International Journal on Advanced Science Engineering Information Technology

Vol.13 (2023) No. 2
ISSN: 2088-5334

Antioxidant Activity and Compound Analysis Using Various Types of Solvents on Cascara Pulp Arabica Gayo Coffee to Treat Skin Aging

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Abstract*— Cascara dry coffee skin (coffee cherry tea) is one of the waste products of dried coffee pulp, which is useful as an inhibitor of free radicals, protects the stomach, and is beneficial for the skin. One that can inhibit free radicals is antioxidants. This study assessed the effect of different solvents on Arabica Gayo coffee pulp cascara's antioxidant activity and analyzed the components of Arabica Gayo coffee pulp compounds. This study's experimental methods include cascara extraction, antioxidant assay, and metabolite identification by GCMS analysis. Water, ethanol, methanol, n-hexane, and ethyl acetate were used to extract the cascara Arabica Gayo coffee, then the various concentration of extract was prepared and tested with DPPH solutions. Extracts also identified their secondary metabolites by using GCMS analysis. The antioxidant assay revealed all extracts showed DPPH reduction with performing by changing color into a yellow color. A high concentration of extract positively correlated with percent DPPH inhibition. The highest antioxidant was the ethanol extract of cascara, followed by n-hexane, water, methanol, and ethyl acetate. The metabolites profile of each extract might cause different antioxidant activities. Metabolite profiles showed caffeine in all extracts, with the highest concentration in the n-hexane extract. Hexadecenoic acid was dominated at n-hexane extract, methanol, and ethyl acetate showed unique compounds, quinic acid in methanol and 1(2H)-Naphthalene, octahydro-4a,8a-dim at ethyl acetate extract. Both reported potential antioxidant activity. In summary, ethanol was recommended solvent with high antioxidant performance.

***Keywords*— Antioxidant activity; *cascara pulp* arabica Gayo coffee; DPPH.**

Manuscript received 19 Mar. 2022; revised 6 Nov. 2022; accepted 5 Dec. 2022. Date of publication 30 Apr. 2023.

I. INTRODUCTION

Indonesia has biodiversity, one of which is coffee, which is one of the export commodities in Indonesia. The Ministry of Agriculture reports that in 2019 674,636 tons of coffee were produced in Indonesia. Aceh, one of the provinces at the tip of Sumatra, has contributed the most in producing the largest Arabica coffee in Indonesia. One of them is in the Gayo highlands area [1]. Aceh has Arabica coffee plantations located in Bener Meriah, Gayo, and Central Aceh with an area of 99,584 Ha with Arabica coffee production of 41,847 tons [2]. Morphologically, coffee consists of several layers, namely: the outermost fruit skin (exocarp), the fruit flesh (mesocarp), the horn skin (endocarp), and seeds (endosperm), which are still wrapped in the epidermis [3]. Coffee processing can be done dry, wet, or both. After that, around 40-60% of waste is produced in the form of liquid and solid waste. However, the utilization of waste has not been optimal, and this is a problem for Indonesia. The most wasted is coffee pulp (cherry rind). This pulp contains 60% carbohydrates, 12% protein, 18% fiber, 1.3% flavonoids (such as caffeine), and phenolic compounds [4], [5].

A study of the therapeutic potential of the five components of coffee, namely ferulic acid, caffeine acid, kahweol, cafestol, and chlorogenic acid, and their mechanism of action was conducted. The results found several pharmacological activities such as antioxidant, cardioprotective, antimicrobial, neuroprotective effects, anti-inflammatory, anti-cancer, and immunomodulatory. Other effects are osteogenesis (kahweol), anti-diabetic (caffeine, chlorogenic acid, and ferulic acid), and hepatoprotective (chlorogenic acid).

However, caffeine harms early brain development and the reproductive system [6].

Several studies have been conducted to process coffee waste into products such as bokashi, pectin, bioethanol, and nata de coffee. However, this research was not optimal due to technology and human resources limitations. The content of this coffee waste consists of nutrients and sources of phytochemicals, namely procyanidin, chlorogenic acid, caffeine, and other polyphenolic compounds, as well as having high antioxidant and fiber content. Polyphenol-based therapies' efficacy and application in skin therapy are still being studied. Anogenital warts can be treated with polyphenols, according to research. Polyphenols can also be utilized to treat alopecia, hyperpigmentation, acne vulgaris, aging skin, and fungal infections, according to other research [7]–[9].

Dried coffee husk / Cascara (coffee cherry tea) is one of the waste products of coffee pulp that has been dried. Cascara coffee is known as coffee skin tea which is currently one of the new drinks. Its benefits include as an inhibitor of free radicals, protecting the stomach, and being beneficial for the skin [10]. Cascara coffee itself contains eight times more inhibiting free radicals than blueberries, so it can prevent cancer and increase body vitality [11]

Antioxidants are chemical components of monohydroxy or polyhydroxy phenol, which function as inhibitors of the oxidation process, even in small concentrations. These antioxidants work by direct chemical reactions or enzymatic free radical scavenging, lipid peroxyl radical scavenging, bonding with metal ions, and repairing oxidative damage. This antioxidant contains vitamin C, beta carotene, vitamin E, selenium, zinc, alpha-lipoic acid, xanthones, and lycopene. In addition, polyphenol compounds function as antioxidants by breaking the chain of the oxidation process [12].

The polyphenolic compounds contained in cascara are chlorogenic acid, flavan-3-ols or catechins, flavanols, and anthocyanidins. Another study reported that there were two dominant phenolic compounds in coffee cascara, namely protocatechuic acid (85.0 mg/L) and chlorogenic acid 69.6 mg/L. These compounds have stability at a temperature of 85ºC, so the antioxidant activity is still relatively large. The antioxidant capacity of Cascara arabica L extract assessed by IC50 via DPPH was 1.082 mg/mL [13].

Besides chronicle aging, human skin aging can also be developed from the cumulative damage of oxidative stress. This oxidative stress affects human skin at the molecular level, leading to the manifestation of aging. Cosmeceutical products containing antioxidants can allow human skin to repair the damage by intensifying skin resistance to oxidative stress [14], [15]. The GCMS identification and antioxidant activity of cascara Gayo Arabica coffee was limited information. Therefore, we performed antioxidant activity and metabolites profile of Gayo Arabica cascara coffee.

II. Material and Method

A. Plant Materials and Extraction of Gayo Arabica Coffee Cascara

Cascara or coffee pulp of Gayo Arabica coffee was used in this study and collected from Gayo, Aceh, Indonesia. Cascara was washed using water and air-dried. Gayo Arabica coffee cascara was grounded by a grinding machine and extracted by water, ethanol, methanol, n-hexane, and ethyl acetate. The extraction was carried out by 1:10 of cascara powder: solvents for overnight maceration. Extract solutions were filtered using filter paper and evaporated at a temperature of 45°C.

B. Antioxidant Activity Test of Cascara Pulp Extract of Gayo Arabica Coffee

The antioxidant activity test of cascara coffee extract was tested by DPPH assay. The DPPH (2,2-diphenyl-1-picrylhydrazyl) was free radicals' analog for the antioxidant test. The reduction of DPPH by the antioxidant extract was characterized by changing the purple color of native DPPH to yellow[16]. The test was initiated by mixing 0.1 mM DPPH in methanol with cascara extracts with several concentrations. The solution was incubated at 37°C in the dark room for 30 minutes, then determined the absorbance at the wavelength 517nm. The percent DPPH inhibition was calculated by the formula [17]:

$$\%\text{Inhibition}=\frac{\text{DPPH Absorbance - Sample Absorbance}}{\text{DPPH Absorbance}}\times 100\% \quad (1)$$

The IC50 value indicated a 50% reducing DPPH activity, calculated by regression curve with the linear regression equation $y=a \pm bx$ [17].

C. Analysis of the Components of the Cascara Pulp of Gayo Arabica Coffee Using Gas Chromatography-Mass Spectroscopy (GC-MS)

Gas Chromatography-Mass Spectroscopy (GC-MS) combines gas chromatography and mass spectroscopy. Identification of the metabolomic profile of samples was carried out by utilizing GC-MS [14]. Coffee has numerous chemical constituents affecting its quality, such as volatile compounds, sugars, proteins, phenolic compounds, caffeine, enzymes, and acids [15].

Volatility and thermal stability are two requirements for GC. The GC is carried out at high temperatures, and the volatility of the components is increased by modifying the chemistry (formation of derivatives). As a result of earlier heated gas, the separation of substances finally flowed into the MS. MS will convert molecules into ions divided into two magnetic and electric fields. Because this technique involves electrons and ions, which will react by being scattered through the air, MS operates under a vacuum. The ion source is heated to keep the molecules in the gas phase. The GC results were illustrated as MS fragments due to the ionization process and the strength of the chemical bonds. [18].

Varied cascara pulp of Gayo arabica coffee extract was filtered using 0.2µm filter paper, and their metabolite profiles were analyzed using GC-MS (Shidmazu, QP2010). The SPME handle was inserted into the hollow tube, and the GC-MS sucked out volatile compounds. The GCMS conditions of this study include as follows:

- Injector temperature of 280˚C.
- Spitless injector mode.
- Column temperature of 40°C (10°C/minute).
- Retention time of 3 minutes (30°C/minute) to reach a temperature of 299°C with a total program time of 29.633 minutes.
- Temperature detector of 280°C.
- Helium gas is used as carrier gas.

- The flow control mode pressure is 4,3367psi.
- The total flow of 8.4mL/m.
- Column current 0.9mL/m; split ratio 5:1.
- The column type is Rtx-5MS.
- The column length is 30.00m.

III. Results and Discussion

Several studies on Arabica coffee cascara have been reported, including the safety of Arabica coffee cascara extract products in instant drinks and infusion coffee (Spanish Tabi Fruit) [19]. Identification of volatile and non-volatile compounds of cascara coffee from El Salvador, Brazil, Papua New Guinea, and Guatemala by using GC-MS was also reported [20]. Coffee also showed a potential product in polymer technology [21]. Coffee also lowered cholesterol levels in medical scopes *in vitro* and *in vivo* studies [22]. Fermented Arabica coffee pulp from West Java was reported to show high antioxidant activity [23], and antioxidant activity was affected by various treatments [24].

A. Antioxidant Activity of Cascara ARABIC Coffee Pulp

The antioxidant activity of the cascara extract of Gayo Arabica coffee pulp, which was extracted using various solvents, namely water, ethanol, methanol, n-hexane, and ethyl acetate, was tested using the DPPH method. Testing the antioxidant activity of the cascara extract of Gayo Arabica coffee pulp was carried out by varying the concentration of the extract 10, 20, 30, 40, and 50. Fig. 1 shows the results of assessing the antioxidant activity of Gayo Arabica coffee pulp cascara extract.

The data revealed a positive correlation between extract concentration with percent inhibition. The results showed that the extract concentration of 50 ppm had a higher percent inhibition value than the extract concentration of <50 ppm. The cascara extract of Gayo Arabica coffee pulp dissolved in ethanol had the lowest IC50 value, with an IC50 value of 4.7, performing the highest antioxidant activity. While the lowest antioxidant activity was cascara extract with ethyl acetate solvent.

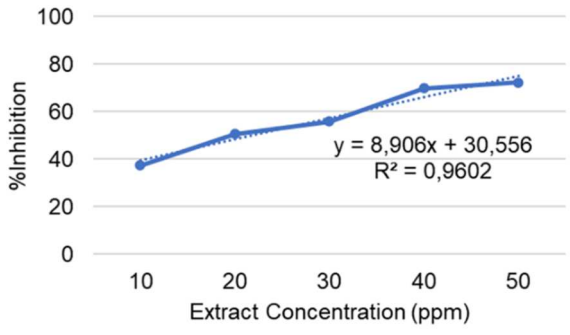
An experimental study on the effect of solvents on pigments and antioxidant activity of Arabica coffee leaf extract by Marcheafave et al. [25] also showed that extracts dissolved in pure ethanol showed the lowest IC50 values compared to extracts dissolved in dichloromethane and hexane (50:50) which were showed the smallest antioxidant activity because it had a higher IC50 value.

The number of polyphenols extracted is heavily influenced by the polarity of the solvent employed for extraction. Because of the interaction between antioxidant chemicals and solvents, optimal extraction of polyphenols was achieved in polar solvents due to their effectiveness in the solvation process. Therefore, a mixture of polar solvents is used to recover the phenolic compounds [25], [26]. It is possible to mix and combine two types of solvents in order to obtain a more efficient solvent for extracting antioxidant compounds, such as water and ethanol [27].

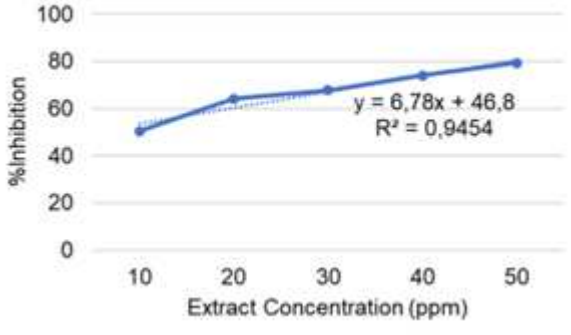
The antioxidant ability of arabica coffee extract occurs by reducing Fe3+ ions to Fe2+ ions through electron transport. Measuring the antioxidant activity of FRAP consists of the antioxidant capacity of the compound to reduce Fe3+ to Fe2+, with this reaction carried out in intervals of 30 minutes. According to Barros et al. [28], two factors that can explain the difference in behavior between methods are that the rate of Fe3+ reduction of some phenolics compound, such as quercetin, is not fast enough to be measured in 30 minutes and longer time intervals required for their total quantification. The type of solvent also plays a great role in determining the rate of reduction of Fe3+. These two factors also rationalize why using ethanol as the solvent for extraction presets a greater number of antioxidants.

In a study conducted by Kiattisin et al. [29], the results of DPPH obtained to test the antioxidant properties of extracts with solvents, it was found that extracts dissolved in ethanol and ethyl acetate had good antioxidant activity compared to hexane solvents. This could be caused by phenolic compounds in the extract extracted with polar and semi-polar solvents.

Ethyl acetate solvent is a solvent with a lower polarity level, while methanol is a high degree of polarity in the extraction method. For solvent extraction, ethyl acetate showed a lower antioxidant capacity than methanol extract. Arabica coffee pulp cascara extract with ethyl acetate solvent also did not show reduced activity compared to methanol extract. This is due to the low solubility of phenolic compounds in less polar solvents or because ethyl acetate cannot extract any iron-containing compounds. The IC50 values obtained from ethyl acetate solvent were 972.95 mg/mL and 6.0 mg/mL in methanol solvent [30].



(a)



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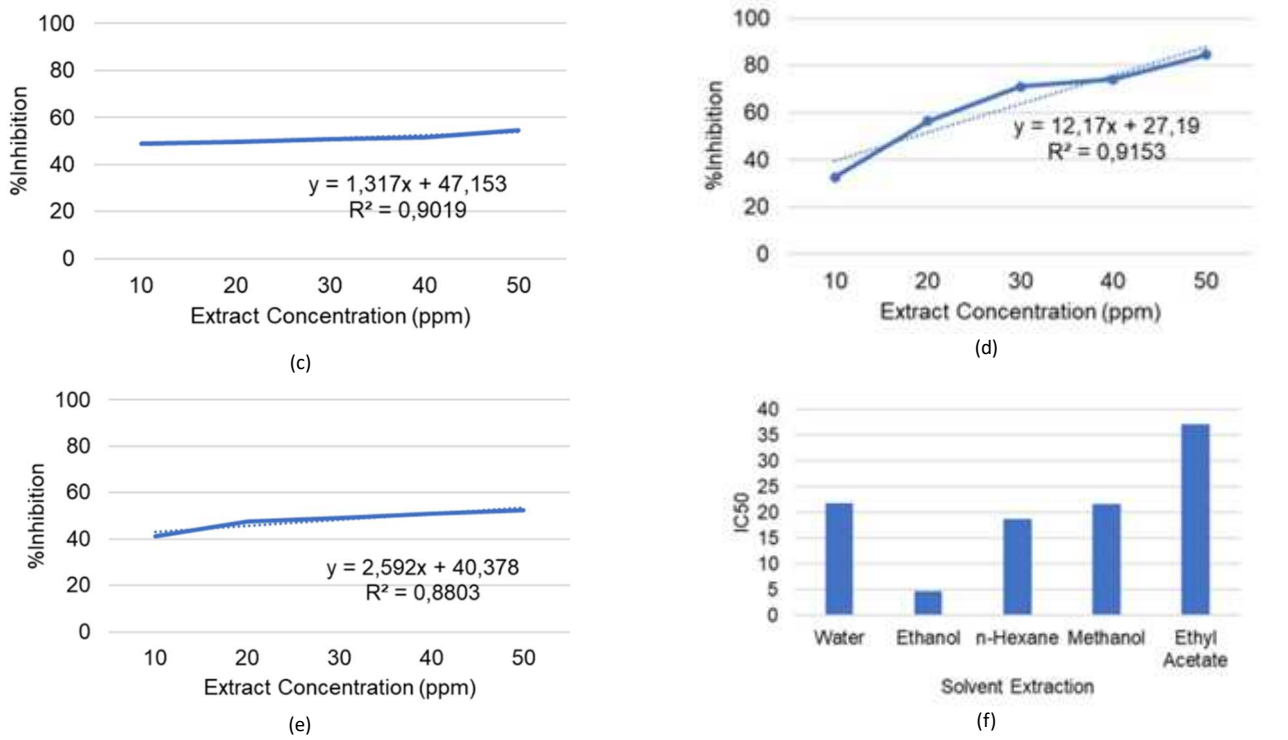

Fig. 1 The DPPH inhibitory and IC50 of Gayo Arabica Coffee Cascara Pulp Extract with varied solvent extractions, A. water extract, B. ethanol extract, C. methanol extract, D. n-hexane extract, E. ethyl acetate extract, F. IC50 revealing antioxidant activity

B. Secondary metabolites profile of cascara Gayo arabica coffee

Various solvents for extraction performed varied antioxidant activities, and varied solvents also affected the metabolites profile. Table 1—3 shows there are several solvents with the same compounds, ethanol, methanol, and ethyl acetate, with 5-Hydroxymethylfurfural compounds that function as antioxidants, antibacterial, and antiproliferative. However, the concentrations of the compounds in the three solvents were different. The highest concentration was found in ethanol solvent, and the lowest concentration of 5-Hydroxymethylfurfural was found in ethyl acetate solvent.

TABLE I
METABOLITE PROFILES OF CASCARA GAYO ARABICA COFFEE IN ETHANOL SOLVENT

Retention time	% Area	Compound	Biological Activity
21.377	22.31	5-Hydroxymethylfurfural	Antioxidant, antibacterial, and antiproliferative [32]
29.277	21.07	Caffeine	Antioxidant and antimicrobial [33]
30.701	12.85	n-Hexadecanoic acid	Anti-cancer, antioxidant, anti-cancer, anti-alopecic, anti-inflammatory [34] 35]
32.435	11.94	Cis-Vaccenic acid	Antibacterial, hypolipidemic effect, antioxidant, anti-inflammatory[36]
28.005	3.93	Hydrazinecarboxamide, 2-(2-methylcyclohe)	Anticonvulsant[37]

TABLE II
METABOLITE PROFILES OF CASCARA GAYO ARABICA COFFEE IN METHANOL SOLVENT

Retention time	% Area	Compound	Biological Activity
21.830	16.61	5-Hydroxymethylfurfural	Antioxidant, antibacterial, and antiproliferative [32]
29.326	12.55	Caffeine	Antioxidant and antimicrobial [33]
28.295	10.59	Quinic Acid	Anti-HIV-1 Antioxidant [38] 37]
30.706	7.38	n-Hexadecanoic acid	Anti-cancer, antioxidant, anti-cancer, anti-alopecic, and anti-inflammatory [34] 35]
32.416	5.71	cis-Vaccenic acid	, antibacterial, hypolipidemic effect, antioxidant, and anti-inflammatory[36]

TABLE III
METABOLITE PROFILES OF CASCARA GAYO ARABICA COFFEE IN N-HEXANE SOLVENT

Retention time	% Area	Compound	Biological Activity
28.934	57.53	Caffeine	Antioxidant and antimicrobial [33]
35.413	5.94	Hexadecanoic acid, 2-hydroxy-1-(hydroxyl)	No activity was reported [40]
30.534	5.26	Palmitic acid n-Hexadecanoic acid	Anti-cancer antioxidant, anti-cancer, anti alopecic, anti-inflammatory [34], [35]
36.947	4.50	9-Octadecenoic acid (Z)-, 2,3-dihydroxypropil ester	Hepatoprotective activity[41], anti-cancer [42], antifungal, antibacterial [43]
30.029	4.34	Hexadecanoic acid, methyl ester (CAS) methyl palmitate	Antioxidant, hemolytic, hypocholesterolemic[43]

In addition, the n-hexadecenoic acid (Table 4) compound, which functions as an antioxidant, is also found in several

solvents, namely ethanol and n-hexane solvents, but with different compound concentrations, the highest concentration of n-hexadecenoic acid compounds is found in ethanol solvents. The caffeine compound that functions as an antioxidant and antimicrobial is also found in several solvents, namely ethanol, methanol, n-Hexane, and ethyl acetate, with caffeine compound concentration found in n-Hexane solvent, 57.53%, and the lowest caffeine concentration was found in methanol solvent, 12.55%.

The water and methanol solvent fractions also have the same compound, quinic acid, which functions as an antioxidant with the highest concentration in the water solvent fraction. The utilization of natural products in the cosmetic application is becoming a great interest nowadays as phenolic compound biological activity proved to have anti-aging activity and the ability to protect skin from ultraviolet radiation [31]. An animal study conducted by Marija et al. [47] shows that a dietary supplement derived from a vegetable from the genus Brassica reduced the effect of skin aging. Puxvadee et al. [48] use *Phyllanthus emblica L*. branch in topical gel to evaluate the efficacy of the anti-aging activity. Clinical improvement was observed in the effect of skin aging, such as reduction of skin wrinkles, enhanced skin elasticity, and lightening skin color.

TABLE IV
METABOLITE PROFILES OF CASCARA GAYO ARABICA COFFEE IN ETHYL ACETATE SOLVENT

Retention time	% Area	Compound	Biological Activity
21.187	13.88	5-Hydroxymethylfurfural	Antioxidant, antibacterial and antiproliferative [32]
29.130	13.81	Caffeine	Antioxidant and antimicrobial [33]
27.307	9.83	1(2H)-Naphthalenone, octahydro-4a,8a-dim	Antioxidant [44]
30.605	8.16	n-Hexadecanoic acid	Anti-cancer, antioxidant, antialopecic, anti-inflammatory [34],[35]
32.347	6.90	Oleic acid	Lower blood pressure, anti-cancer, hepatoprotective, anti-inflammatory [45], [46]

IV. CONCLUSION

In conclusion, various solvents affected the antioxidant and metabolite profiles of cascara Gayo Arabica coffee extracts. The ethanolic extract performs the high antioxidant activity with the lowest IC50 of DPPH inhibition, while the lowest antioxidant activity was ethyl acetate solvent treatment. Polar and non-polar solvents performed different metabolites and shifted the retention time of compounds.

ACKNOWLEDGMENT

The authors thank Syiah Kuala University for all the knowledge and support in every research process and information for the study.

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