

Full Length Research Paper

Acetyl and buteryl cholinesterase inhibitory effect of *Peltophorum pterocarpum* (DC) Backer ex K. Heyne (family Leguminosae)

O. Elufioye Taiwo^{1*}, M. Obuotor Eferè², M. Agbedahunsi Joseph³ and A. Adesanya Saburi⁴

¹Department of Pharmacognosy, Faculty of Pharmacy, University of Ibadan, Nigeria.

²Department of Biochemistry, Obafemi Awolowo University, Ile-Ife, Nigeria.

³Drug Research and Production Unit, Faculty of Pharmacy, Obafemi Awolowo University, Ile Ife Nigeria.

⁴Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria.

Accepted 1 March, 2013

Peltophorum pterocarpum, family Leguminosae is a tree natural to tropical South-Eastern Asia and was brought to Nigeria by immigrants. It has been used traditionally by the South Western people of the country as memory enhancer and anti-ageing. The present study was done to assess the cholinesterase inhibitory activity of the extracts and fractions of *P. pterocarpum* using spectrophotometric and thin layer chromatography (TLC) bioautographic assay methods. Eserin was used as reference cholinesterase inhibitors. The methanolic extract of the leaves, root bark and stem bark were found to be active. The stem-bark gave the highest activity (68.85±3.53%) and better selectivity towards acetylcholinesterase (AChE) at a dose of 42.5 µg/ml followed by the root bark which inhibited both AChE and butyrylcholinesterase (BuChE) at 48.46±4.47 and 51.77±2.20, respectively and then the leaves with values of 47.50±2.41 and 48.91±0.71 against AChE and BuChE, respectively. Fractionation of the various plant parts showed that the active constituent may be moderately polar being mostly extractable in ethyl acetate and that purification leads to improved activity. These results demonstrate that *P. pterocarpum* inhibits cholinesterase and thus may be relevant in the treatment of memory dysfunctions and neurodegenerative disorders such as Alzheimer's disease.

Key words: *Peltophorum pterocarpum*, acetylcholinesterase, butyrylcholinesterase, memory dysfunctions.

INTRODUCTION

Memory loss also referred to as amnesia is an abnormal degree of forgetfulness and/or inability to recall past events. In Nigeria, memory loss is considered a major problem in the traditional setting particularly among the youth who seek memory enhancing remedied to pass examinations. Plants have been used to treat memory related disorder for centuries (Perry et al., 2000). The use of complementary medicines such as plant extracts in dementia therapy however varies according to different cultural traditions (Perry et al., 1996). *Huperzia serata* and *Ginkgo biloba* have been used in Chinese medicine while *Salvia officinalis* and *Salvia lavandulaefolia* have

been used in Europe (Grieve, 1980; Ryman, 1991; Tyler, 1993; Birks et al., 2004).

Inhibition of acetyl cholinesterase, the key enzyme in the breakdown of acetylcholine is considered a promising strategy in the treatment of neurological disorders such as Alzheimer's disease, senile dementia and myasthenia gravis (Blockland, 1996; Crisby et al., 2002). Many plants with a history of use as memory enhancer have been shown to contain cholinesterase inhibitors. Naturally occurring cholinesterases inhibitors continue to be identified in a wide variety of plant species (Tang, 1994; Park et al., 1996). *Peltophorum pterocarpum* (DC)

*Corresponding author. E-mail: toonitaiwo@yahoo.com. Tel: +234 803 385 0773.

Backer ex K. Heyne. (Leguminosae) is commonly referred to as golden flamboyant. It is a wonderful shade tree for a large landscape especially when in full bloom. It is used as astringent to cure or relieve intestinal disorder, after pain at child birth, sprains, bruises and swelling. It is also used as lotion for eye troubles, muscular pains and sores (Sethuraman et al., 1984). It is used as gargles and tooth powders (Sethuraman et al., 1984). Satish et al. (2007) reported the antifungal activity of *P. pterocarpum* against seed borne pathogens of *Aspergillus* species. Sethuraman et al. (1984) reported the anti-inflammatory and antibacterial activities of the flower. The antimicrobial activity was also reported by Duraipandiyani et al. (2006). Although, certain biological activity of *P. pterocarpum* has been reported, its memory enhancing potential has not received due attention. This study therefore explores the potential of *P. pterocarpum* as a cholinesterase inhibitor with a view to examining its folkloric claim as memory enhancer.

MATERIALS AND METHODS

Preparation of plant extract

Fresh sample of the various parts (leave, fruits, stem bark and root bark) of *P. pterocarpum* were collected from Road 7, Obafemi Awolowo University Campus, Ile-Ife, Osun State, Nigeria after proper identification by Mr. T. A Oladele of the Department of Pharmacognosy, Faculty of Pharmacy and authentication by Dr. H. Illoh of Botany Department, Obafemi Awolowo University where voucher specimen was deposited.

Collected samples were macerated with 80% methanol for 72 h. The extracts were concentrated *in vacuo* to dryness at 40°C.

Preliminary phytochemical screening

Preliminary phytochemical analysis of the extracts was carried out using standard methods (Harborne, 1992; Abulude, 2007; Kokate, 1994).

Fractionation of the methanolic extracts

The methanolic extracts of the various parts were partitioned into n-hexane, ethyl acetate and water. The various fractions were concentrated *in vacuo* at 40°C and were tested.

Vacuum liquid chromatography (VLC)

The most active ethyl acetate fraction was subjected to VLC on silica gel using n-hexane, ethyl acetate and water in various ratio as solvent system. Five subfractions were obtained based on thin layer chromatography (TLC) pattern. These subfractions were tested for acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitory activity.

Cholinesterase inhibitory assay

AChE and BuChE inhibitions were determined spectrophotometri-

Table 1. Cholinesterase inhibitory activity of methanolic extract of different plant parts (AChE and BuChE).

Plant part	AChE inhibition (%)	BuChE inhibition (%)
Leaves	47.50 ± 2.41	48.9 ± 0.71
Root bark	48.46 ± 4.47	51.77 ± 2.20
Stem bark	68.85 ± 3.53	3.05 ± 0.58
Fruits	15.68 ± 1.37	4.31 ± 0.22
Eserin	90.31 ± 3.55	84.27 ± 4.72

cally using acetyl thiocholine iodide (ATCI) and buterylcholine chloride (BTCl) as substrate, respectively by the modified method of Ellman (Ellman et al., 1961).

The reaction assay mixture consisted of 2000 ml 100 mM phosphate buffer, pH 8.0, 100 ml of test sample stock solution in methanol (at a final concentration of 42.5 µg/ml), 100 ml of enzyme AChE or BuChE solution at a final concentration of 0.003 and 0.001 µg/ml, respectively. 100 µl of 5,5'-dithiobis-(2-nitrobenzoic) acid (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 initiated by the addition of 100 µl of ATCI or BTCl 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 was then measured for a period of 5 min at room temperature. All assays were carried out in triplicate. Eserin ((-) physostigmine) was used as positive control. The percentage inhibition was calculated as follows:

$$\text{Percentage} = \frac{a-b}{a} \times 100$$

where a = ΔA/min of control; b = ΔA/min of test sample; ΔA = change in absorbance.

The crude methanolic extract, the various fractions, and subfractions were subjected to this test.

RESULTS

Phytochemical screening

The preliminary qualitative phytochemical screening of *P. pterocarpum* revealed the presence of flavonoids, alkaloids and saponins in all the plant parts. Cardiac glycosides was present in the leaves, stem bark and root bark while tannins was positive only in the stem bark and root bark.

Cholinesterase inhibition of methanolic extract

The methanolic extract of *P. pterocarpum* showed inhibitory activity against AChE for all the different plant parts except the fruits as reported in Table 1. However, only the leaves and the root bark showed significant inhibition of BuChE.

Table 2. Cholinesterase inhibitory activity of different fractions (AChE/BuChE).

Fraction	AChE inhibition (%)	BuChE inhibition (%)
Leaves		
N	28.62 ± 1.37	22.01 ± 0.62
E	66.10 ± 0.78	46.32 ± 0.61
Aq	18.00 ± 0.99	19.23 ± 0.86
Stem bark		
N	26.66 ± 1.91	14.44 ± 0.96
E	70.10 ± 0.54	63.84 ± 0.67
Aq	29.14 ± 0.66	21.74 ± 0.39
Fruits		
N	10.90 ± 0.39	12.63 ± 0.57
E	40.58 ± 0.75	22.69 ± 0.68
Aq	31.07 ± 0.52	38.08 ± 0.80
Root bark		
N	34.02 ± 0.93	20.63 ± 0.49
E	69.91 ± 0.91	70.13 ± 0.73
Aq	13.28 ± 0.57	18.15 ± 0.88
Eserin	92.63 ± 1.98	89.30 ± 1.76

N: n-Hexane fractions; E: ethyl acetate fraction; Aq: Aqueous fraction.

Cholinesterase inhibition of various fractions

Cholinesterase inhibition was highest for the ethyl acetate fractions of all the different plant parts. This inhibition was generally on both AChE and BuChE (Table 2).

Cholinesterase inhibition of various subfractions

Five subfractions obtained from the VLC of the most active ethyl acetate fraction were also subjected to cholinesterase assay. The results are as shown in Figures 1 to 5).

DISCUSSION

Memory loss is a common feature in all cultures and plants have always been used for their management. In Chinese culture, the use of such plants as *Ginkgo biloba* which is an effective cholinesterase inhibitor, as memory enhancer has been justified (Atta-ur-Raham et al., 2001) and spectrophotometric assay method has been previously used by several authors to determine the cholinesterase inhibitory activity of many plants (Ellman et al., 1961, Houghton et al., 2004, Oh et al., 2004; Mukherjee et al., 2007).

The study was carried out to evaluate the acetyl and buteryl cholinesterase inhibitory activity of extracts of different plant parts (leaves, root bark, stem bark and fruits) and fractions of leaves of *P. pterocarpum*. Eserin (+ physostigmine) was used as reference standards. The study showed that methanolic extract of the fruits had no inhibitory effect on both AChE and BuChE. However, the extract of the leaves and the root bark inhibited both enzymes, while that of the stem bark selectively inhibited AChE. This result supports the claimed ethnomedical use of *P. pterocarpum* as memory enhancer since an inhibition of the enzymes will lead to an increase in the level of acetylcholine in the brain and hence better cognitive ability.

The plant parts were partitioned into n-hexane, ethyl acetate and water. The various partitioned fractions were also tested. It was observed that the ethyl acetate fraction of the various plant parts were most active. This suggests that the active principle is likely to be moderately polar.

The ethyl acetate fraction of the leaves, being the most regenerative part of the plant, was subjected to VLC and five subfractions (A to E) were obtained which were again tested. The effects of these subfractions at the various concentrations were time dependent. Highest inhibition was obtained within the first 30 s of the reaction. However, as the time increased, the activity of the fractions decreased. This observation was obtained

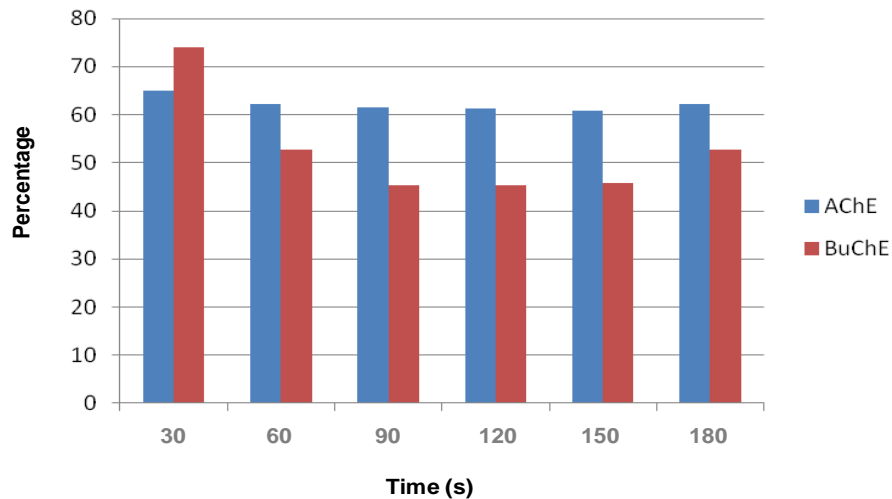


Figure 1. Percentage inhibition of subfraction A.

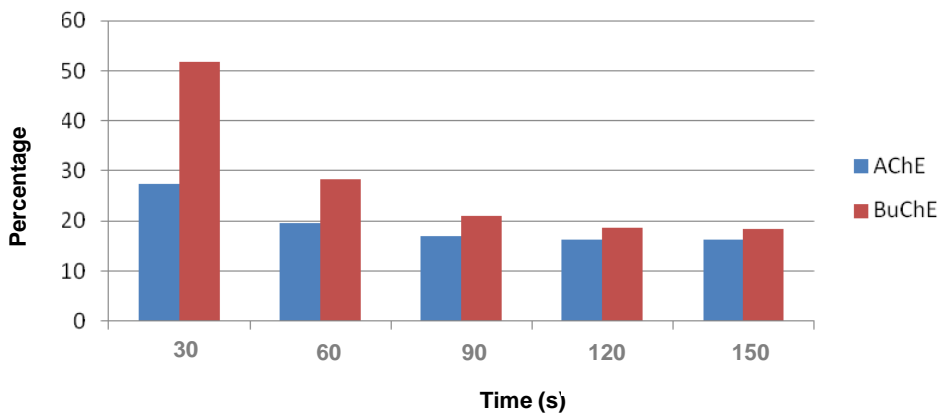


Figure 2. Percentage inhibition of subfraction B.

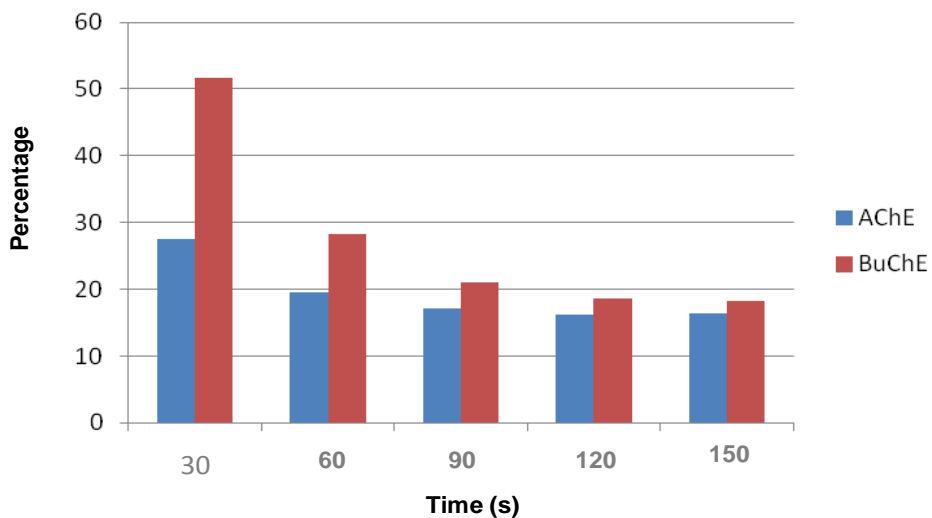


Figure 3. Percentage inhibition of subfraction C.

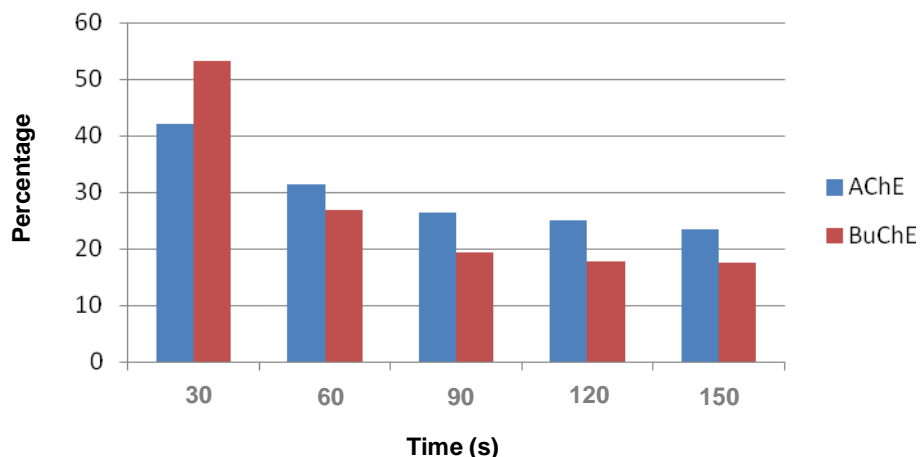


Figure 4. Percentage inhibition of subfraction D.

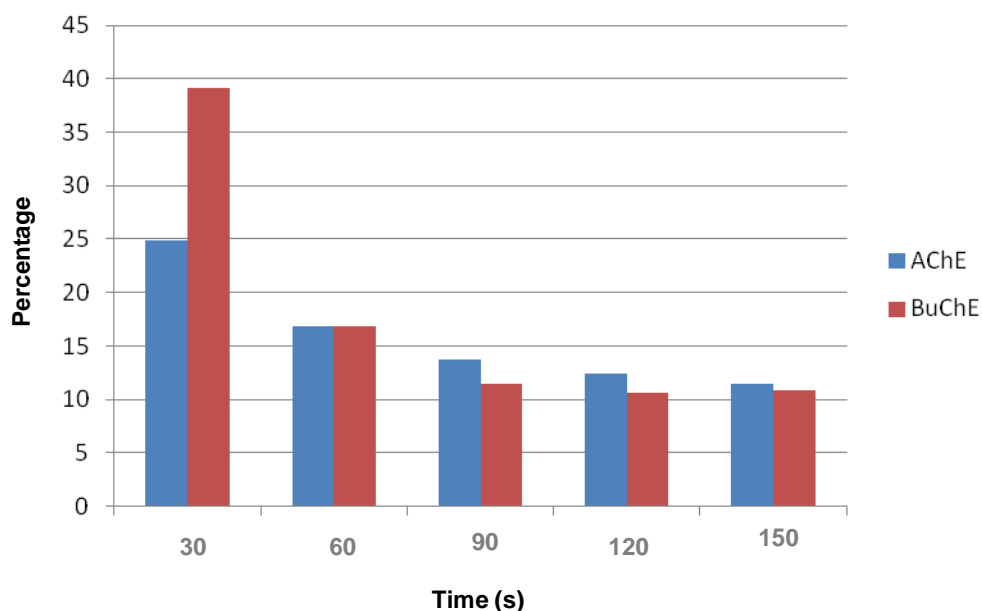


Figure 5. Percentage inhibition of subfraction E.

irrespective of the concentration of the fraction. The results as presented in Figures 1 to 5 showed that subfraction A was most active with a percentage inhibition of 65 and 73.99% against AChE and BuChE, respectively. Activity of subfraction A was time dependent for both enzymes. However, inhibition was more prolonged in AChE. BuChE showed better inhibition at 30 s, but activity dropped more rapidly as the time progressed. Subfraction B showed better BuChE inhibitory activity over the period of time for the reaction. Fractions C to E did not show appreciable activities to both enzymes. It can be seen from the aforementioned that activity increased with purification. It also implicated that the putative AChE and BuChE compounds were likely to be moderately polar.

In conclusion, the findings justified the inclusion of *P. pterocarpum* in therapies for memory loss by traditional medical practitioners and also showed that partial purification enhanced activity of extracts. Further work is however ongoing to identify the active components of the plant.

REFERENCES

- Abulude FO (2007). Phytochemical screening and Mineral Contents of leaves of some Nigerian Woody plants. *Res. J. Phytochem.* 1(1):33-39.
- Atta-ur-Rahman, Shehaz P, Asaad K, Afgan F, M.Iqbal C (2001). Acetyl and Butyrylcholine esterase-inhibiting triterpenoid alkaloids from *Buxus papillosa*. *Phytochem.* 58:963-968.

- Birks J, Grimtey E, Van Dongen M (2004). *Gingko biloba* for cognitive impairments and dementia. Cochrane. Data base system Rev. CD003/20.
- Blockland A (1996). Acetylcholine: A neurotransmitter for learning and memory. Brain Res. Rev. 20:285-300.
- Crisby MI, Carlson L, Winblad B (2002). Statins in the prevention and treatment of Alzheimer's disease. Alzheimer's Dis. Assoc. Disorders 16(13):131-136.
- Duraipandiyar V, Ayaanar M, Ignacimuthu S (2006). Antimicrobial activity of some ethnomedicinal plants used by paliyar tribe from Tamil Madu, India. Complem. Altern. Med. 6:35.
- Ellman GL, Courtney KD, Andres VJR, Feather-stone RM (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol. 7:88-95.
- Grieve M (1980). A modern herbal. Penguin Books, UK. pp. 700-705.
- Gupta GN (1991). Effects of mulching and fertilizer application on initial development of some tree species. Forest Ecol. Manage. 44(2-4):211-221.
- Harborne JB (1992). Phytochemical methods. Chapman and Hall Publications: London, UK. pp. 7-8.
- Houghton PJ, Agbedahunsi JM, Adegbulugbe A (2004). "Choline esterase inhibitory properties of alkaloids from two Nigerian crinum species" Phytochem. 65:2893-2896.
- Kokate CK (1994). Practical Pharmacognosy Vallabh Kubma: Prakashian India.
- Mukherjee PK, Kumar VM, Houghton PJ (2007). Acetylcholinesterase inhibitors from Plants. Phytomedicine: Inter. J. Phytother. Phytopharm. pp. 1-11.
- Oh MH, Houghton PJ, Whang WK, Cho JH (2004). Screening of Korean herbal medicines used to improve cognitive function for anti-cholinesterase activity. Phytomed. 11:544-548.
- Park CH, Kim SH, Choi W, Lee YJ, Kim YS, Kang SS, Suh YH (1996). Novel anticholinesterase and anti-amnesic activities of dehydroevodiamine, a constituent of *Evodia rutaecarpa*. Planta Med. 62:405-409.
- Perry N, Court G, Bidet N, Court Y, Perry EK (1996). Cholinergic activities of European herbs and potential for demential therapy. J. Ger. Psych. 11:1063-1069.
- Perry N, Houghton PJ, Theobald A, Jenner P, Perry EK (2000). In-vitro inhibition of human erythrocyte acetylcholinesterase by *Salvia lavandulaefolia* essential oil and constituent terpenes. J. Pharm. Pharmacol. 52:895-902.
- Ryman D (1991). Aromatherapy. Judy Piakus publishers. U.K pp. 190-194.
- Satish S, Mohana DC, Raghavendra MP, Raveesha KA (2007). Antifungal activity of some plant extracts against important seed borne pathogens of *Aspergillus sp.* J. Agric. Tech. 3(1):109-119.
- Sethuraman MG, Soluchang N, Kameswaran L (1984). Anti-inflammatory and antibacterial activity of *Peltophorum pterocarpum* flowers. Fitoter. 55(3):177-179.
- Tang XC (1994). Neuropharmacological effect of active principle isolated from Chinese medicinal herbs. Neuropsychopharmacology 10(35/2):6915
- Tyler VE (1993). The Honest Herbal. 3rd Edn. Pharmaceutical products press, US. pp. 271-273.