Antibacterial Activities Test of Ethanol Extracts of Kundur Fruit (*Benincasa hispida* Thunb. Cogn) on *Salmonella typhi* Bacteria

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**Abstract.** One of the natural ingredients that can be used as medicinal plants is kundur fruit (*Benincasa hispida* Thunb. Cogn). Kundur is one of the plants that contain compounds that are efficacious in medicine. The people of the Moronene tribe in Southeast Sulawesi used to consume kundur fruit in a shredded way to treat typhoid caused by bacterium *Salmonella typhi* (*S. Typhi*). The general objective of this study was to determine the bioactivity of the ethanol extract of kundur fruit (*B. hispida*) as an antibacterial *S. typhi*. Determination of antibacterial activity was carried out using the broth microdilution method. The results showed that the ethanol extract of kundur fruit had inhibitory activity on the growth of *S. typhi* bacteria with a minimum inhibitory concentration (MIC) of 800 μg/mL. This activity was compared with chloramphenicol with a MIC value of 8 μg/mL. In conclusion, the ethanol extract of kundur fruit has the potential as a source of antibacterial compounds against *S. typhi*.

**Keywords:** Antibacterial, kundur fruit, *Salmonella typhi* Bacteria.

1. Introduction

The use of natural ingredients for treatment is common in Indonesia, this can be seen from many good traditional herbal products which have been processed with modern and simple technologies circulate in the community. From the nature, various kinds of medicines have been obtained such as atropine, various kinds of antibiotics, quinine, reserpine and medicines obtained from their derivatives (Raflizar and Sihombing 2009).

Infectious bacteria usually can be killed using medicines containing synthetic antibiotics. Therapy of infections with synthetic antibiotics could bring problems, namely the bacterial resistance to the antibiotics and symptoms indicating the side effects of them. The search for another alternatives in the treatment of infections is the traditional medicines. Natural antibacterial compounds generally contain steroids, tannins, polyphenols, flavonoids (Rahman et al. 2011), alkaloids, saponins (Ahmed et al. 2008). According to Barnes et al. (1997), asiaticoside compounds (saponin derivatives) are lipophilic...
and can form complex compounds with cell membranes through hydrogen bonds, then destroy the permeability of bacterial cell walls.

One of the natural ingredients that can be used as a medicinal plant is kundur fruit (*Benincasa hispida* Thunb. Cogn). Kundur fruit is one of the plants that contain compounds that are efficacious as medicine. Fruit, fruit peel and seeds of *B. hispida* contain saponins, moreover, the fruit and fruit peels also contain flavonoida and tannins and the seeds also contain polyphenols. Indonesians, especially people in Kendari city, consume kundur fruit as vegetables. According to Indrawati et al. (2014), the Moronene tribe of Southeast Sulawesi used to consume kundur fruit in a shredded way to treat typhoid caused by bacterium *Salmonella typhi* (*S. typhi*).

One of the bacteria which causes typhoid is *Salmonella typhi*. This bacterial infection occurs from food contaminated with feces containing *Salmonella typhi* from the hosts. After entering the digestive tract, these bacteria would attack the intestinal wall which causes damage and inflammation (Jawetz et al. 2001).

The incidence of typhoid fever in Indonesia tends to increase. Treatment for typhoid fever need to be developed to solve the problem. The development needed is the invention of new treatments and alternative medicines. Kundur fruit which has antibacterial properties is expected to combat typhoid fever.

2. **Methodology**

2.1. **Study area**

This is an experimental laboratory research with a one-shot case study design, that is a research design by treating the independent variables followed by observing or measuring the independent variables (Sugiyono 2011). This research was conducted at the Halu Oleo University Pharmacy Laboratory in May 2019. The subject of this study was the kundur fruit (*Benincasa hispida* Thunb. Cogn) ethanol extract which was tested against *Salmonella typhi* bacteria. The independent variable of this study was the variation in concentration of the kundur fruit (*Benincasa hispida* Thunb. Cogn) ethanol extract with a concentration of sample stock solution of 20,000 μg / mL (1000 μg / well), 10,000 μg / mL (500 μg / well), 1000 μg / mL (50 μg / well), and 100 μg / mL (5 μg / well) in 10% DMSO. The dependent variable of this study was the inhibitory zone (clear zone) on the growth of *Salmonella typhi* made using Muller Hinton agar (MHA) media.

2.2. **Procedure**

a) **Material Collection**

Kundur (*Benincasa Hispida* Thunb. Cogn) of medium age (± 3 months) dried, then mashed and sifted using mesh no.20. Kundur fruit powder was characterized based on its water content (no more than 10%, (BPOM 2010).

b) **Kundur fruit extraction (Benincasa hispida Thunb. Cogn)**

Kundur fruit powder which has been mashed to powder then macerated 3 x 24 hours with 95% ethanol, then concentrated with the evaporator until a thick extract obtained. The extract was then calculated by comparing the weight of the extract obtained with the initial weight of the simplicia multiplied by 100%.

c) **Sterilization of Tools**

Equipment to be used was washed and then dried and wrapped in paper. It was then put in a microwave at 150 °C for 15 minutes.
d) *Mueller Hinton Agar (MHA) and Mueller Hinton Broth (MHB) Media*

10 g nutrient agar was dissolved in 500 mL distilled water in the Erlenmeyer flask then stirred and heated till boiled for ± 10 minutes. It was then sterilized in an autoclave at 121°C for 15 minutes. The media then poured into a 10-20 mL petri dish.

e) *Positive Control*

Positive antibacterial control (1 mg / mL in 10% DMSO) was made from 1 mg chloramphenicol and dissolved in 100 μL DMSO then added with 900 μL sterile liquid (MHB) media.

f) *Turbidity Standard (McFarland)*

McFarland standard solution consists of two components, namely 1% BaCl₂ and 1% H₂SO₄. 1.175 g 1% BaCl₂ solution was mixed with 9.95 ml 1% H₂SO₄ solution and stirred until homogeneous. The absorbance value of McFarland 0.5 standard solution is equivalent to bacterial cell suspension with a concentration of 1.5 x 10⁸ CFU / mL (Komansilan et al. 2015).

g) *Test Suspensions*

Before the antimicrobial testing, the bacteria to be tested were suspended into a physiological 0.9% NaCl solution. Turbidity of bacterial suspensions was compared with McFarland 0.5 standard. The McFarland 0.5 standard solution was made with a composition of 0.05 mL BaCl₂ 1% and 9.95 mL 1% H₂SO₄ where the standard solution was equivalent to a bacterial density of 1.5 x 10⁸ CFU / mL (Lalitha 2005).

h) *Antibacterial Activity Testing Screening*

Antibacterial screening used agar well diffusion method:

- The concentration of sample stock solution prepared for screening was 20,000 μg / mL (1000 μg / well), 10,000 μg / mL (500 μg / well), 1000 μg / mL (50 μg / well), and 100 μg / mL (5 μg / well) in 10% DMSO. The volume of solution to the well was 50 μL.
- Growth media used semisolid media: MHA and MHB (Sahidin et al. 2018).

i) *Determination of the MIC*

The value of MIC or KHM was determined using the broth microdilution method.

- 1 ose bacteria which has grown on the MHA growth media was suspended into MHB sterile and its turbidity was equated with the absorbance of McFarland 0.5 standard solution at 620 nm.
- Solution samples prepared (in 10% DMSO and sterile MHB) were 200 μg / mL, 400 μg / mL, 600 μg / mL, 800 μg / mL, 1000 μg / mL, 1200 μg / mL, 1400 μg / mL, 1600 μg / mL, 1800 μg / mL, and 2000 μg / mL.
- Chloramphenicol stock solutions are made in 1 mg / mL (10% DMSO and sterile MHB). The chloramphenicol solution prepared (serial dilution in 10% DMSO) was 512 μg / mL, 256 μg / mL, 128 μg / mL, 64 μg / mL, 32 μg / mL, 16 μg / mL, 8 μg / mL, 4 μg / mL, 2 μg / mL, and 1 μg / mL.
- DMSO solutions prepared in sterile MHB (serial retail) were 5.12%, 2.56%, 1.28%, 0.64%, 0.32%, 0.16%, 0.08%, 0.04%, 0.02%, and 0.01%.
- 100 μL of each solution was dispensed into each well in the 96 welled microplate by order in Figure 6.
- A total of 100 μL of bacterial suspension (McFarland 0.5) was added to the well in column 1 → 11 (lines A → H).
The final concentration of the sample, chloramphenicol, and the solvents after adding 100 μL of bacterial suspension (in sterile MHB) were shown in Figure 7 (CLSI 2006; Wiegand et al. 2008).

The plate was then incubated at 35°C for 20 hours.

MIC values were determined visually by observing turbidity and measuring absorbance (turbidity) using a spectrophotometer at a wavelength of 620 nm.

j) Data Analysis

This study was analyzed descriptively based on the comparison of MIC values between the ethanol extract of kundur fruit and positive control of chloramphenicol.

3. Result and Discussion

3.1. Result

Based on the results of this research conducted in Mei 2019 at the Halu Oleo University Pharmacy Laboratory regarding the effectiveness of the kundur (Benincasa hispida Thunb. Cogn) fruit ethanol extract as an antibacterial for Salmonella typhi, the following results were obtained:

The screening results using the diffusion method to show that at the concentration of ethanol extract of gourd fruit 20,000 μg / mL (1000 μg / well), 10,000 μg / mL (500 μg / well), and 1000 μg / mL (50 μg / well), clear zone were observed. Thus, the MIC value was then determined starting from the concentration of 1000-100 μg / mL.

![Fig. 1. Results of screening for antibacterial activity of kundur fruit ethanol extract at concentrations of 1000 μg / well (A), 500 μg / well (B), 50 μg / well (C), and 5 μg / well (D) against S. typhi.](image)

| Sample                        | KHM (μg/mL) |
|-------------------------------|-------------|
| Kundur fruit ethanol extract  | 800         |
| Chloramphenicol (positive control) | 2          |
Table 2. Effect of variations in the concentration of kundur fruit ethanol extract, chloramphenicol, and DMSO on *S. typhi*

| Sample                              | Concentration (μg/mL) ( % DMSO) | Absorbance (620 nm) ± SD |
|-------------------------------------|---------------------------------|--------------------------|
| Kundur fruit ethanol extract        | 100                             | 0.25925 ± 0.14*          |
|                                     | 200                             | 0.14575 ± 0.46*          |
|                                     | 300                             | 0.16175 ± 0.10*          |
|                                     | 400                             | 0.04412 ± 0.08*          |
|                                     | 500                             | 0.01525 ± 0.14*          |
|                                     | 600                             | 0.02687 ± 0.16*          |
|                                     | 700                             | 0.13487 ± 0.14*          |
|                                     | 800                             | **-0.08612 ± 0.16***      |
|                                     | 900                             | -0.09950 ± 0.17*         |
|                                     | 1000                            | -0.10525 ± 0.16*         |
| Chloramphenicol (positive control)  | 0.5                             | 0.15517 ± 0.17*          |
|                                     | 1                               | 0.04583 ± 0.15*          |
|                                     | **2**                           | **0.00167 ± 0.19***       |
|                                     | 4                               | 0.08933 ± 0.14*          |
|                                     | 8                               | 0.07350 ± 0.19*          |
|                                     | 16                              | 0.00617 ± 0.13*          |
|                                     | 32                              | -0.05533 ± 0.10*         |
|                                     | 64                              | -0.05883 ± 0.08*         |
|                                     | 128                             | -0.05117 ± 0.08*         |
|                                     | 256                             | -0.08867 ± 0.09*         |
| DMSO                                | 0.005                           | 0.58250 ± 0.14**         |
|                                     | 0.01                            | 0.48650 ± 0.10**         |
|                                     | 0.02                            | 0.52000 ± 0.14**         |
|                                     | 0.04                            | 0.51350 ± 0.20**         |
|                                     | 0.08                            | 0.55500 ± 0.16**         |
|                                     | 0.16                            | 0.59550 ± 0.22**         |
|                                     | 0.32                            | 0.67700 ± 0.27**         |
|                                     | 0.64                            | 0.60650 ± 0.28**         |
|                                     | 1.28                            | 0.58650 ± 0.16**         |
|                                     | 2.56                            | 0.35950 ± 0.04**         |

Information: *n=8, *n=6, **n=2.
Table 3. Absorbance value of growth control and sterility control

| Test parameters     | Absorbance (620 nm) ± SD (n=2) | Difference       |
|---------------------|---------------------------------|------------------|
|                     | hour-0                          | hour-20          |                   |
| Growth Control (GC) | 0.23088 ± 0.12                  | 0.57644 ± 0.14   | 0.34556 ± 0.21    |
| Sterility Control (SC) | 0.30044 ± 0.11                  | 0.16133 ± 0.15   | -0.14400 ± 0.23   |

Fig. 2. The effect of the variation in concentration of kundur fruit ethanol extract on *Salmonella typhi* observed by UV-Vis spectroscopic method.

Fig. 3. The effect of the variation in chloramphenicol concentration on *Salmonella typhi* observed by UV-Vis spectroscopic method.

Fig. 4. The effect DMSO (solvent) concentration on *Salmonella typhi* observed by UV-Vis spectroscopic method.
Fig. 5. Control chart of *Salmonella typhi* growth (KP) and sterility control (KS) observed by UV-Vis spectroscopic method.

| Sample | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|--------|---|---|---|---|---|---|---|---|---|----|----|----|
| A      | 2000 μg/mL (100 μL) | 1800 μg/mL (100 μL) | 1600 μg/mL (100 μL) | 1400 μg/mL (100 μL) | 1200 μg/mL (100 μL) | 1000 μg/mL (100 μL) | 800 μg/mL (100 μL) | 600 μg/mL (100 μL) | 400 μg/mL (100 μL) | 200 μg/mL (100 μL) | 100 μL Liquid media | 200 μL Liquid media |
| B      | 2000 μg/mL (100 μL) | 1800 μg/mL (100 μL) | 1600 μg/mL (100 μL) | 1400 μg/mL (100 μL) | 1200 μg/mL (100 μL) | 1000 μg/mL (100 μL) | 800 μg/mL (100 μL) | 600 μg/mL (100 μL) | 400 μg/mL (100 μL) | 200 μg/mL (100 μL) | 100 μL Liquid media | 200 μL Liquid media |
| C      | 2000 μg/mL (100 μL) | 1800 μg/mL (100 μL) | 1600 μg/mL (100 μL) | 1400 μg/mL (100 μL) | 1200 μg/mL (100 μL) | 1000 μg/mL (100 μL) | 800 μg/mL (100 μL) | 600 μg/mL (100 μL) | 400 μg/mL (100 μL) | 200 μg/mL (100 μL) | 100 μL Liquid media | 200 μL Liquid media |
| D      | 2000 μg/mL (100 μL) | 1800 μg/mL (100 μL) | 1600 μg/mL (100 μL) | 1400 μg/mL (100 μL) | 1200 μg/mL (100 μL) | 1000 μg/mL (100 μL) | 800 μg/mL (100 μL) | 600 μg/mL (100 μL) | 400 μg/mL (100 μL) | 200 μg/mL (100 μL) | 100 μL Liquid media | 200 μL Liquid media |
| Chloram | 512 μg/mL (100 μL) | 256 μg/mL (100 μL) | 128 μg/mL (100 μL) | 64 μg/mL (100 μL) | 32 μg/mL (100 μL) | 16 μg/mL (100 μL) | 8 μg/mL (100 μL) | 4 μg/mL (100 μL) | 2 μg/mL (100 μL) | 1 μg/mL (100 μL) | 100 μL Liquid media | 200 μL Liquid media |
| Chloram | 512 μg/mL (100 μL) | 256 μg/mL (100 μL) | 128 μg/mL (100 μL) | 64 μg/mL (100 μL) | 32 μg/mL (100 μL) | 16 μg/mL (100 μL) | 8 μg/mL (100 μL) | 4 μg/mL (100 μL) | 2 μg/mL (100 μL) | 1 μg/mL (100 μL) | 100 μL Liquid media | 200 μL Liquid media |
| DMSO 10% | 5.12% (100 μL) | 2.56% (100 μL) | 1.28% (100 μL) | 0.64% (100 μL) | 0.32% (100 μL) | 0.16% (100 μL) | 0.08% (100 μL) | 0.04% (100 μL) | 0.02% (100 μL) | 0.01% (100 μL) | 100 μL Liquid media | 200 μL Liquid media |

Fig. 6. Composition of sample (kundur fruit ethanol extract), chloramphenicol, 10% DMSO, growth control (KP) and control of sterility (KS) before addition of *S. typhi* suspension.
Fig. 7. Concentration of final sample, chloramphenicol, and DMSO after adding 100 µL suspension of S. typhi.

3.2. Discussion

Kundur or baligo fruit comes from pumpkin family which the people of Moronene tribe of Southeast Sulawesi consume as kundur fruit soup as well as uses to prevent typhoid. Moronene people consume kundur fruit as shredded stuff (Indrawati et al. 2014). By some other people, kundur fruit also believed to be efficacious as an antidote from poisoning and its stem is efficacious as a medicine of dermal disease. Kundur fruit contains several chemicals. Fruit, fruit peel and Benincasa hispida seeds contain saponins, moreover, the fruit and fruit peels also contain flavonoids and tannins and their seeds contain polyphenols (Jahan et al. 2010).

This study were used Kundur fruit (Benincasa hispida Thunb. Cogn) samples. This kundur fruit is made in the form of simplicia before being processed into extract. This material was obtained from the traditional Hukaea-Laea village, Watu-watu Village, Lantari Jaya District, Bombana Regency, Southeast Sulawesi. Fresh kundur fruit of medium age (± 3 months) was wet sorted to separate them from un-needed ingredients, then, washing in running water was purposed to clean the dirts attached to the fruit. Drying out of direct sunlight was purposed to avoid chemical compound damage. The purpose of drying is to reduce the water content to prevent microbial growth. After the simplicia is dried, dry sorting was repeated. The purpose of this dry sorting is to prevent simplicia from being contaminated by unwanted ingredients which can affect the purity of the extract. Smoothing was done to expand the surface of the simplicia to speed up the extracting liquid in attracting chemical components.

Kundur fruit extract was obtained using maceration method. The maceration method is used because this method is a cold method to extract simplicia including contents which unable to stand heating. Maceration was completed by slicing small pieces of kundur and then drying them in a microwave at 40°C. After drying, the pieces then mashed into kundur fruit powder (98.9 g) and then soaked in 95% ethanol for 3 days. The extractor will penetrate the cell wall and enter the cell cavity containing the active substances. The active substances will dissolve; and because of the difference in concentration
between the solution of the active substance in the cell and outside the cell, the concentrated solution is pushed out. The event recurs so that there was an equilibrium concentration inside and outside the cell. This solution was frequently stirred and then filtered. The filtrate obtained is evaporated in the evaporator so that the extract becomes thicker. Then the extract was weighed and obtained an extract weight of 4.97 g.

The antibacterial activity of kundur fruit ethanol extract (Benincasa hispida Thunb. Cogn) against Salmonella thyphi was tested to see which extracts had the highest effectiveness as antibacterial agent. Antibacterial testing used the well diffusion method, the most widely used method because of its superior sensitivity to new antibacterial compounds whose activity is unknown. Absorption of extracts (samples) with this method better than other diffusion methods (Zahro and Agustini 2013). The well diffusion method has the same work principle with the paper disc method, namely the growth inhibition which aimed at the extent of the clear area (inhibition zone) in the form of a clear zone around the paper disk (Brander et al. 1999).

In the antibacterial test, chloramphenicol was used as a positive control. According to Gan and Setiabudi (1987) chloramphenicol is bacteriostatic which works to inhibit the enzyme peptidyl transferase in the process of bacterial protein synthesis. Thus, chloramphenicol was the most suitable compound used as a positive control in this study. The chloramphenicol solution prepared (serial dilution in DMSO%) was 512 μg / mL, 256 μg / mL, 128 μg / mL, 32 μg / mL, 16 μg / mL, 8 μg / mL, 4 μg / mL mL, 2 μg / mL, and 1 μg / mL. Based on observations it was found that at a concentration of 256 μg / mL until a concentration of 32 μg / mL obtained a negative absorbance value which means that at those concentrations there were no bacterial growth (Table 2). Whereas at concentrations of 16 μg / mL, 8 μg / mL, 4 μg / mL, 2 μg / mL bacterial growth began to occur although was still very small. So it can be concluded that chloramphenicol as a positive control has the ability to inhibit Salmonella typhi to a concentration of 2 μg / mL. It can be observed in figure 3 that the greater the concentration of chloramphenicol, the smaller the absorbance (closer to 0 and even minus), which means that the higher the dose of chloramphenicol given, the better it will be to inhibit bacterial growth.

Control of the solvents used, namely dimethyl sulfoxide (DMSO), showed no MIC. DMSO is a colorless organosulfur compound used as an aprotic solvent which dissolves both in polar and non-polar compounds and is also soluble in various organic solvents [16]. The need for testing of DMSO as a solvent is intended to determine whether solvents actually affect or kill bacteria, implying that the activity was caused by the solvents rather than the samples. Table 2 shows that the absorbance value is below 1, indicating that the solvent has no effect on the antibacterial test. As shown on figure 4, the higher the DMSO concentration used, the smaller the absorbance value. So it can be concluded that DMSO does not affect bacterial growth.

Control of bacterial growth (KP) and sterilization control (KS) is intended to keep the research carried out in sterile conditions to avoid contamination from the outside which could affect the desired results. The growth control containing media and bacteria compared with the sterilization control which contained only media, then each control incubated for 20 hours. Figure 5 was shown that sterilization control has a negative absorbance value, reflecting that the work was carried out in sterile conditions.

In this study of the effectiveness of the Kundur fruit ethanol extract (Benincasa hispida Thunb. Cogn) as an antibacterial agent against Salmonella typhi, the well diffusion (diffusion agar method) was made in 4 concentration variations namely 20,000 μg / mL (1000 μg / well), 10,000 μg / mL (500 μg / well), 1000 μg / mL (50 μg / well), and 100 μg / mL (5 μg / well) in DMSO 10%. Screening results for sample concentrations of 20,000 μg / mL (1000 μg / well), 10,000 μg / mL (500 μg / well), and 1000 μg / mL (50 μg / well) showed clear zones (Figure 13 and Figure 14), indicating that the kundur fruit
ethanol extract has bioactivity as an antibacterial agent, which at different concentrations has different levels of antibacterial effectiveness against *Salmonella typhi*.

Based on the testing of *Salmonella typhi*, the value of MIC in the kundur fruit ethanol extract was known to be the lowest sample concentration which can inhibit bacterial growth at a concentration of 800 μg / mL (Table 2), reflecting that at the concentration below 800 μg / mL, extract cannot inhibit bacterial growth as indicated by an increase in the absorbance value. The absorbance read by the UV-Vis spectrophotometry method, where the absorbance as turbidity is the turbidity value of the *S. typhi* culture solution. The results showed that the smaller the concentration of kundur fruit ethanol extract, the more turbid the sample, and the greater the absorbance value. At the concentration below 800 μg / mL, the absorbance value was seen to be higher (Figure 2), indicating the bacterial growth.

At concentrations above 800 μg / mL, the kundur fruit ethanol extract has been shown to have bacteriostatic properties which can inhibit bacterial growth even the absorbance value was minus, which means there was no bacterial growth at that concentration, indicating that the kundur fruit ethanol extract at concentrations above 800 μg / mL, has a "complete bactericidal" property. Thus, the value of the kundur fruit ethanol extract was estimated to be at concentrations above 800 μg / mL.

This study was In line with the research conducted by Kumar and Vimalavathini (2004) stated that *Benincasa hispida* Thunb. Cogn extracted with methanol has antibacterial ability. This extract can inhibit the growth of *Propionibacterium acne* and *Staphylococcus epidermidis*, where both of these bacteria cause inflammation in zits. [18] describe from the results of exploration that baligo contains several phytochemical compounds which include; triterpenene (alnusenol, multiflasenol, isomultiflasenol), flavone (iso-vitesix) and sterol (lupeol, lupeol acetate and β-sitosterol). Some important constituents isolated from *Benincasa hispida* Thub Cogn fruit include: triterpenes, sterols and glycosides and volatile oils. Based on the acute toxicity test conducted by Qodrie et al (2009) on the ethanol extract of *Benincasa hispida* Thunb. Cogn, this extract is not lethal until the use of 5 g / kg bw. There was no symptom of poisoning in albino wistar rats during the study.

4. Conclusion

Bioactivity test of kundur fruit ethanol extract (*Benincasa hispida* Thunb. Cogn) as antibacterial against *Salmonella typhi* using well diffusion method made in 4 variations of concentration namely 20,000 μg / mL (1000 μg / well), 10,000 μg / mL (500 μg / well), 1000 μg / mL (50 μg / well), and 100 μg / mL (5 μg / well) in 10% DMSO. Screening results of sample concentrations were 20,000 μg / mL (1000 μg / well), 10,000 μg / mL (500 μg / well), and 1000 μg / mL (50 μg / well) showed clear zones, which means that ethanol extracts has bioactivity as an antibacterial agent againsts *Salmonella typhi*. The effectiveness of kundur fruit ethanol extract (*Benincasa hispida* Thunb. Cogn) showed a MIC value of 800 μg / mL to inhibit the growth of *Salmonella typhi*. Allegedly at concentrations above 800 μg / mL, this extract has a "complete bactericidal" property so that the minimum killer concentration (KBM) value is above the KHM value.

References

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References

[1] Ahmed F et al 2008 Antibacterial cytotoxic and neuropharmacological activities of Cerbera odollam seeds. Oriental Pharmacy and Experimental Medicine. 8 (4). pp 323-328.
[2] Badan Pengawasan Obat dan Makanan (BPOM), 2010. Acuan Sediaan Herbal. Vol.5 Edisi 1. Badan Pengawas Obat Republik Indonesia, Jakarta.

[3] Barnes BV, Zak DR, Denton SR, Spurr SH. 1997. Forest Ecology. John Wiley and Sons, Inc. New York.

[4] Brander G C et al 1999 Veterinary Applied Pharmacology and Therapeutic. 5th Edition. Brailler Tindal, London.

[5] Clinical and Laboratory Standards Institute (CLSI), 2006. Performance standards for antimicrobial susceptibility testing; sixteenth informational supplement. CLSI document M100-S16CLSI. Wayne, PA.

[6] Gan V H S and Setiabudi P 1987 Farmakologi dan Terapi. Edisi ketiga. Fakultas Kedokteran Univeritas Indonesia, Jakarta.

[7] Harborne J B 1996 Metode Fitokimia: Penuntun Cara Modern Menganalisis Tumbuhan. Cetakan ke-2. Diterjemahkan oleh Padmawinata K & Soediro I. Penerbit ITB Bandung.

[8] Indrawati et al 2014 Pengetahuan dan Pemanfaatan Tumbuhan Obat Tradisional Masyarakat Suku Moronene di Desa Rau-rau Sulawesi Tenggara. Jurnal Biowallacea Vol.1 (1): pp. 39-48, Kendari.

[9] Jahan F I et al 2010 An Evaluation of Antinociceptive Effect of Methanol Extracts of Desmodium Gangeticum (L.) Dc. Stems and Benincasa Hispida (Thunb.) Cogn. Leaves on Acetic Acid-induced Gastric Pain in Mice, Jurnal of Advances in Natural and Applied Sciences, 4(3): 365-369, USA.

[10] Jawetz et al 2001 Mikrobiologi Kedokteran Edisi I (Salemba Medika, Jakarta) pp 196-198.

[11] Kathrin and Roy M 2002 Sulfones and Sulfoxides in Ullmann’s Encyclopedia Of Industrial Chemistry. Wiley-VCH, Weinheim. Doi:10.1002/14356007.a25_487.

[12] Komansilan et al 2015 Daya Hambat Ekstrak Kulit Manggis (Garcinia mangostana L.) terhadap Streptococcus mutans. Jurnal e-GiGi (eG). 3(2) : 309-316.

[13] Kumar A and Vimalavathini R 2004 Possible anorectic effect of methanol extract of Benincasa hispida (Thunb.) Cong, fruit. Indian J Pharmacol, 36 : 348–350.

[14] Lalitha M K 2005 Manual on Antimicrobial Susceptibility Testing. India Association of Medical Microbiologist. Departemen of Microbiology (Vellore, Tamil Nadu).

[15] Mulia H 2011 Pengaruh ekstrak biji bligo (Benincasa hispida) terhadap Penghambatan Efek Toksis Tartrazin dan Rhodamin pada Aktivitas Proliferasi Limfosit Tikus (Bogor : Institut Pertanian Bogor).

[16] Qodrie Z L 2009 Antinocicepative and antipyretic activity of Benincasa hispida Thumb cogn in wistar albino rats. Pak. J. Pharm. Sci. 22 (3): 287-290.

[17] Raflizar and Sihombing M 2009 Dekok Daun Paliasa (Kleinhovia Hospita Linn) Sebagai Obat Radang Hati Akut (Jurnal Ekologi Kesehatan Vol 8 No 2) Badan Litbangkes Depkes RI.

[18] Rahman M D A et al 2011 Antinociceptive Antibacterial & Diuretic Activities of Cerbera odollam Gaertn Roots. Journal of Pharmaceutical. Biological and Chemical Sciences. 2 (3).pp 16-23.

[19] Sahidin I et al 2018 A-Nor steroids from the marine sponge. Clathria species. Malaysian Journal of Analytical Sciences 22: 375 – 382

[20] Sugiyono 2011 Metode Penelitian Kuantitatif Kualitatif dan R&D. Alfabeta.
[21] Wiegand I et al 2008 Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. Nature Protocols, 3: 163 – 175.

[22] Zahro L Agustini R 2013 Uji Efektivitas Antibakteri Ekstrak Kasar Saponin Jamur Tiram Putih (Pleurotus ostreatus) terhadap Staphylococcus aureus dan Escherichia coli. UNESA Journal of Chemistry. 2(3): p. 120-9.