Separation and Purification Technology 118 (2013) 680–689

Contents lists available at ScienceDirect

Separation and Purification Technology

journal homepage: www.elsevier.com/locate/seppur

An efficient preparative procedure for main flavone aglycones from *Equisetum palustre* L. using macroporous resin followed by gel resin flash chromatography

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ARTICLE INFO

Article history:
Received 21 February 2013
Received in revised form 1 July 2013
Accepted 27 July 2013
Available online 3 August 2013

Keywords:
Equisetum palustre L
Flash chromatography
Toyopearl HW-40S gel resin
Flavone aglycones

ABSTRACT

In this study, a simple and efficient procedure for preparation of three flavone aglycones (luteolin, apigenin and genkwanin) from *Equisetum palustre* L. was developed using macroporous resin followed by gel resin flash chromatography. Among six widely used macroporous resins, D101 resin was chosen to enrich three flavone aglycones because of its good enrichment efficiency in static and dynamic tests. After one run treatment with D101 resin, the contents of luteolin, apigenin and genkwanin were 13.3-fold, 12.5-fold and 12.9-fold increased with recovery yields of 83.6%, 78.8% and 81.2%, respectively. The enriched sample was directly subjected to flash chromatography on Toyopearl HW-40S gel resin column. Luteolin, apigenin and genkwanin with purities of more than 97% were produced with recovery yields of 91.8%, 92.3% and 93.1% by one gel resin flash chromatography run. The developed procedure boasts easily reusable solvents, production of high-purity product as well as high recoveries, and allows an easy scale-up. Therefore, it is a promising basis for large-scale preparation of flavone aglycones from *Equisetum palustre* or other plant extracts.

1. Introduction

Equisetum palustre L. is a traditional herbal medicine in many countries and regions. Its aerial parts have been used to treat peptic ulcer and hemorrhoids, and to pass kidney stones [1,2]. The extracts of *E. palustre* possesses various bioactivities including gastroprotective effect, antioxidant, antimicrobial and genotoxicity [3–5]. Previous chemical investigations indicated that the secondary metabolites of *E. palustre* included flavonoids, alkaloids, and essential oils [5–7]. Over the past few decades, flavonoids have received increased attention for their health promoting benefits and were considered as major resources of drugs and food additives [8–10]. Flavone aglycones are flavonoids of particular importance as they are found to possess free radical scavenging activity in foods [11]. Epidemiological studies indicated that their consumption could assure a reduced risk of cancer and cardiovascular disease [12,13]. They have also been reported to exhibit a wide range of bioactivities, such as the anti-inflammatory activity of luteolin, apigenin and genkwanin, the antibacterial activity of apigenin and genkwanin against *Vibrio cholerae* and *Enterococcus faecalis*, the protective effects of apigenin and luteolin on immortalized human keratinocytes (HaCaT) against UV-A damage and so on [14–16]. Moreover, Verbeek et al. [17] found that flavones (luteolin and apigenin included) effectively inhibited the potentially pathogenic function of autoreactive T cells but flavanoles and flavanones did not. Considering the potential use of flavone aglycones, it is extremely necessary to develop a simple and efficient procedure for their preparation separation. However, it is very challenging to separate natural products of interest from plant material because of its compositional complexity.

Silica gel column chromatography was traditionally used for separation of flavone aglycones [18]. However, this method is time consuming and laborious, and has complications such as irreversible adsorption onto the solid support, and tailing of the solute peaks. Recently, high-speed counter-current chromatography, a support-free liquid chromatography technique, was considered to be an alternative for separation of flavone aglycones from plant materials [19–21]. Nevertheless, its application in large-scale preparative separation is limited by its high cost and lower sample load. Macroporous resins, a macromolecular organic polymer with stereo-poriform structure, have been widely used to enrich natural

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http://dx.doi.org/10.1016/j.seppur.2013.07.045

products of interest from traditional Chinese medicine because of their convenience, low cost, easy regeneration, and adjusted selectivity [22]. They selectively adsorb components from aqueous solution as well as non-aqueous systems by electrostatic force, hydrogen bonding interaction, complexation, size sieving action, etc., and are gaining popularity in pharmaceutical applications with chemical inertness, higher capacity and selectivity. At present, macroporous resins have already been applied to enrich lots of compounds, such as flavonoids, alkaloids, and glycosides [23–26]. Flash chromatography, also known as medium pressure chromatography, was popularized as an alternative to slow and often inefficient gravity-fed chromatography several years ago. It is a fast and inexpensive separation technique for the purification of natural products from plant extracts. Compared with traditional gravity-fed chromatography, this technique gives a rapid and high resolution chromatography. As we all know, stationary phase is the key to separation process. Toyopearl HW-40S gel resin is a hydroxylated methacrylic polymer incorporating high mechanical and chemical stability. Compared with traditional stationary phase silica gel, Toyopearl HW-40S gel resin has much better regeneration efficiency and lower back pressure. In previous studies, it exhibited potential capacity for separation and purification of flavonoids [27,28]. Although it is frequently employed as a stationary phase during pre-separations and in open column chromatography systems [28,29], its separation power in flash chromatography is much less prevalent.

In our previous study, we found *E. palustre* was rich in flavonoids, and three main flavone aglycones (luteolin, apigenin and genkwanin) possessing notable bioactivities were isolated for the first time. In this study, we aimed to develop a simple and efficient procedure for preparation of flavone aglycones from *E. palustre* L. using macroporous resin followed by flash chromatography on Toyopearl HW-40S gel resin column (Fig. 1). Toyopearl HW-40S column chromatography can effectively eliminate the complications of silica gel column chromatography, and have very low back pressure which assures the scalability of developed flash chromatography. The developed procedure in this study boasts easily reusable solvents, production of high-purity product as well as high recoveries, and allows an easy scale-up. The chemical structures of these compounds were verified by MS, ^{1}H NMR and ^{13}C NMR. To the best of our knowledge, this is the first report on the purification of luteolin, apigenin and genkwanin by Toyopearl HW-40S gel resin.

2. Experimental

2.1. Reagents

Reagents of HPLC grade including methanol and formic acid were purchased from J & K Chemical LTD. (Beijing, China). The solvents used for extraction and enrichment and separation were analytical grade, and purchased from Beijing Chemistry Corporation (Beijing, China). The macroporous resins used including NKA-9, OU-1, SA-3, D101, FL-3 and AB-8 were obtained from Nankai Hecheng S & T (Tianjin, China) and Bonchem (Hebei, China). Their physical properties were summarized in Table 1. These resins were pretreated according to previous description [30] and their moisture contents were determined by drying the beads at 100 °C to constant weight in a drying oven. Toyopearl HW-40S gel resin and silica gel (300–400 mesh) were purchased from Tosoh Corporation (Tokyo, Japan) and Qingdao Meigao Chemical Co. Ltd. (Qingdao, China), respectively.

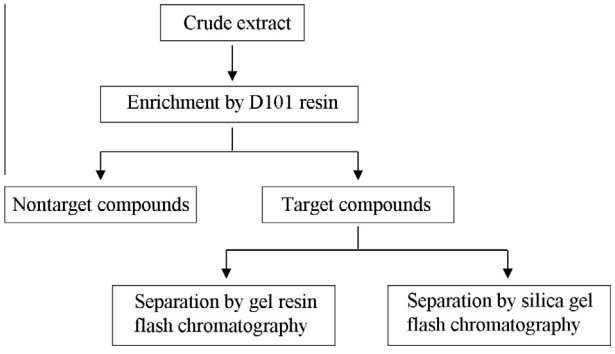

Fig. 1. The purification of three flavone aglycones from *Equisetum palustre* L.

2.2. Preparation of E. palustre extracts

E. palustre was collected in autumn 2011 from Inner Mongolia Autonomous Region, China, and authenticated by Prof. Shao-Quan Nie from the Key Laboratory of Forest Plant Ecology, Ministry of Education, Northeast Forestry University, China. Collected material was dried in shade at room temperature, milled and then stored in dark. Pulverized *E. palustre* (6 kg) was extracted with 80% aqueous ethanol (3 × 42 L) at room temperature for 1 week. The extraction solution was gathered and separated by membrane filtration. The filtrate was concentrated to dryness by removing the ethanol solvent in a rotary evaporator (RE-52AA, Shanghai Huxi Instrument Co., China) at 40 °C, and extracts of *E. palustre* was obtained. 20% aqueous ethanol was added to get sample solution at the concentration of 0.1999 mg/mL for luteolin, 0.0835 mg/mL for apigenin and 0.0482 mg/mL for genkwanin, respectively.

2.3. HPLC detection

The flavone aglycones in samples were analyzed by an Agilent 1200 series liquid chromatography system (Agilent, San Jose, CA, USA) equipped with a G1311A quaternary pump, a G1322A degasser, a G1365B MWD UV detector and a G1328B manual injector. Separation of the analytes was achieved on a Luna C18 reversed-phase column (250 × 4.6 mm i.d., 5 μm, Phenomenex, USA). The mobile phase consisted of 1% formic acid aqueous solution (A) and acetonitrile (B) using the following gradient elution program for separation: 0–15 min, 29% B; 15–25 min, 29–50% B; 25–35 min, 50–60% B. The flow rate was kept at 1.0 mL/min. The column temperature was kept at 30 °C and the injection volume was 5 μL. The wavelength was set at 340 nm. The retention times for luteolin, apigenin and genkwanin were 14.2 min, 22.6 min, and 31.6 min, respectively.

Table 1
Physical properties of the macroporous resins used.

Trade name	Surface area (m^2/g)	Average pore diameter (nm)	Particle diameter (mm)	Polarity
NKA-9	250–290	15.0–16.5	0.3–1.25	Polar
OU-1	100–150	20.0–30.0	0.3–1.0	Strong-polar
SA-3	500–600	15.0–25.0	0.3–1.20	Non-polar
D101	400–600	10.0–12.0	0.2–0.6	Non-polar
FL-3	80–120	15.0–20.0	0.3–1.25	Non-polar
AB-8	480–520	13.0–14.0	0.3–1.25	Weak-polar