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Alkaloids from *Chelidonium majus* L.: Fractionated supercritical CO_2 extraction with co-solvents

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ABSTRACT

Chelidonium majus L. is rich in many isoquinoline alkaloids which are responsible for the anti-inflammatory, antitumor and cytotoxic bioactivities known for this plant. The main alkaloids identified in *C. majus* L. include chelidonine, berberine, coptisine, sanguinarine and chelerythrine, which are present in different parts of the plant. In this work, alkaloids from *C. majus* aerial and terrestrial parts were extracted using a two step extraction procedure which consisted in a first step (SFE) using only supercritical carbon dioxide ($scCO_2$) as solvent followed by a second step (ESE) using $scCO_2$ and a co-solvent mixture composed by an alcohol (ethanol or isopropanol) and diethylamine (alkaline conditions). The effect of operation temperature, pressure, solvent density and solvent pH on extraction yields, kinetic profiles and alkaloids' selectivity was evaluated. Results showed that SFE presents high selectivity for alkaloids (particularly for chelidonine) at solvent densities in the range 813–850 kg/m^3 and for both the aerial and terrestrial parts of the plant. The highest alkaloids extraction yield was observed at higher solvent density conditions using the $scCO_2$/isopropanol/diethylamine mixture (ESE extraction step). Chelidonine was found to be highly soluble in $scCO_2$. Therefore, the fractioned high pressure extraction procedure proposed in this work can be successfully applied to separate enriched chelidonine fractions, while other alkaloids can be obtained using basified isopropanol as co-solvent. This procedure demonstrates that alkaloids fractionation during extraction is an important tool to be used before further purification/isolation steps.

1. Introduction

Chelidonium majus L. (commonly known as "greater celandine") is a perennial plant native from Europe and Western Asia and widely extended to America. A specific feature of this botanical family is the production of outflowing yellow-orange coloured latex, present both in roots and aerial parts, that has been used for centuries in Western phytotherapy and Chinese traditional medicine, mainly due to its antimicrobial and anti-inflammatory properties and its healing activity against skin affections [1,2]. Colombo and Bosisio reviewed the studies reported in the literature concerning the bioactivity of *C. majus* extracts, including anti-inflammatory, antitumor and cytotoxic activity [3]. Authors compared the activity of the plant extracts with their composition (major purified compounds) and concluded that alkaloids are the main responsible for those activities [3,4].

C. majus latex and herbal preparations are rich in many isoquinoline alkaloids (more than 20 have been identified), which belong to three main groups: (a) benzophenanthridines, like chelidonine, sanguinarine and chelerythrine, (b) protopine and derivatives, and (c) protoberberines, like berberine and coptisine [5]. The chemical structures of some of these compounds are shown in Fig. 1. The relative concentration and distribution of these alkaloids in *C. majus* depends on several factors. Typical alkaloid content in aerial parts are between 0.5 and 1.5% (w/w), according to several authors [6,7] while Suchomelová et al. [8] have reported up to 8% (w/w) total quaternary alkaloids in the methanolic extract of *C. majus* roots. It has been reported that chelidonine, berberine and coptisine are the main alkaloids in the aerial parts of the plant, while sanguinarine and chelerythrine are predominant in the roots [9].

Barbosa-Filho et al. have reported an exhaustive review concerning the anti-inflammatory activity of alkaloids, mainly of isoquinoline

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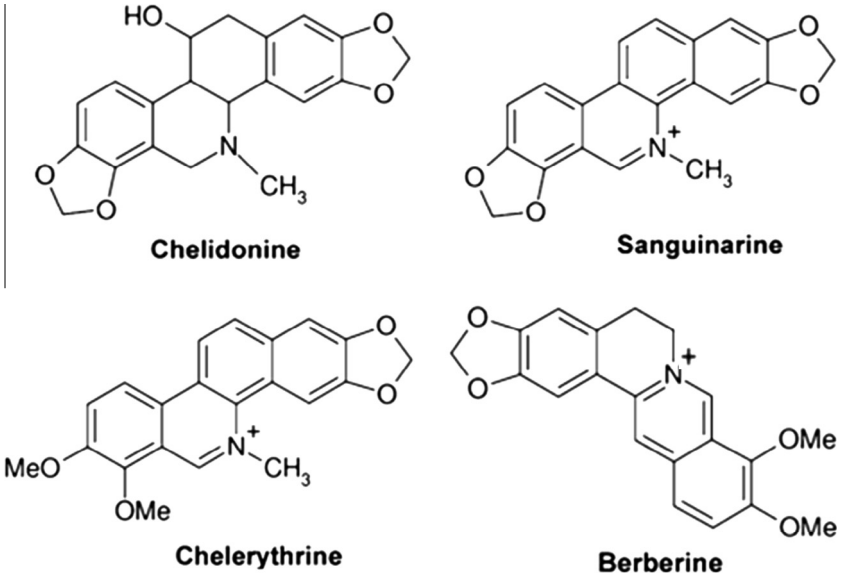

Fig. 1. Chemical structure of main *C. majus* alkaloids.

alkaloids [10]. However, the mechanism responsible for the anti-inflammatory activity of these alkaloids is not yet completely elucidated. Penciková et al. studied the effect of sanguinarine and chelerythrine on gene expression of several pro- and anti-inflammatory cytokines, concluding that sanguinarine presents high anti-inflammatory potential, even comparable to commercial prednisone [11]. Then et al. also reported significant antioxidant activity of *C. majus* ethanolic and aqueous extracts [12].

Due to the promising pharmacological applications of alkaloids, the study and development of techniques for their extraction and purification has become an important research field over the last years. Comparative extraction assays using traditional processes such as infusion, pressing and solvent extraction, as well as novel techniques, such as supercritical fluid extraction (SFE) and microwave assisted extraction have been reported. Then et al. concluded that extract yield, composition and alkaloid content are highly dependent on the extraction method and that SFE using supercritical CO_2 ($scCO_2$) and propylene glycol as co-solvent, and microwave extraction seem to be more selective towards some specific alkaloids, such as coptisine [13].

SFE of alkaloids with pure CO_2 generally presents lower yields than other solvent extraction methods. When polar co-solvents like water or alcohols are used, total extraction yield increases significantly, due to the increase of solvent phase polarity and diffusional properties. Moreover, the choice of a suitable solvent mixture can enhance the extraction selectivity for some specific compounds or group of compounds, with influence on the biological activity of the extracts [14]. General methods to extract/isolate alkaloids are already reported in the literature frequently using an alcohol (methanol or ethanol) as extraction solvent. The obtained solution could be further basified, generally with ammonium hydroxide, and the separation of alkaloids will depend on their differential basicity [15]. Choi et al. have demonstrated that SFE yield of alkaloids (hyoscyamine and scopolamine) from *Scopolia japonica* Maxim. notably increased when a mixture of methanol and diethylamine (10% v/v) was used as co-solvent (operating at 333 K and 34 MPa) [16]. According to these authors, this happens because these alkaloids naturally occur in plant tissues in the form of salts (hydrochlorides) presenting very low solubility in non-polar solvents like $scCO_2$ (which is also acidic). Therefore, the use of a basified co-solvent allows the extraction of alkaloids in the form of free bases, which explains the observed higher alkaloids extraction yields. In fact, quaternary alkaloids exhibit pH-dependent equilibrium between the ionized or iminium form (at pH < pKa) and the neutral or amine form (at pH > pKa), with pKa values typically above 10 [17]. More recently, Xiao et al. tested several co-solvents for the SFE of isoquinoline alkaloids and concluded that the use of diethylamine (10%, v/v), as basifying agent, and water (1%, v/v) as polar co-solvent, enhanced alkaloid extraction yield and selectivity (~50% of the total extract) after 2 h of extraction at 343 K and 20 MPa [18].

Based on these facts, this work aims to study the Supercritical Fluid Extraction (SFE) and Enhanced Solvent Extraction (ESE) of *C. majus* aerial and terrestrial parts in order to obtain fractionated extracts. The effect of temperature, solvent density and basified co-solvents on total extraction yield, kinetic profiles and extract composition was investigated and compared with traditional extraction methods (Soxhlet with ethanol and low pressure solvent extraction with water). Extraction kinetic profiles are also presented and modelled to provide useful information for process design and optimization.

2. Materials and methods

2.1. Samples and chemicals

Samples of *C. majus* were harvested in the district of Guarda, Beira Interior (Portugal). Samples were separated as aerial parts (leaves and stems) and terrestrial parts (roots), dried in an oven at 323 K and comminuted separately originating two distinct raw materials. Particle size distribution was analyzed using a series of sieves and the 18/60 mesh fractions were selected for extraction purposes. The final moisture of the dried samples was determined by thermogravimetric analysis (TGA Q500, TA Instruments, USA).

Carbon dioxide (⩾99.5%, Praxair, Spain), ethanol (⩾99.5%, p.a., Panreac Quimica SA, Spain), isopropanol (⩾99.9%, LC-MS, Fluka, Germany), methanol (⩾99.9%, HPLC grade, Carlo Erba, Italy), ethyl acetate (⩾99.5%, p.a., Panreac Quimica SA, Spain), dichloromethane (⩾99.9%, p.a., Sigma-Aldrich, USA), diethylamine (DEA, ⩾99%, Carlo Erba, Italy), acetonitrile (⩾99.9%, HPLC grade, Carlo Erba, Italy), formic acid (~98%, p.a., Fluka, Switzerland), ammonium acetate (⩾97%, Sigma-Aldrich, Germany), sodium 1-heptanesulfonate (Sigma-Aldrich, Germany), triethylamine