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This theme issue is dedicated to the celebration of three centuries of botany in Vojvodina

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RESEARCH IN PLANT MORPHOANATOMY IN THE PROVINCE OF VOJVODINA IN THE 20TH CENTURY

ABSTRACT**:** In order to understand the diversity of flora and vegetation in a region, the knowledge of the biology of particular plant species is very important. Under the influence of numerios complex biotic and abiotic factors, biological plasticity of the species is reflected in the morphological level through a series of macro and micro adaptations. The subject of the initial research was the analysis of the external plant form, which was implemented in the fields of plant systematics and taxonomy. In the mid-20th century, with the development of the microtechnical equipment, morphoanatomy become a separate scientific area. Numerous researchers contributed to its development. The contribution of some of them is the subject of this paper. This paper gives a general overview of scientific results in different areas of plant morphoanatomy, resulting from researches carred out in the province of Vojvodina during the 20th century, as well as a list of bibliographic units published in scientific journals.

KEYWORDS: morphoanatomy, overview, 20th century, Vojvodina

The first study of the different area of biology and also botany in the province of Vojvodina during the 20th century was connected to different teachers' colleges. Some of them had a tradition longer than one century. The year 2016 was marked by the celebration of the 200th anniversary of the transfer of Serbian Teachers' College (Preparandija) from Szentendre (Hungary) to Sombor (Serbia). At the Teachers' College in Novi Sad, younger than the one in Sombor, biological subjects were taught from 1946 to 1952 by Professor Dr. Živko Slavnić (1910–1975). Prof. Slavnić got his degree in natural sciences from Sorbonne (France) in 1933. A large number of his scientific papers were published in the national and international journals. His most important contribution to the science of biology is in the area of fitocenology. As a recognized scientist, and at the same time an exellent connoisseur of foreign languages, he cooperated with leading European scientists in the area of biosystematics and fitocenology. As a very respected professor he mentored many famous botanists of former

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Yugoslavia. He made a significant influence on the development of plant morphology dealing with infraspecies differentiation, mainly of endemic species.

The Faculty of Agriculture (Novi Sad) was founded in 1954 and botany become a compulsory subject for the first year students. The subject of Botany encompassed plant morphology, plant systematics, and partly plant anatomy. At the bigining, Botany was tought by Prof. Dr. Stevan Jakovljević and Prof. Dr. Milica Ćorović, and later by Prof. Dr. Milan Čanak, Prof. Dr. Stanija Parabućski, Prof. Dr. Vera Janjatović, and Prof. Dr. Marija Anđelić. Professor Dr. Stevan Jakovljević (1890–1962), a member of the Serbian Academy of Sciences and Arts, taught General Botany at the Faculty of Veterinary Medicine from 1937, and also at the Faculty of Pharmacy in Belgrade from 1939. He thought that writting text books was one of the primary tasks of a teacher. He published several text books which are used even today. He was versatile. He gave a contribution to the development of botany by publishing scientific papers in the area of experimenatal plant morphology. The resultats of his doctoral disertation have been cited in the world eminent text books, theses and scientific journals (Uphof 1962; Thurston 1969). The participation in the First Warld War left an indelible mark on his life, and the memories of it were published in *Srpska trilogija* (The Serbian Trilogy) which made him famous as a writer.

The four year programme of biology studies started in 1960 at the Faculty of Phylosophy in Novi Sad. The first lecturer who taught the course of Plant Morphology and Physiology was Prof. Dr. Božidar Stojaković (1899–1976). He taught the courses at this Faculty from 1962 to 1969, when he retired. He published text books and a large number of popular articles. His viewpoint that a good teacher must be informed of developments in science was supported by the fact that he was also subscribed to a large number of scientific journals. He spoke and was able to use four or five foreign languages.The story of Prof. Stojaković as an outstanding teacher has been told by many generations of students.

The Faculty of Sciences was separated from the Faculty of Philosophy in 1969. Since then, more modern equipment has been purchased and it can be said that this was the time when Plant Anatomy started its development and was separated from General Botany. Professor Dr. Marija Anđelić (1930–2012), who got her Ph.D. degree in science at the Faculty of Agriculture, came to teach at the Faculty of Sciences. She was nominated the first professor of Plant Anatomy and Morphology. She published several text books as well as a significant number of scientific papers in national journals. The main subject of her scientific interest was ecoanatomy of plant species in sandy and steppe areas. She showed a particular interest in the influence of environmental factors on the development of plants, such as: salinity, intensity of light, necessary element content in the soil and its mechanical constitution. Professor Dr. Ljiljana Merkulov (1948–) gave an important contribution to the profound understanding of the reaction of plants visible on the anatomical level, and the further development of the subject of Plant Anatomy. Beside the articles in the national and international scientific journals, she also published the text books. The scientific and professional activity of Prof. Merkulov encompassed various areas of

plant anatomy. Her most important contribution, and at the same time the largest number of published articles, was in the field of ecological and taxonomical anatomy.

The first research projects in the field of plant morphoanatomy were primarily related to morphological characteristics. Later on, anatomical studies prevailed, and they served as a base or as a support to other areas. Professor Milorad Janković drew attention to the necessity of anatomical research. Pointing out the inadequacy of the book *Flora SR Srbije* (The Flora of Serbia) just finished at that time, he put an emphases on the insufficient study of the inner structure of species and genera (Janković 1972). According to the subject, anatomical researches could be classified in several groups. The majority of articles belonged to ecological anatomy, applied and physiological anatomy, and a small number belonged to systematical anatomy. The scintific results obtained in the province of Vojvodina during the 20th century were mainly published in the last decades of the same century. The main scientific area of the most authors was ecoanatomical research of plant species. Ecoanatomy makes 44% of the total number of papers. Anatomical research very often found its application in ecophysiological analysis. The specific structural and physiological features, and also functional plant adaptation were analyzed through anatomical characteristics. Hence, a multidisciplinary approach in plant analysis is visible in numerous research papers. The first scientific papers in the area of anatomy were in applied anatomy. The morphoanatomical structure of different plant organs were analyzed in the various domesticated plants which were used by breeders during the plant breeding process to make new genotypes. This scientific area comprises 28% of the total number of papers published in the province of Vojvodina during the 20th century.

A special part of the applied anatomy has been treated in the papers which deal with morphoanatomical characterization of numerous wheat genotypes. Anatomical characterization of autochthonic plants, of some cultivated species, has been done with the aim of using the gene pool of wild varieties in breeding programs. A little less than 25% of the works published in this filed is about physiological anatomy of plants. The changes in the plant structure were studied taking into consideration the conditions of different regime of mineral nutrition, as well as the influence of chemical protection substances.

The articles in the field of physiological ecoanatomy have a significant importance in a more complete interpretation of plant adaptation to the specific conditions of salinity soils. These articles also analyzed seasonal dynamic of osmotic cell potential, water content and mineral substance content in plant tissue. Less numerous are the papers that dealt with such an anatomical analysis which, apart from the better knowledge of some biology species, aim at distinguishing the qualitative characteristics significant for understanding of the taxonomical problems.

A group of researches from the Department of Biology and Ecology in Novi Sad work on the development of plant morphoanatomy in Vojvodina. Through the cooperation with the colleagues from numerous institutions, this research team has been achieving the results that will hopefully play an important role

in the field of botany. This overview of the development and research in the field of plant morphoanatomy has been written with the aim to be used as a preliminary source of data and for the future reviews and analyses of works in this filed.

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ПРЕГЛЕД ИСТРАЖИВАЊА У ОБЛАСТИ БИЉНЕ МОРФО-АНАТОМИЈЕ НА ТЕРИТОРИЈИ ВОЈВОДИНЕ ТОКОМ XX ВЕКА

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РЕЗИМЕ: За познавање диверзитета флоре и вегетације једног региона од великог значаја је познавање биологије појединачних биљних врста. Под утицајем бројних, комплексних биотичких и абиотичких чинилаца, биолошка пластичност врсте огледа се и на морфолошком нивоу кроз читав низ макро- и микроадаптација. Предмет првих истраживања била је анализа спољашње форме биљака која је налазила примену у области систематике и таксономије. Средином XX века, с интезивнијим развојем микротехничке опреме, морфо-анатомија се издваја као посебна дисциплина. Њеном развоју допринели су бројни истраживачи. У овом раду биће приказан допринос неких од ових истраживача. У раду је дат и општи приказ научних резултата у различитим областима морфо-анатомије биљака насталих истраживањима на територији Војводине током XX века, као и списак библиографских јединица публикованих у часописима.

КЉУЧНЕ РЕЧИ: морфо-анатомија, преглед истраживања, XX век, Војводина

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HISTORICAL REVIEW OF BRYOLOGICAL RESEARCH IN FRUŠKA GORA MT. (SERBIA)

ABSTRACT: Fruška Gora is a mountain located in the southern part of Pannonian plane and it is poorly bryologicaly investigated as well as most parts of Serbia. First data on bryophytes, for this region, were published in 1949 by Teodor Soška in his paper *Pregled mahovina i lišajeva u okolini Beograda* (Review of mosses and lichens in the vicinity of Belgrade). Soška listed 34 species of mosses and 8 liverworts species for this region. Later, in 1955, Zlatko Pavletić in his capital work *Prodromus flore briofita Jugoslavije* (Prodromus of Yugoslavian bryophyte flora), stated 26 moss species and 3 liverwort species, without indicating specific localities, but all of them were Soška's original results. In 1966, the second botanist with original findings of bryophytes on Fruška Gora was Popović, who recorded 2 liverwort and 19 moss species, for this mountain, also without specific localities. By far, the most comprehensive data were given by Cvetić and Sabovljević in 2004. Their study *A contribution to the bryophyte flora of Fruška Gora (Vojvodina, Serbia)* counted total number of 118 mosses and 14 liverworts, on 16 localities within National park Fruška Gora. The latest study on bryophytes of Fruška Gora was in 2013, and it is the first and so far, the last study of bryophyte assemblages on grasslands. Authors of this paper recorded 18 species of mosses from 29 releveé.

Complete bryophyte flora of Fruška Gora is still unknown. Considering the fact that this is the region with high biodiversity, dense hydrological network and a rather complex geology, Fruška Gora certainly deserves further bryological researches.

KEYWORDS: Fruška Gora, Liverworts, Moss, Serbia, Vojvodina

INTRODUCTION

Fruška Gora Mt. is located in Northern part of Serbia and southern part of Pannonian plane (Figure 1) between 45°0' – 45°15' N and 16°37' – 18°01' E. This mountain is about 80 km long and stretched between Šarengrad (Croatia) on the west and Stari Slankamen on the East (Butorac 1981). The maximum width

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(15 km) is between Irig and Sremska Kamenica. Fruška Gora is bounded by Danube alluvial plane in the north and east and by two loess plateaus in the south and west. Once, this mountain was an island in the ancient Pannonian Sea. The average altitude is 300–400 m. a. s. l, and the highest peaks are Crveni Čot (539 m. a. s. l.), Isin Čot (524 m. a. s. l.) and Kraljeva Stolica (495 m. a. s. l.).

Figure 1. Position of Fruška Gora Mt. in Serbia

Geologically, Fruška Gora is a rather diverse area. The Paleozoic is represented by phyllites and limestone schist and these rocks are the most widespread on Fruška Gora (Bukurov 1953). Sedimentary rocks, red and gray sandstones, schist and conglomerates date from Mesozoic (Obradović 1966). The most important rocks from Tertiary are volcanic and in many parts of the Mountain they stretch through the surface (Obradović 1966). The Quaternary is represented by loess and alluvial sands (Obradović 1966). Geological history of Fruška Gora is very important for understanding its recent flora and vegetation. The largest part of Fruška Gora is composed of siliceous rocks, and the vegetation dates probably from the Tertiary because glaciation did not have a significant impact on this mountain (Obradović 1966).

There are three soil types on Fruška Gora: chernozem, brown forest soil and brown calcareous soil (Miljković 1975). Chernozem is climatogenous, zonal soil which occurs up to 300 m. a. s. l., with remains of steppe vegetation.

At an altitude higher than 300 meters, there is second zonal soil, brown calcareous soil, with forest vegetation. Third type, brown forest soil, is azonal and is covered with steppe vegetation and thermophilous forests (Butorac 1981).

Fruška Gora is in mild-continental central European climatic region (Cvetić and Sabovljević 2005). Climate varies with an altitude. Higher, as well as the western parts of the mountain are characterized by higher amount of precipitation (833 mm) and narrow temperature fluctuations, due to the impact of humid Atlantic climate (Butorac 1981). Eastern parts are influenced by dry continental climate, temperature fluctuations are wider and amount of precipitation (652 mm) is lower (Butorac 1981). The rainiest months are May–June and September–October (Obradović 1966). The lowest temperature is in January and the highest in July (Butorac 1981).

Fruška Gora is characterized by dense hydrological network. There are a lot of groundwater, karst springs, mineral and thermal springs, streams and some standing water. The eastern and western slopes are relatively arid, while there are many streams on the northern ones (Cvetić and Sabovljević 2005).

Forest is dominant vegetation type on Fruška Gora (Janković and Mišić 1980). The most widespread associations are *Festuco montane – Quercetum petreae* M. Jank. and *Aculeato-Querco-Carpinetum serbicum* B. Jov. as well as *Tilio-Fagetum submontanum* M. Jank. Et V. Miš. (Janković and Mišić 1980). Very interesting association, from bryological point of view, is *Musco-Fagetum submontanum* (B. Jov.) Miš. Et Jank. found on Iriški venac locality (Erdeši 1971; Janković and Mišić 1980).

Due to the exceptional natural and cultural values, Fruška Gora was declared a National park in 1960. The protected area covers 25,393 ha. Under the protection regime of the first, second and third degree are 3.7%, 67% and 29.3% of complete area, respectively. The majority of protected area is under forest vegetation.

The aim of this paper was to show the current state of Fruška Gora bryophyte flora investigations and to determine the least studied or bryologically unresearched areas of the mountain.

FLORISTIC AND BRYOLOGICAL RESEARCH ON FRUŠKA GORA

First floristic data on this region were given in XVIII century (Obradović 1966). The most important botanists, who gave contribution to the floristic studies of Fruška Gora before the World War II were Friedrich Wilhelm von Taube, Pál Kitaibel, Franz Waldstein, Andreas Rafael Wolny, Kosta Petrović, Carl Stoizner, August Kanitz, Vince Borbás, Dragutin Hirc, Josif Pančić, Lajos Zorkóczy and Sándor Jávorka. After the World War II, studies of this area were re continued, resulting in numerous published papers mainly on vascular flora and vegetation of Fruška Gora (Slavnić 1952; Slavnić 1953; Bećarević 1951; Atanacković 1953; Grozdanović 1956; Čolović 1956; 1958; Čerevicki 1959; Obradović 1966; Erdeši 1971; Obradović 1978; Janković and Mišić 1980; Butorac 1981; Stevanović 1984; Butorac 1992). According to these studies, Fruška

Gora is the area of high biodiversity (Obradović 1966; Butorac 1981; Stevanović 1984) with nearly 1,500 species of vascular plants (Nacionalni park Fruška gora, available on: http://www.npfruskagora.co.rs/cir/prirodne-vrednosti/biljke.html).

First data on bryophytes of Fruška Gora were given by Soška in 1949. In the publication *Pregled mahovina i lišajeva u okolini Beograda* (Review of mosses and lichens in the vicinity of Belgrade), he listed 34 moss and 8 liverworts species for localities: Fruška Gora, Stražilovo and Venac (Iriški Venac). Data given in this paper were result of researches and specimen collections from 1907 until 1945. Also, he suggested ecological and geographical groups of bryophytes. Teodor Soška (1876–1948) was inspector in Belgrade Botanical garden. During his carrier, he had collected a large number of specimens, leaving behind a large bryophyte herbarium. One part of his herbarium is located in the Museum of Natural History in Belgrade and the rest is deposited in Belgrade Botanical Garden. Also, Soška used herbarium from Sremski Karlovci for completing bryophyte list. However, unfortunately, a large part of this herbarium with specimens from Fruška Gora was destroyed during the World War II (Čolović 1956). Of all species listed by Soška, moss *Pogonatum urnigerum* (Hedw.) P. Beauv. recorded on Stražilovo locality, was not rediscovered by later researchers. Also, the record of liverwort *Calypogeia trichomanis* Corda (Soška 1949) is doubtful. According to Söderström *et al.* (2002), this name is variously interpreted in Europe for the species *C. azurea* Stotler & Crotz and *C. muelleriana* (Schiffn.) Müll. Frib. The same is for the Mediterranean region including Balkan Peninsula (Ros *et al.* 2007). None of these species have ever been rediscovered on Fruška Gora after Soška (1949) so, the revision of herbarium is necessary, if these herbaria specimens still exist.

Next author who gave some data on Fruška Gora bryophytes was Zlatko Pavletić (1920–1981), in his capital work *Prodromus flore briofita Jugoslavije* (Prodromus of Yugoslavian bryophyte flora) published in 1955. In this book, Pavletić listed all species found on the territory of Yugoslavia (Serbia, Croatia, Slovenia, Bosnia and Herzegovina, Macedonia, Montenegro), by himself and other bryologists. Pavletić (1955) mentioned 29 species of bryophytes (26 moss and 3 liverwort species) on Fruška Gora, but all of them were Soška's original data. As localities, Pavletić (1955) states only Fruška Gora, Venac (Iriški Venac), Rakovac and Beočin.

Stanija Čolović (Parabućski) (1929–2014) studied flora of Fruška Gora, and in one paper (Čolović 1956), she listed 41 bryophyte species for this mountain (33 moss and 8 liverwort species). After revision, total number of species recorded in her study is 40, since *Hypnum purum* L. and *Scleropodium purum* (L.) Limpr. are synonyms and nowadays the accepted name for this taxon is *Pseudoscleropodium purum* (Hedw.) M. Fleisch Čolović (1956) based this bryophyte list only on Teodor Soška's list, made from Sremski Karlovci herbarium data. Localities given by Čolović (1956) were Paragovo–Venac and Sremski Karlovci, each with one liverwort species, Branko Radičević Monument hill with 6 liverwort species and Fruška Gora for all moss species.

Milorad Popović (1923–2010) published the contribution to the bryophyte flora in protected areas of Serbia in 1966. This paper (Popović 1966) includes

results of bryological research in several areas in Serbia (Kopaonik, Oštrozub, Beljanica, Stara Planina, Fruška Gora, Avala, Majdanpečka Domena, Deliblato Sand, Vlasina and Suva Planina). On Fruška Gora, Popović (1966) found 21 species (2 liverwort and 19 moss species) without indicating specific localities. Five species were recorded for the first time on Fruška Gora: *Atrichum undulatum* (Hedw.) P. Beauv., *Ctenidium molluscum* (Hedw.) Mitt., *Eurhynchium striatum* (Hedw.) Schimp., *Rhizomnium punctatum* (Hedw.) T. J. Kop. and *Rhytidiadelphus triquetrus* (Hedw.) Warnst.

Josip Erdeši (1927–2005) was one of the eminent experts in forestry, who gave contribution to the bryophyte flora of Fruška Gora in his PhD thesis on forest phytocoenoses of South-West Srem. Erdeši (1971) listed 5 moss species for the locality of Venac (Iriški Venac). Those species were part of ground flora in association *Musco-Fagetum submontanum* (B. Jov.) Miš. et Jank. For the first time, a specimen belonging to the genus *Hylocomium* Schimp. 1852 was found on Fruška Gora, but it has never been determined to the species level and later confirmed by any bryologist except Janković and Mišić (1980), at the same locality.

Vojislav Mišić (1922–2009) and Milorad Janković (1924–2002) listed 5 bryophyte species for the locality of Venac (Iriški Venac) in association *Musco-Fagetum submontanum* (B. Jov.) Miš. Et Jank. in their publication on forest vegetation of Fruška Gora (Janković and Mišić 1980). These species are the same as previously recorded by Erdeši (1971).

After Janković and Mišić (1980), there had not been any publications about bryophytes on Fruška Gora, until the beginning of XXI century. In the past 14 years, several researches had been done in this area (Sabovljević and Sérgio 2002; Sabovljević 2003; Cvetić and Sabovljević 2005; Širka *at al*., 2013).

Sabovljević and Sérgio (2002) presented the results of bryological research carried out in the period from 1996 to 2002. They listed 7 moss species for the locality Vrdnik and its surroundings and first records of *Dicranella heteromalla* (Hedw.) Schimp., *Pohlia elongata* Hedw. and *Syntrichia ruralis* (Hedw.) F. Weber & D. Mohr on Fruška Gora.

In the study done in 2003, Marko Sabovljević included bryophytes found on Fruška Gora: 2 species of Marchantiohyta and 6 species of Bryophyta, all of them found on the locality Rakovac. *Radula complanata* (L.) Dumort., *Bryum argenteum* Hedw., *Polytrichum formosum* Hedw., were recorded for the first time on Fruška Gora.

Cvetić and Sabovljević (2005) published the most comprehensive study on the Bryophyte flora of Fruška Gora Mountain. Study covered 16 localities: Rim, Vicinity of Rim, Monument to Branko Radičević with surroundings, Rest house Brankovac, Vrdnik and environment, Beočin meadows, Rakovac, quarry near Rakovac, Stražilovo, Petrovaradin, Ležimir, Roman bathhouse ruins, Sremska Mitrovica, Dumbovo rivulet, Dumbovo watefall and Brankovac). Total number of 116 moss and 14 liverwort species were recorded, including previous researches by Pavletić (1955) and Popović (1966). Without previously published data, there were 105 moss and 14 liverwort species (Cvetić and Sabovljević 2005). Among them, 95 bryophyte species were recorded for the

first time on Fruška Gora (84 mosses and 11 liverworts) (Cvetić and Sabovljević 2005). In this study, three species were new for Serbia *Entosthodon obtusus* (Hedw.) Lindb., *Rhynchostegiella curviseta* (Brid.) Limpr. and *Fissidens rivularis* (Spruce) Schimp. Localities with the highest number of species were Dumbovo rivulet and waterfall (Cvetić and Sabovljević 2005).

The latest study on bryophytes from Fruška Gora (Širka *et al*. 2013) is at the same time the first study on grassland bryophyte assemblages in this region. Širka *et al*. (2013) did phytosociological research on several localities (Stari Ledinci, Erdelj, Čerević, Andrevlje, Susek, Ležimir). Total number of 18 moss species were recorded. Five species were new for Fruška Gora: *Aloina aloides* (Koch ex Schultz) Kindb., *Didymodon rigidulus* Hedw., *Pseudocrossidium hornschuchianum* (Schultz) R. H. Zander, *Tortula acaulon* (With.) R.H. Zander and *Physcomitrella patens* (Hedw.) Bruch & Schimp. Širka *et al*. (2013), also gave a valuable insight into the bryophyte vegetation and the most common bryophyte assemblages on dry and wet grasslands.

Considering all previous research on Fruška Gora bryophytes (Soška 1949; Pavletić 1955; Čolović 1956; Popović 1966; Erdeši 1971; Janković and Mišić 1980; Sabovljević and Sérgio 2002; Sabovljević 2003; Svetić and Sabovljević 2005; Širka *et al*. 2013), a total number of 16 liverwort species plus one *Calypogeia trichomanis* (which is a doubtful finding) and 130 moss species were recorded (Table 1 and 2). Nomenclature follows Ros *et al*. (2007), for liverworts (except for the species *Calypogeia trichomanis*) and Ros *et al*. (2013) for mosses. Studied localities were: Monument to Branko Radičević and its surroundings, Stražilovo, Dumbovo stream, Sremska Mitrovica, Rim, Rakovac, Quarry Rakovac, the ruins of the Roman bath, Vrdnik and its surroundings, Brankovac, Ležimir, Beočin meadows, Rim surroundings, Petrovaradin, Dumbovo waterfall, Venac (Iriški venac), Beočin, Stari Ledinci, Čerević, Erdelj, Susek, Andrevlje, Paragovo-Venac and Sremski Karlovci.

Table 1. Historical review of liverwort (Marchantiophyta) species recorded on Fruška Gora mountain

Species	Reference*
Calypogeia trichomanis Corda**	1
Cephaloziella stellulifera (Taylor ex Spruce) Schiffn.	9
Conocephalum conicum (L.) Dumort.	1, 2, 3, 9
Frullania dilatata (L.) Dumort.	1, 3, 8, 9
Frullania tamarisci (L.) Dumort.	1, 2, 3, 9
Lejeunea cavifolia (Ehrh.) Lindb.	9
Lophocolea bidentata (L.) Dumort.	9
Lophocolea heterophylla (Schrad.) Dumort.	9
Marchantia polymorpha L.	3, 4, 9
Metzgeria conjugata Lindb.	9
Metzgeria furcata (L.) Dumort.	1, 3, 9
Pellia endiviifolia (Dicks.) Dumort.	1

Plagiochila asplenioides (L. emend. Taylor) Dumort.	3
Plagiochila porelloides (Torrey ex Nees) Lindenb.	9
Porella cordaeana (Huebener) Moore	9
Porella platyphylla (L.) Pfeiff.	1, 2, 3, 4, 9
Radula complanata (L.) Dumort.	3, 8, 9

[*] 1-Soška, 1949; 2-Pavletić (1955); 3-Čolović (1956); 4-Popović (1966); 5-Erdeši (1971); 6-Janković and Mišić (1980); 7-Sabovljević and Sérgio (2002); 8-Sabovljević (2003); 9-Cvetić and Sabovljević (2005).
[**] Doubtful record

Table 2. Historical review of moss (Bryophyta) species recorded on Fruška Gora mountain

Species	Reference[*]
Abietinella abietina (Hedw.) M. Fleisch.	3, 4, 9
Alleniella besseri (Lobarz.) S. Olsson, Enroth & D. Quandt	9
Alleniella complanata (Hedw.) S. Olsson, Enroth & D. Quandt	1, 2, 3, 9
Aloina aloides (Koch ex Schultz) Kindb.	10
Amblystegium confervoides (Brid.) Schimp.	9
Amblystegium serpens (Hedw.) Schimp.	1, 2, 3, 9
Anomodon attenuatus (Hedw.) Huebener	1, 2, 3, 7, 9
Anomodon viticulosus (Hedw.) Hook. & Taylor	2, 3, 4, 9
Atrichum angustatum (Brid.) Bruch & Schimp.	1, 2, 4, 9
Atrichum tenellum (Röhl.) Bruch & Schimp.	9
Atrichum undulatum (Hedw.) P. Beauv.	4, 7, 8, 9
Barbula convoluta Hedw.	9
Barbula unguiculata Hedw.	9, 10
Brachytheciastrum velutinum (Hedw.) Ignatov & Huttunen	1, 2, 3, 7, 9
Brachythecium glareosum (Bruch ex Spruce) Schimp.	9
Brachythecium mildeanum (Schimp.) Schimp.	9
Brachythecium rivulare Schimp.	1, 2, 3, 8, 9
Brachythecium rutabulum (Hedw.) Schimp.	1, 2, 3, 9
Brachythecium salebrosum (Hoffm. ex F.Weber & D.Mohr) Schimp.	9
Brachythecium tommasinii (Sendtn. ex Boulay) Ignatov & Huttunen	9
Bryoerythrophyllum recurvirostrum (Hedw.) P. C. Chen	9
Bryum argenteum Hedw.	8, 9
Calliergonella cuspidata (Hedw.) Loeske	3, 10
Campyliadelphus chrysophyllus (Brid.) R. S. Chopra	9
Ceratodon purpureus (Hedw.) Brid.	1, 2, 3, 9
Cratoneuron filicinum (Hedw.) Spruce	3, 9
Ctenidium molluscum (Hedw.) Mitt.	4, 5, 6, 9
Dicranella cerviculata (Hedw.) Schimp.	9
Dicranella heteromalla (Hedw.) Schimp.	7, 9
Dicranella subulata (Hedw.) Schimp.	9
Dicranella varia (Hedw.) Schimp.	9, 10

Dicranum scoparium Hedw.	1, 2, 4, 5, 6, 9
Didymodon acutus (Brid.) K. Saito	9, 10
Didymodon insulanus (De Not.) M. O. Hill	9, 10
Didymodon luridus Hornsch.	9, 10
Didymodon rigidulus Hedw.	10
Didymodon vinealis (Brid.) R.H.Zander	9, 10
Drepanocladus aduncus (Hedw.) Warnst.	9
Entosthodon fascicularis (Hedw.) Müll.Hal.	9
Entosthodon obtusus (Hedw.) Lindb.	9
Eurhynchiastrum pulchellum (Hedw.) Ignatov & Huttunen	9
Eurhynchium striatum (Hedw.) Schimp.	4, 9
Fissidens bryoides Hedw.	9
Fissidens dubius P.Beauv.	9
Fissidens gracilifolius Brugg. – Nann. & Nyholm	9
Fissidens rivularis (Spruce) Schimp.	9
Fissidens taxifolius Hedw.	1, 3, 9, 10
Funaria hygrometrica Hedw.	1, 2, 3, 9
Grimmia anodon Bruch & Schimp.	9
Grimmia orbicularis Bruch ex Wilson	3
Grimmia pulvinata (Hedw.) Sm.	9
Grimmia trichophylla Grev.	9
Gymnostomum calcareum Nees & Hornsch.	9
Herzogiella seligeri (Brid.) Z. Iwats.	9
Homalia trichomanoides (Hedw.) Brid.	9
Homalothecium lutescens (Hedw.) H. Rob.	1, 2, 9
Homalothecium sericeum (Hedw.) Schimp.	9
Hygroamblystegium varium (Hedw.) Moönk.	9
Hylocomium sp.	5, 6
Hypnum callichroum Brid.	9
Hypnum cupressiforme Hedw.	1, 2, 3, 4, 8, 9, 10
Hypnum cupressiforme Hedw. var. *lacunosum* Brid.	9
Hypnum cupressiforme Hedw. var. *resupinatum* (Taylor) Schimp.	9
Hypnum recurvatum (Lindb. & Arnell) Kindb.	9
Isothecium alopecuroides (Lam. ex Dubois) Isov.	1, 2, 3, 4, 9
Kindbergia praelonga (Hedw.) Ochyra	1, 3, 9
Leptodictyum riparium (Hedw.) Warnst.	9
Leskea polycarpa Hedw.	1, 2, 3, 9
Leucodon sciuroides (Hedw.) Schwägr.	1, 3, 4, 7, 9
Microeurhynchium pumilum (Wilson) Ignatov & Vanderp.	9
Mnium hornum Hedw.	9
Orthotrichum affine Schrad. ex Brid.	1, 2, 9
Orthotrichum diaphanum Brid.	9
Oxyrrhynchium hians (Hedw.) Loeske	9, 10
Physcomitrella patens (Hedw.) Bruch & Schimp.	10

Plagiomnium cuspidatum (Hedw.) T. J. Kop.	1, 2, 3, 9
Plagiomnium rostratum (Schrad.) T. J. Kop.	9
Plagiomnium undulatum (Hedw.) T. J. Kop.	3, 4, 5, 6
Plagiothecium cavifolium (Brid.) Z. Iwats.	9
Plagiothecium curvifolium Schlieph. ex Limpr.	9
Plagiothecium denticulatum (Hedw.) Schimp.	1, 2, 3, 4, 9
Plagiothecium laetum Schimp.	9
Plagiumnium undulatum (Hedw.) T. J. Kop.	1, 9
Platygyrium repens (Brid.) Schimp.	9
Pleurozium schreberi (Willd. ex Brid.) Mitt.	1, 2, 9
Pogonatum aloides (Hedw.) P. Beauv.	1, 4
Pogonatum nanum (Hedw.) P. Beauv.	9
Pogonatum urnigerum (Hedw.) P. Beauv.	1
Pohlia elongata Hedw.	7
Polytrichum commune Hedw.	1, 2, 3, 4, 5, 6, 9
Polytrichum formosum Hedw.	8, 9
Polytrichum piliferum Hedw.	1, 2, 3, 4, 9
Pseudoamblystegium subtile (Hedw.) Vanderp. & Hedenäs	9
Pseudocampylium radicale (P. Beauv.) Vanderp. & Hedenäs	9
Pseudocrossidium hornschuchianum (Schultz) R. H. Zander	10
Pseudoleskeella catenulata (Brid. ex Schrad.) Kindb.	9
Pseudoleskeella nervosa (Brid.) Nyholm	9
Pseudoscleropodium purum (Hedw.) M. Fleisch.	1, 2, 3, 4, 9
Pterigynandrum filiforme Hedw.	9
Ptychostomum capillare (Hedw.) Holyoak & N. Pedersen	1, 3, 8, 9, 10
Ptychostomum imbricatulum (Müll. Hal.) Holyoak & N. Pedersen	1, 2, 3, 9
Ptychostomum moravicum (Podp.) Ros & Mazimpaka	9
Pylaisia polyantha (Hedw.) Schimp.	1, 3, 4, 9
Rhizomnium pseudopunctatum (Bruch & Schimp.) T. J. Kop.	9
Rhizomnium punctatum (Hedw.) T. J. Kop.	4, 9
Rhynchostegiella curviseta (Brid.) Limpr.	9
Rhynchostegiella tenella (Dicks.) Limpr.	9
Rhynchostegiella teneriffae (Mont.) Dirkse & Bouman	9
Rhynchostegium confertum (Dicks.) Schimp.	9
Rhynchostegium megapolitanum (Bland ex F. W. & D. Mohr) Schimp.	3
Rhynchostegium murale (Hedw.) Schimp.	9
Rhynchostegium riparioides (Hedw.) Cardot	9
Rhytidiadelphus triquetrus (Hedw.) Warnst.	4, 9
Schistidium apocarpum (Hedw.) Bruch & Schimp.	1, 2, 9
Schistidium atrofuscum (Schimp.) Limpr.	9
Scorpiurium circinatum (Bruch) M. Fleisch. & Loeske	9
Syntrichia calcicola J. J. Amann	9
Syntrichia laevipila Brid.	9
Syntrichia montana Nees	9

Syntrichia papillosa (Wilson) Jur.	9
Syntrichia princeps (De Not.) Mitt.	9
Syntrichia ruralis (Hedw.) F. Weber & D. Mohr	7, 9, 10
Thamnobryum alopecurum (Hedw.) Gangulee	1, 9
Thuidium recognitum (Hedw.) Lindb.	3
Thuidium tamariscinum (Hedw.) Schimp.	1, 2, 3, 9
Tortula acaulon (With.) R. H. Zander	10
Tortula caucasica Broth.	9
Tortula muralis Hedw.	9
Tortula subulata Hedw.	1, 2, 3, 9
Trichostomum crispulum Bruch	9, 10

*1-Soška (1949); 2-Pavletić (1955); 3-Čolović (1956); 4-Popović (1966); 5-Erdeši (1971); 6-Janković and Mišić (1980); 7-Sabovljević and Sérgio (2002); 8-Sabovljević (2003); 9-Cvetić and Sabovljević (2005); 10-Širka *et al.* (2013).

In general, considering the number of species listed in all mentioned publications on Fruška Gora bryophytes, the majority of taxa was recorded in two time periods: 1949–1980, and 2002–2013 (Figure 2). From 1980 on, there had been a 22 years long pause in bryological researches on Fruška Gora.

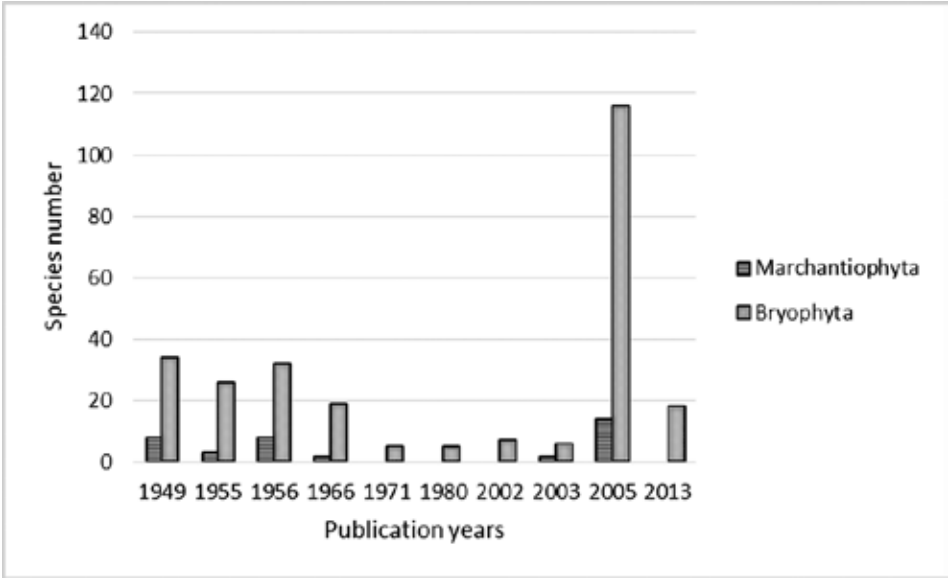

Figure 2. Number of bryophyte species recorded on Fruška Gora 1949–2013.

Although the studies on bryophytes in northern part of Serbia were intensified during the last 14 years (Sabovljević 2002; Sabovljević 2003; Sabovljević 2003a; Sabovljević and Stevanović 2006; Papp and Sabovljević 2010), a more detailed information on mosses and liverworts for the area of Fruška Gora, as well as the rest of the Serbian part of Pannonian plane, is required.

CONCLUSION

Despite the numerous floristic surveys on Fruška Gora Mountain, bryophytes had been neglected during the period before World War II. After that, there were several bryological researches or floristic studies with some notes on bryophytes. The most comprehensive studies on bryophyte flora of this region were published in past 14 years. Considering the number of investigated localities on Fruška Gora, we can conclude that there is a large part of this mountain which has not been bryologically surveyed yet. Complete bryophyte flora of Fruška Gora is still unknown, despite its importance in the structure and ecosystem functions. Considering the fact that this is the region with high biodiversity and that this mountain is characterized by large hydrological network and very complex composition of rocks and soils, Fruška Gora certainly deserves further and more detailed bryological research.

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ИСТОРИЈСКИ ПРЕГЛЕД БРИОЛОШКИХ ИСТРАЖИВАЊА НА ФРУШКОЈ ГОРИ (СРБИЈА)

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РЕЗИМЕ: Фрушка гора је планина смештена у јужном делу Панонске низије, и као и већина Србије бриолошки недовољно истражена. Прве податке за флору маховина овог подручја дао је Теодор Сошка, 1949. године у свом раду „Преглед маховина и лишајева у околини Београда“ (*Гласник Природњачкоī музеја срūске земље*). Сошка наводи 34 врсте правих маховина и осам врста јетрењача, без навођења локалитета. Касније, 1955. године, Златко Павлетић у свом капиталном делу (*Prodromus flore briofita Jugoslavije*) наводи 26 врста правих маховина и три врсте јетрењача, али су сви ови наводи оригинални резултати Т. Сошке. Други ботаничар са оригиналним налазима маховина на Фрушкој гори је М. Поповић, који је забележио две јетрењаче и 19 правих маховина, такође без навођења локалитета. Цветић и Сабовљевић дали су 2004. године најкомплетнији попис флоре маховина на подручју Националног парка „Фрушка гора“, у којем наводе 118 врста правих маховина и 14 врста јетрењача. Последња студија о бриофитама на Фрушкој гори датира из 2013. године и бави се бриофитским заједницама на пашњацима Фрушке горе. Аутори овог рада наводе 18 врста из 29 фитоценолошких снимака.

Детаљан попис маховина на подручју Фрушке горе није још увек завршен. Узевши у обзир да се овај регион одликује великим биодиверзитетом, добро развијеном хидролошком мрежом и веома комплексним саставом земљишта и стена, свакако заслужује даља бриолошка истраживања.

КЉУЧНЕ РЕЧИ: Војводина, јетрењаче, маховине, Србија, Фрушка гора

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PHYSICIANS IN SREM IN XVIII AND XIX CENTURY: DISTINGUISHED BOTANISTS

ABSTRACT: It was necessary for the first physicians to have the knowledge of plants, because phytotherapy was an integral part of medicine from the very beginning. For this reason, botany was a significant part of the curriculum at medical faculties in the XVIII and XIX century. Some professors at these faculties were known in international scientific circles as prominent botanists (Pál Kitaibel, August Kanitz, Giovanni Scopoli, Carl von Linné, etc.). After the liberation from the Turkish rule, flora was insufficiently explored in the territory of today's Vojvodina, which made it an interesting area for botanical studies undertaken by science professors from the universities in Vienna, Pest, and Cluj. A significant contribution to their scientific work was given by researchers from Srem, who in addition to their medical and pharmaceutical work practiced also botany. Some of them had their results published in publications and some became members of the European scientific natural history societies (Andreas Budai, Georgius Streim, Bartholomäus Emmanuel Godra, Mathias Kirchbaum, and Andreas Wolny).

KEYWORDS: botany, medicine, physicians, Srem, Vojvodina, herbarium

INTRODUCTION

Physicians had knowledge of plants from the distant past. The reason for this was the need for mastering the knowledge of plants which, prepared in a special way, were used for treating patients. Even the ancient physicians Hippocrates, Theophrastus, and Galen wrote in their works about herbal therapy. Dioscorides's work on medicines *De materia medica* represents an encyclopedia of ancient botany, pharmacognosy and pharmacology.

Medieval medical schools of Salerno and Montpellier had a significant impact on Serbian medieval monastic medicine. For pharmacological manuscript of *Hilandarski medicinski kodeks* (Hilandar Medical Codex), Relja V.

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Katić, our famous historian of medicine, using comparative analysis proved that it was written under the strong influence of Matthaeus Platearius's *Circa Instans* and *Antidotarius* by Nicolaus Praepositus. *Hilandarski medicinski kodeks* deals mainly with herbal drugs, their composition, pharmacodynamic properties and method of use. For pharmacotherapeutic part of *Hodoški zbornik* (Hodoch Code) was determined to represent an adapted version of *Practica Brevis* by Johannes II Platearius, a well-known professor at medical school of Salerno. In 1749, protomedicus of Austria and the personal physician of Empress Maria Theresa issued a letters patent which initiated reforms of Medical faculty in Vienna. He founded new institutions and departments, as well as botanical garden, acquired various collections and raised the reputation of the faculty to the leading position in Europe (Maksimović 1995). At that time, rationalist and educational work of Zaharija Orfelin was of particular importance for familiarizing uneducated Serbian people with the basics of natural sciences. To this end, in 1768 he launched *Slavenoserbskij magazin* (Slavonic-Serbian Magazine), the first magazine among the South Slavs in which, in addition to literary contributions, there were also articles in natural sciences. The magazine ceased publication after the first issue, but Orfelin published in 1783 his *Večnij kalendar* (Perpetual Calendar) with many articles in physics, geography, astronomy, meteorology and hygiene, as well as *Iskusni podrumar* (The Experienced Wine Cellar Owner) with short list of plants and herbs in Latin and Serbian.

Zaharija Stefanović was born in Vukovar in 1726. His education was irregular and he was largely self-educated. He often changed his place of residence and types of jobs. He was a teacher at Illyrian school in Novi Sad, then a clerk (scribe) in the office of Metropolitan Pavle Nenadović in Karlovci, where he founded his copperplate printing shop, and in 1762 he stayed in the Rakovac Monastery. In 1764/65 he worked in Venice in a printing shop for Cyrillic books, then again in Karlovci, where he was engaged in viticulture and where he created his works. As he could not make a living from copper engraving craft he lived for some time in monasteries Beočin, Grgeteg and Velika Remeta, with the monks who were openly hostile to him because of his rationalist enlightenment ideas. In 1783/84 he worked in Vienna as a press corrector in Kurzbeck printing shop. Gravely ill, he returned from Vienna to Novi Sad, and in 1785 on the estate of Bishop Josif Jovanović Šakabenta died of tuberculosis in material poverty. He was buried at the Almaško cemetery in Novi Sad.

For Orfelin's work *Iskusni podrumar* (published in Vienna, 1783) (Figure 1), Jovan Tucakov believed that, although primarily designed for winegrowers, it was "a real array and plentitude of encyclopedic information about pharmacognosy, pharmacodynamics, pharmacology, galenic pharmacy, ecology and botany". At the end of the book was added a list with 250 names of herbs, different roots and other plant parts that were used for the preparation of medicinal and other beverages. This was of great importance for the development of Serbian botanical terminology. Orfelin was the first who made a scientific classification of medicinal and related herbs and beside Latin names he gave also traditional, Serbian names. The book had four editions: Vienna 1783, Buda 1808, Pančevo 1874, and Pančevo 1885; and two phototype editions of the original

from 1885: Belgrade 1986 and 2010. Orfelin spent a lot of time in Fruška Gora Mountain, where he studied the floristic diversity, and especially medicinal herbs.

Figure 1. Orfelin's *Iskusni podrumar* (The Experienced Wine Cellar Owner).

Among Orfelin's books which were abandoned for many years in the Cathedral Church in Karlovci and transferred to the Patriarchal library at the beginning of the 20th century, Dimitrije Mita Kostić found a herbarium in 1921, which Orfelin himself called *Veliki srpski travnik* (Great Serbian Book of Herbs). According to Kostić, it is an updated and revised German edition of Elizabeth Blackwell's *Herbarium Blackwellianum* (1754–1773), in which Orfelin, beside German and Latin, added Serbian name of each plant. Zaharija Orfelin untied this German-Latin edition with 600 color copper plates and had it bound again in three volumes. He also added Serbian plant names in the top left corner. For the first seven illustrations he gave a description of each plant: genus, charecteristics, where it can be found and the way of preparation. Because of all that, Jovan Tucakov considered Orfelin a pioneer of Serbian botany, herbal medicine, pharmacognosy and pharmacology. Kostić and Tucakov agree in opinion that Orfelin intended to revise this famous European *Herbarium*, which was one of many similar items in scientific circles of Europe at that time. He wanted to publish it as Serbian edition, especially because Orfelin himself was an excellent engraver and possessed in his library extensive botanical literature. That he started to work on it is evidenced by separate numeration of copper plates with Slavonic-Serbian names of plants. What prevented him to finish it has still remained unknown (Maksimović 1995; Tucakov 1971; Kostić 1921) (Figure 2).

Figure 2. Plantain from Orfelin's *Veliki srpski travnik*
(Great Serbian Book of Herbs)

By order of Empress Maria Theresa, protomedicus of Austria Dr. Gerard van Swieten organized in 1769 the establishment of the Medical Faculty in Nagyszombat (today Trnava in Slovakia). Classes were organized according to the model of the Medical Faculty in Vienna, and in compliance with the then rationalist and educational tendencies that were more inclined to natural sciences. There were five departments altogether: *Institutiones* (general subjects), *Anatomia*, *Chirurgia*, *Botanica ac Chymia*, and *Praxis medica*. After the Medical Faculty moved from Nagyszombat to Buda and then to Pest, the organization of teaching remained almost the same. The Department of Botany and Chemistry was one of the most important at the mentioned medical faculties in the XVIII and XIX century. The Department of Pharmacy was established in 1772 (Popov and Antić 1975). In order to learn pharmacotherapy properly, which at that time was based on prescribing medicines and making them of medicinal herbs, a compulsory subject was botany. Therefore, there were many medical doctors who, apart from being physicians, were also actively engaged in the study of botany, and some of them dedicated themselves fully to it.

DISTINGUISHED BOTANIST PHYSICIANS – RESEARCHERS OF FLORA IN VOJVODINA

Pál Kitaibel was a Hungarian botanist, chemist and doctor. He was born in Nagymarton (Ger. Mattersburg), in western Hungary (now Austria) on 3 February 1757. He attended grammar school in Sopron (Ger. Ödenburg) and lyceum in Györ. He studied medicine at the University of Buda, after unsuccessful attempts to study law and theology. After medicine, he studied chemistry and botany. He graduated from Medical Faculty in 1785 when he was 28. After the death of his professor Josef Jacob Winterle, Kitaibel took over the professor's position in 1809 and taught chemistry and botany in Pest. He was also a director of the Botanical garden in Pest. In the period between 1795 and 1815 he carried out 16 research journeys in which he explored the world of plants. Beside plants, he also studied the properties and quality of soil, occupations and illnesses of the local population, minerals, healing mineral waters and economy of the areas that he visited. His biographers speak of him as a botanist, petrographer, and seismologist. Over the two decades, he systematically examined and described 150 mineral springs. He traveled by horse-drawn wagon, carriage, on horseback and on foot. In 1794, he visited Rijeka (Fiume), Trieste and the province of Venice including Piave, and in 1795 he collected a new type of mallow near Vukovar, Beočin and Sremski Karlovci (perhaps together with Budai, *author's note*). In Slavonia, the same plant he located in the vicinity of Našice. It was later named after him *Kitaibelia vitifolia* (Kadivka in Serbian). The following animals and plants were named after Kitaibel: *Ablepharus kitaibelii* (European copper skink), *Cardamine Kitaibelii* (bittercress), *Knautia kitaibelii* (dipsacles), and *Aquilegia kitaibelii* (columbine). Kitaibel went round Baranja in 1799, Fruška Gora Mt. in 1800, and stayed in the vicinity of Zemun. He studied plants also in Banat, Deliblatska peščara, and near Vršac and Oraovica. It can be said that he went across almost the entire territory of today's Vojvodina. In 1802, along with his friend and collaborator Waldstein and cartoonist Schütz, he went on a journey through Croatia, which successfully completed visiting Zagreb, Karlovac, Korenica, Plitvice lakes, and mountains Plješevica and Velebit. They brought a large number of new species of plants, which he planted in the Botanical garden in Pest. He traveled to the High Tatras with Waldstein, and in 1803 spent four months in the Carpathians. In 1798, Kitaibel and Waldstein visited in Berlin Carl Ludwig Willdenow (Berlin, 1765 – Berlin, 1812), a leading botanist of the time. They both sought the advice on a joint scientific botanical work that they were preparing. As a sign of respect to these two researchers, Willdenow introduced in nomenclature new names for the mallow from Srem and the finger-like plant of Mid-Hungarian mountains: *Kitaibelia vitifolia* (chalice flower) and *Waldsteinia geoides* (barren strawberries) (Grmek 1963).

Kitaibel's friend and patron **Franz Adam von Waldstein** (Wartenbur, 1759–1823) was an Austrian officer, Knight of Malta, participant in many battles against the Turks, and the royal chamberlain. After the wars, he dealt with the economics and devoted himself to botany. He had good powers of observation

and a private opinion. His extensive herbarium and botanical literature are kept at the Czech National Museum in Prague. Together with Waldstein, Kitaibel published a major work of classical botanical literature in three volumes: *Descriptiones et icones plantarum rariorum Hungariae* (Wien: M. A. Schmidt, I–III, 1800–1812). The book was written in Latin, modeled on Linné, and could be found in many European bookstores of the time. Kitaibel's contemporaries in Srem were Andreas Budai and Andreas Wolny. Although Kitaibel did not mention them in his writings, it is very probable that during his travels through Srem he was in contact with them and used their botanical contributions for his herbarium and his books. He died on 13 December 1817 in Budapest (*Magyar Lexikon* 1885; Javorka, 1957; Both 2009; *Österr. Biograph. Lexikon* 1965) (Figures 3–6).

Figure 3. Pál Kitaibel (1757–1817)

Figure 4. Stamp representing Pál Kitaibel and chalice flower (*Kitaibelia vitifolia*)

Figure 5. Franz Adam von Waldstein (1759–1823)

Figure 6. Carl Ludwig Willdenow (1765–1812)

August Kanitz was an Austro-Hungarian botanist. He was born in Lugoš, in Banat, on 25 April 1843. At the University of Vienna he studied medicine and natural sciences. He received his Ph.D. degree in 1869 from the University of Tübingen. For a short time he was a science teacher at the Agricultural

High School in Altenburg. For the purpose of establishment of botanical garden and museum at the University he visited Italy in 1871. Already in 1872 he became professor of botany at the newly founded University of Cluj (Klausenburg) where he established botanical garden and herbarium, which was based on a deep knowledge of professional literature, and especially the flora of Romania and Bosnia and Herzegovina. He was the University president (rector) in 1887/88 and the dean of the Faculty twice (1881/82 and 1895/96). He was the first to describe the Hungarian history of botany (*Geschichte der Botanik in Ungarn*, Hannover–Pest 1863). With his friend Josef Armin Knapp, a student of medicine, Kanitz visited the region of Slavonia and Srem in 1864 studying plants. In addition to botanical work, which he presented in one of his publications, Kanitz pointed to the work of prominent physicians, pharmacists and science teachers from Srem and Slavonia and their contribution to botany. Among other things, he said: "Dr. Andreas Budai, a physician in Srem County, for *Plantae sirimienses* in Kitabel's *Geographia botanica* donated 100 plants collected in the vicinity of Vukovar". Kanitz especially praised the work of Andreas Wolny, a botanist and director of the Karlovci Grammar School. For the work of Wolny's successor as the Grammar School director, Andreas Rumi, Kanitz said that his records were like unskilled compilation Wolny's work. Kanitz died in Cluj, on 13 July 1897 (Schulzer *et al*., 1866; *Neue Deutsche Biographie*, 1977; *Österr. Biograph. Lexikon*, 1965a) (Figure 7).

Figure 7. August Kanitz (1843–1897)

Josif Pančić (1814–1888), a prominent Serbian botanist and physician, was born on 5(17) April in the village of Ugrine near Bribir (Croatia). He attended primary school in Gospić, grammar school in Rijeka, and two years of philosophy in Zagreb. He graduated in 1842 from Medical faculty in Pest with doctoral thesis on systematics of plants ("Taxilogia botanica"). At the Univer-

sity of Vienna he attended lectures of a famous botanist Stephan Ladislaus Endlicher. In Vienna he met Vuk Karadžić, who suggested him to go to Serbia and study plants. Pančić arrived in Serbia already in 1846. He studied the flora of the Belgrade area and then put it into the book *Flora u okolini beogradskoj* (Flora of Belgrade surroundings) (Belgrade 1865), which with revisions had several editions in Serbian and Latin. Meanwhile, by order of the ministry he was sent to the glass factory in the village of Belica, Jagodina county, to work as a factory physician and fight against the epidemic of typhoid fever. Soon after that, in 1847, he was appointed a county physician in Jagodina, where he studied the flora of the area in free time. That same year he was transferred to Negotin, and later to Kragujevac, where he also was a county physician. At that time, he was also a spa physician in Bukovačka spa where he began studying mineral waters.

Figure 8. Josif Pančić (1814–1888)*

After the reform of the lyceum in 1853, the third department was established –for natural and technical sciences. Pančić then left Kragujevac but also medicine. He worked under contract as a professor of zoology, botany, mineralogy with geology, and agronomy at the Department of Natural Sciences. In 1863, when the lyceum was transfered into the Higher School, Pančić became full professor of the same subjects. Pančić founded the first natural science laboratories in the school, as well as the Botanical Garden in Belgrade. His large herbarium was the scientific research base for studying the flora of the Balkan Peninsula. He went across mountains in Serbia, Montenegro and Bulgaria. On his journeys he described about five thousand new plant species whose habitat was in the central parts of the Balkan Peninsula. He became known to a wider international and domestic public after he discovered and

* Engraved by Julius Cherny

described a relict spruce, which was named after him in the botanical literature – Serbian or Pančić spruce (*Picea omorika*).

Pančić published his first scientific papers in international journals in German language, because in Serbia at that time there were few professional journals ("Verzeichniss der in Serbien wildwachsenden Phanerogamen, nebst den Diagnosen einiger neuer Arten", *Verh. Zool.-Bot. Ges. Wien*, 6, 1856; "Die Flora der Serpentingebirge in Mittel-Serbien", Ibid. 9, 1859, etc.). As a professor at the the Higher School he published his most important works: *Flora Kneževine Srbije* (Flora of the Principality of Serbia) (Belgrade, 1872) and later *Dodatak flori Kneževine Srbije* (Supplement to the flora of the Principality of Serbia) (Belgrade, 1884), and the aforementioned *Flora u okolini beogradskoj*.

Josif Pančić was six times elected President of the Higher School, and in 1884 he was appointed member of the State Council. He was respected as a scientist, even after retirement, and he taught botany and managed natural sciences laboratory in the School and the botanical garden near the Danube until 1887. Serbian Royal Academy elected him its first president in 1887. He was twice elected Vice President of the National Assembly of the Kingdom of Serbia, in 1870 and 1871 (Vasić 2012). He was a full member of the Serbian Learned Society, a member of the Serbian Royal Academy, a member of the Matica Srpska in Novi Sad, honorary member of the Bavarian society "Pozychia", a member of the Education Council, a corresponding member of the Yugoslav Academy of Sciences and Arts, a member of the Hungarian Academy of Sciences in Pest, Institute of Geology and Zoological-Botanical Society in Vienna, Brandenburg Botanical Society, Cherbourg Society of Natural Sciences, and other biological and medical associations. He was a member of the Serbian Medical Society and one of its fifteen founders. After the outbreak of the Serbo-Turkish Wars (1876-1878) and the Serbo-Bulgarian War (1885/86) he assumed the position of manager of the Reserve military hospital of Serbian Red Cross in Belgrade. He was awarded the Order of St. Sava, the Order of the Cross of Takovo of the third degree and the Order of the Serbian Red Cross (Grmek 1951; *Javor* 1888; Stanojević 1972; Živanović 1893; Đorđević 1897).

Josif Pančić often visited Srem and Karlovci. During one visit to Karlovci and Karlovci Grammar School in 1857 Pančić inspected Wolny's herbarium and corrected some minor errors in determination of plant species. Today, it is known that a part of this herbarium belonged to Pančić including over 400 plant species.

Josif Pančić died in Belgrade in 1888 and was buried in the Old Belgrade Cemetery. Three days before his death, though seriously ill, he sent to the Academy, as its president, his last epistle. In order to fulfill this scientist's wish, mountaineers in cooperation with the Serbian Academy of Sciences and Arts and the Serbian Geographic Society on 4 July 1951 transferred his remains to a crypt in a mausoleum on the highest peak of Kopaonik, which was then renamed Pančić's peak (*Planinarski glasnik* 1999).

Dr. Sava Petrović was born in Šabac on 14 January 1840. He was a physician and botanist, doctor of medicine and surgery, personal physician of King Milan, and medical corps colonel. He finished elementary school and lower

grammar school in his hometown, and upper grammar school and two years of lyceum in Belgrade. He studied medicine in France. At first, he was a county physician in Kruševac, and then he was appointed a military physician in Belgrade. He became a personal physician of King Milan Obrenović who helped him with studying the flora in the area of Niš. Although he was a physician, he dedicated himself to botany and pharmacognosy. On his initiative, King Milan gave an estate in Palilula to the Higher School to establish the Botanical Garden "Jevremovac". He is the author of the following books on botany: *Flora okoline Niša* (Flora of the Niš surrounding area) (1882) *Lekovito bilje u Srbiji* (Medicinal herbs in Serbia) (1883), and *Dodatak flori okoline Niša* (Supplement to the flora of the Niš surrounding area) (1885). In 1884, in the vicinity of Niš, he discovered an endemic species *Ramonda nathaliae* (Natalie's Ramonda or cookie), which he described and named in cooperation with Pančić. He was a full member of the Serbian Learned Society (1869) and corresponding member of the Serbian Royal Academy (1889). He died in Belgrade on 20 January 1889 (Stanojević 1972; Stanojević 1992; Ranđelović 1998).

PHYSICIANS IN SREM – BOTANISTS

Throughout the whole XVIII and XIX century the region of today's Srem was divided into two administrative territories. Srem military district was located along the Sava River and was a part of the Ninth Petrovaradin Border Infantry Regiment based in Sremska Mitrovica, under the direct administration of the Court War Council in Vienna. The other administrative territory, the civil part of Srem (*Provincial*) belonged to the Srem County. The seat of the county was in Vukovar. Each of the two administrative territories had its own special organization of health service. Physicians who woked in the military district graduated from the Josephinum Military Academy of Medicine and Surgery, while in *Provincial* the health service was performed by physicians who graduated from Vienna or Pest Medical Faculty. The seat of the main county physician was in Vukovar (Maksimović 2007).

Andreas Budai was born on 30 November 1759, in Garta in Sopron County. He was of Hungarian nationality. He was a medical doctor, and a physician of Srem County for 41 years. He graduated in 1789 from the Pest Medical Faculty, where he gained his veterinary diploma in the same year. He was appointed a county physician in Vukovar in 1789. In the first few years after the appointment of Dr. Budai for county physician, Srem was hit by a severe plague epidemic, which from July 1795 to February 1796 devastated almost the whole county. Budai spent 12 months in Irig, in the center of the epidemic, but did not get sick, probably because he treated himself with acetic solution. His work and commitment greatly contributed to combating this pest, and his observations about patients and history of the illness was taken over by the University of Pest Professor Dr. Franz von Schraud, who was in charge of the anti-epidemic protection measures during this epidemic. He published them in his well-known work *Historia pestis Syrmiensis*. In addition to the fact

that during many years of work he gained sympathy of the common people, especially when providing assistance during the plague epidemic, Dr. Andreas Budai was respected in scientific circles as an excellent botanist. August Kanitz said that Dr. Budai collected and described about 100 medicinal herbs in the area of Vukovar, and that his herbarium entered the collection "*Plantae Syrmiensis*" in Kitaibel's *Geographia botanica*. Later, Sulzer, Kanitz and Knapp, who studied the flora of this area, when mentioning Kitaibel's fundamental works in this field in their study *Die bisher bekannten Pflanzen Slawoniens*, pointed to Andreas Budai and Andreas Wolny, Kitaibel's contemporaries, who were meritorious researchers of the flora of this region. For his merits and his long-time work Budai was awarded the Grand Gold Medal. He died in Vukovar on 4 March 1816. On his tombstone, which is stored in the City Museum in Vukovar, there is the epitaph: "reLIqVIIs anDreae bVDay phisICI De peste CIVIbVs moto grata fILIa ponIt". When the numerical values of the capitalization on the epitaph are summed up, the year of his death is obtained (Javorka, 1957; Schulzer *et al.*, 1866; Mićić 1968; Mićić 1987; Dorn 1976 Maksimović 2015; Mikić 2004).

Georgius Streim was born in 1803 in Novi Sad, in Bačka County. He was a Catholic. He graduated in 1829 obtaining the title of medical doctor. He moved from Pest to Vukovar in 1829. That same year, the regency demanded that county physicians examined the natural resources of counties in which they were serving ("wild plants, mineral and animal substances, and mainly those that can be used for healing or for technical purposes"). The County entrusted the task to Dr. Georgius Streim, who was then a private physician in Vukovar, "because one physician (Budai) was very old, and the other (Furjaković) suffered from podagra". Shortly after that, in 1831, Streim was appointed secondary physician, and in 1846 a regular county physician. In 1843 he was elected a member of the Royal Medical Society in Pest, and in 1845, a regular member of the Hungarian Society of Natural Sciences. He successfully organized the defense and prevented the spread of cholera (1831). Among other things, Georgius Streim actively practiced botany. Kanitz mentioned that Streim gave his observations about the flora of Srem to botanists of the time. When counties of Srem and Virovitica were merged in 1855, Dr. Georgius Streim moved from Vukovar to Osijek (Schulzer *et al.*, 1866; Mićić 1968).

Mathias Kirchbaum was a pharmacist in Vukovar. He was a son of Felix Kirchbaum, owner of the first pharmacy in Vukovar. Mathias was born in Vukovar on 4 March 1796. He graduated in pharmacy in Vienna in 1826, but was also interested in botany. There were a large number of professional botanical books in his library. He was a member of the Zoological-Botanical Society in Vienna (Verhandlungen der Zoologisch-Botanischen Gesellschaft in Wien) from 1864. He collaborated with a well-known Austrian botanist August Kanitz during his stay in Srem in 1864. Kanitz mentioned him as a renowned pharmacist and a connoisseur of the flora in the area of Vukovar, who informed him about many curiosities and peculiarities of the flora in that area. Mathias died on 19 May 1868 (Schulzer *et al.*, 1866; Mićić 1968).

Andreas Raphael Wolny was born on 10 December 1759 in Chemnitz, in Slovakia. He studied medicine for a short time. In his native town he finished grammar school and then continued his education in Pozsony (Bratislava), where he started medicine, and continued with science and pedagogy. It can be assumed that Wolny started practicing botany during his student days. His older compatriot Stephani Lumnitzer (1749–1806), the author of *Florae Posoniensis* (Leipzig, 1791), adviced Wolny on collecting plants. Upon his arrival in Karlovci, Wolny already had quite an extensive herbarium. At the invitation of Metropolitan Stratimirović and recommendation of Professor Kreil, the Patronage of Karlovci Grammar School appointed Wolny in 1794 to the post of the grammar school professor, and soon after that he was appointed director of the school (Figure 9).

Figure 9. Andreas Wolny (1759–1847)*

During his office in the Grammar School natural sciences had priority. Introducing object-teaching method, he founded natural science laboratories. For two years he compiled a mineralogical collection of 800 pieces of minerals and rocks, and in the area of Karlovci and around Srem he collected plants for his herbaria.

Before Andreas Wolny's herbarium, for teaching purposes in Karlovci Grammar School was used *Herbarium Blackwellianum* from Nuremberg – the first to contain pressed plants and not drawings as before. Beside this herbarium, Andreas Wolny began with the formation of his own herbarium. Next to each plant he wrote its Latin and German name, and next to some of them

* Oil on canvas: unknown author: about 1815 (City Museum of Novi Sad: Sremski Karlovci Homeland Collection, Inv. no. U-46)

also their Serbian name. There is an opinion that the traditional names of some plants in the herbarium were written by Zaharija Orfelin. In 1797 Wolny compiled the first volume ("centuria") of his work *Florae*. One herbarium, which was considerably damaged over time, is now part of the natural science collection of Karlovci Grammar School (*Florae Syrmiensis seu Plantarum in Syrmio sponte nascentium anno 1797. Centuria I et III*), and the other two are placed in museum in Budapest (*Notata botanica ad floram Hungariae et Syrmii spectancia* и *Specimen florae Carloviciensis*). The second centuria from Karlovci was lost during the World War I. In his book *Die bisher bekannten Pflanzen Slavoniens*, August Kanitz, according to Valentin Karl, marked by asterisks all the plants that were in Wolny's herbarium in Budapest (Figure 10). Beside Wolny's "centuriae", in Karlovci Botanical Collection there is a large number of plant specimens contributed by scientists outside Sremski Karlovci. As already stated, the greatest contribution was the one of Josif Pančić with about 400 plants. He examined some herbaria, including Wolny's, during a visit to Karlovci.

Figure 10. Herbarium Wolnyanum

In scientific circles Andreas Wolny was highly respected as a botanist, chemist and mineralogist. The Regensburg Botanical Society (1803) and the Mineralogical Society of Jena elected him an honorary member. In Buda, he published *Historiae naturalis elementa* (1805).

Because of poor financial situation, but already respected as a chemist, he accepted the position of director of a factory in Muszay in Beres County and moved there. After two strokes, he died on 17 October 1827 in Muszay (Petrović 1991; Perić *et al*., 2013; Marčetić and Babić 1952 Jacob, 1999; Stojšić 2010).

Some of Wolny's friends in Karlovci also practiced natural sciences, with a special emphasis on **Ioseph Schanc**, a magistracy clerk in Karlovci. He was Wolny's friend and companion in botanical and mineralogical excursions. He showed great interest in botany, mineralogy and geology and he himself also collected plants for the herbarium. Even today, in the Karlovci Grammar School Botanical Collection there is a herbarium of Ioseph Schanc. Spreading of industrial plants did him credit, so in 1813 he was appointed lieutenant of economics profession in the Military Frontier.

Zuzana Bunke in one her works raises the question of how, when and where Andreas Wolny and Pál Kitaibel met. One possibility is that it was near Pest and Buda, where Kitaibel was assistant in the botanical garden at the University. It seems, however, that Wolny collected plants on his own, and that Kitaibel studied Wolny's herbarium later and put his inscriptions on 66 Wolny's labels and expanded some of them. It is obvious that they were in contact – just by correspondence or personally, it remains unknown.

Studying "Herbarium Wolnyanum" Bunke indicates that for some time Wolny was focused on collecting medicinal herbs, which could be connected with his interest in pharmacognosy, which dates back to his student days, and in medicine, which was evident in introduction of strict hygiene measures and regulations in Karlovci Grammar School at the time of his directorship, concludes further Zuzana Bunke.

Andreas Wolny selflessly helped young researchers and scientists. Bunke cites the example of Joseph Sadler, a young assistant at the Department of Botany at the University of Pest, who defended his dissertation on ferns in 1820. On that occasion, Wolny sent him a multitude of details from his herbarium. For some specimens he even mentioned the place where he had found them, for example: "Ad Carlowitz in Syrmio, in Silva Strazsilovo frequens, item in Slavoniae silvis; in subalpinis AD Erdöd Comitatus szatmariensis" for hart's-tongue fern (*Asplenium scolopendrium*), or "in Rimis saxorum Petrovaradini, et eorum qui iter ex Alba Ecclesia ad Moldaviam facientibus ad sinistram Danubii portam assurgunt. Septemvri, 1817" for rustyback fern (*Asplenium ceterach*) (Bunke 1996).

Karl Georg Rumy was appointed director of the Karlovci Grammar School after Andreas Wolny left. He was born on 18 November 1780 in Spišská Nová Ves (Slovakia) (Figure 11). He spoke Slovak, German, Hungarian, Latin, Greek and Serbian. He practiced in philology, history and natural sciences. Rumi had the intention to continue the work of Wolny on collecting rare flora of Karlovci and Srem. In 1842 he wrote the work *Szerém éghajlata Szlavoniában. A magyar orvosok és természetvizsgálók nagygyűlésének munkálatai* (On the climate of Srem and Slavonia ...). Kanitz characterized this work as uncritical compilation of Wolny' work. Rumy had to leave Karlovci and Karlovci Grammar School in 1821. He died in Esztergom on 5 April 1847 (Schulzer *et al*., 1866; Petrović 1991; Kormošová 1998).

Figure 11. Karl Georg Rumy (1780–1847)

Bartholomäus Emmanuel Godra was a military physician. He was born on 18 June 1834 in Lalić (Lality) in Bačka. His parents were originally from Bohunice in Slovakia. They were teachers at Evangelical school in Lalić, and his father was a director from 1836. After graduating from high school, Bartholomäus studied medicine in Vienna. He graduated from the Josephinum Military Academy of Medicine and Surgery in Vienna in 1867. He was appointed military physician in Sremska Mitrovica, as it was the seat of the Ninth Petrovaradin Border Infantry Regiment in the the area of Srem Military Frontier. He was later promoted to senior military physician and the rank of lieutenant colonel. Apart from being a physician, Bartholomäus Godra was actively engaged in biological and botanical studies in particular, and because of it was distinguished in a broader scientific community. He became a member of the Royal Zoological-Botanical Society in Vienna (Mirković 1961; Zbirka Matičnih knjiga…, 1731/32; 1834; Rodokmeň rodu Godrovcov 2016; Segi 2010; Registry of the deceased Roman Catholics, office in Ruma, 1860–1878; Glesinger 1965).

Apart from the natural resources of the area of Petrovaradin Regiment where he lived and worked, he researched, studied and recorded the way of life and the state of health of the population in this region, as well as economic conditions, cultural and historical circumstances. As a result of his efforts, he published in 1873 in Zemun his most important work, a monograph on Srem (*Monographie von Syrmien: ethnographisch-topographische, kulturhistorische und statistische Skizze des Peterwaradeiner Grenz-Regimentsbezirkes: mit besonderer Berücksichtigung der in Syrmien kultivirten und wildwachsenden Glumaceen: mit einer Karte*) (Figure 12).

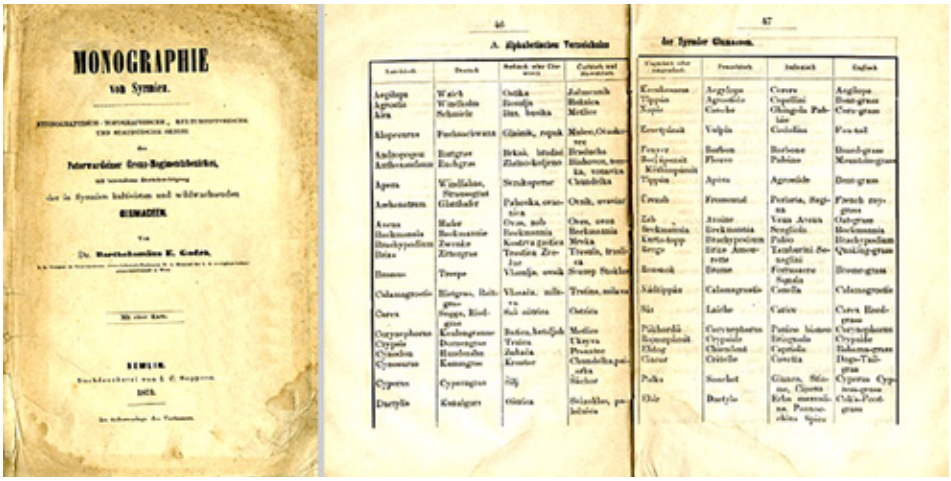

Figure 12. Front page of Godra's monograph on Srem and alphabetical index of Glumaceae of Srem

In the introduction to this book, which is very important for the history and botany of Srem, the writer explained that as a member of the Imperial Royal Zoological and Botanical Society in Vienna he was asked by the Committee to write a paper on botany for the World Exposition. He chose the field of botany which was still insufficiently explored, and which could be useful not only for the residents of the Regiment, but also for the general public. It was an overeview of the plants from this area, belonging to the grass family – then Glumaceae or Glumiflorae and today Gramineae or Poaceae. Given that this area was unknown from botanical perspective, he added a "drawn" herbarium of Glumaceae. It presented the division and description of grass in general, their vegetation units according to the place of discovery and flowering time chronologically given, then the tables of the harvest for the years 1870 and 1871, comparative tables of the weight of cereals, as well as the tables of the nutritional value of the types of cereals. The names of individual species were given in Latin, and for some species there were also traditional names in German, Hungarian, Czech, Slovak, Serbian (Croatian), French, Italian, English and Russian. At the end of the introductory part of the book the author pointed out that it was the love of science and culture of the area that motivated him to write this book, so if the esteemed readers received and evaluated the results of his work favorably, he would understand that his endevour, performed diligently and with joy, was successful. Godra's book is divided into five parts. The first part is devoted to ethnographic, topographical, cultural, historical and statistical description of the area of Regiment. In the second part of the book, entitled "Über Glumaceaen in allgemeinen" (On Glumaceae in general), the author describes the main characteristics of these types of plants, their wide dispersion, species richness and importance to human populations. The third chapter is devoted to the influence of different habitats on the vegetative forms.

The fourth chapter shows the flowering time of Glumaceae in Srem (March-May), as well as their systematics in Latin, German, Serbian or Croatian, and Slovakian language. The last, fifth chapter is devoted to the presentation of the results of harvest of cereals and other crops in Srem in 1870 and 1871, their nutritional value, as well as the need for cereals in the coming years (Godra 1873; Bentham and Hooker 1862–1883).

Bartholomäus Godra published his papers on botanics in periodicals *Obzor* (1870), *Österreichische botanische Zeitschrift* (1877), etc. (Dítě *et al.*, 2016; Mirković 1961; Glesinger 1965; Dugački 2012). Bartholomäus Godra died on 18 May 1874. He was buried in Ruma (Registry of the deceased Roman Catholics, office in Ruma, 1860-1878).

In the XVIII and XIX century, a large number of Czechs and Slovaks, some of them physicians, inhabited the area of the Military Frontier, as well as the civilian part of the southern Hungarian provinces, the territories of today's Vojvodina and Croatia. Many of them stayed in these areas and their good work built a great reputation. Some of them, in addition to being physicians, practiced botany as well. One of them was **Bohuslav Jiruš**. He was born in Prague in 1841. He graduated from Medical Faculty in his hometown in 1865. For a while he worked in the Prague General Hospital, and in 1870 he became an assistant at the Zoochemical institute of Prague. In 1875, Jiruš became a full professor of botany at the newly established University of Zagreb. He organized the Department of Botany and Physiology at the University and donated his herbarium with 10,000 species. He also organized a pharmacy course at the Faculty of Philosophy in 1882 where he taught pharmacognosy. In 1886, he left the position at the University of Zagreb and returned to his hometown, where he became a professor of pharmacology and pharmacognosy at the Czech University. During his stay in Zagreb, he published in professional journals a number of papers on botany and geology, which were results of his research journeys to Dalmatia. He was the editor of *Liječnički vjesnik* (Medical herald), and in 1883 he became a corresponding member of the Croatian Medical Association (Glesinger 1965).

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СРЕМСКИ ЛЕКАРИ XVIII И XIX ВЕКА: ПОЗНАТИ БОТАНИЧАРИ

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РЕЗИМЕ: Познавање биљног света било је неопходно још првим лекарима, јер је фитотерапија од самог почетка била саставни део медицине. Из тог разлога ботаника је била значајан саставни део програма наставе на медицинским факултетима још у 18. и 19. веку. Поједини професори ових факултета истакли су се у светским научним круговима као познати ботаничари (Пал Китајбел, Аугуст Каниц, Ђовани Скополи, Карл Лине и др.). Територија данашње Војводине након ослобођења од Турака што се биљног света тиче била је недовољно истражена, због чега је била интересантно подручје за ботаничка истраживања, која су предузимали професори природних наука Бечког и Пештанског универзитета и Универзитета у Клужу. Значајан допринос њиховом научном делу дали су и истраживачи из Срема који су се, поред свог лекарског и апотекарског посла бавили и ботаничким истраживањима. Поједини су своје резултате објављивали у публикацијама а поједини су постали чланови европских научних природњачких друштава (Андрија Будаји, Ђура Штрајм, Бартоломеј Годра, Матија Кирхбаум, Андрија Волни).

КЉУЧНЕ РЕЧИ: ботаника, медицина, лекари, Срем, Војводина, хербаријум

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ANATOMICAL AND MICRO-MORPHOLOGICAL ANALYSIS OF THE FRUIT AND VEGETATIVE ORGANS OF *Inula oculus-christi* L. IN THE PANNONIAN PART OF SERBIA

ABSTRACT: *Inula oculus-christi* L. is a perennial plant, 20–60 cm high, growing in south-east Europe, central and southern Russia and some parts of Asia. It belongs to Pontic--Pannonian floristic element. In Pannonian part of Serbia this species is extremely rare, with very few recently confirmed literature and herbarium data. According to the field data for the period from 2013 to 2015, only one population of this species was recorded in Pannonian part of Serbia, on the site of Rimski Šanac, which is threatened by anthropogenic activities. Furthermore, anatomical and micro-morphological features of *I. oculus-christi* have not been documented so far. Therefore, in order to get better knowledge of the biology this species, as well as on the basis of the abovementioned, its anatomical and micro-morphological characteristics were investigated. Cross sections of the leaf, stem, rhizome and fruit were obtained using cryotechnique procedure by Leica CM 1850 cryostat. Using a light microscopy, detailed descriptions of anatomical characteristics of the analysed organs were given. The scanning electron microscopy revealed specific qualitative features of leaf and fruit that characterize the species. Obtained data may be useful in determination of this species and represent the valuable contribution to its micro-morphological and anatomical differentiation from other congeneric and related species.

KEYWORDS: anatomy, fruit, leaf, rhizome, stem, Pannonian Plain, Rimski Šanac

INTRODUCTION

Inula L. is a genus with 11 species growing in Serbia (Gajić 1975). These plants are known as medical plants, and their aerial and underground organs are widely used for medical purposes (Topco *et al.*, 1993; Rafi *et al.*, 2005;

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Zhao *et al*., 2006). *Inula* species are of interest to the scientific community, and it is important to increase the available knowledge about the species in this genus. There are limited data in the literature on micro-morphological and anatomical features of *Inula* (Toma *et al*., 2010; Karanović *et al*., 2016).

I. oculus-christi L. is a perennial plant, 20–60 cm in height, belonging to Pontic-Pannonian floristic element (Gajić 1975). It grows in south-east Europe, central and southern Russia and some parts of Asia. Nevertheless, in Pannonian part of Serbia it is extremely rare, with very few recently confirmed literature and herbarium data. According to the field data for the period from 2013 to 2015, only one population of this species was recorded in Pannonian part of Serbia. It was recorded at the site of Rimski Šanac, which is threatened by anthropogenic activities. *I. oculus-christi* is well documented to have cytotoxic effects (Mossaddegh *et al*., 2006), amoebicidal effects (Degerli *et al*., 2011), and antioxidant, antimicrobial and DNA damage protection properties (Berk *et al*., 2011). However, anatomical and micro-morphological features of *I. oculus-christi* have not been documented so far. Therefore, anatomical and micro-morphological characteristics of this species were investigated in order to get better knowledge of its biology.

MATERIAL AND METHODS

Plant material was selected from native population found in Pannonian part of Serbia, at the site of Rimski Šanac, and was collected during the flowering period (June 2015), while the fruit was collected during the fruiting period (July 2015). After the plants were identified, their voucher specimens were deposited in Herbarium of the Department of Biology and Ecology, Faculty of Sciences (BUNS), University of Novi Sad (voucher number 2-2123).

Structure of the leaf, stem, rhizome and fruit was determined using light microscopy. Cross sections from the middle part of the analysed organs were obtained with Leica CM 1850 cryostat at a cutting interval of 40-50μm. Sections were observed using Image Analyzing System Motic 2000.

The micro-morphological characteristics of leaf and cypsela surface were observed using scanning electron microscopy (SEM). Five leaf samples and mature fruits from five different individuals were selected and dry samples were sputter-coated with gold for 180 s, at 30 mA (BAL-TEC SCD 005), and were subsequently viewed using JEOL JSM-6460LV electron microscope at 20 kV acceleration voltage.

RESULTS AND DISCUSSION

In anatomical terms, the leaf of *I. oculus-christi* had a dorsiventral structure (Figure 1A). Mesophyll was differentiated on palisade and spongy tissue. The palisade tissue consisted of elongated, rectangular palisade cells, rich in

chloroplasts and arranged in one or two layers. Large oil bodies were clearly visible in each palisade cell. Spongy cells were round to oval in shape, arranged in several layers. Collateral, closed vascular bundles, surrounded by parenchymatous sheath, were arranged in a line. The main vein was prominent on the abaxial side, and there was a vascular bundle with well-developed surrounding sheath. Epidermis was one-layered, covered with dense indumentum (Figure 1B and C). SEM analysis revealed the presence of two types of trichomes, on both epidermal sides: non-glandular, multicellular, uniseriate trichomes and glandular, multicellular, biseriate trichomes. Our results revealed that the leaf was amphistomatous, with anomocytic stomata (Figure 1D). In the taxonomy, anatomical and micro-morphological features of the leaf are used as discriminative characters at various levels. There are a number of studies on the foliar anatomy and micro-morphology of the family (Adedeji and Jewoola 2008; Karanović *et al*., 2015).

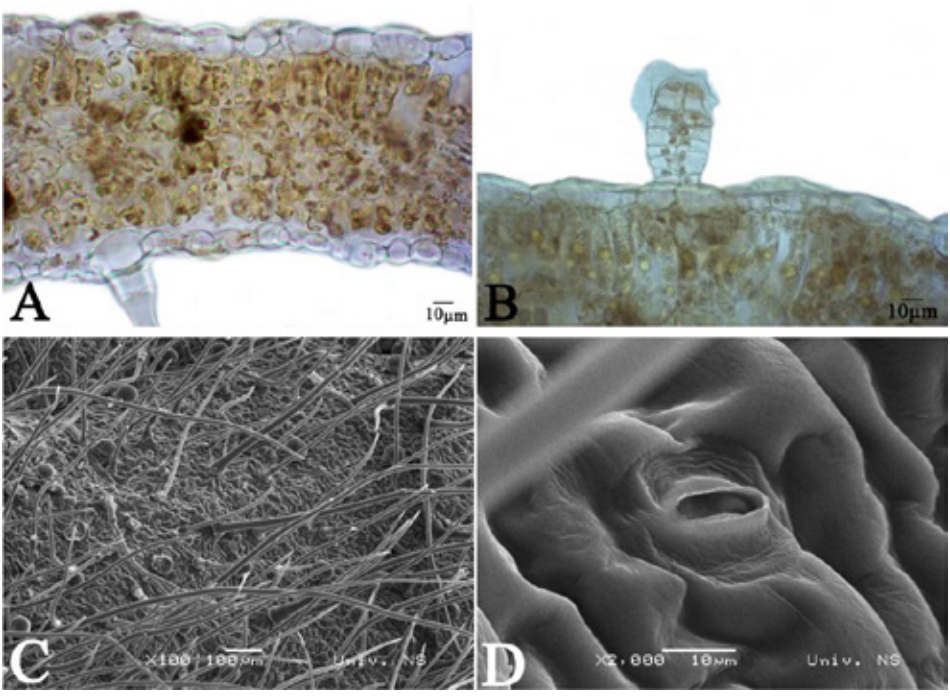

Figure 1. Light (a, b) and scanning electron (c, d) micrographs of *I. oculus-christi* leaf. a. Cross-section at the level of ¼ of the leaf width; b. Multicellular, biseriate glandular trichome; c. Non-glandular and glandular trichomes on the adaxial epidermis; d. Anomocytic stomata on the adaxial epidermis of leaf.

The stem cross-section was mainly round in shape (Figure 2A). The epidermis was composed of small cells with thickened walls, covered with cuticle and trichomes. The type of trichomes found on the leaf was present on the

stem. Under epidermis, one layer of collenchyma tissue was visible, in the form of regularly arranged cells (Figure 2B). Cortex parenchyma cells were arranged in six to ten layers, and the first three layers contained chloroplasts. Numerous vascular bundles were arranged in one row within the central cylinder. Large groups of sclerenchyma cells occurred next to phloem. The central part of the stem was filled with the large pith parenchyma cells.

The rhizome cross-section was regularly round in shape (Figure 2C). Its anatomy was similar to the stem, with the exception of trichomes and cortex chlorenchyma and collenchyma, which were absent. Contrary to the stem, central cylinder in the rhizome was less developed than the cortex. Moreover, in the rhizome cortex secretory ducts rare vascular bundles were observed (Figure 2C and D). Secretory ducts were situated in the internal layer of cortex, between sclerenchyma strands.

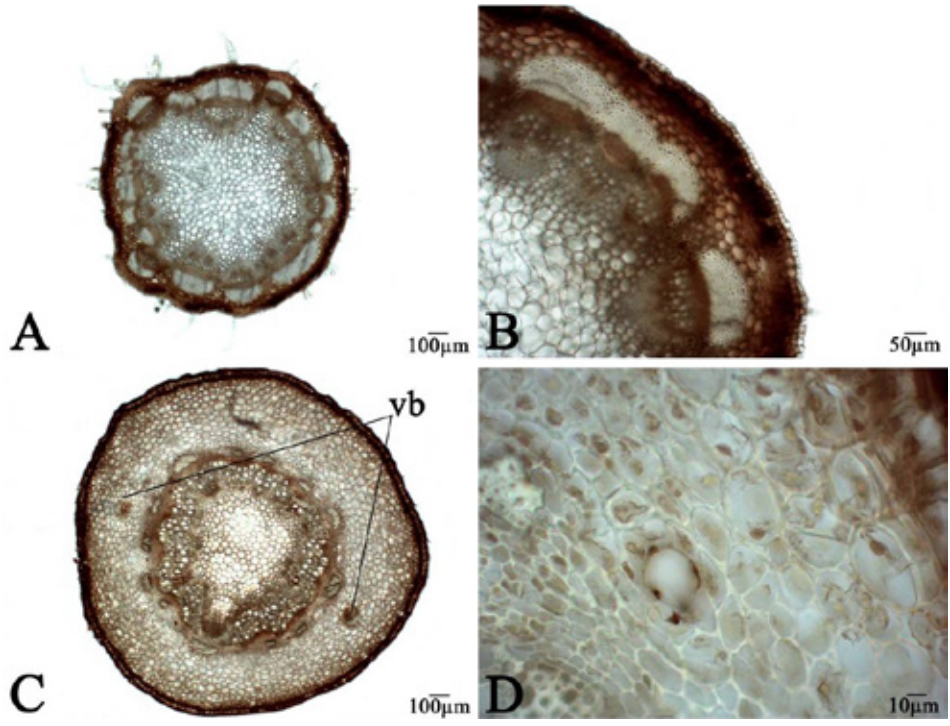

Figure 2. Light micrographs of *I. oculus-christi* stem (a, b) and rhizome (c, d) cross-section. a. Stem cross-section; b. Detail of stem cross-section; c. Rhizome cross-section; d. Detail of rhizome cross-section showing secretory duct. Abbreviations: vb – vascular bundles.

Despite the great importance of the fruit features in taxonomy, little data can be found in the literature on micro-morphological and anatomical characters of *Inula* fruit (Abid and Qaiser 2002; Jana *et al*., 2013; Karanović *et al*., 2016). The mature fruit of *I. oculus-christi* is differentiated into cypsela and pappus

(Figure 3A). Cypsela was narrowly-oblong with more or less conspicuous longitudinal ribs on its surface. Pappus was composed of capillary, barbelate bristles arranged in one whorl. The colour of mature fruit was light to dark brown. According to Karanović *et al.* (2016), the colour of the fruit depends on its ripeness and it cannot be used as reliable taxonomic character.

The epidermal cells of cypsela were rectangular and parallel to the longitudinal fruit axes (Figure 3B). Each epidermal cell contained an elongated, rhomboid, oxalate crystal, which is a characteristic of the subtribe Inulinae (Anderberg 1989). Numerous elongated twin hairs with acute tip, distributed all over the fruit surface, were recorded (Figure 3B). Twin hairs are characteristic features of Compositae, and their presence or absence on the cypsela surface is taxonomically very informative at lower taxonomic levels (Anderberg 1989).

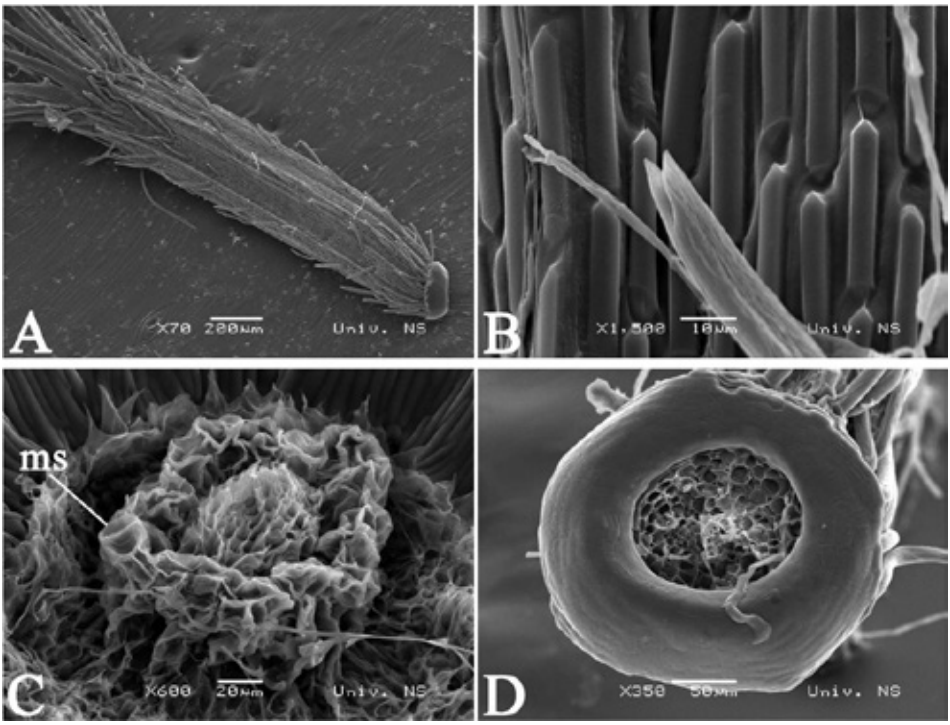

Figure 3. Scanning electron micrographs of *I. oculus-christi* fruit. a. Mature cypsela; b. Detail of pericarp surface with calcium oxalate crystals and twin hair; c. Detail of apical part of cypsela showing remnants of nectariferous tissue; d. Detail of basal part of cypsela showing carpopodium. Abbreviations: ms – modified stomata.

In the centre of the upper part of cypsela, nectary remnants are clearly visible surrounding the base of the style trace. Nectaries were irregular in shape with clearly visible invaginations (Figure 3C). The nectary epidermis contained modified stomata, with large pores between reniform guard cells (Figure 3C), through which nectar was secreted. At the basal part of cypsela, distinct carpopodium

could be seen as a complete, broad ring of cells with thickened outermost walls (Figure 3D). The importance of fruit epidermal cells, nectaries and carpopodial features in taxonomy is emphasised in numerous studies (Anderberg 1989; Bernardello 2007; Karanović *et al*., 2016).

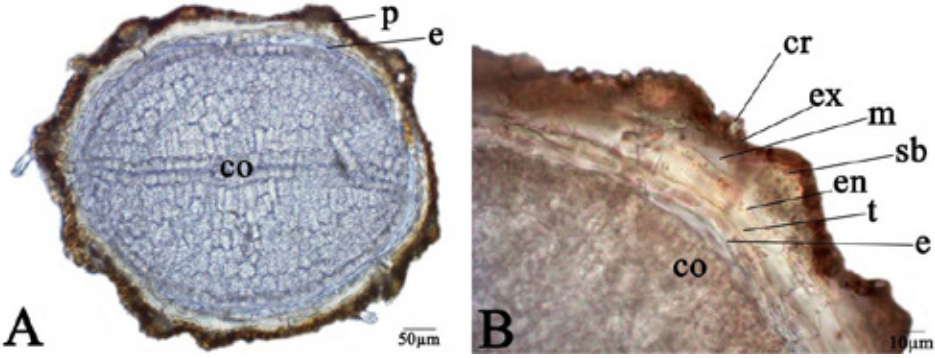

Figure 4. Light micrographs of *I. oculus-christi* fruit cross-section. a. Transverse section through the middle part of cypsela; b. Cypsela transverse section detail. Abbreviations: co – cotyledons, cr – crystal, e – endosperm, en – endocarp, ex – exocarp, m – mesocarp, p – pericarp, sb – sclerenchymatic bundle, t – testa.

The shape of cypsela cross section was circular with prominent ribs. The mature fruit was clearly differentiated into pericarp, testa, endosperm and cotyledons (seed lobes) (Figure. 4A). The pericarp comprised three zones. The outer layer, exocarp, and the innermost layer, endocarp, was uniseriate, made up of compactly arranged, cube shaped cells. The cells between these two layers formed mesocarp, which was mainly composed of parenchyma and sclerenchyma. Parenchymatous tissue was represented by one to three layers of cells. Sclerenchyma tissue was arranged in discrete longitudinal bundles, which were confined to the costae of the cypsela and protruding as ribs. The testa lost its cellular structure and it was represented by a layer of collapsed cells of yellow--brown colour, under the pericarp. Endosperm consisted of one or two layers of thickened, tangentially elongated cells which surround the cotyledons (Figure 4B). The cotyledons were centrally situated and occupied the major part of the cypsela.

CONCLUSION

The presented results on anatomical and micro-morphological characteristics of leaf, stem, rhizome and mature fruit of *I. oculus-christi* may be useful in determination of this species and represent a valuable contribution to its micro-morphological and anatomical differentiation from other congeneric and related species.

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АНАТОМСКА И МИКРОМОРФОЛОШКА АНАЛИЗА ПЛОДА И ВЕГЕТАТИВНИХ ОРГАНА ВРСТЕ *Inula oculus-christi* L. ЗАСТУПЉЕНЕ У ПАНОНСКОМ ДЕЛУ СРБИЈЕ

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РЕЗИМЕ: *Inula oculus-christi* L. је вишегодишња биљка, висока 20–60 cm, распрострањена на подручју југоисточне Европе, централне и јужне Русије, као и у појединим деловима Азије. Припада Понтско-панонском флорном елементу. У панонском делу Србије изузетно је ретка, са свега неколико хербаријумских и литературних референци о њеном присуству. На основу теренских података за период 2013–2015. године, само једна популација ове врсте забележена је на подручју панонског дела Србије, на локалитету Римски Шанац, који је угрожен антропогеним утицајем. Такође, анатомске и микроморфолошке карактеристике врсте *I. oculus-christi* до сада нису описане. Из тог разлога, у циљу доприноса бољем познавању биологије ове врсте, као и на основу горе споменутог, истраживали смо њене анатомске и микроморфолошке карактеристике. Попречни пресеци листа, стабла, ризома и плода, дебљине 40–50 μm, добијени су применом методе криотехнике помоћу Leica CM 1850 криостата. Детаљни описи анатомских карактеристика анализираних органа дати су применом светлосног микроскопа. Скенинг електронском микроскопијом установљени су специфични квалитативни карактери листа и плода који су карактеристични за врсту. Добијени подаци корисни су за детерминацију анализиране врсте. Такође, представљају и значајан допринос њеној анатомској и микроморфолошкој диференцијацији од других, сродних и њој сличних врста.

КЉУЧНЕ РЕЧИ: *Inula oculus-christi*, анатомија, плод, лист, ризом, стабло, Панонска низија, Римски шанац

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THE INVESTIGATION OF MORPHOLOGICAL CHARACTERISTICS OF WILLOW SPECIES IN DIFFERENT ENVIRONMENTAL CONDITIONS

ABSTRACT: Alternative or renewable energy is a modern strategy with a good perspective in the nearest future. There are several directions of renewable energy development that depend on climatic, economic and technological opportunities of a region. The perspective choice for areas with moderate climate is bioenergy. One of the bioenergy directions is agro forestry based on short rotation coppice plantations (SRC) of trees, like willow, poplar and others. The goal of experiments was the assessment of the potential of different willow species for the obtaining of energy in two climatic zones and on two types of soils of Belarus. For this purpose several morphological characteristics were metered: height of plants, biomass, diameter and number of sprouts.

The field experiments were conducted on two types of soils: post-mining peaty soils in Grodno region and on degraded peaty soils in Brest region of Belarus. The same soils are very problematic for growing of traditional agricultural crops, thus willow production is a good alternative for biomass production of energy as well as for the reclamation of these soils. In our experiments the following species of willow were tested (*Salix alba, Salix viminalis, Salix dasyclados, Salix aurita*) that may grow on peaty soils at the natural conditions. The most popular species for modern selection of SRC of willow is *Salix viminalis*. Nevertheless, the most suitable morphological characteristics on post-mining peaty soils were established for plants of *Salix dasyclados* and on degraded peaty soils for the plants of *Salix alba*. The unfavorable parameters at the both type of soils were identified for the plants of *Salix aurita*. However, it is necessary to take into account that the used species are more popular for natural wetlands and in our experiments plants have best results of survival of cutting and rates of growth at the beginning of vegetation. In accordance with these facts *Salix aurita* may not be used for energy plantation directly, but it is interesting for hybridization with other species. Our results have shown that perspective hybrids for peaty soils may be for instance *Salix aurita x Salix dasyclados* and *Salix aurita x Salix alba*.

KEYWORDS: bioenergy, willow, selection, species, morphology parameters

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INTRODUCTION

Alternative or renewable energy is a modern strategy with a good perspective in the nearest future. There are several directions of renewable energy development that depend on climatic, economic and technological opportunities of region (Caslin *et al*., 2010). For example, countries placed near the sea (like Sweden, Denmark, Germany and others) introduce wind energy. The largest photovoltaic station for the use of solar energy is situated in the desert in California State. The perspective choice for areas with moderate climate is bioenergy. One of the bioenergy directions is agro forestry based on short rotation coppice plantations of trees, like willow, poplar and others. The yield of willow biomass crops may achieve 10–15 tons of dried wood or 5–6 tons per hectare. Short rotation coppice plantations (SRC) were introduced in some European countries, the USA, Canada and others. The largest area of SRC is in Sweden, about 12,000 hectares (Rosenqvist *et al*., 2000). This experience testifies about special perspective of willow for introduction of energy plantations, because of intensive biomass growing and broad environmental flexibility.

The economic efficiency of energy crops may be supplied only in the case of high biomass yield. This aspect should be taken into consideration for selection of willow clones that have optimal morphological characteristics for fast biomass accumulation. Most parts of modern European clones were selected on the basis of several species of willow: *Salix viminalis*, *Salix schwerinii, Salix dasyclados, Salix purpurea* (Caslin *et al*., 2010). These species are primeval for Europe and broadly distributed in different ecosystems and they belong to bush types of willow (Parfenov 1986; Tuck 2006; Caslin *et al*., 2012).

Salix viminalis is a branchy shrub that is characterized by fast speed of growth (Levickij 1965; Skvorcov 1968). The height of plant may reach 5–6 or even 10 meters. It is the best species for braiding different handicrafts like baskets, furniture, floor mats and so on. *Salix viminalis* may also be used for soil and bank consolidation against erosion and as food for deer and other ungulate animals.

Salix dasycladus is more often characterized as a bushy type, but also sometimes as a tree type of willow. The height of branches is 5–8 meters, they are flexible and may be used for braiding. *Salix dasycladus* may be used for decoration of green zones, consolidation of soil and as bee plant.

The perspective species of willow that is not as often used for selection as it could be is *Salix alba.* This kind of willow is characterized as both a bushy and a tree type. *Salix alba* has a lot of valuable properties that separate it from other willow species. They are: fast speed of growth; quality of wood that may be used for braiding and construction; high content of salicylic acid and tannin in biomass and its bark may be used for medicine. *Salix alba* also has a huge potential as decorative tree (because of a nice appearance, it has specific varieties for this purpose), as forage for animals and bee plant.

Species of willow *Salix aurita* is particularly interesting for our research. This kind has not been actively used as a selection form, but may successfully grow in specific environmental conditions, like marshes, wetlands, disturbed

areas. *Salix aurita* is characterized by a lot of sprouts and has a huge potential for succession.

As we previously mentioned, these species of willow are adopted for European conditions, but in North America some other kinds are used. Schaff *et al*. (2003) investigate edaphic factors influencing the survival of black willow (*Salix nigra*) cuttings used for stream bank restoration. A two-year field study was conducted at Twentymile Creek, in northern Mississippi. *Salix nigra* is a tree type of willow with the height up to 30 meters and has a good quality of wood.

At a Northern Ontario location, effects of soil types on soil nitrate accumulation, spruce growth and willow growth were estimated (Thevathasan 2000). Willows (*Salix humilis Marsh*.) were established on three soil types (clay, loam and sand). The growth form of plant is a shrub (i.e., a woody plant with several stems growing from the base).

Labrecque and Teodorescu (2001) investigated two species of willow, *Salix discolor* and *S. viminalis*, under short-rotation intensive culture on two abandoned farmland sites: sandy site and clay site in Canada. They found that the best growth performance of two willows was obtained on the clay site. *Salix discolor* is a weak-wooded deciduous shrub or small tree growing to 6m (20ft) tall, with brown shoots. Like other willows, it contains salicylic acid and was used by Native Americans as a painkiller. This species is closely related to *Salix caprea* (European pussy willow).

In New Zealand, the several species and willow hybrids were investigated: *Salix matsudana* x *Salix alba* 'Tangoio', *Salix lucida* spp *lasiandra* x *Salix schwerinii* 'Kinuyanagi' and *Salix lasiolepis*. The best productivity was identified for *Salix matsudana* x *Salix alba* 'Tangoio' (McIvor 2005). *Salix matsudana* (Chinese Willow) is a species of willow native to northeastern China. It is a medium-sized to large, rapidly growing tree up to 6–8 meters tall.

The goal of our experiments was to assess the potential of different willow species for energy production in two climatic zones and on two soil types in Belarus. In the field experiments several morphological characteristics (height and mass of plants, diameter and number of branches) and survival of cutting of willow were metered. Morphological characteristics directly influence the yield of willow wood and the survival of cutting is a very important index because weeds hardly compete with plants during the first period after planting.

MATERIAL AND METHODS

The experiments were conducted in two climatic zones of Belarus. The field experiments are placed on post-mining peaty soils in Grodno region and on degraded peaty soils in Brest region of the country.

Brest region is situated in southwestern part of the country and is characterized by moderate mild winter and sufficient quantity of precipitations. Grodno region is a northwestern part of the country. The climate of this region is a little bit cooler and wetter (Average temperature ^{o}C/year=7.4; Average

humidity %=80) compared to Brest (Average temperature ^{o}C/year=8.2; Average humidity %=76).

The area of post-mining peaty lands in Belarus occupies more than 200 thousands hectares. That area was generated after finishing peat excavation for briquette production. Peat briquettes are the most popular local fuel in Belarus and the optimal use of post-mining peaty lands is one of the basic environmental goals. This type of soil is very heterogenic, poorly drained, with massive structure and poor contents of nutrients. At the result, it is very problematic to grow traditional agricultural crops on those areas that have special demand to soil fertility and perspective direction may be energy crops production such as the use of willows. The problem is absence of adequate technology of willow production for the degraded peaty soils.

Degraded peaty soils appeared as the result of non-optimal agricultural practice when wide-row agricultural crops like maize were grown for several years. The peaty layer decreases and sometimes infertile sandy layers go up to the surface. The area of this type of soils in Belarus is about 190 thousands hectares and the problem is how to conserve them in order to stop further degradation. The characteristics of soils are presented in Table 1.

Table 1. The characteristics of soil of experimental sites in Grodno and Breast region

The experimental site	Soil characteristics					
	Content of ash in the peat %	Decomposition of the peat %	NO_3 mg/kg	P_2O_5 mg/kg	K_2O mg/kg	pH
Grodno	9	65	79.40	20.25	106.40	5.20
Brest	12	45	45.03	220.71	150.1	5.6

The field experiment was designed as 25 m^2 plots in 4 repetitions with 1.40 x 0.70 m spacing. The clones of following willow species were investigated: *Salix viminalis*, *Salix dasycladus*, *Salix aurita* and *Salix alba.*

RESULTS AND DISCUSSION

In accordance with classification, willow is a moisture-loving plant and adequate water supplying is an important factor for its productivity. There are not many publications that cover successful experience of willow cultivation on post-mining peaty soils (Cooper 2000; Kovalchik 1991; Rodzkin 2007).

These lands can be characterized by poor structure and non-stable water supplying. Nevertheless, there is a special interest in willow production of bioenergy on the same type of soils. The reclamation of post-mining peaty soils, production of biomass for energy, supporting of biodiversity are important tasks both from economical and environmental point of view. For these soils, optimal choice of willow species is one of the basic factors. Two months after planting, the best parameters of growth (height of plants, diameter and number of sprouts) and survival were observed for *Salix aurita* (Figure 1).

Figure 1. Seedlings of *Salix aurita* two months after planting

The morphological characteristics of willow species that were grown on the experimental site on post-mining peaty soil after the first year are presented in the Table 2.

Table 2. Morphological characteristics and productivity of willow species after the first year on post-mining peaty soil

Species	Height of plants, cm	Number of branches per plant	Average Diameter of branches, cm	Average mass of the plant, g	Survival of cutting %
Salix viminalis	184.4	3.8	1.5	248.9	78.2
Salix aurita	176.1	4.2	1.3	241.6	89.4
Salix alba	186.2	3.5	1.6	249.3	81.5
Salix dasyclados	197.2	4.2	1.6	257.0	82.5
LSD_{05}	6.63	0.37	1.45	12.2	6.2

To the end of vegetation period the best results were identified for *Salix dasyclados*. The average height of plants of willow type *Salix dasyclados* was about 197.2 cm, number of branches per plant (4.2) and mass of the plant were also the highest, but not significantly important as compared to other types of willows.

The plants of *Salix alba* overcame *Salix aurita* and *Salix viminalis* by parameters of height, mass and diameter but had lower number of branches per plant compared to all species. The plants of *Salix aurita* that had had the best parameters in the beginning of vegetation gradually lost their advantage

and, as a result, their morphological characteristics were lower compared to other species.

In accordance with technology, the best time for harvesting of willow biomass for energy purpose is the third year after planting. The results of morphological characteristics metering are shown in the Table 3.

Table 3. Morphological characteristics and productivity of willow species after the third year on post-mining peaty soil

Species	Height of plants, cm	The diameter of branches, cm	The average mass of the plant, kg
Salix viminalis	347	25.3	2.28
Salix aurita	305	23.2	1.85
Salix alba	338	26.3	2.23
Salix dasyclados	356	25.6	2.39
LSD_{05}	14.7	0.76	0.23

The obtained results show that the highest parameters of height and mass of the plant were identified for *Salix dasyclados* after the first year. The largest diameter of stem was established for the plants of *Salix alba*. This fact may be explained by botanical characteristics of *Salix alba,* because as a rule this species is presented by tree type of plants and consequently has a larger trunk but not so many branches (Rodkin *et al*., 2013).

In accordance with the results, the most perspective species of willow for plantation on area of post-mining peaty lands is *Salix dasyclados.* Nevertheless, as we mentioned before, post-mining peaty lands are problematic for willow cultivation because of chemical and physical characteristic not always optimal for plant growing (Rodzkin *et al*., 2007). Under the same conditions, very important factors are survival rates of cutting and speed of growing at the beginning of vegetation. The fast rates of growing allow willow plants to be more competitive to weeds and it is very important, especially for peaty lands. It means that results of surviving and growing rates obtained for *Salix aurita* identify huge potential of this species. Non-optimal morphological parameters of *Salix aurita* within three years compared to other species signify that this species may not be used for energy plantation directly, but it is interesting for hybridization with other species.

Degraded peaty soils are more fertile compared to post-mining peaty lands. Our earlier results show that these types of soils are more suitable for willow production (Shkutnik *et al*., 2010).

The measurement results of the height of willows during the first year on degraded peaty soils are presented in Figure 1.

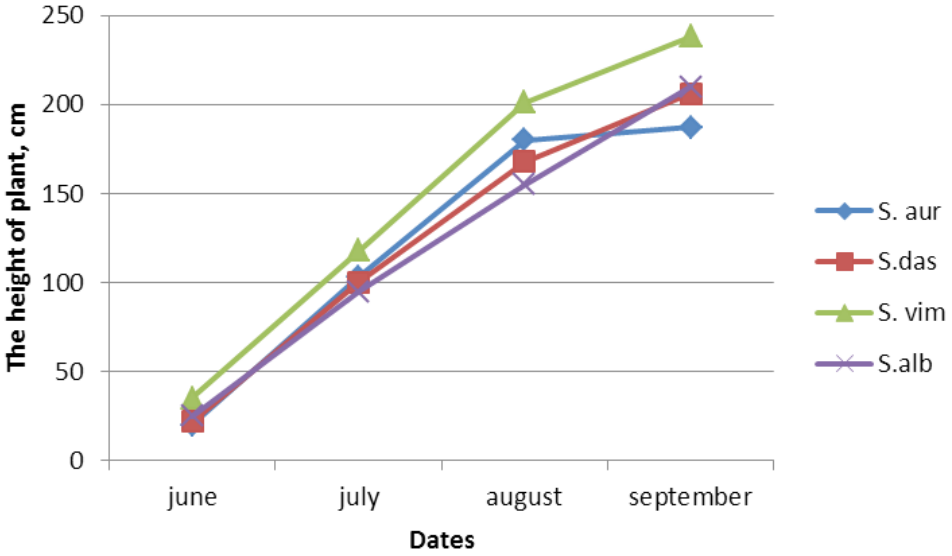

Figure 2. Dynamic of willow height on degraded peaty soils during the first year

After the first year the best results for height were established for willow species *Salix viminalis*. *Salix aurita* had smaller parameter of height to the end of vegetation, but at the beginning of vegetation the plants of this species advanced *Salix dasyclados* and *Salix alba.* It is interesting that plants of Salix *alba* at the first stage had been growing slowly, but to the end of vegetation were higher than *Salix aurita*.

At the following two years the plants of *Salix viminalis* advanced other species on the parameter of height, but the best parameters of diameter of stem and average mass were identified for the plants of *Salix alba.* The plants of *Salix aurita* were the least productive after three years (Table 4).

Table 4. Morphological characteristics and productivity of willow species after the third year on degraded peaty soils

Species	Height of plants, cm	Number of branches per plant	Diameter of branches, cm	Average mass of the plant, kg
Salix aurita	517.1	4.21	4.08	2.14
Salix dasyclados	595.5	4.19	4.57	2.63
Salix viminalis	597.9	4.37	4.67	2.72
Salix alba	581.0	3.91	5.22	2.95
LSD_{05}	17.92	0.29	0.36	0.56

The data presented in Table 4 show that after three years of cultivation, plants are extremely high and are ranked from 517 cm (*S. aurita*) to 597 cm (*S. viminalis*). *S. alba* plants had the best parameters of biomass and diameter of branches. Significantly lowest ranking for height, mass and diameter of branches

was identified for *S. aurita.* In accordance with these facts, *Salix aurita* may not be used for energy plantation, but it is interesting for hybridization because it has the highest survival rate of cuttings.

CONCLUSION

Morphological characteristics of SRC willow is the key factor for plants productivity. Our experiments have shown that the morphological parameters of willow plants depend on the species, types of soils and environmental conditions. The field experiments were conducted on two types of soils: post-mining peaty soils in Grodno region and on degraded peaty soils in Brest region of Belarus. The same soils are very problematic for growing of traditional agricultural crops, thus willow production is a good alternative for biomass production of energy as well as for reclamation of these soils. In our experiments the following species of willows were tested (*Salix alba, Salix viminalis, Salix dasyclados, Salix aurita*) that may grow on peaty soils under the natural conditions. The most popular species for modern selection of SRC of willow is *Salix viminalis.* Nevertheless, the largest biomass on post-mining peaty soils was established for plants of *Salix dasyclados* and on degraded peaty soils for the plants of *Salix alba.* The lowest parameters at the both types of soils were identified for the plants of *Salix aurita.* However, it is necessary to take into account that these species are more popular for natural wetlands and in our experiments plants have the best results of survival of cutting and rates of growth at the beginning of vegetation. In accordance with these facts, *Salix aurita* may not be used for energy plantation directly, but it is interesting for hybridization with other species. Our results have shown that perspective hybrids for peaty soils may be for instance *Salix aurita x Salix dasyclados* and *Salix aurita x Salix alba.*

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ИСПИТИВАЊЕ МОРФОЛОШКИХ КАРАКТЕРИСТИКА РАЗЛИЧИТИХ ВРСТА ВРБА ГАЈЕНИХ У РАЗЛИЧИТИМ УСЛОВИМА СРЕДИНЕ

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РЕЗИМЕ: Алтернативна или обновљива енергија представља модерну стратегију која има добре изгледе за успех у ближој будућности. У зависности од климатских и економских услова као и технолошке развијености подручја, формирано је више различитих праваца развоја обновљивих извора енергије. За области са умереном климом, најбољи избор представља производња биоенергије. Један од праваца развоја биоенергије представља и агрошумарство засновано на засадима за биомасу са кратким турнусима брзорастућих врста попут топола и врба. Принос засада за биомасу врба може да износи 10–15 тона суве масе или 5–6 тона по хектару годишње. Засади са кратким турнусима (СРЦ) увелико се користе у западноевропским земљама, САД и Канади. Највеће површине под тим засадима налазе се у Шведској и захватају површину од око 12.000 хектара. Ова чињеница говори о перспективности гајења врба у овим засадима због њихових високих приноса и широке еколошке валенце. Такође је чињеница да се употребом брзорастућих клонова остварују одговарајући економски ефекти. Резултати истраживања указују да продуктивност врба зависи од станишних услова, али и од врсте врба које се користе. У овом раду представљена су испитивања у две климатске зоне и на два типа земљишта морфометријских карактеристика значајних за добијање биомасе, висине, дијаметра и броја избојака након сече. Истраживање је обухватило следеће врсте врба: *Salix viminalis, Salix dasyclados* и *Salux alba*. Биљке су гајене у различитим еколошким условима на бившим коповима тресета. Резултати су показали да клонови врста *Salix alba* и *Salix dasyclados* као и хибриди *Salix auruta x Salix dasyclados* и *Salix auruta x Salix alba* представљају добре кандидате за производњу биомасе на деградираним тресетним земљиштима.

КЉУЧНЕ РЕЧИ: биоенергија, врбе, селекција, морфометријски параметри

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MICRO-MORPHOLOGICAL FEATURES OF ACHENE OF WILD ANNUAL SUNFLOWERS

ABSTRACT: The aim of this research is to characterize wild annual sunflowers on the basis of achene micro-morphology. Plant material was grown up on an experimental field of the Institute of Field and Vegetable Crops in Novi Sad during 2015. Achene samples were hand-collected at the time of physiological maturity. Morphological measurements of achenes were performed using stereoscopic microscope Leica MZ16 with Leica DFC 320 Camera. The micro-morphological diversity of achenes was assessed using scanning electron microscopy (SEM). Obtained results indicated the presence of some quantitative and qualitative differences in achene characteristics among analyzed species, such as in their size, color, carpopodium and stylopodium shape, and distribution of trichomes on the achene surface. The carpopodium of examined species was asymmetrical at the maturity. Differences in the cuticle and wax ornamentation in different parts of the achenes, on the anticlinal walls of epidermal cells, were identified. The SEM analysis revealed the presence of non-glandular, multicellular bi-seriate trichomes (twin hairs) on the achene surface. This trichome type consisted of two elongated, parallel cells of different length. Considering the distribution of trichomes among the apical, median and basal regions of the fruit, most of the species demonstrated greater trichome density in the apical part.

KEYWORDS: achene, micro-morphology, trichomes

INTRODUCTION

The genus *Helianthus*, which belongs to Asteraceae family, is native to temperate North America and comprises 14 annual and 37 perennial species (Schilling 2006). Its botanical name *Helianthus* comes from the Greek words helios (sun) and anthos (flower). All annual *Helianthus* species, including cultivated sunflower, are diploid (2n = 34) (Kaya *et al.,* 2012).

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The fruit of Asteraceae is named achene, or cypsela, and is developed from bicarpellary ovary. In general, properties of reproductive structures are diagnostically important, and micro-morphological features of fruit surface are especially useful in taxonomy (Zarafshar *et al*., 2010). From taxonomical point of view, value of fruit micro-morphological features has already been recognized for many angiosperm families (Morozowska *et al*., 2012). Zhu *et al.* (2006) reported that a high diversity of fruit structures may provide some important insights into the phylogeny of different angiosperm groups. Anatomical characteristics of Asteraceae fruit have been described in scientific papers (Pandey 1989; Karanović *et al*., 2016), but rare data can be found on micro-morphological features of *Helianthus* fruit (Perez *et al*., 2006; Kocjan 2008). Trichomes are one of the most helpful micro-morphological characters which can be used in angiosperm taxonomy (Carlquist 1961). Also, in Asteraceae family trichomes morphology has already been used to clarify tribal and sectional classifications (Al-Shehbaz *et al*., 2006) and, more recently, it has been used as data in phylogenetic studies (Caruzo *et al*., 2011). The structure of trichomes is genetically controlled and, more or less, it is a stable taxonomic character. The role of non-glandular trichomes relies on their form, location on plant and direction or orientation. Usually, they provide protection, assist in pollen dispersal and reduce mechanical abrasion (Werker *et al*., 2000; Wagner *et al*., 2004). In Asteraceae family, in over 1,000 genera, there are many different types of trichomes (Panero and Funk 2008). The presence of twin or duplex achene hairs is one of characteristic features of the family (Metcalfe and Chalk 1950).

Nectaries occur on different plant organs and nectar is an attractive source of food for many species of flower visiting animals, particularly insects (Freitas *et al*., 2001). The topography, shape and anatomy of nectaries can be significantly different, even within one family (Petanidou *et al*., 2000). In Asteraceae, floral nectaries are annular and multicellular outgrowths at the top of the inferior ovary which surround the style base (Frei 1955).

Since there are only few reports on micro-morphological data of fruits in *Helianthus*, the aim of this research was to compare and characterize annual species of wild sunflower on the basis of morphological and micro-morphological features of their achenes.

MATERIAL AND METHODS

In this study five annual species of wild sunflower were analyzed: *H. annuus* L., *H. argophyllus* Torr. et A. Gray*, H. petiolaris* Nutt., *H. praecox* Engelm et A.Gray, and *H. debilis* Nutt. Mature achenes were collected from the experimental field of the Institute of Field and Vegetable Crops in Novi Sad during 2015 and examined for morphological and micro-morphological characters.

For examination, fifty randomly selected fruits per species were selected. Morphological measurements were performed using stereoscopic microscope Leica MZ16 with Leica DFC 320 camera. The following characters of achene

were studied: shape, surface, color, size (length and width), and also shape and position of carpopodium and stylopodium. Characteristics of achene trichomes, cuticle and wax ornamentation on the anticlinal walls of epidermal cells were not easily discerned using a light microscopy, so they were analyzed by SEM. For scanning electron microscopy, mature and dry achenes from each species were selected and directly mounted on a metallic stub using double adhesive tape and coated with gold for 180 s, at 30 mA (BAL-TEC SCD 005), and were subsequently viewed using JEOL JSM-6460LV electron microscope at 20 kV acceleration voltage.

RESULTS AND DISCUSSION

Most of the tribes of the family Asteraceae have quite similar cypsela features (Abid and Qaiser 2009), with the exception of *Helianthae*, *Eupatorieae* and *Inuleae*, where cypsela epidermal characteristics have been found very useful for tribal delimitation (Bremer 1994). In this study, the examined species shared many achene morphological properties when studied by stereoscopic microscope. Nevertheless, SEM showed that there were some micro-morphological differences. Achenes of all species were homomorphic, and obovate to widely obovate in shape (Figure 1, Table 1).

Table 1. Achene morphological characteristics of wild annual sunflowers.

Species	Shape of achene	Length of achene (mm)	Width of achene (mm)
H. annuus	wide obovate	4.5±0.41	2.3±0.29
H. argophyllus	narrow obovate	4.9±0.45	2.4±0.27
H. petiolaris	elongated	4.7±0.52	2±0.21
H. praecox	elongated	3.8±0.27	2±0.19
H. debilis	narrow obovate	4.4±0.39	2.98±0.32

The mature fruits of examined species were slightly different in size (Table 1). The color of sunflower fruits is determined by the presence or absence of pigments (Pandey 1989). For the examined species the basic color of the fruits was brown, but there were small variations in color. *H. annuus* achenes were golden-brown with two or more dark stripes in direction from base to achene apex. The stripes were variable in width from very narrow to very wide (Figure 1A). In *H. argophyllus* achenes were golden-brown in color with irregularly distributed dark patches (Figure 1B). *H. petiolaris* achenes were light gray with black patches (Figure 1C). *H. praecox* achenes were light brown with a lot of dark patches (Figure 1D) and in *H. debilis* their color was brown with irregularly distributed dark patches (Figure 1E). This variation of brown coloration between the species is correlated with different distribution of phytomelanin pigment in achene pericarp. According to Pandey (1989) phytomelanin in the pericarp of sunflower achenes is an effective mechanism for reducing yield losses due to achene injury by larvae of sunflower moth.

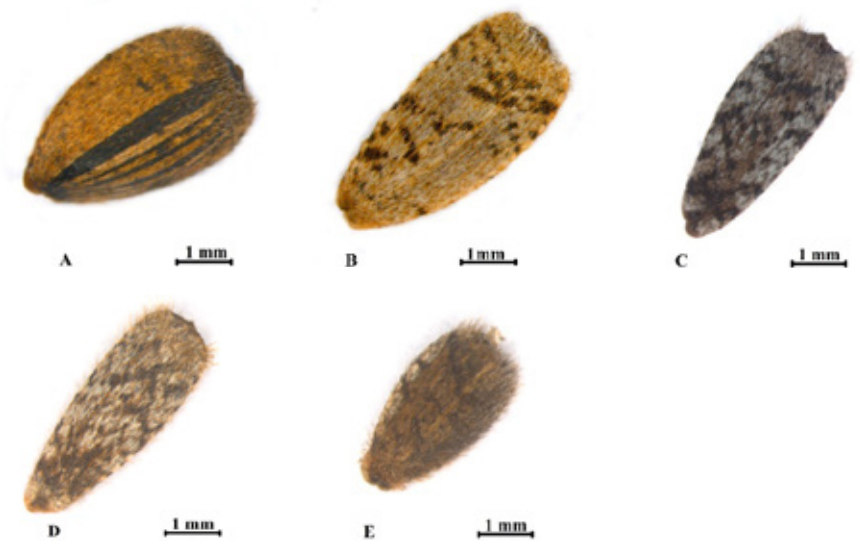

Figure 1. Light micrographs of achenes of wild annual sunflowers. A – *H. annuus*; B – *H. argophyllus*; C – *H. petiolaris*; D – *H. praecox*; E – *H. debilis*.

In all studied species, stylopodium was present at the upper part of achene (Figure 2C). The stylopodium was symmetrical and ring-like in all species. At the basal part of achene the carpopodium was distinct, asymmetrical and did not significantly differ in appearance among the species (Figure 2D). The largest dimensions of carpopodium and stylopodium were recorded in *H. annuus*, while the smallest were observed for *H. petiolaris*. In SEM micrographs of achenes, the cuticular ornamentation of the outer cell walls and the epicuticular wax were distinctly visible in all analyzed species (Figure 2). The fruit surface consisted of irregular epidermal cells which were parallel to the longitudinal fruit axis (Figure 2A and 2B).

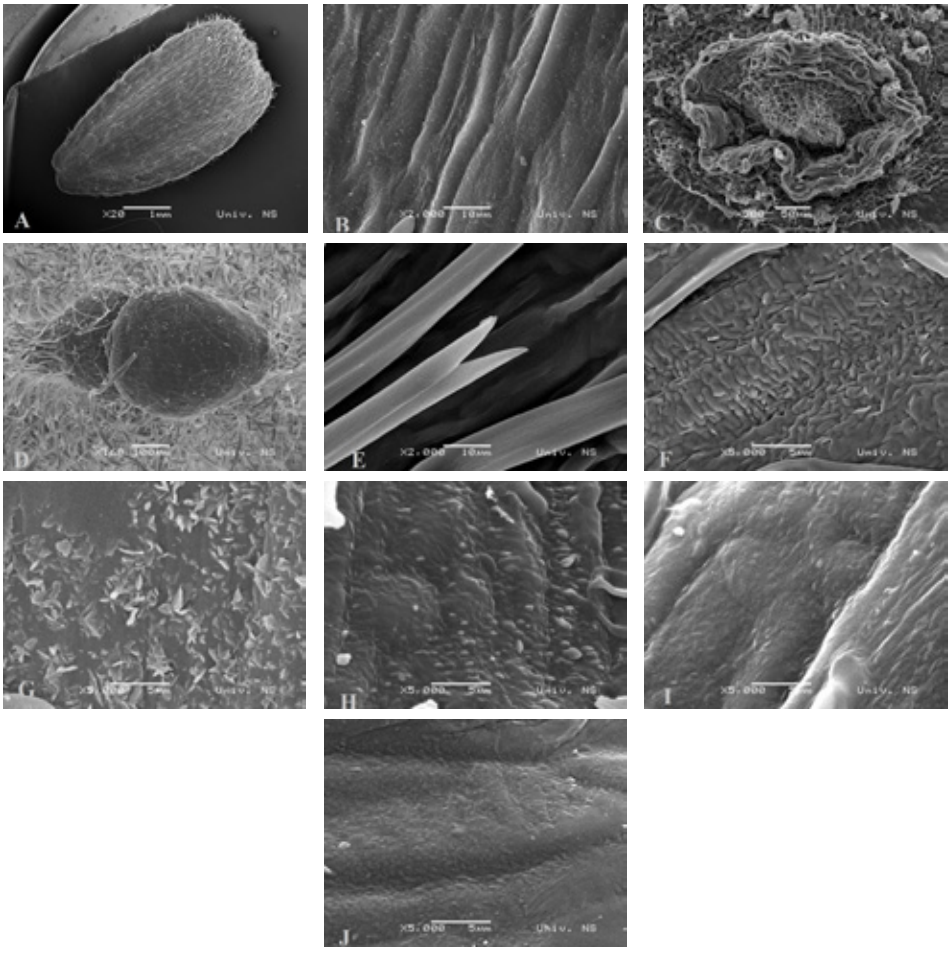

Figure 2. Scanning electron micrographs: A – mature achene (*H. argophyllus*); B – epidermal cells of achene (*H. annuus*); C – apical part of achene – stylopodium and nectariferouse tissue (*H. annuus*); D – basal part of achene – carpopodium (*H. debilis*); E – twin hairs with acute tip (*H. petiolaris*); F–J – epicuticular wax of pericarp (*H. argophyllus, H. debilis, H. annuus, H. praecox, H. petiolaris*).

All species have indistinct borders between epidermal cells, which were covered with smooth cuticle. Cuticular sculptures may serve as excellent diagnostic characters, but their systematic significance for delimitation of categories below the species level is rather limited (Barthlott and Voit 1979). In all species examined in this study, SEM revealed the presence of epicuticular wax in the form of platelets, apart from *H. petiolaris*, whose achenes were without wax. Epicuticular secretions were well developed in *H. argophyllus* and *H. debilis*, but weakly developed in *H. annuus* and *H. praecox* (Figure 2F–J). Wax was distributed throughout the whole fruit surface, but the largest amount was located at the apical part of achene. Wax content can vary between locations

and environmental conditions, also temperature or water stress can enhance cuticular wax synthesis in several plant organs (Morrison 1984).

SEM analysis of mature achenes revealed the presence of multicellular, bi-seriate, non-glandular trichomes (twin hairs) (Figure 2E). Twin hairs are characteristic of Asteraceae and they consist of two elongated and parallel cells, originating from the anticlinal division of an epidermal mother cell (Cron *et al*., 1993). According to our results, in analyzed species hairs were equally distributed across the fruit surface, except for *H. argophyllus,* where trichomes were mainly distributed near the apical part of achene. Twin hairs were elongated and similar in length in all species. Karanović *et al*. (2016) also reported the presence of short, twin hairs in some other taxa of Asteraceae family. In all examined species, the nectaries were present at the apical part of achene. They were ring-shaped, surrounding the stylopodium base. The nectary epidermis was smooth without ornamentation and covered with cuticle. It contained numerous stomata with large pores between large guard cells (Figure 2C). Similar location and description of nectary glands of sunflower was reported by Sammataro *et al*. (1985).

CONCLUSION

The present study on mature fruit of wild annual sunflowers: *H. annuus, H. argophyllus, H. petiolaris., H. praecox,* and *H. debilis* showed that some examined characters such as dimensions of achene, carpopodium and stylopodium morphology, features of epidermal cells, presence of twin trichomes and nectaries morphology were rather similar in analyzed species. However, differences between the species were recorded in pigmentation of achene and development of epicuticular wax.

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МИКРОМОРФОЛОШКЕ КАРАКТЕРИСТИКЕ АХЕНИЈЕ ПОПУЛАЦИЈА ДИВЉИХ, ЈЕДНОГОДИШЊИХ ВРСТА СУНЦОКРЕТА

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РЕЗИМЕ: Циљ истраживања у овом раду је карактеризација једногодишњих врста сунцокрета на основу микроморфолошких параметара ахеније. Биљни материјал узгајан је на експерименталном пољу Института за ратарство и повртарство у Новом Саду. Узорци ахенија сакупљани су ручно током 2015. године, у време физиолошке зрелости. Морфолошка мерења извршена су применом стереоскопског микроскопа Leica M316 са Leica DFC 320 камером. Микроморфолошке карактеристике анализиране су помоћу скенинг електронског микроскопа (CEM). Добијени резултати указују на присуство квалитативних и квантитаивних разлика између анализираних врста, у параметрима као што су величина и боја ахеније, карактеристике карпоподијума и стилоподијума, као и дистрибуција трихома. Пронађена је разлика у орнаментици кутикуле и воска антиклиних зидова епидермалних ћелија на различитим деловима ахеније. CEM анализа указала је на присуство нежлезданих, вишећелијских, бисеријатних трихома (twin hairs). Наведени тип трихома састоји се од две издужене, паралелне ћелије различите дужине. Имајући у виду различиту расподелу трихома на апикалном, медијалном и базалном делу, већина врста се карактерише највећом густином трихома у апикалном делу ахеније.

КЉУЧНЕ РЕЧИ: ахенија, микроморфологија, трихоме

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POLYMORPHISM OF MICROSATELLITE LOCI IN BREAD WHEAT (*Triticum aestivum* L.) AND RELATED SPECIES

ABSTRACT: This study analysed polymorphism of 15 microsatellite loci in the collection comprising of 40 genotypes of bread wheat (*Triticum aestivum* L.), 32 genotypes belonging to other species within *Triticum* genus and 3 genotypes from *Aegilops* genus. The results showed significant differences in the variability of the tested loci in bread wheat and related species. In the collection of bread wheat genotypes, 119 alleles were detected with the average number of 7.9 alleles per locus. In wild and cultivated related species 157 alleles were identified, with the average of 10.5 alleles per locus. All analysed parameters of microsatellite loci variability (PIC value, gene diversity, heterozygosity, etc.) indicated higher level of polymorphism in wild relatives than in the cultivated bread wheat. Analyses of individual genomes indicated that in the bread wheat genetic diversity of the B and D genomes was significantly reduced in relation to the A genome, while the differences in polymorphism between genomes in the wild relatives were significantly lower. The results showed that wild related species can be used as sources for new variability in wheat breeding.

KEYWORDS: *Aegilops* ssp., *Triticum* sp., SSR markers, locus variability

INTRODUCTION

Cultivated wheat is a hexaploid species with genome consisting of three sub-genomes originating from three ancestral species: A genome originates from *Triticum urartu*, B genome originates from *Aegilops speltoides* and D genome originates from *Aegilops tauschii*. The analysis of phylogenetic relations between different *Aegilops* and *Triticum* species, and their relatedness to the cultivated wheat, can contribute to better understanding of the complex wheat genome, as well as the processes that had been carried out throughout the evolution of this important crop.

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One of the preconditions for further improvement of wheat is the assessment of genetic variability of wild relatives for the identification of desirable traits that can be used in breeding. Wild relatives have contributed to the increase of genetic variability in many cultivated species, such as barley, potato, rice, tomato, wheat and many others (Jones *et al.* 2013). It is estimated that the contribution of wild relatives in the genetic improvement of the cultivated species amounts to 1% per year, with a total value of $1 billion in global agriculture (Heywood 2011). Additionally, according to the estimates of Maxted and Kell (2009), 29 cultivated species have benefited from the transfer of useful traits from their wild relatives.

The strategies based on the extensive phenotyping of the germplasm collections, so as to identify wild relatives that can contribute to increased productivity or adaptability of the cultivated plants, are often compared to "finding a needle in a haystack". Molecular evaluation and population genetics were proposed as an alternative approach (Prada 2009). The advantage of using molecular markers and next generation sequencing technologies is the possibility to easily identify the desirable genotypes and alleles that can be used for improvement of certain traits (Kilian and Graner 2012).

The aim of this study was to analyse the genetic variability of microsatellite loci of the bread wheat in comparison to related species from the genera *Triticum* and *Aegilops*. This will give important information on the potential use of wild relatives in wheat breeding.

MATERIAL AND METHODS

In this study, genetic variation of 75 accessions of *Triticum* and *Aegilops* species was evaluated using SSR marker. The collection consists of 40 *T. aestivum* genotypes, 32 accessions of other *Triticum* species and 3 *Aegilops* accessions. Fifteen SSR markers (listed in Table 1) were chosen for the evaluation, 5 for each of the three bread wheat genomes (AA, BB and DD genome).

The genomic DNA was isolated from young leaves using modified CTAB method (Doyle and Doyle 1990). The PCR reactions were carried out according to Röder *et al.* (1998) with specific annealing temperature depending on certain SSR markers. The total amount of PCR reaction was 10 µl, which contained 30 ng of genomic DNA, 1x buffer solution, 2 mM dNTPs, 1.5 mM $MgCl_2$, 10 pmol of each primers and 1 unit of Taq polymerase. The forward primers were labelled with one of the listed fluorescent dyes VIC, 6-FAM, NED or PET. The fragment analysis was performed using capillary electrophoresis at Genetic Analyzer ABI Prism 3130 (Applied Biosystems, Foster City, CA, USA). The electrophoresis data were collected in Data Collection Software v.3.0. and analysed using GeneMapper Software v.4.0 (Applied Biosystems, Foster City, CA, USA).

Descriptive SSR loci parameters, such as number of alleles per locus, allele frequencies, PIC value, heterozygosity, etc., were estimated using GenAlEx 6.5 software (Peakall and Smouse 2012). Significance of the differences in the number of alleles between three wheat genomes was tested with t-test using XLSTAT software (Addinsoft 2007). PowerMarker v.3 software (Liu and Muse 2005) was

used to calculate a shared allele proportion distance matrix among the pairs of genotypes. Visual representation of the distance matrix was performed by UPGMA tree using Dendroscope v.2.7.4 software (Huson *et al.* 2007). Furthermore, principal coordinate analysis (PCoA) was used to depict the relatedness among the genotypes in low-dimensional setting using GenAlEx 6.5. Molecular analysis of variance (AMOVA) was carried out to determine the distribution of the genetic variation among cultivated wheat and related species and within these groups was used Arlequin 3.5 software (Excoffier and Lischer 2010). The significance of φ-statistics was obtained non-parametrically using 1,000 random permutations.

RESULTS AND DISCUSSION

Genetic diversity was investigated in the set of 75 accessions of *Triticum* and *Aegilops* species, using 15 wheat microsatellites. Fragment analyses of PCR products were used for detection of 207 allelic variants in all investigated loci (Table 1). The number of alleles per locus varied from 7 for *Xgwm261* to 21 for *Xbarc12*. The average number of allelic variants in all analysed loci was 13.8, with average PIC (polymorphic information content) value of 0.76. Gene diversity for 15 microsatellite loci varied from 0.69 for *Xgwm261* to 0.91 for *Xbarc12* with an average of 0.79. The gene diversity increased as the number of alleles increased. The obtained results are in agreement with those of Salem et al. (2014) who reported highly significant correlation between gene diversity and the number of alleles per locus ($r = 0.649$, $P<0.01$). They concluded that the number of alleles can be used to evaluate the genetic diversity per loci, genomes and homoeologous group.

Table 1. Polymorphism of 15 microsatellite loci in the collection of 75 *Triticum* and *Aegilops* accessions

Marker	MAF	AN	GD	H	PIC	Genome
cfd65	0.30	17	0.83	0.62	0.81	A
gwm294	0.19	15	0.89	0.00	0.88	A
barc12	0.16	21	0.91	0.09	0.90	A
wmc264	0.19	19	0.89	0.10	0.88	A
cfd71	0.17	19	0.91	0.03	0.90	A
Mean A	0.20	18.2	0.89	0.17	0.88	
taglgap	0.30	15	0.84	0.02	0.83	B
barc164	0.28	10	0.82	0.00	0.79	B
gwm284	0.25	10	0.82	0.06	0.80	B
gpw3071	0.42	17	0.75	0.07	0.73	B
gwm495	0.42	12	0.77	0.08	0.75	B
Mean B	0.33	12.8	0.80	0.05	0.78	
wmc216	0.78	9	0.38	0.00	0.37	D
gwm157	0.46	17	0.71	0.04	0.67	D

gwm261	0.42	7	0.69	0.04	0.64	D
gwm3	0.38	8	0.74	0.26	0.70	D
wmc457	0.32	11	0.82	0.04	0.80	D
Mean D	0.47	10.4	0.67	0.08	0.64	
Total Mean	0.34	13.8	0.79	0.10	0.76	

MAF-Major Allele Frequency, AN – Allele Number, GD – Gene Diversity, H – Heterozygosity, PIC – Polymorphism information content

Analysis of molecular variance (AMOVA) showed that genetic variation was found mainly within populations (89%) while variance among populations of cultivated wheat and wild relatives was only 11%.

In the collection of bread wheat genotypes, 119 alleles were detected with the average number of 7.9 alleles per locus, while in related species 157 alleles were identified with the average of 10.5 alleles per locus (Table 2). The average gene diversity of 0.68 and 0.81 were found in cultivated wheat and relatives, respectively. PIC value ranged in bread wheat from 0.20 (*Xwmc216*) to 0.86 (*Xbarc12* and *Xcfd71*) and in related species from 0.62 (*Xgwm3*) to 0.89 (*Xgwm294* and *Xgwm157*).

Table 2. Polymorphism of 15 microsatellite loci in the collection of 40 *Triticum aestivum* genotypes (cultivated wheat) and 35 related *Triticum* and *Aegilops* species (related species)

Marker	Cultivated wheat					Related species					Genome
	MAF	AN	GD	H	PIC	MAF	AN	GD	H	PIC	
cfd65	0.38	10	0.74	0.90	0.70	0.37	13	0.79	0.38	0.76	A
gwm294	0.31	10	0.84	0.00	0.82	0.18	13	0.89	0.00	0.89	A
barc12	0.23	13	0.87	0.09	0.86	0.25	12	0.87	0.10	0.85	A
wmc264	0.30	10	0.84	0.05	0.82	0.27	14	0.87	0.15	0.86	A
cfd71	0.22	12	0.87	0.07	0.86	0.33	14	0.84	0.00	0.83	A
Mean A	0.29	11.0	0.83	0.22	0.81	0.28	13.2	0.85	0.13	0.84	
taglgap	0.38	7	0.72	0.04	0.67	0.23	12	0.85	0.00	0.84	B
barc164	0.46	7	0.66	0.00	0.61	0.33	8	0.79	0.00	0.76	B
gwm284	0.47	9	0.73	0.10	0.70	0.35	5	0.73	0.00	0.68	B
gpw3071	0.64	6	0.54	0.07	0.50	0.31	15	0.86	0.07	0.85	B
gwm495	0.64	9	0.57	0.07	0.55	0.23	9	0.84	0.10	0.83	B
Mean B	0.52	7.6	0.64	0.06	0.61	0.28	11.7	0.83	0.08	0.82	
wmc216	0.89	5	0.21	0.00	0.20	0.50	6	0.69	0.00	0.66	D
gwm157	0.68	2	0.44	0.00	0.34	0.17	17	0.90	0.10	0.89	D
gwm261	0.54	4	0.61	0.03	0.55	0.34	6	0.74	0.05	0.70	D
gwm3	0.32	6	0.76	0.19	0.72	0.48	6	0.67	0.36	0.62	D
wmc457	0.35	9	0.80	0.03	0.78	0.28	7	0.80	0.06	0.78	D
Mean D	0.55	5.2	0.56	0.05	0.52	0.30	10.7	0.81	0.09	0.79	
Total Mean	0.45	7.9	0.68	0.11	0.65	0.31	10.5	0.81	0.09	0.79	

MAF – Major Allele Frequency, AN – Allele Number, GD – Gene Diversity, H – Heterozygosity, PIC – Polymorphism information content

Different dominant alleles were identified in the collections of bread wheat and related species in 9 out of 15 examined loci. Higher number of alleles in the collection of related species was found in 10 loci (Figure 1), while in three loci (*Xbarc12*, *Xgwm284* and *Xwmc457*) higher number of alleles was found in bread wheat genotypes (Figure 2). The two loci (*Xgwm495* and *Xgwm3*) had the same number of alleles in both groups of genotypes.

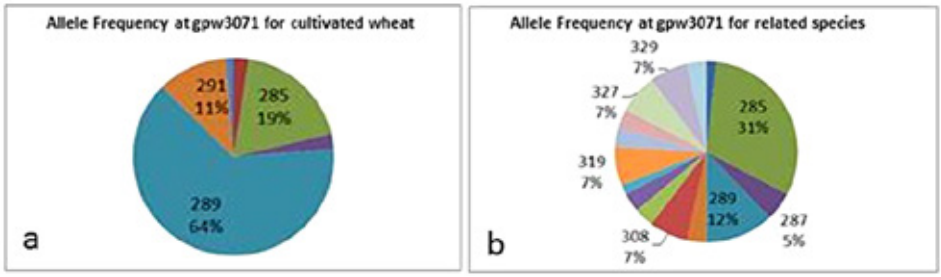

Figure 1. Allele frequencies at the locus Xgpw3071 for cultivated wheat (a) and its relatives (b)

Figure 2. Allele frequencies at the locus Xgwm284 for cultivated wheat (a) and its relatives (b)

All analysed variability parameters showed higher level of polymorphism present in the wild relatives than in the cultivated bread wheat (Table 2). Analyses of individual genomes indicated that in the cultivated wheat genetic diversity of the B and D genomes was significantly reduced in relation to the A genome, while in the related species the differences among the genomes were less pronounced. Thus, in the present study the number of alleles in cultivated wheat for A genome (55) was significantly ($P<0.01$) higher than the B (38) and D (26) genomes. Contrary to our results, some authors found that the B genome chromosomes appear to have high genetic richness compared to the A and D genomes, in various classes of repetitive DNA, particularly microsatellites (Cuadrado and Schwarzacher 1998; Salem *et al.*, 2014). Huang *et al.* (2002) also reported that microsatellite loci of the B genome are more effective than those of the A genome. On the other hand, Roussel *et al.* (2004) concluded that the D genome has a larger number of alleles than the B genome, while the highest number was found in the A genome.

It was possible to distinguish cultivated wheat cultivars from other *Triticum* and *Aegilops* species by using two different methods (PCoA analyses in Figure 3 and UPGMA cluster analyses in Figure 4) for the analysis of molecular data and estimating genetic relationships among genotypes. Similar results were obtained by Yadav *et al.* (2014) in pigeonpea (*Cajanus cajan* (L) Millsp.) and its wild relatives (*C. albicans* and *C. lineatus*). They found that the pattern of genetic divergence obtained by PCA is in close agreement with the results of UPGMA based cluster analysis. In both methods the wild species formed out groups, whereas all the cultivated genotypes showed narrower range of diversity.

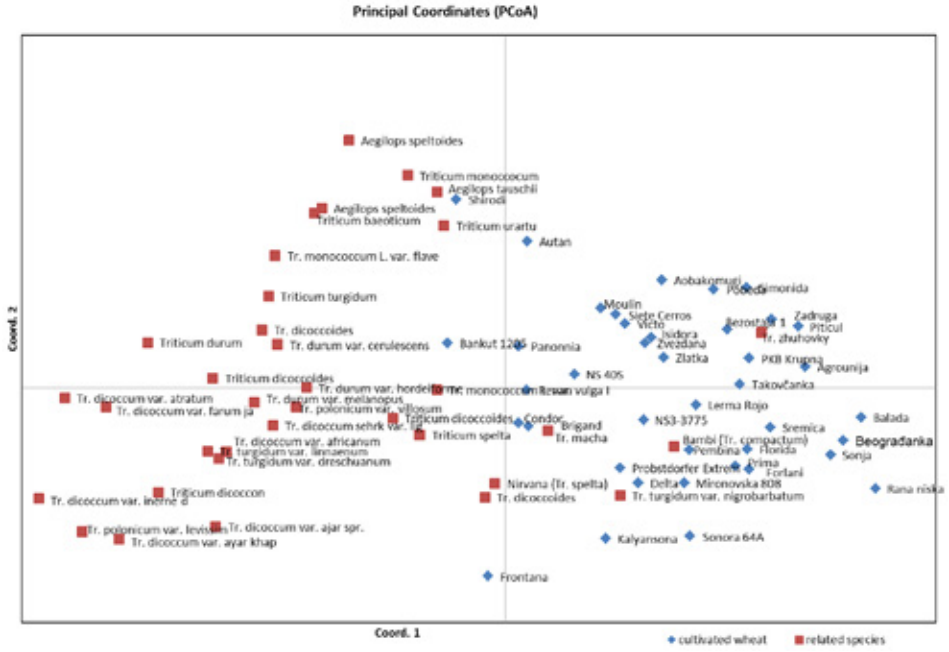

Figure 3. Distribution of 75 accessions of *Triticum* and *Aegilops* species in two-dimensional PCoA system based on molecular data

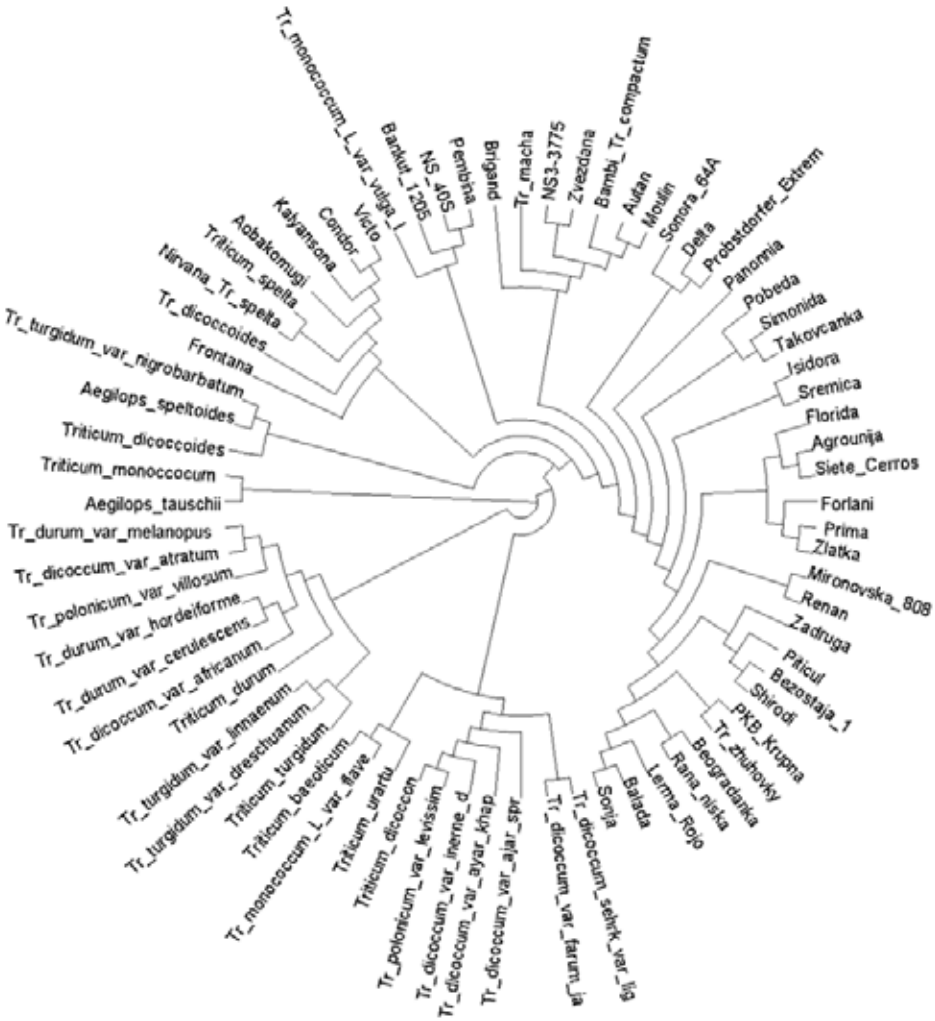

Figure 4. UPGMA tree of 75 accessions of *Triticum* and *Aegilops* species

CONCLUSION

The results showed high diversity present in the collection of cultivated wheat and its relatives. The polymorphism in SSR fragments among different groups of species was shown. It can be concluded that SSR markers can be used in assaying genetic variability of wheat and its wild relatives and establishing of phylogenetic relationships between them. Also, molecular markers can help to organize the genetic variability and expose useful diversity for breeding purposes.

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ПОЛИМОРФНОСТ МИКРОСАТЕЛИТСКИХ ЛОКУСА КОД ГАЈЕНЕ ПШЕНИЦЕ (*Triticum aestivum* L.) И СРОДНИХ ВРСТА

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РЕЗИМЕ: У раду је испитана полиморфност 15 микросателитских локуса у колекцији коју чине 40 генотипова гајене пшенице (*Triticum aestivum* L.), 32 генотипа који припадају другим врстама рода *Triticum* и три генотипа из рода *Aegilops*. Резултати су показали да постоји значајна разлика у варијабилности испитиваних локуса код гајене пшенице и њених сродника. У колекцији генотипова гајене пшенице детектовано је укупно 119 алела, са просеком од 7,9 алела по локусу. Код дивљих и гајених сродника идентификовано је укупно 157 алела, са просеком од 10,5 алела по локусу. Сви израчунати показатељи варијабилности микросателитских локуса (*PIC* вредност, дивергентност гена, хетерозиготност, итд.) указују на већу полиморфност дивљих сродника у односу на гајену пшеницу. Анализа појединачних генома такође је показала да је код гајене пшенице дивергентност Б и Д генома значајно умањена у односу на А геном, док су код дивљих сродника разлике у полиморфности појединачних генома значајно мање. Добијени резултати указују на то да се дивљи сродници могу користити као извори нове варијабилности у оплемењивању пшенице.

КЉУЧНЕ РЕЧИ: *Aegilops* ssp., *Triticum* sp., SSR маркери, варијабилност локуса

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PHENOTYPIC AND MOLECULAR EVALUATION OF GENETIC DIVERSITY IN NS SAFFLOWER (*Carthamus tinctorius* L.) COLLECTION

ABSTRACT: Safflower (*Carthamus tinctorius* L.) belongs to the Asteraceae (Compositae) family. It is primarily grown for seeds used for bird feed or as edible oil. Stamens are used in traditional medicine and nutrition. Breeding for high resistance to dry growing conditions has initiated intensive studies of this plant species in recent years. Six safflower genotypes of different geographical origins (Ukraine, Italy, Turkey) were collected and added to the collection of less cultivated oil plant species of the Institute of Field and Vegetable Crops in Novi Sad. Phenotypic observations during two growing seasons revealed that analysed genotypes differed in flower colour (yellow, orange, red), in the presence of spines, and in seed oil and protein content. Oil and protein content differed between years and genotypes, indicating large influence of genotype and environmental conditions on variations of these quantitative traits that are negatively correlated. Genetic variability of the analysed genotypes was tested by use of molecular markers. Given that sunflower and safflower belong to the same family, the possibility of applying SSR markers developed for sunflower for molecular analysis of safflower was analysed. The obtained results proved that sunflower markers can be successfully transferred to safflower. Future studies should include larger number of markers in order to identify polymorphic and informative ones. Significant variations within a relatively small number of the analysed safflower genotypes justify further work on the evaluation of the collection, taking into account both genetic and environmental factors.

KEYWORDS: oil content, protein content, molecular marker, safflower

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INTRODUCTION

Oil crops are grown all over the world and represent a vital part of the agricultural sector in many economies. Under European agro-ecological conditions traditional oil crops are mostly annual and biannual crops such as sunflower, rapeseed, soybean, castor, poppy or pumpkin seed, but novel or specialty oil crops such as linseed, safflower, false flax, groundnut, sesame and others could be of particular interest regionally. Given their value for diverse food and non-food applications, oils are a highly desired commodity with worldwide consumption increasing by >50% during the past decades (Vollmann and Laimer 2013). Safflower (*Carthamus tinctorius* L.), also known as Dyer's Saffron, American Saffron, Fake/False Saffron, Bastard Saffron, Zaffer, Azafran (Spanish), Hong Hua (Chinese), Kesumba, Qurtum, etc., is of particular interest. Safflower possesses numerous valuable agronomic attributes that make it attractive as an alternative spring-sown crop for tight crop rotations.

Around the world, safflower is mainly grown for its edible oil for cooking, salad oil and margarine. In affluent countries, the demand for the oil increased after researches linking health and diet, because this oil has the highest poly-unsaturated/saturated ratio of all available oils. Safflower oil is stable and its consistency does not change at low temperatures, making it particularly suitable for use in chilled foods. It is nutritionally similar to olive oil, with high levels of linoleic or oleic acid, but much less costly. Safflower oil is sprayed on various edible products to prevent them from absorbing or losing water, and thus extends their shelf life. In China, safflower is grown almost exclusively for its flowers, which are used in treatment of many illnesses as well as in tonic tea. Addition of safflower florets to foods is a widespread and ancient tradition. True saffron is perhaps the world's costliest spice, and safflower is a common adulterant or substitute. Rice, soup, sauces, bread and pickles take on a yellow to bright orange colour from the florets. Health concerns regarding synthetic food colourings may increase demand for safflower-derived food colouring (Mündel and Bergman 2009).

Adapted to arid, semi-arid and saline soils, safflower is commonly grown in such unfavourable conditions where drought and salinity limit seed germination and plant growth. High resistance to dry growing conditions has initiated intensive studies of this plant species in recent years (Özel 2004; Omidi 2012). Phenotyping and evaluation of morphological traits were performed by use of different methods (Atlagić *et al*., 2009; Ada 2013; Hamza 2015), while molecular analysis included the use of various types of molecular analysis (Johnson *et al*., 2007; Yang *et al*., 2007; Amini *et al*., 2008; García-Moreno *et al*., 2010; Panahi *et al*., 2013; Kumar *et al*., 2015; Ambreen *et al*., 2015). Considering that safflower and sunflower belong to the Asteraceae family, several SSR markers developed for sunflower were chosen in order to determine whether these markers could be transferred and applied for molecular analysis of safflower.

MATERIAL AND METHODS

Six safflower genotypes of different geographical origins (Ukraine, Italy, Turkey) were collected and added to the collection of less cultivated oil plant species of the Institute of Field and Vegetable Crops in Novi Sad: Sunčana (Ukraine), Ptica and Liman (Serbia), Remzibey, Dinçer and Yenice (Turkey). Phenotypic observations of flowers and seeds during two growing periods were performed (Figure 1).

Figure 1. Safflower

Each plot in the experiment consisted of 4 rows, 0.25 m apart and 3.6 m long making the harvest area of individual plots equal to 3.6 m^2. Harvest was performed manually and all plants in each plot were threshed together.

Phenotypic observations (flower colour and presence or absence of spines) were conducted according to Dajue and Mündel (1996). At maturity, seed oil and protein content were measured in whole, unpeeled seed. The oil content was determined using the NMR (Nuclear Magnetic Resonance) method, and expressed as a percentage of seed. The protein content was determined using the classical Micro Kjeldahl method and measured only in 2015.

DNA for molecular analysis was extracted from safflower leaves using modified CTAB protocol (Permingeat *et al.*, 1998). Three SSR markers developed for sunflower were applied for molecular analysis: ORS 595, ORS 610, and ORS 1013 (Tang *et al.*, 2002). PCR was performed as described by Dimitrijević *et al.* (2010). Products of PCR amplification were run on 2% agarose gels and visualized in the BIO-Print system (Vilber Lourmat, Marne-La-Vallée, France).

RESULTS AND DISCUSSION

Phenotypic observations showed that analysed genotypes differ in flower colour. Remzibey had yellow, Liman, Ptica and Dinçer orange and Yenice and Sunčana red flowers. Genotype Liman differed from other analysed genotypes of the collection by the absence of spines, which were in all other genotypes present on branches, leaf edges and blossom.

Seed oil and protein content of the analysed safflower genotypes differed between years and genotypes (Figure 2), which indicated the impact of genotype and environmental conditions in variations of these negatively correlated quantitative traits. In the first year, the oil content ranged between 15.75% and 21.20%. The highest oil content was obtained from genotype Yenice.

In the second year, the oil contents were 12.59% to 16.81% and the highest oil content was obtained from genotype Remzibey (Figure 2). In general, the level of oil in the first year was higher than that in the second year. This may be attributed to the lower precipitation in the second year. Oil content values obtained were lower than the values reported by Çamaş *et al.* (2007) and Golkar *et al.* (2012), and higher than the values reported by Marjanović-Jeromela *et al.* (2007).

Seed protein content ranged from 14.29% (Remzibey) to 17.90% (Liman). Similar values were reported by Marjanović-Jeromela *et al.* (2007), while Golkar *et al.* (2012) reported higher protein content.

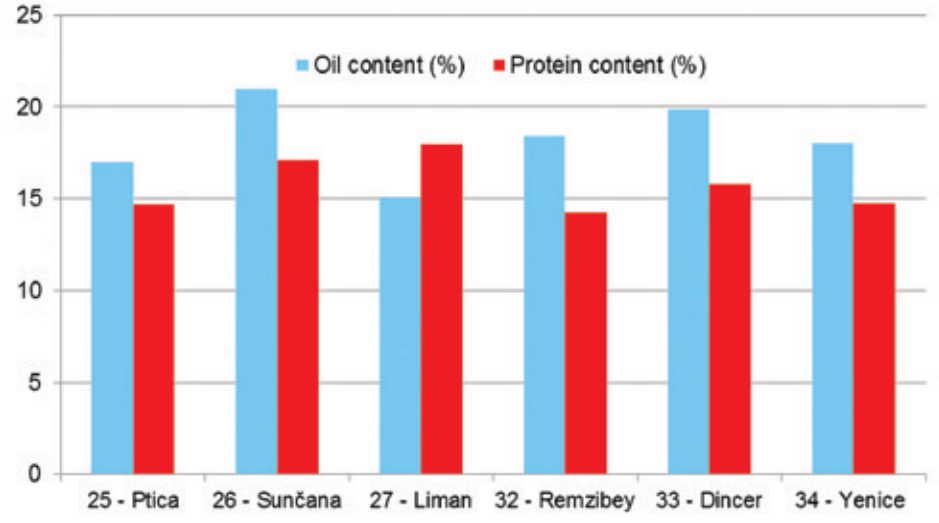

Figure 2. Seed oil and protein content obtained from six safflower genotypes of different geographical origins

Genetic variability of genotypes was analysed by use of SSR molecular markers. Given that sunflower and safflower belong to the same family, the

possibility of using sunflower SSR markers for molecular analysis of safflower was analysed.

All three tested markers amplified bands in the tested safflower genotypes. ORS 595 amplified two bands of different length, 111 bp and 150 bp, while ORS 610 and ORS 1013 were monomorphic, amplifying one band of the same length in all tested genotypes: 73 and 187 bp, respectively (Figure 3). Some unspecific bands were also observed.

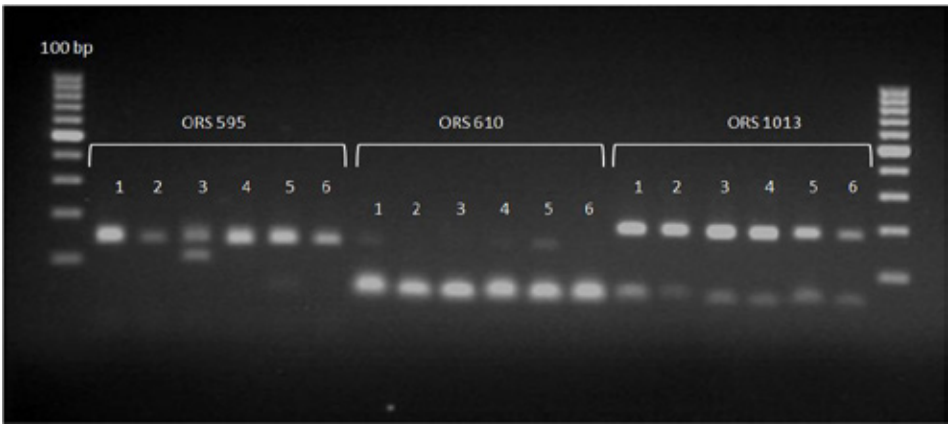

Figure 3. Amplification profiles obtained by use of sunflower SSR markers (ORS 595, ORS 610, ORS 1013). Safflower genotypes: 1 – Sunčana (Ukraine), 2 – Ptica (Serbia), 3 – Liman (Serbia), 4 – Remzibey (Turkey), 5 – Dinçer (Turkey), 6 – Yenice (Turkey). DNA ladder 100 bp (Thermo Scientific).

All tested markers successfully amplified bands in safflower. Only one marker proved to be polymorphic. Opposite to our research, ORS 595 did not amplify bands of sufficient quality for scoring in research reported by García--Moreno *et al.* (2010). In addition, ORS 610 and ORS 1013 were monomorphic, while in the work reported by García-Moreno et al. (2010) these markers were polymorphic. ORS 595 and ORS 610 amplified bands of the similar length (less than 100 bp difference) comparing to bands amplified in sunflower (Dimitrijević *et al.*, 2013). However, when comparing obtained results with those reported by Tang *et al.* (2002), this was only the case with ORS 595.

Seed oil content in safflower usually ranges between 20% and 45% (Vosoughkia *et al.*, 2011), and with decrease in seed coat it can vary between 42% and 50% (Knowles 1982). The results obtained in this trial proved that the evaluated accessions were useful for combined production of oil and proteins, while the collection could be enlarged by introducing breeding material with higher oil content.

Consequently, sunflower markers can be successfully transferred to safflower and future studies should include larger number of markers in order to identify polymorphic and informative ones.

The obtained results indicate significant variation within a relatively small number of the analysed safflower genotypes. This justifies further work on the evaluation of the collection, taking into account both genetic and environmental factors.

ACKNOWLEDGEMENTS

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ФЕНОТИПСКА И МОЛЕКУЛАРНА ЕВАЛУАЦИЈА ГЕНЕТИЧКЕ РАЗНОЛИКОСТИ НС КОЛЕКЦИЈЕ ШАФРАЊИКЕ (*Carthamus tinctorius* L.)

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РЕЗИМЕ. Шафрањика (*Carthamus tinctorius* L.) припада породици Asteraceae (Compositа). Гаји се првенствено за добијање семена које се користи за исхрану птица или производњу јестивог уља. Прашници се користе у традиционалној медицини и исхрани. Висока отпорност на сушне услове гајења иницирала је последњих година интензивнија истраживања ове биљне врсте. За колекцију мање гајених уљаних биљних врста Института за ратарство и повртарство, прикупљено је шест генотипова шафрањике различитог географског порекла (Украјина, Италија, Турска). Фенотипским опажањем у току две вегетационе сезоне утврђено је да се генотипови међу собом разликују у боји цвета (жута, наранџаста, црвена), у присуству бодљи, као и садржају уља и протеина у семену. Садржај уља и протеина се разликовао између година и генотипова, што указује на велики утицај и генотипа и спољашње средине у варирању ових квантитативних својстава која се налазе у негативној корелацији. Генетичка варијабилност генотипова је испитана молекуларним маркерима. С обзиром на то да сунцокрет и шафрањика припадају истој породици, проучавана је могућност употребе SSR маркера сунцокрета за молекуларну анализу шафрањике. Добијени резултати показали су да се маркери сунцокрета могу успешно користити за шафрањику и да будућа истраживања треба да садрже већи број маркера у циљу идентификовања полиморфних и информативних маркера. Значајне разлике у релативно малом броју анализираних генотипова шафрањике оправдавају даљи рад на евалуацији колекције, узимајући у обзир и услове средине и генетичку варијабилност.

КЉУЧНЕ РЕЧИ: садржај уља, садржај протеина, молекуларни маркери, шафрањика

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HEAVY METAL CONTAMINATION OF VEGETABLES FROM GREEN MARKETS IN NOVI SAD

ABSTRACT: Vegetables are valuable source of vitamins, minerals and fibers important for healthy human nutrition. However, an increased level of heavy metals in vegetables has been noticed in recent years. This study was conducted with an aim to analyze content of heavy metals, cadmium (Cd), lead (Pb), and chromium (Cr) in 11 vegetable species which are the most common in human diet. Vegetables were collected from three green markets (Limanska, Futoška and Riblja pijaca) in Novi Sad, during September and October, from 2009 to 2011. Heavy metal contents were analyzed in edible parts of tomato, potato, spinach, onion, beetroot, parsley, parsnip, carrot, cauliflower, pepper and broccoli using atomic absorption spectrophotometer (Varian, AAS 240FS). The results showed statistically significant differences in element concentrations among analyzed vegetables. In general, the highest metal pollution was observed in the year of 2011. Spinach was found to contain the highest metals content – 0.89 μg/g for Cd, 5.81 μg/g for Pb, and 3.67 μg/g for Cr. According to Serbian official regulations, 18.18% of all analyzed species exceeded maximum permissible level for Cd, 9.09% for Pb, while for Cr these limits are not defined. Elevated content of heavy metals in vegetables might be related to soil contamination, atmospheric depositions during transportation and marketing. Thus, a continuous monitoring of vegetables on markets should be performed in order to prevent potential health risks to consumers.

KEYWORDS: accumulation, green market, heavy metals, vegetables

INTRODUCTION

Vegetables are important source of carbohydrates, proteins, as well as vitamins, minerals, fibers and trace elements which are essential for human nutrition and health. Besides the beneficial effects on human diet, vegetables might contain both essential and toxic heavy metals in various ranges of concentrations (Kastori *et al.*, 1997). Heavy metals are non-biodegradable and persistent environmental contaminants, with the specific density over 5 g/cm^3

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and the relative atomic mass above 40 (Seregin and Ivanov 2001). The presence of heavy metals such as cadmium (Cd), lead (Pb) and chromium (Cr) in agricultural soils is mostly influenced by anthropogenic activity (Fytianos *et al*., 2001). Elevated concentrations of these metals inhibit plant growth parameters including respiration, photosynthesis, water and nutrient uptake, resulting in reduction of plant production (Kastori *et al*., 1997; Ali *et al*., 2015). Vegetables take up heavy metals by absorbing them from soil, as well as from depositions of the air polluted environments, and accumulate them in edible plant parts. In recent years, an increased level of heavy metal contamination in vegetables has been noticed, which might have major implications for human health (Arora *et al*., 2008; Pan *et al*., 2016). Elevated concentration of toxic elements are result of different anthropogenic activities, environmental pollution from industrial emission, utilization of waste water for irrigation, certain agricultural practices, application of nitrogen and phosphorous fertilizers, atmospheric depositions during transportation and marketing, etc. (Sharma *et al*., 2006; Nikolić *et al*., 2014). Continuous consumption of vegetables contaminated by heavy metals may lead to their accumulation in humans causing disruption of numerous biochemical processes, leading to cardiovascular, nervous, kidney and bone diseases (Radwan and Salama 2006; Arora *et al*., 2008). The rapid urbanization and industrialization have caused elevation of heavy metals content in urban environments in different developing countries (Radwan and Salama 2006; Sharma *et al*., 2008; Kiende *et al*., 2012). Moreover, increased demands for food encouraged producers to use a variety of cropping practices to achieve higher yields and profit using field or greenhouse cultivation methods. The main source of vegetables are local producers who sell vegetables at green markets, but the data on cultivation, harvest, transport and storage conditions of vegetables is still lacking (Nikolić *et al*., 2014). Thus, several studies have been carried out in order to assess the quality of vegetables purchased from green markets, as well as to evaluate potential health risk to local population (Milačić and Kralj 2003; Radwan and Salama 2006; Kiende *et al*. 2012). Al-Jassir *et al.* (2005) and Sharma *et al*. (2008) have reported the increased levels of heavy metals in vegetables sold in the markets. Therefore, the aim of this study was to analyze heavy metal content (Cd, Pb and Cr) in edible parts of eleven vegetable species purchased from green markets in Novi Sad (Republic of Serbia). The results could reveal a potential risk assessment to consumers and could contribute to preservation of human health.

MATERIAL AND METHODS

During September and October, from 2009 to 2011, samples of fresh vegetables were collected from three green markets in Novi Sad (Limanska, Riblja, and Futoška pijaca). Vegetables which are the most commonly used in human diet in this region were analyzed: tomato (*Lycopersicon esculentum* Mill.), potato (*Solanum tuberosum* L.), spinach (*Spinacia oleracea* L.), beetroot (*Beta vulgaris* L.), parsley (*Petroselinum crispum* (Mill.) Fuss), parsnip (*Pastinaca sativa* L.), carrot (*Daucus carota* L.), cauliflower (*Brassica oleracea* L. var.

botrytis L.), pepper (*Capsicum annuum* L.) and broccoli (*Brassica oleracea* var. sylvestris L.). Vegetable samples (2 kg of each vegetable from each market) were selected using a random sampling procedure, packed into polyethylene bags and transported to the laboratory.

Plant material was prepared for chemical analysis according to standard procedure (Arsenijević-Maksimović and Pajević 2002). Samples were dried at 80 °C, for 24 hours to constant mass and then were powdered in a mixer grinder. The dried samples were measured; 1 g of each vegetable was weighed in three replicates and was heated and mixed with 33% H_2O_2. Dry-aching process was performed at 450 °C for 4 hours to complete mineralization, and then, samples were treated with 25% HCl. Total amounts of Cd, Pb and Cr in edible plant parts were determined using flame atomic absorption spectrophotometry (Varian, AAS240FS). The analysis of each sample was performed in three independent replicates.

The obtained data were expressed as mean of three replicates ± standard deviation (SD). Data were subjected to the analysis of variance (ANOVA) and followed by the Fisher multiple comparison test at a significance level of $p<0.05$. The mean values followed by the same letter within each year of study, showed no significant differences.

RESULTS AND DISCUSSION

Maximum permissible levels (MPC) of Cd, Pb and Cr in dry vegetables are different according to the Official Gazette of the Republic of Serbia (*Službeni glasnik*, 25/2010 and 28/2011) in comparison to values set up by FAO/WHO organization (Food additives and contaminants. Joint Codex Alimentarius Commission, FAO/WHO Standards Programme, 01 12A. 2001) (Table 1).

Table 1. Maximum permissible levels of heavy metal content in dry vegetables

Heavy metal	Cd	Pb	Cr
Permissible levels in Serbia	0.3	3.0	/
Permissible levels in vegetables set up by FAO/WHO	0.2	0.3	2.3

Total concentrations of Cd, Pb and Cr among analyzed vegetables are presented in Figures 1, 2 and 3, respectively. These values were compared with MPC values recommended by Serbian legislation, as well as with MPC values allowed by FAO/WHO. The concentrations of heavy metals in vegetables varied from one species to the other and also differ between years. The average concentrations of heavy metals in all vegetable samples were in the following descending order: Cr > Pb > Cd.

The mean concentrations of Cd among analyzed vegetables during three-year research are summarized in Figure 1. The mean concentrations of Cd among analyzed edible parts of vegetables ranged from 0.035 μg/g to 0.89 μg/g. Generally, the highest Cd content was recorded in spinach 0.5 μg/g, 0.31 μg/g

and 0.89 μg/g in 2009, 2010 and 2011, respectively. These values were above allowed concentrations in dry vegetables. During the first two years, Cd content in edible parts of vegetables exceeded MPC values in spinach (0.5 μg/g), in carrot in the year of 2009 (0.5 μg/g), while in the year of 2011 values of Cd were higher than 0.3 μg/g in tomato, broccoli, pepper and spinach. Furthermore, results revealed that 18.18% of all analyzed species exceeded MPC according to national legislation. At the same time, 30.30% of all vegetables showed higher concentration of MPC values when compared to FAO/WHO regulations. Cadmium is considered to be one of the most abundant and major environmental pollutant and its phytotoxic effect is well established (Ali *et al*., 2015). The transfer of cadmium from soil into the food-chain depends on numerous factors, such as the plant species, type and pH reaction of the soil, and type of fertilizers (Suruchi and Pankaj 2011). In contrast to this work, Radwan and Salama (2006) reported lower range of heavy metals in vegetables sold in markets. They recorded the lowest Cd content in tomatoes (0.005 mg/kg), while the highest content of Cd was registered in cucumber (0.25 mg/kg). Different distribution of heavy metals within the plant was previously reported by Al Jassir *et al*. (2005). The edible parts of leafy and root vegetables showed higher potential to accumulate heavy metals in comparison to storage organs and fruits (Sharma *et al*., 2008). Cadmium is highly mobile element, thus can be easily translocated to the aerial plant parts (Sipter *et al*., 2008). Furthermore, high levels of contamination could be attributed to different factors including the cultivation methods of homegrown vegetables. A field survey by Sipter *et al*. (2008) revealed significant differences between flooded and non-flooded vegetable gardens. They noticed the highest Cd level in carrot (0.81 mg/kg) in flooded gardens. This is slightly higher in comparison to Cd level in carrot recorded in this work.

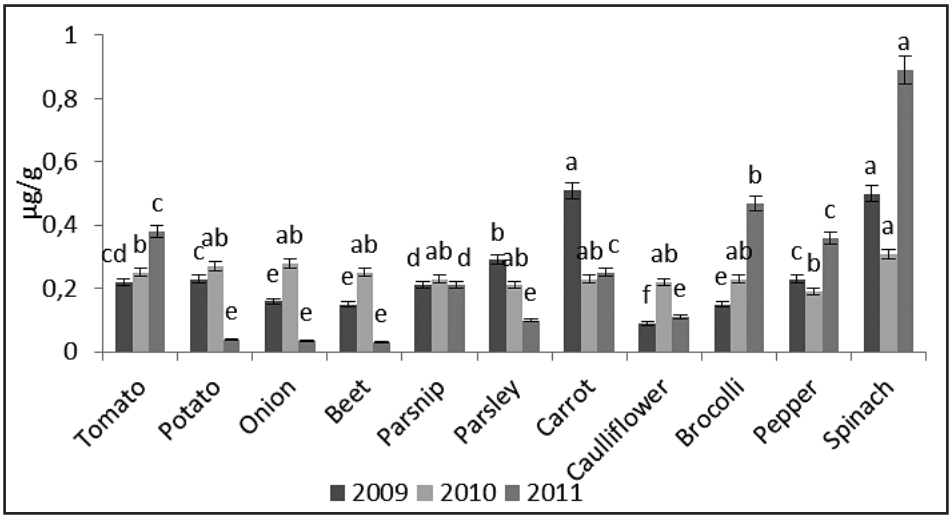

Figure 1. Cadmium concentration in analyzed vegetables.

Lead is one of the most common pollutants in urban area due to the high accumulation via aero pollution and different anthropogenic activity (Sharma *et al*., 2008). Results of this survey showed various variations among analyzed vegetables (Fig. 2). In general, the lowest Pb content was recorded in the second year of research (Fig. 2), while consistent trend between species was not evident. The average of Pb in all analyzed species was above the maximum permissible levels of 0.3 mg/kg permitted by FAO/WHO in every year of research. Results of this work are in line with previous studies. Kudirat and Funmilayo (2011) found that Pb and Cd levels in spinach and tomato were 10 and 2-fold higher than MPC in comparison to Serbian regulations. Similar to this work, Fytianos *et al*. (2001) found the highest Pb content in spinach (0.46 μg/g dry wt.). At the same time, authors observed the lowest Pb content in carrot (0.07 μg/g dry wt.) which is contrary to our findings.

Higher lead concentration in studied vegetables may be attributed to increased air pollution. Previous studies showed that lead contamination in vegetables was significantly increased in the urban environment, near highways and streets, as well as during transportation and marketing at green markets (Fytianos *et al*., 2001; Hough *et al*., 2004). Therefore, vegetables sold in markets should not be allowed to grow on localities with high traffic. Furthermore, Wierzbicka *et al*. (1995) mentioned that a large number of plants usually had ability to accumulate large amounts of lead without visible changes in their appearance or yield. According to MPC values in Serbia (Tab. 1), permissible level of Pb was elevated only in the year of 2011 in tomato (3.53 μg/g), pepper (3.26 μg/g) and spinach (5.81 μg/g). These results could be explained by different cultivation conditions, soil properties, and genotype related specificity of the heavy metals uptake and accumulation.

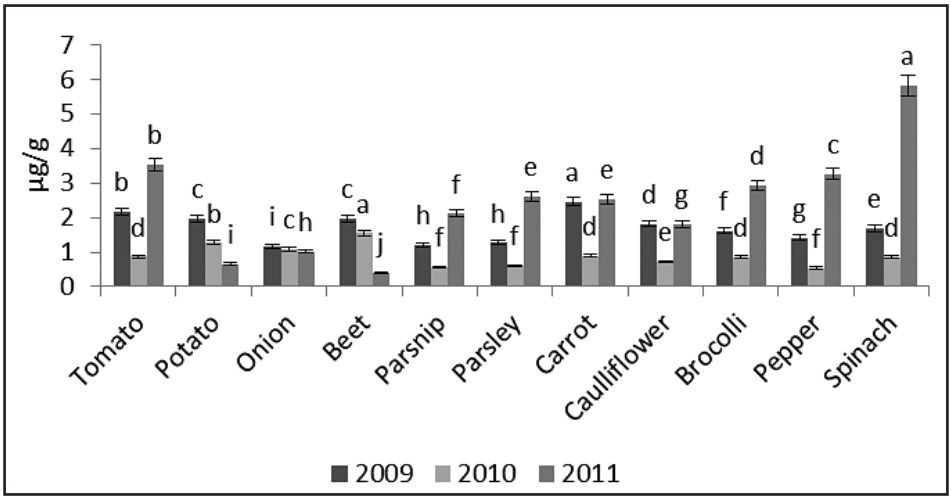

Figure 2. Lead concentration in analyzed vegetables.

Chromium is essential micronutrient for plants, but in high concentration it could be toxic for plants, animals and humans (Chen *et al*., 2014). Besides the positive effects of chromium for human diet, especially in carbohydrates metabolism, FAO set up the limits for Cr content in vegetables, while such regulations in Serbia are still lacking. The content of chromium showed variations between vegetables (Figure 3). Within selected vegetables, the highest content of Cr was found in spinach (3.67 μg/g in 2011, 1.74 μg/g in 2010), whereas in the year of 2009 the highest Cr content was recorded in potato (1.67 μg/g). In general, Cr content was within acceptable level recommended by the FAO/WHO. Exception was spinach, which showed exceeded MPC values in the last year of study (3.67μg/g). Furthermore, 50% of analyzed vegetables showed increased Cr content in 2011, in comparison to previous years of research. Chen *et al*. (2014) recorded the elevated content of Cr in leguminous vegetables (horsebean 10.64 μg/g, and garden pea 7.51 μg/g), while in spinach Cr content was 0.24 μg/g. Authors indicate that leguminous vegetables showed a greater ability to accumulate Cr, in comparison to leafy vegetables which showed tendencies for higher concentrations of Cd and Pb (Chen *et al*., 2014).

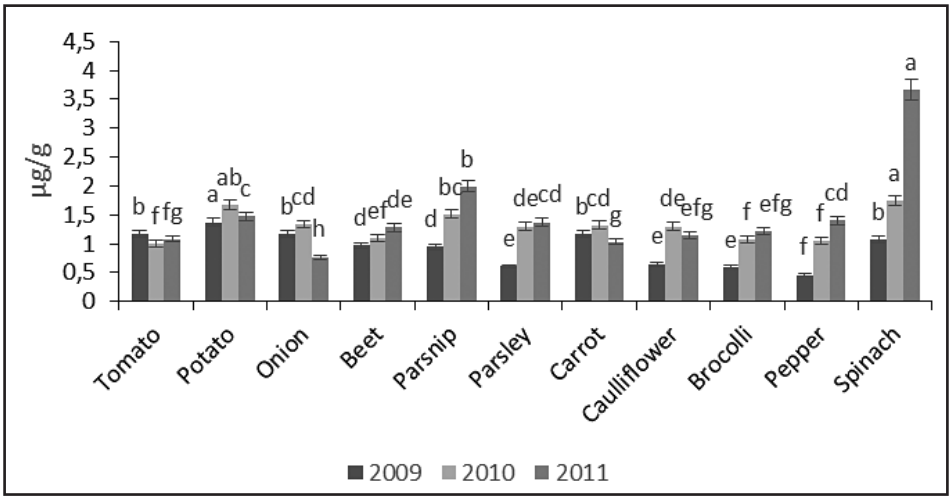

Figure 3. Chromium concentration in analyzed vegetables.

Intake of heavy metals through the soil-crop system has proven to be the dominant route of human exposure to heavy metals (Kastori *et al*., 1997). Toxic effects of these elements are evident in concentrations higher than physiological demand of plants and pose a potential threat to human health. Therefore, the data on distribution of heavy metals in edible parts of plants have a great practical importance in preservation of human health, considering the role of vegetables in human diet. The contamination levels of selected vegetables determined in this study were generally comparable with previous studies (Radwan and Salama 2006; Sharma *et al*., 2008). Results obtained in this work suggest that

different vegetables may accumulate different heavy metals in various ranges of concentrations. This might be related to different uptake capacity of vegetables for different heavy metals through roots, and their translocation to above ground plant parts (Kihampa *et al*., 2011; Ali *et al*., 2015). In general, spinach showed the highest heavy metal pollution among selected vegetables. The possible reason for this can be the large vegetable surfaces and morpho-physiological characteristics (Singh and Kumar 2006). In respect to elevated heavy metal amounts, spinach was determined as good source of essential mineral elements potassium, phosphorus, magnesium and calcium (Nikolić *et al*., 2014). Elevated heavy metal levels in plants are probably caused by several factors, but it is generally accepted that the metal concentration in soil is the main reason for heavy metal contamination of vegetables (Naser *et al*., 2011). Besides the cultivation methods, the non-regular use of fertilizers and quality of water used for irrigation could contribute to elevation of toxic elements in vegetables (Asdeo and Loonker 2011). Most heavy metals are soluble in water, thus they could reach to vegetables through irrigation with polluted water (Suruchi and Pankaj 2011). Moreover, air pollution may pose a threat to post-harvest contamination of vegetables during transportation and marketing, causing increase of the heavy metal levels in vegetables (Kudirat and Funmilayo 2011). Reduction in heavy metal concentrations in vegetables after washing further suggests that atmospheric deposition on vegetables during marketing is one of the major source of heavy metal contamination in urban areas (Divrikli *et al*., 2003; Sharma *et al*., 2008).

CONCLUSION

The present study provides data on heavy metal pollution of vegetables purchased from green markets in Novi Sad. Besides the positive effects on human health, spinach has shown the highest potential for heavy metal contamination among analyzed species. Further analyses should be performed, in order to provide production of safe, healthy food. Also, appropriate precautions should be taken during the transportation and selling vegetables at markets.

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АНАЛИЗА САДРЖАЈА ТЕШКИХ МЕТАЛА У ПОВРЋУ СА ЗЕЛЕНИХ ПИЈАЦА У НОВОМ САДУ

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РЕЗИМЕ: Поврће представља вредан извор витамина, минерала и влакана који су од великог значаја за здраву исхрану. Међутим, последњих година у поврћу забележен је повећан садржај тешких метала. Циљ овог рада био је да се утврди садржај тешких метала: кадмијума (Cd), олова (Pb) и хрома (Cr) у једанаест биљних врста које се најчешће користе у људској исхрани. Поврће је прикупљено са три пијаце у Новом Саду (Лиманска, Футошка и Рибља), током септембра и октобра у периоду од 2009. до 2011. године. Садржај тешких метала анализиран је у јестивим деловима парадајза, кромпира, спанаћа, лука, цвекле, першуна, паштрнака, шаргарепе, карфиола, паприке и броколија, методом атомске апсорпционе спектрофотометрије. Резултати су показали статистички значајне разлике у концентрацији елемената међу анализираним повртарским врстама. Највећи садржај тешких метала забележен је у спанаћу у току 2011. године (0.89 μg/g Cd, 5.81 μg/g Pb 3.67 μg/g Cr). Садржај кадмијума изнад максималне дозвољене концентрације (МДК) забележен је код 18,18% анализираних повртарских врста, док је код 9,09% узорака регистрован повећан садржај олова. Повећан садржај тешких метала у поврћу вероватно је условљен загађењем земљишта, ваздуха, као и контаминацијом у току транспорта и продаје на пијацама. Континуирани мониторинг тешких метала у поврћу са пијаца од изузетног је значаја у контроли квалитета намирница.

КЉУЧНЕ РЕЧИ: акумулација, зелена пијаца, поврће, тешки метали

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BRINE SHRIMP LETHALITY BIOASSAY OF SELECTED GYMNOSPERM AND ANGIOSPERM SPECIES

ABSTRACT: Methanol extracts of selected species of flowering plants *Anthemis cotula*, *A. ruthenica*, *Centaurea dubia* (Asteraceae), *Ajuga genevensis*, *A. chamaepitys*, *A. reptans*, *Micromeria albanica*, *M. cristata*, *M. dalmatica*, *M. juliana*, *Thymus tosevii* (Lamiaceae) and conifers – *Abies alba*, *Picea omorika*, *Pinus heldreichii* (Pinaceae) and *Taxus baccata* (Taxaceae), as well as diethyl ether extracts of ten species *Anthemis cotula*, *A. ruthenica*, *Centaurea dubia*, *Ajuga genevensis*, *A. chamaepitys*, *A. reptans*, *Micromeria albanica*, *M. cristata*, *M. dalmatica* and *M. juliana* from two flowering plant families (Asteraceae and Lamiaceae) were tested for general bioactivity using brine shrimp (*Artemia salina*) lethality test. Lethal concentration (LC_{50}) and 95% confidence intervals were determined by computer program LdP line. Out of fifteen tested methanol extracts, three possessed cytotoxic effect. *Taxus baccata* methanol extract showed the highest effect (LC_{50} = 18.60 μg/ml), while *Thymus tosevii* methanol extract expressed the lowest (LC_{50} = 842.50 μg/ml). All other analyzed species did not express significant cytotoxicity. Also, diethyl ether extracts of all tested species did not show significant cytotoxicity. The obtained results for methanol extracts which show certain cytotoxic effect could be guide for further phytochemical and pharmacological investigations.

KEYWORDS: Asteraceae, brine shrimp lethality bioassay, cytotoxicity, Lamiaceae, Pinaceae, Taxaceae

INTRODUCTION

Over the last decades, interest in plant drugs has been growing constantly. A well known fact is that almost all of medicinal preparations were derived from plants (Ramachandran *et al.*, 2011). Still, medicinal aspect of wild vascular flora throughout the world has not been investigated sufficiently (Gilani

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and Rahman 2005). Also, plants used in folk medicine are more likely to possess pharmacologically active compounds (Hamburger and Hostettmann 1991). The selection of plant species which will be investigated is crucial, while screening and searching for potential biologically active compounds is the main goal.

Artemia salina, brine shrimp, is an invertebrate of saline aquatic ecosystem. It could be used in a laboratory bioassay in order to determine the toxicity of tested extracts by the assessment of the medium lethality concentration (LC_{50}) (Meyer *et al.*, 1982). It is a simple tool to guide screening of physiologically active plant extracts. Brine shrimp has been previously utilized in various bioassay systems. There are a number of reports where brine shrimp has been used for different investigations, such as: environmental studies (Zillioux *et al.*, 1973; Price *et al.*, 1974; Sorgeloos *et al.*, 1978), screening for natural toxins (Harwig and Scott 1971; Reiss 1972) as well as a general screening for bioactive compounds in plant extracts (Meyer *et al.*, 1982; Kumarasamy *et al.*, 2002; Pimentel Montanher *et al.*, 2002; Padmaja *et al.*, 2002; Janaćković *et al.*, 2008; Rajeh *et al.*, 2010; Ramachandran *et al.*, 2011; Tawaha *et al.*, 2015).

The aim of this study is to test methanol extracts of selected species of flowering plants *Anthemis cotula*, *A. ruthenica*, *Centaurea dubia* (Asteraceae), *Ajuga genevensis*, *A. chamaepitys*, *A. reptans*, *Micromeria albanica*, *M. cristata*, *M. dalmatica*, *M. juliana*, *Thymus tosevii* (Lamiaceae) and selected conifer species – *Abies alba*, *Picea omorika*, *Pinus heldreichii* (Pinaceae) and *Taxus baccata* (Taxaceae), as well as diethyl ether extracts of ten species *Anthemis cotula*, *A. ruthenica*, *Centaurea dubia*, *Ajuga genevensis*, *A. chamaepitys*, *A. reptans*, *Micromeria albanica*, *M. cristata*, *M. dalmatica* and *M. juliana* from two flowering plant families (Asteraceae and Lamiaceae) for general bioactivity using brine shrimp lethality test. All the tested plant species have been assumed to contain some of the biologically active constituents (e.g. lactones, flavonoids, iridoids, alkaloids etc.). The aim is also to search for new potential source of biologically active compounds which can be guidance for future research and possible applications in pharmacy, medicine and food industry. Some investigation of citotoxic activities of *A. ruthenica* (Borbala *et al.*, 2007; Hajdu *et al.*, 2010), *A. alba* (Wajs-Bonikowska *et al.*, 2015) and *T. baccata* (Lavelle *et al.* 1993; Guenard *et al.,* 1995; Fumoleau *et al.*, 1997; Jensen *et al.,* 2000; Erdemoglu *et al.*, 2004; Sreekanth *et al.,* 2009; Shokrzadeh *et al.*, 2010; Raghunath *et al.,* 2012; Kajani *et al.*, 2014) are known. There have not been any published data about cytotoxicity by brine shrimp bioassay of these species so far.

MATERIALS AND METHODS

Plant material

Plant material (Table 1) of selected flowering and conifer plants was collected from native habitats in Serbia and Montenegro and from Botanical Garden "Jevremovac". Voucher specimens with accession numbers are deposited

in the Herbarium of the Institute of Botany and Botanical Garden "Jevremovac", University of Belgrade – Faculty of Biology.

Table 1. Investigated plant species

Species	Family	Locality	Voucher specimens, accession numbers
Anthemis cotula L.	Asteraceae	Kladovo, Serbia	ACK02
A. ruthenica M.B.	Asteraceae	Deliblatski pesak, Serbia	ARD02
Centaurea dubia Suter	Asteraceae	Povlen, Serbia	CNP02
Ajuga genevensis L.	Lamiaceae	Košutnjak, Serbia	AGK00
A. chamaepitys (L.) Schreb.	Lamiaceae	Paraćin, Serbia	ACP00
A. reptans L.	Lamiaceae	Košutnjak, Serbia	ARK00
Micromeria albanica (Griesb. ex Maly) Šilić	Lamiaceae	Prizrenska bistrica, Serbia	MAP98
M. cristata (Hoppe) Gris.	Lamiaceae	Sićevačka klisura, Serbia	MCS98
M. dalmatica Benth.	Lamiaceae	Kotor, Montenegro	MDK98
M. juliana (L.) Benth.	Lamiaceae	Kotor, Montenegro	MJK98
Thymus tosevii Vel.	Lamiaceae	Vranje, Serbia	TTV98
Abies alba Mill.	Pinaceae	Botanical Garden "Jevremovac", Serbia	AAJ02
Picea omorika (Pančić) Purk.	Pinaceae	Botanical Garden "Jevremovac", Serbia	POJ02
Pinus heldreichii Christ.	Pinaceae	Botanical Garden "Jevremovac", Serbia	PHJ02
Taxus baccata L.	Taxaceae	Botanical Garden "Jevremovac", Serbia	TBJ02

Sample preparation

Sample preparation was done as described previously (Meyer *et al.*, 1982; Mc Laughlin, 1991). The list of extracts of investigated plant species is shown in Table 2. The percent of fresh weight and dry weight yields of extracts of the tested species is shown in Table 3. Methanol extracts were prepared by mixing 50 mL of MeOH with 5 g of leaves, diethyl ether extracts by mixing 50 mL of Et_2O with 5 g of leaves. Mixtures were extracted in an ultrasonic bath (30 min) and then were kept in the dark for 24 h at room temperature. After filtration, dry residues from extracts were obtained by evaporation in a rotary evaporator (t = 40 ºC). Solutions with starting concentration of 1 mg/mL of methanol extracts and diethyl ether extracts were prepared by dissolving 10 mg of the given extracts in 10 ml of methanol in volumetric flasks. Appropriate amounts of solutions (10, 100, and 1,000 µL, for 10, 100, and 1,000 µg/mL, respectively) were transferred to 1.5 cm disks of filter paper (Schleicher and Schuell, no. 5891, Ø70 mm). The disks were dried in air, placed in 2 dram vials and then dried further *in vacuo* for an hour. Control disks were prepared using potassium dichromate (VI). Five replicates were prepared for each dose level.

Table 2. List of extracts of investigated plant species.

Species	Leaves		Solvent	
	Dry	Fresh	MeOH	ET_2O
Anthemis cotula L.	+		+	+
A. ruthenica M.B.	+		+	+
Centaurea dubia Suter	+		+	+
Ajuga genevensis L.	+		+	+
A. chamaepitys (L.) Schreb.	+		+	+
A. reptans L.	+		+	+
Micromeria albanica (Griesb. ex Maly) Šilić	+		+	+
M. cristata (Hoppe) Gris.	+		+	+
M. dalmatica Benth.	+		+	+
M. juliana (L.) Benth.	+		+	+
Thymus tosevii Vel.	+		+	
Abies alba Mill.		+	+	
Picea omorika (Pančić) Purk.		+	+	
Pinus heldreichii Christ.		+	+	
Taxus baccata L.		+	+	

Table 3. Yield of dry and fresh of extracts of investigated plant species.

Species	Yield of extract (%)	
	MeOH	Et_2O
Ajuga genevensis L.	15.286	1.704
Thymus tosevii Vel.	13.354	np
Abies alba Mill.	12.270	np
Picea omorika (Pančić) Purk.	11.746	np
Ajuga reptans L.	11.460	2.054
Taxus baccata L.	11.196	np
Micromeria dalmatica Benth.	9.212	0.848
M. albanica (Griesb. ex Maly) Šilić	9.158	2.600
Anthemis cotula L.	8.502	7.150
Centaurea dubia Suter	7.000	2.600
Pinus heldreichii Christ.	6.460	np
Anthemis ruthenica M.B.	5.586	2.832
Micromeria juliana (L.) Benth.	5.538	2.180
Ajuga chamaepitys (L.) Schreb.	5.350	1.416
Micromeria cristata (Hoppe)	4.664	4.156

(np): extract not prepared

Brine shrimp cytotoxicity assay

The test was performed as described in Meyer *et al.* (1982) and McLaughlin (1991). Each extract was tested at concentrations of 10, 100, and 1,000 μg/mL. Brine shrimp eggs (*Artemia salina*) were purchased in a pet shop in Belgrade and were hatched in a shallow round dish filled with artificial sea water, which was prepared with a commercial salt and double-distilled water (4 g/L). Temperature of the water was 22 °C. Aeration of the water was performed with electric air pump and aerator. In the dish, a black plastic divider (Plexiglas®) with 2 mm holes was clamped with silicone. Therefore, the aquarium had two unequal compartments. The eggs of brine shrimp (ca. 50 mg) were sprinkled into the darkened larger compartment, while the other compartment was illuminated. After 48 hours the phototropic nauplii were collected by pipette from the lighted side. A suspension of 10 nauplii in artificial sea water was added to each sample vial to a volume of 5 mL. Also, a drop of dry yeast suspension (3 mg in 5 ml artificial sea water) was added as food. The vials were maintained under illumination. The sample vials incubated for 24 h at room temperature. After this period, the number of dead nauplii in each sample vial was counted using a binocular microscope (MBS 9, USSR, 4.8 x). Pure methanol was used as a positive control while artificial sea water was used as a negative. Finney's statistical method of probit analysis (Finney 1971) was used to calculate the concentration of the extract that would kill 50% of brine shrimps within 24 h of exposure, i.e. the LC_{50} with 95% confidence intervals. The extracts were considered as bioactive when LC_{50} was 1,000 μg/mL or less. The value of LC_{50} was determined using the LdP Line® program (Bakr 2007).

RESULTS AND DISCUSSION

As shown in Table 4, general biological activity of selected gymnosperm and angiosperm species belonging to four families was tested using brine shrimp lethality bioassay. Results showed that out of 15 methanol extracts, three possess cytotoxic effect. The methanol extract of *Taxus baccata* shows a highly significant effect (LC_{50} = 18.6 μg/mL). Also, certain cytotoxic effect was showed by methanol extract of *Centaurea dubia* (LC_{50} = 294.4 μg/mL). The lowest cytotoxic effect was found with *Thymus tosevii* (LC_{50} = 842.5). A dose-response curves of these three extracts *T. baccata*, *C. dubia* and *Th. tosevii* are shown in Figures 1, 2, and 3, respectively. Almost negligible cytotoxicity were showed by methanol extracts of *Picea omorika*, *Ajuga genevensis*, *A. reptans*, *A. chamaepitys*, *Anthemis cotula* and *Micromeria juliana*. Their lethal concentration was LC_{50} > 1,000 μg/mL. Methanol extracts of other tested species, as well as diethyl ether extracts of all investigated species, did not show significant cytotoxicity.

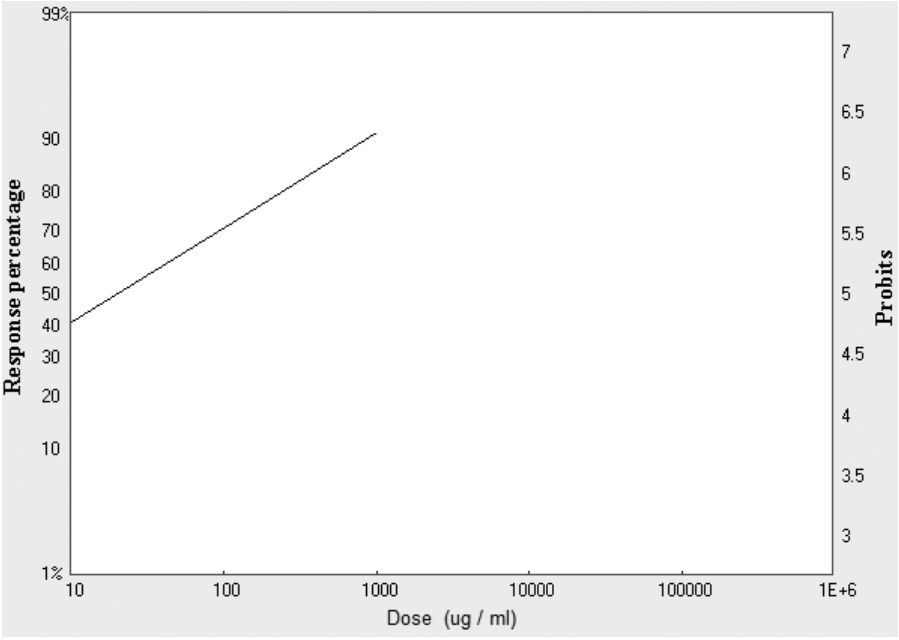

Figure 1. Dose-response curve shows relation between percentage of deaths of brine shrimps nauplii and applied concentration of *T. baccata* methanol extract

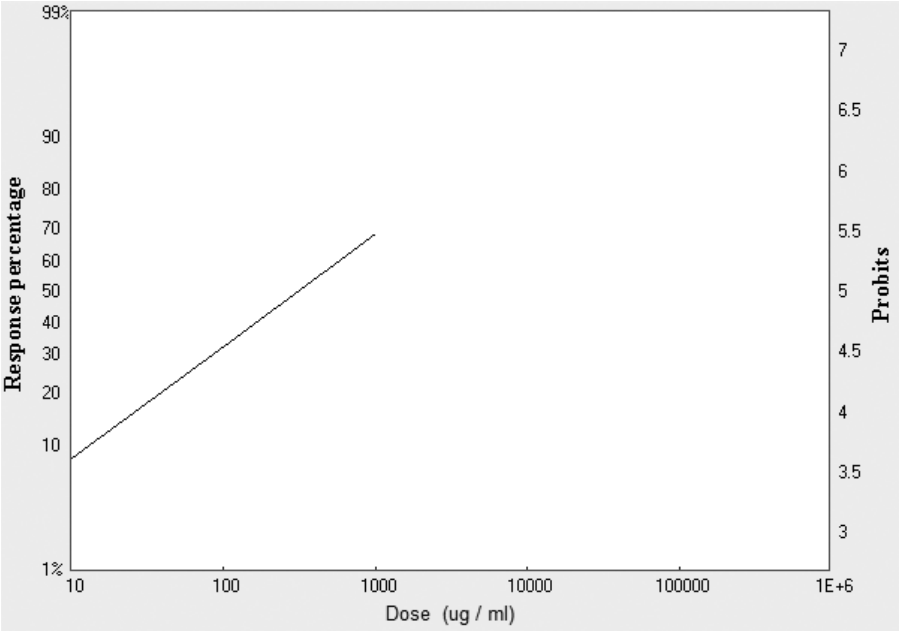

Figure 2. Dose-response curve shows relation between percentage of deaths of brine shrimps nauplii and applied concentration of *C. dubia* methanol extract

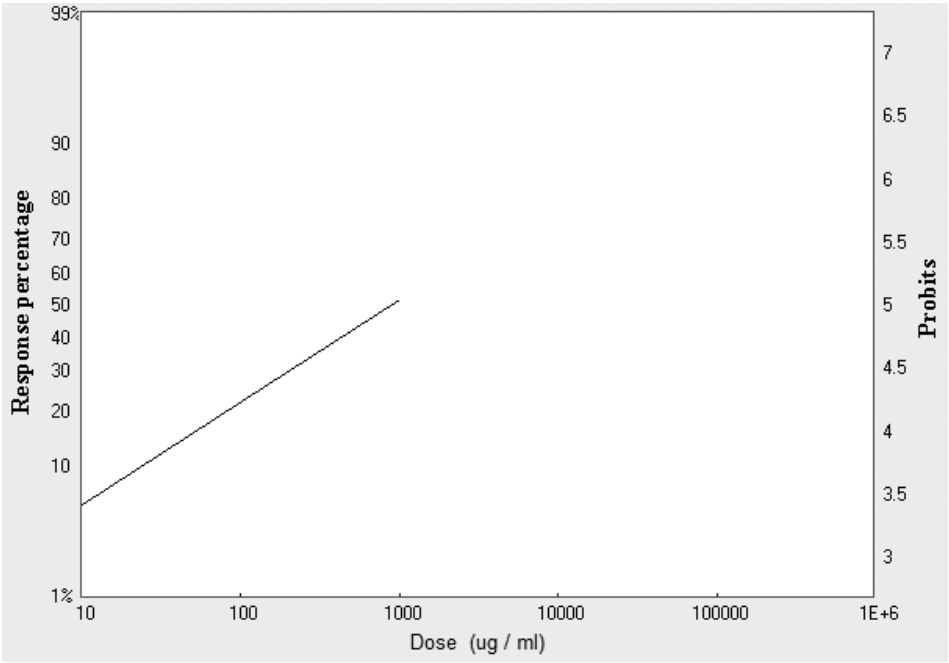

Figure 3. Dose-response curve shows relation between percentage of deaths of brine shrimps nauplii and applied concentration of *Th. tosevii* methanol extract

It is obvious that species *T. baccata* showed the best cytotoxicity, since it produces compounds taxanes (well known anticancer drug) (Yu-Fang *et al*., 2011). The leaves of *T. baccata* are used in the traditional Asiatic Indian (Ayurvedic) medicine system and there is also one reported use in the treatment of "cancer" (Hartwell 1982). Nowadays, investigation is focused on the advances in the production of taxol and related taxanes in *T. baccata,* using cell suspension culture biotechnology (Malik *et al*., 2011). Our result for methanol extract of *Centaurea dubia* (LC_{50} = 294.4 μg/mL) is in correlation with results of investigation of general bioactivity, using brine shrimp lethality test, which covered 15 *Centaurea* L. species and cnicin isolated from *C. derventana* (Janaćković *et al*., 2008). Among these investigated species, diethyl ether extract of *C. splendens* showed significant activity (LC_{50} = 7.3 μg/mL), as did methanol extract of *C. arenaria* (LC_{50} = 12.4 μg/mL). These findings together with our result indicate that genus *Centaurea* possesses compounds which show significant biological activity. Our results of cytotoxicity of methanol extract of *Th. tosevii* confirmed previous findings of biological activities of *Thymus* taxa (Bozkurt *et al.,* 2012).

In fact, better cytotoxicity of methanol extracts compared to diethyl ether extracts, could be explained due to presence of polar (flavonoids and sesquiterpene lactones) and some non-polar compounds (such as polyacetylenes) and other bioactive compounds, as well as of their synergistic effect. Different

activity among investigated taxa also could be caused by different composition of secondary compounds which have an important role in biological activity.

In this way, further research of *Taxus*, *Centaurea* and *Thymus* taxa should be focused on determining pure compounds of certain extracts and their biological activity.

Table 4. Cytotoxic activity of methanol and diethyl ether extracts of investigated species. Potassium dichromate (50 µg/mL) was used as a positive control. (nt): not tested.

Species	Percent deaths at 24 h								
	Methanol extract					Diethyl ether extract			
	10 µg/mL	100 µg/mL	1000 µg/mL	LC_{50} µg/mL	(95% confidence interval)	10 µg/mL	100 µg/mL	1000 µg/mL	LC_{50} µg/mL
Taxus baccata L.	44	67	93	18.6±0.1	9.3-30.9	nt	nt	nt	nt
Centaurea dubia Suter	7	37	67	294.4±0.1	196.9–473.9	0	0	10	> 1000
Thymus tosevii Vel.	4	27	50	842.5±0.1	492.7–1,832.7	nt	nt	nt	nt
Picea omorika (Pančić) Purk.	7	17	37	> 1000	--	nt	nt	nt	nt
Ajuga genevensis L.	0	20	24	> 1000	--	0	0	30	> 1000
A. reptans L.	0	4	17	> 1000	--	0	0	7	> 1000
A. chamaepitys (L.) Schreb.	4	4	20	> 1000	--	0	0	4	> 1000
Anthemis cotula L.	0	7	17	> 1000	--	0	10	20	> 1000
Micromeria juliana (L.) Benth.	7	7	20	> 1000	--	0	0	7	> 1000
M. cristata (Hoppe) Gris.	0	0	7	> 1000	--	0	0	0	> 1000
M. albanica (Griesb. ex Maly) Šilić	0	7	7	> 1000	--	0	0	7	> 1000
M. dalmatica Benth.	0	7	7	> 1000	--	0	0	7	> 1000
Anthemis ruthenica M.B.	0	0	0	> 1000	--	0	0	0	> 1000
Pinus heldreichii Christ.	0	0	7	> 1000	--	nt	nt	nt	nt
Abies alba Mill.	0	0	0	> 1000	--	nt	nt	nt	nt

CONCLUSION

Methanol extracts of *Taxus baccata, Centaurea dubia* and *Thymus tosevii* showed good cytotoxic effect. These findings indicate further pharmacological and phytochemical investigations of these species. Also, for that reason, genera *Taxus*, *Centaurea* and *Thymus*, could be potentially good source of biologically active compounds.

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БИОТЕСТ СОНИ РАЧИЋ ОДАБРАНИХ ВРСТА ГОЛОСЕМЕНИЦА И СКРИВЕНОСЕМЕНИЦА

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РЕЗИМЕ**:** Метанолни екстракти одабраних врста цветница *Anthemis cotula*, *A. ruthenica*, *Centaurea dubia* (Asteraceae), *Ajuga genevensis*, *A. chamaepitys*, *A. reptans*, *Micromeria albanica*, *M. cristata*, *M. dalmatica*, *M. juliana*, *Thymus tosevii* (Lamiaceae), и четинара *Abies alba*, *Picea omorika*, *Pinus heldreichii* (Pinaceae) и *Taxus baccata* (Taxaceae), као и етарски екстракти десет врста: *Anthemis cotula*, *A. ruthenica*, *Centaurea dubia*, *Ajuga genevensis*, *A. chamaepitys*, *A. reptans*, *Micromeria albanica*, *M. cristata*, *M. dalmatica* и *M. juliana* које припадају фамилијама цветница (Asteraceae и Lamiaceae) тестирани су помоћу биотеста сони рачић (*Artemia salina*). Леталне концентрације (LC_{50}) са интервалом поверења од 95% одређиване су помоћу рачунарског програма LdP line. Од 15 тестираних метанолних екстраката, три су показала добар цитотоксични ефекат. Метанолни екстракт врсте *Taxus baccata* показао је највећи ефекат (LC_{50} = 18.60 μg/ml), док је метанолни екстракт врсте *Thymus tosevii* показао најмањи (LC_{50} = 842.50 μg/ml). Метанолни екстракти осталих испитиваних врста нису показали значајнију цитотоксичност. Такође, етарски екстракти свих испитиваних врста нису показали значајнију цитотоксичност. Добијени резултати метанолних екстраката испитиваних врста који су показали биолошку активност указују на даља фитохемијска и фармаколошка истраживања ових врста.

КЉУЧНЕ РЕЧИ: Asteraceae, биотест сони рачић, цитотоксичност, Lamiaceae, Pinaceae, Taxaceae

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RARE AND ENDANGERED PLANT SPECIES AND ASSOCIATIONS IN THE MORAVICA RIVER (SERBIA)

ABSTRACT: The Moravica is a river in the southeast of Banat (Vojvodina Province, Serbia). This relatively small river is characterised by great floristic richness. A total of 87 taxa were found in the Moravica River. It is a sanctuary for some plant species that are rare and endangered both in Serbia and in Europe. Fifty-five species are on the IUCN Red List of Threatened Species and forty-five species are on the European Red List of Vascular Plants. Species *Acorus calamus* L., *Alisma gramineum* Gmel., *Iris pseudacorus* L., *Marsilea quadrifolia* L., *Potamogeton fluitans* Roth. and *Utricularia vulgaris* L. are protected or strictly protected by law in Serbia. Some of these rare species form stands of aquatic and semiaquatic vegetation rare both in Banat and in Serbia in general, such as: *Lemnetum* (*minori*) – *trisulcae* Den Hartog 1963, *Potametum nodosi* Soó (1928) 1960, Segal 1964, *Acoreto – Glycerietum aquaticae* Slavnić 1956, *Rorippo – Oenanthetum* (Soó 1927) Lohm. 1950, Pop 1968, and *Bolboschoenetum maritimi continentale* Soó (1927) 1957 subass. *marsiletosum quadrifoliae* Ljevnaić-Mašić (2010).

Because of its great diversity of flora and vegetation, the Moravica River could be a potential Important Plant Area (IPA) in the future. Unfortunately, strong anthropogenic influence is a threat to this unique flora and vegetation, so appropriate and timely measures for protecting the aquatic ecosystem need to be implemented.

KEYWORDS**:** diversity, flora, rare and protected plant species, the Moravica River, vegetation

INTRODUCTION

Aquatic ecosystems are characterised by diversity of wildlife. They are sanctuaries for many rare and endangered plants, and thus special attention should be paid to preservation of these natural habitats in order to preserve biodiversity (Radović 2005; Nikolić *et al*., 2008; Ljevnaić-Mašić 2010). However, urbanisation, reclamation works, environmental pollution, afforestation

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and other anthropogenic influences have seriously endangered the natural habitats of many species resulting in biodiversity loss, which is an increasing problem in the 21st century (Božа *et al*., 1997a; Božа *et al*., 1997b; Radović 2005). This refers to disappearance of species and their natural habitats. One of the most widely accepted concepts of nature protection is protection of individual areas. Therefore, in order to preserve biodiversity the damaged ecosystems should be preserved and restored, and populations of plant and animal species should be conserved and recovered. Without adequate preservation of habitats there is no adequate protection of biodiversity.

Understanding the biology and ecology of plants is a prerequisite for taking the necessary measures of protecting rare and endangered plant species and plant communities and conservation of unique genetic resources. These findings should be used in planning the protective measures or monitoring of ecosystems. Moreover, knowledge of genetic diversity of rare and endangered species provides important additional information on genetic resources, as well as a basis for planning of conservation strategies.

The aim of this paper is to review plant species and associations which are rare and endangered not only in Serbia but also in the whole of Europe.

MATERIAL AND METHODS

Studied area

The Moravica is a canalled watercourse located in the northeast of Serbia in Banat (Figure 1). It belongs to the network of hydro-amelioration facilities connected into a single hydro-technical unit – the hydro-system Danube-Tisa-Danube (Hs DTD), which solves many amelioration problems in this area. This relatively small river, 17.4 km long, of narrow and shallow riverbed, flows into Serbia from Romania. The amount of water in the Moravica is very variable depending on the period of the year. The highest water level is in the spring, as a result of melting snow from the Carpathians and heavy rainfall. In summer, the water level significantly decreases, so in certain parts of the stream the river dries up. The land through which the Moravica flows is mainly composed of salt marshes (Ljevnaić-Mašić 2010).

Investigation of flora

Floristic and phytocoenological research of the Moravica watercourse was carried out during the vegetation period 2006–2010. Plant species were determined according to Josifović ed. (1970–1977), Sarić ed. (1986–1992), Felföldy (1990), and Javorka and Csapody (1975). Classification of taxa into families was done according to Takhtajan (2009). The conservation status of plant species was given globally (IUCN, 2015), for Europe (Bilz *et al*., 2011), for the area of

Switzerland and the Alps (Landolt 2010), and for Serbia (*Službeni glasnik RS*, 5/2010 and 47/2011; Radulović *et al.*, 2012), Croatia (Nikolić 2016), and Bulgaria according to Red Data Book of the Republic of Bulgaria (Table 1). Syntaxonomic classification of the identified taxa was done according to Soó (1964–1980).

Figure 1. Studied area

This paper shows the distribution of rare and endangered species in the world and Serbia, the basic characteristics of their habitat, and the conservation status and syntaxonomic classification of rare and endangered species identified in the flora of the Moravica in the territory of Serbia (Soó, 1964–1980; Lakušić *et al.*, 2005; Ljevnaić-Mašić, 2010; Bilz *et al.*, 2011; IUCN, 2015; Nikolić 2016; http://e-ecodb.bas.bg/rdb/en/vol1/).

RESULTS AND DISCUSSION

Flora of the Moravica River

During the floristic research of the Moravica watercourse 87 plant species were determined. The largest number of species belong to the families *Poaceae* (9 species; 10.34%), *Asteraceae* (7 species 8.05%), and *Polygonaceae* (6 species, 6.90%) (Table 1). This small river is a sanctuary for species which are rare, endangered and protected not only in Serbia but in the whole of Europe (Table 1). Out of the total number of the identified species, 55 species are on the IUCN Red List of Threatened Species (IUCN, 2015). According to IUCN (2015), 53 species have the status of Last Concern (LC), while the species *Sonchus arvensis* has the status of Near Threatened (NT) and *Alisma gramineum* has the status of Data Deficient (DD) (Table 1). According to the European Red List of Vascular Plants (IUCN-E), 45 species are endangered, including 44 species with the status LC, and *Marsilea quadrifolia* with the status NT (Bilz *et al*., 2011). In addition, a number of identified species are rare and endangered in a significant part of Europe (Switzerland-CH, Jura Mountains-JU, the Central Plateau-MP, the North Alps-NA, and the Central Alps West-WA) (Landolt 2010) (Table 1).

As for the conservation status of the identified taxa in the area of the Balkan Peninsula, the species *Acorus calamus*, *Alisma gramineum*, *Iris pseudoacorus*, *Marsilea quadrifolia*, *Potamogeton fluitans,* and *Utricularia vulgaris* are protected by law in Serbia (*Službeni glasnik RS*, 5/2010 and 47/2011; Radulović *et al*., 2012) (Table 1). *Althaea officinalis* and *Iris pseudacorus* are commercial species and are subject to the provisions of the Regulation on controlling of the use and trade of wild flora and fauna of the Republic of Serbia (*Službeni glasnik RS*, 31/2005, 45/2005-ispr., 22/2007, 38/2008 and 9/2010). In Croatia, the species *Alisma gramineum*, *Lemna gibba,* and *Marsilea quadrifolia* have the status Endangered (EN), the species *Carex vesicaria*, *Clematis integrifolia,* and *Glyceria fluitans* have the status Vulnerable (VU), the species *Bolboschoenus maritimus*, *Butomus umbellatus*, *Heleochloa alopecuroides,* and *Heleochloa schoenoides* have the status NT, while the species *Acorus calamus* and *Serratula tinctoria* have LC status (Table 1). In the Moravica there is *Marsilea quadrifolia* with the status Critically Endangered (CR) and *Nymphoides flava* with the status EN in Bulgaria (Table 1).

Table 1. Flora of the Moravica River

Nº	Family	Taxon	Taxon Vulnerability									
			IUCN	IUCN-E	CH	JU	MP	NA	WA	Cro	Bul	Ser
1.	Acoraceae	*Acorus calamus* L.	LC		VU	EN	VU	EN	EN	LC		PS
2.	Poaceae	*Agropyrum repens* (L.) Beauv.										
3.	Poaceae	*Agrostis alba* L.	LC									
4.	Alismataceae	*Alisma gramineum* Gmel.	DD	LC	LC	CR	EN	RE	-	EN		SPS
5.	Alismataceae	*Alisma plantago-aquatica* L.	LC	LC	LC	LC	LC	LC	VU			
6.	Malvaceae	*Althaea officinalis* L.										
7.	Fabaceae	*Amorpha fruticosa* L.										
8.	Aristolochiaceae	*Aristolochia clematitis* L.										
9.	Asteraceae	*Artemisia vulgaris* L.										
10.	Asteraceae	*Bidens tripartitus* L.										
11.	Cyperaceae	*Bolboschoenus maritimus* (L.) Palla.	LC	LC						NT		
12.	Butomaceae	*Butomus umbellatus* L.	LC	LC	VU	EN	VU	RE	-	NT		
13.	Convolvulaceae	*Calystegia sepium* (L.) R. Br.			LC	LC	LC	LC	LC			
14.	Cyperaceae	*Carex pseudocyperus* L.	LC	LC	VU	EN	VU	EN	EN			
15.	Cyperaceae	*Carex vesicaria* L.	LC	LC	NT	NT	NT	NT	EN	VU		
16.	Ceratophyllaceae	*Ceratophyllum demersum* L.	LC	LC	VU	EN	VU	EN	CR			
17.	Ranunculaceae	*Clematis integrifolia* L.			DD	DD	DD	DD	DD	VU		
18.	Euphorbiaceae	*Euphorbia lucida* W. K.										
19.	Rubiaceae	*Galium mollugo* L.			LC	-	LC	LC	LC			
20.	Rubiaceae	*Galium palustre* L.	LC		LC	LC	LC	LC	LC			
21.	Poaceae	*Glyceria fluitans* (L.) R. Br.	LC	LC	LC	LC	LC	LC	LC	VU		
22.	Poaceae	*Glyceria maxima* (Hartm.) Holm.	LC	LC	VU	CR	VU	CR	-			
23.	Fabaceae	*Glycyrrhiza echinata* L.										
24.	Cyperaceae	*Heleocharis palustris* (L.) R. Br.										
25.	Poaceae	*Heleochloa alopecuroides* (Piller et Mitterp.) Host	LC							NT		
26.	Poaceae	*Heleochloa schoenoides* (L.) Host.	LC							NT		
27.	Hydrocharitaceae	*Hydrocharis morsus-ranae* L.	LC	LC	EN	CR	EN	EN	-			
28.	Asteraceae	*Inula britannica* L.			EN	RE	EN	RE	EN			
29.	Iridaceae	*Iris pseudacorus* L.	LC	LC	LC	LC	LC	LC	NT			PS
30.	Juncaceae	*Juncus compressus* Jacq.	LC	LC	LC	LC	LC	LC	LC			
31.	Juncaceae	*Juncus gerardi* Lois.			LC	LC						
32.	Lemnaceae	*Lemna gibba* L.	LC	LC	RE	-	RE	RE	-	EN		
33.	Lemnaceae	*Lemna minor* L.	LC	LC	RE	-	LC	LC	NT			

34.	Lemnaceae	*Lemna trisulca* L.	LC	LC	NT	VU	NT	VU	EN			
35.	Lamiaceae	*Lycopus europaeus* L.	LC	LC	LC	NT	LC	LC	VU			
36.	Lamiaceae	*Lycopus exaltatus* L.		LC								
37.	Primulaceae	*Lysimachia nummularia* L.	LC	LC	LC	LC	LC	LC	EN			
38.	Primulaceae	*Lysimachia vulgaris* L.	LC	LC	LC	LC	LC	LC	LC			
39.	Primulaceae	*Lysimachia vulgaris* f. *ternifolia* Peterm.	LC	LC	LC	LC	LC	LC	LC			
40.	Lythraceae	*Lythrum virgatum* L.		LC								
41.	Marsileaceae	*Marsilea quadrifolia* L.	LC	NT	EN	RE	EN	RE	-	EN	CR	SPS
42.	Asteraceae	*Matricaria inodora* L.										
43.	Lamiaceae	*Mentha aquatica* L.	LC		LC	LC	LC	LC	NT			
44.	Lamiaceae	*Mentha pulegium* L.	LC	LC								
45.	Haloragaceae	*Myriophyllum spicatum* L.	LC	LC	NT	VU	NT	NT	VU			
46.	Najadaceae	*Najas marina* L.	LC	LC	VU	-	VU	DD	-			
47.	Najadaceae	*Najas minor* All.	LC	LC	EN	-	EN	RE	-			
48.	Menyanthaceae	*Nymphoides flava* Hill.	LC	LC	VU	VU	VU	EN	-		EN	
49.	Apiaceae	*Oenanthe aquatica* (L.) Poiret	LC	LC	EN	CR	EN	-	-			
50.	Poaceae	*Panicum crus-galli* L.			LC	LC	LC	LC	LC			
51.	Poaceae	*Phragmites communis* Trin.	LC	LC	LC	LC	LC	LC	LC			
52.	Plantaginaceae	*Plantago major* L.			LC	LC	LC	LC	LC			
53.	Polygonaceae	*Polygonum amphibium* L.	LC		NT	NT	VU	NT	VU			
54.	Polygonaceae	*Polygonum amphibium* L. var. *aquatica* Leyss.	LC		NT	NT	VU	NT	VU			
55.	Polygonaceae	*Polygonum aviculare* L.			LC	LC	LC	LC	LC			
56.	Polygonaceae	*Polygonum hydropiper* L.	LC		LC	LC	LC	NT	NT			
57.	Polygonaceae	*Polygonum lapathifolium* L.	LC		LC	LC	LC	LC	LC			
58.	Potamogetonaceae	*Potamogeton crispus* L.	LC	LC	LC	VU	LC	NT	VU			
59.	Potamogetonaceae	*Potamogeton fluitans* Roth.	LC	LC	VU	VU	VU	VU	RE			SPS
60.	Potamogetonaceae	*Potamogeton lucens* L.	LC	LC	LC	NT	LC	NT	EN			
61.	Potamogetonaceae	*Potamogeton pectinatus* L.	LC		LC	NT	LC	LC	EN			
62.	Rosaceae	*Potentilla reptans* L.			LC	LC	LC	LC	LC			
63.	Ranunculaceae	*Ranunculus paucistamineus* Tsch.			LC	LC	NT	NT	NT			
64.	Ranunculaceae	*Ranunculus repens* L.		LC	EN	EN	EN	CR	EN			
65.	Brassicaceae	*Rorippa amphibia* (L.) Bess.	LC	LC	VU	EN	VU	RE	RE			
66.	Brassicaceae	*Rorippa kerneri* Menyh.	LC	LC								
67.	Brassicaceae	*Rorippa silvestris* (L.) Bess.			LC	LC	LC	LC	LC			
68.	Rosaceae	*Rubus caesius* L.			LC	LC	LC	LC	LC			
69.	Polygonaceae	*Rumex hydrolapathum* Huds.	LC	LC	EN	EN	EN	EN	DD			
70.	Salicaceae	*Salix alba* L.	LC		LC	LC	LC	LC	LC			
71.	Cyperaceae	*Schoenoplectus lacuster* (L.) Palla			LC	NT	LC	NT	VU			
72.	Asteraceae	*Serratula tinctoria* L.			NT	VU	VU	VU	EN	LC		

73.	Apiaceae	*Sium latifolium* L.		LC	CR	RE	CR	RE	RE			
74.	Asteraceae	*Sonchus arvensis* L.	NT		LC	LC	LC	LC	LC			
75.	Sparganiaceae	*Sparganium ramosum* Huds.	LC	LC	NT	NT	NT	NT	CR			
76.	Lemnaceae	*Spirodela polyrrhiza* (L.) Schleid.	LC		NT	EN	NT	VU	-			
77.	Lamiaceae	*Stachys palustris* L.	LC		NT	NT	NT	NT	VU			
78.	Boraginaceae	*Symphytum officinale* L.			LC	LC	LC	LC	NT			
79.	Ranunculaceae	*Thalictrum lucidum* L.										
80.	Typhaceae	*Typha angustifolia* L.	LC	LC	NT	VU	NT	VU	EN			
81.	Typhaceae	*Typha latifolia* L.	LC	LC	LC	LC	LC	LC	LC			
82.	Poaceae	*Typhoides arundinacea* (L.) Much.	LC	LC	LC	LC	LC	LC	LC			
83.	Urticaceae	*Urtica dioica* L.		LC								
84.	Lentibulariaceae	*Utricularia vulgaris* L.	LC	LC	VU	EN	VU	EN	RE			PS
85.	Scrophulariaceae	*Veronica anagallis-aquatica* L.	LC	LC	LC	NT	LC	LC	VU			
86.	Scrophulariaceae	*Veronica anagalloides* Guss.	LC	LC	CR	-	DD	-	CR			
87.	Asteraceae	*Xanthium italicum* Moretti			EN	-	-	-	-			
			Total of threatened taxa									
			55	**45**	**67**	**60**	**65**	**63**	**54**	**12**	**2**	**6**

Legend:
IUCN – according to International Union for Conservation of Nature; IUCN-E – according to European Red List of Vascular Plants; CH – Switzerland; JU – Jura mountains; MP – Central Plateau; NA – North Alps; WA – Central Alps West; "-"- not occurring in the Flora of Switzerland and the Alps; Cro – Croatia; Bul – Bulgaria, Ser – Serbia; SPS – Strictly Protected Species; PS – protected species**;** LC – Last Concern; NT – Near Threatened; RE – regionally extinct; VU – Vulnerable; EN – Endangered; CR – Critically Endangered; EX – Extinct; DD – Data Deficient

Acorus calamus L.

Population: It is widespread and abundant throughout its native and non-native range in the Old World and in parts of North America.

Habitat and Ecology: The species grows on the margins of standing or slow-flowing water, in river backwaters, canal margins and the margins of ponds and lakes; lukewarm water with a lot of detritus, muddy riverbed, organogenic soil or saline marsh chernozem.

Distribution in Serbia: Pannonian Plain (south-western Banat, pond Obedska Bara, vicinity of Novi Sad, western Bačka, middle Banat, Srem, Deliblatska Peščara, Koviljski Rit, Dubovački Rit, and canals of the Hs DTD.

Threats: No information is available on threats to the species or its habitats.

Conservation Actions: No conservation measures have been undertaken specifically for this species and none are needed.

Conservation Status: IUCN-LC; CH-VU; JU-EN; MP-VU; NA-EN; WA-EN; Croatia (Cro)-LC; Serbia (Ser)-Protected Species (PS).

Syntaxonomic classification of species: *Phragmition* (spec. *Acoretum*).

***Alisma gramineum* Gmel**.

Population: It is one of a number of species that appears to be rare almost throughout its known range. The only area in which the species appears to be abundant is in the Rhine floodplain in Germany and the Netherlands. It is rare in Bulgaria, Croatia and Greece.

Habitat and Ecology: It can occur in a very wide variety of habitat types. Usually, it occurs in calcareous, naturally meso- or eutrophic lowland water bodies (ponds, lakes, fenland, canals, rice and other arable fields, depressions and sandy shorelines).

Distribution in Serbia: Canal of the Hs DTD (Moravica).

Threats: The habitats in which this species occurs are sensitive to hyper-eutrophication, as they are often naturally eutrophic. Many populations are isolated and, therefore, vulnerable to drainage or habitat degradation.

Conservation Actions: It is very rare in many European countries (Austria, Belgium, Denmark, Slovakia, United Kingdom, etc.) and extinct in Sweden. It is very difficult to establish whether or not there is a Europe-wide need for conservation action for this species. In spite of the number of countries in which it is considered to be of conservation concern, it is difficult to identify areas in which there has been a measured decline. For this reason, the main conservation action identified here is research to clarify its status throughout the region.

Conservation Status: IUCN-DD; IUCN-E-LC; CH-LC; JU-CR; MP-EN; NA-Regionally Extinct (RE); Cro-EN; Ser–Strictly Protected Species (SPS).

Syntaxonomic classification of species: *Nanocyperion* (-*Potamion;* var. *angustissimum* (DC.) Hendriks: *Batrachion fluitantis*).

***Iris pseudoacorus* L.**

Population: It is widespread and abundant throughout its European range.

Habitat and Ecology: It occurs in shallow water or saturated soils in marshes, along the shores of lakes, rivers, ponds with stagnant or slow flowing waters, and ditches.

Distribution in Serbia: Riverine forests of willow (*Salix*), alder (*Alnus*) and birch (*Betula*); willow (*Salix*) forests around rivers; Central European forests of white willow (*Salix alba*); marsh forests of narrow-leaf ash (*Fraxinus angustifolia*); and canals of the Hs DTD.

Threats: Past, ongoing or future threats to this species are not known.

Conservation Actions: There are no conservation measures in place or needed.

Conservation Status: IUCN-LC; IUCN-E-LC; CH-LC; JU-LC; MP-LC; NA- C; WA-NT; Ser-PS.

Syntaxonomic classification of species: *Phragmitetea (Salicetea, Alno--Padion).*

***Marsilea quadrifolia* L.**

Population: There is no information on global population trends. It is a common species.

Habitat and Ecology: It grows in still waters such as ponds, rice fields and ditches; on the muddy surface rich in nitrogen, and in the flood zone of rivers and lakes.

Distribution in Serbia: Low river banks and canals on which water stays almost throughout the year; canal of the Hs DTD (Moravica).

Threats: No major threats have been reported.

Conservation Actions: No conservation measures in place.

Conservation Status: IUCN-LC; IUCN-E-NT; CH-EN; JU-RE; MP-EN; NA-RE; Cro-EN; Bulgaria (Bul)-CR; Ser-SPS.

Syntaxonomic classification of species: *Nanocyperion, Hydrocharition, Nymphaeion.*

Potamogeton fluitans Roth.

Population: It is widespread and abundant throughout its known range. There is no detailed information available on population size.

Habitat and Ecology: It occurs in most water body types (lake margins, ponds, temporary pools, streams, larger rivers, river backwaters, and lowland river meanders). It appears to tolerate nutrient enrichment and is most frequent in mesotrophic to eutrophic calcareous waters, and also in calm and slow-flowing waters.

Distribution in Serbia: Aquatic habitat of lowland and mountain areas of Serbia. It is most common in the Danube region in Vojvodina, canals of the Hs DTD, and ponds remaining after river withdrawal in the regions of Mačva and Srem.

Threats: Past, ongoing or future threats to this species are not known.

Conservation Actions: There are no conservation measures in place and none needed.

Conservation Status: IUCN-LC; IUCN-E-LC; CH-VU; JU-VU; MP-VU; NA-VU; WA-RE; Ser-SPS.

Syntaxonomic classification of species: *Lemno-Potametea* (*spec. Batrachion fluitantis*).

Utricularia vulgaris L.

Population: It appears to be widespread and abundant throughout its European range.

Habitat and Ecology: It is found in oligotrophic, eutrophic and mesotrophic, base-rich waters. Habitats include sheltered bays in limestone lakes, ponds, ditches and pools in calcareous fens and grazing marshes, and flooded clay-, marl-, and gravel-pits, as well as shallow water near river banks, of neutral to acid pH.

Distribution in Serbia: Vlasina Lake, Koviljski Rit, canals of the Hs DTD.

Threats: Past, ongoing or future threats to the survival of this species are not known.

Conservation Actions: In France, this species is under regional protection. There are no other conservation measures in place or needed.

Conservation Status: IUCN-LC; IUCN-E-LC; CH-VU; JU-EN; MP-VU; NA-EN; WA-RE; Ser-PS.

Syntaxonomic classification of species: *Lemno-Potametea,* spec. *Hydrochari-Lemnion*.

In addition to great floristic richness, the Moravica River is characterised also by wide diversity of vegetation (Ljevnaić-Mašić 2010). The Moravica vegetation comprises 14 plant associations of aquatic and semi-aquatic vegetation (Ljevnaić-Mašić 2010). Among them there are some plant associations which are rare and endangered in the territory of Serbia and the Balkan Peninsula, including *Lemnetum* (*minori*) – *trisulcae* Den Hartog 1963, *Potametum nodosi* Soó (1928) 1960, Segal 1964, *Acoreto – Glycerietum aquaticae* Slavnić 1956, *Rorippo – Oenanthetum* (Soó 1927) Lohm. 1950, Pop 1968, and *Bolboschoenetum maritimi continentale* Soó (1927) 1957 subass. *marsiletosum quadrifoliae* Ljevnaić-Mašić (2010).

CONCLUSION

The watercourse of the Moravica River (Vojvodina, Serbia) is characterised by great floristic and vegetation richness. The flora of the Moravica comprises a total of 87 plant species, making 14 plant associations of aquatic and semi-aquatic vegetation. The river is a sanctuary for some rare and endangered species protected by law in Serbia and Europe. Fifty-five species are on the IUCN list, while forty-five species are on the European Red List of Vascular Plants. The species *Acorus calamus* L., *Alisma gramineum* Gmel., *Iris pseudacorus* L., *Marsilea quadrifolia* L., *Potamogeton fluitans* Roth., and *Utricularia vulgaris* L. are protected by law in the territory of Serbia. These species make stands of some rare associations of aquatic and semi-aquatic vegetation in the territory of Serbia, including: *Lemnetum* (*minori*) – *trisulcae* Den Hartog 1963, *Potametum nodosi* Soó (1928) 1960, Segal 1964, *Acoreto – Glycerietum aquaticae* Slavnić 1956, *Rorippo – Oenanthetum* (Soó 1927) Lohm. 1950, Pop 1968, and *Bolboschoenetum maritimi continentale* Soó (1927) 1957 subass. *marsiletosum quadrifoliae* Ljevnaić-Mašić (2010).

However, strong anthropogenic influence is a threat to this unique flora and vegetation of the Moravica watercourse. Therefore, appropriate and timely protection measures should be implemented in order to preserve this aquatic ecosystem, so that in future this small river could be a potential Important Plant Area (IPA). Without adequate preservation of habitat there is not adequate protection of biodiversity either at the level of species or the genetic level.

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РЕТКЕ И УГРОЖЕНЕ БИЉНЕ ВРСТЕ И ЗАЈЕДНИЦЕ РЕКЕ МОРАВИЦЕ (СРБИЈА)

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РЕЗИМЕ: Моравица је река у југоисточном делу Баната (Војводина, Србија). Ова релативно мала река одликује се великим флористичким богатством. Флору Моравице чини укупно 87 биљних врста. Уточиште за свој развој овде су нашле неке ретке и угрожене врсте Србије и Европе, уопште. Педесет и пет врста је на IUCN листи, а четрдесет и пет на Европској црвеној листи васкуларних биљака. Врсте *Acorus calamus* L., *Alisma gramineum* Gmel., *Iris pseudacorus* L., *Marsilea quadrifolia* L., *Potamogeton fluitans* Roth. и *Utricularia vulgaris* L. су законом заштићене или строго заштићене у Србији. Неке од ових ретких врста граде састојине ретке акватичне и семиакватичне вегетације у Банату и Србији, уопште, као што су: *Lemnetum* (*minori*) – *trisulcae* Den Hartog 1963, *Potametum nodosi* Soó (1928) 1960, Segal 1964, *Acoreto – Glycerietum aquaticae* Slavnić 1956, *Rorippo – Oenanthetum* (Soó 1927) Lohm. 1950, Pop 1968 и *Bolboschoenetum maritimi continentale* Soó (1927) 1957 subass. *marsiletosum quadrifoliae* Ljevnaić-Mašić (2010).

Због великог флористичког и вегетацијског диверзитета, у будућности би се Моравица могла посматрати као један од потенцијалних значајних подручја за биљке. Нажалост, јак антропогени утицај прети да угрози ову јединствену флору и вегетацију, те би на врема требало применити одговарајуће мере заштите овог акватичног екосистема.

КЉУЧНЕ РЕЧИ: диверзитет, флора, река Моравица, ретке и заштићене биљне врсте, вегетација

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ANALYSIS OF FOREST VEGETATION IN KOVILJSKI RIT: COMPARISON OF HABITATS WITH VARYING DEGREES OF ANTHROPOGENIC INFLUENCE

ABSTRACT: Koviljski Rit is located along the left bank of the Danube River, between the villages of Kovilj and Gardinovci. It is a rare example of pristine nature, where the original features of a swamp biotope are preserved. Because of its exceptional natural value, Koviljski Rit, along with the swamp in Petrovaradin, has been under the protection of the Republic of Serbia since 1998, when it was proclaimed a Special Nature Reserve of the first category. Natural vegetation found in Koviljski Rit includes woodlands composed of willow and poplar. The majority of the reserve consists of forest plantations (55.6%), which include habitats influenced by human activities.

Based on DCA (Detrended correspondence analysis) of forest vegetation in Koviljski Rit, there is a high degree of similarity between natural populations of white willow and stands of anthropogenic origin. However, in contrast, the same analysis indicates clear differences between natural and artificial poplar stands. Moreover, depending on whether they were planted in temporary or permanent flood plain habitats, significant differences are evident in the floristic structures of Euro-American poplar forests.

Ecological conditions in habitats of white and Euro-American poplar (*Populus* x *euroamericana* /Dode/ Guinier) communities were found to be more similar with respect to illumination and soil humidity. However, more importantly, the anthropogenic forests recorded the lowest diversity as a result of regular thinning and measures implemented through forest management.

KEYWORDS: anthropogenic influence, environmental factors, Koviljski Rit, forest vegetation

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INTRODUCTION

Flood plains, swamps and other wetlands are naturally sensitive ecosystems. Koviljski Rit is a remnant of a once wide-spread system of wetlands, and one of only two remaining natural swamp habitats, which marked the landscape of Vojvodina (Milutinović 1982). The Danube River near Kovilj deposits and accumulates sediments, creating an area up to 5 km wide, more commonly known as Koviljski Rit (Kovačević *et al*., 1995).

Koviljski Rit belongs to a forest-steppe climate zone. However, due to the vicinity of the river, low height differences and slightly uneven terrain, plants develop under the influence of its specific soil, groundwater and flood plains.

Forest stands in the swamp can be categorized as indigenous or anthropogenic. Indigenous forest communities are the remains of once wide-spread swamp forests. Unfortunately, because of direct human influence and exploitation, and more recently land reclamation projects, these forest ecosystems have changed dramatically.

Indigenous forest communities are conditioned by specific habitat factors, mainly influenced by the local water regime (flooding and groundwater), as well as soil factors. According to the floristic content and ecological traits of the habitat, Parabućski (1972) divided the forest vegetation of Koviljski Rit into 2 types:

Permanently flooded forests ass. *Salicetum triandrae* Malcult 1929 and ass. *Salicetum albae pannonicum* Parabućski 1972, which developed in areas with an abundance of moisture during the growing season. They are flooded when the Danube is at medium water levels.

Occasionally flooded forests ass. *Salici-Populetum nigrae* Parabućski 1965, ass. *Cratego nigrae – Populetum albae* Parabućski 1965 and ass. *Ulmetum campestrae* Parabućski 1965, developed on the Danube bank and high river beams: flooded only during periods of high water levels of the Danube. Their habitat is characterized by varying degrees of soil humidity.

Over time, due to both human exploitation and disease, indigenous forests have been replaced with resistant clones of Euro-American poplars, which provide excellent production results in forestry.

MATERIAL AND METHODS

Analysis included 113 relevés taken both from the literature and during field research. Relevés were collected using the Braun-Blanquet method (Braun-Blanquet 1932; Horvat *et al*., 1950) at several sites in Koviljski Rit.

Selected representative sites included the following departments and sections: 32/e, 32/s and 33/b GJ "Topolnik" with white poplar as the dominant species; 32/r with black poplar as the dominant species; and 32/r with white willow as the dominant species. Relevés were also taken from artificial stands of Euro-American poplars and white willows: relevés of Euro-American poplar were collected at sections 11/m and 32/h, and for white willow at section 11/o.

Collected plant materials within relevés were deposited in a herbarium using standard methods. Determination of plant taxa was performed with the help of the European flora (Tutin *et al.*, 1964–1993). All phyto-sociological data were analyzed using the TURBOVEG software package for Windows ver. 2.01101 (Hennekens *et al.*, 2001). Data were also analyzed using the software platform JUICE ver. 7.0.84 (Tichý 2002).

Similarities and differences within forest communities of willow and poplar in Koviljski Rit were analyzed with respect to the degree of human influence (natural stands, plantations and fallows) and local environmental conditions. Detrended correspondence analysis (DCA) was conducted using the above software platform and R version 2.10.0, considering species' ecological traits and following the method outlined by Elenberg (1998).

RESULTS AND DISCUSSION

By influencing agriculture, forestry, water management and other industries, human activity is often associated with disturbances in the stability and structure of vegetation and soil. The survival of plant life within plant communities at some biotopes is determined by various abiotic and biotic factors. The main ecological (abiotic) factors that affect the normal growth and development of plant life are humidity, light, temperature, continentality, soil pH and the amount of available nutrients. The relative importance and levels of each of these individual factors vary in different ecological niches.

According to Vasić *et al.* (2012), environmental conditions in forest ecosystems are also influenced by woodcutting, editing, reforestation, and forest restoration. In addition, soil damage, altered hydrological conditions and light regime changes can occur. In fact, a large number of plant species may find these new, altered conditions more favorable for their development. Moreover, such changes can occur in a very short period of time, for example in the first few years after woodcutting.

Adaptation of certain plant or animal species in relation to certain ecological factors in the environments is defined by ecological indices or indicator values. They represent the optimal value of ecological factors that enable the most intense growth and development of a species. Through analyses of the ecological indices of species, a clearer picture of their associated habitat and plant communities can be obtained.

From an ecological perspective, degraded (or disturbed) forest ecosystems should be returned to their original state if possible, or repaired to a state similar to the original. However, a comprehensive analysis of the present state must precede the designing of effective policies for renewing forests and forest ecosystems. These should include research into the origins of forest alterations, evaluation and assessment of the current state, and evaluation of developmental trends in the forest ecosystem, with and without additional anthropogenic influence (Tomić *et al.*, 2011).

In the area of Koviljski Rit, indigenous lowland forests of willow and poplar are being replaced by artificially established stands of Euro-American poplar or willow and poplar clones.

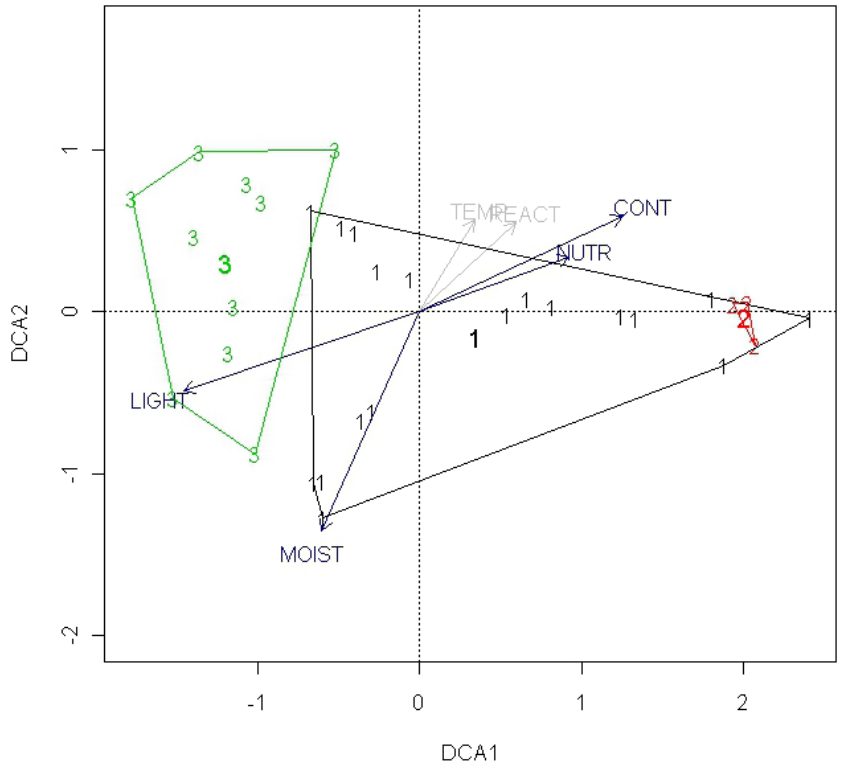

Figure 1. Comparison of willow stands using DCA
(1) natural willow stand (*Salicetum albae pannonicum* Parab. 1972); (2) planted willow stands; (3) willow stands at fallows

Based on DCA, it can bee seen that there is a significant similarity between planted (2) and natural (1) willow stands (Figure 1). In fact, throughout the reserve, natural communities of white willow and stands of anthropogenic origin are sometimes visually indistinguishable.

Deforestation opens a niche for new species which normally thrive in open habitats with more light. This is the reason why stands of trees in cleared areas are visibly different. By opening new habitats through deforestation, the risk of invasive species increases. In fact, cleared areas recorded the highest levels of invasive species, both woody and herbaceous.

Increased influence of anthropogenic factors is also present in renewed forests (Decker *et al.*, 2012), the intensity of light is greater, and due to accelerated decomposition of forest litter, the amount of nutrients in the soil is increased (Huebner and Tobin 2006). These altered conditions can lead to overgrowth

of the renewed forest areas with nitrophilic, ruderal and invasive plant species (Vasić *et al*., 2012).

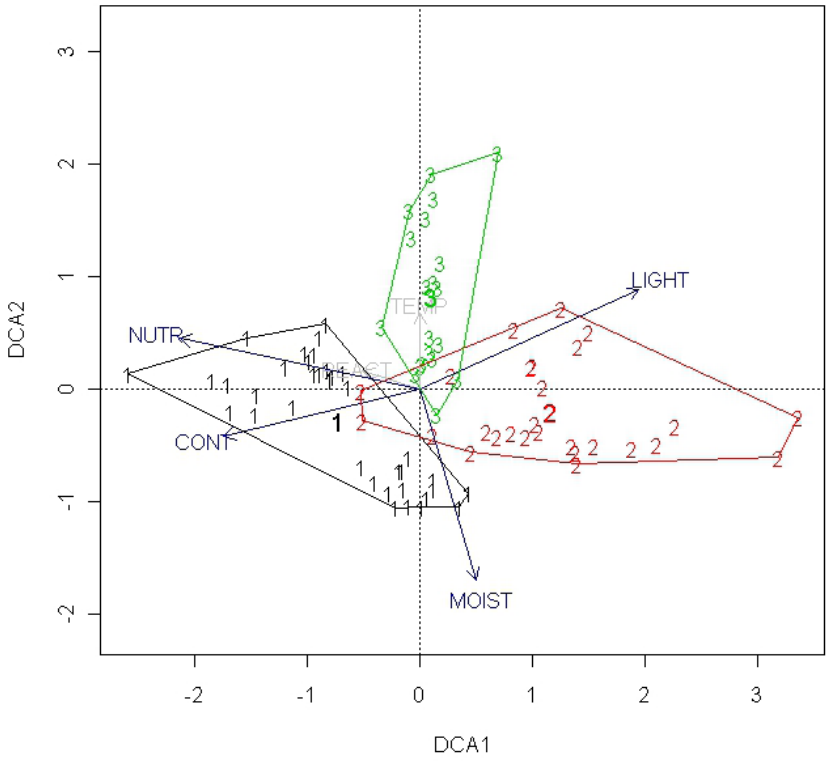

Figure 2. Comparison of poplar stands using DCA
(1) natural poplar stands; (2) planted poplar stands; (3) poplar stands at fallows

In contrast with the results obtained for natural and artificial white willow stands, DCA revealed a clear distinction between natural (1) and anthropogenic (2) poplar communities (Figure 2). Only a couple of relevés of these communities overlap to some extent, while most are inhabited with species with different habitat preferences.

In natural stands of black and white poplar the quantity of nutrients is increased and trees are set more closely. Furthermore, since the analyzed sites lie within a protected nature reserve, floristic diversity is greater.

Black poplar prefer moist well-aerated soil, while white poplar tolerate drier, and even to some extent saline habitats. Both species, but especially white poplar, are characterized by good outgrowing power and the possibility of successful recovery following woodcutting. In fact, their abundant yield potential manages to restore stands generatively, which is particularly important in terms of conservation and improvement of the biodiversity and stability of both species (Tucović *et al*., 1986).

Planted forests of Euro-American poplar are brighter, as expected, since thinning is regularly performed. Also, these hybrids are linked to the appearance of allelopathy, so it is clear that among them less floristic diversity is present.

Tomić *et al.* (2011) states that some potential causes of species diversity reduction in forest ecosystems could be the following: distortion of spatial and age structure of the population by selective tree cutting, inadequate selection of tree species for reforestation, favoring one tree species in plantations, neglecting spontaneous and natural restoration processes, and creating monocultures with low biodiversity.

Fallows of natural poplar forests included in the present study by their characteristics were placed between the two previous groups of relevés. As expected, species found here are those which prefer to inhabit illuminated sites that are somewhat drier vs. the other two groups. An increased number of invasive species was also recorded at these cleared sites compared to planted and natural stands. These altered conditions can lead to overgrowth of the regenerated areas with nitrophilic, ruderal and invasive plant species (Vasić *et al*., 2012).

Vast areas of Koviljsko-petrovaradinski Rit are occupied by planted forests of Euro-American poplars. They grow at different sites, from the highest to the lowest beams, with varying levels of humidity and other factors.

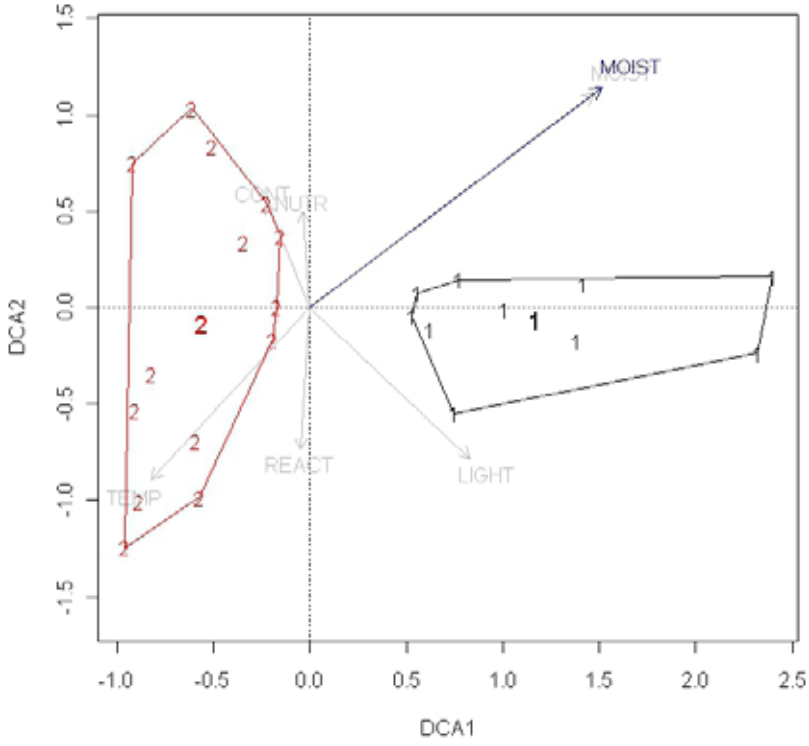

Figure 3. DCA of planted poplar forests at different habitats
(1) habitats of constantly flooded forests; (2) habitats of occasionally flooded forests

Figure 3 clearly reveals differences between ecological factors present at different habitats in poplar forests. Analysis of ecological indices shows that, although they contain the same stands of Euro-American poplar, there are significant differences among them. Constantly flooded forests (1) are planted at sites with high humidity throughout the growing period (flood waters remain longer, and groundwater is very close to the surface). These forests are flooded during periods of medium water levels of the Danube. Sites of constantly flooded forests contain species that are more tolerant to higher soil humidity and that prefer a larger amount of light. Poplar forests that are planted on occasionally flooded sites (2), mostly on high beams, are flooded only during periods of high water levels of the Danube. Their habitats are slightly warmer and have different levels of soil humidity.

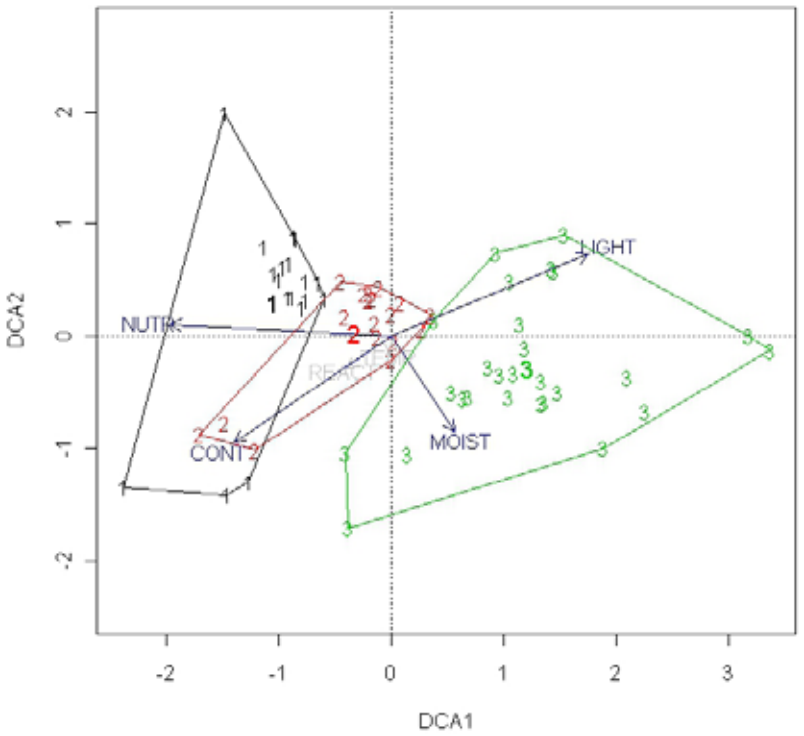

Figure 4. DCA of natural and artificial poplar stands
(1) *Salici-Populetum nigrae* Parab. 1965; (2) *Crataego nigrae-Populetum albae* Parab. 1965; (3) *Populus* x *euramericana* (Dode) Guiner

DCA of natural and artificial stands of poplar (Figure 4**)** shows a clear distinction between natural and artificial stands. If we compare only the natural stands, significant similarities between natural communities of white and black poplar can be noticed, but only for a small percentage of the sample. Since European black (*Populus nigra*) and white poplar (*Populus alba*) occupy

different ecological zones, and can participate in mixed associations, it is possible that there is no significant competition for growth and development of their stands (Tucović *et al.*, 1986).

In the process of regeneration of the natural biocoenosis of floodplains, black and white poplar represent important indicators of biodiversity. They are also important from a social perspective, especially in the control of flooding, groundwater levels and the maintenance and improvement of water quality (Vietto and Chiarabaglio 2004).

In the present study, ecological analyses of relevés clearly revealed differentiation between natural stands of white and black poplar and artificially established communities of Euro-American poplars. Also, ecological conditions associated with these habitats, especially soil humidity and illumination, were more similar at the sites with white and Euro-American poplar than at those with black and Euro-American poplar. Interestingly, sites with black poplar were found to contain the most nutrients. Finally, based on our results, sites receiving the most illumination were artificially planted forests, due to plant spacing and human maintenance of these stands.

CONCLUSIONS

DCA of relevés revealed significant similarities between natural communities of white willow and white willow stands of anthropogenic origin. However, white willow stands located at fallows differed because deforestation created a new niche for the introduction of species that prefer habitats with lots of light. As a result, the greatest presence of invasive species was recorded at fallows, both woody and herbaceous.

DCA also suggest significant differences between natural and artificial stands of poplar. Natural stands of black and white poplar were more closely spaced in comparison to planted forests of Euro-American poplar, where thinning has been performed regularly. Sites of natural stands included in the present study were located in a first-degree protection zone; thus the floristic diversity was found to be greater here than in the artificial forests. Fallows were inhabited with species that prefer higher levels of light, and somewhat drier conditions vs. the other two groups of natural poplar forests. Thus, fallow sites were associated with a greater number of invasive species in comparison to planted and natural stands.

Based on the present study, the floristic composition of the artificially planted Euro-American poplar (*Populus* x *euroamericana* /Dode/ Guinier) forests depended on whether they were planted at constantly or occasionaly flooded sites.

In constantly flooded habitats, forests contained species that are more tolerant to higher soil humidity and that prefer larger amounts of light. Poplar forests planted on occasionaly flooded sites were usually situated on high beams, flooded only during periods of high water level of the Danube. Thus, these habitats were somewhat warmer with different levels of soil humidity.

Certain similarities were noticed between natural stands of black and white poplar, but only for a small percentage**.** Ecological conditions at habitats of white and Euro-American poplar communities are more similar with respect to illumination and soil humidity, when compared to the habitats of black and Euro-American poplars.

The anthropogenic forests recorded the lowest diversity, as well as the smallest percentage of ground cover by invasive species. This is the result of regular thinning and implemented measures performed through human management of these stands.

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АНАЛИЗА ШУМСКЕ ВЕГЕТАЦИЈЕ КОВИЉСКОГ РИТА: ПОРЕЂЕЊЕ СТАНИШТА С РАЗЛИЧИТИМ АНТРОПОГЕНИМ УТИЦАЈИМА

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РЕЗИМЕ: Ковиљски рит налази се уз леву обалу Дунава, између села Ковиљ и Гардиновци. То је редак пример нетакнуте природе, где су очуване оригиналне карактеристике једног мочварног биотопа. Због својих изузетних природних вредности, Ковиљски рит, заједно са мочваром у Петроварадину, под заштитом је Републике Србије од 1998. године, када је проглашен Специјалним резерватом природе прве категорије. Природна вегетација у Ковиљском риту укључује шуме врбе и тополе. Већи део резервата састоји се од шумских засада (55,6%), међу којима су и станишта под утицајем људских активности. Кореспондентна анализа без тренда (DCA – Detrended correspondence analysis) шумске вегетације у Ковиљском риту открива да постоји висок степен сличности између природних популација беле врбе и засада антропогеног порекла. Међутим, насупрот томе, иста анализа указује на јасне разлике између природних и вештачких засада тополе. Даље, у зависности од тога да ли су засађене у привремено или стално плављеним стаништима, значајне разлике евидентне су у флористичким структурама шума евро-америчке тополе. Еколошки услови на стаништима беле и евро-америчке тополе (*Populus x euroamericana* /Dode/ Guinier) сличнији су када је у питању осветљење и влажност земљишта. Међутим, још важније, антропогене шуме најмање су разноврсне, што је резултат редовног прореда и мера које се спроводе кроз управљање шумама.

КЉУЧНЕ РЕЧИ: антропогени утицај, фактори средине, Ковиљски рит, шумска вегетација

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THE DIGITAL DATABASE OF AQUATIC AND SEMIAQUATIC VEGETATION IN SERBIA

ABSTRACT: Despite the absence of a centralized national phytocoenological database of Serbia, over 16,000 relevés of all vegetation types have been gathered and stored in the database "Phytocoenosis of Serbia" as a result of the Project "Habitats of Serbia". However, as the data on aquatic and semiaquatic vegetation comprise only 5% of the collected relevés, the aim of this paper is to expand on this, complete the database and present the data on these vegetation types in Serbia. The work included the expansion of the existing database with the addition of relevant sources, their digitalization, using Flora and Turboveg programs, and their subsequent georeferencing, using OziExplorer and DIVAGIS softwares. Consequently, the phytocoenological database on aquatic and semiaquatic vegetation in Serbia now stores 1,720 relevés from 243 phytocoenological tables, collected and published by 24 authors during the period of 70 years (1940–2010), with the majority of the relevés collected over the last decade (56.40%). *Phragmito-Magno-Caricetea* Klika in Klika et Novák 1941 (Syn: *Phragmitetea communis* R. Tx. et Prsg. 1942), *Potametea* Klika in Klika et Novák 1941 (Syn: *Potametea* R. Tx. et Preising 1942) and *Lemnetea* de Bolós et Masclans 1955 (Syn: *Lemnetea minoris* W. Koch et R. Tx. 1955) have proven to be the most represented classes, while *Scirpo-Phragmitetum* W. Koch 1926 (nomen ambiguum) and *Salvinio natantis-Spirodeletum polyrrhizae* Slavnić 1956 are associations with the highest number of relevés in the database. Accordingly, the most common species (>500 relevés) are *Ceratophyllum demersum* L. subsp. *demersum, Lemna minor* L. and *Spirodela polyrhiza* (L.) Schleiden.

KEYWORDS: aquatic and semiaquatic vegetation, phytocoenological database, Serbia

INTRODUCTION
PHYTOCOENOLOGICAL DATABASES

Vegetation databases have a very wide range of applications, from the classical study of vegetation classification to predictive mapping and tests of

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fundamental ecological hypotheses regarding functional traits, assembly rules and biodiversity patterns (Dengler *et al*., 2008), as well as patterns and processes of global change caused by anthropogenic climate warming, land-use changes and biotic invasions (Schaminée *et al*., 2009).

There are over 80 regional, national and local phytocoenological and other vegetation databases for the territory of Europe (Schaminée *et al*., 2009), with over 4,300,000 relevés, out of which 1,600,000 have already been digitized in the Turboveg format (Dengler *et al*., 2011). Turboveg represents a standard software for the storing of phytocoenological data and is compatible with most of the programs used for their subsequent analysis.

With the aim to standardize them and enable the global exchange of data, The Global Index of Vegetation-Plot Databases (GIVD) was established in 2008 as an internet-based platform (Dengler *et al*., 2011). In addition to the traditional phytocoenological relevés, collected following the Braun-Blanquet method, GIVD also encompasses other vegetation relevés, ranging in size from 1 to 1,000 m^2. By the end of March 2016, GIVD database has encompassed the data from 237 databases, with a total of 3,168,044 relevés. Although a centralized national phytocoenological database for Serbia does not exist (Schaminée *et al*., 2009), over 16,000 phytocoenological relevés, of all vegetation types, were gathered by 2005, as part of the Project „Habitats of Serbia" (Lakušić *et al*., 2005). The relevés were collected in the form of photocopied literature sources and stored in the database called „Phytocoenoses of Serbia", at the Institute for Botany of the Faculty of Biology, University of Belgrade. This database does not hold relevés in an electronic form. A framework for their digitalization, in the form of Excel files, was prepared as part of the project „Habitats of Serbia". The Excel files contain lists of species, with a degree of their frequency from their corresponding phytocoenological tables, information on the name of the phytocoenosis, sintaxonomical affiliation, locality and gathered sources. The aquatic and semiaquatic vegetation comprises only 5% of the relevés in the database, which is far less than their existing number. The database lacks primarily the references for the non-rooted floating vegetation.

Bearing in mind the abovementioned facts, the aim of this paper is to expand, finalize and present the data on the aquatic and semiaquatic vegetation in the database „Phytocoenosis of Serbia", as well as to digitalize these relevés and organize them in a separate database in the Turboveg format.

MATERIAL AND METHODS

DEVELOPMENT OF THE DATABASE

The database „Phytocoenoses of Serbia" (Lakušić *et al*., 2005), has been revised and expanded with the relevant references for the aquatic and semiaquatic vegetation, published until 2010. The phytocoenological data was digitalized first in the format of Excel files, subsequently organized in a separate database, first using the progam package Flora (Karadžić *et al*., 1998), following by the Turboveg software (Hennekens and Schaminée 2001).

THE MAPPING OF DATA

The data were georeferenced in the program OziExplorer (OziExplorer 2009). The distribution maps were produced using the DIVAGIS software (Hijmans *et al*., 2004) and given in UTM map projection. Only those relevés (1,374) from the literature which had a precisely defined locality were georeferenced.

RESULTS
DATABASE FOR AQUATIC AND SEMIAQUATIC VEGETATION

After the revision and data addition, the database of aquatic and semiaquatic vegetation now holds 1,720 phytocoenological relevés, distributed in 243 phytocoenological tables, with a total of 395 species, with an average of 7.63 species per relevé and 13,128 floristic records, while the average number of species per table is 19.68.

The database encompasses the relevés published in the period 1940–2010, in 38 different sources, by 24 authors (Slavnić 1940, 1956; Janković 1953; Babić 1955, 1971; Jovanović 1958, 1965; Danon and Blaženčić 1965; Babić and Parabućski 1971; Horvat *et al.,* 1974; Parabućski and Pekanović 1980; Rauš *et al.,* 1980; Knežević 1980, 1981; Kabić 1985; Vučković 1985; Butorac and Crnčević 1987; Knežević and Boža 1987, 1988; Ranđelović 1988, 2002; Gajić 1989; Stojanović *et al.,* 1990, 1994; Parabućski and Butorac 1994; Butorac *et al.,* 1994; Butorac 1995; Ranđelović and Blaženčić 1997; Radulović 2000, 2005, 2007; Šumatić *et al.,* 2001; Lazić 2003, 2006; Nikolić 2004; Panjković 2005; Polić 2006; Stanković-Kalezić 2006; Jenačković *et al.,* 2010; Ljevnaić-Mašić 2010). The database also holds 26 unpublished relevés (leg.: Vučković Mirjana, period 1994–2000).

The highest percent of relevés in the database originated from Ph.D. theses (57.85%), followed by scientific papers (20.29%) and M.Sc. theses (10.23%). Among the scientific papers, those published in the *Zbornik Matice srpske za prirodne nauke* (*Matica Srpska Journal for Natural Sciences*) are predominant (Slavnić 1956; Babić 1971; Babić and Parabućski 1971; Knežević 1980, 1981; Parabućski and Pekanović 1980; Rauš *et al*., 1980; Butorac and Crnčević 1987; Knežević and Boža 1987), while the Ph.D. theses, M.Sc. theses and final papers have mostly been done at the Department of Biology and Ecology of the Faculty of Sciences, University of Novi Sad (Kabić 1985; Ranđelović 1988; Radulović 2000, 2005; Nikolić 2004; Panjković 2005; Lazić 2006; Polić 2006; Ljevnaić-Mašić 2010).

With regards to the time frame of the data in the database, the highest percent of the relevés (56.40%) was collected after the year 2000 (Figure 1). In the case of data insufficiency, regarding the time of the data collection, the year of the publication was considered as relevant.

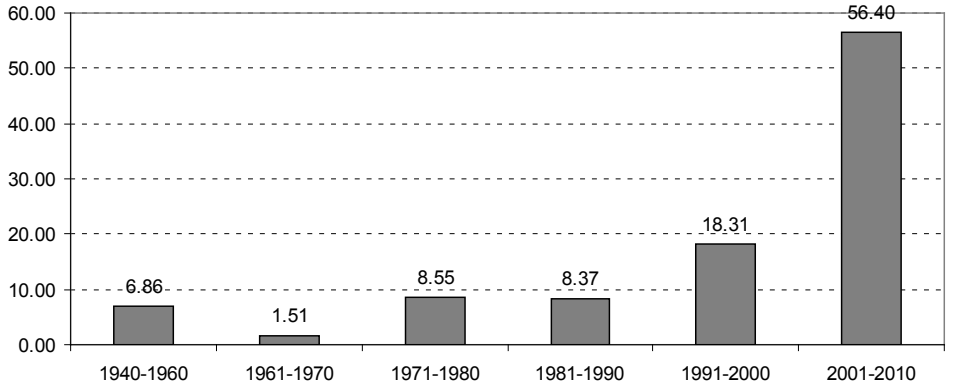

Figure 1. The percent of phytocoenological relevés in the database in decades (%)

Phragmito-Magno-Caricetea Klika in Klika et Novák 1941 (Syn: *Phragmitetea communis* R. Tx. et Prsg. 1942), *Potametea* Klika in Klika et Novák 1941 (Syn: *Potametea* R. Tx. et Preising 1942) and *Lemnetea* de Bolós et Masclans 1955 (Syn: *Lemnetea minoris* W. Koch et R. Tx. 1955) have proven to be the most represented classes, while *Scirpo-Phragmitetum* W. Koch 1926 (nomen ambiguum) and *Salvinio natantis-Spirodeletum polyrrhizae* Slavnić 1956 are associations with the highest number of relevés in the database. Accordingly, the most common species (>500 relevés) are *Ceratophyllum demersum* L. subsp. *demersum, Lemna minor* L. and *Spirodela polyrhiza* (L.) Schleiden.

As expected, the edificators of the typical communities for the area of research are among the most frequent species in the database (Radulović *et al*., 2011). Considering their conservation status (Table 1), out of the total number of 395 species recorded in the database, 45 are considered to be of national conservation concern – 25 as Strictly Protected (6.3%) and 20 species as Protected (5.1%). According to the IUCN assessments, 36.7% of the species are categorized as being of Least Concern by the Red List database, and two species fall into the categories of Near Threatened and Data Deficient (*Elatine alsinastrum* L. and *Alisma gramineum* Lej., respectively). Furthermore, a high frequency of aquatic invasive species in the database, such as *Vallisneria spiralis* and *Azolla filiculoides,* needs to be highlighted. Complying with the Preliminary list of invasive species in Serbia (Lazarević *et al*., 2012), a total of 13 species which are considered invasive have been recorded, the majority of which (8 species) fall under the category of highly invasive species (Table 2).

Table 1. Conservation status of the species in the database

	Number of species	Percent
Total number of species in database	395	
National Conservation Status (NCS)	45	12.7%
SP – Strictly Protected	25	6.3%
P – Protected	20	5.1%
IUCN	147	37.2%
LC – Least Concern	145	36.7%
NT – Near Threatened	1	0.3%
DD – Data Deficient	1	0.3%

Table 2. Overview of the invasiveness status of the species from the database

Invasive species (Lazarević *at al*., 2012)	Number of species	Percent
Total number of invasive species	13	3.29%
Highly invasive	8	2.03%
Sporadically invasive	3	0.76%
Potentially invasive	2	0.51%

The phytocoenological relevés are spread over 210 localities, at an irregular geographical distribution. As high as 87.84 % of the data is concentrated in the Pannonian part of Serbia (Figure 2). The regions which were studied the most are the area of Apatinsko-Monoštorski Rit, Koviljsko-Petrovaradinski Rit, Carska Bara and Stari Begej, and the Vlasina highlands.

Only 6.45% of the relevés contain the data for the soil type, dominated by different forms of fluvisol, humogley and solonetz. The date of the collection of the data is given in 13.31% of relevés, the size of the area in question in 76.86%, the exact locality in 93.66% of the relevés, while the information on the altitude was present in an inconsequential number of relevés. The total cover of the relevés was between 20% and 100%, and their area between 3 and 900 m^2.

DISCUSSION

A well designed and complete phytocoenological database enables scientists to perceive the faults and deficiencies in previous vegetation research and to direct the future studies accordingly (Bell *et al*., 2011; Uğurlu *et al*., 2012; Tozer *et al*., 2010, Radulović *et al*., 2011). The basic limitation of the aquatic and semiaquatic vegetation database formed as part of this study is the disproportionate geographical distribution of the phytocoenological relevés, which is not in proportion with the hydrography of Serbia. The vegetation of aquatic ecosystems south of the Danube has been isufficiently studied.

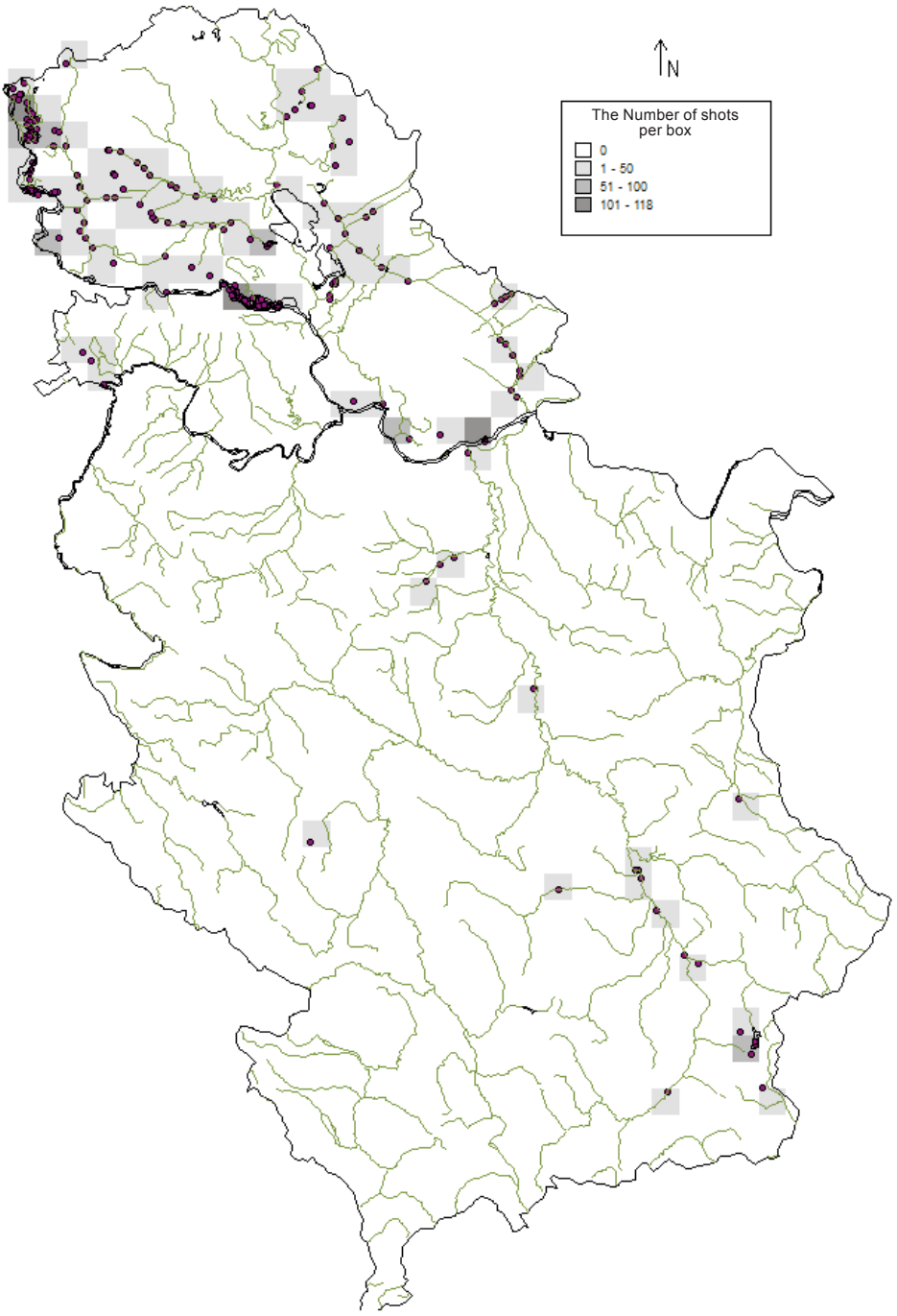

Figure 2. Analysis of the representation of phytocoenological relevés in the database per UTM square grid (10 x 10 km)

From the aspect of the relevance of the database data, all of the used literature sources can be characterized as the so-called „grey literature“, as they generally originate from the scientific papers published in national scientific journals, and Ph.D. and M.Sc. theses. Considering the significant time span (1940–2010) of the collected data, such a bibliography is excpected. A similar relation of sources also exists in the case of the phytocoenological database of the Czech Republic (Chytrý and Rafajová 2003), where, in addition to the „grey literature“, unpublished relevés comprise a significant portion of the database. Regardless of this, all of the data from this database were subjected to some kind of a review, and therefore there was no selection done based on their relevance.

Although a certain number of taxa was only listed to the genus level, the taxonomical resolution and data quality in the database are satisfactory, and due to the selective approach of the authors while collecting the phytocoenological relevés, the cryptogamic flora was mostly eliminated. The lack of general data necessary for phytocoenological relevés, e.g. sampling year, size of the relevés, altitude and soil type, limits the scope of analyses which can be conducted based on the given set of data (Chytrý and Rafajová 2003).

With regards to the time frame of the data in the database, the highest portion of data was collected during the last three decades, which coincides with the period of a rapid transition to the computerised numerical analysis in phytocoenology (Podani 1997). An identical trend can be observed in the example of the phytocoenological database of the Czech Republic (Chytrý and Rafajová 2003).

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ДИГИТАЛНА БАЗА ПОДАТАКА АКВАТИЧНЕ И СЕМИАКВАТИЧНЕ ВЕГЕТАЦИЈЕ СРБИЈЕ

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РЕЗИМЕ: Иако у Србији не постоји централизована национална фитоценолошка дигитална база, до 2005. године, као резултат пројекта „Станишта Србије“ прикупљено је и похрањено у базу података „Фитоценозе Србије“, преко 16.000 фитоценолошких снимака свих типова вегетације. Међутим, како су подаци о акватичној и семиакватичној вегетацији заступљени у бази са само 5% фитоценолошких снимака, циљ овог рада је допуна ових података, комплетирање базе и приказ података о овим типовима вегетације у Србији. Рад је укључио проширење постојеће базе података додавањем одговарајућих референци, уз њихову дигитализацију, применом Flora и Turboveg програма, и након тога њихово георефернцирање, уз примену софтвера OziExplorer и DIVAGIS. Након ревизије и допуне,

фитоценолошка база података о акватичној и семиакватичној вегетацији у Србији сада броји 1.720 фитоценолошких снимака, из 243 фитоценолошке табеле, прикупљене и публиковане од стране 24 аутора, током периода од 70 година (1940–2010), при чему је већина фитоценолошких снимака сакупљена током последње деценије (56,40%). Најзаступљеније класе у бази биле су *Phragmito-Magno-Caricetea* Klika in Klika et Novák 1941 (Syn: *Phragmitetea communis* R. Tx. et Prsg. 1942), *Potametea* Klika in Klika et Novák 1941 (Syn: *Potametea* R. Tx. et Preising 1942) и *Lemnetea* de Bolós et Masclans 1955 (Syn: *Lemnetea minoris* W. Koch et R. Tx. 1955), док су асоцијације са највећим бројем фитоценолошких снимака у бази *Scirpo-Phragmitetum* W. Koch 1926 (nomen ambiguum) и *Salvinio natantis-Spirodeletum polyrrhizae* Slavnić 1956. У складу с тим, најфреквентније врсте у бази (>500 фитоценолошких снимака) биле су *Ceratophyllum demersum* L. subsp. *demersum, Lemna minor* L. и *Spirodela polyrhiza* (L.) Schleiden.

КЉУЧНЕ РЕЧИ: акватична и семиакватична вегетација, фитоценолошка база података, Србија

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ECOLOGICAL ANALYSIS OF STANDS OF ASS. *Asclepiadetum syriacae* LÁNIKOVÁ IN CHYTRÝ 2009 IN BAČKA REGION

ABSTRACT: Perennial adventitious species *Asclepias syriaca* L. which originates from Northern America is spread across Serbia, and especially Vojvodina, along the banks of waterways and borders of floodplain forests. Based on floristic and phytocenological studies in Bačka, the presence of ass. *Asclepiadetum syriacae* Lániková in Chytrý 2009 stands, in which *A. syriaca* is present with the highest degree of presence (V) and with substantial covering value (2850), can be confirmed. The study shows ecological analysis of stands of mentioned association for basic environmental factors (moisture – F, reaction – R, nutrients – N, humus – H, aeration – D, light – L, temperature – T, and continentality – K) based on floristic composition of stands and their ecological indices, considering population and every species coverage in a stand. Given that a combined scale for population and coverage of species in stands, along numerical, has descriptive marks, modification of evaluated values according to Westhoff & van der Maarel scale which is completely numerical was conducted, which enabled data processing. The obtained results of the ecological analysis point to favorable ecological conditions for development of stands of this association, which can help predict the possible directions of spread of this invasive species.

KEYWORDS: *Asclepias syriaca,* ass. *Asclepiadetum syriacae,* ecological analysis.

INTRODUCTION

Flora diversity of an area consists of native elements that are typical for it, but also of a large number of adventive species that have been either deliberately or accidentally introduced by human activities. According to Trinajstić (1977), species introduced by human activities have been marked as anthropomorphic, categorized depending on the period of their introduction. Within that classification, a special attention of botanists is attracted by the species from the category of neophytes that have been introduced since the Second World War

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until today. Species that belong to the last category, i.e. neophytes, are the most aggressive of the introduced species (Vrbničanin *et al*., 2004). The common name for this group of plants is "invasive" (Richardson *et al*., 2000; Pyšek *et al*., 2004). Invasive plants are naturalized, introduced plant taxa that develop in great number in localities that are significantly distant from the parent plant. They have outstanding reproductive power and ability to invade large areas and with a substantial pressure to native flora (Galatowitsch *et al*., 1999; Vrbničanin *et al*., 2004). This plant category includes also *Asclepias syriaca* L. (Lazarević *et al*., 2012).

A. syriaca originates from North America. It is perennial species with adventive buds on the root that serve for over-wintering (Ujvárosi 1973). It is cultivated as honey and caoutchouc (rubber) plant, whose seed is used also in the fiber processing industry (Obradović, 1976). According to Obradović (1976), its presence was for the first time recorded by Kovács (1929) in vegetable crops of Novi Bečej. This invasive species is distributed in Serbia, and especially in Vojvodina, along the banks of waterways and on the edges of floodplain forests (Janković 1973) and other habitats, especially with regard to the wood and sandstone habitats (Bagi, 2008; Anačkov *et al*., 2011) in which it has fully adapted (Igić *et al*., 2003). It is well adapted to ecological conditions as dominant and edificatory species that for the time being forms fragmentary developed communities of ass. *Asclepiadetum syriacae* Lániková in Chytrý 2009 in Bačka.

In this study, the ecological analysis of the mentioned community stands has been presented in order to predict possible directions of spread of this invasive species and its stands. It is based on the demands of the basic environmental factors and stands that they invade, and whose expansion could have extremely negative ecological and economic effect on homogenization of floristic and vegetation composition and preservation of indigenous biodiversity.

MATERIAL AND METHODS

During vegetation period 2013 and 2014, at twelve localities in Bačka (Kać, Novi Sad, Futog, Kovilj, Ratkovo, Silbaš, Bač, Karađorđevo, Bačka Palanka, Molovin and Tavankut), floristic and phytocenological studies of ruderal and weedy vegetation were performed according to the method of Braun-Blanquet (1964). Phytocenological surveys were performed at given localities with detected greater populations of *A. syriaca* on different types of habitats by combined scale for evaluation of weed species number and coverage, according to the given method. Determination of plant species was accomplished according to Josifović (ed.) (1970–1980), floral elements according to Gajić (1980) and plant life forms were determined according to Soó (1980). The ecological analysis of stands of the identified community was performed on the basis of bioindicator values in accordance with Landolt (1977), after transformation of bioindicator values according to Weshoff-van der Maarel scale, which is entirely numerical, unlike the combined descriptive numerical scale (number and coverage) of Braun-Blanquet.

RESULTS AND DISCUSION

In this study, the presence of ass. *Asclepiadetum syriacae* stands, Lániková in Chytrý (2009) was determined in dry-out ruderal sites, with dominating edificator of *A. syriaca* community, an invasive species widespread in the whole of Vojvodina (Igić *et al*., 2003). The stands of this community can be often found near channel banks, on the edges of forests, along roads, field borders, etc.

According to Chytrý (2009), the community *Asclepiadetum syriacae* Lániková in Chytrý (2009) belongs to the association alliance *Dauco carotae – Melilotion* Görs ex Rostański et Gutte (1971), class *Artemisietea vulgaris* Lohmeyer et al. ex von Rochow (1951), i.e. ruderal and weedy vegetation.

The floristic structure of ass. *Asclepiadetum syriacae* comprised 79 plant species. The characteristic type of *Asclepias syriaca* association had the highest level of presence (V) and significant value of coverage (2,850) which made even 37.24% of participation in the whole coverage value of this community (7,652). Of the characteristic types of associations stated by Chytrý (2009), the following were present: *Rubus caesius, Erigeron annuus, Equisetum ramosissimum,* and *Falcaria vulgaris.* Of the species that the same author referred to as a constant in this association stands, the following were found: *Asclepias syriaca, Achillea millefolium, Artemisia vulgaris, Agropyron repens, Falcaria vulgaris, Galium album, G. mollugo, Rubus caesius,* and *Urtica dioica.* When compared to the stands of this association in the Czech Republic (Chytrý 2009), in which dominate species *Artemisia vulgaris, Asclepias syriaca, Agropyron repens, Equisetum ramosissimum,* and *Rubus caesius,* in the studied area, where for the time being fragmentary developed stands of this community, dominate *Asclepias syriaca, Rubus caesius, Galium album*, and *Achillea millefolium*.

ECOLOGICAL ANALYSIS OF ASS. *Asclepiadetum syriacae* LÁNIKOVÁ IN CHYTRÝ 2009

The spectrum of areal types of ass. *Asclepiadetum syriacae* is presented in Figure 1. It is specific for weedy-ruderal communities (Kojić *et. Al*., 1998), and in this community the most common were floral elements of a wide range of distribution, represented with 72% (56 species), among which there were also four diagnostic/characteristic species of this community such as *Asclepias syriaca, Rubus caesius, Erigeron annuus,* and *Equisetum ramosissimum*. Thus, in the stands of the described association, the most dominant were Eurasian species with 27% (21 species), sub-Eurasian with 19% (15 species), and adventive with 11% (9 species). Floral elements of a wide range of distribution were represented in smaller percentages by cosmopolitan (8%, 6 species), circumpolar and sub-south Siberian (with per 3%, 2 species), and one sub-circumpolar (1%).

Table 1. Ass. ***Asclepiadetum syriacae*** Láníková in Chytrý 2009

Stand number			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18		
Surface (m^2)			25	50	50	25	25	50	50	25	25	25	25	25	25	25	50	25	25	50		
Total covering (%)			90	90	100	95	95	90	100	95	70	85	80	80	80	80	70	80	90	95		
Number of plant species			18	18	12	25	16	14	22	12	4	7	7	10	9	6	13	6	12	4		
Fl. El.	LF	Plant species																			SP	PV
Diagnostic/characteristic species of the association																						
Adv.	H	***Asclepias syriaca* L.**	2.1	3.3	5.5	4.4	3.3	2.2	5.5	2.1	1.1	2.1	1.1	1.1	2.1	2.1	+.1	1.1	3.3	3.1	V	2850
Subj.sib.	H	***Rubus caesius* L.**	1.1	+.1		+.1		+.1	3.3	1.1			1.1	1.1			+.1	3.2	1.1		IV	567
Adv.	Th	***Erigeron annuus* (L.) Pers.**		1.1	+.1	1.1	+.1	1.1													II	89
Kosm.	G	***Equisetum ramosissimum* Desf.**							2.2						2.1	1.1					I	222
Pont.ca	Th-TH	***Falcaria vulgaris* Bernh.**							+.1												I	3
Subse.	H	***Galium album* Mill.**	1.1				3.2		1.1	1.1		1.1	1.1				1.1	+.1	+.1		III	381
Adv.	H	*Ambrosia artemisiifolia* L.	2.1	+.1	+.1	1.1	2.1	1.1							+.1	1.1					III	286
Evr.	H	***Achilea millefolium* L.**	1.1	1.1	+.1		+.1		2.2	1.1			1.1	1.1			1.1		+.1		III	272
Evr.	H	***Urtica dioica* L.**								2.1	2.1	1.1	1.1								II	250
Evr.	H	*Carex pilosa* Scop.		1.2	2.2		1.2		+.2												II	156
Evr.	G	***Agropyron repens* L.**		+.1		+.1	1.2	2.2	+.2												II	133
Adv.	M	*Amorpha fruticosa* L.		+.1	+.1	+.1											1.1		2.2		II	133
Evr.	H	*Rumex crispus* L.					+.1		+.1	2.2			1.1								II	131
Cirk.	H (Ch)	***Artemisia vulgaris* L.**	1.1	+.1						1.1			1.1	1.1	+.1	+.1					II	119
Subevr.	H	*Poa trivialis* L.	1.1					+.1		1.1	1.1	1.1			+.1						II	117
Subevr.	Th-TH-H	*Daucus carota* L.	1.1	+.1	+.1	+.1							1.1	1.1							II	92
Subevr.	G	*Cirsium arvense* (L.) Scop.								1.1		1.1	1.1					+.1			II	86
Kosm.	G(H)	*Sorghum halepense* (L.) Pers.	1.1				+.1				1.1		1.1								II	86
Subevr.	Th-TH	*Silene alba* (Mill.) Garc.	1.1	+.1		+.1	+.1	+.1	+.1				1.1								II	69
Kosm.	H	*Polygonum aviculare* L.	1.1			+.1									1.1	+.1					II	61
Kosm.	H-G	*Convolvulus arvensis* L.	1.1				+.1		+.1										1.1		II	61
Subevr.	H	*Dactylis glomerata* L.			+.1		+.2	+.2											1.1		II	36
Subse.	TH-H	*Anchusa officinalis* L.				+.1	+.1	+.1	+.1												II	11
Subm.	Th	*Bromus mollis* L.				2.2	+.1														I	100
Subpont.	Th	*Caucalis platycarpos* L.																		2.2	I	97
Subm.	H(G)	*Aristolochia clematitis* L.		2.1																	I	97

Evr.	H	*Festuca pratensis* L.																2.1					I	97
Kosm.	G(H)	*Cynodon dactylon* (L.) Pers																				2.1	I	97
Subm.	Th	*Bifora radians* L.	1.1								1.1		1.1										I	83
Adv.	Th-TH	*Erigeron canadensis* L.			1.1			+.1														1.1	I	58
Cirk.	H(G)	*Mentha arvensis* L.	1.1						1.1												+.1		I	58
Subse.	Th(H)	*Lamium purpureum* L.							1.1		1.1										+.1		I	58
Evr.	H	*Euphorbia cyparissias* L.		1.1														1.1					I	56
Evr.	H	*Euphorbia esula* L.			1.1			1.1															I	56
Subevr.	H	*Trifolium repens* L.	1.1										1.1										I	56
Evr.	H	*Plantago lanceolata* L.			+.1			+.1										1.1					I	33
Evr.	TH	*Arctium lappa* L.											1.+	+.1							+.1		I	33
Subse.	Th	*Anthemis arvensis* L.											1.+		+.1								I	31
Subevr.	H(TH)	*Cichorium inthybus* L.	1.1															+.1					I	31
Evr.	Th	*Geranium dissectum* L.			+.1													1.1					I	31
Subj.sib.	H-G	*Lathyrus tuberosus* L.										1.1							+.1				I	31
Subevr.	Th	*Sonchus oleraceus* (L.) Gou.	1.1																		+.1		I	31
Subse.	TH	*Carduus acanthoides* L.							1.1														I	28
Subm.	Th	*Centaurea cyanus* L.				1.1																	I	28
Subm.	Th	*Hordeum murinum*L.												1.1									I	28
Evr.	Th-TH-H(Ch)	*Matricaria inodora* L.																				1.1	I	28
Subcirk.	H	*Polygonum lapathifolium* L.	1.1																				I	28
Subse.	M	*Rosa canina* L.											1.+										I	28
Subse.	Th-TH	*Trifolium campestre* Schreber																1.1					I	28

Species with SP – I and small PV (6-3): *Linaria vulgaris* Mill., *Oenothera biennis* L., *Pastinaca sativa* L., *Petrorhagia prolifera* (L.) P. W. Ball & Heyw. *Taraxacum officinale* L. *Trifolium pratense* L., *Verbascum nigrum* L., *Xantium italicum* Mor.**, *Galium mollugo* L.,** *Ailanthus altissima* (Mill.) Swingle, *Avena fatua* L., *Bromus sterilis* L., *Chenopodium album* L., *Coronilla varia* L., *Delphinium consolida* L., *Echinocystis lobata* (Michx.) Torr.& Gray., *Erodium cicutarium* (L.) L'Hér.ex Ait., *Hypericum perforatum* L., *Papaver rhoeas* L., *Plantago maior* L, *Polygonum convolvulus* L. , *Potentilla argentea* L., *Potentilla reptans* L., *Ranunculus repens* L., *Scabiosa ochroleuca* L., *Silene vulgaris* (Moen.) Garcke, *Sonchus arvensis* L. , *Veronica hederifolia* L., *Vicia grandiflora* Scop.

LEGEND: Number of stands: **1,2,3** – Kać, **4,5,6,7** – Novi Sad, **8** – Futog, **9** – Kovilj, **10** – Ratkovo, **11** – Silbaš, **12** – Bač, **13,14** – Mladenovo, **15** – Karađorđevo, **16** – Bačka Palanka, **17** – Molovin, **18** – Tavankut, **SP** – degree of presence, **PV** – covering values. **Fl. El.** – floral element: **Adv.-** Adventive, **Cirk.** – Cirkumpolare, **Evr.** – Eurasian, **Kosm.** – Cosmopolitan, **Pont.-ca**. – Pontic-Central-Asian, **Pont.- is.subm**. – Pontic-East-Submediterranean, **Subcirk**. – Subcirkumpolar, **Subevr. –** Subeurasian**, Subm.** – Submediterranean, **Subj.sib**. – Subsouthsiberian, **Subpont.** – Subpontic, **Subpont.-ca.** – Subpontic-Centralasian, **Subpont.-subm.** – Subpontic-Submediterranean, **Subse.-** Submiddle European. **LF** – life form : **MM** – megafanerofita, **M** – mezofanerofita, **Ch** – Chamaephytes, **H** – hemicryptophytes, **G** – geophytes, **TH** – hemitherophytes, **Th** – therophytes.

The floristic elements of narrow distribution comprised 28% (23 species), among which dominated sub-central European with 14% (11 species), followed by sub-Mediterranean with 6% (5 species), sub-Pontic and Pontic-Central Asian (with per 2 species), while only one species represented Pontic-east-sub-Mediterranean, sub-Pontic-sub-Mediterranean and sub-Pontic-Central Asian floral elements.

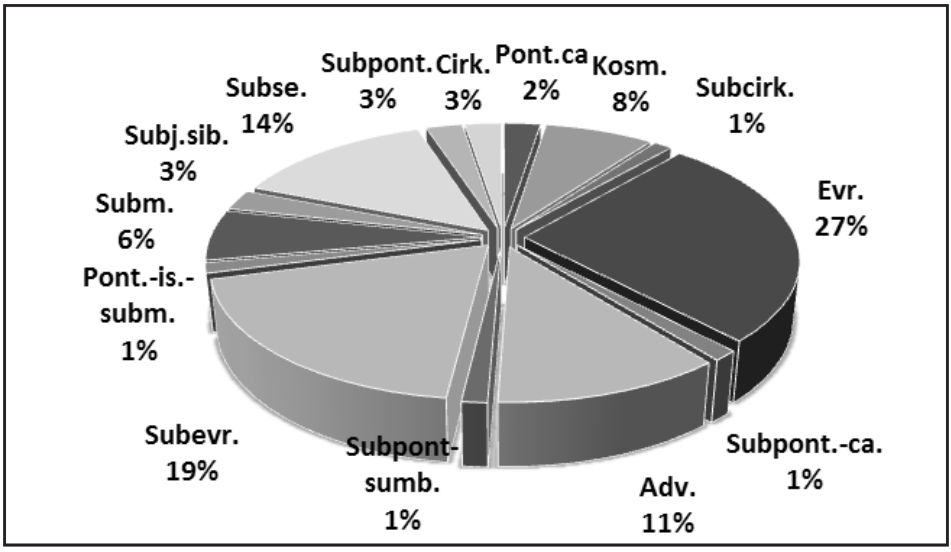

Figure 1. The spectrum of areal types of ass. ***Asclepiadetum syriacae*** Lániková in Chytrý (2009)

The biological spectrum of the association of the established plant community was distinguished by bipolar, hemicryptophyte-therophyte character conditioned by ecological conditions on the studied ruderal habitats. This coincided with the biological spectrum florae of Serbia (Diklić, 1984), and also showed good adaptability of this species to phytosociological model of vegetation in arid and semi-arid areas of Serbia. Thus, in the spectrum of living forms dominated hemicryptophyte with 48% (38 species), together with the edificator species *A. syriaca* and therophyte with the participation of 36% (28 species). Hemitherophyte and geophyte had a significantly lower percentage of participation of 6% (5 species), while phanerophyte were represented by 4% (3 species), of which 2 were meso-phanerophyte (*Amorpha fruticosa* and *Rosa canina)* and only one megaphanerophyte (*Ailanthus altissima*) – Figure 2.

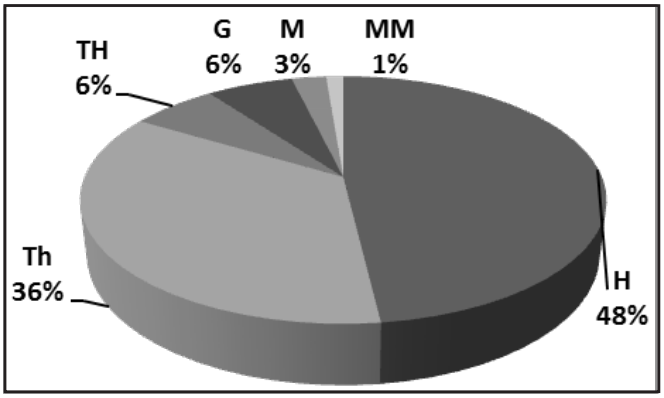

Figure 2. The biological spectrum of ass. *Asclepiadetum syriacae* Lániková in Chytrý (2009)

Results of the ecological analysis of stands of ass. *Asclepiadetum syriacae* are presented in Table 2. The analysis of the ecological moisture index (F) shows that stands of the analyzed community develop in habitats that are characterized by slightly more dry conditions, with the average value of this index of 2.7. The average values of index for the chemical reaction of the environment (R) are quite similar ranging between 2.8 to 3.3, with average of 3.1, which also shows domination of neutrophilic plants in stands of the analyzed community. The analysis of the ecological index for nitrogen and nitrogen matters (N) suggests that stands of ass. *Asclepiadetum syriacae* develop on a medium with moderate content of these matters, which is confirmed also by the middle value of this ecological index (3.5). Also, with regard to humus content (H), these stands have modest demands, as the average values of this index are from 2.6 to 3.4, which indicates lower or medium content of organo-mineral complexes in soil. Stands of the established community prefer well-aerated soils with the middle value of this index (D) of 3.8. Regarding the request for the light (L), plants in the analyzed stands tolerate some shading, although often in well-lighted habitats, because the values of this index are 3 to 4, with average of 3.6. Stands of ass. *Asclepiadetum syriacae* are indicators of warm habitats which is also confirmed by the middle value of this index (T – 4). Based on indices for light and temperature, as well as on the average values of index for continentality (K) of 2.9, it can be concluded that this community is adapted to the conditions of temperate continental climate, which is also characteristic of the studied area.

Table 2. Average values of ecological indices of stands ass. *Asclepiadetum syriacae* Láníková in Chytrý 2009

Ecological indices	Number of stands																		Average
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
F	2.5	2.7	2.8	2.5	2.5	2.4	2.5	2.9	2.6	2.7	2.6	2.5	2.6	2.5	2.8	3.2	3.1	2.4	2.7
R	3.2	3.1	2.9	3.1	2.9	2.9	3.2	3.1	2.8	3.3	3.0	3.0	3.2	3.1	3.1	3.2	3.2	3.2	3.1
N	3.0	3.3	3.3	3.5	3.6	3.5	3.2	3.9	3.9	3.9	3.6	3.5	3.5	3.4	3.2	3.6	3.6	2.9	3.5
H	2.9	2.9	2.9	2.8	2.8	2.7	2.9	3.1	3.4	3.0	3.0	2.9	2.6	2.6	3.1	3.0	3.1	3.0	2.9
D	3.8	3.8	3.6	3.7	3.7	3.5	3.8	4.0	3.8	4.0	3.8	3.9	3.7	3.6	4.0	4.0	4.0	3.6	3.8
L	3.9	3.5	3.5	3.7	3.7	3.7	3.5	3.5	3.4	3.5	3.6	3.6	3.8	4.0	3.7	3.0	3.5	3.8	3.6
T	4.0	4.0	4.4	4.2	4.0	4.0	4.0	3.7	3.9	3.8	3.8	3.9	4.0	4.4	3.6	4.1	4.1	4.7	4.0
K	3.0	2.9	2.6	3.0	3.0	3.0	3.0	2.8	2.8	2.8	3.1	3.0	3.0	2.8	2.9	3.1	2.7	2.7	2.9

In Serbia, in eastern Srem, Kojić *et al.* (2004), and in southern Srem, Jarić *et al.* (2011) established presence of stands of *Asclepietum syriacae* ruderal community, Kojić 2004 from alliance *Arction lappae* Tx. (1937) 1942 em.Gütte 1972, while in Pančevački rit, Stanković-Kalezić *et al.* (2008) within ass. *Convolvulo-Agropyretum repentis* Felf. 1943, on a smaller number of surveys established the presence of stands of the sub-association *Asclepietosum syriaci* in which *Asclepias syriaca* had high values of coverage. However, based on results of these studies, which means on floristic composition and other phytocenological indicators, the ecological analysis of the community, as well as bio-indicator values of dominant, i.e. edificator invasive species *A. syriaca* ($F_3R_3N_3H_3D_4L_4T_5K_2$), that are in accordance with the ecological properties of the community, it can be concluded that in Bačka, for the time being, there are fragmentary developed stands of weedy-ruderal community of *Asclepiadetum syriacae* Láníková in Chytrý (2009).

CONCLUSIONS

In Bačka, invasive species *Asclepias syriaca* L. in dry ruderal habitats finds convenient conditions and fragmentary forms developed stands of ass. *Asclepiadetum syriacae.* Stands of this community in Bačka develop in habitats characterized by slightly dry conditions ($F_{\bar{x}}$ – 2.7), neutral to weak alkaline chemical reaction ($R_{\bar{x}}$ – 3.1), with moderate nitrogen and nitrogen matters content ($N_{\bar{x}}$ – 3.5), medium rich in humus ($H_{\bar{x}}$ – 2.9), well aerated ($D_{\bar{x}}$ – 3.8), with convenient light ($L_{\bar{x}}$ – 3.6) and thermal regime ($T_{\bar{x}}$ – 4.0). The monitoring of phytocenological indicators in characteristic habitats of stands of this community provides possibility to predict the dynamics of common milkweed stands development and take the necessary measures in case of heavier infestation.

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ЕКОЛОШКА АНАЛИЗА САСТОЈИНА ASS. *Asclepiadetum syriacae* LÁNIKOVÁ IN CHYTRÝ 2009 У БАЧКОЈ

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РЕЗИМЕ: Вишегодишња адвентивна врста *Asclepias syriaca* L. пореклом из Северне Америке, распрострањена је у Србији, нарочито у Војводини, дуж насипа, и на рубовима плавних шума. На основу флористичко-фитоценолошких истраживања у Бачкој, констатујемо присуство састојина ass. *Asclepiadetum syriacae* Láníková in Chytrý 2009 у којима се *A. syriaca* јавља с највећим степеном присутности (V) и знатном покровном вредношћу (2850). У раду је приказана еколошка анализа састојина наведене заједнице, за основне факторе животне средине. Састојине ове заједнице у Бачкој развијају се на стаништима која се одликују нешто сушнијим условима ($F_{\bar{x}}$ – 2,7), неутралне до слабо алкалне хемијске реакције ($R_{\bar{x}}$ – 3,1), са умереним садржајем азота и азотних материја ($N_{\bar{x}}$ – 3,5), средњебогатим у садржају хумуса ($H_{\bar{x}}$ – 2,9) и добро аерисаним ($D_{\bar{x}}$ _ 3,8). И климатски показатељи указују на станишта повољног светлосног ($L_{\bar{x}}$ – 3,6) и термичког режима ($T_{\bar{x}}$ – 4,0) што је у складу са умереноконтиненталним условима ($K_{\bar{x}}$ – 2,9) који и карактеришу истраживано подручје. И биоиндикаторске вредности ($F_3R_3N_3H_3D_4L_4T_5K_2$) доминантне и едификаторске инвазивне врсте *A. syriaca*, указују на његову одличну прилагођеност наведеним условима станишта, где се најмасовније и развија. Добијени резултати указују на еколошке услове погодне за развој састојина ове заједнице што ће омогућити предвиђање могућег правца ширења ове инвазивне врсте.

КЉУЧНЕ РЕЧИ: *Asclepias syriaca* L., ass. *Asclepiadetum syriacae,* еколошка анализа

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CHEMICAL CHARACTERIZATION AND CHEMOTAXONOMY OF *Hypericum hirsutum* L. 1753 FROM VOJVODINA (SERBIA)

ABSTRACT: The genus *Hypericum* includes over 500 widely distributed species. The main representative is St. John's wort (*Hypericum perforatum* L. (1753), Hypericaceae), the only approved biological source of *Hyperici herba* by WHO and EMEA monographs. It is frequently used in the form of oil macerate for treatment of burns, scars, eczema and gastrointestinal disorders, as well as in the form of water and alcoholic extracts as clinically proved antidepressant. Available data suggest that the amounts of secondary metabolites in the plant vary depending on ecological factors of the habitat, and consequently affect the quality of herbal drug. The reports show that other species of the genus have similar chemical profile as *H. perforatum*. But, there are also *Hypericum* species in which some of the secondary metabolites of interest occur in higher quantities than in *H. perforatum*. As previous data suggest, *Hypericum hirsutum* L. 1753, could be such example. Therefore, the aim of this study was to chemically characterize water-alcoholic extracts of *H. hirsutum* samples, collected at four localities in Vojvodina (Republic of Serbia) by liquid chromatography (HPLC-DAD). The obtained results suggest a good match (in a term of a presence of investigated compounds) of previously published results describing chemical profile of *H. perforatum* water-alcoholic extracts with examined *H. hirsutum* extracts. Also, chemotaxonomic analysis showed variations in quantity of secondary metabolites in the examined extracts. This opens the door to further investigation of *H. hirsutum* as a new source of bioactive secondary metabolites and additional markers in *Hypericum* chemotaxonomy.

KEYWORDS: chemical characterization, chemotaxonomy, HPLC – DAD, *Hypericum hirsutum*, PCA, secondary metabolites

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INTRODUCTION

St. John's wort (*Hypericum perforatum* L. /1753/, Hypericaceae) is the main representative of the genus *Hypericum* with long history of use in traditional medicine. Usually, it is administered in the form of oil macerate externally for treatments of burns, bruises, eczema, and internally for treatment of gastrointestinal and gallbladder disorders, inflammation of respiratory and urogenital tract (Bombardelli and Morazzoni 1995; Božin *et al.*, 2013). Different water and water-alcoholic extracts exhibit clinically proved antidepressant activity, especially significant in patients suffering from mild to moderate form of depression, comparable to synthetic antidepressants (Brattström 2009). The main active principles of the herbal drug (*Hyperici herba*) are naphtodiantrones (hypericin, pseudohypericin), phloroglucinols (hyperforin, adhyperforin), flavonoids (rutin, quercetin, quercitrin, hyperoside, amentoflavone), phenolic acids and a small amount of essential oil (Kladar *et al.*, 2015b). For registration of phytopreparations 6% of phloroglucinols (hyperforin), 0.1–0.3% of naphtodiantrones (hypericin) and 2–4% of flavonoids (hyperoside) are required (Blumenthal *et al.*, 1998). However, the content of secondary metabolites present in final preparations is directly related to the quality of herbal drug. It is known that the amounts of active principles in plants vary depending of abiotic factors specific for plant habitat (Kladar *et al.*, 2015a). The genus *Hypericum* includes over 500 widely distributed species (Crockett and Robson 2011; Kladar *et al.*, 2015b; Robson 1981). Following the recommendations of WHO and EMEA monographs only *H. perforatum* is marked as the biological source of *Hyperici herba* (European Medicines Agency 2009; World Health Organization 2002). Available studies to date suggest that other representatives of the genus also possess similar profile of chemical constituents as *H. perforatum*. Therefore, a question is whether these species could represent a substitute for the specifically defined biological source of *Hyperici herba*. Some of these representatives contain higher levels of metabolites of significance than *H. perforatum*, which opens a potential door to new biological and pharmacological applications (Kladar *et al.*, 2015a). One of these species could be *Hypericum hirustum* (Hypericaceae) – hairy St. John's wort. The aim of this study was to chemically characterize water alcoholic extracts of *H. hirsutum* collected at four locations with specific sets of ecological factors in Vojvodina, Republic of Serbia. Chemotaxonomic evaluation of the examined samples was applied, to inspect the possible variations in the investigated species, which could reflect healing properties.

MATERIAL AND METHODS

The samples of *Hypericum hirsutum* were collected at four locations in Vojvodina, Republic of Serbia (Table 1). Vouchers are identified and deposited in Herbarium BUNS at the Department of Biology and Ecology, Faculty of

Sciences, University of Novi Sad (Greuter *et al.*, 1986; Holmgren and Holmgren 2003; Robson 1981). Extraction with ethanol (70%, w/w) for 72h was used for obtaining the samples intended for further chemical characterization (European Directorate for the Quality of Medicines & Health Care 2007). After the evaporation of the solvent, the amount of dry extract (d. e.) was quantified and extracts were dissolved in methanol prior to chromatographic analysis.

Table 1. Plant sample information

Sample	Location	GPS coordinates
1	Veternik, Novi Sad	45°13'55.4"N, 19°45'27.9"E
2	Fruška Gora Mt.	45°09'07.2"N, 19°42'54.1"E
3	Zasavica, Ravnje – Banovo Polje road	44°55'48.9"N, 19°26'20.4"E
4	Hrtkovci	44°53'39.6"N, 19°46'01.2"E

Two methods of liquid chromatography (HPLC-DAD) were used for quantification of selected compounds in the examined extracts (Picture 1). The separation was performed on Zorbax CB-C18 column (4.6×150 mm, i.e., 5 μm particle size) held at 25 °C. Method I was used for determination of hypericin (Hpc), hyperforin (Hpf), apigenin (Ap), naringenin (NA), and amentoflavone (Am) (Božin *et al.*, 2013). A modified method by Ziaková and Brandšteterová (2003) was used as Method II for determination of quercetin (Qe), rutin (R), epicatechin (Ec), caffeic (CA), chlorogenic (CHA), ferulic (FA), gallic (GA), and *p*-hydroxybenzoic acid (PHB). Solvent A was 0.1% solution of acetic acid in water and solvent B was 0.1% solution of acetic acid in acetonitrile. The mobile phase was delivered in gradient mode (3.25 min. – 0% B; 8 min. – 12% B, 15 min. – 25% B, 15.8 min. – 30% B, 25 min. – 90% B, 25.4 min. – 100% B) with flow rate of 1 mL/min and detection at wavelength of 280 nm. Before the injection of extracts, calibration curves of chemical standards of quantified compounds were obtained.

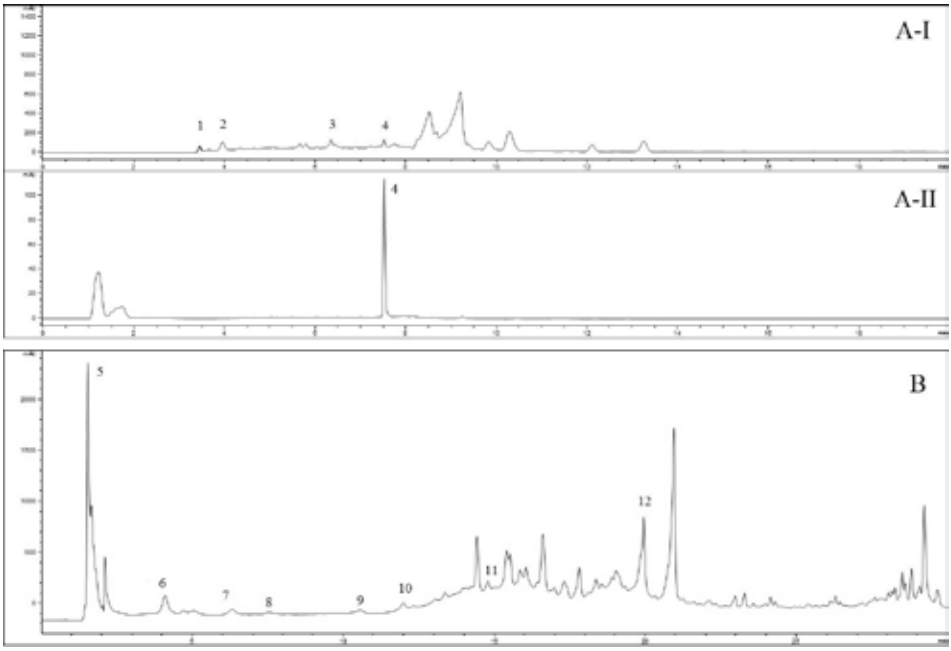

Figure 1. Chromatograms of sample 3 obtained by method I (A-I – detection at 270 nm, A-II – detection at 590 nm) and method II (B – detection at 280 nm) with identified compounds 1 – Ap, 2 – Am, 3 – Hpf, 4 – Hpc, 5 – R, 6 – GA, 7 – CHA, 8 – PHB, 9 – CA, 10 – Ec, 11 – FA, 12 – Qe.

Principal component analysis (PCA) performed by PAST software package was applied for chemotaxonomic analysis and evaluation of differences in the chemical composition of the examined samples (Hammer *et al.*, 2001).

RESULTS AND DISCUSSION

Naphtodiantrones, phloroglucinols, phenolic acids and flavonoids are classes of biologically active compounds present in *Hypericum* species (Brolis *et al.*, 1998). The results of quantification of selected active principles in the extracts of *H. hirsutum*, as well as the amounts of dry extract are given in Table 2. It can be noticed that the amounts of extractable compounds vary between samples (13.73–19.06 %) which could be directly related to the insolation of plant habitat since sample 1 was collected from the habitat with the most sunlight exposure, and sample 2 from the shadowy habitat. Furthermore, differences between the samples in the content of the all compounds except amentoflavone, caffeic, ferulic and para-hydroxybenzoic acid were noticed. The detected levels of hypericin were significantly higher than in the study conducted by Maggi *et al.* (2004) and Smelcerovic *et al.* (2006) which is related to the usage of different extraction procedures and solvents. However,

similarities were noticed with the results in studies by Kitanov (2001) and Smelcerovic *et al.* (2008) in which the plant material was extracted with ethanol, as in the current study. Hypericin was proved to possess antiviral, antidepressant and photodynamic activity (Bombardelli and Morazzoni 1995; Kladar *et al.*, 2015b), and is recognized as one of the most important bioactive compounds present in the St. John's wort. Hyperforin was another biologically active compound quantified in the examined extracts. In contrast to study where hyperforin was not detected (Maggi *et al.*, 2004), the levels in the current study reached as high as 0.86 mg/g of dry herb. This represents significantly higher level than reported in a study conducted by Smelcerovic *et al.* (2006), but comparable to the results of another research by Smelcerovic *et al.* (2008). It is noticeable that the amounts of hypericin and hyperforin in the examined *H. hirsutum* extracts are generally lower than previously reported data for *H. perforatum* where the same extraction procedure as in this study was used (Božin *et al.*, 2013). However, it is important to stress that the amount of hypericin in the examined extracts corresponds to the extracts of *H. maculatum,* which is in some pharmacopoeias listed together with *H. perforatum* as biological source of *Hyperici herba* (Kladar *et al.*, 2015a). The quantified amounts of rutin and quercetin were significantly higher than in a study by Maggi *et al.* (2004). More similarities were noticed with the results reported by Smelcerovic *et al.* (2008), in which the content of rutin corresponded, but the amounts of quercetin in the examined extracts were significantly lower. Furthermore, the estimated amounts of rutin were generally higher than those reported for *H. perforatum* (Božin *et al.*, 2013), and comparable to those reported for *H. maculatum* (Kladar *et al.*, 2015a). The quantified levels of amentoflavone, which is by some authors (Baureithel *et al.*, 1997) mainly responsible for antidepressant activity of *Hyperici herba*, were significantly lower than those reported for *H. perforatum* (Filippini *et al.*, 2010). No previous data describing the amounts of apigenin, naringenin, amentoflavone, epicatechine, caffeic, chlorogenic, ferullic, gallic, and *para*-hydroxybenyoic acid in *H. hirsutum* were found. Therefore, this represents the first report of quantification of these compounds in the hairy St. John's wort.

Table 2. Chemical composition of the examined *H. hirsutum* water-alcoholic extracts.

		mg/g of dry herb													
Sample	**Compounds**	**Hpc**	**Hpf**	**Ap**	**NA**	**Am**	**Qe**	**R**	**Ec**	**CA**	**CHA**	**FA**	**GA**	**PHB**	**% of d. e.**
1	Mean value	0.1383	0.1626	0.0031	n. d.	0.0153	0.2039	1.1853	0.7501	0.0585	0.2227	0.0222	0.1596	0.1487	19.06
	SD	0.0062	0.0119	0.0001	n. d.	0.0014	0.0018	0.0099	0.0541	0.0014	0.0023	0.0017	0.0128	0.0125	
2	Mean value	0.1619	0.0049	n. d.	n. d.	0.0226	0.1548	0.2737	0.8755	0.0447	0.1115	0.0256	0.0596	0.1401	13.73
	SD	0.0119	0.0001	n. d.	n. d.	0.0007	0.0083	0.0089	0.0500	0.0013	0.0047	0.0002	0.0056	0.0079	
3	Mean value	0.5365	0.8689	n. d.	0.1629	0.0116	0.1041	0.6929	0.3702	0.0384	0.0760	0.1033	n. d.	0.1114	15.22
	SD	0.0342	0.0066	n. d.	0.0105	0.0010	0.0038	0.0415	0.0201	0.0006	0.0064	0.0003	n. d.	0.0068	
4	Mean value	0.1223	0.0027	0.0008	n. d.	0.0193	0.3630	1.0314	0.8956	0.0777	0.1803	0.0222	0.0364	0.1749	17.05
	SD	0.0040	0.0002	0.0001	n. d.	0.0014	0.0016	0.0886	0.0845	0.0070	0.0056	0.0004	0.0012	0.0097	

*n. d. – not detected

Performed PCA reveals that the value of the first component is strong, explaining 62.26 % of variance, and together with the second component covers more than 98 % of variance (Figure 2c). This implicates that PCA is a good

and sufficient method for presentation of the size and shape of variations of examined variables and their grouping based on variation resemblance. The load values of the first and second component (Figure 2a and 2b) show that the main compounds responsible for the separation of the samples are the amounts of hyperforin, hypericin and rutin. It is obvious that the drastic separation of sample 3 (Figure 2d) based on the first component is the result of a presence of notably higher amount of hyperforin. The resemblance of samples 1 and 4 is the result of quantities of rutin which are significantly higher than in sample 2. Samples 1, 2 and 4 show similarity in quantity of epicatechin, which occurs in significantly lower amount in sample 3. The amounts of the rest of the quantified secondary metabolites in the examined samples are relatively stable and do not significantly affect the separation. This might indicate that the levels of synthesized hyperforin, hypericin, epicatechin and rutin in *H. hirsutum* are most affected by the sets of ecological factors specific for plant habitat.

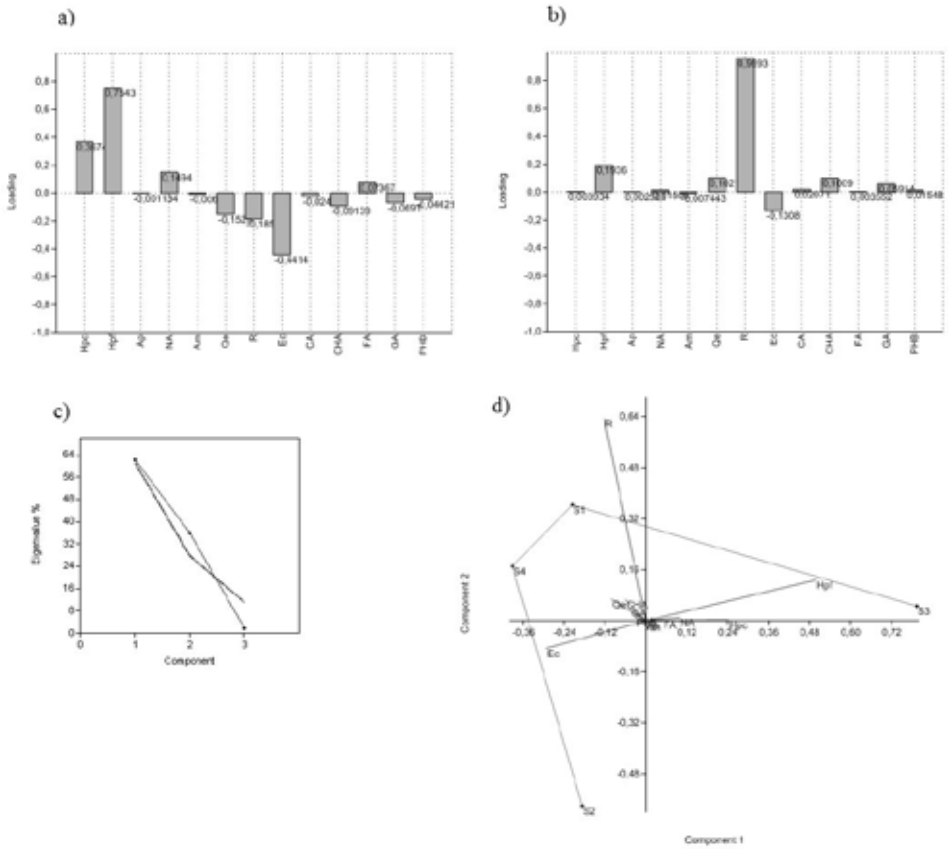

Figure 2. PCA based on the compounds detected in examined *H. hirsutum* extracts (2a and 2b – the load values of the first and second component, respectively, 2c – variance covered by the first and second component, 2d – position of the analyzed variables in the space of the first and second PCA axes.

Consequently, this leads to the conclusion that the origin of plant material is important for the quality of herbal drug since all of these compounds, except epicatechin, are essential for pharmacological effects of St. John's wort.

CONCLUSIONS

The chemical composition of the *H. hirsutum* examined extracts in most of the cases corresponded to those previously reported. When compared to the *H. perforatum* extracts, significantly lower amounts of hypericin and hyperforin were found. However, higher levels of rutin, which is a well-known biologically active compound, were reported. This opens a question of potentially new biological activities of *H. hirsutum*. Furthermore, of particular significance is a resemblance with chemical profile of *H. maculatum*, which is in some pharmacopoeias also listed as a biological source of *Hyperici herba*. Analyses suggested that the main compounds responsible for separation of the samples were hyperforin, hypericin, rutin and epicatechin, which implicates that the production of these secondary metabolites in the plant is highly affected by ecological factors characteristic for plant habitat. This emphasizes the significance of plant collecting locations since all of the mentioned compounds (except epicatechin) are of high importance for so far established pharmacological effects of *Hypericum* species.

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ХЕМИЈСКА КАРАКТЕРИЗАЦИЈА И ХЕМОТАКСОНОМИЈА *Hypericum hirsutum* ИЗ ВОЈВОДИНЕ

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РЕЗИМЕ: Род *Hypericum* убухвата преко 500 широко распрострањених врста. Главни представник рода и према Монографијама СЗО и EMEA, једини биолошки извор биљне дроге је кантарион (*Hypericum perforatum*, Hypericaceae). Кантарион се често користи у облику уљаних мацерата за третирање опекотина, ожиљака, екцема и гастроинтестиналних поремећаја, али и у облику водених или алкохолних екстраката као клинички доказан антидепресив. Према доступним подацима, количине секундарних метаболита у биљкама варирају зависно од еколошких фактора везаних за станиште и последично утичу на квалитет биљне дроге. Такође, претходна истраживања показују да и други припадници рода *Hypericum* поседују сличан профил хемијског састава као и *H. perforatum*. Међутим, постоје и примери да су одређени биомолекули присутни у већим количинама него код *H. perforatum*. Један од таквих примера би могао бити и *H. hirsutum*. Из тог разлога, циљ истраживања био је хемијска карактеризација водено-алкохолних екстраката *H. hirustum* прикупљеног са четири локалитета у Војводини методом течне хроматографије (HPLC-DAD). Добијени резултати хемотаксономске анализе указују на одређене разлике у садржају секундарних метаболита међу испитаним екстрактима. Такође, примећене су и сличности профила хемијског састава (у погледу присутности једињења) водено-алкохолних екстраката *H. perforatum* и *H. hirsutum* чиме се отвара питање даљих истраживања *H. hirsutum* као потенцијалног извора нових биолошки активних секундарних метаболита и додатних хемотаксономских маркера рода *Hypericum*.

КЉУЧНЕ РЕЧИ: хемијска карактеризација, хемотаксономија, HPLC-DAD, *Hypericum hirsutum*, PCA, секундарни метаболити

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ALLELOPATHIC EFFECTS OF *Clinopodium menthifolium* AND *Salvia sclarea* AQUEOUS EXTRACTS

ABSTRACT: Secondary plant biomolecules are the main agents in biochemical interactions between plants and the environment. It is possible to distinguish the role of secondary biomolecules in allelopathic (plant–plant) activity, plant–insect, plant–microbe, plant–herbivore and others. These interactions can significantly affect the productivity of agricultural crops. Application of allelochemicals into agricultural practice may reduce the use of herbicides. Effect of *Salvia sclarea* L. and *Clinopodium menthifolium* (Host) aqueous extracts on lipid peroxidation process, as well as the activity of antioxidant enzymes in leaves and roots of Jimson weed (*Datura stramonium* L.) and soybean (*Glycine max* L.) seedlings were examined 24 h, 72 h and 120 h after the treatment. The third aim was to evaluate effectiveness of aqueous extract as contact toxicant against *Rhyzopertha dominica*. Our results showed that *S. sclarea* aqueous extract induced lipid peroxidation in roots of Jimson weed seedlings 24 h after the treatment. Furthermore, both tested concentrations of *C. menthifolium* aqueous extract induced lipid peroxidation in Jimson weed roots 72 h and 120 h after the treatment. It was observed that *S. sclarea* aqueous extract showed toxic effect against *R. dominica*, with high mortality rate (above 95%).

KEYWORDS: allelochemicals, allelopathy, biopesticides, *Clinopodium menthifolium* Host, *Salvia sclarea* L.

INTRODUCTION

The term allelopathy, originating from the Greek word “allelon” meaning “each other” and “pathos” meaning “suffering”, was coined by plant physiologist Hans Molisch to include biochemical interactions among all types of plants. In 1974, Rice defined allelopathy as the effects of one plant on other plants

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through the release of chemical compounds in the environment (Bhadoria 2011). Chemicals that impose allelopathic influences, called allelochemicals, are secondary plant metabolites that have role in plant–plant, plant–soil, plant–insect and plant–predator interaction (Sharma and Satsangi 2013). They are present in almost all plants and a lot of tissues, like leaves, stems, flowers, fruits, seeds and roots (Gella *et al.*, 2013). Allelochemicals have detrimental effects on the growth of associated crops or, in other words, chemicals produced by invasive plants can inhibit the growth of competing vegetation. Secondary metabolites produced by invasive plants can also contribute to pest resistance (Li *et al.*, 2010). One of the main effects of allelochemicals on target plants is the excess production of reactive oxygen species (ROS), molecules very toxic to cells (Bogatek and Gniazdowska 2007). The accumulation of ROS is accompanied by the activation of the cellular antioxidant system. Enzymes, such as superoxide dismutase (SOD), catalase (CAT) and peroxidases, play important roles in protecting cell against reactive oxygen species (Kuthan *et al.*, 1986).

The aim of this study was to examine the effects of *Clinopodium menthifolium* (Host) and *Salvia sclarea* L. aqueous extract on Jimson weed and soybean antioxidant properties, to explore the potential of these species in weed control as well as to assess the possible side effects when applied as bioherbicide in organic production. This study also aims at evaluating the effectiveness of aqueous extract as contact toxicant against *Rhyzopertha dominica*.

MATERIALS AND METHODS

Plant material and aqueous extract preparation

The wild, aromatic plant, *Clinopodium menthifolium* (Host) was collected at Čanj locality near the Adriatic coast in Montenegro in May, 2012. The aerial parts of the flowering plant *Salvia sclarea* L. were collected in the south of Serbia (Preševo), in July, 2012. Voucher specimen of collected plants was confirmed and deposited at the Herbarium of The Department of Biology and Ecology, Faculty of Science, University of Novi Sad. The air–dried plant materials were ground into powder. The powdered materials (10 g) were extracted in 100 ml distilled water (10% w/v). After 24 h, the extracts were filtered through filter paper and kept at 4 °C until application.

Seedling growth

The Jimson weed (*Datura stramonium* L.) and soybean (*Glycine max* L.) seeds were grown in a controlled climate chamber at 28 ºC, 60% relative humidity, a photoperiod of 18 h, and a light intensity of 10,000 lx, in plastic pots containing sterile sand. After 30 days, the seedlings were transplanted in plastic pots containing 700 ml of Hoagland's solution prepared according to Hoagland

& Arnon, as well as 7 ml and 14 ml of aqueous extract, while control pots contained the same volume of nutrient solution. Seedlings were harvested for determining the investigated biochemical parameters 24 h, 72 h and 120 h after the treatments.

Biochemical assays

For the determination of the oxidative stress parameters, 2 g of fresh plant material were homogenized in 10 ml of phosphate buffer (0.1 M, pH 7.0). Homogenates were centrifuged for 20 minutes at 10,000 x g and filtered. The supernatants were used for biochemical assays.

Lipid peroxidation was measured at 532 nm using the thiobarbituric acid (TBA) test (Mandal *et al.*, 2008). The total amount of TBA–reactive substances was given as nmol malondialdehyde (MDA) equivalents mg^{-1} protein. Catalase (CAT) (EC 1.11.1.6) activity was determined according to Sathya and Bjorn (2010). The decomposition of H_2O_2 was followed as a decrease in absorbance at 240 nm. The activity of the enzyme was expressed as U mg^{-1} of protein. Superoxide dismutase (SOD) (EC 1.15.1.1) activity was assayed according to the method by Mandal *et al.* (2008) slightly modified by measuring its ability to inhibit photochemical reduction of nitro blue tetrazolium (NBT) chloride. One unit of the SOD activity was defined as the amount of enzymes required to inhibit reduction of NBT by 50%. Peroxidase (EC 1.11.1.7) activity was measured using guaiacol (guaiacol peroxidase; GPx) and pyrogallol (pyrogallol peroxidase; PPx) as substrates according to Morkunas and Gmerek (2007). The activity of the enzyme was expressed as U mg^{-1} of protein.

Statistical analyses

Values of the biochemical parameters were expressed as standard error of the mean of determinations made in triplicates and tested by ANOVA followed by comparison of the mean by Duncan's multiple range test ($P<0.05$). Data were analyzed using STATISTICA for Windows version 11.0.

Insects

Rhyzopertha dominica is one of the most important stored grain pests in the world (Guedes *et al.*, 1996). The experiment on the adult of *Rhyzopertha dominica* (Coleoptera: Bostrichidae) was carried out at the Faculty of Agriculture, University of Novi Sad.

Contact test: For assessment of contact insecticidal activity, a method described by Kouninka *et al.* (2007) was used. In glass tubes, previously "rinsed" with plant extracts, 10 adults of *R. dominica* (seven to ten days old) were inserted. The tubes were sealed with parafilm and placed in a horizontal

position so the insects could move along the wetted tube wall. The tubes were incubated in a thermostat at 28 °C in the dark. The mortality was assessed after 24 h and 48 h by counting the number of dead and paralyzed adults.

Contact–digestive test: A contact–digestive insecticidal effect of tested extracts was evaluated in a "No–choice" test, according to Obeng–Ofer and Reichmuth (1997). 40 g of wheat grains were treated with a specific concentration of each aqueous extract, in the ratio of 3 ml per 100 g. The treated grain was left to dry for 2 h at room temperature, and after drying, it was divided into four equal portions, representing four replicates (10 g of grains per replicate) and placed in Petri dishes along with 20 adults. Mortality was evaluated after 48 h and 72 h by counting the number of dead and paralyzed adults.

Statistical analysis: The results were analyzed using Duncan's multiple range test, for confidence interval 95%, in statistical software SPSS 17.

RESULTS

In the leaves of Jimson weed, the significant decrease in activity of SOD was detected in the treatment with both concentrations of *S. sclarea* aqueous extract 72 h after the treatment (Figure 1). The highest activity of GPx was observed in plants 72 h after the treatment with both concentrations of *S. sclarea*. In the roots of Jimson weed, the activity of SOD, GPx and PPx was significantly decreased 72 h after the treatments with *S. sclarea* extract (Figure 2). The significant increase in CAT activity was recorded 72 h after the treatment with *C. menthifolium* extracts (Figure 5). Statistically significant increase in MDA accumulation was recorded in roots of Jimson weed 24 h after the treatment with *S. sclarea* extract and 72 h after the treatments with *C. menthifolium* extract (Figure 2 and Figure 5).

Both tested concentrations of *S. sclarea* aqueous extract showed significant increase in the activity of SOD and CAT after 72 h in soybean roots as compared to control (Figure 3). The significant increase in SOD, CAT and peroxidases activity was recorded 72 h after the treatment with higher concentration of *C. menthifolium* extract (Figure 6). The significant decrease in peroxidases activity and LP intensity was recorded in roots of soybean plants 120 h after the treatment (Figure 3 and Figure 6).

More effective formulation against *R. dominica* was *S. sclarea* aqueous extract with high mortality rate. In contact test the mortality rate of *R. dominica* was almost 100% after 24 h (Figure 7) and above 80% 72 h after the treatment in contact–digestive test (Figure 8). Concentration of 2% *S. sclarea* aqueous extracts exhibited a toxic effect with 100% mortality after 24 h.

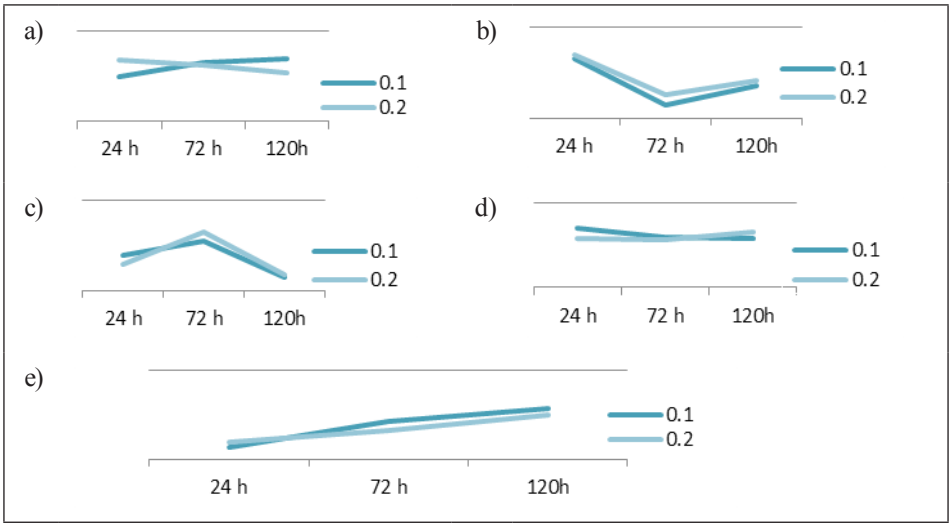

Figure 1. Effect of two concentrations of *S. sclarea* aqueous extracts on CAT(a), SOD(b), GPx(c) and PPx(d) activity (U mg^{-1} protein) and on MDA content (nmol mg^{-1} protein) (e) in leaves of Jimson weed seedlings compared to control group.

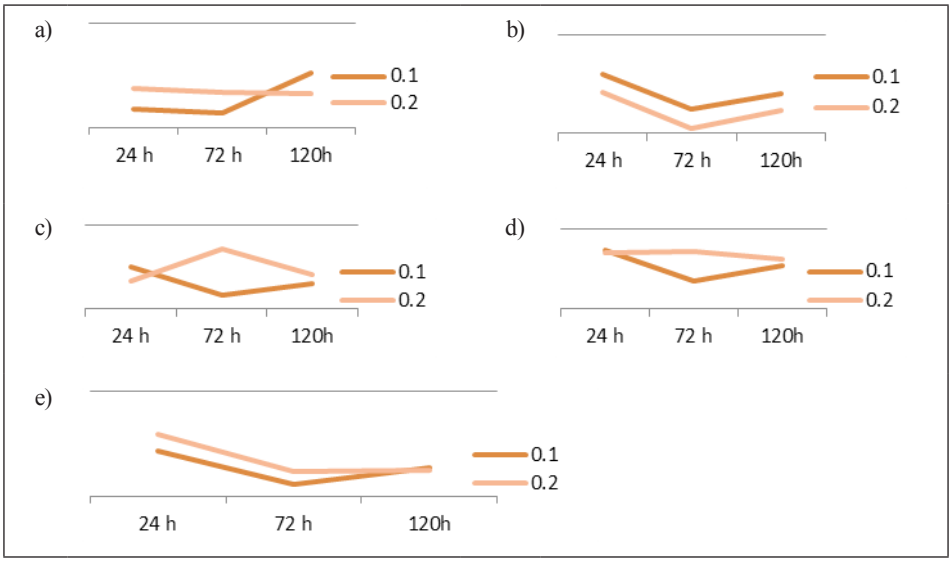

Figure 2. Effect of two concentrations of *S. sclarea* aqueous extracts on CAT (a), SOD (b), GPx (c) and PPx (d) activity (U mg^{-1} protein) and on MDA content (nmol mg^{-1} protein) (e) in roots of Jimson weed seedlings compared to control group.

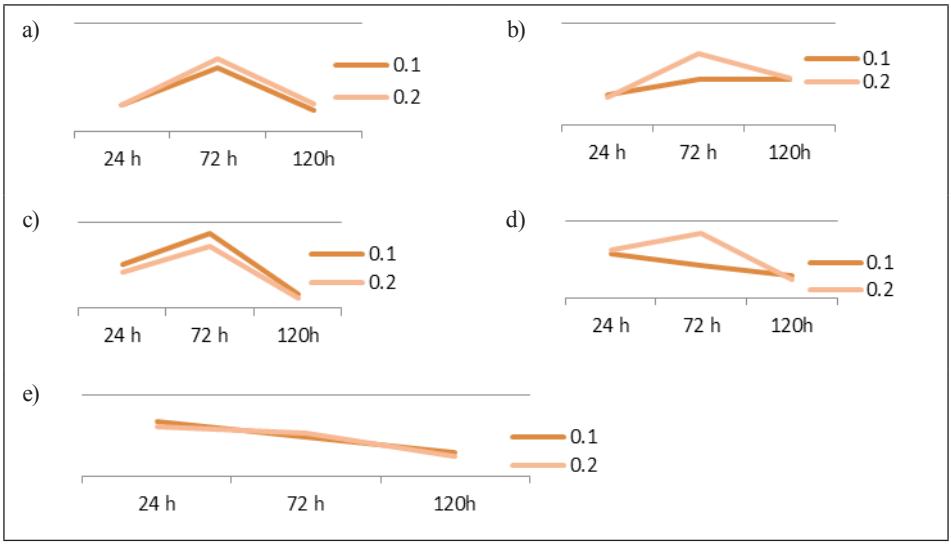

Figure 3. Effect of two concentrations of *S. sclarea* aqueous extracts on CAT (a), SOD (b), GPx (c) and PPx (d) activity (U mg^{-1} protein) and on MDA content (nmol mg^{-1} protein) (e) in roots of soybean seedlings compared to control group.

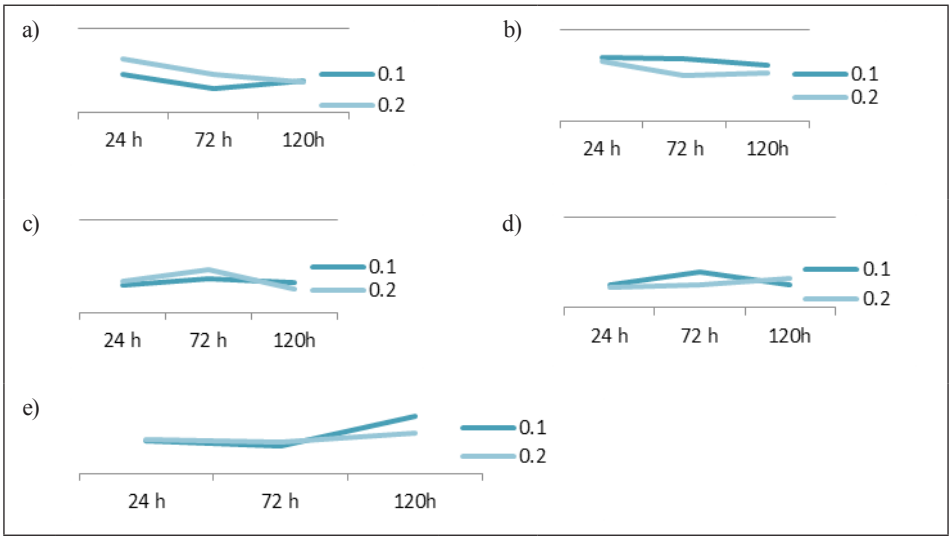

Figure 4. Effect of two concentrations of *C. menthifolium* aqueous extracts on CAT (a), SOD (b), GPx (c) and PPx (d) activity (U mg^{-1} protein) and on MDA content (nmol mg^{-1} protein) (e) in leaves of Jimson weed seedlings compared to control group.

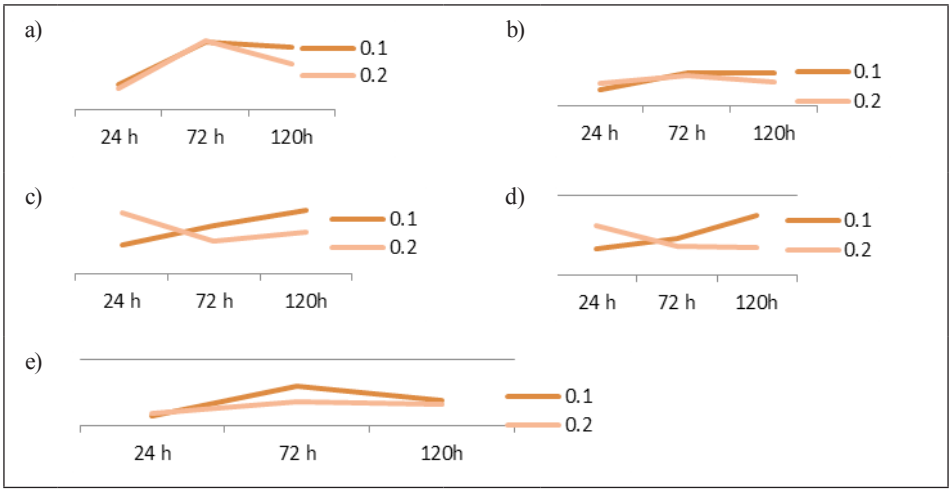

Figure 5. Effect of two concentrations of *C. menthifolium* aqueous extracts on CAT (a), SOD (b), GPx (c) and PPx (d) activity (U mg^{-1} protein) and on MDA content (nmol mg^{-1} protein) (e) in roots of Jimson weed seedlings compared to control group.

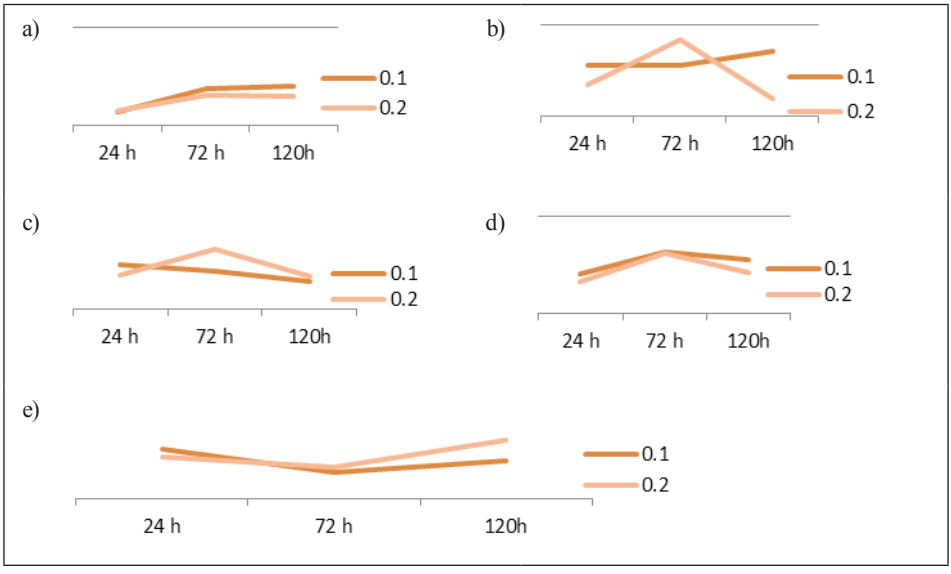

Figure 6. Effect of two concentrations of *C. menthifolium* aqueous extracts on CAT (a), SOD (b), GPx (c) and PPx (d) activity (U mg^{-1} protein) and on MDA content (nmol mg^{-1} protein) (e) in roots of soybean seedlings compared to control group.

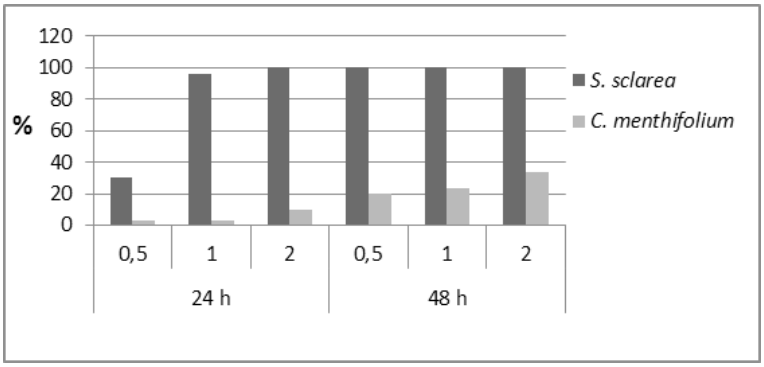

Figure 7. Mortality of *R. dominica* adult treated with formulations containing a known concentration (0.5 %, 1 % and 2 %) of *S. sclarea and C. menthifolium* aqueous extracts (Contact test).

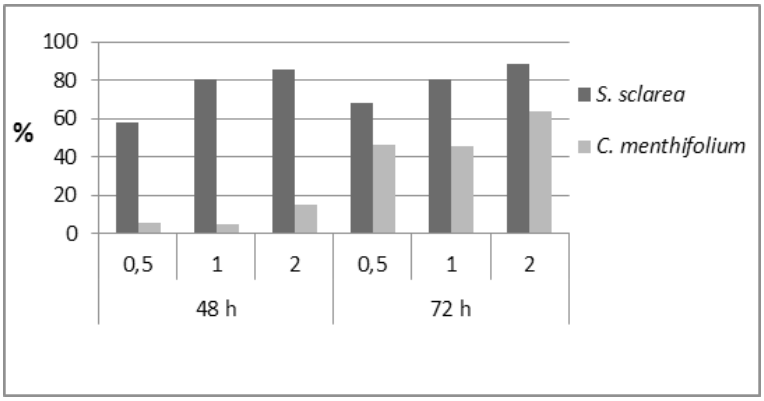

Figure 8. Mortality of *R. dominica* adult treated with formulations containing a known concentration (0.5 %, 1 % and 2 %) of *S. sclarea and C. menthifolium* aqueous extracts (Contact–digestive test).

DISCUSSION

Allelochemicals can cause oxidative damage in target plants, producing ROS during response to allelopathic stress, as evidenced by enhanced activity of ROS–scavenging enzymes (Mutlu *et al.*, 2011; Cruz–Ortega *et al.*, 2007). Therefore, antioxidant enzymes activity can be used as indicator of oxidative stress in plants (An *et al.*, 2005). Antioxidant enzymes activity in roots of Jimson weed was significantly affected by *C. menthifolium* aqueous extract. The significant increases of SOD, CAT and peroxidases activity were detected in the roots of Jimson weed treated with both concentrations of *C. menthifolium* aqueous extract. Some other authors reported changes in anti-

oxidant enzymes activity in plants under oxidative stress (Lara-Nunez 2006). Qian *et al.* (2009) showed that allelochemicals triggered the synthesis of ROS to disrupt the subcellular structure of an aquatic organism such as *Chlorella vulgaris*.

For various plant species under oxidative stress, a significant increase of lipid peroxidation is observed. The accumulation of O_2^- as a result of inhibition of SOD activity in cells also can cause increased membrane lipid peroxidation (Mutlu *et al*., 2011). Malondialdehyde (MDA) content, an end–product of lipid peroxidation process, is used as oxidant biomarker. Our results show that two tested extract concentrations of *C. menthifolium* and *S.sclarea* aqueous extracts affected lipid peroxidation in the roots of Jimson weed. The significantly higher accumulation of MDA in Jimson weed plants treated with plant extracts point to the fact that stress provoked by allelopathic substances was strong enough and scavenging effects of antioxidant enzymes could not prevent oxidative burst and induction of LP. Cruz–Ortega *et al*. (2007) documented the oxidative damage in the target plant caused by aqueous plant extract.

One of the main pests associated to stored grains is the smaller grain borer, *Rhyzopertha dominica*, that can attack wheat, barley, rice and oat which increases the need for efficient control (Guzzo *et al*., 2006). The primary control of *R. dominica* and other stored–product insect populations is primarily dependent upon continued applications of insecticides (Kim *et al*., 2003; Guedes *et al*., 1996). New interest in insect pest elimination in stored products has been shown in plant products (Rajendran and Sriranjini 2008). Use of plants in pest management has been reported all over the world as they are convenient, less expensive, highly effective and safer for the environment (Gandhi and Pillai 2011). In the present work, *C. menthifolium* and *S. sclarea* aqueous extracts were evaluated on *R. dominica*. More effective formulation was *S. sclarea* aqueous extract with mortality above 80%. Results of other authors have shown that different species of the genus *Salvia* have toxic effect on insects (Zavala–Sánchez *et al*. 2013; Tomczyk and Suszko 2011).

CONCLUSIONS

Our results showed that *S. sclarea* aqueous extract induced lipid peroxidation in roots of Jimson weed seedlings 24 h after the treatment. Furthermore, both tested concentrations of *C. menthifolium* aqueous extract induced lipid peroxidation in Jimson weed roots 72 h and 120 h after the treatment. Tested plant extracts did not induce lipid peroxidation process in soybean seedlings. It was observed that *S. sclarea* aqueous extract showed toxic effect with high mortality rate against *R. dominica* (above 95%). The results indicate that the plant extracts and natural substances are good candidates to be developed as sources of natural pesticides.

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АЛЕЛОПАТСКИ ЕФЕКТИ ВОДЕНИХ ЕКСТРАКАТА БИЉАКА *Clinopodium menthifolium* И *Salvia sclarea*

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РЕЗИМЕ: Секундарни биомолекули биљака су основни агенси биохемијске интеракције биљака са спољашњом средином. У том смислу могуће је разликовати улогу секундарних биомолекула у алелопатским односима (биљка–биљка), у односу биљка–инсект, биљка–микроорганизам, биљка–биљојед и др. Овакве интеракције могу значајно да утичу на продуктивност пољопривредних култура, из чега је произишла идеја о алелопатији као перспективној природној стратегији за контролу корова. Због тога је у овом раду вршено испитивање алелопатског деловања водених екстраката самониклих биљака фамилије Lamiaceae, *Salvia sclarea* L. и *Clinopodium menthifolium* Host, на коров татулу (*Datura stramonium* L.) одређивањем активности антиоксидативних ензима и процеса липидне пероксидације у листу и корену третираних биљака. Испитан је утицај екстраката и на сој (*Glycine max* L.) ради утврђивања њиховог утицаја на гајене биљке. Поред испитивања хербицидног ефекта екстраката наведених врста испитана је и њихова инсектицидна активност. Добијени резултати показали су да је значајно повећање интензитета липидне пероксидације забележено у корену татуле након 24 часа у третману са воденим екстрактом *S. sclarea*, док је у третману са воденим екстрак-

том *C. menthifolium* у третираним кореновима татуле забележена већа акумулација MDA у поређењу са нетретираним коренима после 72 и 120 часова. У тестовима за испитивање инсектицидног ефекта на житног кукуљичара већи токсични ефекат испољио је водени екстракт *S. sclarea* (са стопом смртности преко 95%).

КЉУЧНЕ РЕЧИ: алелохемикалије, алелопатија, биопестициди, *Clinopodium menthifolium* Host, *Salvia sclarea* L.

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ANTIOXIDANT PROPERTIES OF *Rubus discolor* LEAF EXTRACTS

ABSTRACT: In this work were examined aqueous, methanol, ethanol and acetone leaf extracts of *Rubus discolor*, wild growing blackberry, for their antioxidant properties and total phenol and flavonoid content. The total phenol content (TPC) varried from 250.05 to 446.61 mg GAE/g of dry extract, while total flavonoid content (TFC) was in range between 22.44 and 61.15 mg QE/g of dry extract. Aqueous extracts were the richest in phenols, as well as in flavonoids. *In vitro* antioxidant capacity of leaf extracts was evaluated by 2,2-diphenyl--1-picrylhydrazil (DPPH), 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) free radical scavenging procedures and ferric reducing ability of plasma (FRAP) assay. Aqueous extracts were the most effective through all antioxidant tests. The total phenol content highly correlated with antioxidant activity of extracts. Moreover, weak correlation was established between total phenol and total flavonoid content. The results presented in this work indicate that phenol compounds contribute to antioxidant ability of extracts.

KEYWORDS: *Rubus discolor*, extracts, flavonoids, phenols, antioxidant activity

INTRODUCTION

The Rosaceae family is a large and diverse family which includes over 3,000 economically important fruits and ornamental species (Potter *et al.*, 2002). The genus *Rubus* counts about 750 species native to all continents except Antarctica (Alice and Campbell 1999). Many *Rubus* species are globally consumed as fresh and frozen fruits or processed in juices, jams and jellies (Kaume *et al.*, 2012). On the other hand, some of them are important not only as ornamental species, but also as invasive weeds, and in early forest succession. Consequently, that gives imense economical and ecological importance to the genus *Rubus* (Alice and Campbell 1999).

Rubus species have been globally very appreciated in traditional medicine due to their therapeutic and healing properties (Hummer 2010). *R. discolor* fruits,

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leaves and roots are used as traditional remedy for nephritis and prostatitis; leaves are also used as antidiarrheals and for wound healing (Kültür 2007).

Blackberries are rich in phenols, particularly in anthocyanins and ellagitannins that are considered to possess antioxidant properties and, therfore, provide many health benefits (Reyes-Carmona *et al*., 2005; Ivanovic *et al*., 2014; Keser *et al*., 2015). Daily consumption of fresh vegetables and fruits, including blackberries, is directly connected with decreased occurence of cancer, coronary hearth diseases and many other disorders (Vendemiale *et al*., 1999; Reyes-Carmona *et al*., 2005).

R. discolor is a shruby plant with high and strong angled brown to purple stems. Leaves consist of 5 leaflets from which teriminal is ovate or suborbicular with truncated base, while basal are on short petioles. Flowers have about 30 mm in diametar and are grouped in pyramidal-truncate and large inflorescence. Fruits are large (Heslop-Harrison 1968). It is widespread in Europe, from France to Mediteranean and Balkan region. In Serbia, it is distributed in hills and mountains in the western part of the country (Tatić 1972).

R. discolor fruits possess higher amounts of bioactive compounds, such as anthocyanins, phenols, and non-flavonoid phenolics, compared to cultivated blackberries and even *R. idaeus* (Dujmović Purgar *et al*., 2012). *R. discolor* extracts exhibit notable antioxidant activity, particularly flower an leaf extracts. Additionally, flower extract has effect on the quantity of antioxidant enzymes, lipophylic vitamins (A and E), cholesterol, glutathion, total protein, and malondialdehyde (Keser *et al*., 2015). Despite the fact that fruits of various *Rubus* species have been the subject of different studies, information about antioxidant properties of blackberry leaves is still scarce. Therefore, the aim of the present study was evaluation of antioxidant properties of leaves collected from natural populations of *R. discolor*, as well as estimation of total phenol and flavonoid content.

MATERIALS

Plant material

Leaves were collected during summer 2012 at two locations: in Belgrade and in Cer Mountain (near the village of Čokešina). Leaves were taken from natural populations during fruiting stage of plants. Voucher specimens have been deposited in the Herbarium of the Institute of Botany and Botanical Garden "Jevremovac", Faculty of Biology, University of Belgrade BEOU; vouchers no. 17084 and 17081.

Chemicals

Organic solvents (methanol, ethanol, acetone) and acids − HCl (concentrated hydrochloric acid) and CH_3COOH (glacial acetic acid) − were purchased

from Zorka Pharma, Šabac (Serbia). Gallic acid, 2,2-dyphenyl-1-picrylhydrazyl (DPPH), iron (III) chloride ($FeCl_3 \times 6H_2O$), iron (II) sulphate heptahydrate ($FeSO_4 \times 7H_2O$), and sodium acetate ($CH_3COONa \times 3H_2O$) were obtained from Sigma Chemicals Co., St. Louis, MO (USA). Folin-Ciocalteu phenol reagent was purchased from Merck, Darmstadt (Germany). Sodium carbonate anhydrous (Na_2CO_3) and L(+)-ascorbic acid (vitamin C) were purchased from AnalaR Normapur, VWR, Geldenaaksebaan, Leuven (Belgium). Aluminum nitrate nonahydrate ($Al(NO_3)_3 \times 9H_2O$) and 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) were purchased from Fluka Chemie AG, Buchs, (Switzerland). Quercetin hydrate was obtained from TCI Europe NV, Boerenveldsweg (Belgium). All chemicals and reagents used in experiments were of analytical grade.

METHODS

Extract preparation, total phenol and flavonoid content

Extracts were prepared identically, by dissolving 5 g of dried powdered leaves in 50 ml of appropriate solvent and sonication in ultrasonic bath for 1 hour. After 24 hours samples were again sonicated for 1 hour, then filtered through Whatman no. 1 filter paper and evaporated by rotatory vacuum evaporator. Samples were left in the fridge at +4 °C till use.

The total phenol (TPC) content was determined spectrophotometrically as suggested by Singleton and Rossi (1965). In brief, 10% Folin-Ciocalteu reagent was added to 0.2 mL aliquots of sample solution. Then, 7.5% sodium-carbonate solution was added to this mixture and left for 2h at room temperature to react. The results were expressed as mg of gallic acid equivalents (GAE) per g of dry extract.

The total flavonoid content (TFC) in *R. discolor* leaves was estimated by JENWAY 6306 UV/VIS spectrophotometer according to the procedure previously described by Park *et al.* (1997) and expressed as mg of quercetin equivalents (QE) per g of dry extract.

All measurements were carried out in triplicate.

Evaluation of antioxidant properties

Antioxidant properties were evaluated spectrophotometrically by three different, previously described, *in vitro* procedures: DPPH (Blois 1958), ABTS (Miller and Rice-Evans 1997), and FRAP (Benzie and Strain 1996).

DPPH is a procedure based on the ability of plant extracts to neutralise 2,2-diphenyl-1-picryllhydrazyl (DPPH) free radicals. The DPPH solution in methanol (0.04 mg/mL) was added to the aliquots of sample of different concentrations. After 30 minutes of incubation in the dark at room temperature,

the absorbance was read at 517 nm against the control which contained methanol instead of sample. The results were expressed as IC_{50} values (μg/mL).

ABTS is another antioxidant method which involves ABTS free radicals to estimate radical scavenging abilities of plant samples. The results were derived from triplicate measurements using JENWAY 6306 UV/VIS spectrophotometer and expressed as IC_{50} values (μg/mL).

FRAP method was used to test capability of samples to reduce iron in complex with tripyridyl-s-triazine (TPTZ) from ferric to ferrous form which is measured by PERKIN ELMER LAMBDA BIO UV/VIS spectrophotometer at 595 nm. The results were calculated from calibration curve of aqueous solution of $FeSO_4x7H_2O$ and expressed as μmol Fe^{2+} equivalents per mg of dry extract.

L-ascorbic acid and BHA, as well-known antioxidants, were used as a control.

RESULTS AND DISCUSSION

Yields, total phenol and flavonoid content

The extraction yields of *R. discolor* leaf extracts, as well as TPC and TFC, are presented in Table 1. The highest yield was found for aqueous and methanol extracts. TPC varied between 250.05 and 446.61 mg GAE/g of dry extract. Aqueous extracts from both localities were the richest in phenols. Despite the fact that samples from Cer had lower yield, TPC was higher in comparison to those from Belgrade.

TFC was in range from 22.44 to 61.15 mg QE/g of dry extract. The highest amount of flavonoids was in acetone (61.15 mg QE/g and 45.35 mg QE/g in sample from Belgrade and Cer, respectively), while the lowest were in aqueous and ethanol extracts.

The total phenol and flavonoid content in leaves of different *Rubus* species was previously investigated by several authors. *R. ulmifolius* extract was the richest in phenols, among 11 tested Sardinian species (Dall'Acqua *et al.*, 2008). Similarly, Conforti *et al.* (2011) investigated bioactive compounds of 70% aqueous ethanol extracts and found the highest amount of phenols in *R. caesius* leaf extract. According to Keser *et al.* (2015), who previously examined variations in TPC among different plant parts of *R. discolor*, flower and leaf extracts were the richest in phenols. These researchers identified several flavonoid compounds such as rutin, apigenin, naringin, naringenin, myricetin and quercetin. Particularly abundant in flower extracts were myricetin and naringin, while rutin and naringin were abundant in leaf extracts.

Table 1. The yield, total phenol and total flavonoid content and antioxidant properties of *R. discolor* leaf extracts

		Yield[1]	Total phenol content[2]	Total flavonoid content[3]	DPPH[4]	ABTS[5]	FRAP[6]
Belgrade	aqueous	8.67	359.19±±9.51	35.63±±0.37	17.31±±0.11	8.00±±0.78	2.24±±0.04
	methanol	10.62	277.19±±2.04	36.74±±0.47	22.46±±0.09	10.10±±0.13	1.03±±0.06
	ethanol	5.23	250.05±±3.90	24.49±±0.27	26.54±±0.25	13.32±±0.42	0.74±±0.56
	acetone	2.78	289.46±±5.80	61.15±±0.60	29.46±±0.80	14.36±±0.22	2.01±±0.01
Cer	aqueous	11.03	446.61±±6.01	22.44±±0.44	15.76±±0.06	4.76±±0.36	2.96±±0.06
	methanol	8.80	341.14±±3.01	30.97±±0.76	17.61±±0.14	7.02±±0.17	1.51±±0.04
	ethanol	3.77	414.05±±1.00	28.95±±0.31	16.65±±0.41	8.03±±0.12	1.90±±0.06
	acetone	1.92	407.68±±27.03	45.35±±1.74	15.86±±0.71	5.36±±0.06	1.44±±0.04

results were expressed as mean ±± standard deviation
[1] %
[2] mg GAE/g d.w.
[3] mg QE/g d.w.
[4] IC_{50} (µg/ml)
[5] IC_{50} (µg/ml)
[6] µmol Fe^{+2}/mg d.w.

Evaluation of antioxidant properties

Antioxidant characteristics of *R. discolor* leaf extracts were examined through three *in vitro* assays and the results are presented in Table 1.

IC_{50} values for DPPH varied from 15.76 µg/ml for aqueous sample from Cer to 29.46 µg/ml for acetone extract of the sample from Belgrade. The most powerful in neutralisation of DPPH free radicals were aqueous extracts with IC_{50} values 15.76 µg/ml and 17.31 µg/ml for samples from Cer and Belgrade, respectively. IC_{50} values for ABTS assay were between 4.76 µg/ml and 14.36 µg/ml. Similarly, aqueous extracts showed the strongest ability to scavenge ABTS free radicals. Possible explanation for lower IC_{50} values obtained in ABTS in comparison to DPPH test could be the ability of ABTS radicals to react with both hydrophilic and lipophilic compounds. Additionally, approach to DPPH free radicals is restricted only to smaller molecules (Magalhães *et al*., 2008; Badarinath *et al*., 2010; Nur Alam *et al*., 2013).

Values obtained for FRAP procedure were in range from 0.74 to 2.96 µmol Fe^{+2}/mg of dry weight. Aqueous extracts exhibited the most promising antioxidant properties among tested samples with values 2.24 and 2.96 µmol Fe^{+2}/mg of dry weight, for samples from Belgrade and Cer, respectively.

Samples from Cer exhibited stronger antioxidant activity through all three used methods, which was probably the consequence of greater amount of phenols present in those samples. The differences among samples could be ascribed

to different environmental conditions and consequently different phytochemical composition of examined extracts.

Antioxidant activity of leaf extracts of related blackberry species was previously reported by some researchers (Dall'Acqua *et al*., 2008; Martini *et al*., 2009; Conforti *et al*., 2011; Keser *et al*., 2015; Veličković *et al*., 2015). The results of this research corresponded to these findings.

The correlation between secondary metabolites content in leaf extract of *R. discolor* and antioxidant properties, expressed by Pearson's correlation coefficient, was also examined. A strong and negative correlation was found between total phenol content and radical scavenging abilities tested by DPPH ($r = -- 0.8532$) and ABTS ($r = -- 0.8631$), which was a consequence of the usage of IC_{50} values to express antioxidant properties. In addition, strong positive correlation was established between total phenol content and FRAP values ($r = 0.7147$), which was expectable because both methods were based on reducing power of extracts. That was also the reason why method used for evaluation of TPC was not so sensitive and specific. On the other hand, total flavonoid content moderately correlated with DPPH and ABTS, and weakly with FRAP values. Weak correlation was established between total phenol and flavonoid content. The results of this study suggest that antioxidant properties of tested samples could be ascribed not only to flavonoid group of phenolic compounds, but also to some other compounds.

CONCLUSIONS

The presented results demonstrate that *R. discolor* leaves possess notable antioxidant activity which is strongly correlated to phenolic compounds. Therefore, *R. discolor* should be considered as a rich source of phytochemicals which could be potentially implemented in food and pharmaceutical industry. Consequently, there is need for further examinations of phytochemical composition of *R. discolor* leaves and their biological activity, particularly through *in vivo* tests.

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АНТИОКСИДАТИВНА СВОЈСТВА ЕКСТРАКТА ЛИСТОВА *Rubus discolor*

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РЕЗИМЕ: У овом раду испитивана су антиоксидативна својства и присуство фенола и флавоноида у воденим, метанолним, етанолним и ацетонским екстрактима листова самоникле купине *Rubus discolor*. Укупан садржај фенола је варирао између 250,05 и 446,61 mg GAE/g сувог екстракта, док се укупан садржај флавоноида кретао у опсегу од 22,44 до 61,15 mg QE/g. Водени екстракти су били најбогатији фенолима и флавоноидима. *In vitro* антиоксидативни капацитет екстраката листова је одређен DPPH, ABTS и FRAP методама, при чему су најефикаснији били водени екстракти. За разлику од флавоноида, феноли су били у јакој корелацији са антиоксидативном активношћу екстраката. Међутим, установљена је слаба корелација између укупне количине фенола и флавоноида. Презентовани резултати указују да је антиоксидативна активност екстраката последица присуства фенолних једињења.

КЉУЧНЕ РЕЧИ: *Rubus discolor*, екстракти, флавоноиди, феноли, антиоксидативна активност

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ESSENTIAL OIL COMPOSITION AND ANTIOXIDANT ACTIVITY OF TWO *Juniperus communis* L. VARIETIES GROWING WILD IN SERBIA

ABSTRACT: The genus *Juniperus* L. (Cupressaceae) consists of ca. 67 species and 34 varieties. *Juniperus communis* L. grows on dry hills or mountainous tracts and is widely distributed in the northern hemisphere. A typical variety *J. communis* L. var. *communis* was collected in Deliblatska peščara (Deliblato Sands) and variety *J. communis* L. var. *saxatilis* Pall. in Kopaonik Mountain. Needle essential oils were obtained using Clevenger apparatus and analyzed using GC/MS and GC/FID. Antioxidant activity of essential oils was evaluated using DPPH assay. A total of 78 compounds were detected and identified. Both oils are characterized by high abundance of monoterpenes. The main constituents of *J. communis* var. *communis* essential oil were sabinene (39.4%), α-pinene (13.3%), myrcene (4.7%) and terpinen-4-ol (3.7%), while *J. communis* var. *saxatilis* essential oil had α-pinene (34.9%), sabinene (20.3%), δ-3-carene (6.4%) and germacrene B (6.3%) as the most abundant components. DPPH test showed IC_{50} values 0.66 mg/ml for *J. communis* var. *communis* and 0.32 mg/ml for *J. communis* var. *saxatilis*. Although antioxidant activity was weaker than used standards (BHT and L-ascorbic acid) it is still significant.

KEYWORDS: antioxidant activity, essential oils composition, *Juniperus,* Serbia

INTRODUCTION

Juniperus communis L. (common juniper) is one of the ca. 67 species that belong to genus *Juniperus* L. (Cupressaceae). It can be found growing on different soils (i.e. limestone, serpentine, volcano rocks, sands) and in different habitats (i.e. rocky cliffs, alpine meadows, semi-deserts and sand dunes, abandoned rural areas, but also in swamps), from sea level up to 2,800 meters above sea level (masl) (Vidaković 1982; Farjon 2001; Adams 2011; Farjon and Filer

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2013). This very high adaptability to a large variety of habitats is followed with great morphological and phytochemical diversity. Even though this taxon is one of the most studied taxa within the *Juniperus* genus, its taxonomic status is still unresolved. According to Farjon, there are five varieties (var. *communis,* var. *depressa,* var. *megistocarpa,* var. *nipponica* and var. *saxatilis*), while Adams recognises two more varieties (var. *charlotensis,* var. *jacki*) (Adams 2011; Farjon and Filer 2013). Furthermore, some authors recognise varieties of this species at the level of subspecies or species (Adams 2011). Two varieties grow in Serbia and the Balkans – *J. communis* L. var. *communis* and *J. communis* L. var. *saxatilis* Pall. (Jovanović 1970; Tutin *et al*., 1976; Vidaković 1982).

Both leaves and berries of the common juniper are used in traditional medicine and for preparation of brandy, thus many papers dealt with biological activity of essential oil obtained from seed cones and needles (Angioni *et al*., 2003; Pepeljnjak *et al*., 2005; Glisić *et al*., 2007; Misharina *et al*., 2009; Martz *et al*., 2009; Abdel-Maksouda and El-Aminb 2011; Tumen *et al*., 2011; Öztürk *et al*., 2011; Vermaak *et al*., 2011; Hădărugă *et al*., 2011; Vourlioti-Arapi *et al*., 2011; Boulogne *et al*., 2012; Haq 2012; Kloucek *et al*., 2012). However, antioxidant activity of essential oil obtained from leaves of common juniper has not been studied in populations originating from the Balkans. The aim of this research was to assess potential antioxidant properties of leaf essential oils obtained from two varieties growing wild in Serbia.

Table 1. Geographical characteristic of two *Juniperus communis* L. collection sites

Juniperus communis	Location	Latitude (N)	Longitude (E)	Altitude (masl)
var. *communis*	Stara Šušara (Deliblatska Peščara)	44° 55.2’	21° 08.9’	175
var. *saxatilis*	Jelica (Kopaonik Mt.)	43° 19.5’	20° 50.7’	1,650

MATERIAL AND METHODS

Plant material was collected in summer 2009 from two very different localities in Serbia regarding their altitude, pedological composition and climate conditions. *Juniperus communis* var. *communis* was collected from the locality of Stara Šušara (Deliblatska Peščara, a part of the Pannonian Plain), while *J. communis* var. *saxatilis* was collected from the locality of Jelica (Kopaonik Mt.). The plant material was frozen until essential oil isolation. The essential oil was submitted to hydrodistillation for 2 h using Clevenger-type apparatus. Part of the oil was dissolved in dichloromethane and analysed using GC/FID and GC/MS, and the other part was used in assessing antioxidant activity of the oil.

The GC-FID and GC/MS analyses were performed with an Agilent 7890A apparatus equipped with an auto-injection system (Agilent 7683B Series), an inert 5975C XL EI/ CI mass-selective detector (MSD) and a flame ionization detector (FID) connected by a capillary flow technology 2-way splitter with make-up, and an HP-5 MS capillary column (30 m/0.25 mm i.e., film thickness

0.25 mm). The oven temperature was programmed to increase linearly from 60 to 300 ° at the rate of 3 °/min and then held isothermal at 300 ° for 10 min; injector temperature – 250 °; detector temperature – 300 °; source temperature – 230 °; quadrupole temperature – 150 °; carrier gas – He (16.255 psi, constant pressure mode). Samples (1 ml) were injected in splitless mode. Electron-impact mass spectra (EI-MS; 70 eV) were acquired over the m/z range 30–550. The solvent delay was 3 min.

DPPH assay was used to assess antioxidant potential of leaf essential oil according to the method of Brand-Williams et al. (1995) with few modifications. The working solution of DDPH (0.04 mg/ml) was prepared by dissolving 4 mg of DPPH in 100 ml of methanol and used promptly. Different concentrations (0.2, 0.3, 0.4, 0.5 w/v) of essential oil were tested in triplicate, and the absorbance of the essential oil and DPPH working solution was measured after 45 minutes. The obtained measures were used to calculate IC_{50} values. Synthetic antioxidants were used as positive control (BHT, L-ascorbic acid).

RESULTS AND DISCUSSION

Essential oil yield and composition. A total of 78 compounds were detected and identified in essential oils. Both oils are characterized by high abundance of monoterpenes, which is characteristic of species belonging to the section *Juniperus* (Rajčević *et al*., 2013). The main constituents of *J. communis* var. *communis* essential oil were sabinene (39.4%), α-pinene (13.3%), myrcene (4.7%) and terpinen-4-ol (3.7%), while *J. communis* var. *saxatilis* essential oil had α-pinene (34.9%), sabinene (20.3%), δ-3-carene (6.4%) and germacrene B (6.3%) as the most abundant compounds. Two varieties also differed in the yield of the essential oil – *J. communis* var. *saxatilis* had twice the amount of essential oil than typical variety. Essential oil composition and yield are given in Table 2.

Table 2. Terpene composition of the needle essential oils of two varieties of *Juniperus communis*

		J. communis	
No.	Compound[a)]	*var. communis*	*var. saxatilis*
1	Hexanal	0.1	0.3
2	[2E] Hexenal	0.5	1.1
3	*α*-Thujene	2.7	1.3
4	*α*-Pinene	13.3	34.9
5	Fenchene	tr	0.3
6	Camphene	0.1	0.2
7	Sabinene	39.4	20.3
8	*β*-Pinene	1.2	2.2
9	Myrcene	4.7	3.2

10	δ-2-Carene	tr	0.3
11	*α*-Phelandrene	0.2	0.4
12	*δ*-3-Carene	tr	6.4
13	*α*-Terpinene	1.0	0.4
14	Cymene	0.3	0.2
15	Limonene	3.2	4.0
16	[E]-*β*-Ocimene	0.5	tr
17	*γ*-Terpinene	2.1	0.8
18	*cis*-Sabinene hydrate	0.4	0.3
19	Terpinolene	2.4	1.7
20	*trans*-Sabinene hydrate	-	0.1
21	Linalool	0.4	0.2
22	*cis*-*p*-Menth-2-en-1-ol	0.2	tr
23	*trans*-Pinocarveol	0.2	-
24	Terpinen-4-ol	3.7	1.6
25	*p*-Cymen-8-ol	tr	tr
26	*α*-Terpineol	0.2	0.3
27	Piperitone	tr	0.2
28	Methyl citronellate	-	0.2
29	Bornyl acetate	0.2	0.3
30	3-Thujanol acetate	0.1	tr
31	Myrtenyl acetate	0.2	0.1
32	*α*-Cubebene	1.0	-
33	*β*-Elemene	0.2	0.1
34	[E]-Caryophyllene	0.4	0.2
35	*α*-Humulene	0.4	0.3
36	*cis*-Cadina-1(6),4-diene	tr	-
37	*trans*-Cadina-1(6),4-diene	0.1	tr
38	*γ*-Muurolene	0.2	tr
39	Germacrene D	0.4	3.2
40	*β*-Selinene	0.1	tr
41	trans-Muurola-4(14),5-diene	0.2	tr
42	Bicyclogermacrene	0.3	0.6
43	*α*-Muurolene	0.6	tr
44	Germacrene A	0.8	0.6
45	*γ*-Cadinene	0.8	tr
46	*δ*-Cadinene	3.5	0.4
47	*α*-Cadinene	0.2	-
48	Elemol	tr	tr
49	Germacrene B	3.3	6.3
50	Germacrene D-4-ol	2.7	0.5
51	Spathulenol	-	0.1
52	Humulene epoxide II	0.1	-
53	1-*epi*-Cubenol	0.1	tr
54	τ cadinol	1.9	0.2

55	*α*-Muurolol (=torreyol)	0.3	tr
56	*α*-Cadinol	3.2	0.5
57	Germacra-4(15),5,10(14)-trien-1-*α*-ol	-	0.2
58	*ent*-Rosa-,15-diene	-	0.2
59	Abieta-8,12-diene	tr	0.1
60	Abietatriene	tr	0.4
61	Abietadiene	tr	2.2
62	Abieta-8(14),13(15)-diene	-	tr
63	Sandracopimarinal	tr	0.2
64	Abietal	0.2	tr
	Total monoterpenes	76.6	80.0
	Monoterpene hydrocarbons	71.3	76.6
	Monoterpenes oxygenated	5.3	3.4
	Total sesquiterpenes	20.9	13.6
	Sesquiterepene hydrocarbons	12.6	12.0
	Sesquiterpenes oxygenated	8.3	1.6
	Total diterpenes	0.4	3.2
	Diterpenes hydrocarbons	0.2	3.1
	Diterpenes oxygenated	0.2	0.1
	Other[b)]	0.6	1.4
	TOTAL	98.5	98.2

[a)] Contents are given as percentages of the total essential oil composition; tr: trace (0.05<tr< 0.10%); –: not detected; compounds with contents <0.05% are not listed. [b)] Others: Aliphatic hydrocarbons, aliphatic aldehydes and alcohols, aliphatic acids, their esters and aldehydes, aromatic esters with aliphatic acids, alkyl-aromatic alcohols, and aryl esters of aromatic acids.

In the past decade, there have been a lot of papers published on the composition of needle essential oil of both varieties across Eurasia. For the typical variety, in most populations, essential oil was dominated by either sabinene or α-pinene, while the essential oil composition of *J. communis* var. *saxatilis* showed higher variability – with sabinene, α-pinene and limonene as the most abundant components (Chatzopoulou and Katsiotis 1993; Caramiello *et al.*, 1995; Ochocka *et al.*, 1997; Adams 1998; Angioni *et al.*, 2003; Gonny *et al.*, 2006; Ottavioli *et al.*, 2009; Markó *et al.*, 2011; Cabral *et al.*, 2012). Furthermore, the results obtained in this study also agree with the results obtained by Milojević *et al.* (2010), also in Kopaonik Mt.

Antioxidant activity. Essential oil showed moderate to high antioxidant activity in comparison to positive controls (Table 3). Essential oil of *J. communis* var. *saxatilis* showed much higher activity than essential oil of the typical variety. Furthermore, the data obtained in this study showed somewhat similar results when compared with available literature data on juniper essential oil antioxidant activity. *J. oxycedrus* and *J. pheonicea* essential oil from Morocco showed the highest activity (0.020 and 0.025 mg/ml, respectively),

while essential oil of *J. phoenicea* from Tunisia had the lowest activity (5.364 mg/ml). The essential oil composition also varied across the samples. Emami *et al*. (2007) studied also the antioxidative activity of several isolated monoterpenes. At a concentration of 4µl/ml, α-pinene showed no activity, sabinene showed low (4.82%), while γ-terpinene had the highest antioxidative activity (17.8%). Since direct comparison with literature data is almost impossible due to very large differences in representation of data and results obtained for positive controls (L-ascorbic acid, quercetin, BHT), IC_{50} value of essential oils was calculated in respect to IC_{50} value of the used positive control (Table 3). When comparing these values, essential oil showed more or less the same activity, varying from 3 to 11% of positive control activity.

Table 3. Antioxidant activity of *Juniperus* essential oil

	Locality	Three most abundant compounds (%)	IC_{50} (mg/ml)	Reference	Rel. act.[b)]
J. communis var. *communis*	Serbia	sabinene (39.4), α-pinene (13.3), myrcene (4.7)	0.660	present study	5
J. communis var. *saxatilis*	Serbia	α-pinene (34.9), sabinene (20.3), δ-3-carene (6.4)	0.320	present study	11
J. communis var. *hemisphaerica*	Iran	sabinene (21.9), α-pinene (12.1), terpinen-4-ol (8.5)	0.009[a)]	Emami *et al.* 2007	9
		sabinene (20.3), α-pinene (15.8), limonene (9.2)	0.020 [a)]	Emami *et al.* 2007	4
J. oblonga	Iran	sabinene (16.4), α-pinene (15.6), germacrene D (8.2)	0.024 [a)]	Emami *et al.* 2007	3
		sabinene (19.5), α-pinene (13.6), [E]-Caryophyllene (6.4)	0.027 [a)]	Emami *et al.* 2007	3
J. excelsa	Iran	α-pinene (67.7), α-cedral (11.5), δ-3-carene (5.2),	0.189	Moein *et al.* 2010	11
J. phoenicea	Tunisia	α-pinene (55.7), δ-3-carene (10.7), gamma-cadinene (2.9)	5.365	Ennajar *et al.* 2009	<1
J. oxycedrus	Morocco	ß-phellandrene (36.8), α-terpinolene (13.2), myrcene (9.1)	0.020	Riahi *et al.* 2013	110
J. phoenicea	Morocco	ß-phellandrene (47.1), α-terpinolene (10.5), myrcene (7.9)	0.025	Riahi *et al.* 2013	100
BHT			0.136	Present study	
L-ascorbic acid			0.034		

[a)] values are given in ml/ml. [b)] Relative activity of essential oil given as percentage, calculated as IC_{50} (positive control)/IC_{50} (sample), values are given as percentage

CONCLUSION

Antioxidant activity of *J. communis* var. *communis* and var. *saxatilis* was similar with results obtained from other junipers throughout the Mediterranean region. Although antioxidant activity is weaker than used standards, it is still significant, especially in the light of potentially harmful effects of artificial antioxidants. Natural compounds with anti-oxidative potential are becoming more and more important, especially in food, cosmetic and drug industries.

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САСТАВ И АНТИОКСИДАТИВНА АКТИВНОСТ ЕТАРСКОГ УЉА ДВА ВАРИЈЕТЕТА *Juniperus communis* L. ИЗ СРБИЈЕ

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САЖЕТАК: Род *Juniperus* L. (Cupressaceae) броји око 67 врста и 34 варијетета. *Juniperus communis* L. настањује ксеротермна отворена станишта, на сувим брдима и планинама широм северне хемисфере. Типски варијетет – *J. communis* L. var. *communis* је сакупљен у Делиблатској пешчари, док је var. *saxatilis* Pall. сакупљен на планини Копаоник. Етарско уље из иглица је добијено коришћењем апаратуре по Клевенџеру и анализирано коришћењем GC/MS и GC-FID. Антиоксидативна активност етарског уља је одређена коришћењем DPPH теста. У укупном уљу, детектовано је и идентификовано 78 једињења. Оба уља карактеришу се високим уделом монотерпена. Главне компоненте етарског уља *J. communis* var. *communis* су сабинен (39,4%), α-пинен (13,3%), мирцен (4,7%) и терпинен-4-ол (3,7%), док у етарском уљу *J. communis* var. *saxatilis* доминирају α-пинен (34,9%), сабинен (20,3%), δ-3-карен (6,4%) и гермакрен Б (6,3%). Резултати DPPH теста су показали IC_{50} вредност од 0,66 mg/ml за етарско уље *J. communis* var. *communis* односно 0,32 mg/ml за уље *J. communis* var. *saxatilis*. Утврђена антиоксидативна активност је нижа од употребљене позитивне контроле али је ипак значајна.

КЉУЧНЕ РЕЧИ: антиоксидативна активност, састав етарског уља, *Juniperus*, Србија

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THE THIRD CENTURY OF BOTANY IN VOJVODINA A VIEW OF THE PAST – A GLIMPSE INTO THE FUTURE

The Botanical Society "Andreas Wolny" from Novi Sad and the Matica Srpska, with the help of the Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad and the Institute for Nature Conservation of Vojvodina Province organized a Symposium THE THIRD CENTURY OF BOTANY IN VOJVODINA. The Symposium was held on April 15, 2016 in the representative premises of the Matica Srpska. More than 90 registered participants participated in this Symposium, thus fulfilling the wish of the organizers to emphasize the tradition of botanical science in plain areas of Vojvodina and institutional engagement in research of botany, as well as to preserve the memory of previous researchers, their scientific works and contribution to the development of botany in this region. This Symposium was held to commemorate the year 1983 when in the same premises was held a major scientific conference titled MAN AND PLANT. In 1986, the proceedings of this conference were published, which was the basis for the celebration of the thirtieth anniversary of the mentioned conference. It is no wonder that such a jubilee was organized by institutions like the Matica Srpska, a guardian of literacy, science, culture and art in this region, and the Botanical Society "Andreas Wolny" which, as one of its activities, defined the preservation of the tradition of botanical research, revitalization of old herbarium collections and monographic publications on the flora.

The Symposium was attended by 60 registered participants. The participants and the idea of the symposium were welcomed by Prof. Dr. Dragan Stanić, the Matica Srpska president, and academician Rudolf Kastori, the Secretary General of the Department of Natural Sciences of Matica Srpska. On behalf of the organizers, the participants were greeted by Prof. Dr. Ružica Igić, president of the Botanical Society "Andreas Wolny", Prof. Dr. Goran Anačkov, Director of the Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, and Dr. Biljana Panjković, Director of the Institute for Nature Conservation of Vojvodina Province. In the spirit of extraordinary collegiality and academicism, and caught up in the mood of this important jubilee, the participants presented the results of their research in the form of 17 oral and 20 poster presentations. This was followed by the presentations on the history of botanical research in Vojvodina, as a kind of remembrance of distinguished flora and vegetation experts, researchers in the field of plant anatomy. Also, the historical connection between botany and medicine was given through a retrospective of the life and work of Srem botanist physicians. However, botany in Vojvodina is not only a reminder of the past, distinguished names and well-known publications. Today, it is reflected in the work of a new generation of botanists, young researchers, who presented the results of their own researches and researches conducted within active teams.

Symposium THE THIRD CENTURY OF BOTANY IN VOJVODINA in Vojvodina was primarily oriented towards young researchers, whose oral and poster presentations confirmed the continuity of intensive work on botanical research in this region.

Many colleagues from other institutions of the University of Novi Sad, University of Belgrade, as well as guests from Hungary and Belarus took part in the Symposium. In a good mood, surrounded by colorfulness so characteristic of botany itself, and in the spirit of collegiality, participants exchanged experiences, information and made plans for the future. The Symposium ended with the idea to become regular with the established dynamics.

Organizing this Symposium, the Botanical Society "Andreas Wolny" and the Matica Srpska gathered without doubt some of the leading names in contemporary Serbian botany. However, this Symposium would not have been organized so well if, aided by co-organizers, there were no dedicated volunteers, members of the Society and the institution like the Matica Srpska. On behalf of the Organizing and Program Committee of the Symposium, organizers and co-organizers give the warmest thanks to all participants and activists involved in this project. We owe our deepest gratitude also to the Institute for Nature Conservation of Vojvodina Province, which, in addition to organizing the event, supported the publication of the second volume of *Matica Srpska Journal for Natural Sciences* in which the presentations were published.

Prof. Dr. Goran Anačkov,
a member of the Program Committee of the Symposium
"The Third Century of Botany in Vojvodina"

ТРЕЋИ ВЕК БОТАНИКЕ У ВОЈВОДИНИ
ОСВРТ НА ПРОШЛОСТ – ПОГЛЕД У БУДУЋНОСТ

Ботаничко друштво „Андреас Волни“ из Новог Сада и Матица српска, уз помоћ Департмана за биологију и екологију Природно-математичког факултета Универзитета у Новом Саду и Покрајинског завода за заштиту природе Војводине организовали су Симпозијум ТРЕЋИ ВЕК БОТАНИКЕ У ВОЈВОДИНИ. Симпозијум је одржан 15. априла 2016. године у репрезентативним просторијама Матице српске. У жељи да истакну традицију ботаничке науке на простору војвођанске равнице, институционално бављење истраживањима у ботаници, као и очувању успомене на претходне истраживаче, њихов научни опус и допринос развоју ботанике на овим просторима, организатори су окупили више од 90 учесника – пријављених за учешће на овом скупу. Сâм Симпозијум одржан је као сећање на 1983. годину када се у истим просторијама одржао велики научни скуп под називом ЧОВЕК И БИЉКА. Године 1986. публикован је зборник радова са овог скупа, што је представљало основу за прославу тридесетогодишњице поменутог скупа. И није чудно што су се у обележавање оваквог јубилеја у организацији окупили Институција која је чувар писмености, науке, културе и уметности на овим просторима – Матица српска и Ботаничко друштво „Андреас Волни“ које је за једну од тачака својих активности дефинисало и очување традиције ботаничких истраживања, ревитализацију старих хербаријумских колекција и монографских приказа флоре.

Учешће на Симпозијуму узело је 60 регистрованих учесника. Скуп и идеју његовог одржавања поздравили су председник Матице српске, проф. др Драган Станић и секретар Одбора Одељења за природне науке Матице српске академик Рудолф Кастори. Скупу су се испред организатора обратили и проф. др Ружица Игић, председница Ботаничког друштва „Андреас Волни“, проф. др Горан Аначков, директор Департмана за биологију и екологију ПМФ Универзитета у Новом Саду, и директор Покрајинског завода за заштиту природе Војводине др Биљана Пањковић. У духу изузетне колегијалности и академизма, а понесени расположењем једног вредног јубилеја, учесници су резултате својих истраживања презентовали у облику 17 усмених и 20 постерских презентација. Уследили су ревијални прилози историје истраживања ботаничког профила на подручју Војводине, подсећање на врсне познаваоце флоре и вегетације, истраживаче из области анатомије биљака као и историјску повезаност ботанике и медицине кроз ретроспективу живота и рада сремских лекара – ботаничара. Међутим, ботаника у Војводини није само подсећање на прошлост, славна имена и познате публикације. Она је данас осликана у раду нове генерације ботаничара, младих истраживача, који су приказали резултате сопствених истраживања и истраживања

која се спроводе у активним тимовима. Симпозијум ТРЕЋИ ВЕК БОТАНИКЕ У ВОЈВОДИНИ првенствено је био окренут ка младим истраживачима, чија су усмена саопштења и постерске презентације потврдиле континуитет интензивног рада на ботаничким истраживањима ових простора.

У раду Симпозијума, учешће су узеле и многе колеге са других институција Универзитета у Новом Саду, Универзитета у Београду, као и гости из Мађарске и Белорусије. У добром расположењу, окружени шаренилом које ботаника сама по себи нуди и у духу колегијалности, размењивана су искуства, информације и правили се планови за будућност. Симпозијум је завршен са идејом да постане традиционалан и да се успостави динамика одржавања сусрета.

Заједничким снагама, Ботаничко друштво „Андреас Волни“ и Матица српска, окупили су засигурно нека од водећих имена савремене српске ботанике организујући овај Симпозијум. Међутим, сâм скуп не би био толико добро организован да, потпомогнутих суорганизаторима, није било пожртвованог рада волонтера, чланова Друштва и институције каква је Матица српска. У име Организационог и Програмског одбора овог Симпозијума организатори и суорганизатори најсрдачније се захваљују свим учесницима и ангажованим активистима у реализацији овог пројекта. Велику захвалност, поред организовања скупа, дугујемо и Покрајинском заводу за заштиту природе Војводине који је потпомогао и издавање друге свеске *Зборника Матице српске за природне науке* у којем су радови објављени.

Проф. др Горан Аначков
члан Научног одбора Симпозијума
„Трећи век ботанике у Војводини“

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Editor-in-Chief

IVANA MAKSIMOVIĆ, Ph.D.

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