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Cholinesterase Inhibitory Potential of Selected Libyan Medicinal Plants

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الفعالية التثبيطية لإنزيم الكولين إستراز في بعض النباتات الطبية الليبية المختارة

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Abstract:
Cholinesterase inhibitors are essential therapeutic agents in the management of neurodegenerative disorders, particularly Alzheimer's disease (AD). This study evaluates the inhibitory effects of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) using extracts from ten Libyan medicinal plants with varying antioxidant capacities. The selected species include Camellia sinensis, Myrtus communis, Alhagi maurorum, Urginea maritima, Olea europaea, Matricaria chamomilla, Hibiscus sabdariffa, Quercus robur, Syzygium aromaticum, and Zingiber officinale. Hot- and cold-water extracts were prepared following traditional Libyan practices, and their cholinesterase inhibitory activity was assessed using spectrophotometric analysis. The plant extracts demonstrated diverse levels of inhibitory potency, with Camellia sinensis and Syzygium aromaticum showing the strongest inhibition toward both enzymes. These findings highlight the potential of several Libyan medicinal plants as promising natural sources of cholinesterase inhibitors for supporting AD management and other neurodegenerative conditions.

Keywords: Cholinesterase inhibition, Libyan medicinal plants, Aqueous plant extracts

الملخص:
تُعدّ مثبّطات إنزيمات الكولينستيراز من العوامل الدوائية الرئيسية المعتمدة في معالجة الاضطرابات التنكسية العصبية، ولا سيّما مرض الزهايمر. يهدف هذا البحث إلى تقييم الفعالية التثبيطية لكلٍّ من إنزيمي الأستيل كولينستيراز (AChE) والبيوتيريل كولينستيراز (BuChE) باستخدام مستخلصات مائية مستخلصة من عشرة نباتات طبية ليبية ذات قدرات متفاوتة في مقاومة الأكسدة، وهي Myrtus communis، Alhagi maurorum، Urginea maritima، Olea europaea، Matricaria chamomilla، Hibiscus sabdariffa، Quercus robur، Syzygium aromaticum، Camellia sinensis. Zingiber officinale. أُعدَّت المستخلصات المائية الباردة والساخنة وفق الطرق التقليدية المتّبعة في الطب الشعبي الليبي، ثم جرى تحديد نشاطها التثبيطي لإنزيمات الكولينستيراز بواسطة التحليل الطيفي. بيّنت المستخلصات النباتية تباينًا ملحوظًا في مستويات نشاطها التثبيطي، حيث أظهر مستخلصا Camellia sinensis وSyzygium aromaticum أعلى فعالية في تثبيط الإنزيمين. تشير النتائج إلى امتلاك عدد من النباتات الطبية الليبية لإمكانات واعدة بوصفها مصادر طبيعية لمثبّطات الكولينستيراز، مما يدعم إمكانية توظيفها في تعزيز إدارة مرض الزهايمر وغيره من الاضطرابات العصبية التنكسية.

الكلمات المفتاحية: تثبيط إنزيمات الكولين إستيراز، النباتات الطبية الليبية، المستخلصات النباتية المائية.

Introduction:

Cholinesterases (ChEs) constitute a subclass of serine hydrolases defined by a conserved catalytic serine residue within the active site. Among these enzymes, acetylcholinesterase (AChE) is functionally distinct from butyrylcholinesterase (BuChE), exhibiting substantially greater affinity and specificity toward the neurotransmitter acetylcholine (ACh) (Quinn, 1987; Giacobini, 2004; Khan, 2009). Acetylcholine is central to cholinergic synaptic transmission: upon arrival of an action potential, ACh is released from presynaptic terminals, traverses the synaptic cleft via diffusion, and engages cholinergic receptors on the postsynaptic membrane. This receptor activation initiates downstream signaling events that modulate multiple cellular processes, including the regulation of potassium (K^+) fluxes across the postsynaptic membrane (Pope et al., 2005; De Boer et al., 2021; Zoli et al., 2018).

Acetylcholinesterase (AChE) catalyzes the rapid hydrolysis of acetylcholine (ACh) into choline and acetic acid under normal physiological conditions, thereby ensuring stringent temporal and spatial regulation of cholinergic neurotransmission. When AChE activity is substantially diminished, ACh accumulates within the synaptic cleft and induces sustained overstimulation of cholinergic receptors. This pathological hyperactivation perturbs neuronal impulse propagation and neuromuscular signaling, manifesting clinically as impaired motor coordination, muscle fasciculations, convulsions, and, at severe levels of exposure, fatality (Quinn, 1987; Pope et al., 2005; Trang and Khandhar, 2019). Owing to its indispensable role at central synapses and neuromuscular junctions, AChE has been widely recognized as a pivotal molecular target for the rational design of mechanism-based inhibitors (Giacobini, 2004; Gajendra et al., 2024).

Alzheimer's disease (AD), the leading cause of dementia among older adults, is a progressive neurodegenerative condition characterized by insidious memory impairment, worsening cognitive dysfunction, and a gradual loss of functional autonomy, typically culminating in death within approximately a decade of diagnosis. A prominent cholinergic deficit constitutes a core neuropathological hallmark of AD and provides the principal rationale for therapeutic approaches aimed at augmenting cholinergic tone, most commonly through pharmacological inhibition of AChE (Ezoulin et al., 2005; Ferreira et al., 2006; Howes and Houghton, 2003; Chen et al., 2022).

To date, cholinesterase inhibitors represent the only drug class with consistent evidence of symptomatic benefit on cognition and daily functioning in patients with AD (Nagabukuro et al., 2004; Chen et al., 2022). Increasing evidence further suggests that dual inhibition of AChE and butyrylcholinesterase (BuChE) may confer added therapeutic value, particularly in later disease stages when BuChE activity tends to rise and may partially compensate for declining AChE function (Giacobini, 2004; Okello et al., 2004; Simchovitz et al., 2017). This perspective reinforces the cholinergic hypothesis which, despite the expansion of alternative mechanistic paradigms, continues to inform a substantial proportion of AD-directed drug discovery and development efforts (Pope et al., 2005; Giacobini, 2004; Chen et al., 2022; Liu et al., 2019).

Clinically approved cholinesterase inhibitors for mild to moderate AD include tacrine, donepezil, rivastigmine, and galantamine. Tacrine, the earliest agent in this class, demonstrated measurable clinical efficacy but was limited by a narrow therapeutic window and

a high incidence of adverse effects, notably hepatotoxicity and gastrointestinal intolerance, which ultimately constrained its clinical utility (Orhan et al., 2004; Hu et al., 2005). Notably, numerous plant taxa biosynthesize alkaloids and other secondary metabolites with anticholinesterase activity; among them, galantamine, initially isolated from the snowdrop (Galanthus nivalis), has been successfully translated into a widely used therapeutic agent for AD (Svedberg et al., 2004; Liu et al., 2019).

In view of the growing demand for safer, cost-effective, and more accessible therapeutic alternatives, research interest has increasingly converged on plant-derived cholinesterase inhibitors. Medicinal plants constitute a rich reservoir of structurally diverse bioactive constituents, including alkaloids, flavonoids, terpenoids, and coumarins, many of which have shown measurable inhibitory activity against cholinesterase enzymes. Accordingly, the present study aims to evaluate the AChE- and BuChE-inhibitory potential of selected Libyan medicinal plants and to appraise their suitability as natural lead sources for the development of novel anticholinesterase agents.

Materials and Methods:

Plant Selection and Extraction:

Ten Libyan medicinal plants were selected for evaluation based on previously reported interspecies differences in antioxidant capacity (Elmestiri et al., 2011). In prior work, these species were classified into three categories—high, moderate, and low antioxidant activity. To align the experimental design with preparation practices commonly used in local herbal medicine, both hot- and cold-water extracts were prepared as described below.

Hot-water extraction

Four grams of air-dried plant material were finely powdered and extracted with freshly boiled deionized water at a 1:5 (w/v) ratio. The suspension was allowed to steep, after which the extract was filtered to remove particulate matter.

Cold-water extraction

An equivalent mass (4 g) of powdered plant material was immersed in deionized water at room temperature using the same 1:5 (w/v) ratio and maintained for an extended maceration period to facilitate extraction of thermolabile constituents. The mixture was subsequently filtered.

Chemicals and Assay Reagents

Acetylthiocholine iodide (ATChI) and butyrylthiocholine iodide (BuTChI) were employed as substrates for acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), respectively, while 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) served as the chromogenic reagent. AChE (from human erythrocytes) and BuChE (from human serum) were used as enzyme sources. All enzymes and analytical-grade reagents were purchased from Sigma Chemical Company (UK).

Enzyme Inhibition Assay

Cholinesterase inhibitory activity was quantified using the Ellman colorimetric method. Briefly, this assay monitors the production of the yellow 5-thio-2-nitrobenzoate (TNB) anion formed when DTNB reacts with thiocholine liberated during enzymatic hydrolysis of ATChI or BuTChI. Absorbance was recorded at 405 nm using a microplate reader. All measurements were performed in triplicate to ensure analytical precision and reproducibility. The percentage inhibition of enzyme activity was calculated using the following relationship:

$$Inhibition\ (I\%) = \frac{(a-b)-(c-d)}{(a-b)} * 100 \quad [1]$$

Where:

- **a:** Control reaction (enzyme + substrate + DTNB + water)
- **b:** Blank reaction (substrate + DTNB + water)
- **c:** Test reaction (enzyme + substrate + DTNB + plant extract)
- **d:** Sample blank (substrate + DTNB + plant extract)

Dose–response curves were generated for extracts showing measurable inhibitory activity, and IC_{50} values (the concentration required to inhibit 50% of enzyme activity) were calculated accordingly.

Results:

AChE and BuChE Inhibitory Activities:

The plant extracts demonstrated a broad spectrum of inhibitory effects against both acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). As shown in Tables 1 and 2, the hot- and cold-water extracts produced varying IC_{50} values and inhibition percentages. Among all species tested, *Camellia sinensis* and *Syzygium aromaticum* exhibited the most potent inhibition of both enzymes. For AChE, their IC_{50} values were 0.068 ± 0.001 mg/mL and

0.075 ± 0.003 mg/mL, respectively, while BuChE inhibition occurred at IC_{50} values of 0.066 ± 0.004 mg/mL and 0.116 ± 0.007 mg/mL.

Urginea maritima showed strong inhibitory activity toward AChE but produced little to no inhibition of BuChE. In contrast, *Matricaria chamomilla* and *Quercus robur* displayed selective activity toward BuChE, with IC_{50} values of 0.109 ± 0.001 mg/mL and 0.055 ± 0.002 mg/mL, respectively.

Activity Categorization:

Based on their observed AChE and BuChE inhibition profiles, the plants were classified into four activity levels:

- **High activity:** *Camellia sinensis*, *Syzygium aromaticum*, *Urginea maritima*
- **Moderate activity:** *Myrtus communis*, *Quercus robur*
- **Low activity:** *Olea europaea*, *Matricaria chamomilla*
- **Negligible or no activity:** *Zingiber officinale*, *Hibiscus sabdariffa*, *Alhagi maurorum.*

Table (1): Inhibition of Human AChE enzyme by selected Libyan medicinal plants.

Plant Scientific Name	Hot Water Extracts	Cold Water Extracts
Camellia sinensis	0.068 ± 0.001 [a]	0.070 ± 0.003 [a]
Myrtus communis	0.15 (44.63 ± 2) [b]	1.31 (40.13 ± 0.90) [b]
Alhagi maurorum	0.172 [c]	0.173 [c]
Urginea maritima	0.200 ± 0.005 [a]	0.114 ± 0.002 [a]
Olea europaea	0.148 [c]	0.118 [c]
Matricaria chamomilla	0.165 [c]	0.178 [c]
Hibiscus sabdariffa	0.279 [c]	0.295 [c]
Quercus robur	0.12 (15.80 ±0.97)[b]	0.11 (34.37 ± 0.92) [b]
Syzygium aromaticum	0.075 ± 0.003 [a]	0.088 ± 0.001 [a]
Zingiber officinale	0.207 [c]	0.110 [c]

Data expressed as mean ± SD (n = 3)
a = IC50 value (mg/ml) (final concentration of inhibitors required for 50% enzyme inhibition, as calculated from the dose response-curve equations).
b = Concentration (mg/ml) providing this percentage inhibition (Inhibition %, inhibitory activity of extracts which did not reach 50% enzyme inhibition).
c = No Inhibition at this concentration (mg/ml).

Table 1 summarizes the inhibitory activity of hot- and cold-water extracts from ten Libyan medicinal plants against human AChE. Overall, only three species achieved 50% inhibition within the tested range, as evidenced by measurable IC_{50} values (superscript a): Camellia sinensis, Syzygium aromaticum, and Urginea maritima. Among these, C. sinensis displayed the highest potency, with nearly identical IC_{50} values for hot and cold extracts (0.068 ± 0.001 and 0.070 ± 0.003 mg/mL, respectively), indicating that its active AChE-inhibitory constituents are effectively recovered by aqueous extraction regardless of temperature and appear relatively stable to heating. Likewise, S. aromaticum exhibited strong inhibition (IC_{50} = 0.075 ± 0.003 mg/mL for hot; 0.088 ± 0.001 mg/mL for cold), supporting its classification as a robust source of polar anticholinesterase components.

A notable extraction-dependent pattern was observed for U. maritima, where the cold-water extract was substantially more potent (IC_{50} = 0.114 ± 0.002 mg/mL) than the hot-water extract (IC_{50} = 0.200 ± 0.005 mg/mL). This divergence suggests that key inhibitory constituents in U. maritima may be thermolabile, susceptible to degradation, or less efficiently preserved under boiling conditions, highlighting the importance of extraction temperature in retaining bioactivity for certain taxa.

In contrast, Myrtus communis and Quercus robur did not reach 50% inhibition at the evaluated concentrations (superscript b), yet both produced measurable partial inhibition. Importantly, M. communis showed a clear temperature effect: the hot extract yielded 44.63 ± 2% inhibition at 0.15 mg/mL, whereas the cold extract required a much higher concentration (1.31 mg/mL) to achieve a comparable inhibition level (40.13 ± 0.90%). This pattern implies that hot-water extraction enhanced the recovery of its active polar inhibitors. For Q. robur, the cold extract provided higher inhibition (34.37 ± 0.92% at 0.11 mg/mL) than the hot extract (15.80 ± 0.97% at 0.12 mg/mL), indicating that extraction conditions may differentially affect the yield or integrity of bioactive compounds.

The remaining species (Alhagi maurorum, Olea europaea, Matricaria chamomilla, Hibiscus sabdariffa, and Zingiber officinale) were classified as non-inhibitory under the tested conditions (superscript c). This outcome does not necessarily exclude anticholinesterase potential; rather, it may reflect (i) insufficient extract concentration, (ii) a predominance of compounds with weak affinity for AChE, or (iii) limited extraction of relevant inhibitors by water alone, suggesting that alternative extraction systems (e.g., hydroalcoholic solvents) or higher dose ranges may be required to reveal activity.

Collectively, these results identify C. sinensis, S. aromaticum, and U. maritima as the most promising candidates for subsequent work, including expanded dose–response profiling, fractionation-guided isolation of active constituents, and mechanistic studies (e.g., competitive vs. non-competitive inhibition). The low standard deviations across replicates further indicate good assay reproducibility and support confidence in the observed potency ranking.

Table (2): Inhibition of Human BuChE enzyme by selected Libyan medicinal plants.

Plant Scientific Name	Hot Water Extracts	Cold Water Extracts
Camellia sinensis	0.066 ± 0.004[a]	0.12 (7.42 ± 0.46) [b]
Myrtus communis	0.148 ± 0.003[a]	0.13 (38.74 ± 1.12) [b]
Alhagi maurorum	0.172 [c]	0.17 (2.89 ± 0.21) [b]
Urginea maritima	0.33 (4.99 ± 0.68)[b]	0.199 [c]
Olea europaea	0.15 (34.79 ± 0.38) [b]	0.12 (22.63 ± 1.2) [b]
Matricaria chamomilla	0.165 [c]	0.109 ± 0.001 [a]
Hibiscus sabdariffa	0.279 [c]	0.29 (21.68± 0.8) [b]
Quercus robur	0.066 ± 0.002 [a]	0.055 ± 0.002 [a]
Syzygium aromaticum	0.116 ± 0.007 [a]	0.15 (44.7 ± 0.72) [b]
Zingiber officinale	0.207 [c]	0.110 [c]

Data expressed as mean ± SD (n = 3)

a = IC50 value (mg/ml) (final concentration of inhibitors required for 50% enzyme inhibition, as calculated from the dose response-curve equations).

b = Concentration (mg/ml) providing this percentage inhibition (Inhibition %, inhibitory activity of extracts which did not reach 50% enzyme inhibition).

c = No Inhibition at this concentration (mg/ml).

Table 2 presents the inhibitory profiles of hot- and cold-water extracts from ten Libyan medicinal plants against human BuChE, revealing marked species- and extraction-dependent differences in potency. Overall, BuChE inhibition reaching 50% (IC_{50}; superscript a) was observed for a limited subset of extracts, indicating that strong BuChE-targeting activity is less widespread than partial inhibition within the screened concentration range. The most potent BuChE inhibition was exhibited by Quercus robur, for which both hot- and cold-water extracts produced IC_{50} values (0.066 ± 0.002 and 0.055 ± 0.002 mg/mL, respectively). The close IC_{50} magnitudes across extraction conditions suggest that the principal BuChE-inhibitory constituents of Q. robur are efficiently extracted in water and remain stable across temperature variation, supporting its candidacy as a robust source of polar BuChE inhibitors.

Several plants demonstrated temperature-sensitive inhibitory behavior, underscoring the importance of extraction conditions for recovering active constituents. Notably, Camellia sinensis showed high potency only in the hot-water extract (IC_{50} = 0.066 ± 0.004 mg/mL), whereas the cold extract produced only weak inhibition (7.42 ± 0.46% at 0.12 mg/mL; superscript b). This pattern may indicate that heating enhances the solubilization of key inhibitory metabolites and/or facilitates release from the plant matrix, while cold maceration may be insufficient to extract these compounds at pharmacologically relevant levels. A similar trend was evident for Syzygium aromaticum and Myrtus communis, where the hot-water extracts achieved IC_{50} values (0.116 ± 0.007 and 0.148 ± 0.003 mg/mL, respectively), whereas the corresponding cold extracts failed to reach 50% inhibition within the tested concentrations, despite showing moderate partial inhibition (44.7 ± 0.72% and 38.74 ± 1.12%; superscript b). Collectively, these results imply that hot-water extraction is more favorable for recovering BuChE-active polar constituents from these taxa.

In contrast, Matricaria chamomilla displayed the opposite behavior: the cold-water extract exhibited a measurable IC_{50} (0.109 ± 0.001 mg/mL), while the hot-water extract was classified as non-inhibitory at the tested concentration (superscript c). This inversion strongly suggests the presence of thermolabile BuChE inhibitors or heat-induced degradation/transformations

that reduce inhibitory activity. The remaining species showed either no detectable inhibition (superscript c; e.g., Zingiber officinale in both preparations) or only limited partial inhibition (superscript b), such as Olea europaea (34.79 ± 0.38% at 0.15 mg/mL for hot; 22.63 ± 1.2% at 0.12 mg/mL for cold) and Hibiscus sabdariffa (21.68 ± 0.8% at 0.29 mg/mL for cold). Particularly weak BuChE inhibition was observed for Urginea maritima (4.99 ± 0.68% at 0.33 mg/mL; hot) and Alhagi maurorum (2.89 ± 0.21% at 0.17 mg/mL; cold), suggesting minimal relevance as BuChE inhibitors under aqueous extraction.

When interpreted alongside typical cholinesterase pharmacology, these findings have practical implications for lead selection: Q. robur appears to be a consistent BuChE inhibitor under both extraction modes, whereas C. sinensis, S. aromaticum, and M. communis exhibit stronger BuChE inhibition predominantly in hot-water preparations, and M. chamomilla emerges as a cold-extract–favored BuChE inhibitor. The reproducibility implied by the low standard deviations supports the reliability of the observed rank order. Future work should prioritize expanded dose–response analyses, bioassay-guided fractionation, and selectivity assessment against AChE to determine whether these extracts offer dual AChE/BuChE inhibition or enzyme-preferential activity relevant to stage-specific therapeutic strategies.

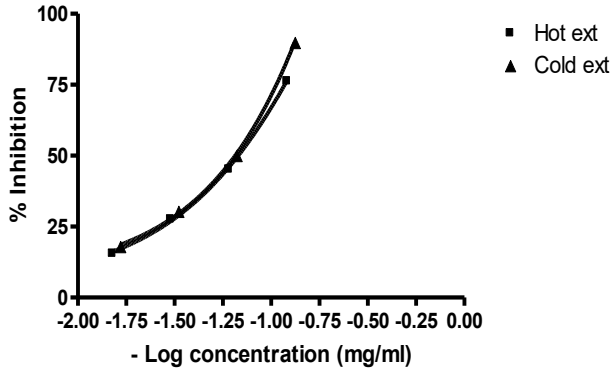

Figure (1): Dose response curve for the hot and the cold *Camellia sinensis* extracts against AChE. The graph was produced using Graphpad Prism to show the sigmoid pattern where concentrations are plotted on a logarithmic scale.

Table (3): Plant extracts categories.

Plant Scientific Name	Hot Water Extracts		Cold Water Extracts	
	AChE	BuChE	AChE	BuChE
Camellia sinensis	+++	+++	+++	+
Myrtus communis	++	+++	++	++
Alhagi maurorum	-	-	-	+
Urginea maritima	+++	+	+++	-
Olea europaea	-	++	-	+
Matricaria chamomilla	-	-	-	+++
Hibiscus sabdariffa	-	-	-	+
Quercus robur	++	+++	+	+++
Syzygium aromaticum	+++	+++	+++	++
Zingiber officinale	-	-	-	-

- = no inhibition.
+ = low activity (1-25% inhibition).
++ = moderate activity (25-50% inhibition).
+++ = good activity (50-100% inhibition).

Discussion:

A substantial body of research indicates that cholinesterase inhibition represents a promising therapeutic approach for managing Alzheimer's disease (AD), as well as other neurological disorders such as Parkinson's disease, age-related cognitive decline, and myasthenia gravis (Liu et al., 2019). The rationale behind this strategy is to prolong the availability of acetylcholine (ACh) within synaptic clefts by preventing its enzymatic degradation, thereby enhancing cholinergic transmission in conditions where receptor activation is insufficient. By inhibiting AChE, the residence time of ACh in synapses increases, partially compensating for deficiencies in neurotransmitter release or reduced cholinergic signalling.

Traditional medicinal plants have long served as a significant source of pharmacologically active compounds, and many modern therapeutic agents originate from such natural sources. Evidence also suggests that butyrylcholinesterase (BuChE) may contribute to the pathophysiology of AD, particularly in later stages of the disease. This has led to the hypothesis that dual targeting of AChE and BuChE may provide greater therapeutic benefit than selective inhibition of AChE alone (Liston et al., 2004; Giacobini, 2004; Nordberg et al., 2009; Li et al., 2017).

In this study, ten medicinal plants commonly used in Libyan ethnobotany, *Camellia sinensis*, *Myrtus communis*, *Alhagi maurorum*, *Urginea maritima*, *Olea europaea*, *Matricaria chamomilla*, *Hibiscus sabdariffa*, *Quercus robur*, *Syzygium aromaticum*, and *Zingiber officinale*, were assessed for their AChE and BuChE inhibitory activities. Extracts were prepared using hot and cold-water methods that closely replicate traditional preparation techniques (Triantaphyllou et al., 2001).

Green tea (*Camellia sinensis*), belonging to the Theaceae family and globally consumed as a staple beverage, has been widely reported for its protective pharmacological properties, including anticancer, antioxidant, and neuroprotective activities (Borek, 2005; Katalinic et al., 2006; Beecher et al., 1999; Benzie and Szeto, 1999; Ali et al., 2016). In the present study, hot-water extracts of *C. sinensis* produced IC_{50} values of 0.068 ± 0.001 mg/mL (AChE) and 0.066 ± 0.004 mg/mL (BuChE). These values, while slightly higher, remain comparable to those reported by Okello et al. (2004), who documented potent inhibitory effects of green tea on both enzymes. Additional studies support these findings, demonstrating that various tea extracts, including white and green tea, as well as seed and pericarp components, possess inhibitory activity against cholinesterases (Okello et al., 2012; Jo et al., 2012). In vivo research also corroborates these results (Raghavendra et al., 2015). Compounds such as thymol (Duke, 1992) and epigallocatechin-3-gallate (EGCG) (Nagle et al., 2006; Okello and Mather, 2020) have been proposed as contributors to this activity.

Similarly, *Syzygium aromaticum* exhibited strong inhibition toward both enzymes in this study. Previous research has shown that clove extracts, particularly hot-water preparations, can inhibit AChE and BuChE by 90% and 73% at 100 µg/mL (Prabha and Anusha, 2015). Variability in inhibitory strength among extract types has also been documented, with essential oils and methanolic extracts showing moderate to strong activity (Dalai et al., 2014; Ali et al., 2013; Phrompittayarat et al., 2014; Napagoda and Jayasinghe, 2022). The weaker activity of cold-water extracts observed in this study may reflect limited solubility of key active constituents at lower temperatures.

Regarding *Myrtus communis*, previous findings from Pakistan reported significant inhibitory effects on both AChE and BuChE (Begum et al., 2012). The moderate-to-high activity observed here may be attributed to compounds such as 1,8-cineole (Duke, 1992), a monoterpene previously recognized as a cholinesterase inhibitor (Perry et al., 2000; Savelev et al., 2003; Miyazawa et al., 1998, 2001). Interestingly, some plants, including *Matricaria chamomilla* and *Quercus robur*, showed selective inhibition toward BuChE, consistent with reports that essential oils from *M. communis* may exhibit stronger activity against BuChE (Maggio et al., 2019). In contrast, *Urginea maritima* displayed pronounced AChE inhibition with minimal BuChE activity, aligning with earlier studies reporting differential inhibition patterns among plant extracts (Orhan et al., 2004). These differences may arise from structural and kinetic variations between AChE and BuChE (Giacobini, 2004), or from differences in how plant constituents interact with each enzyme (Orhan et al., 2004).

The association between antioxidant activity and cholinesterase inhibition has been highlighted in several studies. Extracts of *Hypericum undulatum*, *Melissa officinalis*, *Laurus nobilis*, and *Lavandula pedunculata* have been shown to exhibit both strong antioxidant capacity and cholinesterase inhibitory effects (Ferreira et al., 2006). In agreement with previous antioxidant classifications (Elmestiri et al., 2011), most plant extracts demonstrating notable inhibitory activity in this study, excluding *U. maritima*, belonged to the high antioxidant group. Except for *C. sinensis*, this study reports for the first-time cholinesterase inhibitory potential for several of these Libyan plants.

Collectively, the results reaffirm the potential of *Camellia sinensis* and *Syzygium aromaticum* as strong cholinesterase inhibitors and highlight the therapeutic promise of selected Libyan medicinal plants. The variability in enzyme selectivity underscores the importance of identifying plant-derived compounds suitable for targeted therapeutic applications. Future research should aim to isolate the active phytochemicals responsible for these effects and validate their efficacy and safety through in vivo studies.

Conclusion

Cholinesterases (ChEs) play an essential role in regulating cholinergic neurotransmission by catalyzing the breakdown of acetylcholine (ACh) within synaptic spaces. Impairment or imbalance in the activity of acetylcholinesterase (AChE), the principal enzyme in this process, can lead to excessive accumulation of ACh, contributing to neurotoxicity and the development of neurodegenerative disorders, most notably Alzheimer's disease (AD). Targeting both AChE and butyrylcholinesterase (BuChE) has therefore emerged as an effective therapeutic approach, as inhibiting these enzymes extends the functional lifespan of ACh at neuronal synapses.

Although several approved medications, including donepezil, rivastigmine, and galantamine, provide symptomatic relief, their clinical use is often limited by adverse effects and reduced tolerance in long-term treatment. This reinforces the need to explore safer and more accessible alternatives. The results of this study demonstrate that certain Libyan medicinal plants, particularly *Camellia sinensis* and *Syzygium aromaticum*, possess notable cholinesterase inhibitory activity. These outcomes support the potential of these species as promising natural sources for developing plant-based therapeutic agents aimed at managing AD and other cholinergic dysfunctions.

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