



## Research paper

## Ethnobotanical Investigation of Plants Used for Respiratory Tract Infections in Pirot District (Southeastern Serbia)



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## ABSTRACT

**Introduction:** It is important to evaluate alternative respiratory infection treatments using herbal medicines, whose efficacy and safety have been shown in pharmacological and clinical studies. The study aimed to provide a review that will help screen plants used for respiratory infections that deserve further investigation and eventually develop an effective antiviral agent.

**Methods:** The Pirot District (Serbia) population was surveyed using a semistructured questionnaire to determine how many individuals were familiar with the useful properties of plants against respiratory infections. The surveyed population included 531 individuals from 152 villages, with 1 221 reports on the use of plants against respiratory infections. The most reported taxa are systematised by the most frequent application with the informants' calculated consensus factor.

**Results:** Plants reported for the treatment of respiratory infections (65 plant taxa) were distributed among 31 families, of which the most important were Lamiaceae (14 species) and Rosaceae (10 species), and the most abundant were Lamiaceae (433 reports) and Compositae (274 reports). The most used plant taxa were *Thymus* spp., *Matricaria chamomilla*, and *Sambucus nigra*. The primary indications for use were the common cold (640 reports), cough (330 reports), sore throat (80 reports), and bronchitis (67 reports).

**Discussion/Conclusions:** The use of 21 plant taxa in respiratory disorders has not been reported in other ethnobotanical studies in the Balkans. The presented data may be a good starting point for further phytopharmacological research in the quest for suitable drugs against viral respiratory tract infections.

## Introduction

Respiratory diseases are the most prevalent worldwide and are usually caused by viruses. Antibiotics are often prescribed unnecessarily. Respiratory viruses tend to follow seasonal patterns and have a high rate of spread among humans, especially in the fall and winter. However, they can be active at any time of the year. During this period, it is important to plan and implement strategies to prevent the spread of viruses in the community. Respiratory infections can be severe in older and immunocompromised individuals. Despite healthcare efforts to provide annual seasonal influenza vaccines, influenza causes an annual epidemic in midwinter that varies in severity from year to year, but it can be a major cause of hospitalisation and death (Barrett,

2018; Molinari et al., 2007). The common cold is usually regarded as an annoyance rather than a public health threat. However, even rhinovirus, the least pathogenic cold virus, can be a source of mortality in older people or the immunocompromised (Barrett, 2018; Louie et al., 2005).

More than 200 serologically distinct virus types are associated with colds (Eccles, 2005). The most common virus is the rhinovirus (30–80%), a variety of picornavirus with 99 known serotypes (Palmenberg et al., 2009). Other commonly implicated viruses are human coronaviruses (≈15%) (Goldman and Schafer, 2012). Furthermore, COVID-19, the novel 2019 coronavirus, causes a respiratory disease that can spread from person to person and affect communities worldwide. It is currently considered one of the most dangerous

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diseases in the world. Briefly, COVID-19 is an acute respiratory infection whose symptoms include fever, cough, chills, dizziness, fatigue, and shortness of breath (Wu et al., 2020; Xu et al., 2020). However, it can be fatal, with a mortality rate of 2%, due to massive alveolar damage and progressive respiratory failure (Xu et al., 2020).

It is well known that antibiotics are effective in treating bacterial pneumonia, that antibiotics against tuberculosis are effective in curing tuberculosis, etc. Most viral infections are self-limiting, so no ‘cure’ is needed. Several treatments appear to benefit from reducing the symptoms of respiratory diseases (Allan and Arroll, 2014; Arroll, 2005; Jackson Allen and Simenson, 2013). Regarding prevention, behavioural strategies such as hand washing, smoking cessation, stress reduction training, and regular exercise can effectively reduce the impact of viral acute respiratory infections in the general population (Barrett, 2018; Barrett et al., 2012).

Studies have shown that antibiotics that prevent bacterial complications of common colds and influenza are ineffective (Gadomski, 1993). In addition, antibiotic use is potentially harmful because it increases the risk of colonisation by resistant organisms, leading to subsequent bacterial infection that is not responsive to standard antibiotics (Jain et al., 2001). Conventional palliative therapies with antihistamines, decongestants, and cough suppressants may help mildly with some symptoms but tend to have side effects (Barrett, 2018). Therefore, it is essential to evaluate alternative treatments with herbal medicines whose efficacy and safety are supported by pharmacological and clinical studies.

Aetiologic studies of viral upper respiratory tract infections are important with the advent of new antiviral drugs (Nokso-Koivisto et al., 2006). Safe and effective antiviral treatments for respiratory infections may be available in the future (Barrett, 2018).

Globally, herbal medicines are the mainstay in the treatment of respiratory infections. Herbal extracts are widely used in traditional medicine for their antimicrobial and antiviral effects (De Clercq, 2004), but perhaps also for their impact on common symptoms because of their anti-inflammatory, antitussive, antipyretic, and decongestant activities. Descriptions of herbal therapies of the upper part of the respiratory system have been the subject of discussion by anthropologists and ethnobotanists (Barrett and Kieffer, 2001; Schultes and von Reis, 1995). However, only a few traditional medicines have been adequately tested for their pharmaceutical properties and clinical efficacy (Barrett, 2018).

Several hundred plant species already researched have demonstrated their potential as novel antiviral agents (Jassim and Naji, 2003). However, for most species or preparations of herbal medicines that have been studied so far, systematic studies on their activity against viruses causing respiratory infections have yet to be conducted (Glatthaar-Saalmüller et al., 2011).

The most common groups of diseases in the population of Pirot District in 2016 compared to the previous 5-year period detected in the General Medicine Department were diseases of the respiratory system (19.3%), diseases of the circulatory system (18.8%), and diseases of the musculoskeletal system and connective tissue (10.3%) (Pirot Institute of Public Health, 2016).

The population in rural areas in Southeastern Serbia does not have adequate medical care because they cannot obtain health insurance due to extreme poverty (Matejić et al., 2020). According to the same authors, medicinal plants are the first medical care choice in this underdeveloped region of Serbia. The present study aimed to critically appraise the Pirot District’s local population regarding the plant species used to treat respiratory infections. In this context, it is crucial to obtain answers to the following questions: (1) which are the most commonly used medicinal plants for respiratory infections in the investigated area, (2) which respiratory indications are commonly treated in the four municipalities of the district, (3) are there differences in the use of medicinal plants for respiratory infections between the Serbian and Bulgarian populations, and (4) to present a comparative review of the traditional use of medicinal plants for respiratory infections on the

Balkan Peninsula. Such studies may help identify new antiviral drugs against respiratory infections in the future.

## Materials and Methods

### Study Area

The studied area of Pirot District is in the central part of the Balkan Peninsula. It includes the four municipalities of Pirot, Bela Palanka, Babušnica, and Dimitrovgrad on the border between Serbia and Bulgaria. It covers 2 761 km<sup>2</sup> (Pirot District GIS, 2019) (42.863° N–43.403° N and 22.117° E–23.006° E) and has a total population of 92 479.

The climate of Pirot District is temperate continental, with a transition from a foothill to a mountain climate at altitudes above 600 m (Marković et al., 2010). According to the same authors, Mt. Stara Planina is the most important natural object in the region and represents a mountain rich in resources and medicinal plants. On Mt. Vidlič, which is part of Stara Planina, a community of Oriental hornbeam (*Carpinetum orientalis serbicum* Rudski 1949) was found on steep slopes at altitudes of 400–600 m, and a community of Hungarian and Turkey oak (*Quercetum frainetto-cerridis* Rudski 1949) was found at altitudes 400–1 000 m on the southern exposures (Marković et al., 2015a). Beech forests (*Fagetum moesiaca montanum* Jov. 1953 [non-Rudski 1949]) has been formed at altitudes from 900 to 1 300 m (Marković et al., 2018). Marković et al. (2015b) found dry grasslands and rocky slopes vegetation in the study area. They are the main source of aromatic (Marković et al., 2009) and medicinal plants (Marković et al., 2010).

### Population

The population of Pirot District has a heterogeneous ethnic structure. Nationalities include Serbs (77 379; 83.7%), Bulgarians (6 602; 7.1%), Roma (4 306; 4.7%), and others (4 192; 4.5%). According to the 2011 census, Serbs were in the majority (53 232; 91.9%) in the municipality of Pirot, as well as in the municipalities of Bela Palanka (10 395; 85.7%) and Babušnica (10 933; 88.8%). However, in the municipality of Dimitrovgrad, the ethnic group of Bulgarians was in the majority (5 413; 52.5%) (Statistical Office of the Republic of Serbia, 2011). The particular ethnic population group called ‘Shopi’ or ‘Torlaci’ lives in the study area. Stojković (2010) states they live in western Bulgaria, northeastern North Macedonia, and Southeastern Serbia. The term Torlaks or ‘Torlaci’ is associated with a dialect of the Eastern Balkans (Krstić, 2019).

The language of the people in Pirot District is unique. In Eastern Serbia, the language is divided into two groups of dialects ‘timočko-lužnički’ and ‘svrljiško-zaplanjski’ (Matejić et al., 2020). The language of the people of Pirot District is a specific variant of the ‘timočko-lužnički’ dialect that has many old words (Panajotović, 2007), including the local names of plants. In the study area, 208 medicinal plant taxa and 788 folk plant names were recorded, mainly in the specific dialect of Pirot District (Milojević and Mihajlov, 1985).

### Ethnopharmacological Survey

The study was conducted in 2017–2019 by surveying the population. A total of 631 people were interviewed in this ethnobotanical study in the Pirot District, of which 337 were men and 294 were women; the age range was 16–88. The questionnaire on the knowledge and use of medicinal plants included 152 villages in Pirot District. From the total number of surveyed individuals, 531 mentioned medicinal plants for respiratory infections, of which 445 were Serbs with 1 019 reports, 75 were Bulgarians with 179 reports, and 11 were Roma with 23 reports (Table 1). The detailed ethnic composition of the surveyed population and comparison with the total number of rural inhabitants in the four municipalities of Pirot District are given in Table 1.

**Table 1**  
Overview of the surveyed rural population by nationality and gender in the Pirot District with data on the proportion of respiratory infections.

Municipality/District	Sex	Census data <sup>1</sup>				Interviewed				Rs knowledge <sup>3</sup>				Rs reports <sup>4</sup>			
		SER <sup>2</sup>	BUL	ROM	Σ	SER	BUL	ROM	Σ	SER	BUL	ROM	Σ	SER	BUL	ROM	Σ
Pirot (rural)	Men	9 152	97	353	9 846	163	2	4	169	137	2	3	142	312	4	5	321
	Women	8 645	76	345	9 297	158	1	2	161	123	1	1	125	289	1	1	291
Bela Palanka (rural)	Men	17 797	173	968	19 143	321	3	6	330	260	3	4	267	601	6	5	612
	Women	1 910	1	100	2 090	60	-	4	64	55	-	3	58	122	-	4	126
Babušnica (rural)	Men	1 701	1	101	1 893	61	-	4	61	54	-	3	112	135	-	-	135
	Women	3 611	2	201	3 983	121	-	4	125	109	9	2	53	257	17	4	261
Dimitrovgrad (rural)	Men	3 403	327	112	3 984	50	10	3	63	42	9	2	73	88	17	9	114
	Women	3 216	272	91	3 722	37	7	-	44	31	7	-	51	67	21	-	88
Pirot District (rural)	Men	6 619	599	203	7 706	87	17	3	107	88	16	2	91	155	38	9	202
	Women	464	1 139	3	1 996	2	38	1	41	2	36	1	39	4	85	2	91
Pirot District (rural)	Men	534	916	5	1 844	1	26	1	28	1	20	1	22	2	51	2	55
	Women	998	2 055	8	3 840	3	64	2	69	3	56	2	61	6	136	4	146
Pirot District (rural)	Men	14 929	1 564	568	17 916	275	50	12	337	236	47	9	292	526	106	20	652
	Women	14 096	1 265	542	16 756	257	34	3	294	209	28	2	239	493	73	3	569
	Σ	29 025	2 829	1 110	34 672	532	84	15	631	445	75	11	531	1 019	179	23	1 221

<sup>1</sup> Data according to the census in 2011 (Statistical Office of the Republic of Serbia, 2011).

<sup>2</sup> Nationality codes: SER, Serbian; BUL, Bulgarian; ROM, Roma.

<sup>3</sup> Rs knowledge: number of respondents with knowledge about the use of plants against respiratory tract infections.

<sup>4</sup> Rs reports: number of reports about the use of plants against respiratory tract infections.

Respondents were randomly selected among adult residents, focusing on older adults of the Pirot District villages, who are usually engaged in small-scale farming and pastoral activities. Only those who claimed knowledge of the traditional uses of medicinal plants were interviewed. The study participants were first asked about their gender, age, and nationality by Marija S. Marković and Vesna P. Stankov Jovanović in Serbian, sometimes in the specific dialect spoken by the inhabitants of the study area, especially in remote villages with an elderly population. The detailed data collected from the participants were the common name of the plants, the disease for which the plant is used, the part of the plant used, the form of preparation, and the method of administration. Surveyed respondents from 152 villages mentioned the use of plants against respiratory infections. The distribution of villages visited during the interviews and the knowledge of the local population about medicinal plants for respiratory infections are given in Figure 1. The villages are relatively small in area and number of inhabitants.

According to Jordanov (1963–1979) and Josifović (1970–1986), the plant species collected during the interviews were identified. Taxonomic nomenclature was assigned following the ‘Flora Europaea’ (Tutin et al., 1964–1980) and the checklist from The Plant List database (<http://www.theplantlist.org/>). All species were labelled after identification and deposited in the herbarium collection of the Department of Biology and Ecology, Faculty of Sciences and Mathematics, University of Niš ‘Herbarium Moesiicum Niš.’ The voucher numbers are given in Table 2. The species *Euphrasia stricta* D. Wolff, *Thymus praecox* Opiz. subsp. *jankaе* (Čelak) Jalas, and *Verbascum phlomoides* L. are listed in Table 2 at the genus level because other species from the same genera in the study area can be used and produce the same effect. The questionnaire data obtained during the field study are deposited in the Herbarium Moesiicum Niš herbarium.

#### Data Analysis

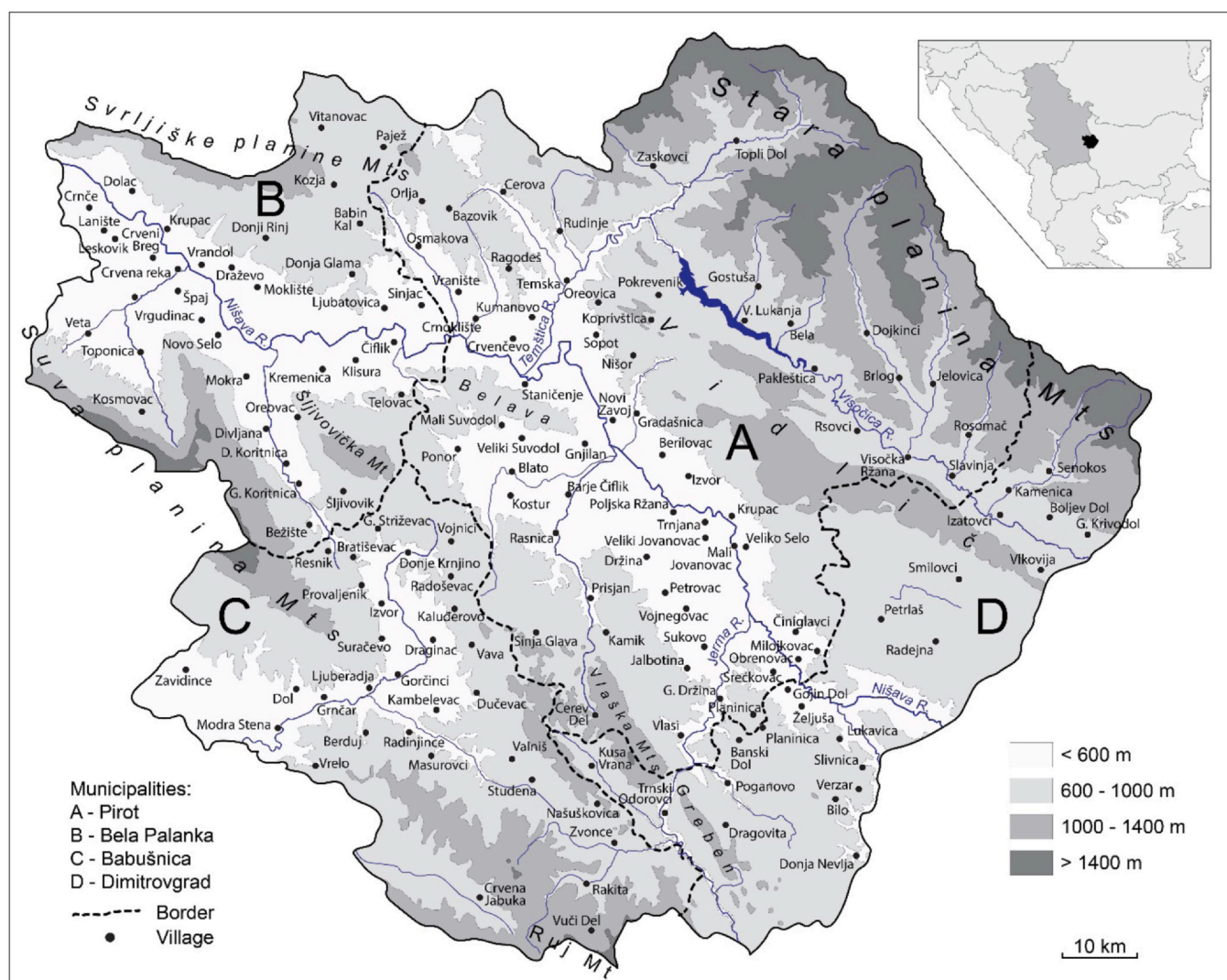
The survey results were systematised in alphabetical order of the scientific name of the plant taxa with additional data: botanical family, voucher specimen, the most common local names, quotation frequency (QF), plant part used, form of application, therapeutic use, method of administration, total number of use reports per species, and use value (UV) of species.

Quotation frequency was calculated for each reported species. It refers to the proportion of the selected species in the total number of reported uses (indications) in the survey (Zlatković et al., 2014).

The UV (Trotter and Logan, 1986), a quantitative calculation showing the relative importance of the species mentioned by informants, was also calculated:  $UV = U/n$ , where UV is the use value of the species; U is the total number of use reports per species; n is the number of informants. The values of UV are close to 0 when there are only a few reports of species use. On the other hand, values are close to 1 when there are many reports of a plant’s use, which indicates that it is important in the studied area. The variance was also calculated for all plants in relation to the total number of respondents and reports of use per species (Weckerle et al., 2018).

The informant consensus factor (FIC) was used to analyse the collected data in the four municipalities and Pirot District. It is the quotient of the number of use reports minus the number of plant taxa used and the number of use reports minus one (Trotter and Logan, 1986). A low value of FIC, close to 0, appears when there are not many reports of a species, and the value can be high, close to 1, when there are many use reports of a species (Matejić et al., 2020). The taxa ratio was also calculated. It is the percentage of reports for the most frequently used plants (Akerreta et al., 2010).

The FIC for nationalities and participant gender for the most common use categories against respiratory tract infections was calculated. The UV matrix for the comparison of two groups of respondents from the Pirot region to different ethnic groups, Bulgarian and Serb, was also presented.



**Fig. 1.** Map of the study area (with mountains and waterbodies highlighted) with villages visited during ethnobotanical interviews in which the local inhabitants mentioned the use of medicinal plants against respiratory infections.

A comparison with previous studies in neighbouring regions was made using the Jaccard and Sørensen similarity indexes. The Jaccard index (JI) was calculated using the following formula:

$$JI = c / 100 / (a + b - c) \text{ (González-Tejero et al., 2008),}$$

and the Sørensen similarity index (QS) was calculated using the formula:

$$QS = 2c / (a + b) \text{ (Sørensen, 1948),}$$

where a is the number of species in area A, b is the number of species in area B, and c is the number of species common to A and B.

#### Use Value Matrix Design

Comparative analysis (Quave and Pieroni, 2015) was performed to determine how the UVs differed in two different and most represented ethnic groups. UVc data for each group were plotted using a standard scatterplot, with group 1 (Bulgarian population) data corresponding to the x-axis and group 2 (Serbian population) data corresponding to the y-axis.

Quadrants were created as an overlay on the scatterplot data. The intersection point was at (UVmax/2), where UVmax corresponded to the maximum UVc value obtained (from either of the two groups studied). For example, if the maximum use value (UVmax) is equal to 1, the quadrants on the x- and y-axes should intersect at (UVmax/2), or 0.5 in this example. If individual informants report multiple uses for the

same species, the UVmax in a data set may be greater than 1.0. The four quadrants were subdivided to obtain a final total of eight regions, numbered I-VIII.

## Results and Discussion

### Quantitative Analyses

The results of the ethnopharmacological survey in Pirot District show that 65 plant taxa are used for practical purposes in treating respiratory infections. The results of the survey are summarised in Table 2, which includes information on each species (scientific name, family, local names, QF, plant part used, use, and administration) with the number of reports, UV of the species, and variance. The higher variance showed that the number of use reports per species was more pronounced. The lower variance demonstrated a lower number of the same use reports for each plant (Table 2). In our study, 21 plant taxa were mentioned for respiratory infections that were not mentioned in published ethnobotanical research in neighbouring regions of the Balkan Peninsula.

Species used in the treatment of respiratory infections in Pirot District were classified into 31 families, with Lamiaceae (14 species), Rosaceae (10 species), and Compositae (6 species) being the most abundant. Families such as Lamiaceae (35.5%, 433 reports),

**Table 2**  
Medicinal plants against respiratory infections reported by respondents in Piroć District.

Latin name, Serbian name (S), Bulgarian name (B), Roma name (R)	Family	Voucher specimen	QF <sup>1</sup> [%]	Part used	Form	Use (number of reports)/ Administration <sup>1</sup>	U <sup>2</sup>	UV <sup>3</sup> <sub>n</sub> = 531	S <sup>2</sup> sample var	Use in neighbouring areas <sup>4</sup>
<i>Acer campestre</i> L. klin (B)	Sapindaceae	14 055	0.08	radix	Decoction	Asthma (1)/1	1	0.002	0.002	*
<i>Achillea clypeolata</i> Sm., žlta mesečina (S), ravnež žlt (B)	Compositae	14 056	0.48	herba	Infusion	Common cold (2)/1	5	0.011	0.009	11
<i>Achillea millefolium</i> L., mesečnjak, bela mesečina, (S), ravnež (B), hajdučica (R)	Compositae	14 057	2.46	herba	Infusion	Cough (3)/1 Bronchial catarrh (1)/1 Asthma (2)/1	30	0.056	0.045	2, 5, 6, 10, 11, 17
<i>Agrimonia eupatoria</i> L., petrovac (S)	Rosaceae	14 061	0.16	herba	Infusion	Bronchitis (1)/1 Chest pain (2)/1 Common cold (16)/1 Cough (5)/1 Sinusitis (1)/1 Sore throat (3)/1	2	0.004	0.003	2, 4, 11, 16
<i>Allium cepa</i> L., crni luk (S)	Amaryllidaceae	14 063	0.08	bulbus	Decoction	Cough (1)/1	1	0.002	0.002	3, 11, 13, 16
<i>Allium ursinum</i> L., sremski (S)	Amaryllidaceae	14 067	0.08	folium	Extract in alcohol	Common cold (1)/1	1	0.002	0.002	3, 13
<i>Althaea officinalis</i> L., beli slez (S, R), bel slez (B)	Malvaceae	14 068	3.60	radix	Macerate in water	Asthma (2)/1	44	0.083	0.065	2, 3, 6, 7, 9, 10, 11, 14, 16
<i>Ballota nigra</i> L., crna kopriiva (S)	Lamiaceae	14 083	0.08	herba	Infusion	Bronchitis (1)/1 Common cold (2)/1 Cough (39)/1	1	0.002	0.002	*
<i>Berberis vulgaris</i> L., žutika, šimsir (S)	Berberidaceae	14 085	0.49	folium	Infusion	Bronchitis/1	1	0.002	0.002	*
<i>Centaurium erythraea</i> Rafin, crven kantarion (S), rozavi kantarion (B)	Gentianaceae	14 096	0.49	herba	Oil extract	Bronchitis (1)/1	5	0.009	0.008	3,11
<i>Chelidonium majus</i> L., rusa (S), lišaivac (B)	Papaveraceae	14 098	0.16	herba	Infusion	Common cold (1)/1 Chest pain (1)/1 Cough (2)/1	2	0.004	0.003	13
<i>Cornus mas</i> L., dren, drenjina (S), drenka (B)	Cornaceae	14 104	0.25	fructus	Decoction	Sore throat (1)/1 Common cold (3)/1	3	0.006	0.005	12
<i>Crataegus pentagyna</i> Waldst. & Kit. ex Willd., crni glog (S)	Rosaceae	14 108	0.16	fructus	Decoction	Common cold (1)/1	2	0.004	0.003	*
<i>Cydonia oblonga</i> Miller, dunja (S)	Rosaceae	14 110	0.25	fructus folium semen	Decoction Infusion Fresh seed	Cough (1)/1 Common cold (1)/1 Cough (1)/1	3	0.006	0.005	11, 12
<i>Drosera rotundifolia</i> L., rosulje (S)	Droseraceae	14 113	0.08	herba	Infusion	Sore throat (1)/1 Tuberculosis (1)/1	1	0.002	0.002	*
<i>Ecballium elaterium</i> (L.) A. Rich., divlje dinjice (S)	Cucurbitaceae	14 115	0.16	fructus	Fresh juice	Sinusitis (2)/E	2	0.004	0.003	*
<i>Eryngium campestre</i> L., magareći trn (S), magareško trnje (B)	Apiaceae	14 120	0.16	herba	Infusion	Cough (2)/1	2	0.004	0.003	*
<i>Euphrasia</i> spp., vidovka (S)	Orobanchaceae	14 121	0.08	herba	Infusion	Cough (1)/1	1	0.002	0.002	*
<i>Fragaria vesca</i> L., šumska jagoda, divlja jagoda (S), diva jagoda (B)	Rosaceae	14 126	0.25	folium	Infusion	Common cold (2)/1 Cough (1)/1	3	0.006	0.005	5, 9, 16

(continued on next page)

Table 2 (continued)

Latin name, Serbian name (S), Bulgarian name (B), Roma name (R)	Family	Voucher specimen	QF <sup>1</sup> [%]	Part used	Form	Use (number of reports)/Administration <sup>1</sup>	U <sup>2</sup>	UV <sup>3</sup> <sub>n</sub> = 531	S <sup>2</sup> sample var	Use in neighbouring areas <sup>4</sup>
<i>Gentiana cruciata</i> L., otodovka (S), otodevka (B)	Gentianaceae	14 132	0.33	herba	Extract in alcohol	Bronchitis (2)/1	4	0.008	0.006	*
<i>Geranium macrorrhizum</i> L., zdravac (S), baštenski zdravac (R)	Geraniaceae	14 134	0.16	herba	Macerate in water Macerate in water Infusion	Common cold (1)/1 Cough (1)/1 Bronchitis (1)/1	2	0.004	0.003	*
<i>Hedera helix</i> L., bršljan (S, B)	Araliaceae	14 137	0.33	folium folium	Extract in alcohol Extract in alcohol	Bronchitis (1)/1 Common cold (1)/1	4	0.008	0.006	8, 14, 15
<i>Hypericum perforatum</i> L., kantarion (S), drenčak, žit kantarion (B)	Hypericaceae	14 142	2.78	herba	Infusion	Cough (3)/1 Asthma (2)/1	34	0.064	0.051	3, 8, 11, 13
<i>Hyssopus officinalis</i> L., izop, miloduh (S)	Lamiaceae	14 143	0.16	herba	Infusion	Bronchitis (3)/1 Common cold (14)/1	2	0.004	0.003	9, 11
<i>Inula helenium</i> L., oman, beli oman (S), oman (R)	Compositae	14 144	0.33	rhizoma	Decoction	Cough (2)/1 Asthma (2)/1	4	0.008	0.006	2, 5, 6, 10, 11, 16
<i>Juglans regia</i> L., orah (S)	Juglandaceae	14 146	0.08	fructus	Fresh fruit	Bronchitis (1)/1	1	0.002	0.002	2, 8, 12, 13
<i>Malva sylvestris</i> L., crni slez (S), crni slez (R)	Malvaceae	14 156	0.33	flos	Infusion	Bronchitis (1)/1	4	0.008	0.006	2, 3, 7, 10, 15
<i>Marrubium peregrynium</i> L., očajnica (S), sosarica (B)	Lamiaceae	14 157	0.16	herba	Infusion	Bronchitis (1)/1	2	0.004	0.003	*
<i>Matricaria chamomilla</i> L., kamilica (S, R), podrumče (B), bela rada (R)	Compositae	14 159	15.72	flos	Infusion	Cough (1)/1 Bronchitis (1)/1	192	0.362	0.212	2, 3, 4, 5, 6, 9, 11, 13, 15, 16
<i>Melissa officinalis</i> L., matičnjak (S)	Lamiaceae	14 160	0.16	folium	Compress Infusion	Common cold (143)/1 Cough (13)/1 Influenza (1) (1)/1 Respiratory diseases (1)/1	2	0.004	0.003	5, 6, 10, 16, 17
<i>Mentha longifolia</i> (L.) L., divlja nana (S)	Lamiaceae	14 161	0.16	herba	Infusion	Sore throat (34)/1 Sinusitis (for inhalation) (1)/E	2	0.004	0.003	3, 7, 8, 10, 16
<i>Mentha x piperita</i> L., pitoma nana, džodžan (S), nana (B)	Lamiaceae	14 162	0.36	folium	Infusion	Common cold (29)/1	41	0.077	0.061	2, 3, 6, 7, 11, 13, 17
				folium folium		Cough (3)/1 Respiratory diseases (1)/1				
				folium		Sore throat (8)/1				(continued on next page)

Table 2 (continued)

Latin name, Serbian name (S), Bulgarian name (B), Roma name (R)	Family	Voucher specimen	QF <sup>1</sup> [%]	Part used	Form	Use (number of reports)/Administration <sup>1</sup>	U <sup>2</sup>	UV <sup>3</sup> n = 531	S <sup>2</sup> sample var	Use in neighbouring areas <sup>4</sup>
<i>Ocimum basilicum</i> L., bosiljak (S), bosiljče (B)	Lamiaceae	14 164	4.83	herba	Infusion	Asthma (1)/1 Common cold (12)/1 Cough (8)/1 Respiratory diseases (3)/1 Sinusitis (for inhalation) (34)/E Sore throat (1)/1 Bronchitis (2)/1	59	0.111	0.085	3, 6, 10, 11, 13, 16, 17
<i>Origanum vulgare</i> L., vraniliova trava, crnovrška (S), crni vrh (B)	Lamiaceae	14 167	1.15	herba	Infusion	Bronchitis (2)/1 Common cold (11)/1 Respiratory diseases (1)/1	14	0.026	0.022	2, 4, 5, 7, 8, 10, 11, 12
<i>Paeonia peregrina</i> Miller, božur (S)	Paeoniaceae	14 168	0.08	flos	Infusion	Asthma (1)/1	1	0.002	0.002	*
<i>Papaver rhoas</i> L., bulka, mak (S)	Papaveraceae	14 171	0.16	flos	Infusion	Asthma (2)/1	2	0.004	0.003	17
<i>Persicaria bistorta</i> (L.) Samp., srčenjak (S)	Polygonaceae	14 181	0.08	rhizoma	Decoction	Cough (1)/1	1	0.002	0.002	*
<i>Plantago major</i> L., bokvica (S), žilovljak (B)	Plantaginaceae	14 178	1.23	folium	Syrup	Bronchitis (2)/1	15	0.028	0.023	2, 3, 4, 5, 6, 9, 11, 13, 14, 17
<i>Primula veris</i> L., jagorčevina (S, R), jagilka (S); jagličje, igličje, žuto jagličje, gorčevina (B), igličje (B, R), igličica (R)	Primulaceae	14 186	4.59	folium folium flos	Infusion Syrup Infusion	Common cold (1)/1 Cough (12)/1 Asthma (5)/1	56	0.105	0.081	1, 5, 6, 9, 10, 11, 12, 16
<i>Prunus spinosa</i> L., trnjina, trn (S) ()	Rosaceae	14 190	0.33	fructus	Decoction	Bronchitis (6)/1 Common cold (5)/1 Cough (40)/1	4	0.008	0.006	*
<i>Pulmonaria officinalis</i> L., plućnjak, medunika (S), meduniče, siskavče (B)	Boraginaceae	14 191	0.82	folium	Infusion	Common cold (4)/1 Cough (10)/1	10	0.019	0.016	3, 10, 11, 13, 16
<i>Pyrus amygdaliformis</i> Vill., divlja kruška ječmenka (S)	Rosaceae	14 192	0.08	fructus	Decoction	Cough (1)/1	1	0.002	0.002	*
<i>Quercus cerris</i> L., hrast cer (S)	Fagaceae	14 194	0.08	succus	Fresh juice from three	Bronchitis (1)/1	1	0.002	0.002	11
<i>Robinia pseudoacacia</i> L., bagrem (S)	Leguminosae	14 196	0.49	flos	Infusion	Bronchitis (2)/1 Common cold (1)/1 Cough (3)/1	6	0.011	0.009	11, 13, 14, 16, 17
<i>Rosa canina</i> L., šipurak, šipak, divlja ruža (S, B, R)	Rosaceae	14 197	5.81	fructus	Decoction	Common cold (68)/1	71	0.134	0.100	1, 2, 5, 6, 7, 8, 10, 11, 12, 13, 16
<i>Rosmarinus officinalis</i> L., ruzmarin (S)	Lamiaceae	14 198	0.16	herba	Infusion	Cough (3)/1 Common cold (1)/1 Cough (1)/1	2	0.004	0.003	*
<i>Rubus caesius</i> L., divlja kupina (S), diva kupina (B)	Rosaceae	14 200	0.82	folium	Infusion	Common cold (2)/1 Cough (6)/1	10	0.019	0.016	*
<i>Rubus idaeus</i> L., malina (S)	Rosaceae	14 201	0.08	fructus	Fresh fruit	Sore throat (2)/1	1	0.002	0.002	4, 6, 13, 16
<i>Sabzia nemorosa</i> L., govnjarnik (B)	Lamiaceae	14 206	0.08	folium	Infusion	Cough (1)/1 Sore throat (1)/1	1	0.002	0.002	*

(Continued on next page)

Table 2 (continued)

Latin name, Serbian name (S), Bulgarian name (B), Roma name (R)	Family	Voucher specimen	QF <sup>1</sup> [%]	Part used	Form	Use (number of reports)/Administration <sup>1</sup>	U <sup>2</sup>	UV <sup>3</sup> n = 531	S <sup>2</sup> sample var	Use in neighbouring areas <sup>4</sup>
<i>Salvia officinalis</i> L., žalfija (S), kaloper (B)	Lamiaceae	14 207	1.8	folium	Infusion	Common cold (2)/E Cough (3)/E Cough (1)/E Respiratory diseases (1)/E Sinusitis (for inhalation) (3)/E Sore throat (12)/E Bronchitis (10)/1	22	0.041	0.034	1, 3, 6, 10, 11, 13, 14, 16
<i>Sambucus nigra</i> L., zova (S), bz (B)	Adoxaceae	14 210	8.76	flos	Infusion	Common cold (65)/1 Cough (30)/1 Respiratory diseases (2)/1	107	0.203	0.141	2, 3, 4, 5, 6, 8, 9, 10, 11, 13, 15
<i>Saponaria officinalis</i> L., sapunjača (S)	Caryophyllaceae	14 213	0.08	herba	Infusion	Cough (1)/1	1	0.002	0.002	7
<i>Satureja montana</i> L., rtański čaj (S, B), dđpikur (B)	Lamiaceae	14 214	4.01	herba	Infusion	Asthma (1)/1	49	0.092	0.072	11, 11, 15, 16
<i>Scilla bifolia</i> L., plavo oko (S)	Asparagaceae	14 215	0.08	bulbus	Compress	Bronchitis (5)/1 Common cold (24)/1 Cough (6)/1	1	0.002	0.002	*
<i>Senperivium tectorum</i> L., čuvarkuća (S)	Crassulaceae	14 218	0.16	folium	Fresh leaf	Respiratory diseases (10)/1 Sore throat (3)/1	2	0.004	0.003	11
<i>Sorbus domestica</i> L., oskoruša (S)	Rosaceae	14 221	0.08	fructus	Decoction	Sore throat (1)/1	1	0.002	0.002	*
<i>Stellaria graminea</i> L., bela mišjakinja (B)	Caryophyllaceae	14 223	0.16	herba	Infusion	Cough (1)/1	2	0.004	0.003	*
<i>Taraxacum campyloides</i> G.E. Haglund, maslačak (S, B)	Compositae	14 224	0.41	flos	Syrup	Cough (2)/1 Bronchitis (2)/1	5	0.009	0.008	9, 11
<i>Teucrium chamaedrys</i> L., podubica (S), parabica (B)	Lamiaceae	14 228	0.16	radix herba	Decoction Syrup Decoction Infusion	Common cold (1)/1 Cough (1)/1 Cough (1)/1 Chest pain (2)/E	2	0.004	0.003	17
<i>Thymus</i> spp., dušičina (S), babina dušica (B)	Lamiaceae	14 290	19.16	herba	Infusion	Asthma (2)/1 Bronchitis (11)/1 Common cold (149)/1 Cough (59)/1 Cough (1)/1 Respiratory diseases (5)/1 Sore throat (7)/1	234	0.441	0.234	3, 4, 5, 6, 7, 9, 10, 11, 16, 17

(continued on next page)

Table 2 (continued)

Latin name, Serbian name (S), Bulgarian name (B), Roma name (R)	Family	Voucher specimen	QF <sup>1</sup> [%]	Part used	Form	Use (number of reports)/ Administration <sup>1</sup>	U <sup>2</sup>	UV <sup>3</sup> n = 531	S <sup>2</sup> sample var	Use in neighbouring areas <sup>4</sup>
<i>Tilia cordata</i> Miller, lipa (S, B, R)	Malvaceae	14 231	7.04	flos	Infusion	Asthma (1)/1	86	0.162	0.118	1, 6, 7, 9, 11, 12, 16, 17
<i>Tussilago farfara</i> L., podbel (S, B)	Compositae	14 233	3.03	flos	Infusion	Bronchitis (2)/1 Common cold (70)/1 Cough (9)/1 Sinusitis (for inhalation) (2)/E Sore throat (2)/1 Asthma (6)/1	37	0.070	0.005	1, 3, 4, 5, 6, 10, 11, 13, 15, 16, 17
<i>Urtica dioica</i> L., kopriiva (S)	Urticaceae	14 236	0.16	flos	Compress Infusion	Bronchitis (6)/1 Cough (23)/1 Cough (1)/E Cough (1)/1				
<i>Verbascum</i> spp., divizma (S)	Scrophulariaceae	14 241	0.39	folium folium flos	Infusion Infusion	Sore throat (1)/1 Common cold (2)/1 Cough (4)/1	2 4	0.004 0.008	0.003 0.006	3, 10, 13, 14 2, 3, 4, 7, 10, 11, 13, 15
<i>Viola odorata</i> L., ljubičica (S)	Violaceae	14 245	0.49	flos radix radix flos	Infusion Decoction Infusion	Bronchitis (1)/1 Bronchitis (1)/1 Common cold (2)/1 Cough (2)/1	6	0.011	0.009	3, 4, 11, 13, 16

Symbol (\*) indicates that use not mentioned in previously conducted ethnobotanical field studies in surrounding regions (1–17).

<sup>1</sup> QF, quotation frequency; administration codes: I, Internal; E, External.

<sup>2</sup> U: total number of use reports per species.

<sup>3</sup> UV: use-value of species.

<sup>4</sup> Numbers refer to the following investigation regions (references): 1. Northern Albanian Alps (Pieroni et al., 2005), 2. Kopaonik Mt. (Jarić et al., 2007), 3. Middle, south and west Bosnia and Herzegovina (Šarić Kundalić et al., 2010), 4. Prokletije Mts. (Menković et al., 2011), 5. Southwestern Serbia–Pešter Plateau, Sandžak (Pieroni et al., 2011), 6. Zlatibor district (Šavikin et al., 2013), 7. Macedonia–Sharr Mts. (Rexhepi et al., 2013), 8. Eastern Albania–Peshkopia (Pieroni et al., 2014), 9. Rtanj Mt. (Zlatković et al., 2014), 10. South Kosovo (Mustafa et al., 2015), 11. Suva Planina Mts. (Jarić et al., 2015), 12. Eastern Albania–Raicë and Mokra (Pieroni et al., 2015), 13. Northeastern Bosnia and Herzegovina (Šarić-Kundalić et al., 2016), 14. Negotin Krajina (Janačković et al., 2019), 15. Northern Greece–Edessa, Naoussa (Tsiotsiou et al., 2019), 16. Svirijig region (Matejić et al., 2020), 17. Timok region (Matejić et al., 2020).

### FAMILIES - THE MOST FREQUENTLY REPORTED

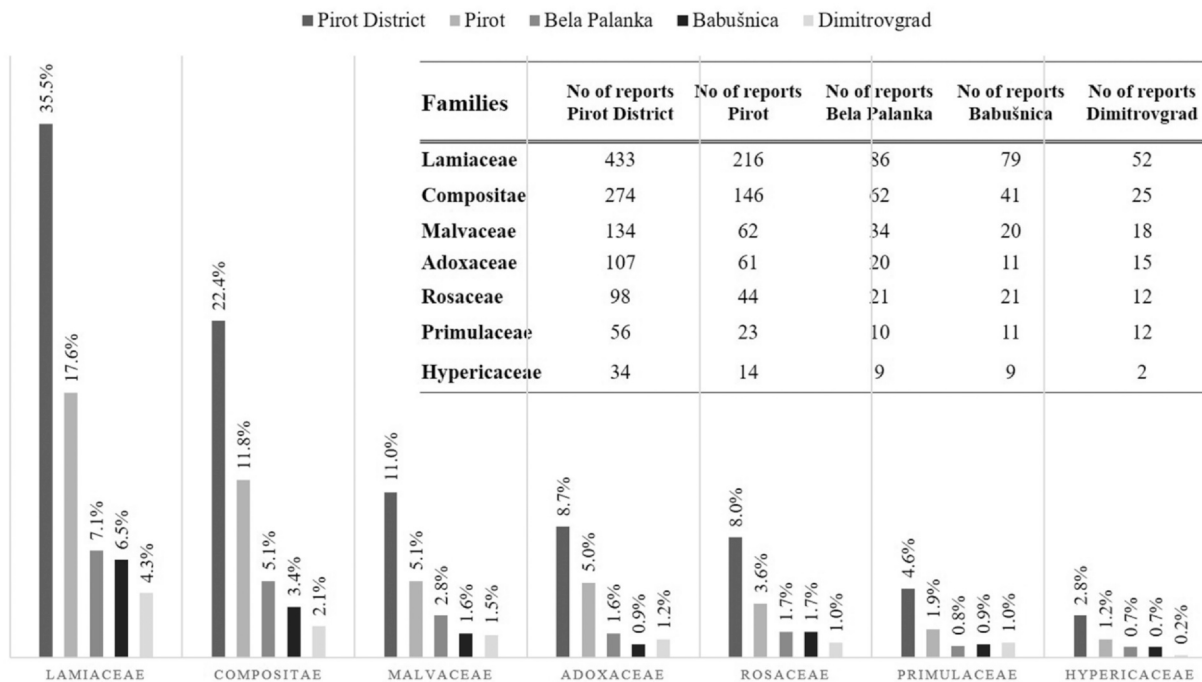


Fig. 2. Comparative analysis of the most frequently reported families of medicinal plants used against respiratory infections in four municipalities of Pirot District.

### PART OF PLANTS - THE MOST FREQUENTLY REPORTED

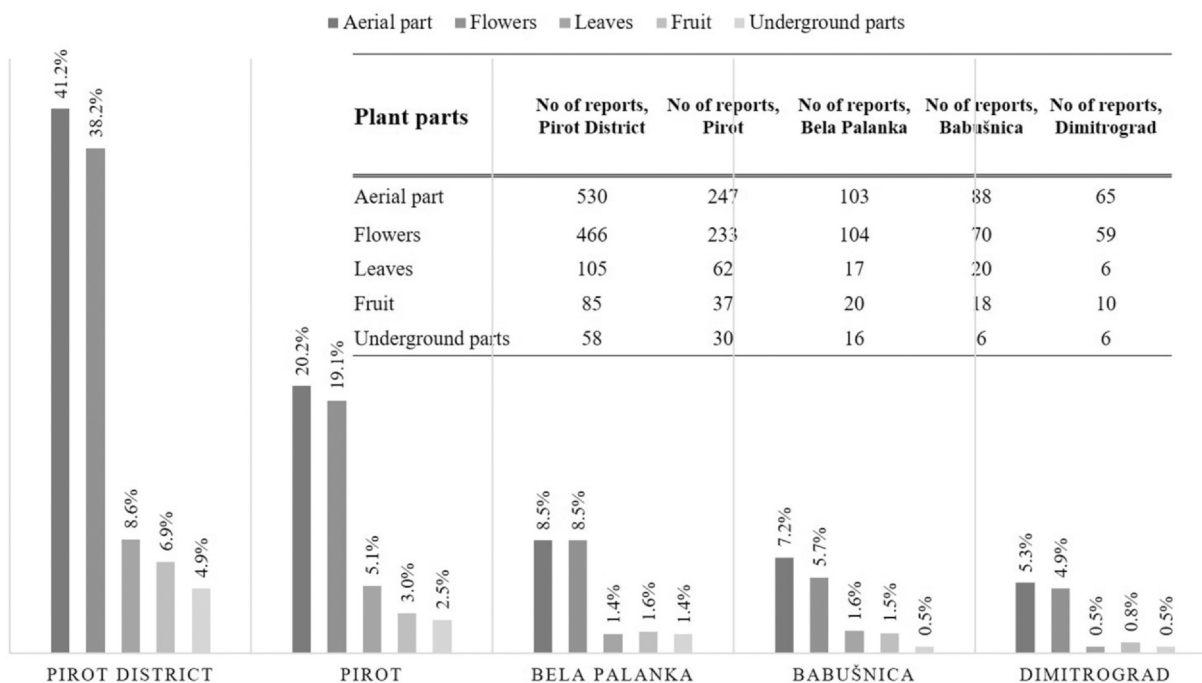


Fig. 3. Comparative analysis of the most frequently reported parts of medicinal plants against respiratory infections in four municipalities of Pirot District.

Compositae (22.4%, 274 reports), Malvaceae (10.9%, 134 reports), Adoxaceae (8.7%, 107 reports), and Rosaceae (8.0%, 98 reports) were the most frequently cited (Fig. 2).

Analysis of the growth forms of medicinal plants used against respiratory tract infections revealed that the majority were herbaceous plants (73.8%), followed by trees (15.4%) and shrubs (10.8%). The most used plant parts were the aerial part (41.2%, 530 reports), floral structures (38.2%, 466 reports), leaves (8.6%, 105 reports), fruits

(6.9%, 85 reports), and underground parts (4.9%, 58 reports) (Fig. 3). Seeds and fresh juice of three (0.16% each) were used in a smaller proportion.

The plants used for respiratory infections are prepared and administered in various forms. Water extraction (the preparation form of tea) was by far the most common preparation—infusions (85.4%, 1 043 reports), decoctions (7.7%, 94 reports), and macerates (3.8%, 46 reports) (Fig. 4). In smaller proportions, syrup (1.3%, 16 reports), extract

## PREPARATION FORMS - THE MOST FREQUENTLY REPORTED

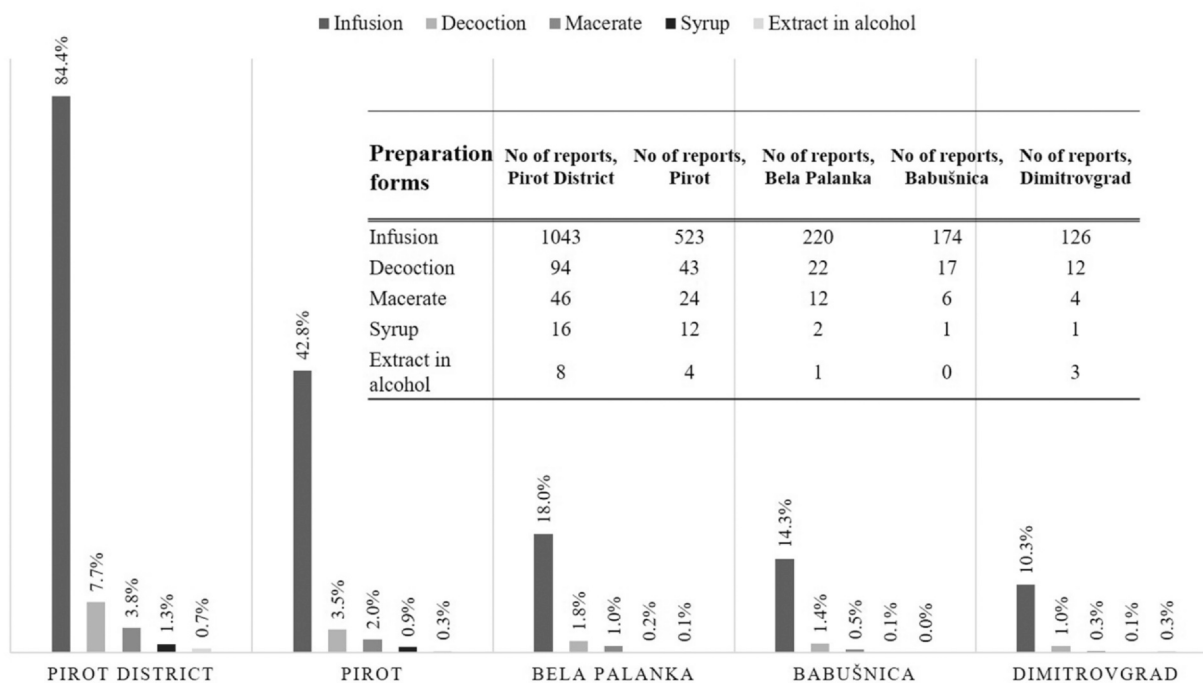


Fig. 4. Comparative analysis of the most common forms of preparation of medicinal plants against respiratory infections in four municipalities of Pirot District.

in alcohol (0.7%, 8 reports), compress, fresh fruit and fresh juice (0.3% each, 3 reports each), fresh leaves and fresh seeds (0.2% each, 2 reports each), and oil extract (0.1%, 1 report) were recorded.

Of the mode of administration, 94.7% (1 156 reports) were taken orally, and 5.3% (65 reports) were applied externally. Individuals used the following plants for external application: *Ecballium elaterium* (2 reports), *Melissa officinalis* (1 report), *Ocimum basilicum* (34 reports), *Salvia officinalis* (22 reports), *Scilla bifolia* (1 report), *Teucrium chamaedrys* (2 reports), *Tilia cordata* (2 reports), and *Tussilago farfara* (1 report). All other plants were used for oral intake.

#### Predominantly Used Plants for Treating Respiratory Infections

Respondents from Pirot District usually used species of the genus *Thymus* (*Thymus* spp.) (234 reports, 19.2%), *Matricaria chamomilla* (193 reports, 15.7%), and *Sambucus nigra* (108 reports, 8.8%) to treat respiratory infections. The ethnomedicinal use of species of the genus *Thymus* in Pirot District was already described by Marković et al. (2020), where the authors determined the following plant taxa: *Thymus longicaulis* C. Presl., *Thymus praecox* Opiz. subsp. *jankaе* (Čelak) Jalas, *Thymus praecox* Opiz. subsp. *polytrichus* (A. Kern. ex Borbas), *Thymus pulegioides* L. subsp. *panonicus* (All.) Kerguelen, *Thymus pulegioides* L. subsp. *pulegioides*, *Thymus odoratissimus* Mill., and *Thymus striatus* Vahl. Moreover, the authors reported that respondents in Pirot District were not aware of the morphological differences among the different species of the genus *Thymus*. Therefore, their use in folk medicine can be interpreted identically.

The most used plant taxa in each region and the most common categories of use against respiratory infections are listed in Table 3. Plants were most often used to treat colds (640 reports, 52.4%), coughs (330 reports, 27.0%), sore throats (80 reports, 6.6%), bronchitis (67 reports, 5.5%), sinusitis (43 reports, 3.5%, of which 40 reports were for inhalation), and asthma (28 reports, 2.3%). The least frequently reported categories of use were respiratory diseases in general without knowledge of specific application (25 reports, 2.1%), chest pain (6 reports, 0.5%), bronchial catarrh (1 report, 0.1%), and tuberculosis (1 report, 0.1%).

The following text includes the number of reports for the plant taxa that respondents used most frequently. The most frequently used taxa for treating the common cold in the four municipalities of the Pirot District were *Thymus* spp. (149), *Mat chamomilla* (143), *Ti cordata* (70), and *Sam nigra* (65). Respondents in the four municipalities mainly reported using *Thymus* spp. (60) *Primula veris* (40), *Althaea officinalis* (39), and *Sambucus nigra* (30) for cough, the typical symptom of respiratory infections. *Pri veris* (40), *Alt officinalis* (39), and *Sam nigra* (30).

The most used plant taxa for respiratory ailments without specific application were *Sat montana* (10), *Thymus* spp. (5), and *O basilicum* (3), *Pri veris* (2), and *Sam nigra* (2). *Achillea millefolium* (2), *Te chamaedrys* (2), *Centaureum erythraea* (1), and *Sc bifolia* (1) were used to treat chest pain. Bronchial catarrh is treated with *Ach clypeolata* (1) and tuberculosis with *Drosera rotundifolia* (1).

The population survey analysis showed that the rural population of the Pirot District is more likely to use medicinal plants to treat respiratory diseases than to seek help from healthcare facilities. Also, residents of the four municipalities mainly use the same herbal medicines to treat colds, coughs, sore throats, sinusitis, and asthma (Table 3). On the other hand, the residents of the different municipalities often use different herbal medicines to treat bronchitis and asthma. In the Pirot municipality, bronchitis is mainly treated with *Thymus* spp., *Tu farfara*, and *Sam nigra*, in Bela Palanka municipality with *Sam nigra*, *Sat montana*, and *Thymus* spp., in Babušnica municipality with *Pri veris*, in Dimitrovgrad municipality with *Sam nigra*, *Gentiana cruciata*, *Pri veris*, and *Sat montana*. In the municipality of Pirot, asthma is treated mainly with *Tu farfara*, *Inula helenium*, *Pri veris*, and *Thymus* spp., in Bela Palanka municipality with *Papaver rhoeas* and *Tu farfara*, in Babušnica municipality with *Pri veris*, and Dimitrovgrad municipality with *Pri veris*, *Acer campestre*, *Ach millefolium*, and *Alt officinalis*.

#### Informant Consensus Factor for Most Frequent Applications by Nationality and Gender

The FIC, considering nationality and gender for the most common applications, is shown in Supplementary Table 4. The highest consensus

**Table 3**  
Plant taxa most employed in each region and most frequently applications against respiratory infections.

Application	Municipality	Reports	Reports ratio [%]	Number of taxa	FIC <sup>1</sup>	Taxa most used (number of reports)	Taxa ratio [%]
Common cold	Pirot	310	25.4	24	0.93	<i>Thymus</i> spp. (82), <i>Matricaria chamomilla</i> (71), <i>Sambucus nigra</i> (30)	61.0
	Bela Palanka	149	12.2	22	0.86	<i>Mat chamomilla</i> (32), <i>Thymus</i> spp. (29), <i>Tilia cordata</i> (20)	54.4
	Babušnica	113	9.3	18	0.84	<i>Mat chamomilla</i> (25), <i>Thymus</i> spp. (19), <i>Rosa canina</i> (17)	54.0
	Dimitrovgrad	68	5.6	9	0.88	<i>Thymus</i> spp. (19), <i>Mat chamomilla</i> (15), <i>Rosa canina</i> (11)	66.1
	Pirot (District)	640	52.4	33	0.95	<i>Thymus</i> spp. (149), <i>Mat chamomilla</i> (143), <i>Ti cordata</i> (70)	56.6
Cough	Pirot	183	15.0	38	0.80	<i>Thymus</i> spp. (28), <i>Althaea officinalis</i> (21), <i>Sam nigra</i> (21)	38.3
	Bela Palanka	60	4.9	23	0.63	<i>Thymus</i> spp. (13), <i>Alt officinalis</i> (10), <i>Primula veris</i> (7)	50.0
	Babušnica	43	3.5	17	0.62	<i>Thymus</i> spp. (10), <i>Pri veris</i> (6), <i>Alt officinalis</i> (5)	48.8
	Dimitrovgrad	44	3.6	15	0.67	<i>Thymus</i> spp. (9), <i>Pri veris</i> (8), <i>Sam nigra</i> (6)	52.3
	Pirot (District)	330	27.0	45	0.87	<i>Thymus</i> spp. (60), <i>Pri veris</i> (40), <i>Alt officinalis</i> (39)	42.1
Sore throat	Pirot	34	2.8	11	0.70	<i>Mat chamomilla</i> (17), <i>Mentha x piperita</i> (3)	58.8
	Bela Palanka	20	1.6	6	0.74	<i>Mat chamomilla</i> (10), <i>Sabia officinalis</i> (4)	70.0
	Babušnica	18	1.5	7	0.65	<i>Mat chamomilla</i> (5), <i>Sal officinalis</i> (5), <i>Mentha x piperita</i> (3)	72.2
	Dimitrovgrad	8	0.7	7	0.14	<i>Mat chamomilla</i> (2)	25.0
	Pirot (District)	80	6.6	15	0.82	<i>Mat chamomilla</i> (34), <i>Sal officinalis</i> (12), <i>Mentha x piperita</i> (8)	67.5
Bronchitis	Pirot	28	2.3	14	0.52	<i>Thymus</i> spp. (7), <i>Sam nigra</i> (4), <i>Tussilago farfara</i> (4)	53.6
	Bela Palanka	16	1.3	13	0.20	<i>Sam nigra</i> (2), <i>Satureja montana</i> (2), <i>Thymus</i> spp. (2)	37.5
	Babušnica	8	0.7	8	0.13	<i>Pri veris</i> (2)	22.2
	Dimitrovgrad	14	1.2	9	0.38	<i>Sam nigra</i> (3), <i>Genitana cruciata</i> (2), <i>Pri veris</i> (2), <i>Sat montana</i> (2)	64.3
	Pirot (District)	67	5.5	25	0.63	<i>Thymus</i> spp. (11), <i>Sam nigra</i> (10), <i>Pri veris</i> (6), <i>Tu farfara</i> (6)	49.3
Sinusitis	Pirot	23	1.9	4	0.95	<i>Ocimum basilicum</i> (18), <i>Sal officinalis</i> (3)	91.3
	Bela Palanka	8	0.7	2	0.71	<i>O basilicum</i> (6), <i>Ti cordata</i> (1), <i>Echallium elaterium</i> (1)	100.0
	Babušnica	10	0.8	2	0.89	<i>O basilicum</i> (9), <i>Melissa officinalis</i> (1)	100.0
	Dimitrovgrad	2	0.2	2	0.00	<i>O basilicum</i> (1), <i>Ti cordata</i> (1)	100.0
	Pirot (District)	43	3.5	6	0.88	<i>O basilicum</i> (34), <i>Sal officinalis</i> (3), <i>Ec elaterium</i> (2), <i>Ti cordata</i> (2)	95.4
Asthma	Pirot	20	1.6	12	0.42	<i>Tu farfara</i> (5), <i>Inula helenium</i> (2), <i>Pri veris</i> (2), <i>Thymus</i> spp. (2)	55.0
	Bela Palanka	2	0.2	2	0.00	<i>Papaver rhoeas</i> (1), <i>Tu farfara</i> (1)	100.0
	Babušnica	1	0.1	1	0.00	<i>Pri veris</i> (1)	100.0
	Dimitrovgrad	5	0.4	4	0.25	<i>Pri veris</i> (2), <i>Acer campestre</i> (1), <i>Achillea millefolium</i> (1), <i>Alt officinalis</i> (1)	100.0
	Pirot (District)	28	2.3	13	0.56	<i>Tu farfara</i> (6), <i>Pri veris</i> (5)	39.3

<sup>1</sup> FIC, Informant consensus factor.

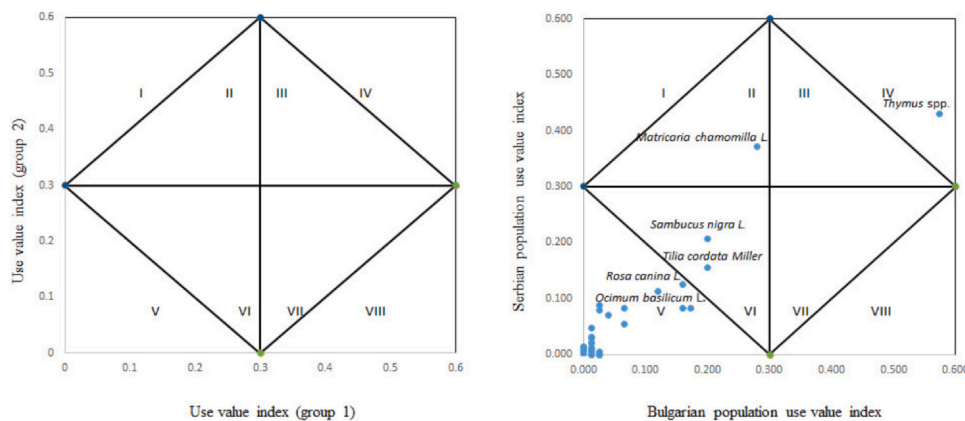


Fig. 5. Use value matrix for comparison of two groups of respondents from the Pirot District who belong to different ethnic groups, namely Bulgarians and Serbs.

(FIC > 0.90) was among Serbs for the common cold. The Serbian population had a greater consensus than the Bulgarian and Roma populations for all application categories, indicating that the Serbs have greater knowledge of plants for respiratory infections.

Generally, men had greater consensus for the common cold, while women had greater consensus for cough, sore throat, bronchitis, sinusitis, and asthma. Women of Serbian and Bulgarian nationality knew a greater variety of plant taxa for the common cold than men. However, the FIC and the number of use reports were lower for women than men. The number of plant taxa used for cough was higher than the number of taxa used for the common cold, although the FIC and the number of use reports for cold were higher. Men are generally better informed about plant taxa for cough and asthma than women, while women know a greater variety of plant taxa for the common cold than men.

#### Use Value Matrix Analysis

UVc data for two of the most represented groups of nationalities are given in Figure 5 as group 1, the Bulgarian population (data on the x-axis), and group 2, the Serbian population (data on the y-axis).

Each quadrant represents the specific relationship between the plant UVs. Values in quadrant I represent a high UVc index according to informants from group 2, otherwise, a low or no value for group 1. Quadrants II and III, VI, and VII are the areas with intermediate UVc indices. The plant taxa found in quadrant IV have a high UV for both groups. In contrast, all values from quadrant V represent moderate values for both groups. High values for group 1 but low or no values for group 2 are found in quadrant VIII. The UV indices presented in the diagram compare plant rankings for the Bulgarian (group 1) and Serbian (group 2) populations. There are no taxa with value in quadrant I. *Mat chamomilla* has intermediate significance. The value is in quadrant II with a higher UV index for Serbian respondents and a lower one for Bulgarian, *Sam nigra*, and *Ti cordata* with the values in quadrant VI. *Thymus* spp. with the value in quadrant IV has important use by both groups of respondents. Among the herbs with moderate use, *Rosa canina* and *O basilicum* can be considered often-used species.

#### Comparative Ethnopharmacological Analysis

In the last two decades, intensive ethnobotanical research has been conducted in the Balkans (Matejić et al., 2020). According to comparative analysis of the researched area with previous research by Janačković et al. (2019), Jarić et al. (2007, 2015), Matejić et al. (2020), Menković et al. (2011), Mustafa et al. (2015), Pieroni et al. (2005, 2011, 2014, 2015), Rexhepi et al. (2013), Šarić Kundalić et al. (2010), Šarić-Kundalić et al. (2016), Šavikin et al. (2013), Tsiotsiou et al. (2019), and Zlatković et al. (2014), it was found that the population of the Balkan Peninsula uses 230 herbal medicines

for the treatment of diseases of the respiratory system, with taxa belonging to 61 families (Supplementary Table 5).

Ethnopharmacological studies can be used to discover new uses for medicinal plants that can form the basis for new drugs (Šavikin et al., 2013). Informants in our study reported using 21 plant taxa that had not been mentioned in any previous studies in the neighbouring Balkan regions. These plant taxa include *Ace campestre*, *Eryngium campestre*, *Berberis vulgaris*, *Stellaria graminea*, *Ec elaterium*, *D rotundifolia*, *Gen cruciata*, *Geranium macrorrhizum*, *Ballota nigra*, *Marrubium peregrinum*, *Rosmarinus officinalis*, *Sal nemorosa*, *Sc bifolia*, *Paeonia peregrina*, *Persicaria bistorta*, *Crataegus pentagyna*, *Prunus spinosa*, *Pyrus amygdaliformis*, *Rubus caesius*, *Sorbus domestica*, and *Euphrasia* spp.

The following text contains plant taxa in Pirot District in terms of forms of preparation for the treatment of respiratory system diseases. It compares previous studies from neighbouring regions of the Balkan Peninsula. Most of the plants, that is, 47 plant species, are used as infusions (Table 2), prepared in the following way: boiling water is poured over dried and chopped medicinal plants and left to stand for some time. From this number, 12 plant taxa were not typical for use against respiratory diseases in the neighbouring regions on the Balkan Peninsula, namely, *Ba nigra*, *Be vulgaris*, *D rotundifolia*, *Er campestre*, *Euphrasia* spp., *Ger macrorrhizum*, *Mar peregrinum*, *Pa peregrina*, *Rosmarinus officinalis*, *Ru caesius*, *Sal nemorosa*, and *St graminea*.

Decoctions are prepared from 12 plant species from the investigated region. Local people prepare decoctions from the underground parts or the fruits (six species each) of plants, which are boiled in water for 5–10 minutes, filtered, and then drunk. The following plants are prepared in the form of decoctions using the underground parts: *Ace campestre*, *Allium cepa*, *I helenium*, *Pe bistorta*, *Taraxacum campyloides*, and *Viola odorata*, while the fruits of the following are used for decoctions: *Cornus mas*, *Cr monogyna*, *Pr spinosa*, *Py amygdaliformis*, *Rosa canina*, and *So domestica*. The above ethnobotanical studies conducted in the surrounding regions do not mention seven plants used to treat respiratory problems. The use of the underground parts of *Ace campestre* and *Pe bistorta* and the fruits of *Cr pentagyna*, *Pr spinosa*, *Py amygdaliformis*, and *So domestica* as decoctions was mentioned in our study but not in other studies from the Balkan Peninsula.

In the traditional medicine of Pirot District, cold macerations of two plants are used to treat respiratory diseases (cough, cold, and bronchitis): the rhizome of *Alt officinalis* (44 use reports) and aerial parts of *Gen cruciata* (2 reports). Boiled water is allowed to cool and is then poured over the rhizome of *Alt officinalis* or aerial parts of *Gen cruciata*. After 2 hours, the preparations are ready for use. Respondents knew that heating the rhizome of *Alt officinalis* or the aerial parts of *Gen cruciata* reduced the active ingredients. *Alt officinalis* was mentioned in most of the above studies in the Balkans for treating respiratory diseases (Supplementary Table 4), while *Gen cruciata* was not.

Syrup for cough and bronchitis in Pirot District is prepared from two plant species: leaves of *Plantago major* (15 reports) and flowers of *Ta campyloides* (2 reports). It is prepared by adding about 1.5 kg of sugar to a glass bottle of strained tea and then boiling for 1 hour. *Pl major* and *Ta campyloides* have been mentioned in previous studies in the Balkans to treat respiratory system diseases (Supplementary Table 4). The leaves of the plant species *All ursinum*, *Hedera helix*, *Sal officinalis*, and the aerial parts of *Gen cruciata* and *Thymus* spp. are soaked in alcohol to form the alcohol extracts used for coughs, colds, and lung diseases. *Gen cruciata* has not been mentioned in neighbouring regions for use against respiratory diseases.

The people of Pirot District prepare compresses from three plant species (*Me officinalis*, *Sc bifolia*, and *Tu farfara*) in the following way: the whole or finely chopped plant is washed thoroughly, then placed on the painful area and covered with a piece of cloth. The use of bulbs of the early-spring plant species *Sc bifolia* for external treatment of chest pain was not mentioned in the neighbouring regions of the Balkan Peninsula. In our study, informants mentioned using fresh fruits of two plant species: *Ru caesius* for sore throats and coughs and *Juglans regia* for bronchitis. *Ru caesius* was not mentioned in studies from surrounding regions. Our respondents mentioned using the fresh juice of two plant species: *Quercus cerris* for bronchitis and *Ec elaterium* for sinusitis. The use of *Ec elaterium* was not reported in other Balkan Peninsula studies.

Two respondents reported the use of fresh leaves of *Sempervivum tectorum* for bronchitis and sore throat. The use of the same plant species against respiratory problems was mentioned by Jarić et al. (2015) only for Mt. Suva Planina for the treatment of otitis and herpes. In our study, the fresh seed of *Cydonia oblonga* was mentioned by respondents for the treatment of common cold, cough, and sore throat. Similar use of *Cy oblonga* was also found on Mt. Suva Planina (Jarić et al., 2015) and in eastern Albania (Pieroni et al., 2015). In our study, the aerial part of *Ce erythraea* was soaked in vegetable oil to produce an oil extract used for bronchitis. A similar use of *Ce erythraea* was also mentioned by informants from Mt. Suva Planina against cold (Jarić et al., 2015) and in Bosnia and Herzegovina (Šarić Kundalić et al., 2010) for the treatment of cough, bronchitis, and asthma. A similar use of the plant species mentioned above (*Se tectorum*, *Cy oblonga*, and *Ce erythraea*) against respiratory diseases, noted for Pirot District and Mt. Suva Planina, is probably due to the proximity of these two study areas in Southeastern Serbia.

We compared our results with previously published data from neighbouring regions by calculating Jaccard's similarity coefficient (JI) and Sørensen's similarity index (QS). Comparative analysis of data from medicinal plants used against respiratory diseases and other regions of the Balkans showed that there are remarkable similarities between Pirot District and Mt. Suva Planina (JI = 36.59, QS = 0.54) and Pirot District and the Svrlijig region (JI = 26.19, QS = 0.42) (Supplementary Table 6), which was expected, given the proximity of these two areas in Southeastern Serbia. The more accessible communication compared to other regions could influence knowledge sharing.

The Jaccard index of similarity (JI) between the use of medicinal plants against respiratory problems in the Pirot District and neighbouring regions ranged from 8.33 to 36.59, and the Sørensen similarity index (QS) ranged from 0.15 to 0.54. The increasing distance between the studied areas is probably the reason for these differences. Nevertheless, the similarity of research data between Pirot District and Negotin Krajina (JI = 8.33, QS = 0.15) (Supplementary Table 6) should be higher in this sense, considering the proximity of these two investigated areas. Despite the proximity of the research areas, the real reason for the low similarity may be that the number of respondents in these two studies differed significantly. Our study had 631 respondents, and the study in Negotin Krajina had only 34 respondents, as shown in Supplementary Table 6. Therefore, there are significant differences in the Jaccard and Sørensen similarity index values even though these two areas are geographically close.

In Pirot District, the following 21 plant species were found to be used to treat respiratory diseases: *Ace campestre*, *Er campestre*,

*Be vulgaris*, *St graminea*, *Ec elaterium*, *D rotundifolia*, *Gen cruciata*, *Ger macrorrhizum*, *Ba nigra*, *Mar peregrinum*, *Rosmarinus officinalis*, *Sal nemorosa*, *Sc bifolia*, *Pa peregrina*, *Pe bistorta*, *Cr pentagyna*, *Pr spinosa*, *Py amygdaliformis*, *Ru caesius*, *So domestica*, and *Euphrasia* spp. At the same time, respondents from neighbouring regions did not report using these plants to treat the same systemic problems, indicating the great diversity of medicinal plants in the studied area compared to other areas on the Balkan Peninsula. Some of them have different uses elsewhere. These are not endemic to this region, but seven are protected by national legislation. *Gen cruciata*, *Ger macrorrhizum*, *Pe bistorta*, *Cr pentagyna*, *Euphrasia* spp., *D rotundifolia*, and *Pa peregrina* are protected taxa according to the regulation 'Code on declaration and protection of strictly protected and protected wild species of plants, animals, and fungi' (Official Gazette of the Republic of Serbia, 2010). According to this legislation, *D rotundifolia* and *Pa peregrina* are strictly protected species and endangered plants of Serbian flora. They almost exclusively cannot be collected and used for any purpose (Stevanović, 1999).

Twenty-one of the taxa mentioned could be potential sources of new medicines for respiratory system diseases, but their uses could be misinterpreted in the transmission of indigenous knowledge. Evidence (laboratory experiments, clinical trials, etc.) shows that 11 of the 21 taxa are responsible for beneficial effects on human health during respiratory infections. Some of these plants have shown inhibitory effects on organisms that cause respiratory infections. At the same time, some are examples of different mechanisms of action, such as anti-inflammatory, antitussive, or antipyretic.

1. *Be vulgaris* is used to ease respiratory tract inflammation, such as pharyngitis, sinusitis, rhinitis, bronchitis, and tuberculosis (Ivancheva et al., 2006).
2. Treatment with *Ec elaterium* may have beneficial effects on lung injuries and therefore has the potential for clinical use (Demir et al., 2016). As in our study, the fresh juice of *Ec elaterium* has been used externally in folk medicine in Turkey to treat sinusitis (Yesilada et al., 1988).
3. Arruda-Silva et al. (2021) investigated the biological functions of *D rotundifolia* *in vitro* after the treatment of bronchial epithelial cells, which are the potential targets of the pharmacological effects of herbal medicine.
4. *Ger macrorrhizum* has been screened for its anti-influenza activity. Essential oil from *G. macrorrhizum* has been used as a complementary and alternative treatment for influenza (Setzer, 2016).
5. Rosmarinic acid from *Rosmarinus officinalis* has therapeutic potential in treating or preventing bronchial asthma (Al-Seretti et al., 1999).
6. According to Ivanova et al. (2011) and Kunduhoglu et al. (2011), *Pa peregrina* was among the most active plants that showed a spectrum of antimicrobial activity against the tested microorganisms. It is, therefore, a potentially good source of antimicrobial agents.
7. Ethanol extracts of *Pe bistorta* exhibited antivirulence activity against the medically important opportunistic pathogen *Pseudomonas aeruginosa* (Jovanović et al., 2020), which can cause respiratory tract infections that usually occur in patients with chronic lung disease.
8. The fruits of *Pr spinosa* are used mainly for mild inflammation of the oral and pharyngeal mucosa, and the flowers are a component of compound herbal formulations traditionally used to treat respiratory tract diseases (Marchelak et al., 2017).
9. The leaves of *Ru caesius* are used in Middle Asia for acute respiratory diseases and angina (Bussmann et al., 2020).
10. Ethnomedicine recommends using species from the genus *Sorbus* against respiratory system diseases (Sołtys et al., 2020). According to Güven et al. (2020), *So domestica* showed antimicrobial, bronchial/vasorelaxant properties.

11. Species of the genus *Euphrasia* have been widely used as an anti-inflammatory agent for hay fever and sinusitis and to treat upper respiratory tract infections (Petrichenko et al., 2006).

To the best of our knowledge, there is no evidence that the following 10 of the 21 plants mentioned are responsible for the beneficial effects on human health for respiratory infections:

1. *Acer campestre*
2. *Eryngium campestre*
3. *Stellaria graminea*
4. *Gentiana cruciata*
5. *Ballota nigra*
6. *Marrubium peregrinum*
7. *Salvia nemorosa*
8. *Scilla bifolia*
9. *Crataegus pentagyna*
10. *Pyrus amygdaliformis*.

The advantage of the method used in the presented paper is the wide variety of data collected. A significant limitation is that we did not ask the respondents about the perceived effectiveness of the herbal remedies. In the future, a 'retrospective treatment-outcome' design, as described by Graz et al. (2005), may be used to explore associations between use and positive outcomes to help prioritise further research.

A fundamental justification for the presented study is the avoidance of antibiotic misuse. The use of plants and phytotherapy in treating respiratory infections does not necessarily have to have an antiviral effect but could also relieve symptoms through other mechanisms of action, such as anti-inflammatory, antitussive, or antipyretic.

We did not consider other research outside the Balkans. There are data in the literature on the use of other plant parts of the same commonly used plants as in our study. For example, the use of *Sam nigra* berries in the treatment of viral respiratory diseases is described by Wieland et al. (2021), while in our study, no one mentioned the use of elderberries, only the use of flowers.

This study contains a large amount of data for application in sustainable rural development and local economies. Species from the genus *Thymus* have the highest QF and UV. In Figure 5, they stand out from the others. In Table 3, they are the most commonly used cough medicines. Thus, we can propose the different species from the genus *Thymus* recorded in Pirot District by Marković et al. (2020) for further clinical trials in treating respiratory infections. Arion et al. (1997) discussed the results of clinical trials of *Thymus* preparations and the perspectives of their therapeutical use. Natural bioactive compounds from *Thymus serpyllum* possess antimicrobial and antispasmodic properties (Jovanovic et al., 2016). Ilić et al. (2017) evaluated the antibacterial and streptomycin-modifying activity of *Thymus glabrescens* essential oil and its components geraniol, geranyl acetate, and thymol. We recommend further clinical trials in the treatment of cough based on combinations of antibiotics and essential oils of the following plant taxa from genus *Thymus* recorded in the Pirot District: *Th longicaulis*, *Th praecox* subsp. *jankae*, *Th praecox* subsp. *polytrichus*, *Th pulegioides* subsp. *panonicus*, *Th pulegioides* subsp. *pulegioides*, *Th Odoratissimus*, and *Th striatus*. Further studies may contribute to the development of controlled pharmacomodulation of antibiotics in treating respiratory infections.

## Conclusions

The study identifies plant species used by the local population of Pirot District for respiratory diseases. Of the 65 plant taxa used against respiratory system diseases, *Thymus* spp., *Mat chamomilla*, and *Sam nigra* are the most commonly known by the rural populations. The percentage of internal use is significantly higher than that of external use, and the most frequently used plant parts are aerial parts. The most

common form of preparation is infusion. Lamiaceae, Compositae, Malvaceae, Adoxaceae, and Rosaceae are the most frequently reported families.

The presented results are unique in the Balkans, as 21 plant species are used against respiratory problems only in the Pirot District, indicating a profound knowledge of medicinal plants to prevent and treat respiratory tract infections. These plant taxa are: *Ace campestre*, *Er campestre*, *Be vulgaris*, *St graminea*, *Ec elaterium*, *D rotundifolia*, *Gen cruciata*, *Ger macrorrhizum*, *Ba nigra*, *Mar peregrinum*, *Rosmarinus officinalis*, *Sal nemorosa*, *Sc bifolia*, *Pa peregrina*, *Pe bistorta*, *Cr pentagyna*, *Pr spinosa*, *Py amygdaliformis*, *Ru caesius*, *So domestica*, and *Euphrasia* spp. They are potential sources of new drugs for respiratory system diseases. Their importance may lie in finding new uses and new herbal remedies for the indications suggested by the respondents in the present study. Interest in the chemical profile and biological activities of the above species is expected to increase. Therefore, it seems promising to encourage further pharmaceutical research in this field in Serbia.

People's knowledge of traditional herbal medicine used to treat respiratory infections in Pirot District is satisfactory, but further improvement is needed. This includes continuous efforts to enhance knowledge about causes, transmission, risk factors, prevention, complications, and the herbal medicines to treat them. New pharmacological and clinical studies are needed for proven herbal medication for the reported respiratory diseases. The data presented could serve as a basis for further research, such as identifying potential antimicrobial and antiviral effects. *In vitro* and/or *in vivo* studies with the herbal extracts of plants identified in our analysis could reveal new antiviral medicinal agents for use against respiratory infections.

## Ethical approval

Ethical approval was not required for this research involving respondents from Pirot District according to institutional requirements and local legislation. The respondents signed an informed consent form to participate in the study.

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The funding source(s) had no involvement.

## Author contributions

**Marija S. Marković:** Conception and design of research, Data collection, Plant material collection, Identification of the plant species, Drafting the article; **Dejan S. Pljevljakušić:** Plant material collection, Reviewing, Editing; **Jelena S. Matejić:** Identification of the plant species, Statistical analysis; **Ljubinko B. Rakonjac:** Analysis, Supervision; **Biljana M. Nikolić:** Data interpretation; **Mrdjan M. Djokić:** Data interpretation assistance, Graphical design; **Vesna P. Stankov Jovanović:** Experimental design, Data collection, Writing assistance.

## Declaration of Competing Interest

The authors declare that they have no competing financial interests or personal relationships that influenced the work presented in this paper.

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## Supplementary materials

Supplementary materials associated with this article can be found in the online version at [doi:10.1016/j.hermed.2023.100743](https://doi.org/10.1016/j.hermed.2023.100743).

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