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Anti-inflammatory and phytochemical properties of twelve medicinal plants used for treating gastro-intestinal ailments in South Africa

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ABSTRACT

Ethnopharmacological relevance: The investigated medicinal plants are commonly used for the treatment of pains and cramps related to gastro-intestinal tract infections in South African traditional medicine.
Aims of the study: This study aimed to evaluate the ability of the plant extracts to inhibit cyclooxygenase enzymes. Phytochemical analysis was also carried out in the quest to determine some plant metabolites that may be responsible for the observed anti-inflammatory activity.
Materials and methods: The cyclooxygenase assay was used to test for the anti-inflammatory activity of the plant extracts using cyclooxygenase-1 and -2 (COX-1 and COX-2) enzymes. Total phenolic compounds including condensed tannins, gallotannins and flavonoids were quantitatively determined using spectrophotometric methods. Qualitative tests for alkaloids and saponins were also carried out.
Results: Most of the plant extracts evaluated showed dose dependent activity against COX-1 and/or COX-2 enzymes. *Agapanthus campanulatus* root dichloromethane extract showed the highest COX-2 inhibitory activity (83.7%) at 62.5 μg/ml. The presence and/or amounts of phenolics, condensed tannins, gallotannins, flavonoids, alkaloids and saponins varied with plant parts and species.
Conclusion: The results support the use of the investigated plant in treating pain and cramp related to gastro-intestinal tract infections. To some extent, the observed anti-inflammatory activity could be attributed to the various plant secondary metabolites detected in the plant materials.

1. Introduction

Gastro-intestinal ailments are associated with inflammation of the gastro-intestinal tract resulting in abdominal pains and cramps of varying degree (Barbara, 1998). Naik and Sketh (1976) defined inflammation as a complex, vascular lymphatic and local tissue reaction elicited in animals by the presence of viable and non-viable irritants. The intestine is vulnerable to muscle spasm in patients suffering from gastro-intestinal infections, and most patients suffering from such conditions often complain of abdominal cramps and pains (Sleisenger and Fordtrand, 1993). *Escherichia coli* and other gastro-intestinal pathogens associated with food poisoning produce enterotoxins that induce watery diarrhoea and abdominal tissue damage through plasmid-encoded invasion factors, resulting in acute or chronic abdominal pains and cramps (Naik and Sketh, 1976; Sleisenger and Fordtrand, 1993).

Non-steroidal anti-inflammatory drugs (NSAIDs) typically relieve inflammation and associated pain by inhibiting cyclooxygenase enzymes involved in the production of prostaglandins. These enzymes exist in two isoforms (COX-1 and COX-2) coded by distinct genes on different chromosomes (Polya, 2003). The two isoforms show about 50% homology and have similar catalytic activity, but are physiologically distinct (Pasinetti, 2001). Compounds that inhibit COX enzymes could therefore be considered to be potential anti-inflammatory drugs. However, many of the commonly used anti-inflammatory agents are becoming less acceptable due to serious adverse reactions such as gastric intolerance, bone marrow depression and water and salt retention, resulting from prolonged use (Xiao et al., 2005). This necessitates the continued search for potent anti-inflammatory agents with reduced or no side-effects. Studies based on the ethnobotanical use of plants have often proved to be a more efficient method of drug discovery than random plant screening (Slish et al., 1999; Khafagi and Dewedar, 2000). Some plant secondary metabolites such as alkaloids, phenols, tannins, glycosides, terpenoids, saponins, flavonoids and steroids have been implicated in their ability to inhibit the formation of pro-inflammatory signalling molecules such as prostaglandin or leukotrienes (Polya, 2003). In the present study, we evaluated 12 medicinal plants used traditionally in the treatment of pain associated with gastro-intestinal infections. The phytochemical components of these plants such as flavonoids, gallotannins, condensed tannins, other phenolic compounds, alkaloids and saponins

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Table 1
Medicinal plants used against gastrointestinal problems in South Africa.

Family	Species	Voucher number	Traditional uses
Agapanthaceae	*Agapanthus campanulatus* Leighton	FAW 4 NU	Root decoctions are taken orally or as enemas for stomach problems in children (Watt and Breyer-Brandwijk, 1962).
Asphodelaceae	*Haworthia limifolia* Marloth	FAW 3 NU	Decoction made from the leaves is used for stomach trouble (Hutchings et al., 1996).
Asteraceae	*Vernonia natalensis* Sch. Bip. ex Walp.	FAW 6 NU	Decoctions from leaves and stems are used for stomach cramps, nervous spasms of the stomach and other stomach ailments (Hutchings et al., 1996).
Cucurbitaceae	*Cucumis hirsutus* Sond	FAW 2 NU	Leaf and root decoctions are used for abdominal pain as well as diarrhoea (Hutchings et al., 1996).
Cyperaceae	*Cyperus textiles* Thunb.	FAW 9 NU	Root infusions are used as enemas for children with various stomach pains and cramps (Hutchings et al., 1996).
Ebenaceae	*Diospyros lycioides* Desf.	FAW 10 NU	Bark and root decoctions are taken for bloody faeces and dysentery (Hutchings et al., 1996).
Euphorbiaceae	*Antidesma venosum* E. Mey.ex Tul.	FAW 7 NU	Leaf decoctions are used for abdominal cramps and dysentery (Hutchings et al., 1996).
Iridaceae	*Gladiolus dalenii* van Geel	FAW 12 NU	Corm ground to a fine meal and mixed with warm water in small quantities to relieve dysentery, diarrhoea and stomach cramps (Margaret, 1990).
	Watsonia tabularis Bak	FAW 5 NU	Corms are used for diarrhoea in humans and calves (Hutchings et al., 1996).
Lamiaceae	*Becium obovatum* E. Mey. Ex Benth.	FAW 8 NU	Warm water infusions of pounded roots and leaves are administered as enemas to treat children with stomach ailments as well as abdominal pain (Pooley, 1998).
Melastomataceae	*Dissotis princeps* (Kunth) Triana	FAW 11 NU	Leaf infusions are administered as enemas for dysentery and diarrhoea (Hutchings et al., 1996).
Proteaceae	*Protea simplex* E. Phillips	FAW 1 NU	Decorticated root and bark infusions are used for dysentery, diarrhoea and stomach pains in humans (Hutchings et al., 1996).

were also evaluated. The antimicrobial and genotoxicity evaluation of the same plant materials have earlier been reported (Fawole et al., 2009).

2. Material and methods

2.1. Plant material

Twelve traditional medicinal plants that are commonly used for the treatment of gastro-intestinal ailments (Table 1) were collected between November (2007) and February (2008) from Mt. Gilboa (29°16.766′S, 30°17.627′E), Midmar (29°29.703′S, 30°12.417′E), University of KwaZulu-Natal Botanical Garden and Pietermaritzburg National Botanical Garden in KwaZulu-Natal Province, South Africa. Due to availability and consideration of potential sustainable harvesting of medicinal plants, the leaves of some plant species were substituted for their roots. Voucher specimens were identified by Prof T.J. Edwards (Curator) and lodged in the University of KwaZulu-Natal Herbarium, Pietermaritzburg. Plant materials were oven-dried at 50 °C, ground into powders and stored in airtight containers at room temperature in the dark.

2.2. Anti-inflammatory activity

2.2.1. Preparation of extracts

Ground plant materials (5 g) were sequentially extracted with 100 ml of petroleum ether (PE), dichloromethane (DCM) and 70% ethanol (EtOH) in a sonication bath (Julabo GMBH, West Germany) at room temperature for 1 h each. The extracts were then filtered under vacuum through Whatman No. 1 filter paper. Water extracts were prepared non-sequentially and freeze-dried while organic extracts were concentrated *in vacuo* using a rotary evaporator at 30 °C. The resultant extracts were air-dried at room temperature.

2.2.2. Cyclooxygenase assays

The cyclooxygenase assays (COX-1 and COX-2), as described by Eldeen and Van Staden (2008), were used to evaluate the anti-inflammatory activity of the extracts. Crude extracts were screened at a concentration of 250 μg/ml for organic extracts and 2 mg/ml for aqueous extract. Extracts showing good (≥50%) COX-2 inhibitory activity were then further evaluated at concentrations of 125 μg/ml and 62.5 μg/ml in both COX-1 and COX-2 assays. Indomethacin (Sigma) (5 μM for COX-1, 200 μM for COX-2) was used as a positive control, while background in which the enzyme was inactivated with HCl before adding [^{14}C] arachidonic acid, and solvent blank was used as negative controls. Percentage inhibition by the extracts was calculated by comparing the amount of radioactivity present in the sample to that in the solvent blank using the equation:

$$\text{COX inhibition}\,(\%) = \left[1 - \left(\frac{\text{DPMsample} - \text{DPMbackground}}{\text{DPMblank} - \text{DPMbackground}}\right)\right] \times 100$$

where DPMsample is the disintegrations per minute for plant extract, DPMbackground the disintegrations per minute in which the enzyme was inactivated and DPMblank is the disintegrations per minute for solvent used in dissolving plant extracts.

Results are expressed as means ± standard errors of two independent experiments, each experiment in duplicate.

2.3. Phytochemical analysis

2.3.1. Preparation of extracts

Phenolic compounds were extracted from plant materials as described by Makkar (1999) with slight modification. Dried plant samples (2 g) were extracted with 10 ml of 50% aqueous methanol by sonication in cold water for 20 min. The extracts were then filtered under vacuum through Whatman No. 1 filter paper.

2.3.2. Determination of total phenolics

The amount of total phenolics in plant samples was determined using the Folin *Ciocalteu* (Folin C.) assay for total phenolics as described by Makkar (1999). Fifty microlitres of each extract from the plant samples were transferred into test tubes and 950 μl distilled water were added to make up to 1 ml, followed by 1N Folin C. reagent (500 μl) and 2% sodium carbonate (2.5 ml). A blank that contained aqueous methanol instead of plant extracts was also prepared. The test mixtures were incubated for 40 min at room temperature and the absorbance read at 725 nm using a UV–vis spectrophotometer (Varian Cary 50). Each extract had three repli-

Table 2
Inhibitory activity (COX-1 and COX-2) of different plant extracts evaluated at 250 μg/ml.

Plant species	Plant part	Percentage inhibition							
		COX-1				COX-2			
		PE	DCM	EtOH	Water	PE	DCM	EtOH	Water
Agapanthus campanulatus	L	92.6 ± 1.1	78.4 ± 7.2	12.8 ± 0.8	74.2 ± 5.9	72.3 ± 9.1	68.1 ± 3.8	16.9 ± 9.5	47.5 ± 3.7
	R	97.7 ± 1.4	98.4 ± 1.0	48.1 ± 9.3	33.4 ± 3.3	78.0 ± 4.4	97.0 ± 1.2	9.1 ± 21.0	28.8 ± 4.0
Antidesma venosum	L	103.0 ± 0.8	72.8 ± 4.3	84.3 ± 7.0	36.2 ± 6.1	46.6 ± 12.3	40.9 ± 9.9	40.9 ± 10.5	0.0
Becium obovatum	R	78.5 ± 4.0	86.4 ± 2.6	4.1 ± 3.0	85.3 ± 1.9	76.6 ± 0.8	62.6 ± 9.3	0.0	1.2 ± 17.0
Cucumis hirsutus	L	91.7 ± 2.1	101.8 ± 1.1	29.1 ± 5.0	26.0 ± 9.4	80.3 ± 3.5	81.5 ± 4.1	0.0	0.0
Cyperus textilis	R	91.7 ± 5.0	88.5 ± 4.9	81.4 ± 8.9	61.3 ± 0.69	75.6 ± 9.8	73.5 ± 2.4	47.9 ± 24.3	0.0
	L	86.3 ± 0.7	88.4 ± 0.5	79.8 ± 8.9	0.0	75.0 ± 1.8	83.0 ± 0.1	32.8 ± 1.0	0.0
Diospyros lycioides	L	92.8 ± 0.9	94.0 ± 5.7	90.4 ± 4.3	37.1 ± 0.2	91.6 ± 1.9	84.8 ± 1.3	72.0 ± 2.3	0.0
	S	73.8 ± 3.6	81.0 ± 2.1	70.6 ± 1.2	13.1 ± 5.7	67.7 ± 0.3	65.9 ± 1.7	37.9 ± 10.1	0.0
Dissotis princeps	L	58.8 ± 1.2	82.7 ± 2.3	87.1 ± 4.0	4.8 ± 2.8	60.6 ± 7.7	67.2 ± 9.5	22.1 ± 1.1	0.0
Gladiolus dalenii	C	88.3 ± 1.5	101.8 ± 0.3	53.1 ± 4.4	34.4 ± 0.1	68.4 ± 5.7	100.6 ± 3.5	35.6 ± 5.7	0.0
Haworthia limifolia	L	88.3 ± 3.8	83.5 ± 1.9	1.3 ± 4.1	30.7 ± 10.5	82.4 ± 2.3	72.3 ± 2.8	0.0	0.0
Protea simplex	L	100.1 ± 0.8	80.6 ± 8.7	23.7 ± 6.1	57.8 ± 14.4	72.4 ± 1.1	68.4 ± 3.7	0.0	20.9 ± 6.9
	B	94.2 ± 3.7	86.1 ± 7.1	89.2 ± 7.8	90.5 ± 1.2	41.0 ± 7.4	35.8 ± 2.4	20.0 ± 2.1	16.7 ± 0.3
Vernonia natalensis	L	88.5 ± 3.2	77.5 ± 3.4	51.2 ± 12.4	38.7 ± 3.3	86.7 ± 0.9	63.4 ± 0.5	0.0	0.0
Watsonia tabularis	C	97.6 ± 5.3	73.9 ± 5.1	90.3 ± 3.4	50.3 ± 1.8	91.5 ± 0.9	80.5 ± 8.7	51.1 ± 8.8	0.0

Percentage inhibition of prostaglandin synthesis by indomethacin was 70 ± 3.3 for COX-1 and 68.9 ± 2.5 for COX-2. B, bark; C, corm; L, leaf; R, root; S, stem.

cates and total phenolic concentrations were expressed as gallic acid equivalents (GAE).

2.3.3. The butanol–HCl assay for condensed tannins

Three millilitres of butanol–HCl reagent (95:5, v/v) were added to 500 μl of each extract, followed by 100 μl ferric reagent (2% ferric ammonium sulphate in 2N HCl). The test combination was vortexed and placed in a boiling water bath for 60 min. The absorbance was then read at 550 nm using a UV–vis spectrophotometer (Varian) against a blank prepared by mixing extract (500 μl) with butanol–HCl reagent (3 ml) and ferric reagent (100 μl), but without heating. Each extract had three replicates. Condensed tannin (% per dry matter) was calculated as equivalent amount of leucocyanidins using the formula developed by Porter et al. (1986):

$$\text{Condensed tannin}\,\% = \frac{A_{550\,\text{nm}} \times 78.26 \times \text{dilution factor}}{\%\,\text{dry matter}}$$

2.3.4. Rhodanine assay for gallotannins

Plant extracts (50 μl) were made up to 1 ml with distilled water. One hundred microlitres of 0.4N sulphuric acid and 600 μl rhodanine were added to the diluted extracts. After 5 min, 200 μl of 0.5N potassium hydroxide were added and 4 ml distilled water after 2.5 min. The mixtures were left for a further 15 min at room temperature, after which the absorbance at 520 nm was read using a UV–vis spectrophotometer against a blank test that contained methanol instead of sample. Each extract was evaluated in replicates and gallotannin concentrations were expressed as gallic acid equivalents (GAE) (Makkar, 1999).

2.3.5. Vanillin assay for flavonoids

Plant extracts (50 μl) (in triplicate), were made up to 1 ml with distilled water in test tubes before adding 2.5 ml methanolic-HCl (95:5, v/v) and 2.5 ml vanillin reagent (1 g/100 ml). Similar preparations of a blank that contained methanol instead of plant extracts were made. After 20 min at room temperature, absorbance at 500 nm was read using a UV–vis spectrophotometer (Varian) against the blank. The flavonoids in the plant extracts were expressed as catechin equivalents (Makkar, 1999).

2.3.6. Thin layer chromatography for alkaloid detection

Ten microlitres each of PE, DCM, EtOH and water extracts (50 mg/ml) (prepared as described in Section 2.2.1) were spotted on thin layer chromatographic (TLC) plates (Silica gel 60 F_{254}, Merck, Germany). The plates were developed using hexane:benzene:ethyl acetate (5:2:3) for PE and DCM extracts, while ethyl acetate:methanol:water (100:16.5:13.5) was used for EtOH and water extracts. After development, the plates were dried, viewed under UV (254, 366 nm) and the fluorescence noted. The presence of alkaloids was indicated by red coloration when the plates were sprayed with Dragendorff reagent (Robert, 1962; Wilfred and Ralph, 2006).

2.3.7. Froth test for saponins

Distilled water (10 ml) was added to 0.1 g of ground plant sample in a test tube. The test tube was corked and vigorously shaken for 2 min. The appearance of stable foam on the liquid surface for 45 min indicated the presence of saponins. To confirm the presence of saponins, 10 drops of olive oil were added to the aqueous extract (2 ml) in a test tube. The test tube was then corked and vigorously shaken. The formation of an emulsion confirmed the presence of saponins (Tadhani and Subhash, 2006).

3. Results and discussion

3.1. Anti-inflammatory activity

The percentage inhibition of COX-1 and COX-2 by all the extracts at 250 μg/ml is presented in Table 2. Plant extracts showing a minimum inhibition of 50% are considered to have good activity (Eldeen and Van Staden, 2008). All the PE and DCM extracts of the plant material (with the exception of *Antidesma venosum* leaf and *Protea simplex* bark) showed good activity against both COX-1 and COX-2 (inhibition of prostaglandin synthesis ranging from 58.8 to 103%). Generally, all the ethanol (except *Diospyros lycioides* leaf and *Watsonia tabularis* corm) and water extracts showed weak or no activity (inhibition < 50%) against COX-2. However, water extracts of *Agapanthus campanulatus* leaf, *Becium obovatum* root, *Cyperus textilis* root, *Protea simplex* leaf and bark, and *Watsonia tabularis* corm exhibited good activity mainly against COX-1 enzyme with inhibition ranging from 50.3 to 90.5%.

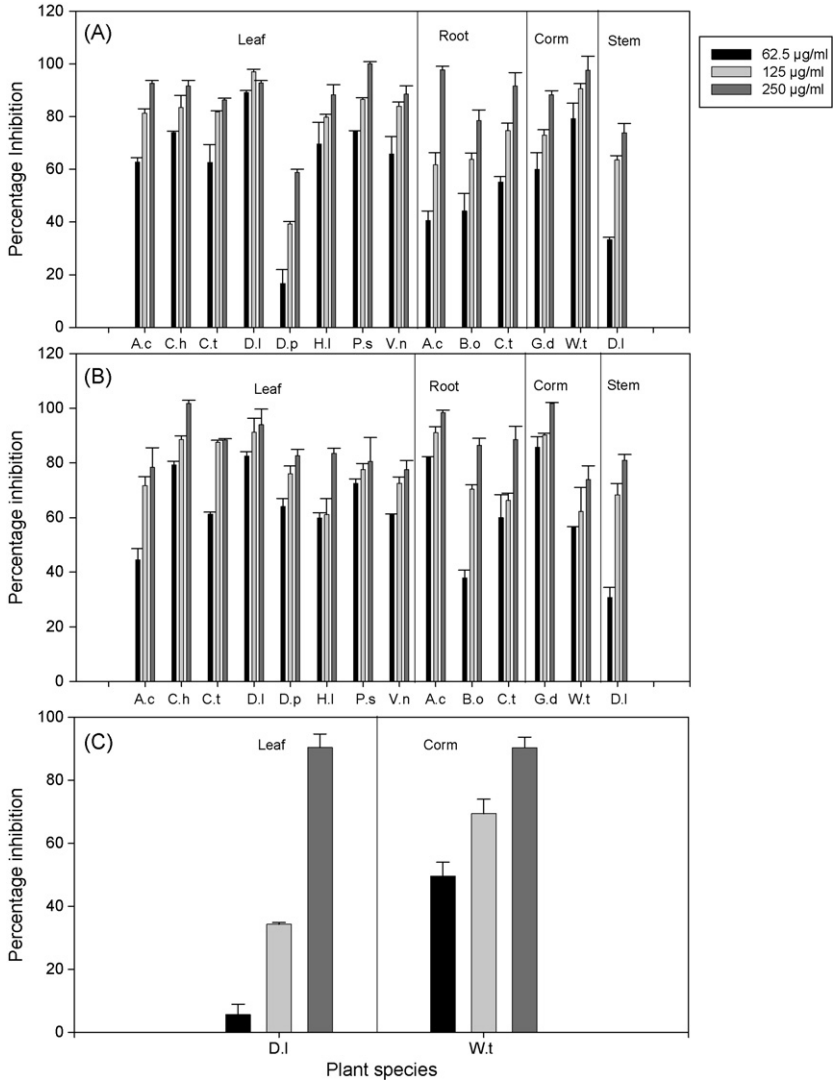

Fig. 1. Dose-dependent COX-1 percentage inhibition by different plant extracts. (A) Petroleum ether extracts, (B) dichloromethane extracts, and (C) ethanol extracts. Percentage inhibition of prostaglandin synthesis by indomethacin was 70 ± 3.3. A.c, *Agapanthus campanulatus*; B.o, *Becium obovatum*; C.h, *Cucumis hirsutus*; C.t, *Cyperus textilis*; D.l, *Diospyros lycioides*; D.p, *Dissotis princeps*; G.d, *Gladiolus dalenii*; H.l, *Haworthia limifolia*; P.s, *Protea simplex*; V.n, *Vernonia natalensis*; W.t, *Watsonia tabularis*.

Most of the plant extracts evaluated showed dose dependent activity against COX-1 and/or COX-2 enzymes (Figs. 1 and 2 respectively). Interestingly, at 62.5 μg/ml concentration, 16 out of 28 PE and DCM extracts evaluated still showed good activity (>50%) against the COX-2 enzyme. Generally, most of the extracts showed higher percentage inhibition for COX-1 than for COX-2 at the highest screening concentration (250 μg/ml). Although COX-2 specific inhibitors have been suggested to be potential classical non-steroid anti-inflammatory drugs due to their reduced or no side-effects, some authors have reported that they also have a non-negligible risk of gastro-intestinal toxicity in some patients (MacAulay and Blackburn, 2002; Bertin, 2004; Warner and Mitchell, 2008). However, the observed activity in many of the extracts supports their uses in South African traditional medicine. In the case of extracts showing weak or no activity in these assays, high dosages of extract are often used in traditional medicine which may result in COX inhibition. Some of these extracts might be active at other sites in the inflammatory pathways and/or contain compounds showing better activity *in vivo* as they undergo metabolic transformation (McGaw et al., 1997; Garcia et al., 2003). In the human inflammatory process, for example, anti-inflammatory activity of medicinal plants could be manifested in the inhibition of nuclear transcriptase factor (NFκB) mediated signalling pathway in immune cells that lead to the production of inducible nitric oxide synthase (iNOS), pro-inflammatory cytokines and inducible cyclooxygenase (iCOX) (Polya, 2003). Moreover, the presence of comparable activities in the leaves and root or stem of *Agapanthus campanulatus*, *Cyperus textilis*, *Diospyros lycioides*, and *Protea simplex* supports the idea of plant part substitution for sustainable use of many highly threatened plants (Zschocke and Van Staden, 2000).

3.2. Phytochemical analysis

3.2.1. Total phenolics composition

Fig. 3 shows the total phenolic compounds' concentrations of the investigated plant species. All the plant species evaluated contained some phenolic compounds. The highest concentration of total phenolics was detected in *Cyperus textilis* leaf (84.5 mg GAE/g). In addition, *Becium obovatum* root, *Protea simplex* leaf and bark, and *Cyperus textilis* root contained total phenolic concentrations ≥ 50 mg GAE/g. Phenolic compounds are of important pharmacological value, some having anti-inflammatory properties (Bruneton, 1995). Different types of phenolic compounds such as flavonoids, condensed tannins, and gallotannins are known to

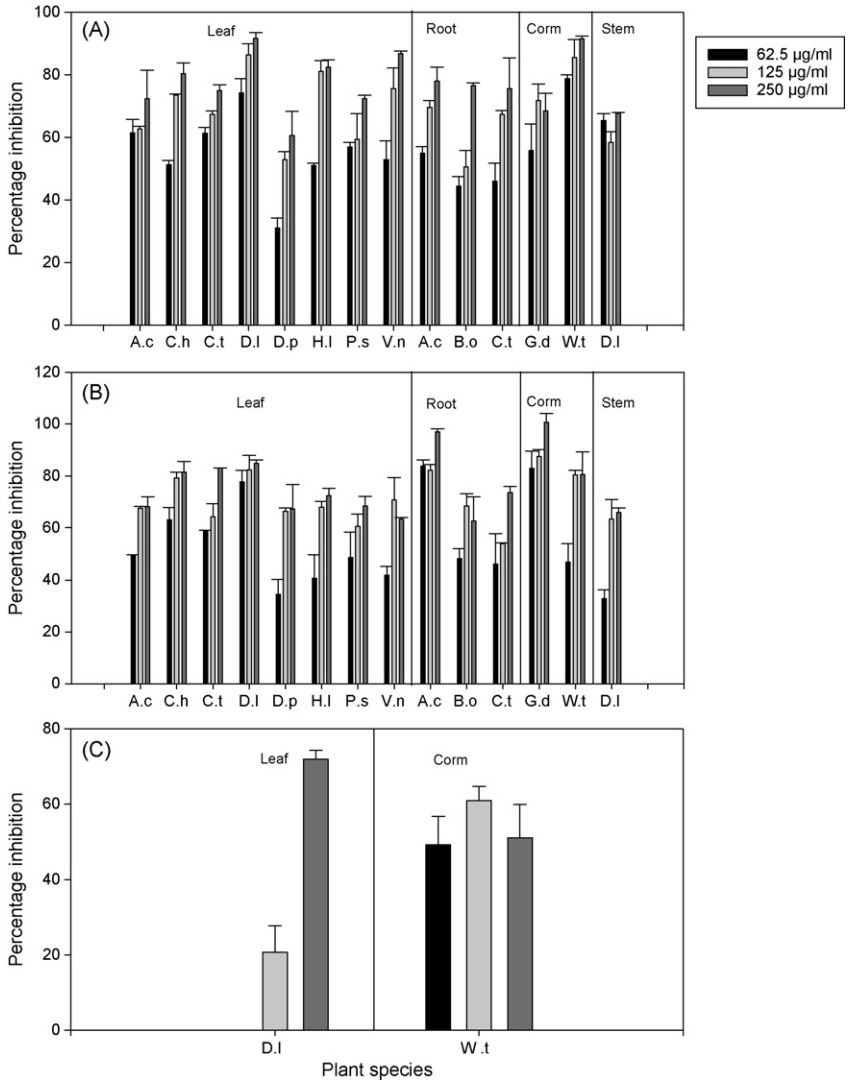

Fig. 2. Dose-dependent COX-2 percentage inhibition by different plant extracts. (A) Petroleum ether extracts, (B) dichloromethane extracts, and (C) ethanol extracts. Percentage inhibition of prostaglandin synthesis by indomethacin was 68.9 ± 2.5. A.c, *Agapanthus campanulatus*; B.o, *Becium obovatum*; C.h, *Cucumis hirsutus*; C.t, *Cyperus textilis*; D.l, *Diospyros lycioides*; D.p, *Dissotis princeps*; G.d, *Gladiolus dalenii*; H.l, *Haworthia limifolia*; P.s, *Protea simplex*; V.n, *Vernonia natalensis*; W.t, *Watsonia tabularis*.

inhibit some molecular targets of pro-inflammatory mediators in inflammatory responses (Sharma et al., 1994; Iwalewa et al., 2007). Specific types of phenolic compounds present in these species were therefore investigated.

3.2.2. Condensed tannins contents

The amounts of condensed tannins expressed as percentage per dry matter are shown in Fig. 4. Highest amounts of condensed tannins were detected in *Cyperus textilis* leaves and roots while low levels were detected in *Gladiolus dalenii* and *Haworthia limifolia*. No condensed tannins were detected in *Becium obovatum* root, *Agapanthus campanulatus* root and *Vernonia natalensis* leaf. Condensed tannins (proanthocyanidins) are essentially derived from (+) gallocatechin, (−) epicatechin, (+) catechin and epigallocatechin, and their derivatives via carbon to carbon (C–C) links. These compounds are antagonists of particular hormone receptors or inhibitors of particular enzymes such as cyclooxygenase enzymes (Polya, 2003).

3.2.3. Rhodanine assay for gallotannins

Fig. 5 presents the amounts of gallotannins present in the investigated plants. With the exceptions of *Agapanthus campanulatus* root and *Gladiolus dalenii* corm, gallotannins were detected in all

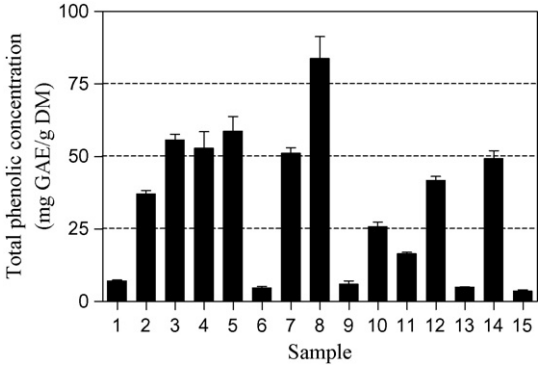

Fig. 3. Total phenolic compounds per dry matter (DM) of 12 medicinal plants traditionally used for treating gastro-intestinal ailments. 1, *Haworthia limifolia* (leaf); 2, *Cucumis hirsutus* (leaf); 3, *Becium obovatum* (root); 4, *Protea simplex* (leaf); 5, *Protea simplex* (bark); 6, *Agapanthus campanulatus* (root); 7, *Cyperus textilis* (root); 8, *Cyperus textilis* (leaf); 9, *Vernonia natalensis* (leaf); 10, *Watsonia tabularis* (corm); 11, *Antidesma venosum* (leaf); 12, *Diospyros lycioides* (leaf); 13, *Diospyros lycioides* (stem); 14, *Dissotis princeps* (leaf); 15, *Gladiolus dalenii* (corm).

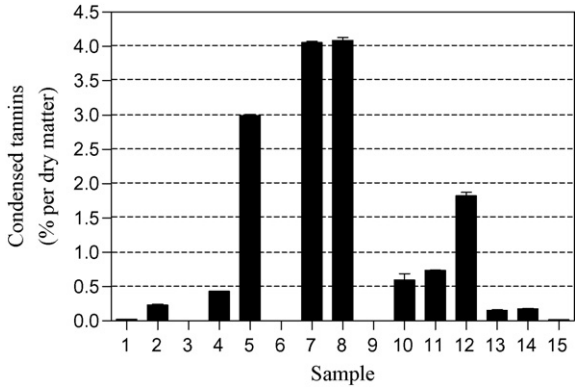

Fig. 4. Percentage condensed tannins as leucocyanidins equivalents of 12 medicinal plants traditionally used for treating gastro-intestinal ailments. 1, *Haworthia limifolia* (leaf); 2, *Cucumis hirsutus* (leaf); 3, *Becium obovatum* (root); 4, *Protea simplex* (leaf); 5, *Protea simplex* (bark); 6, *Agapanthus campanulatus* (root); 7, *Cyperus textilis* (root); 8, *Cyperus textilis* (leaf); 9, *Vernonia natalensis* (leaf); 10, *Watsonia tabularis* (corm); 11, *Antidesma venosum* (leaf); 12, *Diospyros lycioides* (leaf); 13, *Diospyros lycioides* (stem); 14, *Dissotis princeps* (leaf); 15, *Gladiolus dalenii* (corm).

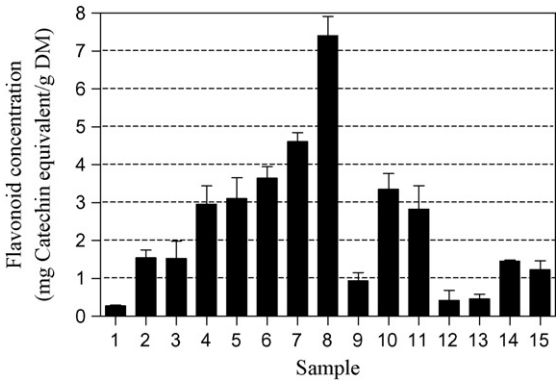

Fig. 6. Flavonoid concentration as catechin equivalent of 12 medicinal plants traditionally used for treating gastro-intestinal ailments. 1, *Haworthia limifolia* (leaf); 2, *Cucumis hirsutus* (leaf); 3, *Becium obovatum* (root); 4, *Protea simplex* (leaf); 5, *Protea simplex* (bark); 6, *Agapanthus campanulatus* (root); 7, *Cyperus textilis* (root); 8, *Cyperus textilis* (leaf); 9, *Vernonia natalensis* (leaf); 10, *Watsonia tabularis* (corm); 11, *Antidesma venosum* (leaf); 12, *Diospyros lycioides* (leaf); 13, *Diospyros lycioides* (stem); 14, *Dissotis princeps* (leaf); 15, *Gladiolus dalenii* (corm).

the investigated species. The highest amount of gallotannin was detected in *Protea simplex* leaf (13.5 μg/g dry matter). Gallotannins exert various biological effects ranging from anti-inflammatory to anticancer and antiviral properties (Erde'lyi et al., 2005). The mechanisms underlying the anti-inflammatory effect of tannins include the scavenging of radicals and inhibition of the expression of inflammatory mediators, such as some cytokines, inducible nitric-oxide synthase, and cyclooxygenase-2 (Polya, 2003; Erde'lyi et al., 2005). The high amounts of gallotannins present in some of the evaluated plant materials could in part be responsible for the observed high anti-inflammatory activity.

3.2.4. Vanillin assay for flavonoids

The flavonoid concentrations present in the investigated plant materials are shown in Fig. 6. The highest (7.4 mg/g) and the lowest (0.24 mg/g) amounts were detected in *Cyperus textilis* and *Haworthia limifolia* leaves respectively. According to Talhouk et al. (2007), flavonoids are known to act on the inflammatory response via many routes and block molecules like COX, iNOS, cytokines, nuclear factor-κB and matrix metalloproteinases. Some flavonoids have been reported to be effective against acute inflammation *in vivo* using a carrageenin-induced mouse paw oedema model (Pelzer et al., 1998).

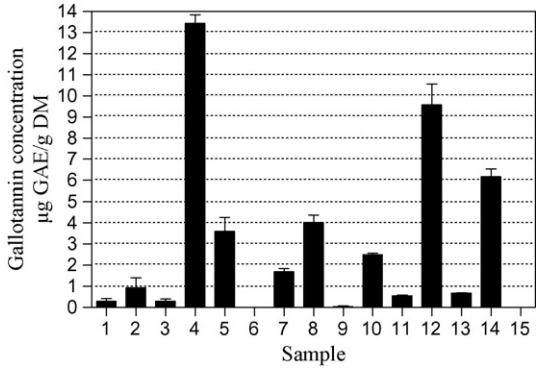

Fig. 5. Gallotannin concentrations per dry matter (DM) of 12 medicinal plants traditionally used for treating gastro-intestinal ailments. 1, *Haworthia limifolia* (leaf); 2, *Cucumis hirsutus* (leaf); 3, *Becium obovatum* (root); 4, *Protea simplex* (leaf); 5, *Protea simplex* (bark); 6, *Agapanthus campanulatus* (root); 7, *Cyperus textilis* (root); 8, *Cyperus textilis* (leaf); 9, *Vernonia natalensis* (leaf); 10, *Watsonia tabularis* (corm); 11, *Antidesma venosum* (leaf); 12, *Diospyros lycioides* (leaf); 13, *Diospyros lycioides* (stem); 14, *Dissotis princeps* (leaf); 15, *Gladiolus dalenii* (corm).

3.2.5. Alkaloids detection

The presence or absence of alkaloids in the investigated plant extracts are summarized in Table 3. Twelve out of 48 extracts evaluated showed the presence of alkaloids. Previous researchers have reported the presence of alkaloids in *Antidesma venosum*, *Vernonia* sp., and *Diospyros lycioides* (Watt and Breyer-Brandwijk, 1962; Hutchings et al., 1996; Ndukwe et al., 2004). Some alkaloids such as isoquinoline, indole and diterpene are known to have good anti-inflammatory activity (Barbosa-Filho et al., 2006).

3.2.6. Saponins detection

All the evaluated plant materials except *Haworthia limifolia*, *Protea simplex*, *Antidesma venosum* and *Dissotis princeps* leaves tested positive for saponins. The anti-inflammatory activities of some saponin derivatives such as triterpenoids saponins have been reported (Sahu and Mahato, 1994). According to Sparg et al. (2004), many saponins extracted from plant sources produce an inhibition of inflammation in the mouse carrageenin-induced oedema assay.

Table 3
Detection of alkaloids in 12 medicinal plants traditionally used for treating gastro-intestinal ailments in South Africa.

Plant name	Plant part	Extracts		
		PE	DCM	EtOH
Agapanthus campanulatus	Leaves	–	–	+
	Roots	–	–	–
Antidesma venosum	Leaves	+	–	–
Becium obovatum	Roots	–	+	–
Cucumis hirsutus	Leaves	–	–	+
Cyperus textiles	Roots	–	+	–
	Leaves	–	–	–
Diospyros lycioides	Leaves	+	–	–
	Stems	–	–	–
Dissotis princeps	Leaves	–	+	–
Gladiolus dalenii	Corms	–	–	–
Haworthia limifolia	Leaves	+	–	+
Protea simplex	Leaves	–	–	+
	Bark	–	–	+
Vernonia natalensis	Leaves	+	–	–
Watsonia tabularis	Corms	–	–	–

–, absence; +, presence.

4. Conclusions

As far as we can ascertain, the anti-inflammatory activity and phytochemical properties of many of the investigated plant species have not been reported, yet they are extensively used in traditional medicine. To a large extent, the results in this study validate the traditional medicinal use of the evaluated plant species in treating stomach pains and cramps associated with gastro-intestinal infections. The study of their phytochemical constituents might be considered sufficient for further studies aimed at isolating and identifying the active principle(s) as well as potential combination effects (if any) of the isolated compounds as some of these plants are frequently included in multiple decoctions.

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