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SADRŽAJ:

New findings and confirmation of the presence of two alien grass species in Croatia: *Cenchrus longisetus* and *Sporobolus indicus*

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izvorni znanstveni rad (original scientific paper)

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Citiranje/Citation[2]

Abstract

This paper provides new data concerning the presence of two alien grass species in Croatia based on a revision of herbarium material from ZA, ZAHO and ZAGR, literature data and on field observations. Two poorly documented alien grass species *Cenchrus longisetus* M. C. Johnst. and *Sporobolus indicus* (L.) R. Br., are confirmed for the country.

Brief information on the species distribution in Croatia and a discussion of the alien and invasive status in the country is provided. Judging from the literature and field observations both species should be considered as naturalized, non-invasive species in Croatia. The text is illustrated with photographs from the new localities of both species. These new confirmed records allow us to better define the European and national distribution of the targeted species and offer new insights into the alien flora of Croatia.

Key words: *Cenchrus*, Croatia, distribution, new record, Poaceae, *Sporobolus*.

Introduction

The genus *Cenchrus* L. (Poaceae) is a tropical genus belonging to subfamily Panicoideae, tribe Paniceae. The genera *Cenchrus* and *Pennisetum* have been formally merged by Chemisquy et al. (2010) in the genus *Cenchrus* and together include approximately 120 species, mostly from warm regions. In the European vascular flora 13 species have been recorded (Valdés and Scholz, 2009), among which only three are present in Croatia: *Cenchrus longisetus* M. C. Johnst., *C. longispinus* (Kneuck.) Fernald and *C. spicatus* (L.) Cav. (Nikolić, 2015).

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The species *Cenchrus longisetus* M. C. Johnst. (syn. *Pennisetum villosum* Fresen) is native to East Africa (Eritrea, Ethiopia, and Somalia). It was introduced for ornament and became naturalized in the Mediterranean region (El Mokni and Verloove, 2019). It is currently known as alien in some European countries: Croatia, France, Germany, Greece, Italy and Spain (Valdés and Scholz, 2009). This species has been reported as occurring in Croatia (Sustipan, Split) by Ruščić (2003), which was not substantiated by herbarium material accessible data in officially registered herbarium collections and has not been confirmed since.

The genus *Sporobolus* R.Br. (Poaceae) is a cosmopolitan genus belonging to subfamily Chloridoideae, tribe Zoysieae (Peterson et al., 2007). It is one of the largest genera within the subfamily, including about 200 species predominantly distributed in the tropical and subtropical areas of the world. In the European vascular flora six species have been recorded (Valdés and Scholz, 2009), among which four are present in Croatia: native *Sporobolus pungens* (Schreb.) Kunth. and three alien species, *S. indicus* (L.) R. Br., *S. neglectus* Nash. and *S. vaginiflorus* (A. Gray) A. W. Wood (Nikolić, 2015).

The species *Sporobolus indicus* (L.) R. Br (syn. *Agrostis indica* L.) is native to North America, currently known as alien in some European countries: Bulgaria, Czech Republic, France, Germany, Greece, Italy, Montenegro, Portugal, Spain and Switzerland (Valdés and Scholz, 2009). It was recorded as new to Slovenia (Glasnović and Jogan, 2009), Serbia (Perić et al., 2013), Austria (Eichberger et al., 2015), Albania (Barina, 2017) and recently Hungary (Bauer and Verloove, 2023). This species has been reported as occurring in Croatia (Istria) by Starmüchler (2003), without precise locality, which was not substantiated by accessible in officially registered herbarium collections and has not been confirmed since.

As no previous records have been mentioned in the Flora Croatica Database (Nikolić 2015), this species is regarded as a new alien for Croatia. Regarding the history of the spread of *S. indicus* in Europe referencing goes to Bauer and Verloove (2023).

The author wanted to report new findings of two poorly documented alien grass species in Croatia (*C. longisetus* and *S. indicus*). The aim of the study is to enable the monitoring of the further spread of the mentioned species, their ecology and potential invasiveness.

Figure.1. *Cenchrus longisetus on the lawns of Park Sustipan (Split), 1 a – naturalized habitat, 1 b – panicle; Sporobolus indicus in the vicinity of Živogošće (car-camp Dole), 2 a – naturalized habitat, 2 b – panicle (Photos by S. Maslo).*

Materials and methods

The material is based on the study of literature data, herbarium material from ZA, ZAHO and ZAGR and on data collected in the field in central Dalmatia, in the period 2007-2023. The specimens were collected and stored in the Herbarium ZA and in the private collection of the author. Digital photographs were taken in the field, while the specimens were identified with the following keys: *Cenchrus longisetus* with Clayton (1980) and *Sporobolus indicus* (Hansen, 1980). The nomenclature follows the Euro-Med checklist (EURO+MED 2006).

Study species

C. longisetus is matforming perennial, rhizomatous grass, up to 60 cm high. Culms are geniculately ascending, or decumbent, with glabrous nodes and sheats. Ligule is very short, membranous, with a row of long hairs. Life-blades are 7-15 cm long and 2-6 mm wide, flat to folded, with ciliate margins. Inflorescence is a compact, terminal panicle, spiciform; oblong, or ovate, white, 2-12 cm long, 1-2 cm wide. Spikelets 1 to 4 in a fascicle. Fascicles are short-peduncled with a tuft of white hairs at base. Involucral bristles are numerous, spreading, the inner ones are very plumose, the longer are 4 -5 cm long. A detailed description of the species can be found in Clayton et al. (2006)

S. indicus, sensu latissimo, is a fairly variable complex and many of separate species have been recognized within the complex (Clayton, 1965). However, if a narrow species concept would apply, then all plants seen from Europe should be assigned to S. indicus (L.) R. Br., and this is the classification currently adopted also in Euro+Med PlantBase (Valdés and Scholz 2009).

S. indicus is a perennial, usually densely tufted, with erect culms, up to 100 cm high. Leaves basal and cauline. Sheaths smooth, often shiny, margin ciliolate, otherwise glabrous. Ligule is a fringe of hairs, up to 0.5 mm long. Leaf-blades folded to more or less flat, linear, filiform in the upper part, gradually tapering to a very fine point, 10-30 cm long, 2-7 mm wide. Panicles contracted and spiciform, then often interrupted at base, to effuse, 10-30 cm long and 0.6-1 cm wide (Fig. 1c,d), Spikelets solitary, lanceolate, subterate, ca 2 mm long, yellowish, pale to nearly blackish green. Lower glume ovate to elliptic, 0.6-1 mm long, membranous, without keels, 1-veined. Upper glume ovate 0.9-1.3 mm long, membranous, without keels, 1-veined. Lemma ovate to oblong, 1.3-2 mm long, 1-3 nerved. Palea elliptic to oblong,1.2-1.9 mm long, not easily split by the seed. Caryopsis with free soft pericarp, oblong, 1-1.3 mm long, obtuse (Clayton, 1965, Hansen, 1980 and Clayton et al. 2006).

Results and discussion

The first finding of Cenchrus longisetus for Croatia comes from Split in December 1998, found on the stony lawns of Park Sustipan (Ruščić, 2003). Upon re-visit of the site twenty-five years later, in February 2023, I observed about twenty tufts growing on waste places and on the stony lawns of Park Sustipan (43°30'04" N; 16°25'28" E, leg. S. Maslo 11.02.2023, private herbarium, Maslo) (Fig. 1a, b). This locality is still the only known finding of the species in Croatia; grown for ornament and sometimes escaped. In the area of Split, the plant is grown as an ornamental, and thus horticulture is considered to have been one of the introduction pathways. Following the framework proposed by

Blackburn et al., 2011, I consider C. longisetus to be a naturalized alien species in the territory of Croatia, currently in the C3 category (Individuals surviving in the wild in the location where introduced, reproduction occurring, and population self-sustaining).

During field research in central Dalmatia in July 2007, the presence of the species Sporobolus indicus in Croatia was confirmed, four years after the first report from the Istrian peninsula. It was recorded in the area of Živogošće (car-camp Dole), 20 km from the city Makarska, (43°10'16" N; 17°11'53" E, leg. S. Maslo 08.07.2009, herbarium ZA 5654). Plenty of plants were observed in a single site of about 200 square meters (Fig. 2a,b), on xeric grassland near sea level with Paspalum dilatatum Poir. with which it co-occurs in places. Approximately at the same time it was registered in the city of Supetar (island Brač), within the city's lawns and the undeveloped habitat within the ruderal flora. Further findings were in the area of Split in 2013. Localities as well as the distribution map can be found in Nikolić (2015).

S. indicus seems to be established in the area now. Since 2007, the plant has managed to survive in the habitats where it was originally observed and has spread along nearby roads and pathways. In July 2011 I observed this neophyte growing even like weeds in the cracks of the concrete next to the camp toilets. Observations during the period between 2007-2022, indicate that this neophyte has been spreading in the area of car-camp. Following the framework proposed by Blackburn et al., 2011, I can consider S. indicus as a naturalized alien species in the territory of Croatia, currently in the C3 category (Individuals surviving in the wild in the location where introduced, reproduction occurring, and population self-sustaining). The area where it was found is frequently visited by many tourists from different countries, so it is likely that it was inadvertently introduced this way.

Conclusion

During the latest field research in the area of central Dalmatia, the presence of 2 lesser-known alien grasses in the flora of Croatia was confirmed. Both species show a stable population and can be safely labeled as naturalized species in Croatia. The presence of the *Cenchrus longisetus* was confirmed after the first finding 25 years ago in the area of Sustipan in Split. About 20 tussocks with numerous culms were recorded. On the other hand, *Sporobolus indicus* was recorded in several localities with the largest population in Dole near Živogošće. In that locality, the species covers several hundred square meters, with a tendency to spread further if it takes on an invasive behavior. The potentially invasive behavior of this species should be monitored in the following years.

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Phenolic compounds in *Geranium dalmaticum* (Beck) Rech. f. and G. *macrorrhizum* L. (Geraniaceae) growing in Croatia

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Abstract

The quantitative analysis of bioactive phenolic compounds was carried out for the first time in above-ground plant parts of *Geranium dalmaticum* (Beck) Rech. f. and *G. macrorrhizum* L. (Geraniaceae) from Croatia by means of UV-Vis spectrophotometric methods and HPLC analysis. Both species had similar contents of polyphenolic substances expressed as mg of standard equivalent /g of dried herbal sample: total polyphenols (217.60 ± 1.08 and 215.53 ± 1.10 mg/g), tannins (155.83 ± 0.60 and 157.73 ± 0.61 mg/g), total flavonoids (5.10 ± 0.10 and 5.53 ± 0.10 mg/g), and total phenolic acids (13.27 ± 1.34 and 15.33 ± 0.45 mg/g), respectively for *G. dalmaticum* and *G. macrorrhizum*. Only the content of phenolic acids was somewhat higher in *G. macrorrhizum* but it was not statistically significant ($p > 0.05$). HPLC analysis showed that, among seven tested phenolic compounds, only quercetin was quantified in *G. dalmaticum* (0.23 % of dry weight), and rutin in *G. macrorrhizum* (1.12 % of dry weight). The obtained results represent a useful basis for further research of phytochemicals and biological activities of *G. dalmaticum* and *G. macrorrhizum* extracts.

Key words: *Geranium*, eastern Adriatic, polyphenols, flavonoids, phenolic acids.

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Introduction

Even today, domestic species still represent a significant source from which specimens can be selected for planting for decorative purposes, especially in urban areas. In the light of current climate changes, species that tolerate climatic extremes, especially drought, are particularly interesting. Domestic species are also important as a food source for many organisms, such as pollinating insects. Furthermore, some of these species are also interesting because of their phytochemical traits. *Geranium* L. species (family Geraniaceae) are aromatic and decorative species, and some of them are used in horticulture as well as in traditional medicine (Şöhretoğlu et al., 2017). One of the most interesting *Geranium* species in Croatia is *Geranium dalmaticum* (Beck) Rech. f. (Fig. 1a–c). It is a perennial herb up to 15 cm high which grows in Mediterranean-mountainous area on screes, at the edges and on the sides of the sinkhole and scrapes, in crevices of limestone and calcareous rocks at altitudes from 200 m to 960 m. *G. dalmaticum* occurs within the eastern Adriatic scrub (*Cisto-Ericetalia* Horvatić, 1958) and black pine forests on dolomite (*Erico-Fraxinion orni* Horvat 1959) at different exposures. It forms small, dense, pure populations in shady or semi-shady places (Šilić, 1990; Nikolić, 2015). The only known population of this species in Croatia is on St. Ilija Mountain, Pelješac peninsula in south Dalmatia. Other several populations are in Montenegro, Kosovo, and Albania (Nikolić, 2015). *Geranium dalmaticum* have small leaves up to 2.5 (– 4.5) cm wide and beautiful dull purplish-red flowers with petals up to 18 mm long (Nikolić, 2015). The chemical composition of the essential oil and the content of macroelements and trace elements in *G. dalmaticum* were investigated by Kremer et al. (2013). *G. macrorrhizum* L. is similar, but larger perennial herb up to 50 cm high with dull purplish-red flowers (Fig. 1d and 1e). *G. macrorrhizum* grows in mountainous area on shallow, calcareous soils from montane to subalpine vegetation belt (Webb and Ferguson, 1978). It inhabits edges of the trail, sinkhole, scrapes, scree, terraces and crevices in limestone and calcareous rocks in shady or semi-shady places.

Geranium species have been used as anti-infective agents (Radulović, 2010). Antioxidant activity, hepatoprotective effect, and antimicrobial properties have also been demonstrated (Miliauskas, 2006; Radulović, 2012). *G. macrorrhizum* is known to be rich in tannins and its extracts were reported to possess a broad spectrum of antimicrobial activities. It also has strong hypotensive and astringent activity, as well as cardiotonic and sedative properties (Bate-Smith, 1981; Ivancheva et al., 1992). It is increasingly cultivated in Europe for its ornamental flowers, as well as for use in aromatherapy and traditional herbal medicine due to its broad spectrum of biological activities. Essential oil of *G. macrorrhizum* is highly valued in perfumery and is also used as a food-flavouring agent (Ćavar Zeljković et al., 2020).

This study aims to get insight into the content of phenolic compounds of *G. dalmaticum* and *G. macrorrhizum* from Croatia, as well as to compare the content of different polyphenolic substances between these two *Geranium* species as a contribution to investigations of their phytotherapeutic potential.

Figure. 1. *Geranium dalmaticum (a, b, c), and G. macrorrhizum (d, e). (Photo: D. Kremer).*

Materials and methods

Plant material

The samples of *Geranium dalmaticum* and *G. macrorrhizum* were collected by K. Karlović and D. Kremer during the blooming period in June 2015 on Sv. Ilija Mountain, Pelješac peninsula (*G. dalmaticum*: 43 00'03.1" N, 17 10'58.9' E, 942 m a.s.l.), and on Velebit Mountain (*G. macrorrhizum*: 44 37'01.2" N, 15 01'45.8' E, 1139 m a.s.l.). Above-ground plant parts of several dozen plants were collected along hiking trails, on a dry day, by random selection. The collected material was mixed to obtain the randomly selected sample. Herbal material was air-dried at 22ºC and 60 % relative humidity in a well-ventilated room and protected from direct sunlight for 20 days. The dried plant material of each plant species was milled with laboratory mill Foss CT 193 Cyclotec (Foss Analytica Co., Ltd., Suzhou, China) into powder and properly stored until phytochemical analysis. Voucher specimens of herbal material were deposited in the "Fran Kušan" Herbarium of the Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia.

Chemicals and apparatus

Folin-Ciocalteu reagent (FCR), naringenin, quercetin, rutin, caffeic acid, coumaric acid, ferulic acid, sinapic acid, tannic acid (Sigma-Aldrich Chemical Co., St. Louis, USA); methanol, ethanol, and HNO_3 (Kemika, Zagreb, Croatia) were used. Absorbance measurements were performed using Agilent 8453 E UV/Vis spectrophotometer equipped with the PC-HP 845x UV-Visible System (both Hewlett Packard, Boeblingen, Germany) and 1 cm quartz cells. HPLC analysis was performed using a C_{18} reversed-phase packing column (Zorbax Eclipse XDB-C_{18}, 150 mm × 4.6 mm, 5 µm; Agilent, Santa Clara, USA) and Agilent 1100 Series HPLC system (Agilent, Santa Clara, USA).

Extract preparation

For spectrophotometric determination of polyphenolic substances, three extractions were made for each polyphenol determination method, i.e., three extractions of total polyphenols/tannins, three of flavonoids, and three extractions of phenolic acids from both *G. dalmaticum* and *G. macrorrhizum*.

Extraction of total polyphenols and tannins

The mass of 0.250 g of powdered plant material (the above ground parts) was extracted with 80 mL of 30 % (V/V) methanol (70°C, water bath, 15 min). After cooling and filtration, each extract was diluted to 100.0 mL with 30 % methanol (basic sample solution, BSS). BSS (2.0 mL) was mixed with 8.0 mL of water and 10.0 mL of acetate buffer (solution TP, S_{TP}). S_{TP} (10.0 mL) was shaken with 50.0 mg of casein during 45 min to allow adsorption of tannins, and then filtrated (solution T, S_T). Solutions S_{TP} and S_T were subjected to quantitative analysis of total polyphenols and tannins.

Extraction of total flavonoids

Powdered plant material (0.20 g) was extracted with 20 mL of acetone, 2 mL of 25 % HCl and 1 mL of 0.5 % hexamethylenetetramine (boiling water bath, 30 min). Each extract was filtered, and extraction of the same herbal material was repeated three times with 20 mL of acetone (boiling water bath, 10 min). After cooling and filtration, each extract was made up to 100.0 mL with acetone (basic sample solution, BSS). 20 mL of BSS was mixed with 20 mL of distilled water and then extracted with ethyl acetate (first with 15 mL and then three times with 10 mL). Ethyl acetate extracts were rinsed two times with distilled water then filtered and made up to 50.0 mL with ethyl acetate (Solution TF, S_{TF}).

Solution S_{TF} was subjected to quantitative analysis of total flavonoids.

Extraction of total phenolic acids

The extraction is performed as follows: to 0.200 g of the powdered drug 80 mL of ethanol (50 %, V/V) was added and boiled in a water-bath under a reflux condenser for 30 min. After cooling and filtration, the filter was rinsed with 10 mL of ethanol (50 %, V/V). The filtrate and the rinsing were combined in a volumetric flask and diluted to 100.0 mL with ethanol (50 %, V/V). Test solutions prepared in the described manner were subjected to quantitative analysis of total phenolic acids.

Phenolic compounds analyses

Total polyphenols and tannins contents (FCR procedure)

Determination of total polyphenols (TP) and tannins (T) was performed by using the prevalidated FCR procedure for polyphenols analysis according to Jurišić Grubešić et al. (2005). This method is based on a reaction with Folin–Ciocalteu's phenol reagent (FCR) and spectrophotometric determination of TP and T (after precipitating with casein) at 720 nm. The standard substance was tannic acid. The contents of TP and T in previously described extracts were evaluated in three independent analyses.

S_{TP} (1.0 mL) was mixed with 0.5 mL of FCR and diluted to 10.0 mL with 33 % $Na_2CO_3 \times 10H_2O$. The same procedure was performed with S_T. After filtration, the absorbance at 720 nm of the final blue solution was measured. Absorbance values obtained for S_{TP} correspond to total polyphenol content. The difference between the absorbance of S_{TP} and S_T corresponds to the concentration of casein-adsorbed tannins in plant samples.

The results were expressed as mg of standard equivalent / g of dried plant sample according to following equations:

$$TP\ (mg/g) = 10A_{TP} / 0{,}025 \quad (1)$$

$$T\ (mg/g) = 10A_{T} / 0{,}025 \quad (2)$$

A_{TP} – absorbance related to TP content; A_T – absorbance related to T content.

Total flavonoids contents (F–Al procedure)

The content of total flavonoids (TF) was obtained by using a prevalidated colorimetric assay with $AlCl_3$ (F–Al procedure) according to Jurišić Grubešić et al. (2007). This method is based on the hydrolysis of glycosides, extraction with ethyl acetate of TF aglycones and complex formation with $AlCl_3$ at 425 nm. The contents of TF in previously described extracts were evaluated in three independent analyses.

In 10 mL of S_{TF} 0.5 mL of 0.5 % solution of sodium citrate and 2 mL of $AlCl_3$ (2 g of $AlCl_3$ in 100,0 mL of 5 % acetic acid solution in methanol) were added and then made up to 25.0 mL with 5 % methanolic solution of acetic acid. After 45 min, yellow solutions were filtered, and the absorbance of the developed complex was measured at 425 nm.

TF concentration was expressed as mg of standard equivalent / g of dried herbal material and calculated as quercetin using the following expression:

$$TF\ (mg/g) = 10A \times 0.772 / m \qquad (3)$$

A – absorbance; m – mass of the dry plant material (g); 0.772 – conversion factor related to specific absorbance of quercetin at 425 nm (i.e., 810).

Total phenolic acids contents (THD procedure)

The content of total phenolic acids (TPA) in plant extracts was obtained using the official pharmacopoeial method (European Pharmacopoeia 2014) for spectrophotometric determination of hydroxycinnamic derivatives, using the nitrite-molybdate reagent of Arnow, in a sodium hydroxide medium (THD procedure) at 505 nm. The contents of TPA in previously described extracts were evaluated in three independent analyses.

One millilitre of the resulting extract was mixed with 2.0 mL of 0.5 M HCl, 2.0 mL of nitrite-molybdate reagent (10 g of sodium nitrite and 10 g of sodium molybdate was dissolved in 100 mL of distilled water), 2.0 mL of 8.5 % sodium hydroxide solution and with distilled water up to 10 mL. A compensatory solution was made by diluting 1.0 mL of extract with distilled water up to 10 mL.

The absorbance of the test solution was measured immediately at 505 nm.

TPA concentration was expressed as mg of standard equivalent / g of dried herbal material and calculated as rosmarinic acid using the following expression:

$$TPA\ (mg/g) = 10A \times 2.5 / m \qquad (4)$$

A – absorbance; m – mass of the dry plant material (g); 2.5 – conversion factor related to specific absorbance of rosmarinic acid at 505 nm (i.e., 400).

HPLC analysis

A powdered herbal material (500 mg) mixed with 20.0 mL of methanol at 25 °C for 25 min was used for the ultrasonic extraction (Čeh et al., 2007). Afterwards, extracts were filtered (filter paper black ribbon, Machery Nagel, Germany), and the obtained filtrates were diluted to 25.0 mL with 80 %

ethanol. Solutions were filtered using a 0.45 µm PTFE 25 mm filter (Restek, Bad Homburg, Germany). Lastly, 5.0 µL of each investigated extract was put into the HPLC instrument for analysis.

The stock solutions of standard compounds (rutin, quercetin, naringenin, sinapic acid, caffeic acid, coumaric acid, ferulic acid) were also prepared according to Čeh et al. (2007). A gradient elution was used for the analysis. The mobile phase was composed of phase A (water, pH = 2.50 adjusted with acetic acid), and phase B (acetonitrile 100 %). Detection was performed by diode array detector at wavelengths of 280 nm (naringenin), 320 nm (sinapic acid, caffeic acid, ferulic acid, coumaric acid), and 370 nm (rutin, quercetin). Individual components were tentatively identified by comparison with retention times of standards and unknown peaks in the samples. The method of standard addition was applied to avoid misinterpretation of the results. HPLC analysis of plant material was performed in triplicate.

Data analysis

All analyses were performed in triplicate and the results were expressed as mean ± SD. The significance of differences between analytical results was checked by the t-test for independent samples using the STATISTICA 7 software (StatSoft Inc., Tulsa, OK, USA).

Results and Discussion

Three different UV-Vis spectrophotometric methods were used for a quantitative analysis of TP, T, TF, and TPA in *G. dalmaticum* and *G. macrorrhizum*, and the results are presented in Table 1. Content of phenolic compounds was similar in both species and according to the t-test there was no statistically significant difference at $p > 0.05$ between species for any group of phenolic compounds. Slightly higher content of TP was recorded in *G. dalmaticum* than in *G. macrorrhizum* (217.60 mg/g dw *vs.* 215.53 mg/g). All other analyzed phenolics showed a tendency of higher concentration in *G. macrorrhizum* than in *G. dalmaticum*, T (157.73 mg/g *vs.* 155.83 mg/g), TF (5.53 mg/g *vs.* 5.10 mg/g), and TPA (15.33 mg/g *vs.* 13.27 mg/g). According to Şöhretoğlu et al. (2017), TP content in two *Geranium L. species from Turkey ranged from* 224.64 to 345.07 mg/g of the dry extract in *G. psilostemon Ledeb.,* and from 208.10 to 389.09 mg/g in *G. stepporum Davis*. In the same study, the TF content was higher in mentioned *Geranium* species compared to our study and ranged from 18.67 to *114.59* mg/g in *G. psilostemon*, and from 7.74 to 116.58 mg/g of the dry extract in *G. stepporum.* According to Radulović et al. (2012), TP content in methanol extracts of *G. macrorrhizum* was 160.2 ± 3.1 mg of gallic acid equivalents (GAE) per g of dry plant material (leaves), which is less than in our study. Ethanol extracts contained even lower, but still significant, amount of phenolics, while extraction with other solvents did not result in high TP values. In the same study, TF content was also the highest for the methanol extracts (44.9 ± 1.1 mg of catechin equivalents per g of dry leaves). TP content in above-ground parts of several *G. macrorrhizum* samples ranged from 35.91 mg/g to 165.95

mg/g GAE (Ćavar Zeljković et al., 2020), which is significantly less than the results obtained in our study. According to the abovementioned study, the results for TF content were very different among the samples and ranged from 1.33 mg/g (significantly less than our results) to 32.17 mg/g GAE (higher than in our study). A possible cause of large differences in TF contents may be the use of different methods for TF evaluation. In our study, a more selective method was applied, which is in a slightly modified form used as the official pharmacopoeial method for determining the flavonoid content (European Pharmacopoeia, 2014).

To the best of the authors' knowledge, there are no published results on the content of hydroxycinnamic acid derivatives (TPA content) in *G. dalmaticum* and *G. macrorrhizum*.

Table 1. *Contents (mg/g) of total polyphenols (TP), tannins (T), total flavonoids (TF), and total phenolic acids (TPA) in Geranium dalmaticum and G. macrorrhizum*

Phenolic compound	***G. dalmaticum***	***G. macrorrhizum***
TP	217.60 ± 1.08a	215.53 ± 1.10a
T	155.83 ± 0.60a	157.73 ± 0.61a
TF	5.10 ± 0.10a	5.53 ± 0.10a
TPA	13.27 ± 1.34a	15.33 ± 0.45a

Data represent mean ± SD of three independent analyses; n = 3.

Only two phenolic compounds were tentatively identified and quantified by HPLC analysis in *G. dalmaticum* and *G. macrorrhizum* (Table 2). Quercetin was identified in *G. dalmaticum* (0.230 %, m/m of dry substance) only, while rutin was determined in *G. macrorrhizum* (1.116 %, m/m of dry substance) only. The contents of the other investigated phenolic substances were below the limit of quantification. Leucuta et al. (2005) used HPLC analysis for investigation of phenolic compounds in *G. sanguineum* L. and they determined quercetin (0.82 mg/g) and rutin (1.71 mg/g), as well as caftaric acid, caffeic acid, hyperoside, isoquercitrin, quercitrin, and kaempferol. TPA content in this study was quantified by UV-Vis spectrophotometric method but the applied HPLC method did not manage to individually quantify investigated phenolic acids (i.e., sinapic acid, caffeic acid, coumaric acid, and ferulic acid). The content of individual phenolic acids could not be quantified by the applied HPLC method due to their individual low concentrations, or some other phenolic acids may be present that are not covered by the applied standard substances.

Table 2. *Contents of phenolic compounds (%, m/m of dry substance ± SD) in Geranium dalmaticum and G. macrorrhizum obtained by HPLC analysis*

Phenolic compound	***G. dalmaticum***	***G. macrorrhizum***
Rutin	BLQ	1.116 ± 0.003a
Quercetin	0.230 ± 0.002a	BLQ

BLQ = below the limit of quantification.

Conclusion

This study provides preliminary results regarding the contents of polyphenolic substances in above-ground plant parts in two *Geranium* species (family Geraniaceae) growing in Croatia, *G. dalmaticum* and *G. macrorrhizum*, by using HPLC analysis and UV-Vis spectrophotometric methods. The following research will be focused on further characterization of other phenolic compounds in *G. dalmaticum* and *G. macrorrhizum*, as well as on investigation of their biological activities with the aim of finding new potential phytotherapeutics.

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Otrovne biljne vrste u dječjim vrtićima u četvrti Trešnjevka – sjever u Zagrebu

Poisonous plant species in kindergartens of Trešnjevka – north district in Zagreb

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Sažetak

Pri oblikovanju otvorenih prostora za djecu potrebno je obratiti pažnju na odabir biljnih vrsta koje za njih ne smiju biti opasne. Cilj ovog istraživanja bio je inventarizirati dendrološku floru na igralištima dječjih vrtića na području gradske četvrti Trešnjevka – sjever te utvrditi zastupljenost otrovnih biljnih vrsta. Istraživanje je provedeno u 13 dječjih vrtića s igralištima. Otrovne drvenaste vrste bile su prisutne na svim analiziranim lokacijama te su obuhvaćale 40% drvenastih vrsta. Ukupno je utvrđeno 29 otrovnih drvenastih vrsta među kojima kao osobito opasne valja izdvojiti lovorvišnju (*Prunus laurocerasus* L.), koja je zabilježena u ukupno sedam vrtića, i tisu (*Taxus baccata* L.), koja je zabilježena u jednom vrtiću. Rezultati su pokazali da je na prostorima dječjih vrtića prisutan velik broj otrovnih vrsta što sugerira da se pri krajobraznom uređenju prostora dječjih vrtića nedovoljno uzima u obzir potencijalna opasnost koju neke ukrasne biljne vrste mogu predstavljati po zdravlje i sigurnost djece.

Ključne riječi: dječji vrtić, igralište, otrovne biljne vrste, sigurnost djece.

Abstract

When designing open spaces for children, it is necessary to pay attention to the selection of plant species that should not be dangerous for them. The aim of this research was to inventory the dendrological flora on the playgrounds of children's kindergartens in the Trešnjevka - north district and to determine the presence of poisonous plant species. The research was conducted in 13 kindergartens

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with playgrounds. Poisonous woody species were present in all analyzed locations and comprised 40% of woody species. A total of 29 poisonous woody species were identified, among which cherry laurel (*Prunus laurocerasus* L.), which was recorded in a total of seven kindergartens, and yew (*Taxus baccata* L.), which was recorded in one kindergarten, are particularly dangerous. The results showed that a large number of poisonous species are present in the area of kindergartens, which suggests that the potential danger which some ornamental plant species can pose to children's health and safety is insufficiently considered when designing such spaces.

Key words: child safety, kindergarten, playground, poisonous plant species.

Uvod

Dekorativne karakteristike jedan su od najvažnijih kriterija pri primjeni ukrasnih biljnih vrsta. Ipak, važno je znati da neke od njih predstavljaju i potencijalnu opasnost za ljudsko zdravlje pa je pri oblikovanju zelenih površina posebnu pažnju potrebno obratiti na prostore namijenjene djeci odnosno na dječja igrališta.

Prema Paravini (1996), dječje igralište je otvoreni, uređeni i oblikovani prostor za spontanu i organiziranu igru, kao i za različite rekreativne i stvaralačke aktivnosti te za druženje, komunikaciju i suradnju. Za djecu od dvije do pet godina, igra je glavni izvor fizičke aktivnosti (Cosco, 2007). Igra na otvorenom prostoru puno je raznovrsnija te ima velik utjecaj na zdravlje i pravilan razvoj djece, no njezin učinak važan je i za mentalno zdravlje te za razvoj samosvijesti i socijalnih vještina (Korg, 2010; Valjan-Vukić, 2012). Prema Shackell et al. (2008), igra djeci omogućuje da razvijaju svoje emocionalne reakcije, kreativnost i sposobnost rješavanja problema. Stoga se prilikom oblikovanja vanjskih prostora odgojno-obrazovnih ustanova ne smije zaboraviti da bilje uz estetsku i ekološku mora također imati i pedagošku ulogu te težiti obrazovanju korisnika (Pirnat, 1952).

Prema Pereković (2017), oblikovanje vanjskih prostora dječjih vrtića ima određene specifičnosti s obzirom da su korisnici djeca do 6 godina pa svi vanjski elementi, kao što su sprave i biljne formacije, moraju biti prilagođeni njihovom uzrastu i ponašanju. Zbog toga na ovim površinama nije poželjna sadnja bilja koje je otrovno i alergeno ili sadrži trnje ili bodlje.

Otrovnim biljkama smatraju se one koje sadrže otrove odnosno tvari koje već i u maloj količini mogu imati štetan utjecaj na ljudsko zdravlje. Otrovnost i otrovanje ovise o brojnim čimbenicima, prvenstveno o onima koji utječu na sadržaj toksičnih tvari u biljci i na njihovu djelotvornost u organizmu. Mnoge otrovne biljke sadrže toksične tvari u svim svojim dijelovima, ali se njihova koncentracija često razlikuje u pojedinim biljnim organima. Na stupanj otrovnosti utječe i godišnje doba pa su tako neke biljke najotrovnije u vrijeme cvatnje ili dozrijevanja plodova (Forenbacher, 1998).

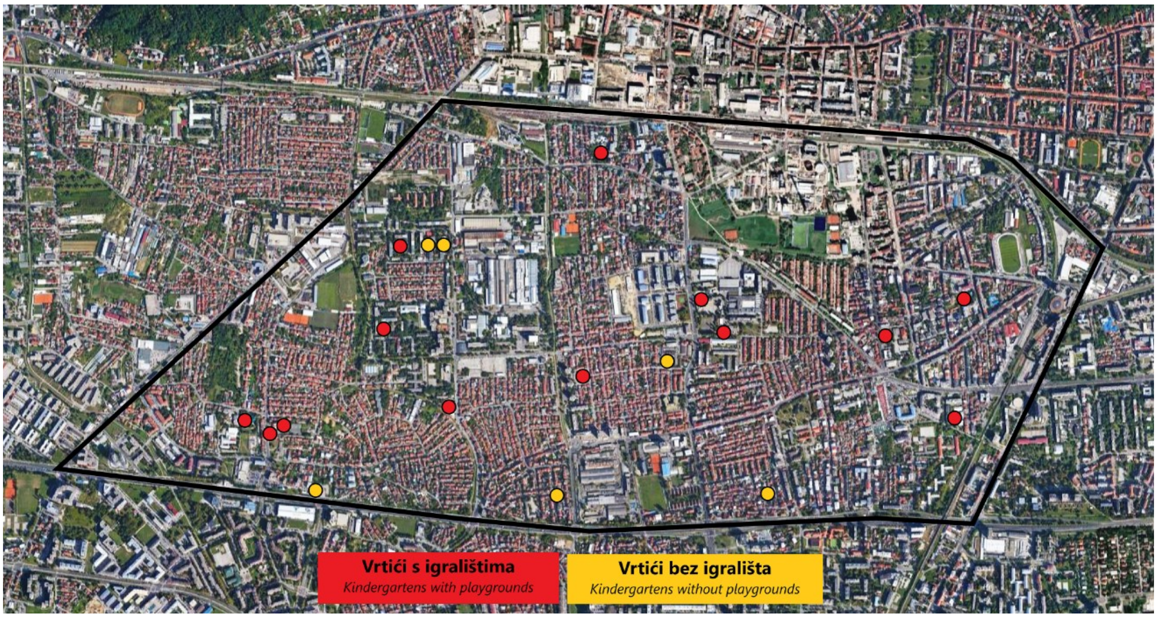
Douglas (2005) procjenjuje da je 3,5 % svih trovanja uzrokovano otrovnim biljkama, a tome su naročito izložena djeca. Pri tome osobit problem predstavljaju plodovi, koji kod mnogih otrovnih biljaka izgledaju vrlo privlačno, a po boji su slični plodovima jestivih vrsta. Iako većina otrovnih plodova ima gorak i odbojan okus, mala djeca mogu biti sklona da ih unatoč tome pojedu. Najčešće žrtve trovanja plodovima biljaka su upravo djeca predškolske dobi, a najviše ih privlače sjajne crvene bobe među kojima ima i onih smrtonosno otrovnih. Prema podacima iz izvještaja Švicarskog centra za toksikološke informacije iz 1978. godine, najviše trovanja kod djece bilo je izazvano plodovima kozokrvine (*Lonicera* sp.), tise (*Taxus baccata* L.) i lovorvišnje (*Prunus laurocerasus* L.) (Centre Suisse d'Information Toxicologique, 1978).

Djeca podnose manje doze otrova od odraslih osoba pa i doze koje djeluju jedva primjetno na odrasle, za djecu mogu biti kobne. Kako bi se spriječilo da dođe do trovanja, djecu je važno informirati o opasnosti koju predstavljaju otrovne biljke te ih naučiti da niti jedan dio biljke ne stavljaju u usta (Grlić, 1984; Filmer, 2012). U Slovačkoj je više od 50 % slučajeva trovanja djece uzrokovano slučajnom konzumacijom biljaka (Plačkova et al., 2006), što pokazuje da je dječje znanje o biljnim vrstama vrlo ograničeno (Fančovičová i Prokop, 2011). S druge strane, pri krajobraznom uređenju otvorenih površina namijenjenih djeci često se premalo pažnje posvećuje odabiru biljnih vrsta te se među njima nerijetko nalaze i vrlo otrovne biljke. Cilj ovog rada bio je inventarizirati dendrološku floru u dječjim vrtićima na području gradske četvrti Trešnjevka - sjever te analizirati zastupljenost otrovnih vrsta.

Materijali i metode

Istraživanje je provedeno u odabranim dječjim vrtićima na području gradske četvrti Trešnjevka-sjever u Zagrebu. Od ukupno 19 dječjih vrtića u četvrti, istraženo je njih 13 (pet centralnih i osam područnih) koji imaju dječja igrališta (slika 1). Istraživanje je bilo usmjereno na vrtiće s igralištima budući da djeca provode određeno vrijeme na otvorenom prostoru gdje mogu doći u kontakt s biljkama.

Uz upotrebu GIS alata dostupnih na https://geoportal.zagreb.hr/Karta za svaki analizirani dječji vrtić izmjerena je ukupna površina parcele (m^2), površina dječjeg igrališta (m^2) te, u slučajevima kada nije bilo moguće izbrojati pojedinačne primjerke biljaka, duljina žive ograde (m).



Slika 1. Satelitska snimka istraživanog područja (izvor: https://www.google.com/maps).

Figure 1. *Satellite image of the investigated area (source: https://www.google.com/maps).*

Terensko istraživanje provedeno je od svibnja do srpnja 2019. godine. U navedenom periodu dječji vrtići su se obilazili nekoliko puta kako bi se prikupila potrebna fotodokumentacija i napravila inventarizacija dendrološke flore. Za determinaciju biljaka i identifikaciju otrovnih biljnih vrsta korištena je standardna znanstvena i stručna literatura: Crvenka (1996), Domac (2002), Forenbacher (1998), Grlić (1984), Idžojtić (2005), Idžojtić (2009), Maretić (1986), Nikolić (2022). Nomenklatura biljnih vrsta usklađena je prema Nikolić (2022).

Nakon identifikacije dendroloških biljnih vrsta, izdvojene su otrovne vrste te je analizirana njihova zastupljenost. Otrovne vrste svrstane su u četiri kategorije toksičnosti (Filmer, 2012):

(1) izrazito otrovne biljne vrste koje mogu uzrokovati ozbiljno trovanje ili smrt;

(2) biljne vrste koje mogu izazvati simptome poput mučnine, povraćanja ili proljeva, no koji nisu opasni po život;

(3) biljne vrste u čijim se dijelovima nalaze kristali kalcijevog oksalata koji mogu iritirati kožu, sluznicu usne šupljine, jezik i grlo, što dovodi do oticanja grla, poteškoća s disanjem, žareće boli i želučanih tegoba;

(4) biljne vrste koje u kontaktu s kožom mogu izazvati osip ili iritaciju kože; takva iritacija može biti vrlo bolna pa je potrebno što prije isprati zahvaćeno područje.

Rezultati i diskusija

Karakteristike dječjih vrtića

Površina parcela analiziranih vrtića kretala se od 1165 m^2 do 7418 m^2, a dječja igrališta zauzimala su prosječno oko 57 % površina parcela. Površina igrališta po djetetu prosječno je iznosila 10,5 m^2, što je više nego što preporučuje Paravina (1996) (4-6 m^2 po djetetu). Živa ograda bila je prisutna u četiri vrtića, a njena prosječna duljina iznosila je 84,50 m (tablica 1).

Tablica 1. Karakteristike 13 analiziranih dječjih vrtića na području gradske četvrti Trešnjevka-sjever.

Table 1. *Characteristics of the 13 analyzed kindergartens in the Trešnjevka-north district.*

Oznaka vrtića*	**Broj djece**	**Površina parcele (m^2)**	**Površina igrališta (m^2)**	**Duljina žive ograde (m)**
C1	317	4601	2914	-
C2	273	6173	3300	84
C3	361	5436	2936	142
C4	283	3318	2471	-
C5	176	3008	2031	-
R1	296	7418	5581	-
R2	81	2103	712	-
R3	85	1165	560	15
R4	188	1748	751	97
R5	99	1875	932	-
R6	191	2991	1538	-
R7	46	2551	1106	-
R8	92	3480	1254	-
Prosjek	**191,38**	**3528,23**	**2006,62**	**84,50**

* C – centralni vrtić, R – područni vrtić

Analiza zastupljenosti otrovnih drvenastih vrsta

Na ukupno 13 analiziranih lokacija dječjih vrtića zabilježene su 73 drvenaste vrste iz 26 porodica, od čega 44 vrste (60 %) drveća te 29 vrsta (40 %) grmlja i penjačica. Ukupno je zabilježeno 29 otrovnih vrsta drveća, grmlja i penjačica, koje su bile zastupljene s ukupno 278 primjeraka i 338 m žive ograde (tablica 2). Otrovne drvenaste vrste bile su prisutne na svim analiziranim lokacijama, a obuhvaćale su 40 % drvenastih vrsta (tablica 2; slika 2). S obzirom na kategoriju otrovnosti, najveći broj otrovnih vrsta pripadao je kategoriji 2 (19 %), dok kategoriji 3 nije pripadala niti jedna zabilježena vrsta (slika 2).

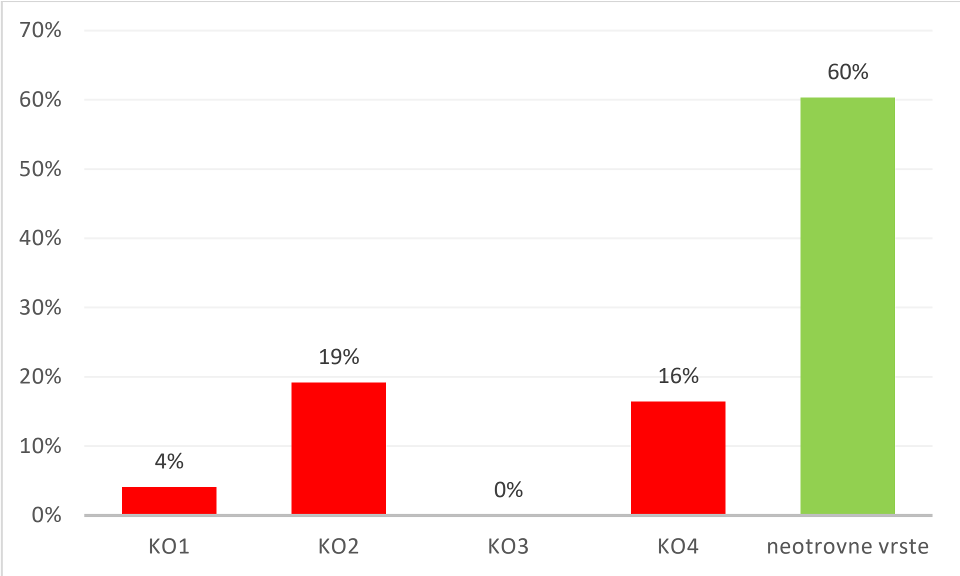
Tablica 2. Popis i zastupljenost (broj primjeraka / duljina žive ograde) otrovnih drvenastih vrsta s navedenim kategorijama otrovnosti (KO) na igralištima 13 vrtića gradske četvrti Trešnjevka – sjever.

Table 2. *List and number of specimens of poisonous woody species with indicated toxicity categories (KO) on the playgrounds of 13 kindergartens in the Trešnjevka – north district. In the case of a hedge, the length (m) is specified.*

Vrsta / Porodica / Narodni naziv	**KO**	**Oznaka vrtića***													**Ukupno**
		C1	C2	C3	C4	C5	R1	R2	R3	R4	R5	R6	R7	R8	
Acer campestre L. / Aceraceae / poljski jasen	4					1									1
Acer negundo L. / Aceraceae / perastolisni javor	4				1	2									3
Acer platanoides L. / Aceraceae / javor mliječ	4	5	4		5	1		2		1		4	3		25
Acer pseudoplatanus L. / Aceraceae / gorski javor	4			1								3			4
Acer saccharinum L. / Aceraceae / srebrnolisni javor	4				3									1	4
Aesculus hippocastanum L. / Hippocastanaceae / divlji kesten	2	2	12												14
Berberis thunbergii DC. / Berberidaceae / žutika	2, 4						1								1
Betula pendula Roth / Betulaceae / obična breza	4	18	10	2	2	10	4			2			5	2	55
Catalpa bignonioides Walt. / Bignoniaceae / južnjačka katalpa	4			21	1										22
Cotoneaster salicifolius Franch. /Rosaceae / vrbolisna mušmulica	2				1										1
Euonymus fortunei (Turcz.) Hand.-Maz. / Celastraceae / kineska kurika	2										1				1
Euonymus japonicus Thunb. / Celastraceae / japanska kurika	2						1			3					4
Fraxinus americana L. / Oleaceae / američki jasen	4		1												1
Fraxinus angustifolia Vahl / Oleaceae / poljski jasen	4											2	3		5

Fraxinus excelsior L. / Oleaceae / bijeli jasen	4		4		6		1			1	4		2	13	31
Fraxinus ornus L. / Oleaceae / crni jasen	4		1												1
Hedera helix L. / Araliaceae / obični bršljan	2, 4			24 m	9		1						1		11 + 24 m
Hydrangea macrophylla (Thunb.) Ser. / Hydrangeaceae / velikolisna hortenzija	1, 4	1		1									1		3
Juglans regia L. / Juglandaceae / obični orah	4						2								2
Juniperus chinensis L. / Cupressaceae / kineska borovica	2													1	1
Ligustrum ovalifolium Hassk. / Oleaceae / zimzelena kalina	2, 4		84 m	118 m					15 m	14 m					231 m
Lonicera frangrantissima Lindl. & Paxton / Caprifoliaceae / zimski orlovi nokti	2					2									2
Lonicera nitida Wilson / Caprifoliaceae / sjajna kozokrvina	2									2			1		3
Lonicera pileata Oliver / Caprifoliaceae / kalinasta kozokrvina	2	5													5
Prunus laurocerasus L. / Rosaceae / lovorvišnja	1		12	4	1	3	1	1					4		26
Quercus robur L. / Fagaceae / hrast lužnjak	2, 4	1												1	2
Quercus rubra L. / Fagaceae / crveni hrast	2, 4		1			1									2
Taxus baccata L. / Taxaceae / tisa	1			6											6
Thuja occidentalis L. / Cupressaceae / zapadnjačka tuja	2, 4	5		8	23	2	3			83 m			1		42 + 83 m
Broj primjeraka / *Number of specimens*		**37**	**45**	**43**	**52**	**22**	**14**	**3**		**9**	**5**	**9**	**21**	**18**	**278 + 338 m**
Broj svojti / *Number of taxa*		**8**	**10**	**10**	**11**	**9**	**8**	**2**	**1**	**7**	**2**	**4**	**9**	**5**	

*C – centralni vrtić, R – područni vrtić.



Slika 2. Postotni udio drvenastih vrsta na igralištima analiziranih dječjih vrtića po kategorijama otrovnosti. Kod vrsta koje pripadaju dvjema kategorijama otrovnosti, u obzir je uzeta samo kategorija koja obuhvaća ozbiljnije simptome. KO – kategorija otrovnosti

Figure 2*. The percentage of woody species on the playgrounds of analysed kindergartens by categories of toxicity. In the case of species that belong to two categories of toxicity, only the category that includes more harmful symptoms was taken into account. KO – toxicity category.*

Na analiziranim lokacijama zabilježene su tri izrazito otrovne vrste (KO=1) (tablica 2), od kojih su osobito opasne lovorvišnja (*Prunus laurocerasus*) i tisa (*Taxus baccata*).

Lovorvišnja je bila zastupljena s ukupno 26 primjeraka te prisutna na čak 7 lokacija. Ova je vrsta na našim prostorima jedna od najčešće zabilježenih opasnih biljaka na igralištima i u dvorištima predškolskih i osnovnoškolskih institucija (Jambrek, 2017; Horvatić, 2018; Židovec et al., 2018; Jarić, 2019). Svi dijelovi biljke su otrovni, a osobito listovi i sjemenke, jer sadrže znatne količine cijanogenih glikozida, uključujući prunazin i amigdalin (Turner i von Aderkas, 2009). Ovi spojevi enzimskom hidrolizom u probavnom sustavu daju otrovnu cijanovodičnu kiselinu (Wink i Van Wyk, 2008). Prilikom trovanja se tipično najprije pojavljuju bolovi u trbuhu, povraćanje, letargija i znojenje, dok u težim slučajevima simptomi obuhvaćaju gubitak svijesti te respiratorni i srčani zastoj (Wink i Van Wyk, 2008; Nelson i Balick, 2020). Simptomi se mogu pojaviti vrlo brzo, a pri konzumaciji većih količina brzo može nastupiti i smrt (Turner i von Aderkas, 2009). Zbog gorčine ploda, otrovanja uzrokovana lovorvišnjom su relativno rijetka (Grlić, 1984; Ivanić et al., 1996). Osim toga, lovorvišnja se u hortikulturi najčešće primjenjuje kao živa ograda pa je relativno udaljena od najaktivnijih zona dječjih igrališta. Plodovi ove vrste (crne koštunice) međutim djeci mogu biti vrlo privlačni, pa bi njenu primjenu u sklopu dječjih igrališta trebalo izbjegavati.

Tisa je zabilježena na području samo jednog dječjeg vrtića, no bila je prisutna u čak šest primjeraka (tablica 2). Ova je vrsta također jedna od u nas najčešćih otrovnih ukrasnih vrsta na vanjskim igralištima dječjih vrtića (Vlahović i Karlović, 2013; Ožbolt, 2016; Mrđan et al., 2017; Horvatić, 2018; Židovec et al., 2018; Jarić, 2019; Kušen et al., 2022), te je ujedno i jedna od najotrovnijih. Osim slatkastog crvenog ovoja sjemenke (arilus), svi ostali dijelovi biljke izrazito su otrovni. Toksičnost listova pripisuje se u prvom redu prisutnosti alkaloida taksina, među kojima osobito snažno djelovanje ima taksin B, te glikozida kao što je taksikatin (Wilson et al., 2001). Konzumacija sjemenki te mastikacija igličastih listova mogu izazvati teška do smrtonosna trovanja (G et al., 2019), a 50 g listova za odrasla je čovjeka letalno (Piskač et al., 2015). Tipični simptomi koji se pojavljuju nakon konzumacije su mučnina, povraćanje, bol u trbuhu, tahikardija i konvulzije, nakon čega slijedi bradikardija i paraliza respiratornih mišića (Willaert et al., 2002). U istraživanju provedenom u Češkoj trovanje ovom vrstom nalazilo se na trećem mjestu gledajući po broju hospitalizirane djece (Vichova i Jahodar, 2003).

U vrlo otrovne biljke često se ubraja i velelisna hortenzija (*Hydrangea macrophylla* (Thunb.) Ser.) (Knight, 2007; Filmer, 2012; Hack, 2022) koja je na području istraživanih vrtića bila zastupljena s ukupno tri primjerka. Ova vrsta sadrži cijanogeni glikozid hidrangin, a otrovnima se smatraju cvjetni pupovi (Nelson i Balick, 2020), ali i drugi dijelovi biljke (Knight, 2007; Hack, 2022). Najčešći simptomi pri konzumaciji listova su gastrointestinalne tegobe, poput bolova u trbuhu, mučnine, povraćanja i proljeva. Komplikacije vezane uz središnji živčani sustav obično se pojavljuju samo ako se konzumiraju velike količine biljke (Hack, 2022). U biljci je također prisutan i izokumarin hidrangenol koji je vjerojatno odgovoran za dermatitis koji se javlja kod nekih ljudi u kontaktu s biljkama (Knight, 2007).

Na području istraživanih vrtića također je utvrđena prisutnost više vrsta koje s obzirom na toksičnost pripadaju kategoriji 2 te su već zabilježene uz dječje vrtiće i osnovne škole na području Novog Sada, Zadra, ali i u drugim gradskim četvrtima grada Zagreba: japanska kurika (*Euonymus japonicus* Thunb.) (Perinčić et al., 2014; Židovec et al., 2018), bršljan (*Hedera helix* L.) (Jambrek, 2017; Mrđan et al., 2017; Kušen et al., 2022), sjajna kozokrvina (*Lonicera nitida* E.H.Wilson) (Perinčić et al., 2014) i kalinasta kozokrvina (*Lonicera pileata* Oliver) (Perinčić et al., 2014; Horvatić, 2018; Židovec et al., 2018). Vrste roda *Euonymus* (kurika) sadrže toksične alkaloide (u svim dijelovima biljke) i kardenolide (u sjemenkama, a u manjoj mjeri i listovima) (Knight, 2007). Svi dijelovi japanske kurike smatraju se otrovnima, a osobito sjemenke i arilus (Parker i Acevedo-Rodriguez, 2017). Bršljan (*Hedera helix)* se obično pojavljuje u sklopu živih ograda kao rezultat slabijeg održavanja. Svi dijelovi bršljana smatraju se otrovnima, a osobito plodovi. Listovi i plodovi sadrže triterpenske saponine (hederasaponine) iz kojih nakon hidrolize nastaju toksični hederini (Turner i von Aderkas, 2009; Quattrocchi, 2012). Saponini se slabo apsorbiraju, no pri konzumaciji većih količina, mogu se javiti gastrointestinalni simptomi poput mučnine, povraćanja, grčeva u trbuhu i proljeva (Nelson i Balick, 2020), a u ekstremnim slučajevima mogu nastupiti koma i smrt (Quattrocchi, 2012). Pored toga, tvari koje sadrži bršljan mogu u osjetljivih ljudi izazvati jaki kontaktni dermatitis (Quattrocchi, 2012). Bršljan bi stoga svakako valjalo redovito uklanjati iz žive ograde. Neke vrste roda *Lonicera* mogu sadržavati toksične tvari kao što su triterpenski saponini slični onima kod bršljana, a koje mogu uzrokovati iritacije gastrointestinalnog sustava (Knight, 2007). Primjerice, konzumacija plodova kalinaste kozokrvine (*Lonicera pileata*) može u djece izazvati gastrointestinalne tegobe kao što su mučnina, povraćanje i proljev (NC State University Extension, 2023). Kalinasta i sjajna kozokrvina relativno su dostupne djeci budući da se obično primjenjuju kao pokrivači tla ili za žive ograde pa bi trebalo paziti pri njihovu smještaju uz dječja igrališta.

Na području istraživanih dječjih vrtića najzastupljenije otrovne biljne vrste bile su obična breza (*Betula pendula* Roth; KO = 4) s ukupno 55 primjerka, zapadnjačka tuja (*Thuja occidentalis* L.; KO = 2) s ukupno 41 pojedinačnim primjerkom i 83 m žive ograde te zimzelena kalina (*Ligustrum ovalifolium* Hassk.; KO = 2) s 231 m žive ograde. Breza u kontaktu s kožom može izazvati dermatitis (Alsop i Karlik, 2016), pa iako je u analiziranim vrtićima bila prisutna u velikom broju primjeraka, u pogledu toksičnosti ne predstavlja veliku opasnost. Sadnju stabala breze u blizini odgojno-obrazovnih institucija bi međutim trebalo izbjegavati jer se radi o vrlo alergenoj vrsti (Biedermann et al., 2019). Zapadnjačka tuja u granama i listovima sadrži monoterpen tujon koji ima neurotoksično djelovanje (EMEA, 1999; Naser i sur., 2005), a može izazvati iritaciju kože i sluznice te djeluje citotoksično na stanice jetre i bubrega (Wink i Van Vyk, 2008). Prema podacima Europske agencije za lijekove simptomi trovanja nakon konzumacije dijelova biljke obično su blagi, a gutanje listića ili grančica u male je djece izazvalo blage gastrointestinalne smetnje i povraćanje (EMEA, 1999). Kod zimzelene kaline svi su dijelovi biljke otrovni, a dodatan oprez potreban je i iz razloga što su njeni plodovi (plavkastocrne bobičaste koštunice) djeci vrlo privlačni (Wink i Van Vyk, 2008; Turner i von Aderkas, 2009). Zimzelena kalina se međutim obično primjenjuje kao živa ograda pa je u većini slučajeva udaljenija od površina namijenjenih igri djece.

Zaključak

Analiza igrališta odabranih dječjih vrtića na području gradske četvrti Trešnjevka-sjever pokazala je da se na njima nalazi velik broj otrovnih vrsta (čak 29) koje su bile zastupljene s ukupno 278 primjeraka i 338 m otrovne žive ograde. Među njima su bile prisutne i izrazito toksične vrste koje mogu uzrokovati ozbiljno trovanje ili smrt, a kao osobito opasne valja izdvojiti lovorvišnju (*Prunus laurocerasus*) i tisu (*Taxus baccata*). Ovi rezultati jasno ukazuju da se krajobraznom uređenju navedenih površina nije pristupalo planski i uz sudjelovanje stručnjaka. Iz tog razloga, odgojitelji bi trebali biti na oprezu, ali i upoznati s tim vrstama odnosno mogućim štetnim posljedicama koje one mogu imati po zdravlje djece, kako bi pravovremeno i na odgovarajući način mogli reagirati. Kako bi se takve situacije spriječile, osim odgojitelja, izuzetno je važno o opasnim biljnim vrstama educirati i djecu.

Pored toga, vrste s izrazito visokim stupnjem otrovnosti, a osobito one koje su smještene uz najaktivnije zone dječje igre, svakako bi trebalo ukloniti te zamijeniti onima koje ne predstavljaju opasnost za djecu.

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Senzorska procjena tjestenine obogaćene maslačkom (*Taraxacum officinale* Weber)

Sensory evaluation of dandelion (*Taraxacum officinale* Weber) enriched pasta

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Sažetak

Zahvaljujući znanstvenim istraživanjima i dokazima koji govore u prilog tomu da su prehrana i zdravlje usko povezani, potrošači se okreću skupini prehrambenih proizvoda koje nazivamo funkcionalnim, a kojoj pripadaju i obogaćeni proizvodi. Tjestenina je popularna i pogodna namirnica za obogaćivanje dodatkom različitih sastojaka. Cilj ovog rada bio je pripremiti svježu tjesteninu, odnosno svježe široke rezance obogaćene različitim udjelima osušenih i pulveriziranih listova maslačka te utvrditi prihvatljivost svježih i kuhanih svježih uzoraka od strane potrošača. U tu svrhu pripremljena su i ocijenjena po 4 (četiri) uzorka širokih rezanaca, svježih i kuhanih svježih, s različitim udjelima listova maslačka. Podaci dobiveni senzorskom procjenom obrađeni su i analizirani u programima Microsoft Excel te IBM SPSS Statistics 25. Rezultati su prikazani kao aritmetička sredina ± standardna devijacija. U analizi podataka korištena je jednosmjerna analiza varijance (ANOVA) uz Tukeyev *post-hoc* test. Tjestenina obogaćena sušenim i pulveriziranim listovima maslačka ocijenjena je visokim ocjenama od strane potrošača. Iz dobivenih rezultata senzorske procjene može se zaključiti da je najprihvatljiviji uzorak kontrolni uzorak, a od širokih rezanaca obogaćenih maslačkom onaj s udjelom 10 % sušenih i pulveriziranih listova maslačka na ukupne suhe sastojke i u slučaju svježih i u slučaju kuhanih svježih uzoraka. Podaci pokazuju da za neka senzorska svojstva postoje statistički značajne razlike između uzoraka, pogotovo za uzorke s najvećim udjelom listova maslačka.

Ključne riječi: obogaćivanje tjestenine, listovi maslačka (*Taraxacum officinale* Weber), senzorska procjena.

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Abstract

Thanks to scientific research and evidence that supports the fact that nutrition and health are closely related, consumers are turning to the group of food products that we call functional foods, which also includes enriched products. Pasta is a popular and suitable food for enrichment by adding different ingredients. The aim of this work was to prepare fresh pasta, i.e. fresh wide noodles enriched with different proportions of dried and pulverized dandelion leaves, and to determine the acceptability of fresh and cooked fresh samples by consumers. For this purpose, 4 (four) samples of wide noodles, fresh and cooked fresh, with different proportions of dandelion leaves in the product recipe were prepared and evaluated. The data obtained from the sensory evaluation were processed and analyzed in Microsoft Excel and IBM SPSS Statistics 25 programs. The results are presented as arithmetic means ± standard deviation. One-way analysis of variance (ANOVA) with Tukey's post-hoc test was used in data analysis. Pasta enriched with dried and pulverized dandelion leaves has been rated highly by consumers. From the obtained results of sensory evaluation, it can be concluded that the most acceptable sample is the control sample, and of the wide noodles enriched with dandelion, the one with a proportion of 10 % of dried and pulverized dandelion leaves to the total dry ingredients, both in the case of fresh and in the case of cooked fresh samples. The data show that for some sensory properties there are statistically significant differences between the samples, especially for the samples with the highest proportion of dandelion leaves.

Key words: pasta enrichment, dandelion (*Taraxacum officinale* Weber) leaves, sensory evaluation.

Uvod

Zahvaljujući znanstvenim istraživanjima i dokazima koji govore u prilog tomu da su prehrana i zdravlje usko povezani, potrošači se okreću skupini proizvoda koje nazivamo funkcionalnim, a karakterizira ih činjenica da pored svoje osnovne nutritivne vrijednosti blagotvorno djeluju na zdravlje (Ćurić i Galić, 2006; Roberfroid, 2000, prema Čalić et al., 2011; Čukelj et al., 2016; Čukelj et al., 2017). Obogaćenim se proizvodima nazivaju oni kojima su dodani novi nutrijenti ili komponente koje nisu prirodno prisutne u određenoj hrani ili je određenom proizvodu dodavanjem povećana količina postojećih nutrijenata (Kotilainen et al., 2006, prema Čalić et al., 2011).

Tjestenina je popularna i pogodna namirnica za obogaćivanje dodatkom različitih sastojaka. Obogaćivanje utječe na nutritivna, senzorska i funkcionalna svojstva tjestenine (Marchylo i Dexter, 2001; Babuskin, 2014; Mercier et al., 2016). Tjestenine se dobivaju miješanjem i oblikovanjem pšenične krupice (krušne ili durum) s vodom, a u recepturu se mogu dodavati različiti sastojci te konačno podvrgnuti različitom završnom oblikovanju i termičkoj obradi o čemu ovisi i naziv pod kojim se prodaju na tržištu (NN 101/2022).

Prema literaturnim podacima tjestenina je obogaćivana izvorima biljnih i životinjskih proteina, izvorima prehrambenih vlakana te drugim nutrijentima i bioaktivnim tvarima (Friganović et al., 2019) na način da je određeni udio brašna za zamjes zamijenjen pulveriziranim ili na drugi način pripremljenim sirovinama. Posljednjih godina autori su radili i na sljedećim temama vezanim za obogaćivanje tjestenine:

Sastojak kojim se obogaćuje/priprema tjestenina		Referenca
Pseudožitarice	heljdino brašno od cjelovitog zrna, hidrotermički tretirano heljdino brašno od cjelovitog zrna	Škrobot et al., 2022
	amarant - proteinski hidrolizat	Valdez-Meza et al., 2019
Mahunarke	brašno žute leće	Bresciani et al., 2021
	manilski tamarind (+ brašna nekoliko žitarica i slanutkovo brašno)	Saha et al., 2021
Voće	liofilizirane maline, crveni i crni ribiz te bobice Boysen	Bustos et al., 2019
Povrće i nusproizvodi prerade povrća	pulverizirani list brokule	Drabińska et al., 2022
	pulverizirani list peršina	Bouasla et al., 2022
	ljuske luka	Michalak-Majewska et al., 2020
	nusproizvodi prerade paprike i rajčice	Teterycz i Sobota, 2023
Lišće stabala	pulverizirano lišće konjske rotkvice (moringe)	Soni i Kumar, 2021
Biomasa mikroalgi	pulverizirana spirulina	Hussein et al., 2021; Raczyk et al., 2022
Gljive	pulverizirane gljive	Szydłowska-Tutaj et al., 2023
Ribe	panga (azijski som) - proteinski izolat	Singh et al., 2021; Reddy Surasani et al., 2019
	lubin	Ainsa et al., 2021
Sjemenke	brašno sjemenki konoplje	Bonacci et al., 2023; Teterycz et al. 2021
	laneno brašno, lanena pogača	Zarzycki et al., 2020

Ljekoviti (obični) maslačak (*Taraxacum officinale* Weber) najpoznatija je vrsta iz porodice glavočika (Asteraceae). Zeljasta je trajnica čiji svi dijelovi sadrže gorak mliječni sok. Listovi su nazubljeni, smješteni pri dnu, a stabljika šuplja sa zlatnožutom cvjetnom glavicom na vrhu koja se dozrijevanjem pretvara u bijel rastresit klobuk. Raste kao korov po livadama, pašnjacima i vrtovima. Mladi se listovi jedu kao salata; medonosna je i ljekovita biljka (za izlučivanje žuči i mokraće, pojačanje teka) (Hulina, 2011). Često se prodaje kao sastavni dio tzv. mišancija na tržnicama u Dalmaciji (Łuczaj et al., 2013) i Istri (plominsko zelje) (Vitasović-Kosić, 2018) i koristi se kao svježe lisnato povrće (Łuczaj et al., 2013). Često je korišten kao lijek u narodnoj medicini zbog povoljnog djelovanja na jetru, a do danas su istraživana njegova diuretska, protuupalna, antioksidativna, antihiperglikemijska, prebiotska (Schütz et al., 2006; Wirngo et al., 2016; Pierzak-Sominka, 2020), antikancerogena (Pierzak-Sominka, 2020; Jalili et al., 2020; Chen et al., 2023), hepatoprotektivna, neuroprotektivna, genoprotektivna (Mahoubi i Mahboubi, 2020; Jalili et al., 2020) i druga svojstva, a upravo zbog protuupalnih, antioksidativnih i antihiperglikemijskih svojstava okarakteriziran je kao antidijabetička i medicinska biljka (Wirngo et al., 2016; Pierzak-Sominka, 2020; Jalili et al., 2020).

Cilj ovog rada bio je pripremiti svježu tjesteninu, odnosno svježe široke rezance obogaćene različitim udjelima osušenih i pulveriziranih listova maslačka te utvrditi prihvatljivost svježih i kuhanih svježih uzoraka od strane potrošača.

Materijali i metode

Svježa tjestenina s maslačkom pripremljena je u Laboratoriju za kemiju i tehnologiju žitarica Veleučilišta "Marko Marulić" u Kninu od durum krupice (Sgambaro, Italija), vode i osušenih i pulveriziranih listova maslačka (*Taraxacum officinale* Weber). Listovi maslačka prikupljeni su na antropogeniziranom području (vrtovi, voćnjaci i pašnjaci) Šibensko–kninske županije te pulverizirani nakon sušenja (konvekcijski, na temperaturi od 60 °C).

Pripremljena su četiri uzorka svježih širokih rezanaca s različitim udjelima osušenih i pulveriziranih listova maslačka u recepturi proizvoda od 0 % (kontrolni uzorak = uzorak br. 1), 5 % (uzorak br. 2), 10 % (uzorak br. 3) i 15 % (uzorak br. 4) na ukupne suhe sastojke.

Za procjenu uzoraka kuhane tjestenine (4 uzorka) svježi uzorci širokih rezanaca pripremljeni su kuhanjem prema prethodno određenom načinu i optimalnom vremenu kuhanja (Trajković et al., 1983).

Za izradu tjestenine koristili smo stolni uređaj (model: Grunberg) s regulatorom za debljinu tijesta, glatkim cilindrom i cilindrom za rezanje širokih rezanaca.

Senzorska procjena svježe i svježe kuhane tjestenine provedena je s ciljem utvrđivanja prihvatljivosti tjestenine obogaćene listovima maslačka (osušenim i pulveriziranim). Od ocjenjivanih svojstava kod svježih širokih rezanaca ocjenjivani su vanjski oblik, izgled i svojstva površine, boja, miris i opća prihvatljivost proizvoda, a kod kuhanih svježih širokih rezanaca ocjenjivani su boja, izgled i svojstva površine, miris, okus, tekstura i opća prihvatljivost proizvoda (Friganović et al., 2019).

Ocjenjivana svojstva pojašnjena su svim ispitanicima prije početka ispitivanja. Svojstva su ocjenjivana ocjenama od 1 do 5, a ocjenjivanje je provodilo 20 neiskusnih članova (Ž i M) između 20 i 47 godina starosti (studenti i nastavnici), redovitih konzumenata tjestenine bez poznatih alergija na sastojke pšenice i/ili maslačka. Svaki ispitanik je prije početka ocjenjivanja dobio olovku i ocjenjivačke listiće. Svježi uzorci tjestenine (po 20 g), jedan po jedan, posluženi su na bijelim plastičnim tanjurima svakom ocjenjivaču. Za procjenu kuhane tjestenine uzorci su, kratko nakon kuhanja, jedan po jedan, posluženi u bijelim plastičnim posudicama. Između procjene pojedinih uzoraka ispitivačima je ponuđena voda.

Dobiveni podaci su obrađeni i analizirani u programima Microsoft Excel (MC, 2010) te IBM SPSS Statistics 25 (IBM, 2017). Rezultati su prikazani kao aritmetička sredina ± standardna devijacija. U analizi podataka korištena je jednosmjerna analiza varijance (ANOVA) uz Tukeyev *post-hoc* test.

Rezultati i diskusija

Rezultati provedene senzorske procjene svježih uzoraka širokih rezanaca obogaćenih osušenim i pulveriziranim listovima maslačka prikazani su u tablici 1.

Tablica 1. Srednje vrijednosti ocjena senzorskih svojstava svježih uzoraka širokih rezanaca obogaćenih osušenim i pulveriziranim listovima maslačka (*Taraxacum officinale* Weber)

Table 1. *Mean values of sensory properties of cooked fresh wide pasta noodle samples enriched with dried pulverized dandelion (Taraxacum officinale* Weber*) leaves*

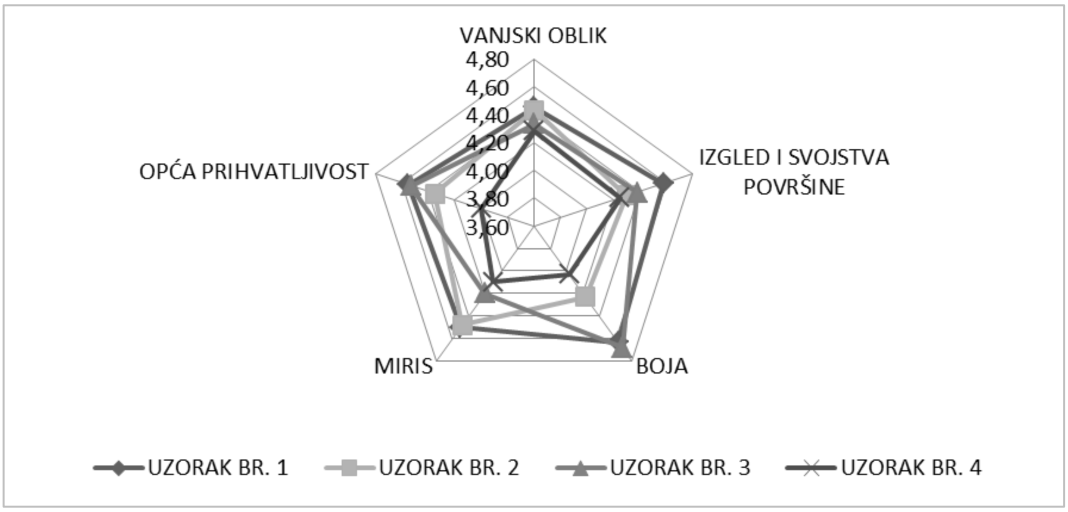
SENZORSKA SVOJSTVA	UZORAK											
	BR. 1			BR. 2			BR. 3			BR. 4		
	Sr. vr.	±	SD	Sr. vr.	±	SD	Sr. vr.	±	SD	Sr. vr.	±	SD
VANJSKI OBLIK	4,45	±	0,81 ^{a}	4,43	±	0,78 ^{a}	4,33	±	0,73 ^{a}	4,28	±	0,68 ^{a}
IZGLED I SVOJSTVA POVRŠINE	4,58	±	0,59 ^{a}	4,30	±	0,61 ^{a}	4,38	±	0,59 ^{a}	4,25	±	0,63 ^{a}
BOJA	4,63	±	0,54 ^{a}	4,23	±	0,66 ^{b}	4,68	±	0,47 ^{a}	4,03	±	0,77 ^{b}
MIRIS	4,50	±	0,55 ^{a}	4,48	±	0,60 ^{ab}	4,20	±	0,65 ^{ab}	4,10	±	0,87 ^{b}
OPĆA PRIHVATLJIVOST	4,55	±	0,64 ^{a}	4,35	±	0,66 ^{ab}	4,53	±	0,60 ^{a}	4,00	±	0,91 ^{b}

Uzorci širokih rezanaca od br. 1. do br. 4. s različitim udjelima osušenih i pulveriziranih listova maslačka u recepturi proizvoda od 0 % (kontrolni uzorak = uzorak br. 1), 5 % (uzorak br. 2), 10 % (uzorak br. 3) i 15 % (uzorak br. 4) na ukupne suhe sastojke.
Vrijednosti u tablici predstavljaju aritmetičke sredine ± standardna devijacija (N = 40, 20 ispitanika, dva ponavljanja). Vrijednosti u istom redu s različitim slovima u eksponentu ($^{a,\, b}$) značajno se razlikuju ($p < 0,05$).
Samples of wide noodles from no. 1. to no. 4. with different proportions of dried pulverized dandelion leaves in the product formulation of 0 % (control sample = sample no. 1), 5 % (sample no. 2), 10 % (sample no. 3) and 15 % (sample no. 4) to total dry ingredients.
The values represent arithmetic means ± standard deviation (N = 40, 20 respondents, two repetitions). The values in a row with different superscript letters ($^{a,\, b}$) are significantly different ($p < 0.05$).

Kao što se vidi iz tablice 1. i slike 1. sva procjenjivana svojstva uzoraka svježih širokih rezanaca ocijenjena su relativno visokim ocjenama, 4,00 ili višim.

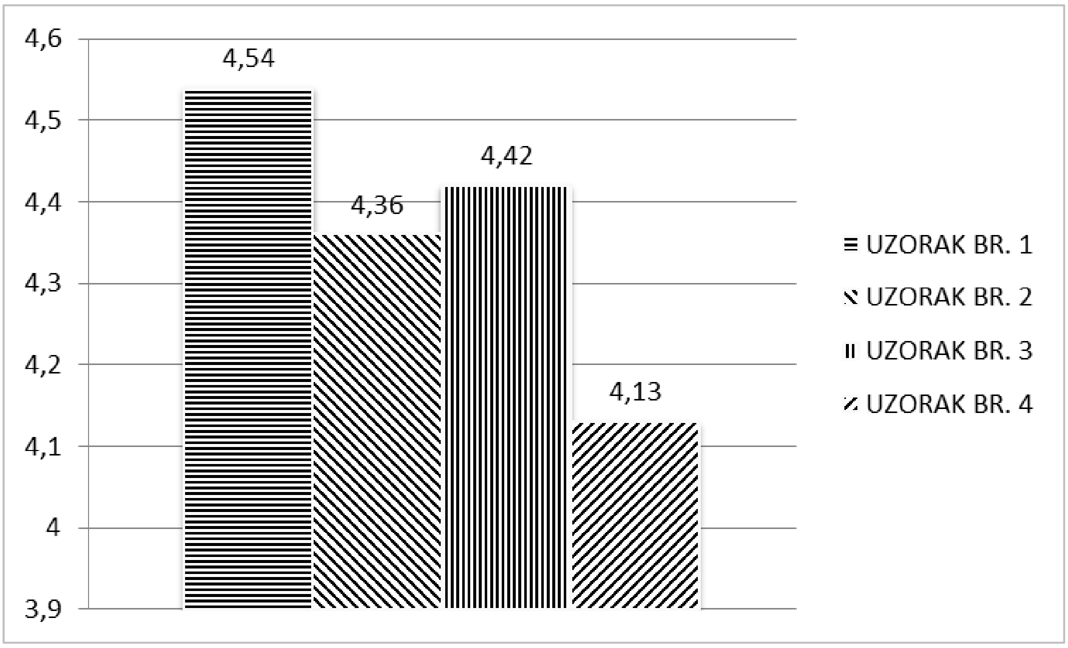
Najveću ocjenu i za vanjski oblik (4,45) i za izgled i svojstva površine (4,58) dobio je kontrolni uzorak, a podaci ne pokazuju da postoje statistički značajne razlike između uzoraka niti za jedno od ta dva svojstva. Najveću ocjenu za boju dobio je uzorak br. 3 (4,68), ali podaci ne pokazuju da postoje statistički značajne razlike između uzorka br. 3 i kontrolnog uzorka. Zanimljivo je da postoji statistički značajna razlika kod obogaćenih uzoraka, između uzorka br. 3 i uzorka br. 2 te uzorka br. 3 i uzorka br. 4. Također, postoji statistički značajna razlika između kontrolnog uzorka i uzorka br. 2 i kontrolnog uzorka i uzorka br. 4. Najveću ocjenu za miris dobio je kontrolni uzorak (4,50), a podaci pokazuju da postoji statistički značajna razlika samo između kontrolnog uzorka i uzorka br. 4. Najveću ocjenu za opću prihvatljivost dobio je kontrolni uzorak (4,55), a uzorak br. 4 se statistički značajno razlikuje od kontrolnog uzorka i uzorka br. 3.

Prema slici 2. koja prikazuje prosječne ocjene senzorske procjene za svježe uzorke širokih rezanaca vidljivo je da je uzorak br. 4 dobio najnižu ocjenu (4,13). Uzorci obogaćeni osušenim i pulveriziranim listovima maslačka ocijenjeni su nižim ocjenama od kontrolnog uzorka. Najprihvatljiviji uzorak je kontrolni s prosječnom ocjenom 4,54, a od obogaćenih proizvoda uzorak br. 3 s prosječnom ocjenom 4,42.



Slika 1. Srednje vrijednosti ocjena senzorskih svojstava svježih uzoraka širokih rezanaca obogaćenih osušenim i pulveriziranim listovima maslačka (*Taraxacum officinale* Weber)

Figure 1. *Mean values of sensory properties of fresh wide pasta noodle samples enriched with dried pulverized dandelion (Taraxacum officinale* Weber*) leaves*



Slika 2. Prosječna ocjena senzorske procjene svježih uzoraka širokih rezanaca obogaćenih osušenim i pulveriziranim listovima maslačka (*Taraxacum officinale* Weber)

Figure 2. *Average grade of sensory evaluation of fresh wide pasta noodle samples enriched with dried pulverized dandelion (Taraxacum officinale* Weber*) leaves*

Rezultati provedene senzorske procjene kuhanih svježih uzoraka širokih rezanaca obogaćenih osušenim i pulveriziranim listovima maslačka prikazani su u Tablici 2.

Tablica 2. Srednje vrijednosti ocjena senzorskih svojstava kuhanih uzoraka svježih širokih rezanaca obogaćenih osušenim i pulveriziranim listovima maslačka (*Taraxacum officinale* Weber)

Table 2. *Mean values of sensory properties of cooked fresh wide pasta noodle samples enriched with dried pulverized dandelion (Taraxacum officinale* Weber*) leaves*

SENZORSKA SVOJSTVA	UZORAK			
	BR. 1	BR. 2	BR. 3	BR. 4
	Sr. vr. ± SD	Sr. vr. ± SD	Sr. vr. ± SD	Sr. vr. ± SD
BOJA	4,70 ± 0,46 ^{a}	4,50 ± 0,55 ^{ab}	4,78 ± 0,53 ^{a}	4,20 ± 0,85 ^{b}
IZGLED I SVOJSTVA POVRŠINE	4,48 ± 0,60 ^{a}	4,20 ± 0,56 ^{ab}	4,25 ± 0,59 ^{ab}	4,08 ± 0,73 ^{b}
MIRIS	4,60 ± 0,50 ^{a}	4,53 ± 0,51 ^{a}	4,45 ± 0,55 ^{ab}	4,15 ± 0,77 ^{b}
OKUS	4,65 ± 0,58 ^{a}	4,45 ± 0,64 ^{a}	4,35 ± 0,58 ^{ab}	4,05 ± 0,85 ^{b}
TEKSTURA	4,50 ± 0,75 ^{ab}	4,58 ± 0,55 ^{a}	4,38 ± 0,63 ^{ab}	4,18 ± 0,75 ^{b}
OPĆA PRIHVATLJIVOST	4,55 ± 0,60 ^{a}	4,28 ± 0,64 ^{ab}	4,43 ± 0,59 ^{a}	4,00 ± 0,85 ^{b}

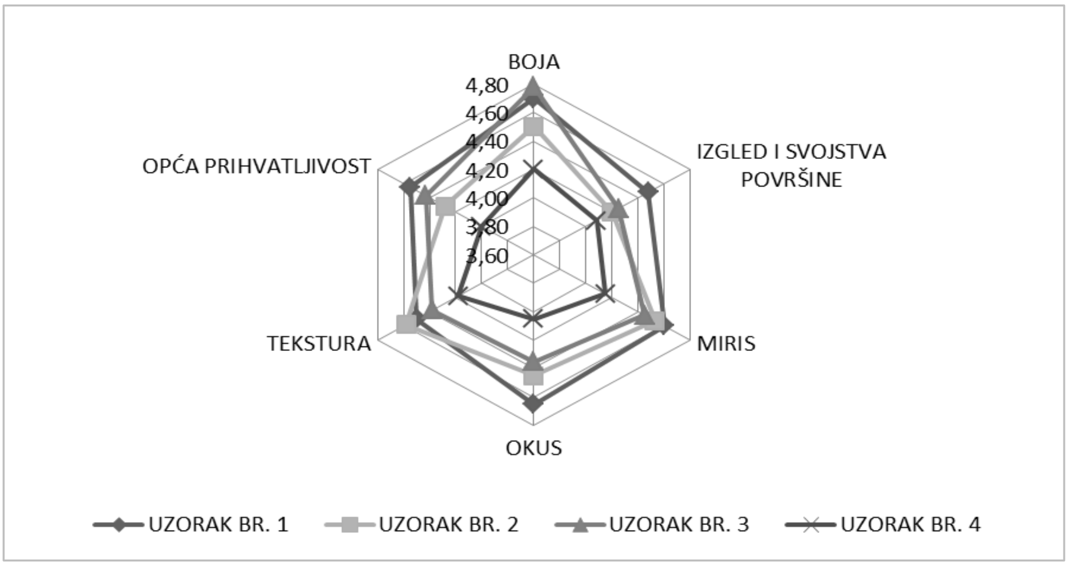
Uzorci širokih rezanaca od br. 1. do br. 4. s različitim udjelima osušenih i pulveriziranih listova maslačka u recepturi proizvoda od 0 % (kontrolni uzorak = uzorak br. 1), 5 % (uzorak br. 2), 10 % (uzorak br. 3) i 15 % (uzorak br. 4) na ukupne suhe sastojke.
Vrijednosti u tablici predstavljaju aritmetičke sredine ± standardna devijacija (N = 40, 20 ispitanika, dvije repeticije). Vrijednosti u redu s različitim slovima u eksponentu ($^{a, b}$) značajno se razlikuju ($p < 0,05$).
Samples of wide noodles from no. 1. to no. 4. with different proportions of dried pulverized dandelion leaves in the product formulation of 0 % (control sample = sample no. 1), 5 % (sample no. 2), 10 % (sample no. 3) and 15 % (sample no. 4) to total dry ingredients.
The values represent arithmetic means ± standard deviation (N = 40, 20 respondents, two repetitions). The values in a row with different superscript letters ($^{a, b}$) are significantly different ($p < 0.05$).

Kao što se vidi iz tablice 2. i slike 3. sva procjenjivana svojstva uzoraka kuhanih svježih širokih rezanaca tjestenine ocijenjena su relativno visokim ocjenama, 4,00 ili višim.

Najveću ocjenu za boju dobio je uzorak br. 3 (4,78), ali podaci ne pokazuju da postoje statistički značajne razlike između uzorka br. 3 i kontrolnog uzorka, međutim, uzorak br. 4 se statistički značajno razlikuje od kontrolnog uzorka i uzorka br. 3. Najveću ocjenu za izgled i svojstva površine dobio je kontrolni uzorak (4,48), a podaci pokazuju da postoje statistički značajne razlike između kontrolnog uzorka i uzorka br. 4. Najveću ocjenu za miris dobio je uzorak kontrolni uzorak (4,60), a podaci pokazuju da postoje statistički značajne razlike između uzorka br. 4 i kontrolnog uzorka i uzorka br. 4 i uzorka br. 2. Najveću ocjenu za okus dobio je uzorak kontrolni uzorak (4,65), a podaci pokazuju da se uzorak br. 4 statistički značajno razlikuje od kontrolnog uzorka i uzorka br. 2. Najveću ocjenu za teksturu dobio je uzorak br. 2 (4,58), ali podaci ne pokazuju da postoje statistički značajne razlike između uzorka br. 2 i kontrolnog uzorka, međutim, uzorak br. 4 se statistički značajno razlikuje od uzorka br. 2. Najveću ocjenu za opću prihvatljivost dobio je kontrolni uzorak (4,55), a uzorak br. 4 se statistički značajno razlikuje od kontrolnog uzorka i uzorka br. 3.

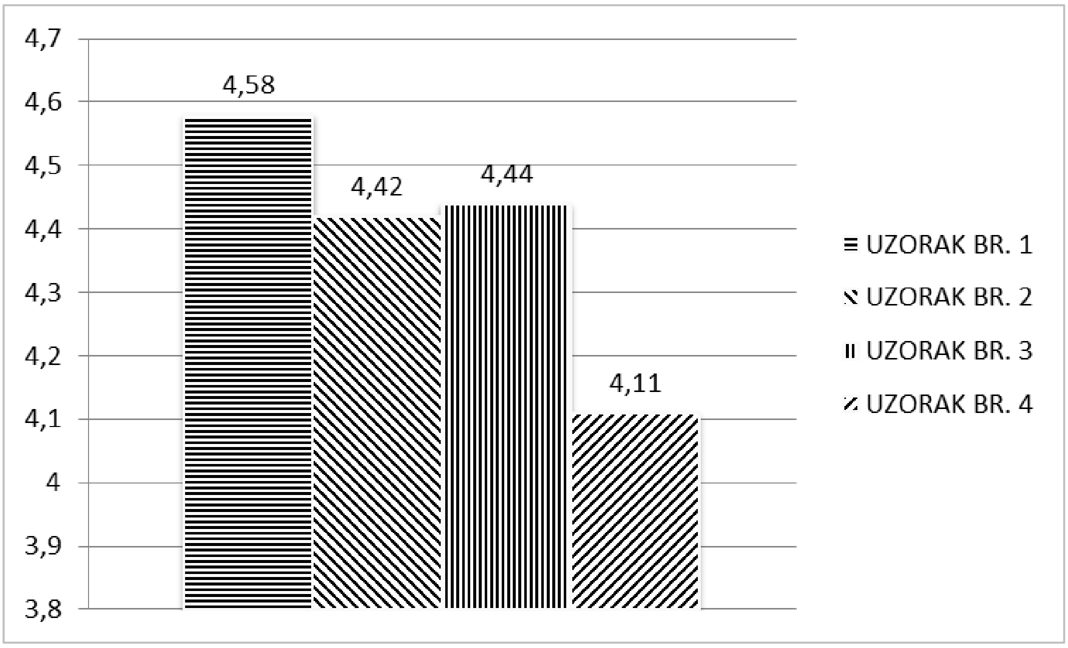
Prema slici 4. koja prikazuje prosječne ocjene senzorske procjene za svježe kuhane uzorke širokih rezanaca vidljivo je da je uzorak br. 4 dobio najnižu ocjenu (4,11). Uzorci obogaćeni osušenim i pulveriziranim listovima maslačka ocijenjeni su nižim ocjenama od kontrolnog uzorka.

Najprihvatljiviji uzorak je kontrolni s ocjenom 4,58, a od obogaćenih proizvoda uzorak br. 3 s prosječnom ocjenom 4,44.



Slika 3. Srednje vrijednosti ocjena senzorskih svojstava kuhanih svježih uzoraka širokih rezanaca obogaćenih osušenim i pulveriziranim listovima maslačka (*Taraxacum officinale* Weber)

Figure 3. *Mean values of sensory properties of cooked fresh wide pasta noodle samples enriched with dried pulverized dandelion (Taraxacum officinale* Weber*) leaves*



Slika 4. Prosječna ocjena senzorske procjene kuhanih uzoraka svježih širokih rezanaca obogaćenih osušenim i pulveriziranim listovima maslačka (*Taraxacum officinale* Weber)

Figure 4. *Average grade of sensory evaluation of cooked fresh wide pasta noodle samples enriched with dried pulverized dandelion (Taraxacum officinale* Weber*) leaves*

Kod ocjenjivanja pojedinačnih senzorskih svojstava kontrolni uzorak dobio je najviše ocjene za gotovo sve parametre, osim što je uzorak br. 3 dobio najvišu ocjenu za boju i u slučaju svježih i u slučaju kuhanih svježih uzoraka, a uzorak br. 2. najvišu ocjenu za teksturu kuhanih svježih uzoraka.

Ocjena boje, mirisa i opće prihvatljivosti proizvoda kod obogaćenih uzoraka (i svježih i svježih kuhanih) te ocjena izgleda i svojstva površine, okusa i teksture svježih kuhanih obogaćenih uzoraka može ovisiti o udjelu listova maslačka, što se pogotovo odnosi na uzorak s najvećim udjelom listova maslačka za kojeg su podaci pokazali da postoje statistički značajne razlike u odnosu na druge uzorke, osim za svojstva vanjski oblik te izgled i svojstva površine kod svježih uzoraka.

Zaključak

Tjestenina obogaćena sušenim i pulveriziranim listovima maslačka ocijenjena je visokim ocjenama od strane potrošača. Iz dobivenih rezultata senzorske procjene po 4 uzorka širokih rezanaca, svježih i kuhanih svježih, s različitim udjelima sušenih listova maslačka u recepturi proizvoda, može se zaključiti da je najprihvatljiviji uzorak kontrolni uzorak, a od širokih rezanaca obogaćenih maslačkom onaj s udjelom 10 % sušenih pulveriziranih listova maslačka na ukupne suhe sastojke i u slučaju svježih i u slučaju kuhanih svježih uzoraka. Podaci pokazuju da za neka senzorska svojstva postoje statistički značajne razlike između uzoraka, pogotovo za uzorke s najvećim udjelom listova maslačka. Rezultati provedenog ispitivanja mogu poslužiti kao osnova za daljnji razvoj obogaćenih tjestenina.

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Ground-ivy (*Glechoma hederacea* L., Lamiaceae) habitats in NE Slovenia: floristic, chorological and syntaxonomic diversity

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Abstract

Ground-ivy (*Glechoma hederacea* L.) is a widespread stoloniferous plant that grows in several ecologically diverse habitats: in the open, at the forest edge, and in understorey. The vegetation of ground-ivy habitats was studied in NE Slovenia in terms of floristic richness and diversity, biological spectrum, phytogeography and syntaxonomy. We identified five clusters of ecologically distinct habitats with the occurrence of ground-ivy, differentiated according to environmental conditions and flora composition: eutrophic forest edges, trampled habitats, forests, which are divided into two groups with different soil moisture, and meadows. The habitats were assessed using Ellenberg indicator values, thus confirming the ecological differentiation of ground-ivy habitats. Ground-ivy coexists with 169 plant species from 49 families. The highest plant species richness and Shannon diversity is found in meadows (100 plant species), while the lowest diversity is found in trampled habitats (12 plant species). Plant species coexisting with ground-ivy belong to 10 geoelements of which circumboreal, sub-cosmopolitan, Euro-Caucasian and Eurasian geoelements are represented in all habitats. The most represented life form is the hemicryptohytes with up to 88 % of all species per relevé. The syntaxon *Molino-Arrhenatheretea* dominates in the meadows, the shrub species of the forest edges belong to the syntaxon *Rhamno-Prunetea*, while *Querco-Fagatea* predominates in the forests. Ruderal species of the syntaxon *Stellarietea mediae* occurred in all habitats except in the understorey where light is a limiting factor. A non-negligible proportion of species belongs to *Galio-Urticetea*, a community characteristic of eutrophic forest edges, an optimal habitat for ground-ivy.

Key words: community composition, Ellenberg indicator values, life form, geoelement, syntaxon.

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Introduction

Ground-ivy (*Glechoma hederacea* L.) is a common European perennial creeping plant. In English, it is known by various names, such as ground-ivy, gill-over-the-ground, creeping Charlie, creeping Jenny, alehoof, tunhoof, catsfoot, field balm, haymaids and run-away-robin (CABI, 2023; Mahr and Stier, 2008). The kidney-shaped leaves with wavy margins are opposite on a square creeping stem that could root at the nodes. They are green and turn slightly purple in the colder months. Purple flowers In appear in spring, arranged in clusters. The flowers are two-lipped, open, tubular corollas with five-lobed bell-shaped calyxes (Figure 1). Each flower may produce up to four dry nutlets each containing one seed (Hutchings and Price, 1999).

Figure 1. *(a) Ground-ivy in the meadow and (b) flowering upright shoots. (Photo: M. Šipek).*

The native range of ground-ivy includes the temperate regions of Europe and Asia (Hutchings and Price, 1999). In North and South America, Southeast Asia, South Australia, and New Zealand, it is considered an alien and invasive plant (Figure 2).

Because of its medicinal and ornamental value, the ground-ivy was introduced to North America in the 19th century. Its ability to tolerate shade and the rapid growth of its creeping stems made the plant very suitable for horticulture as a groundcover (Middleton, 2001; Mahr and Stier, 2008). These characteristics also contribute to the invasive potential of the ground-ivy, which is expressed by high phenotypic plasticity and rapid growth that allows the plant to establish itself in a variety of habitats (Šipek and Šajna, 2021).

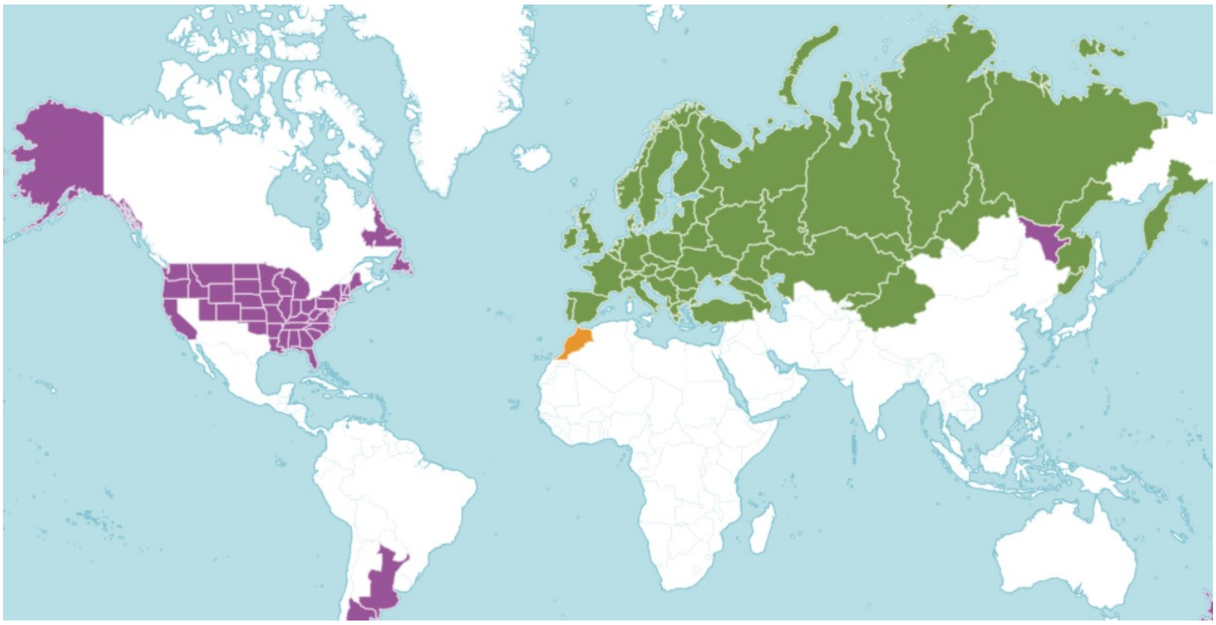

Figure 2. *Ground-ivy global distribution map retrieved from POWO (2023; http://www.plantsoftheworldonline.org/). Green colour indicates native range, purple introduced and orange doubtful presence.*

In addition to its medicinal properties and horticultural value, ground-ivy is also interesting from an ecological point of view as the plant tolerates a wide range of ecological conditions and grows in various habitats with different light and nutrient conditions. Ground-ivy grows in completely open habitats such as meadows and lawns, in semi shaded edge habitats and in shade such as in the forest understorey (Šipek and Šajna, 2021). In response to given environmental conditions, the morphological and reproductive characteristics of ground-ivy are highly variable (Hutchings and Price, 1999; Šipek and Šajna, 2021). In open habitats, ground-ivy cover is usually rather low but seed production is high. In nutrient-rich, partially shaded or shaded habitats, ground-ivy cover increases and can become a dominant component of the community, while seed production decreases as light availability decreases (Šipek and Šajna, 2021).

In the following, an analysis of plant communities in which ground-ivy occurs in northeastern Slovenia is conducted. Communities are analysed in terms of ecological conditions, which are assessed using Ellenberg indicator values, floristic richness and diversity, biological spectrum, phytogeography and syntaxonomy.

Methods

Study site

The study was conducted in northeastern Slovenia, in the Dravsko polje plain and Slovenske gorice hills. The study area belongs to the sub-Pannonian region with a moderate continental climate typical of the lowland and hilly landscape in the eastern and northeastern parts of Slovenia. The average annual temperatures measured at the weather station in Starše on Dravsko polje (240 m above sea level) from 1961 to 2015 are 10.1 °C (ARSO, 2023). The average precipitation in Starše in the same period was 966 mm per year. Although the precipitation regime is favourable, drought is frequent in the summer months.

Vegetation survey

In May 2018, we conducted vegetation surveys in several habitats where ground-ivy occur, in a total of 20 relevés that include covering forests, forest edges, and meadows. Vegetation surveys were conducted using the standard Central European phytosociological method (Braun-Blanquet, 1964). The size of the relevé was smaller than prescribed if the homogeneous area where ground-ivy grows was smaller (Table 1).

Table 1. *List of relevés with size, latitude, longitude and altitude of the site, clusters according to plant species composition, plant species richness and ground-ivy cover.*

Relevé	Relevé size (m^2)	Latitude	Longitude	Altitude (m)	Site	Cluster	Species richness	Ground-ivy cover (%)
I	12	46.53869	15.69907	261	Zrkovci	1	14	37.5
II	8	46.53962	15.69403	246	Brezje	1	16	62.5
III	8	46.54011	15.6913	254	Brezje	1	14	62.5
IV	2.8	46.54028	15.69167	256	Brezje	2	9	5
V	8	46.54025	15.69152	262	Brezje	2	6	15
VI	12	46.5807	15.87603	274	Sv. Trojica	5	38	15
VII	10	46.58291	15.87216	281	Sv. Trojica	5	46	5
VIII	100	46.58816	15.8701	260	Sv. Trojica	3	26	5
IX	10	46.62437	15.88247	267	Drvanja	5	26	15
X	8	46.62218	15.87911	268	Benediški vrh	5	31	37.5
XI	10	46.59192	15.89638	240	Osek	5	40	5
XII	15	45.55508	15.89008	238	Senarska	5	40	5
XIII	10	46.61727	15.88623	304	Benedikt	5	38	15
XIV	8	46.62431	15.8823	260	Drvanja	1	8	87.5
XV	10	46.62451	15.88227	295	Drvanja	5	41	1
XVI	8	46.6227	15.88125	299	Drvanja	1	29	87.5
XVII	100	46.58816	15.87022	261	Sv. Trojica	3	23	37.5
XVIII	6	46.58027	15.64301	389	Ribniško selo	4	17	5
XIX	100	46.5993	15.64226	378	Ribniško selo	4	30	15
XX	100	46.58785	15.86904	340	Sv. Trojica	3	24	15

Environmental conditions in the sampled sites (relevés) were evaluated using average Ellenberg ecological indicator values for light, temperature, continentality, soil moisture, soil reaction, and nutrients calculated from indicator values of plant species growing at each site (relevé).

The nomenclature of plant species and the classification of species into higher taxonomic groups (families) and biological spectrum (Raunkiaer life forms) follow Mala Flora Slovenije (Martinčič et al., 2007). Geoelements were determined according to Pignatti (2005) and syntaxons according to Oberdorfer (1994) and Mucina et al. (1993).

Statistical analysis

Sampled relevés were analyzed using non-metric multidimensional ranking (NMDS), a method for indirect gradient analysis that creates an ordination based on distances or an inequality matrix. Differences between clusters at P < 0.05 were tested with one-way ANOVA (F test) when parametric test assumptions were met, otherwise, the nonparametric Kruskal-Wallis test was used. Significant differences in environmental conditions were determined with the permutation test (P.perm < 0.05). Analyzes were performed using JUICE 7.0.208 software and Microsoft Excel.

Results with discussion

Ecological differentiation of ground-ivy habitats in NE Slovenia

Ground-ivy grows in a wide range of habitats in northeastern Slovenia, including open (meadows, lawns), semi shaded (orchards, vineyards, forest edges), and shaded habitats (nutrient-rich and moist forests). Five clusters of ecologically distinct habitats with ground-ivy occurrence were identified, differentiated by ecological conditions and flora diversity (Figure 3). Eutrophic forest edges are placed in cluster 1 and trampled habitats in cluster 2. Clusters 3 and 4 include moist, nutrient-rich forest habitats, which are most ecologically distinct from cluster 5, which includes meadows. Meadows are habitats with the highest amount of light and the highest temperatures compared to the other clusters. Forests in cluster 3 have higher soil moisture than forests in cluster 4, which is reflected in plant composition, such as the presence of black alder, which grows on moist to wet soils.

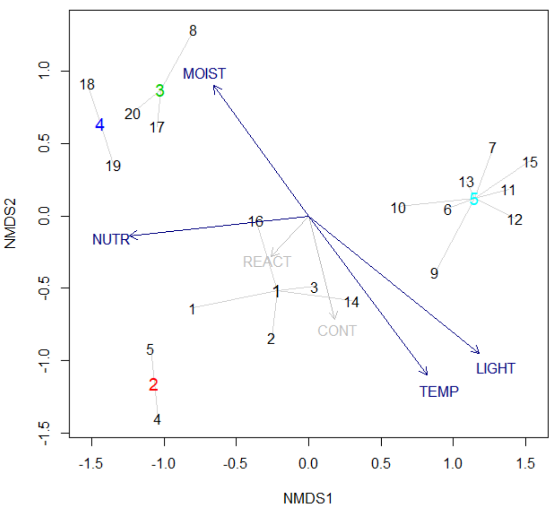

Figure 3. *Nonmetric multidimensional scaling of 20 relevés according to environmental gradient estimated from Ellenberg indicator values, resulting in 5 clusters.*

The clusters identified above were further analyzed to confirm ecological differentiation of ground-ivy habitats. Ellenberg values for light differ significantly among clusters (Figure 4a). Cluster 5 (meadows) has the highest light values, while clusters 3 and 4 (forests) have the lowest values. Ellenberg values for temperature show a similar pattern (Figure 4b). The continentality values differ between clusters: the highest value is obtained in cluster 1, and the lowest in cluster 4 (Figure 4c). The highest soil moisture is obtained in the habitats of clusters 3 and 4 (forests), while the lowest soil moisture is found in cluster 5 (meadows; Figure 4d). The soil reaction is similar in all clusters (Figure 4e). Nutrients are statistically lower in cluster 5, while there are no differences between the other clusters (Figure 4e).

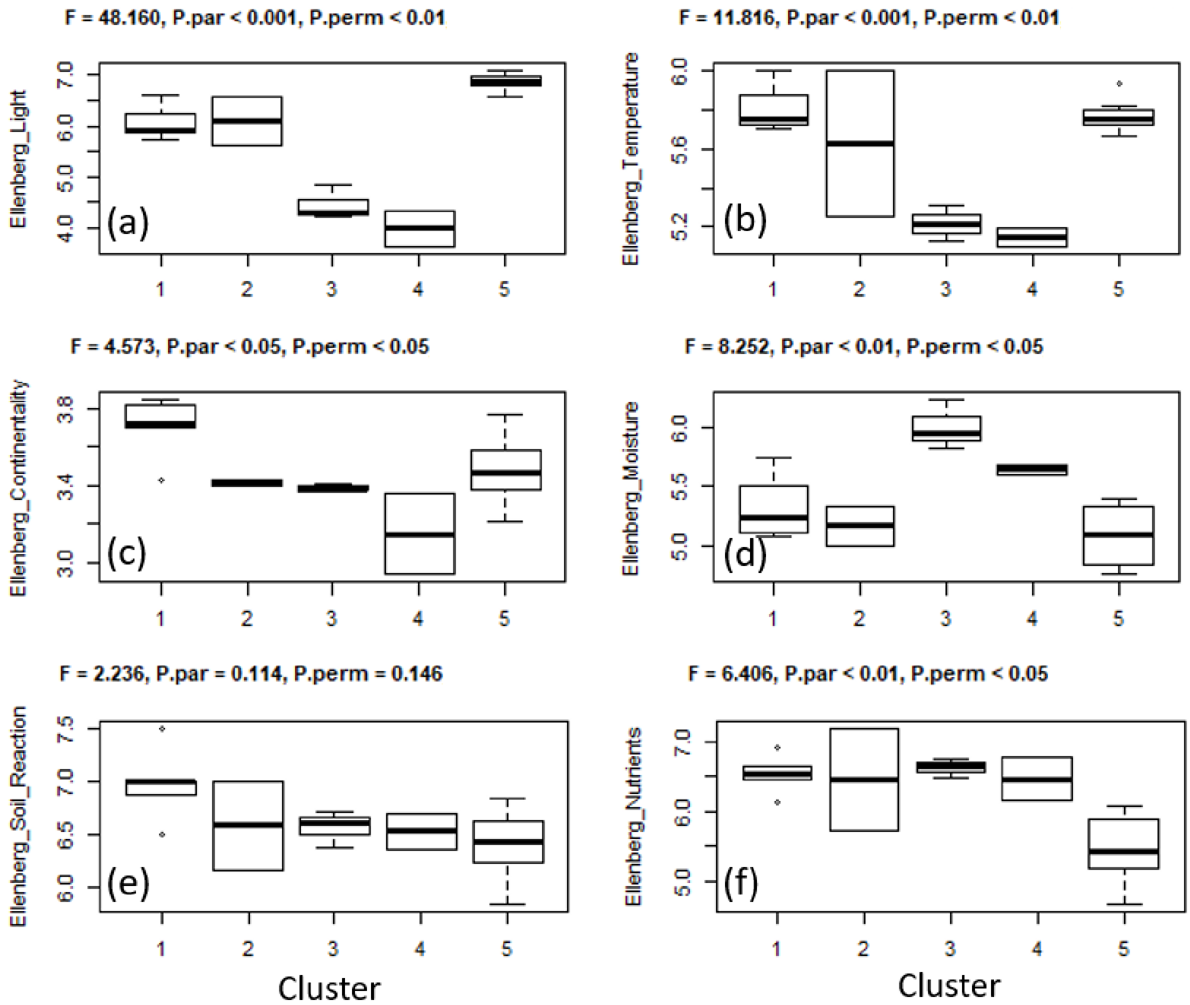

Figure 4. *Ellenberg ecological indicator values for (a) light, (b) temperature, (c) continentality, (d) soil moisture, (e) soil reaction and (d) nutrients in five clusters (habitats). The values above the graphs show the results of the ANOVA and the significance assessed with the permutation test (P.perm).*

Floristic diversity

Floristic analysis of the vegetation of the ground-ivy habitats in NE Slovenia resulted in 169 coexisting plant species, classified into 49 families, of which Poaceae (25), Lamiaceae (10) Fabaceae (10), Caryophyllaceae (10), Asteraceae (9) and Rosaceae (9) contained the highest number of species (Table 2; Figure 5).

Trampled habitats have the lowest species richness and diversity with 12 listed plant species, while meadows are the most species rich and diverse habitats with 100 listed plant species (Table 2).

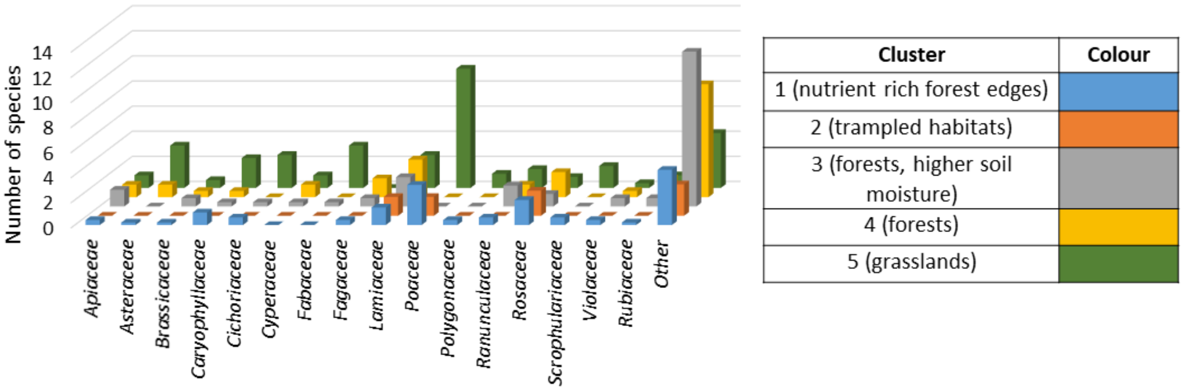

Figure 5. *Phylogenetic diversity of ground-ivy coexisting plant species according to five clusters (habitats).*

Table 2. *Ground-ivy habitat clusters with the average number of species per relevé, total number of species in a cluster, and the average Shannon diversity per relevé.*

Cluster	Average number of species/ relevé	Total number of species	Shannon diversity
1 (nutrient-rich forest edges)	16.2	54	1.602
2 (trampled habitats)	7.5	12	1.310
3 (forests, higher soil moisture)	24	40	2.440
4 (forests)	23	36	2.380
5 (meadows)	37.5	100	2.815

Biological spectrum

The proportion of chamaephytes (ANOVA; $F_{4, 15}$ = 1.06, P = 0.41; Figure 6a) and therophytes (ANOVA; $F_{4, 15}$ = 1.70, P = 0.30; Figure 6e) was similar between clusters. Geophytes dominated in the forests (Kruskal-Wallis test; $\chi 2$= 12.49, P < 0.05; Figure 6b). Hemicryptophytes were represented with the lowest proportion in forests, while their proportion was comparable in other clusters (ANOVA; $F_{4, 15}$ = 4.56, P < 0.05; Figure 6c). Phanerophytes dominated in forests, but were also abundant in forest edges, while they were absent in meadows (ANOVA; $F_{4, 15}$ = 7.33, P < 0.01; Figure 6d).

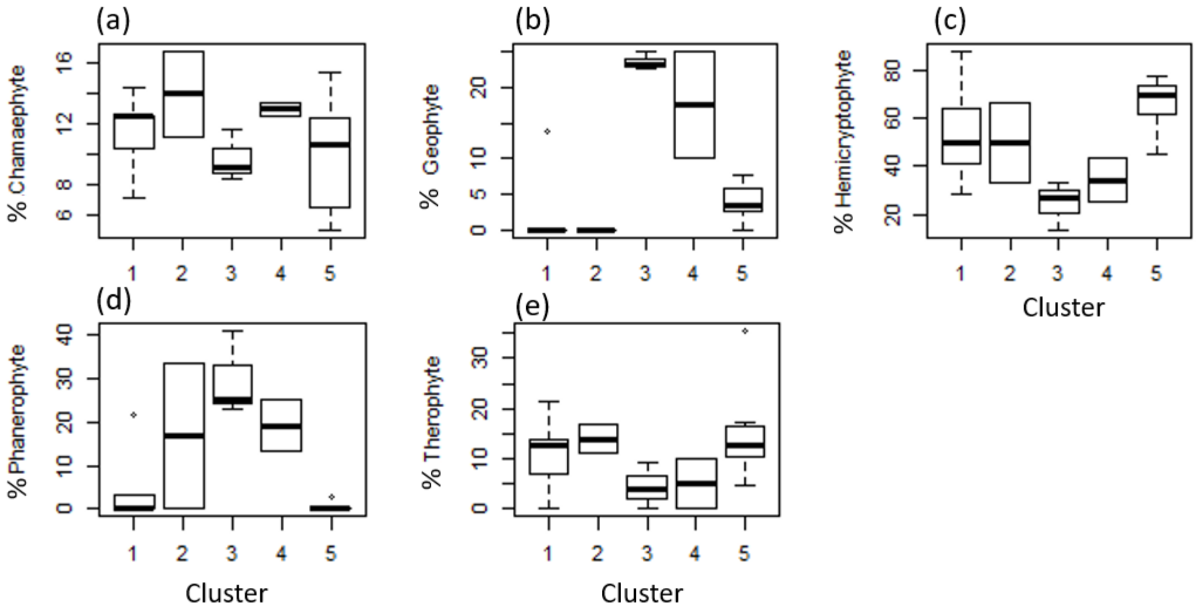

Figure 6. *Percentage incidence of life forms: (a) chamaephytes, (b) geophytes, (c) hemicryptopytes, (d) phanerophytes and (e) therophytes in five clusters (habitats).*

Phytogeographic diversity

Phytogeographic analysis has shown the presence of 10 floral elements, among which the following geoelements were recognized: Eurasian, Euro-Caucasian, circumboreal, paleotemperate, sub-cosmopolitan, cosmopolitan, Central-European, Euro-Siberian, Euromediterranean and adventive (Figure 7).

Species with circumboreal, sub-cosmopolitan, Euro-Caucasian and Eurasian distributions are represented in all clusters. Adventive and cosmopolitan species dominate eutrophic forest edges and trampled habitats, while only a small proportion are found in forests and meadows. Central-European species are represented to a lesser extent in forests. Most Eurimediterranean species are found in meadows, but they also occur in forests in a proportion of less than 5 %.

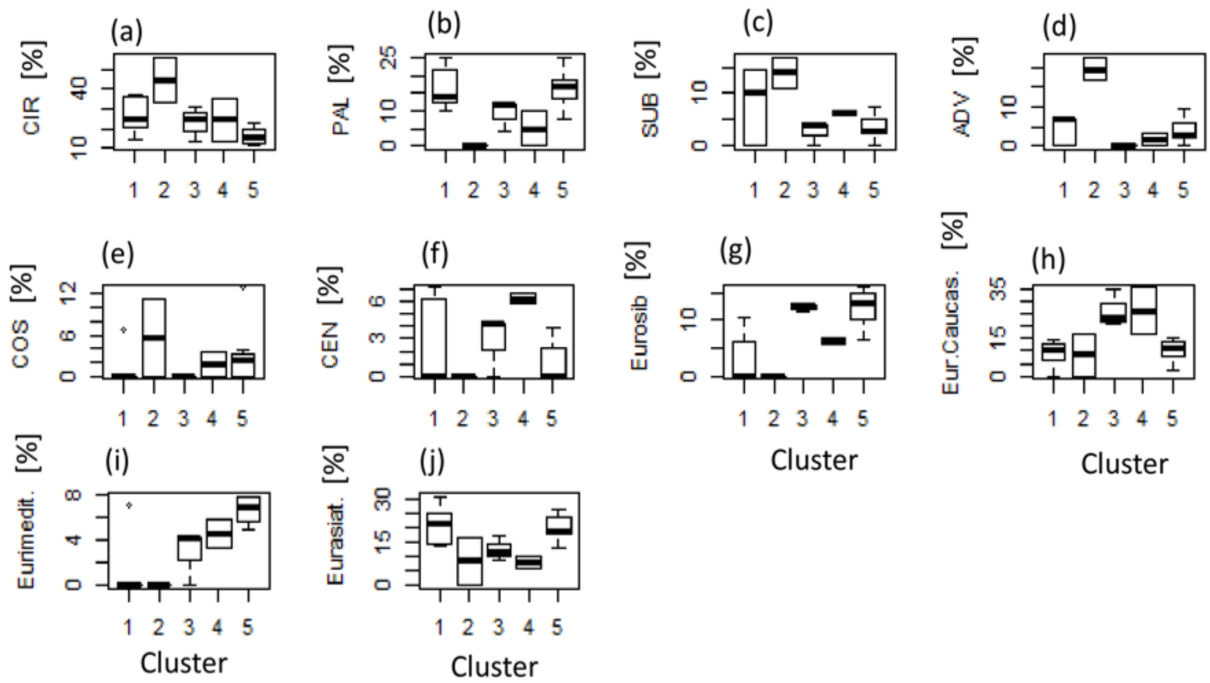

Figure 7. *Chorological spectrum of the ground-ivy habitats: (a) circumboreal, (b) paleotemperate, (c) sub-cosmopolitan, (d) adventive, (e) cosmopolitan, (f) Central-European, (g) Euro-Siberian, (h) Euro-Caucasian, (i) Eurimediterranean and (j) Eurasian floral element.*

Syntaxonomic diversity

We expected the above defined clusters to be dominated by different syntaxons, because the clusters differ ecologically and floristically. The *Molino-Arrhenatheretea* syntaxon dominates cluster 5, which includes 80 % of all species in this cluster. These are typical species of meadows and pastures. In smaller proportions (less than 20 %) these species were also present in other clusters. Shrub species of syntaxon *Rhamno-Prunetea* were represented in clusters 2, 3 and 4, which include forest edges and forests. Ruderal species of syntaxon *Stellarietea mediae* were represented in all habitats except in the forest understorey (cluster 3 and 4) where light is the main limiting factor. The *Querco-Fagatea* syntaxon was defined very broadly, as we included all typical forest species in this group, including species more typical of forests with moist to wet soils. As expected, these species are only represented in clusters that include forest and forest edge habitats. Nitrophilic forest edge species of *Galio-Urticetea* dominated cluster 2, but were also represented to a lesser extent in other habitats, with the exception of meadows where they were absent. Perennial weeds of class *Artemisietea* was most represented in cluster 1, accounting for about 10 %, while it was absent in clusters 2 and 4. Forest edge species of *Trifolio-Geranietea* were found with a small proportion (> 5 %) only in clusters 4 and 5. It is somewhat surprising that the edge species were listed in clusters combining forests and meadows. In this regard, it should be noted that the vegetation survey conducted in the forests included only lighter forests, which are relatively close to the edges and where edge species can also establish. Ground-ivy cannot tolerate conditions in a forest with a completely closed canopy, as light intensity would be too low. On the other hand, cluster 5 also contains samples (relevés) made near forest edges, which explains the occurrence of edge species of *Trifolio-Geranietea* in meadows. As expected, the syntaxon of eutrophic shrub species *Robinietea* was most represented in cluster 2, but even there it did not exceed 10 %. Syntaxon *Polygono-Plantaginetea* is characteristic of trampled habitats and was represented in the largest proportion, but still below 10 %, only in cluster 2 (Figure 8).

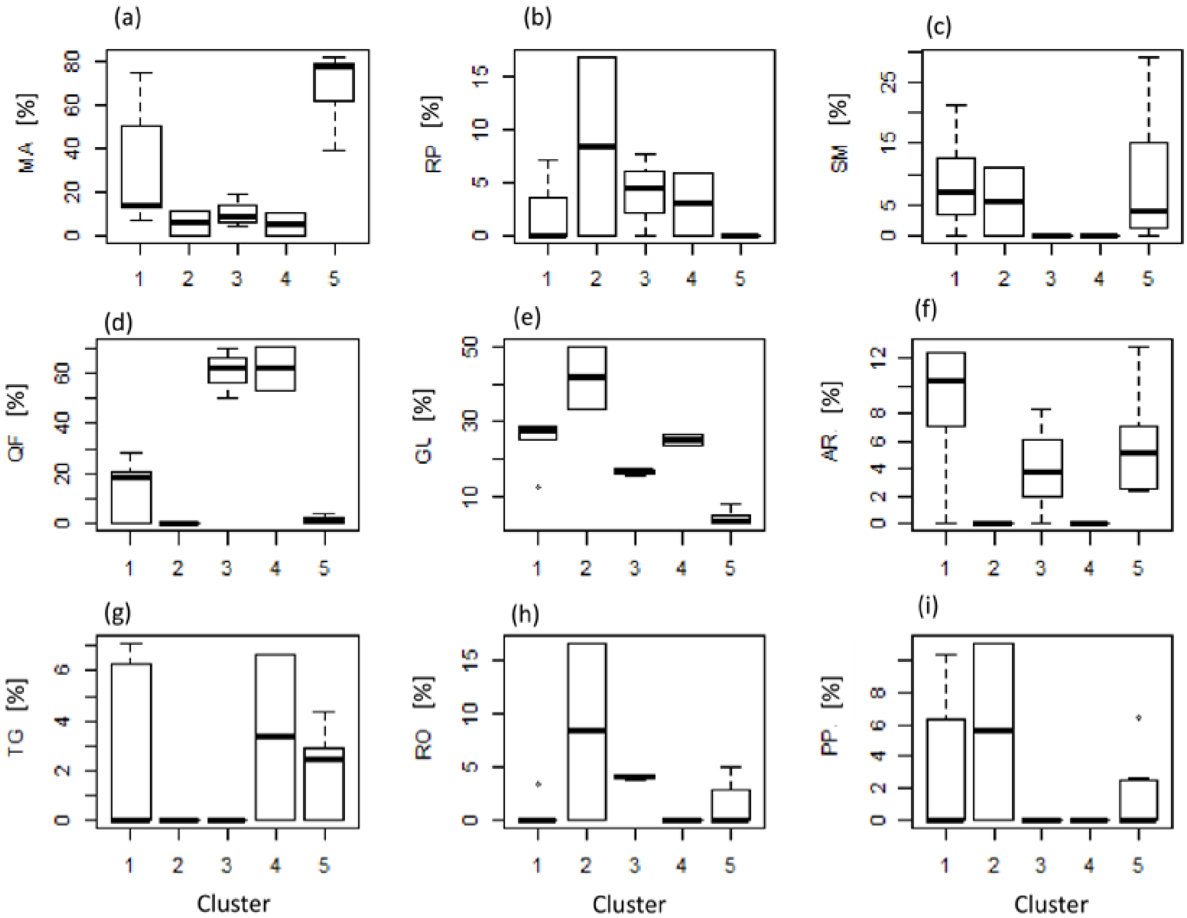

Figure 8. *Percent frequency of syntaxons (a) Molino-Arrhenatheretea, (b) Rhamno-Prunetea (Prunetalia spinose), (c) Stellarietea mediae, (d) Querco-Fagatea, (e) Galio-Urticetea, (f) Artemisietea, (g) Trifolio-Geranietea, (h) Robinietea in (i) Polygono-Plantaginetea in five clusters (habitats).*

Conclusion

Ground-ivy occurs in NE Slovenia in meadows, lawns, trampled habitats, forest edges and moist forest communities. The vegetation survey resulted in 20 relevés which were grouped into five clusters that differ ecologically and floristically. Differences between clusters recognized by floristic composition were confirmed with Ellenberg indicator values for various environmental parameters. We also showed that the proportions of individual life forms, chorotypes and syntaxons differed significantly among the recognized clusters.

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