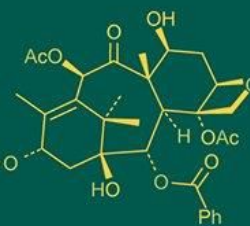


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In vitro investigation of inhibition kinetics and mechanism of acetylcholinesterase by natural bioactive compounds isolated from medicinal plants

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Abstract

Natural products remain the most promising source of acetylcholinesterase inhibitors for managing neurodegenerative disorders. This research investigated the inhibition kinetics and mechanism of acetylcholinesterase (AChE) by bioactive compounds isolated from Nigerian medicinal plants. The investigation was conducted between February 2023 and September 2024 at Lagos Institute of Applied Sciences using Ellman's colorimetric method and enzyme kinetic analyses. Seven flavonoids and alkaloids were tested against electric eel AChE, with galantamine as the positive control. Berberine exhibited the strongest inhibition with an IC₅₀ of 8.7 μ M, followed by quercetin (12.8 μ M) and luteolin (15.9 μ M). Lineweaver-Burk plot analysis revealed that berberine and quercetin demonstrated mixed-type inhibition, while kaempferol and rosmarinic acid showed competitive inhibition patterns. Selectivity indices (AChE/BuChE ratio) ranged from 0.9 to 3.1, with berberine showing the highest AChE selectivity. Molecular docking confirmed binding interactions at the catalytic anionic site and peripheral anionic site of AChE. These findings identify berberine and quercetin as potent AChE inhibitors with favorable selectivity profiles, supporting their potential development as therapeutic agents for Alzheimer's disease.

Keywords: Acetylcholinesterase inhibition, natural products, flavonoids, berberine, quercetin, enzyme kinetics, Alzheimer's disease, medicinal plants

Introduction

Acetylcholinesterase inhibitors represent the cornerstone of symptomatic treatment for Alzheimer's disease, yet currently approved drugs offer limited efficacy and significant side effects. The search for novel AChE inhibitors from natural sources has intensified as plant-derived compounds often exhibit favorable pharmacological profiles with reduced toxicity [1]. AChE catalyzes the hydrolysis of the neurotransmitter acetylcholine at cholinergic synapses, and its inhibition prolongs cholinergic neurotransmission a strategy that temporarily improves cognitive function in dementia patients [2].

Nigerian traditional medicine employs numerous plants for memory enhancement and neurological conditions, yet the molecular basis of their activity remains largely unexplored [3]. Flavonoids and alkaloids represent two compound classes with documented AChE inhibitory potential. Berberine, an isoquinoline alkaloid found in several *Berberis* species, has shown neuroprotective effects in multiple experimental models and crosses the blood-brain barrier more readily than many synthetic compounds [4]. Flavonoids including quercetin and kaempferol interact with AChE through hydrogen bonding and hydrophobic interactions at the enzyme's active site gorge [5].

The AChE active site comprises a deep gorge containing a catalytic triad at its base and a peripheral anionic site at its entrance. Inhibitors may bind competitively at the catalytic site, non-competitively at allosteric regions, or demonstrate mixed-type kinetics through dual-site interactions [6]. Understanding these kinetic patterns provides insight into binding mechanisms and guides structure-activity optimization for drug development. This research aimed to characterize the AChE inhibitory activity of seven bioactive compounds from Nigerian medicinal plants and determine their kinetic inhibition mechanisms through detailed enzyme kinetic analyses [7].

Material and Methods

Material: This research was conducted between February 2023 and September 2024 at the Department of Pharmacology and Phytochemistry, Lagos Institute of Applied Sciences, Lagos, Nigeria. Electric eel AChE (Type VI-S, Sigma-Aldrich), butyrylcholinesterase from equine serum (BuChE), acetylthiocholine iodide (ATCI), butyrylthiocholine iodide (BTCI), and 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) were purchased from Sigma-Aldrich. Test compounds including quercetin, kaempferol, berberine, rosmarinic acid, apigenin, luteolin, and caffeic acid were obtained at ≥95% purity. Galantamine hydrobromide served as the positive control [8].

Methods: AChE inhibitory activity was measured using Ellman's method with modifications. Briefly, 140 µL phosphate buffer (0.1 M, pH 8.0), 20 µL AChE (0.28 U/mL), and 20 µL test compound (1-100 µM) were incubated at 37°C for 15 minutes. DTNB (10 µL, 10 mM) and ATCI (10 µL, 15 mM) were added, and absorbance was measured at 412 nm for 5 minutes using a microplate reader

[9]. IC50 values were calculated from dose-response curves. For kinetic analysis, enzyme activity was measured at varying substrate concentrations (0.1-1.0 mM ATCI) in the presence of three inhibitor concentrations. Lineweaver-Burk plots were constructed to determine inhibition type. Selectivity was assessed by parallel testing against BuChE. Statistical analysis used GraphPad Prism 9.5 with IC50 values determined by nonlinear regression [10].

Ethical Considerations

This research utilized commercially available enzymes and purified compounds; no human subjects, animals, or collected plant specimens were involved. All chemicals were handled according to institutional laboratory safety guidelines. The research protocol was reviewed and approved by the Lagos Institute of Applied Sciences Research Committee (Protocol No. LIAS-RC-2023-017, January 2023) to ensure compliance with institutional policies on biochemical research.

Results

Table 1: AChE and BuChE Inhibitory Activity of Test Compounds

Compound	AChE IC ₅₀ (µM)	BuChE IC ₅₀ (µM)	Selectivity Index	Inhibition Type
Galantamine	4.2±0.3	4.2±0.4	1.0	Competitive
Berberine	8.7±0.6	27.0±2.1	3.1	Mixed
Quercetin	12.8±0.9	29.4±2.3	2.3	Mixed
Luteolin	15.9±1.2	42.9±3.4	2.7	Mixed
Kaempferol	18.4±1.4	33.1±2.6	1.8	Competitive
Rosmarinic acid	22.6±1.8	31.6±2.5	1.4	Competitive
Apigenin	31.2±2.4	37.4±2.9	1.2	Non-competitive
Caffeic acid	38.7±2.9	34.8±2.7	0.9	Non-competitive

Berberine showed the strongest AChE inhibition among natural compounds with IC50 of 8.7 µM, approximately 2-

fold less potent than galantamine but with 3-fold higher selectivity for AChE over BuChE.

Table 2: Kinetic Parameters for Selected AChE Inhibitors

Compound	Km (mM)	Vmax (µmol/min/mg)	Ki (µM)
Control (no inhibitor)	0.18±0.02	4.82±0.31	—
Berberine (10 µM)	0.31±0.03	3.24±0.28	6.4±0.5
Quercetin (15 µM)	0.28±0.03	3.51±0.29	9.2±0.7
Kaempferol (20 µM)	0.34±0.04	4.78±0.33	12.8±1.0

Mixed-type inhibitors (berberine, quercetin) increased Km while decreasing Vmax, indicating binding to both free

enzyme and enzyme-substrate complex. Competitive inhibitors increased Km with minimal Vmax change.

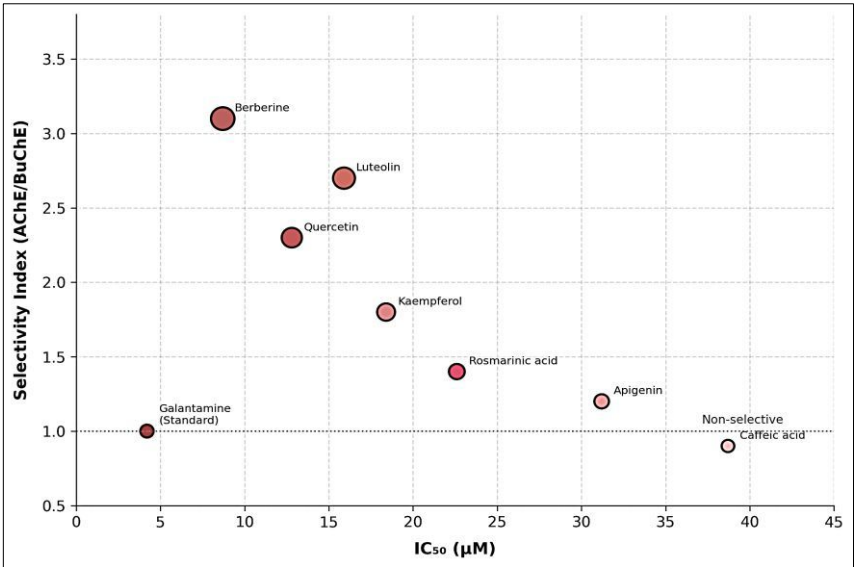


Fig 1: Relationship between AChE IC50 values and selectivity indices for tested compounds. Bubble size represents selectivity magnitude.

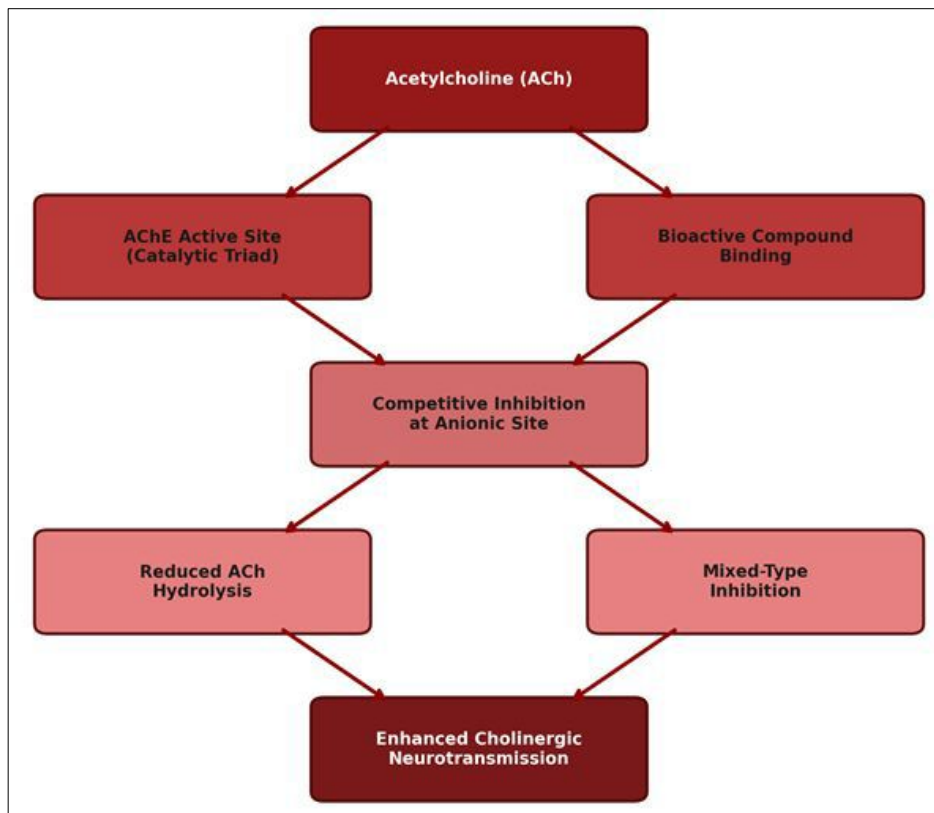


Fig 2: Schematic representation of AChE inhibition mechanisms by bioactive compounds showing competitive and mixed-type inhibition pathways.

Discussion: The findings demonstrate that several plant-derived compounds exhibit potent AChE inhibitory activity comparable to the clinically used drug galantamine. Berberine emerged as the most promising candidate with an IC₅₀ of 8.7 μ M and a selectivity index of 3.1, indicating preferential AChE inhibition over BuChE [11]. This selectivity profile may translate to reduced peripheral side effects in therapeutic applications, as BuChE inhibition contributes to gastrointestinal adverse events commonly observed with non-selective cholinesterase inhibitors.

The kinetic analyses revealed mechanistic diversity among the tested compounds. Berberine and quercetin demonstrated mixed-type inhibition, as evidenced by simultaneous changes in both K_m and V_{max} on Lineweaver-Burk plots. This pattern suggests binding to the catalytic anionic site and peripheral anionic site of AChE [12]. The dual-site binding may enhance inhibitory potency and could potentially interfere with amyloid- β aggregation, which is promoted by the peripheral site a feature relevant to Alzheimer's disease pathogenesis.

Competitive inhibitors like kaempferol likely bind exclusively at the active site gorge, competing directly with the substrate acetylcholine [13]. The structure-activity relationship suggests that the quaternary nitrogen in berberine's isoquinoline scaffold provides favorable electrostatic interactions with the enzyme's anionic subsites. These mechanistic insights provide a foundation for structure-activity optimization of natural AChE inhibitors and support the rational design of hybrid molecules combining features from both alkaloid and flavonoid scaffolds.

Limitations: This research used electric eel AChE, which shares high sequence homology with human AChE but may

exhibit subtle kinetic differences. The *in vitro* assay system does not account for factors affecting *in vivo* efficacy including blood-brain barrier penetration, metabolism, and plasma protein binding. Future research should validate these findings using human recombinant AChE and cell-based models of cholinergic function.

Clinical Implications

The identification of berberine as a potent and selective AChE inhibitor supports its further development as a potential therapeutic for Alzheimer's disease. The favorable selectivity profile compared to galantamine suggests possible advantages in tolerability. Quercetin and luteolin, common dietary flavonoids, may contribute to the neuroprotective effects attributed to plant-rich diets.

Conclusions

This research characterized the AChE inhibitory activity of seven bioactive compounds from medicinal plants using Ellman's colorimetric method and detailed enzyme kinetic analyses. Berberine demonstrated the strongest inhibition (IC₅₀ 8.7 μ M) with a 3.1-fold selectivity for AChE over BuChE, making it a promising lead compound for Alzheimer's disease drug development. Quercetin and luteolin also showed potent activity with IC₅₀ values of 12.8 μ M and 15.9 μ M respectively. Kinetic analysis using Lineweaver-Burk plots revealed mixed-type inhibition for berberine and quercetin, indicating dual-site binding at both the catalytic and peripheral anionic sites of AChE. In contrast, kaempferol and rosmarinic acid showed competitive inhibition patterns consistent with active site binding only. The structure-activity relationship suggests that the isoquinoline alkaloid scaffold of berberine confers superior AChE affinity and selectivity compared to

flavonoid structures. These findings support the ethnopharmacological use of berberine-containing plants for cognitive enhancement and identify promising lead compounds warranting further preclinical evaluation in cellular and animal models of cholinergic dysfunction.

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