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Research Article

Preliminary Data on the Antibacterial Activity of *Coffea Arabica, Coffea Canephora* and *Coffea Liberica*

Madyawati Latief, Heriyanti, Indra Lasmana Tarigan, Sutrisno*

ABSTRACT

The continuous use of synthetic antibiotics will not only kill bacteria but also can accelerate the emergence of resistant pathogenic races. In addition, it can also cause the death of normal flora. Coffee extract is reported to have the potential as a naturally active and non-toxic antibacterial compound suitable for biomedical applications. Extraction using methanol was carried out on green coffee and which had been roasted for three types of Jambi coffee, namely Arabica, Robusta and Liberica coffee and continued with fractionation using hexane and ethyl acetate. The results of the analysis using FTIR showed that OH, C-H, C = C, C = O, C-O, C-N and N-H groups were detected. These functional groups are probably the functional groups that belong to caffeine, trigonelline, nicotinic acid and dehydrocafestol, which are believed to have bacteriostatic effects on some bacteria. The SEM-EDX analysis results of the three types of coffee showed that the dominant elements were O, K and Mg. Potassium and magnesium minerals bind with chlorogenic acid to form salt complexes of chlorogenic acid and magnesium chlorogenic acid complex. The antibacterial activity of the coffee extract and fraction was still low with the diameter of the inhibition zone was still low (0-10 mm). Furthermore, further characterization and tests are needed to confirm the antibiotic potency of the Arabica, Robusta and Liberica coffee ethanol extracts.

Key words: Antibiotics, Coffee, *E. coli.*

Madyawati Latief, Heriyanti, Indra Lasmana Tarigan, Sutrisno*

[1]*Department of Chemistry, Faculty of Science and Technology, Universitas Jambi, INDONESIA.*
[2]*The University Center of Excellence, E2-KOLIM, Universitas Jambi, INDONESIA.*

Correspondence

Sutrisno

Department of Chemistry, Faculty of Science and Technology, Universitas Jambi, INDONESIA.

The University Center of Excellence, E2-KOLIM, Universitas Jambi, INDONESIA.

E-mail: herasutrisno@unja.ac.id

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INTRODUCTION

Humans cannot avoid accidents that can cause minor wound or even life threatening injuries. Wounds can be defined as defects or injuries to living tissue caused by physical or thermal disturbances that arise pathologically and physiologically.[1] The wound healing process will usually take some time and varies in time to heal and depends on the immune system. If the wound is left open there is a possibility of secondary infection. Apart from causing the wound healing to take longer, the infection will also cause pain and be unsightly. Infection is the invasion of the organism's body tissue by infectious agents (pathogens), multiplication of pathogenic agents and the reaction of the host tissue to infectious pathogenic agents and the resulting toxins.[2] Infections are caused by pathogens, namely viruses, fungi and bacteria. Humans can fight infection using their immune system. Infected body tissue reacts to infection with an innate response, often involving inflammation, followed by an adaptive response.[3] Before bacteria multiply and cause symptoms, the immune system can usually kill the pathogenic bacteria. White blood cells attack harmful bacteria and even if symptoms appear, the immune system can usually fight off the infection. However, if the number of harmful bacteria is excessive and the immune system cannot fight back then antibiotics are needed.

Antibiotics are a type of antimicrobial agent that is active against bacteria and is the most important type of antibacterial agent for fighting bacterial infections. Antibiotic drugs are widely used in the treatment and prevention of infections. Antibiotics can kill or inhibit bacterial growth. A small number of antibiotics also have antiprotozoal activity. When an infection is suspected to be the cause of a disease but the type of bacteria has not been identified, empirical therapy is needed. This therapy takes several days and involves administration of broad-spectrum antibiotics.[4] Once the pathogenic microorganism has been identified, definitive therapy can be initiated using narrow spectrum antibiotics. Identification is very important because it can reduce the cost and toxicity of antibiotic therapy and also reduce the possibility of developing antimicrobial resistance.[4]

The emergence of bacterial resistance to antibiotics is a common phenomenon. The emergence of resistance often reflects the evolutionary process that took place during antibiotic therapy. Antibiotic treatment of bacterial strains with physiologically or genetically enhanced capacity to withstand high doses of antibiotics. Under certain conditions, it can cause the growth of resistant bacteria, while the growth of susceptible bacteria is inhibited by drugs.[5] In addition, irregularity, incomplete treatment and uncontrolled use of antibiotics can also lead to resistance if symptoms recur. There are several molecular mechanisms of antibacterial resistance. Intrinsic antibacterial resistance can be part of the genetic makeup of bacterial strains.[6,7] Resistance can come from mutations in the bacterial chromosome or the acquisition of extra-chromosomal DNA. The spread of antibacterial resistance often occurs through vertical transmission of mutations during growth and through genetic recombination of DNA with horizontal genetic exchange.[8] Cross-resistance against multiple antibacterials can also occur when a resistance mechanism encoded by a single gene conveys resistance to more than one antibacterial compound.[9]

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Antibiotics that cause resistance include glycoside acids (dibekasin, gentamicin, kanamycin, netilmicin and tobramycin), cephalosporins (cephalexins, ceftizoxime and ceftriaxone), penicillins (amoxicillin, sulbenicillin, penicillin G and ampicillin) and other groups such as tetracyclines, cotrimicin.[10] Meanwhile, some of the microorganisms that were reported to be resistant were *Candida* sp., S. *epidermidis*, S. *aureus*, Streptococcus sp, *Enterococcus* sp and *E. coli*.[11,12] *E. coli* (superbug) bacteria were identified in the United States as resistant to the colistin antibiotic.[13,14]

Apart from killing bacteria, the continuous use of synthetic antibiotics can also accelerate the emergence of resistant pathogenic races. In addition, it can also cause the death of normal flora. The consideration of resistance and the side effects of using chemical antibiotics is the reason for treatment using natural ingredients to be an option. Several studies related to the treatment of bacterial infections using natural ingredients have been widely applied using natural ingredients because they are easy to use, cheap and have adequate bactericidal or bacteriostatic effects. In addition, natural ingredients rarely cause adverse side effects compared to synthetic materials.[15]

Natural antibiotics derived from natural ingredients can be used to meet the need for new antibiotic sources. Bioactive chemical compounds (secondary metabolite compounds) derived from plants that have medicinal properties play an important role in biological activity, as an indicator of the ability of these natural substances as a source of new medicinal compounds. Scientists take ethnobotany and chemotaxonomic (phytopharmaca) approaches in an effort to search for natural chemical compounds for medicinal sources.[16] Coffee extract is reported to have the potential as a naturally active and non-toxic antibacterial compound suitable for biomedical applications. Significant bacteriostatic effects were seen in Staphylococcus aureus and Staphylococcus epidermidis at short exposure times and became bactericidal after prolonged exposure.[17] A significant cytotoxicity potential was also demonstrated in the evaluation of breast adenocarcinoma MCF7 cells only after 24 hours of exposure and at higher concentrations.[17] Coffee bean powder contains chlorogenic acid which is strongly suspected to have an antioxidant effect so that it can protect the body from the effects of free radicals and anti-bacterial *Methicillin Resistant Staphylococcus aureus* (MRSA) which can cause opportunistic infections in the injured area. Therefore, coffee can be used to accelerate wound healing.

The results of preliminary analysis studies using LC-MS on Liberica, Arabica and Robusta coffees show that the roasting process causes changes in the composition of the compounds contained in coffee. In Liberica coffee after roasting, there are no derivative compounds of chlorogenic acid, quinic acid, and ferulic acid.[18] Green arabica coffee contains chlorogenic acid and green robusta coffee contains ferulic acid derivatives. Meanwhile, roasted robusta coffee contains derivative compounds of ferulic acid and quinic acid. Preliminary studies that have been carried out show that arabica, robusta and liberal coffee contain derivatives of chlorogenic acid, ferulic acid and caffeine which are antioxidants and can be used as anti-aging.[18,19]

The results of SEM-EDX analysis of robusta coffee show that the elements that dominate the three types of coffee roasted at 200oC are elements C, O and K with a percentage of about 56%, 42% and 1% respectively.[18] The main minerals contained in the three types of coffee are potassium, magnesium, calcium, sodium, iron, cuprum and zinc. The mineral content in coffee is chemically related to the main constituent elements of coffee, for example, the mineral potassium binds to chlorogenic acid to form complex salts of chlorogenic acid and others.

Information about the potential of coffee bean compounds against the extract and its fraksinate has not been studied in more depth. Based on these reasons, it is necessary to isolate and characterize the active compounds of antibiotic drugs. In this study, 3 types of coffee extraction and fractionation will be carried out, Arabica, Robusta and Liberica coffee with a multilevel maseration technique based on differences in polarity, followed by the isolation of antimicrobial active compounds guided by antimicrobial bioassays. The pure compounds obtained will be continued with identification through spectroscopy. Spectroscopic methods include UV, IR and ^{1}H, ^{13}C 1 and 2-dimensional NMR and MS. The results are expected to be able to answer the types or groups of active compounds that have antibiotic properties and at the same time know the characteristics of the active compounds as new antibiotic candidates that can overcome the problem of antibiotic resistance.

MATERIALS AND METHODS

Materials

The coffee beans used in this research were robusta, Liberica and arabica coffee collected from Kerinci Regency (Robusta and Arabica) and Liberica from Tanjung Jabung Barat Regency, Jambi Province. The solvents used for extraction and chromatography were technical solvents that have been distilled, namely; methanol, n-hexane, benzene, diethyl ether, methylene chloride, acetone, ethyl acetate and chloroform (Merck). Vacuum liquid chromatography with Merck 60 GF254 silica gel, gravity chromatography with Merck 60 silica gel (230 - 400 mesh), and purity analysis of compounds by thin layer chromatography on plates coated with Merck 60 GF254 silica gel, 0.25 mm will be carried out according to the procedure standard. Determination of the melting point was carried out with the Fisher John melting point apparatus, for the prospective chemical structure determination of compounds as antibiotics, UV-Vis, IR, NMR and MS spectroscopy was required. Petri dish, incubator, autoclave, laminar air flow cabinet, loop and ELISA plate. The incubation site used in the antibiotic activity test was CO_2 incubator. Examination of the effect of active compounds on mice and their effects on the host tissue was carried out under the observation of Electron Microscopy.

Extraction and isolation

A total of 50 g of roasted Arabica, Robusta and Liberica coffee were extracted with methanol as a solvent, followed by fractionation using ethanol, n-hexane, dichloromethane and ethyl acetate as solvents. The initial methanol extract was also tested for antibiotics against Ethanol Fraccinate, n-hexane, dichloromethane and ethyl acetate. For active extracts and Fraccinate, it was continued for determination or phytochemical testing. For Fraccinate that contain chlorogenic acid, condensed proanthocyanidin, quinic acid and ferulic acid and are active as antibiotics followed by isolation of chlorogenic acid compounds, condensed proanthocyanidin, quinic acid and ferulic acid by performing TLC, gravity column chromatography and CVC. Each stage of isolation was guided by activity tests on mice. The isolates were determined by the type and nature of the compounds and their characteristics using spectroscopy, namely UV-Vis, FT-IR, NMR and MS spectroscopy.[20]

Antibacterial activity

The antibacterial activity test was carried out according to previous studies by agar diffusion method, using paper-discs with diameter 6 mm. The medium used for bacterial growth was nutrient agar. The bacteria were inoculated into Mueller Hinton Broth (MHB) medium then incubated for 24 hours at 37°C. The suspension of incubated bacteria was shaken with a centrifuge to make it homogeneous and then measured its transmittance at a wavelength of 580 nm. Transmittance (T) is set at 25% (the number of bacteria is approximately 10^5) by adding a bacterial suspension if the number of cells was too small or by adding a liquid MHB medium if the number of bacteria was too dense. 0.1 ml of 25% T bacterial suspension was put into a petri dish and 10

ml of un-frozen NA medium was added at a temperature of about 40°C. Shake the petri dishes until the bacterial suspension is completely mixed in the medium then let stand until the medium freezes or becomes solid. After that, the disc paper with a diameter of 6 mm was placed on the surface of the frozen culture medium, then aseptically 20 µl of the extract fraction solution was dropped with a concentration of 2% (20 mg / ml) which had been dissolved first in DMSO solution. Then incubated for 24 hours at 37°C. The antibacterial activity test is said to be positive if there is a clear zone around the disc paper that is free of bacterial growth.

RESULT AND DISCUSSION

Extract yield

The research samples were coffee beans from three types of coffee species, Arabica Coffee (*Coffea arabica*), Robusta (*Coffea canephora*) and Liberica (*Coffea Liberica*), which had previously been roasted, grinded and extracted with methanol solvent. The extraction technique used was the maceration method, which was cold extraction by immersing the sample using a solvent. Furthermore, the extract then partition with n-hexane and ethyl acetate. The partition results in the form of the n-hexane fraction, ethyl acetate fraction and the remaining fraction was the methanol fraction.[21]

Phytochemical screening

The screening of secondary metabolites compound which contained in coffee beans was carried out using phytochemical reagents. The results of phytochemical testing for all fractions are shown in Table 2.

Isolation and purification of compounds

The isolation of the compounds from each coffee bean extract was carried out using column chromatography, Liquid Vacuum Chromatography (LVC) and Gravity Column Chromatography (GCC). Before separating the compounds, the column packing was carried out prepare the column with the stationary phase. Then the samples were prepared to be separated by impregnation.

Arabica coffee

The viscous extract from the partition of the Liberica coffee beans was separated by means of LVC. Preparation begins with mixing 15 grams of sample with 15 grams of silica (impregnation). Furthermore, elution was carried out with a gradient of eluent ratios ranging from n-hexane to methanol. The GCC results obtained 21 vials. All vials obtained were tested by TLC. Based on the TLC chromatogram, vials which have the same stain pattern are combined in 1 fraction. From the results of this test, 3 combined fractions were obtained, namely F1: 3-11. F2: 12-17. F3: 18-2. The TLC chromatogram as a result of CVC hexane extract of Arabica coffee can be seen in the following figure. Crystals in the form of a yellow precipitate were screened for phytochemicals for alkaloids, flavonoids and tannins and the results were positive isolates containing alkaloids.

Antibacterial activity

Antibacterial activity test was carried out on the hexane fraction, ethyl acetate and methanol. The inhibition zone of Arabica, Robusta and Liberica coffee presented in Table 3.

The antibacterial activity of the Arabica coffee fraction was tested against *Escherichia coli* bacteria. The inhibition zone data from the antibacterial activity test results are shown in Table 3. The antibacterial activity test was carried out on the hexane, ethyl acetate and methanol fractions of arabica coffee by increasing the test concentration. Based on the diameter of the inhibition zone of all fractions in Arabica coffee, it was shown that the methanol fraction had higher antibacterial activity than other fractions. This is presumably because the methanol fraction contains many polar compounds such as phenolic groups, which generally act as antibacterial. In addition, the spectrum pattern in Figure 2, their main functional groups that characterize the presence of caffeine and other compounds as shown in Table 7. The following table shows the functional groups recorded in green Arabica coffee samples and roasted at 200°C and 230°C.

The peak in the 3300 cm^{-1} region is closely related to the O-H stretch group in green arabica coffee while for roasted arabica coffee it is

Table 1: Extract mass and yields.

Extract	Mass extract (g)	% yield
Arabica		
n-Hexane	92	38.17
Ethyl Acetate	85	35.27
Methanol	62	25.73
Robusta		
n-Hexane	21.81	10.33
Ethyl Acetate	44.59	21.13
Methanol	53.77	25.48
Liberica		
n-Hexane	21.23	9.40
Ethyl Acetate	31.21	13.63
Methanol	46.53	20.32

Table 2: Phytochemical test results of methanol extract of arabica, robusta and Liberica.

Secondary Metabolites	Result		
	Arabica	Robusta	Liberica
Alkaloids	+	+	+
Phenolic	+	+	+
Flavonoid	+	+	+
Terpenoid	+	+	+
Steroids	-	-	-
Saponin	-	-	-

Note: (+) : Presence/(-) : absent

Table 3: The inhibition zone of arabica, robusta and Liberica coffee bean extract against *Escherichia coli*.

Sample	Concentration (%)	Inhibition Zone Diameter (mm)		
		Arabica	Robusta	Liberica
Fraction N-Hexane	10	0.00 ± 0.01	6.72 ± 0.005	2.33 ± 0.01
	20	0.00 ± 0.01	7.29 ± 0.005	2.03 ± 0.01
	30	3.50 ± 0.02	7.26 ± 0.005	2.47 ± 0.01
	40	2.9 ± 0.02	8.69 ± 0.005	3.63 ± 0.01
	50	2.6 ± 0.02	8.6 ± 0.005	3.17 ± 0.01
	+	26.1 ± 0.01	41.53 ± 0.01	26.67 ± 0.01
	-	0.00 ± 0.01	0.00 ± 0.005	0.00 ± 0.005
Fraction Ethyl Acetate	10	2.9 ± 0.01	7.43 ± 0.005	5.2 ± 0.01
	20	5.6 ± 0.01	7.54 ± 0.005	2.9 ± 0.01
	30	5.9 ± 0.01	7.55 ± 0.005	3.3 ± 0.01
	40	6.2 ± 0.001	8.55 ± 0.005	3.7 ± 0.02
	50	5.6 ± 0.002	11.38 ± 0.005	4.27 ± 0.02
	+	34.8 ± 0.002	39.76 ± 0.005	27.27 ± 0.03
	-	0.00 ± 0.001	0.0 ± 0.005	0.00 ± 0.005
Fraction Methanol	10	6.9 ± 0.001	6.67 ± 0.005	1.13 ± 0.005
	20	7.00 ± 0.001	6.83 ± 0.005	2.30 ± 0.005
	30	8.4 ± 0.001	7.63 ± 0.005	3.33 ± 0.005
	40	8.8 ± 0.001	7.79 ± 0.005	4.50 ± 0.005
	50	9.1 ± 0.001	8.35 ± 0.001	5.23 ± 0.003
	+	40.59 ± 0.001	40.59 ± 0.001	25.57 ± 0.002
	-	0.00 ±0.001	0.00 ±0.001	0.00 ± 0.002

Table 4: FT-IR spectrum wave number of roasted arabica coffee.

Coffee Sample	Wavenumber (cm^{-1})	Functional Groups
Green Arabica	3291,92	O-H stretch
	2923,25	C-H stretch
	1641,76	C=C stretch
	1381,39	C-H bending
	1258,41	C-O streching
	1031,45	C-N streching
200°C	3353.72	N-H stretch
	2923.00	C-H stretch
	1734.33	C=O stretch
	1643.76	C=C stretch
	1238.38	C-O stretch
	1192.95	C-N stretch
	713.58	C-H rock
230°C	3349.06	N-H stretch
	2922.89	C-H stretch
	1733.04	C=O stretch
	1643.58	C=C stretch
	1238.20	C-O stretch
	1165.14	C-N stretch
	718.68	C-H rock

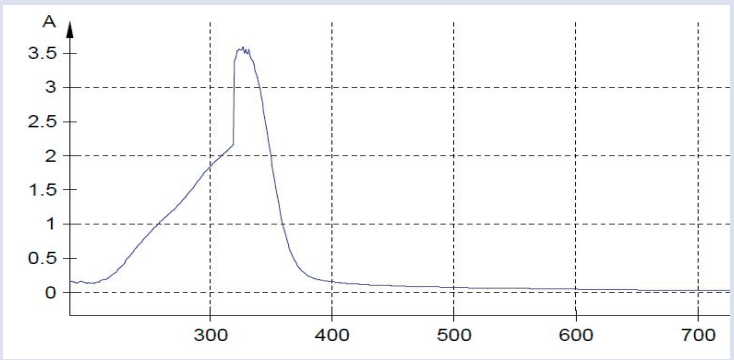

Figure 1: UV-Vis spectrum of isolate F2.

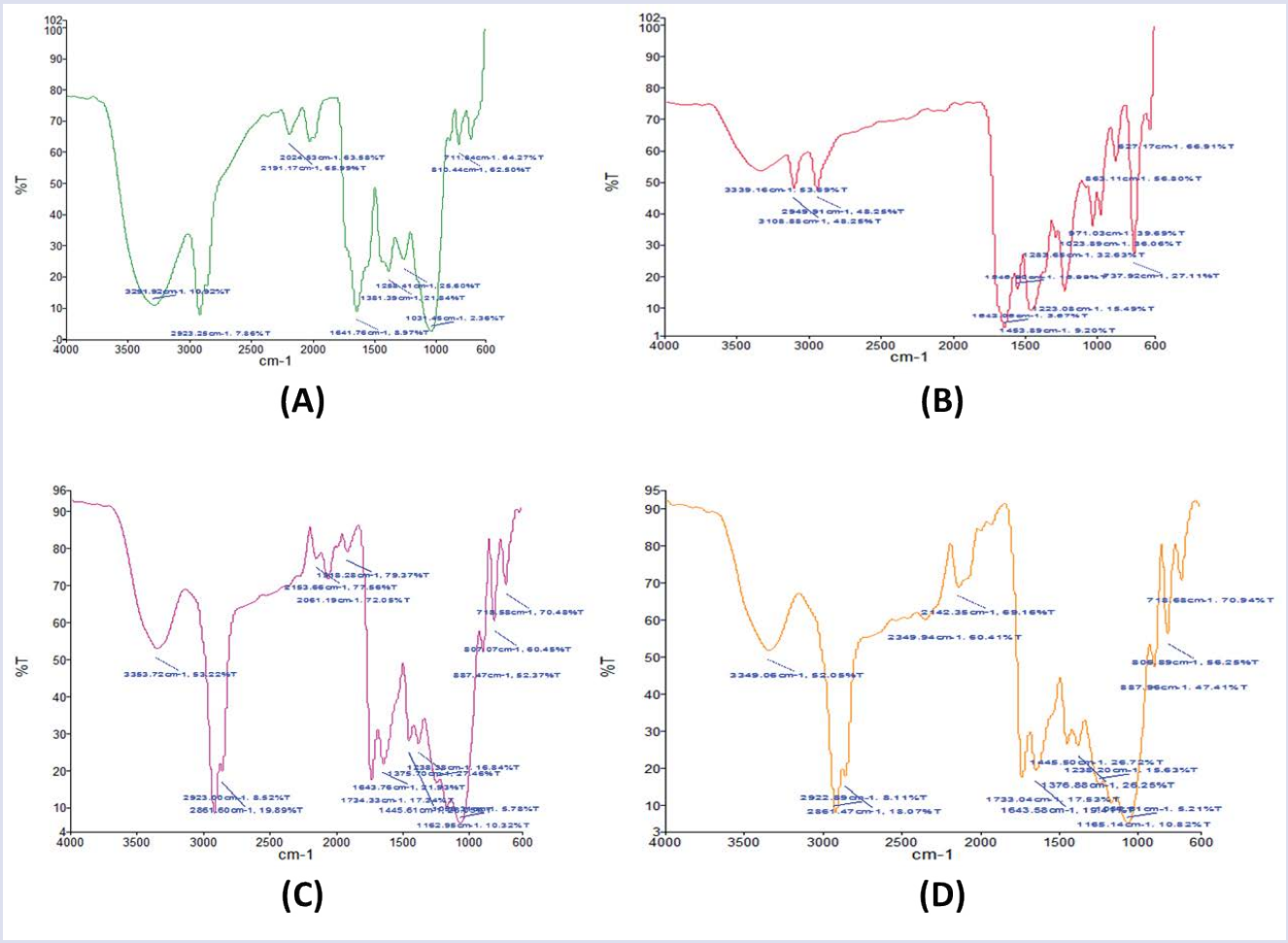

Figure 2: IR spectrum of arabica coffee extract (A) Green Arabica coffee (B) Isolate (C) Roasting 200°C (D) Roasting 230°C

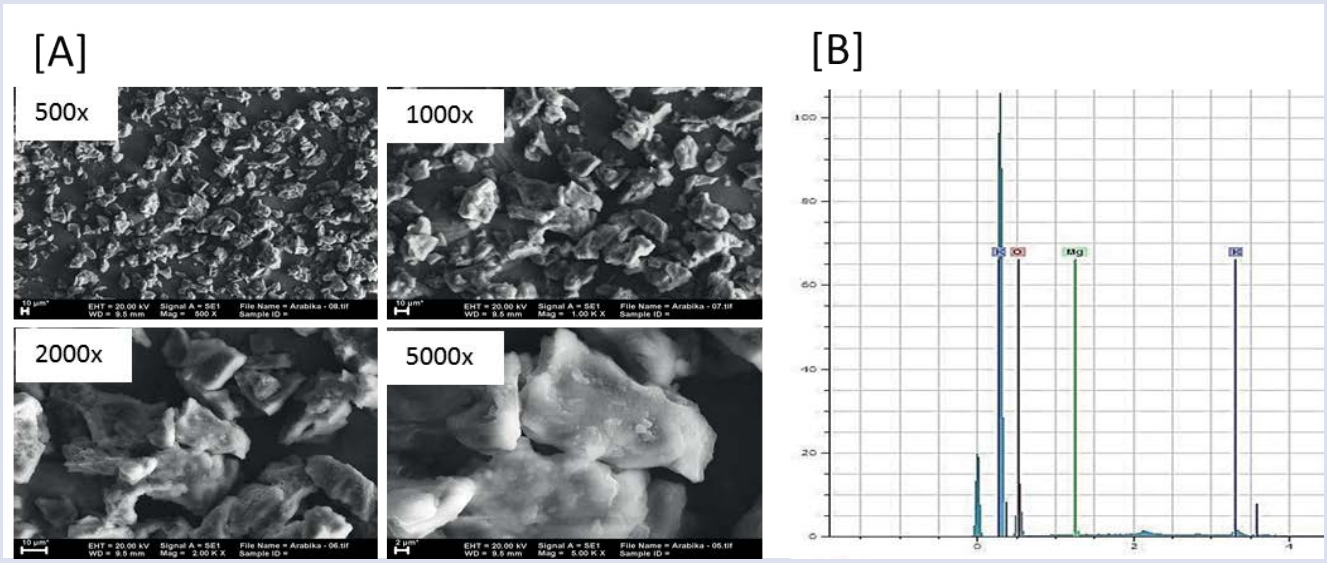

Figure 3: SEM-EDX results of roasted arabica coffee.

associated with the N-H stretch group, then in the 2900 cm^{-1} region it is associated with the C-H stretching. The absorption area of 1730 cm^{-1} was associated with the presence of a carbonyl group (C=O stretch), the band is strongly associated with the presence of caffeine and chlorogenic acid. The absorption area of 1600 cm^{-1} corresponds to C=C stretching, then 1238 cm^{-1} for C-O stretch and 1100 cm^{-1} for C-N stretch. In their result stated that the absorption area between 1194 and 925 cm^{-1} is suitable for C-O bonds in C-O-H bonds such as glycosidic bonds associated with polysaccharide sugars. The FT-IR spectrum isolated from Arabica coffee caffeine has an absorption area of 1180-1360cm^{-1} for CN for amine compounds, then C=C for alkenes in the absorption area of 1640-1680cm^{-1} and CH for compounds. alkanes in the absorption area 2850-2960cm^{-1}. The presence of C=O in the absorption region of 1743 cm^{-1} is associated with carbonyl in lipids or aliphatic esters.

The SEM-EDX results of roasted arabica coffee (Figure 3) show the presence of Mg, O and K metals and show that the entire surface of the roasted coffee is in the form of broken plates and there are several small holes that resemble pores. This is due to the fragility of the cell walls of coffee beans when heated. According to previous studies,[22] green coffee shows a relatively compact morphology compared to roasted coffee. When the coffee bean is heated to the initial crack, several cracks and pores on the surface begin to form. As the roasting continues into the tissue, porousness forms in the coffee. When the sample is baked into the second crack, the expansion of the pores is greater, and the wall thickness is reduced around the pores. The formation of color and flavor compounds increases the volume of coffee beans and changes the ultrastructure of the cell wall and cytoplasm of coffee beans. In particular, it can be assumed that the roasting process changes the cell wall porosity. The mineral content of roasted coffee was Mg, O and K

Coffee Liberica

The Liberica Coffee Fraction was also tested for antibacterial activity. The antibacterial activity test was carried out by the disk diffusion method, with the bacterial growth medium being nutrient agar and the type of test bacteria consisting of *Escherichia coli*. The positive control used was chloramphenicol which is a type of antibiotic. The antibacterial activity test data for Liberica coffee are shown in Table 3. The one that gave stronger antibacterial activity was the ethyl acetate fraction. This shows that compounds that are antibacterial for Liberica coffee are mostly found in the semipolar fraction.

The absorptions at 2900 cm^{-1} and 2800 cm^{-1} indicate the CH stretching absorption band of the methylene (-CH_2-) and methyl (CH_3) groups and are also supported by the appearance of the bending absorption band of the methylene (-CH_2-) and methyl (CH_3) groups at 1300cm^{-1}. Sharp C=O bands around 1700 in roasted coffee are associated with C=O groups in lipids, aliphatic esters and polysaccharides or hemicelluloses as shown in the table 10 that there is C=O absorption at wave numbers 1735 cm^{-1} and 1733 cm^{-1} while in green coffee there is no C=O absorption. The C=O absorption that appears in roasted coffee comes from the coffee oil produced during the roasting process. In addition, the absorption of C = C at wave number 1600 also explains the presence of lipids.

Chlorogenic acid has an absorption of about 1100 cm^{-1} or 1100-1300 cm^{-1}.[23] Acid compounds such as chlorogenic acid, citric acid and caffeic acid are explained by the presence of C-O, O-H and C-O-C absorption at wave numbers 1000-1100, 3000 and 1200 cm^{-1}. In this study, compounds of chlorogenic acid, citric acid and caffeic acid were only found in green Liberica which was indicated by the absorption of C-O at 1030.34 O-H at 3292.70 and C-O-C at 1263.79 cm^{-1}. While acetic acid compounds are only found in green Liberica and roasting 200°C which is indicated by the presence of C-O and O-H groups at wave numbers around 1000-1100 and 3000 cm^{-1}.

Analysis using SEM-EDX on 100 mesh Liberica roasted coffee aims to determine the morphological structure and minerals contained in roasted coffee, which can be shown in the Figure 7. Based on the results of SEM-EDX analysis, the dominating elements are elements O, K and also Mg with percentages of 98.55%, 1.11% and 0.34%. The SEM results found that coffee contained elements of O (30.72%), K (1.87%) and Mg (0.72%).

Coffee robusta

The data shown in Table 11 shows that the ethyl acetate fraction of robusta coffee has higher antibacterial activity than other fractions. This activity is due to the semi-polar fraction of robusta coffee having secondary metabolites that act as antibacterial. Based on the spectrum pattern in Figure 6, there are main functional groups that characterize the presence of caffeine and other compounds as shown in Table 6.

The FT-IR spectrum (Table 6) shows the presence of functional groups recorded in samples of green robusta coffee and robusta coffee after roasting. In the image of the FT-IR spectra of Robusta coffee, it shows absorption in the wave number range of 3500-3200 cm^{-1}, indicating the absorption of the OH group. This indicates a chlorogenic acid compound. This is in accordance with[24] that the O-H and C=C groups are closely related to chlorogenic acid. Furthermore, there are stretch N-H groups in the wave number range of 690-900 cm^{-1} supported by wave numbers in the range 1020-1250 cm^{-1} there are stretching C-N groups. Then the absorption at wave numbers 2922.43 cm^{-1} and 2922.63 cm^{-1} indicates the absorption of aliphatic CH_3 groups, this absorption is supported by the absorption of aliphatic CH_2 groups at wave numbers 2860.31 cm^{-1} and 2860.95 cm^{-1}. This is in accordance with[25] the FT-IR spectrum of caffeine isolation results in the presence of stretched N-H groups, stretched C-N, stretched C-H, then C=C stretched indicated the presence of caffeine compounds. While the groups that indicate the presence of trigonelin compounds are C=C stretched, C-N stretched and C=O stretched. These clusters were found in this study in the range of 2100-2260 cm^{-1} for the C=C group, the range of 1020-1250 cm^{-1} for the C-N group and the range of 1660-1765 cm^{-1} for the C=O group. Furthermore, there is an absorption band in the 900 – 1200 cm^{-1} region as shown in Figure 17 that there are broad features in the 1000 – 1320 cm^{-1} region resulting from CO stretching vibrations on COH bonds such as glycosidic bonds and associated with polysaccharide sugars. The presence of C=O in the range 1660-1765 cm^{-1} is associated with carbonyl in lipids or aliphatic esters. The absorption of C=O that appears in roasted coffee comes from the coffee oil produced during the roasting process.

The analysis using SEM-EDX of the robusta coffee sample aims to determine the morphological structure and the components contained in the robusta coffee sample.

The mineral content in coffee is chemically related to the main constituents of coffee. The minerals potassium and magnesium combine with chlorogenic acid to form salts of chlorogenic acid complexes and magnesium chlorogenic acid complexes. There is no significant difference in the mineral content of green coffee and roasted coffee because during roasting there is no change in minerals.

The characterization of F 1.5 Robusta isolate was carried out by UV-Vis spectrophotometric and Fourier Transform Infrared (FTIR) (Figure 8). The ultraviolet spectrum in Figure 8 shows the absorption at max (log): 315 nm (3.7). This spectral pattern indicates the presence of conjugated C=O. The IR spectrum showed the presence of absorption bands for the chelated carbonyl group (1643 cm^{-1}), –CH absorption.

The ^1H NMR spectrum shows a signal of three 3 protons H 3.38 ppm which belong to a methyl proton signal bound to nitrogen. The shift at 3.56 ppm is for the methyl proton located next to the carbonyl. The 3.970 ppm signal indicates a proton signal from the methyl that

Table 5: The spectrum data of FT-IR Liberica coffee.

No.	Reference[a]	Research Result			Functional Groups
		Green Liberica	Liberica 200°C	Liberica 230°C	
1.	-	712,32	720,91	721,20	C-H
2.	807	809,13	806,72	807,29	C-H
3.	1028	1030,34	1163,38	1167,65	C-O
4.	1239	1263,79	1240,09	-	C-O-C
5.	1376	1378,40	1378,75	1377,40	$-CH_3$
6.	1475	1405,58	1443,70	1446,18	C-H stretch
7.	1556		No detected		C=N stretch
8.	1645	1641,19	1640,92	1642,79	C=C stretch
9.	1743	-	1735,79	1733,47	C=O stretch
10.	2852	2865,28	2861,27	2860,69	C-H
11.	2922	2921,73	2922,98	2922,92	C-H
12.	3391	3292,70			O-H stretch
13.	-	-	3357,22	3363,89	N-H stretch

Note: (-) there is no absorption of functional groups.

Table 6: Identification result of FT-IR Robusta coffee.

No.	Wavenumber (cm^{-1})				Range (cm^{-1}) (Creswell, 2005; Stuart, 2004)	Note
	Green Robusta	Robusta 200°C	Robusta 230°C	Ballesteros et al, 2014		
1.	3293,02	3344,79	3342,63	3391	3200-3500	O-H
2.	2923,96	2922,43	2922,53	2922	3000-3100	C-H
3.	-	2860,31	2860,95	2852	2850-3000	C-H
4.	2164,20	2150,70	2168,28	-	2100-2260	C=C
5.	-	1735,19	1735,54	1743	1660-1765	C=O
6.	1637,88	1641,83	1640,68	1648	1640-1680	C=C
7.	1263,45	1236,43	1235,74	1239	1000-1320	C-O
8.	1032,29	1062,92	1058,47	-	1020-1250	C-N
9.	808,07	806,84	807,48	807	650-1000	C-H
10.	669,04	721,21	720,43	-	690-900	N-H

Table 7: Percentage of elements in the Robusta coffee sample.

Element	No. atom	Percentage (%)	
		Research Result	Previous studies
O	8	98,69	30,72
K	9	1,10	1,87
Mg	12	0,20	0,72

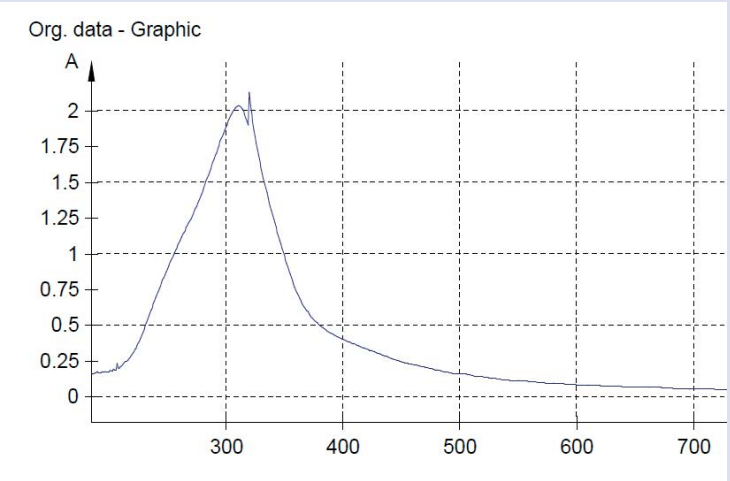

Figure 4: Spectrum UV-vis isolate F14 Liberica.

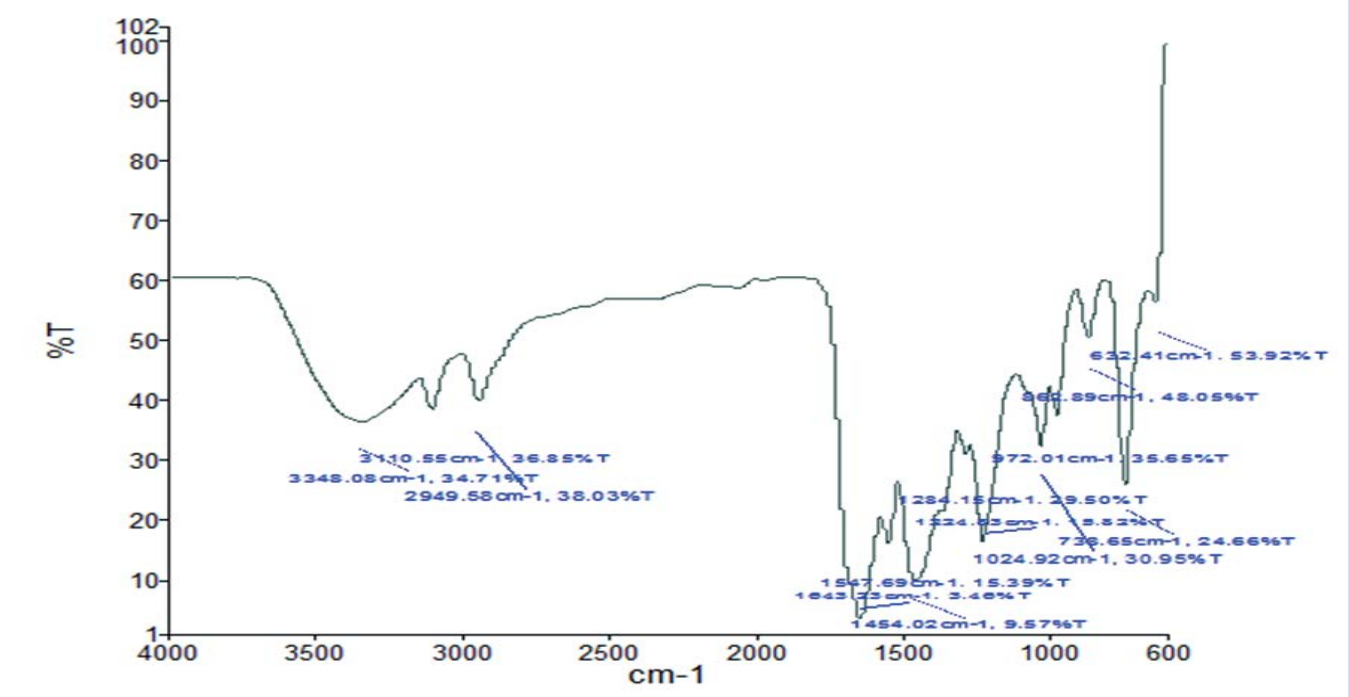

Figure 5: The FT-IR spectrum of isolate F14 Liberica.

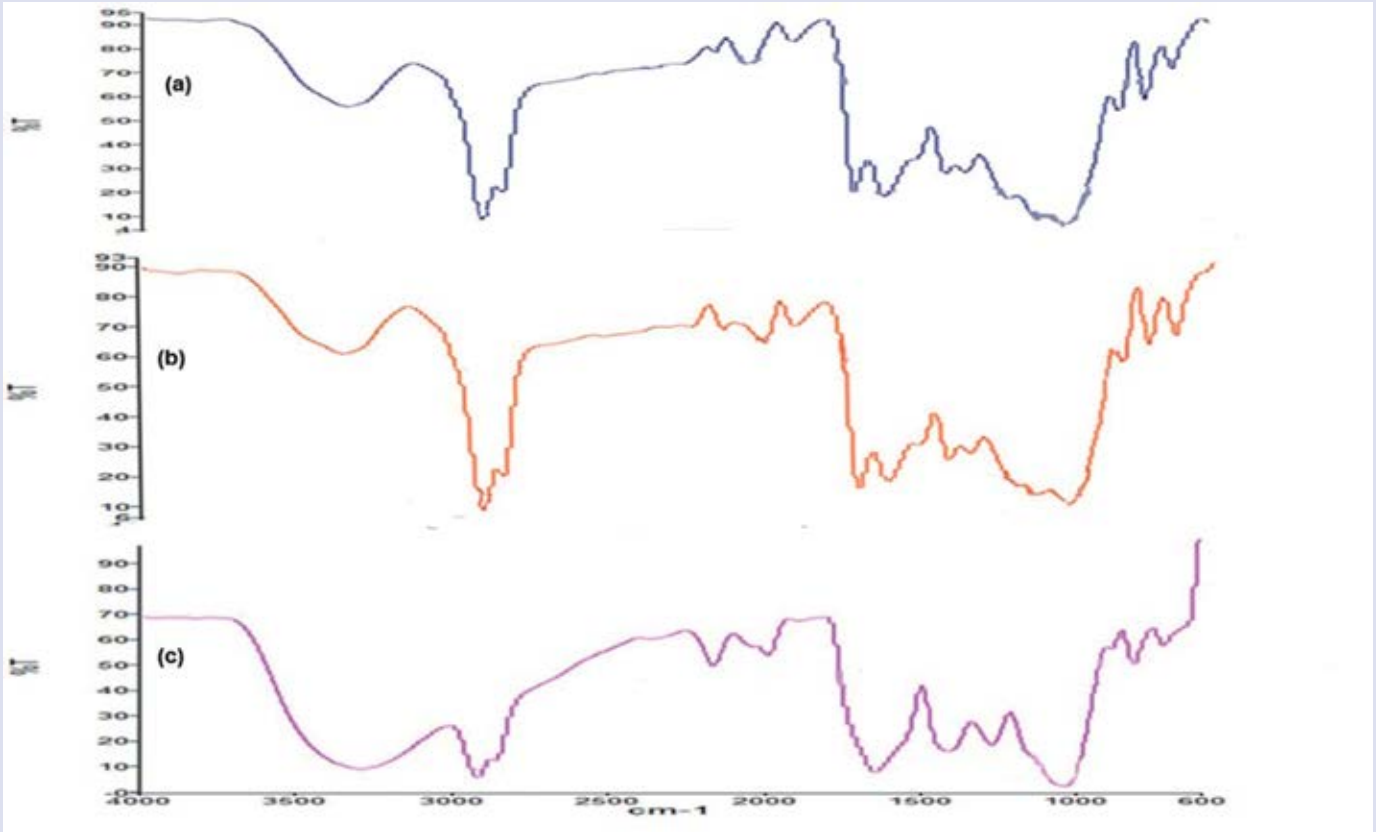

Figure 6: Spectrum of Liberica coffee powder (a) Roasted 200°C (b) Roasted 230°C and (c) Green

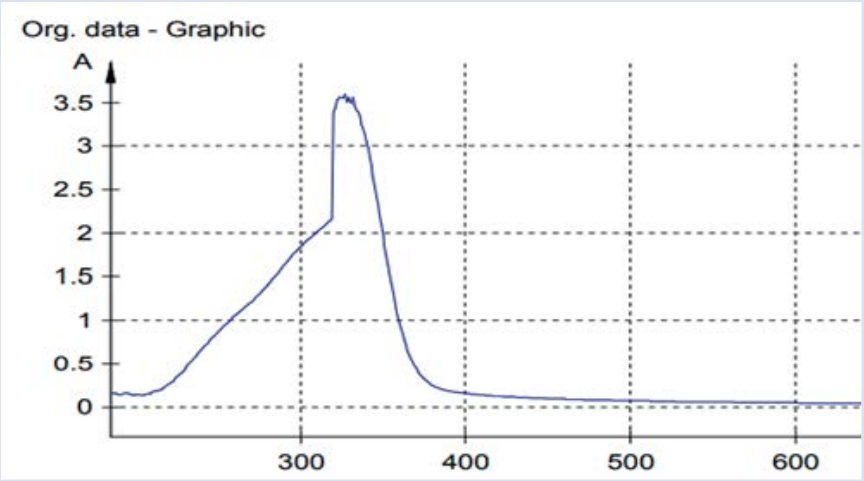

Figure 7: UV-Vis spectrum of F 1.5 robusta isolate.

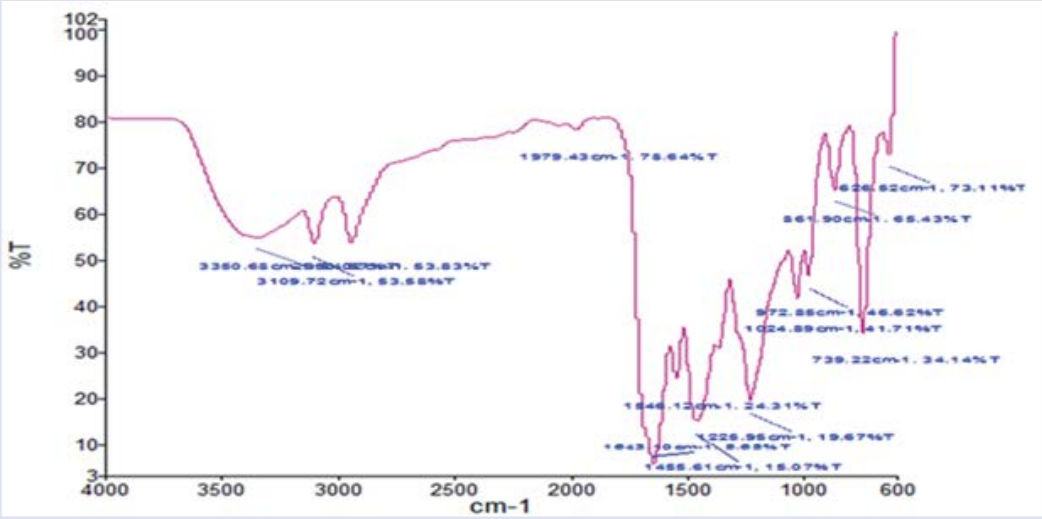

Figure 8: IR spectrum of F 1.5 robusta isolate.

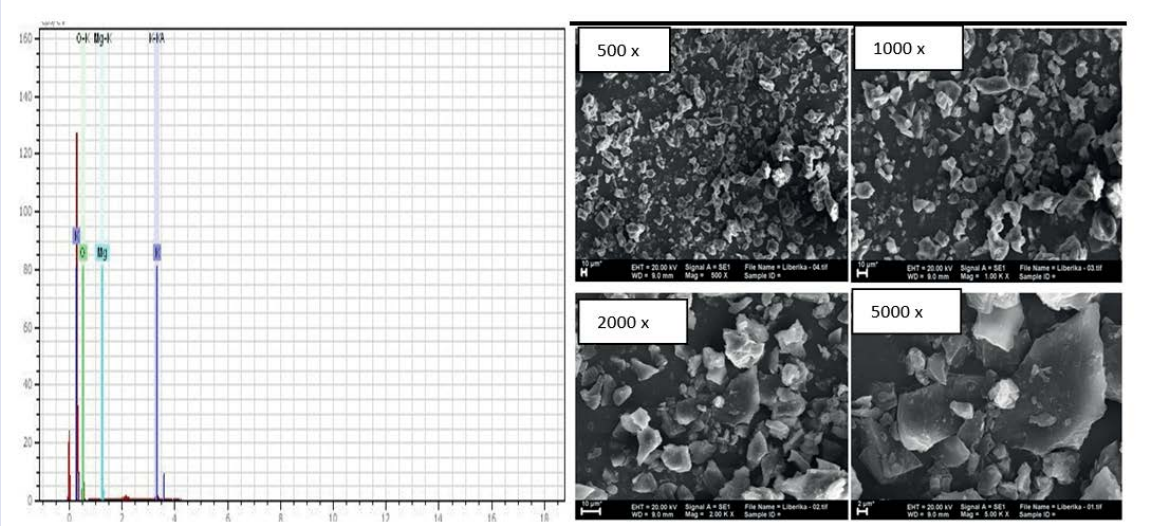

Figure 9: SEM-EDX analysis results of roasting Liberica coffee.

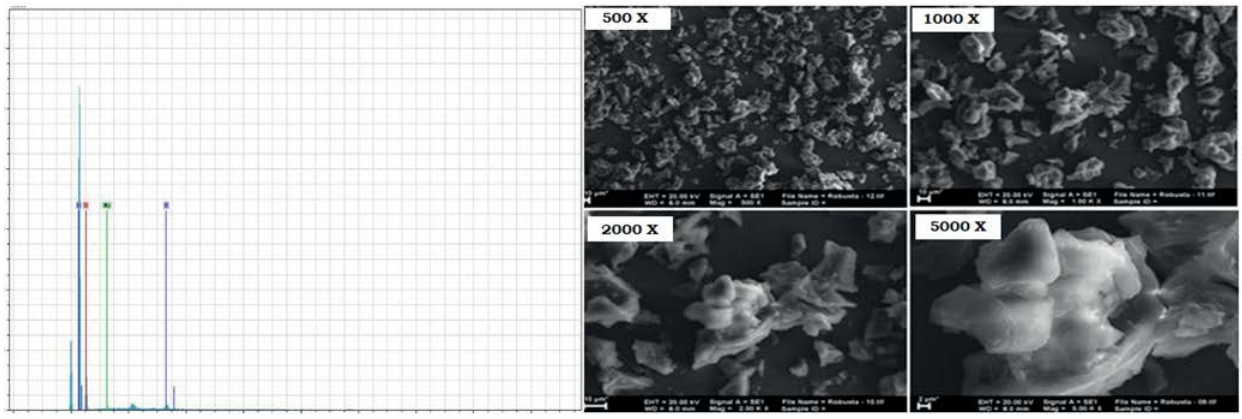

Figure 10: SEM-EDX analysis result of roasting robusta coffee.

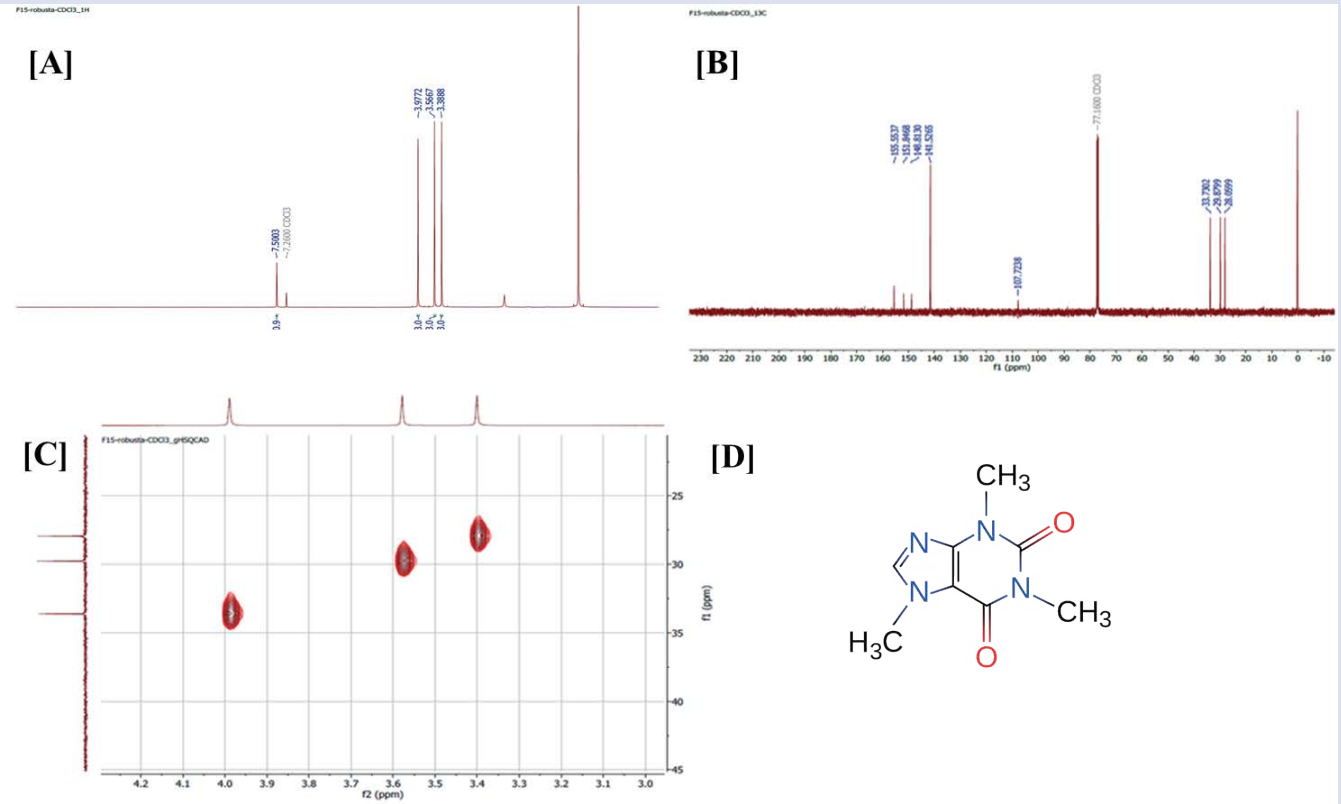

Figure 11: The spectrum of NMR robusta coffee (A) ^{1}H NMR isolat F1.5 robusta ($CDCl_3$, 500 MHz)(B) ^{13}C NMR isolat F 1.5. robusta (CD_3OD, 125 MHz); (C) Spektrum HMQC isolat F1.5. robusta (DMSO, ^{1}H-500 MHz, ^{13}C-125 MHz); (D) Caffeine

lies between the two carbonyls. The ^{13}C NMR spectrum showed the presence of 8 carbon signals consisting of three methyl carbon signals, one methine carbon, two quaternary carbons and 2-carbonyl carbons. The HMQC spectrum shows that the signal at H is 3.3; 3.5 and 3.9 ppm correlated with signals at C 28.06 ppm, 29.87 ppm and 33.73 ppm. From the spectroscopic data of UV, IR, Proton NMR and Carbon NMR, it is suspected that the compound that was successfully isolated was Caffeine with the following structure.

CONCLUSION

Our finding that, In the n-hexane and ethyl acetate fractions, the diameter of the Robusta inhibition zone was better than that of Arabica and Liberica. While the Arabica methanol fraction is better. Moreover, the analysis using FTIR showed that OH. C-H, C = C, C = O, C-O, C-N and N-H groups were detected. These functional groups are probably the functional groups that belong to caffeine, trigonelline, nicotinic acid and dehydrocafestol which are believed to have bacteriostatic effects on some bacteria. The SEM-EDX analysis results of the three types of coffee showed that the dominant elements were O, K and Mg. Potassium and magnesium minerals bind with chlorogenic acid to form salt complexes of chlorogenic acid and magnesium chlorogenic acid complex. The antibacterial activity of the coffee extract and fraction was still low with the diameter of the inhibition zone was still low (0-10 mm). Further characterization and tests are needed to confirm the

antibiotic potency of the Arabica, Robusta and Liberica coffee ethanol extracts.

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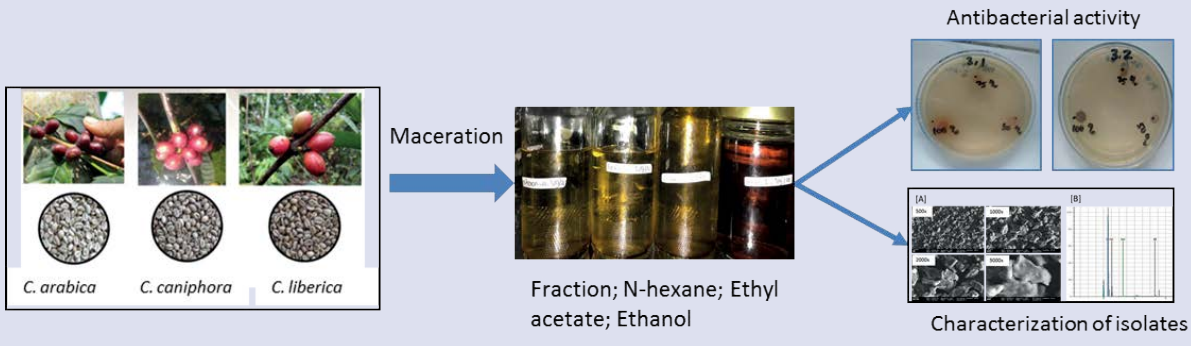
GRAPHICAL ABSTRACT



ABOUT AUTHORS

Madyawati Latief is a Lecturer and Researcher at Department of Chemistry, Faculty of Science and Technology, Universitas Jambi, Jambi, Indonesia. She has experiences in the area of Organic Chemistry, Natural Product, Bioactive Compound, drugs discovery of herbal plants, antibiotic, antioxidant, anti-inflammatory and infectious disease. The central focus of the research is exploration of bioactive compound from Indonesian plant and develop as a new drug candidate.

Heriyanti is a Lecturer and Researcher at Department of Chemistry, Faculty of Science and Technology, Universitas Jambi, Jambi, Indonesia. She has experiences in the area of Polymer and Physical Chemistry, Bioceramics, Synthesis of Hydroxypatite, volatile organic compound identification and Environmental chemistry

Indra Lasmana Tarigan is a Lecturer and Researcher at Department of Chemistry, Faculty of Science and Technology, Universitas Jambi, Jambi, Indonesia. He has experiences in the area of Biological Chemistry, Natural Product, Bioactive Compound, drugs discovery of plant and microbial, antibiotic, antioxidant, anti-inflammatory and infectious disease. The central focus of the research is exploration of bioactive compound from Indonesian plant and develop as a new drug candidate.

Sutrisno Sutrisno is a Professor at Department of Chemistry, Faculty of Science and Technology, Universitas Jambi, Jambi, Indonesia. He has experience in the area of Analytical Chemistry and Instrumentation of Chemistry, working in Applied Chemistry. His research also Coffee chemistry, Volatile Organic Compound identification and Environmental chemistry. Besides that he also has some research in learning development.

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