Journal of Inflammation Research

Dovepress
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ORIGINAL RESEARCH

Inhibition of nitric oxide production and free radical scavenging activities of four South African medicinal plants

This article was published in the following Dove Press journal:
Journal of Inflammation Research

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Introduction: Traditional healing is often the preferred form of therapy especially in rural and resource-limited communities. The extracts of plants are used to treat many diseases such as arthritis and chronic pain. Four medicinal plant species, namely, *Acokanthera oppositifolia, Plantago lanceolata, Conyza canadensis* and *Artemisia vulgaris* used in Southern Africa to treat pain and inflammation-related diseases were selected for evaluation in laboratory-based experiments.

Methods: The selected plant species were evaluated for phytochemical content, antioxidant and anti-inflammatory activities, as well as cytotoxicity effects against mammalian cells in culture.

Results: The results indicated that the *n*-hexane and chloroform extracts of *P. lanceolata* had the best antioxidant activities with an IC_{50}=0.41 µg/mL. Also, the acetone extracts of *P. lanceolata* had 93.76% nitric oxide (NO) inhibition. However, the chloroform and *n*-hexane extracts of *C. canadensis* produced NO inhibition of 98.53% and 99.2%, respectively, at 100 µg/mL with IC_{50}=17.69 µg/mL. Furthermore, the ethyl acetate extracts also had promising NO inhibitory activity (96.33%), but the cytotoxicity results with cell viabilities of 5.31%, 5.7% and 5.89%, respectively, suggested that the observed activity was due to a cytotoxic effect. Acetone extracts of *C. canadensis* were also cytotoxic at 30 µg/mL with 6.07–6.67% cell viabilities compared with the acetone extracts of *P. lanceolata* (99.57%).

Conclusion: The results partially validate the ethnomedicinal uses of the selected plant species used for inflammation-related conditions. However, because some of the extracts had potential cytotoxic effects, caution is advised in their use, especially those consumed orally.

Keywords: nitric oxide, free radicals, medicinal plants, South Africa, inflammation

Introduction

Medicinal plants continue to play a huge role in meeting the primary health care needs of people living in resource-limited areas of developing countries such as South Africa. An estimated 80% of people living in South Africa continue to depend on herbal products for therapeutic purposes.[1] Traditional healing, based on medicinal plants, is often the preferred form of therapy by cultural communities in South Africa,[2] supported by a biodiversity comprising almost 10% of world's higher plant species.[3] Despite the popularity of medicinal plants as sources of remedies, only a few of these abundant natural resources have been fully exploited for their therapeutic and income generation potentials.[4] The ethnomedicinal uses of plants to treat pain and inflammation-related diseases such as arthritis are often used as a motivation for the investigation of the

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DovePress
http://doi.org/10.2147/JIR.S199377

therapeutic efficacy and safety of the plant species.[5] This systematic approach has led to the isolation of potent anti-inflammatory drugs such as quinine, morphine and cocaine in the past.[6]

Four selected plant species, namely, *Acokanthera oppositifolia* (Lamiaceae), *Plantago lanceolata* (Lamiaceae), *Conyza canadensis* (Asteraceae) and *Artemisia vulgaris* (Lamiaceae) are among the plant species used in southern Africa to treat infection, pain and inflammation-related diseases (Table 1). Dried leaves or roots of *Acokanthera oppositifolia*, also known as Bushman's poison, are used to treat headaches, colds and abdominal pains.[7] Leaf infusions are taken orally for abdominal pain in the Northern Cape of South Africa. Colds, anthrax and tapeworm infestation are also treated with aqueous extracts of this plant.[8] Some members of the genus *Acokanthera* are used as sources of extremely toxic hunting arrow poison by Khoi-San people of southern Africa.[8] The leaves are dried, powdered and sniffed, or may be soaked in water and the extract used as a nasal spray to treat headaches.[9] As a snake bite treatment, the leaves or roots are powdered and applied directly to the bite as a paste.[9] *Plantago lanceolata* is used mainly against catarrhs of the respiratory tract and inflammation of the mouth and throat. It is externally used to treat wounds and inflammation of the skin, colds and flu in Chinese modern herbalism.[10] The leaves can be chopped, mashed and placed directly over the problem area. The leaves can be boiled into a strong tea, after which a cloth soaked in the hot tea is placed directly over the affected area. As a poultice, *P. lanceolata* is a highly effective remedy for bites and insect stings, boils and other eruptive skin disorders and any deep-seated infection. *Conyza canadensis* is used to treat rheumatism and as an antidiarrheal and anti-haemorrhoidal agent.[11] Extracts of *Artemisia* species, particularly *Artemisia afra*, are used to treat colds, cough and influenza.[12] Also, the extracts are frequently used to treat inflammation and infections by pathogens.[13]

Nitric oxide (NO) is a signaling molecule that plays a significant role in the prolongation of inflammation and immunological responses.[17] During inflammation, NO is released and sustained at very high levels, leading to inflammation of the joints, lung and gut.[18] The synthesis and release of NO promotes inflammation; therefore, extracts that could act as scavengers of NO or inhibitors of its production, especially with corresponding low cytoxicity, could be used to mitigate the propagation of inflammation by NO.[19] The mechanism of action of the bioactive extracts or their constituents may be via the inhibition of the activity of inducible nitric oxide synthase (iNOs) or by free radical scavenging activities.[20,21] Nitric oxide and singlet oxygen (O^{-}) are among the free radicals that are involved in lipid peroxidation and implicated in the pathogenesis of chronic inflammation.[21,22]

The capacity of plant extracts to modulate NO synthesis and release in lipopolysaccharide (LPS) stimulated RAW cells in vitro has been reported in previous studies.[19–24] Using the selectivity index, it was possible to demonstrate that the biological activity of the plant extracts was not as a result of cytotoxicity. For instance, the leaf acetone extract of *Leucaena leucocephalia, Lippia javanica, Maesa lanceolata* had 97–99% inhibition on NO production at 25–30 µg/mL.[22,24] Similarly, there was no detection of NO production in stimulated human peripheral blood mononuclear cells treated with 25 µg/mL of *Ocimum labiantum* ethyl acetate leaf extracts.[23] These results support the notion that some plant extracts may be useful as natural inhibitors of iNOs and could be developed as templates for the development of alternative therapeutic strategy to prevent over-production of NO.

Table 1 The ethnomedicinal uses of the selected plant species in southern Africa, with pain and inflammation being at least one of the indications for therapeutic use

Plant species	Ethnomedicinal uses	References
Artemisia vulgaris	It is traditionally used for irregular or painful menstruation. It is also used to treat flatulence, distension, colic, diarrhoea, constipation, cramps, worm infestation, hysteria, epilepsy, and vomiting	10
Acokanthera oppositifolia	Decoctions of the leaves and powdered roots are taken orally or used in the form of a snuff to treat headaches and pain, and infusions are used for abdominal pains, convulsions and septicemia	7
Plantago lanceolata	Used for stopping excessive bleeding and wound healing	14
Conyza canadensis	Extracts of the plant are used for rheumatism, and for antidiarrheal and anti-hemorrhoidal use. The extracts also have antiviral and antibacterial activities	11,15,16

This study was aimed at investigating the anti-inflammatory activity, specifically the NO inhibition by extracts of four medicinal plants used to treat pain and inflammation in South Africa. These plants could be useful in the management of inflammation exacerbated by NO overproduction.

Materials and methods

Collection, extraction and preparation of plant materials have been described in a recent publication.[25] In brief, ground leaf (5 g) of each plant species was extracted with 20 mL of acetone, ethyl acetate, chloroform, hexane and water in polyester centrifuge tubes. The tubes were vigorously shaken for 30 mins using an orbital shaker (Velp Scientifica) and centrifuged at 2000 rpm for 10 mins. The supernatant was filtered with Whatman No.1 filter paper and transferred into pre-weighed glass containers. The solvent was dried under a stream of air in a fume hood at room temperature to produce dried extract. The extract was reconstituted in 100% dimethyl sulphoxide (DMSO) (Merck Schuchardt OHG) at 10 mg/mL and used for NO and cytotoxicity assays. Mouse leukemic macrophage cell line (RAW 264.7) was bought commercially (American Type Culture Collection, Rockville, MD, USA) and was used for the NO and cytotoxicity assays.

Free radical scavenging activities

The free radical scavenging activity of the extracts was measured using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) method, modified for working with 96-well microtitre plates. With this method, it was possible to determine the antiradical power of an antioxidant by measuring the decrease in the absorbance of DPPH at 517 nm.

An aliquot of 50 µL of extracts and controls was added into appropriate wells in a 96-well microtitre plate. The microtitre plate was incubated at room temperature in dark for 30 mins. Then, 50 µL of DPPH was added to all the wells in the 96-well microtitre plate. The mixture was shaken vigorously and allowed to stand at room temperature for 30 mins. Absorbance was read at 570 nm using a plate reader. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity.[26]

The percentage (%) free radical scavenging effect calculated using the following equation:

$$\%\ \text{free radical scavenging activity} = 100 - \left(\frac{OD_{extracts} - OD_{blank}}{OD_{negative\ control} - OD_{blank}}\right) \times 100$$

The blank is the absorbance of the control reaction (containing all reagents except the test compound) and sample is the absorbance of the test compound. Extract concentration providing 50% inhibition (IC_{50}) was calculated by plotting inhibition percentage against extract concentration. Ascorbic acid (Vitamin C) and quercetin were used as positive control.

Evaluation of phytochemical constituents of *P. lanceolata* n-hexane extract

The leaves were air-dried at room temperature for two weeks, after which they were ground to a uniform powder. The *n*-hexane extract was prepared by soaking 100 g of the dried ground material in 1 L of *n*-hexane at room temperature for 48 hrs. The extract was filtered after 48 hrs through a Whatmann filter paper number 42 (125 mm). The extract was then concentrated using a rotary evaporator in a water bath at a temperature not exceeding 40°C. The extract was reconstituted in DMSO at 10 mg/mL for all bioassays.

Test for anthraquinones

The leaf extract of *P. lanceolata* (0.5 g) was boiled with 10 mL of concentrated sulfuric acid and filtered through a filter paper while hot. The filtrate was extracted with 5 mL of chloroform. The chloroform layer was pipetted into another test tube and 1 mL of dilute ammonia was added. A pink or red color precipitate indicated the presence of anthraquinones.[26,27]

Test for terpenoids

The extract (0.5 g) was added to 2 mL of chloroform in a clean test tube. Thereafter, 3 mL of concentrated sulfuric acid was carefully added to form a layer. A reddish-brown coloration at the interface indicated the presence of terpenoids.[28]

Test for flavonoids

Three methods were used to test for flavonoids. Firstly, dilute ammonia (5 mL) was added to 5 mL of an aqueous filtrate of the extract. Concentrated sulfuric acid (1 mL) was added. A yellowish coloration that disappears on standing indicates the presence of flavonoids.[26] Secondly, a few drops of 1% of aluminum solution were added to a portion of the filtrate. A yellowish coloration was regarded as a positive indicator of the presence of flavonoids. Lastly, 5 mL of the extract was heated with 10 mL of ethyl acetate over a steam bath for 3 mins. The mixture was filtered through a filter paper and 4 mL of the

filtrate was shaken with 1 mL of dilute ammonia solution. A yellowish coloration indicates the presence of flavonoids.[26]

Test for saponins

Dried extract (0.5 g) was suspended in 5 mL of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, after which it was observed for the formation of an emulsion, which confirms the presence of saponins.[29]

Test for tannins

One gram of ground plant material was weighed in a beaker; 10 mL of distilled water was added before boiling the mixture for 5 mins. Two drops of 5% ferric chloride were added. The presence of a greenish precipitate is an indicator of the presence of tannins.[27]

Test for cardiac glycosides

The hexane extract (0.5 g) was diluted to 5 mL in distilled water and then mixed with 2 mL of glacial acetic acid containing one drop of ferric chloride solution. Concentrated sulfuric acid (1 mL) was carefully added to the solution by the side. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.[30]

Test for steroids

Two milliliters of acetic anhydride were added to 0.5 mL crude plant extract with 2 mL of concentrated sulfuric acid. The color changed from violet to blue or green which indicates the presence of steroids.[31]

Test for quinones

To 1 mL of plant extract filtrate, 1 mL concentrated sulfuric acid was added, leading to the formation of red color to indicate the presence of quinones.[32]

Test for coumarin

Exactly 5 mL of 10% sodium hydroxide was added to 5 mL of plant extract filtrate, followed by the addition of 5 mL of chloroform. The development of yellow color indicates the presence of coumarin.[33]

Nitric oxide assay

Mouse leukemic macrophage cell line (RAW 264.7) stimulated with LPS derived from *Escherichia coli* (*E. coli*) was used to induce NO synthesis.

Preparation of cells for culture

In vitro cell culture system provides a controlled environment where specific cell lines can be incubated under defined conditions and treated with growth factors, drugs, toxins or mechanical stimuli in a reproducible manner, which has now been improved to the use of three-dimensional cell culture systems.[34]

Nitric oxide inhibitory assay

The RAW 264.7 cells were grown in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% heat-inactivated fetal calf serum (FCS) and 5 µg/mL of Penicillin/Streptomycin/Fungizone (PSF), to confluence at 37ºC in a humidified incubator of 5% CO_2. Cells were seeded in 96-well microtitre plates (for nitrite [NO_2] at 5×10^5 cells per well). When the cells were fully adhered after 12 hrs, they were treated with 50 µL of test extracts at 10 mg/mL reconstituted in acetone. After 2 hrs of incubation at 37°C with the test extracts, 50 µL of LPS (5 µg/mL) in DMEM was added to all the wells and further incubated for 24 hrs. After 24 hrs incubation, the supernatants from the cells were collected for NO_2 measurement using the colorimetric Griess reaction method.[35,36] Aliquots (60 µL) of cell supernatant were combined with an equal volume of Griess reagent [1% sulphanilamide/0.1% N-(1-napthyl) ethylene diamine (International Laboratory, USA), each in 2.5% H_3PO_4] in a 96-well plate at room temperature for 10 mins, and the absorbance was taken at 550 nm using a Multiscan plate reader (Genios, Tencan). Absorbance measurements were averaged and converted to µmol/L of NO_2-per well using a standard curve of sodium nitrite.

MTT cell viability assay

RAW264.7 was cultured in DMEM supplemented with 10% FCS and 1% PSF. The cells were harvested with trypsin-EDTA, centrifuged at 600 rpm for 5 mins and the pellet washed with sterile phosphate buffered saline (PBS) at pH 7.2. The cells were seeded in 96-well microtitre plates at 1×10^5 cells per well and allowed to adhere for 1 hr at 37°C in a humidified atmosphere containing 5% CO_2. The medium was then replaced with fresh medium containing plant extracts at various concentrations (2, 10, 30, 100 µg/mL). The microtitre plates were incubated at same conditions for 48 hrs. After 48 hrs, the supernatant was removed and topped up with 200 µL DMEM. To each well, a further 30 µL of 15 mg/mL 3-(4, 5- dimethylthiazol-2-yl)-2, 5-diphenyl tetra-zoliumbromide (MTT) was

added and the microtitre plates were incubated further for 2 hrs. After 2 hrs incubation, the medium was removed, and Isopropanol (containing 0.04 M hydrochloric acid) was added to dissolve the formazan formed. The microtitre plates were read at 570. Hydrogen peroxide (1 mM) was used for positive control.

Results and discussion

Previous studies have indicated that *Plantago lanceolata* has many medicinal applications in many parts of southern Africa.[37] Leaves of the plant are used as astringent, anti-inflammatory agent, cough medicine, topical anodyne, anti-bacterial agent, sore throat treatment and anti-diarrheal medicine. The leaves are also used to treat hemorrhoids, cervicitis, rectal fissures, insect bites, snake bites, cuts and abscess.[38] In a recent report, the aqueous extracts of *P. lanceolata* had 146.4 µg/mL total phenolic contents, which represented the best among the selected plants.[38] The observed antioxidant activity of the methanol/ethanol extracts of *P. lanceolata* was attributed to the high total phenolic contents of the extracts.[38]

Antioxidant activities of the plant extracts

The ability of the plant extracts to scavenge free radicals was evaluated using the DPPH radical scavenging method, with ascorbic acid (vitamin C) as the positive control. Substances capable of donating electrons/hydrogen atoms convert DPPH radical to its non-radical form 1,1′-diphenyl-2-picrylhydrazine, a reaction which can be measured at 517 nm. The *n*-hexane and chloroform extracts of *Plantago lanceolata* had the best antioxidant activities with IC_{50} of 0.41 µg/mL compared with the positive controls (Table 2). This was surprising because antioxidant activity was expected to be higher in polar than in non-polar extracts; however, the antioxidant activity of non-polar extracts has been reported previously.[39] It is important to note that a lower IC_{50} value reflects better protective action. The extracts with the least free radical scavenging activity among those evaluated were the *n*-hexane extracts of *A. vulgaris* (IC_{50}=3.51 µg/mL). The observed antioxidant activity of *P. lanceolata* extracts could be attributed to its terpenoid content. Terpenoids, especially triterpenoids, are widely distributed in higher plants and are reported to exert many biological effects, including free radical scavenging abilities, anti-inflammatory and anti-cancer activities.[40–42]

Therefore, because the best antioxidant activity of *P. lanceolata* was observed in the non-polar extracts, we decided to investigate the classes of phyto-compounds present in the *n*-hexane extracts using qualitative phytochemical screening methods. The presence of other classes of compounds aside of phenolic compounds was recorded (Table 3). For example, terpenoids are known to possess antioxidant, anti-inflammatory and anti-microbial activities.[41] Also, because phenolic compounds were also detected in the *n*-hexane extracts, it is plausible to assume that the relatively higher antioxidant activity of the extract was due to a combined effect of these bioactive compounds. Alkaloids, saponins, tannins and others detected in the *n*-hexane extracts may also contribute to the combined effects of the extracts.[42] Determination of the specific class of phyto-compound responsible for the observed biological activity was beyond the scope of this work.

Inhibition of NO production by the extracts

NO is an important chemical mediator generated by endothelial cells, macrophages, neurons, and are involved in the

Table 2 The antioxidant activity (DPPH radical scavenging activity) of the selected plant species extracted with solvents of varying polarities

Plant species	IC_{50} (µg/mL)				
	Water	Acetone	Ethyl acetate	Chloroform	*n*-Hexane
Artemisia vulgaris	ND	2.10±0.32	2.20±1.03	1.36±0.14	3.51±1.22
Acokanthera oppositifolia	2.23±0.91	0.54±0.72	1.81±0.94	0.87±0.65	2.42±1.05
Plantago lanceolata	1.81±0.24	2.02±0.96	0.56±0.03	0.41±0.77	0.41±0.1 7
Conyza canadensis	0.56±0.07	0.87±0.44	2.42±0.98	2.23±1.92	2.02±1.90
Vitamin C	0.04±0.09				
Quercetin	0.06±0.37				

Abbreviation: ND, Not done.

Table 3 Phytochemical analysis of the *n*-hexane extracts of *P. lanceolata* indicating the presence or absence of some of the secondary metabolites presumably responsible for bioactivity of the extracts.

Phytochemicals	Extracts
Anthraquinones	Present
Terpenoids	Present
Flavonoids	Present
Saponins	Present
Tannins	Present
Quinones	Not detected
Coumarins	Not detected
Cardiac Glycosides	Present
Phlobatannins	Present
Phenols	Present
Alkaloids	Present

Note: The phytochemicals tested were proven to have significant biological activities elsewhere.[38]

regulation of various physiological processes, including inflammation. Excessive production and release of NO is associated with several diseases. NO is generated in biological tissues by specific nitric oxide synthase (NOSs), which metabolizes arginine to citrulline with the formation of NO via a five-electron oxidative reaction.[43] These compounds are responsible for altering the structural and functional behavior of many cellular components. Incubation of sodium nitroprusside solution in phosphate-buffered saline at 25°C for 2 hrs resulted in a linear time-dependent nitrite production. The NO scavenging capacity of the extracts was determined by the decrease in the absorbance at 550 nm, resulting from a reduction of NO production. In Table 4, the ability of the extracts to inhibit NO production in LPS induced RAW 264.7 cells is presented.

The best inhibitory activity on NO synthesis was demonstrated by *Conyza canadensis* extracts. *Conyza canadensis* acetone and ethyl acetate extracts had NO inhibitory activities of 94.3% at 10 mg/mL and 96.3% at 100 µg/mL with IC_{50}<2 µg/mL. *Conyza canadensis* acetone extract also indicates a percentage of inhibition of 97.3% at 10 µg/mL with IC_{50}<2 µg/mL (Table 4). These results are like those reported by others,[44,45] supporting the antioxidant and anti-inflammatory activities of extracts of this plant species.

The phytochemical analysis of *P. lanceolata n*-hexane extract indicated the presence of terpenoids and phenols. Phenolic compounds and terpenoids are associated with antioxidative action in biological systems, acting as scavengers of singlet oxygen and free radicals.[46,47] The NO scavenging activity of phenolic compounds and terpenoids has been reported previously.[48,49]

Cell viability assessments

Mouse Leukemic macrophage cell line (RAW 264.7) was used for cytotoxicity assessments using the MTT methods.

Table 4 Inhibition of NO production in LPS-activated macrophages RAW 264.7 by extracts of *Acokanthera oppositifolia, Plantago lanceolata, Conyza canadensis* and *Artemisia vulgaris*

Plant species	Extracts (µg/mL)	IC_{50} (µg/mL)	Percentage inhibition of nitric oxide				
			Water	Acetone	Ethyl acetate	Chloroform	*n*-Hexane
Acokanthera oppositifolia	100	32.57	ND	86.72±2.25	98.04±1.06	93.27±4.47	86.97±1.97
	30		ND	37.09±2.44	83.97±4.79	50.62±7.76	39.48±1.52
	10		ND	24.31±2.29	37.22±9.25	21.67±1.83	26.88±1.96
	2		ND	16.47±0.94	16.96±5.67	10.35±0.0	23.33±0.68
Artemisia vulgaris	100	41.13	68.24±2.6	100±1.95	97.19±2.82	98.29±0.96	49.15±2.56
	30		38.32±1.5	96.21±1.21	85.8±2.69	87.21±2.25	29.02±1.2
	10		24.9±4.68	68.18±3.39	51.84±1.36	53.13±4.5	25.47±1.87
	2		17.64±9.9	27.79±2.4	23.5±3.5	18.55±7.12	16.35±2.29
Plantago lanceolata	100	44.05	84.4±3.7	93.76±1.17	87.76±7.71	85.74±2.48	84.46±1.26
	30		37.89±1.7	51.78±1.73	50.68±5.56	44.13±1.34	38.81±2.19
	10		21.43±3.6	29.87±2.99	28.1±4.17	26.88±0.48	25.83±1.04
	2		10.66±3.33	21.67±2.01	18.25±1.5	21.67±1.72	18.68±0.78
Conyza canadensis	100	17.69	95.9±2.88	96.88±1.98	96.33±1.17	98.53±0.0	99.20±0.38
	30		53.9±7.04	98.29±1.46	96.94±1.31	97.67±0.57	98.71±0.4
	10		31.71±2.26	97.31±1.67	94.31±4.43	80.11±2.64	71.61±0.77
	2		15.5±6	51.60±1.65	53.80±4.08	48.17±0.69	37.58±1.59

Abbreviations: ND, Not done; NO, Nitric oxide; LPS, Lipopolysaccharide.

Table 5 The cytotoxicity, safety profile and percentage cell viability of the extracts of the selected plant species on RAW 264.7 cells in vitro

Plant species	Extracts (µg/mL)	IC_{50} (µg/mL)	Percentage cell viability				
			Water	Acetone	Ethyl acetate	Chloroform	*n*-Hexane
Acokanthera Oppositifolia	100	32.57	ND	67.98±1.53	48.53±2.60	88.10±4.09	93.33±10.60
	30		ND	76.94±4.37	88.06±7.27	96.55±1.37	86.82±8.84
	10		ND	86.32±3,28	99.92±8.76	74.30±3.99	83.72±7.39
	2		ND	95.16±2.85	85.78±6.64	80.47±6.68	ND
Artemisia vulgaris	100	41.13	84.46±8.17	10.50±1.96	25.00±2.33	40.35±3.3	59.53±4.16
	30		81.09±5.52	54.96±3.55	73.29±4.17	71.78±0.97	71.63±5.00
	10		70.62±6.87	99.34±10.9	91.01±7.83	79.61±3.82	92.09±6.27
	2		74.96±4.61	99.76±6.38	94.88±3.70	88.45±1.99	94.92±10.30
Plantago lanceolata	100	44.05	96.36±19.86	88.80±8.12	94.84±8.60	75.35±2.44	91.90±7.43
	30		94.92±7.20	99.6±10.98	99.16±10.98	91.05±8.87	99.05±10
	10		99.64±11.38	99.57±0.86	99.57±0.86	98.91±4.67	97.93±6.62
	2		99.37±1.42	100±10.10	100±4.98	99.31±3.02	83.99±10.53
Conyza canadensis	100	17.69	85.97±9.83	6.20±0.11	5.89±0.05	5.31±0.15	5.70±0.19
	30		99.65±11.29	6.01±0.05	6.67±0.71	54.22±15.80	98.76±2.41
	10		99.25±16.16	89.57±7.91	89.96±8.62	77.21±0.33	98.18±0.46
	2		100±1.06	78.84±8.53	94.2±11.07	99.72±7.78	81.20±0.56

Conyza canadensis ethyl acetate extracts had promising NO inhibitory activity, but the cytotoxicity evaluation at 100 µg/mL and 30 µg/mL with cell viabilities of 5.89 ±0.05 and 6.67±0.71%, respectively, suggests that the observed activity was due to a cytotoxic effect. *Conyza canadensis* chloroform extract was also cytotoxic at 100 mg/mL with 5.31%±0.15 cell viability (Table 5). *Conyza canadensis* is a plant species that is resistant to a wide range of herbicides,[50] and some of the secondary phyto-compounds it produces could be responsible for the cytotoxicity observed. However, extracts of *Plantago lanceolata* with good free radical scavenging activities indicated a good cell viability and safety profile between 75.35 ±2.44% and 100%.

Conclusions

This study demonstrated that some of the plant species used to treat inflammation-related conditions contained phyto-compounds with reasonable antioxidant and anti-inflammatory activities. However, because some of the extracts had potential cytotoxic effects, caution is advised in their application in therapeutic mixtures. Although, the cytotoxicity assessments were done on cancerous cell lines, confirmation of the cytotoxicity results using other cell types and normal cells is necessary. It is also important to confirm the results in in vivo experiments using animal models. Therefore, further work is required to validate the safe use of extracts with rigorous cytotoxicity assessments and to identify the phyto-compounds responsible for the biological activities.

Availability of data and material

The sources of all the data presented in this manuscript are readily available and can be provided upon official request to the corresponding author.

Acknowledgments

The authors wish to thank Mr. Jason Sampson, the curator for Manie van der Schiff Botanical Gardens, University of Pretoria, for plant collection and identification. This work was carried out at the Department of Biomedical Sciences of the Tshwane University of Technology, Arcadia, and the Department of Paraclinical Sciences, Phytomedicine Programme of the University of Pretoria, Ondersterpoort, by SAA and MO. MO received postgraduate study funding from the University of South Africa (UNISA). LJS is a recipient of a National Research Foundation-incentive grant (103360).

Author contributions

All authors contributed towards data analysis, drafting and critically revising the paper, gave final approval of the

version to be published, and agreed to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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