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

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**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 hydrodistillation 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 ethyl acetate 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 ethyl acetate 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 mg/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.

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### 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 endocrinial 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 (Chiaisson 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...
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, antitumor, 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., 2005) 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 Artabyl/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 Chromatography (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 AlCl₃ 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 blank 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 blank 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 activity of the plant was performed 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 monocyctogenes*, *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 0.5±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 micro- biological 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)-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 (Akpinar 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 (Moghhtader 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<sup>a</sup> | 123.2 ± 0.5<sup>a</sup> |
| | Methanol | 103.6 ± 1.3<sup>b</sup> | 43.9 ± 1.5<sup>b</sup> |
| | Ethylacetate | 569.6 ± 2.9<sup>b</sup> | 32.9 ± 1.6<sup>b</sup> |
| Stem | Ethanol | 48.5 ± 1.3<sup>c</sup> | 19.3 ± 1.2<sup>c</sup> |
| | Methanol | 56.5 ± 0.8<sup>d</sup> | 34.5 ± 0.8<sup>d</sup> |
| | Ethylacetate | 94.7 ± 1.3<sup>c</sup> | 44.6 ± 3.5<sup>c</sup> |
| Root | Ethanol | 64.5 ± 0.6<sup>e</sup> | 8.6 ± 0.3<sup>e</sup> |
| | Methanol | 48.8 ± 1.4<sup>e</sup> | 6.9 ± 0.2<sup>e</sup> |
| | Ethylacetate | 40.2 ± 1.7<sup>f</sup> | 7.6 ± 0.3<sup>f</sup> |

<sup>a</sup>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 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 DPH 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 DPH 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
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 (β-amylase, α-glucosidase for diabetes and cholinesterases for Alzheimer’s 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 (ACH = 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 amylose 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; Ascarli 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 | \(\beta\)-amylase Inhibition (mmol ACAE/g extract) | \(\beta\)-glucosidase Inhibition (mmol GALE/g extract) | AChE Inhibition mg GALE/g extract | BChE Inhibition mg GALE/g extract |
|-------------|----------|-------------------------------------------------|-------------------------------------------------|---------------------------------|---------------------------------|
| Flower      | Ethanol  | 91.38 ± 0.71\(^a\)                              | 8.88 ± 0.02\(^a\)                              | 0.6 ± 0.05\(^d\)               | 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\(^b\)              |
|             | Ethylacetate | 221.54 ± 0.65\(^\alpha\)                       | 7.37 ± 0.06 \(^\alpha\)                      | 0.80 ± 0.06\(^e\)              | 10.80 ± 0.02\(^e\)             |
| Stem        | Ethanol  | 201.86 ± 1.58\(^c\)                             | 6.58 ± 0.08\(^\beta\)                        | 0.74 ± 0.03\(^\delta\)         | 9.46 ± 0.01\(^\epsilon\)       |
|             | Methanol | 109.73 ± 0.36\(^\epsilon\)                      | 7.76 ± 0.17\(^\epsilon\)                      | ND                              | 4.57 ± 0.03\(^\delta\)         |
|             | Ethylacetate | 190.24 ± 0.83\(^\epsilon\)                      | 6.86 ± 0.10\(^\epsilon\)                      | 2.4 ± 0.08\(^\epsilon\)        | 22.35 ± 0.09\(^\epsilon\)       |
| Root        | Ethanol  | 150.57 ± 0.62\(^\beta\)                        | 5.23 ± 0.17\(^\beta\)                        | ND                              | 4.76 ± 0.02\(^\epsilon\)        |
|             | Methanol | 95.03 ± 1.00\(^\beta\)                          | 9.13 ± 0.04\(^\beta\)                        | 0.4 ± 0.01\(^\beta\)           | 5.51 ± 0.03\(^\beta\)          |
|             | Ethylacetate | 113.43 ± 1.46\(^\beta\)                      | 6.23 ± 0.08\(^\beta\)                        | 1.06 ± 0.04\(^\beta\)          | 14.41 ± 0.11\(^\beta\)         |

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 | Choromphenicol | 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. fecalis      | 512              | 8           | 4              | 128        |               |
| L. monocytogenes| 128              | 16          | 2              | 256        |               |
| L. innocua      | 128              | 32          | 128            | 128        |               |
| S. typhiurium   | 128              | 1           | 512            | 8          |               |
| V. Parahaemolyticus | 128  | 8           | 512            | 256        |               |
| P. aeruginosa   | 64               | 4           | 1              | 32         |               |
| Y. enterococita | 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 | Choromphenicol | 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 above-ground 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.

References

Alipinar, K., Yıldırım, N., Üçüncü, O., Yaylı, N., Terzioglu, S., Yaylı, N., 2009. Volatile Constituents of the Flowers and Leaves–Stems of three Doronicum taxa from Turkey. Asian J. Chem. 21 (2), 1225–1229.

Alam, F., Shafique, Z., Amjad, S.T., Bin Asad, M.H.H., 2019. Enzymes inhibitors from natural sources with antidiabetic activity: a review. Phytother. Res. 33, 41–54.

Althaud, M.B., Kirkan, B., Sarikurkcu, C., 2019. Phenolic ingredients and therapeutic potential of Stachys cretica subsp. smyrenae for the management of oxidative stress, Alzheimer’s disease, hyperglycemia, and melisma. Ind. Crops Prod. 127, 82–87.

Bahadori, S., Bahadori, M.B., Karkash, B., Sarikurkcu, C., 2019. Phenolic ingredients and therapeutic potential of Stachys cretica subsp. smyrenae for the management of oxidative stress, Alzheimer’s disease, hyperglycemia, and melisma. Ind. Crops Prod. 127, 82–87.

Bahadori, S., Bahadori, M.B., Zengin, G., Magi, D., Dinaras, D., Aktunsek, A., 2017. Chemical composition profile of the essential oil from Hymenococcus bituminus and its health functionality. Int. J. Food Prop. 20, 972–980.

Borah, A., M., Gogoi, R., Loying, R., Sarma, N., Munda, S., Pandey, S.K., Lal, M., 2019. Chemical composition, antioxidant, anti-inflammatory and in-vitro cytotoxic efficacy of essential oil of Curcuma caesia Roxh. leaves: An endangered medicinal plant of North East India. Ind. Crops Prod. 129, 448–454.

Brookmeyer, R., Johnson, E., Ziegler–Graham, K., Arrighi, H.M., 2007. Forecasting the global burden of Alzheimer’s disease. Alzheimer’s Dement. 3, 186–191.

Candani, F., Unlu, M., Doñer, D., Aydogu, M., Akpulat, H.A., 2019. The effect of methanol extracts treated with supercritical carbon dioxide. Food Chem. 289, 1–7.

Chiasson, J.L., Josse, R.G., Gomis, R., Hanefeld, M., Karasik, A., Laakso, M., STOP-NIDDM Trail Research Group, 2002. Acarbose for prevention of type 2 diabetes mellitus: the STOP-NIDDM randomised trial. Lancet. 359(9293), 2072–2077.

Chiar, J.L., Josse, R.G., Comis, M., Hanefeld, M., Karasik, A., Laakso, M., STOP-NIDDM Trail Research Group, 2002. Acarbose for prevention of type 2 diabetes mellitus: the STOP-NIDDM randomised trial. Lancet. 345(9031), 2072–2077.

Clinical and Laboratory Standards Institute (CLSI), 2017. Performance Standards for Antimicrobial Susceptibility Testing; 27th Informational Supplement. CLSI/NCTCLS, 27th ed.; Clinical and Laboratory Standards Institute: Wayne, PA, USA.

Clinical and Laboratory Standards Institute: Wayne, PA, USA.

Celikbas, O.Y., Bedir, E., Sukan, F.V., 2007. In vitro antioxidant activities of Rosmarinus officinalis extracts treated with supercritical carbon dioxide. Food Chem. 101, 1457–1464.

Davis, P.H., 1965. Flora of Turkey and the East Aegean Islands. Edinburgh University Press, Edinburgh.
Moghtader, M., Afzali, D., 2009. Study of the antimicrobial proprieties of the oil of Lasano, N.F., Hamid, A.H., Karim, R., Dek, M.S.P., Shukri, R., Ramli, N.S., 2019. Biochemical, chemical and toxicological perspectives on aerial and roots of Fillago germanica (L): functional approaches for novel phyto- pharmaceuticals. Food Chem. Toxicol. 123, 363–373.

Salehn, H., Hfar, T.T., Naidu, R., Nawawi, N.S., Ahmad, I., Ashraf, M., Ahern, N., 2019b. Biological, chemical and toxicological perspectives on aerial and roots of Fillago germanica (L): Functional approaches for novel phyto-pharmaceuticals. Food Chem. Toxicol. 123, 363–373.

Shrivastava, S.K., Sinha, S.K., Sivastava, P., Tripathi, P.N., Sharma, P., Tripathi, M.K., Tripathi, A., Choube, P.K., Walker, D.K., Agarwal, L.M., Dixit, M., Khurska, S.C., Gambhir, S., Shankar, S., Srivastava, R.K., 2019. Development and design of novel p-amino benzoic acid derivatives as potential cholinesterase inhibitors for the treatment of Alzheimer’s disease. Bioorg. Chem. 82, 211–223.

Slinkard, K., Singleton, V.L., 1977. Total phenol analysis: automation and comparison with manual methods. Am. J. Enol. Vitic. 28, 49–55.

Usta, C., Yildirim, A.B., Turker, A.U., 2014. Antibacterial and antifungal activities of some plants grown in Turkey. Biotechnol. Biotechnol. Equip. 28 (2), 306–315.

Uysal, S., Senkardes, I., Mollica, A., Zengin, G., Bulut, G., Dogan, A., Glamočlija, J., Soković, M., Lobine, D., Mahoodomolly, F.M., 2018. Biologically active compounds from two members of the Asteraceae family: Tagropogon dubius (L.) Coss. & Dur. and Tussilago farfara L. J. Biol. Med. Struct. Dyn. 24, 1–13.

World Health Organization (WHO), 2016. Improving access to insulin and oral medicines for diabetes, Moldova. Global Reports on Diabetes. 59, WHO Press, Geneva.

Wu, X., Cai, H., Pan, L., Cui, G., Qin, F., Li, Y.C., Gai, Z., 2019. Small Molecule Natural Products and Alzheimer’s Disease. Curr. Top. Med. Chem. 19, 1.

Yang, W.X., Huang, M.Z., Jin, Y.S., Sun, L.N., Song, Y., Chen, H.S., 2012. Phenolics from Bidens bipinnata and their amylase inhibitory activities. Fitoterapia 83, 1169–1172.

Zengin, G., Sarikurkcu, C., Aktumsek, M., Ceylan, R., Ceylan, O., 2014. A comprehensive study on phytochemical characterization of Haplopappus myriophyllus Boiss. endemic to Turkey and its inhibitory potential against key enzymes involved in Alzheimer skin diseases and type II diabetes. Ind. Crop Prod. 53, 244–251.

Zengin, G., Zheleva-Dimitrova, D., Grevova, R., Nedialkov, P., Mocan, A., Ciric, A., Glamočlija, J., Soković, M., Aktumsek, A., Mahoodomolly, M.F., 2018. Identification of phenolic compounds via GC-MS analysis and biological activities of two Centaurea species: C. drabifolia subsp. drabifolia and C. ficopodia. J. Pharm. Biomed. Anal. 149, 436–441.

Zhao, C., Tang, Z., Chung, A.C.K., Wang, H., Cai, Z., 2019. Metabolic perturbation, production and reaction of oxygen species jointly contribute to cytotoxicity of human breast cancer cell induced by tetrabromo and tetrachloro bisphenol A. Ecotoxic. Environ. Safe. 170, 495–501.

Zhu, C., 2013. Aldose reductase inhibitors as potential therapeutic drugs for diabetic complications. In: Diabetes mellitus-insights and perspectives. IUTech.