

Detection Metabolite Volatile Compounds and In Silico Bioactivity of Robusta Leaves (*Coffea canephora*) from Pakusari Village, Jember

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ABSTRACT

Robusta coffee is among the most plentiful varieties, although the utilisation of its foliage remains suboptimal. People believe that coffee leaves help reduce hypertension. The objective of this research was to identify volatile metabolites from robusta coffee foliage utilising GC-MS and this study will contribute to advances in the further development of natural phytopharmaceuticals. This study acts as a preliminary evaluation for the creation of herbal treatments derived from coffee leaves. The primary technique of GC-MS involves identifying volatile metabolites using molecular weight, retention time, and percentage area. The research employed maceration extraction over three days with a leaf sample ratio of 30 g to 300 mL of 96% methanol, chemical identification via GC-MS, and in silico bioactivity assessment using PASS Online. The results of GC-MS detection showed that robusta coffee leaf extract contained 73 metabolite compounds. Dominant compounds in coffee leaf extract included 2-Propanol, 1,1'-oxybis- (peak 18), 1-Propanol, 2-(2-hydroxypropoxy)- (peak 21), Butanedioic acid, monomethyl ester (peak 43), Benzofuran, 2,3-dihydro- (peak 46), Succinimide (peak 49), 5-Hydroxymethylfurfural (peak 50), Phenol, 4-ethenyl-2,6-dimethoxy- (peak 52), Phytol (peak 53), Catechol (peak 57), Niacin (peak 58), n-Hexadecanoic acid (peak 61), Hydroquinone (peak 63), 9-Octadecenoic acid, (E)- [Elaidic acid] (peak 65), Caffeine (peak 67), 9,12-Octadecadienoic acid (Z,Z)- [linoleic acid] (peak 68), Xanthine, 1,3,7,8-tetramethyl- (peak 69), 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (peak 70), dan 1,3-Propanediol, 2-(hydroxymethyl)-2-nitro- (peak 71). The area percentage value of caffeine was 45.37%, and in silico bioactivity showed analeptic characteristics. This analysis could not match the phenomenon in Pakusari Village, where coffee leaves are antihypertensive.

KEYWORDS

Coffea canephora; GC-MS; Metabolite compound, PASS online; Robusta

1. INTRODUCTION

Robusta coffee is a type of coffee that can be grown in lowland areas with favorable temperature and light intensity contours [1]. Robusta coffee is one of the main commodities, but for now the use of coffee leaves, is still not optimal. Coffee leaves contains metabolite compounds such as flavonoids, phenolics, terpenoids, and several other compounds that have potential in natural medicine herbal [2]. Research related to the content of coffee leaf metabolite compounds in the Jember area has not yet been carried out, so the detection of metabolite compounds is very necessary to be the basis for the initial study of phytochemical screening. Based on Indonesia history, coffee leaf herb medicine was actually discovered during the Dutch colonial era. Coffee leaf herbs were first discovered in West Sumatera under the name is "Aia kahwa". The ancestors believed that "Aia kahwa" had beneficial properties for the body [3]. As time goes by, screening or detection of compounds contained in robusta coffee samples can be done using GC-MS [4].

Volatile metabolite compounds in robusta coffee leaves may be recommended to be analyzed with GC-MS as an initial screening. Gas Chromatography-Mass Spectrometry (GC-MS) is a instrumentation can specifically detect volatile compounds that are polar and non-polar based on their molecular weight [5], [6]. Volatile compounds organic metabolites that are volatile at ambient pressures and temperatures [7].

The results of the detection by GC-MS, to be continue followed up with bioinformatics analysis using PASS Online to predict the bioactive potential of metabolite compounds as bioactivity in medicine [8], [9]. The advantage of PASS Online is that it can predict the biological activity of compounds very specific, efficiently and very quickly, with the output from PASS Online is value Probability active and Probability inactive [10]. The use of both methods can be recommended for application to robusta coffee leaves samples, to develop initial metabolomic profiling, and is expected to provide important information for student, researchers, and also the general public.

Observations made by researchers show that many people in Pakusari Village consume coffee leaves herbal in the form of tea. The people in Pakusari believes that coffee leaf herbal medicine has health benefits, especially for lowering high blood pressure (antihypertensive). The local community obtained this information from their ancestors, without any clinical approach. Based on previous research conducted by robusta coffee contains a specific compound, the namely is chlorogenic acid (CGA) compound [11], [12]. CGA has great potential as an antibacterial and antioxidant agent, and other studies have shown that CGA can bind to the proteins DNA gyrase (GyrB) and UDP-N-acetylglucosamine enolpyruvyl transferase (MurA) through molecular docking [13]. In addition to this research, CGA in coffee samples has bioactivity as antioxidant in laboratory tests by inhibiting DPPH solution as a free radical [14]. Based on these studies, follow-up research can be conducted in the Pakusari area to reveal the metabolite compounds contained in robusta coffee leaves. The contribution of this study, in addition to revealing the metabolite compounds in coffee leaf samples, is also to inform the community in Pakusari Village.

2. MATERIALS AND METHODS

2.1. Materials

The ingredients used in this study are robusta coffee leaves (30 g) from Pakusari, Jember Indonesia and 300 mL of 96% methanol. The tools used in this study were GC-MS (QP2010 Plus Shimadzu), rotary evaporator (Buchi), water bath (B-480), 0.2-micron filter, GC-MS 2 mL injection vial bottle, Mass Hunter software, Wiley9.LIB website, NIST.LIB, Way2drug PASS Online website, and PubChem website.

2.2. Extraction

Robusta coffee leaves were taken from the Pakusari Village area, Jember Regency, East Java, Indonesia (coordinate -8.166621, 113.774888, elevation 199 m). Coffee leaves were washed thoroughly with water by shower and dried. As much as 30 g of coffee leaves was weighed, and cutting into small pieces. Then, 300 mL of 96% methanol was poured, and macerated for up to 3 days. Subsequently, the maceration sample was evaporated with a rotary evaporator until crude extract was produced and continue analysis using GC-MS [5], [15].

2.3. GC-MS Analysis

Crude extract was filtered with a 0.2-micron filter, and injected 1-5 μ L into the GC-MS machine. The sample was injected by the split method with the provision that the detector temperature MS was 280 °C. The column used the Restek Rtx-50® column (Crossbond® 5% phenyl-50% methyl polysiloxane) with a deep diameter of 0.25 mm, a length of 30 m and a thickness of 0.25 mm [16]. The carrier gas used in this study using helium pressure 64.1 kP. The result of the detection of this metabolite compound was the peak spectrum mass in the form of a chromatogram. The detection results were compared to the similarity value (%) in bioinformatics using Wiley9.LIB dan NIST library. The results of the detection of GC-MS were in the form of peak chromatography and a list of metabolite compounds that had been read in MS with an area % at Retention Time (RT) [15], [17], [18].

2.4. In Silico PASS Online

Volatile metabolite compounds detected in robusta coffee leaves extract were analyzed in silico bioactivity using PASS Online website. The SMILES of each compound was input data in the PASS Online, and the potential predictions of the metabolite compounds will be detected. The results of the online PASS prediction are in the form of Probability active (Pa) and Probability inactive (Pi) values. $Pa > 0.7$ was

predicted to have strong bioactivity as a drug, Pa 0.5-0.7 has bioactivity but must be validated experimentally, while Pa < 0.5 has weak biological activity [8], [19].

3. RESULTS AND DISCUSSION

3.1. Detection Volatile Compounds of Leaf *C. canephora*

Detection of volatile compounds in robusta coffee leaf extract is presented in the form of chromatographic graphs (Figure 1) and a table of names of detected metabolite compounds (Table 1). Figure 1 shows the chromatography peak of the detection of volatile compounds using GC-MS. The detection process for 30 minutes by detection of 73 metabolite compounds. The compound is detected with GC-MS based on its molecular weight, retention time, and %area, the injected sample will be read in the MS section. Table 1 shows a list of names of volatile metabolite compounds complete with RT, which is the optimal time for the reading of the compound in the MS. The results of the detection of these compounds are influenced by several factors, one of which is the use of a very effective 96% methanol solvent. Methanol is high polarity solvent, it is effective in dissolving polar to semi-polar metabolite compounds [19], [21]. Based on detection of GC-MS, there are several metabolite compounds that were detected to have polar properties, namely is Benzofuran, 2,3-dihydro- (peak 46), HMF (peak 50), Phytol (peak 53), n-Hexadecanoic acid (peak 61), 9-Octadecenoic acid, (E)- (peak 65), 9,12-Octadecadienoic acid (Z, Z)- (peak 68), and several phenolic derivative compounds. In addition to solvent factors, the leaf part of plants physiologically contains primary metabolites and secondary metabolites that play an important role in plant metabolism processes, such as oxidative stress defense [22].

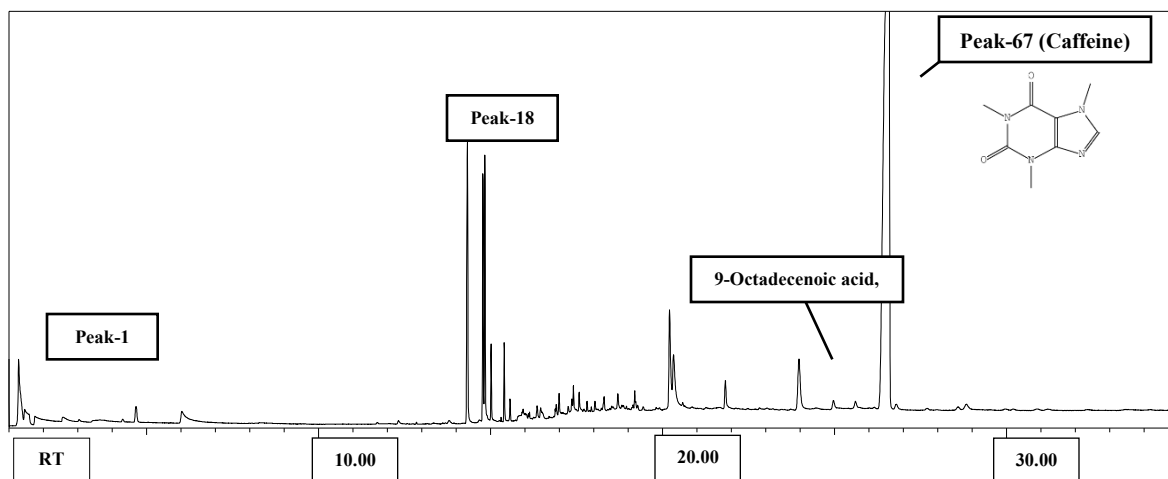


Figure 1. Peak chromatography GC-MS leaf robusta coffee extract.

There are several dominant metabolite compounds in robusta coffee leaves extract, such as 2-Propanol, 1,1'-oxybis- (peak 18), 1-Propanol, 2-(2-hydroxypropoxy)- (peak 21), Butanedioic acid, monomethyl ester (peak 43), Benzofuran, 2,3-dihydro- (peak 46), Succinimide (peak 49), 5-Hydroxymethylfurfural (peak 50), Phenol, 4-ethenyl-2,6-dimethoxy- (peak 52), Phytol (peak 53), Catechol (peak 57), Niacin (peak 58), n-Hexadecanoic acid (peak 61), Hydroquinone (peak 63), 9-Octadecenoic acid, (E)- [Elaidic acid] (peak 65), Caffeine (peak 67), 9,12-Octadecadienoic acid (Z,Z)- [linoleic acid] (peak 68), Xanthine, 1,3,7,8-tetramethyl- (peak 69), 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (peak 70), and 1,3-Propanediol, 2-(hydroxymethyl)-2-nitro- (peak 71). The indicated of dominant compound is by high %area, compared to other detected metabolite compounds [23]. The dominant compound may physiologically affect the benefits of robusta coffee leaves.

The compound that has the largest %area in the robusta coffee leaves extract is caffeine (45.37%), which is certainly a very large figure compared to other volatile metabolite compounds. Caffeine is a specific metabolite compound possessed by coffee plants, this compound is not only detected in coffee

beans, but it has been proven to be detectable in leaf samples [24]. Caffeine is one of the groups of derived from alkaloids compound, these compounds can be effectively expressed with methanol solvents which are polar with a high affinity to weak alkaline compounds such as caffeine [25], [26]. Physiologically, caffeine can be produced in young coffee leaves with the following biosynthesis pathways (Figure 2).

Table 1. Detection volatile compounds of leaf robusta coffee extract using GC-MS.

Peak#	RT (min)	Area%	Compounds
1	1.285	2.50	Carbamic acid, monoammonium salt
2	1.463	0.56	Propanamide, N-methyl-2-amino-
3	1.757	0.33	2-Pentanamine
4	2.567	0.90	Hydrogen isocyanate
5	3.044	0.28	Pyrazine, methyl-
6	4.311	0.29	3-Hexen-1-ol, (Z)-
7	4.696	0.71	Ethanol, 2-butoxy-
8	6.032	1.63	Acetic acid
9	8.282	0.05	Formic acid
10	11.713	0.05	Butanoic acid, 4-hydroxy-
11	12.325	0.13	3-Furanmethanol
12	12.503	0.02	Butanoic acid, 3-methyl-
13	12.851	0.03	Cyclooctasiloxane, hexadecamethyl-
14	13.353	0.02	Naphthalene
15	13.689	0.03	Propanediamide
16	13.794	0.15	Methyl salicylate
17	13.940	0.02	Propanoic acid, 2-hydroxy-, 2-methylpropyl est
18	14.331	4.98	2-Propanol, 1,1'-oxybis-
19	14.679	0.05	Mequinol
20	14.782	3.09	1-Propanol, 2-(2-hydroxypropoxy)-
21	14.839	3.51	1-Propanol, 2-(2-hydroxypropoxy)-
22	15.020	1.21	1-Propanol, 2-(2-hydroxypropoxy)-
23	15.257	0.02	2-Propenamide
24	15.310	0.06	Cyclodecasiloxane, eicosamethyl-
25	15.401	1.06	1-Propanol, 2-(2-hydroxypropoxy)-
26	15.733	0.09	2H-Pyran-2,6(3H)-dione
27	15.877	0.38	Phenol
28	15.920	0.17	Carbonic acid, monoamide, N-butyl-, propargyl
29	15.949	0.29	Furaneol
30	16.010	0.16	Pantolactone
31	16.047	0.17	2-Pyrrolidinone
32	16.107	0.12	2,6-Difluorobenzoic acid, tridec-2-ynyl ester
33	16.255	0.13	2(3H)-Furanone, 5-acetyldihydro-
34	16.461	0.34	Cyclopentanol
35	16.500	0.37	3(2H)-Furanone, 4-hydroxy-5-methyl-
36	16.884	0.15	Cyclononasiloxane, octadecamethyl-
37	16.913	0.34	2-Hydroxy-gamma-butyrolactone
38	16.996	0.71	Phenol, 5-ethenyl-2-methoxy-
39	17.082	0.13	Isopropyl palmitate
40	17.261	0.41	1-Methyl-2-piperidinemethanol
41	17.366	0.63	Phenol, 2,6-dimethoxy-
42	17.413	0.77	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-m

Table 1. Detection volatile compounds of leaf robusta coffee extract using GC-MS (continue).

Peak#	RT (min)	Area%	Compounds
43	17.720	0.46	Butanedioic acid, monomethyl ester
44	17.809	0.45	2-Propanamine, N-methyl-N-nitroso-
45	17.934	0.33	Diethyl Phthalate
46	18.040	0.47	Benzofuran, 2,3-dihydro-
47	18.412	0.37	Dodecanoic acid
48	18.471	0.33	Indole
49	18.568	0.45	Succinimide
50	18.705	1.19	5-Hydroxymethylfurfural (HMF)
51	18.954	0.39	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z)-
52	19.146	0.54	Phenol, 4-ethenyl-2,6-dimethoxy-
53	19.196	0.47	Phytol
54	19.225	0.30	Tetracosamethyl-cyclododecasiloxane
55	19.282	0.55	Hexanoic acid, 2-methoxyethyl ester
56	19.818	0.39	Pentadecanoic acid
57	20.208	3.78	Catechol
58	20.323	3.77	Niacin
59	20.591	0.58	Tetracosamethyl-cyclododecasiloxane
60	21.266	0.46	Nonanedioic acid, monomethyl ester
61	21.830	1.58	n-Hexadecanoic acid
62	22.826	0.50	Tetracosamethyl-cyclododecasiloxane
63	23.971	3.32	Hydroquinone
64	24.972	1.10	Octadecanoic acid
65	25.612	1.12	9-Octadecenoic acid, (E)-
66	26.161	0.60	Bis(2-ethylhexyl) phthalate
67	26.579	45.37	Caffeine
68	26.802	1.02	9,12-Octadecadienoic acid (Z,Z)-
69	27.699	0.64	Xanthine, 1,3,7,8-tetramethyl-
70	28.594	0.74	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-
71	28.844	0.90	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro-
72	30.198	0.26	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl)
73	30.897	0.52	Hydantoin
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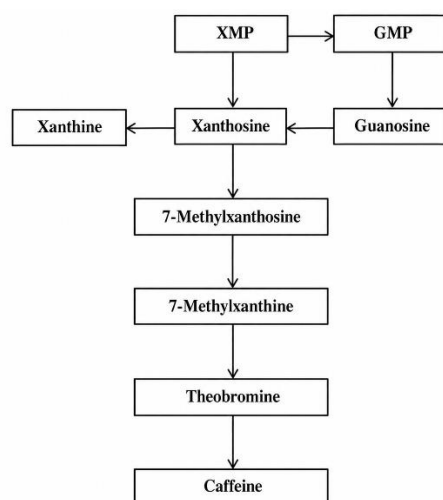


Figure 2. Biosynthesis caffeine of robusta [25].

3.2. Prediction of Bioactivity of Leaf *C. canephora*

The dominant metabolites detected by GC-MS were analyzed for bioactivity in silico using PASS Online. Testing was only conducted on dominant compounds, as they were considered to have the largest %area and potentially greater active properties compared to minor %area metabolites. Bioactive analysis of dominant compounds aims to profile the bioactivity of metabolite compounds detected in robusta coffee leaves for further experimental analysis in the future. The following is Table 2. Results of in silico bioactivity predictions for metabolite compounds using PASS Online.

Table 2. In silico bioactivity of metabolite compounds dominant of leaf *C. canephora*.

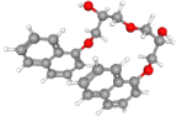
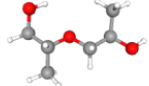
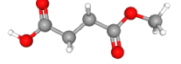
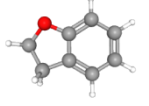
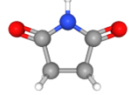
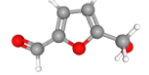
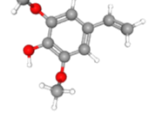
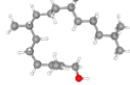
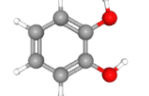
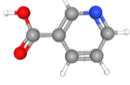
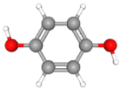
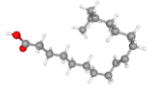
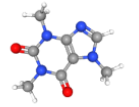
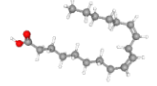
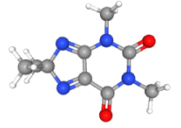
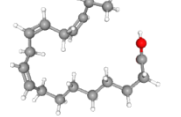
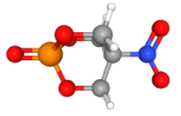
Peak	Metabolite Compound	Prediction Bioactivity	Pa	Structure
18	2-Propanol, 1,1'-oxybis-	Membrane integrity agonist	0,943	
21	1-Propanol, 2-(2-hydroxypropoxy)-	Antidiabetic	0,764	
43	Butanedioic acid, monomethyl ester	Antieczematic	0,855	
46	Benzofuran, 2,3-dihydro-	Antidyskinetic	0,819	
49	Succinimide	Antiviral (Picornavirus)	0,725	
50	5-Hydroxymethylfurfural (HMF)	Sugar-phosphatase inhibitor	0,859	
52	Phenol, 4-ethenyl-2,6-dimethoxy-	Carminative	0,874	
53	Phytol	Phobic disorders treatment	0,893	
57	Catechol	Antifungal	0.961	
58	Niacin	Anti-inflammation, anti-aging, anti-neurodegeneration	0,930	
61	n-Hexadecanoic acid	Anti-eczematic	0,920	

Table 2. In silico bioactivity of metabolite compounds dominant of leaf *C. canephora* (continue).

Peak	Metabolite Compound	Prediction Bioactivity	Pa	Structure
63	Hydroquinone	Antiseborrheic	0.927	
65	9-Octadecenoic acid, (E)- [Elaidic acid]	Antimutagenic	0.852	
67	Caffeine	Analeptic	0.854	
68	9,12-Octadecadienoic acid (Z,Z)- [linoleic acid]	Angiogenesis stimulant	0.832	
69	Xanthine, 1,3,7,8- tetramethyl-	Pterin deaminase inhibitor	0,726	
70	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	Anti- hypercholesterolemic	0,826	
71	1,3-Propanediol, 2- (hydroxymethyl)-2-nitro-	Monodehydroascorbate reductase (NADH) inhibitor	0,852	

Based on PASS online in Table 2, the bioactivity of the dominant metabolite compound shows a strong different predicted bioactivity potential, as indicated by a Pa value (>0.7). Caffeine (peak 67) (a compound characteristic of coffee plant) has the largest %area in Robusta coffee leaf and is predicted to be bioactive as an analeptic ($Pa = 0.854$). Analeptic is a bioactive property closely related to the medulla oblongata central nervous system. Sample with analeptic activity can cause effects such as increased awareness, stimulation of the respiratory system brain, and the ability to reduce drowsiness [27], [28]. Pharmacologically, analeptic properties must be studied in greater depth, because analeptic properties as stimulants cause temporary vasoconstriction and increase blood pressure in the short term [28].

Observations conducted in Pakusari Village, many people of the general public consume coffee leaf herbs as a remedy for high blood pressure. Reviewing the results of Pass Online, several metabolite compounds have the potential to be indirectly linked to high blood pressure, one of which is 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (peak 70), which acts as an anti-hypercholesterolemic agent ($Pa = 0.826$). Hypercholesterolemia is a condition in which cholesterol levels high increase abnormally. This increase in cholesterol is caused by several factors, one of which is excessive fat accumulation [29], [30].

Octadecatrienoic acid, which has potential as an antihypercholesterolemic agent using PASS Online, can indirectly affect blood pressure. This is because high cholesterol, especially low-density lipoprotein (LDL), can trigger the formation of atherosclerotic plaque, which can reduce and inhibit vascular elasticity, potentially leading to blockages in the blood vessels and the heart pumping blood at high pressure (hypertension) [29], [31]. The presence of octadecatrienoic acid, which acts as an anti-hypercholesterolemic agent, can indirectly prevent hypertension by preventing the clumping of cholesterol, according to preliminary in silico studies [32]. Octadecatrienoic acid, which is a derivative of α -linolenic acid, can

suppress PPAR α activation in hepatocytes, and this activation can reduce lipids in the liver. Octadecatrienoic acid or α -linolenic acid can lower blood lipid levels in patients with hyperlipidemia.

Further research conducted on robusta coffee leaves to develop their potential as a natural medicine. Robusta coffee leaves from Ampel Gading, Malang City, Indonesia, have potential as an antidiabetic agent, the test demonstrated by laboratory using Wistar rats. The testing can a reduction in blood sugar levels in diabetic test animals and helped repair pancreatic β cells [33], [34]. Internationally, Robusta coffee leaves have been metabolomics profiling using chromatography instrumentation, with results several sources of metabolites such as flavonoids, phenolics, tannins, and alkaloids that are highly promising in the field of health [35]. In addition, the presence of these metabolite compounds has the potential to act as natural antioxidants, as they can counteract free radicals [36]. This study was unable to detect chlorogenic acid compounds, which act as antioxidants and anti-inflammatories, because these compounds are found in abundance in Robusta and Arabica coffee beans [24], [37], [38].

Based on the GC-MS detection results when correlated with the phenomenon in Pakusari Village, further research needs to be conducted on robusta coffee leaves. The many people in Pakusari believes that robusta coffee leaves can optimally lower high blood pressure, but the detection results show that caffeine metabolites were detected with an area percentage of 45.37%, with in silico bioactive results as analeptics (which can cause short-term high blood pressure). Although there are compounds that have the potential to be indirect anti-hypertensives, the percentage area content of the robusta coffee leaf sample is still dominated by caffeine [39], [40]. However, research on metabolite compounds in robusta coffee leaf extracts with medicinal potential must continue, as several potential compounds have been successfully detected.

4. CONCLUSION

The detection of volatile compounds using GC-MS of Robusta coffee leaf extracts from Pakusari, Jember, revealed 73 metabolite compounds. Caffeine was one of the compounds detected with the highest %area, namely 45.37%. Several dominant compounds detected were subjected to in silico bioactivity using PASS Online, with some producing bioactive properties as analeptics and anti-hypercholesterolemic agents. These analysis results are not in line with the phenomenon occurring in Pakusari Village, which considers coffee leaf herbs to be antihypertensive. Further detection optimization is needed to prove this phenomenon.

AUTHOR CONTRIBUTION

All author contributed equally to the main contributor to this paper. **Abdillah Maulana Farhan:** Writing (original draft), supervision, funding. **Putri Aisiyya Qutlana Munawaroh:** Writing (review & editing), investigation, formal analysis.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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