Antioxidant and acetylcholinesterase inhibitory activity of selected southern African medicinal plants

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Abstract

Alzheimer’s disease (AD) is the most common type of dementia in the aging population. Enhancement of acetylcholine levels in the brain is one means of treating the disease. However, the drugs presently used in the management of the disease have various drawbacks. New treatments are required and in this study, extracts of Salvia tiliifolia Vahl. (whole plant), Chamaecrista mimosoides L. Greene (roots), Buddleja salviifolia (L.) Lam. (whole plant) and Schotia brachypetala Sond. (root and bark) were evaluated to determine their polyphenolic content, antioxidant and acetylcholinesterase inhibitory (AChEI) activity. The DPPH and ABTS assays were used to determine antioxidant activity and Ellman colorimetric method to quantify AChEI activity. Although all four plants showed activity in both assays, the organic extracts of C. mimosoides root was found to contain the highest AChEI activity (IC50 =0.03±0.08 mg/ml) and B. salviifolia whole plant had the highest antioxidant activity (ABTS; IC50 =0.14±0.08 mg/ml and DPPH; IC50 =0.23±0.01 mg/ml). The results suggest that the tested plant species may provide a substantial source of secondary metabolites, which act as natural antioxidants and acetylcholinesterase inhibitors, and may be beneficial in the treatment of AD.

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Keywords: Acetylcholinesterase; Alzheimer’s disease; Antioxidant; Medicinal plants; Neurodegeneration

1. Introduction

Dementia is characterized by the gradual onset and continuing decline of higher cognitive functioning (Dhingra et al., 2005). Alzheimer’s disease (AD), the most common form of dementia (Nie et al., 2009), is a progressive age-related disorder that is characterized by the degeneration of neurological function. The latter is due to the reduction in levels of the neurotransmitter acetylcholine, in the brains of the elderly as the disease progresses, resulting in loss of cognitive ability (Felder et al., 2000). Acetylcholinesterase inhibitors (AChEIs) have been shown to function by increasing acetylcholine within the synaptic region, thereby restoring deficient cholinergic neurotransmission (Giacobini, 1998; Krall et al., 1999).

Selective cholinesterase inhibitors, free of dose-limiting side effects, are not currently available, and current compounds may not allow sufficient modulation of acetylcholine levels to elicit the full therapeutic response (Felder et al., 2000). In addition, some of the synthetic medicines used e.g. tacrine, donepezil and rivastigmine have been reported to cause gastrointestinal disturbances and problems associated with bioavailability (Melzer, 1998; Schulz, 2003). Therefore, the search for new AChEIs, particularly from natural products, with higher efficacy continues.

Oxidative stress, caused by reactive oxygen species (ROS), is known to result in the oxidation of biomolecules, thereby leading to cellular damage and it plays a key pathogenic role in the aging process (Zhu et al., 2004). In recent years, there has been growing interest in finding natural antioxidants in plants because they inhibit oxidative damage and may consequently...
prevent aging and neurodegenerative diseases (Fusco et al., 2007).

In an effort to discover new sources which can potentially be used in the treatment of AD, four plants — *Salvia tiliifolia* Vahl. (Lamiaceae), *Chamaecrista mimosoides* L. Greene (Caesalpinioideae), *Buddleja salviifolia* L. Lam. (Buddlejaceae) and *Schottia brachypetala* Sond. (Fabaceae), traditionally used in the treatment of neurodegenerative diseases (Orhan et al., 2007; Stafford et al., 2008), were evaluated for their AChEI and antioxidant capacity.

### 2. Material and methods

#### 2.1. Chemicals

Acetylthiocholine iodide (ATCI), acetylcholinesterase (AChE) type VI-S, from electric eel, 5,5′-dithiobis[2-nitrobenzoic acid] (DTNB), galanthamine, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and trolox were purchased from Sigma. Methanol and all other organic solvents (analytical grade) were purchased from Merck.

#### 2.2. Plant collection and extract preparation

The plant species; *S. tiliifolia* (whole plant; P03649), *C. mimosoides* (root, P08814), *B. salviifolia* (whole plant, P01281), *S. brachypetala* (bark, P08514) and *S. brachypetala* (root, P06300) were collected in Gauteng Province, South Africa. Identities of the specimens were confirmed by the South African National Biodiversity Institute (SANBI), Tshwane and voucher specimens are deposited at this institution. The plant samples were cut into small pieces and dried in an oven at 30–60 °C for 48 h. Dried material was ground to a coarse powder using a hammer mill and stored at ambient temperature prior to extraction. Six grams of the powdered plant material was extracted with 60 ml of either dichloromethane/methanol (1:1) or distilled water for 24 h. Organic extracts were concentrated by freeze-drying. All extracts were stored at −20 °C prior to analysis. The residues were redissolved in DCM:MeOH or distilled water, respectively to the desired test concentrations.

#### 2.3. Micro-plate assay for inhibition of acetylcholinesterase

Inhibition of acetylcholinesterase activity was determined using Ellman’s colorimetric method as modified by Eldeen et al. (2005). Into a 96-well plate was placed: 25 μl of 15 mM ATCI in water, 125 μl of 3 mM DTNB in Buffer C (50 mM Tris–HCl, pH 8, containing 0.1 M NaCl and 0.02 M MgCl2·6H2O), 50 μl of Buffer B (50 mM, pH 8, containing 0.1% bovine serum albumin) and 25 μl of plant extract (0.25, 0.5, 1 or 2 mg/ml). Absorbance was measured spectrophotometrically (Labsystems Multiscan EX type 355 plate reader) at 405 nm every 45 s, three times consecutively. Thereafter, AChE (0.2 U/ml) was added to the wells and the absorbance measured five times consecutively every 45 s. Galanthamine served as the positive control. Any increase in absorbance due to the spontaneous hydrolysis of the substrate was corrected by subtracting the absorbance before adding the enzyme from the absorbance after adding the enzyme. The percentage inhibition was calculated using the equation:

Inhibition (%) = 1 − (A_{sample} / A_{control}) × 100

where \( A_{sample} \) is the absorbance of the sample extracts and \( A_{control} \) is the absorbance of the blank [methanol in Buffer A (50 mM Tris–HCl, pH 8)]. Extract concentration providing 50% inhibition (IC50) was obtained by plotting the percentage inhibition against extract concentration.

#### 2.4. Determination of total phenolics

Total phenolic content in the extracts were determined by the modified Folin–Ciocalteu method of Wolfe et al. (2003). The extract (1 mg/ml) was mixed with 5 ml Folin–Ciocalteu reagent (diluted with water 1:10 v/v) and 4 ml (75 g/l) sodium carbonate. The mixture was vortexed for 15 s and allowed to stand for 30 min at 40 °C for color development. Absorbance was measured at 765 nm using the Hewlett Packard UV–VIS spectrophotometer. Total phenolic content is expressed as mg/g tannic acid equivalent using the following equation based on the calibration curve: \( y = 0.1216x \), where \( x \) is the absorbance and \( y \) is the tannic acid equivalent (mg/g).

#### 2.5. Determination of total flavonoids

Total flavonoid content was determined using the method of Ordenez et al. (2006). A volume of 0.5 ml of 2% AlCl3 ethanol solution was added to 0.5 ml of sample (1 mg/ml). After one hour at room temperature, the absorbance was measured at 420 nm. A yellow color is indicative of the presence of flavonoids. Total flavonoid content was calculated as quercetin equivalent (mg/g), using the following equation based on the calibration curve: \( y = 0.025x \), where \( x \) is the absorbance and \( y \) is the quercetin equivalent (mg/g).

#### 2.6. Determination of total proanthocyanidins

The procedure reported by Sun et al. (1998) was used to determine the total proanthocyanidin content. A volume of 0.5 ml of 1 mg/ml extract solution was mixed with 3 ml of a 4% vanillin–methanol solution and 1.5 ml hydrochloric acid. The mixture was allowed to stand for 15 min after which the absorbance was measured at 500 nm. Total proanthocyanidin content is expressed as catechin equivalents (mg/g) using the following equation based on the calibration curve: \( y = 0.5825x \), where \( x \) is the absorbance and \( y \) is the catechin equivalent (mg/g).
2.7. Antioxidant activity

2.7.1. DPPH radical scavenging activity

The effect of the extracts on DPPH radical was estimated using the method of Liyana-Pathiranan and Shahidi (2005), with minor modifications. A solution of 0.135 mM DPPH in methanol was prepared and 185 μl of this solution was mixed with 15 μl of varying concentrations of the extract (0.25, 0.5, 1 and 2 mg/ml), in a 96-well plate. The reaction mixture was vortexed and left in the dark for 30 min (room temperature). The absorbance of the mixture was determined at 570 nm using a micro plate reader. Trolox was used as the reference antioxidant compound. The ability to scavenge the DPPH radical was calculated using the equation:

\[
\text{DPPH radical scavenging activity} = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100
\]

where \(A_{\text{control}}\) is the absorbance of DPPH radical + methanol and \(A_{\text{sample}}\) is the absorbance of DPPH radical + sample extract/standard. The extract concentration providing 50% inhibition (IC\(_{50}\)) was obtained by plotting inhibition percentage versus extract concentration.

2.7.2. ABTS radical scavenging activity

The method of Re et al. (1999) was adopted for the ABTS assay. The stock solution which was allowed to stand in the dark for 16 h at room temperature contained equal volumes of 7 mM ABTS salt and 2.4 mM potassium persulfate. The resultant ABTS++ solution was diluted with methanol until an absorbance of 0.706±0.001 at 734 nm was obtained. Varying concentrations (0.25, 0.5, 1 and 2 mg/ml) of the extract were allowed to react with 2 ml of the ABTS++ solution and the absorbance readings were recorded at 734 nm. The ABTS++ scavenging capacity of the extract was compared with that of trolox and the percentage inhibition calculated as:

\[
\text{ABTS radical scavenging activity} = \left( \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100
\]

where \(A_{\text{control}}\) is the absorbance of ABTS radical + methanol and \(A_{\text{sample}}\) is the absorbance of ABTS radical + sample extract/standard. All tests were carried out on three separate occasions. The extract concentration providing 50% inhibition (IC\(_{50}\)) was obtained by plotting inhibition percentage versus extract concentration.

2.8. Statistical analysis

All determinations were done in triplicate, and the results reported as mean±standard deviation (S.D.). Calculation of IC\(_{50}\) values was done using GraphPad Prism Version 4.00 for Windows (GraphPad Software Inc.).

3. Results and discussion

Four plants — S. tiliifolia Vahl. (Lamiaceae), C. mimosoides L. Greene (Caesalpiniaaceae), B. salvifolia (L.) Lam. (Buddlejaeaceae) and S. brachypetala Sond. (Fabaceae), traditionally used in the treatment of neurodegenerative diseases (Orhan et al., 2007; Stafford et al., 2008) were the focus of the current study. Cold water root infusions of C. mimosoides are reported to be taken to remember forgotten dreams by the Zulu (Hulme, 1954). Buddleja species are used together with Heteromorphe trifoliate and Cassonia paniculata by Sotho in South Africa to treat early nervous and mental illnesses (Watt and Breyer-Brandwijk, 1962). The bark and roots of S. brachypetala are reported to be used for nervous conditions (Van Wyk and Gericke, 2000), whereas Salvia species have been reported to be used for memory-enhancing purposes in European folk medicine (Perry et al., 2003). The inclusion of S. tiliifolia and B. salvifolia was a taxonomically informed selection as both Salvia and Buddleja species have been reported to be useful in treatment of neurodegenerative diseases (Perry et al., 2003; Watt and Breyer-Brandwijk, 1962).

The results of the AChE inhibitory activities of the tested plant extracts as well as the positive control, galanthamine, are provided in Fig. 1. All the plant extracts contained some level of inhibitory activity against AChE. Water was used as one of the solvents as the plants investigated are traditionally prepared as either infusions or decoctions (Hulme, 1954; Hutchings et al., 1996; Watt and Breyer-Brandwijk, 1962). However, the DCM:MeOH (1:1) extracts had better activity than the water extracts with C. mimosoides root showing the highest percentage inhibition of AChE. The higher activity of the DCM:MeOH (1:1) extracts may suggest that organic solvents are able to extract more active compounds with possible AChE inhibitory activity than water. The IC\(_{50}\) values of the plant extracts indicating AChE inhibitory activity are presented in Table 1. A Low IC\(_{50}\) value is indicative of good inhibition of the enzyme. The organic extracts of C. mimosoides had the lowest IC\(_{50}\) value, indicating that it contained the best inhibition of the enzyme.

Since a large amount of evidence demonstrates that oxidative stress is intimately involved in age-related neurodegenerative diseases, there have been a great number of studies which have examined the positive benefits of antioxidants to reduce or to block neuronal death occurring in the pathophysiology of these disorders (Ramassamy, 2006). In addition, the antioxidant potential of a compound can be attributed to its radical scavenging ability, and in order to evaluate the ability of the plant extracts to serve as antioxidants, two activities were measured; ability to scavenge DPPH and ABTS radicals. Figs. 2 and 3 depict the dose-dependent ABTS and DPPH radical scavenging activity of the plant extracts expressed as a percentage of the ratio of the decrease in absorbance of the test solution to that of DPPH or ABTS solution without the plant extracts, respectively. All the plant extracts showed a propensity to quench the free radicals, as indicated by the dose-dependent increase in percentage inhibition. This corresponded to a rapid decrease in absorbance in the presence of a plant...
extract, indicating high antioxidant potency of the extracts in terms of electron or hydrogen atom-donating capacity. The IC$_{50}$ values (concentration of the extract that is able to scavenge half of the DPPH or ABTS radical) are presented in Table 3. The organic extracts of the root of *S. brachypetala* had the lowest IC$_{50}$ values in both antioxidant assays, indicative of its good antioxidant potential.

All five extracts contained phenols with the highest amount in the water extract of the bark of *S. brachypetala* (Table 2). Phenolic compounds (Robards et al., 1999). Most of the antioxidant potential of medicinal plants is due to the redox properties of phenolic compounds, which enable them to act as reducing agents, hydrogen donors and singlet oxygen scavengers (Hakkim et al., 2007). Antioxidant activities of plant extracts are presented in Table 3. The organic extracts of the root of *S. brachypetala* had the lowest IC$_{50}$ values in both antioxidant assays, indicative of its good antioxidant potential.

The IC$_{50}$ value for the positive control, galanthamine, was 5.3 × 10$^{-4}$ mg/ml. (Araújo et al., 2008). In addition, the hydrogen-donating substituents (hydroxyl groups) attached to the aromatic ring structures of flavonoids enable them to undergo a redox reaction, which in turn, help them scavenge free radicals (Brand-Williams et al., 1995). The tannins found in proanthocyanidins are also good antioxidant components, as they can reduce metallic ions such as Fe$_{3+}$ to the Fe$_{2+}$ form and can inhibit the 5-lipoxygenase enzyme in arachidonic acid metabolism, which is important in inflammation physiology (Okuda, 2005). The highest level of proanthocyanidins was contained in water extracts of the bark of *S. brachypetala* (Table 2).
A variety of bioactive compounds that could be responsible for the observed bioactivities has been reported in some of the screened medicinal plants or related genera. The essential oil and ethanol extract of *S. officinalis* as well as the essential oil of *S. lavandulaefolia* have been shown to possess anticholinesterase activity (Perry et al., 1996), as have the major components of the essential oil, α-pinene, 1, 8-cineole, and camphor (Perry et al., 2000). *S. brachypetala* showed dose-dependent inhibition of AChE and high antioxidant activity for the organic extracts of the root. This finding is supported by Stafford et al. (2007), who reported good monoamine oxidase (MAO) B inhibitory activity in the aqueous and ethanol extracts of the bark of this plant species. *S. brachypetala* contains stilbenes and phenolics which have been shown to have good radical scavenging activity (Glasby, 1991). The family Caesalpiniaceae has been shown to contain several diterpenes with biological activity. The clerodane diterpenes present in fruit pulp extract of *Detarium microcarpum* Guill. & Perr. showed both antifungal activity and inhibition of acetylcholinesterase (Cavin et al., 2006). The presence of clerodane or similar diterpenes in *C. mimosoides* may be responsible for the good AChE inhibitory activity seen for the organic root extracts. Several plants in the family Caesalpiniaceae have also been reported to contain good antioxidant activity (Motlhanka, 2008), which supports the present finding for the organic root extracts of *C. mimosoides*. The genus *Buddleja* has been reported to contain various terpenoids; monoterpens, sesquiterpenes, diterpenes and triterpenoids (Houghton et al., 2003). Some of the sesquiterpenes have been shown to contain anti-inflammatory activity (Liao et al., 1999). Various species of *Buddleja* have been found to contain luteolin and its glycosides have been shown to contain good antioxidant and anti-inflammatory activity (López-Lázaro, 2009). It is therefore postulated that the presence of these and related compounds in *B. salviifolia* may be responsible for the antioxidant and AChEI activity shown in this study.

### 4. Conclusion

Since AD is pathologically complex, the use of multifunctional drugs is a more rational approach to treatment. Overall, the DCM:MeOH extracts of *C. mimosoides*, *B. salviifolia* and *S. brachypetala* roots showed good antioxidant and cholinesterase inhibitory activity. These plant extracts and their active components could emerge as natural antioxidants, alternative anticholinesterase drugs or serve as starting points for synthesizing more effective AChE inhibitors.

### Table 2

<table>
<thead>
<tr>
<th>Plant</th>
<th>Extract</th>
<th>Total phenols(^a)</th>
<th>Total flavonoids(^b)</th>
<th>Total proanthocyanidins(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. tiliifolia</em></td>
<td>DCM:MeOH (1:1)</td>
<td>129.75±0.02</td>
<td>35.98±0.08</td>
<td>64.08±0.02</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>72.02±0.01</td>
<td>10.65±0.01</td>
<td>17.86±0.10</td>
</tr>
<tr>
<td><em>C. mimosoides</em></td>
<td>DCM:MeOH (1:1)</td>
<td>141.53±0.21</td>
<td>16.86±0.35</td>
<td>98.83±0.01</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>64.16±0.13</td>
<td>5.32±0.38</td>
<td>16.19±0.05</td>
</tr>
<tr>
<td><em>B. salviifolia</em></td>
<td>DCM:MeOH (1:1)</td>
<td>169.66±0.33</td>
<td>23.95±0.11</td>
<td>92.42±0.63</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>77.92±0.91</td>
<td>12.11±0.26</td>
<td>51.80±0.34</td>
</tr>
<tr>
<td><em>S. brachypetala</em></td>
<td>DCM:MeOH (1:1)</td>
<td>303.91±0.92</td>
<td>4.24±0.23</td>
<td>19.65±0.82</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>291.80±0.12</td>
<td>13.44±0.08</td>
<td>12.17±0.07</td>
</tr>
<tr>
<td><em>S. brachypetala</em></td>
<td>bark</td>
<td>DCM:MeOH (1:1)</td>
<td>305.52±0.01</td>
<td>10.97±0.17</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td>337.66±0.12</td>
<td>17.71±0.54</td>
<td>163.04±0.86</td>
</tr>
</tbody>
</table>

Data represent mean±SD.

\(^a\) Expressed as mg tannic acid/g of dry plant material.

\(^b\) Expressed as mg quercetin/g of dry plant material.

\(^c\) Expressed as mg catechin/g of dry plant material.
Table 3
Antioxidant activity, represented by IC_{50} of the plant extracts, measured by the DPPH and ABTS radical scavenging tests.

<table>
<thead>
<tr>
<th>Extract</th>
<th>DPPH test</th>
<th>ABTS test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC_{50} (mg/ml)</td>
<td>IC_{50} (mg/ml)</td>
</tr>
<tr>
<td><strong>S. tilifolia</strong></td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>C. mimosoides</td>
<td>0.72±0.03</td>
<td>0.3±0.05</td>
</tr>
<tr>
<td>B. salvifolia</td>
<td>0.23±0.01</td>
<td>0.14±0.08</td>
</tr>
<tr>
<td>S. brachypetala root</td>
<td>0.05±0.02</td>
<td>3.26×10^{-9}</td>
</tr>
<tr>
<td>S. brachypetala bark</td>
<td>1.90±0.50</td>
<td>0.72±0.03</td>
</tr>
</tbody>
</table>

ND, not determined represents extracts with a maximum inhibition below 50% at the highest tested concentration of 2 mg/ml.

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References


