticaceae)	LT 9	Root	Methanol	–	–	–	–	–	–
			Water	–	–	–	–	–	–
<i>Parinari curatellifolia</i> Planch ex Benth (Chrysoblanaceae)	LT 10	Bark	Methanol	–	–	–	–	–	–
			Water	–	–	–	–	–	–
<i>Peltophorum africanum</i> Sond. (Fabaceae)	NH 1877	Root	Methanol	16.0	–	16.00	–	–	–
			Water	–	–	14.44	14.44	14.44	14.44

Plant (family)	Voucher number	Plant part	Solvent		MIC (mg/ml) <i>Candida albicans</i>				
					Clinical isolates				
				ATCC					
				10231	U1	U7	M42	M43	M44
<i>Piper capense</i> L.f. (Piperaceae)	LT 16	Bark	Methanol	0.56	0.56	0.56	0.56	0.56	0.56
			Water	4.97	4.97	2.48	4.97	1.24	1.24
<i>Rapanea melanophloeos</i> (L.) Mez. (Myrsinaceae)	LT 8	Bark	Methanol	–	–	–	–	–	–
			Water	–	–	–	–	–	–
<i>Rauwolfia caffra</i> Sond. (Apocynaceae)	LT 7	Bark	Methanol	–	–	–	–	–	–
			Water	–	–	–	–	–	–
<i>Rothmannia capensis</i> Thunb. (Rubiaceae)	LT 26	Fruit	Methanol	–	–	–	–	–	–
			Water	–	–	–	–	–	–
<i>Solanum aculeastrum</i> Dun. (Solanaceae)	LT 17	Fruit	Methanol	1.50	0.68	0.68	0.68	0.04	0.04
			Water	8.50	1.88	1.88	1.88	1.88	1.88
<i>Solanum panduriforme</i> E. Mey (Solanaceae)	LT 28	Fruit	Methanol	–	–	–	–	–	–
			Water	–	–	–	–	–	–
<i>Strychnos decussate</i> (Pappe) Gilg. (Loganiaceae)	LT 29	Bark	Methanol	–	–	–	–	–	–
			Water	–	–	–	–	–	–
<i>Syzygium cordatum</i> Hochst. (Myrtaceae)	NH 1880	Bark	Methanol	3.75	–	0.83	1.68	1.68	0.83
			Water	2.50	–	1.11	1.11	1.11	1.11
<i>Tabernaemontana elegans</i> Stapf. (Apocynaceae)	NH 1920	Root	Methanol	–	–	–	–	–	–
			Water	3.30	0.82	0.41	0.41	0.54	0.41
<i>Terminalia sericea</i> Burch. Ex DC. (Combretaceae)	NH 1878	Root	Methanol	2.50	1.10	–	1.10	1.10	–
			Water	2.00	–	2.65	2.65	2.65	2.65

Plant (family)	Voucher number	Plant part	Solvent		MIC (mg/ml) <i>Candida albicans</i>				
				ATCC	Clinical isolates				
				10231	U1	U7	M42	M43	M44
Plant (family)	Voucher number	Plant part	Solvent		MIC (mg/ml) <i>Candida albicans</i>				
				ATCC	Clinical isolates				
				10231	U1	U7	M42	M43	M44
<i>Zantedeschia aethiopica</i> (L.) Spreng. (Araceae)	NH 1923	Root	Methanol	–	–	–	–	–	–
			Water	–	–	–	–	–	–
<i>Zanthoxylum davyi</i> (I. Verd.) P.G. Waterman (Rutaceae)	LT 4	Bark	Methanol	0.50	–	–	–	–	–
			Water	3.25	–	–	–	–	–