Pharmacognostic Specification and Pinocembrin Content of *Boesenbergia rotunda* Root

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Abstract

This study was aimed to investigate the standardization parameters by qualitative and quantitative analyses as well as pinocembrin content of *Boesenbergia rotunda* root. Loss on drying, total ash, acid-insoluble ash, ethanol soluble extractives, water soluble extractives, moisture content and volatile oil content were established by World Health Organization (WHO) guideline. Pinocembrin content was evaluated using TLC densitometry. Loss on drying, total ash, acid-insoluble ash, ethanol soluble extractives, water soluble extractives, moisture content and volatile oil content were respectively found to be 10.11±0.22, 5.93±0.11, 2.87±0.12, 6.72±0.32, 10.50±0.28, 8.87±0.25 and 1.15±0.11 % on dry weight basis. Pinocembrin in ethanol extract was analysed by thin layer chromatography (TLC) using silica gel 60 F254 as stationary phase. In addition, toluene, acetone, methanol and formic acid (9:0.5:0.2:0.1) was used as mobile phase. Pinocembrin content was evaluated using TLC densitometry. The regression line of method was polynomial in range of 0.4-2 µg/spot, and correlation coefficients (R^2) was 0.999. The relative standard deviation of repeatability and intermediate precisions were between 4.39-7.43%. The percent recovery was found to be 101.5-113.3%. The robustness evaluated by slight variation in mobile phase ratio was 0.38% RSD. Limit of detection (LOD) and limit of quantitation (LOQ) were 0.04 and 0.14 µg/spot respectively. The pinocembrin content in *B. rotunda* root was found to be 0.82±0.05% dry basis. This study provided pharmacognostic specification toward fundamental standardization of *B. rotunda* root.

***Keywords*:** *Boesebergia rotunda (L.) Mansf, thin layer chromatography, pinocembrin, pharmacognostic specification*

บทคัดย่อ

การศึกษานี้มีวัตถุประสงค์เพื่อจัดทำข้อกำหนดทางเภสัชเวทและวิเคราะห์หาปริมาณสารพิโนเซมบรินของรากกระชาย ศึกษาคุณสมบัติทางกายภาพและเคมีของรากกระชายโดยหาปริมาณน้ำหนักที่หายไปเมื่อทำให้แห้ง ปริมาณเถ้ารวม เถ้าที่ไม่ละลายในกรด ปริมาณสารสกัดด้วยเอทานอล ปริมาณสารสกัดด้วยน้ำ ปริมาณความชื้นและน้ำมันระเหยด้วยวิธีขององค์การอนามัยโลก *(WHO)* และวิเคราะห์ปริมาณสารพิโนเซมบรินด้วยเทคนิคโครมาโทกราฟีชนิดแผ่นบาง-เด็นซิโทเมทรี จากการศึกษาคุณสมบัติทางกายภาพและเคมีพบว่ารากกระชายมีปริมาณน้ำหนักที่หายไปเมื่อทำให้แห้ง ปริมาณเถ้ารวม เถ้าที่ไม่ละลายในกรด ปริมาณสารสกัดด้วยเอทานอล ปริมาณสารสกัดด้วยน้ำ ปริมาณความชื้นและน้ำมันระเหยเท่ากับร้อยละ *10.11±0.22, 5.93±0.11, 2.87±0.12, 6.72±0.32, 10.50±0.28, 8.87±0.25* และ *1.15±0.11* โดยน้ำหนัก ตามลำดับ วิเคราะห์ปริมาณสารพิโนเซมบรินในสารสกัดแอทานอลของรากกระชายด้วยเทคนิคโครมาโทกราฟีชนิดแผ่นบาง-เด็นซิโทเมทรี โดยมีแผ่นซิลิกาเจล *60* เอฟ *254* เป็นวัฏภาคคงที่ และใช้ตัวทำละลายตัวโทลูอีน อะซิโตน เมทานอล และกรดฟอร์มิก (*9*:0.5:0.2:0.1*)* เป็นวัฏภาคเคลื่อนที่ วิเคราะห์ปริมาณสารพิโนเซมบรินโดยวิธีทางโครมาโทกราฟีชนิดแผ่นบาง-เด็นซิโทเมทรีมีช่วงวิเคราะห์แบบโพลีโนเมียล 0.4-2 ไมโครกรัมต่อจุดและมีค่าสัมประสิทธิ์สหสัมพันธ์ เท่ากับ 0.999 ความเที่ยงของวิธีวิเคราะห์ ประเมินจากค่าสัมประสิทธิ์ของการกระจายมีค่าระหว่างร้อยละ 4.39-7.43 ค่าเฉลี่ยการคืนกลับระหว่างร้อยละ 101.5-*113.3* ขีดจำกัดของการตรวจพบและขีดจำกัดของการหาค่าปริมาณมีค่า *0.04* และ *0.14* วิเคราะห์ปริมาณพิโนเซมบรินของรากกระชาย มีค่าเฉลี่ยร้อยละ 0.82±0.05 จากการศึกษาคุณสมบัติทางกายภาพและเคมีของรากกระชายชี้ให้เห็นว่า ข้อกำหนดทางเภสัชเวทและปริมาณวิเคราะห์สารพิโนเซมบรินจะนำไปสู่การเป็นมาตรฐานของรากกระชายต่อไปในอนาคต

***คำสำคัญ*:** *กระชาย โครมาโทกราฟีชนิดแผ่นบาง พิโนเซมบริน ข้อกำหนดทางเภสัชเวท*

1. Introduction

Boesebergia rotunda (L.) Mansf. (Zingiberaceae) is an ingredient in Traditional Thai medicine remedies and folklore medicine which is used for treatment of several diseases for a long time. It can be used as single drug or remedies which mixed of rheumatism, muscle pain, febrifuge, gout, gastrointestinal

disorders, flatulence, carminative, stomach ache, dyspepsia, and peptic ulcer (Eng-Chong et al., 2012). Pinocembrin is a major flavonoid molecule incorporated as multifunctional in the pharmaceutical industry. Its vast range of pharmacological activities has been well researched including antimicrobial, anti-inflammatory, antioxidant, and anticancer activities (Chahyadi et al., 2014). Conventional method used for qualitative investigation of the active compounds in plant extracts is thin layer chromatography (TLC) (Mongkolrat, Palanuvej, & Ruangrungsi, 2013) and (Phattanawasin, Sotanaphun, & Sriphong, 2008). This method is easy, rapid and cheap methods for screening the active compound in plant extract. Qualitative or semi-quantitative TLC analysis is usually done by visual comparison. Quantitative TLC analysis can be precisely performed by the technique of densitometry (Victoria & Hess, 2007).This study was accomplish to investigate the standardization parameters by qualitative and quantitative analyses as well as pinocembrin content of *B. rotunda* root.

2. Objective

This study was aimed to establish the pharmacognostic specifications of *B. rotunda* root, and investigate the content of pinocembrin using TLC densitometry.

3. Materials and Methods

Plant Materials and Chemicals

B. rotunda roots were collected from 15 various sources throughout Thailand. Voucher specimens were authenticated by one of the authors (Assoc. Prof. Nijsiri Ruangrungsi) and deposited at College of Public Health Sciences, Chulalongkorn University, Thailand. Standard pinocembrin was purchased from Fluka, USA. The chemicals were analytical grade.

Crude Extract Preparation

B. rotunda root was dried and ground into powder. The powders were exhaustively extracted with dichloromethane using Soxhlet apparatus. The extract yields were weighed and recorded.

Macroscopic and Microscopic

Size, colour and other visual inspections of crude drugs were examined. Anatomical and histological characters were determined. Transverse sections and powdered samples (ground and sifted through a 250 micron sieve) were inspected respectively under microscope (Olympus BX41) with a magnification of 4x, 10x and 40x and compared the scale with the 0.01 mm micrometre.

Determination of Total Ash

The ground sample (3 g) were placed in a previously ignited and pre-weighed crucible. The sample was spread in an even layer and ignited by gradually increasing the heat to 500-600°C until white ash was obtained. The ash was then cooled in a desiccator and weighed without delay.

Determination of Acid-Insoluble Ash

The crucible containing the total ash was added with 25 ml of hydrochloric acid (70 g/l), covered with a watch-glass and boiled gently for 5 minutes, and rinsed the watch-glass with 5 ml of hot water in which this liquid was added to the crucible. The insoluble matters were collected on an ashless filter-paper and washed with hot water until the filtrate was neutral. The filter-paper containing the insoluble matters was transferred to the original crucible, dried on a hot plate and re-ignited. The crucible was immediately cooled in desiccator, and weighed.

Determination of Ethanol-Soluble Extractive

The ground sample (5 g) were macerated with 70 ml of 95% ethanol in a closed conical flask for 6 hours in shaking bath and allowed to stand for 18 hours. They were filtered, washed and adjusted to 100 ml with ethanol. Twenty millilitres of the filtrate were evaporated to dryness in a pre-weighed small beaker and dried at 105 ºC to constant weight.

Determination of Water-Soluble Extractives

The ground sample (5 g) were macerated with 70 ml of water in shaking bath and allowed to stand for 18 hours. They were filtered and 20 ml of the filtrate were evaporated to dryness in a pre-weighed small beaker and dried at 105 ºC to constant weight.

Determination of Loss on Drying

The ground sample (5 g) were weighed in a pre-weighed small beaker and dried with heat at 105 °C to constant weight.

Determination of Moisture

The ground sample (50 g) were added with 200 ml of water-saturated toluene and distilled by azeotropic distillation. When water and toluene layers were separated, the volume of water was recorded.

Determination of Volatile Oil Content

The ground sample (100 g) were added with 600 ml of water and distilled by Clevenger apparatus. When volatile oil and water layers were separated, volume of volatile oil was determined.

Thin-layer chromatographic identification

One g of the sample was macerated in powder, with 10 ml of 95% ethanol for 6 hours, and filtered and evaporated to dryness. The residue was dissolved in 1 ml of 95% ethanol. Apply 3-5 µl was applied to the TLC plate, using silica gel GF254 as the coating substance.

TLC Densitometry of Pinocembrin

Two microliter of standard pinocembrin solutions were applied (0.4, 0.8, 1.2, 1.8 and 2.0 µg/ml) and 2 µl of ethanol extract of *B. rotunda* root (10 mg/ml) on a 20 x 10 cm silica gel 60 F254 TLC plate were developed using a mixture of toluene, acetone, methanol and formic acid (9:0.5:0.2:0.1) as mobile phase. After development, the plate was scanned using densitometer (CAMAG TLC Scanner 3, USA). The calibration curve of pinocembrin was prepared by plotting peak areas *versus* concentrations of pinocembrin applied.

Method Validation

The method was validated in terms of its range, limits of detection (LOD), limits of quantification (LOQ), accuracy, precision, and robustness according to ICH guideline.

Data Analysis

The pharmacognostic specification was calculated as grand mean ± pooled standard deviation.

4. Results and Discussion

The whole plant of *B. rodunda* was shown in Figure 1. The dried root crude drugs were bright yellow with brown bark (Figure 2). The smell was a pleasant odor. Anatomical characteristics of dried root was shown in Figure 3. Secretory sac containing volatile oil, oleoresin and starch grain were found. Histological characteristics was composed of parenchyma, periderm, starch grain, reticulate vessel, annular vessel, reticulate vessel, and spiral vessel (Figure 4).

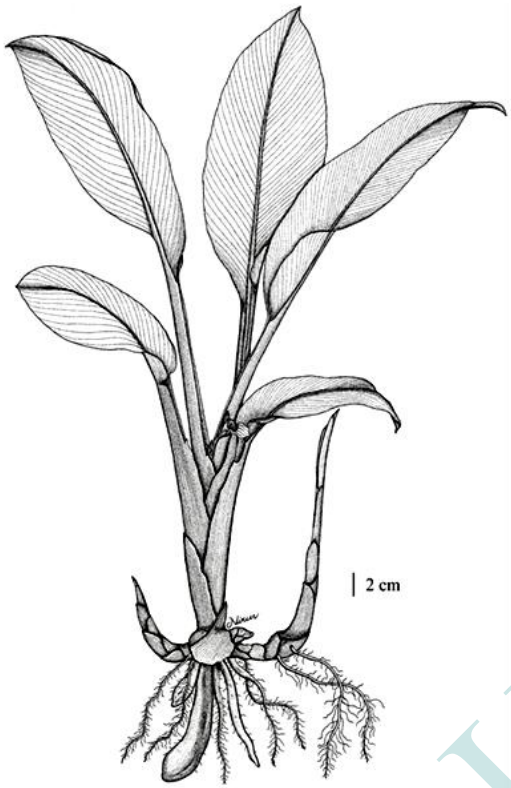
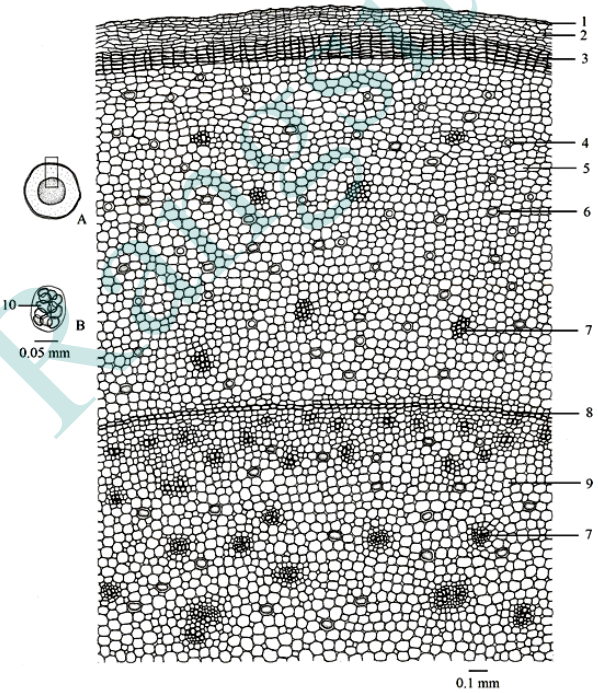


Figure 1 The whole plant of *B. rodunda*

Figure 2 The dried roots of *B. rodunda*

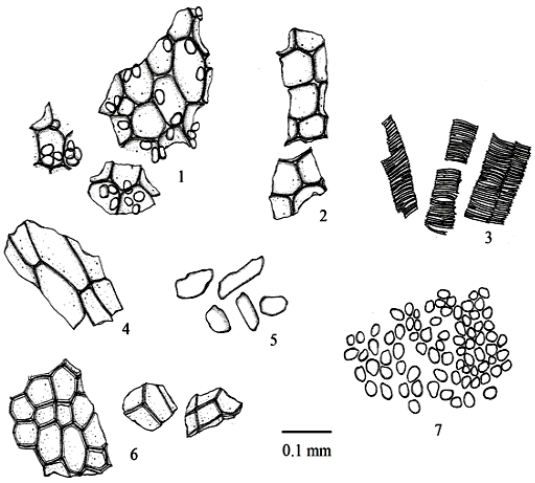


A. Diagram of transverse section

B. Cortical parenchyma

1. Epidermis
2. Hypodermis
3. Cork cell
4. Oil droplet
5. Cortical parenchyma
6. Mass of oleoresin
7. Vascular bundle
8. Endodermis
9. Stele parenchyma
10. Starch grain in parenchyma

Figure 3 Anatomical character of *B. rotunda* (transverse section)



1. Parenchyma containing starch grain
2. Parenchyma, transverse view
3. Fragment of reticulated vessel
4. Parenchyma, longitudinal view
5. Mass of oleoresin
6. Cork, surface view
7. Starch grain

Figure 4 Histological character of *B. rotunda* (powdered)

The pharmacognostic properties of *B. rotunda* roots were determined using loss on drying, total ash, acid-insoluble ash, ethanol soluble extractives, water soluble extractives, moisture contents and volatile oil content which were respectively found to be 10.11±0.22, 5.93±0.11, 2.87±0.12, 6.72±0.32, 10.50±0.28, 8.87±0.25 and 1.15±0.11 % as shown in Table 1. These constant numbers have been reported for the first time in this study. TLC fingerprints of this extract were shown in Figure 5. The presence of dark spot at 254 nm and fluorescing at 365 nm using toluene, ethyl acetate (7.5:2.5) as mobile phase with anisaldehyde as staining reagent was observed.

Table 1 The pharmacognostic parameters of *B. rotunda* root

Specification	Content (% dry weight)*
Loss on drying	10.11±0.22
Total ash	5.93±0.11
Acid-insoluble ash	2.87±0.12
Ethanol soluble extractives	6.72±0.32
Water soluble extractives	10.50±0.28
Moisture content	8.87±0.25
Volatile oil content	1.15±0.11

*The parameters were shown as grand mean ± pooled SD

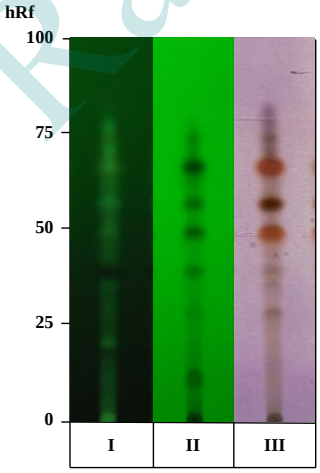


Figure 5 The fingerprint of *B. rotunda* root extract
I = detection under UV light 254 nm
II = detection under UV light 365 nm
III = detection with anisaldehyde staining reagent

Pinocembrin content was evaluated using TLC densitometric method. The regression line of method was polynomial in range of 0.4-2 µg/spot. The repeatability and intermediate precisions were between 4.39-7.43% RSD. The percent recovery was found to be 101.5-113.3%. The robustness evaluated by slightly variation in mobile phase ratio was 0.38% RSD. LOD and LOQ were 0.04 and 0.14 µg/spot respectively. The pinocembrin content in *B. rotunda* root extract was found to be 0.82±0.05% on dry basis, and hRf values of pinocembrin was 62.50.

Table 2 TLC densitometric method validation of pinocembrin content in *B. rotunda* root

Accuracy	101.5-113.3% recovery
Intra-day precision	4.39 % RSD
Inter-day precision	7.43% RSD
Robustness	0.38% RSD
Limit of detection	0.04 µg/spot
Limit of quantitation	0.14 µg/spot

5. Discussion

Pharmacognostic specification is primarily an important tool for identification, authentication and standardization of herbal medicines. The fingerprint of *B. rotunda* root extract showed characteristic profiles which might be used as markers for quality evaluation and standardization of the crude drug. In addition, due to quality of crude drug the constant numbers of total ash, acid insoluble ash, loss on drying and moisture should be not more than 5.93, 2.87, 10.11 and 8.87% of dry weight respectively, whilst ethanol–soluble extractive, water–soluble extractive and volatile oil content should not less than 6.72, 10.50 and 1.15% of dry weight respectively. TLC is easy, rapid and cheap method, and also has promising analytical potential to quantify the chemical constituents in herbal medicine. TLC densitometric analysis which used in this study for quantitative analysis of pinocembrin were validated in term of calibration range, LOD, LOQ, accuracy and precision. Pinocembrin content in dried root was 0.82±0.05% by weight. The method validity was demonstrated in Table 2. The calibration curve was polynomial with the range of 0.4-2 µg/spot ($y= -3790.9\ x^2 + 18542\ x + 5728.5$, $R^2=0.999$). The accuracy was performed by recovery of spiking known three concentrations of standard pinocembrin. The recovery values were within acceptable limits (101.5-113.3%).The repeatability (intra-day precision) and intermediate precision (inter-day precision) were less than 8%. The precision of pinocembrin quantitative analysis was determined by using 3 concentrations (3 replicates) on the same and 3 different days. The LOD and LOQ was calculated using residual standard deviation of regression lines. The LOD value, the lowest concentration of analysis in a sample was found to be 0.04 mg/spot. The LOQ value, the lowest concentration of analysis in a sample was 0.14 mg/spot. The robustness was estimated by analysis of results obtained after deliberate variation of mobile phase ratio which were found to have no differences (%RSD < 5). As the result, this investigation may be useful in the preparation of monograph in Thai medicinal plant.

6. Conclusion

Pharmacognostic specifications is important for herbal drug identification. In present study, quality control and standardization of *B. rotunda* root were established, whereas pinocembrin content was evaluated using TLC densitometry. In conclusion, the pharmacognostic specifications and pinocembrin content from present study could be used for quality control and standardization.

7. Acknowledgement

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8. References

Chahyadi, A., Hartati, R., Wirasutisna, K. R., & Elfahmi. (2014). *Boesenbergia Pandurata* Roxb., An Indonesian Medicinal Plant: Phytochemistry, Biological Activity, Plant Biotechnology. *Procedia Chemistry*, *13*, 13-37.

Eng-Chong, T., Yean-Kee, L., Chin-Fei, C., Choon-Han, H., Sher-Ming, W., Li-Ping, C. T., . . . Yusof, R. (2012). *Boesenbergia rotunda*: From Ethnomedicine to Drug Discovery. *Evid Based Complement Alternat Med, 2012*, 473637.

Mongkolrat, S., Palanuvej, C., & Ruangrungsi, N. (2013). Thin layer chromatography and analysis of seleted liriodenine bearing plant in Thailand *Journal of Health Research*, *27*(2), 72.

Phattanawasin, P., Sotanaphun, U., & Sriphong, L. (2008). Validated TLC-Image Analysis Method for Simultaneous Quantification of Curcuminoids in Curcuma longa. [journal article]. *Chromatographia*, *69*(3), 397-400.

Victoria, A., & Hess, I. (2007). Digitally Enhanced Thin-Layer Chromatography: An Inexpensive, New Technique for Qualitative and Quantitative Analysis. *Journal of Chemical Education 84*(5), 842.