THE PAPAYA LEAF EXTRACT (Carica Papaya L) ON THE GROWTH OF EPIDERMIC STAPHYLOCOCCUS BACTERIA

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INTRODUCTION

The purpose of this research is to see whether papaya leaf extract can inhibit the growth of staphylococcus epidermidis bacteria as an infectious agent that is often found in skin tissue.

Skin infection is still a health problem faced by people in developing countries including Indonesia. One of the common skin diseases is an abscess caused by the bacterium Staphylococcus epidermidis. Many treatments can be used to prevent infection with the Staphylococcus epidermidis bacteria, one of which is by the use of medicinal plants.

The use of medicinal plants as an effort to tackle health problems has been widely applied by the people amid advances in technology and science today. Moreover, the current state of the Indonesian economy has caused the price of medicines to be relatively expensive. One of the plants that can be used for handling health problems is papaya leaves (Carica papaya L.).

Papaya (Carica papaya L.) is a vertical and wet herbaceous plant. Almost all parts of papaya plants can be utilized, such as leaves, stems, fruit, and roots. Papaya is one of the plants which has been using in traditional medicine. The part of the plant that is often to be used as traditional medicine is its leaves because it contains papain enzymes [1].

Papaya is a plant originating from southern Mexico and northern parts of South America. This plant spread to Africa and Asia and India. From India, this plant expanded to various tropical countries, including Indonesia in the 17th century. The Caricaceae tribe has four genera, namely Carica, Jarilla, Jacaranta, and Cylicomorpha [11]. The first three genera are those which are native to southern Mexico and northern parts of South America, while the fourth genus...
is a plant originating from Africa. The Carica clan has 24 types, one of which is papaya [9].

Papaya (Carica papaya L.) is a vertical and wet herbaceous plant. Almost all parts of papaya plants can be utilized, such as leaves, stems, fruit, and roots. Papaya is one of the plants which has been using in traditional medicine [6][12]. The part of the plant that is often to be used as traditional medicine is its leaves because it contains papain enzymes [1]. Papaya leaves contain chemical compounds that are antiseptic, anti-inflammatory, antimalaria, antidiabetic, antifungal, and antibacterial [15][16]. Antibacterial compounds found in papaya leaves include tannins, alkaloids, flavonoids, terpenoids, and saponins [2]. In addition, papaya leaves contain active substances such as carpatine alkaloids, organic acids such as lauric acid, caffeic acid, gentisic acid, and ascorbic acid, and β-sitosterol, flavonoid, saponin, tannin, and polyphenols [3]. The contents included in papaya leaves (Carica papaya L.) are carotain alkaloid compounds, carikakxanthin, papain, saponins, flavonoids, and tannins [4].

One of the bacteria that can cause infectious disease is Staphylococcus epidermidis. Staphylococcus epidermidis generally can cause swelling diseases (abscesses) such as skin infections and acne. Staphylococcus epidermidis bacteria naturally live in the skin membrane and human mucous membranes [8]. It is a round gram-positive bacteria, usually arranged in an irregular sequence such as grapes and a facultative anaerobic. These bacteria are not pathogenic in normal conditions, but the bacteria will turn into invasive if there is an alteration in the condition of the skin [5].

With the surface of the skin as its habitat. This bacteria cause skin infections, bullae, sores, and inflammatory infections. Staphylococcus epidermidis is a Gram-positive, immotile, non-sporous bacterium in a dense culture medium, it looks like an irregular coccus-shaped, arranged like grapes, protruding, sparkling, does not produce pigments, has a color of white porcelain so that Staphylococcus epidermidis is also called as Staphylococcus albus [13]. 1.9 These bacteria grow optimally at temperatures of 30-37°C and grow well at 1-7% NaCl. The colonies are 1-2 mm in diameter, facultative anaerobes which can grow with aerobic respiration or by fermentation. Staphylococcus epidermidis does not have protein A on its cell wall, is negative coagulase, which distinguishes it from Staphylococcus aureus [14]. Staphylococcus epidermidis utilizes glucose, fructose, sucrose, and lactose to form acidic products aerobically, not fermenting manitol [10].

Staphylococcus epidermidis is sensitive to novobiocin, and this test distinguishes it from Staphylococcus saprophyticus, which is also resulted in a negative coagulase, but resistant to novobiocin [5].

Staphylococcus epidermidis produces a type of toxin or toxic substance. This bacterium also produces a kind of mucus that makes it easy to stick everywhere, including on the surface of tools made of plastic or glass. This mucus also makes the bacterium Staphylococcus epidermidis more resistant to phagocytosis (a mechanism of killing of bacteria by the immune system) and certain antibiotics. (Gap Analysis).

Based on the preceding background, the problems examined in this study were formulated as follows: "how to test the effectiveness of papaya leaf extract (Carica papaya L) on the growth of Staphylococcus epidermidis bacteria using diffusion methods at concentrations of 10%, 20%, 30%, 40%, 50 %, 60%, 70%, 80%, 90% and 100% [7].

METODS

The research was conducted in July 2019 at the Clinical Pathology Laboratory, Faculty of Medicine, Prima Indonesia University. The sample used was Papaya leaf (Carica papaya L.) and obtained from the Pajak Pusat, Medan City.

Research Tools

Petri dishes (10), test tubes, filter paper, knives, measuring cups, autoclaves, incubators, paper discs, cotton swabs, tweezers, sterile cotton, calipers, dropper pipettes, Erlenmeyer flasks, analytical scales, test tube racks.
Research Materials

Papaya leaf (Carica L), bacteria (Staphylococcus epidermidis) 70% ethanol, technical 96% ethanol, chloramphenicol, alcohol, aquabidest, physiological NaCl, spiritus, mueller hinton jelly (MHA), nutrient jelly (NA) plastic wrap, aluminum foil.

Data Collections Method

Preparation Phase

a) First, prepare the tools and ingredients, then sterilize in the oven at a temperature of 40-70º C for ± 2 hours. b) Second, the making of the papaya leaf water (Carica papaya L). In the beginning, the papaya leaf (Carica papaya L) is cleaned with water until it is completely clean. c) Then, the papaya leaves (Carica papaya L) were sliced into small pieces and blended. After blending, do filtration until there is no pulp at all.

Making Papaya Extract (Carica papaya L)

Extracts were made using the maceration method. First, the papaya leaves (Carica papaya L) are cleaned with running water until clean. After the papaya leaves (Carica papaya L) are dry, mashed using a blender to form a simplicia powder. From each simplicia, 1 kg sample is taken. Then it is dissolved or soaked using absolute ethanol (96% ethanol) as much as 10 liters per each simplicia. Then stir it every day for one hour. The maceration process is carried out three times.

Tools Preparation

First, prepare the tools that are going to be used. Then, sterilize the device that is going to be utilized; petri dishes (10), test tubes, measuring cups, in an autoclave with a temperature of 121º C for less than 15 minutes. The metal tools are sterilized on an incandescent flame for about 1 minute.

Material Preparation

First, the papaya leaves are washed thoroughly. Then it got dried in a drying cabinet for 3 days. After drying, the next step is to smooth the papaya leaves using a blend. Then, blend it until smooth and become a powder of simplicia.

Making the Concentrate

The ethanol leaf extract which has been evaporated by ethanol is then divided into 10 concentrations (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%) by dissolving it with ethanol 96% technique due to the presence of essential oil content in the extract. a) For a concentration of 10%: 1gram extract + 10 ml technical Ethanol 96%. b) For a concentration of 20%: 2gram extract + 10 ml technical Ethanol 96%. c) For 30% concentration: 3gram extract + 10 technical Ethanol 96%. d) For a concentration of 40%: 4gram extract + 10 technical Ethanol 96%. e) For a concentration of 50%: 5gram extract + 10 technical Ethanol 96%. f) For a concentration of 60%: 6gram extract + 10 technical Ethanol 96%. g) For 70% concentration: 7gram extract + 10 technical Ethanol 96%. h) For concentrations of 80%: 8gram extract + 10 technical Ethanol 96%. i) For a concentration of 90%: 9gram extract + 10 technical Ethanol 96%. j) For 100% concentration: 10gram extract + 10 technical Ethanol 96%.

Making MHA (Mueller Hinton agar) Media

MHA media that has been prepared with bacteria will be tested. Scale MHA as much as 38 grams and dissolve it in 1000 ml of distilled water, then stir it homogeneously with a magnetic hotplate until it dissolves (homogeneous) for one minute, after it is sterilized for 15 minutes in an autoclave with an air pressure of 1.2 atm with a temperature of 121º C. Then pour each 15 ml of petri dish which has been filled with 1 ml of bacterial suspense, and chill it until it got frozen.

Making Bacteria Suspense (Staphylococcus epidermidis)

The bacteria used in this study were obtained from the USU Pharmacy Laboratory. Staphylococcus epidermidis bacteria were taken as 1 ose of bacteria to be put in a tube containing physiological NaCl. Afterward, stir it until the entire bacterial colony dissolves in NaCl.
Bacterial Diffusion Test (*Staphylococcus epidermidis*)

First, take the MHA media that has been frozen, then scratch evenly throughout the surface of the media by using a cotton swab that contains the suspense of *Staphylococcus epidermidis* bacteria. Then take one sterilized blank disk and dip it in a concentration of 10%. Later, place it on the surface of the media that has been etched by *Staphylococcus epidermidis*. And the treatment is then carried out for each concentration and provides a distance to prevent the other inhibition zones from converging. a) All isolates of the test were incubated for 36-48 hours at 37ºC in the incubator. b) After being incubated for 36-48 hours, the inhibition zone is measured using calipers.

RESULTS AND DISCUSSION

Phytochemical Screening

Phytochemical screening results of papaya leaves (*Carica papaya L*) are listed in the following table:

Table 1. Phytochemical Screening Results of Papaya Leaves (*Carica papaya L*)

| No | Measures           | Results | Signs |
|----|--------------------|---------|-------|
| 1  | Alkaloids          | Positive| +     |
| 2  | Tannins            | Positive| +     |
| 3  | Saponins           | Positive| +     |
| 4  | Triterpen/steroids | Negative| -     |
| 5  | Flavonoids         | Positive| +     |
| 6  | Glycosides         | Negative| -     |

Based on the results of phytochemical testing screens, wherein Table 1, it can be seen that papaya leaf extract (*Carica papaya L*) has chemical contents in the form of alkaloids, tannins, saponins, triterpene/steroids, flavonoids, glycosides.

In previous studies, no one had tested papaya leaf extracts against staphylococcus epidermidis bacteria, as was done by Saraswati (6) who used papaya leaf extracts against *Staphylococcus aureus* bacteria. So this research was conducted to find out how papaya leaf extracts against the growth of staphylococcus epidermidis bacteria.

The comparison of Inhibitory Zones of Papaya Leaf Extract (*Carica Papaya L*) Against *Staphylococcus aureus* Bacteria Growth

The examination of the effectiveness of papaya leaf extract (*Carica papaya L*) on the growth of *Staphylococcus epidermidis* bacteria using disk diffusion shown by the presence of inhibition zones or clear zones around the disc paper. Inhibition zones are measured using calipers. The results of the study used a concentration of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80% and 90% and 100%. The obtained inhibitory zones are listed in the following figure and table:

Table 2. The diameter of Inhibitory Zones of Papaya Leaf Extract (*Carica Papaya L*) Against the Growth of *Staphylococcus Epidermidis* Bacteria

| Concentration | The Diameter of Inhibitory Zone (mm) | Mean |
|---------------|--------------------------------------|------|
|               | Petri 1 | Petri 2 | Petri 3 |      |
| 10%           | 6.9     | 0       | 8.4     | 5.10 |
| 20%           | 9.3     | 9.8     | 8.6     | 9.23 |
| 30%           | 7.2     | 7.2     | 8.6     | 7.67 |
| 40%           | 10      | 10      | 9.7     | 9.90 |
| 50%           | 11.1    | 11.9    | 10.2    | 11.07|
| 60%           | 11.1    | 12.73   | 10.7    | 11.51|
| 70%           | 12.6    | 13.6    | 11.9    | 12.70|
| 80%           | 13      | 13.8    | 12.2    | 13.00|
| 90%           | 13      | 14.4    | 12.6    | 13.33|
| 100%          | 13.5    | 16      | 15.4    | 14.97|
| Mean          | 10.77   | 10.94   | 10.83   |      |

Based on the table above, it is known that the papaya leaf extract (*Carica papaya L*) with a concentration of 10% to 100% have the average diameter of the zone which are continuing to increase, except at a concentration of 30% which has a decreased diameter of the inhibitory zone from a concentration of 20% by 9.23 mm to 7.67 mm at a concentration of 30%.

Figure 1. The Inhibitory Zone of Papaya Leaf Extract Against *Staphylococcus Epidermidis* Bacteria Using 10% and 100% Concentrations with Three Repetitions
Figure 2. The Inhibitory Zone of Papaya Leaf Extract Against Staphylococcus Epidermidis Bacteria Using Concentrations of 20%, 40%, 60% and 80% with Three Repetitions

Figure 3. The Inhibitory Zone of Papaya Leaf Extract Against Staphylococcus Epidermidis Bacteria Using 30%, 50%, 70% and 90% Concentrations with Three Repetitions

The average diameter of the lowest inhibitory zone is at a concentration of 20% which is 5.10 mm while the highest diameter of the inhibitory zone is at a concentration of 100% which is 14.97 mm. If the results of the inhibitory zone diameter and the average diameter of the inhibitory zone are displayed in graphical form, the following results are obtained:

Figure 4. The Graph of Inhibitory Zones of Papaya Leaf Extract (Carica Papaya L) Against the Growth of Staphylococcus Epidermidis Bacteria

Figure 5. The Average Curve of Inhibitory Zones of Papaya Leaf Extract (Carica Papaya L) Against the Growth of Staphylococcus Epidermidis Bacteria

Based on the curve above (Figure 5), it can be seen that at concentrations of 10% to 100% the average diameter of the zone continues to increase, except at a concentration of 30%, which has a decrease of the diameter of the inhibitory zone. The average diameter of the inhibitory zone at a concentration of 10% was 5.10 mm, then it got increased at a concentration of 20% to 9.23 mm. At a concentration of 30%, the diameter of the inhibition zone decreases to 7.67 mm. Then the concentration of 40% increased to 9.90 mm. The inhibitory zone diameters at concentrations of 50%, 60%, 70%, 80%, 90% and 100% respectively are 11.07 mm; 11.51 mm; 12.79 mm; 13.00 mm; 13.33 mm and 14.97 mm.

CONCLUSIONS

From the results of this study, it can be concluded that, papaya leaf extract (Carica papaya L) has an effect in inhibiting the growth of Staphylococcus Epidermidis bacteria with the lowest inhibitory zone of 5.10 mm. Papaya leaf extract (Carica papaya L) has an effect in inhibiting the growth of Staphylococcus Epidermidis bacteria with the highest inhibitory zone of 14.97 mm.

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