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Research Article

The Anti-Inflammatory and Anti-Nociceptive Activities of Some Medicinal Plant Species Used to Treat Inflammatory Pain Conditions in Southern Africa

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ABSTRACT
The biological activities of 4 medicinal plants (*Acokanthera oppositifolia, Plantago lanceolata, Artemisia vulgaris* and *Conyza canadensis*) were investigated. These plant extracts were subjected to screening for their possible effects as analgesic, and anti-inflammatory agents. The plant materials (only leaves were used in this study) were used in 4 solvents (acetone, ethyl acetate, chloroform and hexane). *Plantago lanceolata* hexane leaf extract inhibited cyclooxygenase-2 (COX-2) activity with an IC_{50} value of 0.41 µg/mL. Quercetin, positive control in this study inhibited COX-2 with a recorded IC_{50} = 8.39 µg/mL. However, the COX-1 inhibition by same extract indicated an IC_{50} of 68.99 µg/mL compared to the positive control (quercetin), whose activity was represented with an IC_{50} value of 4.6 µg/mL. The lipoxygenase assay indicated that *Plantago lanceolata* hexane extract and *Acokanthera oppositifolia* acetone extract were the most active samples with an IC_{50} of 4.75 µg/mL and 7.73 µg/mL. *Plantago lanceolata* hexane extract was the most active in all enzyme inhibitions, revealing the great potential it presents as a source of new anti-oxidative, analgesic and anti-inflammatory drugs, with less adverse effects.

Keywords: *Plantago lanceolata*, *Conyza canadensis*, *Acokantera oppositifolia*, *Artemisia vulgaris*, anti-inflammatory, lipoxygenase, cyclooxygenase-1 and cyclooxygenase-2.

INTRODUCTION

Pain is a common health problem with substantial socioeconomic impact because of its high incidence. It is a symptom characteristic of many diseases. It is estimated that 80-100% of the world's population experience back pain at least once in their life time[1]. The treatment of pain requires analgesics, including anti-inflammatory products. Most of the so-called non-steroidal anti-inflammatory agents also have analgesic activity. Although many analgesics and anti-inflammatory agents are readily available, modern drug therapy is associated with some adverse effects, like gastrointestinal irritation, fluid retention, bronchospasm and prolonged bleeding time[1,2]. Inflammation is a normal, protective response to tissue injury caused by physical trauma, noxious chemicals or microbiological agents[3]. There are many inflammatory pathways including COX and lipoxygenase (LOX) pathways. Cyclooxygenase-1 (COX-1) and COX-2 are prostaglandin synthases catalysing sequential synthesis of prostaglandins G_2 (PGG_2) and PGH_2 from arachidonic acid (AA) through intrinsic COX and peroxidase activities. Products of 12-LOX are associated with various skin diseases (and 15-LOX products are associated with atherosclerosis[4]. The use of herbal medicines is fast becoming more popular due to toxicity and side-effects of allopathic medicines. Medicinal plants play an important role in the development of potent therapeutic agents. There are over 1.5 million practitioners in the traditional medicine system using medicinal plants in preventive, promotional and curative applications[3]. Inflammation is designed to limit invasions and damage after injury, an essential process for the survival of mankind in the absence of medication such as antibiotics. The process of inflammation is a self-limiting and controlled process of the immune system[5]. The broad aim of the study was to investigate effects of extracts of *Acokanthera oppositifolia, Plantago lanceolata, Conyza canadensis* and *Artemisia vulgaris* on the activity of COX-1 and 2, as well as 15-LOX. The selected medicinal plant species are used in traditional medicine to treat and manage pain associated with inflammatory conditions.

MATERIAL AND METHODS

Plant collection

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Leaves of the 4 plant species: *Acokanthera oppositifolia*, *Plantago lanceolata*, *Conyza canadensis* and *Artemisia vulgaris* were used in this study. *Acokanthera oppositifolia* was collected in June 2012 from the Lowveld Botanival Garden, in Nelspruit. A voucher specimen (PRU/120583/AdebayoSA) was deposited in the herbarium of University of Pretoria. *Plantago lanceolata, Artemisia vulgaris, and Conyza canadensis* were collected in the vicinity of Midrand, Johannesburg. Voucher specimens (Moses TUT1, Moses TUT2, and Moses TUT3) were deposited in the Herbarium of Tshwane University of Technology. The leaves were subsequently dried at room temperature in a ventilated room, milled to a fine powder in an atomy mill Polymax (PC-MFC 90 D) and stored in closed containers until used.

Plant extraction

Plant material (5 g) from each species investigated was separately extracted with 20 mL of acetone, ethyl acetate, chloroform, hexane, and water in polyester centrifuge tubes. The tubes were vigorously shaken for 30 min in an orbital shaker (Velp Scientifica). Tubes were centrifuged at 2 000 rpm for 10 min and the supernatant filtered using Whatman No.1 filter paper before being transferred into pre-weighed glass containers. The solvent was removed by evaporation under a stream of air in a fume hood at room temperature to produce the dried extract. The extract was reconstituted in 100% dimethyl sulphoxide (DMSO) (Merck Schuchardt OHG) at 10 mg/mL and tested in the assays.

Cyclooxygenase-1 and cyclooxygenase-2 assays

A total of 18 samples were used in triplicate. The experiment was done following manufacturer's instructions. The COX assay kit was bought from Cayman chemical company, Ann Arbor, MI (USA). The 96-well microtitre plate was covered with plastic film and incubated for 18 hr at room temperature on an orbital shaker. The microtitre plate was developed according to manufacturer's instruction in the dark for 90 min. The plate was read at 420 nm. Inhibition of enzyme activity was calculated using the formula below:

$$\% \text{ inhibition} = 100 - \left(\frac{OD_{sample} - OD_{blank}}{OD_{control}} \times 100\right)$$

15-Lipoxygenase (15-LOX) assay

Anti-lipoxygenase assay was studied using linoleic acid as substrate and 15-LOX as enzyme[6]. Ten microliters (10 µL) of extracts (10 mg/ml) of *Acokanthera oppositifolia, Plantago lanceolata, Artemisia vulgaris* and *Conyza Canadensis*) were mixed with 90 µL of 15-LOX (400 U/mL). The mixture was incubated in dark for 5 min at 25 °C. The reaction was started by addition of 100 µL of linoleic acid solution (0.4 mM) to each well, followed by a 20 min incubation in the dark at 25°C. Then, 100 µL of freshly prepared ferrous orange xylenol (FOX) reagent was added (90% methanol, 10 µM $FeSO_4$, 100 µM xylenol orange, 30 mM H_2SO_4). After a 30 min incubation at 25°C, absorbance was measured at 560 nm. Indomethacin and quercetin were used as reference standards.

Percentage inhibition was calculated using the equation;

$$\% \text{ inhibition} = 100 - \left(\frac{OD_{sample} - OD_{blank}}{OD_{control}} \times 100\right)$$

The percentage inhibition was plotted as a function of the inhibitor concentration to determine IC_{50} value (concentration at which there was 50% inhibition of enzyme activity).

RESULTS AND DISCUSSION

Cyclooxygenase-1 and cyclooxygenase-2 test

Cyclooxygenase group of enzymes (COXs, prostaglandin endoperoxide synthases) catalyse two reactions, the first being a cyclooxygenase function consisting of the addition of molecular oxygen to arachidonic acid to form PGG_2. The second is the conversion of PGG_2 to PGH_2 by a peroxidase function. Hence, COX performs the critical initial reaction in the arachidonic acid metabolic cascade, leading to the formation of pro-inflammatory prostaglandins, thromboxane and prostacyclin. Prostaglandins regulate smooth muscle contractility, blood pressure and platelet aggregation and induce pain and fever. Inhibition of cyclooxygenase activity is the mechanism by which non-steroidal anti-inflammatory drugs (NSAIDs) exert their analgesic, antipyretic, anti-inflammatory, and antithrombotic effects[7]. The constitutive form COX-1 is responsible for the maintenance of physiological prostanoid biosynthesis. In contrast, COX-2 is an inducible isoform linked to inflammatory cell types and tissues. Prolonged use of NSAIDs is also associated with severe side effects such as gastro-intestinal haemorrhage due to COX-1 inhibition[7]. The new COX-2 selective drugs do not seem to be free of risk either since several COX-2 inhibitors are linked with cardiovascular problems[8]. Steroids have an obvious role in the treatment of inflammatory diseases but, due to their toxicity, they can only be used over short periods of time except in very serious. Consequently, there is a strong need for natural products with minimum side effects. *Plantago lanceolata* hexane extract was less active against COX-1 (IC_{50} = 69 µg/mL) and more active against COX-2 (IC_{50} = 1.96 µg/mL). The inhibitions of COX-1 and COX-2 by positive control (quercetin) were represented by IC_{50} of 4.6 and 8.39 µg/mL, respectively (Figures 1 & 2). A therapeutic advantage, in relation to selective COX-2 inhibitors, is the low ulcer toxicity in the gastrointestinal tracts. Vane and Botting (2001)[9] have suggested a parallel relationship between COX-2 selectivity and gastrointestinal side effects with NSAID treatment, such that COX-2 selective compounds cause fewer ulcers[9]. Masferrer *et al.* (1996)[10] demonstrated that administration of COX-2 selective inhibitors did not produce stomach lesions, in contrast to administration of non-selective NSAIDs.

Lipoxygenase assay

The LOX group of enzyme catalyzes dioxygenation of polyunsaturated fatty acids to yield cis, trans-conjugated diene hydroperoxides. Results for LOX inhibitory activity (IC_{50}) values are shown in Figure 2. The highest inhibitory effect was observed with *Artermisia vulgaris* hexane extract and *Plantago lanceolata* hexane extract with an IC_{50} values of 4.75 and 7.73 µg/mL, respectively compared to the positive controls (quercetin IC_{50} = 0.83 µg/mL). The results reported here suggests that *Artemisia vulgaris* and

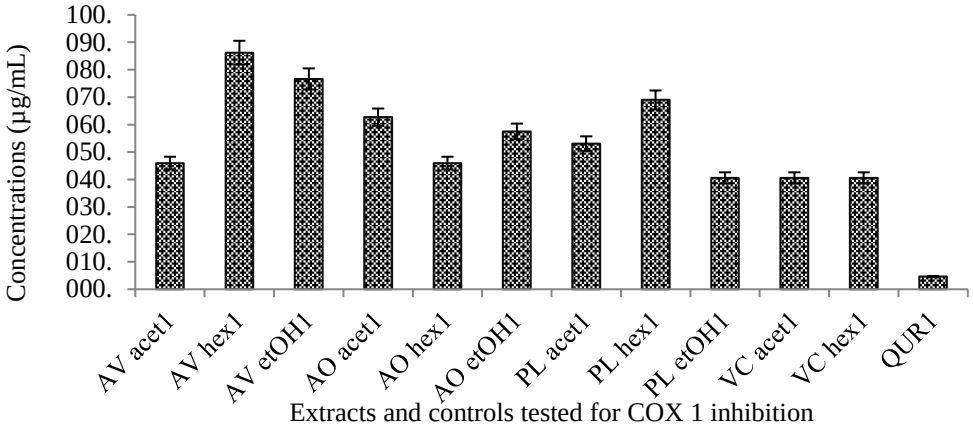

Figure 1: The IC_{50} (µg/mL) values of *Acokanthera oppositifolia*, *Plantago lanceolata*, *Conyza canadensis*, and *Artemisia vulgaris* for COX-1 inhibition. *AV acet1, A. vulgaris* acetone extract; *AV hex1, A. vulgaris n*-hexane extract; *AV etOH1, A. vulgaris* ethylacetate extract; *AO acet1, A. oppositifolia* acetone extract; *AO hex1, A. oppositifolia* hexane extract; *AO etOH1, A. oppositifolia* ethylacetate extract; *PL acet1; P. lanceolata* acetone extract; *PL hex1, P. lanceolata n*-hexane extract; *PL etOH1, P. lanceolata* ethylacetate extract; *VC acet1, C. canadensis* acetone extract; *VC hex1, C. canadensis* hexane extract; QUR, quercetin.

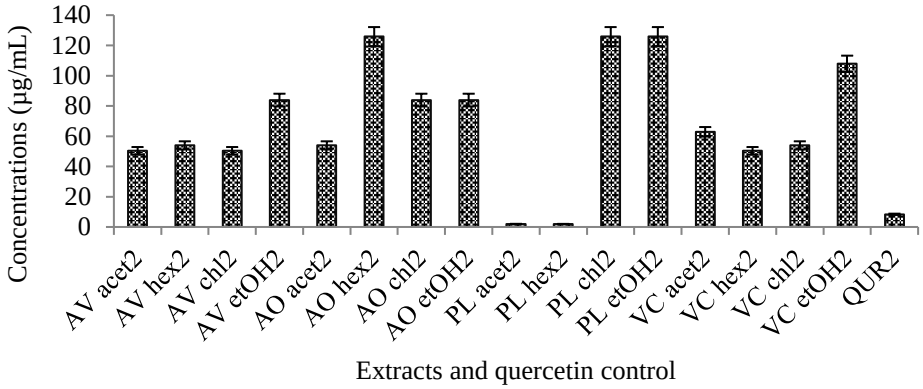

Figure 2: IC_{50} values of *Acokanthera oppositifolia*, *Plantago lanceolata*, *Conyza canadensis*, and *Artemisia vulgaris* tested in COX-2 inhibition. *AV acet2, A. vulgaris* acetone extract; *AV hex2, A. vulgaris n*-hexane extract; *AV chl2, A. vulgaris* chloroform extract; *AV etOH1, A. vulgaris* ethylacetate extract; *AO acet2, A. oppositifolia* acetone extract; *AO hex2, A. oppositifolia* hexane extract; *AO chl2, A. oppositifolia* chloroform extract; *AO etOH2, A. oppositifolia* ethylacetate extract; *PL acet2; P. lanceolata* acetone extract; *PL hex2, P. lanceolata n*-hexane extract; *PL chl2, P. lanceolata* chloroform extract; *PL etOH2, P. lanceolata* ethylacetate extract; *VC acet2, C. canadensis* acetone extract; *VC hex2, C. canadensis* hexane extract; QUR2, quercetin.

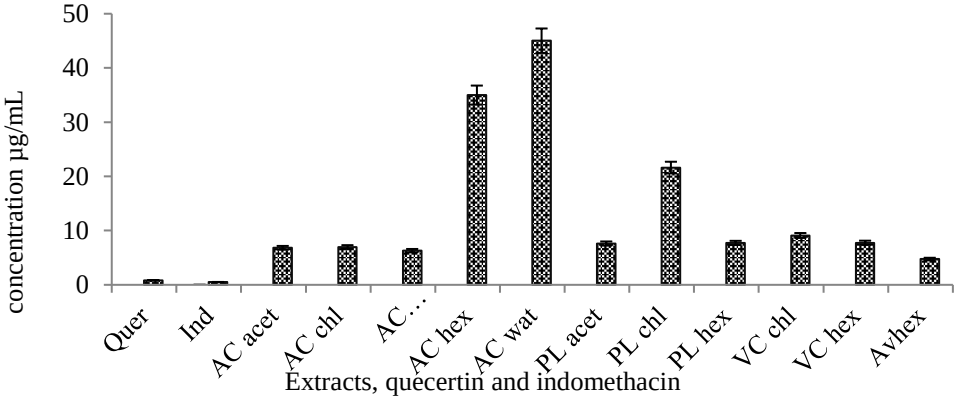

Figure 3: IC_{50} values of lipoxygenase test. Quer, quercetin; Ind, indomethacin; AC acet, *Acokanthera oppositifolia* acetone extract; AC chl, *Acokanthera oppositifolia* chloroform extract; AC etOH2, *Acokanthera oppositifolia* ethyl acetate; AC hex, *Acokanthera oppositifolia* hexane extract; AC wat, *Acokanthera oppositifolia* water extract; PL acet, *Plantago lanceleolata* acetone extract; PL chl, *Plantago lanceleolata* chloroform extract; PL hex, *Plantago lanceleolata* hexane extract; VC chl, *Conyza canadensis* chloroform extract; VC hex, *Conyza canadensis* hexane extract; AV hex, *Artemisia vulgaris* hexane extract.

Plantago lanceolata hexane extracts had promising anti-LOX effect, which might be related to the polyphenolic content and antioxidant property of the extracts. Lipoxygenase plays an important role in the pathophysiology of several inflammatory diseases[11]. Plant derived chemical constituents such as flavonoids, coumarins, quinones, pentacyclic triterpenes, sesquiterpenes, alkaloids and polyacetylates have been reported to inhibit 15-LOX[12]. Lipoxygenases are sensitive to antioxidants and the most of their action may consist of inhibition of lipid hydroperoxide formation due to scavenging of lipidoxy or lipid peroxy-radical formed in course of enzyme peroxidation. This action limits the availability of lipid hydroperoxide substrate necessary for the catalytic cycle of LOX[13]. The results obtained in this study have demonstrated the potential inhibitory effects of hexane extracts of *Plantago lanceolata* on 15-LOX. This is crucial in the search for alternative sources for treatment of inflammatory conditions involving 15-LOX. Chronic inflammation is a pathological condition mediated through production of PGE2 from AA generated by enzyme system PG synthetase, a complex enzyme including COX-2. The enzymatic oxygenation of arachidonic acid via the COX and LOX pathways plays a key role in the mediation of inflammation. As a result, the key enzymes of these pathways COX-1, COX-2, and LOX have become the target for the development of anti-inflammatory drugs[3]. Another group of compounds eliciting inflammatory conditions are leukotrienes which are derived directly from AA by enzymatic action of LOX[14]. For the anti-inflammatory activity, the plant extracts were tested for their anti COX-1 and COX-2 activity, anti-LOX activity, and inhibition of LPS (Lipopolysaccharides). COX-1, an enzyme in the biosynthesis of prostaglandin synthesis, has been extensively used as a tool for studying the anti-inflammatory effects of plant extracts and plant-derived compounds[15]. With the discovery of the iso-enzyme COX-2, whose expression is induced by inflammation mediators, interest in cyclooxygenase inhibitors has grown. The adverse effects observed with traditional nonsteroidal anti-inflammatory drugs (NSAIDs), such as aspirin and indomethacin, are believed to stem from an inhibition of constitutive COX-1 activity, and it is hypothesized that selective COX-2 inhibitors exhibit an improved safety profile. The constitutive form COX-1 is responsible for the maintenance of physiological prostanoid biosynthesis. In contrast, COX-2 is an inducible isoform linked to inflammatory cell types and tissues. Prolonged use of NSAIDs is also associated with severe side effects such as gastro- intestinal haemorrhage due to COX-1 inhibition[7]. The new COX-2 selective drugs do not seem to be free of risk either since several COX-2 inhibitors has been found to cause cardiovascular problems[8]. The Figures 1 and 2 indicate the IC_{50} value of the sample extract used in this study. *Plantago lanceolata* hexane extract indicates the lower IC_{50} in COX-2 inhibition. The IC_{50} value is close to the control (quercetin), indicating that *Plantago lanceleolata* has better inhibitory activity than all other plant species tested. *Plantago lanceolata* hexane extract indicates an IC_{50} 68.99 mg/mL in COX-1 inhibition. This indicates the great interest of the *Plantago lanceolata* hexane extract by the fact that the extract inhibits only the COX-2 enzyme and is without inhibition in COX-1. The biological activity of plant materials was also investigated for the anti-15 LOX activity. Among the 4 plants, *Acokanthera oppositifolia* hexane extract and *Plantago lanceolata* hexane extract with IC_{50} values of 4.75 and 7.73 µg/mL, respectively, were the most active extracts. Lipoxygenases are sensitive to antioxidants as antioxidants are involved in inhibition of lipid hydroperoxide formation due to scavenging of lipidoxy or lipidperoxy radicals. This could lead to less availability of lipid hydroperoxide substrate required for LOX catalysis[16]. Lipoxygenase are the family of the key enzyme in the biosynthesis of leukotrienes which plays an important role in the pathophysiology of several inflammatory diseases. Lipoxygenases are sensitive to antioxidants and the most of their action may consist of inhibition of lipid hydroperoxide formation due to scavenging of lipidoxy or lipidperoxy radicals formed in course of enzymatic peroxidation[17]. *Plantago lanceolata* inhibited the lipoxygenase enzyme. This indicates that *Plantago lanceolata* may have in interest in anti-inflammation studies.

Conclusion and recommendations

The hexane extracts of *Plantago lanceolata* had good inhibitory effects on COX-2 and 15-LOX. The compounds responsible for the observed effects in the extracts are unknown at this stage. Future work will attempt to identify the compounds responsible for the anti-inflammatory effects as well as the mechanisms of their actions.

CONFLICTS OF INTEREST

There is no conflict of interest to be declared.

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