

## Antibacterial, antioxidant and total flavonoid content of *Adenostemma lavenia* (L.)



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**Abstract.** *Adenostemma lavenia* (L.) is one of the medicinal plants on Nias Island that is used to treat many symptoms including cough, fever and diarrhea. Empirical data of this plant suggests that these medicinal benefits can be attributed to its antibacterial and antioxidant properties, as well as the total flavonoids contained within it, but research on this is still limited. *Adenostemma lavenia* (L.) was extracted with ethanol, while the antibacterial properties were measured using the disc method. Antioxidant testing was conducted with the DPPH (1,1-diphenyl-2-picrylhydrazyl) inhibition method. Total flavonoids were assessed using a modified AlCl<sub>3</sub> method with a quercetin standard. Antibacterial test results of *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Propionibacterium acnes*, *Escherichia coli*, *Salmonella typhi*, *Bacillus cereus* and *Shigella sonnei* with mean inhibition were  $8.76 \pm 0.44$ ,  $8.13 \pm 0.72$ ,  $8.77 \pm 0.45$ ,  $6.47 \pm 0.54$ ,  $7.77 \pm 0.60$ ,  $7.33 \pm 0.44$ ,  $12.06 \pm 0.60$  respectively. IC<sub>50</sub> was  $59.844 \pm 3.36$  ppm. Total flavonoids were  $13.566 \pm 0.021$  mcg/g. The results of the antibacterial test of the ethanolic extract of *Adenostemma lavenia* (L.) were classified as moderate. The antioxidant activity and total flavonoids were in the high category, which can be attributed to the capture of free radicals to overcome disease.

**Keywords:** *Adenostemma lavenia*, Ethanol extract, Antibacterial, Antioxidant, Total flavonoid

### INTRODUCTION

Indonesia is one of the countries with the highest biodiversity in the world. It is estimated that there are thousands of species of medicinal plants in Indonesia that have the potential to be used in traditional medicine. Medicinal plants have been an integral part of Indonesia's cultural heritage for many years. The traditional use of medicinal plants has been practiced by local communities for generations. Studying medicinal plant diversity can provide insight into these traditional methods, as well as an understanding of how this knowledge can be integrated into modern medicine. Research on Indonesia's medicinal plant diversity can also contribute to the development of new drugs. Many medicinal plants have active compounds that have pharmacological potential. Further identification and research on these compounds could pave the way for the development of new, more innovative drugs [1]–[4].

*Adenostemma lavenia* (L.) or by its local name Sefe-sefe in Nias is a plant that grows in several tropical regions

including Indonesia. There is some empirical information on its use by the Nias people as a medicinal plant to treat coughs, fever and diarrhea. Based on the experience of the Nias people, *Adenostemma lavenia* (L.) has expectorant properties, which means it can help remove mucus from the respiratory tract. Therefore, local people use this plant as a traditional medicine to relieve coughs. People also use *Adenostemma lavenia* (L.) to treat fever and diarrhea. It is likely that this plant has antimicrobial or anti-inflammatory properties that can help relieve symptoms of fever and diarrhea. Several studies of *Adenostemma lavenia* (L.) have been conducted, namely the potential of this plant as an anti-nociceptive, anti-diarrheal, antipyretic, thrombolytic and anthelmintic. The full knowledge of the antibacterial effect of *Adenostemma lavenia* (L.) is still limited in its investigation [5]–[8].

The prevalence rate of infectious diseases such as dengue fever, diarrhea and thyroid fever still shows a high rate of occurrence, and in 2021 the number of recorded instances of infection reached two million cases in



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Indonesia. Several herbal plants in Indonesia have been studied for antimicrobial activity. Herbal plants are often used in traditional concoctions to treat various types of infections, including respiratory tract infections, skin infections, and gastrointestinal infections. Some pathogenic bacteria that cause infections include *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumonia*, *Propionibacterium acnes*, *Staphylococcus aureus*, *Salmonella typhi*, and *Shigella sonnei*. *Bacillus cereus* causes gastrointestinal infections which can cause diarrhea. *Escherichia coli* is a microbe in feces that can cause diarrhea, dysentery, urinary tract infections, and respiratory infections. *Klebsiella pneumonia* can cause pneumonia, which is an inflammation of the lungs. *Salmonella typhi* causes typhoid fever which also has symptoms of abdominal pain. *Shigella sonnei* is a bacterium that causes bloody diarrhea. *Propionibacterium acnes* and *Staphylococcus aureus* is a type of bacteria that causes acne. Several active compounds in plants such as flavonoids, tannins, and essential oils have been known to have the potential to fight infection-causing microorganisms [9]–[11]

Many medicinal plants that contain antioxidant compounds also have antibacterial effect. For example, flavonoids, tannins and polyphenols in plants can have antimicrobial properties, which help to fight the growth of pathogenic bacteria. Antioxidants play a role in protecting the body's cells from damage caused by free radicals. Free radicals can damage cells and cell membranes, which in turn can trigger various diseases including infections. By minimizing cell damage, antioxidants can support general health and the body's resistance to infections. Antioxidants can support the immune system and help prevent infections. The presence of antioxidants may improve the body's immune response to bacterial invasion and speed up recovery [12]–[14].

Research on the antibacterial activity of *Adenostemma lavenia* (L.) is still limited to insilico research, but its potential to fight against various kinds of bacteria is still very limited, therefore this research is important to investigate. Research on antioxidants and total flavonoids of *Adenostemma lavenia* (L.) also needs to be investigated because the results may vary based on plant variety factors originating from Nias Island.

## METHODOLOGY

### Plant Material

*Adenostemma lavenia* (L.) was obtained from Nias Islands, North Sumatra, Indonesia, and validated by the Indonesian Institute of Sciences (LIPI), Bogor, with parts of the plant being brought to the institute to ensure proper classification.

### Chemical Material

Ethanol (Merck®), Hydrochloric Acid (Merck®), Mayer, Bouchardart, Dragendorff, Methanol (Merck®), Distilled Water, Zinc (Merck®), Molish, Ferric Chloride (Merck®), Liebermann-Bouchard, Hydrochloric Acid (Merck®), Quercetin (Sigma®),

DPPH (Aldrich®), Potassium Acetate (Merck®), Aluminium Chloride (Merck®), Natrium Nitrat (Merck®), Mueller Hinton Agar (MHA), Mueller Hinton Broth (MHB), Nutrient Agar (NA), McFarland Standard.

### Bacterial Strains

Clinical isolates of *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumonia*, *Propionibacterium acnes*, *Salmonella typhi*, *Shigella sonnei*, and *Methicillin Resistant Staphylococcus aureus* (MRSA) were obtained from MERO foundation, Bali.

### Preparation of Adenostemma lavenia (L.)

Fresh *Adenostemma lavenia* (L.) leaves were dried at  $40^{\circ}\text{C} \pm 2^{\circ}\text{C}$  using a drying cabinet that had been modified, then extraction was carried out using the maceration method. Three hundred grams of *Adenostemma lavenia* (L.) folium simplicia was fed with 3 liters of pure ethanol solvent, and soaked for 3 days with periodical stirring. The macerate was then evaporated using a rotary evaporator until all the ethanol was evaporated [15], [16].

### Phytochemical Screening of Extracts

Alkaloid testing: several grams of *Adenostemma lavenia* (L.) extract were dissolved in 2N HCl until the solution was acidic. After that, the mixture was cooled to room temperature in a water bath and filtered by decantation until the residue and filtrate were separated. The filtrate obtained was divided into three parts and then reacted with Mayer, Bouchardart, and Dragendorff reagents. The alkaloid test was said to be positive; there was a white precipitate when dropped with Mayer's reagent and a brown solution when dropped with Bouchardart and Dragendorff [17]. Flavonoid test: several grams of *Adenostemma lavenia* (L.) extracts were dissolved in methanol, then added with magnesium powder, and HCl(p) was added. It was said to be positive if it showed an intense red reaction [18]. Saponin test: when some simplicial was dissolved in hot water and shaken vigorously, it was said to be positive if a stable foam formed; when dropped with HCl, the foam did not disappear. Tannin test: several grams of the extract were dropped with 5% FeCl<sub>3</sub>; it was said to be positive if the solution was blackish blue or brown [19]. Steroid/triterpenoid test: *A. lavenia* simplicia was dissolved using a non-polar solvent such as n-hexane for 2 hours and filtered by decantation, then the filtrate obtained was evaporated until it became a thick extract and dripped with Libermann-Burchard reagent. It was said to be a positive reaction if a green color was formed or a turquoise-blue color [20].

### Preparation of Quercetin Standard Solution

A total of 40 mg of quercetin was dissolved using a pure methanol solvent (100 ml). A concentration of 400 ppm was obtained, and then orientation was carried out to determine the maximum wavelength of quercetin [21].

### Standard Curve for the Determination of Flavonoids Content

The standard quercetin solution (400 ppm) was prepared at 150  $\mu$ L, 200  $\mu$ L, 250  $\mu$ L, 300  $\mu$ L, and 350  $\mu$ L into a flask with a capacity of 10 ml, then distilled water was added (125  $\mu$ L), 5% NaNO<sub>3</sub> (75  $\mu$ L) and stored in a dark place for 6 minutes, then AlCl<sub>3</sub> 10% (150  $\mu$ L) was added and let stand for 2 hours in a place without light. Finally, CH<sub>3</sub>COOK 1% (100  $\mu$ L) and methanol was added to obtain a concentration of 8 ppm, 10 ppm, 12 ppm, and 14 ppm. Absorbance was measured using UV-Visible spectrophotometry at wavelengths 460 nm [22].

### Determination of Total Flavonoid Content

The determination of total flavonoid content in *A. lavenia* (L.) was measured using a method based on a complex formation between AlCl<sub>3</sub> compounds and O<sub>2</sub> atoms at carbons 4 and 5 of the flavonoids. The first step began with storing for 6 minutes without relieving a solution obtained by mixing the extract of *A. lavenia* (L.) with 5% NaNO<sub>3</sub> (75  $\mu$ L). The addition of 10% AlCl<sub>3</sub> (150  $\mu$ L) was added to the solution after the storage process had reached the specified time. Then the mixture was homogenized and kept again without illumination for 120 minutes. CH<sub>3</sub>COOH 1% (100  $\mu$ L) was then added to the solvent and methanol was added to the marking line. It was then homogenized and its absorption measured using UV-visible spectrophotometry. Total flavonoid levels were calculated using the quercetin calibration curve and expressed in mg QE/g and repeated 3 times to obtain more accurate results. The result was expressed in the following formula to obtain the number of flavonoids. To obtain the number of flavonoids in the extract, the resulting absorption was poured into the following formula:

$$X = (A \cdot mo) / (Ao \cdot m)$$

The sample uptake was marked "A" while the standard uptake was marked "Ao". The mass of the extract (mg) was marked 'mo', while the mass of quercetin (mg) was marked 'm' [22].

### Determination Antioxidant Activity

To determine the antioxidant activity of *A. lavenia* (L.) the DPPH (1,1-diphenyl-2-picrihydrazyl) method was used, which is most popularly used because of its stability in the form of free radicals and it is simple to carry out. The DPPH method was measured using a spectrophotometer at room temperature (25°C) with a wavelength of 515 nm. Then a sample stock solution of 400 ppm was made by dissolving 20 mg of extract in 50 ml of pure methanol. Next, dilution was carried out using a pure methanol solvent by varying the concentration, namely 40 ppm, 80 ppm, 120 ppm, and 160 ppm for each sample [23].

A 400-ppm DPPH stock solution was also prepared. Twenty milligrams of DPPH solid was dissolved in 50 ml of pure methanol to create a DPPH stock solution. The next step was to create a comparison solution, or negative control solution, which contained 1 ml of 400

ppm DPPH solution diluted in 10 ml of pure methanol to achieve a concentration of 40 ppm. One milliliter of sample solution, 2 ml, 3 ml, 4 ml of sample solution, and 1 ml of DPPH solution, respectively, were produced for test samples. After being homogenized, the mixture was incubated for 30 minutes at room temperature (25 °C) in the dark until DPPH activity, which was measured at 515 nm using a UV-Visible spectrophotometry, showed a color shift. Every sample was created in triplicate [24], [25].

### Determination Antibacterial Activity

This antibacterial activity test begins with the process of creating a bacterial culture, namely by taking bacteria from the container. Then it was scratched using a special tool that was sterilized on solid media in the form of Nutrient Agar (NA) in an oblique position. Scratching is done 3 times so that the bacteria grows evenly on the media. If a clear mark appears, the name of the bacteria and the date of work is written down. Marking aims to avoid errors in the selection of test bacteria. The temperature is maintained according to the bacteria used in the incubator. Each bacterium has a period to be active so that it is ready for antibacterial activity testing. Even though it is active marked by the appearance of colonies, the media remains in the incubator until the required time [26]. Inoculation is done with all the tools that have been sterilized into a tube to be suspended which contains MHA (10 mL). The sample was homogenized and measured turbidity with a standard solution that has been prepared, namely McFarland. Then the growth was maintained in an incubator at 35±2°C for a day [27].

MHA was used as the media in this antibacterial activity test. This process begins with making MHA media (10 mL) on a sterilized Petri dish, then the samples are mounted and arranged neatly with paper discs on the surface of the media as many as 4 pieces for positive control, repetition 1, repetition 2 and repetition 3 and on paper discs, and tetracycline suspension (antibiotic) is used as a positive control (0.1 mL) and inoculation of test bacteria is carried out (0.1 mL). A petri dish was immediately closed and allowed to stand for half an hour. Growth was maintained by incubating it in an incubator at 35±2°C for a day. The final results appeared in a circle around the hole and the circumference of the circle was measured with a caliper in units of mm. The test was triple repeated to get more accurate data [28].

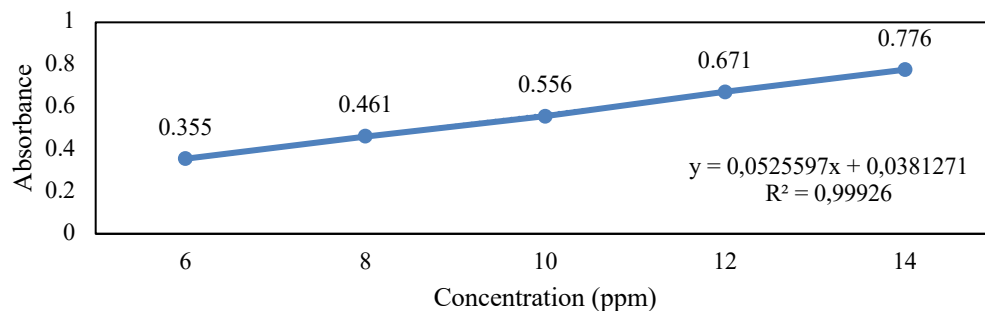
## RESULTS AND DISCUSSION

### Phytochemical Extract Screening Results

Based on the results of the phytochemical screening test from *A. lavenia* (L.) leaf simplicia, it was stated that it contains alkaloids, flavonoids, saponins, tannins, steroids and tripenoids. Flavonoids are a source of antioxidants which can reduce free radicals thereby minimizing the occurrence of oxidase. The screening results can be seen in Table 1.

**Table 1.** Phytochemical screening results

| Content       | Reactor              | Observations      | Results  |
|---------------|----------------------|-------------------|----------|
| Alkaloids     | Dragendroff          | Chocolate         | Positive |
|               | Bouchardart          | Chocolate         | Positive |
|               | Mayer                | White precipitate | Positive |
| Flavonoids    | Zn + HCl(p)          | Red               | Positive |
| Tannin        | FeCl <sub>3</sub> 5% | Blackish green    | Positive |
| Saponins      | Hot water + HCl      | There is foam     | Positive |
| Triterpenoids | Libermann Bouchard   | Turquoise Blue    | Positive |



**Figure 1.** Quercetin Calibration Curve

### Curve Calibration Results

The results of the quercetin calibration curve can be seen in Figure 1. It can be seen that the concentration of 6 ppm produces an absorbance of 0.355, 8 ppm produces an absorbance of 0.461, 10 ppm produces an absorbance of 0.556, 12 ppm produces an absorbance of 0.671 and 14 ppm produces an absorbance of 0.776. The regression line obtained from each concentration against absorbance is  $y = 0.0525597x + 0.0381271$  with  $r^2 = 0.99926$ .

### Total Flavonoid Content Results

The results obtained after determining the total flavonoid content in *A. lavenia* (L.) extract with various concentrations using the aluminum trioxide method are shown in Table 2. This shows that the concentration is directly proportional to the total flavonoid content; in short, the greater the concentration, the higher the flavonoid content.

The range of total flavonoid results from *A. lavenia* (L.) leaves with ethanol solvent was 2.19 - 5.43 mg.QE/g. The same results were carried out by [12] on *A. lavenia* (L.) leaves with ethanol solvent which amounted to 14.40 mg.QE/g. Flavonoids are the most abundant compounds contained in plants. Almost every part of the plant contains flavonoid compounds. From the total amount of food consumed by humans, almost 2/3 of phytonutrients are represented by plant foods. C6-C3-C6 is the core framework owned by flavonoid compounds. Inside the core skeleton is a heterocyclic ring wrapped by two aromatic rings. The two benzo-gamma-pyrone structures found in the core structure of flavonoid compounds are characteristic of heterocyclic rings that have six members with oxygen. There are various subclasses of

flavonoid compounds that have so far been studied, namely flavononols, flavones, flavones, isoflavones, flavonols and anthocyanins with different structures of each subclass and different functional characteristics [29].

There is a hydrogen atom (H) in the hydroxyl group (OH) in the flavonoid structure which makes it a natural antioxidant. Antioxidants donate to the hydrogen atom (H) of free radicals, the chain reaction process caused by free radicals can be stopped optimally so that cell damage caused by free radicals can be prevented. The leaves of *A. lavenia* (L.) possess antioxidant properties because there are flavonoid compounds contained within it [30], [31].

### Antioxidant Activity Results

The maximum wavelength results from DPPH (1,1-diphenyl-2-picrihydrazyl) were 515 nanometers with an absorbance of 1.054. The DPPH test results of *A. lavenia* (L.) extract showed antioxidant activity with an IC<sub>50</sub> value of 59.844 µg/ml. At a concentration of 40 ppm it showed an average absorbance of  $0.591 \pm 0.022$  with a % inhibition of 43.93. At a concentration of 80 ppm it showed an average absorbance of  $0.462 \pm 0.003$  with a % inhibition of 56.17. At a concentration of 120 ppm it showed an average absorbance of  $0.335 \pm 0.016$  with a % inhibition of 68.22. At a concentration of 160 ppm it showed an average absorbance of  $0.211 \pm 0.036$  with a % inhibition of 79.98. This shows that the concentration of the extract is inversely proportional to the DPPH absorbance, where the greater the concentration of the extract, the smaller the DPPH absorbance. The data presented can be seen in Table 3.

IC<sub>50</sub> of *A. lavenia* (L.) leaf extract is 59.844 ± 3.36 ppm. Research conducted by [32] on *A. lavenia* (L.) leaves obtained an IC<sub>50</sub> of 252.02 ± 3.23 ppm from the active compound Ent-11 $\alpha$ -Hydroxy-15-Oxo-Kaur-16-En-19-Oic acid isolated from *A. lavenia* (L.) O. Kuntze.

molecules that donate electrons to reactive free radicals and neutralize them, thus stopping the chain reaction before important molecules are damaged and preventing oxidative damage to target molecules [35], [36].

#### Antibacterial Activity Results

**Table 2.** Total flavonoid content of *A. Lavenia* (L.) extract

| Concentrations (ppm)<br>± SD | Volume (L) ± SI | SampleWeigh (g) ± | Total flavonoid levels<br>(mg.QE/g) ± SD |
|------------------------------|-----------------|-------------------|------------------------------------------|
| 5,494 ± 0,020                | 0.010 ± 0,000   | 0.025 ± 0,000     | 2.19 ± 0,009                             |
| 7,533 ± 0,013                | 0.010 ± 0,000   | 0.025 ± 0,000     | 3.01 ± 0,010                             |
| 10,465 ± 0,014               | 0.010 ± 0,000   | 0.025 ± 0,000     | 4.18 ± 0,005                             |
| 11,654 ± 0,017               | 0.010 ± 0,000   | 0.025 ± 0,000     | 4.66 ± 0,009                             |
| 13,566 ± 0,021               | 0.010 ± 0,000   | 0.025 ± 0,000     | 5.43 ± 0,008                             |

**Table 3.** Antioxidant Activity

| Concentration<br>(ppm) | Absorption    |               |               | Average ± SD  | % Inhibition |
|------------------------|---------------|---------------|---------------|---------------|--------------|
|                        | Replication 1 | Replication 2 | Replication 3 |               |              |
| 0                      | 0             | 0             | 0             | 0 ± 0,000     | 00,00        |
| 40                     | 0.604         | 0.603         | 0.565         | 0.591 ± 0,022 | 43.93        |
| 80                     | 0.469         | 0.465         | 0.462         | 0.462 ± 0,003 | 56.17        |
| 120                    | 0.328         | 0.324         | 0.354         | 0.335 ± 0,016 | 68.22        |
| 160                    | 0.191         | 0.188         | 0.253         | 0.211 ± 0,036 | 79.98        |

**Table 4.** Antibacterial Activity of *A. lavenia* (L.) extract

| Bacteria | Concentration<br>sample (%) | Inhibition (mm) |       |       | Average (mm)<br>±SD | Category |
|----------|-----------------------------|-----------------|-------|-------|---------------------|----------|
|          |                             | 1               | 2     | 3     |                     |          |
| SA       | 5                           | 8,26            | 9,12  | 8,90  | 8,76 ± 0,44         | Moderate |
| KP       | 5                           | 8,09            | 7,43  | 8,87  | 8,13 ± 0,72         | Moderate |
| PA       | 5                           | 8,72            | 9,25  | 8,34  | 8,77 ± 0,45         | Moderate |
| EC       | 5                           | 6,56            | 5,89  | 6,97  | 6,47 ± 0,54         | Moderate |
| ST       | 5                           | 7,88            | 7,12  | 8,32  | 7,77 ± 0,60         | Moderate |
| BC       | 5                           | 8,09            | 7,23  | 7,88  | 7,33 ± 0,44         | Moderate |
| SS       | 5                           | 12,73           | 11,57 | 11,88 | 12,06 ± 0,60        | Strong   |

**Table 5.** Positive Control Antibacterial Activity

| Positive Control<br>(K+) | Inhibition (mm) |          |          |          |        |          |        |
|--------------------------|-----------------|----------|----------|----------|--------|----------|--------|
|                          | SA              | KP       | PC       | EC       | ST     | BC       | SS     |
| Tetracycline             | 13,81           | 8,92     | 8,62     | 8,74     | 12,27  | 8,24     | 10,27  |
| Kategori                 | Strong          | Moderate | Moderate | Moderate | Strong | Moderate | Strong |

Free radicals are unpaired molecules that cause instability in the molecule. When the molecule is unpaired, it will become reactive. The molecule will seek and seize electrons from other molecules so that the molecule becomes stable and the molecule that has taken its electrons will become a new radical. If these free radicals do not bind to antioxidants, the process will continue, causing cell damage such as skin cell damage [33], [34]. Oxidative stress is caused by an imbalance between free radical production and antioxidant defense resulting in oxidative damage to biomolecules such as damage to cells. Antioxidants can reduce oxidative stress that contributes to near-life-threatening diseases and inflammatory diseases. Antioxidants are stable

The results attached from Table 4 are the ability of the extract from the leaves of *A. lavenia* (L.) to inhibit the growth of the bacteria used as the object of research. The research objects referred to in this study are *S. aureus* (SA), *K. pneumoniae* (KP), *P. acnes* (PA), *E. coli* (EC), *S. typhi* (ST), *B. cereus* (BC) and *S. sonnei* (SS). The average ability of *A. lavenia* (L.) extract with a concentration of 5% in inhibiting bacterial growth was 8.76 ± 0.44, 8.13 ± 0.72, 8.77 ± 0.45, 6.47 ± 0.54, 7.77 ± 0.60 and 7.33 ± 0.44 so that it was categorized as moderate except against *S. sonnei*. The inhibition on *S. sonnei* was 12.06 ± 0.60 so it was categorized as strong.

Table 5 attaches the potential of the positive control in inhibiting the bacteria used as the object of research. The ability to inhibit growth against bacteria *K. pneumoniae* (KP), *P. acnes* (PA), *E. coli* (EC) and *B. cereus* (BC) with a range of 8.24 - 8.92 mm so that it is categorized as moderate. While inhibition of growth against bacteria *S. aureus* (SA), *S. typhi* (ST) and *S. sonnei* (SS) with a range of 10.27 - 13.81 mm was categorized as strong.

The results of the antibacterial activity test showed that the leaves of *A. lavenia* (L) can inhibit bacterial growth with moderate category. The extraction of *A. lavenia* (L) leaves used a cold extraction method, namely maceration, because it was necessary to vary the extraction method in order to obtain better results in inhibiting bacterial growth. The results of tetracycline inhibition against test bacteria presented moderate results. This can be due to the type of bacteria used as the object of research which may have already been resistant to tetracycline [37]–[39].

Flavonoid compounds are effective agents in inhibiting bacterial growth. Flavonoids inhibit bacterial growth in many ways, for instance by damaging the bacterial cytoplasm which results in the integrity of the bacterial membrane being damaged so that the stability of the bacteria is disrupted. Flavonoids also interfere with the synthesis of nucleic acids from bacteria which causes bacterial proliferation to become less optimized [40].

A thick wall is characteristic of the identified *S. aureus* bacteria and a blue color is obtained in the gram staining method. *S. aureus* proliferates in the environment and spreads quite significantly because it is rich in amino acids. Being facultative (aerobic and anaerobic), it can live in different temperature conditions which makes it easier for it to multiply indefinitely. Its proclivity for continuous growth causes many clinical manifestations. In small amounts when it contaminates the body it has no significant impact at first. However, if it enters the bloodstream it can cause fatal damage. Transmission is quite easy from human to human through fomites. *S. aureus* causes many clinical manifestations such as urinary tract infections, lung infections and gastroenteritis depending on where it infects the body and the strain involved [41].

*K. pneumoniae* of the Enterobacteriaceae family was discovered in 1882 with a red cell wall in the gram staining method. Resistance to antibiotics has been high due to changes in the core genome of the organism. This bacterium causes many problems in the body such as pneumonia. This is due to contamination from hospitals or communities. In developed countries like the United States, the population infected with this bacteria is 3% - 5%. However, in developing countries such as those in Africa, the mortality is higher at around 15%. Pneumonia can be characterized when the patient has a cough, fever, shortness of breath and fever. The sputum infected by *K. pneumoniae* is characterized by a "currant jelly" appearance. As a result, there is intense inflammation accompanied by necrosis of the surrounding tissue [42].

Acne is a common problem in society, especially for women. *P. acnes* is a bacterium that accounts for 30% of the causes of acne pathology. Acne that appears also produces inflammation. This is because *P. acnes* presents itself by modulating keratinosid diffusion. *P. acnes* also affects the prostate, making it one of the bacteria involved in the pathophysiology of prostate cancer. Despite its adverse effects, *Propionibacterium acnes* also has an important role for the body as it limits bacterial colonies on the skin by interacting with other skin bacteria such as *Staphylococcus* and *Pseudomonas* species [43].

*E. coli* is a common microbiota in the human gut that is colored red in the gram staining method. However, in too large an amount it will become a pathogen that is harmful to the body. Diarrhea and dysentery are the most common diseases caused by uncontrolled *E. coli* colonies. This uncontrolled number of colonies is caused by food or drink consumed because it has been contaminated with *E. coli*. Urinary tract infections (UTIs) are caused by *E. coli* as it travels up the urinary tract and women are more prone to UTIs than men due to the close proximity of the urethra to the anus. However, *E. coli* can ascend into the human respiratory tract and cause pneumonia [44].

Besides *E. coli*, *B. cereus* causes gastrointestinal diseases such as diarrhea. Toxins secreted by these bacteria cause nausea, vomiting and can even cause fatal infections such as respiratory tract infections and eye infections. There are rarely reports of deaths caused by *B. cereus* although humans are susceptible to infection by this bacterium. *B. cereus* primarily contaminates food, and when this contaminated food is consumed, *B. cereus* immediately secretes its toxins and causes food poisoning [45].

Typhoid fever has been a long-standing disease throughout the world, especially in developing countries such as Indonesia. The bacteria responsible for typhoid fever is *S. typhi*. Many efforts have been made by humans over the years to prevent this disease from arising again such as the creation of vaccines that have been made by Almroth Wright. However, typhoid remains one of the more dangerous diseases affecting developing countries around the world. Patients experience abdominal pain, nausea and vomiting during the course of the disease. In addition to abdominal symptoms, fever and influenza are also present as a sign that the patient has typhoid fever [46].

*S. sonnei* is the leading cause of death for people of all ages, resulting in 212,428 deaths per year. Bloody diarrhea is a disease caused by *S. sonnei*. In developed countries, *S. sonnei* has higher mortality rate than in developing countries. This may be because people in developing countries have a higher immune system due to exposure to water contaminated with *P. shigelloides* so that their bodies have specific antigens that are identical to *S. sonnei*. The complications that accompany patients affected by *S. sonnei* include seizures, arthralgia and rectal prolapse. But in other cases, it can be life-

threatening due to sepsis and hemolytic-uremic syndrome (HUS) [47].

Coughing is the most common symptom in people affected by this disease around the world. Approximately 30 million people see a doctor and 40% of them see a lung specialist. Coughing is the body's response to fight pathogens that reside in the body, especially in the respiratory tract. The severity of a disease can be seen in how long the cough occurs. Acute is less than 1 week, subacute is 1-8 weeks and chronic is more than 8 weeks. The most common source is bacterial infection. These bacterial infections cause many respiratory diseases. *S. aureus* can cause acute sinusitis characterized by chronic stones. *K. pneumoniae* causes pneumonia characterized by a prolonged cough. Further examination of this cough is necessary as it is a sign of disease. With the right diagnosis, the patient's illness can be treated quickly and appropriately [48], [49].

Inflammation of the respiratory tract caused by bacteria, triggers receptors at that location so that the body responds by causing a cough. Not only that, the body also responds by producing and releasing chemical compounds called setokines (proteinsignals) and histamine. The release of these compounds causes an itchy sensation that results in coughing [50].

Flavonoid compounds can stop enzymes that trigger the production of compounds called cyclooxygenase (COX) and lipoxygenase (LOX). When the production of these compounds is inhibited, inflammation which is the main contributing factor in triggering a cough can be reduced so that the frequency of coughing decreases [51]. Flavonoids act as receptor antagonists by interacting with flavonoid compounds with inflammation-triggering receptors so that histamine and cytokines are not released, thus resulting in the sensitivity of the receptor to the stimulus being reduced. In addition, histamine and cytokine compounds can be reduced by modulating cellular signaling pathways that are responsible for the release of histamine and cytokine compounds [52].

## CONCLUSION

*A. lavenia* (L) has antibacterial properties against the growth of *S. aureus*, *K. pneumoniae*, *P. acnes*, *E. coli*, *S. typhi*, *B. cereus* with moderate category and *S. sonnei* with strong category. However, *A. lavenia* (L) has high antioxidant activity due to the total number of flavonoids, thus this supports empirical data on the use of this plant as a cough, fever and diarrhea medicine.

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