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Optimization of Extraction Conditions and Quantitative Analysis of Isoquercitrin and Caffeic Acid from *Aster scaber*

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Abstract – To determine the optimum extraction conditions that give the highest yield of isoquercitrin and caffeic acid from *Aster scaber*, the effects of four extraction variables (solvent concentrations, extraction time, number of repeated extraction, and solvent volumes) on isoquercitrin and caffeic acid yield was examined via HPLC-UV. Our results showed that the highest extract and isoquercitrin yield were observed when *A. scaber* was extracted with 450 mL distilled water for 8 hr repeatedly for three times. In case of caffeic acid, the content was higher in the two repeated extracts. Also, content analysis of isoquercitrin in *Aster* species was performed in which *A. fastigiatus, A. ageratoides,* and *A. scaber* exhibited the highest isoquercitrin content at 6.39, 5.68, and 2.79 mg/g extract, respectively. In case of caffeic acid, the highest content of *A. scaber* and *A. glehni* was 0.64 and 0.56 mg/g extract, respectively. This study reports an optimized method for extraction of isoquercitrin and caffeic acid from *A. scaber* and evaluates potential sources of the compounds.
Keywords – *Aster scaber*, high performance liquid chromatography, isoquercitrin, caffeic acid, optimization

Introduction

Chwinamul refers to several *Aster* species found in Korea which are commonly consumed as vegetable and are used in herbal preparations used to ease pain, bruising, and snakebites.[1,2] Some of the species referred to as Chwinamul include *Aster scaber, A. yomena, A. tataricus, Saussurea pulchella,* and *Solidago virgaaurea* var. *asiatica*.[3] Among them, *A. scaber* is focused in this study. *A. scaber* is a perennial plant which grows at a height of 1-1.5 m upon reaching maturity and is known to be widely distributed in the mountainous regions of Korea.[4] Studies have shown that it exhibits biological activities such as anti-viral, neuroprotective, and anti-oxidant effects.[5-7] Phytochemical analysis of *A. scaber* led to the isolation of several bioactive compounds including β-carotene, lutein, zeaxanthin, caffeoylquinic acids, coumarins, saponins, and alkaloids.[8-11]

Isoquercitrin is a flavonoid that was previously purified from *A. scaber* and other plants such as *Opuntia ficus-indica* var. *saboten* and *Brassica oleracea*.[12,13] It has been reported to possess several bioactivities including anti-oxidant, anti-inflammatory, anti-hypertensive, and anti-allergic activities.[7,14-17] However, the number of studies regarding the compound is very limited due to the difficulty in its extraction and its presence at low concentration in different plant sources. Although isoquercitrin is widely distributed, it is difficult to obtain sufficient amounts of the compound in a pure state for its applications in the food and pharmaceutical industry since its content in plant material is extremely low.[18] Caffeic acid is a natural phenol compound found in fruits, vegetables, tea and wine. Caffeic acid is part of a group of chemicals called hydroxycinnamic acids.[19] It has been reported to have broad spectrum of bioactive activities including anti-diabetic, anti-hypertensive, anti-oxidant, immunomodulatory, anti-inflammatory, and neuroprotective properties.[20]

The aim of this study was to optimize the extraction conditions that will give the highest yield of isoquercitrin and caffeic acid from *A. scaber*. Particularly, the effects of

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four extraction variables on isoquercitrin and caffeic acid yield were examined. Moreover, a quantitative analysis of isoquercitrin and caffeic acid in selected *Aster* species was performed via HPLC-UV to screen for potential sources of the compound.

Experimental

Plant materials – *A. scaber* samples were collected on June 2014 at Yangyang, Korea. The methanol (MeOH) extracts of different *Aster* species were purchased from the Korea Research Institute of Bioscience and Biotechnology, Korea.

Chemicals and instruments – Ethyl alcohol (EtOH) was purchased from Samchun Pure Chemicals (Pyeongtaek, Korea). Isoquercitrin was previously isolated from *A. scaber*.[7] Caffeic acid was purchased from Sigma (St. Louis, MO, USA).HPLC grade acetonitrile and MeOH were obtained from J.T. Baker (USA). A Waters 1525 HPLC system equipped with UV/VIS detector and an auto sampler was used (Milford, MA, USA). Evaporation of samples was performed using an Eyela rotary evaporator system (Tokyo, Japan).

Optimization of extraction conditions and isoquercitrin and caffeic acid yield from *A. scaber* – The optimum conditions for the extraction of isoquercitrin and caffeic acid from *A. scaber* were investigated. Fifteen grams of the dried *A. scaber* was prepared and a reflux system was used for the extraction. The influence of varying the solvent concentrations, extraction time, number of repeated extraction, and solvent volumes on the yield of isoquercitrin and caffeic acid was examined. Distilled water and different concentrations of EtOH (30%, 50%, 70%, or 100% EtOH) were used as solvents for the extraction. The extraction time was also varied to a 1, 2, 4, or 8 hr extraction. The extraction was repeated for 1, 2, or 3 times. Solvent volume was varied to 150, 300, or 450 mL. The resulting extracts were evaporated under reduced pressure and were oven dried at 40 ºC for 24 hr to ensure complete dryness.

Preparation of standard and sample solutions for HPLC analysis – A standard stock solution of isoquercitrin and caffeic acid was prepared by dissolving the compound in MeOH (1 mg/mL). The working solutions used to construct the calibration curves were prepared by serially diluting the stock solution to desired concentrations. Each sample was dissolved in MeOH (5 mg/mL). The standard and sample solutions were then filtered using a 0.45 um PVDF filter prior to use.

HPLC-UV conditions for isoquercitrin and caffeic acid analysis – Quantitative analysis of isoquercitrin and caffeic acid was performed using a reverse-phase HPLC system. Chromatographic separation was performed using an INNO C_{18} column (25 cm × 4.6 mm, 5 μm). A gradient elution system using a mobile phase composed of 0.5% acetic acid in water (A) and acetonitrile (B) was followed. The elution system is as follows; 90% A at 0 min, 50% A at 30 min, and 90% A at 35 min. The temperature of the column was maintained at 35 ºC and the flow rate was set at 1 mL/min. The injection volume was 10 μL and was monitored at 270 nm.

Calibration curve – Different concentrations (6.25 - 10 μg/mL) of isoquercitrin and caffeic acid dissolved in MeOH was used to construct the calibration curve. The amount of isoquercitrin and caffeic acid present in the samples was determined from the calibration curve constructed where (Y) corresponds for the peak area and (X) for the concentration of the reference compound (μg/10 μL). Linear regression was used to determine the linearity of the calibration curve.

Result and Discussion

Isoquercitrin is a flavonoid commonly found in medicinal herbs, vegetables, fruits, cereals, and other plant-based beverages (Fig. 1). It has been shown to exhibit chemoprotective effects both *in vitro* and *in vivo*, anti-oxidant, anti-diabetic, and anti-cancer activities.[21] In addition, isoquercitrin from *A. yomena* has been shown to trigger ROS-mediated apotosis in *Candida ablicans* and inhibit IL-6 activity.[22,23] However, the studies regarding isoquercitrin are still limited due to the difficulty in its extraction and its presence in low quantities in plant material. Accordingly, isoquercitrin was previously isolated from *A. scaber* and this study aimed to optimize the conditions for the extraction of isoquercitrin and caffeic acid from *A. scaber.* Particularly, the effects of varying four extraction variables on isoquercitrin and caffeic acid yield were examined. These variables include: solvent concentrations, extraction time, number of repeated extraction, and solvent volumes. The isoquercitrin and caffeic acid yield

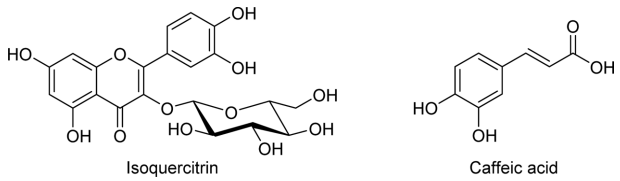


Fig. 1. Structures of isoquercitrin and caffeic acid.

Table 1. Calibration curves of isoquercitrin and caffeic acid

Compound	t_R	Calibration equation [a]	Correlation factor, r^2 [b]
Isoquercitrin	15.71	Y = 1000000X + 16247	0.9982
Caffeic acid	12.83	Y = 2000000X – 5935.4	0.9998

[a] Y = peak area, X = concentration of standard (mg/mL).
[b] r^2 = correlation coefficient for five data points in the calibration curve.

Table 2. Extract yield from *A. scaber* following different extraction conditions

Treatment	Sample	Yield (g)
Solvents concentration	30% EtOH	3.22
	50% EtOH	3.55
	70% EtOH	3.97
	EtOH	1.98
	Water	4.12
Extraction time (hr)	1	1.71
	2	2.04
	4	2.22
	8	2.44
Number of repeated extraction (time)	1	1.84
	2	2.79
	3	3.15
Solvent volumes (mL)	150	1.49
	300	1.81
	450	2.37

was analyzed via a reverse-phase HPLC-UV.

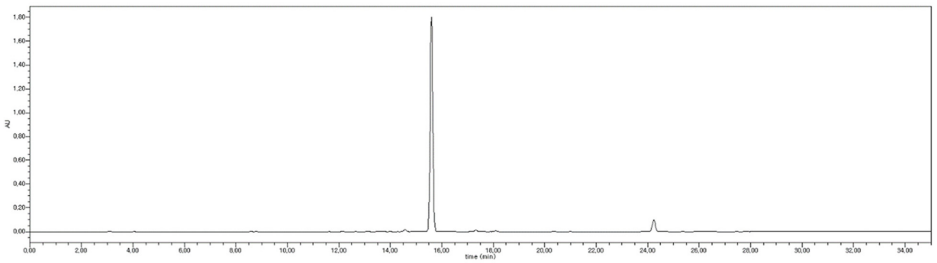
The standard calibration curve for the analysis of isoquercitrin and caffeic acid showed good linearity (r^2 = 0.9982 and r^2 = 0.9998, respectively) within test ranges as shown in Table 1 and Fig. 2. The chromatographic separation in *A. scaber* displayed high resolution (Fig. 3). The results of the experiments are shown in Tables 2 and 3 which summarize the effects of the different extraction conditions on the yield of isoquercitrin and caffeic acid in *A. scaber*. The highest extract and isoquercitrin and caffeic acid yield was obtained when distilled water was used as extraction solvent compared to when EtOH was used. It was also observed that when the extraction time, number of repeated extraction, and solvent volumes were increased, the isoquercitrin and caffeic acid yield increased as well in which an 8 hr, three-time extraction, and 450 mL solvent volume showed the highest extract and isoquercitrin yield, respectively. In case of caffeic acid, it was higher in the repeated two-time extraction, and all the other conditions showed the same high contents. This is consistent with the results of Beom *et al.* in which the highest solid content was observed when water was used as the extraction solvent compared to using EtOH and MeOH for different microwave-assisted extraction.[11] A total of twenty-three polyphenolic compounds were identified and quantified from *A. scaber* leaf extracts. Among them, myricetin was the most dominant compound followed by quercetin and kaempferol. However, we have no detection above compounds in our sample.[24]

The distribution of isoquercitrin and caffeic acid in different *Aster* species was also investigated in this study to evaluate new potential sources of the compound. Quantitative analysis was performed using the same HPLC conditions in which a good chromatographic separation was achieved in the samples analyzed (Fig. 4

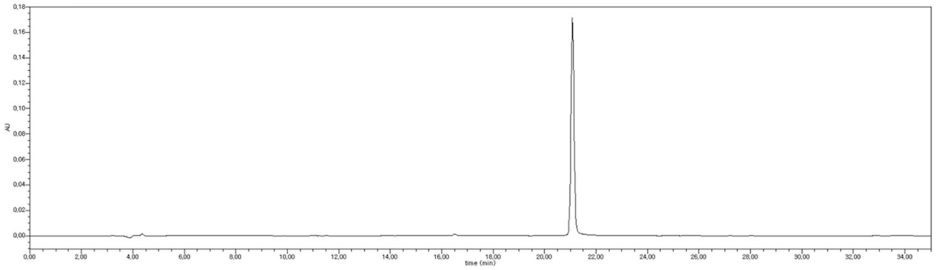
Table 3. Isoquercitrin and caffeic acid yield from *A. scaber* leaves following different extraction conditions

Treatment	Sample	Contents (mg/g DW)	
		Isoquercitrin	Caffeic acid
Solvents concentration	30% EtOH	0.098 ± 0.003	0.019 ± 0.033
	50% EtOH	0.135 ± 0.006	0.021 ± 0.037
	70% EtOH	0.223 ± 0.003	0.028 ± 0.048
	EtOH	0.238 ± 0.003	0.029 ± 0.050
	Water	0.732 ± 0.007	0.322 ± 0.010
Extraction time (hr)	1	0.097 ± 0.001	0.029 ± 0.050
	2	0.107 ± 0.004	0.033 ± 0.057
	4	0.171 ± 0.063	0.035 ± 0.060
	8	0.267 ± 0.005	0.042 ± 0.073
Number of repeated extraction (time)	1	0.173 ± 0.003	0.032 ± 0.056
	2	0.260 ± 0.007	0.053 ± 0.093
	3	0.278 ± 0.032	0.051 ± 0.088
Solvent volumes (mL)	150	0.104 ± 0.035	0.026 ± 0.045
	300	0.094 ± 0.001	0.029 ± 0.051
	450	0.136 ± 0.001	0.033 ± 0.057

Data are represented as mean ± S.D. (n = 3) mg/g DW



(A)



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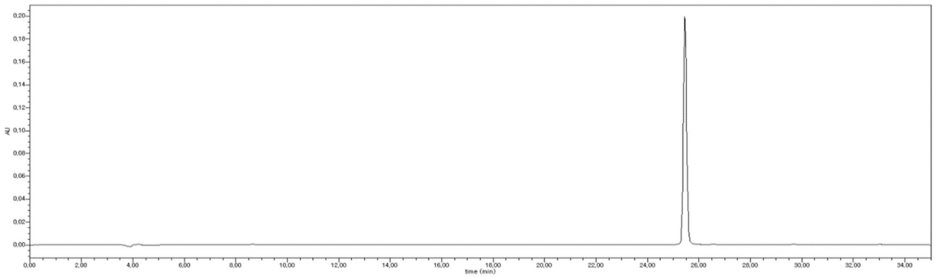

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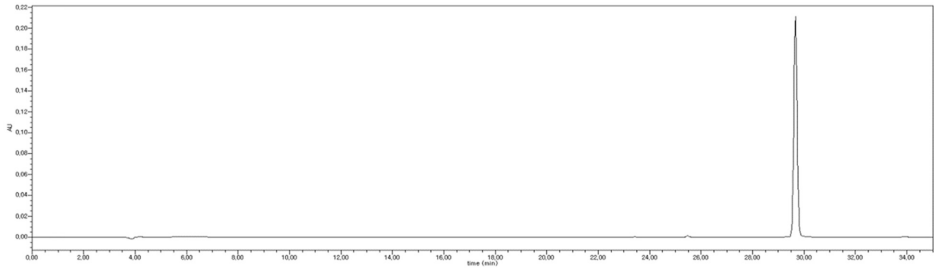
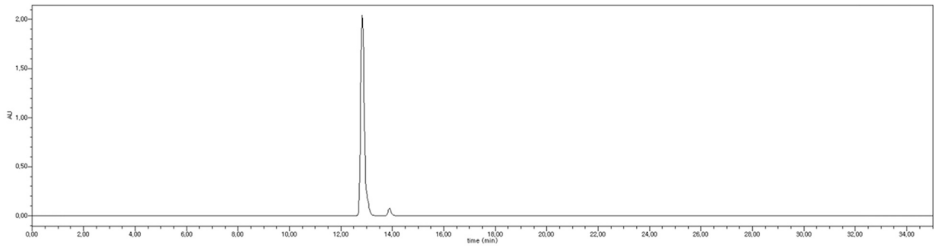
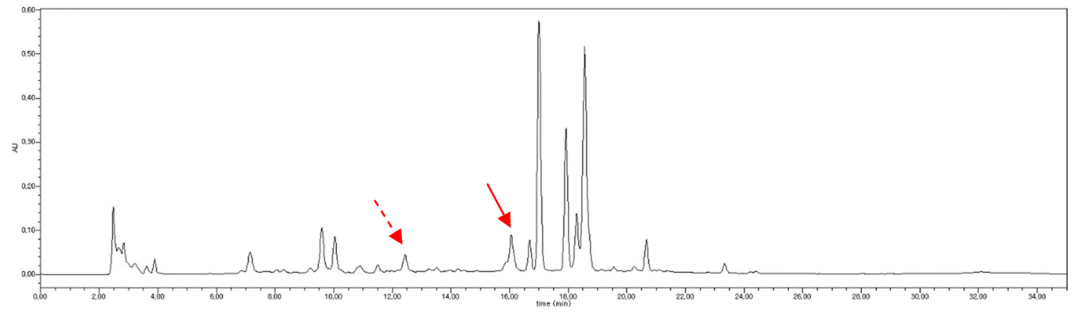


(D)

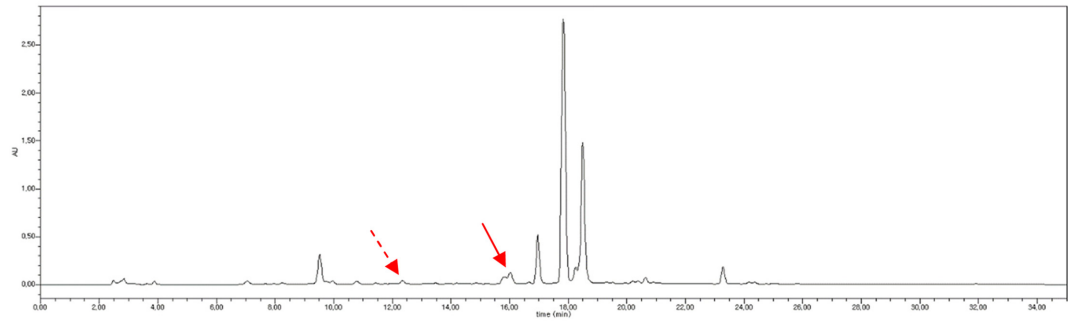


(E)

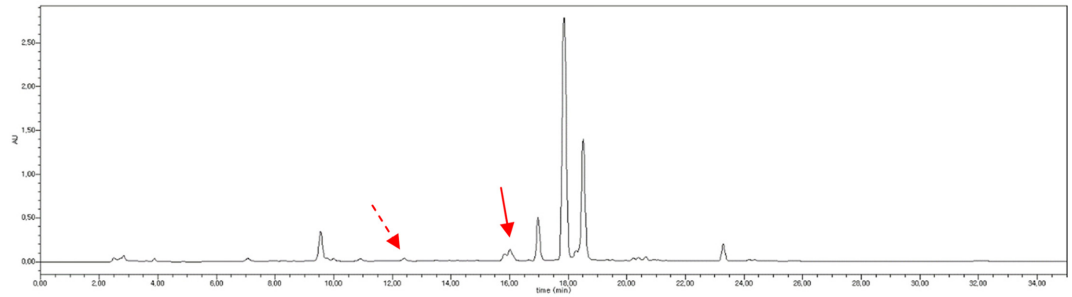
Fig. 2. HPLC chromatograms of isoquercitrin (A), myricetin (B), quercetin (C), kaempferol (D), and caffeic acid (E).



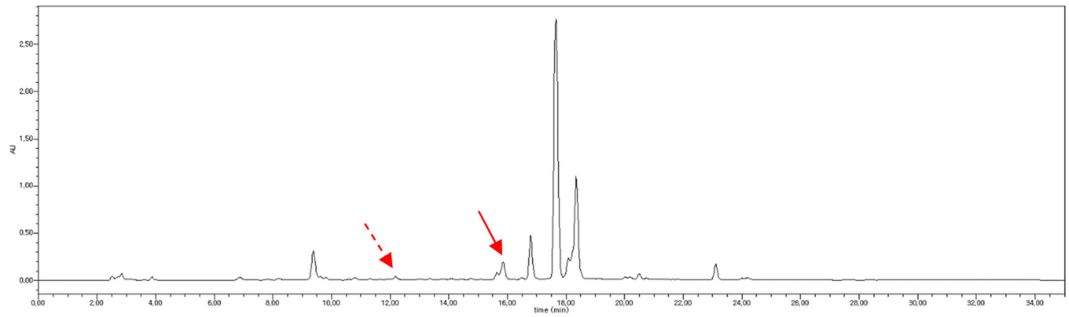
(A)



(B)

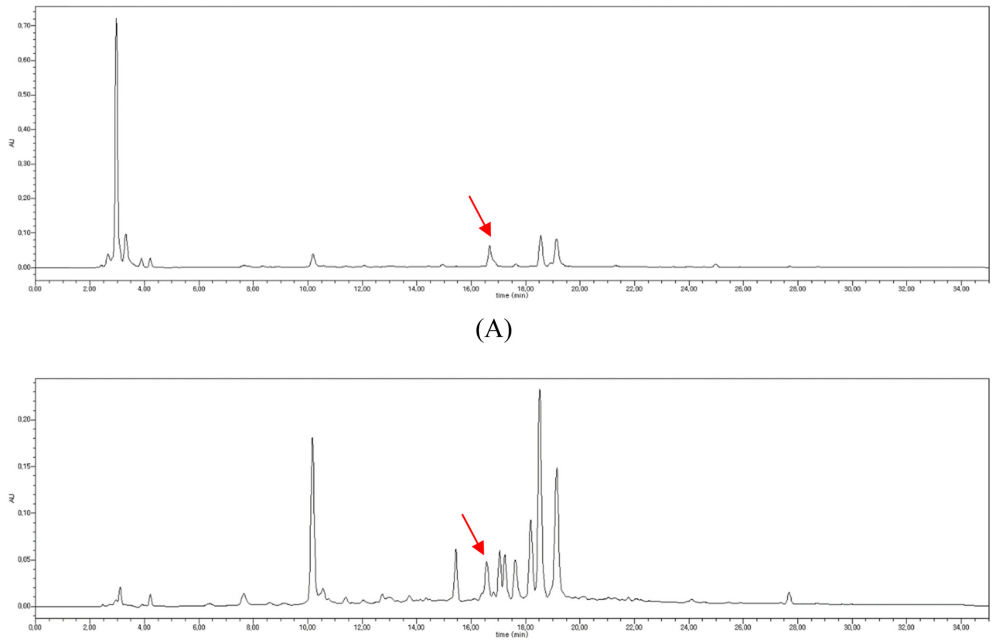


(C)



(D)

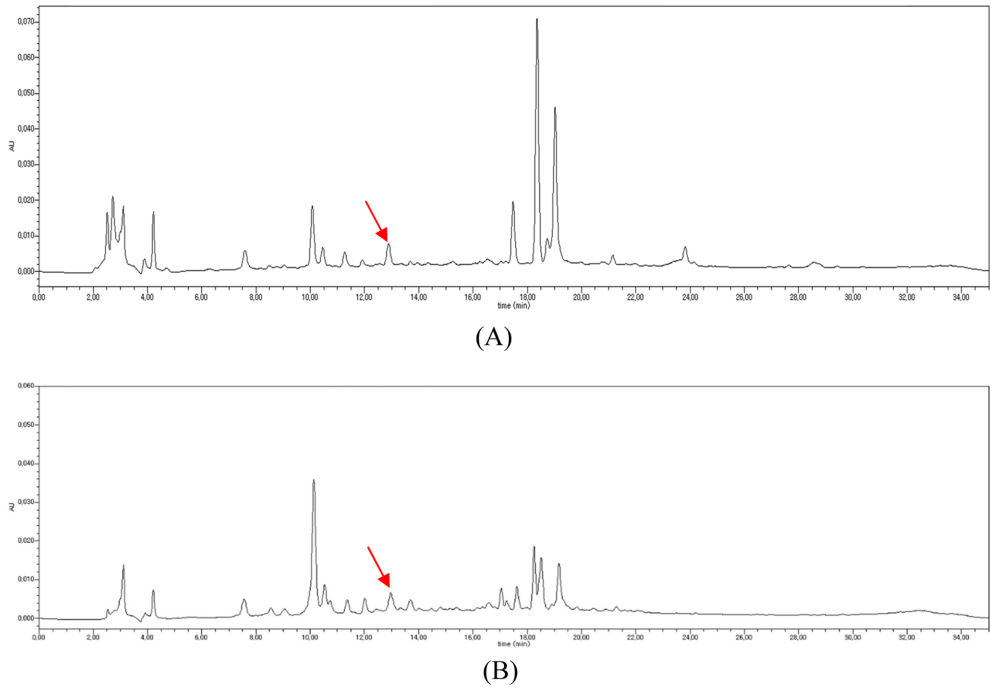
Fig. 3. HPLC chromatograms of samples extracted under different conditions: distilled water (A), 8 hr extraction (B), 3-time extraction (C), and 450 ml solvent extraction (D) showing caffeic acid (dotted arrow) and isoquercitrin peaks (line arrow)



(A)

(B)

Fig. 4. HPLC chromatograms of the MeOH extracts of *A. fastigiatus* (A) and *A. ageratoides* (B) showing isoquercitrin peak (line arrow).



(A)

(B)

Fig. 5. HPLC chromatograms of the MeOH extracts of *A. scaber* (A) and *A. glehni* (B) showing caffeic acid peak (line arrow).

and 5). Isoquercitrin and caffeic acid were present at varying concentrations in the *Aster* species examined (Table 4). Among them, *A. fastigiatus, A. ageratoides,* and, *A. scaber,* exhibited the highest isoquercitrin concentrations at 6.39, 5.68, and 2.79 mg/g extract, respectively. The concentration of caffeic acid was highest in *A. scaber* and *A. glehni* at 0.64 and 0.56 mg/g extract, respectively. A previous study of Kim *et al.* also determined the content of isoquercitrin in different Chwinamul species in which *Synurusexcelsus* and *A. yomena* showed the highest

Table 4. Isoquercitrin and caffeic acid content of the MeOH extracts of selected *Aster* species

Species	Contents (mg/g extract)	
	Isoquercitrin	Caffeic acid
A. ageratoides	5.69 ± 0.24	0.41 ± 0.01
A. altaicus var. *uchiyamae*	ND	0.20 ± 0.03
A. ciliosus	0.63 ± 0.26	0.54 ± 0.00
A. fastigiatus	6.39 ± 0.40	0.32 ± 0.02
A. glehni	ND	0.56 ± 0.03
A. hayatae	0.06 ± 0.01	0.31 ± 0.01
A. incisa	0.04 ± 0.03	0.37 ± 0.02
A. koraiensis	0.42 ± 0.01	0.23 ± 0.02
A. maackii	1.05 ± 0.11	0.25 ± 0.21
A. pekinensis	1.11 ± 0.08	tr
A. pilosus	0.68 ± 0.02	0.44 ± 0.01
A. scaber[*]	2.79 ± 0.06	0.64 ± 0.02
A. spathulifolius	0.14 ± 0.03	0.26 ± 0.00
A. spathulifolius var. *oharae*	0.01 ± 0.10	0.26 ± 0.01
A. tataricus	1.05 ± 0.09	0.27 ± 0.00
A. tripolium	ND	0.19 ± 0.00
A. yomena	0.02 ± 0.04	0.29 ± 0.02

Data are represented as mean ± S.D. (n = 3) in mg/g of the MeOH extracts of the samples
ND = not detected
tr = trace
[*]*A. scaber* was extracted using optimized extraction conditions

isoquercitrin at 44.67 and 34.73 mg/g extract. Moreover, they have measured the isoquercitrin content of *A. ageratoides* to be 15.1 mg/g extract which is three times more than the value measured in this study.[2] Previous studies have confirmed the presence of caffeic acid in *A. scaber*, but did not mention its content.[25] The differences in the results can be attributed to the source and the method of preparation of the samples analyzed, as well as the analytical method employed.

The optimization of the conditions for the extraction of isoquercitrin and caffeic acid from *A. scaber* is reported in this study. Our results showed that the highest yield of isoquercitrin and caffeic acid was observed when *A. scaber* were extracted with 450 mL distilled water for 8 hr repeatedly for three times. Here, a simple and fast analytical method for the quantification of isoquercitrin and caffeic acid in different *Aster* species is reported in which *A. fastigiatus, A. ageratoides, A. glehni*and *A. scaber* showed the highest content of isoquercitrin and caffeic acid among the samples examined, respectively. The results of this study can be used as a guideline to optimize the yield of isoquercitrin and caffeic acid from *A. scaber* for its pharmacological and industrial applications.

Acknowledgments

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