Journal of Research in Pharmacy

Research Article

www.jrespharm.com

Antioxidant and antimicrobial activities of *Hypericum origanifolium* Willd. var. *origanifolium*

Zeynep GÜLCAN[1]*, Fatmanur TUNÇ[1], Nagehan SALTAN[1], Pervin SOYER[2], Yavuz Bülent KÖSE[1]

1 Department of Pharmaceutical Botany, Faculty of Pharmacy, Anadolu University, Eskişehir, Türkiye.
2 Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Anadolu University, Eskişehir, Türkiye.
* Corresponding Author. E-mail: zgulcan@anadolu.edu.tr (Z.G.); Tel. +0222 335 07 50 - 3704.

Received: 17 September 2024 / Revised: 7 November 2024 / Accepted: 9 November 2024

ABSTRACT: *Hypericum origanifolium* Willd. var. *origanifolium*, commonly known as "Lüfer otu". Since the number of studies on this taxon is limited, this study aimed to evaluate its biological activities, especially its antioxidant, and antimicrobial activities. Extracts were prepared with methanol, *n*-hexane and ethyl acetate solvents from the aerial parts of the plant. In the DPPH· assay, the *n*-hexane extract exhibited higher antioxidant activity compared to other extracts, with an IC_{50} value of 0.096±0.021 mg/mL. The highest total phenolic content was observed in the *n*-hexane extract (75.73±0.016 mg GAE/g extract), while the lowest total phenolic content was found in the ethyl acetate extract (40.42±0.003 mg GAE/g extract). The extracts showed antimicrobial activity against all tested microorganisms at values ranging from 7.81-4000 µg/mL. It was proven to have strong antibacterial activity, especially against *S. aureus, B. subtilis,* and *E. faecalis* microorganisms, with its low MIC (minimum inhibitory concentration) value (7.81-62.5 µg/mL). The study's findings show that *H. origanifolium* var. *origanifolium* extracts have the potential to be a good source of free radicals. Targeting particular bacterial illnesses may be possible given the notable antibacterial efficacy against particular strains.

KEYWORDS: Antimicrobial; antioxidant; *Hypericum origanifolium*; phenolic content.

1. INTRODUCTION

Hypericaceae is represented by a single genus, *Hypericum* L., in Türkiye. *Hypericum* species are known in folk medicine as kantaron, kanotu, binbirdelik otu, mide otu, kılıçotu, ülser otu [1,2]. According to the Flora of Turkey [3] and the East Aegean Islands, there were 69 species, but recent data show that there are 95 species and 103 taxa of *Hypericum* in Türkiye. Among these, 47 taxa are endemic, with an endemism rate of 50% [2]. *Hypericum* species have been traditionally used for treating wounds, burns, gastric ulcers, anxiety, and mild to moderate depression. Creams and ointments containing the aerial parts of *Hypericum* possess analgesic and antiseptic properties, accelerating the healing process. Oil extracts prepared by soaking the flowers of *Hypericum* in pure olive oil have been used as an effective remedy for wounds and burns for centuries worldwide. *Hypericum* has also been found useful in alleviating vasomotor symptoms such as hot flashes during menopause [4,5].

H. origanifolium is a perennial herb from the *Hypericaceae* family, typically growing to a height of 5-30 cm with erect or ascending branches. Its leaves are oval to elliptic, with small black glands on the surface, and it produces bright yellow, five-petaled flowers. This species thrives in dry grassy slopes and rocky habitats, demonstrating a remarkable ecological tolerance by growing at altitudes ranging from 50 to 2400 meters. It prefers well-drained, often calcareous soils, which allows it to adapt to relatively arid conditions. *H. origanifolium* exhibits a broad distribution across various regions of Türkiye, including Bilecik, Sakarya, Zonguldak, Kastamonu, Tokat, Eskişehir, Ankara, Kayseri, Sivas, Konya, Içel, and Hatay [3].

Secondary metabolites obtained from *Hypericum* species can be sorted into a minimum of 11 categories, encompassing naphthodianthrones, flavonoids, essential oils, flurogonol derivatives, xanthones, organic acids, amino acids, proanthocyanidins, tannins, along with other water-soluble constituents. Recent research has pointed out that hyperforin plays a important role as the primary chemical compound responsible for the antidepressant effects observed in *Hypericum* extracts. Moreover, the antidepressant and

How to cite this article: Gülcan Z, Tunç F, Saltan N, Soyer P, Köse YB. Antioxidant and antimicrobial activities of Hypericum origanifolium Willd. var. origanifolium . J Res Pharm. 2025; 29(2): 599-604.

ISSN: 2630-6344

https://doi.org/10.12991/jrespharm.1662102

antiviral properties of *Hypericum* extracts have predominantly been ascribed to naphthodianthrones, hypericin, and pseudohypericin [6,7]. Hypericin and pseudoypericin, which have red pigments, have many important biological activities. It has many effects such as antimicrobial, antipsoriatic, antitumoral and antidepressant [8].

Smelcerovic et al., analyzed 17 *Hypericum* species, they can be categorized into nine distinct sections. Every extract examined displayed the seven typical components, except for the extracts from *H. origanifolium* and *H. scabrum*, which were deficient in rutin. The highest concentrations of hypericin and pseudohypericin were detected in the extracts derived from two species (*H. perforatum* and *H. triquetrifolium*) that are members of the *Hypericum* section [9].

In a study, the effects of *H. perforatum, H. montbrettii,* and *H. origanifolium* plant extracts were examined on non-small cell lung cancer (A549), cervical adenocarcinoma (HeLa), and normal cells represented by NIH3T3 fibroblast cells. Methods such as MTT assay, neutral red assay, soft agar colony formation, and acridine orange staining tests were applied. Cytotoxic and antiproliferative effects were observed in all tested plant extracts on all examined cells. This study revealed these effects for the first time for *H. montbrettii* and *H. origanifolium*. Despite some apoptotic features in plant extracts, it was determined that the observed antiproliferative effects were not significantly dependent on apoptotic activity [10].

Among *Hypericum* species, *H. perforatum* is the most researched species. *H. origanifolium* has recently been evaluated as a potential alternative to the well-established *H. perforatum* in the European Union market due to its hypericin and phenolic content [11]. Conventional, the aerial parts of the *H. origanifolium* are brewed in hot water and used for hemorrhoids [12]. Given the paucity of research on *H. origanifolium* var. *origanifolium* (Figure), the objective of this investigation was to assess the biological activities of the taxon, with a particular focus on its cytotoxic, antioxidant, and antibacterial properties.

Figure. *Hypericum origanifolium* Willd. var. *origanifolium*

2. RESULTS AND DISCUSSION

2.1. Extract yields

The dried plant's aerial parts underwent maceration using *n*-hexane, ethyl acetate, and methanol in a shaker, lasting 48h. The quantification of the resulting extracts was determined relative to the dry plant material (g extract /100 g plant). The yields of methanol, ethyl acetate, and *n*-hexane extracts were 31.2%, 10.9%, and 10.1%, respectively.

2.2. Antioxidant activity and total phenolic content

Table 1 displays the data for total phenolic content and antioxidant activity. The *n*-hexane extract of *H. origanifolium* var. *origanifolium* showed the highest antioxidant activity, with an IC_{50} value of 0.096±0.021 mg/ml, according to the results of the DPPH· free radical scavenging experiment. The total phenolic content of the extracts was found to be highest in *n*-hexane (75.73±0,016 mg GAE/g extract) and lowest in ethyl acetate (40.42±0.003 mg GAE/g extract).

Table 1. Total phenolic contents and antioxidant activity of extracts

Extracts	GAE (mg GAE/g extract)	DPPH Test IC_{50} (mg/mL)	ABTS test-TEAC (Mm)
Methanol	40.94±0.028	0.147±0.004	2.14± 0.02
***n*-hexane**	75.73±0.016	0.096±0.021	1.90± 0.08
Ethyl acetate	40.42±0.003	0.172±0.027	1.18± 0.07
Gallic acid (GA)	-	0.0016	2.93± 0.03

GAE: gallic acid equivalent, TEAC: Trolox equivalent antioxidant capacity

In the study conducted by Gül et al., ethanol extract of the flowers and leaves from the *H. origanifolium* was obtained. As a result of the DPPH· antioxidant experiment, antioxidant activity in leaves and flowers was found to be 23.16±0.48 and 17.03±0.24 mg Trolox equivalent /g, respectively. The total amount of phenol was found to be 98.89±2.17 and 144.96±2.95 mg GAE/g extract in the flowers and leaves, respectively [13]. In a study by Güzel et al., the antioxidant activity of *H. origanifolium, H. perforatum,* and *H. montbretii* species was examined. Water extracts showed the lowest level of radical scavenging action, whereas ethyl acetate extracts showed the highest level. At the studied concentrations, the most active plants were *H. montbretii* and *H. origanifolium* flowers and leaves. Their activity was similar to that of the positive control, butylated hydroxytoluene. Still, all extracts except leaves of *H. montbretii* showed weakness [10]. The ethanol extract of *H. origanifolium* was investigated in a 2018 study. It was discovered that the dry extract had a total phenolic content of 93.4 ± 1.6 mg GAE/g. and the IC_{50} values found in the bleaching analyses of β-carotene and DPPH· were 230 ± 0.2 µg/mL and 270 ± 0.1 µg/mL, respectively [14]. In this study, the ABTS antioxidant capacity values for *H. origanifolium* extracts were determined as follows: methanol extract, 2.14 ± 0.02 mM; *n*-hexane extract, 1.90 ± 0.08 mM; and ethyl acetate extract, 1.18 ± 0.07 mM. These values indicated moderate antioxidant activity, with the methanol extract showing the highest TEAC value among the solvents tested. The number of studies on the biological activity of *H. origanifolium* is limited in the literature. In a study conducted by Seyrekoglu et al., the antioxidant capacity of *H. origanifolium* ethanol-water (3:7) extract was reported to be the highest, with a TEAC value of 11.28 ± 0.28 mM/gDW, measured by the ABTS assay [15]. The results of our study are consistent with previous findings.

Hypericin and hyperforin are hydrophobic and virtually insoluble in water, being better soluble in nonpolar solvents [16]. *n*-hexane extract is thought to be more effective due to the nonpolar compounds found in *Hypericum*, which are responsible for many biological activities of the plant. It is impossible to make a clear comparison since many factors such as the methods used in examining antioxidant activity and total phenolic content in previous experiments, standard substances, the part of the plant used, and the solvent changed during the experiment.

2.3. Antimicrobial activity

All of the extracts showed antimicrobial activity against all tested microorganisms varied between 7.81- 4000 µg/mL. The MIC values of plant extracts were given in Table 2. It has been proven with its low MIC value (62.5, 31.25, 15.6, 7.81, µg/mL) that it has strong antibacterial activity especially against *S. aureus, B. subtilis* and *E. faecalis* microorganisms. The strong antibacterial activity seen in *B. subtilis* was observed in *n*-hexane and ethyl acetate extracts. This effect is thought to be due to the nonpolar compounds contained in the extracts effective on *B. subtilis*. The *P. aeruginosa* is more resistant than other bacteria strains and the *Candida* species are also more resistant than bacteria species. According to the extract types, the *n*-hexane exhibited strong antimicrobial activity with lower concentrations.

In comparison between bacterial cultures, all extract types showed the highest effect on *B. subtilis, S. aureus* and *E. feacalis*, respectively. This indicates that *Hypericum* extracts are more effective on Gram positive than on Gram negative bacteria. In a study conducted by Kakouri et al. in 2023, the antimicrobial activity of different *Hypericum* species collected from Greece was compared on both Gram positive and Gram negative bacteria. The results showed that Gram-negative bacteria showed resistance to *Hypericum* extracts, while the development of Gram-positive bacteria was well inhibited [17]. Previously, ethanol extract of *H. origanifolium* leaves and flower parts antimicrobial activity against nine bacterial strains was examined. MIC values were 32 and 64 µg/mL in flower and leaf extracts against *S. aureus* and *P. aeruginosa* strains, respectively, and 32 and 128 µg/mL against the *B. subtilis* strain [13]. Among antifungal tests, *H. origanifolium* var. *origanifolium* extracts showed the highest effect against *C. glabrata.* In the study conducted by Tocci et al. in 2018, *H. hircinum, H. maculatum, H. montanum, H. perforatum,* and *H. hirsutum* extracts were found to be 250-500 µg/mL on *C. glabrata*, while in the present study, except for *n*-hexane extract (1000 µg/mL), all of them showed antifungal activity at 250 and 500 µg/mL [18].

Table 2. MIC values of plant extracts (µg/mL)

H. origanifolium var. *origanifolium* extracts and standards	*E.feacalis*	*P.aeruginosa*	*S.aureus*	*B.subtilis*	*C.albicans*	*C.glabrata*
Methanol	31.25	4000	15.6	15.6	2000	500
Ethyl acetate	62.5	2000	31.25	7.81	500	250
n-hexane	31.25	1000	15.6	7.81	1000	1000
Chloramphenicol	125	62.5	0.97	15.6	-	-
Ketoconazole	-	-	-	-	62.5	15.6

3. CONCLUSION

The study's findings show that *H. origanifolium* var. *origanifolium* extracts have the potential to be a good source of free radicals. The significant relationship between total phenolic content and antioxidant activity highlights the function of phenolic compounds in the effects that have been seen. It has also been found very effective against *E. feacalis, S. aureus,* and *B. subtilis* bacterial strains. The significant antibacterial activity against specific strains indicates the possibility of targeting certain bacterial infections. In conclusion, *H. origanifolium* var. *origanifolium* exhibits promising bioactive properties that deserve further exploration in pharmaceutical and nutraceutical fields.

4. MATERIALS AND METHODS

4.1. Plant material and extraction

The plant (ESSE herbarium no: 16296) was taken in May 2022 from the Seyitgazi district of Eskişehir. The aerial parts were extracted using methanol, *n*-hexane, and ethyl acetate. After weighing ten grams of the material, 100 milliliters of solvent were added. The samples were macerated for 24 hours (8×3) at room temperature in an shaker set to 150 rpm. Following the maceration process, the macerates were filtered and the solvents were extracted using a low-pressure rotary evaporator. Until they were used, the solvent-free extracts were kept in a refrigerator at +4 ^{o}C.

4.2. Determination of antioxidant activity and total phenolic content

The antioxidant activities of the extracts of *H.origanifolium* var. *origanifolium* prepared using different solvents (*n*-hexane, ethyl acetate and methanol) from the aerial parts were determined by ABTS·+ and DPPH· free radical scavenging assay. The methods of Kumarasamy et al. were used for the determination of DPPH· radical scavenging effect. % Inhibition was calculated with the formula given below [19].

$$\% \text{ Inhibition} = [(Abs_{control} - Abs_{sample}) / Abs_{control}] \times 100$$

IC_{50} values were determined with the help of nonlinear regression curves (SigmaPlot 13th version, SPSS Inc., Chicago, IL).

The ABTS·+ (2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging detection was performed as reported by Re et al. (1999) [20]. The antioxidant effects of the samples were determined by comparing them with the ABTS$^{\bullet+}$ radical scavenging effect of Trolox (standard substance). Samples prepared at 1 mg/mL concentration were studied under the same conditions as Trolox (3; 2; 1; 0.5; 0.25 mM).

The extract's total amount of phenol was determined as equivalent to mg Gallic Acid using the Folin-Ciocalteu reagent method. The method based on the Folin-Ciocalteu reagent (FCR), known as the total phenolic reagent, determines the reducing capacity of the sample. Gallic acid is usually used as the standard compound and results are given as gallic acid equivalent (mg/mL). Different solutions of gallic acid were prepared in the concentration range of 1-0.03 mg/mL, absorbance values were recorded at 760 nm, and the calibration equation was determined by drawing the concentration absorbance graph of gallic acid. Samples absorbance measurements at 760 nm were taken and compared with the gallic acid calibration curve. TFC (Total Phenolic Content) was calculated as Gallic Acid Equivalents (GAE) in mg/g dry weight of plant material. A reagent mixture without extract and solvent was used as a control. Three parallel experiments were performed then the results were given as mean values [21,22].

4.3. Microbroth dilution method

By using microbroth dilution method minimum inhibitory concentration (MIC) of extracts were determined against standard microorganisms. The MIC's were defined as the lowest concentrations necessary for the inhibition of growth [23]. Different concentrations of methanol, *n*-hexane, and ethyl acetate extracts were applied to *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* NRRL B478, *Enterococcus faecalis* ATCC 51299, *Candida albicans* ATCC 90028, and *Candida glabrata* ATCC 90030 microorganisms. All plant extracts were weightened and dissolved in the appropriate solvents. The concentrations of plant extracts were prepared by two-fold serial dilution technique in 96-well microplate to 4000, 2000, 1000, 500, 250, 125, 62.5, 31.25, 15.6, 7.81, 3.90, 1.95 µg/mL. The McFarland 0.5 standard (1×10^8 cfu/mL) was adjusted for the microbe cultures overnight in sterile saline (0.85%) solution. 100 µL of each microorganism culture was then injected onto the plates and incubated for 24 hours at 37°C. As a reference control, ketoconazole was utilized for fungus and chloramphenicol for bacteria species. Following the incubation period, wells were stained with 20 µL of resazurin (0.01%) dye in order to see the color differences between living and dead cells (which ranged from pink to blue-green). The experiment was repeated three times and the results were averaged.

Acknowledgements: -
Author contributions: Concept – Z.G., F.T., N.S., P.S., Y.B.K.; Design – Z.G., YBK.; Supervision – Y.B.K.; Resources – Z.G., F.T., N.S., P.S.; Materials – Z.G., F.T., N.S., P.S.; Data Collection and/or Processing –Z.G.; Analysis and/or Interpretation – Z.G., F.T., N.S., P.S.; Literature Search –Z.G.; Writing – Z.G.; Critical Reviews – Z.G., F.T., N.S., P.S., Y.B.K.
Conflict of interest statement: The authors declared no conflict of interest.

REFERENCES

[1] Baytop T, Türkiye'de Bitkiler ile Tedavi (Geçmişte ve Bugün), Nobel Tıp Kitabevleri, İstanbul, 1999, pp. 166-167.
[2] bizimbitkiler.org.tr. https://bizimbitkiler.org.tr/v2/filtre.php?familya=Hypericaceae&taxon=&taxonKriter=0&epitetType=0&epitet=&epitetKriter=0&authorType=0&author=&authorKriter=0&sayfa=1&trkAdi=&trkKriter=0 (accessed March 13, 2024).
[3] Davis P. (Eds). Flora of Turkey and The East Aegean Islands, Volume 2, Edinburgh Univ, Edinburgh, 1967.
[4] Yeşilada E, İyileştiren Bitkiler, Hayy kitap, İstanbul, 2019.
[5] Eatemadnia A, Ansari S, Abedi P, Najar S. The effect of *Hypericum perforatum* on postmenopausal symptoms and depression: A randomized controlled trial. Complement Ther Med. 2019; 45: 109-113. https://doi.org/10.1016/j.ctim.2019.05.028.
[6] Saltan N, Köse YB, Kırcı D, Göger F, Demirci B. Chemical profiling and antimicrobial activity of essential oils from *Hypericum adenotrichum* Spach. An endemic species. J Pharm Res. 2023; 27(4): 1645-1657. http://doi.org/10.29228/jrp.449.
[7] Medina MA, Martinez-Poveda B, Amores-Sanchez MI, Quesada AR. Hyperforin: More than an antidepressant bioactive compound?. Life Sci. 2006; 79: 105–111. https://doi.org/10.1016/j.lfs.2005.12.027.
[8] Guedes RC, Eriksson LA. Theoretical study of hypericin. J Photochem Photobiol A. 2005; 172: 293–299. https://doi.org/10.1016/j.jphotochem.2004.12.025.
[9] Smelcerovic A, Zuehlke S, Spiteller M, Raabe N, Özen T. Phenolic constituents of 17 *Hypericum* species from Turkey. Biochem Syst Ecol. 2008; 36: 316-319. https://doi.org/10.1016/j.bse.2007.09.002.
[10] Güzey G, Ibadova S, Öztürk Y, Öztürk N, Maggi F, Sagratini G, Ricciutelli M, Vittori S. Antiproliferative and antioxidant effects of three *Hypericum* species of Turkish origin: *H. perforatum, H. montbretii* and *H. origanifolium*. Med Aromat Plant Sci Biotechnol. 2011; 5(special issue 1): 91-99.
[11] Bertoli A, Çirak C, Seyis F. *Hypericum origanifolium* Willd.: The essential oil composition of a new valuable species. Ind Crops Prod. 2015; 77: 676-679. https://doi.org/10.1016/j.indcrop.2015.08.014.
[12] Kolit D. Mater's Thesis. Sille (Selçuklu/Konya) ve Çevresinin Etnobotanik Özellikleri. Institute of Science, Gazi University, Ankara, Turkey, 2022.
[13] Gül LB, Özdemir N, Gül O, Çırak C, Can AH. Some bioactive properties and antimicrobial activity of some *Hypericum* species growing wild in Black Sea Region of Turkey. Eur Food Sci Eng. 2021; 2(1): 1-6.
[14] Boran R. Investigations of anti-aging potential of *Hypericum origanifolium* Willd. for skincare formulations. Ind Crops Prod. 2018; 118: 290-295. https://doi.org/10.1016/j.indcrop.2018.03.058.
[15] Seyrekoglu F, Temiz H, Eser F, Yildirim C. Comparison of the antioxidant activities and major constituents of three *Hypericum* species (*H. perforatum, H. scabrum* and *H. origanifolium*) from Turkey. S Afr J. 2022; 146: 723-727. https://doi.org/10.1016/j.sajb.2021.12.012.
[16] Kubin A, Loew HG, Burner U, Jessner G, Kolbabek H, Wierrani F. How to make hypericin water-soluble. Die Pharmazie. 2008; 63: 263–269. https://doi.org/10.1691/ph.2008.7292.

[17] Kakouri E, Daferera D, Trigas P, Charalambous D, Pantelidou M, Tarantilis PA, Kanakis CD. Comparative study of the antibacterial activity, total phenolic and total flavonoid content of nine *Hypericum* species grown in Greece. Appl Sci. 2023; 13(5): 3305. https://doi.org/10.3390/app13053305.

[18] Tocci N, Perenzoni D, Iamonico D, Fava F, Weil T, Mattivi F. Extracts from *Hypericum hircinum* subsp. *majus* exert antifungal activity against a panel of sensitive and drug-resistant clinical strains. Front Pharmacol. 2018; 9: 382. https://doi.org/10.3389/fphar.2018.00382.

[19] Kumarasamy Y, Byres M, Cox PJ, Jaspars M, Nahar, Sarker SD. Screening seeds of some Scottish plants for free radical scavenging activity. Phytother Res. 2007; 21(7): 615-621. https://doi.org/10.1002/ptr.2129.

[20] Re R, Pellegrini N, Proteggente A, Pannalaa A, Yanga M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorisation assay. Free Radic Biol Med. 1999; 26: 1231–1237. https://doi.org/10.1016/s0891-5849(98)00315-3.

[21] Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods Enzymol. 1999; 299: 152-178. https://doi.org/10.1016/S0076-6879(99)99017-1.

[22] Kosar M, Göger F, Can Baser KH. In vitro antioxidant properties and phenolic composition of *Salvia virgata* Jacq. from Turkey. J Agric Food Chem. 2008; 56(7): 2369-2374. https://doi.org/10.1021/jf073516b.

[23] Clinical and Laboratory Standards Institute M07-A9. Reference Methods for Dilution Antimicrobial Susceptibility Tests f or Bacteria That Grow Aerobically; Approved Standard- Ninth Edition, CLSI document M07-A9. 2012; 29(2).