

## COX-1 and COX-2 inhibitory activity in extracts prepared from *Eucomis* species, with further reference to extracts from *E. autumnalis autumnalis*

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Prostaglandins are important mediators of the body's response to pain and inflammation, and are formed from essential fatty acids found in cell membranes. This reaction is catalysed by cyclooxygenase, a membrane-associated enzyme occurring in two isoforms, COX-1 and COX-2. Non-steroidal anti-inflammatory drugs (NSAIDs) act by inhibiting the activity of COX. The investigation of *Eucomis* species (a popular South African medicinal plant) for potential anti-inflammatory activity showed high levels of COX-1 and -2 inhibitory activity associated with several species, specifically *E. autumnalis* and *E. humilis*. Further investigation was

conducted using *E. autumnalis* subspecies *autumnalis*, as this is the species that is most widely utilised by traditional healers in southern Africa. The bulb extracts from this species were characterised by higher  $IC_{50}$  values for both COX-1 and COX-2 inhibition than obtained for the leaf and root extracts. The ratio of COX-2/COX-1 inhibitory activity was, however, similar to that of the root extracts, both values being less than one. The leaf extracts showed higher COX-1 than COX-2 inhibitory activity, with a ratio of 1.9. Most traditional remedies utilising *Eucomis*, are prepared from the bulbs (possibly including some root material) of this species.

### Introduction

Inflammatory and related immune responses are normal defence mechanisms essential to health. These responses are, however, potentially harmful and play a major role in diseases such as rheumatoid arthritis and asthma (Hinman 1973). Inflammation is a complex process with many different mediators, including prostaglandins (Campbell 1990). The inflammatory response involves enzyme activation, mediator release, extravasation of fluid (oedema), cell migration, tissue breakdown and repair (Vane and Botting 1995).

Prostaglandins and related eicosanoids (thromboxanes and leucotrienes) are a family of pharmacologically active, acidic lipids produced from cell membranes (Smith 1990). Eicosanoid precursors (usually arachidonic acid) are transformed by one of two pathways in the body, catalysed by either prostaglandin endoperoxide synthetase or the lipoxigenases (Smith 1990). The synthesis of prostaglandins is a key factor in inflammation and pain. The primary enzyme responsible for prostaglandin synthesis is cyclooxygenase (Wallace and Chin 1997), and this enzyme controls the rate-limiting step in arachidonate metabolism (Taketo 1998). The prostaglandin synthetase enzyme complex (cyclooxygenase) is ubiquitous, with activity varying with tissue type, and exhibits both cyclooxygenase and peroxidase activity (Smith 1990).

The cyclooxygenase enzyme occurs in two isoforms,

COX-1 and COX-2 (Goetzl *et al.* 1995). The constitutively expressed COX-1 is present in cells under physiological conditions and produces protective substances for the stomach and kidney. COX-1 is responsible for the production of prostanoids that maintain mucosal blood flow, promote mucous secretion, inhibit neutrophil adherence and maintain renal blood flow (Wallace and Chin 1997). Suppression of COX-1 results in adverse side-effects including a reduction in mucosal blood flow and mucous secretion, delay in the healing of ulcers and a reduction in renal blood flow (Wallace and Chin 1997). In contrast to COX-1, COX-2 is effectively absent in healthy tissue and is induced in migratory and other cells by proinflammatory agents, such as cytokines, mitogens and endotoxins under pathological conditions such as inflammation (Mitchell *et al.* 1994). Both enzymes have similar active sites, although the active site of the COX-2 enzyme is thought to be larger than that of COX-1. This is suggested by the broader fatty acid substrate specificity of COX-2 and the lower relative affinities of NSAIDs for COX-2 (Taketo 1998). With the onset of inflammation or tissue damage, levels of COX-2 increase substantially (Vane and Botting 1995). Prostanoids produced by COX-2 promote oedema, fever and pain (Wallace and Chin 1997).

Non-steroidal anti-inflammatory drugs (NSAIDs) are a large, chemically diverse group of drugs that act by inhibit-

ing the activity of COX (Vane and Botting 1995). This explains both their effectiveness (in the inhibition of COX-2) and their side effects, manifest in gastrointestinal bleeding and perforation and renal damage (in the inhibition of COX-1) (Mitchell *et al.* 1994).

Aspirin, indomethacin and ibuprofen have been found to be more potent inhibitors of COX-1 than COX-2 in several model test systems. The relative potencies of aspirin and indomethacin vary slightly between models although the  $IC_{50}$  values are different (Mitchell *et al.* 1994). Research has, for the past fifty years, focused on the development of NSAIDs that show reduced toxicity in the gastrointestinal tract and kidney. This is complicated by the mechanistic link between the beneficial and adverse effects of NSAIDs, both of which relate to the ability of the drug to inhibit prostaglandin synthesis (Wallace and Chin 1997). The aim is to develop highly selective COX-2 inhibitors which cause less gastrointestinal damage. A limited number of selective COX-2 inhibitors of natural origin have been isolated (Noreen *et al.* 1998). Investigation of plant extracts in terms of COX-2 inhibition is thus of primary importance, considering the potential value of such compounds.

Plants have been selected and used empirically as drugs for centuries, initially as traditional preparations then as pure active principles (Kamil 1993). Natural products and their derivatives represent more than 50% of all the drugs in clinical use today with 25% originating from higher plants (Balandrin *et al.* 1993). The estimated 500 000 higher plant species known thus form a vast potential pool for the further investigation and exploration of plant products for future use in medicine. Furthermore, the ethnobotanical approach to drug discovery shows great potential for the discovery of new drugs in the fields of dermatological, inflammatory and gastrointestinal illnesses, as these are the most commonly treated ailments in developing countries (Cotton 1996). Testing extracts prepared from widely utilised medicinal plants has become a rapidly growing and successful field in pharmacological science.

*Eucomis* L'Herit (Family Hyacinthaceae) are deciduous geophytes with long, narrow leaves and erect, densely packed flower spikes (Baker 1897, Compton 1990). The bulbs are popular in traditional medicine for the treatment of a variety of ailments, commonly those with underlying symptoms of pain and inflammation (Hutchings *et al.* 1996, Van Wyk *et al.* 1997). An initial screening of the different species for COX-1 and COX-2 inhibitory activity was conducted using the bulb extracts (ethanol). The investigation then focussed on the study of the anti-inflammatory activity (both COX-1 and -2) of plant extracts prepared from the leaves, bulbs and roots, of the heavily utilised species *Eucomis autumnalis* Mill. (Chitt) (subspecies *autumnalis*) (Reyneke 1980). In this study, the relative inhibitory effects of different extracts on the activities of purified cyclooxygenase enzyme preparations (COX-1 in sheep seminal vesicles, COX-2 in sheep placenta) were assessed. *Eucomis autumnalis autumnalis* was chosen as the test species due to its availability and the widely reported usage of the bulbs in traditional medicine from the Zulu, Xhosa and Sotho cultures.

## Materials and Methods

### Plant material

Leaf, bulb and root material was obtained from specimens of *Eucomis* growing in the Botany Department Garden greenhouses (University of Natal, Pietermaritzburg). Voucher specimens (Taylor01-11) were prepared and submitted to the University of Natal Herbarium. Harvested material (summer) was divided into leaves, bulbs and roots. The plant material was cut up, placed in paper bags and dried at 50°C for three days. Dried material was finely ground and extracted in a sonication bath (for the crude ethanol extract) or using a Soxhlet apparatus (serial extraction). Extracts were filtered through Whatman No. 1 filter paper and dried under vacuum at 35°C. The residue was resuspended in the extracting solvent at 10mg ml<sup>-1</sup>.

### Determination of $IC_{50}$ values

A dilution series was prepared for the calculation of the  $IC_{50}$  values of the extracts. These were calculated by regression analysis of the results for four different concentrations of the sample. The  $IC_{50}$  value for indomethacin was determined to be 3.1µM for the COX-1 assay and 188µM for the COX-2 assay, giving a COX-2/COX-1 ratio of 61. This correlates well with the value of 60 recorded in the literature (Vane and Botting 1995, Taketo 1998).

### Serial extraction (Soxhlet)

Dried ground plant material (10g) was placed in a thimble and covered with glass wool. The solvent (150ml) was heated to boiling and maintained for 2.5h. The extraction solution was filtered through Whatman No. 1 filter paper and dried under vacuum at 35°C. The residue was weighed and resuspended at 10mg ml<sup>-1</sup> (a concentration of 250µg ml<sup>-1</sup> in the assay) This procedure was followed for the solvents hexane, ethyl acetate and ethanol.

### Enzyme assays

The basic protocol for the COX-1 and COX-2 assays is the same, allowing a comparison of the inhibitory effects of the extracts on the two enzymes.

The COX-1 assay was performed according to White and Glassman (1974), modified by Jäger *et al.* (1996). The COX-1 enzyme was prepared from sheep seminal vesicles homogenised in potassium phosphate buffer with 1mM EDTA (on ice). The homogenate was centrifuged and the cell debris discarded. The microsomes were isolated by centrifugation of the supernatant at 100 000g for 1h. The microsomal pellet was resuspended in 0.1M K-Pi (pH 7.4) and the enzyme concentration determined by a standard protein assay. Standardised aliquots were stored at -70°C.

Ten microlitres of the standardised enzyme preparation, and 50µl of co-factor solution (per sample) were preincubated for 15min on ice. This solution (60µl) was added to the test solution (2.5µl plant extract + 17.5µl water) and preincubated for 5min at room temperature. <sup>14</sup>C-Arachidonic acid

(20 $\mu$ l) was added to this enzyme-extract mixture and incubated for exactly 8 min in a water bath at 37°C. The reaction was terminated with 10 $\mu$ l 2N HCl.

The COX-2 assay described by Noreen *et al.* (1998), with minor modifications, was followed. Purified COX-2 enzyme (isolated from sheep placental cotyledons) was purchased from Cayman Chemicals. The enzyme (3 units) was activated with 50 $\mu$ l co-factor solution on ice (5min). The enzyme solution (60 $\mu$ l) and sample (2.5 $\mu$ l ethanol extract + 17.5 $\mu$ l water) were preincubated for 5min at room temperature. The reaction was initiated with the addition of 20 $\mu$ l <sup>14</sup>C-arachidonic acid and the solutions were incubated for exactly 10 min in a water bath at 37°C. The reaction was terminated with 10 $\mu$ l 2N HCl.

### Controls

In each test four controls were run (2.5 $\mu$ l ethanol + 17.5 $\mu$ l water). Two were backgrounds in which the enzyme was inactivated with HCl before the addition of <sup>14</sup>C-arachidonic acid, and which were kept on ice during the assay, and two were solvent blanks. In addition, indomethacin standards (5  $\mu$ M for the COX-1 assay and 200 $\mu$ M for the COX-2 assay) were included in each test. A further standard included in the COX-2 assays was nimesulide (200 $\mu$ M)

### Separation of prostaglandins

Unlabelled prostaglandin carrier solution (4 $\mu$ l per sample) was added to the reaction mixture and <sup>14</sup>C-prostaglandins synthesised in the assay were separated from the unmetabolised arachidonic acid by column chromatography using silica columns. Silica gel (Kieselgel 60, Korngrösse 0.063–0.200mm, 70–230 mesh ASTM) in eluent 1 was packed to a height of 3cm in Pasteur pipettes stoppered with glass wool. The assay mixture was applied to the column with 1ml eluent 1. This was followed by an additional 4ml eluent 1 to elute the unreacted arachidonic acid, which was then discarded. The prostaglandins were then eluted into scintillation vials using 3ml eluent 2. Scintillation fluid (4ml) was added and the radioactivity was counted after 1h in the dark, using a Beckman LS3801 scintillation counter.

### Solutions prepared for assays

- Co-factor solution (COX-1): 0.003g L-adrenalin/ l-epinephrine and 0.003g reduced glutathione in 10ml 0.1 Tris buffer, pH 8.2
- Co-factor solution (COX-2): 0.006g L-adrenalin/ l-epinephrine (0.9mM) and 0.003g reduced glutathione (0.49mM) and 1 $\mu$ M hematin in 10ml 0.1 Tris buffer, pH 8.0
- <sup>14</sup>C-Arachidonic acid: 16 Ci/mole, 3mM
- Prostaglandin carrier solution: 0.2mg ml<sup>-1</sup> of unlabelled prostaglandins (PGE<sub>2</sub>:PGF<sub>2</sub> in the ratio 1:1)
- Eluent 1: hexane:1,4-dioxan:acetic acid (350:150:1 v/v/v)
- Eluent 2: ethyl acetate:methanol (85:15 v/v)

### Calculation of inhibition

The percentage inhibition of the extracts was obtained by

measuring the amount of radioactivity in the solutions relative to that of the solvent blank (untreated sample). The radioactivity of the control (background) was subtracted from both the sample and the blank. Statistical analyses were based on the results obtained from 3 different tests. One-way ANOVA and Tukey HSD tests were performed using Minitab Xtra (version 10.52).

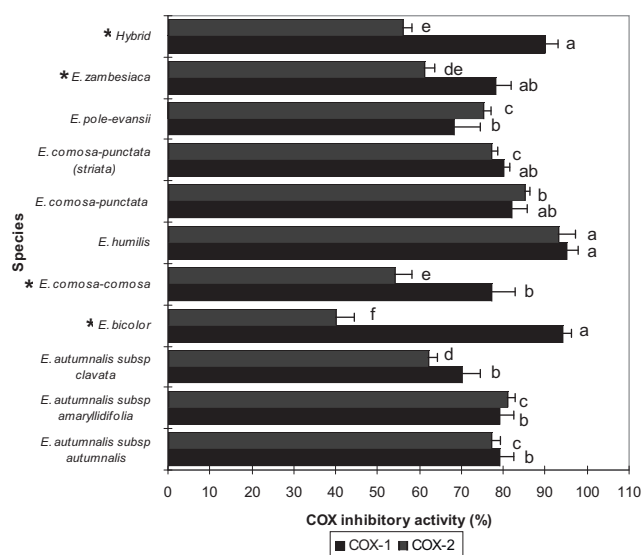
### Method alterations and comment

The addition of co-factors to the enzyme preparation is necessary for the efficient conversion of arachidonic acid to the prostaglandin products by COX-1 and COX-2 (Noreen *et al.* 1998). Reduced glutathione stimulates the biosynthesis of prostaglandin E<sub>2</sub> from arachidonic acid in sheep vesicular microsomes (Raz *et al.* 1976). The purified protein, in the presence of excess reducing substrate, converts arachidonic acid to PGE<sub>2</sub>. In the absence of reducing substrates, prostaglandin synthase rapidly undergoes inactivation by low levels of hydroperoxide. This inactivation during enzymatic turnover is related to the peroxidase activity of the enzyme (Markey *et al.* 1987). The formation of PGE<sub>2</sub> (as well as PGD<sub>2</sub>) increases in the presence of l-epinephrine (Noreen *et al.* 1998). Since PGE<sub>2</sub> is an important mediator of inflammation, high yields of this prostaglandin in the control reaction (solvent blank) increase the efficiency of the assay. The inclusion of a pre-incubation step of the substrate and enzyme (Zshocke pers. comm.), before the addition of the substrate, arachidonic acid, is necessary to detect the presence of time dependent inhibitors (which include indomethacin) of both COX-1 and COX-2 (Quellet and Percival 1995).

### Results and Discussion

A comparison of the COX-1 and COX-2 inhibitory activity from the different *Eucomis* bulb extracts showed that seven species (*E. autumnalis autumnalis*; *E. autumnalis amaryllidifolia*; *E. autumnalis clavata*; *E. humilis*; *E. comosa-punctata (striata)*; *E. comosa-punctata* and *E. pole-evansii*) exhibited almost equipotent COX-1 and COX-2 inhibition, while the remaining four species (*E. bicolor*; *E. comosa-comosa*; *E. zambesiaca* and the hybrid) showed higher levels of COX-1 inhibitory activity (Figure 1). High levels of anti-inflammatory activity were associated with the extracts from *E. humilis*, *E. comosa-punctata (striata)*, *E. pole-evansii*, *E. comosa-punctata* and *E. autumnalis* (subspecies *amaryllidifolia* and *autumnalis*). These are among the commonly utilised medicinal plant species in southern Africa. This is thus a highly significant result in terms of the pharmacological evaluation of the genus, and provides sufficient evidence both to validate the use of this plant in traditional medicine and to warrant further investigation into the nature of the active principle(s).

Previous studies have shown high levels of COX-1 inhibitory activity in ethanol extracts prepared from the dried leaves, bulbs and roots (screened at 250 $\mu$ g ml<sup>-1</sup>) for the various *Eucomis* species, while aqueous extracts (screened at 500 $\mu$ g ml<sup>-1</sup>) showed much lower levels of activity (Taylor and Van Staden 2001). In general, the highest levels of anti-



**Figure 1:** The percentage inhibition of the COX-1 / COX-2 enzymes by bulb extracts (ethanol) of various *Eucomis* species. Screening concentration for crude extracts was 250µg ml<sup>-1</sup>. Error bars represent the S.E., bars bearing different letters are significantly different, P ≤ 0.05. Species marked with \* have significantly different values for COX-1 and COX-2 inhibition, P ≤ 0.05

inflammatory activity were observed for the ethanol bulb and root extracts.

A study of the COX-2 inhibitory activity from the different plant parts of *E. autumnalis autumnalis* (Figure 2) shows that the crude extracts from the leaves and roots have significantly higher activity than the bulb extracts. This suggests that the leaves could serve as a viable and sustainable alternative to the more destructive use of bulb and root material, opening up possibilities for cultivation and thus conservation of the genus.

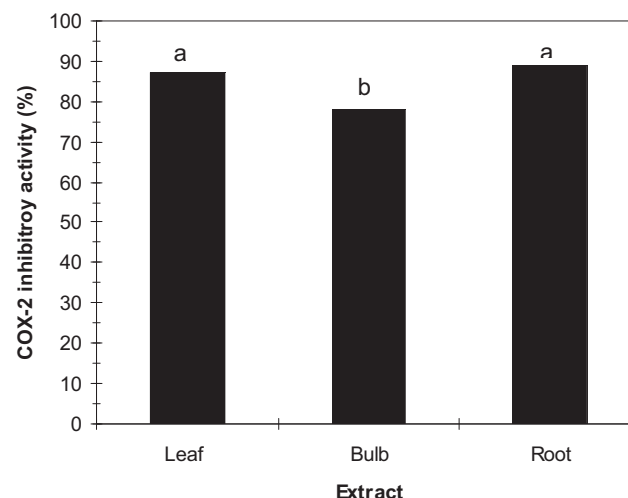
Dose-response curves were drawn (Figure 3) for the crude ethanol extracts of the leaves, bulbs and roots of *E. autumnalis autumnalis* for both assays. From this data, the IC<sub>50</sub> values were calculated by regression analysis (Table 1).

A low COX-2/COX-1 ratio indicates a preferential COX-2 inhibitor, which is valuable pharmacologically. All parts of the plant have reported medicinal usage (Hutchings *et al.* 1996, Roberts 1990), and these results further corroborate previous studies based on the COX-1 inhibitory activity (Taylor and Van Staden 2001).

Serial extraction of the leaf, bulb and root material of *E. autumnalis autumnalis* was thus conducted and the inhibito-

**Table 1:** IC<sub>50</sub> values for COX inhibition by the crude ethanol extracts from the leaves, bulbs and roots of *E. autumnalis autumnalis*

Extract	IC <sub>50</sub> values (µg ml <sup>-1</sup> )		COX-2/COX-1 Ratio
	COX-1	COX-2	
Leaf	15	29	1.9
Bulb	72	55	0.8
Root	27	18	0.7
Indomethacin (µm)	3.1	188	61



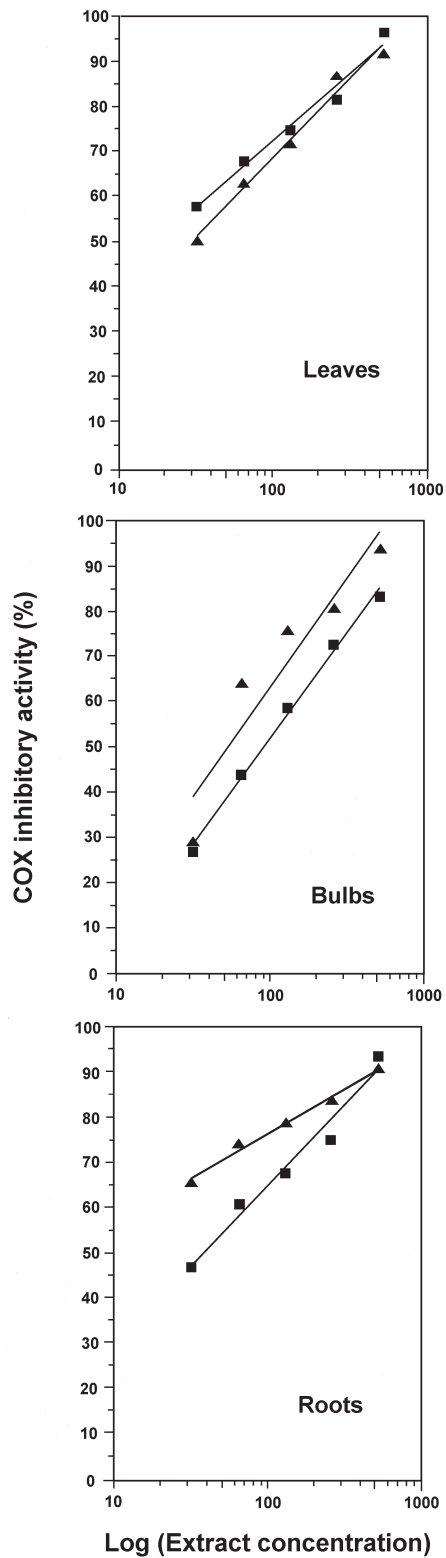
**Figure 2:** The % inhibition of the COX-2 enzyme by ethanol extracts of the leaves, bulbs and roots of *E. autumnalis autumnalis*. Screening concentration of the extracts was 250µg ml<sup>-1</sup>. Bars bearing different letters are significantly different, P ≤ 0.05

ry activity of these extracts compared for COX-1 and COX-2 inhibitory activity (Figure 4).

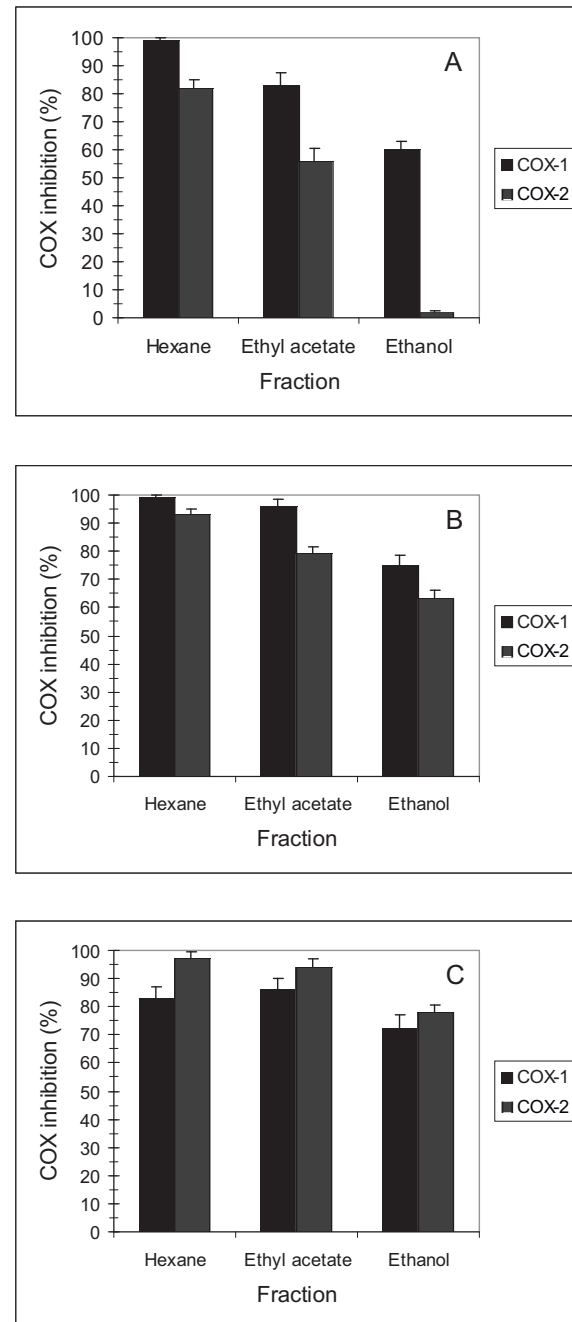
For the leaf extracts, the COX-1 inhibitory activity was significantly higher than that of the COX-2, with no inhibition of the COX-2 enzyme by the ethanol fraction (Figure 4A). The trend of higher COX-1 than COX-2 inhibition was continued in the bulb extracts (Figure 4B), but was reversed for the root extracts (Figure 4C). The difference between the COX-1 and COX-2 inhibition levels in the bulb and root extracts was much less than for the leaf extracts. This pattern of inhibition differs from that of the crude ethanol extracts which show higher COX-2 than COX-1 inhibition for both the bulb and root extracts. This implies that there are different (multiple) compounds, present in the various plant parts tested, that are responsible for the anti-inflammatory of the respective extracts, which leads to the different levels of anti-inflammatory activity in the different solvent extracts.

The screening of plant extracts is motivated by the potential discovery of new drugs that could be developed for use later in clinical medicine. This procedure has the potential of uncovering unexpected chemical structural types as active principles under test conditions. These can then serve as starting points for further elaboration into suitable drugs (Burger 1982). The screening of *Eucomis* extracts for COX inhibitors has revealed the great potential that this genus shows for the discovery of COX-1 and more importantly, selective COX-2 inhibitors.

The ratio of COX-2/COX-1 inhibitory activities for the crude bulb and root extracts were less than one, indicating the preferential COX-2 inhibitory activity of these extracts, which further validates the use of these particular plants in traditional medicine. Even though extracts prepared from *Eucomis* plants, especially the crude extracts, are not high-



**Figure 3:** Regression analyses of the crude extracts (ethanol) prepared from the leaves, bulbs and roots of *E. autumnalis autumnalis*, tested in the COX-1 (n) and COX-2 (t) assays



**Figure 4:** The relative inhibition of COX-1 and COX-2 enzymes by the hexane, ethyl acetate and ethanol fractions from the serial extraction of *E. autumnalis autumnalis* (A) leaves, (B) bulbs and (C) roots. Screening concentration of extracts was  $250\mu\text{g ml}^{-1}$ . Error bars represent the S.E.

ly specific for COX-2 inhibition, the relatively low IC<sub>50</sub> values for COX-2 inhibitory activity makes these extracts valuable medicinal preparations and emphasises the potential that traditional remedies show for the discovery of new drugs. These preparations, which have been utilised for centuries as drugs, show potential pharmacological value that certainly warrants further research to isolate and identify potentially potent COX-1 and/or COX-2 inhibitors. This must then be correlated with clinical trials to ensure the bioavailability of the compounds as potential drugs.

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## References

- Baker (1897) *Eucomis* L'Herit. Flora Capensis VI: 475–478
- Balandrin MF, Kinghorn AD, Farnsworth NR (1993) Plant-derived natural products in drug discovery and development. An overview. In: Kinghorn AD, Balandrin MF (eds) *Human Medicinal Agents from Plants*. ACS Symposium Series 534. American Chemical Society, Washington, USA, pp 2–12. ISBN 0–8412–2705–5
- Burger A (1982) Approaches to screening compounds for pharmacological activity. In: Hamner CE (ed.) *Drug development*. CRC Press, Boca Raton (Florida), USA, pp 65–73. ISBN 0–8493–6310–1
- Campbell WB (1990) Lipid-derived autocooids: Eicosanoids and platelet activating factor In: Gilman AG, Rall TW, Nies AS, Taylor P (eds) *The Pharmacological Basis of Therapeutics*, 8th Edn. Pergamon Press, New York, USA, pp 600–617
- Compton J (1990) *Eucomis* L'Heritier. *Plantsman* 12: 129–139
- Cotton CM (1996) *Ethnobotany. Principles and Applications*. Wiley and Sons, Chichester, UK, pp 242–348. ISBN 0 47195537 X
- Goetzl EJ, An S, Smith WL (1995) Specificity of expression and effects of eicosanoid mediators in normal physiology and human diseases. *FASEB* 9: 1051–1058
- Hinman JW (1973) Round table discussion on inflammation. In: Kahn RH, Lands WEM (eds) *Prostaglandins and Cyclic AMP. Biological actions and clinical applications*. Academic Press, New York, USA, pp 207–210. ISBN 0–12–3944503
- Hutchings A, Scott AH, Lewis G, Cunningham AB (1996) *Zulu Medicinal Plants. An Inventory*. University of Natal Press, pp 42–43. ISBN 0 86980 923 7
- Jäger AK, Hutchings A, Van Staden J (1996) Screening of Zulu medicinal plants for prostaglandin-synthesis inhibitors. *Journal of Ethnopharmacology* 52: 95–100
- Kamil M (1993) Biological and pharmacological implications of flavonoids — A review. In: Govil JN, Singh VK, Hashmi S (eds) *Medicinal Plants: New vistas of research (Part 2)*. Today & Tomorrow's Printers and Publishers, New Delhi, India, pp 377–389. ISBN 1–55528–273–3
- Markey CM, Alward A, Weller PE, Marnett LJ (1987) Quantitative studies of hydroperoxide reduction by prostaglandin synthase. Reducing substrate specificity and the relationship of peroxidase to cyclooxygenase activities. *The Journal of Biological Chemistry* 262: 6266–6279
- Mitchell JA, Akarasereenont P, Thiemermann C, Flower RJ, Vane JR (1994) Selectivity of nonsteroidal anti-inflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. *Proceedings of the National Academy of Science of the United States of America* 90: 11693–11697
- 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
- Quellet M, Percival MD (1995) Effect of inhibitor time-dependency on selectivity towards cyclooxygenase isoforms. *Biochemistry Journal* 306: 247–251
- Raz A, Schwartzman M, Kenig-Wakshal R (1976) Chemical and enzymatic transformations of prostaglandin endoperoxides: Evidence for the predominance of the 15-hydroperoxyl pathway. *European Journal of Biochemistry* 70: 89–96
- Reyneke WF (1980) Three subspecies of *Eucomis autumnalis*. *Bothalia* 13: 140–142
- Roberts M (1990) *Indigenous Healing Plants*. Creda Press, Cape Town, South Africa, pp 251. ISBN 1–86812–317–0
- Smith JB (1990) Prostaglandins and related eicosanoids. In: DiPalma JR, DiGregorio GJ (eds) *Basic Pharmacology in Medicine*, 3rd Ed. McGraw-Hill Publ, New York, USA, pp 181–192. ISBN 0–07–017013–4
- Taketo MM (1998) Cyclooxygenase-2 inhibitors in tumorigenesis (Part I). *Journal of the National Cancer Institute* 90: 1529–1536
- Taylor JLS, Van Staden J (2001) COX-1 inhibitory activity in extracts from *Eucomis* L'Herit. Species. *Journal of Ethnopharmacology* 75: 257–265
- Vane JR, Botting RM (1995) New insights into the mode of action of anti-inflammatory drugs. *Inflammation Research* 44: 1–10
- Van Wyk B-E, Van Oudtshoorn B, Gericke N (1997) *Medicinal Plants of South Africa*. Briza Publications, Pretoria, South Africa, pp 8–22, 130. ISBN 1–875093–09–5
- Wallace JL, Chin BC (1997) New generation NSAIDs: The benefits without the risks? *Drugs of Today* 33: 371–378
- White HL, Glassman AT (1974) A simple radiochemical assay for prostaglandin synthetase. *Prostaglandins* 7: 123–129