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ANTIBACTERIAL ACTIVITY OF ETHANOL EXTRACTS AND *n-hexane* OF (*Centella asiatica* (L.) Urb.) AGAINST THE *Pseudomonas aeruginosa* AND *Bacillus subtilis*

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ABSTRACT---*Centella asiatica (L.) Urb.) Is a plant that has antifungal and antibacterial bioactive compounds. Centella asiatica (L.) Urb.) Is one of the main herbs for treating skin problems, healing wounds and being an antibacterial and antiviral agent. Centella asiatica (L.) Urb.) Leaf samples taken from the manoko bandung plantation were extracted using 96% ethanol and n-hexane, the extract was thickened and a thick extract was obtained which was then tested for its antibacterial activity. Antibacterial activity test uses the paper disk diffusion method and tested against the bacteria Pseudomonas aeruginosa and Bacillus subtilis. The extract concentrations used were 7.5%, 10%, 12.5% and 15%. The positive control used is tetracycline while the negative control used is DMSO. The results showed that ethanol extract was effective in inhibiting the growth of Bacillus subtilis bacteria with an average inhibition zone of 10.41 mm and included in the strong category. As for n-hexane, it is effective in inhibiting the growth of Bacillus subtilis bacteria with inhibition zones 23.19 and categorized as very strong inhibition. The ethanol and n-hexane extracts of Centella asiatica (L.) Urb.) Leaves are effective in inhibiting bacteria from the gram positives group because gram positives have a simpler cell wall with a relatively large number of peptidoglycan.*

Keywords---*antibacterial, paper disk, Centella asiatica (L.) Urb.), B. Subtilis, P.aeruginosa.*

I. INTRODUCTION

Infectious disease is a disease caused by the entry and breeding of microorganisms, a large group of microscopic organisms consisting of one or many cells such as bacteria, fungi, and viruses (Mandell et al., 2013). In this case the cause of bacterial infection is caused by the danger pathogenic bacteria that can attack the stem cells. Bacteria that can cause an infection are Pseudomonas aeruginosa and *Bacillus subtilis*. Pseudomonas aeruginosa can grow with humans without causing disease until the bacteria form a biofilms that overcomes the host's immune system. The formation of the biofilms

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bacteria Pseudomonas aeruginosa colonizes the lungs of cystic fibrosis patients and is the leading cause of death in humans (Tortora, 2012). The bacteria *Bacillus subtilis* is a normal flora also found in the digestive tract of the chicken (Green et al., 2006). *Bacillus subtilis* is a trunk-shaped, gram-positive, non-pathogenic spore-forming organism commonly found in water, air, dust, soil and sediment (Jawetz et al., 2005).

One solution used to remove bacteria and minimize infectious diseases is by looking for compounds that have antibacterial activity. Plants that have been shown to have antibacterial activity is Pegagan leaf. Pegagan is a medicinal plant containing triterpenoid, saponin, tannins, alkaloids, phenolic, steroids, and flavonoids. The triterpenoid compounds, saponins, and tannins are the most powerful compounds (Kristina et al., 2009). Pegagan leaf extract can inhibit the growth of Escherichia coli. In addition the leaves of Centella asiatica can kill the growth of bacteria Escherichia coli and Mycobacterium tuberculosis (Yusran et al., 2016) and Salmonella typhi bacteria (Rahmatina, 2017).

Generally the extraction used is soxhlet extraction. Soxhlet is a method with the principle of heating and immersion samples (Ministry of Health RI, 2006). The solvent used is ethanol and n-hexane. Selection of N-hexane solvents as solvents, because N-hexane is stable and volatile. n-hexane can separate between oils with solvents so it can be known clearly the difference between oil and solvent. In the standard state N-hexane is a colorless fluid that is insoluble in water (Munawaroh, 2010).

Based on this, there is research on the activity test of ethanol extract antibacterial and N-hexane Daun Pegagan (*Centella asiatica (L.) Urb*.) Against Pseudomonas aeruginosa bacteria and *Bacillus subtilis*.

II. METHODS

Determinations of Plant

Determination of PLANT is done in the school laboratory of Biological Science and Technology of Institute Technology Bandung.

Extract Creation

The leaves are extracted using the soxhlet way of using the 96% ethanol solvent and n-hexane. Centella Asiatica leaves that have been mashed in weights and then inserted into the sleeve and then added the ethanol solvent 96% and N-Heksana using a comparison (1:5). Stitch the Soxhlet tool, and then perform the extraction. The Filtrate obtained next to the prohibition by using a rotary evaporator and water bath until the extract of ethanol and n-hexane Centella leaves.

Screening Phytochemistry

Screening Phytochemistry is done against the Simplisia powder and a condensed extract of Centella. The done screening phytochemicals include alkaloid tests, triterpenoids, steroids, tannins, saponins, phenolic, and flavonoids.

Test antibacterial activity

The bacterial test method uses a paper disc disk method. Insert the standard turbidity bacterial suspension Mc. Farland 0.5, applying the bacterial suspension over the medium with sterile reviewed cotton. After that, put each of the paper discs that have been dropped 96% ethanol extract and N-hexane with concentrations (7.5%, 10%, 12.5%, and 15%), paper disks containing tetracycline (positive control), and paper disks containing DMSO (negative control) aseptically

using sterile Pinsent on the surface of the media that has been compacted. Then the media incubated an incubator at the temperature of 37oC for 18-24 hours, after that do observations and measurement of the barrier zone that formed using a wheeled period.

III. RESULTS AND DISCUSSION

Determinations of Plant

Determination of crop is done in the school laboratory of Biological Science and Technology of Bandung Institute of Technology. Based on the results of the determination with the number 924/11. CO 2.2/PL/2019, obtained that the sample used is true *Centella asiatica (L.) Urb*. With a synonym of Hydrocotyle asiatica L.

Extraction

The extraction of the leaf Simplisia powder Pegagan (*Centella asiatica (L.) Urb*.) is carried out using the method of extraction of heat means with soxhlet using ethanol solvent 96% and n-hexane. This simplisia in research is as much as 300 grams with a total of 96% ethanol solvent used as much as 4.5 L of the acquired Randemen value is 44.31 gram whereas for the solvent n-hexane total solvent used as much as 4.5 L Value the obtained yield is 5.10 grams

Screening Phytochemistry

Phytochemical screening is an early stage in this study to give an overview of the group of compounds contained in the plant of the Centella-leaf. The results of the phytochemical cervical in Simplisia and 96% ethanol extract and N-hexane leaf Centella asiatica (*Centella asiatica (L.) Urb*.) can be seen in the following table:

Table 1. The Results of Phytochemistry Screening of Centella-leaves

Secondary Metabolite	Result
Flavonoids	-
Tannins	-
Alkaloids + Dragendrof	+
Alkaloids + Mayer	-
Saponins	+
Polyphenols	+
Steroids	-
Terpenoids	-

Table 2. Results of Screening Phytochemical Extract Ethanol of Centella-leaves

Secondary Metabolite	Result
Flavonoids	-
Tannins	+
Alkaloids + Dragendrof	+

Alkaloids + Mayer	-
Saponins	-
Polyphenols	+
Steroids	-
Terpenoids	-

Table 3. Results of Screening Phytochemistry Extract n-hexane of Centella-leaves

Secondary Metabolite	**Result**
Flavonoids	-
Tannins	-
Alkaloids + Dragendrof	+
Alkaloids + Mayer	-
Saponins	-
Polyphenols	-
Steroids	-
Terpenoids	-

Description: **(+): Positive**

(-): Negative

Based on the results of a phytochemical test that has been carried out with Centella-leaf powder showed positive results in alkaloid compounds, saponin, and polyphenols. Ethanol condensed extract 96% of the Centella leaves show positive results in compounds of tannins, alkaloids, and polyphenols. As for the viscous extracts of n-hexane leaves showed positive results in the alkaloid compounds.

Test antibacterial activity

The antibacterial activity test of 96% ethanol extract and n-hexane leaf Centella asiatica (*Centella asiatica (L.) Urb*.) against Pseudomonas aeruginosa bacteria and *Bacillus subtilis* is performed using the diffusion paper disk method using concentrations of 7.5%, 10 %, 12.5%, and 15%. The positive control used is tetracycline antibiotic and the negative control used is the solvent DMSO. The test results of antibacterial activity are obtained indicating that 96% ethanol extract and N-hexane leaf Centella asiatica (*Centella asiatica (L.) Urb*.) can slow down the growth of the bacteria *Bacillus subtilis*. The results of the measurement of the current zone diameter of the ethanol extract 96% and n-hexane Centella leaves is seen in the table as follows:

Table 4. Average Results Zone Diameter of Inhibition of Ethanol Extract B. Subtilis

Concentration	**Zone diameter Of Inhibition (mm)**	**Zone Of Inhibition Category**
7,5%	10,41±1,83	Strong

10%	8,73±2,10	Intermediate
12,5%	6,83±0,76	Intermediate
15%	8,89±1,01	Intermediate
Control Positive	21,14±4,17	Very Strong
Control Negative	0,00	Negative

The zone diameter of the antibacterial growth is said to be weak in less than 5 mm, the diameter of 5-10 mm belongs to the medium category, diameter 11-20 mm included in a strong category, while for diameter more than equal to 20 included in the Very strong (Schlegel & Schdimt, 1994).

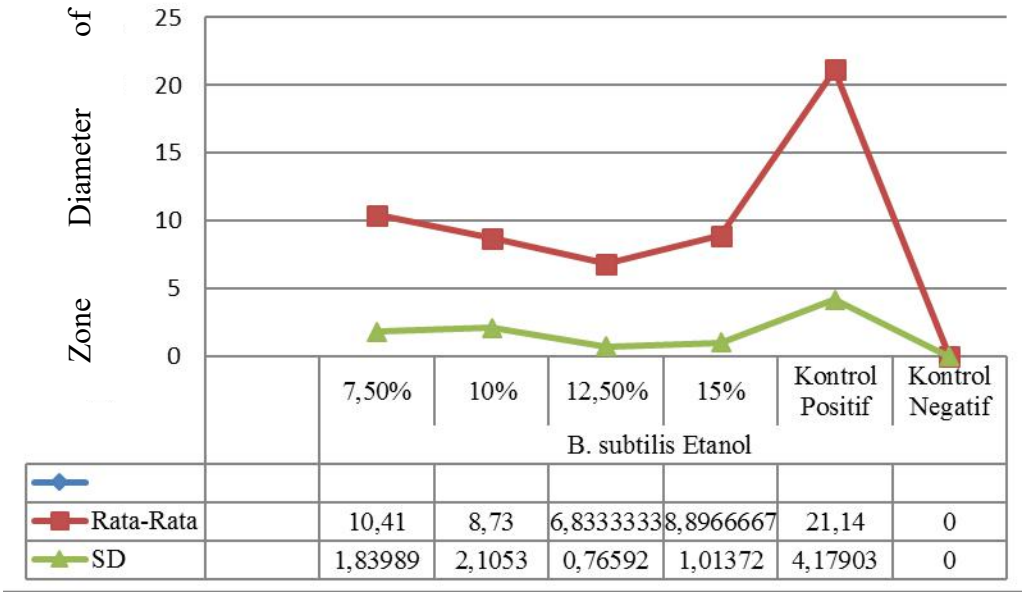
The results of the research that have been done extracts of Centella asiatica ethanol has antibacterial activity on the concentration of 7.5% can slow down the growth of bacteria *Bacillus subtilis* with a diameter of the zone of the strong category of 11-20 mm, at a concentration of 10% 12, 5%, and 15% can inhibit the growth of bacteria *Bacillus subtilis* with the diameter of the medium inhibitory zone of the category is 5-10 mm, the results of control of the tetracycline positive resulting in a barrier zone diameter with a very strong category ≥ 20 mm, while for control Negative (DMSO) Centella Asiatica Ethanol extract does not have a barrier zone against the growth of *Bacillus subtilis*.

Table 5. Average Results Zone Diameter of Inhibition Extract n-Heksana B. Subtilis

Concentration	**Zone Of Inhibition (mm)**	**Zone Of Inhibition Category**
7,5%	14,39±1,09	Strong
10%	23,19±1,48	Very Strong
12,5%	11,71±2,56	Strong
15%	13,79±1,90	Strong
Control Positive	22,40±0,25	Very Strong
Control Negative	0,00	Negative

As for the test of antibacterial activity of n-hexane *Bacillus subtilis* at concentrations of 7.5%, 12.5%, and 15% can inhibit the growth of Baciluus subtilis bacteria with the category of strong inhibitory zone of 5-10 while at a concentration of 10% extract n-hexane Get a result of 11-20 mm i.e. enter into a very strong category, the control of a positive tetracycline antibiotic extract n-hexane Centella-leaves can inhibit the growth of *Bacillus subtilis* with a very strong category of ≥ 20 mm, while for Negative control (DMSO) extract n-hexane of Centella asiatica leaves has no antibacterial activity against *Bacillus subtilis*.

Antibacterial activity of the axtract *Centella asiatica* (L.) URB leaves against *Bacillus subtilis*

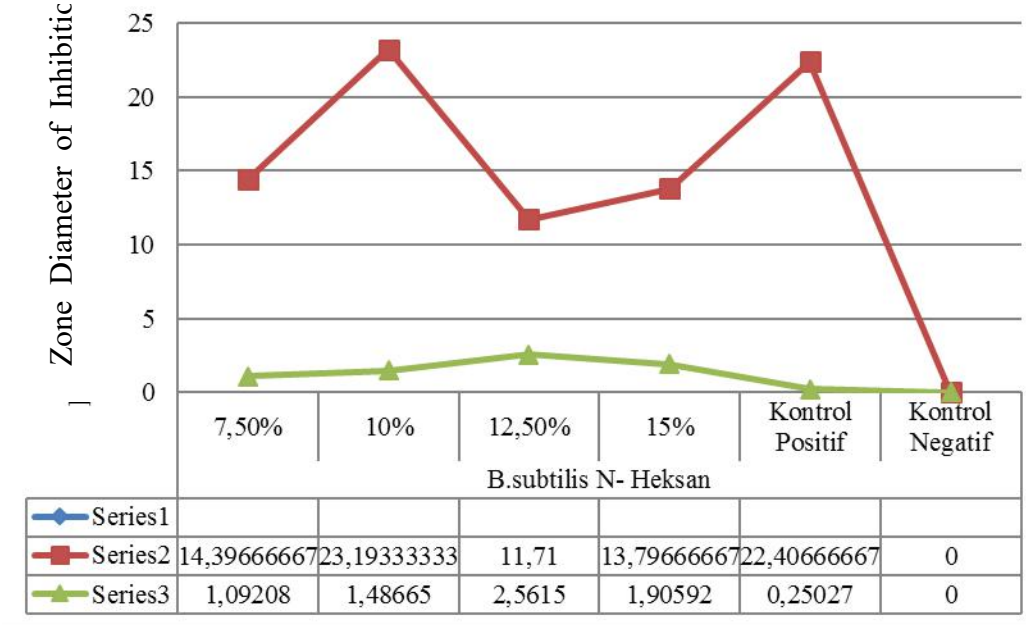


Rata-Rata	10,41	8,73	6,8333333	8,8966667	21,14	0
SD	1,83989	2,1053	0,76592	1,01372	4,17903	0

Figure 1. Test graph antibacterial activity of a Centella leaves extract ethanol against *Bacillus subtilis*

If viewed from Figure 1, the administration of concentrations of extracts greatly affects the growth of *Bacillus subtilis*. The minimum average ethanol extract's Diameter is at a 12.5% concentration of 6.83 mm against the growth of *Bacillus subtilis*. The category of the diameter of the zone of the barrier enters the medium category of 5-10 mm. As for the average of the maximum barrier zone in the get results at a concentration of 7.5% Centella asiatica Ethanol extract received a yield of 10.41 mm to the growth of the *Bacillus subtilis* category diameter Zone of the inhibition entered in the strong category of 11-20 mm.

Antibacterial activity of the axtract n-hexane *Centella asiatica* (L.) URB leaves against *Bacillus subtilis*



Series1						
Series2	14,39666667	23,19333333	11,71	13,79666667	22,40666667	0
Series3	1,09208	1,48665	2,5615	1,90592	0,25027	0

The result of the inhibitory zone of n-hexane extract against *Bacillus subtilis* bacteria indicates that the average inhibitory zone of N-hexane extract of the leaves is found in concentrations of 12.5% i.e. 11.71 mm can already inhibit the growth of bacteria *Bacillus subtilis* . The category of the hate zone enters into a strong category of 10-20 mm. As for the average maximum hate zone inhibition of the Extract n-hexane of Centella asiatica leaves is at a concentration of 10% ie 23.19 mm The category of the Hant zone enters into a very strong category of ≥ 20 mm.

Gram-positive bacteria have a simpler cell wall with a relatively numerous amount of peptidoglycan. The Gram-negative bacterial cell wall has fewer peptidoglycan and is structurally more complex. The outer membrane on a Gram-negative cell wall contains lipopolysaccharides, i.e. carbohydrates associated with lipids (Campbell et al., 2008). Gram-negative bacterial cell walls have an outer membrane that is rich in lipids as a deterrent of enzymes, preventing the inclusion of chemicals from outside the cell-damaging enzymes (Suharni et al., 2008).

IV. CONCLUSION

Based on the results of the research that has been done, it can be concluded that the results of a phytochemical test in ethanol extract have the content of tannins, polyphenols, and alkaloids, whereas on the n-hexane extract only has alkaloid compound content. The results obtained from the influence of solvent used against the bacterial activity of *Bacillus subtilis* are that the N-hexane solvent is more effective in inhibiting the growth of *Bacillus subtilis* which is a group of Gram-positive bacteria. The results of the secondary metabolite leave Centella asiatica on n-hexane solvent indicating that n-hexane contains alkaloids. Alkaloid compounds are antibacterial, by disturbing the constituent components of peptidoglycan in bacterial cells so that the cell wall layer is not formed completely so that it can lead to cell death.

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