Original Article

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Evaluation of Edible Mushroom *Cantharellus lutescens* as Potential Cholinesterase Inhibitors

Yenilebilir Mantar *Cantharellus lutescens*'in Kolinesteraz İnhibitörü Potansiyelinin Değerlendirilmesi

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ABSTRACT

Objective: Alzheimer's disease is one of the most common forms of dementia which affects the cognitive, physical, psychological, and social functions of individuals. Cholinesterase enzymes are known to play a pivotal role in the occurrence and progression of Alzheimer's disease. This study aims to evaluate the anticholinesterase and butyrylcholinesterase inhibitory activities of the mushroom *Cantharellus lutescens*, which can be found in İstanbul and its surrounding regions.

Methods: Extracts of *Cantharellus lutescens* mushroom were prepared by maceration with hexane, ethyl acetate, and methanol at room temperature. Acetylcholinesterase and butyrylcholinesterase inhibitory activities of these three extracts were determined using the Ellman method.

Results: While *Cantharellus lutescens* hexane extract was determined as the most active extract with 48.07±0.51% acetylcholinesterase inhibition at a concentration of 800 µg/mL, ethyl acetate extract showed the highest activity against butyrylcholinesterase with 29.20±0.80% inhibition at the same concentration.

Conclusion: This study evaluates the anticholinesterase activity of an edible mushroom that could be a potential therapeutic agent in the future.

Keywords: *Cantharellus lutescens*, yellow foot, anticholinesterase, Alzheimer's disease

ÖZ

Amaç: Alzheimer hastalığı, bireylerin bilişsel, fiziksel, psikolojik ve sosyal işlevlerini etkileyen en yaygın demans türlerinden biridir. Kolinesteraz enzimlerinin Alzheimer hastalığının ortaya çıkışında ve ilerlemesinde kritik bir rol oynadığı bilinmektedir. Bu çalışma, İstanbul ve çevresinde yetişen *Cantharellus lutescens* mantarının antikolinesteraz ve bütirilkolinesteraz inhibitör aktivitelerinin değerlendirilmesini amaçlamaktadır.

Yöntemler: *Cantharellus lutescens* mantarından hekzan, etil asetat ve metanol ile oda sıcaklığında maserasyon yöntemiyle ekstreler hazırlanmıştır. Bu üç ekstrenin asetilkolinesteraz ve bütirilkolinesteraz inhibitör aktiviteleri Ellman yöntemi ile belirlenmiştir.

Bulgular: *Cantharellus lutescens* hekzan ekstresi, 800 µg/mL konsantrasyonda %48,07±0,51 asetilkolinesteraz inhibisyonu ile en aktif ekstre olarak belirlenirken, etil asetat ekstresi aynı konsantrasyonda %29,20±0,80 bütirilkolinesteraz inhibisyonu ile en yüksek aktiviteyi göstermiştir.

Sonuç: Bu çalışma, gelecekte potansiyel bir terapötik ajan olabilecek yenilebilir bir mantarın antikolinesteraz aktivitesini değerlendirmektedir.

Anahtar Kelimeler: *Cantharellus lutescens*, sarı ayak, antikolinesteraz, Alzheimer hastalığı

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Introduction

Alzheimer's disease (AD), a progressive neurodegenerative disease, is the most prevalent form of dementia (1). According to the Alzheimer Europe report, more than 8 million individuals over the age of 30 were affected by AD in Europe and the USA in 2019, and this number is projected to double by 2050. Based on a survey of over 40,000 participants from 166 countries, conducted by London School of Economics (LSE) as a follow-up to ADI's 2019 survey, the new report examines how perceptions have evolved over five years. The World Alzheimer Report 2024 highlights widespread misconceptions and stigma around dementia. Many health professionals (65%) and the public (80%) wrongly see it as a normal part of ageing, and over a quarter believe it cannot be prevented. Nearly 90% of people with dementia experience discrimination, yet most carers and the public would pursue a diagnosis if treatments existed. Over 80% feel they can influence support through voting, and more than half link dementia to lifestyle factors (2).

Although there are many mechanisms associated with the occurrence of AD, cholinesterase enzymes seem to play a key role in the pathogenesis of the disease (2). Although drugs that exhibit cholinesterase inhibition are widely used as a treatment method, their administration is often associated with adverse side effects. Therefore, investigating the potential of natural products, such as mushrooms, against AD is of considerable importance. The number of studies targeting the anti-Alzheimer potential of mushrooms have been increasing in recent years supporting their involvement in different mechanisms to prevent or treat this neurodegenerative disease (3). *Cantharellus* species are edible mushrooms packed with micronutrients, trace elements and bioactive molecules. Their distinct apricot-like aroma makes them one of the top choices among consumers worldwide (4). A member of the *Cantharellus* genus*, Cantharellus lutescens (C. lutescens),* is commonly called "chick" or "chick"s leg mushroom". *C. lutescens* is characterized by its yellow stem, typically brown, drum-shaped cap and can be found in the forests of İstanbul almost all year long (5,6).

C. lutescens has previously been investigated for its thrombin inhibition activity (7) as well as for the influence of food processing on its antioxidant capacity (8). However, studies focusing on its potential neuroprotective properties remain limited. In this context, the anticholinesterase activities of hexane, ethylacetate, and methanol extracts of *C. lutescens* were systematically evaluated, aiming to provide deeper insights into the possible involvement of mushrooms in the management of neurodegenerative disorders. Such findings may contribute to a broader understanding of the therapeutic potential of *C. lutescens* and highlight its relevance as a source of bioactive compounds in drug discovery research.

Methods

Mushroom Sample Collection and Preparation

Fresh fruit bodies used in this study were collected in January 2024 from Yalova province (Armutlu, Türkiye). A reference sample was deposited with the Department of Medical Biology, Faculty of Medicine, Yalova University accession number SSE24-48, and is available for future reference. The samples identified by Dr. Selime Semra Erol using classical macroscopic and microscopic methods according to the available literature. Specimens identified as *C. lutescens* (Pers.) Fr. 1821 by classical taxonomy were confirmed by molecular taxonomy. The combined morphological and molecular evidence strongly supports the identification of the Turkish isolate as *C. lutescens*. Morphological features such as the funnel-shaped basidiomata, hollow stipe, and orange hymenophore folds are consistent with previous descriptions of the species (9,10). The ITS1-5.8S-ITS2 sequence of the Yalova isolate showed a 98-100% identity with *C. lutescens* voucher A5 from Tunisia (GenBank Accession No. PP533059.1), confirming its taxonomic placement within the *Hydnaceae* (Table 1).

The collected mushrooms were shade-dried at room temperature to prevent thermal degradation of bioactive compounds and then ground into a fine powder using an industrial-scale grinder. The powdered material was stored in airtight containers at 4 °C until further extraction and analytical procedures.

Extraction Process

The dried *C. lutescens* sample (24.57 g) was macerated at room temperature with hexane, ethylacetate, and methanol, successively (Figure 1). The extracts were filtered, and solvents were evaporated under reduced pressure once in every other day until the mushroom material is completely consumed. The maceration step afforded 3 extracts: namely hexane, ethylacetate, and methanol extracts.

Table 1. Comparative table of ITS sequences

Sample	Accession no	Region	Sequence length (bp)	% Identity	Origin
Yalova isolate *(Cantharellus lutescens)*	Pending	ITS1-5.8S-ITS2	507	98-100%	Yalova, Türkiye
Voucher A5 *(Cantharellus lutescens)*	PP533059.1	ITS1-5.8S-ITS2	665	98-100%	Tunisia

Anticholinesterase Activity

To assess the acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activities of *C. lutescens*, Ellman method (11) was followed with slight modifications (12). A reaction mixture was prepared by combining 150 µL of 100 mM sodium phosphate buffer (pH 8.0), 10 µL of the test sample (dissolved in ethanol at varying concentrations), and 20 µL of AChE (5.32×10^{-3} U) or BChE (6.85×10^{-3} U) solution, followed by incubation for 15 min at 25 °C. Subsequently, 10 µL of 0.5 mM DTNB solution were added. The reaction was initiated by adding 10 µL of acetylcholine iodide (0.71 mM) or butyrylthiocholine chloride (0.2 mM). The hydrolysis of these substrates was monitored spectrophotometrically at 412 nm by detecting the formation of the yellow 5-thio-2-nitrobenzoate anion, which results from the reaction between DTNB and the thiocholine released during enzymatic hydrolysis of acetylcholine iodide or butyrylthiocholine chloride. Measurements were performed using a 96-well microplate reader. Galantamine is used as the standard drug. The inhibitory activity was expressed as inhibitory concentration (IC_{50}) values, calculated from the concentration-response curve plotting the percentage of enzyme inhibition against sample concentration.

Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics. The half maximal IC_{50} values obtained from each extract were expressed as mean ± standard deviation (SD) of three independent experiments. Prior to parametric analysis, data were log-transformed ($logIC_{50}$) to improve normality and homogeneity of variance.

The Shapiro-Wilk test was used to assess the normal distribution of the data, and Levene's test was applied to verify the equality of variances. Since the assumptions for normality and homogeneity were met ($p>0.05$), a one-way analysis of variance was conducted to determine whether there were statistically significant differences in IC_{50} values among the extracts. Post-hoc pairwise comparisons were performed using Tukey's honest significant difference and Games-Howell tests to identify specific group differences. The significance level of $p<0.05$ was considered statistically significant.

Ethics Committee Approval

This study does not involve human participants, animal experiments, or private or sensitive data collection. Therefore, ethical approval was not needed. This study does not involve human participants, animal experiments, or private or sensitive data collection. Therefore, informed consent was not needed.

Results

The % AChE inhibition values for hexane, ethyl acetate, and methanol extracts of *C. lutescens* at different concentrations are presented in Table 2 (Figure 2) (separated figures in Supplementary Figure S1-S4). When the AChE inhibitory



Figure 1. Extraction process

Table 2. The acetylcholinesterase inhibitory activity (%) of *Cantharellus lutescens* extracts

Extracts	Concentration (µg/mL)[a]				IC_{50}
	100	200	400	800	µg/mL
CLH (hexane extract)	15.21±0.84	24.00±1.73	30.33±1.53	48.07±0.51	824.56±10.75
CLE (EtOAc extract)	5.39±0.88	8.79±1.55	15.20±0.20	19.46±1.27	2265.72±15.93
CLM (methanol extract)	NA	NA	1.84±0.12	7.20±1.15	1052.86±15.26
Galantamine[b]	68.36±1.10	74.38±0.65	78.59±0.47	80.40±0.9	5.01±0.01

[a]: All results ± standard deviation (three replicate studies), [b]: Galantamine; standard, NA: Not active, IC_{50}: Inhibitory concentration

activity of *C. lutescens* was assessed, all extracts demonstrated a concentration-dependent increase in inhibition as expected. The highest % inhibition among all the extracts was observed for the hexane extract at a concentration of 800 µg/mL (48.07%) which is comparable to the standard drug galantamine (68.36±1.10). While the methanol extract was found to be inactive at 100 and 200 µg/mL concentrations, ethyl acetate extracts showed some activity at all concentrations.

The BChE inhibition values (%) for hexane, ethylacetate, and methanol extracts of *C. lutescens* at different concentrations are shown in Table 3 (Figure 3) (separated figures in Supplementary Figure S5-S7). When the BChE inhibitory activity of *C. lutescens* was assessed, ethyl acetate extract at a concentration of 800 µg/mL exhibited the highest inhibition % value (29.20±0.80) in comparison to the other extracts. Both the hexane and methanol extracts were found to demonstrate show some inhibition, with the methanol extract displaying superior activity at all concentrations.

Discussion

The present study systematically evaluated the AChE and BChE inhibitory activities of *C. lutescens*, contributing to the growing body of literature investigating natural products for AD therapy. Mushrooms are recognized for their diverse bioactive compounds, including polysaccharides from *Armillaria mellea* (13), cordycepin from *Cordyceps militaris* (14), and ganoderic acids A, B, E, and Y from *Ganoderma lucidum* (15), which exhibit neuroprotective, anti-inflammatory, and antioxidant properties. Among these, erinacine A from *Hericium erinaceus* has attracted particular attention due to its neurotrophic activity and is currently undergoing clinical trials (16). In a randomized, double-blind, placebo-controlled pilot study, patients with early-stage AD receiving *Hericium erinaceus* mycelium and erinacine A for 49 weeks exhibited improvements in instrumental activities of daily living, Mini-Mental State Examination scores, and contrast sensitivity compared to controls. These findings underscore the potential of mushroom-derived compounds as multi-target agents, capable of modulating both cognitive function and underlying neuropathology, though further mechanistic studies are warranted.

Within *Cantharellus* species, *C. tubaeformis* has been reported to inhibit AChE, with its water extract showing the highest activity (42.13±0.51%) and its hexane extract demonstrating the

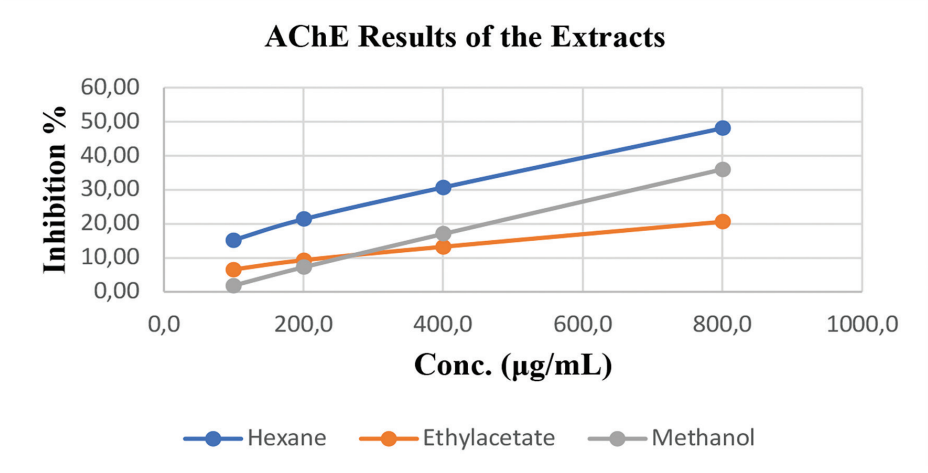

Figure 2. Acetylcholinesterase inhibitory activity of *Cantharellus lutescens* extracts
AChE: Acetylcholinesterase

Table 3. The butyrylcholinesterase inhibitory activity (%) of *Cantharellus lutescens* extracts

Extracts	Concentration (µg/mL)[a]				IC_{50}
	100	200	400	800	µg/mL
CLH (hexane extract)	3.11±0.10	5.01±0.05	7.07±0.05	9.83±0.03	5117.58±0.46
CLE (EtOAc extract)	21.33±1.15	27.40±0.18	28.47±0.10	29.20±0.80	5490.09±6.17
CLM (methanol extract)	8.43±0.08	9.94±0.42	12.81±0.50	17.72±0.93	2999.86±14.30
Galantamine[b]	40.59±2.88	48.73±0.90	65.02±0.44	85.42±0.31	229.45±4.62

[a]: All results ± standard deviation (three replicate studies) [b]: Galantamine; standard, IC_{50}: Inhibitory concentration

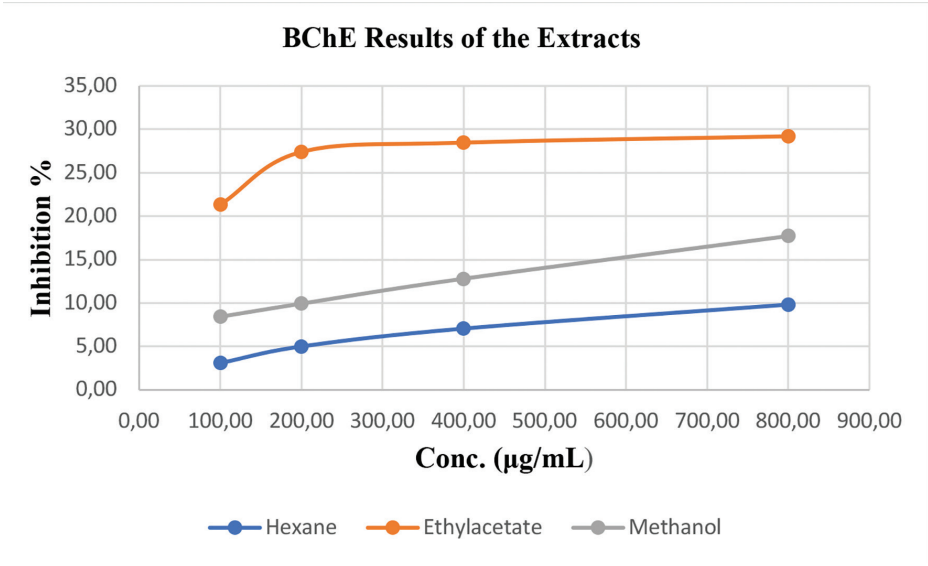

Figure 3. Butyrylcholinesterase inhibitory activity of *Cantharellus lutescens* extracts
BChE: Butyrylcholinesterase

strongest BChE inhibition (29.14±0.04%) (13). Previous studies on *C. lutescens* highlighted its anti-thrombin activity and the influence of industrial processing on antioxidant capacity (17), but its cholinesterase inhibitory potential had not been comprehensively assessed until now.

In the current study, the hexane extract of *C. lutescens* showed 48.07±0.51% AChE inhibition at 800 µg/mL, indicating substantial inhibitory potential, albeit lower than the standard drug galantamine (68.36±1.10% at 100 µg/mL). The ethylacetate extract exhibited 29.20±0.80% BChE inhibition at the same concentration, approaching the activity of galantamine (40.59±2.88% at 100 µg/mL). The methanol extract displayed higher activity than the hexane extract, while other extracts were relatively weak, suggesting that different solvent fractions concentrate distinct bioactive compounds. These observations indicate that *C. lutescens* contains selective cholinesterase inhibitors, supporting its potential as a source of multi-target agents for AD.

Mechanistically, the selective inhibition of AChE and BChE observed in different extracts may reflect the presence of specific terpenoids, phenolics, or fatty acid derivatives, as reported in other *Cantharellus* species. This aligns with the concept that combinatorial bioactivity in mushroom extracts can target multiple pathways relevant to neurodegeneration, including oxidative stress, neuroinflammation, and cholinergic dysfunction. Future studies involving bioassay-guided fractionation, compound isolation, and *in vivo* validation will be essential to identify the active constituents and confirm their therapeutic relevance.

The integration of both morphological and molecular identification of *C. lutescens* strengthens the credibility of the findings and provides a reproducible reference for subsequent studies. Overall, this study highlights the untapped potential of edible and medicinal mushrooms as sources of novel cholinesterase inhibitors, emphasizing the importance of systematic screening, solvent-specific extraction, and mechanistic evaluation in the search for effective natural therapeutics against AD.

Study Limitations

This study, limited to three extracts, offers initial evidence regarding the anti-Alzheimer potential of *C. lutescens*. The isolation and characterization of major bioactive compounds, followed by evaluation of their anticholinesterase activity as well, could provide more targeted and conclusive results.

Conclusion

As a complex disease, multiple mechanisms are thought to play a role in the occurrence and progression of AD. Cholinesterase inhibition is only one mechanism that was the object of this study. Further investigations, including assays on Aβ aggregation inhibition and anti-tau activity, complemented by *in vivo* studies, could provide a more comprehensive understanding of the therapeutic potential of this mushroom, *C. lutescens*. Moreover, as an edible mushroom, conducting cytotoxicity studies on it will be essential to support its potential future use as a therapeutic supplement.

AD is a complex disorder involving multiple pathological mechanisms. Cholinesterase inhibition, which was the focus of this study, represents only one of these pathways. To gain

a more comprehensive understanding of the therapeutic potential of *C. lutescens,* further research should include assays targeting amyloid-β aggregation and tau pathology, supported by *in vivo* studies. In addition, given that it is an edible mushroom, cytotoxicity evaluations will be crucial to confirm its safety and support its prospective use as a therapeutic supplement.

Ethics

Ethics Committee Approval: This study does not involve human participants, animal experiments, or private or sensitive data collection. Therefore, ethical approval was not needed.

Informed Consent: This study does not involve human participants, animal experiments, or private or sensitive data collection. Therefore, informed consent was not needed.

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Authorship Contributions

Concept: E.E., Design: E.E., Data Collection or Processing: E.E., H.K., Analysis or Interpretation: E.E., H.K., S.S.E., Literature Search: E.E., H.K., S.S.E., Writing: E.E., H.K., S.S.E.

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Supplementary Figures: https://d2v96fxpocvxx.cloudfront.net/a7b689d1-7b5b-481e-8a29-9bd45e97266c/content-images/25e024b6-06bb-4020-b6a0-fe3da2e00a35.pdf

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