applied sciences

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Some Bioactivities of Isolated Apigenin-7-O-glucoside and Luteolin-7-O-glucoside

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Abstract: In this study, we aimed to isolate compounds from *Stachys lavandulifolia* (Lamiaceae) by chromatographic methods and perform tyrosinase, acetylcholinesterase, butyrylcholinesterase enzyme-inhibition and antimicrobial activity studies of these compounds by in vitro methods. In addition, a molecular docking study was planned for the molecule with the highest effect. Two flavone glycosides, apigenin-7-O-glucoside and luteolin-7-O-glucoside, were isolated from *S. lavandulifolia*. Both compounds were observed to be effective against *Enterococcus faecalis*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*. The compounds showed weak tyrosinase and butyrylcholine esterase inhibition, while only luteolin-7-O-glucoside showed a more significant inhibitory effect against acetylcholinesterase (65 ± 2%). Therefore, molecular interactions between acetylcholinesterase and luteolin-7-O-glucoside were evaluated. In the docking study, it was observed that the molecule was bound to the enzyme with a low amount of free binding energy (Glide score: −8.31). As a result, the antibacterial effect of apigenin-7-O-glucoside and both antibacterial and acetylcholinesterase-inhibitory effects of luteolin-7-O-glucoside were determined.

Keywords: apigenin-7-O-glucoside; luteolin-7-O-glucoside; antimicrobial activity; enzyme inhibition; molecular docking

Citation: Sezen Karaoğlan, E.; Hancı, H.; Koca, M.; Kazaz, C. Some Bioactivities of Isolated Apigenin-7-O-glucoside and Luteolin-7-O-glucoside. *Appl. Sci.* **2023**, *13*, 1503. https://doi.org/10.3390/app13031503

Academic Editor: Monica Gallo

Received: 6 December 2022
Revised: 12 January 2023
Accepted: 18 January 2023
Published: 23 January 2023

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1. Introduction

Plants have been used for thousands of years for flavoring and conserving food and treating and preventing diseases. Secondary metabolites produced by plants are usually responsible for many biological activities [1]. The genus *Stachys* L. (Lamiaceae) includes about 300 species as herbs or small shrubs that grow in temperate regions. Some species of this genus are used in folk medicine. These species are reported to contain many phytochemicals, such as flavonoids, alkaloids, diterpenes, triterpenes, phenolic acids, fatty acids, phenylpropane glycosides, iridoids, lignans, and phytosterols, and these phytochemicals have some biological activities [2]. Flavonoids are secondary metabolites known to have antioxidative, antiviral, anticancer, anti-inflammatory, and hepatoprotective effects. They have been used in treatment of many diseases for centuries. The biological activity, metabolism, and bioavailability of flavonoids are related to the configuration, hydroxyl group amount, and substitution of functional groups. Flavonoids are recognized as important components in nutraceutical, pharmaceutical, medicinal, and cosmetic fields [3–5]. Apigenin is a yellow crystalline powder and has a hydroxyflavone structure. It is an aglycone of many naturally occurring glycosides [6]. Luteolin is a 3,4,5,7-tetrahydroxy flavone molecule found in fruit, vegetables, and medicinal plants. It has various biological activities [7]. Modifications in the chemical structure, such as hydroxylation, glycosylation, methylation, and acylation, of many flavonoids such as luteolin and apigenin can cause

differences in their biological effects [8,9]. It is important to isolate such flavonoids from plants and to investigate their various biological activities.

Infectious diseases have been an important cause of death throughout history and cost the lives of one-third of the world's population in the 1900s. However, developments in the field of medicine in the 20th century brought success in treatments [10]. For example, salvarsan, the first antibiotic discovered, was introduced in 1910, followed by penicillin in 1928. These developments in the field of modern medicine have brought along a miracle that has lasted for many years, and the average human lifespan has been extended by 23 years. Then, there was a gradual decrease in the discovery of antibiotics, and the problem of antimicrobial resistance began to come to the fore [11]. The problem of resistance to antibiotics is not a new problem. It is an issue that has been on the agenda for more than 50 years. For example, *Staphylococcus aureus* developed resistance in the 1950s to penicillin, which is normally a powerful weapon for infections since its first discovery. However, the development of new drugs such as vancomycin and methicillin in the following years prevented the problem of antibiotic resistance from being a serious concern worldwide for a long time [12]. Antibiotic resistance is a global health problem that has emerged as a result of the transfer of bacteria and resistance genes between humans and all other living things in nature [13]. By definition, antibiotic resistance is the ability of bacteria to resist and escape the effects of antibacterial drugs [14]. Antibiotic resistance causes prolonged hospitalization, increased treatment costs, and most importantly, an increase in death rates [15]. All these negative situations have made it important to obtain safe and inexpensive therapeutic agents from various medicinal plants for the discovery of new and reliable antimicrobials [16].

Tyrosinase (TYR) is an enzyme that takes part in melanin biosynthesis and determines the hair and skin color of humans and animals. Excessive production of melanin causes various pigmentation disorders such as melasma, age spots and sites of actinic damage. In addition, TYR enzyme causes undesirable browning in plant-based foods as well as a decrease in nutritional quality and economic loss of food products. TYR enzyme inhibitors are used to eliminate these problems. All these reasons increase the need for new tyrosinase enzyme inhibitors [17,18].

Alzheimer's disease (AD) is a neurodegenerative disease caused by disruption of the cholinergic system [19]. It progresses due to aging and is common all over the world [20]. Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) enzymes are the two enzymes responsible for this disease [21]. AChE functions by breaking down acetylcholine [22]. BuChE hydrolyzes choline-based esters [23]. AD is treated by inhibiting these two enzymes [24]. Although there are various drugs used for AD today, the invention of new cholinesterase inhibitors is also important.

Molecular docking has become increasingly important in drug design recently. For this, various computer-assisted software programs are used. Molecular docking studies have gained attention in drug design in terms of analyzing interactions, estimating binding affinities, increasing efficacy, and reducing cost [25].

Stachys lavandulifolia is a species belonging to the *Stachys* genus. In this study, we aimed to isolate major flavonoids from *S. lavandulifolia* and investigate some biological activities of isolated compounds. In addition, a molecular docking study was planned for the highly effective compound. Apigenin-7-glucoside (C1) and luteolin-7-glucoside (C2) were isolated from the ethyl acetate extract of *S. lavandulifolia* plant by chromatographic methods. TYR-, AChE-, and BuChE-inhibitory activities and antimicrobial activities of isolated C1 and C2 were studied. As a result of the experiments, the AChE-inhibitory effect of C2 was found to be significant. Therefore, molecular docking studies were performed to examine the interactions between the C2 molecule and the AChE enzyme.

2. Materials and Methods

2.1. Plant Material

S. lavandulifolia was collected from Konaklı Ski Center (Erzurum-Turkey). It was identified by Esen Sezen Karaoğlan. The herbarium sample is in Atatürk University Biodiversity Application and Research Center Herbarium (AUEF 1055).

2.2. Extraction and Isolation Studies

S. lavandulifolia was powdered in a grinder after drying. The powdered plant was macerated for one day with methanol. It was filtered with filter paper. The filtrate was stored. The residue was extracted with methanol in a mantle heater under reflux. The filtrates were combined and dried with an evaporator. Then, this was fractionated by a separatory funnel with hexane, dichloromethane and ethyl acetate. Fractions were dried with an evaporator.

Ethyl acetate extract of aerial parts of *S. lavandulifolia* was applied to the silica gel column (Solvent systems; CH_2Cl_2:MeOH:H_2O (90:10:1 → 50:50:5). A precipitate formed in tubes numbered 18–22. The precipitate was filtered and washed with methanol. Compound 1 (C1) was obtained. Similarly, a different precipitate formed in tubes 44–52. This precipitate was also filtered and washed with methanol. Compound 2 (C2) was obtained.

2.3. Structure Designation

The structure of compounds was determined by ^{1}H NMR and ^{13}C NMR. NMR measurements were performed at Atatürk University, Faculty of Science, Department of Chemistry. The values were confirmed by comparison with literature.

2.4. Antimicrobial Activity

In vitro antibacterial activity tests were done using standard bacteria strains, which were selected from the American Type Culture Collection (ATCC) (*Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 700603), *Proteus mirabilis* (ATCC 29906), *Pseudomonas aeruginosa* (ATCC 9027), *Staphylococcus aureus* (ATCC 29213), and *Enterococcus faecalis* (ATCC 29212)). First, bacteria stored at −80 °C were revived on Mueller–Hinton agar (Oxoid, Italy) to obtain pure colonies.

Microdilution was used for determining the antimicrobial activities of C1 and C2 according to the Clinical and Laboratory Standards Institute using a sterile, U-based 96-well plate [26].

Before starting the dilution step, stock solutions of C1 and C2 were prepared using 10% dimethyl sulfoxide (DMSO) and cation-adjusted Mueller–Hinton broth (CAMHB) (Oxoid, Italy). Then, dilutions of C1 and C2 were prepared with CAMHB starting from the first well in the plate within the range of 1600 µg/mL to 3.12 µg/mL. The last two wells were used as negative and positive controls. Sulfamerazine was used as the reference antibiotic.

All bacterial strains used in the study were diluted with CAMHB to prepare an inoculum with a final concentration of 10^5 CFU/mL. An equal volume of prepared bacterial suspensions was added to the diluted C1 and C2 in each well of the plate. All of the plates were incubated aerobically at 35 °C for 24 h. The compound concentration in the first well without bacterial growth and where clarity was observed was determined as the MIC (minimum inhibitory concentration). The tests were run three times.

2.5. Cholinesterase-Inhibitory Activity

In vitro acetylcholinesterase- and butyrylcholinesterase-inhibitory activities of the compounds were evaluated by a slight modification of the coulometric Ellman method [27]. Donepezil hydrochloride (DH) was used as the reference compound. All test compounds were prepared at concentrations ranging from 5 to 50 µg/mL. Test compounds were dissolved in dimethyl sulfoxide (DMSO) and diluted with Tris buffer solution (50 µM, pH 8.0). First, 125 µL of 3 mM 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) was added to each well. Thereafter, 25 µL (1 (0.2 U/mL) of cholinesterase (AChE or BuChE) was added

to each well except the blank wells. Then, the mixture was incubated at 37 °C for 15 min. Finally, the substrate (acetylthiocholine iodide (ATCI) for AChE or butyrylthiocholine iodide (BTCI) for BuChE) was added. Absorbance was measured three times every 45 s at a wavelength of 412 nm. All experiments were run three times. Percentages of inhibition (I %) are expressed as mean ± SD.

2.6. Tyrosinase-Inhibitory Activity

In the mushroom tyrosinase inhibition activity studies, 3,4-dihydroxyl-L-phenylalanine (L-DOPA) was used as a substrate and kojic acid (KA) was used as a positive control. Various concentrations test substances (40 µL), tyrosinase solution (46 U/mL, 40 µL) and phosphate buffer (pH = 6.8) were applied to a 96-well microplate. It was incubated at 23 °C for 10 min. Then L-DOPA (40 µL) was added to each well and incubated for 10 min. The final amount in each well was 160 µL. Absorbance values at 490 nm were measured with a microplate reader (Bio Tek EL X 800). Percentage of TYR inhibition (%) was calculated with the formula $[[(A - B) - (C - D)]/(A - B)] \times 100$ (A: sample-free absorbance, B: absorbance with sample addition, C: absorbance after incubation, D: absorbance before incubation). Experiments were repeated three times [28].

2.7. In Silico Molecular Docking and Simulation Studies

Docking simulation was performed using the Schrödinger software suite (Maestro 11.9). The crystal structure of AChE (1-EVE) was obtained from the RSCB PDB. The coordinates of the grid box were defined as containing the interaction region of the ligand with the enzyme and were taken as X = 2.8, Y = 64.5, and Z = 67.9. Optimizations such as removing water molecules from the structure part, adding hydrogen atoms to the enzyme structure, and adjusting the pH value to 7 were made using PROPKA software. Energy minimization was performed with optimized potentials for liquid simulations (OPLS3e) force field.

The three-dimensional structure of C2 was obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov, accessed on 15 May 2022). Later, the structure of the compound was minimized via Polak–Ribière conjugate gradient (PRCG) minimization. The compound was docked to the target enzymes by Glide/XP docking protocols (RMSD < 0.5 Å). The docking results were evaluated by considering the docking interactions and the Glide score.

3. Results

3.1. Chemical Structures of the Isolated Compounds

Two flavone glycosides, apigenin-7-O-glucoside (C1) and luteolin-7-O-glucoside (C2), were isolated from ethyl acetate extract of *S. lavandulifolia*. The ^{1}H NMR and ^{13}C NMR values of the compounds are shown below.

Apigenin-7-glucoside (C1), $C_{21}H_{20}O_{10}$, ^{1}H-NMR (400 MHz, DMSO-*d6*) δ: 6.94 (s, H-3),6.44 (d, J 2.2 Hz, H-6), 6.84 (d, J 2.2 Hz, H-8), 8.04 (d, J 8.8 Hz, H-2′,6′), 7.11 (d, J 8.8 Hz, H-3′, 5′), 5.06 (d, J 7.4 Hz, H-1′′), 3.16-3.75 (m, sugar protons). ^{13}C-NMR (100 MHz, DMSO-*d6*) δ: 164.5 (C-2), 104.4 (C-3), 182.7 (C-4), 163.2 (C-5), 95.6 (C-6), 163.7 (C-7), 95.5 (C-8), 157.7 (C-9), 104.4 (C-10), 123.4 (C-1′), 121. (C-2′), 115.3 (C-3′), 161.8 (C-4′), 115.3 (C-5′), 129.1 (C-6′), 100.3 (1′′), 73.8 (C-2′′), 77.8 (C-3′′), 70.2 (C-4′′), 77.1 (C-5′′), 61.3 (C-6′′). The ^{1}H NMR and ^{13}C NMR values are compatible with the literature [29].

Luteolin-7-glucoside (C2), $C_{21}H_{20}O_{11}$, ^{1}H-NMR (400 MHz, CD_3OD) δ: 6.74 (s, H-3),6.42 (d, J 2.2 Hz, H-6), 6.77 (d, J 1.8 Hz, H-8), 7.40 (d, J 2.2 Hz, H-2′), 6.88 (d, J 8.4 Hz, H-5′), 7.43 (dd, J 8.4, 2.2 Hz, H-6′), 5.06 (d, J 7.3 Hz, H-1′′), 3.14-3.70 (sugar protons). ^{13}C-NMR (100 MHz, DMSO-*d6*) δ: 165.1 (C-2), 103.8 (C-3), 182.6 (C-4), 161.8 (C-5), 100.2 (C-6), 163.6 (C-7), 95.3 (C-8), 157.6 (C-9), 106.0 (C-10), 122.5 (C-1′), 114.2 (C-2′), 146.5 (C-3′), 150.6 (C-4′), 116.6 (C-5′), 119.8 (C-6′), 100.5 (C-1′′), 73.8 (C-2′′), 77.0 (C-3′′), 70.2 (C-4′′), 77.8 (C-5′′), 61.3 (C-6′′). The ^{1}H NMR and ^{13}C NMR values are compatible with the literature [30].

3.2. Antimicrobial Activity

According to antimicrobial susceptibility test results, C1 and C2 showed the strongest effect against *S. aureus* (MIC value 3.12 µg/mL). The highest MIC values for both compounds were determined against *P. aeruginosa* and *P. mirabilis.* In general, it was determined that the compounds showed better activity against Gram-positive bacteria. The antimicrobial activity results are shown in Table 1.

Table 1. Antimicrobial activities of components.

Test Samples	*E. coli*	*K. pneumonia*	*P. aeruginosa*	*P. mirabilis*	*S. aureus*	*E. faecalis*
C1	800 µg/mL	25 µg/mL	≥1600 µg/mL	≥1600 µg/mL	3.12 µg/mL	6.25 µg/mL
C2	≥1600 µg/mL	25 µg/mL	≥1600 µg/mL	≥1600 µg/mL	3.12 µg/mL	3.12 µg/mL
Sulfamerazine	≥1600 µg/mL	≥1600 µg/mL	≥1600 µg/mL	≥1600 µg/mL	6.25 µg/mL	3.12 µg/mL

C1; Apigenin-7-glucoside, C2; Luteolin-7-glucoside.

3.3. Tyrosinase- and Cholinesterase-Inhibitory Activity

In enzyme-inhibition studies, C2 showed the highest effect against AChE (65 ± 2). The percentage inhibitory effects of tyrosinase and cholinesterase are shown in Table 2.

Table 2. Percentage inhibitory effect of C1 and C2.

Test Material (100 µg/mL)	% Acetylcholineesterase Inhibition	% Butyrylcholinesterase Inhibition	% Tyrosinase Inhibition
C1	30 ± 3	13 ± 4	24 ± 1.1
C2	65 ± 2	36 ± 3	32 ± 2.4
KA	-	-	92 ± 1.2
DH	84 ± 3	92 ± 2	-

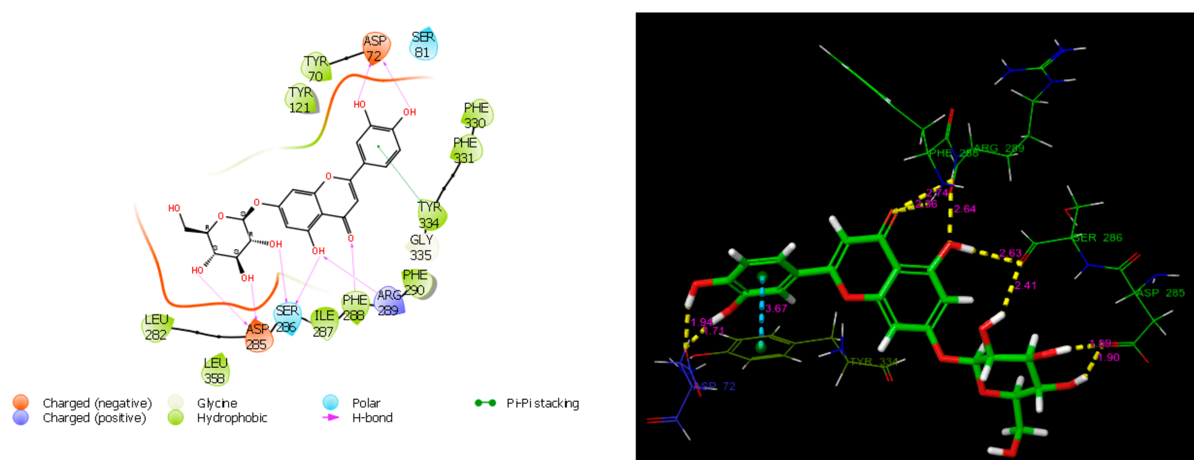
C1; apigenin-7-glucoside, C2; luteolin-7-glucoside, KA: kojic acid (positive control), DH: donepezil hydrochloride (positive control).

3.4. In Silico Molecular Docking and Simulation Studies

According to in silico molecular docking and simulation studies, eight hydrogen bonds and a π–π stacking interaction were formed between C2 and the crystal structure of 1EVE. The interactions between the ligand and the enzyme, bonds formed and bond lengths are summarized in Table 3. The molecular interaction between luteolin-7-glucoside (C2) and acetylcholinesterase is shown in Scheme 1.

Table 3. The interactions between the ligand and the enzyme, bonds formed and bond length.

Ligand-Enzyme	Enzyme Residues	Ligand Interaction Site	Distance (A^0)	Interaction
C2-1EVE	PHE288 (NH_2)	4-Oxo of benzopyran	2.26	H-bond
C2-1EVE	ARG289 (C=O)	5-OH of benzopyran	2.64	H-bond
C2-1EVE	SER286 (C=O)	5-OH of benzopyran	2.63	H-bond
C2-1EVE	SER286 (C=O)	3-OH of glucoside	2.41	H-bond
C2-1EVE	ASP72 (CO-OH)	3-OH of phenyl	1.71	H-bond
C2-1EVE	ASP72 (CO-OH)	4-OH of phenyl	1.94	H-bond
C2-1EVE	ASP285 (CO-OH)	4-OH of glucoside	1.59	H-bond
C2-1EVE	ASP285 (CO-OH)	5-OH of glucoside	1.90	H-bond
C2-1EVE	TYR334 (Ph)	Benzene of 2- (3, 4-dihydroxyphenyl)	3.67	π-π stacking



Scheme 1. Molecular interaction between luteolin-7-glucoside (C2) and acetylcholinesterase (AChE).

4. Discussion

In recent years, studying biological activities with compounds isolated from plants has gained importance. It is valuable to discover new drug molecules and to conduct biological, pharmacological, clinical and toxicological studies on these molecules. Molecular docking studies of drug molecules have also gained in importance recently. For these reasons, we aimed to isolate compounds from *S. lavandulifolia* to test various biological activities and perform molecular docking studies of these compounds.

In this study, two flavone glycosides were obtained from *S. lavandulifolia* ethyl acetate extract by chromatographic methods. With the results of ^{1}H NMR, ^{13}C NMR and literature comparison, it was determined that the substances were apigenin-7-O-glucoside (C1) and luteolin-7-O glucoside (C2). Then, TYR-, AChE-, and BuChE-inhibitory activities and antimicrobial activities of isolated C1 and C2 were assessed. The AChE-inhibitory effect of C2 was found to be significant. Therefore, molecular docking studies were performed to examine the interactions between the C2 molecule and the AChE enzyme.

Compound C1 is a flavone glycoside. Its molecular formula is $C_{21}H_{20}O_{10}$. According to the ^{1}H-NMR spectrum, proton signals 2′-6′ (d), 3′-5′ (d), 3 (s), 8 (d), and 6 (d) were observed at 8.04, 7.11, 6.94, 6.84 and 6.44 ppm. While the anomeric proton signal (H-1″, d) was observed at 5.06 ppm, the signals of other sugar protons were observed in the range of 3.16–3.75 (m) ppm. The signal for the 1″ carbon appeared at 100.3 ppm in the ^{13}C-NMR spectrum. It has been supported by literature that the C1 molecule is apigenin-7-glucoside with 21 carbons [29].

Compound C2 is a flavone glycoside. Its molecular formula is $C_{21}H_{20}O_{11}$. According to the ^{1}H-NMR spectrum, proton signals 6′ (dd), 2′ (d), 5′ (d), 8 (d), 3 (s), and 6 (d) were observed at 7.43, 7.40, 6.88, 6.77, 6.74, and 6.42 ppm. While the anomeric proton signal (H-1″, d) was observed at 5.06 ppm, the signals of other sugar protons were observed in the range of 3.14–3.70 (m) ppm. The signal for the 1″ carbon appeared at 100.5 ppm in the ^{13}C-NMR spectrum. It has been supported by literature that the C2 molecule is luteolin-7-glucoside with 21 carbons [30].

Antibiotic resistance is an important problem that causes prolonged hospitalization, increased treatment costs and increased mortality rates. Therefore, the discovery of new, reliable and inexpensive antimicrobials has gained importance [15,16]. Antimicrobial activity assays were carried out by microdilution method against some important pathogen bacteria (*E. coli*, *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*, *S. aureus*, and *E. faecalis*) by comparison with sulfamerazine in this study. C1 and C2 showed the strongest effect against *S. aureus* (MIC value 3.12 µg/mL). In general, it was determined that the compounds showed better activity against Gram-positive bacteria.

C1 was found to be effective against *E. coli*, *K. pneumoniae*, *S. aureus*, and *E. faecalis*. It was especially effective against *S. aureus* and *E. faecalis*. C1 was observed to be more

effective than sulfamerazine against *S. aureus* and *K. pneumoniae*. In previous literature, it was reported that C1 inhibited the growth of *P. vulgaris*, *P. mirabilis*, *K. pneumonia*, *K. oxytoca*, *M. morgani*, *Streptococcus* sp., *Enterobacter* sp., *Seratia* sp. and the fungi *Candida albicans*. The median diameter of the inhibition zone increased proportionally with the increase in substance concentration [31]. In another study, Aljancic et al. indicated that C1 is especially effective against *Bacillus subtilis* [32]. Although C1 did not show any activity against some microorganisms, it showed a potentially significant effect against some of them. The literature also supports this. The C1 molecule can be evaluated as an alternative antibacterial agent.

C2 was found to be effective againts *S. aureus*, *E. faecalis* and *K. pneumoniae*. C2 was observed to be more effective than sulfamerazine against *S. aureus*. Akroum et al. investigated the antimicrobial effect of C2 against *E. coli*, *S. aureus*, *B. cereus*, *P. aeruginosa*, and *B. subtilis* by diffusion on agar method. They stated that C2 is effective against *S. aureus*, *B. cereus*, and *B. subtilis* [33]. The antibacterial effect of C2 against *S. aureus* indicated in that study is consistent with our study. Although C2 did not show any activity against some microorganisms, it showed a potentially significant effect against some of them. The C2 molecule can also be considered an alternative to antibacterial agents.

Tyrosinase enzyme inhibitors are important as depigmentation agents in the cosmetic and pharmaceutical industries, as well as as antibrowning compounds in the food and agricultural industries [34]. For these reasons, there is a need for new natural tyrosinase enzyme inhibitors. In this study, the TYR enzyme-inhibitory effect of C1 and C2 was tested. Kojic acid was used as positive control. Both C1 (24%) and C2 (32%) showed weak TRY-inhibitory effects. Karioti et al. investigated the tyrosinase enzyme-inhibitory activity of many compounds isolated from *Marrubium velutinum* and *Marrubium cylleneum.* The low effect of C2 (27.35 $\pm$ 1.24) and apigenin glycosides in the study is consistent with our study [35]. Wagle et al. investigated the tyrosinase enzyme-inhibitory effect of some compounds isolated from *Cirsium japonicum* var. *maackii* (Maxim.) Matsum. In the study, C2 showed weak activity (IC_{50} = 177.03 $\pm$ 0.77 μM for l-tyrosine and 399.08 $\pm$ 3.89 μM for l-DOPA), while luteolin 5-O-β-D-glucopyranoside showed high activity (IC_{50} values of 2.95 $\pm$ 0.11 μM for L-tyrosine and 8.22 $\pm$ 0.18 μM for L-DOPA). They stated that a glucose in the 5-OH position increased the activity compared to the 7-OH position [36]. In compounds C1 and C2, the sugar is attached to the seventh carbon. TYR-inhibitory effects were low in both molecules. This is compatible with the literature.

AD is a progressive and widespread neurodegenerative disease associated with aging, and it is an urgent public health problem in many parts of the world [24]. There are many studies in the literature investigating the AChE-inhibitory effects of natural plants [22]. New drug molecule studies are extremely important for this incurable disease. AChE and BuChE enzymes are involved in this disease. In our study, AChE and BuChE enzyme-inhibition activities of C1 and C2 were investigated. According to the results of the study, C1 and C2 showed 30% and 65% AChE inhibition, respectively. Moreover, C1 and C2 showed 13% and 36% BuChE inhibition, respectively. The AChE-inhibitory activity of C2 was the highest. Therefore, the molecular interaction between C2 and AChE enzyme was evaluated in the next step. Liu et al. evaluated the AChE-inhibition effects of extracts of *Drynariae* rhizome and isolated compounds by a bioguided fractionation procedure. Consistently with our results, they reported that C2 showed an AChE-inhibitory effect (17.13 $\pm$ 1.02 μM) [37]. In addition, Tundis et al., investigated the AChE- and BuChE-inhibition effects of *Globularia meridionalis* (Podp.) O. Schwarz and some isolated compounds. They reported that C1 exhibited inhibition against AChE with and IC_{50} value of 172.6 $\pm$ 1.4 μg/mL and against BChE with an IC_{50} value of 221.4 $\pm$ 2.5 μg/mL [38]. In our study, the C2 molecule showed a higher effect than the C1 molecule. This is compatible with the literature. It may be appropriate to conduct more detailed studies on the treatment of AD with the C2 molecule.

Recently, molecular docking studies have gained attention in drug design in terms of analyzing interactions, estimating binding affinities, increasing efficacy and reducing cost [25]. For this reason, the use of similar technological computer programs is very

important. After investigating the inhibition effects of TYR, AChE, and BuChE enzymes of C1 and C2 molecules, the interaction between the significantly effective C2 molecule and the AChE enzyme was investigated by in silico molecular docking studies. The C2 molecule is luteolin-7-O-glucoside, which is in the structure of a flavone glycoside. The interactions between the ligand and the enzyme, the bonds formed and the bond lengths are shown in Table 3 and the molecular interaction between C2 and AChE enzyme is shown in Scheme 1. The benzopyran ring's 4-oxo performed a hydrogen bonding with residue of PHE288. Other hydrogen bonds were observed between the residues of SER286, ARG289, and the 5-OH group of the benzopyran ring. Groups belonging to the glycoside structure of C2 (3, 4, 5-OH) performed much hydrogen bonding with related residues of the enzyme (respectively: SER286, ASP285, and ASP285). The other two hydrogen bonds were observed between the 3,4 hydroxy groups of phenyl and residue of ASP72. π–π charge transfer interaction occurred between the benzene ring at position 2 of the ligand and the benzene ring of TYR334. qqqqHydrophobic interactions (shown in green residues), positive load interaction between ARG289 residue and ligand, negative charge interaction between ASP285 and ASP72 residues and ligand, and polar interactions were observed between SER286 and SER81 residues and ligand (Scheme 1). The ligand was superimposed (glide score: -8.31) into the active part of the enzyme.

In the docking study, no interaction was observed between any of the catalytic triad residues (HİS440, SER200) and the molecule. Many cholinesterase inhibitors, such as donepezil, ambenonium, avd physostigmine, contain the N atom. In previous studies, it was stated that compounds containing the amine group showed strong cholinesterase inhibition [39–42]. A modified new molecule with the C2 skeleton containing an amine group may have stronger cholinesterase inhibition. It may be useful to carry out additional studies on this subject.

5. Conclusions

TYR-, AChE-, and BuChE-inhibitory and antimicrobial effects of isolated apigenin-7-O-glucoside and luteolin-7-O-glucoside were investigated. Both compounds showed potent antibacterial activity, especially against *S. aureus* and *E. faecalis*. We think that our antimicrobial test results can shed light on new research such as antimicrobial formulation studies that can be done in the future with both compounds. Luteolin-7-O-glucoside showed a higher inhibitory effect against AChE. Therefore, molecular docking and simulation studies were conducted between luteolin-7-O-glucoside and AChE. In the docking study, many residues of the enzyme (peripheral anionic side, acyl pocket and anionic side residues) demonstrated interactions such as negative charge, hydrophobic, π–π stacking, and hydrogen bonding with the molecule. Luteolin-7-O-glucoside molecule is thought to be promising for acetylcholinesterase inhibition. Luteolin-7-O-glucoside can be transformed into a more effective molecule with chemical derivatization studies. It may be appropriate to conduct more extensive studies on both molecules.

Author Contributions: Conceptualization, E.S.K.; methodology, E.S.K., H.H., M.K. and C.K.; software, M.K.; validation, M.K., C.K.; formal analysis, E.S.K., H.H.; investi-gation, E.S.K.; resources, E.S.K., H.H., M.K.; data curation, E.S.K., H.H.; writing—original draft preparation, E.S.K., H.H., M.K.; writing—review and editing, E.S.K.; visualization, C.K.; supervision, E.S.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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