Revista Brasileira de Farmacognosia
Brazilian Journal of Pharmacognosy
20(4): 472-477, Ago./Set. 2010
Received 24 March 2009; Accepted 5 August 2009

Artigo

Acetylcholinesterase and butyrylcholinesterase inhibitory activity of some selected Nigerian medicinal plants

Taiwo O. Elufioye,[1] Efere M. Obuotor,[2] Afolake T. Sennuga,[2] Joseph M. Agbedahunsi,[,3] Saburi A. Adesanya[1]*

[1]*Department of Pharmacognosy, Faculty of Pharmacy Obafemi Awolowo University, Ile-Ife, Nigeria,*
[2]*Department of Biochemistry, Obafemi Awolowo University, Ile-Ife, Nigeria,*
[3]*Drug Research and Production Unit, Faculty of Pharmacy Obafemi Awolowo University, Ile-Ife, Nigeria.*

RESUMO: "Atividade inibitória da acetilcolinesterase e butirilcolinesterase de algumas plantas medicinais da Nigéria". As plantas podem ser úteis para estimular a memória, bem como serem usadas para combater o envelhecimento. Vinte e duas plantas, de dezesseis famílias, foram investigadas *in vitro* oara verificar sua atividade inibidora das enzimas acetilcolinesterase (AChE) e butirilcolinesterase (BuChE) pelo método espectrofotométrico de Ellman *in situ* e métodos de bioautografia utilizando fisostigmina como padrão. Pelo menos três partes morfológicas de cada planta foram analisadas e a concentração de ensaio foi de 42,5 µg/mL. Algumas plantas foram ativas em ambas as enzimas, embora com algumas partes mais ativas que outras. A casca da raiz de *Spondias mombin* apresentou a maior atividade as duas enzimas, 64,77% para AChE e 83,94% para BuChE. Outras partes das plantas selecionadas apresentaram boa seletividade em suas ações. As plantas seletivamente ativos contra AChE foram as casca do caule e casca da raiz de *Alchornia laxiflora* (41,12%), e casca da raiz de *Callophyllum inophyllurn* (56,52%). As folhas de *C. jagus* (74,25%), folhas de *Morinda lucida* (40,15%), folhas e casca do caule de *Peltophorum pterocarpum* (49,5% e 68,85%, respectivamente), physiostigmine inibiu 90,31%. Em geral, atividades melhoras foram apresentadas contra BuChE. Folhas, casca da raiz e casca do caule *Bombax bromoposenze* foram particularmente ativos. A inibição foi acima de 80%. Outras partes de algumas espécies também foram seletivas, como as partes aéreas de *Antiaris africana*, *Cissampelos owarensis* (78,96%), folhas e casca do caule de *Combretum molle* (90,42% e 88,13%, respectivamente), casca da raiz e de tubérculos de *Dioscorea dumentorum* (mais de 87%), folhas de G cola, cascas de raiz de *Markhamia tomentosa*, casca do caule de *Pycnanthus angolensis* e folhas de *Tetrapleura tetraptera.* A maioria destas plantas são utilizadas como alimentos ou ingredientes alimentares na Nigéria e podem ser responsáveis pela baixa incidência da doença de Alzheimer no país e desempenhar determinadas funções na mediação da doença.

Unitermos: Plantas medicinais, acetilcolinesterase, butrilcolinesterase, perda da memória.

ABSTRACT: Plants have been found to be useful as memory enhansers as well as antiaging. Twenty two of such plants from sixteen families were investigated for their acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitory activities using the *in vitro* Ellman's spectrophotometric and in situ bioautographic methods with physostigmine as standard. At least three morphological parts were examined for each of the plants investigated and the test concentration was 42.5 µg/mL. Some plants were active on both enzymes though with some morphological parts being more active than others. The root bark of *Spondias mombin* showed the highest activity to the two enzymes; 64.77% and 83.94% on AChE and BuChE respectively. Other plant parts of the selected plants exhibited some remarkable selectivity in their actions. Those selectively active against AChE were *Alchornia laxiflora* stem bark (41.12%) and root bark, *Callophyllum inophyllurn* root bark (56.52%). The leaves of *C. jagus* (74.25%), *Morinda lucida* leaves (40.15%), *Peltophorum pterocarpum* leaves and stem bark (49.5% and 68.85%, respectively), physiostigmine gave 90.31% inhibition. Generally higher activities were found against BuChE. *Bombax bromoposenze* leaves, root bark and stem bark were particularly active. The inhibition was over 80%. Other selective plant parts are the leaves *Antiaris africana, Cissampelos owarensis* aerial parts (78.96%), *Combretum molle* leaves and stem bark (90.42% and 88.13%, respectively), *Dioscorea dumentorum* root bark and tuber (over 87%), *G. kola* leaves, *Markhamia tomentosa* root bark, *Pycnanthus angolensis* stem bark and *Tetrapleura tetraptera* leaves. Most of these plants are taken as food or are food ingredients in Nigeria and may account for the low incidence of Alzheimer's disease in the country and may play certain roles in the mediation of the disease.

Keywords: Ethnomedicinal plants, acetylcholinesterase, butrylcholinesterase, memory loss.

**E mail: jagbedah@oauife.edu.ng, Tel. +234 803 4093594.*

ISSN 0102-695X

INTRODUCTION

In Nigeria ethnomedicine, Alzheimer's disease (AD) is not well defined. However, senile conditions are observed amomg the aged which could be due to neurodegeneration. Some plants used in Indian and Chinese traditional medical practice have been justified as having potentials in the management of CNS related disorder. *Bacopa monniera* and *Gingko biloba* from Indian and Chinese traditional medicine respectively are well documented cognitive enhancers (Das et al, 2002). Phytochemical analysis of such plants led to the characterization of cyanotroside from *Cynanchum atratum* and zeathin from *Fiatoua villosa* as potent AChE inhibitors (Lee et al., 2004, Heo et al., 2002). Houghton et al 2004 has reported the cholinesterase inhibitory activity of two Nigerian *Crinum* species i.e. *Crinum jagus* and *Crinum glaucum*, which could justify their use as memory enhancers. It is also known that physiostigmine, a potent AChE inhibitor, was isolated from *Physiostigma venenosu* (Calabar bean), a medicinal plant native to Nigeria.

Acetylcholine is critical for an adequately functioning memory, and it is the subject of the majority of research looking for treatments for memory defects, like those found in Alzheimer's disease. Any mental health issue that involves memory or lack thereof, directly or indirectly relates to acetylcholine.

Loss of this neurotransmitter plays an instrumental role in the pathogenesis of AD. Postmorterm studies of AD patients consistently have demonstrated the loss of basal forebrain and cortical cholinergic neurons and the depletion of choline acetyltranferase, the enzymes responsible for acetylcholine synthesis. It is also known that the central cholinergic system plays a key role in the retrieval and storage of memory items in the central nervous system (CNS) of mammals (Taylor, 1991, Costa et al., 1999, Okello et al., 2004). There is increasing evidence that cognitive dysfunction due to loss or decline of cholinergic activity in the key areas of the brain, involved in cognition is a normal biological process associated with aging as well as some forms of progressive neurodegenerative disorders such as Alzheimer's disease (AD) (Perry et al., 1996). The decline of cholinergic activity can be ameliorated by agents that restore or enhance cholinergic transmission in the synaptic cleft (Grantham & Geerts, 2002). Current therapeutic strategies for the symptomatic treatment of Alzheimer's disease and other related disorders such as vascular dementia and dementia with Lewy bodies are aimed at enhancing the associated cholinergic deficit by inhibiting acetylcholinesterase (AChE) (Rosler et al., 1999) resulting in a boost in endogenous level of acetylcholine (ACh) in the brain and an improvement of the cognitive function (Costa et al, l991, Nordberg et al., 2001: Giacobini et al., 2002).

Although the precise role of BuChE in the pathogenesis of most dementia disorders have not been unequivocally delineated, it has however been observed that BuChE increases in the brain of AD patients. It has been suggested that inhibition of both enzymes may be important in the management of cognitive deficits associated with the AD (Greig et al., 2001, Ghayur et al., 2008) from our survey (unpublished work) some plants are used as memory enhansers and anti aging. Some of these plant are compiled and investigated for their AChE and BuChE inhibitory activities in order to ascertain their role in the traditional management of memory loss.

MATERIALS AND METHODS

Plant material

The various plant parts were collected from Obafemi Awolowo University (O.A.U), Ile-Ife, Nigeria Campus and authenticated by Dr. H. Iloh of the Botany Department, Obafemi Awolowo University, Ile-Ife. Herberia specimen of all the plants were prepared and voucher specimens deposited at the Herbarium located at the Department of Botany, O.A.U. Other details are as presented on Table1.

Chemicals

Acetylcholinesterase (EC.3.1.1.7) from electrical eel and butrylcholinesterase (EC 3.1.1.8) from horse serum were products of Fluka Co., Germany. Acetylthiocholine iodide (ATChI), butyrylthiocholine chloride (BTChCl), 5:5-dithiobis-2-nitrobenzoic acid (DTNB), eserine and sodium bicarbonate were purchased from Sigma Co. UK. Buffers and other chemicals were of analytical grade.

Extraction of samples

Fresh samples of the various plant parts were oven dried (the leaves and flower at 40 ^{o}C, while root bark and stem bark were at 60 ^{o}C). The different plant parts were separetely milled into powder. The powdered plant parts were soaked with 80% methanol for 72 h; the extracts were filtered and concentrated *in vacuo* using rotary evaporator at 40 ^{o}C. The extracts were reconstituted in methanol to obtain a 1 mg/mL stock solution.

Cholinesterase assay

Acetycholinesterase and butyrylcholinesterase were respectively carried out using the colorimetric method of Ellman et al. (1961). The reaction assay mixture consisted 2000 mL 100mM phosphate butter pH8.0, 100ml of test sample stock solution in methanol (a final concentration of 42.5 µg/mL), 100 mL, of enzyme AChE or BuCHE solution at a final concentration of 0.03 U/mL and 0.01 µ/mL respectively, 100 µL of DTNB (0.3 mM)

prepared in 100 M phosphate buffer pH 7.0 containing 120 mM sodium bicarbonate. The reaction mixture was vortexed and then pre-incubated in a water bath at 37 °C for 30 min. The reaction was then initiated by the addition of 100 µL of ATCI or BTCI at a final concentration of 0.5 mM as a negative control. The inhibitor solution was replaced with methanol. The change in absorbance at λ_{max} 412 nm was then measured for a period of 5 min at ambient temperature. All assays were carried out in triplicate. The final concentration of the sample was 42.5 µg/mL. Eserine (-) physiotigmine) was used as positive control at same concentration. The % inhibition was calculated as follows:

$$\% = \frac{a - b}{a} \times 100$$

Where: a = ΔA/min of control; b = ΔA/min of test sample; ΔA = Change in absorbance.

RESULTS AND DISCUSSION

The selective cholinergic neurodegeneration forms the basis for the so-called cholinergic hypothesis of cognitive hypofunction (Perry et al., 1996; Perry et al., 1998; Bartus et al., 1982), that postulates that many of the cognitive, functional and behavioral symptoms experienced by patients with cognitive dysfunction results from a deficiency in neurotransmitter ACh, and thus in cholinergic neurotransmission. Numerous pharmacological and lesion studies in animals have been shown to support the involvement of central cholinergic systems in learning and memory (Camps & Munoz-Torrero, 2002).

The cholinergic hypothesis for cognitive hypofunction has provided the rationale for the current major therapeutic approach to cognitive dysfunction: which holds that the enhancement or restoration of central cholinergic function may significantly improve the cognitive impairments present in cognitive disorders (Francis et al., 1999). Currently, the only approved therapies for cognitive dysfunction are a group of indirect cholinomimetics which enhance function by inhibiting ACh degradation.

Cholinesterase inhibitors (ChEI), most especially of AChE, however constitute, to date, the most effective approach to treat the cognitive symptoms of AD (Giacobini, 2002). They have shown clear therapeutic utility on both cognitive performances, as well as on the quality of life in these patients (Francis et al, 1999). Indeed, the only drugs currently approved for the treatment of cognitive disorders are AChEI (*e.g*. tacrine, donepezilm rivastigmine and galanthamine). A large amount of AChEI has been developed, which differ among themselves in selectivity for AChE and BuChE, mechanism of inhibition and reversibility.

Twenty two plants from sixteen diferent families *i.e.* Amaryllidaceae, Anacardiaceae, Apocynaceae, Bignoniaceae, Bombacaceae, Combretaceae, Convovulaceae, Dioscoreaceae, Euphorbiaceae, Guttiferaceae, Fabaceae, Menispermaceae, Moraceae, Myristicaceae, Rubiaceae, and Solanaceae were investigated for their AChE and BuChE activities in this study.

Alchornia laxiflora, Croton zambesicus, Jatropha curcas and *Jatropha tangorensis* all belonging to the Euphorbiaceae all showed weak (40-49%) activity towards both AChE and BuChE at 42.5 µg/mL except *Croton zambesicus* whose activity was moderately high (51.29%) towards AChE.

In the Bombaceae family, *Bombax bromoposenze* and *Ceiba pentadra* both showed low activity towards AchE (below 19%). However, *Bombax bromoposenze* showed very high activity towards BuChE (>83%). All the extracts of the different morphorlogical parts tested showed similarly high BuChE activity. This BuChE selective activity was also noted in the Dioscoreaceae family, >87% inhibition of BuChE was observed in the tubers and roots of *Dioscorea dumetorum,* leaves of *Garcinia kola* (86.46%) (Guttiferaceae), *Combretum molle* (Combretaceae), *Cissampelos owarensis* (Menispermaceae), rootbark of *Markhamia tomentosa* (Bignoniaceae) and the leaves of *Tetrapleura tetraptera* (Leguminoceae). Plants studied in this family gave >78% inhibition of BuChE while activity against AChE was rather low (< 24%).

The leaves of *Crinum jagus* (Amaryllidaceae), stembark of *Peltophorum pterocarpum* (Leguminosae), stembark of *Pycnanthus angolensis* (Myristicaceae), rootbark and stembark of *Spondias mombin* (Anarcardiaceae) all gave high AChE inhibition activity (>60%). *Pycnanthus angolensis* and *Spondias mombin* gave high activity in both AChE and BuChE. Their stem bark and root bark gave BuChE inhibition >80%.

Other plants studied, including *Antaiaris africana* (Moraceae), *Capsicum frutensis* (Solanaceae), *Hollarhena floribunda* (Apocynaceae) and *Ipomea involucrata* (Convovulaceae) all gave weak activity (<40%) towards the enzymes.

This non-selectivity of some anti-ChE has been adduced to the structural and functional homology between degrade acetylcholine and the continued use of ChEI with the dual ability to inhibit AChE and BuChE have been implicated in the number of side effects associated with some cholinesterase inhibitors presently in use. However, it has been speculated that this duality could lead to improved clinical outcomes, particularly in respect to the management of Alzheimer's disease (Greig et al, 2001). Thus the development of selective AChE inhibitors has been a primary challenge still confronting anticholinesterase pharmacology to date. While AChE is very specific in its substrate recognition and requirement, BuChE reconizes a broad range of substrates. For this reason, it has been suggested that circulating plasma BuChE could possibly be serving a scavenging function thereby protecting

Table 1. Anticholinesterase activity of the plant extract against acetylcholine esterase (AChE) and buryrylcholine esterase (BuChE).

Plant species (IFE N°) Family	Plant Part	Percentage Inhibition (42.5 µg/mL)	
		AChE	BuChE
Alchornea laxiflora (IFE 2505) Euphorbiaceae	Leaves	25.38+2.44	0
Alchornea laxiflora	Root bark	31.47+1.07	0
Alchornea laxiflora	Stem bark	41.12+1.54	23.71+0.57
Antiaris africana (IFE4187) Moraceae	Leaves	4.39+0.41	78.55+3.97
Antiaris Africana	Root bark	7.02+0.46	31.15+2.40
Antiaris Africana	Stem bark	13.45+0.82	15.61+1.31
Bombax bromoposenze (IFE 2257) Bombacaceae	Leaves	14.33+1.91	83.98+6.26
Bombax bromoposenze	Root bark	0.29+0.01	89.31+9.22
Bombax bromoposenze	Stem bark	11.11+0.54	88.90+7.27
Callophylum inophyllum (IFE 14602) Guttiferaceae	Flowers	7.89+0.17	0.96+0.09
Callophylum inophyllum	Fruits	4.39+0.44	0
Callophylum inophyllum	Leaves	12.28+0.62	0.69+0.08
Callophylum inophyllum	Root bark	56.52+3.97	15.17+0.59
Callophylum inophyllum	Stem bark	31.29+1.22	0
Capsicum frutensis (IFE 12225) Solanaceae	Leaves	10.34+0.83	0.85+0.07
Capsicum frutensis	Stem bark	6.71+0.11	3.01+0.44
Ceiba pentadra (IFE 2260) Bombacaceae	Root bark	18.46+1.15	0
Ceiba pentadra	Leaves	16.30+0.88	9.06+0.21
Ceiba pentadra	Stem bark	18.99+0.42	0
Cissampelos owarensis (IFE 14920) Menispermaceae	Aerial part	19.59+0.81	78.96+7.55
Combretum molle (IFE 1942) Combretaceae	Leaves	12.17+0.15	90.42+5.35
Combretum molle	Root bark	24.72+2.32	88.13+4.21
Combretum molle	Stem bark	12.50+0.67	0
Crinum jagus (IFE 8701) Amaryllidaceae	Bulb	42.87+0.5	48.06+0.66
Crinum jagus	Leaves	74.25+6.42	3.61+0.07
Crinum jagus	Roots	40.64+2.55	91.34+6.25
Croton zambesicus (IFE 2575) Euphorbiaceae	Leaves	51.29+3.86	26.26+1.11
Discorea dumentorum (IFE 8806) Dioscoreaceae	Leaves	15.04+1.17	0.24+0.04
Discorea dumentorum	Root bark	4.22+0.19	87.55+7.24
Discorea dumentorum	Stem bark	17.37+0.95	16.45+0.86
Discorea dumentorum	Tubers	21.74+2.03	87.24+4.23
Garcinia kola (IFE 13184) Guttiferaceae	Leaves	0.29+0.01	86.46+5.04
Garcinia kola	Root bark	30.99+1.44	11.86+0.37
Garcinia kola	Stem bark	11.99+0.84	5.93+0.29
Hollarhena floribunda (IFE 14597) Apocynaceae	Leaves	16.31+0.54	0.47+0.08
Hollarhena floribunda	Root bark	22.39+1.21	0.87+0.56
Hollarhena floribunda	Stem bark	17.12+0.73	25.99+0.82
Ipomea involucrata (IFE 6975) Convovulaceae	Aerial part	25.73+1.41	0
Jatropha curcas (IFE 2699) Euphorbiaceae	Fruits	15.74+1.84	3.25 +0.62
Jatropha curcas	Leaves	23.86+0.96	3.82+0.33
Jatropha curcas	Root bark	22.84+1.91	0.46+0.01
Jatropha curcas	Stem bark	18.27+1.14	4.39+0.01
Jatropha tangorensis (IFE 2700) Euphorbiaceae	Leaves	17.25+1.04	13.23+1.08
Jatropha tangorensis	Stem bark	2.92+0.06	8.16+0.94
Markhamia tomentosa (IFE 7313) Bignoniaceae	Leaves	18.78+2.77	2.96+0.06
Markhamia tomentosa	Root bark	24.37+1.05	78.45+5.67
Markhamia tomentosa	Stem bark	40.61+4.01	24.82+1.77

Morinda lucida (IFE 5672) Rubiaceae	Leaves	4015+2.57	34.09+1.93
Morinda lucida	Root bark	13.20+0.78	0.78 +0.02
Morinda lucida	Stem bark	22.34+0.76	2.50+0.06
Peltophorum pterocarpum (IFE 3171) Leguminoceae	Fruits	15.68+1.37	4.31+0.22
Peltophorum pterocarpum	Leaves	47.5+2.41	4.89+0.71
Peltophorum pterocarpum	Root bark	48.46+4.47	51.77+2.20
Peltophorum pterocarpum	Stem bark	68.85+3.53	3.05+0.58
Pycnanthus angolensis (IFE 13039) Myristicaceae	Fruits	8.44+0.76	9.59+0.32
Pycnanthus angolensis	Leaves	43.96+3.04	43.59+1.77
Pycnanthus angolensis	Root bark	15.51+0.64	0
Pycnanthus angolensis	Stem bark	66.52+5.02	86.05+8.32
Spondias mombin (IFE 9572) Anarcadiaceae	Leaves	48.58+4.56	47.34+2.55
Spondias mombin	Root bark	64.77+2.73	83.94+6.31
Spondias mombin	Stem bark	60.71+3.08	48.72+1.49
Tetrapleura tetraptera (IFE 3391) Leguminosae	Fruits	22.34+1.90	1.04+0.02
Tetrapleura tetraptera	Leaves	1.52+0.01	81.87+3.54
Tetrapleura tetraptera	Root bark	34.77+0.66	0
Tetrapleura tetraptera	Stem bark	24.87+1.32	4.22+0.11
Physostigmine (Standard)		90.31+3.55	84.27+4.72

AChE from naturally occurring inhibitors. In this regard, BuChE could also scavenge anticholinesterase drugs, thereby elevating the dose necessary to achieve reasonable inhibitions at the target region of the brain for the effective therapeutic relief (Perry et al., 1996).

While most researches have been geared towards identifying AChE selective inhibitors, recent results has suggest that inhibition of both AChE and BuChE should be one of the objectives in the treatment of cognitive dysfunctions associated with cases of AD (Greig et al., 2001). Several researches are now aimed at finding plants that inhibits both enzymes (Okello et al., 2004). Thus, plants that show activity towards either or both enzyme may find some use in the management of memory dysfuntion.

CONCLUSION

This work shows that most of the plant parts tested showed cholinesterase inhibitory activity towards either AChE, BuChE or both enzymes and may be considered for further studies in the management of AD. Plants that selectively inhibits AChE may be more useful in early stages of AD while those that inhibits both AChE and BuChE may be of paricular interest in severes cases of AD.

REFERENCES

Bartus RT, Dean RL, Beer B, Lippa AS 1982. The cholinergic hypothesis of geriatric memory dysfunction. *Science 217*: 408-417.

Camps P, Munoz-Torrero D 2002. Cholinergic drugs in pharmacotherapy of Alzheimers disease. *Mini Rev Med Chem 2*: 11-25.

Costa J, Anand R, Cutler N 1991. Correlation between cognitive effects and level of acetylcholine esterase inhibition in a trial with rivastigmine in Alzheimer's patients. *Proc Am Psych Assoc*, Poster NR561.

Das A, Shanker G, Nath C, Pal R, Singh S, Singh HK 2002. A comparative study in rodents of standardize extracts of *Bacopa monniera* and *Ginkgo biloba* anticholinesterase and cognitive enhancing activities. *Pharmacol Biochem Behav 73*: 893-900.

Ellman GL, Courtney KD, Andres V, Fearstherstone RM 1961. A new rapid colorimetric determination of acetyl cholionesterase activity. *Biochem Pharmacol 7*: 88-95.

Francis PT, Palmer AM, Snape M, Wil Cock GK 1999. The cholinengic hypothesis of Alzheimer's disease; a review of progress. *J Neurol Neurosurg Psychiatry 66*: 137-147.

Ghayur MN, Gilani AH, Ahmed T, Khalid A, Nawaz AS, Agbedahunsi JM, Choudhary MI, Houghton PJ 2008. Muscarinic Ca^{++} antagonist and specific butyrylcholinesterase inhibitory activity of dried ginger extract might explain its use in dementia. *J Pharm Pharmacol 60*: 1375-1383.

Giacobini R, Spiegel E, Enz A, Veroff AE, Cutler NR 2002. Inhibition of acetyl and burylcholinesterase in the cerebrospinal fluid of patients with Alzheimer's disease by rivastigmine: correlation with cognitive benefit. *J Neural Transm 109*: 1053-1065.

Grantham C, Geerts H 2002. The rationale behind cholinergic drug treatment for dementia related to cerebrospinal disease. *J Neurol Sci 203*: 131-136.

Greig NH, Utsuki T, Yu QU, Zhu X, Halloway HW, PerryT, Lee B, Ingram DK, Lahiri DK, 2001. New therapeutic target in Alzheimer's disease treatment: Attention to butyrylcholinesterase. *Curr Med Res Opin 17*: 159-165.

Heo HJ, Hong SC,, Cho HY, Hong B, Kim HK, Kim EK, Shin DH 2002. Inhibitory effecy of zeatin isolated from *Fitoua villosa*, on acytycholineterase activity from PC 12 cells.

Mol Cells 13: 113-117.

Houghton JP, Agbedahunsi JM, Adegbulugbe A 2004. Choline esterase inhibitory properties of alkaloids from two Nigerian *Crinum* species. *Phytochemistry 65*: 2893-2896.

Lee YK, Yoon JS, Kim ES, Kang SY, Kim YC 2004. Anti acethycholinesterase and anti-amnessic activities of a pregnane glycoside, cynatroside B, from *Cynanchum atratum. Planta Med 71*: 7-11.

Norberge A, Darreh-Shori T, Svenson A, Quan Z 2001. AchE and BuchE activities in CSF of mild AD patients following 12 months of rivastigmine treatment. *J Neurol Sci 187 (suppl)*: PQ 144.

Okello JE, Savelev SU, Perry KE 2004. In vitro anti-secretase and dual anticholinesterase activity of *Camellia sinensis* L. (tea) relevants to treatment of dementia. *Phytother Res 18*: 624-627.

Perry N, Court G, Bidet N, Court J, Perry EK 1996. Cholinergic activities of European herbs and potential for dementia therapy. *J Ger Psychiatr 11*: 1062-1069.

Perry EK, Pirckering AT, Wang WW, Houghton P, Perry NS 1998. Medicinal plants and Alzheimer's disease: integrating ethnobotanical and contemporary scientific evidence. *J Altern Complement Med (US) 4*: 419-428.

Rösler M, Anand R, Cicin-Sain A, Gauthier S, Agid Y, Dal-Bianco P, Stähelin HB, Hartman R, Gharabawi M, Bayer T 1999. Efficacy and safety of rivastigmine in patients with Alzheimer's disease: international randomised controlled trial. *Brit Med J 318*: 633-640

Taylor P 1991. The cholinesterase. *J Biol Chem 266*: 4025-4028.