Preliminary study on phytochemical screening and antibacterial activity of dry ethanol extract from Kepok Banana Steam Liquid (Musa paradisiaca Linn)

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Abstract
Antibacterial activity on banana plants, has been carried out from the family Musaceae for Kepok Banana type (Musa paradisiaca Linn). In this test ethanol extract is used from the sliced part of the stem liquid. Before the test was carried out, phytochemical screening was done on water extract and ethanol extract. Obtained phytochemical test results indicate the presence of phenolic compounds: Flavonoids, Alkaloids, Tannins, Saponins, Steroids, and Glycosides. The purpose of this study was to evaluate Musa paradisiaca L. (Kepok banana) stem fluid for antibacterial studies with agar diffusion methods and compared with drugs circulating in the Amoxicillin community. Musa paradisiaca ethanol extract showed a spectrum of antibacterial activity in microorganisms tested with the potential for inhibition starting to show active at 10 ppm against Escherichia coli and Staphylococcus aureus.

1. Introduction
Banana plants are plants with arranged fruit groups called "combs" from the family Musaceae with some of the most popular types in Indonesia, among others: Musa acuminate, M. balbisiana and M. paradisiaca is one type of banana in North Sumatra as a type of Kepok banana (Musa paradisiaca Linn).

Musa paradisiaca L., the fruit is the second most widely produced fruit in the world and one of the most important food sources along with rice, wheat and corn [1]. Indonesia as a tropical country that has a climate is very suitable for this plant, so the banana plant is the largest fruit producer throughout the year compared to other fruits that grow in Indonesia. The 2015 statistical data from Badan Pusat Statistik for the production of banana reached 7.29 million tons, the largest data after citrus fruits and mangga.

Banana plants, from different parts are generally used as food on fruit and on flowers, leaves, stems, fruit peels are also used as a popular drug in many Asian countries. These parts, for example flowers, have been studied as antimicrobial and antihyperglycemic [2] [3], antioxidant, anti-inflammatory, and lipase inhibition [3]. From the antibacterial leaf section [4], antiulcer [5] and used for wound healing [6] [7] [8].

From the rind as an antioxidant [9] [10] [11] [12] [13] [14], antidysenteriae [15], anti-bacterial [12] [14] [16] [17] [18], anti-fungal [10] and wound healing [11]. From the skin of the fruit has also been
investigated mineral content including potassium, calcium, sodium, iron, manganese, bromine, rubidium, strontium, zirconium and niobium [12].

Another part of this plant is its fruit other than as a fulfillment of nutrients for food, also as antidiarrheal, antioxidant and anti-microbial [13]. The sap part of this plant has been studied as antidiarrhoel [14], antioxidant and antimicrobial [19].

This paper reports on the determination of phytochemical content and antibacterial test of dried extract of sap from Kepok banana stems (Musa paradisiaca L).

2. Material and Methods
The sap of the sap is obtained by tilting the part of the Musa paradisiaca Linn plant stem and allows the sap to flow freely into a clean cone flask. The liquid is stored in fial-fial and placed in a refrigerator. After 5-6 days the liquid is naturally divided into 3 parts, the top layer of solid form, the middle of the liquid form, and the lower layer of solid form. The three layers were tested for phytochemical screening on water extracts and ethanol extract. Phytochemical screening was carried out for components of alkaloids, flavonoids, tannins, steroids, saponins, and glycosides. From the results of the qualitative preliminary test, the part containing the most components will be tested for antibacterial.

2.1. Phytochemical screening test
Ujidilakukanpadaekstrak air daricairanbatangdnekstraketanoldaricairanbatang.

Test for Flavonoids: To 1ml of the extract was added 3 drops of ammonia solution (NH$_3$) followed by 0.5ml of concentrated HCl. The resultant pale brown colouration of the entire mixture indicated the presence of flavonoids[20].

Test for Alkaloids: To 3ml of the extract was added 1ml of 1% HCL. This resulting mixture was then treated with few drops of Meyer’s reagent. The appearance of a creamy white precipitate confirmed the presence of alkaloids [18].

Test for Tannins: Two drops of 5% FeCl$_3$ was added to 1ml of the plant extract. The appearance of a dirty-green precipitate indicated the presence of tannins [19].

Test for Saponins: Five drops of olive oil was added to 2ml of the plant extract and the mixture shaken vigorously. The formation of a stable emulsion indicated the presence of saponins [19].

Test for Steroids: To 1ml of the plant extract was added 1ml of concentrated tetraoxosulphate (vi) acid (H$_2$SO$_4$). A red colouration confirmed the presence of steroids [19].

Test for Glycosides: To 1ml of the extract was added 2ml of acetic acid and then cooled in an ice bath at 4°C. To this mixture 1ml of concentrated tetraoxosulphate (vi) acid (H$_2$SO$_4$) was added dropwise. The formation of an oil layer on top of solution indicated the presence of glycosides

2.2 Antibacterial Activity Assay
The determination of antibacterial activity was done using the agar well diffusion technique. The organism to be tested was inoculated into sterile nutrient agar. After incubation period of 24h at 37°C, a loop of inoculum was transferred into 5ml of nutrient broth, incubated for 2h at 37°C. This served as fresh suspension inoculum. Wells (5mm diameter) were made in sterile nutrient agar plate using a sterile cork borer (flame sterilized) and inoculum containing 10$^7$ CFU/ml of test bacteria were spread on solid plates with the aid of sterile swab moistened with the bacterial suspension. Then 50μl of aqueous extract or ethanol extract of banana stem liquid from liquid fraction were placed in the wells made in inoculated plates. Controls were set up with 50μl of sterile distilled water or ethanol. The plates were incubated at 37°C for 24h and zones of inhibition if any around the well were evaluated in millimeters (mm) [18].

3. Results and discussions
Kepok banana stem liquid collected in fialial through storage processes for 5-6 in the refrigerator has a separation of three upper layer fractions in the form of solid, middle layer (liquid) and the bottom layer in the form of solids. As in Figure 1.
Figure 1. liquid collection of kepok banana stems (a) liquid first day (b) liquid third day (c) fluid sixth day

Of the three separate sections, phytochemical screening tests for water extracts and ethanol extracts were carried out. The phytochemical extract test results obtained data as shown in Table 1 below.

| Table 1. Phytochemical Screening Test Results of Water Extract and Ethanol Extract of Kepok Banana Stem Liquid |
|--------------------------------------------------|--------------------------------------------------|--------------------------------------------------|--------------------------------------------------|--------------------------------------------------|
| Phytochemical constituent | Water Extract | Ethanol Extract |
|                          | Top | Middle | Low | Top | Middle | Low |
| Flavonoids               | -   | +      | +   | -   | +      | -   |
| Alkaloids                | -   | +      | +   | -   | +      | -   |
| Tanins                   | -   | +      | +   | -   | ++     | -   |
| Saponins                 | -   | ++     | -   | +   | ++     | +   |
| Steroids                 | -   | -      | -   | +   | +      | -   |
| Glycosides               | -   | +      | ++  | -   | -      | -   |

Note: + present, - absent

The phytochemical test revealed that the extract of Kepok banana stem liquid showed flavonoids, alkaloids, tannins, saponins, steroids and glycoside. And from Table 1, it was observed that the most abundant content in the middle ethanol extract of the three fractions formed after storage in the refrigerator.

On that basis the authors tested with ethanol extract middle with antibacterial test starting from a concentration of 2.5 ppm. Amoxicillin is chosen as a positive control because of its widespread use in Indonesian society as an antibiotic. And obtained the results of bacterial testing in Table 2.

| Tabel 2. Antibacterial activity of the ethanol extract from middle fraksion |
|--------------------------------------------------|--------------------------------------------------|--------------------------------------------------|--------------------------------------------------|--------------------------------------------------|
| Zone of inhibition (mm) | Escherichia coli | Staphylococcus aureus | Staphylococcus mutan |
| Blanko                 | 1                | 1                    | -                   |
| Control + Amoksicilin  | 2                | 3.5                  | -                   |
| Extract 2,5 ppm        | 1                | 1                    | -                   |
| Extract 5,0 ppm        | 2                | 1                    | -                   |
The amount of content in the ethanol extract of banana stem liquid, according to several studies from Emomobong Gideon et al. (2016) which conducted a study on parts of Musa paradisiaca plant leave [18]. Ahmed M. et al (2016) extracts from Musa paradisiaca skin carried out a total phenolic test, total tannin and total flavonoids in several solvents [9]. From the skin extracts are antioxidants and antimicrobials. According to Pongsagon Pothavorn et al (2010) the content of several types of banana sap in Thailand, contains several polyphenols namely caffeoylquinic acid, flavones, flavonols, dopamine and N-acetylserotonin [21]. Sunil Jawla. et al (2012) flower extract from Musa paradisiaca is antimicrobial and active as an antihyperglycemic with compounds in phytochemical tests: carbohydrates, proteins, steroids, glicosides, saponins, alkaloids, tannins, phenolic components and flavonoids [2]. Hang T. Vu et al (2018) reviewed Devatkal et al (2014) that the total phenolic content of banana peel type Musa paradisiaca 55.5 mg GAE / g sample. The content with the largest number among various other types [22]. From table.2 that there is bacterial inhibitory power on Escherichia coli and Staphylococcus aureus at a concentration of 10 ppm. Whereas for mutant Staphylococcus bacteria did not show the slightest obstacle. This means that the mutant Staphylococcus bacteria is very resistant to blank, positive control and extract.

4. Conclusions
From the initial phytochemical test, Musa paradisiaca Linn stem fluid contained flavonoids, alkaloids, tannins, saponins, steroids and glycoside. The highest content is in the liquid fraction. And after the antibacterial test has inhibited zones for Escherichia coli and Staphylococcus aureus bacteria. For the mutant Staphylococcus bacteria the extract is resistant.

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References

[1] X. Perrier et al., “Multidisciplinary perspectives on banana (Musa spp.) domestication,” Proc. Natl. Acad. Sci., vol. 108, no. 28, pp. 11311–11318, 2011.

[2] S. Jawla, Y. Kumar, and M. S. Y. Khan, “Antimicrobial and antihyperglycemic activities of Musa paradisiaca flowers,” Asian Pac. J. Trop. Biomed., vol. 2, no. 2, Supplement, pp. S914–S918, 2012.

[3] R. S. Divya, P. Venkatalakshmi, V. Vadivel, and P. Brindha, “In vitro studies on the biological activities of flowers of Musa Paradisiaca L.),” Der Pharm. Lett., vol. 8, no. 10, pp. 238–246, 2016.

[4] P. V Naikwade, S. Gaurav, D. Sharayu, and J. Kailas, “Evaluation of antibacterial properties of Musa paradisiaca L. Leaves,” Biosci. Discov., vol. 6, no. 1, pp. 80–84, 2015.

[5] A. K. Gangwar and A. K. Ghosh, “To estimate the antiulcer activity of leaves of Musa sapientum Linn. by ethanol induced method in rats,” Int. J. Pharmacogn. Phytochem. Res., vol. 6, no. 1, pp. 53–55, 2014.

[6] J. M. dos Santos, E. A. Campesatto, I. C. A. de Omena, L. A. M. Grillo, E. C. de Araújo, and M. L. de A. Bastos, “Potential study of healing Musa paradisiaca L.,” J. Chem. Pharm. Res., vol. 8, no. 8, pp. 182–184, 2016.

[7] P. K. Agarwal, A. Singh, K. Gaurav, S. Goel, H. D. Khanna, and R. K. Goel, “Evaluation of wound healing activity of extracts of plantain banana (Musa sapientum var. paradisiaca) in rats,” Indian J. Exp. Biol., vol. 47, no. 1, pp. 32–40, 2009.

[8] D. Ariandi Candra Putra, H. Lutfiyati, and P. Pribadi, Effectiveness of banana leaves extract (Musa paradisiaca L.) for wound healing, vol. 7. 2017.

[9] A. M. Aboul-enein, Z. A. Salama, A. A. Gaafar, H. F. Aly, A. Faten, and H. A. Ahmed,
“Research Article Identification of phenolic compounds from banana peel (Musa paradisica L.) as antioxidant and antimicrobial agents,” J. Chem. Pharm. Res., vol. 8, no. 4, pp. 46–55, 2016.

[10] M. Z. Imam, S. Akter, M. Md. Ehsanul Hoque, and R. Md. Sohel, Antimicrobial and cytotoxic properties of different extracts of Musa sapientum L. subsp. sylvestris, vol. 2. 2011.

[11] P. Tamri et al., “Evaluation of wound healing activity of hydroalcoholic extract of banana (Musa acuminata) fruit’s peel in rabbit,” Pharmacologyonline, vol. 3, pp. 203–208, 2016.

[12] B. A. Anhwange, “Chemical Composition of Musa sapientum (Banana) Peels,” J. Food Technol., vol. 6, no. 6, pp. 263–266, 2008.

[13] M. S. Hossain et al., “Antidiarrheal, Antioxidant and Antimicrobial Activities of the Musa sapientum Seed,” Avicenna J. Med. Biotechnol., vol. 3, no. 2, pp. 95–105, 2011.

[14] M. T. Yakubu et al., “Antidiarrhoeal Activity of Musa paradisiaca Sap in Wistar Rats,” Evidence-Based Complement. Altern. Med., vol. 2015, no. Article ID 683726, pp. 1–9, 2015.

[15] S. A. Kusuma, M. Febrianti, and A. Saraswati, Comparison of unripe banana peel of kepok (Musa paradisiaca L.) and klutuk (Musa balbisiana colla): Phytochemical and anti-dysenteriae activity, vol. 10. 2018.

[16] G. Ehiowemwenguan, A. O. Emoghene, and J. . Inetianbor, “Antibacterial and phytochemical analysis of Banana fruit peel,” IOSR J. Pharm., vol. 4, no. 8, pp. 18–25, 2014.

[17] S. I. Okorondu, C. O. Akujobi, and I. N. Nwachukwu, “Antifungal properties of Musa paradisiaca ( Plantain ) peel and stalk extracts,” Int. J. Biol. Chem. Sci., vol. 6, no. August, pp. 1527–1534, 2012.

[18] C. E. Udobi, “Antibacterial and toxicity studies of the ethanol extract of Musa paradisiaca leaf AU - Asuquo, Ememobong Gideon,” Cogent Biol., vol. 2, no. 1, p. 1219248, Dec. 2016.

[19] P. R. Kumar, S. Srivastava, K. K. Singh, C. Mathad, and P. S. Thin, “Study of Antioxidant and Antimicrobial Properties, Phytochemical screening and analysis of Sap Extracted from Banana (Musa acuminata) pseudostem,” Int. J. Adv. Biotechnol. Res., vol. 5, no. 4, pp. 649–658, 2014.

[20] G. Ehiowemwenguan, Emoghene, and J. E. Inetianbor, “Antibacterial and phytochemical analysis of Banana fruit peel,” IOSR J. Pharm., vol. 4, no. 8, pp. 2250–3013, 2014.

[21] P. Pothavorn et al., “Sap Phytochemical Compositions of Some Bananas in Thailand,” J. Agric. Food Chem., vol. 58, no. 15, pp. 8782–8787, Aug. 2010.

[22] H. T. Vu, C. J. Scarlett, and Q. V Vuong, “Phenolic compounds within banana peel and their potential uses: A review,” J. Funct. Foods, vol. 40, pp. 238–248, 2018.