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Phytochemical Content Analysis of Different *Lavandula Officinalis* Extracts by LC-ESI-MS/MS and In Silico Molecular Docking Studies

Farklı *Lavandula Officinalis* Ekstraktlarının LC-ESI-MS/MS ile Fitokimyasal İçerik Analizi ve İn Siliko Moleküler Yerleştirme Çalışmaları

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Abstract

Lavandula officinalis (lavender) is an evergreen, shrub-like, flowering, and perennial plant species that is generally distributed in the Mediterranean region. Lavender is rich in secondary metabolites such as essential oil, tannins, anthocyanins, minerals, saponins, flavonoids, and phenolic acids. Secondary metabolites in plants show many biological activities such as antioxidant, antimutagenic, anticarcinogenic, anti-inflammatory, anticancer, antiproliferative, and antimicrobial. In this study, the lavender plant of aboveground part was extracted with 5 different solvents (hexane, chloroform, ethyl acetate, methanol, and water). The resulting extraction was analyzed for phytochemical content by LC-ESI-MS/MS. According to the analysis results, it was seen that the main component of 5 different extracts was the coumarin compound. The interactions of the coumarin compound, determined as the main component of lavender, which is known to have antibacterial and anticancer activity in the literature, with anticancer (topoisomerase II alpha) and antibacterial (glucosamine-6-phosphate) enzymes were calculated theoretically by the molecular docking (MolDock) method. As a result, the moldock score (82.55, 60.26) and binding energies (5.9 kcal mol^{-1}, 6.2 kcal mol^{-1}) from the interactions of the coumarin compound with topoisomerase II alpha, and glucosamine 6-phosphate enzymes were determined, respectively. Thus, this study may provide insight into in vitro studies on the activity of coumarins against these enzymes.

Keywords: Lavandula officinalis, lavender, coumarin, anticancer, antibacterial, MolDock.

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Özet

Lavandula officinalis (lavanta), genel olarak Akdeniz bölgesinde yayılış gösteren, yapraklarını dökmeyen çalı şeklinde çiçekli çok yıllık bir bitki türüdür. Lavanta, uçucu yağ, tanenler, antosiyaninler, mineraller, saponinler, flavonoidler ve fenolik asitler gibi sekendor metebolitler açısından zengin içeriğe sahiptir. Bitkilerdeki sekonder metebolitler, antioksidan, antimutajenik, antikarsinojenik, antiinflamatuar, antikanser, antiproliferatif ve antimikrobiyal gibi birçok biyolojik aktivite göstermektedir. Çalışmamızda lavanta bitkisinin 5 farklı çözücü (hekzan, kloroform, etil asetat, metanol ve su) ile ekstraktsiyonu yapıldı. Elde edilen ekstraksiyonlar LC-ESI-MS/MS ile fitokimyasal içerik analizi yapılmıştır. Analiz sonucuna göre 5 farklı ekstrakta ana bileşenin kumarin olduğu görüldü. Literatürde antibakteriyel ve antianser aktiviteye sahip olduğu bilinen lavantadaki ana bileşen olarak belirlenen kumarin bileşiğinin antikanser (topoizomeraz II, alfa) ve antibakteriyel (glukozamin-6-fosfat) enzimlerle etkileşimleri moleküler modelleme metodu ile teorik olarak hesaplandı. Sonuç olarak kumarin bileşiğinin, topoizomeraz II alfa ve glukozamin 6-fosfat enzimleriyle etkileşimlerinin moldock skoru (82.55, 60.26) ve bağlanma enerjileri (5.9 kcal mol^{-1}, 6.2 kcal mol^{-1}) sırasıyla belirlendi. Böylece bu çalışma ile kumarin bileşiğinin bu enzimlere karşı aktivitesinin *in vitro* çalışmalara fikir verebileceği düşünülmektedir.

Anahtar Kelimeler: *Lavandula officinalis*, lavanta, kumarin, antikanser, antibakteriyel, MolDock.

1. Introduction

Secondary metabolites are compounds found in plants that do not play a role in growth and development, but provide the plant with defense against pests, competition, or adaptation to environmental conditions (1). Secondary metabolites are divided into groups such as alkaloids, terpenoids, phenolics, flavonoids, glycosides, phenolic acids, and iridoids (2). Commonly found in plant roots, leaves, flowers, or fruits. Many plant species rich in secondary metabolites (phytochemicals) are used in traditional medicine due to their properties such as analgesic, antipyretic (fever reducer) or antimicrobial, anti-inflammatory, antiviral, antiallergic, and anticancer (3). Medicinal aromatic plants are plants that contain fragrant essential oils. These plants are used for medicinal or therapeutic purposes and are widely used in fields such as aromatherapy, herbal medicine, traditional medicine, and cosmetics (4, 5).

Lavandula officinalis is a species from the Mediterranean region and is a perennial shrub that is widely grown especially in countries such as France, Spain, and Italy. *L. officinalis* is widely used in a variety of medical, cosmetic, and aromatherapy applications (6, 7). Lavender is widely used among the public due to its effects such as aromatherapy, skin care, digestive problems, sleep, and mental relaxation. Lavender oil, in addition to its stress-reducing, increasing mental relaxation, sleep-regulating, anti-inflammatory, and antiseptic properties. It is widely used in the treatment of disorders such as nausea, digestive disorders, and flatulence (8). The main components of lavender oil have been reported to be linalool, lavandulol, campfer, 1,8-cineole, borneol and terpinen-4-ol (9). In terms of phenolic content, rosmarinic acid, kaempferol, quercetin, coumarin and apigenin are the main components (10). Lavender has been reported to have anti-inflammatory, antioxidant, anticancer, antimicrobial, anxiolytic, relaxing, and analgesic properties (11).

Enzyme inhibition is the phenomenon of reducing or completely stopping the activity of an enzyme. Molecules or compounds that change the activity of the enzyme are called enzyme inhibitors. In the treatment of many diseases, the activity of target enzymes must be inhibited (12). Molecules with biological activity can be designed as inhibitors of specific enzymes in the drug development process and used in the treatment of diseases (13, 14). Molecular docking (MolDock) is a computational method used to predict the binding position and interaction of a drug candidate or a molecule with a receptor molecule using computer simulations. MolDock provides ideas for the design of new drugs by predicting molecule-enzyme interactions in the laboratory environment, without applying biological activity (15, 16). Topoisomerase II alpha; It is involved in solving problems that occur during DNA replication, transcription and chromosome condensation (17). Inhibition of the enzyme topoisomerase II alpha is an important target for cancer treatment, and drugs that inhibit the activity of this enzyme are often used in the treatment of various types of cancer (18). Glucosamine 6-phosphate, which plays an active role in the cell wall synthesis of bacteria, is involved in many mechanisms of antibacterial drugs. Thus, antibacterial compounds or drugs can interfere with its role in cell wall synthesis by inhibiting glucosamine 6-phosphate synthesis (19). *L. officinalis* is known for its anti-cancer and antibacterial effects. The aim was to investigate the effectiveness of the main ingredients in the content analysis on these activities.

In our study, the phytochemical content of 5 different extracts (hexane, chloroform, ethyl acetate, methanol, and water) of *L. officinalis* plant was determined by LC-MS/MS device. The interactions of the coumarin compound detected in the highest amount in *L. Officinalis* extracts according to the LC-MS/MS analysis results with antibacterial (glucosamine-6-phosphate) and anticancer (Topoisomerase II alpha) enzyme were theoretically calculated with the MolDock application.

2. Material and Method

2.1. Plants

L. officinalis, cultivated in the honey forest of Iğdır University Şehit Bülent Yurtseven Campus; was harvested in July 2023. It was identified by Professor Zafer Tel of Botany Department Iğdır University, then dried in an airy and shaded environment.

2.2. Obtaining crude extract

To obtain the crude extract, 20 g of ground plants were macerated in hexane, chloroform, ethyl acetate, methanol, and water for 1 week each in a 1-liter volumetric flask. Then the solvent extract mixture was filtered and the solvent was evaporated in a rotary evaporator. Thus, 5 different extracts were obtained.

2.3. LC- ESI-MS/MS analysis

The phenolic content in hexane, chloroform, ethyl acetate, methanol, and water extracts from L. Officinalis was determined by LC-ESI-MS/MS analysis as explained in our previously published article (20, 21). The results were compared with 43 phenolic standards on the database of the LC-ESI-MS/MS device.

2.4. Molecular docking application

3D structure, and minimum energy of the coumarin were drawn in the ChemDraw program. The enzymes chosen for this docking investigation were glucosamine 6-phosphate (PDB; 1MOQ), topoisomerase II alpha (PDB; 1ZXM), and interactions with coumarin were determined using the Molegro Virtual Docker (MVD) program. 2D and 3D images of the interactions were taken with the BIOVIA Discovery Studio Visualizer program and the AutoDock Vina program was used to calculate the binding affinities (22, 23).

3. Results and Discussion

3.1. Phytochemical content of extracts

Five different crude extracts were obtained by extraction of lavender with different solvents. The different extracts obtained were analyzed by LC-ESI-MS/MS. As a result of the analysis, an in silico Moldock study of coumarin, which was major component detected in 5 different extracts, was performed.

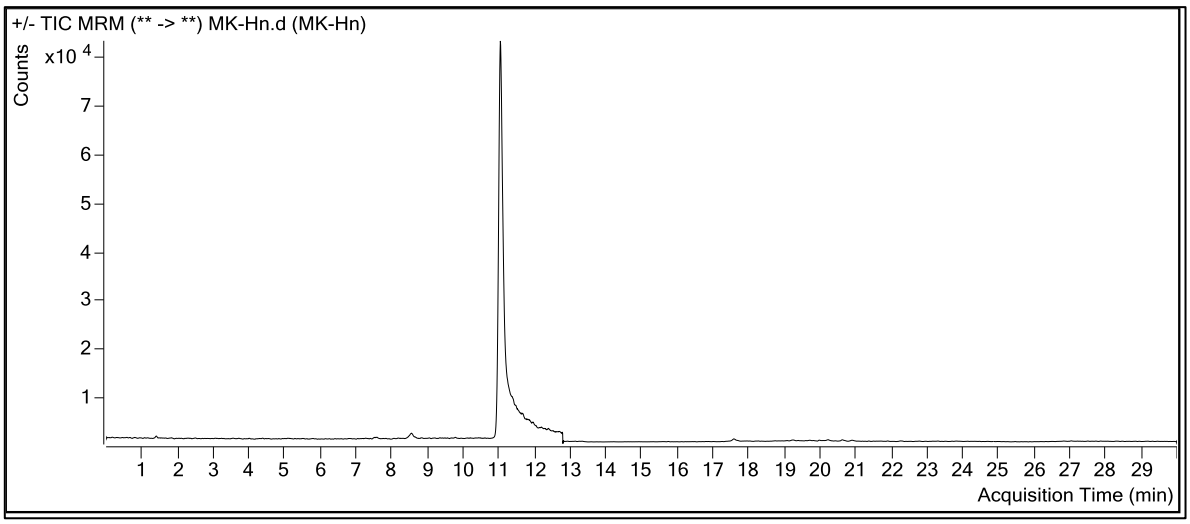

Figure 1. LC-ESI-MS/MS chromatogram of hexane extract

According to LC-ESI-MS/MS analysis results in the hexane extract; coumarin (6214.53 µg g^{-1} extract), syringic acid (18.41 µg g^{-1} extract), and salicylic acid (16.72 µg g^{-1} extract) compounds were detected most (Figure 1-Table 1).

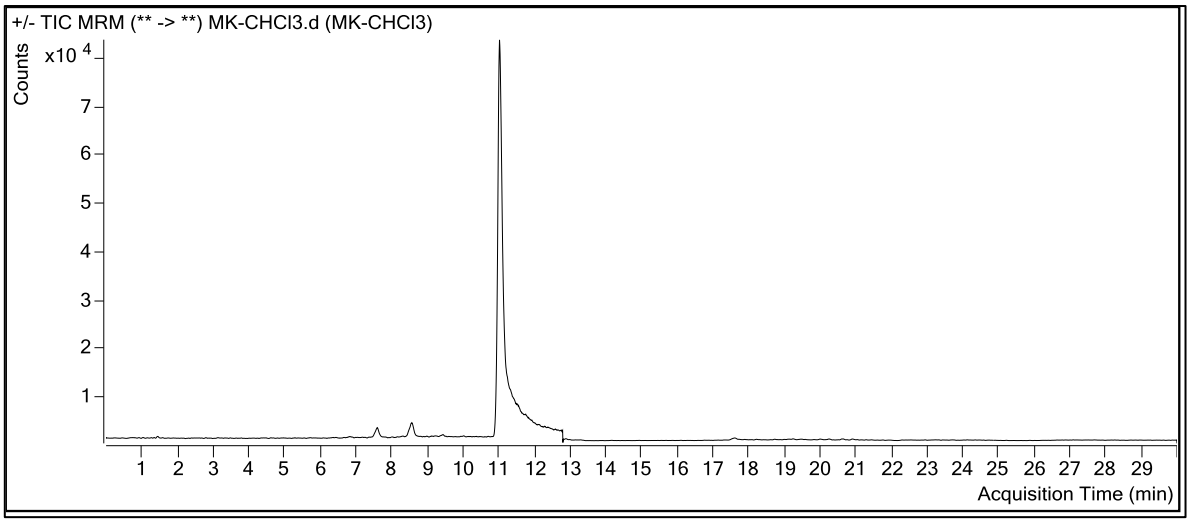

Figure 2. LC-ESI-MS/MS chromatogram of chloroform extract

According to LC-ESI-MS/MS analysis results in the chloroform extract; Coumarin (6763.59 µg g^{-1} extract), syringic acid (41.80 µg g^{-1} extract), and salicylic acid (36.70 µg g^{-1} extract) were main compounds determined in the chloroform extract (Figure 2-Table 1).

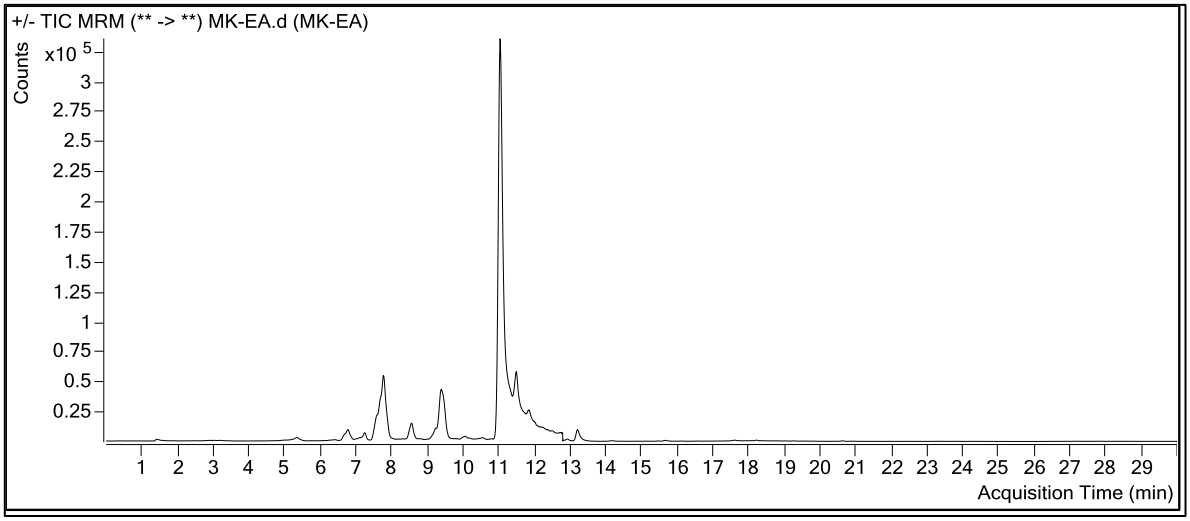

Figure 3. LC-ESI-MS/MS of ethyl acetate extract

According to LC-ESI-MS/MS analysis results in the ethyl acetate extract; Coumarin (27549.75 µg g^{-1} extract), caffeic acid (1638.10 µg g^{-1} extract), salicylic acid (1361.83 µg g^{-1} extract), syringic acid (958.25 µg g^{-1} extract), p-coumaric acid (686.31 µg g^{-1} extract), trans-ferulic acid (677.07 µg g^{-1} extract) vanilic acid (624.17 µg g^{-1} extract), o-coumaric acid (223.95 µg g^{-1} extract), hydroxybenzaldeyde (202.36 µg g^{-1} extract) and protocatechuic acid (142.08 µg g^{-1} extract) were determined in high amounts in the ethyl acetate extract (Figure 3- Table 1).

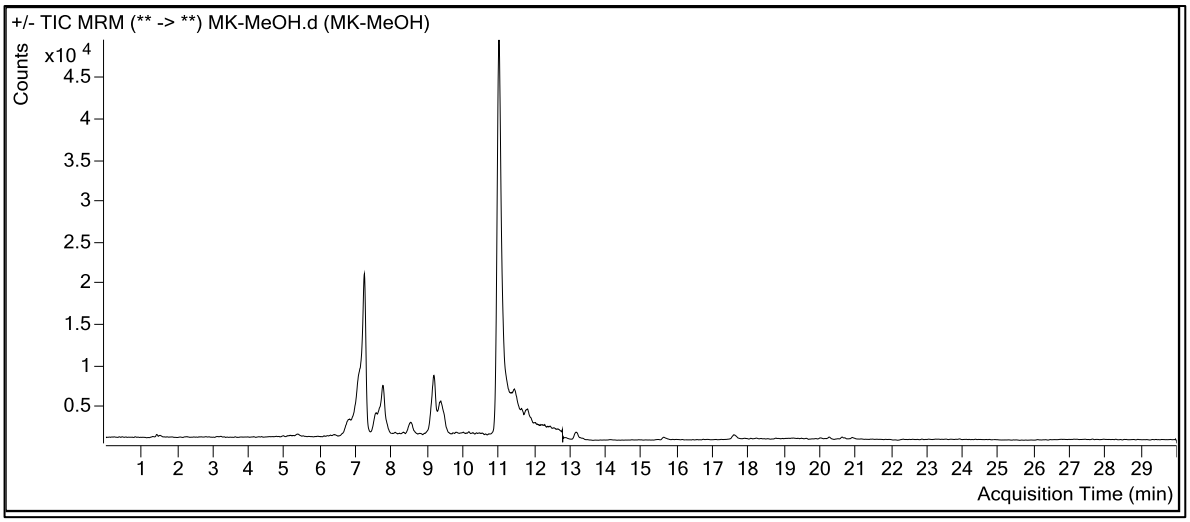

Figure 4. LC-ESI-MS/MS of methanol extract

According to LC-ESI-MS/MS analysis results in the methanol extract; Coumarin (3346.99 µg g^{-1} extract), caffeic acid (154.03 µg g^{-1} extract), salicylic acid (124.12 µg g^{-1} extract) and syringic acid (95.80 µg g^{-1} extract) were detected in high amounts (Figure 4-Table 1).

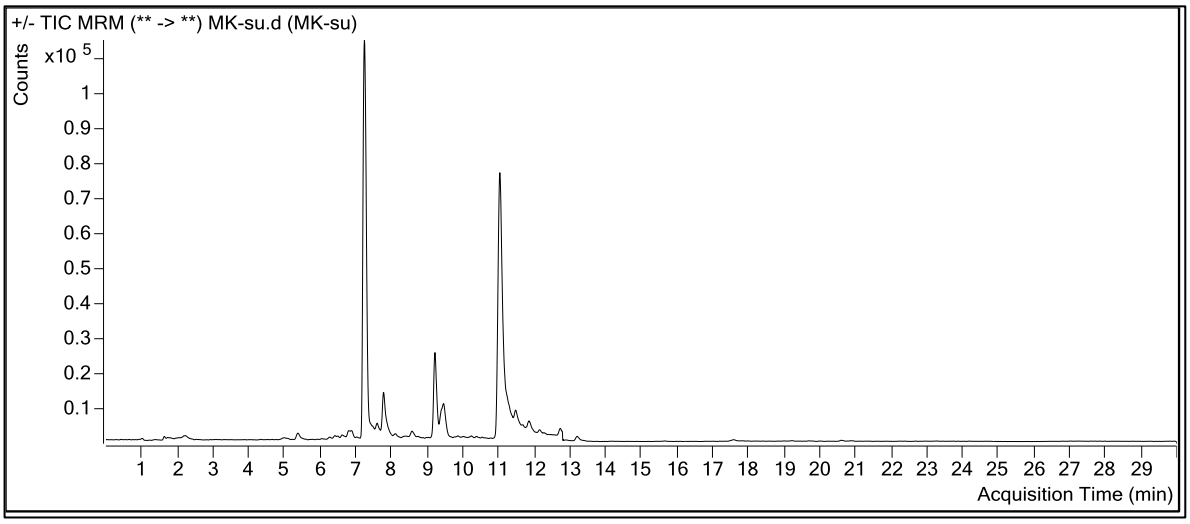

Figure 5. LC-ESI-MS/MS of water extract

According to LC-ESI-MS/MS analysis results of the water extract; Coumarin (3683.85 µg g^{-1} extract), salicylic acid (324.10 µg g^{-1} extract) shikimic acid (254.28 µg g^{-1} extract) caffeic acid (222.74 µg g^{-1} extract), scutellarin (212.99 µg g^{-1} extract) and syringic acid (107.89 µg g^{-1} extract) were observed in high amounts (Figure 5-Table 1). In the phenolic content analysis, the highest percentage in terms of phenolic content was seen in ethyl acetate extraction.

Table 1. Phenolic content analysis of *L. officinalis* extracts (µg g^{-1} extract)

No	Compound	RT	Hexane	Chloroform	Ethyl acetate	Methanol	Water
1	Shikimic acid	1.40	-	-	-	-	254.28
2	Gallic acid	3.21	3.25	3.29	10.84	4.73	3.65
3	Protocatechuic acid	5.38	-	-	142.08	10.77	48.47
4	Epigallocatechin	6.82	-	-	-	-	-
5	Catechin	6.77	-	-	-	-	1.38
6	Chlorogenic acid	7.39	-	-	1.12	0.87	6.27
7	Hydroxybenzaldeyde	7.57	4.55	23.77	202.36	29.49	26.51
8	Vanillic acid	7.85	-	-	624.17	-	-
9	Caffeic Acid	7.76	1.07	1.16	1638.10	154.03	222.74
10	Syringic acid	8.31	18.41	41.80	958.25	95.80	107.89
11	Caffeine	8.45	-	-	-	-	-
12	Vanillin	8.54	10.61	26.47	111.73	14.69	13.92
13	o-coumaric acid	9.35	1.22	1.64	223.95	21.13	30.89
14	Salicylic Acid	9.42	16.72	36.70	1361.83	124.12	324.10
15	Morin	9.68	-	-	-	-	-
16	Resveratrol	9.85	-	-	-	-	6.23
17	Polydatine	9.81	-	-	-	-	-
18	Trans-ferulic acid	10.17	4.24	17.43	677.07	49.65	42.76
19	Sinapic acid	10.49	2.53	2.84	4.67	2.57	3.48
20	Coumarin	11.01	6214.53	6763.59	27549.75	3346.99	3683.85
21	Scutellarin	11.27	-	-	8.30	-	212.99
22	p-coumaric acid	11.45	2.03	2.52	686.31	46.76	79.07
23	Protocatehuic ethyl ester	11.55	-	-	-	-	-
24	Hesperidin	11.83	-	-	-	-	3.76
25	Isoquercitrin	11.82	-	-	72.37	6.33	19.34
26	Rutin	12.29	-	-		-	-
27	Quercetin-3-xyloside	12.44	-	-	-	-	-
28	Kaempferol-3-glucoside	13.17	-	-	36.46	3.89	5.22
29	Fisetin	13.14	1.95	1.97	2.67	1.97	1.97
30	Baicalin	13.72	-	-	-	-	-
31	Chrysin	14.21	-	-	-	-	-
32	Trans-cinnamic acid	14.26	-	-	4.36	-	-
33	Quercetin	14.83	-	-	-	-	-
34	Naringenin	14.97	3.46	3.54	4.75	4.01	3.51
35	Hesperetin	15.73	3.07	3.02	3.01	3.02	3.02
36	Catechin	15.81	-	-	-	-	-
37	Kaempferol	16.46	10.29	11.28	10.64	12.00	11.43
38	Baicalein	17.07	-	-	-	-	-
39	Luteolin	17.90	-	-	-	-	-
40	Biochanin A	17.96	-	-	-	-	-
41	Capsaicin	18.23	-	-	-	-	-
42	Dihydrocapcaicin	18.79	-	-	-	-	-
43	Diosgenin	23.40	1.48	0.41	0.50	0.58-	0.54

According to the content analysis of 5 different extractions, coumarin, salicylic acid, and syringic acid were found to be the main components in all extracts. According to the LC-ESI-MS/MS analysis results, the coumarin compound was determined in the highest amount in 5 different extracts. Coumarin compound, which is the main component of the *L. officinalis* plant with different biological activities; Its interactions with anticancer (topoisomerase II alpha) and antibacterial (glucosamine 6-

phosphate) enzymes were investigated. Coumarin found naturally in some plants, has been reported to have beneficial effects on human health such as antibacterial, antioxidant, antitumor, and anticancer activities (24, 25).

3.2. Molecular docking results

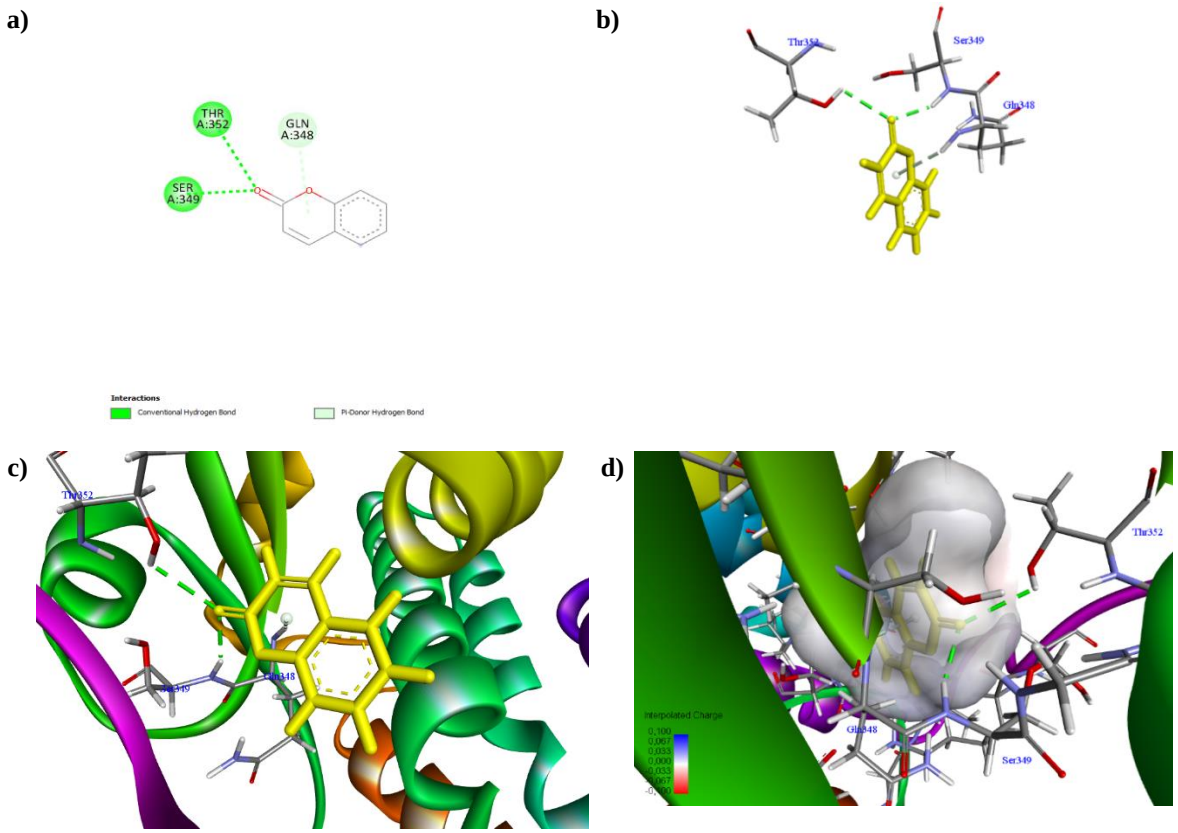

Figure 6. Coumarin-glucosamine 6-phosphate synthase interaction a) 2D images b) general view c) 3D images d) interpolated load view

Coumarin molecule interacted with glucosamine 6-phosphate synthase by two conventional-hydrogen bonds formed with amino acid SER349, THR352, and one pi-donor hydrogen bond with amino acid GLN348 (Figure 6–Table 2).

Table 2. Interaction categories, types, and distances of molecular insertion of the coumarin molecule with glucosamine 6-phosphate

No	Name	Distance	Category	Type	Transmitter	From Chemistry	Receiver	To Chemistı
1	A:SER349:HN -:[001:O2	2.0206	Hydrogen Bond	Conventional Hydrogen Bond	A: SER349:HN	H-Donor	:[001:O2	H-Acceptor
2	A:THR352:HG1 -:[001:O2	2.5842	Hydrogen Bond	Conventional Hydrogen Bond	A: THR352:HG1	H-Donor	:[001:O2	H-Acceptor
3	A:GLN348:HN -:[001	2.9625	Hydrogen Bond	Pi-Donor Hydrogen Bond	A: GLN348:HN	H-Donor	:[001	Pi-Orbitals

Coumarin molecule with glucosamine 6-phosphate synthase interactions was determined as a MolDock score -60.26, binding energies -6.2 kcal mol^{-1}.

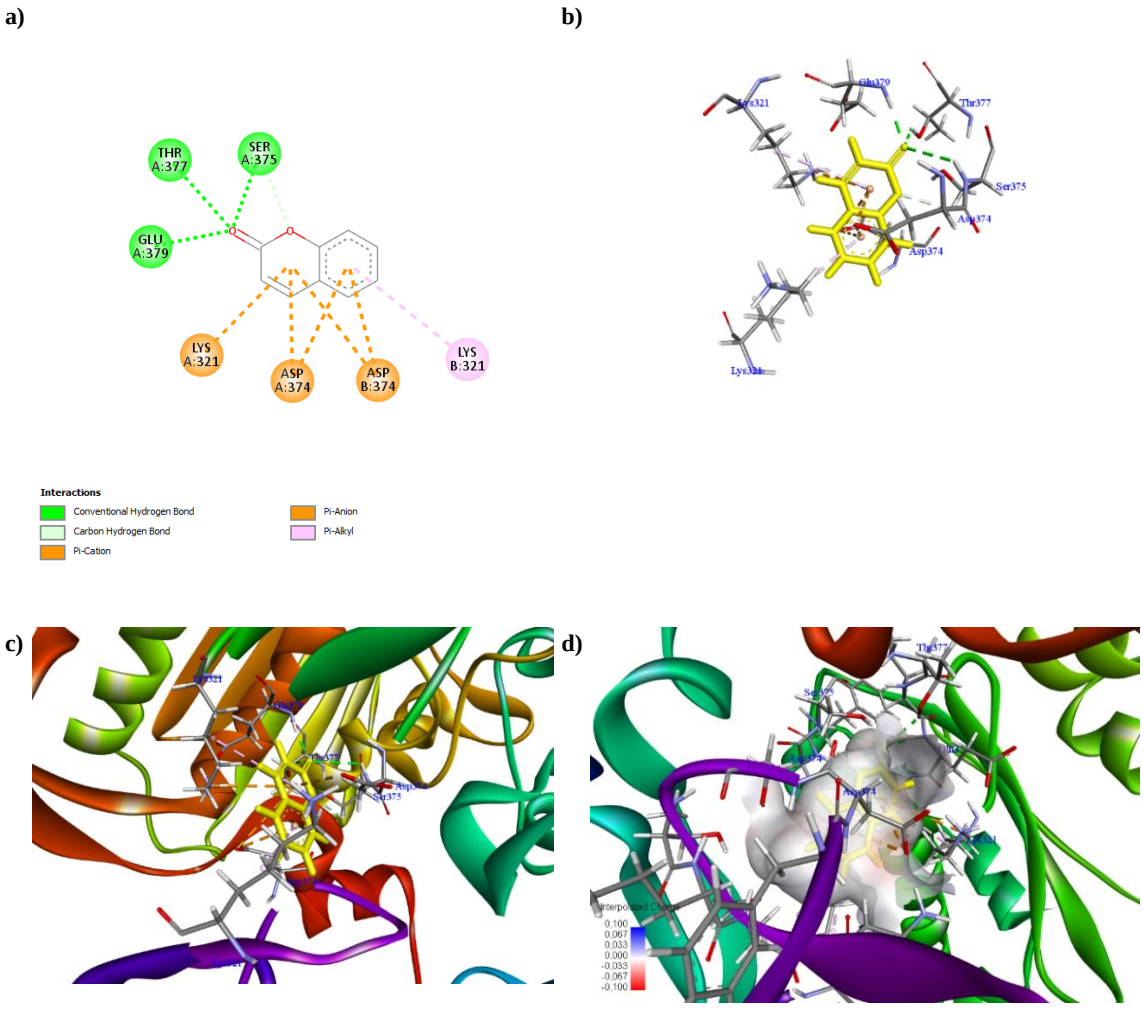

Figure 7. Coumarin-topoisomerase II alpha interaction a) 2D images b) general view c) 3D images d) interpolated load view

Table 3. Interaction categories, types, and distances of molecular insertion of the coumarin with topoisomerase II alpha

No	Name	Distance	Category	Type	Transmitter	From Chemistry	Receiver	To Chemist
1	A:SER375:HN - :[001:O2	2.95712	Hydrogen Bond	Conventional Hydrogen Bond	A: SER375:HN	H-Donor	:[001:O2	H-Acceptor
2	A:THR377:HG1 - :[001:O2	2.36614	Hydrogen Bond	Conventional Hydrogen Bond	A:THR377:HG1	H-Donor	:[001:O2	H-Acceptor
3	A:GLU379:HN - :[001:O2	1.98607	Hydrogen Bond	Conventional Hydrogen Bond	A:GLU379:HN	H-Donor	:[001:O2	H-Acceptor
4	A:SER375:HB2 - :[001:O1	2.77409	Hydrogen Bond	Carbon Hydrogen Bond	A:SER375:HB2	H-Donor	:[001:O1	H-Acceptor
5	A: LYS321:NZ - :[001	4.55168	Electrostatic	Pi-Cation	A: LYS321:NZ	Positive	:[001	Pi-Orbitals
6	A:ASP374:OD2 - :[001	3.83412	Electrostatic	Pi-Anion	A:ASP374:OD2	Negative	:[001	Pi-Orbitals
7	A:ASP374:OD2 - :[001	3.92715	Electrostatic	Pi-Anion	A:ASP374:OD2	Negative	:[001	Pi-Orbitals
8	B:ASP374:OD2 - :[001	3.63606	Electrostatic	Pi-Anion	B:ASP374:OD2	Negative	:[001	Pi-Orbitals
9	B:ASP374:OD2 - :[001	4.55614	Electrostatic	Pi-Anion	B:ASP374:OD2	Negative	:[001	Pi-Orbitals
10	:[001-B:LYS321	5.28446	Hydrophobic	Pi-Alkyl	:[001	Pi-Orbitals	B:LYS321	Alkyl
11	:[001-A:LYS321	4.90323	Hydrophobic	Pi-Alkyl	:[001	Pi-Orbitals	A:LYS321	Alkyl

Coumarin molecule interacted with topoisomerase II alpha by three conventional-hydrogen bonds with amino acid SER375, THR377, GLU379, one carbon-hydrogen bonds with amino acid SER375, one pi-cation with amino acid LYS321, four pi-anion with amino acid ASP374, and two pi-alkyl with amino acid LYS321 (Figure 7–Table 3). Coumarin molecule with topoisomerase II alpha interactions were determined as a MolDock score -82.55, binding energies -5.9 kcal mol^{-1}.

4. Conclusion

L. officinalis plant was extracted with 5 different solvents (hexane, chloroform, ethyl acetate, methanol and water) and the resulting extract was analyzed for phenolic content by LC-ESI-MS/MS. According to the analysis results, the coumarin compound was determined as the main component. Additionally, the interactions of coumarin with topoisomerase II alpha and glucosamine 6-phosphate enzymes were theoretically calculated by in silico MolDock study. Thus, it was concluded that coumarin may be an inhibitor against these enzymes and could be confirmed by *in vitro* studies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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