International Scholars Journals

International Journal of Pharmacy and Pharmacology ISSN: 2326-7267 Vol. 3 (8), pp. 001-007, August, 2012. Available online at www.internationalscholarsjournals.org © International Scholars Journals



*Full Length Research Paper*

# Kinetics of acetylcholinesterase inhibitory activities by aqueous extracts of *Acacia nilotica* (L.) and *Rhamnus prinoides* (L'Hér.)

**Catherine Megan Crowch[1] and Edward Jonathan Okello[2]***

[1]School of Biomedical Sciences, Newcastle University, United Kingdom.

[2]Medicinal Plant Research Group and Institute of Neuroscience, Newcastle University, United Kingdom.

Accepted 13 April, 2012

**Acetylcholinesterase (AChE) is a key target in the treatment of Alzheimer's disease (AD). We studied the potential anti-AChE activities of *Acacia nilotica* (Leguminosae) and *Rhamnus prinoides* (Rhamnaceae) plants that have previously been shown to affect central nervous system activities. Sonicated aqueous extracts of *A. nilotica* and *R. prinoides* displayed significant AChE inhibition by about 56 and 53%, respectively, after 5 min incubation at 0.1mg/ml final assay concentration. Inhibition kinetics showed both plant preparations to be mixed inhibitors (specifically non- competitive uncompetitive type). Galanthamine was assayed as a positive control and was found to be a very potent mixed type (competitive non -competitive) inhibitor; $IC_{50}$ of 0.0004 mg/ml compared to 0.079 mg/ml for *A. nilotica* and 0.201 mg/ml for *R. prinoides*. We conclude that although the AChE inhibition by *A. nilotica* and *R. prinoides* is not as potent as that of galanthamine, in addition to their known antioxidant and anti-inflammatory activities these plants could provide novel poly-pharmacological leads of potential benefit to the treatment of AD and therefore warrant further investigation.**

**Key words:** Alzheimer's disease, acetylcholinesterase, enzyme inhibition, kinetics, *Acacia nilotica*, *Rhamnus prinoides*.

## INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disease affecting the brain. It is the most common cause of dementia, leading to deterioration in vital cognitive processes such as memory (Barnes et al., 2006), understanding (Verdon et al., 2007), and speech (Forbes-McKay et al., 2005). Symptoms can also include unpleasant behavioural changes, such as anxiety and dysphoria (Mega et al., 1996). The aetiology of AD is complex as it involves many factors, all of which contribute and produce damage to the cortical nervous system (Reiman, 2006) . There is evidence that in some people the cause may be due to genetic factors. Four genes have been identified as possible sources of the problem (Parihar and Hemmani, 2004). However, in the majority of cases inheritance does not seem to play a role.Currently there is no cure for AD (Wolf-Klein et al., 2007). To date the most promising target for the symptomatic treatment and slowing of AD progression is cholinesterase inhibitors (Bierer et al., 1995). In AD the destruction of cholinergic neurones causes a depletion of the neurotransmitter, acetylcholine (ACh) (Wenk, 2006). By inhibiting acetylcholinesterase (AChE), the enzyme which catalyses break down of ACh, levels of this neurotransmitter can be elevated and function improved. This approach has a particular success as these cholinergic neurones are found mainly in regions associated with learning and memory - spreading from the basal forebrain (Wu et al., 2005) and hippocampus (Gron et al., 2006) up to the cerebral cortex (Descarries et al., 2005) . However, the benefits of cholinesterase inhibitors do not come without unpleasant side-effects. The most reported risk with AChE inhibitor medication is gastrointestinal problems such as vomiting and diarrhoea (Dunbar et al., 2006). Phytotherapy has been found to

*Corresponding author. E-mail: e.j.okello@ncl.ac.uk. Tel: +44 (0) 191 222 5175. Fax: +44 (0) 191 222 6720.

show potential in the treatment of AD symptoms, especially in the targeting of the cholinergic pathway (Mukherjee et al., 2007). Furthermore, the advantage of using phytotherapy resides in the concoction of chemicals found within plants (Mills and Bone, 2000), which potentially provide protection against a wide range of aetiological factors. For example, *Salvia* species contain different phytochemicals active against not only the ACh deficit found in AD, but the inflammatory and oxidative factors as well (Perry et al., 2003) . In ethno-medicine it is very common for a single plant species to be used to treat various ailments due to the multiplicity of bioactive molecules they contain. In this respect, medicinal plants provide a rich source of biologically active constituents with multiple activities. In this study we continue the search for novel AChE inhibitory activities in plants and look at the anti-AChE potential and inhibition kinetics of *Acacia nilotica* (Leguminosae) and *Rhamnus prinoides (*Rhamnaceae*),* medicinal plants known to possess, *inter alia* , CNS activities (Stafford et al., 2005). The ethyl acetate and ethanol extracts of the leaves and bark of *A. nilotica* have been shown to be weak to moderate inhibitors of AChE, with the ethanolic extract of the bark having no activity (Eldeen et al., 2005). In ethno-medicine the roots of *A. nilotica* and *R. prinoides* are also widely used. The root of *A. nilotica* is reported to have traditional medicinal uses such as appetite enhancer, strength and nutrient supplement, for painful joints, stomach ache and cleaning circumcision wounds. The root of *R. prinoides* on the other hand is used to treat sexually transmitted disease (e.g. syphilis and gonorrhoea), arthritis, flu/cold, back pains, stomach ache, headache, pneumonia and brucellosis (Holford-Walker, 1951; Kokwaro, 1976; Kiringe, 2006).

## MATERIALS AND METHODS

### Plant material

Plant materials were acquired from a registered herbal practitioner in Kenya and identified by botanist Mr Monrinke Ole Njukuna. Voucher specimens CMC-AN01 and CMC- RP01 are deposited at the Medicinal Plant Research laboratory, Newcastle University, United Kingdom.

### Extraction

One gram of dried plant root material was extracted with 10 ml de-ionised water (18.2 M -cm), by sonication for 1 h and filtered using Whatman No.1 filter paper. The residue was re-extracted with 10 ml de-ionised water and filtrates were pooled, frozen, freeze-dried, and re-constituted in de-ionised water prior to assay.

### Inhibition assay

A variation of the Ellman technique (Ellman et al., 1961) was used. Bovine erythrocyte AChE (5 l, 0.03 U/ml (final assay concentration) in pH 8 sodium phosphate buffer (200 l, 0.1 M), DTNB (5 l, 0.3 mM in pH 7 sodium phosphate buffer containing 1.5 mg/ml sodium bicarbonate), and extract (5 l) were added to each well in a 96 well plate. Each extract was run in triplicate, including a blank, containing no enzyme instead extract, and triplicate controls containing no extract instead buffer. The plate was placed into a Multiskan Ascent plate reader and shaken for 10 s, then incubated for 5 or 30 min at 30$^{o}$C, after which substrate (5 l, 0.5 mM ATCI) was added, and the absorbance at 405 nm measured every 20 s for 6 min using a Thermo Labsystems Multiskan Ascent$^{TM}$ plate reader with Ascent$^{TM}$ software. Mean absorbance per minute values were calculated and blank well values were subtracted from the corresponding sample mean. Percentage inhibition was calculated using the equation:

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

The activity of galanthamine dissolved in de-ionised water was also assayed, as a positive control. All chemicals and reagents were purchased from Sigma Co., UK. Galanthamine hydrobromide from *Lycoris* species was of 94% purity.

### Inhibition kinetics

Lineweaver-Burk plots (Palmer, 1995) were drawn from assays using a range of final assay plant extract (inhibitor) concentrations (0, 0.05 and 0.1 mg/ml) at various substrate concentrations (0.5, 0.25, 0.125, 0.0625 and 0.03125 mM ATCI). From these $V_{max}$ and $K_m$ values were calculated for each inhibitor concentration. Re-plots allowed $K_i$ and $K_I$ calculation (1/gradient of Lineweaver-Burk trend line versus inhibitor concentration, and $1/V_{max}$ versus inhibitor concentration, respectively).

## RESULTS

AChE was inhibited in a concentration dependent manner. The $IC_{50}$ values of *A. nilotica* and *R. prinoides*, were 0.079 and 0.201 mg/ml, respectively (Figures 1A and B). Sonicated extracts (5 min incubation) were used in the standard curve serial dilutions as these exhibited comparatively similar inhibitory activities for the two plant species. However, by increasing the incubation time of *R. prinoides* its AChE inhibition decreased, from 52.6% for 5 min incubation to 44% for 30 min incubation whereas that of *A. nilotica* increased from 55.9% for 5 min incubation to 68% for the 30 min incubation period prior to adding the substrate (Table 1). The potency of *A. nilotica* and *R. prinoides* was compared to that of galanthamine, a drug approved for the treatment of AD. Galanthamine was found to have an $IC_{50}$ value of 0.0004 mg/ml (Figure 1C) which is at least 200 times more potent than *A. nilotica* and 500 times more potent than *R. pinoides.*

The enzyme inhibition kinetics of *A. nilotica* and *R. prinoides* aqueous extracts were determined from Lineweaver-Burk plots (Figures 2A and B). The $V_{max}$ and $K_m$ values were derived from the trend line equations of these graphs (Table 2) and $K_i$ and $K_I$ values calculated from the re-plot trend line equations (Table 2). The aqueous extracts of *A. nilotica* and *R. prinoides* both showed non-competitive uncompetitive mixed-inhibition.

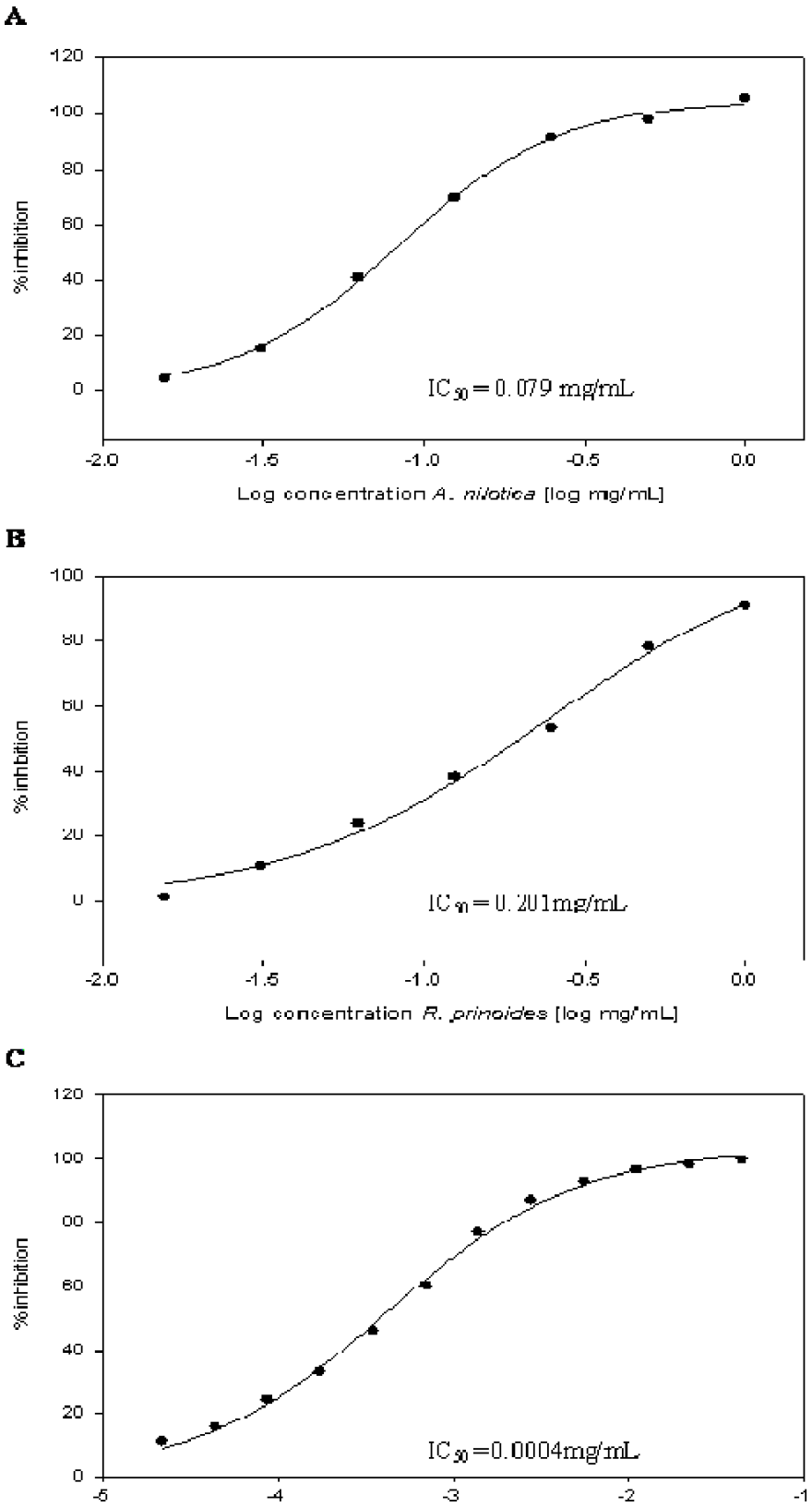


**Figure 1.** AChE inhibition curves for (A) *A. nilotica*, (B) *R. prinoides*, and (C) Galanthamine IC50 values were re-calculated back to mg/mL from log [mg/mL]

**Table 1.** Anti-acetylcholinesterase activities of aqueous extracts of *A. nilotica* and *R. prinoides.*

| Extracts | *Acetylcholinesterase inhibition ± SD (%) Incubation time (min) | | $IC_{50}$ values mg/ml |
|---|---|---|---|
| | 5 | 30 | |
| *A. nilotica* | 55.9 ± 0.01 | 68 ± 0.01 | 0.0790 |
| *R. prinoides* | 52.6 ± 0.01 | 44 ± 0.01 | 0.2010 |
| Galanthamine | | | 0.0004 |

* Final assay concentrations: 0.1mg/ml.

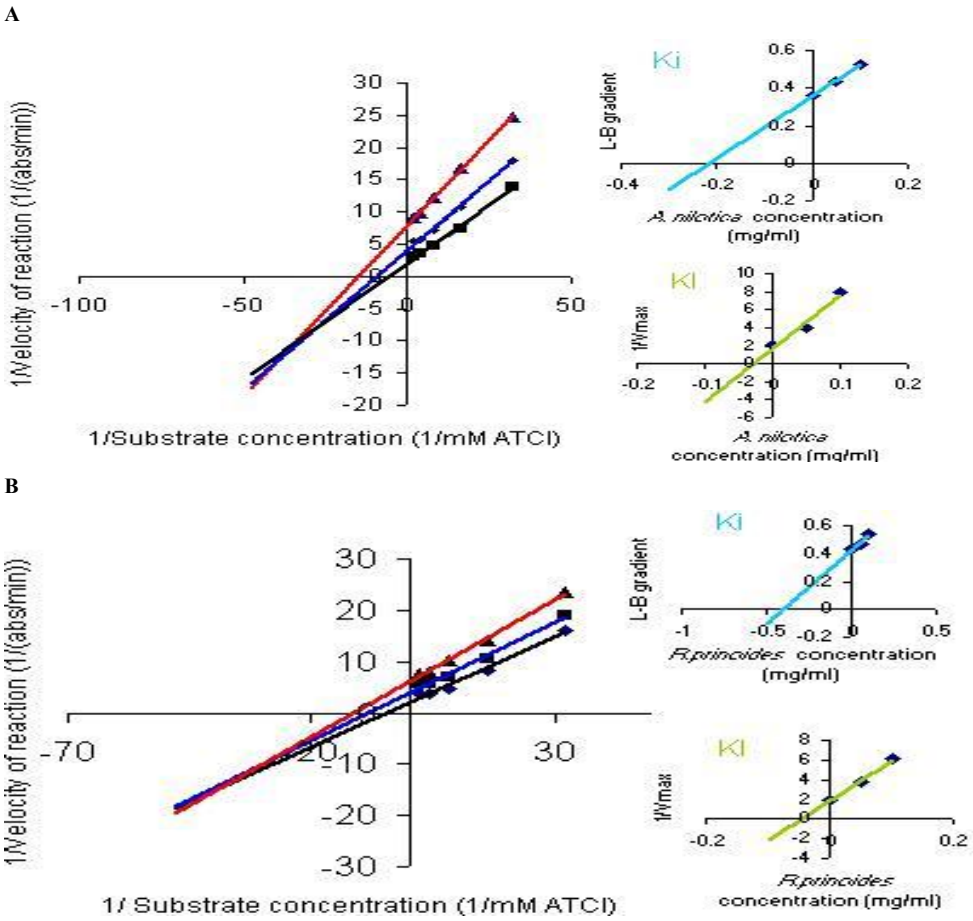


**Figure 2.** Lineweaver-Burk plots for (A) *A. nilotica* and (B) *R. prinoides* aqueous extracts.
0, 0.05 and 0.1 mg/ml inhibitor concentrations (Substrate concentrations used: 0.5, 0.25, 0.125, 0.0625, 0.03125 mM ATCI). Insets show the Lineweaver-Burk re-plots to determine Ki (1/gradient v inhibitor concentration (mg/mL)) and KI (1/Vmax (1/mM ATCI) v inhibitor concentration (mg/ml)). In both cases Ki and KI = (-1*x-axis intercept).

Our results also classify galanthamine as a mixed type inhibitor, but as a competitive non-competitive mixed inhibitor. This is evident from the convergence of the lines on the Lineweaver- Burk plot above the X-axis (Figure 3), and the decreasing $V_{max}$ and increasing $K_m$ values (Table 2) is further confirmed by the higher $K_I$ (1.64) than $K_i$ (0.52) (Palmer, 1995).

## DISCUSSION

A number of plants or plant derived compounds such as *Gingko biloba* and Huperzine A are currently undergoing clinical trials for the symptomatic treatment of dementia of the Alzheimer's type (Vellas et al., 2006; Aisen, 2007). Our data show that aqueous extracts of *A. nilotica* and *R.*

**Table 2.** Inhibition constants [$V_{max}$, $K_m$, $K_i$, $K_I$] for *A. nilotica*, *R. prinoides and galanthamine*

| | | | | | |
|---|---|---|---|---|---|
| | | | | | |
| | | | | | |
| | | | | | |
| | | | | | |

†Units: mM ATCI; ‡ Units: mg/ml extract or nmol/ml galanthamine

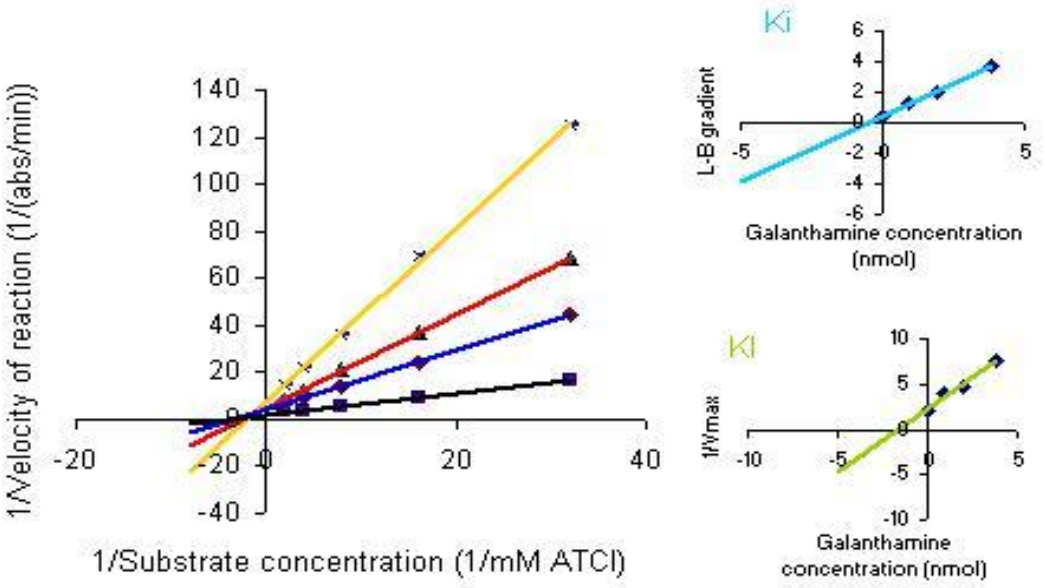


**Figure 3.** Lineweaver-Burk plot for galanthamine. 0, 0.955, 1.91, and 3.82 nmol galanthamine. Ki and KI re-plots are shown in the insets. (Substrate concentrations used: 0.5, 0.25, 0.125, 0.0625 and 0.03125 mM ATCI)

*prinoides* inhibit AChE in a dose dependent manner. *A. nilotica* has already been shown to possess anti-cholinesterase properties (Eldeen et al., 2005). However, our results show that the activity of *A. nilotica* root in an aqueous extraction ($IC_{50}$ 0.079 mg/ml) to be about 10 fold more potent than with leaf ($IC_{50}$ 0.7 and 0.5 mg/ml for ethyl acetate and ethanol extracts, respectively) and bark ($IC_{50}$ 1.3 mg/ml ethyl acetate extraction) results (Eldeen et al., 2005). *R. prinoides* has not previously been investigated for its anti-cholinesterase properties. We found it to have an $IC_{50}$ of 0.2 mg/ml. The inhibitory activity of *R. prinoides* decreased with incubation time probably due to rapid reversibility of inhibition in contrast to that of *A. nilotica*.*A. nilotica* and *R. prinoides* appear to be much more potent than AChE-inhibiting Portugese (Ferreira et al., 2006) and Danish medicinal plants (Adersen et al., 2006). In these studies aqueous extracts were assayed against bovine AChE, but at final enzyme concentrations of 0.007 and 0.002 U/ml respectively. Ferreira et al. (2006) reported *Hypericum undulatum* as the most potent inhibitor, showing 81.7% inhibition at a 5 mg/ml extract concentration. If we consider that this is 50 times greater than our 0.1 mg/ml extract concentration, and the enzyme used was 4.3 fold less concentrated, it

becomes clear that *A. nilotica* and *R. prinoides* are more potent AChE inhibitors. Adersen et al., (2006) reported 0.1 mg/ml extracts of the *Corydalis* species as having significant AChE inhibition, ranging from 92% (*Corydalis cava* bark extract), to 48% inhibition ( *Corydalis solida* leaf extract). Again considering the less final assay units of enzyme used in this study (by 15 fold), these results of *Corydalis* anti-AChE activity would suggest they are not as potent as *A. nilotica* and *R. prinoides* . There is a paucity of data on the kinetics of enzyme inhibition by herbal extracts as used in traditional medicine. This study has demonstrated that herbal extracts are able to display enzyme inhibition kinetics similar to those of single compounds. The kinetic inhibition activities of *A. nilotica* and *R. prinoides* on AChE have not previously been investigated; therefore we provide the first report of their mixed type non-competitive uncompetitive properties. This mixed type inhibition kinetics is typical of some medicinal plants; due to the great variety of compounds they contain all acting in different ways (Mills and Bone, 2000). However, the concentration dependent kinetic pattern which we have shown using the plant extracts has also been demonstrated before for pure synthetic anti-AChE compounds (Rosenfield and Sultatos, 2006). Our findings of galanthamine as a mixed-type inhibitor are unusual, as it is generally recognised as a competitive inhibitor. However, other studies have reported galanthamine as a mixed-type inhibitor (Rahman et al., 2006) and this may be due to the differences in experimental protocol. The deposition and aggregation of -amyloid (A ) peptides into neuro-toxic senile plaques is a recognized hallmark in the pathogenesis of AD. AChE has been shown to form stable complexes with senile plaque components via its peripheral anionic site which might be involved in promoting A fibril formation (Inestrosa et al., 1996; De Ferrari et al., 2001).

Mixed or non-competitive type inhibitors have been put forward as model candidates for inhibiting AChE-induced A aggregation due to their ability to bind to the peripheral anionic site (Bartolini et al., 2003). Other studies also suggest that the A aggregating property of AChE during the onset of AD can be inhibited by mixed or non-competitive type of inhibitors (Choudhary et al., 2005). The AChE inhibition kinetics in the present study indicates a putative mechanism by which the aqueous extract may have a novel therapeutic potential for AD.One of the main benefits of phytotherapy is the wide range of medicinal properties that each plant can offer, whereas pharmaceutical drugs are usually designed attack only a single target (Mills and Bone, 2000). For example, *A. nilotica* is known to possess antioxidant (Al-Fatimi et al., 2007) and anti-inflammatory activities (Eldeen et al., 2005). The toxicity of *A. nilotica* has previously been studied in rats, and even at the high consumption concentrations of 2 and 8% acacia diets, there was no evidence of liver or renal toxicity, although some weight loss was noted after a four weeks experimental period (Al-Mustafa and Dafallah, 2000). Similarly, *R. prinoides* has been found to not pose any notable safety issues (Verschaeve et al., 2004). It can therefore be concluded that *A. nilotica* and *R. prinoides*, in addition to possessing anti-inflammatory and antioxidant agents, also exhibit anti-AChE properties and thus could potentially provide novel leads for poly-pharmacological, multi-target approaches to the treatment of AD. Further studies on the isolation and structure elucidation of the active constituents and their synergistic/ antagonistic interactions are being conducted in our Medicinal Plants Research Laboratory.


## ACKNOWLEDGEMENT

We would like to thank Fiona MacLachlan for assistance with freeze-drying the extract.


### REFERENCES


Adersen A, Gauguin B, Gudiksen L, Jager AK (2006). Screening of plants used in Danish folk medicine to treat memory dysfunction for acetylcholinesterase inhibitory activity. J. Ethnopharmacol., 104: 418-422.

Aisen PS (2007). A Multi-Center, Double-Blind, Placebo-Controlled Therapeutic Trial to Determine Whether Natural Huperzine A Improves Cognitive Function. National Institute on Aging, USA.

Al-Fatimi M, Wurster M, Schröder G, Lindequist U (2007). Antimicrobial, antioxidant and cytotoxic activities of selected medicinal plants from Yemen. J. Ethnopharmacol., 111: 657-666

Al-Mustafa ZH, Dafallah AA (2000). A Study on the toxicology of Acacia nilotica. Am. J. Chin. Med., 28: 123-129.

Barnes LL, Schneider JA, Boyle PA, Bienias JL, Bennett DA (2006). Memory complaints are related to Alzheimer disease pathology in older persons. Neurology., 67: 1581-1585.

Bartolini M, Bertucci C, Cavrini V, Andrisano V (2003). beta-Amyloid aggregation induced by human acetylcholinesterase: inhibition studies. Biochem. Pharmacol., 65: 407-416.

Bierer LM, Haroutunian V, Gabriel S, Knott PJ, Carlin LS, Purohit DP, Perl DP, Schmeidler J, Kanof P, Davis KC (1995). Neurochemical correlates of dementia severity in Alzheimer's disease: Relative importance of the cholinergic deficits. J. Neurochem., 64: 749-760.

Choudhary MI, Nawaz SA, ul -Haq Z, Lodhi MA, Ghayur MN, Jalil S, Riaz N, Yousuf S, Malik A, Gilani AH, ur-Rahman A (2005). Withanolides, a new class of natural cholinesterase inhibitors with calciul antagonistic properties. Biochem. Biophys. Res. Commun., 334: 276-287.

De Ferrari GV, Canales MA, Shin I, Weiner LM, Silman I, Inestrosa NC (2001). A structural motif of acetylcholinesterase that promotes amyloid beta-peptide fibril formation. Biochemistry 40: 10447-10457.

Descarries L, Aznavour N, Hamel E (2005). The acetylcholine innervation of cerebral cortex: new data on its normal development and its fate in the hAPP SW,IND mouse model of Alzheimer's disease. J. Neural. Transm., 112: 149-162.

Dunbar F, Zhu Y, Brashear HR (2006). Post hoc comparison of daily rates of nausea and vomiting with once- and twice-daily galantamine from a double-blind, placebo-controlled, parallel-group, 6-month study. Clin. Ther., 28: 365-372.

Eldeen I MS, Elgorashi EE, Staden J (2005). Antibacterial, anti-inflammatory, anti -cholinesterase and mutagenic effects of extracts from some trees used in South African traditional medicine. J. Ethnopharmacol., 102: 457-464.

Ellman GL, Courtney DK, Andres V, Featherstone RM (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol., 7: 88-95.

Ferreira,A, Proenca C, Serralheiro MLM, Araujo MEM (2006). The in vitro screening for acetylcholinesterase inhibition and antioxidant activity of medicinal plants from Portugal. J. Ethnopharmacol., 108: 31-37.
Forbes-McKay KE, Venneri A (2005). Detecting subtle spontaneous language decline in early Alzheimer's disease with a picture description task. Neurol. Sci., 26: 243-254.
Gron G, Brandenburg I, Wunderlich AP, Riepe MW (2006). Inhibition of hippocampal function in mild cognitive impairment: targeting the cholinergic hypothesis. Neurobiol. Aging. 27: 78-87.
Holford-Walker AF (1951). Herbal medicines and drugs used by the Maasai. File no. DC/NRK/3/1, Nairobi.
Inestrosa NC, Alvarez A, Perez CA, Moreno RD, Vicente M, Linker M, Casanueva Ol, Soto C, Garrido J (1996). Acetylcholinesterase accelerates assembly of amyloid-beta peptides in to Alzheimer's fibrils: possible role of peripheral site of the enzyme. Neuron., 16: 881-891.
Kiringe JW (2006). A Survey of Traditional Health Remedies Used by the Maasai of Southern Kaijiado District, Kenya. Ethnobot. Res. Appl., 4: 61-73.
Kokwaro JO (1976). Prelude Medicinal Plants Database.
Mega MS, Cummings JL, Fiorello T, Gornbein J (1996). The spectrum of behavioural changes in Alzheimers disease. Neurology 46: 130-135.
Mills S, Bone K (2000). Principles and Practice of Phytotherapy: Modern herbal medicine. Edinburgh: Churchill Livingstone.
Mukherjee PK, Kumar V, Mal M, Houghton PJ (2007). Acetylcholinesterase inhibitors from plants. Phytomedicine 14: 289-300.
Palmer T (1995). Understanding Enzymes, 4th edition. London; New York: Prentice Hall/ Ellis Horwood.
Parihar MS, Hemnani T (2004). Alzheimer's disease pathogenesis and therapeutic interventions. J. Clin. Neurosci., 11: 456-467.
Perry NS, Bollen C, Perry EK, Ballard C (2003). Salvia for dementia therapy: review of pharmacological activity and pilot tolerability clinical trial. Pharmacol. Biochem. Behav., 75: 651-659.
Rahman A, Khalid A, Sultana N, Ghayur MN, Mesaik MA, Khan MR, et al. (2006). New natural cholinesterase inhibiting and calcium channel blocking quinoline alkaloids. J. Enzyme. Inhib. Med. Chem. 21: 703 - 710.
Reiman EM (2006). A 100-Year Update on Alzheimer's disease and related disorders. J. Clin. Psychiatry 67: 1784-1800.
Rosenfield CA, Sultatos LG (2006). Concentration-dependent kinetics of acetylcholinesterase inhibition by the organophosphate paraoxon. Toxicol. Sci. 90: 460-469.
Stafford GI, Jäger AK, van Staden J (2005). Activity of traditional South African sedative and potentially CNS-acting plants in the GABA-benzodiazepine receptor assay J. Ethnopharmacol., 100: 210-215.
Vellas B, Andrieu S, Ousset PJ, Ouzid M, Mathiex-Fortunet H (2006). The GuidAge study: Methodological issues. A 5-year double-blind randomized trial of the efficacy of EGb 761(R) for prevention of Alzheimer disease in patients over 70 with a memory complaint. Neurology. 67: S6-11.
Verdon CM, Fossati P, Verny M, Dieudonne B, Teillet L, Nadel J (2007). Social Cognition: An Early impairment in dementia of the Alzheimer type. Alzheimer Dis. Assoc. Disord., 21: 25-30.
Verschaeve L, Kestens V, Taylor JL, Elgorashi EE, Maes A, Van Puyvelde L, De Kimpe N, Van Staden J (2004). Investigation of the antimutagenic effects of selected South African medicinal plant extracts. Toxicol. In Vitro. 18: 29-35.
Wenk GL (2006). Neuropathologic changes in Alzheimer's disease: potential targets for treatment. J. Clin. Psychiatry. 67(3): 3-7.
Wolf-Klein G, Pekmezaris R, Chin L, Weiner J (2007). Conceptualizing Alzheimer's disease as a terminal medical illness. Am. J. Hosp. Palliat. Care. 24: 77-82.
Wu CK, Thal L, Pizzo D, Hansen L, Masliah E, Geula C (2005). Apoptotic signals within the basal forebrain cholinergic neurons in Alzheimer's disease. Exp. Neurol., 195: 484-496.