Pharmaceutical properties of *Pomaria sandersonii*, *Pentanisia prunelloides* and *Alepidea amatymbica* extracts using *in vitro* assays

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The antimicrobial, anti-inflammatory and free radical scavenging activities of root crude acetone extracts and fractions of different polarities from *Pomaria sandersonii* (Fabaceae), *Pentanisia prunelloides* (Rubiaceae) and *Alepidea amatymbica* (Apiaceae) were determined using *in vitro* assays. The antioxidant properties of extracts and fractions were assessed by reduction of 2, 2’-azinobis (3-ethylbenzothiazoline)-6-sulfonic acid and 2, 2-di (4-tert-octylphenyl)-1-picrylhydrazyl radicals which was measured by changes in absorbance using an ultraviolet-visible spectrophotometer. Anti-inflammatory activity of the plant extracts against 15 soybean lipoxygenase enzyme was measured by monitoring the change in absorbance at 234 nm after incubation of 15-LOX with linoleic acid (134 µM) as substrate. The anti-microbial activities were determined by measuring the minimum inhibitory concentrations using a serial dilution microplate method with terazolium violet as a growth indicator. The minimal inhibitory concentration (MIC) value of the dichloromethane (DCM) and ethyl acetate fractions (1 mg/ml) of *P. sandersonii* was 80 µg/ml in each case against *Staphylococcus aureus* and *Escherichia coli*. The inhibition activity of 15 soybean lipoxygenase enzyme by the crude extracts at concentration of 25 µg/ml was 97% for *P. sandersonii*, 79% for *P. prunelloides* and 55% for *A. amatymbica*. This indicates that extracts for these plants can be used as dietary supplements in the management of inflammation related conditions.

**Key words:** Extract, fraction, anti-inflammatory, antibacterial, anti-oxidant, antifungal, antioxidant.

**INTRODUCTION**

The pathogenic bacteria associated with some diseases in many communities/settlements in South Africa include *Escherichia coli* (gastroenteritis); *Enterococcus faecalis* (endocarditis), *Pseudomonas aeruginosa* (inflammation, sepsis in the lungs, urinary tract and kidneys) and *Staphylococcus aureus* (tonsillitis, scarlet fever, minor skin infections, impetigo, boils, abscesses and scalded skin syndrome) (Hamer, 2007). Some of the fungal pathogens also associated with diseases in the community include *Candida albicans*, which are important...
opportunistic yeast involved in oropharyngeal and genital candidiasis (Pfaller and Diekemper, 2004). *Aspergillus fumigatus* is implicated in causing a range of diseases called aspergillosis whose symptoms include cough, fever, wheezing, skin sore and vision problems (Walsh et al., 2008).

In Zululand of South Africa, the use of medicinal plants for prevention and cure of different ailments is well established as part of their cultural heritage. *Alepidia amatymbica* Eckl. & Zeyh. (Apiaceae) is one of the most important species used as medicinal plant in KwaZulu-Natal (KZN) of South Africa and Lesotho (van Wyk, 2008). The rhizomes and roots of *A. amatymbica* (known as Ikathazo) are used for the treatment of colds and chest complaints (Watt and Breyer, 1962). The plant is used in treating gastrointestinal disorder, respiratory tract infection, hypertension and constipation (Hutchings, 1989). The antimicrobial, cyclooxygenase-1 and 2, genotoxicity (Mulaudzi et al., 2009) and antiplasmodial activity (Clarkson et al., 2004) of *A. amatymbica* root extracts have been reported before. Phytochemical investigation of the root and aerial part of the plant revealed the presence of ent-9, (11)-dehydro-16-kauren-19-oic acid, ent-16-kauren-19-oic acid (Rustaiyan and Sadjadi, 1987) which may be the active compounds. Kaurenoic acid isolated from *Helichrysum kraussii* is active against *S. aureus*, *Bacillus cereus*, *Bacillus subtilis* (MIC 10 μg/ml), *Escherichia coli* and *S. marcescens* (1 to 10 μg/ml) (Brenner and Meyer, 2000). However, there is no report on antioxidant activities of extracts and fractions from *A. amatymbica*.

*Pentanisia prunelloides* (Kotzeb ex Eckl & Zeyh.) Walp. (Rubiaceae) also features as a prominent medicinal plant for treating ailment associated with inflammation, microbial infection, muscular contraction, stomach pain, haemorrhoids, snakebite and rheumatism in Zulu traditional medicine (Hutchings et al., 1996). Boiled grated dried bulb is usually taken orally to stop vomiting and diarrhoea in children (Bisi-Johnson et al., 2009). Anti-microbial, cytotoxicity and cyclooxygenase-1 enzyme inhibitory activity of the plant has been reported (Jager et al., 1996). Root and leaf extracts of *P. prunelloides* inhibit COX-1 and the viral replication of the influenza A virus (Yff et al., 2002). Pharmacological investigation of the plant has led to the isolation of palmitic acid as the anti-microbial agent (Yff et al., 2002). *Pomaria sandersonii* (Fabaceae) (Harv) B. B. Simpson and G. P. Lewis is also used in Zulu traditional medicine for pain, inflammation and anti-haemolytic activity (Diamini personal communication). It is endemic to South Africa, grows in the Eastern Cape, KwaZulu-Natal region. It is on the red list of South African plants (Raimondo et al., 2009). This is the first time of recording the ethno pharmacological use of the plant in southern Africa. There is no scientific report on the biological activities of extracts from *P. sandersonii*.

The aim of this study was to scientifically validate the traditional use of *P. prunelloides* (Rubiaceae), *P. sandersonii* (Fabaceae) and *A. amatymbica* (Apiaceae) in treating infections and oxidative stress relating to inflammation. Therefore, this work focuses on the antibacterial, antifungal, antioxidant (DPPH and ABTS radical scavenging assays) and anti-inflammatory activity of the crude extracts and their fractions of different polarities.

**MATERIALS AND METHODS**

**Plant selection and collection**

An ethnobotanical survey based on verbal interviews conducted with nine traditional healers of Mabandla village of Umzimkhulu Local Municipality, Kwa-Zulu Natal, South Africa (30° 15' 45" S, 29° 55' 15" E) for plants used traditionally in the treatment of infectious and inflammatory diseases was carried out. The plants listed in Table 1 were identified and collected with the help of Mr. Sanoyi Paulos Diamini the head of traditional healers from the village. Authentication of plants was done at South Africa National Biodiversity Institute, Pretoria and their voucher specimens are maintained at Pretoria National Botanical Garden.

**Plant treatment**

Plant root and bulb materials collected from Mabandla village, Kwa-Zulu Natal, were washed, air dried at room temperature for three weeks and ground to powder using a Lasec Polymix PX-MFC 90D grinder. Dried plant pulvrised material was stored in glass containers in a cool dry place. Crude extracts were made by shaking fine ground plant powder (200 g) in a ratio of 1 g to 10 ml acetone for 6 h followed by filtering (Eloff, 1998a). Excess solvent was recovered on a rotary vapour until the extract was concentrated. Concentrated slurry was dried at room temperature in the fume hood under an air stream. The crude dried plant extract was stored at 4°C until it is required for biological assays.

**Liquid-liquid extraction (fractionation)**

Dried crude acetone extracts were re-constituted in 200 ml of 70% acetone and extracted sequentially with hexane followed by dichloromethane, ethyl acetate, acetone and methane. The residual water fraction was dried in a conventional oven at 50°C for 96 h. Absolute methanol was added every 24 h to prevent the growth of fungi. The dried water fractions were extracted with acetone and methanol successively to produce fractions of different polarities. Filtered fractions were concentrated on a rotary evaporator and then air dried at room temperature under a fan. The dried fractions were weighed and results are recorded in Table 2.

**Quantitative evaluation of the biological activities of the plant extracts**

**Minimum inhibitory concentrations (MIC)**

Minimum inhibitory concentrations of the crude extracts and fractions were determined by twofold serial dilution using 96-well microtitre plate against *S. aureus* (ATCC 29213), *P. aeruginosa* (ATCC 27853), *E. faecalis* (ATCC 29212) and *E. coli* (ATCC 25922). Tetrazolium violet was used as a growth indicator (Eloff, 1998b). In brief, 100 μL distilled water was placed in each of the wells using a multichannel micropipette. Thereafter, 100 μL of extract (10 mg/ ml) was added to the first well of column and serially diluted to prepare
Table 1. Ethnopharmacological information of the plant use in this study.

<table>
<thead>
<tr>
<th>Botanical name/traditional names</th>
<th>Voucher number</th>
<th>Traditional medicinal use</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alepidea amatymbica</em> Eckl. &amp; Zeyh (Apiaceae) (Ikhathazo (isiZulu), Losoko, (Sesotho))</td>
<td>2116-0</td>
<td>Colds, influenza, stomach and respiratory ailments, rheumatism and wounds (Somova et al., 2001; Stafford et al., 2004; Van Staden et al., 2009).</td>
</tr>
<tr>
<td><em>Pentanisia prunelloides</em>, Klotzeh ex Eckl &amp; Zeyh.) Walp. (Rubiaceae) (Isicimamlilo (isiZulu))</td>
<td>1200-1</td>
<td>Root extract is used to treat aches and pains Kaidoo, T. L. (1997)</td>
</tr>
<tr>
<td><em>Pomaria sandersonii</em> (P.s.) (Harv) B. B. Simpson &amp; G.P. Lewis (Fabaceae) (Istholwane (isiZulu))</td>
<td>14806-0</td>
<td>Root extract is used for post natal care for mothers after child birth for fast recovery Traditional healer, Mabandla Village, Umzimkulu, Kwa-Zulu Natal, South Africa (2009).</td>
</tr>
</tbody>
</table>

Table 2. The percentage yield of the crude extracts and fractions of the plants.

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Crude</th>
<th>H</th>
<th>DCM</th>
<th>ET</th>
<th>AC</th>
<th>METH</th>
<th>W</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. sandersonii</em></td>
<td>7.81±0.78</td>
<td>0.64±0.06</td>
<td>31.33±3.10</td>
<td>16.34±1.60</td>
<td>23.77±2.20</td>
<td>25.18±2.50</td>
<td>-</td>
</tr>
<tr>
<td><em>A. amatymbica</em></td>
<td>7.65±0.76</td>
<td>31.14±3.10</td>
<td>41.31±4.10</td>
<td>0.91±0.09</td>
<td>0.84±0.08</td>
<td>23.73±2.37</td>
<td>-</td>
</tr>
<tr>
<td><em>P. prunelloides</em></td>
<td>5.05±0.49</td>
<td>1.15±0.12</td>
<td>0.82±0.08</td>
<td>-</td>
<td>9.83±0.10</td>
<td>7.05±.08</td>
<td>80.81±8.00</td>
</tr>
</tbody>
</table>

C= crude extract, H= hexane fraction, DCM= dichloromethane fraction, ET = ethyl acetate fraction, AC = acetone fraction and METH= methanol fraction, W= Water fraction.

RESULTS

Yield

The yields of the crude methanol extracts and fractions of various polarities were presented in Table 2. The highest percentage yield of 7.82% was from *P. sandersonii* but had the lowest hexane fraction yield of 0.58%. *A. amatymbica* extract had the highest fraction of non-polar concentration a range between 5.0 and 0.04 mg/ml in the first well and last well respectively. The solvent blank (negative control) and antibiotic (gentamicin for bacteria and amphotericin B for fungi) were included as positive control. From an overnight culture of bacteria grown in diluted Mueller Hinton broth, 100 μL of relevant bacteria dispensed into each well and incubated at 37°C for 18 to 24 h before adding 40 μL (0.2 mg/ml) of iodonitrotetrazolium violet, Sigma (INT) solution. Colour change was noted after 30, 60, and 120 min to determine the lowest concentration which inhibited growth (MIC). MIC of the crude extracts and fractions against *Candida albicans* and *A. fumigatus* were determined using the above-mentioned serial dilution assay with some modifications (Masoko et al., 2005). Fungal stock was prepared using Sabouraud dextrose broth instead of Mueller Hinton broth used for bacteria culture. All measurements were performed in triplicate.

Lipoxygenase inhibition

Inhibitory activity of the plant extracts against 15-soybean lipoxygenase (15-LOX) enzyme was evaluated according to Malterud and Rydland (2000) in borate buffer (0.2 M, pH 9.00). Increase in absorbance at 234 nm was read 5 min at interval of 30 s after addition of 15-LOX, using linoleic acid (134 μM) as substrate. The final enzyme concentration was 167 μg/ml. Test substances were added as dimethyl sulfoxide (DMSO) solutions (final DMSO concentration of 1.6%) while DMSO alone was used as positive control. All measurements were performed in triplicate.

RESULTS
Antimicrobial activities

The minimum inhibitory concentrations of the crude methanol extracts and fractions of various polarities were presented in Figures 2 to 4. The antimicrobial activities of A. amatymbica crude extract and fractions were moderate to good against the bacterial and fungi tested with MICs ranging from 160 to 625 μg/ml. The crude extracts and fractions of P. prunelloides had weak (160 to 320 μg/ml) antibacterial activity (Figure 4). The antifungal activity was low at 625 μg/ml for the crude extract and fractions from P. prunelloides. The crude extract and fractions of P. sandersonii displayed good antimicrobial activity with MICs ranging from 20 to 1250 μg/ml (Figure 3). Dichloromethane and ethyl acetate fractions of P. sandersonii had MIC of 80 μg/ml against S. aureus, E. coli and P. aeruginosa which is comparable to the activities of the antibiotic standard gentamycin. The fractions had good activity against E. faecalis with MIC of 40 μg/ml. The dichloromethane, acetone and methanol fractions of the P. sandersonii also had good antifungal inhibitory activity (20 μg/ml) against C. albicans and A. fumigatus (Figure 3).

Antioxidant and lipoxygenase inhibitory activity

Pentanisia prunelloides

The EC₅₀ values for hexane, DCM and acetone P. prunelloides fractions indicated that they were more reactive with DPPH* radical while there was a comparable activity with both radicals with the methanol fractions with EC₅₀ of 2.619 μg/ml for DPPH* and μg/ml for ABTS** radicals (Table 3). The lipoxygenase inhibitory activity of 79% indicates high anti-inflammatory activity.

Pomaria sandersonii

All the plant extracts and fractions used in this study displayed some anti-radiative activity although, the crude extract DPPH* and ABTS** for P. sandersonii displayed the highest activity compared to all of the plant’s samples. DCM was more reactive towards ABTS** displaying activity of 0.19 μg/ml compared to the methanol extract which had DPPH* displaying activity of 2.61 μg/ml (Table 3). The hexane fraction was more active against DPPH* compared to the reaction with ABTS** radical. The crude extract of P. sandersonii had a high lipoxygenase inhibitory activity of 97% at concentration of 25 μg/ml.

Alepidea amatymbica

The DCM fractions of A. amatymbica also displayed different activities towards the two radicals. EC₅₀ values for the crude and acetone fractions were 4.17 μg/ml towards DPPH* and 5.96 μg/ml towards ABTS** respectively. The lipoxygenase inhibitory activity of 55% at concentration of 25 μg/ml indicated moderate anti-inflammatory activity for A. amatymbica. The results indicate that the A. amatymbica fractions contain antioxidants.

DISCUSSION

Our results demonstrated biological activities of the methanolic crude extracts, hexane, dichloromethane, ethyl acetate and acetone fractions obtained from A. amatymbica, P. prunelloides and P. sandersonii which are used to manage inflammation related conditions. The plants exhibited antibacterial, anti-inflammatory and anti-oxidative properties.

The antimicrobial activity (MIC) of the extracts was determined using the broth microdilution method based on the principle of contact of a test organism to a series of dilutions of test substance. The crude extract and fractions (hexane, dichloromethane, ethyl acetate) of A. amatymbica (Figure 1) displayed good to moderate (160 to 320 μg/ml) anti-bacterial activity against all the organisms tested, however, the methanol fraction

Table 3. Free radical scavenging activity of the crude extract and fractions of varied polarities (μg/ml).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Pomaria sandersonii</th>
<th>Alepidea amatymbica</th>
<th>Pentanisia prunelloides</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DPPH (EC₅₀)</td>
<td>ABTS (EC₅₀)</td>
<td>DPPH (EC₅₀)</td>
</tr>
<tr>
<td>Cr</td>
<td>5.6±0.71</td>
<td>3.24±0.13</td>
<td>7.68±0.59</td>
</tr>
<tr>
<td>H</td>
<td>3.58±0.44</td>
<td>11.31±3.70</td>
<td>19.82±1.74</td>
</tr>
<tr>
<td>DCM</td>
<td>2.93±0.14</td>
<td>7329±117.53</td>
<td>36.25±3.33</td>
</tr>
<tr>
<td>ET</td>
<td>-</td>
<td>88.76±3.50</td>
<td>13.51±1.03</td>
</tr>
<tr>
<td>Ac</td>
<td>70.62±5.38</td>
<td>102.40±15.44</td>
<td>4.17±0.37</td>
</tr>
<tr>
<td>Met</td>
<td>2.61±0.07</td>
<td>0.19±0.04</td>
<td>31.34±2.00</td>
</tr>
</tbody>
</table>

Cr = crude extract, H = hexane fraction, DCM = dichloromethane fraction, ET = ethyl acetate fraction, Ac = acetone fraction, Met = methanol fraction, Positive standards: trolox = 0.04±0.001, ascorbic acid = 0.04±0.001

constituents (72%) that were hexane and DCM soluble. P. prunelloides had the highest water soluble component of 80% yield.
showed a lower MIC (625 µg/ml) against *E. coli*. The activity of the *A. amatymbica* extract and fractions against *C. albicans* was also low (MIC = 625 µg/ml). In a related study, Mulaudzi et al. (2009) reported lower anti-bacterial and anti-fungal activities of polar root extracts and fractions (water and ethanol) from *A. amatymbica*. The

antimicrobial activity of *P. Prunelloides* (Figure 3), which is used traditionally for the treatment of dysmenorrhoea, was good to moderate (160 to 320 µg/ml). Anti-bacterial activity of the *P. prunelloides* acetone fraction was low displaying MIC of 625 µg/ml against *E. faecalis*. Yff et al. (2002), also reported a similar investigation, that antibacterial activity of the water, ethanol and ethyl acetate extracts from *P. prunelloides* root had low bacterial growth.
inhibition (0.39 to 12.5 mg/ml). The low activity values in some of the extracts tested in this study could be due to the impure form and/or low concentration of the active compound(s) in the extracts (Rabe and Van Staden, 1997). DCM and ethyl acetate fractions of *P. sandersonii* (Figure 2) displayed highest inhibitory activities (80 µg/ml in each case) against *S. aureus, E. faecalis, E. coli* and *P. aeruginosa* compared to the other two plants in this study. The more polar fractions (acetone and methanol) had good anti-candidal activity (MIC= 20). The antimicrobial activity of the three plant extracts and fractions were more significant in the non-polar fractions of hexane and dichloromethane. The relatively higher inhibitory activities could be attributed to the presence of terpenoids which had been detected in crude extracts during an earlier qualitative test study, although the compounds have not been isolated and tested individually (Van Wyk, 2008).

Inflammation is an important process involved in the defence mechanisms of an organism against infectious and other deleterious stimuli. However, inflammatory responses to infectious agents can evade control by immuno-regulatory mechanisms. Existing treatment protocols for such inflammation-driven diseases remain less than optimal. In this study, ethanol crude extract of *A. amatymbica* rhizome had 50% LOX inhibition (Figure 4). Although, Mulaudzi et al. (2009) reported that the petroleum ether, DCM and ethanol extracts of *A. amatymbica* rhizome has 90% inhibitory COX-2 activity. The difference in activity could be due to the difference of LOX enzymes and methods used in determining the anti-inflammatory property of the plant. *P. prunelloides* had high 15-LOX inhibitory activity (79%) with EC$_{50}$ value of 15.98 mg/L. The more polar leaf extracts of *P. prunelloides* earlier reported had good COX-1 inhibitory activity (leaf water extract = 74%, root water extract =74%) (Yff et al., 2002). Methanol crude extract of *P. sandersonii* was the most active with 97% LOX inhibitory activity. This is consistent with the fact that the fraction, being the most polar is also rich in polyphenols which are responsible for the anti-inflammatory activity.

Oxidative stress plays a significant role in the pathogenesis of infectious and inflammatory disease. The crude extracts, and more polar fractions of ethyl acetate, acetone and methanol soluble from the three plants have appreciable antioxidant activity. Acetone crude leaf extract, DCM and Ethyl acetate fractions of *P. sandersonii* had higher ABTS** scavenging ability compared to corresponding reaction with the DPPH radical. *P. sandersonii* samples (crude and fractions) were the most active against ABTS** (1.274 to 5.973 µg/ml) compared to all the plants studied (Table 3), except for the hexane fraction which displayed low activity (111.93µg/ml for DPPH) and (3987.33µg/ml for ABTS). The low activity in the hexane fraction could attribute the possible absence of polyphenols in the non-polar fraction as compared to the high activities displayed by the more polar fractions rich in polyphenols. This is the first time reporting biological activities of *P. sandersonii* and are compared with the two relatively more well-known South African medicinal plants.

**Conclusion**

This study revealed that while medicinal plants still play a very vital role in the primary health care of the people of Mabandla village, Zululand, South Africa, the three plants in this study possess significant biological activities as indicated by the *in vitro* assays that were carried out. Taken together, the anti-infectious, anti-inflammatory and antioxidant mechanisms may help to develop better novel pharmacological drugs for various degenerative diseases. In this study, 15-LOX from soybean was used to assess the *in vitro* inhibitory activity. These findings should therefore be cautiously applied to the anti-inflammatory activity in humans because the mechanism of human derived 15 LOX may be different from that which is soybean derived. Other enzymes such as COX-1 and COX-2 should be studied to obtain a more accurate assessment of anti-inflammatory activity of the plants. These medicinal plants have demonstrated broad spectra of activity that might help to discover new pharmacological compounds which could serve as agents for human and animal health maintenance. The results obtained in this study also validate the traditional use of these plants to manage infectious and inflammation related disorders, however, *in vivo* studies are still necessary to completely validate the claims leading to isolation and characterisation of active compounds.

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**Conflicts of interest**

The authors declare that they have no conflicts of interest.

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