

Changes in expression of selected genes in California poppy seedlings (*Eschscholzia californica* Cham.) In relation to alkaloid production

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Abstract **AIM:** Our work deals with the effect of precursor feeding (tyrosine, dopamine and DOPA) on alkaloid production and gene expression of selected enzymes (isoenzymes of stylophine synthase CYP719A2, CYP719A3 and (S)-N-methylcoclaurine-3'-hydroxylase CYP80B1) involved in the benzophenanthridine alkaloid pathway in California poppy seedlings.

MATERIAL/METHODS: Seedlings of California poppy were germinated for 5 days on media containing 0.001 mol l⁻¹ of tyrosine, dopamine and DOPA. The content of alkaloids was determined in methanolic extracts of seedlings by fluorescence spectroscopy at excitation/emission wavelength 324/408 nm for sanguinarine and 294/398 nm for chelerytrine, respectively. Gene expression of selected enzymes in the benzophenanthridine alkaloid pathway was determined by quantitative polymerase chain reaction (qPCR).

RESULTS: The highest amounts of sanguinarine and chelerytrine were determined in seedlings germinated on media containing tyrosine and DOPA. Dopamine elicited the production of both alkaloids markedly lower than previously mentioned precursors. The increased expressions of CYP80B1 were observed in seedlings grown in the presence of all tested precursors. In the case of isoenzymes CYP719A2 and CYP719A3, moderate decrease in the expressions was observed in all seedlings.

CONCLUSIONS: Precursors present in the growth media of seedlings stimulated the formation of sanguinarine and chelerytrine during 5 days of germination. Among the selected enzymes involved in the benzophenanthridine alkaloid pathway, only CYP80B1 exhibited increased expression in seedlings supplied with tyrosine, dopamine and DOPA.

Keywords California poppy – sanguinarine – chelerytrine – gene expression – qPCR

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The genetic resources of medicinal plants in the gene bank of the Slovak Republic

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Abstract

AIM: The aim of the work with genetic resources is their conservation, multiplication, evaluation and dissemination according to National Programme of Conservation of PGR.

MATERIAL/METHODS: All the genotypes are evaluated according to international descriptors for morphological, biological and yield data. All measured data are entered into passport and description databases.

RESULTS: 256 samples of medicinal plants are collected and stored in Gene bank in Piešťany. 254 accessions are in the active collection, 42 accessions are in basic collection and 15 accessions are vegetatively propagated plants in field collection. Abundance within the family is as follows: *Asteraceae* - 64, *Lamiaceae* - 71, *Hypericaceae* - 31, *Plantaginaceae* - 24, *Scrophulariaceae* - 22, *Rhamnaceae* - 19, *Ranunculaceae* - 16, *Droseraceae* - 11, *Caryophyllaceae* - 10, *Liliaceae* - 10. The most abundant species are *Achillea* - 36, *Hypericum* - 31, *Thymus* - 30, *Plantago* - 24, *Ocimum* - 16.

CONCLUSIONS: The obtained results are registered in passport and characterization and evaluation data.

Keywords

genetic resources – medicinal plants – evaluation – descriptor – genus

Acknowledgements

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Lipoxygenase from opium poppy (*Papaver somniferum* L.) and its involvement in the signalling pathway leading to alkaloid biosynthesis

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Abstract

AIM: Opium poppy (*Papaver somniferum* L.) is one of the most important medicinal plants producing a large number of benzylisoquinoline alkaloids. The aim of the present work was to study the possible role of lipoxygenase enzyme (LOX, linoleate: oxygen oxidoreductase, EC 1.13.11.12) in the signal transduction process leading to pharmaceutically utilizable secondary metabolite biosynthesis.

MATERIAL/METHODS: Four-day-old seedlings of *Papaver somniferum* (80 g) were used for extraction and purification procedures. LOX activity was determined by spectrophotometric method at 234 nm and expression of LOX proteins was monitored by immunoblotting. The transcription analysis of selected genes was detected after total RNA isolation and its reverse transcription using the method of quantitative polymerase chain reaction (qPCR).

RESULTS: LOX from *Papaver somniferum* L. seedlings was purified using ammonium sulphate precipitation followed by hydrophobic chromatography (Phenyl-Sepharose CL-4B), ion exchange chromatography (Q-Sepharose) and affinity chromatography (linoleyl-aminopropyl agarose). The identity of the purified LOX was confirmed by immunoblot analysis where a single intense band was obtained. The expression of LOX enzyme and the selected key genes involved in the alkaloid biosynthesis was analysed during the germination of opium poppy seeds using qPCR method.

CONCLUSIONS: The correlation between LOX gene expression and the expression of selected genes was observed. The obtained results indicate that LOX enzyme participates in the signalling pathway, which can affect the biosynthesis of opium alkaloids.

Keywords

Papaver somniferum – lipoxygenase – biochemical characterization – lipoxygenase pathway

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Phytochemical analysis of *Amorpha fruticosa*, Fabaceae and its biological activity

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Abstract **AIM:** The extract of *Amorpha fruticosa*, Fabaceae is applied traditionally to treat hypertension, contusions and hematomas in China. Human macrophages (cell line THP-1) were used for evaluation of anti-inflammatory potential of different parts of *A. fruticosa*. First, the cytotoxicity was tested followed by measurement of ability of extracts to decrease TNF- α secretion in lipopolysaccharide-stimulated cells by enzyme-linked immunosorbent assay (ELISA). Phytochemical analysis of leaf extracts of *A. fruticosa* led to identification of several compounds.

MATERIAL/METHODS: Anti-inflammatory potential was evaluated based on the ability of tested extracts to attenuate lipopolysaccharide-induced expression of TNF- α in the macrophage-like cell line THP-1. The amount of secreted TNF- α was determined by ELISA. The leaf extract was analysed by HPLC-DAD-MS and separated by column chromatography and the semi-preparative HPLC. Isolated compounds were identified by NMR.

RESULTS: In our study, the extract prepared from different parts of *A. fruticosa*, especially leaf extract, inhibited TNF- α production in lipopolysaccharide-stimulated cells by ELISA. The activity was comparable to the standard prednisone. Phytochemical analysis of extracts of *A. fruticosa* led to the identification of several compounds, which could contribute to anti-inflammatory potential of the plant.

CONCLUSIONS: *A. fruticosa*, traditionally used in China, is a perspective plant. Isolated compounds could contribute to anti-inflammatory potential of this plant.

Keywords *Amorpha fruticosa* – HPLC analysis – TNF- α

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Lipoxygenase and transcription of secondary metabolism genes in *Papaver somniferum* L.

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Abstract

AIM: To study connection between lipoxygenase (LOX) signalling cascade and transcription of selected genes involved in the biosynthesis of alkaloids in opium poppy under stress conditions.

MATERIAL/METHODS: The experiments were performed on opium poppy plants. Plants were growing hydroponically for 5 weeks and then were incubated for 24 hours in 50 µM solution of LOX inhibitor – phenidone – or without inhibitor (control), respectively. Then, leaves were mechanically wounded and incubated for 1 or 3 hours. Total RNA was isolated from ground leaves by TRIzol method. Three gene transcripts (coclaurine *N*-methyltransferase, 3-hydroxy-*N*-methylcoclaurine 4-*O*-methyltransferase, salutaridinol 7-*O*-acetyltransferase) involved in benzylisoquinoline alkaloids pathway have been analysed. Real-time qPCR analysis was used to study the expression of transcripts under stress conditions with gene-specific primers.

RESULTS: Real-time qPCR analysis showed a reduction of expression levels in all three transcripts after addition of a specific LOX inhibitor in comparison with the control group. The results indicate that inhibition of LOX may reduce transcription of genes involved in the biosynthesis of alkaloids.

CONCLUSIONS: Inhibition of LOX in *Papaver somniferum* L. changed the transcription level of the selected genes under stress condition. Understanding of cellular mechanisms responsible for production of alkaloids as their defence reaction is important step towards application of genetic engineering in poppy growing practices.

Keywords

Papaver somniferum L. – Lipoxygenase – Defence – 4-OMT – CNMT – SalAT.

Acknowledgements

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Isoflavones transport in *Trifolium pratense*

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Abstract

AIM: The study focuses on isoflavones, which are present in plants from Fabaceae family (*Trifolium pratense*, *Genista tinctoria*) and they act as agonist/antagonist on estrogen receptors (Dixon & Steele, 1999). Their transport within the cell and plant can be explained by application of transporter mechanism (ABC transporters, MATE, vesicular transport) inhibitors (Prieto & Corchette, 2014).

MATERIALS/METHOD: The study was performed with suspension cultures of *T. pratense*, var. DO-8. The culture was incubated with several possible inhibitors (NH₄Cl, probenecid, sodium orthovanadate, verapamil) for 1 hour and cultivated with elicitor metavanadate for 24 hours. The content of isoflavones in methanol extracts was evaluated with the HPLC method.

RESULTS: Only isoflavon glucoside genistin showed significant results after the application of inhibitors. Its concentration was increased by verapamil and orthovanadate treatment in dry mass. NH₄Cl and probenecid did not show any effect on transport.

CONCLUSIONS: Transport of genistin across cell membrane was not altered after the addition of NH₄Cl, but it was affected by ATPase inhibitor orthovanadate. Probenecid (inhibitor of ABC, subclass MRP) had no effect on transport, but genistin concentration was changed after verapamil application (Ca²⁺ channel blocker and inhibitor of ABC, subclass MDR). Nevertheless, more inhibitors will be investigated for better determination of the transport mechanism.

Keywords

Trifolium pratense – Fabaceae – isoflavones – transport mechanism

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Intracellular antioxidant efficacy of *Mentha* rhizomes extracts and their phytochemical analysis

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Abstract

Recently, the rhizomes of mints showed to be an interesting source of phenolic compounds (Fialová *et al.*, 2012).

AIM: To examine the instant antioxidant efficacy of mint rhizomes extracts after/without induction the oxidative stress and to analyse and quantified phenolics in rhizomes extracts.

MATERIAL/METHODS: The mouse embryonic fibroblasts (NIH/3T3) were seeded in a 96-well plate and were allowed to grow for 24 hours. Subsequently, after 1-h incubation with samples tested the PMA was added (finally 100 ng/ml). After 30-min incubation, the cells were stained with DCFH-DA (finally 5 µg/ml) for 35 min. The fluorescence of the dye DCF was measured fluorimetrically ($\lambda = 480/530$ nm). The phytochemical analysis and quantification of secondary metabolites were performed by HPLC-DAD-MS.

RESULTS: Almost all extracts significantly decreased the intracellular ROS without and with PMA-induced oxidative stress compared to control in two concentrations (0.1 and 1 mg/ml), which is comparable to the effect of 0.015 mg/ml ascorbic acid. The concentration ratio of rosmarinic acid (RA) and the extracts tested in the experiment reflected the real concentration of RA in the extracts (approximately 3%). RA did not decrease ROS significantly within the cells without PMA, but decreased the ROS within the cells influenced by PMA. LC-MS analysis revealed the presence of rosmarinic acid, lithospermic acid, salvianolic acid B and hesperetin-7-O-rutinoside as main phenolic compounds in the tested extracts.

CONCLUSIONS: Some extracts decreased the intracellular ROS more effective than the RA. We suppose that antioxidant effect is not only due to RA content extracts but is influenced by other phenolics presented in rhizomes water extracts.

Keywords

phenolics – *Mentha* – rhizome – antioxidant efficacy – HPLC-DAD-MS

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Natural polyphenol quercetin for brain health?

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Abstract

AIM: The normal ageing of the brain is accompanied by chronic inflammation contributing to the risk of development of neurodegenerative diseases. Microglia appear to be the main effectors in this process. A range of studies indicate that dietary flavonoids are capable of mitigating microglia in the brain of aged rodents, thus restoring their functions to the youthful state. Therefore, we evaluated the potential protective effects of flavonoid quercetin (Q) on aged microglia prepared from 22-month-old rat brains.

MATERIAL/METHODS: We focused on parameters of hyperactivation and senescence phenotype of the cells. These involved development of lipofuscin-like autofluorescence, which was determined by spectrofluorimetry, fluorescence microscopy and flow cytometry. Furthermore, we followed isolectin B4 binding for the characterisation of microglia and their reactivity (by means of immunofluorescence analyses and flow cytometry), mitochondrial membrane potential changes (shown by change in red-to-green fluorescence ratio in the cells stained with JC1 dye) and carbonylation of proteins (determined by immunofluorescence analysis).

RESULTS: Q at 10 μ M concentration enhanced viability and cell yields in old microglia-enriched culture. Q protected mitochondria by increasing mitochondrial membrane potential. In addition, Q suppressed lipofuscin-like autofluorescence as well as protein oxidation and decreased microglial activation state compared to controls.

CONCLUSIONS: The flavonoid, quercetin, protected functions of the aged rat microglia. Therefore, Q might have potential in the prevention and management of neurodegenerative and ageing-related diseases.

Keywords

quercetin – microglia – ageing – neurodegeneration

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Plant pigments from selected culinary herbs

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Abstract **AIM:** Plant pigment is a generic expression used to designate a large number of coloured molecules. On the basis of their chemical structure, they can be classed into five groups: tetrapyrroles, carotenoids, phenolic compounds and *N*-heterocyclic compounds (Schoefs, 2002). The aim of this study was to determine the content of plant pigments (chlorophyll *a*, chlorophyll *b* and carotenoids) from culinary herbs that were grown as pot plants under greenhouse conditions.

MATERIALS AND METHODS: Twelve kinds of different culinary herbs (flat leaf parsley, common chives, mint, lovage, rosemary, sweet basil, coriander, curly leaf parsley, thyme, marjoram, lemon balm and oregano) were purchased from Bylinky Ltd. (Suchbátka u Miroslavi). The aerial parts of plants were harvested, dried and used to determine the content of photosynthetic pigments (chlorophyll *a*, chlorophyll *b* and carotenoids). These plants were extracted using a microwave extraction system (*Start E Milestone*) in acetone at 60 °C. After 24 hours, extracts were analysed using a Specord 50 PLUS, Analytik Jena spectrophotometer. Absorbance was measured at a wavelength of 644 nm for chlorophyll *a*, 662 nm for chlorophyll *b* and 440 nm for carotenoids.

RESULTS: The highest content of chlorophyll *a* and chlorophyll *b* was determined in the basil, while the lowest values showed rosemary. The carotenoid content decreased in the following order: rosemary – marjoram – common chives – thyme – lovage – lemon balm – flat leaf parsley – oregano – sweet basil – mint – curly leaf parsley – coriander.

CONCLUSIONS: The results of our work shows that culinary herbs, especially basil and coriander, have high levels of plant pigments and they can be used as a source of plant pigments in the diet.

Keywords carotenoids – chlorophyll *a* – chlorophyll *b* – culinary herbs – plant pigments – pot plants

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Evaluation of antioxidants in early flowering edible flowers

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Abstract **AIM:** The aim of this study is evaluation of the antioxidant activity of early flowering edible flowers (*Bellis perennis* L., *Viola odorata* L., *Tussilago farfara* L. and *Lamium purpureum* L.).

MATERIALS AND METHODS: The concentration of ascorbic acid (AA) was determined by RP-HPLC using UV-VIS detector. The total antioxidant capacity (TAC) was determined by the DPPH method. The total phenolic content (TPC) was determined with Folin–Ciocalteu's reagents. The total flavonoid content (TFC) was measured using a colorimetric method (Shan *et al.*, 2005, Zloch *et al.*, 2004).

RESULTS: The content of AA was between 5.10 mg kg⁻¹ (*Lamium purpureum* L.) and 979.85 mg.kg⁻¹ (*Viola odorata* L.). The TAC was between 1.288 mM TE.100 g⁻¹ (*Tussilago farfara* L.) to 1.749 mM TE.100 g⁻¹ (*Lamium purpureum* L.) d.w. Values of TPC were between 1888.0 mg GAE.100 g⁻¹ (*Lamium purpureum* L.) and 2,272.0 mg GAE.100g⁻¹ (*Viola odorata* L.) d.w. Finally, the TFC was between 377.0 mg CE.100g⁻¹ (*Viola odorata* L.) to 1 722.0 mg CE.100g⁻¹ (*Tussilago farfara* L.) d.w.

CONCLUSIONS: Our analysis of early flowering edible flowers shows that foremost flowers of *Viola odorata* L. could be used as a new source of antioxidants.

Keywords edible flowers – AA – TAC – TPC – TFC

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Mentha × villosa Huds. – the source of phenolic antioxidants

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Abstract

AIM: To examine antioxidant-active phenolic compounds in leaf pairs of *Mentha × villosa* Huds., which seems to be a promising agent in prevention of tissue injury caused by oxidative stress (Fialová *et al.*, 2015; Fialová *et al.*, 2012).

MATERIAL/METHODS: Acetone, methanol and water extracts were prepared from dry leave pairs of *M. × villosa* grown in Garden of Medicinal Plants in Bratislava. The determinations of flavonoids, hydroxycinnamic derivatives and polyphenols were performed by spectrophotometric pharmacopoeial (Ph. Eur. 8) methods. The antioxidant activity was determined by the method with DPPH radicals and expressed as SC_{50} [μg/ml].

RESULTS: The highest antioxidant activity was detected in water leaves extracts (SC_{50} = 10.60–16.87 μg/ml), slightly lower in methanol extracts (10.73–21.61 μg/ml) and the lowest in ethyl acetate part of acetone extract (18.75–32.12 μg/ml).

CONCLUSIONS: The most antioxidant active were extracts of young apical leaves but high antioxidant activity was expressed stems of basal leaves too. The lowest antioxidant activity was found in leaves from middle part of the stem, which are usually most abundant from the whole leaves mass of aerial part. The examined antioxidant activity corresponds to the contents of phenolic compounds, especially with the hydroxycinnamic derivatives.

Keywords

Mentha × villosa – leaves pairs – DPPH – flavonoids – hydroxycinnamic derivatives – polyphenols

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Antioxidative properties of *Sambucus nigra* extracts

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Abstract

AIM: The purpose of this study was to determine anti-oxidative capacity of *Sambucus nigra* extracts as a whole, in order to explore their potential as a food supplement or as a natural ingredient.

MATERIALS/METHODS: To evoke free-radical-mediated hyaluronan (HA) degradation, we applied Weissberger's biogenic oxidative system. Time-dependent decrease in HA dynamic viscosity was recorded by rotational viscometry (RV). Free-radical scavenging capability of extracts was investigated by ABTS and reverse ABTS assay. The oxygen consumption of the HA oxidative system in the absence and presence of the extracts in the HA reaction system was determined. The total volume of anthocyanins was determined.

RESULTS: RV revealed that addition of the extracts to the HA reaction mixture exerted a dose-dependent decrease in dynamic viscosity of the HA solutions. Significant radical scavenging capacity of extracts towards ABTS⁺ was demonstrated. Extracts significantly lowered the oxygen consumption of the HA reactive system.

CONCLUSION: Our study proved the antioxidative properties *Sambucus nigra* extracts, which means that the extracts are suitable for use in health-enhancing foods.

Keywords

Hyaluronan – rotational viscometry – *Sambucus nigra*

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