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Antibacterial Activity of Papaya Seeds (*Carica papaya* L.) Ethanol Extract Against *Escherichia coli*

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Abstract

Escherichia coli is a normal flora found in the large intestine of humans and is opportunistic. Acute diarrhea is the discharge of watery or loose stools in larger amounts than normal, lasting less than 14 days. Acute diarrhea is caused by several microbes, one of which is Escherichia coli. Treatment of acute diarrhea caused by bacteria can be given antibiotics, but the relatively high use of antibiotics can lead to resistance. One of the natural ingredients known to treat acute diarrhea is papaya seeds (Carica papaya L.). Papaya seeds are known to have antibacterial activity because they contain flavonoids (quercetin), alkaloids, saponins, and tannins. This study aims to determine and analyze the antibacterial activity of the ethanol extract of papaya seeds on the growth of Escherichia coli. This research is an experimental study with a post-test only with control group research design using papaya seeds (Carica papaya L.). The test bacteria used in this study were Escherichia coli with the dilution method to determine the Minimum Inhibitory Concentration (MIC) and the spread plate to determine the Minimum Bactericidal Concentration (MBC). The results showed that MIC was present at a concentration of 2%, while MBC was at a concentration of 5%. It was concluded that the ethanol extract of papaya seeds (Carica papaya L.) had antibacterial activity against Escherichia coli.
Keywords: *Antibacterial, Diarrhea, Papaya seeds extract, Escherichia coli, Minimum bactericidal concentration, Minimum inhibitory concentration.*

Abstrak

Escherichia coli termasuk flora normal yang terdapat di dalam usus besar manusia dan bersifat oportunis. Diare akut merupakan keluarnya feses yang cair atau lembek dengan jumlah lebih banyak dari normal, berlangsung kurang dari 14 hari. Diare akut disebabkan oleh beberapa mikroba, salah satunya adalah Escherichia coli. Pengobatan diare akut yang disebabkan oleh bakteri dapat diberikan antibiotik, namun penggunaan antibiotik yang relatif tinggi dapat menimbulkan terjadinya resistensi. Salah satu bahan alam yang diketahui dapat mengobati diare akut adalah biji papaya (Carica papaya L.). Biji papaya diketahui memiliki aktivitas antibakteri karena memiliki kandungan seperti flavonoid (kuersetin), alkaloid, saponin, dan tanin. Penelitian ini bertujuan untuk mengetahui dan menganalisis aktivitas antibakteri ekstrak etanol biji papaya terhadap pertumbuhan Escherichia coli. Penelitian ini merupakan penelitian eksperimental dengan rancangan penelitian post-test only with control group dengan menggunakan sampel biji papaya (Carica papaya L.). Bakteri uji yang digunakan dalam penelitian ini adalah Escherichia coli dengan metode dilusi untuk mengetahui Kadar Hambat Minimum (KHM) dan spread plate untuk mengetahui Kadar Bakterisidal Minimum (KBM). Hasil penelitian didapatkan bahwa KHM terdapat pada konsentrasi 2%, sedangkan KBM pada konsentrasi 5%. Disimpulkan bahwa ekstrak etanol biji papaya (Carica papaya L.) memiliki aktivitas antibakteri terhadap Escherichia coli.
Kata kunci*: Antibakteri, Diare, Ekstrak biji papaya, Escherichia coli, Kadar bunuh minimum, Kadar hambat minimum*

1. Pendahuluan

Acute diarrhea is the discharge of watery or loose stools in larger amounts than normal, lasting less than 14 days. Cases of acute diarrhea in adults are estimated at 99,000,000 cases every year. In the United States, it is estimated that as many as 8,000,000 patients go to the doctor and more than 250,000 patients are hospitalized each year (Sudoyo *et al.*, 2014). Cases of acute diarrhea in Riau Province are still relatively high, namely 92.3%. In 2018 the number of sufferers of acute diarrhea at all ages was 80,498 (42,9%) in health facilities. In 2019, the number of sufferers of acute diarrhea at all ages was 70,348 (37,4%) in health facilities. The national incidence of diarrhea for all ages is 270/1,000 population (Rahayu *et al.*, 2019).

In general, acute diarrhea is caused by several microbes, including bacteria (*Shigella*, *Escherichia coli*, *Salmonella*, *Staphylococcus aureus*, *Streptococcus*, *Klebsiella*, *Pseudomonas*, *Aeromonas*, *Campylobacter jejuni*,

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etc.), parasites (Protozoa, *Entamoeba histolytica*, *Giardia lamblia*, *Cryptosporidium parvum*, *Balantidium coli*), and viruses (Rotavirus, Adenovirus, Norwalk virus, Cytomegalovirus (CMV), Echovirus, HIV) (Sudoyo *et al.*, 2014). *Escherichia coli* is a Gram negative bacterium, cocobacilli form, and has a size of 0.4-0.7µm x 1.4µm, is motile and some strains have a capsule and include normal flora in the large intestine. *Escherichia coli* can cause diarrhea because it produces enterotoxins, namely LT toxin (Thermo labile) and ST toxin (Thermo stabile) (Syahrurachman, 2019).

Treatment of acute diarrhea caused by bacteria can be given antibiotics. Consuming relatively high antibiotics can cause various problems, especially bacterial resistance to antibiotics, especially for *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Escherichia coli* bacteria. The results of the research from the Depkes RI (2011) were obtained from 781 patients who were hospitalized, found that 81% of *Escherichia coli* were resistant to antibiotics including ampicillin (73%), cotrimoxazole (56%), chloramphenicol (43%), ciprofloxacin (22%), and gentamicin (18%). The problem of antibiotic resistance has made researchers look for antibacterial sources by using natural ingredients that function to minimize the side effects of using antibiotics. One of the natural ingredients that have the ability as an antibacterial is papaya seeds (*Carica papaya* L.) (Jaipah, Saraswati and Hapsari, 2017).

Papaya seeds (*Carica papaya* L.) are known to contain active compounds such as flavonoids (quercetin), alkaloids, saponins and tannins. The mechanism of action of flavonoids (quercetin) is by inhibiting the synthesis of bacterial cell walls, alkaloids by interfering with the peptidoglycan component, saponins by interfering with the permeability of the bacterial outer membrane, and tannins by shrinking the bacterial cell wall (Arabski *et al.*, 2012; Wijayanti and Febrinasari, 2017; Restyana, Ihtiramidina and Kristianingsih, 2019).

Previous research, Subekti, Molek, and Sim (2018) found that papaya seed extract (*Carica papaya* L.) was able to inhibit the growth of *Streptococcus mitis* at concentrations of 0.5%, 1%, 2%, 3%, and 4% with a minimum inhibitory concentration (MIC) was present at a concentration of 1% with an average colony count of 927.5±5.260 and a minimum bactericidal concentration (MBC) at a concentration of 4%. Based on the above background, this study aims to determine and analyze the antibacterial activity of the ethanol extract of papaya seeds on the growth of *Escherichia coli*.

2. Methods

2.1. Time and Location

This research was conducted in June 2021 at the Abdurrab University Microbiology and Parasitology Laboratory and Abdurrab Vocational School Laboratory.

2.2. Tools and Materials

The instrument used in this study was a spectrophotometer. The materials used in this study were papaya seeds and Escherichia coli ATCC 25922 bacteria

2.3. Papaya Seeds Extract

Papaya seed extract was prepared following the procedure of Subekti, Molek, and Sim (2018). 800g of fresh papaya seeds were washed and dried in an oven to obtain 300g of dried papaya seeds. Dried papaya seeds are then blended until they become powder. Papaya seed powder as much as 100g was macerated with 96% ethanol in a ratio of 1:10 and allowed to stand for 3-4 days with daily stirring. The solution obtained is then removed using an infusion tube into a plastic bottle container. Next, the solution was evaporated using a rotary vacuum evaporator for 30 minutes to obtain a thick extract of papaya seeds.

2.4. Dilution of Papaya Seed Extract Concentration

The thick extract of 96% papaya seeds was diluted into concentrations of 2%, 3%, 4%, 5%, and 6% using the

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following formula V1 x M1 = V2 x M2

2.5. Phytochemical Screening

Phytochemical screening includes tests for flavonoids, alkaloids, saponins, and tannins. The flavonoid test was carried out by means of a thick extract of 0.5g papaya seeds dissolved in ethanol, then put into a test tube and added Mg and HCl powder. Furthermore, amyl alcohol is added, then shaken vigorously and allowed to separate. The presence of flavonoids is indicated by the formation of a red or brown color (Mauti, Rini, and Rante 2018).

The alkaloid test was carried out by means of a thick extract of 0.5g of papaya seeds mixed with 1ml of 2N HCl and 9ml of hot distilled water. The solution was heated for 2 minutes, then cooled and filtered and the filtrate was divided into two. The first filtrate was dropped on filter paper and then sprayed with Dragendorf's reagent, the rest was put in a test tube and Dragendorf's reagent was added. The presence of alkaloids is indicated by the formation of a red or orange color (Mauti, Rini and Rante, 2018).

The saponin test was carried out by means of a thick extract of 0.5g papaya seeds mixed with 10 ml of hot water, then cooled and shaken until foam appeared. The solution was allowed to stand for 2 minutes, then 2N HCl was added. If there are saponin compounds in the extract, a steady foam will be formed for 10 minutes. The tannin test was made by means of 1g of thick papaya seed extract mixed with 10ml of hot distilled water and heated for about one hour. Then, cooled and filtered with filter paper. A total of 5 ml of 1% FeCl3 solution was added and the color was observed, if a dark blue or blackish green color is formed, it indicates the presence of tannins (Mauti, Rini and Rante, 2018).

2.6. Papaya Seeds Extract Antibacterial Activity Test against Escherichia coli

The antibacterial activity test was carried out using the dilution method based on (Nasution, 2013). The first step was to make a suspension of Escherichia coli bacteria by inoculating the bacteria into 9ml NaCl 0.9% so that it was equivalent to the 0.5% McFarland standard. Next, the bacterial suspension obtained was diluted to 107CFU/ml by taking 1ml of the bacterial suspension from 108CFU/ml which was inoculated into 9ml of 0.9% NaCl. Then, it was diluted again to 106CFU/ml by taking 1ml of bacterial suspension from 107CFU/ml which was inoculated into 9ml NB. The bacterial suspension is ready to be tested for MIC and MBC.

The MIC test was carried out by taking 1ml of papaya seed extract with concentrations of 2%, 3%, 4%, 5%, and 6% and put it in a test tube containing 1ml of Escherichia coli bacteria culture. The bacterial control treatment contained 2ml of Escherichia coli bacterial suspension, while the solvent control treatment contained 1ml of 10% ethanol extract of papaya seeds to which 1ml of distilled water was added. All treatments were homogenized and incubated at 37oC for 24 hours. After incubation time, 2ml of treatment was taken and the value of Optical Density (bacterial density seen as turbidity in the medium) was measured using a UV-Vis spectrophotometer (λ630nm). The MIC value was obtained from the lowest extract concentration which was indicated by the clarity of the solution in the test tube.

Furthermore, to determine the MBC value, it was carried out using the spread plate method on NA medium, each of which came from 2%, 3%, 4%, 5%, and 6% MIC tubes using a micropipette. Then, it was incubated at 37˚C for 24 hours. After incubation, the MBC value was obtained from the absence of bacterial colony growth at the lowest concentration. This research was conducted 3 times replication.

2.7. Data Analysis

The data obtained were analyzed descriptively, then presented in the form of tables and figures.

3. Results and Discussion

The results of phytochemical screening showed that papaya seed extract contains active compounds such as flavonoids (quercetin), alkaloids, saponins, and tannins (Table 1). This is in accordance with the research of

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Mauti, Rini, and Rante (2018) who found that papaya seeds contain active compounds such as flavonoids (quercetin), alkaloids, saponins, and tannins that act as antibacterial.

Table 1. Phytochemical screening result

Compound	Result
Flavoids (qurcetin)	+
Alkaoids	+
Saponins	+
Tannins	+

Flavonoids (quercetin) have a mechanism by inhibiting the action of DNA-gyrase from bacteria (Murtiningsih, Nurbaeni and Kusharyanti, 2014). Alkaloids work as antibacterial by interfering with the peptidoglycan component in bacterial cells so that the cell wall layer is not fully formed and inhibits the work of the topoisomerase enzyme which causes cell death (Campbell and Reece, 2012).

Furthermore, saponin compounds work by interfering with the permeability of the bacterial outer membrane. Thus, damage to the cell membrane causes the release of important components from inside the bacterial cell such as nucleic acids, proteins and nucleotides which causes the bacterial cell to lyse (Arabski et al., 2012). Tannins work by shrinking the cell wall so that it interferes with the permeability of bacterial cells. As a result, they cannot carry out living activities and their growth is stunted or even dead (Ajizah, 2004 cited by Wijayanti and Febrinasari 2017).

The results of the MIC test showed that papaya seed extract with concentrations of 2%, 3%, 4%, 5%, and 6% had antibacterial activity, where the MIC value started at a concentration of 2% as indicated by the clear suspension in the test tube (Figure 1; Table 2). The MIC value in this study was determined qualitatively by observing the turbidity level of each extract concentration and measuring the absorbance value using a spectrophotometer. This is in accordance with Nasution's research (2014) that the higher the concentration of the extract, the level of turbidity in the test tube will decrease or the test tube will appear clearer or closer to the control solvent.

Tabel 2. Absorbance value of papaya seed extract on Escherichia coli growth

Treatment	Amount	*Min*	*Max*	*Mean* ±SD
Solvent control	3	0,120	0,203	0,16±0,042
Bacteria control	3	1,200	1,255	1,23±0,027
2% concentration	3	0,789	0,936	0,85±0,074
3% concentration	3	0,759	0,797	0,77±0,024
4% concentration	3	0,687	0,698	0,69±0,005
5% concentration	3	0,420	0,584	0,49±0,082
6% concentration	3	0,256	0,398	0,34±0,074

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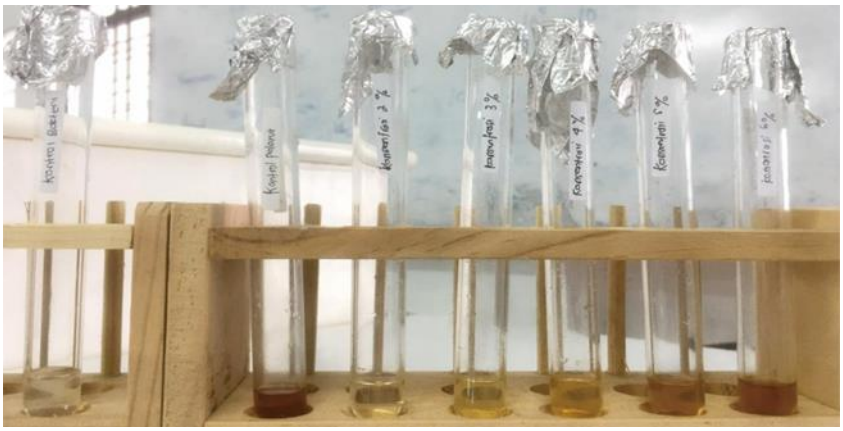

Figure 1. Results of MIC test of papaya seed extract on Escherichia coli growth at concentrations of 2%, 3%, 4%, 5% and 6%

The results of the KBM test of papaya seed extract were obtained at a concentration of 5% which was indicated by the absence of Escherichia coli bacterial colony growth on NA medium. The bacterial control showed the growth of Escherichia coli, while the solvent control did not show the growth of Escherichia coli (Figure 2). Paramesti's research (2014) showed that 96% ethanol extract of papaya seeds was able to inhibit the growth of Escherichia coli bacteria by the agar diffusion method with an average diameter of the inhibition zone formed at a concentration of 5% which was 8.25mm and 75% concentration was 14.75mm.

Another study, Subekti, Molek, and Sim (2018) found that papaya seed infusion extract could inhibit the growth of Streptococcus mitis with a MIC value at a concentration of 1%, while at a concentration of 4% there was no bacterial growth indicating the MBC value. The difference in the results obtained may be influenced by several factors such as the type and origin of the papaya seeds so that it affects the percentage of active compounds contained in papaya seeds, as well as ineffective drying and extraction methods causing the active compounds in papaya seeds to not reach levels. In addition, the solvent used for extraction also has an effect on producing a higher quality and quantity of extract. Therefore, ethanol as a solvent is generally used in the extraction process of papaya seeds by the maceration method (Dewanti and Wahyudi, 2013).

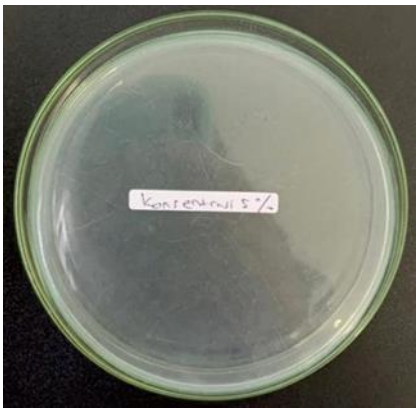

Figure 2. Results of KBM papaya seed extract on the growth of Escherichia coli at a concentration of 5% in NA medium

4. Conclusions

Papaya seeds have active compounds such as flavonoids, alkaloids, saponins and tannins. The results of the

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MIC test were obtained at a concentration of 2% which was indicated by the apparent clarity of the suspension, while the MBC test was obtained at a concentration of 5% which was indicated by the absence of growth of Escherichia coli bacteria on NA medium

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