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The Antioxidant Activity of Combined Green Arabica Coffee Bean and Dahu Leaf Extract using DPPH Radical Scavenger Method

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Abstract: Coffee (*Coffea arabica* L.) and Dahu (*Dracontomelon dao* (Blanco) are combined to enrich the types of functional herbal drink with antioxidant properties. Dahu leaves and green coffee beans contain secondary metabolites with antioxidant properties. This research aims to investigate the interaction between the ethanolic extract of coffee (CA) and dahu (DD) and its effect on their antioxidant activity. The antioxidant activity test was performed using DPPH radical scavenging method on 2 control groups and 5 treatment groups, consisting of the CA sample group, the DD sample group, and 3 coffee and dahu (CA: DD) combination groups with different ratios. The research showed that antioxidant activities (%), expressed in mean ± SEM, for the control, DD, CA, CA:DD (1:1), CA:DD (3:1), and CA:DD (9:1) were (80.1 ± 0.4), (65.3 ± 1.5), (37.4 ± 2.4), (47.3 ± 0.4), (34.0 ± 1.4) and (33.4 ± 1.1) respectively. The coffee and dahu combination groups had lower antioxidant activities than single dahu extract, thus indicating an antagonistic interaction between coffee and dahu extracts.

Keywords: *Coffee arabica*, *Dracontomelon dao*, dahu, antioxidant, DPPH

1. Introduction

The bioactive compound in the food we eat can affect the body's health. The process of preserving food often affects the quality of the food itself. Sometimes, this process will decrease the quality due to the oxidation process leading to changes in taste and metabolite composition of each food. However, this change can be prevented by the addition of antioxidants (Sula et al., 2018). Antioxidants are molecules that are capable of suppressing the oxidation of other molecules. In terms of foods, antioxidants were defined as any substance that significantly delays or inhibits the oxidation of another substance even if it presents at a lower concentration than the oxidizable substance (Gulcin, 2020).

Pacific walnut (*Dracontomelon dao* (Blanco) Merr. & Rolfe) or traditionally known as Sengkuang or Dahu, is one of the traditional plants known to have antioxidant properties. Dahu is a woody plant that belongs to the *Anacardia* family and can be easily found in the plains of Malaysia, East India, Thailand, Cambodia, Philippines, Papua New Guinea, and Indonesia (Dapar, 2021). The trunk is classified as commercial wood for household furniture. The fruits and leaves can be eaten or used as seasonings by the local community (Fiqa et al., 2019). As a traditional medicine, Dahu treats dysentery, abdominal pain, and diabetes (Yusro et al., 2014).

Ragasa et al. (2017) reported the isolation of anacardic acid, β-sitosteryl-3β-glucopyranoside-6'-O-fatty acid esters, β-sitosterol, phytol, phytyl fatty acid esters, β-sitosteryl fatty acid esters, chlorophyll a, squalene, long-chain fatty alcohols, and long-chain hydrocarbons from the leaves of *D. dao*. The antioxidant ability of dahu leaves were proven by the presence of polyphenol, flavonoid, and terpenoid with the stronger DPPH radical scavenger ability than curcumin and vitamin E (Novita, 2011 in Zaman, 2021). Methanolic extract of dahu also have the ability to inhibit α-glucosidase in vitro and prevent human colon epithelial FPCK-1-1 cells damage (Yusro et al., 2016)

Another plant that is widely known to have antioxidant properties is coffee. Coffee is one of the most popular non-alcoholic beverages globally, with significantly increased consumption over recent years. The popularity of this beverage is due to its pleasing taste sensation, psychostimulant effect, and appealing beneficial health effects (Dong et al., 2019). The primary cultivated and marketed species of coffee are Arabica (*Coffea arabica*) and Robusta (*Coffea canephora*). Still, Arabica coffee is preferred since it has lower bitterness and better aroma characteristics. Arabica coffee provides more than 95% of coffee worldwide, although Robusta dominates the Indonesian coffee market (Zhu et al., 2021; Gumulya & Helmi, 2017). Coffee is known to have potent antioxidant, antibacterial, and cell-protective activities in ex vivo experiments. There are four substances known to play a role in the main clinical effects of coffee consumption, such as cafestol, kahweol, chlorogenic acid, and caffeine (Franca & Oliveira, 2016).

Numerous researchers found that the actions of food bioactive compounds alone are not comparable to the benefits of diets rich in fruits, vegetables, or legumes. This finding signifies that phytochemicals and other bioactive substances in whole food form synergistically working complexes, intensifying the benefit effect (Chen et al., 2021). There are various innovations made in the presentation of coffee, one of which was combining herbal ingredients to increase the desired effect of coffee. Several studies have tried to combine coffee with pandan leaves, mangosteen peel, gambir, and ginseng, which generally aim to increase the antioxidant properties of coffee (Apriani et al., 2016; Putri et al., 2021; Retnaningtyas et al., 2017; Sari et al., 2021). Based on previous studies mentioned before, research is conducted to investigate the antioxidant activity of combined green coffee beans extract and dahu leaves extract, which is expected to have higher antioxidant activity.

2. Materials and Methods

2.1 Material

The specimens used were green *Coffea arabica* L. beans from Kutai Kartanegara, *Dracontomelon dao* (Blanco) Merr. & Rolfe leaves from Samarinda. The coffee and Dao specimens identified by dendrologist at Faculty of Forestry, Mulawarman University and its validity is stated in the specimen determination letter No. 99/UN17.4.08/LL/2019 and 03/UN17.4.08/LL/2021 respectively.

The materials and tools used beside the plant specimens were DPPH (1,1-diphenyl-2-picrylhydrazil) powder (Sigma-Aldrich®), 70% ethanol, aquabidest, filter paper (Whatman®), Buchner funnel (Haldenwanger®), measuring cup (Pyrex®), rotary evaporator model RV06-ML1-B (ILA®) with a vacuum pump, micropipette (Eppendorf®), vortex (Thermolyne®), beaker glass (Pyrex®), reaction tube (Pyrex®), tube rack, and GENESYS™ 20 visible spectrophotometer (Thermo Fischer Scientific®).

2.2 Methods

2.2.1 Sample extraction

The extraction of both samples was done by maceration. Five hundred (500) grams of green coffee beans and 350 grams of Dahu leaves were soaked with 70% ethanol (b/v 1:10) and stored in closed dark glass containers. The extracts were left for 5 days in a sun-shaded location to avoid oxidation and evaporation, stirred for 5 minutes a time a day. After day 5, the macerate was filtered with the Buchner funnel, and the dregs re-macerated with the same ratio of solvent. Macerate was evaporated with a rotary evaporator until it became viscous extract. The viscous extract was then restored in a transparent glass container and placed in the oven at 50^{o}C to evaporate the remaining solvent until it became a thick, semi-solid extract.

2.2.2 Test preparation

Two (2) mg DPPH powder diluted in 50 ml ethanol to make the DPPH solution. The solution is stored in a dark glass bottle and only used once. Ethanol was used as the blank solution and rutin was used as a standard for high antioxidant activity (positive control). The prime rutin solution was made from 25 mg powder diluted in 100 ml ethanol (250 ppm). The prime extract solution for the DPPH assay was made from 100 mg thick extract diluted in 100 ml ethanol (1000 ppm).

2.2.3 Determination of Antioxidant Activity

The antioxidant assay was done by DPPH radical scavenger method. The extract solution with preferred concentration taken as much as 2 mL was added with 500 mL DPPH solution. The concentration used for all types of extract is 8 ppm. This mixture is then called sample solution. The sample solution was homogenized with vortex and located in a sun-shaded place at 37^{o}C for 30 minutes. Spectrophotometer with 517 nm wavelength used to measure the absorbance of DPPH, blank, rutin, and samples solutions. Antioxidant activity was determined with quantitative analysis using radical scavenger percentage (%RS). Antioxidant activity calculated with formula (1) as follows:

$$\%\ \text{radical scavenger} = \frac{DPPH\ absorbance - sample\ absorbance}{DPPH\ absorbance} x\ 100\% \qquad (1)$$

The DPPH absorbance is the absorbance of the DPPH solution subtracted by the absorbance of the blank solution. The sample absorbance is the absorbance of the sample solution.

2.2.4 Statistical Analysis

The result obtained from the experiment was analyzed using SigmaPlot for Windows version 14.0 (AD Instrument®). The data will be presented in graphics and valued as mean ± SEM. The statistical calculation were based on t-test ($p < 0.05$).

3. Results and Discussion

The extracted weight is divided by simplicia weight to get the rendemen value. The extract rendemen was 10.2% (CA) and 11.4% (DD). DPPH radical scavenging assay was used to test the antioxidant activity of ethanol extract of coffee, dahu, and their combination. DPPH is a free radical, so by reacting the sample with DPPH reagent, the sample's scavenging ability can be seen as proof of antioxidant activity. The parameter used to determine the antioxidant activity of the sample is radical scavenging ability (%).

This experiment did not use the concentration variance but the ratio variance with the uniform concentration of 8 ppm. The sample solution ratio used is presented in Table 1. The result from the study shows in Figure 1.

Table 1. The Ratio of Extract Combination

Solution Group	Solute Volume (µL)	Solvent Volume (µL)	Total Volume (µL)
Rutin	32	1936	2000
Coffee arabica	16	1984	2000
Dracontomelon dao	16	1984	2000
CA:DD (1:1)	8 (CA) + 8 (DD)	1984	2000
CA:DD (3:1)	12 (CA) + 4 (DD)	1984	2000
CA:DD (9:1)	14.4 (CA) + 1.6 (DD)	1984	2000

As seen in Figure 1, the greater to weaker group based on their antioxidant activity was R > DD > CA:DD (1:1) > CA > CA:DD (3:1) > CA:DD (9:1). The sample groups had lesser antioxidant activity compared to the control group. Coffee also had weaker activity than dahu and seemed to weaken dahu's antioxidant activity in the combination extract groups—the greater concentration of coffee in the combination extract, the lesser activity of antioxidants.

Phytochemical antioxidants inhibit oxidative stress mainly by acting as radical scavengers and lipid peroxidation inhibitors. Reactive oxygen species (ROS) such as superoxide anions, hydroxyls, and singlet oxygen are the waste products of cellular respiration, protein folding, and many other metabolic reactions. Physiologically, ROS are vital elements for diverse signaling pathways, as stem cell proliferation and autophagy, and regulate many physiological activities (Siche et al., 2016).

Dracontomelon dao (Blanco) Merr. & Rolfe leaf is known to contain polyphenolic compounds and flavonoids, which have antioxidant abilities by donating hydrogen ions or single electrons. Based on several studies, the leaf of *Dracontomelon dao* (Blanco) Merr. & Rolfe possesses great potential as an antibacterial and antifungal agent primarily due to its tannins,

saponin, sterol, flavonoids, and triterpenoids content. Liu et al. (2014) and Zhao et al. (2015) testified that the ethyl acetate fraction of dahu leaves extracts demonstrated an antibacterial effect on *S. aureus* and *E. coli* (Dapar, 2021). In the study of the antimicrobial efficacy using the microcalorimetric analysis method by Li et al. (2017), the active compound of dahu leaf consists of various flavonoids in the form of luteolin, L-epicatechin, cyanidanol, and quercetin are reported.

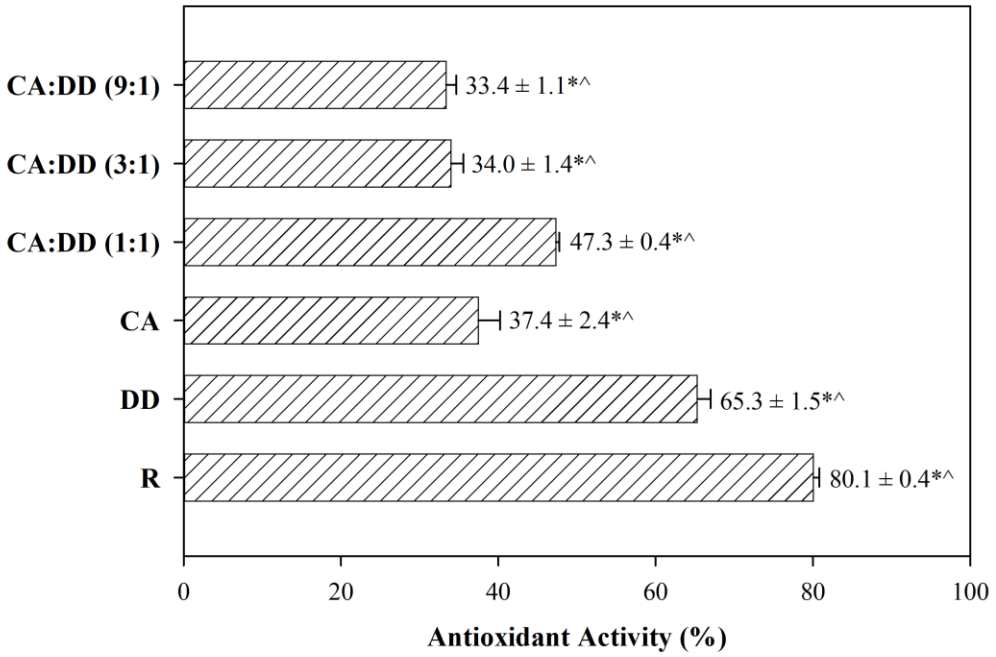

Figure 1. Comparison of Antioxidant Activity between Groups

Note. n = 6 groups with 3 reduplications. Data presented in mean ± SEM. Statistic analysis using t-test. R = rutin as the positive control. DD = *Dracontomelon dao*. CA = *Coffea arabica*. CA:DD = combination of *Coffea arabica* and *Dracontomelon dao* followed with the ratio. * = significantly different compared to the control group. ^ = significantly different compared to the DD group.

Another study found methyl gallate as the primary acting compound for vasodilator properties of dahu leaf (Ismail, 2015). Methyl gallate is a methyl ester derivative of gallic acid, one of the prevalent phenolic compounds found in various plants (Farhoosh & Nyström, 2018). Numerous studies have proven the antioxidant and tissue-protective properties of methyl gallate (Ahmed et al., 2021; Ng et al., 2018). It is reported that both gallic acid and methyl gallate possess great antiradical scavenging activities, although gallic acid solubility in lipid made it a slightly better antioxidant than methyl gallate (Mamat et al., 2020). Studies conducted by Ragasa et al. (2017) also found anacardic acid as a major constituent in dahu leaf. Anacardic acid is a phenolic lipid abundant in cashew nut shell liquid (Bastos et al., 2019). Anacardic acid has anti-inflammatory and anti-nociceptive properties due to its antioxidant mechanism (Gomes Júnior et al., 2020).

Green coffee consists of a composite arrangement of lipids, sterols, fatty acids, phenolic acids, monosaccharides, polysaccharides, polyphenols, alkaloids, proteins, free amino acids, vitamins, and minerals. The abundance of phenolic acids, such as chlorogenic acids, hydroxycinnamic acids, and caffeic acid, and alkaloids, such as caffeine and its derivates, contributes to the antioxidant and radical scavenger properties of coffee (Şemen et al., 2017).

Chlorogenic acids (CGA) consist of major phenolic compounds. CGAs in coffee are mainly in quinic ester acid with caffeic, ferulic, or coumaric acids. The abundance of CGAs isomers frequently found in coffees is caffeoylquinic acids, di-caffeoylquinic acids, ferulyolquinic acids, *p*-coumaroylquinic acids, and others (Farah & Lima, 2019). CGAs intake from coffee consumption has many advantageous healthy effects such as antioxidant, antidiabetic, anticarcinogenic, cardioprotective, antibacterial, and others (Tomac et al., 2020). The antioxidant properties of CGAs proven by the ability to scavenge radicals such as hydroxyl, superoxide, DPPH, and ABTS in a dose-dependent manner. Two plausible mechanisms are underlying the radical scavenging activity of CGA: (1) a hydrogen-atom-transfer (HAT) reaction where the free radical binds a hydrogen atom from CGA and (2) a radical adduct formation (RAF) mechanism where the free radical transform into radical intermediate with the addition of CGA (Nabavi et al., 2016).

Caffeine is one of the best-known bioactive compounds in coffee. This heterocyclic organic compound has a purine base called xanthine, which is based on a pyrimidine ring conjoined to an imidazole ring hence categorized as purine alkaloid, but some researchers call it pseudo-alkaloid since it does not have the incorporation of an amino acid in its biosynthesis (Depaula & Farah, 2019). Numerous studies stated the beneficial effect of caffeine on human health with antioxidant properties as one of the effects possessed by this neuro-stimulant compound, yet when there are many deemed caffeine as a good antioxidant, and some others doubted the effective role of caffeine against oxidative stress-induced injury. However, a recent study demonstrated caffein effectivity as an antioxidant, where caffeine inhibits adenine degradation as an impact of oxidative stress. It is believed that this protection is based on caffeine ability to scavenge hydroxyl radicals (Vieira et al., 2020)

The antioxidant properties of caffeine and other methylxanthine derivatives are considered based on their ability to reduce Cu(II) ions to Cu(I). Other studies have reported the antioxidant potential of caffeine through its ability to protect against oxidative damage to mice's lenses by increasing levels of glutathione (GSH) and reducing hydroxyl free radicals. The effect of caffeine on the antioxidant system in the body may also be due to the action of the molecular target of caffeine, namely the adenosine receptor, that plays a role in the regulation of ROS production. Caffeine is an adenosine receptor antagonist that can reduce dopaminergic toxicity, reduce inflammation, and upregulate A1 receptors (Stefanello et al., 2019)

Many can categorize interactions of the bioactive compound into additive, synergy, and antagonism interactions. The additive interaction applies if the combined effects of multiple substances are equal to the sum of each substance. The synergism is applied if the combined effects are more enhanced than the sum of each substance. In contrast, antagonism is applied if

the combined effect is inferior to the additive effect. Many antioxidant interactions resulting from the combination of bioactive compounds have been confirmed over the last decade (Chen et al., 2021).

The variance in polarity and solubility of antioxidants play an important role in their interaction. In a homogeneous system, hydrophilic antioxidants react directly in the lipid phase. In polyphase systems, the solubility of antioxidants must be considered. Low-polarity hydrophilic antioxidants easily collect on the surface of the oil/water system. This phenomenon is significant for synergistic antioxidant activity (Phan et al., 2018).

CGA is reported to have poor lipophilicity; meanwhile, caffeine is often considered a lipophilic compound, although it is more accurately characterized as an amphiphilic compound (Wang et al., 2022; Willson, 2018). Anacardic acid in dahu leaf may also be a lipophilic compound since it possesses antifungal activity. This effect is directly related to the lipophilicity of bioactive compounds, an essential trait for penetrating the lipophilic layer of microorganism cell membranes (Morais et al., 2017). Meanwhile, methyl gallate occupies both hydrophilic hydroxyl and hydrophobic alkyl chains. However, it has a shorter alkyl chain than other gallates such as propyl gallate and octyl gallate, thus having the weakest hydrophobicity among them (Luo et al., 2019).

Based on the knowledge, the combination of these four components shows a tendency toward lipophilic compounds in the mixture. If the conjecture is precise, then the phenomenon in this study is in accordance. The combination of coffee and dahu has antagonistic interactions, particularly if the lipophilic component of coffee is more abundant in the mixture. If the ratio of dahu is more prominent than coffee, different results may appear since the interaction between antioxidants also differs with combined concentration or ratio. For example, synergistic effects are usually found if hydrophilic compounds have a bigger ratio. Conversely, antagonistic effects are found if lipophilic compounds dominate the mixtures (Pan et al., 2018).

Another thing to consider is the caffeine-polyphenols complex. Caffeine forms hydrophobic complexes with chlorogenic acid, especially 5-caffeoylquinic and 3,5-dicaffeoylquinic acids, where the imidazole ring of caffeine is linked with the quinic group of chlorogenic acid (Depaula & Farah, 2019). This phenomenon was confirmed with the association between increasing caffeine content and decreasing polyphenol status in the experimental study conducted by Górecki & Hallmann (2020). This complex most likely reduces the bioavailability of CGA, which has prominent antioxidant properties compared to caffeine. This conjecture is supported by previous studies where decaffeinated coffee has higher antioxidant activity than other coffees due to increased levels of 3-CQA, 4-CQA, and 5-CQA, which may be due to the lixiviation process (Jeszka-Skowron et al., 2016).

The findings above may explain the result that occurred in this study. The caffeine tendency to bind the hydroxyl group of phenolic compounds makes the antioxidant potential of the extract not fully measured due to the decrease in the bioavailability of phenol compounds in the extract. This was further supported by the decrease in antioxidant activity as the coffee ratio was bigger than the combined extract. In light of this invention, it can be concluded that in order

to obtain more potent antioxidant properties, the dahu extract should be consumed as is without the addition of coffee extract. However, if one wants to consume a combination of coffee and dahu extract, the mixture's ratio should not exceed the 1:1 ratio. It may be more efficacious if the dahu extract is greater in the combination than the coffee, but this matter certainly needs further studies.

Conclusion

Based on the result obtained, it is known that the antioxidant activity of the combination of coffee and dahu decreases when the concentration of coffee bean extract is greater. The more the concentration of coffee bean extract in combination, the antioxidant activity of dahu leaf extract will be further suppressed.

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Conflict of Interest

There is no conflict of interest, and all of the authors agree with the manuscript's content.

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