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Journal of Ethnopharmacology 56 (1997) 81–87

Journal of ETHNO-PHARMACOLOGY

Antibacterial activity of South African plants used for medicinal purposes

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Received 10 September 1996; received in revised form 12 January 1997; accepted 21 January 1997

Abstract

Crude extracts from 21 South African medicinal plants, traditionally used for ailments of an infectious or septic nature, were screened for in vitro antibacterial activity using the agar diffusion and dilution methods. Almost all the activity exhibited was against Gram-positive bacteria, with 12 of the 21 plant species tested showing some activity against *Bacillus subtilis*. Only the *Warburgia salutaris* methanol extract inhibited the growth of *Escherichia coli*. None of the extracts had any activity against *Klebsiella pneumoniae*. The highest activity was found in the methanol extracts from *Bidens pilosa*, *Psidium guajava*, *Artemisia afra* and *Warburgia salutaris*. The majority of the antibacterial activity was present in the methanolic, rather than the aqueous extracts. © 1997 Elsevier Science Ireland Ltd.

Keywords: Antibacterial activity; Plant extract; Screening; South Africa; Traditional medicine

1. Introduction

Traditional medicine is practised by a large proportion of the South African population for their physical and psychological health needs. Medicinal plants have become the focus of intense study recently in terms of conservation and as to whether their traditional uses are supported by actual pharmacological effects or merely based on folklore (Cunningham, 1988; Locher et al., 1995; Williams, 1996; Jäger et al., 1996). With the increasing acceptance of traditional medicine as an alternative form of health care, the screening of medicinal plants for active compounds is very important.

The purpose of this study was to investigate South African plants for potential antibiotic activity by preliminary bioassay screening. The selection of plants for evaluation was based on traditional use (Watt and Breyer-Brandwijk, 1962; Gelfand et al., 1985; Pujol, 1990; Mander et al., 1995) for treatment of symptoms such as wounds,

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PII S0378-8741(96)01515-2

Table 1
South African medicinal plants investigated for antibacterial activity

Family and botanical name (voucher specimen[a])	Plant part[b]	Administration/traditional uses
AMARYLLIDACEAE		
Boophane disticha (L.f.) Herb. (JÄGER7UN)	BL	Bulb scales applied to wounds, boils, sores, septic cuts and inflammatory conditions (Watt and Breyer-Brandwijk, 1962; Gelfand et al., 1985)
Crinum macowanii Bak.	BL	Infected sores, boils, fever and pus diseases (Pujol, 1990)
ASCLEPIADACEAE		
Xysmalobium undulatum (L.) Ait.f. (HUTCHINGS3417ZUL)	RT	Decoction as lotion for infected sores and wounds (Watt and Breyer-Brandwijk, 1962)
ASTERACEAE		
Artemisia afra Jacq. ex Willd. (TR5UN)	LF	Infusion/poultice used for haemorrhoids, wounds, boils, earache, toothache, fever, sinusitis, head colds and respiratory complaints (Watt and Breyer-Brandwijk, 1962)
Bidens pilosa L. (TR13UN)	LF	Infusion used for dysentry and diarrhoea (Watt and Breyer-Brandwijk, 1962)
CANELLACEAE		
Warburgia salutaris (Bertol.f.) Chiov. (JÄGER19UN)	BK	Decoction drunk for colds, sinuses, influenza and other chest complaints; powder applied to sores (Mander et al., 1995)
COMBRETACEAE		
Terminalia sericea Burch. ex DC.	BK	Powder applied to wounds, diarrhoea (Gelfand et al., 1985)
DIOSCOREACEAE		
Dioscorea sylvatica Ecklon (TR10UN)	TB	Decoction used to treat cuts, wounds and sores (Mander et al., 1995)
LEGUMINOSAE		
Erythrina lysistemon Hutch.	BK	Poultice used to treat swelling and abscesses, ash of burnt bark disinfects open wounds (Pujol, 1990; Roberts, 1990)
Acacia sieberiana DC. (TR3UN)	BK	Decoction used to treat gonorrhoea (Watt and Breyer-Brandwijk, 1962)
	RT	Infusion used as an antiseptic (Gelfand et al., 1985)
Dalbergia obovata E.Mey. (TR11UN)	RT	Infusion used for stomach ache and toothache (Watt and Breyer-Brandwijk, 1962)
LILIACEAE		
Bulbine frutescens (L.) Willd. (TR8UN)	LF	Juice applied to wounds, burns, sores, cutaneous inflammation (Pujol, 1990)
Eucomis autumnalis (Mill.) Chitt.	BL	Decoction used for respiratory and urinary problems (Mander et al., 1995)
	LF	Used as a bandage on festering sores and boils (Roberts, 1990)
Scilla natalensis Planch. (TR6UN)	BL	Dry powder rubbed into wounds and sprains; hot compress applied to boils and sores (Watt and Breyer-Brandwijk, 1962; Mander et al., 1995)
MELIACEAE		
Ekebergia capensis Sparrm. (JÄGER18UN)	BK	Decoction used to wash sores; powder applied to abscesses and boils (Pujol, 1990)

Table 1 (continued)

Family and botanical name (voucher specimen[a])	Plant part[b]	Administration/traditional uses
MORACEAE		
Ficus natalensis Hochst. (TR7UN)	LF	Poultice applied to sores and abscesses (Pujol, 1990)
	RT	Infusion used to treat boils and carbuncles (Pujol, 1990)
MYRTACEAE		
Psidium guajava L. (TR1UN)	LF	Decoction for boils (Gelfand et al., 1985)
RHAMNACEAE		
Ziziphus mucronata Willd. (TR9UN)	LF	Poultice applied to boils, abscesses and septic swellings of the skin; powder also applied to wounds and decoction taken for pneumonia (Watt and Breyer-Brandwijk, 1962; Gelfand et al., 1985)
SOLANACEAE		
Datura stramonium L. (TR2UN)	LF	Poultice applied to wounds, sores, boils, abscesses and ulcers (Watt and Breyer-Brandwijk, 1962; Gelfand et al., 1985)
Nicotiana tabacum L. (TR12UN)	LF	Powder applied to wounds (Gelfand et al., 1985)
URTICACEAE		
Pouzolzia mixta Solms (TR4UN)	RT	Paste applied to burns; infusion taken for venereal diseases; fibre used to stitch open wounds to promote healing (Gelfand et al., 1985)

[a]Voucher specimens: UN, Herbarium of the University of Natal Pietermaritzburg; ZUL, Herbarium of the University of Zululand.
[b]Plant part: LF, leaf; RT, root; BL, bulb; BK, bark; TB, tuber.

boils and purulent sores, with which the bacteria tested are routinely associated (Isenberg and D'Amato, 1991). In total, 54 plant extracts, some from different parts of the same plant, were tested for antibacterial activity by the filterpaper disc bioassay.

2. Materials and methods

2.1. Plant material

Plants were collected from the KwaZulu/Natal region of South Africa. Information on traditional medicinal usage of the plants was obtained by a review of the available literature presented in Table 1. Voucher specimens (cited in Table 1) were deposited at the herbarium of the University of Natal, Pietermaritzburg. Plant material was dried in an oven at 50°C and stored at room temperature until further processing.

2.2. Preparation of extracts

Dried plant material (5.0 g) was finely ground and extracted with 50 ml of water or 80% methanol for 30 min in an ultrasound bath (Branson 2210, 47 kHz). The plant extracts were macerated overnight, filtered, and the clear filtrates evaporated to dryness under reduced pressure at a temperature of 40°C. The residues were resuspended in water or 80% methanol to give 100 mg residue/ml.

2.3. Antibacterial activity

The disc-diffusion assay (Rasoanaivo and Ratsimamanga-Urverg, 1993) was used to determine the growth inhibition of bacteria by the plant extracts. The following bacteria were used: *Staphylococcus aureus*, *Staphylococcus epidermis*, *Bacillus subtilis*, *Escherichia coli* and *Klebsiella*

Table 2
Antibacterial[a] activity of the South African plant extracts

Botanical binomial	Plant part[b]	Extract	Bacteria tested[c]				
			S.a.	S.e.	B.s.	K.p.	E.c.
Acacia sieberiana	BK	Water	0	0.38	0.15	0	0
		Methanol	0	0.39	0.33	0	0
	LF	Water	0	0	0	0	0
		Methanol	0	0	0	0	0
Artemisia afra	LF	Water	0	0	0	0	0
		Methanol	1.25	0	0.59	0	0
Bidens pilosa	LF	Water	0	0	0	0	0
		Methanol	1.15	0.38	0.55	0	0
Boophane disticha	BL	Water	0	0	0	0	0
		Methanol	0	0	0	0	0
Bulbine frutescens	LF	Water	0	0	0	0	0
		Methanol	0	0	0	0	0
Crinum macowanii	BL	Water	0	0	0	0	0
		Methanol	0	0	0	0	0
Dalbergia obovata	LF	Water	0	0	0	0	0
		Methanol	0	0	0	0	0
Datura stramonium	LF	Water	0	0	0	0	0
		Methanol	0	0	0.13	0	0
Dioscorea sylvatica	TB	Water	0	0	0	0	0
		Methanol	0	0	0	0	0
Ekebergia capensis	BK	Water	0	0	0	0	0
		Methanol	0.83	0.48	0.30	0	0
Erythrina lysistemon	BK	Water	0	0	0	0	0
		Methanol	0	0	0	0	0
Eucomis autumnalis	BL	Water	0	0	0	0	0
		Methanol	0	0	0.13	0	0
	LF	Water	0	0	0	0	0
		Methanol	0	0	0.17	0	0
Ficus natalensis	RT	Water	0	0	0	0	0
		Methanol	0.71	0.50	0.30	0	0
	LF	Water	0	0	0	0	0
		Methanol	0	0	0	0	0
Nicotiana tabacum	LF	Water	0	0	0	0	0
		Methanol	0.26	0	0.32	0	0
Pouzolzia mixta	RT	Water	0.10	0.18	0.06	0	0
		Methanol	0.70	0.62	0.32	0	0
	LF	Water	0	0	0	0	0
		Methanol	0.58	0.33	0.28	0	0
	ST	Water	0	0	0	0	0
		Methanol	0.23	0	0.08	0	0
Psidium guajava	LF	Water	0.52	0.33	0.26	0	0
		Methanol	1.20	0.45	0.28	0	0
Scilla natalensis	BL	Water	0	0	0	0	0
		Methanol	0	0	0	0	0
Terminalia sericea	BK	Water	0	0	0	0	0
		Methanol	0	0	0	0	0
Warburgia salutaris	BK	Water	0.38	0.15	0.36	0	0
		Methanol	1.34	0.25	0.78	0	0.25

Table 2 (continued)

Botanical binomial	Plant part[b]	Extract	Bacteria tested[c]				
			S.a.	S.e.	B.s.	K.p.	E.c.
Xysmalobium undulatum	RT	Water	0	0	0	0	0
		Methanol	0	0	0	0	0
Ziziphus mucronata	LF	Water	0	0	0	0	0
		Methanol	0.30	0.13	0.14	0	0
	BK	Water	0	0	0	0	0
		Methanol	0	0	0	0	0

[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 200–500 μg/ml).
[b]Plant part: LF, leaf; ST, stem; RT, root; BK, bark; BL, bulb; TB, tuber.
[c]Bacteria: S.a., *Staphylococcus aureus*; S.e., *Staphylococcus epidermis*; B.s., *Bacillus subtilis*; K.p., *Klebsiella pneumoniae*; E.c., *Escherichia coli*.

pneumoniae. Bacteria were maintained at 4°C on nutrient agar (NA) plates.

Base plates were prepared by pouring 10 ml Mueller-Hinton (MH) agar (Biolab) into sterile Petri dishes (9 cm) and allowed to set. Molten MH agar held at 48°C was inoculated with a broth culture (10^6–10^8 bacteria/ml) of the test organism and poured over the base plates forming a homogenous top layer. Ten μl of plant extract were applied per filter paper disc (Whatman No.3, 6 mm diameter) so that each disc contained 1 mg of material. The discs were air-dried and placed onto the seeded top layer of the agar plates. Each extract was tested in quadruplicate (4 discs/plate), with a neomycin (200–500 μg/ml) disc as reference or positive control. Methanol saturated discs (air-dried) were used as negative controls. The plates were evaluated after incubation at 37°C for 18 h. 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). The activity of neomycin was included in this equation to adjust for plate-to-plate variations in the sensitivity of a particular bacterial strain.

Minimal inhibiting concentrations (MIC) were determined for extracts with antibacterial activity >0.60. The agar dilution method (Sahm and Washington II, 1991) was used against *S. aureus*, *S. epidermis* and *B. subtilis*, with two-fold serial dilutions of plant extracts from 8.0–0.25 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. Neomycin was used as the reference and appropriate controls with no extract and solvent were used.

3. Results and discussion

A total of 54 extracts representing 21 plant species distributed among 15 families were investigated. Table 2 shows the botanical names, plant parts tested and the results of the antibacterial screening.

The antibacterial activity of the plant extracts tested was found mainly against the Gram-positive bacteria. The *Warburgia salutaris* methanol extract was the only one to show activity against *Escherichia coli*, a Gram-negative bacterium. None of the extracts showed any activity against the other Gram-negative bacterium, *Klebsiella pneumoniae*. The negative results obtained against the Gram-negative bacteria were not surprising as, in general, these bacteria are more resistant than Gram-positive ones (Martin, 1995; Paz et al., 1995; Vlietinck et al., 1995).

Of the 21 plant species tested, 10, 8, and 12 of the species showed activity against *Staphylococcus aureus*, *Staphylococcus epidermis* and *Bacillus subtilis*, respectively. It was interesting to note that

Table 3
The minimum inhibitory concentration (MIC) of methanolic plant extracts with antibacterial activity

Plant name	Plant part	Bacteria used (MIC (mg/ml))		
		S. aureus	*S. epidermis*	*B. subtilis*
A. afra	LF	2.0	4.0	4.0
A. sieberiana	BK	>2.0	2.0	>2.0
B. pilosa	LF	2.0	8.0	4.0
E. capensis	BK	4.0	2.0	2.0
F. natalensis	RT	4.0	4.0	8.0
P. guajava	LF	4.0	4.0	4.0
P. mixta	RT	4.0	2.0	2.0
W. salutaris	BK	0.5	2.0	0.5
Neomycin		4.0×10^{-3}	1.2×10^{-4}	1.2×10^{-4}

the majority of the antibacterial activity was present in the methanol extracts. Traditionally, plant extracts are prepared with water (for example, infusions, decoctions, and poultices), so it would seem unlikely that the traditional healer extracts those compounds which are responsible for activity in the methanol extracts.

P. guajava has known antibacterial action with the leaf yielding three active substances: quercetin, avicularin and guaijaverin (Khadem and Mohammed, 1958; Watt and Breyer-Brandwijk, 1962; Oliver-Bever, 1986). Therefore, the methanolic leaf extract of *P. guajava* was used to compare the activity of the other plant extracts against, as it would be expected that plant extracts with a similar degree of antibacterial activity to that of *P. guajava*, might be worth pursuing further. Plants showing the highest antibacterial activity were *Bidens pilosa*, *Psidium guajava*, *Artemisia afra* and *Warburgia salutaris*, in order of increasing activity. The volatile oil of *A. afra* has greater activity as an antimycotic than as an antibacterial agent (Graven et al., 1992). The high activity in the methanolic extract of *W. salutaris* may be due to sesquiterpene dialdehydes, which have been isolated from the related species *W. ugandensis* and *W. stuhlmannii* (Kubo et al., 1976). These drimane-type sesquiterpenoids are known to have antibacterial, antifungal, cytotoxic, insect-antifeedant and molluscicidal activities (Fukuyama et al., 1982; Taniguchi and Kubo, 1993).

The MIC values for some of the more positive extracts are shown in Table 3. These extracts do not have a good potency level based on their high MIC values, implying the active compounds would probably not be pharmaceutically useful (Rios et al., 1988). Reasons for the relatively high MIC values could be that the extracts tested are still in an impure form, or that the active compound/s are present in very low concentrations. Nevertheless certain of the plant extracts warrant further investigation using bioassay-guided fractionation to characterise the active constituents. The results of this study support to a certain degree the traditional medicinal uses of the plants evaluated and reinforce the concept that the ethnobotanical approach (Cox and Balick, 1994) to screening plants as potential sources of bioactive substances is successful.

Acknowledgements

We would like to thank Richard Symmonds of Silverglen Nature Reserve, Durban and Janet Gates of the Pietermaritzburg Botanical Gardens, for supplying plant material. The Departments of Microbiology, University of Natal, Pietermaritzburg and University of Durban-Westville, are also thanked for providing bacterial strains. This work was financially supported by the Foundation for Research Development and the University of Natal Research Fund.

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