UDK 582.661.51:547.56
DOI 10.54652/RSF.2022.V52.I1.367

ORIGINAL SCIENTIFIC PAPER

Total phenolic content, antioxidant and antimicrobial activity of *Silene sendtneri* Boiss. (*Caryophyllaceae*)

Sadržaj ukupnih fenola, antioksidativna i antimikrobna aktivnost *Silene sendtneri* Boiss. (*Caryophyllaceae*)

Sabina Rastoder[1], Rasim Duraković[2], Mirel Subašić[3], Neđad Bašić[3,4], Adisa Parić[4,5], Fatima Pustahija[3,4,*]

[1] JKP "Gradska groblja" d. o. o. Visoko, Mule Hodžića 108, 71300 Visoko, Bosnia and Herzegovina
[2] Krupska bb, 77245 Bužim, Bosnia and Herzegovina
[3] University of Sarajevo, Faculty of Forestry, Zagrebačka 20, 71000 Sarajevo, Bosnia and Herzegovina
[4] University of Sarajevo, Faculty of Science, Department of Biology, Laboratory for Research and Protection of Endemic Resources, Zmaja od Bosne 33-35, 71000 Sarajevo, Bosnia and Herzegovina
[5] University of Sarajevo, Faculty of Science, Department of Biology, Laboratory for Plant Physiology, Zmaja od Bosne 33-35, 71000 Sarajevo, Bosnia and Herzegovina

ABSTRACT

Genus *Silene* (*Caryophyllaceae*) is very rich in secondary metabolites and has antiviral, antimalarial, antitumor, antibacterial and antioxidant properties, but Balkan endemic *Silene sendtneri* (Sendtner's campion) is not analyzed from the aspect of phenolic composition and their biological activities. Evaluation of total phenolic contents (TPC), total flavonoid contents (TFC), and antioxidant and antimicrobial activities of hydromethanolic extracts from inflorescences, stem, rhizome, and seeds of *S. sendtneri*, was done in this study for the first time. The TPC, TFC, and antioxidant activity (DPPH; 2,2-diphenyl-1-picrylhydrazyl) were determined by UV/VIS spectrophotometry. Antimicrobial activity was estimated against selected test microorganisms (*Staphylococcus epidermididis*, *Staphylococcus aureus* subsp. *aureus*, *Salmonella abony, Escherichia coli,* and *Candida albicans*) using a disc diffusion assay. The inflorescences had the highest (11.587 mg $GAEg^{-1}$ DW) and rhizome the lowest TPC (2.017 mg $GAEg^{-1}$ DW). The inflorescences extract exhibited the highest TFC (69.824 mg CEg^{-1} DW), while TFC was not detected in the rhizome extract. The stem extract had the highest antioxidant activity (IC_{50}; 20.51%), while the rhizome had the lowest (61.89%). All extracts showed moderate antibacterial activity against *Staphylococcus epidermididis* and low activity against the three remaining tested organisms. The antifungal activity of inflorescence and rhizome extracts was moderate. Obtained results provide a basis for further investigations of various *S. sendtneri* extracts, which can be a potential natural antioxidant and antimicrobial agent.

Key words: *antimicrobial, Balkan, Bosnia and Herzegovina, secondary metabolites, Sendtner's campion*

* Corresponding author: Fatima Pustahija, f.pustahija@sfsa.unsa.ba

INTRODUCTION – *Uvod*

The growth and development of a plant, as well as its survival and communication with the environment, are regulated by a large number of plant secondary metabolites (Bennett and Wallsgrove, 2006; Hartman, 2007; Kliebenstei, 2013). Secondary metabolites are highly chemically diverse and complex low molecular weight compounds with a wide range of biological and pharmaceutical activity. To date, more than 200,000 secondary metabolites have been identified that are differently distributed in the plant world with diverse and important functions (Wink, 2008; Ribera and Zúñiga, 2012; Kabera et al., 2014). Among them, phenolics and phenolic-related compounds show very pronounced antioxidant, antimicrobial, and anti-inflammatory properties (Mamadalieva et al., 2014; Santos-Sánchez et al., 2019; Hassanuzaman et al., 2020; Aygun et al., 2022).

Genus *Silene* L. (*Caryophyllaceae*), with 887 accepted species, is distributed almost all over the world (Royal Botanic Gardens, online). The genus includes predominantly herbaceous species (annuals, biennials, and perennials), less often smaller shrubs, many of which are cultivated as horticultural species (Eggens, 2006). Also, some of the *Silene* species are used in gastronomy (Alarcon et al., 2006) and traditional medicine (Golovko and Bushneva, 2007); some others, due to the richness of saponins in the root, for soap production (Ahmad et al., 1998); and some are bioindicators of soils rich with salts and heavy metals (Nadgórska-Socha et al., 2011; Filippidis et al., 2012; Karalija et al., 2021). Genus *Silene* is very rich in secondary metabolites and has antiviral, antimalarial, antitumor, antibacterial and antioxidant properties (Mamadalieva et al., 2004; Alarcon et al., 2006; Kucukboyaci et al., 2010; Karamian and Gasemlou, 2013; Mamadalieva et al., 2014; Aygun et al., 2022).

The Sendtner's campion (*Silene sendtneri* Boiss.; syn. *Otites sendtneri* (Boiss.) Holub, *S. velenovskyana* Jordanov & Panov, and *S. schlosseri* Vuk.) is endemic in Albania, Bulgaria, Bosnia and Herzegovina, Croatia, Greece, Montenegro, North Macedonia and Serbia (Marhold, 2011; Đug et al., 2013). According to Grlić (1990) and Mišić and Lakušić (1990), the young leaves of most Balkan *Silene* species are edible as salads, but the plants are also recognized as honey-bearing, medical and good fodder. The total content of phenolic compounds and their antioxidant and antimicrobial activities were analyzed in a number of species of the *Silene* genus, but not in *S. sendtneri*.

The aims of this study were to: 1) quantify total phenolic and flavonoid contents, 2) evaluate antioxidant activity, and 3) antimicrobial activity in methanol extracts of different plant parts of *Silene sendtneri* from the natural population in Bosnia and Herzegovina.

MATERIAL AND METHODS – *Materijal i metode*

Plant material

Plant material was collected from seven individuals in the locality of Pješčana Ravan, Mt. Ozren near Sarajevo in Bosnia and Herzegovina (43°54'191" N, 18°27'170" E; 1,302 m a.s.l., SE exposure, limestone, slope 0-3°). Determination of taxa was done according to Šilić (1988). Vouchers are deposited in the Herbarium of the Faculty of Forestry, University of Sarajevo.

Plant parts (inflorescences, leaves and stems, rhizomes, and seeds) were separated, rinsed with running tap water and dried at a temperature of 50-60°C for 48h. Dried samples were ground and stored in glass bottles until extraction.

Chemicals and reagents

Gallic acid, catechin and DPPH (1,1-diphenyl-2-picrylhydrazyl) are HPLC purity. All chemicals and reagents used were of analytical grade (Sigma-Aldrich, Steinheim, Germany).

Quantitative determination of total phenolics and flavonoids

80 mg of plant material was extracted, using an ultrasonic bath (Elma sonic S 60 H) for 30 min, at 30°C, in two replicates with 12.5 mL of 80% methanol. After evaporation to dry material, the concentration was adjusted to 10 mg mL^{-1}. The obtained extracts were stored at -20°C until further analyses.

For determination of total phenolic and flavonoid contents and antioxidant activity of methanol extracts Shimadzu UVmini-1240 UV-VIS spectrophotometer was used.

Total phenolic content (TPC) was determined using the Folin-Ciocalteu method (Wolfe et al., 2003). 20µL of the methanol extract's aliquot was mixed with distilled water and then with 100 µL of Folin-Ciocalteu's reagent, and 300 µL of freshly prepared sodium carbonate solution (7.5%). After mixing, the tubes were incubated in a water bath, in the dark, for 30 min at 45°C until blue color has developed. Absorbance was measured spectrophotometrically at 765 nm. TPC was expressed as the gallic acid equivalent per gram of dry material (mg GAE/g; $y=0.005x$; $R^2=0.975$).

Total flavonoid content (TFC) was done by the method of Ordoñez et al. (2006). An amount of 20 µL of leaves' or 60 µL of stem's and inflorescences' methanol

extracts were mixed with 25 µL of 10% $AlCl_3$ water solution (w/v), then 25 µL of 1M sodium acetate water solution, 375 µL 96% ethanol, and distilled water to the 1.250 µL of total volume. After homogenization and incubation at room temperature (24°C) for 20 min, the absorbance was taken at 415 nm and TFC was expressed as the catechin equivalent per gram of dry matter (mg CE/g; y=0.0473x; R^2=0.9711).

Antioxidant activity

The DPPH free-radical scavenging activity was determined by the method of Meda et al. (2005). Freshly prepared methanol DPPH solution was mixed with aliquots of diluted methanol extracts (100, 80, 60, 40, and 20 µL) and incubated for 30 min at room temperature in the dark. The change in absorbance was determined at 517 nm against the methanol, and results were expressed as percent inhibition (IC_{50}), calculated graphically based on the calibration curves for each sample. Lower IC_{50} values indicated higher antioxidant activity.

Antimicrobial activity

The disc diffusion method (Bauer et al., 1966) was used to analyze the antimicrobial potential of methanol extracts against selected microorganisms. The antibacterial activity of extracts of different parts of the *S. sendtneri* was determined on gram-positive (*Staphylococcus epidermididis* ATCC® 8739™ and *Staphylococcus aureus* subsp. *aureus* ATCC® 6538™) and gram-negative (*Salmonella abony* NCTC® 6017™ and *Escherichia coli* ATCC® 8739™) bacteria, and the antifungal activity of the extracts on the *Candida albicans* ATCC® 10231™.

Inoculums were prepared by diluting overnight grown microbial cultures with 0.9% NaCl, and suspensions were standardized to the spectrophotometric equivalence of density against 0.5% McFarland standard. 1 mL of bacterial and fungal suspension was spread on sterile Müeller Hinton and Sabouraud dextrose agar plate, respectively. Sterile paper discs (5 mm in diameter) were soaked with 25 µL of methanol extracts. Amoxicillin (HiMedia) and nystatin (Semikem d.o.o.) were used as a positive control for bacteria and *C. albicans*, respectively, while 80% methanol was used as a negative control. The inoculated plates with discs were incubated at 37°C for 24 and 48 hours to allow maximum growth of the bacteria and the fungus, respectively. Antimicrobial activity was expressed as zone of inhibition in mm.

Statistical analysis

The results were presented as means of triplicates ± standard deviation. Data was analyzed using a one-way ANOVA, followed by Duncan's multiple range test (Statistica 8.0 for Windows; ©Copyright StatSoft, Inc. 1984-2007), considering $p<0.01$ as significant.

RESULTS – *Rezultati*

The determined values of total phenols and flavonoid contents in methanol extracts of *S. sendtneri* are presented in Table 1. The highest mean values of TPC were noticed in inflorescences (11.587 mg GAE g^{-1} DW), and the lowest in the rhizome (2.017 mg GAE g^{-1} DW). The inflorescences had a quite high amount of TFC (69.824 mg CE g^{-1} DW), the stem and seeds had a lower amount (42.682 and 23.594 mg CE g^{-1} DW), while its presence was not detected in the rhizome.

Analysis of variance indicated the presence of significant differences in concentrations of TPC and TFC between the analyzed plant parts ($p<0.01$). Duncan's test confirmed that the inflorescence and rhizome differ significantly from the other analyzed plant parts based on TPC (Table 2).

The highest antioxidant activity was recorded for the stem methanol extract of *S. sendtneri*, and the lowest for the rhizome extract (Table 3). All analyzed extracts of *S. sendtneri* shoot showed weak to moderate antibacterial activity against the analyzed microorganisms in comparison to positive control amoxicillin, except against the gram-positive *Staphylococcus epidermididis*. Antifungal activity against *Candida albicans* was moderate.

DISCUSSION – *Diskusija*

Research into the functionality and phytochemical composition of plants is of great interest due to their nutritional and medicinal properties, and the aim is to determine their biological activity (Fabricant and Farnsworth 2001). Thus, according to Verpoorte (2000), the phytochemical composition was determined for only 15% of the total number of plants on Earth, and biological activity was observed in only 6% of them.

The *Caryophyllaceae* family is characterized by the presence of saponins in larger quantities (Ahmad et al., 1998), while more than 450 secondary metabolites have been isolated in the genus *Silene*, mainly phytoecdisteroids, triterpenes, saponins, other terpenoids, phenols and fatty acids (Mamadalieva et al., 2004; Alarcon et al., 2006; Karamian and Ghasemlou, 2013; Mamadalieva, 2012; Mamadalieva et al., 2014).

Total phenolic and flavonoid content and antioxidant activity

It is considered that phenolic compounds have the greatest potential for neutralizing free radicals, whereby the phenolic composition of the plant determines its pharmacological properties. Phenylpropanoid metabolism depends on the genotype, phenophase, and ecological conditions (altitude, light, temperature and soil) in which the individual lives (Dixon and Paiva, 1995; Hossain and Shah, 2015; Sharma et al., 2019; Kandoudi and Németh-Zámboriné, 2022; Medda et al., 2022). However, the biological activity of natural plant extracts depends on the plant compounds themselves, their mixture, and the type of solvents for extraction, as well as the isolation procedure itself (Zheng and Wang, 2001; Oreopoulou, 2003; Bastola et al., 2017; Aygun et al., 2022).

Aerial parts of *S. sendtneri* had higher TPC and TFC compared to extracts of the rhizome. The amount of TPC in the methanol extracts of Sendtner's campion has relatively similar values to those of *S. inflata* (Mouffouk et al. 2019), but moderate to low values in comparison to data of Karamian and Ghasemlou (2013) and Aygun et al. (2022) for five other *Silene* species. But, TFC values in this study were mostly higher, especially for inflorescences, compared to the data of Karamian and Ghasemlou (2014) and Aygun et al. (2022), which was not the case with the rhizome. Possible reasons for this clear deviation can be found in the species themselves, the ecological conditions in which they live, but also in the use of different methods, standards, and solvents for extraction.

Many *Silene* species are characterized by the possession of complex systems of antioxidant and antimicrobial activity, while the same has not been analyzed in *S. sendtneri* (Taskin and Bitis, 2013; Mihajilov-Krstev et al., 2015; Mouffouk et al., 2019; Aygun et al., 2022).

Due to the fact that each plant contains different groups of phenolic compounds, the antioxidant activity of their extracts is very different, although, most often, it is significantly correlated with the presence of phenols and flavonoids. The antioxidant activity of plant extracts may be a consequence of the presence of certain phenolic compounds, their concentrations, and chemical structure, but also the possible presence of synergisms/antagonisms between them (Shahidi et al., 1992; Kähkönen et al., 1999; Zheng and Wang, 2001; Cai et al., 2004; Spiridon et al., 2011; Jakimiuk et al., 2022). Also, total phenols often do not include all antioxidants present in the analyzed extracts (e.g. ascorbic acid, tocopherol, pigments, and some minerals) (Kähkönen et al., 1999; Singelton et al., 1999; Karamian and Ghasemlou, 2013; Cai et al., 2004; Spiridon et al., 2011; Hossain and Shah, 2015).

Research on the antioxidant activity of extracts of a number of species of campions showed that some species contain relatively large amounts of phenols and flavonoids and that there is a good correlation between antioxidant activity and their content (Conforti et al., 2011; Karamian and Ghasemlou, 2013; Taskin and Bitis, 2013; Mouffouk et al., 2019; Aygun et al., 2022; Jakimiuk et al., 2022). However, the results obtained in this study indicate a strong and moderate anti-radical activity of the analyzed extracts of Sendtner's campion. Therefore, it can be assumed that the antioxidant activity of the analyzed extracts probably can be attributed to some other types of secondary metabolites, which needs to be better studied.

Antibacterial and antifungal activity

The antibacterial activity of plants is a consequence of the action of various chemical agents in the extracts, which is why there is a need for constant development and discovery of potential antimicrobial drugs. In the majority of phytochemical studies, it was discovered that the presence of phenols, flavonoids, tannins, glycosides, saponins, terpenoids, alkaloids, and anthocyanins most often contributes to the antimicrobial activity of many plants (Ertürk et al., 2006; Kaur and Mondal, 2014).

A number of authors state that some *Silene* species have moderate antibacterial activity against both gram-positive and gram-negative bacteria, which can be explained by the presence and synergistic action of phenolic compounds and monoterpenes (Ertürk et al., 2006; Bajpai et al., 2008; Kucukboyaci et al., 2010; Mamadalieva et al., 2010a, 2010b; Mamadalieva, 2012; Karamian and Ghasemlou, 2013; Mamadalieva et al., 2013).

The results obtained in this study expand the knowledge about the effect of extracts of *Silene* species on microbial organisms. Antibacterial activity of methanol extracts of Sendtner's campion was low against all investigated bacteria, whereby the seeds extract showed greater activity against *Staphylococcus epidermidis*. The obtained data could not be correlated with antioxidant activity and/or TPC and TFC, although the presence of some unknown bioactive organic compounds in the seeds extract of *S. sendtneri* is possible.

Weak to moderate antibacterial activity of the tested *S. sendtneri* extracts, in comparison to positive control amoxicillin, can be explained by the presence/absence of appropriate bioactive compounds, their concentrati-

ons and mutual interactions, and also solvent and extraction procedure used (Bajpai et al., 2008; Mouffouk et al., 2019, and references therein).

According to the available literature, only three *Silene* taxa showed antifungal activity against *Candida albicans* (Ertürk et al., 2006; Kucukboyaci et al., 2010; Mamadalieva et al., 2014), and the results obtained in this study represent an important contribution to the understanding of this genus. Rhizome and inflorescences extracts of *S. sendtneri* have been shown to be moderately potent against *C. albicans*, which probably does not depend on the phenolic compounds present but on some other secondary metabolites, because plants produce diverse bioactive compounds against fungal infection depending on the species itself, the analyzed plant part and environmental conditions (Morrisey and Osbourn, 1999; Selitrennikoff, 2001; Webster et al., 2008).

CONCLUSION – *Zaključak*

The results of this study suggested that the analyzed extracts of *Silene sendtneri* have lower TPC and higher TFC, and higher to moderate antioxidant activity in comparison to other *Silene* species. Also, weak and moderate antimicrobial activity against four strains of gram-positive and gram-negative bacteria and the fungus *Candida albicans*, was proved. This activity can be explained by the presence and mutual interactions between phenols, flavonoids and some other, in this study, unidentified compounds. Therefore, more detailed investigations of different types of *S. sendtneri* extracts are necessary, in order to both isolate and identify its potential bioactive compounds and better understand their action mechanisms.

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Table 1. Descriptive parameters of secondary metabolites for *Silene sendtneri* (TPC – total phenolic content, TFC – total flavonoid content, $\bar{X}$ – average, SD – standard deviation, CV – coefficient of variation)

*Tabela 1: Opisni parametri sekundarnih metabolita za **Silene sendtneri** (TPC – ukupni sadržaj fenola, TFC – ukupni sadržaj flavonoida, $\bar{X}$ – srednja vrijednost, SD – standardna devijacija, CV – koeficijent varijacije)*

	TPC (mg g^{-1} GAE)				TFC (mg g^{-1} CE)		
	Stem	*Inflorescence*	*Seed*	*Rhizome*	*Stem*	*Inflorescence*	*Seed*
Min	5.907	11.045	5.450	1.174	32.803	60.516	21.462
Max	8.383	12.823	8.800	2.970	53.538	79.657	26.183
$\bar{X}$	7.134	11.587	7.040	2.017	42.682	69.824	23.594
SD	0.871	0.595	1.137	0.680	6.775	7.432	1.874
CV (%)	12.210	5.133	16.146	33.703	15.874	10.644	7.943

GAE – gallic acid equivalent; CE – catechin equivalent

Table 2. Intrapopulation differences of TPC (total phenolic content) and TFC (total flavonoid content) according to Duncan's multiple range test ($p<0.01$)

Tabela 2: Intrapopulacijske razlike TPC (ukupni sadržaj fenola) i TFC (ukupni sadržaj flavonoida) na osnovu Duncanovog testa višestrukog raspona ($p<0,01$)

TPC (mg g^{-1} GAE)				TFC (mg g^{-1} CE)		
	Shoot	***Inflorescence***	***Seed***		***Shoot***	***Inflorescence***
Inflorescence	**0.000152**			***Inflorescence***	**0.000161**	
Seed	0.836984	**0.000065**		***Seed***	**0.000168**	**0.000075**
Rhizome	**0.000065**	**0.000054**	**0.000152**			

GAE – gallic acid equivalent; CE – catechin equivalent

Table 3. Antioxidative activity (IC_{50}) and antimicrobial activity (expressed as zone of inhibition) of methanol extracts of *Silene sendtneri* against selected microbial strains (mean ± SD, n = 3)

*Tabela 3: Antioksidativna aktivnost (IC_{50}) i antimikrobna aktivnost (izražena kao zona ihibicije) metanolnih ekstrakata **Silene sendtneri** protiv odabranih sojeva mikroorganizama (srednja vrijednost ± SD, n = 3)*

Extract	IC_{50}	The diameter of the inhibition zone (mm)				
		SE	SAu	SA	EC	CA
Amoxicillin		12±0.10	38±0.20	23±0.30	20±0.20	
Nystatin						20±0.20
Stem	20.51	7±0.15	6±0.20	8±0.15	8±0.15	8±0.15
Inflorescence	40.47	8±0.10	6±0.10	6±0.15	7±0.15	11±0.20
Seed	40.44	11±0.20	6±0.10	6±0.15	7±0.10	8±0.10
Rhizome	61.89	9±0.15	7±0.10	8±0.20	6±0.15	11±0.20

SE – *Staphylococcus epidermididis* ATCC® 8739™; SAu – *Staphylococcus aureus* subsp. *aureus* ATCC® 6538™; SA – *Salmonella aboni* NCTC® 6017™; EC – *Escherichia coli* ATCC® 8739™; CA – *Candida albicans* ATCC® 10231™

SAŽETAK

U ovoj studiji urađena je procjena ukupnog sadržaja fenola (TPC) i flavonoida (TFC) te antioksidativnog i antimikrobnog djelovanja hidrometanolnih ekstrakata iz cvasti, stabljike, rizoma i sjemenki *Silene sendtneri* (Sendtnerova pušina, *Caryophyllaceae*). TPC, TFC i antioksidativna aktivnost (DPPH; 2,2-difenil-1-pikrilhidrazil) određeni su UV/VIS spektrofotometrijom. Antimikrobna aktivnost procijenjena je protiv odabranih testnih mikroorganizama (*Staphylococcus epidermididis*, *Staphylococcus aureus* subsp. *aureus*, *Salmonella abony* i *Escherichia coli*, te *Candida albicans*) pomoću disk difuzijskog testa. Cvasti su imale najviši (11,587 mg $GAEg^{-1}$ SM), a rizom najniži ukupni sadržaj fenola (TPC) (2,017 mg $GAEg^{-1}$ SM). Najbogatiji sa ukupnim flavonoidima (TFC) bili su ekstrakti cvasti (69,824 mg CEg^{-1} DW), dok TFC nije detektovan u ekstraktu rizoma. Najveću antioksidativnu aktivnost (IC_{50}; 20,51%) imao je ekstrakt stabljike, a najmanju rizoma (61,89%). Svi ekstrakti su pokazali umjerenu antibakterijsku aktivnost samo protiv *Staphylococcus epidermididis,* te nisku aktivnost protiv tri preostala testirana organizma. Antifungalna aktivnost ekstrakata cvasti i rizoma bila je umjerena. Dobiveni rezultati daju osnovu za daljnja istraživanja različitih ekstrakata *S. sendtneri*, koji mogu biti potencijalni prirodni antioksidanti i antimikrobni agensi.