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In Vitro Antioxidant Assessment of Methanol and Water Extracts of *Anthemis kotschyana* Boiss. var. *kotschyana*

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ABSTRACT

The genus *Anthemis*, with approximately 130 species globally, is significant within the Asteraceae family and is widely used in traditional medicine. Studies on the biological activity of *Anthemis kotschyana*, an endemic species found in Turkey, are limited. This study investigated the chemical constituents and antioxidant activity of *Anthemis kotschyana* using two different extracts, methanol and water. The major components of the plant were determined by GC–MS. According to the data obtained, the major component was found to be "2-propenoic acid, a tridecyl ester" in the water extract and "cyclododecane" in the methanol extract. When the DPPH and ABTS radical scavenging activities were analysed, the methanol extract was found to be more active than the water extract in the DPPH experiment, while the opposite was observed in the ABTS experiment. The TPC was greater than the TFC based on the acquired results. The TPC of the water extract was 83.7 ± 15.6 mg GAE/g, the TPC of the MeOH extract was 170.7 ± 17.4 mg GAE/g, and the TFC of the water extract was 24.3 ± 7.2 QE/g, while the TFC of the MeOH extract was 65.6 ± 5.3 QE/g. For both substances, the amount of methanol extract was greater than that of the water extract. In conclusion, further studies on this species, known for its antioxidant activity and potential as a source of phenolic compounds, could support its use as a therapeutic agent in the future.

Keywords: *Anthemis kotschyana*, Antioxidant, DPPH, ABTS, Medicinal plant.

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Introduction

The Asteraceae family is widely distributed throughout the planet, with the exception of Antarctica. It ranks first among flowering plants in terms of diversity, having more than 20,000 species and more than 1,100 genera. This family contains both annual and perennial species. The capitulum, a flower structure, is the most distinctive feature of this family [1]. The majority of plant life is found in glades, open meadowlands, and mountain vegetation. In moist tropical forests, they are less common [2]. Within the Asteraceae family, the genus *Anthemis* includes 51 species and 81 taxa that are registered in the Turkish flora [3,4]. A total of 36.3% of these species are endemic [5].

In traditional medicine, many species of this genus—especially flower organs—are widely used as sedatives, anti-inflammatory agents, gastrointestinal spasmolytics, and digestive aids. They are also applied externally to reduce pain and irritation as well as to cure wounds and ulcers [6]. It can be proposed that this therapeutic effect of *Anthemis* is related to the secondary metabolites it produces.

Sesquiterpene lactones (tatridin A, nobilin, 1-epi-tatridin B), phenolics (caffeoylquinic acid derivatives, 3-hydroxybenzoic acids, gallic and ferulic acids), and flavonoids (salvigenin, quercetin, naringenin, apigenin, pectolinarigenin, eupatilin and rutin) are the primary secondary metabolites of *Anthemis* according to phytochemical investigations [7-9]. This genus is also known to possess antibacterial, antispasmodic, antiproliferative, anticholinesterase and antioxidant properties [10-14].

Different effects on metabolic processes may emerge from variations in the kind, density, and interaction of antioxidant chemicals [15]. Exogenous antioxidants may help the healing process when a pathological outcome arises because antioxidants functioning in the defense mechanism of cells might not be enough to stifle free radicals. To improve metabolic defense in such situations, antioxidant-rich medicinal plants can be employed [16-18].

This study investigated the chemical composition and antioxidant properties of water and methanol (MeOH) extracts from *Anthemis kotschyana* plants that were grown in Turkey. In addition, this study is the first in the literature to involve the use of methanol extracts from plants. It is thought that the acquired data will benefit phytotherapeutic research.

Materials and Methods

Plant Material and Extract Preparation

Anthemis kotschyana plants were collected from Sivas Province in May 2019 in a meadow area at an average altitude of 1100 m. The general distribution of the plants was reported to be *Pinus* and *Quercus* forests, calcareous

rocks and slopes at an average altitude of 400-2745 m [3]. After being harvested, the plants were shade-dried and sized appropriately for extraction using a lab-style grinder. The Biology Department of Sivas Cumhuriyet University identified the plant.

The plants were ground in a blender (blue house) after being dried in direct sunlight until they reached a constant weight. For 24 hours, with sporadic shaking, 10 g of dried plant material was soaked in 50 mL of MeOH (Sigma) and water. Next, No. 1 Whatman filter paper was used to filter the extract. A rotary evaporator operating at 40 °C and lower pressure was used three times to concentrate the filtrate.

The components and relative percentages of the extracts that were filter-dried in a rotary evaporator were ascertained by GC–MS [19]. The carrier gas, helium gas, was used at a steady flow rate of 1.5 mL/minute. For two minutes following the run, the splitless mode injection volume of 1 µl was set at 300 °C and designed to be 5 per minute between 80 and 300. The total running time was one hour [20]. Several libraries were used to investigate the chemical composition of the extract obtained from the dried leaves (W9N11. L, NIST05a. L and wiley7n.l).

In vitro Antioxidant Activity

2,2-Diphenyl-1picrylhydrazyl (DPPH) Assay

The antioxidant activity of the extracts was quantified using the DPPH radical scavenging assay, as previously described [21]. The plant extracts (water and MeOH) were dissolved in DMSO, and 180 µL of DPPH solution (made in MeOH at 40 µg/mL) was combined with 20 µl of test solutions at different concentrations in a 96-well plate. After 15 minutes in the dark, the absorbance levels on the plates were measured with a microplate reader at 540 nm. The standard and control were butylated hydroxytoluene (BHT) and DMSO, respectively. The experiments were conducted in triplicate, and the standard deviation (SD) was computed to assess the outcomes.

2,2′-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay

With a few minor adjustments, the ABTS cation radical decolorization activity of the extracts was assessed using the methodology of Re et al. [22]. The preparation of the test samples followed the DPPH protocol. 15 mL of 7 mM ABTS and 264 µL of 140 mM potassium persulfate solution were allowed to react in the dark at room temperature for 16 hours before the experiment to create a stock solution of ABTS + radical. The stock solution was diluted with MeOH to an absorbance of 0.70±0.02 at 734 nm, which was the starting point for the preparation of the ABTS working solution. Fifty microliters of the sample solution and 100 µL of the ABTS working solution were combined on a 96-well plate. After ten minutes of standing at room temperature, the absorbance of the mixture at 734 nm was measured. The ABTS·+ scavenging activity was compared using BHT as the antioxidant standard. The concentrations of the extracts utilized in the antioxidant tests ranged from 0–1 mg/mL.

Total phenol content

The total amount of phenol in the extract was ascertained as a result of the reaction with Folin–Ciocalteu (F–C) reagent. Following the appropriate dilution of the extract with 10 µL of DMSO, it was mixed with 100 µL of distilled water and newly diluted F–C reagent (10-fold). Five minutes later, 100 µL of 7.5% Na_2CO_3 was added, and after sixty minutes, the absorbance at 650 nm was measured. With the proper references (gallic acid in DMSO) and blanks (DMSO), parallel runs were carried out. The total amount of phenol was calculated using the sample absorbance values [21].

Total flavonoid content

A colorimetric method with aluminum chloride was used to determine the total flavonoid content of the extracts. For calibration, serial dilutions of 0.0625 mg/mL, 0.125 mg/mL, 0.25 mg/mL, 0.5 mg/mL, and 1 mg/mL were made using a 10 mg/mL quercetin stock solution. The ethanol-based test solution (150 µL, 0.3 mg/mL) was mixed with 2% $AlCl_3$ in a 96-well plate. The absorbance at 435 nm was measured following a 15-minute incubation period at room temperature. The total flavonoid concentration was calculated by multiplying the extract's dry weight by the mg equivalent of quercetin [23].

Results and Discussion

Chemical Composition

The chemical contents of the water and MeOH extracts of *Anthemis kotschyana* were determined by GC–MS (Table 1). The chemical contents of the water and MeOH extracts of plants showed significant differences in terms of a variety of components. According to the data obtained, 2-propenoic acid, a tridecyl ester, was found to constitute 24.07% of the major component of the water extract. The content of ethyl methyl ethylphosphonate was 17.17%. When we evaluated the data obtained from the MeOH extract, "cyclododecane", with 16.72% as the major component, was found. Octadecane was the second major component at 9.15%.

Table 1. Chemical components of the water and methanol extracts of *A. kotschyana*

R.T.	Components	Area (%)	
		Water	MeOH
15.034	o-Hydroxyanisole	4.79	
15.063	Guasol		1.21
22.616	4-vinyl-2-methoxy-phenol		1.29
28.258	Tridecanol	11.16	
28.281	Dodecanol		5.80
29.374	Phenol, 2,4-bis(1,1-dimethylethyl)	9.09	3.25
32.166	Megastigmatrienone		1.50
33.041	Ethyl methyl ethylphosphonate	17.17	
33.453	2-Propenoic acid, tridecyl ester	24.07	
33.476	Cyclododecane		16.72
35.164	(-)-Loliolide		3.34
36.354	Hexahydropseudoionone		1.56
37.722	Hexadecanoic acid, methyl ester	4.88	6.64
38.420	Palmitinic acid		1.91
39.193	1-Hexadecanol	1.60	
40.463	Methyl linoleate		7.79
40.537	Oleic acid, methyl ester	1.27	
40.549	Methyl 7-octadecenoate		6.10
40.915	Methyl stearate	2.72	1.81
44.388	Oleic acid amide	2.54	
46.059	Octadecane		9.15
Total		79.29	68.07

This study represents the first investigation of the MeOH extract of *Anthemis kotschyana*. To date, studies utilizing different extracts of this plant are very limited. In 2020, a study was conducted with water and ethanol extracts of this plant, which revealed the major components to be hamnetin and quinic acid [12].

Antioxidant Activity

When the antioxidant activity of the *Anthemis kotschyana* extracts was assessed, the two extracts displayed varying levels of antioxidant activity in different *in vitro* systems. The greater the ability of the extracts to scavenge radicals was, the lower their IC_{50} value was. Both extracts displayed comparable levels of radical scavenging activity in DPPH and ABTS. The MeOH extract outperformed the water extract in the DPPH experiment (IC_{50} value: 31.04 µg/mL for water; IC_{50} value: 28.44 µg/mL for MeOH). Conversely, in the ABTS experiment, the water extract outperformed the MeOH extract (IC_{50} values: 108.9 µg/mL for MeOH and 103.8 µg/mL for water) (Figure 1). Nevertheless, in the water and MeOH extracts for DPPH and ABTS, the radical scavenging activity was less than that of the reference substance BHT (IC_{50} value: 7,7 µg/mL).

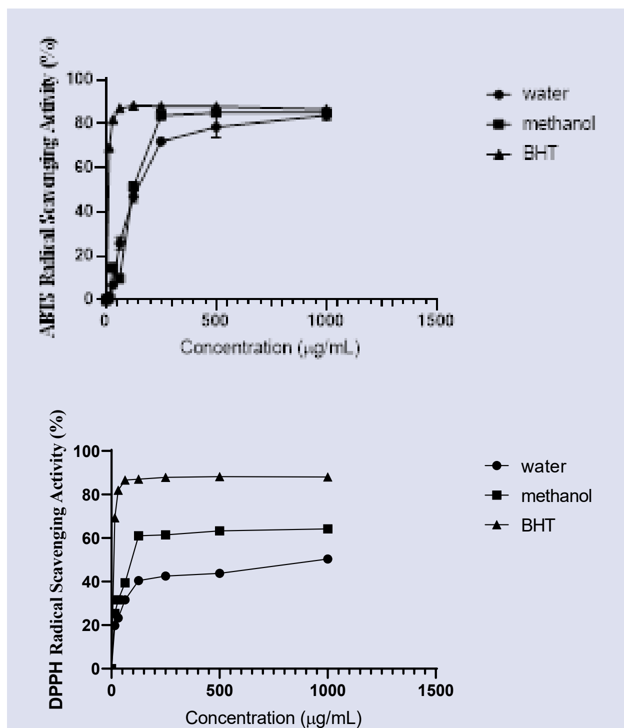

Figure 1. ABTS and DPPH radical scavenging activity of *A. kotschyana* extracts and reference BHT.

Bursal et al. (2020) reported that the DPPH radical scavenging activity of water and ethanol extracts of *A. kotschyana* was lower than that of the reference substance BHT. This result is consistent with the findings of the present study. Additionally, the water extract exhibited radical scavenging activity comparable to that of the ethanol extract, although the latter was more effective [12]. The data obtained from both studies suggest that the presence of bioactive compounds in *A. kotschyana* may play a role in its antioxidant activity.

Total Phenol and Flavonoid Content (TPC and TFC)

Phenolic compounds are important because of their antioxidant activity. The TPC and TFC of both the water and MeOH extracts of *A. kotschyana* were evaluated. The TPC was greater than the TFC, and the TFC of the MEOH extract was greater than that of the water extract (83.7 ± 15.6 mg GAE/g for the TPC of the water extract and 170.7 ± 17.4 mg GAE/g for the TPC of the MeOH extract; 24.3 ± 7.2 QE/g for the TFC of the water extract and 65.6 ± 5.3 QE/g for the TFC of the MeOH extract) (Figure 2). The observation that phenolics were present in greater quantities in the MeOH extract than in the water extract indicates that the choice of solvent has an impact on the ability to identify the phenolic compounds present in the plant.

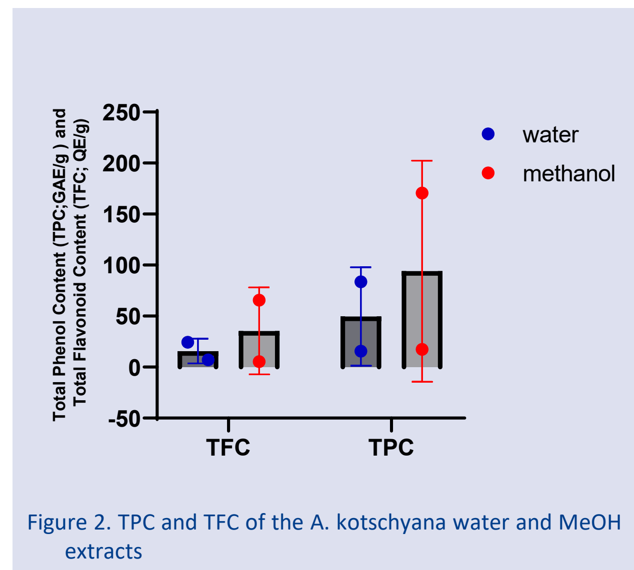

Figure 2. TPC and TFC of the A. kotschyana water and MeOH extracts

Bursal et al. (2020) employed LC–MS/MS to analyse the phenolic and flavonoid compound contents of *A. kotschyana* [12]. The results indicated that both the TFC and TPC of *A. kotschyana* were high. Two significant compounds out of twenty-seven standard compounds for *A. kotschyana* were found by LC MS/MS analysis to be quinic acid and rhamnetin. Furthermore, the *in vitro* antioxidant data indicated that *A. kotschyana* extracts possess effective antioxidant potential. However, a comparison of the water and ethanol extracts was not conducted.

Due to the limited number of studies on *A. kotschyana*, the data obtained were also compared with those of different species of this genus. In a 2020 study, the total phenolic and flavonoid contents of the MeOH extract of *Anthemis tinctoria* var. *tinctoria* were examined by LC–MS/MS, and 30 different phenolic compounds were detected [7]. In a separate study, the antioxidant activities of MeOH extracts of *A. cretica* and *A. tinctoria* were evaluated using the DPPH assay. Furthermore, in the same

study, the total phenol and flavonoid contents of two species of the genus *Anthemis* were investigated with three different extracts (EA, MeOH and aqueous). The total phenol and flavonoid contents of the MeOH extract of *A. tinctoria* were greater than those of the MeOH extract of *A. cretica* [24]. A further study on *A. tinctoria* indicated that the ethanol extract exhibited high antioxidant activity, as evidenced by its ability to scavenge DPPH and ABTS radicals [13]. In a separate study investigating the antioxidant activity of various extracts of *Anthemis aciphylla* Boiss. var. *aciphylla*, it was reported that the methanol extract exhibited high DPPH, ABTS, superoxide radical, and nitric oxide scavenging activities [14]. This corroborates the notion that the antioxidant activity of different species belonging to the genus *Anthemis* is considerable.

Conclusion

The findings of this study indicate that the MeOH and water extracts of *A. kotschyana* demonstrate antioxidant properties, as evidenced by their ability to scavenge free radicals (DPPH, ABTS) and to inhibit the oxidation of phenolic and flavonoid compounds. According to the GC–MS analysis, if the major components 2-propenoic acid, tridecyl ester (water) and cyclododecane (MeOH) are obtained by fractionation, this will provide a different direction for studies on the antioxidant activity of this species.

In conclusion, the extension of the biological properties of this species, which has antioxidant activity and is a good potential source for phenolic compounds, with more comprehensive studies may allow the use of this plant as a therapeutic agent in the future.

Conflicts of interest

No conflicts of interest have been declared by the authors.

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