Potential polysaccharide gel from *Durio zibethinus* var. Raja galuh rind extract towards *Klebsiella pneumoniae* bacteria

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Abstract. Pneumonia is commonly caused by *Klebsiella pneumonia* (gram negative bacteria), which is prevalent in developing countries. In 2014, from 4,300,143 toddlers in West Java, 48.06% of them suffered from pneumonia. Especially in Cirebon, 70.9% of toddlers were diagnosed with pneumonia. The Rind of *Durio zibethinus* extract phenolic compounds which are believed to be able to inhibit the growth of *Klebsiella pneumoniae*. This experimental in vitro study used 12 group samples with an experimental post-test only group design, with the following concentrations: 100%, 50%, 25%, 12.5%, 6.25%, 3.13%, 1.56%, 0.78%, 0.39%, and 0.20%. MIC test results on the tube contain non-motile gram-negative bacteria, not encapsulated, large colonies, and very mucus and tend to collect. and total plate positive control account showed growth of bacteria number 23,66, group of 100% durian rind extract as much 25,66, group 50% that is 26,00, group 25% 22,67, group 12.5% that is 18,34, group of 6.25% is 18,00, group 3.13% is 16.34, group 1.56% is 13.67, group 0.78% is 10.67, group 0.39% 8.67, group 0.20% is 8.34 with statistical analysis p value 0.00 (significant) concentration 0.39% and 0.20% is Minimum Inhibitory Concentration (MIC) bacterium *Klebsiella pneumoniae*.

1. Introduction

Indonesia has an ever-increasing spread of infectious diseases, one of which is pneumonia. Pneumonia has a high prevalence rate in developing countries [1]. Pneumonia is the cause of 15% toddlers’ death, which is estimated to be equal to 922,000 toddlers in 2015. Populations that are vulnerable towards pneumonia include children younger than 2 years old, individuals older than 65 years old and people with health problems (malnutrition, immunological disorders). In 2014, the prevalence of pneumonia in toddlers stayed the same, which was about 20-30%. In 2015, there was an increase in this number, reaching 63.45%. In 2015, based on 2013 Riskesdas data, the prevalence varied for each province, and nationally it was 3.55%.

According to the Department of Health of containing 2014, the population of toddlers in West Java was 4,300,143. The number of toddlers diagnosed with pneumonia in West Java was 48.06%, while in Cirebon District there was 70.9% toddlers diagnosed with pneumonia [3]. By time, the prevalence of pneumonia in West Java kept increasing. In West Java province, especially in Cirebon, there is a diverse group of fruits, one of which is durian. Cirebon is one of the centers for durians in West Java,
and the type of durian in Cirebon is Raja Galuh durian, which has a sweet taste and a large size. The rind of this durian usually goes to waste, but if used, this durian rind extract contains flavonoid, tannin, alkaloid, and triterpenoid [4].

In herbal medicine, the properties of bioactive compounds from plants depend on the part of the plant concerned (root, leaf stalk, pulp, or fruit) and the type of extract used. The number of health protective effects of phenolic compounds have been reported due to their antioxidant, antimitagenic, anticarcinogenic, anti-inflammatory, antimicrobial, and other biological properties [5, 6, 7]. Activities to antimicrobial leaf extract Durio zibethinus showed Klebsiella Pneumoniae, Streptococcus pyogenes, against Escherichia coli and Staphylococcus aureus.

Microorganisms have created resistance to various antibiotics, and this has developed immense clinical difficulty in the curing of contagious illness. The enlarge in resistance of microbes due to indiscriminate utilization of commercial antimicrobial medicines supported scientists to investigate for modern antimicrobial substances from several sources including medicinal plants [8].

These contents have the potential as antimicrobials. Nowadays, the society interest to the usage of nature produces are increasing because they are easier to obtain, more cost-effective and has less side effects. There is currently no standardized concentration for Durio rind extract as an antimicrobial. Therefore, there is a need to test the Minimum Inhibitory Concentration (MIC) to test the standard concentration in which microbes can no longer grow or reproduce. Thus, this study is performed to test the polysaccharide gel from Durio zibethinus var. Raja Galuh rind extract towards Klebsiella pneumoniae Bacteria.

2. Material and Methods

The study was designed as an experimental descriptive study and has an explorative nature using Klebsiella pneumoniae as the study object. The study used 12 groups, which were 1 negative control group, 1 positive control group, and 10 treatment groups. The study groups were as the following concentration: 100%, 50%, 25%, 12%, 5%, 6%, 25%, 3%, 13%, 1%, 56%, 0%, 78%, 0%, 39%, 0%, 20%. Positive control group which was Klebsiella pneumoniae bacteria that were given levofloxacin 750mg. and Negative control group which was Klebsiella pneumoniae bacteria that were given 1ml DMSO 10%. The number of repetitions was 3 times for each group.

2.1 Durio zibethinus Rind Extract

Polysaccharide gel extraction from rind of durian Extraction of PG was previously reported (Pongsamart and Panmaung, 1998). Briefly, polysaccharide gel was extracted from the dried rind of durian with boiling water. The polysaccharide gel extracted water was concentrated under reduced pressure and precipitated by its addition into acidified aqueous ethanol, filtered, dried and ground. The pale beige colored powder of durian polysaccharide was used in the experiment.

2.2 Quantitative phytochemical screening-Determination of total phenolic content

Extract phenolic content was identified by modified spectrophotometric method. The sample was prepared in various concentrations ranging from 0.005 mg/ml to 0.02 mg/ml. The reaction was composed of the sample (100 μl), Folin-Ciocaltéu’s reagent (100 μl) and 2.5% sodium carbonate solution (2 ml) and it was kept for incubation at room temperature for 30 minutes. The absorbance was taken at 750 nm. The procedure was repeated for standard Gallic acid (GA). The calibration curve was constructed. From the measured absorbance value, the phenolic content was expressed as GA equivalent in mg/g (GA mg/g).

2.3 McFarland 0.5 Suspension

As much as 0.5 barium chloride solution 0.048 M (BaCl2 2H2O 1.175%) was mixed with 9.5 sulfuric acid solution 0, 18 M (H2SO4 1% b/v) in a beaker glass and homogenized. This suspension was used as a standard solution to compare the turbidity of bacteria suspension during testing.
2.4 Klebsiella pneumoniae Suspension

*Klebsiella pneumoniae* was obtained from the Microbiology Lab of the Faculty of Medicine, Indonesia University. Culture was performed by incubating them for 2-6 hours in 37°C temperature, and then turbidity was equalized to the McFarland 1.5x10^8 CFU/mL standard.

2.5 Determination of Minimum Inhibitory Concentration (MIC)

The determination of MIC was performed by putting in the durian fruit rind extract at 100%, 50%, 25%, 12.5%, 6.25%, 3.13%, 1.56%, 0.78%, 0.39%, and 0.20% concentrations into test tubes that have been filled with the testing bacteria. Then, for positive control, 50μg/ml levofloxacin was added into the tube that has been filled with bacteria suspension; while for negative control, poured in 1 ml DMSO was added into the tube that has been filled with bacteria suspension. Afterwards, tubes were vortexed until homogeneity and incubated at a temperature of 37°C for 24 hours in an incubator, then the tubes’ clarity were compared with the control tube. MIC was marked by the purity of the tubes. The Effect of Giving Raja Galuh Durian Fruit (*Durio zibethinus* Murr.) Rind Extract towards *Klebsiella pneumoniae* these bacteria study aimed to see the effect of the lowest concentration of Raja Galuh durian fruit (*Durio zibethinus* Murr.) rind extract towards *Klebsiella pneumoniae*.

2.6 Antibacterial assay for *Durio zibethinus* Murr rind extract

The bacterial colonies from Muller Hinton agar were suspended in sterile 0.9% NaCl, diluted in NaThrium Broth and the turbidity was adjusted equivalent to a 0.5 McFarland Standard before use. Klebsiella pneumonia strain was grown in Muller Hinton agar. The colonies from this agar were suspended in normal NaThrium Broth and the turbidity was adjusted by the same method as described for the preparation of bacterial suspension. In vitro antimicrobial activities of polysaccharide gel Agar diffusion test (Brock et al., 1994; Lorian, 1991). Agar diffusion testing was performed as follows: serial two-fold dilutions of various concentrations of polysaccharide gel (PG), 100%, 50%, 25%, 12.5%, 6.25%, 3.13%, 1.56%, 0.78%, 0.39%, and 0.20%. Place 0.1 mL of the undiluted homogenate onto a Petri-dish (-1 dilution) spread plate. Total plate count was determined by pour plate method. After 48 hrs of incubation colonies were counted by using colony counter and results were expressed as CFU/ml. Media without PG and using DMSO were used was performed as a culture control. The plates were incubated at 37ºC for 24 hours.

3. Result and Discussion

Bacteria are in use in getting from the Laboratory of Microbiology, Faculty of Medicine, University of Indonesia. The medium used was Mueller Hinton (MH), which serves to grow the test bacteria on a petri dish with a temperature of 37º C for 2-6 hours. *Klebsiella pneumoniae* that has been cultured on MH media shows the growth of cream-colored colonies, round, smooth and smooth. Identification of the microscope showed *Klebsiella pneumoniae* isolate in the form of bacillus or stem, red in color, not moving and has a capsule.

Levene's test shows a significance value with a sig value of 0.179 where> 0.05 so as to show the variant variation between groups has a significant difference.

The rind has weighs more than half of the total fruit weight, with greenish to yellowish brown in color, thick and semi-woody with sharply pointed pyramidal thorns [9]. Colonized bacteria in the form of mucous membranes are the result of adhesion between bacterial capsules and host mucus [10, 11, 12, 13]. Only one-third of durian is edible, whereas the seeds (20–25%) and the shells are usually thrown away. Due to the high consumption of durians, massive amounts of the peels (as waste products) are disposed, causing a severe problem in the community. In the interest of the environment, we propose to use this agricultural waste into good use for health, as an antimicrobial activity.

Some chemicals (photo chemicals) of durian fruit are also used in drug formulations and phenolic compounds contained can be used as anti-oxidants, anti-inflammatory, antimicrobial, anti-carcinogenic and other biological contents. Utilization of durian fruit rind in daily life by the
compounds it contains is a first-class flavor and health-promoting compounds such as flavonoids, phenolic and carotenoids contents [5].

Table 1. Annova test result of Minimum Inhibitory Concentration (MIC) bacterium *Klebsiella pneumoniae*

| Total Plate account | Concentration | MIC (Mg/ML) | df | F     | P value |
|---------------------|---------------|-------------|----|-------|---------|
| Control positive    |               | 23,667      |    | 36,722| 0,00    |
| Control negative DMSO 10% |       | 4,333       |    | 0,5774|         |
| *Durio zibethinus* Husk extract 100% | | 25,667 | 1,5275 |
| *Durio zibethinus* Husk extract 50% | | 26 | 3 |
| *Durio zibethinus* Husk extract 25% | | 22,667 | 1,5275 |
| *Durio zibethinus* Husk extract 12,5% | | 18,333 | 2,0817 |
| *Durio zibethinus* Husk extract 6,25% | | 18 | 3,6056 |
| *Durio zibethinus* Husk extract 3,13% | | 16,333 | 3,0551 |
| *Durio zibethinus* Husk extract 1,56% | | 13,667 | 2,5166 |
| *Durio zibethinus* Husk extract 0,78% | | 10,667 | 1,5275 |
| *Durio zibethinus* Husk extract 0,39% | | 8,667 | 1,1547 |
| *Durio zibethinus* Husk extract 0,20% | | 8,333 | 1,1547 |

Result isolated of *Durio zibethinus* rind extract called polysaccharides with function is antimicrobial properties and has also been investigated for its antibacterial activity against a variety of bacteria [14]. Substances of polysaccharides can be development of a new antibacterial agent and it could be possibly having the advantage of being a new kind with water-soluble antibacterial agent and interesting substance [15]. Moreover, pectin polysaccharides for rind extracts have with immunomodulatic activity [16].

*Klebsiella pneumonia* bacteria were inhibition through pathogenic factors through Capsular polysaccharide (CPS), lipopolysaccharide (LPS) and fimbriae [10]. LPS is a major component of the Gram-negative bacterial outer membrane consisting of A-lipids, core oligosaccharides and long chain polysaccharides (O-antigens) and responsible for bacterial resistance in humans mediated by complement [11, 12].

*Klebsiella pneumonia* bacterial growth can be inhibited through the beta lactamase enzyme produced by Klebsiella Pneumonia. The enzyme can hydrolyze the beta lactam ring found in beta lactam antibiotics and cause resistance to the antibiotic [17]. In addition, the enzyme urease and enzyme citrate permease and the enzyme ESBL (Extended Beta Spectrum Lactamase) causes
resistance to penicillin antibiotics, cephalosporins and aztreonam. Whereas the gel-shaped durian fruit extract can penetrate the polysaccharide capsule that surrounds these bacteria protect against the action of phagocytosis and serum bactericidal and can be considered as the most important virulence factor of Klebsiella pneumonia.

Results show that Rind many antioxidant compounds such as phenolic acids, flavonoids, alkaloids, and saponins. These components have been shown to prevent oxidative stress and inflammation by reducing reactive oxygen species (ROS) [18]. Extract Durio zibethinus rind extract demonstrated antimicrobial activities of leaf Durio zibethinus seem to be mediated by phenolic compounds content. Altogether, these reports provided evidence that Durio zibethinus with antibacterial effects might be considered as source of natural biomolecules for producing synthetic bactericides presented the most efficient compares durio leaves. This beneficial effect might be correlated with the high total concentration of alkaloid.

Based on this study, we have shown that Durio zibethinus Murr. Rind extract at varying concentrations are able to inhibit the growth of Klebsiella pneumonia. This study showed that the lowest concentration of Durio zibethinus Murr rind extract, which is 0.20%, has been proven to be effective in inhibiting the growth of Klebsiella pneumoniae. The inhibition of Klebsiella pneumoniae bacteria in this study was due to the contents of Raja Galuh durian fruit (Durio zibethinus Murr.) rind extract, which are active anti-bacterial compounds that can inhibit the growth of Klebsiella pneumoniae in Mueller Hinton agar plates.

The active compounds include flavonoid, tannin, alkaloid and triterpenoid. Flavonoid is a reducing agent, which inhibits many oxidation reactions using enzymes or non-enzymes. Flavonoid has antibacterial properties by inhibiting cell membrane function, nucleic acid synthesis, and energy metabolism. In addition, tannins can also inhibit the reverse transcriptase and DNA topoisomerase enzymes, the inhibition of reverse transcriptase enzymes can cause no reverse transcription process to occur so that the DNA is not formed and bacteria will die [19]. Tannin can shrink cell walls, thus disrupting their permeability which causes cells to not be able to perform their live activities, inhibit their growth and leads to death. Alkaloid inhibits the formation of peptidoglycan in bacterial cells, thus their cell wall is not completely formed and causing cell death [20].

Flavonoids are reducing compounds, which inhibit many oxidation reactions enzymes and non-enzymes. Flavonoids have antibacterial properties by inhibiting the function of cell membranes, inhibiting nucleic acid synthesis, inhibiting energy metabolism [21]. The phenolic compounds with the side chain of C3 at a lower level of oxidation and absences of oxygen have reported being responsible for the antimicrobial assay. The partial hydrophobic nature of phenolic compounds also an antimicrobial factor. The toxicity mechanism of polyphenols against pathogens could be related to inhibition of hydrolytic enzymes, especially proteases or different interactions that inactivate the microbial adhesions, cell envelope transport proteins, and non-specific interactions with carbohydrates [22].

Triterpenoid can react with porin (a transmembrane protein) which is found in the outer cell membrane of bacterial cell wall and forms a strong polymer bond and disrupting porin, thus decreasing bacterial cell wall permeability and causing the cell to lack nutrition and inhibits its growth. This bacterial growth inhibition occurs within only 24 hours because the Raja Galuh durian (Durio zibethinus Murr.) rind extract has the form similar to a gel that contains polysaccharide as the immune system of the Klebsiella pneumoniae bacteria, thus after 24 hours the Klebsiella pneumoniae bacteria will grow again.

4. Conclusion

Durio zibethinus Murr Rind extract effective can inhibit the growth of Klebsiella pneumoniae bacteria. With the lowest concentration 0, 39% and 0, 20% of polysaccharide gel from Durio zibethinus var.Raja Galuh rind extract towards Klebsiella pneumonia, Durio zibethinus Murr Rind extract have Phenol compound Alkaloid, Tanin, flavonoid can inhibit with LPS mechanism.
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