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Zulu medicinal plants with antibacterial activity

Jonathan E. Kelmanson, Anna K. Jäger *, Johannes van Staden

Research Center for Plant Growth and Development, School of Botany and Zoology, University of Natal Pietermaritzburg, Private Bag X01, Scottsville, 3209, South Africa

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Abstract

Aqueous, methanolic and ethyl acetate extracts of 14 plants used in traditional Zulu medicine for treatment of ailments of an infectious nature were screened for antibacterial activity. Most of the activity detected was against Gram-positive bacteria. Tuber bark extracts of *Dioscorea sylvatica* had activity against Gram-negative *Escherichia coli* and extracts of *Dioscorea dregeana*, *Cheilanthes viridis* and *Vernonia colorata* were active against *Pseudomonas aeruginosa*. The highest antibacterial activity was found in extracts of *C. viridis*, *D. dregeana*, *D. silvatica*, *Melianthus comosus* and *V. colorata*. In general, methanolic extracts exhibited higher activity than aqueous and ethyl acetate extracts.

Keywords: Antibacterial activity; Medicinal plants; Plant extracts; South Africa; Traditional medicine

1. Introduction

Traditional healing is widely practised in South Africa. It is estimated that 80% of the black population is consulting with traditional healers (Jäger et al., 1996). Recently, some major Medical Aid Schemes have included traditional healing in their cover. This further raises the need for a scientific evaluation of the methods used by these healers. It is necessary to establish the efficiency and safety of traditional treatments.

Previously, 21 species used medicinally in South Africa were screened for antibacterial activity (Rabe and van Staden, 1997). *Helichrysum* species used to treat circumcision wounds have been investigated for antimicrobial activity and an antimicrobial compound, 3,5,7-trihydroxyflavone, has been isolated from *Helichrysum aureonitens* (Meyer and Afolayan, 1995; Meyer and Dilika, 1996; Afolayan and Meyer, 1997; Dilika et al., 1997).

In this study, extracts made of plants used in traditional medicine for various ailments such as sores, wounds and burns, which could indicate a possible antimicrobial property, were tested for antibacterial activity.

* Corresponding author.
E-mail address: jager@botany.unp.ac.za (A.K. Jäger)

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2. Methodology

2.1. Ethnological data

The information on plant usage in this paper was based on literature surveys, and data collection previously done by Hutchings et al. (1996) by consulting with practicing Zulu traditional healers, Inyangas and Sangomas, in the Pietermaritzburg and Durban areas.

2.2. Plant material

The botanical names and families, Zulu names, voucher specimen numbers, collection sites and traditional usage for the plant material are given in Table 1. The plant material was dried in an oven at 50°C for 48 h, whereafter it was ground to a fine powder and stored in airtight glass containers in the dark until extraction.

2.3. Extraction of plant material

Dried plant material (1 g) was extracted with 10 ml water, methanol or ethyl acetate for 30 min in an ultra sound bath. The extracts were then filtered using a Büchner funnel and the filtrates taken to dryness in front of a fan. The extracts were resuspended in water or methanol to yield 100 mg residue/ml solvent.

2.4. Disc-diffusion assay

The plant extracts were tested for antibacterial activity in the disc-diffusion assay (Rasoanaivo and Ratsimamanga-Urverg, 1993), using seven strains of bacteria, *Bacillus subtilis*, *Escherichia coli*, *Kleibsiella pneumoniae*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Staphylococcus epidermidis* obtained from the bacterial collection of the Microbiology Department, University of Natal, Pietermaritzburg. Bacteria were maintained on Mueller–Hinton (MH) nutrient agar at 4°C. Molten MH agar (10 ml) was inoculated with a broth culture (1 ml, containing 10^6–10^8 bacteria/ml) of the respective bacterial strains and poured over base plates containing 10 ml MH agar in sterile 9 cm Petri dishes. Ten microliters (1 mg) of plant extract were applied to a sterile filter paper disc (Whatman No. 3, 6 mm in diameter). The discs were allowed to dry before being placed onto the seeded top layer of the agar plates. Each plate contained four paper discs with plant extract and a disc with a neomycin control (2 μg). Each extract was tested in quadruplicate. The plates were incubated at 37°C for 12 h, whereafter inhibition zones were recorded. Antibacterial activity was expressed as the ratio of the inhibition zone (mm) produced by the plant extract and the inhibition zone caused by the reference (Vlietinck et al., 1995).

2.5. Minimal inhibitory concentration

Minimal inhibitory concentrations (MIC) were determined for plant extracts showing antibacterial activity in the disc diffusion assay. The agar dilution method (Sahm and Washington II, 1991) was employed for *B. subtilis*, *E. coli*, *M. luteus*, *P. aeruginosa*, *S. aureus* and *S. epidermidis* with two-fold serial dilution of plant extracts from 8.0 to 0.25 mg/ml. MIC values were taken as the lowest concentration of extract that completely inhibited bacterial growth after 18 h of incubation at 37°C.

3. Results and discussion

Samples of 14 plants used for various ailments that are normally caused by bacterial infection were collected (Table 1). Voucher specimens are lodged in The Herbarium, School of Botany and Zoology, University of Natal, Pietermaritzburg. Water, methanol and ethyl acetate extracts were made from the different samples of plant parts of the species collected. A total of 78 extracts were tested against seven bacterial strains, four Gram-positive and three Gram-negative. The results of the screening are given in Table 2. No antibacterial activity was associated with the extracts from *Crinum bulbispermum* (upper and lower leaves), *Equisetum ramosissimum* (leaves), *Heteropogon contortus* (leaves), *Leonotis leonurus* (leaves), *Leonotis ocymifolia* (leaves), *Senecio serratuloides* (leaves, stems, roots), *Sida dregei* (leaves, stems,

Table 1
Zulu medicinal plants investigated for antibacterial activity[a]

Botanical names and family	Zulu names	Voucher specimen	Collection sites	Traditional usage
C. viridis (Forssk.) Swartz Adiantaceae	Ikhambi lesilonda	KELMANSON 7UN	1	Household remedy used for treatment of sores and other skin complaints
C. bulbispermum (Burm. f.) Milne-Redh. & Schweick. Amaryllidaceae	Umduze	KELMANSON 2UN	3	Roasted bulbs are applied to aching joint, rheumatism, varicose veins and backache and are used as poultices for septic sores, also used for colds
D. dregeana (Kunth) Dur. and Schinz Dioscoreaceae	Ilabatheka		9	Water heated in the scooped out tuber is used to treat sores and wounds in humans and animals
D. sylvatica (Kunth) Eckl. var. *paniculata* (Dümmer) Burkill Dioscoreaceae	Ilabetheka	KELMANSON 6UN	1	as above
E. ramosissimum Desf. Equisetaceae	Ishobalehashi		5	Sap from plant is used to relieve tooth ache, and applied to the wounds after tooth extraction.
H. contortus (L.) Roem. & Shult. Poaceae		KELMANSON 10UN	2	Used for treatment of burns, wounds and rheumatism
L. leonurus (L.) R. Br. Laminaceae	Imunyamunya	KELMANSON 1UN	6	Used as an emetic for snakebite. Infusions of leaves are taken for dysentery and colds.
L. ocymifolia (Burm. f.) Iwarsson Laminaceae	Umunyane	KELMANSON 5UN	3	Pounded leaf infusion is taken for colds
M. comosus Vahl Melianthaceae	Ibonya	KELMANSON 8UN	3	Poultices are applied to bad sores and for snake bites
R. capensis Thunb. Rosaceae	Omkhulu		8	Used for treatment of burns, wounds and rheumatism
S. serratuloides DC. var. *serratuloides* Asteraceae	Insukumbili	KELMANSON 3UN	4	Tea made from the leaves is taken in case of infections while leaves are applied directly to purulent sores. Leaf decoction is taken as a blood purifier in case of skin eruptions, while powdered leaves or roots are applied to burns or sores
S. dregei Burtt Davy Malvaceae	Umdiza wethafa	KELMANSON 11UN	7	Leaf paste is applied to sores
V. colorata (Willd.) Drake Asteraceae	Ibozane	KELMANSON 4UN	4	Roots are used as tonics and to treat boils
Z. davyi (Verdoorn) Waterm. Rutaceae	Amabelintombi	KELMANSON 9UN	3	Leaves are used to heal sores as a poultice

[a] Collection sites: 1, Garden of the Botany Department, University of Natal Pietermaritzburg; 2, Ukalinga Research Farm, University of Natal Pietermaritzburg; 3, Botanical Gardens, Pietermaritzburg; 4, Silverglen Nursery, Durban; 5, Umgeni Valley Nature Reserve, Howick; 6, Queen Elizabeth Park, Pietermaritzburg; 7, Dargle District, Natal Midlands; 8, Crammond; 9, East Street Muthi market, Pietermaritzburg; 10, Voucher specimens held in The Herbarium, University of Natal, Pietermaritzburg.

Table 2
Results of antibacterial test of Zulu medicinal plants

Botanical name	Plant part	Solvent	Test results[a]						
			S.e	S.a	M.l	P.a	E.c	B.s	K.p
C. viridis	Leaves	Water	0	0	0	0	0	0	0
		Methanol	0	0.77	0.46	0.46	0	0.73	0
		Ethyl acetate	0	0	0	0	0	0	0
	Stems	Water	0	0	0	0	0	0	0
		Methanol	0.63	0.65	0.57	0.36	0	0.69	0
		Ethyl acetate	0	0	0	0	0	0	0
D. dregeana	Tuber	Water	0	0	0	0	0	0	0
		Methanol	0	0.81	0.45	0.39	0	0	0
		Ethyl acetate	0	0	0	0	0	0	0
D. sylvatica	Roots	Water	0	0	0	0	0	0	0
		Methanol	0	0	0	0	0	0.55	0
		Ethyl acetate	0	0	0	0	0	0	0
	Tuber	Water	0	0	0	0	0	0	0
		Methanol	0	0	0.49	0	0	0	0
		Ethyl acetate	0	0	0	0	0	0	0
	Tuber bark	Water	0	0	0	0	0	0	0
		Methanol	0.52	BacStat	0.59	0	0.54	0.45	0
		Ethyl acetate	0.40	BacStat	0.68	0	0	0.41	0
M. comosus	Leaves	Water	0	0	0.85	0	0	0.64	0
		Methanol	0.46	0.95	0.98	0	0	0.43	0
		Ethyl acetate	0	0	0	0	0	0	0
	Stems	Water	0	0	0.63	0	0	0	0
		Methanol	0.47	0.60	0.50	0	0	0.65	0
		Ethyl acetate	0	0	0	0	0	0	0
R. capensis	Leaves	Water	0	0	0	0	0	0	0
		Methanol	0	0	0	0	0	0	0
		Ethyl acetate	0	0	0	0	0	0	0
	Stems	Water	0	0	0	0	0	0	BacStat
		Methanol	0	0	0	0	0	0	0
		Ethyl acetate	0	0	0	0	0	0	0
	Roots	Water	0	0	0	0	BacStat	0	BacStat
		Methanol	0	0	0	0	0	0	0
		Ethyl acetate	0	0	0	0	0	0	0
V. colorata	Leaves	Water	0	0	0	0	0	0	0
		Methanol	BacStat	1.21	0.57	0.62	BacStat	0.77	BacStat
		Ethyl acetate	BacStat	1.36	0.52	0.63	0	0.72	BacStat
	Stems	Water	0	0	0	0	0	0	0
		Methanol	BacStat	0.68	0	0	0	BacStat	BacStat
		Ethyl acetate	BacStat	0.88	0	0	0	BacStat	BacStat
	Roots	Water	0	0	0	0	0	0	0
		Methanol	0	BacStat	0	0	0	0	BacStat
		Ethyl acetate	0	0.63	0	0	0	BacStat	BacStat

[a] The antibacterial activity is expressed as the ratio of the inhibition zone of the extract (1 mg/ml) to the inhibition zone of the reference (neomycin 200 μg/ml). Bacteria: S.e: *S. epidermis*; S.a: *S. aureus*; M.l: *M. luteus*; P.a: *P. aeruginosa*; E.c: *E. coli*; B.s: *B. subtilis*; K.p: *K. pneumoniae*.

Table 3
Minimal inhibitory concentration (MIC) of plant extracts with antibacterial activity(Effective concentration of extract against a particular bacterium in mg ml^{-1})

Plant name	Plant part	Extract	*S. aureus*	*S. epidermis*	*B. subtilis*	*P. aeruginosa*	*E. coli*	*M. luteus*
C. viridis	Leaves	MeOH	4.0	–	8.0	4.0	–	4.0
D. sylvatica	Tuber bark	MeOH	–	2.0	2.0	–	4.0	4.0
M. comosus	Leaves	MeOH	2.0	4.0	8.0	–	–	4.0
V. colorata	Leaves	MeOH	1.0	–	2.0	4.0		4.0
		EtOAc	0.5	–	2.0	4.0		4.0
Neomycin			4.0×10^{-3}	1.2×10^{-4}	1.2×10^{-4}	2.5×10^{-3}	5.0×10^{-4}	2.0×10^{-3}

roots) and *Zanthoxylum davyi* (leaves, bark). These results were therefore omitted from Table 2. Antibacterial activity was found in the methanolic extracts of the leaves and stems of *Cheilanthes viridis*, and *Dioscorea dregeana* tubers, both methanol and ethyl acetate extracts of *Dioscorea sylvatica* tuber bark, water and methanol extracts of leaves and stems of *Melianthus comosus*, and methanol and ethyl acetate extracts of leaves, stems and roots of *Vernonia colorata*. In general, these extracts were most active against Gram-positive bacteria, though tuber bark extract of *D. sylvatica* was active against *E. coli*, while extracts of *C. viridis*, *D. dregeana* and *V. colorata* were active against *P. aeruginosa*. Extracts of *Rothmannia capensis* and *V. colorata* had bacteriostatic activity against both *E. coli* and *K. pneumoniae*. These results are in line with those from previous screenings of medicinal plants for antimicrobial activity, where most of the active plants showed activity against Gram-positive strains only (Vlietinck et al., 1995; Rabe and van Staden, 1997). MIC was determined for extracts of the four most active plants, *C. viridis*, *D. sylvatica*, *M. comosus* and *V. colorata* (Table 3). Since MIC values are relatively high, but active compounds in the extracts may be present in low concentrations, further investigation using bioassay-guided fractionation will be justified for a number of species.

The fern *Cheilanthes viridis* has not been investigated before, but another species *Cheilanthes pteridioides* from Greece was found to have antibacteriophage properties (Skaltsa et al., 1991). In spite of the long use of *Dioscorea* species in medicine as a source of steroids, little work has been done on the antimicrobial properties of this genus. Two compounds with antifungal activity, 2,5-dihydroxy-4-methoxy-9,10-dihydrophenanthrene and 7-hydroxy-2,4,6-trimethoxyphenanthrene, were isolated from the tuber of *Dioscorea rotundata*, but were absent from the flesh (Ogundana et al., 1984). In this study the tuber bark of *D. sylvatica* exhibited higher antibacterial activity than any other plant part. It seems logical that the tuber bark should possess the highest antifungal and antibacterial activity as this is the part of the plant that is in constant contact with soil.

No pharmacological studies have been reported on *M. comosus* or other members of the genus. *M. comosus* is, however, known to be toxic and have caused human death (Watt and Breyer-Brandwijk, 1962), which could be due to bufadienolides, among them, melianthusigenin (Anderson and Koekemoer, 1969).

Antibacterial activity is well documented in the genus *Vernonia*, such as in *Vernonia karaguensis*, *Vernonia thomsoniana* (Mungarulire, 1990) and in *Vernonia venosa* and *Vernonia adoensis* (Al Magboul et al., 1988). Six sesquiterpene lactones isolated from *Vernonia amygdalina* strongly inhibited *B. subtilis* and *M. luteus*, but not the Gram-negative *E. coli* and *Agrobacterium tumefaciens* (Jisaka et al., 1993). However, sesquiterpene lactones from *V. amygdalina* has subsequently been found to possess activity against Gram-negative bacteria as well as against fungi (Al Magboul et al., 1997). Extracts of *Vernonia kotschyana* exhibited antibacterial activity against three Gram-positive and four Gram-negative bacteria. Fractions exhibiting antibacterial activity tested positive for alkaloids (Deeni and Hussain, 1994). A sesquiterpene lactone isolated from *Vernonia nitidula* displayed marked

inhibitory activity against several pathogenic bacteria, both Gram-positive and negative (Montanaro et al., 1996); these results support the fact that *V. colorata* investigated in this study also showed antibacterial activity.

It is worth noting that only in the case of *M. comosus* did aqueous extracts have activity. Drugs used by traditional healers are mostly prepared by some form of extraction 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 compound(s) that might be present in the plant and consequently the prepared drug would not contain all the pharmacologically active compounds. This study has identified six plants with some antibacterial activity. This finding lends some support to traditional knowledge and can serve as a basis for selecting the most active medicinal plants to use in traditional medicine practices in the future.

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