Antimicrobial activity of moringa leaf (Moringa oleifera L.) extract against the growth of Staphylococcus epidermidis

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Abstract. The study was aimed to determine the antimicrobial activity of Moringa oleifera L. leaf extract against the growth of Staphylococcus epidermidis. This study was conducted at the Microbiology Laboratory of the Department of Pharmacy, Ministry of Health Polytechnic Makassar, South Sulawesi. Three different concentrations of Moringa leaf extract were used, namely 2% b/v, 4% b/v and 8% b/v which were tested on S. epidermidis bacteria using the paper disk diffusion method. The results showed that Moringa oleifera L. extract could inhibit the growth of S. epidermidis by showing the inhibition zone around the extract. The biggest inhibition zone at a concentration of 8% b/v was 14 mm, for a concentration of 4% b/v was 10.8 mm and the smallest zone at a concentration of 2% b/v was 9.3 mm.

1. Introduction
Infection disease is the most suffered disease by the population of developing countries including Indonesia. One of the causes is bacteria [1]. The term infection describes the growth of replication of microorganism in the host’s body. The disease grows when the infection produces changes to the normal body physiology [2].

The research conducted by the WHO in 14 countries around the world shows that 8.7% of the hospital patients have an infection during hospital treatment [3]. Bacteria that often cause the infection are Escherichia coli, Staphylococcus aureus, Pseudomonas, and S. epidermidis. The treatment of patients in bacterial infections is antibiotics. The selection of antibiotics used as an infectious therapy has an important role in patient recovery. Furthermore, in the National Nosocomial Infection Surveillance (NINS) was explained that therapy with dose and duration that is not appropriate, or the dose that is not educate, can cause the change of the bacteria pattern and resistance to various antibiotics [1].

S. epidermidis is one of the genus staphylococcus that is most commonly found in clinical interest. These bacteria are gram-positive bacteria in white or yellow colonies that are facultative anaerobes and include staphylococcus with negative coagulation [1]. Most of these bacteria are normal flora on the skin and mucous membranes in human. Previously, these organisms rarely caused significant infection. But, by the increasing use of catheter or implants and prosthetic devices [4]. S. epidermidis
becomes an important agent that cause nosocomial infections. The treatment for this bacterium becomes very difficult due to the increased resistance to various microbial agents and its ability to form biofilms. About 70% of isolated S. epidermidis has been resistance to penicillin, oxacillin, cefazolin, ciprofloxacin, and ciprofloxacin. The high rate of resistance will be difficult in the treatment of the infections [5].

One of the alternative treatments for infections caused by bacteria is by using natural ingredients, such as Moringa oleifera L. plants. This plant is referred to as the world’s most valuable multipurpose and miracle tree because all parts of the plant are useful for food, medicine, cosmetics, or water purified [6]. Moringa oleifera L. leaf pharmacologically has benefits as antimicrobial, antifungal, antihypertensive, antihyperglycemic, antitumor, anticancer, anti-inflammatory [7]. One of the most prominent effect is antioxidants. Based on phytochemical tests, Moringa oleifera L. leaves contain tannins, steroids, triterpenoids, flavonoids, saponins, interquinones, and alkaloids that all are antioxidants [8]. Based on a research by Fuglie, fresh moringa leaves has antioxidant strength seven times higher than vitamin C [9]. One of the flavonoid group that moringa has, is quercetin. It contains antioxidant 4 to 5 times higher than vitamin C and vitamin E [10].

It is known that tannin and flavonoids are active compounds in Moringa oleifera L. moringa leaf extract with ethanol solvent are able to inhibit the formation of S. aureus, and have antibacterial properties against Pseudomonas aerogenosa and E. coli. However, there is no scientific evidence yet that supports the effectiveness of Moringa oleifera L. ethanolic extract against S. epidermidis. Based on these description, an antimicrobial activity of Moringa oleifera L. leaf extract was conducted on S. epidermidis.

2. Methodology
The study was an experimental research. It used static-group comparison design. It was conducted in May 2018 at microbiology laboratory Pharmacy Department, The Ministry of Health Polytechnic Makassar, South Sulawesi.

2.1. Extraction procedure
Moringa oleifera L. leaves extract was produced by maceration method. The simplicia weighed as much as 200 gram, then put into the maceration container and soaked with 70% ethanol, then stirred until homogeneous. Subsequently, it closed immediately then stored in a room that is protected from the sun light for five days while stirring it occasionally. After soaking for five days, the extract is filtered by using filter paper to obtained filtrate (liquid extract). Next, the liquid extract is collected in a container and then evaporated. The Moringa leaves extract were made in three levels, namely 2% b/v and 8% b/v. Moringa oleifera L. leaf extract was weighed 0.2 gram for 2% b/v, 0.4 gram for 4% b/v, and 0.8 for 8% b/v in the petri dish, then each level dissolved with Na CMC up to 10 ml and stirred until it is homogeneous, then labelled.

2.2. Bacterial preparation
The bacteria used was S. epidermidis. The pure bacterial stock was taken from NA slant media and then incubated for 1x24 hours at 37°C. The tested bacterial were taken from one culture and then suspended with 10 ml of sterile aquadest.

2.3. Antimicrobial activity of moringa leaf extract
The antimicrobial activity test of Moringa oleifera L. leaf extract was carried out by using paper disk diffusion. The suspended bacterial test was inoculated on the surface of a sterile NA medium using sterile swab. After that the paper disk which has been soaked in Moringa oleifera L. leaves extract with each concentration of 2% b/v, 4% b/v, and 8% b/v is placed aseptically using sterile tweezers on the surface of the medium with 2-3 cm space among the paper disk from the edge of the petri dish. Likewise for the positive control (Tetracycline 30bhp) and negative control (Na CMC). Then, it was inoculated for 1x24 hours at 37°C.
2.4. Observation of antimicrobial activity
Antimicrobial activity was determined by measuring Diameter of Inhibition Zones (DIZ) in mm after incubation for 24 hours. The inhibition zone formed was measured using a caliper. The data obtained were then analyzed using the ANOVA test and followed by the LSD test.

3. Results and discussion
The research result showed that from the three concentrations of *Moringa oleifera* L. leaf extract can inhibit the growth of *S. Epidermidis* which is characterized by the formation of transparent and circular inhibition zones around the paper disk for incubation period along 24 hours at 37°C (figure 1). The transparent circle around the reservoir is caused by the extract of *Moringa oleifera* L. leaf which inhibits the growth of bacteria that cause infection.

![Figure 1](image)

**Figure 1.** The antimicrobial activity of *Moringa oleifera* L. leaf extract against *S. epidermidis* growth at incubation time 1x24 hours: (a) 8% b/v, (b) 4% b/v, (c) 2% b/v, (d) positive control and (e) negative control.

The measurement of inhibition zone of *Moringa oleifera* L. Leaf extract against *S. Epidermidis* growth can be seen in table 1.

**Table 1.** The measurement result of inhibition zone diameter of *Moringa oleifera* L. Leaf extract against *S. Epidermidis* growth

| Replication | Concentration (% b/v) | Positive control | Total |
|-------------|-----------------------|------------------|-------|
|             | Negative control      |                  |       |
| I           | 2                     | 9                | 13    | 32 | 65 |
| II          | 0                     | 11               | 17    | 30 | 69 |
| III         | 0                     | 8                | 12    | 28 | 58 |
| **Total**   | **0**                 | **28**           | **42**| **90** | **192** |
| **Average** | **0**                 | **9.3**          | **10.6**| **14** | **30** | **64.3** |

Table 1 shows the average resistance diameter for the concentration of 2% b/v is 9.3 mm, for the concentration of 4% b/v is 20.8 mm, for the concentration 8% b/v is 14 mm. Then, the positive control (tetracycline) is 30.11 mm while in the negative control using Na CMC there is no inhibition zones was formed. This result is appropriate and greater than the study of the antimicrobial activity of water.
extract of moringa leaf at 8.75 mm [11] and 7 mm indicated by moringa leaf hexane extract [10] against S. Aureus bacteria [12]. Based on the observation, the average of moringa leaf extract diameter of 8% b/v concentration was greater than the concentration of 4% b/v, and 2% b/v. Although the diameter difference was not too large, but the significance showed the differences between the three concentrations.

The calculation obtained using statistical calculations show the effectiveness of Moringa oleifera L. leaf extract in inhibiting the growth of bacteria where the calculated value (Fh) is 127.946 greater than the table value (Ft) which is 3.48 at the $\alpha = 0.05$ level.

As a follow-up test to support the result of statistical data using LSD shows a significant difference in level ($\alpha = 0.05$) between moringa leaf extract concentration 2% b/v, 4% b/v, 8% b/v, or negative and positive control. From the results of LSD, there is a significant difference between the concentration of 2% b/v, 4% b/v, 8% b/v and positive control. It shows that the three concentrations had different inhibition zones. So, it could be concluded that the greatest concentration in inhibiting S. epidermidis growth is the highest concentration of 8% b/v ad positive control using tetracycline.

4. Conclusion

Based on the research result, it could be concluded that Moringa oleifera L. leaf extract can inhibit the growth of S. epidermidis. For the concentration of 2% b/v it can inhibit the growth of S. epidermidis in the amount of 9.3 mm, for the concentration of 4% b/v in the amount of 10.8mm, for the concentration of 8% b/v in the amount of 14 mm, while for positive control it is 30.11 mm. the biggest power inhibition is in the concentration of 8% b/v in the amount of 14mm. the statistical result shows a significant difference among treatments.

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