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RESEARCH ARTICLE **Open Access**

Phytochemical analysis of *Robinia pseudoacacia* flowers and leaf: quantitative analysis of natural compounds and molecular docking application

Robinia pseudoacacia çiçekleri ve yaprağının fitokimyasal analizi: doğal bileşiklerin kantitatif analizi ve moleküler doking uygulaması

Yunus BAŞAR[a] *, İbrahim HOSAFLIOĞLU[a], Ramazan ERENLER[b]

[a] *Research Laboratory Practice and Research Center, Iğdır University, 7600, Iğdır, Türkiye*

[b] *Department of Chemistry, Faculty of Arts and Sciences, Tokat Gaziosmanpaşa University, 60240 Tokat, Türkiye*

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**Corresponding author*:
e-mail: yunus.basar@igdir.edu.tr
ORCID: 0000-0002-7785-3242

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ABSTRACT

Phenolic compounds are widely found and well-known secondary metabolites in plants. Identification, and quantification of phenolic compounds, and determination of their biological activities reveal the unknown secrets of plants. *Robinia pseudoacacia* (RP) is known as the white-flowered false acacia and is distributed in Northern Anatolia in Turkey. Spectrophotometric and chromatographic techniques are used to identify the presence and amount of phenolics. In this study, RP flowers and leaves were extracted with methanol and analyzed by LC-MS/MS to determine their phytochemical content. Salicylic acid and syringic acid were found as major products in leaves and flowers. RP extracts have been reported to have antibacterial activity and BChE inhibitory properties. Therefore, the BChE and DD peptidase enzyme inhibitory properties of the main components salicylic acid and syringic acid were investigated by molecule docking (MolDock). According to MolDock results, syringic acid interacted with BChE and DD peptidase and was calculated as a MolDock score of -79.38, and -71.25, with binding energies -5.90, and -5.40 kcal/mol respectively. Salicylic acid interacted with BChE and DD peptidase and was calculated as a MolDock score of -63.54, and -66.18, with binding energies of -6.10, and -5.70 kcal/mol respectively. As a result, salicylic acid had higher binding energy in its interactions with BChE and DD peptidase enzymes. In theory, salicylic acid can be used as a good BChE and DD peptidase inhibitor.

ÖZ

Fenolik bileşikler bitkilerde yaygın olarak bulunan ve iyi bilinen sekonder metabolitlerdir. Fenolik bileşiklerin tanımlanması, miktarlarının belirlenmesi ve biyolojik aktivitelerinin belirlenmesi, bitkilerin bilinmeyen sırlarını ortaya çıkarmaktadır. *Robinia pseudoacacia* (RP), beyaz çiçekli sahte akasya olarak bilinir ve Türkiye'de Kuzey Anadolu'da yayılış gösterir. Fenoliklerin varlığını ve miktarını belirlemek için spektrofotometrik ve kromatografik teknikler kullanılır. Bu çalışmada, RP çiçekleri ve yaprağı metanol ile ekstrakte edilmiş ve fitokimyasal içeriğini belirlemek için LC-MS/MS ile analiz edilmiştir. Yaprak ve çiçeklerde salisilik asit ve siringik asit ana ürünler olarak bulunmuştur. RP ekstraktlarının antibakteriyel aktiviteye ve BChE inhibitör özelliklerine sahip olduğu rapor edilmiştir. Bu nedenle, ana bileşenler olan salisilik asit ve siringik asidin BChE ve DD peptidaz enzimini inhibe edici özellikleri, molekül yerleştirme (MolDock) yöntemiyle araştırıldı. MolDock sonuçlarına göre, siringik asit, BChE ve DD peptidaz etkileşimi sonucu MolDock skoru -79.38, -71.25 ve bağlanma enerjileri -5.90, -5.40 kcal/mol olarak sırasıyla hesaplandı. BChE ve DD peptidaz ile etkileşimi sonucu salisilik asitin, MolDock skoru -63.54, -66.18 ve bağlanma enerjileri -6.10,-5.70 kcal/mol olarak sırasıyla hesaplandı. Sonuç olarak salisilik asidin BChE ve DD peptidaz enzimleri ile etkileşimlerinde daha yüksek bağlanma enerjisine sahip olduğu görüldü. Teorik olarak salisilik asit iyi bir BChE ve DD peptidaz inhibitörü olarak kullanılabilir.

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1. INTRODUCTION

Herbal medicines, one of the most important components of traditional medicine, have unique variations in chemical components (Topçu et al., 1999; Elmastas et al., 2004; Demirtas et al., 2013; Sahin Yaglioglu et al., 2013). For this reason, compounds effective in the treatment of many diseases have been isolated from plants with a wide range of biological activities and introduced to the pharmaceutical industry (Aksit et al., 2014; Bayir et al., 2014; Erenler et al., 2014; Türkmen et al., 2014). In recent studies, it has been shown that phytochemical natural compounds (steroids, carotenoids, phenolic compounds, alkaloids, and vitamins) treat diseases and prevent the formation of diseases in humans and animals. Treatments of existing diseases in the world are evaluated in many ways with the developing technology and solutions are sought (Erenler et al., 2015; Aydin et al., 2016; Elmastas et al., 2016; Erenler, et al., 2016a; Erenler, et al., 2016b). Interest in medicinal plants, which are widely used among the public in Turkey, is increasing day by day. For this reason, with the developing technology, the research of plants used for medicinal purposes in our country and the detection of active ingredients contribute greatly to the country's economy (Erenler, 2016c; Erenler et al., 2016d; Karan et al., 2016; Erenler et al., 2017a; Erenler et al., 2017b). Natural products have been used commonly in nanotechnology, basically green synthesis of nanoparticles (Erenler et al., 2023a; Erenler et al., 2023b; Erenler et al., 2023c; Erenler & Hosaflioglu 2023; Karan et al., 2024a; Karan et al., 2024b).

Robinia species is a perennial plant belonging to the Fabaceae family, popularly called acacia in our country. *Robinia pseudoacacia* (RP) is known as the white-flowered false acacia and is distributed in Northern Anatolia in Turkey. RP is rich in compounds such as polyphenolics, tannins, and monoterpenes. Additionally, different extracts have been reported to have antibacterial activity and AChE and BChE enzyme inhibitory properties. It has a strong backup potential thanks to the polyphenols contained in the flowers. It is used among the public as a stomach acid reducer, sedative, and antispasmodic (Vítková et al., 2017).

Enzyme Inhibitors are a control mechanism in biological systems. It is known that enzyme inhibitors are effective in the treatment of many diseases. There are some herbs commonly used in traditional medicine to reduce the symptoms of Alzheimer's disease. Acetylcholinesterase (AChE) inhibitors inhibit the breakdown of acetylcholine, thus strengthening central and peripheral cholinergic function. One of the most commonly used approaches in the treatment of Alzheimer's, senile dementia, ataxia, myasthenia gravis, and Parkinson's diseases is AChE inhibition (Mukherjee et al., 2007; Atalar et al., 2023). Butyrylcholinesterase (BChE); is found in the pancreas, central nervous system, liver, and serum. The esterase activity of BChE is important in removing organophosphate and carbamate inhibitors from the circulation before they reach AChE, and in controlling cholinergic neurotransmission in AChE deficiency (Çokuğraş et al., 2003; Rao et al., 2007). Bacterial diseases occur when bacteria enter the body, destroying healthy bacteria, or beginning to grow in sterile tissues (Aissous et al., 2023). DD-peptidase enzyme (penicillin-binding) catalyzes the final reaction in the biosynthesis of the cell wall of the bacterium. The main approach to the treatment of bacterial infections is the use of antibiotics. The importance of discovering new drugs containing natural molecules without side effects is increasing. Therefore, natural compounds can be medicines; Their interactions with enzymes are observed with programs such as molecular docking. Molecular docking is a web-based method that predicts binding or non-binding orientation in the ligand-protein complex. It is an in silico approach used to theoretically predict the binding affinity and activity of the molecule to protein targets (Boussaha et al., 2024).

RP flowers and leaf methanol extracted were analyzed by LC-MS/MS to determine their phytochemical content. Biological activity applications are a costly and time-consuming process. Enzyme inhibitory properties of molecules are calculated theoretically through molecular docking applications. Therefore, Syringic acid and salicylic acid, which are determined as the common and main components in the flower and leaf; BChE and DD peptidase inhibitory properties, were investigated. Thus, the accuracy of this plant's antibacterial properties as well as its use in the treatment of memory loss and central nervous system disorders was determined.

2. MATERIALS AND METHODS

2.1. Plants

RP was harvested in the city of Van and identified by Dr. Murat Ünal, Van Yüzüncü Yıl University, a voucher specimen was deposited in the Herbarium of the same university (VANF20432).

2.2. Extraction

Sex- Each leaf and flower of a plant (30 g) were extracted with methanol (500 mL) in a flask separately. The solvent extract mixture was filtered and the solvent was evaporated using a rotary evaporator to yield the crude extract (Gecer & Erenler, 2023).

2.3. Quantitative analysis

The phenolic contents and amounts of RP leaf and flower of methanol extract were determined by LC-ESI-MS/MS analysis (Erenler et al., 2023c; İpek et al., 2024). Forty-one phenolic standards were employed for LC- ESI-MS/MS analysis (Figure 1).

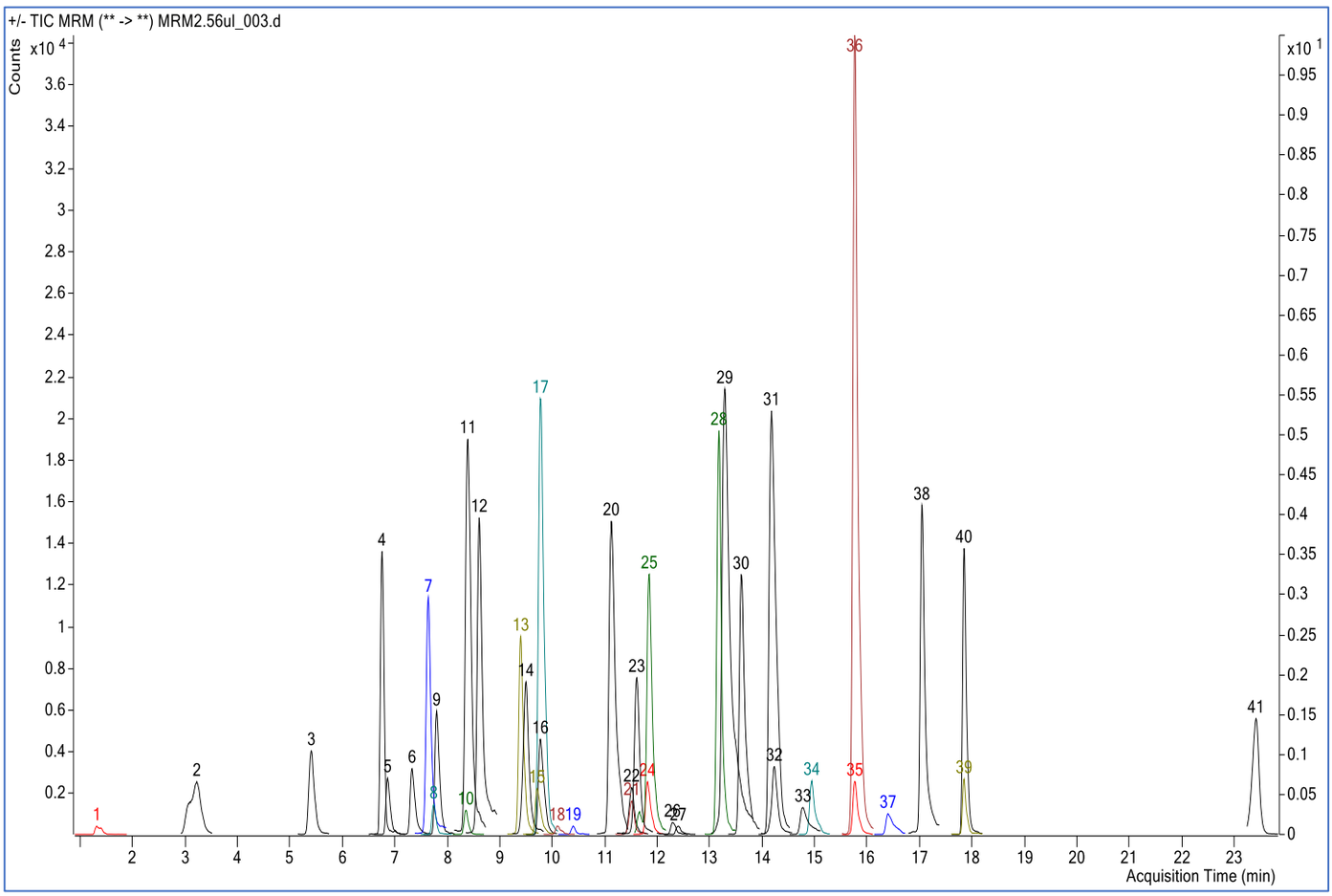

Figure 1. LC-MS/MS standard chromatogram: Shikimic acid (1), Gallic acid (2), Protocatechuic acid (3), Epigallocatechin (4), Catechin (5), Chlorogenic acid (6), Hydroxybenzaldeyde (7), Vanillic acid (8), Caffeic Acid (9), Syringic acid (10), Caffein (11), Vanillin (12), o-Coumaric acid (13), Salicylic acid (14), Taxifolin (15), Resveratrol (16), Polydatine (17), *Trans*-ferulic acid (18), Sinapic acid (19), Scutellarin (20), p-Coumaric acid (21), Coumarin (22), Protocatehuic ethyl ester (23), Hesperidin (24), Isoquercitrin (25), Rutin (26), Quarcetin-3-xyloside (27), Kaempferol-3-glucoside (28), Fisetin (29), Baicalin (30), Chrysin (31), *Trans*-cinnamic acid (32), Quercetin (33), Naringenin (34), Hesperetin (35), Morin (36), Kaempferol (37), Baicalein (38), Luteolin (39), Biochanin A (40), Diosgenin (41)

2.4. Molecular docking application

The drawing, 3D structure, and minimum energy of the syringic acid and salicylic acid were calculated in the ChemDraw software. The enzymes chosen for this docking study were BChE [PDB ID: 1P0I] and DD peptidase [PDB ID:3PTE]. Syringic acid and salicylic acid molecules, DD peptidase, and BChE interactions were determined using the Molegro Virtual Docker (MVD) program (Başar et al., 2024). 2D and 3D images of the interactions were taken with the BIOVIA Discovery Studio Visualizer program. Also, The AutoDock Vina program was used to calculate the binding affinities (Başar et al., 2023; Yenigün et al., 2023).

3. RESULTS AND DISCUSSIONS

3.1. Quantitative analysis of bioactive compounds

When using the plant for medicinal purposes, its phytochemical content must be known (Erenler et al., 2023d). For this purpose, the quantitative analysis of bioactive compounds (phenolics, flavonoids, etc.) in plants is determined by devices such as HPLC, LC-MS, and LC-MS/MS (Erenler et al., 2018). In this study, a quantitative analysis of phenolic compounds in the methanol extract of the leaf and flower parts of the RP plant was performed (Table 1). According to the analysis results, on the leaf; syringic acid (24.78 µg/g extract), salicylic acid (14.86 µg/g extract), and kaempferol (10.30 µg/g extract), in flower; rutin (199.74 µg/g extract), salicylic acid (26.41 µg/g extract), and syringic acid (19.23 µg/g extract) were detected in the highest amount (Table 1). Syringic acid and salicylic acid are the main components in the flower and leaf. Syringic acid is a naturally occurring phenolic compound found in a wide variety of herbal products (Erenler et al., 2023e). It has biological activities such as antioxidant, antimicrobial, anticancer, antidiabetic, and anti-inflammation. Moreover, it is known to be friendly to the central nervous system, brain, heart, and liver. Salicylic acid is a *β*-hydroxy acid derived from salicin. Salicylic acid has been reported to have biological activities such as anti-inflammatory and anti-tumor, anti-cancer, anti-bacterial, and anti-oxidant (Randjelović et al., 2015).

Table 1. Quantitative analysis of natural compounds of RP leaf and flowers (µg/g extract)

No	Compound	RT	RPL	RPF
1	Gallic acid	3.23	3.39	4.37
2	Protocatechuic acid	6.08	-	2.46
3	Chlorogenic acid	7.11	-	1.10
4	Hydroxybenzaldeyde	7.60	1.46	1.96
5	Caffeic Acid	7.77	-	1.11
6	Syringic acid	9.11	24.78	19.23
7	Vanillin	9.26	2.12	2.49
8	o-coumaric acid	9.39	1.26	1.32
9	Salicylic Acid	9.54	14.86	26.41
10	*Trans*-ferulic acid	10.12	5.13	4.019
11	Sinapic acid	10.77	2.79	2.56
12	p-coumaric acid	11.54	1.35	1.30
13	Hesperidin	11.84	-	67.29
14	Isoquercitrin	11.81	-	4.30
15	Rutin	13.09	-	199.74
16	Kaempferol-3-glucoside	13.29	-	11.05
17	Fisetin	13.44	1.95	2.19
18	Naringenin	15.07	3.47	3.58
19	Hesperetin	15.87	3.08	3.01
20	Kaempferol	16.12	10.30	10.69
21	Luteolin	17.88	10.14	-
22	Diosgenin	23.56	1.48	-

3.2. Molecular Docking Result

According to the LC-MS/MS analysis results; syringic acid and salicylic acid are the common and most abundant compounds in leaves and flowers. In the literature, RP plant extracts have been reported to have anti-alzheimer and antibacterial activity (Vítková et al., 2017). Therefore, the BChE (anti-Alzheimer) and DD peptidase (antibacterial) inhibitory properties of syringic acid and salicylic acid molecules, which are the main components of the RP plant extract, were investigated (Figure 2-Figure 3).

Syringic acid molecule interacted with BChE; Five conventional hydrogen bonds with amino acid THR120, TYR128, GLU197, TRP82, one carbon-hydrogen bond with amino acid TRP82, one pi-sigma with amino acid TRP82, pi-pi-stacked with amino acid TRP82, alkyl with amino acid with LEU125 and pi-alkyl amino acid with TRP82 (Figure 2-Table2). Syringic acid with BChE was calculated as a MolDock score of -79.38, with binding energies of -5.90 kcal/mol.

Table 2. Interaction categories, types, and distances of molecular insertion of the syringic acid molecule with BChE

No	Name	Distance	Category	Type	Transmitter	From Chemistry	Receiver	To Chemistry
1	A:THR120:OG1- :[001:O4	3.02952	Hydrogen Bond	Conventional Hydrogen Bond	A:THR120:OG1	H-Donor	:[001:O4	H-Acceptor
2	A:THR120:OG1- :[001:O5	3.14928	Hydrogen Bond	Conventional Hydrogen Bond	A:THR120:OG1	H-Donor	:[001:O5	H-Acceptor
3	A:TYR128:OH - :[001:O3	3.09903	Hydrogen Bond	Conventional Hydrogen Bond	A:TYR128:OH	H-Donor	:[001:O3	H-Acceptor
4	:[001:H3 - A:GLU197:OE1	2.09025	Hydrogen Bond	Conventional Hydrogen Bond	:[001:H3	H-Donor	A:GLU197:OE1	H-Acceptor
5	:[001:H7 - A:TRP82:O	2.06705	Hydrogen Bond	Conventional Hydrogen Bond	:[001:H7	H-Donor	A:TRP82:O	H-Acceptor
6	:[001:H5 - A:TRP82:O	2.62529	Hydrogen Bond	Carbon Hydrogen Bond	:[001:H5	H-Donor	A:TRP82:O	H-Acceptor
7	A:TRP82:CB - :[001	3.88238	Hydrophobic	Pi-Sigma	A:TRP82:CB	C-H	:[001	Pi-Orbitals
8	A:TRP82 - :[001	4.99669	Hydrophobic	Pi-Pi Stacked	A:TRP82	Pi-Orbitals	:[001	Pi-Orbitals
9	A:TRP82 - :[001	4.97275	Hydrophobic	Pi-Pi Stacked	A:TRP82	Pi-Orbitals	:[001	Pi-Orbitals
10	:[001:C8 - A:LEU125	3.44254	Hydrophobic	Alkyl	:[001:C8	Alkyl	A:LEU125	Alkyl
11	A:TRP82 - :[001:C9	5.26866	Hydrophobic	Pi-Alkyl	A:TRP82	Pi-Orbitals	:[001:C9	Alkyl
12	A:TYR128 - :[001:C8	5.01949	Hydrophobic	Pi-Alkyl	A:TYR128	Pi-Orbitals	:[001:C8	Alkyl

Syringic acid molecule interacted with DD peptidase; Three carbon-hydrogen bonds with amino acid VAL302, SER326, THR123, one pi sigma with amino acid PHE120, one amide-pi stacked with amino acid VAL302, and one pi-alkyl with amino acid TYR306 (Figure 2-Table 3). Syringic acid with DD peptidase was calculated as a MolDock score of -71.25, with binding energies of -5.40 kcal/mol.

Table 3. Interaction categories, types, and distances of molecular insertion of the syringic acid molecule with DD peptidase

No	Name	Distance	Category	Type	Transmitter	From Chemistry	Receiver	To Chemistry
1	A:VAL302:HA - :[001:O2	2.73206	Hydrogen Bond	Carbon Hydrogen Bond	A:VAL302:HA	H-Donor	:[001:O2	H-Acceptor
2	A:SER326:HB2 - :[001:O1	2.43792	Hydrogen Bond	Carbon Hydrogen Bond	A:SER326:HB2	H-Donor	:[001:O1	H-Acceptor
3	:[001:H9 - A:THR123:O	2.35551	Hydrogen Bond	Carbon Hydrogen Bond	:[001:H9	H-Donor	A:THR123:O	H-Acceptor
4	:[001:H7 - A:PHE120	2.82865	Hydrophobic	Pi-Sigma	:[001:H7	C-H	A:PHE120	Pi-Orbitals
5	A:VAL302:C,O;GLN303:N-:[001	4.56129	Hydrophobic	Amide-Pi Stacked	A:VAL302:C,O;GLN303:N	Amide	:[001	Pi-Orbitals
6	A:TYR306 - :[001:C1	5.1516	Hydrophobic	Pi-Alkyl	A:TYR306	Pi-Orbitals	:[001:C1	Alkyl

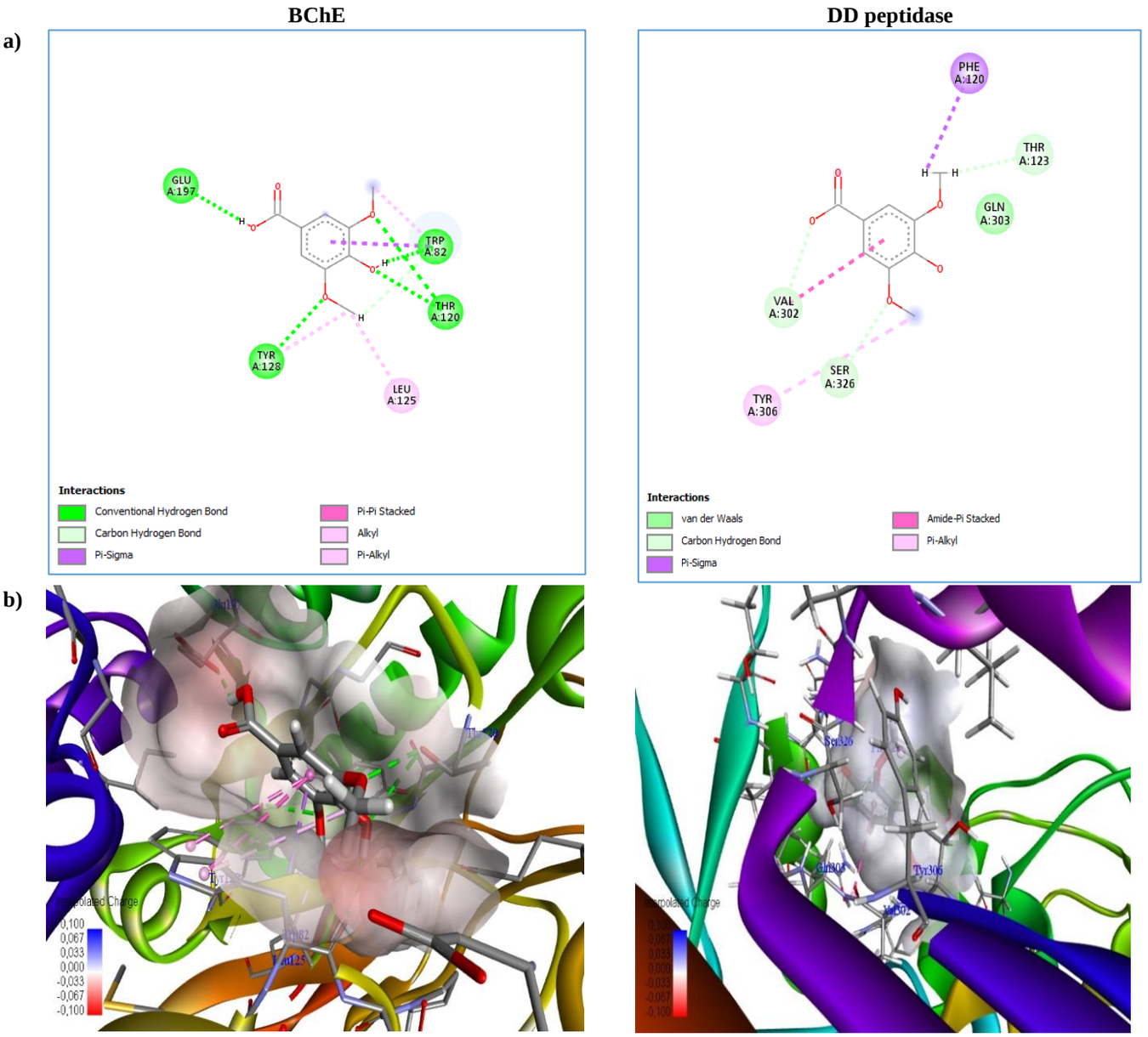

Figure 2. Syringic acid interaction with BChE and DD peptidase, a) 2D images b) interpolated load view

Salicylic acid molecule interacted with BChE; Five conventional hydrogen bonds with amino acid GLY116, GLY117, SER198, HIS438, three pi-pi-t shaped with amino acid TRP231, PHE329, and pi-alkyl with amino acid LEU286 (Figure 3-Table 4). Salicylic acid with BChE was calculated as a MolDock score of -63.54, with binding energies of -6.10 kcal/mol.

Table 4. Interaction categories, types, and distances of molecular insertion of the salicylic acid molecule with BChE

No	Name	Distance	Category	Type	Transmitter	From Chemistry	Receiver	To Chemistry
1	A:GLY116:N - :[001:O1	3,03493	Hydrogen Bond	Conventional Hydrogen Bond	A:GLY116:N	H-Donor	:[001:O1	H-Acceptor
2	A:GLY117:N - :[001:O1	2,87395	Hydrogen Bond	Conventional Hydrogen Bond	A:GLY117:N	H-Donor	:[001:O1	H-Acceptor
3	:[001:H5 - A:SER198:OG	2,41903	Hydrogen Bond	Conventional Hydrogen Bond	:[001:H5	H-Donor	A:SER198:OG	H-Acceptor
4	:[001:H5 - A:HIS438:NE2	1,85719	Hydrogen Bond	Conventional Hydrogen Bond	:[001:H5	H-Donor	A:HIS438:NE2	H-Acceptor
5	:[001:H6 - A:SER198:OG	1,7192	Hydrogen Bond	Conventional Hydrogen Bond	:[001:H6	H-Donor	A:SER198:OG	H-Acceptor
6	A:TRP231 - :[001	4,97577	Hydrophobic	Pi-Pi T-shaped	A:TRP231	Pi-Orbitals	:[001	Pi-Orbitals
7	A:TRP231 - :[001	4,92386	Hydrophobic	Pi-Pi T-shaped	A:TRP231	Pi-Orbitals	:[001	Pi-Orbitals
8	A:PHE329 - :[001	5,68254	Hydrophobic	Pi-Pi T-shaped	A:PHE329	Pi-Orbitals	:[001	Pi-Orbitals
9	:[001 - A:LEU286	5,07008	Hydrophobic	Pi-Alkyl	:[001	Pi-Orbitals	A:LEU286	Alkyl

Salicylic acid molecule interacted with DD peptidase; two conventional hydrogen bonds with amino acid GLN303, THR123, one carbon-hydrogen bond with amino acid THR123, and one pi-pi-t shaped with amino acid THR123 (Figure 3- Table 5). Salicylic acid with DD peptidase was calculated as a MolDock score of -66.18, with binding energies of -5.70 kcal/mol.

4. CONCLUSION

RP, also known as white-flowered false acacia; is distributed in Northern Anatolia in Turkey. RP's different extracts have been reported to have antibacterial activity and BChE enzyme inhibitory properties. RP flowers and leaf was extracted with methanol and analyzed by LC-MS/MS to determine their phytochemical content. According to the LC-MS/MS result, salicylic acid and syringic acid were the main components. The interactions of syringic acid and salicylic acid molecules with BChE and DD-phosphate enzymes were calculated theoretically by molecular docking application. The binding affinity of the molecular interaction of the salicylic acid molecule with BChE was determined to be higher than that of syringic acid. However, the mold lock score of the molecular interaction of the salicylic acid molecule with BChE was observed to be lower than that of the syringic acid molecule. Moreover, the binding affinity of the molecular interaction of the salicylic acid molecule with DD peptidase was observed to be higher than that of the syringic acid molecule, and the MolDock score was lower. As a result, salicylic acid has higher BChE and DD peptidase enzyme binding affinity. Therefore, the BChE and DD peptidase inhibitory properties of these molecules can be investigated *in vitro*.

Table 5. Interaction categories, types, and distances of molecular insertion of the salicylic acid molecule with DD peptidase

No	Name	Distance	Category	Type	Transmitter	From Chemistry	Receiver	To Chemistry
1	A:GLN303:HE22-:[001:O2	2,25256	Hydrogen Bond	Conventional Hydrogen Bond	A:GLN303:HE22	H-Donor	:[001:O2	H-Acceptor
2	:[001:H5 - A:THR123:O	2,23203	Hydrogen Bond	Conventional Hydrogen Bond	:[001:H5	H-Donor	A:THR123:O	H-Acceptor
3	A:THR123:HA - :[001:O1	2,73968	Hydrogen Bond	Carbon Hydrogen Bond	A:THR123:HA	H-Donor	:[001:O1	H-Acceptor
4	A:PHE120 - :[001	5,19155	Hydrophobic	Pi-Pi T-shaped	A:PHE120	Pi-Orbitals	:[001	Pi-Orbitals

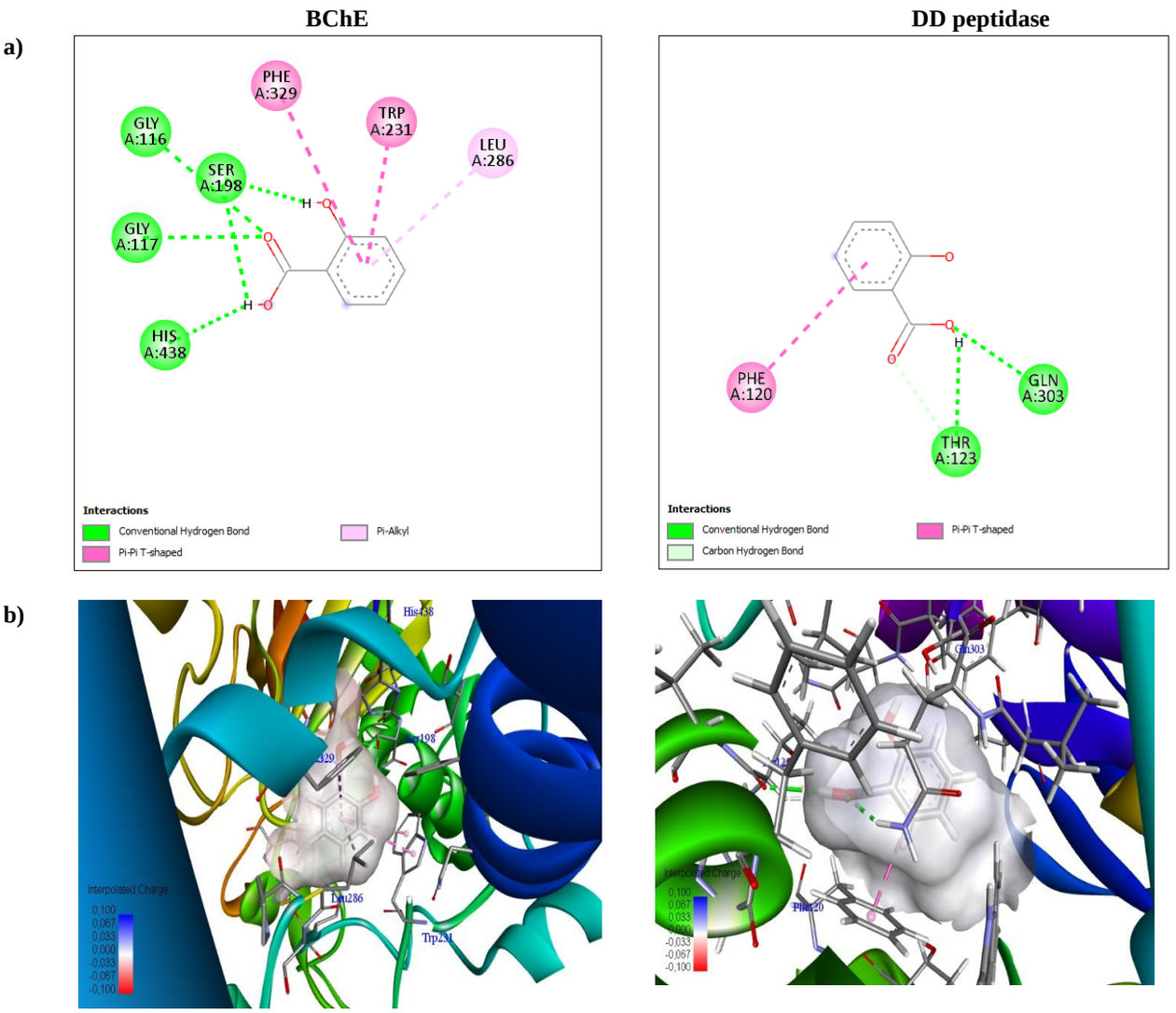

Figure 3. Salicylic acid interaction with BChE and DD peptidase a) 2D images b) interpolated load view

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