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Effect of Extraction and Centrifugation on Caffeine Levels in Urine of Robusta Coffee Drinkers Using the Method of Uv-Vis Spectrophotometry

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ABSTRACT

Caffeine is a heterocyclic alkaloid compound in the methylxanthine group that has a bitter taste that works as a mild diuretic and psychoactive stimulant. These compounds are naturally found in more than 60 types of plants, including coffee plants. Coffee is known as a drink that has a high enough caffeine content. Caffeine content in robusta coffee raw beans is higher than Arabica coffee which is only about 1.2%. The content of caffeine in the body is broken down in the liver by the enzyme cytochrome P-450 into metabolic 3-dimethylxanthin which is then excreted in the urine. Analysis of caffeine in urine was carried out quantitatively by means of UV-Vis Spectrophotometry. The analysis process is carried out by extraction and centrifugation. Based on this study, a study was conducted to determine the differences in extraction and centrifugation methods on caffeine levels in the urine of robusta coffee drinkers using the UV-Vis spectrophotometric method. The results were analyzed using the Paired T-Test through SPSS. The results showed that there was significant difference in the levels of caffeine extracted and centrifuged in the urine of robusta coffee drinkers using the UV-Vis Spectrophotometry method. The caffeine content in the urine sample was obtained with the lowest extraction at 29.60 ppm and the highest caffeine content at 50.18 ppm. Meanwhile, in urine samples by centrifugation, the lowest caffeine content was 35.71 ppm and the highest caffeine content was 50.45 ppm.

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INTRODUCTION

Coffee is one of the plantation crops that has a high economic value and has long been cultivated. Coffee plants are a species of woody tree plants that belong to the Rubiaceae family and the genus Coffea (Rahmawati, 2018). Most of Indonesia's coffee production and exports are dominated by robusta coffee types with the number of cultivated plants reaching 90%. It is not surprising that the need for superior clone Robusta coffee seeds continues (Ibrahim and Sri, 2017).

From various circles of society, coffee is one of the most popular drinks. Coffee is currently the world's most preferred drink after water and tea (Cornelis, 2019). The results of the study mentioned that few nutrients are contained in coffee, but there are more than thousands of natural chemicals such as carbohydrates, lipids, nitrogen compounds, vitamins, minerals, alkaloids and phenolic compounds. Some of these are potentially healthy and some are potentially harmful. One of the potentially harmful alkaloid

compounds for health is caffeine (Spiller in Wachamo, 2017). The caffeine content in coffee is known to be quite high (Muhibatul, 2014).

Caffeine is an organic compound that has other names "caffeine", "tein", or 1,3,7-trimethylxanthine. Caffeine crystals in water are luminous needles. When not containing water, caffeine melts at 234°C - 239°C and sublimes at lower temperatures. Caffeine has a similar chemical structure with three alkaloid compounds, namely xanthin, theophylline, and theobromine C8H10N4O (Abraham, 2010). Caffeine is known to have benefits when consumed by humans and also has adverse effects on the body if consumed during certain body conditions and in high levels of caffeine. Caffeine consumption is useful for increasing alertness, eliminating drowsiness and improving mood. Caffeine also aids physical performance by increasing endurance and enhancing muscle contraction (Ennis, 2014).

In addition to positive effects, excessive caffeine use can cause addiction if consumed in large quantities and regularly (Wilson, 2018). Excessive caffeine consumption can also cause tooth discoloration, bad breath, increased stress and blood pressure if consumed in the morning, insomnia, heart attack, stroke, male infertility, indigestion, addiction and even premature aging (Farida et al., 2013). Furthermore, excessive caffeine consumption can have negative effects such as abnormal heartbeat, headaches, anxiety, tremors, restlessness and memory loss (Özpalas and Özer, 2017). Therefore, it is highly recommended to consume caffeine at permissible levels. According to SNI 01-7152-2006, the maximum limit for consuming caffeine either directly or mixed in food or beverages is 150 mg/day or 50 mg/serving.

It is known that as much as 99% of caffeine has an absorbance time of 45 minutes after the caffeine is consumed, 20% at the digestive level and most of it is absorbed in the small intestine. In the study of Alsabri et al., (2018) stated that caffeine has a volume of distribution of 0.5-0.75 L/kg, and does not show accumulation in many specific tissues. It is added by other sources that caffeine accumulates in the body within a few hours after consumption (Wanyika, 2010).

In previous studies, the determination of caffeine levels in coffee has been carried out by the extraction process. Caffeine levels will be higher the longer the extraction time on the sample. The extraction process is necessary because this process is a process of withdrawing soluble chemical content so that it is separated from soluble materials with a liquid solvent (Directorate General of POM, 2000).

The rapid and reliable measurement of caffeine demands a method that is simple, easy to handle and inexpensive. UV spectrophotometry with its various constituents is a clear alternative to existing methods. The UV Spectrophotometric method is one of the simple, rapid, and validated methods for the estimation of caffeine in drugs, serum, and urine that shows stability. The method is based on measuring the native absorption of caffeine in acidified alcohol. The method proved to be sensitive, selective, accurate and precise (Kumar et al., 2019).

Based on the description above, the need for research by taking the title "Differences in Extraction and Centrifugation Methods on Caffeine Levels in Urine of Robusta Coffee Drinkers Using UV-Vis Spectrophotometric Method" to determine whether there are differences in caffeine content in biological fluid samples (urine). The purpose of this study was to determine the differences in extraction and centrifugation methods on caffeine levels in the urine of robusta coffee drinkers using the uv-vis spectrophotometric method.

METHODOLOGY

The type of research design used is descriptive experimental research using a UV-Vis Spectrophotometer as a quantitative test. The sampling location is at Putri Cempo cafe, Kebomas District, Gresik Regency, East Java 61124. This research begins with a survey of coffee shops with more than 20 visitors per day. Based on the survey results there are about 16 coffee shops that provide robusta coffee (filter coffee) and arabica coffee (packaged coffee). Furthermore, 16 samples were taken from the urine of visitors who were willing to be respondents and filled out an informed consent form by previously fulfilling the requirements provided, namely not consuming caffeinated food / drinks for two days.

Tools and Materials

The tools used in this study are PC UV-1600 Spectrophotometer with 10 mm quartz cells. Other equipment are analytical balance (adventurer OHAUS), volumetric flask (volume 100 ml, 25 ml, 10 ml), push ball, beaker, dropper, 250 ml volume separatory funnel, stirring rod, volume pipette (1 ml, 2 ml, 3 ml, 4 ml, 5 ml), erlenmeyer (volume 100 ml), centrifuge, hotplate, porcelain cup, pH meter, watch glass, test tube and tube rack.

Preparation of Carbonate Buffer

Weighing 8.4 mg of sodium bicarbonate (NaHCO3) and 10.6 mg of sodium carbonate (Na2CO3) and then added with 250 ml of distilled water, then adjusted to pH 9.7 with sodium hydroxide (NaOH).

Preparation of Standard Solution of Caffeine 100 ppm

Weighing as much as 0.1 gram of standard caffeine was put into a beaker and dissolved with 30 mL of sour alcohol. Then the solution was put into a 100 mL volumetric flask, enough with alcohol acid to the limit mark so that the concentration of this solution became 1000 ppm. Next, take 10 mL of 1000 ppm solution and then put it into a 100 mL volumetric flask, enough with acidic alcohol to the limit mark so that a 100 ppm solution is obtained.

Sample Preparation

Samples taken from respondents were urine at one time that was not specifically determined. Urine samples were taken after the respondent consumed coffee, before which the respondent was not allowed to consume caffeinated food / drinks for two days. Then the urine is collected in a sterile urine pot container with a volume of about 10-20 ml. After being collected, the next process was carried out, namely extraction and without extraction of caffeine levels (Gandasoebrata, 2007).

Determination of Maximum Wavelength

A total of 10 mL of 100 ppm standard solution was pipetted into a 25 mL volumetric flask, then dissolved with acidic alcohol until the limit mark, so that a standard solution of 40 ppm was obtained. Then the absorbance was measured using a UV spectrophotometer at a wavelength of 200-400 nm. Caffeine detection was carried out using acidified alcohol as a blank.

Preparation of Caffeine Standard Curve

The standard curve was prepared by pipetting as much as 2.0; 2.5; 3.0; 3.5; 4.0; 4.5 and 5.0 mL of 100 ppm caffeine standard solution and diluted in a 10 mL volumetric flask so that the concentration of the standard solution obtained was 20, 25, 30, 35, 40, 45 and 50 ppm respectively. Furthermore, this caffeine standard solution was measured for absorbance using a UV-Vis spectrophotometer at a wavelength of 275 nm (maximum wavelength). Concentrations were read from the respective standard curves or calculated from regression equations derived using Beer's law data.

Determination of Caffeine Level in Urine of Coffee Drinkers

Extraction

The extraction process carried out in this study is to take 5 ml of urine sample pipetted and put into a separating funnel, then added with 5 ml of diethyl ether, 5 ml of carbonate buffer (pH 9.7) and extracted by gently shaking for 15-20 minutes. After standing for a while, two layers will form, namely the top layer and the bottom layer. The upper layer is a non-aqueous aliquod ether which is accommodated, while the aqueous lower layer is added with 5 ml of diethyl ether for re-extraction. The extraction procedure was carried out three times and the resulting ether layers were combined and then evaporated at room temperature.

Centrifugation

The centrifugation process is carried out by pipetting 5 ml of urine sample into a test tube and then centrifuged for 15 minutes at 3000 rpm. After standing for a while, two layers will form, namely the top layer (supernatant) whose color is clearer and the bottom layer (sediment) is more turbid in color. The supernatant is added with 5 ml of acid alcohol which will then be used in the next stage of analysis, namely the absorbance test on a spectrophotometer.

The residue resulting from the extraction process is then dissolved with 5 ml of acidic alcohol in a 10 ml volumetric flask until the limit mark, the same thing is done with the centrifuged urine supernatant. Subsequently, it was inserted in a UV cuvette and the absorbance was read using a Spectrophotometer. The above mentioned procedure was then followed to analyze caffeine. The nominal caffeine content was estimated from the corresponding regression equation (Kumar et al., 2019).

Data Analysis

Data analysis in this study was carried out by statistical calculations calculated using SPSS (Statistical Product Services Solution) software version 16, one way to determine parametric and non-parametric tests is to conduct a normality test. The normality test was carried out with the Kolmogorov-Smirnov test. In this study, the data were normally distributed so that statistical calculations continued using parametric tests, namely using the Paired Sample T-Test.

RESULTS

Descriptive statistical analysis was carried out on the variables used in this study, namely Leverage, Profitability, Dividend Policy and Stock Price. The description of each variable of this study is from the food and beverage industry listed on the Indonesia Stock Exchange during the 2021-2023 period which is explained in the descriptive statistical analysis as follows:

This study began with the collection of urine samples from respondents who were willing to be treated by consuming 250 ml of robusta coffee drinks for each of the 16 respondents. After 45-60 minutes each respondent was asked to urinate. It is known that caffeine accumulates in the body with a period of 45 minutes after the caffeine is consumed (Wanyika, 2010). Urine samples were selected in this study because caffeine is reabsorbed in the renal tubules and excreted through urine (Alsabri et al., 2018).

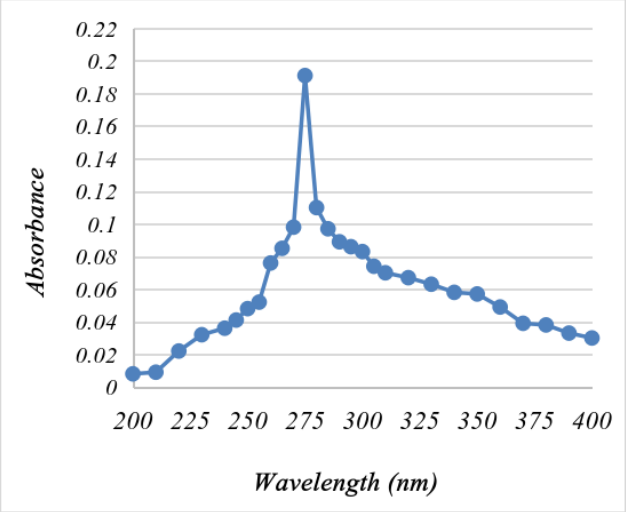

Figure 1 Graph of Maximum Wavelength Determination

The study was conducted by determining the maximum wavelength using UV-Vis spectrophotometry. Based on the measurement results, the maximum wavelength was 275 nm with an absorbance of 0.191 as shown in table 5.1. This is in accordance with what was reported by Egan (1981), in Fitri (2008) that the absorbance is in the maximum wavelength range of 272-276 nm.

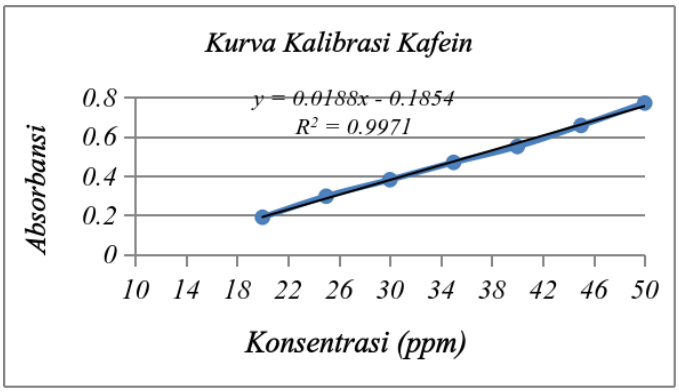

Figure 2 Caffeine Solution Standard Curve

Furthermore, the determination of caffeine standard curve with consecutive concentrations of 20 ppm; 25 ppm; 30 ppm; 35 ppm; 40 ppm; 45 ppm; and 50 ppm. The measurement results of the standard standard curve are shown in table 5.2, where the absorbance is 0.189; 0.296; 0.380; 0.468; 0.549; 0.656; and 0.770, respectively. The absorbance value of the light passed is proportional to the concentration of the solution in the cuvette. From the data, the regression equation y = 0.0188x - 0.1854 was obtained with a value of R2 = 0.9971. The value of the correlation coefficient that meets the requirements is ≥ 0.9970 and ≤ 1. The higher the concentration of a compound in the solution, the more light is absorbed. This is in accordance with Lambert Beer's Law.

Table 1. Caffeine Content of Urine Samples

Jenis Sampel	Kode Sampel	Absorbansi	Kadar Kafein (ppm)
Ekstraksi	U A	0,652	44,54
	U B	0,675	45,77

	U C	0,692	46,67
	U D	0,758	50,18
	U E	0,419	32,15
	U F	0,522	37,63
	U G	0,457	34,17
	U H	0,371	29,60
Sentrifugasi	U 1	0,747	49,60
	U 2	0,624	43,05
	U 3	0,674	45,71
	U 4	0,763	50,45
	U 5	0,664	45,18
	U 6	0,593	41,40
	U 7	0,486	35,71
	U 8	0,506	36,78

Determination of caffeine levels in urine samples carried out by extraction is useful for the withdrawal or separation of a compound with its mixture with the help of a solvent. The extraction method used in this study is liquid-liquid extraction. The mixture of carrier fluid and solvent is heterogeneous, if separated there will be two phases, namely the diluent phase (raffinate) and the solvent phase (extract). In this extraction, the solute is separated from the carrier liquid (diluent) using a liquid solvent (Wibawa, 2012). Therefore, it is important to use a solvent with the appropriate polarity. Based on table 5.3, it can be seen that the determination of caffeine levels in urine samples with the lowest extraction method is 32.15 ppm (mg/L) and the highest caffeine level is 50.18 ppm (mg/L).

Determination of caffeine levels in urine samples carried out by centrifugation is useful for the separation of a compound using centrifugal force as a driving force. This separation is done based on the difference in mass density of a liquid. Two liquids separated in this way will usually form two emulsified liquid phases (Djauhari, 2010). Table 5.3 shows the results of the determination of caffeine levels in urine samples by centrifugation, the lowest is 35.71 ppm (mg/L) and the highest caffeine level is 50.45 ppm (mg/L).

CONCLUSION

From this study it can be concluded that caffeine levels in urine samples of robusta coffee drinkers with the lowest extraction method is 32.15 ppm (mg/L) and the highest caffeine level is 50.18 ppm (mg/L). While the results of the determination of caffeine levels in urine samples by centrifugation the lowest is 35.71 ppm (mg/L) and the highest caffeine level is 50.45 ppm (mg/L).

There is a significant difference between extraction and centrifugation methods on caffeine levels in urine samples of robusta coffee drinkers using the UV-Vis spectrophotometric method.

RECOMMENDATION

Based on the research that has been done, it is suggested that future researchers can analyze the differences in extraction methods and centrifugation on the effect of extracting caffeine levels in the urine of robusta coffee drinkers with other more suitable solvents.

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