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ANTIOXIDANT SCAVENGING ACTIVITY OF ARABICA COFFEE (*Coffea arabica*) AND AVOCADO PEELS (*Persea americana* Mill) MIX

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ABSTRACT

This study aims to examine the antioxidant activity of a mixture of coffee beans and avocado peels in various combinations. Avocado peels were obtained from the subdistrict of *Lampineung*, Banda Aceh, Indonesia while the samples of Arabica coffee beans were collected from a local coffee farmer in *Pantan Cuaca*, district of *Gayo Lues*, Aceh, Indonesia at the coordinate of 4°10'52.8"N 97°13'12.9"E. The avocado peels were dried by wind-dry method to maintain the secondary metabolite compounds and divided into two parts rough avocado peel and avocado peel powder. Raw coffee beans were also divided into two parts, to be roasted together with avocado peel and pure coffee beans. Each sample was extracted by maceration method for 48 hours using 96% ethanol and then evaporated with a rotary evaporator to obtain a thick extract. The antioxidant assay was carried out by using DPPH (1,1-diphenyl-2-picrylhydrazyl) technique. The antioxidant activity test showed that the raw avocado peels had better antioxidant power with an IC_{50} value of 4.03 µg.mL^{-1} approaching the IC_{50} value of the standard ($C_6H_8O_6$, CAS No. 50-81-7) 2.96 µg/mL. Meanwhile, roasted coffee and avocado peels have an IC_{50} value of 4.92 µg/mL and are included in the medium category. This value is better than the IC_{50} of pure coffee powder 9.71 µg.mL^{-1}. The addition of avocado peels to the coffee can significantly elevate its antioxidant activity.

Keywords: Avocado Peel, Coffee Arabica, Antioxidant Essay, IC_{50}, DPPH.

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INTRODUCTION

Free radicals are atoms or molecules without an electron pair in their outer orbital, unstable and highly reactive. Oxygen metabolism produces non-radical *OH, O*-, and H_2O_2. while the *OH radical is very reactive, thus readily reacts with biological molecules such as DNA, proteins, and lipids which will result in changes in the chemical bonds of these molecules[1] and if it is not deactivated with an antioxidant, then a radical can be harmful to the human body. Antioxidants are electron-donor compounds that can donate electrons to oxidant compounds to inhibit their activity.[2] Furthermore, antioxidants can also inhibit oxidation reactions of macromolecules such as lipids, proteins, and nucleic acids.[3] Basically, the human body has natural antioxidants produced by the body such as the enzyme superoxide dismutase (SOD), catalase, and glutathione.[4] However, the antioxidants produced by enzymes are often insufficient because the production of reactive oxygen species (ROS) from outside the body is getting higher so additional antioxidants from the intake of fruits and vegetables that contain naturally occurring antioxidants such as vitamin C, beta-carotene, phenolic compounds, and vitamin E are needed.[5] Avocado (*Persea Americana* Mill) is a popular fruit not only because it tastes delicious and fresh but avocado also contains important nutrients such as carbohydrates, protein, and fibers.[6,7] In addition, some micronutrients including vitamins, minerals, and polyphenols are also found in avocados.[8] Usually, most people consume only the flesh of the fruit, while the peel is discarded or used as animal feed. In fact, a lot of research revealed that the seed and peel of avocado also contain antioxidants.[9] The avocado peel contains secondary metabolites, including flavonoids, carotenoids, vitamin C, and phenolics[10] which can prevent cell oxidation and inhibit the development of cancer and liver disease. By using avocado peel as raw material, it is not only reducing the amount of natural solid waste but also increasing the value through combination with other components such as tea or coffee.

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Coffee is the most popular beverage that is loved by almost the entire population of the world. Instead of using coffee seeds, there are communities that make coffee leaves as a substitute for tea by brewing the leaves in hot water. Coffee plants are included in the coffee genus in the *Rubiaceae* family. Although there are more than 70 known species of coffee in the world, Coffee *canephora var Robusta* and Arabica coffee are the among the most widely consumed worldwide. In Indonesia, these two species of coffee are also the most planted species in various regions such as Toraja, Lampung, Flores, Central Java, and Aceh. Arabica coffee seeds have unique characteristics that make them superior to Robusta coffee, including taste, caffeine content, sugar content, price, and environmental growing conditions. Arabica coffee seeds also have a high selling value in the international market. Basically, coffee beans not only contain caffeine but also contain useful secondary metabolites such as alkaloids, flavonoids, and polyphenols[12,13], but the content of these compounds is partially damaged due to the roasting process. Therefore, to maintain the antioxidant content in the coffee beverage, fortification with the addition of synthetic or natural active ingredients is necessary. This study was conducted to investigate the antioxidant levels of coffee powder, avocado peel powder, and a mixture of both. Specifically, this study wanted to analyze whether there were differences in the antioxidant power of the following five different combinations: raw avocado peels, avocado peel powder, coffee grounds, roasted coffee, avocado peels, and coffee-avocado peels powder as depicted in Table-1.

EXPERIMENTAL

Materials and Equipment

Chemicals including ethanol (C_2H_5OH), DMSO, ascorbic acid ($C_6H_8O_6$), and the DPPH or 1,1-diphenyl-2-picrylhydrazil were purchased from Sigma-Aldrich, Singapore. Equipment used were a rotary evaporator (Buchi, Switzerland), UV-Vis spectrophotometer (UV-1800, Shimadzu, Japan), dry raw material chopper, analytical balance (Ohaus), coffee grinder (Didacta, Italy), and coffee roaster (Gene café, Korea).

Sample Preparation

500 grams of the coffee bean was obtained from a coffee farm in *Pantan Cuaca* sub-district, *Gayo Lues* district, and 5 kg of avocado peel from *Lampineung* sub-district, Banda Aceh. Preparation of avocado peel powder refers to Rotta[14] with minor modifications. 5 kg of avocado peel was cleaned of remnants of fruit flesh, washed thoroughly with distilled water, and then air-dried for 7 days. The air-dry method was carried out to maintain the content of chemical compounds in the avocado peel.[15] The dried avocado peel was separated into two parts; rough avocado peel (sample No.1), and powdered avocado peel (sample No. 2). Avocado peel powder was mashed using a dry chopper. The coffee beans were separated into 3 parts; pure coffee powder (sample No. 3), roasted coffee and avocado peel (sample No. 4), and coffee powder mixed with finely ground avocado peel powder (sample No. 5), the sample criteria can be seen in Table-1.

Table-1: Variations of Coffee and Avocado Peel Samples

No	Sample	Code
1	Raw Avocado Peels	RAP
2	Avocado Peel Powder	APP
3	Coffee Grounds	CG
4	Roasted Coffee and Avocado Peels	RCAP
5	Coffee and Avocado Peels Powder	CAPP

The maceration method was used to extract the samples by immersing the five samples in 96% ethanol solvent. Samples were soaked for 48 hours, and the solvent was replaced every 24 hours and subsequently filtered using filter paper. The macerated samples were protected from direct sunlight to prevent a photocatalytic reaction that could change the color of the sample. All extracts were evaporated with a rotary evaporator at a temperature of 65ºC at 40 rpm and the obtained extracts were concentrated using a hair dryer. The extracts were then diluted for antioxidant assay.

Antioxidant Essay

The DPPH ($C_{18}H_{12}N_5O_6$, MW = 394.33 $gmol^{-1}$) free radical scavenging method was originally developed by Blois[16] and has been further improved by many researchers to increase its sensitivity and validity.[17] The procedure for assessing the antioxidant power of samples is described as follows: The solution of 0.1 mM DPPH was prepared by dissolving 8 mg of DPPH (Sigma-Aldrich, Singapore) in 200 mL of ethanol. Then

varied volumes (from 2 to 10 µL) of extracts and the positive standard ascorbic acid (AA) were diluted to 40 µL with DMSO and 2.96 mL of previously prepared DPPH solution was added. The reaction mixture was incubated in a dark room at ambient temperature for 20 minutes. The absorbance of the mixture was measured at 517 nm. As a negative control, 3 ml of DPPH was used, while ascorbic acid was used as a positive control. The following formula was used to calculate the % radical scavenging activity (RSA) of the extracts:

$$\%\text{RSA} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \text{x } 100$$

RESULTS AND DISCUSSION

Both samples of coffee and avocado peels were prepared according to table 1. The variations were intended to investigate the form of samples with better antioxidant activity. The image of the samples is depicted in Fig.-1.

Fig.-1: Samples with Different Combination (a) CG, (b) RAP, (c), CAPP, (d) APP, (e) RCAP

Figure-1 shows the image of 5 different forms of samples. The avocado peel was prepared in two different forms (raw peel and powdered peel) while the mixture of avocado peel and coffee was prepared as coffee & avocado peel powder, and roasted coffee and avocado peel. Those combinations were formulated and tested for a better IC_{50} value. The inhibition profile of all samples is tabulated in Fig.-2.

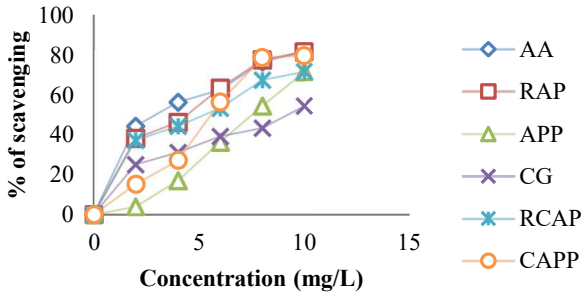

Fig.-2: Antioxidant Activity of Avocado Peel and Coffee Mix evaluated by using the DPPH Method

Figure-2 indicates that as the concentration increase, the power of inhibiting ability against DPPH increases. The inhibiting power of RAP and CAPP are almost like AA at both 8 mg/L and 10 mg/L. However, the

RCAP and RAP inhibition profiles are similar to the profile of Vitamin-C and more linear compared to other samples. Therefore, the IC_{50} of RAP and RCAP is lower and closer to the IC_{50} value of AA. These findings proved that the avocado peel is a rich source of antioxidants, with a total of phenolic content up to 6.8 (mg GAE/g sample) for the fresh peel and up to 120.3 mg GAE/g for the dry avocado peel.[18] Rotta[14], Tremocoldi[19] also reported bioactive compounds present in avocado peels and it is very potential to be utilized as natural resources of antibacterial agents.[18] Therefore, the RAP shows better antioxidant activity when compared to other forms of the sample. In addition, all samples with avocado peels show a steady increase in the percent of DPPH scavenging as the concentration increase. On the other hand, the CG which was raw coffee grounds shows fewer increments in the percent of DPPH scavenging as the concentration increase. Even though the CG shows less antioxidant activity, Choi and Koh[20] reported that both roasted coffee and spent coffee grounds still contain several antioxidative compounds such as phenolic, and tannins as proven in this research that the CG shows the IC_{50} of about 9 µg/mg sample.

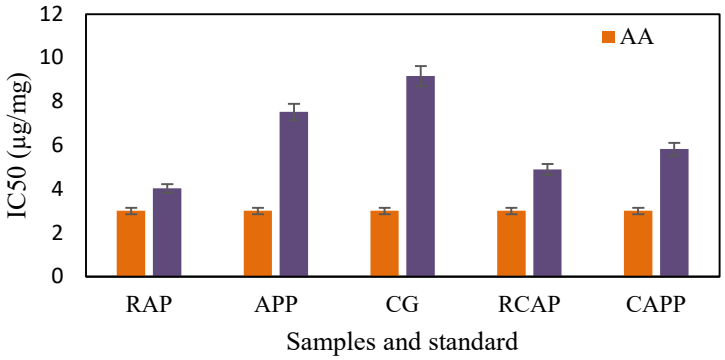

Fig.-3: IC_{50} Value of Coffee Bean and Avocado Mix Compared to the Positive Standard (Ascorbic Acid)

Figure-3 shows the IC_{50} of five different samples compared to a positive standard. The sample of RAP, RCAP, and CAPP shows better IC_{50} as are approaching the standard's values when compared to APP and CG. The data also indicates that the mixture of coffee and avocado peels exhibits slightly better IC_{50}. RAP has the strongest activity with IC_{50} of 4 µg/mL. Furthermore, the test of antioxidant activity also revealed that the raw peel of avocado shows better activity as compared to both coffee grounds and three other mixtures of coffee and treated avocado peel. The test also revealed that generally, the samples containing avocado peel either pure or mixture show better IC_{50} as compared to the CG. This finding, therefore, is proving that the antioxidant activity was dominantly contributed by the avocado peels. The IC_{50} is the concentration of the sample where 50% of DPPH is inhibited.[21] The lower the IC_{50} value the better the quality of the antioxidant because the IC_{50} value is important to determine the dosage of the extract or antioxidant needed to inhibit all DPPH molecules in the solution. The IC_{50} value also determines how effective is the extract could hinder or eliminate free radicals.[22]

CONCLUSION

The results reveal that mixing the avocado peels in the coffee with various forms could significantly improve the antioxidant activity and the value of IC_{50}.

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