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**Ethanol Extract Of *Centella Asiatica (L.) Urban* Leaves Effectively Inhibit *Streptococcus pyogenes* and *Pseudomonas aeruginosa* by Invitro Test****Dini Aulia Azmi, \*Nurlailah, Ratih Dewi Dwiyantri**

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**Abstract:** *Streptococcus pyogenes* and *Pseudomonas aeruginosa* are some of the causes of infectious diseases. *Centella asiatica (L.) Urban* has many benefits for humans, including overcoming fever, anti-bacterial, and anti-inflammatory. This study aims to determine the inhibition of *Centella asiatica (L.) Urban* leaves ethanol extract on the growth of *Streptococcus pyogenes* and *Pseudomonas aeruginosa*. This research is the initial stage of the development of herbal medicines to treat *Streptococcus pyogenes* and *Pseudomonas aeruginosa* infections. The independent variable was the concentration of ethanol extract of *Centella asiatica (L.) Urban* leaves and the dependent variable was the growth of *Streptococcus pyogenes* and *Pseudomonas aeruginosa*. The anti-bacterial activity test was carried out by the liquid dilution method. The concentrations used are 20%, 40%, 60%, 80%, 100%. The results showed that the minimum inhibitory concentration (MIC) against *Streptococcus pyogenes*: 40% and *Pseudomonas aeruginosa*: 40%. Minimum bactericidal concentration (MBC) results for *Streptococcus pyogenes*: 60% and *Pseudomonas aeruginosa*: 60%. So it can be concluded that there is inhibition of the ethanol extract of *Centella asiatica (L.) Urban* leaves on the growth of *Streptococcus pyogenes* and *Pseudomonas aeruginosa*. *Centella Asiatica (L.) Urban* extract has potential as herbal medicine against bacterial infections but requires further research to determine its effect in vivo.

**Keywords:** *Centella asiatica (L.) Urban*; *Streptococcus pyogenes*; *Pseudomonas aeruginosa*.

**INTRODUCTION**

Death rates caused by infectious diseases in Indonesia are still quite high and very alarming. Causes of infectious diseases include bacteria, viruses, fungi, and protozoa. The bacteria that most often cause such infections are gram-positive and gram-negative bacteria. *Streptococcus pyogenes* and *Pseudomonas aeruginosa* are some of the causes of infectious diseases<sup>1</sup>. Bacteria *Streptococcus pyogenes* (*Streptococcus*  $\beta$  hemolyticus group A) is one of the causes of respiratory tract infections. These bacteria often accumulate in the tonsils or can also attack the pharynx<sup>2</sup>. While the bacterium *Pseudomonas aeruginosa* is an opportunistic pathogenic bacterium, infection occurs when the host's immune system declines. *Pseudomonas aeruginosa* can cause urinary tract infections, breathing, digestion, especially in patients with severe burns, cancer, and AIDS sufferers<sup>3</sup>.

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Various types of antibiotics have been widely used, but the cure rate is still not satisfactory. The emergence of antimicrobial resistance is one of the factors causing failure in handling infection cases. The high price of antibiotics also causes the economically weak community not to afford it, so various plants in the treatment of infectious diseases can be an option for Indonesia's people<sup>4</sup>. One of the plants that have potential as traditional medicine is *Centella asiatica (L.) Urban*<sup>5</sup>. *Centella asiatica (L.) Urban* is a plant that often found in open and damp places such as fields, paddy fields, even the edges of walls or fences. *Centella asiatica (L.) Urban* has long been used as traditional medicine both in the form of fresh, dry ingredients, and herbs<sup>6</sup>. *Centella asiatica (L.)* The community uses an urban leaf as a wound healer, inflammation, rheumatism, asthma, hemorrhoids, tuberculosis, leprosy, dysentery, fever, and blood-booster<sup>7,8</sup>.

*Centella asiatica (L.) Urban* plants have been known to have chemical properties beneficial to health, including triterpenoids, flavonoids, tannins, and saponins<sup>9,10</sup>. Its triterpenoid content consists of asiatic acid, madecassic acid, asiaticoside, and madecassoside<sup>11</sup>. This content has many pharmacological effects on wound healing, namely an anti-inflammatory, anti-bacterial, encouraging angiogenesis and type I collagen synthesis. While flavonoids, tannins, and saponins have anti-bacterial properties<sup>12</sup>. Previous research conducted by Azzahra and Hayati found that *Centella asiatica (L.) Urban* leaves at extract concentrations of 10%, 20%, 40%, 60%, and 80% were effective in inhibiting the growth of *Streptococcus mutans* with an average inhibition zone 10.03 mm, 10.08 mm, 13.00 mm, 15.30 mm, 19.50 mm<sup>1</sup>.

Another study conducted in 2011 found that *Centella asiatica (L.) Urban* ethanol extract was the most effective among petroleum ether and chloroform extracts in inhibiting *Proteus vulgaris* bacteria with an average inhibition zone of 17 mm, *Staphylococcus aureus* 17 mm, *Bacillus subtilis* 19 mm and *Escherichia coli* 16 mm. Previous studies have shown that *Centella asiatica (L.) Urban* data have greater barriers to Gram-positive bacteria than Gram-negative bacteria. But among the many bacteria studied, the effect of *Centella asiatica (L.) Urban* on *Streptococcus pyogenes* and *Pseudomonas aeruginosa* has not been widely expressed. This study aims to determine the inhibitory effect of *Centella asiatica (L.) Urban* leaf extracts on the growth of *Streptococcus pyogenes* and *Pseudomonas aeruginosa*<sup>13</sup>.

## MATERIALS AND METHODS

Experimental research with Posttest Only Control Group Design done by examining MIC and MBC of ethanol extract of *Centella asiatica (L.) Urban* leaves at concentrations of 20%, 40%, 60%, 80%, and 100% on the growth of *Streptococcus pyogenes* and *Pseudomonas aeruginosa*. This study has eight treatment groups with three repetitions. Positive control in the form of bacterial suspension with chloramphenicol while negative control in bacterial suspension with Tryptic Soy Broth (TSB). The sample in this study was *Centella asiatica (L.) Urban* leaf, which grew in the Tanjung area, Tabalong Regency, South Kalimantan Province, Indonesia. Green leaves, kidney-shaped, serrated edges, and slightly hairy. The independent variable in this study was the concentration of ethanol extract of *Centella asiatica (L.) Urban* leaves. This study's dependent variable was the growth of *Streptococcus pyogenes* and *Pseudomonas aeruginosa* based on Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC). The determination test of *Urban Centella asiatica (L.)* plant was carried out at the FMIPA Laboratory of Lambung Mangkurat University.

500 gram *Centella asiatica* (L.) Urban leaves are washed with running water then dry for  $\pm$  two weeks. The leaves are made into powder with a 60 mesh sieve. The maceration process of *Centella asiatica* (L.) Urban leaves with 96% ethanol (1: 4) for three days. Macerate concentrated using a rotary evaporator at 55 ° C until a thick extract obtained. The concentrated extract then dissolved with a sterile aqua dest ratio of 2: 1 to obtain a concentration of 200%. Making a concentration of 160%, 120%, 80%, 40% is done using a 200% concentration and aqua dest. Suspension of *Streptococcus pyogenes* and *Pseudomonas aeruginosa* using bacterial incubation results for 24 hours at 37 ° C.

MIC test was carried out by mixing 1 mL of *Centella asiatica* (L.) Urban leaf ethanol extract and 1 mL of bacterial suspension by repetition three times. After adding the bacterial suspension, the final concentration of the test solution is half of the initial concentration so that the concentration of the test solution becomes 100%, 80%, 60%, 40%, 20%. MIC results after 24 hours incubation at 37°C were determined in a solution containing the lowest extract content but still able to inhibit bacteria (clear solution). The solution showing the MIC taken 20 ul spread on the surface of the Nutrient agar incubation plate 24 hours at 37°C. MBC was demonstrated with Nutrient agar plates, which had no growth in bacterial colonies.

## RESULT AND DISCUSSION

Anti-bacterial testing of *Centella asiatica* (L.) Urban ethanol extract on the growth of *Streptococcus pyogenes* using the Minimum inhibitory concentration (MIC) determination showed the level of clarity at the lowest concentration of 40%. MIC results can be seen in table 1.

Table 1. Minimum inhibitory concentration (MIC) results of Garlic Ethanol Extract on *Streptococcus pyogenes* Growth

| The concentration of Ethanol Extract from <i>Centella asiatica</i> (L.) Urban Leaves | Repetition |        |        | Conclusion |
|--|------------|--------|--------|------------|
|  | I          | II     | III    |            |
| 20%  | Turbid     | Turbid | Clear  | Turbid     |
| 40%  | Clear      | Clear  | Clear  | Clear      |
| 60%  | Clear      | Clear  | Clear  | Clear      |
| 80%  | Clear      | Clear  | Clear  | Clear      |
| 100%   | Clear      | Clear  | Clear  | Clear      |
| Control (+)  | Clear      | Clear  | Clear  | Clear      |
| Control (-)  | Turbid     | Turbid | Turbid | Turbid     |
| <i>Centella asiatica</i> (L.) Urban Leaves Control                                   | Clear      | Clear  | Clear  | Clear      |

Based on the determination of the minimum bactericidal concentration (MBC), which is characterized by the absence of colony growth at the lowest concentration for *Streptococcus pyogenes* occurs at a concentration of 60%. The results can be seen in Table 2 as follows.

Table 2. Minimum bactericidal concentration (MBC) yields of *Centella asiatica* (L.) Urban Ethanol Extract on *Streptococcus pyogenes* Growth

| The concentration of Ethanol Extract from <i>Centella asiatica</i> (L.) Urban Leaves | Repetition |    |     | Colony Average |
|--|------------|----|-----|----------------|
|  | I          | II | III |                |
| 20%  | 25         | 27 | 23  | 18             |
| 40%  | 1          | 2  | 0   | 1              |
| 60%  | 0          | 0  | 0   | 0              |
| 80%  | 0          | 0  | 0   | 0              |
| 100%   | 0          | 0  | 0   | 0              |
| Control (+)  | 0          | 0  | 0   | 0              |
| Control (-)  | ∞          | ∞  | ∞   | ∞              |
| <i>Centella asiatica</i> (L.) Urban Leaves Control                                   | 0          | 0  | 0   | 0              |

The average number of *Streptococcus pyogenes* colonies for each concentration on the Minimum bactericidal concentration determination is presented in Figure 1.

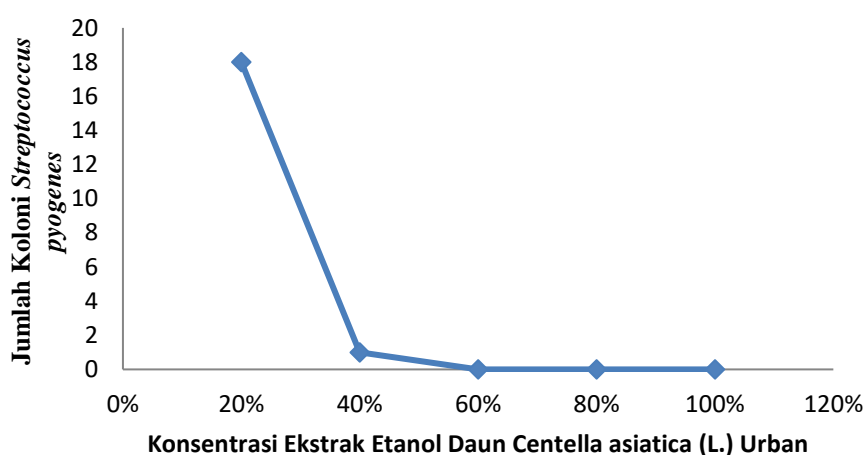


Figure 1. Graph of Average Number of *Streptococcus pyogenes* Colonies in Various Concentrations on Determination of Minimum Bactericidal Concentration (MBC)

Anti-bacterial testing of *Centella asiatica* (L.) Urban ethanol extract on the growth of *Pseudomonas aeruginosa* using the Minimum inhibitory concentration (MIC) determination showed the level of clarity at the lowest concentration of 40%. MIC results can be seen in table 3.

Based on the determination of the minimum bactericidal concentration (MBC), which is characterized by the absence of colony growth at the lowest concentration for *Pseudomonas aeruginosa* occurs at a concentration of 60%. The results can be seen in table 4.

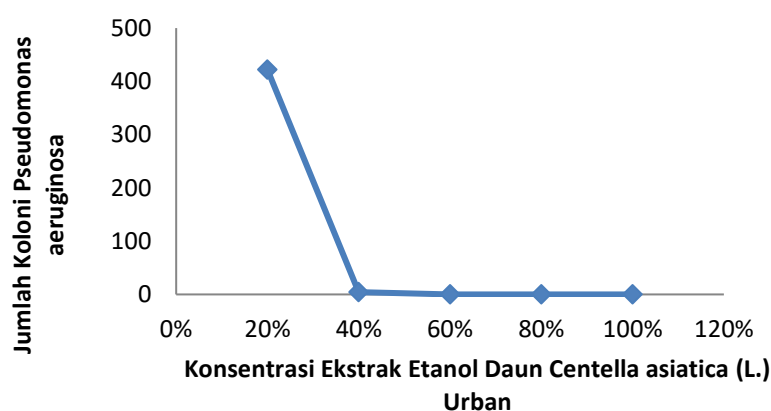
The results of the average number of *Pseudomonas aeruginosa* colonies for each concentration on the determination of the Minimum bactericidal concentration are presented in Figure 2.

Tabel 3. Hasil Minimum inhibitory concentration (MIC) Ekstrak Etanol Daun *Centella asiatica* (L.) Urban terhadap Pertumbuhan *Pseudomonas aeruginosa*

| The concentration of Ethanol Extract from <i>Centella asiatica</i> (L.) Urban Leaves | Repetition |        |        | Conclusion |
|--|------------|--------|--------|------------|
|  | I          | II     | III    |            |
| 20%  | Turbid     | Turbid | Turbid | Turbid     |
| 40%  | Clear      | Clear  | Clear  | Clear      |
| 60%  | Clear      | Clear  | Clear  | Clear      |
| 80%  | Clear      | Clear  | Clear  | Clear      |
| 100%   | Clear      | Clear  | Clear  | Clear      |
| Control (+)  | Clear      | Clear  | Clear  | Clear      |
| Control (-)  | Turbid     | Turbid | Turbid | Turbid     |
| <i>Centella asiatica</i> (L.) Urban Leaves Control                                   | Clear      | Clear  | Clear  | Clear      |

Table 4. Minimum bactericidal concentration (MBC) yields of *Centella asiatica* (L.) Urban Ethanol Extract on *Pseudomonas aeruginosa* Growth

| The concentration of Ethanol Extract from <i>Centella asiatica</i> (L.) Urban Leaves | Repetition |     |     | Colony Average |
|--|------------|-----|-----|----------------|
|  | I          | II  | III |                |
| 20%  | 440        | 400 | 427 | 330            |
| 40%  | 6          | 2   | 3   | 3              |
| 60%  | 0          | 0   | 0   | 0              |
| 80%  | 0          | 0   | 0   | 0              |
| 100%   | 0          | 0   | 0   | 0              |
| Control (+)  | 0          | 0   | 0   | 0              |
| Control (-)  | ∞          | ∞   | ∞   | ∞              |
| <i>Centella asiatica</i> (L.) Urban Leaves Control                                   | 0          | 0   | 0   | 0              |

Figure 2. Graph of the Average Number of *Pseudomonas aeruginosa* Colonies in Various Concentrations on the Determination of MBC

This study's results indicate that the higher the level of ethanol extract of *Centella asiatica* (L.) *Urban* leaves, the higher the inhibitory and killing power of the growth of *Streptococcus pyogenes* and *Pseudomonas aeruginosa*. This marked by the occurrence of clarity in Tryptic Soy Broth (TSB) media and a decrease in the number of *Streptococcus pyogenes* and *Pseudomonas aeruginosa* colonies growing on the surface of agar nutrient media. The anti-bacterial properties of the *Centella asiatica* (L.) *Urban* leaves are affected because in the *Centella asiatica* (L.) *Urban* leaves there are triterpenoid compounds, flavonoids, tannins, and saponins. The mechanism of action of *Centella asiatica* (L.) *Urban* leaf flavonoids as an anti-bacterial that inhibits the synthesis of nucleic acids in bacterial cells inhibit the function of bacterial cell membranes and inhibit bacterial cell energy metabolism. *Centella asiatica* (L.) *Urban* leaves also contain saponins, which can increase the permeability of bacterial cell membranes so that they can change the structure and function of the membrane, causing denaturation of membrane proteins so that cell membranes will be damaged and lysis<sup>14</sup>.

According to Robinson, triterpenoids' mechanism as an anti-bacterial is to react with porin (transmembrane protein) on the outer membrane of bacterial cell walls, forming strong polymeric bonds that cause damage to porin. Damage to the porin, which is the entrance and exit of the compound, will reduce the bacterial cell wall's permeability, which will cause the bacterial cell to be deficient in nutrients so that bacterial growth is inhibited or dead. At the same time, tannins are one of the phenol group's active compounds that can inhibit bacterial growth by precipitation and denaturation of bacterial proteins<sup>15</sup>. Tannins have targets against cell wall polypeptides that damage bacterial cell walls<sup>16</sup>. In previous studies conducted by Azzahra and Hayati, the leaves of *Centella asiatica* (L.) *Urban* at extract concentrations of 10%, 20%, 40%, 60%, and 80% with the disc diffusion method effectively inhibited the growth of *Streptococcus mutans* with inhibition zone averages 10.03 mm, 10.08 mm, 13.00 mm, 15.30 mm, 19.50 mm. The resulting inhibition zone is in a strong category<sup>14</sup>.

Another study conducted by Mpila, about the activity of *Centella asiatica* (L.) *Urban* extracts against the growth of *Streptococcus mutans* by diffusion method showed that the inhibitory power obtained at a concentration of 15% with inhibition zones of 15.64 mm, which means classified as anti-bacterial with the ability which is very strong<sup>17</sup>. The difference in research results between researchers and other researchers can be caused by the methods and results of different secondary metabolites which according to Runtungok and budiarmo bioactive substances contained in *Centella asiatica* (L.) *Urban* leaf plants have differences influenced by the environment, such as climate, sunlight, moisture, and soil fertility. Based on descriptive analysis seen in tables 2 and 4 the average number of colonies in nutrient agar media, for the growth of *Streptococcus pyogenes* at 40% concentration is one colony, and 20% is 18 colonies while the growth of *Pseudomonas aeruginosa* at 40% concentration is 3 and 20% as much 330 colonies. These results indicate that the ethanol extract of *Centella asiatica* (L.) *Urban* leaves are more effective against *Streptococcus pyogenes*<sup>18</sup>.

The results showed that the test solution inhibited Gram-positive bacteria more efficiently than Gram-negative bacteria, meaning that Gram-positive bacteria were more susceptible to chemical compounds than Gram-negative. This probably caused by differences in the composition and structure of cell walls in Gram-positive and Gram-negative bacteria. The structure of the cell wall of Gram-positive bacteria is more straightforward, which is a single layer with low lipid content (1-4%), making it

easier for bioactive material to enter the cell. The structure of the Gram-negative bacterial cell wall is more complex, consisting of three layers consisting of the outer layer of lipoprotein, the middle layer of lipopolysaccharide which acts as a barrier to the entry of anti-bacterial bioactive material, and the inner layer of peptidoglycan with high lipid content (11-12%)<sup>19</sup>.

The limitation of this study is that no Minimum Bactericidal Concentration test between concentrations of 40% -60%, so the distance between these concentrations makes the MBC value more precisely unknown. MBC values may be at concentrations below 60%.

## CONCLUSION

Minimum inhibitory concentration (MIC) of *Centella asiatica* (L.) Urban ethanol extract against *Streptococcus pyogenes*: 40% and *Pseudomonas aeruginosa*: 40%. Minimum bactericidal concentration (MBC) of *Centella asiatica* (L.) Urban ethanol extract against *Streptococcus pyogenes*: 60% and *Pseudomonas aeruginosa*: 60%. *Centella Asiatica* (L.) Urban extract has potential as herbal medicine against bacterial infections but requires further research to determine its effect in vivo.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest and have not received any funds for this study.

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