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IN VITRO BUTYRYLCHOLINESTERASE INHIBITORY ACTIVITY OF *DETARIUM MICROCARPUM*, *EPIPREMNUM AUREUM,* AND *PROSOPIS AFRICANA*

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ABSTRACT
Background: *Alzheimer's disease, a progressive neurodegenerative condition, is primarily associated with reduced cholinergic neurotransmission and notable memory loss. Cholinesterase inhibitors are considered valuable in the discovery of anti-Alzheimer's agents. Recently, butyrylcholinesterase (BChE) inhibitors garnered significant attention in the development of anti-Alzheimer's drugs due to their role in modulating cholinergic neurotransmission, and they are believed to hold promise in Alzheimer's drug development, offering a complementary approach to traditional acetylcholinesterase (AChE) inhibitors.*
Objective: *The study investigates the anti-Alzheimer's potential of Detarium microcarpum, Epipremnum aureum and Prosopis africana extracts.*
Method: *Extracts from the three medicinal plants were evaluated using an in vitro BChE inhibitory assay, and the most active extract was fractioned to identify the activity-rich fraction. Result: All extracts exhibited varying degrees of BChE inhibition. D. microcarpum demonstrated the most potent activity, with 87.26 ± 2.67% inhibition at 1 mg/mL and an IC_{50} of 0.10 ± 0.04 µg/mL. In contrast, E. aureum and P. africana showed weaker inhibitory effects (16.15 ± 1.37% and 23.62 ± 1.91%, respectively) compared to D. microcarpum and were not pursued further. Among the fractions of D. microcarpum, the ethyl acetate and hexane fractions exhibited strong BChE inhibition (87.32 ± 2.06% and 72.18 ± 1.33%, respectively), with IC_{50} values of 0.10 ± 0.04 µg/mL and 0.27 ± 0.08 µg/mL, comparable to galantamine (90.37 ± 1.62%, IC_{50}: 0.10 ± 0.07 µg/mL).*
Conclusion: *The stem bark of Detarium microcarpum demonstrates potent BChE inhibitory properties, suggesting its potential as an anti-Alzheimer agent.*

Keywords: *Detarium microcarpum*, Alzheimer's disease, butyrylcholinesterase inhibitory, *Epipremnum aureum*, *Prosopis africana.*

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurological disorder marked by significant cognitive decline, particularly memory loss, impairing daily social and occupational activities. As the leading cause of dementia worldwide, AD affects over 20 million people globally (Loizzo *et al*., 2008). Improved living conditions and an ageing population have contributed to a rise in AD cases(Jacobsen *et al*., 2005). Approximately 60% of dementia cases among individuals over 65 years of age are attributed to AD, characterised by cholinergic neurotransmitter deficits and significant memory impairments (Saleem *et al*., 2016).

The cognitive decline associated with AD is primarily linked to diminished acetylcholine (ACh) levels, which are crucial for memory processing within the brain's cortical and hippocampal regions (Hasselmo and Giocomo, 2006). While acetylcholinesterase (AChE) levels drop significantly in late-stage AD, butyrylcholinesterase (BChE) levels remain stable or increase, compensating for lost AChE activity(Lane *et al*., 2024). Butyrylcholinesterase (BChE) inhibitors are crucial in developing anti-Alzheimer medications due to their ability to modulate cholinergic neurotransmission (Kassab *et al*., 2024). As Alzheimer's progresses, AChE levels decrease, while BChE levels stabilise or increase, compensating for the loss of AChE activity (Sharma *et al*., 2024). Blocking BChE may help maintain stable acetylcholine levels, potentially boosting cognitive performance (Alkanad *et al*., 2024).

Recent advancements have identified natural sources of BChE inhibitors as promising anti-Alzheimer's treatments, particularly during AD's advanced stages when BChE activity predominates (Orioli *et al*., 2024). These inhibitors, derived from plants, marine organisms, and other natural products, prevent ACh degradation, thus improving brain function (Khadem and Marles, 2024). Phytochemical investigations and structure-activity analyses continue to inform the design of multi-target ligands and bioscavengers, paving the way for innovative therapies. Despite their potential, current treatments such as tacrine, rivastigmine, and donepezil mainly target AChE and are effective only for mild AD cases, often accompanied by side effects (Gajendra *et al*., 2024). Since BChE activity rises in advanced AD, selective BChE inhibitors may provide benefits with a different, potentially safer, side effect profile (Xing *et al*., 2021). The development of BChE inhibitors could address this gap, with medicinal plants serving as a rich source of bioactive compounds for new drugs (Chaachouay and Zidane, 2024)

This study evaluates the BChE inhibitory potential of *Detarium microcarpum*, *Epipremnum aureum,* and *Prosopis africana*, medicinal plants traditionally used for cognitive enhancement Adewusi *et al*., 2011; Kanti *et al*., 2021; Orisakwe *et al*., 2024. Extracts from these plants were selected for their historical use and regional availability in southwest and north-central Nigeria.

MATERIALS AND METHOD (METHODOLOGY)

Plant Selection, Collection and Authentication

Aerial parts of *Epipremnum aureum* were collected in August 2023 from the botanical garden of the University of Ibadan, situated in Southwestern Nigeria. During the same period, fruits of *Prosopis africana* and stem bark of *Detarium microcarpum* were obtained from the main campus of the University of Ilorin, North-central Nigeria. The taxonomic identity of each specimen was confirmed at the Herbarium Unit of the Forest Research Institute of Nigeria (FRIN), Ibadan, and

voucher specimens were deposited with the Forest Herbarium Ibadan under the accession numbers FHI-112371 (*E. aureum*), FHI-111954 (*P. africana*), and FHI-111953 (*D. microcarpum*).

Chemicals and Reagents

All organic solvents employed in the extraction and partitioning procedures, including methanol (70% aqueous), hexane, dichloromethane (DCM), and ethyl acetate, were of analytical grade and procured from Merck (Darmstadt, Germany). Reagents used for preliminary phytochemical screening, including those for alkaloids, tannins, flavonoids, and saponins, were obtained from BDH Chemicals Ltd. (Poole, UK) and prepared according to standard pharmacognostic protocols.

The butyrylcholinesterase (BChE) inhibition assay was carried out using horse serum butyrylcholinesterase (EC 3.1.1.8), acetylthiocholine iodide, butyrylthiocholine chloride, and 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), all purchased from Sigma-Aldrich (St. Louis, MO, USA). Galantamine (≥98% purity), used as a reference standard, was also sourced from Sigma-Aldrich. Sodium phosphate buffer (100 mM, pH 8.0) was prepared using analytical-grade reagents. All other chemicals and reagents used in this study were of analytical grade and used as received without further purification.

Spectrophotometric measurements were performed using a microtiter plate reader (SpectraMax, Molecular Devices, Sunnyvale, CA, USA), with absorbance monitored at 412 nm.

Preparation of Extract and Fractions

Two hundred grams of air-dried powdered plant material were extracted through cold maceration with 70% aqueous methanol for 72 hours at room temperature (26-33 °C). The extracts were filtered, concentrated under reduced pressure at 45 °C, and stored at 4 °C. Based on preliminary screening, the most active extract *(Detarium microcarpum)* was fractionated via stepwise liquid-liquid partitioning using solvents of increasing polarity (hexane → DCM → EtOAc), with the residual aqueous fraction retained. This polarity-guided separation isolated distinct phytochemical classes: non-polar terpenoids (hexane), moderately polar alkaloids (DCM), and semi-polar flavonoids (EtOAc), streamlining the identification of bioactive fractions. Yield percentages were determined for all crude extracts and *D. microcarpum* fractions.

Qualitative Phytochemical Analysis

Qualitative phytochemical screening was conducted on the crude extracts to detect major classes of secondary metabolites, including saponins, tannins, alkaloids, flavonoids, terpenoids, cardiac glycosides, and anthraquinones, using standard methods as described by Evans (2009) (Evans, 2009).

Butyrylcholinesterase Inhibitory Assay

The assay was conducted using a modified Ellman's method, focusing solely on **butyrylcholinesterase (BChE)** inhibition as described in the butyrylcholinesterase section of Parveen *et al*. (2001) (Parveen *et al*., 2001).

The enzyme used was **horse serum butyrylcholinesterase** (Sigma, St. Louis, MO), and the substrate was **butyrylthiocholine chloride** (0.5 mM).

The chromogenic reagent used was **5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB, 0.3 mM).** The reaction mixture was prepared in **100 mM sodium phosphate buffer (pH 8.0)** at 25 °C in a 96-well microplate.

For each well, 140 µL of phosphate buffer was mixed with 20 µL of the test sample (crude extract or fraction at 1 mg/mL in 5% DMSO) or **galantamine (1 mg/mL, positive control)** and incubated for 30 minutes. After incubation, 10 µL of DTNB solution and 10 µL of butyrylthiocholine chloride were sequentially added to initiate the reaction.

The formation of the yellow 5-thio-2-nitrobenzoate anion was monitored **spectrophotometrically at 412 nm** over 15 minutes using a microplate reader (Molecular Devices, USA). The percentage inhibition was calculated, and extracts/fractions showing more than 50% inhibition were selected for IC_{50} determination.

All assays were conducted in **triplicate across two independent experiments (n = 6)**. IC_{50} values were determined using **GraphPad Prism 9.0** by nonlinear regression (log concentration vs. response).

Galantamine was used as the positive control at a concentration of 1 mg/mL. Extracts and fractions with over 50% inhibition at 1 mg/mL were deemed active, and subsequent dilutions (1.6, 8.0, 40.0, 200.0 and 1000.0 µg/mL) were prepared to calculate the concentration required for 50% inhibition (IC50). Each experiment was performed in triplicate to ensure reproducibility.

RESULTS

Extract Yield of Selected Plants

A total of 200 g each of dried powdered plant materials were extracted using 70% aqueous methanol. The extraction yielded varying percentages among the three plants. *Detarium microcarpum* exhibited the highest extract yield (17.0% w/w), producing a reddish-brown, crispy solid upon drying. *Epipremnum aureum* yielded 15.5% w/w of a greasy, greenish-black extract, while *Prosopis africana* gave the lowest yield (6.0% w/w), appearing as a greasy, dark brown extract with a characteristic honey-like aroma.

Phytochemical Screening Evaluation of Selected Plants

The extracts exhibited various phytochemicals, including glycosides, alkaloids, and polyphenolic compounds. Specifically, the *E. aureum* extract contained saponins, tannins, alkaloids, flavonoids, and both free and combined anthraquinones. Similarly, the *P. africana* extract was found to include saponins, tannins, alkaloids, flavonoids, free and combined anthraquinones, as well as terpenes. In the case of *D. microcarpum* stem bark, the extract revealed the presence of saponins, tannins, flavonoids, and free and combined anthraquinones, alongside terpenes, as summarised in Table 1. None of the extracts contained cardiac glycosides, and while *D. microcarpum* tested negative for alkaloids, both *E. aureum* and *P. africana* tested positive for them.

Table 1: Selected Medicinal Plants and Phytochemical Screening of Selected Medicinal Plants

Selected Plants / Test	***Epipremnum aureus* aerial part**	***Prosopis africana* Fruit**	***Detarium microcarpum* stem bark**
Voucher Number	FHI 112371	FHI 111954	FHI 111953
Family	Araceae	Leguminosae	Caesalpiniaceae
Collection Site	Botanical Garden, University of Ibadan	University of Ilorin main campus	University of Ilorin main campus
Appearance of Extract	Grassy greenish-black extract	Greasy, dark brownish extract	Crispy reddish brown extract
Saponins	+++	+++	++
Tannins	++	++	+++
Alkaloids	++	++	-
Cardiac Glycosides	-	-	-
Free Anthraquinones	+	+	+
Combined Anthraquinones	++	+	+
Flavonoids	++	++	+++
Terpenoids	-	++	+++

Key: +++ = strong presence; ++ = moderate presence; + = weak presence;– = not detected

Butyrylcholinesterase Inhibitory Activity of Selected Plants

The butyrylcholinesterase inhibitory activities of the three extracts are summarised in Table 2. While all extracts demonstrated varying degrees of enzyme inhibition, *D. microcarpum* showed the most significant activity, achieving 87.26±2.67% inhibition at a concentration of 1 mg/L. In contrast, *E. aureum* and *P. africana* exhibited weaker inhibitory effects, with percentage inhibitions of 16.15±1.37% and 23.62±1.91%, respectively, as outlined in Table 2. Given the promising results for *D. microcarpum*, its crude extract was fractionated into hexane, dichloromethane (DCM), ethyl acetate (EtOAc), and aqueous portions. Among these, the ethyl acetate fraction provided the highest yield (45.6% w/w), while the hexane fraction yielded the least (0.3% w/w). Notably, the hexane (72.18±1.33% inhibition) and ethyl acetate (87.32±2.06% inhibition) fractions displayed strong inhibitory activities.

Further analysis revealed IC50 values of 0.1±0.04 µg/mL for the crude extract, 0.27±0.08 µg/mL for the hexane fraction, and 0.1±0.07 µg/mL for the ethyl acetate fraction, comparable to the standard drug galantamine (0.1±0.07 µg/mL). Both the crude extract and the ethyl acetate fraction exhibited inhibitory activity similar to that of galantamine.

Table 2: Butyrylcholinesterase Inhibitory Activities of Extracts and Fractions of Selected Plants

Sample	Percentage yield (%w/w) of extract / fraction	% inhibition at Conc. of 1mg/mL	IC_{50}±SEM (µg/mL)
EAA	15.5	16.15±1.37	NT
PAF	6.0	23.62±1.91	NT
DMS	17.0	87.26±2.67	0.1±0.04*
Hexane fraction*	0.3	72.18±1.33	0.27±0.08
DCM fraction*	4.8	27.43± 1.28	NT
EtOAc fraction*	45.6	87.32±2.06*	0.1±0.04*
Aqueous residue*	35.6	31.78±1.48	NT
Galantamine†	-	90.37±1.62	0.1±0.07

Values were expressed as mean ± S.E.M. (n=6), * p < 0.05 compared to vehicle (5% DMSO control), † = standard drug (galantamine)

Key: EAA = *Epipremnum aureum* aerial part, PAF = Prosopis africana Fruit, DMS = Detarium microcarpum stem bark, NT = Not Tested, * = fractions of Detarium microcarpum stem bark, **= standard drug

DISCUSSION

Medicinal plants, including Gingko biloba, Panax ginseng and Curcuma longa, have shown efficacy in the management of AD by inhibiting the butyrylcholinesterase enzyme (Singhal *et al*., 2012). It is also imperative to study medicinal plants commonly found in the environment for new activity, including their ability to inhibit the butyrylcholinesterase enzyme (Gajendra *et al*., 2024).

The extracts of *D. microcarpum* stem bark displayed the highest yield of extract and displayed strong colour change that signifies the presence of large amounts of saponins, tannins, flavonoids and terpenoids. The finding from this work corroborates the findings of other researchers who reported that the aqueous extract of the stembark shows the presence of tannins, carbohydrates, terpenoids, cardiac glycosides, saponins and similar alkaloids were not reported (Gambo *et al*., 2024). Interestingly, alkaloids have been thought of and reported in the literature as the primary class of phytochemicals that possess anti-Alzheimer's activities, and they have been one of the most attractive groups of compounds that are considered when

searching for new Alzheimer's drugs (Vrabec *et al*., 2023). However, *D. microcarpum* extracts with the highest inhibitory activity (comparable to galantamine, alkaloid and the standard drug) showed positive responses for the presence of polyphenolic compounds. Medicinal plants with high polyphenolic content (tannins and flavonoids) have been reported to possess high antioxidant and anti-inflammatory effects and are effective in combating AD (Singhal *et al*., 2012). Polyphenolic compounds such as resveratrol found in grapes and other fruits, and several others have been reported to possess neuroprotective impact and anti-Alzheimer's activities (Koul *et al*., 2023).

The extracts of *D. microcarpum* have been reported in other studies to display anti-inflammatory (Yaro *et al*., 2017) and antioxidant (Rouamba *et al*., 2017) activities. To further investigate the activity of *D. microcarpum*, the crude extract was fractionated, and the ethyl acetate fraction displayed the highest yield (45.6 % w/w), while hexane (IC_{50} of 0.27±0.08 µg/mL) and ethyl acetate (IC_{50} of 0.1±0.07 µg/mL) fractions displayed powerful butyrylcholinesterase inhibitory activities. The ethyl acetate fraction displayed the most powerful butyrylcholinesterase-inhibitory activity comparable to Galantamine.

Detarium microcarpum has not been reported or known to be used traditionally for the treatment of AD, and this is the first report of butyrylcholinesterase-inhibitory activity of *D. microcarpum* extract and fractions.

The finding here suggest need for more chemical and biological evaluation of the extract and ethyl acetate fraction for the treatment AD and isolation of bioactive compounds with butrylcholinesterase inhibitory activity is ongoing.

CONCLUSIONS

Detarium microcarpum stem bark collected from Northcentral Nigeria displayed **potent butyrylcholinesterase inhibitory activity**, comparable to galantamine, a standard anti-Alzheimer's drug. The ethyl acetate fraction demonstrated the highest inhibitory potency, suggesting potential for further development in managing Alzheimer's disease.

LIMITATIONS

This study was limited to the *in vitro* evaluation of enzyme inhibition. The bioavailability, toxicity, and *in vivo* efficacy of the extract and fractions were not assessed. Further pharmacological and toxicological studies are required to validate their therapeutic relevance.

DECLARATION OF CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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