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Phytochemical Analysis of Eight *Genista* L. taxa (Fabaceae) from Natural Populations in Bosnia and Herzegovina

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Abstract: Phytochemical analysis of aerial parts of eight autochthonous *Genista* L. taxa (brooms; Fabaceae; *G. germanica*, *G. januensis*, *G. pilosa*, *G. radiata*, *G. sagittalis*, *G. sericea*, *G. sylvestris* ssp. *dalmatica* and *G. tinctoria*) from natural populations in Bosnia and Herzegovina was performed in this study. Using fast phytochemical methods, for the first time, emodin was identified in the genus; coumarins, fatty acids, saponins, steroids, tannins and terpenoids in some taxa, but also the presence of phenolic compounds or the absence of anthocyanins in all studied taxa. The analysis of total phenol (TPC), flavonoid (TFC), phenolic acids (TPA) and alkaloid (TA) contents and antioxidant activity (DPPH), determined by spectrophotometry method, indicated the existence of differences between the studied taxa ($p<0.01$). The taxa differed significantly from each other in TPA and TA, and the least in terms of antioxidant activity. There is a positive correlation between TPA, TPC and TFC in one hand, and TA and antioxidative activity in other ($p<0.01$). The Euclidean dendrogram indicates two main clusters: the first cluster includes *G. januensis* and *G. pilosa*, and the second is derived from the remaining six taxa. Obtained PCA clusters were more diffused than those generated by Euclidean distance dendrogram but in a good agreement with them. The obtained data indicate the need for further phytochemical and pharmacological research of the genus *Genista*, as a very interesting source of natural active compounds, as well as population research with special emphasis on the influence of microclimate on SMs content.

INTRODUCTION

Plants produce different groups of secondary metabolites (SMs) that play important roles in stabilizing cellular structures, plant adaptation, and defence reactions to biotic and abiotic stresses. They are low molecular weight compounds that are chemically very diverse and complex, with different pathways of biosynthesis as well as biological and pharmaceutical activity. Secondary metabolites are distributed differently in the plant kingdom, with diverse and important functions (protection from herbivores, insects, pathogens, UV radiation, or stress; attract pollinators and seeds-spreading animals; mediate in plant-plant competition; and exhibit antioxidative properties). They vary in quality and quantity for certain plant species that grow in different ecological conditions (He, He, Farrar,*et al*., 2017; Santos-Sánchez, Salas-Coronado, Villanueva-Cañongo,*et al*., 2019).

The genus *Genista* L. (brooms; Fabaceae) includes about 140 taxa of shrubs and herbs which can slow down/prevent soil erosion, and with three nitrogen-fixing species (Lewis, Schrire, Mackinder, *et al*., 2005; Andrews and Andrews, 2017). The genus shows a very large variety of SMs, especially flavonoids, isoflavonoids, and alkaloids with various biological activities. Brooms have been of interest to human study since ancient times and were used in folk medicine (Kerkatou, Menad, Sarri, *et al.*, 2013; Wink, 2013; Grafakou, Barda, Tomou, *et al*., 2021).

The aims of this study were to: 1) do a fast phytochemical screening for the presence of nine groups of SMs (anthocyanins, coumarins, emodins, fatty acids, phenols, saponins, steroids, tannins, and terpenoids) in aqueous extracts, 2) quantify total phenolic, flavonoid, phenolic acids and alkaloid contents in methanol extracts, and 3) evaluate antioxidant activities in methanol extracts of different aerial parts of analyzed eight autochthonous *Genista* taxa from natural populations in Bosnia and Herzegovina.

MATERIAL AND METHODS

Site characteristics, plant material and authentication

In the area of the inner and outer Dinaric Alps, there are eight localities where plant material was collected in natural populations in Bosnia and Herzegovina during 2019-2021. (Table 1). Determination of taxa was done according to Flora Europaea (Gibbs, 1992) and Index Florae Bosna et Hercegovinae (Mišić and Šoljan, 2014). Vouchers are deposited in Herbarium of Faculty of Forestry University of Sarajevo.

Table 1: Origin of analyzed *Genista* taxa with data about site geological substrate, rhizosphere soil types and their physicochemical characteristics (Soil Map of B&H, 2013).

Taxon	Latitude	Longitude	Altitude (m)	Exposure	Substrate	Soil type
G. germanica	43°55'52.97" N	17°46'21.35" E	1774	SE	Shale	Dystric Leptosol, Dystric Cambisol
G. januensis	43°15'30.25" N	18°20'59.21" E	937	S-SE	Limestone	Lithic Leptosol
G. pilosa	44°08'47.26" N	17°34'51.24" E	876	S	Shale	Eutric Leptosol
G. radiata	44°21'11.29" N	17°30'46.31" E	1132	SW	Conglomerate	Rendzic Leptosol
G. sagittalis	43°32'07.05" N	18°35'14.53" E	650		Limestone	Lithic Leptosol, Rendzic Leptosol
G. sericea	42°48'19.05" N	18°24'31.32" E	571	E	Limestone	Rendzic Leptosol
G. sylvestris* ssp. *dalmatica	43°50'10.56" N	17°00'10.19" E	935	S	Limestone	Rendzic Leptosol, Cambisol
G. tinctoria	43°47'54.33" N	18°05'22.58" E	661	SW	Philite	Dystric Leptosol, Dystric Cambisol

The stems and inflorescences were immediately separated and stored in paper bags in the field. Collected materials were then rinsed with running tap water and dried at room temperature (20°C), in dark and well-ventilated area, for seven days. The dried samples were then grinded into powder, and stored in glass vials, at room temperature, until extraction.

Soil samples

Soil samples (individual plant rhizosphere) were cleaned, air-dried and sieved through a 1mm sieve. The texture, gravel content (%), drainage and water holding capacity (WHC) were determined during field studies. The soil organic carbon (SOC; ISO14235), carbonate content ($CaCO_3$; ISO10693), and pH values in water and 1M KCl reagent solute (ISO10390) were determined during laboratory studies.

Chemicals and reagents

Atropine, caffeic acid, gallic acid, rutin, and DPPH (1,1-diphenyl-2-picryl-hydrazyl) are of HPLC purity. All other used chemicals and reagents were of analytical grade (Sigma-Aldrich, Deinheim, Germany).

Fast phytochemical screening

After homogenization of the crushed plant material with 50 mL of sterile distilled water, the solutions were transferred to a water bath (temperature 50° C, incubation 30 min). The contents were then filtered through Whatman No.1 filter papers, and the filtrates were centrifuged for 15 min at 2,500 rpm. The isolated supernatants were immediately used for phytochemical screening for the presence of SMs.

Qualitative phytochemical analysis of aqueous extracts of aerial parts of studied *Genista* taxa were performed to determine the presence of:

Anthocyanins: In 2 mL of aqueous extract 2 mL of 2N hydrochloric acid and 2 mL of ammonia was added. The presence of anthocyanins is proven by discoloration of red-pink colour in the blue-violet (Paris and Moyse, 1969).

Coumarins: The volume of 3 mL of 10% sodium hydroxide was add to 2 mL of the aqueous extract. The presence of coumarins is proven by the appearance of a yellow color (Rizk, 1982).

Emodin: Mix 2 mL of ammonium hydroxide and 3 mL of benzene was mixed with 2 mL of the aqueous extract. The appearance of red color indicates the presence of emodin (Rizk, 1982).

Fatty acids: The volume of 5 mL of ether was added to 0.5 mL of the aqueous extract. The solution was poured on filter paper and dried. The presence of fatty acids is indicated by the transparency of the filter paper (Savithramma, Linga Rao, and Ankanna, 2012).

Phenolics: The volume of 2 mL of 2% iron (III) chloride solution was mixed with2 mL of aqueous extract. The presence of phenols is proven by the appearance of a blue-green or black color (modified Gibbs, 1974).

Saponins: The volume of 5 mL of the aqueous extract was diluted with 20 mL of distilled water. Resulting solution was mixed for 15 minutes. Formation of foam indicates the presence of saponins (Kumar, Ilavarsn, Jayachandran, *et al.*, 2009).

Steroids: The volume of 1 mL of the aqueous extract was added to 10 mL of chloroform, and then 10 mL of concentrated sulphuric acid to the edges of the test tube was added. If the upper layer of the solution (chloroform)

turns red, and the lower layer (sulphuric acid) turns yellow with green fluorescence, then the presence of steroids is indicated (Gibbs, 1974). *Tannins*: A few drops of 1% lead acetate were added to 2 mL of the aqueous extract. The presence of tannins is indicated by the appearance of a yellowish precipitate (Trease and Evans, 1983). *Terpenoids*: The volume of 2 mL of chloroform was added to 0.5 ml of aqueous extract. The volume of 3 mL of concentrated H_2SO_4 was added to form a layer. The presence of terpenoids is indicated by the appearance of a reddish-brown color (Ayoola, Coker, Adesegun, *et al*., 2008).

Quantitative determination of phenolic compounds and alkaloids

Per 0.5 g of ground plant material was extracted with 25 mL of 80% methanol in two replicates using an ultrasonic bath (Elma sonic S 60 H) for 30 min, and then were centrifuged at 1,800 rpm for 10 min. The supernatants were stored at +4°C for further analysis.

For determination of polyphenolic and alkaloid contents, and antioxidant activity of all methanol extracts Lambda 25 UV/VIS W/WINLAB V4 Perkin Elmer spectrophotometer was used. All values are presented as means of triplicates.

Total phenolic content (TPC) was determined by the Folin-Ciocalteu method (Luthria, Mukhopadhyay and Krizek, 2006). Absorbance was measured spectrophotometrically at 765 nm against the blank. TPC was expressed as the gallic acid equivalent per gram of dry plant material (mg GAE/g DW).

The total flavonoid content (TFC) was performed by the method of Quettier-Deleu, Gressier, Vasseur, *et al*. (2000). The absorbance of coloured samples was taken at 415 nm against the blank. TFCwas expressed as the rutin equivalent per gram of dry plant material (mg RE/g DW).

Total phenolic acids content (TPA) was determined by Arnow method (Szaufer-Haydrich and Goślińska, 2004). The absorbance at 490 nm against the blank was measured. TPAwas expressed as the caffeic acid equivalent per gram of dry plant material (mg CAE/g DW).

Total alkaloid content (TA) was determined by method of Patel, Patel and Trivedi (2015). Absorbance was measured at 415 nm against blank immediately after collection of the chlorophorm layer. TA content was expressed as the atropine equivalent per gram of dry plant material (mg AE/g DW).

Antioxidant activity

The DPPH free-radical scavenging activity (methanolic solutions of 100, 80, 60, 40, and 20 μL) was estimated by method of Thaipong, Boonprakob, Crosby, *et al*. (2006). The results were expressed as percent inhibition (IC_{50}) calculated from the control, where lower IC_{50} values indicate higher antioxidant activity.

Statistical analysis

The results were expressed as the mean of three replicates ± standard deviation. The data was analyzed using a one-way ANOVA, followed by Duncan's multiple range test, Pearson's correlation coefficient and Euclidean distance (r; IBM SPSS Statistics version 20, IBM Corp., Armonk, NY, USA), considering $p<0.01$ as very significant. The Principal Component Analysis (PCA) was performed in RStudio Team (2020).

RESULTS

Soil

General observations corresponding to all sites are soil shallowness, coarse texture, high percentage of gravel content, high water conductivity, and low water holding capacity in each site (Table 2). Most soil samples had neutral to alkali reaction, except soil sample number 1 which was extremely acidic. **Fast screening for secondary metabolites**

The presence of phenolics, tannins and terpenoids in the aboveground parts of all analyzed taxa was determined by fast screening methods (Table 3). Coumarins were present in aboveground parts of *G. pilosa* and *G. sagittalis*, in the stem of *G. sericea*, and in the inflorescences of *G. radiata*. Emodin was present only in the aboveground parts of *G. pilosa*. Only *G. sericea* had fatty acids in stem and inflorescences, and saponins in the stem. All analyzed taxa, except *G. sericea*, had steroids in aerial parts. On the other hand, presence of anthocyanins was not proven for any of the analyzed *Genista* taxa

Table 2: Soil traits at sampling site. Water Holding Capacity (WHC); Soil Organic Carbon (SOC); carbonate content ($CaCO_3$); pH value in water (pH_{H2O}); pH value in 1M KCl reagent solute (pH_{KCl}).

Taxon	Texture	Gravel (%)	Drainage	WHC	SOC (%)	$CaCO_3$ (g kg^{-1})	pH_{H2O}	pH_{KCl}
G. germanica	Sandy loam	0	Excessive	Weak	9.41	0.00	4.29	3.69
G. januensis	Loamy sand	10-20	Excessive	Weak	2.00	10-20	6.5	7.11
G. pilosa	Sandy loam	50-55	Excessive	Weak	1.09	20-80	7.01	5.92
G. radiata	Sand	0	Strong	Weak	2.43	80-160	7.26	7.00
G. sagittalis	Sandy loam	0	Strong	Weak	3.25	160-200	7.11	6.81
G. sericea	Sandy loam	5-10	Excessive	Weak	2.55	20-40	6.30	6.44
G. sylvestris* ssp. *dalmatica	Loam	70-80	Excessive	Weak	6.64	10-20	7.25	6.45
G. tinctoria	Loamy sand	50-60	Excessive	Weak	1.95	0.00	6.80	6.40

Table 3: Results of phytochemical analysis of secondary metabolites presence by rapid screening methods for analyzed *Genista* taxa.

Taxon	Plant part	Secondary metabolites															
		Coumarins		Emodin		Fatty acids		Phenols		Saponins		Steroids		Tannins		Terpenoids	
		OD	LD	OD	LD	OD	LD	OD	LD	OD	LD	OD	LD	OD	LD	OD	LD
G. germanica	**S**	–	–	–	–	–	–	+	+	–	–	+	–	+	–	+	–
	I	–	–	–	–	–	–	+	+	–	–	+	–	+	–	+	–
G. januensis	**S**	–	–	–	–	–	–	+	+	–	–	+	–	+	–	+	–
	I	–	–	–	–	–	–	+	+	–	–	+	–	+	–	+	–
G. pilosa	**S**	+	–	+	–	–	–	+	+	–	–	+	–	+	–	+	–
	I	+	–	+	–	–	–	+	+	–	–	+	–	+	–	+	–
G. radiata	**S**	–	–	–	–	–	–	+	+	–	–	+	–	+	–	+	–
	I	+	–	–	–	–	–	+	+	–	–	+	–	+	–	+	–
G. sagittalis	**S**	+	–	–	–	–	–	+	+	–	–	+	–	+	–	+	–
	I	+	–	–	–	–	–	+	+	–	–	+	–	+	–	+	–
G. sericea	**S**	+	–	–	–	+	–	+	–	+	–	–	–	+	–	+	–
	I	–	–	–	–	+	–	+	–	–	–	–	–	+	–	+	–
G. sylvestris **ssp. *dalmatica***	**S**	–	–	–	–	–	–	+	+	–	–	+	–	+	–	+	–
	I	–	–	–	–	–	–	+	+	–	–	+	–	+	–	+	–
G. tinctoria	**S**	–	–	–	–	–	–	+	+	–	–	+	–	+	–	+	–
	I	–	–	–	–	–	–	+	+	–	–	+	–	+	–	+	–

Note: S – stem and leaves; I – inflorescence; (–) – negative result; (+) – positive result; OD – our data; LD – literature data.

Table 4: Total phenolic, flavonoid, phenolic acids and alkaloid contents and DPPH scavenging activity in stem and leaves (S) and inflorescences (I) of eight analyzed *Genista* taxa.

Taxa	Sample	Phenols (mg GAE/g DW)	Flavonoids (mg RE/g DW)	Phenolic acids (mg CAE/g DW)	Alkaloids (mg AE/g DW)	IC_{50} (mg/mL)
G. germanica	S	26.128 ±0.16^{e}	36.265±0.02 ^{f}	7.220±0.06^{c}	0.419±0.03^{c}	0.223±0.04^{a}
	I	16.621±0.12^{a,b}	13.118±0.07^{b}	4.421±0.08^{a}	0.902±0.02^{f}	0.259±0.04^{a}
G. januensis	S	43.411 ±0.86^{g}	75.566±2.89^{h}	32.291±0.31^{i}	1.258±0.01^{g}	0.187±0.05^{a}
	I	43.859 ±1.22 ^{g}	64.972±2.20^{i}	29.633±0.85^{h}	0.598±0.03^{d,e}	0.198±0.04^{a}
G. pilosa	S	58.027 ±1.12^{h}	32.498±1.20^{f}	51.031±0.55^{j}	0.195±0.01^{a}	0.174±0.05^{a}
	I	55.030 ±8.03 ^{h}	36.547±0.67^{e}	55.388±0.89^{k}	0.208±0.01^{a}	0.164±0.10^{a}
G. radiata	S	16.938 ±0.48^{a,b}	9.068±0.40^{c}	4.732±0.08^{a}	6.508±0.04^{k}	1.783±0.31^{e}
	I	26.703 ±0.99^{e,f}	16.111±0.83^{a}	6.525±0.12^{b,c}	1.337±0.04^{h}	1.348±0.08^{d}
G. sagittalis	S	17.410±1.05^{a,b}	19.818±0.20^{d}	7.554±0.11^{c}	0.653±0.01^{e}	0.976±0.32^{c}
	I	18.468±0.31^{a,b,c}	20.879±0.49^{d}	9.648±0.33^{d}	0.228±0.01^{a}	0.597±0.12^{b}
G. sericea	S	18.409±0.07^{a,b,c}	35.989±0.15^{e}	4.184±0.03^{a}	2.345±0.07^{i}	1.073±0.01^{c,d}
	I	14.115 ±0.18^{a}	32.058±0.19^{f}	5.923±0.12^{b}	2.494±0.03^{j}	0.625±0.01^{b}
G. sylvestris **ssp. *dalmatica***	S	19.637±0.81^{b,c,d}	20.086±0.60^{g}	13.155±0.39^{e}	0.559±0.01 ^{d}	0.477±0.15^{a,b}
	I	31.168 ±1.02^{f}	41.198±1.36^{d}	16.498±0.24^{f}	0.328±0.01 ^{b}	0.347±0.06^{a,b}
G. tinctoria	S	24.240 ±0.16^{d,e}	41.668±2.09^{d}	17.795±1.16^{g}	0.415±0.01^{c}	0.371±0.07^{a,b}
	I	23.192±1.12^{c,d,e}	21.685±0.77^{g}	10.282±0.19^{d}	0.324±0.01^{b}	0.605±0.13^{a}

Note: GAE– Expressed as mg of gallic acid per g of dry plant material; RE– Expressed as mg rutin per g of dry plant material; CAE– Expressed as mg of caffeic acid per g of dry plant material; AE– Expressed as mg of atropine per mL of dry plant material; Values are expressed as means ± standard deviations (n = 3); Means in the same column with different letters in superscript are significantly different at $p<0.01$.

Active compounds: total phenols, flavonoids, phenolic acids, and alkaloids

The TPC, TFC and TA values of analyzed *Genista* taxa are shown in Table 4. Analyzed taxa had relatively high and fairly uniform amounts of TPC, where *G. pilosa* and *G. januensis* had the highest values and *G. radiata* (stem) and *G. sericea* (inflorescences) the lowest. On the contrary, TFC values are very variable and in a wide range. The amount of TPA is relatively low and uniform in most of the analysed taxa, except in *G. pilosa* and *G. januensis*. Based on available literature data, TA spectrophotometric determination was done for the first time in this study. The stem of *G.radiata* had a very high content of TA, followed by *G. sericea* and *G. januensis*. Among the inflorescence extracts, the highest content of TA was recorded in *G. sericea* and *G. radiata*, while *G. pilosa* had the lowest amount of alkaloids in both stem and inflorescence.

DPPH free radical scavenging

Genista pilosa and *G. januensis* showed the highest antioxidant activity (Table 4), while *G. radiata*, *G. sericea* and *G. sagittalis* had the lowest antioxidant activity.

Multivariate analysis of phytochemical characteristics of *Genista* taxa

ANOVA showed that all the studied characters indicate the presence of mutual differences ($p<0.01$; Table 4). The results of the Duncan's test confirmed statistically significant differences in individual comparisons of the studied taxa. The largest number of significant interspecies differences is related to testing of TPA and TA, while the smallest number of interspecies differences was found in the antioxidant activity. Also, significant differences were observed in the analysed samples of stem/leaves and inflorescences in the individuals of the same taxon. Further data analysis showed a positive relationship between TPA, TPC and TFC, and TA and antioxidative activity at $p<0.01$ level. The Euclidean distance dendrogram showed two main clusters, where the first cluster includes *G. januensis* and *G. pilosa*, and the second is formed from the remaining six taxa (Figure 1). Taxa within the second cluster are relatively close although they are grouped into three subclusters.

According to principal component analysis (PCA), the first two principal components accounted for 82.90% of total variability between taxa (Figure2). The PC1 correlated with TPC, TPA and TFC, while PC2 correlated with TA and antioxidant activity. Obtained PCA clusters were more diffused than those generated by Euclidean distance dendrogram but in a good agreement with them.

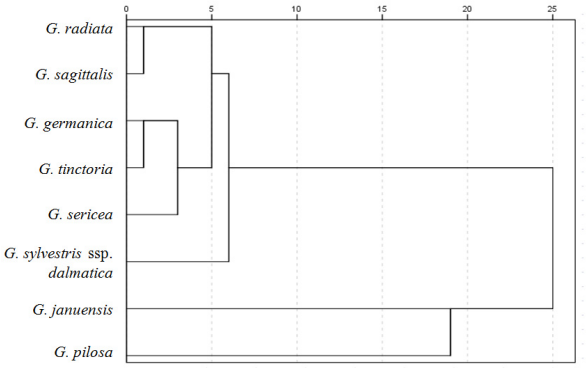

Figure 1. Dendrogram resulting from a cluster analysis selecting the Euclidean distance as similarity measurement of means of total phenol, flavonoid, phenolic acids and alkaloid contents, and antioxidant activity of studied *Genista* taxa.

DISCUSSION

Fabaceae species possess a distinctive and specific chemical composition characterized by variable bioactive phenolic compounds, especially flavonoids. Namely, certain genera or species are rich in different types of SMs whose concentrations vary significantly in different plant parts, seasonally or in relation to environmental conditions, especially from sun's radiation and temperature (Wink, 2013; Tsypysheva, Petrova, Baykova, *et al*., 2014).

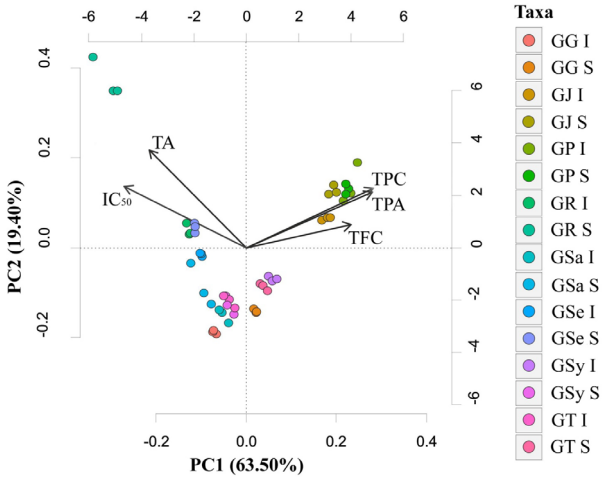

Figure 2. Principal component analysis (PCA) of the different phytochemicals of *Genista* taxa methanolic extracts. Principal component 1 (PC1) = 63.50% and component 2 (PC2) = 19.40%. I – inflorescences; S – stem and leaves; GG – *G. germanica*; GJ – *G. januensis*; GP – *G. pilosa*; GR – *G. radiata*; GSa – *G. sagittalis*; GSe – *G. sericea*; GSy – *G. sylvestris* ssp. *dalmatica*; GT – *G. tinctoria*.

Preliminary qualitative tests for SMs in eight *Genista* taxa showed the presence of steroids in all taxa (except for *G. sericea*), tannins and terpenoids for the first time, while the occurrence of phenolic compounds was consistent with published data. For some of the analysed taxa, the presence of coumarins, emodin, fatty acids and saponins was recorded for the first time. These results indicate the potential use of the investigated *Genista* taxa as a source of bioactive compounds that have not yet been sufficiently investigated. Despite its significant roles in protecting plants from many biotic and abiotic stressors, preliminary qualitative tests have shown that there are no anthocyanins in the aboveground parts of all analysed taxa. According to the literature, only Lrhorfi, Dahmani, Elyahyoui, *et al*. (2016) reported that *G. quadriflora* possess the anthocyanins and leukoanthocyanidins.

Coumarins and flavonoids are known to have a number of beneficial effects on human health, which are related to antioxidant activity as previously demonstrated (Lrhorfi, Dahmani, Elyahyoui, *et al*., 2016). In this work, coumarins were detected in the stem of *G. sericea*, inflorescences of *G. radiata* while *G. pilosa* and *G. sagittalis* had coumarins in all aboveground parts. Emodin was found only in *G. pilosa* while fatty acids in *G. sericea*. Previous results showed the presence of fatty acids in essential oils of *G. numidica* and *G. saharae* (Lograda, Chaker, Chalard, *et al.*, 2009).

Phenols were present in the aboveground parts of all analyzed taxa with a content that was relatively high and fairly uniform. In the genus *Genista* the presence of phenols has already been proven (Boukaabache, Boumaza, Mekkiou, *et al.*, 2015; Guetaff, Abidli, Kariche, *et al.*, 2016; Hanganu, Olah, Benedec, *et al.*, 2016; Lrhorfi, Dahmani, Elyahyoui, *et al.*, 2016; Ati, Salima and Warda, 2017; Barek, Rahmoun, Aissaoui, *et al.*, 2020) and their content varies depending, among other things, on solvents used, which may indicate the polyphenolic composition of the extracts (Zhang, Cai and Cheng, ~~*et al.*,~~ 2022). Saponins and steroids were detected only in *G. sericea* but they were also detected in

aboveground parts of some other *Genista* taxa (Boutaghane, Voutquenne-Nazabadioko, Harakat, *et al*., 2013; Boukaabache, Boumaza, Mekkiou, *et al.*, 2015; Guetaff, Abidli, Kariche, *et al.*, 2016). In the aboveground parts of all investigated taxa, except in *G. sericea*, the presence of steroids was confirmed for the first time in this study. Tannins, which provide protection of plants from insects, pests and herbivores, were previously detected in several *Genista* species. Tannins and some phenolic compounds are usually associated with antioxidant activity (Guetaff, Abidli, Kariche, *et al.*, 2016; Lrhorfi, Dahmani, Elyahyoui, *et al.*, 2016; Ati, Salima and Warda, 2017). In this study, for the first time, the presence of tannins in the aboveground parts of all analyzed *Genista* taxa was confirmed. Terpenoids play very important roles in all life processes (Adamski, Blythe, Milella, *et al.*, 2020), and have previously been identified only in other four *Genista* taxa (Lograda, Chaker, Chalard, *et al*., 2009; Boukaabache, Boumaza, Mekkiou, *et al.*, 2015; Guetaff, Abidli, Kariche, *et al.*, 2016; Lrhorfi, Dahmani, Elyahyoui, *et al.*, 2016). Terpenoids were noticed in the aboveground parts in all investigated *Genista* taxa for the first time in this study.

Alkaloids have different biological activities, but in plants they act as allelopathic compounds, and have a defensive role against herbivores and pathogens (Wink, 2013). In Fabaceae family, quinolizidine alkaloids and some piperidine alkaloids are principal SMs for almost all taxa of the Genistoid clade (Wink, 2008; Küçükboyaci, Özkan and Tosun, 2012; Wink, 2013). The presence of alkaloids, mainly of quinolizidine alkaloids, was detected in 23 *Genista* taxa, of which only two of the eight taxa were analyzed in this study (*G. tinctoria* and *G. sagittalis*) (Kirch, Veit, Waetzig, *et al.*, 1995; Christov and Evstatieva, 2000; Tero-Vescan, Vari and Vlase, 2014; Küçükboyaci, Özkan and Tosun, 2020). Tsypysheva, Petrova, Baykova, *et al.* (2014, and references therein) found that the alkaloids concentration in *G. tinctoria* was highest in twigs during the flowering period, while Tero-Vescan, Vari and Vlase (2014) found that extracts of *G. tinctoria* and *G. sagittalis* have very low content of alkaloids. Some studies have shown that alkaloid compositions in *Genista* were similar although very different alkaloid values were determined (Küçükboyaci, Özkan and Tosun, 2012; Tero-Vescan, Vari and Vlase, 2014). The TA content varied both among the taxa and between the analyzed plant parts in this study. Most of the analyzed taxa had more-or-less approximate TA concentrations in stem/leaves and inflorescences, except *G. radiata*, *G. sericea* and *G. januensis*. Different studies showed that *Genista* taxa are rich in alkaloids, but data about TA contents we could not find (Wink, 2013; Ati, Salima and Warda, 2017).

Natural antioxidant compounds have a key defensive role against the free radicals in prevention of the antioxidation processes. Due to the complex chemical composition of plant extracts, it is difficult to estimate correlations between their active compounds and antioxidant activities. Many authors are agreed that antioxidant activity depends on the plant species, plant part, concentration, structure, and synergism or antagonism of present antioxidants in the extracts (Zheng and Wang, 2001; Barek, Rahmoun, Aissaoui, *et al.*, 2020). However, most of them agree that different groups of phenolic compounds, especially phenols and flavonoids (Zheng and Wang, 2001; Aoruhaon, Fazouane, Benayache, *et al*., 2019), or their structures (Kaur and Mondal, 2014) are most responsible for the antioxidant activity. Using DPPH free radical scavenging method, we found a fairly high to moderate antioxidant activity in almost all analyzed methanol extracts. Many studies show that the main contributors to antioxidant activity of numerous *Genista* taxa were phenols and/or flavonoids in the extracts (Rauter, Martins, Lopes, *et al.*, 2009; Serrilli, Graziosi, Ballero, *et al.*, 2010; Orhan, Tosun, Tamer, *et al.*, 2011; Kerkatou, Menad, Sarri, *et al.*, 2013; Meriane, Genta-Jouve, Kaabeche, *et al*., 2014; Guettaf, Abidli, Kariche, *et al.*, 2016; Hanganu, Olha, Benedec, *et al.*, 2016; Ati, Salima and Warda, 2017; Aourahoun, Fazouane, Benayache, *et al.*, 2019; Barek, Rahmoun, Aissaoui, *et al.*, 2020; Simões, Pinto, Neves, *et al.*, 2020; Wafaand Sofiane, 2021). In contrast, Chebbah, Marchioni, Sarri, *et al.* (2016) found that antioxidant activity is probably due to the presence of phenolic acids, and Bouchouka, Djilani and Bekkouche (2012) reported no correlations between antioxidant activity and TPC and flavonoid contents in extracts of *G. saharae*. Among the investigated taxa, only for *G. sagittalis* and *G. tinctoria* there are data on the antioxidant activity of aboveground parts (Hanganu, Olah, Benedec, *et al.*, 2016), and their values were significantly lower than those recorded in this study. High antioxidant activity of *G. pilosa* and *G. januensis* in this study can be associated with the presence of high concentrations of TPC, TFC and TPA. All these results indicate that antioxidant compounds in higher concentrations contribute and act through multiple mechanisms, directly or indirectly, which requires further chemical and pharmacological investigations.

Based on cluster analysis, it is possible to describe the structure of data and determine natural groups within the data set. Thus, based on the similarity of analyzed total phenolic compounds, total alkaloids and antioxidant activity, the studied *Genista* taxa can be divided into two major groups. The PCA clusters, in comparison with Euclidean distance dendrogram, were more diffused but still in a good agreement with them. According to those analyses, *G. januensis* and *G. pilosa* separated from others based on TPA, TPC and TFC, while other *Genista* taxa are grouped in three subclusters without the possibility of clear mutual discrimination.

CONCLUSIONS

The use of qualitative fast phytochemical screenings of eight *Genista* taxa has proven the presence of new groups of SMs both in the genus and in certain taxa, which represent a potential source of bioactive compounds. Also, the presence of significant differences in the analyzed samples of the studied *Genista* taxa was found for TPA and TA, and the lowest for antioxidant activity. The results obtained in this study are promising, and it would be desirable and very interesting to continue in two directions: 1) phytochemical and pharmacological research of the potential alternative sources of bioactive natural compounds; and 2) population analyzes with

special emphasis on correlations of microclimatic conditions and SMs content.

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Summary/Sažetak

U ovom radu urađena je fitohemijska analiza nadzemnih dijelova osam autohtonih svojti roda *Genista* L. (žutilovke; Fabaceae; *G. germanica*, *G. januensis*, *G. pilosa*, *G. radiata*, *G. sagittalis*, *G. sericea*, *G. sylvestris* ssp. *dalmatica* i *G. tinctoria*) iz prirodnih populacija u Bosni i Hercegovini. Kvalitativne fitohemijske metode pokazale su se korisnim jer su neki od spojeva po prvi put identificirani u rodu (emodin) i u svojtama (kumarini, masne kiseline, saponini, steroidi, tanini i terpenoidi), kao i potvrđena prisutnost fenolnih spojeva ili odsutnost antocijanina u svim proučavanim svojtama. Analiza sadržaja ukupnih fenola (TPC), flavonoida (TFC), fenolnih kiselina (TPA) i alkaloida (TA) te antioksidativnog djelovanja (DPPH), određena spektrofotometrijskim očitanjem metanolnih ekstrakata, pokazala je da postoje razlike između proučavanih svojti ($p<0,01$). Svojte su se međusobno značajno razlikovale u TPA i TA, a najmanje po antioksidativnom djelovanju. Korelacijska analiza provedena u ovoj studiji pokazala je pozitivan odnos između TPA, TPC i TFC s jedne strane te TA i antioksidativne aktivnosti s druge strane ($p<0,01$). Dendrogram Euklidske udaljenosti, na temelju sličnosti hemijskog sastava i antioksidativnog djelovanja analiziranih svojti *Genista*, ukazuje na dva glavna klastera: prvi klaster uključuje *G. januensis* i *G. pilosa*, a drugi je izveden od preostalih šest svojti. Dobiveni klasteri PCA bili su raspršeniji od onih generiranih dendrogramom Euklidske udaljenosti, ali su se dobro podudarali s njima. Dobiveni podaci ukazuju na potrebu daljnjih fitohemijskih i farmakoloških istraživanja roda *Genista*, vrlo zanimljivog izvora prirodnih aktivnih spojeva, kao i populacijskih istraživanja s posebnim naglaskom na uticaj mikroklime na sadržaj sekundarnih metabolita.