Bütünleyici ve Anadolu Tıbbı Dergisi & J. Integrative Anatolian Med., vol. 5, no. 2, pp. 10-24, 2024.
DOI: 10.53445/batd.1513403

Journal of Integrative and Anatolian Medicine
Bütünleyici ve Anadolu Tıbbı Dergisi

Cilt/ Volume: 5 Sayı / Issue: 2
Yıl/Year: 2024

Yayıncı / Publisher
Sağlık Bilimleri Üniversitesi
University of Health Sciences Turkey

Phytochemical Content and *In Silico* Molecular Docking Studies of *Achillea biebersteinii* and *A. millefolium* Plants

Fatih Gül [1]*, Yunus Başar [1], İbrahim Demirtas [1,2]

[1] Research Laboratories Application and Research Center (ALUM) Iğdır University, 76000, Iğdır, Türkiye
[2] Department of Pharmaceutical Chemistry, Faculty of Pharmacy Ondokuz Mayıs University, 55139, Samsun, Türkiye

RESEARCH ARTICLE

ARTICLE INFO

Article history:
Received 09 July 2024
Accepted 31 July 2024

Available online 31 August 2024

Keywords:
A. biebersteinii
A. millefolium
Phytochemical analysis
Chlorogenic acid
Xanthine oxidase

ABSTRACT

The genus *Achillea* L. belongs to *Asteraceae*, the largest family of vascular plants. *Achillea* species is a medicinal plant widely used in traditional medicine due to the bioactive compounds it contains that are widely distributed worldwide. In this study, we aimed to examine the biological activity potential of the plants by analyzing the phytochemical content of two *Achillea* species growing on the land of Iğdır University campus. In this study, the volatile and phenolic contents of flower, and stem-leaf parts of *A. biebersteinii* and *A. millefolium* harvested in different periods were determined by chromatographic methods (GC-MS/MS and LC-MS/MS). As a result of the GC-MS analysis, it was observed that the presence of high levels of eucalyptol (43.22%) in the *A. millefolium* plant and that the harvest time dramatically changed the rates of volatile components in the *A. biebersteinii* plants. The LC-MS/MS analysis showed that the main constituent in all plant materials was chlorogenic acid. In addition, the main component, chlorogenic acid interactions with the xanthine oxidase enzyme were determined by *in silico* molecular docking. Chlorogenic acid interaction with xanthine oxidase was calculated of binding energies (-8.0 kcal/mol) and MolDock score (-130.96).

2024

Achillea biebersteinii ve *A. millefolium* Bitkilerinin Fitokimyasal İçeriği ve *In Silico* Moleküler Doking Çalışmaları

ARAŞTIRMA MAKALESİ

MAKALE BİLGİSİ

Makale Geçmişi:
Geliş Tarihi 09 Temmuz 2024
Kabul Tarihi 31 Temmuz 2024

Çevrimiçi yayın 31 Ağustos 2024

Anahtar kelimeler:
A. biebersteinii
A. millefolium
Fitokimyasal analiz
Klorojenik asit
Ksantin oksidaz

ÖZET

Achillea L. cinsi, damarlı bitkilerin en büyük familyası olan *Asteraceae* familyasına aittir. *Achillea* türleri, dünya çapında yaygın olarak bulunan, içerdiği biyoaktif bileşikler nedeniyle geleneksel tıpta yaygın olarak kullanılan tıbbi bir bitkidir. Bu çalışmada Iğdır Üniversitesi kampüsü arazisinde yetişen iki *Achillea* türünün fitokimyasal içeriğini analiz ederek bitkilerin biyolojik aktivite potansiyelini incelemeyi amaçladık. Bu çalışmada, farklı dönemlerde hasat edilen *A. biebersteinii* ve *A. millefolium*'un çiçek, gövde-yaprak kısımlarının uçucu ve fenolik içerikleri kromatografik yöntemlerle (GC-MS/MS ve LC-MS/MS) belirlendi. GC-MS analizi sonucunda *A. millefolium* bitkisinde yüksek düzeyde eucalyptol (%43.22) varlığının olduğu ve hasat zamanının *A. biebersteinii* bitkisinde uçucu bileşenlerin oranlarını önemli ölçüde değiştiği görüldü. LC-MS/MS analizinde, tüm bitki materyallerindeki ana bileşenin klorojenik asit olduğunu gösterdi. Ayrıca ana bileşen, klorojenik asit ile ksantin oksidaz enzimi etkileşimleri, *in silico* moleküler yerleştirme yöntemi ile belirlendi. Ksantin oksidaz ile klorojenik asit etkileşiminin, bağlanma enerjisi (-8,0 kcal/mol) ve MolDock skoru (-130,96) hesaplandı.

2024

Gül, F., Başar, Y., & Demirtas, İ. (2004). Phytochemical Content and In Silico Molecular Docking Studies of *Achillea biebersteinii* and *A. millefolium* Plants. Bütünleyici Ve Anadolu Tıbbı Dergisi, 5(2), 10-24. https://doi.org/10.53445/batd.1513403

*Corresponding author; e-mail: fatihgul3018@gmail.com

1. INTRODUCTION

Plants have developed an effective defense system against their enemies and pests. This defense is based on various phytochemical compounds (Wallander & Albert, 2001). Plants have been used for centuries as medicine in the treatment of human diseases, and the compounds extracted from these plants are known as secondary metabolites (Demirci et al., 2018).

The *Asteraceae* family is found almost everywhere in the world. It is widespread in America and Mexico, in the Brazilian Andes, in the Mediterranean region, on the Asian continent, and in South Africa and Australia (Bremer, 1993). The *Asteraceae* family consists of 136 genera and 1195 species in the flora of Türkiye and there are a total of 446 endemic species (Arabaci, 2012). The genus *Achillea L.* belongs to the Anthemideae of the *Asteracea*e family. Although the genus *Achillea* occurs in almost all regions of our country, it is particularly widespread in the region of northern and eastern Anatolia. The endemic species are widespread in the Eastern Anatolia region (Güneş & Ozhatay, 2000). *Achillea* is commonly known to the public as "yarrow". In Turkey, the genus *Achillea* consists of a total of 46 species belonging to 6 sections. (Arabaci, 2012). *Achillea* species growing in Turkey; wound healing, anti-inflammatory, antipyretic, antidepressant, antihypertensive, astringent, antiseptic, antimicrobial, diuretic, in the treatment of respiratory diseases (in pregnant women), antispermatogenic and antidiabetic, antitumor and hepatoprotective, as well as analgesic, muscle relaxant, digestive, kidney-strengthening and It is reported to be used in the treatment of liver diseases, as an appetite stimulant, carminative, menstrual regulator, for the treatment of hemorrhoids and protection against pests (Candan et al., 2003; Karamenderes et al., 2002; Popovic et al., 2002; Saeidnia et al., 2011).

Among the *Achillea* species, *A. biebersteinii* and *A. millefolium* species grow in the Ağrı Mountain flora of Iğdır province in Turkey (Zeynalov, Y., & Türkoğlu, M., 2016). The main volatile components of *A. biebersteine* volatiles have been reported to be 1,8-cineole, camphor, piperitone, p-cymene, β-edesmol, and ascaridol (Bariş et al., 2006). Moreover in *A. millefolium*, it has been reported that the main volatile components are eucalyptol, camphor, α-terpineol, β-pinene, and borneol (Candan et al., 2003).

Xanthine oxidase is a key enzyme in purine catabolism, in which xanthine and hypoxanthine are converted to uric acid (Page et al., 1998). It is known to reduce oxygen, forming reactive oxygen sources such as hydrogen peroxide and superoxide. It also plays a role in the formation of peroxynitrite, which has a strong antimicrobial effect by reducing nitrite to nitrite oxide (Martin et al., 2004). Xanthine oxidase can catalyze the reduction of molecular oxygen to superoxide anion and hydrogen peroxide. This reagent (superoxide anion, hydrogen peroxide) is present in various oxygen sources. It has been reported to cause ischemia-reperfusion injury in tissues (Granger et al., 1986). It has also been reported that hyperactivity of this enzyme and deficiency of detoxification mechanisms are associated with diseases such as oxidative stress, inflammation, diabetes, and cardiovascular diseases (Abd-ElGawad et al., 2024).

In this study, the phenolic content (LC-MS/MS) and the volatile oil content (GC-MS/MS) of the flower stem/leaf parts of the species *A. biebersteinii* and *A. millefolium* were determined. In addition, the interactions of the molecule, which was identified as the main component in the LC-MS/MS analysis of these species and which is said to have an anti-inflammatory effect, with xanthine oxidase were theoretically calculated by molecular

docking. It is anticipated that investigating the phytochemical content and inhibitory properties of *A. biebersteinii* and *A. millefolium* will provide insight into areas such as pharmaceuticals and cosmetics.

2. MATERIAL and METHODS

Plants

A. biebersteinii (15 may, 15 June 2023) and *A. millefolium* (15 June 2023) harvested at the Iğdır University Şehit Bülent Yurtseven campus (Figure 1). Species identification was made by Prof. Dr. Ahmet Zafer Tel at the Faculty of Agriculture, Department of Agricultural Biotechnology. The collected plants were dried in a cool place without sunlight.

a) **b)**

Figure 1. Natural appearance of *Achillea*, a) *A. millefolium*, b) *A. biebersteinii* (Dr. Yunus BAŞAR)

Extraction

Dried 3 different samples of *A. biebersteinii* May (AB1), *A. biebersteinii* June (AB2) and *A. millefolium* flowers and stem leaves were ground to powder. 100 grams of each samples were placed in separate 1 liter bottles and ethyl acetate was added. The extraction process was continued for 1 week. Then the solvent-extract mixture was filtered and the solvent was removed using a rotary evaporator. The crude extract was then obtained.

GC-MS/MS analysis

The volatile content of *A. biebersteinii* and *A. millefolium* was determined using GC-MS analysis using Agilent (7890 A GC System, 5975 C by Triple-Axis Detector MS) device. The instrument had an Agilent J&W HP-5 ms UltraInert 5%-phenyl)-methylpolysiloxane (30 m × 0.25 mm × 0.25 µm) GC column (Başar et al., 2024). For headspace analysis, samples were placed in the oven of the instrument (Agilent 7000 A GC/MS Triple Quad with 7890 GC, 7693 Autosampler, and 7697A Headspace Sampler). Headspace conditions: The oven temperature was set to 130 °C, the loop temperature was set to 140 °C, the transfer line temperature was set to 145 °C, and the

vial equilibration was set to 30 minutes. GC conditions: The initial column oven temperature was set to 60 °C and then increased to 220 °C at a rate of increase of 4 °C/min and held for 10 minutes. The Wiley software, the NIST library, and NIST MS Search 2.2 were used for the qualitative determination of the components (Yenigün et al., 2024a).

LC-MS/MS analysis

An LC-MS/MS instrument with mass spectrometry (MS) in combination with liquid chromatography (LC) under the brand name Agilent 6460 Triple Quad was used to determine the phenolic content of *A. biebersteinii* and *A. millefolium* of flowers and steam-leafs of ethyl acetate extracts. A flow rate of 0.400 mL/min was set for eluents A (pure water + 5 mM NH_4HCO_2 + 0.1% CH_2O_2) and B (MeOH + 5 mM NH_4HCO_2 + 0.1% CH_2O_2). 50 mg of each sample was weighed. It was then dissolved in 1 mL methanol and 1 mL hexane was added (to remove non-polar solvents) and waited for phase formation. 100 µL of the methanol phase was removed and filtered into the vial, and then 900 µL of methanol was added the monitoring process was performed and the samples were placed in the apparatus (Başar et al., 2023).

Molecular Docking Studies

The interaction of chlorogenic acid with xanthine oxidase (PBD ID: 3NRZ) was investigated theoretically. The structure of chlorogenic acid was drawn with Chem-Draw, refined to the lowest energy, and saved in Mol2 format. Molecule-enzyme interactions were observed using the program Molegro Virtual Docker 6.0.1 (MVD). 2D and 3D representations of the interactions were obtained using the BIOVIA Discovery Studio Visualizer (İpek et al., 2024; Yenigun et al., 2024b).

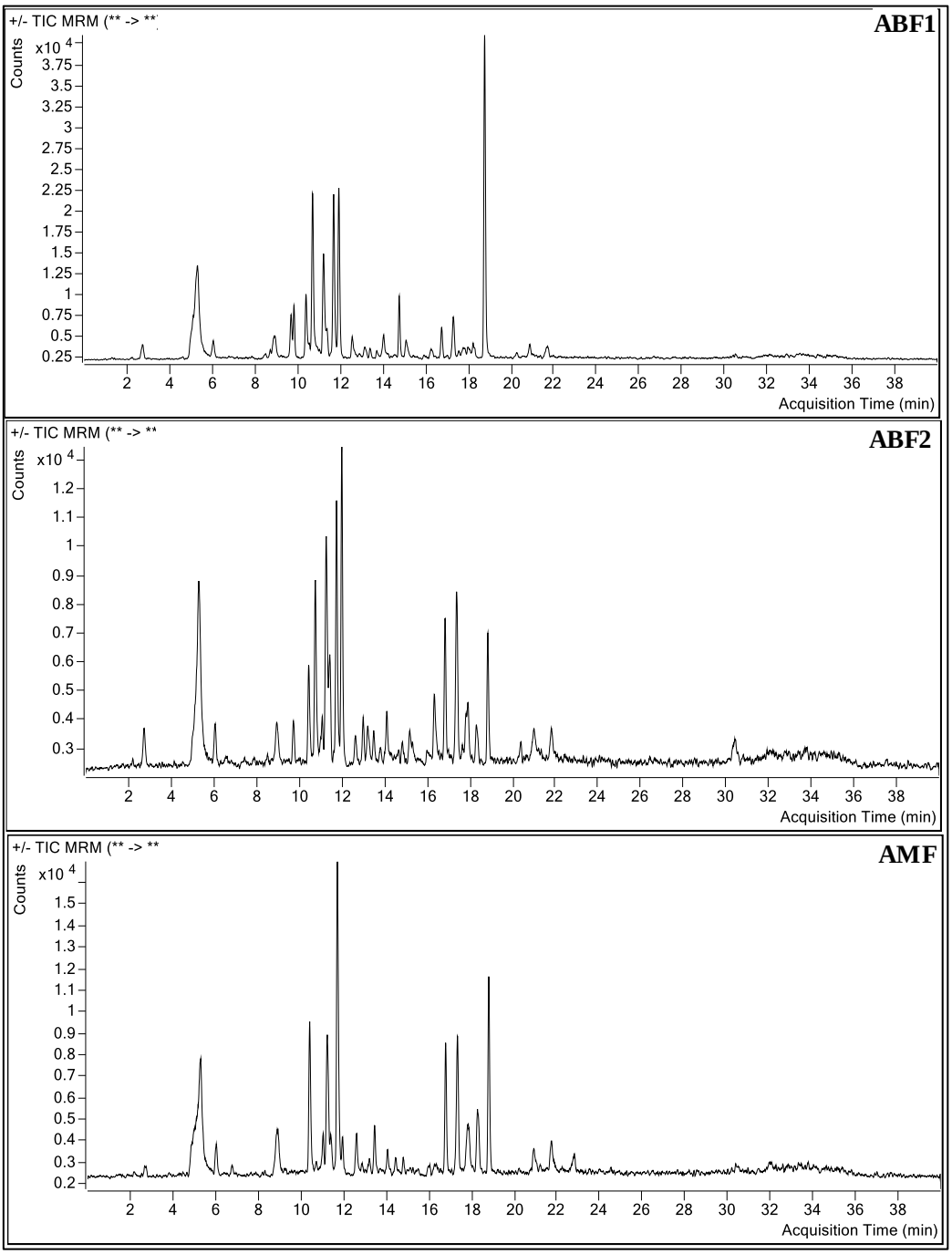
3. RESULTS and DISCUSSIONS

Ethyl acetate extracts were obtained from flower, stem, and leaf parts of the species *A. biebersteinii* and *A. millefolium* collected at different times. The phytochemical content of the extracts was analyzed by LC-MS/MS. In addition, the volatile oil content of the flower parts was determined using the headspace method on the GC-MS/MS instrument. The results of the interactions of chlorogenic acid, which was determined as the main component in the analysis of phenolic content, with BSA were theoretically determined by molecular docking.

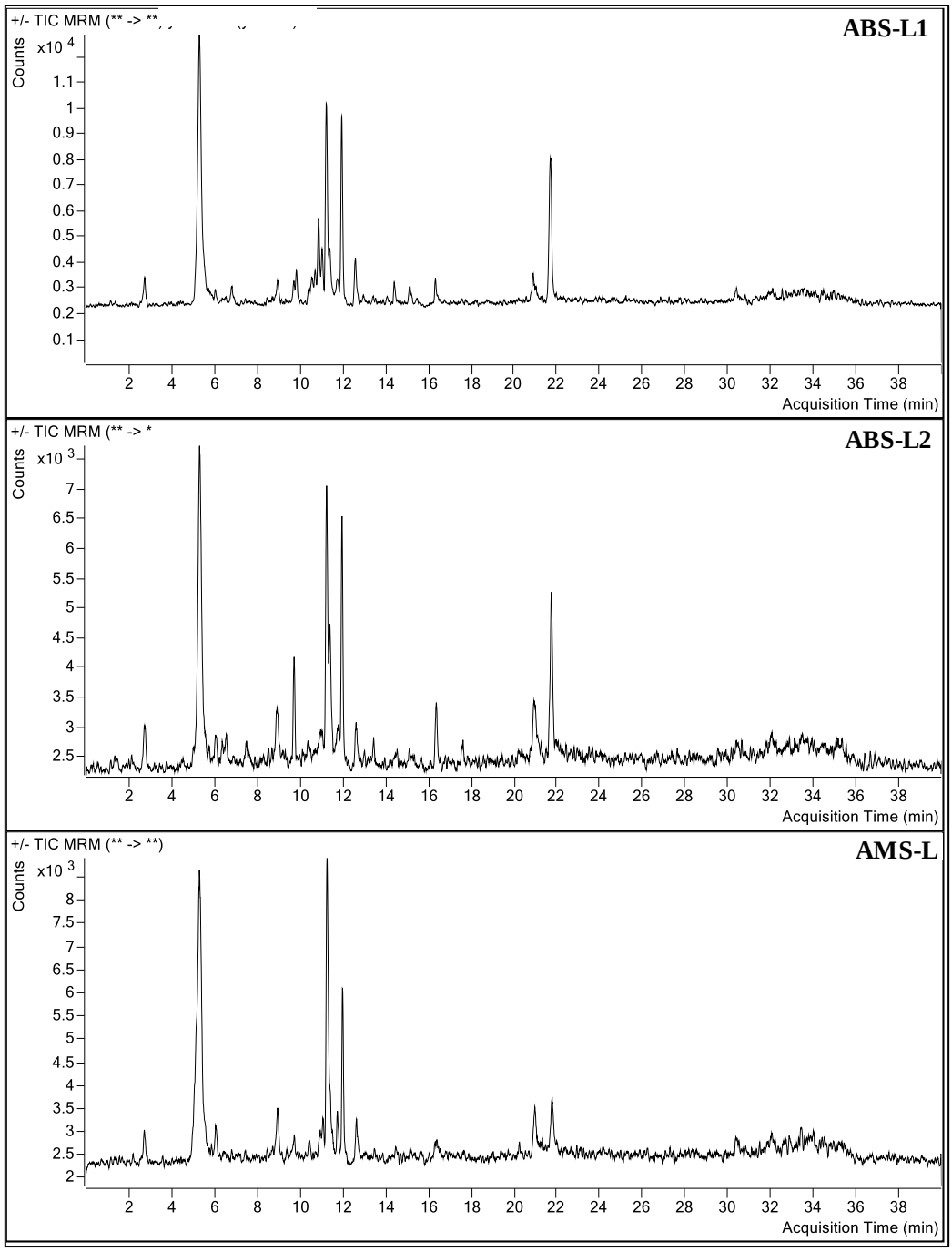
LC-MS/MS results

The phenolic content of different parts of *A. biebersteinii* and *A. millefolium* plants was determined using LC-MS/MS (Figure 2-Figure 3). According to the results, in ABF1; chlorogenic acid (8535.71 µg/g extract), quercimetrin (597.71 µg/g extract), shikimic acid (73.08 µg/g extract), in ABS-L1; chlorogenic acid (2872.09 µg/g extract), cynarin (100.17 µg/g extract), shikimic acid (53.04 µg/g extract) were detected in the highest amount (Table 1). In ABF2; chlorogenic acid (4035.84 µg/g extract), cynarin (208.11 µg/g extract), quercimetrin (192.52 µg/g extract), in ABS-L2; chlorogenic acid (6174.62 µg/g extract), cynarin (67.20 µg/g extract), shikimic acid (53.80 µg/g extract) were determined in the highest amount (Table 1). In AMF; chlorogenic acid (2848.87 µg/g

extract), cynarin (70.22 µg/g extract), shikimic acid (53.04 µg/g extract), in AMS-L; chlorogenic acid (4502.28 µg/g extract), cynarin (245.60 µg/g extract), shikimic acid (53.24 µg/g extract) were detected in the highest amount (Table 1). Similar studies have confirmed that the main component in the different parts of the two species is chlorogenic acid. (Kostevski et al., 2016). In addition to its antioxidant, antibacterial, antitumor, and anti-inflammatory effects, chlorogenic acid is also reported to have liver-protecting, kidney-protecting, nerve-protecting, and vascular-protecting effects (Wang et al., 2022).



ABF1: *A. biebersteinii* flower may, **ABF2:** *A. biebersteinii* flower june, **AMF:** *A. millefolium* flower
Figure 2. LC-MS/MS chromatogram of *A. biebersteinii* and *A. millefolium* of flowers



ABS-L1: *A. biebersteinii* stem-leaf may, **ABS-L2:** *A. biebersteinii* stem-leaf june,
AMS-L: *A. millefolium* stem-leaf

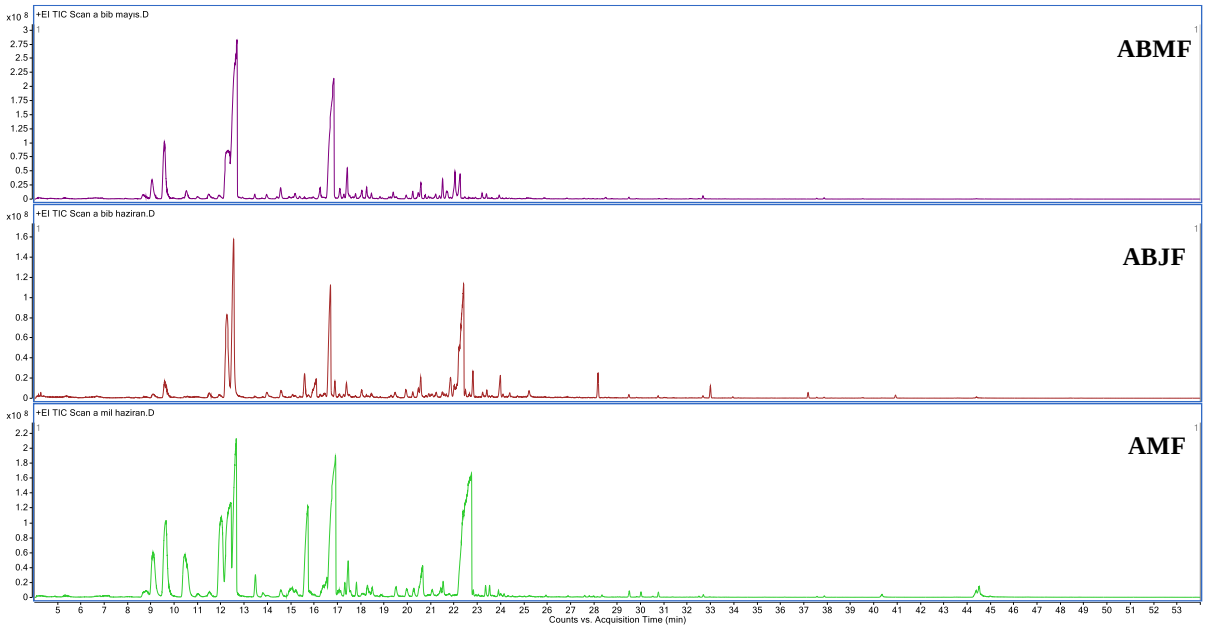
Figure 3. LC-MS/MS chromatogram of *A. biebersteinii* and *A. millefolium* of stem-leaf

Table 1. Phenolic content of *A. biebersteinii* and *A. millefolium* flower and leaf-stem (µg/g extract)

No	*Compound*	*RT (min.)*	*ABF1*	*ABF2*	*AMF*	*ABS-L1*	*ABS-L2*	*AMS-L*
1	*Ascorbic Acid*	*1.29*	*16.64*	*17.36*	*21.74*	*20.78*	*22.10*	*16.62*
2	*Shikimic acid*	*1.18*	*73.08*	*60.94*	*53.16*	*53.04*	*53.80*	*53.24*
3	*Protocatechuic acid*	*2.75*	*17.45*	*22.11*	*2.40*	*6.97*	*1.40*	*4.61*
4	*Gentisic acid*	*3.28*	*8.06*	*7.01*	*7.17*	*7.49*	*7.26*	*6.85*
5	*Catechin*	*4.14*	*9.87*	*10.99*	*11.97*	*10.33*	*10.29*	*10.65*
6	*Chlorogenic acid*	*5.29*	*8535.71*	*4035.84*	*2848.87*	*2872.09*	*6174.62*	*4502.28*
7	*4-Hydroxybenzaldehyde*	*5.76*	*1.07*	*1.58*	*1.45*	*1.03*	*0.83*	*0.69*
8	*Vanillic acid*	*5.98*	*-*	*3.54*	*-*	*-*	*-*	*-*
9	*Caffeic Acid*	*6.07*	*25.55*	*14.20*	*16.30*	*2.40*	*0.77*	*3.72*
10	*Epicatechin*	*6.83*	*5.60*	*5.84*	*5.89*	*6.30*	*5.65*	*6.07*
11	*Syringic acid*	*6.98*	*29.41*	*29.92*	*28.74*	*32.20*	*28.14*	*28.00*
12	*Salicylic Acid*	*8.95*	*39.97*	*13.12*	*29.73*	*-*	*-*	*0.45*
13	*trans-Ferulic acid*	*9.57*	*4.66*	*5.34*	*3.54*	*3.77*	*3.29*	*3.35*
14	*Quercimetrin*	*10.75*	*597.71*	*192.52*	*12.29*	*1.44*	*29.25*	*1.30*
15	*Coumarin*	*10.62*	*8.64*	*3.12*	*3.33*	*3.23*	*3.51*	*4.33*
16	*Scutellarin*	*11.20*	*-*	*-*	*15.60*	*-*	*-*	*1.98*
17	*Cynarin*	*11.43*	*62.37*	*208.11*	*70.22*	*100.17*	*67.20*	*245.60*
18	*Hyperocide*	*11.52*	*-*	*-*	*2.71*	*1.60*	*-*	*3.14*
19	*Quercetin-3-glucoside*	*11.99*	*55.95*	*30.29*	*0.01*	*-*	*20.02*	*1.11*
20	*Isoquercitrin*	*11.99*	*56.11*	*30.24*	*3.68*	*11.64*	*20.24*	*8.12*
21	*Quercetin*	*15.19*	*52.90*	*23.88*	*-*	*2.41*	*16.07*	*1.88*
22	*Naringenin*	*15.30*	*8.55*	*12.89*	*3.35*	*-*	*0.78*	*1.13*
23	*Tamarixetin*	*17.65*	*16.32*	*9.26*	*5.70*	*7.99*	*4.29*	*1.72*
24	*Chrysin*	*21.00*	*19.83*	*23.57*	*19.22*	*21.49*	*17.85*	*15.86*
25	*Flavon*	*21.81*	*2.72*	*2.04*	*2.74*	*4.91*	*10.95*	*2.22*

RT: Retention time, **ABF1:** *A. biebersteinii* flower may, **ABF2:** *A. biebersteinii* flower june, **AMF:** *A. millefolium flower*, **ABS-L1:** *A. biebersteinii* leaf-stem may, **ABS-L1:** *A. biebersteinii* leaf-stem june, **AMS-L:** *A. millefolium* leaf-stem

GC-MS/MS Results



***ABMF**: A. biebersteinii May flower, **ABJF**: A. biebersteinii June flower, **AMF:** A. millefolium flowers.*

Figure 4. GC-MS/MS chromatogram of *A. biebersteinii* and *A. millefolium* of flowers

The volatile oil content of flower parts of *A. biebersteinii* and *A. millefolium* species was determined by the headspace method in GC-MS/MS (Figure 4 and Table 2). According to the results in AB1; isoascaridol (30.15%), camphor (17.39%), eucalyptol (12.89%), m-cymene (8.82%), in AB2; isoascaridol (22.91%), camphor (15.54%), o-cymene+ p-cymene (10.73%), eucalyptol (10.60%), 4-carene (7.53%), camphene (6.84%), sabinene (4.72%), α-pinene (4.14%), in A. millefolium; eucalyptol (43.22%), cis-chrysanthenyl acetate (7.93%), camphor (7.87%), sabinene (3.39%), m-cymene (3.26%), α-pinene-isomer (3.03%) were determined in the highest amount. The main components of the two species were consistent with similar studies (Kostevski et al., 2016).

Table 2. Volatile oil of A. biebersteinii and *A. millefolium* flower (%)

No	***RT***	***Compound Name***	***ABF1***	***ABF2***	***AMF***
1	*5.33*	*Hexanal*	*-*	*0.16*	*-*
2	*8.67*	*Tricyclene*	*0.18*	*0.75*	*0.73*
3	*9.05*	*α-Pinene-isomer*	*0.79*	*-*	*3.03*
4	*9.56*	*α-Pinene*	*2.06*	*4.14*	*-*
5	*9.63*	*Camphene*	*-*	*6.84*	*-*
6	*10.53*	*Sabinene*	*0.12*	*4.72*	*3.39*
7	*11.00*	*3,3-Dimethyl-6-methylenecyclohexene*	*0.18*	*-*	*-*
8	*11.46*	*α-Phellandrene*	*0.32*	*0.35*	*-*
9	*11.91*	*α-Terpinene*	*0.39*	*-*	*-*
10	*12.03*	*4-Carene*	*-*	*7.53*	*-*
11	*12.21*	*m-Cymene*	*8.82*		*3.26*
12	*12.43*	*o-Cymene + p-Cymene*	*-*	*10.73*	*-*
13	*12.47*	*Eucalyptol*	*12.89*	*10.60*	*43.22*
14	*13.44*	*γ-Terpinene*	*0.09*	*0.78*	*0.67*
15	*13.70*	*Sabinene hydrate, cis*	*-*	*0.19*	*1.00*
16	*13.94*	*Linalool oxide*	*0.41*	*-*	*-*
17	*14.55*	*p-Cymenene*	*0.67*	*0.36*	*0.29*
18	*14.91*	*Linalool*	*0.26*	*-*	*0.70*

No	*RT*	*Compound Name*	*ABF1*	*ABF2*	*AMF*
19	*15.08*	*Nonanal*	*-*	*-*	*0.45*
20	*15.17*	*Thujone*	*1.34*	*-*	*-*
21	*15.38*	*1,5,8-p-Menthatriene*	*0.15*	*-*	*-*
22	*15.56*	*Chrysanthone*	*0.15*	*-*	*0.54*
23	*15.73*	*α-Thujone*	*-*	*5.84*	*-*
24	*15.76*	*cis-2-Menthenol*	*0.29*	*-*	*0.69*
25	*15.96*	*2-Propylcyclohexanone*	*2.78*	*-*	*-*
26	*16.26*	*1,2,3,4,5,8-Hexahydronaphthalene*	*0.24*	*-*	*-*
27	*16.39*	*trans-Pinocarveol*	*-*	*-*	*1.19*
28	*16.40*	*Cyclooctanone*	*-*	*0.62*	*-*
29	*16.68*	*Camphor*	*17.39*	*15.54*	*7.87*
30	*17.09*	*Sabinaketone*	*-*	*-*	*0.74*
31	*17.10*	*trans-Chrysanthemol*	*1.34*	*-*	*-*
32	*17.26*	*Pinocarvone*	*0.18*	*0.30*	*1.97*
33	*17.38*	*endo-Borneol*	*2.42*	*1.10*	*1.86*
34	*17.51*	*2-Caren-4-ol*	*0.46*	*-*	*-*
35	*17.76*	*4-Terpinenol*	*0.16*	*0.25*	*0.67*
36	*18.03*	*p-Cymen-8-ol*	*1.38*	*-*	*1.07*
37	*18.23*	*α-Terpineol*	*0.48*	*0.29*	*2.17*
38	*18.45*	*Myrtenal*	*0.20*	*-*	*0.76*
39	*18.50*	*α-Thujenal*	*-*	*0.18*	*-*
40	*18.82*	*cis-Piperitol*	*0.15*	*-*	*-*
41	*18.92*	*Verbenone*	*0.12*	*-*	*0.39*
42	*19.37*	*2-Hydroxycineole*	*0.26*	*-*	*0.51*

No	*RT*	*Compound Name*	*ABF1*	*ABF2*	*AMF*
43	*19.45*	*Ascaridole*	*0.74*	*-*	*-*
44	*19.50*	*Ascaridole epoxide*	*-*	*0.31*	*-*
45	*19.93*	*Cuminal*	*0.53*	*-*	*0.51*
46	*19.97*	*Dihydro carveol neo*	*-*	*0.27*	*-*
47	*20.23*	*Ascaridole-isomer*	*0.25*	*-*	*-*
48	*20.27*	*Ascaridole*	*-*	*0.24*	*-*
49	*20.46*	*Piperitone oxide*	*0.56*	*-*	*-*
50	*20.50*	*Piperitone oxide isomer + 7-Oxabicyclo[4.1.0]heptan-2-one, 3-methyl-6-(1-methylethyl)-*	*-*	*1.83*	*-*
51	*20.55*	*6-Isopropyl-3-methyl-7-oxabicyclo[4.1.0]heptan-2-one*	*0.89*	*0.22*	*-*
52	*20.66*	*cis-Chrysanthenyl acetate*	*-*	*-*	*7.93*
53	*20.75*	*2,5-Bornanedione*	*0.31*	*-*	*-*
54	*21.03*	*trans-Ascaridol glycol*	*1.01*	*0.31*	*0.31*
55	*21.11*	*4-Thujen-2-α-yl acetate*	*-*	*-*	*0.93*
56	*21.20*	*1,7-Octadiene-3,6-diol, 2,6-dimethyl-*	*0.53*	*-*	*-*
57	*21.39*	*Cyclopropanemethanol, 2,2-dimethyl-3-(2-methyl propenyl)-, acetate, trans-*	*1.18*	*-*	*-*
58	*21.51*	*Bornyl acetate*	*1.12*	*0.45*	*1.42*
59	*21.64*	*Thymol*	*0.16*	*-*	*0.77*
60	*21.81*	*Bicyclo[3.1.1]hept-2-en-4-ol, 2,6,6-trimethyl-, acetate*	*2.71*	*-*	*0.49*
61	*21.85*	*Carvacrol*	*0.49*	*-*	*-*
62	*22.39*	*Isoascaridol*	*30.15*	*22.91*	*0.96*
63	*22.65*	*2-Methyl-5-(propan-2-ylidene)cyclohexane-1,4-diol*	*0.41*	*-*	*-*
64	*22.81*	*Cyclopentaneacetaldehyde, 2-formyl-3-methyl-α-methylene-*	*-*	*-*	*0.49*
65	*23.36*	*γ-Terpineol acetate*		*0.25*	

No	*RT*	*Compound Name*	*ABF1*	*ABF2*	*AMF*
66	*23.85*	*Eugenol*	*0.09*	*0.14*	*-*
67	*25.90*	*Caryophyllene*	*-*	*-*	*0.95*
68	*26.96*	*Humulene*	*-*	*-*	*0.10*
69	*27.77*	*Germacrene D*	*0.06*	*-*	*0.55*
70	*28.52*	*β-Bisabolene*	*-*	*-*	*0.22*
71	*29.51*	*Terpinyl propionate*	*0.25*	*-*	*-*
72	*29.80*	*Nerolidol*	*-*	*-*	*0.51*
73	*29.84*	*Terpinyl propionate-isomer*	*0.07*	*0.16*	*-*
74	*29.93*	*Caryophyllene oxide*	*-*	*-*	*0.45*
75	*30.02*	*Benzyl angelate*	*-*	*0.12*	*-*
76	*30.08*	*Viridiflorol*	*-*	*-*	*1.39*
77	*30.64*	*Spathulenol*	*0.06*	*-*	*0.32*
78	*30.75*	*Piperityl angelate, cis*	*0.19*	*-*	*-*
79	*30.81*	*Patchoulane*	*-*	*-*	*1.09*
80	*32.20*	*α-acorenol*	*0.19*	*-*	*0.71*
81	*32.30*	*Caryophylladienol II*	*-*	*-*	*-*
82	*32.50*	*Methyl jasmonate*	*0.10*	*-*	*-*
83	*32.68*	*13-Tetradecanolide*	*0.20*	*-*	*0.62*
84	*32.75*	*α-Eudesmol*	*-*	*-*	*1.02*
85	*33.22*	*α-Santalal*	*-*	*-*	*0.18*
86	*33.64*	*ent-Germacra-4(15),5,10(14)-trien-1β-ol*	*-*	*-*	*0.67*
87	*37.56*	*Hexahydrofarnesyl acetone*	*0.06*	*-*	*0.24*
88	*37.88*	*Palmitoleic acid*	*0.12*	*-*	*-*
89	*40.34*	*Gerany-p-cymene*	*0.06*	*-*	*-*

Molecular Docking Results

Chlorogenic acid has been reported to have antioxidant, antibacterial, antitumor, and anti-inflammatory effects as well as hepatoprotective, renoprotective, neuroprotective, and vasoprotective effects (Wang et al., 2022). Chlorogenic acid is known to have an antioxidant effect; its interaction with xanthine oxidase was determined by molecular docking. The inhibitory properties were investigated by calculating mold score and binding energies. Its inhibitory properties were investigated by calculating the MolDock score and binding energies.

Table 3. Interaction categories, types, and distances of molecular insertion of the chlorogenic acid with xanthine oxidase

No	Name	Distance	Category	Type	From Chemistry	To Chemistry
1	L:LYS754:HZ1 - :[001:O5	2.0507	Hydrogen Bond	Conventional Hydrogen Bond	H-Donor	H-Acceptor
2	:[001:H8 - :[001:O2	1.90921	Hydrogen Bond	Conventional Hydrogen Bond	H-Donor	H-Acceptor
3	:[001:H16 - C:VAL764:O	2.06686	Hydrogen Bond	Conventional Hydrogen Bond	H-Donor	H-Acceptor
4	:[001:H17 - C:ARG793:O	1.73615	Hydrogen Bond	Conventional Hydrogen Bond	H-Donor	H-Acceptor
5	:[001:H18 - L:LYS754:O	1.92604	Hydrogen Bond	Conventional Hydrogen Bond	H-Donor	H-Acceptor
6	C:ILE596:HA - :[001:O8	2.24509	Hydrogen Bond	Carbon Hydrogen Bond	H-Donor	H-Acceptor
7	L:LYS754:HA - :[001:O1	2.66327	Hydrogen Bond	Carbon Hydrogen Bond	H-Donor	H-Acceptor
8	:[001:H3 - L:TYR599:OH	2.38793	Hydrogen Bond	Carbon Hydrogen Bond	H-Donor	H-Acceptor
9	C:LYS792:HN - :[001	2.72295	Hydrogen Bond	Pi-Donor Hydrogen Bond	H-Donor	Pi-Orbitals
10	:[001 - C:CYS593	4.74597	Hydrophobic	Pi-Alkyl	Pi-Orbitals	Alkyl
11	:[001 - C:LYS792	4.52128	Hydrophobic	Pi-Alkyl	Pi-Orbitals	Alkyl

Chlorogenic acid interacted with xanthine oxidase by five conventional hydrogen bonds with amino acid LYS754, VAL764, ARG793, three carbon-hydrogen bonds with amino acid ILE596, LYS754, TYR599, one pi-donor hydrogen bond with amino acid LYS792, and two alkyls with amino acid CYS593, LYS792 (Figure 5 – Table 3). Chlorogenic acid interaction with xanthine oxidase was calculated as a MolDock score of -130.96, with binding energies of -8.0 kcal/mol.

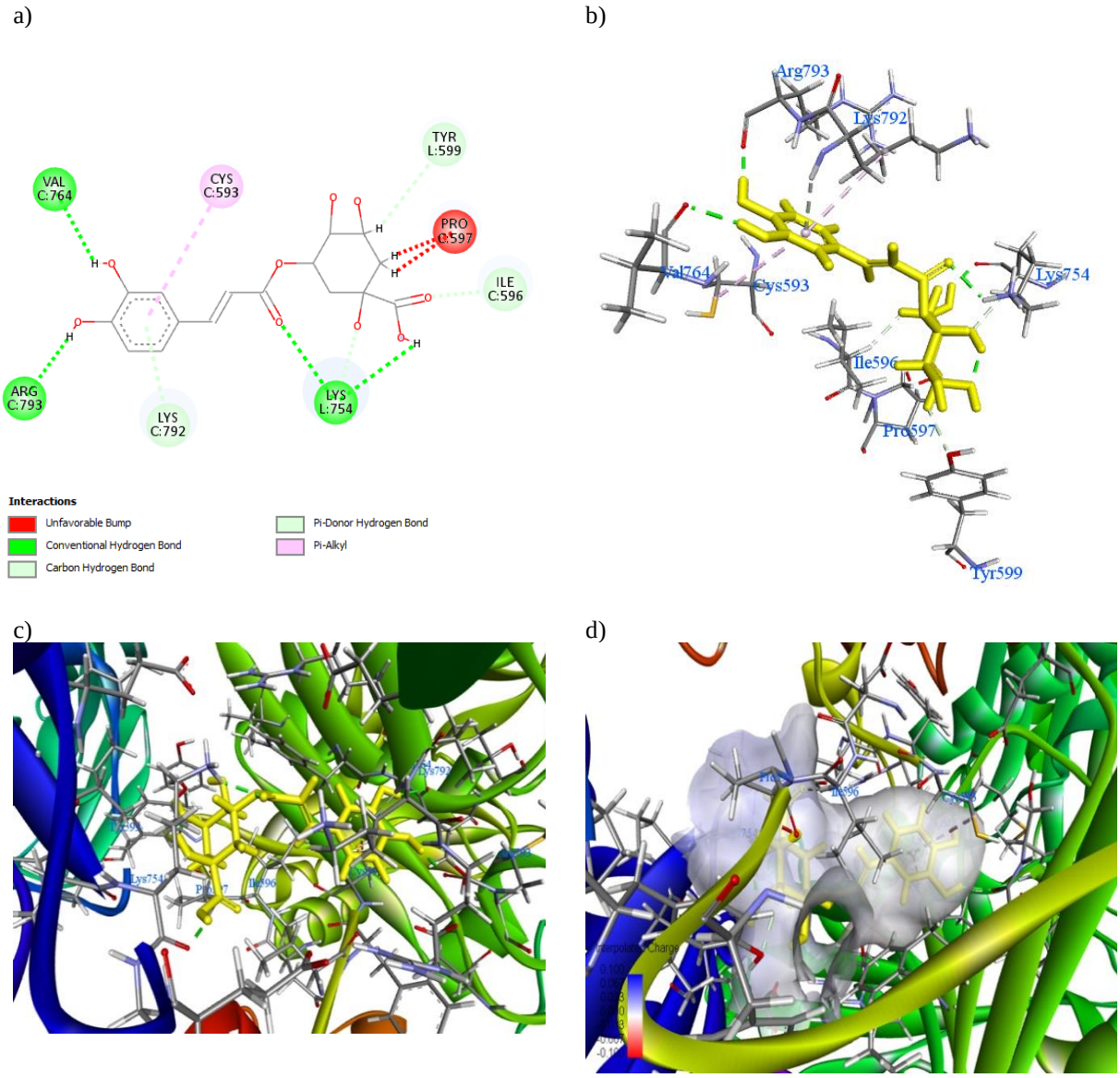


Figure 5. Chlorogenic acid interaction with xanthine oxidase, a) 2D images, b) interaction images c) 3D images, d) interpolated load view

4. CONCLUSION

Achillea is commonly known to the public as "yarrow". It has a high content of volatile oils and phenols. In our study, the volatile and phenolic components of the leaves-stems, and flowers of two different Achillea species were determined by chromatographic methods. According to the results of LC-MS/MS analysis of A. biebersteinii and A. millefolium, the main component in the leaves, stems and flowers was found to be chlorogenic acid. In addition, GC-MS analysis revealed that the presence of high content of eucalyptol (43.22%) in the A. millefolium plant and the time of harvest (May and June) drastically altered the levels of volatiles (especially m-cymene, α-thujone, 4-carene) in the A. biebersteinii plants. The ability of chlorogenic acid to be an inhibitor of the enzyme xanthine oxidase was investigated by an in silico molecular docking application. According to the results of molecular docking, chlorogenic acid could be an inhibitor of xanthine oxidase, but it is expected that these results will be supported by *in vitro* and *in vivo* studies in future studies.

Author Contributions

All the authors equally contributed to this work. They all read and approved the final version of the paper.

Conflict of Interest

All the authors declare no conflict of interest.

Acknowledgment

The authors thank the High-Value Added Agricultural Products Specialization Program, Production of Value-Added Raw Materials from Agricultural Products by Supercritical Carbon Dioxide Extraction Method (Project No: YİP0723İ01).

5. REFERENCES

Abd-ElGawad, A., Essa, A., Taher, R., Souid, A., El-Newary, S., Sarker, T. C., El Gendy, A. E. N., Assaeed, A., Dar, B., & Elshamy, A. (2024). Chemical components and antioxidant potential of essential oil of Rhanterium epapposum growing in sandy habitat in Saudi Arabia: In silico study on cytochrome P450 1A1 and xanthine oxidase. *Journal of Essential Oil Bearing Plants*, *27*. https://doi.org/10.1080/0972060X.2024.2358894

Arabaci, T. (2012). Achillea hamzaoglui (Asteraceae), a New Species from Turkey. *Annales Botanici Fennici*, *46*, 459-463. https://doi.org/10.5735/085.046.0516

Bariş, Ö., Güllüce, M., Sahin, F., Ozer, H., Kilic, H., Özkan, H., Sökmen, M., & Özbek, T. (2006). Biological activities of the essential oil and methanol extract of Achillea biebersteinii Afan. (Asteraceae). *Turkish Journal of Biology*, *30*, 65-73.

Başar, Y., Yenigün, S., İpek, Y., Behçet, L., Gül, F., Özen, T., & Demirtaş, İ. (2023). DNA protection, molecular docking, enzyme inhibition and enzyme kinetic studies of 1,5,9-epideoxyloganic acid isolated from Nepeta aristata with bio-guided fractionation. *J Biomol Struct Dyn*, 1-14. https://doi.org/10.1080/07391102.2023.2250461

Başar, Y., Yenigün, S., Gül, F., Özen, T., Demirtas, I., Mehmet, A., & Temel, S. (2024). Phytochemical profiling, molecular docking and ADMET prediction of crude extract of Atriplex nitens Schkuhr for the screening of antioxidant and urease inhibitory. *International Journal of Chemistry and Technology*, *8*. https://doi.org/10.32571/ijct.1389719

Bremer, K. r. (1993). Generic monograph of the Asteraceae-Anthemideae. *Bulletin of the Natural History Museum. Botany series*, *23*(2), 71-177. https://www.biodiversitylibrary.org/part/224587

Candan, F., Unlu, M., Tepe, B., Daferera, D., Polissiou, M., Sökmen, A., & Akpulat, H. A. (2003). Antioxidant and antimicrobial activity of the essential oil and methanol extracts of Achillea millefolium subsp. millefolium Afan. (Asteraceae). *J Ethnopharmacol*, *87*(2-3), 215-220. https://doi.org/10.1016/s0378-8741(03)00149-1

Candan, F., Unlu, M., Tepe, B., Daferera, D., Polissiou, M., Sökmen, A., & Akpulat, H. A. (2003). Antioxidant and antimicrobial activity of the essential oil and methanol extracts of Achillea millefolium subsp. millefolium Afan. (Asteraceae). *Journal of Ethnopharmacology*, *87*(2), 215-220. https://doi.org/https://doi.org/10.1016/S0378-8741(03)00149-1

Demirci, M. A., Ipek, Y., Gul, F., Ozen, T., & Demirtas, I. (2018). Extraction, isolation of heat-resistance phenolic compounds, antioxidant properties, characterization and purification of 5-hydroxymaltol from Turkish apple pulps. *Food Chem*, *269*, 111-117. https://doi.org/10.1016/j.foodchem.2018.06.147

Granger, D. N., Höllwarth, M. E., & Parks, D. A. (1986). Ischemia-reperfusion injury: role of oxygen-derived free radicals. *Acta Physiol Scand Suppl*, *548*, 47-63.

Güneş, F., & Ozhatay, N. (2000). Flora of Turkey and the East Aegean Islands. In (Vol. 11, pp. 92-94).

İpek, Y., Başar, Y., Yenigün, S., Behçet, L., Özen, T., & Demirtas, I. (2024). In vitro bioactivities and in silico enzyme interactions of abietatrien-3β-ol by bio-guided isolation from Nepeta italica subsp. italica. *Journal of Biomolecular Structure and Dynamics*. https://doi.org/10.1080/07391102.2024.2322626

Karamenderes, C., Karabay, N. U., & Zeybek, U. (2002). Composition and antimicrobial activity of the essential oils of some Achillea L. Species in Turkey. *Acta Pharmaceutica Turcica*, *44*, 221-225.

Kostevski, I. R., Petrović, G. M., Stojanović, G. S., Stamenković, J. G., & Zlatković, B. K. (2016). Essential Oil Chemical Composition and Headspace Volatiles Profile of Achillea coarctata from Serbia. *Natural Product Communications*, *11*(4), 1934578X1601100431. https://doi.org/10.1177/1934578x1601100431

Martin, H. M., Hancock, J. T., Salisbury, V., & Harrison, R. (2004). Role of xanthine oxidoreductase as an antimicrobial agent. *Infect Immun*, *72*(9), 4933-4939. https://doi.org/10.1128/iai.72.9.4933-4939.2004

Page, S., Powell, D., Benboubetra, M., Stevens, C. R., Blake, D. R., Selase, F., Wolstenholme, A. J., & Harrison, R. (1998). Xanthine oxidoreductase in human mammary epithelial cells: activation in response to inflammatory cytokines. *Biochim Biophys Acta*, *1381*(2), 191-202. https://doi.org/10.1016/s0304-4165(98)00028-2

Popovic, M., Jakovljevic, V., Bursac, M., Mitic, R., Raskovic, A., & Kaurinovic, B. (2002). Biochemical investigation of yarrow extracts (Achillea millefolium L.). *Oxidation Communications*, *25*, 469-475.

Saeidnia, S., Gohari, A., Mokhber-Dezfuli, N., & Kiuchi, F. (2011). A review on phytochemistry and medicinal properties of the genus Achillea. *Daru*, *19*(3), 173-186.

Wallander, E., & Albert, V. (2001). Phylogeny and classification of Oleaceae based on RPS16 and TRNL-F sequence data. *American journal of botany*, *87*, 1827-1841. https://doi.org/10.2307/2656836

Wang, L., Pan, X., Jiang, L., Chu, Y., Gao, S., Jiang, X., Zhang, Y., Chen, Y., Luo, S., & Peng, C. (2022). The Biological Activity Mechanism of Chlorogenic Acid and Its Applications in Food Industry: A Review. *Front Nutr*, *9*, 943911. https://doi.org/10.3389/fnut.2022.943911

Yenigün, S., Başar, Y., Gül, F., Marah, S., Behcet, L., Demirtas, I., & Özen, T. (2024a). Chemical Constituents and Bioactivities of Nepeta Taxa Essential Oils from Turkey: Principal Component Analysis, Molecular Docking Study, Molecular Dynamics, MM-PBSA and Drug-Likeness Estimation. *ChemistrySelect*, *9*. https://doi.org/10.1002/slct.202400583

Yenigun, S., Basar, Y., Ipek, Y., Gok, M., Behcet, L., Ozen, T., & Demirtas, I. (2024b). A potential DNA protector, enzyme inhibitor and in silico studies of daucosterol isolated from six Nepeta species. *Process Biochemistry*, *143*, 234-247. https://doi.org/https://doi.org/10.1016/j.procbio.2024.04.039

Zeynalov, Y., & Türkoğlu, M. (2016). *The Flora of Agri mountain*. Turkish Ministry of Forestry and Water Affairs.