Journal of Ethnopharmacology 127 (2010) 235–241

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Anti-inflammatory, anticholinesterase, antioxidant and phytochemical properties of medicinal plants used for pain-related ailments in South Africa

O.A. Fawole, S.O. Amoo, A.R. Ndhlala, M.E. Light, J.F. Finnie, J. Van Staden*

Research Centre for Plant Growth and Development, School of Biological and Conservation Sciences, University of KwaZulu-Natal Pietermaritzburg, Private Bag X01, Scottsville 3209, South Africa

ARTICLE INFO

Article history:
Received 29 September 2009
Received in revised form
12 November 2009
Accepted 13 November 2009
Available online 20 November 2009

Keywords:
Acetylcholinesterase
Anti-inflammatory
Antioxidant
Cyclooxygenase
DPPH

ABSTRACT

Ethnopharmacological relevance: Extracts of seven South African medicinal plants used traditionally for the treatment of pain-related ailments were evaluated.
Aims of the study: The study was aimed at evaluating medicinal and therapeutic potentials of the investigated traditional medicinal plants. Plant extracts were evaluated for anti-inflammatory activity and other pharmacological properties such as anticholinesterase and antioxidant activities. Phytochemical analysis of total phenolic contents, condensed tannins, gallotannins and flavonoids in the aqueous methanol extracts of the medicinal plants were also carried out.
Materials and methods: The evaluation of anti-inflammatory activity of 50% methanol (50% MeOH), petroleum ether (PE), dichloromethane (DCM) and ethanol (EtOH) plant extracts was done against cyclooxygenase-1 and -2 (COX-1 and COX-2) enzymes. 50% MeOH, PE, DCM and EtOH extracts were tested for acetylcholinesterase (AChE) inhibition, while 50% MeOH extracts were tested for 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging activity and ferric-reducing power in the antioxidant assays. Total phenolic compounds, condensed tannins, gallotannins and flavonoids were quantitatively determined using spectrophotometric methods.
Results: At the screening assay concentration (0.25 μg/μl), 13 extracts showed good COX-1 inhibitory activity (>50%), while good activity was observed in 15 extracts against COX-2 enzyme. All the extracts of *Crinum moorei* (bulbs) showed good inhibition against both COX-1 and COX-2 enzymes. Though not significantly different ($P = 0.05$), the highest COX-1 percentage inhibition (100%) was shown by *Aloe ferox* leaf PE and *Colocasia antiquorum* tuber DCM extracts, while *Colocasia antiquorum* tuber PE extract exhibited the highest (92.7%) percentage inhibition against COX-2. *Crinum moorei* bulb DCM extract showed the lowest EC_{50} value (2.9 μg/ml) in the AChE assay. In addition, good to moderate bioactivities were observed in some extracts of *Aloe ferox* (leaves), *Crinum moorei* (bulbs) and *Pycnostachys reticulata* (leaves) in all the assays. The presence and/or amounts of phenolic compounds varied with plant species.
Conclusion: The results obtained in this study validate the use of the investigated medicinal plants in South African traditional medicine for pain-related ailments.
© 2009 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Many degenerative diseases such as rheumatoid arthritis, shoulder tendonitis, gouty arthritis, polymyalgia rheumatica, heart disease, asthma, cancer, and inflammatory bowel disease are often associated with inflammatory processes (Polya, 2003; Iwalewa et al., 2007). During inflammatory responses, the activation of phospholipase A_2 induces the mobilization of fatty acids, in particular arachidonic acid from the membrane lipid pool (Fiorucci et al., 2001). Arachidonic acid is then oxidized by constitutive cyclooxygenase-1 (COX-1) or inducible cyclooxygenase-2 (COX-2) enzymes, leading to the production of prostaglandins (Fiorucci et al., 2001; Polya, 2003). Prostaglandins are a group of inflammatory mediators, implicated in many pain-related ailments (Rang and Dale, 1987).

In addition, oxidants such as reactive oxygen species (ROS) generated from activated neutrophils and macrophages have been reported to play an important role in the pathogenesis of various pain-related diseases, including neurodegenerative disorders, cancer and atherosclerosis (Winrow et al., 1993; Confortia et al., 2007). Furthermore, oxidative and inflammatory processes are among the pathological features associated with the central nervous system in Alzheimer's disease (AD) (Howes and Houghton, 2003). The brain of patients suffering from AD is said to be under oxidative stress as

* Corresponding author. Tel.: +27 33 2605130; fax: +27 33 2605897.
E-mail address: rcpgd@ukzn.ac.za (J. Van Staden).

0378-8741/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2009.11.015

Table 1
Medicinal plants traditionally used to treat pain-related ailments in South Africa.

Species (Family)	Part used	Voucher number	Ethnobotanical use
Aloe ferox Mill. (Asphodelaceae)	Leaves	FAW 19 NU	Boiled leaves are taken for the cure of arthritis, hypertension and strains (van Wyk and Gericke, 2000).
Carpobrotus dimidiatus (Haw.) L. Bolus (Mesembryanthemareae)	Leaves	FAW 13 NU	The leaf juice is used to treat wounds, burns and toothache (van Wyk and Gericke, 2000).
Colocasia antiquorum Schott (Araceae)	Tubers	FAW 18 NU	Tubers are used as poultices for snakebite and rheumatism (Hutchings et al., 1996).
Crinum moorei Hook. f. (Amaryllidaceae)	Bulbs	FAW 17 NU	Bulb decoctions are taken for swelling of the body (Hutchings et al., 1996).
Pycnostachys reticulata (E. Mey.) Benth (Lamiaceae)	Roots	FAW 15 NU	Root decoctions are used as mouthwashes for teeth aching from neuralgia (Hutchings et al., 1996).
Senecio serratuloides DC. (Asteraceae)	Leaves	FAW 16 NU	Powdered leaves are used to treat burns, cuts, sores, swollen gums and chest pains (Hutchings et al., 1996; van Wyk and Gericke, 2000).
Turraea floribunda Hochst (Meliaceae)	Roots	FAW 14 NU	Root infusions are taken as a cure for rheumatism, swollen and painful joints (Hutchings et al., 1996).

a result of perturbed ionic calcium balances within their neurons and mitochondria (Emilien et al., 2000; Tabet, 2006). Moreover, there is evidence that acetylcholinesterase (AChE) inhibitors have an anti-inflammatory role through action against free radicals and amyloid toxicity, as well as through decreasing release of cytokines from activated microglia in the brain and blood (Tabet, 2006). There is an established link between the cholinergic system and inflammation as acetylcholine, the principle neurotransmitter, is reported to attenuate the release of cytokines in the parasympathetic anti-inflammatory pathway by which the brain modulates systemic inflammatory responses to endotoxin (Borovikova et al., 2000).

Anti-inflammatory medications such as non-steroidal anti-inflammatory drugs often relieve inflammation and associated pain by inhibiting specific enzymes that the body needs in order to produce pro-inflammatory eicosanoids (Rang and Dale, 1987). According to Howes and Houghton (2003), the use of anti-inflammatory drugs has been proposed as a therapy for AD due to their ability to delay the advancement or reduce the risk of AD. In addition, the intake of anti-inflammatory drugs, oestrogenic steroids and a high level of antioxidants have been reported to result in lower incidences of AD (Houghton et al., 2007).

Due to the fast growing interest in the anti-inflammatory activity of medicinal plants by pharmaceutical companies and scientific research on the discovery of novel anti-inflammatory compounds, medicinal plants could potentially serve as leads in the production of new drugs for treating pain-related ailments with reduced or no side-effects. A variety of chemical types are often present in a plant extract, each having different biological or pharmacological activities such as anthelminthic, antioxidant, antimicrobial, anti-inflammatory and anticholinesterase properties. These chemical types possibly act in a synergistic manner resulting in the overall clinical effect. It is thus important to screen medicinal plant extracts in different *in vitro* assays because there is a possibility of losing other potentially useful bioactive compounds when only a single biological activity is investigated (Rates, 2001; Houghton et al., 2007).

Table 2
Yield (%) of plant extracts prepared from seven South African medicinal plants.

Plant name (part)	Yield (%)			
	50% MeOH	PE	DCM	EtOH
Aloe ferox (L)	22.0	1.5	0.6	1.3
Carpobrotus dimidiatus (L)	15.2	1.2	0.5	1.2
Colocasia antiquorum (T)	8.0	1.8	0.9	0.3
Crinum moorei (B)	6.7	1.6	1.4	0.6
Pycnostachys reticulata (L)	30.1	0.8	0.4	1.9
Senecio serratuloides (L)	24.1	0.8	1.2	1.8
Turraea floribunda (L)	22.8	1.2	0.7	1.0

50% MeOH, 50% methanol; PE, petroleum ether; DCM, dichloromethane; EtOH, ethanol; B, bulbs; L, leaves and T, tubers.

The study was aimed at evaluating medicinal and therapeutic potentials of seven traditional medicinal plants used for pain-related ailments. The anti-inflammatory, anticholinesterase and antioxidant properties of the medicinal plant extracts were investigated. Phytochemical analysis, for the total phenolic, condensed tannin, gallotannin and flavonoid contents, was also carried out using spectrophotometric methods.

2. Materials and methods

2.1. Plant selection and preparation

The selection of medicinal plants was based on indigenous ethnopharmacological records. Medicinal plants (Table 1) were collected from the University of KwaZulu-Natal Botanical Garden, Pietermaritzburg, South Africa. Voucher specimens were prepared, identified and were lodged in the University of KwaZulu-Natal herbarium for record purposes. Plant materials were oven-dried at 50 °C and ground into fine powders through a 1 mm ring sieve using a Retsch® ZM200 ultracentrifugal mill (Rheinische straße B6, Germany).

2.2. Plant extracts

Each ground plant sample (5 g) was first extracted with 50% methanol (50% MeOH) (100 ml) by sonication for 1 h, followed by sequential extraction of the same plant material with 100 ml each of petroleum ether (PE), dichloromethane (DCM) and ethanol (EtOH). Plant extracts were filtered *in vacuo* through Whatman No. 1 filter paper and concentrated using a rotary evaporator (Büchi, Switzerland) at 30 °C. The extracts were completely dried under a stream of air. Percentage yields of all plant extracts were obtained (Table 2).

2.3. Anti-inflammatory evaluation (cyclooxygenase assays)

The cyclooxygenase assays (COX-1 and COX-2) were performed as described by Eldeen and Van Staden (2008). Plant extracts were screened at a concentration of 0.25 μg/μl. Indomethacin (Sigma–Aldrich, USA) (5 μM for COX-1, 200 μM for COX-2) was used as a positive control, while background samples in which the enzyme was inactivated with HCl before adding [^{14}C] arachidonic acid (16 mCi/mmol; 3.0 μM) and a solvent blank (EtOH) were 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 below:

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

where DPMsample is the disintegrations per minute for plant extract, DPMbackground is the disintegrations per minute where the enzyme was inactivated and DPMblank is the disintegrations per minute for solvent used in dissolving plant extracts. Results are presented as means ± standard errors of two independent experiments, each experiment was done in duplicate.

2.4. Acetylcholinesterase enzyme inhibitory activity

2.4.1. Reagents for the acetylcholinesterase assay

The acetylcholinesterase enzyme activity was measured by spectrophotometric observation of the increase in a yellow colour produced from thiocholine when it reacts with the dithiobis-nitrobenzoate ion. The following buffers were used: buffer A: 50 mM Tris–HCl, pH 8; buffer B: 50 mM Tris–HCl, pH 8, containing 0.1% bovine serum albumin (BSA); buffer C: 50 mM Tris–HCl, pH 8, containing 0.1 M NaCl and 0.02 M $MgCl_2 \cdot 6H_2O$. Acetylthiocholine iodide (ATCI), galanthamine, 5,5-dithiobis-2-nitrobenzoic acid (DTNB) and acetylcholinesterase (AChE) enzyme from electric eels (type VI-S lypophilized powder) were obtained from Sigma–Aldrich.

2.4.2. Thin layer chromatography-bioautography (TLC-B) for acetylcholinesterase inhibition

Plant extracts (10 μl, 10 mg/ml) and galanthamine (5 μl, 0.2 mM) were each applied to Silica gel 60 F_{254} on a polyester plate (10 cm × 20 cm) (Merck, Germany) and developed with ethyl acetate:methanol:water (100:16.5:13.5) in a pre-saturated chromatographic chamber. After drying, the TLC plate was sprayed with 5 mM ATCI and 5 mM DTNB dissolved in buffer A until the TLC plate was saturated. The plates were allowed to slightly dry and then sprayed with 3 U/ml AChE dissolved in buffer A. After 2–5 min, a yellow background appeared with white spots indicating the presence of AChE inhibiting compounds. The white spots were observed and recorded within 5 min. To eliminate false-positive reactions, the enzyme and substrate were mixed before spraying onto the TLC plate (Ellman et al., 1961).

2.4.3. The microplate assay for acetylcholinesterase inhibition

Inhibition of AChE by plant extracts was done as described by Ellman et al. (1961) with some modifications. In a 96-well plate, 25 μl of plant extracts, at an initial concentration of 10 mg/ml, were added to the first well and serially diluted two-fold down the plate. To the diluted sample, 25 μl of 15 mM ATCI in water, and 125 μl of 3 mM DTNB in buffer C were added, followed by 50 μl of buffer B. The absorbance of the reaction mixture was then measured three times at 405 nm every 45 s using a microplate reader (Opsys MR™, Dynex Technologies Inc.). After the third reading, 25 μl of 0.2 U/ml AChE in buffer A was added. The final concentration of the plant extract in the first well was 1.0 mg/ml. The absorbance was measured again every 45 s for a further five times. Galanthamine, at 0.12, 0.23, 0.46, 0.92, 1.84, 3.68 and 7.37 μg/ml, and water were used as positive and negative controls, respectively. The increase in absorbance due to the spontaneous hydrolysis of the substrate was corrected by subtracting the rate of reaction before adding the enzyme from the rate after adding the enzyme. Percentage inhibition was calculated by comparing the reaction rates for the sample to the negative control. Results are presented as means ± standard errors of the experiment in duplicate. The EC_{50} values of plant extracts showing percentage inhibition >50% at the highest concentration (1.0 mg/ml) were calculated.

2.5. Antioxidant evaluation

The radical scavenging activity of the plant materials was determined using the DPPH assay as described by Karioti et al. (2004) with some modifications. Methanolic extracts of each plant at different concentrations (15 μl) were diluted with methanol (735 μl) and then added to a methanolic DPPH solution (750 μl, 0.1 mM). The reaction mixtures were prepared under dim light. Each plant extract was tested in triplicate. After incubation at room temperature for 30 min in the dark, the decrease in the purple colouration of the reaction mixtures due to the bleaching of the DPPH colour was read using a UV–vis spectrophotometer (Varian Cary 50) at 517 nm. Ascorbic acid was used as a positive control and methanol as a negative control. In order to correct the absorbance due to colour of the extracts, the absorbance of extracts without DPPH was measured and subtracted from the corresponding readings with DPPH. The free radical scavenging activity (RSA) as determined by the decolouration of the DPPH solution was calculated according to the formula

$$\%\,\text{RSA} = 100 \times \left(\frac{1 - A_E}{A_D}\right)$$

where A_E is the absorbance of the reaction mixture containing the standard antioxidant or extract, and A_D is the absorbance of the negative control. The EC_{50} values, representing the concentration of extract required to decrease the absorbance of DPPH by 50% were calculated. Results are represented as means ± standard errors of the EC_{50} values.

The reducing powers of the extracts were determined as described by Lim et al. (2009) with some modifications. Each extract (30 μl) or the standard, ascorbic acid (6.25 mg/ml) dissolved in methanol was serially diluted in a 96-well microplate in triplicate, followed by the addition of potassium phosphate buffer (40 μl, 0.2 M, pH 7.2) and potassium ferricyanide (40 μl, 1% w/v). The reaction mixtures were incubated at 50 °C for 20 min before adding trichloroacetic acid (40 μl, 10% w/v), distilled water (150 μl) and $FeCl_3$ (30 μl, 0.1% w/v). The reaction mixtures were further incubated at room temperature for 30 min in the dark. Absorbance readings were measured using a microplate reader (Opsys MR™, Dynex Technologies Inc.) at 630 nm. The absorbance of each extract were plotted against different concentrations and compared with the standard.

2.6. Phytochemical analysis

Phenolic compounds were extracted from dry plant materials as described by Makkar (1999). Ground plant samples (2 g) were extracted with 10 ml of 50% aqueous MeOH by sonication in cold water for 20 min. The extracts were then filtered *in vacuo* through Whatman No. 1 filter paper. Fresh sample extracts were used for each assay.

The Folin Ciocalteu (Folin C.) assay was employed as described by Makkar (1999) for the determination of total phenolics. Total phenolic concentrations were expressed as gallic acid (GAE) equivalents.

Condensed tannins were quantified using the method as described by Makkar (1999). Condensed tannin (% per dry matter) was calculated as equivalent amount of leucocyanidins using the formula developed by Porter et al. (1985):

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

For flavonoid quantification, the assay was performed as described in Fawole et al. (2009). The flavonoids in the plant extracts were expressed as catechin equivalents (CE).

Table 3
Inhibition (%) of cyclooxygenase enzymes (COX-1 and COX-2) by extracts of South African medicinal plants.

	COX-1 inhibition (%)				COX-2 inhibition (%)			
	50% MeOH	PE	DCM	EtOH	50% MeOH	PE	DCM	EtOH
Aloe ferox (L)	96.7 ± 3.6[e]	100 ± 2.8[d]	96.4 ± 1.5[c]	95.1 ± 1.4[c]	20.8 ± 1.5[a]	49.3 ± 5.2[ab]	68.4 ± 9.4[b]	74.8 ± 0.9[c]
Carpobrotus dimidiatus (L)	28.6 ± 2.3[a]	12.1 ± 5.8[a]	38.2 ± 1.0[bc]	12.8 ± 7.0[b]	65.4 ± 1.5[b]	25.8 ± 6.9[a]	25.8 ± 5.9[a]	0[a]
Colocasia antiquorum (T)	48.2 ± 8.4[cd]	98.4 ± 6.8[d]	100 ± 3.1[c]	98.1 ± 2.3[c]	15.3 ± 9.1[a]	92.7 ± 3.7[b]	77.3 ± 3.5[b]	79.9 ± 1.4[c]
Crinum moorei (B)	99.2 ± 0.96[e]	97.1 ± 0.2[d]	84.0 ± 0.4[c]	95.6 ± 3.2[c]	75.5 ± 1.5[b]	74.6 ± 1.5[b]	52.0 ± 3.2[ab]	71.6 ± 3.6[c]
Pycnostachys reticulata (L)	29.7 ± 0.3[ab]	20.0 ± 4.4[ab]	0[a]	0[a]	68.7 ± 2.4[b]	30.3 ± 0.1[a]	61.0 ± 8.1[b]	0[a]
Senecio serratuloides (L)	38.6 ± 0.8[bc]	62.0 ± 0.2[cd]	32.6 ± 12.2[bc]	0[a]	71.6 ± 1.9[b]	41.9 ± 10.1[ab]	40.2 ± 18.3[ab]	23.7 ± 6.4[b]
Turraea floribunda (L)	56.3 ± 6.1[d]	38.9 ± 14.6[bc]	21.8 ± 1.4[b]	0[a]	87.3 ± 4.9[b]	29.1 ± 13.3[a]	56.5 ± 8.3[ab]	30.6 ± 9.6[b]

50% MeOH, 50% methanol; PE, petroleum ether; DCM, dichloromethane; EtOH, ethanol; B, bulbs; L, leaves and T, tubers. Indomethacin COX-1 = 82.0 ± 2.6%, COX-2 = 85.28 ± 1.37%. Means in the same column followed by different letter(s) are significantly different ($P = 0.05$).

The amounts of gallotannin in the plant extracts were determined according to Makkar (1999). Gallotannin concentrations were expressed as gallic acid equivalents (GAE).

2.7. Statistical analysis

Percentage inhibition values were log-transformed before being subjected to statistical analysis. Data were subjected to one-way analysis of variance (ANOVA) using SPSS version 10 software (SPSS Inc., Chicago, USA). Where there was statistical significance ($P = 0.05$), the means were further separated using Duncan's Multiple Range Test. The EC_{50} values for DPPH and AChE assays were calculated from the logarithmic non-linear regression curve derived from the plotted data using GraphPad Prism software version 4.03 (GraphPad Software, Inc., San Diego, USA).

3. Results

3.1. Cyclooxygenase enzyme inhibition

Table 3 shows the percentage inhibitory activity of COX-1 and COX-2 enzymes by the extracts. Some degree of COX-inhibitory activity was observed in all the evaluated extracts. Considering plant extracts with a minimum percentage inhibition of 50% as showing good activity (Eldeen and Van Staden, 2008), at the screening assay concentration of 0.25 μg/μl, out of 28 plant extracts evaluated against both enzymes (COX-1 and -2), 13 showed good COX-1 inhibitory activity, while good activity was observed in 15 extracts against the COX-2 enzyme. Ten extracts showed good inhibition (>50%) against both COX-1 and -2 enzymes. All of the extracts of *Pycnostachys reticulata* (leaves) and *Carpobrotus dimidiatus* (leaves) showed poor or no COX-1 inhibition. Good inhibition against COX-1 and -2 enzymes was exhibited by all extracts of *Crinum moorei* (bulbs). *Carpobrotus dimidiatus* leaf (50% MeOH), *Pycnostachys reticulata* leaf (50% MeOH and DCM), *Senecio serratuloides* leaf (50% MeOH) and *Turraea floribunda* leaf (DCM) extracts showed higher COX-2 inhibition than COX-1 inhibition. Although not significantly different, *Colocasia antiquorum* tuber PE extract showed the highest COX-2 enzyme inhibition (92.7%), while the highest COX-1 inhibition (100%) was observed with *Aloe ferox* leaf PE and *Colocasia antiquorum* tuber DCM extracts. Generally, a higher number of PE extracts showed good COX-1 enzyme inhibition while 50% MeOH and DCM extracts showed good activity against COX-2 compared to the other solvent extracts.

3.2. Acetylcholinesterase enzyme inhibition

The AChE inhibitory activity of the evaluated plant extracts at different concentrations (0.0625, 0.25 and 1.0 mg/ml) is shown in Fig. 1. All the plant extracts evaluated showed some level of inhibitory activity against the AChE. At the highest concentration (1.0 mg/ml), 12 out of 28 plant extracts evaluated showed percentage inhibition greater than 50% against AChE. Generally, higher numbers of aqueous 50% MeOH extracts showed good AChE inhibitory activity (>50%). With the exception of *Carpobrotus dimidiatus* leaf (DCM), *Colocasia antiquorum* tuber (EtOH), *Turraea floribunda* leaf (DCM and EtOH) and *Pycnostachys reticulata* leaf (PE and DCM) extracts, all the plant extracts showed a dose-dependent activity. The lowest EC_{50} value (2.9 μg/ml) was observed in *Crinum moorei* bulb DCM extract (Table 4). On the TLC confirmatory assay, all the plant extracts showed AChE inhibition as evident by the appearance of white spots against a yellow background on the TLC plate. No false-positive activity was observed in any of the plant extracts.

Table 4
Effective inhibition concentration (EC_{50}) against AChE by extracts of South African medicinal plants.

Plant species	Extract	EC_{50} (μg/ml)
Aloe ferox (L)	50% MeOH	84.0 ± 1.0
	PE	37.7 ± 5.0
	DCM	62.6 ± 7.5
Colocasia antiquorum (T)	50% MeOH	7.9 ± 0.3
	PE	6.4 ± 2.5
	DCM	168.1 ± 28.9
Crinum moorei (B)	50% MeOH	21.5 ± 8.4
	PE	18.9 ± 8.7
	DCM	2.9 ± 0.5
	EtOH	22.5 ± 6.5
Pycnostachys reticulata (L)	50% MeOH	28.8 ± 0.3
	EtOH	8.8 ± 5.3

EC_{50} values were calculated for plants that showed percentage inhibition >50% at 1.0 mg/ml. EC_{50} value for galanthamine was 0.33 ± 0.1 μg/ml. 50% MeOH, 50% methanol; PE, petroleum ether; DCM, dichloromethane; EtOH, ethanol; B, bulbs; L, leaves and T, tubers.

Table 5
DPPH radical scavenging activity (EC_{50}) of 50% MeOH extracts of South African medicinal plants used to treat pain-related ailments.

Plant name (part)	EC_{50} (μg/ml)
Aloe ferox (L)	10.45 ± 0.45[d]
Carpobrotus dimidiatus (L)	7.63 ± 0.37[bc]
Colocasia antiquorum (T)	8.34 ± 0.51[cd]
Crinum moorei (B)	5.06 ± 1.54[ab]
Pycnostachys reticulata (L)	2.40 ± 0.16[a]
Senecio serratuloides (L)	10.40 ± 0.42[d]
Turraea floribunda (L)	5.04 ± 1.63[ab]
Ascorbic acid	5.06 ± 0.20[ab]

Means in the same column followed by different letter(s) are significantly different ($P = 0.05$). B, bulbs; L, leaves and T, tubers.

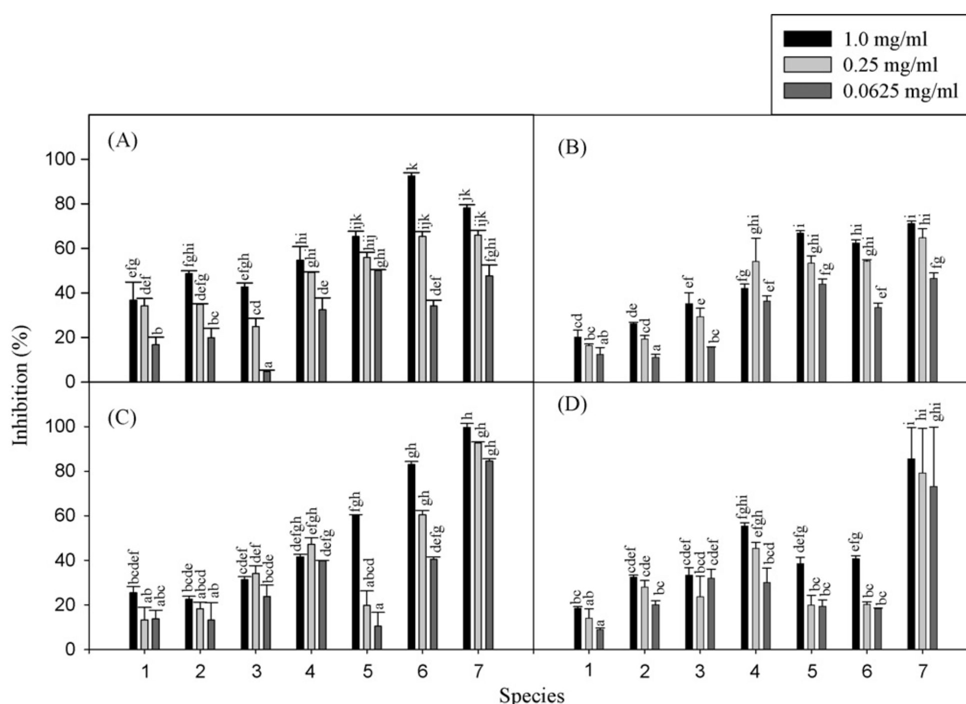

Fig. 1. Inhibition (%) of acetylcholinesterase enzyme by plants extracts (0.0625, 0.25 and 1.0 mg/ml) used for pain-related ailments in South Africa: (A) 50% aqueous methanol; (B) petroleum ether; (C) dichloromethane; (D) ethanol extracts. 1, *Carpobrotus dimidiatus* (leaves); 2, *Senecio serratuloides* (leaves); 3, *Turraea floribunda* (leaves); 4, *Pycnostachys reticulata* (leaves); 5, *Colocasia antiquorum* (tubers); 6, *Aloe ferox* (leaves); 7, *Crinum moorei* (bulbs). The percentage inhibition of the positive control, galanthamine (20 μM) was 89.5 ± 3.4. Bars in each graph followed by different letter(s) are significantly different (*P* = 0.05) according to Duncan's Multiple Range Test.

3.3. Antioxidant evaluation

Table 5 shows the EC_{50} values of DPPH radical scavenging activity of the aqueous 50% MeOH plant extracts and ascorbic acid (positive control). All plant extracts showed good radical scavenging activity. Although there was no significant difference between the EC_{50} values of *Crinum moorei*, *Pycnostachys reticulata* and *Turraea floribunda* extracts compared with ascorbic acid, *Pycnostachys reticulata* extract had the lowest EC_{50} value (2.40 μg/ml).

The ferric-reducing power (FRAP) of aqueous 50% MeOH plant extracts and ascorbic acid are presented in Fig. 2. In the FRAP assay, the antioxidants contained in the samples react as reductants in a redox-linked colourimetric reaction and the value reflects the reducing power of the antioxidants (Li et al., 2006). All the plant extracts evaluated showed a dose-dependent reducing activity. None of the plant extracts had absorbance higher than the standard (ascorbic acid) at the lowest concentration. The absorbance of the plant extracts at the lowest screening concentration of 4.4 μg/ml was in the order *Crinum moorei* > *Pycnostachys reticulata* > *Aloe ferox* > *Turraea floribunda* > *Colocasia antiquorum* > *Senecio serratuloides* > *Carpobrotus dimidiatus*.

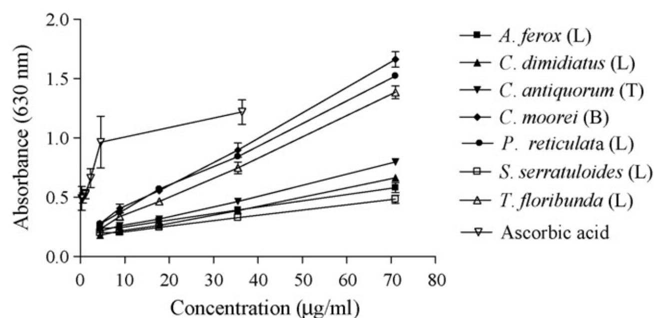

Fig. 2. Ferric-reducing power of extracts of medicinal plants used for pain-related ailments in South Africa. B, Bulbs; L, leaves and T, tubers.

3.4. Phytochemical analysis

The phenolic content in all the plant species ranged from 0.64 to 4.5 mg GAE/g dry matter (Fig. 3A). No condensed tannins were detected in *Colocasia antiquorum* tuber and *Crinum moorei* bulb extracts (Fig. 3B). The highest amount (0.39 mg CE/g dry matter) of flavonoids was observed in *Crinum moorei* bulb extract while the lowest amount (0.22 mg CE/g dry matter) was detected in *Turraea floribunda* leaf extract (Fig. 3C). Gallotannins were detected in all the plant extracts tested (Fig. 3D). The highest amount of gallotannins was observed in *Colocasia antiquorum* tuber extract with 40.8 μg GAE/g dry matter.

4. Discussion

All the evaluated medicinal plants in this study are used traditionally for the treatment of pain-related ailments in South Africa. Some of the evaluated plant extracts showed COX-inhibitory activity and other pharmacological activities. A variety of bioactive compounds that could be responsible for the observed bioactivities have been reported in some of the screened medicinal plants. *Aloe ferox* leaf (DCM and EtOH) showed good anti-inflammatory activity while a moderate antioxidant activity was exhibited by the 50% MeOH extract. Antimicrobial compounds, such as aloe-emodin, chrysophanol and aloin as well as an anti-inflammatory compound aloeresin-I have been identified in *Aloe ferox* leaves (Kambizi et al., 2005; Speranza et al., 2005). Furthermore, aloeresin-I from *Aloe ferox* leaves was reported to reduce an oedematous response induced by croton oil in the mouse ear *in vivo* test (Speranza et al., 2005). Aloeresin-I could be a major compound responsible for the anti-inflammatory activity observed in some *Aloe ferox* leaf extracts. Moreover, compounds such as larvicidal tetranortriter-

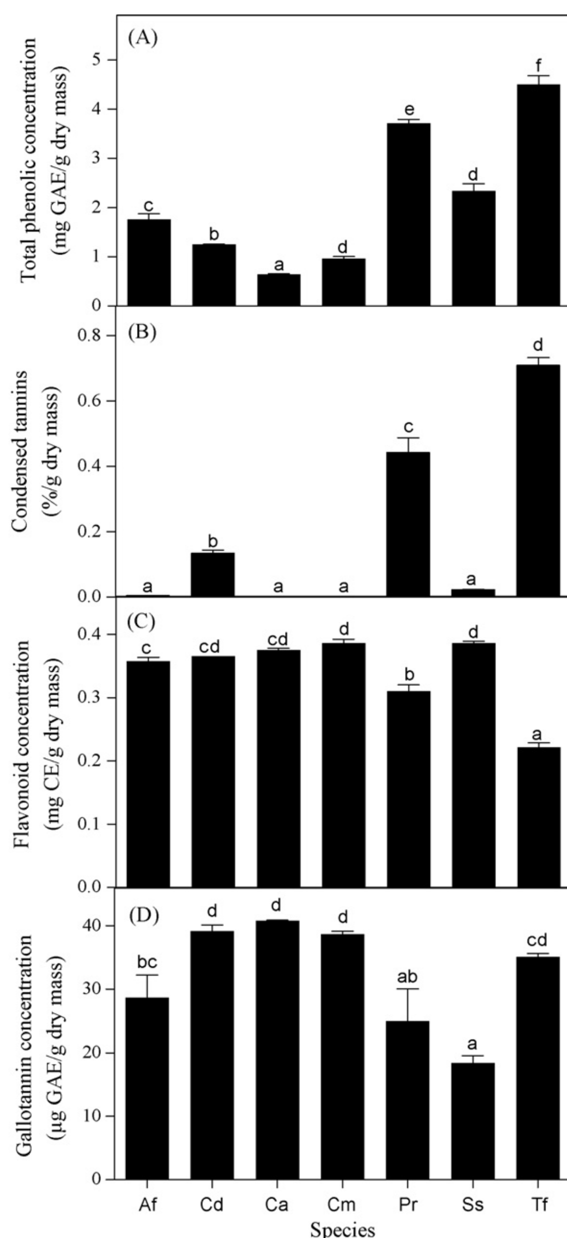

Fig. 3. Phytochemical contents (per dry mass) of traditional medicinal plants used to treat pain-related ailments in South Africa. (A) Total phenolic concentration (mg GAE/g dry mass); (B) condensed tannins (%/g dry mass); (C) flavonoid concentration (mg CE/g dry mass); (D) gallotannin concentration (μg GAE/g dry mass). Af, *Aloe ferox* (leaves); Cd, *Carpobrotus dimidiatus* (leaves); Ca, *Colocasia antiquorum* (tubers); Cm, *Crinum moorei* (bulbs); Pr, *Pycnostachys reticulata* (leaves); Ss, *Senecio serratuloides* (leaves); Tf, *Turraea floribunda* (leaves). Bars in each graph followed by different letter(s) are significantly different ($P = 0.05$) according to Duncan's Multiple Range Test.

penoids in *Turraea floribunda* root and limonoids in the seed extract have been reported (Mulholland et al., 1998; McFarland et al., 2004; Ndung'u et al., 2004). In addition, a high inhibitory activity against COX enzymes has been reported in *Turraea floribunda* root EtOH extract (McGaw et al., 1998). In this study, *Turraea floribunda* leaves were evaluated. The leaf 50% MeOH extract showed a high inhibition against COX-2 enzyme and a good antioxidant activity. To promote sustainable harvesting of *Turraea floribunda*, the leaves can be used as substitute for the root. Other compounds reported in some of the evaluated plant species include an antifungal compound, 9,12,13-trihydroxy-10-octadecenoic acid in *Colocasia antiquorum* tubers (Kuo et al., 2001), as well as antitumor, antispasmodic and anti-inflammatory compounds such as pyrrolizidine alkaloids, senecionine and senecionine-N-oxide in *Senecio* species (Hutchings et al., 1996). Good antioxidant activity and the lowest EC_{50} in the AChE assay were observed with *Crinum moorei* 50% MeOH and DCM bulb extracts, respectively. Moreover, most of the bulb extracts evaluated in the COX assay showed good activity against COX-1 and -2. Amaryllidaceae alkaloids such as crinine, epibuphanisine, epivittatine, crinamidine, 1-oacetyllycorine and cherylline are reported in *Crinum moorei*. These alkaloids have good AChE inhibitory effects (Elgorashi et al., 2001). The relationship between some or all of these alkaloids in synergism could perhaps be responsible for to the bioactivities exhibited by *Crinum moorei* bulb extracts in this study.

Although the plants tested in this study are used traditionally for the treatment of pain-related ailments, *Crinum moorei* bulbs extracts exhibited good anti-inflammatory, antioxidant and AChE inhibitory activities. Good to moderate bioactivities were also observed in some of the evaluated plant extracts in all the assays. Phytochemicals, in particular phenolics, were observed in all the plant materials. Phenolic compounds such as gallotannins, condensed tannins and flavonoids are known to inhibit some molecular targets of pro-inflammatory mediators in inflammatory responses. They also act as antioxidants by scavenging radicals and thereby attenuate the inflammatory process (Polya, 2003; Iwalewa et al., 2007). Various *in vitro* and *in vivo* bioactivities such as anticancer, anti-inflammatory, and anti-allergic activities by flavonoids have been reported. According to Chi et al. (2001), anti-inflammatory activity of flavonoids is mediated by the inhibition of the arachidonic acid metabolizing enzymes, as well as by their antioxidant properties. Anti-inflammatory activity of 19 isolated prenylated flavonoids from some medicinal plants against COX and lipoxygenase enzymes, and the AChE inhibition by four flavonoids from *Agrimonia pilosa* have been reported (Chi et al., 2001; Jung and Park, 2007). The flavonoid content observed in all the investigated plant species could, in this study, be of importance in the pharmacological activities exhibited by their extracts.

Generally, the results obtained in this study validate the use of the investigated medicinal plants in South African traditional medicine for pain-related ailments. Other pharmacological conditions linked to inflammation were also investigated to establish other potential activities of the medicinal plant extracts. The results obtained in this study provide scientific information that could aid in the isolation of potential pharmacologically active compounds from some of these medicinal plants in future research.

Acknowledgements

The authors thank Mrs Alison Young (Horticulturist) of the University of KwaZulu-Natal Botanical Garden, for her assistance in plant collection. The National Research Foundation and the University of KwaZulu-Natal are gratefully acknowledged for financial assistance.

References

Borovikova, L.V., Ivanova, S., Zhang, M., 2000. Vagus nerve stimulation attenuates the systemic inflammatory response to endo-toxin. Nature 405, 458–462.

Chi, Y.S., Jong, H.G., Son, K.H., Chang, H.W., Kang, S.S., Kim, H.P., 2001. Effects of naturally occurring prenylated flavonoids on enzymes metabolizing arachidonic acid: cyclooxygenases and lipoxygenases. Biochemical Pharmacology 62, 1185–1191.

Confortia, F., Sosa, S., Marrelli, M., Menichini, F., Statti, G., Uzunov, D., Tubaro, A., Menichini, F., Loggia, R.D., 2007. *In vivo* anti-inflammatory and *in vitro* antioxidant activities of Mediterranean dietary plants. Journal of Ethnopharmacology 116, 144–151.

Eldeen, I.M.S., Van Staden, J., 2008. Cyclooxygenase inhibition and antimycobacterial effects of extracts from Sudanese medicinal plants. South African Journal of Botany 74, 224–229.

Elgorashi, E.E., Drewes, S.E., Van Staden, J., 2001. Alkaloids from *Crinum moorei*. Phytochemistry 56, 637–640.
Ellman, G.L., Courtney, D., Andies, V., Featherstone, R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology 7, 88–95.
Emilien, G., Beyreuther, K., Master, C.L., Maloteaux, J.M., 2000. Prospects for pharmacological intervention in Alzheimer disease. Archives of Neurology 57, 454–459.
Fawole, O.A., Ndhlala, A.R., Amoo, S.O., Finnie, J.F., Van Staden, J., 2009. Anti-inflammatory and phytochemical properties of twelve medicinal plants used for treating gastro-intestinal ailments in South Africa. Journal of Ethnopharmacology 123, 237–243.
Fiorucci, S., Meli, R., Bucci, M., Cirino, G., 2001. Dual inhibitors of cyclooxygenase and 5-lipoxygenase. A new avenue in anti-inflammatory therapy? Biological Pharmacology 62, 1433–1438.
Houghton, P.J., Howes, M.-J., Lee, C.C., Steventon, G., 2007. Uses and abuses of *in vitro* tests in ethnopharmacology: visualizing an elephant. Journal of Ethnopharmacology 110, 391–400.
Howes, M.-J.R., Houghton, P.J., 2003. Plants used in Chinese and Indian traditional medicine for improvement of memory and cognitive function. Pharmacology, Biochemistry and Behavior 75, 513–527.
Hutchings, A., Scott, A.H., Lewis, G., Cunningham, A., 1996. Zulu Medicinal Plants. An Inventory. University of Natal Press, Pietermaritzburg.
Iwalewa, E.O., McGaw, L.J., Naidoo, V., Eloff, J.N., 2007. Inflammation: the foundation of diseases and disorders. A review of phytomedicines of South African origin used to treat pain and inflammatory conditions. African Journal of Biotechnology 6, 2868–2885.
Jung, M., Park, M., 2007. Acetylcholinesterase inhibition by flavonoids from *Agrimonia pilosa*. Molecules 12, 2130–2139.
Kambizi, L., Sultana, N., Afolayan, A.J., 2005. Bioactive compounds isolated from *Aloe ferox*: a plant traditionally used for the treatment of sexually transmitted infections in the Eastern Cape, South Africa. Pharmaceutical Biology 42, 636–639.
Karioti, A., Hadjipavlou-Litina, D., Mensah, M.L.K., Fleischer, T.C., Saltsa, H., 2004. Composition and antioxidant activity of the essential oils of *Xylopia aethiopica* (Dun) A. Rich. (Annonaceae) leaves, stem bark, root bark, and fresh and dried fruits, growing in Ghana. Journal of Agricultural and Food Chemistry 52, 8094–8098.
Kuo, T.M., Kim, H., Hou, C.T., 2001. Production of a novel compound, 7,10,12-trihydroxy-8(*E*)-octadecenoic acid from ricinoleic acid by *Pseudomonas aeruginosa* PR3. Current Microbiology 43, 198–203.
Li, Y., Guo, C., Yang, J., Wei, J., Xu, J., Cheng, S., 2006. Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. Food Chemistry 96, 254–260.
Lim, T.Y., Lim, Y.Y., Yule, C.M., 2009. Evaluation of antioxidant, antibacterial and anti-tyrosinase activities of four *Macaranga* species. Food Chemistry 114, 594–599.
Makkar, H.P.S., 1999. Quantification of Tannins in Tree Foliage: A Laboratory Manual for the FAO/IAEA Co-ordinated Research Project on Use of Nuclear and Related Techniques to Develop Simple Tannins Assay for Predicting and Improving the Safety and Efficiency of Feeding Ruminants on the Tanniferous Tree Foliage. Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, Austria.
McFarland, K., Mulholland, D.A., Fraser, L.A., 2004. Limonoids from *Turraea floribunda* (Meliaceae). Phytochemistry 65, 2031–2037.
McGaw, L.J., Jäger, A.K., Van Staden, J., 1998. Prostaglandin synthesis inhibitory activity in Zulu, Xhosa and Sotho medicinal plants. Phytotherapy Research 11, 113–117.
Mulholland, D.A., Monkhe, T.V., Coombes, P.H., Rajab, M.S., 1998. Limonoids from *Turraea holstii* and *Turraea floribunda*. Phytochemistry 49, 2585–2590.
Ndung'u, M.W., Kaoneka, B., Hassanali, A., Lwande, W., Hooper, A.M., Tayman, F., Zerbe, O., Torto, B., 2004. New mosquito larvicidal tetranortriterpenoids from *Turraea wakefieldii* and *Turraea floribunda*. Journal of Agricultural and Food Chemistry 52, 5027–5031.
Polya, G.M., 2003. Biochemical targets of plant bioactive compounds. In: A Pharmacological Reference Guide to Sites of Action and Biological Effects. CRC Press, Florida.
Porter, L.J., Hrstich, L.N., Chan, B.G., 1985. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. Phytochemistry 25, 223–230.
Rang, H.P., Dale, M.M., 1987. Pharmacology. Churchill Livingstone, Edinburgh.
Rates, S.M.K., 2001. Plants as source of drugs. Toxicon 39, 603–613.
Speranza, G., Morelli, C.F., Tubaro, A., Altinier, G., Duri, L., Manitto, P., 2005. Aloeresin I, an anti-inflammatory 5-methylchromone from Cape aloe. Planta Medica 71, 79–81.
Tabet, N., 2006. Acetylcholinesterase inhibitors for Alzheimer's disease: anti-inflammatories in acetylcholine clothing! Age and Ageing 35, 336–338.
van Wyk, B.-E., Gericke, N., 2000. Peoples Plants. Briza Publications, Pretoria.
Winrow, V.R., Winyard, P.G., Morris, C.J., Blake, D.R., 1993. Free radicals in inflammation: second messengers and mediators of tissue destruction. British Medical Bulletin 49, 506–522.