EFFECT OF EXTRACTION CONDITIONS ON YIELD AND BIOACTIVE COMPOUNDS OF COFFEE PULP EXTRACT

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ABSTRACT: Coffee pulp is a by-product of coffee processing that contains a considerable amount of bioactive compounds. The extraction conditions have a great effect on the yield and bioactive compounds of coffee pulp extract. This study aimed to investigate the effect of extraction conditions for shaking-assisted maceration and Soxhlet extraction on the yield and bioactive compounds of coffee pulp from West Java, Indonesia. For shaking-assisted maceration, extraction time (30, 60, 90, and 120 min), shaking speed (30, 60, 90, and 120 movement/min), and concentration of citric acid (0, 3, 5, and 8 % weight per volume) were varied whereas for Soxhlet extraction, sample to solvent ratio (1:7, 1:10, 1:12, and 1:15 weight per weight), concentration of ethanol (0, 70, and 96 % volume/volume), and concentration of citric acid (0, 3, 5, and 8% weight per volume) were varied during the extraction process to determine their influence on the yield and bioactive components of the coffee pulp extract. The highest yield of coffee pulp extract (27.6% weight/weight) was obtained when the coffee pulp was extracted by Soxhlet extraction using 8% citric acid concentration in 96% ethanol with a material to solvent ratio of 1:15. Bioactive compounds in the coffee pulp extract had been determined and found to contain total phenolic compound of 20.3-21.3 mg GAE/g, anthocyanin content of 6.0-13.8 mg/L, vitamin C content of 1.5-1.7 mg/g, and flavonoid content of 3.4-3.8%. The coffee pulp extract also demonstrated an antioxidant activity with IC_{50} values of 277.89 - 253.57 ppm.

KEYWORDS: *Bioactive Compounds, Coffee Pulp, Shaking-assisted Maceration, Soxhlet Extraction, and Yield.*

1. INTRODUCTION

Coffee is one of the world's most valuable commodities, only second to petroleum, as reported by Bae et al. [1]. Data from the International Coffee Organization (ICO) [2] shows that in 2020, Indonesia occupied the fourth largest coffee producer globally with 12,1 million bags produced annually. The ICO also reports that the Asian market continues to show stable growth [3]. As demand grows, coffee production will be an even more lucrative business. However, as it is common in most processing of agricultural goods, coffee beans generate organic waste. The coffee industry only uses around 10% of the coffee bean, discarding the rest [4] comprising of the pulp and husk [5]. While agricultural wastes are generally used as feedstock, coffee cherry waste contains considerable amount of bioactive

compounds such as tannins and caffeine, which are not ideal for animal consumption in significant quantities [6]. However, these bioactive compounds such as anthocyanins [7] and chlorogenic acid [8] are beneficial for human health.

Coffee cherry waste also contains substantial amounts of polyphenolic and flavonoid phytochemicals [8-9]. It has been reported that diets rich in phytochemicals are associated with decreased risk of non-communicable diseases [10]. As such have sparked the interest of many researchers to extract the bioactive compounds from coffee cherry waste. The value of these biological compounds has made the extraction of coffee waste the interest of many recent studies. Murthy & Naidu [8] investigated the extraction of coffee silver skin, spent waste and cherry husk from India using a Soxhlet apparatus with isopropanol and water. The study reported that the antioxidant capacity of the wastes reached up to 1.5-2 mmol Trolox/100 g. In another study, Vijayalaxmi et al. [9] examined the effect of using different solvents to extract bioactive compounds in coffee pulps originating from India. The study concluded that combination of methanol and water were the most effective solvents followed by ethanol and water and the extract contain significant amounts of polyphenol, tannin, and flavonoid.

Murthy et al. [7] reported that coffee pulp from India extracted via maceration using 0.01 M HCl solutions in methanol contained 25 mg of anthocyanins per 100 g of fresh pulp on a dry weight basis. In another study, Heeger et al. [11] evaluated the extraction of coffee pulp of different varieties, origin (Salvador, Congo, Honduras) and type of process using hot water as a solvent. The study found that the aqueous extract of coffee pulp contains an antioxidant capacity of 51-92 μg Trolox equivalent/g dry matter and a total polyphenol concentration of 4.9-9.2 mg GAE/g dry matter. In addition, Oktaviani et al. [12] investigated the effect of natural fermentation of coffee pulp from Indonesia with simultaneous aeration and determined the phenolic content and antioxidant activity of coffee pulp extracted with methanol using a maceration method. The study reported that at the optimum extraction conditions (4.2 hours, 31.8°C), the phenolic content and antioxidant activity were 6.72% and 27.6%, respectively.

All relevant studies that have been carried out highlighted that coffee waste contains a considerable amount of bioactive compounds. Valorization of the waste to produce valuable bioproducts may help coffee farmers to generate more income apart from the sales of the existing coffee beans [13]. Systematic studies that investigate the effect of different extraction conditions on the yield and bioactive compound of coffee pulp are still scarce. Hence, this study was carried out to determine the effect of different operating conditions for extracting bioactive compounds from Indonesian coffee pulp using shaking-assisted maceration and Soxhlet extraction methods. In addition, this study also determined the polyphenol, flavonoid, and anthocyanin content as well as antioxidant activity of the coffee pulp extract.

2. METHODS

2.1. Raw Material Preparation

The coffee pulp ("Mahkota Java Coffee Garut") used in this study was obtained from Garut, West Java, Indonesia. The coffee pulp had an initial moisture content of 12% and crushed into smaller sizes before used for shaking-assisted maceration (0.05-0.10 cm in diameter) and Soxhlet extraction (0.2 cm in diameter).

2.2. Shaking-Assisted Maceration

Shaking-assisted maceration was carried out according to the procedures as suggested by Oktaviani et al [12]. To facilitate mass transfer from the materials to the solvent, shaking-assisted maceration was performed using a laboratory shaker incubator. A ratio of 1:2 on a weight basis (w:w) of coffee pulp to solvent was used with ethanol (96 %) as the solvent. Extraction time (30, 60, 90, and 120 min), shaking speed (30, 60, 90, and 120 movements/min), and concentration of citric acid (0, 3, 5, and 8 % weight per volume, w/v) were varied during the extraction process to determine their influence on the yield and bioactive components of the coffee pulp extract. After extraction, the liquid extract was filtered, followed by evaporation using a rotary vacuum evaporator at 40°C and 17.5 kPa to obtain a coffee pulp extract and the yield was calculated using Eq. (1).

$$\text{Yield (\%)} = \frac{m_e}{m_c} \times 100\% \quad (1)$$

where m_e is the mass of coffee pulp extract (g) and m_c is the mass of coffee pulp (g).

2.3. Soxhlet Extraction

Soxhlet extraction of coffee pulp was conducted based on the methods suggested by Murthy & Naidu [8]. In this study, preliminary experiments were carried out to determine the number of cycles for each Soxhlet extraction. The experiments were carried out by repeating Soxhlet extraction cycles until a clear solvent was obtained particularly after three cycles as determined in this study. Therefore, all Soxhlet extractions in this study were performed for three cycles. The extractions were carried out at 78.5°C using approximately 12 g of sample with ethanol and water as the solvent. Sample to solvent ratio (1:7, 1:10, 1:12, and 1:15 w/w), concentration of ethanol (0, 70, and 96 % v/v), and concentration of citric acid (0, 3, 5, and 8 % w/v) were varied during the extraction process to determine their influence on the yield and bioactive compounds of the coffee pulp. After the third cycle, the solvent was evaporated using a rotary vacuum evaporator at 60°C and 17.5 kPa to obtain the coffee pulp extract and the yield was calculated using Eq. (1).

2.4. Determination of Bioactive Compounds

Total phenolic content of the coffee pulp extract was measured using a Folin-Ciocalteu method as described by Orak [14] with slight modification. Anthocyanin content was determined using a pH difference method described by Wrolstad et al. [15]. Vitamin C content was measured using iodometric titration recommended by AOAC [16]. Flavonoid content was determined by colorimetry using $AlCl_3$ as a reagent described by Chang et al. [17]. Detailed procedures to determine vitamin C as well as total phenolic, anthocyanin and flavonoid content have been described elsewhere [14-16].

Determination of antioxidant activity was based on the procedures by Leu et al. [18] which measured antioxidant activity in terms of inhibition against oxidation by 1,1-diphenyl-2-picrylhydrazyl (DPPH). The data presented in this study is expressed as IC_{50}, which is the concentration of sample that resulted in 50% inhibition of DPPH. The antioxidant activity determined in this study is expressed as DPPH scavenging activity which can be calculated using Eq. (2).

$$\text{Scavenging activity (\%)} = \frac{A_C - A_S}{A_C} \quad (2)$$

where A_c is the absorbance of the DPPH solution while A_s is the absorbance of the sample.

3. RESULTS

3.1. Extraction of Coffee Pulp

3.1.1 Shaking-assisted Maceration

Initially, shaking-assisted maceration was carried out using 96% v/v ethanol with 8% w/v citric acid for 120 min, with the shaking speed was varied from 30 to 120 movements/min. The highest speed at 120 movements/min produced the highest yield (13.17 ± 0.26% w/w), followed by a shaking speed of 90 movements/min (9.40 ± 0.11% w/w), 60 movements/min (9.91 ± 0.36% w/w) respectively while the lowest speed of 30 movements/min produced the lowest yield of 9.06 ± 0.08% w/w. Next, experiments were carried out using a shaking speed of 120 movements/min while the extraction time was varied from 30-120 min. An extraction time of 120 min produced the highest yield of 14.14 ± 0.11% w/w, followed by 90, 60, and 30 min with an extraction yield of 12.42 ± 0.20, 11.62 ± 0.11, and 11.11 ± 0.08% w/w, respectively. Based on the pre-determined shaking extraction time and extraction time that gave the highest yield, further experiments were carried out to determine the concentration of citric acid that lead to a greater yield. Citric acid was added to the solvent to break down polysaccharides [19] in the coffee pulp to facilitate mass transfer during the extraction process. The highest concentration of 8% w/v citric acid produced the highest yield of 17.90%, while lower concentrations of 5 and 3 % produced the yields of 15.43 ± 0.69 and 14.12 ± 0.60% w/w, respectively. The sample extracted without citric acid produced a yield of 13.17 ± 0.17% w/w.

3.1.2 Soxhlet Extraction

Initially, Soxhlet extraction was carried out with a material to solvent ratio of 1:12 w/w and 8 % w/v of citric acid concentration in the solvent. The results show that ethanol concentration of 96% v/v produced a yield of 24.42 ± 0.94% w/w, compared to 20.83 ± 0.35 and 17.21 ± 0.41% w/w for 70 and 0 % ethanol concentrations, respectively. Based on this result, the next step was to determine the effect of citric concentration using ethanol 96% v/v as the solvent. The highest concentration of citric acid (8% w/v) produced the highest yield of 24.42 ± 0.94% w/w, followed by 5, 3, and 0% w/v of citric acid with a corresponding yield of 24.38 ± 0.29, 22.99 ± 1.30, and 23.08 ± 0.71% w/w, respectively. Based on the pre-determined ethanol and citric acid concentration that gave the highest yield, further experiments were carried out to determine the material to solvent ratio that lead to a greater yield. As the material to solvent ratio increased from 1:7, 1:10, 1:12, and 1:15 w/v, the yield also increased from 21.54 ± 0.53, 24.54 ± 0.76, 24.42 ± 0.94, and 27.58 ± 1.53 % w/w, respectively.

3.2. Determination of Bioactive Compounds

The coffee pulp extract that had the highest yield for both shaking-assisted maceration (120 movement/min, 90 min, 8% w/v of citric acid) and Soxhlet extraction (ethanol 96%, material to solvent ratio of 1:15, 8% w/v of citric acid) were analyzed in terms of antioxidant activity as well as flavonoid, polyphenol, vitamin C and anthocyanin content and compared with the control samples and the results are shown in Table 1.

Table 1: Antioxidant activity, bioactive compounds, and yield of coffee pulp extract

Method	**Condition**	**Antioxidant activity (ppm)**	**Flavonoid (%)**	**Polyphenol (mg GAE/g)**	**Vitamin C (mg/100 g)**	**Anthocyanin (mg/L)**	**Yield (%w/w)**
Shaking-assisted maceration	Speed: 120 movement/min, time: 90 min, citric acid: 8 %w/v	277.89 ± 8.44	3.07 ± 0.04	18.2 ± 0.91	133.76 ± 29.87	10.34 ± 2.27	12.42 ± 0.2
	Speed: 120 movement/min, time: 120 min, citric acid: 8 %w/v	270.66 ± 18.61	3.35 ± 0.31	20.27 ± 1.01	154.88 ± 39.82	13.81 ± 2.05	17.9 ± 0.39
Soxhlet extraction	Ethanol 96%, material to solvent ratio: 1:15, time: 310 min, citric acid: 8 %w/v	253.57 ± 9.92	3.99 ± 0.0.2	21.64 ± 0.29	183.04 ± 19.91	5.26 ± 1.40	27.58 ± 1.53
	Ethanol 96%, material to solvent ratio: 1:12, time: 180 min, citric acid: 8 %w/v	268.85 ± 4.36	3.77 ± 0.02	20.96 ± 0.44	161.92 ± 29.97	6.81 ± 0.33	24.42 ± 0.94

4. DISCUSSION

4.1. Effects of Extraction Conditions on the Yield of Coffee Pulp Extract

A higher coffee pulp extract yield was obtained when the shaking-assisted maceration was carried out with a shaking speed of 120 movements/min, extraction time of 120 min, and 8% w/v citric acid. A higher speed during the extraction helped to reach the mass transfer equilibrium within a shorter amount of time [18]. The results also show that a longer extraction time produced a higher yield because it allows a longer contact between the solvent and material, increasing the mass transfer and allowing the mixture to reach an equilibrium. The results obtained in this study agrees with a previous study by Lu et al. [19] which found that the extraction yield increases with the addition of citric acid. There is a possibility that the extraction yield will keep increasing as the concentration of citric acid increases. Investigating the optimum citric acid concentration might be of interest for future studies.

The highest coffee pulp extract yield was obtained when the Soxhlet extraction of coffee pulp was carried out using 96% v/v ethanol as solvent, 8% w/v citric acid, and a 1:15 w/w ratio of material to solvent. Overall, the amount of yield obtained using Soxhlet extraction produced higher yields compared to extraction by shaking-assisted maceration. This is most likely due to more considerable amount of solvent used for Soxhlet extraction, facilitating a better mass transfer and longer extraction time as compared to the shaking-assisted maceration, allowing the mixture to reach an equilibrium with a higher yield.

4.2. Bioactive Compounds and Antioxidant Activity of Coffee Pulp Extract

The amount of polyphenol, vitamin C, and flavonoid extracted from coffee pulps were consistently higher in coffee pulp extracts obtained by Soxhlet extraction than the samples obtained by shaking-assisted maceration. This is most likely due to a higher amount of solvent used for Soxhlet extraction in comparison to the shaking-assisted maceration. The shaking-assisted maceration only used solvent twice the amount of coffee pulp, whereas Soxhlet extraction used up to 15 times the amount of coffee pulp. The extraction time for Soxhlet extraction was also much longer than the shaking-assisted maceration, thus allowing a better mass transfer from the pulp to the solvent.

Total phenolic content of the coffee pulp extract ranged from 18.2 ± 0.91 to 21.64 ± 0.29 mg GAE/g extract with the lowest concentration produced by the shaking-assisted maceration for 90 min and the highest concentration produced by Soxhlet extraction with 1:15 w/w solid to solvent ratio. In this study, the amount of total polyphenol in coffee pulp extract exceeded those of extracts from common vegetables such as onion, broccoli, carrot, tomato, and cabbages as reported by Faller and Fialho [21]. The total polyphenol content of coffee pulp extracted determined in the study is comparable with the total polyphenol content of several herb-tea extracts such as wolfberry and balsam pear tea, but less than traditional teas such as oolong and jasmine tea as reported by Toda [22].

Vitamin C content of the coffee pulp extract ranged from 133.76 ± 29.87 mg/100 g to 183.04 ± 19.91 g/100 mg. These values are considered as rich in vitamin C compared to the amount measured in exotic fruits, as was reported by Valente et al. [23]. Fruits such as kiwi, lime, grapefruit, and guava had vitamin concentrations of 91, 21, 35.5, and 65.8 mg/100 g, respectively. However, the measured anthocyanin content in the coffee pulp extract obtained from both shaking-assisted maceration and Soxhlet extraction were lower than reported in previous studies. Murthy et al. [7] reported an amount of 24 mg/100 g dry weight of Indian

coffee pulp while Prata and Oliveira [24] reported an average of 19.2 mg/100 g fresh Brazilian coffee pulp.

The difference may be attributed to differences in the varieties as well geographical factors. The low anthocyanin content could also be caused by degradation of bioactive compounds during the drying and processing of the coffee pulp used in this study. Xiong et al. reported that degradation of anthocyanin could be affected by many factors, including temperature, oxygen content, enzymes, and exposure to light [25]. The coffee pulp used in this study was dried prior to use, which means that the enzymatic factor was not likely to be the factor at play. The effect of temperature on anthocyanin degradation has been documented by Patras et al. [26], with higher temperatures accommodating a faster degradation rate by triggering anthocyanin cleavage, resulting in the production of colorless polyphenolic compounds. A study by Aramwit et al. [27] showed that anthocyanin in mulberry significantly degraded after heating at a temperature of 70°C. In addition, Wu et al. [28] reported that 78.52% of the anthocyanin in roselle extract diminished after 30 minutes of processing at a temperature of 80°C, which is closer to the temperature used in this study. The same study cited that, acidic conditions stabilize anthocyanin even after heat processing, turning the degradation rate to 39.79% after 2 h of heating. The addition of citric acid to the solvent used in this study might have prevented a greater loss of anthocyanin but could not eliminate the effect of heat.

Coffee pulp extract obtained from Soxhlet extraction showed a slightly greater antioxidant activities than the coffee pulp extract obtained by shaking-assisted maceration despite the low amount of anthocyanin with the values of IC_{50} ranged from 253.57 ± 9.92 ppm to 277.89 ± 8.44 ppm. This suggests that the compounds generated by the cleavage of anthocyanin can also possess antioxidant activity, as was previously mentioned by Patras et al. [26]. The values of IC_{50} obtained in this study are comparable with an IC_{50} of around 200 ppm for coffee pulp from India as reported by Murthy and Naidu [8]. However, these values are not as strong as the antioxidant activity of ascorbic acid that possess an IC_{50} value of 8.9 ± 0.1 ppm [29]. The relatively lower values of antioxidant activity of coffee pulp extract despite of containing substantial amount of polyphenols may suggest that the polyphenols in coffee pulp extract are mainly composed of compounds with a weaker capability of reducing radical DPPH, as different polyphenols have different reducing capacities depending on the number of available hydroxyl groups and steric structure [30].

5. CONCLUSIONS

The effect of processing conditions for shaking-assisted maceration and Soxhlet extraction on the yield and bioactive compounds of coffee pulp extract from West Java, Indonesia was determined in this study. The coffee pulp contains a considerable amount of polyphenol, flavonoid, vitamin C and anthocyanin but with a relatively lower antioxidant activity. Soxhlet extraction produced a higher yield and extracted more bioactive compounds particularly flavonoid, polyphenol and vitamin C as well having a slightly greater antioxidant activity as compared to the coffee pulp extract obtained from the shaking-assisted maceration. However, Soxhlet extraction requires more than sevenfold the amount of solvent than required by the shaking-assisted maceration. When economic and environmental factors are considered, the solvent factor may outweigh the benefit of gaining more yield and bioactive content.

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