Seasonal variation in the cyclooxygenase inhibitory activities of four South African medicinal bulbs

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Abstract

Due to the critical role of cyclooxygenase (COX) enzymes in the complex process of inflammation, their inhibition has formed part of the basic therapeutics used in the treatment of inflammation in humans. Medicinal plants have been widely explored in the search for remedies for pain-related ailments. The bulb/corm and leaf extracts of four medicinal bulbs, *Tulbaghia violacea*, *Hypoxis hemerocallidea*, *Drimia elata* and *Merwilla plumbea*, commonly used in the treatment of pain-related ailments in South African traditional medicine, were evaluated for their ability to inhibit cyclooxygenase (COX-1 and COX-2) enzymes. The plant materials were collected in spring, summer, autumn and winter seasons, with a view of assessing the dynamics of their medicinal properties in different seasons. The dried plant materials were extracted with petroleum ether (PE), dichloromethane (DCM), 80% ethanol (EtOH) and water. All the PE and DCM extracts (at final concentrations of 0.25 mg/ml) in all the tested plant samples recorded between moderate (40–70%) and high (>70%) COX-1 and COX-2 inhibition levels across all seasons. The ethanol extracts of corms of *H. hemerocallidea* also demonstrated moderate to high inhibitory activity against COX-1 enzyme across all seasons. The ethanol extracts of bulb and leaf samples of *T. violacea* showed selective inhibitory activity for COX-2 enzyme in all the seasons. The highest COX inhibitory levels were recorded against COX-2 from the PE leaf (spring) and bulb (autumn) extracts of *T. violacea*, with both recording 100% inhibitory activity. Thus the material collected in any season can be considered to be similarly effective.

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1. Introduction

Plants cannot escape from the environmental extremes of light, temperature, and drought, nor move to regions with better nutritional conditions, and have thus evolved highly complex mechanisms to integrate physiology and metabolism in order to adapt to the conditions to which they are exposed. Secondary metabolites form an integral component of these adaptive mechanisms. External factors quantitatively affect secondary metabolic processes through their effects on plant development, growth rates and partitioning of metabolites to the secondary metabolite of interest. These factors can also trigger abrupt activation of qualitative changes in secondary metabolite production (Laughlin, 1993; Lommen et al., 2008). Climatic (abiotic) factors often have an especially large influence on the biosynthetic levels and quality of secondary metabolites in plants (Coley, 1987). Since medicinal plant extracts derive their therapeutic effects from these secondary metabolites, an insight into the seasonal dynamics of the pharmacological properties of medicinal plants is basic to the understanding of when to collect them for medicinal use. Coinciding medicinal plant harvesting with maximum biological activity of a particular plant species would serve as a way for the more effective utilisation of plant-based medicine.

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Based on the compelling evidence that COX enzymes are involved in inflammatory processes, these enzymes have since become the research targets for drug development in the treatment of inflammation. Prostanoid biosynthesis is inhibited by nonsteroidal anti-inflammatory drugs (NSAIDs) that are widely prescribed as analgesics and anti-inflammatory agents. Treatment with NSAIDs inhibits the production of prostaglandins and down-regulates inflammation-related pathological symptoms such as pain and swelling. The use of NSAIDs however, results in major changes to the pathophysiological functions of the body leading to some side effects such as gastric and renal ulceration, irritation and bleeding (Botting, 2006; Oshima et al., 1996). It is for these undesirable side effects associated with the use of NSAIDs that research interest into alternative forms of anti-inflammatory agents has been stimulated. Medicinal plants offer a vast biogenic resource base for exploitation in the discovery of innovative anti-inflammatory agents. Several natural plant-derived compounds such as flavonoids, saponins, tannins, alkaloids and essential oils have possess some anti-inflammatory activities (Gurib-Fakim, 2006; Just et al., 1998). Plant-derived natural products are therefore important in the search for anti-inflammatory agents.

In view of this fact, four medicinal bulbs used in the treatment of pain-related ailments in South African traditional medicine were evaluated for their seasonal variation in the inhibition of COX-1 and COX-2 enzymes, which are involved in the biosynthesis of prostaglandins in inflammation processes.

2. Materials and methods

2.1. Plant material

The plant materials used in this study were collected in Summer (December), Autumn (March), Winter (June) and Spring (September) from the University of KwaZulu-Natal Botanical Garden, Pietermaritzburg, South Africa and separated into bulbs and leaves. Voucher specimens (Table 1) were deposited in the University of KwaZulu-Natal Herbarium (NU), Pietermaritzburg. The samples were then dried in an oven at a constant temperature of 50 °C for five days and ground into fine powders.

2.2. Preparation of plant extracts

The samples were sequentially extracted with 20 ml/g of petroleum ether (PE), dichloromethane (DCM), 80% ethanol (EtOH) and water in a sonication bath containing ice for 1 h. The crude extracts were then filtered under vacuum through Whatman No. 1 filter paper and the organic extracts were concentrated in vacuo at 35 °C using a rotary evaporator. The concentrated extracts were subsequently dried at room temperature under a stream of air. Water extracts were freeze dried and kept in airtight containers.

2.3. Cyclooxygenase (COX-1 and COX-2) inhibitory bioassays

The COX inhibitory activity of plant extracts were evaluated against COX-1 and COX-2 as described in Jäger et al. (1996) and Zschocke and Van Staden (2000) respectively. The COX-1 and COX-2 (Sigma-Aldrich) enzymes were activated with a co-factor solution and pre-incubated on ice for 5 min. The enzyme/co-factor solution (60 μl) was added to the sample solution (2.5 μl, 10 mg/ml) and pre-incubated for 5 min at room temperature. To the test samples, [14C] arachidonic acid (20 μl) was added and incubated at 37 °C for 10 min. After incubation, the reaction was terminated by adding HCl (10 μl, 2N). Four controls were included. Two were background in which the enzyme was inactivated with HCl before the addition of [14C] arachidonic acid, and two were solvent blanks. Indomethacin was used as a positive control at a final concentration of 5 μM for COX-1 and 200 μM for COX-2. Organic extracts were evaluated at a final concentration of 0.25 mg/ml and water extract at 2 mg/ml. Percentage inhibition of the extracts was calculated by comparing the amount of radioactivity present in the sample to that in the blank solvent. Results are presented as means±standard errors of two independent experiments; each experiment was done in duplicate.

2.4. Statistical analysis

Data on percentage inhibition activity for each extracting solvent in each plant sample in the four different seasons were arcsine transformed and subjected to one-way analysis of variance (ANOVA) using GenStat 12th edition (VSN

Table 1

| Medicinal plants used in this study and their traditional medicinal uses. |
| :---: |
| **Family** | **Species** | **Voucher number** | **Medicinal uses** |
| Alliaceae | *Tulbaghia violacea* Harv. | NCUBE 04 NU | Bulbs and leaves are used in the treatment of stomach pains, asthma, constipation, oesophageal cancer, tuberculosis, colds and fever, HIV/AIDS opportunistic infections (Hutchings et al., 1996; Crouch et al., 2006; Van Wyk et al., 2009; Klos et al., 2009). |
| Hypoxidaceae | *Hypoxis hemerocallidea* Fisch. Mey & Avé-Lall | NCUBE 01 NU | Plant decoctions have purging effects and boost the immune system. Corms are used for the treatment of inflammation, testicular tumours, urinary complaints, cancer and HIV/AIDS opportunistic infections (Watt and Breyer-Brandwijk, 1962; Crouch et al., 2006). |
| Hyacinthaceae | *Drimia elata* Jacq | NCUBE 03 NU | Bulbs are used to treat urinary infections, diseases of the uterus, pain, feverish colds, coughs, urinary infections and diseases of the uterus (Hutchings et al., 1996; Van Wyk et al., 2009). |
| Hyacinthaceae | *Mervilla plumbea* (Lindl.) Speta | NCUBE 02 NU | Decoctions are used for wound healing, boils, sores, sprains, removing scar tissue, cleaning and rejuvenating the body. Enhances, male potency and libido (Crouch et al., 2006; Van Wyk et al., 2009). |

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International, UK). Significantly different means were separated using the Least Significant Difference (LSD) technique ($P \leq 0.05$) and results presented as means±standard errors.

3. Results and discussion

The percentage inhibition of the COX-1 enzyme by four bulb and four leaf extracts from material collected in different seasons is presented in Table 2. There were no leaf samples for *D. elata* in winter because the plant shed its leaves during this period. The COX inhibitory activity was defined at four levels, with activity below 20% being considered insignificant, 20–40% low, 40–70% moderate, and 70–100% high (Tunón et al., 1995). With the exception of the corm extracts of *H. hemerocallidea* and bulb extracts of *M. plumbea* in autumn, all the PE extracts showed high inhibition (%) levels (>70%) in all seasons. Of the DCM extracts, only extracts from *T. violacea* bulbs and *D. elata* leaves showed high inhibitory activity across all the seasons. All the other DCM extracts displayed moderate to high (>40%) inhibitory activity in all seasons. The highest COX-1 inhibitory activity (99.8%) was recorded from the PE extracts of *T. violacea* bulbs in winter.

In all the extracts that showed some level of inhibition, there was significant variation in this activity from season to season. Although the inhibitory activity of PE and DCM extracts against COX-1 enzyme varied significantly from season to season, the activity fluctuated between moderate to high levels. As an adaptation for survival and growth within changing environmental conditions, plants have evolved different morphological and chemical traits (Wink, 2003). This has resulted in different types and quantities of chemical compounds being produced by plants in different seasons (Ncube et al., 2011). Pharmacological and phytochemical screening of *M. plumbea* bulbs revealed that dichloromethane and hexane extracts have good COX-1 and COX-2 inhibitory properties, owing to the presence of saponins and bufadienolides (Sparg et al., 2002). The concentrations of active compound(s) responsible for the anti-inflammatory activity could therefore fluctuate with seasons.

### Table 2

| Plant species         | Plant part | Extract | Inhibition (%) |   |   |   |   |
|-----------------------|------------|---------|----------------|---|---|---|---|
|                       |            |         | Spring         | Summer | Autumn | Winter |
| *Tulbaghia violacea*  | Bulb       | PE      | 88.5±1.25     | 96.5±1.43 | 91.8±2.61 | 99.8±1.18 |
|                       |            | DCM     | 90.4±2.54     | 95.1±4.60 | 88.7±1.58 | 94.3±3.80 |
|                       |            | EtOH    | 0.00          | 0.00      | 0.00      | 0.00    |
|                       |            | Water   | 0.00          | 0.00      | 0.00      | 0.00    |
|                       | Leaf       | PE      | 94.7±2.02     | 88.2±3.73 | 79.5±3.20 | 81.8±3.74 |
|                       |            | DCM     | 89.1±1.66     | 50.3±5.55 | 88.1±3.92 | 91.4±4.31 |
|                       |            | EtOH    | 25.6±2.22     | 27.3±2.13 | 33.1±1.11 | 29.3±3.50 |
|                       |            | Water   | 0.00          | 0.00      | 0.00      | 0.00    |
| *Hypoxis hemerocallidea* | Corn    | PE      | 90.5±2.89     | 89.3±1.37 | 49.5±2.54 | 87.2±1.58 |
|                       |            | DCM     | 77.4±2.96     | 52.3±4.57 | 78.8±0.71 | 71.6±3.90 |
|                       |            | EtOH    | 66.0±3.32     | 67.7±5.14 | 74.5±4.12 | 86.9±5.97 |
|                       |            | Water   | 0.00          | 0.00      | 0.00      | 0.00    |
|                       | Leaf       | PE      | 77.5±4.12     | 80.2±0.07 | 70.8±1.24 | 76.0±2.91 |
|                       |            | DCM     | 87.9±2.18     | 60.0±2.61 | 70.6±2.37 | 57.2±3.02 |
|                       |            | EtOH    | 12.8±3.60     | 10.8±3.50 | 13.6±4.22 | 8.80±3.11 |
|                       |            | Water   | 0.00          | 0.00      | 0.00      | 0.00    |
| *Drimia elata*        | Bulb       | PE      | 84.6±1.73     | 88.1±1.99 | 73.7±3.12 | 94.1±2.42 |
|                       |            | DCM     | 68.2±2.41     | 59.2±1.93 | 58.0±1.65 | 83.1±1.78 |
|                       |            | EtOH    | 7.10±0.99     | 9.80±3.23 | 3.42±1.03 | 1.67±0.18 |
|                       |            | Water   | 12.2±0.36     | 9.10±2.11 | 11.1±1.21 | 5.12±0.56 |
|                       | Leaf       | PE      | 99.9±4.57     | 90.9±0.30 | 75.4±3.29 | –        |
|                       |            | DCM     | 73.8±4.36     | 70.3±0.88 | 75.5±0.53 | –        |
|                       |            | EtOH    | 1.22±0.14     | 0.00      | 0.00      | –       |
|                       |            | Water   | 0.00          | 0.00      | 0.00      | –       |
| *Merwilla plumbea*    | Bulb       | PE      | 85.0±3.67     | 82.0±2.16 | 68.9±1.40 | 78.2±0.87 |
|                       |            | DCM     | 82.4±4.42     | 83.9±1.35 | 68.3±1.31 | 43.6±2.51 |
|                       |            | EtOH    | 0.00          | 0.00      | 0.00      | 0.00    |
|                       |            | Water   | 0.00          | 0.00      | 0.00      | 0.00    |
|                       | Leaf       | PE      | 90.0±2.20     | 91.1±2.19 | 93.0±1.31 | 83.9±2.16 |
|                       |            | DCM     | 83.3±3.48     | 55.8±2.57 | 85.2±2.31 | 62.1±0.85 |
|                       |            | EtOH    | 0.00          | 3.70±3.95 | 4.10±0.93 | 0.00    |
|                       |            | Water   | 0.00          | 0.00      | 4.11±0.07 | 1.30±0.11 |

Values in a row with different letters are significantly different at $P \leq 0.05$ ($n=4$).

PE = petroleum ether, DCM = dichloromethane, EtOH = 80% ethanol.

COX-1 inhibition (%) by indomethacin at a final concentration of 5 μM was 61.3±2.18%.

Organic extracts were tested at a final concentration of 0.25 mg/ml and water extracts at 2 mg/ml.
Interestingly, however, of all the extracts that showed good activity (moderate to high levels) in this study, the activity was maintained at these levels (>40%) in all seasons. This trend, therefore, may justify the collection and use of these extracts for treatment of pain-related ailments in traditional medicine in any of the seasons.

Although water extracts were tested at a higher concentration (2 mg/ml) than organic extracts (0.25 mg/ml) in this bioassay, all water and ethanol extracts, except for the ethanol extracts of *H. hemerocallidea* corms and *T. violacea* leaves, displayed insignificant to low COX-1 inhibitory activity. The extracts of *H. hemerocallidea* (corm) were the only ethanol extracts that showed moderate to high activity across all the seasons. The low activity shown by the water and ethanol extracts in this study is consistent with other previous findings on similar and other plant species (Gaidamashvili and Van Staden, 2006; Jäger et al., 1996; Sparg et al., 2002). The moderate to high inhibitory activity of PE and DCM extracts towards COX-1, suggest that lipophilic compounds are involved in the enzyme inhibition process. Lipophilic compounds have good resorption through the cell membrane even at lower concentrations compared to polar compounds (Zschocke and Van Staden, 2000). These compounds are seldom found, and if they are, they are often in very minute quantities in water extracts (Tunón et al., 1995). This therefore, explains the high activity of these extracts in spite of the low extract yields obtained when using lipophilic extraction solvents.

Inflammation is a complex pathophysiological process that involves an interaction of a variety of signalling molecules and mediators in a series of enzyme catalysed reactions (White, 1999). Lack of inhibitory activity of the water extracts in the COX-1 inhibitory assay does not necessarily disqualify these extracts as possibly having anti-inflammatory activity. Plant extracts exert their enzyme inhibitory effects through a spectrum of different modes of action and target sites (Capone et al., 2007). It is possible, therefore, that the active compounds in the water extracts could have an effect at alternative sites in the complex inflammation process. A number of other studies with different plant species have reported good COX-1 inhibitory activity from water extracts (Jäger and Van Staden, 2005; Taylor and Van Staden, 2001).

The COX-1 enzyme is expressed constitutively in most tissues and catalyses the production of prostaglandins involved in the prostanoid-mediated physiological functions such as gastric cytoprotection, maintenance of renal homeostasis, and normal platelet functions (Morita, 2002). Findings suggest that COX-1 has an important role in pain processing and sensitisation in the rat spinal cord after surgery (Zhu et al., 2003). Complete COX-1 inhibition is generally associated with some detrimental side effects. Because of these side effects, such as the damage to the gastrointestinal tract, anti-inflammatory agents with high COX-1 inhibitory activity are less desirable (Anderson et al., 1996). Although plants screened in this study showed good COX-1 inhibition, considering these side effects, extracts with moderate activity may be preferable to use rather than those with high activity. Prolonged use of plant extracts with high inhibitory activity may result in the manifestation of the damaging side effects (Smith et al., 1998).

Table 3 shows the COX-2 inhibitory activity of plant extracts. The PE and DCM extracts of both bulb and leaf extracts of all the screened plant extracts showed good inhibitory activity (>40%) in all seasons. However, the PE and DCM bulb extracts of *T. violacea* and *D. elata* and DCM corm extracts of *H. hemerocallidea* displayed high inhibitory levels in all the seasons. Although the inhibition levels varied significantly from season to season, extracts from bulbs of *T. violacea* (PE, DCM, EtOH) and *D. elata* (PE, DCM) showed consistently high (73.7% to 100%) inhibition levels in all seasons.

The highest inhibitory activity was recorded in the PE extracts of *T. violacea* bulbs and leaves with both having 100% inhibition in autumn and spring, respectively. The results for the COX-2 inhibitory activity followed a somewhat similar trend as those of COX-1. Among the ethanol extracts, good COX-2 inhibitory activity was recorded from the *T. violacea* bulb and leaf extracts, with both exhibiting consistently high and moderate activity respectively, across all seasons. All water and a significant number of ethanol extracts showed insignificant activity. In most of the plant species, extracts of the plant material gathered in winter appeared to have lower activity compared to the activity of those gathered in the other seasons. This could have been due to the senescing leaf tissues and the onset of dormancy in the bulbs/corms. The biochemical activity rate, and consequently the production of compounds in plants, decreases drastically during dormancy and tissue senescence (Bidwell, 1974).

The inducible COX-2 enzyme is thought to be responsible for the accumulation of prostaglandins in most acute inflammations (Vane et al., 1998). However, there is increasing evidence that in some tissues such as the brain, reproductive organs (ovaries, uterus), kidney, and placenta, COX-2 is also synthesised at a constant rate, and is responsible for the synthesis of prostanoids responsible for the regulatory and homeostatic functions in these tissues (Hinz and Brune, 2002; Mitchell and Warner, 2006). Anti-inflammatory agents with selective COX-2 inhibition are often more desirable as they attenuate the damaging side effects associated with the inhibition of COX-1. Interestingly, ethanol extracts of both bulbs and leaves of *T. violacea* showed higher inhibitory activity (51.2% to 83.9%) towards COX-2 compared to very low to insignificant levels (0 to 33.1%) for COX-1. The ethanol bulb extracts of *T. violacea* maintained high inhibitory levels (79.7 to 83.9%) in all seasons, while leaf extracts had moderate (51.2 to 63.6%) activity. The two extracts could contribute to the development of remedies with specific COX-2 activity as this remains a considerable challenge. The important pharmacological and biological differences between COX-1 and COX-2 enzymes are attributed to the small differences in their structure. The active site of COX-2 is larger than that of COX-1 (Gieryse et al., 1996). The development of COX-2 specific inhibitors therefore, in part, utilises this characteristic (Habeeb et al., 2001). High COX-2 inhibitory activity in the ethanol extracts of *T. violacea* bulbs and leaves compared to COX-1, may be an indication that the active compound(s) in these extracts are specific targets of the
COX-2 active site. COX-2 selective inhibitors are believed to induce selectivity through interaction with the secondary pocket of COX-2 which is absent in COX-1 (Habeeb et al., 2001). The secondary pocket present in COX-2 has been attributed to the presence of isoleucine (Ile523) in COX-1 relative to the smaller valine (Val523) in COX-2 (Luong et al., 1996). It is therefore, an interaction between the active site chemistry and that of the molecular inhibitor that determines selectivity. Ethanol extracts of *T. violacea* may thus be promising anti-inflammatory agents with a reduced risk of serious gastrointestinal side effects. The active compounds in these extracts may be inhibiting the COX-2 enzyme through this unique secondary pocket site.

### 4. Conclusions

In all plant species screened in this study, the inhibitory activity of both bulb and leaf extracts were comparable, with both plant parts exhibiting good activity (moderate to high levels) in PE and DCM extracts. The results from this study supports the traditional use of the four plant species in the treatment of pain-related ailments such as, gastro-intestinal ailments, stomach ache, wounds, urinary tract infections and cancer. The COX bioassay is an example of a mechanism-based assay that utilises subcellular structures (enzymes) to detect inhibitors of inflammation (Noreen et al., 1998). The actual inflammation process in living tissues is, however, very complex and involves a series of mediators and various other enzymes. In this regard, the *in vitro* effects of an extract should be appreciated as supporting evidence only, since the *in vivo* effects may be complicated by a plethora of chemical, physical and physiological factors. *In vivo* tests are required to validate the effects of these extracts in living organisms. Ethanol extracts of *T. violacea* offer prospects for the development of COX-2 selective inhibitors. Research aimed at identification and possible isolation of the active compound(s) in these extracts, and testing them further in *in vivo* models could be a significant breakthrough in alleviating the detrimental effects of COX-1 inhibition by NSAIDs. Considering the aspect of conservation of these medicinal plants, and in light of the comparable anti-inflammatory

| Plant species           | Plant part | Extract | Inhibition (%) |
|------------------------|------------|---------|----------------|
| *Tulbaghia violacea*   | Bulb       | PE      | 94.3 ± 0.90<sup>a</sup> |
|                        |            | DCM     | 97.9 ± 3.49<sup>b</sup> |
|                        |            | EtOH    | 83.5 ± 3.18<sup>b</sup> |
|                        |            | Water   | 0.00±0.00       |
|                        | Leaf       | PE      | 100±0.00        |
|                        |            | DCM     | 91.5±2.38<sup>e</sup> |
|                        |            | EtOH    | 63.6±2.06<sup>e</sup> |
|                        |            | Water   | 0.00±0.00       |
| *Hypoxis hemerocallidea* | Corm      | PE      | 85.7±1.61<sup>d</sup> |
|                        |            | DCM     | 86.5±0.33<sup>b</sup> |
|                        |            | EtOH    | 0.00±0.00       |
|                        |            | Water   | 12.4±0.56       |
| *Drimia elata*         | Bulb       | PE      | 73.7±2.58<sup>a</sup> |
|                        |            | DCM     | 90.8±1.51<sup>b</sup> |
|                        |            | EtOH    | 5.41±1.25<sup>a</sup> |
|                        |            | Water   | 0.00±0.00       |
| *Merwilla plumbea*     | Bulb       | PE      | 80.1±6.40<sup>c</sup> |
|                        |            | DCM     | 76.1±1.59<sup>c</sup> |
|                        |            | EtOH    | 0.00±0.00       |
|                        |            | Water   | 0.00±0.00       |
|                        | Leaf       | PE      | 85.1±1.00<sup>b</sup> |
|                        |            | DCM     | 78.3±0.27<sup>d</sup> |
|                        |            | EtOH    | 0.00±0.00       |
|                        |            | Water   | 0.00±0.00       |

Values in a row with different letters are significantly different at *P* ≤ 0.05 (*n*=4).

PE = petroleum ether, DCM = dichloromethane, EtOH = 80% ethanol.

COX-2 inhibition (%) by indomethacin at a final concentration of 200 μM was 65.2 ± 2.74%.

Organic extracts were tested at a final concentration of 0.25 mg/ml and water extracts at 2 mg/ml.
activity of their bulb/corn and leaf extracts, leaves may thus substitute for bulbs in the treatment of inflammation ailments. This could offer a practical strategy in the conservation of these medicinal plants from the wild. Harvesting of leaves is considered less destructive than the underground parts, although intensive pruning can affect reproductive performance. Based on these results, both leaves and bulbs/corns of these plants can be harvested for use in the treatment of pain-related ailments irrespective of the season.

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References

Anderson, G.D., Hauser, S.D., McGarity, K.L., Bremer, M.E., Isakson, P.C., Gregory, S., 1996. Selective inhibition of cyclooxygenase (COX-2) reverses inflammation and expression of COX-2 and interleukin 6 in adjuvant arthritis. Journal of Clinical Investigation 97, 2672–2679.
Bidwell, R.G.S., 1974. Plant Physiology. Macmillan, New York.
Botting, R.M., 2006. Cyclooxygenase: past, present and future. A tribute to John R. Van (1927–2004). Journal of Thermal Biology 31, 208–219.
Capone, M.L., Tacconelli, S., Francesco, L.D., Sacchetti, A., Sciulli, M.G., Patrignani, P., 2007. Pharmacodynamic of cyclooxygenase inhibitors in humans. Prostaglandins & Other Lipid Mediators 82, 85–94.
Coley, P.D., 1987. Interspecific variation in plant anti-herbivore properties: the role of habitat quality and rate of disturbance. In: Rorison, I.H., Grime, J.P., Hunt, R., Hendry, G.A.F., Lewis, D.H. (Eds.), Frontiers of Comparative Plant Ecology. Academic Press, London.
Crouch, N., Symmonds, R., Spring, W., Diederichs, N., 2006. Fact sheets for growing popular medicinal plant species. In: Diederichs, N. (Ed.), Commercialising Medicinal Plants. A Southern African Guide. Sun Press, Stellenbosch.
Gaidamashvili, M., Van Staden, J., 2006. Prostaglandin inhibitory activity by lectin-like proteins from South African medicinal plants. South African Journal of Botany 72, 661–663.
Giers, J.K., McDonald, J.J., Hauser, S.D., Rangwala, S.H., Koboldt, C.M., Seibert, K., 1996. A single amino acid difference between cyclooxygenase-1 (COX-1) and -2 (COX-2) reverses the selectivity of COX-2 specific inhibitors. Journal of Biological Chemistry 271, 15810–15814.
Gurib-Fakim, A., 2006. Medicinal plants: traditions of yesterday and drugs of tomorrow. Molecular Aspects of Medicine 27, 1–93.
Habeeb, A.G., Rao, P.N.P., Knaus, E.E., 2001. Design and synthesis of celecoxib and rofecoxib analogues as selective cyclooxygenase-2 (COX-2) inhibitors: replacement of sulfonamide and methylsulfonyl pharmacophores by an azido bioisostere. Journal of Medical Chemistry 44, 3039–3042.
Hinz, B., Brune, K., 2002. Cyclooxygenase-2: 10 years later. The Journal of Pharmacology and Experimental Therapeutics 300, 367–375.
Hutchings, A., Scott, A.H., Lewis, G., Cunningham, A., 1996. Zulu Medicinal Plants. An Inventory. University of Natal Press, Pietermaritzburg.
Jäger, A.K., Van Staden, J., 2005. Cyclooxygenase inhibitory activity of South African plants used against inflammation. Phytochemistry Reviews 4, 39–46.
Jäger, A.K., Hutchings, A., Van Staden, J., 1996. Screening of Zulu medicinal plants for prostaglandin-synthesis inhibitors. Journal of Ethnopharmacology 52, 95–100.
Just, M.J., Recio, M.C., Giner, R.M., Cuéllar, M.J., Mañez, S., Bilia, A.R., Rios, J. L., 1998. Anti-inflammatory activity of unusual lupane saponins from Bupleurum frutescens. Planta Medica 64, 404–407.
Klos, M., Van de Venter, M., Milne, P.J., Traore, H.N., Meyer, D., Oosthuizen, V., 2009. In vitro anti-HIV activity of five selected South African medicinal plant extracts. Journal of Ethnopharmacology 124, 182–188.
Laughlin, J.C., 1993. Effect of agronomic practices on plant yield and antimalarial constituents of Artemisia annua L. Acta Horticulturae 331, 53–61.
Lommen, W.J.M., Bouwmeester, H.J., Schenk, E., Verstappen, F.W.A., Elzinga, S., Struijk, P.C., 2008. Modelling processes determining and limiting the production of secondary metabolites during crop growth: the example of antimalarial Artemisinin produced in Artemisia annua. Acta Horticultrae 765, 87–94.
Luong, C., Miller, A., Barnett, J., Chow, J., Ramesha, C., Browner, M.F., 1996. Flexibility of the NSAID binding site in the structure of human cyclooxygenase-2. Nature Structural Biology 3, 927–933.
Mitchell, J.A., Warner, T., 2006. COX isozymes in the cardio-vascular system: understanding the activities of non-steroidal anti-inflammatory drugs. Nature 5, 75–85.
Morita, I., 2002. Distinct functions of COX-1 and COX-2. Prostaglandins & Other Lipid Mediators 68–69, 165–175.
Ncube, B., Finnie, J.F., Van Staden, J., 2011. Seasonal variation in antimicrobial and phytochemical properties of frequently used medicinal bulbous plants from South Africa. South African Journal of Botany 77, 387–396.
Noreen, Y., Ringbom, T., Perera, P., Danielson, H., Bohlin, L., 1998. Development of a radiochemical cyclooxygenase-1 and -2 in vitro assay for identification of natural products as inhibitors of prostaglandin biosynthesis. Journal of Natural Products 61, 2–7.
Oshima, M., Dinchuk, J.E., Kargman, S.L., Oshima, H., Hancock, B., Kwong, E., 1996. Suppression of intestinal polyposis in Ape delta716 knock out mice by inhibition of cyclooxygenase-2 (COX-2). Cell 87, 803–809.
Smith, C.J., Zhang, Y., Koboldt, C.M., Muhammad, J., Zweifel, B.S., Shaffer, A., Talley, J.J., Masferrer, J.L., Seibert, K., Isakson, P.C., 1998. Pharmacological analysis of cyclooxygenase-1 in inflammation. Proceedings of the National Academy of Sciences 95, 13313–13318.
Sparg, S.G., Van Staden, J., Jäger, A.K., 2002. Pharmacological and phytochemical screening of two Hyacinthaceae species: Sella natalensis and Ledebouria ovatifolia. Journal of Ethnopharmacology 80, 95–101.
Taylor, J.L.S., Van Staden, J., 2001. COX-1 inhibitory activity in extracts from Eucomis L’Herit. species. Journal of Ethnopharmacology 75, 257–265.
Tunón, H., Olavsdotter, C., Bohlin, L., 1995. Evaluation of anti-inflammatory activity of some Swedish medicinal plants. Inhibition of prostaglandin biosynthesis and PAF-induced exocytosis. Journal of Ethnopharmacology 48, 61–76.
Van Wyk, B.-E., Van Oudtshoorn, B., Geracie, N., 2009. Medicinal Plants of South Africa. Briza Publications, Pretoria.
Van, J.R., Bakhle, Y.S., Botting, R.M., 1998. Cyclooxygenase 1 and 2. Annual Review of Pharmacology and Toxicology 38, 97–120.
Watt, J.M., Breyer-Brandwijk, M.G., 1962. The Medicinal and Poisonous Plants of Southern and Eastern Africa. Livingstone, London.
White, M.J., 1999. Mediators of inflammation and inflammatory process. The Journal of Allergy and Clinical Immunology 103, S378–S381.
Wink, M., 2003. Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. Phytochemistry 64, 3–19.
Zhu, X., Conklin, D., Eisenach, J.C., 2003. Cyclooxygenase-1 in the spinal cord plays an important role in postoperative pain. Pain 104, 15–23.
Zwetke, S., Van Staden, J., 2000. Cryptocaryu species-substitute plants for Ocotea bulbata? A pharmacological investigation in terms of cyclooxygenase-1 and -2 inhibition. Journal of Ethnopharmacology 71, 473–478.