Saudi Pharmaceutical Journal 28 (2020) 95–100

Contents lists available at ScienceDirect

Saudi Pharmaceutical Journal

journal homepage: www.sciencedirect.com

Original article

Antibacterial, antioxidant and enzyme inhibition activity capacities of *Doronicum macrolepis* (FREYN&SINT): An endemic plant from Turkey

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ARTICLE INFO

Article history:
Received 15 April 2019
Accepted 29 November 2019
Available online 7 December 2019

Keywords:
Antimicrobial
Antioxidant
Doronicum macrolepis
Enzyme inhibitory activity

ABSTRACT

In the present study, the antioxidant, enzyme inhibition (α-amylase, α-glucosidase, and cholinesterase) and antimicrobial (MIC) activities of three different solvent (ethanol, methanol, or ethyl acetate) extracts of stem, root, and flower of *Doronicum macrolepis* plant were investigated. In addition to this, the chemical composition and the antimicrobial activity of the essential oil were determined. Antioxidant activity was detected using ABTS and DPPH assays. Antimicrobial activity evaluated by microdilution method against to nineteen microorganisms. Also, enzyme inhibition activities were determined by colorimetric methods. Essential oil of the plant extracted by hydrodistilation and characterized using GC/MS. The antioxidant properties of the flower were determined to be higher than those of the other segments of this plant. Moreover, the total phenolic and flavonoid contents were also found to be higher in the flower parts. The highest enzyme inhibition activity was observed to be α-amylase (221.54 mmol ACAE/g extract) in flower ethylacetate extract, α-glucosidase (15.32 mmol ACAE/g extract) in flower ethanol extract, and cholinesterase (AChE: 2.4 and BChE: 22.35 mg GALE/g extract) in stem ethylacetate extract. Besides them, the antimicrobial activity of the essential oil was found to be higher than the extracts. It showed a high level of inhibition especially on *E. coli* at 4 μg/ml concentration. Moreover, remarkable inhibition was observed for two candida strains tested. In conclusion, the results suggest that, because of its bioactivity including the antioxidant, antimicrobial, and enzyme inhibition properties, the *D. macrolepis* can be accepted as a promising and natural source for the industrial applications. The present study is the first study, in which the bioactive components and the antioxidant, antimicrobial, and enzyme inhibition properties of endemic *D. macrolepis* plant were determined.

1. Introduction

Thanks to the rapidly advancing technology, the living standards of the people have significantly increased and, thus, the average life has prolonged. Besides that, the prevalence of chronic diseases such as diabetes and Alzheimer's disease has increased depending on the aging (Qiu and Folstein, 2006).

Diabetes (hyperglycemia) is an endocrinal disease incorporating the disorders in carbohydrate, lipid, and protein metabolism and arising from the deterioration in insulin secretion or its activity in the target cell. It is known that this disease is very common throughout the world and it is projected that the prevalence of this disease will increase in forthcoming years (Zhu, 2013; WHO, 2016; Alam et al., 2019). On the other hand, the Alzheimer's disease (AD) is a lethal and neurodegenerative disease that arises with the symptoms such as loss of memory, cognitive disorders, and dementia and it is projected that the number of peoples influenced by the AD will increase in near future (Brookmeyer et al., 2007; Wu et al., 2019). It was reported that these diseases might be cured by inhibiting specific enzymes (Bahadori et al., 2019). From this aspect, the inhibition of the key enzymes related with the disorder is considered to be an effective method for eliminating these disorders (Bahadori et al., 2017). However, the synthetic medications used in inhibiting these key enzymes have many adverse effects (Chiasson et al., 2002; Lasano et al., 2019). In order to overcome this problem, the researchers initiated incentives aiming to find an alternative natural product that has less or no adverse effect. Within this context, the plants became more popular among the

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jsps.2019.11.010
1319-0164/

studies on natural bioactive compounds (Denev et al., 2019). In the previous studies, it was reported that, with the secondary metabolites they have, the plants are responsible many biological activities including the antioxidant, antimicrobial, anti-inflammatory, anticancer, and enzyme inhibition activities (Echiburu-Chau et al., 2017; Demirci et al., 2017; Djihane et al., 2017; Özcan and Acet, 2018; Alothman et al., 2018; Saleem et al., 2019; Elansary et al., 2019; Borah et al., 2019).

Under favor of its various height and climate conditions, Turkey has a highly diversified flora and many of these plants are aromatic and widely used by the public in treating various diseases (Altindal, 2019). Besides that, the information about which parts of these plants should be used in treating which disorder and how to use them is very limited. For this reason, it is very important to support the traditionally used plants with new studies and scientific knowledge and to develop new natural and healthy products against the damages of synthetic products.

Asteraceae is the largest flowering plant family and it consists of 27 taxa, 15 of which are endemic and widely found in Anatolia (Davis et al., 1988; Guner et al., 2000). It is known that the Asteraceae species are used for nourishing and medical purposes in extract and essential oil forms (Roig, 1965; Denisow-Pietrzyk et al., 2019; Kladar et al., 2015). *Doronicum macrolepis* Freyn. & Sint is an endemic species belonging to that family and is widely used in treating various diseases in folk medicine (Edmondson et al., 1975). In literature, there are few studies on *D. macrolepis* (Akpinar et al., 2009) but no study on the biological activities of this plant was found.

The objective of the present study is to reveal the phytochemical profile of the essential oil of *D. macrolepis*, as well as determining the biological activities such as antioxidant, antimicrobial, and enzyme inhibition (α-amylase, α-glucosidase, acetylcholinesterase, and butyrylcholinesterase). The present study is the first comprehensive study carried out on the biological activities of *D. macrolepis*, which is an endemic species.

2. Materials and methods

2.1. Plant material

Doronicum macrolepis Freyn & Sint (Asteraceae) plants were collected in August 2017 from Artabel/Gümüşhane at 2800–2900 m altitude during the blooming season. The identification of this plant was performed according to 'Flora of Turkey and the East Aegean Islands' (Davis, 1965). By preparing witness herbarium sample, it was kept in Gümüşhane University (Turkey) (TA1703).

2.2. Preparation of the extract

The plants dried at shadow were ground to powder form. The extraction was performed by using ethanol, methanol, or ethyl acetate. 10 g plant powder was added to 200 ml solvent and shaken at 125 rpm for 8 h at the temperature not exceeding beyond 40 °C. Then, it was filtered in order to remove the plant particles. The solvent was removed from the filtrate by using vacuum and evaporator at the temperature not exceeding 40 °C, and the raw extracts were achieved. The extracts were stored at −20 °C for the experiments.

2.3. Essential oil isolation

The essential oil of the plant was obtained in 3 h by using the hydro-distillation method with a Clevenger-type apparatus. 200 g ground plant and 800 ml pure water were used for the extraction. The essential oil that was obtained was stored at −20 °C and in a dark environment until used in analyses.

2.4. GC-FID and GC–MS analysis

Gas Chromotography (GC) analysis was performed with HP-5 MS capillary column (30 m × 0.32 mm i.d., film thickness 0.25 μm) and HP 5973 mass selective detector Hewlett Packard 6890 N model GC-FID and GC–MS (Gas Chromatography-Mass Spectrometer). For detection of GC–MS, electron ionization system with 70 eV ionization energy was used. Helium was used as the carrier gas and the flow rate was set at 1 ml/min. In the splitless method 1.0 μL diluted sample (1/100 hexane, v/v) was injected automatically (Kaya et al., 2017). The characterization of the components in the essential oil is made using electronic libraries (Adams, Wiley, NIST)

2.5. Determining the total phenolic and flavonoid contents

The total phenolic content was determined by using the Folin-Ciocalteu method and the total flavonoid content was determined spectrally by using $AlCl_3$ method (Slinkard and Singleton, 1977; Moreno et al., 2000; Özcan and Acet, 2018). The total phenolic content was expressed as gallic acid equivalent and the total flavonoid content as quercetin equivalent.

2.6. Antioxidant activity

The antioxidant activity was determined by making minor modifications in ABTS (Re et al., 1999) and DPPH (Kirby and Schmidt) methods. In sum, for the DPPH assay, 125 μL plant extract was mixed with 0.1 mM DPPH solution at the same volume and the measurement was made at 490 nm after waiting for 45 min. For the ABTS assay, 80 μL plant extract was mixed with 160 μL ABTS solution and the measurement was performed at 750 nm after waiting for 6 min. In both methods, the results were expressed as trolox equivalent.

2.7. Enzyme inhibition activity

2.7.1. α-Amylase inhibition

The α-Amylase inhibition activity was determined by using Caraway-Somogyi iodure/ potassium iodide (IKI) method (Yang et al., 2012). The sample solutions (25 μL) were mixed with α-amylase solution (50 μL) in phosphate buffer (pH 6.9, 6 mM sodium chloride) on micro-plate with 96 wells. The mixture was incubated for 10 min at 37 °C. After the preliminary incubation, the reaction was initiated after adding starch solution (50 μL, 0.05%). Similarly, a blind solution containing no enzyme was prepared. The reaction mixture was incubated for 10 min at 37 °C and the reaction was stopped after adding HCl (25 μL, 1 M). Then, the iodine–potassium iodide (100 μL) solution was added. The samples and blank absorbance values were read at 630 nm. The results of α-amylase inhibition activity were expressed as acarbose equivalent.

2.7.2. α-Glucosidase inhibition

The α-Glucosidase inhibition activity was determined by making minor modifications in the method of Palanisamy et al. (2011). The sample solution (50 μL), glutathione (50 μL), α-glucosidase solution (50 μL), phosphate buffer (pH 6.8), and PNPG (4-Nitrophenyl β-D-glucuronide) (50 μL) solvent were mixed on a micro-plate with 96 wells and incubated for 15 min at 37 °C temperature. Similarly, a blank specimen containing no enzyme was prepared. The reaction was stopped after adding sodium carbonate (50 μL, 0.2 M). The sample and blind absorbance values were read

at 400 nm. The α-glucosidase inhibition activity was expressed as acarbose equivalent.

2.7.3. Anticholinesterase activity

The cholinesterase (ChE) inhibition activity was determined by making minor modifications in Ellman's method (Zengin et al., 2014). In sum, the sample solution (50 μL), DTNB (125 μL), and anticholinesterase (or butyrylcholinesterase) solution (25 μL) was mixed in Tris-HCl buffer (pH 8.0). Then, it was incubated for 15 min in a microplate with 96 wells at 25 °C. The reaction was initiated by adding acetylcholine iodure (or butyrylcholine iodure). Similarly, a blank mixture containing no enzyme was prepared. The absorbance values of the samples and blank specimens were read at 405 nm after 10 min of incubation at 25 °C. The cholinesterase inhibition activity was expressed as galanthamine equivalent.

2.8. Antimicrobial activity

2.8.1. Test organisms

The antimicrobial activities of the plant against 19 standard microorganisms were determined by using the micro-dilution method. The test organisms used were *Enterococcus faecium* DSMZ 13590, *Enterococcus faecalis* ATCC 29212, *Bacillus cereus* RSKK 709, MRSA ATCC 43300, *Staphylococcus aureus* ATCC 6538, *Enterococcus hirae* ATCC 10541, *Staphylococcus epidermidis* ATCC 12228, *Listeria monocytogenes*, *Listeria innocua* ATCC 33090, *Klebsiella pneumoniae* ATCC 13883, *Salmonella typhimurium* CCM 5445, *Escherichia coli* ATCC 29998, *Pseudomonas aeruginosa* ATCC 27853, *Vibrio parahaemolyticus* ATCC 17802, *Yersinia enterocolitica* ATCC 27729, *Yersinia pseudotuberculosis* ATCC 911, *Proteus vulgaris* ATCC 13315, *Candida albicans* DSMZ 5817, *Candida tropicalis* NRRLL YB-366.

2.8.2. Minimum inhibition concentration

The minimum inhibition concentrations of the samples were determined by using broth dilution method (CLSI, 2017) on a microtiter plate (with 96 wells). The samples were dissolved in DMSO and the obtained serial dilution concentration was used for determining 0.512–0.001 mg/ml MIC values. First of all, the samples were added to the wells at determined concentrations, and then the suspensions of the test microorganisms (0.5 MacFarland) inoculated to the wells. After 48 h of incubation, the microbial growth was determined by using a microplate absorbance reader. The MIC value was reported as the lowest plant extract concentration preventing the microbial growth. Chloramphenicol, novobiocin, nalidixic acid, and nystatin were used as positive control.

2.9. Statistical analyses

The statistical analyses were conducted after the experiments and the results were expressed as mean values ± SD of the triplicated measurements. ANOVA was used in order to identify the variations between various extracts ($p < 0.05$). The statistical calculations were carried out by SPSS version 20.0 programs (IBM).

3. Results and discussion

3.1. Phytochemical composition

In the present study, the total bioactive components of the flower, stem, and root extracts of the plant were spectrophotometrically examined in terms of the total phenolic and flavonoid contents. According to the results obtained, the highest total phenolic content was found in flower ethyl acetate extract, followed by the flower ethanol extract and the flower methanol extract. The total flavonoid content of the flower ethanol extract was higher than the other extracts (Table 1). Moreover, the essential oil was obtained by using the hydro-distillation method and the phytochemical composition was determined by using GC/GC–MS (Table 2). The oil was found to contain high amounts of (E,E)-α-Farnesene (21.5%), *trans*-β-Ocimen (12.8%), δ-Cadinene (9.5%), Caryophyllene oxide (8.2%), and thymol (4.4%). The essential oil content of the flower and stem parts of *D. macrolepis* collected from a different region (Akpınar et al., 2009) was reported to have a similar phytochemical profile, whereas the percentages of the compounds were found to be different. These differences in chemical composition were thought to be because of various factors such as the ecological conditions under which the plants were collected (Salehi et al., 2018), the collection timing, and the extracting method (Moghtader and Afzali, 2009; Celiktas et al., 2007; Okoh et al., 2010).

Table 1
Total bioactive components of the extracts.

Plant parts	Extracts	Total phenolic content (TPC) (mg GAE/g extract)	Total flavonoid content (TFC) (mg QE/g extract)
Flower	Ethanol	273.8 ± 0.7^{b}	123.2 ± 0.5^{a}
	Methanol	103.6 ± 1.3^{c}	43.9 ± 1.5^{b}
	Ethylacetate	569.6 ± 2.9^{a}	32.9 ± 1.6^{c}
Stem	Ethanol	48.5 ± 1.3^{g}	19.3 ± 1.2^{d}
	Methanol	56.5 ± 0.8^{f}	34.5 ± 0.8^{c}
	Ethylacetate	94.7 ± 1.3^{d}	44.6 ± 3.5^{b}
Root	Ethanol	64.5 ± 0.6^{e}	8.6 ± 0.3^{e}
	Methanol	48.8 ± 1.4^{g}	6.9 ± 0.2^{e}
	Ethylacetate	40.2 ± 1.7^{h}	7.6 ± 0.3^{e}

*Values expressed are means ± SD of three different measurements. GAE, gallic acid equivalents; QE, quercetin equivalents. The data shown with different letters in the same column refer to statistically significant differences between the extracts ($p < 0.05$).

3.2. Antioxidant activity

Scavenging the free radicals accumulating within the tissues is very important for protecting the organisms from many diseases. The free radicals play role in many diseases by causing damage and aging in the cells (Seo et al., 2019; Zhao et al., 2019). It is known that the plants have high antioxidant properties (Bahadori et al., 2019). In the present study, the in vitro antioxidant activity of *Doronicum macrolepis* extracts were determined by using DPH and ABTS methods, which yield economic, accurate, and exact results and are used in herbal experiments. According to the results obtained, it was determined that the highest scavenging efficiencies were observed in ABTS method for flower ethyl acetate extract (262.4 mg TE/g extract) and DPPH method for stem ethanol extract (486.5 mg TE/g extract) and root ethylacetate extracts (480.6 mg TE/g extract). Besides that, all the extracts including the stem extracts were found to have strong antioxidant activities (Table 3).

When comparing Tables 1 And 3, it was determined that there was a correlation between the total phenolic content and the antioxidant activity determined by using ABTS method, whereas there was no correlation between the total phenolic content and the antioxidant activity determined by using DPPH method. Although there are similar results reported in the literature (Karadeniz et al., 2015), it was also observed that there was no correlation (Javanmardi et al. 2003; Özcan and Acet, 2018).

3.3. Enzyme inhibition activity

Diabetes and Alzheimer's disease were among the most important global health problems. The synthetic medications are widely

Table 2
Phytochemical composition of the essential oil of the plant.

No	compounds	% area	RI	RI $_{literature}$
1	n-Hexanal	0.9	773	780
2	2-Hexenal	0.4	844	847
3	Fenchene	0.1	957	953
4	2-Heptenal, (Z)-	0.2	970	964
5	Benzaldehyde	0.2	972	968
6	β-Thujene	0.4	988	987
7	β-Pinene	0.2	990	991
8	Furan, 2-pentyl-	2.5	1010	1001
9	α-Phellandrene	0.3	1023	1014
10	3-Carene	0.3	1029	1025
11	o-Cymene	0.6	1047	1036
12	β-Phellandrene	2.7	1051	1043
13	Eucalyptol	0.4	1054	1049
14	*trans*-β-Ocimen	12.8	1063	1062
15	Hyacinthin	0.7	1069	1063
16	β-*cis*-Ocimene	0.5	1075	1063
17	γ-Terpinene	0.5	1086	1074
18	1-Octanol	0.7	1102	1091
19	*trans*-thujone	0.1	1120	1114
20	Nonanal	0.7	1139	1142
21	Camphor	0.1	1159	1146
22	*p*-Acetyltoluene	0.1	1179	1183
23	Ethyl caprylate	0.2	1201	1202
24	Safranal	0.4	1245	1206
25	Carvacrol	0.1	1294	1297
26	Undecanal	0.1	1309	1307
27	(2E,4E)-Decadienal	0.1	1314	1317
28	2,4-Decadienal	0.1	1318	1323
29	Thymol	4.4	1350	1318
30	α-Cubebene	6.4	1358	1352
31	α-Copaene	0.3	1375	1377
32	β-Cubebene	0.2	1384	1388
33	(E)-Caryophyllene	0.2	1411	1419
34	α-Caryophyllene	0.6	1451	1455
35	(E)-β-Farnesene	1.2	1460	1457
36	Germacrene D	0.1	1484	1485
37	Aromandendrene	1.9	1490	1441
38	(E,E)-α- Farnesene	21.5	1508	1506
39	δ-Cadinene	9.5	1531	1523
40	Caryophyllene oxide	8.2	1561	1579
41	β-Bisabolene	1.4	1577	1531
42	Oplopenone	3.7	1608	1608
43	α-Cadinol	0.3	1630	1654
44	Pentadecanal	2.7	1713	1714
45	Hexahydrofarnesyl acetone	1.4	1847	1847
46	Tricosane	1.2	2304	2300
	Total area	91.2		

Table 3
Antioxidant activities of the extracts.

Plant parts	Extracts	ABTS scavenging mg TE/g extract	DPPH scavenging mg TE/g extract
Flower	Ethanol	185.7 ± 1.5^{e}	21.6 ± 0.9^{g}
	Methanol	248.6 ± 0.1^{b}	347.5 ± 2.1^{d}
	Ethylacetate	262.4 ± 0.8^{a}	159.1 ± 1.8^{f}
Stem	Ethanol	198.8 ± 1.6^{d}	486.5 ± 4.0^{a}
	Methanol	101.6 ± 1.4^{f}	273.3 ± 2.8^{e}
	Ethylacetate	248.1 ± 1.8^{b}	347.6 ± 3.1^{d}
Root	Ethanol	249.3 ± 1.5^{b}	371.8 ± 3.8^{c}
	Methanol	100.2 ± 1.9^{f}	402.5 ± 3.5^{b}
	Ethylacetate	235.7 ± 2.2^{c}	480.6 ± 2.0^{a}

* Values expressed are means ± SD of three different measurements. TE, Trolox equivalents. The data shown with different letters in the same column refer to statistically significant differences between the extracts ($p < 0.05$).

used in treatments of these patients. It was also revealed in studies that these chemical medications have many adverse effects on the tissues and organs. In the recent period, researchers started to investigate the potential active compounds from herbal sources, which would not cause these adverse effects (Howes and Houghton, 2003; Hu et al., 2013; Lasano et al., 2019). In the present study, the potential of plant extracts for controlling diabetes and Alzheimer's disease was determined with in vitro methods by inhibiting the key enzymes (α-amylase, α-glucasidase for diabetes and cholinesterases for Alzeimer' disease) playing role in the management of these disorders (Figueiredo-González et al., 2019; Shrivastava et al., 2019). The enzyme inhibition activity results of plant extracts are presented in Table 4. Accordingly, the flower ethyl acetate (221.54 mmol ACAE/g extract), flower methanol (15.32 mmol ACAE/g extract), and stem ethyl acetate (AChE = 2.4 mg GALE/g extract; BChE = 22.35 mg GALE/g extract) extracts were found to have highest α-amylase, α-glucosidase, and acetylcholinesterase inhibition activities, respectively. When considering the plant parts, it was determined that the flower ethyl acetate, stem, and root methanol extracts were found to have the highest amylase inhibition when compared to the other extracts, whereas the highest glucosidase inhibition effect was observed in flower, stem, and root methanol extracts. The root, stem, and flower ethyl acetate extracts of the plant were found to have higher cholinesterase inhibition activity when compared to the ethanol and methanol extracts. In general, the extract of plant parts (except for the stem methanol and root ethanol extracts) showed remarkable enzyme inhibition activities. Given Table 4, it was determined that there was a correlation between the cholinesterase and α-glucosidase inhibition activities and the solvent used in extraction. In many studies, it was reported that the compounds of plants such as phenolic and flavonoid contents are responsible for various biological activities. It is thought that the enzyme inhibition activity in the present study arises from the phytochemical content of the plant. Among the main compounds, the thymol was reported to have anti-diabetic (Saravanan and Pari, 2016) and cholinesterase (Duke, 2007) inhibition activity in various studies. Besides the dominant compounds, the minor compounds may also create many activities when together. In literature, no study examining the amylase, glucosidase, and cholinesterase inhibition activities of *Doronicum* species was found. However, the enzyme inhibition activity of many plants from the Asteraceae, which includes also *Doronicum*, was investigated before (Saoud et al., 2019; Uysal et al., 2018; Ascari et al., 2019; Zengin et al., 2018; Saleem et al., 2019).

3.4. Antimicrobial activity

The antimicrobial activity of the plant extracts and oil was investigated with the microdilution method by using 17 bacteria and 2 yeast strains. It was determined that they showed weak antimicrobial activity against all the tested microorganisms (>512 μg/mL), whereas the essential oil of the plant showed remarkable activity on all the microorganisms (Table 5–6). The oil showed a very strong effect on *E. coli* at 4 μg/mL concentration, and remarkable antimicrobial effect on *S. epidermidis*, vancomycin-resistant *E. faecium*, *Y. pseudotuberculosis, C. albicans,* and *C. tropicalis* at ≤32 μg/mL concentration. These results suggest that the essential oil of this plant could be used as an antimicrobial agent. In previous studies carried out on similar species, it was reported that the essential oils of the plants showed higher antimicrobial activity when compared to the plant extracts (Politi et al., 2016; Shirazi et al., 2014; Candan et al., 2003).

The results obtained here are in corroboration with the antimicrobial activity results reported in the literature for the other *Doronicum* species. In a similar study carried out on the antimicrobial and antitumoral activity of the ethanol, methanol, and water extracts of *Doronicum orientale*, both of these activities were found to be weak (Usta et al., 2014). In another study, *Doronicum hookeri* root dichloromethane:methanol (1:1, v/v) extract (500 μg/mL con-

Table 4
Enzyme inhibitory activities of the extract.

Plant parts	Extracts	α-amylase Inhibition (mmol ACAE/g extract)	α-glucosidase Inhibition (mmol ACAE/g extract)	AChE Inhibition mg GALE/g extract	BChE Inhibition mg GALE/g extract
Flower	Ethanol	91.38 ± 0.71^{i}	8.88 ± 0.02^{c}	0.6 ± 0.05^{e}	7.27 ± 0.05^{f}
	Methanol	218.44 ± 0.59^{b}	15.32 ± 0.09^{a}	0.68 ± 0.02^{e}	7.65 ± 0.03^{e}
	Ethylacetate	221.54 ± 0.65^{a}	7.37 ± 0.06 ^{e}	0.80 ± 0.06^{c}	10.80 ± 0.02^{c}
Stem	Ethanol	201.86 ± 1.58^{c}	6.58 ± 0.08^{g}	0.74 ± 0.03^{d}	9.46 ± 0.01^{d}
	Methanol	109.73 ± 0.36^{g}	7.76 ± 0.17^{d}	ND	4.57 ± 0.03^{i}
	Ethylacetate	190.24 ± 0.83^{d}	6.86 ± 0.10^{f}	2.4 ± 0.08^{a}	22.35 ± 0.09^{a}
Root	Ethanol	150.57 ± 0.82^{e}	5.23 ± 0.17^{i}	ND	4.76 ± 0.02^{h}
	Methanol	95.03 ± 1.00^{h}	9.13 ± 0.04^{b}	0.4 ± 0.01^{f}	5.51 ± 0.03^{g}
	Ethylacetate	113.43 ± 1.46^{f}	6.23 ± 0.08^{h}	1.06 ± 0.04^{b}	14.41 ± 0.11^{b}

Values expressed are means ± SD of three different measurements. ACAE, acarbose equivalents; GALE, galantamine equivalents. The data shown with different letters in the same column indicate statistically significant differences between the extracts ($p < 0.05$).

Table 5
Antibacterial activities of the essential oil.

Test organisms	MIC value (μg/mL)			
	Volatile oil	Chloromphenicol	Novobiocin	Nalidixic acid
E. hirae	64	8	1	256
B. cereus	128	2	1	4
S. epidermidis	32	16	4	4
S. aureus	128	64	128	32
MRSA	128	32	1	64
E. faecium	32	4	1	256
E. feacalis	512	8	4	128
L. monocytogenesis	128	16	2	256
L. innocua	128	32	2	128
S. typhimurium	128	1	512	8
V. Parahaemolyticus	128	8	512	256
P. aeruginosa	64	4	1	32
Y. enterocolitica	64	2	512	4
K. pneumoniae	128	4	2	1
E. coli	4	32	512	4
Y. pseudotuberculosis	32	16	4	4
P. vulgaris	128	16	4	8

Table 6
Anticandidal activity of the essential oil.

Test organisms	MIC value (μg/mL)			
	Volatile oil	Chloromphenicol	Nystatine	Nalidixic acid
C. albicans	16	16	16	8
C. tropicalis	32	16	16	16

centration) was tested and it showed inhibition only on *Candida albicans* among 14 microorganisms, for which it was tested (Kumar et al., 2006).

4. Conclusion

Because of the positive effects of active compounds they contain, the plants are widely used for therapeutic purposes by the humans. Diabetes and Alzheimer's diseases are among the metabolic diseases affecting many people, and the increasing number of people suffers from the negative effects of these diseases. Although many synthetic medications are used in the management of these disorders, these medications might have undesired adverse effects. For this reason, the plants constitute an important source for the natural active compounds that have very low or no adverse effect. However, it became necessary to reveal if the plants, which are used for therapeutic purposes, have the effects that are in parallel with the intended use. The biological activity of *D. macrolepis*, which is an endemic plant, was determined for the first time. When considering the study results from a holistic aspect, it was found that the ethyl acetate extracts of the plant's aboveground and belowground parts were superior to the other extracts in terms of the characteristics investigated in the present study. Besides the plant extracts, also the antimicrobial effects of the essential oil were determined and it was found that the essential oil is more effective. Moreover, since the plant has the potential of usage in the management of diseases such as diabetes and Alzheimer's disease, it is thought that the further studies including purifying and revealing the active contents in order to determine the source of its biological activity.

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