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Original Article

High-performance liquid chromatographic determination of β-carotene content in four varieties of lotus stamens

Nithida Phonkot[1], Preeya Puangsomlee Wangsomnuk[2] and Chantana Aromdee[1]*

[1] *Faculty of Pharmaceutical Sciences,*

[2] *Department of Biology, Faculty of Science,*
Khon Kaen University, Muaeng, Khon Kaen, 40002 Thailand.

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Abstract

A high-performance liquid chromatography method was developed to determine β-carotene content in lotus stamens. The stamens of four varieties of *Nelumbo nucifera* (Gaertn.) and ten samples bought from traditional drug stores were determined. Validation of the method was carried out, the linearity of the β-carotene concentrations range from 6.50-58.50 μg.mL^{-1} were 0.9997-0.9998. The repeatability and intermediate precision were 0.29 %CV (n=9) and 7.48 %CV (n=9), respectively; the accuracy was 100.10 %w/w, the detection limit was 9.83 ng.mL^{-1} and the quantitation limit was 29.80 ng.mL^{-1}. The β-carotene of the four varieties were 465.77-1150.80 mg% (n=3), and of the store samples were 4.70-41.73 mg% (n=3). The results demonstrated that the contents varied according to the variety. The contents in the samples obtained from traditional drug stores were much lower that those dried in our laboratory, this might due to the source of the stamens, duration, and conditions of storages.

Key words: *Nelumbo nucifera* (Gaertn.), carotenes in lotus stamens, HPLC method

1. Introductions

Three commonly used and well known recipes of Thai herbal medicine are Kae-sorn-thang-ha (The Total 5 Types Stamens), Kae-sorn-thang-Jed (The Total 7 Types Stamens) and Kae-sorn-thang-Kao (The Total 9 Types Stamens). In these recipes Lotus stamen is one of the compounds. The indications of all the recipes are antipyretic, nerve and heart tonic, and refreshing (Bunyapraphatsara, 1998). In Thailand, there are at least four varieties of commonly available *Nelumbo nucifera* that are used in Thai herbal medicine, Pathum, Boontharik, Sattabongkot, and Sattabutre (Picheansoonthon, 1999). The main chemical components of the lotus stamens are flavonoids, alkaloids, and carotenoids (Jung *et al.*, 2003). Carotenoids act as scavengers of alkyl radicals, thus no initiation of lipid peroxidation occurs, which could cause many diseases (Belitz and Grosch, 1999).

DNA and TLC fingerprints of the haxane extract using β-carotene as a marker of these lotus stamens were documented as well as their antioxidant activity (Aromdee and Phonkot, 2005; Phonkot *et al.*, 2008). The total carotene content in mixed solvent extract was determined by a column chromatography technique applied from AOAC (Horwitz, 2000). The total carotenes in four varieties of lotus stamens varied in the range 33.94-69.16 mg% (Phonkot and Aromdee, 2006). Quantitative determination by a column chromatography method is time consuming and does not provide good replications. This method was also very tedious and required a skillful analyst. There are various methods employed for β-carotene determination; for example, Souri *et al.* (2005)

* Corresponding author.
Email address: chaaro@kku.ac.th

used a third derivative UV-absorption method to determine anthocyanin and β-carotene simultaneously in pharmaceutical preparation. Schierle *et al.* (2004) determined β-carotene in supplements and raw materials by a reversed-phase liquid chromatography. Although this method can be used to determine all-*trans*, 9-*cis*, 13-*cis*, 15-*cis*?-β-carotene, β-carotene, cryptoxanthin, lutein, lycopene, and zeaxanthin, the compositions of the mobile phases are complicated and one of the mobile phases contains acetonitrile, which is always unavailable in the market and considerable expensive. Besides that the running time of the method is quite long (30 mins). Thus it was our aim to develop a simple, comparably fast, and low cost method.

This work is a part of the scheme for establishing standards and specifications for herbal medicines. An analytical method and quality survey of specimens obtained from herbal drug stores from various parts of the country were examined. A simple gradient normal phase HPLC method was developed, and validated according to the ICH guidelines (2005). The method was used to analyze four varieties of lotus stamens and samples obtained from traditional drug stores. The content of β-carotene of each sample was determined.

2. Materials and Methods

2.1 Chemicals and plant materials

β-carotene was purchased from Fluka, and all solvents are analar grade. Four varieties of fully grown lotus (*Nelumbo nucifera* Gaertn.) flowers were used. Pathum and Boontharik collected from Nakorn Pathom Province and Sattabongkot, Sattabutre were collected in Khon Kaen Province (see Figure 1). Stamens were picked out and air dried and turned over occasionally for 7 days. Ten samples of dried lotus stamens were randomly bought from drug stores from various parts of Thailand (see Figure 2). From visual inspection of the samples, it was found that the color of the samples varied from yellow, light brown, dark brown, and even greyish brown, reflecting the conditions and durations of the storage of the samples.

2.2 HPLC system

High-performance liquid chromatographic system composes of a 4 channel pump (Agilent, USA) equipped with an online degasser (HP1100), auto sampler (HP1100), column heater (HP1100), and a diode array UV-Visible detector (HP1100) was used in the experiment. Standards and samples, 20 μL, were injected on a silica column, 5 μm, 4.6×150 mm (Agilent), at 27°C, with the detection wavelength

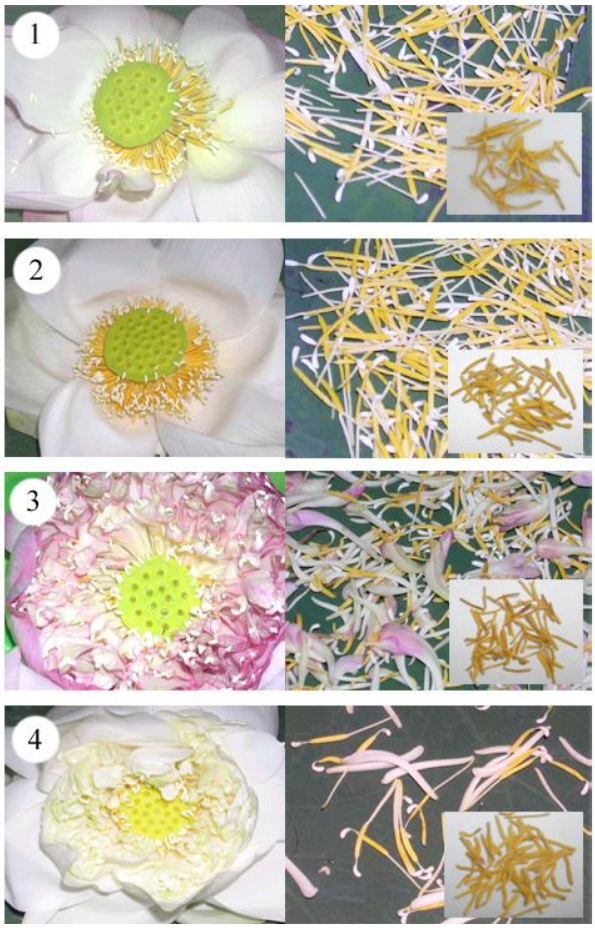

Figure 1. Four varieties of lotus flowers: Lotus flowers of Pathum (1), Boontharik (2), Sattabongkot (3), and Sattabutre (4).

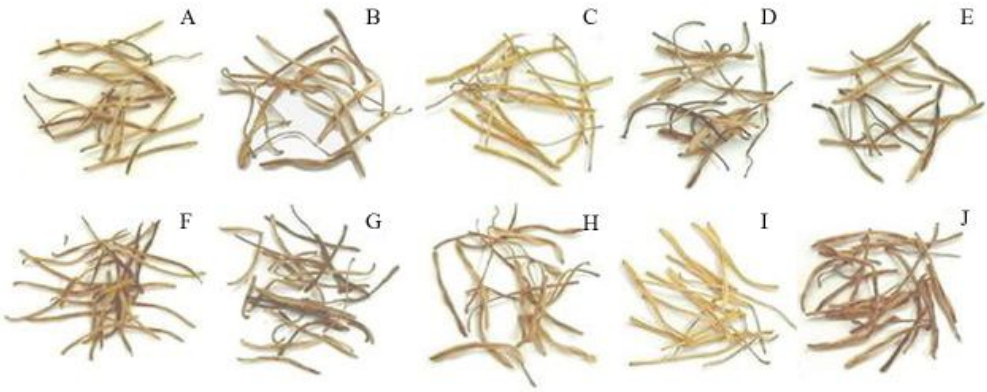

Figure 2. Lotus stamen samples purchased from herbal medicine drug stores.

at 450 nm. The separation was obtained at a flow rate of 1 $mL.min^{-1}$ with the following gradient program: 99% hexane and 1% acetone for 5 min, then 1% acetone was increase to 100% in 1 min and then the column was washed with isopropanol for 2 min.

2.3 Preparation of standard and samples solutions

β-carotene stock standard solution containing 0.65 $mg.mL^{-1}$ in a mixed solvent (hexane-acetone-methanol-toluene=10:7:6:7) was prepared. A serial of standard solutions were prepared by diluting the standard stock solution 0.1, 0.3, 0.5, 0.7, 0.9 mL to 10 mL with the same solvent.

Four varieties of lotus stamens and ten purchased samples were ground. The obtained powder, 0.5 g, was accurately weighed and transferred into a 10-mL volumetric flask, adjusted to the volume with the mixed solvent and then mixed well. The solution was allowed to stand in the dark for 24 hrs, and then filtered. The samples of four varieties samples were diluted to ten times from their original concentration.

2.4 Validation parameters

2.4.1 Linearity

The linearity of the detector response for the prepared standards was assessed by means of linear regression regarding the amount of β-carotene solutions and the peak areas of the corresponding peaks on the chromatograms (n=5).

2.4.2 Specificity

Specificity was checked by comparing retention times of the β-carotene peak of standard and samples. To confirm the specificity and identity of the peak obtained from sample, the λ_{max} and λ_{min} of UV absorption as well as their ratios were compared with that of standard β-carotene.

2.4.3 Precision

The repeatability and intermediate precision were carried out at three concentrations β-carotene solutions at 6.5, 32.5, and 58.5 $mg.mL^{-1}$. The repeatability was determined by injecting each concentration trice within a day. The intermediate precision was determined the same way in three consecutive days. The repeatability and intermediate precision were calculated as %CV.

2.4.4 Accuracy

The accuracy of the method was determined by analyzing the percentage of recovery of β-carotene. A sample was spiked with five concentrations of β-carotene solutions. A graph of the found amounts and the spiked concentrations presented the β-carotene in a sample at the concentration 0 $mg.mL^{-1}$ of the standard.

2.4.5 Detection and quantitation limit

Detection and quantitation limits (DL and QL) were calculated as (3.3s)/S and (10s)/S, respectively, where s is the standard deviation of the *y*-intercept and S is the slope of regression line.

2.4.6 β-Carotene determination

The β-carotene content of fourteen samples were analyzed by the validated method (n = 3).

3. Results and Discussion

The developed HPLC method was validated, and the linearity range of the β-carotene from the concentrations of 6.50-58.50 $\mu g.mL^{-1}$ was 0.9997-0.9998. The repeatability and intermediate precision were 0.29 and 7.48%, respectively (n=9). The percent recovery was 100.10% w/w. The DL and QL were 9.83 and 29.80 $ng.mL^{-1}$, respectively. The retention times of the peak of β-carotene of the standard and samples varied from 1.846-1.913 min. For qualitative purpose, the specificity and identity of the UV spectra of β-carotene of standard and sample were compared. The maximum absorption wavelengths were 425, 451, and 482 nm, corresponding to that described by Harborne (1998). This indicated the identity of the peaks obtained from standard and samples, as shown in Figure 3. The absorbance ratio of peak and valley of the samples were 1.0204 (λ_1/λ_4) and 1.0341 (λ_2/λ_5), which also corresponded to those of the standard β-carotene; 1.0236 (λ_1/λ_4), 1.0393 (λ_2/λ_5). The relative percent difference of each ratio of sample and standard are 0.31% and 0.50%, respectively. The advantages of the method are that a silica column was used, which is cheaper than a C-18 column and that the running time is shorter.

In this study, the β-carotene in mixed solvent of fourteen samples of lotus stamens is giving the main peak, and it

Table 1. Validation parameters: linearity, specificity, precision, accuracy, detection limit (DL), and quantitation limit (QL).

Parameters	Values
Linearity; R^2	0.9997-0.998
Specificity; retention times (min)	1.846-1.913
Precision (% C.V.)	
- repeatability	0.29
- intermediate	7.48
Accuracy (% w/w)	100.10
DL ($ng.mL^{-1}$)	9.83
QL ($ng.mL^{-1}$)	29.80

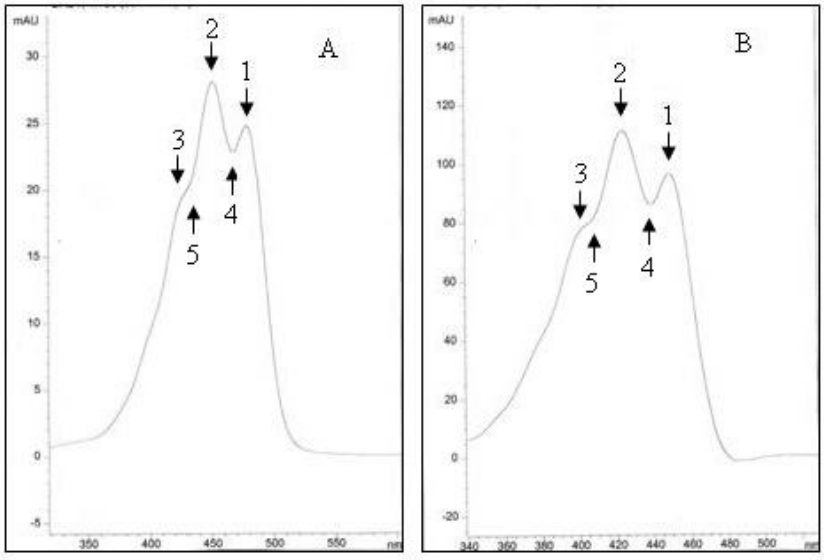

Figure 3. UV spectra of β-carotene (A) and a sample obtained from traditional drug stores (B).

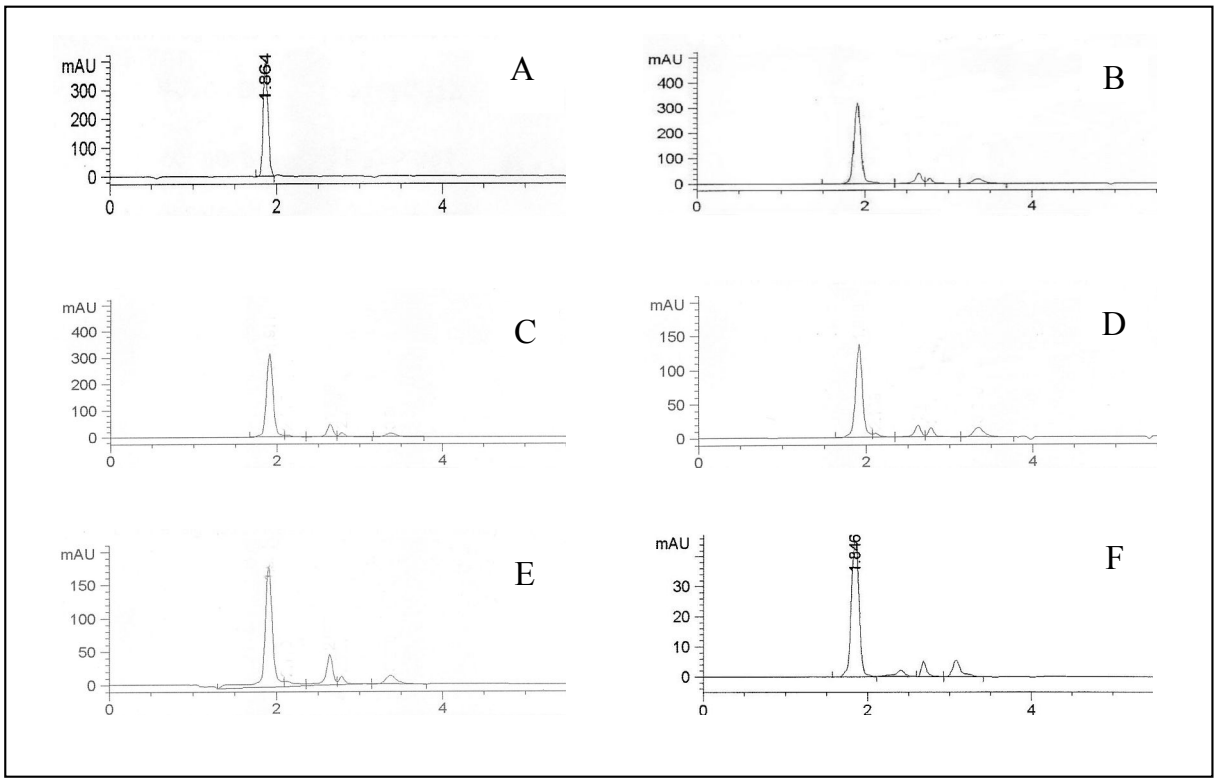

Figure 4. HPLC chromatograms of β-carotene (A) and four varieties; Pathum (B), Boontharik (C), Sattabongkot (D), Sattabutre (E) and a sample obtained from traditional drug stores (F).

can be detected in every sample. The HPLC chromatograms of β-carotene and of the four varieties were shown in Figure 4. The β-carotene content in four varieties and ten samples from drug stores were expressed in mg% w/w, as shown in Figure 5. From our study, Pathum gave the highest content of β-carotene followed by Boontharik, Sattabutre, and Sattabongkot. Whereas the β-carotene content of samples obtained from drug stores were very low, about 40-100 times of freshly dried samples. The results implied there is hardly any carotene left in lotus stamens purchased from drug stores. The definite constituents in lotus stamens, which are responsible for the remedies in the Thai herbal recipes, are

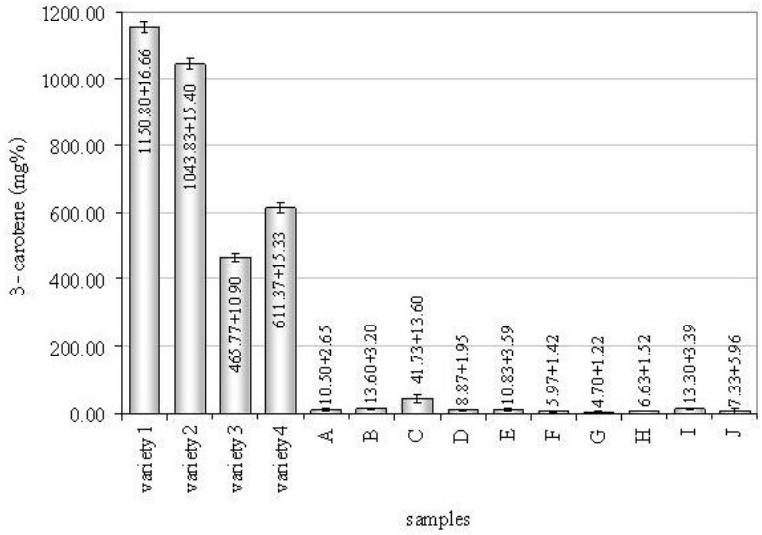

Figure 5. β-carotene of four varieties, Pathum (1), Boontharik (2), Sattabongkot (3), Sattabutre (4), and ten samples from drug stores (A-J).

still unknown. If carotenoids are responsible, then the stamen should be freshly prepared by picking and drying before use. Carotenoids are well known for their instability. They can be easily oxidized and also undergo *trans-cis* isomerisation (Harborne, 1998). Another advantage of self-preparation of the stamens is that the age and variety of the lotus can be selected.

4. Conclusion

A simple HPLC method was developed and used to determine β-carotene, an indigenous constituent in lotus stamens. The β-carotene content in four varieties of lotus stamens and ten samples from drug stores varies, depending on variety, source origin, and storage conditions.

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