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Free Radical and Fungal Inhibition of Methanol Soluble Extract of Mixed Coffee Parchment (*Coffea arabica* and *Coffea robusta*) Grown in Yogyakarta, Indonesia

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Abstract— In Suroloyo Yogyakarta, Coffea robusta and Coffea arabica were planted to produce coffee powder as a local business. However, the utilization discarded coffee parchment as residue. This study aims to evaluate the inhibitory potential of coffee parchment residue against DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals and the white-rot fungus (Phanerochaete chrysosporium). The mixed coffee parchment from both aforementioned species was extracted with methanol at ambient temperature over a period of three days. The methanol soluble extract was then subjected to total phenolic content, total flavonoid content, inhibition of DPPH and fungal growth of white-rot fungus, and GC-MS analysis. The total phenolic and flavonoid contents were 0.491 µmol/mg and 0.006 µmol/mg, respectively. The IC50 of the antioxidant and antifungal samples were 1257.1 µg/mL and 1166.7 µg/mL, respectively, GC-MS analysis revealed that fatty acids were the predominant constituents. In addition, caffeine was also detected in the coffee parchment of mixed C. arabica and C. robusta. It can be concluded that the presence of DPPH and antifungal activities in the sample was due to the total phenolic and flavonoid contents, along with caffeine, in the methanol-soluble extract of coffee parchment.

Keywords—antifungal, antioxidant, caffeine, coffee parchment, total phenolic content, total flavonoid content

Abstrak— Di Suroloyo, Yogyakarta, Coffea robusta dan Coffea arabica ditanam untuk menghasilkan bubuk kopi sebagai usaha lokal. Namun, pemanfaatannya menghasilkan limbah berupa kulit tanduk kopi (coffee parchment). Penelitian ini bertujuan untuk mengevaluasi potensi penghambatan residu kulit tanduk kopi terhadap radikal DPPH (2,2-difenil-1-pikrilhidrazil) dan jamur pelapuk putih (Phanerochaete chrysosporium). Campuran kulit tanduk kopi dari kedua spesies tersebut diekstraksi dengan metanol pada suhu ruang selama tiga hari. Ekstrak larut metanol kemudian dianalisis kandungan total fenolik, total flavonoid, aktivitas penghambatan terhadap DPPH dan pertumbuhan jamur pelapuk putih, serta dilakukan analisis GC-MS. Kandungan total fenolik dan flavonoid masing-masing adalah 0,491 µmol/mg dan 0,006 µmol/mg. Nilai IC50 sampel antioksidan dan antijamur masing-masing sebesar 1257,1 µg/mL dan 1166,7 µg/mL. Analisis GC-MS menunjukkan bahwa asam lemak merupakan konstituen yang dominan. Selain itu, kafein juga terdeteksi pada kulit tanduk kopi campuran C. arabica dan C. robusta. Dapat disimpulkan bahwa aktivitas antioksidan (DPPH) dan antijamur pada sampel disebabkan oleh kandungan fenolik total, flavonoid, serta kafein dalam ekstrak larut metanol dari kulit tanduk kopi.

Kata Kunci—antijamur, antioksidan, kafein, kulit tanduk kopi, kandungan fenolik total, kandungan flavonoid total

1. INTRODUCTION

Coffee, as a globally popular beverage, is produced on a large scale. In Yogyakarta, coffee is one of the beverages commonly found in local businesses, as evidenced by the numerous angkringan (local markets) and cafes that are always crowded with visitors (Iban et al., 2019). Kulon Progo, located in the Special Region of Yogyakarta, is known as a coffee-producing area of *Coffea arabica* L. and *Coffea robusta* (*Coffea canephora* Pierre ex A. Froehner). This coffee commodity is spread across several regions, such as Kapanewon (sub-district) Samigaluh, Girimulyo, Kalibawang, and others. Suroloyo Coffee from Kapanewon Samigaluh is the most popular among the community. The coffee varieties produced include Arabica line S, Arabica Kartika, and Robusta. This coffee is self-grown, self-picked, and self-processed by the Suroloyo Coffee Farmers Group (Puspita-

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sari et al., 2022).

In general, the processing of coffee produces substantial amounts of organic solid waste, primarily from the depulping process. This waste, including coffee husk, pulp, and parchment, is often poorly managed, leading to environmental pollution. However, they can be repurposed for bioethanol, biogas, compost, and animal feed. In addition, coffee by-products—such as pulp, husk, silverskin, and parchment—are abundant in carbohydrates, proteins, dietary fiber, lipids, caffeine, and phenolic compounds Aguilera et al., 2019; Alvarado-Ambriz et al., 2020; Hasballah et al., 2022; Yousef & Amina et al., 2018). The coffee parchment part especially is an underutilized fibrous endocarp on the outer layer of coffee bean, mainly composed of lignocellulose, ash, as well as secondary metabolites such as phenolics and caffeine (Mirón-Mérida et al., 2019). Although less studied than other coffee bean parts, several bioactivities have been reported from recent studies such as antioxidant (Machado et al., 2023) and anti-obesity properties (Benyelles et al., 2023). Thus, it might be utilized as material for cosmetic, food, or pharmaceutical products.

It has been considered that various phenolic and caffeine compounds with phytochemical bioactivity from coffee have the potential to contribute to human health when included in food or skin product mixtures (Damat et al., 2019; Prihadi et al., 2020). Phenolic compounds and flavonoids can act as antioxidants and possess antimicrobial, antifungal, antiallergic, and anti-hypertensive properties. In previous works, the polyphenol compounds in coffee were also reported as antioxidant agents, which play a crucial role in human health by capturing free radicals (Fu et al., 2021; Sholichah et al., 2019) and inhibiting bacterial and fungal growth (Alvarado-Ambriz et al., 2020; Bouhlal et al., 2020; Mirón-Mérida et al., 2019; Sangta et al., 2021).

Based on those potential applications, the present study aims to evaluate the total phenolic content, total flavonoid content, caffeine concentration, as well as the antioxidant and antifungal activities of coffee parchment derived from a blend of *Coffea arabica* and *Coffea robusta*. The total phenolic and flavonoid contents were quantified using colorimetric assays. Antioxidant and antifungal activities were assessed through the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay and by monitoring the growth inhibition of *Phanerochaete chrysosporium* (a white-rot fungus), respectively. Additionally, caffeine content was determined using gas chromatography–mass spectrometry (GC-MS) analysis. Based on the results, the relationship among total phenolic content, flavonoid content, caffeine content, and antioxidants or antifungal activity was discussed.

2. Materials and Methods

2.1 Materials and Equipments

The materials utilized in this study included methanol, coffee parchment derived from two species (*C. arabica* and *C. robusta*) obtained from a local processing facility in Suroloyo, Kapanewon Samigaluh, Yogyakarta, Indonesia (7°38'S, 110°11'E), Folin-Ciocalteu reagent, sodium carbonate, gallic acid, $AlCl_3 \cdot 6H_2O$, DPPH (2,2-diphenyl-1-picrylhydrazyl), quercetin, *Phanerochaete chrysosporium* (a white-rot fungus), potato dextrose agar medium, pyridine, N,O-bis(trimethylsilyl)acetamide, and trimethylchlorosilane.

The equipments used were grinder, Whatman paper No. 2, rotary evaporator (IKA RV 10 digital, Bresgau, Germany), visible spectrophotometer (SP-3000 Nano, Optima, Tokyo, Japan), Petri dish, and GC-MS machine (GCMS-QP 2010, Shimadzu, Japan).

2.2 Sample Collection and Extraction

Coffee parchment samples derived from two species (*C. arabica* and *C. robusta*) were sourced from a local processing facility located in Suroloyo, Kapanewon Samigaluh, Yogyakarta, Indonesia (7°38'S, 110°11'E). The parchment was air dried at room temperature for seven days, ground into 40 mesh size, and mixed in a 1:1 (g/g) ratio. The air-dried sample (100 g) was extracted with methanol at room temperature for 72 hours. The methanol-soluble extract was filtered using Whatman No. 2 filter paper and concentrated using a rotary evaporator. The obtained extract was weighed as methanol extract (percentage of an air-dried sample).

2.3 Total Phenolic Content

To determine the total phenolic content, 500 µL of the sample (1,000 µg/mL) was transferred into a test tube, followed by the addition of 2.5 mL of Folin–Ciocalteu reagent diluted with water (1:9, v/v). After 2 minutes, 2.0 mL of sodium carbonate (7.5 g in 100 mL of water) was added. The mixture was incubated for 30 minutes. Absorbance was measured at 765 nm using a visible spectrophotometer. A gallic acid calibration curve was prepared at concentrations of 0.147, 0.294, and 0.588 µmol/mL in methanol. Results were expressed as µmol gallic acid equivalents (GAE) per mg of air-dried extract (Diouf et al., 2009).

2.4 Total Flavonoid Content

The total flavonoid content was assessed following the method outlined by Brighente et al. (2007). The total flavonoid content was assessed by mixing 2.0 mL of the sample (1,000 µg/mL) with a 2 % solution of $AlCl_3 \cdot 6H_2O$ in methanol. The reaction mixture was allowed to stand at 20 °C for one hour. Absorbance was measured at 415 nm using a visible spectrophotometer. A calibration curve was constructed using quercetin standards at concentrations of 0.006, 0.013, and 0.026 µmol/mL in methanol. Total flavonoid content was expressed as µmol quercetin equivalents (QE) per mg of air-dried extract.

2.5 Antioxidant Activity

The assessment of antioxidants was conducted using a published work (Baba & Malik et al., 2015). The antioxidant activity was determined using DPPH radical scavenging assay. A total of 0.1 mL of the sample was mixed with 3.0 mL of 0.1 mM DPPH solution. To evaluate the IC_{50} value, the samples were prepared in different concentrations. All experiments were performed in triplicate. The antioxidant activity was calculated using Equation (1):

$$\text{Antioxidant activity}\ (\%) = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 is the absorbance of the blank and A_1 is the sample absorbance.

2.6 Antifungal Activity

Phanerochaete chrysosporium was chosen for mycelium inhibition. Briefly, 300 µL of methanol extract sample with

concentrations ranging from 500 to 2000 µg/mL was distributed on the surface of potato dextrose agar medium (20 mL) in a Petri dish. After drying for one hour, the fungus was inoculated into the medium containing extracts. A blank sample was prepared by spreading methanol solvent onto the medium. The growth inhibition was calculated using Equation (2):

$$\text{Growth inhibition}\ (\%) = \frac{A_1}{A_0} \times 100$$

Fungal growth area (A) was calculated using the formula $A = \pi \times (d/2)^2$, where d represents the diameter of fungal growth. A_0 denotes the growth area in the control (blank), while A_1 represents the growth area in the presence of the sample. The antifungal assay was performed in triplicate, and the IC_{50} value was determined (Lukmandaru et al., 2013).

2.7 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

For identification of low molecular weight in the methanol extract, the methanol extract was silylated according to the method described in a previous work (Lina et al., 2016). A total of 1 mg of extract was dissolved in 50 µL of pyridine. Then, 100 µL of a derivatizing reagent—comprising 99 µL of N,O-bis(trimethylsilyl)acetamide (BSA) and 1 µL of trimethylchlorosilane (TMCS)—was added. The reaction mixture was incubated at 70 °C for 30 minutes, cooled, and re-dissolved in methanol. For GC-MS analysis, 1 µL of the derivatized sample was injected into a GCMS-QP 2010 system (Shimadzu, Japan). The detector temperature was maintained at 280 °C, and the column temperature was programmed from 170 °C (1 minute) to 280 °C at a ramp rate of 5 °C/min. The injection temperature was set at 200 °C. Helium served as the carrier gas, and data acquisition was conducted over a mass range of 50–500 atomic mass units. The mass spectra were compared to the NIST11 library with a similarity index threshold of 80 %. The quantity of each compound was calculated using the peak-relative method (Masendra et al., 2021).

3. Results

3.1 Amount of Methanol Extract, Total Phenolic and Flavonoid Content

The coffee parchment from two species (*C. arabica* and *C. robusta*) was extracted using methanol. The obtained extract amounted to 2.1 % of the air-dried sample. The total phenolic content was determined at 765 nm and expressed as µmol gallic acid equivalents (GAE) per mg of air-dried extract. The total phenolic content of coffee parchment was 0.491 $\pm$ 0.005 µmol/mg. The total flavonoid content was measured at 415 nm and expressed as µmol quercetin equivalents (QE) per mg of air-dried extract. The total flavonoid content was 0.006 $\pm$ 0.001 µmol/mg.

3.2 Antioxidants and Antifungals

The antioxidant activity was evaluated through the determination of IC_{50} values in triplicate. The IC_{50} for antioxidant activity of coffee parchment was 1257.1 $\pm$ 14.7 µg/mL. Antifungal activity was assessed using *P. chrysosporium* inoculated into the medium containing extracts. Growth inhibition was calculated in triplicate, with an IC_{50} value of 1166.7 $\pm$ 24.8 µg/mL.

3.3 GC-MS Analysis

The methanol extract was analyzed using GC-MS (GCMS-QP 2010, Shimadzu, Japan). The mass spectra were compared to the NIST11 library with a similarity index threshold of 80 %. Compounds were quantified using the peak-relative method. The analysis results of methanol extractives and their chromatograms are presented in Table 1 and Figure 1, respectively.

A total of 20 constituents were identified. The dominant constituents were fatty acids, fatty alcohols, and hydrocarbons, including linoleic acid chloride (17.68 %), 17-octadecynoic acid (16.78 %), and palmitic acid (16.75 %) (Figure 2). In addition, caffeine (4.08 %) was also detected in the coffee parchment mixture of *C. arabica* and *C. robusta*.

4. Discussion

Coffee beans, husk, pulp, and parchment are rich in polyphenols. Previously, it was reported that total phenolic and flavonoid contents were found in *C. arabica* and *C. robusta* bean and husks planted in Indonesia (Pebriati & Diana, n.d.; Virginia et al., 2024). Furthermore, *C. arabica* parchments planted in Teocelo, Veracruz (Mexico) ranged 0.002 to 0.017 µmol/mg for total phenolic contents extracted by ethanol using reflux method (Mirón-Mérida et al., 2019), planted in Nicaragua (Central America) ranged 0.011 to 0.014 µmol/mg for total phenolic contents and 0.0044 to 0.0067 µmol/mg for total flavonoid contents extracted by water in reflux (Aguilera et al., 2019), *C. arabica* husk and bean planted in Chapada Diamantina (Brazil) ranged 0.023 to 0.039 µmol/mg for total phenolic contents and 0.00023 to 0.00046 µmol/mg for total flavonoid contents extracted by hot water using reflux method (Das Neves et al., 2019), *C. arabica* husk planted in Taquaritinga do Norte, PE and State of Minas Gerais (Brazil) ranged 0.010 to 0.058 µmol/mg for total phenolic contents and 0.0007 to 0.0519 µmol/mg for total flavonoid contents extracted by ethanol and water using ultrasound and reflux methods (Ribeiro et al., 2019; Silva et al., 2021). Despite variations in solvents and extraction methods used in previous studies, the total flavonoid content observed in this study was within the reported range for *Coffea* species beans, husks, and parchment. Conversely, the total phenolic content identified in this study was comparatively higher than those reported in earlier literature (Das Neves et al., 2019; Mirón-Mérida et al., 2019; Pebriati & Diana, n.d.; Ribeiro et al., 2019; Silva et al., 2021; Virginia et al., 2024). Therefore, it suggests that the use of methanol extraction at room temperature for 72 hours in the present study might result in high total phenolic contents in coffee parchment.

Polyphenols found in coffee, including the bean, parchment, pulp, and husk, are potential sources of antioxidant agents (Aguilera et al., 2019; Damat et al., 2019; Pebriati & Diana, 2023; Prihadi et al., 2020). For example, the antioxidant activity (IC and EC_{50}) of *C. robusta* husks planted in Indonesia was 96.5 µg/mL (Amini et al., 2022) and 2.73 to 15.09 mg/mL from *C. arabica* husks and beans planted in Brazil (Das Neves et al., 2019). Further, the mean values of IC_{50} from *C. arabica* beans and husk planted in Indonesia were 26.41 and 20.00 µg/mL, respectively (Pebriati & Diana, 2023; Prihadi et al., 2020). The result of DPPH inhibition from this study was relatively greater compared to a previous

TABLE 1: METHANOL EXTRACTIVES OF MIXED COFFEE PARCHMENT (*C. arabica* AND *C. robusta*)

No	Retention Time (min)	Constituents	Concentration (%)	Similarity Index (%)
1	26.5	Caffeine	4.08	97
2	27.4	Methyl palmitate	3.76	95
3	28.5	Palmitic acid	16.75	94
4	30.2	Methyl 9,12-octadecadienoate	3.60	96
5	30.3	Methyl 10-octadecenoate	1.65	97
6	30.6	Methyl stearate	1.07	95
7	31.2	17-Octadecynoic acid	16.78	91
8	31.5	Octadecanoic acid	2.73	93
9	33.3	Dipalmitin	14.86	84
10	33.5	Methyl 2-propylhexadecanoate	1.29	80
11	33.6	Methyl 18-methylnonadecanoate	0.74	94
12	35.1	β-Monolinolein	2.10	90
13	35.8	Linoleic acid chloride	17.68	87
14	35.9	6,9-Pentadecadien-1-ol	2.13	89
15	36.1	Dipalmitin (isomer)	3.05	83
16	38.7	Dipalmitoyl	1.06	82
17	39.6	Thiaheneicosane	1.41	80
18	41.9	9,17-Octadecadienal	1.11	87
19	44.7	14-Methyl-8-hexadecyn-1-ol	2.73	88
20	47.1	16-Hentriacontanone	1.42	94

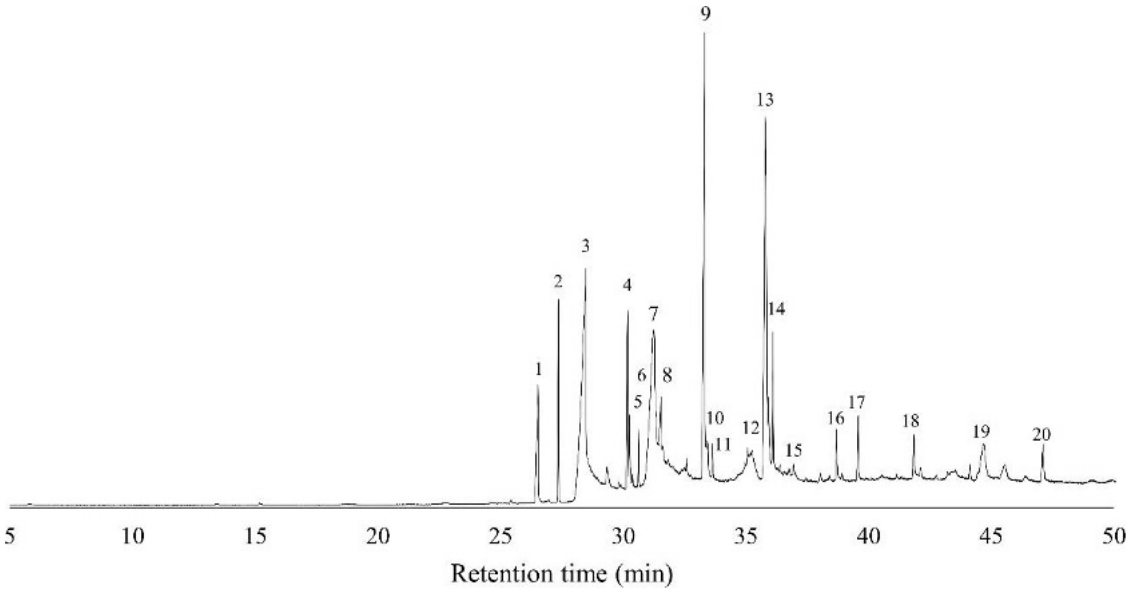

Figure 1: GC–MS chromatogram of methanol soluble extractives from coffee parchment (*C. arabica* and *C. robusta*). Peaks: 1. Caffeine (26.5 min), 2. Methyl palmitate (27.4 min), 3. Palmitic acid (28.5 min), 4. Methyl 9,12-octadecadienoate (30.2 min), 5. Methyl 10-octadecenoate (30.3 min), 6. Methyl stearate (30.6 min), 7. 17-Octadecynoic acid (31.2 min), 8. Octadecanoic acid (31.5 min), 9. Dipalmitin (33.3 min), 10. Methyl 2-propylhexadecanoate (33.5 min), 11. Methyl 18-methylnonadecanoate (33.6 min), 12. β-Monolinolein (35.1 min), 13. Linoleic acid chloride (35.8 min), 14. 6,9-Pentadecadien-1-ol (35.9 min), 15. Dipalmitin (isomer) (36.1 min), 16. Dipalmitoyl (38.7 min), 17. Thiaheneicosane (39.6 min), 18. 9,17-Octadecadienal (41.9 min), 19. 14-Methyl-8-hexadecyn-1-ol (44.7 min), 20. 16-Hentriacontanone (47.1 min).

study (Das Neves et al., 2019). In contrast, it was relatively low compared to those results reported in previous studies (Amini et al., 2022; Pebriati & Diana, 2023; Prihadi et al., 2020).

In addition to their roles as antioxidant agents, it has been investigated that the coffee beans, pulps, parchment, and husks also possess potential antifungal and antibacterial properties (Alvarado-Ambriz et al., 2020; Bouhlal et al., 2020; Mirón-Mérida et al., 2019; Sangta et al., 2021). Mirón-Mérida et al. (2019) reported that *C. arabica* parchments exhibit inhibitory effects against fungi such as *Fusarium verticillioides*, *Fusarium* species, and *Colletotrichum gloeosporioides*. Additionally, extracts from *C. arabica* pulp and husk have demonstrated antifungal activity against *Aspergillus niger*, *Botrytis cinerea*, *Rhizopus stolonifer*, *Alternaria brassicicola*, *Pestalotiopsis* species, and *Paramyrothecium breviseta* (Alvarado-Ambriz et al., 2020; Sangta et al., 2021). These facts suggest the methanol extract of mixed *C. arabica* and

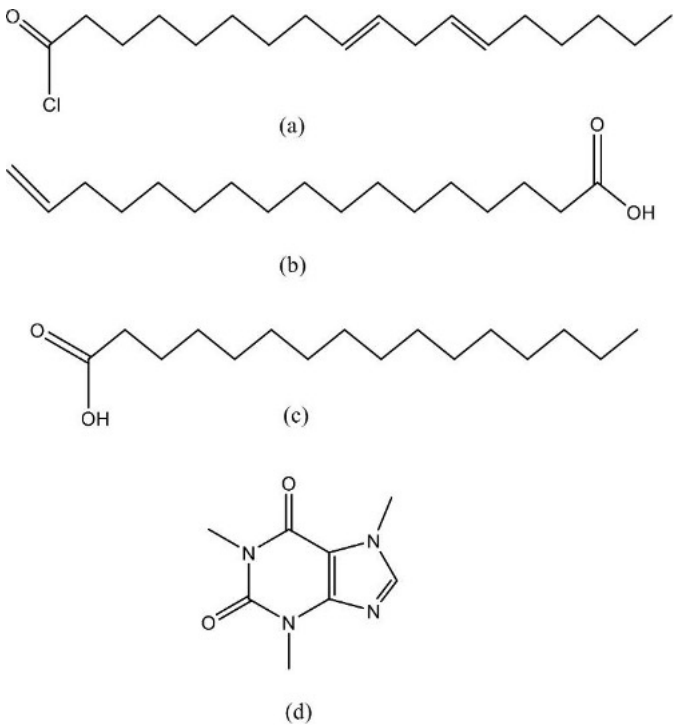

Figure 2: Chemical structure of linoleic acid chloride (a), 17-octadecynoic acid (b), palmitic acid (c), and caffeine (d).

C. robusta parchment in this study might be potent as an antifungal against *P. chrysosporium*.

The GC-MS results showed that the coffee parchment contained caffeine as well as lipophilic compounds e.g. fatty acids and hydrocarbons (Table 1). In comparison, research exhibited that fatty acids, fatty alcohols, hydrocarbons, and caffeine were also detected in the coffee beans of mixed *C. arabica* and *C. robusta* (Bouhlal et al., 2020), coffee parchment waste in Brazil (Coura et al., 2024), *C. arabica* seeds and husks (Di Stefano et al., 2023; Dippong et al., 2022), and *C. robusta* pulps (Buck et al., 2021). Therefore, the identification of fatty acids, fatty alcohols, hydrocarbons, and caffeine in this study aligns with the findings reported in previous research (Bouhlal et al., 2020; Buck et al., 2021; Coura et al., 2024; Di Stefano et al., 2023).

Phenolic compounds, including flavonoids, are well known for their antiradical and antifungal activities (Aguilera et al., 2019; Alvarado-Ambriz et al., 2020; Calheiros et al., 2023; Mirón-Mérida et al., 2019; Sangta et al., 2021). These results indicate that the inhibition of DPPH radicals and *Phanerochaete chrysosporium* observed in this study may be attributed to the samples' total phenolic and flavonoid contents. Moreover, previous reports have identified caffeine and chlorogenic acid as the primary compounds contributing to the antioxidant and antifungal properties in coffee, including its parchment (Calheiros et al., 2023; Mirón-Mérida et al., 2019). In the present study, on the other hand, the antioxidant and antifungal activities were relatively low. This might be due to the low concentration of caffeine (4.08 %) in the coffee parchment mixed with *C. arabica* and *C. robusta*, as shown in Table 1. In Table 1, it also shows that the dominant compounds of the coffee parchment methanol extract were fatty acids. This finding suggests that the low antioxidant and antifungal activity of the sample might be due to the fact that these compounds (fatty acids) did not act as DPPH and *P. chrysosporium* inhibitors. The presence of these compounds in the coffee parchment might physiologically provide energy support for the coffee seeds.

5. Conclusion

This study examined the antioxidant and antifungal activities of mixed coffee parchment (*C. arabica* and *C. robusta*). Total phenolic content, total flavonoid content, and GC-MS analysis were also clarified. The IC_{50} of the antioxidant and antifungal samples were 1257.1 µg/mL and 1166.7 µg/mL, respectively. The total phenolic and flavonoid contents were 0.491 µmol/mg and 0.006 µmol/mg, respectively. The GC-MS analysis showed the detection of fatty acids (linoleic acid chloride, 17-octadecynoic acid, and palmitic acid) and caffeine as major and minor compounds, respectively. The presence of those methanol extractives in the sample might have antioxidant and antifungal activity. In conclusion, the mixed *C. arabica* and *C. robusta* parchment from Kulonprogo, Yogyakarta, Indonesia, has potential as a caffeine source that can be used for antioxidant and antifungal agents.

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