Pak. J. Bot., 57(5): DOI: http://dx.doi.org/10.30848/PJB2025-5(30)

COMPARISON OF ANTIMICROBIAL, ANTIOXIDANT ACTIVITIES AND CHEMICAL CONTENTS OF ENDEMIC *HYPERİCUM MALATYANUM* AND *HYPERİCUM PERFORATUM* SPECIES GROWING IN MALATYA

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Abstract

Plants are being used for various purposes since ancient times and have an important place in improving human health. Studies have shown that plant components contain any phytochemical compounds with strong antioxidant and antimicrobial activity. Plants and their essential oils have been studied in number of research fields in terms of their antimicrobial and antioxidant effects, especially since 1940 and important results have been obtained. *Hypericum perforatum* L, also known as St. John's wort, is one of these medicinally important plants, traditionally widely used and recognized as a valuable herbal medicine for more than 2000 years. In this study, the antimicrobial properties and antioxidant capacity of *H. perforatum* L. (St. John's wort) were determined and compared with *Hypericum malatyanum* Peşmen, a species endemic to Malatya. The antimicrobial activity of the extracts of these plants were tested and compared against the microorganisms consisting of selected bacteria and yeasts. It was found that they were more effective against bacteria. The best results in antioxidant activity tested by DPPH radical scavenging activity were recorded as 90. 80 % in Malatya St. John' s wort at a concentration of 1000 µl. The chemical content of the samples of *H. perforatum* and *H. malatyanum* plant extracts were determined by GC/MS method and as a result, 14 of the 30 different chemical components identified in both plants showed differences.

Keywords: St. John's wort, Malatya St. John's wort, Endemic plant, Biological activities.

Introduction

Recently, interest in naturally occurring antioxidants and plant antimicrobials has increased considerably. The reasons for this are that pathogenic microorganisms develop resistance to antibiotics, antibiotics may have some undesirable side effects and oxidative stress has been found to play a role in the epidemiology of many diseases such as cancer and diabetes (Düzgüner *et al.*, 2020).

Hypericum perforatum L. (St. John's wort) and *Hypericum malatyanum* Peşmen (Malatya St. John's wort) belong to the family Hypericeae, and 350- 400 species of the genus *Hypericum* are known worldwide (Baytop, 1999; Müller, 2005). *H. perforatum* is the most widely cultivated perennial plant in the genus *Hypericum*, with yellow flowers, sessile, glabrous leaves, usually with a woody structure at the base (Zou *et al.*, 2004; Ernst, 2013). *H. perforatum* L is known worldwide as St. John's Wort. This plant, which has a wide distribution in temperate and tropical climates of the world, grows naturally in areas up to 2500 m above sea level (Güner *et al.*, 2000; Altan *et al.*, 2015). Turkey is an important center for *Hypericum* species and 46 of the 96 species are endemic (Güner *et al.*, 2012; Özkan, 2013; Özhatay *et al.*, 2011). One of these endemic species, *H. malatyanum* Peşmen is an obligate rocky species. It grows on the limestone rocks of the canyon at an elevation of 1900-2000 m in the vicinity of Meletbaşı Hamlet in Eskiköy, Doğanşehir, Malatya. This species is restricted to this area. In 1980 and was discovered by Hasan Peşmen and introduced to the world of science (Yıldırım, 2015; Robson, 1988). The stem of *H. malatyanum* is 5-12 cm high, woody at the base, lying on the soil, leaves 3-7(-9) mm, obovate-elliptic, margins smooth or more or less hardened, naked, dull bluish green, with intramarginal and superficial glands. Inflorescence (1-)2-3-flowered (Peşmen, 1980).

H. perforatum has been known to have antiseptic, antispasmodic, sedative and de-worming effects since time immemorial. It is especially effective in the treatment of burn wounds (Baytop, 1999; Baytop, 1984) and has been used as herbal medicine for more than 2000 years (Curtis & Levsten, 1990). In some studies, conducted in recent years, the plant's anti-depression and liver protective effect has been proven, as well as its pain-relieving effect.

Hypericum species contain a large number of secondary metabolites in 11 different classes including naprodianthrones, flurogonol derivatives, flavonoids, organic acids, essential oils, amino acids, xanthones, tannins, proxyanidins and other water-soluble compounds (Greeson *et al.*, 2001; Gitea *et al.*, 2018). *H. perforatum* L, contains different phenolic compounds such as flavonoids and phenolic acids in the polyphenols group of compounds, has been the subject of many studies because of the presence of natural antioxidant properties of these compounds (Jürgenliemk & Nahrstedt, 2002; Silva *et al.*, 2008). In addition, important phenolic compounds that prevent oxidation of many foods are present in significant amounts in this plant (Sánchez-Muniz *et al.*, 2012; Becker *et al.*, 2016; Camas *et al.*, 2014; Bilia, 2002).

Hypericum plant extracts are used as antidepressant drugs in many parts of the world, especially in Germany, America and other European countries, as an alternative to synthetic antidepressants because they are natural, safe and have fewer side effects. The antidepressant effect is due to the hypericin and hyperforin compounds in *Hypericum* extracts. The World Health Organization (WHO) declared *H. perforatum* as a medicinal plant in 2002 and gave it a wide coverage in its monographs as Hyperici herba. These monographs include the plant's antidepressant, antibacterial, antiviral, protein kinase-C inhibitor and wound healing effects (Butterweck, 2002).

The natural population areas of many of the species belonging to the genus *Hypericum*, especially endemic species, are rapidly decreasing. For this reason, there is a need for scientific studies to determine the chemical compounds of *Hypericum* species, which are neglected especially for our country, to reveal their pharmacological and chemical properties, and to protect and mass produce by culturing them (Çırak & Kurt, 2014). Among *Hypericum* species, *H. perforatum*, which is the best known and subject to more research, is consumed in different forms (tea, oil, etc.), but studies on the phenolic compound contents, antioxidant capacity and antimicrobial effect of the endemic species *H. malatyanum* are very few (Özkan *et al.,* 2018). Considering that climate and soil conditions affect the amount and variety of phytochemicals in plants, It is thought that the findings obtained from such a study with *Hypericum* species growing in the ecological conditions of Malatya will contribute to the literature.

Materials and Methods

Hypericum perforatum L., was collected from the medicinal and aromatic plants garden of Malatya Turgut Özal University Battalgazi Vocational High School, and *Hypericum malatyanum* was collected from the rocky slopes at an altitude of 1680 m in Malatya Doganşehir Eskiköy Meletbaşı Hamlet in the first week of July. Plant samples were dried in the shade at room temperature and ground into powder.

Preparation of plant extracts: The powdered plant samples were weighed 10 g each, 150 ml of methanol was added to them and the extract was kept in an ultrasonic water bath for 30 minutes. It was then filtered through filter paper. The filtrate were concentrated in an evaporator and stored at +4ºC until analyzed.

Measurement of antimicrobial activity: Bacterial strains such as *Staphlococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis, Saccharomyces cerevisiae* and *Candida albicans* were used as test microorganisms for the measurement of antimicrobial activity. After the methanol of the plant extracts was evaporated and the residue was dissolved in DMSO (Dimethyle sulfoxide), the antimicrobial activity was tested by disk diffusion method. Sterile blank antibiotic discs were impregnated with 20 µl of each extract and Cefadroxil (CFR 30) antibiotic discs were used as positive control. Bacterial strains to be tested were activated in Nutrient Broth and yeasts were activated in Sabouroud Dextrose Broth and cultured for 1-3 days. Microorganisms taken from stocks were incubated in Nutrient Broth medium for 24 hours at 37 ºC and 10^{-1} dilution of the active culture was made with sterile swabs on the previously prepared solid Nutrient Agar and Sabouroud Dextrose Agar medium. Each petri dish was placed with discs saturated with each plant extract and bacteria were incubated at 37 ºC for one day and yeasts were incubated at 28 ºC for 3 days. The zones of inhibition were measured in mm and the analyses were performed in triplicate and the mean values were used.

Determination of antioxidant activity: Plant extracts of *Hypericum* species were centrifuged at 5000 rpm for 10 m in and the supernatant was used or antioxidant capacity analysis. The antioxidant capacity of the samples was determined using DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) radical scavenging method. For this purpose, 1 mM DPPH solution was prepared and kept in dark and cool environment. The DPPH solution was added to the plant extract (100, 200, 400, 800 and 1000 µl) and methanol mixtures prepared at different concentrations and incubated for 30 minutes and the absorbances were measured at 517 nm wavelength in a spectrophotometer. The decrease in absorbance gives the amount of DPPH removed, i.e. the free radical scavenging activity. DPPH scavenging activity was calculated using the following formula (Kıvrak, 2018).

$$\% \text{ DPPH removal activity} = \frac{\text{Absorbancecontrol- Absorbancesample}}{\text{Absorbancecontrol}} \text{ x100}$$

Biochemical analyses: The phytochemical content of plant extracts of *H. perforatum* and *H. malatyanum* was determined using Agilent Technologies 5973 mass selective detector (Agilent G3180B Two-Ways Splitters) GC-MS. The analysis, was performed on a Cp WAX 52 CB capillary column (50 m x 0.32 mm ID, df: 1.2 µm) using He gas.

Results

H. perforatum plant extract showed maximum antimicrobial activity on *P. aeruginosa* and *B. subtilis* among the selected microorganisms. While *H. malatyanum* did not show activity on *E. coli* and *S. aureus*, however, antimicrobial activity was detected in *P. aeruginosa* and *B. subtilis* bacteria. *H. perforatum* and *H. malatyanum* plant extracts did not show any antimicrobial activity on yeast fungi such as *S. cerevisiae* and *C. albicans* (Table 1).

In this study, the antioxidant activity of methanol extract of *H. perforatum* was compared with that of endemic *H. malatyanum* extract. As a result, it was noted that the % DPPH scavenging capacity of both plant species increased with concentration and the highest % DPPH scavenging capacity of *H. malatyanum* was 90.345% in 1000 µl samples (Fig. 1).

Based on these data, it can be said that *H. perforatum* and *H. malatyanum* species are both free oxygen radical inhibitors. The chemical contents of plant extracts obtained from endemic *H. malatyanum* and *H. perforatum* growing in the ecological conditions of Malatya were compared by GC/MS analysis. In both the species, 14 of the 30 chemical contents showed differences. Accordingly, the compounds with the highest % area value in *H. perforatum* were Octadecenoic Acid, Oleic Acid - (71.856-6.41) and N-Hexadecanoic Acid, Ascorbic Acid-(67.776- 4.97). In *H. malatyanum*, N-Hexadecanoic Acid -(67.776-4.96) and Octadecenoic Acid, Oleic Acid- (71.862- 6.54) were recorded (Tables 2, 3).

Table 1. Antimicrobial activity results of *H. perforatum* and *H. malatyanum* (Mean + Standard deviation, NA: Not active).

Micro-organisms	Zone diameter (mm)		
	H. perforatum	*H. malatyanum*	Positive control (Cefadroxil)
E. coli	1.5 ± 0.47	NA	66 ± 0.00
B. subtilis	3.6 ± 0.00	2.1 ± 0.05	2.5 ± 0.07
S. aureus	2.3 ± 0.04	NA	5.2 ± 0.03
P. aeruginosa	3.75 ± 0.35	2.1 ± 0.05	10.5 ± 0.07
S. cerevisiae	NA	NA	NA
C. albicans	NA	NA	NA

Table 2. GC/MS results of the chemical content of *H. perforatum* plant extract.

Component	RT	% Area
Hexaoxacycloıcosane	5.327	0.02
Chloropropenoat	16. 960	0.01
Pyrazole	21. 217	0.01
Sodıum Phenoxıde	35. 138	0.06
Benzopyran	54. 215	0.05
Benzofuran	56. 870	0.02
BenzoıcAcıd	57. 351	0.02
Cyclopentadecanone	58. 850	0.01
Oleic acid	59. 199	0.01
Octadecenoic acid	60. 492	0.03
Octadecadien	60. 756	0.01
Hexadecenal	61. 820	0.05
N-Hexadecanoic acid AscorbicAcid	67. 776	4.97
Isobutyric acid	68. 864	2.52
Heptaethylene Glycol Monododecyl	70. 729	0.78
Octadecenoic acid Oleic acid	71. 856	6.41
Octadecadienoic acid	72. 886	3.74
Octaethylene Glycol Monododecyl Hexaoxacyclononadecane	75. 553	1.09
Methylpropanoate	77. 681	0.58
Tetradecyl Ester	78. 202	0.25
Hexaoxacyclononadecane	80. 616	1.38
Tridecyl Ester	85. 137	0.73
Tridecyl 2-Methylpropanoate	90. 401	0.11
Pentaoxacyclohexadecane	91. 179	0.07

Table 3. GC/MS results of the chemical content of *H. malatyanum* plant extract.

Component	RT	% Area
Methıonıne	5.327	0.02
Caryophyllene	16. 639	0.01
Furancarboxaldehyde	21. 125	0.01
Phenol	35. 029	0.11
Chlororesorcınol	50. 456	0.01
Heptadecenoic acid	54. 707	0.03
Benzenecarboxylic acid	57. 236	0.03
Octadecadienoic acid Linoleic acid	57. 997	0.01
Enoic acid Cyclopentadecanone	60. 464	0.06
Cyclohexene	61. 047	0.02
Cyclohexylidene-N-Butanol	62. 054	0.05
Dihydroxyisopropyl	63. 090	0.16
N-Hexadecanoic acid	67. 776	4.96
Octaethylene Glycol Monododecyl	69. 041	0.70
Hexaoxacyclononadecane	69. 539	1.24
Octadecenoic acid Oleic acid	71. 862	6.54
Eicosadienoic acid Octadecadienoic acid	72. 886	3.95
Tetradecyl Ester	74. 311	0.91
Dihydroxyisopropyl	75. 518	1.14
Hexadecyl Ester	76. 703	3.17
Methylpropanoate	79.152	2.46
Isobutyric acid Tridecyl 2-Methylpropanoate	83.775	1.88
Pentaoxacyclohexadecane	86.470	1.85

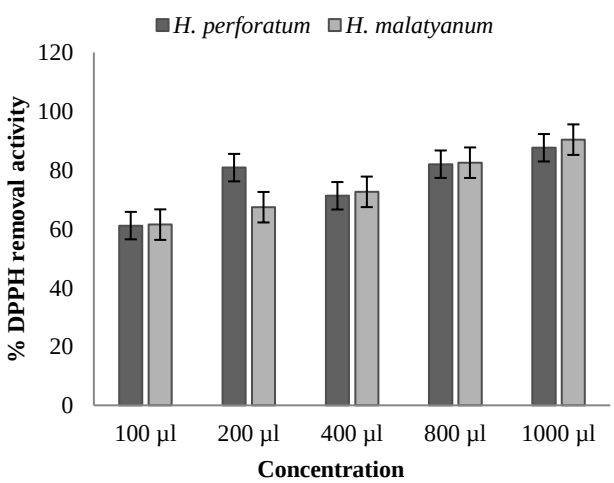

Fig. 1. % DPPH removal capacities of *H. perforatum* and *H. malatyanum* at different concentrations.

Discussion

In this study, the antioxidant, antimicrobial activities and chemical contents of methanol extracts of *Hypericum* species collected from certain regions of Malatya were compared and it was found that our findings were consistent with the literature.

The use of *H. perforatum* as a wound healer since time immemorial has been primarily attributed to its antibacterial action (Sarıkürkçü *et al.*, 2020). It is known that doctors in the Middle Ages treated infected wounds with *H. perforatum* oil. Fluoroglucinol derivative compounds such as deoxyhyperforin, furohyperforin, furoadhyperforin, furohyperforin A, pyranohyperforin, hyperforin isolated from *H. perforatum* species were tested for antifungal activity against *C. albicans* strain and it was found that hyperforin, urohyperforin did not show antifungal activity (Vajs *et al.*, 2003). Similarly, no

antifungal effect as found in this study. In a study, the antibacterial activity of *H. perforatum* extract and amoxicillin was compared, and it was found that the extract was 227 % more effective than amoxicillin against *S.aureus* strain and141% more effective against *P. aeruginosa* strain. In another study investigating the antimicrobial activity of some endemic species of the genus *Hypericum* and *H. perforatum*, it was observed that the extract showed activity against the tested Gram-positive bacteria (*S. aureus*, Methicillin-resistant *S. aureus* and *Streptococcus epidermidis*). The highest activity was obtained in *H. neurocalycinum* and *H. malatyanum*. In addition, some extracts of *H. spectabile* and *H. pseudolaeve* were found to have antifungal activity against *C. albicans* (Özkan *et al.*, 2018). Again, in a study on *Hypericum* species, the antifungal activity of *H. alpinum*, *H. barbatum*, *H. rumeliacum*, *H. maculatum*, *H. perforatum*, *H. hirsutum* essential oils was investigated in *C. albicans* and it was found that *H. alpinum* and *H. hirsutum* essential oils did not show any activity and the others were weakly effective (Saroglou *et al*., 2007). In this study, the maximum antimicrobial activity for *H. perforatum* was found to be 56% and 58% higher than *H. malatyanum*, respectively.

It is known that antioxidant activity is important for the use of herbal products both as food and for their therapeutic properties (Tusevski *et al.*, 2019). In recent years, treatment with plants has become important in addition to natural nutrition. For this purpose, the biochemical effects of the plants used among the people are examined and it is investigated whether these uses have a scientific basis. There is evidence that the curative effects of plants on some diseases are due to natural antioxidants. For this reason, studies on the antioxidant activity of widely used plant extracts and the elucidation of the structures of the compounds responsible for this activity have gained momentum in studies. In a study conducted to evaluate the free oxygen radical scavenging effect and antioxidant effect of the flavonoid extract of *H. perforatum*, it was found that the extract rich in flavonoid content showed strong antioxidant effect and it was stated that it has the potential to be a nutritional support or medicine that can be recommended in heart diseases (Zou *et al*., 2004; Zdunic *et al*., 2017; Eroğlu *et al.*, 2019). In addition, the antioxidant activity and phenolic compounds of roots, flowers and non-flowering shoots of St. John's wort grown in Macedonia were determined using DPPH, Phosphomolybdenum assay, copper ion reducing antioxidant capacity and power reduction methods. Accordingly, the highest amount of phenolic substances was determined in the flowering part (107. 38mg/g) (Tusevski *et al.*, 2019). Although similar plant sand methods were used in the studies, different values were obtained. *Hypericum* species have high antioxidant activity thanks to the large number of different phenolic compounds they contain (Mir *et al.*, 2016; Gitea *et al.*, 2018). This shows that the ecological characteristics of the environment in which the plants grow may be effective on the antioxidant activity of plant parts (Sarıkürkçü *et al.*, 2020; Mir *et al.*, 2016). In our study with *Hypericum* species grown in the ecological conditions of Malatya, it was determined that the scavenging effect of DPPH radical was higher in *H. malatyanum* depending on the concentration.

It is known that many factors effect in the formation of the chemical composition of aromatic plants. In a study, 27 components were identified in *H. perforatum* L., oil, constituting 98.7% of the total composition. The main components identified in this study were α-pinene (61.7%), 3-carene (7.5%), β-caryophyllin (5.5%), myrcene (3.6%), and cadalene (3.2%) (Çakir, 1997). It is assumed that these existing compounds may contribute to antimicrobial activity (Ultee *et al.*, 2002). In addition, the compositional content of the essential oil of this plant was studied and the presence of different compounds was determined (Deveci, 2014). This difference is thought to be related to the different ecological conditions where the plant grows (Zdunic, 2017). Accordingly, in our study, 14 compounds differed in the chemical composition of the plants, while other phytochemicals were found in similar proportions (Tables 2, 3).

Acknowledgements

I would like to thank Şükrü KARAKUŞ, lecturer at İnönü University for his contribution to the collection of *H. malatyanum* from the plants used in the study. In addition, this study was supported by Inonu University Scientific Research Projects with project number 2571.

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(Received for publication 31 July 2024)