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Evaluation of Acetylcholinesterase Inhibitory Activities of Selected Plants in the Light of Ethnobotanical and Phytochemical Perspectives

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ABSTRACT

Alzheimer's disease (AD) is the most common form of dementia, and treatment options only relieve the progression of the disease. Thus, the search for new therapeutic agents for AD remains an ongoing challenge. Utilizing the richness of chemical structures in natural sources is one of the most effective approaches in discovering new drug candidate molecules. For this purpose, this study aimed to evaluate the acetylcholinesterase enzyme inhibitory activities of *Dysphania botrys* (L.) Mosyakin & Clemants, *Lotus corniculatus* L. var. *tenuifolius* L., *Noaea mucronata* (Forssk.) Aschers. et Schweinf. ssp. *mucronata*, *Vicia cracca* L. ssp. *stenophylla* Vel. to assess their potential as natural sources. Ellman's colorimetric assay was used for the determination of enzyme inhibitory activity of *n*-hexane, dichloromethane, ethyl acetate, and *n*-butanol extracts of the plants, which were prepared by the partition of crude methanolic extracts by the corresponding solvents and the remaining aqueous phase.The dichloromethane extract of *D. botrys* between the 30-90 µg/ml concentrations exerted the highest inhibitory activity, with a maximum inhibitory value of 49.9%. Metabolites of active extracts that may be responsible for the activity deserve to be investigated, and further studies are needed.

Keywords: Alzheimer's disease, Ellman's method, Enzyme inhibition, Natural sources.

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Introduction

Neurodegenerative disorders, particularly Alzheimer's disease (AD), pose significant global health challenges. A hallmark of AD is a progressive and common brain disorder, representing the most prevalent cause of dementia, accounting for 50%-75%. It is the deficiency of acetylcholine, a neurotransmitter essential for memory and learning, and is characterized by a gradual decline in cognitive functions, which leads to disabilities in daily tasks [1, 2]. The disease not only has a destructive impact on the patient but also imposes a significant economic burden on patients, caregivers, and governments. Additionally, caregivers often experience stress, depression, anxiety, and social isolation. This highlights the importance of effectively treating the disease [3]. However, there is no definitive cure for AD. Today, FDA-approved drugs, acetylcholinesterase (AChE) inhibitors (galantamine, donepezil, and rivastigmine), and an NMDA receptor antagonist (memantine), are used to mitigate the cognitive impairment of patients without alleviating the progression of the disease [4]. Therefore, research on finding new candidates for the treatment of AD still retains its relevance and significance.

The cholinergic hypothesis suggests that the primary cause of AD is the reduced synthesis of acetylcholine. Accordingly, enhancing the acetylcholine level by inhibiting the AChE may be a strategic approach to alleviating the symptoms of AD [5].

Traditional medicinal plants, one of the main sources of drug discovery research, offer a reservoir of bioactive compounds and have long been explored for their potential neuroprotective properties. One effective approach is to utilize ethnopharmacological studies to identify bioactive principles [6]. Additionally, serendipity can play a role in drug discovery, as exemplified by the accidental identification of etoposide and vinblastine as effective anticancer agents derived from plant sources [7]. As natural resources, plant materials can produce unique bioactive molecules due to their unlimited biosynthesis capacity. Therefore, regardless of the starting point, natural sources offer amazing opportunities for discoveries.

Infusion and decoction of the seeds of *Dysphania botrys* (L.) Mosyakin & Clemants (Amaranthaceae) are used for asthma, cough, wounds, fever, pain, liver, respiratory, urinary, and gastric complaints, as an antiseptic, and for wound healing in folk medicine [8]. This species is also reported to be used for several conditions worldwide, including nervous disorders, as an antiasthmatic, diuretic, antispasmodic, anticonvulsant, anthelmintic, and antidiarrheic [9]. *Lotus corniculatus* L.

var. *tenuifolius* L. (Fabaceae) is used as a sedative, antihemorrhoidal, and antineuralgic [10]; *Vicia cracca* L. ssp. *stenophylla* Vel. (Fabaceae) is used for the treatment of cough [11] in traditional folk medicine in Türkiye. *Noaea mucronata* (Forssk.) Aschers. et Schweinf. ssp. *mucronata* (Amaranthaceae) has been identified as a dominant species in regions with traditional medicinal practices, and the leaves and flowers of *N. mucronata* are used as an anticalculus for kidney problems [12]. Phytochemical screening revealed the presence of phenolic compounds, especially high flavonoid content in *D. botrys* and *L. corniculatus* [9, 13-16].

This study aims to determine the AChE inhibitory activity of different solvent extracts of *D. botrys*, *L. corniculatus*, *N. mucronata*, *V. cracca* for their neuroprotective potentials.

Materials and Methods

Chemicals and Instruments

The AChE enzyme, 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), acetylthiocholine iodide, and the solvents used for extractions—*n*-hexane, dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc), and *n*-butanol (*n*-BuOH)—were purchased from Sigma in Germany. A *Buchi* R-210 extractor was employed for the extraction procedures, and absorbance monitoring was conducted using a BIO-TEK µQuant Spectrophotometer.

Plant Materials

Plant materials used in this study were collected from Ankara, Türkiye, and authenticated by Prof. Dr. Galip Akaydın, Hacettepe University, Faculty of Education, Department of Biology Education. The detailed information about the plant samples is provided in Table 1.

Table 1. Collection sites and voucher numbers of plant samples

Plants	Site of Collection	No of Herbarium
Dysphania botrys (L.) Mosyakin & Clemants	A4 Ankara: Kızılcahamam	Akaydın 13505
Lotus corniculatus L. var. *tenuifolius* L.	B4 Ankara: İdris Dağı	Akaydın 13437
Noaea mucronata (Forssk.) Aschers. et Schweinf. ssp. *mucronata*	A4 Ankara: Kızılcahamam	Akaydın 13504
Vicia cracca L. ssp. *stenophylla* Vel.	A4 Ankara: Kızılcahamam	Akaydın 13488

Extraction and Organic Solvent Partitioning

Air-dried and powdered aerial parts of each plant sample were extracted with methanol (100 mL x 3) in a water bath at 40 °C using a rotary extractor. The corresponding extracts were then filtered and combined, followed by concentration *in vacuo* to yield crude extracts. The extracts were suspended in water and sequentially partitioned with *n*-hexane, CH_2Cl_2, EtOAc, and *n*-BuOH to obtain related extracts.

Acetylcholinesterase Inhibitory Activity

Anti-acetylcholinesterase inhibitory activity of the extracts was assessed using the colorimetric Ellman method [17] with modifications we described previously [18]. Each extract, at concentrations of 30–120 µg/mL, was incubated with the enzyme (Electric eel AChE, Type-VI-S, EC 3.1.1.7; 0.01 U/µL) and DTNB (1.25 mM) in phosphate buffer (pH 8, 500 mM) for 10 min. at 25 °C. The reaction was initiated by adding the substrate (acetylthiocholine iodide, 7.5 mM). The change in absorbance was monitored for 10 min. at 412 nm using a microplate reader. The results were expressed as a percentage of the enzyme inhibition.

Results

The AChE inhibitory activities of different solvent extracts of *D. botrys*, *L. corniculatus*, *N. mucronata*, and *V. cracca* were determined by the colorimetric Ellman method. The results were given as the percentage of inhibition in Table 2 and Figure 1.

Table 2. AChE inhibition rates of plant extracts

	Inhibition (%)			
Samples	30 µg/ml	60 µg/ml	90 µg/ml	120 µg/ml
D. botrys - *n*-Hexane	16.4	25.9	22.0	29.4
D. botrys - CH_2Cl_2	42.2	42.7	46.5	49.9
D. botrys - EtOAc	12.6	10.4	8.0	12.4
D. botrys - *n*-BuOH	10.4	7.5	6.3	3.2
D. botrys - Aqueous phase	0.0	3.5	4.9	0.7
L. corniculatus - *n*-Hexane	10.0	2.0	6.9	7.4
L. corniculatus - CH_2Cl_2	24.1	25.9	8.9	9.9
L. corniculatus - EtOAc	16.2	14.3	7.5	6.7
L. corniculatus - *n*-BuOH	6.9	1.4	0.7	4.3
L. corniculatus - Aqueous phase	0.0	4.3	11.0	13.9
N. mucronata - *n*-Hexane	1.2	6.5	26.2	7.4
N. mucronata - CH_2Cl_2	0.3	8.5	16.0	14.0
N. mucronata - MeOH	7.1	5.2	0.3	5.8
V. cracca – *n*-Hexane	0.0	9.3	5.8	24.3
V. cracca - CH_2Cl_2	15.1	19.8	6.0	0.0
V. cracca - EtOAc	21.6	6.9	1.0	11.3
V. cracca - *n*-BuOH	2.2	8.3	14.8	20.2
V. cracca - Aqueous phase	12.6	1.5	7.1	14.0

The CH_2Cl_2 extract of *D. botrys* was found to be the most active, with a 49.9% inhibition rate at 120 µg/mL. This activity was attributed to the presence of medium-polarity phytochemicals capable of modulating cholinergic neurotransmission. The use of Ellman's assay in this study provided a standardized and reliable screening tool for enzyme inhibition, establishing a foundation for further phytochemical analysis. *D. botrys*-*n*-hexane, *L. corniculatus*-CH_2Cl_2, *V. cracca*-*n*-hexane, and *V. cracca*-EtOAc extracts displayed weak inhibitory activities at various concentrations.

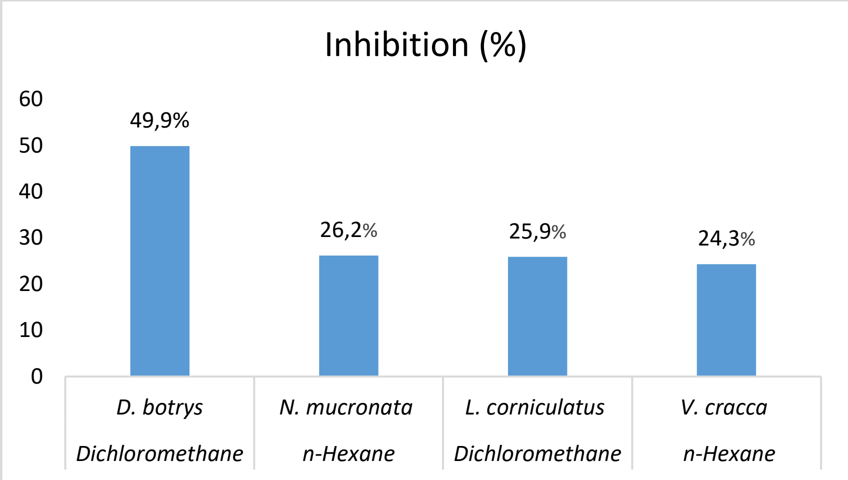

Figure 1. AChE inhibition percentages of selected plant extracts

Discussion

There are some studies on *D. botrys* regarding its cholinesterase inhibitory activity. Iqbal et al. examined the cholinesterase inhibitory activity of *Chenopodium botrys* (Synonym of *D. botrys*). They prepared the crude methanol extract and then fractionated it with *n*-hexane, chloroform, and ethyl acetate to obtain the corresponding extracts and the remaining aqueous fraction. The AChE inhibitory potential of the extracts was assessed in the following order: chloroform > EtOAc ≅ MeOH > water > *n*-hexane, with inhibitory values of 50.16%, 30.00%, 59.64%, 50.61%, and 40.56%, respectively, at a concentration of 125 µg/mL. Further insight was provided by kinetic analyses conducted on methanolic fractions of *D. botrys*. This experiment revealed an inhibition mechanism for AChE, suggesting that the bioactive compounds in the plant bind to both the active site and allosteric region of the enzyme. This mechanistic understanding adds depth to the pharmacological evaluation of *D. botrys* and supports its potential use as a multitarget agent in cholinesterase-based therapy [19].

Ethanol and water extracts of *C. botrys* were determined to have AChE inhibitory activity with values of 50.87 µg and 113.69 µg galantamine equivalent/g extract. These extracts exhibited high phenolic content, correlating with their antioxidant and neuroprotective potential. BChE inhibition activity was also evaluated in this study. This dual inhibition profile is particularly relevant, as BChE activity tends to increase in the later stages of AD, complementing the role of AChE inhibition in early-stage therapy [14].

Another study investigated the AChE inhibitory activity of the essential oils, which are rich in volatile constituents such as monoterpenes and sesquiterpenes, of three different *C. botrys* samples collected from different geographic regions. The essential oils were ascertained to have inhibitory activity on the enzyme in the range of 0.54–0.87 mg galantamine equivalent/g essential oil. These essential oils demonstrated substantial AChE inhibition, indicating that non-polar, volatile components may contribute significantly to the plant's neuroactive properties. The chemical composition of the essential oils varied depending on geographic origin, highlighting the need for chemotypic standardization in future studies [13].

Tüfekçi et al. also assessed the AChE inhibitory activity of the plant's essential oil along with other extracts. For this purpose, after obtaining the essential oil, the water extract was subsequently partitioned with EtOAc and *n*-BuOH. The aqueous phase remaining after partitioning and the *n*-BuOH fraction exhibited enzyme inhibitory activity, with IC_{50} values of 460 µg/mL and 376 µg/mL, respectively [20].

The therapeutic potential of *D. botrys* in the management of AD has been investigated through multiple experimental models, with particular focus on its cholinesterase inhibitory activity. Among the most studied targets in Alzheimer's pharmacotherapy are AChE and BChE enzymes responsible for the hydrolysis of acetylcholine. Several studies have explored the ability of *D. botrys* extracts and essential oils to inhibit these enzymes, indicating a promising role for this species in neurodegenerative disease management. According to our results and literature survey, *D. botrys* may have inhibitory potential on the AChE enzyme. However, the simultaneous determination of metabolites responsible for the activity would be the most innovative approach, since factors such as the collection place and time of the plant samples, as well as extraction conditions, could affect the biological activity.

In the study, *L. corniculatus* aerial parts were extracted sequentially using solvents of increasing polarity, *n*-hexane, CH_2Cl_2, EtOAc, *n*-BuOH, and MeOH. The extracts of *L. corniculatus* also demonstrated measurable AChE inhibition compared with other plant species. This suggested the presence of moderately active polar and non-polar constituents, though the inhibition rates were lower than those observed in some of the other tested species. In contrast, Yerlikaya et al. [16] took a focused approach by evaluating the enzyme inhibitory activities of three extract types of *L. corniculatus*: EtOAc, MeOH, and water extracts. Among these, the EtOAc extract exhibited significant AChE and BChE inhibition. A point that should be emphasized is that the EtOAc extract obtained after the liquid–liquid partitioning process in our study was less active than that obtained by the direct extraction process performed by Yerlikaya et al. [16].

The activities of *N. mucronata* and *V. cracca* were relatively lower but still measurable. To the best of our knowledge, these species have not been previously investigated for their AChE inhibitory activity. The preliminary findings on the AChE inhibitory activity of *N. mucronata* and *V. cracca* are promising, indicating their potential as a natural source of neuroprotective agents. However, comprehensive phytochemical studies are necessary to isolate and identify the specific compounds responsible for this activity. Further research, including *in vivo* assessments, the mechanism of action, and clinical trials, is essential to fully understand the efficacy and safety profile in the context of neurodegenerative diseases.

Conclusion

Neurodegenerative diseases such as AD are closely associated with the progressive loss of cholinergic neurotransmission, particularly due to the degradation of acetylcholine by AChE. The inhibition of AChE remains one of the most validated therapeutic strategies for managing symptoms of AD, and the search for effective, safe, and natural AChE inhibitors continues to drive pharmacological research. Ethnobotanical practices have long utilized various plants for cognitive and neurological ailments, suggesting the presence of compounds that may modulate neurotransmitter systems. With the growing demand for safer and natural alternatives, several plant species have been explored for their potential cholinesterase inhibitory properties.

This study demonstrates the potential AChE inhibitory activities of *D. botrys*, *L. corniculatus*, *N. mucronata*, and *V. cracca*. These findings support their traditional usage and highlight their potential in developing natural treatments for neurodegenerative diseases such as AD. The AChE inhibitory potential observed aligns with the traditional uses of the selected species.

D. botrys, traditionally used for nervous disorders, contains flavonoids and other phenolics that may contribute to its neuroprotective effects. Similarly, *L. corniculatus* has been employed as a sedative and antihaemorrhoidal agent in folk medicine, supporting its potential role in modulating cholinergic function. *N. mucronata*, though less studied, has been used traditionally for kidney ailments and as a diuretic. Its moderate AChE inhibition suggests the presence of bioactive compounds, warranting further investigation. *V. cracca*, known for its use in treating cough problems, also demonstrated notable AChE inhibitory activity, indicating potential neuromodulatory properties.

The high flavonoid content found in *D. botrys* and *L. corniculatus* may be responsible for their neuroprotective activities, as flavonoids have been reported to modulate neuronal signaling and oxidative stress. The correlation between the presence of flavonoids and AChE inhibition underscores the importance of these compounds in neuroprotection. Further studies are needed for all tested species to isolate and characterize the active constituents and validate their *in vivo* efficacy.

Conflicts of interest

There are no conflicts of interest in this work.

References

[1] Alzheimer’s Association, What Is Alzheimer’s Disease? Available at: https://www.alz.org/alzheimers-dementia/what-is-alzheimers#symptoms. Retrieved May 15, 2025.

[2] National Institute on Aging, Alzheimer’s and Dementia. Available at: https://www.nia.nih.gov/health/alzheimers-and-dementia. Retrieved May 15, 2025.

[3] Alzheimer’s Association. Alzheimer’s Disease Facts and Figures. Available at: https://www.alz.org/alzheimers-dementia/facts-figures. Retrieved May 15, 2025.

[4] Sharma A., Rudrawar S., Bharate S.B., Jadhav H.R., Recent Advancements in the Therapeutic Approaches for Alzheimer’s Disease Treatment: Current and Future Perspective, *RSC Med. Chem*., 16(2) (2025) 652-693.

[5] Sharma K., Cholinesterase Inhibitors as Alzheimer’s Therapeutics (Review), *Mol. Med. Rep*., 20(2) (2019). 1479-1487.

[6] Süntar I., Importance of Ethnopharmacological Studies in Drug Discovery: Role of Medicinal Plants, *Phytochem. Rev*., 19(5) (2020) 1199-1209.

[7] Prasad S., Gupta S.C., Aggarwal B.B., Serendipity in Cancer Drug Discovery: Rational or Coincidence? *Trends Pharmacol. Sci*., 37(6) (2016) 435-450.

[8] Dagni A., Hegheș S.C., Suharoschi R., Pop O.L., Fodor A., Vulturar R., Cozma A., Aniq Filali O., Vodnar D.C., Soukri A., El Khalfi, B., Essential Oils from *Dysphania* Genus: Traditional Uses, Chemical Composition, Toxicology, and Health Benefits, *Front. Pharmacol*., 13 (2022) 1024274.

[9] Morteza-Semnani K., A Review on *Chenopodium botrys* L.: Traditional Uses, Chemical Composition and Biological Activities, *Pharm. Biomed. Res*., 1(2) (2015) 1-9.

[10] Altundag E., Ozturk M., Ethnomedicinal Studies on the Plant Resources of East Anatolia, Turkey, *Procedia Soc. Behav. Sci.,* 19 (2011) 756-777.

[11] Hayta S., Polat R., Selvi S., Traditional Uses of Medicinal Plants in Elazığ (Turkey), *J. Ethnopharmacol*., 154(3) (2014) 613-623.

[12] Ghasemi Pirbalouti, A., Momeni M., Bahmani M., Ethnobotanical Study of Medicinal Plants Used by Kurd Tribe in Dehloran and Abdanan Districts, Ilam Province, Iran, *Afr. J. Tradit. Complement. Altern. Med.*, 10(2) (2013) 368-385.

[13] Ozer M.S., Sarikurkcu C., Ceylan O., Akdeniz I., Tepe, B., A Comprehensive Study on Chemical Composition, Antioxidant and Enzyme Inhibition Activities of the Essential Oils of *Chenopodium botrys* Collected from Three Different Parts of Turkey, *Ind. Crops Prod*., 107 (2017) 326-331.

[14] Ozer M.S., Sarikurkcu C., Tepe B., Phenolic Composition, Antioxidant and Enzyme Inhibitory Activities of Ethanol and Water Extracts of *Chenopodium botrys*, *RSC Adv*., 6(69) (2016) 64986-64992.

[15] Şimşek Sezer, E.N., Uysal T., Phenolic Screening and Biological Activities of *Chenopodium botrys* L. Extracts, *Ant. J. Bot*., 5(2) (2021) 78-83.

[16] Yerlikaya S., Baloglu M.C., Diuzheva A., Jekő J., Cziáky Z., Zengin G., Investigation of Chemical Profile, Biological Properties of *Lotus corniculatus* L. Extracts and Their Apoptotic-Autophagic Effects on Breast Cancer Cells, *J. Pharm. Biomed. Anal*., 174 (2019) 286-299.

[17] Ellman G.L., Courtney K.D., Andres V., Feather-Stone R.M., A New and Rapid Colorimetric Determination of Acetylcholinesterase Activity, *Biochem. Pharmacol*., 7(2) (1961) 88-95.

[18] Kahraman Ç., Baysal İ., Çankaya İ.İ., Göger F., Kırımer N., Akdemir Z.Ş., Acetylcholinesterase Inhibitory Activities and LC-MS Analysis of the Antioxidant *Ferula caspica* M. Bieb. and *F. halophila* Pesmen Extracts, *J. Res. Pharm.*, 23(3) (2019) 543-551.

[19] Iqbal N., Ullah F., Sadiq A., Shahid M., Ayaz M., Cholinesterase's Enzymes Inhibition and Michaelis-Menten Kinetics Studies on Ethnomedicinally Important Plant *Chenopodium botrys*, *Journal of Food Health and Bioenvironmental Science*, 13(3) (2023) 40-46.

[20] Tüfekçi A.R., Akşit H., Şimşek S., Karakoç Ö.C., Adem Ş., Hameed Z.A., Atalar M.N., Topkara A.R., Evaluation of Insecticidal and Enzyme Activity Potentials of Essential Oils and Extracts of *Chenopodium botrys* Against Storage Products Pests, *Bull. Biotechnol*., 4(1) (2023) 7-12.