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COMPARATIVE ANALYSIS OF BIOCHEMICAL CONTENT, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF *HYPERICUM PERFORATUM* L. SPECIES IS GROWN IN TÜRKİYE

VOLKAN GUL[1*], HATICE CETINKAYA[2], BURCU SECKIN DINLER[2] AND FIRAT SEFAOGLU[3]

[1]Bayburt University, Aydintepe Vocational School of Higher Education, Food Processing, Food Technology Program, Aydintepe/Bayburt, Türkiye
[2]Sinop University, Faculty of Arts and Sciences, Department of Biology, Sinop, Türkiye
[3]Kastamonu University, Faculty of Engineering and Architecture, Department of Genetics and Bioengineering, Kastamonu, Türkiye
[*]Corresponding author's email: volkangul555@ gmail.com

Abstract

Many external factors, such as climatic conditions, geographical differences, and altitude, directly affect the primary and secondary metabolites and therapeutic use of St. John's Wort (*Hypericum perforatum* L.), an important plant in alternative medicine. This study was carried out to identify the antioxidant and antimicrobial activities and the content of proline, malondialdehyde, hydrogen peroxide, flavonoid, anthocyanin, and total phenolic in the St. John's Wort growing naturally in different regions of Bayburt. The St. John's Wort samples were collected from 11 different locations. As a result of the analyses carried out on the plant samples, it was found that the content of proline was within the range of 4.6-8.2 µmol g^{-1} DW, the amount of malondialdehyde was within the range of 0.69-1.10 nmol g^{-1} DW, the amount of hydrogen peroxide was within the range of 79.89-155.49 µmol g^{-1} DW, the amount of flavonoids was within the range of 91.38%-100.27%, the amount of anthocyanin was within the range of 0.15-1.11 mkmol g^{-1} DW, the total phenolic content was within the range of 15.82-45.22 mg GAE g^{-1}, and the antioxidant activity was within the range of 6.07-105.60 µg mL^{-1}.

In conclusion, although the biochemical contents and nonenzymatic antioxidant activities showed a wide variety, the antioxidant activity was found to be higher in samples 5, 8, and 11. According to the antimicrobial activity results, some plant extracts were effective on bacteria and fungi, especially samples 5, 6, 8, and 11 showed an effect at the concentration of 200 µg mL^{-1}. This study, the first regional study on this subject, will contribute to future research and clinical trials in the health field.

Key words: *Hypericum perforatum* L., Antimicrobial activity, Antioxidant activity, Biochemical analysis, Total phenolic content.

Introduction

From the past to the present, medicinal and aromatic plants found in nature have been used for food, clothing, shelter, and most importantly for overcoming some health problems. St. John's Wort (*Hypericum perforatum* L.), which grows in many parts of the world, has been used in alternative medicine for centuries. It is an important herbal medicine used for therapeutic purposes in a wide range of areas thanks to its soothing, wound healing, antimicrobial, antidepressant, antibiotic, anti-inflammatory, anticancer, antiviral, and antibacterial effects (Cirak *et al*., 2006; Birt *et al*., 2009; Caraci *et al*., 2011; Gonence *et al*., 2020). St. John's Wort, which belongs to the *Hypericaceae* family, has more than 500 species distributed in various parts of the world. There are 108 known species of *Hypericum* in Turkey. In Turkey, the locals call *Hypericum perfortum* L., known as St. John's Wort in the world, by various names such as St. John's Wort, *lamb breaker, blood grass* and *bloodgrass* (Baytop, 1999; Dogan *et al*., 2017; Ozbek *et al*., 2019).

Local people widely use St. John's Wort in the treatment of depression, wounds, and burns. In general, the above-ground parts of the plant are collected during the flowering period and used as a tincture or oil in wounds and burns and as a sedative tea by brewing its flowers. *H. perforatum* is very rich in phenolic compounds, has an important effect in healing wounds, and can be used as an agent in antidepressants. Moreover, its flavonoid components help increase the effectiveness of other activities in treatments, although not directly (Butterweck *et al*., 2003). Phenolic compounds prevent oxidation, reduce oxygen concentration which has a negative effect on the body, and exhibit antioxidant properties thanks to their antimicrobial, anti-inflammatory, and antiviral properties (Kaur & Mondal, 2014; Zhang *et al*., 2014; Zhang & Tsao, 2016). In their study examining the species of *H. perforatum* L., *H. maculatum Cr.*, *H. hirsutum* L., and *H. tetrapterum Fr* obtained from various parts of the northwest part of Transylvania, Romania, Gitea *et al*., (2018) reported that *H. maculatum* and *H. perforatum* had the highest polyphenolic and antioxidant properties among all species. In a previous study, Odabas *et al*., (2009) reported that temperature and light intensity increased the phenolic content in *Hypericum perforatum.* Moreover, Yao *et al*., (2019) reported that the contents of bioactive compounds (flavonoids and phenolics) and the antioxidant capacity increased significantly depending on the temperature in *Hypericum perforatum*. In another study, it was reported that vermicompost application to *H. perforatum* under heat stress stimulated carbon metabolism and caused an increase in secondary metabolites in the plant (Kaundal *et al*., 2021). On the other hand, it is remarkable that Torun *et al*., (2021) reported an increase in the content of proline in the leaves of *H. perforatum* due to drought stress.

In the therapeutic use of St. John's Wort, which is very important in alternative and modern medicine, many external factors such as climatic conditions, geographical differences, altitude, harvest time, and drying method directly affect its components such as oil, phenolic compounds, flavonides, antioxidant capacity, and anthocyanin. There are many *Hypericum* species in Turkey, and all *Hypericum perforatum* L. species do not have the same effect due to their different contents. This gives rise to misconceptions about the plant. The purpose of this study, which is the first original study on this subject, is to identify some of the chemical profiles and basic components of St. John's Wort that grow naturally in the flora of Bayburt, compare the results with previous studies, to lay the basis for future research, and contribute to clinical studies.

Material and Methods

Study area and collection of plant material: The plant samples used in the study were collected from the province of Bayburt and its districts, villages, and road routes. The Province of Bayburt is located in the Eastern Black Sea Region and has an average surface area of 3739 km^2 and an altitude of 1550 m. It is located between the latitudes 40°37' north and 39°52’ south and the longitudes 40°45' east and 39°37' west. Since Bayburt is located at the junction of the north and the east, it has a transitional climate dominated by continental climate characteristics with cold and snowy winters and hot and dry summers. It is one of the untouched regions of Turkey and has many medicinal and aromatic plants thanks to its diverse vegetation. St. John's Wort, one of the important medicinal and aromatic plants growing naturally in Bayburt, was used in the study. The plant samples were collected from the following 11 locations within the borders of Bayburt during the flowering period: the village of Gez (A1), Toki Çaykara road (A2), Demirözü road (A3), Pamuktaş (A4), the village of Mutlu (A5), Nişantaşı (A6), Old İspir road (A7), Maden (A8), Baberti road (A9), Sanayi (A10), and Aydıntepe (A11) (Fig. 1).

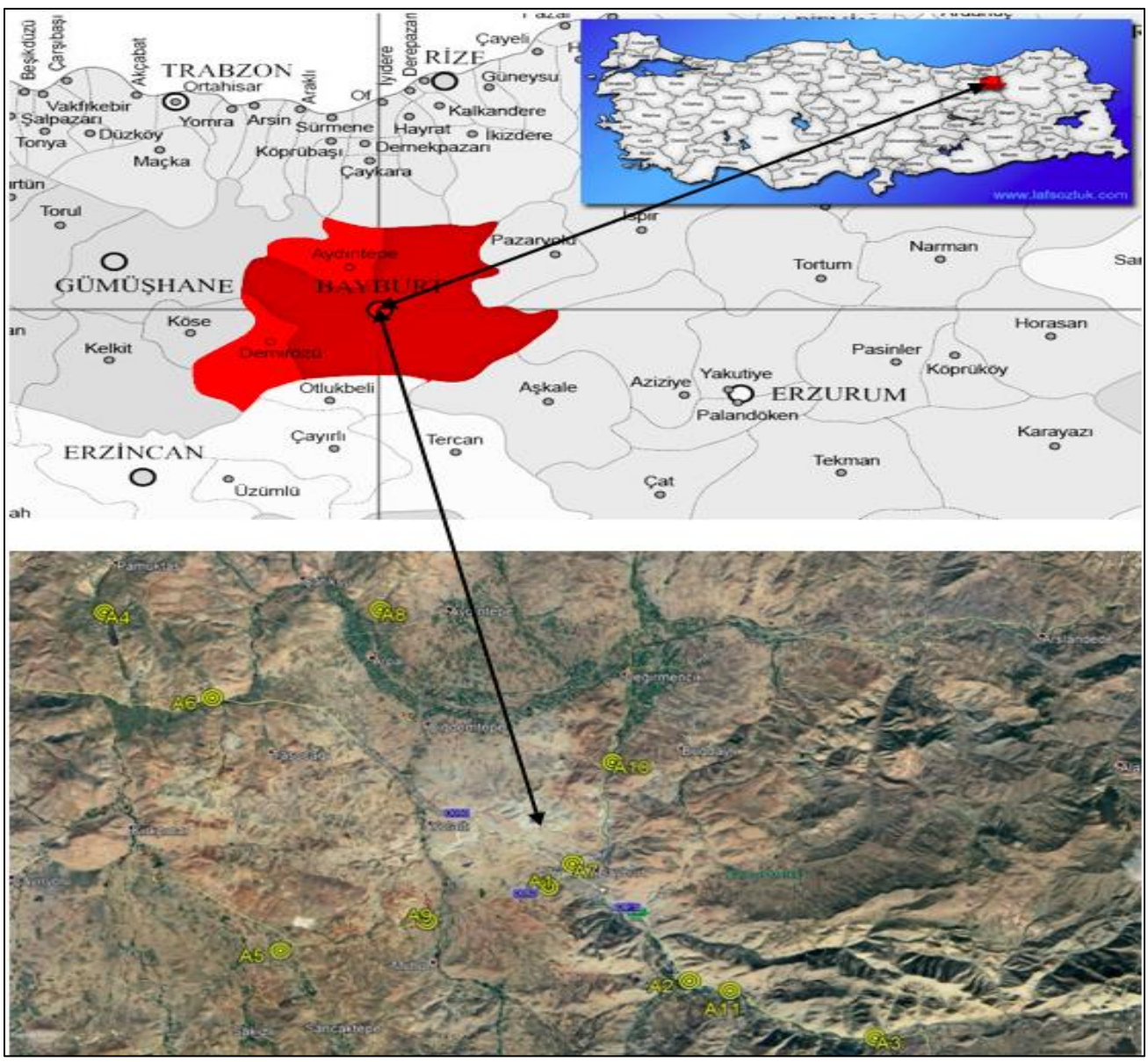

Fig. 1. The location of the study area on the map of Turkey and the sampling points.

Preparation of the samples: The plant samples were dried in the open air out of the sun, thoroughly cleaned, homogenized by grinding using a grinding mill, passed through steel sieves, and stored until use (Kacar *et al*., 2013).

Biochemical analyses

Determination of the content of proline: The amount of free proline was determined using the procedure reported by Bates *et al.,* (1973) about 20 g of the dry plant was homogenized with 0.1% (w v^{-1}) trichloroacetic acid and centrifuged for 30 minutes (15000 x g, 4°C). The homogenates were filtered using filter paper and mixed by adding 1 ml of ninhydrin and 1 ml of glacial acetic acid to the supernatants. After the resulting mixture was kept in a 100°C water bath for 1 hour, the reaction mixture was read at 520 nm in a supernatant spectrophotometer containing 4 ml of toluene.

Determination of the content of hydrogen peroxide: The content of H_2O_2 was determined using the method reported by Velikova *et al*., (2000) 0.5 g of dry plant sample from each group was homogenized by adding 5 ml TCA (trichloroacetic acid) and centrifuged for 30 minutes (12000 x g, 4°C). The supernatants were mixed with 0.5 ml buffer solution (potassium phosphate 10mM, pH:7) and 0.5 ml and 1 ml KI buffer. The absorbances were measured at 390 nm using a Thermo Scientific Genesys (10S UV-VIS) spectrophotometer.

Determination of the lipid peroxidation level: The lipid peroxidation levels were determined using the method reported by Madhava Rao & Sresty (2000). 0.5 g of dry plant sample from each group was homogenized by adding 2.5 ml TCA (trichloroacetic acid). The homogenates were centrifuged for 5 minutes (10000 g, +4°C). The reaction mixture containing TBA (thiobarbituric acid) and TCA was added to the obtained supernatants, and then the mixture was heated at 95 °C for 30 minutes and centrifuged for 15 minutes (10000 g). The absorbances of the supernatant were read at 532 and 600 nm. Malondialdehyde (MDA) concentration was computed using the extinction coefficient (155 mM cm^{-1}).

Nonenzymatic antioxidant activities

Total phenolic content: The total phenolic content was determined using Folin-Ciocalteu chlorometric method in line with the procedure reported by Singleton & Rossi (1965). Dry plant samples were centrifuged for 10 minutes (5000 g, +4°C) with 80% methanol extraction. 50 µL of supernatant, 450µL of DI H_2O, and 2.5 mL of 0.2 N Folin-Ciocalteu reagents were added, and then the mixture was left for 5 minutes. Then, 2 mL of sodium carbonate (75 g L^1) was added to the mixture. The measurements were made using a spectrophotometer at 765nm according to the standard curve formed with gallic acid dissolved in 80% methanol. This analysis was conducted at Sinop University Scientific and Technological Research Application and Research Center (SUBITAM).

Determination of the amount of anthocyanin: 0.5 g of dry plant material was homogenized with 79% methanol, 20% distilled water, and 1% HCl. The obtained homogenates were centrifuged for 20 minutes (10000 g, +4°C). The amount of anthocyanin was measured at 530 and 657 nm using the spectrophotometric method (Mancinelli *et al*., 1975). This was calculated as below.

$$\text{Anthocyanin} = A_{530}-(A_{657}/3)$$

Determination of the amount of flavonoid: The total flavonoid amount was determined using the method reported by Mirecki & Teramura (1984). Dry plant samples were homogenized with 90% methanol, 20% distilled water, and 1% HCl. The obtained homogenates were centrifuged for 20 minutes (10000 g, +4°C). The obtained supernatant was measured at 300 nm. The results were expressed in %.

Measurement of the antimicrobial and antioxidant activity

Preparation of the plant extracts: Each of the powdered materials (~30 g) was extracted with 300 mL of methanol using a shaker for 24 hours. The combined extracts were filtered and concentrated under a vacuum to obtain a crude extract. The methods used for antimicrobial and antioxidant activity studies were described in the previous study by the researcher (Elkiran & Avsar, 2020). These methods are mentioned below.

Microorganisms: The antimicrobial studies were carried out using 3 Gram-positive bacteria *(Staphylococcus aureus* ATCC 6538, *Bacillus cereus* ATCC 7064, *Enterococcus faecalis* ATCC 51299), 4 Gram-negative bacteria (*Escherichia coli* ATCC 11293, *Klebsiella pneumonia* ATCC 27889, *Pseudomonas aeruginosa* ATCC 27853, *Shigella flexneri* ATCC 12022), and 2 fungi *(Candida parapisilosis* ATCC 22019 and *Candida krusei* ATCC 6258) which were stored as pure culture in Microbiology Laboratory, Department of Biology, Faculty of Science and Letters, Sinop University.

Evaluation of the antimicrobial activity: The antimicrobial activity of the extracts was evaluated using the disc diffusion method (Bauer *et al*., 1966). Before the test, the bacteria were transferred to Muller Hinton Broth (MHB) and the fungi to Sabouraud Dextrose Broth (SDB), and then they all were incubated throughout the night at 37°C. The turbidity was adjusted to the equivalent of 0.5 McFarland standard. 100 µL of fresh microorganism culture was spread on the sterile agar surface, and 25 µL of extract (4000 µg/1 mL 12.5% DMSO) impregnated onto 6 mm sterile blank antibiotic discs were placed on the surface of the medium. The medium was incubated at 35±0.5°C for 18-24 hours (for the bacteria) and at 25±1°C for 48-72 hours (for the fungi). Antibiotic susceptibility discs containing erythromycin (E15), ampicillin (AM10), and cycloheximide were used as the positive control and 12.5% DMSO was used as a negative control. Antimicrobial activity was assessed by measuring the zone diameter. The tests were carried out in two repetitions.

The Minimum Inhibitory Concentration (MIC) was determined using the serial tube dilution method. The first stock solution was produced by dissolving the methanol extracts (4000 µg) of the plants in 12.5% DMSO. Glass tubes with 0.9 mL of MHB and SDB were prepared to adjust for dilute concentrations of the stock (200-12.5 µg mL^{-1}). All the tubes were inoculated with 50 µl of standardized inoculum of each organism and incubated for 18-24 hours at 35±0.5°C (for the bacteria) and 48-72 hours at 25±1°C (for the fungi). MIC was defined as the lowest concentration at which no growth was observed. The tests were carried out in two repetitions.

Measurement of the antioxidant activity: Antioxidant activities of the extracts were determined on the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) using the methods reported by Blois (1958) & Kumar *et al*., (2011). The extracts were produced at different concentrations (µg ml^{-1}) using the serial dilution method. 1 mL of ethanol solution of each concentration of the extracts was mixed with 4 mL of 0.1 mM DPPH solution, and the samples were kept in the dark for 30 minutes at room temperature. The absorbances were measured at 567 nm and the scavenging activity on the DPPH radical was computed using the following.

Equation: $\% \text{ Inhibition} = [(A_B - A_S) / A_B] \times 100$

where A_B refers to the absorbance of the control reaction and A_S to the absorbance of the test compound, ascorbic acid was used as the positive control. The non-standard or non-sample compound was used as a control. The scavenging activity was expressed as the median inhibitory concentration (IC50), which represents the extract concentration (µg ml^{-1}) required to inhibit 50% of the free radical scavenging activity. Graphs were created using MS Office Excel 2013.

Statistical analysis

SPSS for Windows (v.22.0) was used to analyze the data. The treatments were compared using Duncan's multiple range test at the significance level of $p<0.05$ (Steel & Torrie, 1980).

Results

Content of proline: It was found that the differences between the locations where the samples were collected, in terms of the amount of proline were statistically significant ($p\leq0.01$, Table 1). The samples collected from the 5th location were found to have the highest amount of proline (8.2 µmol g^{-1} DW), and those from the 4th location were found to have the lowest amount of proline (4.6 µmol g^{-1} DW). The amounts of proline were found to be 7.7, 7.5, 7.3, 6.8, 6.3, 5.9, 5.0, 4.9 and 4.7 µmol g^{-1} in the samples collected from the locations 6, 1, 2, 11, 3, 9, 7, 8, and 10, respectively.

Content of malondialdehyde: The differences between the plant samples collected from 11 locations were found to be statistically significant in terms of the amount of malondialdehyde ($p\leq0.01$) (Table 1). The amounts of malondialdehyde were observed to range from 0.69 to 1.10 nmol g^{-1} DW. The lowest amount of MDA was found to be in the plant samples collected from the 5th location, while the highest MDA was in those collected from the 8th location. This result is in line with the values obtained in the proline analysis.

Content of hydrogen peroxide: The differences between the locations were found to be statistically very significant in terms of the content of hydrogen peroxide ($p<0.01$) (Table 1). It was found that the lowest amount of hydrogen peroxide (79.89 µmol g^{-1} DW) was in the samples collected from the 8th location, while the highest (155.49 µmol g^{-1} DW) was in those collected from the 3th location. As for the other locations, the amounts of hydrogen peroxide in the samples collected from locations 4, 1, 9, 7, 6, 10, 5, 2, and 11 were found to be 150.51, 127.82, 126.08, 124.75, 120.31, 107.49, 104.64, 99.33, and 84.97 µmol g^{-1} DW, respectively.

Table 1. Biochemical analyzes of *Hypericum perforatum* plant taken from 11 different regions.

Plant example/ values	Anthocynin (µmol g^{-1} DW)	Flavonoid (%)	Hydrogen peroxide (µmol g^{-1} DW)	MDA (nmol g^{-1} DW)	Proline (µmol g^{-1} DW)	Total Phenolic content (mg GA g^{-1})	Antioxidant capacity (µg ml^{-1})
1	1.113a	100.267a	12.7824b	0,991b	7.50abc	40.557ab	34.767f
2	0.149d	92.030cd	9.9330cde	0,803cd	7.30abc	39.253bc	18.700h
3	0.966ab	97.500b	15.5491a	0,830c	6.32bcd	42.473ab	61.167c
4	0.901b	98.327b	15.0509a	0,796cd	4.56e	45.223a	20.900g
5	0.178d	91.380d	10.4639bcd	0,697d	8.20a	31.557de	7.453i
6	1.049ab	99.102ab	12.0306bc	0,758cd	7.78ab	32.707de	105.600a
7	0.663c	97.342b	12.4750b	0,961b	5.06de	32.080de	40.267e
8	0.183d	92.820cd	7.9898e	1,103a	49.5de	15.823g	6.067j
9	0.738c	98,609ab	12.6083b	0,956b	5.99cde	27.350ef	68.233b
10	1.001ab	98.331b	10.7491bcd	1,027ab	4.71e	25.703f	49.200d
11	0.199d	93.667c	8.4972de	0,782cd	6.83abc	34.840cd	15.067ı
F value	58,970**	31,730**	9,947**	14,363**	7,554**	21,545**	41458,390**

There is a difference between the means indicated by different letters in the same column, significant at $p\leq0.01$

Table 2. Inhibition zones (mm) of the methanolic plant extracts against tested microorganisms using disc diffusion method.

Plant extracts	A	B	C	D	E	F	G	H	I
1	-	-	10	-	-	-	7	-	-
2	-	-	-	-	-	-	-	-	-
3	-	-	10	-	7	-	7	-	-
4	-	-	-	-	-	-	7	-	-
5	-	-	10	-	-	-	-	-	-
6	-	-	10	-	9	-	8	-	-
7	-	-	9	-	8	-	8	-	-
8	-	-	-	-	-	-	-	-	-
9	-	-	9	-	7	-	8	-	-
10	-	-	7	-	-	-	8	-	-
11	-	-	-	-	-	-	-	-	-
DMSO	-	-	-	-	-	-	-	-	-
Ery	*	*	*	24	9	12	*	10	12
Amp	*	*	19	*	18	15	23	*	*
Cyc	43	40	*	*	*	*	*	*	*

(-) not effect, (*) not tested, A: *C. krusei* ATCC 6258, B: *C. parapsilosis* ATCC 22019 C: *S. aureus* ATCC 6538, D: *B. cereus* ATTC 7064, E: *E. faecalis* ATCC 51299, F: *E. coli* ATCC 11293, G: *P. aeruginosa* ATCC 27853, H: *K. pneumonia* ATCC 27889, I: *S. flexneri* ATCC 12022

Table 3. MIC values (µg mL^{-1}) of the methanolic plant extracts against tested microorganisms using microdilution procedure.

Plant extracts	A	B	C	D	E	F	G	H	I
1	-	-	-	-	-	-	-	-	-
2	-	-	-	-	200	-	200	-	-
3	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	-	-
5	-	-	-	-	200	-	200	-	-
6	-	-	-	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-
8	-	-	-	-	200	-	-	-	-
9	-	-	200	-	200	-	-	-	-
10	-	-	-	-	-	-	-	-	-
11	200	-	-	-	200	-	200	-	-

(-) not effect, **A:** *C. krusei* ATCC 6258, **B:** *C. parapsilosis* ATCC 22019 **C:** *S. aureus* ATCC 6538, **D:** *B. cereus* ATTC 7064, **E:** *E. faecalis* ATCC 51299, **F:** *E. coli* ATCC 11293, **G:** *P. aeruginosa* ATCC 27853, **H:** *K. pneumonia* ATCC 27889, I: *S. flexneri* ATCC 12022

Content of flavonoid: It was found that the differences between the samples collected from different locations were statistically very significant in terms of the amount of flavonoid (p<0.01) (Table 1). While the highest amount of flavonoid (100.27%) was found to be in the samples collected from the 1st location, the amounts of flavonoid in the samples collected from locations 6, 9, 10, 4, 3, 7, 11, 8, 2, and 5 were found to be 99.10%, 98.61%, 98.33%, 98.33%, 97.50%, 97.34%, 93.67%, 92.87%, 92.03%, and 91.38%, respectively.

Content of anthocyanin: It was found that the differences between the plant samples collected from different locations were statistically very significant in terms of the amount of anthocyanin (p<0.01) (Table 1). The amounts of anthocyanin were found to be in the range of 0.15-1.11 mkmol g^{-1} DW in the plant samples collected from the 11 locations. While the highest amount of anthocyanin was observed in the plant samples collected from the 1st location, the lowest was in those collected from the 2nd location.

Total phenolic content: When the results were examined, it was found that the lowest total phenolic content (15.82 mg^{-1} GAE g^{-1}) was in the samples collected from the 8th location, while the highest (45.22 mg^{-1} GAE g^{-1}) in those collected from the 4th location. These results are in line with the antioxidant activity results. In addition, according to the variance analysis results, these differences were statistically significant at the significance level of p<0.01 (Table 1).

Antioxidant activity results: According to the antioxidant results, the differences between the samples collected from different locations were found to be statistically very significant (p<0.01) (Table 1). The samples collected from locations 5 (7.45 µg mL^{-1}), 8 (6.1 µg mL^{1}), and 11 (15.1 µg mL^{-1}) were found to have an effect close to the standard ascorbic acid; so, their antioxidant activities were higher than the other samples. On the other hand, the samples collected from location 6 (105.6 µg mL^{-1}) were found to have the lowest antioxidant activity.

Antimicrobial activity results: When the disk-diffusion test results of the plant extracts were evaluated, it was found that they showed a relatively low effect (Table 2). All the samples, except those collected from locations 2, 8, and 11, were found to be effective against at least one of the Gram-positive bacteria *S. aureus* (7-10 mm) and *E. faecalis* (7-9 mm) or the Gram-negative bacterium *P. aeruginosa* (7-8 mm). However, they were ineffective against other bacteria and fungi (Table 2). It was observed that the plant samples collected from locations 3, 6, 7, and 9 were effective against all three isolates, but those collected from location 6 were more effective than the others.

When the MIC test results shown in Table 3 are examined, it can be seen that the extracts of the plant samples collected from locations 2, 5, 8, 9, and 11 were effective against at least one of the Gram-positive Bacteria *S. aureus* and *E. faecalis* or the Gram-negative Bacterium *P. aeruginosa* at the concentration of 200 µg mL^{-1}, likewise the case in the disc-diffusion technique. Moreover, it was observed that the samples collected from location 11 were effective against the fungus *C. krusei* at the concentration of 200 µg mL^{-1}. *E. faecalis* was the one against which the highest number of extracts showed MIC activity.

Discussion

Proline is one of the important amino acids produced by plants under stress conditions (Ghosh *et al*., 2021). In this study, the amounts of proline varied between 4.56 and 8.20 µmol gr^{-1}DW (Table 1). In plants, the increase in the amount of proline is directly proportional to the tolerance mechanism against stress. Based on this information, it can be argued that the plants collected from location 5, which were found to have the highest proline content, were under more stress (heavy metal, exhaust, polluted air) than those collected from other locations. In their study, Torun *et al*., (2021) reported that the amount of proline in the leaves of *Hypericum perforatum* was within the range of 0-10 µmol gr^{-1} FW, which is in line with the result of the present study.

The amount of malondialdehyde is the product of lipid peroxidation formed by plants under stress (Pan *et al*., 2021). In the present study, the lowest MDA content was found to be in the plant samples collected from location 5. This can be interpreted as the St. John's Worts growing in location 5 were more tolerant than those in other locations (Table 1). It is quite remarkable that the plants collected from location 8 had the highest MDA. Based on these results, it can be asserted that the St. John's Wort plants growing in location 8 might have been exposed to environmental stress factors. This result was also in agreement with the proline results. The location with the highest amount of proline had the lowest amount of MDA. Nazari *et al*., (2022) and Franklin *et al*., (2009) also reported similar results for the MDA content of the Hypericum under selenium and biotic stress.

Hydrogen peroxide is one of the active oxygen types that occur in plants under stress. Superoxide radical is efficiently converted to hydrogen peroxide by the action of enzyme superoxide dismutase (SOD; EC 1.15.1.1). If the hydrogen peroxide is not converted to H_2O, it has the potential to damage proteins, lipids, and molecules (Raja *et al*., 2017). The plant samples collected from the 3rd location were found to have the highest amount of hydrogen peroxide (Table 1). When this result is evaluated together with the MDA results for the same location (8.30), it can be asserted that the plants in this location were damaged. So, it can also be asserted that the plants in this location were affected by environmental factors more than the plants in other locations.

Flavonoids are categorized as phenolic compounds in plant cells. These compounds accumulate in tissues under the effect of environmental stimuli and serve a wide variety of functions in plant growth, development, propagation, and protection against various negative factors (Li *et al*., 2021). Hypericum species contain a large number of secondary metabolites from at least 11 different classes, such as naphthodianthrones, phloroglucinols, flavonoids, organic acids, essential oils, amino acids, xanthones, tannins, procyanidins, and other water-soluble components (Greeson *et al*., 2001). Flavonoids are defined as a group of bioactive phenolics found in plants. The flavonoid results for the plant samples from different locations were found to be close to each other (91.38-100%) (Table 1). The result closest to that of the control group was observed in the 6th location. Similarly, in their study, Kazlauskas & Bagdonaite (2004) reported that *Hypericum perforatum* L., had the highest flavonoid accumulation potential among different varieties of St. John's Wort. Besides this, in recent years, Makarova *et al*., (2021) marked that harvest time affects the flavonoid content in *Hypericum perforatum* L., flowers. Additionally, this result showed us that the differences in flavonoid content in this study might be related to harvesting time.

In the literature, there are some studies reporting that anthocyanins have an antioxidant effect, reduce the risk for chronic heart diseases, and improve visual activity and antiviral activity (Delgado-Vargas & Paredes-López, 2003). Anthocyanins are water-soluble pigments derived from flavonoids via shikimic acid. They are a group of secondary metabolites synthesized by the phenylpropanoid pathway. These compounds accumulate in tissues under the effect of environmental stimuli and function as active oxygen species (AOS) scavengers due to the hydroxyl groups in their structure (Chutipaijit *et al*., 2009, Cirillo *et al*., 2021). In the present study, it was found that the highest amount of anthocyanin was in the 1st location with 1.113 mg DW^{-1}. This value is quite high compared to that (4.10^{-5} mg FW^{-1}) reported by Fornasiero *et al*., (1998) for the leaves of *Hypericum perforatum* (Table 1). We are of the opinion that this difference is due to the fact that in the present study, the samples were collected from the whole plant, not only from the leaves. In the present study, the anthocyanin results were in agreement with the flavonoid results (5th region with the lowest values).

The antioxidant activity of a plant is defined as the scavenging capacity of the active oxygen species formed by stress promotion (Dogru, 2020). The lowest antioxidant activity was observed in the samples collected from location 6. In their study on *Hypericum perforatum* L., Kul *et al*., (2021) examined the soil

samples collected from the 6th location and reported that the Fe and Pb contents were the highest, even if they did not reach toxic levels as per the standards set by FAO/WHO. Similarly, Vuko *et al*., (2021) introduced that high antioxidant activity in the essential oil of *Hypericum peroforatum* plants was observed. This result was in agreement with our study.

Phenolic compounds are phytochemicals that are important in terms of many aspects such as showing antimicrobial and antioxidative effects, causing enzyme inhibition, and being used as control criteria for purity in some foods (Van Der Sluis *et al*., 2002, Zamuz *et al*., 2021). In the present study, it was found that the highest total amount of phenolic compound was in location 4 with 45.22 mg $^{-1}$GAE g^{-1}and the lowest in location 8 with 15.82 mg $^{-1}$GAE g^{-1} (Table 1). In their studies on *Hypericum perforatum*, Gioti *et al*., (2009) and Fathi & Ebrahimzade (2013) reported the total phenolic content as 228.2 and 505.7 mg $^{-1}$GAE g^{-1}, respectively. Compared to this result, the phenolic content of St. John's Wort growing in Bayburt was quite low. But it was also found by Pogorzelska-Nowicka (2021) that the total phenolic content was 56.95 mg^{-1}GAE g^{-1} in *Hypericum perforatum* plants. From this result, it could be suggested that our plants from location 4 have a medium capacity of phenolic content. The plants collected from Bayburt were also found to have the highest antioxidant activity and hydrogen peroxide. These results prove that the plants in this region can cope with the environmental conditions. In their study, Kul *et al*., (2021) reported that the amount of Fe^{+2} was higher in the Hypericum plants collected from location 4 than in the plants collected from other locations. We are of the opinion that the high iron content in location 4 might have caused an increase in the stress in the plants.

When the antimicrobial activities of the plant extracts are evaluated, it can be asserted that they were not highly effective (Tables 2 and 3). Still, there were differences between the samples of the same plants collected from different locations. We will discuss some of the studies on *Hypericum* below. However, it should be noted that the plant extracts were obtained using different solvents or the extract concentrations used to observe the antimicrobial effect were different in different studies. In their study examining the antimicrobial activity of ethanolic extracts of *Hypericum perforatum*, Milosevic *et al*., (2007) reported that the extract concentration of 2.5-25 mg mL^{-1} showed the highest activity against *Pseudomonas glycinea* and *Azotobacter chrococcum*, but had no effect against *K. pneumoniae*, which is in line with the result we found in the present study. Suntar *et al*., (2016) examined the H. *perforatum* extracts obtained using various solvents in terms of their antimicrobial effect against oral bacteria and reported that the extracts had strong activity against various bacteria, and the ethanol and water extracts were effective against *E. faecalis* at the concentration of 16 µgmL^{-1}, which supports our results. Meral & Karabay (2002) tested the effectiveness of the methanol extracts obtained from three genera of *Hypericum* against Gram-positive and -negative bacteria. They reported that all the plant extracts were effective against 4 Gram-positive and 4 Gram-negative bacteria. They reported a higher antibacterial activity compared to our study. Dadgar *et al*., (2006) examined the antibacterial effect of ethanol and water extracts of *Hypericum perforatum* collected in Iran on methicillin-resistant and susceptible *S. aureus* and reported that the ethanolic extracts were highly effective. They observed that the methanolic extract was more effective against *S. aureus* than against other bacteria. In another study, Okmen & Balpinar (2017) tested the effectiveness of the methanolic extract of the *H. perforatum* collected in Izmir against *S. aureus* and coagulase-negative Staphylococci and reported that plant extract had an antibacterial activity and a strong antioxidant activity. In their study examining the antimicrobial effect of water and acetone: water extracts of the H. perforatum collected in Muğla, Celen *et al*., (2008) reported that 0.1 mL of the acetone: water extract was more effective against *P. aeruginosa*, *E. coli*, *S. aureus*, *Bacillus subtilis* and *C. krusei* isolates (within the range of 14- 21 mm) than the water extract. Dall'agnol *et al*., (2003) examined the antibacterial effect of methanolic extracts obtained from six *Hypericum* species collected in Brazil and reported that no extract was effective against *E. coli*, but the most effective plant extract had activity against *S. aureus*, which supports the result of our study.

Conclusion

In this study, some chemical contents, chemical profiles, and basic components determining the bioactivity of *Hypericum perforatum* plants collected from 11 different locations were examined. As a result of the study, it was found that the plant samples collected from location 8 had the highest antioxidant activity, those collected from location 1 had the highest flavonoid and anthocyanin contents, and those collected from location 5 had the highest amount of proline. It is quite noteworthy that, in location 4, the soil had the highest Fe content, and the plant samples had the highest amount of phenolic and the lowest amount of proline. This result confirms that "this plant" can build a strong defense mechanism under stress conditions.

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