

# Phospholipase A<sub>2</sub> relationship to the opium poppy secondary metabolism

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## Abstract

**AIM:** Phospholipase A<sub>2</sub> (PLA<sub>2</sub> EC 3.1.1.4) releases fatty acids and lysophospholipids from membrane phospholipids. These secondary mediators are involved in the response to changes of external and internal environment. An example of plant-defensive responses is the accumulation of secondary metabolites such as alkaloids in mechanically damaged tissue. The thesis is focused on studying the impact of 24-hour exposure of a specific PLA<sub>2</sub> inhibitor – aristolochic acid at a concentration of 50 µmol.l<sup>-1</sup> on benzyloquinoline alkaloid (BIAs) biosynthesis.

**MATERIALS/METHODS:** The content of BIAs and mRNA transcription of selected genes (CNMT, 4-OMT and SalAT) involved in their biosynthesis were determined in intact or mechanically wounded 5-week-old poppy seedlings (*Papaver somniferum* L.).

**RESULTS:** After inhibitor application in intact plants, real-time PCR analysis showed an increase in expression level of CNMT, but after injury, the expression rate was reduced. In uninjured and injured plants incubated with aristolochic acid the expression of 4-OMT and SalAT was decreased. Using LC-MS method, we have determined the level of five poppy alkaloids. After inhibitor application, morphine levels were higher before and after wounding. The level of reticuline, salutaridine and codeine increased in the wounded plant. The amount of thebaine was accumulated to a greater extent after the injury. Based on the results, we assume the existence of several regulatory mechanisms participating in the biosynthesis of secondary metabolites.

## Keywords

Benzyloquinoline alkaloids – Phospholipase A<sub>2</sub> – Opium poppy

## Acknowledgements

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# The effect of the natural excipients in topical drug delivery systems

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**Abstract** **AIM:** The objective of any formulation is to deliver drug to the patient in the definite amount, at the required rate and maintain the stability of drug over the product's shelf-life. The use of natural excipients to deliver bioactive agents has been hampered by the synthetic materials (Ogaji *et al.*, 2012). Their growing role and application in the pharmaceutical industry may be attributable not only to the fact that they are biodegradable and toxicologically harmless raw materials available at low cost and of relative abundance compared to their and synthetic counterparts, but also because natural resources are renewable and if cultivated or harvested in a sustainable manner, they can provide a constant supply of raw material. Furthermore, their extensive applications in drug delivery have been realised because, they offer unique properties which so far have not been attained by any other materials (Bahadur *et al.*, 2014).

In our work, we investigate the influence of natural polymers on the drug release.

**MATERIALS AND METHODS:** The different formulations of micro-emulsions and hydrogels were prepared depending on the nature of polymers – xanthan gum, chitosan, carrageenan and alginate.

**RESULTS:** In all the cases, the natural polymers have influenced the release of the drug from formulations of microemulsion.

**CONCLUSION:** Novel drug delivery systems are developed to address the challenges of drug development such as bioavailability, permeability and poor solubility. This work helps to comprehend various natural excipients and their role in pharmaceutical drug delivery systems.

**Keywords** *natural polymeric materials – pharmaceutical excipients – biodegradable – biocompatibility*

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## Medicinal plants – an important source of honey-bee forage

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### Abstract

**AIM:** The importance of medicinal plants as a part of honey-bee forage is nowadays underestimated, although these plants represent one of the main sources of honey-bee forage during early spring and late summer. This article provides an overview of medicinal plants species, which are attractive for bees and their possible uses on different types of settings.

**MATERIAL/METHODS:** The literature references as well as the authors' own observations and experiences were used.

**RESULTS:** The examples of medicinal plant species attractive for bees, which are cultivated on large areas, the species suitable for growing on smaller areas in the gardens, near apiaries and residential areas of towns and villages, and the species suitable for the regeneration of the countryside, founding of flowering meadows, or for the establishment of anti-erosion belts in the landscape are presented and its importance as the source of nectar and/or pollen is taken into account in the article.

**CONCLUSIONS:** The use of medicinal plants for apiculture purposes offers another opportunity to extend their cultivation and application. Almost all kinds of medicinal plants are interesting, the cultivated ones as well as these wild growing, which form part of different habitats and landscapes. A huge variability of species, resistant to abiotic and biotic stress and modest growing conditions mean positive disposition for the cultivation of these plants and for their return to the cultural landscape as a form of plantations or as a part of meadow communities (flowering meadows, blooming belts, bio-corridors).

### Keywords

*Medicinal plants – bee pasture – pollen – nectar*

### Acknowledgements

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## Leaves of selected Cornaceae species – phytochemical screening and bioactivity testing

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### Abstract

**AIM:** *Cornus officinalis* fruits are used as a part of remedial mixtures in traditional Asian medicines. In Slovakia, *Cornus mas* fruits are still used for culinary purposes and 'drienkovica' distillation. But there is little information about the leaves of these shrubs/trees, so that our aim was to evaluate bioactivities of leaf water infusions from five *Cornus* species (cultivated in the Slovak region) and to isolate some of mainly represented chemical substances in *C. officinalis* leaves.

**METHODS:** The evaluation of biological activities (antiproliferative and immunomodulatory) was based upon the incubation of leaf water infusions with particular cell lines (MCF-7 and human leukocytes). Anti-oxidant activity was tested by DPPH method. Isolation and identification of chemical compounds present in leaves is performed by chromatographic and spectral methods.

**RESULTS:** We have tested the anti-proliferative, immunomodulatory, anti-microbial (against *E. coli* and *C. albicans*) and anti-oxidant activity (Šeršeň *et al.*, 2005) of water infusions prepared from the freeze-dried leaves of *Cornus officinalis*, *C. kousa*, *C. sericea* 'Flaviramea', *C. mas* and *C. alba in vitro*. The most potent species are *C. officinalis* (anti-proliferative and anti-oxidative), *C. sericea* 'Flaviramea' (immunomodulatory), *C. mas* (antimicrobial). Leaves are rich in iridoids and polyphenolic substances, such as tannins. Some of them were isolated (gallic acid, rutin, and common substances glucose and sucrose). Currently isolation and identification of other compounds is under progress.

**CONCLUSIONS:** The above mentioned members of Cornaceae family appear to be generally prospective and interesting in the current trend of searching for new natural drug sources.

### Keywords

Cornaceae – leaves – biological activities – chemical substances

### Acknowledgements

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## *In vitro* testing of antiplatelet activity of flavonoids

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### Abstract

**AIM:** To test the anti-platelet activities of 29 flavonoids on three steps of arachidonic acid (AA)-based pathway.

**MATERIAL/METHODS:** A commercial set from Cayman Chemical Company was used for the determination of cyclooxygenase-1 (COX-1) inhibition. Thromboxane A<sub>2</sub> synthase inhibition was evaluated according to the method of Chang et al. (1997), with minor modifications. Antagonism at the thromboxane A<sub>2</sub> (TXA<sub>2</sub>) receptors was performed by turbidimetry using a Chrono-log 500-Ca aggregometer connected to a computer (Aggro/Link software, Chrono-log Corp). For all experiments, human platelet-rich plasma with a platelet concentration of  $3.5 \times 10^8$  per mL was used for comparable results. Blood samples were collected from 31 healthy non-smoking volunteers who did not use anti-platelet drugs.

**RESULTS:** Several flavonoids acted as antagonists on TXA<sub>2</sub> receptors and the most active compounds, the isoflavonoids genistein and daidzein, are moreover better inhibitors of ovine COX-1 than ASA. Although their effects were lower in comparison with ASA in human platelets, such activity seems to be clinically relevant.

**CONCLUSIONS:** The consumption of flavonoid-rich food, especially with content of the isoflavonoids genistein and daidzein, may decrease platelet aggregation and thus have a positive effect on cardiovascular diseases associated with enhanced platelet activity.

### Keywords

flavonoid – aggregation – platelet – arachidonic acid – cyclooxygenase-1 – thromboxane A<sub>2</sub>

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## *Sideritis raeseri* Boiss. & Heldr. – secondary metabolites quantification

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### Abstract

**AIM:** The aim of this work was the quantitative determination of secondary metabolites in *Sideritis raeseri herba*.

**MATERIALS AND METHODS:** Herbal drugs – *Sideritis raeseri herba* – were collected in June (2011, 2012 and 2013) in Greece (Othrys mountain). Spectrophotometric methods were used for the determination of secondary metabolites (total polyphenols, tannins, flavonoids, hydroxycinnamic derivatives, coumarins).

**RESULTS:** The content of total polyphenols expressed as pyrogallol were 1.68–1.99 %. The content of tannins expressed as pyrogallol were 0.22–0.59 %. The content of total flavonoids expressed as hyperoside were 1.31–1.63 %. The content of hydroxycinnamic derivatives expressed as rosmarinic acid were 1.15–0.47%. The content of coumarins expressed as umbelliferone were 0.22 –0.33 %. Volatile oils were isolated (from dry plant materials) by hydrodistillation. The content of essential oil in *Sideritis raeseri herba* were 60–65 ml/kg.

**CONCLUSIONS:** This pilot study quantified some secondary metabolites of the *Sideritis raeseri herba*.

**Keywords** *Sideritis raeseri* – tannins – flavonoids – hydroxycinnamic derivatives – coumarins – essential oil

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# Hyaluronidase inhibitors from selected plants of Lamiaceae and Asteraceae family

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## Abstract

**AIM:** Degradation of hyaluronic acid by hyaluronidase not only regulates several normal physiologic functions but can be also involved in disease process such as cancer progression, atherosclerosis and inflammation. The aim of this work was to introduce a hyaluronidase inhibition assay and to evaluate plant extracts that are researched at the Department of Natural Drugs. Phytochemical analysis of tested extracts led to the identification of active compounds, which could contribute to inhibitory activity.

**MATERIAL/METHODS:** Hyaluronidase inhibitory activity was measured spectrophotometrically according to the Morgan–Elson method with some modifications. Methanolic plant extracts were analysed by HPLC-DAD-MS and separated by semi-preparative HPLC. Isolated compounds were identified by co-HPLC with standards or by NMR.

**RESULTS:** Many naturally occurring compounds were reported as inhibitors of hyaluronidase (Isoyama *et al.*, 2006). In our study, especially species of the genus *Tagetes* and *Agrimonia* demonstrated significant anti-hyaluronidase activity due to catechins and flavonoids (patulitrin, quercetin and its glycosides), which may act as anti-inflammatory and anti-cancer agents. Extracts from *Plectranthus* species, with rosmarinic acid as the dominant compound, were moderate inhibitors.

**CONCLUSIONS:** Species of *Agrimonia* and *Tagetes* genus are rich in the contents of polyphenols and can have high potential to maintain the homeostasis of hyaluronic acid.

## Keywords

*Hyaluronic acid – hyaluronidase inhibitors – HPLC analysis*

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# The effect of chosen plant extracts on diabetic vascular complications

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## Abstract

**AIM:** *Diabetes mellitus* (DM) is often diagnosed at a time when patients already suffer from several complications associated with diabetes – diabetic vascular complications (DVC). These complications develop through different pathways. The aim of this study was to evaluate the potential mechanism of action of two medicinal plants, agrimony (*Agrimonia eupatoria* L. – AE) and artichoke (*Cynara cardunculus* L. – CC), in the prevention and/or adjuvant therapy of diabetes and its complications.

**MATERIAL/METHODS:** HPLC-MS analysis of water infusions was performed. Furthermore, the anti-oxidant effects were compared as well. To extend the evidence of possible protective activities in DM and DVC, DM-related parameters were studied in a STZ-induced diabetic rat model. Changes in blood glucose and body weight were monitored during a five-week period. After this period, rats were sacrificed to determine the changes in the reactivity of aortas and the activity of BuChE in livers. To complete the panel of experiments, the inhibition of  $\alpha$ -glucosidase and aldose-reductase, as well as antiglycation activity, was assessed *in vitro*.

**RESULTS:** During the HPLC-MS analysis, approximately 8% of polyphenols were identified. Both extracts showed excellent anti-oxidant activities and good anti-diabetic properties.

**CONCLUSIONS:** The differences in the behaviour of the two medicinal plants with almost the same amount of total polyphenols may be due to different phytochemical composition. In conclusion, these results indicate the clinical potential of agrimony and artichoke in the prevention and/or adjuvant therapy of developing complications related to DM and diseases associated with oxidative stress.

## Keywords

agrimony – artichoke – antioxidants – oxidative stress – diabetes mellitus – diabetic vascular complications

## Acknowledgements

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# Evaluation of anti-microbial activity of different plant extracts

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## Abstract

Bacterial resistance is closely related to over-consumption of antibiotics in human, agricultural and veterinary practice (Porsby *et al.*, 2011). Therefore, there is a huge challenge to find new or alternative sources of anti-microbially effective drugs.

**AIM:** The aim of our study was to test the anti-microbial activity of water extracts of *Cotinus goggygia* (CCW) leaves, *Origanum vulgare* (OV) leaves, *Mentha spicata* (M1), *M. longifolia* (M4), and *M. longifolia* var. *lavanduliodora* (M7) rhizomes.

**METHODS:** The *in vitro* anti-microbial activity of water extracts was examined on five different strains of bacteria (methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-sensitive *S. aureus* (MSSA), *Enterococcus faecalis*, *Escherichia coli* and *Pseudomonas aeruginosa*) using the broth-microdilution method.

**RESULTS:** All extracts showed excellent bactericidal effect against MRSA and MSSA strains. CCW extract had cidal activity also against *E. faecalis*, but only bacteriostatic effect against *E. coli* and *P. aeruginosa*. Rhizomes of mints, recently expressing a good anti-oxidant activity (Fialová *et al.*, 2012), have been evaluated for their anti-microbial effects for the first time. Except for the anti-staphylococcal activity, they showed bacteriostatic activity against *E. faecalis*, but no or only very low bacteriostatic activity against Gram-negative bacteria (*E. coli*, *P. aeruginosa*).

**CONCLUSIONS:** The results of our study demonstrate that all tested extracts seem to be prospective anti-microbial agents.

## Keywords

*Cotinus* – *Origanum* – *Mentha* – antibacterial activity

## Acknowledgements

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## The microscopic evaluation of *Cannabis sativa* L.

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**Abstract** **AIM:** Trichomes, specially the capitate-stalked glandular trichomes, are well known as the main sites of cannabinoid and essential oil production of *Cannabis sativa* L. (Happyana *et al.*, 2013). For quality assessment of herbal material, the various types of *C. sativa* L. trichomes have been investigated microscopically.

**MATERIAL/METHODS:** *Cannabis sativa* L., varieties NL1A and SL1A were grown from seeds and cultivated indoors under standardised conditions. Cannabis leaves and flower tops were cleared with chloral hydrate solution. Photomicrographs were prepared using an Olympus BX41 light microscope coupled to Olympus Camedia 7070 and evaluated by QuickPhoto Micro 2.3. Deep Focus 3.1. software.

**RESULTS:** The characteristic trichomes are of different types. The glandular trichomes, i.e. the champignon-like capitate-stalked glandular trichomes are present, especially on bracts and floral leaves. They differ in size, up to 500 µm large, according to the variety of *Cannabis*. The small sessile bulbous glandular trichomes are present as well. The non-glandular trichomes – trichomes approximately 100 µm in length – are with enlarged base where cystoliths of calcium carbonate occur. Conical trichomes without cystoliths can be also found.

**CONCLUSIONS:** The *Cannabis sativa* L., variety NL1A and SL1A has typical microscopic characteristics as *Cannabis sativa* L. with small difference in size and appearance of trichomes.

**Keywords** *Cannabis sativa* – microscopy – trichomes

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## Potential therapeutic applications of prenylated phenols

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**Abstract** **AIM:** The flavonoids are plant pigments containing benzopyrane substituted with a phenyl ring at position 2 or 3. Their aglycones can be lipophilic; their lipophilicity can be further enhanced by methylating the hydroxyl groups, or by prenylation or geranylation at different positions on the skeleton. The prenyl or geranyl moiety may be modified in different ways and enhances the interaction with organism.  
**MATERIAL/METHODS:** Different chromatographic methods were used for the isolation of series of prenylated phenols. Experimental *in vitro* and *in vivo* studies have revealed many biological and pharmacological activities of flavonoids. We worked with prenylated compounds from *Morus alba* and *Paulownia tomentosa*.  
**RESULTS:** Inflammation is a multiple and complex response by the body to infection or injury. Prenylated compounds show pleiotropic effects and can modulate a broad spectrum of inflammatory regulatory nodes. Their anti-phlogistic action combines many particular effects: it can be mediated by several pathways: *via* anti-oxidant and pro-oxidant effects, by interacting directly with pro-inflammatory proteins and by interacting with signal pathways and inhibiting the expression of inflammation-related genes. *In vivo* tests have confirmed all of the effects of flavonoids previously observed in *in vitro* experiments. The anti-bacterial effect of prenylated substances was confirmed, and some synergy with standard antibiotics was observed against MRSA.  
**CONCLUSIONS:** Prenylated phenols are good candidates for further research to discover new therapeutics for the treatment of diseases connected with bacterial infection and inflammatory disorders.

**Keywords** *Paulownia tomentosa* – flavonoid – inflammation – antibacterial

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# Plants of genus *Opuntia*, *Epiphyllum*, *Hylocereus* – volatile compounds analysis

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## Abstract

**Introduction:** Cacti are known mostly as ornamental plants. Cactus fruits are important part of diet in several regions of America. The active compounds include betaxanthins, phenolic compounds and terpenes.

**Aim:** The aim of this work was the analysis of genus *Opuntia*, *Epiphyllum*, *Hylocereus* volatile constituents.

**Materials and Methods:** *Opuntia* fructus, *Epiphylli* fructus and *Hylocerei* fructus were analysed and collected in the Botanical Garden in Bratislava (2012). The volatile constituents were evaluated using SPME GCMS.

**Results:** In purple *Opuntia* fructus, we have identified eight compounds (e.g. nonaldehyde (43.5%), ethylcaprylate (12.1%) and ethylpalmitate (9.4%)). In orange *Opuntia* fructus, we have identified eight compounds; e.g. nonaldehyde (11.8%), ethylcaprylate (35.1%) and octadiene (21.9%). In purple *Epiphylli* fructus, we have identified 14 compounds, e.g. nonadecene (5.4%), butane-2,3-diol (27.1%) and (*E*)-anethole (0.6%). In pink *Epiphylli* fructus, we have identified 13 compounds (e.g. nonadecene (14.0%) and (*E*)-anethole (28.1%)). In green *Epiphylli* fructus, we have identified 10 compounds (e.g. (*E*)-anethole (20.5%), eugenol methyl ether (14.7%) and ethylpalmitate (13.2%)). In red or white *Hylocerei* fructus, we identified three or four constituents. In largest percentage of both were hexadecan-1-ol (26.9%), cetylalcohol (60.5%) and octadecan-1-ol (6.4%).

**Conclusions:** This pilot study identified some lipophilic constituents of the fruits. Further analyses will be carried out to identify nitrogen-containing as well as flavour-bearing compounds to compare with other cacti fruits.

## Keywords

*Opuntia* fructus – *Epiphylli* fructus – *Hylocerei* fructus – SPME GC-MS – volatile compounds

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