Memory-Enhancing and Memory-Related Beneficial Effects of Selected Medicinal Plants from the Nigerian Flora

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Taiwo O. Elufioye

Abstract

The cholinesterase inhibitory activity and the memory-enhancing effects of 22 Nigerian medicinal plants belonging to 16 different families were investigated. The acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitory potentials of extracts, fractions, and isolated compounds were evaluated by Ellman colorimetric and thin-layer chromatography (TLC) bioautographic assay techniques. Bioactivity-directed phytochemistry, as well as spectroscopic analysis, was carried out. Morris water maze test was used to assess the cognitive enhancing potential of some of the most active plants. Some plants such as *Morinda lucida, Spondias mombin, Pycnanthus angolensis*, *and Peltophorum pterocarpum* showed inhibitory activity on both enzymes, while others exhibited some remarkable selectivity in their actions. *Alchornea laxiflora* stem bark and root bark, *Calophyllum inophyllum* root bark, and *Crinum jagus* leaves were selectively active against AChE, while *Antiaris africana*, *Bombax bromoposenze*, *Combretum molle*, and *Garcinia kola* were selectively active against BuChE. Activity-directed phytochemistry led to the isolation of bioactive compounds which may lead to drug development. The in vivo effect of four most active plants, *S. mombin, P. angolensis, P. pterocarpum*, *and M. lucida*, on scopolamine-induced memory loss was also confirmed.

Keywords

Alzheimer's disease • Anticholinesterase • Medicinal plants • Memory-enhancing effects • Morris water maze

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Contents

15.1 Introduction 489
15.2 Plant Materials 489
15.3 Extraction 489
15.4 Anticholinesterase Assay Procedures 489
 15.4.1 Spectrophotometric Analysis 489
 15.4.2 Thin-Layer Chromatography (TLC) Bioautographic Assay 491
 15.4.3 Fractionation of Methanolic Extracts 491
 15.4.4 Ethyl Acetate Extraction and Precipitation Studies 491
 15.4.5 Phytochemical and TLC Cholinesterase Analysis of the Selected Plants 493
 15.4.6 *Peltophorum pterocarpum* 494
 15.4.7 *Pycnanthus angolensis* 495
 15.4.8 *Spondias mombin* L 498
 15.4.9 *Morinda lucida* 501
 15.4.10 Cognitive Enhancement Study 502
15.5 Conclusion 505
References 507

Abbreviations

AChE	Acetylcholinesterase
AD	Alzheimer's disease
APT	Attached proton test
ATCHI	Acetylthiocholine iodide
BUCHCL	Butyrylcholine chloride
BuChE	Butyrylcholinesterase
COSY	Correlation spectroscopy
DEPT	Distortionless enhancement by polarization transfer
DMSO	Dimethyl sulfoxide
DTNB	5,5′-Dithiobis-(2-nitrobenzoic acid)
H2SO4	Sulfuric acid
HMBC	Heteronuclear multiple-bond correlation spectroscopy
HMQC	Heteronuclear single quantum correlation spectroscopy
IMRAT	Institute of Advanced Medical Research and Training
NMR	Nuclear magnetic resonance
NOESY	Nuclear Overhauser effect spectroscopy
OAU	Obafemi Awolowo University
PTLC	Preparative thin-layer chromatography
TLC	Thin-layer chromatography
VLC	Vacuum liquid chromatography

15.1 Introduction

Apart from pathological memory loss of neurodegenerative diseases, memory impairment and dementia are on the increase due to an increase in aging population. Medicinal plants have significant therapeutic value in the treatment of the above disorders (Li and Vederas 2009; Silverman and Holladay 2014; Link et al. 2015), and memory-related diseases have been managed with plant remedies for centuries (Perry et al. 2000). Alzheimer's disease (AD) is a neurodegenerative disease characterized by cholinergic neurodegeneration in the brain leading to cognitive deficit and memory impairment (Murraya et al. 2013)**.** Cholinesterase inhibitory activity of plants, used traditionally for managing memory loss, has been reported by many researchers (Ingkaninan et al. 2003; Oh et al. 2004; Elufioye et al. 2010). Scopolamine-induced amnesic animal model has been widely used for screening compounds for anti-dementia effects (Lee et al. 2009; Rubaj et al. 2003). Morris water maze test is widely accepted for the assessment of spatial memory in an experimental animal model (Lee et al. 2009; Moris 1984; Kim et al. 2003). Our findings on cholinesterase inhibitory and memory-enhancing potentials of selected species of the Nigerian flora are presented in this chapter.

15.2 Plant Materials

The plant parts used were collected from various locations (Table 15.1) and were properly identified.

15.3 Extraction

The powdered parts of the different plants were macerated separately with 80% methanol for 72 h and extracts concentrated in vacuo at 40 °C.

15.4 Anticholinesterase Assay Procedures

15.4.1 Spectrophotometric Analysis

The inhibitions of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) were determined spectrophotometrically using acetylthiocholine iodide (ATCHI) and butyrylcholine chloride (BUCHCL) as substrates, respectively (Ellman et al. 1961). Used as a positive control was physostigmine (eserine). In the assay, 2.0 ml of 100 mM of sodium phosphate buffer (pH 8.0), 100 μl of enzyme preparation (2.55×10^{-3} units/μl), and 100 μl of test samples (10 mg/ml) dissolved in methanol were mixed and incubated for 30 min. One hundred microliter of DTNB was then added to the mixture, and the reaction started with the addition of 100 μl of appropriate substrate dissolved in buffer. The hydrolysis of acetylthiocholine and

Table 15.1 Medicinal plants selected for the screening of anticholinesterase activity

Plant species	Family	Collection site
Tetrapleura tetraptera	Leguminosae	Medicinal farm, OAU
Markhamia tomentosa	Bignoniaceae	Ede road, Ile Ife
Jatropha curcas	Euphorbiaceae	Medicinal farm, OAU
Spondias mombin	Anacardiaceae	Medicinal farm, OAU
Alchornea laxiflora	Euphorbiaceae	Medicinal farm, OAU
Morinda lucida	Rubiaceae	Medicinal farm, OAU
Peltophorum pterocarpum	Leguminosae	Road 7, OAU campus
Dioscorea dumetorum	Dioscoreaceae	Medicinal farm, OAU
Capsicum frutescens	Solanaceae	Medicinal farm, OAU
Ceiba pentandra	Bombacaceae	Medicinal farm, OAU
Combretum molle	Combretaceae	Road 1, OAU campus
Holarrhena floribunda	Apocynaceae	Medicinal farm, OAU
Pycnanthus angolensis	Myristicaceae	Road 7, OAU campus
Bombax bromoposenze	Bombacaceae	Medicinal farm, OAU
Garcinia kola	Guttiferaceae	Medicinal farm, OAU
Antiaris africana	Moraceae	Medicinal farm, OAU
Calophyllum inophyllum	Guttiferaceae	Road 7, OAU campus
Crinum jagus	Amaryllidaceae	Medicinal farm, OAU
Jatropha tanjorensis	Euphorbiaceae	Medicinal farm, OAU
Cissampelos owariensis	Menispermaceae	Road 1, OAU campus
Croton zambesicus	Euphorbiaceae	Ede road, Ile Ife
Ipomea involucrata	Convolvulaceae	Road 1, OAU campus

OAU Obafemi Awolowo University

butyrylthiocholine was determined spectrophotometrically at 412 nm by the formation of yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholine catalyzed by enzymes. Negative control was methanol, and all assays were carried out in triplicates. Percentage enzyme inhibition was calculated as

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

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

For the IC_{50} study, 2 ml of phosphate buffer (100 mM, pH = 8.0) and varying concentration of extracts were added together, followed by the addition of 100 μl of the enzyme. The resulting mixture was vortexed and incubated for 30 min at 37 °C. After 30 min, 100 μl of DTNB was added, and the reaction was initiated by the addition of 100 μl of the substrate. The change in absorbance was monitored spectrophotometrically for 4 min at 412 nm. The data recording from the spectrophotometer was subjected to a linear regression analysis using the SigmaPlot Graphical Software, Version 1.02, to obtain the change in absorbance per minute (ΔA/min) which was used to calculate the percentage inhibitions.

The anticholinesterase activity of extracts of several plant species against acetylcholine esterase (AChE) and butyrylcholine esterase (BuChE) has been reported earlier (Elufioye et al. 2010).

15.4.2 Thin-Layer Chromatography (TLC) Bioautographic Assay

The TLC bioautographic assay was performed according to Rhee et al. (2001a). Crude extracts, fractions, and subfractions were spotted on the TLC plates followed by the development of appropriate solvent systems. The developed plates were air-dried and sprayed first with 2.55×10^{-3} units/ml of AChE until saturated and then incubated at 37 °C for at least 20 min before spraying with 0.5 mM of the substrate (ATCHI), and then DTNB. Eserine (physostigmine) was co-chromatographed as standard AChE inhibitors.

A duplicate plate was run to detect false-positive effects due to the interaction between the components of the extract chromogenic reagents, according to the method cited by Rhee et al. (2001b). Developed TLC plates were sprayed with DTNB/ATCHI reagent (ImM DTNB and ImM ATCHI in phosphate buffer) until the plates were saturated. The plates were allowed to air-dry for about 5 min before they were sprayed with the enzyme solution. A yellow background appeared with white spots caused by inhibiting compounds. Thus, it could be established whether the inhibition was in the enzymatic reaction or in the chemical reaction between thiocholine and DTNB.

15.4.3 Fractionation of Methanolic Extracts

The different parts of eight active plants selected for fractionation were partitioned into n-hexane, ethyl acetate, and water. The various fractions were concentrated in vacuo at 40 °C and assayed for AChE and BuChE inhibitory action (Table 15.2).

15.4.4 Ethyl Acetate Extraction and Precipitation Studies

The leaves of four most active plants (i.e., *Pycnanthus angolensis, Morinda lucida, Spondias mombin,* and *Peltophorum pterocarpum*) were selected for further investigation. They were bulk extracted separately with 100%, ethyl acetate and extracts were concentrated in vacuo. Lipid constituent of the ethyl acetate extracts was precipitated out by gradual addition of methanol.

Table 15.2 Anticholinesterase activity of fractions of selected plants against acetylcholine esterase (AChE) and butyrylcholine esterase (BuChE)

Samples		% Inhibition (AChE)			% Inhibition (BuChE)		
Plant species	Plant part	Hexane	Ethyl acetate	Aqueous	Hexane	Ethyl acetate	Aqueous
Eserine		92.63			89.30		
Crinum jagus	Bulb	45.31			44.71		
Croton zambesicus	Leaves	21.29	64.81	16.79	12.66	58.34	20.11
Pycnanthus angolensis	Root	24.92	68.48	23.46	13.19	49.66	30.21
Pycnanthus angolensis	Stem	21.74	66.70	34.46	13.28	40.02	6.98
Pycnanthus angolensis	Fruits	20.02	55.59	33.16	17.39	43.14	19.40
Pycnanthus angolensis	Leaves	23.94	65.66	48.80	11.49	49.38	42.17
Spondias mombin	Stem bark	17.24	67.80	30.08	23.00	68.51	19.93
Spondias mombin	Root bark	14.60	88.13	57.10	21.08	76.38	49.66
Spondias mombin	Leaves	36.16	58.10	23.00	24.86	52.66	18.84
Calophyllum inophyllum	Stem bark	27.18	47.81	11.75	14.06	21.11	8.67
Calophyllum inophyllum	Leaves	10.46	46.04	18.89	4.88	23.12	13.28
Calophyllum inophyllum	Flowers	10.69	38.15	10.53	9.32	24.67	18.28
Calophyllum inophyllum	Root bark	16.19	44.92	19.77	14.63	43.43	18.11
Calophyllum inophyllum	Fruits	10.69	28.15	13.53	10.10	29.68	5.04
Tetrapleura tetraptera	Fruits	10.19	44.32	15.77	17.38	48.13	19.37
Tetrapleura tetraptera	Leaves	10.15	39.85	19.37	7.94	43.20	11.49
Tetrapleura tetraptera	Root bark	7.94	46.48	10.17	5.80	42.00	12.50
Tetrapleura tetraptera	Stem bark	14.08	64.30	19.40	13.23	49.11	13.28
Ipomea involucrata	Aerial part	10.55	38.00	10.13	9.08	43.19	11.69
Alchornea laxiflora	Stem bark	12.31	28.10	10.69	4.02	16.60	13.33
Alchornea laxiflora	Root bark	13.10	25.04	12.55	18.46	15.68	13.88
Alchornea laxiflora	Leaves	10.69	34.20	17.38	7.73	18.15	4.88
Peltophorum pterocarpum	Leaves	28.62	66.10	18.00	22.01	46.32	19.23

(continued)

Table 15.2 (continued)

Samples		% Inhibition (AChE)			% Inhibition (BuChE)		
Plant species	Plant part	Hexane	Ethyl acetate	Aqueous	Hexane	Ethyl acetate	Aqueous
Peltophorum pterocarpum	Stem	26.66	70.10	29.14	14.44	63.84	21.74
Peltophorum pterocarpum	Fruits	10.90	40.58	31.07	12.63	22.69	38.08
Peltophorum pterocarpum	Root	34.02	69.91	13.28	20.63	70.13	18.15

15.4.5 Phytochemical and TLC Cholinesterase Analysis of the Selected Plants

The TLC of both the precipitates and the supernatant of the selected most active plants were carried out using chloroform-hexane 7:3 as the solvent system. The developed plates were sprayed with different phytochemical reagents such as vanillin/sulfuric acid, antimony trichloride, Dragendorff's reagent, and anisaldehyde spray reagents. Some of the developed plates were also subjected to TLC autobiographic enzyme assay. After spraying with vanillin/H_2SO_4, it was observed that supernatant of most of the plants gave better color reaction to the spraying reagent. Harborne (1973) showed that concentrated sulfuric acid is useful in the general detection of organic compounds such as steroids, terpenes, and lipids. Vanillin/H_2SO_4 is also used in the detection of essential oils with positive detection indicated by several different colors (Pothier 2000).

Spraying with Dragendorff's reagent indicated the presence of alkaloid in some of the plants. Alkaloids have been implicated as cholinesterase inhibitor by several researchers (Houghton et al. 2004). Both eserine from *Physostigma venenosum* and galanthamine from *Crinum* are alkaloids which have been reported as AChE inhibitors. Alkaloidal spots were observed as orange-brown zones against a yellow background (Pothier 2000).

Antimony trichloride is used for detecting cardiac glycosides and saponins (Pothier 2000). Precipitates of *S. mombin, M. lucida,* and *P. Pterocarpum* and the supernatant of *C. zambesicus* and *S. mombin* showed positive results with antimony trichloride.

Spraying with anisaldehyde is useful for the detection of terpenoids (usually purple, blue or red) and some other compounds such as ligands, sugar, and flavonoids (Pothier 2000). A number of terpenoid spots were observed in the tested extracts.

Both the precipitates and the supernatants were also subjected to quantitative and qualitative AChE inhibitory activities. The activity was higher in the supernatant when compared with the precipitate (Table 15.3).

Table 15.3 Cholinesterase inhibitory activity of precipitate and supernatant of the four most active plants

Name of plants	Plant part	Methanolic extracts (AChE)	Methanolic extracts (BuChE)	Ethyl acetate extracts	Weight (g)	% AChE inhibition
Morinda lucida	Leaves	40.15 ± 2.57	34.09 ± 1.93	Precipitate	28.95	53.20
				Supernatant	38.66	82.35
Spondias mombin	Leaves	48.58 ± 4.56	47.34 ± 2.55	Precipitate	18.09	71.52
				Supernatant	19.20	87.33
Peltophorum pterocarpum	Leaves	47.5 ± 2.41	48.9 ± 0.71	Precipitate	36.71	42.65
				Supernatant	28.45	86.25
Pycnanthus angolensis	Leaves	43.96 ± 3.04	43.59 ± 1.77	Precipitate	23.31	72.60
				Supernatant	40.78	77.44
Crinum jagus	Internal standard			Extract	38.93	42.83

15.4.6 *Peltophorum pterocarpum*

Peltophorum pterocarpum is a deciduous tree from the family Leguminosae and subfamily Caesalpiniaceae. Several pharmacological activities have been reported for the plant including hepatoprotective (Kaushik et al. 2010) and antioxidant effects (Sridharamurthy et al. 2012). Several bioactive compounds have also been isolated. One new derivative of peltogynoid ophioglonin and a new 2-phenoxychromone with its 3′-O-β-D-glucoside derivative have been reported in the dichloromethane leaf extract (Polasek et al. 2013). Terrestribisamide (Karunai et al. 2012) and sitosterol-β-D-glucopyranoside tetraacetate (Pathipati et al. 2014) have also been isolated from the plant.

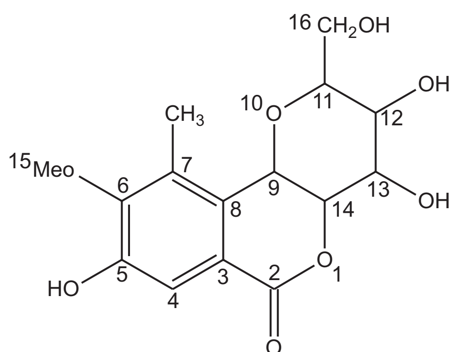
15.4.6.1 Isolation of Bioactive Components

The powdered sample (1 kg) was extracted with 80% methanol and concentrated in vacuo. The methanol extract (44.34 g) was successively partitioned into n-hexane, ethyl acetate, and water. Vacuum liquid chromatography (VLC) of the ethyl acetate fraction on silica gel with gradient elution from n-hexane through ethyl acetate to methanol yielded five subfractions. These were tested, and the most active fraction was further purified by column chromatography on silica gel 60 with gradient elution from n-hexane in ethyl acetate through 100% ethyl acetate to 100% methanol. This yielded 112 subfractions bulked into 25 based on their TLC patterns. One compound was isolated following repeated crystallizations in methanol of subfractions d and e pulled together (Elufioye et al. 2016).

15.4.6.2 Spectroscopy Analysis

^{1}H and ^{13}C NMR (in both methanol and acetone), COSY, NOESY, and HMBC were recorded on a 600 MHz instrument.

Fig. 15.1 Chemical structure of bergenin



15.4.6.3 Spectra Data

^{13}C NMR: δ 165 (C-2), 119 (C-3), 111 (C-4), 152 (C-5), 142 (C-6), 149 (C-7) 117 (C-8), 74 (C-9), 83 (C-11), 71 (C-12), 75 (C-13), 81 (C-14), 60 (C-15), and 62 (C-16). ^{1}H NMR: δ 7.08 (s), 4.95 (d), 4.90 (dd), 4.06 (dd), 3.94 (s), 3.80 (dd) 3.70 (m), and 3.49 (dd).

Following data analysis and comparison with literature (Nunomura et al. 2009), the compound was identified as bergenin (Fig. 15.1) with an IC_{50} of 13.17 μM toward AChE and 14.60 μM toward BuChE.

15.4.7 *Pycnanthus angolensis*

Pycnanthus angolensis (African nutmeg) is an evergreen tree from Myristicaceae family. Flavonoids with cytotoxic effect have been isolated from the plant (Mansoor et al. 2011). Analgesic and anti-inflammatory fatty acids have also been reported (Brill et al. 2004). Other reported activities include antioxidant (Oladimeji and Akpan 2015), antimalarial (Ancolio et al. 2002), antihelminthic (Onocha and Otunla 2010), cholesterol lowering (Leonard 2004), and antinociceptive/antiulcer effects (Sofidiya and Awolesi 2015).

15.4.7.1 Isolation of Bioactive Components

The supernatant (120.36 g) was subjected to vacuum liquid chromatography (VLC) on silica gel using hexane, dichloromethane, and methanol mixtures as the solvent system. A total of 53 fractions were collected and bulked into 6 based on their TLC profile. The bulked fractions were subjected to TLC autobiographic assay, and fractions showing activity were further purified by repeated VLC and PTLC leading to the isolation of the compounds.

15.4.7.2 Spectroscopic Analysis

Both 1D and 2D NMR spectroscopic analyses were carried out. Structure elucidation was done based on ^{1}H and ^{13}C NMR, COSY, HMQC, and HMBC spectra data.

15.4.7.2.1 Spectra Data for Compound 1

Compound **1** was brownish yellow in color and oily. The ^{1}H NMR spectrum, ($CDCl_3$, 300 Hz) showed the following signals – δ6.4(s), δ5.4 (t), δ4.1(d), δ2.0(d), δ1.4(m), δ0.85(m), δ0.87(m), δ0.9(m), δ 1.70(s), and δ1.60(s) – while the ^{13}C NMR ($CDCl_3$, 300 Hz) data are δ 59.63 (C-1), 123.30 (C-2), 140.50 (C-3), 40.08 (C-4), 26.93 (C-5), 37.51 (C-6), 33.90 (C-7), 37.64 (C-8), 25.35 (C-9), 39.95 (C-10), 33.01 (C-11), 39.58 (C-12), 25.00 (C-13), 37.50 (C-14), 28.19 (C-15), 29.91 (C-16), 36.88 (C-17), 135.50 (C-18), 123.48 (C-19), 24.68 (C-20), 16,23 (C-21), 16.38 (C-22), 19.96 (C-23), 22.83 (C-24), 22.92 (C-25), and 19.93 (C-26).

The signal at 5.4 (t) is an olefinic proton assigned to the protons on C-2 and C-19. The signal at δ4.1 (d) represents an alcohol proton and is assigned to the proton residing on C-1. There is a multiplet at δ1.40 to δ1.35 which represents the methylene protons on C-7, C-11, and C-15, while multiplets at δ 1.30 to δ 1.00 were assigned to the protons on C-6, C-8, C-9, C-10, C-12, C-13, C-14, C-16, and C-17. The signal at δ 1.60 (s) was assigned to the methyl protons on C-22 and C-26, while the signal at δ 1.70 was assigned to the OH group. Other assignments include the signals at δ 0.85 (m), δ 0.87 (m), and δ 0.9 (m) which were assigned to the methyl protons on C-21, C-23, C-24, and C-25.

Compound **1** appears to be a C-26 carbon compound since the ^{13}C spectrum showed that there were 6CH_3, 13CH_2, 5CH, and 2C. Characteristic are the oxygenated terminal methylene carbon resonating at δ 59.39 (C-1), the methine carbons resonating at δ_c 123.39 and δ_c 123.48 (C-2 and C-19), respectively, and the quaternary carbons C-3 and C-19 resonating at δ_c 140.50, and δ_c 135.50, respectively. The tertiary methyl groups (C-22 and C-26) on C-3 and C-18 resonated at δ_c 16.38 and δ_c 19.93, respectively; the secondary methyl groups (C-23, C-24, and C-25) resonated at δ_c 19.96, δ_c 22.83, and δ_c 22.92; while the terminal methyl group (C-21) resonated at δ_c16.23.

On critical examination of the spectra, compound 1 appears to be an extension of phytol by additional double bound and methyl groups. Phytol is a C-20 compound, while compound **1** is a C-26 compound with additional CH_3 at C-22; CH_2 at C-16, C- 17, and C-20; CH at C-19; and Cq at C-18. This compound with IUPAC name (2*E*, 18*E*)-3,7,11,15,18-pentamethylhenicosa-2,18-dien-1-ol, and named eluptol (Fig. 15.2), appears new, and it is also being reported for cholinesterase inhibitory activity for the first time with an IC_{50} of 22.26 μg/ml (AChE) and 34.61 μg/ml (BuChE).

15.4.7.2.2 Spectra Data for Compound 2

1H NMR ($CDCl_3$, 300 Hz): δ6.6(s), δ6.4(s), δ6.0(t), δ5.1(m), δ4.2(t), δ3.1(d), δ2.6(dd), δ2.2(m), δ2.0(m), δ1.6(m), and δ1.2(m). ^{13}C NMR ($CDCl_3$, 300 Hz): δ 132.30 (C-1),124.73 (C-2), 132.42(C-3), 134.77 (C-4), 139.94 (C-5), 123.74 (C-6), 145.69 (C-7), 118.32 (C-8), 173.52 (C-9), 68.33 (C-10), 28.11 (C-11), 26.56 (C-12), 28.38 (C-13), 29.29 (C-14), 34.76 (C-15), 29.91 (C-16), 29.58 (C-17), 27.76 (C-18), 39.26 (C-19), 25.85 (C-20), 39.79 (C-21), 146.10 (C-22), 133.33 (C-23), 130.91 (C-24), 148.68 (C-25), 188.18 (C-26), 16.11 (C-27), 17.88 (C-28), 16.15 (C-29), and 16.28 (C-30).

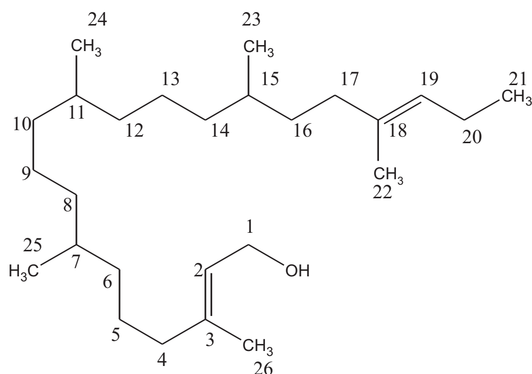


Fig. 15.2 Chemical structure of eluptol [(2*E*, 18*E*)-3,7,11,15,18-pentamethylhenicosa-2,18-dien-1-ol]

The ^{13}C spectrum showed $4CH_3$, $11CH_2$, 7CH, and 8C. Thus, the compound is a C-30 compound. Diagnostic are the carbonyl carbons C-9 and C-26 resonating at δ_c 173.52 and δ_c 188.18, respectively. Also important is the oxygenated methylene carbon at C-10 that acts as a bridge between the two aromatic ring systems and resonated at δ_c 68.33. Also, the methine carbons C-7 and C-8 resonated at δ_c 145.69 and δ_c 118.32, respectively, which are in HMBC correlating with the carbonyl at C-9. The hydroxyl group on C-25 (δ_c 148.68) which made it absorb at a higher value differentiated it from that at C-24 (δ_c 130.91) even though both are quaternary carbons. The secondary methyl carbon C-28 resonated at δ_c 17.88, while tertiary methyl groups C-27, C-29, and C-30 resonated at δ_c 16.11, δ_c 16.15, and δ_c 16.28.

The proton signal at δ 6.6 (s) represents the methylene proton on C-23 resonating at δ 133.33 in the HMQC, while that at δ 6.4 (s) resides on C-3 at δ 132.42. The triplet at δ 6.0 was shown to reside on the carbon signal at δ 145.69 assigned as C-7.

In the HMQC spectra, the multiplet at δ 5.1 showed correlation with the carbons at δ 124.73 (C-2), δ 123.74 (C-6), and δ 118.32 (C-8), while the signal at δ 4.2 (t) showed correlation with the diagnostic OCH_2 carbon at δ 68.33 and is thus assigned to C-10. The signal at δ3.1 (d) correlated with the carbon at δ 27.76 assigned to C-18, and the multiplet at δ 2,2 to δ 2.0 correlated with carbon signals at δ 26.56, δ 34.76, δ 29.58, and δ 39.79 and was assigned to carbons C-12, C-15, C-17, and C-21. The multiplet at δ 1,6 to δ 1,2 were assigned to the methyl groups at C-27, C-28, C-29, and C-30.

In the HMBC, the CH_2 at C-10 showed correlation with the CH_2 signal at δ 28.11 which was assigned to C-11 is diagnostic. Also, the CH_2 at δ 39.79 (C-21) couples to the quaternary carbon at δ 146.10 (C-22), while the carbonyl carbon at δ 188.18 (C-26) is coupled to the carbon resonating at δ 133.33 (C-23). The HMBC spectra also showed that the CH at δ 145.69 (C-7) coupled with the quaternary carbon at δ 173.52 (C-9) (Fig. 15.3).

Upon comparison with literature, (Renmin et al. 2004; Venkateswara et al. 2011), compound **2** appears to be a cinnamic acid derivative with differences at C-4 and C-5 of the isolated compound and cinnamic acid because of the 4,5-dimethyl substitution on compound 2 which made the carbons absorb at a higher δ values (δ 134.77 and δ 139.94), respectively. Most common cinnamic acid derivative in

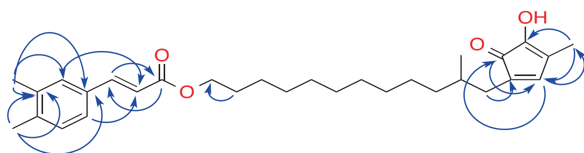


Fig. 15.3 HMBC correlations of compound 2

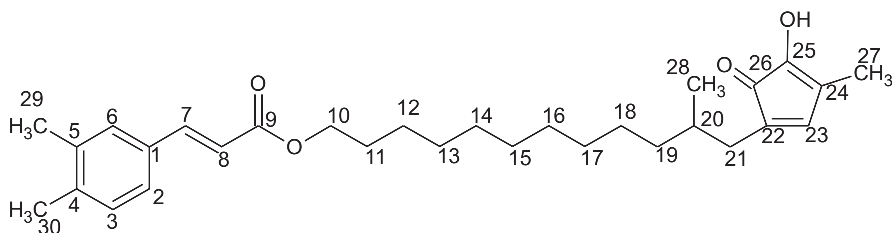


Fig. 15.4 Chemical structure of omifoate A

literature are 2,3-dimethoxy or 2,3-dihydroxy, unlike the isolated compound which is a 2,3-dimethyl derivative. Also, the attached group to the cinnamic acid through the ester linkage appears new. Thus compound 2,[12-(4-hydroxy-3-methyl-oxo-cyclopenta-1,3-dien-1yl)-11-methyl-dodecyl] (*E*)-3-(3,4-dimethylphenyl)prop-2-enoate, named omifoate A (Fig. 15.4), appears to be new, and it is being reported as cholinesterase inhibitor for the first time with an IC_{50} of 6.51 μg/ml (AChE), 9.07 μg/ml (BuChE).

15.4.8 *Spondias mombin* L.

Spondias mombin is a medium-sized, occasionally large deciduous tree of the family Anacardiaceae. Biological activities reported on the plant include antiviral (Corthout et al. 1991, 1992, 1994), antifertility (Uchendu and Isek 2008), molluscicidal (Corthout et al. 1994; Abo et al. 1999), β-lactamase inhibitory (Coates et al. (1994), anti-inflammatory (Abad et al. 1996), hematinic (Asuquo et al. 2013), anticonvulsant, antipsychotic, and sedative properties (Ayoka et al. 2005a, b) and abortifacient (Offiah and Anyanwu (1989), oxytocic (Nworu et al. 2007), antimicrobial (Amadi et al. 2007), antigonadotropic (Asuquo et al. 2012), antioxidant (Maduka et al. 2014), and antidiabetic actions (Moke et al. 2015). Isolated compounds include caryophyllene, myrcene, hexanal, 3-hexenol, and (ε) –2-hexenal (Ceva-Antunes et al. 2003), cinnamic acid, 4-hydroxycinnamic acid, 3-methoxy-4-hydrocinnamic acid, 3-methoxy-4-hydroxycinnamic acid, benzaldehyde, linalool, hexanoic acid, alpha-terpineol, palmitic acid and octanoic acid (Adedeji et al. 1991) anacardic acid (Coates et al. 1994), phytosterols mombintane I and II (Olugbuyiro et al. 2013), coumarin, and new flavonoids mombinrin, mombincone, mombinoate, and mombinol, respectively (Olugbuyiro and Moody 2013).

15.4.8.1 Isolation of Bioactive Components

Vacuum liquid chromatography (VLC) of *Spondias mombin* supernatant (19.20 g) was carried out on silica gel 60 with n-hexane, dichloromethane, and methanol. Monitoring of fractions was by thin-layer chromatography (TLC) on pre-coated silica gel 60 F254 (0.25 mm) plates and spraying with vanillin/sulfuric acid reagent. The subfractions collected (103) were bulked into six based on their TLC pattern. The bulked samples were tested for AChE inhibitory activity using TLC bioautographic assay method. Active subfractions further purified using VLC and three bioactive compounds were isolated by preparative thin layer chromatography (PTLC).

15.4.8.2 Spectroscopic Analysis

The isolated compounds were analyzed spectroscopically (^{1}H, ^{13}C, DEPT, COSY, APT, HMQC, HMBC). TLC analysis in different solvent systems, solubility in water, and determination of IC_{50} was also carried out.

15.4.8.3 Spectra Data

Compound (1) 35 mg was a white powder with R_f of 0.46 in hexane: chloroform 3:7 and R_f of 0.35 in 100% chloroform. It gave purple color to both vanillin and H_2SO_4 and anisaldehyde spray reagent indicating the steroidal nature of the compound (Osman et al. 2015).

The ^{1}H NMR spectrum ($CDCl_3$, 300 Hz) gave signals at δ7.8(m), δ7.75(m), δ5.45(t), δ4.6(s), and δ4.5(d), and the ^{13}C NMR spectrum ($CDCl_3$, 300 Hz) gave signals at 38.71 (C-1), 20.90 (C-2), 78.83 (C-3), 35.57 (C-4), 55.24 (C-5), 18.30 (C-6), 34.06 (C-7), 39.35 (C-8), 54.96 (C-9), 37.34(C-10), 27.22 (C-11), 24.92 (C-12), 37.83(C-13), 39.99 (C-14), 27.19 (C-15), 29.48 (C-16), 47.08 (C-17), 50.22 (C-18), 48.97 (C-19), 150.8 (C-20), 29.66 (C-21), 36.65 (C-22), 27.92 (C-23), 15.96 (C-24), 15.46 (C-25), 16.64 (C-26), 14.33 (C-27), 59.41 (C-28), 109.40 (C-29), and 19.70 (C-30). The DEPT experiment showed that there were $6CH_3$, $11CH_2$, 6CH, and 7C. Thus, compound 1 appeared as a C-30 carbon compound.

In the proton NMR, there was a proton at δ4.5 (d) geminal to the hydroxyl group, with a corresponding carbon chemical shift at δ59.41. It also had an olefinic proton at δ4.6 which resided on the carbon at δ109.40. This proton is a terminal CH_2 and was assigned to C-22. In comparison with literature data (Tolstikov et al. 2005; Sharma et al. 2010; Uddin et al. 2011), compound 1 was identified as betulin (Fig. 15.5). Betulin has been reported previously in many plant species for various biological activities (Tolstikov et al. 2005). However, its cholinesterase inhibitory activity is reported here for the first time with an IC_{50} of 0.88 μg/ml against AChE and 4.67 μg/ml against BuChE. However, previous researchers (Kim et al. 2006) have reported the activity of some oleanane triterpene saponin compounds in the treatment of dementia and mild cognitive impairment.

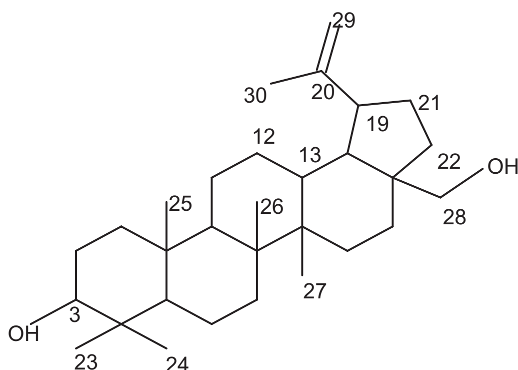


Fig. 15.5 Chemical structure of betulin

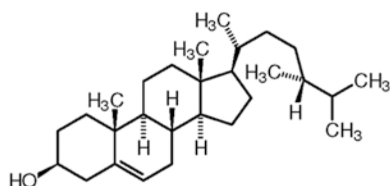


Fig. 15.6 Chemical structure of campesterol

15.4.8.4 Spectra Data of Compound 2

Compound 2 20 mg gave purple color to both vanillin and H_2SO_4 and anisaldehyde spray reagent and had R_f values of 0.2 and 0.27 in hexane: chloroform 2:8 and 100% chloroform, respectively.

The ^{13}C NMR data are 36.92 (C-1), 34.35 (C-2), 72.22 (C-3), 42.73 (C-4), 141.17 (C-5), 122.14 (C-6), 28.67 (C-7), 32.80 (C-8), 50.53 (C-9), 32.33 (C-10), 21.50 (C-11), 37.66 (C-12), 40.18 (C-13), 57.17 (C-14), 23.42 (C-15), 26.45 (C-16), 56.45 (C-17), 12.26 (C-18), 19.82 (C-19), 36.56 (C-20), 19.44 (C-21), 32.31 (C-22), 24.72 (C-23), 46.23 (C-24), 29.54 (C-25), 20.25 (C-26), 19.20 (C-27), and 12.40 (C-28).

^{13}C NMR spectral data of compound 2 suggested that it is a C-28 compound with the APT experiment revealing three quaternary (3 C), ten methylene (10 CH_2), six methyl (6 CH_3), and nine methine (9 CH) carbons. The proton NMR showed an olefinic proton at δ5.40 with a corresponding carbon chemical shift of δ121.14 in the HMQC spectrum as well as an oxygenated methylene proton at δ3.5. In the HMBC data, the diagnostic olefinic proton and the proton geminal to the OH had connectivity with the quaternary carbon resonating at 141.17. From the summary of ^{1}H, ^{13}C NMR, APT, HMQC, and HMBC data as well as comparison with literature (Jaju et al. 2010; Jain and Bari 2010), compound 2 was identified as campesterol (Fig. 15.6) with an IC_{50} of 1.89 μg/ml (AChE), 4.08 μg/ml (BuChE),

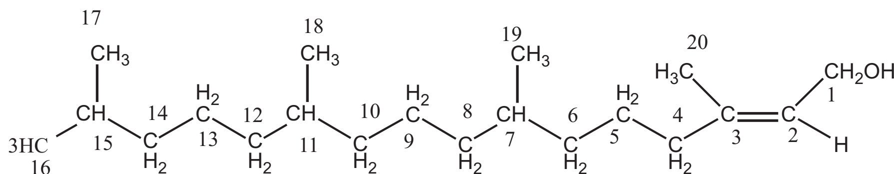


Fig. 15.7 Chemical structure of phytol (3,7.11.15-tetramethyl-2-hexadecen-1-ol)

Campesterol was earlier reported in many plant species such as soybean oil (*Glycine max*) (Shi et al. 2010), rapeseed oil (*Brassica napa*), (Amar et al. 2008), and wheat germ oil (*Triticum* spp.) (Ruibal-Mendieta et al. 2004), but it is being reported for cholinesterase inhibitory activity for the first time with an IC_{50} of 1.89 µg/ml (AChE) and 4.08 µg/ml (BuChE).

15.4.8.5 Spectra Data of Compound 3

Compound 3 19 mg was isolated as a yellowish liquid and had Rf of 0.64 in hexane: chloroform 1:1 and 0.51 in chloroform 100%. It gave pink color to anisaldehyde spray reagent and purple color with vanillin/H_2SO_4.

^{13}C NMR spectral had signals at 59.85 (C-1), 123.48 (C-2), 130.92 (C-3), 40.29 (C-4), 25.55 (C-5) 33.21 (C-6) 30.13 (C-7), 37.78 (C-8), 24.89 (C-9), 37.08 (C-10), 33.11 (C-11), 37.70 (C-12), 25.22 (C-13), 39.79 (C-14), 28.40 (C-15), 23.15 (C-16), 23.05 (C-17), 20.17 (C-18), 20.14 (C-19), and 16.86 (C-20) and revealed $5CH_3$, $10CH_2$, 3CH, and 1C=C suggesting a C-20 compound. The ^1H NMR had a signal at δ 5.4(t) which is an olefinic proton assigned to C-2. The alcoholic proton at δ 4.1(d) was assigned to the proton residing on C-1while, the triplet at δ1.98 was assigned to the proton on C-4. The multiplets at δ 1.44 and δ1.35 were the methine protons on C-7 and C-11. Also, the multiplets at δ1.30 to δ1.03 were assigned to protons residing on C-6, C-8, C-9, C-10, C-12, and C-13, while the signal at δ1.65 (s) is the methyl proton on C-20. The signal at δ1.66 represents the OH group. Analysis of the spectral data and comparison with literature (Arigoni et al. 1999) showed compound 3 as phytol (Fig. 15.7). Phytol has been previously reported for its cholinesterase inhibitory activity (Elufioye et al. 2015).

15.4.9 *Morinda lucida*

Morinda lucida from the family Rubiaceae is a medium-sized tree that grows in tropical West Africa rainforest. Activities reported for the plant include hepatotoxicity and nephrotoxicity (Oduola et al. 2010) and antimalarial (Makinde and Obih

1984) and molluscicide properties (Adewumi and Adesogan 1983). Adesogan (1973) reported the isolation of 18 anthraquinones and its derivatives: lucidin, soranjidiol, damnacanthal, nordamnacanthal, morindin, munjistin, and purpuroxanthin from the wood and bark of *Morinda lucida.* In addition, tannins, flavonoids, and saponosides have been isolated. Adewumi and Adesogan (1983) reported the isolation of anthraquinones and oruwacin from the roots of *Morinda lucida*. Two known triterpenic acids (ursolic and oleanolic acids) were isolated from the leaves (Cimanga et al. 2006). Koumaglo et al. (1992) reported the isolation of three compounds (digitolutein, rubiadin 1-methyl ether, and damnacanthal) from the stem bark of *Morinda lucida*.

15.4.9.1 Isolation of Bioactive Constituents

Morinda lucida supernatant (36.66 g) was subjected to repeated vacuum liquid chromatography (VLC) on silica gel with n-hexane, dichloromethane, and methanol as the solvent system. One hundred thirteen subfractions collected were bulked into 7 based on their chromatographic pattern. The bulked fractions were assayed for AChE inhibitory activity using TLC bioautographic method. Fraction M_1 showing highest activity was further purified by VLC. The active subfraction ($M1_b$) was subjected to PTLC and active compound isolated (Elufioye et al. 2015).

15.4.9.2 Spectroscopic Analysis

The isolated compound was analyzed spectroscopically (^{1}H NMR, ^{13}C NMR).

15.4.9.2.1 Spectral Data

^{13}C NMR: 59.65 (C-1), 123.30 (C-2), 140.55 (C-3), 40.10 (C-4), 25.36 (C-5), 36.89 (C-6), 32.92 (C-7), 37.66 (C-8), 24.70 (C-9), 37.51 (C-10), 33.01 (C-11), 37.59 (C-12), 25.02 (C-13), 39.59 (C-14), 28.20 (C-15), 22.94 (C-16), 22.85 (C-17), 19.97 (C-18), 19.94 (C-19), and 16.41 (C-20).

The ^{13}C NMR data of ML-2 showed 5CH_3, 10CH_2, 3CH, and 1C=C making compound ML-2 a C-20 carbon compound. The ^{1}H NMR had signals at δ 5.4(t), δ 4.1(d), δ1.98 (d), δ1.65 (s), δ1.52. δ 1.44 (m), δ1.35 (m), and δ1.30(m) to δ1.03 (m). Analysis of the spectra showed that compound ML-2 is phytol when compared with literature data (Arigoni et al. 1999) and has been previously reported (Elufioye et al. 2015).

15.4.10 Cognitive Enhancement Study

15.4.10.1 Animals

Sixty-five albino mice purchased from the Institute of Advanced Medical Research and Training (IMRAT), College of Medicine, University of Ibadan, were used for this study.

15.4.10.2 Administration of Doses for the Various Groups

The mice were labeled, weighed and grouped into 13 groups of five animals each. All animals were preinjected with 3 mg/kg scopolamine intraperitoneally. Groups 1–3, 4–6, 7–9, and 10–12 were given 0.2 ml equivalent doses of 4 mg/kg, 6 mg/kg, and 8 mg/kg of the extracts of *Morinda lucida*, *Peltophorum pterocarpum, Pycnanthus angolensis*, and *Spondias mombin*, respectively, while the control group 13 was given 0.2 ml of distilled water for 3 consecutive days.

15.4.10.3 Morris Water Maze Test Procedure

Morris water maze is a test usually done to assess spatial memory function. In this study, it was carried out according to the method of Morris (1984) as described by Kim et al. (2003) and Lee et al. (2009). The water maze is made up of a circular pool (90 cm in diameter and 45 cm in height) filled with a mixture of water and evaporated milk to a height of 30 cm. The pool was usually divided into four quadrants with a platform submerged at 1 cm below the water level in one of the quadrants. On day 1 of the assays, the animals were trained to swim for 60 s without the platform. Thereafter, the animals were given two swimming trial sessions per day for 4 consecutive days with the platform in place. Average escape latencies were calculated for each trial session by measuring the locations of each animal from starting position to the platform. After locating it, each mouse was allowed to stay on the platform for 10 s. However, any animal which failed to locate the platform after 120 s was also placed on the platform for 10 s before taking away from the pool. A 30 min interval was observed between daily trials. The point of entry into the pool for the animals and the location of the platform were changed on a daily basis but remained unchanged between daily trials. Changes in the escape latency from day to day represent long-term or reference memory, while changes from trial 1 to trial 2 on the same day represent working or short-term memory. Amnesia was induced in all animals by intraperitoneal injection of 3 mg/kg scopolamine dissolved in water/DMSO. To establish amnesia, all the animals were assessed for spatial memory 24 h after the administration of scopolamine. Treatment with different doses commenced after establishing amnesia in the animals. The control group was given 0.2 mL distilled water instead of extracts.

15.4.10.4 Histopathology

At the end of the experiments, all animals were sacrificed by cervical dislocation. The brains were removed and preserved in phosphate formalin. Slides of the forebrain and hippocampus were prepared and observed under a light microscope with photomicrographs taken and the number of cells in the CA1 region of the hippocampus estimated.

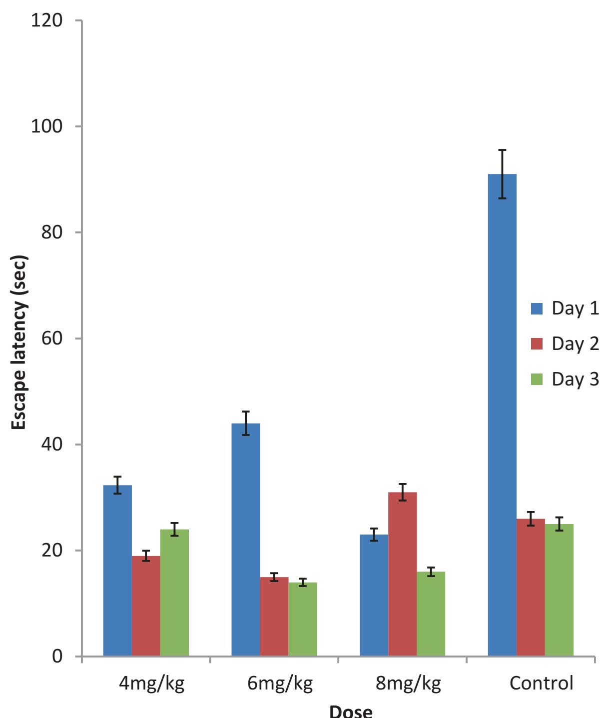


Fig. 15.8 Escape latency time of *Morinda lucida* extract

15.4.10.5 Observations

Impairment of memory and learning is the most characteristic manifestation of cognitive dysfunction, and it can be induced chemically in people (Broks et al. 1988) as well as in experimental animals by scopolamine, a cholinergic antagonist known to interfere with acetylcholine transmission in the central nervous system (Misane and Ogren 2003). The effect of *Morinda lucida* (Fig. 15.8), *Peltophorum pterocarpum* (Fig. 15.9), *Spondias mombin* (Fig. 15.10), and *Pycnanthus angolensis* (Fig. 15.11) showed that the escape latency time of animals induced by scopolamine was significantly reduced by ethyl acetate extracts of the plants when compared with the control group that received distilled water.

The extracts showed dose-dependent cognitive enhancing activity. The histopathology study revealed no significant change in the histology of the brain. However, a reduction in density of cells in the hippocampus of the control mice pretreated with scopolamine only and an increase in the number and density of cells in the animals treated with extracts were observed.

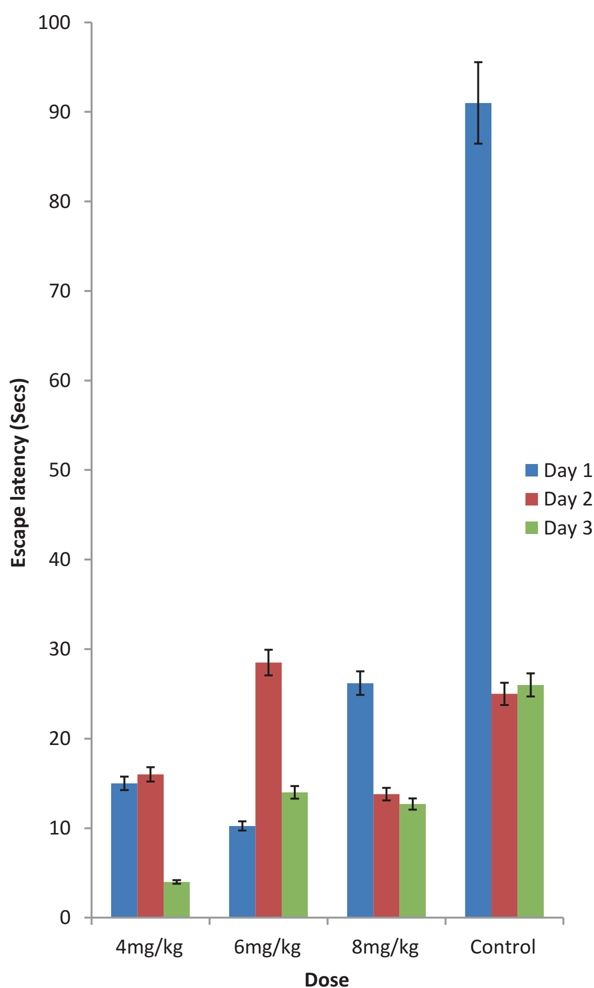


Fig. 15.9 Escape latency time of *Peltophorum pterocarpum* extract

15.5 Conclusion

Several plants have been used in many traditional medical systems all over the world for the management of memory-related problems. This study showed the potential of *S. mombin, P. angolensis, P. pterocarpum*, and *M. lucida* as cholinesterase inhibitors as well as memory enhancers. Thus, the inclusion of these plants in remedies used for managing memory dysfunctions in Nigerian ethnomedicine is justified.

Fig. 15.10 Escape latency time of *Spondias mombin* extract

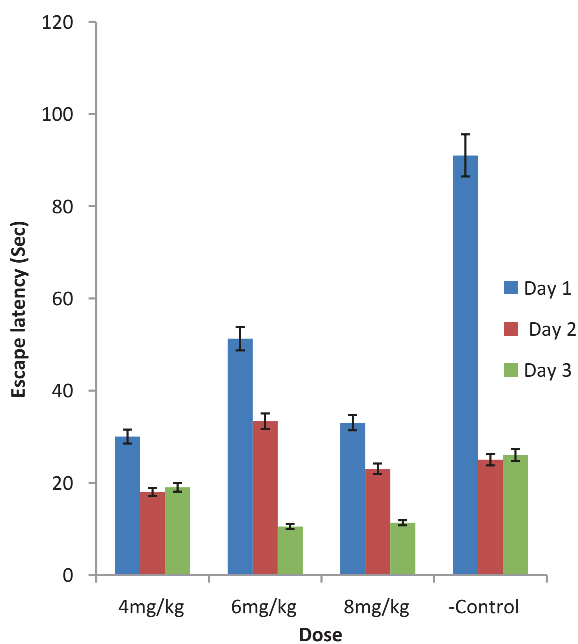
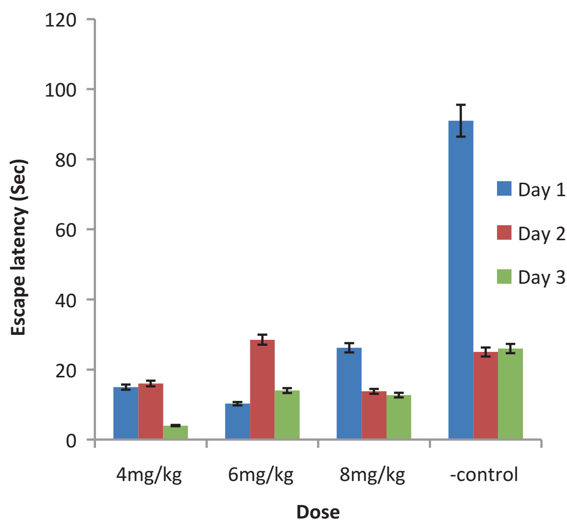


Fig. 15.11 Escape latency time of *Pycnanthus angolensis* extract



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