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The Antimicrobial and Antioxidant Effects of *Equisetum arvense* Extracts

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Abstract: *Equisetum arvense* L, also known as horsetail, is a medicinal plant used in traditional medicine. Especially, it is used in the treatment of bleeding, antiseptic, anti-inflammatory, urethritis, jaundice and hepatitis. In the study, the antimicrobial and antioxidant activities of extracts obtained from different solvents of *E. arvense* were investigated. Antimicrobial activity of *E. arvense* extracts was determined using the disc diffusion method. The antimicrobial activity was determined utilizing the pathogenic microorganisms *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Bacillus megaterium*, *Candida albicans* and *Candida glabrata*. In the results obtained, it was determined that the ethanol extract of *E. arvense* at 500 µg concentration showed antimicrobial activity at different rates (14.3-28.0). Ethanol extract showed the highest antimicrobial activity against *Candida glabrata* (28.0 mm) at the same concentration. It was detected that the chloroform extract showed antimicrobial activity (7.3-10.6 mm) against the microorganisms used. The antioxidant activity of the aerial parts of *E. arvense* at different concentrations of methanol extract was determined according to the 2,2-diphenyl-1-picrylhydrazil radical scavenging capacity method. The highest radical scavenging capacity of the methanol extract was observed at a concentration of 10mg/mL (91.5%). The IC_{50} value of the methanol extract of *E. arvense* was calculated as 3.13 mg/mL.

Key words: *Equisetum arvense*, antimicrobial, antioxidant, medicinal plant.

Equisetum arvense Ekstraktlarının Antimikrobiyal ve Antioksidan Etkileri

Öz: Atkuyruğu olarak bilinen *Equisetum arvense* L. geleneksel tıpta kullanılan tıbbi bir bitkidir. Özellikle; kanama, antiseptik, antiinflamatuar, üretrit, sarılık ve hepatit tedavisinde kullanılır. Çalışmada *E. arvense*'nin farklı solventlerinden elde edilen ekstraktların antimikrobiyal ve antioksidan aktiviteleri araştırılmıştır. *E. arvense* ekstraktlarının antimikrobiyal aktivitesi disk difüzyon yöntemi kullanılarak tespit edilmiştir. Antimikrobiyal aktivite patojenik mikroorganizmalar *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Bacillus megaterium*, *Candida albicans* ve *Candida glabrata* kullanılarak belirlendi. Elde edilen sonuçlarda *E. arvense*'nin 500 µg konsantrasonda etanol ekstraktının farklı oranlarda (14,3-28,0) antimikrobiyal aktivite gösterdiği belirlendi. Etanol ekstraktı aynı konsantrasyonda *Candida glabrata*'ya (28,0 mm) karşı en yüksek antimikrobiyal aktivite göstermiştir. Kloroform ekstraktının kullanılan mikroorganizmalara karşı antimikrobiyal aktivite gösterdiği (7,3-10,6 mm) tespit edilmiştir. *E. arvense*'nin toprak üstü kısımlarının metanol ekstraktının farklı konsantrasyonlarındaki antioksidan aktivitesi, 2,2-diphenyl-1-picrilhydrazyl radikal temizleme kapasitesi yöntemine göre tespit edilmiştir. Metanol ekstraktının en yüksek radikal temizleme kapasitesi 10mg/mL (%91,5) konsantrasyonda görülmüştür. *E. arvense*'nin metanol ekstraktının IC_{50} değeri 3,13 mg/mL olarak hesaplandı.

Anahtar kelimeler: *Equisetum arvense*, antimikrobiyal, antioksidan, tıbbi bitki.

1. Introduction

Medicinal aromatic plants have been used since the existence of humanity to prevent and cure diseases and to maintain health. In addition, it is used in many industries such as food, perfume and cosmetics, and the importance given to medicinal plants is increasing [1, 2]. For this reason, the majority of the population in the world finds herbal medicines more reliable [3]. Studies on the antimicrobial and antioxidant effects of some components in herbal sources are increasing rapidly [3-4]. Today, considering that bacteria have important power over antibiotics, different alternative ways are sought. In particular, thanks to the secondary metabolites of plants, this can be prevented [5, 3]. In addition, plants protect the organism against oxidation thanks to these components. For this reason, plants, which are one of the natural antioxidant sources, have started to be used more than synthetic antioxidants [2-4].

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Equisetum arvense (field horsetail) L. belongs to the *Equisetaceae*. It is a northern hemisphere herbaceous perennial plant that has long been utilized for medicinal reasons. This species are found across Canada, Europe, the United States (except for the southeast) southern Asia, and Africa, including Turkey, the Himalayas, Iran, Japan, China (except for the southeast), and Korea [6-8]. The horsetail has been used as an anti-inflammatory agent in Europe, Asia and America, as well as an antiseptic agent in Turkey and in the United States for years [9-12].

Thanks to its anti-inflammatory effect, *E. arvense* is known to be used in kidney stones, gout, and prostate disease. Additionally, its tea is good for mouth and gum infections [7]. In addition, it is used among the public as a hemostatic agent in the treatment of tuberculosis and menstrual bleeding [8]. Studies have confirmed various biological effects of *E. arvense*, such as sedative, anticonvulsive, hepatoprotective, antioxidant, antibacterial and antifungal activity [13-16]. *E. arvense* is used in food supplements and alternative medicine thanks to the phytochemicals it contains. Its biological activity is related to the content of various classes of secondary metabolites such as phenolics (flavonoids, styryl pyrones and phenolic acids), alkaloids (equisetin, nicotine, palustrine), phytosterols (campesterol), and minerals (silica, calcium, magnesium, selenium, iron, potassium, zinc, etc.) [8, 15-16].

The aim of the present study was to evaluate the antimicrobial effect of ethanol, methanol and chloroform extraction of the aerial parts of *E. arvense* and to evaluate the 2,2-diphenyl-1-picrylhydrazine (DPPH) radical scavenging effect of methanol extract.

2. Material and Method

2.1. Collecting and Obtaining of Sample

E. arvense plant was obtained from herbalists in 2020. Taxonomic description of plant material was carried out by the systematics-botany expert Prof. Dr. Şemsettin Civelek of Fırat University using the book Flora of Turkey. The powdered dry plant material weighed 0.5 g. 100 mL of solvent 96% methanol (MeOH), ethanol (EtOH) and chloroform were added to the weighed plant. It was then stirred on a rotary shaker in a dark environment at room temperature for 72 hours and filtered using Whatman filter paper. The prepared extracts were stored at +4°C.

2.2. Antimicrobial Assay

The antimicrobial activities of *Equisetum arvense*'s chloroform, ethanol and methanol extracts were performed according to the method specified by Collins and Lyne [17]. Prepared broth cultures yeast (*Candida albicans* and *Candida glabrata*) and bacterial (*Staphylococcus aureus* ATCC25923, *Klebsiella pneumoniae* ATCC 700603, *Escherichia coli* ATCC25322, *Bacillus megaterium* DSM32) were cultured on Sabouraud Dextrose Agar (Difco) and Müeller Hinton Agar (Difco), respectively inoculated at 1% (10^6 bacteria/mL, 10^4 yeast/mL) and placed in sterile petri dishes. Antimicrobial discs (6 mm diameter), each impregnated with 100 µl (500 µg) of different extracts, were gently transferred on agar medium. Following incubation for 1.5-2 hours at 4°C, the bacteria and yeast were transferred onto plates and incubated for 24 hours at 37 ± 0.1°C, and for 72 hours at 25 ± 0.1°C, respectively. Nystatin (30 µg/disc) (for yeast) and Streptomycin sulfate (10 µg/disc) (for bacteria) were used as standard discs. The zones (mm) were then measured.

2.3. Antioxidant Assay

The antioxidant assay was found by using 2,2-diphenyl-1-picrylhydrazine (DPPH) radical scavenging capacity of [18,19]. Plant extracts were prepared in methanol at concentrations of 1.25, 2.5, 5, and 10 mg/mL. 3 ml of the DPPH solution (0.004%) was prepared before the study was taken and 30 µL of plant extracts were added to it and left in the dark for 30 minutes. The activity was measured spectrophotometrically at 517 nm. Antioxidant activity was performed in triplicate. Methanol and butylated hydroxyanisole (BHA) were utilized as control. The percent scavenging effect of the DPPH radical was measured using the formula (1).

$$\%\text{DPPH inhibit} = [(\text{Abs Control} - \text{AbsSample}) \div \text{AbsControl}] \times 100 \quad (1)$$

2.4. Statistical Analysis

SPSS Statistics (version 22) was used to perform the statistical analysis and generate the figures. Analysis of variance (ANOVA) and Student's t-test were performed, and $p < 0.01$ was considered significant.

3. Results and Discussion

3.1. Antimicrobial Assay

The antimicrobial effects of the aerial parts of *E. arvense* are shown in Table 1. In the results obtained, the inhibition zones of methanol extract against *B. megaterium*, *K. pneumoniae*, *S. aures*, *E. coli*, *C. glabrata* and *C. albican*s were determined 20.3, 20.3, 20.3, 20.0, 21.6 and 20.6, respectively. Ethanol extract showed antimicrobial effect against the same microorganisms at different rates (14.3-28.0 mm). It was detected that the chloroform extract showed antimicrobial activity (7.3-10.6 mm) against *C. glabrata*, *C. albicans*, *S. aureus*, *E. coli* and *B. megaterium*, but not against *K. pneumoniae* (Table 1).

Table 1. Inhibition zones of *E. arvense* extracts (mm).

		Extracts		
Microorganisms	**Control**	***E. arvense*-M***	***E. arvense*-E***	***E. arvense*-C***
E. coli	10.6 ± 0.33	20.0 ± 0.57	15.3 ± 0.66	7.6 ± 0.33
K. pneumoniae	17.0 ± 0.57	20.3 ± 0.33	15.0 ± 0.57	-
C. albicans	9.6 ± 0.33	20.6 ± 0.34	14.3 ± 0.66	7.3 ± 0.33
C. glabrata	22.6 ± 0.33	21.6 ± 0.33	28.0 ± 0.57	9.6 ± 0.33
S. aureus	14.6 ± 0.34	20.3 ± 0.33	18.3 ± 0.33	10.6 ± 0.34
B.megaterium	9.6 ± 0.33	20.3 ± 0.34	20.3 ± 0.33	7.6 ± 0.33

**E. arvense*-M: methanol extract of *Equisetum arvense*; *E. arvense*-E: ethanol extract of *Equisetum arvense*; *E. arvense*-C: chloroform extract of *Equisetum arvense.*

In the previous study, while the water extract of *E. arvense* showed an antimicrobial effect against *S. aureus*, *S. pneumoniae* and *S. pyogenes* at a concentration of 100 mg/mL (8mm-11mm), it did not show an antimicrobial effect against *C. albicans* and *E. coli* [20]. Kukrić et al.[15] determined that the methanol extract of *E. arvense* L. had the highest antibacterial activity against *S. aureus* with MIC and MBC (11.14 and 22.28 mg/mL). Inhibition zones of hydro-alcoholic extracts of *E. arvense* against *E. coli*, *K. pneumoniae*, *S. aureus* and, *C. albicans*, were reported as 12.1 mm, 11.3 mm, 11.7 mm and 13.1 mm, respectively [21]. Ethyl acetate, aqueous extracts and chloroform from the same plant did not show antimicrobial activity against *E. coli*. However, ethyl acetate and chloroform extracts created a 9 mm zone of inhibition against *S. aureus* [22]. The ethanol extracts of *E. arvense* at 1000 µg concentration were reported as 11 mm, 18 mm,14 mm and 18 mm zones of inhibition against *P. aeruginosa, K. pneumoniae*, *S. aureus* and *E. faecalis,* respectively [23]. The methanol extract of *E. arvense* showed antimicrobial activity against *E. coli* at a concentration of 1mg/mL [24]. The zone diameters of 4 different extracts of *E. arvense* (hexane, ethyl acetate, ethanol and methanol) were determined to be 9-15 mm. The highest antimicrobial activity of the plant extracts was observed against 15 mm *C. albicans* yeasts [25]. It was determined that *E. arvense* ethanol extracts did not have an antimicrobial effect against *E. coli* at concentrations of 5 µL and 10 µL [26]. The minimum concentration values of *E. arvense* shoots methyl and ethyl extracts against clinical isolates *S. aureus* and *S. aureus* were determined in the range of (20.58, 15.5 mg/mL), respectively. It has been reported that the minimum concentration values of methyl and ethyl extracts against *E. coli* are in the range of 15.41- 12.58 mg/mL [27]. It was reported that the essential oil of *E. arvense* had the highest antimicrobial effect against *K. pneumoniae* (37 mm) and *S. enteritidis* (35 mm) [28]. The same species formed 14.67 mm, 15.33 mm and 14.33 mm inhibition zones against *E. coli*, *Y. enterocolitica* and *S. enterica*, respectively [29]. When the results obtained are compared with previous studies, it is seen that the results obtained show similarities with literature studies [21, 23, 25, 26, 28, 29] It is seen that the results vary depending on the solvents and concentrations used.

3.2. Antioxidant Assay

The DPPH radical scavenging activities of the aboveground parts of the methanol extract of *E. arvense* at concentrations of 1.25 mg/mL, 2.5 mg/mL, 5 mg/mL and 10 mg/mL are shown in Figure 1. Methanol extract showed a DPPH radical scavenging effect of 24.5% at 1.25 mg/mL concentration, 46.6% at 2.5 mg/mL concentration, 81.7% at 5 mg/mL concentration and 91.5% at 10 mg/mL concentration. It was observed that the DPPH radical scavenging effect of *E. arvense* increased with increasing concentrations. The IC_{50} value of the methanol extract of *E. arvense* was calculated as 3.13 mg/mL. The DPPH radical scavenging activity of the aerial parts of the methanol extracts of *E. arvense* at different concentrations is shown in Figure 1.

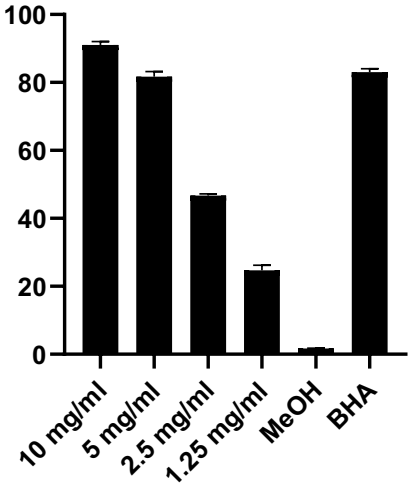

Figure 1. Percent inhibition of the DPPH radical of *E.arvense*.

In previous studies, it was reported that the EtOAc extract of *E. arvense* has a scavenging effect on DPPH radicals of 2.37 µg/mL, and the water extract is 37.20 µg/mL. Ethanol extract has a higher antioxidant effect than water extract in this study.This is explained by the fact that ethanol extract contains significant amounts of quercetin 3-*O*-glucoside (isoquercitrin), apigenin 5-*O*-glucoside, and kaempferol 3-*O*-glucoside [30]. It was determined that the pectins obtained from the stems of *E. arvense* have a 63% scavenging effect on the DPPH radical [31]. It was reported that the antioxidant effect of *E. arvense* sterile stem extracts was 87.30%. In the same study, epicatechin, which is known to have antioxidant effects, was detected in *E. arvense* sterile stem extracts [16]. Free radical scavenging effects of *E. arvense*'s foliage and central stalk were determined as 71.37%-96.22% mg/mL at different concentrations. In the same study, scavenging activity for rhizomatous stem and root was reported as 70.55-94.66% mg/mL [32]. The horsetail n-butanol extract has been reported to DPPH (EC_{50}=0.65 mg/mL) and hydroxyl radical scavenging activities (EC_{50}=0.74 mg/mL) respectively. It has been reported that there may be a relationship between the high phenolic content of n-Butanol extract and its antioxidant effect. [14]. Wang et al. [33] determined that the IC_{50} value of the antioxidant effect of *E. arvense* extract was 12.3 µg/ml. In the antioxidant study of the essential oil of *E. arvense* using RSA and FIC methods, IC_{50} values were found to be 952.7 and 1, 282.7 µg/mL, respectively. It was determined that the IC_{50} value of the scavenged DPPH of *E. arvense* extract was 15.2 µg/mL [34]. The scavenging effect of methanol, ethanol and water extracts of *E. arvense* on DPPH radical was calculated as 1847, 2217 and 374 µmol TE g^{-1}, respectively. Ethanol extract was found to be rich in flavonoids, flavonoid-*O*-glycosides, phytosterols, phenolic and fatty acids, as well as in minerals and mainly in K, Ca, Mg, Si and P [35]. The highest DPPH radical scavenging effect of *E. arvense* was found in the ethanol extract (IC_{50}=2.37 µg/mL) [30]. The IC_{50} value of the antioxidant effect of *E. arvense* was reported as 13.5 µg/mL [15]. The percent inhibition of the DPPH radical scavenging effect of *E. arvense* was calculated as 87.50 [16]. The results obtained are compared with previous studies. [16, 30-33], [14], it is known that the results differ depending on the methods used, the concentrations used, the solvent used, and the phytochemicals they contain and their amounts.

4. Conclusion

The antimicrobial and DPPH radical scavenging effects of the aerial parts of *E. arvense* on some pathogenic microorganisms were investigated. It was found that the methanol extract different solvents 20.3, 20.3, 20.3, 20.0, 21.6 and 20.6, and mm inhibition zones against *B. megaterium*, *S.aureus*, *K.pneumoniae*, *E.coli, C.glabrata* and *C.albicans*. *E. arvense*'s ethanol extract showed the highest antimicrobial activity against *C. glabrata* (28.0 mm). The highest DPPH radical scavenging effect of methanol extract was at 10mg/ml (%91.5) concentration. Considering that *E. arvense* is responsible for the bioactivity of the biochemicals it contains, the results of the study may be important in terms of the literature.

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