Lekovito bilje i fitoterapija

Medicinal Plants and Phytotherapy

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INVESTIGATION OF ANTIBACTERIAL POTENTIAL OF THE PHENOLICS DERIVED FROM *CISTUS INCANUS* L. BY MEANS OF TLC-DB

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Thin-layer chromatography (TLC) combined with direct bioautography (DB) (i.e.,TLC-DB) is a quick and effective method for preliminary assessment of biological activity of the medicinal plant extracts. *Cistusincanus* L. (the *Cistaceae* family) is popular in eastern parts of the Mediterranean basin and in the Middle East, and it has been known for centuries as an important medicinal herb. Antimicrobial activity of the *C. incanus* extractsis attributed mainly to the phenolics contained in this plant. The main objective of this study was to obtain the TLC fingerprint of fraction I (supposed to contain flavonoid aglycons alone) derived by selective multi-step extraction of the phenolics from the plant sample, and then to screen it by means of TLC-DB in the search for the fingerprint zones with antibacterial activity.

The main analytical technique: thin layer chromatography hyphenated with direct bioautography (TLC-DB). The supplementary technique: HPLC-DAD-ESI-MS.

The antibacterially active zones were found by means of TLC-DB and proved inhibiting both, the Gram positive *B. subtilis* and the Gram negative *A. fischeri* bacterial strains. Then an attempt was undertaken to identify at least some phenolics from active zones and it was revealed that fraction I contained not only flavonoid aglycons, but surprisingly enough flavonoid glycosides as well. Among the identified antibacterial components, apigenin, kaempferide andacylated kaempferol glycosides were found.

Once again, thin-layer chromatography with direct bioautography (TLC-DB) has proved efficient in screening extracts derived from medicinal plants in the search for natural antibiotics of botanical origin.

HEMIJSKI SASTAV I ANTIOKSIDACIONA AKTIVNOST EKSTRAKATA IVANJSKOG CVEĆA, *GALIUM VERUM* L.

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Ivanjsko cveće (*Galium verum*) je višegodišnja zeljasta biljka iz roda *Galium*, poznata od davnina po terapijskom dejstvu. Međutim njen hemijski sastav, kao i biološka aktivnost nisu u potpunosti razjašnjeni. Stoga je cilj našeg istraživanja bio da se utvrdi ukupan sadržaj fenola i flavonoida acetonskog i vodenog ekstrakta ivanjskog cveća, kao i procena njihove antioksidacione aktivnosti.

Acetonski i vodeni ekstrakti su zasebno pripremljeni ekstrakcijom nadzemnog dela (150 g) biljke sa 500 ml odgovarajućeg rastvarača ekstrakcijom pod refluksom. Ukupan sadržaj fenola određen je metodom *Folin-Ciocalteu* i izražen kao mg ekvivalenta galne kiseline po gramu ekstrakta (mg GAE /g ekstrakta). Procenjen je i ukupan sadržaj flavonoida, a rezultat je izražen kao mg ekvivalenta kvercetina na gram ekstrakta (mg kvercetina/g ekstrakta). *In vitro* antioksidaciona aktivnost je testirana spektrofotometrijski pomoću DPPH (1,1-difenil-2-pikrilhidrazil) testa. Sposobnost neutralisanja DPPH radikala je izražena kao IC50 vrednost.

Naši rezultati su u skladu sa rezultatima drugih studija i pokazali su da je da ukupan sadržaj fenola acetonskog ekstrakta ivanjskog cveća iznosio 101,58 ± 7,96 mg GAE/g ekstrakta. Ova vrednost je niža od vrednosti za vodeni ekstrakt, čiji je sadržaj iznosio127,57 ± 11,25 mg GAE/g ekstrakta. Ukupan sadržaj flavonoida bio je 30,51 ±3,37 i 61,24 ± 6,11 mg kvercetina/g acetonskog i vodenog ekstrakta, redom. Pored toga, ispitivani ekstrakti su smanjili DPPH uz IC50 11,65 ± 1,16 μg/ml za acetonski i 9,63 ± 0,32 μg/ml za vodeni ekstrakt.

Naši rezultati su pokazali da oba ekstrakta ivanjskog cveća predstavljaju vredan izvor fenola i flavonoida i ukazali na racionalnu osnovu za tradicionalnu upotrebu ove biljne vrste u ublažavanju oksidativnog stresa. Ova studija može biti polazna osnova za dalja istraživanja koja bi u potpunosti ispitala hemijski sastav, kao i farmakološke efekte ivanjskog cveća.

CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF *GALIUM VERUM* L. EXTRACTS

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Galium verum L. is a perennial herbaceous plant belonging to the genus *Galium*, renowned for its therapeutic potential since ancient times. However, its chemical composition as well as biological activities haven't been fully clarified. Therefore, the aim of our study was to determine the total content of phenols and flavonoids of acetone and aqueous extracts of *G. verum* as well as to estimate their antioxidant activity.

Aqueous and acetone extracts were prepared separately extracting aerial part (150 g) with 500 ml of appropriate solvent by heat reflux extraction. Total phenolic content was determined by Folin-Ciocalteu's method and expressed as mg gallic acid equivalents per gram of extract (mg GAE/g extract). The total flavonoid content was estimated as well and result was expressed as mg of quercetin equivalent per gram of extract (mg of quercetin/g of extract). *In vitro* antioxidant activity was evaluated spectrophotometrically by DPPH (1,1-diphenyl-2-picrylhydrazyl) assay. The DPPH scavenging ability was expressed as IC50 value.

Our results are in accordance with the results of other studies, and illustrated that total phenol content of acetone extract of aerial part of *G. verum* was 101.58 ± 7.96 mg GAE/g extract. This value is lower when compared to aqueous extract, whose content was 127.57± 11.25 mg GAE/g extract. Total flavonoid content was 30.51 ± 3.37 and 61.24 ± 6.11 mg of quercetin/g of acetone and aqueous extracts, respectively. Furthermore examined extracts reduced DPPH with an IC_{50} 11.65 ± 1.16 μg/ml of acetone and 9.63 ± 0.32 μg/ml of aqueous extract.

These finding demonstrated that both extracts of *G. verum* respresents a valuable source of phenols and flavonoids and indicated the rational basis for the traditional uses of this plant species in alleviation of oxidative stress. This study may be a starting point for further researches which would fully examine their chemical composition as well as pharmacological effects.

PHYTOCHEMICAL STUDY OF THE GREEK ENDEMIC SPECIES *INULA SUBFLOCCOSA* RECH. F. (ASTERACEAE)

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The genus *Inula* L. (Asteraceae) comprises *c.* 90 species, about 19 of which are native to Europe. They are perennial herbs, rarely shrubs, sometimes with an unpleasant smell. Several *Inula* species are used as traditional herbal medicines to treat a broad spectrum of disorders, mainly respiratory, digestive, inflammatory, dermatological, as well as microbial infections. Sesquiterpenes, mostly sesquiterpene lactones including eudesmane, guaiane, pseudoguaiane and germacrane derivatives are characteristic components of *Inula* species, many of which have exhibited a wide range of biological activities, particularly anti-tumor and anti-inflammatory. The chemical profile of *Inula subfloccosa* Rech. f., an endemic plant of Greece that is restricted to cipolin and marbles, has not been previously studied.

Aerial parts of *I. subfloccosa* were collected during the flowering period. The air-dried plant material was exhaustively extracted with CH_2Cl_2/MeOH at room temperature and the resulting crude extract was fractionated with a series of chromatographic separations.

So far, 9 sesquiterpene lactones have been isolated from the aerial parts of *I. subfloccosa*. Detailed analyses of 1D and 2D NMR and MS data led to the identification of four eudesmanolides, four guaianolides, and one pseudoguaianolide.

Among the isolated compounds, the bioactive metabolites 4$^{\alpha}$,5α-epoxy-10α,14*H*-1-*epi*- inuviscolide and isoalantolactone were the major constituents, whereas the pseudoguainolide derivative is a new natural product.

VOLATILE CONSTITUENTS OF DIFFERENT PLANT PARTS OF *JUNIPERUS OXYCEDRUS* SSP. *MACROCARPA* (SIBTH. & SM.) BALL. FROM GREECE

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The family Cupressaceae is represented in Europe by five genera namely, *Cupressus, Chamaecyparis*, *Thuja*, *Tetraclinis* and *Juniperus*. Their representatives are monoecious or dioecious resiniferous trees or shrubs. *Juniperus* berries have traditionally been used for dyspepsia, acute and chronic cystitis, arteriosclerosis, gout and inflammations, while essential oils of *Juniperus* species are used as fragrance ingredients in cosmetics. The aim of the present study was the analysis of essential oils obtained from different plant parts of *Juniperus oxycedrus* ssp. *Macrocarpa* (Sibth. & Sm.) Ball.

Aerial parts of *J. oxycedrus* ssp. *macrocarpa* were collected from the Greek islands Chryssi and Elafonissos. After hydrodistillation of the berry-like fruits, leaves and branches, the essential oils were obtained, respectively. The chemical composition of each essential oil was determined by gas chromatography (GC-FID, GC-MS).

A total of 87 components were identified in the essential oil of berry-like fruits, representing 91.7% of the total oil composition. The major compound was *α*-pinene (30.5%), followed by *α*-cedrol (10.4%), myrcene (10.1%) and germacrene D (9.7%). In the essential oil of leaves 113 constituents were identified, representing 97.3% of the total oil composition. The main constituent was *α*-pinene (28.2%), followed by manool oxide (12.5%) and *α*-cedrol (9.8%). Finally, 107 compounds were identified in the essential oil of the branches, representing 92.5% of the total oil composition. The major compound was manool oxide (20.5%), followed by *α*-cedrol (14.4%) and *α*-pinene (11.2%). Comparison of our results to the literature data showed mainly quantitative differences.

ANATOMSKA GRAĐA I HEMIJSKA ANALIZA ETARSKOG ULJA *PIMPINELLA SAXIFRAGA* L. (APIACEAE)

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Koren vrste *Pimpinella saxifraga* L. se tradicionalno koristi kao ekspektorans, bronhosekretolitik i antiflogistik. Cilj ovog rada je ispitivanje anatomske građe, sadržaja i sastava etarskog ulja vegetativnih organa i ploda *P. saxifraga*. Biljni materijal je sakupljan u jugoistočnoj Srbiji: Bojanine vode (Suva planina) i Ostrovica (Sićevačka klisura). Anatomska analiza izvršena je na trajnim preparatima dobijenim standardnom metodom pripreme za posmatranje pod svetlosnim mikroskopom. Etarska ulja su izolovana iz herbe u cvetu, ploda, korena u fazi cvetanja i plodonošenja, destilacijom vodenom parom i analizirana GC-FID i GC-MS metodama.

Rezultati anatomske analize su pokazali da je koren sekundarne građe a stablo primarne sa zatvorenim kolateralnim sprovodnim snopićima. Listovi su dorziventralni, hipoamfistomatski, lisna drška je sa lučno raspređenim sprovodnim snopićima, a plodovi (merikarpi) su polukružnog oblika sa slabo izraženim rebrima, bez trihoma. Retke, nežlezdane, višećelijske, uniserijatne trihome sa oštrim vrhom su uočene na stablu, listovima i lisnoj dršci. Sekretorni kanali su brojni u svim organima.

Najviši sadržaj etarskog ulja određen je u plodu (1,48-1,52%), dok su herba (0,13-0,21%) i koren (0,50-0,53%) sadržali manju količinu ulja (*v/m*). Etarska ulja istih organa sa različitih lokaliteta su sličnog kvalitativnog sastava sa manjim kvantitativnim razlikama. U uljima herbe i ploda dominantna jedinjenja su β-bisabolen (28,8-76,0%) i epoksi-pseudoizoeugenil-2-metilbutirat (7,7-21,8 %). Najzastupljenija jedinjenja u uljima korena, u obe faze, su azuleni (31,4-35,7%) i pregeijeren (13,7-18,8%). U ispitivanim etarskim uljima različitih organa *P. saxifraga* utvrđeno je prisustvo trinorseskviterpena (azulena i geijerena) i fenilpropanoidnih jedinjenja pseudoizoeugenil tipa, koja su karakteristični hemijski markeri za etarska ulja biljaka ovog roda.

Istraživanje je podržalo Ministarstvo prosvete, nauke i tehnološkog razvoja (Projekat ON 173021).

ANATOMY AND CHEMICAL ANALYSIS OF ESSENTIAL OIL OF *PIMPINELLA SAXIFRAGA* L. (APIACEAE)

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Root of *Pimpinella saxifraga* L. is traditionally used as expectorant, bronchosecretolytic and antiphlogistic. The aim was investigation of anatomy, content and composition of the essential oils of vegetative organs and fruits. Plant material was collected in South-Eastern Serbia: Bojanine vode (Mt. Suva planina) and Ostrovica (Sićevo gorge). The anatomical analysis was conducted on permanent slides obtained by standard method of preparation. The essential oils obtained by hydrodistillation from flowering aerial parts, fruits and roots (from flowering and fruiting period) were analysed by GC-FID and GC-MS.

The anatomical analysis revealed a secondary structure of root and primary structure of stem with closed collateral vascular bundles. The leaves are dorsiventral, hypoamphistomatic and petiole is with arched vascular bundles. The fruit (mericarp) is semi-circular and lightly ribbed in cross section without trichomes. Rare, non-glandular, multicellular, uniseriate, cuneate trihomes were noted on the stem, leaves and petiole. Secretory channels are numerous in all organs.

The highest content of essential oil was determined in the fruits (1.48-1.52%), while aerial parts (0.13-0.21%) and roots (0.50-0.53%) contained a lower amount of oil (*v/w*). The oils from the same organs originated from different localities were similar in qualitative composition with some quantitative differences. The most dominant constituents in the oils from aerial parts and fruits were β-bisabolene (28.8-76.0%) and epoxy-pseudoisoeugenyl-2-methylbutyrate (7.7-21.8 %), whereas those of the oils from roots, in both stages, were azulenes (31.4-35,7%) and pregeijerene (13.7-18.8%). Trinorsesquiterpenes (azulenes and geijerenes) and phenylpropanoid compounds of the pseudoisoegenyl type, which are characteristic chemical markers of the essential oils of the plants of this genus, were present in the all analyzed oils.

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ANTIMIKROBNA AKTIVNOST SOKA PLODA ARONIJE (*ARONIA MELANOCARPA* (MICHX.) ELLIOTT)

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Neosporna je činjenica da se otpornost mikroorganizama na poznate antimikrobne lekove povećava. Stoga su različiti biljni proizvodi i njihovi aktivni sastojci u fokusu istraživanja kao potencijalni prirodni antimikrobni agensi. Plodovi biljne vrste *Aronia melanocarpa* (Michx.) Elliott (aronija) predstavljaju bogat izvor farmakološki aktivnih jedinjenja kao što su polifenoli, što se povezuje sa antimikrobnom aktivnošću koju ispoljavaju. Cilj rada bio je utvrđivanje antimikrobne aktivnosti soka aronije.

Plodovi aronije sakupljeni su sa plantaža na planini Suvobor u Srbiji. Sveže bobice su zdrobljene i isceđene i dobijeni sok je filtriran. Antimikrobna aktivnost soka aronije procenjena je korišćenjem laboratorijskih kontrolnih sojeva mikroorganizama. Određivanje antimikrobne i antifungalne aktivnosti (minimalna inhibitorna koncentracija [MIC] i minimalna baktericidna/fungicidna koncentracija [MBC/MFC]) dobijena je mikrodilucionom metodom prema NCCLS (2003). Minimalna koncentracija u kojoj nije bilo vidljivog rasta definisana je kao minimalna inhibitorna koncentracija (MIC). Minimalna baktericidna/fungicidna koncentracija (MBC/MFC) je definisana kao najniža koncentracija uzorka koja je ubila 99,9% ćelija mikroorganizama. Kontrole su uključivale: medijum sa rastvaračem (negativnom kontrolom) i medijum sa hloramfenikolom, streptomicinom i nistatinom (pozitivna kontrola).

Rezultati pokazuju da je sok aronije ispoljio antibakterijsku aktivnost protiv Gram-pozitivnih bakterija *Listeria mono*cytogenes, *Bacillus cereus* i *Staphylococcus aureus*, kao i Gram-negativnih bakterija *Salmonella enteritidis* i *Escherichia coli*. Sok nije imao uticaja na *Pseudomonas aeruginosa* i sojeve gljiva (*Candida albicans* i *Aspergillus niger*). Minimalne inhibitorne i mikrobicidne koncentracije variraju od MIC/MBC = 12,5 do 100 mg/mL. Podaci iz literature potvrđuju antibakterijsku aktivnost plodova aronije protiv *Escherichia coli*, *Bacillus cereus*, *Staphylococcus aureus* i *Pseudomonas aeruginosa*. Sok aronije može biti koristan u prevenciji i lečenju bolesti izazvanih bakterijskim infekcijama.

ANTIMICROBIAL ACTIVITY OF CHOKEBERRY JUICE (*ARONIA MELANOCARPA* (MICHX.) ELLIOTT)

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It is an indisputable fact that the microbial resistance to known antimicrobial drugs increases. Therefore, the various plant products and their active ingredients are in the focus of the research as a possible natural antimicrobial agent. The berries of *Aronia melanocarpa* (Michx.) Elliott (chokeberry) are the rich source of pharmacological active compounds such as polyphenols and that may indicate their antimicrobial activity. The aim of the research was to evaluate the antimicrobial activity of the chokeberry juice.

Fruits of black chokeberry were collected from a plantation field on mountain Suvobor, Serbia. Fresh berries were crushed and squeezed and the juice was filtered. The antimicrobial activity of black chokeberry juice was assessed using laboratory control microorganism strains. The determination of antimicrobial and antifungal activity (the minimum inhibitory concentration [MIC] and minimum bactericidal/fungicidal concentration [MBC/MFC]) was carried out by microwell dilution method according to the NCCLS (2003). The minimal concentration where there was no visible growth was defined as the minimal inhibitory concentration (MIC). The minimal bactericidal/fungicidal concentration (MBC/MFC) was defined as the lowest concentration of the sample that had killed 99.9% of microorganism cells. The controls included: medium with solvent (negative) and medium with chloramphenicol, streptomycin and nystatin (positive).

Results showed that chokeberry juice exhibited antibacterial activity against Gram-positive bacteria *Listeria monocytogenes*, *Bacillus cereus* and *Staphylococcus aureus*, as well as Gram–negative bacteria *Salmonella enteritidis* and *Escherichia coli*. The juice did not have influence on Pseudomonas aeruginosa and the fungal strains (*Candida albicans* and *Aspergillus niger*). The minimum inhibitory and microbicidal concentrations ranged from MIC/MBC = 12.5 to 100 mg/mL. Literature data showed antibacterial activity of chokeberries against *Escherichia coli*, *Bacillus cereus*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Chokeberry juice could be useful in the prevention and treatment of the disease induced by bacterial infections.

FITOHEMIJSKA ANALIZA I ODREĐIVANJE SADRŽAJA FLAVONOIDA U *ALLIUM URSINUM* L., ALLIACEAE

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Kako bi se odredio sadržaj flavonida i antioksidativna svojstva etanolnih i vodenih ekstrakata suhog i svježeg lista sa dva različita staništa srijemuša *Allium ursinum* L. u Bosni i Hercegovini, biljni uzorci su sakupljeni na dvije lokacije: Rogoj (1212 m nadmorske visine) i Neretvica (850 m nadmorske visine).

Esktrakti su pripremljeni od suhog i svježeg lista koristeći ekstrakciju na povišenoj temperaturi i ultrazvučnu ekstrakciju. Voda i 80% etanol su korišteni kao otapala. Korištene su $AlCl_3$ i DPPH metoda zaodređivanje antioksidativnog potencijala. Nađeno je da sadržaj flavonoida ovisi o vrsti ekstrakcije i korištenom otapalu. Za detekciju flavonoida korištena je TLC analiza. TLC analiza je potvrdila prisustvo kvercetina, rutina, hiperozida, izokvercetina i kempferola.

TLC analiza potvrdila je značajnije prisustvo kvercetina u etanolnim ekstraktima. Sadržaj ukupnih flavonida kretao se u rasponu 11,31-79,60 mg QE/g (suhog lista) i 0,023-32,054 mg QE/g (svježeg lista). Najveći sadržaj flavonoida pronađen je u etanolnom ekstraktu (suhog lista) 79,6 mgQE/g. Uzorak je prikupljen u Rogoju i pripremljen ultrazvučnom ekstrakcijom. Najmanji sadržaj flavonoida pronađen je u vodenom ekstraktu (svježeg lista) 0,023 mg QE/g. Uzorak je prikupljen u Neretvici i pripremljen ekstrakcijom na povišenoj temperaturi.

Etanolni ekstrakti suhog lista ispoljili su najveći postotak inhibicije DPPH (30,80%). Uzorak je prikupljen u Rogoju. Etanolni ekstrakt svježeg lista ispoljio je najmanji postotak inhibicije DPPH radikala (11,49 %). Uzorak je prikupljen u Rogoju.

PHYTOCHEMICAL ANALYSIS AND FLAVONOID CONTENT DETERMINATION IN *ALLIUM URSINUM* L., ALLIACEAE

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In order to estimate the amount of flavonoids and antioxidative properties of ethanolic and water extracts from dried and fresh leaves of two different ecotypes of wild garlic (*Allium ursinum* L.) in Bosna and Herzegovina plant samples were collected on two loctions Rogoj (altitude 1212 m) and Neretvica (altitude 850 m).

Extracts were prepared from dry and fresh leaves using extraction at elevated temperature and ultrasound extraction. Water and 80 % ethanol were used as solvents. $AlCl_3$ and DPPH radical scavenging assay were used. It was found that the type of extraction and solvent affect the value of flavonoids. For determination of flavonoids is used TLC. TLC analysis confirmed the presence of quercetin, rutin, hyperoside, isoquercetin, kaempherol.

The spot of quercetin was more intense in etanolic extracts. Total flavonoid content was in range 11.31-79.60 mg QE/g (dried leaves) and 0.023-32.054 mg QE/g (fresh leaves). The biggest flavonoid content was found in ethanolic extract (dry leaves) 79.6 mg QE/g. Sample was collected in Rogoj and prepared by ultrasound extraction. The smallest flavonoid content was found in water extract (fresh leaves) 0.023 mg QE/g. Sample was collected in Neretvica and prepared by extraction at elevated temperature. No matter which method of extraction was used, higher flavonoid content were in ethanolic extracts.

Ethanolic extract of dry leaves had the biggest percent of inhibition of DPPH radical (30.80%). Sample was collected in Rogoj. Ethanolic extract of fresh leaves had the smallest percent inhibiton of DPPH radical (11.49 %). Sample was collected in Rogoj.

ISPITIVANJE KVALITETA BILJNIH DROGA KOJE ULAZE U SASTAV MONOKOMPONENTNIH ČAJEVA

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Shodno propisima i metodama datim u važećoj Evropskoj farmakopeji u poglavlju Metode farmakognozijske analize, kao i pojedinim monografijama ispitivanih droga, predmet ove studije bio je ispitati kvalitet četiri biljne droga koje se koriste za izradu raznih čajnih smjesa, a mogu biti samostalne, kao monokomponentni čajevi, shodno propisima i metodama datim u Evropskoj farmakopeji u poglavlju Farmakognostička ispitivanja.

Ispitivanje je provedeno na četri biljne droge, nabavljene iz dva različita izvora, te na biljnom materijalu koje se može naći u slobodnoj prodaji pod identičnim imenom kao ranije spomenute biljne droge. Ispitivane biljne droge bile su: *Absinthii herba* (*Artemisia absinthium* L.), *Melissae folium (Melissa officinalis* L.), *Menthae piperitae folium (Mentha piperita* L.) i *Thymi herba (Thymus vulgaris* L.). Opće metode koje su korištene za analizu bile su: makroskopska analiza, mikroskopska analiza, određivanje sadržaja stranih supstanci, određivanje sadržaja vode i određivanje sadržaja pepela. Specijalne metode koje su korištene za analizu bile su: određivanje sadržaja eteričnih ulja, određivanje gorčine te tankoslojna hromatografija.

Ispitivane biljne droge, nabavljene u zvaničnoj prodaji, pokazale su se izuzetno kvalitetnim, s obzirom da su vrijednosti dobijene ispitivanjem parametara kvalitete zadovoljavale farmakopejom definirane kriterijume. Biljni materijal nabavljen u slobodnoj, nezvaničnoj prodaji pokazao je najviše propusta u pogledu ispitivanja kvalitete.

Dobijeni rezultati ukazuju na opravdanost i važnost farmakognozijske analize u pogledu kvalitete biljnih materijala koji se koriste u službenoj farmaciji i medicini.

QUALITY TESTING OF HERBAL DRUGS INCLUDED IN THE COMPOSITION OF MONOCOMPONENT TEAS

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The subject of this study was in accordance with the regulations and methods given by the currently valid European Pharmacopoeia in the chapter Pharmacognostic examinations, as well as certain monographs of investigated drugs: Examine the quality of the four types of drugs, which are in composition of various tea mixtures and may be self-contained as mono-constituent teas, according to the regulations and methods given by European Pharmacopoeia in the chapter Pharmacognosy.

The study was conducted on four herbal drugs from two different sources and on herbal material which can be obtained on a free market by the name equivalent to the previously mentioned herbal drugs. Tested drugs were: *Absinthii herba* (*Artemisia absinthium* L.), *Melissae folium* (*Melissa officinalis* L.), *Menthae piperitae folium* (*Mentha piperita* L.) and *Thymi herba* (*Thymus vulgaris* L.). General methods used for analysis were: macroscopical analysis, microscopical analysis, determination of foreign matters, determination of water content and determination of ash. Specific methods used were: determination of essential oils content, determination of bitterness and thin layer chromatography.

Herbal drugs from official sources shown a very good quality rate, since the obtained values by examination of quality parameters met pharmacopoeias defined criteria. Herbal material from free sale and non-official source, shown the lowest quality and was not in accordance with a most of Pharmacopoeial demands. Obtained results indicates the validity and importance of pharmacognostic analysis regarding the quality of herbal materials and drugs used in official pharmacy and medicine.

FITOHEMIJSKO ISPITIVANJE TRITERPENSKIH SAPONINA SA POTENCIJALNIM FARMAKOLOŠKIM DJELOVANJEM U VRSTAMA PORODICE LAMIACEAE

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Triterpenski derivati prisutni u biljkama su često spojevi nosioci farmakološkog djelovanja. Ova grupa obuhvata različite spojeve od kojih su najvažniji pentaciklični drivati oleana, ursana, lupana i tetraciklični damarani. Ispitivane biljne vrste su dobro poznate i koriste se jer sadrže eterično ulje, fenolne spojeve i druge metabolite ali ne zbog prisustva betulina, betulinske, oleanolne, ursolne kiseline i lupeola. Prisustvo ovih važnih spojeva bi moglo odrediti neke plejotropne farmakološke učinke ovih biljnih droga.

U ovom radu je provedena kvalitativna ii kvantitativna analiza triterpenskih spojeva u biljnim vrstama porodice Lamiaceae. Cilj studije bio pronaći najbolje uslove kvantitativne ekstrakcije ispitivanih triterpenskih supstanci što je postignuto sukcesivnom ekstrakcijom otapalima različite polarnosti. Identifikacija i kvantifikacija ciljnih spojeva u različitim frakcijama je postignuta primjenom metoda TLC i HPLC. Standardi triterpenskih supstanci (betulin, betulinska, oleanolna, ursolna kiselina i lupeol) i pojedine frakcije su nanošne koristeći CAMAG Linomat 5 aparat. Sistem za razvijanje (benzen: etilacetat: mravlja kisleina – 36:12:5) i uslovi HPLC metode (ConstaMetric®3000 sistem, Hypersil ODS (Agilent Technologies) kolona, 4.6 x 250 mm, 5µm, mobilna faza acetonitril/voda (700/300) acidifikovana sa orto-fosfornom kiselinom) su se pokazali optimalni za separaciju triterpenskih supstanci, koji su vrlo slične hemijske strukture.

Dobijeni rezultati potvrđuju prisustvo ovih važnih supstanci u biljnim vrstama koje pripadaju porodici Lamiaceae, sakupljene na području Bosne i Hercegovine. Najveći sadržaj betulina (4,19 µg/ml) i betulinske kiseline (51,58 µg/ml) pronađeni su u heksanskoj frakciji lista ruzmarina. Hloroformski ekstrakt cvijeta lavande je pokazao najveću koncentraciju ursolne kiseline (0,14 mg/ml). Oleanolna kiselina je bila prisutna samo u heksanskom ekstraktu lista ruzmarina (1,01 µg/ml). List ruzmarina predstavlja potencijalnu farmakognostičku drogu sa stanovišta prisustva triterpenskih saponina.

PHYTOCHEMICAL INVESTIGATION OF TRITERPENE SAPONINS WITH POTENTIAL PHARMACOLOGICAL ACTIVITY IN PLANT SPECIES BELONGING TO LAMIACEAE FAMILY

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Triterpene derivatives in plants are compounds that are frequently bearers of pharmacological activities. This group comprise different compounds, among which the most important are pentacyclic derivatives of olean, ursane, lupane and tetracyclic damaranes. Investigated plant species are well known and used for their content in essential oil, phenol compounds, and other metabolomes, but not for the presence of betulinic, oleanolic, lupeol, and ursolic acid. Identification of this compounds could determinate some pleiotropic pharmacological activity of this herbal drugs. Qualitative and quantitative analysis of triterpenes, present in plant species belonging to Lamiacae family was performed in this study. The aim of this study was to find out the best conditions for quantitative extraction of investigated triterpene substances which has been achieved by successive extraction using different polar solvents.

Identification and quantification of target compounds in different fractions was obtained using TLC and HPLC method. Triterpene standards (betulin, betulinic, oleanolic, lupeol, and ursolic acid) and different fractions were applied on TLC plates using »CAMAG Linomat 5« device. The Systems used for developing in TLC (benzene: ethyl acetate: formic acid (36:12:5) and HPLC method (ConstaMetric®3000 system for solvent release, Hypersil ODS (Agilent Technologies) column, 4.6 x 250 mm, 5µm, mobile phase acetonitril/aqua (700/300) acidified with ortho-phosphoric acid) were proved to be optimal for separation of tested triterpene substances, otherwise having very similar chemical structure.

The results confirm the presence of this important compounds in the plant species belonging to Lamiaceae family collected from the Bosnia and Herzegovina region. The highest amount of betulin (4.19 µg /ml) and betulinic acid (51.58 µg/ml) were found in hexane extract of *Rosmarini folium.* Chloroform extract of *Lavandulae flos* show the highest amount of ursolic acid (0.14 mg/ml). Oleanolic acid was found only in the hexane extract of *Rosmarini folium* (1.01 µg/ml). Due to the presence of triterpene substances *Rosmarini folium* could represent potential pharmacognostic drug.

ISPITIVANJE MAKROSKOPSKIH, MIKROSKOPSKIH OSOBINA I ETARSKOG ULJA LISTA MATIČNJAKA, *MELISSA OFFICINALIS* L.

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Tradicionalno list matičnjaka, *Melissae folium*, koristi se za ublažavanje blagih simptoma mentalnog stresa i olakšavanje uspavljivanja, kao i u simptomatskoj terapiji blagih gastrointestinalnih poremećaja. Etarsko ulje matičnjaka je veoma cenjeno u prehrambenoj i kozmetičkoj industriji. Cilj rada je bio ispitivanje makroskopskih i mikroskopskih osobina uzoraka lista samoniklog i gajenog matičnjaka sa različitih lokaliteta i komercijalno dostupnih čajeva, kao i ispitivanja sastava njihovih etarskih ulja.

Za analizu etarskih ulja (16 uzoraka), dobijenih destilacijom vodenom parom, primenjena je gasna hromatografija (GC-FID) i gasna hromatografija sa masenom spektrometrijom (GC-MS). Klaster analizom ispitivane su razlike između etarskih ulja.

Makroskopske i mikroskopske odlike svih uzoraka su odgovarale monografiji *Melissae folium* u Ph. Eur. 7.0, ali je u 2 od 5 komercijalnih uzoraka bio veći sadržaj stranih materija. U svim ispitivanim etarskim uljima glavne komponenate su predstavljali: geranial (15,58-53,33%), neral (10,73-36,64 %), kariofilen oksid (0,76-35,57 %), (*E*)-kariofilen (tragovi-16,72 %) i citronelal (0,00-12,64 %). Sadržaj geraniala i nerala u ispitivanim uzorcima je bio u opsegu vrednosti dobijenih u prethodnim ispitivanjima.

Klaster analizom uzorci etarskog ulja su podeljeni na dva klastera. U prvom klasteru su bila etarska ulja sa visokim sadržajem geraniala i nerala (31,87-53,33% i 19,81-36,64%): etarska ulja lista i herbe samoniklog i gajenog matičnjaka u različitim fazama razvoja i sa različitih lokaliteta i dva komercijalna uzorka. U drugi klaster su svrstana etarska ulja sa nižim sadržajem geraniala i nerala (15,58-26,67 % i 10,73-15,48 %) i većim udelom kariofilen-oksida (17,79-35,57 %): tri komercijalna uzorka i etarsko ulje herbe. List samoniklog matičnjaka u različitim fazama razvoja, sadrži visok procenat geraniala i nerala u etarskom ulju, ne razlikuje se značajno od gajenih uzoraka i može predstavljati izvor kvalitetne droge.

Istraživanje je podržalo Ministarstvo prosvete, nauke i tehnološkog razvoja (Projekat ON 173021).

INVESTIGATION OF MACROSCOPIC AND MICROSCOPIC CHARACTERISTICS AND ESSENTIAL OIL OF THE LEMON BALM LEAF, *MELISSA OFFICINALIS* L.

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Traditional herbal remedies based on lemon balm leaf, *Melissae folium* are used for relief of mild symptoms of mental stress, to aid sleep and for symptomatic treatment of mild gastrointestinal complaints. The essential oil of lemon balm is very appreciated in food and cosmetic industry. The aim was to examine the macroscopic and microscopic characteristics of lemon balm leaves of cultivated and wild plants from different localities, commercially available tea samples and to determinate the composition of their essential oils. The essential oils obtained by hydrodistillation (16 samples), were analysed by gas chromatography (GC-FID) and gas chromatography with mass spectrometry (GC-MS). The differences between essential oils were examined by cluster analysis.

Macroscopic and microscopic characteristics corresponded to the monograph *Melissae folium* in Ph. Eur. 7.0, while 2 of 5 commercial samples contained higher content of foreign matter.

The main components in investigated essential oils were: geranial (15.58-53.33 %), neral (10.73-36.64 %), caryophyllene oxide (0.76-35.57 %), (*E*)-caryophyllene (traces-16.72 %) and citronellal (0.00-12.64 %). The content of geranial and neral was in the range of values obtained in previous investigations.

Cluster analysis divided samples of essential oils into two clusters. Essential oils with high geranial and neral content (31.87-53.33 % and 19.81-36.64 %) were in the first cluster: essential oils of leaf and aerial parts of wild and cultivated lemon balm from different development phases and localities and two commercial samples. The essential oils with lower geranial and neral content (15.58-26.67 % and 10.73-15.48 %) and higher caryophyllene oxide (17.79-35.57 %) were separated to the second cluster: three commercial samples and essential oil of aerial parts.The wild lemon balm leaf in different phases of development contains essential oil with high amounts of geranial and neral. It is not significantly different from cultivated samples and could represent a source of quality herbal substance.

The study was supported by the Ministry of Education, Science and Technological Development (Project ON 173021).

MASNE KISELINE, STEROLI I TRITERPENI MASNIH ULJA PLODOVA OSAM TAKSONA RODA *HERACLEUM* L. IZ JUGOISTOČNE EVROPE

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Taksoni roda *Heracleum* L. su rasprostranjeni predstavnici familije Apiaceae, koji bi mogli biti od značaja u raznim granama industrije. Predmet ovog ispitivanja su masne kiseline, steroli i triterpeni masnih ulja plodova *H. sphondylium* L., *H. sibiricum* L., *H. montanum* Schleich. ex Gaudin, *H. ternatum* Velen., *H. pyrenaicum* subsp. *pollinianum* (Bertol.) F. Pedrotti & Pignatti, *H. pyrenaicum* subsp. *orsinii* (Guss.) F. Pedrotti & Pignatti, *H. verticillatum* Pančić i *H. orphanidis* Boiss., sakupljenih u Srbiji, Makedoniji, Crnoj Gori i Sloveniji.

Osušeni i samleveni plodovi ekstrahovani su dihlormetanom postupkom bimaceracije na sobnoj temperaturi. Rastvarač je uklonjen pod sniženim pritiskom, ekstrakti filtrirani i dobijeni supernatanti podvrgnuti postupku saponifikacije. Saponifikovane frakcije, bogate masnim kiselinama, su metilovane, a nesaponifikovane frakcije, koje prvenstveno sadrže sterole i triterpene, silanizovane, u cilju dobijanja isparljivih derivata, koji su analizirani gasnom hromatografijom (GC-FID i GC-MS). Komponente su identifikovane pomoću komercijalnih standarda i biblioteka masenih spektara.

Šesnaest od ukupno osamnaest identifikovanih masnih kiselina su detektovane u svim saponifikovanim frakcijama, a dominantne su bile petroselinska (42,8-56,5 %), linolna (20,3-33,3 %) i oleinska (12,3-13,7 %) kiselina. U svakoj nesaponifikovanoj frakciji identifikovani su triterpen α-amirin (0,8-6,0 %) i devet istih sterola, od kojih je najzastupljeniji bio β-sitosterol (44,9-56,9 %), a sledili su ga po količini stigmasterol (15,7-25,0 %), Δ^7-stigmastenol (6,6-12,5 %) i kampesterol (5,2-8,1 %). Kao najznačajnije može se istaći prisustvo petroselinske kiseline, potencijalno važne sirovine za farmaceutsku, kozmetičku, prehrambenu i hemijsku industriju.

U ovom radu, masne kiseline, steroli i triterpeni masnih ulja plodova *H. ternatum*, *H. pyrenaicum* subsp. *pollinianum*, *H. verticillatum* i *H. orphanidis* ispitivani su po prvi put, a u slučaju *H. sphondylium*, *H. sibiricum*, *H. montanum* i *H. pyrenaicum* subsp. *orsinii*, postojeći podaci o ovim sastojcima masnih ulja su značajno dopunjeni. Dobijeni rezultati pružaju dobru osnovu za dalja istraživanja, u cilju primene ovih biljaka kao potencijalnih novih izvora industrijski značajnih masnih ulja.

Istraživanje je podržalo Ministarstvo prosvete, nauke i tehnološkog razvoja (Projekti ON 173021; ON 172053).

FATTY ACIDS, STEROLS AND TRITERPENES OF THE FRUIT FATTY OILS OF EIGHT *HERACLEUM* L. TAXA FROM SOUTHEASTERN EUROPE

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Heracleum L. taxa are widespread members of Apiaceae family, with potential application in different industries. Focus of this study was on fatty acids, sterols and triterpenes of the fruit fatty oils of *H. sphondylium* L., *H. sibiricum* L., *H. montanum* Schleich. ex Gaudin, *H. ternatum* Velen., *H. pyrenaicum* subsp. *pollinianum* (Bertol.) F. Pedrotti & Pignatti, *H. pyrenaicum* subsp. *orsinii* (Guss.) F. Pedrotti & Pignatti, *H. verticillatum* Pančić and *H. orphanidis* Boiss., collected in Serbia, Macedonia, Montenegro and Slovenia.

Air-dried and powdered fruits were bimacerated with dichloromethane at room temperature. Solvent was evaporated under reduced pressure, extracts were filtered and obtained oily residues were subjected to saponification. Saponifiable fractions, rich in fatty acids, were further subjected to methylation, and unsaponifiable fractions, mostly containing sterols and triterpenes, to silanization, to obtain volatile derivatives that were analyzed by GC-FID and GC-MS. Compounds were identified using commercial standards and mass spectra libraries.

Sixteen of the total of eighteen identified fatty acids were detected in all saponifiable fractions, with petroselinic (42.8-56.5 %), linoleic (20.3-33.3 %) and oleic (12.3-13.7 %) acids being the most abundant. In every unsaponifiable fraction, a triterpene α-amyrin (0.8-6.0 %) and the same nine sterols, predominantly β-sitosterol (44.9-56.9 %), followed by stigmasterol (15.7-25.0 %), Δ^7-stigmastenol (6.6-12.5 %) and campesterol (5.2-8.1 %), were identified. The most notably, petroselinic acid can be utilized in pharmaceutical, cosmetic, food and chemical industries.

In this research, fatty acids, sterols and triterpenes of the fruit fatty oils of *H. ternatum*, *H. pyrenaicum* subsp. *pollinianum*, *H. verticillatum* and *H. orphanidis* were investigated for the first time, while in the case of *H. sphondylium*, *H. sibiricum*, *H. montanum* and *H. pyrenaicum* subsp. *orsinii*, the knowledge about their fatty oils constituents was enhanced. Obtained results provide a good basis for further investigations, aiming to establish these plants as potential sources of valuable novel raw materials.

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ANTIINFLAMATORNI EFEKAT EKSTRAKTA MUSKATNE ŽALFIJE (*SALVIA SCLAREA* L.)

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Salvia sclarea L. je aromatična biljna vrsta čija se lekovitost zasniva na odličnim antimikrobnim, antiinflamatornim, antioksidativnim i spazmolitičkim efektima. Najčešće se u suvom ili svežem obliku koristi kao stomahik, kao i u tretmanu gingivitisa, stomatitisa i afti. Cilj rada je utvrditi antiinflamatornu aktivnost ekstrakta *S. sclarea* na modelu eksperimenatalno indukovanog periodontitisa pacova.

Inflamacija periodoncijuma indukovana je lipopolisaharidom (LPS) u prostoru između prvog i drugog desnog maksilarnog molara pacova. Etanolni ekstrakt muskatne žalfije, pripremljen ultrazvučnom ekstrakcijom herbe, primenjivan je *per os* (200 mg/kg) dva puta dnevno tokom deset dana. Grupu I i II činili su pacovi kojima je ubrizgan fiziološki rastvor, a tretirani vodom ili ekstraktom, dok je grupama III, IV i V ubrizgan LPS rastvor, a tretirani su vodom, ekstraktom ili ekstraktom preventivno tri dana pre indukovanja inflmacije, redom. Status inflamacije praćen je merenjem proinflamatornih citokina u gingivalnom tkivu ELISA testovima: interleukina-1*beta* (IL-1*beta*), interleukina-6 (IL-6) i faktora tumorske nekroze-*alfa* (TNF-*alfa*). Ekstrakt je hemijski okarakterisan HPLC hromatografijom.

U grupi pacova koja je bila tretirana ekstraktom (IV i V) je značajno došlo do redukovanja nivoa citokina u poređenju sa netretiranom grupom (III): IL-1*beta* (1,15 i 0,54 vs. 2,16 pg/mg), IL-6 (5,58 i 5,05 vs. 6,93 pg/mg) i TNF-*alfa* (0,27 i 0,29 vs. 4,35 pg/mg). Preventivna aplikacija ekstrakta nije bila od značaja. U grupama I i II nisu zabeleženi povećani nivoi citokina. U ekstraktu je ruzmarinska kiselina bila predominantna (165,30 ug/mg), a u manjoj količini su bile zastupljene i kafena kiselina, luteolin, luteolin-7-*O*-glukozid, apigenin i apigenin-7-*O*-glukozid. Ekstrakt muskatne žalfije ispoljava antiinflamatorni efekat na modelu LPS-indukovanog periodontitisa kod pacova, pa stoga može imati ulogu potencijalnog i značajnog terapijskog sredstva u tretmanu periodontalnih bolesti.

Istraživanje je podržalo Ministarstvo prosvete, nauke i tehnološkog razvoja (III41018 i III46013).

THE ANTI-INFLAMATORY EFFECT OF THE CLARY SAGE EXTRACT (*SALVIA SCLAREA* L.)

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Salvia sclarea L. is an aromatic plant species whose healing properties are based on excellent antimicrobial, anti-inflammatory, antioxidant and spasmolytic effects. It is most often used dry or fresh as a stomachic, but also in gingivitis, stomatitis and aphthae treatment. The aim of the paper is to determine the anti-inflammatory activity of a *S. sclarea* extract on the experimentally induced periodontitis in rats.

Periodontitis was induced by lipopolysaccharide (LPS) in the area between the first and the second right maxillary rat molars. An ethanol extract of sage, prepared by ultrasonic extraction of the herb, was administered*per os* (200 mg/kg) twice daily for ten days. Group I and II were injected with saline and treated with water or the extract, while groups III, IV and V were injected with LPS and treated with water, the extract or the extract three days before inflammation induction, respectively. The inflammation status was monitored by proinflammatory cytokines measurements (ELISA) in the gingival tissue: interleukin-1*beta* (IL-1*beta*), interleukin-6 (IL-6) and tumor necrosis factor-*alfa* (TNF-*alfa*). The extract was chemically characterized by HPLC.

A significant reduction in cytokine levels was observed in the rats treated with the extract (IV and V), compared to the untreated group (III): IL-1*beta* (1.15 and 0.54 vs. 2.16 pg/mg), IL-6 (5.58 and 5.05 vs. 6.93 pg/mg) and TNF-*alfa* (0.27 and 0.29 vs. 4.35 pg/mg). The preventive application of the extract was not significant. In the group I and II cytokines levels were not increased. Rosmarinic acid was predominant (165.30 ug/mg) in the extract, while caffeic acid, luteolin, luteolin-7-*O*-glucoside, apigenin and apigenin-7-*O*-glucoside were present in smaller amounts. The clary sage extract exhibits an anti-inflammatory effect on the LPS-induced periodontitis in rats, and can therefore be used as a potential and significant therapeutic agent in the treatment of periodontal diseases.

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KONTROLA KVALITETA KORE I SPRAŠENE KORE CIMETA NA TRŽIŠTU SRBIJE

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In vitro i *in vivo* istraživanja cimeta ukazuju na visok medicinski potencijal ovog začina. Značajna delovanja su antimikrobno, antiinflamatorno, uticaj na sniženje nivoa holesterola, a nalazi se i kao sastojak dijetetskih suplemenata namenjenih za koterapiju i prevenciju šećerne bolesti. Zvanični ispitani izvor ove droge je *Cinnamomum verum (zeylanicum)*-cejlonski cimet, dok se na tržištu najčešće nalazi *Cinnamomum cassia*-kineski cimet i *Cinnamomum burmanii* - indonežanski cimet. Ove vrste se razlikuju po kvalitetu i bezbednosti upotrebe. Cilj ovog rada bio je da se ispita kvalitet cimeta koji je dostupan na tržištu Srbije prema Pravilniku o kvalitetu začina.

U ovom radu za izolaciju etarskog ulja primenjena je metoda hidrodestilacije po Ph. Jug. V. Količina etarskog ulja izrazi se kao prosečna vrednost prinosa iz tri merenja po masi suvog biljnog materijala (g/100 g). Ukupan pepeo i pepeo nerastvorljiv u HCl određen je prema Ph. Jug. V. Izražen je u procentima. Rezultati su poređeni sa propisima važećeg Pravilnika o kvalitetu začina Republike Srbije.

Od sedam ispitivanih uzoraka samo sprašena kora organski gajenog cejlonskog cimeta zadovoljava propis u pogledu sadržaja etarskog ulja, dok s druge strane kora cejlonskog cimeta odstupa od propisanog minimalnog sadržaja cimeta čak 7,5 puta, najviše od svih ostalih uzoraka (kora i sprašena kora indonežanskog cimeta kora kineskog cimeta). Primetna je razlika u sadržaju ulja kod Indonežanskog cimeta u prahu koji je čuvan u najlonskoj ambalaži (0,36 %) i onog koji je čuvan u papirnoj ambalaži (0,15 %). Svih sedam uzoraka zadovoljava propis za maksimalan sadržaj ukupnog pepela i pepela nerstvornog u HCl.

S obzirom na široku upotrebu cimeta, kako kao začina, tako i kao dodatka ishrani s ciljem postizanja određenih zdravstvenih benefita, njegov uvoz i identifikacija trebala bi da podleže strožijim kriterijumima kako bi se izbegao potencijalan rizik po zdravlje.

QUALITY CONTROL OF CINNAMON'S BARK AND POWDERED BARK FROM THE SERBIAN MARKET

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In vitro and *in vivo* research indicate the high medical potential of this spice. The problem with the use of this plant is that there are hundreds of species and subspecies of cinnamon plant. The official source is *Cinnamomum verum (zeylanicum)* - Ceylon cinnamon, while *Cinnamomum cassia* - Chinese cinnamon and *Cinnamomum burmanii*- Indonesian cinnamon, are commonly found on the market. It is important to emphasize that they differ in quality and safety of use. The aim of this paper was to examine the quality of cinnamon that is available on the Serbian market according to the Rules for the quality of spices.

In this study essential oil was isolated using hydrodestilation method according to *Ph. Yug.* V, whose quantity is expressed in percentages. Total ash and ash insoluble in HCl was determined according to *Ph. Yug.* V. It is expressed in percentages, too.

Only the powder of the organic Ceylon cinnamon meets the standard of the regulation for the content of essential oil, while on the other side, bark of the Ceylonian cinnamon has the biggest deviation from the prescribed minimum content, most of all other samples (Indonesian powder and bark and Chinese bark). There is a significant difference in the oil content of Indonesian cinnamon powder stored in nylon packaging (0.36 %) and the one stored in paper packaging (0.15 %). All seven samples have satisfied the regulation for the maximum content of total ash and ash insoluble in HCl.

Cinnamon is widely used as an addition to nutrition for achieving health benefits. Therefore, its imports and identification should be subject to stricter criteria in order to avoid potential health risks.

VAZORELAKSANTNA AKTIVNOST TERPINOLENA

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Terpeni predstavljaju veoma važnu grupu hemijskih jedinjenja kako zbog industrijske upotrebe, tako i zbog svojih značajnih bioloških efekata koji se mogu iskoristiti u medicini. Jedna su od najbrojnijih grupa sekundarnih metabolita biljaka, a naziv ove klase jedinjenja potiče od terpentina (terpentinskog ulja), tečnog proizvoda destilacije oleorezina bora. Terpinolen (*p*-menta-2,4(8)-dien) je bezbojna ili bledo žuto obojena tečnost aromatičnog mirisa. Ovaj monociklični, monoterpenski alken je široko zastupljen u biljnim tkivima različitih četinara, paškanata, konoplje, čajevca, listu kurkume i peršuna, a čest je sastojak etarskog ulja *Citrus*, *Mentha*, *Juniperus* i *Myristica* vrsta. Jedna je od glavnih komponenata etarskog ulja nadzemnih delova endemične vrste *Seseli gracile* Waldst. & Kit. (6,1-57,5 %). Terpinolen poseduje potvrđenu antiradikalsku, antihiperalgezijsku i antiedematoznu aktivnost. S obzirom da su neka strukturno slična monoterpenska jedinjenja pokazala vazorelaksantnu aktivnost, cilj ovog istraživanja je da se istraži vazorelaksantni potencijal terpinolena.

U ovom istraživanju ispitivan je efekat standarda terpinolena (43905 Sigma-Aldrich) na izolovanoj renalnoj arteriji pacova Wistar soja. Korišćeni su prstenasti segmenti renalne arterije dužine 3-5 mm sa očuvanim endotelom. Arterijski segmenti su postavljani u vodeno kupatilo u Krebs-Ringerov rastvor, na 37°C i aerisani kontinuiranim dovođenjem smeše 95 % O_2 i 5 % CO_2. Kontrakcija krvnih sudova je izazvana primjenom fenilefrina (10^{-6} M). Integritet endotela je potvrđen farmakološki, primjenom acetilholina (10^{-6} M). Na stabilan tonus krvnog suda izazvan fenilefrinom su dodavane rastuće koncentracije terpinolena, pripremljenog od standarda razblaživanjem u 5 % karboksi-metil celuloze (0,2 % - 33,3 %, kumulativno). Svaka sledeća koncentracija je dodavana tek nakon što se završi efekat prethodno primjenjene koncentracije, tj. otprilike nakon 15 minuta od prethodne. U kontrolnoj seriji eksperimenata je na isti način dodavan rastvarač (6 dodavanja kumulativno, sa vremenskim razmakom od 15 minuta).

Utvrđeno je da terpinolen u kocentraciji od 11,1% dovodi do statistički značajne vazorelaksacije u odnosu na rastvarač kao kontrolu ($F=2,584$, $p<0,05$; Two-way ANOVA). Naši rezultati opravdavaju nastavak ispitivanja potencijalne primene terpinolena u terapiji kardiovaskularnih oboljenja.

Istraživanje je podržalo Ministarstvo prosvete, nauke i tehnološkog razvoja (Projekat ON 173021).

VASORELAXANT ACTIVITY OF TERPINOLENE

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Terpenes represent a very important group of chemical compounds because of their industrial use, as well as medical use which is possible due to their significant biological effects. They are one of the largest groups of secondary plant metabolites. The name of this class of compounds comes from turpentine (oil of turpentine), a liquid product of pine oleoresin distillation process.

Terpinolene (p-menth-2,4 (8)-diene) is a colorless or pale yellow colored liquid with aromatic odor. This monocyclic, monoterpenic alkene is widely represented in plant tissues of various pines, pashkans, cannabis, tea tree, turmeric and parsley leaves, and it is often a component of essential oils obtained from *Citrus*, *Mentha*, *Juniperus* and *Myristica* species. It is one of the main components of the essential oil of aerial parts of the endemic species *Seseli gracile* Waldst. & Kit. (6.1-57.5%). Terpinolen possesses confirmed antiradical, antihyperalgesic and antiedematous activity. Since some structurally similar monoterpenic compounds have previously shown vasorelaxant activity, the aim of this study was to investigate the vasorelaxant potential of terpinolene.

In the present study, the effects of standard terpinolene (43905 Sigma-Aldrich) on the isolated renal artery of Wistar rats were investigated. Three to five mm long arterial ring tissue segments with preserved endothelium were used. The arterial segments were placed in water bath in the Krebs-Ringer solution, at 37° C and continuously aerated by 95% O_2 and 5% CO_2. Contractions of the blood vessels were induced with 10^{-6} M of phenylephrine. The endothelium integrity was confirmed pharmacologically, by using the acetylcholine (10^{-6}M). After accomplishing tonic phase of the contraction, increasing concentration of terpinolene (0.2 % - 33.3 %, cumulatively) in 5 % carboxymethyl cellulose (CMC) solution were added to the organ bath. Each subsequent concentration was added only after the end of effect of the previously applied concentration, i.e. approximately 15 minutes from the previous one. In the control series of experiments, a solvent was added in the same manner.

It was found that terpinolene at the concentration of 11.1% exibits statistically significant vasorelaxation in comparison to the solvent control ($F = 2.584$, $p < 0.05$; Two-way ANOVA). Our results justify a continuation for the further studies of terpinolene's potential in the treatment of cardiovascular diseases.

The study was supported by the Ministry of Education, Science and Technological Development (Project ON 173021).

APITERAPIJA U MENOPAUZI

Lidija Jerotijević

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Apiterapija predstavlja primenu pčelinjih proizvoda sa ciljem očuvanja zdravlja ljudi i/ili njihovog lečenja. Svi pčelinji proizvodi imaju široku upotrebu u tradicionalnoj medicini. Menopauza je fiziološko stanje žene koje predstavlja kraj njene reproduktivne faze. Do menopauze dolazi zbog naglog pada nivoa estrogena u organizmu. Ovo stanje često je praćeno brojnim neprijatnim simptomima: vazomotorna nestabilnost, atrofija sluzokože i kože urogenitalnog trakta, promene u skeletnom sistemu, promene u kardiovaskularnom sistemu, psihički problemi, promena kvaliteta kože, estetske promene. Konvencionalna medicina i farmakologija kao rešenje ovog problema nude hormonsku supstitucionu terapiju. Ova terapija sa sobom nosi višestruke rizike i neželjene efekte koji često prevazilaze dobit za pacijentkinju. Cilj ovog rada je da izučavanjem dostupne literature potvrdi delotvornost i neškodljivost apiterapije u rešavanju problema koji prate ovo stanje.

U simptomatskoj terapiji menopauze opravdana je upotreba svih pčelinjih proizvoda. Matični mleč i pčelinji polen mogu se koristiti kao kauzalna terapija za otklanjanje i ublažavanje menopauzalnih problema. Matični mleč sadrži 10-hidroksi-2-decensku kiselinu i njene derivate. Ovi molekuli imaju mogućnost vezivanja za estrogenske receptore. Pri tome mogu da ispolje agonistički ili antagonistički efekat u odnosu na humani estrogen zavisno od koncentracije estrogena u organizmu. Ovaj rad navodi mehanizme agonističkog i antagonističkog dejstva ovih molekula. U radu je preporučen i način upotrebe matičnog mleča, doza, navedeni su mogući neželjeni efekti, interakcije i kontraindikacije. Pčelinji polen u svom sastavu sadrži fitoestrogene. U radu se obrađuju mehanizmi dejstva fitoestrogena, navodi se preporučeni način upotrebe polena, mogući neželjeni efekti do kojih dovodi, interakcije sa lekovima i kontraindikacije. Modulatorni efekat matičnog mleča i pčelinjeg polena na estrogenske receptore omogućava njihovu efikasnu i neškodljivu upotrebu u terapiji menopauzalnih simptoma. Kao pomoć u menopauzi apiterapeuti preporučuju ženama upotrebu matičnog mleča i polena zasebno ili u kombinaciji sa drugim pčelinjim proizvodima, kao i kombinaciju sa fitoterapijom.

APITHERAPY IN MENOPAUSE

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Apitherapy is the application of bee products with the goal of preserving the health of people and/or their treatment. All bee products are used in traditional medicine. Menopause is the physiological state of woman and it represents the end of her reproductive phase. Menopause is due to a sudden drop in estrogen levels in the body. This condition is often accompanied by numerous unpleasant symptoms: vasomotor instability, mucosal and skin atrophy in the urogenital tract, changes in the skeletal system, changes in skin quality, aesthetic changes. Conventional medicine and pharmacology offer hormon replacement therapy as a solution for this problem. This therapy carries multiple risks and side effects that often outweigh the benefits for a patient. The aim of this paper is to study the available literature to confirm the effectiveness and harmlessness of apitherapy in solving problems that follow this condition.

All bee products could be used in symptomatic menopause therapy. Royal jelly and bee pollen can be used as causal therapy for the elimination and alleviation of menopausal problems. Royal jelly contains 10-hydroxy-2decenoic acid and its derivatives. These molecules have the ability binding to estrogen receptors. In doing so, they may exhibit agonistic or antagonistic effects in relation to human estrogen, depending on the estrogen concentrations in the body. Mechanisms of agonistic and antagonistic action of these molecules are processed in zhis paper. Also, it is written how to use royal jelly, in which dose, possible side effects, drug interactions and contraindications are listed as well. The modulatory effect of royal jelly and bee pollen on estrogen receptors enables their effective and innocuous use in the treatment of menopausal symptoms. As an aid to menopause, apitherapy recommends to women the use of royal jelly, pollen and bee pollen separately or in combination with other bee products or combinations with phytotherapy.

ANTIMIKROBNA I ANTIOKSIDATIVNA AKTIVNOST METANOLNOG EKSTRAKTA *ALCHEMILLA VIRIDIFLORA* ROTHM. (ROSACEAE)

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Rod *Alchemilla* L. (Rosaceae) čine višegodišnje zeljaste biljke koje rastu na vlažnim livadama širom Evrope, Azije i Severne Amerike. Do sada, najdetaljnije istražena vrsta je virak, *A. vulgaris* L., čija se herba na našim prostorima tradicionalno koristi za lečenje digestivnih i ginekoloških problema. Ova droga sadrži značajnu količinu tanina, i stoga poseduje adstrigentno, antioksidativno, antimutageno dejstvo i ostvaruje pozitivan efekat na zarastanje rana dokazan *in vitro*. Međutim, ostale vrste ovog roda su znatno manje ispitane. Stoga, cilj ovog rada je ispitivanje antimikrobne i antioksidativne aktivnost metanolnog ekstrakta vrste *A. viridiflora* Rothm. koja dosada nije istražena.

Nadzemni delovi *A. viridiflora* prikupljeni su u julu 2013. godine na Suvoj Planini (Srbija), nakon čega su osušeni prirodnim putem (320 g), ekstrahovani 70% metanolom, pri čemu je dobijen metanolni ektrakt (80 g). Sadržaj ukupnih polifenola u metanolnom ekstraktu određen je spektrofotometrijski *Folin-Ciocalteu* metodom. Sposobnost uklanjanja slobodnih radikala metanolnog ekstrakta je ispitivana standardnim DPPH testom, i izražena kao koncentracija koja dovodi do uklanjanja 50% DPPH radikala (IC_{50}) i poređena sa standardnim supstancama. Antimikrobna aktivnost metanolnog ektrakta određena je bujon dilucionom metodom na devet bakterijskih sojeva.

Utvrđeni sadržaj ukupnih polifenola u metanolnom ekstraktu iznosi 205,8 μg galne kiseline/g suvog ektrakta, dok je dobijena IC_{50} vrednost u DPPH testu bila 7,8 μg/ml. Vrednosti IC_{50} za vitamin C iznosi 3,8 μg/ml i za rutin 5,75 μg/ml. Metanolni ekstrakt inhibira rast svih testiranih bakterijskih sojeva pri minimalnoj inhibitornoj koncentraciji (MIC) od 125 μg/ml, osim bakterijskog soja *Salmonella abony* (MIC 62,2 μg/ml).

Metanolni ekstrakt vrste *A. viridiflora* ispoljava snažan antioksidativni potencijal, približan standardnim antioksidansima, koji je u korelaciji sa visokim sadržajem ukupnih polifenola u ekstraktu. Međutim, ispitivani ekstrakt pokazuje umerenu antimikrobnu aktivnost na testirane bakterijske sojeve.

Istraživanje je podržalo Ministarstvo prosvete, nauke i tehnološkog razvoja (Projekat ON 173021).

ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF METHANOL EXTRACT OF *ALCHEMILLA VIRIDIFLORA* ROTHM. (ROSACEAE)

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Plants from genus *Alchemilla* L. (Rosaceae) are a perennial herbaceous that grow throughout Europe, Asia and North America. The most thoroughly studied species is the *A. vulgaris* L., whose herb is used traditionally for digestive and gynecological problems. This drug contains tannins, and therefore possesses an astringent, antioxidant, antimutagenic effect and produces a positive effect on wound healing. However, other species of this genus are less investigated. Therefore, the aim of this study is to investigate the antimicrobial and antioxidant potential of the methanol extract of *A. viridiflora* Rothm. which has not been studied so far.

Areal parts of *A. viridiflora* were collected in July 2013 at Mt. Suva Planina, dried naturally, extracted with 70% methanol, where methanol extract was obtained. The content of total polyphenols was determined by spectrophotometric *Folin-Ciocalteu* method. The ability of the extract to remove free radicals was tested by standard DPPH test, and expressed as a concentration that removes 50% of DPPH radical (IC_{50}) and compared to standard substances. The antimicrobial activity was determined by the broth microdilution method on nine bacterial strains.

The determined content of the total polyphenolic compounds in the methanol extract was 205.8 μg of gallic acid/g of dry extract, while obtained IC_{50} in DPPH test was 7.8 μg/mL. IC_{50} values for vitamin C were 3.8 μg/ml and for rutin 5.75 μg/mL. The extract inhibits the growth of all tested bacterial strains with minimum inhibitory concentration (MIC) of 125 μg/mL, apart from the bacterial strain of *Salmonella abony* (MIC of 62.2 μg/mL).

Methanol extract of *A. viridiflora* exhibits a strong antioxidant potential, comparable with standard antioxidants, which was correlated with the high content of polyphenols in the extract. However, extract shows a mild antimicrobial activity against tested bacterial strains.

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SADRŽAJ ESTRAGOLA U ETARSKOM ULJU I INFUZU HERBE BOSILJKA, *OCIMUM BASILICUM* L.

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Herba bosiljka se tradicionalno koristi kao karminativ, spazmolitik, blag sedativ i laktagog. Etarsko ulje herbe bosiljka sadrži fenilpropanoid estragol, koji je kancerogen i genotoksičan, a čija količina zavisi od hemotipa bosiljka. Količina estragola koja se unosi putem biljnih lekovitih proizvoda, prema preporuci Evropske agencije za lekove (EMA), ograničena je na 0,5 mg dnevno tokom 14 dana. Cilj rada bio je ispitivanje hemijskog sastava etarskih ulja, sadržaja estragola u etarskim uljima i infuzima herbe bosiljka, dostupne u Srbiji.

Ispitivani su komercijalni uzorci monokomponentnih čajeva i uzorci herbe bosiljka gajenog u domaćinstvima u različitim delovima Srbije. Infuzi su pripremljeni prelivanjem uzoraka ključalom vodom (2 g/150 ml) i ceđenjem nakon 15 min. Izolovanje etarskog ulja iz herbe, kao i izolovanje estragola iz infuza izvršeno je destilacijom vodenom parom u aparaturi po Klevendžeru. Analiza etarskih ulja i destilata infuza izvršena je GC-FID-MS metodom. Sadržaj estragola u etarskim uljima i infuzima određen je metodom eksternog standarda.

Sastav svih ispitivanih etarskih ulja odgovara evropskom hemotipu bosiljka, kojeg karakteriše visok sadržaj linalola ili linalola i estragola. Sadržaj estragola u ispitivanim etarskim uljima iznosio je 2,1-565,4 mg/ml, a u infuzima 0,5-11,3 μg/ml. Primenom ispitivanih infuza prema preporuci *Deutsche Arzneimittel-Codex* (DAC) (2 g/150 ml, tri puta dnevno), dnevni unos estragola iznosio bi 0,2-5,1 mg.

Primenom infuza pojedinih uzoraka herbe bosiljka postoji mogućnost prekoračenja dozvoljenog dnevnog unosa estragola. Dobijeni rezultati ukazuju na značaj utvrđivanja sadržaja estragola u herbi bosiljka koja se koristi u obliku biljnih lekovitih proizvoda.

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THE CONTENT OF ESTRAGOLE IN ESSENTIAL OIL AND INFUSION OF BASIL HERB, *OCIMUM BASILICUM* L.

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Basil herb is traditionally used as carminative, spasmolytic, mild sedative and lactagogue. Basil essential oil contains phenylpropanoide estragole, which is carcinogenic and genotoxic, and its amount depends on Basil chemotype. The amount of estragole administered through herbal medicinal products, according to the recommendation of the European Medicines Agency (EMA), is limited to 0.5 mg daily during 14 days. The aim of this work was examination of chemical composition of essential oils, as well as determination of estragole content in essential oils and infusions of Basil herb available in Serbia.

We have analyzed commercial samples of monocomponent Basil herbal teas and samples of Basil herb cultivated in different parts of Serbia. Infusions were prepared by steeping samples in boiling water (2 g/150 ml) for 15 min and subsequent filtering. Isolation of the herb essential oil, as well as isolation of estragole from infusions was carried out by hydrodistillation using a Clevenger-type apparatus. Analyses of essential oils and infusion distillates were performed using GC-FID-MS. The content of estragole in essential oils and infusions was determined using external standard method.

Chemical composition of all investigated essential oils corresponds to European chemotype of Basil that is characterized by high content of linalool or linalool and estragole. The content of estragole in essential oils ranged from 2.1 to 565.4 mg/ml, whereas infusions contained 0.5-11.3 μg/ml of this compound. If the examined infusions are administered according to the recommendation of *Deutsche Arzneimittel-Codex* (DAC) (2 g/150 ml, three times daily), the daily intake of estragole would be 0.2-5.1 mg.

By administration of infusions of some Basil herb samples, there is a possibility of exceeding the permitted daily intake of estragole. The obtained results indicate the importance of determining the content of estragole in Basil herb used in the form of herbal medicinal products.

The study was supported by the Ministry of Education, Science and Technological Development (Project ON 173021).

ISPITIVANJE TRITERPENA U DIHLORMETANSKIM EKSTRAKTIMA 28 VRSTA RODA *HIERACIUM* L. (ASTERACEAE) SA BALKANSKOG POLUOSTRVA

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Rod *Hieracium* L. s. str. (Asteraceae) predstavlja jedan od najbrojnijih i najkompleksnijih rodova skrivenosemenica. Na Balkanskom poluostrvu, najveći broj vrsta ovog roda zabeležen je na Dinaridima, naročito na Durmitoru u Crnoj Gori. Cilj ovog istraživanja bio je da se utvrdi i međusobno uporedi profil triterpenskih jedinjenja u dihlormetanskim ekstraktima nadzemnih delova u cvetu 28 vrsta ovog roda poreklom iz Crne Gore i Srbije, i to: *H. gymnocephalum* Griseb. ex Pant., *H. orieni* A. Kern., *H. blecicii* Niketić, *H. paratrichum* Niketić, *H. spirocaule* Niketić, *H. mokragorae* (Nägeli & Peter) Nägeli & Peter, *H. pannosum* Boiss. s.l., *H. plumulosum* A. Kern., *H. villosum* Jacq., *H. pilosum* Froel., *H. pseudoschenkii* (Rohlena & Zahn) Niketić, *H. naegelianum* Pančić, *H. anastrum* (Degen & Zahn) Niketić, *H. calophyllum* R. Uechtr., *H. scheppigianum* Freyn, *H. durmitoricum* (Rohlena & Zahn) Niketić, *H. guentheri-beckii* Zahn, *H. mirificissimum* Rohlena & Zahn, *H. coloriscapum* Rohlena & Zahn, *H. pyricephalum* Niketić, *H. albopellitum* (Zahn) Niketić, *H. glabratum* Willd., *H. scorzonerifolium* Vill. s.l., *H. dentatum* Hoppe s.l., *H. valdepilosum* Vill. s.l., *H. neilreichii* Beck, *H. tommasinianum* K. Malý i *H. macrodontoides* (Zahn) Zahn.

U suvim dihlormetanskim ekstraktima nadzemnih delova biljaka u cvetu vršena je komparativna kvalitativna i kvantitativna GC-FID-MS analiza triterpena. Struktura triterpena utvrđena je poređenjem sa komercijalnim standardima, kao i na osnovu 1D i 2D NMR spektara odgovarajućih frakcija.

U ispitivanim vrstama identifikovano je ukupno pet triterpena, i to dva derivata ursana (α-amirin i njegov acetat), dva derivata oleana (β-amirin i njegov acetat) i jedan derivat lupana (lupeol-acetat). Dominantni triterpeni, u većini ispitivanih vrsta, bili su derivati ursana, a praćeni su jedinjenjima tipa oleana.

U ovom radu je, za većinu (26) odabranih biljnih vrsta, ispitivanje triterpena sprovedeno po prvi put. Na osnovu dobijenih rezultata, značajno su upotpunjeni podaci o prisustvu i kompoziciji triterpenskih jedinjenja u vrstama roda *Hieracium*.

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INVESTIGATION OF TRITERPENES IN THE DICHLOROMETHANE EXTRACTS OF 28 *HIERACIUM* L. SPECIES (ASTERACEAE) FROM THE BALKAN PENINSULA

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The genus *Hieracium* L. s. str. (Asteraceae) represents one of the most numerous and most complex genera of flowering plants. In the Balkan Peninsula the largest number of species of this genus are recorded on Mt Durmitor in Montenegro. The objective of this study was to determine and compare triterpene profile of flowering aerial parts dichloromethane extracts of 28 *Hieracium* species from Montenegro and Serbia: *H. gymnocephalum* Griseb. ex Pant., *H. orieni* A. Kern., *H. blecicii* Niketić, *H. paratrichum* Niketić, *H. spirocaule* Niketić, *H. mokragorae* (Nägeli & Peter) Nägeli & Peter, *H. pannosum* Boiss. s.l., *H. plumulosum* A. Kern., *H. villosum* Jacq., *H. pilosum* Froel., *H. pseudoschenkii* (Rohlena & Zahn) Niketić, *H. naegelianum* Pančić, *H. anastrum* (Degen & Zahn) Niketić, *H. calophyllum* R. Uechtr., *H. scheppigianum* Freyn, *H. durmitoricum* (Rohlena & Zahn) Niketić, *H. guentheri-beckii* Zahn, *H. mirificissimum* Rohlena & Zahn, *H. coloriscapum* Rohlena & Zahn, *H. pyricephalum* Niketić, *H. albopellitum* (Zahn) Niketić, *H. glabratum* Willd., *H. scorzonerifolium* Vill. s.l., *H. dentatum* Hoppe s.l., *H. valdepilosum* Vill. s.l., *H. neilreichii* Beck, *H. tommasinianum* K. Malý and *H. macrodontoides* (Zahn) Zahn.

In dried dichloromethane extracts of flowering aerial plants parts comparative qualitative and quantitative GC-FID-MS analysis of triterpenes was performed. The structures of triterpenes were elucidated comparing to commercial standards, and on the basis of 1D and 2D NMR spectra of corresponding fractions.

In investigated species, overall five triterpenes, including two of ursane (α-amyrin and its acetate), two of oleane (β-amyrin and its acetate) and one of lupane (lupeol acetate) type, were identified. Dominant triterpenes, for the majority of species, were of ursane type, followed by representatives of oleane type.

In this paper, for the majority (26) of selected plant species, triterpenes were tested for the first time. Based on obtained results, the data on the presence and composition of triterpen compounds in *Hieracium* species have been significantly supplemented.

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