

Nano-Herb of Meniran (*Phyllanthus niruri*) as Antibacteria against *Escherichia coli*

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ABSTRACT

Introduction: *Escherichia coli* infection cause death and declining production of broiler farm in Indonesia frequently. Its infection occurs systemic and lead to bacteremia.

Objective: The aim of this study was to reveal nano-herb of meniran (*Phyllanthus niruri*) extract capability as antibacterial against *Escherichia coli*.

Methods: Obtained data was tested parametrically using prohibit analyzes. The method for the research was dilution method. Before the substance was diluted, phytochemical screening on the substance was conducted.

Results: The result of the screening revealed that meniran extract contains some compounds such as alkaloid, tannin, flavonoid, saponin, and steroid. The result of MIC assay was not capable to be observed due to blackish colour of the extract which covered the medium. MBC assay showed that nano-herb of meniran extract 5% of doze was capable to against growth of *Escherichia coli*.

Conclusion: In conclusion, meniran nano-herb has capability as antibacterial against APEC.

Keywords: Antibacteria, *Escherichia coli*, nanoparticle, *Phyllanthus niruri*.

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INTRODUCTION

Escherichia coli infection cause death and declining production of broiler farm in Indonesia frequently. Its infection occurs systemic and lead to bacteremia¹. Due to their ability of growing and form colony in many organs, avian pathogenic *Escherichia coli* (APEC) cause several effects such as pericarditis, perihepatitis, airsacculitis, cellulitis, salpingitis, synovitis, and omphalitis². Antibiotic administration through feeding is conducted initially as method to eradicate APEC yet it leaves residue and cause resistance. It worsens the condition, so medication become difficult³.

In addition, Indonesia is an archipelago with approximately 17,508 islands and is covered by tropical rain forest, seasonal forest, swamp, subalpine shrub vegetation, coastal vegetation, and mountain vegetation. With its reflective mixture of Asian and Australian native species, Indonesia is stated to possess the second largest biodiversity in the world, with around 40,000 endemic plant species including 6,000 medicinal plants¹. Consequently, Indonesia is rich in medicinal plants which were used by its population traditionally from generation to generation in curing diseases^{4,5,6,7,8,9,10}.

Meniran (*P. niruri*) is an herb belongs to genus *Phyllanthus* known as *P. niruri* which has antibacterial activity against APEC¹¹. Meniran contains several chemical substances such as lignin, flavonoid, alkaloid, terpenoid, saponin and tannin^{12,13}. Lignin has function as antioxidant in food. Flavonoid has function as antioxidant, antibacterial, and immunostimulatory. Alkaloid has function as antimicrobial, antimalaria, antidiarrhea and antidiabetic. Tannin has function as defender for the plant and has antioxidant activity hinders tumor activity. Terpenoid is capable to hinder

APEC and *Staphylococcus aureus* activities, while saponin has capability as antimicrobial¹⁴. To optimize the effect of herb-medicine. It is needed to arrange formulation which capable to increase solubility, stability, bioavailability, and system that focus on effectivity of simplicial application. Nanotechnology is commonly used to observe the effectivity of simplicial application. It generates small particle size of the simplicial (1-100 nm)¹⁵. This method makes the simplicial are easy to be dissolved and increase the efficiency of absorption in intestine. Moreover, nanoparticle extract diffuses in blood easier and more accurate reaching the medication¹⁶. Nano ball mill method is a method to grinding powder into smaller particle and mixture¹⁷. Therefore, in this study, we aimed to reveal nano-herb of meniran extract capability as antibacterial against APEC.

Materials and Methods

Meniran nano herb

The simplicial of meniran was diluted with 96% methanol in 500 ml of Erlenmeyer flask then stored for 72 hours. The mixture was filtered then evaporated using rotary evaporator on temperature of 50 °C. Obtained extract was diluted with 1% of CMC Na. The diluter was made by mixing 1 g of CMC Na with 20 ml of hot water stirred until they turn into mucilage. The mucilage was poured into steam cup then grinded with meniran extract till 100 ml final concentration. It was stored in murky bottle. Dose of meniran simplicial were 3%, 5%, 10%, 20%, 25%. The simplicial was turned into nanoparticle ball mill by mixing the extract with feed mineral with composition 2 ml of the extract in each 300-feed mineral. The ball mill was conducted for 30 minutes.

Phytochemistry screening analyses**Alkaloid test:**

It was conducted using Mayer, Wagner and Dragendorff methods. 3 ml of simplicial was mixed with 5 mL HCl 2 M. It was stirred then stored in room temperature. After the temperature of the mixture decreased, 0,5 g NaCl was added then stirred. Three drops of 2 M HCl was added into the filtrate. The filtrate was divided into 4 parts (A, B, C, and D). A filtrate as the blank, B filtrate was added with Mayer reagent, C filtrate was added with Wagner reagent, and D filtrate was used for confirmation test. If sediment was observed on both Mayer and Wagner tests, it meant that the simplicial contained alkaloid. Confirmation test was conducted by adding 25% of ammonia on D filtrate until its PH 8-9. Chloroform was added to the mixture then evaporated on water bath. The formation of sediment meant the simplicial contains alkaloid.

Tannin test: 3 ml of the simplicial was poured by hot distilled water then stored until the temperature decrease. Five drops of 10% NaCl was added to the mixture then it was filtered. The filtrate was divided into 3 parts (A, B, and C). A filtrate was used as the blank. Three drops of FeCl₃ was added into B filtrate and gelatin salt was added into C filtrate. The reaction was observed.

Saponin test: Saponin test was conducted using Forth method. 2 ml of the sample was mixed with 10 ml of distilled water then shaken for 30 seconds. The alteration of the reaction was observed. If foam was observed and last for 30 seconds, it meant that the sample contains saponin. Confirmation test was conducted by evaporating the sample until it completely dry then rinse it using hexane until the filtrate turned to be clear. The residue was added with chloroform. They were stirred for 5 minutes. After that, anhydrate Na₂SO₄ was added. They were mixed then filtered. The filtrate was divided into two parts (A and B). A filtrate as blank. Anhydrate acetate was dropped into B filtrate then stirred gentle then it was added with high viscosity H₂SO₄. They were shaken. If reddish or brownish ring-like formation observed, it meant the sample contains saponin.

Flavonoid test: Three milliliter of samples was evaporated then rinsed using hexane until the filtrate turned to be clear. Residue of the filtrate was diluted with 20 ml of ethanol then filtrated. The filtrate was divided into 4 parts (A, B, C, and D). A filtrate as the blank. B filtrate was added with 0,5 mL high viscosity HCl then warmed on water bath then observed. If the color of the filtrate changed into redness or violate. It meant the sample contains of flavonoid (Bate Smith-Metchalf

method). C filtrate was added with 0,5 mL of HCl and Mg then observed. If the color changed into red or orange color, it meant the sample contains flavonoid too (Wilstater method). Red or orange color was given by flavone compound while dark red was given by flavanol or flavanone. Green and blue color were given by aglycon or glycoside.

Steroid and triterpenoid tests: Steroid and triterpenoid tests used Liebermann-Bouchard method, simplicial was diluted using chloroform then Liebermann-Bouchard reagent was added. The color of sample will turn to be Red-brown and brown-violate if the sample contains steroid and triterpenoid respectively. The reaction between triterpenoid and Liebermann reagent resulted red-violate color while the reaction of it with steroid showed green-blue color. Triterpenoid and steroid will produce color through their reaction with H₂SO₄ in acetate acid anhydride. It happened because both chemical compounds has different group on Carbon number 4.

Dilution method

It consists of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) assays. Minimum Inhibitory Concentration (MIC) assay is used to know the minimum concentration of antibacterial could hamper bacterial growth. While Minimum Bactericidal Concentration (MBC) assay is used to know the minimum concentration of antibacterial could eradicate all bacterial¹⁸.

Nano-herb of meniran dose 2%, 3%, 5%, 10%, 20%, 25% and 30% were poured into PBS which cultured with Avian Pathogenic *Escherichia coli* 10⁸. Then the turbidity of the media and clarity of MIC were observed. Then the result of MIC was processed for MBC assay by culturing them into MHA media by streak. They were incubated in 37 °C. The colony formation was observed.

Statistical analysis

Obtained result was analyzed using SPSS 23.0 for windows. To know the sensitivity of meniran nano-herb parametric test was conducted using probit analyzes.

Results**Phytochemistry analyzes of meniran**

According to the phytochemistry analyzes, meniran extract contains several compounds such as alkaloid, tannin, flavonoid, saponin, and steroid/ triterpenoid. The result was presented on Table 1 and Figure 1.

Table 1. The result of alkaloid test, tannin test, flavonoid test, saponin test, and steroid test.

No	Test	Result	Description
1	Alkaloid (Wagner reagent)	+	Brown residue formation
2	Tannin	+	Color changing to be blackish green
3	Flavonoid	+	The presence of color changing
4	Saponin	+	Presence of constant foam
5	Steroid/triterpenoid	+	Color changing to be green

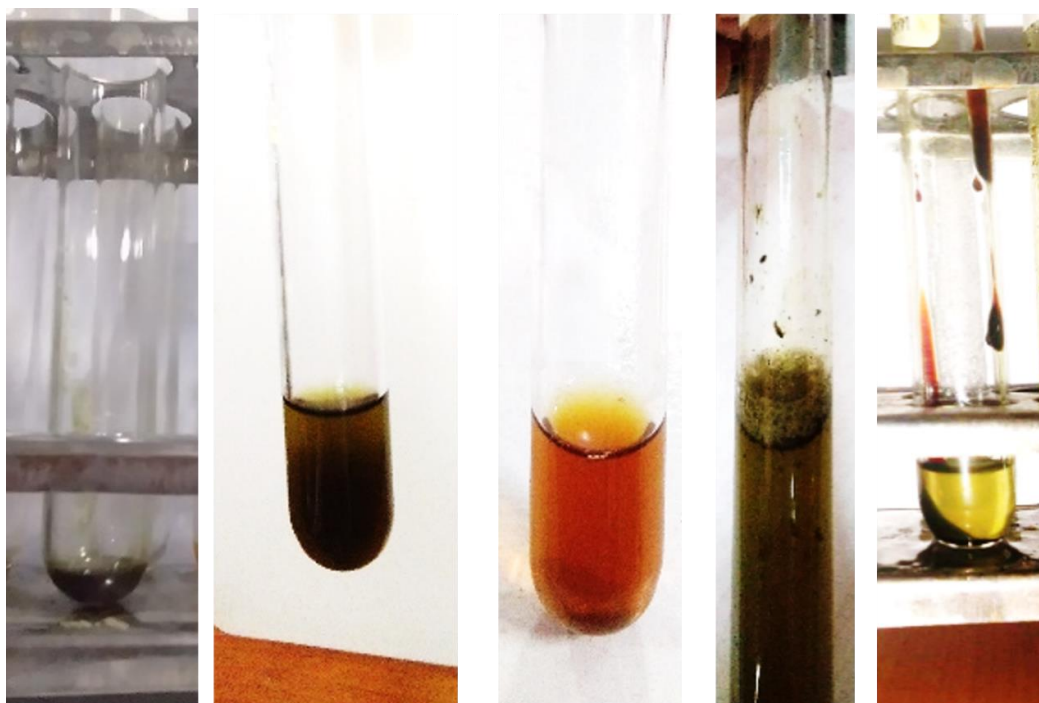


Figure 1. The result of alkaloid, tannin, flavonoid, saponin, and steroid/triterpenoid tests.

Nano ball mill

One of method to make nano-herb of meniran is nano ball mill. The basic of ball mill machine is mashing and

grinding the particle. Length of the process of mashing and grinding influence the measure of nano-herb¹⁹.

Tabel 2. The result of nano-herb production.

Sample	Time	Z-Average
1 hour 1:10 5	1 hour	305.3
1:5 3	1 hour	595.8
1 hour 1:10 1	1 hour	356
1:1 C 5	1 hour	859.2
D1	30 minutes	662.5
1:10 B4	1 hour	445.8
E3	>1 hour	357.8

Anti-bacterial activity of meniran nano-herb against APEC

According to the observation, 5% of meniran extract could stop the growth of APEC (Table 3 and Figure 3).

Table 3. APEC Bacteria growth on meniran nano-herb dose of 2%, 3%, 5%, 10%, 20%, 25%, and 30%.

Dose	Bacteria growth
2%	+
3%	+
5%	-
10%	-
20%	-
25%	-
30%	-
Control +	+
Control -	-

*Note: (+) presence of APEC bacteria growth (-) absence of APEC bacteria growth.

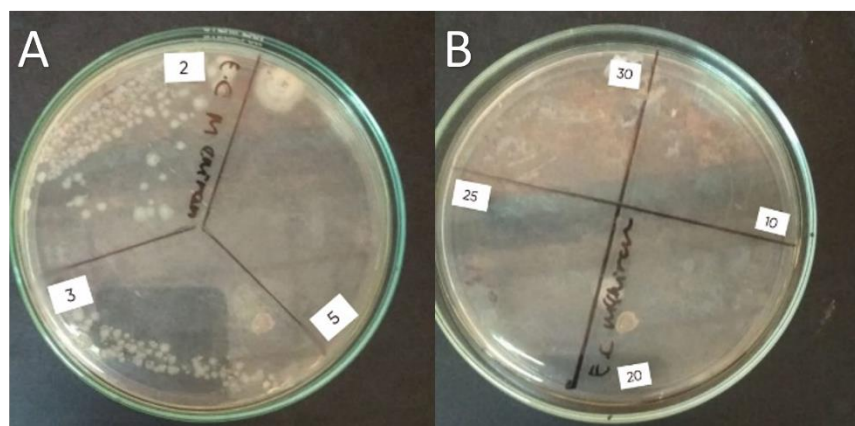


Figure 3. A. Dose 2%, 3%, and 5%; B. Dose 10%, 20%, 25%, and 30%.

Discussion

According to result, administration of 2% and 3% of meniran extract could not eradicate APEC bacterial growth. While administration of 5%, 10%, 20%, 25%, and 30% of meniran nano-herb could eradicate APEC bacterial growth.

In Indonesia, herbal medicines are widely used and very often are the only treatment option for more than 70 percent of the population, particularly in rural areas^{20,21,22,23}. Over the years the potential chemotherapeutic property of herbal drug and its ability to counteract the antibiotic resistance has led to numerous innovations using herbal molecules. meniran is one of the indigenous herbs which have been used in Indonesia for treatment of various ailments^{24,25,26,27,28}.

Furthermore, the in vitro antimicrobial efficacy of meniran extracted in water, methanol and dichloromethane were bacteriolytic on various pathogenic bacteria (*Bacillus pumilus*, *Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus luteus*, *Escherichia coli*, and *Klebsiella pneumonia*) and on fungus *Candida albicans*. The antibacterial action observed against the pathogens *Bacillus pumilus* with methanolic and dichloromethane extracts exhibited wider zones of inhibition measuring 18-20 mm²⁹. In addition, different concentrations of ethanolic and aqueous extracts of meniran on *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa*, and *Klebsiella aerogenes*. *Pseudomonas aeruginosa* was found to be most susceptible among the ethanolic extracts³⁰.

Conclusion

In conclusion, meniran nano-herb has capability as antibacterial against APEC. Moreover, the best dose according this research is 5%.

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Conflict of Interest

The authors declare no conflicts of interest.

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