Development and validation of HPLC analysis for banana bunch extract

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Abstract

The \textit{KluayNamwa} is a variety of banana common in Thailand, grown in all regions and available throughout the year. Outer parts (peel, hand and bunch) of banana are usually left and disposed from industrial process where the banana peel accounts for 40 \%w/w of total fruit. Many studies showed that banana peel contained high value of phytochemicals for using in pharmaceutical, cosmetic and food industries such as gallocatechin, tannin and provitamin A. Ingkaninan and colleague studied the antioxidant activity of banana peel, hand and bunch extract. The extracts were determined percent yield and antioxidant activity by using 4 methods, Folin–Ciocalteu method, DPPH assay, ABTS assay, and FRAP assay. The banana bunch extract have shown the highest antioxidant activity and the greatest percentage yield of three extracts.

Our study was interested with the components including gallic acid, gallocatechin, catechin, epicatechin and epigallocatechin gallate in the banana bunch extract, which can be used in the pharmaceutical, cosmetic and food industries. This study can add value to the banana bunch usage and increase the revenue income of the banana cultivating farmers. The aim of this study was to use high performance liquid chromatography (HPLC) analysis to determine the gallic acid and catechins in banana bunch extract compared to standard.

High performance liquid chromatography analysis for banana bunch extract with an isocratic elution was developed. There are 2 systems of mobile phase; 1) 0.1\% formic acid and acetonitrile and 2) 0.1M phosphoric acid and acetonitrile. Gallic acid (GA), gallocatechin (GC), catechin (C), epicatechin (EC) and epigallocatechin gallate (EGCG) were used as standard compounds for this study. The suitable system of HPLC analysis for banana bunch extract was 0.1M phosphoric acid: acetonitrile at ratio 90:10. From 5 standards in this study, gallic acid only, was detected from banana bunch extract.

Keywords HPLC, gallic acid, catechins, banana, banana bunch extract

Introduction

The \textit{KluayNamwa} is a variety of banana common in Thailand, grown in all regions and available throughout the year. According to data from the Thai Department of Agriculture, in 2011, some 781,683 tons was produced, representing total revenue from sales of more than 8,680 million Baht (\textit{กล้วยน ้ำว้ำ พืชเศรษฐกิจหมื่นล้ำน}, 15 August 2013). This revenue was achieved from the sale of natural bananas and did not include revenue derived from processed banana products. The fruit of the banana is processed into saleable products, leaving the peel and the stems of the hand and the bunch. The weight of the left–over banana peel, after processing, is 40\%w/w of the whole banana (Anhwange, Ugye, & Nyiaatagher, 2009).
The *KluayNamwa* is the variety *Musa sapientum* L. in the type AAB (triploids) which belongs to the Musaceae family. After cutting of the bunches, the bananas are either sold as natural fruit, or prepared for processing into further products, such as sun-dried bananas. The left-over peels are essentially a waste product of the processing activity. (Wilaipol, 2009). However, banana peel is known to have medicinal properties such as providing protection against stomach ulcers, (Lewis & Shaw, 2001), being an antioxidant (Mokbel & Hashinaga, 2005), and having antibacterial (J. Chen, Liu, Ren, Li, & Jiang, 2004; Mokbel & Hashinaga, 2005) and wound healing properties (Agarwal et al., 2009; Someya, Yoshiki, & Okubo, 2002). Many studies showed that banana peel contained high value of phytochemicals for using in pharmaceutical and food industries such as α-carotene (Davey, Keulemans, & Swennen, 2006), β-carotene, vitamin C, oxalate, phytic acid, tannin (Nagarajaiah & Prakash, 2011) and gallocatechins (Someya, et al., 2002). Ingkaninan and colleague studied the antioxidant activity of banana peel, hand and bunch extract. The extracts were determined percent yield and antioxidant activity by using 4 methods, Folin–Ciocalteu method, DPPH assay, ABTS assay, and FRAP assay. The banana bunch extract have shown the highest antioxidant activity and the greatest percentage yield of three extracts (Waranuch, Ingkaninan, Viyoch, Kritsunankul, & Sittichokechaiwut, 2014).

Our study was interested with the components including gallic acid, gallocatechin, catechin, epicatechin and epigallocatechin gallate in the banana bunch extract, which can be used in the pharmaceutical, cosmetic and food industries. This study can add value to the banana bunch usage and increase the revenue income of the banana cultivating farmers. The aim of this study was to use high performance liquid chromatography (HPLC) analysis to determine the gallic acid and catechins in banana bunch extract compared to standard.

**Materials and methods**

**Materials and instruments**

Gallic acid, gallocatechin, catechin, epicatechin, and epigallocatechin gallate were purchased from Sigma (Steinheim). Methanol, ortho–phosphoric acid 85% and acetonitrile were purchased from RCI Labscan (Bangkok, Thailand). Banana bunch extract was obtained from Assoc. Prof. Dr. Kornkanok Ingkaninan.

HPLC instrument (LC-10AT VP) consists of UV –Vis detector (SPD–10A VP,), an autosampler (SIL–20AC HT,) and column oven (CTO–10AS VP) were products of Shimadzu, Kyoto, Japan. Analytical column and guard column were Vertisep™ pH endure, products of Vertical Chromatography, Thailand.
Methods

Chromatography separation were carried out on a reverse–phase Vertisep™ C18 column with 5 µm and 4.6x250 mm diameter. The injection volume was 20 microliters and the temperature of column was thermostated at 25 degree Celsius. The mobile phases were used 2 systems; 1) 0.1% formic acid and acetonitrile (85:15) and 2) 0.1M phosphoric acid: acetonitrile (83:17). The flow rate was set at 1.0 ml/min and time of analysis was 15 minutes. The detector’s wavelength was set at 275 and 210 nm for each system, respectively. Determination was performed in triplicates.

Gallic acid (GA), gallocatechin (GC), catechin (C), epicatechin (EC) and epigallocatechin gallate (EGCG) were used as standard compounds for this study. The standard stock solutions were prepared by weighing 10 milligrams of each standard into each 10 milliliters volumetric flasks. The volumes were adjusted with methanol and then mixed it well. All solutions were kept at −20 degree Celsius.

Ten milligrams of banana bunch extract was precisely weighed and added to 1 milliliter of methanol mixed until cleared solution was obtained. Mobile phase, 9 milliliters were added to the portion mixture then mixed it well.

A validation method was performed according to US Food and Drug Adminitration volume II– methods, method verification and validation ORA–LAB.5.4.5 (USFDA, 2014).

Linearity

Eleven additional calibration levels were prepared by 2-fold serial dilution with mobile phase in the range of 25 to 0.0244 µg/ml.

Limit of detection (LOD) and Limit of quantification (LOQ),

An estimation of the limits can be achieved by the determination of the signal/noise ratios of 3:1 (LOD) and 10:1 (LOQ).

Precision

Precision of the system was determined as intra- and inter-day precision. The intra-day precision was analyzed by triplicate analysis at 3 concentrations of the sample on the same day. The inter-day precision was analyzed at 3 concentrations on 3 consecutive days.

Accuracy

Accuracy was determined by using spiked technique, the standard gallic acid was spiked in matrix a comparison of the theoretical concentration of standards added to sample solution and those obtained within the HPLC analysis. The theoretical concentration of gallic acid were 6.25, 1.56 and 0.39 µg/ml. Each sample solution was injected in triplicate.
Result

HPLC analysis for GA, GC, C, EC and EGCG in banana extract with an isocratic elution was developed. There are 2 systems of mobile phase; 1) 0.1% formic acid and acetonitrile and 2) 0.1M phosphoric acid and acetonitrile. Both systems were compared the sensitivity of the systems on standards and extracts to select the suitable system.

![Chromatogram A and B were analyzed by using 0.1% formic acid and acetonitrile (85:15). Chromatogram C and D were analyzed by using 0.1M phosphoric acid: acetonitrile (83:17). The chromatogram A and C were showed the peaks of the standards; GA, GC, C, EC and EGCG. The chromatogram B and D were showed the peaks of the banana bunch extract.](image)

Chromatogram A and C represented the peak of standards; GA, GC, C, EC, and EGCG, respectively. Chromatogram B and D represented the peak of extract. The elution system of chromatograms, A and B were 0.1% formic acid and acetonitrile (85:15) whereas those for chromatogram C and D were 0.1M phosphoric acid: acetonitrile (83:17). From the chromatograms, 0.1M phosphoric acid: acetonitrile was selected to analyze the compounds in banana bunch extract. The peak of gallic acid in the banana peel extract was shown with retention time of 3.521 which was in the range of dead space, thus the system was developed by changing the ratio of 0.1M phosphoric acid: acetonitrile from 83:17 to 90:10. The retention time was shifted from 3.521 to 4.921 in the Fig. 2. The calibration data of standard gallic acid was shown in the Table 1. Linearity of all response
was obtained in the range of 0.0976–25 µg/ml. The detection limit determine at signal to noise ratio was 0.0244.

![Chromatogram of standard gallic acid at retention time of 4.921 minutes.](image)

**Fig. 2** Chromatogram of standard gallic acid at retention time of 4.921 minutes.

<table>
<thead>
<tr>
<th>Table 1 Calibration data of the standard gallic acid</th>
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</thead>
<tbody>
<tr>
<td><strong>Linearity range (µ g/ml)</strong></td>
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<tr>
<td><strong>Regression equation</strong></td>
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<tr>
<td><strong>Correlation coefficient</strong></td>
</tr>
<tr>
<td><strong>Limit of detection (µ g/ml)</strong></td>
</tr>
<tr>
<td><strong>Limit of quantification (µ g/ml)</strong></td>
</tr>
</tbody>
</table>

The accuracy of this method was determined by used the standard addition method. Three different concentration of standard gallic acid were added to a sample and analyzed. The determination was performed in triplicates for each set. The results were shown in the Table 2.

The intra- and inter-day precision of the method was obtained from 3 dilutions of standard gallic acid for 3 consecutive days. The inter- and intra-day precision can be determined from percentage of RSD (%RSD).
The analytical amount of gallic acid was $3.145 \, \mu\text{g/ml}$ in the banana bunch extract at concentration $1 \, \text{mg/ml}$.

**Table 2** Accuracy data of the HPLC analyzes of gallic acid by spiked technique

<table>
<thead>
<tr>
<th>Theoretical concentration ((\mu\text{g/ml}))</th>
<th>%Accuracy (± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.25</td>
<td>102.69 ± 0.39</td>
</tr>
<tr>
<td>1.56</td>
<td>98.77 ± 1.12</td>
</tr>
<tr>
<td>0.39</td>
<td>99.77 ± 0.33</td>
</tr>
</tbody>
</table>

**Table 3** Intra–and inter–day precision of the HPLC analyzes of standard gallic acid

<table>
<thead>
<tr>
<th>Concentration ((\mu\text{g/ml}))</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Intra-day (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.25</td>
<td>6.45</td>
<td>6.38</td>
<td>6.65</td>
<td>6.43</td>
</tr>
<tr>
<td></td>
<td>(0.25)</td>
<td>(0.02)</td>
<td>(0.05)</td>
<td>(0.21)</td>
</tr>
<tr>
<td>1.56</td>
<td>1.54</td>
<td>1.53</td>
<td>1.63</td>
<td>1.53</td>
</tr>
<tr>
<td></td>
<td>(0.04)</td>
<td>(0.04)</td>
<td>(0.03)</td>
<td>(0.03)</td>
</tr>
<tr>
<td>0.39</td>
<td>0.39</td>
<td>0.38</td>
<td>0.39</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>(0.01)</td>
<td>(0.01)</td>
<td>(0.01)</td>
<td>(0.00)</td>
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</tbody>
</table>

**Discussion**

The mobile phase systems in this study were 1.) 0.1% formic acid and acetonitrile and 2.) 0.1M phosphoric acid and acetonitrile. The determined wavelength were 275 nm for the system 1 and 210 nm for the system 2. From the literature review, the addition of acid into mobile phase is essential to enable a complete separation of catechins in mixture standard and eliminate the tailing of the peak as well and the determination wavelength 210 and 275–280 nm showed the maximum absorbance of gallic acid and catechins (Dalluge, Nelson, Brown Thomas, & Sander, 1998). From the result of our experiment, 0.1 M phosphoric acid and acetonitrile at ratio 90 to 10 and determination wavelength at 210 nm showed the greater sensitivity of the determination than the system 1. The results are shown in similar manner with Wang, H. et. al. and Wisuitiprot, W. et. al. Wang H. et. al. used isocratic elution system for determination of catechins, caffeine and gallic acid at wavelength 210 and 280 nm. They found that at the wavelength of 210 nm with addition of orthophosphoric acid shown complete separation (Wang, Helliwell, & You, 2000). Wisuitiprot, W.et. al. used mobile phase consisted of 0.05% trifluoroacetic acid in water : acetonitrile and detection was performed at 210 nm to analyze catechins in green tea extract (Wisuitiprot, Somsiri, Ingkaninan, & Waranuch, 2011).
The correlation of the method and concentration was shown in the regression line and the line was estimated for the degree of linearity. The correlation coefficient of determination ($r^2$) for linearity of calibration curve must be $\geq 0.995$. From the result was, $r^2=0.9997$.

The accuracy of this method was determined by standard addition method. The percentage of accuracy should be in the range of 80–120%. From the result was within 97.65–103.08.

Table 3 showed the precision of assay. Intra- and inter-day precision data was expressed by the %RSD of less than 20%.

From all of the results (the correlation coefficient, accuracy and intra- and inter-day precision) were showed the suitability and sensitivity of the system to the gallic acid in the banana bunch extract under the guideline of USFDA.

**Conclusion and suggestion**

This study was aimed to develop and validate method of HPLC analysis for banana bunch extract. The method was validated according to US Food and Drug Administration volumm II– methods, method verification and validation ORA–LAB.5.4.5 (USFDA, 2014). The method was accurate, precise, selective and inexpensive. The suitable system of HPLC analysis for banana bunch extract was 0.1M phosphoric acid: acetonitrile at ratio 90:10. In this study, only gallic acid was detectable in the banana bunch extract.

**Acknowledgement**

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**References**


Waranuch, N., Ingkaninan, K., Viyoch, J., Kritsunankul, O., & Sittichokechaiwut, A. (2014). การพัฒนาผลิตภัณฑ์เพื่อสุขภาพและความงามจากส่วนเปลือก หวีหรือเครือกล้วยว้าว (Development of health and beauty products from peel, hand or bunch of Cultivated banana (Musa sapientum Linn.)) (pp. 10–49).
