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Research Article

Biological activities and phenolic content of endemic *Helichrysum artvinense* P.H. Davis et Kupicha (Asteraceae)

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Abstract: Species from the Asteraceae family have been extensively utilized in traditional medicine and as food sources for centuries. They also exhibit important biological activities attributed to their diverse array of phytochemical compounds. This research aimed to determine total phenolic and flavonoid contents of ethanol (EtOH) and methanol (MeOH) extracts of endemic *Helichrysum artvinense* and to reveal its antioxidant, antimicrobial enzyme inhibitory (*α*-glucosidase, *α*-amylase, and tyrosinase) and DNA protective activities. In addition, phenolic compound analyses were conducted using high-performance liquid chromatography (HPLC), establishing a correlation with the aforementioned biological activities. Based on the obtained data, the ethanol (EtOH) extract of the plant demonstrated greater prominence in terms of the screened biological activities. This extract was found to contain significant phenolic components, including epicatechin, chlorogenic acid, and luteolin. Consequently, it appears that the plant has the potential to serve as a natural alternative in both food and pharmacological applications. However, further studies to elucidate the mechanisms underlying the observed biological activities would be beneficial for the product development phase.

1. INTRODUCTION

The rapidly changing living conditions of today have introduced various stressors, leading to an increased incidence of numerous diseases (Pakpour *et al.*, 2021). Various stress-induced reactive oxygen species (ROS) disrupt cellular homeostasis in living organisms (Anwar *et al.*, 2022). The increase of ROS leads to various metabolic diseases such as cancer (with DNA damage), diabetes, aging, inflammation and neurodegenerative diseases (Maritim *et al.*, 2003; Tsao & Deng, 2004; Tepe *et al.*, 2005; Lee *et al.*, 2010; Fu *et al.*, 2011). Therefore, it is crucial to remove these reactive oxygen species (ROS) by natural means. Plants can accomplish this with bioactive compounds (Ebrahimzadeh & Tavassoli, 2015). Plants have already been used throughout history to alleviate or prevent many diseases and have become part of traditional medicine worldwide (Nebrigić *et al.*, 2023). Although the use of synthetic drugs has increased in the modern age, people still prefer natural herbal products to treat diseases due to the negative effects they cause (Gonçalves *et al.*, 2017; Zengin *et al.*, 2018). In fact, studies have shown that

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polyphenols of plants have many biological activities and antioxidant properties (Annadurai *et al.*, 2021; Birsan *et al.*, 2021).

During this period of pronounced global climate change, increased exposure to ultraviolet (UV) rays from the sun can result in advanced pigmentation in humans, leading to serious skin problems (aging, cancer, etc.) (Sheng *et al.*, 2022). UV radiation can stimulate the activity of tyrosinase, leading to an overproduction of melanin in the skin. While it normally helps conserve the skin from UV, excessive melanin synthesis can result in hyperpigmentation, age spots, inflammation, and even skin cancer (Brenner & Hearing, 2008; Pillaiyar *et al.*, 2017). To prevent these problems, it is vital to develop new and effective tyrosinase inhibitors that can help regulate melanin production and prevent its excessive accumulation in the skin. On the other hand, inhibition of enzymes such as alpha-amylase and glucosidase, which are responsible for the digestion of carbohydrates, is also important for solving diabetes, one of the most common health problems of our time (Ogunyemi *et al.*, 2022). Undoubtedly, another of the biggest dangers is the spread of pathogenic microorganisms or their becoming resistant to antibiotics (Gan *et al.*, 2024). Faced with all these problems, it has become essential to identify natural compounds, especially those obtained from plants, and to reveal their potential for use as enzyme inhibitors or antimicrobial agents. Because, considering the possible side effects of the synthetic agents used, the use of plants seems much safer.

Türkiye hosts a remarkable diversity of plant species, with the genus *Helichrysum* from the Asteraceae family being especially prominent (Lahlou *et al.*, 2024). These species have been variously named by the public (e.g. goldenrod, highland flower or immortelle) and are consumed mainly as herbal teas (Acet *et al.*, 2020). *Helichrysum* genus and its members are ethnobotanically valuable and are used as antioxidants, antimicrobials and to alleviate kidney disorders (Sezik *et al.*, 2001; Sala *et al.*, 2003; Albayrak *et al.*, 2010). In recent decades, *Helichrysum* species have been recognized as one of the promising medicinal plants. *Helichrysum* species occupy an important place in the literature with extensive research on their phytochemicals and biological activities. For instance, they have huge properties including cytotoxic, antimicrobial, antioxidant, anti-inflammatory, enzyme inhibitory activities for metabolic and neurodegenerative diseases, and anti-aging (Tepe *et al.*, 2005; Aslan *et al.*, 2007; Gouveia-Figueira *et al.*, 2014; Popoola *et al.*, 2015; Gonçalves *et al.*, 2017; Özcan & Acet, 2018; Acet *et al.*, 2020). Although there are many studies on *Helichrysum* species in the literature, there is limited research on the therapeutic properties of endemic *Helichrysum artvinense* in Artvin region (Eroğlu *et al.*, 2009; Albayrak *et al.*, 2010). Hence, in the present research we proposed to determine the biological activities of EtOH and MeOH extracts of *Helichrysum artvinense* such as *i*- antioxidant activity, *ii*- antimicrobial activity, *iii*- enzyme inhibitory activity, *iv*- DNA protective activity (for the first time), also *v*- phenolic component analysis by HPLC.

2. MATERIAL and METHODS

2.1. Collection of Samples

The plant materials of Helichrysum artvinense P.H. Davis & Kupicha examined in this study were collected from their natural habitat in Artvin Province, Türkiye. Specifically, samples were collected from Ardanuç: Cehennem Deresi Canyon upper sections, along roadsides, and on rocky slopes at an elevation of 660 m (41°08'35.0"N, 42°02'39.0"E), under the collection number Aksu 409, on 3 August 2022. The taxonomic identification and verification of the species were conducted by Dr. Nurşen Aksu Kalmuk, following the taxonomic keys for Helichrysum species as described by Davis (1975) in Flora of Türkiye and the East Aegean Islands. Voucher specimens are preserved at the Medicinal and Aromatic Plants Application and Research Center, Artvin Çoruh University. 5 g of dried and powdered aerial parts of plant were taken and kept in a shaker with 100 mL of EtOH and MeOH for 24 hours, and the solvent was evaporated with an evaporator at 37 °C (Bozkır *et al.*, 2022). For further experiments, the extracts were stored at -20°C.

2.2. Total Phenolic (TPC) and Flavonoid (TFC) Contents

Total phenolic and flavonoid contents in the extracts were determined according to Acet et al. (2020). The results obtained were determined as gallic acid (GAE) and quercetin equivalence (QUE), respectively.

2.3. Determination of Antioxidant Activity

ABTS [(2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] radical scavenging activity of the extracts was assessed by modified method developed by Re *et al*., (1998). Accordingly, 80 µL of the sample was combined with 160 µL of ABTS, allowed to react for 6 minutes, and then measured at 750 nm using a microplate reader. Results were expressed as trolox equivalent (mg TE/g extract). On the other hand, DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of the extracts was determined by modifying the method developed by Kirby & Schmidt, (1997). 125 µL of DPPH was added to 125 µL of extract solution and after waiting for 45 minutes at room temperature, measurement was made at 490 nm. The results were calculated as trolox equivalent (mg TE/g extract).

The activity of the EtOH and MeOH extracts was determined using the following equation, and then, expressed with IC_{50} value.

$$\%\text{Inhibition} = [(A_{\text{Control}} - A_{\text{Extract}})/A\text{Control}] * 100$$

2.4. Determination of Some Enzyme Inhibitory Activities

Enzyme inhibition activities of plant extracts associated with diabetes were determined using the assay of Acet et al. (2020). The results were stated as acarbose equivalent (mmol g/ extract). Tyrosinase inhibitory activity was determined according to Sarıkürkçü and Zengin (2020). Tyrosinase inhibition activity was given equivalents as kojic acid.

2.5. Determination of Antimicrobial Activity

Disc diffusion and microdilution methods were preferred to detect the antimicrobial activity of the plant extracts (CLSI, 2017). *Salmonella typhimurium* CCM5445, *Escherichia coli* ATCC 29998, *Bacillus cereus* RSKK 709, *Bacillus subtilis* IMG 22, *Enterococcus faecalis* ATCC 29212, *Klebsiella pneumoniae* ATCC 13883, methicillin resistant *Staphylococcus aureus* (MRSA) ATCC 43300, *Enterococcus hirae* ATCC 10541, vancomicine resistance *Enterococcus faecium* DSMZ 13590, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 6538, *Yersinia pseudotuberculosis* ATCC 911, *Streptococcus pyogenes* ATCC 12344, *Candida tropicalis* NRRLL YB-366, *Clavibacter michiganensis* subsp. *michiganensis*, *Xanthomonas* spp. were used.

In both tests, the microorganism concentration was prepared at 0.5 McFarland turbidity. At the end of 48 hours of incubation at 37°C, the zones formed around the discs were measured in the disc diffusion test, while the concentration at which there was no microbial growth in the microdilution test was determined as the MIC value.

2.5. Determination of DNA Protective Activity

DNA protective activity of the extracts was performed by making minor changes to the Çelik Altunoğlu et al. (2022). Firstly, pUC19 plasmid DNA was damaged by using the Fenton solution. Extracts at different doses such as 1, 5, 10, 20 and 40 mg per mL were used. The presence or absence of activity was also observed by electrophoresis of DNA samples in 1% agarose gel.

2.6. Phenolic Compounds

HPLC (Shimadzu, Japan) was used to screen the presence of 20 different phenolic compounds commonly found in this family in the extract. The results of gallic acid, catechin, p-hydroxy benzoic acid, chlorogenic acid, caffeic acid, epicatechin, sirinic acid, vanillin, p-coumaric acid, ferulic acid, benzoic acid, rutin, hesperidin, trans-cinnamic acid, luteolin, kaempferol contents are given as µg/g extract.

2.7. Statistical Analyses

All trials were designed in triplicates. The results were calculated by the SPSS program (One-way ANOVA) (p<0.05 values mean significant).

3. RESULTS and DISCUSSION

TPC and TFC of EtOH and MeOH extracts of *Helichrysum artvinense* were determined by spectrophotometric methods (Table 1). In accordance with the results obtained, EtOH extracts were richer in TPC (62.74 mg GAE/g extract) and TFC (80.75 mg QUE/g extract) than MeOH extracts.

In a study on 15 diverse *Helichrysum* species, including *H. artvinense* species were investigated, revealing that the total phenolic content (TPC) of the methanol (MeOH) extracts was lowest in *H. peshmenianum* (66.75 mg GAE/g extract) and highest in *H. noeanum*(160.63 mg GAE/g extract). Also, the TPC of the MeOH extract obtained from the aerial parts of *H. artvinense* determined as 83.98 mg GAE/g extract (Albayrak *et al.*, 2010). In other study, the TPC and TFC of the ethanol extracts of flower of *H. plicatum* and *H. chionophilum* collected from Gümüşhane were found 535.3 and 424.6 mg GAE/g extract, and 50.9 and 73.6 mg QE/g extract, respectively. In ethanol extracts of the same plants, the TPC was identified as 536.9 and 537.7 mg GAE/g extract and the TFC was 55.4 and 44.6 mg QE/g extract, respectively (Acet *et al.*, 2020). In another study, Gouveia-Figueira (2014) investigated four different *Helichrysum* species and the TPC -TFC were found to be 0.04-121.4 mg GAE/g extract and 0.02-8.2 mg RUE/g extract, respectively. On the other hand, Ebrahimzadeh and Tavassoli (2015) reported the TPC (22.7 mg GAE/g extract) and TFC (9.6 mg QE/g extract) of MeOH extract of *H. pseudoplicatum.* In a study they conducted with *H. pseudoplicatum,* Ebrahimzadeh and Tavassoli (2015) reported that the TPC of the MeOH extract of the plant was 22.7 mg GAE/g extract and the TFC was 9.6 mg QE/g extract. When compared with the literature, it is seen that the TPC and TFC in the present research is lower or over than some studies. However, these differences are thought to be due to the differences in the solvents used. There is limited studies used ethanol as a solvent in the same species. In addition, different secondary metabolite contents in the same plant species may be related to various factors such as climate, collection period, altitude and soil structure.

Table 1. TFC and TPC of plant extracts.

Extracts	TPC (mg GAE/g extract)	TFC (mg QUE/g extract)
EtOH	62.74±0.7[a]	80.75±0.1[a]
MeOH	51.15±0.6[b]	65.03±0.6[b]

Values expressed are the mean ± *SD* of three different measurements. Data shown with different letters in the same column indicate statistically significant differences between the extracts (p<0.05).

Antioxidants are structures that prevent or delay oxidation caused by free radicals (Popoola *et al.*, 2015; Aguilera *et al.*, 2016). ABTS and DPPH methods are frequently used to determine the potential of plants to scavenge free radicals. The results of these experiments are shown in Table 2. As seen in this table, it was detected that the ABTS activity of the ethanol extract (508.036 mgTE/g extract) was higher than that of the methanol extract (450.350 mgTE/g extract). ABTS IC_{50} values also showed parallelism with the equivalence results. While the IC_{50} value of ethanol extract was 0.0284 mg/mL, methanol was determined as 0.0321 mg/mL. On the other hand, in DPPH analysis found that ethanol extract (66.533 mgTE/g extract) was higher than methanol extract (16.024 mgTE/g extract). DPPH IC_{50} values were also in parallel with the equivalence results. While the IC_{50} value of ethanol extract was 0.133 mg/mL, methanol was determined as 0.225 mg/mL. In a study on similar species, ABTS activity in ethanol extracts of flower of *H. plicatum* and *H. chionophilum* collected from Gümüşhane was determined as 89.5 and 66.2 mgTE/g extract, respectively, and IC_{50} values were 0.446 and 0.051 mg/mL. ABTS values in the EtOH extracts of the same plants were found to be 52.6 and 355.3

mgTE/g extract, respectively, and IC_{50} values were 0.574 and 0.348 mg/mL (Acet *et al.*, 2020). Data from the current study indicate that ABTS scavenging activity of extracts is stronger than in the literature. If the DPPH activities in Acet *et al.*, (2020) are compared, it can be seen that the DPPH activity in the EtOH extracts of flower of *H. plicatum* and *H. chionophilum* is 7.9 and 17.7 mgTE/g extract, respectively, and the IC_{50} values are 0.234 and 0.87 mg/mL. DPPH values in the stem ethanol extract of the same plants were found to be 5.8 and 22.0 mgTE/g extract, respectively, and IC_{50} values were 0.381 and 0.679 mg/mL (Acet *et al.*, 2020). The data obtained from the current study show that the DPPH scavenging activity, especially of the EtOH extract, is stronger than the literature. Similarly, the antioxidant capacity of *H. plicatum, H. chionophilum* and *H. arenarium* methanol extracts taken from the Sivas region was reported using the DPPH assay (IC_{50} = 0.0405 and 0.047.6 mg/mL, respectively) (Tepe *et al.*, 2005). In other study, the DPPH IC_{50} value of the MeOH extract obtained using the aerial parts of *H.artvinense* was stated as 0.021 mg/mL (Albayrak *et al.*, 2010). Differences in these values may be due to the location where the plant was taken or the experimental conditions.

Table 2. Antioxidant properties of plant extracts.

Extracts	ABTS (mgTE/g extract)	ABTS IC_{50} (mg/mL)	DPPH (mgTE/g extract)	DPPH IC_{50} (mg/mL)
EtOH	508.036±1.5^{a}	0.0284±0.05^{b}	66.533±0.8^{a}	0.133±0.003^{b}
MeOH	450.350±2.25^{b}	0.0321±0.03^{c}	16.024±0.05^{b}	0.225±0.005^{c}
Trolox	-	0.009539±0.005^{a}	-	0.00528±0.025^{a}

Values expressed are the mean ± *SD* of three different measurements. Data shown with different letters in the same column indicate statistically significant differences between the extracts (p<0.05).

Type-II diabetes, a metabolic disease related to blood sugar levels, is a major health problem (Pari ve Srinivasan, 2010). Around 250 million individuals are projected to be affected by this disease by 2030 (Hwang *et al.*, 2012). From this point of view, the necessity of proper management of this disorder becomes evident. In this regard, control of carbohydrate hydrolyzing enzymes (amylase and glucosidase) is one of the important strategies to cope with the disease (Hu *et al.*, 2013). For this purpose, various synthetic enzyme inhibitors such as acarbose have been produced (Chiasson *et al.*, 2002). However, since these compounds cause significant side effects such as tissue and organ damage in humans, the discovery and use of natural inhibitors have become popular in recent years (Lasano *et al.*, 2019). In addition, tyrosinase inhibitors are used in the treatment of skin disorders such as hyperpigmentation and plants constitute an important source in this regard (Bozkır *et al.*, 2022). In this study, some enzyme inhibition activities of the aerial part of EtOH and MeOH extracts of *Helichrysum artvinense* were investigated. The results are shown in Table 3.

Table 3. Enzyme inhibitory activity of plant extracts.

Extracts	α-amylase inh. (mmolACAE/g extract)	α-glucosidase inh. (mmolACAE/g extract)	Tyrosinase inh. (mgKAE/g extract)
EtOH	533.27±5.5^{a}	24.92±1.5^{ab}	86.05±3.5^{a}
MeOH	489.02±3.5^{b}	25.33±1.25^{a}	22.45±1.05^{b}

Values expressed are the mean ± *SD* of three different measurements. Data shown with different letters in the same column indicate statistically significant differences between the extracts (p<0.05).

There is some research in the literature on the inhibition activities related to diabetes of the Asteraceae family (Spinola ve Castilho, 2017); additionally, studies on the enzyme inhibition activities of *Helichrysum* species were also examined. Accordingly, the α-amylase and α-glucosidase inhibition activity of the ethanol extract of the *Helichrysum stoechas* subsp. *barrelieri*, which was extracted similarly, was found to be 0.59 and 1.63 mmol ACAE/g extract, respectively. Additionally, in this study, tyrosinase enzyme inhibition activity was found to be 183.32 mg KAE/g extract (Zengin *et al.*, 2020). Accordingly, when the data obtained from the current study is compared with the literature, the enzyme inhibition results of the ethanol extract

of the *Helichrysum artvinense*, except tyrosinase, are quite high. In a different study on *H. chionophilum* and *H. plicatum*, the α-amylase enzyme inhibition activity of ethanol extracts of flower was found to be 156.53 and 105.35 mmolACAE/g extract, respectively. On the other hand, the α-glucosidase enzyme inhibition activity of ethanol extracts of stem was determined as 193.36 and 105.12 mmol ACAE/g extract, respectively. Similar evaluations are valid for α-glucosidase inhibition activities. According to the data obtained from the present study, it is seen that ethanol extract is more effective in inhibiting diabetes-related enzymes. As a result, it can be said that the findings are compatible with the literature.

The antimicrobial activity of the extracts was determined by disc diffusion and microdilution tests, and the results are shown in Table 4 (a and b). It was found that the extracts showed antimicrobial activity against all tested microorganisms except *B. cereus*. In addition, it was observed that both plant extracts showed strong antimicrobial activity against *E. faecalis* with MIC value of 16 µg/mL. In a previous study, it was stated that *H. chionophilum* extracts had no activity against *K. pneumoniae*, while *H. plicatum* had an antimicrobial effect against this organism (MIC value 512 µg/mL) (Acet et al., 2020).In the current research, EtOH and MeOH extracts were found to have a similar antimicrobial effect against *K. pneumoniae.* In this context, the results obtained are consistent with the literature. In addition, in a study conducted with *H. artvinense*, generally similar (disk diffusion) activities were observed (Albayrak *et al.*, 2010). MIC values were reported for the first time in this study.

The phenolic components of the extracts were screened using HPLC (Figures 1 and Figure 2) and the findings are given in Table 5. Since the ethanol extract of the plant was more effective than methanol extracts, phenolic content analysis of the ethanol extract was performed. Accordingly, the major phenolic components of the ethanol extract were found to be Epicatechin (2190.5 µg/g extract), Chlorogenic acid (1738.6 µg/g extract) and Luteonin (910.8 µg/g extract). While routine and hesperidin were not found in this extract, other standard phenolics were detected at minor levels. As per the literature, chlorogenic acid and its derivatives are the predominant phenolic acids identified in the plant samples under analysis (Nicolle *et al.*, 2004; Mattila and Hellström, 2007). In the present study, these compounds were also detected in substantial quantities in the tested plant. In this regard, the results taken from the study are compatible with the other scientific studies and it is supposed that the high biological activities exhibited by the plant is due to the phenolic compounds they contain.

The DNA protective activity of the extracts was evaluated by damaging the pUC19 plasmid using Fenton's solution (Figure 3). Different concentrations of extracts were used in the study and no activity was noticed in the MeOH extract. However, DNA protective activity was partially seen at a 20 mg/mL concentration of the EtOH extract. Some studies show that plants have protective activities against DNA damage at various doses (Giri *et al.*, 2017; Bozkır *et al.*, 2022). It has been suggested that phenolic components are related to DNA protective activity (Petersen, 2013). It is possible that some phenolic compounds, also detected in the current study, are responsible for this activity.

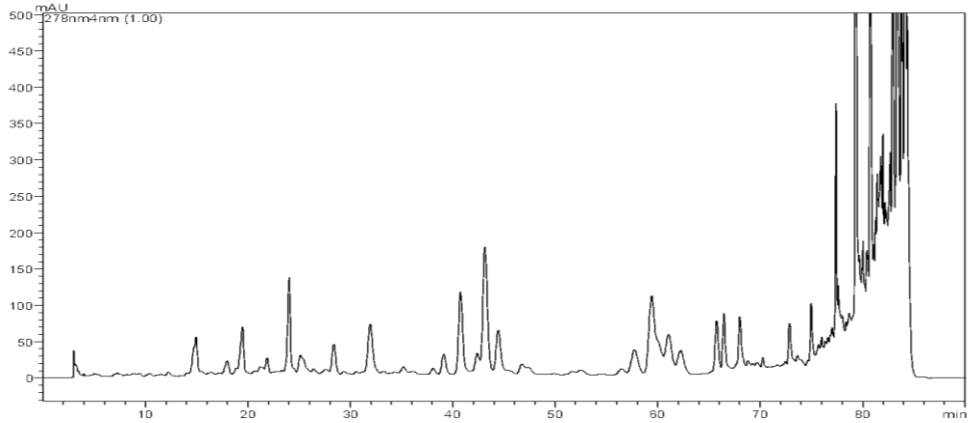

Figure 1. Chromatogram of EtOH extract.

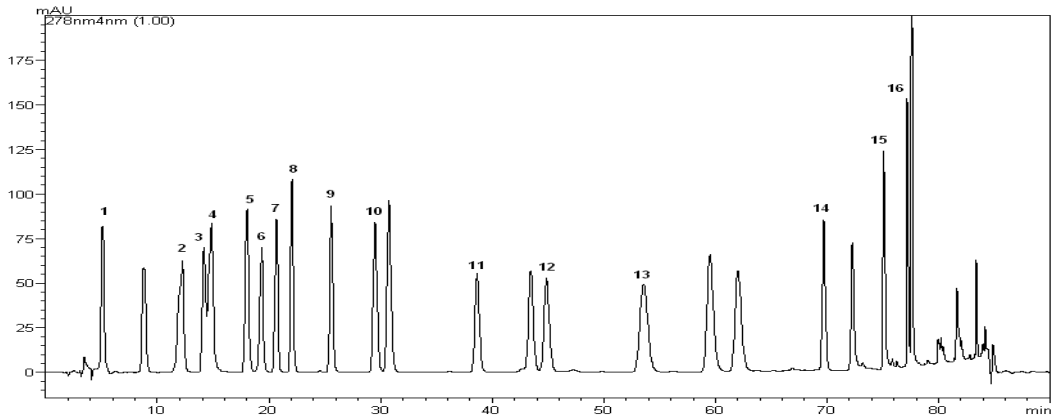

Figure 2. Standard chromatogram**.** Standard chromatogram, 1:gallic acid 2:catechin 3:p-hydroxy benzoic acid 4:chlorogenic acid 5:caffeic acid 6:epicatechin 7:syringic acid 8:vanillin 9:p-coumaric acid 10:ferulic acid 11:benzoic acid 12:rutin 13:hesperidin 14:cinnamic acid 15:luteolin 16:campferol.

Table 4. Antimicrobial activity of *H. artvinense.*
a. Antimicrobial activity (MIC) findings (µg/mL)

	Extracts	
Microorganisms	Ethanol	Methanol
B. cereus	ND	ND
E. coli	64	64
S. thyphi	64	64
K. pneumoniae	512	512
P. aeruginosa	512	512
S. aureus	16	32
MRSA	32	32
C. tropicalis	512	256
B. subtilis	128	128
E. faecium	512	512
E. faecalis	16	16
E. hirae	32	32
Y. pseudotuberculosis	512	128
X. spp.	16	128
C. michiganensis sups. m.	512	256
S. pyogenes	1000	1000

b. Antimicrobial activity (Disc diffusion) findings of the extracts (mm)-800 µg/mL per disc

	Extracts	
Microorganisms	Ethanol	Methanol
B. cereus	13	12
E. coli	-	-
S. thyphi	13	12
K. pneumoniae	13	12
P. aeruginosa	-	-
S. aureus	-	-
MRSA	12	11
C. tropicalis	14	12
B. subtilis	-	-
E. faecium	12	11
E. faecalis	11	12
E. hirae	12	-
Y. pseudotuberculosis	13	12
X. spp.	14	15
C. michiganensis sups. m.	-	-
S. pyogenes	20	17

Table 5. Phenolic compounds of ethanol extract.

No	Phenolic components	Amount (µg/g extract)
		EtOH
1	Gallic acid	34.7±0.001
2	Catechin	265.5±0.5
3	Chlorogenic acid	1738.6±0.4
4	Epicatechin	2190.5±0.05
5	Caffeic acid	224.4±0.02
6	Syringic acid	80.2±0.01
7	*p*-Coumaric acid	84.4±0.25
8	Ferulic acid	71.9±0.1
9	Rutin	*
10	Hesperidin	*
11	*t*-Cinnamic acid	28.0±0.02
12	Luteolin	910.8 ±0.01
13	Kaempferol	385.4±0.006
14	*p*-hydroxy benzoic acid	56.1±0.06
15	Vanilin	150.6 ±0.02
16	Benzoic acid	982.0±0.5

Values expressed are the mean ± SD of three different measurements. *Not detected

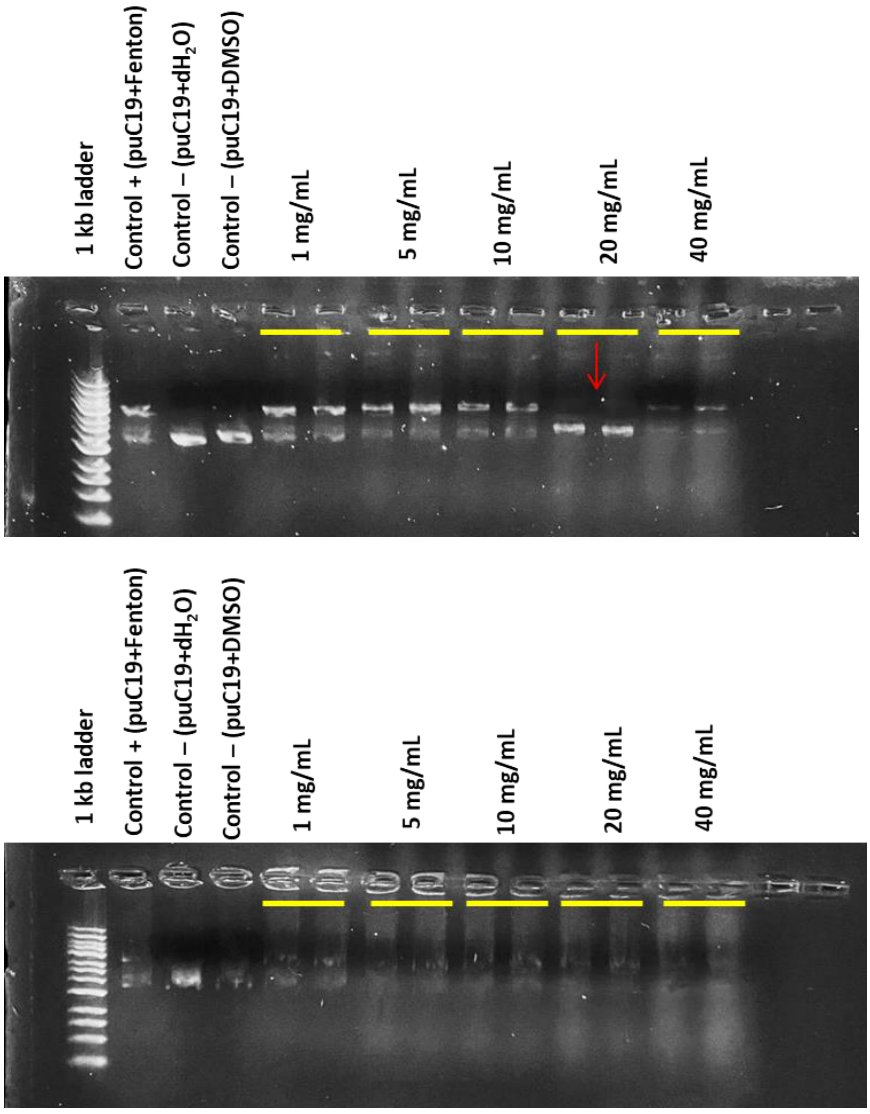

Figure 3. DNA protective activity **a-** ethanol extract, **b-** methanol extract.

4. CONCLUSION

In the study, some biological activities of endemic *Helichrysum artvinense* collected from Artvin province were investigated. With this study, the DNA protective activity of the plant was revealed for the first time. In the study where EtOH and MeOH extracts of the aerial parts of plant were used, it was observed that EtOH extracts were more prominent in terms of the screened properties. Therefore, phenolic components of EtOH extract were revealed by HPLC analysis. Accordingly, epicatechin, chlorogenic acid and luteolin were determined as basic phenolics. At the same time, extracts were demonstrated a high antimicrobial activity against some organisms with a MIC value of 16 µg/mL. It is thought that the high biological activities determined are due to the major and/or minor components of the plant. As a result, the plant in question has the potential to be used in both food and pharmacological fields. However, it seems useful to support the detected biological activity with in vivo studies.

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Declaration of Conflicting Interests and Ethics

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

Authorship Contribution Statement

Tuba Acet provided financial support for this study with project and contributed to the experimental studies, data analysis, preparation of manuscript and proofreading; **Kadriye Özcan** participated in antimicrobial experiments and the writing and proofreading; **Nurşen Aksu Kalmuk** provided and determined the plant material used. She also contributed to the critical reading of the article.

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