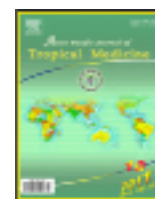


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## *In vitro* screening for acetylcholinesterase inhibition and antioxidant activity of medicinal plants from southern Africa

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## ABSTRACT

**Objective:** To determine the acetylcholinesterase inhibitory (AChEI) and antioxidant activity of the ethyl acetate and methanol extracts of 12 traditional medicinal plants used in the treatment of neurological disorders. **Methods:** AChEI activity was determined spectrophotometrically using the Ellman's colorimetric method. Antioxidant activity was carried out by determining the ability of the extracts to scavenge 2,2-diphenyl-1-picryl hydrazyl (DPPH) and 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radicals. The levels of total phenols, flavonoids and flavonols were determined quantitatively using spectrophotometric methods. **Results:** AChEI was observed to be dose-dependent. *Lannea schweinfurthii* (*L. schweinfurthii*) (Engl.) Engl. and *Scadoxus puniceus* (*S. puniceus*) (L.) Friis & I. Nordal. root extracts showed the lowest IC<sub>50</sub> value of 0.0003 mg/mL for the ethyl acetate extracts while *Zanthoxylum davyi* (*Z. davyi*) (I. Verd.) P.G. Watermann had the lowest IC<sub>50</sub> value of 0.01 mg/mL for the methanol extracts in the AChEI assay. The roots of *Piper capense* (*P. capense*) L.f., *L. schweinfurthii*, *Ziziphus mucronata* (*Z. mucronata*) Willd., *Z. davyi* and *Crinum bulbispermum* (*C. bulbispermum*) (Burm.f.) Milne-Redh. & Schweick. showed noteworthy radical scavenging activity and good AChEI activity. **Conclusions:** Five plants show good antioxidant and AChEI activity. These findings support the traditional use of the plants for treating neurological disorders especially where a cholinesterase mechanism and reactive oxygen species (ROS) are involved.

### 1. Introduction

Neurological disorders primarily affect the elderly population. Alzheimer's disease (AD), the most common neurodegenerative disorder is characterized clinically by progressive memory deficits and impaired cognitive function[1,2]. AD is estimated to account for between 50 and 60% of dementia cases in persons over 65 years of age and according to the United Nations, the number of people suffering from age-related neurodegeneration, particularly from AD, will exponentially increase from 25.5 million in 2000 to an estimated 114 million in 2050[3]. It is a major public health concern in developed countries due to the increasing number of sufferers, placing strains on caregivers

as well as on financial resources[2].

A deficiency in levels of the neurotransmitter acetylcholine (ACh) has been observed in the brains of AD patients, and inhibition of acetylcholinesterase (AChE), the key enzyme which hydrolyses ACh, is a major treatment option for AD[4]. Traditionally used plants have been shown to be good options in the search for AChE inhibitors. Galantamine, originally isolated from plants of the Amaryllidaceae family, has become an important treatment of AD[5]. The AChE inhibitory activity of this drug is the principal mode of action to provide symptomatic relief. Galantamine increases the availability of ACh in the cholinergic synapse by competitively inhibiting the enzyme responsible for its breakdown, AChE. The binding of galantamine to AChE slows down the catabolism of ACh and, as a consequence, ACh levels in the synaptic cleft are increased[6–9]. It is licensed in Europe for AD treatment and was well tolerated and significantly improved cognitive function when administered to AD patients in multi-center randomized-controlled trials[10]. To date, several plants

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have been identified as containing acetylcholinesterase inhibitory (AChEI) activity<sup>[11]</sup>.

Reactive oxygen species (ROS) generated from activated neutrophils and macrophages have been reported to play an important role in the pathogenesis of various diseases, including neurodegenerative disorders, cancer and atherosclerosis<sup>[12,13]</sup>. Oxidative processes are among the pathological features associated with the central nervous system in AD. Oxidative stress causes cellular damage and subsequent cell death especially in organs such as the brain. The brain in particular is highly vulnerable to oxidative damage as it consumes about 20% of the body's total oxygen, has a high content of polyunsaturated fatty acids and lower levels of endogenous antioxidant activity relative to other tissues<sup>[14–16]</sup>. The brain of patients suffering from AD is said to be under oxidative stress as a result of perturbed ionic calcium balances within their neurons and mitochondria<sup>[17,18]</sup>. Herbal products are reported to possess the ability to act as antioxidants, thereby reducing oxidative damage<sup>[19]</sup>. Among the natural phytochemicals identified from plants, flavonoids together with flavonols, and phenols represent important and interesting classes of biologically active compounds. Evidence suggests that these compounds are effective in the protection of various cell types from oxidative injury<sup>[20]</sup>.

The aim of the present study was to determine the AChEI and antioxidant activity of the ethyl acetate and methanol extracts of 12 plants, traditionally used in the treatment of neurological disorders.

## 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

Specimens investigated in this study were identified and voucher specimens deposited at the South African National Biodiversity Institute (SANBI), Tshwane. The plant samples were cut into small pieces and air-dried at room temperature. Dried material was ground to a fine powder and stored at ambient temperature till use. Six grams of the powdered plant material was extracted with 60 mL of either methanol or ethyl acetate for 24 h while shaking. The extracts were filtered, concentrated using a rotary vacuum evaporator and then further dried in vacuo at ambient

temperature for 24 h. All extracts were stored at  $-20\text{ }^{\circ}\text{C}$  prior to analysis. The residues were redissolved in either MeOH or ethyl acetate 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<sup>[21]</sup> as modified by Eldeen *et al*<sup>[22]</sup>. Into a 96-well plate was placed: 25  $\mu\text{L}$  of 15 mmol/L ATCI in water, 125  $\mu\text{L}$  of 3 mmol/L DTNB in Buffer A (50 mmol/L Tris-HCl, pH 8, containing 0.1 mol/L NaCl and 0.02 mol/L  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ ), 50  $\mu\text{L}$  of Buffer B (50 mmol/L, pH 8, containing 0.1 % bovine serum albumin) and 25  $\mu\text{L}$  of plant extract (0.007 mg/mL, 0.016 mg/mL, 0.031 mg/mL, 0.063 mg/mL or 0.125 mg/mL). Absorbance was determined spectrophotometrically (Labsystems Multiscan EX type 355 plate reader) at 405 nm at 45 s intervals, three times consecutively. Thereafter, AChE (0.2 U/mL) was added to the wells and the absorbance measured five times consecutively every 45 s. Galantamine 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:

$$\text{Inhibition (\%)} = 1 - (A_{\text{sample}}/A_{\text{control}}) \times 100$$

Where  $A_{\text{sample}}$  is the absorbance of the sample extracts and  $A_{\text{control}}$  is the absorbance of the blank [methanol/ethyl acetate in 50 mmol/L Tris-HCl, (pH 8)]. Extract concentration providing 50% inhibition ( $\text{IC}_{50}$ ) was obtained by plotting the percentage inhibition against extract concentration.

### 2.4. Determination of total phenolics

Total phenolic content was determined using the modified Folin-Ciocalteu method of Wolfe *et al*<sup>[23]</sup>. 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  $40\text{ }^{\circ}\text{C}$  for color development. Absorbance was measured at 765 nm using a Hewlett Packard UV-VIS spectrophotometer. Total phenolic content is expressed as mg/g gallic acid equivalent and was determined using the equation based on the calibration curve:  $Y = 6.993X + 0.037$ , where X is the absorbance and Y is the gallic acid equivalent (mg/g).

### 2.5. Determination of total flavonoids

Total flavonoid content was determined using the method of Ordonez *et al*<sup>[24]</sup>. A volume of 0.5 mL of 2%  $\text{AlCl}_3$  ethanol solution was added to 0.5 mL of sample solution (1 mg/mL). After one hour at room temperature, the absorbance was measured at 420 nm using a Hewlett Packard UV-VIS spectrophotometer. A yellow color is indicative of the presence of flavonoids. Total flavonoid content was

calculated as quercetin equivalent (mg/g), using the 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 flavonols

Total flavonol content was assessed using the method of Kumaran and Karunakaran[25]. To 2 mL of sample (1 mg/mL), 2 mL of 2%  $AlCl_3$  ethanol and 3 mL (50 g/L) sodium acetate solution were added. The samples were incubated for 2.5 h at 20 °C after which absorbance was determined at 440 nm. Total flavonoid content was calculated using the equation based on the calibration curve:  $Y = 0.0255X$ , where  $X$  was the absorbance and  $Y$  is the quercetin 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–Pathirana and Shahidi[26], with minor modifications. A solution of 0.135 mmol/L DPPH in methanol was prepared and 185  $\mu$ L of this solution was mixed with 15  $\mu$ L of varying concentrations of the extract (0.007 mg/mL, 0.016 mg/mL, 0.031 mg/mL, 0.063 mg/mL or 0.125 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 microplate reader. Trolox was used as the reference antioxidant compound. The ability to scavenge the DPPH radical was calculated using the equation:

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

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*[27] 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 mmol/L ABTS salt and 2.4 mmol/L potassium persulfate. The resultant  $ABTS^{*+}$  solution was diluted with methanol until an absorbance of  $0.706 \pm 0.001$  at 734 nm was obtained. Varying concentrations (0.007 mg/mL, 0.016 mg/mL, 0.031 mg/mL, 0.063 mg/mL or 0.125 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:

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

where  $A_{\text{control}}$  is the absorbance of ABTS radical + methanol

and  $A_{\text{sample}}$  is the absorbance of ABTS radical + sample extract/standard. The extract concentration providing 50% inhibition ( $IC_{50}$ ) was obtained by plotting inhibition percentage versus extract concentration.

## 2.8. Statistical analysis

All determinations were carried out on three occasions in triplicate. The results are reported as mean  $\pm$  standard deviation (S.D.). Calculation of  $IC_{50}$  values was done using GraphPad Prism Version 4.00 for Windows (GraphPad Software Inc.).

## 3. Results

Twelve plant species: roots of *Adenia gummifera* (*A. gummifera*) (Harv.) Harms (Passifloraceae), *Piper capense* (*P. capense*) L.f. (Piperaceae); *Zanthoxylum davyi* (*Z. davyi*) (I. Verd.) P.G. Watermann (Rutaceae), *Xysmalobium undulatum* (*X. undulatum*) (L.)W.T.Aiton. (Apocynaceae), *Lannea schweinfurthii* (*L. schweinfurthii*) (Engl.) Engl. (Anacardiaceae), *Terminalia sericea* (*T. sericea*) Burch. ex DC. (Combretaceae), *Ziziphus mucronata* (*Z. mucronata*) Willd. (Rhamnaceae), *Tabernaemontana elegans* (*T. elegans*) Stapf. (Apocynaceae), *Crinum bulbispermum* (*C. bulbispermum*) (Burm.f.) Milne–Redh. & Schweick. (Amaryllidaceae), *Scadoxus puniceus* (*S. puniceus*) (L.) Friis & I. Nordal. (Amaryllidaceae), *Tulbaghia violacea* (*T. violacea*) Harv. (Alliaceae) and fruits of *Ficus capensis* (*F. capensis*) Thunb. (Moraceae) were investigated for AChEI as these plants have been reported to treat various neurological conditions[28–39]. Ten of the plant species showed some level of inhibitory activity against AChE as indicated by their  $IC_{50}$  values (Table 1). At the highest concentration (0.125 mg/ml), 40% showed good (>50% inhibition), 50% moderate (30–50% inhibition) and 10% low (<30% inhibition) AChE inhibition[40]. *L. schweinfurthii* and *S. puniceus* root extracts showed the lowest  $IC_{50}$  values for the ethyl acetate extracts while *Z. davyi* had the lowest  $IC_{50}$  value for the methanol extracts (Table 1). Generally, inhibition of AChE was dose dependent and the ethyl acetate extracts were more active than the methanol extracts.

The ethyl acetate extracts of all the plants with the exception of *T. sericea* showed either no activity or very low radical scavenging activity in both the DPPH and ABTS assays as indicated by their  $IC_{50}$  values (Table 1). As the methanol extract showed higher activity, it would appear as if very polar solvents are able to extract compounds containing antioxidant activity. Methanol extracts of the roots of five plants and ethyl acetate of one plant showed radical scavenging activity < 50%.

The extracts which showed good DPPH and ABTS radical scavenging ability (> 60%) were further evaluated for their phenolic composition (Table 2). The levels of these phenolic

**Table 1**

AChEI, ABTS and DPPH radical scavenging activity of methanol and ethyl acetate extracts.

| Species                  | Extraction solvent (plant part) | AChE inhibition IC <sub>50</sub> (mg/mL) | ABTS radical inhibition IC <sub>50</sub> (mg/mL) | DPPH radical inhibition IC <sub>50</sub> (mg/mL) |
|--------------------------|---------------------------------|--|--|--|
| <i>A. gummifera</i>      | Ethyl acetate (root)            | 0.018 9±0.005                            | *  | *  |
| <i>P. capense</i>        | Methanol (root)                 | *  | 0.040 2±0.003                                    | 0.044 3±0.010                                    |
|                          | Ethyl acetate (root)            | 0.040 7±0.012                            | *  | *  |
| <i>Z. davyi</i>          | Methanol (root)                 | 0.010 0±0.004                            | 0.075 2±0.021                                    | *  |
|                          | Ethyl acetate (root)            | 0.011 6±0.002                            | *  | *  |
| <i>X. undulatum</i>      | Ethyl acetate (root)            | 0.000 5±0.000                            | *  | *  |
| <i>L. schweinfurthii</i> | Methanol (root)                 | *  | 0.003 6±0.001                                    | 0.015 1±0.004                                    |
|                          | Ethyl acetate (root)            | 0.000 3±0.000                            | *  | *  |
| <i>T. sericea</i>        | Methanol (root)                 | *  | 0.003 1±0.001                                    | 0.014 7±0.006                                    |
|                          | Ethyl acetate (root)            | *  | 0.074 6±0.017                                    | *  |
| <i>Z. mucronata</i>      | Methanol (root)                 | *  | 0.018 7±0.020                                    | 0.029 1±0.051                                    |
|                          | Ethyl acetate (root)            | 0.011 2±0.003                            | *  | *  |
| <i>F. capensis</i>       | Ethyl acetate (fruit)           | 0.031 9±0.005                            | *  | *  |
| <i>S. puniceus</i>       | Ethyl acetate (bulb)            | 0.000 3±0.000                            | *  | *  |
| <i>C. bulbispermum</i>   | Ethyl acetate (root)            | 0.039 3±0.014                            | *  | *  |
|                          | Methanol (bulb)                 | 0.014 8±0.039                            | 0.068 5±0.041                                    | *  |
|                          | Ethyl acetate (bulb)            | 0.002 1±0.007                            | *  | *  |
| Galanthamine             | N/A                             | 5.3×10 <sup>-5</sup>                     | N/A  | N/A  |
| Trolox                   | N/A                             | N/A                                      | 0.013 1  | 9.6×10 <sup>-6</sup>                             |

\*Represents extracts with maximum inhibition below 50% at the highest tested concentration of 0.125 mg/mL.

**Table 2**

Total phenol, flavonoid and flavonol contents of the methanolic plant extracts with antioxidant activity (&gt; 60%).

| Plant and part                 | Total phenol <sup>a</sup> | Total flavonoid <sup>b</sup> | Total flavonol <sup>b</sup> |
|--------------------------------|---------------------------|------------------------------|-----------------------------|
| <i>Z. davyi</i> roots          | 97.26±0.40                | 8.66±0.40                    | 22.84±0.10                  |
| <i>L. schweinfurthii</i> roots | 101.27±0.10               | 13.58±0.30                   | 17.29±0.60                  |
| <i>T. sericea</i> roots        | 36.73±0.21                | 73.05±0.40                   | 28.78±0.50                  |
| <i>Z. mucronata</i> roots      | 73.86±0.25                | 17.76±0.20                   | 15.53±0.30                  |
| <i>C. bulbispermum</i> roots   | 202.38±0.50               | 9.18±0.50                    | 20.79±0.10                  |
| <i>P. capense</i> roots        | 237.60±0.12               | 18.14±0.20                   | 12.90±0.10                  |

<sup>a</sup>Expressed as mg tannic acid/g of extract. <sup>b</sup>Expressed as mg quercetin/g of extract.

compounds are an indication of the potential antioxidant activity of the plant extracts. The methanol extracts of *T. sericea* roots contained the highest flavonoid and flavonol content.

#### 4. Discussion

*Z. davyi* roots showed good AChEI with IC<sub>50</sub> values of 0.01 mg/mL and 0.012 mg/mL for the methanol and ethyl acetate extracts respectively. Seven benzo[c]phenanthridine alkaloids have been isolated from the stem–bark of *Z. davyi*<sup>[41]</sup>, and these or similar alkaloids may be responsible for its observed inhibition of acetylcholinesterase. Also,

anticonvulsant activity has been reported for both the methanol and aqueous leaf extracts of *Z. capense*<sup>[42]</sup>. As convulsion is a neurologic disorder, similar compounds present in the roots of *Z. davyi* may be responsible for its activity and this supports the traditional use of the plant in the treatment of neurologic diseases. *Z. capense* leaves have also been shown to contain triterpene steroids and saponins and these compounds are known to exhibit neuroprotective activity<sup>[43]</sup>. The ethyl acetate extracts of *C. bulbispermum* bulbs showed an IC<sub>50</sub> value of 0.039 mg/ml for AChEI, which may be ascribed to several alkaloids which have been isolated from the plant<sup>[44]</sup>. In addition alkaloidal extracts from *Crinum jagus* and *C. glaucum* have been demonstrated to possess AChEI activity which

has been ascribed to hamayne ( $IC_{50}$ –250  $\mu$  mol/L) and lycorine ( $IC_{50}$ –450  $\mu$  mol/L)[45]. Furthermore, the alkaloids; haemanthamine and lycorine, isolated from *C. ornatum*, have been shown to contain anticonvulsant activity[46]. It is possible that the presence of these or similar alkaloids may be responsible for the activity observed. The ethyl acetate extract of *Piper capense* was observed to show inhibition of AChEI with an  $IC_{50}$  value of 0.041 mg/mL. Amide alkaloids with activity in the CNS have been identified from the roots of *P. guineense*[28]. *P. methysticum* has been reported to possess local anaesthetic, sedating, anticonvulsive, muscle-relaxant and sleep-stimulating effects which are due to the presence of kavopyrones[28]. *P. capense* contains the amide alkaloids; piperine and 4,5-dihydropiperine, which have previously been shown to have CNS activity[47]. Also, piperine has been reported to improve memory impairment and neurodegeneration in the hippocampus of animal models with AD[48]. The ethanol extracts of *X. undulatum* were found to exhibit good antidepressant-like effects in three animal models[49]. The leaves of this plant have also been reported to have good selective serotonin re-uptake inhibitory activity[50]. The neuroprotective effect of the plant has been ascribed to several glycosides[29], which may be responsible for its observed activity as its ethyl acetate extracts showed inhibition of the enzyme with  $IC_{50}$  value of 0.000 5 mg/mL. Glycosides are among the class of compounds which show neuroprotective activity. Four pregnane glycosides; cynatoside A, cynatoside B, cynatoside C and cynascyroside D, have been isolated from *C. atratum*[51–53]. These glycosides showed AChE inhibition with  $IC_{50}$  values varying between 3.6  $\mu$  mol/L for cynatoside B and 152.9  $\mu$  mol/L for cynascyroside D[51–53].

Polar solvents have been reported to extract compounds including alkaloids which show cholinesterase inhibitory activity[22]. This explains the use of methanol and ethyl acetate as solvents for extraction in this study. As the ethyl acetate extracts showed better activity for most of the plants, it may appear as if the solvent is able to extract more of the compounds which inhibit AChE.

Several Anacardiaceae species including *Lannea velutina*, *Sclerocarya birrea* and *Harpophyllum caffrum* have been shown to be a source of natural antioxidants. This activity has been ascribed to the high levels of proanthocyanidins and gallotannins present in the plants[54]. As *L. schweinfurthii*, belongs to the same family, similar compounds could be present and therefore responsible for its good antioxidant activity, as its methanol extracts showed an  $IC_{50}$  value of 0.003 6 mg/mL for inhibition of ABTS radicals. *P. capense* showed good antioxidant activity ( $IC_{50}$  value of 0.040 2 mg/mL and 0.044 3 mg/mL for inhibition of ABTS and DPPH radicals) which has also been reported for other *Piper* species; *P. arboreum* and *P. tuberculatum*[55–57]. This activity has been ascribed to the flavonols; quercetin

and quercitrin[58]. The leaves and roots of *T. sericea* are reported to be used traditionally in treating several infections and diseases. Sericoside, the triterpenoidal saponin found in *T. sericea* has been reported to have anti-inflammatory and antioxidant activity[59]. Sericoside acts by reducing neutrophil infiltration and decreasing superoxide generation due to its radical scavenging activity[60] and it may be responsible for the antioxidant activity of the plant as observed in the study. *C. ornatum* bulbs have been shown to contain good inhibition of DPPH radicals and hydrogen peroxide as well as being able to inhibit peroxidation of tissue lipids in the malonaldehyde test[30]. Similar to the AChEI activity, lycorine and haemanthamine have been reported to be responsible for the antioxidant activity[46].

The total phenolic content of the methanol extracts of *P. capense* and *C. bulbispermum* roots were relatively high for both solvents tested. Phenolic compounds contribute to the antioxidant activity of plant extracts and they are well known as radical scavengers, metal chelators, reducing agents, hydrogen donors and singlet oxygen quenchers[60].

Flavonoids have been reported to be partly responsible for antioxidant activity, as they act on enzymes and pathways involved in anti-inflammatory processes[61]. Furthermore, the hydrogen-donating substituents (hydroxyl groups) attached to the aromatic ring structures of flavonoids enable them to undergo a redox reaction, which in turn, helps them scavenge free radicals[62].

Flavonols are phytochemical compounds found in high concentrations in a variety of plant-based foods and beverages[58]. Consumption of flavonols has been associated with a variety of beneficial effects including an increase in erythrocyte superoxide dismutase activity, decrease in lymphocyte DNA damage, decrease in urinary 8-hydroxy-2'-deoxyguanosine, and an increase in plasma antioxidant capacity[58].

The roots of *P. capense*, *Z. capense*, *L. schweinfurthii*, *Z. mucronata* and *C. bulbispermum* showed good antioxidant and cholinesterase inhibitory activity. These findings support the traditional use of the plants for treating neurological disorders especially those where a cholinesterase mechanism and reactive oxygen species are involved. These novel leads require further investigation.

### Conflict of interest statement

We declare that we have no conflict of interest.

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## References

- [1] Mattson MP. Pathways towards and away from Alzheimer's disease. *Nature* 2004; **430**: 631–639.
- [2] Houghton PJ, Howes MJ. Natural products and derivatives affecting neurotransmission relevant to Alzheimer's and Parkinson's disease. *Neurosignals* 2005; **14**: 6–22.
- [3] Wimo A, Winblad B, Aguero-Torres H, von Strauss E. The magnitude of dementia occurrence in the world. *Alzheimer Dis Assoc Disord* 2003; **17**(2): 63–67.
- [4] Şenol FS, Orhan İ, Yılmaz G, Çiçek M, Şener B. Acetylcholinesterase, butyrylcholinesterase, and tyrosinase inhibition studies and antioxidant activities of 33 *Scutellaria* L. taxa from Turkey. *Food Chem Toxicol* 2010; **48**: 781–788.
- [5] Eckert GP. Traditional used plants against cognitive decline and Alzheimer's disease. *Front Pharmacol* 2010; doi: 10.3389/fphar.2010.00138
- [6] Thomsen T, Zendeh B, Fischer JP, Kewitz H. *In vitro* effects of various cholinesterase inhibitors on acetyl- and butyrylcholinesterase of healthy volunteers. *Biochem Pharmacol* 1991a; **41**: 139–41.
- [7] Thomsen T, Kaden B, Fischer P, Bickel U, Barz H, Gusztory G, et al. Inhibition of acetylcholinesterase activity in human brain tissue and erythrocytes by galanthamine, physostigmine and tacrine. *Eur J Clin Biochem* 1991b; **29**: 487–92.
- [8] Bores GM, Huger FP, Petko W, Mutlib AE, Camacho F, Rush DK, et al. Pharmacological evaluation of novel Alzheimer's disease therapeutics: acetylcholinesterase inhibitors related to galanthamine. *J Pharmacol Exp Ther* 1996; **277**(2): 728–38.
- [9] Heinrich M, Teoh HL. Galanthamine from snowdrop – the development of a modern drug against Alzheimer's disease from local Caucasian knowledge. *J Ethnopharmacol* 2004; **92**: 147–162.
- [10] Lopez S, Bastida J, Viladomat F, Codina C. Acetylcholinesterase inhibitory activity of some Amaryllidaceae alkaloids and *Narcissus* extracts. *Life Sci* 2002; **71**(21): 2521–2529.
- [11] Adewusi AE, Moodley N, Steenkamp V. Medicinal plants with cholinesterase inhibitory activity: a review. *Afr J Biotechnol* 2010; **9**(49): 8257–8276.
- [12] Winrow VR, Winyard PG, Morris CJ, Blake DR. Free radicals in inflammation: second messengers and mediators of tissue destruction. *Brit Med Bull* 1993; **49**(3): 506–522.
- [13] Confortia F, Sosa S, Marrelli M, Menichini F, Statti G, Uzunov D, et al. *In vivo* anti-inflammatory and *in vitro* antioxidant activities of Mediterranean dietary plants. *J Ethnopharmacol* 2007; **116**(1): 144–151.
- [14] Halliwell B, Gutteridge JMC. Oxygen radicals in the nervous system. *Trends Neurosci* 1985; **8**: 22–6.
- [15] Floyd RA, Hensley K. Oxidative stress in brain aging. Implications for therapeutics of Neurodegenerative diseases. *Neurobiol Aging* 2002; **23**: 795–807.
- [16] Shulman RG, Rothman DL, Behar KL, Hyder F. Energetic basis of brain activity: implications for neuroimaging. *Trends Neurosci* 2004; **27**: 489–495.
- [17] Emilien G, Beyreuther K, Master CL, Maloteaux JM. Prospects for pharmacological intervention in Alzheimer's disease. *Arch Neurol* 2000; **57**(4): 454–459.
- [18] Tabet N. Acetylcholinesterase inhibitors for Alzheimer's disease: anti-inflammatories in acetylcholine clothing. *Age Ageing* 2006; **35**(4): 336–338.
- [19] Siddhuraju P, Becker K. The antioxidant and free radical scavenging activities of processed cowpea (*Vigna unguiculata* (L.) Walp.) seed extracts. *Food Chem* 2007; **101**: 10–19.
- [20] Zou Y-P, Lu Y-H, Wei D-Z. Protective effects of a flavonoid-rich extract of *Hypericum perforatum* L. against hydrogen peroxide-induced apoptosis in PC12 cells. *Phytother Res* 2010; **24**(Suppl 1): S6–S10.
- [21] Ellman GL, Courtney D, Andies V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol* 1961; **7**: 88–95.
- [22] Eldeen IMS, Elgorashi EE, van Staden J. Antibacterial, anti-inflammatory, anti-cholinesterase and mutagenic effects of extracts obtained from some trees used in South African traditional medicine. *J Ethnopharmacol* 2005; **102**: 457–464.
- [23] Wolfe K, Wu X, Liu RH. Antioxidant activity of apple peels. *J Agric Food Chem* 2003; **51**(3): 609–614.
- [24] Ordonez AAL, Gomez JD, Vattuone MA, Isla MI. Antioxidant activities of *Sechium edule* (Jacq.) Swart extracts. *Food Chem* 2006; **97**: 452–458.
- [25] Kumaran A, Karunakaran RJ. *In vitro* antioxidant activities of methanol extracts of *Phyllanthus* species from India. *LWT* 2007; **40**: 344–352.
- [26] Liyana-Pathiranan CM, Shahidi F. Antioxidant activity of commercial soft and hard wheat (*Triticum aestivum* L.) as affected by gastric pH conditions. *J Agric Food Chem* 2005; **53**: 2433–2440.
- [27] Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med* 1999; **26**: 1231–1237.
- [28] Gomes NGM, Campos MG, Órfão JMC, Ribeiro CAF. Plants with neurobiological activity as potential targets for drug discovery. *Prog Neuro-Psychopharmacol Biol Psychiatry* 2009; **33**: 1372–1389.
- [29] Hutchings A, Scott AH, Lewis G, Cunningham AB. *Zulu medicinal plants: an inventory*. Pietermaritzburg: University of Natal Press; 1996.
- [30] Oloyede GK, Farombi EO. Antioxidant properties of *Crinum ornatum* bulb extract. *World J Chem* 2010; **5**: 32–36.
- [31] Bryant AT. *Zulu medicine and medicine-men*. Cape Town: Struik; 1996.
- [32] Gelfand M, Mavi S, Drummond RB, Ndemera B. *The traditional medical practitioner in Zimbabwe*. Qweru, Zimbabwe: Mambo

- Press; 1985.
- [33]van Wyk B, Gericke N. *Peoples plants*. Pretoria, South Africa: Briza Publications; 2000.
- [34]Watt JM, Breyer-Brandwijk MG. *The medicinal and poisonous plants of Southern and Eastern Africa*. 2nd ed. London: E and S Livingstone Ltd; 1962.
- [35]Mabogo DEN. The ethnobotany of the Vhavenda. MSc thesis, University of Pretoria; 1990.
- [36]Taesotikul T, Panthong A, Kanjanapothi D, Verpoorte R, Scheffer JJ. Neuropharmacological activities of the crude alkaloidal fraction from the stems of *Tabernaemontana pandacaqui* Poir. *J Ethnopharmacol* 1998; **62**: 229–234.
- [37]Ingkaninan K, Temkitthawon P, Chuenchom K, Yuyaem T, Thongnoi W. Screening for acetylcholinesterase inhibitory activity in plants used in Thai traditional rejuvenating and neurotonic remedies. *J Ethnopharmacol* 2003; **89**: 261–264.
- [38]Gamaliel K, Amos S, Chindo B, Wambebe C, Vongtau H, Olusola A. Behavioral effects of the methanol extract of *Ficus platyphylla* bark in mice and rats. *J Neurosci* 2000; **3**: 17–23.
- [39]Veale DJH, Furman KI, Oliver DW. South African traditional herbal medicines used during pregnancy and child-birth. *J Ethnopharmacol* 1992; **36**: 185–191.
- [40]Vinutha B, Prashanth D, Salma K, Sreeja SL, Pratiti D, Padmaja R, et al. Screening of selected Indian medicinal plants for acetylcholinesterase inhibitory activity. *J Ethnopharmacol* 2007; **109**(2), 359–363.
- [41]Amabeoku GJ, Kinyua CG. Evaluation of the anticonvulsant activity of *Zanthoxylum capense* (Thumb.) Harv. (Rutaceae) in mice. *Int J Pharmacol* 2010; **6**: 844–853.
- [42]Chauhan AK, Dobhal MP, Joshi BC. A review of medicinal plants showing anticonvulsant activity. *J Ethnopharmacol* 1988; **22**(1): 11–23.
- [43]Tarus PK, Coombes PH, Crouch NR, Mulholland DA. Benzo[e]phenanthridine alkaloids from stem bark of the Forest Knobwood, *Zanthoxylum davyi* (Rutaceae). *South Afri J Bot* 2006; **72**: 555–558.
- [44]Elgorashi EE, Stafford GI, van Staden J. Acetylcholinesterase enzyme inhibitory effects of Amaryllidaceae alkaloids. *Planta Medica* 2004; **70**: 260–262.
- [45]Houghton PJ, Agbedahunsi JM, Adegbulugbe A. Cholinesterase inhibitory properties of alkaloids from two Nigerian *Crinum* species. *Phytochemistry* 2004; **65**(21): 2893–2896.
- [46]Oloyede GK, Oke JM, Raji Y, Olugbade TA. Antioxidant and anticonvulsant alkaloids in *Crinum ornatum* bulb extract. *World J Chem* 2010; **5**(1): 26–31.
- [47]Pedersen ME, Metzler B, Stafford GI, van Staden J, Jäger AK, Rasmussen HB. Amides from *Piper capense* with CNS activity—a preliminary SAR analysis. *Molecules* 2009; **14**(9): 3833–3843.
- [48]Chonpathompikunlert P, Wattanathorn J, Muchimapura S. Piperine, the main alkaloid of Thai black pepper, protects against neurodegeneration and cognitive impairment in animal model of cognitive deficit like condition of Alzheimer's disease. *Food Chem Toxicol* 2010; **48**: 798–802.
- [49]Pedersen ME, Szewczyk B, Stachowicz K, Wieronska J, Andersen J, Stafford GI, et al. Effects of South African traditional medicine in animal models for depression. *J Ethnopharmacol* 2008; **119**: 542–548.
- [50]Nielsen ND, Sandager M, Stafford GI, van Staden J, Jäger AK. Screening of indigenous plants from South Africa for affinity to the serotonin reuptake transport protein. *J Ethnopharmacol* 2004; **94**: 159–163.
- [51]Maiga A, Malterud EK, Mathisen HG, Paulsen ER, Thomas-Oates J, Bergström E, et al. Cell protective antioxidants from the root bark of *Lannea velutina* A. Rich., a Malian medicinal plant. *J Med Plants Res* 2007; **1**: 66–79.
- [52]Lee KY, Sung SH, Kim YC. New acetylcholinesterase-inhibitory pregnane glycoside of *Cynanchum atratum* roots. *Helv Chem Acta* 2003; **86**: 474–83.
- [53]Lee KY, Yoon YS, Kim ES, Kang SY, Kim YC. Anti-acetylcholinesterase and anti-amnesic activities of a pregnane glycoside, cynatroside B, from *Cynanchum atratum*. *Planta Med* 2005; **71**: 7–11.
- [54]Hostettmann K, Borloz A, Urbain A, Marston A. Natural product inhibitors of acetylcholinesterase. *Curr Org Chem* 2006; **10**: 825–847.
- [55]Regasini LO, Cotinguiba F, Siqueira RJ, Bolzani SV, Silva SHD, Furlan M, et al. Radical scavenging capacity of *Piper arboreum* and *Piper tuberculatum* (Piperaceae). *Lat Am J Pharm* 2008; **27**: 900–903.
- [56]Erukainure OL, Ajiboye JA, Adejobi RO, Okafor OY, Adenekan SO. Protective effect of pineapple (*Ananas cosmosus*) peel extract on alcohol-induced oxidative stress in brain tissues of male albino rats. *Asian Pac J Trop Dis* 2011; **1**(1): 5–9.
- [57]Thirumalai T, Therasa SV, Elumalai EK, David E. Intense and exhaustive exercise induce oxidative stress in skeletal muscle. *Asian Pac J Trop Dis* 2011; **1**(1): 63–66.
- [58]Williamson G, Manach C. Bioavailability and bioefficacy of polyphenols in humans. II. Review of 93 intervention studies. *Am J Clin Nutr* 2005; **81**: 243S–255S.
- [59]Mochizuki M, Hasegawa N. Anti-inflammatory effect of extract of *Terminalia sericea* roots in an experimental model of colitis. *J Health Sci* 2007; **53**: 329–331.
- [60]Khadri A, Neffati M, Smiti S, Falé P, Lino RL, Serralheiro MLM, et al. Antioxidant, antiacetylcholinesterase and antimicrobial activities of *Cymbopogon schoenanthus* L. Spreng (lemon grass) from Tunisia. *LWT* 2010; **43**: 331–336.
- [61]Araújo TAS, Alencar NL, de Amorim ELC, de Albuquerque UP. A new approach to study medicinal plants with tannins and flavonoid contents from the local knowledge. *J Ethnopharmacol* 2008; **120**: 72–80.
- [62]Brand-Williams W, Cuvelier ME, Berset C. Use of a free-radical method to evaluate antioxidant activity. *LWT* 1995; **28**: 25–30.