IJBCS
ISSN: 2147-1428

Original Article

International Journal of Basic and Clinical Studies (IJBCS)
2020; 9(1): 40-55 Ersoy E. et all

In vitro Biological Activities of *Origanum onites* L. (Turkish Oregano) with Chemical Composition by LC-MS/MS

Ezgi Ersoy[1,*] Ercan Cinar[2] Mehmet Boga[3] Emel Mataraci Kara[4]
Yeter Yesil[5] Mustafa Abdullah Yilmaz[6] Esra Eroglu Ozkan[1]

[1]Istanbul University, Faculty of Pharmacy, Department of Pharmacognosy, Istanbul, Turkey
[2]Department of Nursing, School of Health Science, Batman University, Batman, Turkey
[3]Dicle University, Faculty of Pharmacy, Department of Analytical Chemistry, Diyarbakır, Turkey
[4] Istanbul University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology, Istanbul, Turkey
[5]Istanbul University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Istanbul, Turkey
[6] Dicle University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, Diyarbakır, Turkey

*Corresponding Author: Dr. Ezgi Ersoy
Istanbul University, Faculty of Pharmacy, Department of Pharmacognosy, Istanbul, Turkey
e-mail: ecz.ezgiersoy@gmail.com Tel: +905353953264

Abstract

Origanum onites L., known as "Turkish oregano" in the world, is an edible plant that has been used as a spicy food ingredient since ancient times. *O. onites* is a plant of great commercial importance for Turkey, incidentally it is the most exported *Origanum* species among the others. In this study, chemical composition of ethanol extract prepared from *O. onites* was collected from Mugla-Turkey was investigated by LC-MS/MS. 14 metabolites were detected, and the extract was found to be quite rich in naringenin (1495±77.89 µg/g extract). Besides, apigenin (507.56±32.99 µg/g extract), caffeic acid (496.03±17.56 µg/g extract), and fumaric acid (466.41±5.78 µg/g extract) were also abundant in the extract. Total phenolic (48.29±0.75 µg PEs/mg extract), and total flavonoid contents (10.45±0.93 µg QEs/mg extract) were determined as pyrocatechol and quercetin equivalents, respectively. Antioxidant activities of the species were carried out by using DPPH free radical scavenging, ABTS cation radical scavenging, and CUPRAC activities. The extract showed good antioxidant activity in all antioxidant activity assays. Antiacetylcholinesterase, antibutyrylcholinesterase, antiurease, antityrosinase, and antimicrobial activities were also investigated. The studied extract showed weak activity against acetylcholinesterase (22.36±0.48%) and moderate activity against butyrylcholinesterase (55.71±1.00%). The extract also exhibited moderate antimicrobial activity against *C. tropicalis* with an MIC value of 19.53 µg/mL. The obtained results suggest that *O. onites* is a plant rich in antioxidants with further beneficial health effects, and safe to consume as an herb to enhance the flavor of the foods.

Keywords: *Origanum onites* L., Turkish oregano, LC-MS/MS, antioxidant, anticholinesterase

IJBCS
ISSN: 2147-1428

Original Article

International Journal of Basic and Clinical Studies (IJBCS)
2020; 9(1): 40-55 Ersoy E. et all

1. Introduction

The genus *Origanum* is a member of Lamiaceae family, represented by 49 species, and 17 hybrids evaluated under 10 sections. Being widely used annual, biennial, or perennial strongly aromatic and medicinal plants, they can be found mainly in the Mediterranean area (1). *Origanum* genus is highly important for Turkey, because 23 species and 8 hybrids are distributed throughout the Mediterranean region of the country, and 20 taxa of them are endemic (2).

Origanum onites L., is commonly known as the "Turkish oregano" in the world. It should be mentioned that the common term "oregano" is referred to various plants from different families and genera, such as *Origanum vulgare* L. (Greek oregano), *Lippia graveolens* HBK (Mexican oregano), or *Coridothymus capitatus* L. (Spanish oregano). Being strongly aromatic plants, they are extensively used for culinary purposes, as it happens, it can be said they are one of the mostly used culinary herbs worldwide (3).

The dried herbs of the *Origanum* species can be consumed as cooking ingredients or herbal teas directly. Also, especially in Turkey, and also Greece, the essential oils and the hydrosols of these plants are commonly used. They are used for different therapeutic purposes including the treatment of ulcers, diarrhea, pain in rheumatism traditionally. In some areas they have been used to decrease the blood glucose and cholesterol levels by locals (2).

O. onites is a medicinal plant with woody stems up to 65 cm tall and branches up to 13 cm long. It is known with different names such as "kekik", "İzmir kekiği, "bilyalı kekik", "beyaz kekik", "tokalı kekik", "arı kekiği", "taş kekiği" in different regions in Turkey. Based on bibliographies and ethnobotanical research, it has been used as a spicy herb in gastronomy for thousands of years. Furthermore, in recent years, investigations on the plant has provided evidence for its potential value in the pharmaceutical and cosmetic industries, therefore it has gained more commercial importance (4,5). It must be noted that *O. onites* is the most exported *Origanum* species among others, with approximately 80% of market share in Turkey. It has been traditionally used against digestive disorders for its antispasmodic, and carminative effects, to treat coughing with its expectorant effect, also used topically for its antiseptic and astringent effects (6). As it is extremely important to develop new plant-derived products as sources of medications and foods to mankind, it has been crucial to do further research on *O. onites* and investigate its potential biological activities.

There have been studies conducted to determine the chemical composition of the essential oil of *O. onites*, and accordingly carvacrol was found to be the major component (7). Moreover, several biological activity assays have been carried out, and it has been showed to possess antioxidant, analgesic, anti-spasmodic, larvicidal, acaricidal, antibacterial, and antifungal effects (8,9,10). Additionally, it has been reported that it has beneficial effects in patients with mild hyperlipidemia, including endothelial functions and antioxidative status (11). As the search for natural agents to fight cancer continues, results of the study that investigated its anti-angiogenic and anti-tumoral potentials showed that it was found to have anti-angiogenic activity which might be useful in cancer prevention (12). In another study,

IJBCS
ISSN: 2147-1428

Original Article

International Journal of Basic and Clinical Studies (IJBCS)
2020; 9(1): 40-55 Ersoy E. et all

the antioxidant and anticancer properties of its essential oil and its two major phenolic components (carvacrol and thymol) was evaluated, and they were showed to exhibit antioxidant activity with carcinogenesis-reducing potential (13).

Among the numerous articles about the biological effects of different Lamiaceae plants, many of them focused on their neurobiological effects including cholinesterase inhibitory activities to investigate their anti-Alzheimer potential (14). Besides, antioxidant molecules were also found to have many pernicious health effects, and the shortage of antioxidant compounds is shown to be related to neurodegenerative diseases. For that reason, it is important to investigate the antioxidant activities of the plants, aiming to discover new natural sources to use in cosmetic, food and pharmaceutical industries (15).

Bearing all this mind, our study is designed to investigate the antioxidant, anticholinesterase, and antimicrobial activities of *O. onites* that was collected from Mugla-Turkey. To determine its phytochemical compounds, an LC-MS/MS analysis was performed. On top of that, tyrosinase and urease inhibitory activities of the plant were also investigated within this current study. We aim to contribute new insights for understanding the biological effects and chemical constituents of one of Turkey's most important commercial plants, *O. onites*.

2. Materials and Methods

2.1. Chemicals and instruments

Chemical composition of *O. onites* ethanol extract was determined by using LC-MS/MS (Shimadzu, Kyoto, Japan). For *in vitro* biological activity assays, a UV spectrophotometer from Shimadzu and a microplate reader from BioTek PowerWave XS (USA) were used. All compounds which were used for the LC-MS/MS analysis and *in vitro* biological activity assays were purchased from Merck (Germany), Sigma (Germany); and Fluka (Germany). All solvents were of analytical grade.

2.2. Plant Material

Samples of *Origanum onites* L. were collected from Koycegiz - Mugla (400 m), Turkey by Dr. Yeter Yesil and Dr. Mehmet Boga in July 2014, and determined by Dr. Yeter Yesil (Department of Pharmaceutical Botany, Faculty of Pharmacy, Istanbul University). A voucher specimen was kept in the Herbarium of Faculty of Pharmacy of Istanbul University (ISTE number: 116047).

2.3. Preparation of the Extracts for Bioactivity Assays and LC-MS/MS Analysis

The aerial parts of the plant were weighed (10 g) and macerated in ethanol (100 mL) for 24h at room temperature. After the filtration using Whatman no 1 filter paper, the residue was gathered, and this procedure was repeated for two more times under the same conditions. To evaporate the ethanol, the obtained filtrates were concentrated in a vacuum at 35 ºC. The extract was stored at -20 ºC until the assessments. Prior to the LC-MS/MS analysis, the stored dry filtrates were diluted to 250 mg/L with ethanol and filtrated again using a 0.2 µm microfiber filter.

IJBCS ISSN: 2147-1428

Original Article

International Journal of Basic and Clinical Studies (IJBCS)
2020; 9(1): 40-55 Ersoy E. et all

2.4. LC-MS/MS Analysis

The LC-MS/MS analysis of *O. onites* ethanol extract was carried out according to a previously validated method (16). Quantitative analyses of 37 phenolic compounds were performed by using a Nexera model UHPLC (Shimadzu) coupled to a tandem MS instrument. The data gathered from LC-ESI-MS/MS was processed by using Shimadzu Lab Solutions software.

2.5. Total Phenolic and Flavonoid Contents of the Extracts

Total phenolic content of the ethanol extract of *O. onites* determined as micrograms of pyrocatechol equivalents (PEs) by using the method reported by Boga et al. (17). Total flavonoid content of the plant was measured by using the method designed by Moreno et al. (18), and the results here were expressed as micrograms of quercetin equivalents (QEs).

The following equations were used to calculate total phenolic and flavonoid contents of the ethanol extracts of *O. onites*:

Absorbance = 0.0409x + 0.0495 (R^2 = 0.9975)

Absorbance = 0.0347x + 0.1174 (R^2 = 0.9992)

2.6. Antioxidant Activity Assays

Antioxidant activity of *O. onites* ethanol extract was investigated by using DPPH free radical scavenging, ABTS cation radical scavenging, and CUPRAC activity methods.

2.6.1. DPPH free radical scavenging activity

The DPPH free radical scavenging activity of the studied plant was determined by using a procedure described by Blois (19) and modified Boga et al. (17). The percentage inhibition of absorbance at 517 nm was calculated for each concentration relative to ethanol (blank absorbance)

2.6.2. ABTS cation radical decolorization (scavenging) assay

ABTS cation radical decolorization activity of *O. onites* was conducted by using the method designed by Re et al. (20) and modified by Boga et al (17). The change in absorbance was measured at 734 nm.

2.6.3. Cupric reducing antioxidant capacity (CUPRAC)

The cupric reducing antioxidant capacity (CUPRAC) of the *O. onites* ethanol extract was determined using the method summarized by Apak et al. (21). The change in absorbance was measured at 450 nm, and the results here were given as absorbance values.

2.7. Anticholinesterase Activity Assays

To determine the anticholinesterase activity of *O. onites* ethanol extract, acetylcholinesterase and butyrylcholinesterase inhibitory activity assays were performed based on the method designed by Ellman et al. (22). The change in absorbance was measured at 412 nm. For the positive control, galantamine was used. The following equation was used to calculate the anticholinesterase activity:

Anticholinesterase activity (Inhibition %)

$$(A_{control} - A_{sample})/A_{control} \times 100$$

$A_{control}$ = Absorbance of the control

A_{sample} = Absorbance of the samples

2.8. Anti-tyrosinase Activity

IJBCS
ISSN: 2147-1428

Original Article

International Journal of Basic and Clinical Studies (IJBCS)
2020; 9(1): 40-55 Ersoy E. et all

To determine the tyrosinase inhibition activity of the *O. onites* ethanol extract, the method designed by Hearing and Jimenez (23) was used. The change in absorbance was measured at 475 nm at 37ºC. For the positive control, kojic acid was used.
The following equation was used to calculate the antityrosinase activity:
Antityrosinase activity (Inhibition %)

$$(A_{control} - A_{sample})/A_{control} \times 100$$

$A_{control}$ = Absorbance of the control
A_{sample} = Absorbance of the samples

2.9. Antiurease Activity
To determine the urease inhibition activity of the *O. onites* ethanol extract, the protocol reported by Zahid et al. (24) was followed. The change in absorbance was measured at 630 nm and thiourea was used for the positive control.
The following equation was used to calculate the antiurease activity:
Antiurease activity (Inhibition %)

$$(A_{control} - A_{sample})/A_{control} \times 100$$

$A_{control}$ = Absorbance of the control
A_{sample} = Absorbance of the samples

2.10. Antimicrobial Activity
10 different human pathogenic microbial strains including *Staphylococcus aureus* ATCC 29213, *S. epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 4352, *Proteus mirabilis* ATCC 14153, *Candida albicans* ATCC 10231, *C. parapsilosis* ATCC 22019, and *C. tropicalis* ATCC 750 were used in this study. Antimicrobial activity assay of the ethanol extract of *O. onites* and standard compounds against these strains were carried out using the microbroth dilution technique as described by the Clinical and Laboratory Standards Institute (25, 26, 27). Minimum inhibitory concentrations (MICs) of the extract against the strains were determined by microbroth dilution technique as described by the CLSI (25, 26). The following antibacterial and antifungal agents were used as standards: Cefuroxime-sodium, cefuroxime, ceftazidime, amikacin, amphotericin B, and clotrimazole.
2.11. Statistical Analysis
The results of the enzyme inhibition assays were mean ± SD of three parallel measurements. The statistical significance was estimated using a Student's t-test,
p values$\leq$0.05 were regarded as significant.

3. Results and Discussion

3.1. LC-MS/MS Analysis
In the current study, chemical composition of the ethanol extract prepared from aerial parts of *O. onites* collected from Mugla – Turkey was determined by LC-MS/MS. The analysis was performed according to a method developed, optimized, and validated by Yilmaz et al. (16). The results were given in Table 1. Analytical parameters of the LC–MS/MS method for the phytochemicals were given in Table 2.

IJBCS
ISSN: 2147-1428

Original Article

International Journal of Basic and Clinical Studies (IJBCS)
2020; 9(1): 40-55 Ersoy E. et all

Table 1. Chemical composition of *Origanum onites* ethanol extract

No	Analytes	RT[a]	Parent ion (m/z)	Daughter Ions	Ion. Mode	Quantification (µg analyte/g extract)[b]
1	Coumarin	17.40	147.05	91.0-103.2	Poz	N.D.
2	Hesperidin	12.67	610.90	303.1-465.1	Poz	N.D.
3	*p*-Coumaric acid	11.53	162.95	119.25-93.25	Neg	81.38±4.20
4	*o*- Coumaric acid	15.45	162.95	119.35-93.25	Neg	N.D.
5	Gallic acid	3.00	168.85	125.2-79.2	Neg	N.D.
6	Caffeic acid	8.80	178.95	135.2-134.3	Neg	496.03±17.56
7	Vanilic acid	8.57	166.90	152.25-108.25	Neg	N.D.
8	Salicylic acid	11.16	136.95	93.3-65.3	Neg	43±1.41
9	Quinic acid	1.13	190.95	85.3-93.3	Neg	N.D.
10	4-OH-benzoic acid	7.39	136.95	93.3-65.3	Neg	N.D.
11	Ferulic acid	12.62	192.95	178.3	Neg	N.D.
12	Chlorogenic acid	7.13	353.15	191.2	Neg	21.92±0.15
13	Rosmarinic acid	14.54	359	161.2-197.2	Neg	N.D.
14	Protocatechuic acid	4.93	152.95	108.3	Neg	96.13±3.95
15	Cinnamic acid	25.61	147.00	103.15-77.3	Neg	N.D.
16	Sinapinic acid	12.66	222.95	208.3-149.2	Neg	N.D.
17	Fumaric acid	1.48	115.00	71.4	Neg	466.41±5.78
18	Vanillin	10.87	151.00	136.3-92.2	Neg	1.2±0.03
19	Pyrocatechol	6.48	109.00	108.35-91.25	Neg	N.D.
20	Malic acid	1.23	133.00	115.2-71.3	Neg	138.37±1.56
21	Syringic acid	9.02	196.95	182.2-167.3	Neg	N.D.
22	Hesperetin	31.76	300.95	164.2-136.2	Neg	22.01±1.24
23	Naringenin	30.68	270.95	151.2-119.3	Neg	1495±77.89
24	Rutin	12.61	609.05	300.1-271.1	Neg	N.D.
25	Quercetin	28.17	300.90	151.2-179.2	Neg	14.58±0.79
26	Quercitrin	16.41	447.15	301.15-255.15	Neg	N.D.
27	Apigenin	31.43	268.95	117.3-151.2	Neg	507.56±32.99
28	Chrysin	36.65	252.95	143.3-119.4	Neg	9.29±0.19
29	Liquitrigenin	25.62	254.95	119.25-135.15	Neg	N.D.
30	Isoquercitrin	13.42	463.00	300.15-271.15	Neg	N.D.
31	Apigetrin	16.59	431.00	268.2-239.2	Neg	N.D.
32	Rhoifolin	16.11	577.05	269.2-211.15	Neg	N.D.
33	Nicotiflorin	14.68	593.05	285.1-255.2	Neg	N.D.
34	Fisetin	19.30	284.95	135.2-121.25	Neg	M13±0.19
35	Luteolin	28.27	284.75	133.2-151.2	Neg	N.D.
36	Myricetin	18.72	317.00	179.15-151.25	Neg	N.D.
37	Kaempferol	31.88	284.75	255.1-117.3	Neg	N.D.

[a]RT: Retention time,
[b]Values in µg/g (w/w) of plant extracts.
N.D: not detected

IJBCS
ISSN: 2147-1428

Original Article

International Journal of Basic and Clinical Studies (IJBCS)
2020; 9(1): 40-55 Ersoy E. et all

Table 2. Analytical parameters of the LC–MS/MS method for the phytochemicals.

No	Analytes	RT[a]	Parent ion (m/z)[b]	Daughter Ions	Ion. Mode	Equation	R[2c]	RSD%[d]		Linearity Range (µg/L)	LOD/LOQ (µg/L)[e]	Recovery (%)		U[f]
								Intraday	Interday			Intraday	Interday	
1	Coumarin	17,40	147,05	91,0-103,2	Poz	y=33.6376×-897.142	0.994	0.01306	0.01239	1000-20000	208.49/228.38	0.99947	1.00081	0.0237
2	Hesperidin	12,67	610,90	303,1-465,1	Poz	y=1340.27×-43769.1	0.998	0.00945	0.01126	25-1000	3.41/4.17	1.01733	1.01263	0.0262
3	p-Couumaric acid	11,53	162,95	119,25-93,25	Neg	y=3199.2×+130019	0.992	0.01820	0.01727	25-1000	7.31/9.07	1.00617	1.01224	0.0516
4	o- Couumaric acid	15,45	162,95	119,35-93,25	Neg	y=1219.34×-10915.7	0.999	0.02730	0.02566	25-1000	24.36/31.10	0.98344	0.99061	0.0513
5	Gallic acid	3,00	168,85	125,2-79,2	Neg	y=226.763×+38152.3	0.998	0.01601	0.01443	250-10000	95.45/106.85	1.00004	1.00454	0.0282
6	Caffeic acid	8,80	178,95	135,2-134,3	Neg	y=3963.32×+178156	0.998	0.01454	0.01469	25-1000	18.44/22.43	1.00917	0.98826	0.0354
7	Vanilic acid	8,57	166,90	152,25-108,25	Neg	y=35.8398×-12097.9	0.999	0.00528	0.00619	1000-20000	122.21/139.70	1.00093	1.04095	0.0508
8	Salicylic acid	11,16	136,95	93,3-65,3	Neg	y=5286.26×+309192	0.989	0.01016	0.01242	25-1000	5.00/6.53	1.00989	0.99013	0.0329
9	Quinic acid	1,13	190,95	85,3-93,3	Neg	y=41.0559×+10671.6	0.996	0.00259	0.00274	250-10000	75.75/79.41	1.00288	0.98778	0.0082
10	4-OH-benzoic acid	7,39	136,95	93,3-65,3	Neg	y=409.028×+112079	0.998	0.01284	0.01538	250-10000	33.18/37.95	0.99662	1.00058	0.0289
11	Ferrulic acid	12,62	192,95	178,3	Neg	y=80.453×-31782.5	0.997	0.00708	0.00619	250-10000	36.61/42.00	0.99987	1.00289	0.0494
12	Chlorogenic acid	7,13	353,15	191,2	Neg	y=781.364×-18697.9	0.998	0.00058	0.00076	25-1000	6.19/8.11	1.00806	0.99965	0.0069
13	Rosmarinic acid	14,54	359	161,2-197,2	Neg	y=909.672×-201692	0.994	0.02014	0.01751	100-5000	6.60/8.84	0.99206	1.03431	0.0713
14	Protocatechuic acid	4,93	152,95	108,3	Neg	y=297.752×+30590.7	0.995	0.01236	0.01296	100-5000	28.24/31.39	0.99404	1.01070	0.0411
15	Cinnamic acid	25,61	147,00	103,15-77,3	Neg	y=9.06322×-12403.2	0.996	0.00648	0.00816	5000-20000	839.84/915.08	1.00051	0.99927	0.0143
16	Sinapinic acid	12,66	222,95	208,3-149,2	Neg	y=141.955×-73293.9	0.992	0.01446	0.01517	250-10000	77.59/85.51	1.00164	0.99962	0.0281
17	Fumaric acid	1,48	115,00	71,4	Neg	y=64.9967×-11592.4	0.997	0.00536	0.00460	100-5000	26.05/28.03	0.99748	0.99867	0.0124
18	Vanillin	10,87	151,00	136,3-92,2	Neg	y=446.102×+70934.3	0.998	0.00696	0.00793	250-10000	48.39/64.23	0.99679	0.99611	0.0280

IJBCS
ISSN: 2147-1428

Original Article

International Journal of Basic and Clinical Studies (IJBCS)
2020; 9(1): 40-55 Ersoy E. et all

Continue to Table 2

No	Analytes	RT[a]	Parent ion (m/z)[b]	Daughter Ions	Ion. Mode	Equation	R^{2c}	RSD%[d]		Lineerite Range (µg/L)	LOD/LOQ (µg/L)[e]	Recovery (%)		U[f]
								Intraday	nterday			Intraday	Interday	
19	Pyrocatechol	6,48	109,00	108,35-91,25	Neg	y=30.6141×+14735.5	0.996	0.01313	0.01339	1000-20000	272.81/315.87	0.99987	0.99936	0.0235
20	Malic acid	1,23	133,00	115,2-71,3	Neg	y=316.948×-42041.3	0.999	0.00477	0.00527	250-10000	58.65/74.98	1.01266	0.99836	0.0113
21	Syringic acid	9,02	196,95	182,2-167,3	Neg	y=42.3309×-52547.4	0.996	0.01049	0.01345	1000-20000	219.92/257.54	0.99922	0.99977	0.0238
22	Hesperetin	31,76	300,95	164,2-136,2	Neg	y=876.665×+48916.5	0.997	0.03209	0.02605	25-1000	5.26/5.76	0.98850	0.99435	0.0562
23	Naringenin	30,68	270,95	151,2-119,3	Neg	y=4315.1×+178410	0.995	0.02054	0.02019	25-1000	5.24/5.99	0.99883	1.01002	0.0521
24	Rutin	12,61	609,05	300,1-271,1	Neg	y=561.912×-16879.1	0.997	0.00473	0.00624	25-1000	5.16/5.66	1.00994	0.98017	0.0159
25	Quercetin	28,17	300,90	151,2-179,2	Neg	y=1198.48×+480562	0.990	0.01589	0.01360	100-5000	22.48/30.45	0.98470	1.00103	0.0543
26	Quercitrin	16,41	447,15	301,15-255,15	Neg	y=339.385×+38910	0.999	0.01528	0.02320	100-5000	22.38/26.44	0.99726	1.00620	0.0279
27	Apigenin	31,43	268,95	117,3-151,2	Neg	y=4548.36×+295252	0.990	0.02304	0.02204	25-1000	5.43/6.62	1.01444	1.01331	0.0650
28	Chrysin	36,65	252,95	143,3-119,4	Neg	y=2032.13×+95593.8	0,993	0.00490	0.00630	25-1000	5.44/6.08	1.00338	1.00437	0.0283
29	Liquitrigenin	25,62	254,95	119,25-135,15	Neg	y=2384.96×+59141.1	0,996	0.01849	0.01738	25-1000	5.61/7.09	1.00333	0.99957	0.0341
30	Isoquercitrin	13,42	463,00	300,15-271,15	Neg	y=803.233×+4981.43	0,999	0.00682	0.00515	25-1000	5.39/6.45	1.00594	1.00722	0.0133
31	Apigetrin	16,59	431,00	268,2-239,2	Neg	y=1775.55×+91121.7	0,993	0.01797	0.01607	25-1000	5.58/6.71	1.01394	1.00419	0.0597
32	Rhoifolin	16,11	577,05	269,2-211,15	Neg	y=237.15×+11887.8	0,999	0.00747	0.01528	100-5000	25.59/34.51	1.01046	1.01739	0.0941
33	Nicotiflorin	14,68	593,05	285,1-255,2	Neg	y=498.381×+79274.4	0,991	0.00737	0.00875	100-5000	26.78/38.54	1.02558	1.00970	0.0276
34	Fisetin	19,30	284,95	135,2-121,25	Neg	y=547.459×+274791	0,991	0.00557	0.00820	250-10000	58.82/68.45	0.99877	1.00031	0.0148
35	Luteolin	28,27	284,75	133,2-151,2	Neg	y=3272.65×+150557	0,997	0.00575	0.00696	25-1000	5.16/5.97	1.00772	0.99524	0.0174
36	Myricetin	18,72	317,00	179,15-151,25	Neg	y=583.548×+205727	0,999	0.00652	0.00711	250-10000	57.08/67.80	0.99982	1.00042	0.0126
37	Kaempferol	31,88	284,75	255,1-117,3	Neg	y=26.292×+87558.2	0,992	0.01436	0.01070	1000-20000	211.94/227.76	0.99971	0.99851	0.0209

a RT: Retention time.
b Mother ion(m/z): Molecular ions of the standard compounds (m/z ratio).
c R^2: Coefficient of determination.
d RSD: Relative standard deviation.
e LOD/LOQ (µg/L): Limit of detection/quantification.
f U (%): percent relative uncertainty at 95% confidence level (k=2).

Among the analyzed 37 phytochemicals, 14 of them were found to be present in the extract. Naringenin, a compound which belongs to the class of flavonoids called the flavanones, was detected as the major compound with 1495±77.89 µg/g extract. The extract was found to be rich in apigenin, a flavone, with 507.56±32.99 µg/g extract. Caffeic acid was also determined in the extract with 496.03±17.56 µg/g extract. Another abundant constituent of the extract is fumaric acid with 466.41±5.78 µg/g extract. Other identified phytochemicals are, *p*-coumaric acid (81.38±4.20 µg/g extract), salicylic acid (43±1.41 µg/g extract), chlorogenic acid (21.92±0.15 µg/g extract), protocatechuic acid (96.13±3.95 µg/g extract), vanillin (1.2±0.03 µg/g extract), malic acid (138.37±1.56 µg/g extract), hesperidin (22.01±1.24 µg/g

extract), quercetin (14.58±0.79 µg/g extract), chrysin (9.29±0.19 µg/g extract), and fisetin (13±0.19 µg/g extract).

Although there are numerous studies focused on the chemical composition of the essential oil of the *O. onites*, to our best knowledge, this is the first report about the identification of the phenolic compounds in its ethanol extract by LC-MS/MS. On the other hand, there are a few studies carried out on other *Origanum* species to investigate their constituents by using LC-MS/MS methods. For instance, in a previous study, caffeic acid was found to be the most abundant compound in *O. laevigatum* Boiss methanol extract. In the acetone extract of the plant, rosmarinic acid was found to be the major constituent (28). In 2017, Yilmaz et al. conducted a study about investigations on chemical compounds of 6 different *Origanum* species by using LC-MS/MS. Accordingly, kaempferol, rutin, and luteolin were found in the methanol extracts of the six studied species (2). In another study, again six *Origanum* species were evaluated for their chemical compounds by LC-MS/MS, and kaempferol, ferulic acid and chlorogenic acid were present in the methanol extracts of the studied plants (29). In methanol extract of *O. onites* collected from Crete/Greece, caffeic acid and rosmarinic acid were identified by HPLC (30). It must be noted that, phytochemical profiles of different species belonging to the same genus can be quite different, as a matter of fact, there can be important changes in phytochemical profiles of the same species also, due to specific physiological, environmental, climatic, and geopolitical conditions (31). Incidentally, Ozkan et al. designed a study to investigate the influence of harvest time on the chemical constituents and antioxidant properties of *O. onites*, and they reported that, chemical compositions, free radical scavenging activities and reducing/antioxidant capacities of extracts of the plant changed significantly depending on vegetative periods of growing season. In their study, naringenin, and apigenin were the two compounds detected in the extracts at all different harvest times (32).

3.2. Total Phenolic and Flavonoid Contents of the Extracts

Total phenolic and flavonoid contents of the ethanol extract of the aerial parts of the studied *O. onites* were determined as pyrocatechol (PEs), and quercetin (QEs) equivalents, respectively. (y = 0.0409 pyrocatechol (µg) + 0.0495, (R^2 = 0.9975), and y = 0.0347 quercetin (µg) + 0.1174, (R^2 = 0.9992). The results were shown in Table 3. Total phenolic content of the ethanol extract of the plant was reported to be 48.29±0.75 (µg PEs/mg extract), and total flavonoid content of the same extract was calculated as 10.45±0.93 (µg QEs/mg extract). In a previous study, total phenolic content of the extract from *O.onites* at different harvest times were found to be between 106.13 and 149.40 mg GAE/mg extract (32).

IJBCS
ISSN: 2147-1428

Original Article

International Journal of Basic and Clinical Studies (IJBCS)
2020; 9(1): 40-55 Ersoy E. et all

Table 3: Total phenolic-flavonoid contents of *Origanum onites* ethanol extract

Sample	Total Phenolic Content (μg PEs/mg extract)[a]	Total Flavonoid Content (μg QEs/mg extract)[b]
Origanum onites	48.29±0.75	10.45±0.93

Values expressed are means ± standard deviation of three parallel measurements ($p<0.05$)
[a] PEs, pyrocatechol equivalents ($y = 0.0409x + 0.0495$, $R^2 = 0.9975$).
[b] QEs, quercetin equivalents ($y = 0.0347x + 0.1174$, $R^2 = 0.9992$)

3.3. Antioxidant Activity

Considering there are different oxidation aspects in all systems, to determine the antioxidant activity of the plants more accurately, it is necessary to use more than only one method (15). In this context, three different antioxidant activity assays, namely DPPH free radical scavenging, ABTS cation radical scavenging, and CUPRAC activity methods were used to investigate the antioxidant potential of *O. onites* ethanol extract. DPPH free radical scavenging and ABTS cation radical scavenging results are given in Table 4, CUPRAC activity results are given in Table 5.

Table 4: DPPH free radical scavenging and ABTS cation radical scavenging activity results of *Origanum onites* ethanol extract

	IC_{50} (µg/mL)	
Samples	**DPPH Free Radical**	**ABTS Cation Radical**
Origanum onites	55.57±1.11	6.89±0.02
BHA*	7.88±0.20	2.74±0.03
α-TOC*	16.30±0.79	10.20±0.05
BHT*	58.86±0.50	3.16±0.06

The results are given as IC_{50} values
Values expressed are means ± standard deviation of three parallel measurements ($p<0.05$)
*Standard compounds

Table 5: CUPRAC activity results of *Origanum onites* ethanol extract

Samples	10 µg/mL	25 µg/mL	50 µg/mL	100µg/mL
Origanum onites	0.63±0.015	1.05±0.190	1.86±0.148	3.03±0.251
BHT*	0.944±0.035	2.108±0.094	3.169±0.119	3.990±0.000
BHA*	1.148±0.136	2.420±0.006	3.913±0.023	3.990±0.000
α-TOC*	0.388±0.041	0.770±0.009	1.478±0.028	2.601±0.070

The results are given as absorbance values

IJBCS ISSN: 2147-1428

Original Article

International Journal of Basic and Clinical Studies (IJBCS)
2020; 9(1): 40-55 Ersoy E. et all

Values expressed are means ± standard deviation of three parallel measurements
*Standard compounds

Based on the IC_{50} values, ethanol extract of *O. onites* exhibited 55.57±1.11 µg/mL in DPPH free radical scavenging assay. As already known, lower IC_{50} values indicate higher antioxidant effects. Three different standard antioxidants were used as positive control, and *O. onites* showed stronger activity than BHT (with the IC_{50} value of 58.86±0.50 µg/mL). Other standard compounds were found to exert better activities than the extract (BHA: 7.88±0.20 µg/mL, α-TOC: 16.30±0.79 µg/mL). According to the results of ABTS cation decolorization assay, *O. onites* was found to exhibit better antioxidant activity (6.89±0.02 µg/mL) than α-TOC (10.20±0.05 µg/mL). Other standard compounds were found to be stronger antioxidants due to this assay (BHA: 2.74±0.03 µg/mL, BHT: 3.16±0.06 µg/mL). In CUPRAC assay, *O. onites* showed good antioxidant activity compared to the standard compounds as well.

Thus far, several studies have been published on antioxidant properties of the essential oils of *Origanum* species (33, 34, 35). On the report of LC-MS/MS analysis, the ethanol extract of the studied *O. onites* was found to be rich in terms of some phenolic compounds with strong antioxidant activities including naringenin, and apigenin. Naringenin, (4,5,7-trihydroxyflavanone) is a flavanone, that was shown to possess high antioxidant capacity and radical scavenger efficiency (36). Moreover, naringenin was reported to protect the cells from heavy metal induced oxidative stress (37)

3.4. Anticholinesterase Activity

The inhibition of the two enzymes, acetylcholinesterase and butyrylcholinesterase, which are known as the key enzymes in the breakdown of two important neurotransmitters, acetylcholine and butyrylcholine, respectively, has been a promising approach for the treatment of Alzheimer's disease. Therefore, in the recent years, anticholinesterase activity of the plants is under investigation by many scientists all over the world. Within this study, anticholinesterase activity assay was performed to determine the anti-Alzheimer potential of *O. onites* ethanol extract. The results were shown in Table 6.

Table 6: Anticholinesterase activity results of *Origanum onites* ethanol extract

Samples	Acetylcholinesterase (Inhibition %)	Butyrylcholinesterase (Inhibition %)
Origanum onites	22.36±0.48	55.71±1.00
Galanthamine*	83.31±0.09	86.38±0.10

Cholinesterase inhibitory activity of the extracts was tested against acetylcholinesterase and butyrylcholinesterase at 200 mg/mL concentration. Values expressed are means ± SD of three parallel measurements * Standard compound

IJBCS
ISSN: 2147-1428

Original Article

International Journal of Basic and Clinical Studies (IJBCS)
2020; 9(1): 40-55 Ersoy E. et all

According to the results, at 200 µg/mL concentration, *O. onites* showed weak acetylcholinesterase inhibitory activity with 22.36±0.48%, and moderate butyrylcholinesterase inhibitory activity with 55.71±1.00% compared to the standard compound galantamine.

There are a few studies about the anticholinesterase potential of other members of *Origanum* species. To the best knowledge so far, *O. acutidens, O. haussknechtii, O. husnucan-baseri, O. leptocladum*, and *O. rotundifolium* extracts showed moderate antibutyrylcholinesterase activity with no activity against acetylcholinesterase, only *O. brevidens* extracts showed weak acetylcholinesterase inhibitory activity (2). In another study, the essential oil of *O. majorana* was evaluated for its antiacetylcholinesterase potential, and it showed better activity than the standard compound, galantamine. It must be noted that the chemical compositions of the essential oils of *Origanum* species are rather different than the phytochemicals identified in their extracts. This strong activity was attributed to the monoterpene hydrocarbons, and oxygenated monoterpenes found in the essential oil (38). In fact, there is a study conducted on the essential oils of different *Origanum* species including *O. onites* as well, and the results were found to be very promising. In particular, *O. onites* appeared to be extremely effective with 96.3% inhibition towards acetylcholinesterase (39). Consequently, it can be said that the essential oils of the *Origanum* species have more potential for the treatment of Alzheimer's disease compared to the extracts prepared from them.

3.5. Antityrosinase and Antiurease Activities

No activity was observed according to the results of antityrosinase and antiurease activity assays (Table 7).

Table 7: Antityrosinase and antiurease activity results of *Origanum onites* ethanol extract

Samples	Tyrosinase (Inhibition %)	Urease (Inhibition %)
Origanum onites	NA	NA
Kojik acid*	95.26±0.23	-
Tiyourea*	-	88.61±1.16

Tyrosinase and urease inhibitory activities of the extract were tested against tryosinase and urease at 200 mg/mL concentration. Values expressed are means ± SD of three parallel measurements * Standard compound NA: Not active

There are well-known natural tyrosinase inhibitors such as gallic acid, and quinic acid (40). Nevertheless, these compounds were not present in the extract. Likewise, there are phenolic metabolites with proven antiurease effect, including myricetin, myricitrin, rutin and luteolin (15). The studied plant did not contain any of these compounds, therefore it can be suggested that, this is the main reason

for them being inactive against these enzymes.

3.6. Antimicrobial Activity

The MIC values of the *O. onites* ethanol extract and standard compounds can be seen in Table 8.

Table 8: Antimicrobial activity results of *Origanum onites* ethanol extract

Microorganisms	MIC values of (µg/mL) the extract
P. aeruginosa ATCC 27853	NA
E. coli ATCC 25922	NA
K. pneumoniae ATCC 4352	NA
P. mirabilis ATCC 14153	NA
S. aureus ATCC 29213	NA
S. epidermidis ATCC 12228	312.5
E. faecalis ATCC 29212	1250
C. albicans ATCC 10231	NA
C. parapsilosis ATCC	NA
C. tropicalis ATCC	19.53

NA: Not active
Standards; Cefuroxime-Na: 1.2 µg/ml for *S. aureus* ATCC 29213,
Cefuroxime 9.8 µg/ml for *S. epidermidis* ATCC 12228,
Amikacin 128 µg/ml for *E. faecalis* ATCC 29212,
Ceftazidime 2.4 µg/ml for *P. aeruginosa* ATCC 27853,
Cefuroxime-Na: 4.9 µg/ml for *E. coli* ATCC 25922 and *K. pneumoniae* 4352,
Cefuroxime-Na 2.4 µg/ml for *P. mirabilis* ATCC 14153,
Clotrimazole 4.9 µg/ml for *C. albicans* ATCC 10231,
Amphotericin B 0.5 µg/ml for *C. parapsilosis* ATCC 22019,
Amphotericin B 1 µg/ml for *C. tropicalis* ATCC 750.

The results indicated that the extract exhibited moderate activity against *C. tropicalis* with an MIC value of 19.53 µg/mL. Additionally, the extract showed weak antibacterial activity against *S. epidermidis* with 312.5 µg/mL, and *E. faecalis* with 1250 µg/mL. Although there are a great number of studies about the antimicrobial activity of the essential oils of the *Origanum* species, further research on the antimicrobial effect of their extracts is needed.

4. Conclusion

Origanum onites L., widely known as "Turkish oregano", being one of the most exported plants of Turkey, has gained more commercial importance in the recent years. Although there are studies carried out to investigate the chemical compounds and biological activities of *O. onites*, they have been mostly performed with the essential oil of the plant. Within this study, an ethanol extract was prepared from the aerial parts of the plant to evaluate its biological activities with a focus on its chemical composition by LC-MS/MS. It has been shown that the plant is rich in terms of several phenolic compounds such as naringenin, apigenin, and caffeic acid. Due to the mixture of such phenolic compounds, the extracts exhibited important *in vitro* biological activities, including good antioxidant activity. Phenolic compounds are known to be quite beneficial to human health. Consequently, the results suggest that the

extract could also be a promising source for the treatment of several diseases related to oxidation.

References

1. Jedrzejczyk I. Study on genetic diversity between *Origanum* L. species based on genome size and ISSR markers. Industrial Crops and Products. 2018; 126: 201-207.
2. Yilmaz H, Carikci S, Kilic T, Dirmenci T, Arabaci T, Goren AC, et al. Screening of chemical composition, antioxidant, and anticholinesterase activity of section Brevifilamentum of *Origanum* (L.) species. Records of Natural Products. 2017; 11(5):439-455
3. Economou G, Panagopoulos G, Tarantilis P, Kalivas D, Kotoulas V, Travlos IS, et al. Variability in essential oil content and composition of *Origanum hirtum* L., *Origanum onites* L., *Coridothymus capitatus* (L.) and *Satureja thymbra* L. populations from the Greek island Ikaria. Industrial Crops and Products. 2011; 33(1): 236-241.
4. Gonuz A, Ozorgucu B. An investigation on the morphology, anatomy and ecology of *Origanum onites* L. Turkish Journal of Botany. 1999; 23(1): 19-32.
5. Tepe B, Cakir A, Sihoglu Tepe,A. Medicinal uses, phytochemistry, and pharmacology of *Origanum onites* (L.): A Review. Chemistry & Biodiversity. 2016; 13(5): 504-520.
6. Tonk FA, Yuce S, Bayram E, Giachino RRA, Sonmez C, Telci I, et al. Chemical and genetic variability of selected Turkish oregano (Origanum onites L.) clones. Plant systematics and evolution. 2010; 288(3-4): 157-165.
7. Sevki C, Girisgin O, Kurkcuoglu M, Malyer H, Girisgin AO, Kinmer N, et al. Acaricidal efficacy of *Origanum onites* L. essential oil against *Rhipicephalus turanicus* (Ixodidae). Parasitology Research. 2008; 103(2):259-261.
8. Aydin S, Ozturk Y, Beis R, Baser KHC. Investigation of *Origanum onites*, *Sideritis congesta* and *Satureja cuneifolia* essential oils for analgesic activity. Phytotherapy Research. 1996; 10(4): 342-344.
9. Cetin H, Erler F, Yanikoglu, A. Toxicity of essential oils extracted from *Origanum onites* L. and *Citrus aurentium* L. against the pine processionary moth, *Thaumetopoea wilkinsoni* Tams. Folia Biologica. 2006; 54(3-4): 153-157.
10. Chishti S, Kaloo ZA, Sultan P. Medicinal importance of genus *Origanum*: A review. Journal of Pharmacognosy and Phytotherapy. 2013; 5(10): 170-177.
11. Ozdemir B, Ekbul A, Topal NB, Sarandol E, Sag S, Baser KHC, et al. Effects of *Origanum onites* on endothelial function and serum biochemical markers in hyperlipidaemic patients. Journal of International Medical Research. 2008; 36(6): 1326-1334.
12. Bostancioglu RB, Kurkcuoglu M, Baser KHC, Koparal AT. Assessment of anti-angiogenic and anti-tumoral potentials of *Origanum onites* L. essential oil. Food and Chemical Toxicology. 2012; 50(6): 2002-2008.
13. Ozkan A, Erdogan A. A comparative evaluation of antioxidant and anticancer activity of essential oil from *Origanum onites* (Lamiaceae) and its two major phenolic components. Turkish Journal of Biology. 2011; 35(6): 735-742.
14. Gurbuz P, Martinez A, Pérez C, Martínez-González, Goger F, Ayran, I. Potential anti-Alzheimer effects of selected Lamiaceae plants through polypharmacology on glycogen synthase kinase-3β, β-secretase, and casein kinase 1δ. Industrial Crops and Products. 2019; 138: 111431.
15. Ersoy E, Ozkan EE, Boga M, Mat A. Evaluation of *in vitro* biological activities of three *Hypericum* species (*H. calycinum*, *H. confertum*, and *H. perforatum*) from Turkey. South African Journal of Botany. 2020; 130: 141-147.
16. Yilmaz MA, Ertas A, Yener I, Akdeniz M, Cakir O, Altun M. et al. A comprehensive LC–MS/MS method validation for the quantitative investigation of 37 fingerprint phytochemicals in *Achillea* species: A detailed examination of *A. coarctata* and *A. monocephala*. Journal of Pharmaceutical and Biomedical Analysis. 2018; 154: 413-424.

17. Boga M, Ertas A, Eroglu-Ozkan E, Kizil M, Ceken B, Topcu, G. Phytochemical analysis, antioxidant, antimicrobial, anticholinesterase and DNA protective effects of *Hypericum capitatum* var. *capitatum* extracts. South African Journal of Botany. 2016; 104: 249–257.
18. Moreno MIN, Isla MI, Sampietro AR, Vattuone MA. Comparison of the free radical-scavenging activity of propolis from several regions of Argentina. Journal of Ethnopharmacology. 2000; 71(1–2): 109–114.
19. Blois MSJN. Antioxidant determinations by the use of a stable free radical. Nature. 1958; 181(4617): 1199–1200
20. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans CJF. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine. 1999; 26(9–10): 1231–1237.
21. Apak R, Guclu K, Ozyurek M, Karademir SE. 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. 2004; 52(26): 7970–7981.
22. Ellman GL, Courtney KD, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical Pharmacology. 1961; 7(2): 88–95.
23. Hearing VJ, Jiménez M. Mammalian tyrosinase-the critical regulatory control point in melanocyte pigmentation. International Journal of Biochemistry. 1987; 19(12): 1141–1147.
24. Zahid H, Rizwani GH, Kamil A, Shareef H, Tasleem S, Khan, A. Anti-urease activity of *Mimusops elengi* Linn (Sapotaceae). European Journal of Medicinal Plants. 2015; 6(4): 223–230.
25. Clinical and Laboratory Standards Institute. Reference method for broth dilution antifungal susceptibility testing of yeasts; Approved standard–Second Edition. 1997; M27-A2. Wayne, PA: Author.
26. Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 7th ed. 2006; Approved standard M7-A7. Wayne, PA: Author.
27. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing: 7th informational supplement. 2010; M100-S20. Wayne, PA: Author.
28. Carikci, S, Kilic, T, Ozer Z, Dirmenci T, Arabacı T, Goren AC. Quantitative determination of some phenolics in *Origanum laevigatum* Boiss extracts via validated LC-MS/MS method and antioxidant activity. Journal of Chemical Metrology. 2018; 12(2): 121-127.
29. Ozer Z, Goren AC, Kilic T, Oncu M, Carikci S, Dirmenci T. The phenolic contents, antioxidant and anticholinesterase activity of section Amaracus (Gled.) Vogel and Anatolicon Ietsw. of *Origanum* L. species. Arabian Journal of Chemistry. 2020; 13(4): 5027-5039.
30. Pizzale L, Bortolomeazzi R, Vichi S, Uberegger E, Conte LS. Antioxidant activity of sage (*Salvia officinalis* and *S. fruticosa*) and oregano (*Origanum onites* and *O. indercedens*) extracts related to their phenolic compound content. Journal of the Science of Food and Agriculture. 2002; 82(14): 1645-1651.
31. Figueiredo AC, Barroso JG, Pedro LG, Scheffer JJ. Factors affecting secondary metabolite production in plants: volatile components and essential oils. Flavour and Fragrance journal. 2008; 23(4): 213-226.
32. Ozkan G, Baydar H, Erbas S. The influence of harvest time on essential oil composition, phenolic constituents and antioxidant properties of Turkish oregano (*Origanum onites* L.). Journal of the Science of Food and Agriculture. 2010; 90(2): 205-209.
33. Kulisic T, Radonic A, Katalinic V, Milos M. Use of different methods for testing antioxidative activity of oregano essential oil. Food Chemistry. 2004; 85(4): 633–640.

34. Viuda-Martos M, Navajas YR, Zapata ES, Fernandez-Lopez Perez-Alvarez JJA. Antioxidant activity of essential oils of five spice plants widely used in a Mediterranean diet. Flavour and Fragrance Journal. 2010; 25(1): 13–19.
35. Baranauskaite J, Kubiliene A, Marksa M, Petrikaite V, Vitkevičius K, Baranauskas A. The influence of different oregano species on the antioxidant activity determined using HPLC postcolumn DPPH method and anticancer activity of carvacrol and rosmarinic acid. BioMed research international. 2017. https://doi.org/10.1155/2017/1681392
36. Cavia-Saiz M, Busto MD, Pilar-Izquierdo MC, Ortega N, Perez-Mateos M. et al. Antioxidant properties, radical scavenging activity and biomolecule protection capacity of flavonoid naringenin and its glycoside naringin: a comparative study. Journal of the Science of Food and Agriculture. 2010; 90(7): 1238-1244.
37. Karim N, Jia Z, Zheng X, Cui S, Chen,WA. Recent review of citrus flavanone naringenin on metabolic diseases and its potential sources for high yield-production. Trends in Food Science & Technology. 2018; 79, 35-54.
38. Hajlaoui H, Mighri H, Aouni M, Gharsallah N, Kadri A. Chemical composition and *in vitro* evaluation of antioxidant, antimicrobial, cytotoxicity and anti-acetylcholinesterase properties of Tunisian *Origanum majorana* L. essential oil. Microbial Pathogenesis. 2016; 95: 86-94.
39. Orhan I, Kartal M, Kan Y, Sener, B. Activity of essential oils and individual components against acetyland butyrylcholinesterase. Zeitschrift fuer Naturforschung C. 2008; 63(7-8): 547-553.
40. Ersoy E, Ozkan EE, Boga M, Yilmaz MA, Mat A. Anti-aging potential and anti-tyrosinase activity of three *Hypericum* species with focus on phytochemical composition by LC–MS/MS. Industrial Crops and Products. 2019; 141, 111735.