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Identification of bioactive compounds of banana corm *(Musa paradisiaca)* using GC-MS and its inhibitory effect against pathogenic bacteria

NURUL FAJRIH[1,4], KOMANG GEDE WIRYAWAN[1,♥], SUMIATI[1], SURAYA KAFFI SYAHPURA[3], WIWIN WINARSIH[2]

[1]Department of Nutrition and Feed Technology, Faculty of Animal Science, Institut Pertanian Bogor. Jl. Agatis, Kampus IPB Darmaga, Bogor 16680, West Java, Indonesia. Tel.: +62-251-8622841, ♥email: kgwiryawan61@gmail.com
[2]Department of Veterinary Clinic Reproduction and Pathology, Faculty of Veterinary Medicine, Institut Pertanian Bogor. Jl. Agatis, Kampus IPB Darmaga, Bogor 16680, West Java, Indonesia
[3]Department of Animal Husbandry, Politeknik Negeri Lampung. Jl. Soekarno Hatta, Kota Bandar Lampung 35141, Lampung, Indonesia
[4]Department of Animal Husbandry, Faculty of Agriculture, Fisheries and Animal Husbandry, Universitas Nahdlatul Ulama Lampung. Jl. Lintas Pantai Timur, Lampung Timur 34192, Lampung, Indonesia

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Abstract. *Fajrih N, Wiryawan KG, Sumiati, Syahpura SK, Winarsih W. 2022. Identification of bioactive compounds of banana corm (*Musa paradisiaca*) using GC-MS and its inhibitory effect against pathogenic bacteria. Biodiversitas 23: 195-204.* The banana corm is an agricultural waste that is abundant in nature but is rarely used even though it has many benefits including as an antibacterial agent. Therefore, this study aims to identify and isolate antibacterial compounds in banana corm and evaluate their antibacterial activity against pathogenic bacteria (*E. coli*, *S. aureus*, *S. typhi*, *B. cereus*) that generally infect livestock. The extraction procedure was carried out by maceration using ethanol and fractionation through the liquid-liquid method using ethyl acetate. The ethyl acetate fraction was further analyzed using column chromatography using silica gel as a stationary phase and eluted with 100% of $CHCl_3$, 20% of MeOH/$CHCl_3$, 3% of MeOH/$CHCl_3$ and 100% of MeOH, respectively. Identification of antibacterial compounds was carried out using GC-MS. Growth inhibitory activity against pathogenic bacteria was conducted by the well agar diffusion method. Based on the GC-MS chromatogram results, several compounds with antibacterial properties compounds were identified in the banana corm, namely *Octadeca-9,12-Dienoic* and *Hexadecanoic acids.* The extract of banana corm has antibacterial activity against *E. coli*, *Salmonella typhi*, *S. aureus*, and *B. cereus* at the concentration of 5% with inhibitory zones of 5.88 mm (moderate), 9.13 mm (moderate), 13.86 (strong), and 14.66 (strong), respectively. It is concluded that banana corm extract contains antibacterial compounds and can inhibit the growth of several pathogenic bacteria.

Keywords: Antibacterial, banana corm, GC-MS analysis, *Musa paradisiaca*

INTRODUCTION

Bacterial infections cause health problems or diseases in livestock, including in poultry. Several bacteria in the digestive tract of poultry include *Escherichia coli*, *Salmonella* sp., *Clostridium* sp, and *Enterococcus* sp. The most common pathogenic bacteria in the digestive tract of chickens and secondary agents that often follow other diseases such as respiratory or digestive diseases is *E. coli*. *Salmonella* sp. is a pathogen that generally infects the gastrointestinal tract such as the stomach, small, and large intestines of chickens.

Digestive tract health is one of the important factors that play a major role in increasing poultry productivity. A healthy digestive tract is required to achieve optimal growth. It is closely related to the external environment where pathogenic micro-organisms cause the gastrointestinal tract to be easily infected by bacteria, either through feed or the environment. It can interfere with nutrient absorption and has implications in decreasing growth and productivity. Therefore, the use of antibiotics is necessary to overcome infectious diseases. However, the continuous use of antibiotics causes bacterial resistance due to their evolution (Savitri et al. 2019) and the presence of residues in the product. Therefore, it is necessary to search for antibacterial compounds that are safer to replace resistant antibiotics to overcome infectious diseases, such as the utilization of banana corm.

Previous studies on banana corms have been carried out. A study by Wenas et al. (2020) showed that the ethanol extract of *muli* banana corm has good antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* with a minimum inhibitory concentration (MIC) value of 5.25%. A study by Azizah and Antarti (2019) also reported that *kepok* banana corm extract had antibacterial activity against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* with the widest diameter of inhibition (10.57±0,35 mm) against *Pseudomonas aeruginosa*. Furthermore, Kusuma et al. (2019) reported that the ethanolic extract of the *klutuk* banana corm has antibacterial activity *against Staphylococcus* epidermidis, at concentrations of 100%, 50%, 25%, and 12.5% with the diameter of inhibition of 17.05 mm (strong), 10.75 mm (strong), 9.25 mm (medium) and 8.57 mm (medium). respectively. Harlina et al. (2019) also reported that stems and corms of banana plants extracted using methanol had

antibacterial activity because they were proven to be able to inhibit the growth of *Staphylococcus aureus* bacteria. Although several similar studies have been carried out, the components of the active compounds with antibacterial properties in banana corm are still unknown. Therefore, further study is needed to isolate and identify the active compounds in banana corm.

MATERIALS AND METHODS

Sample preparation and extraction

Kepok banana corm *(Musa paradisiaca)* was obtained from Tanggamus Regency, Lampung Province, Indonesia. Wet banana corms were cleaned under running water to remove any adhering dirt. The clean banana corms were sliced with a thickness of approximately 0.5 cm and dried under the sun for 3 days or in the oven at 60ºC for 15 hours. The dried corms were ground using a grinder into flour. Banana corm flour was extracted based on the method modified by (Zhang et al. 2018) and (Subeki et al. 2005). Extraction was carried out by maceration with a ratio of 1:10, namely 500 g of corm flour was macerated with 5 liters of ethanol (EtOH) for 3 days and stirred daily for 5 minutes. The sample was then filtered using a filter cloth. The ethanol extract was evaporated using a rotary vacuum evaporator to obtain a concentrated extract.

Fractionation

Fractionation was carried out by liquid-liquid method using ethyl acetate (EtOAc) solvent based on a modified method by Subeki et al. (2005). The purpose of fractionation was to separate the components of the active compounds of the extract. The concentrated extract was dissolved in EtOAc and water (H2O) in a ratio of 1:1 as much as 1 L. Then, it was placed into a flask separating funnel and it was shaken slowly, and allowed to settle until there was a separation between the EtOAc and EtOH-H2O fractions. The EtOAc fraction was collected. This procedure was repeated several times until the solution became clear. The ethyl acetate fraction was evaporated using a rotary vacuum evaporator to obtain a concentrated ethyl acetate fraction. The viscous fraction was then re-evaporated to dryness. Subsequently, the dry extract was first dissolved with a small amount of ethyl acetate then put into column chromatography using silica gel as stationary phase and eluted with 100% chloroform ($CHCl_3$), 20% of MeOH/$CHCl_3$, 3% of MeOH/$CHCl_3$ and 100% of MeOH. The sub-fractions from column chromatography were analyzed using GC-MS (Gas Chromatography-Mass Spectroscopy) to determine the chemical compounds in each eluent/sub-fraction. The process of extraction and fractionation are presented in the following flow chart.

Identification of chemical compounds

The fractionation results of the banana corm extract, namely 100% of $CHCl_3$, 20% of MeOH/$CHCl_3$, 3% of MeOH/$CHCl_3$, and 100% of MeOH were analyzed using GC-MS (Gas Chromatography-Mass Spectroscopy). The GC-MS analysis was carried out to identify chemical compounds in banana corm that may contribute to antibacterial activity. The GC-MS analysis was carried out using Shimadzu QP 5000 type and using a method by Velmurugan and Anand (2017). The GC was equipped with a CP-Sil 5CB capillary column with a length of 25 m, 0.25 mm in diameter and 0.25 m in thickness Sample of 1 µL was injected into the GC. It was programmed as follows: oven temperature from 70 to 270°C, a rising rate of 10°C/min, with a flow rate of 1.0 mL/min while a total rate of 30 mL/min and with 12 kPa helium carrier gas pressure and a split ratio of 1:50 with a molecular weight range of 50-500. The eluted component was detected on the mass detector, and compounds identification was carried out using the Wiley7/LIB Library software.

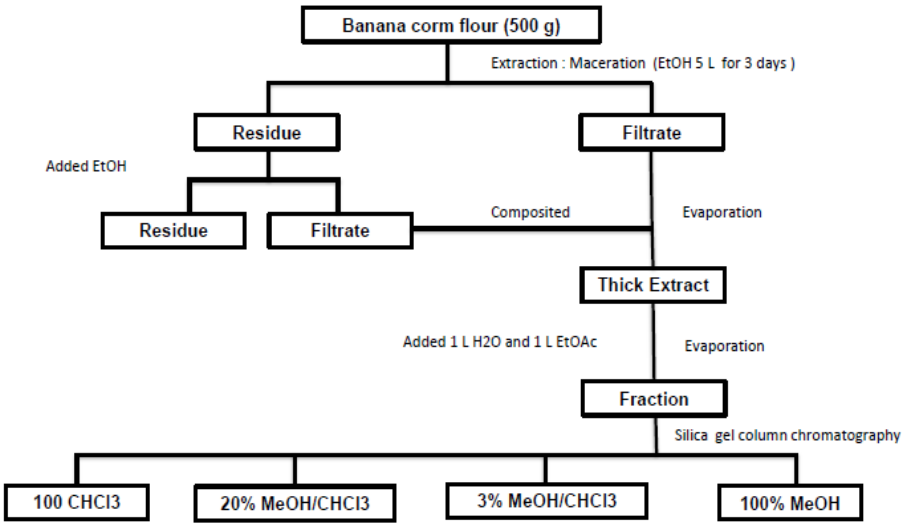

Figure 1. Flowchart of extraction and fractionation of banana corm

Antibacterial inhibitory test

An inhibitory test was carried out using the agar well diffusion method by Gonelimali et al. (2018). The bacterial cultures of *Escherichia coli, Salmonella typhi, Staphylococcus aureus,* and *Bacillus cereus* were suspended with NaCl and then adjusted for 0.5 McFarland's turbidity. The positive control solution was made from crushed tetracycline tablets, with a concentration of 10%, that is 100 mg dissolved in 1 mL of sterile distilled water while the negative control was sterile distilled water. Growth medium was the basic nutrient agar medium that was poured into a 15 mL petri dish and allowed to semi-solidify. The bacterial suspension was poured into the petri dish until it solidified, then the agar wells were prepared using a cork borer with a diameter of 6 mm for the test solution. Subsequently, the wells were filled with a control solution and a test solution for the banana corm extract fraction (100% $CHCl_3$) with 3 concentrations, i.e., 1%, 2%, and 3%. The positive and negative controls were dispenced as much as 50 µL in different wells. Petri dishes were incubated in an incubator at 37°C for 24 hours. Each test was repeated three times for each concentration. The inhibitory activity of the banana corm extract against *Escherichia coli, Salmonella typhi, Staphylococcus aureus,* and *Bacillus cereus* was indicated by the formation of the inhibitory zone. It was observed and measured using a caliper.

RESULTS AND DISCUSSION

The GC-MS chromatograms of the banana corm extract showed several identified compounds. Based on the results, three fractions (fractions 1/100% $CHCl_3$, fraction2 /20% MeOH/$CHCl_3$, and fraction 3/3% MeOH/$CHCl_3$ contained the active compounds, which are Octadeca-9,12-Dienoic acid methyl ester; Hexadecanoic acid / Palmitic acid; Hexadecanoic acid, methyl ester / Methyl palmitate; 9-Octadecenoic acid methyl ester; 1,4,8-Dodecatriene, (E,E,E)-; Octadecanoic acid, methyl ester/Methyl stearate; 1,2-Benzenedicarboxylic acid, dioctyl ester phthalate; Ergost-25-ene-3,5,6,12-tetrol; 4,8,13-Cyclotetradecatriene-1,3-diol; 1,5,9-trimethyl-12-(1-methyl ethyl, 9,19-Cyclolanost-25-en-3-ol, 24-methyl-, (3.beta.,24S) and Cycloeucalenol. The identified chemical compounds of each fraction are shown in Tables 1, 2, and 3. Meanwhile, the chemical compounds of fraction 4 (100% MeOH) were not identified, but only the solvent was identified. The MS spectrum of each peak of the fraction was compared to the mass spectra in the Wiley database to characterize and identify. GC-MS analysis is a method generally used to determine the unknown chemical components of plants (Revathi et al. 2014) and is widely used to identify various components of plant bioactive compounds that not possibly be carried out by ordinary phytochemical screening (Elezabeth and Subramanian 2014).

The identified chemical compound in fraction 1 (100% $CHCl_3$)

GC-MS chromatogram of fraction 1 using 100% $CHCl_2$ showed 7 identified chemical compounds (Table 1).

Octadeca-9,12-Dienoic acid methyl ester

The most dominant identified compound (6.45%) in fraction 1 was Octadeca-9,12-Dienoic acid methyl ester, with a retention time of 21,253 minutes. It is identical to Octadeca-9,12-Dienoic acid methyl ester in the Wiley7.LIB database. The mass spectra are shown in Figure 2. This compound is also known as sterculic acid, is vegetable oil with the molecular formula C19H3402 that is used in various industries such as cosmetics, soaps, shampoos, fabric softeners, paints, and plastics (Astiti and Ramona 2021). This compound was also reported to be present in 4.16% VCO (Virgin Coconut Oil) and has antibacterial activity against Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) bacteria (Mena et al. 2020). This compound has been known to have antibacterial and antifungal activity (Godwin et al. 2015), Satheesh Naik et al. (2018) also stated that the methanol extract of *Eclipta alba* roots contained Octadeca-9,12-Dienoic acid methyl ester, Starlin et al. (2019) reported that ethanol extract of *Tylophora pauciflora* contained and one of them was identified as Octadeca-9,12-Dienoic acid methyl ester.

Table 1. Identified chemical compounds of fraction 1 (100% $CHCl_3$)

R. Time (minutes)	Compound	Chemical formula	Molecular weight	Area (%)
21.253	Octadeca-9,12-Dienoic acid methyl ester	C19H34O2	294	6.45
18.844	Hexadecanoic acid (CAS) Palmitic acid	C16H32O2	256	4.27
18.070	Hexadecanoic acid, methyl ester (CAS) Methyl palmitate	C17H34O2	270	2.60
20.575	9-Octadecenoic acid, methyl ester	C19H34O2	294	2.23
20.605	1,4,8-Dodecatriene, (E,E,E)- (CAS)	C19H36O2	162	1.37
20.892	Octadecanoic acid, methyl ester (CAS) Methyl stearate	C19H38O2	298	1.93
25.768	1,2-Benzenedicarboxylic acid, dioctyl ester (CAS) phthalate	C24H38O4	390	0,18

Hexadecanoic acid /Palmitic acid

The second highest identified compound was Hexadecanoic acid or Palmitic acid (4.27%) (Figure 3) based on the Mass Spectral in the Wiley7.LIB database. This compound is a group of fatty acids with a retention time of 18.844 minutes. Hexadecanoic acid / Palmitic acid is also found in VCO oil (2.55%) (Mena et al. 2020). Palmitic acid is reported to have antimicrobial activity and is used as a flavoring, cosmetic ingredient, fragrances, or perfumes (Gideon 2015). Tyagi and Agarwal (2017) reported that Hexadecanoic acid has antitumor activity. The roots of *Bruguiera gymnorrhiza* contain 18.52% of palmitic acid and are reported to work synergistically with various other active compounds, hence it can increase its antibacterial activity (Dia et al. 2019).

Hexadecanoic acid, methyl ester / Methyl palmitate

Hexadecanoic acid, methyl ester / Methyl palmitate (2.60%) was also identified based on the Mass Spectral in Wiley7.LIB database (Figure 4), and has a retention time of 18.070 minutes. Its mass spectrum was identical to the mass spectrum of Hexadecanoic acid, methyl ester. This compound has a molecular weight of 270 with the chemical formula of C17H34O2 and is classified as a fatty acid group. The mechanism of the antibacterial activity of methyl palmitate was by damaging the walls and membranes of bacterial cells (Astiti and Ramona 2021). Methyl palmitate is present in *Cirsium arvense* plants (15.572%) which were also reported to have potential as antimicrobials, pesticides, and nematicides (Hema et al. 2015; Banaras et al. 2017). Mint (*Mentha spicata)* contained methyl palmitate by 31.51% and was reported to have potential as an antioxidant with an IC_{50} value of 65.13±1.29 g/mL using the DPPH method & 52.31±0.81g/m by ABTS method (Abdel-Hady et al. 2018).

9-Octadecenoic acid, methyl ester

Banana corm fraction contained 9-Octadecenoic acid, methyl ester (2.23%) that was detected at a retention time of 20,575 minutes (Figure 5). This compound is classified as unsaturated fatty acids that also commonly known as oleic acid (Belakhdar et al. 2015). Oleic acid was very abundant in lemuru fish oil (35.59%) which was detected at a retention time of 20.00 minutes (Kosasih et al. 2021). The difference in retention time may be due to the different types of GC-MS equipment used. This compound is potential as an anticancer and anti-inflammatory (Gideon 2015).

1,4,8-Dodecatriene, (E,E,E)-

The peak at a retention time of 20,605 minutes was detected to be identical to the mass spectrum of 1,4,8-Dodecatriene (Figure 6), at the concentration of. 1.37% in the banana corm extract fraction. It has a molecular weight of 162 with the chemical formula C19H36O2. It has been reported to present in seaweed (*Hormophysa cuneiformis*) extract in smaller amounts by 0.46% at a retention time of 11,681 minutes and is reported as an antifungal. Furthermore, this compound can prevent the growth of pathogenic fungi in humans and fungi that infect crops (Samar et al. 2019). The compound 1,4,8-Dodecatriene, (E,E,E)- is also found in lemuru fish oil by 3.52% (Kosasih et al. 2021).

Octadecanoic acid, methyl ester / Methyl stearate

The next identified compound is octadecanoic acid, methyl ester, or usually known as Methyl stearate (1.93%). at a retention time of 20,892 minutes. It is identical to the mass spectrum of GCMS in the Wiley7.LIB database (Figure 7). This compound has the chemical formula $C_{19}H_{38}O_2$ similar to steroids used for lubricants and plasticizers as well as has properties as fragrances (Asghar et al. 2011). Octadecanoic acid, methyl ester (CAS), or Methyl stearate was also reported to be present in *Pseudoglochidion anamalayanum* (Gideon 2015) and *Albizia adianthifolia* extracts at the concentration of 5.89% (Abubakar and Majinda 2016). Perumalsamy et al. (2015) reported that this compound functions as an insecticide and is effective for controlling *Aedes aegypti* and *Culex pipiens*. It is also effective for controlling the growth of bacteria and fungi (Rangel-Sánchez et al. 2014).

1,2-Benzenedicarboxylic acid, dioctyl ester

1,2-Benzenedicarboxylic acid, a dioctyl ester (0.18%) is a chemical with a molecular weight of 390 with the chemical formula of $C_{16}H_{22}O_4$ which is widely used in industrial applications. The peak of this compound was identified at a retention time of 25,768 minutes according to the mass spectrum in the database (Figure 8). This compound is used as a cosmetic ingredient, as a plasticizer in products such as nail polish and hair spray, as well as a solvent, perfume fixative, and many other products. This compound was also identified in mint plants (0.46%) (Abdel-Hady et al. 2018), and 4.10% in the extract of *Cenchrus setigerus* herbal plant (Arora and Kumar 2017), and large amounts (12.37%) is found in the leaf extract of *Andrographis paniculatas* (Krishnamoorthy and Kalaiselvan 2016).

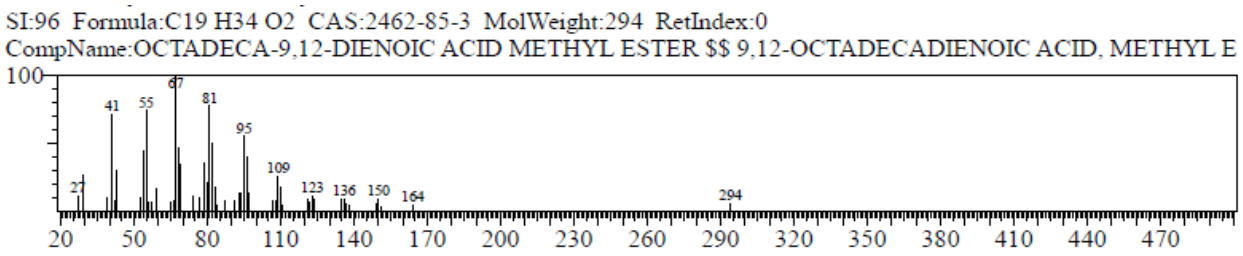

Figure 2. The mass spectra of Octadeca-9,12-Dienoic acid methyl ester

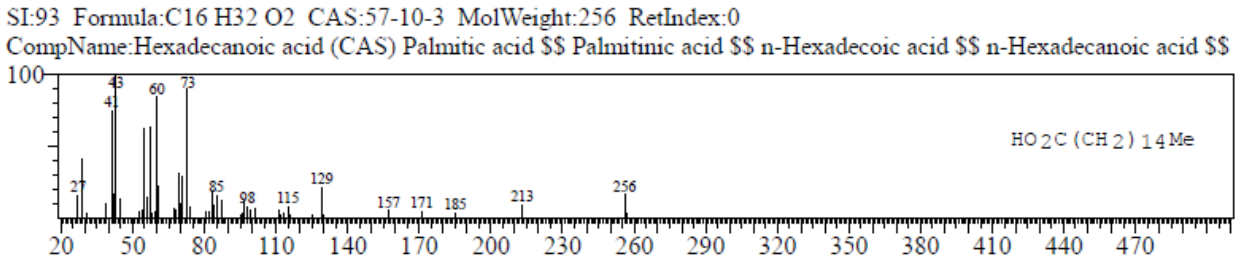

Figure 3. The mass spectra of Hexadecanoic acid / Palmitic acid

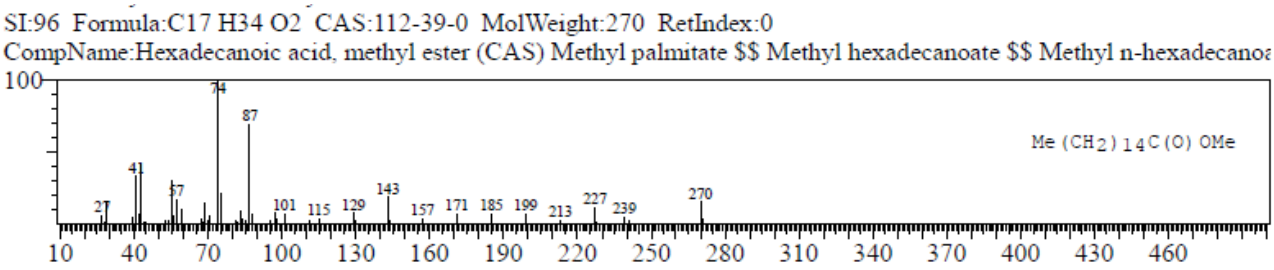

Figure 4. The mass spectra of Hexadecanoic acid, methyl ester / Methyl palmitate

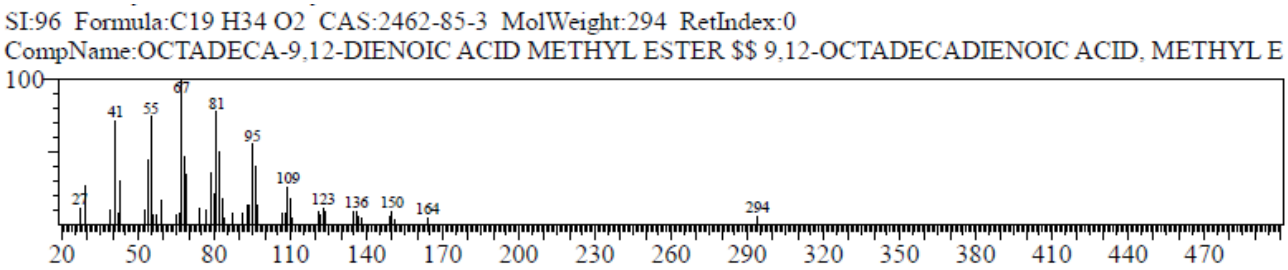

Figure 5. The mass spectra of *9-Octadecenoic acid, methyl ester*

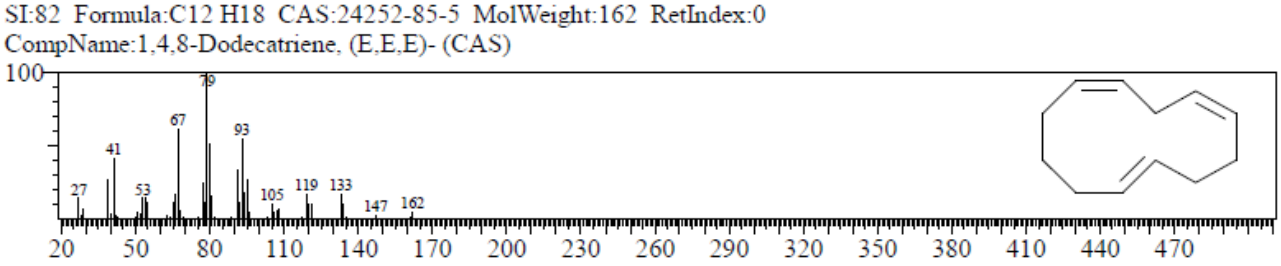

Figure 6. The mass spectra of *1,4,8-Dodecatriene, (E,E,E)-*

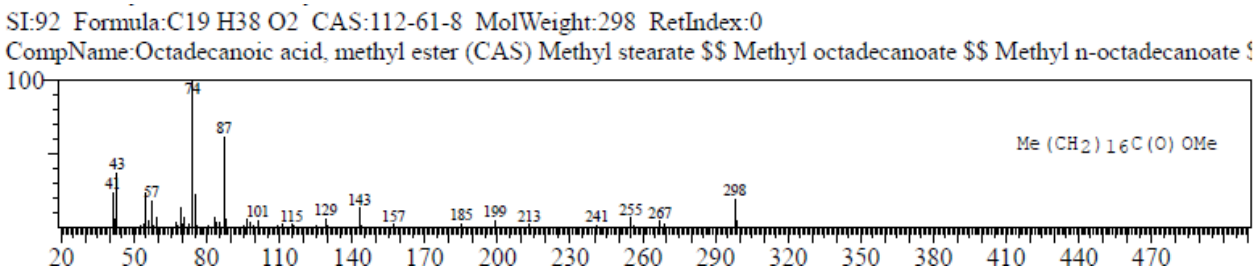

Figure 7. The mass spectra of octadecanoic acid, methyl ester / Methyl stearate

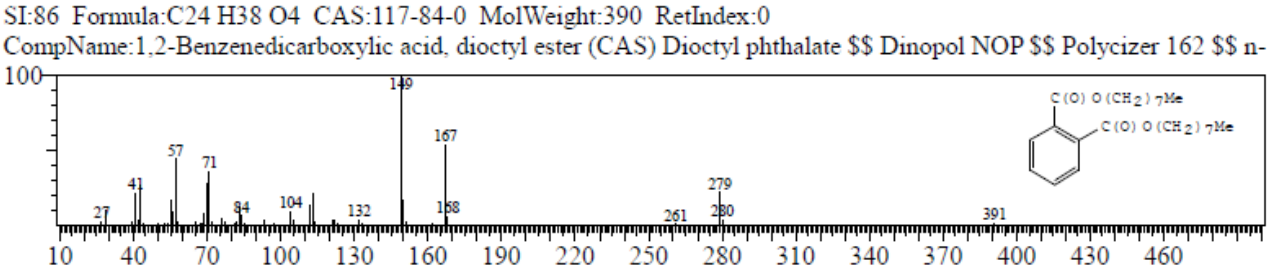

Figure 8. The mass spectra of 1,2-Benzenedicarboxylic acid, dioctyl ester

The identified chemical compounds in fraction 2 (20% MeOH/$CHCl_3$)

There were only 2 identified chemical compounds in fraction 2 and very small amounts (Table 2).

Ergost-25-ene-3,5,6,12-tetrol

The first identified chemical compound in the fraction was *Ergost-25-ene-3,5,6,12-tetrol* (1.96%) (Figure 9) at a retention time of 28.125 minutes. It has a molecular weight of 448 with the chemical formula of $C_{28}H_{48}O_4$. It was also found in the brotowali plant extract at a higher amount (10.46%). The compound was used as an antibacterial and was reported to have inhibitory activity against *P. syringae* bacteria of 36.3 mm (methanolic extract), 40 mm (n-hexane extract), 30.2 (ethyl acetate extract), 29 mm (chloroform extract) (Akbar et al. 2020).

4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl

The second identified compound was *4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl* (0.36%) (Figure 10) at a retention time of 28.170 minutes. It was also found in *Curcuma raktakanda* extract (1.67%). It was reported to have anticarcinogenic activity against cancer cells. (Mishra et al. 2019)

The identified chemical compounds in fraction 3 (3% MeOH /$CHCl_3$)

Fraction 3 contained 2 identified chemical compounds as presented in Table 3.

9,19-Cyclolanost-25-en-3-ol, 24-methyl-, (3.beta.,24S)

The peak of this compound was identical to *9,19-Cyclolanost-25-en-3-ol, 24-methyl-, (3.beta.,24S)* in the mass spectrum of the Wiley7.LIB database (Figure 11). This compound was identified at a retention time of 28,170 minutes at the amount of 2.38%. It has the chemical formula of $C_{30}H_{50}O$ which is widely applied in the health, food, and chemical industries. It was found at a high amount in the *Trigona* spp. propolis fraction (49.91%) at a retention time of 40.25 minutes and was reported to have the potent antibacterial activity against *E. coli* (Hasan et al. 2014).

Cycloeucalenol

Cycloeucalenol was identified in fraction 3 but it was present in very small amounts (0.17%) (Figure 12). The peak of this compound was identified at a retention time of 28,572 minutes. This is a *pentacyclic triterpenoid* with the chemical formula of $C_{31}H_{52}O$. It was previously isolated from several plant species including *Turraeanthus* and *Tillandsia*, however, this compound has not been previously reported to be present in the banana corm.

Antibacterial activity of fraction 1

In this study, fraction 1 (100% CHCl3) of banana corm extract was selected for antibacterial activity assay. As previously explained, fraction 1 contained 7 chemical compounds that have antibacterial activity. Therefore, further analysis is necessary to determine its effect on the tested bacteria. The well diffusion method is more effective in inhibiting the growth of bacteria because the active substance can diffuse directly without a paper disc barrier as in the Kirby Bauer method (Kusuma et al. 2019). The antibacterial activity of the banana corm fraction 1 is presented in Table 4.

The results of the antibacterial test showed that the banana corm (*Musa paradisiaca*) fraction had antibacterial activity against *E. coli*, *Salmonella typhi*, *S. aureus* and *B. subtilis* that is characterized by the formation of a clear or inhibitory zone as shown in Figure 12. Table 4 showed fraction concentration of 5% concentration had a wider diameter of the inhibitory zone than the 1% and 3% concentrations. The results showed that fraction 1 had strong antibacterial activity against *B. subtilis* (14.66 mm), *S. aureus* (13.86 mm), and moderate activity against *S. typhi*. However, the widest inhibitory zone against *E. coli* (7.73mm) was obtained at a concentration of 3% which was classified as moderate. The criteria for antibacterial activity refer to (Davis and Stout 1971), as follows: low antibacterial activity (< 5 mm), medium (5-10 mm), strong (10-19 mm), and very strong (>20 mm). The higher the concentration of the corm fraction, the wider the diameter of the inhibition. The diameter of the inhibitory zone is an indication of the sensitivity of the test bacteria where the wider the inhibitory zone, the better the antibacterial activity (Bhargav et al. 2016).

Table 2. Identified chemical compounds of fraction 2 (20% MeOH/$CHCl_3$)

R. Time (minutes)	Compounds	Chemical formula	Molecular weight	Area (%)
28.152	*Ergost-25-ene-3,5,6,12-tetrol*	C28H48O4	448	1.96
28.577	*4,8,13-Cyclotetradecatriene-1,3-diol,1,5,9-trimethyl-12-(1-methylethyl*	C20H34O2	306	0.36

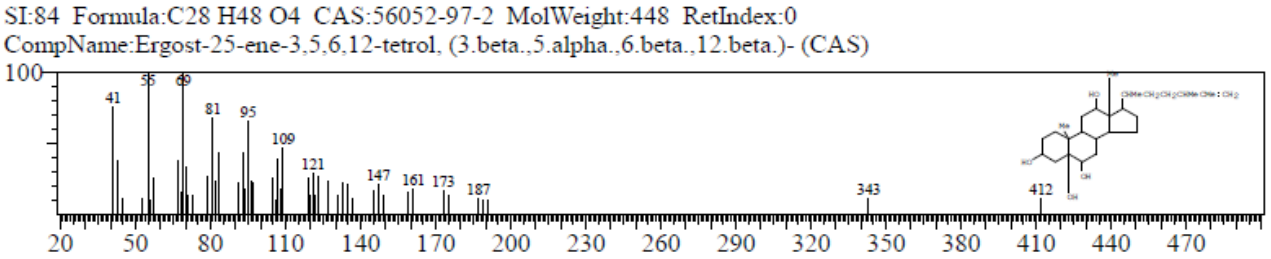

Figure 9. The mass spectra of *Ergost-25-ene-3,5,6,12-tetrol*

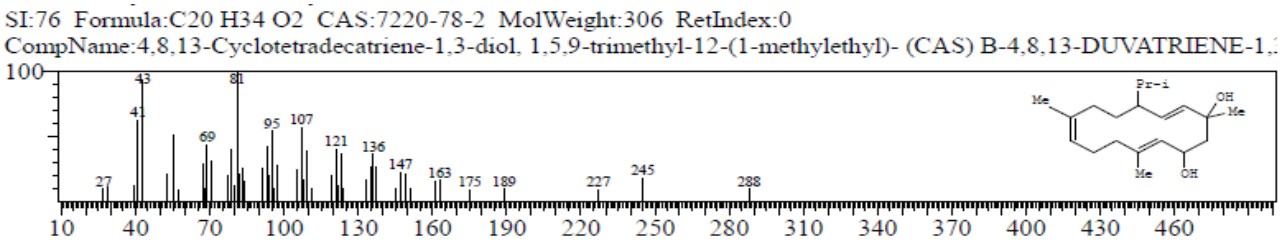

Figure 10. The mass spectra of 4,8,13-Cyclotetradecatriene-1,3-diol,1,5,9-trimethyl

Table 3. Identified chemical compounds of fraction 3 (3% MeOH/$CHCl_3$)

R. Time (minutes)	Compounds	Chemical formula	Molecular weight	Area (%)
28.170	9,19-Cyclolanost-25-en-3-ol, 24-methyl-, (3.beta.,24S)	C30H50O	440	2.38
28.572	Cycloeucalenol	C31H52O	462	0.17

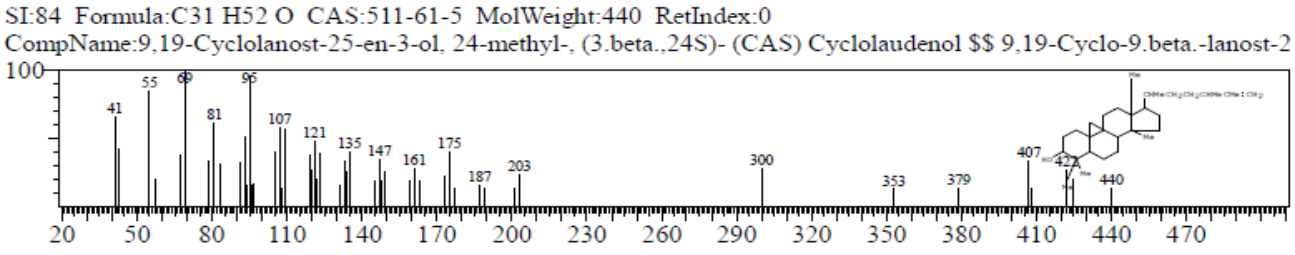

Figure 11. The mass spectra of 9,19-Cyclolanost-25-en-3-ol, 24-methyl-, (3.beta.,24S)

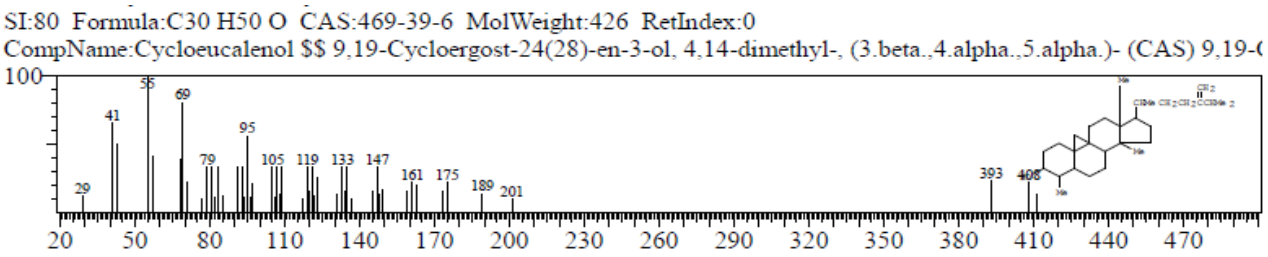

Figure 12. The mass spectra of *cycloeucalenol*

Table 4 showed that each concentration of banana corm fraction had inhibitory activity against 4 bacterial isolates except for the negative control treatment. Furthermore, it can be seen that the higher the concentration of the banana corm fraction, the wider the inhibitory zone. It might be due to the presence of many compounds which have antibacterial activity including *Octadeca-9,12-Dienoic acid methyl ester, Hexadecanoic acid (CAS) Palmitic acid, Hexadecanoic acid, methyl ester (CAS) Methyl palmitate, 9-Octadecenoic acid, methyl ester, 1,4,8-Dodecatriene, (E,E,E)- (CAS), Octadecanoic acid, methyl ester (CAS) Methyl stearate, and 1,2-Benzenedicarboxylic acid, dioctyl ester (CAS) phthala*. The presence of these compounds might work synergistically in inhibiting bacteria. The results are supported by Azizah and Antarti (2019) who reported that the kepok banana corm extract was able to inhibit the growth of *S. aureus* and *E. coli* because it contains saponins, flavonoids, and alkaloids with antibacterial activity. Wenas et al. (2020) showed that the ethanol extract of muli banana corm has good antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

The widest zone of inhibition was obtained against *S. aureus* and *B. cereus* and the smallest against *E. coli* and *Salmonella typhi* (Figure 13). The difference in the inhibitory properties of the test bacteria was caused by the sensitivity of each bacterium. The sensitivity of bacteria to antibacterial substances depends on the type of micro-organism (Balouiri et al. 2016). *Escherichia coli* is a Gram-negative bacteria that have 3 layers of the cell wall, namely lipoprotein, outer membrane, and lipopolysaccharide, with very high fat or lipid content reaching 11-22%. Therefore, it was difficult for antibacterial substances to penetrate. The fat or lipid content in Gram-positive bacteria, i.e. *S. aureus* and *B. subtilis* is 1- 4% so that it might easier for antibacterial substances to penetrate the cell wall of Gram-positive bacteria. Meanwhile, the diameter of the greatest inhibition among all treatments occurred in the Positive control treatment had the widest inhibitory zone (22.93 mm -25.93 mm) that was classified as very strong antibacterial activity. The magnitude of inhibitory activity in the positive control is due to tetracycline antibiotics has a broad-spectrum antibacterial. The mechanism of antibacterial activity of tetracycline was by inhibiting bacterial cell wall synthesis (Azizah and Antarti 2019) and is very effective in inhibiting the growth of Gram-positive, Gram-negative bacteria, and other microorganisms such as mushroom or fungus (Kusuma et al. 2019). In general, the findings of this study showed that banana corm contained several antibacterial compounds. Furthermore, despite their presence in small amounts, these compounds can inhibit *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, and *Bacillus cereus* at the concentration of 5% with inhibitory zones of 5.88 mm (moderate), 9.13 mm (moderate), 13.86 strong) and 14.66 (strong), respectively.

Table 4. Average diameter of inhibition of banana corm fraction1 (100% CHCl3) against bacteria

Species of bacteria	The diameter zone of inhibition (mm)				
	Control (-)	Concentration 1%	Concentration 3%	Concentration 5%	Control (+)
E. coli	0.00	5.18	7.73	5.88	22.93
S. typhi	0.00	6.06	8.20	9.13	24.47
S. aureus	0.00	6.26	10.79	13.86	25.27
B. subtilis	0.00	7.65	11.27	14.66	25.93

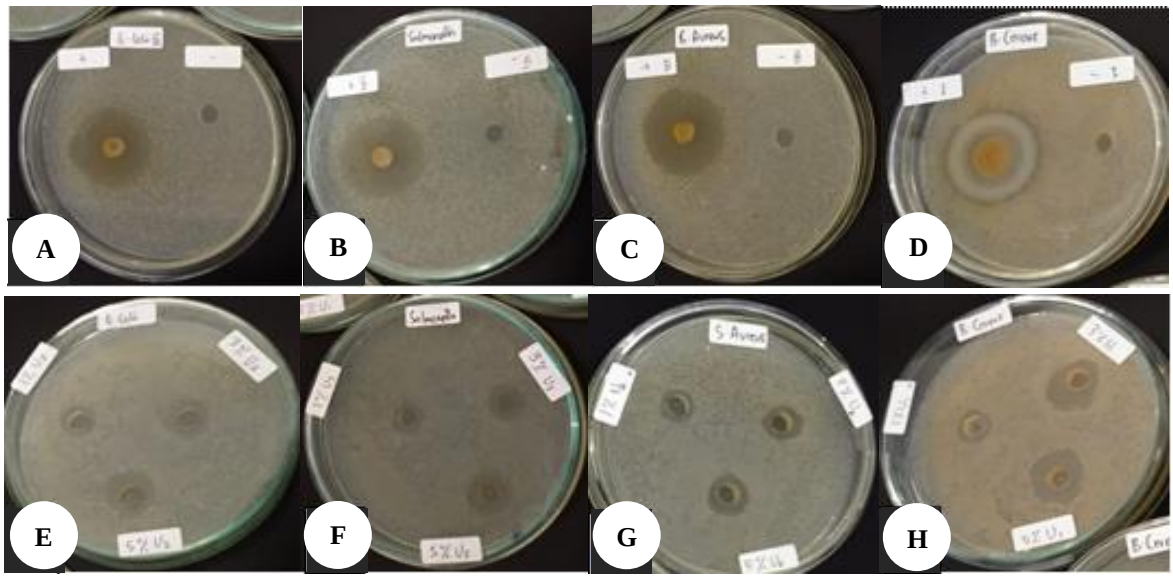

Figure 13. Inhibitory zone of banana corm fraction. A. control against *E. coli*. B. control against *S. typhi*. C. control against *S. aureus*. D. control against *B. subtilis*. E. various concentrations against *E. coli*. F. various concentrations against *S. typhi*. G. various concentrations against *S. aureus*. H. various concentrations against *B. subtilis*

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