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# Phytochemicals and Antioxidant Activities in Methanol Extracts of Endemic *Haplophyllum* Species from Türkiye

Cennet YAMAN *1

## Abstract

The aim of the current study is to determine the total flavonoids, phenolics and antioxidant activities of methanol extracts from aerial parts of four endemic *Haplophyllum* species to Türkiye (*H. myrtifolium, H. vulcanicum, H. pumiliforme*, and *H. sahinii*). There are two populations collected from different regions belonging to *H. myrtifolium* and *H. pumiliforme*. Antioxidant activities were measured by radical scavenging activity such as the 2, 2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH), and compared with synthetic standards such as trolox, ascorbic acid and butylated hydroxytoluene (BHT). The highest total bioactive contents were recorded as *H. sahinii* for total phenolic content (66.4 mg GAE/g extract) and *H. myrtifolium* for total flavonoid content (40.8 mg QE/g extract), but *H. vulcanicum* displayed the lowest amount for both contents (38.0 mg GAE/g extract and 34.5 mg QE/g extract, respectively). Among the species, *H. myrtifolium* exhibited the strongest DPPH and ABTS radical scavenging activity followed by *H. pumiliforme*, *H. sahinii* and *H. vulcanicum*. All *Haplophyllum* species showed higher antioxidant activity for these two radical scavenging activities than trolox and BHT. The phytochemicals and antioxidant activities in methanol extracts from these endemic *Haplophyllum* species is reported for the first time. The diversity of the findings is to be discussed as likely consequence of the different species and regions.

**Keywords**: *Haplophyllum* L, phenolic, flavonoids, DPPH, ABTS

## 1. INTRODUCTION

*Haplophyllum*, belonging to Rutaceae family, herbaceous perennial and fragrant plants is a genus containing approximately 68 species, and has the most species diversity in the flora of Türkiye and Iran in terms of its spread over the world [1]. Türkiye is an important gene center for the Haplophyllum genus which is represented in the flora of Türkiye by 17 taxa belonging to 14 species with 52% endemism [2]. Recently, two new species (*Haplophyllum sahinii and H. ermenekense*) was described by Tugay and Ulukuş, Ulukuş and Tugay [3, 4], Türkiye has 18 *Haplophyllum* taxa, 11 (58%) of which are endemic. This genus taxa are foetid perennial herbs, which grows mainly on rocky hills, steppes, slopes, rocky place on limestone, especially near pine forests, or sandy soils [2].

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Members of the Rutaceae family are of great economic importance, including wood, food, cosmetic and medicinal uses [5]. *Haplophyllum* species of this family are traditionally used actively in the treatment of different diseases in many countries. It is used in the treatment of malaria, rheumatoid arthritis and gynecological diseases in Saudi Arabia [6]. The herb part is used as an antispasmodic in the treatment of allergic rhinitis and gynecological diseases, asthma and respiratory distress in Sudan [7], while its leaves are used for skin infections in Oman [8]. Infusion samples of the herbal part are used to treat gynecological problems and digestive problems such as rheumatoid arthritis as well as constipation and diarrhea [9]. Moreover, *Haplophyllum* species, analyzed in previous studies, have been reported to exhibit incredible levels of biological activities including antimicrobial [10, 11], antioxidant [12, 13], anti-inflammatory [14], and especieally anti-cancer [15-17]. The potential of these activities is based on phytochemicals in the plant. Many scientists reported that various *Haplophyllum* species contained important characteristic classes of phytochemical such as phenolics, flavonoids, flavonols, coumarins, alkaloids and important compounds of lignans [4, 15, 18].

Many compound classes or compounds with high antioxidant properties are preferred for preservative and additive purposes in industries such as food, medicine, pharmacology and cosmetics. These compounds are mostly synthetic products due to their cheapness and quick availability. As a result of the researches, it was revealed that synthetic compounds have toxic and carcinogenic effects, and instead, natural products/preparations with high antioxidant activity have become popular [19, 20]. Natural products of plant origin provide alternatives to synthetic antioxidants. Therefore, natural antioxidant products have been developed from aromatic plants, spices, and fruit powder and are still being developed [21, 22].

The biosynthesis of these phytochemicals in plant and therefore their biological activities are affected by external factors such as environmental factors (soil, light intensity and climatic conditions) [23], as well as by internal factors such as the biotype and chemo-type of the plant [24], physiological and genetic aspects [25]. Therefore, it is an important first step to identify the preparation/extract or a specific compound that can be used for commercial purposes from nature.

The high pharmaceutical effects of *Haplophyllum* species suggest that there may be species with high antioxidant activity among the species. In this context, this study aims to investigate the antioxidant activities of endemic *Haplophyllum* species to Türkiye, of which there are very few studies, and to compare them with synthetic antioxidant standards. The results of this study are important in terms of the use of *Haplophyllum* species as a potential source of natural antioxidants in food and pharmaceutical products.

## 2. MATERIALS AND METHODS

### 2.1. Material

The erial parts of *Haplophyllum* species (*H. myrtifolium, H. vulcanicum, H.pumiliform*e and *H. sahinii*) respresenting a total of 40 shoots were collected according to completely randomized design at full flowering period (Figure 1). The species were identified by Prof. Dr. Osman Tugay, Faculty of Farmacy, Department of Pharmaceutical Botany, Selçuk University, Konya, Türkiye. The locations belonging to each taxon were recorded as following (Table 1).

### 2.2. Extraction

The dried and finely ground samples (about 4g) of the aerial parts of *Haplophyllum* species were extracted in methanol at 40°C for 24 h. The resulting solutions were filtered through whatman paper and solvent was separated with a rotary evaporator (Heidolph, laborota 4000), and extract yields were calculated as %. Then, extracts were dissolved in methanol. Each extraction process was repeated three times.

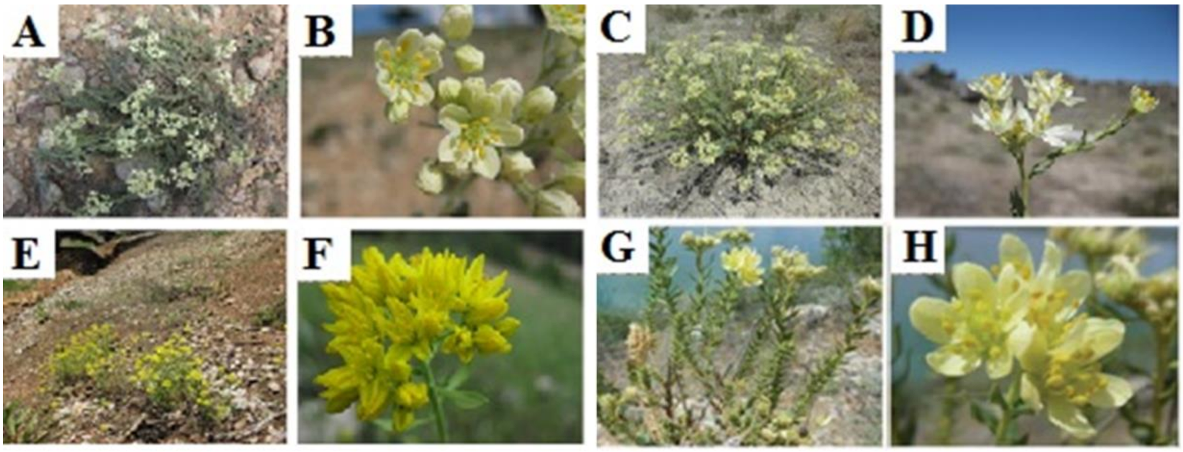


Figure 1 General view of habit and flowers of endemik *Haplophyllum* species (*H. myrtifolium* (A&B), *H. vulcanicum* (C&D), *H.pumiliforme* (E&F) and *H. sahinii* (G&H))

Table 1 Habitats of *H. myrtifolium, H. vulcanicum, H. pumiliforme* and *H. sahinii* from Türkiye

| Plant Name | Abbreviated Names | Hazard Category[1] | Altitude (m) | Collection Site | Collector Number |
|---|---|---|---|---|---|
| *Haplophyllum myrtifolium* Boiss. | HM1 | EN | 1070 | C4[2] Konya; Çumra, Apasaraycık Köyü, taşlı yerler | OT-9264-DU[3] |
| | HM2 | | 1090 | C4 Konya; Çumra, Apasaraycık Köyü | OT-7392-DU |
| *Haplophyllum vulcanicum* Boiss. & Heldr. | HV2 | VU | 1200 | C4 Karaman; Karadağ | OT-9614-DU |
| *Haplophyllum pumiliforme* Hub.-Mor. & Reese | HP1 | VU | 1450 | C3 Konya; Derebucak, Soğukoluk yolu | OT-7495-DU |
| | HP2 | | 1470 | C3 Konya; Derebucak | OT-7481-DU |
| *Haplophyllum sahinii* Tugay & Ulukuş | HS | EN | 1090 | C4 Konya; Çumra, Apasaraycık-Apa köyü, kayalık alan | OT-7410-DU |

## 2.3. Phytochemical Contents

### 2.3.1. Total phenolic

Total phenolic content in the methanol extracts of *Haplophyllum* species will be measure using the Folin–Ciocalteu reagent method as described by Yaman et al. [13]. The total phenolic contents of the samples were expressed as mg gallic acid equivalent (GAE) / g extract according to the equation obtained from the standard gallic acid graph. The experiment was done in triplicates with two replicates.

### 2.3.2. Total flavonoid

Total flavonoid content in the methanol extracts of *Haplophyllum* species will be determine using colorimetric method as described by Yaman et al. [13]. The total flavonoid contents will be calculate from the calibration curve and express as mg quercetin equivalent (QE) / g extract according to the equation obtained from the standard quercetin graph. The experiment will be done in triplicates with two replicates.

## 2.4. Radical Scavenging Activity

### 2.4.1. DPPH free radical scavenging activity

Measurement of DPPH (2, 2-diphenyl-1-picryl-hydrazyl) radical scavenging properties of the methanol extracts from *Haplophyllum* species will be carry out according to the method described by Yaman et al. [13] with some modifications. Trolox, Ascorbic acid (AA), butylated hydroxytoluene (BHT) will be used as positive control. The experiments will be doing in triplicates with two replicates. The results of the radical scavenging activity were calculated according to the following equation as % inhibition of the DPPH radical.

$$\%\ inhibisyon = \frac{(Abscontrol - \ Abssample)}{Abscontrol} \times 100$$

### 2.4.2. ABTS radical cation scavenging activity

Measurement of ABTS (2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging properties of the methanol extracts from *Haplophyllum* species will be carry out according to the method described by Yaman et al. [13] with some modifications. Trolox and AA will be used as positive control. The experiments will be doing in triplicates with two replicates. Results of radical scavenging activity were denoted as % inhibition of ABTS radical. The % inhibition of ABTS radical cation scavenging activity was calculated according to the following equation:

$$\%\ inhibisyon = \frac{(Abscontrol - \ Abssample)}{Abscontrol} \times 100$$

## 2.5. Statistical Analysis

The findings were statistically analyzed using one-way ANOVA in SPSS statistical program, and comparison of the means was evaluated by Duncan’s multiple range tests at a significance level of 0.05. Data were given as the mean ± standard deviation.

# 3. RESULTS AND DISCUSSIONS

## 3.1. Extract Yield

The methanol extract yields of *Haplophyllum* species used in the project were analyzed and are given in Table 2 as %.

Table 2 The methanol extract yields of endemic *Haplophyllum* species from Türkiye

| Species | Extract Yield (%) | Standard Error |
|---|---|---|
| HM1 | 13.98[a] | 1.54 |
| HM2 | 5.54[e] | 3.18 |
| HV1 | 9.92[b] | 2.70 |
| HP1 | 6.91[d] | 0.73 |
| HP2 | 8.28[c] | 0.51 |
| HS | 9.32[b] | 2.43 |

HM, *H. myrtifolium*; HV, *H. vulcanicum*; HP, *H.pumiliforme*; HS, *H. sahinii*

Generally, in the extraction of plants, methanol solvent provides higher extract yield than other solvents [26, 27]. Yaman et al. [13] investigated ethanol extracts of similar species in their study, and reported their extract yields lower than the methanol extract yield in the current study.

HM1 gives the best extraction yield an average of 13.9%, while HM2 collected from a second region had the lowest yield (5.54 % on average). HV and HS had a statistically similar extract yield with 9.92% and 9.32% whereas HP from two regions was lower with 8.28% - 6.9%.

### 3.2. Phytochemical contents

The methanol extracts of endemic *Haplophyllum* species collected from different localities were investigated for their phytochemical contents such as total phenolic and flavonoid contents (Table 3). Results was calculated from the calibration curve ($R^2$ = 0.999 for total phenolic content and $R^2$ = 0.9997 for total flavonoid content). Differences for the species were showed in findings of this study. The great distinction between the same species collected from different regions appears due to different environmental and climatic conditions [13].

Table 3 Total bioactive contents in methanol extracts of endemic *Haplophyllum* species from Türkiye

| Species | Total phenolic content (mg GAE/g extract) | Total flavonoid content (mg QE/g extract) |
|---|---|---|
| **HM1** | 48.0±0.4$^{d}$ | 39.7±0.2$^{b}$ |
| **HM2** | 56.1±0.8$^{c}$ | 40.8±0.2$^{a}$ |
| **HV** | 38.0±0.3$^{f}$ | 34.5±0.1$^{f}$ |
| **HP1** | 40.1±0.2$^{e}$ | 35.3±0.1$^{d}$ |
| **HP2** | 60.1±1.0$^{b}$ | 36.1±0.2$^{c}$ |
| **HS** | 66.4±0.2$^{a}$ | 35.0±0.2$^{e}$ |

HM, *H. myrtifolium*; HV, *H. vulcanicum*; HP, *H.pumiliforme*; HS, *H. sahinii*

The results revealed that the HS is very rich in phenolic compounds with 66.4 mg of GAE/g of extract, whereas HV is lowest with 38.0 mg of GAE/g of extract (Table 2). Interestingly, Yaman et al. [14] reported that ethanol extracts of the *Haplophyllum vulcanicum* (HV) species contained higher total phenolic than other *Haplophyllum* species. HV may differ from other species in terms of phytochemical content or biosynthesis ability, or affected by environmental factors.

When Table 3 was examined, the total flavonoid amounts of extracts varied from 34.5 to 40.8 mg QE/g extract. The highest levels of the total flavonoid were found in HM2 and HM1, respectively. Yaman et al. [13] also reported a similar finding for ethanol extracts of *Haplophyllum myrtifolium* (HM).

The collection of species from different regions affected their phytochemical contents and compositions, especially total phenolic content (Table 2). Different results have been observed in populations at the different regions of one species. As a result of these differences, populations belonging to different regions of each species have ecological conditions (abiotic and biotic differentiators) and habitat (rocky, slopes etc. and diversity of flora). Such differences have also been identified by many researchers [28-31]. Rawat et al. [30] indicated that total phenol contents among the populations of *Hedychium spicatum* ranged were a significantly significant different. The present findings indicate to have a significant effect on different species and regions for total bioactive components.

### 3.3. Radical Scavenging Activity

Various phytochemicals (secondary metabolites) of plants such as flavonoids, polyphenols and other phenolics, tannins are the main group of components that serve as primary free radical scavengers [32, 33]. The extracts obtained from endemic *Haplophyllum* species collected from different localities were investigated for their two radical scavenging activities, namely ABTS and DPPH. Results were also expressed as % inhibition (Figure 2 and Figure 3).

The DPPH radical is largely used in the evaluation of free radical scavenger activity due to the ease of the reaction. When the DPPH radical is cleaned by a compound of antioxidant via hydrogen donation to form a stable DPPH-H molecule, the color of the solution is return from purple to yellow. All extracts in current study were determinate to reduce the stable violet DPPH radical to yellow. DPPH antioxidant activity ranged between 43.9-86.5% at a concentration of 100 μg of all the assessed extracts and standards

(Figure 2). Among extracts of *Haplophyllum* species, HP1, HP2 and HM1 exhibited maximum DPPH free radical scavenging activity and statistically the same DPPH activity with 85.0-85.1%, followed by HM2 (83.1%) and HS (71.7%) and HV (68.3%). The scavenging effect of different species on the DPPH radical for methanol solvent generally decreased in the order of HP ≥HM >HS >HV (Figure 2).

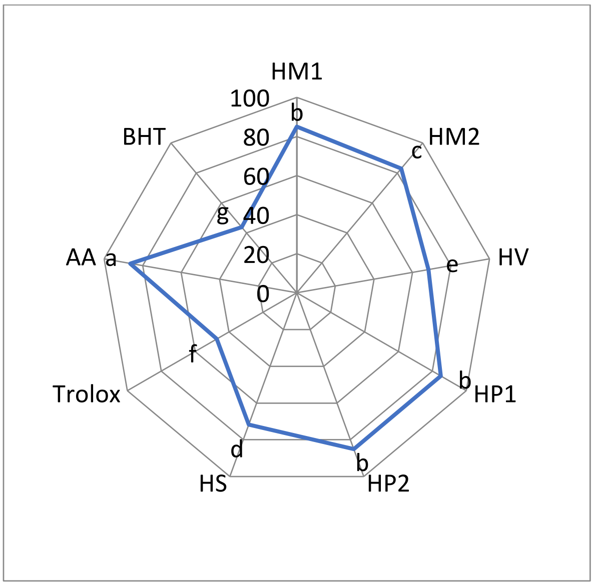


Figure 2 The DPPH free radical activities of methanol extracts of endemic *Haplophyllum* species from Türkiye (HM, *H. myrtifolium*; HV, *H. vulcanicum*; HP, *H.pumiliforme*; HS, *H. sahinii*)

The ABTS radical is blue, but transforms from blue to the colorless form through an antioxidant compound. As seen in Figure 3, ABTS radical scavenging activity ranged between 39.5%-89.9% at a concentration of 200 µg of all the assessed extracts and standards.

All values of methanol extracts from *Haplophyllum* species were statistically in different groups. Among *Haplophyllum* species, samples at both different regions of HM displayed the highest ABTS activity (HM1:76.5% and HM2:71.1%), followed by HP (HP2: 66.7%, HP1: 61.3%) and HS (56.5%) and HV (47.2%).

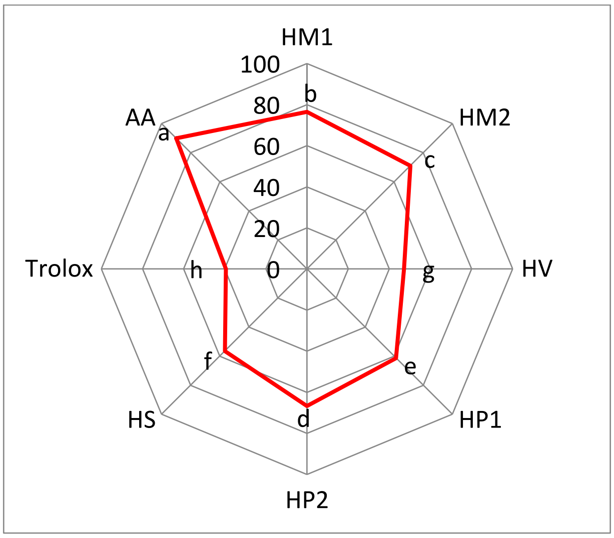


Figure 3 The ABTS free radical activities of methanol extracts of endemic *Haplophyllum* species from Türkiye (HM, *H. myrtifolium*; HV, *H. vulcanicum*; HP, *H.pumiliforme*; HS, *H. sahinii*)

Interestingly, DPPH and ABTS activities of all *Haplophyllum* species were lower than AA as the synthetic antioxidant standard, but stronger than the trolox standard. Also, DPPH activities of all *Haplophyllum* species had the higher than BHT standard. This finding indicates that the analyzed *Haplophyllum* species exhibit very powerful radical scavenging activity. In the other hand, the results between different localities of the species were close to each other.

Antioxidant activities of these *Haplophyllum* species have been reported very little in some previous studies [13, 34], but, for the first time, methanol extracts of endemic *Haplophyllum* species from Türkiye were compared among themselves and with synthetic standards in this study.

Bioactive compounds defined as secondary metabolites in plants have been taken the evidence as natural functional components, and various medicines have been developed all over the World [35, 36]. These compounds include total flavonoids, other phenolic compounds (phenolic acids, tocopherols, stilbenes, alcohols etc.) and polyphenolics (condensed and hydrolysable tannins, saponin, lignin), vitamins, terpenoids, carotenoids, essential oils [37]. These compounds could use as a potential source of natural antioxidants [38]. Many researchers reported that found a correlation between the antioxidant activity and phenolics, in particular flavonoids [39-42]. Moreover, flavonoids are the most important and abundant polyphenols, more than 5000 reported up to today [43]. So, former studies have indicated that the amount of bioactive compounds in plants and their antioxidant activities depend on both biological factors (genetic, organ etc.) and environmental (precipitation, temperature, altitude, light intensity etc.) conditions [44]. Also, stress conditions may induce various flavonoid biosynthetic genes. The biotic and abiotic stresses such as drought, temperature, wounding, nutrient deprivation, metal toxicity and can increase the levels of flavonoids in the plants as a part of their defense strategy [45]. So, the antioxidant activity of flavonoids is principal gone on their ability to donate the electrons or hydrogen atoms [46]. The variety of phenolic and flavonoid compounds is as important as amount of the compounds, because effect of antioxidant activity of each compound is different.

## 4. CONCLUSIONS

Less than 10% of the world's biological diversity has been assessed for potential biological activity and there are many more natural compounds awaiting exploration to achieve this natural chemical variety. When both radical scavenging activities are taken into consideration, HM and HP have been exhibited the strongest antioxidant activity, especially the antioxidant activities of both species are very close to those of ascorbic acid. The findings suggest that these endemic species show much stronger antioxidant activities, involved significantly high levels of total phenolic and flavonoid contents, and could be a potential source of natural antioxidants. However, there is little research about antioxidant activities and total bioactive components of the species evaluated in this study. Further chemical investigations are required to isolate the elements of active phenolic and flavonoid components of the plants that show a broad spectrum of pharmacological activity.

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***The Declaration of Conflict of Interest/ Common Interest***

No conflict of interest or common interest has been declared by the authors.

***The Declaration of Ethics Committee Approval***

This study does not require ethics committee permission or any special permission.

***The Declaration of Research and Publication Ethics***

The authors of the paper declare that they comply with the scientific, ethical and quotation rules of SAUJS in all processes of the paper and that they do not make any falsification on the data collected. In addition, they declare that Sakarya University Journal of Science and its editorial board have no responsibility for any ethical violations that may be encountered, and that this study has not been evaluated in any academic publication environment other than Sakarya University Journal of Science.

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