Antibacterial Analysis of *Talinum triangulare* Against *Staphylococcus aureus* and *Escherichia coli*

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Abstract. This study aims to determine whether Talinum triangulare can inhibit the growth of Staphylococcus aureus and Escherichia coli. The T. triangulare used in this research were the leaves, stems and roots. Simplicia leaves, stems and roots of T. triangulare were extracted by maceration using 96% ethanol solvent, then filtered and thickened using a rotary evaporator. Testing of T. triangulare against S. aureus and E. coli using the agar well diffusion method. The test solutions used were concentrations of 20%, 40%, 60%, 80%, 100% as well as positive and negative controls as comparisons. The positive control used amoxicillin while the negative used sterile distilled water. The results of the antibacterial activity test were shown by the formation of a growth inhibition zone (ZHP) for S. aureus and E. coli. Statistical tests using One way ANOVA followed by Post Hoc tests in the form of LSD. Research data shows that leaf extract is the most effective in inhibiting the growth of S. aureus and E. coli compared to roots and stems. The most effective concentration in inhibiting the growth of S. aureus and E. coli bacteria is 100%. The increase in concentration is directly proportional to the extent of the clear zone formed around the well. T. triangulare leaf, stem and root extracts were more effective in inhibiting S. aureus than E. coli.

1 INTRODUCTION

Bacteria are single-celled microorganisms that are several micrometers long and have a morphology ranging from a rod (bacilli), cocci to a spiral shape [1]. Microorganisms that become one of the causes of infection with several diseases are bacteria. High levels of infection can cause death. Infection is included in the top 10 diseases with a high mortality rate [2]. According to the world health agency (WHO) *Staphylocccus aureus* and *Escherichia coli* are two microorganisms that are included in the 12 most dangerous bacteria that infect humans [3].

S. aureus and *E. coli* can cause disease in humans. S. aureus can cause infectious diseases such as boils, pimples, impetigo, and wound infections. More severe infections include

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pneumonia, mastitis, phlebitis, meningitis, urinary tract, osteomyelitis and endocarditis. *S. aureus* is also a major cause of nosocomial infections, food poisoning and toxic shock syndrome. Food poisoning can be caused by enterotoxin contamination from *S. aureus.* The amount of toxin that can cause poisoning is 1.0 µg/gr of food. Symptoms of poisoning are characterized by severe nausea, vomiting and diarrhea without fever. *E. coli* becomes a pathogen if the number of these bacteria in the digestive tract increases or is outside the intestine. *E. coli* produces enterotoxins which cause several cases of diarrhea (Faculty of Medicine, University of Indonesia, 1994).

The bacteria *S. aureus* and *E. coli* were included in the list of the 12 most dangerous bacteria released by WHO in February 2017 because of their resistance to antibiotics. Dr. Marie Paule Kieny, WHO director general for health systems and innovation, suggests developing effective antibacterials to kill or inhibit these two bacteria in the near future. Antibacterials using natural ingredients are really needed, because the side effects are mild, they are cheap and easy to obtain.

Plants that are considered as weeds but have active substances that can be beneficial to humans, namely *Talinum triangulare. T. triangulare* is a plant that has a distinctive morphology. This plant is commonly known as Javanese ginseng by Indonesians, because it has enlarged (bubbled) roots. The leaves of *T. triangulare* are thick/fleshy which are light green in color. The flower of this plant has 2 green sepals (petals). There are 5 petals (crown) of this plant and they are purple-red. The fruit is a round capsule which is yellowish green in color. The seeds of this plant have a flat shape and are shiny black in color [4] *T. triangulare* contains flavonoids, alkoloids, glycosides, saponins, proteins, carbohydrates, fats, oils, tannins, steroids, essential oils and terpenes [5]. This plant contains almost the same content as *P.oleracea,* namely carbohydrates, proteins, alkaloids, glycosides, flavonoids, tannins, essential oils and steroids/triterpenoids [6]. In addition, *T. triangulare* also has almost the same content as *T. paniculatum*. *P. oleracea* and *T. paniculatum* can be used as antibacterials for *Staphylococcus aureus* and *Escherichia coli* [7]. This is evidenced by the formation of an inhibition zone on the media overgrown by the two bacteria. The inhibition zone was formed due to the presence of secondary metabolite compounds contained in *P. oleracea* and *T. paniculatum*. This research aims to determine whether *T. triangulare* leaves, stems and roots can inhibit the growth of *Staphylococcus aureus* and *Escherichia coli.*

2 Research Methods

This research was conducted in several places, namely in Gedung Agung Village, Jatiagung District, South Lampung Regency, Unila Biochemical Laboratory and UPTD Regional Health Center of Lampung Province. Samples of three types of purslane plants were taken in Gedung Agung Village and processed to become simplicia. Simplisia is processed at the Unila Biochemistry Laboratory to become a thick extract. The viscous extract became the experimental material for observing antibacterial effectiveness, carried out at the UPTD Regional Health Office of Lampung Province.

2.1 Sample Preparation

Plant samples were taken in Gedung Agung Village, Jati Agung District, South Lampung Regency in fresh condition. T. triangulare was separated into leaves, stems and roots. The plants that have been selected are then washed until clean, then chopped and dried in the sun until dry. The dried samples were made into powder by blending.

2.2 Test the Content of Secondary Metabolite Compounds

The content of secondary metabolite compounds that were tested simply was flavonoids, tannins and saponins. Flavonoids were tested by adding 0.2 grams of simplicia then heating it with 10 ml of ethyl acetate and boiling for 3 minutes. After heating the mixture, it is filtered. 4 ml of the filtrate was taken then 1 ml of 1% AlCl3 was added. The bright yellow color seen in the sediment indicates the presence of flavonoids.

Saponins were tested by inserting 0.1 gram of simplicia into a test tube then adding 5 ml of distilled water and heating for 5 minutes. The mixture that has been heated is then filtered. The filter results are divided into 2 for testing using olive oil and distilled water. 2 drops of olive oil added to 1 ml of the filtrate and then shaken, if it forms oil formation, it indicates the presence of saponin compounds. 5 ml of distilled water was added to a test tube containing 1 ml of filtrate, shaken and if there was a stable foam, it indicated the presence of saponin compounds. Tannin was tested by adding 2 g of simplicia into a test tube, then adding 5 ml of 45% ethanol for 5 minutes. The mixture is cooled then filtered. 1 ml of filtrate was diluted with distilled water then 2 drops of ferric chloride were added and if the color changed to black-green it indicated the presence of tannin compounds [5].

2.3 Make Krokot Extract

T. triangulare plant extract was prepared using the maceration method. Maceration was carried out using 96% ethanol solvent. Simplisia as much as 500 grams is put into a vessel plus 2 liters of solvent and soaked for 1 day. The simplistic marinade is stirred every 6 hours, then filtered. The resulting maserate is then collected to be concentrated using a rotary evaporator to obtain a thick extract. The yield obtained was weighed and recorded.

2.4 Preparation Of Test Solusions

Test solutions were prepared in different concentration ranges between 20 to 100%. The stock solution concentration is 100% using 10 ml of thick extract. The thick extract of the purslane plant is diluted using distilled water to obtain the desired concentration. The concentration of the treatment group used 5 test solution concentrations, namely 20%, 40%, 60%, 80% and 100%. Dilute the 80% sample concentration by adding 8 ml of the viscous extract into a test tube and adding sterile distilled water until the volume reaches 10 ml. The amoxicillin solution used was 100 µg/ml. How to make an amoxicillin solution by weighing 1 mg of amoxicillin powder then dissolving it in 10 ml of sterile distilled water.

2.5 Antibacterial Activity Test

Rejuvenated bacteria were taken 1 ose then put into a bottle containing 5 ml of physiological NaCl solution. Then the turbidity was calculated using the standard Mc. Farland. Standard 0.5 Mc Farland equivalent to the number of bacteria 1.5 x 108 CFU/ml. The bacterial suspension was taken with a sterile swab stick. The bacteria that are on the sterile swab stick (sterile cotton) are rubbed onto the surface of the solidified agar using a continuous technique. Media containing bacteria were incubated for 24 hours at 370C. The antibacterial activity test of purslane plant extract was carried out using the well method. The media containing the bacteria in the petri dish was perforated using a blue tip. Then given the T. triangulare extract. The media was incubated at 370C for 24 hours. Observations were made after 24 hours and measured the inhibition zone formed using a caliper in millimeters (mm).

2.6 Data Analysis Technique

Analysis of data obtained from the growth inhibition zone (ZHP) of S. aureus and E. coli was tested using one-way Annova (Analysis of Variance) followed by further Post Hoc LSD testing. One-way Annova uses SPSS (Statistical Program for Social Sciences) version 17.

3 Results And Discussion

3.1 Results

Simplisia has a yield of less than 50% viscous extract (Table 1). Leaf, stem and root extracts contain different amounts of secondary metabolites in the form of flavonoids, saponins and tannins (Table 2).

Table 1. Yield of Simplicia Leaf, Stems and Roots Triangulare

Test Material	Wet Weight (grams)	Simplicity (gram)	Condensed Extract (ml)	Yield (%)
Leaf	5500	520	150	28,84
Stems	4000	500	230	46
Roots	3500	500	210	42

Table 2. Phytochemical Screening Test Results for *T. Triangulare*

Extract	Flavonoid	Saponin	Tanin
Leaf	+++	+	+
Stems	+	-	+
Roots	++	++	+

Description: - no secondary metabolite compounds detected, + The content is less than the others, ++ The content is moderate, +++ The content is greater than the others.

The results showed that there were clear zones around the wells with different sizes (Figure 1 dan Figure 2). The results of the One-way ANOVA statistical test show that the values are significant for all data at $p<0.05$, so it is known that the extract can inhibit bacterial growth. The data was continued with a Post Hoc LSD further test. Post Hoc follow-up test results in the form of LSD with a 95% confidence level regarding the growth inhibition zone (ZHP) of *S. aureus* with leaf extract showed that the control (+) was significantly different from the other test groups. Likewise with the control (-) and 20% concentration. The 40% concentration was not significantly different from the 60% treatment. Meanwhile, the 80% concentration was not significantly different from the 100% treatment (Table 3). *T. triangulare* stems with control (+) were significantly different from other treatments. Control (-) was not significantly different only from treatment concentrations of 20% and 40%. The 60% concentration was significantly different from all test treatments. Meanwhile, the 100% concentration was not significantly different from only the 80% concentration. The roots of *T. triangulare* tested against S. aureus showed that the (+) control was significantly different from the other treatments. Control (-) was not significantly different only at concentrations of 20% and 40%. While the concentration of 60% was significantly different from other treatments. The 100% concentration was not significantly different with only 80% concentration.

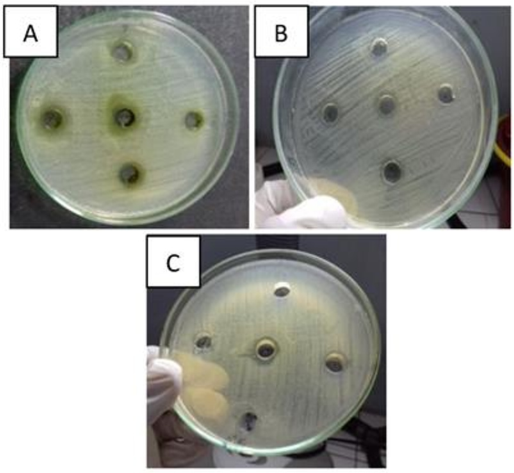

Fig. 1. Results of antibacterial activity test of leaves (A), stems (B), and roots (C) of *Talinum triangulare* against **Staphylococcus aureus** ATCC 25923

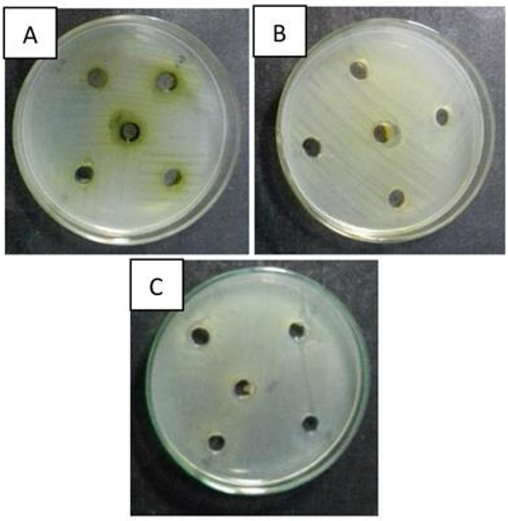

Fig. 2. Test results for the antibacterial activity of leaves (A), stems (B), and roots (C) of *Talinum triangulare* against *Escherichia coli* ATCC 25922

Table 3. Effectiveness test of *T. triangulare* leaves, Stems, and Roots compared with control against *S.aureus*

No	Treatment	Growth Inhibition Zone (mm)		
		Leaves	Stems	Roots
1	Kontrol (+)	14,79 ^{a} ± 4,73	14,79 ^{a} ± 4,73	14,79 ^{a} ± 4,73
2	Kontrol (-)	0,00^{b} ± 0,00	0,00^{b} ± 0,00	0,00^{b} ± 0,00
3	20%	1,55^{bc} ± 0,69	0,00^{b} ± 0,00	0,00^{b} ± 0,00

4	40%	4,17^{cd} ± 0,22	0,00^{b} ± 0,00	1,52^{b} ± 0,61
5	60%	4,69^{cd} ± 0,16	3,24^{bc} ± 0,49	2,81^{bc} ± 1,08
6	80%	5,20^{d} ± 0,29	3,94^{c} ± 0,43	3,44^{c} ± 1,05
7	100%	6,57^{d} ± 0,44	4,58^{c} ± 0,18	6,40^{c} ± 0,29

Table 4. Effectiveness test of *T. triangulare* leaves, Stems, and Roots compared with control against *E. coli*

No	Treatment	Growth Inhibition Zone (mm)		
		Leaves	Stems	Roots
1	Kontrol (+)	9,00^{a} ± 0,66	9,00^{a} ± 0,66	9,00^{a} ± 0,66
2	Kontrol (-)	0,00^{b} ± 0,00	0,00^{b} ± 0,00	0,00^{b} ± 0,00
3	20%	0,00^{b} ± 0,00	0,00^{b} ± 0,00	0,00^{b} ± 0,00
4	40%	0,00^{b} ± 0,00	0,00^{b} ± 0,00	0,00^{b} ± 0,00
5	60%	0,00^{b} ± 0,00	0,00^{b} ± 0,00	0,00^{b} ± 0,00
6	80%	0,00^{b} ± 0,00	0,00^{b} ± 0,00	0,00^{b} ± 0,00
7	100%	4,88^{c} ± 0,46	3,32^{c} ± 0,27	3,57^{c} ± 0,09

Table 5. Comparison Test of Leaves, Stems and Roots against *S. aureus* and *E. coli*

No	Extract	Growth Inhibition Zone (mm)	
		S. aureus	*E. coli*
1	Leaves	4,43^{a} ± 0,06	0,97^{a} ± 0,09
2	Stems	2,35^{b} ± 0,23	0,66^{b} ± 0,05
3	Roots	2,83^{b} ± 0,52	0,71^{b} ± 0,02

T. triangulare leaves with control (+) were significantly different from other treatments for E. coli bacteria. Control (-) significantly different only with control (+) and 100% concentration. The 100% concentration was significantly different from other treatments (Table 4). Likewise with stems and roots which have the same notation in the Post Hoc follow-up test in the form of LSD. *T. triangulare* leaves have a significantly different average ZHP of S. aureus when compared to stems and roots. The stems and roots of *T. triangulare* had an average ZHP of S. aureus that was not significantly different (Table 5). Leaves have a ZHP against *E. coli* that is significantly different from roots and stems. Stems and roots did not have significantly different average ZHP of *E. coli.*

3.2 Discussion

Based on research results and statistical test data, it shows that *T. triangulare* extract can inhibit the growth of *S. aureus* and *E. coli* bacteria. This is due to the formation of a clear zone around the well area. The widest clear zone is found in the inhibition of *S. aureus* by leaves, namely 6.57 mm. Stems with a concentration of 100% have an average ZHP of 4.58 mm. Meanwhile, roots at 100% concentration had an average zone of inhibition against S. aureus of 6.40 mm. Research on *E. coli* bacteria shows that the concentration that can inhibit its growth is 100%. Leaves have an average zone of inhibition of 4.88 mm, stems 3.57 mm and roots 3.57 mm at 100% concentration. Leaves, stems and roots with concentrations of 20% -80% did not show the presence of ZHP *E. coli* bacteria around the wells. This could happen because the bacteria are resistant to T. triangulare extract. Apart from that, it can also be caused by errors when testing antibacterial activity or it can also be caused by a lack of accuracy when measuring. The clear zone or barrier area is formed due to the antibacterial activity shown by the *T. triangulare* plant. The leaves and roots of *T. triangulare* can be classified as a strong antibacterial at a concentration of 100% and the stems are classified as moderate in inhibiting *S. aureus.* Meanwhile, the inhibition of *E. coli*, both leaves, stems and roots, is classified as moderate antibacterial.

Table 6. Categories of Antimicrobial Inhibition Based on Inhibition Zone Diameter

Diameter (mm)	Growth Barrier Response
0-3	Weak
3-6	Medium
>6	Strong

Different clear zones can be caused by the presence of secondary metabolites contained in these plants. The results of the phytochemical screening test showed that *T. triangulare* leaves contained more flavonoids than saponins and tannins. *T. triangulare* leaves contain flavonoids, alkoloids, glycosides, saponins, proteins, carbohydrates, fats and oils, tannins, steroids, resins and terpenoids [5]. The stems of T. triangulare contained small amounts of flavonoids and tannins, but no saponins were detected. The roots contain moderate amounts of flavonoids and saponins and little tannins. Leaves have the largest flavonoid content compared to stems and roots. Stems contain the least amount of flavonoids than leaves and roots. The highest saponin content is found in the roots.

Flavonoids are a group of aromatic compounds which include polyphenols and contain antioxidants. Flavonoids, which are classified as phenolic compounds, can damage cell membranes, precipitate proteins, and deactivate enzymes that play a role in secondary metabolic processes. . This bond causes the denaturation of structural proteins in the bacterial cell wall. This results in damage to the structure of the bacterial cell wall. Damaged bacterial walls result in cell membranes having no protection, so their semipermeability can decrease. Furthermore, nutrients and enzymes will leave the cells, causing obstacles in metabolism and decreased ATP production. Metabolism and decreased ATP production result in inhibited cell growth so that cell death can occur [8]. Saponins can inhibit bacterial growth by reducing the surface tension of the bacterial cell wall and damaging the permeability of the membrane because they are like soap. The mechanism of action is by diffusing through the outer membrane and cell walls which are vulnerable because they have been damaged by flavonoids. Saponin that enters then binds to the cytoplasmic membrane, disrupting and damaging the stability of the cell membrane. Saponin-bound and unstable membranes cause cytoplasm to leak out of the cell [9]. The leaky cytoplasmic membrane causes the release of various important components from inside the bacterial cell such as proteins, nucleic acids and nucleotides. This membrane also works to maintain and regulate the entry and exit of certain materials. In addition, the cytoplasmic membrane also provides the biochemical apparatus for moving mineral ions, sugars, amino acids, electrons, and other metabolites across the membrane. Damage to the membrane will result in inhibited cell growth and even bacterial cell death [10].

Tannins can inhibit bacterial growth by coagulating the protoplasm. The mechanism for inhibiting tannins is by entering bacterial cells whose walls have been damaged by flavonoids and saponins. Tannins that enter the cells can coagulate the protoplasm of the bacteria [7]. Coagulated protoplasm can cause lysis, resulting in inhibited cell metabolism and causing cell death. Apart from that, tannins can also react with proteins to form copolymers that are not soluble in water. This disrupts the absorption of protein by body fluids because it inhibits proteolytic breakdown of protein into amino acids. Inhibition of protein absorption can inhibit cell metabolism.

Leaves have larger ZHP S. aureus and E. coli than roots and stems. Stems had the smallest ZHP *S. aureus* and *E. coli* compared to leaves and roots. The leaves are more effective in inhibiting *S. aureus* than *E. coli.* Stems are more effective at inhibiting *S. aureus* than *E. coli*. Roots are also more effective in inhibiting *S. aureus* than *E. coli.* Control (+) using amoxicillin was still more effective than *T. triangulare* leaf, stem and root extract against both *S. aureus* and *E. coli*. Amoxicillin can inhibit protein synthesis from bacterial cells. The mechanism of action is by blocking the binding of RNA (aminoacyl transfer RNA) to specific sites on the ribosome, during peptide chain elongation. As a result, protein synthesis is inhibited [11].

Previous studies have also shown that S. aureus is more susceptible than *E. coli* so that the inhibition zone formed is wider. As research conducted by Karlina (2013) shows that Portulaca oleracea is more effective at inhibiting *S. aureus* than *E. coli.* Testing the inhibitory power of yaki betel nut extract by Rundengan (2017) also proved that S. aureus has a larger zone of inhibition compared to *E. coli.* Research on betel leaf extract conducted by Hermanto (2007) can also inhibit the growth of *S. aureus* to a greater extent than *E. coli*. Moningka (2015) also stated that cat's tail leaf extract can provide greater inhibitory power *against S. aureus* than *E. coli*. Likewise, Fatisa (2013) stated that fruit extracts and seeds have a larger zone of inhibition for *S. aureus* compared to *E. coli.* S. aureus has a larger zone of inhibition than E. coli due to differences in the composition of its peptidoglycan. *S. aureus* bacteria have a thicker peptidoglycan layer compared to *E. coli*. The peptidoglycan layer of *S. aureus* is 1580 nm thick, while that of *E. coli* is 10-15 nm. However, the lipid content of *S. aureus* is low, only 1-4%, while that of *E. coli* is high, namely 11-12%. *S. aureus* has teichoic acid which is soluble in water and is polar. *E. coli* does not contain teichoic acid [11]. Thus, it can be seen that *E. coli* has a more complex peptidoglycan layer than S. aureus, even though this gram-positive bacterium has a thicker peptidoglycan layer. The clear zone that forms in media that grows *S. aureus* and *E. coli* is caused by the presence of substances that inhibit bacterial growth. The bacterial growth inhibitors found in T. triangulare extract are secondary metabolite compounds, namely flavonoids, saponins and tannins. Flavonoids can provide a larger inhibition zone against S. aureus than E. coli. This is because flavonoids have the same properties as S. aureus peptidoglycan, namely polar. Peptidoglycan of E. coli is more nonpolar because of its high lipid content and does not contain teichoic acid. Therefore, the active substances of flavonoids penetrate and damage the peptidoglycan of S. aureus bacteria more easily than E. coli. Likewise, saponins can reduce the surface tension of cell walls. Saponins more easily reduce the surface tension of the cell walls of S. aureus bacteria because they are polar compared to non-polar E. coli. After the peptidoglycan layer is damaged, then the tannins can enter the bacterial cell. If bacterial cells are difficult to damage or penetrate because their lipid content is thick, then the tannins that enter the bacterial cells cannot be optimal, and vice versa. Flavonoids can penetrate and damage the peptidoglycan layer of S. aureus bacteria quickly because they are polar, so tannins can enter cells optimally. Thus, the inhibition zone formed on the media overgrown with S. aureus bacteria was wider than that of E. coli.

4 Conclusion

Based on these data, it can be concluded that Talinum triangulare leaf, stem and root extracts can inhibit the growth of both Staphylococcus aureus and Escherichia coli bacteria. T. triangulare was more effective in inhibiting S. aureus than E. coli in both leaves, stems and roots. The leaves of the T. triangulare plant are more effective in inhibiting bacterial growth. This is because the content of secondary metabolite compounds is greater than in the stems and roots. Secondary metabolite compounds contained in T. triangulare include flavonoids, tannins and saponins which effectively inhibit bacterial growth.

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