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Short Communication

In vitro antibacterial activity of Venda medicinal plants

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Ethanolic and aqueous extracts of ten medicinal plants used in folklore medicine in Venda (South Africa) were screened for their *in vitro* activity against some Gram-positive and Gram-negative pathogenic bacteria using the disc diffusion method. Root and stembark extracts of *Datura stramonium* at a concentration of 50mg ml^{-1} were inhibitory to most of the organisms with a diameter of zone of inhibition of growth ranging from 12–19mm. *Warburgia salutaris* was also inhibitory to most of the bacteria with a diameter of zone of inhibition of growth ranging from 8–17mm at a concentration of 50mg ml^{-1}. A range of 10–16mm was observed for *Peltophorum africanum* at a concentration of 40mg ml^{-1} against the Gram-negative bacteria tested. Other plants showed moderate or no activity, compared to a 30µg ml^{-1} oxytetracycline control antibiotic. The use of these plants by the indigenes of Venda against diseases apparently caused by these organisms may be of some value.

The continuous evolution of bacteria resistant to currently available antibiotics has necessitated the search for novel and more effective antibacterial compounds. Efforts in this regard have focused on plants because of their use historically and the fact that a good portion of the world's population, particularly in developing countries, rely on plants for the treatment of infectious and non-infectious diseases (Arnold and Gulumian 1984, Martinez *et al*. 1996). Globally, plant extracts are employed for their antibacterial, antifungal and antiviral activities (Meyer *et al*. 1996, Verastegui *et al*. 1996). Some plant decoctions are of great value in the treatment of diarrhoea or gastrointestinal disorders, urinary tract infections, cervicitis, vaginitis, skin infections, infertility, wounds and cutaneous abscesses (Caceres *et al*. 1990, Olukoya *et al*. 1993, Meyer *et al*. 1996). In spite of this, there is still a lack of scientific experimental studies authenticating the potential antimicrobial activities of a wide array of medicinal plants (Dimayuga and Garcia 1991).

Previous *in vitro* studies have revealed the activity of several South African medicinal plants against a good number of bacteria and viruses pathogenic to man (Meyer *et al*. 1996, Salie *et al*. 1996, Eloff 1999, Lall and Meyer 2000, Eloff *et al*. 2001). In this study, ten medicinal plants which are employed in the treatment of diseases of probable bacterial etiology by the Vendas in South Africa were screened for activity against some pathogenic bacteria. Aqueous and ethanolic extracts of different plant parts were investigated.

The required plant parts, namely roots, stem bark and leaves, were collected from various communities within the Venda region between August 1999 and January 2001. Plant species were identified by Mr P Tshikawe, Botany Unit, Department of Biological Sciences, University of Venda for Science and Technology, South Africa. Voucher specimens were deposited at the Herbarium of the Department of Biological Sciences. Plant material was washed with distilled water, dried at room temperature for two weeks and ground into a fine powder. The ethnobotanical information available on the studied plants is presented in Table 1 (Retief and Herman 1997, Van Wyk *et al*. 1997).

Aqueous extracts were prepared by soaking 100g of ground material for two days in one litre of sterile distilled water. The extract was filtered through a mesh and Whatman filter paper No. 8 successively and evaporated to dryness in a rotatory evaporator at 60°C. The ethanolic extracts were obtained by soaking the ground plant material in one litre ethanol (Merck) for 12 hours. The extract was filtered as above and evaporated to dryness at 40°C. The residual extract was stored at 4°C for further use. The bacterial species tested were *Staphylococcus aureus* (ATCC 6538), *Streptococcus pyogenes* (ATCC 10389), *Bacillus cereus* (ATCC 10876), *Pseudomonas aeruginosa* (ATCC 9027), *Escherichia coli* (ATCC 8739), *Salmonella typhi* (SABS 47), *Shigella sonnei* (ATCC 11060). These were obtained from the South African Bureau of Standards (SABS). *Campylobacter jejuni* and *Aeromonas hydrophila* were obtained from the Department of Medical Microbiology,

Table 1: Data on the ethnobotany of the plants investigated

Plant species	Family	Voucher number	Plant part used	Common uses
Warburgia salutaris (Bertol. f.) Chiov	Canellaceae	0081	Leaves	Used for gonorrhoea, septic wounds, coughs
Adansonia digitata (L.)	Bombaceae	0010	Fruit / bark	Used for fevers, diarrhoea
Aloe ferox Mill.	Asphodelaceae	0119	Leaves, flowers	Used as laxative, for conjunctivitis, hypertension, diarrhoea, fodder, insect repellent
Euclea divinorum Hiern	Ebenaceae	5101	Fruits, roots, stem bark	Treatment of gastrointestinal disorders, sore throat, skin infections and used as a laxative
Rhus lancea (L.f.)	Anacardiaceae	1810	Roots, leaves, stem bark	Used to treat skin diseases
Piper auritum (L.)	Piperaceae	1630	Leaves	Treatment for stomach pains, diarrhoea, ulcers
Peltophorum africanum (Sond*)*	Fabaceae	1520	Roots, stem bark	Sore throat, diarrhoea, dysentery, sore throat, tooth ache
Vernonia stipulacea Klatt	Asteraceae	2220	Roots	Stomach disorders, skin diseases
Datura stramonium (L.)	Solanaceae	4001	Roots, stem bark	Rheumatic pains, bronchitis, gonnorhea
Withania somnifera (L.) Dunal	Solanaceae	i0074	Roots	Skin infections

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The antibacterial activity of the extracts was investigated using the disc diffusion method. The aqueous and ethanolic extracts were dissolved in sterile distilled water and ethanol respectively, and filtered through 0.22µm filters before use in each assay. For the disc diffusion method the previously reported technique of Essawi and Srour (2000) was used. The microorganisms were cultured overnight at 35°C on nutrient agar (Oxoid). Saline suspensions of bacterial cultures corresponding to an optical density of McFarland 0.5 were spread evenly over the entire surface of Mueller-Hinton agar (Difco, Detroit, MI), in petri dishes, using sterile cotton wool swabs. The plates were allowed to dry for about 5–10 minutes. Sterile 6mm diameter filter paper discs impregnated with either a 40mg ml^{-1} or 50mg ml^{-1} concentration of the extracts were placed on the seeded plates. Diameters of zones of inhibition of growth were measured in millimetres after 24 hours of incubation at 37°C. Discs saturated with 30µg ml^{-1} oxytetracycline (Sigma) were included in each test as a positive control (Brantner *et al*. 1996, Essawi and Srour 2000). The negative controls consisted of two 6mm filter paper discs impregnated with the extracting solvents (water and ethanol). Experiments were performed three times in their entirety.

The results obtained are shown in Table 2. The ethanolic extracts of the leaves, stem bark and roots of *Euclea divinorum* demonstrated activity against *Staphylococcus aureus, Streptococcus pyogenes, Shigella sonnei, Escherichia coli* and *Campylobacter jejuni*. The diameter of the zones of inhibition ranged from 10–14mm. No activity was noted against *Bacillus cereus, Pseudomonas aeruginosa*, and *Salmonella typhi*. The aqueous extracts had activity against *Staphylococcus aureus, Shigella sonnei, Escherichia coli* and *Campylobacter jejuni* with zones of inhibition of growth ranging between 10–14mm, but no activity against *Streptococcus pyogenes, Bacillus cereus, Pseudomonas aeruginosa,* and *Salmonella typhi*, except for the root extract that had activity against *Streptococcus pyogenes* (13mm). The aqueous and ethanolic extracts of the stem bark of *Rhus lancea* and the leaves of *Piper auritum* equally demonstrated antibacterial activity against *Staphylococcus aureus, Streptococcus pyogenes*, *Salmonella typhi, Shigella sonnei*, *Escherichia coli* and *Campylobacter jejuni*, with a range of zone diameter of inhibition of growth of 8–16mm. The ethanolic and aqueous extracts of the leaves of *Piper auritum* showed no activity against *Bacillus cereus*, *Pseudomonas aeruginosa* and *Aeromonas hydrophila*. Ethanolic and aqueous extracts of the roots and stem of *Warburgia salutaris*, *Datura stramonium* and *Vernonia stipulacea* were active against *Staphylococcus aureus, Streptococcus pyogenes*, *Bacillus cereus*, *Escherichia coli, Salmonella typhi* and *Shigella sonnei*. *Peltophorum africanum* showed activity against *Salmonella typhi*, *Shigella sonnei*, *Escherichia coli, Campylobacter jejuni* and *Aeromonas hydrophila*, whereas *Withania somnifera* was active against all the tested bacteria with zone diameters of inhibition of growth ranging from 10mm to 15mm. *Aloe ferox* and *Adansonia digitata* showed no activity. Comparing the observed zones of inhibition of the extracts with the positive control (30µg ml^{-1} oxytetracycline disc) which produced a 17mm zone of inhibition against *S. aureus,* the ethanolic and aqueous extracts of the stem bark of *Datura stramonium* and the ethanolic extract of the stem bark of *Warburgia salutaris* showed greater activity against this organism with zones of inhibition of 19mm and 17mm respectively. Likewise, the ethanolic leaf extracts of *Piper auritum*, and the stem bark of *Warburgia salutaris* showed significant activity against *Streptococcus pyogenes* with zones of inhibition of 16mm and 15mm respectively compared to 15mm for oxytetracycline. Equally, the ethanolic extracts of the root and stem of *Datura stramonium*, and the ethanolic and aqueous extracts of the roots of *Vernonia stipulacea* showed good activity against *Escherichia coli* with zones of diameter of inhibition between 15–16mm compared to 17mm for oxytetracycline. The ethanolic stembark extract of *Peltophorum africanum* also showed marked antibacterial activity against *Campylobacter jejuni* with a zone diameter of inhibition of 16mm compared to 15mm for the control antibiotic. Bacterial growth flourished in the negative controls.

The results of this study have revealed the *in vitro* antibacterial activity of leaves, bark and roots of *Euclea divinorum*, the leaves and bark of *Rhus lancea* and the leaves of *Piper auritum* against *Staphylococcus aureus* and *Streptococcus pyogenes. Datura stramonium, Warburgia*

Table 2: *In vitro* activities of selected medicinal plants against some pathogenic bacteria

Plant species	Part of plant investigated	Extract	Concentration mg ml^{-1}	Antibacterial activity of plant extracts: diameter of inhibition zone (mm)[1]								
				Sa (+)	Sp (+)	Bc (+)	Pa (-)	St (-)	Sh (-)	Ec (-)	Cj (-)	Ah (-)
Euclea divinorum	Leaves	Ethanolic	50	12	13	–	–	–	11	10	11	ND
		Aqueous	50	10	–	–	–	–	9	12	10	ND
	Stem bark	Ethanolic	50	12	10	–	–	–	13	13	14	ND
		Aqueous	50	11	–	–	–	–	13	10	11	ND
	Roots	Ethanolic	50	10	12	–	–	–	12	10	11	ND
		Aqueous	50	14	13	–	–	–	10	9	11	ND
Rhus lancea	Leaves	Ethanolic	40	–	–	–	–	–	–	–	–	–
		Aqueous	50	–	–	–	–	–	–	–	–	–
	Stem bark	Ethanolic	50	10	10	9	ND	12	11	10	ND	ND
		Aqueous	50	10	13	10	ND	10	9	10	ND	ND
Piper auritum	Leaves	Ethanolic	50	14	16	–	–	9	11	12	10	–
		Aqueous	50	10	12	–	–	9	10	9	8	–
Aloe ferox	Leaves	Ethanolic	50	ND	ND	ND	–	–	–	–	–	–
		Aqueous	50	ND	ND	ND	–	–	–	–	–	–
Adansonia digitata	Stem bark	Ethanolic	50	ND	ND	ND	–	–	–	–	–	–
		Aqueous	50	ND	ND	ND	–	–	–	–	–	–
Datura stramonium	Roots	Ethanolic	50	16	13	16	–	15	15	16	ND	ND
		Aqueous	50	15	12	15	–	11	13	14	ND	ND
	Stem bark	Ethanolic	50	19	13	18	–	17	14	16	ND	ND
		Aqueous	50	17	10	18	–	12	12	13	ND	ND
Peltophorum africanum	Stem bark	Ethanolic	40	ND	ND	ND	ND	10	12	14	16	14
		Aqueous	40	ND	ND	ND	ND	13	10	13	14	11
	Roots	Ethanolic	40	ND	ND	ND	ND	12	11	13	15	13
		Aqueous	40	ND	ND	ND	ND	9	9	13	10	12
Warburgia salutaris	Stem bark	Ethanolic	50	17	15	11	12	10	9	10	ND	13
		Aqueous	50	12	11	10	11	9	9	8	ND	10
Vernonia stipulacea	Roots	Ethanolic	40	14	11	12	10	11	13	16	ND	ND
		Aqueous	40	12	9	10	11	9	10	15	ND	ND
Withania somnifera	Roots	Ethanolic	40	15	10	11	9	10	14	14	10	8
		Aqueous	40	12	10	9	9	13	11	11	8	8
Oxytetracycline				17	15	16	16	16	19	17	15	15

[1] Diameters of the zones of inhibition in millimetres are the means of three replicates given to the nearest whole number

Sa = *Staphylococcus aureus*, Sp = *Streptococcus pyogenes*, Bc = *Bacillus cereus*, Pa = *Pseudomonas aeruginosa*, St = *Salmonella typhi*, Sh = *Shigella sonnei*, Ec = *Escherichia coli*, Cj = *Campylobacter jejuni*, Ah= *Aeromonas hydrophila*

(+) or (-) = Gram reaction, ND = not done, – = no activity

salutaris and *Vernonia stipulacea* showed marked antibacterial activity against all the Gram-positive bacteria and the Gram-negatives *Salmonella typhi, Shigella sonnei* and *Escherichia coli*, with the exception of *Pseudomonas aeruginosa*, which was not inhibited by *Datura stramonium*. *Staphylococcus aureus* is the bacterial etiologic agent of skin diseases, food poisoning, blepharitis, osteomyelitis, toxic shock syndrome, staphylococcal scalded skin syndrome and lymphadenitis. *Streptococcus pyogenes* is implicated in cases of glomerulonephritis, rheumatic fever, sore throat, scarlet fever and skin infections. The demonstrated antibacterial activity of the extracts of *Euclea divinorum* and *Rhus lancea* against these two pathogens supports their traditional use in the treatment of diseases potentially caused by *Staphylococcus aureus* and *Streptococcus pyogenes*. Likewise, the observed activity of extracts of *Warburgia salutaris*, *Withania somnifera*, *Datura stramonium* and *Vernonia stipulacea* against most of the test organisms shows their usefulness in the management of a variety of infections including those caused by enteropathogens such as *Escherichia coli, Salmonella* spp. and *Shigella sonnei*. These enteropathogens are causes of dysentery, diarrhoea, ulcers and gastrointestinal disorders (Blom *et al*. 1999, Rowe and Kirk 2001). *Withania somnifera*, *Warburgia salutaris, Rhus lancea* and *Vernonia stipulacea* may also be of future importance in antiseptic and disinfectant formulations as well as in chemotherapy because of their wide range of antimicrobial activity (Olukoya *et al*. 1993). In this study, ethanolic and aqueous extracts from the same plants demonstrated varying activity but generally ethanolic extracts showed greater activity than aqueous extracts, which is in harmony with a previous report (Olano *et al*. 1996).

Specifically, extracts from *Warbugia salutaris*, *Datura stramonium*, *Peltophorum africanum* showed the best antibacterial activities in this study. The antibacterial property of *Datura stramonium* has already been elucidated. Rabe and Van Staden (1997, 2000) have demonstrated the activity of *Warburgia salutaris* against a wide range of Gram-positive bacteria, and identified muzigadial as a potent antibacterial agent. In another vein, aqueous suspensions of *Warburgia salutaris* have also been shown to have molluscidal properties (Clark and Appleton 1997). *Datura stramonium* is used traditionally in Central Italy as an antiparasitic and repellent (Guarrera 1999). Several reports have revealed its toxicity to humans and animals (Schulman and Bolton 1998, Hamouda *et al*. 2000, Onen *et al.* 2002, Tostes 2002). In this study *Aloe ferox* did not show any activity against the test organisms. However, acetone extracts of the leaf of *Aloe ferox* have been shown to have antifungal activity (Afolayan *et al*. 2002). The antiplasmodial activity of some species of *Aloe* has also been documented (Van Zyl and Viljoen 2002). We are not aware of any reports on the antimicrobial activity of *Peltophorum africanum*.

It is concluded that the demonstration of inhibitory activities of the tested plants revealed their value in traditional medicine and supports once more the enormous role of medicinal plants in primary health care.

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