South African Journal of Botany 167 (2024) 322–332

Contents lists available at ScienceDirect

South African Journal of Botany

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

# Anti-inflammatory activity of seven plant species with potential use as livestock feed additives

M.M. Lebeloane[a,b], I.M. Famuyide[a], K.G. Kgosana[c], E. Elgorashi[b], K.K. Ndivhuwo[d], V. Maharaj[d], L.J. McGaw[a,*]

[a] Phytomedicine Programme, Department of Paraclinical Sciences, University of Pretoria, Private Bag X04 Onderstepoort 0110, Pretoria 0001, South Africa
[b] Toxicology and Ethnoveterinary Medicine, ARC-Onderstepoort Veterinary Research, Onderstepoort, South Africa
[c] Department of Chemical Pathology, Faculty of Medicine, Sefako Makgatho Health Sciences University, South Africa
[d] Biodiscovery Centre, Department of Chemistry, Faculty of Natural and Agricultural Sciences, University of Pretoria, Lynnwood, South Africa

ARTICLE INFO

*Article History:*
Received 27 November 2023
Accepted 9 February 2024
Available online 23 February 2024

Edited by Dr S.C. Pendota

*Keywords:*
Antioxidant
Anti-inflammatory
Cyclooxygenase
Nitric oxide
Reactive oxygen species (ROS)

ABSTRACT

Inflammatory conditions and associated oxidative stress are common in livestock. The present study aimed to investigate in depth the anti-inflammatory activity of seven medicinal plants (*Dichrostachys cinerea, Ehretia rigida, Salix babylonica, Vachellia erioloba, V. gerrardii, V. sieberiana* and *V. tortilis*) traditionally used to treat inflammatory disorders in South Africa. These plants are being investigated for possible inclusion in animal feed as additives to support health and promote growth. The anti-inflammatory potential of plant extracts was evaluated in terms of inhibition of the 15-lipoxygenase (15-LOX) enzyme and nitric oxide (NO) release in LPS-induced RAW 264.7 macrophages, together with cytotoxicity studies. The effect of plant extracts in modulating the expression of pro-inflammatory mediators including cyclooxygenase (COX), inducible nitric oxide synthase (iNOS), and the cytokine interleukin-6 (IL-6) was evaluated using quantitative reverse transcription polymerase chain reaction (qRT-PCR). *In vitro* antioxidant activity was investigated using chemical assays. ULPC-MS and ICP-MS were used to detect potentially bioactive phytochemicals and concentrations of essential elements respectively. Methanol and acetone extracts of *D. cinerea* had the best activity against 15-LOX ($IC_{50}$ values of 0.80 and 0.64 mg/ml, respectively). The methanol extracts of *E. rigida, V. tortilis* and *V. sieberiana* inhibited NO with $IC_{50}$ of 90.11, 101.52 and 94.11 $\mu$g/ml respectively, higher than that of the positive control ($IC_{50}$ = 30.00 $\mu$g/ml). Furthermore, these plants had low cytotoxicity ($LC_{50} \geq 70$ $\mu$g/ml) against RAW 264.7 macrophages. Interestingly, *E. rigida, V. sieberiana* and *V. tortilis* extracts decreased the expression of iNOS, COX and IL-6. There was a positive correlation between the inhibition of NO release from macrophages by plant extracts and the downregulation of iNOS mRNA levels. There was no significant difference in the antioxidant activity (ABTS) of *D. cinerea* methanolic extract with $IC_{50}$ = 5.37 $\mu$g/ml compared to the positive controls, Trolox ($IC_{50}$ = 4.42 $\mu$g/ml) and ascorbic acid ($IC_{50}$ = 4.43 $\mu$g/ml). Flavonoids, fatty acids and essential trace elements such as Fe, Zn and Mn were detected in *E. rigida*, and these are likely to be responsible for the good anti-inflammatory effect of the extracts. This study provides the first evidence of the regulation of pro-inflammatory mediator and cytokine genes by the selected plant extracts. These results support the use of the plants, in particular *E. rigida*, as potential feed additives to reduce inflammation associated with many diseases.



## 1. Introduction

In livestock production, animals are constantly exposed to various external stressors such as pathogens, abnormal climate, inadequate feeding, poor hygienic environment, injury and internal stressors such as oxidative stress (Li et al., 2012; Seckin et al., 2018). These factors cause a decrease in natural immunity, which, in turn, enables microbial infection (Seckin et al., 2018). Inflammation and oxidative stress (OS) disorders affect cattle health and productivity resulting in economic losses (Keller et al., 2021). According to Gessner et al.

*Abbreviations:* AGP, Antimicrobial growth promoters; NSAIDs, nonsteroidal anti-inflammatory drugs; IL-6, interleukin-6; COX, cyclooxygenase; LOX, lipoxygenase; ROS, reactive oxygen species; LPS, lipopolysaccharide; iNOS, inducible nitric oxide synthetase; TFC, total flavonoid content; TPC, total phenolic content; ABTS, 2′-azino-bis (3-ethyl benzothiazoline-6-sulfonic acid; ATCC, American Type Culture Collection; DMEM, Dulbecco's Modified Eagle's Medium; DMSO, dimethyl sulfoxide; DPPH, 2, 2-diphenyl-1-picrylhydrazyl; FCS, foetal calf serum; GAE, gallic acid equivalent; MTT, 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide; PBS, phosphate buffered saline

* Corresponding author.
*E-mail address:* lyndy.mcgaw@up.ac.za (L.J. McGaw).

https://doi.org/10.1016/j.sajb.2024.02.017

(2017), inflammation is a complex but defensive temporary biological response of the innate immune system to infectious pathogens or physical injury, but prolonged inflammation is undesirable. Inflammatory mediators in the animal diet can suppress appetite, reduce nutrient availability for production, and activate the metabolism of the host tissue (Niewold, 2007; Broom and Kogut, 2018).

Lipopolysaccharides (LPS) are structural components of Gram-negative bacteria which activate macrophages during inflammation (Pepe et al., 2015). Macrophages synthesize and release cytokines, affecting innate immunity; other pro-inflammatory mediators such as nitric oxide (NO) also play an important role in pathogen-induced tissue damage (Amirghofran et al., 2011; Hall et al., 2017). Nitric oxide (NO) is a highly reactive molecule generated endogenously from l-arginine using NADPH and molecular oxygen expressed by a variety of mammalian cells, including macrophages and endothelial cells (Amirghofran et al., 2011; Yang et al., 2019). There are three main isoenzymes of NO: endothelial, neuronal, and inducible NO synthase. Inducible NO (iNOS) is present primarily in macrophages and is able to synthesize high concentrations of NO in many cells stimulated with bacterial toxins (Amirghofran et al., 2011). Furthermore, NO overproduction induces tissue damage associated with acute or chronic inflammation (Taira et al., 2009; Joo et al., 2014).

Cyclooxygenase (COX) and lipoxygenase (LOX) are two common arachidonic acid (AA) inflammatory pathway enzymes implicated in many diseases (Jacob et al., 2018; Mukhopadhyay et al., 2023). COX isoenzymes, COX 1 and COX 2 and COX-3, catalyze AA conversion to form hydroperoxide prostaglandin G2 (PPG2) that reduces hydroperoxide to prostaglandin H2 (PGH2) by peroxidase activity (Eldeen and Van Staden, 2008; Amessis-Ouchemoukh et al., 2014; Mukhopadhyay et al., 2023). These isomers have been reported to be significantly different in their distribution in the body, as well as in their roles in health and disease (Simon, 1999). COX-1 is predominantly constitutive, distributed throughout the body and, more importantly, provides homeostasis (Simon, 1999; Attiq et al., 2018). In contrast, COX-2 is inducible, expressed in inflammatory cells, and activity is largely responsive to adverse stimuli, including pro-inflammatory cytokines, interleukin-1 (IL-1), IL-6, tumor necrosis factor- $\alpha$ (TNF-$\alpha$), lipopolysaccharide (LPS) and stress (Simon, 1999; Yuhas et al., 2005; Attiq et al., 2018). COX-3, which is a variant of COX-1, is sensitive to inhibition by acetaminophen (paracetamol) (Elgorashi and McGaw, 2019). Interleukin-6 (IL-6) is not only involved in pathological inflammation and infectious response, but also regulates metabolic, regenerative and neural processes (Seckin et al., 2018). In particular, it plays an important role by linking the innate with the acquired immune response (Tanaka et al., 2014).

Lipoxygenases comprise a group of non-heme iron atoms implicated in the regulation of inflammatory responses by generating pro-inflammatory mediators known as leukotrienes (LT) or anti-inflammatory mediators such as lipoxins (Wisastra and Dekker, 2014). Leukotrienes are produced largely by inflammatory cells like macrophages (Chensue and Kunkel, 1983). LOX enzymes are described as dioxygenases that catalyze metabolism of polyunsaturated fatty acids (PUFA) including AA and linoleic acid (Mashima and Okuyama, 2015). They catalyze the formation of hydroperoxyl eicosatetraenoic acids (HPETES) from AA, which can be transformed into eicosanoids (Wisastra and Dekker, 2014). Eicosanoids play a fundamental role in physiological processes and the immune response. In humans and mice, there are a few distinctive isoforms of LOX, including 5-LOX, 8-LOX, 12-LOX and 15-LOX, classified according to their selectivity to oxygenate fatty acids in a specific position (Brash et al., 1999; Mashima and Okuyama, 2015; Mukhopadhyay et al., 2023). Overexpression of LOX and its pro-inflammatory products is associated with acute and chronic diseases (Wisastra and Dekker, 2014).

The levels of reactive oxygen species (ROS) modulate immune cells in scavenging free radicals and lead to inflammation and adverse effects on animal production and reduced reproductive performance (Ciliberti et al., 2020). ROS are reactive oxygen metabolites (free radicals) generated by cellular enzymes known as nicotinamide adenine dinucleotide phosphate (NADPH) oxidases (Bardaweel et al., 2018; Lee and Song, 2021). They include nitric oxide ($NO^-$), superoxide radicals ($O_2^-$), hydrogen peroxide ($H_2O_2$), hydroxyl radicals (OH) and singlet oxygen ($^1O_2$) (Pizzino et al., 2017; Navarro-Yepes et al., 2014). Their overproduction may lead to oxidative stress (OS) which is an imbalance between systematic ROS production and the ability of cells to detoxify the reactive intermediate to restore balance with antioxidants. It has negative effects on cellular structures including lipids, proteins and nucleic acids (Auten and Davis, 2009; Pizzino et al., 2017). Cellular sources of ROS include mitochondria, endothelial cells, COX, LOX, NOS and others (Izyumov et al., 2010; Al-Gubory et al., 2012). Oxidative damage remains an inevitable outcome of aerobic life.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are commonly used as a treatment for inflammation by inhibiting COX or LOX enzymes (Vonkeman and van de Laar, 2010; Amessis-Ouchemoukh et al., 2014). However, other studies have reported that NSAIDs have detrimental effects on animal organs such as the gastrointestinal tract, cardiovascular system and liver (Piao et al., 2023). Additionally, antimicrobial growth promoters (AGPs) are added to animal feed for their anti-inflammatory effects, especially in controlling the intestinal microbiota (Niewold, 2007; Broom and Kogut, 2018). However, the ban on synthetic antimicrobials in many countries as feed additives to prevent or cure microbial diseases in animals, and the detrimental effects of NSAIDs as therapeutic treatment for inflammatory conditions have prompted the investigation of other sources such as plant phytochemicals (Jaiswal et al., 2020).

Plant extracts have been used for generations as natural therapeutic agents against inflammation, characterized by the overproduction of inflammatory mediators such as ROS and pro-inflammatory cytokines (Rodríguez-Yoldi, 2021). According to Iwalewa et al. (2007), more than 115 plant species from 60 families have been traditionally used to treat diseases of inflammation and pain in humans and animals. A review by Elgorashi and McGaw (2019) provides an overview of several plants with *in vitro* anti-inflammation activities, including *Vachellia* (*Acacia*) species, *Ehretia rigida*, and *Dichrostachys cinerea*. The plant species selected for this study belonging to the genera *Dichrostachys* and *Vachellia* are indigenous to South Africa and have been reported to be used traditionally treat inflammatory diseases (Kiki et al., 2022). Previous work showed that aerial parts and fruits of *D. cinerea* possess antioxidant, anti-inflammatory and anti-ulcer effects *in vivo* due to the presence of polyphenolic compounds such as flavan-3-ols, proanthocyanidins and quercetin derivatives, apigenin, kaempferol, and flavone glycosides (Zeid et al., 2010; Shandukani et al., 2018; Kiki et al., 2022). *Acacia* (*Vachellia)* spp. have good antibacterial, antioxidant, immunoregulating and anti-radical activities (Ramde-Tiendrebeogo et al., 2019; Ahovègbé et al., 2021; More et al., 2021). Furthermore, *Salix babylonica* crude extract and compounds have been used to treat skin ulcers and relieve pains (Wahab et al., 2018, 2022) and possess pharmacological activities that include anti-inflammatory, antioxidant and cytotoxic (Wahab et al., 2018; Wahab et al., 2022). Flavonoids such as quercetin and other phenolic components of plants have been reported to have anti-inflammatory, antioxidant and immunostimulant activities (Tungmunnithum et al., 2018; Yahfoufi et al., 2018). A positive correlation has been established between antioxidant, anti-inflammatory activities, and total phenolic compounds (Diaz et al., 2012). Apart from plant bioactive molecules, it is observed that essential trace elements such as Zn, Fe and Se have noticeable antioxidant, immunomodulating and anti-inflammatory activities (Tamoli et al., 2022; Yatoo et al., 2013; Yazdankhah et al., 2014).

The rationale for the present study was to establish the regulatory effect of methanol and acetone extracts of seven plants on the transcription of the inflammatory markers IL-6, COX, iNOS and *in vitro*

NO, in cultured LPS-activated RAW 264.7 macrophages. Furthermore, the antioxidant activity and phenolic concentration of the plant extracts were determined to identify a plant feed additive that could be used as an immunoregulator in animal production.

## 2. Material and methods

### 2.1. Plant extracts

The leaf materials were collected from the Onderstepoort communal area, Pretoria North, Gauteng Province South Africa, and are presented in Table 1. A voucher specimen of each plant was deposited at the HGWJ Schweickerdt Herbarium of the University of Pretoria for identification. Ten g of oven-dried, ground leaf material was separately mixed with 100 ml of methanol or acetone as extraction solvents under shaking conditions for 24 h. Previous reports have shown that methanol and acetone are able to extract phenolic compounds, including flavonoids, that are responsible for the antioxidant and anti-inflammatory activities of various medicinal plants (Ibrahim et al., 2015; Susithra and Jayakumari, 2018; Truong et al., 2019). After solvent removal, the dried plant material was stored for further analysis.

### 2.2. Determination of 15-lipoxygenase inhibitory assay

The assay was performed according to the method of del Carmen Pinto et al. (2007), with modifications (Dzoyem and Eloff, 2015). The assay is based on measuring the formation of the iron or xylenol orange complex in terms of absorbance at 560 nm using a spectrophotometer. Briefly, lipoxygenase (15-LOX) was incubated with 25 $\mu$l extracts (final concentration 3.6–454.5 $\mu$g/mL) or a standard inhibitor (quercetin, final concentration 0.36–45.5 $\mu$g/mL) at 25 °C for 5 min. Then 50 $\mu$L of 140 mM linoleic acid was added in 50 mM Tris–HCl buffer (pH 7.4) and the mixture was incubated at 25 °C for 20 min in the dark. Furthermore, an aliquot of 100 $\mu$L of FOX reagent consisting of 30 mM sulfuric acid, 100 mM xylenol orange and 100 mM iron (II) sulfate in methanol/water (9:1) was added. For the negative control, only the LOX solution and buffer were pipetted into the wells. The blanks contained the enzyme LOX, the substrate (linoleic acid), and the FOX reagent. After 30 min at 25 °C incubation, the inhibitory activity of lipoxygenase was estimated by calculating the percentage of inhibition of hydroperoxide production from changes in absorbance values at 560 nm using a spectrophotometer. The percentage inhibition was calculated as follows:

$$\text{Percentage inhibition } (\%) = [(\text{Acontrol} - \text{Ablank}) - (\text{Asample} - \text{Ablank})]/((\text{Acontrol} - \text{Ablank})) \times 100$$

Where, Acontrol is the absorbance of the control well, Ablank is the absorbance of the blank well, and Asample is the absorbance of the sample well. Thereafter, the $IC_{50}$ value was estimated using the nonlinear regression curve, that is, $y = mx + c$.

**Table 1**
Selected plant species belonging to *Vachellia, Ehretia, Salix* and *Dichrostachys* that are traditionally used in inflammation conditions.

| Plant family | Scientific name (Voucher number) | Common name | Traditional usage | Biological activity | Phytochemistry | Reference |
|---|---|---|---|---|---|---|
| Boraginaceae | *Ehretia rigida* (Thunb.) Druce (PRU131533) | Puzzle bush (English), Morobe (Northern Sotho) | Abdominal pain<br>Chest pain | Antioxidant (DPPH, FRAP, ABTS)<br>Anti-inflammatory (15-LOX, NO)<br>Anti-arthritics<br>Anti-allergic | Phenolic acids<br>Flavonoids<br>Tannins | (Shukla and Kaur, 2018)<br>(Maroyi, 2023a)<br>(Dzoyem and Eloff, 2015) |
| Fabaceae | *Dichrostachys cinerea* (L.) Wight & Arn. (PRU131904) | Sickle bush (English), uGagane (Zulu) | Local anesthesia, sore eyes and toothache, Headache Rheumatoid arthritis | Anti-inflammatory *in vivo*<br>Antioxidant (DPPH)<br>Analgesic<br>Immunomodulation | Phenols<br>Flavonoids<br>Tannins<br>Flavan-3-ols<br>Proanthocyanidins<br>Flavone glycosides | (Susithra and Jayakumari, 2018)<br>(Kiki et al., 2022)<br>(Mazimba et al., 2022)<br>(Shandukani et al., 2018)<br>(Agyare et al., 2013)<br>(Agbonlahor et al., 2017)<br>(Sahoo et al., 2011)<br>(Rodríguez-Mesa et al., 2023) |
| | *Vachellia erioloba* (E. Mey) P.J.H Hunter (PRU131903) | Camel thorn (English) | Fever<br>Sore throat<br>Stomachache<br>Hemorrhage<br>Rheumatism | Anti-inflammatory<br>Nitric oxide inhibition<br>Interleukin and COX-2 suppression<br>Antioxidant (DPPH, ABTS)<br>Immunomodulating/ immune stimulating | Phenols<br>Flavonoids<br>Anthocyanins<br>Quercetin<br>Flavonol<br>Flavone glycosides, aglycones,<br>Flavan-3-ols, Flavan-3,4-diols, - | (More et al., 2021)<br>(Taha et al., 2022)<br>(Ngaffo et al., 2020)<br>(Tchatchedre et al., 2019)<br>(Atiya et al., 2022)<br>(Ahovègbé et al., 2021) |
| | *Vachellia gerrardii* subsp. *gerrardii*. (PRU124571) | Red Thorn (Eng.) Moki. (Tswana) | | | | |
| | *Vachellia sieberiana* (DC.) Kyal. & Boatwr. var. *woodii* (Burtt Davy) Kyal. & Boatwr (PRU125469) | Paperbark thorn (English), Mosetlha (Tswana) | | | | |
| | *Vachellia tortilis* (Forssk) Gallasso & Banfi (PRU125473) | Umbrella thorn (English), Mosu (Tswana) | | | | |
| Salicaceae | *Salix babylonica* L. (PRU131536) | Weeping willow (English) | Relieve pain, analgesic antipyretic<br>Skin ulcers<br>Sore and burns | Antioxidant activity (ABTS)<br>Anti-rheumatic<br>Anti-analgesic<br>Antipyretic<br>Cytotoxic | Phenolic compounds<br>Salicin compound<br>Flavonoids | (Wahab et al., 2018; Wahab et al., 2022)<br>(Shah et al., 2016)<br>(Tawfeek et al., 2021) |

### 2.3. Cell culture

The RAW 264.7 macrophage cells obtained from the American Type Culture Collection (ATCC® TIB-71™, Rockville, MD, USA) were cultured in plastic culture flasks containing Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10 % heat-inactivated fetal calf serum (FCS) and 5 $\mu$g/mL of streptomycin, to confluence at 37 °C in a humidified incubator with 5 % $CO_2$ and split twice a week.

### 2.4. MTT assay against RAW 264.7 macrophages

To ensure that the active extracts were not cytotoxic, the colorimetric tetrazolium-based cytotoxicity assay (Mosmann, 1983) was performed, with a few modifications (Ondua et al., 2019). Cells were seeded in 96-well microtitre plates at a concentration of $1 \times 10^5$ cells/mL for 24 h at 37 °C to allow cell attachment. The medium was then replaced with fresh DMEM containing different concentrations (1–0.0075 mg/ml) of plant extracts for 48 h. Doxorubicin and acetone (diluted with DMEM) served as positive and negative controls, respectively, and untreated cells were used as a blank control. Subsequently, the wells were washed with phosphate buffered saline (PBS) and 200 $\mu$l of fresh medium was added to each well. Then 30 $\mu$l of MTT (5 mg/ml in PBS) was added to each well and the plates were incubated for 4 h at 37 °C. After this, the medium of the wells was aspirated and 50 $\mu$l of DMSO was added to the wells to solubilize the formed formazan crystals. The absorbance was measured on a microplate reader (SpectraMax 190, Molecular Devices) at a wavelength of 570 nm. The activity of each extract concentration was determined in triplicate, and the assay was repeated three times. The concentration causing 50 % inhibition of cell viability ($LC_{50}$) was calculated with the following equation:

$$\text{Percentage viability} = ((\text{ODSample} - \text{ODblankcontrol})/\text{ODblankcontrol}) \times 100$$

### 2.5. Measurement of nitrite

The concentration of nitrite released from macrophages in the culture supernatant was evaluated using the Griess reagent (Joo et al., 2014; Dzoyem and Eloff, 2015). Cells were seeded in 96-well microtitre plates (for nitrite [$NO_2$] at $5 \times 10^5$ cells per well). After 24 h of incubation, the cells were fully attached and treated with 50 $\mu$l of plant extracts at various concentrations (100–0.78 $\mu$g/ml). After 2 h of incubation at 37 °C, 50 $\mu$l of LPS (5 $\mu$g/mL) in DMEM was added to all wells and further incubated for 24 h. After incubation, cell supernatants were collected for $NO_2$ measurement using the Griess colorimetric reaction method. A 60 $\mu$L aliquot of cell supernatant was combined with an equal volume of Griess reagent [1 % sulphanilamide/0.1 % N-(1-napthyl) ethylene diamine, each in 2.5 % $H_3PO_4$] in a 96-well plate at room temperature for 10 min, and the absorbance was measured at 550 nm using a microplate reader (Biotek, USA). The percentage inhibition was calculated based on the ability of the extracts to inhibit the formation of nitric oxide by cells compared to the control (cells in media without extracts containing triggering agents and DMSO), which was considered 0 % inhibition.

### 2.6. Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) analysis of NO, IL-6 and COX expression in induced macrophages

Firstly, RAW 264.7 macrophages ($5 \times 10^6$ cells/flask) were allowed to attach overnight at 5 % $CO_2$ and 37 °C. Then the medium was removed and replaced with 2 ml of plant extracts (at a concentration of 2 x $IC_{50}$ NO dissolved in DMEM). An aliquot of 2 ml of LPS (1 $\mu$g/ml) was added to activate macrophages. Quercetin and LPS only were used as controls. Subsequently, the RNA was extracted from activated RAW 264.7 cells according to the Zymo RNA extraction kit protocol (Inqaba biotec) and the RNA was reverse transcribed using qPCR. About 20–100 ng of mRNA were converted into cDNA by reverse transcription using a Gene Amp PCR thermal cycler. To 10 $\mu$l of synthesized cDNA, 30 $\mu$l of nuclease-free water was added. The final 5 $\mu$l reaction mixture containing primer, cDNA template (0.39 $\mu$l), and Greenmaster mix (Biolabs) was prepared in tubes. Subsequently, a one-step qPCR analysis was performed using specific primers for COX, IL-6, and iNOS (Table 2) according to Boesch-Saadatmandi et al. (2011) and Kiselova-Kaneva et al. (2012). The difference between samples and controls was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001). As a standard housekeeping gene, the $\beta$-actin gene was used. Expression levels of mRNA were presented as relative units (RU) compared to the untreated control group of cells, where the levels of mRNA expression were equal to 1. The experiments were carried out in triplicate and included all cell groups in one plate while testing the expression of each gene.

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

The DPPH assay was conducted 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 $\mu$l) was added to 40 $\mu$l of different crude extracts at different concentrations (3 –1000 $\mu$g/ml). The mixture was incubated in the dark for 30 min, and the absorbance was measured at 517 nm using a microplate reader (Biotek, USA). Lower absorbance of the solution indicates higher free radical scavenging activity. The percentage of scavenging activity was calculated using as follows:

$$\text{Percentage scavenging activity } (\%) = [(\text{A0} - \text{As})/\text{A0}] \times 100$$

Where A0 is the absorbance of the control (DPPH solution without sample), As is the absorbance of the tested sample (DPPH plus sample). The 50 % inhibitory concentration ($IC_{50}$) values of extracts or fractions was determined using a non-linear regression curve of the percentage of scavenging activity against the logarithm of concentrations. Ascorbic acid and Trolox were used as positive controls. Each test was repeated thrice and results presented as mean ± standard deviation (SD).

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

The radical scavenging ability of the plant extracts was determined by the ABTS method described by Re et al. (1999) with

**Table 2**
Primer sequences used for qPCR analysis.

| Gene | Forward primer (5′–3′) | Reverse primer (5′–3′) | Annealing temperature ( °C) |
|---|---|---|---|
| IL-6 | AGTTGCCTTCTTGGGACTGA | CAGAATTGCCATTGCACAAC | 55 |
| iNOS | GGCAGCCTGTGAGACCTTTG | GCATTGGAAGTGAAGCGTTTC | 57 |
| -Actin | GACAGGATGCAGAAGGAGATTACT | GACAGGATGCAGAAGGAGATTACT | 55 |
| COX | TGAGCAACTATTCCAAACCAGC | GCACGTAGTCTTCGATCACTATC | 55–60 |

modifications (Dzoyem and Eloff, 2015). The stock solution of the ABTS radical was prepared by mixing ABTS (7 mM) with potassium persulfate (2.45 mM) at room temperature in the dark for 12–16 h before the experiment. The working solution was obtained by calibrating the stock solution to obtain an optical density (OD) = 0.70 at 734 nm. An aliquot of 160 $\mu$l ABTS working solution was mixed with the plant extract (40 $\mu$l) (final concentration 1–0.14 mg/ml) and the absorbance was measured after 5 min at 734 nm using a microplate reader (Biotek, USA). The percentage of scavenging activity was calculated using the formula above. The 50 % inhibitory concentration ($IC_{50}$) values of plant extracts were evaluated using a non-linear regression curve of a 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 presented as mean ± standard deviation (SD).

### 2.9. ULPC-MS analysis of phytochemicals in E. rigida extracts

One mg of methanol and acetone was dissolved in 1 ml of methanol to produce a final concentration of 1 mg/ml. Then, the methanol extracts were reconstituted in ultra-pure LC water:methanol (1:1) and centrifuged. An ultra-performance liquid chromatography system coupled to a Waters® SynaptG2 quadrupole time-of-flight mass spectrometer (UPLC-QTOF-MS/MS) was used to tentatively identify compounds through comparison of their fragmentation patterns and accurate masses to those recorded in databases using UNIFI Scientific Information System (Van Wyngaard et al., 2023).

### 2.10. Trace elements analysis in E. rigida

The trace elements selenium (Se), zinc (Zn), manganese (Mn), cobalt (Co), copper (Cu), iron (Fe), and molybdenum (Mo), in dried leaf powder of *E. rigida* digested with perchloric acid ($HClO_4$) and nitric acid ($HNO_3$) at 180 °C temperature, were determined using inductively coupled plasma mass spectrometry ICP-MS (Agilent 7500 s, Agilent Technologies, Germany). On the other hand, magnesium (Mg) concentration was estimated by ICP coupled with optical emission spectroscopy (OES) (Shimadzu).

## 3. Results

### 3.1. Inhibition of 15-LOX and NO

The effect of plant extracts on 15-LOX, NO and cell viability was established and presented in Table 3. Although the acetone extract of *V. gerrardii* exhibited a low $IC_{50}$ of 0.39 mg/ml against 15-LOX, both the methanol and acetone extracts of *D. cinerea* had good 15-LOX inhibitory activity ($IC_{50}$ of 0.64 mg/ml and 0.80 mg / ml, respectively). The concentrations that inhibited 50 % of NO were not cytotoxic with $LC_{50}$ ranging from 78.22 $\mu$g/ml to 1 mg/ml against RAW 264.7 macrophages; however, the *V. gerrardii* methanol extract showed a low $LC_{50}$ of 78.22 $\mu$g/ml compared to other extracts. The methanol extract of *V. erioloba* was the least cytotoxic with an $IC_{50}$ of >1 000 $\mu$g/ml. Three extracts, including the methanol extract of *E. rigida* (90.11 $\mu$g/ml), *V. sieberiana* (94.11 $\mu$g/ml), and *V. tortilis* (101.52 $\mu$g/ml) were found to have a good NO inhibitory effect.

### 3.2. Effects of three extracts on LPS-induced expression of the iNOS, IL-6, and COX gene in RAW 256 macrophage cells

Fig. 1 shows the gene expression of the cytokine IL-6 and pro-inflammatory enzymes (COX and iNOS) produced by cultured RAW 264.7 macrophages at the transcriptional level after 24 h exposure to concentration ($IC_{50}$ NO) of *E. rigida* (90.11 $\mu$g/ml), *V. tortilis* (101 $\mu$g/ml) and the acetone extract of *V. sieberiana* (93.28 $\mu$g/ml). -Actin was used as a housekeeping gene and was highly expressed in all samples. The selected plants down-regulated the expression of iNOS (0.0001–0.0003 RU mRNA), IL-6 (0.1–0.14 RU mRNA) and COX (0.03–0.6 RU mRNA) by reducing their mRNA quantified by qRT-PCR. The methanol extract of *E. rigida* showed outstanding inhibitory activity of the proinflammatory mediator iNOS and the cytokine IL-6 compared to the two species of *Vachellia* and the positive control, quercetin.

### 3.3. UPLC-MS analysis of active phytochemicals with anti-inflammatory activity in E. rigida extracts

The UPLC chromatograms of methanol and acetone extract are shown in Fig. 2. The identified principal compounds belonging to phenolic compounds and fatty acids included quercetin-3-O-$\alpha$-l-rhamnopyranosyl-(1–>6)-$\beta$-d-galactopyranoside (Peak 1) in Table 4, isorhamnetin-3-O-rutinoside (Peak 2), eupatin (Peak 3) and vicenin (Peak 4) in Table 5.

### 3.4. The level of trace elements E. rigida

The results of estimation of trace elements levels in *E. rigida* using the ICP-MS method are presented in Table 6. Among all metals, Fe was the highest with value of 262 mg/kg DM followed by Zn (58.90 mg/kg DM) and Mn (45.10 mg/kg DM). Several trace elements

**Table 3**
Effect of methanol and acetone extracts on 15-LOX activity, cell viability and NO expressed by LPS-activated macrophages.

| Plant species | Extraction solvent | 15-LOX $IC_{50}$ (mg/ml) | Cell viability ($LC_{50}$ $\mu$g/ml) | NO ($IC_{50}$ $\mu$g/ml) |
|---|---|---|---|---|
| *D. cinerea* | Acetone | 0.80 | 283.69 | 125.26 |
| | Methanol | 0.64 | 291.89 | 119.66 |
| *E. rigida* | Acetone | 1.39 | 667.05 | 118.83 |
| | Methanol | 1.13 | 125.41 | 90.11 |
| *S. babylonica* | Acetone | 2.00 | 722.61 | 107.86 |
| | Methanol | 2.00 | 384.29 | 109.81 |
| *V. erioloba* | Acetone | 2.00 | 559.01 | 122.42 |
| | Methanol | 1.55 | >1 000 | 200.63 |
| *V. gerrardii* | Acetone | 0.39 | 317.36 | 120.49 |
| | Methanol | 1.82 | 78.22 | 326.66 |
| *V. sieberiana* | Acetone | 2.00 | 267.99 | 111.26 |
| | Methanol | 2.00 | 396.85 | 94.11 |
| *V. tortilis* | Acetone | 2.00 | 323.46 | 118.00 |
| | Methanol | 2.00 | 429.00 | 101.52 |
| Quercetin | | 0.035 | nd | 30.00 |
| Doxorubicin | | nd | 0.04 | nd |

nd denotes not determined.

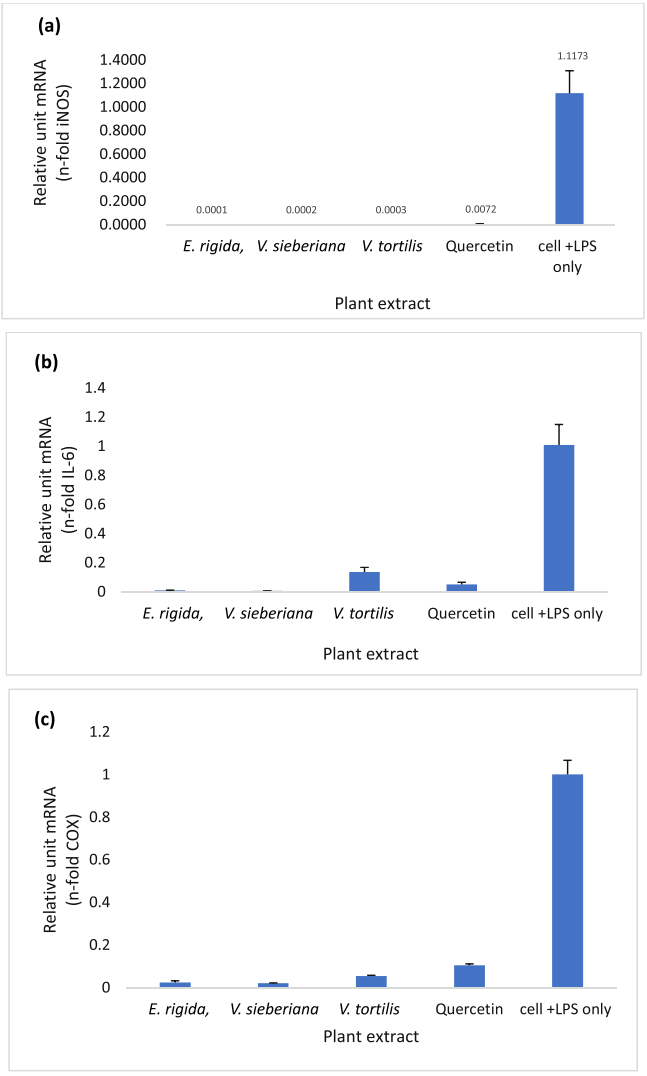



**Fig. 1.** Expression of (**a**) iNOS, (**b**) IL-6 and (**c**) COX in LPS activated RAW 256 macrophages after exposure to *E. rigida*, *V. sieberiana* and *V. tortilis*. Quercetin was used as a positive control.

including Se, Co, Mo and Mg were found in extremely low levels > 1.00 mg/kg DM, however, Cu showed significant levels of 11.50 mg/kg DM.

## 4. Discussion

The maintenance of animal health and the prevention of disease are of paramount importance in sustainable livestock production. Phytogenic extracts are known to be sustainable feed additives with antioxidant, anti-inflammatory and immunostimulant properties for animal health. Although many studies have investigated the anti-inflammatory and antioxidant activity of some of the selected medicinal plants used to treat inflammation conditions, their effects on the expression of pro-inflammatory genes, particularly iNOS, IL-6 and COX using qPCR is a novel area of research.

In this study, the potential inhibitory ability of the seven plant extracts on the AA pathway and the activity of 15-LOX that plays an important role in the biosynthesis of leukotrienes (LT) implicated in the pathophysiology of many inflammatory diseases, was examined. All extracts revealed a low 15-LOX inhibition activity with $IC_{50}$ values ranging from 0.39 to ≥ 2 mg/ml in comparison with the quercetin positive control ($IC_{50}$ = 35 $\mu$g/ml). Dzoyem and Eloff (2015) demonstrated that *E. rigida* acetone extract inhibited 15-LOX with an $IC_{50}$ value of 62.99 $\mu$g/ml, with quercetin ($IC_{50}$= 35.85 $\mu$g/ml) as a positive control, however, in the current study the *E. rigida* acetone extract had a high $IC_{50}$ of > 1000 $\mu$g/ml. Furthermore, indigenous South African *Vachellia* species have been reported to have inhibitory activity against the pro-inflammatory enzyme, LOX, as well as iNOS (Mamba et al., 2016; Maroyi, 2017) with a low $IC_{50}$ of 62.42 $\mu$g/ml by an ethanol extract in the 15-LOX assay compared to the high values in this present study (Mamba et al., 2016). The difference may be attributed to the geographical area of plant collection, or the amount and type of phenolic compounds or flavonoids in the plant. This study is the first to report the 15-LOX inhibitory activity by methanol and acetone extracts of *D. cinerea, V. sieberiana* and *S. babylonica*.

Nitrate production in LPS-induced RAW 264.7 macrophages was quantified using the Griess reaction spectrophotometric assay. The crude extracts of *E. rigida, V. sieberiana* and *V. tortilis* significantly decreased the accumulation of nitrate in LPS-stimulated macrophages with $IC_{50}$ values of 90.11, 94.11 and 101.52 respectively. There is insufficient information available on NO inhibition by the selected plants. However, a previous study indicated that the acetone extract of *E. rigida* inhibited NO by 86.3 to 92.6 % with cell viability of 34.3 to 63.6 % in a dose-dependent manner (Dzoyem and Eloff, 2015). Regarding cytotoxicity against RAW 264.7 macrophages, extracts had high $LC_{50}$ values ranging from 90 to 1000 $\mu$g/ml. According to the guidelines of the National Cancer Institute, the methanol and acetone extracts of the selected plants were not toxic because their $LC_{50}$ was higher than 20 $\mu$g/ml. It is crucial to reduce NO overproduction, which is a free radical molecule produced endogenously because NO induces tissue damage associated with acute and chronic inflammation (Joo et al., 2014).

Lipopolysaccharide-induced macrophages produce excessive inflammatory mediators COX and iNOS, as well as cytokines such as IL-6 (Nkadimeng et al., 2020). The expression of COX, IL-6, and iNOS mRNA in three extracts that showed good NO inhibition of the untreated control macrophages stimulated with LPS was evaluated by qPCR. Plants with good NO inhibition were selected for further molecular analysis because the expression of inducible NOS is responsible for increasing the level of NO important in the pathogenesis of a variety of inflammatory diseases (Yang et al., 2019). Our findings also suggested a positive correlation between the decrease in expression of iNOS and decrease in inhibition of NO by LPS-induced macrophages treated with plant extracts with the positive control quercetin. Several studies have demonstrated that NO production is dependent on the iNOS expression in the cell (Broadbelt et al., 2007; Anavi and Tirosh, 2020; Madhu et al., 2016). For instance, Broadbelt et al. (2007) showed that the upregulation of iNOS mRNA expression increases NO metabolism in epithelial cells *in vitro*. In the present study, untreated macrophages showed <1-fold increase in gene expression of COX, IL-6, and iNOS mRNA; however, treatment with plant extracts decreased gene expression of these inflammatory mediators and cytokines compared to the quercetin positive control. COX was expressed in a relative unit mRNA of 0.02 to 0.06 higher by all plant extracts compared to quercetin (0.01 relative mRNA). *Vachellia* spp. showed $IC_{50}$ inhibition of COX 1 and COX 2 in an *in vivo* model (Burnett et al., 2007). In this study *E. rigida* showed good regulatory effects on COX and iNOS. On the other hand, the methanol extract of *V. tortilis* had a higher 0.12 relative unit of IL-6 mRNA. It is essential to regulate IL-6 because high levels could induce tissue damage, reduce growth hormone secretion, change metabolic energy and protein and negatively affect animal performance (Li et al., 2012). Previous studies have shown that *Acacia* (*Vachellia*) spp. inhibit tumor progression by regulating inflammatory TNF, iNOS, COX-2, IL-2 and IL-6 *in vivo* (Susithra and Jayakumari, 2018).

The commonly used antioxidant colorimetric tests such as ABTS and DPPH are inexpensive, simple and useful techniques to

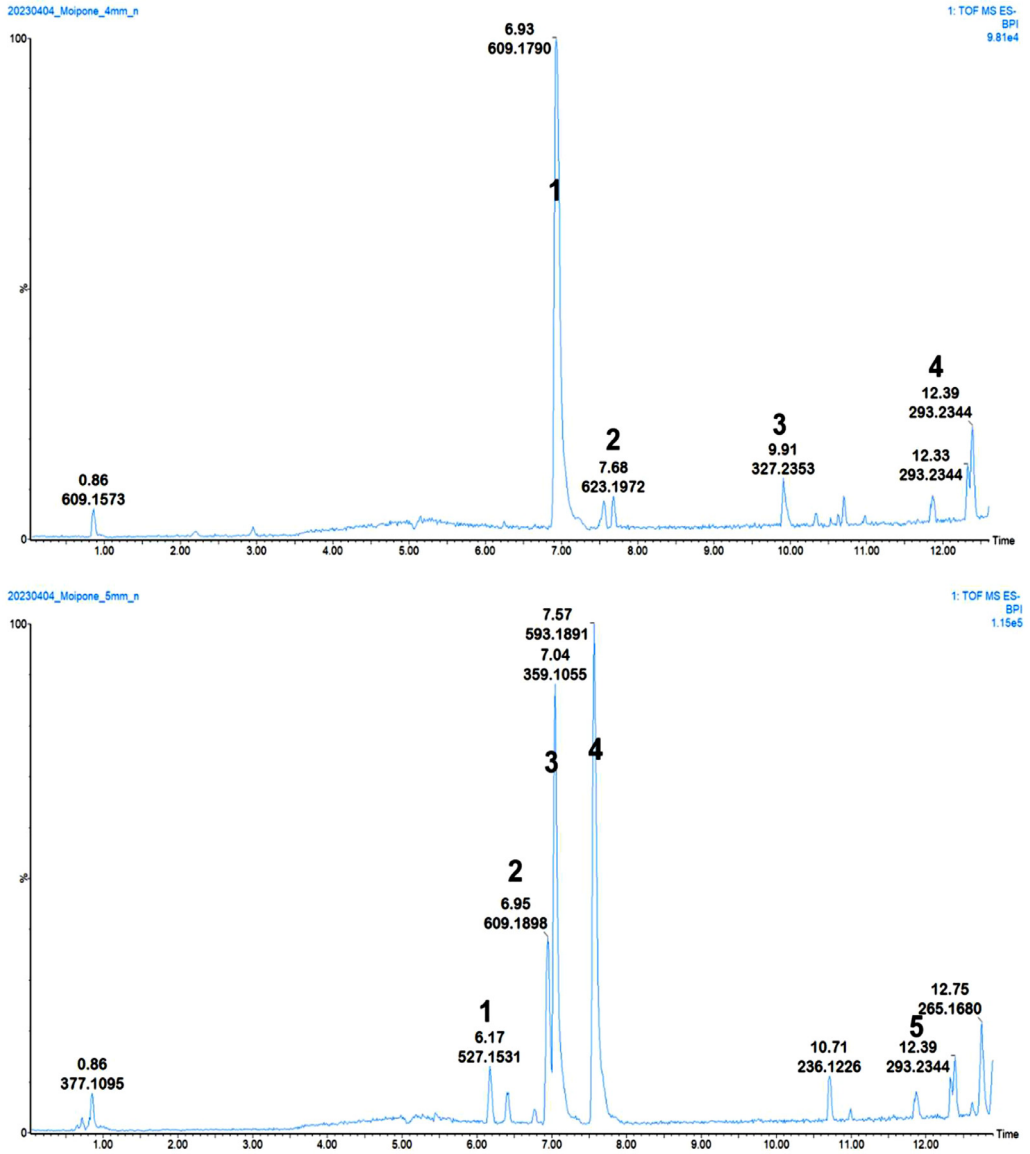



**Fig. 2.** UPLC-MS chromatogram of *E. rigida* methanol extract (A) and acetone (B) in ESI negative mode.

determine the radical scavenging activity of plant extracts. According to Jumina et al. (2019), a plant extract with $IC_{50}$ < 100 $\mu$g/ml has good antioxidant activity, while one with $IC_{50}$ < 50 $\mu$g/ml is considered a very strong antioxidant agent. In this regard, the methanol extract of *D. cinerea* and the acetone extract of *V. erioloba* had strong antioxidant capacity using the ABTS and DPPH mechanisms. Similarly, several studies have reported that the extracts and fractions of *D. cinerea* fruits, roots and leaves have good antioxidant potential *in vitro* (Bolleddu et al., 2019; Irie-N'guessan et al., 2018).

Trace elements greatly impact cattle health and performance, particularly by influencing the inflammatory, antioxidant and immune status of the animal. More importantly, reports have shown that Fe plays a role in the inflammation response in livestock and horses (van Emon et al., 2020; Ceciliani et al., 2012). The current study showed that Fe, Zn, Mn and Cu were the most abundant trace elements with values of 262, 58.90, 45.10 and 11.50 mg/kg DM respectively, and relatively lower Mg and Se in *E. rigida* collected from Onderstepoort, Gauteng province. Sebolai (2018) collected leaves from the Eastern Cape province and these contained 85 g/100 g DM of Fe, 7.10 g/100 g DM of Cu, 25.70 g/100 g of Zn and 78.00 g/100 g Mn. The discrepancy may be attributed to the geographical and climatic conditions where *E. rigida* was growing. According to van Emon et al. (2020), supplementation of Mn, Zn and Cu increases the total antioxidant capacity in cows. Selenium, which is a major component of the antioxidant system to maintain health status of animals, was found in low concentrations.

Flavonoids function to decrease ROS synthesis, inhibit enzymes, chelate trace elements, scavenge ROS, and improve the oxidant defense (Halliwell and Gutteridge, 2015). Several researchers have isolated and identified phytochemicals in bark, leaf and roots responsible for various biological activities including antioxidant and anti-inflammation in the *Ehretia* genus (Maroyi, 2023b; Shukla and Kaur, 2018; Li et al., 2008). Those phytochemicals include benzoquinones, glycosides, fatty acids, flavonoids, alkaloids, phenolic acids, and others. This study showed the presence of four flavonoids (quercetin-3-O-$\alpha$-l-rhamnopyranosyl-(1−>6)-$\beta$-d-galactopyranoside, isorhamnetin-3-O-rutinoside, eupatin and vicenin) and one fatty acid (9-oxo-10,12-octadecadienoic acid) as well as two unknown molecules with molecular weights of 328.22 *m/z* ($C_{18}H_{32}O_5$) and 528.13 *m/z* ($C_{26}H_{24}O_{12}$). According to Dong et al. (2000), 9-oxo-10,12-octadecadienoic acid and other isomers were isolated from *E. dicksonii* and reported to have potent anti-inflammatory activity *in vivo* at 500 mg

**Table 4**
Tentative identification of phytochemicals in methanolic crude extract of *E. rigida*.

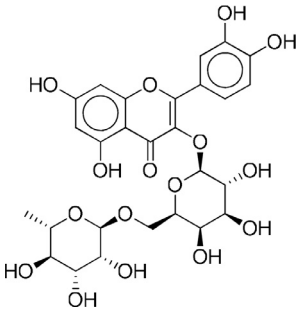
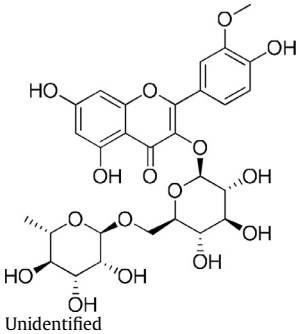
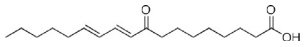
| Peak | Retention time(min) | Acquired [M-H] - *m/z*. | Adduct | Mass error (ppm) | Formula of possible compound. | Calculated accurate mass. | Possible compound | Possible Structure |
|---|---|---|---|---|---|---|---|---|
| 1 | 6.93 | 609.1790 | $[M-H]^-$ | 0.2 | $C_{27}H_{30}O_{16}$ | 610.153390 | Quercetin-3-O-$\alpha$-l-rhamnopyrano-syl-(1−>6)-$\beta$-d-galactopyranoside |  |
| 2 | 7.68 | 623.1972 | $[M-H]^-$ | −2.1 | $C_{28}H_{32}O_{16}$ | 624.169040 | Isorhamnetin-3-O-rutinoside |  |
| 3 | 9.91 | 327.2353 | [M-H]- | 2.8 | $C_{18}H_{32}O_5$ | 328.224975 | Unidentified | Unidentified |
| 4 | 12.39 | 293.2344 | $[M-H]^-$ | 2.9 | $C_{18}H_{30}O_3$ | 294.219495 | 9-Oxo-10,12-octa-decadienoic acid |  |

**Table 5**
Tentative identification of phytochemicals in acetone crude extract of *E. rigida*.

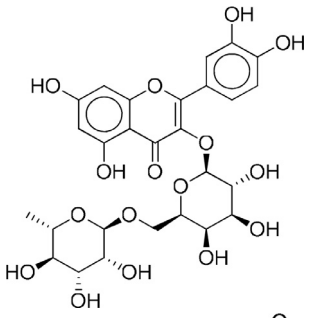
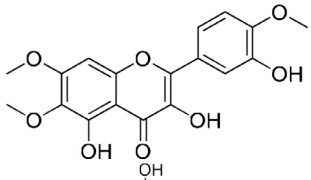
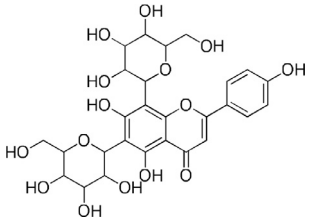
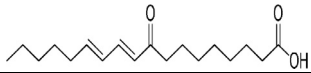
| Peak | Retention time(min) | Acquired [M-H] - *m/z*. | Adduct | Mass error (ppm) | Formula of possible compound. | Calculated accurate mass. | Possible compound | Possible Structure |
|---|---|---|---|---|---|---|---|---|
| 1 | 6.17 | 527.1531 | [M-H]- | 2.8 | $C_{26}H_{24}O_{12}$ | 528.126780 | Unidentified | Unidentified |
| 2 | 6.95 | 609.1898 | $[M-H]^-$ | 0.2 | $C_{27}H_{30}O_{16}$ | 610.153390 | Quercetin-3-O-$\alpha$-l-rhamnopyrano-syl-(1−>6)-$\beta$-d-galactopyranoside |  |
| 3 | 7.04 | 359.1055 | $[M-H]^-$ | 1.2 | $C_{18}H_{16}O_8$ | 360.084520 | Eupatin |  |
| 4 | 7.57 | 593.1891 | [M-H]- | 0.2 | $C_{27}H_{30}O_{15}$ | 594.158475 | Vicenin Ⅱ |  |
| 5 | 12.39 | 293.2344 | $[M-H]^-$ | 4.4 | $C_{18}H_{30}O_3$ | 294.219495 | 9-Oxo-10,12-octa-decadienoic acid |  |

**Table 6**
The trace element content in *Ehretia rigida*.

| Plant | Mg | Co | Cu | Fe | Mn | Mo | Se | Zn |
|---|---|---|---|---|---|---|---|---|
| *E. rigida* | 0.60 | 0.19 | 11.50 | 262 | 45.10 | 0.44 | < 1.00 | 58.90 |

dose. Furthermore, quercetin-3-O-$\alpha$-D arabinoside, quercetin-3-*O*-$\beta$-*D*-glucopyranoside, and quercetin-3-*O*-arabinosylgalactoside have been identified in *E. thrysiflora* (Li et al., 2008; Shukla and Kaur, 2018). According to a review by Chagas et al. (2022), quercetin is a potent anti-inflammatory molecule which has been extensively studied *in vivo* and *in vitro*. Vicenin-2 is a c-glycoside that is present in many plants and has shown to exert anti-inflammatory effects by suppressing the production of tumor necrosis factor-$\alpha$ (TNF-$\alpha$) or interleukin (IL)−6 and the activation of nuclear factor-$\kappa$B (NF-$\kappa$B) by LPS (Kang et al., 2015). Moreover, this molecule was found to significantly inhibit NO production and reduce iNOS and COX-2 expression in LPS-stimulated RAW 264.7 cells at 100 $\mu$g/ml (Kim et al., 2016).

In summary, this study provides novel evidence that plant extracts including *E. rigida, V. tortilis* and *V. sieberiana* have anti-inflammatory activities by inhibiting the production of cytokines and expression of inflammatory markers involved in disease pathogenesis in animal production. The findings are consistent with previous reports, as selected plant extracts possess flavonoids and trace elements known to have antioxidant and anti-inflammatory effects.

## 5. Conclusions

The present study was designed to evaluate the *in vitro* inflammatory and antioxidant activity and identify active compounds as well as essential trace elements in seven medicinal plants traditionally used to treat inflammation and pain in South Africa. Novel molecular studies demonstrated that the methanolic extract of *E. rigida* is a promising source of good anti-inflammatory activity by inhibiting NO and depressing mRNA expression of COX, IL-6 and NO, and they were also non-toxic to LPS-induced macrophages. However, extracts of this plant showed relatively low antioxidant activity. ULPC-MS and ICP-MS analysis revealed the presence of flavonoids, fatty acids and essential elements responsible for anti-inflammatory activity. Further *in vivo* studies are recommended for testing the active compound(s) or extracts before development of a feed additive with immunomodulating, anti-inflammatory and antioxidant properties for animal health.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

The National Research Council (NRF) South Africa and Onderstepoort Veterinary Institute (OVI) provided doctoral scholarship support to MML. The University of Pretoria provided postdoctoral support to IMF.

## Funding

The project was supported by the National Research Foundation (grant number 105993 to LJM) and Red Meat Research and Development South Africa.

## References

Agbonlahor, O., Godswill, N., Ozolua, R., 2017. Analgesic and anti-inflammatory effects of the aqueous leaf extract of *Dichrostachys cinerea*. J. Appl. Sci. Environmen. Manage. 21 (5), 821–825.
Agyare, C., Obiri, D.D., Boakye, Y.D., Osafo, N., 2013. 19 - anti-inflammatory and analgesic activities of African medicinal plants. In: Kuete, V. (Ed.), Medicinal Plant Research in Africa. Elsevier, Oxford.
Ahovègbé, L., Ogwang, P., Peter, E., Mtewa, A., Kasali, F., Tolo, C., Gbenoudon, J., Weisheit, A., Pakoyo, K., 2021. Therapeutic potentials of *Vachellia nilotica* (L.) extracts in Hepatitis C infection: a review. Scient. African 13, e00918. https://doi.org/10.1016/j.sciaf.2021.e00918.
Al-Gubory, K.H., Garrel, C., Faure, P., Sugino, N., 2012. Roles of antioxidant enzymes in corpus luteum rescue from reactive oxygen species-induced oxidative stress. Reprod. Biomed. Online 25 (6), 551–560.
Amessis-Ouchemoukh, N., Madani, K., Falé, P.L.V., Serralheiro, M.L., Araújo, M.E.M., 2014. Antioxidant capacity and phenolic contents of some Mediterranean medicinal plants and their potential role in the inhibition of cyclooxygenase-1 and acetylcholinesterase activities. Ind. Crops Prod. https://doi.org/10.1016/j.indcrop.2013.12.008 536-15.
Amirghofran, Z., Malek-Hosseini, S., Golmoghaddam, H., Kalantar, F., Shabani, M., 2011. Inhibition of nitric oxide production and proinflammatory cytokines by several medicinal plants. Iranian J. Immunol. 8 (3), 159–169.
Anavi, S., Tirosh, O., 2020. iNOS as a metabolic enzyme under stress conditions. Free Radical Biol. Med. 146, 16–35. https://doi.org/10.1016/j.freeradbiomed.2019.10.411.
Atiya, A., Majrashi, T., Akhtar, S., Khan, A.A., Asiri, A.M.S., Al-Zahrania, H.J., Alnami, R.S., Alsharif, S.A., Amer, T., Faiz, Z.A., Malyahya, S.A., Saeedhabtar, S., 2022. The value of Genus *Acacia* in arid and semi-arid environments for the treatment of chronic inflammatory diseases. Phytomed. Plus 2 (3), 100315. https://doi.org/10.1016/j.phyplu.2022.100315.
Attiq, A., Jalil, J., Husain, K., Ahmad, W., 2018. Raging the war against inflammation with natural products. Front. Pharmacol. 99, 76. http://doi.org/10.3389/fphar.2018.00976.
Auten, R.L., Davis, J.M., 2009. Oxygen toxicity and reactive oxygen species: the devil is in the details. Pediatr. Res. 66 (2), 121–127.
Bardaweel, S.K., Gul, M., Alzweiri, M., Ishaqat, A., Alsalamat, H.A., Bashatwah, R.M., 2018. Reactive oxygen species: the dual role in physiological and pathological conditions of the human body. Eurasian J. Med 50 (3), 193–201. http://doi.org/10.5152/eurasianjmed.2018.17397.
Boesch-Saadatmandi, C., Loboda, A., Wagner, A.E., Stachurska, A., Jozkowicz, A., Dulak, J., Döring, F., Wolffram, S., Rimbach, G., 2011. Effect of quercetin and its metabolites isorhamnetin and quercetin-3-glucuronide on inflammatory gene expression: role of miR-155. J. Nutr. Biochem. 22 (3), 293–299. http://doi.org/10.1016/j.jnutbio.2010.02.008.
Bolleddu, R., Venkatesh, S., Rao, M., Shyamsunder, R., 2019. Investigation of the pharmacognostical, phytochemical, and antioxidant studies of various fractions of *Dichrostachys cinerea* root. J. Nat. Sci. Med. 2 (3), 141–146.
Brash, A.R., Jisaka, M., Boeglin, W.E., Chang, M.S., Keeney, D.S., Nanney, L.B., Kasper, S., Matusik, R.J., Olson, S.J., Shappell, S.B., 1999. Investigation of a Second 15 S-lipoxygenase in Humans and Its Expression in Epithelial Tissues. Springer, Boston, MA.
Broadbelt, N.V., Stahl, P.J., Chen, J., Mizrahi, M., Lal, A., Bozkurt, A., Poppas, D.P., Felsen, D., 2007. Early upregulation of iNOS mRNA expression and increase in NO metabolites in pressurized renal epithelial cells. American J. Physiol.-Renal Physiol. 293 (6), F1877–F1888. http://doi.org/10.1152/ajprenal.00238.2007.
Broom, L.J., Kogut, M.H., 2018. Gut immunity: its development and reasons and opportunities for modulation in monogastric production animals. Animal Heal. Res. Rev. 19 (1), 46–52.
Burnett, B.P., Jia, Q., Zhao, Y., Levy, R.M., 2007. A medicinal extract of *Scutellaria baicalensis* and *Acacia catechu* acts as a dual inhibitor of cyclooxygenase and 5-lipoxygenase to reduce inflammation. J. Med. Food 10 (3), 442–451. http://doi.org/10.1089/jmf.2006.255.
Ceciliani, F., Ceron, J.J., Eckersall, P.D., Sauerwein, H., 2012. Acute phase proteins in ruminants. J. Proteomics 75 (14), 4207–4231. https://doi.org/10.1016/j.jprot.2012.04.004.
Chagas, M.D.S.S., Behrens, M.D., Moragas-Tellis, C.J., Penedo, G.X.M., Silva, A.R., Gonçalves-De-Albuquerque, C.F., 2022. Flavonols and flavones as potential anti-inflammatory, antioxidant, and antibacterial compounds. Oxidative Med.& Cellular Long. 2022, 9966750. http://doi.org/10.1155/2022/9966750.
Chensue, S.W., Kunkel, S.L., 1983. Arachidonic acid metabolism and macrophage activation. Clin. Lab. Med. 3 (4), 677–694.
Ciliberti, M.G., Albenzio, M., De Palo, P., Santillo, A., Caroprese, M., 2020. Nexus between immune responses and oxidative stress: the role of dietary hydrolyzed lignin in *ex vivo* bovine peripheral blood mononuclear cell response. Front. Veterin. Sci. 7. http://doi.org/10.3389/fvets.2020.00009.
Del Carmen Pinto, M., Tejeda, A., Duque, A.L., Macías, P., 2007. Determination of lipoxygenase activity in plant extracts using a modified ferrous oxidation– xylenol orange assay. J. Agricul. Food Chem. 55 (15), 5956–5959.
Diaz, P., Jeong, S.C., Lee, S., Khoo, C., Koyyalamudi, S.R., 2012. Antioxidant and anti-inflammatory activities of selected medicinal plants and fungi containing phenolic and flavonoid compounds. Chin. Med 7 (1), 26. http://doi.org/10.1186/1749-8546-7-26.
Dong, M., Oda, Y., Hirota, M., 2000. (10E,12Z,15Z)-9-hydroxy-10,12,15-octadecatrienoic acid methyl ester as an anti-inflammatory compound from *Ehretia dicksonii*. Biosci., Biotechnol. Biochem. 64 (4), 882–886. http://doi.org/10.1271/bbb.64.882.

Dzoyem, J.P., Eloff, J.N., 2015. Anti-inflammatory, anticholinesterase and antioxidant activity of leaf extracts of twelve plants used traditionally to alleviate pain and inflammation in South Africa. J. Ethnopharmacol 160, 194–201. https://doi.org/10.1016/j.jep.2014.11.034.
Eldeen, I., Van Staden, J., 2008. Cyclooxygenase inhibition and antimycobacterial effects of extracts from Sudanese medicinal plants. South African J. Botany 74 (2), 225–229.
Elgorashi, E.E., McGaw, L.J., 2019. African plants with *in vitro* anti-inflammatory activities: a review. South African J. Botany 126, 142–169. https://doi.org/10.1016/j.sajb.2019.06.034.
Gessner, D.K., Ringseis, R., Eder, K., 2017. Potential of plant polyphenols to combat oxidative stress and inflammatory processes in farm animals. J. Animal Physiol. Animal Nutrition (Berlin) 101 (4), 605–628. http://doi.org/10.1111/jpn.12579.
Gyamfi, M.A., Yonamine, M., Aniya, Y., 1999. Free-radical scavenging action of medicinal herbs from Ghana: *Thonningia sanguinea* on experimentally-induced liver injuries. General Pharmacol. 32 (6), 661–667.
Hall, B.M., Balan, V., Gleiberman, A.S., Strom, E., Krasnov, P., Virtuoso, L.P., Rydkina, E., Vujcic, S., Balan, K., Gitlin, Ii, Leonova, K.I., Consiglio, C.R., Gollnick, S.O., Chernova, O.B., Gudkov, A.V., 2017. p16(Ink4a) and senescence-associated $\beta$-galactosidase can be induced in macrophages as part of a reversible response to physiological stimuli. Aging (Albany NY) 9 (8), 1867–1884. https://doi.org/10.18632/aging.101268.
Halliwell, B., Gutteridge, J.M., 2015. Free Radicals in Biology and Medicine. Oxford university press, USA.
Ibrahim, E.A., Gaafar, A.A., Salama, Z.A., El Baz, F.K., 2015. Anti-inflammatory and antioxidant activity of *Solenostemma argel* extract. Int. J. Res. Pharmacol. Phytochem. 7 (4), 635–641.
Irie-N'guessan, A.G., Kouakou, S.L., Koua, K., Effo, K., Djadji, A., Diarrassouba, N., Kouakou-Siransy, N., 2018. Anticonvulsant and analgesic assessment of *Dichrostachys cinerea* root bark, an Ivorian anti-asthmatic herbal, in mice. J. Pharmacol. Clin. Res. 6, 1–6.
Iwalewa, E.O., McGaw, L., Naidoo, V., Eloff, J., 2007. Inflammation: the foundation of diseases and disorders. A review of phytomedicines of South African origin used to treat pain and inflammatory conditions. Afr. J. Biotechnol. 6 (25).
Izyumov, D., Domnina, L., Nepryakhina, O., Avetisyan, A., Golyshev, S., Ivanova, O.Y., Korotetskaya, M., Lyamzaev, K., Pletjushkina, O.Y., Popova, E., 2010. Mitochondria as source of reactive oxygen species under oxidative stress. Study with novel mitochondria-targeted antioxidants—the "Skulachev-ion" derivatives. Biochemistry (Moscow) 75, 123–129.
Jacob, P.J., Manju, S.L., Ethiraj, K.R., Elias, G., 2018. Safer anti-inflammatory therapy through dual COX-2/5-LOX inhibitors: a structure-based approach. Europ. J. Pharmac. Sci. 121, 356–381. https://doi.org/10.1016/j.ejps.2018.06.003.
Jaiswal, L., Ismail, H., Worku, M., 2020. A review of the effect of plant-derived bioactive substances on the inflammatory response of ruminants (sheep, cattle, and goats). Int. J. Veterin. Animal Med. 3, 130.
Joo, T., Sowndhararajan, K., Hong, S., Lee, J., Park, S.-Y., Kim, S., Jhoo, J.-W., 2014. Inhibition of nitric oxide production in LPS-stimulated RAW 264.7 cells by stem bark of *Ulmus pumila* L. Saudi J. Biol. Sci 21 (5), 427–435.
Jumina, J., Siswanta, D., Zulkarnain, K., Triono, S., Priatmoko, P., Yuanita, E., Fatmasari, N., Nursalim, I., 2019. Development of C-arylcalix[4]resorcinarenes and C-Arylcalix[4]pyrogallolarenes as antioxidant and UV-B protector. Indones. J. Chem. 19, 273. http://doi.org/10.22146/ijc.26868.
Kang, H., Ku, S.-K., Jung, B., Bae, J.-S., 2015. Anti-inflammatory effects of vicenin-2 and scolymoside *in vitro* and *in vivo*. Inflamm. Res. 64 (12), 1005–1021. http://doi.org/10.1007/s00011-015-0886-x.
Keller, M., Manzocchi, E., Rentsch, D., Lugarà, R., Giller, K., 2021. Antioxidant and inflammatory gene expression profiles of bovine peripheral blood mononuclear cells in response to arthrospira platensis before and after lps challenge. Antioxidants 10 (5), 814.
Kiki, G., Pop, R., Sabin, O., Bocsan, I., Chedea, V., Socaci, S., Pârvu, A., Finsia, E., Francis, T., Mathieu, Z., Buzoianu, A.D., 2022. Polyphenols from *Dichrostachys cinerea* fruits anti-inflammatory, analgesic, and antioxidant capacity in Freund's adjuvant-induced arthritic rat model. Molecules 27 (17), 5445. http://doi.org/10.3390/molecules27175445.
Kim, M.K., Yun, K.J., Lim, D.H., Kim, J., Jang, Y.P., 2016. Anti-inflammatory properties of flavone di-C-glycosides as active principles of *Camellia mistletoe, Korthalsella japonica*. Biomolec. Therapeutics (Seoul) 24 (6), 630–637. http://doi.org/10.4062/biomolther.2016.019.
Kiselova-Kaneva, Y., Tasinov, O., Vankova, D., Ivanova, D., 2012. Ethanol induces IL-6 and TNF-alpha cytokine and iNOS and COX-2 enzyme gene expression in 3T3-L1 preadipocytes. Scripta Scientifica Medica 44, 31–35. http://doi.org/10.14748/ssm.v44i2.354.
Lee, J., Song, C.H., 2021. Effect of reactive oxygen species on the endoplasmic reticulum and mitochondria during intracellular pathogen infection of mammalian cells. Antioxidants 10 (6), 872. http://doi.org/10.3390/antiox10060872.
Li, L., Peng, Y., Xu, L.-J., Li, M.-H., Xiao, P.-G., 2008. Flavonoid glycosides and phenolic acids from *Ehretia thyrsiflora*. Biochem. System. Ecol. 36 (12), 915–918.
Li, P., Piao, X., Ru, Y., Han, X., Xue, L., Zhang, H., 2012. Effects of adding essential oil to the diet of weaned pigs on performance, nutrient utilization, immune response and intestinal health. Asian-australas. J. Anim. Sci. 25 (11), 1617.
Madhu, B.P., Singh, K.P., Saminathan, M., Singh, R., Tiwari, A.K., Manjunatha, V., Harish, C., Manjunathareddy, G.B., 2016. Correlation of inducible nitric oxide synthase (iNOS) inhibition with TNF-$\alpha$, caspase-1, FasL and TLR-3 in pathogenesis of rabies in mouse model. Virus Genes 52 (1), 61–70. http://doi.org/10.1007/s11262-015-1265-y.
Mamba, P., Adebayo, S.A., Tshikalange, T.E., 2016. Anti-microbial, Anti-Inflammatory and HIV-1 Reverse Transcriptase Activity of Selected South African Plants Used to Treat Sexually Transmitted Diseases. University of Pretoria.
Maroyi, A., 2017. *Acacia karroo* Hayne: ethnomedicinal uses, phytochemistry and pharmacology of an important medicinal plant in southern Africa. Asian Pac. J. Trop. Med 10 (4), 351–360. https://doi.org/10.1016/j.apjtm.2017.03.021.
Maroyi, A., 2023a. Medicinal uses of the Asteraceae family in Zimbabwe: a historical and ecological perspective. Ethnobotany Res. Applic. 25, 1–30.
Maroyi, A., 2023b. A systematic review on biological and medicinal properties of *Ehretia rigida* (Thunb.) Druce (Ehretiaceae) in Southern Africa. Plant Sci. Today. http://doi.org/10.14719/pst.2359.
Mashima, R., Okuyama, T., 2015. The role of lipoxygenases in pathophysiology; new insights and future perspectives. Redox Biol. 6, 297–310. https://doi.org/10.1016/j.redox.2015.08.006.
Mazimba, O., Kwape, T., Gaobotse, G., 2022. *Dichrostachys cinerea*: ethnomedicinal uses, phytochemistry and pharmacological activities - a review. Natural Products J. 12. https://doi.org/10.2174/2210315511666210806144540.
More, G.K., Chokwe, C.R. & Meddows-Taylor, S., (2021). The attenuation of antibiotic resistant non-*albicans Candida* species, cytotoxicity, anti-inflammatory effects and phytochemical profiles of five *Vachellia* species by FTIR and UHPLC−Q/Orbitrap/MS. *Heliyon, 7*(11).
Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods 65 (1–2), 55–63. https://doi.org/10.1016/0022-1759(83)90303-4.
Mukhopadhyay, N., Shukla, A., Makhal, P.N., Kaki, V.R., 2023. Natural product-driven dual COX-LOX inhibitors: overview of recent studies on the development of novel anti-inflammatory agents. Heliyon 9 (3), e14569. https://doi.org/10.1016/j.heliyon.2023.e14569.
Navarro-Yepes, J., Zavala-Flores, L., Anandhan, A., Wang, F., Skotak, M., Chandra, N., Li, M., Pappa, A., Martinez-Fong, D., Del Razo, L.M., Quintanilla-Vega, B., Franco, R., 2014. Antioxidant gene therapy against neuronal cell death. Pharmacol. Ther. 142 (2), 206–230. https://doi.org/10.1016/j.pharmthera.2013.12.007.
Ngaffo, C.M.N., Tchangna, R.S.V., Mbaveng, A.T., Kamga, J., Harvey, F.M., Ngadjui, B.T., Bochet, C.G., Kuete, V., 2020. Botanicals from the leaves of *Acacia sieberiana* had better cytotoxic effects than isolated phytochemicals towards MDR cancer cells lines. Heliyon 6 (11), e05412. http://doi.org/10.1016/j.heliyon.2020.e05412.
Niewold, T., 2007. The nonantibiotic anti-inflammatory effect of antimicrobial growth promoters, the real mode of action? A hypothesis. Poult. Sci. 86 (4), 605–609.
Nkadimeng, S.M., Nabatanzi, A., Steinmann, C.M., Eloff, J.N., 2020. Phytochemical, cytotoxicity, antioxidant and anti-inflammatory effects of *Psilocybe natalensis* magic mushroom. Plants 9 (9), 1127.
Ondua, M., Njoya, E.M., Abdalla, M.A., McGaw, L.J., 2019. Anti-inflammatory and antioxidant properties of leaf extracts of eleven South African medicinal plants used traditionally to treat inflammation. J. Ethnopharmacol 234, 27–35. https://doi.org/10.1016/j.jep.2018.12.030.
Pepe, G., Sommella, E., Manfra, M., De Nisco, M., Tenore, G.C., Scopa, A., Sofo, A., Marzocco, S., Adesso, S., Novellino, T., 2015. Evaluation of anti-inflammatory activity and fast UHPLC−DAD−IT-TOF profiling of polyphenolic compounds extracted from green lettuce (*Lactuca sativa* L.; var. Maravilla de Verano). Food Chem 167, 153–161.
Piao, M., Tu, Y., Zhang, N., Diao, Q., Bi, Y., 2023. Advances in the application of phytogenic extracts as antioxidants and their potential mechanisms in ruminants. Antioxidants 12 (4), 879.
Pizzino, G., Irrera, N., Cucinotta, M., Pallio, G., Mannino, F., Arcoraci, V., Squadrito, F., Altavilla, D., Bitto, A., 2017. Oxidative stress: harms and benefits for human health. Oxidat. Med. Cellular Longev. 2017, 8416763. http://doi.org/10.1155/2017/8416763.
Ramde-Tiendrebeogo, A., Koala, M., Ouattara, N., Lompo, M., Guissou, I.P., 2019. A comparative study of phytochemical profile and antioxidant activity of Sahelian plants used in the treatment of infectious diseases in northern part of Burkina Faso: *Acacia seyal* Delile and *Acacia tortilis* (Forssk.) Hayne subsp. *raddiana* (Savi). J. Pharmacog. Phytother. 11 (3), 74–79.
Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biol. Med. 26 (9–10), 1231–1237.
Rodríguez-Mesa, X.M., Contreras Bolaños, L.A., Mejía, A., Pombo, L.M., Modesti Costa, G., Santander González, S.P., 2023. Immunomodulatory properties of natural extracts and compounds derived from *Bidens pilosa* L.: literature review. Pharmaceutics 15 (5), 1491.
Rodríguez-Yoldi, M.J., 2021. Anti-inflammatory and antioxidant properties of plant extracts. Antioxidants 10 (6), 921. http://doi.org/10.3390/antiox10060921.
Sahoo, N., Manchikanti, P., Dey, S.H., 2011. Herbal drug patenting in India: IP potential. J. Ethnopharmacol 137 (1), 289–297.
Sebolai, T.M., 2018. Nutritional Characterization of Browse Plants Harvested At Different Browsing Heights in Eastern Cape Province. North-West University (South Africa).
Seckin, C., Alpun Kalayci, G., Turan, N., Yilmaz, A., Cizmecigil, U.Y., Aydin, O., Richt, J.A., Yilmaz, H., 2018. Immunomodulatory effects of *Echinacea* and *Pelargonium* on the innate and adoptive immunity in calves. Food Agric. Immunol 29 (1), 744–761.
Shah, Z.A., Hameed, A., Ahmed, A., Simjee, S.U., Jabeen, A., Ullah, A., Shaheen, F., 2016. Cytotoxic and anti-inflammatory salicin glycosides from leaves of *Salix acmophylla*. Phytochem. Lett 17, 107–113. https://doi.org/10.1016/j.phytol.2016.07.013.
Shandukani, P.D., Tshidino, S.C., Masoko, P., Moganedi, K.M., 2018. Antibacterial activity and *in situ* efficacy of *Bidens pilosa* Linn and *Dichrostachys cinerea* Wight et Arn extracts against common diarrhoea-causing waterborne bacteria. BMC Complem. Altern. Med. 18 (1), 171. https://doi.org/10.1186/s12906-018-2230-9.

Shukla, A., Kaur, A., 2018. A systematic review of traditional uses bioactive phytoconstituents of genus *Ehretia*. Asian J. Pharmac. Clin. Res. 11 (6), 88–100.
Simon, L.S., 1999. Role and regulation of cyclooxygenase-2 during inflammation. Am. J. Med. 106 (5b), 37s–42s. http://doi.org/10.1016/s0002-9343(99)00115-1.
Susithra, E., Jayakumari, S., 2018. Analgesic and anti-inflammatory activities of *Dichrostachys cinerea* (L.) Wight and Arn. Drug Inven. Today 10, 361–366.
Taha, D., El Hajjaji, S., Mourabit, Y., Bouyahya, A., Lee, L.-H., El Menyiy, N., Tarik, A., Benali, T., El Moudden, H., Gallo, M., Iba, N., Bourais, I., 2022. Traditional knowledge, phytochemistry, and biological properties of *Vachellia tortilis*. Plants 11 (23), 3348. http://doi.org/10.3390/plants11233348.
Taira, J., Nanbu, H., Ueda, K., 2009. Nitric oxide-scavenging compounds in *Agrimonia pilosa* Ledeb on LPS-induced RAW264. 7 macrophages. Food Chem 115 (4), 1221–1227.
Tamoli, S., Gokarn, V., Ibrahim, M., Ahmad, S., 2022. Comparative investigation of Ashwagandha FMB extract and standardized extract for their antioxidant, anti-inflammatory and immunomodulatory potential. Annals Phytomed. 11 (1), 405–412.
Tanaka, T., Narazaki, M., Kishimoto, T., 2014. IL-6 in inflammation, immunity, and disease. Cold Spring Harb Perspect. Biol. 6 (10), a016295. http://doi.org/10.1101/cshperspect.a016295.
Tawfeek, N., Mahmoud, M.F., Hamdan, D.I., Sobeh, M., Farrag, N., Wink, M., El-Shazly, A.M., 2021. Phytochemistry, pharmacology and medicinal uses of plants of the genus *Salix*: an updated review. Front. Pharmacol article 12, 593856.
Tchatchedre, M., Amoussa, A.M., Houinato, M., Yedomonhan, H., Lagnika, L., 2019. Ethnobotanical survey and phytogeographical study of plants species from genus *Acacia* in Benin. J. Medic. Plants Res. 13 (9), 199–212.
Truong, D.-H., Nguyen, D.H., Ta, N.T.A., Bui, A.V., Do, T.H., Nguyen, H.C., 2019. Evaluation of the use of different solvents for phytochemical constituents, antioxidants, and in vitro anti-inflammatory activities of *Severinia buxifolia*. J Food Qual 2019, 8178294.
Tungmunnithum, D., Thongboonyou, A., Pholboon, A., Yangsabai, A., 2018. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: an overview. Medicines (Basel) 5 (3). http://doi.org/10.3390/medicines5030093.
Van Emon, M., Sanford, C., Mccoski, S., 2020. Impacts of bovine trace mineral supplementation on maternal and offspring production and health. Animals (Basel) 10 (12). http://doi.org/10.3390/ani10122404.
Vonkeman, H.E., Van De Laar, M.A., 2010. Nonsteroidal anti-inflammatory drugs: adverse effects and their prevention. Seminar. Arthr. Rheum. 39 (4), 294–312. http://doi.org/10.1016/j.semarthrit.2008.08.001.
Van Wyngaard, J., Famuyide, I.M., Invernizzi, L., Ndivhuwo, K.K., Tordiffe, A.S.W., Maharaj, V.J., McGaw, L.J., 2023. Optimizing extraction of *Pelargonium sidoides* roots: impact of ethanol concentration on biological activity of extracts. South African J. Botany 162, 667–679.
Wahab, G.A., Sallam, A., Elgaml, A., Lahloub, M.F., Afifi, M.S., 2018. Antioxidant and antimicrobial activities of *Salix babylonica* extracts. World J. Pharmac. Sci. 1–6.
Wahab, G.A., Sallam, A., Elgaml, A., Lahloub, M.F., Afifi, M.S., 2022. Antioxidant, antimicrobial and anti-quorum sensing compounds from *Salix babylonica*. South African J. Botany 147, 774–781.
Wisastra, R., Dekker, F.J., 2014. Inflammation, cancer and oxidative lipoxygenase activity are intimately linked. Cancers (Basel) 6 (3), 1500–1521. http://doi.org/10.3390/cancers6031500.
Yahfoufi, N., Alsadi, N., Jambi, M., Matar, C., 2018. The immunomodulatory and anti-inflammatory role of polyphenols. Nutrients 10 (11), 1618. http://doi.org/10.3390/nu10111618.
Yang, Z.-L., Zhao, Q., He, Q., 2019. Nitric oxide. Medknow.
Yatoo, D.M., Saxena, A., Pm, D., Habeab, B., Devi, S., Jatav, R., Dimri, U., 2013. Role of trace elements in animals: a review. Vet World 6, 963–967. http://doi.org/10.14202/vetworld.2013.963-967.
Yazdankhah, S., Rudi, K., Bernhoft, A., 2014. Zinc and copper in animal feed - development of resistance and co-resistance to antimicrobial agents in bacteria of animal origin. Microb. Ecol. Heal. Disease 25. http://doi.org/10.3402/mehd.v25.25862.
Yuhas, Y., Weizman, A., Vanichkin, A., Ashkenazi, S., 2005. Involvement of prostaglandins in an animal model of *Shigella*-related seizures. J. Neuroimmunol. 168 (1), 34–39. https://doi.org/10.1016/j.jneuroim.2005.06.029.
Zeid, A.H.A., Hifnawy, M.S. & Mohammed, R.S. Phenolic compounds and biological activities of *Dichrostachys cinerea* L. 2010.