DOI: 10.19184/ICL.v4i1.5688

Identification of The Chemical Compound From Ethyl Acetate and Ethanol Fraction of Banana Peel Extract (*Musa Acuminata* Colla)

Denok Risky Ayu Paramita[a], Hadi Barru Hakam Fajar Siddiq*[a], Agnis Pondineka Ria Aditama[a], Rosida[a]

Abstract: Bananas are broadly utilized as an alternative nourishment, whereas banana peels, as squander, are right now as it were utilized as creature nourish added additives. Banana peel contains flavonoids, saponins, glycosides, alkaloids, tannins, and catechins. Flavonoids are phenolic compounds (phenolic hydroxyl) that can act as cancer prevention agents and are by and large found in plants. This inquiry will recognize the chemical compounds of the ethyl acetic acid derivation division and the ethanol division of the banana peel extract (Musa acuminata Colla). This sort of investigation is a test research facility investigation with a Quasi-Experimental Plan investigate plan. In this consider, two sorts of fractionation were performed on the banana peel extract, specifically ethanol fractionation and ethyl acetate extraction fractionation. Besides, the formation of the fractionation was analyzed utilizing the LC-HRMS strategy. The comes about appears that the ethanol division of the banana peel extract contained different chemical compounds, including 4-Methoxycinnamaldehyde, Choline, and catechin. Within the ethyl acetic acid derivation division, the banana peel extract contained a few chemical compounds, including 5-Hydroxymethyl-2-furaldehyde, Diisobutylphthalate, and Pyrogallol. These compounds appear in a huge region within the scope of HPLC-MS investigation.

Keywords: banana peel, fractionation, LC-HRMS.

INTRODUCTION

Banana plants belong to the Musaceae family. Banana plants come from three genera (Musa, Ensette, and Musella) in the same family, but generally consist of various species within Musa. Almost all known banana cultivars come from two diploid species, namely Musa acuminata (genome A) and Musa balbisiana (genome B). Cultivars within Musa acuminata are generally sweet bananas that are edible and easy to peel (Mathew & Negi, 2017).

Every day, tons of banana peel waste are generated from fruit markets and household waste, which causes unpleasant odors due to anaerobic digestion of biomass, producing gases that disrupt the natural air balance. Although ripe bananas are eaten raw, large quantities of bananas are industrially processed into banana flour, chips, and other processed products, resulting in a large amount of banana peel waste. In the past, banana peels were thrown into the trash by the food processing industry. The agricultural sector would benefit economically from converting banana peels into valuable products (Mohd Zaini et al., 2022).

Fruit peels have been conventionally used as medicine for various diseases such as burns, anemia, diarrhea, boils, inflammation, diabetes, cough, snake bites, and excessive menstruation (Pereira & Maraschin, 2015). Fruit peels are rich in dietary fiber and phenolic compounds, and have significant antioxidant, antibacterial, and antibiotic activities (Fidrianny & Insanu 2014). Therefore, fruit peels are a very potential source, which encourages their use in the nutraceutical and pharmaceutical sectors.

Mas banana peel (Musa acuminata Colla) has been analyzed in various studies, including banana peel extract can increase VEGF expression and accelerate the re-epithelialization process in white rat skin as a burn wound model (Khotib et al., 2014). Furthermore, testing the antioxidant activity of mas banana peel extract (Musa acuminata Colla) using the DPPH method showed an IC50 value of 70.41 mg/L (Rosida dan Diyan, 2015). Mas banana peel extract is also able to lower blood sugar levels (Dewi et al., 2016).

On the other hand, one of the steps in the processing and analysis of natural material compounds phytochemically is the extraction stage. In Extraction, a mixture of substances is broken down by dissolving each component using one or more solvents, resulting in two phases – Raffinate Phase (rich in Feed Solvent) and Extract Phase (rich in Solute) (Komal Patel et al., 2019). Extraction methods include solvent extraction, distillation, pressing, and sublimation according to the principles of extraction. Solvent extraction is the most commonly applied technique. Extraction of natural materials occurs through the following steps: (1) the solvent enters the solid matrix; (2) the solute dissolves into the solvent; (3) the solute diffuses out of the solid matrix; (4) the solute that has been successfully extracted is collected. Any element that increases solubility and diffusivity in the above process will facilitate extraction (Hidayat & Patricia Wulandari, 2021).

The characteristics of the extraction solvent, the particle size of the raw material, the ratio of solvent to solid, the extraction temperature, and the extraction time will affect the extraction efficiency. The selection of solvents plays a crucial role in solvent extraction. The selection of solvents must consider selectivity, solubility, cost, and safety. According to the law of similarity and intermissibility, solvents with polarity values close to the polarity of the solute generally show better performance, and vice versa. EtOH, MeOH, and ethyl acetate are universal solvents in solvent extraction for phytochemical research. (Hidayat & Patricia Wulandari, 2021).

[a] DRA. Paramita, HBHF. Siddiq, APR. Aditama, Rosida
Diploma III Farmasi, Politeknik Kesehatan Jember, Jember 68125, Indonesia
*e-mail: hakamfajar@gmail.com

In this study, the partition method was applied through a two-stage extraction process, namely solid-liquid extraction by maceration, which aims to extract all plant components using ethanol as a solvent. Ethanol solvent is a polar solvent that is often used to extract polar compounds such as flavonoids, saponins, tannins, and alkaloids. Furthermore, ethanol extraction was carried out through a liquid-liquid extraction process using ethyl acetate solvent. Referring to this explanation, researchers are interested in continuing from the extraction phase to the fractionation phase. Fractionation is the separation of compounds based on their polarity.

METHODS

Materials and Tools

The equipment used in this study included Ohaus CP 214 analytical balance, macerator, RV 10 IKA Scientific rotary evaporator, mercury thermometer, stir bar, blender, mortar, stamper, measuring cup, and Liquid Chromatography – High-Resolution Mass Spectrometry (LC-HRMS) Thermo Scientific Dionex Ultimate 3000 RSLCnano with microflow meter and commonly used glassware. The materials used in this study were ripe banana peels, ethanol p.a.(E.Merck), n-hexane p.a.(E.Merck), ethyl acetate p.a.(E.Merck), and aquadest.

Extraction of mas banana peel (*Musa acuminata* Colla)

Bananas are ripe. The fruit skin is separated manually, then cut into small pieces (±2 mm), dried, and made into powder. Dried banana peels (50 g) were extracted with 250 ml of 70% ethanol at room temperature for 5 days. The extract was then concentrated using a rotary evaporator. The crude extract is weighed and then stored in a dark glass bottle in a freezer dryer for further use (Khotib et al., 2014).

Fractionation of banana peel extract

The viscous ethanol extract was extracted by partitioning using n-hexane solvent twice, so that two layers were produced, namely the top layer (n-hexane extract) and the bottom layer (ethanol extract I), because different polarities influenced them. Extraction with n-hexane aims to remove fat and other non-polar compounds to facilitate the process of separating flavonoid compounds. Then, the ethanol extract was further partitioned with ethyl acetate twice to obtain ethyl acetate extract and ethanol extract II. To help separate these two fractions, water is added. Furthermore, the ethyl acetate extract was evaporated with a rotary vacuum evaporator to obtain a thick ethyl acetate extract. The ethyl acetate extract that has been condensed is weighed by mass to calculate the % yield, which is suspected to contain flavonoid compounds (Engida et al., 2015).

LC-HRMS analysis

The extracted sample was diluted according to the solvent. Dilution was done by looking at the concentration of the sample (not too concentrated and not too dilute) with a final volume of 1500 μL. Then the sample was vortexed at 2000 rpm for about 2 min. Then spin down at 6000 rpm for about 2 minutes to get the supernatant. Then the supernatant was taken and filtered with a 0.22 μm syringe filter and put into a vial. The sample in the vial is ready to be inserted into the autosampler and then injected into the LC-HRMS according to the desired injection method.

RESULT AND DISCUSSION

Fractionation of banana peel extract

In this study, the banana peel extract was fractionated in two stages, namely n-hexane fractionation using ethanol and ethyl acetate fractionation with ethanol. The initial fractionation stage is n-hexane fractionation carried out using the n-hexane extraction method using ethanol. The solid extract from the banana peel was obtained through the use of two solvents, n-hexane and ethanol, with a ratio of 1:1. This extraction was carried out to separate fat and other non-polar compounds so that the process of separating flavonoid compounds becomes easier. From the extraction process, two fractions were obtained, namely the n-hexane phase (upper part) and the ethanol fraction (lower part), as shown in Figure 1a. The second fractionation stage is ethyl acetate fractionation using the ethyl acetate-ethanol extraction method with a ratio of 1:1. This stage is carried out to obtain compounds that can be dissolved in ethanol and ethyl acetate solvents. The fractions produced from the fractionation are the ethyl acetate fraction (upper part) and the ethanol fraction (lower part), as shown in Figure 1b. These two results were then concentrated and analyzed using the LC-HRMS (Liquid Chromatography – High Resolution Mass Spectrometry) method.

(a)

(b)

Figure 1. a) Results of fractionation of n-hexane with ethanol, b) Results of fractionation of ethyl acetate with ethanol

Fractionation is a way to separate substances from an extract using two solvents that do not mix. Solvents that are often used for separation are methanol and ethyl acetate. Ethyl acetate is used to separate semi-polar compounds, while methanol is used to separate polar compounds (Putri et al., 2023). The results of the extraction yield of banana peel samples, ethanol fractionation, and ethyl acetate fractionation are shown in Table 1. These results indicate that the yield in the ethanol fraction is higher than the ethyl acetate fraction, meaning that the compounds dissolved in the ethanol solvent are more than the compounds dissolved in the ethyl acetate solvent.

Table 1. Weight and yield of extract, ethanol fraction, ethyl acetate fraction in 50 g banana peel sample

Materials	Weight (g)	Yield (%)
Extract	4,65	9,3
Etanol fraction	1,027	22,086
Etil asetat fraction	0,567	10,043

Results of LC-HMRS Analysis

Derivatives of ethyl acetate and ethanol were obtained from the Organic Science Research Institute, Brawijaya University, to be analyzed using LC-HRMS (Liquid Chromatography - High

Resolution Mass Spectrometry). The results of LC-HRMS analysis on ethyl acetate derivatives from banana peel extract (*Musa acuminata* Colla) showed that ethyl acetate derivatives from banana peel extract (*Musa acuminata* Colla) contained various chemical compounds, namely 5-Hydroxymethyl-2-furaldehyde, Diisobutylphthalate, and Pyrogallol, as seen in Table 2. The chromatogram of 5-Hydroxymethyl-2-furaldehyde is shown in Figure 2.

Table 2. Compounds resulting from ethyl acetate fractionation of banana peel extract (*Musa acuminata Colla*)

No	Name	Formula	RT (Min)	Area (Max)
1	5-Hydroxymethyl-2-furaldehyde	$C_6H_6O_3$	2.019	3,798,079,652.97
2	Diisobutylphthalate	$C_{16}H_{22}O_4$	18.558	841,612,960.30
3	Pyrogallol	$C_6H_6O_3$	2.829	504,946,459.09

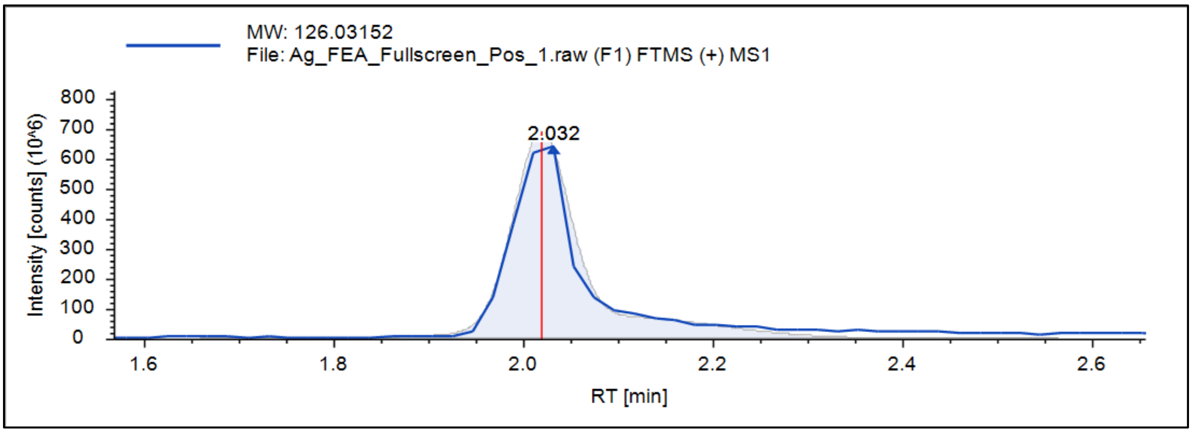

Figure 2. Chromatogram of 5-Hydroxymethyl-2-furaldehyde

5-Hydroxymethyl-2-furfural can be a six-carbon heterocyclic aldehyde, a derivative of furan, which has an aldehyde and a liquid (hydroxymethyl functional bond). The ring structure is based on the furan group, where two utilitarian bonds, namely the formyl and hydroxymethyl bonds, are connected at positions 2 and 5 on the ring (Figure 3). Due to its composition and presence in carbohydrate and agricultural sources, HMF can be seen as a renewable material that acts as a bridge or a key particle between "green chemistry" and natural chemistry based on mineral oils. (Kowalski et al., 2013).

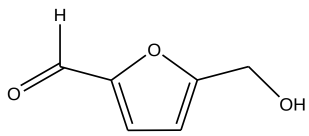

Figure 3. Structure of 5-Hydroxymethyl-2-furfural

Pyrogallol, also known as pyrogallic acid or 1,2,3-trihydroxybenzene, is an organic phenolic compound found naturally in various plants such as oak, eucalyptus, Terminalia chebula, and Myriophyllum spicatum. Pyrogallol is a phenolic compound found naturally in oak, hardwood, and some fruits such as apricots, avocados, and bananas. This substance has antibacterial, antipsoriatic, and antifungal characteristics. However, the oxidative nature of pyrogallol has prompted several studies regarding the potential health risks of its consumption. However, oxidizing agents can be used to treat several diseases, such as malaria. Based on studies that have been conducted, several antimalarial drugs, such as primaquine, chloroquine, and artemisinin derivatives, can kill parasites by producing oxidative stress caused by free radicals. (Alfaqih and Abu-Bakar, 2020).

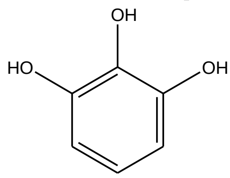

Figure 4. Structure of Pyrogallol

On the other hand, LC-HRMS investigation on ethanol extract of banana peel (Musa acuminata Colla) contained various chemical compounds, including Choline, catechin, and 4-Methoxycinnamaldehyde, as listed in Table 3. The substances contained in the ethanol division have polar properties. The resulting chromatogram of catechin compounds can be seen in Figure 5.

Table 3. Compounds resulting from ethanol fractionation of banana peel extract (*Musa acuminata Colla*)

No	Name	Formula	RT (Min)	Area (Max)
1	Choline	$C_5H_{13}NO$	0.908	5,688,271,071.17
2	Catechin		0.96	1,378,063,128.96
3	4-Methoxycinnamaldehyde	$C_{10}H_{10}O_2$	1.01	9,538,315,003.00

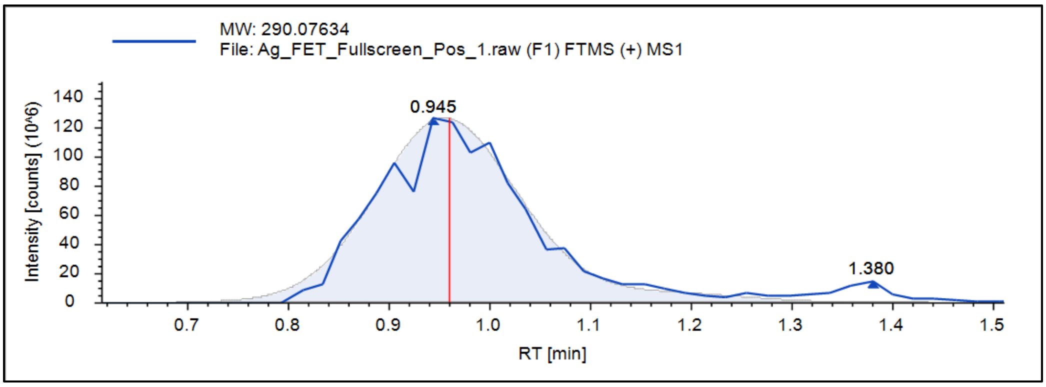

Figure 5. Chromatogram of Catechin

Choline (trimethyl-beta-hydroxyethylammonium) may be an essential nutrient for the normal functioning of all cells. Choline can be considered a quaternary ammonium compound that requires an ester bond and has three methyl groups, which are essential for a group of metabolic responses. Although cholineis often associated with vitamin B12, its properties still show that choline is more than just a vital vitamin (Biswas & Giri, 2015).

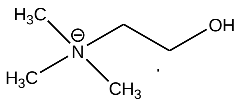

Figure 6. Structure of Choline

Catechins are the main compounds in tea leaves and have excellent antioxidant properties and physiological effects. Catechins offer several health benefits by scavenging free radicals and preventing damage to extracellular structures caused by sunlight (UV) radiation and pollution(Shi et al., 2016). Catechins specifically affect the skin by stimulating collagen synthesis and inhibiting the production of tissue metalloproteinase proteins. Due to the presence of hydroxyl groups in gallate, EGCG and ECG are highly effective as free radical scavengers compared to many other common antioxidants, such as ascorbic acid, tocopherol, and trolox(Matsubara et al., 2013) (Kim et al., 2018). Along with these beneficial activities, tea catechins are increasingly being utilized in therapeutic, pharmaceutical, and cosmetic products and are being considered effective in various methods.

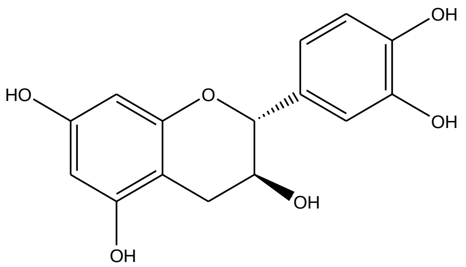

Figure 7. Structure of Catechin

CONCLUSION

Based on the results of the inquiry about what has been done, it can be concluded that the ethyl acetic acid derivation division of banana peel extract (*Musa acuminata* Colla) contains different compounds, including 5-Hydroxymethyl-2-furaldehyde, Diisobutylphthalate, and Pyrogallol. The ethanol division ofthe mas banana peel extract (Musa acuminata Colla) contains different compounds, including 4-Methoxycinnamaldehyde, Choline, and Catechin.

ACKNOWLEDGEMENT

The authors thank Jember Pharmacy Academy for providing funding assistance and facilities at Laboratorium for this research.

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