Anti-inflammatory and antioxidant properties of leaf extracts of eleven South African medicinal plants used traditionally to treat inflammation

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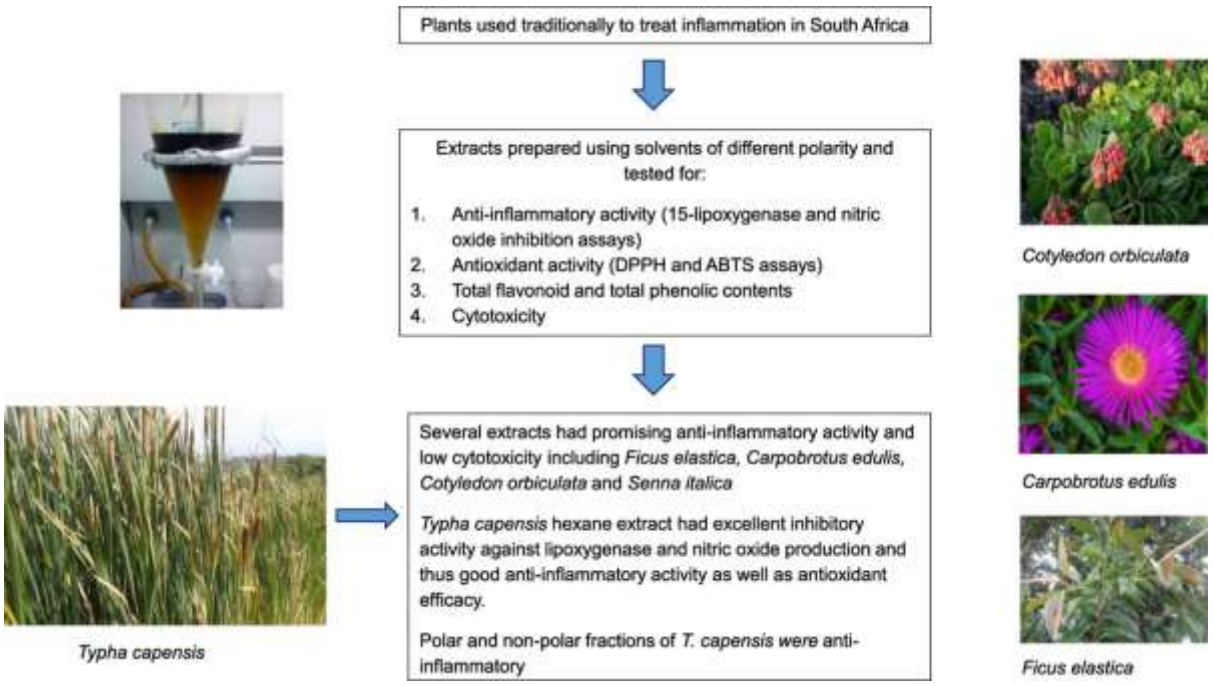
Graphical abstract



Abstract

Ethnopharmacological relevance: Inflammation is a complex mechanism employed by the body to promote healing and restoration to normal function in the event of injury. Eleven plant species were selected in this study based on their use in traditional medicine against inflammation in South Africa.

Methods: Hexane, acetone, ethanol, methanol and water extracts of the powdered plants were prepared and a total of fifty-five extracts were tested for their anti-inflammatory and antioxidant activities. The anti-inflammatory activity of extracts was evaluated via the 15-lipoxygenase (15-LOX) inhibitory and the nitric oxide (NO) inhibition assays using lipopolysaccharide (LPS)-

activated RAW 264.7 murine macrophages. Total flavonoid and total phenolic contents were determined. The antioxidant activity of the extracts was performed using radical scavenging DPPH (2, 2-diphenyl-1-picrylhydrazyl) and electron reducing ABTS (2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) assays.

Results: The hexane extract of *Typha capensis* (TC) had good lipoxygenase inhibitory activity with IC_{50} of 4.65 µg/mL, significantly ($p<0.05$) higher than that of the positive control quercetin (IC_{50} = 24.60). The same extract also had good nitric oxide inhibitory activity with 86% NO inhibition and cell viability of 97% at 50 µg/mL. The TC acetone extract had the best antioxidant activity with IC_{50} of 7.11 and 1.91 µg/mL respectively in the DPPH and ABTS assays. Following fractionation of the TC plant material, the ethyl acetate fraction had interesting antioxidant activity and the methanol/water (35%) and hexane fractions had good 15-LOX inhibitory activity. The antioxidant and anti-inflammatory activities therefore resided in both polar and more non-polar fractions.

Conclusion: The acetone extract of *Typha capensis* and its fractions had good anti-inflammatory and antioxidant activities, supporting the medicinal use of this species against inflammation. Other species including *Ficus elastica, Carpobrotus edulis, Cotyledon orbiculata* and *Senna italica* also had good activity worthy of further investigation.

Keywords: anti-inflammatory; antioxidant; 15-lipoxygenase; nitric oxide; total phenolic content; total flavonoid content; *Typha capensis*; South Africa.

Abbreviations: ABTS = 2,2′-azino-bis (3-ethyl benzothiazoline-6-sulfonic acid), ATCC = American Type Culture Collection, COX = cyclooxygenase, DMEM = Dulbecco's Modified Eagle's Medium, DMSO = dimethyl sulfoxide, DNA = deoxyribonucleic acid, DPPH = 2, 2-diphenyl-1-picrylhydrazyl, EHA = egg hatching assay, FCS = foetal calf serum, GAE = gallic acid equivalent, IL-1β = interleukin one beta, iNOS = inducible nitric oxide synthase, LDA = larval development assay, LOX = lipoxygenase, MAPKs = mitogen-activated protein kinases, MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, NF-κB = nuclear factor κappa B, NO = nitric oxide, NOS = nitric oxide synthase, $OONO^-$ = peroxynitrite, PBS = phosphate buffer solution, PSF = penicillin/streptomycin/fungizone, RE = rutin equivalent, RNS = reactive nitrogen species, ROS = reactive oxygen species, TC = *Typha capensis*, TFC = total flavonoid content, TNF-α = tumour necrosis factor alpha, TPC = total phenolic content

1. Introduction

Since time immemorial, plant-based drugs have been used for various ailments ranging from the common cold to cancer. Traditional herbal medicine is an important component of primary health care systems in developing countries (Sowjanya et al., 2013). Medicinal plants play a significant role in the daily lives of many people and are an important part of the South African cultural heritage (Van Wyk et al., 2009). The World Health Organization (WHO) has estimated that 80% of the world's inhabitants utilize traditional medicine for their primary health care needs and the majority of this therapy requires the use of herbal extracts and their active components. It is estimated that there are 20 000 herbal plants used for medicinal purposes in the world (Baytop, 1999). There are 30 000 species of higher plants in South Africa and, about 350 species are traded as medicinal plants (Van Wyck et al., 2009). In recent years, there has beeen an increasing interest in botanical description and biological activities from plants used traditionally to treat many ailments and those used as food (Erdem et al., 2017; Erecevit and Kırbağ, 2017). Various medicinal plant bioactive extracts and their isolated active constituents have shown a variety of medicinal pharmacological properties against acute and chronic diseases and disorders (WHO, 2005; Gothai et al., 2016). Pain is a direct response to an untoward event associated with tissue damage, such as injury, inflammation or cancer (Rang et al., 2012). Inflammation is one of the first physiological and immune responses, playing a defensive function from inside the body against potentially harmful stimuli such as metabolic stress, injury or pathogens (Torres et al., 2015; Antonelli and Kushner, 2017). Phytomedicines containing substances like phytoestrogens, flavonoids and their derivatives can inhibit molecular targets of pro-inflammatory mediators in inflammatory responses (Iwalewa et al., 2007). Some of the causative organisms and factors responsible for initiating and promoting inflammation could be removed or neutralised to suppress the expression of pro-inflammatory agents (Iwalewa et al., 2007).

There are many inflammatory pathways including cyclooxygenase (COX) and lipoxygenase (LOX) pathways. COX-1 and COX-2 are prostaglandin synthases catalysing sequential synthesis of prostaglandin G2 (PGG2) and prostaglandin H2 (PGH2) from arachidonic acid (AA) through intrinsic COX and peroxidase activities (Figure 1). Lipoxygenases are a group of dioxygenases involved in the insertion of one molecule of oxygen in different sites in arachidonic acid, and they are key enzymes responsible for the biosynthesis of leukotrienes from arachidonic acid. LOXs are postulated to play an important role in the physiology of several inflammatory diseases. The catalytic activity of COX-2 and LOX enzymes are activated by prostaglandins, leukotrienes, nitrogen species, interleukin 1-β (IL-1β), tumour necrosis factor-α (TNF-α), and reactive oxygen species (ROS) (Rackova et al., 2007; Rajan et al., 2016). The enzyme 15-lipoxygenase (15-LOX) is involved in the pathogenesis of

rheumatoid arthritis via the nuclear factor κappa B (NF-κB) pathway. It has been reported that the NF-κB pathway is activated in the early stage of joint inflammation and NF-κB (intracellular mediator and transcriptional regulator in inflammatory process) DNA binding activity is increased in rheumatoid arthritis patients (Asahara et al., 1995; Wang and Kaufman, 2016). Nitric oxide (NO) is a major inflammatory mediator during the immuno-inflammatory reaction. NO is a membrane-permeable signalling molecule involved in a broad array of biological processes through its ability to modify proteins, lipids and DNA, and it even alters their functions and immunogenicity (Oates, 2010) . Appropriate levels of NO assist in mounting an effective defence against invading microbes, whereas the inability to generate NO results in serious and even fatal susceptibility to infections. Furthermore, overproduction of NO has been implicated in the pathogenesis of many disorders, including atherosclerosis, neurodegenerative diseases, inflammatory autoimmune diseases and cancers (Karpuzoglu and Ahmed, 2006). Therefore, NO has the potential to behave like a “double-edged” biological sword, depending on its level within the physiological system (Karpuzoglu and Ahmed, 2006).

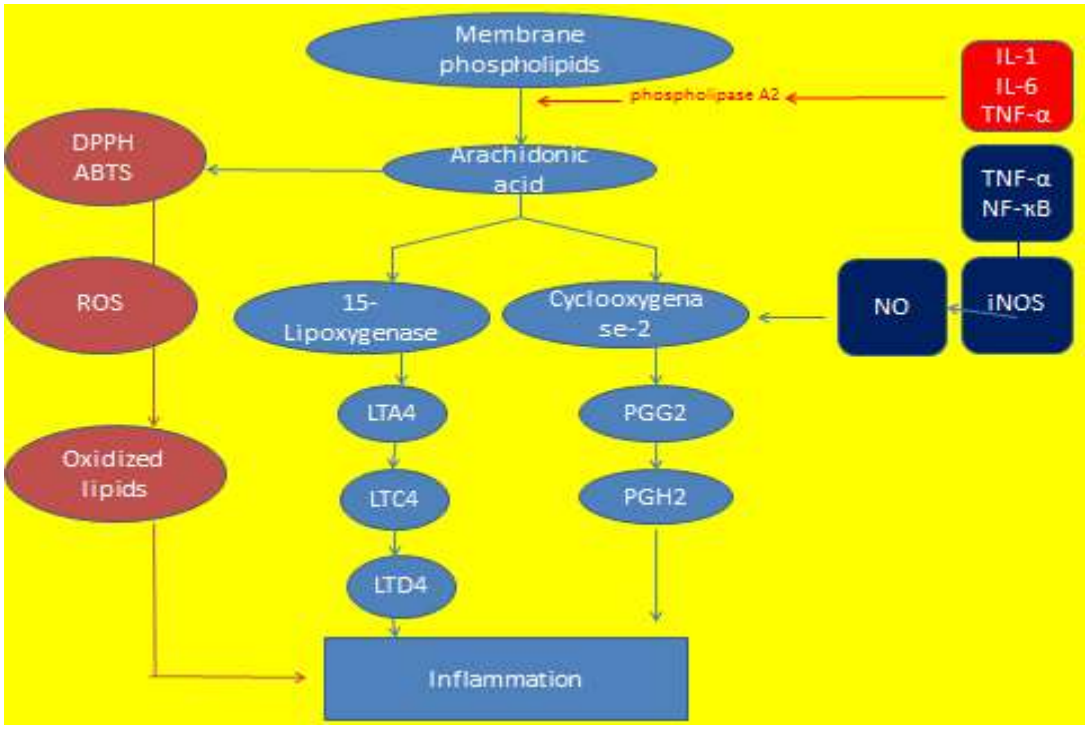


Figure 1. Mechanism of inflammation, involving antioxidant, 15-lipoxygenase, cyclooxygenase-2, nitric oxide and cytokines.

The mechanism of action of many anti-inflammatory compounds such as flavonoids, phenols, tannins, and curcumins is thought to be via their free radical scavenging activities or inhibition of pro-inflammatory enzymes such as COX and LOX. Besides having antioxidant activities, flavonoids and phenolic compounds also exert an effective role as anti-inflammatory factors.

The anti-inflammatory activities of natural compounds have been reported in several studies and have been observed in numerous preclinical studies (Ravipati et al., 2012). Bioactive extracts and their constituent compounds may exert their biological properties by blocking components of major signalling pathways such as NF-κB and mitogen-activated protein kinases (MAPKs) which have a main role in the production of various pro-inflammatory mediators (Arulselvan et al., 2016).

Typha capensis, Carpobrotus edulis, Cotyledon orbiculata, Gomphocarpus fruticosus, Rumex crispus, and Ricinus communis are indigenous South African plants used traditionally to treat inflammation. Alien plants such as *Senna italica, Amaranthus hybridus, Datura stramonium, Ficus elastica* and *Oxalis pes-caprae* are also used by traditional healers in South Africa, in Asia, and in Europe to treat inflammation and other ailments (for global distribution and medicinal uses, see Table 1). In the present study, we evaluated the antioxidant and anti-inflammatory activity of extracts from eleven plant species selected based on their traditional uses against inflammation (Table 1). Extracts were prepared using different solvents in an effort to determine the best solvent for extraction of anti-inflammatory compounds. In addition, fractions of the most active extracts were tested for their biological activities to determine correlations with the traditional use of these plants in the management of pain relief and against inflammation.

Table 1. List of plants with medicinal uses and their herbarium specimen numbers

Plant species	Family name	Herbarium specimen number	Common names	Global distribution	Medicinal uses	References
Amaranthus hybridus L.	Amaranthaceae	PRU 123538	Pigweed, Amaranth	Widely distributed accros the world (Africa, Europa, Asia, America)	Knee pain, laxative, antioxidant, cicatrisation properties, stomach aches, diarrhoea, and dysentery	Nacoulma, 1996; Akin-Idowu et al., 2017, Li et al., 2018
Carpobrotus edulis (L.) N.E.Br.	Aizoaceae	PRU 123546	Sour fig	Indigenous South African plant (Cape Town. Also present in Europe, America.	Eczema, wounds, toothache, earache, source of natural antioxidants	Van Wyk et al., 2009, Hafsa et al., 2018, Roiloa and Ruterto, 2016
Cotyledon orbiculata L.	Crassulaceae	PRU 123542	Pig ears	Western Cape, South Africa, America, Europe	Earache and toothache	Rood, 2008, Van Wyck et al., 2009.
Datura stramonium L.	Solanaceae	PRU 123550	Thornapple	America, Africa, Asia, Europe	Antioxidant, pain, rheumatism, gout, wounds	Watt and Breyer-Brandwijk, 1962; Sreenivasa et al., 2012. Al-Snafi, 2017.
Ficus elastica Roxb. ex Hornem.	Moraceae	PRU 123544	Fig	Africa, Europe, Asia, America	Inflammation and cancer	Teinkela et al., 2015
Gomphocarpus fruticosus (L.) W.T.Aiton	Apocynaceae	PRU 123539	Milkweed	Widely distributed in South Africa, indigenous plant	Stomach pain, general pain	Pujol, 1990, Mulaudzi et al, 2015
Oxalis pes-caprae L.	Oxalidaceae	PRU 123540	African wood-sorrel	Europe, Africa, Asia	Diuretic, tapeworm and other worms	Watt and Breyer-Brandwijk, 1962
Ricinus communis L.	Euphorbiaceae	PRU 123549	Castor oil plant	Throughout Africa, Europe, America, Asia	Stomach ache, wounds, sores, boils	Watt and Breyer-Brandwijk, 1962
Rumex crispus L.	Polygonaceae	PRU 123547	Curly dock	Indigenous from Eastern Cape, South Africa, present in Europe, Asia, America	Abscesses, boils and tumours	Pujol, 1990, Vasas et al., 2015
Senna italica Mill.	Fabaceae	PRU 123545	Wild senna	Africa, Europa, Asia, America	Abdominal pain, ulcers, fever	Gelfand et al., 1985; Ghorbani et al., 2014, Khalaf et al., 2018.
Typha capensis (Rohrb.) N.E.Br.	Typhaceae	PRU 123548	Bulrush	Indigenous South African plant, also found in Europe, Asia and America	Postpartum, abdominal pain, stomach pain, and abscesses	Yeung, 1985. Van Wyk et al., 2009.

2. Materials and methods

2.1. Plant collection and extraction

Fresh leaves of eleven plant species were collected in Countryview, Vorna Valley, President Park, Midrand (25° 57' 49" South, 28° 8' 16" East, Gauteng, South Africa) in February 2016

and dried at room temperature in a well-ventilated room for one to eight weeks. Herbarium voucher specimens for each of the plant species were prepared and deposited at the HGWJ Schweickerdt Herbarium (PRU), University of Pretoria, South Africa (Table 1). The dried plant material was ground to a fine powder using a grinder (MF 10, Merck Chemical (Pty) Ltd). Twenty grams of each leaf powder were separately extracted in 100 mL of different solvents (hexane, acetone, ethanol, methanol, and water) and were macerated at room temperature (25^{o}C) for 48h. The mixture was then filtered through Whatman No. 1 filter paper and the organic solvents were evaporated to dryness. The extraction was repeated twice on the same plant material and the solvents were removed under a stream of air in a fume hood at room temperature to obtain the dried crude extract. A total of 55 crude extracts (eleven plants each extracted using five different solvents) were obtained and kept in a cold room (4^{o}C) until use. Depending on the biological assay, aliquots of the crude extract were reconstituted in dimethylsulfoxide (DMSO), methanol, or phosphate buffer solution (PBS).

2.2. Fractionation of acetone leaf extracts of *Typha capensis* and *Ficus elastica*

Based on their interesting biological activity, the acetone leaf extracts of *T. capensis* and *F. elastica* were selected for further solvent-solvent fractionation. The acetone extracts (42 g for each plant) were partitioned into equal volumes (300 mL) of chloroform/water (1:1). This was extracted using chloroform ($CHCl_3$) three times and then ethyl acetate (ETOAc) three times to obtain chloroform and ethyl acetate fractions.

The aqueous phase was extracted with an equal volume of butanol in a separating funnel to yield the water and butanol fractions. The chloroform fraction was dried in a rotary evaporator under reduced pressure and partitioned between (1:1) hexane : 35% water in methanol. The hexane fraction was collected and the 35% water in methanol fraction was again extracted with chloroform to yield $CHCl_3$ and 35% water in methanol fractions (Figure 2).

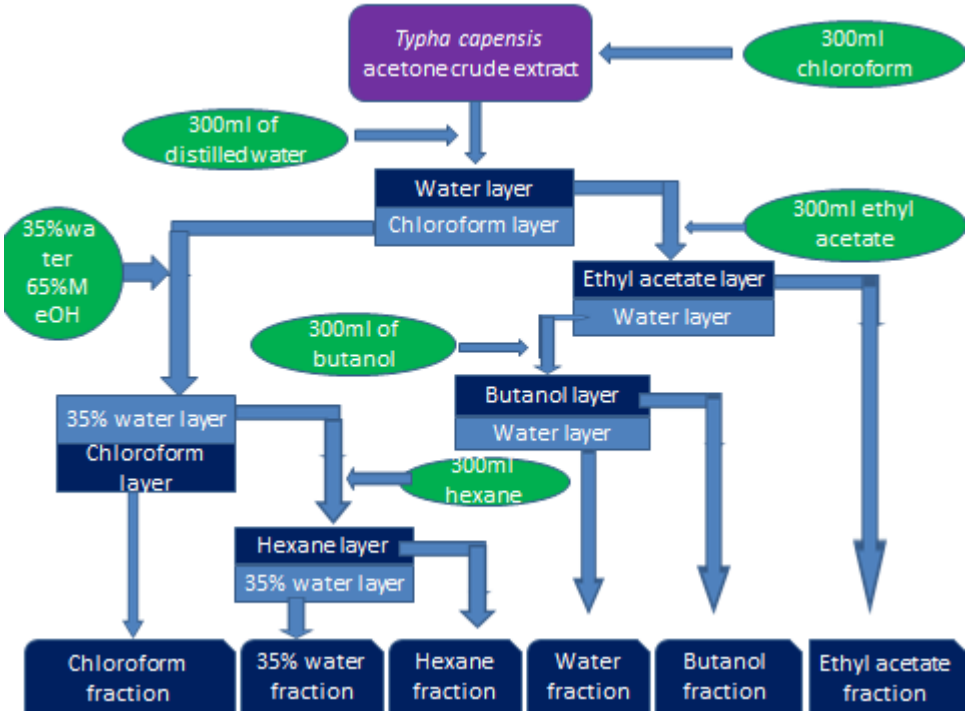


Figure 2. Solvent-solvent partitioning of *Typha capensis*

2.3. Polyphenolic compound analysis of crude extracts and fractions

2.3.1. Total phenolic content (TPC)

The total phenolic content (TPC) of different crude extracts of the eleven plant species and the fractions of *Typha capensis* and *F. elastica* was determined according to the Folin Ciocalteu method (Folin and Ciocalteu, 1927). The reaction mixture was prepared by adding the extract (20 µL at 5 mg/mL in DMSO), 100 µl Folin Ciocalteu reagent (1 mL of Folin Ciocalteu in 9 mL of distilled water) and 80 µL of 7.5% Na_2CO_3 solution in deionized water. The solution mixture was placed in the dark at room temperature for 30 min and the absorbance was read at 765 nm on a microplate reader (Epoch, BioTek). The total phenolic concentration was calculated from a calibration curve prepared with a standard solution of gallic acid (10-100 mg/L). Data were expressed as gallic acid equivalent (GAE)/g of extract or fraction obtained from three measurements.

2.3.2. Total flavonoid content (TFC)

The total flavonoid content (TFC) of different crude extracts of the plant species and the fractions of *T. capensis* and *F. elastica* was determined according to the aluminium chloride spectrophotometric method which is based on the formation of aluminium chloride complexes (Chang et al., 2002). The crude extracts and fractions of *T. capensis* and *F. elastica* were prepared (0.3 mg of each crude extract/fraction in 1 mL methanol) and mixed with 0.1 mL

aluminium chloride hexahydrate solution (10% aqueous $AlCl_3$ solution), 0.1 mL 1M sodium acetate and 2.8 mL of deionized water. The mixture was shaken and incubated at room temperature (25^{o}C) for 20 min and 200 µL of each mixture was transferred to a 96-well microtitre plate and the absorbance was read at 415 nm in a microtitre plate reader (Epoch, BioteK). A standard curve was plotted using the solution of rutin (0.005-0.1 mg/mL) and the total flavonoid content (TFC) was expressed in milligram rutin equivalent (RE) per gram of dry extract/fraction (Chang et al., 2002).

2.4. Radical scavenging assays

2.4.1. The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH assay was done according to Gyamfi et al. (1999) with slight modifications. Firstly, the optical density (OD) of the DPPH solution was calibrated at 517 nm to between 0.9-1.00. Then, the DPPH solution (160 µL) was added to 40 µL of different crude extracts and fractions at different concentrations (3.125-200 µg/mL). The mixture was incubated in the dark for 30 min, and the absorbance was measured at 517 nm using a microplate reader (Epoch, Biotek). Lower absorbance of the solution indicated higher free radical scavenging activity. The percentage of scavenging activity was calculated using the formula (1) below:

% scavenging activity = [(A0 −As)/A0]×100 formula (1)

Where A0 is absorbance of control (DPPH solution without sample), As is absorbance of tested sample (DPPH plus sample).

The 50% inhibitory concentration (IC_{50}) values of extracts or fractions were determined using a non-linear regression curve of percentage of scavenging activity against the logarithm of concentrations. Ascorbic acid and Trolox were used as positive controls. Each test was done in triplicate and results are presented as mean ± standard deviation (SD).

2.4.2. The 2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay

The stock solution of the ABTS radical was prepared by mixing the ABTS (7 mM) with potassium persulfate (2.45 mM) at room temperature in the dark for 12-16h (Re et al., 1999). The working solution was obtained by calibrating the stock solution to obtain an optical density (OD) of 0.70±0.02 at 734 nm. The ABTS working solution (160 µL) was mixed with the samples (40 µL) at different concentrations and the absorbance was measured after 5 min at 734 nm using a microplate reader (Epoch, BioTek). The percentage of scavenging activity was calculated using the formula (1) above. The 50% inhibitory concentration (IC_{50}) values of samples were determined using a non-linear regression curve of percentage of scavenging

activity against the logarithm of concentrations. Ascorbic acid and trolox were used as positive controls. Each test was done in triplicate and results are presented as mean ± standard deviation (SD).

2.5. Anti-inflammatory assays

2.5.1. The 15-lipoxygenase (15-LOX) inhibitory assay

A spectrophotometric assay for determination of LOX activity was used according to Pinto del Carmen et al. (2007) with slight modifications. This assay evaluated the inhibition of soybean 15-lipoxygenase activity by samples in the presence of linoleic acid based on the formation of the complex Fe^{3+}/xylenol orange with absorption at 560 nm. The substrate linoleic acid (final concentration, 140 µM) was prepared in Tris-HCl buffer (50 mM, pH 7.4). All crude extracts and fractions (10 mg/mL) were prepared in 100% DMSO and further diluted to 2 mg/mL in Tris-HCl buffer, except the water extracts which were directly prepared at 2 mg/mL in the Tris-HCl buffer. Forty microliters of the enzyme (15-LOX), diluted in ice-cold Tris-HCl buffer (final concentration, 0.2 U/mL), was mixed with 20 µL of different concentrations (100 to 0.78 µg/mL) of test samples or quercetin (positive control) at 25°C for 5 min. Linoleic acid (40 µL) was added to the mixture and was further incubated at 25°C for 20 min in the dark. The assay was terminated by the addition of 100 µL of freshly prepared FOX reagent [sulfuric acid (30 mM), xylenol orange (100 µM), iron (II) sulfate (100 µM) in methanol/water (9:1)]. The negative control was made of the enzyme 15-LOX solution, Tris-HCl buffer, substrate and FOX reagent while the blanks contained the enzyme 15-LOX and buffer, but the substrate was added after the FOX reagent. The lipoxygenase inhibitory activity was determined by calculating the percentage of the inhibition of hydroperoxide production from the changes in absorbance values at 560 nm after 30 min at 25°C as indicated in the formula (2) below.

$$\text{Percentage } 15-\text{LOX inhibition } (\%) = \frac{\text{Absorbance (control)} - \text{Absorbance (sample)}}{\text{Absorbance (control)}}\ x\ 100$$ Formula (2).

The 50% inhibitory concentrations (IC_{50}) were determined using the non-linear regression curve of the percentage (15-LOX) inhibition against the logarithm of concentrations tested.

2.5.2. Inhibition of nitric oxide (NO) production

2.5.2.1. Cell culture and treatment with samples

RAW 264.7 macrophages obtained from the American Type Culture Collection (ATCC) (Rockville, MD, USA) were grown at 37°C with 5% CO_2 in Dulbecco's Modified Eagle's

Medium (DMEM) containing 4.5 g/L of glucose and 4 mM of L-glutamine (Hyclone™) supplemented with 10% foetal calf serum (FCS) (Capricorn Scientific Gmbh, South America) and 1% penicillin/streptomycin/fungizone (PSF). The cells were sub-cultured three times a week.

One hundred microliters of a cell suspension (2×10^6 cells/mL) of RAW 264.7 cells at the logarithmic phase of growth (about 80% confluence) were seeded in 96-well microtitre plate and incubated overnight at 37°C with 5% CO_2 to allow attachment. The RAW 264.7 cells were activated by incubation in a medium containing 5 µg/mL of lipopolysaccharide (LPS) alone (control) and treated simultaneously with different concentrations of the samples dissolved in DMSO and further diluted in culture medium. The concentration of DMSO in the experiment did not exceed 0.5%.

2.5.2.2. Measurement of nitrites and determination of percentage of nitric oxide inhibition

After 24h of incubation at 37°C with 5% CO_2, nitric oxide released from RAW 264.7 macrophages was assessed by determination of nitrite concentration in supernatant using the Griess reagent (Adebayo et al., 2015). Briefly, 100 µL of cell supernatant from each well was transferred into a new 96-well microtitre plate and the same volume of Griess reagent was added. After 15 min of incubation in the dark at room temperature, the absorbance was recorded at 550 nm on a microtitre plate reader (Epoch Biotek). The concentrations of the nitrites were calculated from the standard curve using serial dilutions of sodium nitrite (0-50 µM). Percentage of NO inhibition was calculated based on the ability of extracts or fractions to inhibit nitric oxide formation by RAW 264.7 macrophages compared with the control (cells treated with LPS without samples).

2.6. Determination of cell viability

The cytotoxicity of crude extracts and fractions was determined using the 3-(4,5-dimethythiazol- 2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay as described by Mosmann (1983). The culture medium was removed from the wells and after washing with 200 µL of phosphate buffered saline (PBS), 200 µL of fresh culture medium and 30 µL of MTT solution (5 mg/mL in PBS) were added to all wells and the plates were incubated at 37°C with 5% CO_2 for 4h. After incubation, the culture medium was carefully aspirated using a suction pump (Integra, USA) and 50 µL of dimethylsulfoxide (DMSO) was added to all wells. The absorbance was read on a microplate reader (Biotek Synergy, USA) at the wavelength of 570 nm and a reference wavelength of 630 nm. The percentage of cell viability was calculated by comparing

the absorbance of the samples to the negative control (cells treated only with LPS were considered as 100% viable).

2.7. Statistical analysis

All results are presented as mean ± standard deviation (SD) of triplicate experiments using linear regression.

3. Results and discussion

3.1. Yield of crude extracts

The yield of crude extracts is presented in Table 2. The highest extraction yield was obtained with water followed by methanol, ethanol, acetone, and hexane. Among all the solvents used in this study, the water extract had the highest average yield percentage extract (4.65% yield extract). This means that the higher polarity of solvent increases the extraction of more substances from the plant, or polar substances with a higher mass.

Table 2. Yield of extract, total flavonoid and phenolic contents, antioxidant potential and 15-lipoxygenase inhibitory activity of extracts

Plant name	Extract	Yield of extract		TFC mgRE/g	TPC mgGAE/g	IC_{50} in µg/mL		
		mg	%			DPPH	ABTS	15-LOX
A. hybridus	Hexane	10	0.5	22.14±1.17	0.54±0.32	>100	40.77±3.54	59.29±2.42
	Acetone	14	0.7	113.37±5.93	4.96±0.83	>100	27.93±4.36	>100
	Ethanol	18	0.9	72.80±4.16	7.65±2.77	>100	30.19±1.65	>100
	Methanol	34	1.7	30.10±1.13	6.42±0.38	>100	28.92±7.06	>100
	Water	74	3.7	14.71±0.79	14.10±0.95	67.73±8.65	9.6±0.58	>100
C. eduli.	Hexane	30	1.5	7.37±0.87	2.09±0.12	>100	>100	>100
	Acetone	14	0.7	31.12±0.96	23.95±5.36	21.94±3.32	5.37±3.26	56.75±1.68
	Ethanol	56	2.8	7.95±0.39	15.01±0.63	2.97±0.22	1.11±0.87	30.47±0.71
	Methanol	108	5.4	6.97±0.44	14.30±1.02	6.3±0.16	2.57±0.19	9.84±1.65
	Water	106	5.3	9.28±0.79	8.36±8.36	>100	77.42±6.01	>100
C. orbiculata	Hexane	14	0.7	9.47±0.09	1.34±0.34	>100	>100	>100
	Acetone	12	0.6	73.73±4.23	14.60±0.84	23.52±0.70	6.86±1.11	18.10±1.16
	Ethanol	18	0.9	54.26±0.04	18.84±0.33	16.2±0.33	1.96±0.4	41.6±3.02
	Methanol	20	1	65.06±3.14	23.93±0.13	3.76±2.69	3.35±0.18	63.93±6.89
	Water	112	5.6	3.82±3.81	7.96±5.64	>100	>100	>100
D. stramonium	Hexane	10	0.5	27.36±0.82	0.75±0.33	51.80±2.76	>100	30.61±0.53
	Acetone	18	0.9	65.71±1.53	7.99±0.28	31.83±2.31	>100	8.66±0.50
	Ethanol	20	1	78.88±1.13	9.49±0.00	21.17±2.48	>100	57.54±0.57
	Methanol	48	2.4	20.26±0.09	10.04±0.09	24.75±2.02	42.51±3.40	>100
	Water	102	5.1	14.83±0.26	13.22±1.54	31.60±0.36	>100	>100
F. elastica	Hexane	10	0.5	35.26±5.5	3.54±0.49	>100	20.02±1.54	>100
	Acetone	14	0.7	55.71±1.11	11.70±0.89	7.87±0.03	1.80±1.56	7.89±4.11
	Ethanol	22	1.1	41.43±2.86	34.70±0.79	2.16±0.22	0.22±1.18	9.53±2.61
	Methanol	48	2.4	20.17±1.00	43.42±0.16	3.55±0.16	1.26±0.38	3.47±0.07
	Water	42	2.1	24.80±1.18	44.33±1.89	27.33±7.25	11.74±2.26	>100
G. fruticosus	Hexane	24	1.2	12.55±0	4.81±0.74	>100	>100	59.34±2.43
	Acetone	26	1.3	40.72±1.88	8.08±2.06	>100	32.52±1.25	84.98±0.77
	Ethanol	48	2.4	74.04±0.57	22.30±0.30	26.52±7.37	24.21±0.06	>100

	Methanol	82	4.1	54.01±4.36	18.78±0.44	20.22±5.1	11.4±3.4	>100
	Water	80	4	35.29±2.84	42.24±4.03	>100	5.12±4.7	>100
O. pes-caprae	Hexane	14	0.7	28.32±0.04	2.88±0.25	>100	>100	70.53±0.78
	Acetone	14	0.7	76.32±1.79	2.33±0.03	5.22±1.44	24.71±1.14	>100
	Ethanol	26	1.3	75.33±3.01	12.65±0.19	73.22±2.79	26.53±0.35	>100
	Methanol	48	2.4	73.42±0.65	15.66±2.66	17.33±1.48	20.22±0.91	>100
	Water	68	3.4	25.14±0.78	20.58±0.48	31.39±2.16	24.5±2.91	>100
R. communis	Hexane	12	0.6	39.45±0	0.74±0.05	>100	33.94±0.39	40.60±1.53
	Acetone	18	0.9	53.61±0.92	11.30±0.13	>100	>100	15.4±0.3
	Ethanol	24	1.2	64.69±0.87	13.02±0.04	>100	>100	74.05±2.8
	Methanol	56	2.8	52.69±2.23	8.26±0.64	49.27±4.24	12.36±1.08	78.49±2.24
	Water	88	4.4	24.74±4.58	31.86±0.62	>100	3.15±0.1	>100
R. crispus	Hexane	16	0.8	30.44±0.35	2.11±1.54	>100	39.09±6.5	2.17±1.03
	Acetone	22	1.1	122.17±4.76	8.15±0.01	>100	16.00±0.3	>100
	Ethanol	20	1	64.51±3.40	9.00±0.26	>100	19.35±5.12	>100
	Methanol	50	2.58	91.90±4.10	25.46±1.81	38.69±1.91	14.27±4.3	>100
	Water	100	5	10.30±1.09	7.39±0.73	27.33±7.25	3.43±1.48	>100
S. italica	Hexane	16	0.8	26.03±0.83	16.04±0.36	>100	>100	85.75±0.22
	Acetone	22	1.1	83.76±1.66	13.33±0.00	27.33±2.49	9.44±0.13	46.93±2.69
	Ethanol	28	1.4	107.85±4.23	21.84±1.23	21.44±1.14	11.11±0.33	73.22±0.37
	Methanol	52	2.6	53.80±2.05	20.38±1.58	26.95±4.14	1.13±0.09	>100
	Water	98	4.9	15.70±0.52	18.64±1.66	>100	15.65±2.34	>100
T. capensis	Hexane	12	0.6	12.80±0.95	6.29±0.56	>100	>100	4.65±0.03
	Acetone	20	1	27.73±2.44	30.97±0.50	7.11±1.71	1.91±0.06	18.00±4.23
	Ethanol	28	1.4	36.68±2.69	45.29±0.86	7.68±5.06	1.45±0.16	9.16±1.51
	Methanol	22	1.1	19.55±3.01	22.55±0.10	20.48±2.74	0.6±0.25	20.60±2.68
	Water	36	1.8	30.48±1.88	33.03±4.06	55.87±3.32	36.96±2.65	>100
Vit C		NA	NA	NA	NA	2.13±0.91	0.32±0.59	NA
Trolox		NA	NA	NA	NA	2.49±2.02	0.69±0.28	NA
Quercetin		NA	NA	NA	NA	NA	NA	24.60±0.70

3.2. Total phenolic and flavonoid contents

The highest total phenolic content (TPC) was obtained with the ethanol crude extract of *Typha capensis* (Table 2) with the values of 45.29 ± 0.86 mg GAE /g of extract. TPC was determined for *Typha capensis* fractions and the results indicated that the ethyl acetate fraction of *T. capensis* (37.79 ± 1.94 mg GAE /g of fraction) had the highest TPC, followed by the butanol fraction (27.45 ± 1.67 mg GAE /g of fraction). Regarding the total flavonoid content (TFC), the acetone crude extract of *Rumex crispus* had the highest TFC with 122.17 ± 4.76 mg RE /g of extract followed by *Amaranthus hybridus* acetone crude extract with 113.37 ± 5.93 mg RE/g extract (Table 2). The results showed poor correlation between phenolic and flavonoid contents.

Reports on the phytochemical composition of *Typha capensis* indicated the presence of several flavones and phenolic compounds, long chain hydrocarbons as well as various triterpenoids with a steroidal skeleton such as typhasterol that has been isolated from this plant (Van Wyk et al., 2009). Secondary metabolites from plants, such as phenolic compounds, may have anti-inflammatory activity via inhibition of ROS, reactive nitrogen species (RNS), 15-LOX, and COX pathways. ROS and RNS act as secondary messengers, closely associated to both acute and chronic types of inflammation (Nishio et al., 2013; Brune

et al., 2013; Mittal et al., 2014). Flavonoids are able to increase the function of the body against viruses and carcinogenic agents, and have good anti-inflammatory activity; these activities are due to the phenolic hydroxyl groups linked to the flavonoid structures (Yamamoto and Gaynor, 2001; Pereira et al., 2013). There is increasing interest in flavonoids as possible therapeutic agents against free radical-mediated diseases (Peng et al., 2014; Liang et al., 2014). *Typha capensis* had good phenolic and flavonoid contents, thus potentially good activity against free radicals.

3.3. Antioxidant activity of crude extracts and fractions

A free radical is a molecule or atom that carries one or more unpaired electrons and is able to exist independently. Examples of free radicals are hydroxyl free radical, superoxide free radical anion, lipid peroxyl, lipid peroxide, and lipid alkoxyl. Reactive oxygen species (ROS) are radical derivatives such as singlet oxygen and hydrogen peroxide (Poyton et al., 2009). The results of the antioxidant activity assays are presented in Table 2. In this study, the acetone crude extract of *Typha capensis* had good antioxidant activity against DPPH and ABTS with IC_{50} values of 7.11 ± 1.71 µg/mL and 1.91 ± 0.06 µg/mL respectively compared to trolox and ascorbic acid positive controls with IC_{50} values of 2.13 ± 0.91µg/mL and 2.49 ± 2.02 µg/mL. The ethyl acetate and butanol fractions of *T. capensis* had good antioxidant activities against DPPH and ABTS with IC_{50} of 5.61 ± 0.18 µg/mL and 9.98 ± 1.05 µg/mL respectively (Table 3). The total phenolic content of the ethanol extracts of *Typha capensis* leaves was high (45.29 ± 0.86 mg GAE/g of extract). The ethyl acetate fraction of this plant also had high total phenolic content (Table 4). Phenolic compounds contribute to the antioxidant activity of plant extracts and they are well known as radical scavengers, metal chelators, reducing agents, hydrogen donors and singlet oxygen quenchers (Khadri et al., 2010).

Table 3. Antioxidant and anti-inflammatory activities of fractions from acetone extracts of *Ficus elastica* and *Typha capensis*

Plant name	Fractions	IC_{50} in µg/mL		
		DPPH	**ABTS**	**15-LOX**
Ficus elastica	Chloroform	>100	44.89±0.15	18.31±1.02
	Ethyl acetate	1.10±0.26	0.27±0.01	100
	Butanol	0.26±0.05	0.54±0.06	>100
	MeOH/Water (35%)	7.15±0.76	4.68±0.59	>100
	Hexane	0.55±0.39	62.05±2.65	10.15±4.12
Typha capensis	Chloroform	83.27±1.43	11.95±1.77	35.17±3.00
	Ethyl acetate	5.61±0.18	5.56±2.47	48.11±3.92
	Butanol	9.98±1.05	0.89±0.34	27.47±3.48
	MeOH/Water (35%)	62.88±4.13	55.56±0.36	9.15±0.0
	Hexane	>100	63.39±2.02	10.19±1.39
Controls	Vit C	2.13±0.91	0.32±0.59	NA
	Trolox	2.49±2.02	0.69±0.28	NA
	Quercetin	NA	NA	24.6±0.7

Table 4. Total phenolic content (TPC) and total flavonoid content (TFC) of *Typha capensis* fractions

Plant name	Fraction	TFC (mgRE/g)	TPC (mgGAE/g)
Typha capensis fractions	Chloroform	134.63±2.14	1.73±0.39
	Ethyl acetate	19.89±0.17	37.79±1.94
	Butanol	71.08±0.13	27.44±1.67
	35% methanol	71.01±0.04	26.73±2.09
	Hexane	69.19±0.79	0.94±0.87

3.4. Anti-inflammatory activities of extracts and fractions

The 15-lipoxygenase (15-LOX) and nitric oxide (NO) inhibitory assays were used to determine the anti-inflammatory activities of different crude extracts and the IC_{50} values are presented in Table 5. The hexane extract of *Typha capensis* (IC_{50} of 4.65 ± 0.03 µg/mL) and the methanolic extract of *Ficus elastica* (IC_{50} of 3.47 ± 0.07 µg/mL) had promising 15-LOX inhibitory potential compared to the positive control, quercetin (IC_{50} of 24.60 ± 0.07 µg/mL). These crude extracts may contain compounds with higher activity than quercetin. The 35% methanol/water fraction of *T. capensis* had the highest activity against 15-LOX (IC_{50} of 9.15 ± 0.00 µg/mL) followed by the hexane fraction with IC_{50} of 10.19 ± 1.39 µg/mL.

Table 5. Percentage of nitric oxide inhibition and cell viability of acetone extracts from *Typha capensis, Ficus elastica, Carpobrotus edulis* and *Cotyledon orbiculata* using LPS-stimulated RAW 264.7 macrophage cells

Plant name	Concentration (µg/mL)	NO inhibition (% of LPS)	Cell viability (% of DMSO)
Typha capensis	100	86.10± 0.24	96.58± 2.53
	50	79.00± 0.96	100.00± 2.23
	25	58.05± 0.48	99.84± 2.30
	12.5	43.32± 0.38	100.00± 0.04
Ficus elastica	100	97.06±0.07	90.50±2.29
	50	93.10±0.20	86.65±1.21
	25	72.24±0.61	88.17±1.28
	12.5	52.83±0.75	101.74±2.84
Carpobrotus edulis	100	97.88±0.10	112.15±1.14
	50	83.25±0.55	84.05±2.67
	25	54.14±0.65	72.82±1.07
	12.5	39.07±0.24	92.46±1.23
Cotyledon orbiculata	100	99.37±0.14	92.08±0.23
	50	84.31±0.14	78.35±1.98
	25	51.34±0.03	77.48±1.67
	12.5	34.25±0.44	98.26±1.15
Quercetin	100	99.54±0.62	41.59±2.00
	50	102.97±0.23	80.83±1.01
	25	99.43±0.15	79.42±0.59
	12.5	90.39±0.85	93.63±1.08

The inhibitory activity of the acetone extracts of *C. edulis, C. orbiculata, F. elastica,* and *T. capensis* as well as fractions obtained from *T. capensis* on LPS-activated RAW 264.7 macrophages and inhibition of NO production is presented in Tables 5 and 6. The acetone extracts of *C. orbiculata, C. edulis, F. elastica,* and *T. capensis* exhibited very good inhibitory activity against NO production (99.37%, 97.88%, 97.06%, and 86.10% respectively).

From all the plant extracts selected in this study, *T. capensis* had the best antioxidant (DPPH, ABTS), and anti-inflammatory (15-LOX) activity and was fractionated for further studies. The butanol fraction of *T. capensis* was the most active with 94.6% of NO inhibition at 100 µg/mL and 77.39% at 50 µg/ml with cell viability of 100% at 12.5 µg/ml (Table 6). Extracts with good inhibitory activity on NO production and lower toxicity are the most useful to decrease inflammation. NO is a signalling molecule which plays a role in mediating vasodilatation, neurotransmission, as well as inhibition of platelet adherence, and is synthesized by iNOS in response to LPS which acts as a pro-inflammatory mediator (Son et al., 2000; Faro et al., 2014; Lambden et al., 2016). There are many other pro-inflammatory mediators such as bradykinins, and histamine produced by NO released by endothelial cells (Kodela, 2012). The inhibitory activity of iNOS or NOS by plant-derived compounds or crude extracts may be responsible for their anti-inflammatory activity, and many natural products from plants have

been described as inhibitors of iNOS in LPS activated by macrophages (Son et al., 2000; Ryu et al., 2003; Lu et al., 2008). Macrophages stimulated by LPS result in accumulation of NO and superoxide anion. Excessive accumulation of NO and superoxide anion may increase the formation of peroxynitrite ($OONO^-$), resulting in inflammation disorders. The inhibition of any molecular target involved in the NO pathway of inflammation such as ROS, iNOS, and NO itself could have potential to inhibit inflammatory responses (Sekhar et al., 2015).

Table 6. Percentage of nitric oxide inhibition and cell viability of isolated fractions from acetone extracts *Typha capensis*

Typha capensis **Fraction**	**Concentration (µg/mL)**	**NO inhibition (% of LPS)**	**Cell viability (% of DMSO)**
MeOH/Water (35%)	100	93.58±0.26	105.48±1.38
	50	72.26±0.51	76.88±3.96
	25	48.36±4.18	73.63±1.51
	12.5	36.75±0.87	89.11±4.26
Butanol	100	94.60±0.24	94.75±1.92
	50	77.39±1.13	72.17±3.06
	25	54.69±1.86	66.29±1.53
	12.5	41.42±1.05	88.36±2.52
Ethyl acetate	100	60.71±0.01	84.97±1.33
	50	42.40±1.54	69.11±1.53
	25	35.02±0.77	61.77±2.72
	12.5	25.19±0.74	94.71±1.45
Chloroform	100	93.85±0.81	96.78±1.40
	50	84.19±1.21	92.21±1.26
	25	58.86±0.97	84.69±1.46
	12.5	35.76±3.39	99.65±3.59
Hexane	100	90.19±1.46	46.20±1.52
	50	8248±1.19	77.86±4.19
	25	62.86±3.88	73.54±3.32
	12.5	39.65±1.82	88.45±2.32
Quercetin	100	99.54±1.22	41.59±2.01
	50	102.97±0.85	80.83±0.59
	25	99.43±1.01	79.42±1.02
	12.5	90.39±0.22	93.63±1.99

LOX enzymes are sensitive to antioxidants as the latter are involved in inhibition of lipid hydroperoxide formation due to scavenging of lipidoxy- or lipidperoxy-radicals. This could lead to lower availability of the lipid hydroperoxide substrate required for LOX catalysis (Ammon et al., 1992). There are three groups of lipoxygenase enzymes (5-, 12-, and 15-lipoxygenases) which play an important role in inflammatory disorders. The 15-LOX is an important enzyme involved in the synthesis of leukotriene from arachidonic acid. Leukotrienes are mediators of many pro-inflammatory compounds and targeting 15-LOX is considered as one of the therapeutic strategies in the management of inflammatory conditions (Schneider and Bucar, 2005). Extracts or compounds from plants inhibiting the pro-inflammatory activities of the lipoxygenase enzyme may lead to potent anti-inflammatory drugs (Adebayo et al., 2015).

Flavonoids play a key role in decreasing the arachidonic acid cascade via cyclooxygenase or lipoxygenase pathways which leads to a reduced inflammatory response (Mulaudzi et al., 2013). Secondary metabolites from natural products with high phenolic contents have been reported to inhibit the inflammatory response via molecular targets of inflammation such as 15-LOX, and ROS/RNS pathways (Mittal et al., 2014). *T. capensis* had very good activity against DPPH and ABTS followed by *F. elastica*, *C. edulis*, *C. orbiculata* and *S. italica*. In the anti-inflammatory studies, *T. capensis*, and *F. elastica* showed very good activity against 15-LOX and NO. *Typha capensis* is used traditionally in South Africa to treat dysmenorrhea, diarrhoea and dysentery, to enhance male potency and libido, to treat genital problems, to improve circulation, to promote fertility in women, to strengthen uterine contraction in childbirth, and promote expulsion of the placenta (Pujol et al., 1990; Hutchings et al., 1996). Several flavonoids and other phenolic compounds have been found in *Typha capensis* species, but of particular interest are various triterpenoids with a steroidal skeleton, such as typhasterols. Compounds such as typhaphthalide, typharin, sitosterol and the flavonoids afzelekin, epiafzelekin, (+)-catechin, and (-)-epicatechin have been isolated from rhizomes of *Typha capensis* (Shode et al., 2002). Several flavones and phenolic compounds have been isolated from the *Typha* genus. These phenolic compounds are long-chain hydrocarbons; triterpenoids have also been isolated and identified with a steroidal skeleton of typhasterol (Chapman and Hall, 2000; Woo et al., 1993). Some studies have indicated antimicrobial activity of *Typha capensis* (Masoko et al., 2007). Sitosterol, isolated from *T. capensis* was shown to prevent obesity-related chronic inflammation, with a strong negative Pearson correlation between pro-inflammatory cytokines such as TNF-α and IL-6 and the tested serum sistosterol in animal and clinical studies (Kurano et al., 2018). (−)-*Epi*afzelechin, is a flavan-3-ol compound isolated from *T. capensis* which has antioxidant and anti-inflammatory activities (Min et al., 1999; Wong et al., 2014). Several studies on (-)-epicatechin (also isolated from *T. capensis*) using LPS-stimulated RAW 264.7 macrophages can decrease the expression of pro-inflammatory cytokines (TNF-α, IL-6) protected the renal dysfunction induced by LPS, and consequently, reduced the inflammatory damage. We can speculate that those compounds isolated from *T. capensis* might be responsible for the anti-inflammatory activity of the crude extract and fractions. Further work is required to identify and characterize the pure compounds responsible for the 15-LOX and nitric oxide inhibition in this study. After compound isolation (from plants with the most promising activity against inflammation), the mechanism of antioxidant and anti-inflammatory activities of those compounds will be elucidated, using some of the pathways of inflammation such as COX-2, iNOS, pro and anti-inflammatory cytokines, and NF-κB.

4. Conclusion

The results showed that from eleven plants used in southern Africa against inflammation, the acetone crude extract of *Typha capensis* had the best antioxidant and anti-inflammatory activities and can potentially be developed into a useful anti-inflammatory remedy. Further work is required to isolate and characterize the bioactive compounds that are responsible for the antioxidant and anti-inflammatory activities. These may act as chemical markers if a potential extract or fraction of the extract is developed into a therapeutic remedy, or as lead compounds for chemical modification to enhance activity.

Author contributions

MO (onduam@yahoo.fr) carried out the experiments with the assistance of EMN (njoyamfotie@gmail.com) and MAA (munalsamahoni@yahoo.com). LJM (lyndy.mcgaw@up.ac.za) supervised the work and provided funding and facilities. All authors were involved in writing and editing the manuscript.

Acknowledgements

The National Research Foundation (grant number 105993) is thanked for providing research funding. The NRF and University of Pretoria are acknowledged for doctoral fellowships to OM. Ms Elsa van Wyk and Ms Magda Nel are thanked for assisting with preparation of herbarium voucher specimens.

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