Ptaeroxylon obliquum leaf extracts, fractions and isolated compounds as potential inhibitors of 15-lipoxygenase and lipopolysaccharide-induced nitric oxide production in RAW 264.7 macrophage cells

T.E. Ramadwa [*,a,b] , J.P. Dzoyem [b] , S.A. Adebayo [b] , J.N. Eloff [b]

[a]Department of Life and Consumer Sciences, College of Agriculture and Environmental Sciences, Florida Campus, University of South Africa, Private Bag X6, Florida, 1710, South Africa

[b]Phytomedicine Programme, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, Pretoria, South Africa

* Corresponding author. Department of Life and Consumer Sciences, College of Agriculture and Environmental Sciences, Florida Campus, University of South Africa, Private Bag X6, Florida, 1710, South Africa

Phytomedicine Programme, Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort 0110, Pretoria, South Africa. Email: ramadwa.te@gmail.com

Highlights

- The three isolated compounds (obliquumol, lupeol and β-amyrin mixture, and eranthin) had promising 15-LOX inhibitory activity with LC_{50} which were comparable to the positive control quercetin.
- Eranthin had the most promising anti-inflammatory activity with good percentage inhibition of NO production in LPS-induced RAW 264.7 cells and with good cell viability.

Abstract

Ptaeroxylon obliquum is used traditionally to treat inflammatory diseases and related symptoms such as arthritis, rheumatism, fever and headache. The aim of the study was to determine the anti-inflammatory activities of the crude extracts, fractions and isolated compounds from *P. obliquum* leaves. Nitric oxide (NO) production in lipopolysaccharide (LPS)-induced RAW 264.7 macrophages and soybean 15-lipoxygenase (15-LOX) inhibitory assays were used to evaluate the anti-inflammatory activity. The acetone crude extracts and fractions had weak 15-LOX inhibitory activity and also had some toxicity against the RAW 264.7 macrophage cells. The three isolated compounds, obliquumol, lupeol and β-amyrin mixture, and eranthin had good 15-LOX inhibitory activity with IC_{50} values ranging from 7.4 to 13.9 µg/mL which was in the same order as the positive control quercetin (2.1 µg/mL). The high percentage inhibition of NO production by the crude extracts, fractions and isolated compounds appeared to be due to the toxicity to the macrophage cells. Eranthin had the most promising activity with 71.1% inhibition and 89.1% cell viability at a 0.5 µg/mL concentration. The 15-LOX inhibitory activity of obliquumol, lupeol and β-amyrin mixture and eranthin may explain the traditional uses of *P. obliquum* to treat various inflammatory diseases even though the possibility that the anti-inflammatory activity might still be exerted through other inflammatory mechanisms.

Key words: *Ptaeroxylon obliquum;* Inflammation; Nitric oxide; Lipoxygenase; Obliquumol; Eranthin

Abbreviations

(MeOH) - Methanol; (H_2O) - water; (SANBI) - South African National Biodiversity Institute; (DMSO) - dimethyl sulphoxide; (AIDS) - Acquired immunodeficiency syndrome; (NO) - nitric oxide; (DMEM) - Dulbecco's Modified Eagle's Medium; (LPS) - lipopolysaccharide; (4,5-dimethylthiazol-2-yl)-2, 3-; (MTT) - 5-diphenyl tetrazolium bromide; (15-LOX) - 15-lipoxygenase

1. Introduction

Inflammation can be defined as an innate protective response of cells/tissues of the body to allergic or chemical irritation, injury and/or invasion by pathogens (Calixto et al., 2003; Iwalewa et al., 2007; Muniandy et al., 2018). Inflammation is a hallmark of many diseases and disorders, and its persistence may lead to various degenerative diseases. It is associated with acute and chronic inflammation in diseases such as rheumatoid arthritis, atherosclerosis, type-2 diabetes, sepsis, heart diseases, Alzheimer's, asthma, Acquired Immunodeficiency Syndrome (AIDS), cancer, congestive heart failure, multiple sclerosis, gout, inflammatory bowel disease (IBD), aging and other neurodegenerative central nervous system depressions (O'Byrne and Dalgleish, 2001; Dalgleish and O'Byrne, 2002; Tilg and Moschem, 2006). It is characterized by pain, heat, redness, swelling and loss of function that result from dilation of the blood vessels leading to an increased intercellular spaces resulting in the movement of leukocytes, protein and fluids into inflamed regions (Parham, 2000). These changes in the affected tissue serve to reduce the effects of the injury thereby limiting the threat to the body (Levine and Reichling, 1999). As the body recognizes the injury and prepares to repair the damage, chemical mediators are released in response to the problem that binds to specific target receptors on the cells. Pro-inflammatory mediators are substances released as plasma proteins or from cells like mast cells, platelets, neutrophils and monocytes/macrophages that promote inflammation. Examples of pro-inflammatory mediators include NO, prostaglandins, leukotrienes, vasoactive amines and cytokines (Iwalewa et al., 2007).

Macrophages play an important role in the generation of pro-inflammatory mediators like Nitric oxide (NO). NO is a short lived free radical gas which is synthesized by the enzyme nitric oxide synthase (Heras et al., 2001). After stimulation with bacterial LPS, many cells including macrophages express inducible nitric oxide synthase (iNOS) which is responsible for the production of high level of NO and plays an important role in immunoregulation. Overproduction of NO can result in tissue destruction and immunological and inflammatory diseases (Xie et al., 1994; Saha et al., 2004). Substances that can inhibit NO release have potential therapeutic effects for inflammatory diseases (Chen et al., 2014).

Lipoxygenase are the most studied and considered to be key enzymes in the biosynthesis of leukotrienes which play an important role in pathophysiology for number of inflammatory diseases (Roussaki et al., 2010; Attiq et al., 2018). Lipoxygenase are expressed in epithelial, tumor and immune cells that show variety of physiological functions, including inflammation

and skin disorders (Singh and Rao, 2019). Mammalian lipoxygenases are classified as 5-, 12- and 15-LOX depending on the position of oxygen insertion into the arachidonic acid (Schneider and Bucar, 2005). They are expressed in a tissue-specific fashion in humans; 15-LOX is mainly expressed in reticulocytes, eosinophils, and microphages (Klil-Drori and Ariel, 2013). There is a pressing need to develop specific inhibitors of 15-LOX in order to manage inflammatory diseases. Soybean lipoxygenase are used in many assays to detect potential substances which might act as inhibitors on mammalian lipoxygenase because they also catalyze oxidation of linoleic acid, arachidonic acid and other unsaturated fatty acids (Komoda et al., 2004; Kumari et al., 2011).

Many anti-inflammatory drugs that have been used successfully over the years in the treatment of various inflammatory related diseases are classified as steroidal or non-steroidal chemical therapeutics. Unfortunately, some of these drugs have severe and adverse side effects and long term administration is frequently required to treat chronic inflammation (Conforti et al., 2008). Plants have been used for centuries to treat many diseases including inflammatory diseases or related symptoms. *Ptaeroxylon obliquum* (Thunb). Radlk a member of the Rutaceae family commonly known as sneezewood is traditionally used in southern Africa to treat a wide range of diseases including inflammation-related diseases and symptoms such as arthritis, rheumatism, fever and headache (Iwalewa et al., 2007; Moyo and Masika, 2009; Ribeiro et al., 2010; Ramadwa et al., 2021). Isolated compounds from medicinal plants may have a potential to serve as template for the synthesis of novel anti-inflammatory agents with high pharmacological activity and fewer side effects. The aim of this study was to determine the anti-inflammatory activity of *P. obliquum* leaf extracts, fractions and isolated compounds as potential inhibitors of 15-LOX and LPS-induced NO production from RAW 264.7 macrophage cells.

2. Materials and methods

2.1. Plant selection, collection, storage and extraction

The plant leaves of *P. obliquum* used in this study were collected from trees growing in the Pretoria National Botanical Garden of SANBI in the summer season. The leaves were collected in open woven orange bags, dried at room temperature in the shade and powdered using a mill. The powders were stored in closed containers in the dark until needed. Voucher specimens were prepared and kept at the HGW Schweikert Herbarium of the University of Pretoria (PRU 96709).

2.2. Preparations of the crude extracts, fractions and isolated compounds

The plant collection, extraction procedure and isolation of bioactive compounds were carried out as described and led to isolation of obliquumol, lupeol and β-amyrin mixture, and eranthin from *P. obliquum* acetone leaf extract (Ramadwa et al., 2019). Our further studies on the synthesis of obliquumol and its derivatives revealed that the correct structure of obliquumol is not the angular compound (eranthin acetate) with excellent antifungal activity as previously reported (van Wyk et al., 2018), but the linear isomer ptaeroxylinol acetate (Malefo et al., 2020). The acetone crude extracts, the five fractions (hexane,

chloroform, 30% H_2O in methanol (MeOH), butanol and H_2O fractions), and the isolated pure compounds obliquumol, lupeol and β-amyrin mixture, and eranthin were reconstituted in dimethyl sulphoxide (DMSO) for the anti-inflammatory assays.

2.3. Anti-inflammatory assays

The anti-inflammatory activities of *P. obliquum* leaf extracts, fractions and its bioactive compounds were evaluated on the basis of their ability to inhibit 15-LOX, NO production inhibiting effect in LPS activated RAW 264.7 macrophages and the cellular viability.

2.3.1. Soybean lipoxygenase inhibition assay

The assay was performed according to previously described procedure (Pinto et al., 2007) with slight modifications. The assay is based on the formation of the complex Fe^{3+} xylenol orange with absorption at 560 nm. The 15-LOX from *Glycine* max was incubated with the tested samples at 25 °C for 5 min. Linoleic acid (final concentration, 140 mM) in Tris–HCl buffer (50 mM, pH7.4) was added and the mixture was then incubated at 25 °C for 20 min in the dark. The assay was terminated by the addition of 100 mL of FOX reagent [sulphuric acid (30 mM), xylenol orange (100 mM), iron (II) sulfate (100 mM), methanol/water (9:1)]. The standard inhibitor quercetin was used as a positive control and DMSO as the negative control. The 15-LOX inhibitory activity was evaluated by calculating the percentage of the inhibition of hydroperoxide production from the changes in absorbance values at 560 nm after 30 min at 25 °C. The% Inhibition= [(Absorbance of control -Absorbance of test sample)/Absorbance control] × 100. The results were expressed as IC_{50} values which were defined as the test extracts, fractions and compounds concentration necessary to produce 50% inhibition of hydroperoxide-release.

2.3.2. Nitric oxide (NO) inhibition assay

2.3.2.1. Cell culture

The RAW 264.7 cells were grown in plastic culture flask in Dulbecco's Modified Eagle's Medium (DMEM) with phenol red containing HEPES, l-glutamine supplemented with 10% foetal bovine serum (FBS) and 1% antibiotic/antimycotic solution (Gibco/BRL) under 5% CO_2 at 37 °C. After 4–5 days cells were removed from the culture flask by scraping and centrifuged for 10 min at 3000 rpm at 4 °C. The medium was then removed and the cells were resuspended with fresh DMEM without phenol red containing HEPES, l-glutamine with same supplements. Cell counts and viability was performed using a standard Trypan blue cell counting technique. The cell concentration was adjusted to 10^6 cells/mL in the same medium. Apart from the normal cell controls all cells were cultured in the above media (50 µL) which was used when containing 10 µg/mL of LPS seeded into wells of 96-well tissue culture microtitre plates. Then, 50 µL of serially diluted samples in media containing DMSO were dispensed into wells of the cell plates to yield a final concentration of DMSO at 0.1% per well. Samples were used in triplicate cells and then incubated for 24 h at 37 °C, 5% CO_2 in a fully humidified incubator. Controls used included media only, cells in media containing LPS and 0.1% DMSO.

2.3.2.2. Effects on production of NO in LPS-stimulated macrophage RAW 264.7 cells

The stable conversion product of NO, nitrite (NO^{2-}) was measured using the Griess reagent to determine the NO concentration (Chi et al., 2001). After 24 h incubation 50 µL of supernatant from each well of cell culture plates were transferred into 96-well microplates and equal volume of Griess reagent (1% sulphanilamide, 0.1% *N*-(1-naphthyl)-ethyline diamine hydrochloride, 2.5% phosphoric acid) was then added to the supernatant at room temperature. The absorbance at 550 nm was determined in a BioTek Synergy microplate reader after 10 min. The concentrations of nitrite were derived from regression analysis and sodium nitrite was used as a standard. Percentage inhibition was calculated based on the ability of the tested samples to inhibit nitric oxide formation by cells compared with the control (cells in media without samples containing triggering agents) and DMSO, which were considered as 0% inhibition.

2.3.2.3. Measurement of cell viability

To determine whether NO inhibition was a result of false positives due to cytotoxic effects, a cytotoxicity assay was also performed on macrophage RAW 264.7 cells according the method described by Heras et al. (2001). After removal of media, the cells were topped up with 100 µL complete DMEM. To each well 20 µL of 5 mg/mL 3-(4, 5-dimethylthiazol-2-yl)−2, 5-diphenyl tetrazolium bromide (MTT) in PBS 7.2 was added. The cells were incubated at 37 °C with 5% CO_2 for 4 h. The medium was then carefully removed and the formed formazan salts were dissolved in DMSO. The absorbance was read at 570 nm. The absorbance of formazan formed in control (cells without the tested samples containing LPS, and DMSO) cells were taken as 100% viability.

3. Results and discussion

3.1. Lipoxygenase inhibitory activity of the acetone extracts, fractions, and isolated compounds from P. obliquum

The 15-LOX enzyme is key in leukotriene biosynthesis and catalyzes the first steps in the conversion of arachidonic acid to biologically active leukotrienes which are considered as potent mediators of inflammatory and allergic reactions and are important in the treatment of inflammation-related diseases (Schneider and Bucar, 2005). The crude extracts and five fractions had weak inhibitory activity with IC_{50} values of 1.61 mg/mL and 2.55–12.43 mg/mL respectively (Table 1). The fractionation of the crude extract resulted in reduced 15-LOX inhibitory activity since all the fractions had IC_{50} >1.61 mg/mL of the crude extract. The lipoxygenase activity was associated with the non-polar compounds with very low activity in the water extract.

Table 1. Inhibition (IC_{50}) of 15-LOX by the acetone crude extracts, fractions and isolated compounds from *P. obliquum.*

Samples	15-lipoxygenase IC_{50} (mg/mL)
Crude extract	1.61
Hexane fraction	2.55
Chloroform fraction	3.03
30% H_20 in MeOH fraction	5.24
Butanol fraction	6.55
Water fraction	12.43
Obliquumol	0.0139
Lupeol & β-amyrin mixture	0.0074
Eranthin	0.0075
Quercetin	0.0021

The three isolated compounds (obliquumol, lupeol and β-amyrin mixture and eranthin) from the non-polar fraction had good inhibitory activity with IC_{50} values ranging from 0.0074 to 13.9 mg/mL which were comparable to that of the positive control, quercetin (0.0021 mg/mL). The lupeol and β-amyrin mixture and eranthin had the best inhibitory activity with IC_{50} values of 0.0074 mg/mL and 0.0075 mg/mL, respectively. Lupeol is known to modulate the expression of several inflammation-associated molecules such as soybean 15-LOX, macrophages, prostaglandin, cytokines, myeloperoxidase, tumor necrosis factor, interleukin, and T-lymphocytes (Akihisa et al., 1996; Vidya et al., 2002; Fernández et al., 2001a, 2001b; Moreira et al., 2001; Bani et al., 2006; Ding et al., 2009; Nguemfo et al., 2009). This study reports for the first time the 15-LOX inhibitory activity of *P. obliquum* acetone leaf extract, fractions and the two isolated bioactive compounds (obliquumol and eranthin).

3.2. Inhibition of nitric oxide production from LPS-induced RAW 264.7 cells by the crude extract, fractions and isolated compounds from P. obliquum

NO are key mediators for the pathogenesis of rheumatoid arthritis and inflammation-related diseases. The inhibition of NO is an important strategy for the treatment of these inflammatory conditions and consequently, we evaluated the anti-inflammatory activity of the crude extract, fractions and isolated compounds from *P. obliquum* leaves using exposure to LPS-induced macrophage RAW264.7 cell line. This method usually involves the simultaneous evaluation of the crude extracts, fractions or isolated compounds for their potential cytotoxicity. The results are carefully interpreted by taking cytotoxicity results into consideration to indicate that the resultant anti-inflammatory activity is not due to a cytotoxic effect (Adebayo and Amoo, 2019). The crude extracts, fractions and compounds had high percentage of inhibition on nitric oxide production in induced cells. However, it appeared as if the high percentage of inhibition may have been as a result of toxicity of the crude, fractions and compounds on the macrophages (Table 2). The crude extracts and fractions had some level of toxicity in all the concentrations tested with the highest cell viability of only 66.8%. The crude extract together with hexane and chloroform fractions led to cell viability of less than 3% and 30% H_2O in MeOH fraction led to 14.6% cell viability at the highest concentration tested. The crude extract had a 77.8% inhibition when tested at 30 µg/mL concentration, but there was only 47.6% cell viability. The same trend was observed on all the five fractions at 30 µg/mL and 10 µg/mL concentrations where there

was good percentage of inhibition but poor cell viability. Obliquumol had 85.2–81.8% inhibition at concentrations from 20 to 2 µg/mL with cell viability ranging from 33.2 to 67% cell viability. Lupeol and β-amyrin mixture had 74.2% inhibition with 55% cell viability at the highest concentration µg/mL. Eranthin had the most promising activity compared to all the samples tested in the study with good percentage of inhibition (71.1%) and cell viability (89.1%) at 0.5 µg/mL concentration. Quercetin (Positive control) had cell viability ranging from 77.1% to 92.4% when tested at 5–0.5 µg/mL concentrations. Previous studies on obliquumol indicated that it was not toxic against both Vero African green monkey kidney cell and human liver (C3A) cell at the highest concentration tested of 200 µg/mL and had good selectivity index values against *C. albicans* and *Cryptococcus neoformans* (Ramadwa et al., 2021). No report in the literature was found on the inhibitory activity of *P. obliquum* acetone leaf extract, fractions and the two pure compounds (obliquumol and ptaeroxylinol) on NO production in LPS-activated RAW 264.7 cells.

Table 2. Inhibitory effects of the crude extract, fractions and isolated compounds from *P. obliquum* on NO production in LPS-induced RAW 264.7 cells and their viability against macrophage RAW 264.7 cells.

Samples	Concentration (µg/mL)	NO Inhibition (%)	Cell viability (%)
Crude extract	100	80.7	1.7
	30	77.8	47.6
	10	69.7	48.5
	2	36.9	50.2
Hexane fraction	100	80.5	2.1
	30	79.4	51.9
	10	72.8	53.5
	2	41.3	66.8
Chloroform fraction	100	83.3	2.7
	30	83.2	44.2
	10	80.9	49.2
	2	44.2	53.3
30% H_2O in MeOH	100	83.7	14.6
	30	79.2	55.2
	10	71.4	59.9
	2	29.4	61.3
Butanol fraction	100	82.2	44.8
	30	77.3	54.5
	10	48.8	58.1
	2	9.8	63.0
Water fraction	100	76.1	40.7
	30	55.5	43.6
	10	36.3	43.5
	2	9.8	49.5
Obliquumol	20	85.2	33.2
	5	82.9	59.7
	2	81.8	65.0
	0.5	64.0	67.0
Lupeol & β-amyrin mixture	20	74.2	55.0
	5	49.0	62.2
	2	26.5	65.0
	0.5	12.9	74.5
Eranthin	20	85.0	38.9
	5	84.0	50.8

	2	82.4	55.3
	0.5	71.1	89.1
Quercetin	20	72.5	48.2
	5	63.2	77.1
	2	42.0	81.9
	0.5	11.0	92.4

4. Conclusion

P. obliquum is traditionally used to treat various ailments including inflammatory diseases or inflammatory related symptoms. There were reasonable activities in the non-polar fractions but very low activities in the polar fractions of leaf extracts. If traditional leaders have mainly water available as extractant, it is difficult to reconcile the efficacy with our results. The *P. obliquum* acetone leaf extract and fractions had weak 15-LOX inhibitory activity and also had some toxicity against the macrophages. The three isolated compounds had promising 15-LOX inhibitory activity with LC_{50} which were comparable to the positive control quercetin. The 15-LOX inhibitory activity of obliquumol, lupeol and β-amyrin mixture and eranthin may explain the traditional uses of *P. obliquum* to treat various inflammatory diseases. The NO production inhibition by the crude extracts, fractions and compounds seems to be due to its cytotoxic effects to macrophages. Eranthin the deacetylated form of obliquumol had the most promising anti-inflammatory activity with good percentage inhibition of NO production in LPS-induced RAW 264.7 cells and with good cell viability. The possibility that the anti-inflammatory activity of *P. obliquum* acetone leaf extracts and fractions might still be exerted through other mechanisms such prostaglandins, vasoactive amines and cytokines which were not investigated in this study cannot be ruled out. This is the first report on the anti-inflammatory activities of *P. obliquum* acetone leaf extracts, fractions and isolated compounds against 15-LOX and NO production in LPS-induced macrophage. Another explanation for the efficacy of the traditional use may be due to the use of bark and also possibly the application as a snuff leading to the common name of sneezewood. It does not appear as if the antifungal compounds present in leaves play a major role in the anti-inflammatory activity of *P. obliquum* products (Fig. 1).

Additional data

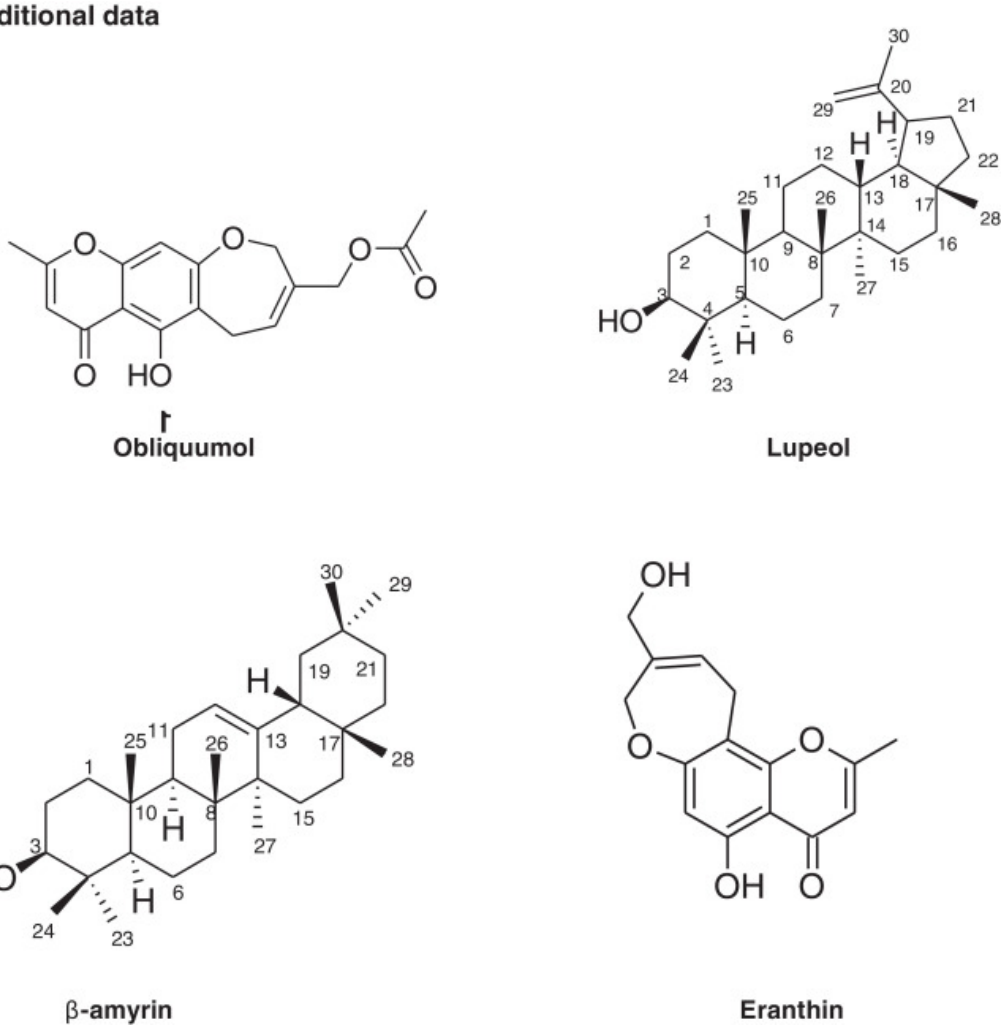

Fig. 1. The structures of the isolated obliquumol, lupeol and β-amyrin mixture and eranthin isolated from *P. obliquum* leaf extracts evaluated for anti-inflammatory activity.

Declaration of Competing Interest

The authors declare that there is no conflict of interest.

Authors' contributions

TER carried out the experiments, analysed the data and wrote the first draft; JPD and SAA assisted nitric oxide work and SAA helped with 15-LOX assay. JNE was involved in the conception, design of the study and supervised the whole study. All authors read and approved the final manuscript.

Acknowledgements

The National Research Foundation (NRF) and the University of Pretoria provided funding for the research.

References

Adebayo, A.A., Amoo, S.O., 2019. South African botanical resources: a gold mine of natural pro-inflammatory enzymes inhibitors? S. Afr. J. Bot. 123, 214–227.

Akihisa, T., Yasukawa, K., Oinuma, H., Kasahara, Y., Yamanouchi, S., Takido, M., 1996. Triterpene alcohols from the flowers of Compositae and their anti-inflammatory effects. Phytochemistry 43, 1255–1260.

Attiq, A., Jalil, J., Husain, K., Ahmad, W., 2018. Raging the war against inflammation with natural products. Front Pharmacol 9, 976.

Bani, S., Kaul, A., Khan, B., Ahmad, S.F., Suri, K.A., Gupta, B.D., 2006. Suppression of T lymphocyte activity by lupeol isolated from *Crataeva religiosa*. Phytother. Res. 20, 279–287.

Calixto, J.B., Otuki, M.F., Santos, A.R.S., 2003. Anti-inflammatory compounds of plant origin. Part 1. Action of Arachiodonic Acid Pathway, Nitric oxide and nuclear factor kB (NF- kB). Planta Med. 69, 973–985.

Chen, H., Chen, B., Liu, C.T., Zhao, Z., Shao, W.H., Yuan, H., Bi, K.J., Liu, J.Y., Sun, Q.Y., Zhang, W.D., 2014. Synthesis and structure activity relationships of guaiane-type sesquiterpene lactone derivatives with respect to inhibiting NO production in lipopolysaccharide-induced RAW 264.7 macrophages. Eur. J. Med. Chem. 83, 307–316.

Chi, Y.S., Cheon, B.S., Kim, H.P., 2001. Effect of wogonin, a plant flavone from *Scutellaria radix*, on the suppression of cyclooxygenase-2 and the induction of inducible nitric oxide synthase in lipopolysaccharide-treated RAW 264.7 cell. Biochem. Pharmacol. 61, 1195–1203.

Conforti, F., Sosa, S., Marrelli, M., Menichini, F., Statti, G.A., Uzunov, D., Tubaro, A., Menichini, F., Loggia, R.D., 2008. In vivo anti-inflammatory and in vitro antioxidant activities of Mediterranean dietary plants. J. Ethnopharmacol. 116, 144–151.

Dalgleish, A.G., O'Byrne, K.J., 2002. Chronic immune activation and inflammation in the pathogenesis of AIDS and cancer. Adv. Cancer Res. 84, 231–276.

Ding, Y., Nguyen, H.T., Kim, S.I., Kim, H.W., Kim, Y.H., 2009. The regulation of inflammatory cytokine secretion in macrophage cell line by the chemical constituents of *Rhus sylvestris*. Bioorg. Med. Chem. Lett. 19, 3607–3610.

Fernández, A., Alvarez, A., García, M.D., Sáenz, M.T., 2001a. Anti-inflammatory effect of *Pimenta racemosa var. ozua* and isolation of the triterpene lupeol. Il Farmaco 56, 335–338.

Fernández, M.A., de las Heras, B., García, M.D., Sáenz, M.T., Villar, A., 2001b. New insights into the mechanism of action of the anti-inflammatory triterpene Lupeol. J. Pharm. Pharmacol. 53, 1533–1539.

Heras, B.D.L., Abad, M.J., Silvan, A.M., Pascual, R., Bermejo, P., Rodriguez, B., Villar, A.M., 2001. Effects of six diterpenes on macrophage eicosanoid biosynthesis. Life Sci. 70, 269–278.

Klil-Drori, A.j., Ariel, A., 2013. 15-Lipoxygenases in cancer: a double-edged sword? Prostagl. Lipid Mediat. 106, 16–22.

Komoda, T., Kishi, M., Abe, N., Sugiyama, Y., Hirata, A., 2004. Novel lipoxygenase inhibitors, tertrapetalone B, C and D from Streptomyces sp. Biosci. Biotechnol. Biochem. 68, 903–908.

Kumari, R., Meyyappan, A., Selvamani, P., Mukherjee, J., Jaisankar, P., 2011. Lipoxygenase inhibitory activity of crude bark extracts and isolated compounds from *Commiphora berryi*. J. Ethnopharmacol. 138, 256–259.

Iwalewa, E.O., McGaw, L.J., Naidoo, V., Eloff, J.N., 2007. Inflammation: the foundation of diseases and disorders. A review of phytomedicines of South African origin used to treat pain and inflammatory conditions. Afr. J. Biotechnol. 6 (25), 2868–2885.

Levine, J.D., Reichling, D.B., 1999. Peripheral mechanisms of inflammatory pain. In: Wall, P.D., Melzack, R. (Eds.), Textbook of pain; Fourth edition. Church Livingstone, London, pp. 59–84.

Malefo, M.S., Ramadwa, T.E., Famuyide, I.M., McGaw, L.J., Eloff, J.N., Sonopo, M.S., Selepe, M.A., 2020. Synthesis and antifungal activity of chromones and benzoxepines from the leaves of *Ptaeroxylon obliquum*. J. Nat. Prod. 83 (8), 2508–2517.

Moreira, R.R., Carlos, I.Z., Vilega, W., 2001. Release of intermediate reactive hydrogen peroxide by macrophage cells activated by natural products. Biol. Pharm. Bull. 24, 201–204.

Moyo, B., Masika, P.J., 2009. Tick control methods used by resource-limited farmers and the effect of ticks on cattle in rural areas of the Eastern Cape Province, South Africa. Trop. Anim. Health Prod. 41, 517–523.

Nguemfo, E.L., Dimo, T., Dongmo, A.B., Azebaze, A.G., Alaoui, K., Asongalem, A.E., 2009. Antioxidative and anti-inflammatory activities of some isolated constituents from the stem bark of Allanblackia monticola Staner L.C [Guttiferae]. Inflammopharmacology 17, 37–41.

Muniandy, K., Gothai, S., Badran, K.M., Suresh Kumar, S., Esa, N.M., Arulselvan, P., 2018. Suppression of proinflammatory cytokines and mediators in LPS-induced RAW 264.7 macrophages by stem extract of alternanthera sessilis via the inhibition of the NF-kB pathway. J. Immunol. Res. 2018.

O'Byrne, K.J., Dalgleish, A.G., 2001. Chronic immune activation and inflammation as the cause of malignancy. Br. J. Cancer 85, 473–483.

Parham, P., 2000. The Immune system, Chapter 1. Elements of the Immune Systems and Their Roles in Defense. Garland Publishing, New York, pp. 1–31.

Pinto, M.D.C., Tejeda, A., Duque, A.L., Macias, P., 2007. Determination of lipoxygenase activity in plant extracts using a modified ferrous oxidation-xylenol orange assay. J. Agric. Food Chem. 55, 5956–5959.

Ramadwa, T.E., Awouafack, M.D., Sonopo, M.S., Eloff, J.N., 2019. Antibacterial and antimycobacterial activity of crude extracts, fractions and isolated compounds from *Ptaeroxylon obliquum* (Rutaceae). Nat. Prod. Commun. 14 (9), 1–7.

Ramadwa, T.E., McGaw, L.J., Adamu, M., Madikizela, B., Eloff, J.N., 2021. Anthelmintic, antimycobacterial, antifungal, larvicidal and cytotoxic activities of acetone leaf extracts, fractions and isolated compounds from *Ptaeroxylon obliquum* (Rutaceae). J. Ethnopharmacol. 280, 114365.

Ribeiro, A., Romeiras, M.M., Tavares, J., Faria, M.T., 2010. Ethnobotanical survey in Canhane village, district of Massingir, Mozambique: medicinal plants and traditional knowledge. J. Ethnobiol. Ethnomed. 6, 33–48.

Roussaki, M., Kontogiorgis, C.A., Hadjipavlou-Litina, D., Hamilakis, S., Detsi, A., 2010. A novel synthesis of 3-aryl coumarins and evaluation of their antioxidant and lipoxygenase inhibitory activity. Bioorg. Med. Chem. Lett. 20, 3889–3892.

Saha, K., Lajis, N.H., Israf, D.A., Hamzah, A.S., Khozirah, S., Khamis, S., Syahida, A., 2004. Evaluation of antioxidant and nitric oxide inhibitory activities of selected Malaysian medicinal plants. J. Ethnopharmacol. 92, 263–267.

Schneider, I., Bucar, F., 2005. Lipoxygenase inhibitors from natural plant sources. Part1: medicinal plants with inhibitory activity on arachidonate 5-lipoxygenase and 5-lipoxygenase/cyclooxygenase. Phytother. Res. 19, 81–102.

Singh, N.K., Rao, G.N., 2019. Emerging role of 12/15-Lipoxygenase (ALOX15) in human pathologies. Prog. Lipid Res. 73, 28–45.

Tilg, H., Moschen, A.R., 2006. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. Nat. Rev. Immunol. 6, 772–783.

van Wyk, C., Botha, F., Vleggaar, R., Eloff, J.N., 2018. Obliquumol, a novel compound, isolated from the leaves of sneeze wood (*Ptaeroxylon obliquum*) has excellent activity against Candida albicans and may present a new antifungal scaffold molecule". South Afr. J. Sci. Technol. 37, 1.

Vidya, L., Lenin, M., Varalakshmi, P., 2002. Evaluation of the effect of triterpenes on urinary risk factors of stone formation in pyridoxine deficient hyperoxaluric rats. Phytother. Res. 16, 514–518.

Xie, Q.W., Nathan, C., 1994. Nitric oxide synthases: roles, tolls, and controls. Cell 78 (6), 915–918.