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Off-line coupling of new generation centrifugal partition chromatography device with preparative high pressure liquid chromatography-mass spectrometry triggering fraction collection applied to the recovery of secoiridoid glycosides from *Centaurium erythraea* Rafn. (Gentianaceae)☆

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ABSTRACT

A purification sequence including a Gilson CPC 250 PRO device coupled to PrepHPLC hyphenated with a MS triggering fraction collector was applied to isolate secoiridoid glycosides from a complex methanolic extract of *Centaurium erythraea*. This species is widely used for ethnomedicinal purposes around the Mediterranean Sea.

The solvent system ethyle acetate/ethanol/water 7.5/3/5 was determined using shake-flask method targeting swertiamarin, the major secoiridoid of the extract. Optimization of CPC experimental parameters enabled the injection of 4 g of extract with a flow rate of 40 mL/min at 3000 rpm to provide a secoiridoid glycosides enriched fraction. 130 mg of this latter was submitted to a second step of purification by preparative HPLC (gradient water/formic acid (19:1) (A) and methanol (B) as follows: 0 min, 85% A; 8 min, 60% A; 12 min, 55% A; 35 min, 55% A; 40 min, 10% A; 50 min, 10% A; 52 min, 85% A; 55 min, 85% A) to give swertiamarin (36 mg, yield 27.7%, purity 98.2%). Other secoiridoid glycosides (sweroside, gentiopicroside, secologanol, secoxyloganin) were also isolated in minor amounts. As these monoterpene derivatives are responsible for several biological activities, their quick recovery with high yield and purity may serve as a model for further scale-up and industrial development.

1. Introduction

Centaurium erythraea Rafn. (Gentianaceae) is widely used around the Mediterranean Sea for various ethnomedicinal purposes, especially for the treatment of pathologies that affect the digestive tract and the carbohydrate and lipid metabolism. For example, in Algeria, this species is recommended in case of digestive disorders, hyperglycemia, and as antipyretic, in Turkey to treat stomach ache, ulcers and enteritis [1,2]. In Serbia and FYROM, the prescriptions are related to gastritis and other digestive symptoms, and to diabetes [3–5]. These observations were confirmed in vivo; hepaprotective and gastroprotective effects of *C. erythraea* extracts against aspirin or acetaminophen damages, steatosis and induced type-2 diabetes were demonstrated on animal models [5–8].

Secoiridoid glycosides (SG), a class of polyfunctionnalized monoterpenes are with flavones and xanthones the major metabolites of *C. erythraea* [5,9]. The most abundant are swertiamarin (**1**), gentiopicroside (**2**) and sweroside (**3**) (Fig. 1). They are considered as at least partially responsible for the biological activities mentioned above and were also evaluated after isolation for their anti-bacterial properties [10,11]. Several papers describe their identification and quantification within this plant species, using various methods, including hyphenated techniques [9,11–14]. Other reports were dedicated to the specific obtainment of SG with high yield from callus, root, shoot and cell suspension cultures of *C. erythraea*. [14–17]

Thus, efficient recovery of **1**-**3** is currently considered as a challenging task. Centrifugal partition chromatography (CPC), a

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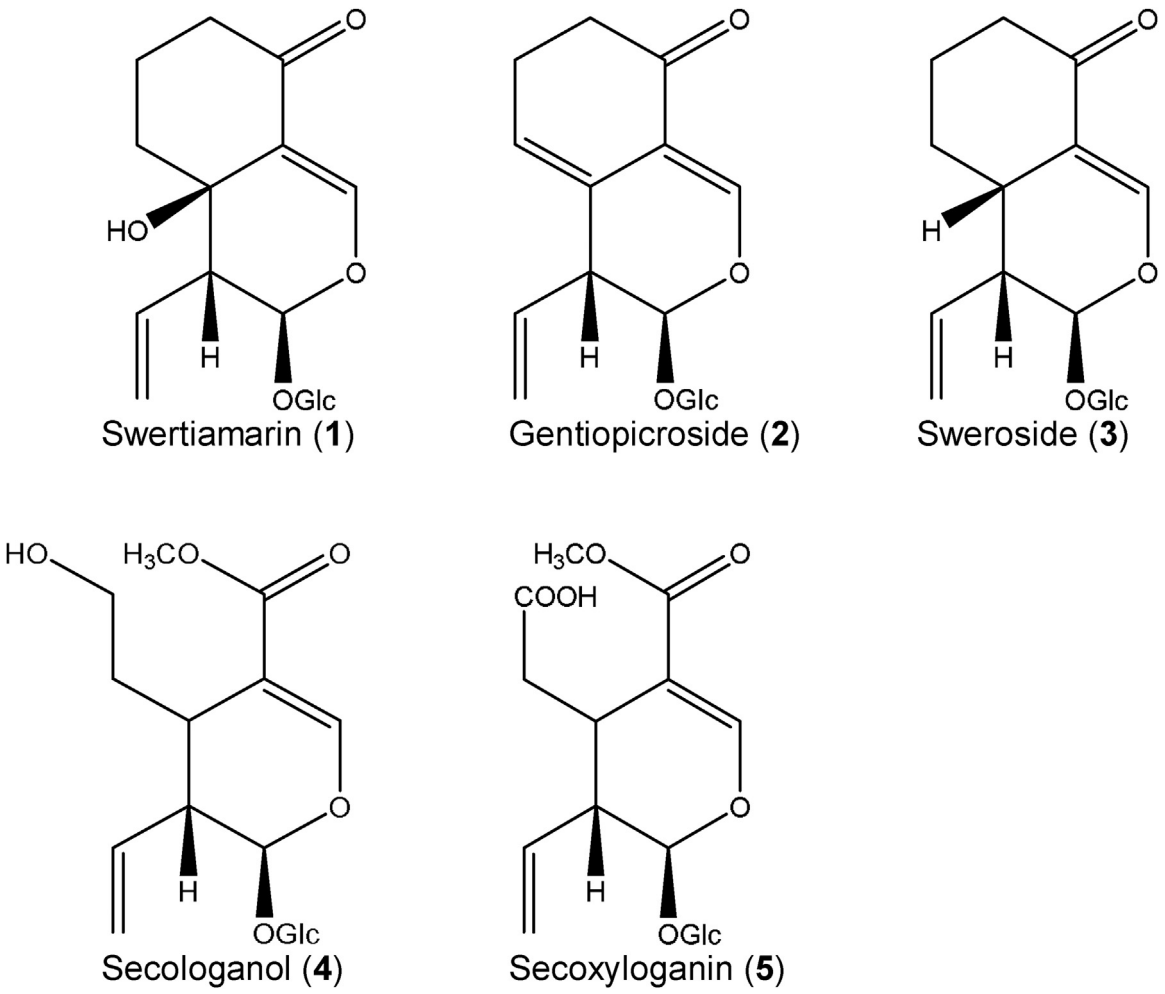

Fig. 1. Structures of the secoiridoid glycosides isolated from *C. erythraea* methanolic extract.

liquid-liquid chromatography based on the partition of the analytes between two immiscible phases, has proven since long to be a useful technique for the isolation of pure natural products [18,19]. A wide variety of solvent systems has been developed aiming to target specific class of metabolites, including iridoid and secoiridoid glycosides [20–22]. Nevertheless, CPC or CCC may also be regarded as tools dedicated to the quick fractionation and enrichment of extracts. Then, coupling procedure with another purification technique such as liquid chromatography could give access to pure compounds [23–26]. Moreover, mass spectrometry has benefited in the last decades of increasing consideration, mainly due to the application in metabolomic studies and fingerprinting. Besides this research and drug quality control purposes, mass spectrometry used as a detection method coupled to liquid chromatography does not require chromophore, is very sensitive, and therefore is well-adapted to the detection of poor UV-sensitive compounds, such as SG [9,14].

In this context, a strategy based on the off-line coupling of a Gilson CPC 250 PRO, a new device for ultra-fast separation and high injection capacity, with PrepHPLC-Mass Spectrometry triggering fraction collection has been developed and applied to the isolation of **1**, **2** and **3** from a methanolic extract of *C. erythraea*. Previous attempts to separate **1** and/or **2** and/or **3** were made using CCC [27–30]. In these cases, biphasic solvent systems were for example *n*-butanol/ethyl acetate/methanol/1% acetic acid water (7.5:0.5:0.5:3.5, v/v/v/v) or *n*-butanol/0.1% aqueous trifluoroacetic acid (1:1, v/v).

2. Materials and methods

2.1. Reagents

Methanol for extraction (ACS RPE grade), deuterated methanol for NMR, methanol for HPLC (HPLC grade) and formic acid (ACS reagent grade) were purchased from Acros Organic, Fisher Scientific (Illkirch, France). Ethanol and ethyl acetate were purchased from Carlo Erba-SDS. Cyclohexane and ethyl acetate were distilled before use. Water was filtered from deionized water.

Merck precoated silica gel $60F_{254}$ plates, 0.25 mm thickness, were used for analytical thin-layer chromatography. The spots on TLC plates were visualized by exposure to UV 254 nm and by spraying vanillin solution and heating.

2.2. Plant material and preparation of the crude extract

10 kg of aerial parts of *C. erythraea* were purchased from SAS Cailleau Herboristerie (Chemillé en Anjou, France). A voucher specimen has been deposited in the laboratory of Pharmacognosy, Paris Descartes University, under the reference TSVM03.

The plant material (150 g) was macerated for 24 h in MeOH (1L). After paper filtration, the solvent was evaporated under reduced pressure to furnish 23.4 g of crude extract (yield 15%).

2.3. HPLC-UV-MS

HPLC-UV-MS profiles were done on a UHPLC focused BUL300 Thermo Scientific equipped with a pump, an autosampler, a column