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Screening of Zulu medicinal plants for prostaglandin-synthesis inhibitors

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Abstract

Aqueous and ethanolic extracts of 39 plants used in traditional Zulu medicine to treat headache or inflammatory diseases were screened for prostaglandin-synthesis inhibitors. Extracts were tested in an in vitro assay for cyclooxygenase inhibitors. In general, ethanolic extracts caused higher inhibition than aqueous extracts. Two-thirds of the plants screened had high inhibitory activity. The highest inhibition was obtained with ethanolic extracts of *Bidens pilosa, Eucomis autumnalis, Harpephyllum caffrum, Helichrysum nudifolium, Leonotis intermedia, L. leonorus, Ocotea bullata, Rumex saggitatus, Solanum mauritianum, Synadenium cupulare* and *Trichilia dregeana.*

Keywords: Anti-inflammatory; Cyclooxygenase; Headache; Inhibitors; Prostaglandin-synthesis; Screening

1. Introduction

Traditional healing is widely practised in South Africa and it has been estimated that up to 80% of Zulu patients seen by medical practitioners also consult traditional healers (Gumede, 1989). Unfortunately, however, traditional healers have not officially been recognised by the state authorities in South Africa. The situation is now changing and there has for some time been active movement towards the integration of traditional healing into the official health care system (Holdstock, 1979; De Vos, 1988; Gumede, 1989; Pick, 1992). This correlates well with the philosophy underlying the new government's Reconstruction and Development Plan. There is thus an urgent need for an evaluation of traditional methods of treatment to facilitate the procedure of integration.

In this paper, we investigate plants used for either headache or inflammatory ailments (Hutchings and Van Staden, 1994) by screening them for prostaglandin-synthesis inhibitory activity. Prostaglandins are involved in the complex processes of inflammation and are responsible for the sensation of pain.

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Table 1
Zulu medicinal plants screened for prostaglandin-synthesis inhibitors

Plant name and family	Voucher specimen no.	Collection site	Plant part used	Ailment plant is used against	Administration	% Inhibition	
						H_2O	EtOH
Acokanthera oppositifolia (Lam.) Codd (Apocynaceae)	JÄGER22UN	1	Root	H	Snuffed	21%	78%
Albizia adianthifolia (Schumach.) W.F. Wright (Mimosaceae)	JÄGER13UN	1	Bark	H	Snuffed	7%	69%
Aptenia cordifolia (L.f.) Schwant. var. *cordifolia* (Mesembryanthemaceae)	HUTCHINGS3402ZUL	3	Aerial parts	I	Rubbed into cuts	15%	73%
Artemisia afra Jacq. ex Willd. (Asteraceae)		3	Aerial parts	H	Snuffed, steam inhaled	19%	65%
Asclepias physocarpa L. (Asclepiadaceae)	JÄGER1UN	4	Leaves	H	Snuffed	20%	41%
Bidens pilosa L. (Asteraceae)	JÄGER2UN	4	Leaves	I	Chewed	22%	90%
Boophane distica (L.f.) Herb (Amaryllidaceae)	JÄGER7UN	2	Bulb	H	Oral decoction, enema	0%	55 %
Bowiea volubilis Harv. ex Hook. (Hyacinthaceae)	JÄGER12UN	2	Bulb	H		45%	30%
Bulbine latifolia (L.F.) (Liliaceae)		3	Aerial parts	I	Rubbed into cuts	46%	88%
Clematis brachiata Thunb. (Ranunculaceae)	HUTCHINGS3412ZUL	3	Stem	H	Snuffed	23%	73%
Clerodendrum glabrum E. Mey (Verbenaceae)	JÄGER23UN	1	Root	I	Emetic	4%	88%
Croton silvaticus Hochst. (Euphorbiaceae)	JÄGER14UN	1	Bark	I	Rubbed into cuts	9%	59%
Drimia elata Jacq. (Liliaceae)	HUTCHINGS3418ZUL	3	Bulb		Rubbed into skin	8%	46%
Ekebergia capensis Sparrm. (Meliaceae)	JÄGER18UN	1	Root	H	Oral decoction	0%	82%
Eucomis autumnalis (Mill.) Chitt. (Hyacintaceae)		1	Bulb	H/I	Oral decoction/enema	73%	90%
Gnidia capitata L. (Thymelaceae)	JÄGER29UN	5	Aerial parts	H		75%	87%
Gnidia macropetala Meisn. (Thymelaceae)	JÄGER30UN	6	Aerial parts	H		65%	88%
Harpephyllum caffrum Bern. ex Krauss (Anacardiaceae)	JÄGER17UN	1	Bark	I	Rubbed into cuts	42%	93%
Helichrysum nudifolium (L.) Less. (Asteraceae)	HUTCHINGS3415ZUL	3	Leaves	H	Smoke inhaled	34%	96%
Helichrysum subglomerata Less. (Asteraceae)	JÄGER31UN	7	Aerial parts		Smoke inhaled	22%	69%

Hypoxis hemerocallidea Fisch. & Mey (Hypoxidaceae)		2	Corm			16%	48%
Lippia javanica (Burm. f.) Spreng. (Vermenaceae)	HUTCHINGS3416ZUL	3	Leaves	H/I	Oral decoction/rubbed into cuts	50%	53%
Leonotis intermedia Lindl. (Laminaceae)	JÄGER5UN	2	Leaves	H	Oral decoction	23%	97%
Leonotis leonorus (L.) (Laminaceae)	JÄGER9UN	2	Leaves	H	Oral decoction	2%	90%
Ocotea bullata (Burch.) Baill. (Lauraceae)	JÄGER16UN	1	Bark	H/I	Snuffed, smoke inhaled/ rubbed into cuts	82%	97%
Pittosporum viridiflorum Sims (Pittosporaceae)	JÄGER20UN	1	Bark	I	Oral decoction	0%	86%
Plumbago auriculata Lam. (Plumbaginaceae)	JÄGER4UN	2	Leaves	H	Snuffed	9%	45%
			Root	H	Snuffed	36%	—
Protorhus longifolia (Bernh.) Engl. (Anacardiaceae)	JÄGER21UN	1	Bark	I	Rubbed into cuts	62%	35%
Rumex sagittatus Thunb. (Polygonaceae)	JÄGER10UN	2	Root	H	Snuffed	76%	95%
Scilla natalensis Planch. (Liliaceae)		2	Bulb	I		11%	81%
Solanum mauritianum Scop. (Solanaceae)	JÄGER26UN	6	Leaves	H	Snuffed	0%	97%
Stangeria eriopus (Kunze) Baille. (Stangeniaceae)		8	Root	H/I	Burned, ash snuffed	56%	75%
Stapellia gigantea N.E. Br. (Asclepiadaceae)		3	Aerial parts	I	Rubbed into cuts	48%	75%
Synadenium cupulare (Boiss.) L.C. Wheeler (Euphorbiaceae)	JÄGER8UN	2	Leaves	H	Inhaled	28%	93%
Tetradenia riparia (Hochst.) Cood. (Laminaceae)	JÄGER6UN	2	Leaves	H	Inhaled	45%	86%
Trichilia dregeana Sond. (Meliaceae)	JÄGER15UN	1	Bark	I	Enema	30%	100%
Warburgia salutaris (Bertol.f.) Chiov. (Cannelaceae)	JÄGER19UN	1	Bark	H		14%	11%
Xysmalobium undulatum (L.) Ait.f. (Asclepiadaceae)	HUTCHINGS3417ZUL	3	Root	H		12%	72%
Ziziphus mucronata Willd. (Rhamnaceae)	JÄGER25UN	1	Leaves	*i*	Oral decoction	22%	89%

Indomethacin (0.5 μg) 66,5 ±2,5%

Water and ethanol extracts were tested for cycloxygenase inhibitory activity. Voucher specimens: UN, Herbarium of the University of Natal Pietermaritzburg. Collection sites: 1, Silverglen Nature Reserve, Durban; 2, Garden of the Botany Department, University of Natal Pietermaritzburg; 3, Garden of the Botany Department, University of Zululand, Empangeni; 4, Cleland, Pietermaritzburg; 5, Hayfields, Pietermaritzburg; 6, Ferncliff, Pietermaritzburg; 7, Sani Pass Border Station, Lesotho; 8, Chemistry Department, University of Natal, Durban. Ailments: H, Headache; I, Inflammation. Inhibitory activity of ethanol extract *P. auriculata* roots could not be determined due to high quenching.

2. Methodology

2.1. Ethnological data

The information on plant usage in this paper is based on literature surveys and interviews with traditional healers as described by Hutchings (1989a, 1989b) and Hutchings and Van Staden (1994).

2.2. Chemicals

Adrenalin, arachidonic acid, glutathione (reduced form), indomethacin, prostaglandin E_2 and F_2 were purchased from Sigma. [^{14}C]]arachidonic acid was obtained from Amersham and silica gel 60 (0.040–0.063 mm) from Merck.

2.3. Plant material

The botanical names, plant part used, voucher specimen numbers and collection sites for the plant material are given in Table 1.

The plant material was dried in an oven at 50°C and stored at room temperature in brown paper bags in the dark until extraction.

2.4. Extraction of plant material

Dried material (500 mg) was ground and extracted with 5 ml water or ethanol for 30 min in an ultrasound bath. The extraction mixtures were centrifuged, the supernatants decanted, or filtered when necessary, and then taken to dryness under vacuum. The residues were resuspended in water or ethanol, respectively, giving 2.5 mg residue/ml water and 20 mg residue/ml ethanol.

2.5. Preparation of sheep seminal vesicle microsomal fraction

All procedures were carried out at 4°C. Sheep seminal vesicles (100 g) were homogenised in 150 ml 0.1 M K-Pi, pH 7.4, containing 1 mM EDTA. The homogenate was centrifuged at 4000 × *g* for 15 min; the supernatant was further centrifuged at 17 000 × *g* for 10 min. The microsomes were isolated by centrifugation at 100 000 × *g* for 1 h. The microsomal pellet was resuspended in 0.1 M K-Pi, pH 7.4 and adjusted to 10 mg protein/ml. Aliqouts were stored at −70°C. Protein determinations were performed with the Bio-Rad protein assay kit.

2.6. Cyclooxygenase assay

The bioassays, with slight modifications, were performed according to the method of White and Glassman (1974). Ten microlitres (0.3 μg protein) of sheep seminal vesicle microsomes and 50 μl of co-factor solution (L-adrenalin and reduced glutathione, 0.3 mg/ml each in 0.1 M Tris buffer, pH 8.2) were preincubated in an ice bath for 15 min. Twenty microlitres of solvent, test solution or standard solution (20 μl of aqueous solutions; 2.5 μl of ethanolic solutions + 17.5 μl water; 2.5 μl of a 8×10^{-4} M ethanolic indomethacin solution + 17.5 μl water) and 20 μl [^{14}C]arachidonic acid (16 Ci/mole, 30 mM) were added and the assay mixture incubated at 37°C for 10 min. The reaction was terminated by adding 10 μl 2 N HCl. A blank was kept in the ice bath. After incubation 5 μl of a 0.2 mg/ml carrier solution of unlabelled prostaglandins (PGE_2:PGF_2 1:1) was added.

The prostaglandins were separated from unmetabolized arachidonic acid by column chromatography. Silica gel in eluent 1 (hexane–dioxane–acetic acid 350:150:1) was packed to a height of 3 cm in pasteur pipettes. One millilitre of eluent 1 was added to the assay mixture and this mixture was applied to the column. The reaction vial was washed with 1 ml of eluent 1, which was then applied to the column. The arachidonic acid was eluted with a further 3 ml of eluent 1. The prostaglandins were subsequently eluted with 3 ml eluent 2 (ethyl acetate–methanol 85:15) directly into scintillation vials. After mixing with scintillation solution the radioactivity was counted. All experiments were performed in duplicate.

3. Results and discussion

The results of the screening for cyclooxygenase inhibitors are given in Table 1.

Most of the plant remedies used for headache are ground powders taken as a snuff. Nasal application has the advantage that it avoids first-pass

metabolism and as such is a much more efficient way of administration than orally taken drugs. The sniffed drug is distributed over the nasal mucosa where active substances dissolve in the watery mucosa and pass directly into the blood. This way of administration can basically be viewed as an aqueous extraction. In order to simulate this, aqueous extracts as well as ethanolic extracts were prepared. Water and ethanol are the solvents most commonly available to traditional healers.

The indomethacin standard inhibited cyclooxygenase to a level of 66.5%. To classify a plant extract as active we decided on a minimum inhibition criterion that should be met. For water extracts, this was 50% and for ethanolic extracts 70% inhibition. Indomethacin (0.5 μg) or 50 μg plant extract residue was added to the assay, respectively. Thus, a compound with activity similar to indomethacin should constitute 1% of the residue or, if present in lower quantities, be more active than indomethacin. The minimum inhibition criterion serves as a working model to select plants for further investigation. Using these criteria, two-thirds of the plants screened could be classified as active. The high number of active plants in our screening shows that the ethno-approach is of considerable value.

The highest inhibition was obtained with ethanolic extracts of *Bidens pilosa*, *Eucomis autumnalis*, *Harpephyllum caffrum*, *Helichrysum nudifolium*, *Leonotis intermedia*, *L. leonorus*, *Ocotea bullata*, *Rumex saggitatus*, *Solanum mauritianum*, *Synadenium cupulare* and *Trichilia dregeana*.

Phytochemical information about most of the plants we tested is limited. Some work has been carried out on species from the same genus as those in our screening. It is likely that when active compounds are found in one species, more species of the same genus contain active compounds of a similar nature. In addition, when a species contains a compound with known anti-inflammatory activity that species should also be active.

Anti-inflammatory activity has been reported in species belonging to similar genera as the species we found to have inhibitory activity against cyclooxygenase: *Bidens campylotheca* (Redl et al., 1994), *Clematis* (Lewis, 1989), *Clerodendrum cyrtophyllum* (Lewis, 1989), *Helichrysum stoechas* (L.) (Reico et al., 1991), *Ziziphus sativa* (Shah et al., 1989) and *Z. spinachristi* (L.) Willd. (Tanira et al., 1988).

Solanum mauritianum contains solasodine (Drewes, 1994), a compound with known anti-inflammatory activity (Lewis, 1989). This could explain our finding that the ethanolic extract from this plant was active.

Essential oils of *Artemisia* species have been shown to possess anti-inflammatory activity (Lewis, 1989), therefore the relatively low activity we obtained with *A. afra* could be due to the extraction process, which was not aimed at extracting essential oils. Alternatively, *A. afra* contains essential oil of a different composition.

Croton silvaticus-extracts did not inhibit cyclooxygenase, but extracts from *C. cajucara* are reported to inhibit phospholipase A_2 in vitro (Ichihara et al., 1992), an earlier enzyme in the arachidonate pathway; so there might well be a rationale for the ethnopharmacological claim that *C. silvaticus* possesses anti-inflammatory properties.

A negative result in the cyclooxygenase assay does not necessarily mean that the plant is without anti-inflammatory activity: the active compound(s) could work at other sites in the complex processes of inflammation.

It is essential that any knowledge obtained from ethnopharmacologically studies is transferred back to the traditional practitioners. A project aimed at the compilation of a primary health care manual, written for and with traditional healers in collaboration with university departments, has recently been initiated.

Research is underway to isolate the active compounds by bioassay-guided fractionation from some of the species that showed the highest inhibitory activity.

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References

De Vos, A. (1988) Scientists and Sangomas. *Odyssey* August/ September, 18–19.

Drewes, F.E. (1994) Extraction, purification and determination of solasodine in cultures of *Solanum mauritianum* Scop. *Ph.D. Thesis*, University of Natal, Pietermaritzburg.

Gumede, M.V. (1989) Traditional healers — a Medical Doctor's Perspective. Skotaville, Johannesburg.

Holdstock, T.L. (1979) Indigenous healing in South Africa: a neglected potential. *South African Journal of Psychology* 9, 118–124.

Hutchings, A. (1989a) A survey and analysis of traditional medicinal plants as used by the Zulu, Xhosa and Sotho. *Bothalia* 19, 111–123.

Hutchings, A. (1989b) Observations on plant usage in Xhosa and Zulu medicine. *Bothalia* 19, 225–235.

Hutchings, A. and van Staden J. (1994) Plants used for stress-related ailments in traditional Zulu, Xhosa and Sotho medicine. Part 1: Plants used for headaches. *Journal of Ethnopharmacology* 43, 89–124.

Ichihara, Y., Takeya, K., Hitothuyanagi, Y., Morita, H., Okuyama, S., Suganuma, M., Fujiki, H., Motidome, M. and Itokawa, H. (1992) Cajucarinolide and isocajucarinolide: anti-inflammatory diterpenes from *Croton cajucara*. *Planta Medica* 58, 549–551.

Lewis, D.A. (1989) *Anti-inflammatory Drugs from Plant and Marine Sources. Agents and Actions Supplements*, Vol. 27. Birkhäuser, Basel, Boston, Berlin.

Pick, W.M. (1992) Primary health care — some lessons for South Africa. *South African Medical Journal* 82, 300–301.

Redl, K., Breu, W., Davis, B. and Bauer, R. (1994) Anti-inflammatory active polyacetylenes from *Bidens campylotheca*. *Planta Medica* 60, 58–62.

Reico, M.C., Giner, R., Terencio, M.C., Sanz, M.J. and Rios, J.L. (1991) Anti-inflammatory activity of *Helichrysum stoechas*. *Planta Medica* 57, Suppl. Issue 2, A56.

Shah, A.H., Al-Bekairi, A.M., Qureshi, S. and Ageel, A.M. (1989) *Ziziphus sativa* fruits: evaluation of some biological activities and toxicity. *Phytotherapy Research* 3, 232–236.

Tanira, M.O.M., Ageel, A.M., Tarig, M., Moshin, A. and Shah, A.H. (1988) Evaluation of some pharmacological, microbiological and physical properties of *Ziziphus spina-christi*. *International Journal of Crude Drug Research* 26, 56–60.

White, H.L. and Glassman, A.T. (1974) A simple radiochemical assay for prostaglandin synthetase. *Prostaglandins* 7, 123–129.