

# Antioxidant and Antibacterial Potential of Water Extracts of Selected Plant Species from Tuzla Region (Bosnia and Herzegovina)

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## Abstract

This study aims to determine the antioxidant and antibacterial potential of aqueous extracts of six different plant species, which are widely used in traditional medicine: *Salvia officinalis* L., *Mentha longifolia* (L.) Huds., *Urtica dioica* L., *Rosmarinus officinalis* L., *Rubus fruticosus* L. and *Achillea millefolium* L. The samples were collected in the Tuzla region between March and July 2025. All of the mentioned plants are important in traditional medicine and have been previously reported to have biological activity. The antioxidant activity was tested by the DPPH and FRAP methods, while the antibacterial potential was tested by the diffusion technique on reference bacterial strains from the WDCM collection. The extracts showed high antioxidant activity, with nettle and sage extracts showing the most potent in neutralizing DPPH radicals. Weak to moderate antibacterial activity was recorded in the case of nettle, sage, mint and rosemary extracts, and a complete absence of activity in the case of yarrow and blackberry leaf extracts.

**Keywords:** Nettle; Sage; Mentha; Rosemary; Yarrow; Blackberry leaf.

## INTRODUCTION

Damage to cells and organs caused by reactive oxygen species (ROS) occurs when the body is unable to adequately detoxify these molecules or repair the harm they cause. This imbalance is called oxidative stress (Sies et al., 2017). A consequence of oxidative stress can be manifested through many diseases such as cancers, cardiovascular diseases such as hypertension and atherosclerosis, neurodegenerative diseases such as Parkinson's disease and Alzheimer's dementias, diabetes etc. (Sies et al., 2017). An effective antioxidant system is essential for maintaining the redox balance within cells. Plant-derived compounds such as flavonoids, phenolic acids, and phenolic diterpenes are natural source of effective antioxidants (Guo et al., 2020). Reactive oxygen species are byproducts of normal intracellular metabolism (Guo et al., 2020). Endogenous antioxidants which are involved in defense mechanisms from reactive oxygen species damage are vitamin E, vitamin C, glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) (Guo et al., 2020). A significant role in maintaining the redox balance within cells have the polyphenolic compounds from plant species (Guo et al., 2020). Aside from being strong antioxidants, polyphenolic compounds show antimicrobial and antifungal activities (Lourenço et al.,

2019). Polyphenols show differences in structure from simple phenolic acids like caffeic acid to very complex, high-molecular weight tannins (Dragsted, 2023). For this study 6 different traditionally used medicinal plants were chosen. The antioxidative activity of *Salvia officinalis* L. has been attributed mainly to rosmarinic acid (Bettaieb et al., 2011). Rosmarinic acid is a caffeic acid ester found in a variety of plants, but it is especially abundant in *Lamiaceae* family (Bakota et al., 2015). The concentration of rosmarinic acid in Sage can vary, depending on environmental factors (Bakota et al., 2015). Rosmarinic acid has the capability to inhibit the complement pathway of the immune system, thereby suppressing inflammation (Englberger et al., 1988; Sahu et al., 1999). *Urtica dioica* L. is widely known and used in traditional medicine, especially beneficial for blood health (Đurović et al., 2024). Compounds like syringic acid, myricetin, quercetin, kaempferol, rutin, ellagic acid, isorhamnetin, *p*-coumaric acid, ferulic acid, and naringin were found in Nettle extracts (Đurović et al., 2024). *Mentha longifolia* (L.) Huds. shows several bioactivities such as antibacterial, antifungal, antioxidant, anticandidal, pesticidal, insecticidal, antimutagenic, anticancer, calcium channel blocking, cyclooxygenase, and HIV 1 inhibitory properties have been reported for this species (Bahadori et al., 2018). The *Mentha* genus is generally famous for its high essential oil composition

(Bahadori et al., 2018). *Mentha longifolia* water extracts contain high amounts of sinapic acid, rosmarinic acid, hesperidin, and o-coumaric acid (Bahadori et al., 2018). *Rosmarinus officinalis* L. is a very famous plant from Mediterranean region, used in medicine and culinary (Moore et al., 2016). *Rosmarinus officinalis* L. has many medicinal values, especially as rubefacients, increasing the blood circulation which is beneficial for cold extremities and hair loss problems (Begum et al., 2013). The main polyphenols found in rosemary extract (RE) include the diterpenes carnosic acid (CA) and rosmarinic acid (RA) (Moore et al., 2016). *Rubus fruticosus* L. is used in traditional medicine as an antidiarrhoeic since it has strong astringent effect (Verma et al., 2014). *Rubus fruticosus* L. contains high levels of ellagitannins, flavonoids, salicylic acid, and ellagic acid (Četojević-Simin et al., 2017). *Achillea millefolium* L. is famous for its beneficial effects on gastrointestinal system, such as treatment of hemorrhoids, dyspepsia, dysmenorrhea and gastritis (Miraldi et al., 2001). *Achillea millefolium* L. contains many different polyphenols such as chlorogenic acid, caffeic acid, ferulic acid (Ali et al., 2017).

The aim of this study was to determine antioxidant potential of 6 different plant species, widely used in traditional medicine: *Salvia officinalis* L., *Mentha longifolia* (L.) Huds., *Urtica dioica* L., *Rosmarinus officinalis* L., *Rubus fruticosus* L. and *Achillea millefolium* L. All listed plants have significance in traditional medicine and are previously reported to have antioxidant activity.

## MATERIALS AND METHODS

### Chemicals and plant material

All chemicals used were of p.a. purity and were used without further purification. Demineralized water was used to prepare extracts and aqueous solutions of reagents used for spectrometric measurements.

The samples of plant species were collected in the Tuzla region from March to July 2025. The aerial plant material was dried in a dark and dry place at room temperature for two to three weeks. The dried samples were then ground in an electric mill into a fine powder to prepare the extracts.

### Preparation of extracts

Extracts for testing antioxidant potential were prepared by mixing 1 gram of chopped plant material with 100 mL of demineralized boiling water. The mixture was mixed for five minutes on a vibromix and then filtered. The extracts thus prepared were allowed to cool at room temperature and analyzed.

Extracts for testing antibacterial activity were prepared by mixing 5 grams of chopped plant material with 100 mL of demineralized boiling water. The mixture was mixed for five minutes on a vibromix and then filtered. The extracts thus prepared were allowed to

cool at room temperature after which they were evaporated on a Buchi R-210 rotary vacuum evaporator. The dry residue after evaporation was dissolved in dimethyl sulfoxide.

### Determination of total phenolic content (TPC)

Total phenolic compounds present in water extracts of the examined plant species were quantified spectrophotometrically through the Folin-Ciocalteu test following the protocol (Singleton et al., 1999). 200  $\mu$ L of extracts was mixed with 2.54 mL of 10% Folin-Ciocalteu reagent. After 5 min 420  $\mu$ L of 10% sodium carbonate was added. 910  $\mu$ L of distilled water was added to each sample prior to measuring. The absorbance of the resulting blue-coloured solution was measured at 765 nm.

### Determination of total flavonoid content (TFC)

Total flavonoid content of extracts was determined by the previously described method (Olajire and Azeez, 2011), with some modifications. 1 mL of extract solution was mixed with 0.3 mL of 5% sodium nitrite. 0.3 mL of 10% aluminium chloride was added after 5 minutes. After 6 minutes incubation at room temperature, 1 mL of 1 M sodium hydroxide was added to the reaction mixture. Immediately, the final volume was made up to 10 mL with distilled water. Absorbance of the sample was measured against the blank at 510 nm.

### Analysis of antioxidant activity

The antioxidant potential of plant extracts was tested by the FRAP and DPPH methods. The FRAP (Ferric-reducing antioxidant power) test is based on testing the efficiency of extracts to reduce  $\text{Fe}^{3+}$  ions to  $\text{Fe}^{2+}$  ions, which is indicated by a change in color from yellow to purple. The reducing powers of the extracts were determined following the protocol (Benzie and Strain, 1999). 3 mL of prepared FRAP reagent is mixed with 100  $\mu$ L of extracts. Absorbance at 593 nm is recorded after a 30 min incubation at 37 °C.

DPPH (2,2-diphenyl-1-picryl-hydrazyl) method was performed according to earlier described method (Horozić et al., 2019). A series of dilution was made by mixing different volume extracts with methanol, to a volume of 2 mL. The tube was then added 0.5 mL of 0.5 mM DPPH radical solution. The samples incubated 30 minutes in dark space after which their absorbance was measured at 517 nm.

### Analysis of antibacterial activity

Antimicrobial activity was examined at the reference bacterial strains of the Collection from the Gram-positive bacteria, as prescribed by Clinical and Laboratory Institute, 2009. Reference bacterial strains were cultivated overnight in BHI broth at 37 °C, aerobic. The suspension of the turbidity of 0.5 McFarland (density of  $10^7$ - $10^8$  CFU/mL) in a sterile physiological solution was prepared. The strains

were then applied to the mueller-hinton agar substrate surface, spilled in sterile Petri plates, which was 4 mm thickness. In the agar, the recesses of the 10 mm diameter, which is added 100  $\mu$ L extracts were added. Once the plates were left at room temperature for 15 minutes, that the substance diffuses the agar, they have been placed on incubation at 37 °C/24 hours. After the incubation period, the size of the inhibitory zone was measured. The concentration of extract in this analysis was 50 mg/mL.

## RESULTS AND DISCUSSION

The results of the content of total polyphenols, total flavonoids and antioxidant activity are shown in Tables 1 and 2. A graphical representation of the results of this study is shown in Figure 1. Labels E-1 to E-6 denote the extracts of *Urtica dioica* L., *Salvia officinalis* L., *Mentha longifolia* (L.) Huds., *Rosmarinus officinalis* L., *Achillea millefolium* L. and *Rubus fruticosus* L., respectively. The results obtained in this study for all the analyzed plants confirm the correlation between antioxidant activity and total phenolic and flavonoid content of selected plants. All the obtained results confirm the presence of antioxidant activity reported in previous studies, with differences in values that are strongly connected with extraction conditions and plant origins.

**Table 1.** Results of total polyphenols and flavonoids content.

Plant species	TPC [mg GAE/g]	TFC [mg QE/g]
<i>Urtica dioica</i> L.	68.50	9.55
<i>Salvia officinalis</i> L.	140.62	14.1
<i>Mentha longifolia</i> (L.) Huds.	16.44	2.44
<i>Rosmarinus officinalis</i> L.	19.45	5.12
<i>Achillea millefolium</i> L.	21.22	8.25
<i>Rubus fruticosus</i> L.	18.22	5.02

Among the six medicinal plants analyzed in this study, *Salvia officinalis* L. demonstrated the highest antioxidant activity, as evidenced by all applied assays: total phenolic content (140.62 mg GAE/L), total flavonoid content (14.1 mg QE/L), DPPH radical scavenging activity (0.040 mg/mL), and FRAP reducing capacity (1702.0  $\mu$ mol/L). In addition, these results are consistent with previously reported data indicating *S. officinalis* as a rich source of bioactive phenolic compounds and antioxidants (Hamrouni-Sellami et al., 2013; Ben Farhat et al., 2009). Among the reviewed published values for the DPPH activity of *Salvia officinalis* L. extracts, our result lies within the moderate range. Hamrouni-Sellami et al. (2013) achieved 0.01349 mg/mL for DPPH using microwave-dried Sage extracted with methanol. This difference could be attributed to the solvent polarity and drying method. Furthermore, their use of methanol, a more polar organic solvent than water, potentially contributed to the extract yield (Alara et al., 2021). Microwave drying is proven to

affect the extraction process, resulting in plant extracts with higher polyphenolic content compared to air drying methods (Snoussi et al., 2021; Khodja et al., 2020-). Results obtained by Ben Farhat et al. (2009), a DPPH value of 0.01628 mg/mL in methanolic extracts of cultivated sage, show the possible effect of environmental conditions on the antioxidant activity of *Salvia officinalis*. Namely, cultivated plants tend to accumulate higher levels of antioxidants under optimized growing conditions, meaning that environmental factors can impact the polyphenolic content of plants (Brahmi et al., 2020). In the study of Issa-Issa et al. (2019), total phenolic content and total flavonoid content were significantly higher compared to our study results (TPC 492 mg GAE/L, TFC 240 mg QE/L). This could be due to the plant origin and solvent difference. Other studies using different extraction techniques, such as sonication (Mokhtari et al., 2023; DPPH = 0.07721 mg/mL), reported slightly lower antioxidant activities, which could be explained by thermal or mechanical degradation of phenolic compounds during the extraction process. Long periods of sonication greater than 40 minutes at a higher energy level that is above 20 kHz could seriously affect the extracted phytochemicals during sonication extraction process (Annegowda et al., 2010; Wang et al., 2008). Our result for FRAP value (1702.0  $\mu$ mol/L) was significantly higher than result reported by Francik et al. (2020), who observed 496  $\mu$ mol/L in aqueous Sage leaf extracts obtained by sequential extraction. The moderate FRAP value may be attributed to factors such as plant origin, drying method, or extraction conditions.

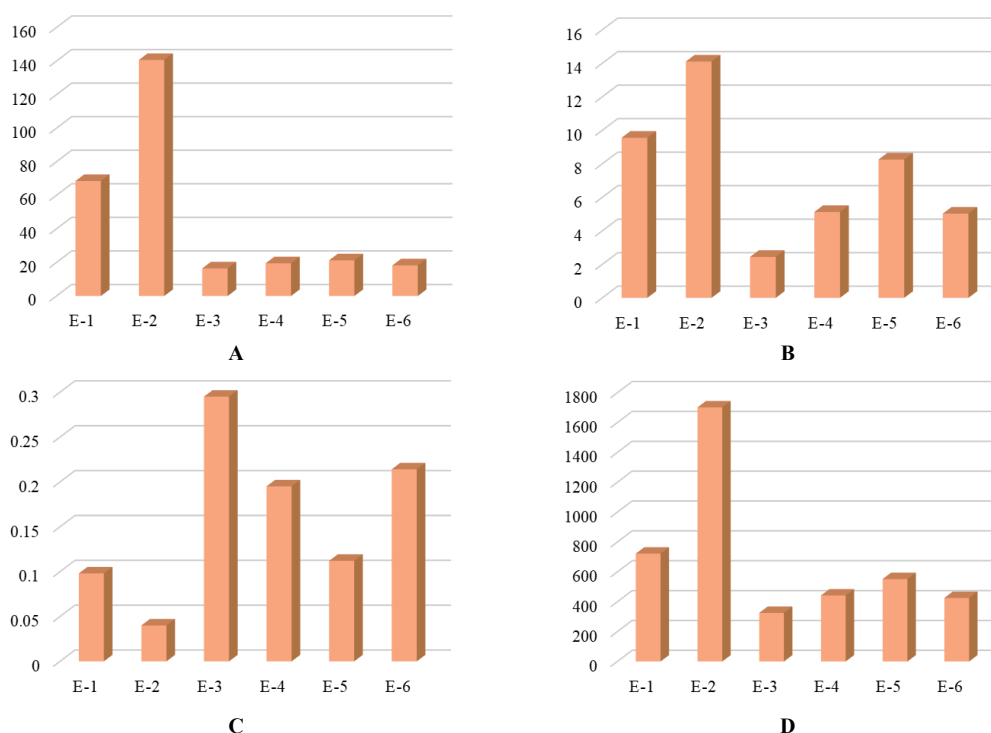
**Table 2.** Results of antioxidant activity *in vitro*.

Plant species	IC <sub>50</sub> value [mg/mL]	FRAP value [ $\mu$ mol/g]
<i>Urtica dioica</i> L.	0.098	722.5
<i>Salvia officinalis</i> L.	0.040	1702.0
<i>Mentha longifolia</i> (L.) Huds.	0.295	325.5
<i>Rosmarinus officinalis</i> L.	0.195	441.7
<i>Achillea millefolium</i> L.	0.112	552.1
<i>Rubus fruticosus</i> L.	0.214	425.4

The lowest antioxidant activity in our study was detected for *Mentha longifolia* L. extracts. In comparison to previously reported results, our results are lower. The antioxidant activity of *Mentha longifolia* L. assessed through the DPPH radical scavenging method in our study resulted in a value of 0.295 mg/mL for the aqueous extract. This finding is consistent with the result reported by Tourabi et al. (2023), who found 0.306 mg/mL for a similar aqueous extract obtained after prolonged maceration. These comparable values support the conclusion that water can effectively extract antioxidant compounds from *M. longifolia*, especially under certain extraction conditions, despite its relatively weaker solvation capacity compared to organic solvents. In contrast, more efficient DPPH values were reported in studies that used polar organic solvents. For example,

Janifer et al. (2010) reported a DPPH value of 0.0153 mg/mL for an 85% methanol extract of *M. longifolia* collected in India. Similarly, Bouali et al. (2024) demonstrated higher antioxidant capacities with ethyl acetate (0.03576 mg/mL) and chloroform (0.12386 mg/mL) extracts from cultivated plants. These

differences further confirm the well-documented observation that polar organic solvents are more efficient than water for extracting phenolic and flavonoid compounds, because of the high solubility of polyphenols in such solvents (Alara et al., 2021).



**Figure 1.** Graphical representation of: (A) total polyphenol content, (B) total flavonoid content, (C) IC<sub>50</sub> values for neutralization of DPPH radicals and (D) reducing capacity of extracts.

Using the conventional extraction technique in our study for preparing the extracts of *Rosmarinus officinalis* L. might not be the most efficient technique. This is proven by the much higher results for the FRAP value of 1703.3  $\mu\text{mol/L}$  reported by Škugor Rončević et al. (2025) using microwave-assisted extraction. The high FRAP activity in their study highlights the efficiency of microwaves in breaking down plant cell walls and improving the solubility of plant antioxidants (Oracz et al., 2023). The total phenolic content obtained in our study (19.45 mg GAE/L) was substantially lower than the 947.8 mg GAE/L obtained in the microwave-assisted extraction conducted by Škugor Rončević et al. (2025). Different plant parts show differences in antioxidant activity. Hence, when comparing the DPPH radical scavenging activity of *Rubus fruticosus* L., our result (0.214 mg/mL) is less effective than the values reported by Mirza (2021) and Sedlák et al. (2025), who reported DPPH IC<sub>50</sub> values of 0.04885 mg/mL and 0.0400 mg/mL, respectively. Mirza et al. (2021) employed a prolonged water-based extraction over three days at room temperature with root material, followed by methanol resuspension, while Sedlák et al. used ethanol-based

percolation for extraction of fruit. Both studies used different plant parts (roots and fruits); hence, the greater antioxidant activity suggests that either the bioactive compound concentration is higher in those plant parts, or different extraction protocols were more efficient in extracting antioxidant components. Total phenolic content in our *Achillea millefolium* L. extract (21.22 mg GAE/L) was also considerably lower than the values reported in other studies. Generalić-Mekinić et al. (2014) recorded a total phenolic content of 2097.9 mg GAE/L using an ethanol/water (80:20 v/v) extract prepared at 60°C for one hour. This vast difference underscores the importance of solvent polarity and temperature in efficiently extracting polyphenolic compounds from *A. millefolium*. In terms of flavonoid content, our extract of *Achillea millefolium* L. yielded 8.25 mg QE/L, which is significantly lower than the 73.63 mg QE/L reported by Belščak-Cvitanović et al. (2017). Their extract was prepared by simulating traditional household use, with hot water extraction at 80°C. Despite the simplicity of the method, the high flavonoid content suggests that thermal conditions and prolonged extraction time can be highly effective in case of *Achillea millefolium* L.. The

flavonoid content of *Urtica dioica* L. extract in our study, 9.55 mg QE/L, indicates a moderate presence of this compound class. It is notably lower than the 715 mg QE/L reported by Salević et al. (2017), who applied aqueous extraction under optimized temperature (80 °C) and time (30 min) parameters. The vast difference in flavonoid content, once more, points to the importance of controlled thermal extraction and solvent interaction in maximizing yield. While maceration and room-temperature soaking may preserve thermolabile compounds, they appear less effective in liberating flavonoid-bound complexes from the leaf matrix.

Table 2 shows the results of the antibacterial effect of water extracts of the examined plant species. The results show that aqueous extracts of selected plant species show

weak to moderate antibacterial activity. Slightly higher sensitivity was shown by *S. aureus* and *E. faecalis*, with inhibition zones of 11 to 15 mm, depending on the plant species. A complete absence of antibacterial activity was recorded for water extracts of *Achillea millefolium* L. and *Rubus fruticosus* L. Ciprofloxacin, which was used as a control antibiotic, showed greater antibacterial activity than all tested extracts, with inhibition zones greater than 20 mm. The results of antibacterial activity indicate that aqueous extracts of selected plant species have weaker antibacterial activity compared to methanol and ethanol extracts, since lower alcohols have a greater ability to extract bioactive components that are responsible for the antibacterial activity of some plant species.

**Table 3.** Results of antioxidant activity *in vitro*.

Plant species	Inhibition zone [mm]		
	<i>Staphylococcus aureus</i> WDCM 00034	<i>Enterococcus faecalis</i> WDCM 00087	<i>Listeria monocitogenes</i> WDCM 00109
<i>Urtica dioica</i> L.	11	14	12
<i>Salvia officinalis</i> L.	12	12	-
<i>Mentha longifolia</i> (L.) Huds.	12	11	11
<i>Rosmarinus officinalis</i> L.	15	13	11
<i>Achillea millefolium</i> L.	-	-	-
<i>Rubus fruticosus</i> L.	-	-	-

## CONCLUSIONS

The results showed that water has a high efficiency in extracting bioactive components from the plant species used in this study. This is also reflected in the efficiency of neutralizing free radicals, i.e. the reducing ability of the extract. The aqueous extract of *Salvia officinalis* L. showed the highest antioxidant activity, in contrast to the extract of *Mentha longifolia* (L.) Huds. for which the weakest antioxidant effect was recorded. Weak to moderate antibacterial activity was recorded for most of the tested extracts. These results are significant because they show what antioxidant and antibacterial potential the plant species from the Tuzla region show, since they are often used by the local population. Differences in the results of antioxidant activity can be explained by the different geographical origin of the samples, the quality of the soil on which the plant species grows, but also the extraction conditions.

**Authors' Contributions:** Lejla Mekić participated in the experimental determination of the content of total polyphenols and writing the scientific paper. Edina Huseinović and Jasmina Dedić experimentally determined the content of total flavonoids and tested the antioxidant activity of the extracts. Darja Husejnagić examined the antibacterial potential of extracts. Emir Horozić designed the study, participated in the collection

of plant material, prepared the extracts, processed the results and participated in writing the scientific paper.

**Competing Interests:** The authors declare that they do not have any conflict of interest.

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