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Short communication

Screening of medicinal plants used in Lesotho for anti-bacterial and anti-inflammatory activity

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Abstract

Traditional healers and herbalists from Lesotho were interviewed about plants used in traditional remedies by the Sotho. Plant roots are most often used to make water extracts. Mainly high altitude plants are used, with lowland healers obtaining most of their plant material from the highlands, either by collecting them or buying them from highland gatherers. As a result of ethnobotanical data obtained, leaves and roots of 12 plants were extracted using hexane, methanol and water, respectively and the extracts screened for anti-inflammatory activity using the cyclo-oxygenase bioassay. Six species yielded inhibitory activity above 90%. Hexane and methanol leaf and root extracts were the most active. Leaves and roots of 16 plants were extracted using hexane, methanol and water and the respective extracts screened for anti-bacterial activity using the disc-diffusion assay. Six species displayed very high anti-bacterial activity against both gram-positive and gram-negative bacteria. A number of plant extracts had medium inhibitory activity, mostly against gram-positive bacteria. The activity was mainly found in the root extracts.

Keywords: Lesotho; Traditional medicinal plants; Anti-inflammatory activity; Anti-bacterial activity

1. Introduction

Plants have long provided mankind with a source of medicinal agents, with natural products once serving as the source of all drugs (Balandrin et al., 1993). Dependence on plants as the source of medicine is prevalent in developing countries where traditional medicine plays a major role in health care (Farnsworth, 1994; Srivastava et al., 1996). The rural population of a country is more disposed to traditional ways of treatment because of its easy availability and cheaper cost (Banquar, 1993). Herbal therapy, although still an unwritten science, is well established in some cultures and traditions, and has become a way of life in almost 80% of the people in rural areas, especially those

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in Asia, Latin America and Africa (Banquar, 1993).

Infectious and inflammatory diseases are among those treated using traditional remedies. Infectious diseases are enhanced by factors such as inadequate sanitation, poor hygiene and overcrowded living conditions (Rasoanaivo and Ratsimamanga-Urverg, 1993; Kerr and Lacey, 1995). The diseases may occur as local purulences (e.g. furuncles, ulcers, phlegmous, inflammation of the pharynx and tonsilitis), which can subsequently become generalised as a blood infection (Brantner and Grein, 1994). Many inflammatory diseases are associated with prostaglandins, a family of C_{20} fatty acids found widely distributed in almost all living animal cells, tissues and glands (Hickock et al., 1985; Rang and Dale, 1987). The principal substrate for prostaglandins is arachidonic acid, found as a constituent of phospholipids. Arachidonic acid is oxidised by the enzyme cyclo-oxygenase to prostaglandins (Rang and Dale, 1987). Prostaglandins induce redness (erythema) and swelling (edema) associated with heat and pain. These result in the development of inflammatory diseases such as asthma, articular rheumatism and psoriasis (Zurier, 1982).

Lesotho is a country in southern Africa where the majority of the population are Sotho speaking. As a developing country, it experiences the situation where traditional medicines play a vital role towards the well-being of the rural population, with knowledge being passed on by traditional healers and herbalists.

The present research focused on interviewing traditional healers and herbalists from different areas in Lesotho about their role in community health care and on plants used in traditional remedies to treat inflammations and bacterial infections. The plants were tested for the active compounds using the cyclo-oxygenase bioassay for anti-inflammatory activity and the disc-diffusion assay for anti-bacterial activity.

2. Materials and methods

2.1. Collection of ethnobotanical data

Twenty traditional healers and herbalists were interviewed by questionnaire. Ten were based in Mohale's Hoek district, a lowland area (altitude, 1860 m), and 10 were from Qacha's Nek district, a highland area (altitude, 2500 m). The questions asked ranged from the plants used for treating infections and inflammatory diseases, plant parts used, methods of extraction, extracting solvents, forms and dosage of medications, the storage and cultivation of plants for future use. Other aspects covered in the questionnaire included training and acquisition of knowledge, the period of study and training, number of patients treated per month, the cost of treatment and the extent of co-operation with Western doctors.

2.2. Plant material

Twenty-three plants used for the treatment of inflammation and bacterial infections were collected from Mohale's Hoek and Qacha's Nek districts in Lesotho during Spring (March) 1998. The interviewed traditional healers and herbalists assisted in the plant collection and information on plant usage. Voucher specimens of the plants were deposited at the herbarium at the Natal University, Pietermarizburg (Table 1). Plant material was dried at 50°C and stored at room temperature for future use.

2.3. The cyclo-oxygenase bioassay

Dried, powdered leaves and roots (500 mg) were extracted with 5 ml hexane, methanol or water, respectively, for 30 min in an ultrasound bath. The extracts were centrifuged, the supernatants decanted and air dried. The water extracts were resuspended in water (1 mg ml^{-1}) and the hexane and methanol extracts in ethanol (8 mg ml^{-1}).

The inhibition of prostaglandin biosynthesis by the plant extracts was investigated using the in vitro cyclo-oxygenase assay as described by Jäger et al. (1996), where sheep seminal vesicle microsomes were used as a source of the enzyme. The conversion of ^{14}C-arachidonic acid to prostagladins was measured to determine the degree of inhibition by the plant extracts.

Table 1
Traditional medicinal plants used by the Sotho to treat inflammatory diseases and bacterial infections[a]

Family	Species/voucher specimen	Plant part used	Traditional uses
Amaryllidaceae	*Boophane distica* (L.f) Herb. (SHALE 11 UN)	Bulb	Infusion used on external sores and wounds. The application also relieves rheumatic pain and draws out pus and pain
Asparagaceae	*Protasparagus microraphis* (Kunth) Oberm.(SHALE 15 UN)	Roots	Decoction drunk for rheumatism and menstrual pains
Asteraceae	*Aster bakeranus* Burtt Davy & C.A Sm (SHALE 6 UN)	Roots	Decoction drunk to treat syphilis and used to bathe syphilitic sores. Powder snuffed for headaches
	Dicoma anomala Sond. (SHALE 10 UN)	Roots	Decoction used a remedy for diarrhoea, stomach cramps and skin lesions. Dried roasted and ground plant material is mixed with fat and used as ointment for wounds and sores
	Haplocarpha scaposa Harv. (SHALE 2 UN)	Roots	Decoction drunk for internal sores and paste applied to infected sore ears
	Schkuhria pinnata (Lam.) Cabr. (SHALE 23 UN)	Leaves	Infusion used to treat stomach-aches
	Senecio sp. (SHALE 3 UN)	Whole plant	An infusion used as a gargle for throats and mouth sores. It is used in hot baths for treatment of rheumatic arthritic joints
	Xanthium spinosum L. (SHALE 17 UN)	Whole plant	Decoction used to treat gonorrhoea and syphilis
Euphorbiaceae	*Euphorbia clavarioides* Boiss (E. basutica Marl.) (SHALE 9 UN)	Roots	Infusion used externally to treat body rash, sores and wounds
Geraniaceae	*Monsonia brevirostrata* Knuth (SHALE 16 UN)	Whole plant	Powdered plant material applied on to sores resulting from sexually transmitted diseases (gonorrhoea)
	Pelargonium alchemilloides (L.) L' Hèrit (SHALE 1 UN)	Roots	Dried powder mixed with fat and applied to sore ears
Hypoxidaceae	*Hypoxis rigidula* Bak. var. rigidula (SHALE 13 UN)	Bulb	Infusion used for treating wounds and itching rash. Decoction drunk for asthma and arthritis
Lamiaceae	*Salvia repens* Burch. ex Benth. (SHALE 19 UN)	Whole plant	Used for stomach-aches and appendix problems
Leguminosae	*Rhynchosia adenoides*E. & Z. (SHALE 12 UN)	Roots	Decoction used to treat rheumatic pains, menstrual pains and dysentery
Malvaceae	*Malva parviflora* L. (SHALE 21 UN)	Whole plant	Infusion used as a lotion for bathing bruised limbs or to treat a broken limb. The dried powder is applied onto clean wounds
Periplocaceae	*Pachycarpus rigidus* E. Mey. (SHALE 4 UN)	Roots	Powder snuffed for the relief of headaches. Decoction drunk for colds and stomach-aches
Poaceae	*Festuca caprina* Nees. (SHALE 7 UN)	Roots	Infusion used for stomach-aches
Polygonaceae	*Rumex acetosella* L. (SHALE 14 UN)	Roots	Decoction used to bathe wounds and bruises
Solanaceae	*Solanum aculeatissimum* Jacq (SHALE 8 UN)	Whole plant	Decoction given after a miscarriage for internal cleaning. Dry powder rubbed into wounds or placed on painful teeth
	Solanum nigrum L. (SHALE 18 UN)	Whole plant	Decoction drunk for body pains, heart problems and to treat rash
	Aster sp. (SHALE 22 UN)	Whole plant	Infusion used to treat wounds and sores
–	*Cheilanthes sp*. (SHALE 24 UN)	Whole plant	Slightly roasted powdered plant material applied to wounds
–	*Chenopodium sp*.(SHALE 20 UN)	Whole plant	Decoction used to treat wounds and sores

[a] Voucher specimens: UN, Herbarium of the University of Natal Pietermaritzburg.

2.4. Anti-bacterial disc-diffusion bioassay

Dried, powdered leaves and roots (2 g) were extracted with hexane, methanol or water, respectively, for 30 min in an ultrasound bath. The plant extracts were left overnight, filtered and the filtrates evaporated to dryness. The residues were resuspended in 100 mg ml^{-1} hexane, methanol or water.

The disc-diffusion assay as described by Rasoanaivo and Ratsimamanga-Urverg (1993) and Rabe and van Staden (1997) was used to determine the growth inhibition of bacteria by the plant extracts. The test bacteria used were: *Micrococcus luteus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. These were maintained at 4°C on Molten Mueller-Hinton (MH). Ten microliters of the extracts were applied per filter paper disc (Whatman no. 3.6 mm diameter). Each extract was tested in quadruplicate (4 discs/plate). A neomycin (200–500 μg ml^{-1}) disc was used as a positive control. The screening was performed in duplicate.

3. Results

3.1. Traditional healers and herbalists' responses to the questionnaire

Twenty three plant species belonging to 13 families were collected with the assistance of the traditional healers and herbalists. The healers and herbalists also provided information on use and administration of the plant material and/or extracts (Table 1).

The healers and herbalists indicated that plants roots are the most commonly used part of the plant. Water is mostly used to extract the active compounds. The dosage of the prescribed medication depends on the strength of the medication and age of the patient. Orally taken medication ranges from four teaspoons per day for children to four cups per day for adults. Other means of administration are indicated in Table 1. Plants are normally air dried at room temperature and stored indoors for up to 5 years. Attempts to cultivate medicinal plants have been made. Some plants are easily cultivated, while others are unable to survive outside their natural habitat. Mainly highland plants are used in traditional remedies. Lowland healers obtain most of their plant material from highland areas, either by collecting them or buying them from highland gatherers.

Traditional healers acquire their knowledge from their ancestors/gods who are believed to convey the healing knowledge to the trainee through experienced healers. The training may last for up to 10 years depending on the individual. Herbalists acquire their healing knowledge through experience, which involves meetings and discussions with other herbalists and healers. Learning is a continuous process.

An experienced healer and herbalist may attend up to 100 patients per month. Charges range from R10.00 (approximately US$1.50) to a cow (live animal or money equivalent to the cost of a cow) depending on the extent of the treatment. Traditional healers and herbalists do sometimes refer their patients to modern doctors on occasions such as bone fracture, tuberculosis and extreme sexual diseases. However, traditional healers and herbalists have indicated that patients have not been referred to them by Western doctors.

3.2. Anti-inflammatory activity

Six of the 12 plants screened had anti-inflammatory activity above 90% (Table 2). Hexane and methanol extracts were the most active, while water extracts usually gave lower activity. There were a few exceptional cases where water extracts showed high inhibitory activity, e.g. *Protasparagus microraphis*, *Rhynchosia adenoides* and *Watsonia* sp. Both leaf and root extracts showed high inhibitory activity. Most of the other plant extracts had inhibitory activity between 60 and 90%, except for *Senecio* sp., which gave low activity from all the extracts tested.

3.3. Anti-bacterial activity

Extracts were considered highly active if their inhibition zone was between 0.70 and 1.00,

Table 2
Anti-inflammatory activity of hexane, methanol and water extracts from medicinal plants traditionally used in Lesotho[a]

Plant name	Plant parts analysed	Inhibition (%)		
		Hexane	Methanol	Water
Boophane disticha	Leaves	55	62	65
	Roots	44	24	7
Dicoma anomala	Leaves	86	85	5
	Roots	79	27	7
Festuca caprina	Leaves	78	85	69
	Roots	85	64	53
Malva parviflora	Leaves	62	57	23
	Roots	98	40	28
Pachycarpus rigidus	Leaves	85	91	41
	Roots	90	41	32
Protasparagus microraphis	Leaves	89	97	73
	Roots	97	69	73
Rhynchosia adenoides	Leaves	95	89	84
	Roots	93	66	88
Salvia repens	Leaves	0	83	47
	Roots	45	30	44
Schkuhria pinnata	Leaves	93	62	49
	Roots	80	79	45
Solanum nigrum	Leaves	83	81	35
	Roots	92	76	9
Senecio sp.	Leaves	20	52	44
	Roots	29	23	32
Watsonia sp.	Leaves	83	74	72
	Roots	54	56	83
Indomenthacin standard (20 μM)		87		

[a] The final screening concentration was 200 μg ml^{-1} for all the samples tested.

medium inhibitory activity with an inhibition zone between 0.30 and >0.70, and low or no inhibitory activity with an inhibition zone between 0.00 and >0.30.

Extracts from *Aster bakeranus* (roots), *Haplocarpha scaposa* (leaves and roots), *Malva parviflora* (roots), *Rumex acetosella (*leaves), *Solanum aceleatissimum* (leaves) and *Chenopodium* sp. (roots) had extreme highly inhibitory activity (Table 3). Methanol and water extracts were the most active against both gram-positive and gram-negative bacteria. Hexane extracts inhibited few bacteria. *Malva parviflora* extracts inhibited most of the tested bacteria including *E. coli*. This bacterium had great resistance to most of the tested extracts. Water extracts from *Chenopodium* sp. (roots) also showed high inhibitory activity against *E. coli*.

R. acetosella, *S. aceleatissimum*, *Chenopodium* sp., *Aster* sp. and *Senecio* sp. extracts showed medium inhibitory activity. The activity was mainly from root extracts and was most active against gram-positive bacteria. *Euphorbia clavariodes* and *Hypoxis rigidula* extracts did not have inhibitory activity against most bacteria. They did, however, show low inhibition against *B. subtilis* and *S. epidermis* from root and bulb extracts. *Monsonia brevirostrata* showed no anti-bacterial activity from all the tested extracts.

4. Discussion

The preliminary screening of plants for anti-inflammatory and anti-bacterial activity indicated that most of the plants tested were highly active. This supports their use in traditional remedies.

Table 3
Anti-bacterial activity of plants used in Lesotho by traditional healers and herbalists[a]

Species	Plant part analysed	Extraction solvent	Microorganisms tested[b]						
			Gram-positive				Gram-negative		
			M.I.	B.s.	S.a.	S.e.	E.c	P.a	K.p
Aster bakeranus	Leaves	Methanol	0.00	0.47	0.00	0.00	0.50	0.00	0.00
		Water	0.00	0.00	0.50	0.59	0.00	0.56	0.47
	Roots	Hexane	0.00	0.31	0.58	0.80	0.00	0.33	0.44
		Methanol	0.00	0.31	0.58	0.80	0.00	0.33	0.44
		Water	0.42	0.44	0.72	0.00	0.46	0.00	0.00
Haplocarpha scaposa	Leaves	Hexane	0.01	0.00	0.31	0.42	0.50	0.75	0.32
		Methanol	0.34	0.00	0.46	0.65	0.00	0.40	0.57
		Water	0.60	0.43	0.00	0.83	0.00	0.54	0.31
	Roots	Hexane	0.01	0.00	0.31	0.42	0.50	0.75	0.32
		Methanol	0.00	0.50	0.41	0.58	0.00	0.62	0.00
		Water	0.00	0.60	0.29	0.00	0.29	0.43	0.00
Xanthium spinosum	Leaves	Water	0.00	0.39	0.43	0.33	0.00	0.43	0.44
	Roots	Methanol	0.43	0.14	0.46	0.00	0.00	0.00	0.00
		Water	0.22	0.11	0.44	0.00	0.67	0.50	0.44
Euphorbia clavarioides	Roots	Hexane	0.00	0.43	0.00	0.00	0.00	0.00	0.00
		Methanol	0.22	0.00	0.00	0.33	0.00	0.22	0.00
Pelargonium alchemilloides	Leaves	Hexane	0.00	0.00	0.25	0.00	0.00	0.00	0.33
		Water	0.00	0.33	0.00	0.00	0.00	0.00	0.00
	Roots	Hexane	0.33	0.17	0.00	0.00	0.42	0.00	0.00
Hypoxis rigidula	Bulb	Hexane	0.00	0.43	0.00	0.00	0.00	0.00	0.00
		Methanol	0.22	0.00	0.00	0.33	0.00	0.22	0.00
Malva parviflora	Leaves	Methanol	0.00	0.00	0.34	0.00	0.40	0.00	0.00
	Roots	Hexane	0.00	0.50	0.94	0.00	0.00	0.56	0.55
		Methanol	0.00	0.53	0.00	0.06	0.00	0.60	0.63
		Water	0.38	0.75	0.58	0.00	0.70	0.70	0.44
Rumex acetosella	Leaves	Hexane	0.22	0.10	0.31	0.60	0.09	0.17	0.33
		Methanol	0.00	0.29	0.35	0.25	0.53	0.80	0.28
		Water	0.00	0.22	0.25	0.46	0.19	0.71	0.33
	Roots	Hexane	0.00	0.21	0.67	0.54	0.58	0.00	0.00
Solanum aceleatissimum	Leaves	Hexane	0.00	0.38	0.00	0.00	0.00	0.00	0.00
		Methanol	0.00	0.25	0.00	0.79	0.00	0.58	0.66
		Water	0.00	0.25	0.00	0.67	0.00	0.66	0.00
	Roots	Hexane	0.00	0.00	0.00	0.00	0.00	0.36	0.29
		Methanol	0.22	0.00	0.22	0.67	0.39	0.38	0.00
	Fruits	Hexane	0.50	0.00	0.00	0.00	0.57	0.00	0.00
		Methanol	0.41	0.38	0.00	0.00	0.58	0.33	0.00
		Water	0.00	0.50	0.00	0.46	0.50	0.00	0.00
Cheilanthes sp.	Whole plant	Water	0.00	0.64	0.00	0.00	0.67	0.00	0.00
Chenopodium sp.	Leaves	Methanol	0.60	0.00	0.00	0.00	0.00	0.25	0.00
		Water	0.00	0.00	0.00	0.00	0.00	0.50	0.00
	Roots	Water	0.63	0.38	0.00	0.75	0.81	0.00	0.00
Aster sp.	Leaves	Methanol	0.00	0.00	0.00	0.00	0.50	0.00	0.00
		Water	0.00	0.00	0.00	0.00	0.50	0.00	0.00
	Roots	Hexane	0.00	0.41	0.00	0.00	0.31	0.00	0.00
		Methanol	0.00	0.00	0.34	0.75	0.28	0.00	0.00
Senecio sp.	Leaves	Water	0.00	0.25	0.00	0.00	0.25	0.00	0.65
	Roots	Hexane	0.42	0.00	0.00	0.45	0.25	0.00	0.35
		Methanol	0.55	0.33	0.00	0.42	0.67	0.00	0.00

[a] All plants and plant parts were extracted with hexane, methanol and water. Extracts with no or little inhibitory activity (0.00–0.30) have been omitted. Only positive results are reported.

[b] M.l, *Micrococcus luteus*, B.s, *Bacillus subtilis*, S.a, *Staphylococcus aureus*, S.e, *Staphylococcus epidermis*, E.c, *Escherichia coli*, P.a, *Pseudomonas aeruginosa*, K.p, *Klebesiella pneumoniae*.

The screening in both assays (Tables 2 and 3) showed that most inhibitory activity was recorded with root extracts. This confirmed the leads from the interviewed healers and herbalists who use roots in preference to leaves. Other plant extracts which showed moderate or low anti-inflammatory activity in the cyclo-oxygenase bioassay may have active compounds but probably in smaller amounts and/or the screened crude extracts could yield more potent compounds once they had undergone some purification (Fabry et al., 1998). Also, extracts which showed medium or no anti-bacterial activity in the disc-diffusion bioassay may be active against other bacteria which were not tested. The knowledge of the traditional healers and herbalists should not to be underestimated or considered inferior to Western methods of treatment (Banquar, 1993). Incorporation of tradition healing into the health care system may promote the useful elements of traditional knowledge of the healers and herbalists (Sindinga, 1995).

Although water is reported by the traditional healers and herbalists to be the most commonly used solvent to extract the active compounds due to its easy availability, anti-inflammatory and anti-bacterial screening of the plants (Tables 2 and 3) generally resulted in higher inhibitory activity from hexane and methanol extracts compared with the water extracts. This suggests that water is not the most effective solvent at extracting the active compounds from plants. However, considering the prescribed dosage which may be as high as four cups per day per adult, water extracts can still be considered as an appropriate extracting solvent for traditional remedies. The same dosage from hexane and methanol extracts would be more concentrated, potentially becoming dangerous to the patients unless smaller dosages were prescribed. Hexane and methanol may also extract other compounds in higher concentrations that may cause the crude extracts to be toxic. The results indicated that a high number of plants used traditionally for remedies belong to the Asteraceae. Species from this family contain polyacetylene derivatives which have potent insecticidal effects when sensitized by near-ultraviolet light (phototoxicity) due to the production of activated oxygen species or other radicals, that damage the lipid membranes (Christensen and Lam, 1990; Guillet et al., 1997). This phototoxicity may also affect the results presented in this work but the same effects would be encountered by the traditional healers using these plants.

Although traditional healers and herbalists refer patients to Western doctors on certain occasions, the referral is one way as Western doctors do not send patients to traditional healers and herbalists. This indicates that there is little co-operation between Western doctors and traditional healers and herbalists. The incorporation of traditional healing in health care could improve the way of life for many people, especially those in rural areas. Traditional healers and herbalists need to be educated about certain Western diseases such as cancer and cardiovascular illness which are unlikely to be easily diagnosed by indigenous people (Cox, 1994). The diseases that are likely to be recognized by indigenous people include gastrointestinal maladies, inflammations, skin infections and certain viral diseases (Cox, 1994).

Geographically, Lesotho is surrounded by South Africa. This means that plants which are found in Lesotho are likely to occur in South Africa. Some plants used traditionally in South Africa by Zulu and Xhosa people for bacterial infections and inflammations are reported to be used for similar ailments by traditional healers in Lesotho. These include the same or similar species such as *Boophane distica*, *Cheilanthes eckloniana*, *Rhynchosia curibaea* and *Rumex sagitatus* (Hutchings, 1992), which are used by the Zulu for anti-inflammation. Screening of these plants using the cyclo-oxygenase bioassay resulted in detection of inhibitory activity (Jäger et al., 1996).

This preliminary screening of crude extracts made from plants used by traditional healers and herbalists in Lesotho showed that most of the screened plants are potentially a rich source of

anti-inflammatory and anti-bacterial agents. This demonstrates their importance in traditional remedies in rural populations where Western medicine is not readily available. The healers and herbalists traditional knowledge is a valuable guide in the selection of plants which can be used to isolate and identify active compounds. Work is currently being undertaken to isolate the active compound(s) by bioassay guided fractionation from some of the species that showed high inhibitory activity in this preliminary screening.

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