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Antibacterial and antifungal activity of traditional medicinal plants used against venereal diseases in South Africa

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Abstract

Aqueous, ethanolic and ethyl acetate extracts of 13 plants used in South Africa for the treatment of venereal diseases were screened for antibacterial and antifungal activity. Among the plants tested, *Gunnera perpensa*, *Harpephyllum caffrum*, *Hypoxis latifolia* and *Ledebouria ovatifolia* showed the best antibacterial activity. The aqueous extracts of *Gunnera perpensa* and *Harpephyllum caffrum* were most active against all the tested bacteria. In antifungal screening, good activity was shown by the ethanolic extracts of *Bersama lucens* and *Harpephyllum caffrum*. Only in the case of *Harpephyllum caffrum* did aqueous extracts have activity against *Candida albicans*.

Keywords: Venereal diseases; Antibacterial; Antifungal; Traditional medicine

1. Introduction

Throughout the history of mankind, many infectious diseases have been treated with plant extracts. Venereal infections are one such disease and are regarded as conditions that are highly responsive to traditional treatment. Venereal diseases, also referred to as sexually transmitted diseases (STDs), are infections that are usually acquired during sexual intercourse. WHO estimated that 340 million new cases of STDs occurred globally in 1999 (WHO, 2001), with at least, 111 million occurring in young people under 25 years of age. The largest number of new infections occurred in South and Southeast Asia, followed by sub-Saharan Africa and Latin America and the Caribbean (WHO, 2001). The most common or most reported venereal diseases are gonorrhoea and syphilis. These diseases are caused by pathogens that can thrive in warm, moist dark areas including the genital area, anus and mouth. These pathogens include bacteria, fungi and viruses and they produce a variety of manifestations. Common pathogens include the bacteria *Escherichia coli*, *Staphylococcus saprophyticus*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Enterobacter* species, and the fungus *Candida albicans*. Although other microorganisms do not cause venereal diseases, those with compromised immune systems such as occurs with HIV+ patients, have been known to get skin infections caused by *Micrococcus luteus* and *Staphylococcus aureus*. Moreover, if venereal diseases are left untreated, they can lead to other diseases, e.g. conjunctivitis which is caused by *Bacillus subtilis*.

There is substantial evidence demonstrating that the presence of other venereal diseases increases the chances of both acquiring and transmitting HIV (Wasserheit, 1992; Fleming and Wasserheit, 1999) and they may be partly responsible for the growing HIV epidemic in Africa (Grosskurth et al., 1995). A number of studies undertaken (Grosskurth et al., 1995; Gilson et al., 1997; Mayaud et al., 1997) have shown that a proper therapy for other STDs, be they ulcerative or non-ulcerative, is an important strategy for HIV control.

The aims of this study were to screen medicinal plants used by South African traditional healers for the treatment of venereal diseases for the presence of antibacterial and antifungal activities.

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2. Materials and methods

2.1. Plant collection

Some plants were collected through consultations with a traditional healer and herbalist from the Eastern Cape and others were collected on the basis of a literature survey from the KwaZulu-Natal region of South Africa. Voucher specimens for each plant were deposited at the herbarium of the University of KwaZulu-Natal, Pietermaritzburg (Table 1).

2.2. Plant extraction

Plant material was dried in an oven at 50 °C and ground to fine powders using a blender. Three separate samples of 1 g each were extracted with 10 ml water, 100% ethanol and ethyl acetate, respectively. Extraction was performed by sonication for 30 min in a Julabo ultrasound bath. The plant extracts were filtered through Whatman No. 1 filter paper into pill vials. The filtrates were taken to total dryness in front of a fan until a constant dry weight of each extract was obtained. This was done by weighing plant extracts daily. The residues were stored at 10 °C.

2.3. Antibacterial screening

The following bacteria were used: *Bacillus subtilis* (ATCC No. 6051), *Escherichia coli* (ATCC No. 11775), *Klebsiella pneumoniae* (ATCC No. 13883), and *Staphylococcus aureus* (ATCC No. 12600).

The microplate method of Eloff (1998) was used to determine the minimal inhibitory concentration (MIC) values for plant extracts with antibacterial activity. Residues of plant extracts were dissolved at 50 mg/ml with the extracting solvents in the case of water and ethanol. Ethyl acetate extracts were dissolved in ethanol. All extracts were initially tested at 12.5 mg/ml in 96-well microplates and serially diluted two-fold to 0.38 μg/ml, after which 100 μl bacterial culture were added to each well. The antibiotic neomycin was included as reference in each assay. Extract-free solution was used as a blank control. The microplates were incubated overnight at 37 °C. As an indicator of bacterial growth, 40 μl *p*-iodonitrotetrazolium violet (INT) dissolved in water were added to the wells and incubated at 37 °C for 30 min. MIC values are recorded as the lowest concentration of the extract that completely inhibited bacterial growth, i.e. a clear well. The colourless tetrazolium salt acts as an electron acceptor and is reduced to a red-coloured formazan product by biologically active organisms (Eloff, 1998). Where bacterial growth was inhibited, the solution in the well remained clear after incubation with INT.

2.4. Antifungal screening

A standard strain of *Candida albicans* (ATCC 10231) was obtained from the South African Bureau of Standards. The water extract residues were redissolved in water and the organic solvent extract residues were dissolved in dimethyl sulfoxide

Table 1
South African medicinal plants used in the treatment of venereal diseases

Family/botanical name/voucher numbers/collection site[a]	Vernacular names in Xhosa (X) and Zulu (Z)	Plant part used	Traditional uses and administration
Amaryllidaceae/*Cyrtanthus obliquus* Ait./BUWA 6 UKZN/Eastern Cape	Umathunga (X)	Bulbs	Decoctions taken for venereal diseases
Anacardiaceae/*Harpephyllum caffrum* Bernh. ex Krauss/BUWA 8 UKZN/Eastern Cape	Umgwenya (X)	Stem bark	Decoctions taken orally for gonorrhoea
Asclepiadaceae/*Xysmalobium undulatum* (L.) Ait.f./BUWA 12 UKZN/Eastern Cape	Ishongwe (Z)	Roots	Decoctions taken for syphilis
Caesalpinaceae/*Albizia gummifera* (J.F. Gmel.) C.A. Smith/BUWA 1 UKZN/KwaZulu-Natal	Umgadankawu (Z)	Stem bark	Decoctions used for venereal diseases
Capparidaceae/*Capparis tomentosa* Lam./BUWA 5 UKZN/Eastern Cape	Intsihlo (X)	Roots	Infusion used as steam bath against lice
Gunneraceae/*Gunnera perpensa* Linn./BUWA 7 UKZN/KwaZulu-Natal	Iphuzi (Z)	Roots	Decoctions used for gonorrhoea, syphilis and urinary infections
Hyacinthaceae/*Bowiea volubilis* Harv. ex Hook./BUWA 4 UKZN/KwaZulu-Natal	Umagaqana (X)	Bulbs	Poultice for the treatment of syphilis
Hyacinthaceae/*Ledebouria ovatifolia* (Bak.) Jessop/BUWA 11 UKZN/KwaZulu-Natal	Icubudwana (Z)	Bulbs	Decoctions taken orally for venereal diseases
Hypoxidaceae/*Hypoxis latifolia* Hook./BUWA 9 UKZN/Eastern Cape	Ilabathela (X)	Roots and corm	Decoctions used as steam bath against lice and taken orally for urinary infections
Liliaceae/*Albuca nelsonii* N.E. Br./BUWA 2 UKZN/Eastern Cape	Inqwebeba (X/Z)	Bulbs	Decoctions used for gonorrhoea
Melianthaceae/*Bersama lucens* (Hochst.) Szyszyl./BUWA 3 UKZN/KwaZulu-Natal	Isindiyandiya/Undiyaza (Z)	Stem bark	Decoctions used against lice
Ranunculaceae/*Knowltonia bracteata* Harv. ex Zahlbr./BUWA 10 UKZN/Eastern Cape	Umvuthuza (X)	Roots and leaves	Decoctions used as steam bath and/or taken orally against lice
Rutaceae/*Zanthoxylum capense* (Thunb.) Harv./BUWA 13 UKZN/Eastern Cape	Umlungumabele (X)	Leaves	Infusions used for syphilis

[a] Voucher numbers: UKZN, Herbarium of the University of KwaZulu-Natal Pietermaritzburg.

(DMSO). All extracts were dissolved to a concentration of 100 mg/ml. A modification of the NCCLS proposed method (M27-P) broth microdilution test was performed (Espinel-Ingroff and Pfaller, 1995). Four millilitres of sterile saline were added to approximately 400 μl of 24 h old *Candida* cultures. The absorbance was read at 530 nm and adjusted with sterile saline to match that of a 0.5 McFarland standard solution. From the prepared stock yeast culture, a 1:1000 dilution with broth (e.g. 10 μl stock yeast culture: 10 ml broth) was prepared.

One hundred microlitres of broth were added to each well of a 96-well microplate. One hundred microlitres of the water extract were added to well (A) and serially diluted from (A) by taking 100 μl into (B). This two-fold dilution was continued down the plate and 100 μl from the last well (H) were discarded. In the case

Table 2
Screening of crude extracts from South African medicinal plants for antibacterial and antifungal activity (MIC recorded in mg/ml)

Plant	Plant part extracted	Extraction solvent	Extract yield (mg)	Bacteria tested				*Candida albicans*
				B.s. MIC	E.c. MIC	K.p. MIC	S.a. MIC	MIC
Albizia gummifera	Bark	W	103	>12.5	>12.5	>12.5	>12.5	>25
		E	18	3.125	3.125	3.125	0.39	3.125
		EA	16	1.56	3.125	3.125	1.56	>6.25
Albuca nelsonii	Bulb	W	222	>12.5	>12.5	>12.5	>12.5	12.5
		E	15	6.25	3.125	3.125	6.25	6.25
		EA	11	3.125	3.125	3.125	6.25	6.25
Bersama lucens	Bark	W	45	>12.5	>12.5	>12.5	>12.5	12.5
		E	17	3.125	3.125	3.125	3.125	0.78
		EA	22	–	–	–	–	3.125
Bowiea volubilis	Bulb	W	766	>12.5	>12.5	>12.5	>12.5	>25
		E	24	3.125	3.125	3.125	3.125	–
		EA	14	–	–	–	–	3.125
Capparis tomentosa	Root	W	96	>12.5	>12.5	6.25	>12.5	>25
		E	14	3.125	3.125	3.125	6.25	6.25
		EA	26	6.25	6.25	3.125	12.5	3.125
Cyrtanthus obliquus	Bulb	W	13	>12.5	12.5	>12.5	>12.5	>25
		E	155	6.25	6.25	6.25	6.25	6.25
		EA	22	1.56	3.125	3.125	1.56	6.25
Gunnera perpensa	Root	W	57	12.5	0.78	0.78	0.78	25
		E	84	3.125	1.56	1.56	6.25	–
		EA	19	3.125	6.25	6.25	3.125	6.25
Harpephyllum caffrum	Bark	W	132	0.39	1.56	1.56	0.39	1.56
		E	108	0.098	0.78	1.56	0.195	0.78
		EA	12	3.125	3.125	3.125	3.125	3.125
Hypoxis latifolia	Root	W	26	>12.5	>12.5	>12.5	>12.5	–
		E	28	3.125	3.125	3.125	3.125	–
		EA	12	3.125	6.25	6.25	6.25	–
	Tuber	W	108	>12.5	1.56	0.78	1.56	12.5
		E	88	6.25	6.25	3.125	12.5	6.25
		EA	13	–	–	–	–	6.25
Knowltonia bracteata	Leaf	W	181	>12.5	3.125	3.125	3.125	12.5
		E	77	3.125	1.56	1.56	3.125	–
		EA	9	3.125	3.125	3.125	3.125	–
	Root	W	130	>12.5	1.56	1.56	1.56	–
		E	104	6.25	6.25	6.25	6.25	–
		EA	19	3.125	1.56	1.56	3.125	–
Ledebouria ovatifolia	Bulb	W	144	12.5	6.25	12.5	12.5	25
		E	41	0.78	3.125	3.125	1.56	3.125
		EA	35	0.39	1.56	1.56	0.78	3.125
Xysmalobium undulatum	Root	W	121	>12.5	12.5	12.5	12.5	12.5
		E	130	3.125	3.125	3.125	3.125	–
		EA	44	–	–	–	–	–
Zanthoxylum capense	Leaf	W	76	>12.5	12.5	12.5	>12.5	–
		E	49	6.25	3.125	3.125	6.25	–
		EA	41	–	–	–	–	–
Reference[a]				9.8×10^{-2}	3.9×10^{-1}	3.9×10^{-1}	2.0×10^{-1}	0.195

Extract: W, water; E, ethanol; EA, ethyl acetate. Bacteria: B.s., *Bacillus subtilis*; E.c., *Escherischia coli*; K.p., *Klebsiella pneumoniae*; M.l., *Micrococcus luteus*; S.a., *Staphylococcus aureus*. Dif: results obtained in the disc-diffusion assays. Antibacterial activity is expressed as the ratio of the inhibition diameter around the extract to the inhibition zone around the reference neomycin antibiotic. 0, indicates no activity, i.e. no inhibition zone around the extract discs. MIC: results obtained in the dilution assays. Antibacterial and antifungal activity is expressed as the minimum inhibitory concentration (mg/ml). –, extract not tested.

[a] Neomycin was used as a reference for antibacterial activity, whereas Amphotericin B was used for antifungal activity.

of organic solvent extracts 25 μl of the extracts were added to 175 μl broth and serially diluted. Three replicates were prepared for each extract. All the wells were then filled with 100 μl of stock yeast culture. Amphotericin B was used as a reference for this experiment and the following controls were prepared: wells containing broth only, fungal strain with no extract, and serial dilutions of Amphotericin B with the fungi at the recommended inhibitory concentrations. The plates were then read at 630 nm in an ELISA reader, covered with parafilm and incubated at 33 °C overnight, whereafter their absorbance was reread.

3. Results and discussion

The results for the general screening for antibacterial activity are shown in Table 2. A total of 45 extracts belonging to 13 plant species were investigated.

MIC values of active extracts are shown in Table 2. Among the plants tested, *Gunnera perpensa*, *Harpephyllum caffrum*, *Hypoxis latifolia* and *Ledebouria ovatifolia* showed the best antibacterial activity. Poor inhibitory activity was detected against Gram-negative bacteria with most of the plant extracts tested. The aqueous and ethanolic extracts of *Gunnera perpensa* had the highest inhibitory activity against both the Gram-negative bacteria. The MIC values in water extracts were high for both *Escherichia coli* and *Klebsiella pneumoniae* (0.78 mg/ml), and one Gram-positive bacterium *Staphylococcus aureus* (0.78 mg/ml).

Harpephyllum caffrum plant extracts showed the best MIC values for the ethanolic extracts, where the extract showed activity against the four bacterial strains used. Good antibacterial activity was also detected with aqueous extracts. The MIC values in aqueous extracts were high, especially against *Bacillus subtilis* and *Staphylococcus aureus* (0.39 mg/ml). The ethyl acetate extract showed slight activity. *Harpephyllum caffrum* is reported to contain phenolic compounds which may be responsible for its biological activity (El Sherbeiny and El Ansari, 1976).

Hypoxis latifolia aqueous corm extracts exhibited very good MIC values against *Klebsiella pneumoniae* (0.78 mg/ml).

The results of the antifungal screening are presented in Table 2. Among the plants tested, the ethanolic extracts of *Bersama lucens* and *Harpephyllum caffrum* displayed the best activity against *Candida albicans* with an MIC value of 0.78 mg/ml. The candidal strain was found to be resistant to most plant extracts screened.

Most of the plant extracts tested showed some level of antibacterial activity. This supported the observations made by other investigators that plant pathogenic fungi are more resistant to plant extracts than plant pathogenic bacteria (Leven et al., 1979; Naqvi et al., 1991; Heisey and Gorham, 1992). Heisey and Gorham (1992) observed that 13 extracts inhibited the growth of bacteria, while only 5 extracts inhibited fungal growth.

It is worth noting that only in the case of *Gunnera perpensa* and *Harpephyllum caffrum* did aqueous extracts have activity. Drugs used by traditional healers are mostly prepared with water, as the healers do not usually have access to other more lipophilic solvents. This is of concern, as it is possible that healers do not extract all the active compounds that might be present in the plant. Dosage is important with regard to which solvent is being used. If water is used, the dosage would be higher, whereas the same dosage using a lipophilic solvent may be dangerous.

The screening of crude extracts made from medicinal plants have shown that some of the screened plants are potentially rich sources of antibacterial and antifungal agents. Determining the antibacterial and antifungal properties of plants used in traditional medicine is helpful to the rural communities and informal settlements. Work is currently being undertaken to isolate the active compound(s) by bioassay-guided fractionation from the species that showed high inhibitory activity during screening.

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