Journal of Chromatography B, 1014 (2016) 45–55

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Application of ionic liquids in vacuum microwave-assisted extraction followed by macroporous resin isolation of three flavonoids rutin, hyperoside and hesperidin from *Sorbus tianschanica* leaves

Huiyan Gu*, Fengli Chen, Qiang Zhang, Jing Zang

School of Forestry, Northeast Forestry University, Harbin 150040, China

ARTICLE INFO

Article history:
Received 16 September 2015
Received in revised form 27 January 2016
Accepted 30 January 2016
Available online 2 February 2016

Keywords:
Ionic liquid
Vacuum microwave-assisted extraction
Macroporous resin
Rutin
Hyperoside
Hesperidin
Sorbus tianschanica

ABSTRACT

Rutin, hyperoside and hesperidin were effectively extracted from *Sorbus tianschanica* leaves by an ionic liquid vacuum microwave-assisted method. A series of ionic liquids with various anions and alkyl chain length of the cations were studied and the extraction was performed in $[C_6mim][BF_4]$ aqueous solution. After optimization by a factorial design and response surface methodology, total extraction yield of 2.37 mg/g with an error of 0.12 mg/g (0.71 ± 0.04 mg/g, 1.18 ± 0.06 mg/g and 0.48 ± 0.02 for rutin, hyperoside and hesperidin, respectively) was achieved under −0.08 MPa for vacuum, 19 min and 420 W for microwave irradiation time and power, and 15 mL/g for liquid–solid ratio. The proposed method here is more efficient and needs a shorter extraction time for rutin, hyperoside and hesperidin from *S. tianschanica* leaves than reference extraction techniques. In stability studies performed with standard rutin, hyperoside and hesperidin, the target analytes were stable under the optimum conditions. The proposed method had a high reproducibility and precision. In addition, separation of rutin, hyperoside and hesperidin from $[C_6mim][BF_4]$ extraction solution was completed effectively by AB-8 macroporous resin adsorption and desorption process. Ionic liquid vacuum microwave-assisted extraction is a simple, rapid and efficient sample extraction technique.

1. Introduction

Sorbus is a genus of the family Rosaceae, which composed of many kinds of deciduous trees or shrubs occurring in cool-temperate regions of the northern hemisphere [1,2]. *Sorbus tianschanica* Rupr., a deciduous tree in Rosaceae family, is distributed and cultivated in mountainous regions of Central Asia and in the northern region of China, especially in Xinjiang Uygur autonomous region [3]. It has been used as a pharmacologically valuable plant to treat tuberculosis, asthma, and gastritis [4]. Chemical analysis shows that it contains terpenoid, organic acids, and flavonoids. Phytochemical research shows that flavonoids are the major biologically active compounds in this plant, such as hesperidin, rutin, and hyperoside [5,6]. For other known sources, the content of rutin in soybean is 0.44 mg/g [7], and the contents of rutin and hyperoside are 0.355 mg/g and 1.528 mg/g for *Houttuynia cordata* Thunb [8]. For hesperidin, most of it has been extracted from *Citrus sinensis*, *Citrus reticulate* etc., plants [9,10]. Based on the references [6], the contents of rutin, hyperoside and hesperidin were found to be 0.44 mg/g, 1.13 mg/g and 0.43 mg/g, respectively. Due to the leaves of *S. tianschanica* are very exuberant, and it is a renewable resource, thus extraction of these bioactive components from *S. tianschanica* leaves would be useful.

Hesperidin (hesperitin-7-rhamnoglucoside, a flavonoids compound) exhibits several pharmacological actions such as antihypertensive, antidiabetic activities, anti-cancer [11], liver protection [12], anti-inflammatory and antioxidant activity [13]. In addition, it has effective influence in inhabitation of cholesterol synthesis and absorption [14], stimulation of intestinal BCMO1 activity [15], protection hypoxia-ischemic brain injury [16]. Rutin (Quercetin-3-O-rutinoside, a flavonol glycosides compound) play an important role in antioxidant activity [17], anticonvulsant [18], liver protection [19], lung protection [20], improvement of spatial memory [21], heart protection [22], anti-inflammatory [23] and improvement of circulation [24], thus has attracted increasing attention. Hyperoside (Quercetin-3-O-galactopyranoside, a flavonol glycosides compound) has various pharmacological and physiological effects such as antinociceptive activity [25], anti-inflammatory [26], antibiofilm activity [27], antioxidant [28], Anti-melanogenesis

* Corresponding author at: School of Forestry, Northeast Forestry University, Hexing Road 26, Harbin, Heilongjiang, China.
E-mail address: hygnefu@126.com (H. Gu).

http://dx.doi.org/10.1016/j.jchromb.2016.01.045
1570-0232/

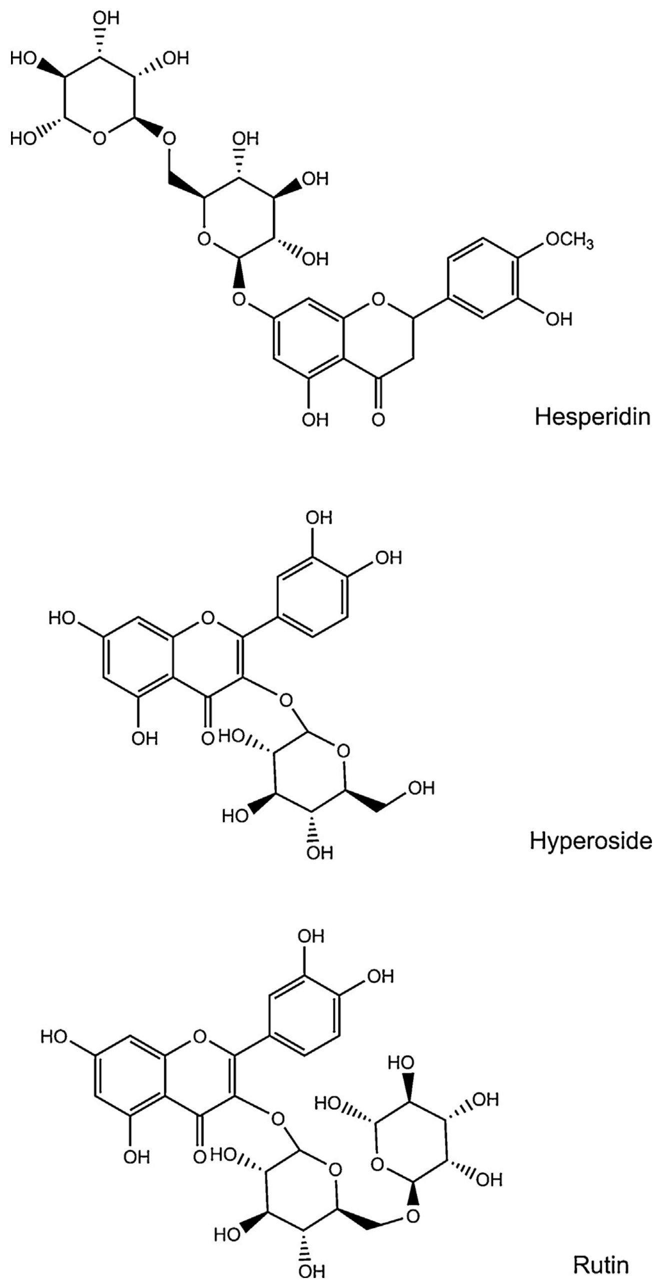

Fig. 1. The molecular structures of rutin, hyperoside and hesperidin.

properties [29], antiviral [30], antidepressant [31] and protection of ucose-mediated protein damage [32]. The molecular structures of rutin, hyperoside and hesperidin are presented in Fig. 1. Due to these potential pharmacological values existed in the three natural compounds, it is significant to separate these three components from plant matrixes.

Conventionally, some common extraction methods with organic solvents were applied in extraction of flavonoids from plant materials, including maceration extraction, heating reflux extraction (HRE) and ultrasound-assisted extraction. Unfortunately, the longer time needed, larger volumes of toxic organic solvents required and lower extraction yield obtained by these methods result in unsatisfactory recoveries, leading to these methods inefficient. Microwave-assisted extraction (MAE) requires shorter time to obtain higher extraction yields for the target analytes, thus it has been accepted as a potential and powerful alternative to traditional extraction techniques in extraction of active constituents widely. However, the high temperature for MAE will lead to condensation, hydrolyzation, degradation, isomerization or oxidation of flavonoids during extraction process [33], especially for the thermo- and oxygen-sensitive constituents. For reduction of the loss of rutin, hyperoside and hesperidin, a method with low-toxic extraction solvent and efficient extraction yield was required urgently.

Vacuum microwave-assisted extraction (VMAE) apparatus that combines conventional MAE with a vacuum pump was developed [34,35], which can avoid or reduce degradation of thermo- and oxygen-sensitive constituents compared to conventional open MAE [36,37]. In VMAE, the effect of vacuum was to remove the air from the extraction reaction system, so that it can lead to lower boiling point of the extraction solvent. The extraction of thermo- and oxygen-sensitive constituents in the system with lower temperature was always accompanied by higher extraction yield.

Ionic liquids, also known as molten salts, which are composed of bulky organic cations and inorganic or organic anions, are liquid near room temperature. In recent years, ionic liquids used as alternative to conventional extraction solvents have attracted much interest in various applications thanks to their excellent chemical and physical properties, including wide liquid range, negligible vapor pressure, tunable viscosity, good stability and miscibility with water and organic solvents, and good solubility and extractability for various organic compounds [38]. Ionic liquids have been applied in preparation of active constituents from plant materials successfully, such as lignan [39], coumarin [40], glycoside [41], triterpenoid [42], flavonoid [42], procyanidins [43], alkaloid [38,44] and organic acid [45,46]. However, separation target analytes from ionic liquid extraction solution has been rarely reported in the literatures [47–49].

In the present work, an ionic liquid vacuum microwave-assisted extraction (ILVMAE) technique was developed for extraction of rutin, hyperoside and hesperidin from *S. tianschanica* leaves. A factorial design and response surface methodology (RSM) with a Box–Behnken design (BBD) were applied to investigate the influences of changing the kinds of anion and carbon chain length of cation, the ionic liquid aqueous solution concentration, microwave irradiation time, microwave irradiation power, liquid–solid ratio and vacuum on the extraction yield. In addition, these target compounds was preliminary separated from ionic liquid extraction solution using macroporous resin.

2. Experimental

2.1. Materials and chemicals

The leaves of *S. tianschanica* Rupr. (Rosaceae family) were collected in September 2014 in Tianshan Mountain in Urumqi of China. A voucher specimen was deposited in the herbarium of this Key Laboratory. Its voucher specimen number is 006110232592012. The leaves were dried, grinded using a pulverizer, sieved (60–80 mesh) and stored at 4 °C before use. The same batch of sample was used in all experiments.

Reference compounds of rutin, hyperoside and hesperidin were purchased from Sigma–Aldrich Inc. (St. Louis, MO, USA). All ionic liquids ([C_4mim]Cl, [C_4mim]Br, [C_4mim][BF_4], [C_4mim][NO_3], [C_4mim][HSO_4], [C_4mim][ClO_4], [C_2mim][BF_4], [C_6mim][BF_4], [C_8mim][BF_4], where C_2 = 1-ethyl, C_4 = 1-butyl, C_6 = 1-hexyl, C_8 = 1-octyl, mim = 3-methyl-imidazolium) were purchased from Chengjie (Shanghai, China) and used without further purification. Methanol in chromatographic grade was bound from J&K Chemical Ltd. (Beijing, China). All the other reagents in analytical grade were purchased from Beijing Chemical Reagents Co. (Beijing, China). Deionized water was purified by a Milli-Q water purifi-