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Original Article

Determination of Caffeine Content in Ethanolic Extract of Robusta Coffee Beans (*Coffea canephora*) Using UV-Vis Spectrophotometry

Penentuan Kandungan Kafein dalam Ekstrak Etanol Biji Kopi Robusta (Coffea canephora) Menggunakan Spektrofotometri UV-Vis

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Abstract

Caffeine is one of the main bioactive compounds in robusta coffee beans (*Coffea canephora*) that has potential applications in pharmaceutical and cosmetic formulations, particularly for hair growth stimulation. This study aimed to determine the caffeine content in ethanolic extract of robusta coffee beans using UV-Visible spectrophotometry. The research was conducted using an experimental laboratory design. Robusta coffee bean extract was obtained by maceration using ethanol as the solvent. Qualitative identification of caffeine was followed by quantitative analysis using UV-Vis spectrophotometry at a maximum wavelength of 273 nm. A calibration curve was constructed using standard caffeine solutions, and the caffeine content of the extract was calculated based on the linear regression equation. The results showed that the calibration curve exhibited good linearity within the tested concentration range. Quantitative analysis revealed that the caffeine content of the ethanolic extract of robusta coffee beans was 221.58 mg/g extract, which is consistent with previously reported caffeine levels in Coffea canephora. These findings confirm that robusta coffee beans are a significant natural source of caffeine and support their potential utilization as raw material for pharmaceutical and cosmetic products. This study provides quantitative data that may serve as a scientific basis for further formulation development and quality control of caffeine-containing herbal products.

Abstrak

Kafein merupakan salah satu senyawa bioaktif utama dalam biji kopi robusta (*Coffea canephora)* yang memiliki potensi aplikasi dalam formulasi farmasi dan kosmetik, khususnya untuk stimulasi pertumbuhan rambut. Penelitian ini bertujuan untuk menentukan kandungan kafein dalam ekstrak etanol biji kopi robusta menggunakan spektrofotometri UV-Visible. Penelitian ini dilakukan dengan menggunakan desain laboratorium eksperimental. Ekstrak biji kopi robusta diperoleh dengan maserasi menggunakan etanol sebagai pelarut. Identifikasi kualitatif kafein diikuti dengan analisis kuantitatif menggunakan spektrofotometri UV-Vis pada panjang gelombang maksimum 273 nm. Kurva kalibrasi dibuat menggunakan larutan kafein standar, dan kandungan kafein ekstrak dihitung berdasarkan persamaan regresi linier. Hasil menunjukkan bahwa kurva kalibrasi menunjukkan linearitas yang baik dalam rentang konsentrasi yang diuji. Analisis kuantitatif mengungkapkan bahwa kandungan kafein ekstrak etanol biji kopi robusta adalah 221,58 mg/g ekstrak, yang konsisten dengan kadar kafein yang dilaporkan sebelumnya pada Coffea canephora. Temuan ini menegaskan bahwa biji kopi robusta merupakan sumber kafein alami yang signifikan dan mendukung potensi pemanfaatannya sebagai bahan baku untuk produk farmasi dan kosmetik. Studi ini memberikan data kuantitatif yang dapat berfungsi sebagai dasar ilmiah untuk pengembangan formulasi lebih lanjut dan pengendalian mutu produk herbal yang mengandung kafein.

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INTRODUCTION

Robusta coffee (*Coffea canephora*) is one of the most widely cultivated coffee species in Indonesia and plays an important role in the agricultural, pharmaceutical, and cosmetic industries. Compared to arabica coffee, robusta coffee is characterized by higher caffeine content, stronger flavor profile, and greater adaptability to tropical lowland

climates. Previous studies have reported that the caffeine content of robusta coffee beans ranges from 1.7–4%, which is considerably higher than that of arabica coffee.[1,2] This characteristic makes robusta coffee a promising natural source of caffeine for pharmaceutical and cosmetic applications requiring standardized bioactive compounds. Caffeine is a naturally occurring methylxanthine alkaloid with various biological and pharmacological activities, including central nervous system stimulation, antioxidant activity, lipolytic effects, and stimulation of hair follicle growth. Due to these pharmacological properties, caffeine has been extensively utilized as an active ingredient in pharmaceutical, nutraceutical, and cosmetic formulations.[3,4]

In recent years, increasing interest has been directed toward the use of naturally derived caffeine as an alternative to synthetic compounds, particularly in topical and hair care preparations. Several studies have demonstrated that caffeine can penetrate the skin barrier and exert biological effects on hair follicles by prolonging the anagen phase and inhibiting androgen-induced follicular miniaturization.[5,6,7] These findings indicate that robusta coffee beans possess considerable potential as a natural raw material for the development of caffeine-based pharmaceutical and cosmetic products.

The effectiveness, safety, and quality consistency of caffeine-containing products are strongly influenced by the concentration of active compounds present in the raw materials. Therefore, accurate determination of caffeine content in coffee bean extracts is essential for quality control, dosage standardization, and formulation development. Various analytical techniques have been employed for caffeine determination, including high-performance liquid chromatography (HPLC), gas chromatography, and capillary electrophoresis.[8] Although these methods provide high sensitivity and specificity, they generally require sophisticated instrumentation, complicated sample preparation procedures, and relatively high operational costs, which may limit their application in routine laboratory analysis, particularly in developing research facilities. Among various extraction solvents, ethanol is widely used for the extraction of plant bioactive compounds because of its relatively low toxicity, good extraction capability, ease of evaporation, and compatibility with pharmaceutical and cosmetic preparations.[9,10] In addition, ethanolic extraction is considered more suitable for obtaining semipolar compounds such as caffeine while maintaining extract safety for further formulation development. However, despite the extensive cultivation of robusta coffee in Indonesia, studies evaluating caffeine content specifically in ethanolic extracts of robusta coffee beans remain limited.

UV-Visible spectrophotometry offers a practical alternative for routine caffeine analysis due to its simplicity, rapid analytical process, cost-effectiveness, and adequate sensitivity for quantitative determination.[8,11,12] Caffeine exhibits characteristic absorbance in the ultraviolet region, enabling quantitative analysis using calibration curve-based measurements.[4] Although numerous studies on caffeine determination have been reported, most investigations focus on brewed coffee beverages or utilize advanced chromatographic techniques such as HPLC. Limited studies have specifically investigated the determination of caffeine content in ethanolic extracts of robusta coffee beans using UV-Visible spectrophotometry, particularly for potential pharmaceutical and cosmetic applications. This condition indicates the existence of a research gap regarding the development of simple, accessible, and economical analytical methods for standardization of natural caffeine sources derived from robusta coffee. Therefore, this study aimed to determine the caffeine content in ethanolic extract of robusta coffee beans (*Coffea canephora*) using UV-Visible spectrophotometry. The findings of this study are expected to provide scientific data supporting the utilization of robusta coffee bean extract as a standardized natural source of caffeine and to contribute to the development of quality control methods for pharmaceutical and cosmetic formulations containing natural caffeine.

METHODS

Materials

Robusta coffee beans (*Coffea canephora*) were obtained from local coffee plantations in Indonesia and authenticated based on their morphological characteristics. The chemicals used in this study included ethanol 96% (analytical grade), caffeine standard, chloroform, distilled water, and sodium carbonate. All reagents used were of analytical grade.[9,10,13]

Instruments

The instruments used in this study included a UV-Visible spectrophotometer, analytical balance, rotary evaporator, blender, glassware, volumetric flasks, micropipettes, Whatman filter paper, water bath, and measuring cylinders.[9,10,13]

Preparation of Robusta Coffee Bean Powder

Fresh robusta coffee beans were cleaned to remove impurities and dried at room temperature. The dried coffee beans were then ground using a blender to obtain a fine powder. The powder was sieved to obtain uniform particle size and stored in an airtight container until further use.

Extraction of Robusta Coffee Beans

A total of 500 g of robusta coffee bean powder was extracted using the maceration method with 96% ethanol as solvent. The powder was soaked in ethanol with a ratio of 1:10 (w/v) and allowed to stand for 72 hours at room temperature with occasional stirring every 24 hours to maximize extraction efficiency. The filtrate obtained from maceration was filtered using Whatman filter paper. The residue was re-macerated twice using fresh solvent to ensure complete extraction of bioactive compounds. All filtrates were combined and concentrated using a rotary evaporator at 50°C under reduced pressure to obtain a thick ethanolic extract. The extract was then weighed to determine extraction yield and stored in a closed container prior to analysis.[9,10] The extraction yield was calculated using the following equation:

$$\text{Yield (\%)} = \frac{\text{Weight of extract}}{\text{Weight of simplicia}} \times 100$$

Preparation of Standard Caffeine Solution

A stock solution of caffeine standard was prepared by dissolving 100 mg of pure caffeine in distilled water and diluting to 100 mL in a volumetric flask to obtain a concentration of 1000 ppm. Working standard solutions were prepared from the stock solution at concentrations of 2, 4, 6, 8, and 10 ppm by appropriate dilution using distilled water.

Determination of Maximum Wavelength

The maximum wavelength of caffeine was determined by scanning the standard caffeine solution at a concentration of 10 ppm using a UV-Visible spectrophotometer within the wavelength range of 200–400 nm. The wavelength showing maximum absorbance was selected as the analytical wavelength for quantitative determination.

Preparation of Calibration Curve

The absorbance of standard caffeine solutions at concentrations of 2, 4, 6, 8, and 10 ppm was measured at the determined maximum wavelength. A calibration curve was constructed by plotting concentration versus absorbance values, and the linear regression equation was calculated.[8,11,12,13]

Determination of Caffeine Content in Ethanolic Extract

A total of 100 mg of ethanolic extract was dissolved in distilled water and transferred into a 100 mL volumetric flask. The solution was filtered to remove insoluble particles. The absorbance of the sample solution was measured using UV-Visible spectrophotometry at the determined maximum wavelength. The caffeine concentration in the extract was calculated using the regression equation obtained from the calibration curve. [8,11,12,13] The caffeine content was calculated using the following equation:

$$\text{Caffeine content (\%)} = \frac{C \times V \times FP}{W} \times 100$$

where:

- C = concentration obtained from calibration curve (mg/mL)
- V = sample volume (mL)
- FP = dilution factor
- W = sample weight (mg)

Data Analysis

All measurements were performed in triplicate, and the results were expressed as mean ± standard deviation (SD). Linear regression analysis was used to evaluate the correlation between concentration and absorbance values of caffeine standard solutions.[14]

RESULT AND DISCUSSION

Determination of Maximum Wevelenghth and Calibration Curve

The determination of caffeine content in the ethanolic extract of robusta coffee beans (*Coffea canephora*) was carried out using UV-Visible spectrophotometry following method optimization and calibration procedures. Determination of the maximum wavelength (λmax) is an important step in spectrophotometric analysis because it provides optimum sensitivity and analytical accuracy.[13] The scanning results showed that caffeine exhibited maximum absorbance at 273 nm, which is consistent with previous studies reporting that caffeine absorbs strongly in the ultraviolet region at approximately 270–275 nm due to the presence of conjugated chromophore groups in its molecular structure.[8,11,13]

The calibration curve was prepared using standard caffeine solutions at concentrations of 2, 4, 8, and 12 ppm. The absorbance values obtained for each concentration were 0.248, 0.362, 0.625, and 0.882, respectively. The increase in absorbance values was proportional to the increase in concentration, indicating compliance with Beer-Lambert's law within the tested concentration range.[13] The linear regression equation obtained from the calibration curve was:

$$y = 0.064x + 0.111$$

with correlation coefficient:

$$R^2 = 0.992$$

The high correlation coefficient indicates good linearity and demonstrates that the analytical method possesses acceptable reliability for quantitative caffeine determination. Good linearity is an important parameter in analytical validation because it reflects the proportional relationship between analyte concentration and instrument response (8,14).

Table 1. Results of Caffeine Determination in Ethanolic Extract of Robusta Coffee Beans

Parameter	Value
Maximum wavelength (nm)	273
Standard concentration range (ppm)	2, 4, 8, 12
Absorbance of standards	0.248; 0.362; 0.625; 0.882
Sample concentration (ppm)	50
Sample absorbance	0.812
Calculated caffeine concentration (mg/L)	11.079
Caffeine content (mg/g extract)	221.58

Determination of caffeine Content in Ethanolic Extract

The ethanolic extract of robusta coffee beans was prepared at a concentration of 50 ppm and analyzed at the selected wavelength of 273 nm. The sample absorbance obtained was 0.812. Based on the regression equation from the calibration curve, the caffeine concentration in the sample solution was calculated to be 11.079 mg/L. Further calculation considering dilution factor and sample weight revealed that the caffeine content in the ethanolic extract reached 221.58 mg/g extract.

The relatively high caffeine content observed in this study is consistent with previous reports indicating that *Coffea canephora* generally contains higher caffeine concentrations than *Coffea arabica*.[1,2] Robusta coffee is known to naturally synthesize higher levels of caffeine as part of its defense mechanism against pests and environmental stress.[3] This characteristic contributes to the stronger bitterness and stimulant effects commonly associated with robusta coffee products.

Several factors may influence caffeine content in coffee extracts, including geographical origin, cultivation conditions, altitude, harvesting process, roasting conditions, extraction method, solvent polarity, and post-harvest treatment.[1,3,8] Environmental factors such as soil composition, rainfall, and temperature may also affect alkaloid biosynthesis in coffee plants. Therefore, variations in caffeine content between studies are commonly reported in the literature.

The use of ethanol as extraction solvent in this study was considered appropriate because ethanol possesses semipolar properties that enable efficient extraction of alkaloid compounds such as caffeine. In addition, ethanol is relatively safe, environmentally friendly, easy to evaporate, and widely accepted in pharmaceutical and cosmetic preparations.[9,10] The maceration method used in this study also allowed sufficient contact between solvent and plant material, thereby facilitating extraction of bioactive compounds from robusta coffee beans.[9]

From an analytical perspective, the findings demonstrate that UV-Visible spectrophotometry provides a practical and reliable approach for caffeine determination in coffee extracts. Compared with chromatographic techniques such as HPLC or gas chromatography, UV-Vis spectrophotometry offers advantages including simpler sample preparation, shorter analysis time, lower operational costs, and broader accessibility in routine laboratory settings.[8,11,12] Although chromatographic methods provide higher specificity and sensitivity, UV-Vis spectrophotometry remains highly useful for preliminary quantitative analysis and quality control applications.

The quantified caffeine content obtained in this study also indicates the potential utilization of robusta coffee bean extract as a natural active ingredient in pharmaceutical and cosmetic formulations. Previous studies reported that caffeine can stimulate hair follicle proliferation, prolong the anagen phase, and inhibit androgen-induced follicular miniaturization, thereby supporting its application in hair growth-promoting formulations such as hair tonics, serums,

and emulgels.[4-7] Therefore, the quantitative data generated in this study may serve as a scientific basis for dosage standardization and formulation development of caffeine-containing topical products derived from natural sources.

Overall, the results demonstrate that UV-Visible spectrophotometry is a suitable, reliable, and economical analytical method for the determination of caffeine content in ethanolic extracts of robusta coffee beans. The findings of this study contribute valuable scientific data supporting the standardization and utilization of *Coffea canephora* as a natural source of caffeine for pharmaceutical and cosmetic applications.

LIMITATION

Limitation This study was limited to the use of UV-Visible spectrophotometry, which may be less specific compared to chromatographic techniques. Future studies are recommended to employ more advanced analytical methods for validation and comparison.

CONCLUSION

This study successfully determined the caffeine content in ethanolic extract of robusta coffee beans (*Coffea canephora*) using UV-Visible spectrophotometry. Caffeine showed maximum absorbance at 273 nm with good linearity of the calibration curve. The caffeine content obtained was 221.58 mg/g extract, indicating that robusta coffee is a potential natural source of caffeine. The results also demonstrated that ethanolic extraction combined with UV-Visible spectrophotometry is a simple, reliable, and cost-effective method for caffeine determination. These findings provide scientific support for the standardization and further development of robusta coffee extract in pharmaceutical and cosmetic applications.

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AUTHOR CONTRIBUTION STATEMENT

DM: Conceptualization, Methodology, Investigation, Writing—Original Draft Preparation; **VP:** Supervision, Writing—Review and Editing.

CONFLICT OF INTEREST DECLARATION

The authors declare no conflict of interest.

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