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Anti-inflammatory, antioxidant, anti-cholinesterase activity and mutagenicity of South African medicinal orchids

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ABSTRACT

While the role of various processes in inflammatory-related degenerative disorders is still being researched, many avenues of research have concentrated on the treatment and/or prevention of these disorders. Inflammatory-responses, the cholinergic system and oxidative stress have often been linked to the symptoms prevalent in aged persons and Alzheimer's patients. The current research explored the selective inhibition of cyclooxygenase (COX) enzymes, antioxidant and anti-cholinesterase activities of selected South African orchid extracts, currently traded in herbal markets along the east coast of South Africa. Out of a total of 53 evaluated extracts, significant anti-inflammatory activity was observed in nearly 40% of extracts in the COX-1 assay and 25% of extracts in the COX-2 assay. Overall, the DCM root extract of *Ansellia africana* was the most potent, the DCM tuber extract of *Eulophia hereroensis* was the only extract to significantly inhibit both COX enzymes, while all *Bulbophyllum scaberulum* organic root extracts exhibited COX-2 selective inhibitory activity. *Bulbophyllum scaberulum* DCM root extract was also the most effective anti-cholinesterase extract, performing better than galanthamine. In the single electron transfer (SET) (2,2′-diphenylpicrylhydrazyl (DPPH) free radical scavenging assay and ferric reducing antioxidant power (FRAP) reaction based assays, *E. petersii* pseudobulb and *A. africana* root extracts performed better than other extracts. In the hydrogen atom transfer (HAT) (β-carotene/linoleic acid assay) reaction-based assay, the leaf extract of *Tridactyle tridentata* and root extracts of *Cyrtorchis arcuata* and *E. hereroensis* exhibited the best antioxidant effects. The mutagenicity (*Salmonella*/microsome assay) was also determined. Organic leaf and root extracts of *C. arcuata* produced no genotoxic effects in comparison to the other tested species. None of the crude extracts tested demonstrated mutagenic effects using *S. typhimurium* strain TA98 with metabolic activation. The results obtained in this study validate the use of certain orchid species in South African traditional medicine for inflammation-related degenerative disorders.

1. Introduction

Since the detection of prostaglandins (PGEs) in the brain, the function, distribution and expression of all constituents including cyclooxygenase (COX) enzymes in the inflammatory process have been researched (Kaufmann et al., 1997). While a more comprehensive understanding is needed, the role of anti-inflammatory agents in the prevention or treatment of Alzheimer's has been the focus of current research (Moore and O'Banion, 2002). According to Rich et al. (1995) and DeKosky (2003) certain non-steroidal anti-inflammatory drugs (NSAIDs) when administered over a long time exhibited a decreased risk of Alzheimer's. The varying effects of different classes of anti-inflammatory agents in different trials suggest the use of anti-inflammatories as a preventative measure and not as a treatment (DeKosky, 2003). Montine et al. (1999) demonstrated that while COX activity in persons susceptible to Alzheimer's was comparable to that of control persons; concentration levels of PGE_2 in cerebrospinal fluid of susceptible patients had increased five times. Their study demonstrated the potential of COX-inhibitors in the treatment of Alzheimer's. By selectively inhibiting the inducible COX-2, one could possibly produce an efficient and tolerable anti-inflammatory regime that could be used to treat symptoms of common inflammatory disorders such as pain and fever; and that could be used to prevent the onset and/or treat symptoms of diseases affecting the CNS.

According to Borovikova et al. (2000) there is an established link between the cholinergic system and inflammation, with acetylcholine (ACh) playing a role in cytokine release. Tabet (2006) reported on evidence that acetylcholinesterase (AChE) inhibitors have an anti-inflammatory role by indirectly increasing the production of antioxidants in the brain, thereby acting against free radicals, amyloid toxicity

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and a reduction in release of cytokines from activated microglia in the brain and blood. Oxidative stress has been associated with several degenerative disorders; which include cancer, arteriosclerosis, inflammatory disorders and neurodegenerative diseases. Processes such as lipid peroxidation have been implicated in neurodegenerative diseases such as Alzheimer's (Markesbury and Carney, 1999). The ageing process has also been associated with oxidative stress (Markesbury and Carney, 1999). Tabet (2006) also suggested that the anti-inflammatory action of antioxidant compounds has a positive effect in Alzheimer's patients. Selkoe (2005) suggested the use of antioxidants and free radical scavengers as possible treatment options for certain features of Alzheimer's.

Natural plant products have been used as poultices and/or anti-inflammatories and as antioxidants for years. Bohlin (1995) listed flavonoids, naphthoquinones, alkylamides, phenolic phenyl-propane derivatives, among others, as those compounds responsible for COX inhibition in certain natural products. In the southern African region approximately 494 orchid species occupy restricted distribution ranges, with 75% endemism. From the literature, it has been ascertained that approximately 14 South African orchid species from 7 genera are used for medicinal purposes in the practice of South African traditional medicine (Chinsamy et al., 2011). Following the review which revealed the pharmacological potential for South African medicinal orchid species, this research article demonstrates the mutagenicity and validity of medicinal use of certain South African orchid species for inflammatory-related degenerative disorders.

2. Materials and methods

2.1. Plant selection and extract preparation

Various plant parts of seven indigenous orchid species; *Ansellia africana* Lindl. (MC 01NU), *Bulbophyllum scaberulum* (Rolfe) Bolus (MC 02NU), *Cyrtorchis arcuata* (Lindl.) Schltr. (MC 03NU), *Eulophia hereroensis* Schltr. (MC 04NU), *Eulophia petersii* (Rchb.f.) Rchb.f. (MC 05NU), *Polystachya pubescens* (Lindl.) Rchb.f. (MC 06NU) and *Tridactyle tridentata* (Harv.) Schltr. (MC 07NU) were purchased from herbal markets in Nongoma, Pietermaritzburg and Umlazi (KwaZulu-Natal, South Africa). Voucher specimens were deposited at the University of KwaZulu-Natal Herbarium. All plant material was dried at 37 °C for 72 h, milled into fine powders through a 1 mm ring sieve (Ultra-Centrifugal Mill ZM 200, Retsch®, Germany) and stored in airtight containers in the dark at room temperature. For extraction solvents; petroleum ether (PE), dichloromethane (DCM), ethanol (EtOH) and water were used in a sequential extraction process. In order of increasing polarity, 5 g powdered plant material were extracted in 100–200 ml cold solvent, sonicated for 1 h in an ultrasonic water bath (Julabo GMBH, West Germany), left to saturate overnight, and then filtered under vacuum using Whatman No. 1 filter paper. Resultant organic and aqueous extracts was concentrated using a rotary evaporator (Büchi, Germany) at 30 °C and a freeze-drier respectively.

2.2. Anti-inflammatory evaluation

An enzyme-based cyclooxygenase assay (COX-1 and COX-2) as described by Jäger et al. (1996) and Eldeen and Van Staden (2008) was performed. Plant extracts were tested at a concentration of 250 μg/ml per test solution. Indomethacin (Sigma-Aldrich, USA) (5 μM for COX-1 and 200 μM for COX-2) served as a positive control, while background samples with inactivated enzymes before adding [^{14}C] arachidonic acid (16 mCi/mmol; 3.0 μM) and a solvent blank (EtOH) served as negative controls. Each assay was repeated three times with a duplicate set of samples per assay. The anti-inflammatory effect of the plant extracts, that is: percentage inhibition of prostaglandin synthesis, was determined by comparing the amounts of radioactivity (disintegrations per minute (DPM)) in a solvent blank to those of the samples. Inhibition was indicated by a decline in PGE_2 formation. This was calculated using the following formula:

$$\text{Cyclooxygenase Inhibition}(\%) = \left[1-\left(\frac{\text{DPM}_{\text{sample}}-\text{DPM}_{\text{background}}}{\text{DPM}_{\text{solvent blank}}-\text{DPM}_{\text{background}}}\right)\right]\times 100$$

where $\text{DPM}_{\text{sample}}$ $\text{DPM}_{\text{background}}$ and $\text{DPM}_{\text{solvent blank}}$ is the radioactivity for plant extracts, inactivated enzyme sample and EtOH sample respectively. The EC_{50} (mg/ml) of extracts considered significantly active were also determined using extracts within a range of 250–4.625 μg/ml using a two-fold serial dilution.

2.3. Acetylcholinesterase (AChE) enzyme inhibitory activity

2.3.1. Reagents for the AChE assay

Acetylcholinesterase enzyme activity was measured by spectrophotometric observation of the increase in a yellow colour produced from thiocholine when it reacts with the dithiobisnitrobenzoate ion. Three buffers (Buffer A: 50 mM Tris–HCl, pH 8; Buffer B: 0.5 g (0.1%) bovine serum albumin (BSA) in 500 ml Buffer A; Buffer C: 2.92 g NaCl and 2.03 g $MgCl_2 \cdot 6H_2O$ in 500 ml Buffer A) were prepared with Millipore water. The substrate acetylthiocholine iodide (ATCI), 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) or Ellman's reagent, enzyme AChE (isolated from electric eel- type VI-S lypophilized powder) and galanthamine were purchased from Sigma-Aldrich.

2.3.2. The microplate assay for acetylcholinesterase inhibition

The capacity of crude orchid extracts to inhibit AChE enzyme was determined using the protocol outlined in Ellman et al. (1961). Using a 96-well microtitre plate on ice, samples (25 μl, 10 mg/ml) and the solvent blank methanol were added in triplicate to the last well and serially diluted in a 2-fold dilution up the plate. The effective concentration of samples ranged between 0.03 and 1.0 mg/ml. Positive control, galanthamine (20 μM, 50 μl) was also prepared in a 2-fold serial dilution up the plate. To this 25 μl of substrate ATCI (15 mM), 125 μl of DTNB Ellman's reagent (3 mM in Buffer C) and 50 μl of Buffer B were added in sequence to all wells. The absorbance of the reaction mixture was read at 405 nm every 45 s using an ELISA microplate reader (Opsys MR™, Dynex Technologies Inc.). Each plate was read three times to obtain a stable background or baseline value, after which, 25 μl of AChE enzyme (0.2 U/ml in Buffer A) was added to each well. The absorbance was read once again at 405 nm at 45 s intervals, five times. The effect of spontaneous hydrolysis of the substrate was corrected by subtracting the rate of reaction before adding the enzyme from the rate after the addition. Percentage inhibition of the enzyme AChE by each sample was calculated using the formula:

$$\text{Inhibition}\ (\%) = \left[1-\left(\frac{\text{Reaction rate}_{\text{sample}}}{\text{Reaction rate}_{\text{blank}}}\right)\right]\times 100$$

2.4. Antioxidant evaluation

The free radical scavenging antioxidant activity of crude orchid extracts were determined using the protocol outlined in Karioti et al. (2004) with slight modifications. The 2,2′-diphenylpicrylhydrazyl (DPPH) free radical scavenging assay is based on a single electron transfer (SET) reaction-based assay. The DPPH radical used in this assay is a substrate that can be reduced to DPPH-H by antioxidants that have the ability to donate a hydrogen atom to the DPPH radical. Methanolic plant samples were prepared at different concentrations (0.065–50 mg/ml). Each sample (15 μl) was diluted with 735 μl methanol, to which 750 μl of a methanolic DPPH solution (0.1 mM) (Sharma and