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Analysis of piperine content in cabe jawa extracts (*Piper retrofractum Vahl*) using UV spectrophotometry and HPLC

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Abstract. Cabe Jawa (*Piper retrofractum Vahl*) is an important commodity for herbal industries in Indonesia. One of the bioactive compounds in this herb is piperine. In this study, concentration of piperine in Cabe Jawa was analysed using HPLC and UV spectrophotometry methods. Cabe Jawa samples were prepared using two different methods. The first sample (S1) was dried from fresh *Piper retrofractum*, while the second sample (S2) was dried from boiled *Piper retrofractum*. Both samples were extracted using Soxhlet, followed by solvent evaporation to obtain the concentrated extract. Piperine concentration was measured using UV spectrophotometry and HPLC via internal standard method. The yields of extract of S1 and S2 were 23.0% and 22.1%, respectively. Piperine UV spectrophotometry standard curve equation was y = 0.1107*(X)-0.1578, R2 = 0.9999, whereas HPLC standard curve equation was y = 105916*(X) + 28100.9, R2 = 0.9978. Piperine concentration in S1 and S2 extracts quantified using UV spectrophotometry were 19.0% and 18.1%., respectively. The values for S were much higher compared to values obtained by HPLC, which were 6.8% (S1) and 7.3% (S2). Therefore, the use of UV spectrophotometry for analysis of piperine is not recommended due to the interference of other compounds, which are absorbed at the same wavelength.
Keywords: Cabe Jawa, HPLC, Piper retrofractum, Piperine, UV spectrophotometry

1. Introduction

Cabe Jawa fruit (*Piper retrofraktrum* Vahl, Fig. 1) is a typical horticultural commodity from Indonesia [1]. This plant has been reported to increase blood testosterone levels [2], anti-obesity [3] and anti-bacteria. These activities are generally indicated by the presence of piperine in *Piper retroftactum* as the major bioactive compound [4].

Figure 1. *Piper retrofractum Vahl.*

Piperine is an indole alkaloid compound. In Cabe Jawaes, other indole alkaloids have been reported, such as dihydropiperine, dihydropiperolonguminine, piperolonguminine, pipersida, pelitorin, and piperiline [5]. The presence of these derivative compounds needs attention because they can interfere with the determination of piperine content in the sample. The Indonesian National Standard uses UV spectrophotometric method for piperine analysis. It has to be noted that the SNI is a standard method established based on agreements among stakeholders (industry, government and society) to become a method that can be applied for routine analysis. There are strong indications that compounds other than piperine can absorb ultraviolet rays at the same absorption wavelength as piperine (about 340 nm). In such cases, piperine analysis using UV spectrophotometry needs to be re-evaluated. In this study, piperine analysis using UV spectrophotometry is compared against HPLC method. In the HPLC method, it is hypothesized that the piperine compound is separated from other interfering compounds before being captured by the detector; hence, the analysis of piperine content is not interfered by other compounds.

2. Materials and Methods

2.1. Materials.

Cabe Jawa Fruit (*Piper retrofractum*) was obtained from medicinal plant farmers in Pracimantoro, Wonogiri, Central Java Province, Indonesia. Piperine (≥ 99% purity) was obtained from Sigma Aldrich Chemical Company, Germany. Ethanol (Merck) was used as solvent. Equipment: Shimadzu UV-1601 UV-Vis Spectrophotometer and HPLC Shimadzu LC-20AD.

2.2. Piperine Extraction

Fresh Cabe Jawa (10 kg) was divided into two equal parts and prepared as samples. The first sample (S1) was fresh Javan chilli that was directly dried, while the second sample (S2) was fresh Cabe Jawa that was boiled for 5 min before drying. The drying process was performed by farmers by aerating the sample for 6 days. All of the samples (Fig. 2) were then prepared in powder form by grinding. The water content of S1 was 10.2% and S2 was 10.6%. Dried Cabe Jawa powder (S1 and S2) was extracted using a Soxhlet tool. Extraction was carried out using 96% ethanol solvent until the solvent was clear (approximately 58 h). The filtrate was collected and concentrated using a rotary evaporator until a viscous ethanol extract was obtained.

Figure 2. *Piper retrofractum Vahl* Samples.

2.3. Quantitative Piperine Analysis Using UV-Vis Spectrophotometry

2.3.1. Preparation of Calibration Curve from Standard Piperine Solution. Stock of piperine solution was prepared by dissolving 10 mg of piperine into 10 mL of methanol. Standard solution was prepared from the stock at concentrations of 4 mg L^{-1} until 20 mg L^{-1}. Absorbance of standard piperine solution was measured at maximum piperine wavelength, using ethanol as blank [6].

2.3.2. Determination of Piperine Content in Extract. A total of 0.1000 g S1 and S2 extracts, each dissolved into 10 mL of ethanol and diluted as needed was measured for absorbance at maximum wavelength (343 nm). Piperine content in both samples was calculated by entering the absorbance value in the regression equation obtained from the calibration curve.

2.4. Quantitative Piperine Analysis using HPLC

Measurement of piperine content was performed using HPLC with C18 column following the method of Hamrapurkar *et al.* [7] and Upadhyay *et al.* [8] with modifications. Eluent used was acetonitrile/water/acetic acid at ratio of (60/39.5/0.5). Isocratic elution with flow rate of 1 mL/min was performed. Detection was carried out at wavelength of 340 nm.

2.4.1. Preparation of calibration curve standard piperine solution. Standard solution of piperine was prepared by dissolving 10 mg piperine into 10 mL of methanol, then further diluted to 20-100 mgl^{-1}. The linear regression equation derived from calibration was made by distributing the area (Y axis) and concentration (X axis).

2.4.2. Determination of Piperine Content in Extracts. A total of 0.1000 g S1 and S2 extracts, each dissolved in 10 mL methanol, were diluted as needed, and piperine content was analysed using HPLC. Piperine content in both samples was calculated by entering the value of area of the regression equation obtained from the calibration curve

3. Results and Discussion

Heat extraction of dried Cabe Jawa powder using Soxhlet method results in the dark brown Cabe Jawa extract known for its spicy taste and distinctive smell. The yield of extract in S1 is 23.0%, and in S2 is 22.1%; this indicates that the test sample is a good sample according to the Indonesian Herbal Pharmacopoeia (minimum 12.0% yield). It can also be informed that redemption reduces only 0.9% in the yield of the extract.

3.1. Quantitative Piperine Analysis of Cabe Jawa using UV Spectrophotometry

Piperine is an alkaloid compound belonging to the pyridine group. It contains six conjugated double bonds which are believed to be the system absorbing at wavelength of around 343 nm, thus allowing measurement using spectrophotometer. Plotting the absorbance values against various concentrations (Fig. 3) produced a calibration curve with a linear regression line equation of y = 0,1107x-0.1578, with coefficient of determination (r^2) of 0.99. Based on the regression line equation prepared from the standard curve, piperine content of S_1 and S_2 was 19.1% per gram of extract and 18.1% per gram of extract, respectively

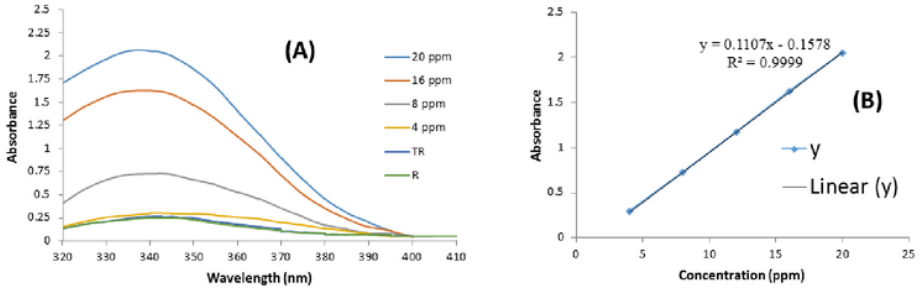

Figure 3. UV spectrum of piperine standard at different concentrations (A), Calibration Curve from Standard Piperine Solution (B).

3.2. Quantitative Piperine Analysis of Cabe Jawa using HPLC

HPLC chromatogram profiles of standard piperine at various concentrations and of S1 and S2 at 20 ppm concentration are shown in Fig. 4. Retention time of piperine under the applied conditions was 8.195 min. From the chromatograms, a calibration curve was generated by plotting the value of area against concentration. A linear regression equation of y = 105916x + 28100.9 was obtained, with correlation coefficient equal to 0.9989 and determination coefficient of 0.9978.

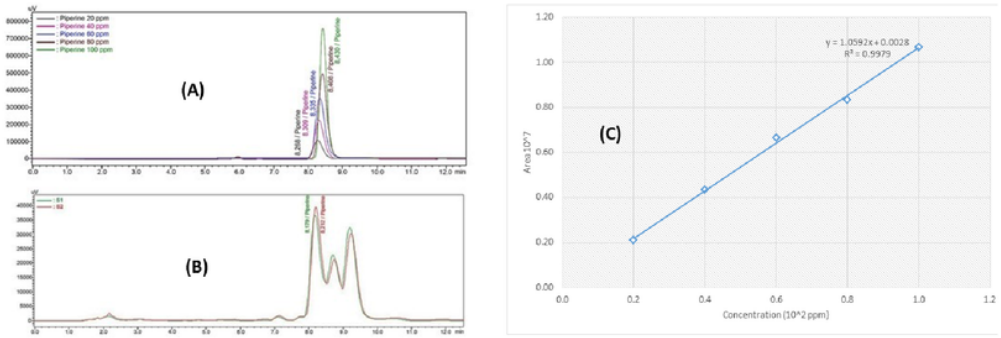

Figure 4. HPLC chromatogram: standard piperine (A), extract S1 and S2 (B), Calibration Curve Standard Piperine Solution (C).

Piperine content in S1 and S2 samples were determined using HPLC, using the equation obtained from piperine standard calibration curve. Piperine content in S1 and S2 samples were determined as 6.8% and 7.3%, respectively. The HPLC chromatogram of both samples presented more than 7 peaks. The inclusion of some HPLC chromatogram peaks in the quantitation step results in a piperine content of 19.1% for S1 and 18.5% for S2.

It can be presumed that other types of alkaloids in *Piper retrofractum Vahl*, such as dihydropiperin (G) and piperilin pipersida (D) absorb at the same wavelength as piperine, due to presence of the same conjugation system with piperine. The interference of these other alkaloid compounds can be presumed to be the cause of the higher value of piperine when analysed using UV spectroscopy. Other compounds contained in Cabe Jawa(Fig. 5) such as dihydropiperine (B), dihydropiperolonguminin (C), piperside (E) and pelitorin (F) [9] also contribute to absorption at wavelength of 343 nm.

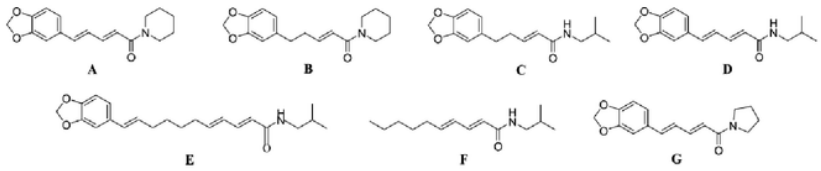

Figure 5. Indole alkaloids present in Cabe Jawa.

4. Conclusion

Piper retrofractum Vahl extracts analysed using UV spectrophotometry resulted in higher piperine content values compared to values obtained from HPLC analysis. The presence of compounds interfering with UV absorption in the sample causes this difference in piperine value. Therefore, HPLC is recommended for the analysis of piperine content in Cabe Jawa

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The authors declare no conflict of interest.

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References

[1] Sholikhah E N 2016 Indonesian medicinal plants as sources of secondary metabolites for pharmaceutical industry *J. Med. Sci.* **48** 4 226-39

[2] Midoen Y H, Anggraeni D R, Suryandari D A and Freisleben H-J 2017 Finding the Minimal Effective Dose of a Combination of Depot Medroxyprogesterone Acetate and Javanese Long Pepper for Male Contraception on Testicular Fertility Parameters in Male Sprague Dawley Rats *Biomedical and Pharmacology Journal* **10** 2 725-37

[3] Kim K J, Lee M-S, Jo K and Hwang J-K 2011 Piperidine alkaloids from Piperretrofractum Vahl. protect against high-fat diet-induced obesity by regulating lipid metabolism and activating AMP-activated protein kinase *Biochem. Biophys. Res. Commun.* **411** 1 219-25

[4] Indonesia K K R 2009 *Farmakope Herbal Indonesia* (Jakarta: Penerbit Menteri Kesehatan Republik Indonesia, Jakarta)

[5] Scott I M, Jensen H R, Philogène B J and Arnason J T 2008 A review of Piper spp.(Piperaceae) phytochemistry, insecticidal activity and mode of action *Phytochem. Rev.* **7** 1 65

[6] Genest C, Smith D M and Chapman D 1963 Pepper Analysis, A Critical Study of Two Procedures for the Determination of Piperine in Black and White Pepper *J. Agric. Food Chem.* **11** 6 508-12

[7] Hamrapurkar P, Jadhav K and Zine S 2011 Quantitative estimation of piperine in Piper nigrum and Piper longum using high performance thin layer chromatography *J. Appl. Pharm. Sci.* **1** 3 117-20

[8] Upadhyay V, Sharma N, Joshi H M, Malik A, Mishra M, Singh B and Tripathi S 2013 Development and validation of rapid RPHPLC, method for estimation of piperine in Piper nigrum L *Int. J. Herb. Med.* **1** 6-9

[9] Parmar V S, Jain S C, Bisht K S, Jain R, Taneja P, Jha A, Tyagi O D, Prasad A K, Wengel J and Olsen C E 1997 Phytochemistry of the genus Piper *Phytochem.* **46** 4 597-673

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