

ANTIOXIDANT AND ACETYLCHOLINESTERASE ACTIVITIES OF THREE SPECIES OF THE FAMILY LAMIACEAE

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Abstract

Antioxidant and acetylcholinesterase inhibitory activity of the three species namely, *Endostemon obtusifolius* (E. Mey. ex Benth.) N. E. Br, *Plectranthus zuluensis* (T. Cooke) and *Tetradenia riparia* (Hochst.) Codd. were evaluated. Maximum antioxidant activity was exhibited by *E. obtusifolius* (IC₅₀ 130 µg/ml) followed by *T. riparia* (IC₅₀ 142 µg/ml) and *P. zuluensis* (IC₅₀ 169 µg/ml). Acetylcholinesterase inhibition (AChEI) was highest in *P. zuluensis* (IC₅₀ 290 µg/ml) followed by *E. obtusifolius* (IC₅₀ 470 µg/ml) and *T. riparia* (IC₅₀ 750 µg/ml). The results suggest that these three species possess natural antioxidants and acetylcholinesterase inhibitors, which may be beneficial for the treatment of Alzheimer's disease, that require high concentration of these compounds.

Introduction

In the symptomatic treatment of Alzheimer's disease (AD), enhanced cholinergic activity is achieved when the key enzyme responsible for ACh hydrolysis, acetylcholinesterase (AChE), is inhibited (Adewusi *et al.* 2011). Acetylcholine inhibitors (AChEIs) should function by preventing acetylcholine hydrolysis, thereby restoring ACh levels at the synapse (Giacobini 1998, Krall *et al.* 1999). Presently, AChE inhibitors cause adverse side effects therefore the drug discovery has now been directed to alternative AChE inhibitors from natural sources (Adewusi *et al.* 2011).

Oxidative stress, triggered by reactive oxygen species (ROS), is regarded as one of the earliest occurrences in AD patients. Currently, there is increased interest in antioxidants from plants to prevent oxidative damage and attenuate the effects of neurodegenerative disorders (Fusco *et al.* 2007, Amoo *et al.* 2012).

Studies indicate that decoctions of the leaves of *Plectranthus barbatus* contain rosmarinic acid, which has pronounced acetylcholinesterase and antioxidant activity (Fale *et al.* 2009). However, there is no record of the activity of *Plectranthus zuluensis* and *Endostemon obtusifolius* extracts. Decoctions of *Tetradenia riparia* have been widely utilized in traditional medicine, but its' cholinergic and antioxidant effect is yet to be investigated (Gairola *et al.* 2009). Plants in the family Lamiaceae are regarded as having great antioxidant potential and play a significant role in the protection against free radicals (Dorman *et al.* 2004, Erdemoglu *et al.* 2006, Orhan *et al.* 2007).

In this study the ethanolic extracts of three species of the Lamiaceae: *E. obtusifolius*, *P. zuluensis* and *T. riparia* were evaluated for their antioxidant and acetylcholinesterase activity.

Materials and Methods

P. zuluensis (Coll by R.G. Strey 7350000), *E. obtusifolius* (Coll by C.J. Ward 7345010) and *T. riparia* (Coll by C.J. Ward 7357a) were collected in Durban, KwaZulu-Natal during March 2011. Voucher specimens for all these plants were prepared and deposited at the Ward Herbarium, School of Life Sciences, University of KwaZulu-Natal, Durban, South Africa.

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Leaves from the three species were air dried at room temperature and then milled to powder form. The leaf powder (250 g) was extracted using a Soxhlet apparatus with ethanol. The resultant extracts were filtered and concentrated to dryness under reduced pressure in a rotary evaporator and stored at 4°C for further use.

The free radical scavenging activity of the crude extracts was determined by the 2,2-diphenylpicrylhydrazyl (DPPH) assay described by Blois (1958) with slight modification. A 0.1 mM solution of DPPH in methanol was prepared and 4 ml of this solution were added to 1 ml of sample solution in methanol at different concentrations. All the assays were carried out in triplicate. The absorbance was read at 510 nm. Percentage DPPH scavenging activity was determined as follows:

$$\text{DPPH scavenging activity (\%)} = (A_0 - A_1) / A_0 \times 100$$

where A_0 is absorbance of standard DPPH solution only and A_1 is the absorbance of the reaction mixture or standard antioxidant.

A male Wistar rat (150 g) was sacrificed by cervical dislocation; the brain was immediately excised and homogenized with cold 0.1 mM sodium phosphate buffer (pH 7.0). The homogenate was stored at -80°C. Acetylcholinesterase inhibitory activity was measured following the method of Ellman *et al.* (1961). The percentage of AChE inhibition was determined with the help of the following equation:

$$\text{Percentage inhibition} = \frac{\text{Control} - \text{Extract} \times 100}{\text{Control}}$$

The total phenolics content was determined by the Folin-Ciocalteu assay (Singleton *et al.* 1965). Absorbance was determined at 550 nm with an UV-Visible spectrophotometer. Different gallic acid standard solutions (20, 40, 60, 80 and 100 µg/ml) were used for obtaining a standard curve (Singleton *et al.* 1965). Total phenolic content was expressed as mg of gallic acid equivalents (GAE) per gram of extract.

Total flavonoid content was measured by the aluminum chloride colorimetric assay (Zhishen *et al.* 1999). The solution was mixed and the absorbance measured against the blank at 510 nm. The total flavonoid content was expressed as mg quercetin equivalents (QE).

Results and Discussion

The antioxidant and acetylcholinesterase inhibitory activities of three species of Lamiaceae were evaluated in this study. Disturbance and insufficient cholinergic functions are identified among the pathological features of central nervous system disorders of the test rats. The most important changes observed in the brain are a decrease in cortical levels of the neurotransmitter acetylcholine. Inhibition of acetylcholinesterase activity, therefore, can restore levels of acetylcholine in the brain. The ethanolic extracts of *E. obtusifolius*, *P. zuluensis* and *T. riparia* (Table 1) showed good antioxidant and acetyl cholinesterase inhibitory activities. Acetyl cholinesterase inhibition was highest in *P. zuluensis* (IC₅₀ 290 µg/ml) followed by *E. obtusifolius* (IC₅₀ 470 µg/ml) and *T. riparia* (IC₅₀ 750 µg/ml). Plants have traditionally been used to enhance cognitive function and to alleviate other symptoms associated with Alzheimer's disease (Howes and Houghton 2003). Similar species in Lamiaceae, such as *Salvia*, has been traditionally used in European folk medicine to improve memory (Adewusi *et al.* 2011). The degeneration of neurons by oxidative stress is a significant symptom at the onset of Alzheimer's disease. Therefore, the ability to scavenge free radicals is an important mechanism to treat patients suffering from degenerative diseases. The hydrogen donating capacity of the polyphenolic compounds is

responsible for inhibition of free radicals. The potency of the extracts to scavenge radicals is inversely proportional to the inhibitory concentration. Maximum antioxidant activity was exhibited by *E. obtusifolius* (IC₅₀ 130 µg/ml), followed by *T. riparia* (IC₅₀ 142 µg/ml) and *P. zuluensis*, (IC₅₀ 169 µg/ml), compared to standard BHA (Butylated hydroxyanisole) in the DPPH assay (Table 1). The efficacy of the antioxidant potential of the above extracts is affected by many factors: the part of the plant analysed, as well as time of harvesting, climatic conditions, storage and processing of the plant material (Jayachitra and Krithiga 2012). Further research needs to be conducted to elucidate the precise compounds in the ethanolic extract that could be responsible for the relative DPPH activities.

Studies have shown that antioxidant activity correlated strongly to aromatic, phenolic and flavonoid contents since these compounds can undergo redox reactions and therefore scavenge free radicals (Adewusi *et al.* 2011, Ghimire *et al.* 2011). Phenolic compounds such as gallotannins, condensed tannins and flavonoids contribute directly to the antioxidant activity of plant extracts (Proestos *et al.* 2006). The total phenolic content was least in *E. obtusifolius* (15.1 µg), intermediate in *P. zuluensis* (17.5 µg) and greatest in *T. riparia* (26.2 µg) (Fig. 1). Since any phenolic compound (including aromatic amines), ascorbic acid and sugar reduce the Folin-Ciocalteu reagent; over-estimation of total phenolic content by this method could have affected the results of this assay (Siow and Hui 2013). Consequently, the high phenolic content of *T. riparia* resulted in a relatively intermediate antioxidant activity, compared to *E. obtusifolius* and *P. zuluensis*. Similarly, a low antioxidant activity (Table 1) was exhibited by *P. zuluensis* extracts, although there is an intermediate concentration of total phenolic content (Fig. 1). These results suggest that there may be other molecules responsible for conferring antioxidant activity in these species (Ghimire *et al.* 2011). Other classes of compounds that may have free radical scavenging capabilities, such as alkaloids, are currently used to treat Alzheimer's disease (Adewusi *et al.* 2011, Dell'Acqua 2013).

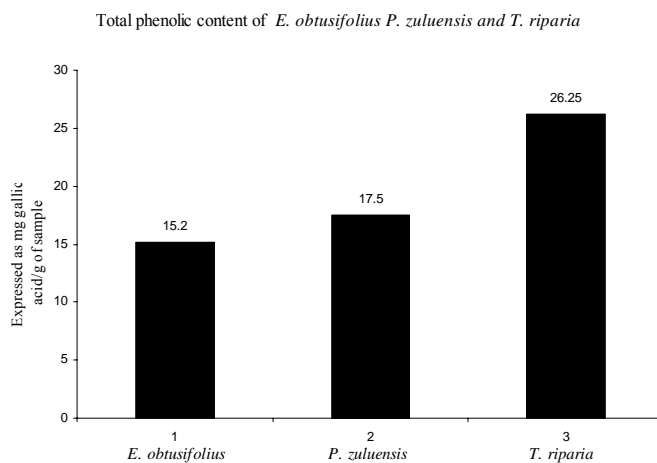


Fig. 1. Mean total phenolic content expressed as gallic acid equivalent of ethanolic leaf extracts of *E. obtusifolius*, *P. zuluensis* and *T. riparia*.

The flavonoid content was greatest in *E. obtusifolius* (7.0 µg), intermediate in *T. riparia* (0.6 µg) and least in *P. zuluensis* (0.42 µg) (Fig. 2). The high flavonoid content is possibly responsible for the high antioxidant activity in *E. obtusifolius* (Table 1).

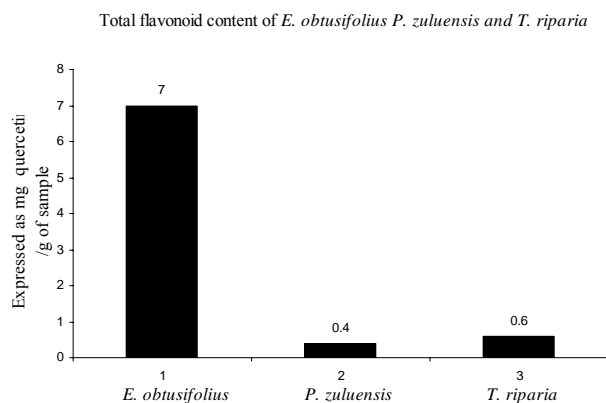


Fig. 2. Mean total flavonoid content expressed as quercetin equivalent of ethanolic leaf extracts of *E. obtusifolius*, *P. zuluensis* and *T. riparia*.

Table 1. DPPH free radical scavenging activity and AChE inhibition of ethanolic leaf extracts of *E. obtusifolius*, *P. zuluensis* and *T. ripari* using BHA and neostigmine as standards.

Extracts	DPPH IC ₅₀ µg/ml	AChE inhibition IC ₅₀ µg/ml
<i>E. obtusifolius</i>	130	470
<i>P. zuluensis</i>	169	290
<i>T. riparia</i>	142	750
Neostigmine	-	37.5
BHA	10	-

Plant extracts used in dementia therapy vary according to culture and tradition. Several phyto-constituents can be exploited for their antioxidant and anti cholinesterase activities based on their use in traditional medicines. The extracts from these three plant species exhibited good antioxidant and acetyl cholinesterase inhibition. The data suggest that these species could be used as natural sources of antioxidant and acetyl cholinesterase inhibitors, and could possibly be used in the treatment of Alzheimer's disease. However, their potential beneficial effects and efficacy in humans require further clinical research.

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