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Combining metabolomic analysis and microarray gene expression analysis in the characterization of the medicinal plant *Chelidonium majus* L

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ABSTRACT

Background and objective: Even though herbal medicines have played an important role in disease management and health for many centuries, their present frequent use is challenged by the necessity to determine their complex composition and their multitarget mode of action. In the present study, modern methods were investigated towards their potential in the characterization of herbal substances. As a model the herbal substance Chelidonii herba was used, for which several reports on liver toxicities exist. Extracts of Chelidonii herba with different solvents were characterized phytochemically and functionally by experiments with HepG2 liver cells.

Methods: Chelidonii herba was extracted with four solvents of different polarity (dichloromethane, water, ethanol, and ethanol 50% (V/V); four replicates each). The different extracts were characterized metabolomically by ^{1}H-NMR fingerprinting analysis and principal component analysis (PCA). The content of alkaloids was additionally determined by RP-HPLC. Functional characterization was achieved by the determination of cell proliferation and by transcriptomics techniques (Whole Genome Gene Expression Microarrays v2, Agilent Technologies) in HepG2 cells after exposure to the different extracts (four experimental replicates each).

Results: Based on data from ^{1}H-NMR fingerprints and RP-HPLC analyses the different extracts showed a divergent composition of constituents depending on the solvent used. HepG2 liver cells responded differentially to the four extracts. Microarray analysis revealed a significant regulation of genes and signal cascades related to biotransformation. Also liver-toxic signal cascades were activated. Neither the activated genes nor the proliferation response could be clearly related to the differing alkaloid content of the extracts.

Conclusion: Different manufacturing processes lead to different herbal preparations. A systems biology approach combining a metabolomic plant analysis with a functional characterization by gene expression profiling in HepG2 cells is an appropriate strategy to characterize variations in plant extracts. Safety assessments of herbal substances may benefit from such complementary analyses.

Abbreviations: IPA, Ingenuity pathway analysis; PCA, Principal component analysis; qRT-PCR, quantitative real-time PCR.

Abbreviations: AhRR, Aryl-hydrocarbon receptor repressor; AKR1B10, Aldo-keto reductase family 1 member B10; AKR1C4, Aldo-keto reductase family 1 member C4; BAAT, Bile Acid-CoA:Amino Acid N-Acyltransferase; CHST10, Carbohydrate sulfotransferase 10; CHST14, Carbohydrate sulfotransferase 14; Cyp1A1, Cytochrome P450 family 1 member A1; CYP19A1, Cytochrome P450 family 19 member A1; Cyp1B1, Cytochrome P450 family 1 member B1; CYP24A1, Cytochrome P450 family 24 member A1 (1,25-dihydroxyvitamin D_3 24-hydroxylase); CYP26A1, Cytochrome P450 family 26 member A1; CYP27C1, Cytochrome P450 family 27 member C1; CYP2A7, Cytochrome P450 family 2 member A7; CYP2B6, Cytochrome P450 family 2 member B6; CYP2A7, Cytochrome P450 family 2 member A7; CYP2C18, Cytochrome P450 family 2 member C18; CYP3A4, Cytochrome P450 family 3 member A4; CYP3A7, Cytochrome P450 family 3 member A7; CYP4V2, Cytochrome P450 family 4 member V2; CYP8B1, Cytochrome P450 family 8 member B1; EGR2, Early growth response protein 2; GLYATL1, Glycine-N-Acyltransferase-Like 1; GSTO2, Glutathione S-transferase omega 2; GSTP1, Glutathione S-transferase P; HMOX1, heme oxygenase (decycling) 1; HS3ST3A1, Heparan sulfate glucosamine 3-O-sulfotransferase 3A1; HTR3A, 5-hydroxytryptamine receptor 3A; IGFBP1, Insulin-like growth factor-binding protein 1; KCNAB3, Voltage-gated potassium channel subunit beta-3; SULT1C3, Sulfotransferase 1C3; SULT1E1, Sulfotransferase 1C3; SULT4A1, Sulfotransferase 1C3; UGT2A3, Uridine 5'-diphospho-glucuronosyltransferase 2A3; UGT2B11, Uridine 5'-diphospho-glucuronosyltransferase 2B11.

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Introduction

Medicinal plants and products derived thereof have a long tradition of therapeutic use and are widely accepted in the population. The quality of medicinal plants is a basic requirement for the safety of herbal preparations. In terms of quality requirements, the authentication of herbal substances is indispensable. In this context molecular biological methods were tested successfully for their applicability in the identification of herbal substances (Kersten et al., 2008). For safety assessment, several established *in-vitro* and *in-vivo* "toxicological" methods are available: for example the comet assay determines DNA strand breakage or the T-cell-dependent antibody response assesses the antibody response to immunization. However, in the field of regulatory affairs there exists the necessity to investigate novel technologies, including functional genomics, proteomics, metabolomics, high-throughput screening and systems biology in order to replace current toxicology assays used for drug approval (Hamburg, 2011). In future investigations it is important to characterize the toxicant/s with additional complementary methods. Thus, the chemical profile of a plant-derived extract, together with data on its cellular responses, toxicological findings and extrapolated effects of dose-response investigations have to be taken into account for the compilation of an overall picture (Hartung and McBride, 2011; Krewski et al., 2010).

In contrast to single substances, plant extracts present a challenge, as they are complex mixtures. Often, plant extracts are characterized by marker substances used for the standardization of the respective herbal substances. But multi-component mixtures can have synergistic effects, which may arise due to the combination of many components of an herbal preparation (Wagner, 2006) not covered by the determination of marker substances alone.

Chelidonium majus L. (Papaveraceae) is a biennial or perennial plant native to Europe, North America and Western Asia and commonly known as swallow wort, rock poppy or greater celandine. *C. majus* has been used in traditional Chinese medicine, western phytotherapy, homeopathy and anthroposophy. The German Commission E indicated Chelidonii herba for the use in spastic discomfort of bile ducts and gastrointestinal tract. The fresh latex was externally used in the treatment of warts but also for other skin complaints such as corns, tinea infections, eczema and tumours of the skin. According to the German Comission D monograph on *C. majus*, the herbal substance is used in homeopathy for different disorders of the liver and the gallbladder, inflammation of the respiratory organs and the pleura and in rheumatism. According to the Chinese medicine, *C. majus* is mainly used to treat blood stasis, to relieve pain, to promote diuresis in oedema and ascites, to treat jaundice and to relieve cough (Gilca et al., 2010).

Several *in-vivo* and *in-vitro* studies suggest anti-viral, anti-microbial, anti-spasmodic, choleretic, anti-inflammatory, immunomodulatory, analgesic and anti-tumour effects for preparations containing *C. majus* or isolated constituents thereof (Gilca et al., 2010).

In this study Chelidonii herba was used as a model for our investigations. In literature, there are several reports on liver toxicity associated with application of herbal preparations derived from *C. majus* (Teschke et al., 2012; Stickel et al., 2003; De Smet, 2002; Strahl et al., 1998).

The secondary metabolites most abundant in *C. majus* L. are alkaloids, more than 20 of which are chemically identified. The yellow-orange alkaloid-containing latex is present throughout the entire plant. The most important alkaloids (Fig. 1) are the benzophenanthridine alkaloids (sanguinarine, chelerythrine and chelidonine) and protoberberines (berberine, coptisine). But also organic acids like chelidonic acid (Shen et al., 2001), citric acid, malic acid, or succinic acid were isolated from *C. majus* L. (Slavik, 1955), as well as saponins, choline and histamine (Kwasniewski, 1955), ferulic acid, caffeic acid and *p*-coumaric acid (Hahn and Nahrstedt, 1993) are present.

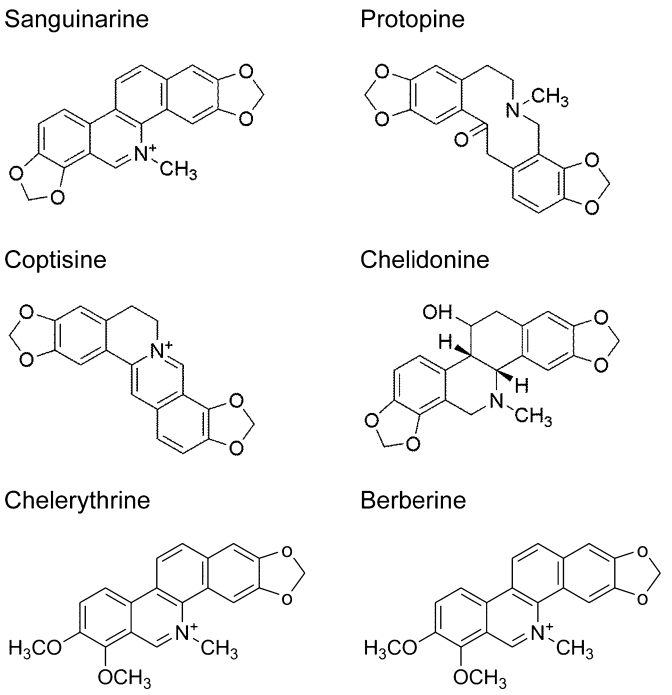

Fig. 1. Chemical structure of typical alkaloids in *C. majus* L.

In the European Pharmacopoeia monograph, the aerial parts of *C. majus* L. are used. On Chelidonii herba there is a minimum content of 0.6% of alkaloids specified, calculated as chelidonine. For the investigations performed in the current study four extracts with solvents of different polarities were used. The complex mixture of phytochemical constituents was characterized by ^{1}H-NMR fingerprint analysis, which is a comprehensive approach in the characterization of the plant metabolome (Daniel et al., 2008). The content of the alkaloids chelidonine, protopine, coptisine, berberine, sanguinarine and chelerythrine was quantified by RP-HPLC-DAD. The chemical profile was correlated to effects on liver cells. Therefore, liver cell proliferation was investigated in response to the different extracts and effects were further characterized by a transcriptomics-based approach. Alterations in gene expression were monitored by microarrays, permitting the assessment of complete gene expression profiles induced by different compounds or extracts. By systems biological data evaluation it was possible to place our data into a biological context.

Materials and methods

Extraction of Chelidonii herba

The dried aerial parts, purchased from a local pharmacy were used. The herbal substance was complying with the monograph in Ph. Eur. 7.5/1861. Chelidonii herba was powdered and extracted with four different extraction solvents (ethanol, ethanol 50% (V/V), dichloromethane and water). Respectively, 1.0 g of plant material was extracted with 10 ml of the respective solvent, frequently stirring at room temperature for 10 min. After filtering, the extraction procedure was repeated. The solvents were eliminated under reduced pressure and the dried extract was resolved in 500 μl deuterated dimethyl sulphoxide. Each extract was performed in four replicates. The sample material Chelidonii herba in analogy to