Bioactivity and Phytochemical Constituents of Extract Etanol from Stem *Musa paradisiaca* Linn

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Abstract: The search for active ingredients from plants that are secondary metabolites as a defense compound from plants has been carried out. This study was conducted to investigate the phytochemical constituents of *Musa paradisiaca* Linn's pseudo-stem, such as alkaloids, flavonoids, steroids, terpenoids, and saponins. In this study, we estimated the content of terpenoids and saponins and determined the activity of 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging. Ethanol extract of *Musa paradisiaca* Linn's pseudo-stem, active as an antioxidant (IC 50 = 494.2) with a comparison of Ascorbic acid. Chemical constituents of ethanol extract of *Musa paradisiaca* Linn's pseudo-stem are characterized by GC-MS, which shows that they contain triterpenoid organic compounds, such as: Corticosterone, Stigmasterol, Obtusifoliol, Lupeol, and 9-Cyclolanost-24-en-3-ol

1 INTRODUCTION

Indonesia's geographical location has a tropical climate with high average rainfall throughout the year so that Indonesia has very famous natural resources. Various types of plants that can thrive throughout the archipelago do not know the season. The use of these plants as well as food ingredients is also used as traditional medicine. Research on the chemistry of natural materials is increasingly being exploited today as a medicinal ingredient and for the benefit of other fields. The chemical structure diversity produced by these plants also reduces abandoned and easily available side effects.

Banana plants, plants that are easy to breed in tropical climates. One of the varieties known is kepok banana plant (*Musa paradisiaca* Linn). Some parts of this plant have benefits, one of which is as an antioxidant.

The use of diphenylpicrylhydrazyl (DPPH) as a stable free radical can estimate antioxidant activity with IC50 parameters. As a good recommendation for testing and evaluating data (Molyneux, 2003). Dopamine and L-dopa compounds contained in banana peels are significantly active as antioxidants. The banana peel extract from the varieties (Cavendish and Dream) had been analyzed by DPPH inhibitory activity 26.55% to 52.66% (Fatemeh et al, 2012). The compound content of banana peel extract (Musa Cavendish) has been identified as Gallocatechin the most, which is a strong antioxidant (158 mg / 100 g dry weight) (Someya et al, 2002).

Extract of Banana peel (Musa acuminata Colla AAA) from 4 types of banana peel has been ana-lyzed has a high capacity to scavenge 2,2-diphenyl-1-pikrillhidrazil (DPPH). DPPH scavenging activity of acetone extract and methanol from banana peel showed greater value than ethanol and water ex-tract (Aboul-Enein, 2016). Peel extract from all nine banana varieties showed significant antioxidant and phytochemical activity. Antioxidant activity of fresh green and yellow banana peel from fruit (Musa, cv. Cavendish) was treated with 70% acetone, which was partitioned with chloroform ($CHCl_3$) and ethyl acetate (EtOAc), which was evaluated (Mok-bel et al., 2005).

Pseudo-stem sap Banana has several special properties related to various phenomena such as browning of fruit after harvest, permanent coloring of fabrics and fibers, antioxidant, antimicrobial and antihemorrhagic properties. All aqueous pseudo-stem

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extracts, methanol and ethanol have been found to contain good amounts of antioxidants along with different phytochemical compounds such as carbohydrates, proteins and phenolic compounds (Kumar et al., 2014).

This research has been carried out phytochemical screening test and determined the compounds contained in the ethanol extract of kepok banana stem (*Musa paradisiaca* Linn) as well as knowing the antioxidant activity of the crude extract.

2 MATERIALS AND METHODS

2.1 Collection and Processing of Kepok Banana Pseudo-stem (*Musa Paradisiaca* Linn)

Kepok Banana pseudo-stems were collected from the Kotamadya Medan which has a stem diameter of about 5-10 cm collected in February 2019. Sample was washed with tap water and dried in drying cabinet at 500 C for 3 days, crushed with a blender into powder. And preparations were immediately made for crude extract ethanol.

2.2 Extraction Procedures

Kepok banana stem powder (250 gr) was extracted with ethanol 96% immersion for 72 hours and occasionally stirring. Filtration was carried out, the filtrate was collected and the precipitate was soaked with ethanol 96% for 48 hours and filtered again. The first and second filtrates were combined and then concentrated at 70ºC using a rotary flash evaporator. The crude extract obtained is stored in the dark at 4ºC for further testing.

2.3 Phytochemical Tests

Introduction Phytochemical analysis is carried out to determine the presence of various phytochemicals.

2.3.1 Phenolic Compounds

The extract (500 mg) was dissolved in 5 ml of distilled water. For this, a few drops of neutral 5% ferric chloride solution is added. Dark green indicates the presence of phenolic compounds (Ingonga et al., 2015).

2.3.2 Terpenoids and Steroids

Steroids (Liebermann-Burchard reaction). The 200 mg extract material was added in 10 mL of chloroform. Acetic anhydride is added in a 1: 1 ratio which results in a blue-green ring formation pointing towards the presence of steroids.

Terpenoid (Salkowski test). For 200 mg of extract material, 2 mL of chloroform ($CHCl_3$) and 3 mL of concentrated sulfuric acid (H_2SO_4) were added carefully. Reddish brown marks the presence of terpenoids (Ingonga et al., 2015).

2.3.3 Phytochemical Tests

200 mg of extract is diluted to 10 mL with Methanol, boiled and filtered. For 5 mL of filtrate, 2 mL of dilute ammonia is added. 5 mL Chloroform is add-ed and gently shaken the alkaloid base extract. The chloroform layer was extracted with 1 mL of acetic acid. This is divided into two parts. Mayer reagent was added to one part and Draggendorff reagent to the other. The formation of cream (with Mayer reagent) or reddish-brown precipitate (with Draggendorff reagent) is considered positive for the presence of alkaloids (Abdallah, 2016).

Mayer reagent & Wagner reagent confirmed the presence of alkaloids in the extract. Plant extracts are heated with 2% H_2SO_4 for two minutes. It is filtered and a few drops of reagent are added separately. With a few drops of Reagent Mayer creamy white precipitation appears a positive result. Indi-cates the presence of alkaloid compounds. Wagner's reagent, the precipitate of reddish brown appeared which also confirmed the presence of alkaloids in the extract (Rdhia et al, 2018).

2.3.4 Saponins

Crude extract (2 g) boiled in 20 mL of distilled water in a water bath and filtered. The filtrate was shaken violently for stable froth which was considered positive for the presence of saponins (Moubayed et al., 2017).

2.3.4 Flavonoids

In the aqueous filtrate, 5 mL of dilute ammonia solution is added, followed by concentrated H_2SO_4. yellow staining indicates the presence of flavonoids (Ingonga et al., 2015).

2.4 Gas Chromatography and Mass

The analysis was carried out using Shimadzu GCMS-QP2010S with Detector: 0.85 kV + 0.00 kV. Electron Energy: 10 to 200 eV with helium at 1.51 ml for 1 minute as a carrier gas. The mass spectrometer is operated in electron (El) impact mode at 70 eV in the scanning range 50-500 m / z. Column Flow: 1.03 mL / min Separation ratio is adjusted to 1:10. The injector temperature is 200◦C, and the oven temperature is maintained at 70◦C for 3 minutes, rising to 200◦C. Identification of peak extracts of raw banana plant extracts was carried out by comparison with standard retention times, and mass spectra obtained compared to those available in the NIST library (NIST 14 - MassSpectral Library, 2014 version).

2.5 Activities for DPPH Radical Scavengers

Scavenging 1,1-diphenyl-2-pikrillhidrazil (DPPH) Radicals by the sample were monitored according to the modified Yen and Chen (1995) method. Briefly, 2 mL aliquots of the test sample were added to 1 mL of DPPH 0.4 mM methanol solution. Mix the vortex for 1 minute and then leave the room temperature for 30 minutes in dark conditions, and the absorbance is read at 517 nm (Moubayed et al., 2017). Synthetic antioxidant ascorbic acid is used as a positive control.

The ability of test samples to scavenge DPPH radicals is calculated using the following equation (Wu el al., 2009):

Inhibition percent = [(AB - AA) / AB] x 100 (1)

Where: AB = absorbance value of blank sample, AA = absorbance value of test sample

3 RESULTS AND DISCUSSION

3.1 Phytochemical Screening

Important phytochemicals, such as alkaloids, triterpenoids, steroids, phenolics, flavonoids and saponins for their presence and are presented in Table 1.

From the results of the phytochemical tests carried out, it is clear that the high content of the banana plant stems is terpenoids, steroids and saponins. Further testing of crude extracts in GC-MS was carried out to determine the compounds present in the plant extract.

Table 1: Phytochemical test results of crude ethanol extract of Kepok Banana pseudo-stem (*Musa paradisiaca* Linn).

Secondary metabolite	Ethanol extract
Phenolic	-
Terpenoids	++
Steroids	++
Alkaloids	-
Saponins	++
Flavonoids	-

+ : The presence of secondary metabolite
- : The absence of secondary metabolite

3.2 GC-MS of Crude Extract

Table 2: Chemical constituents of ethanolic crude extract from *Musa paradisiaca* Linn pseudo-stem.

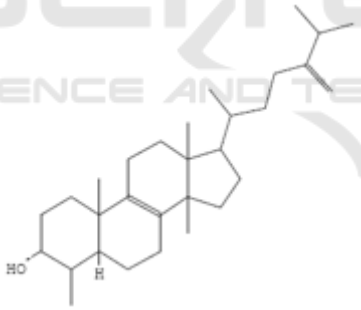
Compound's name	RT	Peak area (%)
1,3,5-triazine-2,4,6-triamine	12.49	0.17
Trihloroacetic acid, tridecyl ester	13.31	0.08
Phytol	19.07	0.04
Corticosterone	22.27	0.19
Heptasiloxane tetradecamethyl	28.29	54.05
Corticosterone 21-acetate	28.78	10.95
Stigmasterol	29.05	4.63
Obtusifoliol	29.33	8.47
Butyl-4-[(trimethylsilyl)amino]benzoate	29.77	3.93
Propionic acid, 3-(benzol[1,3])dioxol-5-yl)-3-(4-methylbenzoylamino)	29.89	1.67
Sebacic acid, 4-bromo-2,6-difluorobenzyl isobutyl ester	30.02	1.59
Lupeol	30.37	4.14
9,19-Cyclolanost-24-en-3-ol, (3-beta)-	30.63	3.69
9,19-Cyclolanost-3-ol, 24-methylene-,	31.22	5.49
Pentasiloxane, 1,1,3,3,5,5,7,7,9,9-decamethyl	31.65	0.22
Trimethylsilyl-2-(5H-chromenol[2,3-b]pyridine	31.96	0.33
Ethanethioic acid, S-[8-(diethylphosphono)octyl	33.69	0.34

The consequences associated with GC-MS investigations led to the recognition of many

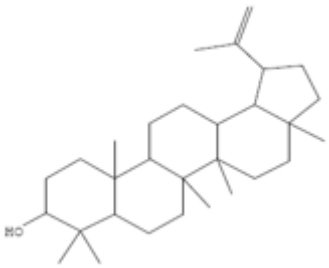
compounds from GC from the ethanol extract of the *Musa paradisiaca* Linn. This compound is recognized through the mass spectrum assembled with GC. The active principle with their retention time [RT], molecular formula (MF), molecular weight (MW) and concentration (%) can be accessed in Table 2.

Seventeen chemical compounds identified from ethanol extract from the stem of *Musa paradisiaca* Linn by GC-MS analysis. The occurrence of various bioactive compounds confirms the application of the stem of *Musa paradisiaca* Linn to various diseases, by traditional practitioners, a number of compounds previously reported from a number of other plant species.

From the data above (Table 2), there are eight of the most chemical compounds: Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl (RT 28.29; 54.05%); Corticosterone 21-acetate (RT 28.78; 10.95%); Obtusifoliol (RT 29.33; 8.47%); 9,19-Cyclolanostan-3-ol, 24-methylene -, (RT 31.22; 5.49%); Stigmasterol (RT 29.05; 4.63%); Lupeol (RT 30.37; 4.14%); Butyl 4 - [(trimethylsilyl) amino] benzoate (RT 29.77; 3.93%); 9,19-Cyclolanost-24-en-3-ol, (3.beta.) - (RT 30.63; 3.69%). And the bioactive compounds in the extract have the following chemical structures:



(a)



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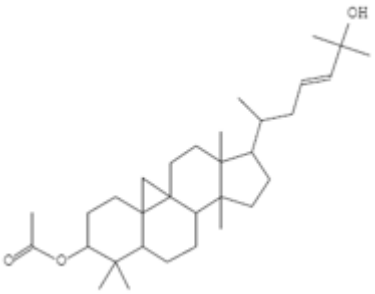

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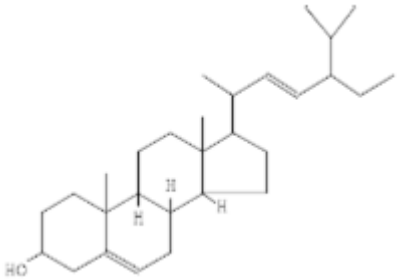

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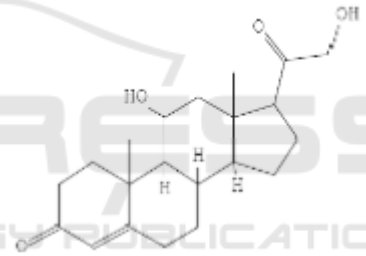

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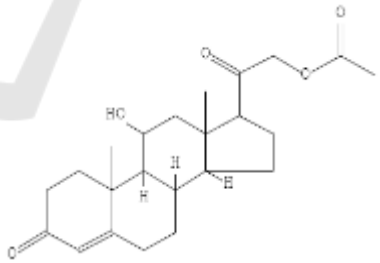

(f)

Figure 1: Chemical structures of (a) Obtusifoliol (b) Lupeol (c) Stigmasterol (d)9,19-Cyclolanostan-3-ol, 24-methylene- (3β. (e) Corticosterone (f) Corticosterone acetate.

The obtusifoliol content of 8.47%, the type of steroid contained in stem ethanol extract is very potential as a compound that can inhibit the proliferation of MCF-7 and MDA-MB231 breast cancer cells through the cell cycle which is stopped

the development and induction of apoptosis. (Aghaei et al., 2016)

Lupeol obtained from GC-MS results as much as 4.14%. Lupeol is a pharmacologically active penta-cyclic triterpenoid found in several medicinal plants throughout the world. This compound shows a hepatoprotective effect on Aflotoxin B1-induced damage in mice. In addition, lupeol has a hepato-protective effect on CCl_4 poisoning. The protective effect of the Lupeol compound will improve kidney injury associated with hypercholesterolemia, namely the presence of cardioprotective which can be beneficial in the condition of hypercholesterolemia because it minimizes lipid abnormalities and ab-normal biochemical changes caused by cholesterol and mice fed colic acid (Mbaveng et al., 2014).

Stigmasterol in the crude extract of ethanol stem as much as 4.63%. The presence of cholesterol-lowering activities by Stigmasterol, other bioactivities are ascribed to plant sterol compounds, one of which has the potential to cause anti-inflammatory effects. To investigate the effects of stigmasterol, plant sterols, on inflammatory mediators and metalloproteinases produced by chondrocytes (Gabay et al., 2010).

9,19-Cyclolanostan-3-ol, 24-methylene- (3β.) In this extract contained 9.08%, were triterpenes and n-Hexadecanoic acid, have been reported to have antimicrobial activity. It has also been reported that plant sterols are a good therapeutic choice for the management of hypercholesterolemia (Ameachi and Chijioke, 2018).

Some antibacterial compounds, such as 24, 24-dimethyl-9,19-cyclolanostan-3 b-ol, daucosterol, allantoin, and D-mannitol have been reported in aerial parts (Phthalides et al., 2018). 9.19-cyclolanostan-3-ol.24-methylene-3.beta acting as an anti-HIV compound, used to prevent the HIV virus. (Arora and Kumar, 2017). Corticosterone is a compound that can act as a metabolism (low to moderate levels) and stress hormones (high levels) and, can affect reproductive (Apfelbeck et al., 2017).

3.3 Antioxidant Test

Effect of ethanol extract of *Musa parasiaca* Linn containing active compounds reacting with DPPH free radicals will change to 1,1-diphenyl-2-picrylhydrazine which is non-radical. Look at the color changes that occur from DPPH which is purple to yellow and proven by the smaller absorbance value (Molyneux, 2003). The decrease in absorbance along with the increase in plant ethanol extract was added. So that we can determine% inhibition of each concentration and we can determine the linear regression equation in the Figure 2.

Based on Figure 2, the linear line equation is obtained and from this equation is used to calculate the IC50 value of the ethanol extract of the sample. The results of calculation of IC50 values were obtained 494.209. And from Figure 3 as a comparison is ascorbic acid with an IC50 value of 5.492.

The ability of extracts to ward off DPPH is thought to be the presence of Lupeol compounds. The statement that Lupeol as a natural active constituent is well-known for its anti-inflammatory, antioxidant and neuroprotective activities (Kaundal, 2017).

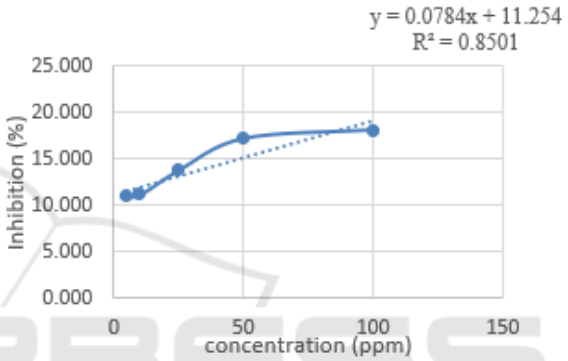

Figure 2: Curve of % inhibition of *Musa paradisiaca* Linn extract.

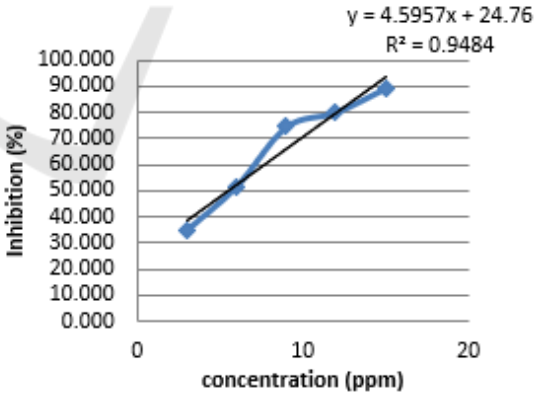

Figure 3: Curve of % inhibition of ascorbic acid.

Besides lupeol, there is also the presence of Stigmasterol, also known as Stigmasterin or the Wulzen anti-stiffness factor, a non-saturated plant sterol found in various medicinal plants. Stigmasterol is used in a number of chemical processes designed to produce various synthetic and semi-synthetic compounds for the pharmaceutical industry. It acts as a precursor in the synthesis of

progesterone and acts as an intermediary in androgen biosynthesis, estrogen, corticoids 1 and in the synthesis of vitamin D and Stigmasterol has also been investigated for its pharmacological prospects such as antiosteoarthritis, antihypercholestrolemic, cytotoxic, antitumor, hypoglycemic, antimutagenic, antioxidant, and anti-inflammatory (Chaudhary et al., 2011).

4 CONCLUSIONS

Crude ethanol extract of *Musa paradisiaca* Linn from 250 g of dried powder phytochemical screening test was carried out and continued with compound analysis by GC-MS. Some active compounds are obtained which are thought to be very beneficial for biological activities. The presence of antioxidant activity extracts has been evaluated. As far as we know, this research is the first report on the bioactivity of plant ethanol extract *Musa Paradisiaca* Linn Further phytochemical research is needed to identify the active principle responsible for activity antioxidant. This investigation presents sufficient data about phytochemical constituents in one polar solvent. Bioactive compounds found in the stem of *Musa paradisiaca* Linn plant hope to be applied as natural antioxidants and can be extrapolated for clinical studies.

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