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Abstract

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Kinetine Induced Changes in Quercetin, Naringenin, Hesperitin and Rutin content in *Knautia sarajevensis* (G. Beck) Szabó Shoot Cultures

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Keywords:
Knautia sarajevensis,
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Abstract: *Knautia sarajevensis*, Dipsacaceae, is an endemic species found at wood margins and meadows only on mountains of Dinaric Alps. Members of this family are widely used in traditional medicine as rich sources of pharmacologically important substances. Since it is well known that flavonoid compounds are carriers of biological activities of plant extracts, the aim of this study was to investigate cytokinin effects in concentration changes of flavonoid constituents. Four different flavonoid constituents were analysed: quercetin, naringenin, hesperitin and rutin in extracts of *K. sarajevenis* shoots cultivated on three *in vitro* treatments (control, 1.0 mg L^{-1} kinetine and 10.0 mg L^{-1} kinetine). All extracts were prepared using dried material and 80% methanol HPLC grade. Analysis of four flavonoid constituents indicated that high cytokinin concentrations did induce improvement of quercetin, naringenin and rutin content, but these concentrations are still lower than those recorded for control treatment. Heseperitin showed cytokinin depended decrease in concentration, and control treatment had the highest hesperitin concentration compared to cytokinin treatments. Further analysis using different types and concentrations of cytokinins are necessary to establish a pattern of cytokinin induced concentration changes in content of these four investigated flavonoids in *Knautia sarajevensis*.

Sažetak

Knautia sarajevensis, Dipsacaceae, je endemična vrsta koja raste na rubovima šuma i livadama dinaridskih Alpa. Pripadnici ove porodice koriste se u narodnoj medicini kao izvor farmakološki značajnih supstanci. S obzirom da su flavonoidi nosioci bioloških aktivnosti biljnih ekstrakata cilj ove studije bio je istražiti djelovanje citokinina na promjene koncentracije flavonoidnih konstituenata. Četiri različita flavonoida su analizirana: kvercetin, naringenin, hesperitin i rutin, u ekstraktima izdanaka *K. sarajevenis* kultiviranim na tri različita *in vitro* tretmana (kontrola, 1.0 mg L^{-1} kinetina i 10.0 mg L^{-1} kinetina). Svi ekstrakti su pripremljeni od sušenog biljnog materijala uz korištenje 80% metanola, HPLC čistoće. Analiza četiri flavonoidna konstituenta pokazala je da visoke koncentracije citokinina induciraju povećnaje koncentracije kvercetina, naringenina i rutina, ali su te koncentrtacije i dalje niže od kontrole. Sniženje koncentracije hesperitina bilo je ovisno o koncentraciji citokinina, te je kontrola imala najveću koncentraciju hesperitina. Dodatne analize, sa različitim tipovima i koncentracijama citokinina, su neophodne da bi se uspostavio model citokinin-indukovane ovisnosti promjene koncentracije istraživanih flavonoidnih konstituenata kod vrste *Knautia sarajevensis*.

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Abstract

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Total phenolic content variation in Herzegovinian populations of *Hypericum perforatum* L.

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Keywords:
total phenolics,
Hypericum perforatum,
St. John's wort.

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Abstract: *Hypericum perforatum* L. (St. John's wort), Hypericaceae, is a perennial herb, which above-ground parts are used in traditional medicine. Concentrations of total phenolic content in above-ground organs of two *Hypericum perforatum* infraspecific taxa from four Herzegovinian populations were determined. Three populations were typical *H. perforatum* subspecies and one was infraspecific taxa with unresolved taxonomic position (*H. perforatum* ssp. *angustifolia*). The lowest concentrations of total phenolic content were recorded for *H. perforatum* ssp. *perforatum* population Hutovo Blato (6.62 and 8.97 mg/g DW in flowers and leaves respectively), and the highest in *H. perforatum* ssp. *angustifolia* population Podveležje (14.86 and 21.79 mg/g DW in flowers and leaves respectively). Newman-Keuls test showed significant interpopulation and infraspecific differences. The observed chemical variations among plant parts were detected. Interpopulation differences in *H. perforatum* ssp. *perforatum* could be related to different habitats conditions. Observed differences between two taxa from the same population Podveležje, where environmental effects were excluded, could support opinions about *H. perforatum* ssp. *angustifolia* as separate taxon. Further investigations are necessary for confirmation of such findings.

Sažetak

Hypericum perforatum L. (kantarion, Gospina trava), Hypericaceae, je višegodišnja biljka, čiji se nadzemni dijelovi koriste u tradicionalnoj medicini. Određene su koncentracije ukupnih fenola u nadzemnim organima dva infraspecijska taksona *H. perforatum* iz četiri hercegovačke populacije. Tri populacije su tipična podvrsta *H. perforatum*, a jedna je infraspecijski takson sa neriješenim statusom (*H. perforatum* ssp. *angustifolia*). Najniže koncentracije ukupnih fenola su utvrđene za *H. perforatum* ssp. *perforatum* u populaciji Hutovo Blato (6.62 mg/g DW cvijet i 8.97 mg/g DW list), a najveće za *H. perforatum* ssp. *angustifolia* u populaciji Podveležje (14.86 mg/g DW cvijet i 21.79 mg/g DW list). Newman-Keuls test pokazao je značajne interpopulacijske i infraspecijske razlike. Uočene su hemijske varijacije između istraživanih biljnih dijelova. Interpopulacijske razlike taksona *H. perforatum* ssp. *perforatum* mogu se dovesti u vezu sa različitim uslovima staništa. Uočene razlike između dva taksona iz iste populacije Podveležje, gdje je isključeno djelovanje različitih ekoloških faktora, mogu ići u prilog mišljenjima da je *H. perforatum* ssp. *angustifolia* poseban takson. Neophodna su daljnja istraživanja da bi se potvrdili ovi nalazi.

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Abstract

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Potentiometric characterization and determination of amino acids and their mixtures in non-aqueous media

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Keywords:
L-histidine,
β-alanine,
non-aqueous titration

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Abstract: L-histidine is an α-amino acid with an imidazole functional group. It is a common coordinating ligand in metalloproteins, is a part of catalytic sites in certain enzymes, and has important role in hemoglobin. Another α-amino acid, β-alanine, is not used in the biosynthesis of any major proteins or enzymes. It is formed *in vivo* by the degradation of dihydrouracil and carnosine, and is a component of the naturally occurring peptides carnosine and anserine and also of pantothenic acid, which itself is a component of coenzyme A.

In this paper L-histidine and β-alanine were studied pottentiometrically, as single and in a binary mixture (1:1). Their acid-base properties were studied by use of potentiometric titrations that were carried out using non-aqueous solvents,-tetrabutylammonium hydroxide in toluene/methanol solution, perchloric acid in acetic acid anhydride, and perchloric acid standard solution in acetic acid anhydride. Amino acids were dissolved in glacial acetic acid solution and acetonitrile in 1:10 ratio. The generated potentiometric data were used for defining buffering capacities (buffer strength) for both amino acids and for determination of the distributions diagrams of corresponding species.

Sažetak

L-histidin je α-amino kiselina sa imidazolnom funkcionalnom grupom. Obično je koordinirajući ligand kod metaloproteina i aktivno je katalitičko mjesto nekih enzima, te ima važnu ulogu kod hemoglobina. β-Alanin se ne koristi kod biosinteze važnih enzima ili proteina. L-histidin nastaje *in vivo* raspadanjem dihidrouracila i karnozina. U sastavu je prirodnih peptida karnozina i anserina, te pantotenske kiseline koja je komponenta koenzima A.

U ovom istraživanju određivane su potenciometrijskim metodama aminokiseline L-histidin i β-alanin, pojedinačno i u smjesi (1:1). Njihova kiselo-bazna svojstva okarakterizirana su- potenciometrijskim titracijama u nevodenim otapalima – tetrabutilamonijev hidroksid u otopini toluena/metanola, perklorna kiselina i anhidrid octene kiseline, standardna otopina perklorne kiseline u anhidridu octene kiseline. Ledena octena kiselina i acetonitril u omjeru 1:10 korišteni su kao otapalo za aminokiseline. Generirani podaci dobiveni direktnom potenciometrijom poslužili su za definiranje kapaciteta (jakost pufera) za obje aminokiseline i za određivanje odgovarajuće distribucije specija.

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Antioxidant Activity of *Achillea clypeolata* Sm.

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Keywords:
Achillea clypeolata,
antioxidant activity,
flavonoid content,
phenolic content

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Abstract: *Achillea clypeolata* Sm. commonly known as yellow yarrow is an endemic species of the genus *Achillea*. Different species of genus *Achillea* are known for their antioxidant activities, but *A. clypeolata* antioxidant activity is investigated for the first time in this paper. Antioxidant activity of leaf, flower and root methanolic extracts is determined by DPPH and total reducing power assay. Total phenolic and total flavonoid content were estimated using standard chemical assay procedures. Highest total phenolic and flavonoid content was observed for flower methanolic extracts (64.70 μg GAE/ml and 1.09 μg RE/ml, respectively). Total reducing power ranged between 10.66 mg AAE/ml for root extract to 11.90 mg AAE/ml flower extract. Antioxidant activity was analyzed *in vitro* using DPPH reagent and expressed as %RSC. All extracts showed similar antioxidant activities. Significant positive correlations between antioxidant activity assays and total flavonoid and phenolic content indicate that these compounds contribute to antioxidant activity of plants. According to these results, *A. clypeolata* can be used as potential natural antioxidants source.

Sažetak

Achillea clypeolata Sm. u narodu poznata kao žuta hajdučka trava je endemska vrsta iz roda *Achillea*. Iako su biljne vrste iz ovog roda poznate po svojoj antioksidativnoj aktivnosti, antioksidativna aktivnost vrste *A. clypeolata* nije do sada određivana. Antioksidativna aktivnost metanolnih ekstrakata lista, cveta i korena određena je DPPH i metodom za određivanje ukupne redukcione moći. Sadržaj fenolnih jedinjenja i flavonoida određen je standardnim metodama, koje su u širokoj upotrebi. Najveći sadržaj flavonoida i fenolnih jedinjenja detektovan je kod metanolnog ekstrakta cveta (64.70 μg GAE/ml i 1.09 μg RE/ml, respektivno). Ukupna redukciona moć kreće se između 10.66 mg AAE/ml za ekstrakt korena, do 11.90 mg AAE/ml za ekstrakt cveta. Antioksidativna aktivnost, određena DPPH metodom, izražena je kao %RSC, i prema ovoj metodi svi ekstrakti pokazali su sličnu aktivnost. Značajna korelacija između metoda određivanja antioksidativne aktivnosti i metoda za određivanje ukupnih fenola i flavonoida ukazuje na to da ova jedinjenja imaju doprinos u ukupnoj antioksidativnoj aktivnosti biljaka. Prema dobijenim rezultatima, *A. clypeolata* se može smatrati potencijalnim izvorom prirodnih antioksidanasa.

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Cd influence on accumulation of compounds of ascorbate-glutathione cycle during vegetation of barley

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Keywords:
cadmium,
compounds of ascorbate-glutathione cycle,
ascorbic acid, glutathione,
vegetation of barley

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Abstract: The analysis of the experimental data has shown the interrelation of the ascorbate-glutathione cycle components with the malondialdehyde (MDA) content, with oxidative reactions in barley (Hordeum vulgare L.) being enhanced with the increase in Cd exposure time. Particularly pronounced oxidative processes related to MDA accumulation were reported on day 80 of vegetation due to rather inactivation of the ascorbate-glutathione cycle components. Only on day 20 of Cd exposure, an increase was noted in the ascorbic acid content, whereas in the following periods the contents of ascorbic acid and glutathione were reduced.

Sažetak

Analiza eksperimentalnih podataka je pokazala interkorelaciju komponenti askorbinsko-glutationskog ciklusa i sadržaja malondialdehida (MDA) sa oksidativnim reakcijama u ječmu (Hordeum vulgare L.) pojačanim sa povišenjem izlaganja kadmiju. Naročito izraženi oksidativni procesi povezani sa MDA akumulacijom su zabilježeni u osamdesetom danu vegetacije usljed inaktivacije komponenti askorbinsko-glutationskog ciklusa. Povišenje sadržaja askorbinske kiseline je je primjećenou dvadesetom danu izlaganja kadmiju, dok je u ostalom period sadržaj askorbinske kiseline I glutationa bio umanjen.

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Expression of caveolin-1 on bladder smooth muscle under psychological stress in male rats

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Keywords:
Caveolin-1,
western blot,
immunohistochemistry,
water avoidance stress.

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Abstract: Stress can generate and worsen urinary symptoms and functional urinary disorders such as interstitial cystitis (IC). Recently, it has been suggested that bladder smooth muscle caveolae might regulate pivotal signaling processes involved in contraction. Altered expression of caveolin proteins may lead to bladder dysfunction. In this study, we aimed to study caveolin-1 expression on bladder smooth muscle in water avoidance stress (WAS) model in male rats.

Twelve Wistar rats were divided into 2 groups: Control group (n: 6): which had no intervention and chronic stress group (WAS, n: 6): underwent 2 hour daily WAS for 5 days. At the end of the study intracardiac blood was withdrawn for determination of serum cortisol levels. Urinary bladders were also removed for determination of caveolin -1 expression by immunohistochemistry and western blot analyses. Chronic stress group revealed significantly higher levels of serum cortisol when compared to controls. Expression and localization of caveolin-1 showed no significance between the groups according to immunohistochemistry and western blot analyses. Our results revealed that WAS does not cause an obvious alteration in caveolin-1 expression in the bladder smooth muscle of male rats. Therefore, more studies focusing on the molecular mechanism of stress induced IC are warranted to figure out its pathogenesis.

Sažetak

Stres može uzrokovati I pogoršati simptome funkcionalnih urinarnih oboljenja kao što je interstitialni cistitis (IC). Dokazano je da kaveola glatkog mišićnog tkiva mjehura može regulirati process ključne signalizacije koji predstavlja dio kontrakcije. Promijenjena ekspresija proteina kaveolina može dovesti do disfunkcije mokraćnog mjehura. U ovom radu proučavali smo ekspresiju kaveolin-1 na glatke mišiće mokraćnog mjehura na "water avoidance stress" (WAS) model stresa u mužjacima štakora.

Dvanaest Wistar štakora je podijeljeno u dvije grupe: kontrola grupa (n:6) koja nije imala intervenciju I grupa sa hroničnim stresom (WAS, n:6): koja je bila pod WAS uticajem 2 sata dnevno, 5 dana. Na kraju studije, uzeta je intrakardijalnim krv za određivanje nivoa kortizola u serumu. Mokraćni mjehuri štakora su uzete za imunohemijsko određivanje ekspresije kaveolin-1 I za wester blot analizu.

Hronični strest je uzrokovao znatno više nivoe kortizola u serumu u poređenju sa kontrolama. Ekspresija I lokalizacija kaveolina-1 nije pokazala značajne razlike između grupa kada je riječ o imunohemijskim I western blot analizama.

Naši rezultati su pokazali da WAS ne uzrokuje vidljive alteracije u ekspresiji kaveolina-1 u glatkim mišićima mokraćnog mjehura mužjaka štakora. Zbog toga je neophodan veći broj studija o molekularnomk mehanizmu stresa induciranog sa IC koje će osigurati razumijevanje njegove patogeneze.