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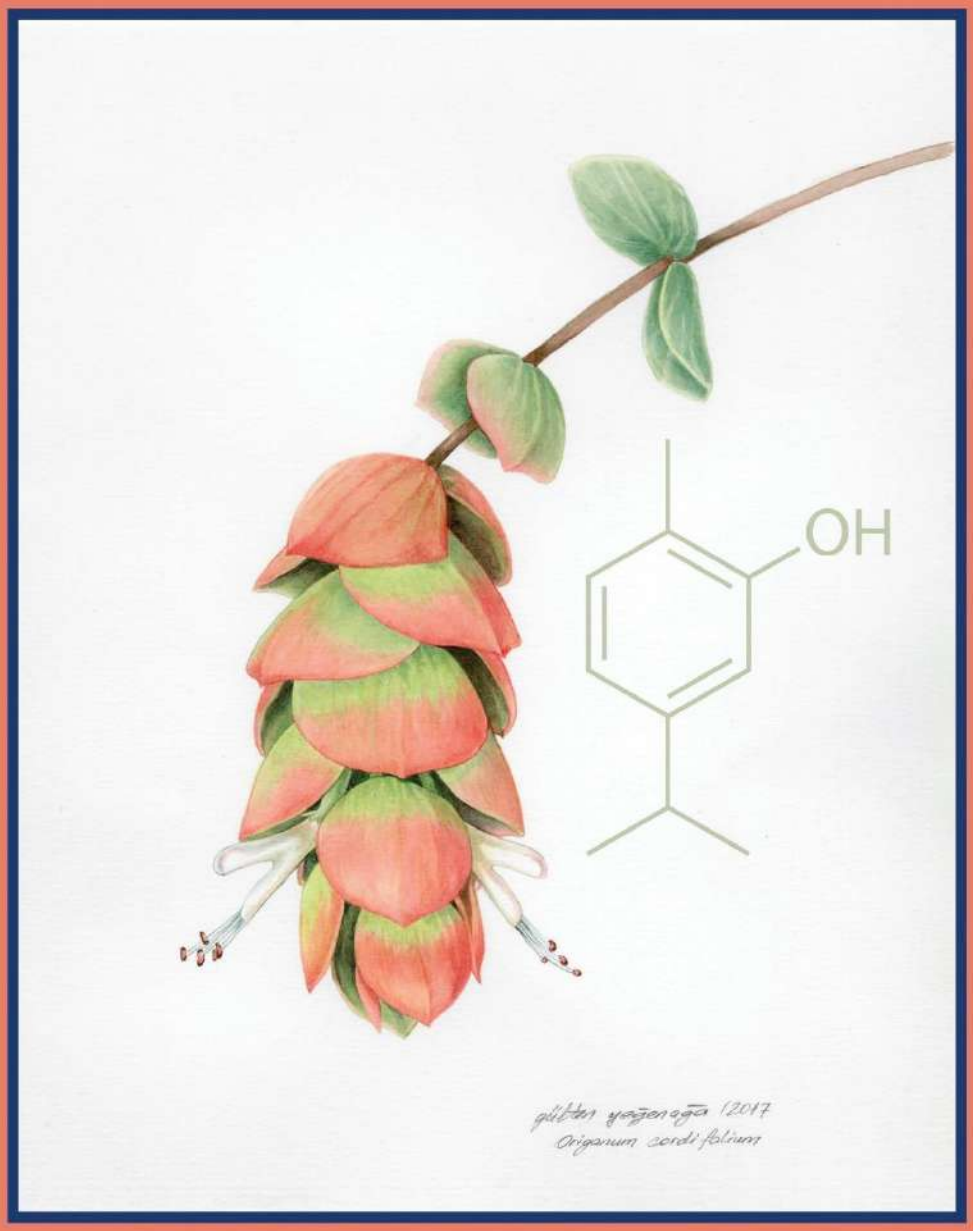

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MESSAGE FROM EDITOR IN CHIEF

Dear Colleagues,

It is our pleasure and privilege to present the first issue of the *EMU Journal of Pharmaceutical Sciences* for the year 2025. This issue features four original research articles and one review article, each contributing valuable insights to the field.

Established in 2018, our journal has consistently published three issues annually, reaching a total of 12 issues to date. We are preparing to release the second issue of 2025 soon, with the third issue scheduled for publication towards the end of the year.

EMU Journal of Pharmaceutical Sciences is an internationally oriented publication. It is indexed in DergiPark Akademik, a platform operated by the Scientific and Technological Research Council of Türkiye (TÜBİTAK). Submission guidelines and updates are available on our official website: https://dergipark.org.tr/en/pub/emujpharmsci.

Our editorial team is working diligently to have the journal included in additional international indexing databases.

We sincerely thank you in advance for your valuable contributions and continued support.

Best regards,

Prof. Dr. H. Ozan Gulcan

Dean of Faculty of Pharmacy
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Eastern Mediterranean University
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EMU Journal of Pharmaceutical Sciences (EMUJPharmSci) covers the research on all aspects of Pharmacy presented as original articles, short reports and reviews.

EMU Journal of Pharmaceutical Sciences is published three times (March, August, December) in a year. It is an open access and peer-reviewed journal.

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Original articles: These are limited to 15 typewritten pages in addition to supplementary materials (schemes, tables, figures, etc.).

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Reviews: They are limited to 20 pages in addition to supplementary materials (schemes, tables, figures, etc.).

- The original manuscript must be arranged as follows: Title page (including the title, authors and correspondence address), abstract, key words, introduction, materials and methods, results and discussion, acknowledgements and references.
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a) All manuscripts can only be submitted electronically via DergiPark.
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a) Give a brief and clear description of the materials and methods used. Subtitles can be given as appropriate.
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A combined Results and Discussion section is often appropriate. Results should be concise.

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Discussion should not repeat the results.

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D. Acknowledgement

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Electronic resources:

World Nuclear Association (WNA) (2014). Radioisotopes in Medicine, http://www.world-nuclear.org/info/ Accessed 13.10.2014.

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CONTENTS

Research articles

Reviews

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Investigation of the Stability of Acetylsalicylic Acid Solution at Different Temperatures

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Abstract

This study investigated the stability of acetylsalicylic acid (ASA) in aqueous citric acid solutions at various temperatures (21°C, 37°C, 45°C, and 60°C). The degradation of ASA was monitored using a titrimetric method to quantify its hydrolysis product, salicylic acid. Kinetic analysis based on the Arrhenius equation was performed to evaluate the stability profile of ASA under different storage conditions. The findings indicate that hydrolysis occurs even at room temperature, leading to a significant decrease in ASA concentration over time. The activation energy was calculated as 7.48 kcal, suggesting a rapid degradation process. The results highlight the instability of ASA in aqueous formulations, making it unsuitable for liquid dosage forms. To improve stability and prevent hydrolysis, alternative solvents such as propylene glycol and polyethylene glycol may be used instead of water. Additionally, microencapsulation techniques can offer a protective barrier against degradation, ensuring extended shelf life and improved pharmaceutical efficacy. These findings provide crucial insights for the formulation of stable liquid aspirin preparations and emphasize the necessity of selecting appropriate solvents and excipients in pharmaceutical development.

Keywords

Acetylsalicylic acid, arrhenius equation, citric acid, hydrolysis, stability, pharmaceutical formulation.

Article History

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INTRODUCTION

The formulation of pharmaceutical products must ensure that all characteristics comply with predefined specifications and remain within acceptable limits throughout their entire lifecycle, from manufacturing to patient use, without undergoing significant changes. Therefore, when developing a pharmaceutical product, careful consideration is given not only to the active ingredient(s) but also to all excipients and even the packaging materials to maximize stability over time. The designed dosage form should maintain the integrity of the formulation, enhance the stability of the active ingredient(s), and minimize potential interactions with other active or excipient components. Ensuring long-term stability is a critical aspect of pharmaceutical development, as it directly affects the efficacy, safety, and overall quality of the final product (González et al., 2022; Chinchole et al., 2014).

Acetylsalicylic acid, widely recognized as aspirin, is a crucial substance in pharmaceutical science due to its multifaceted therapeutic effects, including analgesic, antipyretic, anti-inflammatory, and antithrombotic properties. Its mechanism of action involves irreversible inhibition of cyclooxygenase (COX-1 and COX-2) enzymes, leading to the suppression of prostaglandin synthesis, which plays a key role in pain, fever, and inflammation. Aspirin is extensively prescribed for the treatment of various inflammatory conditions such as rheumatoid arthritis and osteoarthritis, as well as for symptomatic relief of mild to moderate pain and fever. Beyond its conventional uses, aspirin's role in cardiovascular health is well established, particularly in the prevention of thrombosis, myocardial infarction, and ischemic stroke. Recent researches also suggest its potential application in cancer chemoprevention and neurodegenerative diseases. Despite its broad clinical utility, aspirin's stability in solution remains a critical challenge, as hydrolysis leads to the formation of salicylic acid, affecting its efficacy and safety in pharmaceutical formulations (Dominiak et al., 2022; Merimi et al., 2023).

The stability of pharmaceutical compounds in solution is a critical factor affecting their efficacy, safety, and shelf life. Chemical degradation in aqueous environments can lead to loss of therapeutic activity and the formation of potentially harmful by-products. Factors such as temperature, pH, light exposure, and oxidative conditions significantly affect the stability of active pharmaceutical ingredients (APIs). Hydrolysis, oxidation, and

photodegradation are among the most common degradation pathways in solution. Hydrolysis, especially in ester- and amide-containing drugs such as acetylsalicylic acid, results from interaction with water and leads to the degradation of the parent compound to hydrolyzed derivatives. Oxidative degradation, usually catalyzed by dissolved oxygen or transition metals, can generate reactive oxygen species that accelerate the degradation of sensitive functional groups. Understanding these mechanisms and designing solutions of active substances, especially acetylsalicylic acid, as stable pharmaceutical formulations is important to optimize storage conditions and ensure consistent therapeutic performance (Kowalska et al., 2022).

Citric acid, a widely utilized weak organic acid in pharmaceutical formulations, serves as an effective buffering agent in aqueous solutions. Its primary function is to regulate and stabilize pH levels, which is crucial for preserving the integrity of pH-sensitive APIs. In solution, citric acid establishes an equilibrium with its conjugate bases (citrate ions), forming a buffer system that mitigates drastic fluctuations in pH. This buffering capability helps to reduce hydrolytic degradation, oxidative breakdown, and other pH-dependent chemical reactions that may compromise drug stability. Additionally, citric acid acts as a chelating agent by binding metal ions that can otherwise catalyze oxidative degradation. Through these combined effects, sodium citrate, a sodium salt of citric acid, can contribute to the enhanced stability of pharmaceuticals such as acetylsalicylic acid, potentially slowing its hydrolysis into salicylic acid and acetic acid, thereby extending its shelf life and therapeutic efficacy (Ciaramitaro et al., 2023).

This study focuses on evaluating the stability of acetylsalicylic acid in an aqueous sodium citrate solution under various storage temperatures. By analyzing the degradation kinetics at 21°C, 37°C, 45°C, and 60°C, the study aims to quantify the hydrolysis rate and identify the temperature at which acetylsalicylic acid demonstrates the highest instability. The results will offer valuable insights into the impact of temperature on the degradation process of acetylsalicylic acid and assess the potential role of citric acid as a stabilizing agent in liquid pharmaceutical formulations.

MATERIALS AND METHODS

Materials

Pharmaceutical-grade acetylsalicylic acid, sodium citrate, sodium hydroxide, and phenolphthalein were obtained from Sigma-Aldrich®. Purified water was used for the preparation of all solutions to ensure consistency and quality in the experimental procedures.

Methods

Preparation of acetylsalicylic acid solution

A solution containing 3.6 g of acetylsalicylic acid and 10.8 g of sodium citrate was prepared in 200 mL of purified water under continuous stirring. The mixture was stirred at room temperature using a magnetic stirrer until complete dissolution was achieved, ensuring homogeneity and stability for further analysis.

Determination of acetylsalicylic acid by titrimetric method

For the quantification of acetylsalicylic acid, 10 mL of the prepared solution was taken, and 2–3 drops of phenolphthalein indicator were added. The solution was then titrated with 0.1N NaOH solution until a persistent color change was observed, remaining stable for at least 1 minute, indicating the endpoint of the titration (Khouri et al.,2024).

The concentration of acetylsalicylic acid in the solution was calculated using the following formula.

$$Remining\ acetylsalicylic\ acid\ concentration = (2A - B)x100/A$$

A: The amount (ml) of 0.1 N NaOH consumed when acetylsalicylic acid is 100% present in the medium before hydrolysis starts.

B: It is the amount (ml) of 0.1N NaOH that neutralizes the hydrolyzed and unhydrolyzed acetylsalicylic acid in the sample after a certain time.

Stability studies

The prepared acetylsalicylic acid solution was divided into four separate beakers, each containing 50 mL of solution, and stored at 21°C (room temperature), 37°C, 45°C, and 60°C. To determine the initial concentration of acetylsalicylic acid before degradation, a sample was analyzed with titrimetric method immediately after preparation (at zero time). Subsequently, at 30, 60, 90, and 120 minutes, aliquots were taken from the solutions stored at each temperature, and the acetylsalicylic acid concentration was determined. The method allowed the assessment of the remaining stable acetylsalicylic acid concentration over time at different storage temperatures (Yenda et al., 2023).

RESULTS AND DISCUSSION

Determination of acetylsalicylic acid by titrimetric method

The concentrations (%) of acetylsalicylic acid remaining without degradation in solutions held at four different temperatures for up to 120 minutes, along with the natural logarithm (ln) values of these concentrations, are shown in Table 1.

Table 1: The concentrations of acetylsalicylic acid remaining without degradation in solutions at different temperatures.

	21 °C			37 °C			45 °C			60 °C		
t (min)	B (mL)*	C (%)*	In C	B (mL)*	C (%)*	In C	B (mL)*	C (%)*	In C	B (mL)*	C (%)*	In C
0	9.5	100	4.6	9.5	100	4.6	9.5	100	4.6	9.5	100	4.6
30	9.6	98.95	4.59	9.6	98.95	4.59	10	94.74	4.55	10.1	93.68	4.54
60	9.9	95.79	4.56	9.8	96.84	4.57	10.8	86.32	4.46	11.5	78.95	4.37
90	10.1	93.68	4.54	10.2	92.63	4.53	11	84.21	4.43	11.7	76.84	4.34
120	10.3	91.58	4.52	10.5	89.47	4.49	11.5	78.95	4.37	12.5	68.42	4.23

*B: The amount (ml) of 0.1N NaOH that

Determination of degradation equations for each temperature condition

The graphs of time (t) values as independent variables (x) and ln concentration values as dependent variables (y), were plotted as shown in Table 1. The slope (m), intercept (n), and determination coefficient (r^2) of the resulting lines were calculated. Since four different neutralizes the hydrolyzed and unhydrolyzed acetylsalicylic acid in the sample; C: The concentrations (%) of acetylsalicylic acid remaining without degradation in solutions. Temperatures were used, four separate lines were obtained. These equations are shown in Table 2 and Table 3 according to temperatures.

Table 2: First-order degradation kinetics equations at different temperatures.

Temperature (°C)	Degredation Equation
21	$y= 4.604-7.10^{-4} x$, r= -0.9922
37	$y= 4.612-9,33.10^{-4} x$, r= -0.971
45	$y=4.598-1,93.10^{-3} x$, r= -0.9906
60	$y=4.604- 3,13. 10^{-3} x$, r= -0.9815

Degradation kinetics and Arrhenius Equation

The relationship between reaction rate and temperature is defined by the Arrhenius equation below. According to Table 3, the activation energy (Ea) was calculated from the slope of the line that was calculated, and the frequency factor, which gives the frequency of molecules colliding with each other, was calculated from the intersection value. According to Table 3, the equation y = -5.3911 + 3,765.71x was obtained. Here, the Ea/R value, where R is a gas constant, can be read as 3,765.71.

Table 3: Details of the degradation equations with respect to temperatures.

Temperature °C	Temperature °K	1/T (x)	Slope (k)	In k (y)
21	294	$3{,}4.10^{-3}$	7.10^{-4}	7.26
37	310	$3{,}23.10^{-3}$	$9.3.10^{-4}$	6.98
45	318	$3{,}14.10^{-3}$	$1.9.10^{-3}$	6.25
60	333	3.10^{-3}	$3.1.10^{-3}$	5.77

CONCLUSION

The Arrhenius equation plays a crucial role in stability studies, as it allows for the prediction of the degradation rate of pharmaceutical compounds at different temperatures. By establishing a relationship between the reaction rate constant (k) and temperature (T), the equation helps to estimate a drug's shelf life and determine optimal storage conditions. In stability studies, accelerated stability testing is conducted at elevated temperatures, and the degradation rates are extrapolated to predict long-term stability at room temperature. This approach significantly reduces the time required for stability assessments while ensuring the reliability and safety of pharmaceutical products over their intended shelf life.

In the study, based on the values in Table 3, the degradation reaction was determined using the Arrhenius equation as: $y = -5.3911+3{,}765.71x$.

In this equation, the Ea/R value was found to be 3,765.71, and the activation energy (Ea) was calculated as 7.48 Kcal. It is well-known that if the activation energy of a degradation reaction is below 10 Kcal, the degradation process occurs easily. The prepared acetylsalicylic acid solution was found to be unstable at all studied temperatures (21°C, 37°C, 45°C, and 60°C), including room temperature, indicating a stability issue.

To enhance the stability of acetylsalicylic acid solutions and prevent hydrolysis, alternative solvents such as propylene glycol and polyethylene glycol can be used instead of water. Additionally, the preparation of microcapsules containing the active ingredient can create a protective barrier against degradation, making this approach a viable option for formulation development.

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Research Article EMUJPharmSci ISSN 2651-3587 https://dergipark.org.tr/emujpharmsci

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Synthesis of 7-(3-(4-(2,3-dichlorophenyl)piperazin-1-yl)propoxy)-3,4-dihydroquinolin-2(1H)-one and Screening Its Cholinesterase Inhibitor Properties

Manijeh Dehnabi, Acelya Mavideniz*, Tugba Ercetin, Hayrettin Ozan Gulcan

[1] Eastern Mediterranean University, Faculty of Pharmacy, Famagusta, North Cyprus via Mersin 10, Türkiye.

Abstract

Aripiprazole is a well-known atypical antipsychotic drug [i.e., 7-(4-(4-(2,3-dichlorophenyl)piperazin-1-yl)butoxy)-3,4-dihydroquinolin-2(1*H*)-one]. The pharmacological effects of aripiprazole are primarily attributed to its partial agonist activity at dopamine and serotonin receptors, although the exact mechanism of action remains unclear. However, it is generally accepted that aripiprazole exerts its therapeutic effects by modulating dopaminergic and serotoninergic neurotransmission. This study aimed to synthesize a modified derivative of aripiprazole and to investigate the cholinesterase inhibitory potential of this compound. The cholinesterase inhibition potential of the synthesized compound was evaluated using the Ellman's method. The inhibitory activities against various cholinesterases were determined. The results indicated that the scaffold employed might be used for further cholinesterase inhibitor molecule design.

Keywords

Alzheimer's disease, antioxidant activity, aripiprazole, cholinesterase inhibition, modified ellman's method.

Article History

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Article Info

***Corresponding author:** Acelya Mavideniz **email:** acelya.mavideniz@emu.edu.tr

Research Article:

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INTRODUCTION

Aripiprazole is an FDA (the US Food and Drug Administration)-approved atypical antipsychotic, with a unique mechanism of action. It is widely used in the treatment of schizophrenia, bipolar disorder, and major depressive disorder. While it acts as a partial agonist at D2 and D3 dopamine receptors and 5HT1A serotonin receptors, it also interacts with 5-HT2A and 5-HT2C receptors. The pharmacological effects of aripiprazole are primarily attributed to its partial agonist activity at dopamine and serotonin receptors, although the exact mechanism of action remains unclear. However, it is generally accepted that aripiprazole exerts its therapeutic effects by modulating dopaminergic and serotoninergic neurotransmission (Burris et al., 2002). Its clinical effectiveness in treating both positive and negative symptoms of schizophrenia with a lower incidence of side effects is attributed to its 'dopamine stabilization' effect and functional selectivity (Shapiro et al., 2003). Pharmacological profiling has shown that aripiprazole has moderate to high affinity for a variety of G protein-coupled receptors, ion channels, and other molecular targets. These findings support the idea that aripiprazole is not a typical partial agonist but may act as an antagonist depending on the functional context.

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by memory loss, cognitive decline, and behavioral changes which is caused by the deficit of acetylcholine in the brain. In the context of AD, cholinesterase inhibition is a key therapeutic strategy. Cholinesterases, particularly acetylcholinesterase (AChE), is a critical enzyme involved in maintaining the proper function of the nervous system in humans and other animals (Fishel, 2016). Cholinesterase inhibitors help increase acetylcholine levels, providing evidence that these agents are a viable strategy for the treatment of AD (Birks, 2006; Lane et al., 2004).

This study aimed to synthesize a modified derivative of aripiprazole with the chemical formula 7-(3-(4-(2,3-dichlorophenyl)piperazin-1-yl)propoxy)-3,4-dihydroquinolin-2(1*H*)-one and to investigate the cholinesterase inhibitory potential of the compound. The synthesis was performed in two steps. Firstly, the intermediate compound 7-(3-chloropropoxy)-3,4-dihydroquinolin-2(1*H*)-one was synthesized and this intermediate was reacted with 1-(2,3-dichlorophenyl)piperazine in the presence of K_2CO_3, acetonitrile, and sodium iodide under microwave irradiation to obtain the target aripiprazole derivative. The

cholinesterase inhibition potential of the synthesized compound was evaluated using the Ellman's method. The inhibitory activities against Electrophorus electricus acetylcholinesterase, recombinant human acetylcholinesterase, and horse serum butyrylcholinesterase were determined. At concentrations of 20 µM and 40 µM, the compound exhibited selective inhibition against the recombinant human acetylcholinesterase. The half maximal inhibitory concentrations (IC_{50}), as a key biochemical parameter, were also determined.

MATERIALS AND METHODS

Materials and equipment

7-hydroxy-3,4-dihydroquinolin-2(1*H*)-one, DMF, NaH, 1-bromo-3-chloropropane, ethyl acetate, n-hexane, potassium carbonate, sodium iodide, acetonitrile, 1-(2,3-dichlorophenyl)piperazine, were purchased from Sigma–Aldrich and were used without further purification. Thin-layer chromatography (TLC) studies were conducted on Merck aluminum-packed silica gel plates employing ethyl acetate / n-hexane as mobile phase at 2:1 ratio. For the biological activity investigations, Varioskan Flash model multi-plate reader (a thermoscientific spectrofluorometer) was employed.

Synthesis of 7-(3-chloropropoxy)-3,4-dihydroquinolin-2(1*H*)-one (X1)

A solution of 7-hydroxy-3,4-dihydroquinolin-2(1*H*)-one (3.5 g, 23.1 mmol) in anhydrous N,N-dimethylformamide (DMF, 45 mL) was prepared and added to a reaction flask. Sodium hydride (0.825 g, 69 mmol) was then added portion-wise to the solution with continuous stirring. After that, 1-bromo-3-chloropropane (5.5 mL, 57.75 mmol) was added dropwise to the reaction mixture. The mixture was then stirred at ambient temperature for 6 hours. After completion of the reaction, the precipitate was washed with ice-cold water and filtered off. The crude product was obtained as a white powder. TLC was employed both to monitor the progress of the reaction and to observe the products formed upon completion.

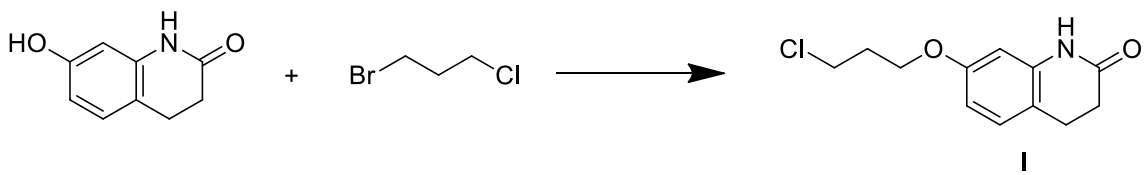

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Figure 1: The synthetic scheme of 7-(3-chloropropoxy)-3,4-dihydroquinolin-2(1*H*)-one (I).

Synthesis of 7-(3-(4-(2,3-dichlorophenyl)piperazin-1-yl)propoxy)-3,4-dihydroquinolin-2(1*H*)-one (II)

In the initial step of the synthesis, 0.43 g (1.73 mmol) of 7-(3-chloropropoxy)-3,4-dihydroquinolin-2(1*H*)-one was mixed with 2.75 mmol of potassium carbonate (K_2CO_3). Separately, 0.38 g (2.56 mmol) of sodium iodide (NaI) was dissolved in 15 ml of acetonitrile (ACN) and subsequently added to the reaction mixture. Following this, 1.2 g (5.2 mmol) of 1-(2,3-dichlorophenyl)piperazine was added. The reaction was carried out under microwave-assisted conditions at 107 °C for 30 minutes. Upon completion of the microwave irradiation, the solvent (ACN) was removed using a rotary evaporator. The residue was then treated with 20 ml of 5% K_2CO_3 solution and stirred at 60 °C for one more hour. After completion of the reaction, the crude product was obtained as a white.

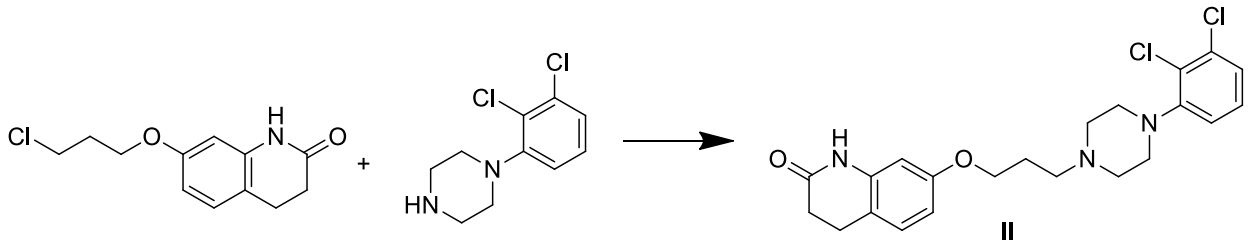

Figure 2: The synthetic scheme of 7-(3-(4-(2,3-dichlorophenyl)piperazin-1-yl)propoxy)-3,4-dihydroquinolin-2(1*H*)-one (II).

Measuring the cholinesterase inhibitor potential of II

Cholinesterase activity was assessed following the spectrophotometric method developed by Ellman et al (Ellman et al., 1961). The reaction was monitored using a Varioskan spectrophotometer equipped with SkanIt RE software, calibrated for absorbance readings at 412 nm. The test compounds were dissolved in methanol. DTNB was prepared by dissolving it in a buffer solution containing sodium chloride (NaCl) and magnesium chloride ($MgCl_2$). The reaction buffer comprised Tris(hydroxymethyl)aminomethane (Tris) and bovine serum albumin (BSA) dissolved in distilled water, adjusted to pH 8.0 to optimize enzymatic activity. Acetylcholinesterase inhibitors (AChEIs) and butyrylcholinesterase inhibitors (BChEIs) were individually dissolved in distilled water and stored in separate flacons. Different concentrations of compound were tested. Enzymatic sources included AChE from Electrophorus electricus and butyrylcholinesterase (BChE) from equine serum. All experiments were performed in triplicate to ensure reproducibility and statistical reliability.

Measuring the antioxidant activity of II

The compounds' antioxidant activity was assessed using the ORAC assay (Shukur at al 2020). Consequently, experiments were conducted at pH 7.4 in a 200 µl volume of 75 mM phosphate buffer. After that, 120 µl of fluorescein (at the final concentration of 150 nM) and 20 µl of test chemicals (at the final concentration of 10 µM) were incubated for 20 minutes at 37°C. 60 µl of 2,2C-azobis (2-methylpropionamidine) dihydrochloride (12 mM final concentration) was added to each solution in the well following the incubation period. Fluorescence readings (i.e., excitation at 485 nm and emission at 535 nm in a Thermo Scientific Varioskan Flash Multimode Reader) were recorded at 5-minute intervals for 90 minutes in order to conduct the measurements. Additionally, Trolox final concentrations ranging from 0.5 to 8 µM were employed as standards. Three duplicates of each experiment were performed. The ORAC values were represented as µmol Trolox equivalents per µmol of compounds and were computed as the difference between the areas under the fluoresceine quenching curves of the blank and the sample.

RESULTS AND DISCUSSION

The IR and 1HNMR spectrum of the compound displayed the following features: 3368 cm^{-1} (brs, N–H), 3109 cm^{-1} (aromatic C–H), 2944 cm^{-1} (aliphatic C–H), 1677 cm^{-1} (C=O), 1594-1445 cm^{-1} (aromatic C=C), 1174 cm^{-1} (C–N), 779 cm^{-1} (C–Cl). ^{1}H NMR (DMSO-d6, 400 MHz): 7.05-7.29 (m, 4H); 6.78(d, 1H); 6.57 (d, 1H); 4.11 (t, 3H); 3.48–3.40 (m, 6H); 3.34-3.27 (m, 4H); 2.85 (t, 2H); 2.50-2.45 (m, 4H);1.84 (m, 2H). The anticholinesterase and antioxidant activities obtained are displayed in Table 1.

Table 1: Biological activity results obtained.

Compound	AChE IC50 (µM)	BuChE IC50 (µM)	ORAC (µmol Trolox equivalent/µmol of test compound)
II	7.19 ± 0.4	19.20 ± 1.0	3.09 ± 0.01
Rivastigmine	11.25 ± 0.1	26.41 ± 0.9	NT*

*NT: Not tested.

Accordingly, the test compound displayed comparable cholinesterase inhibitor property with respect to the activity results obtained for rivastigmine. Moreover, the antioxidant activity has been found significant.

CONCLUSION

Aripiprazole, itself, possesses CNS penetrating properties. Therefore, the employment of a scaffold bearing drug-like properties can have important effect in novel drug design. From this perspective, within this study, aripiprazole has been modified and checked for its cholinesterase inhibitor and antioxidant activities. The results indicated that the compound tested might be a promising molecule for upcoming design works to obtain active cholinesterase inhibitor properties bearing additional activity properties.

ACKNOWLEDGEMENT

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In vitro Evaluation of Antibacterial Activity of 2-benzoxazolinone and Its Combination Interaction with Ciprofloxacin

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Abstract

In the present study, it was aimed to demonstrate the antibacterial and synergistic activities of 2-benzoxazolinone, a promising allelochemical, against *Klebsiella pneumoniae* ATCC 700603, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, and *Staphylococcus aureus* ATCC 25923 *via* broth microdilution method. 2-benzoxazolinone exhibited promising antibacterial activity against all tested bacteria with 4 mg/mL against *E. coli* and *E. faecalis*, and *K. pneumoniae* and *S. aureus* 2 mg/mL against. When used in combination with ciprofloxacin, no antagonistic activity was observed. However, the combination interaction revealed indifferent activity against all tested strains. Overall, the antibacterial data obtained from the study demonstrated that 2-benzoxazolinone can further be used as a potential antibacterial agent in the treatment of bacterial infections.

Keywords

2-benzoxazolinone, antibacterial, checkerboard, ciprofloxacin.

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INTRODUCTION

As the antimicrobial resistance crisis continues, there is an inevitable need for the development of new antimicrobial options. 2-benzoxazolinone, a secondary metabolite produced by plants, algae, and microorganisms, possess variety of pharmacological activities including antioxidant, anti-inflammatory, and anticancer (Verma and Silakari, 2018). Furthermore, it has been previously demonstrated that 2-benzoxazolinone compounds revealed promising antimicrobial activities against pathogenic microorganisms (Bravo et al., 1997; Koksal et al., 2002; Siugzdaite et al., 2024).

Koksal et al. screened the antimicrobial activity of 2-benzoxazolinone derivatives against various microorganisms. The antimicrobial results revealed promising activity against two Gram negative bacteria: *E. coli* and *Pseudomonas aeruginosa*, one Gram positive bacteria: *S. aureus*, and three fungi: *Candida albicans*, *C. krusei*, *C. parapsilosis* (Koksal et al., 2002). Additionally, novel 2-benzoxazolinone derivatives were shown to possess significant antibacterial activity against various pathogenic bacteria. The most sensitive strains against the compounds were shown to be *E. coli* and *Bacillus subtilis*. However, *S. aureus* and *Salmonella* Enteritidis were shown to be more resistant in comparison to the sensitive strains with higher MIC values (Siugzdaite et al., 2024).

In this study, we aim to evaluate the antibacterial activity of 2-benzoxazolinone, while also evaluating its combination interaction with ciprofloxacin, one of the most commonly prescribed antibiotic, against selected pathogenic bacteria.

MATERIALS AND METHODS

Inoculum preparation

The evaluation of antibacterial activity of 2-benzoxazolinone (Sigma-Aldrich) was conducted against *Klebsiella pneumoniae* ATCC 700603, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, and *Staphylococcus aureus* ATCC 25923. Each inoculum of bacteria were sub-cultured on Mueller Hinton Agar (MHA) at 37 ^{o}C for 24 hours prior to the antibacterial experiments. Each inoculum was adjusted to 0.5 McFarland (1.5 x 10^8 cfu/mL) within Mueller Hinton Broth (MHB).

Evaluation of minimum inhibitory concentration

Broth microdilution method was conducted to evaluate the antibacterial activity of 2-

benzoxazolinone (Wikler, 2006). The final concentrations of 2-benzoxazolinone ranged from 0.06 – 4 mg/mL. The highest concentration of 2-benzoxazolinone in MHB was used as regular sterility control and ciprofloxacin was used as the positive control. Incubation of 96-well plate was done at 37 ^{o}C for 18 hours. The MIC for each bacterium was accepted as the minimum concentration of 2-benzoxazolinone that clearly prevented the growth of each tested bacteria.

Evaluation of minimum bactericidal concentration

10 µL of 2-benzoxazolinone from the wells that clearly prevented the growth of each tested bacteria and was loaded onto MHA. Incubation of media was done at 37 ^{o}C for 18 hours. MBC was accepted for the lowest concentration that has completely eliminated the bacteria on the MHA.

Evaluation of the interaction of 2-benzoxazolinone with ciprofloxacin

The evaluation of the combination interaction of 2-benzoxazolinone with ciprofloxacin was done *via* checkerboard assay as previously described (Bellio et al., 2021). The final concentrations of the 2-benzoxazolinone ranged from 0.03 to 4 mg/mL, whereas ciprofloxacin ranged from 0.001 to 1 mg/L. Incubation of 96-well plate was done at 37 ^{o}C for 18 hours.

The combination interaction of the 2-benzoxazolinone and ciprofloxacin was determined *via* fractional inhibitory concentration (FIC) index calculation (FICI = A / MIC_A + B / MIC_B). 'A' and 'B' in the calculation are the MIC of each antibacterial agent in combination within a single well plate; and MIC_A and MIC_B in the calculation are the MIC of each agent individually. The interaction is accepted as synergistic if the FIC index was < 0.5; additive between 0.5-0.9; indifference between 1-4; and antagonistic if it was > 4, respectively.

Statistical analyses

The antibacterial experiments were conducted three times for each bacteria. The analyses were done *via* Students t-test using Excel.

RESULTS AND DISCUSSION

Antibacterial activity of 2-benzoxazolinone

The antibacterial activities of 2-benzoxazolinone were evaluated using microdilution method against pathogenic bacteria: *K. pneumoniae*, *E. coli*, *E. faecalis*, and *S. aureus*. MIC of each bacteria of 2-Benzoxazolinone is shown in Table 1.

Table 1: Minimum inhibitory concentrations (MICs) of 2-benzoxazolinone against tested bacteria.

Agents		Gram positive bacteria		Gram negative bacteria	
		S. aureus **ATCC 25923**	***E. faecalis*** **ATCC 29212**	***E. coli*** **ATCC 25922**	***K. pneumoniae*** **ATCC 700603**
Sample (mg/mL)	2-benzoxazolinone	2 ± 0	4 ± 0	4 ± 0	2 ± 0
Control (mg/L)	Ciprofloxacin	0. 25 ± 0	1 ± 0.	0.015 ± 0	0.06 ± 0

*Data represented as the standard error of mean (±S.E.M).

2-benzoxazolinone showed promising antibacterial activity against all tested Gram positive and Gram negative bacteria with 2 mg/mL against *S. aureus* and *K. pneumoniae*, whereas, 4 mg/mL against *E. faecalis* and *E. coli*, respectively. Furthermore, MBC assays revealed no bactericidal effect even at the highest tested concentration for each bacterial strain. However, there was a decrease in the colonies of *K. pneumoniae* treated with 4 mg/mL in comparison to 2 mg/mL of 2-benzoxazolinone as shown in Figure 1. Similarly, 2-benzoxazolinone derivatives were investigated where their antimicrobial properties demonstrated notable activity against two Gram negative bacteria: *E. coli* and *P. aeruginosa*, as well as one Gram positive bacterium: *S. aureus* (Koksal et al., 2002). In a more recent study, it was reported that novel 2-benzoxazolinone derivatives exhibited substantial antibacterial activity against various pathogenic bacterial strains. Among these, *E. coli* and *B. subtilis* were identified as the most susceptible, whereas *S. aureus* and *S. Enteritidis* displayed comparatively higher MICs (Siugzdaite et al., 2024). Furthermore, Ozalp et al. screened the antimicrobial activity of several 2-benzoxazolinone derivatives against various pathogenic microorganisms and found promising results, particularly against *E. faecalis* (Ozalp et al., 2001). These findings collectively suggest that 2-benzoxazolinone hold promise as potential leads for the development of new antibacterial agents.

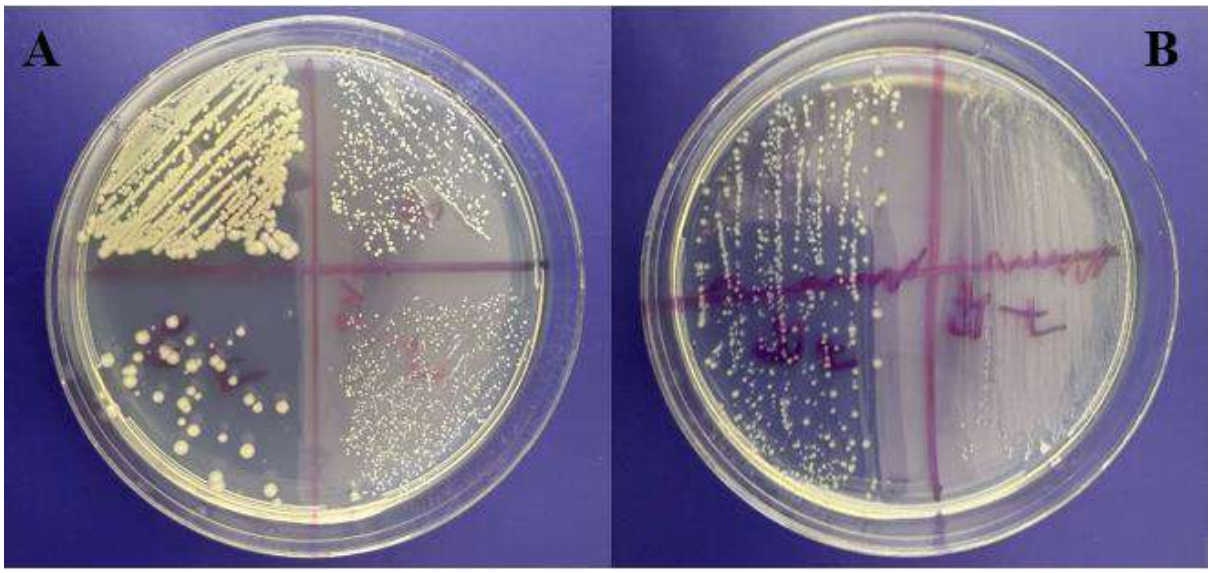

Figure 1: Minimum bactericidal concentration (MBC) results of 2-benzoxazolinone from the wells that clearly prevented the growth of each tested bacteria. **A-** 4 mg/mL (*bottom left*) and 2 mg/mL (*top left*) of 2-benzoxazolinone against *K. pneumoniae*; 4 mg/mL (*bottom right*) and 2 mg/mL (*top right*) against *S. aureus*. **B-** 4 mg/mL (*left*) of 2-benzoxazolinone against *E. coli*; 4 mg/mL (*right*) against *E. faecalis*.

Furthermore, when binary combinations of 2-benzoxazolinone and ciprofloxacin were tested against selected bacteria, no antagonistic activity was observed as shown in Table 2. Furthermore, all of the results revealed indifference effect against tested bacterial strains.

Table 2: Fractional inhibitory concentration index (FICI) of 2-benzoxazolinone and ciprofloxacin combinations against tested bacteria.

Samples	Best Combination		FICI Index	
	Ciprofloxacin (mg/L)	2-benzoxazolinone (mg/mL)	< 0.5	> 0.5
S. aureus	0.001	2		1.004 (I)
E. faecalis	0.001	4		1.001 (I)
E. coli	0.001	4		1.067 (I)
K. pneumoniae	0.001	2		1.017 (I)

I: Indifference.

CONCLUSION

2-benzoxazolinone, a promising allelochemical, is known for its antioxidant, anti-inflammatory, and anticancer properties. In addition, antimicrobial screenings with the compound and its derivatives revealed promising antimicrobial activities against various pathogenic microorganisms. In our study, the MIC values were determined were promising against all tested Gram positive and Gram negative bacteria. Furthermore, the combination of the compound with ciprofloxacin revealed no antagonistic activity against all tested strains.

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Microscopic Evaluation and Qualitative Phytochemical Screening of Leaves and Fruits of *Lycium ferocissimum* Miers.

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Abstract

Lycium ferocissimum Miers. is a plant species belonging to the Solanaceae family. This study aimed to investigate the microscopic structure and phytochemical composition of the leaf, green fruit, and red fruit of *L. ferrocissimum*. Plant materials were oven-dried at 50 °C, powdered, and prepared for microscopic analysis using chloral hydrate. Microscopy revealed stomata, epidermis, crystals, and secretory hairs in leaves; and parenchyma, pigment cells, and stone cells in fruits. Phytochemical screening was conducted using standard biochemical reactions for primary and secondary metabolites. Alkaloids were confirmed by Dragendorff's test, producing a reddish-brown precipitate. Carbohydrates were detected using Fehling, Molisch, and Seliwanoff tests, all giving positive results. Flavonoid glycosides were identified via Cyanidin test; red fruit gave a strong orange color and zinc confirmed the presence of flavonoid. Lipid detection using Sudan III resulted in orange spots in both red and green fruits. Carotenoids were identified by pH-dependent color changes. No saponins were detected.

Keywords

Lycium ferocissimum, phytochemical analysis, microscopy, flavonoids, medicinal plant

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INTRODUCTION

The genus *Lycium*, belonging to the Solanaceae family, grows in temperate and subtropical climatic regions. Approximately 80 species of the genus have been identified globally. *Lycium* species are distributed across South America, South Africa, Europe, and Asia (Yao et al., 2011). The genus holds significant importance in Traditional Chinese Medicine. In particular, two species, *Lycium barbarum* and *Lycium chinense*, have been widely used for centuries as traditional medicinal plants in China, with *L. barbarum* being commonly cultivated there. The parts of *Lycium* species that are used both as food and medicine are primarily the fruits. In addition, the leaves and root bark are also utilized, and in some cases, the whole plant is used. The fruits are typically consumed fresh or dried, while the fresh leaves are either cooked as food or brewed as tea. Among the most frequently reported species in the literature are *L. barbarum*, *L. chinense*, and *Lycium ruthenicum* from China; *Lycium europaeum*, *Lycium intricatum*, and *Lycium shawii* from the Mediterranean and the Middle East; *Lycium pallidum* from North America; and *Lycium afrum* from Africa (Yao et al., 2018). The traditional uses of *Lycium* species among the public include the treatment of eye diseases, cough (Lev and Amar, 2006), digestive and gastric inflammations (Trillo et al., 2010), headache, rheumatism, kidney disorders (Dhar et al., 2011), toothache, and chickenpox (Yao, 2018).

The traditional uses of *Lycium* species have attracted the attention of many researchers. Numerous phytochemical studies have been conducted on these species. These studies have revealed that *Lycium* species contain polysaccharides, lipids, terpenes, and phenolic compounds, with a particular focus on the types and effects of polysaccharides (Jiang et al., 2021). The chemical constituents reported in *Lycium* species include glycerogalactolipids, phenylpropanoids, coumarins, lignans, flavonoids, amides, alkaloids, anthraquinones, organic acids, terpenoids, sterols, steroids, and their derivatives (Qian et al., 2017). Phytochemical studies on *Lycium* species have identified various chemical constituents, including alkaloids, cyclopeptides, lignans, anthraquinones, coumarins, flavonoids, terpenoids, sterols, and others (Yang et al., 2018). In *L. barbarum* fruits, derivatives of luteolin such as luteolin-7-*O*-glucuronide, luteolin-7-*O*-glucoside, apigenin including apigenin-7-*O*-glucoside and acacetin have been detected (Zhang et al., 2019). The fruits also contain quercetin diglycoside, rutin, kaempferol-*O*-rutinoside, and

phenolic acids such as chlorogenic, caffeoylquinic, and caffeic acids (Wu et al., 2012). In a study carried out on the root bark and leaves of *L. barbarum*, N-trans-caffeoylphenethylamine, N-trans-caffeoyltryptamine, and N-trans-feruloylphenethylamine were identified in the root bark and it was reported that the leaves contained N-trans-caffeoyltyramine and N-trans-feruloylphenethylamine (Wang et al., 2018). Compounds isolated from *L. chinense* include acacetin, apigenin, kaempferide, caffeic acid, luteolin, and vanillic acid, along with flavonoids such as kaempferol and isoscopoletin (Chen et al., 2016). An 80% ethanol extract from the leaves and stems of *L. chinense* also revealed several phenolic compounds, including gallic acid, catechin, chlorogenic acid, and rutin (Liu et al., 2017).

MATERIALS AND METHODS

Plant Material and Extraction

Mature (unripe) and ripe fruits, as well as leaf samples of *Lycium ferocissimum*, were collected from the Salamis region of Northern Cyprus, Famagusta. The collected plant materials were dried, ground into powder, and extracted with 80% ethanol using a shaker at room temperature. Additionally, 5% aqueous decoctions were prepared from each plant part. The resulting extracts were concentrated using a rotary evaporator, water was removed with freeze drying method and stored at −18°C until further analysis. The extracts were labeled as follows: GFE for mature fruit 80% ethanol extract, GFD for mature fruit water extract (decoction), RFE for ripe fruit 80% ethanol extract, RFD for ripe fruit water extract (decoction), LE for leaf 80% ethanol extract, and LD for leaf water extract (decoction).

Microscopic Evaluation of *Lycium ferocissimum* Miers.

For microscopic analysis, leaf, green fruit, and red fruit samples were dried in an oven at 50°C. After one day of drying, each sample was separately ground using a mortar and pestle. The ground samples were then transferred to three microscope slides using needles, and a few drops of chloral hydrate were added as a reagent to enhance clarity for microscopic observation. A cover slip was placed on top of each specimen. The microscope slides were then heated using a Bunsen burner to fix the samples. The slides were then examined under the microscope one by one to observe and document the particulate structures.

Phytochemical Screening of *Lycium ferocissimum* Miers.

Test for Alkaloids

A 60% hydrochloric acid ethanol solution (in 6% HCl, 20 mL) was prepared, and 0.5

g of dried leaves and 1.8 g of fruits were added into the solution. The mixture was then boiled and filtered. For the alkaloid extraction, a liquid-liquid extraction method was employed. First, a basic solution (10% ammonium hydroxide) was added, and the pH was checked using litmus paper. The alkaloids were then extracted from the aqueous layer by adding 10 mL chloroform. The resulting extraction solution was treated with 10% acetic acid, 10 mL of water, and 1.5 mL of acetic acid. To separate the acetic acid from the upper layer, the bottom layer was discarded. Dragendorff's reagent was subsequently added to test for alkaloids. The presence of alkaloids was indicated by the formation of an orange or reddish-brown color precipitation.

Test for Carbohydrates

Seven grams of the plant sample were weighed and finely crushed using a mortar and pestle, after which the powder was transferred into a beaker. Forty milliliters of distilled water were added to the crushed plant material in the beaker and stirred for 5 minutes. The aqueous portion of the suspension was then filtered. To the filtrate, 3.5 mL of 10% lead acetate solution was added dropwise, and the mixture was re-filtered. This step resulted in the precipitation and separation of compounds such as chlorophyll, flavonoids, and tannins from the aqueous extract. Subsequently, 4 mL of 2.5% disodium hydrogen phosphate solution was added dropwise to the filtrate, and the solution was filtered once more. The obtained extract was used for the subsequent determination tests.

Fehling's Test: One milliliter of the extract was placed in a test tube, followed by the addition of 2 mL of Fehling A solution and 2 mL of Fehling B solution. The mixture was heated using a Bunsen burner. The formation of a red-colored precipitate (Cu_2O) indicates the presence of carbohydrates.

Molisch's Test: To 1 mL of the extract, 5 drops of 5% alcoholic α-naphthol solution were added in a test tube. The tube was slightly tilted, and concentrated sulfuric acid was carefully added down the side of the tube. The formation of a violet-purple ring confirms the presence of carbohydrates.

Seliwanoff's Test: To 1 mL of the extract, 2.5 mL of Seliwanoff reagent was added in a test tube. The solution was then heated to boiling. The presence of a ketose is indicated by the formation of a red color, while aldoses produce a delayed reaction and form a light red color. Saccharides such as pentoses result in a blue-green coloration.

Test for Carotenoids

The soluble components of the plant sample were extracted by dissolving the material in ethanol or methanol. If the sample was in

solid form, it was ground using a mortar and pestle or subjected to ultrasonic treatment to accelerate the extraction process. The resulting extract was divided into three equal portions and transferred into separate test tubes. To the first and second test tube, 1% hydrochloric acid (HCl) and 1% sodium hydroxide (NaOH) was added, respectively. The third test tube was left untreated and served as a control. Solutions containing anthocyanins exhibited a red or pink coloration under acidic conditions (HCl) and a green or blue coloration under basic conditions (NaOH). In instances where carotenoids were present and oxidized into anthocyanin-like compounds, similar color changes were also observed.

Test for Flavonoids

Two grams each of red and green fruits were accurately weighed and subsequently soaked to facilitate the extraction of soluble compounds. The samples were then homogenized with 5 mL of ethanol to ensure the efficient dissolution of target metabolites. The resulting mixture was filtered to separate the solid residues from the liquid extract. To isolate the flavonoid components, ethyl acetate was added to the filtrate using a separation funnel, allowing for phase separation. The upper organic layer, enriched with flavonoids, was collected and evaporated under controlled conditions to obtain a concentrated residue. The residue was subsequently re-dissolved in 2–3 drops of distilled water for further analysis. A solvent mixture of methanol and water (1:1:1) was prepared and added to the solution, followed by the introduction of a small quantity of magnesium or zinc powder. Color change observed.

Test for Lipids

Sudan III solution was used as a reagent to visualize lipids and plant fats. To prepare the solution, 0.25 g of Sudan III powder was weighed, and 35 mL of ethanol along with 15 mL of water were added to a beaker. The mixture was stirred thoroughly and heated until a homogeneous solution was obtained. Subsequently, 1 g of dried powder from red and green leaves was weighed and transferred into separate test tubes. To each tube, 10 mL of hexane was added, and the mixtures were stirred using a magnetic stirrer for 5–10 minutes. Following stirring, the mixtures were filtered, and the resulting filtrates were subjected to gentle heating. A portion of each filtered solution was then applied onto individual filter papers, which were left to dry completely. Once dried, the filter papers were sprayed with the prepared Sudan III solution for lipid detection.

Test for Saponins

Foam Test: Approximately 0.5 g of the powdered plant material was placed into a test tube containing 10 mL of hot water. After allowing the mixture to cool to room temperature, it was shaken vigorously for about 10 seconds. The formation of a stable

foam layer measuring between 1 to 10 cm in height, which persists for at least 10 minutes and remains unaffected by the addition of 1–2 drops of 2N HCl, indicates the presence of saponins.

RESULTS

Microscopic Evaluation of *Lycium ferocissimum* Miers

The powdered leaf sample was prepared using chloral hydrate as a mounting reagent. Microscopic examination revealed the presence of various anatomical structures, including stoma, epidermis, calcium oxalate crystals, covering hair and secretory hair (Figure 1).

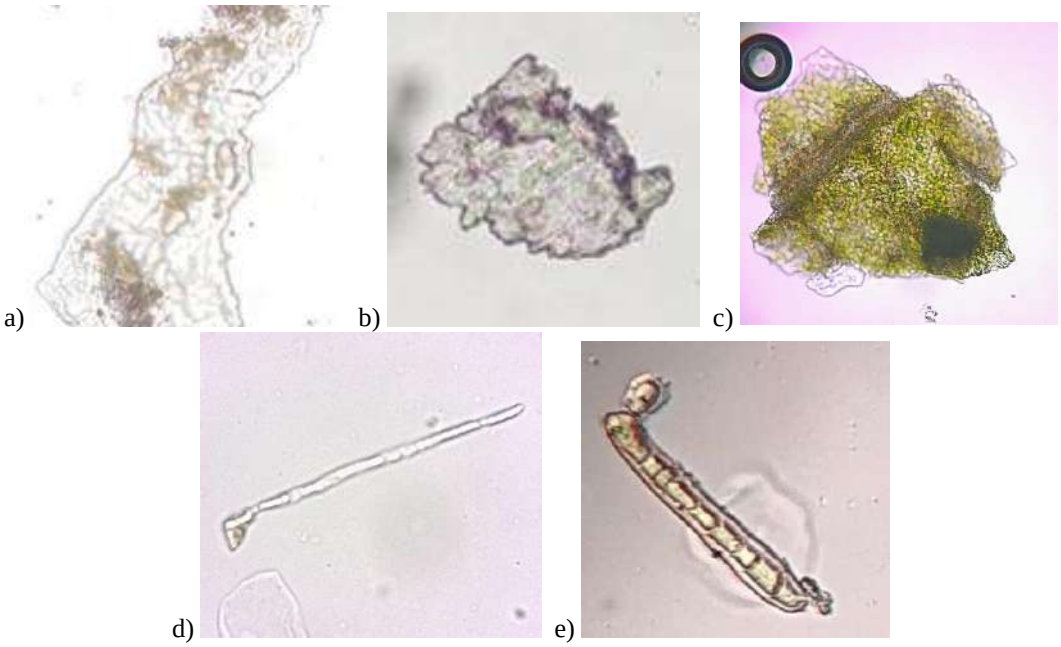

Figure 1: Microscopic images of *L. ferocissimum* leaves (a: stoma, b: epidermis, c: calcium oxalate crystals, d: covering hair, e: secretory hair).

Microscopic examination of the green fruit revealed the presence of various anatomical structures, including parenchyma, stone cells, pigment cells and exocarp (Figure 2).

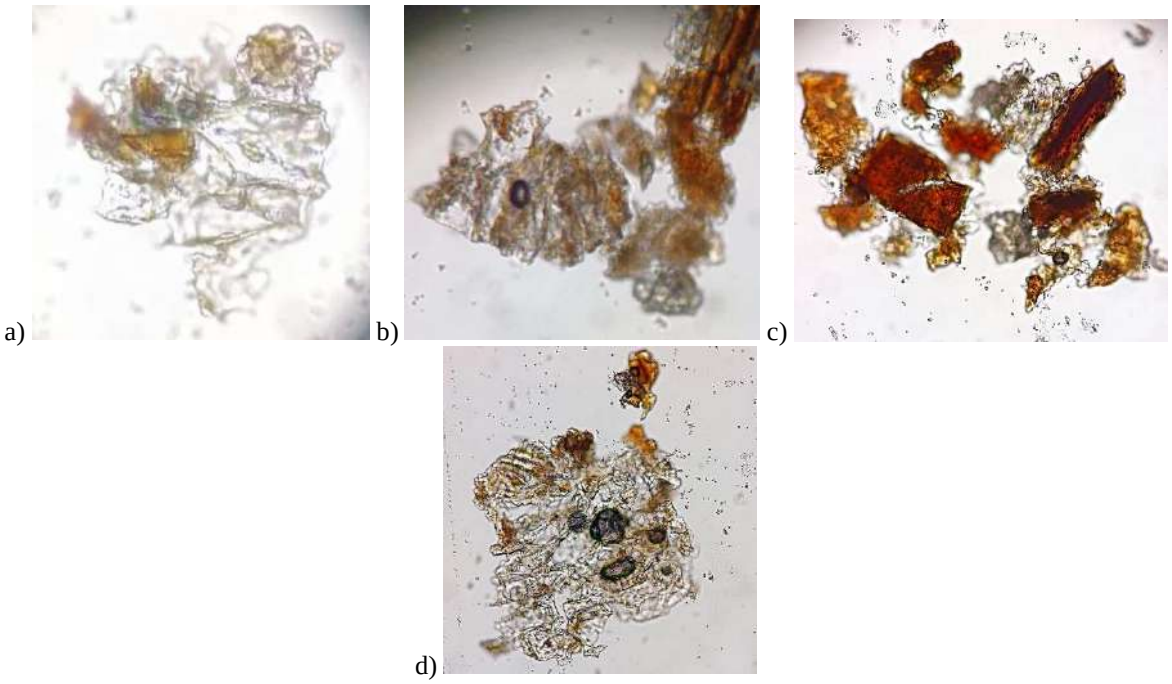

Figure 2. Microscopic images of *L. ferocissimum* green fruits (a: parenchyma, b: stone cells, c: pigment cells, d: exocarp).

Various anatomical structures, including endocarp, stone cells and exocarp were observed as a result of microsvopic examination of red fruit samples (Figure 3).

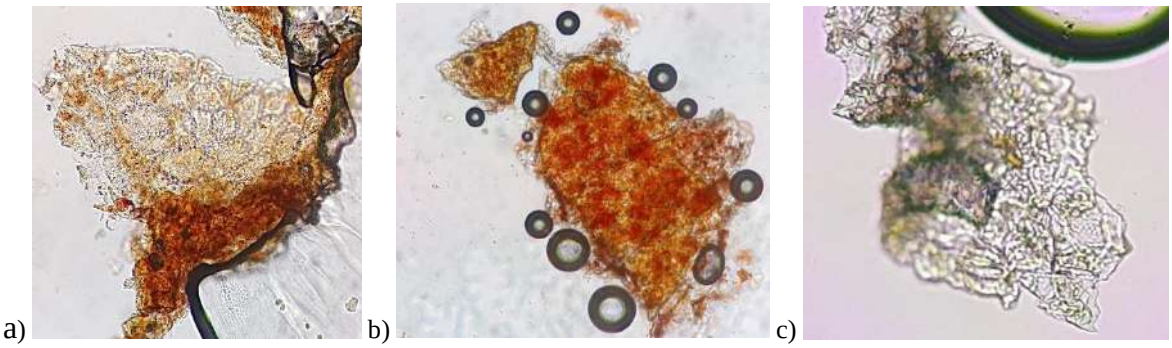

Figure 3. Microscopic images of *L. ferocissimum* red fruits (a: endocarp, b: stone cells, c: exocarp)

Phytochemical Screening of *Lycium ferocissimum* Miers

Phytochemical screening was conducted to identify novel sources of bioactive compounds with potential therapeutic and industrial applications, particularly those derived from medicinal plants. Both primary and secondary metabolites were qualitatively analyzed. The phytochemical analysis demonstrated the presence of several active constituents, including alkaloids, carbohydrates, carotenoids, flavonoids, and lipids (Table 1).

Table 1: Qualitative phytochemical screening of *L. ferocissimum* leaves, green fruits, and red fruits.

Primary/Secondary metabolites	Leaf	Green Fruit	Red Fruit	Name of tests
Alkaloids	+	+	+	Precipitation test (Dragendorff reagent)
Carbohydrates	+	+	+	Fehling test, Molisch test, Seliwanoff test
Carotenoids	ND*	+	+	Anthocyanin test
Flavonoids	ND*	+	+	Cyanidin test
Lipids	ND*	+	+	Sudan III test
Saponins	-	-	-	Foam test

*ND: Not determined

DISCUSSION

In the present study including microscopic examination of the red fruits of *Lycium barbarum*, anatomical structures such as the epidermis, stomata, hypodermis, mesocarp, cuticle, endocarp, and endosperm were investigated (Konarska, 2018). In another study conducted on 11 *Lycium* species, leaf analysis was performed using microscopy. The examined anatomical features included calcium oxalate crystals, glandular and non-glandular trichomes, palisade parenchyma, spongy parenchyma, stomata, and epidermal cells (Joubert et al., 1984). A comparative anatomical investigation of *Lycium europaeum*, *Lycium shawii*, and *Lycium schweinfurthii* var. *aschersonii* focused on several structural features, including the adaxial and abaxial epidermal layers, type of mesophyll, presence of crystals, the number of palisade and spongy parenchyma layers, and the organization of the vascular system (Ragab et al., 2023).

In our previous study with *L. ferocissimum*, phenolic acids and flavonoids were detected from leaves and fruits (Kosar et al., 2024). In another study investigating the leaf and stem extracts of *Lycium ruthenicum*, the presence of 69 compounds, including steroids, terpenes, fatty acids, esters, phenolics, aldehydes, furans, and pyridine derivatives were indicated (Kumar et al., 2022). Studies conducted on *Lycium* species have identified the presence of various phytochemicals, including alkaloids, flavonoids, carotenoids, polysaccharides, terpenes, lignans, coumarins, and anthraquinones (Yao et al., 2011). Because, only a limited number of microscopic and phytochemical investigations have been carried out on this species, such analyses are crucial for accurate identification and characterization of plant taxa. Further comprehensive studies are required to uncover additional properties of *L. ferocissimum*.

CONCLUSION

In conclusion, the present study provides insight into the characteristics and phytochemical profile of *L. ferocissimum* leaves, green fruits, and red fruits. Future research focusing on the pharmacognostic analysis of the leaves may establish specific diagnostic criteria for precise identification of the species.

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Use of Medicinal Plants in Alzheimer's Disease

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Abstract

Alzheimer's disease (AD) is a chronic and progressively worsening condition that is primarily characterized by the loss of synapses and neurons in various regions of the brain, particularly in the hippocampus. The prevalence of Alzheimer's disease has increased due to the growing elderly population. The disease is triggered by a combination of molecular, genetic, and other risk factors. Key molecular contributors include amyloid-beta plaque accumulation and the hyperphosphorylation of tau proteins. These pathological changes lead to significant brain alterations, such as atherosclerotic changes, neurofibrillary tangles, neuritic plaques, synaptic and neuronal loss, granulovacuolar degeneration, and cholinergic cell loss. Therefore, the primary approach in AD treatment focuses on inhibiting amyloid-beta accumulation and tau protein hyperphosphorylation. Additionally, stimulation of the cholinergic system is considered a key therapeutic strategy. In the present study, 18 medicinal plants were reviewed based on their potential effects on AD. These plants are valuable drug candidates with various pharmacological properties, including antiplatelet, antioxidant, antidepressant, inhibition of amyloid-beta accumulation, cholinergic system stimulation, and neuroprotective effects, all of which are crucial in AD management. Due to the limited understanding of the disease's exact causes, phytotherapy is currently used only to decelerate the progression of the disease or provide preventive benefits rather than as a definitive cure.

Keywords

Alzheimer's Disease, brain disorders, hippocampus, medicinal plant, phytotherapy.

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INTRODUCTION

Neuropathologist Alois Alzheimer was the first to identify Alzheimer's disease (AD) in a 51-year-old patient named Auguste Deter. Clinical findings in Deter included memory impairment, disorientation, and difficulty with reading and writing. As the disease progressed, cognitive decline worsened, accompanied by hallucinations. After Deter's passing in 1906, Alois Alzheimer conducted a microscopic examination of her brain, leading to the discovery of the pathological hallmarks of the disease (Graeber, 1998).

AD is a genetic, irreversible, progressive disorder of central nervous system (Graeber, 1998). These pathological evidences include atherosclerotic changes, neurofibrillary tangles, neurotic plaques, loss of synapses, loss of neurons, degeneration of granulovacuolar and cholinergic cells in the basal nucleus (Sheppard, 2020).

The brain is a crucial part of the nervous system and is divided into three main regions: the forebrain, midbrain, and hindbrain. The forebrain, located at the front and upper part of the brain, includes the thalamus, hypothalamus, limbic system, cerebral cortex, and basal ganglia. The limbic system plays a key role in motivation, learning, memory, and emotions, making it particularly important in AD, where memory impairment and distortion are common. In AD, memory loss is a significant clinical concern (Hopper, 1976). Momentarily confuse of routes which are familiar from before and placing stuffs in locations which are inappropriate are some examples of events which are reported frequently by the patients and their caregivers (Monacelli, 2003). By comprising three interconnected central cerebral structures (the septum, amygdale, and hippocampus), limbic system allows adaptation to a changing environment (Hopper, 1976). The medial temporal lobes (MTL), particularly the hippocampus, play a crucial role in episodic memory function. AD, the most common form of amnesia, is directly associated with lesions in these structures. While other cortical regions of the brain, which are involved in a wide variety of functions, also contribute to AD, recent studies focusing on the anatomical basis of memory impairment have primarily concentrated on the MTL, especially the hippocampus (Wolk, 2011). The hippocampus is closely linked to memory, particularly short-term memory, which refers to the ability to store new information. Therefore, regardless of the underlying mechanism, without the presence of both the left and right hippocampus, it is impossible to

consolidate long-term memories. The right hippocampus is primarily involved in visual memory, while the left hippocampus is more active in verbal memory. Lesions in these regions can lead to the loss of the corresponding types of memory. Scientists widely agree that the hippocampus plays a crucial role in forming new memories, and some view the hipocampus as part of a broader memory system located within the medial temporal lobe. Episodic memory, which records thousands of daily experiences, is heavily influenced by the extensive network of connections within the hippocampal region. Significant damage to the hippocampus can severely impair the formation of new memories, while memories formed before the damage often remain affected, especially those from the few years leading up to the event (Izci, 2015).

A healthy vascular system is essential for the normal functioning of the brain. Vascular changes in the central nervous system can trigger and accelerate neurodegeneration, loss of brain connectivity, and neuronal injury. The human brain is a highly complex structure, containing approximately 644 kilometers of blood vessels that supply brain cells with nutrients, oxygen, and energy metabolites. These blood vessels also remove metabolic waste products and carbon dioxide from the brain, transferring them to the systemic circulation. The smallest blood vessels in the brain, the capillaries, form the majority of the blood-brain barrier (BBB) and account for about 85% of the total cerebral vessel length. The BBB plays a crucial role in regulating the chemical composition of the brain's interstitial fluid. Therefore, maintaining the integrity of the BBB is vital for neuronal connectivity, information processing, and synaptic function. When the BBB is compromised, vascular permeability increases, leading to reduced cerebral blood flow and impaired hemodynamic responses. Furthermore, when the BBB breaks down, toxic blood-derived molecules, microbial agents, and immune cells can enter the brain, triggering immune and inflammatory responses that may initiate neurodegeneration (Sweeney et al, 2017).

In a comparison between AD patients and the patients who are healthy, it is observed that Alzheimer’s patients have *amyloid plaque accumulations*, *cortical superficial siderosis*, and *lobar microbleeds* in their brain (Sweeney et al, 2017).

In the literature, the risk factors for AD include age, family history, genetic factors, Down syndrome, gender, socioeconomic status, education level, occupation, mental activity, physical activity, smoking, alcohol consumption, exposure to neurotoxins, head trauma, diet and nutrition, dyslipidemia, cardiovascular diseases,

vascular factors, hypertension, depression, stress, and elevated homocysteine levels (Anstey, 2008).

Treatment of Alzheimer's Disease

Currently, there is no adequate or complete prophylactic treatment for AD, as its pathogenesis and pathophysiology are still not fully understood. Treatment choices should be based on the patient's cognitive abilities, any psychiatric disorders that develop during the course of the disease, and the education level of both the patient and caregiver, with the primary goal being the protection and improvement of quality of life. The main issue in AD is the loss of neurons and reduced cholinergic system activity. Enhancing cholinergic activity can improve the patient's quality of life and slow the progression of the disease; however, neuronal loss cannot be reversed (Hampel, 2018).

Cholinergic drugs, such as acetylcholinesterase inhibitors, cholinergic agonists, and muscarinic receptor agonists are commonly used in the conventional treatment of AD. Additionally, drugs with metabolic and vascular effects, including antioxidants and nootropics, are also prescribed for patients. In conventional therapy, other medications, such as amyloid-beta inhibitors, monoamine oxidase (MAO) inhibitors, and nonsteroidal anti-inflammatory drugs (NSAIDs), may also be utilized.

Plants used for Alzheimer's Disease

***Acorus calamus* L.**

Usage part: Rhizome.

Chemical composition: Acorenone, galgravin, epieudesmin (Balakumbahan et al, 2010).

Effects of plant on AD: Galgravin which is found in the chemical composition of *A. calamus* show neuroprotective activity by preventing the death of neurons and by stimulating the growth of neurons (Balakumbahan et al, 2010). It is used to treat the loss of memory. (Shukla et al, 2002)

***Angelica archangelica* L.**

Usage part of plant: Root.

Chemical composition: α-pinene, δ-3-carene, limonene, sabinene (Nivinskiene, 2003).

Effects of plant on AD: It shows inhibitory activity on acetylcholine esterase enzyme, thus increases acetylcholine amount in brain which is necessary in cerebral diseases especially in AD (Dastmalchi et al, 2007).

***Convolvulus pluricaulis* Chois.**

Usage part of plant: Whole plant.

Chemical composition: Steroids, flavonol glycosides, triterpenoids, and anthocyanins (Akram et al, 2017).

Effects of plant on AD: It regulates the production of the stress hormones of the body. This property leads to calm nerves,

that's why patient have memory- enhancing ability and it is used as nootropic. It shows cognitive and memory function improvement (Rao, 2012).

***Crocus sativus* L.**

Usage part of plant: Stigma.

Chemical composition: Crocin, crocetin (carotenoids) (Dastmalchi et al, 2007).

Effects of plant on AD: When fibrils of amyloid-beta peptides are oxidized, fibrilogenesis has been formed. The carotenoid contents of *C. sativus* inhibit the amyloid-beta fibrilogenesis (Bhargava, 2011).

Impaired plasticity of hippocampal synapses are inhibited by crocin content. Thus, impairment of memory and learning improved (Dastmalchi et al, 2007).

***Curcuma longa* L.**

Usage part of plant: Rhizomes.

Chemical composition: Curcumin, turmerin, and turmerone (Rao et al, 2012).

Effects of plant on AD: Curcumin has ability to inhibit spontaneous formation of amyloid-beta by binding to it. Thus, deposition of the plaques in the brain has been reduced. By its strong antioxidant and anti-inflammatory action, pathology of amyloid and oxidative stress are significantly reduced (Akram et al, 2017).

***Ginkgo biloba* L.**

Usage part of plant: Leaf.

Chemical composition: Gingko-flavone glycosides and terpenoids (Diamond et al, 2013).

Effects of plant on AD: It is an antioxidant that has a strong action against AD. It is neuroprotective, activation of caspase-3 and aggregation of amyloid beta are inhibited (Vellas et al, 2012). Due to its anti-platelet activity, it facilitates blood flow and enhances supply of blood to the brain which improves cognitive performance and functioning and this ability is one of the most significant advantages of *Ginkgo biloba* (Dastmalchi et al, 2007).

***Huperzia serrata* Trevis.**

Usage part of plant: Whole plant.

Chemical composition: Huperzine A (Dong et al, 2014).

Effects of plant on AD: Huperzine A is a potent acetylcholine esterase inhibitor that increases cholinergic neurotransmission without showing any toxicity to cholinergic system. Such an ability improves memory and learning functions (Ferreira et al, 2016). Huperzine A can also regulate protein kinase C, promote nonamyloidogenic pathway of APP metabolism and reduce alpha-beta amyloid production. Therefore, it can modulate APP's processing and improve memory functions (Wu et al, 2011).

***Hypericum perforatum* L.**

Usage part of plant: Aerial part.

Chemical composition: Hypericin and hyperforin, kaempherol, and quercetin (Silva et al, 2005).

Effects of plant on AD: Due to its hypericin and hyperforin content, *H. perforatum* shows antioxidant and anti-inflammatory action. Thus, it has neuroprotective activity. Acetylcholine degradation rate can be reduced by *H. perforatum* leading to improvement in learning and memory impairments (Dastmalchi et al, 2007). Researches have shown that hypericin and hyperforin are the major constituents for antidepressant activity of St. John's Wort which is very important case during the progression of AD (Barnes et al, 2001).

***Ilex paraguariensis* A. St.-Hil.**

Usage part of plant: Leaf.

Chemical composition: Purine alkaloids, flavonoids, cinnamic acid hydroxylated derivatives, and caffeoyl derivatives (Isolabella, 2010). Vitamin A, vitamin B complex, vitamin C, and vitamin E were also identified (Bracesco, 2011).

Effects of plant on AD: Development of neurodegenerative disease risk is reduced due to the antioxidant activity (Bortoli, 2018). It is a good memory enhancer that improves both short- and long-term memory (Akram, 2017).

***Melissa officinalis* L.**

Usage part of plant: Leaf.

Chemical composition: Monoterpenes that are oxygenated, polyphenolic substances, and sesquiterpene hydrocarbons, citral, citronellal, naringin, and caffeic acid. (Dastmalchi et al, 2007; Moradkhani et al, 2010)

Effects of plant on AD: *M. officinalis* has a neuroprotective action by treating PC12 cells that protects them from H_2O_2 toxicity. With antioxidant property, it prevents oxidative stress and amyloid-beta toxicity. Citral inhibits acetylcholine esterase enzyme and improves mood, memory, and cognition (Shakeri et al, 2016).

***Myristica fragrans* Houtt.**

Usage part of plant: Seed.

Chemical composition: Myristicin, eugenol, elimicin, and safrole (Akram et al, 2017).

Effects of plant on AD: By its antioxidant and antidepressant properties, *M. fragrans* is a very useful plant in the management of AD. It also acts as a potent acetylcholine esterase enzyme inhibitor that enhances memory and learning (Cuong et al, 2019).

***Panax ginseng* C.A.May.**

Usage part of plant: Root.

Chemical composition: Panaxadiol, protopanaxadiol, and protopanaxatriol (Ginsenosides) (Choi, 2008).

Effects of plant on AD: With its ginsenoside contents, *P. ginseng* supports cognitive

performance, increases ability of learning, attention and memory (Choi, 2008).

***Piper methysticum* Frost.**

Usage part of plant: Rhizome.

Chemical composition: Methysticin (kavalactones), dihydro-5,6-dehydrokavain (DDK), and desmethoxyagonin (Xuan et al, 2008).

Effects of plant on AD: Reversible inhibition of MAO-B, cyclooxygenase inhibition, ligand binding increase to GAMA-A receptor, voltage dependent calcium channel blockage, decrease reuptake of noradrenalin from neurons and voltage gated sodium ion channel blockage are the pharmacological mechanisms of Kava which increases the mood and cognitive performance (LaPorte et al, 2011).

***Salvia lavandulifolia* Vahl.**

Usage part of plant: Leaf.

Chemical composition: α-pinene, camphene, β-pinene, and 1,8-cineole (eucalyptol) (Tildesley et al, 2003).

Effects of plant on AD: Acetylcholine esterase enzyme inhibitory action of Spanish sage in the hippocampus improves memory, attention, learning, and cognition (Tildesley, 2003). Eucalyptol content of *S. lavandulifolia* has a neuroprotective activity which enhances locomotor activity (Martinez et al, 2015).

***Salvia miltiorrhiza* Bung.**

Usage part of plant: Dried root and rhizome.

Chemical composition: Active components are examined in two groups; salvianolic acids which are hydrophilic compounds and tanshinones and diterpenoids which are lipophilic compounds. (Wang, 2010)

Effects of plant on AD: Acetylcholine esterase enzyme inhibition ability of tanshinone content increases the level of acetylcholine in the brain. This improves the cognitive dysfunction (ChunYan, 2015).

***Salvia officinalis* L.**

Usage part of plant: Leaf, flower, and stem.

Chemical composition: Humulene, thujene, camphene, and camphor are the contents of the leaves; cineol and alpha-pinene are contents of the flowers and linalool is the major content of stem part (Hamidpour et al, 2014).

Effects of plant on AD: Neurotoxicity which is induced by amyloid-beta is protected by sage which do cognitive and memory enhancement. Speed of memory and mood are increased. Antioxidant property is assisting these properties in the management of Alzheimer's Disease (Dastmalchi et al, 2007).

***Withania somnifera* L.**

Usage part of plant: Root

Chemical composition: It contains withanolides A to Y, withasomniferin A, withasomniferols A to C, withaferin A, and withanone (Rao et al, 2012).

Effects of plant on AD: *W. somnifera* has capability to increase cholinergic activity which increases the level of acetylcholine. Thus, cognition and memory are improved. It has central nervous system calming effect. It reduces concentrate inability, stress, and forgetfulness. Withanamide A and C prevent formation of fibrils by binding to amyloid-beta (Rao et al, 2012).

***Zingiber officinale* Roscoe.**

Usage part of plant: Rhizome.

Chemical composition: alpha-zingiberene, zingiberol, and gingerol (Rehman et al, 2010).

Effects of plant on AD: Due to its antioxidant and acetylcholine esterase inhibitory activity property, it decreases the level of free radicals and increases the acetylcholine level, respectively. Thus, memory impairment is improved by *Z. officinale* (Akram et al, 2017).

CONCLUSION

AD was first identified by Dr. Alois Alzheimer and is a neurodegenerative disorder characterized by the progressive loss of synapses and neurons in various regions of the central nervous system. The disease severely affects cognitive functions, leading to an increased dependence on caregivers. With the rising elderly population worldwide, including Northern Cyprus and Turkey, the prevalence of AD has also been increasing. The etiology of AD is both molecular and genetic. At molecular level, the accumulation of amyloid-beta plaques and the hyperphosphorylation of Tau proteins are the primary pathological hallmarks of the disease. Genetically, AD can be categorized as either familial or sporadic, both of which result in increased amyloid-beta production in the brain. Additionally, several risk factors, including advanced age, alcohol consumption, head trauma, smoking, depression, and obesity contribute to the onset and progression of the disease. These molecular, genetic, and environmental factors collectively lead to significant pathological changes in the brain, such as atherosclerosis, neurofibrillary tangles, neurotic plaques, synaptic loss, neuronal degeneration, granulocyte degeneration, and cholinergic cell loss. Despite extensive research, the exact cause of AD remains unknown. Consequently, current treatments focus

primarily on slowing the disease progression and alleviating symptoms rather than providing a definitive cure.

Conventional Treatment Approaches

The first-line treatment for AD consists of acetylcholinesterase inhibitors, which can be classified as reversible (Donepezil, Rivastigmine, Galantamine) and irreversible (Physostigmine, Synapton, Meftrionate). These medications act via inhibition of acetylcholinesterase enzyme, thereby increasing acetylcholine levels in the brain to support the cholinergic system. Other conventional treatments include cholinergic-muscarinic receptor agonists and Memantine, which regulates glutamate levels to prevent excitotoxicity.

Medicinal Plants in the Management of Alzheimer's Disease

This study reviewed medicinal plants that may have therapeutic potential in treating, slowing down, or preventing AD. Various plants exhibit properties such as anti-inflammatory, antioxidant, antimicrobial, antidiabetic, antihypertensive, antispasmodic, and neuroprotective effects, all of which contribute to the management of AD.

For effective treatment, several pharmacological activities are essential, including:

Neuroprotective activity: Preventing neuronal degeneration and promoting neuron growth.

Antioxidant activity: Reducing oxidative stress, which plays a key role in AD progression.

Acetylcholinesterase inhibition: Increasing acetylcholine levels to support cholinergic function.

Stress hormone regulation: Calming down the nervous system and supporting cognitive function.

Inhibition of amyloid-beta formation: Preventing plaque accumulation and neurotoxicity.

Antiplatelet activity: Enhancing cerebral blood flow, ensuring better oxygen and nutrient supply.

Antidepressant effects: Reducing depression symptoms which are common in AD patients.

Key Findings

The present study reviewed 18 medicinal plants, evaluating their pharmacological effects in AD management. The most notable findings include:

Acetylcholinesterase inhibitors: *Angelica archangelica, Huperzia serrata, Melissa officinalis, Myristica fragrans, Salvia lavandulifolia, Salvia miltiorrhiza,* and *Zingiber officinale.*

Neuroprotective properties: *Acorus calamus, Ginkgo biloba, Hypericum perforatum, Melissa officinalis, Salvia lavandulifolia,* and *Withania somnifera.*

Reduction of amyloid-beta accumulation: *Crocus sativus, Curcuma*

longa, Ginkgo biloba, Huperzia serrata, and *Zingiber officinale.*

Antioxidant properties: *Curcuma longa, Hypericum perforatum, Ilex paraguariensis, Melissa officinalis, Myristica fragrans, Panax ginseng,* and *Withania somnifera.*

Antiplatelet activity: *Ginkgo biloba* and *Panax ginseng* (enhances cerebral blood flow but may interact with anticoagulant medications).

Stress hormone regulation: *Convolvulus pluricaulis* (calms the nervous system and enhances memory function).

GABA receptor binding enhancement: *Piper methysticum* (a promising drug candidate due to its monoamine oxidase B and cyclooxygenase inhibition).

Antidepressant effects: *Curcuma longa, Hypericum perforatum,* and *Myristica fragrans.*

Despite significant advancements in Alzheimer's research since Dr. Alois Alzheimer has identified the disease, the exact cause remains elusive. As a result, current treatments focus on slowing the progression of the disease rather than offering a definitive cure. This study reviewed 18 medicinal plants, highlighting their mechanisms of action and potential therapeutic applications for AD. While these plants show promise as complementary treatments, they are not as potent as first-line acetylcholinesterase inhibitors. Therefore, they should be considered as adjunct therapies to slow disease progression and manage symptoms. Moreover, due to the potential antiplatelet and anticoagulant effects of various plants, careful dosage adjustments and physician consultation are essential to prevent adverse drug interactions. Continued research into the medicinal plants may help identify novel treatment options for AD in the future.

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