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Original Article

Essential oil content, *in-vitro* and *in-silico* activities of *Hypericum triquetrifolium* Turra, *H. empetrifolium* subsp. *empetrifolium* Willd., and *H. pruinatum* Boiss. & Balansa species

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ABSTRACT

Background and Aims: The importance of *Hypericum* species that are used traditionally against many diseases is increasing day by day.

Methods: In this study, the essential oil contents of *Hypericum triquetrifolium*, *H. empetrifolium* subsp. *empetrifolium*, and *H. pruinatum* species were determined with GC-MS/FID. This is the first study on the antioxidant, anticholinesterase, antiurease, antityrosinase, antielastase, and anticollagenase activities of these species. Also, *in silico* and *in vitro* enzyme inhibitory activities of the major compounds in the essential oil samples of the species have been evaluated. In addition, the cytotoxic effects of the essential oils were determined by the MTT method.

Results: According to GC-MS/FID results, the major compounds were determined as caryophyllene oxide (16.76%) for *H. triquetrifolium*, α-pinene (21.67%) for *H. empetrifolium* subsp. *empetrifolium*, and germacrene D (22.47%) for *H. pruinatum*. Especially, *H. pruinatum* sample showed a high cytotoxic effect (IC_{50}: 34.78±0.22 and 29.06±0.40 µg/mL, respectively) on HT-29 and MCF-7 cell lines. It was determined that the same sample showed a promising inhibitory activity on acetyl (18.33±2.79, 36.48±2.40, and 56.97±0.94, respectively) and butyryl (71.63±2.78, 73.88±1.16, and 56.97±0.97, respectively) cholinesterase enzymes.

Conclusion: Results of the *in-vitro* activity studies indicated that *H. pruinatum* essential oil could be used in the pharmaceutical industry.

Keywords: *Hypericum triquetrifolium, H. empetrifolium, H. pruinatum*, essential oil, cytotoxicity, anti-aging, *in silico*

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INTRODUCTION

Hypericum L. genus, belonging to the Hypericaceae family; has about 500 species known in the world and 97 species in Turkey (Babacan, Aytac, & Pinar, 2017). They are called "sarı kantaron, binbirdelik otu, koyunkıran, and kılıç otu" in Anatolia (Baytop, 1984). Extracts obtained from *Hypericum* species are traditionally used against depression, stomach ailments (gastritis, ulcer), loss of appetite, jaundice, athlete's foot, gingivitis (gargling), sinusitis, intestinal inflammation, hemorrhoids, and fever, and as an inflammation dryer in external wounds (by making an ointment), expectorant, and blood production enhancer in folk medicine (Volz, 1997; Karatoprak et al., 2019). There are many studies in the literature on the essential oil contents of *Hypericum* species (Bertoli, Menichini, Mazzetti, Spinelli, & Morelli, 2003; Cirak & Bertoli, 2013; Sajjadi, S. E., Mehregan, I., & Taheri, 2015; Akdeniz et al., 2020; Grafakou et al.,2020; Silva, Taofiq, Ferreira, & Barros, 2021) Hydrocarbons such as 2-methyloctane, nonane, and undecane, monoterpenes such as *α*-pinene, limonene, *β*-myrcene, and cis-*β*-ocimene, and sesquiterpenes such as caryophyllene and caryophyllene oxide are present in essential oils of *Hypericum* species (Akdeniz et al., 2020). While there are many studies in the literature on the *in vitro* activities of various extracts of species belonging to the genus *Hypericum*, in particular, there are few studies on the *in vitro* activities of essential oils (Akdeniz et al., 2020; Tahir et al., 2019).

The essential oil contents of *Hypericum triquetrifolium* Turra., *H. empetrifolium* subsp. *empetrifolium* Willd., and *H. pruinatum* Boiss. & Balansa obtained by hydrodistillation were determined by GC-MS/FID. Antioxidant (DPPH, ABTS, and CUPRAC), acetyl- and butyryl-cholinesterase inhibitory, antiurease, antityrosinase, antielastase, and anticollagenase activities of these essential oils, which had not been studied before, were investigated. Also, the toxic effects of these essential oils on healthy cell line (PDF) and their cytotoxic effects on cancerous HT-29 (colon cancer) and MCF-7 (breast cancer) cell lines were determined by the MTT method. Both *in silico* and *in vitro* enzyme studies of the major compounds, which were detected at high percentages according to the GC-MS results, were carried out to reveal the content-activity relationship.

MATERIAL AND METHODS

Plant material

H. triquetrifolium (S1) was collected by Dr. Abdulselam Ertas from Diyarbakır in July 2014 and identified by Dr. Yeter Yeşil (Istanbul University). *H. empetrifolium* subsp. *empetrifolium* (S2) was collected from Muğla and *H. pruinatum* (S3) from Trabzon and they were identified by Dr. Yeter Yeşil in July 2015. The herbarium numbers of the samples are ISTE 98926, ISTE 113613, and ISTE 110750, respectively.

Studies of GC-MS analysis

Essential oils of studied samples (shadow dried 100g aerial parts) obtained by a hydrodistilation method using Clevenger apparatus, and the yields of essential oils (*H. triquetrifolium* (S1): 0.65%, *H. empetrifolium* subsp. *empetrifolium* (S2): 0.21% and *H. pruinatum* (S3): 0.45%) were calculated. 20 µL of pure essential oil samples were taken and diluted with hexane to a total volume of 1000 µL. The GC-MS conditions are the same as those given in the relevant references and are detailed below.

Analysis of GC-MS/FID was carried out using Agilent Tecnolocies 7890A GC-FID and 5977B MS detectors, respectively. HP-5MS UI capillary column (30 m–0.25 mm i.d. and 0.25 µm film thickness) was used. The injector temperature was adjusted to 250 °C. Split flow and split ratio were 25 mL/min and 25:1, respectively. The injection volume was 1.0 µL. Mass spectra were detected at 70 eV, and mass range was *m/z* 40–500 amu. GC oven temperature started at 50°C and was held at this temperature for 4 mins and then increased to 240°C by a rate of 3°C per minute and held at final temperature for 5 mins. The MSD and FID detectors temperature were 230°C and 300°C, respectively. Helium gas as carrier has a flow rate of 1 mL/min. The compounds were identified by comparing their retention times and mass spectra with those obtained from authentic samples and/or the NIST and Wiley spectra as well as data from the published literature (Akdeniz et al., 2020; Bakir et al., 2020).

Antioxidant and cytotoxic activities

ABTS cation radical (Re et al., 1999), CUPRAC (Copper (II) ion reducing antioxidant capacity) (Apak, Guclu, Ozyurek, & Karademir, 2004), and DPPH free radical scavenging (Blois, 1958) methods were used to determine the antioxidant activities of the samples. Additionally, on the healthy cell lines (PDF) and on the cancerous MCF-7 and HT-29 cell lines were used by the MTT method to determine the cytotoxic and toxic effects of the samples (Mojarraba, Langzian, Emamic, Asilic, & Tayarani-Najaranb, (2013). IC_{50} values were calculated using the different concentrations (250, 100, 50, 25, 10 and 1 µg/mL concentrations) of the samples (Ertas Yener, 2020).

Enzyme inhibitory activities

A spectrophotometric method based on acetyl- (AChE: from electric eel, Type-VI-S, Sigma) and butyryl-cholinesterase (BChE: from horse serum, Sigma), urease (from Canavalia ensiformis, Type III, Sigma), tyrosinase (from *mushroom*, Sigma), elastase (from *Porcine pancreas,* Type I, Sigma) and collagenase (from *Clostridium histolyticum*, Type I, Sigma) inhibitory activities developed by Ellman, Courtney, Andres, & Featherstone (1961), Hina et al. (2015), Hearing & Jimenez (1978), Kraunsoe, Claridge, & Lowe (1996) and Thring, Hili, & Naughton (2009) with slight modifications, respectively were used to determine the enzyme inhibitory activities. Ethanol (99.9%, Merck) was used to prepare the stock solutions and to dilute the solutions in all enzyme experiments (Ertas et al., 2021; Yener et al., 2018).

Molecular docking

The compatibility of *α*-pinene, caryophyllene oxide and germacrene D, which were determined as the major compounds in the essential oils, to the active site of cholinesterase, tyrosinase, elastase, and collagenase enzymes were determined using the Dock 6.5 program. The coordinates of the protein mentioned above were taken from the Protein Data Bank (4cex.pdb for urease, 5i38.pdb for tyrosinase, 4bbz.pdb for BuChE, 2x8b.pdb for AChE). Crystallographic water molecules were removed from all structures. In 4cex.pdb, 5i38.pdb, 4bbz.pdb, 2x8b.pdb, the missing coordinates were modelled using XLEAP and ff99SB force fields. (Kohno, Hochigai, Yamashita, Tsukihara,

& Kanaoka, 2006; Lang et al., 2007; Carletti et al., 2010; Carletti, Colletier, Schopfer, Santoni, & Masson, 2013; Deri et al., 2016; Nakanishi, Kinoshita, Sato, & Tada, 2020). Molecular modeling study was not performed for urease enzyme because the anti-urease activities of the samples were found to be insignificant. The details related to the current *in silico* studies were given in previous studies (Yener et al., 2020).

RESULTS AND DISCUSSION

Essential oil content and *in vitro* activities

The essential oils of the shade-dried samples of *H. triquetrifolium* (S1), *H. empetrifolium* subsp. *empetrifolium* (S2), and *H. pruinatum* (S3) species were determined by hydrodistillation with GC-MS and GC-FID (Table 1 and Figure 1). The contents of the essential oils of three samples were 90.50, 95.55, and 91.68%, respectively, and 61 compounds were determined in total (Table 1). Essential oils of the samples have been found to contain monoterpene hydrocarbons (18.50, 32.10, and 3.12%, respectively), oxygenated monoterpenes (2.45, 1.31, and 0.25%, respectively) sesquiterpene hydrocarbons (28.7, 17.95, and 66.62%), oxygenated sesquiterpenes (26.82, 10.54, and 16.08%) and other hydrocarbons (14.03, 33.65, and 5.61%) (Table 1 and Figure 1). It was determined that *H. triquetrifolium* and *H. pruinatum* are rich in sesquiterpenes and *H. empetrifolium* subsp. *empetrifolium* is rich in other hydrocarbons. The major components are determined as caryophyllene oxide (16.76%), *α*-pinene (9.92%), and 2-methyloctane (6.58%), for S1, *α*-pinene (21.67%), humulene (15.00%), and 2-methyloctane (12.10%) for S2, germacrene D (22.47%), *α*-cadinol (12.73%), and *δ*-cadinene (9.59%) for S3.

In the previous studies, germacrene-D (21.7%), *β*-caryophyllene (18.3%), and *δ*-cadinene (6.4%) (Sajjadi et al., 2015), myrcene (16%), *α*-pinene (13%), sabinene (13%), germacrene-D (10%), *β*-pinene (8%), and caryophyllene oxide (5%) (Bertoli, Menichini, Mazzetti, Spinelli, & Morelli, 2003) were determined as the main components of the *H. triquetrifolium* essential oil.

In the presented study, major components (caryophyllene oxide: 16.76%, oxygenated sesquiterpenes; *α*-pinene: 9.92%, monoterpene hydrocarbons; 2-methyloctane: 6.58%, others; *β*-caryophyllene: 5.39%, sesquiterpene hydrocarbons; germacrene-D: 4.80%, sesquiterpene hydrocarbons and *δ*-cadinene: 3.67%, sesquiterpene hydrocarbons) of *H. triquetrifolium* essential oil were determined in parallel with the literature (Sajjadi et al., 2015; Al-Snafi 2018). In the literature, the major constituents of the essential oil of *H. empetrifolium* subsp. *empetrifolium* were determined as *α*-pinene (19.0%), *β*-pinene (8.7%), and germacrene D (12.5%) (Grafakou et al., 2020), and *β*-selinene (15%), caryophyllene oxide (9%), *β*-caryophyllene (8%), and *γ*-muurolene (7%) were determined from *H. pruinatum* essential oil (Cirak & Bertoli, 2013). In another study on essential oil of *H. empetrifolium* subsp. *empetrifolium*, *α*-pinene (35.60%) and γ-gurjunene (10.50%) were determined as the major components (Petrakis et al., 2005).

The antioxidant activity results of the essential oils of the samples are given in Table 2. In general, the DPPH free radical scavenging activity of all samples was determined to be low (Table 2). ABTS radical scavenging activities of the S1 and S3 were significant (IC_{50}: 36.65±0.78 and 77.58±1.15 µg/mL, respectively), and the S2 sample was low (IC_{50}: 858.77±1.45 µg/mL). In the CUPRAC method, the results of three samples were found to be close to each other with moderate antioxidant activity. The toxic effects of the samples on the healthy cell–lines (PDF) and the cytotoxic effects on the cancerous MCF-7 and HT-29 cell–lines were determined by the MTT method (Table 2). All samples appear to have toxic effects on the healthy cell–lines (PDF) at high concentrations. In particular, it was determined that the toxic effect of the S3 sample was lower than the others (IC_{50}: 76.09±0.34 µg/mL) on PDF cell-lines and highly cytotoxic (IC_{50}: 34.78±0.22 and 29.06±0.40 µg/mL, respectively) on HT-29 and MCF-7 cell-lines. It was determined that the BChE inhibitory activity of all samples was promising (inhibition%: 71.63±2.78, 73.88±1.16 and 82.08±1.99, respectively), but only the S3 sample had moderate AChE inhibitory activity (inhibition%: 56.97±0.94). Urease, tyrosinase, elastase and collagenase enzyme inhibitory activities potentials of all samples were determined to be low (Table 2).

In silico and *in vitro* studies of the major components

When the *in vitro* enzyme activity of *α*-pinene, caryophyllene oxide and germacrene D at 100 µg/mL concentration were examined, it was determined that they showed low-moderate activity. *α*-Pinene, caryophyllene oxide and germacrene D, which are the main components of the samples, showed moderate AChE (inhibition%: 47.89±0.91, 52.34±0.41 and 49.58±0.21, respectively), BChE (inhibition%: 11.43±0.06, 34.39±0.17 and 43.72±0.26), elastase (inhibition%: 16.31±0.11, 28.30±0.27 and 35.80±0.42) and collagenase (inhibition%: 12.86±0.10, 18.03±0.20 and 32.93±0.52) enzyme inhibitory activities. In the AChE and BChE methods, galanthamine were used (inhibition%: 91.45±0.84 and 78.92±0.65) as a standard reference. The thiourea (inhibition%: 96.75±0.42), kojic acid (93.47±0.48), oleanolic acid (43.25±0.68), epicatechin gallate (84.52±1.98) were used in urease, tyrosinase, elastase and collagenase enzyme activities as standard references, respectively.

According to the results of *in silico* studies, enzyme-inhibitor interactions were assessed with help of docking calculations where binding free energy was recorded at each possible position. The molecular docking result of AChE complexation energy was observed in range from -16.86 kcal/mol to -21.69 kcal/mol, the result of BChE complexation energy was observed in range from -24.18 kcal/mol to -32.36 kcal/mol, the result of tyrosinase complexation energy was observed in range from -20.75 kcal/mol to -25.34 kcal/mol, the result of elastase complexation energy was observed in range from -17.07 kcal/mol to -28.93 kcal/mol, the result of collagenase complexation energy was observed in range from -22.35 kcal/mol to -28.87 kcal/mol, respectively (Table 3). Since the major components have similar chemical structures, it was determined that they showed activity with similar interactions in all the enzymes studied, with Van der Waals and pi alkyl interactions being dominant (Figure 2).

AChE and BChE enzymes bind to ligands in their active sites with the help of similar amino acid residues, especially Trp, Hie,

Table 1. Essential oil composition of aerial parts of *Hypericum* species.

No	RI[a]	Constituents[b]	S1[c]	S2[c]	S3[c]	95% RI range in literatured	No	RI[a]	Constituents[b]	S1[c]	S2[c]	S3[c]	95% RI range in literatured
1	848	2-Hexenal	0.43	0.12	0.11	817–853	34	1457	α-Himachalene	0.29	-	-	1445- 1664
2	859	2-methyloctane	6.58	12.10	0.10	-	35	1462	Humulene	0.53	15.00	2.20	1425–1472
3	899	Nonane	2.65	1.06	0.12	-	36	1468	Alloaromadendrene	-	-	1.19	-
4	935	α-Pinene	9.92	21.67	1.98	912–948	37	1473	Cyclodecane	0.17	1.44	-	-
5	950	Camphene	0.25	0.37	0.12	928–964	38	1483	γ-Muurolene	2.93	0.23	5.47	1450–1501
6	967	3-Methylnonane	3.92	2.46	0.21	-	39	1490	Germacrene D	4.80	0.11	22.47	1463–1499
7	981	β-Pinene	1.75	2.93	0.10	962–989	40	1495	β-Selinene	1.26	-	1.38	1458–1502
8	991	β-Myrcene	0.60	0.11	0.13	975–998	41	1501	Valencene	4.17	-	-	1492- 1728
9	999	Decane	-	0.14	-	-	42	1503	α-Selinene	-	-	2.04	1467–1512
10	1026	*p*-Cymene	0.25	0.72	0.10	1010–1034	43	1506	α-Muurolene	0.61	-	4.47	1473–1506
11	1030	Limonene	0.28	1.38	0.14	1014–1040	44	1509	α-Farnesene	-	-	3.15	1479–1518
12	1033	Eucalyptol	-	0.27	-		45	1521	γ-Cadinene	2.90	0.12	3.52	1480–1526
13	1036	*trans*-β-Ocimene	0.12	0.11	0.06	1022–1049	46	1530	δ-Cadinene	3.67	0.44	9.59	1497–1529
14	1047	*cis*-β-Ocimene	0.11	0.12	0.15	1035–1058	47	1540	Cubenene	-	-	0.40	-
15	1059	γ-Terpinene	0.1	0.11	0.13	1043–1073	48	1551	α-Calacorene	0.85	-	0.28	1514–1543
16	1062	2-Methyldecane	2.07	5.72	tr	-	49	1566	Nerolidol	-	0.47	-	1536–1565
17	1099	Undecane	2.08	3.03	5.22	-	50	1587	Spathulenol	2.86	1.68	0.81	1545–1581
18	1104	Nonanal	0.22	0.51	-	-	51	1594	Caryophyllene oxide	16.76	6.69	0.12	1560–1596
19	1169	*endo*-Borneol	1.03	0.54	-	1134–1180	52	1602	Viridiflorol	-	-	0.49	1569–1604
20	1193	α-Terpineol	0.22	0.78	0.25	1163–1207	53	1613	Globulol	3.20	-	0.98	1559–1595
21	1200	Myrtenol	-	0.26	-	1168–1200	54	1620	Bisabolene epoxide	3.26	1.70	-	-
22	1263	2-Methyldodecane	-	1.05	-	-	55	1630	Junenol	-	-	0.95	-
23	1299	Tridecane	-	0.28	0.06	-	56	1640	1-Dodecanol	-	5.32	-	1473-1959
24	1301	Thymol	2.23	-	-	1272–1304	57	1649	*tau*-Cadinol	0.74	tr	tr	1618–1669
25	1354	α-Cubebene	-	-	0.39	1332–1381	58	1663	α-Cadinol	-	tr	12.73	1618–1669
26	1370	β-Cubebene	0.34	0.92	-	1364–1395	59	1676	1-Tetradecene	-	2.11	-	-

Table 1. Continue.

No	RI[a]	Constituents[b]	S1[c]	S2[c]	S3[c]	95% RI range in literatured	No	RI[a]	Constituents[b]	S1[c]	S2[c]	S3[c]	95% RI range in literatured
27	1376	α-Ylangene	-	-	0.31	1370-1484	60	1690	α-Bisabolol	-	-	0.53	1682- 2213
28	1382	α-Copaene	0.51	0.10	1.38	1355–1395	61	1846	Hexahydrofarnesyl acetone	-	2.35	-	1817–1850
29	1391	β-Bourbonene	0.12	0.18	0.12	1362–1405			Total identified (%)	90.50	95.55	91.68	
30	1396	β-Elemene	0.33	-	3.29	1370–1404			Monoterpene hydrocarbons	18.50	32.10	3.12	
31	1422	α-Cedrene	-	0.42	-	1412-1583			Oxygenated monoterpenes	2.45	1.31	0.25	
32	1427	Caryophyllene	5.39	0.43	4.73	1397–1449			Sesquiterpene hydrocarbons	28.70	17.95	66.62	
33	1446	Aromadendrene	-	-	0.24	1419–1464			Oxygenated sesquiterpenes	26.82	10.54	16.08	
									Others	14.03	33.65	5.61	

[a]Retention index on HP–5MS fused silica column, [b]A nonpolar Agilent HP-5MS fused silica column, [c]S1: *H. triquetrifolium* collected in Diyarbakır, S2: *H. empetrifolium* subsp. *empetrifolium* collected in Muğla, S3: *H. pruinatum* collected in Trabzon, [d]Retention indices for most frequently (95%) reported essential oil compounds in literature, [tr] trace

Table 2. *In vitro* activities of essential oil of aerial parts of *Hypericum* species

	Antioxidant activity[1]			Cytotoxic activity[1]			Enzyme activity (100 µg/mL)[1]					
	IC_{50} (µg/mL)		$A_{0.5}$ (µg/mL)	IC_{50} (µg/mL)			Inhibition (%)					
Samples[2]	DPPH	ABTS	CUPRAC	HT-29	MCF-7	PDF	AChE	BChE	Urease	Tyrosinase	Elastase	Collagenase
S1	≥250[a]	36.65±0.78[a]	102.30±3.32[a]	112.19±1.20[a]	109.42±1.17[a]	39.03±0.50[a]	18.33±2.79[a]	71.63±2.78[a]	NA[a]	2.35±0.31[a]	NA[a]	8.88±0.03[a]
S2	≥250[a]	≥250[b]	197.59±2.53[b]	86.36±2.21[b]	110.82±0.89[a]	48.89±1.52[b]	36.48±2.40[b]	73.88±1.16[a]	NA[a]	2.56±0.82[a]	NA[a]	11.07±0.08[b]
S3	≥250[a]	77.58±1.15[c]	164.22±3.54[c]	34.78±0.22[c]	29.06±0.40[b]	76.09±0.34[c]	56.97±0.94[c]	82.08±1.99[b]	NA[a]	2.16±0.17[a]	NA[a]	14.92±0.01[c]
α-Pinene	-	-	-	-	-	-	47.89±0.91[d]	11.43±0.06[c]	38.62±0.31[b]	NA[b]	16.31±0.11[b]	12.86±0.10[d]
Caryophyllene oxide	-	-	-	-	-	-	52.34±0.41[e]	34.39±0.17[d]	14.70±0.23[c]	NA[b]	28.30±0.27[c]	18.03±0.20[e]
Germacrene D	-	-	-	-	-	-	49.58±0.21[f]	43.72±0.26[e]	27.58±0.17[d]	NA[b]	35.80±0.42[d]	32.93±0.52[f]
BHT[3]	54.68±0.47[b]	15.24±0.63[d]	8.42±0.25[d]	-	-	-	-	-	-	-	-	-
α-TOC[3]	14.55±0.26[c]	9.52±0.36[e]	19.53±0.34[e]	-	-	-	-	-	-	-	-	-
Galanthamine[3]	-	-	-	-	-	-	91.45±0.84[g]	78.92±0.65[f]		-	-	-
Thiourea[3]	-	-	-	-	-	-	-	-	96.75±0.42[e]	-	-	-
Kojic acid[3]	-	-	-	-	-	-	-	-	-	93.47±0.48[c]	-	-
Oleanolic acid[3]	-	-	-	-	-	-	-	-	-	-	43.25±0.68[e]	-
Epicatechin gallate[3]	-	-	-	-	-	-	-	-	-	-	-	84.52±1.98[g]

[1]Values expressed are means ± S.D. of three parallel measurements and values were calculated according to negative control Values with different letters in the same column were significantly different ($p < 0.05$), [2]S1: *H. triquetrifolium* collected in Diyarbakır, S2: *H. empetrifolium* subsp. *empetrifolium* collected in Muğla, S3: *H. pruinatum* collected in Trabzon, [3]Standard compound, NA: Not active

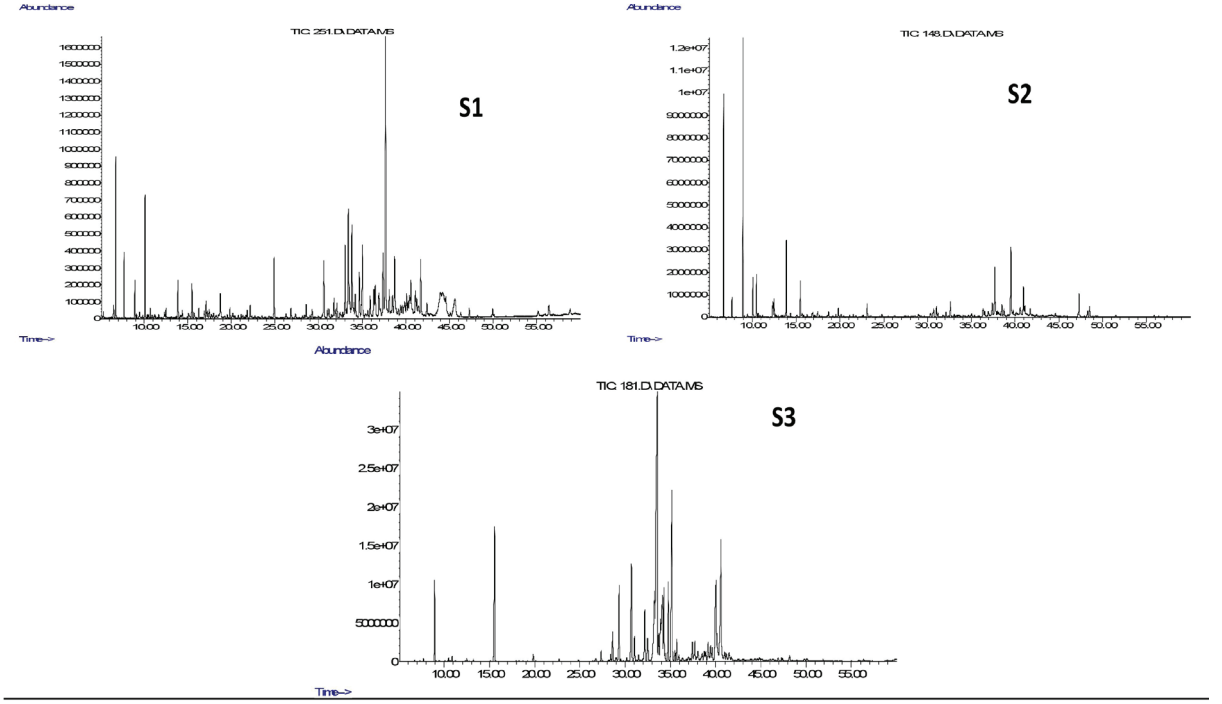

Figure 1. TIC chromatograms of essential oil of studied Hypericum species by GC-MS.
S1: H. triquetrifolium collected in Diyarbakır, S2: H. empetrifolium subsp. empetrifolium collected in Muğla, S3: H. pruinatum collected in Trabzon

Phe, Tyr. It is predicted that π-π and alkyl-π interactions between ligands and aromatic rings in the catalytic region and van der Waals interactions between alkyl groups play an important role. It is thought that the presence of donor oxygen and nitrogen atoms of the galantamine molecule, which is used as a reference, differs from the natural components, making a difference in its effectiveness. The π bonds of the germacren D molecule are thought to play an important role in its effectiveness.

Interactions with Phe, Asn, Val and Ser amino acids are observed in the active binding site of the tyrosinase enzyme. In addition to the -OH groups Vdw and π-π interactions of the reference kojic acid molecule, hydrogen bonds with Met amino acid residues in the active region in particular. It is remarkable that natural components with relatively high activity have a strong effect on the enzyme with non-covalent interactions.

Electrostatic interactions and hydrogen bonds with ligands of Ser, Asn, Gly, His and Cys amino acids in the active site of the elastase enzyme appear to play a role in the activity. The reference compound olenaoic acid has a high affinity with its carboxylic acid functional group, which differs from natural components. It has been determined that it shows activity by forming a large number of hydrogen bonds, especially to Ser and Hie active amino acids.

Collagenase enzyme shows chemical activity with Gly, Leu, Ala, Glu, Tyr and Pro amino acid residues in its active site with natural components and reference molecule. H-bond interactions between the ligands and the enzyme were generally observed with Leu, Ala, Glu, His. The epicatechin gallate used as a reference in the study has a large number of –OH groups and oxygen atoms, unlike the natural components that show activity. It is clearly seen in the results that both the non-covalent interactions of the methyl groups in the *α*-pinene molecule and the effect of the π electrons in the germacren and caryoxyphlene oxide compounds are not that strong.

The *in vitro* test results of the compounds identified as major ones in the essential oils of the species show parallelism with the *in silico* results. In particular, it is observed that the BChE activity of all studied samples is high, but the major compounds are moderately active. However, it is seen that the BChE activity of the essential oil samples, in which these major compounds are mixed in certain proportions, is quite high. It can be suggested that the high BChE enzyme activity of essential oils is due to the synergistic effect of the components. It was determined that the major component germacrene D in the S3 sample was moderately effective in elastase and collagenase (Inhibition%: 35.80±0.42 and 32.93±0.52, respectively) enzyme inhibitory activities, while the relevant sample showed low activity. Therefore, the major component of the S3 sample might have an antagonist effect against these enzymes with other components.

CONCLUSION

In the last decade, more than three thousand studies have been published on this genus, mainly *H. perforatum* L. (Silva, Taofiq, Ferreira, & Barros, 2021). In particular, many *in vivo* and *in vitro* activities related to the cosmetic field of different extracts (methanol, ethanol and water etc.) of the genus were investigated (Silva, Taofiq, Ferreira, & Barros, 2021). However, there are few studies on the *in vitro* activities of the essential oil of *Hypericum* species. The aerial parts of *H. triquetrifolium* (S1), *H.*

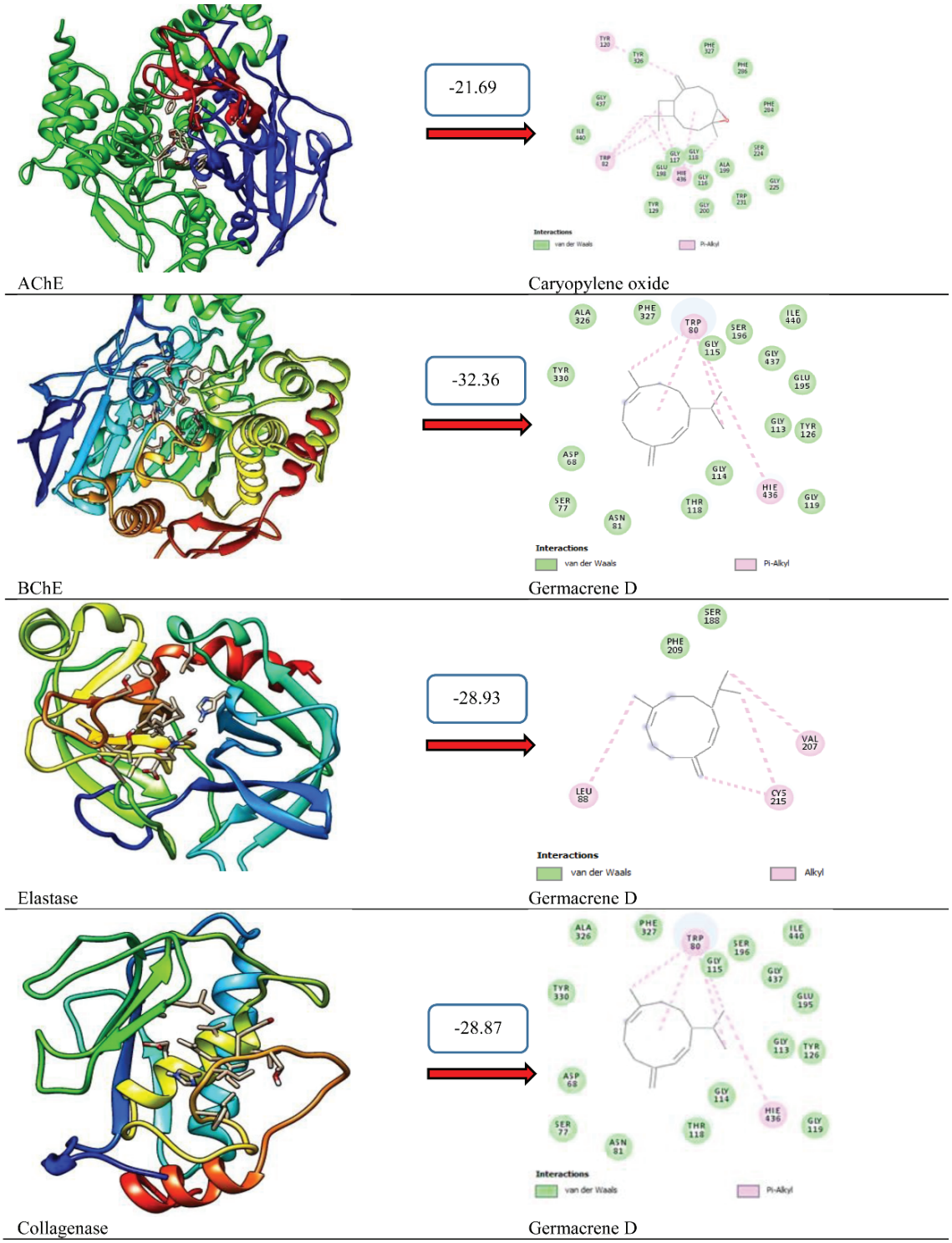

Figure 2. Ribbon representation of the active site pocket enzymes with the bound ligands. The wide opening of the binding site pocket allows the compounds to adopt flexible conformation in this area.

empetrifolium subsp. *empetrifolium* (S2) and *H. pruinatum* (S3) samples collected in this framework were dried in the shade, and the content of their essential oils obtained by hydrodistilation method was indicated by GC-MS/FID. At the same time, cytotoxic, antioxidant, cholinesterase, urease, tyrosinase, elastase and collagenase enzyme inhibitory activities of essential oils were evaluated. It was determined that *H. triquetrifolium* and *H. pruinatum* species are rich in sesquiterpene, and *H. empetrifolium* subsp. *empetrifolium* species are rich in other hydrocarbons. The major components are determined as caryophyllene oxide (16.76%) for S1, *α*-pinene (21.67%) for S2, and germacrene D (22.47%) for S3. This is the first study on the *in*

Table 3. Calculated thermodynamic parameters for complexation of ligands by docking method.

Compounds	AChE			BChE			Tyrosinase			Elastase			Collagenase		
	VdW	es	DockS	VdW	es	DockS	VdW	es	DockS	VdW	es	DockS	VdW	es	DockS
α- pinene	-16.77	-0.09	-16.86	-23.99	-0.18	-24.18	-21.85	-0.03	-21.88	-16.95	-0.12	-17.07	-22.16	-0.18	-22.35
Caryophylene oxide	20.96	-0.65	-21.69	-31.63	-0.05	-31.63	-20.29	-0.46	-20.75	-22.33	-0.07	-22.40	-25.36	-0.46	-25.82
Germacrene D	-17.23	-0.16	-17.40	-32.28	-0.08	-32.36	-25.21	-0.12	-25.34	-28.80	-0.13	-28.93	-28.73	-0.14	-28.87
Galantamine	-66.53	-11.68	-78.21	-63.05	-7.14	-71.19	-	-	-	-	-	-	-	-	-
Kojic acid	-	-	-	-	-	-	-69.19	-10.97	-80.16	-	-	-	-	-	-
Oleanolic acid	-	-	-	-	-	-	-	-	-	-31.39	-0.86	-32.25	-	-	-
Epicatechin gallate	-	-	-	-	-	-	-	-	-	-	-	-	-7.20	-53.90	-61.11

vitro activities of essential oils of *H. empetrifolium* subsp. *empetrifolium, H. pruinatum,* and *H. triquetrifolium*.

It has been determined that the essential oil of *H. pruinatum* has a high cytotoxic effect on HT-29 and MCF-7 and a high inhibitory effect on both AChE and BChE enzymes. The essential oil of *H. pruinatum* might be used in the pharmaceutical industry in treatment of diseases such as memory loss, colon cancer, and breast cancer.

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REFERENCES

- Akdeniz, M., Yilmaz, M. A., Ertas, A., Yener, I., Firat, M., Aydin, F., & Kolak, U. (2020). Method validation of 15 phytochemicals in *Hypericum lysimachioides* var. *spathulatum* by LC–MS/MS, and fatty acid, essential oil, and aroma profiles with biological activities. *Journal of Food Measurement and Characterization, 14*, 3194–3205. https://doi.org/10.1007/s11694-020-00562-6
- Al-Snafi, A. E. (2018). Chemical constituents and pharmacological effects of *Hypericum triquetrifolium*. Indo American Journal of Pharmaceutical Sciences, 5, 1757-1765. https://doi.org/10.5281/zenodo.1210525
- Apak, R., Guclu, K., Ozyurek, M., & Karademir, S.E. (2004). Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. *Journal of Agricultural and Food Chemistry, 52*, 7970-7981. https://doi.org/10.1021/jf048741x
- Babacan, E.Y., Aytac, Z., & Pinar, M. (2017). *Hypericum ekerii* (Hypericaceae) a new species from Turkey. *Pakistan Journal of Botany, 49*, 1763-1768.
- Bakir, D., Akdeniz, M., Ertas, A., Yilmaz, M. A., Yener, I., Firat, M., & Kolak, U. (2020). A GC–MS method validation for quantitative investigation of some chemical markers in *Salvia hypargeia* Fisch. & C.A. Mey. of Turkey: Enzyme inhibitory potential of ferruginol. *Journal of Food Biochemistry, 44,* e13350. https://doi.org/10.1111/jfbc.13350
- Baytop, T. (1984). Treatment with plants in Turkey, Istanbul University Publications, Istanbul, Turkey, 3255.
- Bertoli, A., Menichini, F., Mazzetti, M., Spinelli, G., & Morelli, I. (2003). Volatile constituents of the leaves and flowers of *Hypericum triquetrifolium* Turra. *Flavour and Fragrance Journal, 18*, 91-94. https://doi.org/10.1002/ffj.1161
- Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature, 181,* 1199-1200. https://doi.org/10.1038/1811199a0
- Carletti, E., Colletier, J. P., Dupeux, F., Trovaslet, M., Masson, P., & Nachon, F. (2010). Structural evidence that human acetylcholinesterase inhibited by tabun ages through O-dealkylation. *Journal of Medicinal Chemistry, 53,* 4002-4008. https://doi.org/10.1021/jm901853b
- Carletti, E., Colletier, J. P., Schopfer, L. M., Santoni, G., & Masson, P. (2013). Inhibition pathways of the potent organophosphate

CBDP with cholinesterases revealed by X-ray crystallographic snapshots and mass spectrometry. *Chemical Research in Toxicology, 26,* 280-289. https://doi.org/10.1021/tx3004505

- Cirak, C., & Bertoli, A. (2013). Aromatic profiling of wild and rare species growing in Turkey: *Hypericum aviculariifolium* Jaub. And Spach subsp. *Depilatum* (Freyn and Bornm.) Robson var. *Depilatum* and *Hypericum pruinatum* Boiss. And Bal. *Natural Product Research, 27*, 100-107. https://doi.org/10.1080/14786419.2012.660633
- Deri, B., Kanteev, M., Goldfeder, M., Lecina, D., Guallar, V., Adir, N., & Fishman, A. (2016). The unravelling of the complex pattern of tyrosinase inhibition. *Scientific Reports, 6,* 34993.
- Ellman, G. L., Courtney, K. D., Andres, V., & Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology, 7,* 88-95. https://doi.org/10.1016/0006-2952(61)90145-9
- Ertas, A., & Yener, I. (2020). A comprehensive study on chemical and biological profiles of three herbal teas in Anatolia; rosmarinic and chlorogenic acids. *South African Journal of Botany, 130,* 274-281. https://doi.org/10.1016/j.sajb.2020.01.008
- Ertas, A., Cakırca, H., Yener, I., Akdeniz, M., Fırat, M., Topcu, G., & Kolak, U. (2021). Bioguided fraction and isolation of secondary metabolites from *Salvia cerino-pruinosa* Rech. F. var. *Cerino-pruinosa*. *Records of Natural Products, 15*, 585-592. https://doi.org/10.25135/rnp.248.21.01.1933
- Grafakou, M. E., Diamanti, A., Antaloudaki, E., Kypriotakis, Z., Ciric, A., Sokovic, M., & Skaltsa, H. (2020). Chemical composition and antimicrobial activity of the essential oils of three closely related *Hypericum* species growing Wild on the Island of Crete, Greece. *Applied Sciences, 10,* 2823. https://doi.org/10.3390/app10082823
- Hearing, V. J., & Jiménez, M. (1987). Mammalian tyrosinase-the critical regulatory control point in melanocyte pigmentation. *International Journal of Biochemistry, 19,* 1141-1147. https://doi.org/10.1016/0020-711X(87)90095-4
- Hina, Z., Ghazala, H. R., Arfa, K., Huma, S., Sabiha, T., & Ajmal, K. (2015). Anti-urease activity of *Mimusops elengi* Linn (Sapotaceae). *European Journal of Medicinal Plants, 6,* 223-230. https://doi.org/10.9734/EJMP/2015/12240
- Karatoprak, G. S., Yucel, C., Kaytan, H.C., Ilgun, S., Safak, E. K., & Kosar, M. (2019). Antioxidant and cytotoxic activities of aerial and underground parts of *Hypericum scabrum* L. *Iranian Journal of Science and Technology, Transactions A: Science, 43,* 2107–2113 https://doi.org/10.1007/s40995-019-00717-1
- Kohno, T., Hochigai, H., Yamashita, E., Tsukihara, T., & Kanaoka, M. (2006). Crystal structures of the catalytic domain of human stromelysin-1 (MMP-3) and collagenase-3 (MMP-13) with a hydroxamic acid inhibitor SM-25453. *Biochemical and Biophysical Research Communications, 344,* 315-322. https://doi.org/10.1016/j.bbrc.2006.03.098
- Kraunsoe, J. A. E., Claridge, T. D. W., & Lowe, G. (1996). Inhibition of human leukocyte and porcine pancreatic elastase by homologues of bovine pancreatic trypsin inhibitor. *Biochemistry, 35,* 9090-9096. https://doi.org/10.1021/bi953013b
- Lang, P. T., Moustakas, D., Brozell, S., Carrascal, N., Mukherjee, S., Pegg, S. … Kuntz, I. (2007). DOCK 6.1. University of California, San Francisco. http://dock.compbio.ucsf.edu/
- Mojarraba, M., Langzian, M.S., Emamic, S.A., Asilic, J., & Tayarani-Najaranb, Z. (2013). *In vitro* anti-proliferative and apoptotic activity of different fractions of *Artemisia armeniaca*. *Revista Brasileira de Farmacognosia, 23,*783-7888. https://doi.org/10.1590/S0102-695X2013000500010
- Nakanishi, I., Kinoshita, T., Sato, A., & Tada, T. (2020). Structure of porcine pancreatic elastase complexed with FR901277, a novel macrocyclic inhibitor of elastases, at 1.6Å resolution. *Biopolymers 53,* 434-445. https://doi.org/10.1002/(SICI)1097-0282(20000415)53:5<434::AID-BIP7>3.0.CO;2-5
- Petrakis, P. V. Couladis, M., Couladis, M., & Roussis, V. (2005). A method for detecting the biosystematic significance of the essential oil composition: The case of five Hellenic *Hypericum* L. species. *Biochemical Systematics and Ecology,* 33, 873-898. https://doi.org/10.1016/j.bse.2005.02.002
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26,* 1231-1237. https://doi.org/10.1016/S0891-5849(98)00315-3
- Sajjadi, S. E., Mehregan, I., & Taheri, M. (2015). Essential oil composition of *Hypericum triquetrifolium* Turra growing wild in Iran. *Research in Pharmaceutical Sciences, 10,* 90-94.
- Silva, A. R., Taofiq, O., Ferreira, I. C. F. R., & Barros, L. (2021). *Hypericum* genus cosmeceutical application: A decade comprehensive review on its multifunctional biological properties. *Industrial Crops and Products, 159,* 113053. https://doi.org/10.1016/j.indcrop.2020.113053
- Tahir, N. A. R., Azeez, H. A., Muhammad, K. A., Faqe, S. A., & Omer, D. A. (2019). Exploring of bioactive compounds in essential oil acquired from the stem and root derivatives of *Hypericum triquetrifolium* callus cultures. *Natural Product Research, 33,* 1504-1508. https://doi.org/10.1080/14786419.2017.1419228
- Thring, T. S. A., Hili, P., & Naughton, D.P. (2009). Anti-collagenase, anti-elastase and anti-oxidant activities of extracts from 21 plants. *BMC Complementary and Alternative Medicine, 9,* 1-11. https://doi.org/10.1186/1472-6882-9-27
- Volz, H.P. (1997) Controlled clinical trials of *Hypericum* extracts in depressed patients: An overview. *Pharmacopsychiatry, 30,* 72–76. https://doi.org/10.1055/s-2007-979522
- Yener, I., Ozhan Kocakaya, S., Ertas, A., Ercan, B., Kaplaner, E., Varhan Oral, E., Yilmaz-Ozden, T., Yilmaz, M. A., Ozturk, M., & Kolak, U. (2020). Selective *in vitro* and in *silico* enzymes inhibitory activities of phenolic acids and flavonoids of food plants: Relations with oxidative stress. *Food Chemistry, 327,* 127045. https://doi.org/10.1016/j.foodchem.2020.127045
- Yener, I., Tokul-Olmez, O., Ertas, A., Yilmaz, M. A., Firat, M., Irtegun-Kandemir, S., Ozturk, M., Kolak, U., & Temel, H. (2018). A detailed study on chemical and biological profile of nine *Euphorbia* species from Turkey with chemometric approach: Remarkable cytotoxicity of *E. fistulasa* and promising tannic acid content of *E. eriophor*a. *Industrial Crops and Products, 123,* 442-453. https://doi.org/10.1016/j.indcrop.2018.07.007