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Global proteomic analysis of *Chelidonium majus* and *Corydalis cava* (Papaveraceae) extracts revealed similar defense-related protein compositions

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ABSTRACT

Chelidonium majus and *Corydalis cava* are phylogenetically closely related (Papaveraceae family). The medicinal and pharmaceutical interest in these plants is based on their synthesis of pharmaceutically important compounds, such as alkaloids, flavonoids, phenolic acids and proteins. *C. majus* shoot and *C. cava* tuber extracts have been used in traditional folk medicine to treat many diseases, such as fungal, bacterial and viral infections, liver disorders, fever, post-traumatic, colic, abdominal and menstrual pains and even cancer. This study attempts to perform a global comparative proteomic analysis of pharmacologically important extracts from these two closely related unsequenced plant species to gain insights into the protein basis of these plant organs and to compare their common and specific proteomic compositions. We used a shotgun proteomic approach combined with label-free protein quantitation according to the exponentially modified protein abundance index (emPAI). In total, a mean number of 228 protein identification results were recorded in *C. cava* tuber extracts and about 1240 in *C. majus* shoot extracts. Comparative analysis revealed a similar stress and defense-related protein composition of pharmacologically active plant species and showed the presence of different pathogenesis-related and low molecular inducible antimicrobial peptides. These findings could form the basis for further elucidation of the mechanism of the strong pharmacological activities of these medicinal plant extracts.

1. Introduction

Greater Celandine (*Chelidonium majus* L.) and Hollowroot (*Corydalis cava* Schweigg. & Koerte) plants are phylogenetically closely related. They both belong to the Papaveraceae family and are a rich source of various biologically active substances. These two species are perennial herbaceous plants, which grow in similar soil conditions; therefore, they could be subjected to similar pathogenic stress [1,2].

Our focus was to compare *C. majus* and *C. cava* extracts due to their common applications in traditional folk medicine and contemporary pharmacology in the light of their antiviral, antifungal, sedative, and anticancer activities. The medicinal and pharmaceutical interest in these plants is based on their synthesis of pharmaceutically important compounds, such as alkaloids, flavonoids or phenolic acids. *C. majus* contains alkaloids such as chelidonine, sanguinarine, cheleritrine and berberine of cytostatic activities. Extracts and the milky sap of Greater Celandine are used in traditional folk medicine to treat papillae, warts, condylomas, which are visible effects of human papilloma virus (HPV) infections. *C. majus* plant extracts are also used to treat liver disorders and fight fever [3]. It has been shown that *C. majus* extracts have antimicrobial, antiviral, antitumor, cytotoxic, anti-inflammatory, antifungal, and fungistatic properties [1,3,4]. *C. cava* develops

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sizeable subterraneous tubers derived from rhizomes, which are empty inside and scantily covered by fibrous roots [2]. The tuber of *Corydalis* contains isoquinoline alkaloids of apomorphine type, e.g. bulbocapnine, corydaline, which manifest analgetic, sedative and narcotic effects [5]. The Hollowroot plant is used for the treatment of severe neurological disorders and mental diseases. It is also used in cases of insomnia, tension and anxiety conditions [5]. Some species of *Corydalis* are used in East Asia as analgetic drugs: in traditional Chinese medicine the species *Corydalis yanhusuo* was used to alleviate post-traumatic, colic, abdominal and menstrual pains [5]. Moreover, extracts of the same species demonstrated anti-cancer metastasis effects in vitro [6]. The anti-tumor activity of the *Corydalis* species has also been reported for the Korean *Corydalis turtschaninovii*, which is effective for the treatment of inflammatory and allergic diseases and tumors [7].

Our previous studies revealed that the biological activity of Greater Celandine and Hollowroot extracts may depend not only on their alkaloid content but also on the presence of different pathogenesis- and stress-related proteins [4,8–10]. Various biologically active compounds and proteins are present in parts of those plants used in traditional folk medicine: mainly shoots (stem with leaves) for *C. majus* and tubers for *C. cava*. Plant defense proteins and other compounds are deposited in the laticifer system of *C. majus*, which is formed throughout the whole plant — in shoots as well as in roots [11]. Similarly, in laticifer-free *Corydalis* such compounds are stored in rhizome-derived tubers, which develop from the stem of the plant. Recent findings also show that, as a part of defense response, the plants produce a high number of toxic molecules, including antimicrobial peptides (AMPs), that kill pathogens by interaction with phospholipids and membrane permeabilization [12].

Therefore, protein extracts from both *C. majus* shoots and *C. cava* tubers, the parts that are the source of many pharmacologically active substances, were subjected to comparative proteomic analysis to gain insights into the protein basis of these pharmacologically active plant organs and to compare their common and specific proteomic composition. To date, there is also a lack of information in the literature on such a global proteomic analysis of pharmacologically important plant species. We decided to use a tandem mass spectrometry identification (nano-LC–MS/MS) approach combined with label-free protein quantitation (emPAI) [13,14] to compare the protein composition of these two unsequenced plant species. Discussion is focused on elucidation of the stress and defense response proteins present in both plants.

2. Experimental

2.1. Preparation of plant protein extracts

C. cava plants were collected in the neighborhood of Poznan during flowering in April. *C. majus* plants were collected in the neighborhood of Poznan in June. Plant shoots were collected from adult *C. majus* plants of similar developmental stage (height of the plant ca. 50 cm). The protein extracts were prepared from *C. cava* tubers and *C. majus* shoots (stem with leaves), dissolved in 0.1 M Tris–Cl buffer, pH 8.0, containing 10% glycerol (extract: buffer ratio was 1:1). Extract (50% v/v) samples were separated into a supernatant and a pellet fraction by centrifugation at 10,000 *g* for 20 min at 4 °C as described [9]. Protein concentration was determined according to Bradford [15].

Voucher specimens of both plants were deposited at the Department of Molecular Virology, Faculty of Biology, Adam Mickiewicz University in Poznan, Poland.

2.2. Analysis by SDS-PAGE

In order to verify the protein composition of protein samples, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was conducted in a slab mini-gel apparatus according to Laemmli [16], using 10% polyacrylamide as the separating gel and 5% polyacrylamide as the stacking gel. The proteins were denatured by heating them to 100 °C in the presence of 2-mercaptoethanol for 5 min. 50 μg of each sample was put into the gel (4 replicates — 2 biological and 2 technical). After SDS-PAGE the gels were fixed and stained using sensitive Coomassie Blue Staining [17].

2.3. Sample preparation for LC–MS

Stained protein bands were excised from the gel. Separate lanes were cut to each form 10 different bands. The excised bands were chopped into gel pieces of approx. 1 × 1 mm to enlarge the contact surface of gel pieces with subsequent solvents. Gel pieces originating from one gel band were treated together in one tube. Gel particles were equilibrated with 20 mM NH_4HCO_3 for 5 min, briefly vortexed and spun down and the liquid was discarded. Then, gel particles were shrunk by adding acetonitrile (corresponding to approximately 3 times the total volume of gel pieces), incubated for 5 min, spun down and the liquid was discarded. Both steps were repeated. The remaining particles were swelled in 200 μl of reduction buffer (10 mM dithiothreitol in 20 mM NH_4HCO_3) and incubated for 20 min at 56 °C to reduce the disulfide bridges of in-gel proteins. After shrinking the gel pieces with acetonitrile, the latter was replaced with 200 μl of alkylation buffer (55 mM iodoacetamide in 20 mM NH_4HCO_3) and incubated for 20 min at room temperature in the dark to alkylate free cysteines. Then, the iodoacetamide solution was discarded, gel particles were washed with 100 μl of 20 mM NH_4HCO_3 for 5 min and shrunk using acetonitrile. The step was repeated twice. Proteins were further digested by adding a digestion buffer (20 mM NH_4HCO_3) containing 12.5 ng/μl sequencing grade trypsin (Promega) and incubated at 37 °C overnight. After trypsin digestion, peptides were acidified to pH 2 and extracted from the gel matrix by addition of 0.1 volumes of 10% TFA to a final concentration of 1% TFA. After 10 minutes' incubation, the remaining gel particles were spun down and the collected supernatant contained tryptic peptides. All samples were subsequently desalted and concentrated using C_{18}-StageTips (Thermo Scientific, http://www.proxeon.com) [18].

2.4. Liquid chromatography and tandem mass spectrometry (LC–MS/MS)

Tryptic peptide mixtures were analyzed by LC–MS/MS using nanoflow HPLC and an LTQ-Orbitrap XL (Thermo Fisher Scientific, http://www.thermoscientific.com) as the mass