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RESEARCH ARTICLE **Open Access**

Phytochemical analysis of *Silybum marianum* flowers: Quantitative analysis of natural compounds and molecular docking application

Silybum marianum çiçeklerinin fitokimyasal analizi: Doğal bileşiklerin kantitatif analizi ve moleküler yerleştirme uygulaması

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ABSTRACT

Secondary metabolites in plants, identifying, quantifying, and determining the biological activity of plants enables the use of plants in different fields such as pharmacology, food, and cosmetics. Different chromatographic methods such as GC-MS/MS (volatile compounds, fatty acid) and LC-MS/MS (phenolic compounds) are used to identify and quantify these secondary metabolites. *Silybum marianum* is a member of the Asteraceae family and grows naturally. It is known among the public by names such as Thistle, Virgin Mary Thorn, and Milky Kengel. In this study, *S. marianum* hexane extract was analyzed by GC-MS/MS, and the methanol-chloroform (1:1 v/v) extract was analyzed by LC-MS/MS. Palmitic acid methyl ester (17.96%), linoleic acid methyl ester (14.20%), and sesquicineole (10.22%) were determined by GC-MS/MS. Moreover, LC-MS/MS analysis resulted in the quantification of chlorogenic acid (250.171 µg/g extract), salicylic acid (234.95 µg/g extract), isoquercitrin (210.65 µg/g extract), and rutin (102.05 µg/g extract). According to the analysis results, palmitic acid and chlorogenic acid were detected as the main components of fatty acid and phenolic compound respectively. Molecular docking was applied to determine their interaction with the urease enzyme. Palmitic acid and chlorogenic acid interaction with urease were calculated as a MolDock score of -104.63, and -113.21, with binding energies of -3.70, and -6.50 kcal/mol respectively. According to the results, chlorogenic acid may be a urease enzyme inhibitor.

ÖZ

Bitkilerdeki sekonder metabolitler, Bitkilerin biyolojik aktivitesinin tanımlanması, ölçülmesi ve belirlenmesi, bitkilerin farmakoloji, gıda ve kozmetik gibi farklı alanlarda kullanılmasına olanak sağlamaktadır. Bu ikincil metabolitleri tanımlamak ve ölçmek için GC-MS/MS (esansiyel yağ, yağ asidi) ve LC-MS/MS (fenolik bileşikler) gibi farklı kromatografik yöntemler kullanılır. *Silybum marianum* Asteraceae familyasının bir üyesidir ve doğal olarak yetişir. Halk arasında Devedikeni, Meryem Dikeni, Sütlü Kengel gibi isimlerle tanınır. Bu çalışmada, *S. marianum* heksan ekstraktı GC-MS/MS ile, metanol-kloroform (1:1 v/v) ekstraktı ise LC-MS/MS ile analiz edildi. Palmitik asit metil ester (%17.96), linoleik asit metil ester (%14.20), seskisinol (%10.22) bileşikleri GC-MS/MS ile belirlendi. Ayrıca LC-MS/MS analizi, klorojenik asit (250.171 µg/g ekstrakt), salisilik asit (234.95 µg/g ekstrakt), izokersitrin (210.65 µg/g ekstrakt) ve rutinin (102.05 µg/g ekstrakt) miktarlarının belirlenmesiyle sonuçlandı. Analiz sonuçlarına göre ana bileşenler olarak sırasıyla yağ asidi olarak palmitik asit ve fenolik bileşik olarak klorojenik asit belirlendi. Üreaz enzimi ile etkileşimlerini belirlemek için moleküler doking uygulandı. Palmitik asit ve klorojenik asitin üreaz ile etkileşimi, sırasıyla -103,16 ve -113,21 olarak belirlendi. Bağlanma enerjisi ise sırasıyla -3.70 ve -6.50 kcal/mol olarak hesaplandı. Sonuçlara göre klorojenik asit bir üreaz enzim inhibitörü olabilir.

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1. INTRODUCTION

Throughout human history, plants have been used for food, fuel, and pharmaceutical purposes (Elmastas et al., 2004; Topçu et al., 1999). Medicinal plants used in traditional treatments have become known as alternative medicine with the development of modern medicine (Erenler et al., 2016). Due to the side effects of drugs derived from synthetic substances, the tendency towards drugs with natural bioactive components is increasing steadily (Demirtas et al., 2013). Research on plants to identify natural bioactive components and to find new natural drug raw materials is increasing currently (Aksit et al., 2014; Bayir et al., 2014; Erenler et al., 2014; Sahin Yaglioglu et al., 2013). After the improvement of spectroscopy, bioactive compounds began to be isolated from natural sources (Erenler et al., 2017). It has been reported that many medicinal plants show biological activity due to secondary metabolites and studies on the flora of Turkey have gained great interest in identifying new plant species that can be used in medicines (Beğen Akyıldırım & Eminağaoğlu, 2022; Önal & Eminağaoğlu, 2022; Palaşoğlu & Eminağaoğlu, 2022).

Milk thistle (*S. marianum*) is a member of the Asteraceae family and grows naturally in North Africa, Anatolia, Southern Europe, and Northern Russia (Demirezer et al., 2007). Thistle grows uncontrolled on roadsides, in meadows, and in all kinds of terrain. It consists of a head part, spiny and light green leaves, and small purple flowers. There are dark-colored seeds in the head. It ripens in July-August. It is known among the public by names such as Camel Obstacle, Virgin Mary Thorn, and Milky Kengel (Corchete, 2008). Its structure contains compounds such as flavonolignan (silymarin etc.), flavonoids (apigenin, kaemferol, naringerin, quercetin), fatty acids (linoleic acid, linolenic acid, oleic acid, palmitic acid), protein, tocopherol and sterol (AbouZid, 2012). It has been reported that milk thistle seeds protect the liver from the harmful effects of chemical drugs, poisonous mushrooms, and alcohol. In addition, milk thistle seed is used especially in the treatment of such as stomach, liver, and gall bladder disorders (Agarwal et al., 2006). Among the public, the aboveground part is used as a diuretic, antipyretic, sedative, appetite stimulant, and for rheumatism pain (Baytop, 1999).

The molecular docking method is a method that examines the binding of two molecules to each other in stable conformations. The key-lock model is used to describe compounds that bind to proteins. Here, the protein can be defined as the lock, and the ligand to be bound can be defined as the key. Similarly, docking examines the binding possibilities of the inhibitor or substrate to suitable spaces in the enzyme (Yenigün et al., 2023). Studies on urease inhibitors are very important to develop drugs to be used in the treatment of diseases caused by urease-containing pathogens in humans and animals and to repair these negative effects on the environment (Amtul et al., 2004).
In this study, the fatty acid content of the hexane extract of the flower was analyzed by GC-MS/MS, and the phenolic content of the methanol-chloroform extract was analyzed by LC-MS/MS. A molecular docking study was conducted for the major compounds of *S. marianum* to detect their interaction with the urease enzyme. Thus, the interactions of the palmitic acid and chlorogenic acid molecules with the urease enzyme were determined.

2. MATERIALS AND METHODS

2.1. Plants

S. marianum plant was collected on 11 August at Iğdır University Şehit Bülent Yurtseven campus. Identification of plant was carried out by Prof. Dr. Ahmet Zafer Tel at Iğdır University Faculty of Agriculture, Department of Agricultural Biotechnology.

2.2. Extraction

The flowers of the dried *S. marianum* plant were ground with a blender. Then, 300 grams of sample was weighed and transferred to the volumetric flask. Hexane solvent was added to the flask and extracted for 2 days. Afterward, the hexane was removed by reduced pressure to yield the crude extract to be used for fatty acid analysis. The flowers that were extracted with hexane were extracted with the methanol-chloroform (1:1, v/v) for 2 days. After filtration and evaporation of the solvent, crude extract was acquired for phenolic compounds analysis (Elmastas et al., 2016).

2.3. GC-MS/MS Analysis

GC-MS/MS analysis of hexane extract of *S. marianum* was determined using an Agilent (7890A GC System, 5975C by Triple-Axis Detector MS), a built-in-Autosampler, HP-5MS capillary column (30 m × 0.25 mm × 0.25 µm). Helium was the transporter gas at a flow rate of 1.0 mL min^{-1}. The injector temperature was set at 250°C. The column temperature was 50°C and raised to 270°C at a rate of 4°C/min. The material was diluted with hexane to 1.0 L split/splitless (10:1) and transferred to the column. Molecules were identified through comparison with the Willey and NIST library (Başar et al., 2024a).

2.4. LC- ESI-MS/MS Analysis

We determined the phenolic contents and amounts of *S. marianum* flower of methanol-chloroform extract by LC-ESI-MS/MS analysis. An Agilent 6460 Triple Quad device combined with liquid chromatography (HPLC) and mass spectrometry (MS) was used to analyze the chemical as explained in our previously published article (Erenler et al., 2023; İpek et al., 2024). It was analyzed with 34 phenolic standards on the LC- ESI-MS/MS device.

2.5. Molecular Docking Application

The drawing, 3D structure, and minimum energy of the palmitic acid and chlorogenic acid were conducted by the ChemDraw program. The enzymes chosen for this docking investigation were urease, and urease interactions with palmitic acid and chlorogenic acid molecules were determined using the Molegro Virtual Docker (MVD) program (Başar et al., 2024b). 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).

3. RESULTS AND DISCUSSIONS

The flower part of the *S. marianum* was ground with its seeds, and extraction was performed with hexane and methanol-chloroform sequentially. Then, the hexane extract was determined for fatty acids by GC-MS/MS, and the phenolic content of the methanol-chloroform extract was determined by LC-ESI-MS/MS. Phytochemical analysis is very important for food, pharmacy, and biotechnology. The isolation, identification, and evaluation of bioactive compounds contribute to the new drug invention. In addition, synthetic chemists continue to work intensively to synthesize compounds with the frame of natural products (Cakmak et al., 2006; Çelik et al., 2003; Erenler, 2011; Erenler & Biellmann, 2005; Erenler & Biellmann, 2007; Erenler & Cakmak, 2004; Erenler et al., 2009; Lu et al., 2014).

3.1. GC-MS/MS Analysis Results

Phytochemicals are naturally occurring chemical compounds found in plants that have biological activity. It is found in different parts of plants such as roots, stems, leaves, flowers, fruits, and seeds (Costa et al., 1999; Erenler et al., 2015). In this study, natural compounds analysis of the flowers of *S. marianum* hexane extract was performed (Figure 1).

According to the analysis results, a total of 68 volatile compounds and fatty acid compositions were detected. Palmitic acid methyl ester (17.96%), linoleic acid methyl ester (14.20%), sesquicineole (10.22%), oleic acid methyl ester (6.74%), stearic acid methyl ester (5.59%), nonacosane (4.20%) and 2-ethylhexanol (3.28%) were determined in the highest amount (Figure 3-Table 1). Therefore, fatty acid components were found to be in higher amounts.

In a similar study on *S. marianum* seeds, palmitic acid (8.25%), stearic acid (6.67%), oleic acid (31.58%), linoleic acid (45.36%), arachidic acid (4.11%) were determined to be the main components (Hasanloo et al., 2008).

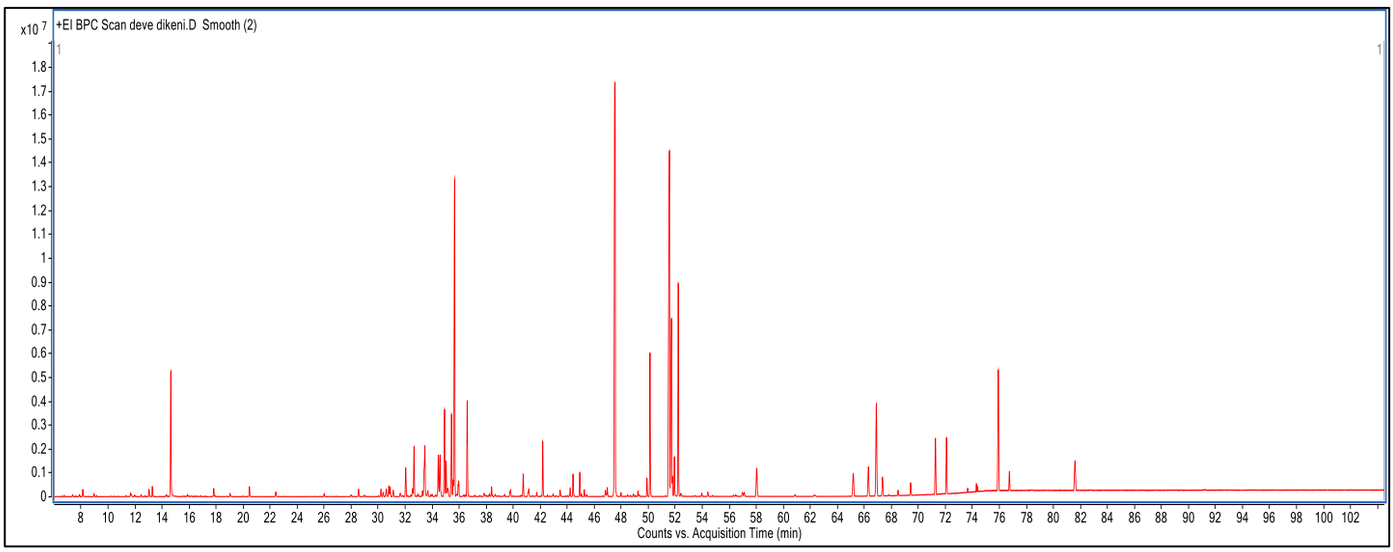

Figure 1. GC-MS/MS analysis chromatogram of hexane extract from *S. marianum* flowers

In another study, the main components in the seed part were linoleic acid (53.3%), oleic acid (21.3%) palmitic acid (9.4%), and stearic acid (6.6%) (El-Mallah et al., 2003). The results in our study are generally similar to the literature. But changes in rates in content analysis; The climate where the plant grows, soil structure, altitude, and different parts of the plant affect it. However, the main components identified in the literature and our study are almost common.

Table 1. GC-MS/MS content analysis results of hexane extract from *S. marianum* flower

Peak	Compound	RT	RI	%Content
1	Ethylbenzene	8.13	855	0.19
2	p-Ethyltoluene	13.04	954	0.20
3	Decane	13.28	1000	0.25
4	2-Ethylhexanol	14.65	1030	3.38
5	Undecane	17.83	1100	0.23
6	Caprylic acid methyl ester	19.04	1126	0.10
7	Dodecane	22.43	1200	0.14
8	Elemene isomer	28.55	1344	0.23
9	Copaene	30.21	1376	0.23
10	Daucene	30.37	1381	0.14
11	β-Bourbonene	30.60	1384	0.24
12	7-epi-Sesquithujene	30.79	1391	0.31
13	β-Elemene	30.89	1391	0.29
14	Tetradecane	31.12	1400	0.19
15	α-Gurjunene	31.64	1409	0.14
16	Caryophyllene	32.05	1419	0.88
17	β-Gurjunene	32.55	1432	0.23
18	trans-α-Bergamotene	32.66	1435	1.48
19	Guaia-6,9-diene	33.28	1443	0.16
20	Humulene+β-Famesene	33.46	1457	2.21
21	Alloaromadendrene	33.68	1461	0.22
22	Germacrene D	34.46	1481	1.18
23	Aristolochene	34.58	1487	1.25
24	Valencene	34.92	1492	2.52
25	β-Cyclogermacrane	35.03	1495	0.87
26	β-Bisabolene	35.43	1509	2.52

27	Sesquicineole	35.66	1516	10.22
28	Lauric acid, methyl ester	35.93	1526	0.24
29	β-Cadinene	35.97	1518	0.35
30	cis-Sesquisabinene hydrate	36.60	1543	2.61
31	2.6.10-Trimethyltetradecane	38.40	1539	0.25
32	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methyl ethylidene)-trans-	39.76	1544	0.10
33	Guaia-3,9-diene	39.80	1556	0.17
34	trans-Sesquisabinene hydrate	40.74	1581	0.63
35	Viridiflorol	41.16	1591	0.25
36	Norphytane	41.75	1687	0.10
37	Myristic acid, methyl ester	42.19	1725	1.42
38	1(2H)-Naphthalenone, 3,4,4a,5,6,7-hexahydro-4a,5-dimethyl-3-(1-methyl ethenyl)-	43.48	1756	0.18
39	Octadecane	44.22	1800	0.22
40	Dehydrosaussurea lactone	44.43	1838	0.61
41	Pentadecanoic acid methyl ester	44.93	1820	0.61
42	Neophytadiene	45.27	1837	0.16
43	Methyl 6,9,12-hexadecatrienoate	46.83	1871	0.17
44	Palmitoleic acid, methyl ester	46.97	1899	0.23
45	Palmitic acid, methyl ester	47.53	1926	17.96
46	Eicosane	49.25	2000	0.14
47	Margaric acid methyl ester	49.91	2028	0.44
48	Linoleic acid, methyl ester	51.57	2092	14.20
49	Oleic acid, methyl ester	51.72	2091	6.74
50	Oleic acid, methyl ester-isomer	51.78		0.38
51	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	51.93	2116	0.95
52	Stearic acid, methyl ester	52.22	2128	5.59
53	cis-10-Nonadecenoic acid, methyl ester	54.41	2209	0.15
54	Tricosane	56.97	2300	0.16
55	cis-Methyl 11-eicosenoate	57.10	2306	0.15
56	Arachidic acid methyl ester	58.03	2329	1.18
57	Pentacosane	65.18	2600	1.01
58	Behenic acid, methyl ester	66.30	2528	1.18
59	2-Methylpentacosane	67.35	2561	0.72
60	Hexacosane	68.50	2600	0.16
61	Tricosanoic acid, methyl ester	69.43	2628	0.39
62	Heptacosane	71.27	2700	1.84
63	Lignoceric acid methyl ester	72.08	2728	1.81
64	Squalene	74.30	2832	0.16
65	Pentacosanoic acid, methyl ester	74.39	2823	0.10
66	Nonacosane	75.92	2900	4.20
67	Cerotic acid methyl ester	76.74	2935	0.53
68	Triacontane	81.60	3000	1.32

RT: Retention time, **RI:** Retention index

3.2. LC-ESI-MS/MS Analysis of Bioactive Compounds

In LC-MS/MS analysis, 22 compounds were detected (Figure 2). Chlorogenic acid (250.171 µg/g extract), salicylic acid (234.95 µg/g extract), isoquercitrin (210.65 µg/g extract), and rutin (102.05 µg/g extract) were determined in the highest amount (Figure 3-Table 2). Chlorogenic acid is a polyphenol that shows important therapeutic and biological activities such as antibacterial, hepatoprotective, radical scavenger, central nervous system stimulant cardioprotective, anti-inflammatory, antipyretic, neuroprotective, anti-obesity, anti-viral, anti-microbial, anti-hypertension (Naveed et al., 2018). Salicylic acid is a phenolic compound and has beneficial

effects on human health. Salicylic acid has anti-inflammatory and antioxidant activity. Also, it is effective in treating cardiovascular disease (Randjelovic et al., 2015). Isoquercitrin is the monoglucoside of the quercetin. It is widely found in the plant kingdom, but its quantity is small. Isoquercitrin has biological activities such as oxidative stress, antioxidants, cancer, cardiovascular disorders, diabetes, and chemoprotective (Valentová et al., 2014). Rutin is a flavonoid with pharmacological properties such as antimicrobial, anti-inflammatory, anticancer, and antidiabetic (Gullón et al., 2017). Therefore, *S. marianum* is a plant species that contains phenolic and flavonoid compounds with biological and pharmacological activities.

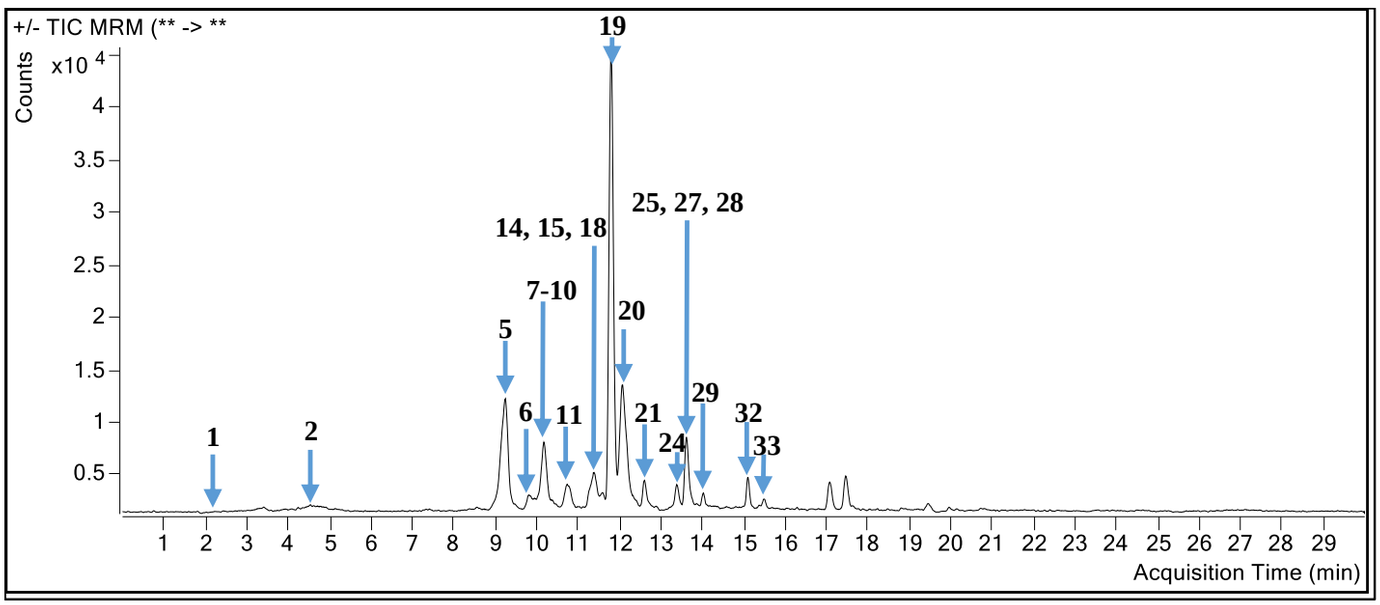

Figure 2. LC- ESI-MS/MS analysis chromatogram of methanol-chloroform extract from *S. marianum* flowers. Shikimic acid (1), Gallic acid (2), Epigallocatechin (3), Catechin (4), Chlorogenic acid (5), Caffeine (6), Vanillic acid (7), Caffeic Acid (8), Hydroxybenzaldeyde (9), Syringic acid (10), Vanillin (11), Polydatine (12), Resveratrol (13), o-Coumaric acid (14), Trans-ferulic acid (15), Taxifolin (16), Sinapic acid (17), Salicylic acid (18), Rutin (19), Isoquercitrin (20), Hesperidin (21), Quercetin-3-xyloside (22), Protocatechuic ethyl ester (23), Quercetin (24), Naringenin (25), Hesperetin (26), Morin (27), Trans-cinnamic acid (28), Kaempferol (29), Baicalein (30), Biochanin A (31), Luteolin (32), Chrysin (33), Diosgenin (34)

Table 2. LC-ESI-MS/MS analysis result of methanol-chloroform extract (µg/g extract) from *S. marianum* flowers

No	Compound	RT	Quantity
1	Shikimic acid	2.16	24.48
2	Gallic acid	4.69	2.27
3	Chlorogenic acid	9.13	250.71
4	Caffeine	9.82	31.26
5	Vanillic acid	10.03	63.51
6	Caffeic acid	10.12	22.43
7	Hydroxybenzaldeyde	10.19	28.08
8	Syringic acid	10.35	79.10
9	Vanillin	10.80	8.78
10	o-coumaric acid	11.39	13.10
11	trans-ferulic acid	11.58	31.94
12	Salicylic acid	11.80	234.95
13	Rutin	12.02	102.05
14	Isoquercitrin	12.10	210.65
15	Hesperidin	12.20	57.05

16	Quercetin	13.38	86.99
17	Naringenin	13.62	24.63
18	Morin	13.70	29.70
19	trans-cinnamic acid	13.73	15.30
20	Kaempferol	14.03	76.93
21	Luteolin	14.96	0.99
22	Chrysin	15.09	12.57

RT: Retention time

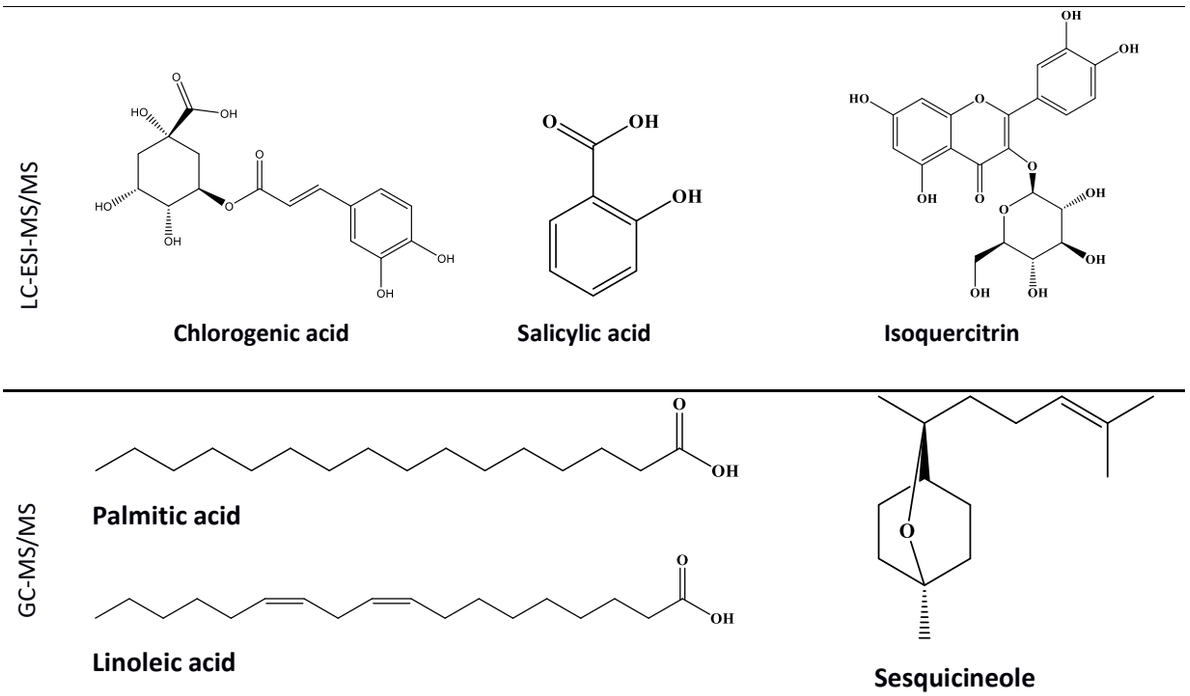

Figure 3. The molecular structures of the main components determined by LC-MS/MS and GC-MS/MS analyses

3.3. Molecular Docking Results

S. marianum plant is used among the public in stomach treatment. The urease enzyme produced by Helicobacter pylori catalyzes the hydrolysis reaction of urea into ammonia and carbon dioxide. Ammonia formed as a result of catalysis neutralizes stomach acid and causes bacteria to form colonies (Demiray & Yılmaz, 2007).
Therefore, inhibitors are needed, which inhibit the action of the urease enzyme. According to GC-MS and LC-MS/MS results, Palmitic acid and chlorogenic acid molecules were determined as the main components; Their interactions with the urease enzyme were observed. Moreover, the urease inhibitor properties (Moldock score, binding energy) of the molecules were calculated theoretically.

Palmitic acid molecules interacted with urease by two conventional hydrogen bonds with amino acid LYS709, ASP730, six alkyls with amino acid VAL36, ALA37, LYS716, PRO743, and three pi-alkyl with amino acid TYR32, PHE712 (Figure 4-Table 3).
Palmitic acid with urease was calculated as a MolDock score of -104.63, with binding energies of -3.70 kcal/mol.

Chlorogenic acid molecules interacted with urease by seven conventional hydrogen bonds with amino acid LYS716, MET746, THR33, TYR32, ASP730, THR715, two carbon-hydrogen bond with amino acid LYS745, one alkyl with amino acid VAL744, five pi-alkyl with amino acid TYR32, PHE712, LYS716, PRO717, MET746 (Figure 5-Table 4).

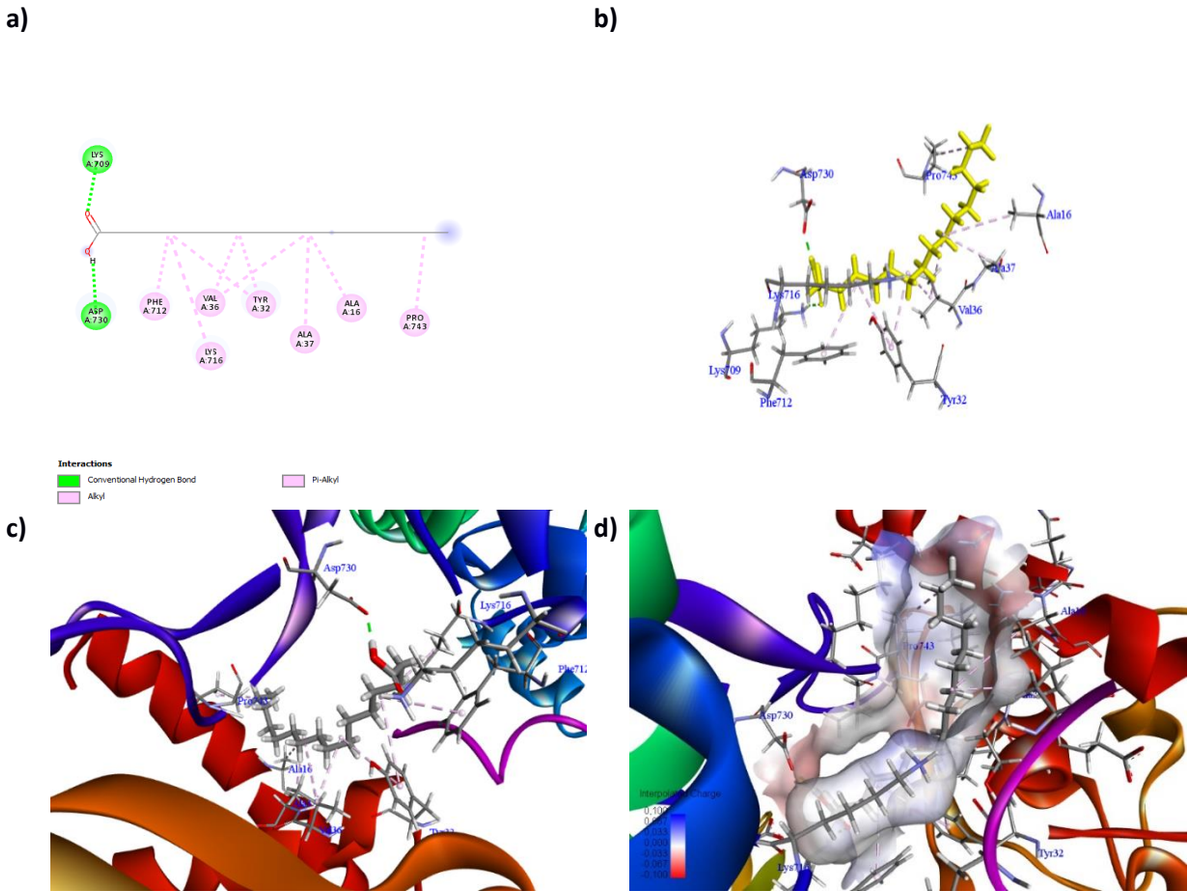

Figure 4. Palmitic acid interaction with urease, a) 2D images, b) interaction images c) 3D images, d) interpolated load view

Table 3. Interaction categories, types, and distances of molecular insertion of the palmitic acid molecule with urease

No	Name	Distance	Category	Type	Transmitter	From Chemistry	Receiver	To Chemistry
1	A:LYS709:HZ1- :[001:O2	2.28934	Hydrogen Bond	Conventional Hydrogen Bond	A:LYS709:HZ1	H-Donor	:[001:O2	H-Acceptor
2	:[001:H13-A:ASP730:OD2	1.98395	Hydrogen Bond	Conventional Hydrogen Bond	:[001:H13	H-Donor	A:ASP730:OD2	H-Acceptor
3	A:ALA16 - :[001	5.34477	Hydrophobic	Alkyl	A:ALA16	Alkyl	:[001	Alkyl
4	A:VAL36 - :[001	4.09893	Hydrophobic	Alkyl	A:VAL36	Alkyl	:[001	Alkyl
5	A:VAL36 - :[001	5.20353	Hydrophobic	Alkyl	A:VAL36	Alkyl	:[001	Alkyl
6	A:ALA37 - :[001	4.58918	Hydrophobic	Alkyl	A:ALA37	Alkyl	:[001	Alkyl
7	A:LYS716 - :[001	5.1705	Hydrophobic	Alkyl	A:LYS716	Alkyl	:[001	Alkyl
8	:[001:C15 - A:PRO743	4.69701	Hydrophobic	Alkyl	:[001:C15	Alkyl	A:PRO743	Alkyl
9	A:TYR32 - :[001	4.79773	Hydrophobic	Pi-Alkyl	A:TYR32	Pi-Orbitals	:[001	Alkyl
10	A:TYR32 - :[001	5.02732	Hydrophobic	Pi-Alkyl	A:TYR32	Pi-Orbitals	:[001	Alkyl
11	A:PHE712 - :[001	4.66364	Hydrophobic	Pi-Alkyl	A:PHE712	Pi-Orbitals	:[001	Alkyl

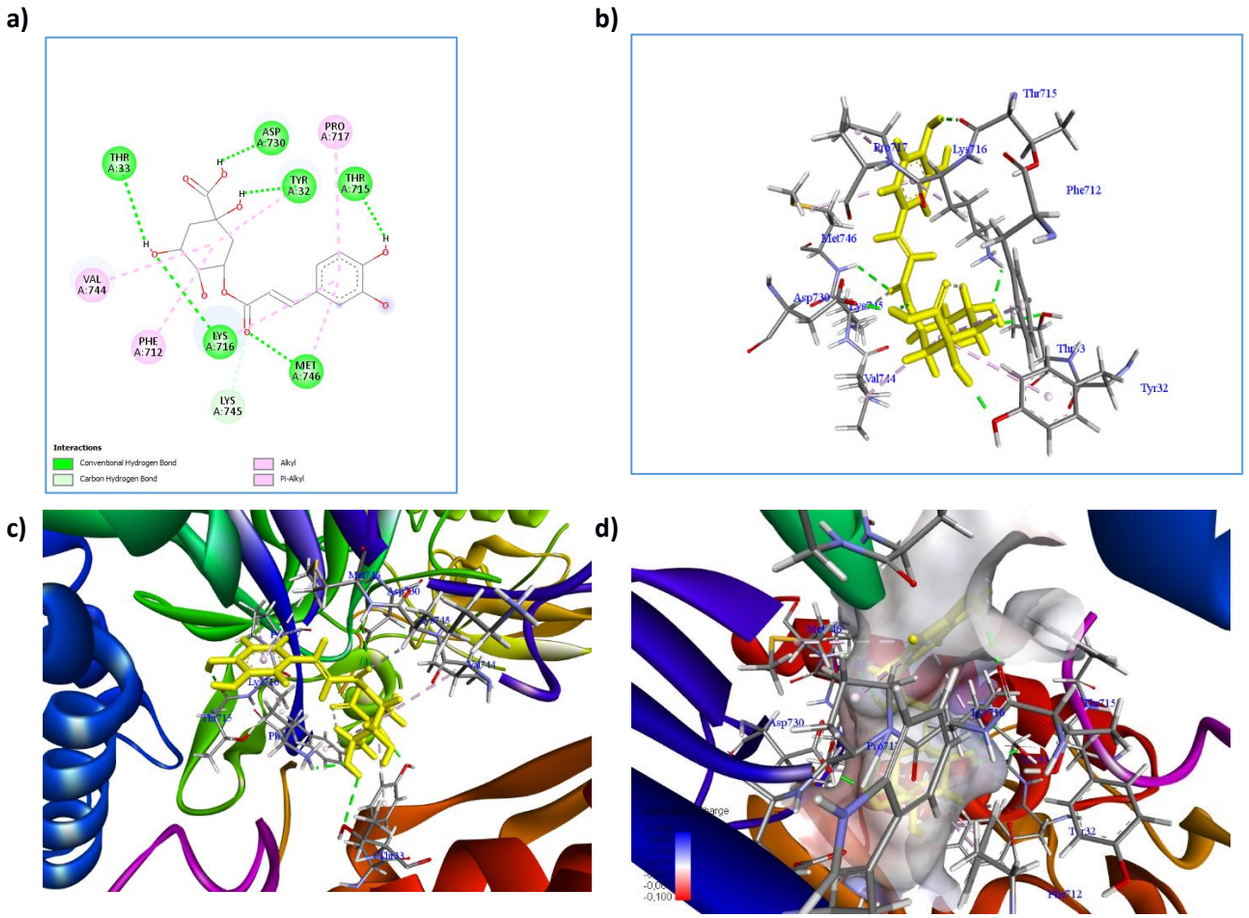

Figure 5. Chlorogenic acid interaction with urease, a) 2D images, b) interaction images c) 3D images, d) interpolated load view

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

No	Name	Distance	Category	Type	Transmitter	From Chemistr	Receiver	To Chemistry
1	A:LYS716:HZ1 - :[001:O1	2.08972	Hydrogen Bond	Conventional Hydrogen Bond	A: LYS716:HZ1	H-Donor	:[001:O1	H-Acceptor
2	A:MET746:HN - :[001:O7	2.73541	Hydrogen Bond	Conventional Hydrogen Bond	A: MET746:HN	H-Donor	:[001:O7	H-Acceptor
3	:[001:H8 - A:THR33:OG1	2.86661	Hydrogen Bond	Conventional Hydrogen Bond	:[001:H8	H-Donor	A: THR33:OG1	H-Acceptor
4	:[001:H10 - A:TYR32:OH	1.98119	Hydrogen Bond	Conventional Hydrogen Bond	:[001:H10	H-Donor	A: TYR32:OH	H-Acceptor
5	:[001:H11 - A:ASP730:OD2	1.68883	Hydrogen Bond	Conventional Hydrogen Bond	:[001:H11	H-Donor	A: ASP730:OD2	H-Acceptor
6	:[001:H11 - :[001:O4	2.85831	Hydrogen Bond	Conventional Hydrogen Bond	:[001:H11	H-Donor	:[001:O4	H-Acceptor
7	:[001:H18 - A:THR715:O	2.19567	Hydrogen Bond	Conventional Hydrogen Bond	:[001:H18	H-Donor	A: THR715:O	H-Acceptor
8	A:LYS745:HA - :[001:O7	2.41686	Hydrogen Bond	Carbon Hydrogen Bond	A: LYS745:HA	H-Donor	:[001:O7	H-Acceptor
9	:[001:H3 - :[001:O6	2.53425	Hydrogen Bond	Carbon Hydrogen Bond	:[001:H3	H-Donor	:[001:O6	H-Acceptor
10	A:VAL744 - :[001	5.15651	Hydrophobic	Alkyl	A:VAL744	Alkyl	:[001	Alkyl
11	A:TYR32 - :[001	5.48771	Hydrophobic	Pi-Alkyl	A: TYR32	Pi-Orbitals	:[001	Alkyl
12	A:PHE712 - :[001	5.44746	Hydrophobic	Pi-Alkyl	A: PHE712	Pi-Orbitals	:[001	Alkyl
13	:[001 - A:LYS716	3.55539	Hydrophobic	Pi-Alkyl	:[001	Pi-Orbitals	A: LYS716	Alkyl
14	:[001 - A:PRO717	5.44682	Hydrophobic	Pi-Alkyl	:[001	Pi-Orbitals	A: PRO717	Alkyl
15	:[001 - A:MET746	5.18975	Hydrophobic	Pi-Alkyl	:[001	Pi-Orbitals	A: MET746	Alkyl

Chlorogenic acid with urease was calculated as a MolDock score of -113.21, with binding energies of -6.50 kcal/mol.

4. CONCLUSION

S. marianum is a member of the Asteraceae family and grows naturally. In our study, *S. marianum* hexane extract was analyzed by GC-MS/MS, and the methanol-chloroform (1:1 v/v) fraction was analyzed by LC-MS/MS. GC-MS/MS analysis resulted in the determination of palmitic acid methyl ester (17.96%) as a chief compound. Chlorogenic acid (250.171 µg/g extract) was detected as a major compound in LC-MS/MS analysis. Molecular docking (MolDock) was applied theoretically to determine the interactions of the main components, palmitic acid, and chlorogenic acid molecules with the urease enzyme. The interactions, binding energies, and MolDock score of palmitic acid and chlorogenic acid with urease were calculated. The analysis showed that the chlorogenic acid molecule could be a urease enzyme inhibitor. Hence, *S. marianum*, with its high secondary metabolite content, may further increase its use in sectors such as pharmacology and food.

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