ANTIBACTERIAL ACTIVITY OF THE ISOLATION ETHYL ACETATE-SOLUBLE EXTRACT NONI FRUIT (Morinda citrifolia L.) AGAINST MEAT BACTERIAL DECAY

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Abstract. Noni (Morinda citrifolia L.) is native to Indonesia which have medicinal properties. One of them as an antibacterial. This study aims to determine the antibacterial activity of isolates from the ethanol extract noni fruit to bacterial decay meat is Bacillus licheniformis, Klebsiella pneumonia, Bacillus alvei, Acinetobacter calcoaceticus, and Staphylococcus saprophyticus. The extraction process using the maceration method, and then made a partition by centrifugation ethyl acetate. Soluble part partition showed bacterial growth inhibition activity of the strong to very strong. Furthermore, the ethyl acetate soluble partition on preparative thin layer chromatography produced 5 isolates. Isolates obtained antibacterial activity test performed with a concentration of 20% and 30%. The results of antibacterial test against bacteria test isolates, showing isolates A can not inhibit the growth of bacteria, isolates B and C have medium activity and strong, isolates D and E isolates have activity against bacteria that were tested. MIC and MBC test results showed that the isolates B gives an inhibitory effect (bacteriostatic) against all bacteria. Content analysis of compounds by TLC using the reagents cerium (IV) sulfate indicates a phenol group. Isolates B contains a major compound which can be used as an antibacterial candidate in food preservation replace chemical preservatives.

1. Introduction
Meat is a food of high nutritional value because it is rich in protein, fat, minerals and other substances that are needed the body [1]. Foodstuffs are often decay and rancidity [2]. The process caused by the biochemical reaction that comes from within and outside of foodstuffs such as microbial activity [3]. The microbial contaminant is one cause of reduced quality of the meat so as to be unsafe for consumption. Contamination of microbial spoilage leading to degradation of the protein-protein breakdown process into simple molecules such as amino acids that cause cells to become damaged flesh [4].
According to Purwani et al. (2008) [5], there are some bacteria that live in fresh meat, namely Bacillus licheniformis, Klebsiella pneumoniae, Bacillus alvei, Acinetobacter calcoaceticus, and Staphylococcus saprophyticus. The bacteria can potentially as spoilage bacteria capable of degrading protein for meat used as a nutrient for the growth and development of the bacteria. Noni (Morinda citrifolia L.) is one of the plants that produce active compounds that can be used as an alternative to natural preservatives. This plant is native to Indonesia being very abundant. Part of these plants contains many active compounds found in stems, roots, leaves or fruit [6]. According to Peter (2005) [7], in part noni plant there are several compounds that can be used as antibacterials, such as flavonoids, glycosides and scopoletin.

According to [8], the ethanol extract of noni is able to inhibit the growth of spoilage bacteria on meat and fresh fish. The minimum inhibitory ethanol extract of Noni fruit by 70%. To optimize its inhibitory ability is necessary to do advanced processes such as partitioning and preparative thin layer chromatography to obtain pure isolates are active and have greater power resistor. This study uses the antibacterial activity test method to determine the potential of the results of partitioning and preparative thin layer chromatography ethanol extract of noni. The final analysis of the study by measuring Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) to determine the inhibition of the growth of the active isolates, against spoilage bacteria on meat.

2. Experimental
2.1 Isolation of Section Ethyl Acetate with Preparative Thin Layer Chromatography (TLC)
Isolation was done by Thin Layer Chromatography (TLC). Silica gel plate of GF254 was made by comparison of 7 grams of powdered silica and 14 mL of distilled water (1: 2), then put on glass plates and do activation in oven with temperature of 50 °C for ± 1 hour. Then, samples were spotted on TLC glass plate of like made ribbon until approximately 3 times. The next step was elution of TLC glass plate with mobile phase (chloroform: ethyl acetate 3: 1). Spot formed scraped to obtain isolates. Isolates of silica gel separated with methanol: chloroform in the ratio 1: 1, then filtered the isolates to obtain filtrate and residue. The filtrate was dried to be powder, then was weighed.

2.2 Thin Layer Chromatography and Determination Compound of Isolation Results
Isolates of the isolation results was detected by TLC. The stationary phase used silica gel plate of GF254 while the mobile phase using chloroform: ethyl acetate = 3: 1. The results of separation identified under UV light (254 nm and 366 nm). Furthermore, the TLC plate was sprayed with cerium (IV) sulphate to detect the general presence of compounds and with specific sprayers FeCl$_3$ and AlCl$_3$ then calculated value of Rf (retention factor).

2.3 Test of Antibacterial of Isolates Noni Fruit (M. citrifolia L.)
2.3.1 Well Diffusion Method
Sterile media of MHA 10 mL and inoculum poured in petri dish where inoculated with the bacteria of Bacillus licheniformis, Klebsiella pneumoniae, Bacillus alvei, Acinetobacter calcoaceticus, and Staphylococcus saprophyticus. Then made sinks with perforator. 25 microliters sample with concentration of 20 and 30% put on pitting, each concentration of 2 replicates. Furthermore, incubated at 37 °C for 24 hours.

2.3.2 Test of MIC and MBC
Isolates with optimum concentration made stock 40 mg / mL (2x of the optimum concentration). 50 mL of isolates and 50 mL inoculum were mixed in eppendorf. Then, incubated for 24 hours at 37 °C. After 24 hours, result of incubation displacement in a microplate which contains media of MHA, 25 mL for each repetition, then leveled with glass spreader. Then, incubated for 24 hours at 37 °C. If showed bacterial growth were expressed as the Minimum Inhibitory Concentration (MIC). While the absence of bacterial growth were expressed as the Minimum Bactericidal Concentration (MBC) [9].
3. Result and Discussion
3.1 Separation and Isolation Compound
Some parts of dissolved ethyl acetate separation and isolation with TLCp. The result of the isolation of soluble ethyl acetate 70% by UV_{254} showed 5 isolates could be separated and further isolates marked as A, B, C, D, and E. Isolate B has the greatest weight to the HRF 77 that allegedly contains major compound in noni fruit (M. citrifolia L.).

![Figure 1. Profile chromatogram of the isolated section of noni dissolved ethyl acetate with 254 detection (I), UV_{366} (II). Isolates (A), (B), (C), (D) and (E).](image)

Isolates have then tested the antibacterial against five species of meat spoilage bacteria such as Bacillus licheniformis, Klebsiella pneumoniae, Bacillus alvei, Acinetobacter calcoaceticus, and Staphylococcus saprophyticus.

3.2 Antibacterial Test
3.2.1 Well Diffusion Method
Isolates that have made the series based on the concentration of the effective concentration of the test partition that is 20 and 30% tested in five Bacillus licheniformis bacterium, Klebsiella pneumoniae, Bacillus alvei, Acinetobacter calcoaceticus, and Staphylococcus saprophyticus. Test plate was incubated at 37°C for 24 hours using amoxicillin controls 0.25%, CMC 1% and 70% ethyl acetate. Table 1 as a whole shows that the isolated B has a diameter greater inhibition than the other isolates. This is because there is a major B isolate in a compound which can inhibit the growth of bacteria. These isolates can be used as a broad-spectrum antibacterial candidate because it can inhibit the growth of gram-positive and negative bacteria.

| Table 1. The diameter of inhibition (mm) test isolate bacterial growth results TLCp |
|---------------------------------|-------------------------------|----------------------------|----------------------------|
| Species                        | Control X 20 % | Control Y 30 % | Isolate A 20 % | Isolate B 30 % | Isolate C 20 % | Isolate C 30 % |
| Bacillus alvei                 | 36 - - -       | 17             | 8             | 6             | 7             |
| S. saprophyticus               | 18 - - -       | 7              | 5             | 5             | 10            |
| Acinetobacter calcoaceticus    | 19 - - -       | 9              | 10            | 7             | 11            |
| Klebsiella pneumonia           | 20 - - -       | 7              | 13            | 7             | 10            |
| Bacillus licheniformis         | 19 - - -       | 10             | 10            | 5             | 8             |
| Species          | Control | Isolate D | Isolate E |
|-----------------|---------|-----------|-----------|
|                 | X       | Y         | Z         | 20 % | 30 % | 20 % | 30 % |
| B. alvei        | 36      | -         | -         | 7    | 8    | 6    | 9    |
| S. saprophyticus| 18      | -         | -         | 5    | 9    | -    | -    |
| A. calcoaceticus| 19      | -         | -         | 7    | 9    | 5    | 8    |
| K. pneumonia    | 20      | -         | -         | 8    | 6    | -    | -    |
| B. licheniformis| 19      | -         | -         | 6    | 8    | 9    | 6    |

Note:
X = Control Amoxicillin 0:25%, Y = Control Ethyl Acetate 70%, Z = Control CMC 1%

Each isolate has the potential inhibition of growth of bacteria that is different. The greater concentration of isolates greater inhibitory power. This is consistent with the statement [11], that the higher the concentration of an antibacterial material than the stronger antibacterial activity. The results of measuring the diameter of the inhibition isolate soluble partition results have inhibitory weak to strong to bacteria B. licheniformis, K. pneumonia, B. alvei, A. calcoaceticus and S. Saprophyticus. Species for gram-positive bacteria have an average zone of inhibition greater than negative bacteria, it is influenced by the structure of the bacterial cell wall. Differences in cell wall structure determine the penetration, binding and antibacterial activity of the compounds [12]. Gram-positive bacteria have a cell wall structure with more peptidoglycan, a little lipid and cell wall polysaccharides (teikoat acid). Teikoat acid is a water-soluble polymer, which serves as a positive ion transport to leave or enter. The nature of this water soluble indicating that the cell wall of gram-positive bacteria is more polar. While the gram-negative bacteria more lipid, a little peptigoglycan, formed the outer membrane bilayer (functioning as selective defense compounds exit or enter the cell and cause toxic effects). The outer membrane is composed of phospholipids (inner lining), and lipopolysaccharide (outer layer) composed of lipid A, which is nonpolar. This causes the antibacterial compounds contained in the noni fruit isolates it more difficult to enter the cell so that the antibacterial activity is weaker than the gram-positive bacteria.

3.2.2 MIC dan MBC Test
In the current study used a concentration of 20%, where the selection is based on the concentration of effective concentration in the inhibition test. From the results obtained that isolates B, C, D, and E show the growth of bacteria that is different.

Figure 2. (a) Growth B.licheniformis to isolate B concentration of 20% in media MHA. (b) Controls
Table 2. The test results MIC/MBC isolates results TLCp incubation at 37°C

| Species            | Control | Mass (g) | Area (cm²) | Isolate B | Mass (g) | Area (cm²) |
|--------------------|---------|----------|------------|-----------|----------|------------|
| B. alvei           | Grow    | 0.0010   | 13.63      | Grow      | 0.0002   | 2.83       |
| S. saprophyticus   | Grow    | 0.0014   | 2.27       | Grow      | 0.0005   | 2.54       |
| A. calcoaceticus   | Grow    | 0.0027   | 4.65       | Grow      | 0.0009   | 1.77       |
| K. pneumonia       | Grow    | 0.0028   | 3.25       | Grow      | 0.0008   | 0.84       |
| B. licheniformis   | Grow    | 0.0015   | 6.01       | Grow      | 0.0006   | 2.54       |

Based on Table 2. It can be seen that the isolated B concentration of 20% showed bacterial growth by various of percentage of growth. Isolates B bacteriostatis by inhibiting the growth of test bacteria. B. alvei growth inhibition of 20%, 35% S. saprophyticus, A. calcoaceticus by 30%, K. pneumoniae at 28%, B. licheniformis by 40%.

3.3 Detection of compounds in Isolates with Reagent Spray and UV rays

Detection of compounds with spray reagent FeCl₃, AlCl₃ and UV rays performed on all isolates to suspect compounds in each isolate. Results of detection isolate A, B, C, D, E as shown in Table 3.

Table 3. Results of detection of isolates A, B, C, D, E with a reagent spray

| No | Isolate | Reagent Spray | Hypothesis                  |
|----|---------|---------------|------------------------------|
|    |         | FeCl₃ | AlCl₃ | AlCl₃ dan UV₂₅₄ | Fenol                           |
| 1  | A       | Light yellow | -    | -               | Fenol                           |
| 2  | B       | Black brown  | Yellow| blue phosphorescent | Flavonoid or coumarin |
| 3  | C       | -              | Yellow| blue phosphorescent | Flavonoid or coumarin |
| 4  | D       | -              | -    | Blue            | Flavonoid or coumarin |
| 5  | E       | -              | -    | Blue            | Flavonoid or coumarin |

Figure 3. Profile chromatogram compound detection results.

Information: 1. Detection of isolates A, B, C, D, E with UV₂₅₄
2. Detection of isolates A, B, C, D, E with UV₂₅₄
3. Detection of isolates A, B, C, D, E with Cerium (IV) sulfate
4. Section dissolved ethyl acetate to 70% with the detection of FeCl₃
5. Isolate A, B, C, D, E with detection FeCl₃
6. Section dissolved ethyl acetate to 70% of visible light detection AlCl₃
7. Section dissolved ethyl acetate 70% with AlCl₃ detection and UV 366
8. Isolate A, B, C, D, E, and UV 366 detection AlCl₃

Stationary phase: Silica gel 60 GF 254
Mobile phase: chloroform: ethyl acetate 3:1 (v/v)

The results of the detection reagent compound with FeCl₃ spray against isolates of A, B, showed positive results (+) phenol compounds, but to isolate the C, D, and E showed a negative result (-). Detection of compounds with the spray reagent AlCl₃ followed UV 366 showed positive results (+) to isolate B (Table 2), so that suspected isolates B contains flavonoids. Wagner (1983) [13] mentions that the compounds detected by UV 365 blue fluorescence or blue-green indicate that these compounds including flavonoids or coumarin also depend on the chemical structure of the compound is still in a class of phenolic. E isolates also showed blue luminescence under UV365 rays that can be suspected to contain coumarin compounds or flavonoids as well, but the allegations need to be further analyzed to determine the type of compounds.

Flavonoids and coumarin synthesized from two main pathways for biosynthesis of an aromatic ring, namely the shikimic pathway and acetate malonate. Coumarin is a phenol compound derived from the shikimic pathway while flavonoid derived from a combination of both [14]. Coumarin showed to have a broad spectrum of biological activities such as antibacterial activity, antifungal, anticoagulant, anti-inflammatory, antitumor and anti-HIV [15]. The survey results revealed that a compound coumarin and xanthones, has the ability to inhibit the growth of bacteria Staphylococcus aureus but not for a number of bacteria Escherichia coli, Acinobacter spp, and Crypto Coccus neoformus [16]. While the flavonoids reported to having activity inhibiting pathogenic agents in humans and are antibiotic that can inhibit gram-positive bacteria and negative [17]. these conditions indicate that isolates thought to have the content of flavonoid compounds or compound coumarin which are candidates antibacterial, especially isolates B. Detection of compounds must be strengthened with the identification using either UV-Vis spectrophotometry, IR, or NMR in order to obtain a clear chemical structure of the compound antibacterial candidate.

4. Conclusion
The antibacterial activity of isolates (B, C, D, E) of the 70% ethyl acetate soluble portion had inhibitory activity against 5 bacteria test. The inhibitory activity showed isolates B in B. alvei and isolates C in A. calcoaceticus bacteria. Concentration of 20% ethyl acetate isolates soluble part based antibacterial activity test was enough optimum inhibit growth bacterial of B. licheniformis, K. pneumonia, B. alvei, A. calcoaceticus and S. saprophyticus. Inhibitory activity against bacteria of this test is bacteriostatic. Detection of active compounds showed that isolates contains flavonoids and coumarin which both included in the class of phenol compounds.

Reference
[1] Yanti H, Hidayati and Elfwafati 2008 Jurnal Peternakan 5(1) 22-27.
[2] Amalia P R 2008 Pengaruh Penambahan Bakteriosin dari Lactobacillus sp. Galur SCG 1223 Asal Susu Sapi terhadap Karakteristik Mikrobiologis Daging Dada Ayam Segar IPB Bogor
[3] Barus P 2009 Pemanfaatan bahan pengawet dan antioksidan alami pada industri bahan makanan Medan: Universitas Sumatera Utara.
[4] Usmiati S and Richana N 2011 Buletin Teknologi Pascapanen Pertanian 7(2).
[5] Purwani E, Retnaningtyas, Dyah Widowati 2008 Laporan penelitian Fakultas Ilmu Kedokteran Universitas Muhammadiyah Surakarta.
[6] Kumala S and Endro B S 2007 Microbiology Indonesia 1(3) 145-148
[7] Peter 2005 Chemical Constituents and Noni’s Function Noni News Indian Magazine Edisi Oktober (2) X.
[8] Retnaningtyas E, E Purwani, and T Purwoko 2009 Pemanfaatan Ekstrak Buah Mengkudu (Morinda citrifolia L) dan daun Pandan (Pandanus amaryllifolius Roxb.) sebagai Pengawet Alami Daging dan Ikan Segar LPPM UNS: Surakarta.
[9] Zakaria Z A, A M Desa, K Ramasamy, N Ahmat, A S Mohamad, D A Israf and M R Sulaiman 2010 African Journal of Microbiology Research 4(1) 071-075.
[10] Pelczar M J and E C S Chan 1988 Dasar-dasar Mikrobiologi 2 Jakarta: UI Press.
[11] Jawetz E, J L Melnick, and E A Adelberg 2001 Mikrobiologi untuk Profesi Kesehatan (Review of Medical Microbiology) Translate by H. Tomang Jakarta: EGC.
[12] Wagner H 1983 Plant Drug Analysis Translation of the German Edition
[13] Kristanti A N, N S Aminah, M Tanjung, and B Kurniadi 2008 Fitokimia. Department Kimia FMIPA UNS: Surakarta.
[14] Dekic B R, N S Radulovic, V S Dekic, R D Vukicevic, and R M Palic 2010 Molecules 15 2246-2256.
[15] Pattalung P N, Thongtheeraparp W, Wiriyachitra P, and Taylor W C 1994 Planta Med. 60 365-368.
[16] Verotta L, Lovaglio E, Vidari G, Finzi P V, Neri M G, Raimondi A, Parapini S, Tara melli D, Riva A, and Bombardelli E 2004 Phytochemistry 2867-2879.
[17] Bylka W, I Matlawska, and N N Pilewski 2004 Review Article JANA 7(2) 24-31.