



Purification of Three Flavones from *Chamaecyparis obtusa* by Multi-Phase Extraction using Anion-Exchange Imidazolium Silica

Y.R. LEE and K.H. ROW*

Department of Chemistry and Chemical Engineering, Inha University, Incheon 402-751, Republic of Korea

*Corresponding author: Fax: +82 32 8720959; Tel: +82 32 8607470; E-mail: rowkho@inha.ac.kr

Received: 24 September 2013;

Accepted: 27 December 2013;

Published online: 16 September 2014;

AJC-15935

This paper reports the application of a multi-phase extraction (MPE) method for the purification of three flavones from *Chamaecyparis obtusa* using silica-confined ionic liquids as the sorbent. 0.1 g silica, SilprImCl, SilprMImCl, SilprEImCl and SilprBImCl sorbents were packed into separate empty polypropylene cartridges with 0.1 g *C. obtusa* powder. The multi-phase extraction conditions was water using a washing step and methanol using an elution step. When using different SiILs, SilprBImCl showed the best recovery. The amounts of quercetrin, myricetin and amentoflavone obtained were 33.69, 4.09 and 27.38 $\mu\text{g g}^{-1}$, respectively.

Keywords: Multi-phase extraction, Ionic liquid, Anion exchange, Purification, Flavone.

INTRODUCTION

Chamaecyparis obtusa (*C. obtusa*) is a member of the cypress family *Cupressaceae*, which is commonly known as Kiso-Hinoki in Japan¹. The essential oils extracted from the leaves of the *C. obtusa* tree are generally used as a functional additive with a good fragrance in soaps, toothpastes and cosmetics². *C. obtusa* plants have been found to contain bioactive compounds, such as quercetrin, myricetin and amentoflavone (Fig. 1), some of which exhibit antioxidant³, antibacterial, antitumor, antifungal and anti-mite^{4,5}. Ionic liquids (ILs) have attractive properties, such as hydrophobicity, miscibility with several inorganic/organic solvents and π - π interactions between the anlyte and functional groups of the ionic liquids. The ionic liquids are used in chemical reactions and separation⁶. Ionic liquids are also used as stationary phases in HPLC and solid-phase extraction (SPE) sorbents, bonding them to silica and hence constituting silica-confined ionic liquids (SiILs)⁷.

Solid-phase extraction used widely for the clean-up of analytical samples, as well as for the purification of range of chemicals in the 1970s⁸. On the other hand, SPE has disadvantages, such as the low interactions between sorbent and target compounds, which results in, a loss of the target compounds and low selectivity⁹. To overcome the disadvantages of SPE, multi-phase extraction (MPE) was developed to clean-up solid samples. The solid sample and sorbent were packed into a SPE cartridge. The first step was removing the impurities. This method uses the classical solid-liquid or SPEs. Solid-phase extraction generally involves extracting, loading, washing and

eluting, but the MPE method combines the extraction and loading steps to decrease the processing time¹⁰.

This paper, reports the use of differnt SiILs used as an MPE sorbent to extract and purify quercetrin, myricetin and amentoflavone from *C. obtusa*. The interactions between ttarget compounds and sorbent were examined.

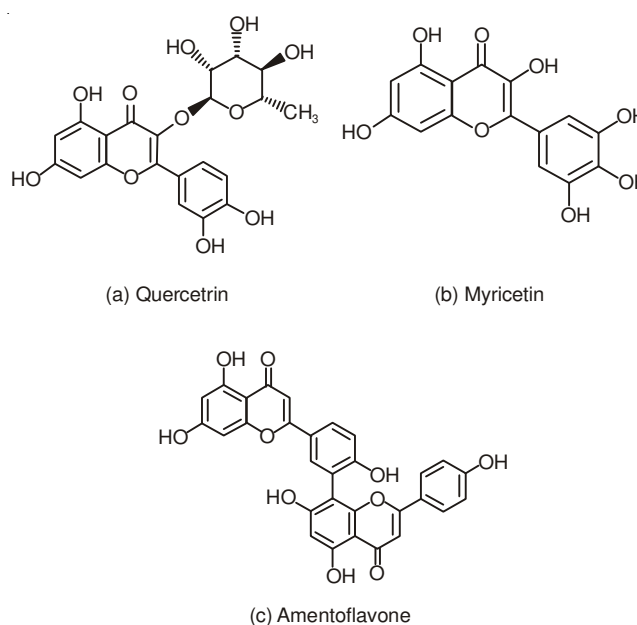


Fig. 1. Structure of the three flavones (a) quercetrin, (b) myricetin and (c) amentoflavone

EXPERIMENTAL

Quercetrin, myricetin and amentoflavone were obtained from Sigma-Aldrich (Milwaukee, WI, USA). Imidazole (99 %) and 1-methylimidazole (99 %) were supplied by Aldrich (Milwaukee). 1-Ethylimidazole (>98 %) and 1-butylimidazole (>98 %) were acquired from Tokyo Chemical Industry (Tokyo, Japan). (3-Chloropropyl)trimethoxysilane (97 %) was purchased from Sigma. Acetonitrile (ACN), methanol (MeOH) and trifluoroacetic acid were obtained from Duksan Pure Chemical Co., Ltd. (Ansan, Korea). All other reagents used in the experiment were of the HPLC grade. Double distilled water was filtered using a vacuum pump (Division of Millipore, Waters, USA) and filter (HA-0.45, Division of Millipore, Waters, USA) prior to use. All samples were filtered through a filter (MFS-25, 0.2 mm TF, Whatman, USA) before being injected into the high performance liquid chromatography (HPLC) system.

Chromatographic condition: HPLC was performed using a Waters 1525 Binary HPLC pump and Waters 2489 variable wavelength, dual-channel UV detector (Waters Associates, Milford, MA, USA). A commercial C₁₈ column (4.6 × 250 mm, 5 μm) purchased from RStech Co. (Daejeon, Korea) was used. The flavones were separated by HPLC using acetonitrile/water/trifluoroacetic acid (35:65:0.1, v/v/v) as the mobile phase. The flow rate, UV wavelength and injection volume were set to 0.5 mL min⁻¹, 372 nm and 10 μL, respectively.

Preparation of silica-confined ionic liquids: The SiILs were synthesized using a slight modification of the procedure reported by Bi *et al.*¹⁰. Silica was first immersed in hydrochloric acid for 12 h with constant stirring. Subsequently, the material was washed with deionized water until it was neutral and dried at 100 °C for 8 h. A solution of 5 g activated silica and excess of 5 mL 3-chloropropyltrimethoxysilane in 50 mL dry toluene was used to obtain chloropropyl silica (SilprCl).

The substrate mixture was heated under reflux for 12 h. After the reaction, the mixture was allowed to cool naturally to room temperature. The particles were washed sequentially with toluene, deionized water and methanol. Finally, the particles were dried at 60 °C for 10 h. Solutions containing 5 g dry chloropropyl silica and a large excess of either imidazole, 1-methylimidazole, 1-ethylmethylimidazole or 1-butylimidazole in 50 mL dry toluene were prepared to obtain SilprImCl, SilprMImCl, SilprEImCl and SilprBImCl, respectively. The mixture was heated under reflux with stirring for 18 h. After the reaction, the mixture was allowed to cool naturally to room temperature. The particles were washed sequentially with toluene, deionized water and methanol. Finally, the silica chemically bonded to imidazolium, 1-methylimidazole, 1-ethylmethylimidazole and 1-butylimidazole were dried at 60 °C for 10 h.

Preparation of standard and procedure of MPE: Stock solutions of the three target compounds (0.1-100 μg mL⁻¹)

were prepared in 1 mL methanol. 0.1 g silica, SilprImCl, SilprMImCl, SilprEImCl and SilprBImCl sorbent was packed separately into empty polypropylene cartridges (diameter 0.9 cm, 3 mL) with 0.1 g *C. obtusa* powder. Unlike SPE, there was no need for a sample loading step in MPE. The cartridges were washed with water and eluted with methanol.

RESULTS AND DISCUSSION

Calibration curves were constructed using the areas of the chromatography peaks measured at five different concentrations ranging from, 0.1 to 100.0 μg mL⁻¹. The limit of detection (LOD) was determined by gradually diluting the standard solution until it could not be detected, Table-1 lists the linear ranges, regression equations and LODs of quercetrin, myricetin and amentoflavone. Here, Y and X represent the peak area of analytes and the concentration of the analytes in the *C. obtusa* leaves, respectively.

Properties of SiILs: In Fig. 2, the grafting reactions were monitored by Fourier transform infrared spectroscopy, which clearly revealed the presence of single imidazole ring stretching vibration absorption bands at 1,670 cm⁻¹, a broad peak for the hydroxyl telescopic vibrations at 3,400 cm⁻¹ v_{as}(-OH) and the strong absorption bands at 1,100 cm⁻¹ (Si-O-Si), which are available for the linking of ionic liquids and silica.

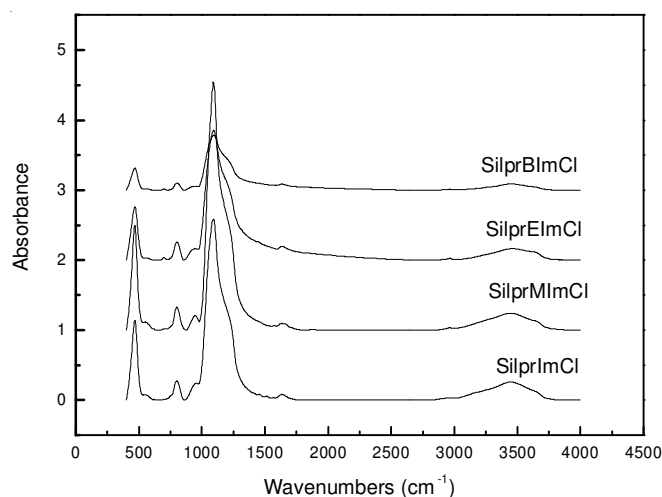


Fig. 2. FT-IR spectra of the silica-confined ionic liquids

Table-2 summarizes the carbon, hydrogen and nitrogen elemental contents and surface coverage of SilprCl, SilprImCl, SilprMImCl, SilprEImCl and SilprBImCl. The surface coverage was calculated using the method reported by Qiu *et al.*¹¹. Based on the nitrogen percentage, the bonding densities for SilprImCl, SilprMImCl, SilprEImCl and SilprBImCl were 3.23, 2.93, 2.60 and 1.63 μmol m⁻², respectively. Elemental analysis revealed an increase in the number of alkyl branches of imidazoles due to a decrease in the bonding density of imidazolium. This was

TABLE-1
REGRESSION EQUATIONS AND DETECTION LIMITS OF QUERCETRIN, MYRICETIN, AND AMENTOFLAVONE

Target compounds	Regression equation	Linear range (μg mL ⁻¹)	r ²	LOD (μg mL ⁻¹)
Quercetrin	Y = 2E + 08x + 0.2498	0.1-100.0	0.9998	0.02
Myricetin	Y = 7E + 08x - 46095	0.1-100.0	0.9977	0.04
Amentoflavone	Y = 3E + 08 x - 7035.3	0.1-100.0	0.9998	0.13

TABLE-2
CHEMICAL STRUCTURES AND THE RESULTS OF ELEMENT ANALYSIS OF THE SILICA-CONFINED IONIC LIQUIDS

Name	Structure	Elemental analysis (%)			Coverage ($\mu\text{mol m}^{-2}$)
		C	H	N	
SilprImCl		11.81	1.95	4.02	3.23
SilprMimCl		12.34	1.98	3.64	2.93
SilprEImCl		12.49	1.99	3.24	2.60
SilprBImCl		12.33	1.97	2.06	1.63

TABLE-3
AMOUNT OF THREE FLAVONES AFTER MULTI-PHASE EXTRACTION (MPE)

	Sorbent	Amount ($\mu\text{g g}^{-1}$)		
		Quercetin	Myricetin	Ametoflavone
Washing (water)	Silica	24.32	-	-
	SilprImCl	26.89	-	-
	SilprMimCl	21.43	-	-
	SilprEImCl	3.57	-	-
	SilprBImCl	8.98	-	-
Elution (Methanol)	Silica	14.25	2.68	23.39
	SilprImCl	19.52	4.25	18.84
	SilprMimCl	24.42	4.78	21.80
	SilprEImCl	11.79	2.28	8.41
	SilprBImCl	33.69	4.09	27.38

-: not detection

attributed to steric hindrance of the alkyl chain. On the other hand, separation of the target was dominated by the influence of the bonding density rather than by the interactions between the target and SiILs¹⁰.

Purification of the three flavones by MPE: Table-3 lists the amount of the three flavones obtained after MPE. After packing the *C. obtusa* powder and SiILs, a solvent was required to wash the weakly interacting interference. According to previous studies⁵, water was used as the washing solvent to avoid unnecessary interference of the sample and environment by the organic solvents. Most of the interference was removed with 2 mL of water. After the washing stage, the largest amounts of the target compounds were extracted in the elution stage using methanol as the elution solvent at 2 mL. Under SilprBImCl, the amounts of quercetin, myricetin and amentoflavone obtained were 33.69, 4.09 and 27.38 $\mu\text{g g}^{-1}$, respectively. The anion-exchange interaction between the target compounds and SilprBImCl was one of the main interactions.

Conclusion

Silica-confined ionic liquids were developed for the MPE of three flavones (quercetin, myricetin and amentoflavone) from *C. obtusa*. Of the different SiILs used, SilprBImCl showed the best recovery. The amounts of quercetin, myricetin and

amentoflavone obtained were 33.69, 4.09 and 27.38 $\mu\text{g g}^{-1}$, respectively. The SiILs used anion metathesis with other interactions to enhance the adsorption capacity and selectivity of the target compounds.

ACKNOWLEDGEMENTS

This research was supported by Basic Science Research Program through the National Research Foundation (NRF) of Korea funded by the Ministry of Education, Science and Technology (2014-002046)

REFERENCES

- G.S. Lee, E.J. Hong, K.S. Gwak, M.J. Park, K.C. Choi, I.G. Choi, J.W. Jang and E.B. Jeung, *Fitoterapia*, **81**, 17 (2010).
- E.J. Hong, K.J. Na, I.G. Choi, K.C. Choi and E.B. Jeung, *Biol. Pharm. Bull.*, **27**, 863 (2004).
- Y.M. Zhang, J. Xu, S. Xiao, G.Z. Zeng, Z.H. Sun and N.H. Tan, *Molecules*, **18**, 1255 (2013).
- C.H. Chen, H.C. Chan, Y.T. Chu, H.Y. Ho, P.Y. Chen, T.H. Lee and C.K. Lee, *Molecules*, **14**, 2947 (2009).
- M. Tian, D.D. Han and K.H. Row, *Anal. Lett.*, **44**, 737 (2011).
- J.P. Hallett and T. Welton, *Chem. Rev.*, **111**, 3508 (2011).
- H. Qiu, X. Liang, M. Sun and S. Jiang, *Anal. Bioanal. Chem.*, **399**, 3307 (2011).
- C.W. Huck and G.K. Bonn, *J. Chromatogr. A*, **885**, 51 (2000).
- M. Tian, W. Bi and K.H. Row, *Phytochem. Anal.*, **24**, 81 (2013).
- W. Bi, M. Tian and K.H. Row, *J. Chromatogr. B*, **880**, 108 (2012).
- H. Qiu, Q. Jiang, Z. Wei, X. Wang, X. Liu and S. Jiang, *J. Chromatogr. A*, **1163**, 63 (2007).