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ORIGINAL SCIENTIFIC PAPER

Study of Antioxidative and Antibacterial Activity of Extracts of Plant Species *Lysimachia vulgaris L.* and *Lythrum salicaria L.*

Ispitivanje antioksidativne i antibakterijske aktivnosti ekstrakata biljnih vrsta *Lysimachia vulgaris L.* i *Lythrum salicaria L.*

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ABSTRACT

Two highly invasive plant species, *Lysimachia vulgaris* and *Lythrum salicaria* are well-known for their anti-inflammatory, hemostatic, and antidiarrheal activity. These plants are most widespread on the territory of Euroasia, where their traditional medicinal use has been reported. Due to their invasiveness, they are undesirable plants in other parts of the world. To this day, many studies have been conducted regarding the biological and pharmacological activity of *L. salicaria*. In this study, the polyphenol content of plant extracts was examined using the Folin-Ciocalteou method. The antioxidant activity of selected plant species was also determined, using DPPH and FRAP methods. Extracts of different polarities were prepared using methanol, water, and acetone. Extraction was performed by maceration and ultrasonic extraction. The results of the study show that both plant species possess antioxidant activity. *Lythrum salicaria* extracts show a significant polyphenol content and antioxidant capacity, with results notably higher than the results of studies conducted so far. The different antioxidant activity of the prepared extracts confirms the influence of solvents and extraction methods on the utilization of the antioxidant potential of plants. Additionally, for the aqueous extracts prepared by the ultrasonic extraction method, an in vitro study of antibacterial activity was conducted. Both plant species show antibacterial activity, with an emphasis on the very strong antibacterial activity of *L. salicaria* extracts against selected bacterial strains.

Keywords: *Lysimachia vulgaris, Lythrum salicaria, antioxidative activity, antibacterial activity*

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INTRODUCTION – *Uvod*

Lysimachia vulgaris is a rhizomatous perennial plant from the *Myrsinaceae* family (Yildirim et al, 2017). In recent years, the genus Lysimachia has been moved from the family *Primulaceae*, which includes mainly species growing in temperate regions, to the family *Myrsinaceae*, typical of tropical and subtropical areas. The medicinal properties of many species of *Lysimachia* are well known (Hanganu et al, 2016). Due to its astringent properties, it is used to treat gastrointestinal conditions such as diarrhoea and dysentery, to stop internal and external bleeding and for wound cleaning in traditional medicine (Turker & Guner, 2013; Podolak et al, 2013). It is used in the treatment of fever, ulcers, diarrhoea and wounds in folk medicine. It also has analgesic, expectorant, astringent and anti-inflammatory effects. It is used as a mouthwash in the treatment of sore gums and mouth ulcers (Turker & Guner, 2013). In Chinese folk medicine, *Lysimachia vulgaris* is used to treat high blood pressure (Yasukawa & Takido, 1988). There are reports of antileishmanial and anthelmintic properties and use as a means of treating cholecystitis (Hanganu et al, 2016). Recent studies have revealed that *L. vulgaris* has antifungal, antibacterial, antitumor, and antioxidant effects (Son et al, 2021).

Lythrum salicaria is a perennial plant (Piwarowski et al, 2015). Its medicinal use has been known since ancient Greek and Roman times and has been an important medicine for centuries (Piwarowski et al, 2015). The whole flowering plant and the tops of the flowering branches of this plant are used in folk medicine and pharmacy (Humadi & Istudor, 2009). Beekeepers also used it as a honey plant (Thompson et al, 1987; Pellet, 1977). The seed has been included in a commercial "wildflower" seed mix (Tunalier et al, 2007). Many biological and pharmacological activities of *L. salicaria* have been studied (Manayi et al, 2013). Although *L. salicaria* is today considered an invasive plant, it has a long use in folk medicine as a medicinal plant due to its significant biologically active compounds (Šutovská et al, 2012). In external use, due to its astringent, anti-inflammatory, and hemostatic properties, this plant species is useful in the treatment of eczema, bleeding gums, eye inflammation, sinusitis (rinsing the nose with a diluted tincture), varicose veins, haemorrhoids, menorrhagia, haemorrhages, leukorrhea, and ulceration. Moreover, Campardon (1878) recommended *Lythrum salicaria* in the treatment of chronic and acute vaginitis and pruritus of various etiologies (Tunalier et al, 2007; Piwowarski et al, 2015). Internal use of this plant is intended for the treatment of diarrhoea, chronic intestinal catarrh and dysentery (Tunalier et al, 2007; Çoban et al, 2003). There are individual reports on the use of *Lythrum salicaria* in rheumatism, benign prostatic hyperplasia, infections and irritation of the urinary system mucosa, rabies, and as a tonic and fever remedy (Piwarowski et al, 2015). For oral and external use it is recommended to prepare infusions or decocts of the herb (Shakeneva 2019). The aim of this study is to determine the antioxidant activity and antimicrobial activity of invasive plant species, *Lythrum salicaria L. and Lysimachia vulgaris L.*, and to understand the effects of using the different solvents and extraction methods on final antioxidant activity and antimicrobial activity.

MATERIALS AND METHODS – *Materijal i metode*

For the purpose of this research, the plant material was collected in the area of Banovići (Bosnia and Herzegovina) in August 2024. The plants were cleaned and air-dried with natural airflow, in a ventilated area, for 2 days at a temperature of 37°C. The dried plants were ground in an electric mill and used to prepare extracts. All chemicals used were of analytical grade and were used as received without any further purification. Chemicals were purchased from Merck (Darmstadt, Germany) and Sigma Chemical Co. (St. Louis, Missouri, USA).

Preparation of extracts

For the evaluation of polyphenol content and antioxidant capacity, extracts were prepared by mixing 5 grams of pulverated plant material with 50 mL of solvent (methanol, water and acetone). The extraction was performed using 2 different techniques, in an ultrasonic bath and maceration in Vibromix (at 300 rpm) for one hour. The extracts were filtered through a blue dot filter paper. After filtering, the extracts were evaporated to dry extracts. The dry extract was dissolved in dimethyl sulfoxide and used to test the antibacterial activity. Extracts obtained by maceration are marked with (M) and extracts prepared by ultrasonic extraction with (UE). For the antimicrobial testing, solely water extracts were prepared by maceration in Vibromix (at 300rpm) for one hour.

Determination of total phenolic content (TPC)

Total phenolic compounds were quantified by the spectrophotometric method using the Folin-Ciocalteu test according to the protocol (Singleton et al, 1999), with some modifications. 100 µL of the extract was mixed with 1270 µL of 10% Folin-Ciocalteu reagent. After 5 minutes, 210 µL of 10% sodium carbonate was added. After incubation for an hour, 455 µL of distilled

water was added to the incubated solution. Absorbance was measured on a spectrophotometer at a wavelength of 765 nm. Quantitative analysis was performed based on the gallic acid standard calibration curve. Total phenolic content is expressed as gallic acid equivalent (GAE) in milligrams per gram of sample.

Determination of antioxidant capacity

The antioxidant activity of the extracts was tested *in vitro* using the DPPH and FRAP methods. The 2,2-diphenyl-1-picryl-hydrazyl (DPPH) method was carried out according to the described method (Horozić et al., 2019). 0.1 mL of the extract was added to the test tube and made up to 2 mL with methanol. Then 0.5 mL of 0.5 mM DPPH solution was added. The samples were incubated for 30 minutes in a dark place at room temperature. Absorbance was measured at 517 nm with methanol as a blank. The radical scavenging effect (%) or DPPH radical inhibition percentage was calculated according to the equation:

$$[(Ac - As) / Ac] \times 100$$

where As is the absorbance of the sample solution at 517 nm and Ac is the absorbance of the control.

The FRAP (Ferric-Reducing Antioxidant Power) method is based on the ability of the extract to reduce Fe(III) to Fe(II) ions. The test was conducted according to a published protocol (Benzie and Strain, 1999). 3 mL of prepared FRAP reagent was mixed with 100 µL of diluted extracts. Absorbance at 593 nm was recorded after incubation for 30 minutes at 37 °C. The FRAP value was calculated from the calibration curve of ferrous-sulfate-heptahydrate.

Determination of antibacterial activity

Antibacterial activity was tested using the diffusion method on reference bacterial strains from the ATCC collection, from the group of Gram-positive (*Staphylococcus aureus* and *Enterococcus faecalis*) and Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) as prescribed by the Clinical and Laboratory Standards Institute, 2009. Turbidity suspensions of 0.5 McFarland (density 10^7-10^8 CFU/mL, depending on the strain) were prepared from overnight culture of bacterial strains. The strains were then applied to the surface of the nutrient medium - Mueller-Hinton agar (MH), spread in sterile Petri plates, where the thickness of the medium was 4 mm. Indentations ("wells") were made in the agar using sterile drills and 100 µL of extract was added. After the plates were left at room temperature for 15 minutes to allow the extract to diffuse into the agar, they were incubated at 37°C/24 hours. After the incubation period, the zone of inhibition was measured. Ciprofloxacin was used as the positive control, with an inhibition zone >20mm.

RESULTS AND DISCUSSION - *Rezultati i diskusija*

Polyphenol content

The content of polyphenols in the extracts of the plant species *L. salicaria* and *L. vulgaris* is shown in Table 1. According to the obtained results, it can be concluded that the content of polyphenols in *L. salicaria* is very high, for all applied solvents and extraction methods. All previously conducted studies, covered in this discussion, on this plant species had a somewhat lower level of polyphenols, which can be justified by a number of factors such as: the geographical origin of the plant, degree of comminution of the plant material, types of solvents and extraction, extraction conditions, time of collection of the plant species for analysis, methods of drying and preservation of plant species, and solvent concentration. The results obtained in this study show that the methanol extracts of *L. salicaria* have the highest content of polyphenols. A more efficient extraction method is maceration, with the obtained polyphenol content of extracts being 848.86 mg GAE/g. A possible reason for the somewhat lower content of polyphenols in the methanolic extracts of *L. salicaria* obtained by ultrasonic extraction (603.15 mg GAE/g) is the known degrading effect of ultrasonic waves (stronger than 20 kHz) on active plant components (Vuleta et al, 2006; Tiwari et al, 2011). The content of polyphenols in terms of the different solvents used is in the following order: methanolic extracts > aqueous extracts > acetone extracts. The effect of solvent polarity on the content of phenolic compounds can be noticed, where acetone, as the least polar solvent used, shows the lowest extraction efficiency. The efficiency of acetone extraction can be increased by combining acetone with water, where the dielectric constant of the solvent is lowered. In addition, water causes the plant material to swell and facilitates the penetration of the organic solvent into the plant material. Lopes et al., (2016) examined the level of polyphenols in water-acetone extracts of *L. salicaria*, obtained by maceration (overnight, under stirring, at room temperature, 16h RT), whose content was 278 ± 3.04 mg GAE/g. However, this content is lower than the content of polyphenols obtained in this research, which is 392.63 mg GAE/g for acetone extracts obtained by maceration and 434.55 mg GAE/g for extracts obtained by ultrasonic extraction. It is known that *L. salica-*

ria blooms from June to September, but the highest content of active components is associated with August (Benscik et all). The different levels of polyphenols for these two studies can be attributed to the period in which the plant species was collected, which was the month of June for the study by Lopes et al, (2016). The reason for such results may be different extraction methods, the level of fragmentation of the plant material and the concentration of the solvent. A study conducted with methanol extracts, obtained by maceration, by Srećković et al. (2020) shows a lower level of polyphenols compared to the results obtained in our study (201.50 ± 11.49 mg GAE/g). It should be emphasized that such results may be the result of a different geographical origin of the plant (Serbia), different concentrations of methanol used and different conditions of maceration (3 times with 30 mL of methanol each, 24h with occasional mixing in the study of Srećković et al.). In the same study, a higher content of polyphenols was obtained in the root compared to the green part of the plant (326.36 + 10.25 mg GAE/g), which indicates the possibility of using the root of *L. salicaria* for medicinal purposes, in contrast to more often use of aerial parts of this species. Water-methanol extracts obtained by the percolation method, by Manayi et al (2013), showed a polyphenol level of 331 ± 3.7 mg GAE/g, which is lower than the obtained results in this study. A selection of solvent, extraction technique and the time period when the plant species was collected, which for the Manayi et al study was May, affected the total polyphenol content of plant species.

Table 1. Results of polyphenol content in extracts of plant species *L. salicaria* and *L. vulgaris*

Tabela 1. Rezultati mjerenja sadržaja polifenola u ekstraktima biljnih vrsta L.salicaria i L. vulgaris

Sample	Polyphenol content [mg GAE/g of sample]	
	L. salicaria	***L. vulgaris***
Methanol extract (M)	848.86	77.95
Aqueous extract (M)	434.43	70.06
Acetone extract (M)	392.63	34.17
Methanol extract (UE)	603.15	86.19
Aqueous extract (UE)	525.39	65.59
Acetone extract (UE)	434.55	37.99

All extracts of *L. vulgaris* showed a polyphenol content greater than 20 mg GAE/g. The results show that methanol extracts contain the highest concentration of polyphenols. The methanol extract obtained by ultrasonic extraction has the highest content of polyphenols (86.19 mg GAE/g). The higher content of polyphenols, obtained by ultrasonic extraction compared to maceration, indicates that active components of this plant species are possibly resistant to degrading effects of ultrasonic waves. It is also noticeable that the polyphenol content in this case is also closely related to the polarity of the solvent, where the acetone extracts obtained by maceration show the lowest content of polyphenols (34.17 mg GAE/g). A study conducted by Yildirim et al., (2017) shows a higher level of polyphenols for aqueous extracts of *L. vulgaris* (88.69 ± 0.0 mg GAE/g). These are extracts obtained by extraction in a water bath at 45°C for 12 hours. It is understandable that the higher content of polyphenols in these extracts was obtained because of the action of high temperature, where high temperature enhances the thermal movement of molecules in the liquid phase, so the diffusion rate is higher (Vuleta et al., 2006). In the same study, ethanolic extracts were also prepared using the Soxhlet extraction method, which showed twice the polyphenol content (161.42 mg GAE/g) compared to the aqueous extracts obtained by maceration and ultrasonic extraction in this study (70.06 mg GAE/ g and 65.59 mg GAE/g respectively).

Antioxidant activity

Tables 2 and 3 show the results of the antioxidant activity of *L. salicaria* and *L. vulgaris* extracts obtained by maceration and ultrasonic extraction. According to the results obtained by the DPPH method, the methanol extract of *L. salicaria* obtained by maceration has the highest percentage of DPPH radical quenching, thus the highest antioxidant capacity, while the acetone extract obtained by maceration has the lowest antioxidant capacity. The results obtained by the FRAP method are in correlation with the results obtained by the DPPH method. The results of the antioxidant activity indicate a correlation between the level of polyphenols in the plant species and the strength of its antioxidant activity. The highest level of polyphenols was shown by the methanol extract obtained by maceration, which also has the highest antioxidant activity (% inhibition of DPPH radicals 71.73%; FRAP 4052.1 μmol/g). Water extracts obtained by ultrasonic extraction (% inhibition of DPPH radicals 39.89%) and maceration (% inhibition of DPPH radicals 35.87%) showed somewhat lower antioxidant activity, compared to methanol extracts. In this study, the optimal extraction method for the plant species *Lythrum salicaria* is maceration with methanol or water as solvent.

Table 2. Results of antioxidant activity of *L. salicaria* extracts

Tabela 2. Rezultati mjerenja aktioksidativne aktivnosti ekstrakata L. salicaria

Sample	FRAP [µmol/g of sample]	Inhibition of DPPH radical [%]
Methanol extract (M)	4052.1	71.73
Aqueous extract (M)	2853.9	35.87
Acetone extract (M)	960.48	2.06
Methanol extract (UE)	3597.58	60.99
Aqueous extract (UE)	2994.01	39.89
Acetone extract (UE)	1235.92	5.73

The highest antioxidant capacity, for the plant species *Lysimachia vulgaris*, was recorded in the methanol extract obtained by the ultrasonic extraction method. This is supported by the results obtained by the DPPH and FRAP methods, where the DPPH radical quenching percentage is 55.32% and the result obtained by the FRAP method is 1836.35 µmol/g. Acetone extracts showed the lowest antioxidant activity, with a quenching percentage for the acetone extract obtained by maceration of only 0.112 %. The value of the antioxidant activity measured by the FRAP method for the same sample is 116.89 µmol/g.

Table 3. Results of antioxidant activity of *L. vulgaris* extracts

Tabela 3. Rezultati mjerenja aktioksidativne aktivnosti ekstrakata L. vulgaris

Sample	FRAP [µmol/g of sample]	Inhibition of DPPH radical [%]
Methanol extract (M)	1555.89	52.59
Aqueous extract (M)	1468.79	46.79
Acetone extract (M)	116.89	0.112
Methanol extract (UE)	1836.25	55.32
Aqueous extract (UE)	1170.13	37.74
Acetone extract (UE)	142.51	2.79

Antibacterial activity

Water extracts with a concentration of 1 mg/mL were used to test the antibacterial activity of the plant species *Lythrum salicaria* and *Lysimachia vulgaris*. The test was conducted on strains of gram-positive (*Enterococcus faecalis* and *Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) with ciprofloxacin as positive control. Aqueous extracts of both plant species showed antibacterial activity, with a very high sensitivity of bacteria to *L. salicaria* extracts (inhibition zones in the interval of 18-23 mm) and medium sensitivity of bacteria to *L. vulgaris* extracts (inhibition zones in the interval of 11-13 mm). The absence of antibacterial activity is present only in the extract of *L. salicaria* on *Enterococcus faeacalis*. The results of antibacterial activity are presented in Table 4.

Table 4. Antibacterial activity of extracts of plant species *L. salicaria* and *L. vulgaris*

Tabela 4. Rezultati mjerenja antibakterijske aktivnosti ekstrakata biljnih vrsta L. salicaria I L. vulgaris

Sample	Inhibition zone [mm]			
	S. aureus	*E. faecalis*	*E. coli*	*P. aeruginosa*
L. salicaria extract	22	-	23	18
L. vulgaris extract	13	11	12	11
Ciprofloxacin (positive control)	>20	>20	>20	>20

On the *Escherichia coli* strain, *L. salicaria* extract showed an inhibition zone of 23 mm, which indicates the highest sensitivity of the microorganism. These results confirm the results of previous studies, where *L.salicaria* showed good antimicrobial activity against the *E. coli* strain (Çitoglu & Altanlar, 2003; Borchardt et al, 2008). The study conducted by Srećković et al. Srećković et al., (2021) showed the absence of antibacterial activity of *L. salicaria* on the *E. coli* strain, which points to the influence of the difference in the extraction conditions. *Pseudomonas aeruginosa* belongs to MDR strains (multidrug resistant strains). Today, there are very few antibiotics that can be used to treat infections caused by this bacteria (Guclu et al, 2014). *L. salicaria* extract showed promising results in this research with an inhibition zone of 18 mm, which indicates a good sensitivity of the bacteria. This result is confirmed by the results of a study conducted on MDR strains. Extracts of *L. salicaria* showed a strong ability to inhibit the growth of *Pseudomonas aeruginosa* and *Acinetobacter baumanni* strains isolated from infected patients (inhibition zones of 16.09 mm and 18.3 mm, respectively) (Guclu et al, 2014).

The *Staphilococcus aureus* strain also showed the highest sensitivity to the aqueous extract of *L. salicaria*, with an inhibition zone of 22 mm. Good antibacterial activity

against the *S. aureus* strain was also shown in other studies (Çitoglu & Altanlar, 2003; Rauha et al, 2000; Borchardt et al, 2008). Water extracts of *Lysimachia vulgaris* showed a weaker ability to inhibit the growth of all tested bacterial strains, with inhibition zones of 13 mm for the *S. aureus* strain, 11 mm for the *E. faecalis* strain, 12 mm for the *E. coli* strain and 11 mm for the *P. aeruginosa* strain. These results are confirmation of the study of the antimicrobial activity of *L. vulgaris* conducted by Yildirim et al., (2017). In other studies, good antimicrobial activity of *L. salicaria* against *L. monocytogenes* and *L. innocua* strains was confirmed (Altanlar et al, 2006). *Bacillus cereus* and *Mycobacterium smegmatis* strains showed sensitivity to *L. salicaria* extracts (Dugler et al, 2004). Very good activity of *L.salicaria* extract against *Candida albicans* has also been established (Rauha et al, 2000; Borchardt et al, 2008). Becker et al (2005) isolated the active components of *L. salicaria* by thin-layer chromatography, of which oleanolic and ursolic acids were found to be responsible for antifungal activity, and vescalgin was the active component responsible for the antibacterial activity of this plant species.

CONCLUSIONS – *Zaključak*

In this research, it was observed that the plant species *L. salicaria* and *L. vulgaris* show antioxidant and antimicrobial effects. *L. salicaria* extracts showed very strong antioxidant and antibacterial activity. Namely, the methanol extract obtained by the maceration method showed the strongest antioxidant activity and the highest level of polyphenols. In the case of *Lysimachia vulgaris*, the methanol extract obtained by ultrasonic extraction showed the highest antioxidant activity. It can be observed that choosing an adequate solvent, method of extraction and extraction conditions are the most important factors in the utilization of the biological activity of these plant species. According to the results of other studies conducted on *L. vulgaris*, it can be observed that the extraction method and conditions of extraction in this study were not satisfactory for the adequate extraction of polyphenolic compounds. Aqueous extracts of *L. salicaria* showed a very strong ability to inhibit the growth of *E. coli* and *S. aureus* strains, and aqueous extracts of *L. vulgaris* showed a medium strong ability to inhibit the growth of all strains of bacteria used. The good antibacterial activity of the aqueous extract of *L. salicaria* against strains of *Pseudomonas aeruginosa*, a bacterium that belongs to MDR strains, is particularly significant. Based on the results of this research, but also on the results of other scientific works, there is a good basis for further pharmacokinetic and pharmacodynamic research of the active compounds in *Lythrum salicaria*.

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SAŽETAK

Biljne vrste *Lysimachia vulgaris L. i Lythrum salicaria L.* su invazivne biljne vrste, čija su ljekovita svojstva poznata u tradicionalnoj medicini Evrope i Azije. Ove biljne vrste pokazale su blagotvorne efekte u tretmanu kožnih oboljenja, dijareje, proširenih vena, sinuzitisa, različitih vrsta krvarenja. Cilj ovog istraživanja bio je ispitivanje antioksidativnog i antibakterijskog potencijala ekstrakata navedenih biljnih vrsta. Pripremljeni su ekstrakti različite polarnosti korištenjem metoda maceracije i ultrazvučne ekstrakcije. Za ispitivanje sadržaja polifenola korištena je metoda Folin-Ciocalteou, a putem FRAP i DPPH metode ispitan je antioksidativni potencijal. Prema dobivenim rezultatima, možemo uočiti dobro poznat značaj odabira pravog otapala i metode ekstrakcije za maksimalno iskorištenje ljekovitog potencijala biljne vrste. Rezultati ispitivanja pokazali su prisustvo antioksidativne aktivnosti ekstrakata obje biljne vrste, što je evidentirano u tabeli 1, 2 i 3. Od naročitog značaja jeste upečatljivi rezultat antioksidativne aktivnosti metanolnog ekstrakta *Lythrum salicaria L.* dobivenog metodom maceracije. Antibakterijska aktivnost ispitana je za vodene ekstrakte dobivene metodom ultrazvučne ekstrakcije. Poseban akcenat stavlja se na rezultat antibakterijskog dejstva ekstrakta *Lythrum salicaria L.* na sojeve bakterije *Pseudomonas aeruginosa,* koja pripada MDR sojevima. Prema dobivenim rezultatima studije, evidentno je da biljna vrsta *Lythrum salicaria L.* ima naročit potencijal za dalja farmakokinetička i farmakodinamična ispitivanja njenih biološki aktivnih komponenti.

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