International Journal of Secondary Metabolite
2023, Vol. 10, No. 1, 71–85
https://doi.org/10.21448/ijsm.1132698
Published at https://dergipark.org.tr/en/pub/ijsm
Research Article

Analysis of phytochemical composition and biological activities of *Helichrysum pallasii* (Sprengel) ledeb leaves

Nejmi Işık[1], Muzaffer Mukemre[2], Rabia Sena Turker[3], Gokhan Zengin[4], Abdullah Dalar[1,*]

[1]Van Yuzuncu Yil University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Van, 65100, Türkiye
[2] Hakkari University, Yuksekova Vocational School, Department of Plant and Animal Production, Hakkari, 30100, Türkiye
[3]Van Yuzuncu Yil University, Faculty of Pharmacy, Department of Pharmacology, Van, 65100, Türkiye
[4]Selcuk University, Faculty of Science, Department of Biology, Konya, 42130, Türkiye

Abstract: This study aimed at the investigation of *in vitro* biological activities (antioxidant and enzyme inhibition) and phytochemical composition of various extracts (ethanol maceration, aqueous infusion, and aqueous decoction) obtained from leaf organs of *Helichrysum pallasii*, which has been traditionally used as herbal tea and medicine in Eastern Anatolia.

Experimental analysis showed that ethanol-based extract had higher total phenolic content (TPC: 103 mg GAE/gE) and showed superior antioxidant potentials (FRAP: 2205 µmol Fe2+/gE; ORAC: 2540 µmol Trolox Eq./gE; DPPH: IC50=0.58 mg/ml; CUPRAC: IC50=0.37 mg/ml; Phosphomolybdenum: IC50=1.34 mg/ml ve metal chelation: IC50=1.42 mg/ml) and enzyme inhibition (Acetylcholinesterase: IC50=1.49 mg/ml; Butyrylcholinesterase: IC50=1.98 mg/ml; Tyrosinase: IC50=0.68 mg/ml; Alpha-amylase: IC50=2.09 mg/ml; Alpha-glucosidase: IC50=0.51 mg/ml; and Pancreatic lipase: IC50=42.5 µg/ml) and contained higher amounts of phenolic (chlorogenic acid isomers and rutin) and fatty acid (palmitic, linoleic, and linolenic acids) compounds than traditional preparations (infusion and decoction).

The current study's findings indicate that the leaves of the *Helichrysum pallasii* are a source of phytochemicals with strong antioxidant and enzyme inhibitory properties, implying that it could be a candidate for biotherapeutic agent research and development.

ARTICLE HISTORY

Received: Jan. 18, 2022
Accepted: Feb. 14, 2023

KEYWORDS

Helichrysum pallasii,
Antioxidant,
Enzyme inhibition,
Phenolics, Biotherapeutic.

1. INTRODUCTION

The ethnobotanical knowledge in the Anatolian area consists of the blending of the ethnobotanical knowledge of the native and immigrant peoples due to the rich vegetation, the high rate of endemic plants, and the migration of various tribes and nations from other lands to Anatolia, along with their knowledge and usage patterns. Especially the Eastern Anatolia

*CONTACT: Abdullah Dalar dalar.abdullah@gmail.com Department of Pharmaceutical Botany, Faculty of Pharmacy, Van Yuzuncu Yil University, Van, Türkiye

ISSN-e: 2148-6905 / **© IJSM 2023**

Region is home to a vibrant culture in terms of ethnobotany (Altundag and Ozturk, 2011; Dalar *et al.*, 2018).

This study chose *Helichrysum pallasii* (Asteraceae) (Figure 1), which has been used in traditional medical treatment for a long time in the Eastern Anatolia Region. In Eastern Anatolia, *Helichrysum pallasii* is called as Ölmez çiçek, Altın çiçek, Altın otu, Herdemtaze, and Herdemcan. It is commonly used as folk medicine to treat diabetes, epilepsy, colds, and respiratory disorders. Taxa of *the Helichrysum* genus in Anatolia are used internally, primarily by preparing herbal tea by infusion or decoction (Baytop, 1999; Mükemre *et al.*, 2015; Dalar *et al*., 2018, Dalar and Mükemre, 2020).

Herbal teas have been widely used in the daily lives of various cultures for their health-promoting activities. They are among the primary sources of dietary antioxidants in the diet of many cultures due to the presence of phenolics, vitamins, and carotenoids (Piljac-Žegarac *et al*., 2013). Herbal tea process is among the oldest and simplest forms of drug administration known in herbal therapy. The plants used as herbal tea are collected during the vegetation period, dried in the dark and stored for later use (Altundag and Ozturk, 2011; Dalar *et al.*, 2018; Dalar and Mükemre, 2020). Bioactive components can interact more effectively with organic solvents. These solvents are thought to deform the cell wall and vacuole membrane more effectively, enabling the release of compounds in the plant sap. Dai and Mumper (2010) propounded that ethanol solvent is highly effective in extracting plant bioactive components.

Despite extensive utilization as infusion and decoction, there is only limited data regarding the chemical composition and biological activities of organic solvent-based extracts obtained from *the Helichrysum* genus in the literature (Carini *et al*., 2001; Süzgeç *et al*., 2005; Albayrak *et al*., 2008; Kolaylı *et al.*, 2010; Albayrak *et al*., 2010; Jahromi *et al*., 2017). Therefore, this study aimed to (i) identify phytochemical composition by using HPLC-MS/MS and GC-MS and (ii) measure total phenolic levels and antioxidant capacities by using complementary methods (FRAP, ORAC, DPPH, Metal chelation, CUPRAC, and Phosphomolybdenum) of traditional preparation methods (infusion and decoction) and ethanol-based extracts obtained from *Helichrysum pallasii* leaves comparatively.

2. MATERIAL and METHODS

2.1. Plant materials

Leaf samples of *Helichrysum pallasii* (Figure 1a) were collected from the rocky slope, steppe habitats in the hamlet of Batkan (Tanriverdi), the village of Konalga, Çatak, Van, Türkiye on June 24th, 2019 (Global Positioning System (GPS) coordinates 37° 50' 080" N, 43° 12' 412" E, 2163 m) and transferred to the laboratory. Botanical identification of the plant samples was carried out at Van Pharmaceutical Herbarium (VPH), Faculty of Pharmacy, Van Yuzuncu Yil University, Turkey, and the voucher specimens were stored at VPH (Herbarium code: VPH-548, Collector Code: DM-345) (Figure 1b). Plant materials were divided, adequately cleaned of dust and contaminants, and left at room temperature in the dark until dry (Figure 1c). Subsequently, the samples were ground into a fine powder using a grinding mill (Isolab laboratuar mill 602, Interlab, İstanbul, Turkey) and stored at -20°C for a maximum of 4 weeks until analysis.

Figure 1. *Helichrysum pallasii* (Sprengel) Ledeb. **a.** General view of the plant; **b.** Herbarium specimen; **c.** Dried leaf samples.

a	b	c

2.2. Chemicals

Unless otherwise stated, all chemicals were obtained from Sigma-Aldrich, Inc. (St Louis, MO, USA) and were of analytical or HPLC grade.

2.3. Preparation of Extracts

2.3.1. *Ethanol extract*

The ethanol-based lyophilized extract was prepared as described previously (Dalar and Konczak, 2013). Briefly, the ground plant material was mixed with a 10-fold volume of acidified ethanol (80% ethanol and 1% HCl (v/v) in water) shaken for 2 h at room temperature (22°C), and centrifuged at 15,320 x g (10000 rpm in a Beckman JA14 (137 mm) rotor, Sorvall RC-5B centrifuge, Wilmington, DE, USA) at 4°C for 20 min, after which the supernatant was collected. Subsequently, the supernatant was individually evaporated using reduced pressure at 37°C and a rotary evaporator (Rotavapor R-205, Buchi, Flawil, Switzerland). The concentrated fraction was freeze-dried using a lyophilizator (Alpha 1-2 LDplus, Christ, Osterode am Harz, Germany) under a vacuum at -51°C to fine lyophilized powder stored at -20°C for a maximum of 4 weeks until being analyzed.

2.3.2. *Herbal infusion extract*

The herbal infusion was prepared according to Baytop (1999). Briefly, plant materials (leaf) were mixed with a 10-fold volume (g/ml) of preboiled distilled water and incubated for 10 min. The mixture was filtered using cotton and vacuum filtering (45 µm). The filtrates were evaporated and freeze-dried as previously described.

2.3.3. *Herbal decoction extract*

The decoction was prepared according to Baytop (1999). Briefly, the leaf powder was mixed with a 10-fold volume (g/ml) of cold distilled water and heated to boiling. The mixture simmered for 3 min, then was placed aside to cool over the next 10 min and processed like the herbal infusion.

2.4. Total Phenolic Content

The extracts' phenolic content was measured using the Folin-Ciocalteu method, as described previously by Ainsworth and Gillespie (2007). It was expressed as mg gallic acid equivalents per gram of dry weight of the lyophilized extracts, based on the gallic acid standard curve and against a blank control. The analyses were conducted in triplicate.

2.5. Antioxidant Capacity

Ferric reducing antioxidant power (namely total reducing; FRAP) assay was conducted according to Benzie and Strain (1996), and total reducing capacities of the extracts were expressed as µmol of iron (Fe^{2+}) per gram of dry weight of lyophilized extracts based on an iron sulphate standard (Fe_2SO_4) curve against a blank control. The extracts' oxygen radical scavenging (ORAC) capacities were measured as described previously by Dalar & Konczak (2013). Based on a Trolox standard curve, the results were expressed as µmol Trolox equivalent per gram of dry weight of the lyophilized extract.

The total antioxidant (TAC; phosphomolybdenum method), DPPH radical scavenging, ABTS radical cation scavenging, cupric ion reducing (CUPRAC), and metal chelating activities of the extracts were determined as previously described by Uysal *et al*. (2017) and the results were expressed as IC_{50}-half maximal inhibitory concentration (mg extract/ml).

2.6. Enzyme Inhibitory Activities

Cholinesterase (ChE), α-amylase, α-glucosidase, and tyrosinase inhibitory activities of the extracts were determined according to Zengin (2016) and expressed as IC_{50}-half maximal inhibitory concentration (mg extract/ml). The pancreatic lipase activity was assayed as described previously (Dalar and Konczak, 2013) using 4-methylumbelliferyl oleate (0.1 mmol) as a substrate, and the results were expressed as IC_{50}-half maximal inhibitory concentration (mg extract/ml). All inhibitory enzyme analyses were conducted in triplicate.

2.7. Chemical Composition

2.7.1. *HPLC-MS/MS analysis*

Identification and quantification of phenolic compounds by high-performance liquid chromatography-diode array detector (HPLC-DAD) and liquid chromatography-photo-diode array–mass spectrometry (LC-PDA–MS/MS) (Thermo Fisher Scientific, Waltham, MA, USA) analysis were conducted as described previously (Dalar and Konczak, 2013). The composition of phenolic compounds was characterized based on their UV spectrum, retention time, co-chromatography with commercial standards, when available, and MS/MS fragmentation patterns. MS experiments in the full scan (parent and product-specific) and the selected reaction monitoring (SRM) mode were conducted. All analyses were conducted in triplicate.

2.7.2. *GC-MS Analysis*

Volatile compounds and fatty acids were analyzed by gas chromatography-mass spectrometry (GC/MS) (3800 Varian GC, Agilent Technologies) using a headspace solid-phase microextraction and identified by the fragment ions and relative retention indices of their peaks with those of the MS library standards as described previously (Uzun *et al*., 2017). All analyses were conducted in triplicate.

2.8. Data Analysis

The mean values were calculated based on at least three determinations (n=3). One-way ANOVA followed by the Bonferroni *posthoc* test was performed to assess differences between the samples at $p<0.05$ through Graphpad Prism 5 (Graphpad Software, CA, USA).

3. RESULTS and DISCUSSION

3.1. Phytochemical Composition

The highest extraction yield was determined in infusion (24.4%) and decoction (26.2%) extracts, and the lowest yield in ethanol extract (16.2%); however, when the results of antioxidant and enzyme inhibition activities were examined, it was observed that the highest values were in ethanol extracts. The phytochemical composition of the extracts was analyzed

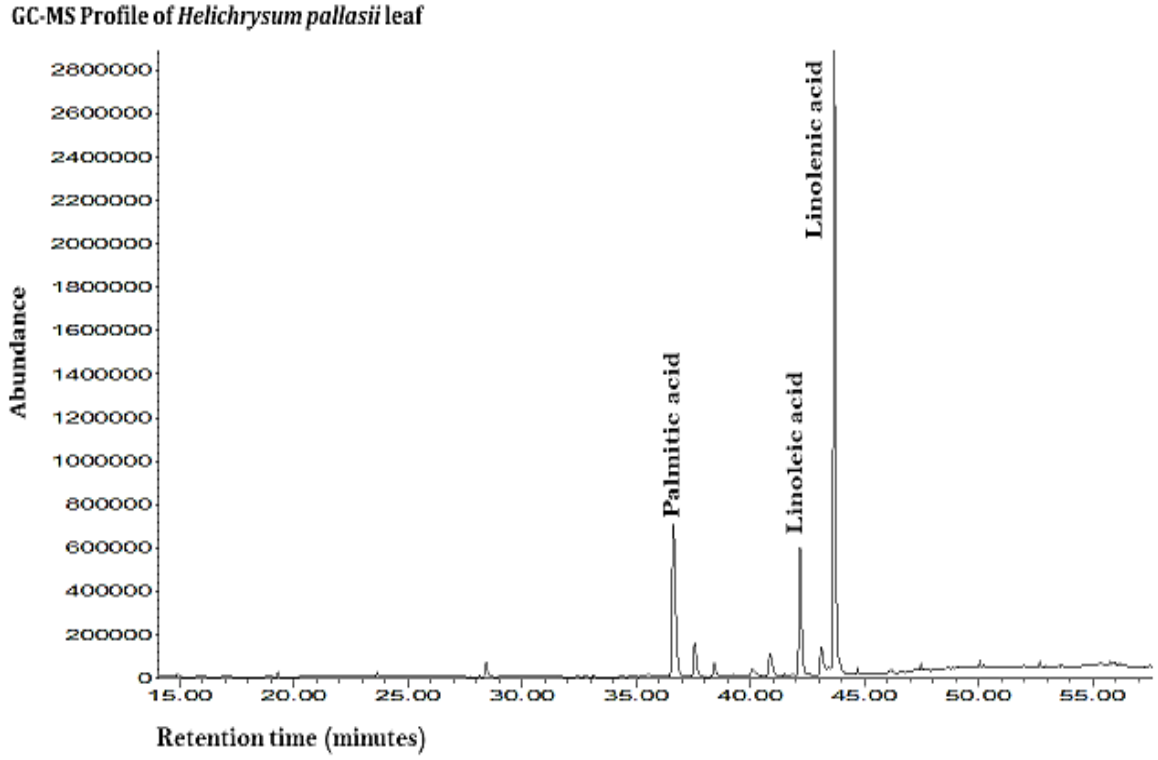
to detect significant contributors to antioxidant and enzyme inhibition activities. The fatty acid compositions of the extracts were determined using GC-MS, and the phenolic compound compositions were determined using HPLC-MS/MS. As presented in Table 1 and Figure 2, the fatty acid compositions of the extracts consist of three main components, namely palmitic, linoleic, and linolenic acids in the ethanol extract; and two fatty acid compounds, palmitic and linolenic acids were detected in infusion and decoction extracts. Palmitic and linolenic acids were determined as the most dominant fatty acids in the extracts. These findings are consistent with previous chromatographic results of *Helichrysum* species (Albayrak *et al*., 2008). This finding showed that *Helichrysum* species are among the well-tolerated plant species to environmental stress factors such as UV and soil. Although the levels of fatty acid compounds were detected at low levels in the extracts, they may contribute to biological activities such as reducing the risk of hypertension and cancer and lowering levels of serum cholesterol, triglycerides, and LDL cholesterol (Kim *et al*., 2014; Shen *et al*., 2012) as reported previously.

Table 1. GC-MS profile of *Helichrysum pallasii* leaves.

	Retention time	Compound	Relative concentration (%)		
			Ethanol	Infusion	Decoction
1	36.77	Palmitic acid	21.1±0.2^{c}	76.3±2.6^{a}	65.8±1.1^{b}
2	42.47	Linoleic acid	14.9±0.2^{a}	-	-
3	43.77	Linolenic acid	53.8±1.4^{a}	19.8±0.5^{c}	34.1±0.8^{b}

Means with different letters in the same column were significantly different at $p < 0.05$; all data were determined as a result of at least three independent experiments.

Figure 2. GC-MS profile of *Helichrysum pallasii* leaves



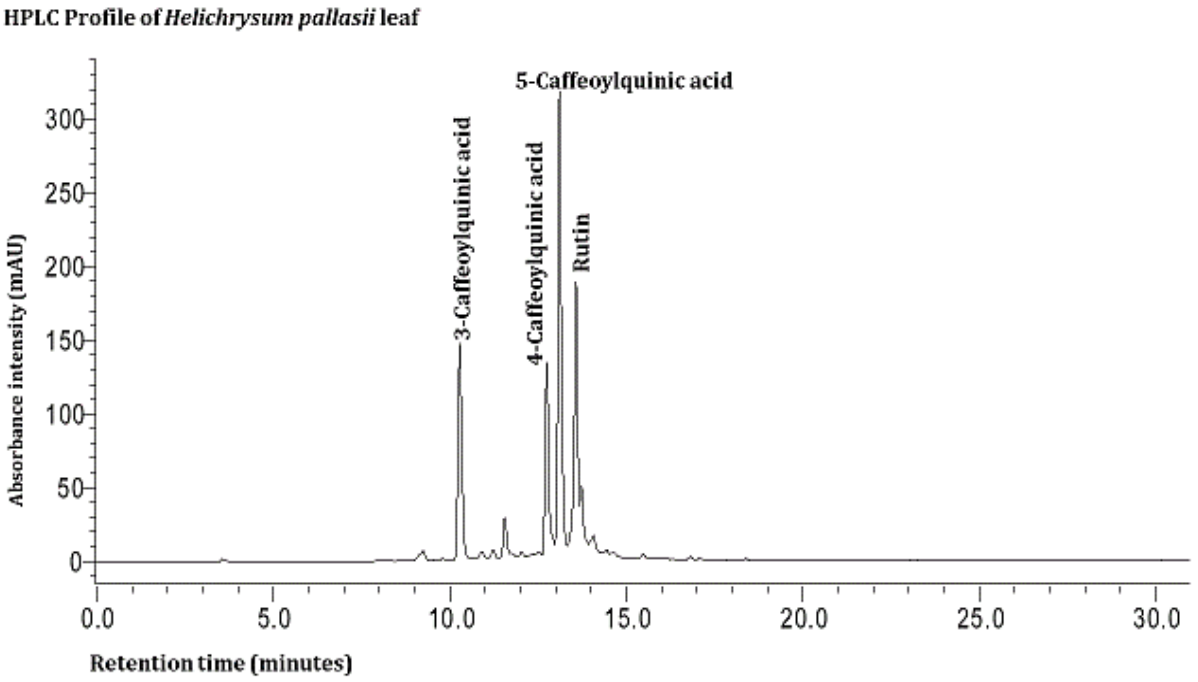
The identities of phenolic compounds were obtained using HPLC-PDA-MS/MS, and results are presented in Table 2 and Figure 3. As a result of the analysis, four compounds were determined. According to co-chromatography, UV spectrum properties, molecular weights, fragment ions, neutral loss, selective reaction imaging, and esterification reaction studies, the predominant compounds in the extracts were chlorogenic acid isomers (3-caffeoylquinic, 4-caffeoylquinic, and 5-caffeoylquinic acids) and the rutin.

Table 2. HPLC-MS/MS profile of *Helichrysum pallasii* leaf.

Individual phenolic compounds	MS/MS		Concentration (µg/mg extract)		
	-/[M-1]$^-$	Fragments (m/z) (+/-)	Ethanol	Infusion	Decoction
3-Caffeoylquinic acid	-/353	-/191, 179	14.9±0.2^a	9.1±0.1^b	7.5±0.1^c
4-Caffeoylquinic acid	-/353	-/191, 179, 173	9.2±0.1^a	5.2±0.1^b	4.7±0.1^c
5-Caffeoylquinic acid	-/353	-/191	42.5±1.2^a	25.8±0.9^b	19.9±1.0^c
Rutin	-/609	303/301	22.1±3.2^a	6.9±0.4^b	6.1±0.3^c

Means with different letters in the same raw were significantly different at the level ($p< 0.05$); n=3.

Figure 3. HPLC profile of *Helichrysum pallasii* leaf.



Various studies have been carried out on the beneficial effects of chlorogenic acid isomers and rutin until now. The most abundant form of chlorogenic acid in nature is 5-caffeoylquinic acid (Olthof *et al*., 2001). Chlorogenic acid isomers (3-caffeoylquinic, 4-caffeoylquinic, and 5-caffeoylquinic acids) show many biological activities such as antibacterial, antioxidant, anticarcinogenic, hypoglycaemic, hypolipidemic, hepatoprotective, antihistamine antimutagenic, anti-obesity, antidiabetic, antiviral, anti-Alzheimer, neuroprotective (Clifford *et al*., 2017; Hamed *et al*., 2020; Matthews *et al*., 2020; Torres *et al*., 2021). Rutin is a flavonoid found in many plants, also considered a non-toxic chemical that may be useful in biomedical applications and showing biological activities such as anti-inflammatory, anticarcinogenic,

antioxidant, neuroprotective, cytoprotective, vasoprotective, cardioprotective, and hepatoprotective (Ghorbani, 2017; Shahi *et al*., 2019).

The findings agree with the phytochemical composition results in species belonging to the *Helichrysum* genus. For example, Albayrak *et al.* (2008) reported the presence of p-hydroxybenzoic acid, chlorogenic acid, caffeic acid, syringic acid, p-coumaric acid, ferulic acid, hesperidin, apigenin-7-glucoside, naringenin, and apigenin compounds in the methanol extract prepared from the aerial part of *H. pallasii*. In other studies, significant compounds have been reported as chlorogenic acid, naringenin glucosides, quercetin, and apigenin glycosides in *H. stoechas* capitulum (Carini *et al*., 2001); apigenin, apigenin-7-glucoside, luteolin, naringenin and quercetin in *H. compactum* (Süzgeç *et al*., 2005); chlorogenic acid, caffeic acid, ferulic acid, p-coumaric acid, p-hydroxybenzoic acid, syringic acid, apigenin, apigenin-7-glucoside, epicatechin, hesperidin, luteolin, naringenin and resveratrol in *H. arenarium* subsp. *erzincanicum*, *H. arenarium* subsp. *rubicundum*, *H. armenium* subsp. *araxinum*, and *H. plicatum* subsp. *pseudoplicatum* (Albayrak *et al*., 2010); p-hydroxybenzoic, caffeic acid, chlorogenic acid, 5,7-dihydroxy-3,6,8-trimethoxyflavone, isoquercitrin, quersetagetin-7-O glucopyranoside in the methanol extract of *Helichrysum stoechas* Moench plant (Les *et al*., 2017). The solvent structure of ethanol can explain the high levels of the phenolic compounds detected in the ethanol extract and its ability to extract rutin and chlorogenic acid compounds more effectively than other polar solvents (Naczk and Shahidi, 2006).

3.2. Biological Activities

3.2.1. *Antioxidant potential*

Oxidative stress can damage lipids, structural proteins, carbohydrates and the DNA chain, which occurs when the balance between free radicals and antioxidants is disrupted. Oxidative stress also constitutes the molecular basis for the occurrence of many degenerative disorders such as cancer, arterial diseases, neurodegeneration, diabetes, autoimmune disorders, rheumatoid arthritis, and kidney disease (Di Meo *et al*., 2016). Antioxidants play a significant role in the prevention of damage that may occur as a result of oxidative stress when the endogenous defence system in the organism is interrupted, or free radicals are produced in excessive amounts for various reasons, exceeding the capacity of the defence system (Andries *et al*., 2020; Pisoschi *et al*., 2021).

In this study, the antioxidant results were determined by Ferric reducing power (FRAP), Oxygen radical absorbance capacity (ORAC), DPPH radical scavenging activity, ABTS radical cation scavenging, Cupric ion reducing activity (CUPRAC), Phosphomolybdate total antioxidant capacity, and Metal chelation activity methods. Antioxidant capacity results and total phenolic contents of the extracts prepared in the leaf part of *Helichrysum pallasii* are presented in Table 3.

Table 3. Total phenolic contents and antioxidant activities of *Helichrysum pallasii* leaf.

7		*Helichrysum pallasii* leaf			Positive control (Synthetic antioxidants)			
		Ethanol	Infusion	Decoction	Ascorbic acid	Butylated hydroxyanisol	Trolox	Ethylenediamine tetraacetic acid
	Total phenolics [1]	103.0±5.1b	68.5±5.0c	51.8±1.8d	384±9a	-	-	-
Antioxidant activity	Ferric reducing antioxidant power [2]	2205.6±16.2b	1660.6±29.5c	1289.7±4.8d	5049±15a	-	-	-
	Oxygen radical absorbance capacity [3]	2540.5±42.5b	1800.7±42.2c	1278.9±6.9d	-	5991±74a	-	-
	DPPH radical scavenging activity [4]	0.58±0.01b	0.75±0.02c	0.87±0.19d	-	-	0.06±0.01a	-
	ABTS radical scavenging activity [4]	0.85±0.02b	1.14±0.03c	1.30±0.01d	-	-	0.18±0.02a	-
	Cupric ion reducing antioxidant capacity [4]	0.37±0.01b	0.56±0.01c	0.65±0.01d	-	-	0.11±0.01a	-
	Phosphomolybdenum total antioxidant capacity [4]	1.34±0.03b	1.61±0.02c	1.73±0.05d	-	-	0.55±0.1a	-
	Metal chelation activity [4]	1.42±0.02b	1.51±0.02c	1.72±0.07d	-	-	-	0.03±0.0a

Means with different letters in the same row were significantly different at the level ($p< 0.05$). All data represent the mean ± standard deviation of at least three independent experiments (n=3). [1] mg Gallic acid Equivalent/g extract, [2] µ mol Fe^{2+}/g extract, [3] µ mol Trolox Equivalent/g extract, [4] IC_{50}-half maximal inhibitory concentration (mg extract /ml).

Considering the obtained results, ethanol > infusion > decoction ranking was revealed in terms of antioxidative potential. Although the ethanol, infusion, and decoction extracts were found as effective antioxidant sources capable of both single electron and hydrogen atom transfer, as well as metal binding mechanisms. They showed weaker antioxidant activities than synthetic antioxidant agents such as gallic acid, butylated hydroxyanisole, trolox, and ethylenediamine tetraacetic acid, which were used as positive controls in the present study (Table 3).

It was determined that the total phenolic values of the extracts of *Helichrysum pallasii* leaves ranged between 51.8-103 mg Gallic acid/g extract. Albayrak *et al.* (2008) reported 94.1 mg GAE/g extract in methanol extract prepared from the aerial part of the *H. pallasii* plant. In another study, Albayrak *et al.* (2010) reported the total phenolic contents of methanol extracts prepared in the aerial parts of *H. arenarium* subsp. *erzincanicum, H. arenarium* subsp. *rubicundum, H. armenium* subsp. *Araxinum,* and *H. plicatum* subsp. *pseudoplicatum* plants at a range of 71.8-144.5 mg GAE/g extract. Also, a very similar total phenolic content (108.33 mg GAE/g extract) compatible with our finding was found by Özkan et al. (2004) in the methanolic extract of *H. chasmolycicum*.

The FRAP values of the extracts were determined in the range of 2205.6-1289.7 µmol Fe2+/g extract, and the highest FRAP value was found in the ethanol extract (Table 3). FRAP values of the *Helichrysum* genus used for treatment have been reported in various studies. For example, Zengin *et al.* (2020) the FRAP value of 80% above-ground methanol extract of *Helichrysum stoechas* plant as 662.87mg TE/g; Kolaylı *et al.* (2010) the FRAP value of the extract prepared in the leaf part of *H. plicatum* plant as 336.25 Trolox/ 100 g DW; Bojilov *et al.* (2019) the FRAP value of the ethanol extract of the aerial part of *H. italicum* as 106.92 mM TE/g DW were reported. Like FRAP results, ethanol extract had superior ORAC, DPPH, ABTS, CUPRAC, Phosphomolybdate total antioxidant, and metal chelation activities. The literature search (Albayrak *et al*., 2008; Albayrak *et al*., 2010; Kolaylı *et al*., 2010; Jahromi *et al*., 2017; Bojilov *et al*., 2019; Zengin *et al*., 2020) revealed similar DPPH (IC_{50} =26.23 µg/ml for *H.pallasii*; IC_{50} =23-39 µg/ml for *H. arenarium* subsp. *erzincanicum, H. arenarium* subsp. *rubicundum, H. armenium* subsp. *araxinum* and *H. plicatum* subsp. *pseudoplicatum*; IC_{50} =58 µg/ml for *H. plicatum* and IC_{50} =61 µg/ml for *H. leucocephalum*), ABTS (107.51 mM TE/g DW for *H. italicum*), CUPRAC (06.86 mM TE/g DW for *H. italicum* and 927.39 mM TE/g DW for *H. stoechas*), and phosphomolybdate (118.99 mg AAE/g extract for *H. pallasii*) results which are in agreement with our findings.

Considering all data obtained, ethanol solvent and low-temperature usage might provide higher levels of antioxidant phenolic compounds from plant matrix. Chlorogenic acid was one of the most capable antioxidant compounds among phenolics regarding hydrogen atom transfer mechanism (Ou *et al*., 2001), confirmed *in vitro* and *in vivo* (Liang and Kitts, 2016). Caffeoylquinic acid derivatives prevent many diseases with their protective roles against oxidative stress in the human body (Yoshimoto *et al*., 2002). The major phenolic compounds of *Helichrysum pallasii* identified within the present study, chlorogenic acid isomers and rutin, were reported as effective antioxidant agents.

3.2.2. *Enzyme inhibitory abilities*

Molecules that can partially or entirely bind to the target enzyme, slowing down or inhibiting its action, are called 'Enzyme Inhibitors'. Phytochemical compounds are efficient inhibitors as they are widely used to treat metabolic diseases. Active research areas of enzyme inhibitors are pharmaceutical chemistry, pharmacology, biochemistry and biotechnology, whose main goals are the design, discovery, and development of enzyme inhibitors (Bhagavan *et al*., 2015; Gonçalves and Romano, 2017; Patadiya *et al.*, 2021).

Enzyme inhibitory findings showed a similar pattern of activity (Table 4) which reveals ethanol solvent for the extraction of phenolic compounds that can bind enzyme protein. Scientific literature reports the superior inhibitory activity of ethanol extracts obtained from several *Helichrysum* species compared to water-based extracts (Orhan *et al.*, 2016; Gonçalves *et al.*, 2017). Water-based preparations (infusion and decoction) were found active only in pancreatic lipase inhibition. Total phenolic and individual phenolic concentration results positively correlate with suppressive enzyme activities.

Table 4. Enzyme inhibitory activities of *Helichrysum pallasii* leaf.

			Ethanol	Infusion	Decoction
Enzyme inhibitory activity	Acetylcholinesterase	IC_{50} (mg/ml)	1.49±0.10	NA	NA
		Galanthamine Equivalent (mg/g extract) *	1.98±0.11	NA	NA
	Butyrylcholinesterase	IC_{50} (mg/ml)	1.66±0.26	NA	NA
		Galanthamine Equivalent (mg/g extract) *	1.86±0.46	NA	NA
	Tyrosinase	IC_{50} (mg/ml)	0.68±0.01	≥3	≥3
		Kojic acid Equivalent (mg/g extract) *	123.99±0.24	12.45±2.78	12.09±2.32
	Alpha-Amylase	IC_{50} (mg/ml)	2.09±0.24	≥3	≥3
		Acarbose Equivalent (mmol/g extract) *	0.51±0.07	0.07±0.01	0.08±0.01
	Alpha-glucosidase	IC_{50} (mg/ml)	0.51±0.01	≥3	≥3
		Acarbose Equivalent (mmol/g extract) *	11.7±0.06	1.33±0.05	1.11±0.23
	Pancreatic Lipase	IC_{50} (µg/ml)	42.5±4.2	56.8±3.9	62.1±3.4
		Orlistat Equivalent (µmol/g extract) *	88.6±5.1	67.2±2.1	59.1±2.1

Means with different letters in the same row were significantly different at the level ($p< 0.05$); All data represent the mean ± standard deviation of at least three independent experiments (n=3). NA: not active (no inhibition was observed). *The equivalent of commercial standards calculated based on a standard curve and against control.

Ethanol extract containing a high amount of antioxidant phenolic compounds may be a candidate for biotherapeutic agents that can be used for antidiabetic purposes due to its low alpha-amylase and high alpha-glucosidase activities, which can minimize the accumulation of undigested carbohydrates in the large intestine (Exteberria *et al*., 2012). Obesity is a disease that occurs with the accumulation of abnormal or excessive fat in the body in a way that impairs health and negatively affects the quality of life, which is associated with diabetes, cardiovascular diseases, gastrointestinal system diseases and some types of cancer (Weiss *et al.*, 2013). Phenolic-rich herbal extracts were reported as promising candidates for anti-obesity agents whose mechanism is based on inhibitory of pancreatic lipase *in vitro* and *in vivo* (Cho *et al*., 2010; Karthikesan *et al*., 2010; Dalar *et al*., 2014; Zhang *et al*., 2015).

The dominant phenolic compounds of the *Helichrysum pallasii* extracts were chlorogenic acid isomers which were previously reported as potent antidiabetic agents in vitro (α-glucosidase; IC_{50}: 9.1 µg/ml and α-amylase; IC_{50}: 9.2 µg/ml) (Oboh *et al*., 2015) and *in vivo* (Jung *et al*., 2006; Kamalakkannan and Prince, 2006; Ong *et al*., 2012; Torres *et al.*, 2021).

The primary mode of inhibition determined for the digestive enzymes of polyphenolic compounds is the non-competitive mode. The structure of proteins plays an essential role in protein-phenolic compound interaction. Therefore, the formation of protein-phenolic compound complexes that inhibit enzyme activity depends on the molecular structure of a

phenolic compound and on the amino acids that make up the protein structure. For instance, pancreatic lipase mainly comprises four pancreatic lipase amino acids binding sites such as Phe^{78}, Tyr^{115}, His^{152} and Phe^{216}, for binding polyphenolic compounds (Cho *et al*., 2010; Martinez-Gonzalez *et al*., 2017). Hu *et al.* (2015) reported that caffeoylquinic acid isomers could effectively bind the sites of His^{263}, Asp^{176} and Ser^{15}, which indicate the high lipase inhibitory activities of *H. pallasii* extracts.

4. CONCLUSION

The phytochemical composition and biological activities of traditional preparation methods (infusion and decoction) and ethanol-based extract prepared from *H. pallasii* leaves were assessed. The ethanol-based extract had higher phenolic fatty acid content and antioxidant and enzyme suppressive activities. The phenolic composition comprised chlorogenic acid isomers (3-caffeoylquinic, 4-caffeoylquinic, and 5-caffeoylquinic acids) and the rutin. The lipophilic composition consisted of fatty acids, including palmitic, linoleic, and linolenic acids. These findings contribute to the scientific literature on developing and investigating biotherapeutic agents that can be used in pharmaceutical and therapeutic industries.

Acknowledgments

None of the authors has a commercial interest, financial interest, and/or other relationship with manufacturers of pharmaceuticals, laboratory supplies and/or medical devices or with commercial providers of medical services. This study represents a part of Nejmi Isık MSc thesis.

Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Nejmi Isik: Investigation, Resources, Visualization, Software, Formal Analysis, and Writing - original draft. **Muzaffer Mukemre**: Methodology, Investigation. **Rabia Sena Turker**: Methodology, Investigation. **Gokhan Zengin**: Methodology, Investigation. **Abdullah Dalar**: Investigation, Resources, Visualization, Software, Formal Analysis, and Writing -original draft.

Orcid

Nejmi Isik https://orcid.org/0000-0002-8632-5919
Muzaffer Mukemre https://orcid.org/0000-0001-6154-6603
Rabia Sena Turker https://orcid.org/0000-0002-2017-7159
Gokhan Zengin https://orcid.org/0000-0002-8901-6484
Abdullah Dalar https://orcid.org/0000-0002-0080-2519

REFERENCES

Ainsworth, E.A., & Gillespie, K.M. (2007). Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nature Protocols*, *2*(4), 875-877.

Albayrak, S., Sagdic, O., Aksoy, A., & Hamzaoglu, H. (2008). Antimicrobial and antioxidant activities of *Helichrysum* species from the Mediterranean region of Turkey. *Asian Journal of Chemistry*, *20*, 3143–3152.

Albayrak, S., Aksoy, A., Sagdic, O., & Hamzaoglu, E. (2010). Compositions, antioxidant and antimicrobial activities of *Helichrysum* (Asteraceae) species collected from Turkey. *Food chemistry*, *119*(1), 114-122.

Altundag, E., & Ozturk, M. (2011). Ethnomedicinal studies on the plant resources of east Anatolia, Turkey. *Procedia - Social and Behavioral Sciences, 19*, 756-777.

Andries, A., Rozenski, J., Vermeersch, P., Mekahli, D., & Schepdael, A.V. (2020). Recent progress in the LC–MS/MS analysis of oxidative stress biomarkers. *Electrophoresis, 42*(4): 402-428.

Bhagavan, N.V., & Ha Chung-Eun. (2015). Enzymes and Enzyme Regulation. *Essentials of Medical Biochemistr,* 63- 84.

Baytop, T. (1999). *Türkiye'de Bitkiler ile Tedavi*. Nobel Tıb Kitabevleri, İstanbul, 480.

Benzie, I.F.F., & Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry*, *239*, 70-76.

Bojilov, D.G., Manolov, S.P., Ivanov, I.I., & Mollova, S.L. (2019). Investigation of Antioxidant Activity of Different Extracts of *Helichrysum italicum* from Bulgaria. *Journal of International Scientific Publications, 13*, 41-249.

Carini, M., Aldini, G., Furlanetto, S., Stefani, R., & Facino, R.M. (2001). LC coupled to ion-trap MS for the rapid screening and detection of polyphenol antioxidants from *Helichrysum stoechas. Journal of Pharmaceutical and Biomedical Analysis*, *24*(3), 517–526.

Cho, AS., Jeon, SM., Kim, MJ., Yeo, J., Seo, KI., Choi, MS., & Lee, MK. (2010). Chlorogenic acid exhibits anti-obesity property and improves lipid metabolism in high-fat diet-induced-obese mice. *Food and Chemical Toxicology, 48*(3), 937–943.

Clifford, M.N., Jaganath, I.B., Ludwig, I.A., & Crozier, A. (2017). Chlorogenic acids and the acyl-quinic acids: Discovery, biosynthesis, bioavailability and bioactivity. *Natural Product Reports, 34*, 1391–1421.

Dai, J., & Mumper, RJ. (2010). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules. 15*, 7313-7352.

Dalar, A., & Konczak, I. (2013). Phenolic contents, antioxidant capacities and inhibitory activities against key metabolic syndrome relevant enzymes of herbal teas from Eastern Anatolia. *Industrial Crops and Products, 44*, 383-390.

Dalar, A., Türker, M., Zabaras, D., & Konczak, I. (2014). Phenolic composition, antioxidant and enzyme inhibitory activities of *Eryngium bornmuelleri* leaf. *Plant foods for human nutrition (Dordrecht, Netherlands), 69*(1), 30–36.

Dalar, A., Mukemre, M., Unal, M., & Ozgokce, F. (2018). Traditional medicinal plants of Ağrı Province, Turkey. *Journal of Ethnopharmacology, 226*, 56-72.

Dalar, A., & Mükemre, M. (2020). Traditional Medicinal Plants of Van Province, Eastern Anatolia. New York: NOVA Science Publishers Inc.

Di Meo, S., Reed, T.T., Venditti, P., & Victor, V.M. (2016). Harmful and Beneficial Role of ROS. *Oxidative Medicine and Cellular Longevity*, 7909186.

Exteberria, U., Garza, A. L., Campión, J., Martinez, J. A., & Milagro, F. I. (2012). Antidiabetic effects of natural plant extracts via inhibition of carbohydrate hydrolysis enzymes with emphasis on pancreatic alpha amylase. *Expert opinion on therapeutic targets*, *16*(3), 269-297.

Ghorbani, A. (2017). Mechanisms of antidiabetic effects of flavonoid rutin. *Biomedecine & pharmacotherapie*, *96*, 305-312.

Gonçalves, S., & Romano, A. (2017). Phenolic Compounds: Biological Activity**.** Soto-Hernandez, M., M. Palma-Tenango and M.D.R. GarciaMateos (Eds.). Rijeka, Croatia: InTech Publisher; Chapter 6, Inhibitory Properties of Phenolic Compounds Against Enzymes Linked with Human Diseases; p. 99-118.

Gonçalves, S., Moreira, E., Grosso, C., Andrade, PB., Valentão, P., & Romano, A. (2017). Phenolic profile, antioxidant activity and enzyme inhibitory activities of extracts from aromatic plants used in Mediterranean diet. *Journal of food science and technology*, *54*(1), 219–227.

Hamed, Y.S., Abdin, M., Chen, G., Akhtar, H.M.S. & Zeng, X. (2020) Effects of impregnate temperature on extraction of caffeoylquinic acid derivatives from Moringa oleifera leaves and evaluation of inhibitory activity on digestive enzyme, antioxidant, anti-proliferative and antibacterial activities of the extract. *International Journal of Food Science & Technology, 55*, 3082–3090.

Hu, B., Cui, F., Yin, F., Zeng, X., Sun, Y., & Li, Y. (2015). Caffeoylquinic acids competitively inhibit pancreatic lipase through binding to the catalytic triad. *International journal of biological macromolecules*, *80*, 529–535.

Jahromi, M.A.F., Dehshahri, S., & Samani, SF. (2017). Volatile Composition, Antimicrobial and free radical scavenging activities of essential oil and total extract of *Helichrysum leucocephalum* Boiss. *Trends in Pharmaceutical Sciences, 3*(3), 193-200.

Jung, U.J., Lee, M.K., Park, Y.B., Jeon, S.M., & Choi, M.S. (2006). Antihyperglycemic and antioxidant properties of caffeic acid in db/db mice. *The Journal of Pharmacology and Experimental Therapeutics*, *318*(2), 476–83.

Kamalakkannan, N., & Prince, P. S. (2006). Antihyperglycaemic and antioxidant effect of rutin, a polyphenolic flavonoid, in streptozotocin-induced diabetic wistar rats. *Basic & Clinical Pharmacology & Toxicology*, *98*(1), 97–103.

Karthikesan, K., Pari, L., & Menon, V. P. (2010). Antihyperlipidemic effect of chlorogenic acid and tetrahydrocurcumin in rats subjected to diabetogenic agents. *Chemico-Biological Interactions*, *188*(3), 643–650.

Kim, K.B., Nam, Y.A., Kim, H.S., Hayes, A.W., & Lee, B.M. (2014). α-Linolenic acid: Nutraceutical, pharmacological and toxicological evaluation. *Food and Chemical Toxicology*, *70*, 163–178.

Kolaylı, S., Şahin, H., Ulusoy, E., Tarhan, Ö., 2010. Phenolic Composition and Antioxidant Capacities of *Helichrysum plicatum*. *Hacettepe Journal Biology & Chemistry 38*(4), 269-276.

Les, F., Venditti, A., Casedas, G., Frezza, C., Guiso, M., Sciubba, F., Serafini, M., Bianco, A., Valero, M.S., & López, V. (2017). Everlasting flower (*Helichrysum stoechas* Moench) as a potential source of bioactive molecules with antiproliferative, antioxidant, antidiabetic and neuroprotective properties. *Industrial Crops and Products, 108*, 295-302.

Liang, N. & Kitts, D.D. (2016). Role of chlorogenic acids in controlling oxidative and inflammatory stress conditions. *Nutrients, 8*, 16.

Martinez-Gonzalez, A.I., Diaz-Sanchez, A.G., de la Rosa, L.A., Vargas-Requena, C.L., Bustos-Jaimes, I., & Alvarez-Parrilla, E. (2017). Polyphenolic compounds and digestive enzymes: *in vitro* non-covalent interactions. *Molecules, 22*, 669.

Matthews, D.G., Caruso, M., Alcazar Magana, A., Wright, K.M., Maier, C.S., Stevens, J.F., Gray, N.E., Quinn, J.F., & Soumyanath, A. (2020). Caffeoylquinic Acids in *Centella asiatica* Reverse Cognitive Deficits in Male 5XFAD Alzheimer's Disease Model Mice. *Nutrients*, *12*(11), 3488.

Mükemre, M., Behçet, L., & Çakılcıoglu, U. (2015). Ethnobotanical study on medicinal plants in villages of Çatak (Van-Turkey). *Journal of Ethnopharmacology, 166*, 361–374.

Naczk, M., & Shahidi, F. (2006). Phenolics in cereals, fruits and vegetables: Occurrence, extraction and analysis. *Journal of Pharmaceutical and Biomedical Analysis, 41*, 1523-1542.

Oboh, G., Agunloye, O.M., Adefegha, S.A., Akinyemi, A.J., & Ademiluyi, A.O. (2015). Caffeic and chlorogenic acids inhibit key enzymes linked to type 2 diabetes (in vitro): a comparative study. *Journal of Basic and Clinical Physiology and Pharmacology*, *26*(2), 165–170.

Olthof, M.R., Hollman, P.C., & Katan, M.B. (2001). Chlorogenic acid and caffeic acid are absorbed in humans. *The Journal of Nutrition*, *131*(1), 66–71.

Ong, K.W., Hsu, A., & Tan, B.K. (2012). Chlorogenic acid stimulates glucose transport in

skeletal muscle via AMPK activation: a contributor to the beneficial effects of coffee on diabetes. *PLoS One*, *7*(3).

Orhan, N., Hosbas, S., Orhan, D.D., Aslan, M., & Ergun, F. (2016). Enzyme inhibitory and radical scavenging effects of some antidiabetic plants of Turkey. *Iran Journal of Basic Medicinal Sciences, 17*, 426-32.

Ou, B., Hampsch-Woodill, M., & Prior, R.L. (2001). Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *Journal of Agricultural and Food Chemistry, 49*, 4619–26.

Özkan, G., Sagdıç, O., & Özçelik, H. (2004). Some Turkish endemic herb extracts as antimicrobial and antioxidant agents. 4th International Conggress Environmental Micropaleontology, Microbiology and Meiobenthology; 13-18 September, 2004; Isparta, Turkey; p. 151-154.

Patadiya, N., Panchal, N., & Vaghel, V. (2021). A Review on Enzyme Inhibitors. *International Research Journal of Pharmacy*,*12*(6):12.

Piljac-Žegarac, J., Šamec, D., & Piljac, A (2013). Chapter 11 - *Herbal Teas: A Focus on Antioxidant Properties*, Editor(s): Victor R. Preedy, Tea in Health and Disease Prevention, Academic Press, 129-140.

Pisoschi, A.M., Pop, A., Iordache, F., Stanca, L., Predoi, G., & Serban, A.I. (2021). Oxidative stress mitigation by antioxidants - An overview on their chemistry and influences on health status. *European Journal of Medicinal Chemistry*, *209*, 112891.

Shahi, S., Ahmadian, E., Fathi, N., Eftekhari, A., Khalilov, R., & Dizaj, S.M. (2019). Preparation and physicochemical assessment of rutin-loaded gelatin nanoparticles. *Journal of Advanced Chemical and Pharmaceutical Materials (JACPM)*, *2*(2), 138–142.

Shen, X.J., Zhou, J.D., Dong, J.Y., Ding, W.Q., & Wu, J.C. (2012). Dietary intake of n-3 fatty acids and colorectal cancer risk: a meta-analysis of data from 489 000 individuals. *The British Journal of Nutrition*, *108*(9), 1550–1556.

Süzgeç, S., Meriçli, A.H., Houghton, P.J., & Çubukçu, B. (2005). Flavonoids of H*elichrysum compactum* and their antioxidant and antibacterial activity. *Fitoterapia*, *76*(2), 269-272.

Torres, A., Noriega, L.G., Delgadillo-Puga, C., Tovar, A.R., & Navarro-Ocaña, A. (2021). Caffeoylquinic Acid Derivatives of Purple Sweet Potato as Modulators of Mitochondrial Function in Mouse Primary Hepatocytes. *Molecules*, *26*(2), 319.

Uysal, S., Zengin, G., Locatelli, M., Bahadori, M.B., Mocan, A., Bellagamba, G., & Aktumsek, A. (2017). Cytotoxic and enzyme inhibitory potential of two *Potentilla* species (*P. speciosa* L. and *P. reptans* Willd.) and their chemical composition. *Frontiers in Pharmacology*, *23*(8), 290.

Uzun, Y., Dalar, A., & Konczak, I. (2017). *Sempervivum davisii*: phytochemical composition, antioxidant and lipase-inhibitory activities. *Pharmaceutical Biology, 55*, 532-540.

Weiss, R., Bremer, A., & Lusting, R. (2013). What is metabolic syndrome, and why children getting it?. *Annals of New York Academy of Science, 1281*, 123-40.

Yoshimoto, M., Yahara, S., Okuno, S., Islam, M. S., Ishiguro, K., & Yamakawa, O. (2002). Antimutagenicity of mono-, di-, and tricaffeoylquinic acid derivatives isolated from sweetpotato (Ipomoea batatas L.) leaf. *Bioscience, Biotechnology, and Biochemistry*, *66*(11), 2336–2341.

Zhang, B., Deng, Z., Ramdath, DD., Tang, Y., Chen, PX., Liu, R., Liu, Q., & Tsao, R. (2015). Phenolic profiles of 20 Canadian lentil cultivars and their contribution to antioxidant activity and inhibitory effects on α-glucosidase and pancreatic lipase. *Food Chemistry*, *172*, 862-872.

Zengin, G. (2016). A study on in vitro enzyme inhibitory properties of *Asphodeline anatolica*: new sources of natural inhibitors for public health problems. *Industrial Crops and Products*. *83*, 39-43.

Zengin, G., Cvetanović, A., Gašić, U., Tešić, Z., Stupar, A., Bulut, G., et al. (2020). A comparative exploration of the phytochemical profiles and bio-pharmaceutical potential of *Helichrysum stoechas* subsp. *barrelieri* extracts obtained via five extraction techniques. *Process Biochemistry, 91*, 113-125.