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Covalent Immobilization of Lactase onto UV-Curable Polymeric Matrix

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Keywords:

Lactase,
 β -Galactosidase,
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Immobilization

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Abstract: Lactase (β -Galactosidase; EC 3.2.1.23; from *E. coli*), was immobilized on to glycidyl methacrylate monomer containing epoxy functionality by covalent binding. The response surface methodology (RSM) was applied to optimize the immobilization conditions, enzyme concentration (25-100 μ g/mL), reaction time (2–8 h), and reaction pH (6.0-8.0). The results indicated that enzyme concentration, pH and immobilization time were the significant factors on the immobilization of lactase. The morphology of the polymeric support was characterized by scanning electron microscopy (SEM) and FT-IR. By immobilization, the temperature resistance of the enzyme was improved and showed maximum activity at 60 °C. pH dependent activities of the free and immobilized enzymes were also investigated, and it was found that the pH of maximum activity for the free enzyme was 6.8, while for the optimal pH of the immobilized enzyme was 6.5. The immobilized enzyme retained 65% of its activity after 20 runs. Free enzyme lost its activity completely within 30 days, while immobilized enzyme lost only 18.7% of its activity in 30 days. V_{max} values for the free and immobilized enzymes were calculated as 1.0 and 0.077 mg/mL/min, respectively.

Sažetak: Laktaza (β -Galaktozidaza; EC 3.2.1.23; iz *E. coli*), je imobilizirana na glicidil metakrilatni monomer sa epoksi osobinama kada se kovalentno veže. Metodologija odgovora površine (RSM) je korištena za optimizaciju imobilizacionih uslovia: koncentracija enzima (25-100 μ g/mL), vrijeme reakcije (2–8 h) i pH reakcije (6.0-8.0). Rezultati upućuju da su koncentracija enzima, pH i vrijeme imobilizacije značajni faktori u imobilizaciji laktaze. Morfologija polimerne potpore je okarkaterizirana uz pomoć skenirajućeg elektronskog mikroskopa (SEM) i FT-IR. Imobilizacijom je poboljšana otpornost enzima na temperature, a maksimalna aktivnost je dostignuta na 60 °C. pH ovisnost aktivnosti slobodnog i imobiliziranog enzima je također ispitana i nađeno je da je pH maksimalne aktivnosti slobodnog enzima 6.8, dok je optimalna pH za imobilizirani enzim 6.5. Imobilizirani enzim je zadržao 65 % svoje aktivnosti nakon 20 ispitivanja. Slobodni enzim je u potpunosti izgubio svoju aktivnost nakon 30 dana, dok je imobilizirani enzim izgubio tek 18.7 % svoje aktivnosti u periodu od 30 dana. V_{max} vrijednosti za slobodne i imobilizirane enzime su izračunati kao 1.0 i 0.077 mg/mL/min, respektivno.



Covalent Immobilization of Lipase onto Sol-Gel Hybrid Coating Films

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Keywords:

Lipase,
Candida rugosa,
Epoxy-silica polymer,
Immobilization

Abstract: In this study UV-curable hybrid epoxy-silica polymer films were prepared via sol-gel method. Lipase (E.C. 3.1.1.3) from *Candida rugosa* was covalently immobilized onto hybrid epoxy-silica polymer films. The response surface methodology (RSM) was applied to optimize the immobilization conditions: enzyme concentration (25-100 µg/mL), reaction time (2-8 h), and reaction pH (6.0-8.0). Immobilization yield was found as 1.54 mg/g of polymer films. The morphology of the polymeric support was characterized by scanning electron microscopy (SEM) and FT-IR. Immobilized and free enzymes were used in two different reaction systems: hydrolysis of *p*-nitrophenyl palmitate (*p*NPP) in aqueous medium and synthesis of *p*NPP (from *p*-nitrophenol and palmitic acid) in hexane medium. The effects of pH on hydrolytic activities was found that the pH of maximum activity for the free enzyme was 6.5, while for the optimal pH of the immobilized enzyme was 6.0. The effects of pH on synthetic activities were found that for both free and immobilized enzyme was found to be 6.5. The characteristic properties of the immobilized and native enzyme, such as kinetic activity, reusability and storage stability were also studied at optimum pH and temperature. Immobilized enzyme exhibited better reusability and storage stability than the free one.

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Sažetak: U ovom istraživanju UV-ljekoviti hibridni epoksi-silika polimerni filmovi su pripremljeni pomoću sol-gel metode. Lipaza (E.C. 3.1.1.3) iz *Candida rugosa* je kovalentno imobilizirana na hibridne epoksi-silika polimerne filmove. Metodologija odgovora površine (RSM) je korištena da se optimiziraju imobilizacioni uslovi: koncentracija enzima (25-100 µg/mL), vrijeme reakcije (2-8 h), i pH reakcije (6.0-8.0). Imobilizacioni prinos polimernog filma je bio 1.54 mg/g. Morfologija polimerne potpore je okarakterizirana uz pomoć skenirajućeg elektronskog mikroskopa (SEM) i FT-IR. Imobilizirani i slobodni enzimi su korišteni u dva različita reakciona sistema: hidroliza *p*-nitrofenil palmitata (*p*NPP) u vodenoj sredini i sinteza *p*NPP (iz *p*-nitrofenola i palmitinske kiseline) u heksanskoj sredini. Utvrđeni efekti pH na hidrolitičke aktivnosti su takvi da pH maksimalne aktivnosti za slobodan enzim iznosi 6.5, dok optimalna pH za imobilizirani enzim iznosi 6.0. Efekti pH na sintetske aktivnosti kod slobodnog i imobiliziranog enzima ustanovljeni su pri pH 6.5. Karakteristična svojstva imobiliziranog i nativnog enzima kao što su kinetička aktivnost, mogućnost ponovnog korištenja i stabilnost pri skladištenju su također ispitana pri optimalnoj pH i temperaturi. Imobilizirani enzim je pokazao bolju stabilnost pri skladištenju kao i mogućnost ponovnog korištenja u odnosu na slobodni.



Effects of Chard (*Beta vulgaris* L. var. cicla) on Pancreas Damage in Valproic Acid Induced Toxicity

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Keywords:

Chard,
Pancreas,
Valproic Acid

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Abstract: Valproic acid (VPA) is one of the most widely used anticonvulsants in adults and children. However, its usefulness may be compromised due to its potential adverse effects on the gastrointestinal, neurological, hematological and reproductive systems. It is possibly the most common cause of drug induced pancreatitis. Pancreatitis with VPA was first recognized in 1979. Chard (*Beta vulgaris* L. var. cicla) is a low cost plant and has a widespread use in many traditional dishes. It has been demonstrated that chard has antioxidant, antiacetylcholinesterase, antidiabetic, antitumor and hepatoprotective effects. The aim of this study is to evaluate whether VPA and/or chard might interfere with oxidative metabolism in pancreas. Female rats were divided into four groups as intact control animals, VPA (0.5 g/kg/day, i.p.), chard (100 mg/kg/day, oral) and VPA+chard (in same dose and time) given groups for seven days. Chard extract was given 1 h prior to the administration of VPA. On the 8th day the animals were sacrificed under anesthesia and pancreas samples were homogenized in saline. Oxidant-antioxidant biochemical parameters were determined in homogenized pancreas samples. Results were evaluated statistically and discussed.

Sažetak

Valproična kiselina ("Valproic acid"-VPA) je najčešće korišten antikonvulziv kod odraslih osoba i djece. Međutim, njenu korist umanjuju štetni uticaji na gastrointestinalni, neurološki, hematološki i reproduktivni system. Lijekovi sa valproičnom kiselinom su najčešći uzrok nastanka pankreatitisa. 1979 godine prvi put je nastanak pankreatitisa povezan sa valproičnom kiselinom. Blitva (*Beta vulgaris* L. var. cicla) je biljka niskih troškova uzgoja koja je jako zastupljena u različitim tradicionalnim kuhinjama. Dokazano je da ova biljka ima antioksidativna, antiacetylholinesterazna, antidijabetska, antitumorska i hepatoprotektivna svojstva. Cilj ove studije je dokazati da li VPA i/ili blitva mogu interferirati sa oksidativnim metabolizmom pankreasa. Ženke štakora su podijeljene u četiri grupe kao intakt kontrolne životinje, VPA (0.5 g/kg/dan, i.p.), blitva (100 mg/kg/dan, oralno) i VPA+blitva (u istoj dozi i vremenu) su date životinjama u periodu od sedam dana. Ekstrakt blitve je dat životinjama 1 sat prije VPA. Osmog dana uzorci pankreasa su uzeti od uspavanih životinja. Biohemijski parametric oksidans-antioksidans su određivani u homogeniziranim uzorcima. Rezultati su obrađeni statistički.



An Oxovanadium (IV) Complex Based on Thiosemicarbazone Reduces Glycoprotein Components and Oxidative Lung Injury in Experimental Diabetes

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Keywords:

Lung,
vanadium complex,
glycoprotein,
Diabetes mellitus

Abstract: Vanadium derivatives have been shown to possess insulin-mimetic and antidiabetic activities in the animal models and diabetic people. The aim of this study was to investigate the protective effect of an oxovanadium (IV) complex based on thiosemicarbazone (VOL) [L: (N(1)-2,4-dihydroxybenzylidene-N-(4)-2-hydroxybenzylidene-S-methyl-thiosemicarbazidato-oxovanadium (IV)] on glycoprotein components levels and oxidative lung injury of streptozotocin (STZ)-induced diabetic rats. The male animals were divided into four groups. Group I: control (intact) animals. Group II: control animals administered with VOL. Group III: STZ-induced diabetic rats. Group IV: STZ-induced diabetic animals administered VOL. VOL was given to some of the experimental animals by gavage at a dose of 0.2 mM/kg body weight every day for 12 days. Diabetes was induced by single intraperitoneal injection of STZ (65 mg/kg body weight). On the 12th day, lung tissue samples were taken. Hydroxyproline, advanced oxidation protein products and protein carbonyl levels and arginase, prolidase activities and glycoprotein components significantly increased and carbonic anhydrase, Na⁺/K⁺-ATPase and arylesterase activities decreased in lung tissue of diabetic rats. Treatment with VOL reversed these effects showing a beneficial effect of vanadium. In conclusion, the present study shows that VOL has a protective effect against diabetes-induced lung damage and on abnormal glycoprotein component levels.

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Sažetak

Dokazano je da derivati vanadija posjeduju inzulimimetičke i antidijabetičke aktivnosti na životinjskim modelima i kod dijabetičara. Cilj ovog rada je bio ispitivanje zaštitnih osobina oksovanadij(IV) kompleksa baziranog na tiosemikarbazonu (VOL) [L: (N(1)-2,4-dihidroksibenziledin-N-(4)-2-hidroksibenziledin-S-metil-tiosemikarbazidato-oksovanadij (IV)] na nivoe glikoproteinskih komponenta i oksidativne povrede pluća dijabetičnih štakora kojima je dat streptozotocin (STZ). Mužjaci su podijeljeni u četiri grupe. Grupa I su bile kontrolne životinje. Grupa II su bile kontrolne životinje kojima je dat VOL. Grupa III su bili STZ-inducirani dijabetični štakori. Grupa IV su bili STZ inducirani dijabetični štakori kojima je dat VOL. VOL je dat eksperimentalnim životinjama u dozi od 0.2 mM/kg tjelesne težine svaki dan u periodu od 12 dana. Dijabetes je induciran jednom intraperitonealnom injekcijom STZa (65 mg/kg tjelesne težine). Uzorci tkiva pluća su uzeti dvanaestog dana. Proteinski proizvodi hidroksiprolinske oksidacije i karbonilni proteinski nivoi kao aktivnosti arginaze i prolidaze te glikoproteinske komponente su se značajno povisile dok su se aktivnosti karbonilne anhidraze, Na⁺/K⁺-ATPaze i arilesteraze snizile u plućnom tkivu dijabetičnih štakora. Tretman sa VOL je obrnuo procese pokazujući bitnu ulogu vanadija. Rad je pokazao da VOL ima zaštitnu ulogu kod oštećenja pluća uzrokovanih dijabetesom i na abnormalno visoke nivoe glikoproteina.



Effects of Chard on Some Biochemical Parameters in the Testicular Tissue of Streptozotocin-Induced Diabetic Rats

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Keywords:

Diabetes mellitus,
testis,
rat,
chard.

Abstract: Chard (*Beta vulgaris* L. var. *cicla*) is widely spread in Turkey. This plant is used as antidiabetic in traditional medicine in Turkey. The aim of this study was to investigate the protective effect of chard on enzyme activities and glycoprotein components of streptozotocin (STZ) - induced diabetic rat testis. Male Sprague Dawley rats were divided into three groups. Group I; Control animals fed with citrate buffer, Group II; STZ - diabetic animals, Group III; STZ - diabetic animals fed with chard extract. Diabetes was induced by intraperitoneal injection of STZ in a single dose of 60 mg/kg body weight. The chard extract was administrated by gavage technique to rats at a dose of 2 g/kg every day for 45 days, 15 days after animals were made diabetic. At the end of the experimental period, testis tissue was taken after decapitation. The testis tissues were homogenized. Myeloperoxidase activity and protein carbonyl, advanced oxidation protein products, hydroxyproline contents and glycoprotein components were increased, while paraoxonase activity was decreased in testis tissues of diabetic rats. After administration of chard to diabetic rats, these changes were reversed. These results suggest that chard is potentially beneficial agent to reduce testicular damage in diabetic rats.

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Sažetak

Blitva (*Beta vulgaris* L. var. *cicla*) je široko rasprostranjena u Turskoj. Koristi kao antidijabetik u turskoj tradicionalnoj medicini. Cilj ovog istraživanja bio je ispitivanje uticaja blitve na aktivnosti enzima i glikoproteina streptozotocinom (STZ) koji izaziva dijabetični rat testisa. Mužjaci Sprague Dawley štakora korišteni u ovom eksperimentu su bili podijeljeni u tri grupe. Grupa I; Kontrolni uzorci hranjeni citratnim puferom, Grupa II; STZ – dijabetičarski uzorci životinja, Grupa III; STZ – dijabetičarski uzorci životinja hranjeni ekstraktom blitve. Dijabetes je izazvan intraperitonealno za STZ u dozi od 60 mg/kg tjelesne težine. Ekstrakt blitve je davan štakorima u dozi od 2 g/kg svaki dan u toku 45 dana, 15 dana nakon što su životinja dijagnosticirane kao dijabetičari. Tkivo testisa je odstranjeno i homogenizirano na kraju ekperimentalnog perioda. Aktivnost mijeloperoksidaze i proteina karbonila, napredne oksidacije proizvoda proteina, te sadržaj hidroksiprolina i komponenti glikoproteina su povećani, dok aktivnost paraoksonaze je smanjena u tkivima testisa štakora dijabetičara. Ove promjene su bile obrnute nakon administracije blitve u štakora dijabetičara. Ovi rezultati ukazuju da je blitva korisno sredstvo pri smanjenju oštećenja testisa u štakora dijabetičara.



Antioxidant Activities of Various Plant Oils

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Keywords:

Plant oils,
Antioxidant

Abstract: Fats and oils present in many foods may easily deteriorate due to oxidation, in a chain of reactions in which free radicals are formed, propagated, and finally converted into stable oxygenated compounds, which are responsible for off-flavors and other undesirable characteristics. Synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene, and propyl gallate have been used in food industries, but there are some arguments about the safety and adverse effects of these substances when used as food additives. Thus, there is a need for identifying alternative natural and safe sources of food antioxidants. The objective of this study was to measure the antioxidant capacity of twenty five different plant oils such as rose, lavender, jasmine, garlic, corn, olive and clove oils. 2,2-Diphenyl-1-picryl-hydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activities were used to evaluate the antioxidant activities. The results were compared with natural and synthetic antioxidants. Antioxidant activity was increased with increasing oil concentration in all plant oils. All plant oils showed a potential antioxidant activity. We can conclude that these oils can be used in pharmacy and food industries due to their excellent antioxidant activity.

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Sažetak

Masti i ulja, prisutni u različitoj hrani mogu vrlo lako da se razgrade usljed oksidacije, u seriji reakcija u kojima se formiraju slobodni radikali, propagiraju i na kraju pređu u stabilne oksigenizirane spojeve, koji su odgovorni za loš miris i druge neželjene karakteristike. Sintetski antioksidansi kao što su butilirani hidroksianisol, butilirani hidroksitoluen i propil galat se koriste u prehrambenoj industriji, ali postoje određeni dokazi o sigurnosnim i štetnim efektima ovih tvari kada se koriste kao aditivi u hrani. Stoga postoji potreba da se pronađu alternativni prirodni i sigurniji izvori antioksidanasa za hranu. Cilj ovog istraživanja je bio da se izmjeri antioksidativni kapacitet dvadeset pet različitih biljnih ulja kao što su ružino, lavandino, ulje jasmína, ulje bijelog luka, kukuruzno, maslinovo i ulje klinčića. 2,2-difenil-1-pikril-hidrazil (DPPH) i 2,2'-azino-bis (3-etilbenzotiazolin-6-sulfonska kiselina) (ABTS) metode aktivnosti radikalskog istiskivanja su korištene da bi se procijenila antioksidativna aktivnost. Rezultati su upoređivani sa prirodnim i sintetskim antioksidansima. Antioksidativna aktivnost se povećavala sa povećanjem koncentracije ulja u svim biljnim uljima. Sva biljna ulja su pokazala potencijalnu antioksidativnu aktivnost. Možemo zaključiti da se ova ulja mogu koristiti u farmaceutskoj i prehrambenoj industriji zbog svoje odlične antioksidativne aktivnosti.



Antioxidant and Antiacetylcholinesterase Activities of *Sorbus torminalis* (L.) Crantz Fruits

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Keywords:

acetylcholinesterase,
antioxidant activity,
free radicals,
Sorbus torminalis (L.) Crantz,

Abstract: In this study, the antioxidant and antiacetylcholinesterase activities of *Sorbus torminalis* (L.) Crantz (wild service tree) fruits were evaluated. Total phenolic and flavonoid compounds, 2,2'-azino-bis (3-ethylbenzothioazoline-6-sulphonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and superoxide anion (O_2^-) radicals scavenging activities and ferric-reducing antioxidant power (FRAP) of water, ethyl acetate, acetone and methanol extracts were determined for the measurement of the antioxidant activity. Quercetin and α -tocopherol were used as standard antioxidants. The inhibitory effect of the water extract on acetylcholinesterase (AChE) was evaluated using Ellman method and galantamine was used as a standard. The results showed that water extract had the highest total phenolic content and the strongest antioxidant activity followed by ethyl acetate and acetone extracts whereas methanol extract had the lowest phenolic content and the weakest antioxidant activity. Moreover, water extract showed moderate ability to inhibit AChE. It was concluded that fruits of *S. torminalis* had antioxidant and antiacetylcholinesterase activities and that the plant might be a natural source of antioxidants and AChE inhibitors.

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Sažetak

U ovom radu je ispitivana antioksidativna i antiacetylholinesterazna aktivnost voća *Sorbus torminalis* (L.) Crantz (brekinja, "wild service tree"). Sadržaj ukupnih fenola i flavonoidnih jedinjenja, 2,2'-azino-bis (3-etilbenzotioazolina-6-sulfonska kiseline) (ABTS), 2,2-difenil-1-pikrilhidrazil (DPPH) i superoksidnog anionskog (O_2^-) inhibitora aktivnosti slobodnih radikala, antioksidativna aktivnost ispitivana sa sa željezom (FRAP), vodenih, etil acetatnih, acetonskih i metanolnih ekstrakata je određivana mjerenjem antioksidativne aktivnosti. Kvercetin i α -tokoferol su korišteni kao standardi antioksidansa. Inhibitorski uticaj vodenog ekstrakta na acetylholinesterazu (AChE) je određivan Ellmanovom metodom i galantaminom kao standardom. Rezultati su pokazali da je vodeni ekstrakt imao najviši sadržaj fenolnih jedinjenja i najjaču antioksidativnu aktivnost. Etil acetatni i acetonski ekstrakt su imali niže vrijednosti fenolnih jedinjenja i antioksidativne aktivnosti dok je metanolni ekstrakt imao najniži sadržaj fenolnih jedinjenja i najslabiju antioksidativnu aktivnost. Također, vodeni ekstrakt je pokazao umjerenu sposobnost inhibicije AChE. Zaključeno je da voće roda *S. torminalis* posjeduje antioksidativnu i antiacetylholinesteraznu aktivnost i da ova biljka može predstavljati prirodni izvor antioksidansa i AChE inhibitora.



Zinc Supplementation Ameliorates Oxidative Stress Changes in the Lung of Streptozotocin Induced Diabetic Rats

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Keywords:

Diabetes mellitus,
lung,
zinc,
rat.

Abstract: Diabetes mellitus is an endocrine and metabolic disease that affects almost every organ of the body. The lung is a target organ for diabetic microangiopathy. Hyperglycemia causes physiologically important losses of Zn from body. Zn may improve glycemia and helping to prevent complications associated with diabetes. The present study was undertaken in order to evaluate the effect of Zn in lung tissues of streptozotocin-induced diabetic rats. Female Swiss albino rats were divided into 4 groups. Group I, control; Group II, control + zinc sulfate; Group III, STZ-diabetic; Group IV, diabetic + zinc sulfate. Diabetes was induced by intraperitoneal injection of STZ (65 mg/kg body weight). Zinc sulfate was given daily by gavage at a dose of 100 mg/kg body weight every day for 60 days to groups II and IV. At the last day of the experiment, rats were sacrificed and lung tissues were taken. Lung glutathione levels and glutathione peroxidase and aryl esterase activities were decreased, while lipid peroxidation levels, catalase, superoxide dismutase, myeloperoxidase and adenosine desaminase activities were increased in diabetic group. Treatment with Zn reversed these effects. In conclusion, the present study shows that zinc has a protective effect against diabetes-induced lung damage by downregulating oxidative stress.

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Sažetak

Diabetes mellitus je endokrina i metabolička bolest koja zahvata gotovo svaki organ ljudskog tijela. Pluća predstavljaju glavni organ zahvaćen dijabetičkom microangiopatijom. Hiperglikemija uzrokuje fiziološki važne gubitke Zn iz tijela. Zn može poboljšati glikemiju i pomoći u prevenciji komplikacija povezanih sa dijabetesom. Cilj ovog rada je bio evaluacija efekata Zn u plućnom tkivu streptozotocin-induciranih dijabetičnih štakora. Ženke Švicarskih albino štakora su podijeljene u 4 grupe. Grupa I – kontrola; grupa II –kontrola+ cink sulfat; grupa III – STZ dijabetični štakori; grupa IV – dijabetični štakori+cink sulfat. Dijabetes je indiciran STZ intraperitonealnom injekcijom (65 mg/kg tjelesne težine). Cink sulfat je indiciran dnevno u dozi od 100 mg/kg tjelesne težine u period od 60 dana grupama II i IV. Zadnjeg dana eksperimenta, uzeti su uzorci pluća životinja. Nivoi aktivnosti glutaciona u plućima, glutatoin peroksidaze i aril esterase su se smanjili dok su nivoi aktivnosti lipidne peroksidacije, katalaze, superoksid dizmutase, mieloperoksidaze i adenozin desaminaze povećali u dijabetičnim grupama. Tretman sa Zn je obrnuo ove uticaje. Ova studija je pokazala da cink pokazuje protektivni uticaj kod oštećenja pluća nastalih dijabetesom regulacijom oksidativnog stresa.



Quantification of Proline in Honey Using HPLC–ED

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Keywords:

amino acid
imino acid
proline
honey
HPLC – ED
chromatography

Abstract: Proline is a cyclic aliphatic amino acid (AA), one of the major components of collagen, which is the predominant protein of bone, cartilage and connective tissue in the human body. Reduced levels of this AA have been observed in some athletes, especially in runners. It is known that honey is one of many natural nutrients, which has a big contribution to health. These contributions, are also due to free AAs, among which proline is the most common. The aim of this study was to quantify proline in different types of honey from Bosnia and Herzegovina using the high performance liquid chromatography with electrochemical detection (HPLC-ED). For the calculation of proline, the equation of calibration curve was used: $\text{integrated area peak} = f[\text{proline}]_{\text{standard solution}}$. The mobile phase was EDTA, methanol, acetic acid, sodium acetate and water. Experimental parameters of the instrument were: range 50 nA, working potential +0.75 V, filter 0.02 Hz, mobile phase flow rate 1 mL/min, column ODS Hypersil 5 μm , 250x4.6 mm, Phenomenex. 43 samples of honey were analysed. The highest content of proline was in meadow honey (6.02 mg/100 g) and the lowest was in honey from linden (0.70 mg/100 g).

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Sažetak

Prolin je ciklična alifatska aminokiselina (AK), jedna od glavnih gradivnih komponenti kolagena, kao najzastupljenijeg proteina kostiju, hrskavice i vezivnog tkiva ljudskog organizma. Sniženi nivoi ove AK uočeni su kod nekih sportista, a posebno kod maratonaca. Poznato je da se pčelinji med ubraja u prirodni nutrijent sa brojnim doprinosima za zdravlje. Ti doprinosi su, pored ostalog, zahvaljujući sadržaju slobodnih AK, od kojih je prolin najzastupljenija. Ovo istraživanje imalo je za cilj da se primjenom metode tačne hromatografije visoke efikasnosti, uz elektrohemijску detekciju (HPLC–ED), odredi sadržaj prolina u različitim vrstama meda sa prostora Bosne i Hercegovine. Za izračunavanje sadržaja prolina korištena je jednačina kalibracionog pravca: površina integrala pika = $f[\text{prolin}]_{\text{standarda otopina}}$. Mobilna faza je bila EDTA, acetatna kiselina, metanol, natrij-acetat i voda. Eksperimentalni uslovi metode bili su: range 50 nA, radni potencijal +0,75 V, filter 0,02 Hz, brzina protoka mobilne faze 1 mL/min, kolona ODS Hypersil 5 μm ; 250x4,6 mm, Phenomenex. Analizirana su 43 uzorka meda. Najviše prolina zastupljeno je u livadskom medu (6,02 mg/100 g), a najmanje u medu od lipe (0,70 mg/100 g).



Identification of Proline in Honey Using HPLC-ED Method

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Keywords:

Proline
Honey
HPLC-ED
Amino acid

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Abstract: Proline is a hydrophobic amino acid with an aliphatic cyclic structure in side chain. It is not an essential amino acid in the organism, but it is an important component of collagen. Proline is the most dominant amino acid in honey, used for the determination of its quality. The aim of this work was to identify proline in samples of different types of natural bee honey and from different locations using high-performance liquid chromatography with electrochemical detection (HPLC-ED). The investigation was performed with 43 honey samples. Three different mobile phases at working potential of 750 mV were used for optimization of the method. The working potential was optimized in the interval 350 – 850 mV. The mobile phase composed of EDTA (372 mg/L):sodium acetate (13.61 g/L):methanol (50%, 63.3 ml/L):concentrated acetic acid (7.9 ml/L) was chosen as the most appropriate for the assay. The other experimental conditions were: working potential of 750 mV, mobile phase flow rate 1.00 ml/min, range 50 nA, filter 0.02 Hz, column ODS Hypersil 5 µm; 250x4.6 mm, Phenomenex. Retention time for the detection of proline at these conditions in the samples was 5.80±0.17 min, and 5.89±0.10 min for the standard of proline.

Sažetak

Prolin je hidrofobna aminokiselina sa alifatskom cikličnom strukturom u bočnom lancu. Nije esencijalna aminokiselina, ali je važan sastojak kolagena. Prolin je najdominantnija aminokiselina u medu i koristi se za određivanje njegovog kvaliteta. Cilj ovog rada je identifikacija prolina u uzorcima prirodnog pčelinjeg meda različitih vrsta i sa različitih lokacija metodom tečne hromatografije visoke efikasnosti uz elektrohemijski detektor (HPLC-ED). Analiza je vršena na 43 uzorka meda. Za optimizaciju metode korištene su tri različite mobilne faze pri radnom potencijalu od 750 mV. Radni potencijal je optimiziran u intervalu 350-850 mV. Mobilna faza sastava EDTA (372 mg/L):natrijum-acetat (13,61 g/L):metanol (50%, 63,3 ml/L):koncentrirana acetatna kiselina (7,9 ml/L) pokazala se kao najprikladnija za ovu vrstu detekcije. Ostali radni uslovi bili su: radni potencijal 750 mV, brzina protoka mobilne faze 1,00 ml/min, range: 50 nA, filter: 0,02 Hz, kolona: ODS Hypersil 5 µm; 250x4,6 mm, Phenomenex. Pri navedenim uslovima dobijeno je da se prolin detektuje u uzorcima pri retencionom vremenu 5,80±0,17 min, te pri 5,89±0,10 min. kao standard.



Fluorimetric Determination of Thiamine in Baby Cereal Food

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Keywords:

baby cereal-based food,
grains,
fortification, thiamin,
fluorimetric thiochrome method

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Abstract: Grains are used as processed products and therefore have a lower nutritional content. Fortification of the products is a useful procedure which increases the daily intake of vitamin B group. Thiamine is crucial in intercellular glucose metabolism and essential for normal growth and development, helps to maintain proper functioning of the heart, nervous and digestive systems. Thiamine content was determined in twelve, commercially available, baby cereal food using fluorimetric method. Combination of acid digestion and enzymatic degradation released vitamin bound to protein. Samples were hydrolyzed with 0.1 M sulfuric acid (H₂SO₄) at 105-110°C to break protein complexes and effectively liberate thiamin. Phosphorylated thiamin esters were converted to a free thiamin using enzyme clarase. The digest was incubated at 45°C for 60 min and filtrated. An aliquot of extract was oxidized with 1% potassium ferricyanide in 15% sodium hydroxide to form highly fluorescent thiochrome. Thiochrome was extracted with isobutanol and the fluorescence was measured at excitation and emission wavelengths of 375 nm and 430 nm, respectively. Total thiamin content in 12 baby cereal food samples, analyzed with fluorimetric thiochrome method, varied from 0,59 mg/100 g to 1,87 mg/100 g. Free thiamine content varied from 0,32-1,73 mg/100 g. Content of thiamin diphosphate made up 4,84-51,26 % of total thiamin.

Sažetak

Upotreba prerađenih žitarica za posljedicu ima nižu nutritivnu vrijednost prehrambenih proizvoda. Fortifikacija takvih proizvoda je korisna procedura kojom se povećava dnevni unos vitamina B grupe. Tiamin je važan u intercelijskom metabolizmu glukoze i esencijalan za normalan rast i razvoj, a pomaže održavanje pravilnog funkcionisanja srca, nervnog i probavnog sistema. Fluorimetrijskom metodom je određivan sadržaj tiamina u dvanaest komercijalno dostupnih uzoraka dječije hrane na bazi žitarica. Kombinacijom kiselinske i enzimске hidrolize oslobađa se za proteine vezani tiamin, kao i tiamin iz fosfatnih formi. Hidroliza sa 0,1 M H₂SO₄ kiselinom pri 105-110°C kida proteinske komplekse, čime se oslobađa tiamin. Enzim klaraza je korišten za prevođenje fosfatiranih tiaminskih formi u slobodan tiamin, inkubacijom na 45°C, u trajanju od 60 minuta. Nakon filtracije, ekstrakt uzorka se oksidira sa 1% kalijheksacijanoferatom u 15%-oj vodenoj otopini NaOH, čime se formira visoko fluorescirajući tihrom. Tihrom se ekstrahuje sa izobutanolom i potom se mjeri intenzitet fluorescencije pri talasnoj dužini ekscitacije od 375 nm i talasnoj dužini emisije od 430 nm. Sadržaj ukupnog tiamina u uzorcima dječije hrane na bazi žitarica, koji su analizirani tihrom metodom, bio je u intervalu od 0,59 mg/100 g do 1,87 mg/100 g, dok je interval za slobodni tiamin iznosio 0,32-1,73 mg/100 g. Sadržaj tiamin difosfata čini od 4,84-51,26% ukupnog tiamina.



Levels of Erythropoietin in Patients with Varying Degrees of Renal Insufficiency and Anemia

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Keywords:

erythropoietin,
renal insufficiency,
anemia,
creatinine clearance,
hemoglobin

Abstract: Chronic renal insufficiency leads to hyporegenerative anemia due to the lack of erythropoietin (Epo). In order to determine the value of Epo in patients with various kidney diseases, research was conducted on 300 patients with varying degrees of renal impairment in the Nephrology Department and Nephrology counseling center. These subjects were divided into four groups according to the degree of renal impairment. For these studies, human urine, serum and whole blood samples were used. Creatinine clearance (CC) and hemoglobin (Hb) were also measured. To obtain the reference group, 109 healthy subjects were sampled as well. Epo was determined by the chemiluminescent enzyme immunometric method of solid phase by IMMULITE/IMMULITE 1000 from Siemens. The concentration of Hb was determined by a blood cell counter (Abbott Cell Dyn 3700 system), which uses a modified hemoglobin-cyanide or hemoglobin-hydroxylamine colorimetric method. CC was determined by the kinetic method based on the Jaffe reaction using auto-analyzer Dimension RxL from Siemens. The results show that the determinations of Epo, CC, and Hb are good laboratory indicators of renal insufficiency and anemia.

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Sažetak

Hronična renalna insuficijencija dovodi do hiporegenerativne anemije zbog nedostatka eritropoetina (Epo). Da bi se utvrdile vrijednosti Epo kod oboljelih od različitih bubrežnih oboljenja sprovedeno je ispitivanje na 300 osoba sa različitim stepenom bubrežnog oštećenja sa nefrološkog odjeljenja i nefrološkog savjetovaništa. Ovi ispitanici su podijeljeni u četiri grupe prema stepenu bubrežnog oštećenja. Za ova ispitivanja korišteni su humani uzorci i to: urin, serum i puna krv. Određivane su i vrijednosti klirensa kreatinina (KK) i hemoglobina (Hb). Za dobijanje referentne grupe uzeti su uzorci 109 zdravih ispitanika. Epo je određivan enzimatsko-kemiluminiscentnom imunometrijskom metodom na čvrstoj fazi na IMMULITE/IMMULITE 1000 kompanije Siemens. Za određivanje koncentracije Hb koristio se brojač krvnih elemenata (Abbott Cell Dyn 3700 system), koji koristi modificiranu hemoglobin-cijanidnu ili hemoglobin-hidroksilaminsku kolorimetrijsku metodu. KK je određivan kinetičkom metodom na bazi Jaffeove reakcije na autoanalizatoru Dimension RxL kompanije Siemens. Dobiveni rezultati pokazuju da su određivanja Epo, KK i Hb dobri laboratorijski pokazatelji renalne insuficijencije i anemije.



Isolation, amplification and profiling DNA from blood stains treated with different reagents

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Keywords:

Profiling DNA,
blood stains,
reagents,
DNA degradation,
Identification.

Abstract: In these modern times, forensic analysis and establishment identity of the person - used for various purposes, is unimaginable without DNA profiling. The aim of this paper was to examine the influence of different types of agents-which in realistic cases can be found on the place of biological trace discovery-in the aspects of isolation, amplification and DNA profiling. The biological trace used in this research was a blood smear applied on filter paper and treated with the following agents: : 0,1 M NaOH, 50 % CH₃COOH, olive oil, detergent and gasoline. The samples were situated in a laboratory for 10 days, on the temperature of 20°C. DNA isolation was performed by *Miller's salting out procedure*, quantification with gel electrophoresis, and the amplification with the *PowerPlex®16 System* commercial kit. Detection was made by *ABI PRISM® 310* genetic analyzer which is based on the capillary electrophoresis, combined with *310 Collecting Data Software*. The samples of the former treatment were compared to the undisputed buccal mucus sample. The sample treated with 0,1 M NaOH gave a complete DNA profile, expected because of the fact that pH=13 makes DNA resistant to hydrolysis. Remaining four treated samples gave partial DNK profiles, and the sample treated with detergent also gave a mixed profile which pointed to DNA degradation, contamination or some other interaction of the used agents with DNA. We can conclude that, based on the results, the sample treated with 0,1 M NaOH can be undeniably used for identification, while the other samples need to be combined with other evidence.

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Sažetak

Forenzička analiza i utvrđivanje identiteta osobe, u različite svrhe, u današnje vrijeme je nezamislivo bez DNK profiliranja. Cilj ovog rada je bio ispitati utjecaj različitih vrsta agenasa, koji se u realnim slučajevima mogu naći na mjestu pronalaska biološkog traga, na izolaciju, amplifikaciju i profiliranje DNK. Kao biološki trag korištena je krvna mrlja nanešena na filter papir i tretirana sljedećim agensima : 0,1 M NaOH, 50 % CH₃COOH, maslinovim uljem, deterdžentom i benzinom. Uzorci su smješteni u laboratorij i čuvani 10 dana, na 20°C. Izolacija DNA je izvršena *Millerovim postupkom isolvavanja*, kvantifikacija postupkom gel elektroforeze, a amplifikacija pomoću *PowerPlex®16* komercijalnog kita. Detekcija je vršena pomoću *ABI PRISM® 310* genetičkog analizatora koji se zasniva na principu kapilarne elektroforeze, u kombinaciji sa *310 Collecting Data Software*. Uzorci su upoređivani sa nespornim uzorkom brisa bukalne sluznice. U ovisnosti od korištenog reagensa dobiveni su i različiti rezultati. Uzorak tretiran 0,1 M NaOH dao je potpun DNK profil, što se i očekivalo zbog činjenice da je pri datom pH DNK otporna na hidrolizu. Preostala četiri tretirana uzorka dala su parcijalne DNK profile, a kod uzorka tretiranog deterdžentom dobijen je i mješani profil što ukazuje na degradaciju DNK, kontaminaciju ili eventualno neku drugu interakciju korištenih agenasa sa DNK. Na osnovu dobivenih rezultata moglo bi se zaključiti da se uzorak tretiran sa 0,1 M NaOH nedvojbeno može iskoristiti za identifikaciju, dok se preostala četiri uzorka mogu upotrijebiti tek u kombinaciji sa drugim dokaznim materijalom.



GC-MS analysis of sertraline in human urine

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Keywords:

Sertraline,
Gas Chromatography-Mass
Spectrometry;
Liquid-Liquid Extraction;
Solid-Phase Extraction.

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Abstract: Sertraline (Zoloft) is a selective serotonin reuptake inhibitor that is a commonly prescribed drug for the treatment of depression, as well as obsessive-compulsive disorder, panic disorder, social anxiety disorder, premenstrual dysphoric disorder, and post-traumatic stress disorder. A gas chromatographic-mass spectrometric (GC-MS) method was developed for detection of sertraline in urine. Liquid-liquid and solid phase extraction were applied to urine samples using methadon as an internal standard (IS). The GC-MS analysis were carried out using HP-5MS capillary column. The linearity ranges of the method were 0,025-2,0 mg/L for both LLE and SPE. The detection limits were achieved under 0,097 mg/L. The range of recoveries were 75-110% by LLE and 71-116% by SPE for analyte. The GC-MS method developed for detection of sertraline in urine appears to be very precise, it shows good linearity and a wide range of detection. The developed method allowed clinical and toxicological analysis of sertraline in urine samples. With this method it is possible to determine the presence of antidepressants and other psychoactive substances at the same time.

Sažetak

Sertralin (Zoloft) je selektivni inhibitor povrata serotonina koji se često koristi za liječenje depresije, opsesivno kompulzivnih poremećaja, paničnih napada, kod poremećaja socijalne anksioznosti i PTSP-a. Za detekciju sertralina je razvijena GC-MS metoda. Tečno-tečno ekstrakcija (LLE) i ekstrakcija na čvrstoj fazi (SPE) su korištene u pripremanju uzoraka urina, koristeći metadon kao interni standard. Za GC-MS analize korištena je P-5MS kapilarna kolona. Linearnost metode je bila u području 0,025-2,0 mg/L za tečno-tečnu ekstrakciju kao i za ekstrakciju na čvrstom nosaču. Limit detekcije je bio ispod 0,097 mg/L. Efikasnost ekstrakcije za analit je bila 75-110% za LLE i 71-116% za SPE. GC-MS metoda razvijena za detekciju sertralina u urinu je veoma precizna, pokazuje dobru linearnost i širok raspon određivanja. Razvijena metoda je uspješno korištena za kliničke i toksikološke analize sertralina u uzorcima urina. Pokazalo se da je metoda primjenjiva za istovremeno određivanje antidepressiva i drugih psihoaktivnih supstanci.



Antioxidant Activity of *Ornithogalum sigmoideum* Frey et Sint.

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Keywords:

Antioxidant activity,
Ornithogalum sigmoideum.

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Abstract: *Ornithogalum sigmoideum* Frey et Sint. is native to Balkan Peninsula, Caucasus, Turkey and northern Iran. This plant is widely used in Black Sea region of Turkey for consumption in the daily diet. Moreover, it is used as an antiinflammatory and anticancer agent and might help improve liver and immune system functions in people with hepatitis. In the present study, different antioxidant tests were employed in order to evaluate the antioxidant activities of water, ethanol, and acetone extracts of *O. sigmoideum*, namely, reducing power, free radical scavenging (DPPH), hydroxyl radical scavenging, ABTS radical scavenging, metal chelating activities. The results were compared with natural and synthetic antioxidants, e.g. α -tocopherol, butylated hydroxyanisole, butylated hydroxytoluene. The level of total phenolics and total flavonoids of the extracts were also determined. It was found that antioxidant activity increased with increased concentrations of the extracts. It can be concluded that because of the highest antioxidant activity of acetone extract, which was rich in flavonoids, there could be a relation between flavonoids and antioxidant activity. This study showed that *O. sigmoideum* extracts exhibited antioxidant activity in all tests and that the extracts could be considered as a source of natural antioxidants.

Sažetak

Ornithogalum sigmoideum Frey et Sint. je rasprostranjen na Balkanskom poluotoku, Kavkazu, Turskoj i sjevernom Iranu. Ova biljka se naširoko koristi u crnomorskoj regiji Turske u svakodnevnoj prehrani. Nadalje, koristi se kao antiupalni i antikancerogeni agens i može da potpomogne poboljšanju funkcije jetre i imunog sistema kod ljudi koji boluju od hepatitisa. U ovom radu, upotrijebljeni su različiti antioksidativni testovi s ciljem da se ocjeni antioksidativna aktivnost vodenog, etanolskog i acetonskog ekstrakta *O. sigmoideum*, tj. redukcijska moć, istiskivanje slobodnih radikala (DPPH), istiskivanje hidroksil radikala, istiskivanje ABTS radikala i metal helatne aktivnosti. Rezultati su upoređeni sa prirodnim i sintetskim antioksidansima, npr. α -tokoferol, butilirani hidroksianisol, butilirani hidroksitoluen. Također su utvrđeni ukupni fenoli i ukupni flavonoidi u ekstraktima. Nađeno je da se antioksidativna aktivnost povećava s povećanjem koncentracije u ekstraktima. Može se zaključiti da zbog najveće antioksidativne aktivnosti acetonskog ekstrakta, koji je bogat flavonoidima, može postojati veza između flavonoida i antioksidativne aktivnosti. Ovo istraživanje je pokazalo da su *O. sigmoideum* ekstrakti pokazali antioksidativnu aktivnost u svim testovima i da se ekstrakti mogu uzeti u obzir kao izvori prirodnih antioksidansa.



Antioxidant Activity of Phytoestrogens

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Keywords:

Antioxidant activity,
Phytoestrogens,
Phloretin,
Phloridzin.

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Abstract: Phytoestrogens are a group of compounds that display estrogen like properties. Phloretin is a type of flavonoid that has beneficial anticancer properties and primarily derived from various species of apple leaves. It is commonly used as a new type of whitening agent in cosmetics. Phloridzin belongs to the chemical dihydrophenylpropanoids with structures closely related to those of the immediate flavonoid precursors, the calcones. Phloridzin and its derivatives have been widely used in medicine and for physiological studies on biological membranes. Antioxidants are natural molecules important for life because they that help protect the body from harmful free radicals. Due to these facts, most of scientific research is based on antioxidants. In the present study, we have established the antioxidant activities of phloridzin and phloretin which are phytoestrogens used in menopausal symptoms, cardiovascular diseases, prostate cancers, diabetes and other various diseases. These tests used for antioxidant activity are; reducing power, DPPH radical scavenging, ABTS radical scavenging, DMPD radical scavenging activities and Cuprac test. The results were compared to natural and synthetic antioxidants. It was found that antioxidant activity increased with increased concentrations of phloretin and phloridzin. It was determined that both phloridzin and phloretin show antioxidant activity in all tests. For reason, these two products could also be used an antioxidants.

Sažetak

Fitoestrogeni su grupa jedinjenja koja pokazuju ponašanje slično estrogenima. Floretin je vrsta falonoida koji ima antikancer osobine i koji se dobiva iz lišća različitih vrsta jabuka. Često se koristi i kao sredstvo za bijeljenje u kozmetici. Floridzin pripada dihidrofenilpropanoidima sa strukturom koja odgovara flavonoidnim prekursorima, kalconima. Floridzin i njegovi derivati se jako koriste u medicine i za fiziološke studije na biološkim membranama. Antioksidansi su prirodne molekule važne za život zbog toga što štite tijelo od štetnih slobodnih radikala. Zbog toga je veliki broj naučnih istraživanja baziran na antioksidansima. U ovom radu smo odredili antioksidativne aktivnosti floridzina i floretina, fitoestrogena, koji se koriste za prevenciju simptoma menopause, kardiovaskularnih oboljenja, tretmanu kancera prostate, dijabetesa i drugih oboljenja. Testovi korišteni za određivanje antioksidativne aktivnosti su bili; stepen redukcije, DPPH inhibicija radikala, ABTS inhibicija radikala, DMPD inhibicija radikal i Cuprac test. Rezultati su upoređeni sa prirodnim i sintetskim antioksidansima. Utvrđeno je da se antioksidativna aktivnost povećava sa povećanjem koncentracije floretina i floridzina. Utvrđeno je da floridzin i floretin pokazuju antioksidativnu aktivnost u svim testovima. Zbog toga se oba ova produkta mogu koristiti kao antioksidansi.



Spectrophotometric determination of total phenolics and total flavonoids contents in extracts of different *Ephedra* species

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Abstract: Extracts of *Ephedra*, an evergreen shrub-like plant, have been used in traditional medicine to treat conditions such as colds, asthma and nasal congestion. Phenolic compounds and flavonoids as their subgroup represent aromatic secondary metabolites in plants and include a diverse group of molecules that are essential for their growth, reproduction and defense against pathogens. Considerable attention has been drawn to their potentially preventive role in human chronic diseases and antioxidant activity. The aim of this study was to determine the content of total phenolics and total flavonoids in herb extracts of different *Ephedra* species. The total phenolics content was determined spectrophotometrically using the Folin-Ciocalteu reagent. Gallic acid was used as a standard. Analyses were performed in cold water extracts and in water-organic solvent mixture (methanol-water-acetic acid) extracts. The total flavonoids content was also determined spectrophotometrically using the aluminium chloride method in water-organic solvent extracts only. Quercetin was used as standard. Results showed that the total phenolics content was higher in water-organic solvent extracts and there the values ranged between 0.67 mg GAE/g_{dry weight (d.w.)} and 3.13 mg GAE/g_{d.w.}. The total flavonoids content in the species of interest varied between 0.48 mg QE/g_{d.w.} and 2.13 mg QE/g_{d.w.}.

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Sažetak

Ekstrakti efedre, zimzelene biljke nalik na grm, koristili su se u tradicionalnoj medicini za stanja kao što su prehlade, astma i nazalne kongestije. Fenolni spojevi i flavonoidi kao njihova podgrupa predstavljaju sekundarne aromatske metabolite biljaka i obuhvataju raznovrsnu grupu molekula značajnih za njihov razvoj, reprodukciju i zaštitu od patogena. Značajna pažnja je poklonjena njihovoj potencijalno preventivnoj ulozi u hroničnim oboljenjima kod ljudi, te antioksidativnoj aktivnosti. Cilj ovog rada je bio odrediti sadržaj ukupnih fenola i ukupnih flavonoida u ekstraktima herbe različitih *Ephedra* vrsta. Sadržaj ukupnih fenola određen je spektrofotometrijskom metodom upotrebom Folin-Ciocalteu reagensa i galne kiseline kao standarda. Analize su izvedene u hladnim vodenim ekstraktima i ekstraktima iz smjese voda-organsko otapalo (metanol-voda-acetatna kiselina). Sadržaj ukupnih flavonoida (samo u ekstraktima iz smjese voda-organsko otapalo) također je određen spektrofotometrijski upotrebom metode sa aluminij hloridom i kvercetina kao standarda. Rezultati su pokazali da je sadržaj ukupnih fenola bio viši u ekstraktima iz smjese voda-organsko otapalo (0,67-3,13 mg GAE/g_{suhe droge (s.d.)}). Sadržaj ukupnih flavonoida u vrstama od interesa varirao je u intervalu 0,48-2,13 mg QE/g_{s.d.}.



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Production of phenylpropanoids and naphthodiantrones in callus cultures of *Hypericum perforatum* L. elicited with dextran

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Keywords:

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Abstract: *Hypericum perforatum* L. callus cultures were evaluated for their growth, antioxidant potential and ability to produce phenylpropanoids (phenolics, flavonoids, flavanols and anthocyanins) and naphthodiantrones (hypericin and pseudohypericin) after elicitation with different dextran concentrations (50-200 mg/L). Non-enzymatic antioxidant properties (NEAOP) and specific activity of antioxidant enzymes such as peroxidase (POD) and catalase (CAT) were observed in callus culture extracts. The activities of two key enzymes of the phenylpropanoid/flavonoid pathways, phenylalanine ammonia lyase (PAL) and chalcone isomerase (CHI) were also monitored to estimate channeling in the different metabolic pathways. Treatment with dextran did not affect the growth of *H. perforatum* calli after 21 day of elicitation. Exogenously applied dextran induced NEAOP, POD and CAT activities in treated calli suggesting a strong perturbation of the cell redox system leading to the activation of defense responses. The elevated activities of PAL and CHI confirmed an efficient activation of the phenylpropanoid/flavonoid pathway. Furthermore, naphthodianthrone and phenylpropanoid productions were globally stimulated upon elicitation with all tested concentrations of dextran. Therefore, dextran application seems to be a promising approach for enhancement of secondary metabolite productions in callus cultures and can also be used to further extend other plant culture models.

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Sažetak

Hypericum perforatum L. callus kulturama je ispitivan rast i razvoj, antioksidativni potencijal i sposobnost proizvodnje fenilpropanoida (fenola, flavonoida, flavanola i antocijanina) i naftodiantrona (hipericina i pseudohipericina) nakon elicitacije sa različitim koncentracijama dekstrana (50-200 mg/L). Ne-enzimatske antioksidativne osobine (NEAOP) i specifična aktivnost antioksidativnih enzima kao što su peroksidaze (POD) i katalaze (CAT) su praćene u ekstraktima callus kultura. Aktivnosti dva ključna enzima fenilpropanoidnog/flavonoidnog reakcionog puta, fenilalanine amonij liaze (PAL) i kalkon izomeraze (CHI) su također praćene u cilju procjene kanaliziranja u različitim metaboličkim procesima.

Tretman sa dekstranom nije uticao na rast *H. perforatum* calli nakon 21 dana od elicitacije. Egzogeno primjenjeni dekstran je inducirao NEAOP, POD i CAT aktivnosti u calli ukazujući na jaku perturbaciju ćelijskog redoks sistema koja dovodi do aktivacije mehanijske ćelijske odbrane. Povišene aktivnosti PAL i CHI su potvrdile efikasnu aktivaciju fenilpropanoidnog/flavonoidnog reakcionog puta. Također, proizvodnja naftodiantrona i fenilpropanoida su jako stimulirane nakon elicitacije sa svim testnim koncentracijama dekstrana. Zbog toga, primjena dekstrana predstavlja obećavajući pristup za poboljšanje proizvodnje sekundarnih metabolite u callus kulturama i može se koristiti za primjenu na druge kulture biljnih modela.



Acetylcholinesterase and butyrylcholinesterase inhibitory activity of extracts from medicinal plants

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Abstract: Inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), enzymes which breakdown acetylcholine and butyrylcholine, are considered as a promising strategy for the treatment of Alzheimer's disease (AD). Alzheimer's disease is chronic neurological disorder characterized by memory impairment, cognitive dysfunction and behavioral disturbances. A potential source of AChE and BChE inhibitors is provided by the abundance of plants in nature. In the present study, we selected five plants used in traditional medicine to treat different disorders of the central nervous system. Aqueous and methanolic extracts of sage (*Salvia officinalis* L.), arnica (*Arnica montana* L.), rue (*Ruta graveolens* L.), St John's wort (*Hypericum perforatum* L.) and aronia (*Aronia melanocarpa* (Michx.) Elliot.) were tested for the AChE and BChE inhibitory activity using Ellman's colorimetric method. Galanthamine hydrobromide was used as positive control. The results show that extracts from the aerial parts of St John's wort, sage, and rue and flowers of arnica could inhibit the activity of AChE or BChE or both. The best inhibition effect was observed using the methanolic extracts of St John's wort and arnica at concentration of 400 µg mL⁻¹.

Sažetak

Inhibicija enzima koji razgrađuju acetilkolin, acetilkolinesteraze (AChE) i butirilkolinesteraze (BChE), smatra se obećavajućom strategijom za liječenje Alzheimerove bolesti (AD). Alzheimerova bolest je kronični neurološki poremećaj koji se očituje u smanjenju pamćenja, kognitivne disfunkcije i poremećajem ponašanja. Obilje biljaka u prirodi pruža potencijalni izvor inhibitora za AChE i BChE. U ovom istraživanju odabrali smo pet biljaka koje se koriste u tradicionalnoj medicini za liječenje različitih poremećaja središnjeg živčanog sustava. Pomoću Ellmanove kolorimetrijske metode testiran je inhibicijski učinak vodenih i metanolnih ekstrakata kadulje (*Salvia officinalis* L.), arnike (*Arnica montana* L.), rute (*Ruta graveolens* L.), gospine trave (*Hypericum perforatum* L.) i aronije (*Aronia melanocarpa* (Michx.) Elliot.) na AChE i BChE. Kao pozitivna kontrola korišten je galntamin hidrobromid. Rezultati ukazuju da ekstrakti nadzemnih dijelova gospine trave, kadulje i rute i cvjetova arnike mogu inhibirati aktivnost AChE ili BChE, ili oboje. Najjači inhibicijski učinak uočen je kod metanolnih ekstrakata gospine trave i arnike pri koncentraciji od 400 µg mL⁻¹.