South African Journal of Botany 2003, 69(4): 595–598
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SOUTH AFRICAN JOURNAL OF BOTANY
EISSN 1727–9321

Short Communication

Evaluation of antibacterial activity of traditionally prepared South African remedies for infections

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Received 27 February 2003, accepted in revised form 14 July 2003

Seven traditional remedies used in South Africa for bacterial infections were prepared according to the traditional method of preparation. The remedies consisted of 1–3 plants of the following species: *Acorus calamus, Artemisia afra, Alepidea amatymbica, Jatropha zeyheri, Pentanisia prunelloides, Tetradenia riparia* and *Warburgia salutaris* and were prepared as decoctions or infusions. The aqueous extracts were partitioned onto ethyl acetate. The aqueous extracts and the ethyl acetate phases were tested for antibacterial activity in the microtitre plate assay. The aqueous extracts had no detectable activity, whereas several of the ethyl acetate phases had some activity. When relating this activity to the daily dose of the remedies it becomes questionable whether the remedies have any antibacterial effect.

Traditional medicine is a very important part of primary health care in South Africa. It is a holistic healing system comprising both a spiritual/psychological and a medical component. While the first cannot be scrutinised in a scientific way, the medicinal treatment can be scientifically evaluated.

To directly improve traditional medicine it is necessary to evaluate the medicines in their entirety as they are prepared traditionally.

Traditional healers in most parts of the world prepare plant medicines by some form of extraction with water. Scientists who investigate traditional medicine often discard the aqueous extracts, partly because higher activity is found in more apolar extracts and aqueous extracts are technically more difficult to work with.

Many studies have investigated antibacterial activities of South African medicinal plant species (Rabe and Van Staden 1997, Mathekga and Meyer 1998, Lin *et al*. 1999, Eloff 1999, Kelmanson *et al*. 2000, McGaw *et al*. 2000, McGaw *et al*. 2001, Pillay *et al*. 2001). In these studies plant material has typically been extracted with solvents of varying polarity and subsequently been subjected to antibacterial testing with little regard to how traditional healers prepare and use extracts. In this study remedies to treat various bacterial infections were prepared according to traditional practice and tested for antibacterial activity.

Plant material was collected in the University of Natal Botanical Gardens, Pietermaritzburg or obtained from Silverglen Nursery, Durban or from a muthi shop in Pietermaritzburg. The material was dried at 50°C and kept in brown paper bags. Before extraction, it was ground into a fine powder. Voucher specimens are lodged in the University of Natal Herbarium, Pietermaritzburg.

Preparation of extracts followed the traditional methods (Felhaber 1997) outlined in Table 1. One teaspoon (5ml) of each of the ground materials weighed: *Artemisia afra*: 0.71g; *Alepidea amatymbica*: 2.19g; *Acorus calamus*: 1.20g; *Jatropha zeyheri*: 1.88g; *Pentanisia prunelloides*: 2.65g; *Tetradenia riparia*: 0.59g and *Warburgia salutaris*: 2.89g. Infusions and decoctions were made with distilled water and filtered through Whatman No. 1 filter paper when luke warm, except the decoction of *A. afra* + *W. salutaris*, which was filtered while hot. One daily dose (1Tbs = 15ml; 1 cup = 250ml; 1 full wineglass = 250ml) was taken to dryness. Extracts that had been taken to dryness either by freeze-drying or under vacuum could not be redissolved, therefore an aliquot was reduced in volume, but never taken to dryness, and readjusted to 4mg ml^{-1}, which was the highest concentration that would remain in solution.

Another daily dose was partitioned three times against equal parts of ethyl acetate. The ethyl acetate phases were combined and dried over anhydrous sodium sulphate before being taken to dryness under reduced pressure. The extracts were redissolved in DMSO to 8mg ml^{-1} before bioassaying.

The microplate method of Eloff (1998) was used to determine the MIC values for plant extracts. Cultures of the following bacteria were used: *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 6538. Aqueous extracts and

Table 1: Traditional remedies used to treat infections (Felhaber 1997)

Vernacular names (Zulu)	Species	Plant part	Traditional usage	Preparation	Administration and dosage
Umhlonyane ikhathazo	*Artemisia afra* *Alepidea amatymbica*	Leaves Root	Colds and flu	Boil 1tsp of each, powdered, in 1l water for 5min	Drink ½ cup 3 times a day
Umhlonyane Isibaha	*A. afra* *Warburgia salutaris*	Leaves Bark	Acute bronchitis	Add ½l boiling water to ½tsp of each, powdered, let stand for ½h	Drink ¼ cup 3 times a day
Umhlonyane Isibaha	*A. afra* *W. salutaris*	Leaves Bark	Coughs from cold or flu	Boil ½ teaspoon of each, powdered, in 1l water for 3min. Strain while hot	Take 2Tbs every 4–6h
Ikalamuzi Isibaha	*Acorus calamus* *W. salutaris*	Rhizome Bark	Pneumonia	Add ½l boiling water to ½tsp of each, powdered, let stand for 10min	Drink ½ cup 3 times a day
Icimamilo	*Pentanisia prunelloides*	Tuber	Boils	Boil 1tsp, powdered, in 1l water for ½h	Drink ½ cup 3 times a day
Ugodide Icimamilo Isibaha	*Jatropha zeyheri* *P. prunelloides* *W. salutaris*	Root Tuber Bark	Cuts, blisters and burns	Boil 1tsp of each, powdered, in 2l water for ½h	Drink 1 full wineglass 3 times a day for 4 days
Iboza	*Tetradenia riparia*	Leaves	Coughs from cold or flu	Add 4 cups boiling water to ½tsp, powdered, let stand for 5min	Drink ½ cup 3 times a day

the ethyl acetate phases were initially tested at 2mg ml^{-1} and serially diluted two-fold to 0.016mg ml^{-1}. The antibiotic neomycin was included as standard in each assay and extract-free solvent was used as blank controls. The microplates were incubated overnight at 37°C. As an indicator of bacterial growth, *p*-iodonitrotetrazolium was added and the microplates were incubated for 30min at 37°C. MIC values were recorded as the lowest concentration of extract that completely inhibited bacterial growth. Experiments were done in duplicate.

Extracts were tested for hemolytic activity in the blood agar plate assay (Luyt *et al*. 1999).

Several of the plants tested in this study have been investigated previously. Aqueous and solvent extracts of individual plants have been tested for antibacterial activity. Aqueous and ethyl acetate extracts of the rhizome of *A. calamus* were found to have activity against *B. subtilis*, but not against other Gram-positive or negative test bacteria (McGaw *et al*. 2000). In the same study McGaw *et al*. (2000) found that ethanol extracts of *A. afra* leaves exhibited activity against *B. subtilis* and *S. aureus*, but not against Gram-negative bacteria. Rabe and Van Staden (1997) found similar results with a methanol extract of leaves of *A. afra*. The essential oil of *A. afra*, which consisted of a- and b-thujone and 1,8-cineole, was found to exhibit activity against a range of Gram-positive and -negative bacteria (Mangena and Muyima 1999). Aqueous extracts of roots of *P. prunelloides* had some activity and ethanol and ethyl acetate extracts good activity against both Gram-negative and -positive bacteria (Yff *et al*. 2002). A mixture of fatty acids with palmitic acid being the main component was isolated by bioassay-guided fractionation from *P. prunelloides* as the main antibacterial agent (Yff *et al*. 2002). An aqueous bark extract of *W. salutaris* showed very high activity against Gram-positive bacteria and the methanol extract further also against *E. coli* (Rabe and Van Staden 1997). The sesquiterpenoid muzigadial was isolated by bioassay-guided fractionation of an ethyl acetate extract as the main antibacterial compound (Rabe and Van Staden 2000), but other drimanes polygoidal and warburganal were also active (Jansen and De Groot 1991). Leaf and stem extracts of *T. riparia* prepared with 80% methanol had activity against *S. aureus*, but not against Gram-negative bacteria (Vlietinck *et al*. 1995). An ethanolic extract *of T. riparia* was active against several mycobacteria (Van Puyvelde *et al*. 1994); the active compound was 8(14),15-sandaracopimaradiene-7α,18-diol (De Kimpe *et al*. 1982). A daphnane diterpene, jaherin, with activity against *Streptococcus pyrogenes*, but inactive against *S. aureus* and Gram-negative bacteria, was isolated from a non-aqueous extract of *J. zeyheri* (Dekker *et al*. 1987). Ethanolic extracts of *A. amatymbica* were active against both Gram-negative and -positive bacteria (pers. comm. Gary Stafford). It is evident that active compounds are present in the non-polar extracts of the plants tested in this study, but reports on activity in aqueous extracts are lacking.

The aqueous extracts in this study did not exhibit any antibacterial activity (Table 2). The lack of activity could possibly be related to a problem with dissolving the aqueous extracts at an adequately high concentration. Aqueous extracts contain a high amount of inert sugars and amino acids and other polar compounds.

A daily dose of the aqueous extracts was partitioned onto ethyl acetate. Only a small fraction (2.3–16.7%) of the dry mass of the aqueous extract passed over onto the lipophilic phase. When the ethyl acetate extracts were tested the preparations of *A. afra* + *A. amatymbica*, *A. afra* + *W. salutaris* (both infusion and decoction), *A. calamus* + *W. salutaris* and *J. zeyheri* + *P. prunelloides* + *W. salutaris* had some activity against *S. aureus* (Table 2). Ethyl acetate extracts from *T. riparia* or *P. prunelloides* did not have activity. None

Table 2: Minimal inhibitory concentration and total activity of aqueous extracts of remedies prepared according to traditional healing practices; directly and after partitioning with ethyl acetate

Species, family, voucher No. and collection site	Extract	Yield (mg)		MIC (mg ml^{-1})		Total activity (ml) against Sa	
		one daily dose	1g plant material	Bs	Sa	(yield of daily dose/MIC)	(yield from 1g material/MIC)
Artemisia afra Jacq. Ex Willd., Asteraceae, (McGaw30 NU), (a) *Alepidea amatymbica* Eckl. & Zeyh. var. amatymbica, Apiaceae, (Stafford1NU), (b)	Water	288	265	>2	>2	<2 304	<2 120
	EtOAc-phase	20	18.4	>1	0.125	160	147
A. afra *Warburgia salutaris* (Bertol. F.) Chiov., Canellaceae	Water (infusion)	138	204	>2	>2	<69	<102
	EtOAc-phase	16.7	24.7	>1	0.5	33	50
A. afra *W. salutaris*	Water (decoction)	69	214	>2	>2	<35	<107
	EtOAc-phase	10	30.9	>1	0.5	20	62
Acorus calamus L., Araceae, (McGaw56NU), (a) *W. salutaris*	Water	331	215	>2	>2	<166	<107
	EtOAc-phase	36.8	23.8	>1	0.5	74	48
Pentanisia prunelloides Walp., Rubiaceae	Water	479	482	>2	>2	<240	<241
	EtOAc-phase	20.3	20.4	>1	>1	<20	<10
Jatropha zeyheri Sond., Euphorbiaceae *P. prunelloides* *W. salutaris*	Water	957	516	>2	>2	<479	<258
	EtOAc-phase	22.1	20.4	>1	0.5	44	41
Tetradenia riparia (Hochst.) Codd, Lamiaceae, (McGaw31NU)	Water	39	347	>2	>2	<20	<174
	EtOAc-phase	6.5	57.8	>1	>1	<7	<58
Neomycin (µg ml^{-1})				3.125	1.56		

(a) = University of Natal Botanical Gardens, Pietermaritzburg, (b) = Silverglen Nursery, Chatworth, Durban
Sa = *Staphylococcus aureus*, Bs = *Bacillus subtilis*

$$\text{Total activity from 1g} = \frac{\text{Extraction volume (ml) x yield ethyl acetate phase (mg)}}{\text{Material extracted (g) x daily dose (ml) x MIC (mg ml}^{-1}\text{)}}$$

of the ethyl acetate phases had activity against *B. subtilis*.

It has been suggested by Eloff (2000) that antimicrobial activity should be expressed as total activity in order to compare the effectiveness of various plant materials. When comparing the total activity extracted from 1g of plant material (Table 2) with results presented by Eloff (2000) and Eloff *et al*. (2001) the traditionally prepared extracts in this study compare favourably, though not being among the highest values obtained. It is surprising that aqueous extracts can obtain values comparable to acetone extracts, as used in the Eloff studies, and must indicate that these plants contain strong antibacterial compounds. The *A. afra* + *A. amatymbica* preparation had the highest total activity (Table 2). A comparison of the total activity contained in one daily dose showed that the *A. afra* + *A. amatymbica* was the preparation that contained the highest activity, followed by the *A. calamus* + *W. salutaris* preparation (Table 2).

The intention of this study was to evaluate traditional preparations of antibacterial remedies. No activity could be demonstrated in the aqueous extracts, but some active compounds were extracted as was evident from the results after partition onto a lipophilic phase. In order to answer whether the amount of compound extracted will be able to exert a medicinal effect, one could look at a theoretical distribution volume. The total activity of one daily dose also represents the volume (in ml) into which the daily dose maximally could be diluted, or distributed in the body, still having antibacterial activity. The highest value was 160ml, obtained with the *A. afra* + *A. amatymbica* extract (Table 2). It therefore seems unlikely that the preparations could have any clinical antibacterial effect. However, in most cases the active compounds in the plants analysed in this study are not known, so the possibility of a compound acting as a prodrug, being metabolised to an active compound in the body, cannot be ruled out.

The *A. afra* + *W. salutaris* (both infusion and decoction), *A. calamus* + *W. salutaris* and *J. zeyheri* + *P. prunelloides* + *W. salutaris* extracts all produced a stable foam. When investigating the plants individually, *W. salutaris*, a component of all four foaming extracts, was found to be responsible for the foaming. An aqueous extract of this species as well as the four foaming extracts could not hemolyse blood. Although the ability to hemolyse blood is a characteristic of saponins, not all saponins are able to do this. At present, it is not

known whether saponins are present in *W. salutaris*. Saponin-containing plant extracts are used in western medicine in Europe as expectorants for cough-related ailments, although the mechanism of action remains unknown (Bruneton 1999). Saponins could, however, also improve the solubility of other, more lipophilic, compounds, thus improving the extraction process.

W. salutaris is the third most frequently sold plant in the Durban muthi markets (Mander 1998), likely reflecting the situation in South Africa. The sale of *W. salutaris* increases during winter months when people are suffering from respiratory ailments (Mander 1998). Indications are coughs from cold and flu, pneumonia and acute bronchitis (Felhaber 1997). Is it possible that *W. salutaris* is used as an expectorant, due to saponins extracted into a decoction or infusion, rather than for its documented antibacterial effect of solvent extracts and the highly antibacterial, but toxic, compound muzigadial? Gerstner (1938) wrote that isiBaha (*W. salutaris*) bark was 'used all over and sold by the Native herbalists as one of the most famous expectorants'. Van Wyk and Gericke (2000) also mention the use of *Warburgia* as an expectorant in coughs, based on a pers. comm. from well-known healer Solomon Mahlaba. On the other hand, the well-known healer Elliot Ndlovu of Kamberg, KwaZulu-Natal, is of the opinion that *W. salutaris* is used for its antimicrobial activity and not as an expectorant (pers. comm.).

The results from this study raises concern that the traditional way of preparing medicinal plants may not lead to very active extracts, and in the process most of the active compounds are left behind in the plant material. This could be seen as waste of scarce plant resources — *W. salutaris* is listed as Endangered (Scott-Shaw 1999) — better utilisation of the material could help in conservation efforts.

Acknowledgements — Miss Heidi L Døring is thanked for technical assistance with the bioassays. The University of Natal donated plant material.

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Edited by JN Eloff