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The effects of *Equisetum arvense* L. extracts prepared using different solvents and extraction methods for antioxidant and antimicrobial activity

Şule BARAN, Sarah ALLAZAWI, Alican Bahadır SEMERCİ

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Sakarya University, Faculty of Arts and Sciences, Department of Biology, Sakarya, Türkiye

ORCID IDs of the authors:
Ş.B. 0000-0003-2497-5876
S.A. 0000-0002-9242-0349
A.B.S. 0000-0001-9502-9321

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Correspondence:
Şule BARAN
E-mail: sbaran@sakarya.edu.tr

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ABSTRACT

Our study aimed to determine the solvent and extraction method that reveals the biological activities and phenolic contents of the plant *Equisetum arvense* at the maximum rate. Leaf and stem extracts of *Equisetum arvense* were prepared using the soxhlet and maceration method in the presence of 5 different solvents. The extracts' total phenolic content (TPC) was determined using the Folin-Ciocalteu method, the antioxidant activity by the DPPH radical scavenging test, and the antimicrobial activity by the disc diffusion method. When the antibacterial activity results were examined, the methanolic leaf extract prepared by the maceration method showed the inhibition zones as 14.5 mm on *S. aureus*, 14.2 mm on *S. epidermidis* and 14 mm on *E. faecalis*. From leaf parts, it was determined that the methanolic extract prepared by the soxhlet method was 85.1%, the acetonic extract 84.5%, the methanolic extract prepared by the maceration method 83%, and the acetonic extract 84.1% scavenged DPPH radical. As a result of the study, it was determined that the maceration method showed better results in evaluating the total phenolic substance amount and antimicrobial activity and the soxhlet method in determining the antioxidant activity. It has been determined that methanol and acetone were the ideal solvents for TPC antioxidant and antimicrobial activity studies to be carried out with *E. arvense* plant. In this study, plant leaves and stems were studied separately for the first time and their biological activities were compared. In addition, our study provides integrative data investigating and comparing the antioxidant and antimicrobial activities of the *E. arvense* plant in detail with various solvents and methods.

Keywords: *Equisetum arvense*, Antibacterial activity, DPPH radical

Introduction

It has been known for many years that plants, fruits and vegetables have antioxidant, antimicrobial and anti-inflammatory effects due to their phytochemical composition. They are used in medicine and nutrition, including the cosmetic industry (Tan & Lim, 2015; Pallag et al., 2018). Herbaceous plants of Equisetum (horsetail) are widely distributed worldwide, excluding Australia. *E. arvense* L. is a perennial fern from the Equisetaceae family. In traditional medicine, horsetail plants have long been used (Patova et al., 2019). As a skin antiseptic, *E. arvense* is used in folk medicine to treat kidney and stomach diseases (Badole & Kotwal, 2015). Many *E. arvense* herbal products are mainly used against urinary and kidney disorders. It is also used in skin, hair and nail care due to the species' potentially high silica content (Saslis-Lagoudakis et al., 2015).

The antioxidant activities of plant extracts depend on the extraction efficiency of the bioactive components and the composition of the extract. Extraction with solvents is frequently used for the isolation of antioxidant compounds, and both the extraction efficiency and antioxidant activity of the extracts are closely related to the used solvent, mainly due to the different polarities of the obtained compounds (Moure et al., 2001; Tran et al., 2020). Organic solvents (petroleum ether, hexane, chloroform, methanol, etc.) are widely used to extract phenolic compounds as antioxidants (Oniszczuk et al., 2014). The choice of the most suitable solvent is a determining factor of the extract properties, and due to the different composition and structure, each matrix-solvent system exhibits a specific unpredictable behaviour (Fu et al., 2016).

There are various studies on the active ingredient contents and biological effects of *E. arvense.* The effect of extraction parameters on extract composition has been evaluated in the literature. Still, differences in biological activity based on composition changes have rarely been considered in extraction studies. Active phenolic compounds and the effect of these parameters on biological activities can be altered by changing the extraction method and the solvent used. Extensive studies comparing extractions and biological activities are needed to isolate active phenolic compounds of extracts and elucidate their effects on biological activity. In this context, the study aims to evaluate the effect of the total phenolic content antimicrobial and antioxidant activity of 20 different extracts obtained from the leaf and stem of *E. arvense* (using two different extraction methods and five different solvents).

Materials and Methods

Chemicals and Reagents

All the chemicals used in this study (petroleum ether, ethyl acetate, chloroform, methanol and acetone) and reagents (2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu, Mueller Hinton Agar, Tryptic Soy Broth, Ascorbic Acid, Sodium Carbonate, Gallic Acid) were of analytical grade and obtained from Sigma or Merck Company, Germany.

Plant Material and Preparation of Plant Extracts

Plant samples were collected by Şule Baran in May-June 2021 (Kazımpaşa-Serdivan/Sakarya). After the samples were sorted and cleaned, they were dried at room temperature in the shade for seven days. The dried stem and leave samples were grinded separately in an electric grinder. Scanning electron microscopy revealed a morphological examination of dried leaf samples (Figure 1).

The dried samples were extracted by soxhlet and maceration method. Maceration method: 15 grams of sample was mixed with 150 mL of solvent (ethyl acetate, petroleum ether, chloroform, methanol and acetone) and kept at room temperature in the dark for 72 hours. Then, the extracts were filtered on filter paper. Soxhlet method: Powdered samples (15 g) were put into soxhlet cartridges and extracted in 150 mL of solvents (petroleum ether, ethyl acetate, chloroform, methanol and acetone) for eight hours. All extracts were evaporated under a vacuum at 45°C, and the obtained extracts were stored at -20°C until performing chemical and biological analyses. The extracts' yield was calculated as the dry weight of the extract relative to the raw material weight: yield (%) = m/M×100 (M is the raw material weight, and m is solvent-free extract mass).

Determining of Total Phenolic Content

TPC (Total phenolic content) determination was done using the Folin-Ciocalteu method developed by Singleton and Rossi (1965). 0.1 mL of the prepared extract (1mg/mL) was taken, 0.2 mL of 50% Folin Ciocalteu reagent was added and left for 3 minutes. 1 mL of 2% Na_2CO_3 solution was added to it, and it was left to rest in the dark for 1 hour. At the end of the period, absorbances were measured at 760 nm in the spectrophotometer. A calibration curve was employed for gallic standard, and TPC extract was expressed as mg per gr (mg GA/g).

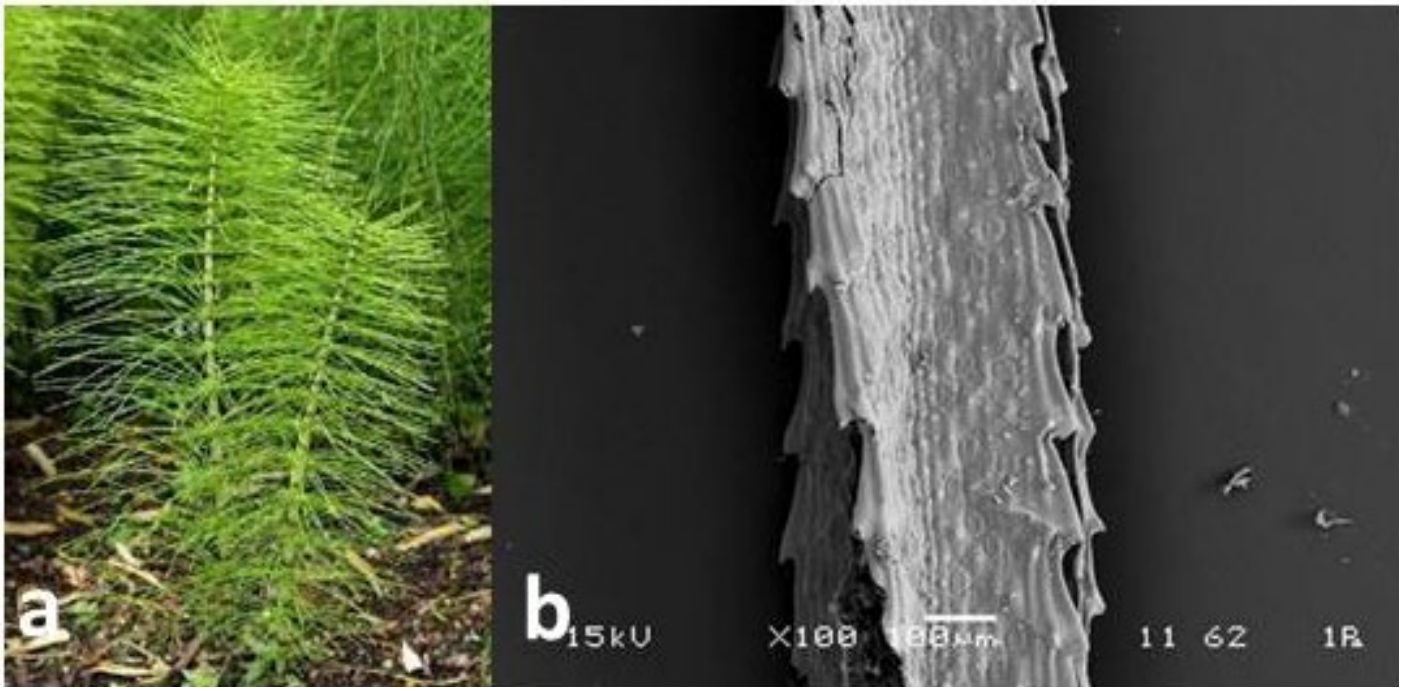

Figure1. a) General image b) SEM image of *E. arvense* leaf surface (X100)

Antioxidant Activity

The modified Blois method was used to investigate the antioxidant activity of *E. arvense* extracts. (Blois, 1958). 1 mL of 0.004% solution of DPPH radical in ethanol was mixed with 1 mL of extract solution (100 µg/mL). The mixture was held in a dark place for 30 minutes, and then optical density was measured at 517 nm in a spectrophotometer. Ethanol was used as a blank. For determination of the % DPPH radical scavenging activity the following equation was used: %DPPH radical scavenging = [(control absorbance-extract absorbance)/control absorbance] x 100

Antibacterial Activity

Staphylococcus epidermidis ATCC 12228, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 8739, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, *Salmonella typhimurium* ATCC 14028 were used as test microorganisms for determination of antibacterial of *E. arvense*. Overnight bacterial cultures were utilised to prepare the bacterial suspensions (adjusted to 0.5 McFarland using a densitometer). The disc diffusion method absorbed 20 µL of the previously prepared extracts (1 mg/mL concentration) into a 6 mm diameter sterile disc. density-adjusted bacteria suspensions were inoculated to Mueller Hinton Agars (MHA) using sterile swabs. Impregnated discs were periodically placed on inoculated MHA. After a 24-hour incubation at 37°C, the inhibition zone (IZs) diameters were measured with a digital calliper. Solvent (petroleum ether, ethyl acetate, chloroform, methanol and acetone) impregnated discs were used as a negative control, and commercial antibiotic discs (Gentamicin (10 mcg) were utilised as a positive control.

Statistical Analysis

All analyses were carried out in triplicate, and results were given as mean ± SD. Statistical analyses were performed using the SPSS 20.0 program. Statistical data analyses were obtained using the Duncan test ($P < 0.05$) and One-way ANOVA.

Results and Discussion

Various methods of extraction of natural compounds have different extraction yields and efficiency. Our extraction yield results, which were evaluated with *E. arvense* stem and leaf parts using 2 different extraction methods and 5 different solvents, are shown in Figure 2.

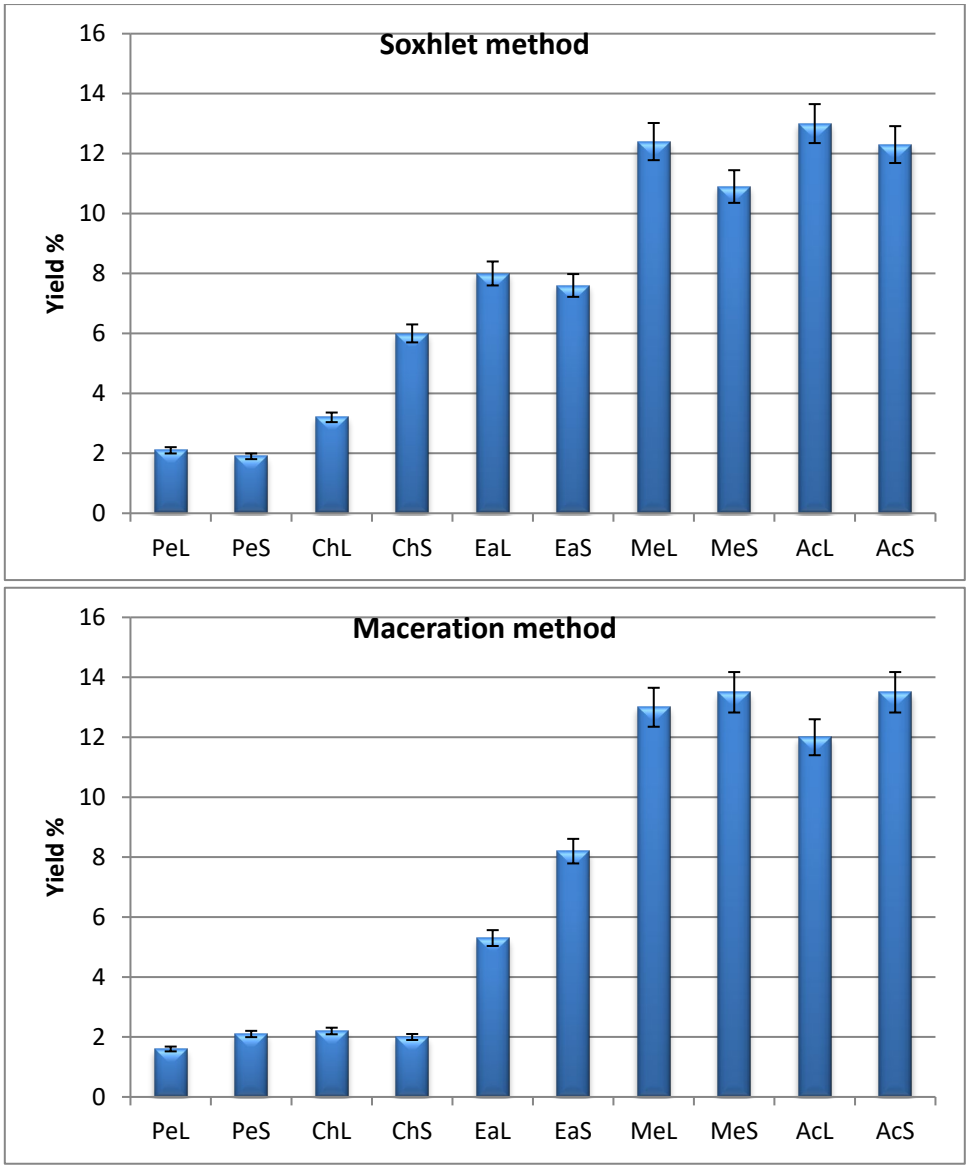

Figure 2. Extraction yield (%) of applications with maceration and soxhlet method (PeL: petroleum ether leaf, PeS: petroleum ether stem, ChL: Chloroform leaf, ChS: Chloroform stem, EaL: Ethyl acetate leaf, EaS: Ethyl acetate stem, MeL: Methanol leaf, MeS: Methanol stem, AcL: Acetone leaf, AcS: Acetone stem)

When the soxhlet and maceration extraction methods were compared, it was determined that ethyl acetate and chloroform solvents produced higher yields in the soxhlet method. For *E. arvense* plant, the Soxhlet method produced higher yields than the Maceration method. It has been shown once again in our study that the solute used directly affects the yield during extraction. When the extract yields were compared in both methods, it was seen that the yield increased in the form of petroleum ether, chloroform, ethyl acetate, methanol and acetone, respectively. This indicates that the yield in the extracts in our study is related to the polarity of the solution used and that the efficiency increases depending on the increasing polarity, and this supports us in the data in the literature (Do et al., 2014; Ng et al., 2020). Differences in extract yields from the plant leaf and stem parts tested in the present analysis may be due to the production and storage of various chemical compounds in different parts of the plant (Sultana et al., 2009).

It is admitted that phenolic compounds of plants can play an essential role in shaping the biological properties of the plant, including antioxidant properties (Zlotek et al., 2016). The total phenolic content (TPC) of the extracts obtained from the leaf and stem of *E. arvense* are given in Table 1.

It was seen in Table 1 that the extracts using the maceration method contained higher TPC values. The maceration technique is an extraction method at room temperature without any heating. It can prevent phenolic degradation but may not produce a high yield, as seen in our study (Dahmoune et al., 2015; Dewi et al., 2022). However, although the efficiency of the soxhlet method is high, the extracted phenolic substances may have been adversely affected since it was extracted under reflux at temperature.

In a study conducted in Serbia, the TPC values of the extracts obtained from the plant *E. arvense* were reported as: petroleum ether 0 mgGA/g, chloroform 2.86 mgGA/g, Ethyl acetate 43.6 mgGA/g, and methanol 79.52 mgGA/g (Četojević-Simin et al., 2010). In another similar study, TPC amounts were shown to be petroleum ether < chloroform < ethyl acetate in the solvents. Studies have shown that the solvent's polarity affects phenolic compounds' solubility. Phenolic compounds generally show polar properties depending on the -OH groups in their structure (Boungo Teboukeu et al., 2018; Aryal et al., 2019). Our study results observed that the phenolic substance increased as the polarity of the solvent used in the extraction increased. In addition, it was determined that the extracts prepared with petroleum ether from the plant *E. arvense* were unsuitable for TPC isolation.

A wide variety of in vitro assays are used to determine the antioxidant activity of the plant extract. The DPPH radical scavenging activity test is one of them. DPPH radical is one of the most commonly used substrates for rapid antioxidant activity evaluation because of its stability and simplicity of the assay (Bozin et al., 2008). It was determined that petroleum ether, ethyl acetate and chloroform extracts obtained in the soxhlet method showed higher antioxidant activity (Figure 3). When the leaf and stem extracts obtained with the same solvent were compared, it was determined that they showed higher antioxidant properties. The highest DPPH scavenging activity was determined in leaf methanolic extract (85%). Notably, leaf methanol and acetone extracts show antioxidant activity close to ascorbic acid, which we use as a standard antioxidant. Methanol and acetone solutions were the most suitable solvents to reveal the antioxidant properties of the plant. On the other hand, the antioxidant activity rates of petroleum ether and chlorophyll solvents remained the lowest.

There are some studies on the antioxidant activities of *E. arvense* plants in the literature. However, it is impossible to make a healthy comparison due to differences in used methods, solvents and plant parts. In this respect, in our study, by comparing the antioxidant activity of the extracts prepared using two different methods and 5 different solvents, significant complementary information is provided to the literature. In the literature, studies evaluate the DPPH scavenging and antioxidant activity of the plant *E. arvense*, some of which are given in Table 2.

Table 1. TPC values of prepared *E. arvense* extracts

Extract	TPC (*mgGA/g*)			
	Maceration		Soxhlet	
	Leaf	Stem	Leaf	Stem
P. ether	18.3^{a}±1.2	24.5^{a}±0.2	20.47^{a}±0.5	23.8^{a}±4.8
Chloroform	156.21^{b}±2	59.1^{b}±0.8	134.04^{b}±2.20	63.09^{b}±0.2
Ethyl acetate	219.76^{c}±1.6	114.28^{c}±1.6	213.3^{c}±4.6	150.47^{c}±2.9
Methanol	234.04^{c}±0.9	238.09^{d}±4.3	139.52^{b}±0.7	135.23^{c}±3.2
Acetone	507.61^{d}±8.3	471.42^{e}±0.5	252.1^{d}±6.2	466.6^{d}±4.6

The results were performed in triplicate, and the average was calculated. Using the ANOVA Duncan test, different letters symbolised significant differences ($P < 0.05$).

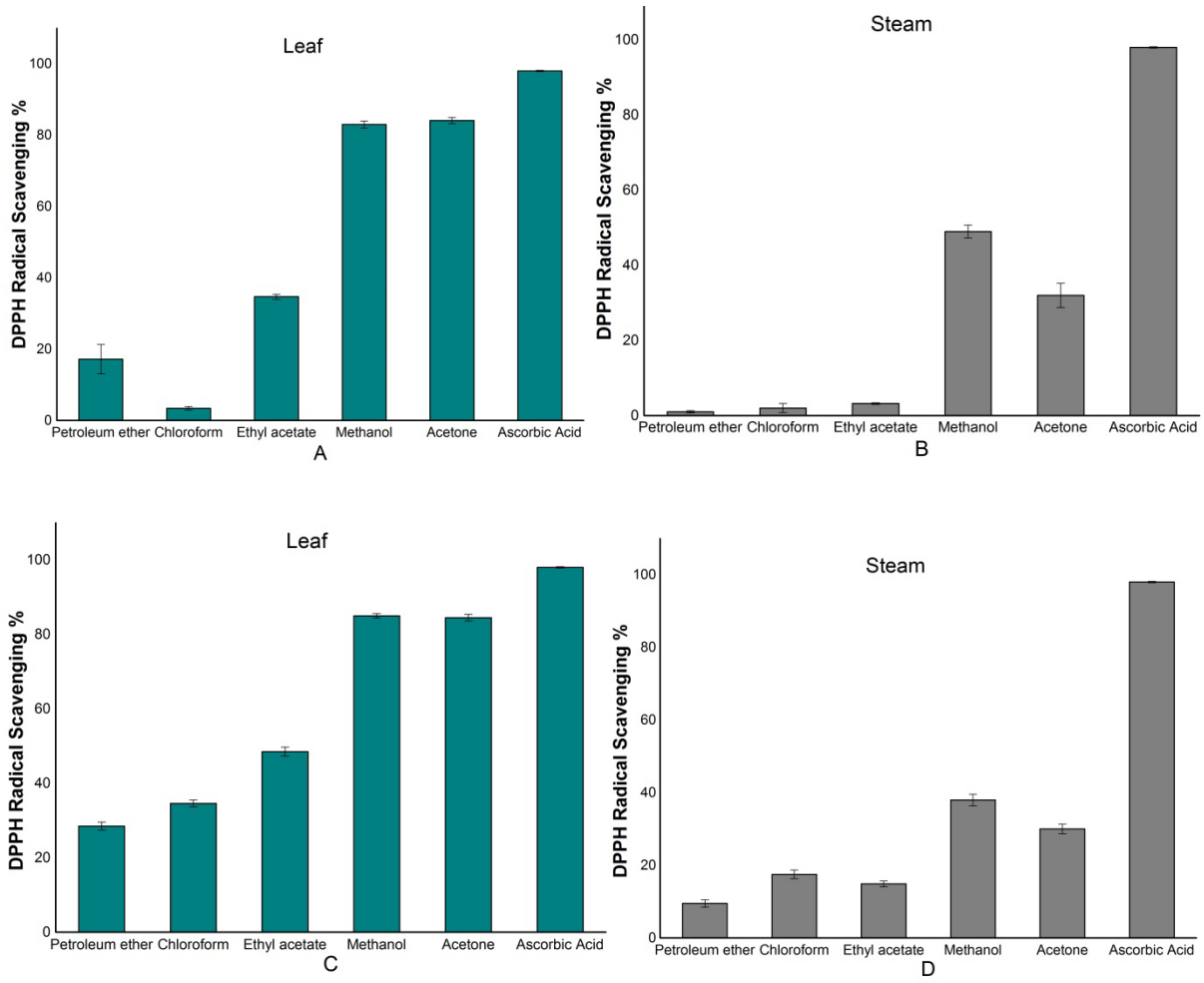

Figure 3. DPPH scavenging activity (%) of different extracts prepared from *E. arvense*
A-B: Maceration methods, C-D: Soxhlet methods

Table 2. DPPH scavenging activities of *E. arvense* extracts in the literature

Used of parts	Methods	Solvents	Antioxidant activity (DPPH methods)	Reference
Stem	Maceration	Ethyl acetate Butanol	IC50=2.37µg/mL IC50=7.16 µg/mL	Mimica-Dukic et. al.2008
All parts	Microwave-assisted extraction	Ethanol-water	1 mg/mL for* %33	Milutinović et al 2014
Stem	Modified of Maceration	Methanol	1 mg/mL for *%87.5	Pallag et al. 2016
Aerial parts	Maceration	Ethyl acetate Bütanol	1mg/mL for* %10 %70	Čanadanović-Brunet et al 2009
All parts	Maceration	Methanol	0.2 mg/mL for* %52.4	Necip and Işık 2019

*: %DPPH radical scavenging, IC50: concentratıon requıred for 50 percent DPPH radical scavenging

In the study of Čanadanović-Brunet et al. (2009), in parallel with our study, it was observed that the extract obtained from ethyl acetate showed deficient antioxidant activity compared to alcohol. In the given literature, it has been reported that *E. arvense* plant shows antioxidant activity. As in our study, extracts prepared with alcohol groups such as methanol are more effective in revealing antioxidant activity (Table 2). As seen from Table 2, there are different results among some studies in the literature, depending on the different parts of the plant used. Depending on the place where the plants are collected, the collection time, and the environmental conditions of the area where they are collected, secondary metabolites of different types and rates can be produced. Accordingly, there may be differences in biological activities.

The presence of antioxidant activity of *E. arvense*, which has been represented in the literature to contain many bioavailable, bioactive compounds such as vitamins B1, B2, B6, C, E, nicotinic acid, folic acid, pantothenic acid (Nagai et al.,2006), was also confirmed in our study.

Recently, there has been a growing interest in herbal products and plant compounds. It is known that many diseases, including infectious diseases, have been treated with herbal medicines throughout human history. Standardised and reliable antimicrobial activities are needed to examine the potential antimicrobial properties of plant-derived phytochemicals (Othman et al., 2011; Vanlalveni et al., 2021). In order to obtain bioavailable compounds such as phenolic compounds in herbal extracts at a maximum rate and with minimum degradation, an effective extraction method and solvent selection should be made.

In our study, the antibacterial activity results of the extracts obtained from the plant *E. arvense* by maceration and soxhlet method are given in Tables 3 and 4. When the study results were examined, it was seen that the highest antibacterial activity was in the methanolic leaf extract prepared by the maceration method. This extract had a broad-spectrum effect on the test bacteria *(S. aureus* 14.5 mm, *S. epidermidis* 14.2 mm, *E. faecalis* 14 mm, *B. subtilis* 13.5 mm inhibition zone diameter). This extract was comparable to gentamicin, a standard antibiotic on *S. aureus, E. faecalis* and *S. epidermidis*. Its vigorous antibacterial activity on *S. aureus*, one of the most common Gram-positive bacteria in food poisoning, is valuable data for the literature. The extract obtained from the leaf part of the E. arvense plant was more effective on the test bacteria than the stem extracts. It was determined that the acetonic extract prepared by the maceration method among the extracts obtained from the stem produced the highest activity in *S. aureus* with an inhibition zone diameter of 11 mm. In addition, it was determined that only methanolic and acetonic stem extracts prepared by the maceration method showed activity on *S. aureus*. In the soxhlet method, it was observed that only the methanolic extract formed a 10 mm inhibition zone on *E. faecalis*.

Table 3. Antibacterial activity of extracts obtained by maceration method

Extracts		Test bacteria (Inhibition Zones (mm) (±SD))					
		Bs	**Ec**	**Ef**	**Sa**	**Se**	**St**
Leaf	**P. ether**	0	0	0	8	0	0
	Ethyl acetate	0	0	0	0	0	0
	Chloroform	0	0	0	0	0	0
	Methanol	13.5 ±0.5	0	14 ±0.1	14.5 ±0.4	14.2 ±0.2	0
	Acetone	8 ±0.1	0	9.5 ±0.5	9.5 ±0.5	8 ±0.3	0
Stem	**P. ether**	9 ±0.1	0	8	0	0	0
	Ethyl acetate	0	0	0	0	0	0
	Chloroform	0	0	0	0	0	0
	Methanol	0	0	0	9	0	0
	Acetone	0	0	0	11 ±0.9	0	0
Gentamicin 10 mcg		20	18	19	22	21	20
N. Control		0	0	0	0	0	0

Bs-*Bacillus subtilis*, Ec-*Escherichia coli*, Ef-*Enterecoccus faecalis*, Sa-*Staphylococcus aureus*, Se-*Staphylococcus epidermidis*, St-*Salmonella typhimurium*.

Table 4. Antibacterial activity of extracts obtained by Soxhlet method

Extracts		Test bacteria (Inhibition Zones (mm) (±SD))					
		Bs	Ec	Ef	Sa	Se	St
Leaf	P. ether	0	0	0	0	0	0
	Ethyl acetate	0	0	9.5 ±0.4	0	0	0
	Chloroform	0	0	0	0	0	0
	Methanol	9 ±0.1	0	8	0	0	0
	Acetone	0	0	8	0	0	0
Stem	P. ether	0	0	0	0	0	0
	Ethyl acetate	0	0	0	0	0	0
	Chloroform	0	0	0	0	0	0
	Methanol	0	0	10 ±0.1	0	0	0
	Acetone	0	0	0	0	0	0
Gentamicin 10 mcg		20	18	19	22	21	20
N. Control		0	0	0	0	0	0

Bs-*Bacillus subtilis*, Ec-*Escherichia coli*, Ef-*Enterecoccus faecalis*, Sa-*Staphylococcus aureus*, Se-*Staphylococcus epidermidis*, St-*Salmonella typhimurium*

It is determined that all the extracts used in the study don't have antibacterial activity on the gram-negative bacteria *E. coli*. Čanadanović-Brunet et al. (2009) reported that chloroform and petroleum ether extracts, which they extracted from the plant *E. arvense* by the maceration method, did not have antibacterial activity on the bacteria they used; our data also support this. In another study, *E. arvense* extract was shown to be ineffective on *E. coli* (Pallag et al., 2018). The difference in cell wall structure and permeability of Gram-positive and Gram-negative bacteria causes different sensitivities (Kosanic et al., 2015). In Gram-negative bacteria, the outer membrane is a barrier to many substances, including antibiotics. Gram-negative bacteria have an outer phospholipidic membrane that carries the lipopolysaccharide components. Gram-positive bacteria, on the other hand, only have an outer peptidoglycan layer that does not have an effective permeability barrier (Kosanic et al., 2012; Albouchi et al., 2013).

Recent studies have confirmed the in vitro antimicrobial activity of the plant *E. arvense* (Radulović et al., 2006; Čanadanović-Brunet et al., 2009; Uslu et al., 2013). Studies have also reported that Petroleum ether and chloroform solvents are unsuitable for antibacterial activity studies of *E. arvense*.

Conclusion

As a result of the study, it was determined that the method and solvent used in preparing the extract are essential in revealing the chemical content and exhibiting the activity. Generally, extracts produced by the maceration method exhibited higher antibacterial activity, while those obtained by the soxhlet method showed higher antioxidant activity. It was determined that the qualitative efficiency was not directly related to the extract yield values. Leaf extracts generally showed higher antioxidant and antibacterial activity than stem extracts. In addition, the highest antioxidant and antibacterial activity was found in the methanolic leaf extract. It has been determined that methanol and acetone are the ideal solvents for TPC antioxidant and antimicrobial activity studies to be carried out with *E. arvense.* In addition, the importance of pre-experiment optimisation to find the appropriate method and solvent while investigating the biological activities of herbal extracts has been demonstrated in our study. As a result, horsetail is not only healthy food but also helpful in protecting from various diseases due to its antioxidant and antibacterial activity. We believe it will bring a high added value to the scientific world by examining the effects of the bioactive components on various diseases in detail in future studies.

Compliance with Ethical Standards

Conflict of interests: The author(s) declares that for this article, they have no actual, potential, or perceived conflict of interest.

Ethics committee approval: Authors declare that this study includes no experiments with human or animal subjects.

Data availability: Data will be made available on request.

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Disclosure: -

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