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Profile of secondary metabolite compounds in methanol extract of robusta coffee husk *(Coffea canephora Pierre ex A. Froehner)* from Jember, East Java, based on GC-MS analysis

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ABSTRACT

Robusta coffee husk (Coffea canephora Pierre ex A.Froehner), which has long been regarded as a low-value agricultural waste, contains bioactive compounds with promising potential for development into functional products. This study aims to identify the secondary metabolite profile of the methanol extract from robusta coffee husk originating from Jember using GC-MS analysis. Extraction was conducted by maceration with methanol, yielding a concentrated extract containing various bioactive constituents. Sixteen compounds were identified, with three major components: caffeine (55.22%), quinic acid (18.72%), and lactic acid (18.49%). These compounds exhibit diverse biological activities, including central nervous system stimulation, anticancer potential, and metabolic functions. In addition, several other compounds with antioxidant, anti-inflammatory, and antimicrobial properties were also detected. The findings indicate that robusta coffee husk from Jember has significant potential as a source of bioactive compounds for further development in functional food or pharmaceutical applications.

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INTRODUCTION

Coffee processing generates several by-products, including pulp, husk, mucilage, and silverskin. These materials are often treated as low-value residues, although they can be converted into value-added products when managed through appropriate recovery and valorization strategies (Murthy and Madhava Naidu 2012, Lee et al. 2023). Coffee husk, or cascara, has attracted increasing attention because it contains bioactive constituents such as phenolic compounds, methylxanthines, organic acids, and dietary fiber-associated components (Silva et al. 2021, Ali and Bhowmik 2025). In Indonesia, arabica and robusta coffee by-products have also been reported as sources of polyphenols with antioxidant and antibacterial potential (Sholichah et al. 2019).

Previous studies have shown that the extraction method and solvent strongly influence the recovery of coffee-husk metabolites. (Silva et al. 2021), for example, evaluated extraction strategies for Coffea arabica husk and showed that extraction conditions affected phenolic content and antioxidant capacity. Ethanolic extracts of Coffea arabica cascara from the Gayo Highlands have also been profiled by GC-MS and linked to phenolic and flavonoid constituents (Lestari et al. 2023). At the regulatory level, dried coffee husk from Coffea arabica has been assessed as safe as a novel food under specific conditions of use in the European Union (Turck et al. 2022). These findings support the broader potential of coffee-husk valorization and indicate that source, solvent, and processing conditions must be clearly reported.

Despite this progress, most phytochemical profiling has focused on Coffea arabica or on general coffee by-products. In contrast, the metabolite profile of robusta coffee husk (Coffea canephora Pierre ex A. Froehner) remains less documented. This gap is important because coffee metabolite composition is affected by botanical species, geographical origin, altitude, climate, light exposure, processing, and other management factors (Alonso-Salces et al. 2009, Olechno et al. 2021, Ahmed et al. 2021). Robusta coffee is also known to differ from arabica in its methylxanthine composition, particularly in caffeine content (Alonso-Salces et al. 2009, Olechno et al. 2021). Therefore, locality-specific profiling is needed before robusta husk can be developed further as a raw material for functional ingredients or other value-added products.

Coffee husk, or cascara, has potential as a value-added food ingredient due to its bioactive phytochemicals. The European Food Safety Authority has stated that dried coffee husk from Coffea arabica L. is safe as a novel food under specific conditions of use (Turck et al. 2022), supporting further development of cascara-based food products. Previous studies also showed that C. arabica cascara contains phenolic and flavonoid compounds, including those identified through GC-MS analysis of cascara extract from the Gayo Highlands, Aceh (Lestari et al. 2023).

However, existing studies are still dominated by C. arabica, while the phytometabolite profile of robusta coffee husk (Coffea canephora Pierre ex A. Froehner) remains less explored. A study on Lampung robusta cascara essential oil reported the presence of caffeine and phenolic compounds (Damanik 2025). Still, it did not specifically evaluate the methanol extract or samples from different agroecological origins. This gap is important because solvent type, coffee species, and growing location can influence the metabolites detected.

Jember, East Java, is an important Robusta Argopuro coffee-producing area that generates substantial coffee husk as a by-product. Therefore, identifying the metabolite profile of Jember robusta coffee husk is necessary to support its valorization as a functional food ingredient. To the best of our knowledge, GC-MS profiling of the methanol extract from Jember robusta coffee husk has not been reported. Thus, this study aims to characterize the phytometabolite profile of the methanol extract of Jember robusta coffee husk using GC-MS analysis.

METHOD

Extraction

A total of 2.6 kg of dried robusta coffee husk powder from Jember, East Java, was divided into 11 jars, each containing approximately 250 g of sample. The sample was macerated using 99% methanol at a sample-to-solvent ratio of 1:5 (w/v) for 48 h at room temperature. Methanol was selected because polar organic solvents are commonly used to recover polar and semi-polar phytochemicals, including phenolic compounds, alkaloids, and organic acids, whereas cold maceration is a simple extraction approach that

minimizes thermal degradation of heat-sensitive compounds (Do et al. 2014, Zhang et al. 2018). The mixture was filtered to obtain 7.8 L of filtrate. Approximately 7.1 L of filtrate was concentrated using a rotary evaporator to remove the solvent, yielding approximately 0.355 L of thick crude extract. The recorded crude extract recovery was approximately 13.65% based on the starting dry material and the measured crude extract volume; for true gravimetric yield, the concentrated extract should be weighed after complete solvent removal.

GC-MS Analysis

Liquid sample injection GC-MS analysis of the methanol extract identified volatile and semi-volatile constituents. Before analysis, the concentrated extract was filtered through a membrane filter. A 1 µL filtered sample was injected into a Shimadzu GCMS-QP2020 NX instrument equipped with an AOC-6000 Plus autosampler at an injector temperature of 250 °C using split mode. The MS ion source temperature was 280 °C, and the interface temperature was 250 °C. The column used was a Shimadzu SH-PolarWax-MS column (crossbond carbowax polyethylene glycol; 30 m × 0.25 mm; 0.25 µm film thickness). Helium was used as the carrier gas at 100 kPa. The oven temperature program started at 100 °C with a 10 min hold, then increased at 20 °C/min to 250 °C, followed by a 20 min final hold. Chromatographic and mass spectral data were compared with the Wiley9.LIB database.

Data interpretation

Compound identification was based on mass spectral library matching and similarity scores; thus, the listed compounds should be interpreted as putative identifications unless confirmed with authentic standards. Peak areas were normalized and expressed as relative peak-area percentages. These values are semi-quantitative indicators of relative abundance. They must not be interpreted as absolute concentrations because no external calibration curves, internal standards, response-factor correction, or replicate validation were applied (Ruiz-Hernández et al. 2018). GC-MS is useful for profiling small volatile and semi-volatile metabolites, but very polar, non-volatile, thermolabile, or high-molecular-weight constituents can be underrepresented without derivatization or complementary LC-MS/HPLC analysis (Fiehn 2016).

RESULTS AND DISCUSSION

The concentrated extract yielded 13.65% (w/w). GC-MS analysis generated a chromatogram with multiple peaks, indicating the presence of diverse volatile and semi-volatile constituents in the methanol extract (Figure 1). The chromatographic profile was then interpreted using mass spectral library matching, and the putatively identified compounds are summarized in Table 1.

The spectral results indicate that the robusta coffee husk waste from Jember contains a wide range of active compounds, as evidenced by multiple peaks in the chromatogram. Each peak represents a distinct chemical compound detected in the extract. A total of sixteen compounds were putatively identified. The three largest peaks were caffeine (55.22%), quinic acid (18.72%), and (S)-2-hydroxypropanoic acid (lactic acid; 18.49%). The use of the term 'largest' refers to normalized peak-area percentage, not absolute concentration. Similarity scores ranged from 76 to 97; therefore, compounds with lower similarity scores, especially those below 80, should be considered tentative and require confirmation with reference standards.

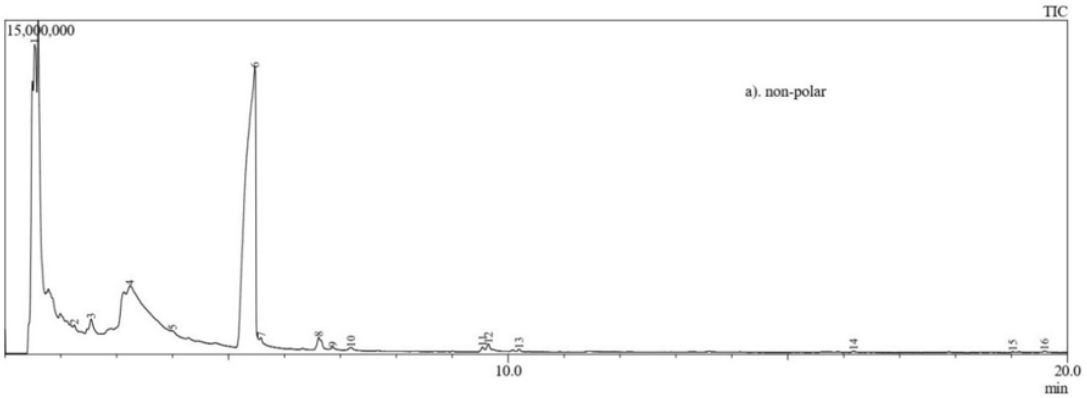


Figure 1 GC-MS chromatogram of robusta coffee husk methanol extract from Jember

Table 1 Bioactive Compounds Identified from Non-Polar GC-MS Analysis

Peak	RT (min)	Area (%)	Similarity	Putative compound	Chemical class
1	1.534	18.49	94	(S)-2-Hydroxypropanoic acid	Organic acid
2	2.242	1.44	85	Benzeneethanol-4-hydroxy-	Phenolic alcohol
3	2.542	1.52	78	2-Octenoic acid	Organic/fatty acid
4	3.234	18.72	79	Quinic acid	Organic acid
5	4.000	0.07	76	Phenol, 4-(3-hydroxy-1-propenyl)-2-methoxy-	Phenylpropanoid/monolignol derivative
6	5.471	55.22	97	Caffeine	Methylxanthine alkaloid
7	5.585	1.16	92	Theobromine	Methylxanthine alkaloid
8	6.616	1.25	82	Tetradecanoic acid	Fatty acid
9	6.869	0.27	90	2H-1-Benzopyran-2-one, 7-hydroxy-6-methoxy-	Coumarin derivative
10	7.194	0.32	89	1H-Purine-2,6-dione, 3,7-dihydro-1,3-dimethyl-	Methylxanthine derivative
11	9.544	0.33	92	9,12-Octadecadienoic acid, methyl ester	Fatty acid ester
12	9.649	0.84	90	9,12,15-Octadecatrien-1-ol	Fatty alcohol derivative
13	10.199	0.15	96	Ethyl linoleate	Fatty acid ester
14	16.181	0.08	90	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	Fatty acid ester
15	19.026	0.06	90	9,12-Octadecadienoic acid (Z,Z)-	Fatty acid
16	19.594	0.07	78	Pregnenolone	Steroidal compound

Note: Relative area (%) represents normalized peak area and should be interpreted semi-quantitatively.

GC-MS analysis of robusta coffee husk extract revealed sixteen metabolites, consisting of organic acids, alkaloids, phenolic compounds, fatty acids and their derivatives, and a steroidal compound (Table 2). The three metabolites with the highest relative abundance were caffeine (55.22%), quinic acid (18.72%), and (S)-2-hydroxypropanoic acid (lactic acid; 18.49%), indicating that alkaloids and organic acids were the dominant chemical groups detected in the extract.

Organic acids included (S)-2-hydroxypropanoic acid (lactic acid; $C_3H_6O_3$, MW 90), an intermediate in plant metabolism (Sitorus et al. 2015), and quinic acid ($C_7H_{12}O_6$, MW 192), a chemical scaffold with relevance in anticancer drug synthesis (Jayaraj and Kanagaraj 2025). Other acids contributing to aroma or biological activities were 2-octenoic acid ($C_8H_{14}O_2$; MW 142) and tetradecanoic acid ($C_{14}H_{28}O_2$; MW 228), which has antiviral activity (Juárez-Rodríguez et al. 2021, Kaczmarek et al. 2025).

Alkaloids included caffeine ($C_8H_{10}N_4O_2$; MW 194), a central nervous system stimulant enhancing alertness and cognition (Sharma et al. 2023), and theobromine ($C_7H_8N_4O_2$; MW 180), known to regulate lipid metabolism, gut microbiota, and neuroprotection (Aslan and Goktaş 2025). The high caffeine abundance may reflect factors such as robusta variety, agroecological conditions, and the extraction method, consistent with prior observations in robusta husk from Lampung and East Java (Damanik 2025, Purnomo et al. 2025).

Phenolics and related compounds included p-hydroxyphenethyl alcohol ($C_8H_{10}O_2$; MW 138), an antioxidant (CHEBI 2025), coniferyl alcohol ($C_{10}H_{12}O_3$; MW 180), a lignin precursor (Shigetomi and Uraki 2025), and isoscopoletin ($C_{10}H_8O_4$; MW 192), which exhibits antioxidant, anti-inflammatory, and anticancer properties (Barbosa et al. 2020).

Fatty acids and derivatives were represented by linoleic acid (C21H38O4; MW 354), linolenyl alcohol (C18H32O; MW 264), ethyl linoleolate ($C_{20}H_{36}O_2$; MW 308), and hexadecanoic acid ester ($C_{19}H_{38}O_4$; MW 330), all of which contribute to lipid metabolism, cardiovascular health, immune function, or anti-inflammatory activity (Bertsch et al. 2005, Rayar and Manivannan 2015, Yokota et al. 2018, NMPP Database 2024, NIST Chemistry WebBook 2025).

Finally, pregnenolone ($C_{21}H_{32}O_2$; MW 316) was identified as a steroidal prohormone that influences corticosteroid and sex hormone synthesis and exhibits neurosteroid activity (Butenandt and Westphal 1934).

Overall, these results provide an integrative view of the bioactive composition of robusta coffee husk, highlighting the predominance of alkaloids (caffeine, theobromine), organic acids, and fatty acids, while also confirming previously reported compounds in robusta husk and expanding knowledge on less-explored metabolites. Comparative analysis with prior studies indicates that solvent type, processing, and regional variation likely influence the observed metabolite profiles (Lestari et al. 2023, Damanik 2025).

Table 2 Phytometabolite profile of robusta coffee husk extract identified by GC-MS analysis

Compound	Category	Abundance (%)	Molecular Formula	MW	Reference
(S)-2-hydroxypropanoic acid (lactic acid)	Organic acid	18.49	$C_3H_6O_3$	90	(Sitorus et al. 2015)
p-Hydroxyphenethyl alcohol	Phenolic	–	$C_8H_{10}O_2$	138	(CHEBI 2025)
2-Octenoic acid	Organic acid	–	$C_8H_{14}O_2$	142	(Kaczmarek et al. 2025)
Quinic acid	Organic acid	18.72	$C_7H_{12}O_6$	192	(Jayaraj and Kanagaraj 2025)
Coniferyl alcohol	Phenolic	–	$C_{10}H_{12}O_3$	180	(Shigetomi and Uraki 2025)
Caffeine	Alkaloid	55.22	$C_8H_{10}N_4O_2$	194	(Sharma et al. 2023)
Theobromine	Alkaloid	–	$C_7H_8N_4O_2$	180	(Aslan and Goktaş 2025)
Tetradecanoic acid	Fatty acid	–	$C_{14}H_{28}O_2$	228	(Juárez-Rodríguez et al. 2021)
Isoscopoletin	Phenolic	–	$C_{10}H_8O_4$	192	(Barbosa et al. 2020)
1H-purine-2,6-dione, 3,7-dihydro-1,3-dimethyl	Alkaloid	–	$C_7H_8N_4O_2$	180	(Sreeja S. 2023)
9,12-Octadecadienoic acid, methyl ester	Fatty acid	–	$C_{19}H_{34}O_2$	294	(Abulila and Sabry 2024)
9,12,15-Octadecatrien-1-ol (linolenyl alcohol)	Fatty acid derivative	–	$C_{18}H_{32}O$	264	(Bertsch et al. 2005)
Ethyl linoleate	Fatty acid derivative	–	$C_{20}H_{36}O_2$	308	(Rayar and Manivannan 2015, Yokota et al. 2018)
Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	Fatty acid derivative	–	$C_{19}H_{38}O_4$	330	(NMPP Database 2024)
9,12-Octadecadienoic acid (linoleic acid)	Fatty acid	–	$C_{21}H_{38}O_4$	354	(NIST Chemistry WebBook 2025)
Pregnenolone	Steroid	–	$C_{21}H_{32}O_2$	316	(Butenandt and Westphal 1934)

CONCLUSION

This study showed that Jember robusta coffee husk contains diverse metabolites that may support its valorization as a bioactive by-product. GC-MS analysis of the methanol extract identified sixteen compounds, with caffeine (55.22%), quinic acid (18.72%), and lactic acid (18.49%) as the dominant metabolites. The detected compounds included alkaloids, organic acids, phenolic-related compounds, fatty acids, and steroidal compounds, indicating a chemically diverse phytometabolite profile. These findings provide preliminary evidence for the potential use of Jember robusta coffee husk as a source of functional compounds; however, further targeted quantification, compound confirmation, and bioactivity assays are required to validate its practical application.

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