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Effect of crude extract of *Acalypha hispida* and *A. indica* leaves on the growth of *Staphylococcus aureus* bacteria in vitro

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Abstract. *Kartika RPT, Purwoko T, Sunarto. 2018. Effect of crude extract of* Acalypha hispida *and* A. indica *leaves on the growth of* Staphylococcus aureus *bacteria in vitro. Biofarmasi J Nat Prod Biochem 16: 29-35. Staphylococcus aureus* is one of the bacteria causing infectious diseases. One alternative to dealing with the infectious diseases caused by *S. aureus* is using natural plant materials, often called traditional medicine. *Ekor kucing* (*Acalypha hispida* Brum.f.) and *anting-anting* (*Acalypha indica* L.) plants are typical herbs producing useful chemical substances in medicine, such as saponin, tannin, flavonoid, acalyphin, and volatile oil functioning as an antibacterial agent, among others. The aims of this research are to (i) to examine the inhibitory potential of crude extracts of *A. hispida*, and *A. indica* leaves against the growth of *S. aureus* bacteria, and (ii) to find out the comparison of potential bacterial inhibitory activity between crude extracts of *A. hispida* and *A. indica* leaves. The bacterial inhibition potential testing was conducted using Poisoned Food Techniques (PFT) method. Crude extracting with 70% ethanol solvent was done on *A. hispida* and *A. indica* leaf and the extract was dissolved serially using aquadest solvent to obtain the various extract concentrations: 0,5%, 0.4%, 0.3%, 0.2%, 0.1% and 0% (control). Then, the colony width was measured using the gravimetry method. The Completely Random Design was used to analyze the data result of this experiment. The crude extract of *A. hispida* and *A. indica* leaves at all concentrations can inhibit the *S. aureus* bacteria growth. The crude extract of *A. hispida* is more able to pursue the bacterium of *S. aureus* than the crude extract of *A. indica*.

Keywords: *Acalypha hispida, Acalypha indica*, antibacterial, *Staphylococcus aureus*

INTRODUCTION

Infectious diseases are a high cause of morbidity and mortality worldwide, especially in developing countries such as Indonesia (Guntur 2007). Infectious diseases can be transmitted from one organism to another by various microorganisms, one of which is bacteria (Gibson 1996). It was reported from Guntur's research (2007) that the factors causing infection were mostly Gram-positive and negative bacteria, including the genus *Staphylococcus*.

Staphylococcus aureus is one of the bacteria that cause various infections from the genus *Staphylococcus*. These pathogenic bacteria cause skin, lower respiratory tract, digestive tract infections, bones, joints, mucous membranes, and eczema, causing acne, boils, and pneumonia. The *S. aureus* is a normal microflora on the skin, but when there is an increase in the number, this bacterium can cause infection (Taukhid et al. 2002).

One alternative to treat infectious diseases caused by *S. aureus* is to use natural plant materials or traditional medicines (Lestari et al. 2006). The soaring prices of synthetic drugs and their side effects on health have increased the community's use of traditional medicines by utilizing the natural resources around them. The advantages of using natural ingredients include being more environmentally friendly, easy to obtain, inexpensive, and having relatively smaller side effects when used correctly and appropriately, both in the right dose, time of use, method of use, the accuracy of material selection, and accuracy of selection of traditional medicines for indications certain (Nugroho et al. 1999).

Ekor kucing (*Acalypha hispida* Brum.f.) and *anting-anting* (*Acalypha indica* L.) plants are a type of herb that produces chemical compounds that are useful in medicine, including saponins, tannins, flavonoids, acalyphin, and essential oils, one of which functions as an antibacterial (Hutapea 1993; Villes and Reese 1995; Akintola and Ande 2006). In traditional medicine, the efficacy of this *A. hispida* plant is as a hemostatic drug, coughing up blood, treatment of white patches on the skin, burns, intestinal inflammation, intestinal worms, vomiting blood, efficacious as a wound cover, and laxative urine (Dalimarta 1991). The *A. hispida* is an ornamental plant usually found in house yards. At the same time, *A. indica* is a weed commonly found growing wild on roadsides, grass fields, or mountain slopes. In the Malay peninsula, the leaves of *A. indica* are used for laxatives and eye pain medications (Hutapea 1993). The benefit of this plant as traditional medicine is an added value to improve the function of *Acalypha* so that it is not just a weed or ornamental plant. Cahyanti's research (2004) shows that the root and shoot extract of *A. indica* can inhibit growth and reduce the chlorophyll content of purslane (*Portulaca oleracea* L.).

Besides being easy to obtain and useful for natural herbicides, it turns out that secondary metabolites of plants *A. hispida* and *A. indica* can be used as ingredients for traditional medicines, so it is suspected that they have potential as alternative materials for controlling bacterial

diseases. So far, there have been many studies to determine the antibacterial power of various species of the genus *Acalypha* against various kinds of bacteria. In addition, although *A. hispida* and *A. indica* plants are widely used as traditional medicinal plants for various diseases, it is not widely known how much antibacterial effectiveness these two plants inhibit or kill bacteria that cause disease.

Therefore, it is necessary to compare the antibacterial power of crude extracts of *A. indica,* and *A. hispida* leaves against *S. aureus* bacteria to find out which is more effective in inhibiting or killing *S. aureus* bacteria and to find alternative materials for controlling bacterial diseases, especially those caused by *S. aureus* bacteria.

The aims of this research are to (i) to examine the inhibitory potential of crude extracts of *A. hispida,* and *A. indica* leaves against the growth of *S. aureus* bacteria, and (ii) to find out the comparison of potential bacterial inhibitory activity between crude extracts of *A. hispida* and *A. indica* leaves.

MATERIALS AND METHODS

Materials

The ingredients were leaves of *A. hispida,* and *A. indica* obtained from B2P2TO2T (*Balai Besar Penelitian dan Pengembangan Tanaman Obat dan Obat Tradisional*) Tawangmangu, Karanganyar, Central Java, Indonesia, while pure culture of *S. aureus* bacteria obtained from the Faculty of Pharmacy, Universitas Setia Budi, Surakarta, Central Java, Indonesia.

Research procedure

Sterilization of tools and materials

Petri dishes, test tubes, Erlenmeyer, NA media, and all tools and materials (except *A. hispida* and *A. indica* leaf powder extract) to be used were sterilized in an autoclave for 30 minutes by setting the pressure at 1 atm (15 dyne/cm) and a temperature of 121°C after previously being washed, dried and wrapped in paper.

Production of bacterial suspension stock

The bacterial suspension was made to increase the stock by inoculating 1 ose of pure culture into 5 mL of NA media, then incubating at 37°C for 24 hours in an incubator.

Leaf powder making

The plant parts used for extracting were the leaves of *A. hispida* and *A. indica*. from each plant, healthy and fresh leaves with optimum growth with uniform age and size were selected, then washed with running water to remove dust and other impurities. After that, the leaves are drained, then dried in indirect sunlight by covering them with a black cloth. The purpose of drying is to obtain an extract that is not easily damaged so that it can be stored longer. The dried leaves are made into powder with an electric blender, then stored in a closed container. The dried leaf powder will be used to make the extract.

Extract preparation

Two hundred and fifty grams of dried *A. hispida* and *A. indica* leaf powders were soaked in 500 mL of 70% ethanol and then shaken for 24 hours at 120 rpm. The extract was filtered using a Buchner funnel, and the filtrate was taken. Furthermore, the filtrate and solvent that are still mixed are dried with a rotary evaporator at a maximum temperature of 50°C to obtain a dry extract. The extract obtained was serially diluted with distilled water to obtain extract concentrations of 0.5%, 0.4%, 0.3%, 0.2%, 0.1% and 0% (control) (Modification of Sunarto et al. 1999; Ogbebor and Adekunle 2005).

Bacterial inoculum preparation

Pure cultures of *S. aureus* were rejuvenated in NA media. The liquid NA media (at 50°C), mixed with one bacterial loop in a test tube, was vortexed for the growth of bacteria to be evenly distributed. Then it was poured into a petri dish and incubated for 48 hours at 37°C. For the antibacterial test, the bacteria grown were molded with a cork drill with a diameter of 0.5 cm and a thickness of 1-2 mm, then inoculated right in the middle of the agar media.

Inhibition potential test

First, a comparative test of negative control (sterile aquades) and positive control (1% ethanol) was conducted to determine any inhibitory activity of ethanol solvent in NA media. Then *S. aureus* bacteria inoculum was inoculated on each control and incubated for 7 x 24 hours. The results of bacterial cultures were photographed to compare with the growth of bacteria tested with the extract. Next, 6 g of NA media plus 2 g of agar was dissolved in 300 mL of sterile distilled water and boiled at 100°C. After boiling, the media was put into test tubes for as much as 10 ml each and then autoclaved for 15 minutes at 121°C, with a pressure of 1 atm.

Furthermore, the media was cooled to a temperature of 50°C, mixed with the extract, and poured into a petri dish aseptically. Bacterial mold was inoculated right in the middle of the test medium aseptically. After the bacterial culture was about 7 days old, the diameter of the colony was calculated. The process of calculating the colony diameter for each concentration started after bacterial growth; the results were then compared with the control. The treatment was repeated 3 times for each concentration (Modification of Sunarto et al. 1999).

Determination of bacterial colony growth area

The colony area was measured by the gravimetric method by making a replica of the growth using paper. However, before making a replica, the paper used to make the replica is weighed, and its area is measured so that the paper's initial weight and the original paper's area are known. After that, a replica of the growth of bacterial colonies was made, and the weight of the replica was weighed. The formula can calculate the width of bacterial colonies:

$$\text{Bacterial colony width} = \frac{\text{weight of replica x initial width of paper}}{\text{initial weight}}$$

The choice of this calculation method is based on the results of the study that the area of bacterial colony growth is not completely circular, so it has different radii from each side.

Percentage determination

The percentage of growth inhibition of *S. aureus* bacteria is calculated by the following formula (Ogbebor and Adekunle 2005):

% inhibition = [(control-treatment) / control] x 100 %

Data collection technique

The qualitative research data shows the size of the bacterial culture colony diameter (expressed in mm^2) at the five levels of extract concentration (expressed in %). The experimental design used a factorial Completely Randomized Design (CRD), with the following conditions:

E: Extract 70% ethanol *A. hispida*, and extract 70% ethanol *A. indica*

K: Concentration, 6 levels, i.e.: 0%(control), 0.1%, 0.2%, 0.3%, 0.4%, 0.5%

So, there were 14 combinations, each treatment combination with 3 replications.

Data analysis

The data in this study was quantitative data showing the size of the growth area of *S. aureus* bacterial colonies (expressed in mm^2). First, the data was analyzed by General Linear Model (GLM) to determine whether there were differences in each treatment. Then, the data was tested with Tamhane at a 5% level test to compare the results.

RESULTS AND DISCUSSION

The *A. hispida* plants are a type of herb that produces chemical compounds useful in medicine, including saponins, tannins, flavonoids, acalyphin and essential oils which function as an antibacterial (Dalimartha 1991; Hutapea 1993; Akintola and Ande 2006). Besides being easy to obtain, secondary metabolites from *Acalypha* can be used as a potential source of traditional medicinal ingredients, so it is suspected that *Acalypha* also has potential as an alternative material for controlling bacterial diseases. Therefore, the benefit of this plant as traditional medicine is an added value to improve the function of *Acalypha* so that it is not just a weed or ornamental plant.

So far, although *A. hispida* and *A. indica* plants are widely used as traditional medicinal plants for bacterial diseases, the amount of antibacterial activity of these plants is not widely known, so it is necessary to do a comparative test of the antibacterial power of leaf crude extract of *A. indica* and *A. hispida* against changes in *S. aureus* bacteria to find out which is more effective in inhibiting or killing *S. aureus* bacteria.

Inhibitory potential test

The potential inhibitory test of leaves crude extracts of *A. indica* and *A. hispida* on the growth of *S. aureus* was carried out using the Poisoned Food Techniques (PFT) method, which aims to examine the inhibitory potential and compare the effect of leaves crude extracts of *A. indica* and *A. hispida* on the growth of *S. aureus*. In this test, crude leaf extracts of *A. indica* and *A. hispida* were obtained from the extraction using 70% ethanol and then dissolved and diluted with distilled water to obtain concentrations of 0.5%, 0.4%, 0.3%, 0, 2%, and 0.1%. The choice of solvent for ethanol and aquadest is because they both have similar polarities. Ethanol is a polar solvent, and this solution obtains the chemical content of *A. indica* and *A. hispida* leaves which are also polar, such as tannins, saponins, volatile oils, acalyphins, and flavonoids. So that these compounds can be mutually soluble with the principle of "like dissolved like," which means that a polar compound will dissolve in a polar solvent and vice versa (Noerono 1994).

Using distilled water as a solvent was based on preliminary research, which showed that aquadest did not affect bacterial growth, so only the leaf extracts of *A. indica* and *A. hispida* were affected.

Table 1 shows the test results of leaves crude extract of *A. indica* at various concentration levels in the form of the growth area of *S. aureus* bacteria. From Table 1, it is known that crude extract of *A. indica* leaves can inhibit the growth of *S. aureus* with an average bacterial growth colony area ranging from 42,150-54,810 mm^2. While in the control plate, the average bacterial growth area was around 557,340 mm^2. While the crude extract of *A. hispida* leaves is presented in Table 2. In the administration of *A. hispida* leaf extract, the average area was only around 1000-4170 mm^2 (Table 2).

In this study, there was a significant broad difference in the administration of the two extracts compared to the control. It is supported by the statistical analysis of the Tamhane follow-up test at a 5% level, where there is a significant difference between the control and the test concentration. However, for each test concentration for a crude extract of *A. indica* leaf, there was no significant difference, as well as for each test concentration for the crude extract of *A. hispida* leaf. Perhaps this is due to the concentration range not being too far away.

All test concentrations of the two extracts showed the potential for inhibition of bacterial growth. The similarity of the effects given by the two extracts was closely related to the chemical content of the extracted leaves in each leaf extract. This similarity is thought to be due to the similarity of the compounds with antibacterial properties, such as tannins, saponins, flavonoids, essential oils, and acalyphins (Dalimartha 1991; Hutapea 1993; Villes and Reese 1995; Akintola and Ande 2006).

From the two extracts tested, it was generally seen that the crude extract of *A. hispida* leaves was more able to inhibit the growth of *S. aureus* bacteria. This difference is thought to be due to the different concentrations of the active ingredients in the two extracts.

The histogram above shows that the area of bacterial colony growth with *A. indica* leaf extract and *A. hispida* leaf crude extract was significantly different; namely, the bacterial growth area with *A. indica* leaf extract was higher than *A. hispida* leaf extract. It indicates that the leaves of *A. hispida* were more effective in inhibiting the growth of *S. aureus* bacteria. This difference is due to the levels of chemical compounds in the two plants. The percentage of inhibition of growth of *S. aureus* bacterial colonies from Tables 1 and 2 are presented in Table 3.

Table 1. Average area (mm^2) of colony growth of *Staphylococcus aureus* bacteria by administration of leaves crude extract of *A. indica* at five concentrations (%) compared to control

Concentrations (%)	Growth area (mm^2)
0%	557,340a
0.1%	54,810b
0.2%	47,500b
0.3%	46,520b
0.4%	44,040b
0.5%	42,150b

Note: The numbers followed by the same letter are not significantly different from the 5% Tamhane test

Table 2. Average area (mm^2) of colony growth of *Staphylococcus aureus* bacteria by administration of leaves crude extract of *A.hispida* at five concentrations (%) compared to control

Concentrations (%)	Growth area (mm^2)
0%	557,340a
0.1%	4,170b
0.2%	2,580b
0.3%	2,550b
0.4%	2,040b
0.5%	1,000b

Note: The numbers followed by the same letter are not significantly different from the 5% Tamhane test

Table 3. Percentage of inhibition of bacterial colony growth of *Staphylococcus aureus* (%) by administering a crude extract of *A. indica* and *A. hispida* leaves at various concentration levels (%)

Concentration (%)	Percentage of inhibition (%)	
	Extract of *A. indica* leaf	Extract of *A. hispida* leaf
0 (Control)	0.000	0.000
0.1	90.171	99.233
0.2	91.454	99.528
0.3	91.475	99.540
0.4	91.621	99.631
0.5	92.420	99.822

Based on Table 3, the difference between the crude extract of *A. indica* and *A. hispida* leaves can be seen. The percentage of inhibition of the leaf extract of *A. hispida* had a higher inhibitory value than that of *A. indica*. The administration of crude leaf extract of *A. indica* and leaf crude extract of *A. hispida* with a concentration of 0.5% had the highest percentage of inhibition. In comparison, a concentration of 0.1% had the lowest percentage of inhibition. From Table 3, it can be seen that the higher the concentration of the extract, the greater the inhibitory power of bacteria. Sunarto et al. (1999) stated that increasing the extract's concentration will increase the percentage of growth inhibition. It is because the concentration of chemical compounds is also getting higher.

Staphylococcus aureus isolates

The *S. aureus* is a Gram-positive, immobile, found singly, in pairs, short chains, or clusters. It is the cause of various kinds of infections such as acne, boils, pneumonia, and abscesses on any part of the body (Fardiaz 1993; Schegel 1994; Pratama 2005).

From the results of observations, colonies of *S. aureus* were obtained, as shown in Figures 1-2.

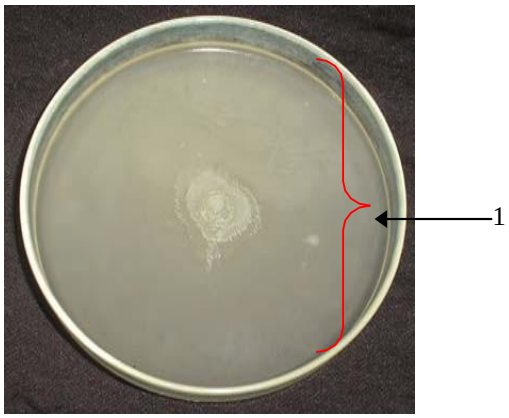

Figure 1. *Staphylococcus aureus* colonies on NA media (7 days incubation). Note: 1. Colonies of *S. aureus* bacteria

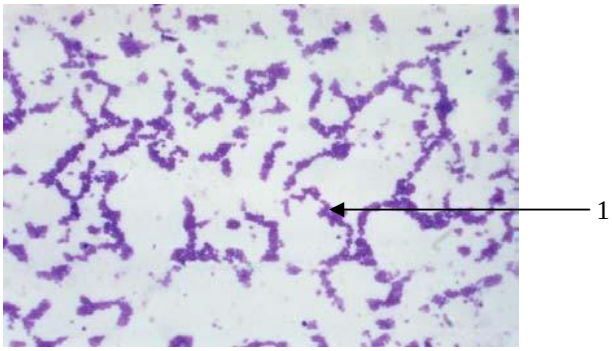

Figure 2. Colony of *Staphylococcus aureus* with gram stain, magnification 100x. Note: 1. Colonies of *S. aureus* bacteria

With gram staining, *S. aureus* appears purple because these bacteria are resistant to alcohol during staining, so they bind to the first paint and do not bind to the second paint. By observing using a visible light microscope, *S. aureus* bacteria are spherical and form clusters like grapes (Syahrurachman et al. 1993; Pratama 2005).

Growth inhibition of *Staphylococcus aureus*

The two types of extracts generally showed that all concentrations of crude extract of *A. indica* and *A. hispida* leaves could only inhibit the growth of bacterial colonies but did not kill bacteria. It can be seen from the difference in the area of bacterial colonies. In general, increasing the extract's concentration will increase the growth inhibition percentage, although the response is not always linear (Sunarto et al. 1999). The condition caused this increase in inhibitory power that the amount of extract concentration would affect the amount of chemical content, which played a role in inhibiting the growth of *S. aureus* bacteria.

The two extracts tested found that the crude extract of *A. hispida* leaf was more able to inhibit the growth of *S. aureus* bacteria than the crude extract of the *A. indica* leaf. This difference is thought to be due to the different concentrations of the active ingredients in the two extracts. In addition, it is influenced by environmental factors such as temperature, the altitude where it grows, humidity of air and soil, light intensity, and availability of water. A good growing environment for a plant will also improve its growth so that the metabolism of secondary metabolites in the plant is also more optimal. For the environment where plants live, *A. hispida* plants are usually found living in the highlands, while *A. indica* plants mostly live in the lowlands (Dalimartha 1991; Hutapea 1993).

Each chemical compound agent has its mechanism to inhibit or kill bacteria. However, one of the weaknesses of natural extracts for antimicrobial substances is the inconsistent effect because the type and content of the active ingredients obtained from each extraction are not always the same, depending on the extraction method, age, the part of the plant organ extracted and the environment in which the plant grows. In addition, the process of inhibiting bacterial growth can be carried out by all types of active ingredients in the extract, not depending on only one type (Sunarto et al. 1999).

The type and content of the active ingredients of the leaf extract are also determined by the source of the extract and the age of the source of the extract. The part of the plant used for extracting is the leaves because it contains tannins, saponins, flavonoids, essential oils, and acalyphin. The leaves of older plants have relatively higher metabolites than younger ones (Hutapea 1993).

Tannins have properties as a chelating agent with a spasmolytic effect, which can shrink cell walls or cell membranes so that they interfere with the permeability of the cell itself. Due to the disruption of permeability, cells cannot carry out living activities, so their growth is inhibited or even dies (Harborne 1996). According to Masduki (1996), tannins also have antibacterial power by precipitation of protein because it is suspected that tannins have the same effect as phenolic compounds. Saponins are secondary metabolites belonging to the glycoside group (Robinson 1991). Saponin compounds damage the cytoplasmic membrane and kill cells (Assani 1994). Flavonoid compounds have a mechanism of action: denaturing bacterial cell proteases and damaging cell membranes beyond repair (Naim 2003; Subroto and Saputro 2006). Essential oils can inhibit the growth or kill bacteria by interfering with the process of forming cell walls, or cell walls are not formed or formed imperfectly (Ajizah 2004).

Acalyphin is an active ingredient found in the leaves of *A. hispida* which has a cyanide chain (HCN) toxic, so it is suspected that this compound is the most active in killing bacteria. The content of this compound in *A. indica* leaf is 0.03%. While in the leaf of *A. hispida*, it is not yet known, if seen in Table 2, it is known that the leaf extract of *A. hispida* is more inhibiting than the leaf of *A. indica*, so it is suspected that the acalyphin content in the leaf of *A. hispida* is greater than that of the leaf of *A. indica* (Lenny 2006).

The solvent used to make the extract also affects the levels of chemical compounds. This test uses ethanol as a solvent because it is a universal polar solvent that can dissolve polar compounds. According to Cowan (1999), ethanol can dissolve active compounds such as tannins, polyphenols, polyacytelin, terpenoids, sterols, alkaloids, essential oils, volatiles, curcumin, anthraquinones, flavonoids, steroids, resins, and chlorophyll, and improve the stability of soluble substances, can inhibit enzyme activity, and is effective in producing an optimal amount of active ingredient, where the free material slightly enters the extracting fluid. Still, it does not cause swelling of the cell membrane (Voight 1995).

Meanwhile, water is a dilution solvent because the ethanol extract is easily soluble in water due to its polarity. Water can dissolve alkaloids, saponins, terpenoids, volatile oils, glycosides, tannins, sugars, gums, starch, proteins, mucus, enzymes, waxes, fats, pectin, dyes, and organic acids (Cowan 1999).

Inhibition mechanism

The compounds thought to be antibacterial in the crude extract of the leaves of *A. indica* and *A. hispida* were tannins, saponins, essential oils, flavonoids, and acalyphin. According to Assani (1994), tannins have antibacterial power by precipitation of bacterial cell proteins so that bacterial protein synthesis will be disrupted or lead to enzyme inactivation reactions and destruction or inactivation of genetic material functions. Saponins are surface-active compounds that are antibacterial by lowering the surface tension that can bind lipids so that antibacterial compounds can enter through the membrane and will damage the cytoplasmic membrane and kill cells. Finally, essential oils can inhibit the growth or kill bacteria by interfering with forming a membrane or cell wall so that the membrane or cell wall is not formed or is formed imperfectly (Ajizah 2004).

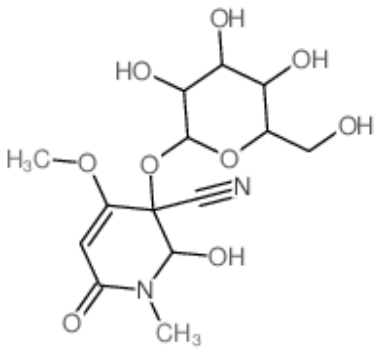

Figure 3. Chemical structure of acalyphin

According to Masduki (1996), flavonoid compounds are antibacterial with their mechanism of action is to damage cell membranes beyond repair and degrade bacterial cell proteins. In addition, flavonoids are thought to be able to inhibit the growth of *S. aureus* bacteria because of the phenolic effect of flavonoids. While the most active compound from the leaves of *A. indica* and *A. hispida* is acalyphin, this compound presented in Figure 3.

Although only 0.03% in *A. indica* leaves, acalyphin has a cyanide chain (HCN) which is toxic, so it is suspected that cyanide enters the cell structure of *S. aureus* and poisons it so that it interferes with metabolic processes in cells and even kills cells (Lenny 2006). According to Jawetz et al. (2001), inhibited bacterial growth or death due to an antibacterial substance can be caused by inhibition of the cell wall, cell membrane function, protein synthesis, or nucleic acid synthesis.

Antibacterial compounds that diffuse into the agar media can cause inhibition of cell wall formation so that cells are only limited by a thin membrane and can be lysed (Madigan and Martinko 1997). Antimicrobial compounds will damage the cell wall's structure by inhibiting the cell wall's growth. The mechanism of the destruction of the cell wall is by lysing the cell membrane, which is the structure of the cell wall. Fessenden and Fessenden (1999) say that the cell membrane is formed from embedded proteins and fused with a double layer (bilayer) of phosphoglyceride molecules, with the hydrophobic ends facing in and the hydrophilic ends facing out. The function of these proteins is to allow the entry of water, ions, and compounds. Compounds with high concentrations will diffuse and be captured by hydrophilic sensors. Hydrophilic components will bind to compound molecules which eventually causes the lysis of the entire lipoprotein membrane to inhibit the growth of the cell wall. If the cell wall, which is a protective barrier for cells, is damaged, it will cause the death of microbial cells.

Antimicrobial compounds, according to Pelczar and Chan (1988), will work to affect the permeability of the cell cytoplasmic membrane, where the cytoplasm functions to regulate the entry and exit of substances between cells and the outside environment. The cytoplasmic membrane is also the site of enzyme reactions. The *S. aureus* bacteria are Gram-positive bacteria with a structure with lots of peptidoglycans and relatively little lipid, so the bacterial cell wall becomes hydrated during treatment with ethanol. Peptidoglycan plays a role in hardness and gives cell shape. The ethanolic extracts of the leaves of *A. hispida* and *A. indica* will easily dissolve in water because they are polar. Therefore, the compounds in the leaves of the two plants are generally polar. At the same time, S. aureus has teichoic and trichuronic acids, which are polymers that are soluble in water (in peptidoglycan) so that polar compounds easily penetrate the walls.

Ethanol is relatively polar, so the compound extracted is relatively polar. The polarity of these compounds causes the compounds to more easily penetrate the cell walls of Gram-positive bacteria (Hugo and Russell 1998; Gopalakrishnan et al. 2000). Therefore, the destruction of cytoplasmic membrane permeability and protein will inhibit the growth of *S. aureus* bacteria.

Inhibition also occurs in the process of protein synthesis or the synthesis of nucleic acids. Therefore, it is suspected that tannin compounds have an inactivation mechanism of genetic material. According to Pelczar and Chan (1982), the process of inhibition of protein synthesis occurs in the transcription process and the transition of genetic material, where there is a translation error, so that the amino acids produced are misplaced in the peptide chain and produce non-functioning proteins.

Inhibition by antimicrobial compounds can also occur against enzymes that work in cells. According to Pelczar and Chan (1988), enzymes are potential targets for the work of an antimicrobial agent. Furthermore, it is stated that the inhibition of antimicrobial substances is generally irreversible; that is, a change occurs, so the enzyme is not active. The inhibition or cessation of enzyme activity can cause the mechanism of enzyme work to be disrupted. For example, *S. aureus* has a coagulase enzyme that can clot plasma supplemented with oxalate or in the presence of a factor or serum. It also has the enzyme catalase, which can convert hydrogen peroxide into water and oxygen. With these enzymes, *S. aureus* will produce an enterotoxin, leukocidin, exfoliatin, and lysostaphin toxins which can cause red blood cell lysis and cause infection. Disruption of the working enzyme mechanism will affect the formation of bacterial cells and bacterial growth.

The combined activity of several antibacterial compounds can be more effective than the work of each compound (Jawetz et al. 2001). But it is also possible that antibacterial compounds with the largest percentage can affect the effectiveness of their work. On the other hand, the combined working activity of several antibacterial compounds can also be less effective than the work of each compound (Kusumaningrum 2002).

Judging from the antibacterial activity of each chemical compound in the leaves of *A. indica* and *A. hispida*, the inhibition of the growth of *S. aureus* bacteria may be carried out by all chemical compounds or only one chemical compound. However, this can not be ascertained because it is unclear how many levels of each chemical compound are contained in the leaves of *A. indica* and *A. hispida.*

In conclusion, the crude extract of the leaves of *A. hispida* at a concentration of 0.1%, 0.2%, 0.3%, 0.4%, and 0.5% were able to inhibit the growth of *S. aureus* bacteria. Crude extract of the leaves of *A. hispida* at a concentration of 0.1%, 0.2%, 0.3%, 0.4%, and 0.5% were able to inhibit the growth of *S. aureus* bacteria. Crude extract of *A. hispida* leaf was more effective in inhibiting *S. aureus* bacteria than crude extract of *A. indica* leaf.

REFERENCES

Ajizah A. 2004. Sensivitas *Salmonella typhimurium* terhadap Ekstrak Daun *Psidium guajava* L. Bioscientiae 1 (1): 31-38. DOI: 10.20527/b.v1i1.130. [Indonesian]

Akintola AJ, Ande AT. 2006. Aspect of the biology of *Rastrococcus* sp. (Hemiptera: Pseudococcidae) on *acayipha hispida* in Southern Guine Savanna of Nigeria. Afr J Agric Res 1 (2): 21-23.

Assani S. 1994. Ultra Struktur, Morfologi dan Pewarnaan Kuman, dalam Buku Ajar Mikrobiologi Kedokteran. Binarupa Aksara, Jakarta. [Indonesian]

Cahyanti ID. 2004. Pengaruh Ekstrak Anting-Anting (*Acalypha indica* L.) Terhadap Pertumbuhan, Kadar klorofil dan Nitrogen Total Gulma Krokot (*Portulaca olevacea* Linn.). [Skripsi]. Universitas Sebelas Maret, Surakarta. [Indonesian]

Cowan MM. 1999. Clinical Microbiology Review: Plants Product as Anti Microbial Agents. Miami University, Miami, FL.

Dalimartha S. 1991. Atlas Tumbuhan Indonesia. Jilid 1. Departemen Kesehatan Republik Indonesia, Jakarta. [Indonesian]

Fardiaz S. 1993. Analisis Mikrobiologi Pangan. PT Raja Grafindo Persada, Jakarta. [Indonesian]

Fessenden R, Fessenden JS. 1999. Kimia Organik. Jilid II. Penerjemah: Pudjaatmaka AH. Erlangga, Jakarta. [Indonesian]

Gibson JM. 1996. Mikrobiologi dan Patologi Modern untuk Perawat. EGC, Jakarta. [Indonesian]

Gopalakrishnan V, Rao KNV, Loganathan V, Shanmuganathan S, Bollu VK, Sharma TB. 2000. Antimicrobial activity of extracts of *Acalypha indica* Linn. Indian J Pharm Sci 62 (5): 347-350.

Guntur A. 2007. The Role of cefepime empirical treatment in critical illness. J Dexa Media 2 (20): 59-62. DOI: 10.1080/09332480.2007.10722834. [Indonesian]

Harborne JB. 1996. Metode Fitokimia Penuntun Cara Modern Menganalisis Tumbuhan. Terbitan Kedua. Institut Teknologi Bandung, Bandung. [Indonesian]

Hugo WB, Russell AD. 1998. Pharmaceutical Microbiology, Sixth Edition, Blackwell Science, Oxford.

Hutapea IR. 1993. Inventaris Tanaman Obat Indonesia. Departemen Kesehatan Indonesia, Jakarta. [Indonesian]

Jawetz E, Melnick GE, Adelberg CA. 2001. Mikrobiologi Kedokteran. Edisi 1. Diterjemahkan oleh Bagian Mikrobiologi Fakultas Kedokteran Universitas Airlangga. Salemba Medika, Surabaya. [Indonesian]

Kusumaningrum GD. 2002. Aktivitas Penghambatan Minyak Atsiri dan Ekstrak Kasar biji Pala (*Myristica fragnans* Houtt dan *Myristica fattua* Houtt) Tethadap Pertumbuhan Bakteri *Xanthomonas campestris* Oammel Asal Tanaman Brokoli (*Brastica oleracea* var. Italica). [Skripsi]. Universitas Sebelas Maret, Surakarta. [Indonesian]

Lenny S. 2006. Senyawa Flavonoid, Fenilpropanoid dan Alkaloid. Universitas Sumatra Utara, Medan. [Indonesian]

Lestari U, Pangastuti A, Susilowati A. 2006. Penghambatan produksi enzim eksoprotease *Aeromonas hydrophila* oleh ekstrak rimpang temu lawak (*Curcuma xanthorrhiza* Roxb.). Biofarmasi 4 (2): 45-54. DOI: 10.13057/biofar/f040202.

Madigan MT, Martinko J. !997. Biology of Microorganism. 8th Edition. Prentice-Hall, New York.

Masduki I. 1996. Efek antibakteri ekstrak biji pinang (*Areca catechu*) terhadap *S. aureus* dan *E. coli*. Cermin Dunia Kedokteran 109: 4-21.

Naim R. 2003. Cara kerja dan Mekanisme Resistensi Antibiotik. Institut Pertanian Bogor Press, Bogor. [Indonesian]

Noerono S. 1994. Pelajaran Teknologi Farmasi. Universitas Indonesia Press, Jakarta. [Indonesian]

Nugroho BW, Dadang, Prijono D. 1999. Bahan Pelatihan Pengembangan dan Pemanfatan Insektisida Alami. Pusat Kajian Pengendalian Hama Terpadu Institut Pertanian Bogor, Bogor. [Indonesian]

Ogbebor N, Adekunle AT. 2005. Inhibition of conidial germination and mycelial growth of *Corynespora cassiicola* (Berk & Curt) of rubber (*Hevea brasiliensis* Muell. Arg.) using extracts of some plants. Afr J Biotechnol 4 (9): 996-1000.

Pelczar MJ, Chan ECS. 1988. Dasar-Dasar Mikrobiologi II. (diterjemahkan oleh R. S. Hadioetomo, Teja Imam, SW. S. Tjitrosomo dan Sri Lestari Angka). Universitas Indonesia Press, Jakarta. [Indonesian]

Pratama MR. 2005. Pengaruh Ekstrak Serbuk Kayu Siwak (*Salvadora persica*) Terhadap Pertumbuhan *Streptococcus mutans Dan Staphylococcus aureus* Dengan Metode Difusi Agar. [Skripsi]. Institut Teknologi Sepuluh November, Surabaya. [Indonesian]

Robinson T. 1991. Kandungan Organik Tumbuhan Tinggi. Institut Teknologi Bandung, Bandung. [Indonesian]

Schegel HG. 1994. Mikrobiologi Umum. Edisi Keenam. Universitas Gadjah Mada Press, Yogyakarta. [Indonesian]

Subroto MA, Saputro H. 2006. Gempur Penyakit dengan Sarang Semut. Penebar Swadana, Jakarta. [Indonesian]

Sunarto, Solichatun, Listyawati S, Etikawati N, Susilowati A. 1999. Aktivitas antifungal ekstrak kasar daun dan bunga cengkeh (*Syzigium aromaticum* L.) pada pertumbuhan cendawan perusak kayu. BioSMART 2: 20-27. [Indonesian]

Syahrurachman A, Chatim A, Asmono N. 1993. Mikrobiologi Kedokteran. Binarupa Aksara, Jakarta. [Indonesian]

Taukhid P, Taufik, Mundriyanto H. 2002. Respon histologis tubuh kodok (*Rana catesbeiana* Shaw) terhadap Infeksi bakteri patogen dan potensi *Saccharomyces cereviceae* sebagai immunostimulan. J Penelitian Perikanan Indonesia 8 (3): 53-63. DOI: 10.15578/jppi.8.3.2002.53-63. [Indonesian]

Villes AL, Reese RN. 1995. Allelopathic potential of *Echinacea angustifolia* DC. Environ Exp Bot 36: 39-43.

Voight R. 1995. Buku Petunjuk Teknologi Farmasi. Uiversitas Gadja Mada Press, Yogyakarta. [Indonesian]