RESEARCH ARTICLE

Identifying metabolite profiling in unripe fruits of Kayu Banana (Musa paradisiaca L. var.Kayu) by Using LCMS instruments in different extraction methods

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Abstract The unripe fruit of a wood banana (Moses paradisiaca L. Var. Wood) is used in traditional medicine as an antidiarrheal drug in Lumajang Regency, East Java which has antidiarrheal activity. Phenolic compounds in the unripe fruit of wood bananas have antidiarrheal activity in vivo and in vitro. This study aims to prove the influence of extraction methods on the acquisition of phenolic acid levels of unripe fruit of wood bananas (Musa paradisiaca L. Var. Wood) and its secondary metabolite profile. The research method used is an experimentally causative method The extraction method used in this study is 2, namely: cold extraction (remaseration and maceration) and heat extraction (reflux and soxhlet). The unripe fruit of a wood banana (Moses paradisiaca L. Var. Wood) is distracted using four methods namely remaseration, maceration, reflux and soxlet. The extract obtained was tested for the total phenolic content (TPC). Analysis of secondary metabolite profiles using the UPLC-Q-TOF/MS instrument. Total phenolic data obtained were followed by ANOVA analysis. The result obtained in the extraction method with the remaseration extraction method was 99.31 ± 1.11 mgGAE / gram. Based on the data from the interpretation of compound content analysis using UPLC-QToFMS, it can be seen that there are 10 mayor compounds in the remaseration method, 17 mayor compounds in the maceration method, 15 mayor compounds in the reflux method, and 13 mayor compounds in the soxlet method.

Keywords: metabolite profiling, unripe fruits of Kayu Banana, Musa paradisiaca L. var.Kayu, LCMS, extraction methods, Diarrhoeal diseases.

Introduction

Diarrheal Disease is an endemic disease that has the potential to cause Extraordinary Events (KLB) and is still a contributor to the mortality rate in Indonesia, especially in toddlers (KEMENKES RI, 2021). Based on WHO 2019 Diarrhea is one of the diseases with the highest incidence and mortality rate in the world. There are reportedly around 1.7 trillion cases annually (Shen et al., 2019). Diarrheal disease is the second leading cause of death in children under five years of age, and each year it can kill about 525,000 children. Diarrhea can last several days, and can leave the body without the water and salt

necessary for survival(Putri et al., 2019).
The island of Java, especially the province of East Java, there is one city that has received the nickname "Banana City" which is none other than Lumajang City. One of them is the wooden banana, which has a special feature based on the empirical experience of the people of Senduro village, Lumajang, East Java, raw wood bananas can be used as an antidiarrheal medicine whose use is by burning, steaming, and boiling. research conducted by Ningsih, et al., in 2019 stated that ethanol extract of the raw fruit of wood banana has an antidiarrheal effect and has been tested on male mice of balb-C strains that make diarrhea(Ningsih, 2019).
Phenolics are a group of compounds consisting of aromatic rings containing one or more hydroxyl groups (Çoklar & Akbulut, 2017). All plants contain phenolic compounds in the form of glycosides and bind to proteins and then form a complex bond through hydrogen bonds (Rivai et al., 2010). Phenolic compounds in the unripe fruit of wood bananas have pharmacological activity as antidiarrheals in vitro and in vivo. The extraction method used may affect the concentration or loss of therapeutic effect of the extract due to the breakdown of the compounds and the decomposing contained during the extraction process(Hmidani et al., 2019).
The technique for obtaining phenolic compounds can use several extraction methods coldly and hotly. The cold extraction method uses remaseration and maceration extraction, while the hot extraction uses the soxhlet and reflux extraction method(Zhou Xu 1,†, Shiling Feng 1,†, Jipeng Qu 1, 2, Ming Yuan 1 & Ding, 2019). Several major industries have long applied continuous filtering so that they are efficient in terms of time, nutrient savings, and more extracted raw materials (Susanty & Bachmid, 2016). In general, various methods of extraction of bioactive compounds have their advantages and disadvantages therefore it is necessary to determine the most appropriate method of extraction of bioactive compounds taking into account factors such as temperature, extraction time, cost, yield and purity(Akyıl et al., 2020).
The choice of extraction method greatly affects the content of chemical compounds contained in a plant, especially compounds that are efficacious as antidiarrheals and affect the antidiarrheal activity produced. Based on the above background, a test will be carried out on the effect of the extraction method on phenolic levels and metabolite profiles in raw wood banana fruit (Musa paradisiaca L. Var. wood) with UPLC / Q-TOF MS analysis to characterize the differences in secondary metabolite profiles. This research is focused on profiling secondary metabolites of extracts with different extract methods and their phenolic content(Lee et al., 2014).

Material And Methods

Materials

The tools used in this study are oven, aluminum foil, scissors, blender, sieve No. 100, plastic jar, knife, glass jar, stirrer, filter paper, bunsen, hotplate, Buchner funnel, test tube, drip pipette, volume pipette, measuring pipette, measuring flask, porcelain cup, analytical balance, measuring cup, watch glass, glass beaker glass, erlenmeyer, glass funnel, macerator, rotary evaporator, drip plate, basin container, UV – vis spectrophotometry, UPLC-MS instrument, Ultrasonic Cleaner (Sonica) and cuvette.
The ingredients used in this study were raw wood bananas (Moses paradisiaca L. Var. Wood) which are characterized by banana peel that is still green and the fruit is still hard and is approximately 3 months old after the flower comes out, 96% ethanol, sterile aquadest, folin-ciocaltuae reagent, mayer reagent, dragendoorf, bouchardate, gelatin salt, HCl 2 N, magnesium, concentrated HCl, iron (III) chloride, CH3COOH, concentrated H2SO4, gallic acid, ethyl acetate, n-butanol, and methanol, methanol (hypergrade for UPLC), formic acid (ultrapure for UPLC), acetonitrile (hypergrade for UPLC), and water injection 0.05% for UPLC.

Plant Determination

The determination of the raw fruit of wood bananas was carried out at LIPI Purwodadi and the Food and Agricultural Security Service of Lumajang Regency.

Making Simplisia Unripe Fruit Banana Wood (Musa paradisiaca L.var. kayu)

A total of 12,959.9 g of unripe bananas were washed under running water until clean, drained, and weighed wet weight. Then the cutting is carried out, dried in a drying rack at a temperature of 50ºC, dry disortation, and weighed dry weight. The dry sample is then blended and then sifted and stored in a plastic container(Ningsih, 2019).

Extract Making

Remaseration: As much as 500 grams of simplicia powder of unripe fruit of wood banana (*Musa paradisiaca* L.var. Kayu) is macerated using 96% ethanol solvent at room temperature and stirring is carried out. The powder is soaked for 24 hours. Remaseration is carried out 2 times, filtering is carried out to separate residues and filtrates. The resulting maserat is then evaporated with a rotary evaporator at a temperature of 50ºC and evaporated until it becomes a viscous extract (Ningsih et al., 2020). Maceration: 500 grams of simplicia powder of raw fruit of banana wood is added with 96% ethanol solvent as much as 3,750 ml and maceration is carried out. After the whole powder is soaked, then stirring is done slowly and soaked for 5 days with stirring. The resulting maserat is then evaporated with a rotary evaporator at a temperature of 50ºC(Ningsih et al., 2020). Soxhlet :A total of 500 grams of simplicia powder is wrapped in filter paper and tied up and then put into the soxhlet extractor. A 96% ethanol solvent of 1.5 liters is put into a round base flask, then a soxhlet tool is assembled with a condenser. Extraction is carried out at a temperature of 60-80ºC until the liquid is colorless. The extract obtained was evaporated using a rotary evaporator at a temperature of 50ºC(Mokoginta et al., 2013). Reflux: A total of 500 grams of simplicia powder is put into the round base flask, then a 96% ethanol solvent is added. Assemble the reflux device, then the sample is extracted at a temperature of 50ºC for 2 hours. The solution obtained is filtered using filter paper and evaporated using a rotary evaporator at a temperature of 50ºC(Susanty & Bachmid, 2016).

Phytochemical Screening

Alkaloid Test: A total of 0.5 grams of wood banana fruit extract was added 1 ml of 2 N hydrochloric acid and 9 ml of distilled water, heated on a water bath for 2 minutes cooled and filtered. The filtrate is divided into 3 parts, each of which is added Mayer, Dragendorf, and Wagner reagents. Shows a positive result of alkaloids if with Mayer a white or yellow precipitate is formed, with Wagner a reddish-brown precipitate is formed and with Dragendorf a reddish-brown precipitate is formed(Endarini, 2016) (RI, 1980). Saponin Test: A total of 0.5 grams of powder is put into a test tube, 10 ml of hot water is added, cooled and then shaken for 10 seconds. If a stable foam of 1 to 10 cm high is formed for not less than 10 minutes and does not disappear with the addition of 1 drop of hydrochloric acid 2 N indicates the presence of saponins(Bonggol, 2018). Flavonoid Test: 0.5 grams of wood banana raw fruit extract is added to 20 ml of hot water, simmered for 10 minutes, and filtered hot. 5 ml of filtrate plus 0.1 g of Mg powder, 2 ml of amyl alcohol and 1 ml of concentrated hydrochloric acid, shaken and allowed to separate. The formation of a red, yellow, or orange color formed on the amyl alcohol layer indicates a positive presence of flavonoids (Ningsih et al., 2020). Tannin Test: 0.5 grams of raw fruit extract of banana wood is added with 10 ml of aquades. Then it is allowed to stand for 5 minutes and filtering is carried out. The filtrate is diluted with water until it is colorless. Next, the solution is taken as much as 2 ml added with 1 to 2 drops of 1 1% FeCl3%. Discoloration to green, blue or blackish indicates a positive result of tannins(Ningsih et al., 2020). Polyphenol Test: A total of 1 gram of raw fruit extract of banana wood was extracted with 15 ml of hot aquades then added 10% NaCl and filtered, the filtrate is divided into 3 parts (A, B, C). Filtrate A as a blank, filtrate B plus 3 drops of FeCl3 and filtrate C plus gelatin salt. Discoloration from green to blue-black indicates the presence of phenolic compounds(Hanani, 2015). Triterpenoid and Steroid Assay: A total of 0.1 gram of extract was added 3 drops of concentrated HCl and 1 drop of H2SO4. If red or purple color is formed then the positive contains terpenoids. If a green color is formed then positively contains steroids(Ergina et al., 2014). Anthraquinone Test: A total of 0.3 grams of the extract was extracted with 10 ml of aquades, then the filtrate was extracted with 3 ml of toluene and added ammonia. There is a change in color to red indicating positive anthraquinone(Muthia et al., 2019). Glycoside Test: 1 gram of viscous extract dissolved with ethanol, evaporated on a water bath, then dissolved in 5 ml of anhydrous acetic acid P. and added 10 drops of sulfuric acid P. blue or green color formed indicates the presence of glycosides(Program, 2014).

Determination of the total phenolic content of unripe wood banana fruit extract (Musa paradisiaca L. Var. Kayu)

The extract solution is picketed as much as 1 mL of raw wood banana fruit extract solution, then the sample is added with 0.4 mL of FolinCiocalteau reagent whipped and left for 4-8 minutes, add 4.0 mL of 7% Na2CO3 solution shake until homogeneous. Add aquades up to 10 mL and let stand for 2 hours at room temperature. Measure absorption at a maximum absorption wavelength of 750 nm. Do 3 repetitions so that the phenol levels obtained by the results are obtained as mg of gallic acid equivalent / g of extract(Campos et al., 2022).

$$TPC = \frac{x.v.fp}{g}$$

Information:
x = Phenolic concentration (ppm)
v = Extract volume used (mL)
fp = Dilution factor
g= Sample weight used (g)

Metabolite Profiling Using UPLC/Q-TOF MS

Thoroughly weighed 10.00 mg of extract then dissolved with methanol into a 10 ml measuring flask. The extract in methanol is taken with a microsyringe of 5 µl to be further injected into the sample site and entered into the UPLC column. Replication is carried out 4 times. The sample in the form of a liquid will be converted into droplets through the needle that has been given a positive ESI charge (+). The ions that have been generated by the detector will then be separated by a Q-ToF analyzer. The eluent used was a mixture of (A) water: formic acid (99.9:0.1) and (B) acetonitrile: formic acid (99.9:0.1) with a gradient elution system as listed in table 2 with an eluent flow velocity of 0.2 ml/min. A chromatogram with a polar compound will appear first then be followed by a compound whose polarity is lower. The separation results are then read by the QToF-MS detector resulting in a chromatogram peak. Peak chromatograms are then interpreted using the Masslynx application (Gong et al., 2020).

Results and Discussion

Plant determination

Sampling was carried out in Lumajang Regency, East Java. The selection of fruits taken is fresh and unripe fruit with a green color that has no yellow color, hard and aged 3 months after the flower tandan comes out. The determination of the raw fruit plant of wood bananas was carried out at LIPI Purwodadi and the raw fruit of wood bananas is indeed a wood variety by proving the determination of the raw fruit of wood bananas at the Food Security and Agriculture Office of Lumajang Regency.

Manufacture of simplisia

Table 1. Characteristic results of simplisia powdered unripe fruit banana wood

Simplisia	Temperature	Fresh Simplisia weight (gr)	Weight of dry Simplisia (gr)	% Shrinkage drying	Moisture content	Organoleptic
Unripe fruit of banana wood	Temperature 50^0C	12959,9 gram	4152,6 gram	32,04%	2,74%	Aromatic characteristic smell, ivory-white color, powder-shaped

The unripe fruit of wood bananas as much as 12,959.9 grams was washed thoroughly with running water to remove dirt and sap that was still attached to the wood banana fruit. The washed wooden banana fruit is then dried by aerating, then the wooden banana fruit is cut into thin strips to facilitate a drying process. Drying is carried out using a food dehydrator oven at a temperature of 50º and dry simplicia results are obtained. The dried simplisia is then mashed using a blender until it becomes a fine powder and the powder produced by 4152.6 grams.

Extract making

Simplisia extraction uses 2 extraction methods, namely cold and hot methods for cold extraction methods use remaseration and maceration while the hot extraction method uses reflux and Soxhlet uses 96% ethanol solvent with a temperature of 500 C according to table results 4.1% the highest amendment using the remaceration extraction method with results16.45%.

Table 2. % yield of amendments and phenolic total levels of wood banana raw fruit extract

No.	Extraction Methods	Powder Weight	Extract Weight	% Extract Amendments	KTFe±SD	Organoleptic Extract
1	Remaseration	750 gram	123,4 gram	16,45%	99.31±1.11	Color: brownish green Smell: slightly pungent Taste: slightly bitter and astringent
2	Maceration	750 gram	106,3 gram	14,17%	53.96±0.81	Color: brownish green Smell: slightly pungent Taste: slightly astringent and very bitter
3	Reflux	750 gram	85,7 gram	11,42%	54.65±0.80	Color: green-black Smell: slightly pungent Taste: slightly bitter and slightly astringent
4	Soxhlet	750 gram	76,8 gram	10,24%	54.47±0.65	Color: green-black Smell: very pungent Taste: bitter

Phytochemical screening examination

Table 3. Phytochemical screening results of unripe fruit of the wood banana

Organoleptic Examination	Extraction Method			
	Remaseration	Maceration	Reflux	Soxhlet
Alkaloid	+	+	+	+
Saponin	+	+	+	+
Flavonoid	+	+	+	+
Tanin	+	+	+	+
Polyphenol	+	+	+	+
Anthraquinone	+	+	+	+
Glikosida	-	-	-	-
Steroid	-	-	-	-
Triterpenoid	-	-	+	+

Phytochemical screening is carried out to determine the content of secondary metabolites contained in the extract of unripe fruit of banana wood. Phytochemical screening is carried out, namely tannins, alkaloids, saponins, flavonoids, polyphenols, glycosides, anthraquinones, terpenoids and steroids. Based on the results of phytochemical screening tests with the extraction method of remaseration and maceration, it produces a significant content of secondary metabolite compounds, namely containing alkaloids, saponins, flavonoids, tannins, polyphenols, and anthraquinones. Whereas in the reflux extraction method and Soxhlet produces compounds as secondary metabolites of alkaloids, saponins, flavonoids, tannins, polyphenols, anthraquinones, and triterpenoids. In this study, the results of phytochemical screening produced different secondary metabolite compounds because time and temperature greatly affect the number of compounds extracted so the extraction method by heating where the extraction method will provide an opportunity to obtain maximum secondary metabolite compounds.

DISCUSSION

The remaseration method is a modification method of the maceration method where the remaseration method is carried out by adding solvent repeatedly after the first extract filtering. In this study, the extract yield from the remaseration extraction method was 16.45% and for the results of obtaining phenolic compound levels produced 99.31±1.11 mgGAE / gram, it was suspected to produce the highest levels of amendments and phenolic compounds because at room temperature and protected from sunlight and heat, the withdrawal of active compounds for 2 days was carried out by soaking the simplicia powder with the appropriate solvent for 2 days and changing the solvent every time day. When it reaches the equilibrium phase, the plant cell will be entered by the solvent by passing through the cell wall. The equilibrium process occurs by exiting secondary metabolite compounds inside the cell because the concentration inside the cell is different from the concentration outside the cell. The equilibrium process occurs because there is a diffusion process caused by a difference in concentration where the concentration inside the cell is higher will cause secondary metabolite compounds to come out and be replaced by solvent liquids outside the cell whose concentration is lower(Zhou Xu 1,†, Shiling Feng 1,†, Jipeng Qu 1, 2, Ming Yuan 1 & Ding, 2019). The event occurs repeatedly until there is a balance of concentration outside and inside the cell. During the remaseration process, a replacement of the igniting fluid is carried out every day for 2 days so that the effectiveness of the withdrawal will be maximized(Qiu et al., 2021).

The maceration method is a method of extraction of the cold way and the simplest method where the soldering liquid will penetrate the cell wall of the plant and will enter the cell cavity containing the active substance so that the active substance which is a sealed solution will be urged out of the cell because of the difference in concentration between the active substance solution inside the cell and the one outside the cell(Hasnaeni, Wisdawati, 2019). The resulting extract yield was 14.17% and the results of obtaining phenolic compound levels produced 53.96±0.81 mgGAE / gram. The yield is high because the time used is quite long and stirring is carried out many times, therefore the compounds contained in the unripe fruit of wood bananas are attracted quite a lot. The yield of the amendment is high enough that the level of phenolic compounds that should be produced must be high, but the principle of the maceration extraction method is not carried out by repeating the addition of solvents so that the active compounds contained in the raw fruit of wood bananas are not attracted to the maximum, therefore the levels of phenolic compounds produced are low.

The reflux method is a method of extraction with the help of heating. The thing that greatly affects extraction using reflux is the addition of heating and the solvent used will remain fresh due to the re-evaporation that is submerged in the material. Reflux extraction is used to extract materials that are heat-resistant and have a rough texture(Hasnaeni, Wisdawati, 2019). The resulting extract yield was 11.42% and the resulting phenolic content of 54.65±0.80 mgGAE / gram. High levels of phenolic compounds should produce high levels of amendments, but the time made for extraction is quite short, namely for 2 hours so that the withdrawal process of secondary metabolite compounds is not interested in the maximum, but the levels of phenolic compounds produced are quite high due to the heating process, causing the cell walls of banana wood fruit powder to open larger, in addition, the heating process also results in the viscosity of the solvent decreasing so that the ability of the solvent to penetrate the cell wall becomes easier and the amount of phenolic compounds extracted becomes high.

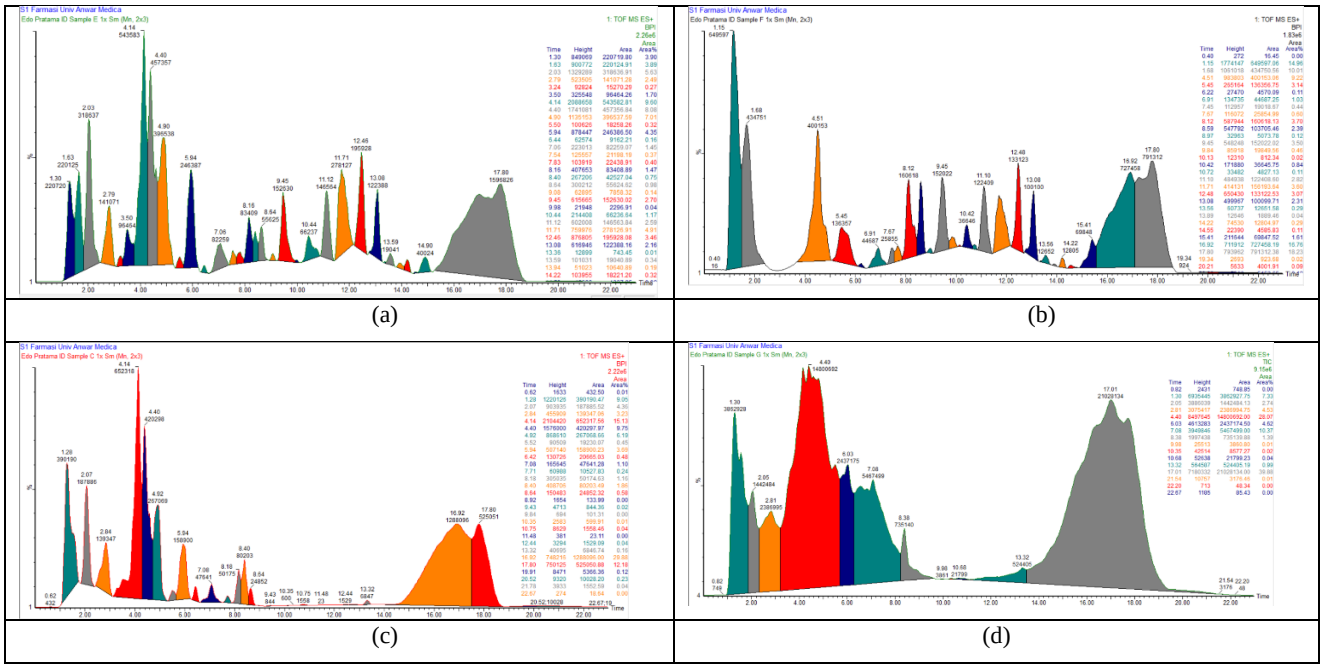
The soxhlet method is a heat extraction method. At this extraction, the solvent and the sample are placed separately. The principle of the soxhlet method is that it is carried out continuously using relatively few solvents. When the extraction is complete then the solvent can be evaporated so that an extract will be obtained. Usually, the solvent used is volatile or has a low boiling point(Susanty & Bachmid, 2016). The resulting extract yield was 10.24% and the resulting phenolic compound content was 54.47.47±.0.65 mgGAE / gram. The Soxhlet extraction method produces high yield levels but produces a fairly high level of phenolic compounds because the extraction process is quite long, which is 5 hours, and takes 3 days to be able to extract 750 grams of raw fruit powder of wood banana so that the time in the extraction process greatly affects the right extraction time will produce optimal compounds, but if the extraction time is too long it will damage the compound active and if the extraction time is too short it will result in low extract yield levels. The results of high phenolic compounds, it is suspected to be caused by the heating process so that the cell walls of banana powder can break and can secrete active compounds that can withstand heating.

Based on the results of phytochemical screening of extracts by extraction, the positive heat method contains triterpenoid compounds while in the cold way it does not contain triterpenoid compounds, where these triterpenoid compounds have antidiarrheal activity so that the extract with the soxhlet extraction method provides higher antidiarrheal activity than the maceration method. The reflux method has an average cross-marker length in the intestines of mice that is lower than the soxhlet

method, the length of extraction will affect the attraction of chemical compounds. The soxhlet method takes quite a long time for extraction so the soxhlet method can attract more than the reflux method.

Based on the results of the one-way ANOVA test on phenolic level testing, it can be concluded that the results of hypothesis testing have significant differences in performance between types of extraction methods. This result is indicated by a Ftable value of 2.8951073 and a probability value (sig) of 0.000 H0 rejected, where the Fhitung ≥ Ftabel and its probability (sig) ≤ 0.05. The calculated F value from the ANOVA table is 3404,728 and the Ftabel value is 2.8951073 to 3404.728 ≥ 2.8951073 and the probability value (sig) in the ANOVA table is 0.000 while the signification level α = 0.05 to 0.000≤0.05. It can be concluded that the test results have significant differences in the results of phenolic compound levels.

According to research by Hernanz, et all (2021) (Rebollo-Hernanz et al., 2021), The amendment of phenolic extraction from cocoa peels increases by modifying the extraction parameters including temperature, time, acidity, and S/L ratio. In the results of this study, the difference in extraction methods in a cold way produced the highest yield compared to the extraction method in a hot way because the temperature would affect the stability of the compound content. In the cold extraction method, namely, remaseration has the highest percentage of extract yield and the highest phenolic content.



Picture. Chromatogram UPLC-MS extract by method of (a) maceration, (b) reflux, (c) remaseration, (d) soxlet

Analysis of the profile of the unripe fruit of the wood banana (Musa paradisiaca L.Var.Kayu) in this study using UPLC-MS. UPLC is one of the developmental techniques of liquid chromatography used for the segregation of different components in a mixture with a molecular level reaching two microns of analyte particles. The analysis method using UPLC can reduce the consumption of the phase of motion by up to 80% in a relatively shorter time of about 1.5 minutes than using HPLC. The UPLC-MS used in this study used an MS detector with an ESI ion source (+) and an MS analyst in the form of Q-ToF. Such instruments have several advantages, that is, selective and sensitive with high and fast resolution performance so that the analysis time is faster. The profile analysis of the metabolite of *Musa paradisiaca* L.Var.Kayu begins by injecting the sample, then the sample will enter the column so that a process of separation of metabolite components occurs. In this study, the silent phase used was column C18 or octimethyl silica. The advantage of octadecyl silica as a stationary phase is that this phase can separate compounds ranging from low, medium, to high polarity

Table 4. Results of interpretation of metabolite data profiling extract by remaseration method

No	Rt (min)	m/z	Molecular Formula	Predictions Compound Name	% Area
1	16,92	960,8978	C61H117NO6	1,15-Bis[[1-(3-pentyloctoxy)cyclohexyl]oxy]pentadecan-8-yl 4-pyrrolidin-1-ylbutanoate	29,88
2	4,14	222,15	C13H19NO2	Ethyl-4-Butylamineobenzoate	15,13
3	17,8	960,8975	C61H117NO6	1,15-Bis[[1-(3-pentyloctoxy)cyclohexyl]oxy]pentadecan-8-yl 4-pyrrolidin-1-ylbutanoate	12,18
4	4,40	256,1339	C16H17NO2	4-(2-(5-Ethylpyridin-2-yl)ethoxy)benzaldehyde	9,75
5	1,280	381,0798	C9H20N2O12S	Unknown	9,05
6	4,9	236,164	C14H21NO2	2,6-Di-tert-butyl-4-nitrosophenol	6,19
7	2,07	178,0839	C10H11NO2	2-(3,4-dimethoxyphenyl)acetonitrile	4,36
8	5,94	322,2019	C18H27NO4	1'-Methylspiro(adamantane-2,3'-pyrrolidine)maleate	3,69
9	2,84	230,0938	C12H11N3O2	2-((Pyridin-4-ylmethyl)amino)nicotinic acid	3,23
10	8,40	454,2294	C19H35NO11	5-Aminopentyl 3-O-(2-O-acetyl-6-deoxy-α-L-talopyranosyl)-β-D-glucopyranoside	1,86
11	8,18	269,1759	C15H24O4	1,9-Nonanediol Diacrylate	1,16
12	7,08	247,1329	C15H18O3	Loxoprofen	1,10

Table 5. Results of interpretation of metabolite data profiling extracts by maceration method

NO	Rt (min)	m/z	Molecular Formula	Predictions Compound Name	% Area
1	4.094	222.1499	C13H19NO2	Ethyl 4 – Butylaminobenzoate	9,60
2	4.395	256.1331	C16H17NO2	4-(2-(5-Ethylpyridin-2-yl)ethoxy)benzaldehyde	8,08
3	4,902	279.1135	C17H14N2O2	2,6-Di-tert-butyl-4-nitrosophenol	7,01
4	2.027	178.0868	C10H11NO2	2-(3,4-dimethoxyphenyl)acetonitrile	5,63
5	11.715	518.3247	C24H39N9O4	N-Acetyl-L-phenylalanyl-N-{(1E,2S)-1-(carbamoylhydrazono)-5-[(diaminomethylene)amino]-2-pentanyl}-L-leucinamide	4,91
6	5.935	336.2160	C19H29NO4	decyl 3-acetamido-4-hydroxybenzoate	4,35
7	1.303	381.0786	C9H20N2O12S	Unknown	3,90
8	1.633	180.1019	C10H13NO2	propan-2-yl *N*-phenylcarbamate	3,89
9	12,459	520.3436	C22H45N7O7	(2S,3R)-2-amino-3-[(2S)-2-amino-5-(diaminomethylideneamino)pentanoyl]oxybutanoic acid;[(2S)-2-amino-4-methylpentanoyl] (2S)-2-amino-4-methylpentanoate	3,46
10	9.451	291.1955	C18H26O3	2-ethylhexyl (*E*)-3-(4-methoxyphenyl)prop-2-enoate	2,70
11	11,145	295.2263	C14H34N2S2	N,N'-(Disulfanediyldi-2,1-ethanediyl)bis(N,N-dimethyl-2-propanaminium	2,59
12	2,792	230.0927	C12H11N3O2	2-((Pyridin-4-ylmethyl)amino)nicotinic acid	2,49
13	13.079	496.3411	C20H45N7O7	N,N-bis(2-aminoethyl)nitrous amide;tert-butyl (2-methylpropan-2-yl)oxycarbonyl carbonate;N-[2-(methylamino)ethyl]-N-propylnitrous amide	2,16
14	3,496	180.0658	C9H9NO3	2-benzamidoacetic acid	1,70
15	8.157	275.2021	C15H30O2S	3-(Dodecylthio)propanoic acid	1,47
16	7,061	442.2656	C19H39NO10	1-Amino-3,6,9,12,15,18,21,24-octaoxaheptacosan-27-oic acid	1,45
17	10,442	277.2178	C18H28O2	Phenyl laurate	1,17

Table 6. Results of Interpretation of metabolite data profiling extracts by the soxlet method

No	Rt (min)	m/z	Molecular Formula	Predictions Compound Name	% Area
1	16,99	960,8997	C36H8N4O25S2	1,15-Bis[[1-(3-pentyloctoxy)cyclohexyl]oxy]pentadecan-8-yl 4-pyrrolidin-1-ylbutanoate	29,92
2	17,80	960,8969	C26H12N2O36S	1,15-Bis[[1-(3-pentyloctoxy)cyclohexyl]oxy]pentadecan-8-yl 4-pyrrolidin-1-ylbutanoate	13,35
3	4,14	222,1493	C13H19NO2	Ethyl 4-Butylaminobenzoate	11,79
4	4,40	256,1349	C16H17NO2	4-(2-(5-Ethylpyridin-2-yl)ethoxy)benzaldehyde	8,47
5	4,82	434,2018	C19H31NO10	21-(2,5-Dihydro-2,5-dioxo-1H-pyrrol-1-yl)-4,7,10,13,16,19-hexaoxaheneicosanoic acid	8,13
6	2,07	178,0875	C10H11NO2	2-(3,4-dimethoxyphenyl)acetonitrile	7,38
7	1,28	381,0808	C9H20N2O12S	Unknown	5,57
8	1,56	180,1037	C10H13NO2	propan-2-yl *N*-phenylcarbamate	4,05
9	5,85	197,1179	C11H16O3	1-carboxy-3-hydroxyadamantane	3,47
10	2,84	230,0937	C12H11N3O2	2-((Pyridin-4-ylmethyl)amino)nicotinic acid	3,00
11	8,40	454,2294	C19H35NO11	5-Aminopentyl 3-O-(2-O-acetyl-6-deoxy-α-L-talopyranosyl)-β-D-glucopyranoside	1,70
12	3,50	180,0660	C9H9NO3	2-benzamidoacetic acid	1,32
13	7,08	247,1335	C15H18O3	(3*S*,3*aS*,5*aS*,9*bS*)-3,5*a*,9-trimethyl-3*a*,4,5,9*b*-tetrahydro-3*H*-benzo[g][1]benzofuran-2,8-dione	1,17

Table 7. Results of Interpretation of metabolite data profiling extracts by reflux method

NO	Rt (min)	m/z	Molecular Formula	Predictions Compound Name	% Area
1	17.803	960.8945	C61H117NO6	1,15-Bis[[1-(3-pentyloctoxy)cyclohexyl]oxy]pentadecan-8-yl 4-pyrrolidin-1-ylbutanoate	18,23
2	16.925	960.9003	C81H117NO6	1,15-Bis[[1-(3-pentyloctoxy)cyclohexyl]oxy]pentadecan-8-yl 4-pyrrolidin-1-ylbutanoate	16,76
3	1.148	381.0803	$C_9H_{20}N_2O_{12}S$	Unknown	14,96
4	1.675	256.1341	$C_{12}H_{13}N_7$	2-{[4-Amino-6-(dimethylamino)-1,3,5-triazin-2-yl]amino}benzonitrile	10,01
5	4.508	236.1636	$C_{14}H_{21}NO_2$	Padimate A	9,22
6	8.115	275.2010	$C_{15}H_{30}O_2S$	3-(Dodecylthio)propanoic acid	3,70
7	11.715	518.3239	C24H39N9O4	N-Acetyl-L-phenylalanyl-N-{(1E,2S)-1-(carbamoylhydrazono)-5-[(diaminomethylene)amino]-2-pentanyl}-L-leucinamide	3,60
8	9.451	291.1956	C18H26O3	2-ethylhexyl (*E*)-3-(4-methoxyphenyl)prop-2-enoate	3,50
9	5.450	197.1175	$C_{11}H_{16}O_3$	1-carboxy-3-hydroxyadamantane	3,14
10	12.480	520.3423	$C_{22}H_{45}N_7O_7$	(2S,3R)-2-amino-3-[(2S)-2-amino-5-(diaminomethylideneamino)pentanoyl]oxybutanoic acid;[(2S)-2-amino-4-methylpentanoyl] (2S)-2-amino-4-methylpentanoate	3,07
11	11.103	295.2264	C14H34N2S2	N,N'-(Disulfanediyldi-2,1-ethanediyl)bis(N,N-dimethyl-2-propanaminium	2,82
12	8.593	277.2168	$C_{18}H_{28}O_2$	Phenyl laurate	2,39
13	13.079	496.3407	C20H45N7O7	N,N-bis(2-aminoethyl)nitrous amide;tert-butyl (2-methylpropan-2-yl)oxycarbonyl carbonate;N-[2-(methylamino)ethyl]-N-propylnitrous amide	2,31
14	15.406	378.2218	$C_{28}H_{27}N$	4,4'-Bis(1-Phenylethyl)Diphenylamine	1,61
15	6.906	247.1324	$C_{15}H_{18}O_3$	(3*S*,3*aS*,5*aS*,9*bS*)-3,5*a*,9-trimethyl-3*a*,4,5,9*b*-tetrahydro-3*H*-benzo[g][1]benzofuran-2,8-dione	1,03

The eluent used is a mixture of water: formic acid (99.9:0,1) (v:v) and acetonitrile: formic acid (99.9:0.1) (v:v) with a gradient elution system, which is an elution system in which the eluent used changes its composition every time. The mixture of water and formic acid with acetonitrile and formic acid is an eluene mixture that facilitates the separation process in the column in a fast period, which is less than 10-15 minutes. A chromatogram with a polar compound will appear first then be followed by a compound whose polarity is lower. Next, the eluation results in the column go to the MS detector so that the results can be read easily. The sample in the form of a liquid will be converted into droplets and then pass through a needle that has been assigned an ESI (+) charge to produce ions that will be read by the MS detector. The result of the separation will appear as a chromatogram which is then processed using the Masslynx 4.1 application so that the spectra of each chromatogram peak can be known. Figure 1 is a chromatogram of the results of the analysis of the metabolite profile of Moses paradisiaca L.var.wood. The chromatogram is then processed using the Masslynx 4.1 application so that it can be known and predictable the molecular formula of each compound. Each peak of a chromatogram indicates one compound. Based on the measured mass and calculated mass values on the spectra, it can be known the prediction of the molecular formula of the spectra. The value of measured mass and calculated mass must also be reduced by the mass of 1 H atom, which is 1.0078 because at the time of separation using the column there is an addition of H atoms derived from the firing of ESI ions (+). The prediction of the molecular formula that appears in the data is then selected as whose difference between measured mass and calculated mass ± 0.0005. Predictions of the molecular formula that have been selected are then searched with the help of the chemspider.com website. The molecular formula written on this website must be reduced by 1 H atom first because in the separation process there is an addition of 1 H atom derived from firing ESI ions (+). After the search is completed, the compound ID number is selected based on the number of publications, then the name ADC / IUPAC is selected to be further converted using the Chemdraw Ultra 12.0 application so that the structure of the desired compound can be known. Based on the data from the interpretation of compound content analysis using UPLC-QToFMS, it can be seen that there are 136 compounds in the remaseration method, 133 compounds in the maceration method, 126 compounds in the reflux method, and 136 compounds in the soxhlet method (table 1-table 4). Based on these data, it is known that there are differences in metabolite profiles in different extraction methods characterized by differences and the number of types of compounds contained in each extract. Based on the results of the interpretation of the data that has been obtained, it can be known several major compounds, namely compounds that have a higher percentage area compared to other compounds. The major compounds in the remaseration method are 15.13% ethyl-4-butylaminobenzoate, 29.88% 1,15-Bis[[1-(3-pentyloctoxy)cyclohexyl]oxy]pentadecan-8-yl 4-pyrrolidin-1-ylbutanoate, and 12.18% 1,15-Bis[[1-(3-pentyloctoxy)cyclohexyl]oxy]pentadecan-8-yl 4-pyrrolidin-1-ylbutanoate. The major compounds in the maceration method are 9.6% ethyl-4-butylaminobenzoate, 8.08% 4-(2-(5-Ethylpyridin-2-yl)ethoxy)benzaldehyde, and 7.01% 2,6-Di-tert-butyl-4-nitrosophenol. The major compounds in the soxhlet method are 11.79% ethyl-4-butylaminobenzoate, 29.92% 1,15-Bis[[1-(3-pentyloctoxy)cyclohexyl]oxy]pentadecan-8-yl 4-pyrrolidin-1-ylbutanoate, and 13.35% 1,15-Bis[[1-(3-pentyloctoxy)cyclohexyl]oxy]pentadecan-8-yl 4-pyrrolidin-1-ylbutanoate. The major compounds in the reflux method are 14.96% unknown compounds, 16.76%% 1,15-Bis[[1-(3-pentyloctoxy)cyclohexyl]oxy]pentadecan-8-yl 4-pyrrolidin-1-ylbutanoate and 18.23% 1,15-Bis[[1-(3-pentyloctoxy)cyclohexyl]oxy]pentadecan-8-yl 4-pyrrolidin-1-ylbutanoate compounds.

Conclusions

Based on the results of the research that has been carried out, it can be concluded that differences in extraction methods affect phenolic levels and metabolite profiles in the unripe fruit of wood bananas (Musa paradisiaca L.Var.Kayu). In the remaseration extraction method, the highest phenolic content is 99,31 In the remaseration method, it was found that there were 12 compounds, the maceration method of 17 compounds, the reflux method of 13 compounds, and the soxhlet method of 15 compounds.

Conflicts of Interest

The authors state there is no conflict of interest.

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