Araştırma Makalesi/Original Article
Makale Dili "İngilizce"/Article Language "English"

Türk Hijyen ve Deneysel Biyoloji Dergisi

Analytical insights into edible plant volatiles: SPME-GC/MS analysis and antioxidant/anticancer evaluation with *in vitro* and *in silico* methods

Yenilebilir bitki uçucu maddelerine ilişkin analitik bilgiler: SPME-GC/MS analizi ve *in vitro* ve *in silico* yöntemlerle antioksidan/antikanser değerlendirme

Emine ATEŞ ARSLAN[1] (ID), Nilüfer VURAL[2,3] (ID), Emine Kübra İNAL[4] (ID), Sibel KAYMAK[2] (ID), Mehmet Abdülkadir AKAY[4] (ID), Mustafa TAŞTEKİN[4] (ID)

ABSTRACT

Objective: Due to the secondary metabolites, edible plants have been a recent focus in the literature for their biotherapeutic effects and functional food potentials. Discovering the aromatic bioactive components of plants consumed as food is of great importance in an ethnobotanical vision. In this context, the study investigated *Helichrysum arenarium* L., *Origanum sipyleum* L., *Plantago major* L., and *Rumex* spp. species both *in vitro* and *in silico*.

Methods: The volatile composition and identification of these plants were determined using carboxen-polydimethylsiloxane / solid-phase microextraction (CAR-PDMS/SPME) fiber with gas chromatography/mass spectrometry (GC/MS) method. Moreover, the antioxidant activities of the plants were determined using a 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical and ß-carotene-linoleic acid assays. To determine their antioxidant activities and inhibitory effects on oral cancer, molecular docking analysis was conducted. Also, ADMET (absorption, distribution, metabolism, elimination and toxicity) properties were identified, the

ÖZET

Amaç: Yenilebilir bitkiler, sekonder metabolitleri kaynaklı, biyoterapötik etkileri ve fonksiyonel gıda potansiyelleri açısından literatürde son zamanların odak noktası olmuştur. Gıda olarak tüketilen bitkilerin aromatik biyoaktif bileşenlerinin keşfedilmesi etnobotanik vizyonda büyük önem taşımaktadır. Bu çalışmada, *Helichrysum arenarium* L., *Origanum sipyleum* L., *Plantago major* L. ve *Rumex* spp. türleri hem *in vitro* hem de *in siliko* olarak araştırılmıştır.

Yöntem: Bu bitkilerin uçucu bileşimi ve tanımlanması karboksen-polidimetilsiloksan/katı faz mikroekstraksiyon (CAR-PDMS/SPME) fiber ile gaz kromatografisi/kütle spektrometresi (GC/MS) yöntemi kullanılarak gerçekleştirilmiştir. Ayrıca bitkilerin antioksidan aktiviteleri 2,2'-difenil-1-pikrilhidrazil (DPPH) radikali ve ß-karoten-linoleik asit analizleri kullanılarak belirlenmiştir. Antioksidan aktiviteleri ve oral kansere yönelik inhibitör etkilerini belirlemek amacıyla moleküler docking (yanaştırma) analizleri gerçekleştirilmiştir. Ayrıca, ADMET (absorbsiyon, dağılım, metabolizma, eliminasyon ve toksisite) özellikleri belirlenmiş,

[1]Ministry of Health, Turkish Pharmaceuticals and Medical Devices Agency, Ankara, Türkiye
[2]Ankara Yıldırım Beyazit University, Institute of Public Health, Department of Traditional, Complementary and Integrative Medicine, Biotherapeutic Products Research and Development Program, Ankara, Türkiye
[3]Ankara Yıldırım Beyazit University, Vocational School of Technical Sciences, Department of Food Processing, Ankara, Türkiye
[4]Ankara University, Faculty of Science, Department of Chemistry, Ankara, Türkiye

İletişim / Corresponding Author : Nilüfer VURAL
Ankara Yıldırım Beyazıt Üniversitesi Etlik, Ankara - Türkiye
E-posta / E-mail : nilufervural@aybu.edu.tr

Geliş Tarihi / Received : 21.07.2024
Kabul Tarihi / Accepted : 29.11.2024

DOI ID : 10.5505/TurkHijyen.2025.65289

Ateş Arslan E, Vural N, İnal EK, Kaymak S, Akay MA, Taştekin M. Analytical insights into edible plant volatiles: SPME-GC/MS analysis and antioxidant/anticancer evaluation with *in vitro* and *in silico* methods. Turk Hij Den Biyol Derg, 2025; 82(1): 67 - 84

presence of carcinogenic and mutagenic components was investigated, and their potentials for various diseases were revealed through PASS (prediction of activity spectra for substances) analysis.

Results: The results indicate that the plants exhibit a rich profile of volatile bioactive compounds, with the highest bioactive compound content found in *Rumex* spp. Compared with other plants, *Helichrysum arenarium* was found to have the highest antioxidant potential and in silico anticancer potential. Additionally, it was determined that the various volatile compounds of the plants could have protective effects on the gastrointestinal system and could be used particularly for stomach complaints.

Conclusion: This investigation shows that these plants may have pharmaceutical and dietary potential as functional foods.

Key Words: Gas chromatography-mass spectroscopy, edible plants, functional food, molecular docking simulation

karsinojenik ve mutajenik bileşenlerin varlığı araştırılmış ve çeşitli hastalıklara yönelik potansiyelleri PASS (maddelerin aktivite spektrumlarının tahmini) analizi ile ortaya konulmuştur.

Bulgular: Sonuçlar, bitkilerin zengin bir uçucu biyoaktif bileşen profili sergilediğini ve en yüksek biyoaktif bileşen içeriğinin *Rumex* spp.'de bulunduğunu göstermiştir. Diğer bitkilerle karşılaştırıldığında, *Helichrysum arenarium*'un en yüksek antioksidan ve in siliko antikanser potansiyeline sahip olduğu bulunmuştur. Ayrıca, bitkilerin çeşitli uçucu bileşenlerinin gastrointestinal sistem üzerinde koruyucu etkileri olabileceği ve özellikle mide şikayetlerinde kullanılabileceği belirlenmiştir.

Sonuç: Bu araştırmada kullanılan bitkilerin fonksiyonel gıdalar olarak farmasötik ve diyet potansiyeline sahip olabileceğini göstermektedir.

Anahtar Kelimeler: Gaz kromatografi-kütle spektrometresi, yenilebilir bitkiler, fonksiyonel gıda, moleküler docking simülasyonu

INTRODUCTION

The exploration of plants and their significance in therapeutic approaches dates to the early stages of human civilization. These natural resources contain various bioactive compounds resulting from the production of plant secondary metabolites. Ethnopharmacology and pharmacognosy stand out as two major scientific fields dedicated to uncovering new natural remedies from these plants (1). In addition, an emerging discipline, reverse pharmacognosy, uses computational tools to enhance the efficiency of drug discovery and design processes. In addition, the field of traditional phytotherapy, which encompasses the medicinal use of plants, stands as both a historical and contemporary precursor among disease treatment options (2). Nowadays, in less-developed countries, over 80% of the population primarily relies on herbal products to obtain healing benefits. Plant-derived components that provide these health benefits are referred to as phytochemicals, which are also considered micronutrients in diets (3). Phytochemicals exhibit not only high antioxidant activities but also possess properties such as chelation of metal ions, stimulation of detoxifying enzymes, and inhibition of transcription factors that initiate and support tumor development. Simultaneously, they have a wide range of diverse features, including preventive effects on degenerative diseases, anti-allergenic, anti-inflammatory, antimicrobial, antithrombotic, anticarcinogenic, antiatherogenic, antiulcer, and vasodilator effects (4). Embracing a diet rich in edible plants not only fulfills nutritional requirements but also serves as a proactive approach to promoting health and well-being through the powerful influence of phytochemicals. According to the plant database system of the Republic of Turkey Ministry of Agriculture and Forestry, Türkiye is home to 12,141 different plant species, most of which are actively consumed in diets. Among the taxonomic

diversity, Ankara, ranking seventh, has several Anatolian culinary cultures based on these plants (5).

Türkiye has a rich diversity of species within the *Lamiaceae* family, with 49 genera and 629 species naturally distributed across the country. In addition, this family harbors 360 endemic taxa in Türkiye. Among these, *Origanum* stands out with 23 species and 27 taxa naturally occurring in Türkiye with great biotherapeutic potentials such as antimicrobial, antioxidant, and antiviral properties (6). On the other hand, the *Helichrysum* species is represented in the Turkish flora by 27 taxa, 15 of which are endemic and commonly found in Anatolia. These plant species are known for their antibacterial, antifungal, anti-inflammatory, antiproliferative, antiradical, cholinergic, hepatoprotective, and detoxifying properties (7). *Rumex* is another plant group with a wide distribution in Türkiye (22 species) and is known for its appetite stimulant and blood purifier features in traditional uses. Among the properties tested on people in our healing books from the 15th century, there are different biotherapeutic effects of the plants, such as preventing intestinal worms, antipyretic, and diuretic effects when consumed raw (8). Lastly, *Plantago* leaves, belonging to the *Plantagiaceae* family, have been used as a wound-healing herbal agent in traditional medicine for many years. *P. major* contains high concentrations of mucilaginous carbohydrates and is frequently used to treat intestinal diseases (9). In addition, it has been traditionally used as an analgesic and antipyretic in wound healing and other skin diseases, for treating infectious diseases, in problems related to digestive and respiratory organs, and in reproductive and circulatory problems (8). One of the important steps in benefiting from plants considering their traditional use is the correct selection of plant species. Most of the time, the same plant species named in different regions differ from each other. Because the basis of regional naming is that people living in the region recognize the plants and use them for different purposes, plants that are similar in appearance, taste, and smell can sometimes receive the same name. Although this makes a great contribution ethnobotanically, it can sometimes cause confusion (10). Such differently named edible plants constitute the basis of antioxidant sources for consumers. While green edible plants are used to increase the organoleptic qualities (aroma, flavor, and taste) of many food products, their possible health-promoting effects are one of the popular research topics. Phytochemicals in edible plants can include hundreds of different volatile and non-volatile compounds such as polyphenols, terpenes, and alkaloids. In addition to providing “sweetness”, “spicy”, “floral” and “herbal” properties in plant species, volatile compounds can also have a strong pharmaceutical effect (11). *Rumex, Plantago* and other plant species, which are very popular edible plants around the world, may some of their components have a great bioactive volatile compound potential. Therefore, in this study, four different edible plant species were collected and analyzed by solid phase microextraction- gas chromatography/mass spectrometry (SPME-GC/MS). Additionally, *in vitro* antioxidant and *in silico* anticancer properties were investigated.

MATERIAL and METHOD

Chemicals and reagents

All the analytical grade chemicals were used to perform both chemical and biological experiments. Ethanol, methanol, hexane, acetone, and other solvents were purchased from Merck. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Aldrich. Milli-Q water was used for the preparation of solutions.

Plant materials

Fresh plant samples (*Helichrysum arenarium* L., *Origanum sipyleum* L., *Plantago major* L., and *Rumex* spp.) consumed by local people in meals were purchased from the local bazaar in Ayas, a region in Ankara Province in Central Anatolia Region, Türkiye, in June-August 2023. The aerial parts of the plants

were washed, cleaned, and shade-dried at room temperature without an airflow (25 °C) for 15 days.

Isolation of the volatiles by SPME

First, the carboxen/polydimethylsiloxane (CAR-PDMS) SPME fiber was conditioned in the GC/MS device injection port at 220°C for 10 min. The conditioned fiber was inserted into glass vials; the mouth was tightly closed with a crimple containing 0.5 g of plant sample through an SPME holder. Afterward, plant volatile components were allowed to be absorbed into the SPME fiber for 30 min at 80°C. Finally, the fiber was drawn into the holder, taken from the vial, and placed into the injection port of the device (12).

GC/MS analysis

The volatile compounds were analyzed via GC using the Shimadzu GC-17A/QP5000 system equipped with both a flame ionization detector (FID) and a mass selective detector (MSD) (12). A supelcowax-10 capillary column (30 m × 0.32 mm i.d., 0.25 film thickness) was employed, with helium serving as the carrier gas at a flow rate of 1.8 mL/min. The temperature program involved an initial hold at 40°C for two minutes, followed by a gradual increase of 2°C per minute until reaching 220°C, where it was maintained for 30 minutes. The injector and MSD transfer line temperatures were set at 200°C and 250°C, respectively, while the FID temperature was maintained at 300°C. The analytes were detected via electron impact ionization (70 eV) in SCAN mode over the mass range of m/z 50 to 550. Compound identification relied on comparing spectra and retention times against standards. This comparison encompassed relative retention times to a C8–C32 n-alkanes mixture, mass spectra from various sources including NBS75K, Wiley 7, NIST MS search 2.0 library data, and literature references. All analyses were conducted in triplicate.

Total antioxidant activities

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ß-carotene-linoleic acid assays were used to determine the antioxidant activities of aqueous plant extracts (13). For DPPH activity, 500 µL of 120 µM DPPH ethanol solution and 500 µL of pure ethanol were added to 200 µL of plant extracts prepared at different concentrations, and the resulting mixture was incubated at room temperature and in the dark for 30 min. As a result of the incubation, the intensity of the purple color (resulting from the reaction) in all samples was determined by absorbance measurements against blank (200 µL dH2O, 500 µL DPPH, 500 µL ethyl alcohol) at 517 nm. The results were evaluated using the calibration curve created with different concentration values of standard gallic acid solutions. Antioxidant capacity was also determined by investigating the inhibition of volatile organic compounds and conjugated diene hydroperoxides resulting from the oxidation of linoleic acid. This method relies on the alteration of the yellow color of ß-carotene, depending on to its reaction with radicals generated during linoleic acid oxidation which the velocity of ß-carotene bleaching is attenuated in the presence of antioxidants. This alteration serves as the basis for evaluating the antioxidant activity of the plant samples in comparison to well-established synthetic and natural antioxidants, such as butylated hydroxytoluene (BHT). The antioxidative capacities of the aqueous plant extracts were measured against BHT at equivalent concentrations, with a blank comprising only 350 µL of ethanol. All experiments were replicated three times for robustness and reliability.

In silico studies

Molecular docking

Structures of target proteins were retrieved from the Protein Data Bank (PDB) (Table 1). Each protein structure was used as a receptor and remained rigid. For the docking preparation procedure of proteins, the following steps were applied: (i) energy minimization was performed with 100 steepest descent steps with a 0.02 Å step size and an update interval of 10, (ii) water molecules non-complex ions were removed, and (iii) polar hydrogen atom and AM1-BCC charges were added. For ligand structure retrieval and preparation, chemical structures were downloaded

Table 1. Molecular docking parameters

Structure Name	PDB ID	Target Activity	Grid Box Center Coordinates	Grid Box Size
Kelch-Neh2 complex	2FLU	Antioxidant Activity	center_x = 18 center_y = 17 center_z = 8	size_x = 21 size_y = 21 size_z = 21
EGFR kinase domain	1XKK	Oral Cancer	center_x = 24 center_y = 37 center_z = 36	size_x = 32 size_y = 26 size_z = 26

from the PubChem database in .sdf format. To determine anticancer and antioxidant properties at the molecular level, semi-flexible molecular docking simulations were performed by using AutoDock Vina (14). The binding energy poses of each protein were visualized with Discovery Studio Visualizer (15).

Carcinogenicity and mutagenicity prediction

The potential of any chemical to induce carcinogenicity and mutagenicity in humans and animals were predicted computationally by using CarcinoPred-EL (16) and Lazar (17) prediction web-based tools. To enable the system to perform predictions computationally, the compounds in chemical SMILES format were initially introduced into the system sequentially, and predictions were made by selecting appropriate in silico analysis conditions.

PASS prediction

The PASS online web tool helps predict the expected biological function profile of a chemical substance with similarities to a drug molecule. Computational predictions can be obtained by inserting chemical SMILES (Simplified Molecular Input Line Entry System) formats of the chemical structures. The PASS tool prediction results in 2 category labels of “probability to be active” (Pa) or “probability to be inactive” (Pi) as biological activity (18).

ADME/T analysis

ADME/T analysis was performed as the last step of the in silico experiments. SwissADME (19) web tool and literature data were used for these predictions. For analysis, the SMILES chemical data format of all of the bioactive compounds was also retrieved from the PubChem database.

Statistical analysis

The data underwent statistical analysis using a one-way analysis of variance (ANOVA) with SPSS software (version 27.0.0). To assess differences in biochemical parameters across groups, a post-hoc Tukey test was applied. Statistical significance was assigned to p values below 0.05. Results are presented as the mean ± standard error of the mean for each test group.

RESULTS

Volatile profiles of plants

Unlike other chemical extraction methods of volatiles, SPME fiber can quantify the highly volatile compounds of the plant samples. The bioactive volatile composition of the plants is given in Table 2. A total of 112 volatile compounds were identified from four different plants. Compared to other plant species, the highest emission of volatile compounds was observed in *Rumex* spp. The component acetic acid was found to be common for all four plants (Figure 1). Particularly, a significant amount of shared volatile components was identified between *P. major* and *Rumex* spp.

Antioxidant activities

The antioxidant analysis results obtained from both experimental methods are presented in Table 3. As can be understood from the results, the highest antioxidant activity was found in *H. arenarium* according to the DPPH test, while the results from the other test indicate that *P. major* exhibits the highest antioxidant activity).

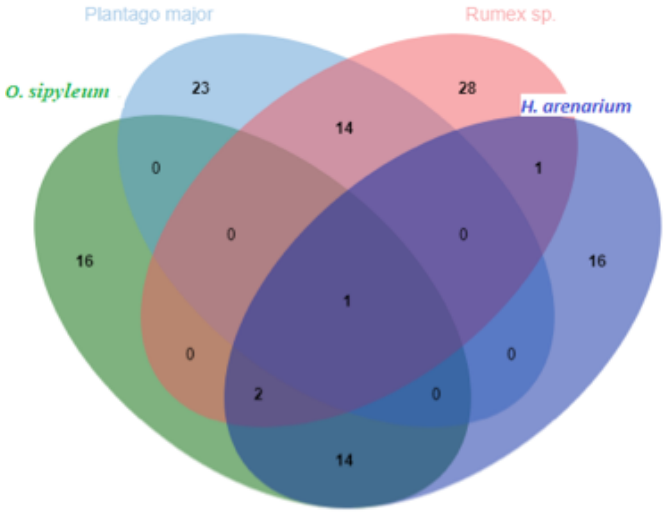

Figure 1. Venn diagram distribution of the plant volatiles

Table 2. Volatile component analysis results with GC-MS after CAR-PDMS/SPME extraction

	Volatile compounds of Plants	CAR-PDMS/SPME fibre				
		Helichrysum arenarium L.	*Origanum sipyleum* L.	*Plantago major* L.	*Rumex* spp.	
	Compound	%	%	%	%	Identification Method
1	2-Pentadecyn-1-ol	-	-	2,47±0,03	-	a
2	Acetaldehyde	0.11±0.01	1.02±0.02	-	0.98±0.03	a
3	Mercaptomethane	-	-	-	0.15±0.00	a
4	Acetone	0.70±0.02	0.27±0.02	-	0.19±0.01	a
5	n-Butanal	0.06±0.01	-	0.11±0.01	0.29±0.00	a,b
6	Isobutanal	-	-	-	0.40±0.03	a,b
7	2-Methylbutanal	-	-	0.22±0.01	0.68±0.03	a,b
8	Isobutyric anhydride	0.15±0.01	-	-	-	a
9	Methyl Isobutyl Ketone	-	-	-	0.15±0.01	a
10	Acetic acid	0.08±0.01	0.11±0.00	0.24±0.05	3.39±0.05	a,b
11	2-Methylpentanol	0.16±0.01	-	-	-	a
12	Furfural	-	-	2.68±0.02	-	a
13	Alpha-pinene	1.65±0.08	0.39±0.02	-	-	a
14	Camphene	0.09±0.01	0.21±0.01	-	-	a
15	n-Pentanal	-	-	0.18±0.03	-	a

Table 2 (cont.) Volatile component analysis results with GC-MS after CAR-PDMS/SPME extraction

	Volatile compounds of Plants	CAR-PDMS/SPME fibre				
		Helichrysum arenarium L.	*Origanum sipyleum* L.	*Plantago major* L.	*Rumex* spp.	
	Compound	%	%	%	%	Identification Method
16	2,4-Pentadienal-	-	-	0.27±0.02	-	a
17	2-Pentenal, (E)-	-	-	0.20±0.05	-	a
18	Sabinene	-	0.39±0.02	-	-	a,b
19	2-Hexenal	-	-	0.16±0.01	-	a,b
20	Furfuryl alcohol	-	-	-	0.56±0.02	a
21	1,3-Cyclopentenedione	-	-	-	0.53±0.03	a
22	2-.Beta.-pinene	0.06±0.04	0.29±0.01	-	-	a,b
23	Beta.-mycrene	0.95±0.01	6.15±0.00	-	-	a,b
24	2-Acetyl furan	-	-	-	0.61±0.02	a
25	Pyrazine, 2,6-dimethyl-	-	-	-	0.39±0.03	a
26	Benzaldehyde	-	-	1.75±0.03	0.66±0.05	a,b
27	5-Methyl furfural	-	-	1.62±0.03	0.57±0.03	a
28	3-Pentanone, 2,4-dimethyl-	-	-	0.31±0.02	-	a
29	1-Octen-3-ol	1.24±0.08	0.65±0.02	-	-	a,b
30	Heptanoic acid	0.26±0.01	-	-	-	a,b
31	6-Methylhept-5-en-2-one	1.09±0.05	-	-	-	a
32	n-Octanal	-	-	-	0.16±0.01	a,b
33	2,4-Heptadienal, (E,E)-	-	-	3.78±0.05	0.36±0.02	a,b
34	Benzeneacetaldehyde	-	-	3.24±0.02	0.38±0.02	a
35	.Beta.-Phellandrene	-	0.17±0.02	-	-	a,b
36	.Alpha.-Terpinene	0.39±0.04	-	-	-	a,b
37	Cymol	2.58±0.50	9.95±0.12	-	-	a,b
38	.Beta.-Terpinyl acetate	13.24±0.3	-	-	-	a
39	1,8-Cineole	7.75±0.21	1.47±0.01	-	-	a,b
40	l-Limonene	-	1.44±0.02	-	-	a,b
41	Ethanone, 1-(1H-pyrrol-2-yl)-	-	-	-	1.56±0.05	a
42	1-Octanol	-	-	-	0.18±0.02	a
43	1,3,6-Octatriene, 3,7-dimethyl-, (Z)-beta-ocimene	1.15±0.08	0.59±0.03	-	-	a
44	.Gamma.-Terpinene	0.99±0.01	17.36±0.28	-	-	a,b
45	Thymol	-	20.18±0.32	-	-	a,b
46	Carvacrol	-	10.18±0.24	-	-	a,b
47	.Alpha.-Cubebene	-	0.24±0.01	-	-	a
48	.Alpha.-Copaene	-	2.67±0.03	-	-	a
49	3-Decanone	0.28±0.01	-	-	-	a
50	Alpha-terpinolene	0.75±0.01	1.45±0.02	-	-	a

Table 2 (cont). Volatile component analysis results with GC-MS after CAR-PDMS/SPME extraction

	Volatile compounds of Plants	CAR-PDMS/SPME fibre				
		Helichrysum arenarium L.	*Origanum sipyleum* L.	*Plantago major* L.	*Rumex* spp.	
	Compound	%	%	%	%	Identification Method
51	Linalool	0.55±0.01	0.28±0.01	-	-	a,b
52	Heptanoic acid	-	-	-	0.23±0.02	a
53	Nonanal	-	-	0.38±0.02	1.65±0.05	a
54	1H-Pyrrole-2-carboxaldehyde, 1-methyl-	-	-	-	0.67±0.03	
55	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	-	-	-	7.12±0.07	a
56	p-Cymene	-	0.63±0.03	-	-	a,b
57	D-Fenchyl alcohol	0.17±0.01	-	-	-	a,b
58	Borneol	0.41±0.03	1.66±0.03	-	-	a
59	Phenylethyl Alcohol	-	-	1.56±0.06	-	a
60	Alpha-Terpineol	0.45±0.03	0.78±0.02	-	-	a
61	Octanoic acid	-	-	0.68±0.00	-	a
62	Linalyl propionate	0.35±0.02	-	-	-	a
63	Trans-2,cis-6-Nonadienal	-	-	0.25±0.01	-	a
64	2-Allylphenol	-	-	0.69±0.02	-	a
65	Decanal	-	-	0.32±0.01	-	a,b
66	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-	-	-	-	1.12±0.05	a
67	Benzeneacetaldehyde,. alpha.-ethylidene-	-	-	-	0.39±0.03	a
68	Benzofuran, 2,3-dihydro-	-	-	-	2.57±0.05	a
69	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	-	-	2.30±0.05	5.27±0.07	a
70	Nonanoic acid	-	-	0.67±0.03	2.98±0.05	a
71	Trans-Caryophyllene	21.02±0.31	2.98±0.03	-	-	a,b
72	2,6-Dimethyl-3(2-methyl-1-butyl) pyrazine	-	-	-	0.98±0.04	a
73	.Beta.-Cyclocitral	-	-	0.14±0.01	-	a
74	2-Methoxy-4-vinylphenol	-	-	0.13±0.01	6.27±0.07	a
75	1-Octanol, 2,7-dimethyl-	-	-	0.18±0.03	-	a
76	Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester	-	-	0.38±0.03	-	a
77	Decanoic acid	-	-	-	0.45±0.02	a,b
78	Limonene oxide	-	-	-	0.32±0.01	a
79	Naphthalene, 1,2-dihydro-1,1,6-trimethyl-	-	-	1.73±0.04	-	a
80	Dodecane, 2,6,11-trimethyl-	-	-	0.48±0.06	-	a

Table 2 (cont.) Volatile component analysis results with GC-MS after CAR-PDMS/SPME extraction

	Volatile compounds of Plants	CAR-PDMS/SPME fibre				
		Helichrysum arenarium L.	*Origanum sipyleum* L.	*Plantago major* L.	*Rumex* spp.	
	Compound	%	%	%	%	Identification Method
81	Dodecan-2-one	-	-	0.95±0.02	-	a
82	(E)-Geranylacetone	-	-	0.58±0.03	1.55±0.05	a
83	Aromadendrene	0.25±0.01	0.23±0.02	-	-	a
84	.Alpha.-Humulene	30.77±0.31	0.65±0.01	-	-	a
85	.beta.-Ionone	-	-	2.62±0.03	1.38±0.06	a
86	Naphthalene, 1,2-dihydro-2,5,8-trimethyl-	-	-	1.54±0.02		a
87	5-Methyl-2-phenyl-2-hexenal	-	-	-	0.85±0.02	a
88	Germacrene	-	0.44±0.01	-	-	a,b
89	1-Hexadecanol	-	-	-	0.28±0.03	a
90	Hexadecanal	-	-	-	0.38±0.04	a,ba
91	Methanone, dicyclohexyl-	-	-	-	0.82±0.03	a
92	Methyl pentadecanoate	-	-	-	0.25±0.03	a
93	Neophytadiene	-	-	12.45±0.13	1.25±0.03	a
94	ß-Farnesene	0.28±0.02	-	-	-	a
95	.Gamma.-Cadinene	0.39±0.03	-	-	-	a
96	Eudesma-4(14),11-diene	2.28±0.02	-	-	-	a
97	Phytol	-	-	7.32±0.18	0.96±0.03	a
98	2-Undecanone, 6,10-dimethyl-	-	-	8.95±0.13	-	a
99	Farnesylacetone	-	-	0.23±0.00	4.36±0.07	a
100	(+) Spathulenol	-	0.63±0.01	-	-	a
101	(-)-Caryophyllene oxide	-	0.83±0.02	-	-	a
102	1-Decanol, 2-hexyl-	-	-	1.75±0.02	-	a
103	Unknown	-	-	2.32±0.02	-	a
104	Hexadecane	-	-	-	0.26±0.02	a,b
105	n-Hexadecanoic acid	-	-	-	2.49±0.05	a,b
106	Isophytol	-	-	0.21±0.00	-	a
107	.Beta.-selinene	1.04±0.02	-	-	-	a
108	Heptadecane		0.25±0.00	-	-	a
109	.Delta.-Cadinene	1.24±0.03	-	-	-	a
110	Hexadecanoic acid, methyl ester	-	-	0.29±0.03	-	a
111	9-Octadecenoic acid	-	-	-	0.18±0.02	a
112	n-Nonadecane	-	-	-	0.43±0.05	a

a : Compounds listed in order of elution from a DB-5 column.

b : Identification of components based on standard compounds

All values are mean ± standard deviation of triplicates ($p<0.05$), Percentage concentrations obtained by peak area normalization

Table 3. Antioxidant activities of the aqueous plant extracts

Sample	Inhibition IC50 (mg/mL) (DPPH)	Inhibition % (-carotene- Linoleic acid)
Helichrysum arenarium L.	0.037±0.010	18.50 ±0.09
Origanum sipyleum L.	1.043±0.094	85.40±1.98
Plantago major L.	0.085±0.003	89.07±0.75
Rumex spp.	1.985±0.125	56.43±0.25
BHT	3.457±0.092	97.55±1.45

BHT: Standard antioxidant

*Values are mean of three replicate determinations (n=3) ± standard error. Mean values followed by different letters in a column are significantly different ($p<0.05$).

In silico analysis

When mechanically applied force is exerted during the chewing of plant-based foods, plant tissues, including cell walls and other structures, undergo fragmentation. This mechanical disruption leads to the release of volatile compounds from plant cells. Considering that areas exposed to volatile compounds include the oral cavity, the potential anticancer effects of these volatile compounds have been evaluated *in silico*. The relevant ligands and analysis results were presented in the heatmap clustering provided in Figure 2 and Figure 3.

The computational analysis results regarding the probability of volatile compounds being mutagenic and carcinogenic are presented in Table 4. The average values calculated using the XGBoost method for highly carcinogenic compounds are close to 1, whereas the values obtained from plant volatiles remain below the average carcinogenicity (0.5). These bioactive compounds, currently utilized in the pharmaceutical and cosmetic industries, as evident from the results, do not possess carcinogenic or mutagenic effects at moderate concentrations.

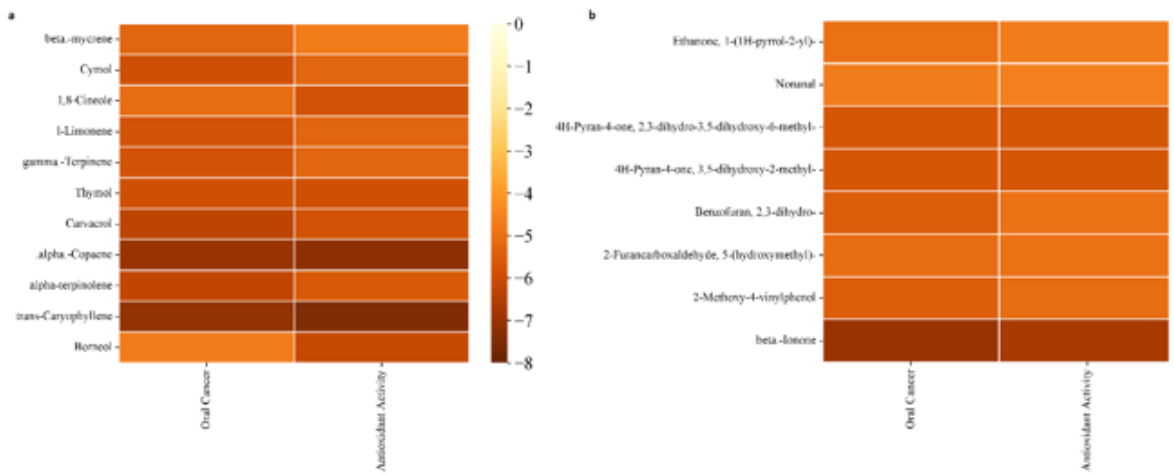

Figure 2. Clustered hierarchical heatmap showing binding affinities from a) *O. sipyleum* and b) *Rumex* spp.

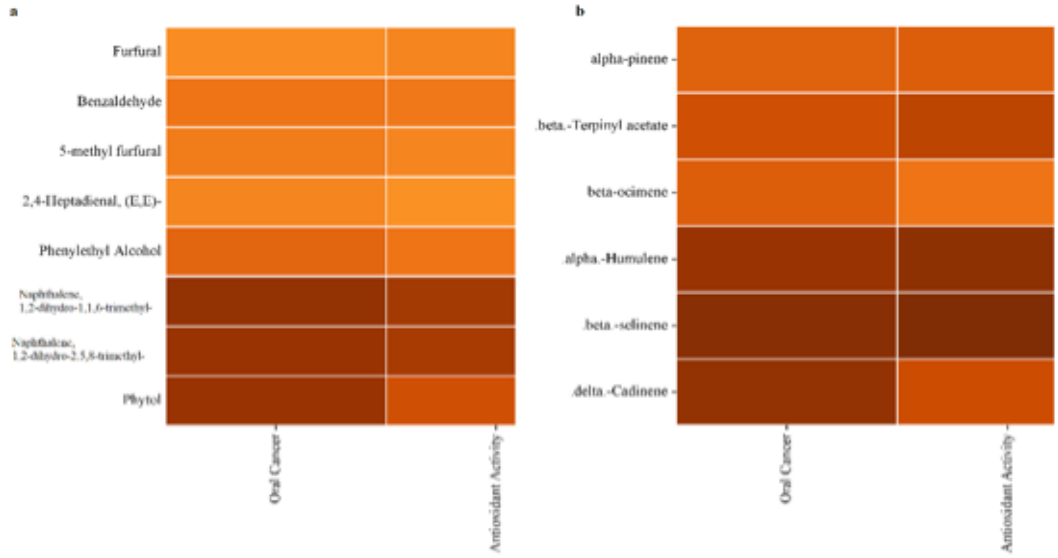

Figure 3. Clustered hierarchical heatmap showing binding affinities from a) *P. major* and b) *H. arenarium*

Table 4. Carcinogenicity and mutagenicity prediction results

Compounds	Carcinogenicity Score (CarcinoPred -EL/ XGBoost Method)	Predicted Result	Mutagenicity Prediction	Acute toxicity (Daphnia magna) (mg/L)
Thymol	0.49	Non-Carcinogen	non-mutagenic	7.4
.Gamma. -Terpinene	0.49	Non-Carcinogen	non-mutagenic	1.85
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	0.43	Non-Carcinogen	mutagenic	-
2-Methoxy-4-vinylphenol	0.49	Non-Carcinogen	non-mutagenic	2.13
Neophytadiene	0.45	Non-Carcinogen	non-mutagenic	0.473
2-Undecanone, 6,10-dimethyl-	0.46	Non-Carcinogen	non-mutagenic	0.329
.Alpha.-Humulene	0.47	Non-Carcinogen	non-mutagenic	0.57
Trans-Caryophyllene	0.49	Non-Carcinogen	non-mutagenic	4.8

In this study, analysis of the possibilities of plant volatiles activity through PASS online tool revealed that all the most abundant plant volatiles exhibit anticancer and have similarities with digestive system drugs related activities (Table 5). According to the results of the obtained ADMET analysis, all analyzed volatile components have the potential to be used as drugs according to Lipinski's Rule of 5 (Table 6).

Table 5. PASS predictions

	Antimetastatic		Antineoplastic		Anti-inflammatory		Mucomembranous protector		Gastrin inhibitor	
Compounds	**Pa**	**Pi**	**Pa**	**Pi**	**Pa**	**Pi**	**Pa**	**Pi**	**Pa**	**Pi**
Thymol	0.28	0.08	0.29	0.09	0.54	0.04	0.92	0.004	0.64	0.01
.Gamma.-Terpinene	0.47	0.02	0.55	0.05	0.7	0.01	0.39	0.17	0.25	0.24
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	0.44	0.03	0.54	0.05	0.47	0.06	-	-	-	-
2-Methoxy-4-vinylphenol	0.34	0.06	0.52	0.06	0.35	0.122	-	-	-	-
Neophytadiene	0.36	0.05	0.46	0.08	0.24	0.223	-	-	-	-
2-Undecanone, 6,10-dimethyl-	0.5	0.01	0.55	0.005	0.41	0.01	0.87	0.005	0.6	0.02
.Alpha.-Humulene	0.56	0.008	0.83	0.008	0.74	0.01	0.39	0.17	0.25	0.23
Trans-Caryophyllene	0.57	0.008	0.91	0.005	0.74	0.01	0.44	0.15	0.25	0.24

Table 6. ADMET prediction

Compound	Molecular Weight (g/mol)	Lipophilicity (ilogP)	Solubility (mg/ml)	GI (gastrointestinal) absorption	BBB (blood-brain barrier)	Lipinski Ro5
Thymol	150.22	2.32	9.74E-02	High	Yes	Yes
.Gamma.-Terpinene	136.23	2.73	4.79E-02	Low	Yes	Yes
4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	144.13	1.19	4.50E+01	High	No	Yes
2-Methoxy-4-vinylphenol	150.17	2.14	2.31E-01	High	Yes	Yes
Neophytadiene	278.52	5.05	4.74E-05	Low	No	Yes
2-Undecanone, 6,10-dimethyl-	198.34	3.22	7.98E-02	High	Yes	Yes
.Alpha.-Humulene	204.35	3.29	2.17E-02	Low	No	Yes
Trans-Caryophyllene	204.35	3.25	2.78E-02	Low	No	Yes

DISCUSSION

Volatile profiles of plants

In a study focused on the chemical composition of *O. sipyleum* essential oil, our findings revealed the presence of 10 common compounds, including thymol, linalool, and carvacrol (20). In another study of the same species, Semiz et al. (21), reported p-cymene as the highest essential oil volatile component, and 15 of the 34 volatile compounds that analyzed were determined to be the same as the volatile compounds in our study. On the other hand, Judzentiene et al. (22), reported palmitic acid (23.8 ± 1.13) and myristic acid (14.9 ± 1.05) are the highest volatile constituents of *H. arenarium* essential oil. It was determined that 6 out of 20 compounds, including beta-selinene were common with our analysis. According to the results of essential oil analysis of species collected from three different regions in Bulgaria, α-pinene (34.64–44.35%), sabinene (10.63–11.1%), germacrene D (3.56–4.86%) and ß-gurjunene (3.61%) components were dominant (23). In the analysis of *H. arenarium* essential oil collected from the Caucasus region, the largest group of compounds was determined to be aliphatic acids (34.6%), and 11.9% dodecanoic acid and 9.8% decanoic acid were reported (24). On the other hand, Lemberkovics et al. (25), reported that the dominant compound was methyl palmitate (21.7-28.5%) from Poland and Hungary regions. These discrepancies in chemical profiles may be a consequence of different environmental factors, such as plant harvesting strategies, soil type, precipitation, etc. For the *Rumex* spp., as a result of the volatile component analysis of the hydroalcoholic extracts of the *R. obtusifolius*, 1, 2, 3-benzenetriol (pyrogallol) (62.8%) and for the *R. crispus* 5-eicosene (31.7%) were reported as the dominant volatile compound (26,27). Among the four plants that were analyzed, *P. major* emerges as perhaps the species with the most changeable chemical content ecologically. In the Iranian region, Haghighi et al. (28), reported the volatile component 2-dodecen-1-yl (-) succinic anhydride (15.29%) as the main compound of the *P. major*. Jamilah et al. (29), reported volatiles of *P. major* differ with varying solvents. The main constituents in petroleum ether extract were reported as phytol 13.22%, and for methanol diglycerol, 30.31% and glycol l18.91%; ethyl acetate extract was glycerine 30.70%, benzene 21.81% and for aqueous extract were ethno-phenol 27.47% and diathiapentene 14.53% .

Antioxidant activities

Popa et al. (30), reported the DPPH activity in aqueous extracts of *H. arenarium* obtained from Romania as 0.151 mg/ml while Czinner et al. (31), found a similar IC50 value of 0.14 for samples from the Hungarian region. The DPPH value reported by Karima et al. (32), for Algerian *P. major* is 0.1 mg/mL, while the inhibition value in the ß-carotene/linoleate test is 50.16%. The results indicate a higher antioxidant activity in the plant species collected from the Ankara region. It is known that *Origanum* species generally exhibit high antioxidant activity and obtained results confirm this assertion for the species. Most of the antioxidant studies on *O. sipyleum* have been conducted on samples from Türkiye, and these studies generally report IC50 values ranging around 0.1 mg/mL and an average inhibition activity of approximately 80% in the ß-carotene/linoleic acid assay (33). On the other hand, in the literature, *Rumex* species have been reported to exhibit highly variable results in terms of antioxidant activities. The antioxidant analysis results conducted on edible wild *Rumex* species in Russia have reported IC50 values ranging from 3 to 69 mg/mL (34).

In silico analysis

Molecular docking

The heatmap was plotted by http://www.bioinformatics.com.cn/srplot, an online platform for data analysis and visualization. Based on the results of the clustered hierarchical heatmap, the highest antioxidant activity was detected in the volatiles of *H. arenarium* species. Although *O. sipyleum* exhibited the most potent antioxidant activity with trans-caryophyllene (-7.5 kcal/mol), the synergistic

antioxidant effect of volatile components was found to be the highest in this species. It has been determined that the volatile components of edible plants exhibit moderate binding affinities against oral cancer with an average of -6 kcal/mol binding affinity scores. The selected target in oral cancer has been preferred due to its promising discovery of upregulation in recent drug development studies (35). As a result of our *in silico* analyses, docking scores and binding positions that were obtained showed similarities with FDA (Food and Drug Administration)-approved kinase inhibitors such as afatinib, dacomitinib, and sapitinib. We could not find a literature study directly investigating the effects of these volatile compounds on oral cancer (Table 2). However, in some studies exploring the potential of essential oils and volatile components for oral cancer, including aromadendrene, among other compounds, have been identified (36,37). When molecular-level analyses are examined in 2D, it appears that alkyl bonds are predominant. Since the formation of alkyl bonds is dependent on hydrophobicity, it is determined that this interaction between the ligand and the protein arises from the protein's hydrophobicity. Unlike other molecular bindings, the observed pi-sigma bond in the naphthalene, 1,2-dihydro-1,1,6-trimethyl- ligand originates from the ring structure's active region (Figure 4).

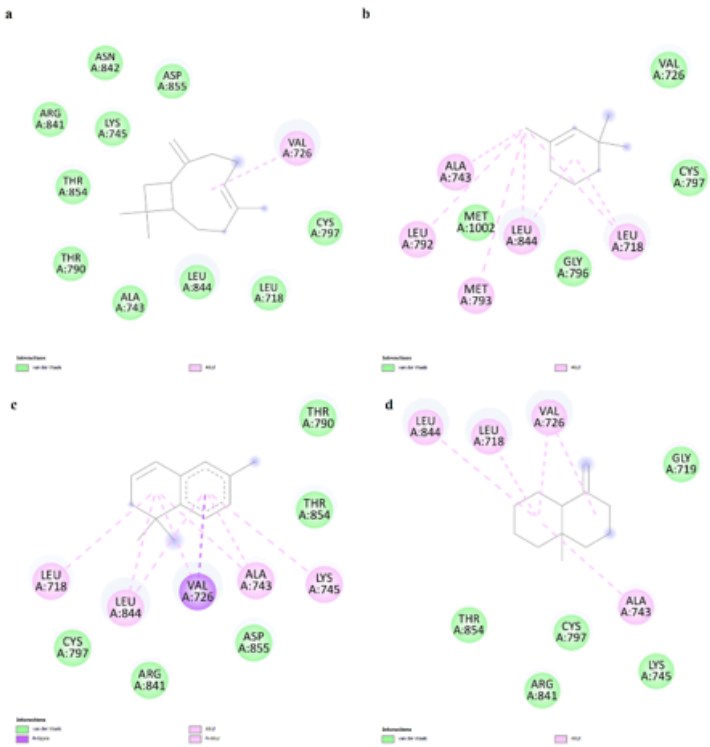

Figure 4. Two dimensional binding geometry of
a) trans-Caryophyllene (*O. sipyleum*), b) .beta.-Ionone (*Rumex* spp.),
c) naphthalene, 1,2-dihydro-1,1,6-trimethyl- (*P. major*) and d) .beta.-selinene (*H. arenarium*)

PASS prediction and ADMET results

It is known that Pa values above 60% indicate strong similarity, as known from previous studies (38). In addition to the absence of a structure resembling digestive drugs in approximately 50% of the compounds constituting the *Rumex* spp., there was also an average resemblance to anticancer drugs. However, notably high similarities in antineoplastic and anti-inflammatory activity were observed in *P. major* and *H. arenarium* species (Pa>0.7). These findings parallel the potential ethnomedicinal use of both plant species in addressing issues related to digestion and indigestion (39).

In addition to drug similarities, these natural volatile components themselves exhibit characteristic pharmacological properties (40). In this study, which sheds light on the potential of natural herbal volatiles as drug molecules for oral cancer, it is expected that the components provide permeability across the BBB and exert an effect on the target organ. The obtained results indicate that while some of the volatile components abundant in plants provide permeability, others do not. Having synergistic blood-brain barrier permeability for plant volatiles is a significant advantage in preventing adverse effects on the brain and prolonged exposure to the active substance.

In conclusion, investigating the pharmacological potentials of edible plants is crucial for both pharmaceutical and food research. These plants are known by different names in various regions of our country and are actively consumed. During the mechanical breakdown of edible plants in the mouth, volatile compounds are also exposed directly to the oral cavity. Analysis conducted in the study revealed that all four plants are rich in volatile compounds, which exhibit *in vitro* and *in silico* antioxidant properties. Molecular-level analyses on oral cancer also indicated that the volatiles of these plants harbor anticancer potential without exhibiting carcinogenic or mutagenic properties. It has been determined that the species exhibiting the highest pharmacological activity is *H. arenarium*. This research evaluates in silico anticancer activity of natural plant volatiles by targeting oral cancer and antioxidant proteins. Further *in vitro* and *in vivo* validations are necessary to investigate their safety profile and potential interactions with other drugs.

ETHICS COMMITTEE APPROVAL

* This study does not require Ethics Committee Approval.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Leonti M, Casu L, de Oliveira Martins DT, Rodrigues E, Benítez G. Ecological theories and major hypotheses in ethnobotany: their relevance for ethnopharmacology and pharmacognosy in the context of historical data. Rev Bras Farmacog, 2020;30(4):451-66.
2. Pirintsos S, Panagiotopoulos A, Bariotakis M, Daskalakis V, Lionis C, Sourvinos G, et al. From traditional ethnopharmacology to modern natural drug discovery: a methodology discussion and specific examples. Molecules, 2022;27(13):4060.
3. Godswill AG, Somtochukwu IV, Ikechukwu AO, Kate EC. Health benefits of micronutrients (vitamins and minerals) and their associated deficiency diseases: a systematic review. Int J Food Sci, 2020;3(1):1-32.
4. Leitzmann C. Characteristics and health benefits of phytochemicals. Forschende Komplementärmedizin/Research in Complementary Medicine, Forsch Komplementmed, 2016;23(2):69-74.
5. Acıbuca V, Budak DB. Dünya'da ve Türkiye'de tıbbi ve aromatik bitkilerin yeri ve önemi. Çukurova Tarım Gıda Bil Derg, 2018;33(1):37-44.
6. Selvi S, Polat R, Çakilcioğlu U, Celep F, Dirmenci T, Ertuğ ZF. An ethnobotanical review on medicinal plants of the Lamiaceae family in Turkey. Turk J Botany, 2022;46(4):283-332.
7. Trivellini A, Lucchesini M, Maggini R, Mosadegh H, Villamarin TS, Vernieri P, Mensuali-Sodi A, Pardossi A. Lamiaceae phenols as multifaceted compounds: bioactivity, industrial prospects and role of "positive-stress". Indust Crop Product, 2016 May 1;83:241-54.
8. Cinbilgel I, Kurt Y, Johm J. Oregano and/or marjoram: Traditional oil production and ethnomedical utilization of Origanum species in southern Turkiye. 2019;16:100257.
9. Akinyede KA, Cupido CN, Hughes GD, Oguntibeju OO, Ekpo OEJP. Medicinal properties and In vitro biological activities of selected Helichrysum species from South Africa: a review. 2021;10(8):1566.
10. Minina EA, Staal J, Alvarez VE, Berges JA, Berman-Frank I, Beyaert R, et al. Classification and nomenclature of metacaspases and paracaspases: no more confusion with caspases. Molecular Cell, 2020;77(5):927-9.
11. Pinto T, Aires A, Cosme F, Bacelar E, Morais MC, Oliveira I, et al. Bioactive (poly) phenols, volatile compounds from vegetables, medicinal and aromatic plants. Foods, 2021;10(1):106.
12. Vural N. Chemometrics data analysis and controversial carcinogenic effect of Ocimum basilicum L. rich in methyl eugenol. J Food Measure Charact, 2021;15(5):4825-37.
13. Göze İ, Kaymak S, Vural N. Chemical profiling of essential oils: investigations into modulating milk production in dairy cows using in silico methods. Sci Phytochem, 2024;3(1):27-37.
14. Huey R, Morris GM, Forli S. Using AutoDock 4 and AutoDock Vina with AutoDockTools: A Tutorial. California: The Scripps Research Institute Molecular Graphics Laboratory. 2011.
15. Biovia Discovery Studio Modeling Environment. USA: Dassault Systèmes. 2015.
16. Zhang L, Ai H, Chen W, Yin Z, Hu H, Zhu J, et al. CarcinoPred-EL: novel models for predicting the carcinogenicity of chemicals using molecular fingerprints and ensemble learning methods. Sci Rep, 2017;7(1):2118.
17. Maunz A, Gütlein M, Rautenberg M, Vorgrimmler D, Gebele D, Helma C. Lazar: a modular predictive toxicology framework. Front Pharmacol, 2013;4:38.
18. Filimonov D, Lagunin A, Gloriozova T, Rudik A, Druzhilovskii D, Pogodin P, et al. Prediction of the biological activity spectra of organic compounds using the PASS online web resource. Chem Heterocycl Compd, 2014;50:444-57.

19. Daina A, Michielin O, Zoete VJS. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep, 2017;7(1):42717.

20. Oluk EA, Çakır A, Yaşa I, Çapanlar S, Kırmızıgül S. Comparison of the antimicrobial activity and essential oil content of wild and micropropagated Origanum sipyleum L.: A medicinal herb native to Turkiye. J Med Plant Res, 2013;7(6):230-3.

21. Semiz G, Semiz A, Mercan-Doğan N. Essential oil composition, total phenolic content, antioxidant and antibiofilm activities of four Origanum species from southeastern Turkiye. Int J Food Prop, 2018;21(1):194-204.

22. Judzentiene A, Budiene J, Nedveckyte I, Garjonyte R. Antioxidant and toxic activity of Helichrysum arenarium (L.) Moench and Helichrysum italicum (Roth) G. don essential oils and extracts. Molecules, 2022;27(4):1311.

23. Zheljazkov VD, Semerdjieva I, Yankova-Tsvetkova E, Astatkie T, Stanev S, Dincheva I, et al. Chemical profile and antimicrobial activity of the essential oils of Helichrysum arenarium (L.) Moench. and Helichrysum italicum (Roth.) G. Don. Plants (Basel), 2022;11(7):951.

24. Czinner E, Lemberkovics É, Bihátsi-Karsai E, Vitányi G, Lelik L. Composition of the essential oil from the inflorescence of Helichrysum arenarium (L.) Moench. J Essent Oil Res, 2000;12(6):728-30.

25. Lemberkovics E, Czinner E, Balázs A, Bihátsi-Karsai E, Vitanyi G, Lelik L, et al. New data on composition of esssential oil from inflorescence of everlasting (Helichrysum arenarium (L.) Moench.). Acta Pharm Hung, 2001;71(2):187-91.

26. Nazari H, Bakhshandeh N, Gholami M, Mehrzad J, Bineshian F. Anti-Candida activities and GC mass analysis of seeds hydroalcohlic extract of Rumex obtusifolius. Jundishapur J Microbiol, 2017;10(7).

27. Idris OA, Wintola OA, Afolayan AJ. Comparison of the proximate composition, vitamins (ascorbic acid, α-tocopherol and retinol), anti-nutrients (phytate and oxalate) and the GC-MS analysis of the essential oil of the root and leaf of Rumex crispus L. Plants (Basel), 2019;8(3):51.

28. Haghighi SR, Yazdinezhad A, Bagheri K, Sharafi AJP, Research B. Volatile constituents and toxicity of essential oils extracted from aerial parts of Plantago Lanceolata and Plantago Major growing in Iran. Pharm Biomed Res, 2022; 8(3): 205-24. 2022.

29. Jamilah J, Sharifa A, Sharifah N. GC-MS analysis of various extracts from leaf of Plantago major used as traditional medicine. World Appl Sci J, 2012;17:67-70.

30. Popa G, Danaila-Gıidea S, Damian E, Margarit G, Gropoşila-Constantinesu D. Evaluation of Bioactive Compounds With Antioxidant Activity of Helichrysum arenarium (L.) Moench. In Florescens Extracts. 2023;67(2)

31. Czinner E, Hagymási K, Blázovics A, Kéry Á, Szőke É, Lemberkovics É. In vitro antioxidant properties of Helichrysum arenarium (L.) Moench. J Ethnopharm, 2000;73(3):437-43.

32. Karima S, Farida S, Mihoub Z. Antioxidant and antimicrobial activities of Plantago major. Int J Pharmacy Pharm Sci, 2015;7(5):58-64.

33. Kaska A. The phenolic content, antioxidant and cytotoxic activities of Origanum sipyleum from Turkiye. Int J Sec Metab, 2018;5(4):343-52.

34. Nakiboglu M, Urek RO, Kayali HA, Tarhan L. Antioxidant capacities of endemic Sideritis sipylea and Origanum sipyleum from Turkiye. Food Chem, 2007;104(2):630-5.

35. Feduraev P, Skrypnik L, Nebreeva S, Dzhobadze G, Vatagina A, Kalinina E, et al. Variability of phenolic compound accumulation and antioxidant activity in wild plants of some Rumex species (Polygonaceae). Antioxidants (Basel), 2022;11(2):311.

36. Santi MD, Zhang M, Liu N, Viet CT, Xie T, Jensen DD, et al. Repurposing EGFR inhibitors for oral Cancer Pain and Opioid Tolerance. Pharmaceuticals, 2023;16(11):1558.

37. Alam A, Jawaid T, Alsanad SM, Kamal M, Balaha MF. Composition, Antibacterial Efficacy, and Anticancer Activity of Essential Oil Extracted from Psidium guajava (L.) Leaves, 2023;12(2):246.

38. Ray A, Jena S, Sahoo A, Kamila PK, Das PK, Mohanty S, et al. Chemical composition, antioxidant, anti-inflammatory and anticancer activities of bark essential oil of Cryptocarya amygdalina from India. J Essent Oil-Bear Plants, 2021;24(3):617-31.

39. Rudik AV, Dmitriev AV, Lagunin AA, Filimonov DA, Poroikov V, Joci VJ. Metabolism site prediction based on xenobiotic structural formulas and PASS prediction algorithm. J Chem Inf Model, 2014;54(2):498-507.

40. Gonçalves S, Moreira E, Andrade PB, Valentão P, Romano A. JEFR Technology. Effect of in vitro gastrointestinal digestion on the total phenolic contents and antioxidant activity of wild Mediterranean edible plant extracts. Eur Food Res Technol, 2019;245:753-62.