Antimicrobial and cyclooxygenase enzyme inhibitory activities of Sclerocarya birrea and Harpephyllum caffrum (Anacardiaceae) plant extracts

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Abstract

The growing demand of medicinal plants is posing a major threat to biodiversity conservation. Sclerocarya birrea and Harpephyllum caffrum (Anacardiaceae) are traditionally used to treat numerous ailments increasing their susceptibility to overexploitation. The aim of the study was to evaluate the medicinal efficacy of renewable aerial parts of the plants using in vitro assays, thus providing a conservation measure by plant part substitution. Plant samples were sequentially extracted with petroleum ether, dichloromethane and 80% ethanol, sonicated on ice and concentrated under vacuum. Antimicrobial activities were determined by the minimum inhibition concentration (MIC) using the micro-dilution assay. The ability of extracts to inhibit COX-1 and COX-2 enzymes was used to evaluate anti-inflammatory activity. Ethanolic extracts of S. birrea and H. caffrum exhibited high antibacterial activity (MIC \( < 1.0 \text{ mg/ml} \)). S. birrea twig extract was the most active with a total activity of 1609.1 ml/g against Bacillus subtilis and an MIC of 0.098 mg/ml. Petroleum ether and dichloromethane extracts exhibited high COX-1 (90.7–99.8%) and COX-2 (69–92.6%) enzyme inhibition at a concentration of 250 \( \mu \text{g/ml} \). The extracts of S. birrea and H. caffrum exhibited high antimicrobial and anti-inflammatory properties. Based on these results, plant part substitution can be a practical conservation strategy for the two species.

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Keywords: Anacardiaceae; Antimicrobial; Anti-inflammatory; Cyclooxygenase; Plant part substitution

1. Introduction

In most parts of Africa, including South Africa, the utilisation of plants for medicinal purposes is a well established practice. The growing demand for medicinal plants, possibly arising from the high levels of poverty and rising costs of modern medicine, continues to exert sustained pressure on the limited plant resource base, posing a major threat to biodiversity conservation. A major concern emanating from this growing demand for medicinal plants is the decimation of tree species, which have long regeneration cycles, through the harvesting of mature bark and roots. The over-exploitation of medicinal plants from their natural habitats has long been a global challenge (Jäger and Van Staden, 2005). In KwaZulu-Natal, South Africa, bark from primary tree trunks and roots from mature trees accounted for a combined total of 54% (27% each) of the medicinal market products sold by traders (Mander, 1998). Other studies in South Africa have also reported high utilisation of trees for medicinal purposes (32%) and a corresponding high preference (83%) for mature bark by traders (Zschocke et al., 2000a). A number of conservation options have been proposed to curtail the destructive utilisation of medicinal plants, for example, establishment of conservation areas, law enforcement against bark collections, large-scale cultivation of popular species (Zschocke et al., 2000a), plant and plant part substitution (Jäger and Van Staden, 2005). However, most of these conservation strategies are long-term and have to be implemented inclusively with short-term options to cater for a sustained supply of medicinal remedies. Plant substitution, though practical, may be resisted by consumers who may not readily accept alternative plants (Zschocke et al., 2000a). Instead, a practical alternative strategy could be plant part substitution.
substitution through the utilisation of renewable parts, such as twigs and leaves which could lead to non-destructive harvesting (Jäger and Van Staden, 2005). Thus, as part of an overall conservation strategy, medicinal plant research should include investigations into parts that are less traditionally used such as twigs and leaves (Zschocke et al., 2000a).

Anacardiaceae tree species are commonly used as bio-resources for medicinal remedies. For example, Spondias mombin has been reported to be an important component of traditional health care in the West Indies, southern Mexico, Peru, Brazil, Equatorial Guinea, Cote D’Ivoire, Nigeria and Sierra Leone (Ayoka et al., 2006). Lima et al. (2006) reported on the medicinal uses of the bark, leaves, roots and flowers of Mangifera indica in the treatment of diarrhoea, fever, gastritis and ulcers. In South Africa, Sclerocarya birrea (A. Rich.) Hochst. subsp. caffra (Sond.) Kokwaro and Harpephyllum caffrum Bernh. ex Krauss, are used extensively in folk medicine for a wide range of ailments, including diarrhoea, dysentery, malaria, fevers, headaches and inflammations (Buwa and Van Staden, 2006; Eloff, 2001; McGaw et al., 2000; Ojevole, 2003). The main part used in the treatment of bacterial and inflammation conditions is the mature bark of both S. birrea (McGaw et al., 2000) and H. caffrum (Buwa and Van Staden, 2006). Mature bark of S. birrea and H. caffrum was the preferred plant part sold by medicinal traders for both species in KwaZulu-Natal (Mander, 1998). S. birrea and H. caffrum accounted for approximately 7% and 5%, respectively, of medicinal plants sold by traders in KwaZulu-Natal (Mander, 1998). Though the two species are not extinct, continued unchecked harvesting of the bark for medicinal purposes poses a major threat to their conservation. The purpose of this study was to evaluate renewable plant parts (twigs and leaves) of S. birrea and H. caffrum for antimicrobial bioactivity; and inhibition of cyclooxygenase-1 and -2 enzymes, which are primarily involved in the biosynthesis of prostaglandins in inflammation processes.

2. Materials and methods

2.1. Chemicals

Yeast malt (YM) broth, Mueller-Hinton (MH) broth, neomycin, amphotericin B, adrenaline, glutathione (reduced form), prostaglandin E2, indomethacin, cyclooxygenase-1 (COX-1, isolated from ram seminal vesicles), cyclooxygenase-2 (COX-2, human recombinant expressed in Sf 21 cells), arachidonic acid, [(14)C] arachidonic acid and p-iodonitrotetrazolium chloride (INT) were purchased from Sigma-Aldrich (Sigma Chemical Co., Steinheim, Germany). Silica gel 60 (particle size 0.063–0.200, 70–230 mesh ASTM), petroleum ether (PE), ethanol (EtOH), dichloromethane (DCM) and dimethyl sulfoxide (DMSO) were obtained from Merck KGaA (Darmstadt, Germany). All chemicals used in the assays were of analytical grade.

2.2. Plant material

H. caffrum plant material was collected in December 2008 from the University of KwaZulu-Natal Botanical Gardens, Pietermaritzburg, South Africa. Plant parts were separated into leaves and the bark was stripped off the twigs and oven dried at 50 °C for three days. S. birrea plant material was obtained from St. Lucia, KwaZulu-Natal, South Africa in November 2008. Plant parts were separated into leaves and twigs and oven dried at 50 °C for three days. Voucher specimens, MOYO 1 and MOYO 2 for S. birrea and H. caffrum, respectively, were identified and deposited in the Natal University (NU) Herbarium at the University of KwaZulu-Natal, Pietermaritzburg.

2.3. Preparation of plant samples

The dried samples were ground into fine powders using a blender (IKA® All basic, Wilmington, USA), and extracted sequentially with petroleum ether, dichloromethane and 80% ethanol. Extractions were done on ice in a sonication bath (Branson model 5210, Branson Ultrasonics B.V., Soest, The Netherlands) using 20 ml of extraction solvent per g of plant extract for 1 h. The extracts were vacuum-filtered through Whatman No. 1 filter paper, concentrated under vacuum (Rotavapor-R, Büchi, Switzerland) at 30 °C and dried at room temperature under a stream of cold air until a constant dry weight was obtained. The dried extracts were stored in sample bottles at 10 °C in the dark.

2.4. Antibacterial microdilution assay

The minimum inhibition concentration (MIC) of the plant extracts was determined using the serial micro-dilution assay (Eloff, 1998) as outlined by Amoo et al. (2009). Four bacteria consisting of two Gram-positive (Bacillus subtilis ATCC 6051 and Staphylococcus aureus ATCC 12600) and two Gram-negative (Escherichia coli ATCC 11775 and Klebsiella pneumoniae ATCC 13883) strains were tested. Overnight bacterial cultures were diluted with sterile Mueller-Hinton (MH) broth to a final inoculum concentration of 10⁶ colony forming units (CFU)/ml. The plant extracts were re-suspended in 80% ethanol to a concentration of 50 mg/ml. A positive control, neomycin (100 μl, 100 μg/ml in the first well) was used against each bacterial strain. Distilled water and 80% ethanol served as the negative and solvent controls respectively. The minimum bacterial concentration (MBC) was determined by adding MH broth (50 μl) to each of the clear wells (no bacterial growth), followed by further incubation at 37 °C for 24 h (Kuete et al., 2008; Pavithra et al., 2009). The assay was done in duplicate and repeated twice.

2.5. Antifungal assay

The micro-dilution assay (Eloff, 1998) with modifications (Masoko et al., 2007) was used in the determination of antifungal activity against a standard strain of Candida albicans (ATCC 10231) as detailed by Ndhlala et al. (2009). An overnight culture of C. albicans in Yeast Malt (YM) broth was incubated at 37 °C in a water bath on an orbital shaker and used to prepare final inoculums of approximately 10⁶ CFU/ml. All plant extracts were re-suspended in 80% ethanol to a concentration of 50 mg/ml. The positive control, amphotericin B was prepared in DMSO and diluted 10 times with sterile distilled water to a final concentration.
of 0.25 mg/ml. Water and 80% ethanol served as the negative and solvent controls respectively. The minimum fungicidal concentration (MFC) was determined by adding YM broth (50 μl) to each of the clear wells, followed by a further incubation at 37 °C for 24 h. The assay was done in duplicate and repeated twice.

### 2.6. Anti-inflammatory activity using cyclooxygenase (COX-1, COX-2) assays

Anti-inflammatory activities of the plant extracts were evaluated on their ability to inhibit prostaglandin synthesis using COX-1 and COX-2 enzyme assays (Jäger et al., 1996; White and Glassman, 1974) and as outlined by Zschocke and Van Staden (2000). Controls for both COX-1 and COX-2 assays included a solvent blank, background and an indomethacin standard. Inhibition of prostaglandin synthesis was calculated by comparing the radioactivity of test reactions to that of the solvent blank using the formula:

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

where DMP(sample), DMP(background) and DMP(solvent blank) represent the radioactivity counts of the plant extract, background and solvent blank, respectively.

### 3. Results and discussion

#### 3.1. Antibacterial activity

The results of the antibacterial assay (MIC and MBC) are presented in Table 1. Extracts of *S. birrea* showed high bioactivity against Gram-positive bacteria (*B. subtilis* and *S. aureus*). For *H. caffrum*, ethanolic extracts were most active (MIC ≤ 0.195 mg/ml) against Gram-positive bacteria when compared to petroleum ether and dichloromethane extracts. The highest activity against Gram-positive bacteria (MIC ≤ 0.195 mg/ml) was recorded for *S. birrea* twig, and *H. caffrum* twig bark extracts. The highest activity (MIC < 1.0 mg/ml) against Gram-negative bacteria (*E. coli* and *K. pneumoniae*) was achieved with ethanolic fractions of *S. birrea* twig, *S. birrea* leaf and *H. caffrum* twig bark extracts. Using *S. birrea* mature bark ethanolic extracts, McGaw et al. (2000) reported high antibacterial activity against the Gram-positive strains of *B. subtilis* (0.012 mg/ml) and *S. aureus* (0.049 mg/ml). Buwa and Van Staden (2006) reported high antibacterial activities of *H. caffrum* ethanolic extracts against *B. subtilis* (0.098 mg/ml), *S. aureus* (0.195 mg/ml) and *E. coli* (0.78 mg/ml). The results of previous studies using mature bark are comparable to the current research findings using renewable plant parts such as twigs. In both cases, Gram-positive bacteria were more sensitive to the extracts than Gram-negative bacteria. In general, Gram-negative bacteria are more resistant and impermeable to certain antibacterial compounds compared to Gram-positive bacteria due to the complex and unique multilayered organization of the cell wall containing lipopolysaccharides and a peptidoglycan-containing matrix (Cos et al., 2006; Risco and Pinto da Silva, 1998). This structural feature may explain the reported difference in sensitivity to plant extracts between Gram-positive and Gram-negative bacteria (Eldeen et al., 2005; Rabe and Van Staden, 1997). The MIC values of the different extracts were translated to total antibacterial activity by dividing the quantity extracted per g by the MIC value (Eloff, 2001). Based on the most susceptible bacterial strain, ethanolic extracts were the most active with total activities in the order, *S. birrea* twig (1609.1) > *H. caffrum* twig.

<table>
<thead>
<tr>
<th>Plant part</th>
<th>Solvent</th>
<th>Yield (mg/g of sample)</th>
<th>MIC (mg/ml)</th>
<th>Total activity (ml/g)</th>
<th>MBC (mg/ml)</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td><em>B. s</em></td>
<td><em>S. a</em></td>
<td><em>E. c</em></td>
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<td>6.25</td>
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* Total activity was calculated for each plant extract based on the bacterial strain with the lowest MIC value.
* *B. s* = *Bacillus subtilis*; *S. a* = *Staphylococcus aureus*; *E. c* = *Escherichia coli*; *K. p* = *Klebsiella pneumoniae*.
* Extracts with values in bold font are considered very active (MIC < 1.0 mg/ml).
bark (1276.9) > S. birea leaf (792.8) > H. caffrum leaf (379.4) > S. birea opercula (21.6 ml/g) (Table 1). For example, the bioactive compounds present in 1 g of H. caffrum twig extracts would still inhibit B. subtilis growth when diluted to 1609.1 ml. The potency of S. birea twigs was twice that of leaf extracts. H. caffrum twig bark extract was 3.3 times more potent than the leaf extract. Conversely, Eloff (2001) reported that the total activity of S. birea leaf extracts was 11 times less active than stem bark extracts. The present results suggest that S. birea leaves can effectively be used for the treatment of bacterial ailments. In the bactericidal evaluation, ethanolic fractions of S. birea twig, leaf, and opercula extracts were most active (MBC = 1.56 mg/ml) against Gram-positive bacteria.

### 3.2. Antifungal activity

Antifungal activity of the plant extracts was evaluated using C. albicans and the obtained MIC and MFC values are presented in Table 2. The therapy of fungal infections caused by opportunistic pathogens such as C. albicans remains a major medical challenge (Sangetha et al., 2009). Infection by C. albicans leads to the formation of a biofilm which is resistant to the penetration of antifungal agents (Sangetha et al., 2009). The most active extract was S. birea twig ethanolic fraction (MIC = 1.56 mg/ml and MFC = 1.56 mg/ml), H. caffrum leaf and all S. birea ethanolic extracts recorded MIC and MFC values of 3.125 mg/ml against C. albicans. The MIC value for H. caffrum twig bark extracts (3.125 mg/ml) is still comparable to that reported by Buwa and Van Staden (2007) for mature bark extracts (1.56 mg/ml). Based on total activity, ethanolic fractions had the highest potency against C. albicans in the order, S. birea twig (101.0) > S. birea leaf (98.9) > H. caffrum twig bark (79.6) > H. caffrum leaf (47.3 mg/ml). The results indicate that S. birea leaves are as effective as twig extracts. Almost twice the dose of H. caffrum leaf extract is required for an equal effect as exhibited by H. caffrum twig bark extract against C. albicans. Buwa and Van Staden (2006) obtained comparable antimicrobial MIC results for ethanolic H. caffrum mature bark extracts. Using an ultrastructural approach, Sangetha et al. (2009) showed that Cassia spectabilis leaf extract prevented formation of C. albicans biofilm at an MIC of 6.25 mg/ml. Findings of the present study suggest that S. birea and H. caffrum extracts may also function by interfering with formation of a C. albicans biofilm.

### 3.3. Cyclooxygenase enzyme assays for anti-inflammatory activity

The bioactivity of plant extracts in the inflammation process was evaluated on their ability to inhibit COX-1 and COX-2 enzymes which are involved in the biosynthesis of prostaglandins. Results on percentage inhibition of COX-1 and COX-2 enzymes by plant extracts at 250 µg/ml in the assay are presented in Figs. 1 and 2. For all extracts petroleum ether and dichloromethane fractions showed high COX-1 enzyme inhibition (90.7–99.8%). Petroleum ether and dichloromethane fractions exhibited high inhibition against COX-2 enzyme (69.0–92.6%). Due to the high activity of the extracts in the COX-2 assay, they were evaluated at lower doses (125 and 62.5 µg/ml) against both cyclooxygenase

![Fig. 1. Dose-dependent prostaglandin synthesis inhibitory activity (% of H. caffrum and S. birea (Anacardiaceae) plant extracts on COX-1 enzyme. (A) Petroleum ether extracts and (B) dichloromethane extracts. H.c. B: H. caffrum twig bark; H.c. L: H. caffrum leaf; S.b. B: S. birea twigs; S. birea leaf; S.b. O: S. birea opercula. Percentage inhibition by indomethacin was 67.1±2.74%.](image)
Percentage inhibition by indomethacin was 67.1±2.62%.

Using an animal experimental model, Ojewole (2003) reported that extracts. Both Figs. 1 and 2 show an increase in prostaglandin synthesis inhibition (%) when evaluated against the COX-1 enzyme. Fig. 1 depicts the dose-dependent prostaglandin synthesis inhibition (%) of the polar extract against COX-2 enzyme suggests some specific anti-inflammatory activity (Zschocke et al., 2000b). The inactivity of the non-polar extracts is likely due to lipophilic compounds, which are active in low concentrations (Zschocke and Van Staden, 2000). Specific cyclooxygenase inhibitors acting on their own or in synergism with other active compounds in the extracts are responsible for the anti-inflammatory activity (Zschocke et al., 2000b). The inactivity of the non-polar extracts is likely due to lipophilic compounds, which are active in low concentrations (Zschocke and Van Staden, 2000). Specific cyclooxygenase inhibitors acting on their own or in synergism with other active compounds in the extracts are responsible for the anti-inflammatory activity (Zschocke et al., 2000b). The inactivity of the non-polar extracts is likely due to lipophilic compounds, which are active in low concentrations (Zschocke and Van Staden, 2000). Specific cyclooxygenase inhibitors acting on their own or in synergism with other active compounds in the extracts are responsible for the anti-inflammatory activity (Zschocke et al., 2000b). The inactivity of the non-polar extracts is likely due to lipophilic compounds, which are active in low concentrations (Zschocke and Van Staden, 2000). Specific cyclooxygenase inhibitors acting on their own or in synergism with other active compounds in the extracts are responsible for the anti-inflammatory activity (Zschocke et al., 2000b). The inactivity of the non-polar extracts is likely due to lipophilic compounds, which are active in low concentrations (Zschocke and Van Staden, 2000). Specific cyclooxygenase inhibitors acting on their own or in synergism with other active compounds in the extracts are responsible for the anti-inflammatory activity (Zschocke et al., 2000b). The inactivity of the non-polar extracts is likely due to lipophilic compounds, which are active in low concentrations (Zschocke and Van Staden, 2000). Specific cyclooxygenase inhibitors acting on their own or in synergism with other active compounds in the extracts are responsible for the anti-inflammatory activity (Zschocke et al., 2000b). The inactivity of the non-polar extracts is likely due to lipophilic compounds, which are active in low concentrations (Zschocke and Van Staden, 2000). Specific cyclooxygenase inhibitors acting on their own or in synergism with other active compounds in the extracts are responsible for the anti-inflammatory activity (Zschocke et al., 2000b). The inactivity of the non-polar extracts is likely due to lipophilic compounds, which are active in low concentrations (Zschocke and Van Staden, 2000). Specific cyclooxygenase inhibitors acting on their own or in synergism with other active compounds in the extracts are responsible for the anti-inflammatory activity (Zschocke et al., 2000b). The inactivity of the non-polar extracts is likely due to lipophilic compounds, which are active in low concentrations (Zschocke and Van Staden, 2000). Specific cyclooxygenase inhibitors acting on their own or in synergism with other active compounds in the extracts are responsible for the anti-inflammatory activity (Zschocke et al., 2000b).

4. Conclusions

Plant part substitution as suggested by Zschocke et al. (2000b) and Jäger and Van Staden (2005) can be a practical conservation strategy for medicinal plants. Based on the demonstrated high microbial and anti-inflammatory bioactivities of renewable aerial parts of both S. birrea and H. caffra, non-destructive harvesting of these species can be achieved. Though the species are at present neither extinct nor threatened, their multipurpose uses in traditional medicine makes them highly vulnerable to over-exploitation, hence the need to protect the available wild populations.

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References


