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Solvent-Dependent Butyrylcholinesterase Inhibition and Antioxidant Activity of Cucurbitaceous Seed Extracts and *Hydrocotyle asiatica*

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Abstract

Background: Oxidative stress and impaired cholinergic neurotransmission are central features of Alzheimer's disease. Plant-derived inhibitors of butyrylcholinesterase (BChE) may provide useful leads for neuroprotective research. **Objective:** To compare the influence of plant species and extraction-solvent polarity on the BChE inhibitory and DPPH radical-scavenging activities of cucurbitaceous seed extracts and *Hydrocotyle asiatica*. **Methods:** Seeds of *Cucurbita maxima*, *Cucumis melo*, *Cucumis sativus*, and *Citrullus lanatus*, together with whole-plant material of *H. asiatica*, were sequentially extracted with petroleum ether, chloroform, ethyl acetate, and methanol. BChE inhibition and DPPH scavenging were evaluated in triplicate at 0.2, 0.5, and 1.0 mg/mL, using galantamine and ascorbic acid as reference compounds. **Results:** The methanolic extract of *C. lanatus* was reported to inhibit BChE by 81.19% at 1.0 mg/mL (IC50 = 98 µg/mL). The ethyl acetate extract of *C. maxima* produced 81.77% inhibition, whereas the chloroform extract of *H. asiatica* reached 88.88% inhibition at 1.0 mg/mL. In the DPPH assay, the chloroform extract of *C. sativus* showed the strongest seed-derived activity (63.78% at 1.0 mg/mL; IC50 = 642 µg/mL), followed by the ethyl acetate extract of *C. lanatus* (IC50 = 708 µg/mL). **Conclusion:** Bioactivity depended strongly on both species and solvent. The most active fractions warrant bioassay-guided isolation, mechanistic enzyme-kinetic studies, and in vivo validation; the present findings should be interpreted as preliminary in vitro evidence rather than proof of therapeutic efficacy.

Keywords: Alzheimer's disease; butyrylcholinesterase; DPPH; cucurbitaceous seeds; solvent polarity; *Hydrocotyle asiatica*

Highlights

- Sequential extraction revealed strong solvent-dependent differences in BChE inhibition and antioxidant activity.
- Methanolic *Citrullus lanatus*, ethyl acetate *Cucurbita maxima*, and chloroform *Hydrocotyle asiatica* were among the most active BChE-inhibitory fractions.
- The study identifies plant–solvent combinations for future phytochemical isolation rather than establishing clinical efficacy against Alzheimer's disease.

1. Introduction

Alzheimer's disease (AD) is a progressive and multifactorial neurodegenerative disorder that gradually impairs memory, executive function, orientation, communication, and the ability to perform daily activities. Its pathology involves interacting processes, including extracellular amyloid-beta deposition, intracellular tau abnormalities, synaptic failure, mitochondrial dysfunction, neuroinflammation, oxidative injury, and progressive neuronal loss. Although pharmacological treatments can provide symptomatic benefit and newer disease-modifying approaches may slow progression in selected patients, therapeutic responses remain variable and the underlying biological

complexity of AD continues to justify the search for additional molecular leads [1,2]. Natural products are especially relevant at the discovery stage because they provide structurally diverse metabolites capable of interacting with enzymes, receptors, membranes, and redox pathways.

The cholinergic system is closely associated with learning, attention, and memory. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) terminate cholinergic signaling by hydrolyzing acetylcholine and related choline esters. AChE is the predominant cholinesterase in the healthy brain, whereas the relative contribution of BChE becomes more prominent as AD advances and AChE activity declines. BChE is therefore increasingly considered a complementary pharmacological target, particularly for maintaining acetylcholine availability during later disease stages [3-5,13,14]. Inhibition of BChE does not by itself establish disease-modifying efficacy, but it provides a rational biochemical screening endpoint for identifying extracts and molecules that may support subsequent neuropharmacological investigation.

Oxidative stress represents another important component of AD pathogenesis. Excessive generation of reactive oxygen and nitrogen species can damage membrane lipids, proteins, nucleic acids, mitochondria, and cellular signaling systems. The brain is particularly susceptible because of its high oxygen consumption, abundant polyunsaturated lipids, and comparatively limited regenerative capacity. Antioxidant compounds may reduce chemically reactive species or strengthen endogenous defensive pathways, although simple in vitro radical-scavenging activity cannot be directly equated with clinical neuroprotection [6]. For this reason, combining a cholinesterase assay with a chemical antioxidant assay can provide a broader preliminary assessment than either test alone, while still requiring careful interpretation and later confirmation in biological models.

Plant-derived secondary metabolites have long served as sources of enzyme inhibitors, antioxidants, anti-inflammatory agents, and lead structures for drug development. Their recovery is strongly influenced by extraction conditions, particularly solvent polarity. Petroleum ether preferentially extracts lipophilic constituents, chloroform recovers relatively non-polar to moderately polar compounds, ethyl acetate concentrates many semi-polar metabolites, and methanol generally extracts a broad range of polar phytochemicals. Sequential extraction therefore separates crude plant material into chemically different fractions and can reveal whether biological activity is associated with particular polarity ranges. Such comparative fractionation is valuable for prioritizing samples before more expensive chromatographic, spectrometric, and mechanistic studies [9-12,16,17].

Seeds from cucurbitaceous plants are nutritionally rich but remain less extensively investigated for neuropharmacological activity than edible fruit tissues, leaves, or peels. The present work examined seeds of *Cucurbita maxima* (pumpkin), *Cucumis melo* (melon), *Cucumis sativus* (cucumber), and *Citrullus lanatus* (watermelon). These seeds contain oils, sterols, phenolic constituents, flavonoid-related compounds, proteins, and other metabolites whose distribution differs among species and extraction solvents. Previous studies have demonstrated antioxidant or related biological activities in cucurbit fruits and extracts, but direct comparison of sequential seed fractions for BChE inhibition remains comparatively limited [15,19-21]. Evaluating these underused plant materials may therefore identify fractions suitable for bioassay-guided isolation and more focused chemical characterization.

The study also included *Hydrocotyle asiatica* as a medicinal comparison material. Preparations of this plant have been investigated for antioxidant and traditional neurological applications, and triterpenoid-rich fractions are frequently discussed in relation to its biological properties [18]. Including *H. asiatica* alongside cucurbitaceous seeds provided a useful pharmacological context and allowed the solvent-dependent responses of an established medicinal plant to be compared with those of commonly available food-derived seed materials. Importantly, the comparison was intended as an exploratory in vitro screen rather than evidence that any crude extract is an effective treatment for AD.

A central knowledge gap addressed by this work was whether plant species and solvent polarity jointly determine the balance between BChE inhibition and radical-scavenging capacity. Extracts that perform strongly in both assays may contain multifunctional constituents, whereas extracts showing selective activity in only one assay may help distinguish enzyme-directed effects from general chemical antioxidant behavior. A systematic plant-by-solvent comparison also provides a practical ranking framework for deciding which fractions should proceed to chromatographic separation, compound identification, kinetic analysis, and biological validation.

Accordingly, the present study comparatively evaluated the BChE inhibitory and DPPH radical-scavenging activities of sequential petroleum ether, chloroform, ethyl acetate, and methanol extracts prepared from four cucurbitaceous seeds and whole-plant *H. asiatica*. Activities were assessed at 0.2, 0.5, and 1.0 mg/mL, with galantamine and ascorbic acid used as reference compounds for the respective assays. The working hypothesis was that differences in plant chemistry and solvent polarity would produce distinct concentration-dependent activity profiles and identify a limited number of high-priority plant-solvent combinations for future phytochemical and neuropharmacological research.

2. Materials and Methods

2.1 Plant materials and authentication

Seeds of *Cucurbita maxima* (pumpkin), *Cucumis sativus* (cucumber), *Cucumis melo* (melon), and *Citrullus lanatus* (watermelon), together with whole-plant material of *Hydrocotyle asiatica*, were obtained from a local market in Gujrat, Punjab, Pakistan. The materials were identified by a herbarium specialist in the Department of Botany, University of Gujrat, using available botanical reference materials. A voucher specimen number was not reported in the source record and should be supplied if available.

2.2 Sequential solvent extraction

Plant materials were shade-dried at room temperature and ground to a fine powder. Six grams of each material were sequentially extracted with petroleum ether, chloroform, ethyl acetate, and methanol. Each extraction was performed in a shaking incubator at room temperature for 24 h. After filtration, the organic phase was concentrated under reduced pressure using a rotary evaporator. The residue remaining after each extraction step was subsequently extracted with the next solvent in the sequence. Concentrated extracts were adjusted to 1.0 mg/mL and diluted to 0.5 and 0.2 mg/mL for bioactivity testing.

2.3 Butyrylcholinesterase inhibitory assay

BChE inhibitory activity was determined using a modification of the Ellman colorimetric method [7,8]. A DTNB running solution was prepared by combining 3.6 mL of stock solution containing 20 mM sodium bicarbonate and 10 mM DTNB in 0.1 M phosphate buffer (pH 7.0) with 96.4 mL of 0.1 M phosphate buffer (pH 8.0). The reaction mixture contained 1.35 mL of buffered DTNB solution, 50 µL of BChE from equine serum (0.4 U), 50 µL of test extract at 0.2, 0.5, or 1.0 mg/mL, and 50 µL of 4 mM butyrylthiocholine chloride, giving a final volume of 1.5 mL. Following incubation for 15 min at room temperature, absorbance was measured at 412 nm using a 1-cm quartz cuvette. Galantamine hydrobromide was used as the positive control. All measurements were performed in triplicate. Percentage inhibition was calculated as $(E - S)/E \times 100$, where E represents enzyme activity without extract and S represents activity in the presence of extract.

2.4 DPPH radical-scavenging assay

DPPH radical-scavenging activity was assessed using a modified spectrophotometric procedure [9,10]. A 50-µL aliquot of each extract at 0.2, 0.5, or 1.0 mg/mL was mixed with 2 mL of 0.1 mM DPPH prepared in ethanol. The mixture was incubated for 20 min at 37 °C, and absorbance was recorded at 517 nm. Ethanol served as the blank and ascorbic acid as the reference antioxidant. Reactions were performed in triplicate. Radical-scavenging activity was calculated as $(C - S)/C \times 100$, where C is the absorbance of DPPH without extract and S is the absorbance in the presence of extract.

2.5 Data presentation and IC50 estimation

Results are presented as percentage inhibition with error bars representing variation among triplicate measurements. IC50 values reported in the source dataset were derived from concentration–response relationships. No inferential statistical procedure was documented; consequently, this reformatted article avoids the term “statistically significant” unless supported by an appropriate analysis of the original raw data.

The five plant materials examined, the plant part used in each case, and the sequential extraction scheme applied throughout are summarized in Table 1; the solvent abbreviations introduced there (PE, CHCl3, EtOAc, MeOH) are used throughout the Results and Discussion.

Table 1. Plant materials, plant parts, and extraction sequence. PE, petroleum ether; CHCl3, chloroform; EtOAc, ethyl acetate; MeOH, methanol.

Species	Common name	Material tested	Sequential solvents
Cucurbita maxima	Pumpkin	Seed	PE → CHCl3 → EtOAc → MeOH
Cucumis melo	Melon	Seed	PE → CHCl3 → EtOAc → MeOH
Cucumis sativus	Cucumber	Seed	PE → CHCl3 → EtOAc → MeOH

Species	Common name	Material tested	Sequential solvents
Citrullus lanatus	Watermelon	Seed	PE → CHCl3 → EtOAc → MeOH
Hydrocotyle asiatica	—	Whole plant	PE → CHCl3 → EtOAc → MeOH

3. Results

3.1 Solvent-dependent BChE inhibition

BChE inhibition varied markedly according to plant species and extraction solvent (Figure 1). Petroleum ether fractions generally produced low-to-moderate inhibition, although the *C. melo* seed fraction was reported to reach an IC50 of 565 µg/mL. Chloroform extracts from cucurbitaceous seeds were comparatively weak, whereas chloroform-extracted *H. asiatica* produced 88.88% inhibition at 1.0 mg/mL and a reported IC50 of 161 µg/mL.

Ethyl acetate fractions showed stronger activity across several seed samples. The ethyl acetate extract of *C. maxima* produced 81.77% BChE inhibition at 1.0 mg/mL. Methanolic fractions also showed consistently high activity, and the methanolic *C. lanatus* extract was reported to produce 81.19% inhibition at 1.0 mg/mL with an IC50 of 98 µg/mL. Galantamine produced substantially greater potency than the plant extracts (IC50 = 3.6 ± 0.1 µg/mL).

Considered across all four solvents, the *H. asiatica* fractions shown in Figure 1E were consistently active at 1.0 mg/mL: the petroleum ether, chloroform, and ethyl acetate fractions all exceeded 80% inhibition, and only the methanolic fraction fell appreciably lower, at approximately 66%. This broad, less solvent-selective response contrasts with the cucurbitaceous seeds, where high inhibition was largely confined to the ethyl acetate and methanol fractions, and suggests that the BChE-inhibitory constituents of *H. asiatica* are distributed across a wider polarity range than those of the seed matrices. The leading extracts from each species, together with the reference compound, are consolidated in Table 2 for direct comparison; the corresponding DPPH results are presented in Section 3.2.

Table 2. Reported high-activity extracts and reference compounds.

Assay	Plant/reference	Solvent	Maximum reported response	IC50 (µg/mL)
BChE	*Citrullus lanatus*	Methanol	81.19% at 1.0 mg/mL	98
BChE	*Hydrocotyle asiatica*	Chloroform	88.88% at 1.0 mg/mL	161
BChE	*Cucurbita maxima*	Ethyl acetate	81.77% at 1.0 mg/mL	Not reported
BChE	Galantamine	Reference	Positive control	3.6 ± 0.1
DPPH	*Cucumis sativus*	Chloroform	63.78% at 1.0 mg/mL	642
DPPH	*Citrullus lanatus*	Ethyl acetate	Reported as second-most active	708
DPPH	Ascorbic acid	Reference	Positive control	4.2 ± 0.2

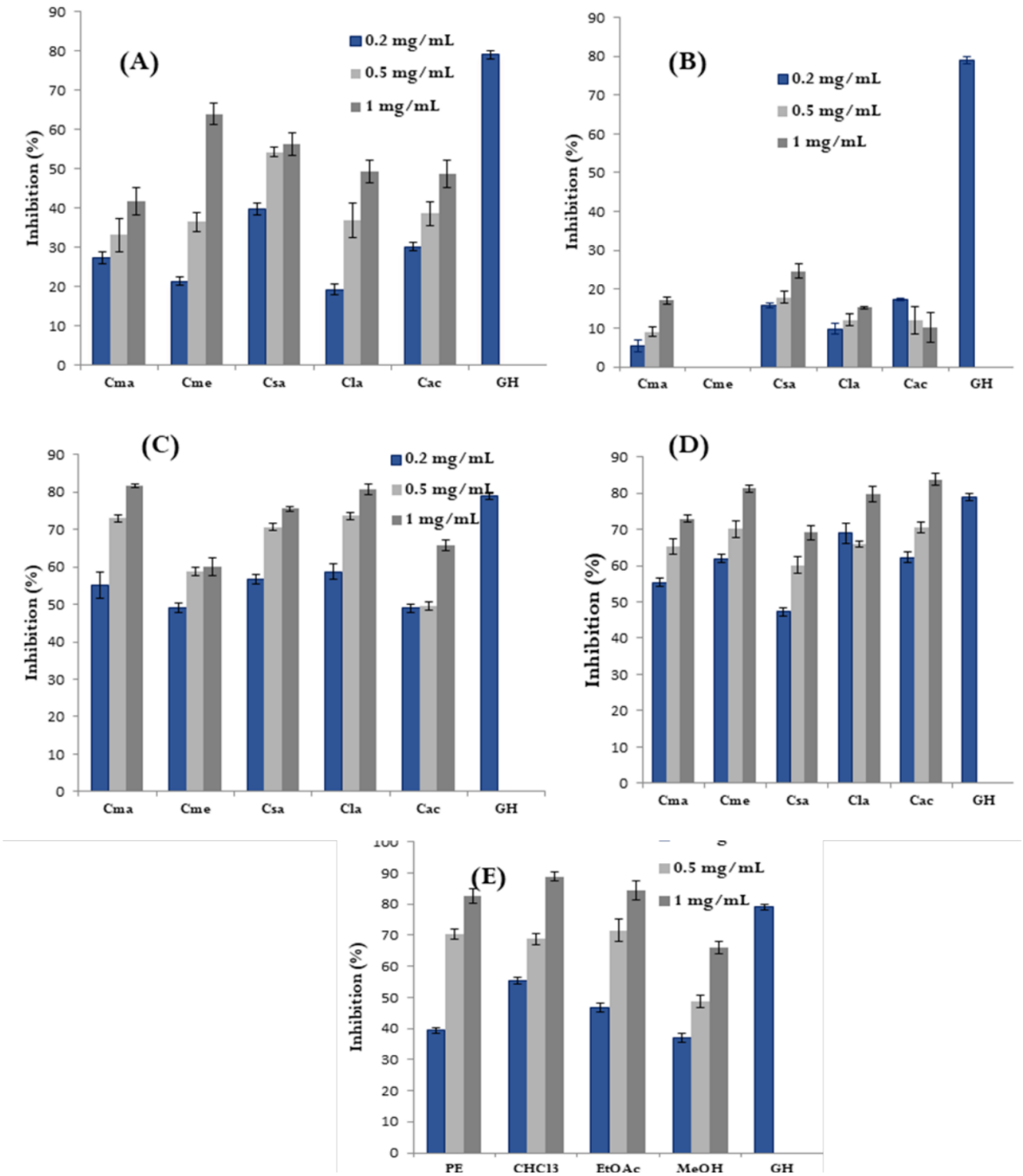

Figure 1. Butyrylcholinesterase inhibition by sequential solvent extracts. Panels A–D show petroleum ether, chloroform, ethyl acetate, and methanol seed fractions, respectively; panel E compares the four solvent fractions of Hydrocotyle asiatica. Cma, Cucurbita maxima; Cme, Cucumis melo; Csa, Cucumis sativus; Cla, Citrullus lanatus; Cac/Mac, combined cucurbitaceous seed extract as labeled in the source figure; GH, galantamine hydrobromide. Values are shown as mean with error bars from triplicate measurements.

3.2 DPPH radical-scavenging activity

The antioxidant response also depended on both species and solvent (Figure 2). Most plant extracts were less active than ascorbic acid, and the pattern did not directly parallel BChE inhibition. Among the seed fractions, chloroform-extracted *C. sativus* showed the strongest reported DPPH activity, reaching 63.78% inhibition at 1.0 mg/mL with an IC50 of 642 µg/mL. The ethyl acetate fraction of *C. lanatus* was the next most potent based on its reported IC50 of 708 µg/mL. The reference antioxidant ascorbic acid showed an IC50 of 4.2 ± 0.2 µg/mL.

The relatively weak antioxidant activity of several fractions indicates that BChE inhibition was not solely explained by general radical-scavenging capacity. Instead, different phytochemical classes may contribute independently to enzyme inhibition and antioxidant effects.

A similar comparison across the four *H. asiatica* fractions in Figure 2E shows a more solvent-selective pattern for radical scavenging than for BChE inhibition: the ethyl acetate fraction was the strongest antioxidant, reaching

approximately 51% inhibition at 1.0 mg/mL, while the chloroform and methanolic fractions were comparatively weak. Because chloroform was the leading BChE-inhibitory fraction for this species (Section 3.1), this divergence within a single plant reinforces the broader divergence between the two assays discussed in Section 4.4.

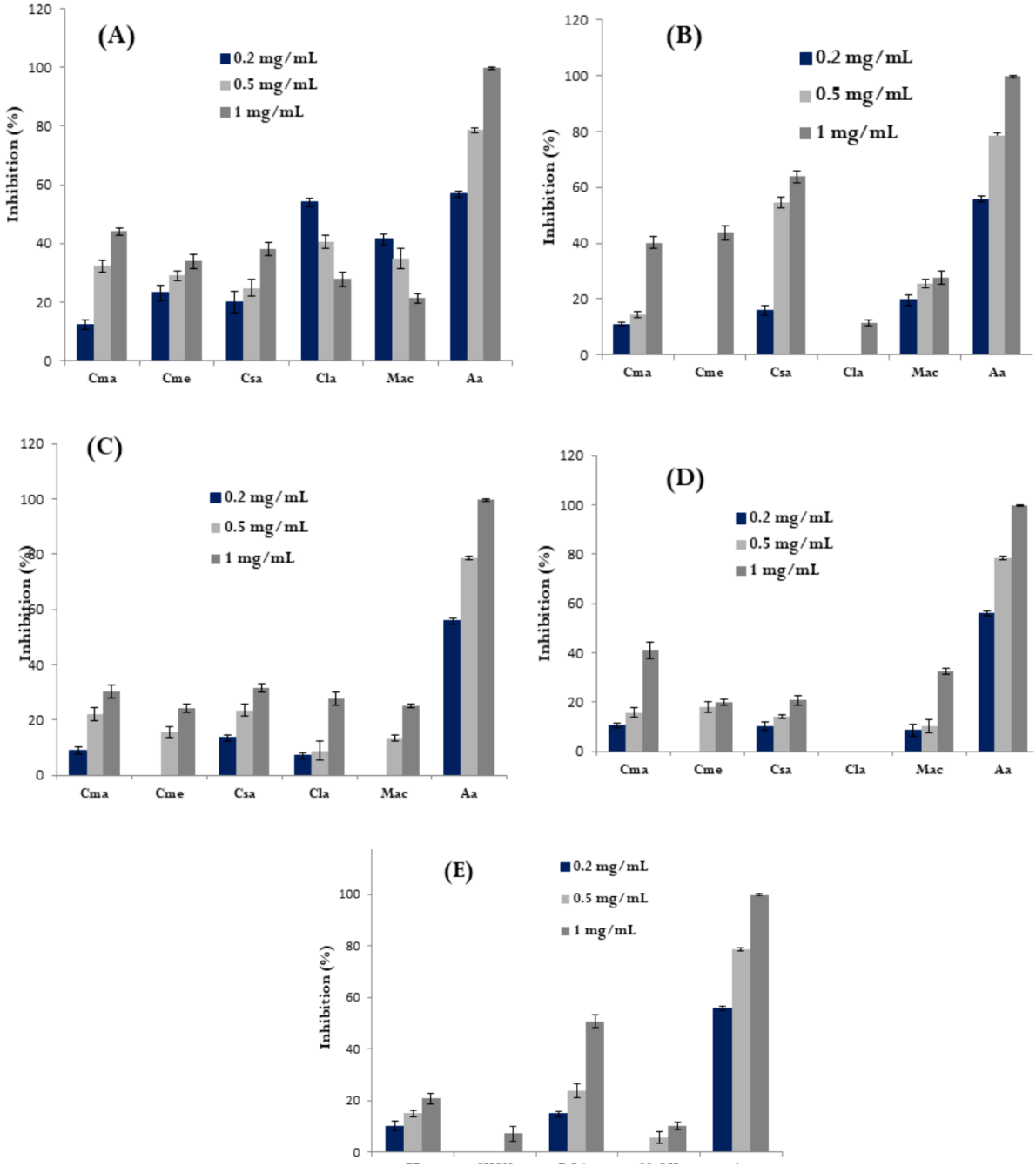

Figure 2. DPPH radical-scavenging activity of sequential solvent extracts. Panels A–D show petroleum ether, chloroform, ethyl acetate, and methanol seed fractions, respectively; panel E compares the four solvent fractions of Hydrocotyle asiatica. Cma, Cucurbita maxima; Cme, Cucumis melo; Csa, Cucumis sativus; Cla, Citrullus lanatus; Mac, combined cucurbitaceous seed extract as labeled in the source figure; Aa, ascorbic acid. Values are shown as mean with error bars from triplicate measurements.

4. Discussion

4.1 BChE inhibition and solvent polarity

The present findings demonstrate that BChE inhibition was strongly influenced by both solvent polarity and plant identity. Petroleum ether and chloroform seed fractions generally displayed lower inhibition than ethyl acetate and methanol fractions, whereas chloroform *H. asiatica* was notably active. This pattern suggests that the compounds contributing to BChE inhibition were not confined to a single polarity class. Rather, the activity may reflect several chemically distinct groups distributed differently among the plant materials. Sequential extraction was therefore useful not only for recovering constituents but also for separating broad chemical classes and exposing activity that could have been diluted or masked in a single crude extract.

The comparatively strong responses of several ethyl acetate and methanol fractions are consistent with the ability of these solvents to recover semi-polar and polar metabolites, including many phenolic, flavonoid-related, nitrogen-containing, and glycosylated compounds. However, qualitative solvent behavior alone cannot identify the active constituents, and the strong chloroform response of *H. asiatica* indicates that less-polar metabolites may also contribute. Similar variability has been observed in plant cholinesterase screens, where activity depends on the botanical source, plant part, extraction protocol, enzyme source, substrate, and assay conditions [11-13]. The present data should therefore be viewed as a comparative ranking within this experimental system rather than as an absolute potency classification across studies.

The concentration-dependent increases observed in many fractions support the presence of genuine inhibitory activity rather than a single-point experimental effect. Nevertheless, percentage inhibition at a high extract concentration can be influenced by sample color, turbidity, non-specific protein interactions, or interference with the chromogenic reaction. Future confirmation should include extract blanks at every concentration, full concentration-response curves, nonlinear regression with confidence intervals, and enzyme kinetic experiments to determine whether inhibition is competitive, non-competitive, mixed, or time dependent. Counter-screening against AChE would also clarify whether the active fractions are selective for BChE or act broadly against both cholinesterases [7,8,13,14].

4.2 Species-specific patterns and priority extracts

Among the tested seed samples, methanolic *C. lanatus* emerged as a leading BChE-inhibitory fraction, while ethyl acetate *C. maxima* also produced high inhibition at 1.0 mg/mL. These results indicate that different cucurbit species may concentrate active metabolites in different polarity fractions. The high response of methanolic *C. lanatus* suggests that polar constituents deserve priority, whereas the activity of ethyl acetate *C. maxima* points toward semi-polar compounds. In contrast, the lower activity of several chloroform seed fractions emphasizes that a solvent effective for one plant cannot be assumed to be optimal for another.

The whole-plant *H. asiatica* fractions displayed a distinctive pattern, remaining active across all four solvents and reaching the greatest potency in the chloroform fraction (Figure 1E). This result is important because it demonstrates that the medicinal comparison plant did not simply reproduce the solvent-selective profile of the cucurbit seeds. It also supports a targeted strategy in which the chloroform fraction is separated further by chromatographic methods and repeatedly tested during fractionation. Such bioassay-guided work would help determine whether the observed response is produced by one dominant constituent, several additive constituents, or synergistic interactions within the fraction [16,18].

Despite their promising percentage inhibition, the crude extracts remained substantially less potent than galantamine on an IC50 basis: the leading fraction, methanolic *C. lanatus* (IC50 = 98 µg/mL), was approximately 27-fold less potent than galantamine (IC50 = 3.6 ± 0.1 µg/mL), as summarized in Table 2. This difference is expected because a purified reference inhibitor is compared with chemically complex mixtures containing both active and inactive constituents. Accordingly, the value of the present screening lies in identifying starting materials for isolation rather than proposing direct replacement of established medicines. Priority for subsequent work should be given to methanolic *C. lanatus*, ethyl acetate *C. maxima*, and chloroform *H. asiatica*, followed by confirmation with independently prepared extracts and standardized enzyme assays.

4.3 Antioxidant activity and assay interpretation

DPPH radical-scavenging activity was generally modest and followed a solvent pattern that differed from BChE inhibition. The chloroform fraction of *C. sativus* displayed the strongest seed-derived antioxidant response, while selected *C. lanatus* fractions also showed measurable activity. Most extracts were considerably less potent than ascorbic acid — the strongest seed-derived fraction, chloroform *C. sativus* (IC50 = 642 µg/mL), was approximately 150-fold less potent than ascorbic acid (IC50 = 4.2 ± 0.2 µg/mL; Table 2) — indicating that they should be described as preliminary antioxidant leads rather than strong antioxidants. The different response patterns across petroleum ether, chloroform, ethyl acetate, and methanol fractions further demonstrate that antioxidant behavior cannot be predicted solely from broad solvent polarity.

The DPPH method measures the capacity of a sample to donate an electron or hydrogen atom to a stable synthetic radical. It is rapid, reproducible, and useful for comparative screening, but it does not reproduce the complexity of oxidative injury in neurons, where membrane localization, metabolism, bioavailability, metal chelation, endogenous antioxidant enzymes, and mitochondrial processes influence biological outcomes [9,10,17]. Colored extracts may also contribute to absorbance interference at 517 nm if appropriate sample blanks are not included. Therefore, DPPH activity should be interpreted as chemical radical-scavenging capacity under the assay conditions, not as direct proof of neuroprotection.

The relatively stronger activity of the chloroform *C. sativus* fraction is compatible with previous reports that cucumber-derived materials contain compounds capable of reducing free radicals, although the present study examined seed fractions rather than fruit tissues [19,21]. Differences between the current seed data and published fruit or whole-plant studies may arise from plant part, maturity, cultivation conditions, extraction method, and assay concentration. Additional antioxidant tests, including ABTS, ferric-reducing power, lipid-peroxidation inhibition, and cellular oxidative-stress models, would provide a more complete assessment and help determine whether the leading DPPH fractions retain activity in biologically relevant systems.

4.4 Relationship between BChE inhibition and antioxidant capacity

A notable outcome of the study was the absence of a simple parallel relationship between BChE inhibition and DPPH scavenging. Some fractions were strong enzyme inhibitors but only modest radical scavengers, whereas others showed better antioxidant activity without equivalent BChE inhibition. This divergence indicates that the two assays probably respond to different constituents or different chemical features within the same fraction. It also argues against attributing BChE inhibition merely to non-specific antioxidant capacity.

From a discovery perspective, selective and dual-activity fractions are both valuable. A selective BChE-inhibitory fraction may contain molecules with direct affinity for the enzyme, while a fraction active in both assays may provide compounds with complementary biochemical properties. Future studies should calculate correlations using the complete concentration-response dataset, test purified subfractions in both assays, and examine whether combinations of isolated compounds produce additive or synergistic effects. Such work would establish whether the apparent dual activity arises from a single multifunctional molecule or from different constituents coexisting in the crude extract [6,16,17].

4.5 Scientific significance, limitations, and future directions

A major strength of the study is its comparative design. Four sequential solvents were applied across several cucurbitaceous seed species and *H. asiatica*, and the resulting fractions were examined in two mechanistically different screening assays. This plant-by-solvent matrix provides more information than testing a single crude extract because it identifies not only which species are active but also which polarity fractions should be prioritized. The use of concentration-dependent measurements, triplicate testing, and recognized reference compounds further supports the value of the dataset as an exploratory screening platform.

Several limitations should nevertheless be considered. The active constituents were not isolated or structurally characterized, extraction yields were not reported, and the available dataset did not include complete raw absorbance values or detailed statistical comparisons among all extracts. Voucher specimen numbers were not provided, and the use of market-derived material may introduce variability related to cultivar, geographic origin, storage, and processing. The study also relied on a single cholinesterase target and a single chemical antioxidant assay; it did not examine AChE selectivity, enzyme kinetics, cytotoxicity, blood-brain barrier permeability, cellular neuroprotection, pharmacokinetics, or in vivo efficacy.

The next stage should begin with independent replication using authenticated plant material, documented voucher specimens, extraction-yield measurements, and standardized sample preparation. The leading fractions should then undergo bioassay-guided chromatography, followed by LC-MS/MS, HPLC, GC-MS where appropriate, and spectroscopic structure elucidation. Confirmed constituents should be tested in full BChE and AChE concentration-response assays, with kinetic analysis and orthogonal methods to exclude color or aggregation artifacts. Molecular docking may help generate mechanistic hypotheses, but predicted binding should be verified experimentally rather than treated as proof of inhibition.

Biological validation should proceed stepwise. Initial work may include neuronal or glial cell models of oxidative stress, assessment of mitochondrial function and membrane integrity, and measurement of inflammatory or redox biomarkers. Fractions or purified compounds showing reproducible activity and acceptable cytotoxicity could then be evaluated in suitable animal models, with attention to bioavailability, metabolism, brain exposure, and safety. This staged approach would determine whether the current in vitro signals translate into meaningful neuropharmacological effects while avoiding premature therapeutic claims.

Within these boundaries, the study contributes a useful comparative dataset on underexplored cucurbitaceous seeds and a medicinal reference plant. Its principal significance is the identification of a small number of high-

priority fractions and the demonstration that solvent choice materially changes the biological profile obtained from the same plant material. These findings provide a rational foundation for more rigorous chemical and pharmacological investigation of plant-derived BChE inhibitors and antioxidant constituents relevant to neurodegenerative disease research.

5. Conclusions

Sequential solvent extraction revealed clear species- and solvent-dependent differences in BChE inhibition and DPPH radical-scavenging activity. Methanolic *C. lanatus*, ethyl acetate *C. maxima*, and chloroform *H. asiatica* were the most notable BChE-inhibitory fractions, whereas chloroform *C. sativus* showed the strongest seed-derived DPPH response. The different rankings obtained in the two assays indicate that enzyme inhibition and chemical antioxidant activity were not governed by a single common pattern and may arise from distinct phytochemical constituents. The results support the use of cucurbitaceous seeds as underexplored sources of bioactive metabolites and confirm the importance of solvent selection during preliminary screening. At the same time, the crude extracts were much less potent than the reference compounds, and the assays were limited to in vitro biochemical measurements. The findings therefore identify promising research leads rather than demonstrating clinical efficacy, disease modification, or suitability for direct therapeutic use in Alzheimer's disease. Future studies should prioritize reproducible preparation and bioassay-guided fractionation of the leading extracts, followed by chemical identification, BChE/AChE selectivity testing, enzyme kinetics, broader antioxidant evaluation, cytotoxicity assessment, and validation in cellular and in vivo models. By narrowing a large set of plant-solvent combinations to a manageable group of priority fractions, this study provides a practical starting point for discovering and characterizing plant-derived compounds with potential relevance to cholinergic dysfunction and oxidative stress in neurodegenerative research.

Declarations

Author contributions: Sankara Narayanan Senthilkumar: Conceptualization, Methodology, Investigation, Formal analysis, Writing – Original Draft. Mohamed Isthiyak M: Investigation, Validation, Data curation, Writing – Review & Editing. Bilqees Fatima: Literature review, Visualization, Writing – Review & Editing. Balaji Nagarajan: Conceptualization, Supervision, Project administration, Resources, Writing – Review & Editing. All authors reviewed and approved the final manuscript, including the revised title, text, figures, declarations, and authorship order.

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Informed consent statement: Not applicable.

Data availability: The data supporting the findings of this study are available from the corresponding author upon reasonable request. The raw data are securely maintained by the Post Graduate Department of Biotechnology, Microbiology & Bioinformatics, National College, Tiruchirapalli, Tamil Nadu, India, and will be made available in accordance with institutional policies.

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Use of artificial intelligence tools: AI-assisted editorial tools were used for language improvement, structural reorganization, and journal formatting. The authors remain responsible for the accuracy of the scientific content, data, citations, authorship, and final conclusions.

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