



ANTIBACTERIAL ACTIVITY OF NAMNAM FRUIT JUICE FROM SOUTH KALIMANTAN AGAINST STAPHYLOCOCCUS AUREUS AND ESCHERICHIA COLI

AKTIVITAS ANTIBAKTERI SARI BUAH NAMNAM ASAL KALIMANTAN SELATAN TERHADAP STAPHYLOCOCCUS AUREUS DAN ESCHERICHIA COLI

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ABSTRACT

Background: Namnam (*Cynometra cauliflora* L.) has been reported to contain bioactive compounds that are beneficial as being antifungal, antibacterial, antiviral, and antioxidant. Namnam fruit (*Cynometra cauliflora* L.) is a herbal plant that has antibacterial properties because it contains chemicals in the form of tannins, saponins, and flavonoids. **Purpose:** To determine the content of phytochemical compounds contained in them and their activity against *Staphylococcus aureus* and *Escherichia coli*. **Method:** The method used in this study was agar diffusion using blank disks and the measurement of the inhibition of the test bacteria. **Result:** The results showed that Namnam fruit juice (*Cynometra cauliflora* L.) was proven to contain alkaloids, phenol, flavonoid, and saponin phytochemicals. The results of the inhibition zone measurement for *S. aureus* bacteria at a 100% concentration showed 6 mm and for *E. coli* bacteria at a 100% concentration showed 7.33 mm, which means that the inhibition zone of the two bacteria was quite intense. It meant that the higher the concentration, the greater the inhibition zone and the stronger the antibacterial activity produced. Namnam fruit juice activity in inhibiting the growth of *E. coli* bacteria was more substantial when compared to *S. aureus* bacteria. **Conclusion:** The result of this study concluded that Namnam fruit juice had the potential to be an antibacterial agent.

ABSTRAK

Latar belakang: Namnam (*Cynometra cauliflora* L.) telah dilaporkan sebelumnya mengandung senyawa bioaktif yang bermanfaat sebagai antifungal, antibakteri, antivirus, dan antioksidan. Buah namnam (*Cynometra cauliflora* L.) adalah tanaman herbal yang mempunyai sifat antibakteri karena memiliki kandungan kimia berupa tanin, saponin, dan flavonoid. **Tujuan:** Untuk mengetahui kandungan senyawa fitokimia yang terkandung dalam sari buah namnam dan aktivitasnya terhadap *Staphylococcus aureus* dan *Escherichia coli*. **Metode:** Metode yang digunakan dalam penelitian ini adalah difusi agar menggunakan kertas cakram (*disk blank*) dan dilakukan pengukuran daya hambatnya terhadap bakteri uji. Hasil penelitian menunjukkan bahwa sari buah namnam (*Cynometra cauliflora* L.) terbukti memiliki kandungan fitokimia alkaloid, fenol, flavonoid, dan saponin. **Hasil:** Hasil pengukuran zona hambat terhadap bakteri *S. aureus* pada konsentrasi 100% menunjukkan hasil 6 mm dan terhadap bakteri *E. coli* pada konsentrasi 100% menunjukkan nilai 7,33 mm yang berarti zona hambat kedua bakteri tersebut cukup kuat. Hal ini menunjukkan bahwa semakin tinggi konsentrasi, maka zona hambat yang dihasilkan semakin besar dan aktivitas antibakterinya semakin kuat. Aktivitas sari buah namnam dalam menghambat pertumbuhan bakteri *E. coli* lebih besar bila dibandingkan dengan bakteri *S. aureus*. **Kesimpulan:** Hasil penelitian ini disimpulkan bahwa sari buah namnam mempunyai potensi yang dapat dimanfaatkan sebagai antibakteri.

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INTRODUCTION

Indonesia has wide biodiversity which is an unlimited source of chemical compounds. Therefore, biodiversity is comprised of the diversity of natural chemicals that can be utilized to be medicines, insecticides, cosmetics, and base ingredients for synthetical compounds. Traditional medication derived from plants and pure natural ingredients have side effects, danger levels, and risks much lower than chemical drugs when consumed for long term (Sunaryanti *et al.*, 2012).

Namnam fruit (*Cynometra cauliflora* L.) is a herbal plant that has antibacterial characteristics because it contains chemical compounds in the form of tannins, saponins, and flavonoids (Tarasti, 2020). Moreover, it produces phenolic compounds substituted by hydroxyl groups, especially the oligostilbenoid group. These oligostilbenoid compounds have a biological activity such as antioxidant, anti-HIV, antibacterial, antifungal, and antihepatotoxic (Kristanti *et al.*, 2006). Some studies of antibacterial activity in vitro on namnam fruit with the extraction method had been done, and the results showed that the ethanol extract of the namnam fruit contains compounds, triterpenoids, flavonoids and saponins. The ethanol extract of namnam fruit has activity as an antibacterial agent against *S. aureus* and *E. coli* (Adawiyah *et al.*, 2012). In addition, a previous study conducted by Anliza *et al.* (2022) indicated that the ethanol extract of namnam leaves effectively inhibited the growth of *S. aureus* bacteria in hand sanitizer gel formulations, while another study conducted by Ulpiyah *et al.* (2019) showed that namnam leaf extract had inhibitory power against *Porphyromonas gingivalis* of 11.43 mm at a concentration of 100%.

The results of the phytochemical tests that have been held showed that namnam fruit juice contained high phenolic compounds when compared to other fruit juices such as lemon, tomato, and lime, and the phenolic content was lower in sapota, pomegranate, and cashew fruit juices (Adawiah *et al.*, 2015). Namnam fruit is commonly consumed by the public as a medicine to cure diarrhea, a source of vitamin C, to improve digestion, etc (Gesha, 2019). Hence, the namnam fruit use, especially its juice, for antibacterial testing is considered significant. In South Kalimantan, namnam fruit can be either consumed directly or made into a fruit salad. Also, it can be freely found for sale in traditional markets, but not many, because no residents cultivate these plants. The purpose of using fruit juice compared to other extraction methods is that fruit juice is easier for the community to get compared to other methods such as maceration or infusion.

Therefore, terpenoid are a group of compounds that have bioactivity as antibacterial bioactive compounds because they have lipophilic compounds that can damage bacterial membranes (Lestari *et al.*, 2020). Flavonoid compounds form complex compounds with extracellular and dissolved could damage cell membranes

bacteria and accompanied by the release of intracellular compounds (Ngajow *et al.*, 2013). The function of saponins as an antibacterial will work by breaking the porin on the outer membrane of the bacterial cell wall. Bacterial growth is inhibited or dies due to damage to the porin as a bridge for compounds to enter and leave, resulting in a nutritional crisis in the bacterial cell. One of the causes of infectious diseases is bacteria. *S. aureus* is capable of causing various infections with different levels of severity in soft tissue, bone tissue, respiratory organs, and endovascular tissue (Erikawati *et al.*, 2016). Meanwhile, *E. coli* bacteria commonly cause diarrhea, urinary tract infections, pneumonia, wound infections, especially in the abdomen, and meningitis (Suryati *et al.*, 2017).

Diseases caused by bacteria are treated with antibiotics. However, antimicrobial resistance against some bacteria is rapidly increasing. Various efforts to find alternative treatments continue to be improved, one of which is with plants as traditional medication. The aim of the study was to determine the content of phytochemical compounds contained in namnam fruit juice and their activity against *S. aureus* and *E. coli* bacteria. Therefore, the present study aims to provide scientific information to the public about the antibacterial activity of namnam fruit juice (*Cynometra cauliflora* L.) on bacterial growth.

MATERIAL AND METHOD

The materials used in the study were ripe namnam fruit obtained from South Kalimantan and determined at the ULM Basic Laboratory. The present study took place at the Laboratory of Pharmaceutical Microbiology and Chemistry, University of Borneo Lestari. Namnam fruit is washed with salt water, so that the fruit does not oxidize quickly. The method used to produce namnam juice is by squeezing. The concentration series of namnam fruit juice made were concentrations of 20%, 40%, 60%, 80%, and 100% in a total of 15 mL of solution. For the negative control test in the form of sterile aquadest and positive control form in 0.1 mg/mL chloramphenicol.

In the phytochemical screening test, the content of bioactive compounds was alkaloids, 2 mL of the sample was added by 2-3 drops each of Mayer's reagent, Dragendorff's reagent, and Wagner's reagent. Flavonoids, 2 mL of sample added 2 mg of Mg powder and 1 mL of concentrated HCL. Saponins, 2 mL of sample added to 10 mL of distilled water, left for 10 minutes, added 1 mL of HCl 2 N. Phenol, namely as much as 2 mL of sample added by 2 drops of 10% FeCl₃ solution. Terpenoids, 2 mL of sample added by 2 mL of chloroform and 10 drops of anhydrous acetic acid and 2-3 drops of concentrated H₂SO₄ (Lieberman Burchard reagent). Tannins, 2 mL of sample added with 1% NaCl and gelatin solution (Ainia, 2017; Khairiah *et al.*, 2018; Ramadhan *et al.*, 2020).

Antibacterial activity on Gram-positive bacteria namely *S. aureus* and Gram-negative bacteria namely *E. coli*, used the paper disc diffusion (*disk blank*) on *Mulleur Hinton Agar* media with the *Kirby Bauer* method (Surjowardojo et al., 2015). On the antibacterial activity test, the first step to be done is rejuvenate the bacteria on the nutrient agar slant media, then make a bacterial suspension by inserting one dose of bacterial colonies in the media using 0.9% NaCl until the turbidity meets the standard of *Mc. Farland* 0.5% (1.5×10^8 bacteria/mL). Then the sterile swab stick was put into a tube containing the suspension of test bacteria and streaked evenly on the MHA media. Disk blanks that had previously been soaked for 30-45 minutes in each series of control concentrations of namnam fruit juice and placed on MHA media containing the test bacteria and incubated at 37°C for 1 x 24 hours. Each test was conducted repeatedly for three times. The clear zone formed around the disk indicated inhibition of bacterial growth. Hence, the diameter could be measured using a caliper and then compared to the positive control.

RESULT

Phytochemical test results of namnam fruit juice (*Cynometra cauliflora* L.)

A qualitative phytochemical test was conducted to ensure that namnam fruit juice contained chemical compounds that had antibacteria activity. The results showed that namnam fruit juice contained alkaloids, flavonoids, saponin, and phenolic compounds. The results of the phytochemical test of namnam fruit juice are shows Table 3.

Antibacterial test results of namnam fruit juice against *S. aureus* and *E. coli*

The results found out that all concentrations of namnam fruit juice had an antibacterial effect on *S. aureus* and *E. coli*. The results of the study of the antibacterial activity of various concentrations of namnam fruit juice (*Cynometra cauliflora* L.) against the growth inhibition zone of *S. aureus* and *E. coli* bacteria in vitro are in the following Figure 1 and Figure 2. The inhibition zone against *S. aureus* and *E. coli* bacteria in vitro using namnam fruit juice from a concentration of 20%, 40%, 60%, 80%, and 100% indicated that the greater the concentration, the greater the zone the inhibition seen from the clear zone produced (Table 1 dan Table 2).

A previous study conducted by Surjowardojo et al. (2015) discovered that if the inhibition zone with a diameter of <5 mm had a weak inhibition strength, 6-10 mm had sufficient inhibition strength, 11-20 mm had an intense inhibition strength, and if >20 mm had very strong inhibition. The results of the inhibition zone measurement for *S. aureus* in the positive control had

the largest average inhibition zone, which was 18.67 mm, while the lowest was 0 mm in the negative control using sterile aquadest. At concentrations of 20%, 40%, 60% and 80%, the average inhibition zone is below 5 mm, this result indicated that it had a weak inhibition category. Whereas at a concentration of 100% it had an average inhibition zone of 6 mm which means it was in the medium inhibition zone category.

Table 1. Results of measurement of inhibition zone for *S. aureus* bacteria

Treatment	Inhibition zone diameter (mm) deuteronomy			Rate-rate (mm)
	1	2	3	
	Negative control	0	0	
Positive control	24	17	15	18.67
Concentration 20%	2	1	1	1.67
Concentration 40%	2	3	3	2.67
Concentration 60%	3	3	4	3.33
Concentration 80%	5	5	4	4.67
Concentration 100%	7	5	6	6

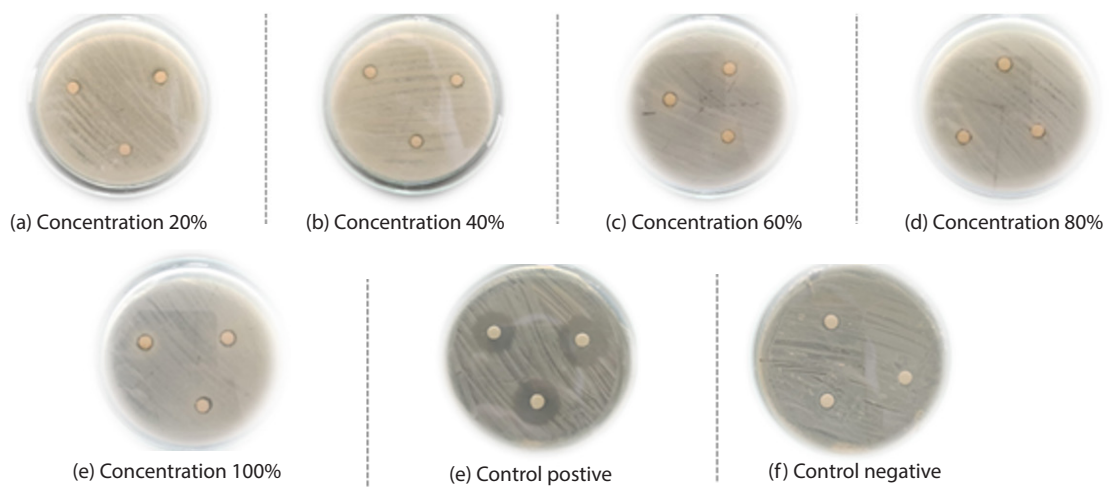
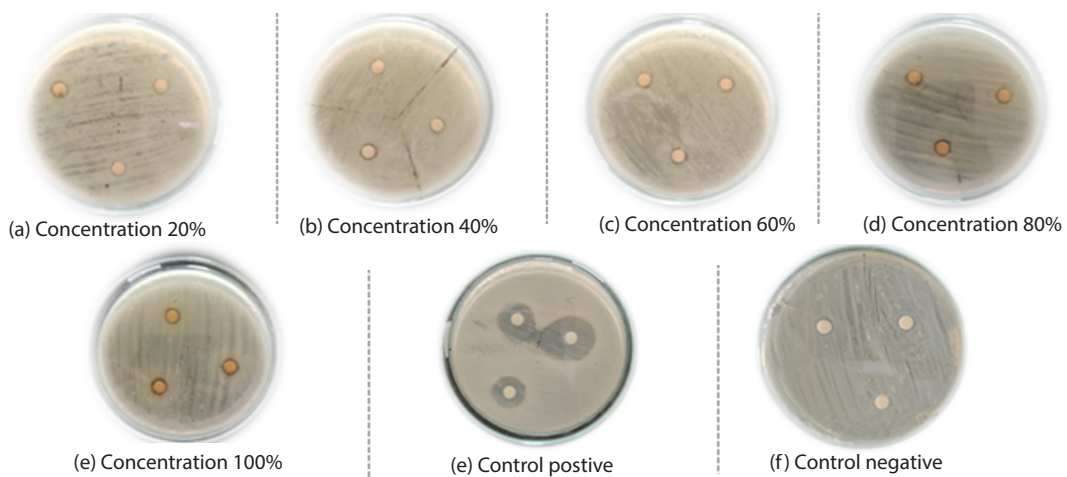
Table 2. Results of measurement of inhibition zone for *E. coli* bacteria

Treatment	Inhibition zone diameter (mm) deuteronomy			Rate-rate (mm)
	1	2	3	
	Negative control	0	0	
Positive control	27	25	22	24.67
Concentration 20%	1	1	1	1
Concentration 40%	2	1	1	1.33
Concentration 60%	4	4	2	3.33
Concentration 80%	6	6	5	5.67
Concentration 100%	7	8	7	7.33

The results of the inhibition zone measurement on *E. coli* bacteria in the positive control had the largest average inhibition zone, which was 24.67 mm. This indicated that the positive control had a very strong inhibition zone strength, while the lowest was 0 mm in the negative control using sterile aquadest. At concentrations of 20%, 40% and 60%, the average inhibition zone was below 5 mm, and this result indicated that they had a weak inhibition category. Meanwhile, at concentrations of 80% and 100%, the average inhibition zone was above 5 mm and below 10 mm, this result indicated that it was in the medium inhibition zone category. The average bacterial inhibition zone can be seen in Figure 3.

Table 3. Phytochemical test results

Testing	Reagent	Results	Information
Alkaloid	HCl + Reagent Mayer	A white precipitate formed	+ (positive)
	HCl + Reagent Dragendoff	An orange precipitate formed	+ (positive)
	HCl + Reagent Wagner	No brown precipitate formed	- (negative)
Flavonoid	Mg + HCl concentrated	Color change to yellow	+ (positive)
Saponin	Aquadest + HCl	Formed foam as high as 1 cm	+ (positive)
Fenol	FeCl ₃	There is a black discoloration	+ (positive)
Terpenoid	Chloroform + Anhydrous acetic acid + H ₂ SO ₄ concentrated	No change in color to red	- (negative)
Tanin	NaCl + Gelatin 1%	No white precipitate formed	- (negative)

**Figure 1.** Inhibition zone results for *S. aureus***Figure 2.** Inhibition zone results for *E. coli*

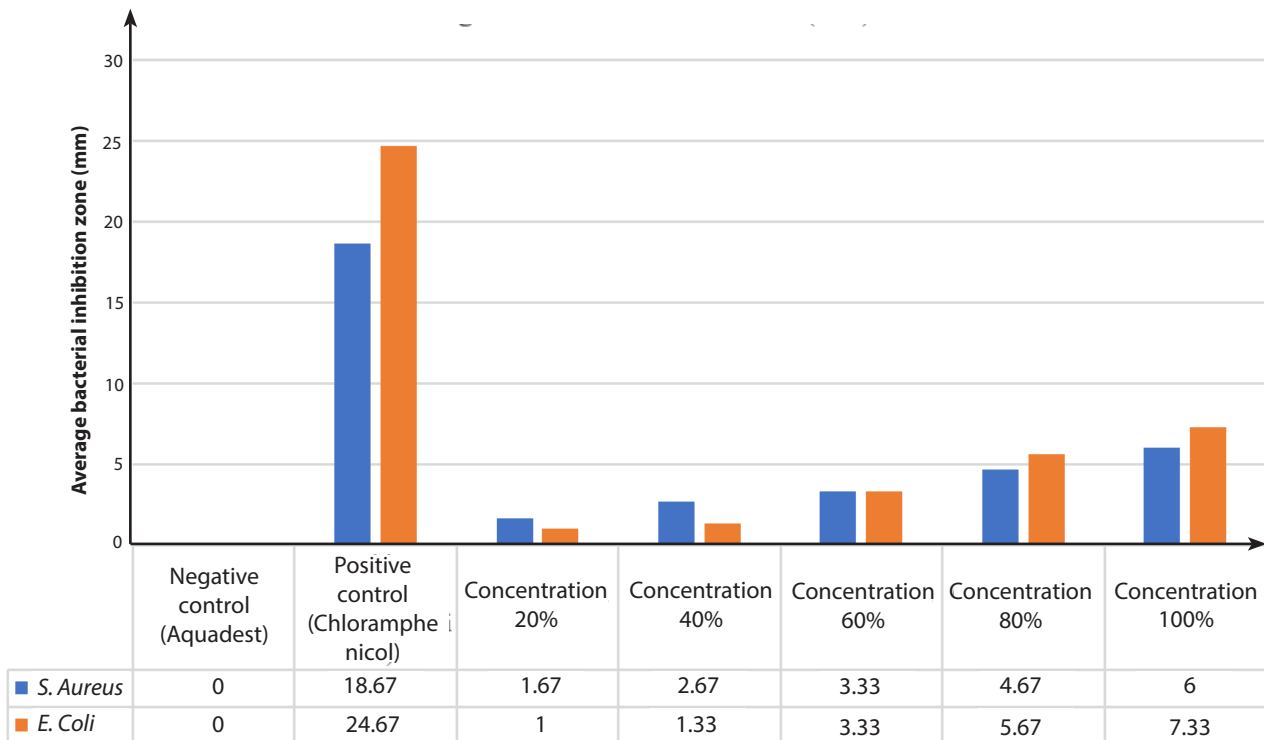


Figure 3. Average bacterial inhibition zone

DISCUSSION

The results of the inhibition zone test on *S. aureus* and *E. coli* bacteria in vitro using namnam fruit juice (*Cynometra cauliflora* L.) from a concentration of 20%, 40%, 60%, 80%, and 100% indicated that the greater the concentration, the greater the inhibition zone seen from resulting clear zone. A comparison of the effects at specific concentrations in this study showed that all extract concentrations had activity in inhibiting the growth of *S. aureus* and *E. coli* bacteria. The greatest activity against *S. aureus* bacteria was shown by a concentration of 100% with an average yield of 6 mm and for *E. coli* bacteria was shown by a concentration of 100% with an average yield of 7.33 mm. Although its activity is still under positive control, this showed that namnam fruit juice had antibacterial properties. The results of these inhibitions depended on the type and the strength of the antibacterial compounds from the components contained in the namnam fruit.

The choice of chloramphenicol as a positive control is because *S. aureus* and *E. coli* bacteria have experienced resistance to several antibiotics. Further, this antibiotic is bacteriostatic with a broad spectrum active against Gram-positive bacteria, which are able to inhibit the attachment of amino acids from bacteria, so that it can inhibit the growth of *S. aureus* and *E. coli* (Suciari et al., 2017). While the negative control is aquadest in which it does not have antibacterial activity. Antibacterial testing showed antibacterial activity marked by the formation of a clear area around the disc paper, after incubation for 1 x 24 hours at 37°C. Based on the tests, they showed that the antibacterial activity of namnam fruit juice with

the formation of a clear zone around the paper disc in a sample with a particular diameter was called the inhibition zone. Antibacterial activity can be affected by several factors, including the concentration of namnam fruit juice, the amount antibacterial compounds, the type and number of bacteria (Jawetz et al., n.d.).

The results of the inhibition zone test indicated that the largest inhibition zone was at a 100% concentration of namnam fruit juice. It means that the higher concentration, the greater the inhibition zone produced. The higher the concentration of an antibacterial substance, the stronger the antibacterial activity. It can be stated that the diameter of the inhibition zone is directly correlated to the level of concentration (Azzahra et al., 2019). The content of the active substance compounds in namnam fruit juice including alkaloids, flavonoids, saponins, and phenols, can inhibit the growth of *S. aureus* and *E. coli* bacteria. One of the mechanisms of action of secondary metabolites as antibacterials is conducted by interfering with peptidoglycan synthesis. Thus, the cell wall formation becomes incomplete. This situation will cause the bacterial cells to lyse due to osmotic and physical pressure lead to cells to die (Pertiwi et al., 2022). Changes in the structure of the cell wall cause changes in function that interfere with cell metabolism which results in decreased cell wall permeability, uncontrolled entry and exit of molecules, and disturbed ATP formation. ATP in bacteria serves as a source for bacterial growth, so that when ATP is reduced, the growth process in bacteria is inhibited, which results in bacterial death (Mambang and Rosidah, 2014).

The content of flavonoids and phenolic compounds can be antibacterial. In Adawiah *et al.* (2015) study, the results showed that the content of phenolic compounds in namnam fruit juice was 996.03 mg/L, and flavonoid compounds was 421.09 mg/L. Phenol has been stated as a disinfectant that has broad spectrum antibacterial activity against Gram-positive and Gram-negative bacteria. Phenol in high concentrations can penetrate and disrupt the bacterial cell wall and precipitate proteins in the bacterial cell. A lower phenol concentration can inactivate significant enzyme systems in bacterial cells (Oliver *et al.*, 2001). Flavonoid compounds are synthesized in plants and used as a defense system and in response to infection by microorganisms. Therefore, flavonoid compounds are effective as antimicrobial compounds against several microorganisms (Parubak, 2019).

Alkaloid has antibacterial activity by damaging the cell wall through the constituent components of peptidoglycan in bacterial cells. Meanwhile, saponins suppress bacterial growth by lowering the surface tension of the cell wall and interacting with the bacterial cell wall resulting in lysis (Azzahra *et al.*, 2019). Phenol compounds work by interacting with bacterial cells through an absorption process involving hydrogen bonds and interfering with the action in the cytoplasmic membrane, including interfering with active transport and proton strength.

The difference in the diameter of the inhibition zone at each concentration is the mechanism of action. The mechanism of action in *S. aureus* and *E. coli* bacteria, inhibiting protein synthesis by binding to the 50 subunit ribosome of *S. aureus* bacteria are Gram-positive bacteria, which have a thick and rigid peptidoglycan layer on their cell walls, *E. coli* bacteria are Gram-negative bacteria that has a thin peptidoglycan layer (Sudarmi *et al.*, 2017). Diameter of inhibitory shows disinfectant and antibacterial properties with several levels in which the diameter >20 mm indicates very strong inhibition, 10-20 mm diameter indicates strong inhibition, 5-10 mm indicates sufficient inhibition (medium), and the diameter of <5 mm indicates weak inhibition (Rini and Nugraheni, 2018). This indicates that namnam juice with a concentration of 100% was on medium level of inhibition.

Namnam fruit juice can inhibit the growth of *S. aureus* and *E. coli* bacteria, which are characterized by an inhibition zone formation around the disc paper. The results of this study indicated that the inhibition zone formed on *E. coli* bacteria was larger than *S. aureus*. Because *S. aureus* and *E. coli* came from different groups of bacteria, namely *S. aureus* as Gram-positive and *E. coli* bacteria as Gram-negative. It was due to differences in the structure of the cell wall of the two bacteria.

The cell wall of *S. aureus* bacteria consists of several peptidoglycan layers which form a thick and rigid structure and contains a cell wall substance called teichoic acid, while the cell wall of *E. coli* bacteria consists of one or thinner layers and a membrane on the outside

of the peptidoglycan layer. It only contains a small amount of peptidoglycan layer and does not contain teichoic acid, the cell wall of the *E. coli* bacteria is more susceptible to physical damage, such as administration of antibiotics or other antibacterial agents. It is what causes the inhibitory activity of Gram-negative bacteria, *E. coli* greater than Gram-positive bacteria *S. aureus* (Mpila *et al.*, 2012).

CONCLUSION

Based on the study, it can be concluded that namnam fruit juice contains secondary metabolites including alkaloids, flavonoids, saponins and phenols. Also, namnam fruit juice has antibacterial activity antibacterial activity againsts *S. aureus* and *E. coli*. Further research is recommended to use maseration ethanolic extraction and measure the level of metabolite compounds, as well as using other plant parts.

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