Dampit Robusta coffee leaf tea (*Coffea canephora*) potential for kidney stone therapy

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Abstract. Robusta coffee leaf (*Coffea canephora*) is part of coffee plants that have not been utilized optimally. Coffee leaves contain flavonoids, alkaloids, caffeine, saponins, and other polyphenols that may contribute to prevent degenerative diseases such as kidney stone disease. This study aims to determine the dissolved calcium oxalate levels in-vitro as affected by coffee leaf tea. The experiment was conducted by Completely Randomized Block Design with 2 factors; namely leaf type (old non-oxidative leaf, old oxidative leaf, young non-oxidative leaf, young oxidative leaf) and the second factor was the concentration of coffee leaf tea powder (1%, 3%, 5%). The results showed that differences in leaf type and concentration of coffee leaf tea powder significantly affected the decay of calcium oxalate. The non-oxidative old leaf types with concentration of 5% shows the highest ability to dissolve calcium oxalate.

1. Introduction

Robusta coffee leaf (*Coffea canephora*) is part of the coffee plant that has not been fully utilized. According to data from [1] levels of coffee production in East Java in 2014-2016 reached 8.393 tons, mostly from Malang, Dampit area. Coffee plants to be pruned regularly, where each pruning will be leaves that is not utilized. The content of phenolic compounds in the leaves of Robusta coffee is much higher than Arabica coffee leaves [1] Robusta coffee leaf contains flavonoids that act as antioxidants and there are also other compounds such as alkaloids, saponins, and other polyphenols that have health benefits that can prevent degenerative disease. [2].

One of the degenerative disease, often experienced by people in Indonesia is kidney stone disease. Kidney stone is caused by deposition of excess mineral in the body. Kidney stones are composed of different constituent content, there were 70-80% of kidney stones are composed of calcium oxalate, the remaining 10% is composed of struvite, 10% are composed of uric acid, and less than 1% of kidney stones are composed of cysteine [2]. Coffee leaves contain flavonoid that can help to reduce the risk of kidney stones in which the OH groups contained in flavonoids will bind to calcium on the calcium oxalate to form a chelate complex between the water soluble and excreted through the kidneys [2].

The purpose of this study was to know the effect of leaf type and concentration of coffee leaf tea against the decay of calcium oxalate. This study is expected to be another alternative for the treatment of kidney stones by examining the effects of coffee leaves tea in dissolving kidney stones, especially calcium oxalate in vitro by measuring the levels of soluble calcium using atomic absorption spectrophotometry.
2. Materials and methods

2.1 Tools and materials
Materials used in this research are coffee leaf tea, CaCl2, (NH4) 2C2O4 (PA), distilled water, standard solution 1000 ppm Ca, 0.2 mM DPPH, methanol, Folin-ciocalteau, NaNO2, 1M NaOH, AlCl3, Na2CO3, standards gallic acid, and quercetin.

The tools used in this study are cabinet dryer, oven, electric cooker, thermometer, desiccator, a pH meter, analytical balance, uv-vis spectrophotometer, vortex, atomic absorption spectrophotometer variants with Ca cathode lamps, colour reader.

2.2 Methods
Data analysis method used was a completely random block design with two factors of types of leaves with 4 levels (young leaves oxidative, young leaves non-oxidative, old leaves oxidative, and old leaves non-oxidative) and the second factor is the concentration of powdered tea from coffee leaf (1 %, 3% and 5%). Observation is done by soaking a 100 mg calcium oxalate into 100 mL of coffee leaf tea according to the factor and incubated at 37°C for 3 hours with shaking every 20 minutes for 5 minutes using a shaker of 60 rpm speed. The outcome of the incubation process is soluble and insoluble calcium oxalate. Both parts were separated by decantation so the dissolved calcium oxalate in the filtrate can be analysed using atomic absorption spectrophotometer to determine the concentration of dissolved calcium, whereas insoluble calcium oxalate dried in an oven and weighed. The responses were observed by physic-chemical analysis, including colour, total phenolic content, total flavonoids, antioxidant activity, and solubility of calcium with SSA.

2.2.1 Total flavonoid analysis. Total flavonoid analysis was carried out by weighing 0.025 grams of sample which was then treated with a solvent in a 5 ml volumetric flask to obtain a stock solution of 5000 mg/ml. One ml of solution was added by 0.5 ml of 5% NaNO2 solution then mixed by vortex and incubated for 5 minutes. Afterward, 0.5 ml of 10% AlCl3 solution was added and mixed well by vortex and waited for 6 minutes. The solution of NaOH (1 M, 5mL) was also added and mixed by vortex. Then incubated for 15 minutes at room temperature in dark conditions. The absorbance was measured at a wavelength of 447 nm. Then calibrated with the standard curve equation to obtain the total flavonoids.

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\text{Total Flavonoid} = \frac{\text{Quercetin equality x sample volume x dilution factor}}{\text{Sample mass}}
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2.2.2 Total phenolic content analysis. Total phenolic content analysis was conducted by first taking 0.5 ml of sample solution and mixing with 2.5 ml of Folin-ciocalteau reagent (diluted 1:10). The solution was mixed by vortex and waited for 5 minutes before the next steps. Afterwards 4 ml of 7.5% Na2CO3 was added. The solution was mixed by vortex again and allowed to stand for 30 minutes in a dark room. The absorbance with a wavelength of 765 nm was measured by spectrophotometer. The readings were plotted against gallic acid standard curves to obtain total phenolic compound in mg GAE / L.

2.2.3 pH analysis. The pH is analysed by means of a homogeneous sample taken as much as 30 ml and placed in a 50 ml glass beaker. Previously, the pH meter was calibrated with a buffer of pH 4 and pH 7 first. Then the mouth of the pH meter is dipped into the sample and the pH of the sample is measured around 10 minutes.

2.3 Research design
The experiment was conducted by Completely Randomized Block Design with 2 factors; namely leaf type (old non-oxidative leaf (T1), old oxidative leaf (T2), young non-oxidative leaf (T3), young oxidative leaf (T4)) and the second factor was the concentration of coffee leaf tea powder (1%, 3%, 5%),
as described in Table 1. The combination of two factors of leaves and coffee leaf tea powder concentration was conducted on 17 Minitab software application program.

| Leaf type | Concentration Coffee Leaf Tea Powder |
|-----------|--------------------------------------|
|           | K1 (1%)  | K2 (3%)  | K3 (5%)  |
| T1        | T1K1     | T1K2     | T1K3     |
| T2        | T2K1     | T2K2     | T2K3     |
| T3        | T3K1     | T3K2     | T3K3     |
| T4        | T4K1     | T4K2     | T4K3     |

3. Results and discussion

3.1 Total phenolic content

Figure 1 shows that the lowest average of total phenol obtained from young leaves oxidative concentration of 1%, while the highest average of total phenol obtained from the old leaves non-oxidative concentration of 5%. The older the leaves and the more concentration is used, there will be an increase in total phenol. On the leaves undergo oxidation in line with the increase in concentration, the total yield of phenol will be lower than the leaves that do not undergo oxidation.

The reduction in total phenol affected by catechin's reduction with increasing time of withering or oxidation through activity of the enzyme polyphenol oxidase [3]. The content of total phenols are also affected by the age of leaves where the old coffee leaf contains phenol levels higher than young leaves, because the old leaves have higher sensitivity defence against pests, so the production of phenolic compounds on old leaves is higher than in young leaves [4]. The concentration of the tea powder affects the total phenol. The higher the concentration used, there is increase tendency of total phenol. Polyphenols are compounds that are soluble in water, the higher the concentration of powdered tea used, the more also polyphenols extracted [5].
3.2 Total Flavonoids

Figure 2 shows that the lowest total flavonoids average is young leaves oxidative at a concentration of 1%, while the highest average total flavonoids is old leaves non-oxidative concentration of 5%. The older the leaves and more concentration used, will increase the total flavonoids. Older leaves have higher total flavonoids than young leaves. The factors that affects the level of total flavonoids in the leaf is leaf morphology and age, which will affect the secondary metabolites and bioactive compounds produced [6]. Figure 2 shows the higher concentration of coffee leaf tea powder, the higher total flavonoid produced. This is caused by the more the concentration of the tea leaf powder, water-soluble flavonoid compounds such as catechins, which are more dissolved in water steeping tea [7]. Similarly, total phenols, factors that affect the number of total flavonoids in the samples are leaf morphology and age will affect bioactive secondary metabolites and the resulting compound [6]. Types of flavonoids found in coffee leaves is catechins, quersetin, and kaempferol [8].

3.3 pH

Figure 3 shows that the highest the concentration of the sample used, there is a downward trend in pH values, and the oxidative leaf samples have a lower pH of the non – oxidative leaf. pH value of an old non-oxidative leaf is higher than the other. Boiling gives effect to the increasing number of components are extracted as theaflavin and thearubigin. It is appropriate where the higher the concentration, the higher the extracted components such as theaflavin and thearubigin that causes tea to become more acidic and dense [9].

Based on Figure 3 can be seen that the higher the concentration of the sample used, there is a downward trend in pH values, and the oxidative leaf samples have a lower pH of the non – oxidative leaf. pH value of an old non-oxidative leaf is higher than the other. Boiling gives effect to the increasing number of components are extracted as theaflavin and thearubigin. It is appropriate where the higher the concentration, the higher the extracted components such as theaflavin and thearubigin that causes tea to become more acidic and dense [9].

In the processing of the tea can undergo oxidation polyphenol components that generate theaflavin and thearubigin. The more thearubigin are formed will decrease the pH, because theaflavin is weakly
acidic and thearubigin is strongly acidic [5]. In samples with low pH also had a higher total phenol. This is because at a lower pH, oxidation of catechins are suppressed so that the total phenol in samples that have an acidic pH has higher total phenols than at a higher pH samples. In acidic conditions, the vacuole cell would easily break, so it easily extracts phenolic compounds by solvent [10].

3.4 Measurement of calcium levels

![Figure 4](image.png)

Figure 4. Effect of leaf type and concentration coffee leaf tea to calcium level

Figure 4 shows that the lowest calcium levels obtained from young leaves oxidative concentration of 1%, while the highest calcium levels obtained from the old leaves non-oxidative concentration of 5%. Old leaves non-oxidative is a type of leaf that has the highest concentration of dissolved calcium. The older the leaf, the non-oxidative leaf type increased levels of calcium than other samples. Increased calcium levels indicate the amount of calcium that is dissolved by the sample. Older leaves have a higher content of flavonoid compounds from young leaves that may affect the levels of calcium [11].

The ability of coffee leaf tea to shed calcium oxalate into calcium ions are affected by the flavonoids content. The more flavonoid, the greater calcium will decay. This occurs due to the OH groups contained in the flavonoid compound able to react and bind to calcium in the calcium oxalate and will form a chelate complex compound Ca-flavonoid so that the compound will be soluble in water. Besides the diuretic properties of the flavonoids also help the solubility of the calcium so that add the potential for dissolving calcium oxalate [2]. The active compounds are able to shed calcium kidney stones are flavonoid compounds because they are polar and contains many hydroxyl groups on the aromatic chain flavonoids [12].

4. Conclusions

Result shows that different types of leaves and concentration of coffee leaf tea powder have significant effect on the decay of calcium oxalate. The non-oxidative old leaves with concentration of 5% has the highest ability in the decay of calcium oxalate. Thus, it may suggest that to some extent the coffee leaves tea is potential to be further explored and investigated as functional drink to treat kidney stone.

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