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In Vitro Antibacterial Activities of Noni Fruit (*Morinda citrifolia L.*) Ethanolic Extract against *Escherichia coli* and *Salmonella typhimurium*

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Abstract

This Research aimed to examine the in vitro antibacterial activity of noni fruit extract (NE) against the growth of *Escherichia coli* and *Salmonella typhymurium* bacteria. The Research was carried out for 3 weeks from April to May 2022. The Research used Completely Randomized Design (CRD) which consisted of 6 treatments and each treatment was replicated 3 times so that there were 18 experimental units. The treatments used were: P1 = 5% NE concentration P2 = 15% NE concentration, P3 = 25% NE concentration, P4 = 35% NE concentration, P5 = NE concentration 45%, and P6 = 55% NE concentration. The parameters observed in this Research were the physical characteristics of noni fruit extract and its antibacterial activity. The results of the Research showed that noni fruit extract had a significant effect ($P<0.05$) on the growth of *Escherichia coli* and *Salmonella typhymurium* bacteria. A concentration of 55% was the most effective concentration in inhibiting the growth of the two tested bacteria.

Keywords: Noni fruit, antibacterial, *Escherichia coli, Salmonella typhimurium*

A. Introduction

Microbiota such as viruses and bacteria are found in air, feed, and water and are potentially harmful to animals. One of the possible contaminations by bacteria is meat and egg contamination intended for human consumption. Some of the prevention efforts have been applied such as sanitizing and antibiotics use. Those efforts pose some side effects, one of them is the building up resistance of antibiotics by some strains of bacteria. The use of antibiotics especially in developed countries is controlled by strict regulations. Indonesia also has the regulation of antibiotics use mentioned in Agriculture Ministry Regulation No. 14 the Year 2017 about Animal Medications Classification, Chapter 15 Verse 1 about the restriction of animal medications use in animals intended for human consumption which was first implemented on January 1, 2018. The regulation caused farmers to start applying herbal medication to farm animals.

Many herbal plants are considered to contain antibacterial activity and are more economical due to their abundance and low price. One of the antibacterial herbal plants is noni fruit (*Morinda citrifolia L.*). Noni fruit contains terpenoids, flavonoids, anthraquinones, saponins, and alkaloids which show antibacterial activities (Sogandi & Rabima. 2019). Research is necessary to determine the antibacterial activity of noni fruit extract against *Escherichia coli* and *Salmonella typhimurium* in vitro.

B. Methodology

1. Location and Time to Research

The Research was conducted at the Unit Laboratory of Feed Analysis and Unit Laboratory of Feed Technology, Faculty of Animal Science, Laboratory of Microbiology, Faculty of Medicine, and UPT Integrated Laboratories of Halu Oleo University.

2. Materials of Research

The main materials used in this Research were five kilograms of nearly-ripe noni fruit, Dimethyl Sulfoxide (DMSO), chloramphenicol, *E. coli* culture, and *S. typhimurium* culture. Other equipment and materials used for this Research were 96% ethanol, Nutrient Agar, Nutrient Broth, distilled water, alcohol, a digital scale, 65^0C oven, blender, 30-mesh sieve, glass jar, glass funnel, Erlenmeyer, paper discs, sterile 5 ml pipet, heater, petri discs, beaker glass, Whatman filter paper aluminum foil, evaporator, stirrer, furnace, incubator, test tube, glass spatula, bacteria spreader, burner, autoclave, caliper, wrapping plastic, and tissue.

3. Procedures of Research

a) Sample drying and simplicial materials producing

Five kilograms of fresh noni fruit samples were cleaned and washed in running water, then cut into small pieces and dried under the sun followed by a 65^0C oven for 24 hours until dried completely. The dried simplicial materials were then blended until fine powder formed. The powder was then sieved by a 30-mesh sieve. The sieved powder was then kept in a dry place protected from direct sunlight (Sogandi & Nilasari, 2019).

b) Extraction Process

The extraction of noni fruit was done by the maceration process. One kilogram of noni fruit powder was put in a glass jar and 96% ethanol was added with a 1:10 ratio (W/V). The maceration process was done for 72 hours and stirred three times. The macerate was then evaporated by a rotary evaporator at 45-50^{o}C until a thick extract was produced (Sogandi & Nilasari, 2019). The extract was then used to make different concentrations for the antibacterial activities test.

c) Nutrient Agar Making

The Nutrient Agar (NA) media was made by weighing 2.8 g NA powder and then dissolving it in 100 mL distilled water. The NA solution was then heated on a hotplate until fully dissolved and homogenous. The solution was then autoclaved at 121^{o}C for 15 minutes and the media was poured into a sterile petri dish at the temperature of 40^{o}C and left to solidify at room temperature (Juriah & Sari, 2018).

d) Negative Control Making

The negative control used in this Research was DMSO (Dimethyl sulfide). Five ml of DMSO stock solution was kept in a sterile tube. The negative control was used as a comparison and solvent for positive control and treatment solutions (Kumayas et al., 2015).

e) Positive Control Making

The positive control was made from 250 mg *Chloramphenicol* capsules*.* One capsule of *Chloramphenicol* was opened and 30 mg of its powder was used. The powder was then diluted in 5 ml of distilled water to achieve a *Chloramphenicol* stock solution of 250 µg/50µL (Kumayas et al., 2015).

4. Design of Research

The design used in this Research was a completely randomized design consisting of 6 treatments and each treatment was replicated 3 times. The treatments applied were P1 = 5% noni fruit extract, P2 = 15% noni fruit extract, P3 = 25% noni fruit extract, P4 = 35% noni fruit extract, P5 = 45% noni fruit extract, P6 = 55% noni fruit extract, negative control = DMSO, and positive control = *Chloramphenicol.*

5. Parameters of Research

The parameters examined in this Research were the physical characteristics of noni fruit extract and the antibacterial activities of noni fruit extract against *Escherichia coli* and *Salmonella typhimurium.*

6. Data Analysis

The analysis used in this Research was Analysis of Variance (ANOVA) and descriptive analysis followed by the Duncan Multiple Range Test. The application used was SPSS Version 16 to analyze the difference between treatments.

C. Result and Discussion

1. Noni Fruit Extract Physical Characteristics

The physical characteristics examination was done to evaluate the consistency, color, and aroma of the extract produced. The physical characteristics of noni fruit extract are shown in Table 1.

Table1. Noni fruit extract physical characteristics

Physical characteristic	Sample
Consistency	Thick
Color	Dark brown
Aroma	Noni fruit aroma

The physical characteristics of noni fruit extract showed thick consistency; the dark brown color acquired resembled the dried noni fruit; and the aroma was those of noni fruit aroma. The aroma and color of noni fruit extract were affected by the concentration of the extract itself. The higher the extract concentration, the thicker the aroma and color of the extract (Juwita *et al*., 2013). The standard physical characteristics of herbal extract were dark in color, thick consistency, having an herbal aroma, and being water-soluble (BSN, 2016).

2. Test of Antibacterial Activity Against Escherichia coli

The result of the antibacterial activity of noni fruit extract against *E. coli* by diffusion disc method showed different restriction zone diameters between treatments. The results are presented in Table 2 and Figure 1.

Table 2. Restriction zone diameter (mm) of noni fruit extract against *Escherichia coli*

Treatment (concentration)	Restriction zone diameter (mm)
P1 (5%)	17.50 ± 1.81^{bc}
P2 (15%)	12.67 ± 9.76^{c}
P3 (25%)	26.17 ± 3.52^{a}
P4 (35%)	23.67 ± 1.26^{ab}
P5 (45%)	28.00 ± 4.23^{a}
P6 (55%)	30.67 ± 2.76^{a}
Positive control (Chloramphenicol)	29.30 ± 3.15^{a}
Negative control (DMSO)	0^{d}

Note: Different superscripts in the same column indicate significant differences between treatments (P<0.05).

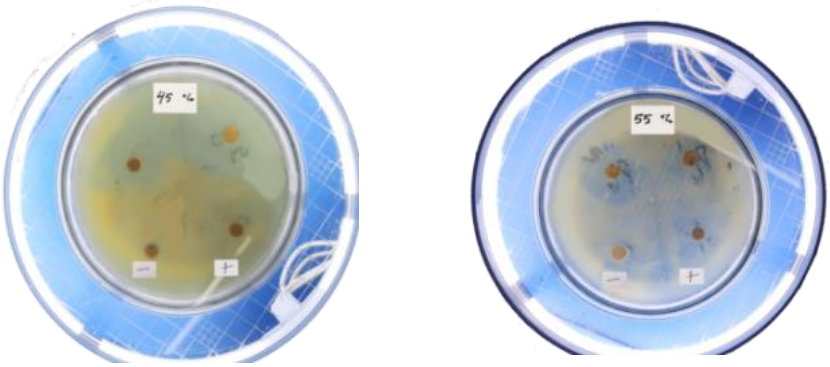
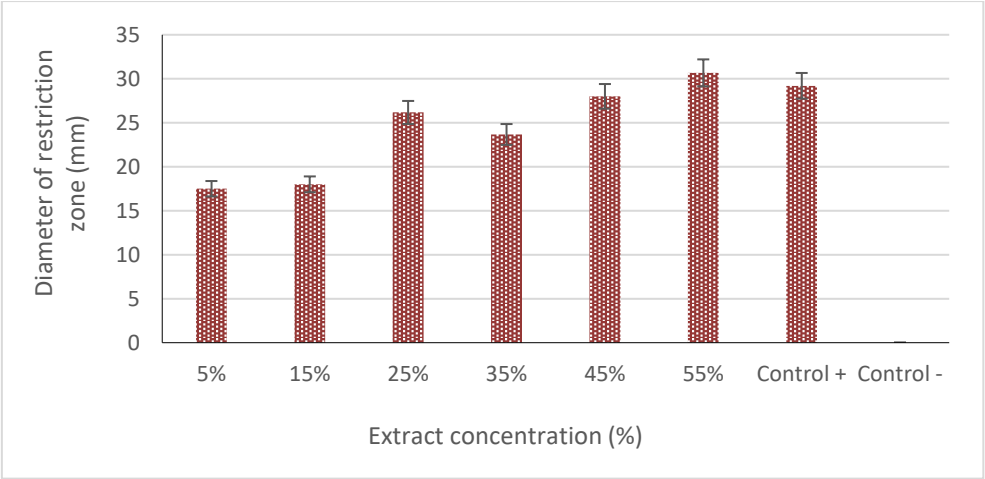


Figure 1. Antibacterial activity of noni fruit ethanolic extract against *Escherichia coli*

The data analysis showed that the diameter of the bacterial restriction zone was significantly different between treatments. The different concentrations of noni fruit extract show antibacterial activity similar to chloramphenicol against *Escherichia coli*, a Gram-negative bacterium. It was suggested that the alkaloids, flavonoids, saponins, tannins, steroids, and phenolics content in noni fruit are responsible for restricting the growth of *E. coli* (Sogandi & Rabima. 2019).

The Duncan Test results showed that the restriction zone of the negative control (DMSO) was significantly different from the treatments and positive control. The positive control using chloramphenicol showed significant antibacterial activity as well as treatments P3, P4, P5, and P6. Chloramphenicol is broad spectrum antibacterial with bacteriostatic properties but can be bacteriocidic when used in high concentrations (Nabila *et al*., 2021).

The restriction zone test against *E. coli* showed a significant difference between treatments with 5%, 15%, 25%, 35%, 45%, and 55%. This proved that different concentrations of noni fruit extract possessed different antibacterial activity against *E. coli*. The best antibacterial activity was recorded at 55% concentration and even the 5% concentration was able to restrict *E. coli* growth. The restriction zone of each concentration is presented in Figure 2.



(Figure 2. The correlation of noni fruit extract concentration and diameter of restriction zone against *E. coli*

The antibacterial activity of noni extract improves as the concentration increases. The lowest concentration showing antibacterial activity is 5% concentration and continues to increase in restriction zone diameter until 55% concentration. The restriction zones of bacterial growth on 5% and 15% extract concentrations are categorized as strong (17.50±1.81 mm and 12.67±9.76 mm, respectively). The 25%, 35%, 45%, and 55% noni extract concentrations are categorized as a very strong restriction against *E. coli* with the restriction zones of 26.17±3.52; 23.67±1.26; 28.00±4.2; and 30.67±2.76 mm, respectfully. Noni fruit contains antibacterial compounds such as flavonoids, alizarin, and aucubin. The flavonoids may restrict bacterial growth by cell protein denaturation and destructing the cell membrane (Malinggas *et al*., 2015).

3. Test of Antibacterial activity against *Salmonella typhimurium*

The result of the antibacterial activity of noni fruit extract against *Salmonella typhimurium* by diffusion disc method showed different restriction zone diameters between treatments. The results are presented in Table 3 and Figure 3.

Table 3. The diameter of restriction zone (mm) of noni fruit extract against *Salmonella typhimurium* growth.

Treatment (Concentration)	Diameter of restriction Zone (mm)
P1 (5%)	16.17±2.09^{c}
P2 (15%)	19.17±0.77^{c}
P3 (25%)	24.00±0.50^{b}
P4 (35%)	25.33±0.29^{b}
P5 (45%)	26.33±2.57^{b}
P6 (55%)	29.67±1.89^{a}
Positive control (Chloramphenicol)	29.67±3.37^{a}
Negative Control (DMSO)	0^{d}

Note: different superscript on the same column shows a significant difference in treatments

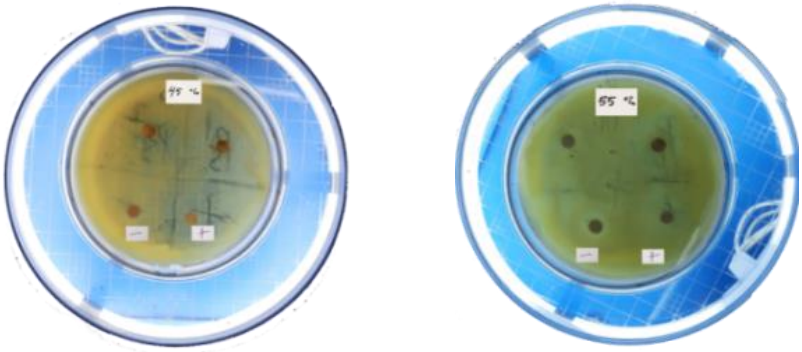


Figure 3. The antibacterial activity of noni fruit extract against *Salmonella typhimurium*.

The analysis test results showed that the restriction zone diameters of *Salmonella typhimurium* on all treatments (5%, 15%, 25%, 35%, 45%, and 55%) and positive control are significantly different from the negative control. Noni fruit extract is proven to be effective in restricting the growth of *Salmonella typhimurium.*

The Duncan Test results showed that the negative control has no restriction zone against *S. thyphimurium,* unlike the positive control and noni fruit extract treatments. This is because the negative control has no antibacterial activity (Auza *et al*., 2021). The lower concentration of noni fruit extracts (5%, 15%, 25%, 35%, and 45%) showed a significant difference in the restriction zone compared to the positive control. The positive control used chloramphenicol which is a broad-spectrum antibacterial that is effective against either Gram-negative or Gram-positive bacterium. Chloramphenicol is effective in controlling infection caused by *Neisseria meningitides, Haemophilus influenza, Rickettsiae, Escherichia coli, Staphylococcus aureus,* and *Salmonella typhmurium* (Rahmitasari *et al*., 2020).

The restriction zone diameter obtained by various concentrations showed that the noni fruit extract possesses antibacterial activity against *Salmonella thyphimurium*. The 55% concentration showed similar results as chloramphenicol as a positive control in restricting the growth of *S. thyphimurium*. The diameters of the restriction zone increased alongside the increase in extract concentration. The antibacterial property of an extract is highly dependent on the concentration of the extract used (Sudarmi *et al*., 2017). The correlation between the concentration of noni fruit extract and the diameter of the restriction zone produced against *S. thyphimurium* is presented in Figure 4.

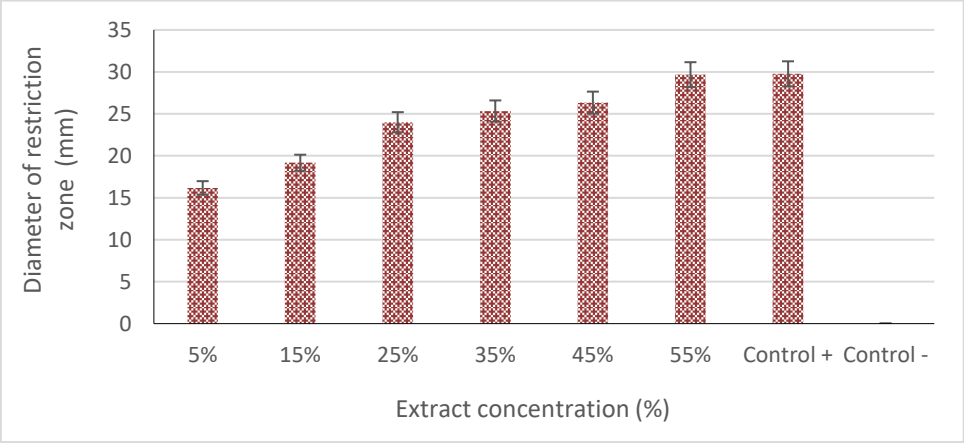


Figure 4. The correlation of noni fruit extract concentration and diameter of restriction zone against *Salmonella thyphimurium*.

The noni fruit extract of 5% and 15% concentrations are categorized as a strong restriction, this is shown by the diameter produced of 16.17±2.09 mm and 19.17±0.77 mm, respectively. The diameters of the restriction zone produced by 25% (24.00±0.50 mm), 35% (25.33±0.29 mm), 45% (26.33±2.57 mm), and 55% (29.67±1.89 mm) noni fruit extract concentration are categorized as very strong. The antibacterial properties of noni fruit extract against *S. thyphimurium* are thought to be due to the chemical compositions of noni fruit. Noni fruit contains phenol and flavonoids which acts as natural antibiotics, and the highest phenol compositions are found in ripened noni fruit (Purwatuningsih et al., 2014).

D. Conclusion

The noni fruit (*Morinda citrifolia* L.) extract shows antibacterial activities against *Escherichia coli* and *Salmonella typhimurium.* The 55% concentration is the most effective extract concentration in restricting the growth of both bacteria.

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