Short communication

Antiplasmodial, acetylcholinesterase and alpha-glucosidase inhibitory and cytotoxicity properties of Buddleja saligna

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ABSTRACT

The crude ethanolic extract, hexane, dichloromethane, ethyl acetate and aqueous fractions of Buddleja saligna leaves were investigated for antiplasmodial, alpha-glucosidase and acetylcholinesterase inhibitory and cytotoxicity properties. Although the crude extract displayed some level of activity, the hexane fraction demonstrated the best antiplasmodial (IC50 = 8.5 μg/ml), alpha-glucosidase inhibitory (IC50 = 260 μg/ml) and anticholinesterase (IC50 = 124.8 μg/ml) activities. The safety index values of the hexane fraction [antiplasmodial (0.2), alpha-glucosidase inhibition (0.007) and anticholinesterase (0.01)] revealed that the fraction is cytotoxic. These findings highlight the importance of reporting the SI values of biologically active samples together with their biological activities. It remains to be determined whether the toxicity observed is caused by the compounds responsible for the biological activities investigated or not.

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1. Introduction

In South Africa, plants have always and still remain a vital source of therapeutics for various illnesses such as malaria, diabetes and mental disorders in traditional medicine. One such plant is Buddleja saligna Willd (Loganiaceae), also known as “umceba” and “igqeba-elimhlophe” in Zulu. It is a small to medium-sized evergreen tree (10 m) growing in warm moist areas but usually 4–5 m at high elevation (Aubrey, 2002). As members of the genus have many healing properties, this species is widely used in traditional medicine. Traditionally, it is used as a purgative, to treat coughs, colds, diabetes, tuberculosis, thrush and sores, anasarca and chest pains (Hutchings et al., 1996; Anonymous, 2013). An unspecified species of Buddleja is used by the Sotho people in South Africa to treat early nervous and mental illness (Watt and Breyer-Brandwijk, 1962).

A number of biological activities have been reported for B. saligna. These include anti-myco bacterial (Ramumba et al., 2008), antibacterial, antioxidant (Adedapo et al., 2009), and antimitogenic (Verschaeve and van Staden, 2008) properties. In line with the traditional use of B. saligna, we report here the antiplasmodial, alpha-glucosidase (antidiabetic), anticholinesterase and cytotoxicity properties of B. saligna crude extract and its fractions.

2. Materials and methods

2.1. Plant collection

Leaves of B. saligna were collected in May, 2013 from the Botanical Garden of the University of KwaZulu-Natal, Pietermaritzburg, South Africa. After identification by the Curator, a voucher specimen (Chukwujekwu #7 NU) was deposited in the Herbarium of the University of KwaZulu-Natal, Pietermaritzburg. Plant material was dried at 50 °C, powdered and stored in paper containers at ambient temperature for less than 24 h prior to extraction.

2.2. Extraction and fractionation

The leaf powders (14.2 g) were extracted using 80% ethanol (300 ml) with sonication for 1 h and then left overnight with stirring. The extract was filtered through a Büchner funnel using Whatman no. 1 filter paper. The extraction and filtration were subsequently repeated three times with 300 ml 80% ethanol, and the solvent from the combined extracts evaporated under reduced pressure at 30 °C. Liquid–
liquid partitioning was done by dissolving the crude extract (4.1 g) in aqueous methanol (300 ml, 80% v/v) followed by sequential extraction with hexane, dichloromethane, ethyl acetate and water as described by Chukwujekwu et al. (2013). All four fractions and crude extract were used in subsequent assays.

### 2.3. Antiplasmodial assay

Samples were tested for antiplasmodial activity in triplicate against chloroquine-sensitive (CQS) strain of *Plasmodium falciparum* (NF54) as described by Chukwujekwu et al. (2014). The reference drugs used were chloroquine diphosphate (CQ) (Sigma) and artesunate (Sigma).

### 2.4. Anticholinesterase assay

The acetylcholinesterase (AChE) inhibitory property of the crude extracts and fractions was determined using a 96-well microplate colorimetric method (Ellman et al., 1961) as detailed by Fawole et al. (2010). Galanthamine was used as a positive control. Each sample was tested in triplicate. The rate of reaction was calculated for each sample and the percentage inhibition was determined as follows:

\[
\text{AChE inhibition (\%)} = \frac{[1 - (\text{sample reaction rate/blank reaction rate})]}{1} \times 100
\]

### 2.5. Alpha-glucosidase inhibitory activity

Alpha-glucosidase inhibitory activity was determined as described by Rengasamy et al. (2013). Acarbose was used as a positive control. The negative control had the same volume of phosphate buffer in place of the sample volume. Each determination was done in triplicate. Percentage inhibition by each sample was calculated using the equation:

\[
\text{Percentage inhibition} = \left[ \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \right] \times 100
\]

where \(A_{\text{control}}\) and \(A_{\text{sample}}\) are the absorbance values of the negative control and sample, respectively.

### 2.6. Cytotoxicity assay

The cytotoxicity assay was performed as previously described by Chukwujekwu et al. (2014). Only the hexane fraction was selected for cytotoxicity testing as better biological activity was displayed by this fraction when compared to the crude extract and other fractions.

### 2.7. Data analysis

For the determination of IC\(_{50}\) a non-linear regression analysis was done using GraphPad Prism (version 4.03) software. Data were subjected to analysis of variance (ANOVA) followed by Duncan’s multiple range test (where appropriate) using SPSS (version 10.0) software in order to determine any significant difference between the mean values.

### 3. Results and discussion

Table 1 presents the antimalarial and cytotoxicity properties as well as the selective index (SI) of *B. saligna* crude extract and its fractions. With the exception of water and ethyl acetate fractions, there was an improvement in antimalarial activity of the other fractions over the crude extract. A two to three times better antimalarial activity was recorded with dichloromethane and hexane fractions, respectively when compared to the crude extract. Considering the good antimalarial activity displayed by the hexane fraction, it was tested for cytotoxicity. The SI of the hexane fraction (Table 1) with regards to antimalarial activity shows that this fraction was toxic. Previous studies have reported antimalarial activity in certain *Buddleja* species with or without SI values. For example, a dichloromethane extract of *B. salvifolia* exhibited moderate antimalarial activity (IC\(_{50}\): 22 \(\mu\)g/ml) against a chloroquine-sensitive strain of *P. falciparum* (3D7) with a high SI value (>9) (Jonville et al., 2011). Using a multi-drug resistant strain of *P. falciparum* (K1), Debenedetti et al. (2002) observed good antimalarial activity (IC\(_{50}\): 8.9 \(\mu\)g/ml) with *Buddleja globosa* water extract but there was no information on the cytotoxicity of the extract. The current study highlighted the need to incorporate safety evaluations when determining the biological activity of plant extracts.

In a previous investigation, a hexane fraction of *B. saligna* was reported to possess antimycobacterial activity and oleanolic acid was identified as the antimycobacterial compound (Bamuamba et al., 2008). This compound had an IC\(_{50}\) value greater than 100 \(\mu\)g/ml with regards to its cytotoxicity activity using CHO cells (Bamuamba et al., 2008). Oleandric acid was inactive against a multi-drug resistant strain of *P. falciparum* (K1) (Suksamrarn et al., 2003) but moderately active against a chloroquine-sensitive strain (3D7) (Steele et al., 1999; Sairafianpour et al., 2003). Since oleandric acid is known to exert very moderate antimalarial activity against a chloroquine-sensitive strain of *P. falciparum*, it could be that the antimalarial activity of the hexane fraction in this study was enhanced through a synergistic interaction of oleandric acid with other constituents of the fraction. However, the very low SI (0.2) recorded for the fraction is not encouraging. The antimalarial activity exhibited by the hexane fraction could be due to toxicity of the fraction on the parasites.

The anticholinesterase, alpha-glucosidase inhibitory activities, and SI values of *B. saligna* crude extract and its fractions are presented in Table 2. A significantly higher anticholinesterase activity (lower IC\(_{50}\) values) was observed with the crude extract, hexane and ethyl acetate fractions when compared to dichloromethane and water fractions. Although the hexane fraction displayed the highest activity, its very low SI value suggests that it is very toxic with regards to anticholinesterase activity. According to Fan et al. (2008), a methanol leaf extract of *B. davidi* exhibited anticholinesterase inhibitory activity from which the acetylcholinesterase inhibitor linarin was isolated. In the current study, it is not known at this stage which phytochemical(s) is/are responsible for the observed anticholinesterase activity. *B. saligna* has never been reported to contain the phytochemical linarin. However, being a related species to *B. davidii*, it may well be that it contains linarin which might be responsible for the observed activity. Hopefully, further study of the potent fractions will clarify which phytochemical(s) are responsible for the activity.

Alpha-glucosidase inhibitors are known to be very active in reducing postprandial glucose by suppressing the absorption of glucose and are effective in the treatment and management of diabetes and obesity (DeMelo et al., 2006). The evaluation of alpha-glucosidase inhibitory activity of *B. saligna* extracts revealed that the hexane fraction displayed the highest activity (the lowest IC\(_{50}\) value). An IC\(_{50}\) value could not be computed for the crude extract and dichloromethane fraction due to

<table>
<thead>
<tr>
<th>Sample</th>
<th>NF54: IC(_{50}) ((\mu)g/ml)</th>
<th>CHO: IC(_{50}) ((\mu)g/ml)</th>
<th>SI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>27.0 ± 3.9</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Hexane fraction</td>
<td>8.5 ± 1.6</td>
<td>1.7 ± 0.31</td>
<td>0.2</td>
</tr>
<tr>
<td>Dichloromethane</td>
<td>13.3 ± 3.1</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>73.1 ± 4.0</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Aqueous fraction</td>
<td>120 ± ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>0.0058 ± 0.0004</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Artesunate</td>
<td>0.0041 ± 0.0003</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Emetine</td>
<td>0.035 ± 0.02</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Selectivity index (SI) = IC\(_{50}\) CHO/IC\(_{50}\) NF54; ND = not determined.
the fact that an inhibitory activity less than 20% was observed at the highest concentration tested (1.2 mg/ml). The IC50 value of the hexane fraction (0.26 mg/ml) was comparable to that of the positive control (0.20 mg/ml). Although this is very promising, the very low SI value of this fraction with reference to alpha-glucosidase inhibition suggests that it may be very toxic. It remains to be seen whether the toxicity observed is caused by the compounds responsible for alpha-glucosidase inhibition or other constituent phytochemical(s).

4. Conclusions

The present study demonstrated the antiplasmodial, anticholinesterase and alpha-glucosidase inhibitory properties of B. saligna crude extract and its fractions. On fractionation of the crude extract, the hexane fraction stood out among all fractions with regards to the biological activities determined. There was an improvement in biological activity of the hexane fraction over the crude extract. However, the SI value of this fraction in each biological activity investigated revealed that this fraction may be toxic. This reinforces the importance of reporting the SI values of biologically active samples together with its biological activities. As fractions are mixtures of different constituents, which are not known at this stage, future studies will help to unravel the constituent(s) and their biological activities. To the best of our knowledge, this is the first report of antiplasmodial, anticholinesterase and alpha-glucosidase inhibitory properties of B. saligna. The safety of using this plant species in traditional medicine for treating diabetes and mental-related ailments must be carefully monitored.

Acknowledgements

The University of KwaZulu-Natal is thanked for financial assistance.

References


Table 2

Anticholinesterase and alpha-glucosidase inhibitory properties of B. saligna crude extracts.

<table>
<thead>
<tr>
<th>Sample</th>
<th>AChE inhibition IC50 (μg/ml)</th>
<th>SI</th>
<th>R² value</th>
<th>Alpha-glucosidase inhibition IC50 (μg/ml)</th>
<th>SI</th>
<th>R² value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>149.7 ± 21.34 a</td>
<td>ND</td>
<td>0.9771</td>
<td>ND</td>
<td>ND</td>
<td>0.9688</td>
</tr>
<tr>
<td>Hexane fraction</td>
<td>124.8 ± 5.12 a</td>
<td>0.01</td>
<td>0.9876</td>
<td>0.26 ± 0.112 a</td>
<td>ND</td>
<td>0.8206</td>
</tr>
<tr>
<td>Dichloromethane fraction</td>
<td>212.9 ± 5.87 b</td>
<td>ND</td>
<td>0.9748</td>
<td>0.58 ± 0.102 b</td>
<td>ND</td>
<td>0.9444</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>140.7 ± 15.43 a</td>
<td>ND</td>
<td>0.9901</td>
<td>2.02 ± 0.015 c</td>
<td>ND</td>
<td>0.9798</td>
</tr>
<tr>
<td>Water fraction</td>
<td>206.4 ± 13.41 b</td>
<td>ND</td>
<td>0.9767</td>
<td>0.20 ± 0.015</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive controls</td>
<td>0.22 ± 0.019</td>
<td></td>
<td></td>
<td>0.9767</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Galanthamine (μg/ml)</td>
<td>0.22 ± 0.019</td>
<td></td>
<td></td>
<td>0.9767</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acarbose (mg/ml)</td>
<td>0.22 ± 0.019</td>
<td></td>
<td></td>
<td>0.9767</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean values followed by different letters in the same column are significantly different (P = 0.05) according to Duncan’s multiple range Test. R² is the coefficient of determination for the goodness of fit of non-linear regression curve.

Selectivity index (SI) = IC50 CHO/IC50 (biological activity); ND = not determined.