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Centrifugal partition chromatography directly interfaced with mass spectrometry for the fast screening and fractionation of major xanthones in *Garcina mangostana*

E. Destandau [a,*], A. Toribio [a], M. Lafosse [a], V. Pecher [b], C. Lamy [b], P. André [b]

[a] *ICOA, Université d'Orléans, UMR CNRS 6005, 45067 Orléans Cedex 2, France*
[b] *Active Ingredient Department, LVMH Recherche, 45800 Saint Jean de Braye, France*

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ABSTRACT

Xanthones are well known for their interesting phytochemical properties, which make them attractive to the pharmaceutical and medicinal industry. We have therefore developed a method to analyse the major xanthones in *Garcina mangostana*. The xanthones were extracted by pressurized liquid extraction with ethanol and separated at the semi-preparative scale by centrifugal partition chromatography (CPC) with a biphasic solvent system consisting of heptane/ethyl acetate/methanol/water (2:1:2:1, v/v/v/v). A CPC-electrospray ionisation MS coupling was performed and used to simultaneously separate and identify the compounds. Thanks to a variable flow splitter and an additional stream of ethanol/1 mol L^{-1} ammonium acetate (95:5, v/v), all the compounds were ionised, detected and monitored whatever the solvents used in mobile phase for the CPC separation. The dual mode or elution–extrusion which are less solvent-consuming and faster than the elution mode were used without loss of ionisation and detection.

1. Introduction

It is widely recognized that the consumption of fruits and vegetables can reduce the incidence of degenerative diseases including cancer, heart disease, inflammation, immune system decline and brain dysfunction. These protective effects are considered to be mainly due to the presence of various antioxidants in fruits and vegetables [1].

Garcinia mangostana, commonly known as *mangosteen*, is a tropical tree which can attain 6–25 m in height and is mainly found in India, Sri Lanka, Thailand and Indonesia. *Mangosteen* has dark-purple to red-purple fruits. The edible fruit is white, soft, and juicy with a sweet, slightly acid taste and pleasant aroma [2]. The fruit hull of *mangosteen* has been used for hundreds of years in Southeast Asia to treat skin infections, wounds, and diarrhea. Phytochemical studies have shown that these plant species are rich in a variety of prenylated xanthones and the constituents have demonstrated a number of bioactivities (antioxidant, antifungal, anti-inflammatory, antimicrobial, antibacterial, etc.) [3].

Xanthones are a kind of polyphenolic compounds that contain a distinctive chemical structure with a tricyclic aromatic ring. Most often these ring moieties are substituted with a variety of isoprene, phenolic, methoxy groups that give rise to a large variety of possible structures with a wide range of polarity.

Six xanthones have already been extracted from *Garcina mangostana*, separated and identified by HPLC-DAD (photodiode array detection) [4] or HPLC-electrospray ionisation (ESI) MS [5] methods. However only a few methods have been reported for directly interfacing counter-current chromatography (CCC) with mass spectrometry (MS) [6–13]. They demonstrate that combining the preparative capacities of CCC with the low detection limits of mass spectrometry is a versatile tool for 'target'-oriented and rapid compound screening in isolation procedure.

In a new approach, the aim of this study is to develop a method coupling centrifugal partition chromatography (CPC) with ESI-MS with a variable flow splitter to simultaneously isolate and identify the main natural xanthones extracted from the pericarp of *mangosteen*, in order to achieve fast screening of the crude extract and to obtain some standards of pure xanthone or at least enriched fractions to study the bioactivity of each xanthone independently.

2. Experimental

2.1. Reagents and apparatus

Organic solvents, acetonitrile, heptane, ethyl acetate, methanol, ethanol used for extraction, CPC and HPLC were of analytical grade from SDS Carlo Erba (Val-de-Reuil, France). Ultrapure water (resis-

* Corresponding author.
E-mail address: emilie.destandau@univ-orleans.fr (E. Destandau).

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tance < 18 MΩ) was provided by an Elgastat UHQ II apparatus (Elga, Antony, France).

Lyophilised pericarp of *G. mangostana* was provided by LVMH collaborators.

Pressurized liquid extraction (PLE) was performed with an ASE 100 system from Dionex (Voisins le Bretonneux, France). HPLC analyses were carried out with an Agilent HP 1100 chromatographic system (Waldbronn, Germany) equipped with a quaternary pump, a 20 μL sample loop, and a UV detector Kontron (Zurich, Switzerland) set at 320 nm. The column used was a LiChrospher RP-18 (150 mm × 4.6 mm, 5 μm) from VWR (Fontenay-sous-bois, France).

The preparative CPC instrument used in the present study was a Semi-Preparative FCPC from Kromaton (Angers, France), equipped with a 200 mL or a 50 mL rotor depending on the experiment.

The mass spectrometer was a Quattro Ultima triple quadrupole equipped with a Z-Spray dual orthogonal electrospray source from Waters. MassLynx1 4.0 software was used to process the data.

The splitters used to connect the CPC to the ESI ionisation source were purchased for the micro valve T-splitter from Upchurch Scientific (Oak Harbor, USA) and for the variable flow splitter (VFS) from Rheodyne (Rohnert Park, CA, USA).

2.2. Extraction of xanthones

Xanthones are often extracted from plants by using high amounts of organic solvent such as hexane [4], acetone [5] or methanol [1]. The dried fruit is extracted by maceration in solvent at room or higher temperature for several hours. After filtration and evaporation of the extraction solvent, the crude extract is then redissolved in a convenient solvent for analysis.

In our method xanthones were extracted from the lyophilised pericarp of *mangosteen* with ethanol (a biodegradable solvent) by PLE. PLE accelerates the traditional extraction process and reduces the amount of solvent by using solvent at elevated temperature and pressure. Pressure is applied to the sample extraction cell to maintain the heated solvent in a liquid state during extraction. After heating, the extract is flushed from the sample cell into a collection bottle and is ready for analysis. The operating conditions were: temperature of the extraction cell 100 °C, static solvent extraction time: 5 min, flushed volume 60%, 1 static cycle, purge time 100 s. From 2 g of lyophilised pericarp an amount of 964 mg of dry extract was collected in 15 min with about 50 mL of ethanol.

This method was compared to a classical ultrasonic extraction (UE) method: 2 g of lyophilised pericarp was weighed in a centrifuge tube filled with 15 mL of ethanol. The mixture was then sonicated during 20 min in an ultrasonic bath and centrifuged. The supernatant was transferred to evaporation and the same steps were repeated eight times until the amount extracted was considered as negligible. After evaporation of the 120 mL of solvent, an amount of 464 mg of dry extract was obtained in at least 3 h. The extraction yield with PLE was twice as good as that obtained by UE; moreover both the solvent consumption and the extraction time were reduced.

2.3. HPLC procedure

All HPLC analyses were carried out on a LiChrospher RP-18 (150 mm × 4.6 mm, 5 μm) column, the mobile phase was (80:20, v/v) acetonitrile/water mixture at room temperature with a 1 mL min^{-1} flow rate and a 20 μL injection loop. Detection was performed with a UV detector at 320 nm. This wavelength was a good compromise, since the tricyclic aromatic structure of xanthones enables good absorption at this wavelength, at which only a small number of other compounds absorb, making 320 nm a selective wavelength for xanthone detection.

2.4. Crude extract partitioning by centrifugal partition chromatography

2.4.1. Centrifugal partition chromatography

CPC was chosen to fractionate the crude extract and isolate xanthones. CPC is a separation method that uses no solid stationary phase support. Instead, two non-miscible liquids are used: the first one is the stationary phase and the second one is the mobile phase. The CPC apparatus consists of a single rotation axis column which is in hydrostatic equilibrium. The CPC column contains a stacked circular disk made of a succession of small cells linked in cascade by capillary ducts. The stationary phase is retained inside the column thanks to the centrifugal field, while the mobile phase is pumped through the stationary phase. The compounds elute and are separated through the column according to their respective partition coefficient defined as the concentration of solute in the upper phase on the concentration of solute in the lower phase ($K_D = C_{\text{upper phase}}/C_{\text{lower phase}}$). The compounds that have a stronger affinity for the stationary phase are retained inside the column for a longer period of time, whereas those that have a stronger affinity for the mobile phase are eluted faster out of the column [14,15].

The main advantage of CPC over HPLC is the absence of solid phase, which avoids any irreversible adsorption of solutes in the column. If the retention volumes of solutes become too high, the dual mode can be used. The role of the two phases can be reversed at any time; the stationary phase becomes the mobile phase and vice versa. Consequently the compounds remaining in the stationary phase can be eluted in the mobile phase. The elution–extrusion procedure is another way to extrude compounds from the column without consuming large amounts of solvent. The method comprises two steps: the first one is a regular chromatographic elution process. Next, the stationary phase containing the partially separated hydrophobic solutes is extruded out of the column continuously using fresh liquid stationary phase pumped in the same descending or ascending mode as the one used during the first step [16].

2.4.2. Measurement of partition coefficient and choice of a biphasic system

The first step in CPC separation is selection of the biphasic solvent system and determination of the partition coefficients (K_D) of the analytes. In optimal conditions, where $K \approx 1$, the solutes are partitioned equally between the two phases and a satisfactory separation may occur.

The partition coefficient of the extract was evaluated by calculating the partition coefficient of the major solute in several biphasic solvent systems. Crude extract (0.5 mg) was dissolved in a biphasic system (5 mL) in a test tube. The upper and the lower phases were then injected in HPLC. The partition coefficient was calculated as the ratio of the major solute peak area in each phase. Two-phase solvent systems were chosen according to the moderate polarity of xanthones. First, ternary solvent systems such as heptane/MeOH/H_2O or EtOAc/MeOH/H_2O were tested. However, since the large polarity gap between the two phases does not allow the partition of the extract ($K \ll 1$ or $K \gg 1$), the Arizona liquid system was evaluated [17]. This quaternary system was found to be highly suitable when developing a strategy to find a liquid system that could be used for the separation and purification of a wide variety of natural products. The Arizona liquid system consists of 23 varying, proportions of heptane/EtOAc/MeOH/H_2O, from polar to non-polar systems labelled A–Z (except E, I and O). Four moderately non-polar systems were chosen and the partition coefficients were calculated (see Table 1).

The 5/2/5/2 system provided the optimal partition coefficient, and was therefore the first one tested in CPC. However, the 2/1/2/1 system which allowed both better retention in the stationary phase