Efficiency for extracting icariin from *Epimedium koreanum* Nakai by temperature and solvent variations

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ABSTRACT

To improve industrial scale extraction method for extraction of icariin from *Epimedium koreanum* Nakai, the yields under different extracting conditions such as solvent, temperature, duration and solvent to plant material weight ratio were compared. Regarding extracting solution, highest extracts and icariin yield could be achieved when 10% EtOH was used. In case of plant material to extracting solvent ratio, no significant differences could be observed from 1/10 to 1/50, indicating 1/10 was the most efficient. Extracting temperature significantly affected extracts and icariin yields in that 90℃ increased the collected extracts and icariin contents up to 29.6% and 0.76%, respectively, compared to 27.2%, 0.33% at 70℃. The yield of extracts was less dependent upon extracting temperature compared to icariin yield. Regarding extraction time, 4 hr and 6 hr resulted in high extracts and icariin yield, respectively. We found extracting *Epimedium koreanum* Nakai in 10 times volume of 10% EtOH for 4 and 6 hr at 90℃ seem to be relatively efficient methods for extracts and icariin, respectively.

Key words **:** *Epimedium koreanum*, icariin, extracts

INTRODUCTION

Epimedium koreanum Nakai is a perennial medicinal plant native in middle mountainous areas in Korea. It is also called as "Sam-Ji-Gu-Yop-Cho" due to its morphological characteristics; *i.e.*, it has three twigs and nine leaves.

As a traditional oriental medicinal herb plant, the root, stem and leaf of *Epimedium koreanum* have been widely used for tonic and regain for vigor, and recent reports revealed its various physiology-related functions such as antigenotoxic, hypotensive, hepatoprotective, immunostimulating, and antioxidative activities (Kim *et al.*, 1992; Chun *et al.*, 2000; Choi *et al.*, 1995; Lee *et al.*, 2002; Lee *et al.*, 1995).

The major functional compounds in *Epimedium koreanum* are quercetin, magnoflorine, phytosteryl glucoside, icariin and epimedoside A. Among these compounds, the icariin is widely used as a chemical standard for *Epimedium koreanum* specially (Kang *et al.*, 1988; Kang *et al.*, 1990; Kim *et al.*, 2001).

Based upon enhanced standard of living and average

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span of human life, more public concerns are focused on health, and consequently various functional foods and health-promoting compounds are being industrialized. As an oriental medicinal herb plant, the extract of *Epimedium koreanum* also has been widely used as an ingredient for developing various functional food products. The increasing demand of *Epimedium koreanum* induced many researches for developing breeding and cultivation practices (Kang *et al.*, 1994; Shin *et al*, 1996; Kang and Kim, 1991), and present production of *Epimedium koreanum* in Korea is estimated about 39 ton per year. Recently, however, the import of *Epimedium koreanum* from China with relatively low price is increasing and becoming a threat to Korean *Epimedium koreanum* farmers. To overcome such problems, studies comparing Korean and Chinese *Epimedium koreanum* have been conducted and the fact that Korean *Epimedium koreanum* have higher contents of functional compounds such as icariin than Chinese has been reported (Kang *et al.*, 1994; Shin *et al.*, 1996).

In contrast to breeding and cultivation-related researches, the processing technique is relatively underdeveloped in Korea and consequently some companies utilize the imported extracts rather than using plant materials produced in Korea. This research was conducted as a part of developing efficient methods to increase icariin and extracts yield from *Epimedium koreanum* by comparing yields under different extracting conditions such as solvents, temperature, duration, and the ratio of plant material to extracting solution.

MATERIALS AND METHODS

Plant materials

Epimedium koreanum plants which had been collected from Kyonggi and Kangwon (South Korea) area and cultivated in Kyonggi-Do Agricultural Researches and Extension Services as a part of breeding program was used. Prior to extraction, harvested plants were dried in shade, ground to powders and passed through a 0.5 mm sieve. To prepare homogeneous plant materials, sieved powders were pooled, uniformly mixed, and a part of the pool was used in each extraction replication.

Extraction methods

The extraction process consisted of 4 major factors (1) solvent (0 to 30% EtOH) (2) ratio of plant dry weight (1g) to solvent volume (10 to 50 mL) (3) extraction temperature (70 and 90℃) (4) extraction duration (2 to 8 hrs). To clarify the sole impacts of pre-described 4 factors, a basic standard extraction condition as followings was established based upon Kang *et al* (1994); 1 g of dry *Epimedium koreanum* was added into 50 mL of 20% EtOH and extracted for 8 hours at 90℃, and the resultant extracts and icariin yields were used as control. To compare the efficiency of extraction, the yields of extracts and icariin were evaluated.

Measurement of extracts and icariin contents

After extraction, all the plant materials and solvents were vacuum - filtrated through Whatman No. 6 filter paper, and the final volume was adjusted to 200 mL by adding the same solvent used in extraction. For the measurement of extracts yield, the half (100 mL) of this final solution was transferred into a pre-weighed flask, and heated at 105℃ until no further weight changes could be found, cooled at room temperature, and the extracts contents (%) were calculated based upon the weight of residues.

For analysis of icariin content, 10 mL of pre-described final solution was transferred into a separatory funnel, mixed with 150 mL of distilled water and 300 mL of dichloromethane. After shaking,

dichloromethane layer was removed. This process was repeated two times. To the aqueous layers, 300 mL ethylacetate was added and after shaking, ethylacetate layers were collected. After repeating this process one more time, pooled ethylacetate layers were passed through Na_2SO_4, and concentrated in a rotary evaporator at 60℃. The residue was suspended in MeOH and filtered through 0.2 ㎛ syringe filter and injected into an HPLC (Spectraphysics 8800, USA). For icariin analysis Lichrosorb RP-18 (10 ㎛, 250 × 46 mm) column was used and the mobile phase conditions were as followings: time (min) -H_2O(%)-MeOH(%) = (0-50-50), (10-40-60), (15-30-70), with flow rate of 1.5 mL/min. The icariin peaks detected at 350 nm were quantitated against authentic standard and the resultant chromatograms obtained from standard and sample icariin analyses are shown in Fig. 1.

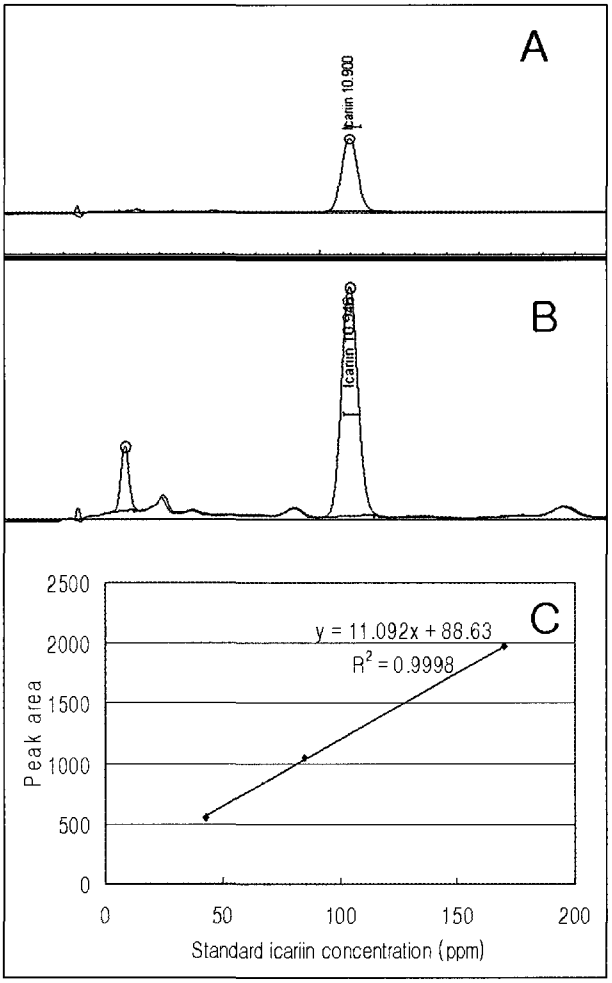

Fig.1. HPLC chromatogram of icariin standard (42.5 ppm, A), *Epimedium koreanum* Nakai plant sample (108 ppm, B), and calibration curve of icariin standard (C).

Statistical data analysis

Each extraction was repeated at least three times, and the collected data from each extraction method were analyzed by SPSS (SPSS Inc, ver 10.0, U.S.A.) statistics software for Duncan's multiple range tests at 5% significance level.

RESULTS AND DISCUSSION

Extracting solvent conditions

The water is generally used for industrial extraction of icariin and extracts from *Epimedium koreanum*, although icariin favors more alcoholic conditions. To clarify the effects of EtOH concentration in solvents on yields of extracts and icariin, 0, 10, 20, and 30% of EtOH was tested for extraction. When 10% EtOH was used, extracts yield significantly increased up to 30.83% compared to control (29.08%). However, when the

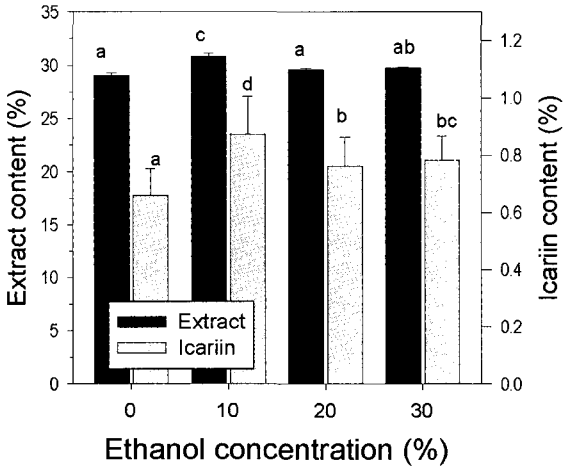

Fig. 2. Effects of ethanol concentration in extracting solution on yields of icariin and extract contents of *Epimedium koreanum* Nakai.

EtOH concentration was increased to 20 and 30%, the extracts yields became lower than 10% EtOH (Fig. 2). The same results could be observed in icariin yield in that 10% EtOH exhibited yield of 0.87%, which was higher than control (0.66%), 20% (0.76%), and 30% (0.78%) EtOH. These results suggest that low concentration of 10% EtOH is the optimal solvent condition for both extracts and icariin extractions from *Epimedium koreanum*.

The ratio of plant material to extracting solvent

In general, better extraction and higher yield could be estimated when higher volume of extracting solvents are used to a given amount of plant materials to be extracted. Increasing the volume of solvents, however, reduces the efficiency of extraction in terms of expenses. Consequently, finding out the minimum volume of extracting solvent without losing the yield efficiency is an important factor, especially in industrial cases. When 1 g of *Epimedium koreanum* was extracted in 10 to 50 mL of 20% EtOH, the increment in solvent volume induced the tendency of extracts yield increase, and the highest yield in extracts (29.6%) and icariin (0.88%) could be observed in 50 mL and 40 mL EtOH; i.e., when the ratio of plant material to volume was 1/50 and 1/40, respectively (Fig. 3). No statistical significance, however, could be observed within the rage of 1/10 to 1/50. Based upon these results, the minimum ratio of 1/10 seems to be enough to effectively extract icariin and extracts from *Epimedium koreanum* when 20% EtOH was used for extraction. However, to overcome the fact that 1/10 was the lowest ratio tested in this experiment, yield tests under the conditions of lower ratio of plant material to solvent volume needs to be tested.

Extracting temperature

To clarify the effects of extracting temperature on yields of extracts and icariin content, 70℃ and 90℃ was compared. As shown in Fig. 4 extracting temperature significantly affected extracts and icariin yields in that higher temperature was more efficient. At 90℃, the yield of icariin content was 0.76%, which was 140% increment compared to 0.33% at 70℃. Although temperature-dependency of extracts yield was not as prominent as icariin yield, 90℃ conditions increased the yield of extract contents up to 29.6%, which was 9% increment compared to 27.2% at 70℃. These results

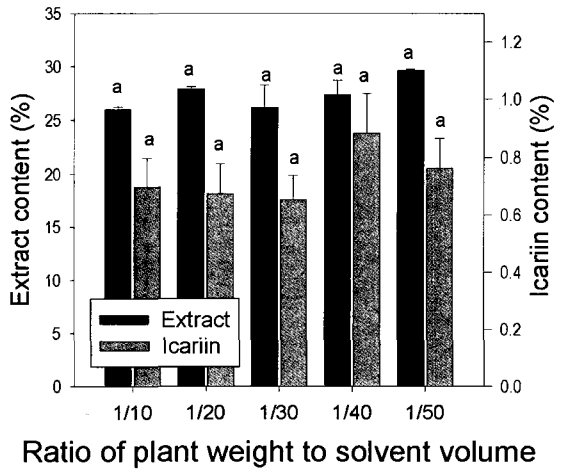

Fig. 3. The yield of icariin and extract contents under different ratio of *Epimedii koreanum* Nakai weight to solvent volume.

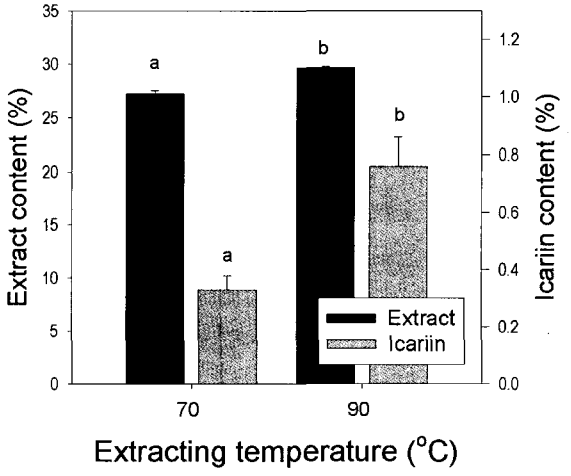

Fig. 4. Effects of extracting temperature on yields of icariin and extract contents of *Epimedium koreanum* Nakai.

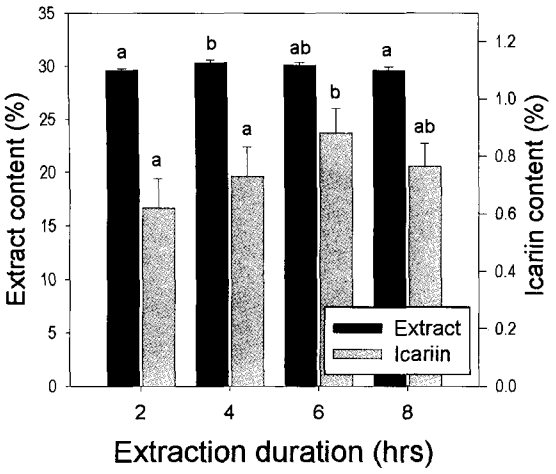

Fig. 5. Effects of extracting duration on yields of icariin and extract contents of *Epimedium koreanum* Nakai.

suggested that higher temperature is required to enhance the extraction yield of icariin and extracts, and especially more concern should be given to temperature when extracting icariin from *Epimedium koreanum*.

Extracting time

Along with extraction temperature, the duration of extraction is an important factor determining the efficiency of extraction in both yield and economic concerns. The effect of extracting duration was examined by altering the duration from 2 to 8 hours. The yield of extract and icariin contents under 2 hours extraction conditions were 29.58% and 0.62%, respectively (Fig. 5), which were raised up to 30.33% and 0.73%, respectively when the extraction was extended to 4 hours. Compared to 4 hours of extractions, 6 hours significantly increased the yield of icariin content up to 0.88%, but slightly decreased the yield of extracts down to 30.1%. Extended extractions for 8 hours, however, resulted in decrement in both icariin (0.76%) and extracts (29.6%) compared to 6 hours. These results suggest that optimal time duration for extracting icariin and extracts from *Epimedium koreanum* is 6 hours and 4 hours, respectively.

CONCLUSION

When the yields of icariin and extract contents from *Epimedium koreanum* Nakai were compared under various extraction conditions, the optimal solvent and extraction temperature were 10% EtOH and 90℃ for both icariin and extracts, respectively. And the ratio of 1/10 as plant material weight to solvent volume seemed to be enough for efficient extractions of both icariin and extracts. For the yield of increased icariin and extracts 6 hours and 4 hours, respectively, proved to be efficient.

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