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 charactherized 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% $BaCl_2$ and 1% H_2SO_4. 1.175 g 1% $BaCl_2$ solution was mixed with 9.95 ml 1% H_2SO_4 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 $BaCl_2$ 1% and 9.95 mL 1% H_2SO_4 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, 32 µg / mL, 16 µg / mL, 16 µg / mL, 8 µg / mL, 4 µg / mL 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^{o}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 determinated starting from the concentration of 1000-100 µg / mL.

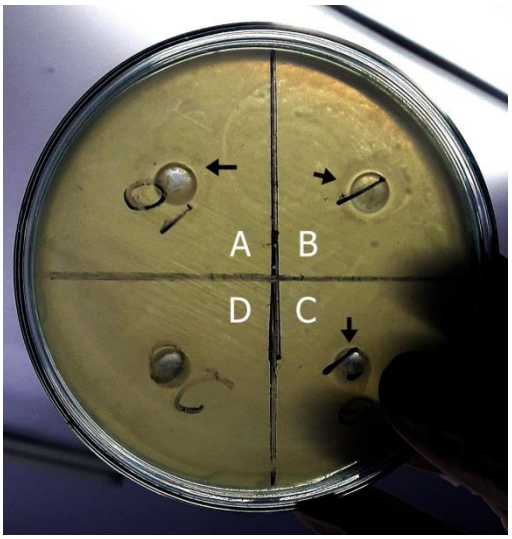

Fig. 1. Results of screening for antibacterial activity of kundur fruit ethanol extract at concentrations of 1000 µg / well (A), 500 µg / well (B), 50 µg / well (C), and 5 µg / well (D) against *S. typhi.*

Table 1. The antibacterial test results of the kundur fruit ethanol extract using the microdilution method

Sampel	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

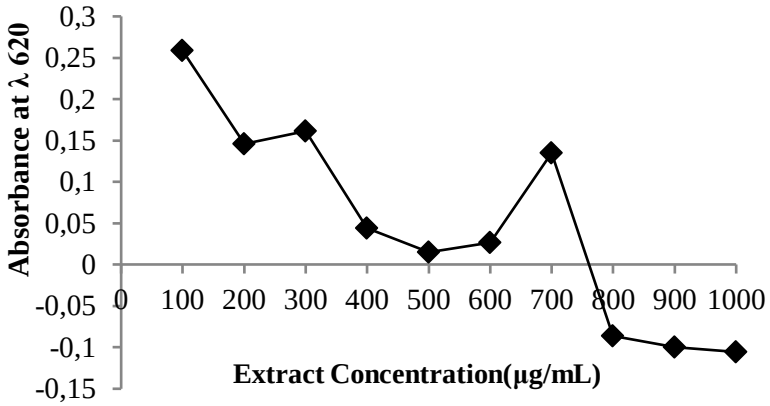

Fig. 2. The effect of the variation in concentration of kundur fruit ethanol extract on *Salmonella typhi* observed by UV-Vis spectroscopic method.

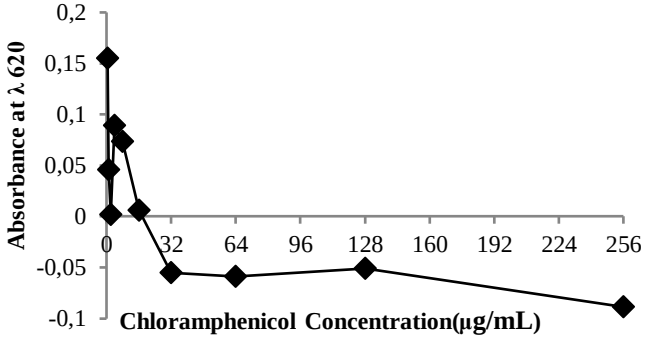

Fig. 3. The effect of the variation in chloramphenicol concentration on *Salmonella typhi* observed by UV-Vis spectroscopic method.

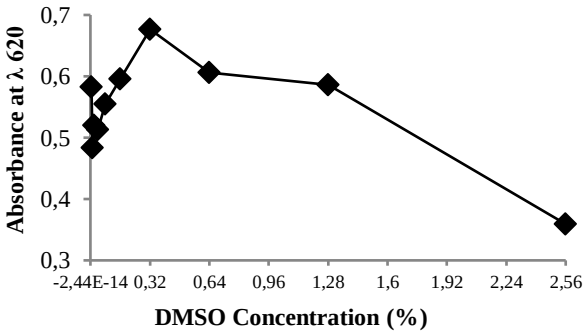

Fig. 4. The effect DMSO (solvent) concentration on *Salmonella typhi* observed by UV-Vis spectroscopic method.

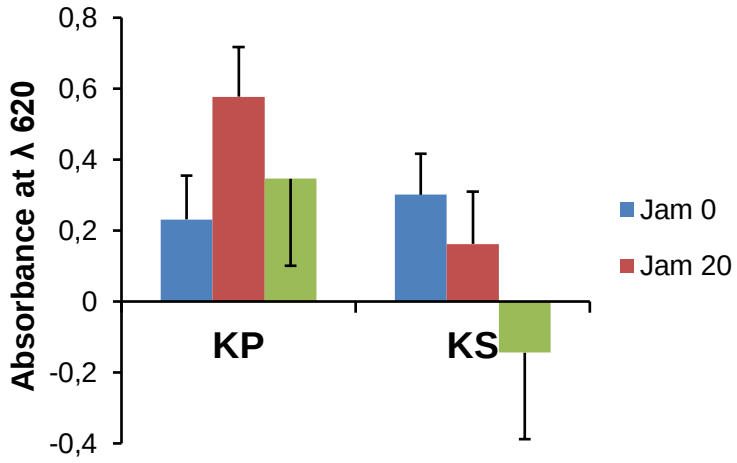

Fig. 5. Control chart of *Salmonella typhi* growth (KP) and sterility control (KS) observed by UV-Vis spectroscopic method.

		1	2	3	4	5	6	7	8	9	10	11	12
Sample	**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
Sample	**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
Sample	**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
Sample	**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	**E**	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	**F**	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	**G**	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%	**H**	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.

		1	2	3	4	5	6	7	8	9	10	11	12
Sampel	**A**	1000 µg/mL	900 µg/mL	800 µg/mL	700 µg/mL	600 µg/mL	500 µg/mL	400 µg/mL	300 µg/mL	200 µg/mL	100 µg/mL	**KP**	**KS**
Sampel	**B**	1000 µg/mL	900 µg/mL	800 µg/mL	700 µg/mL	600 µg/mL	500 µg/mL	400 µg/mL	300 µg/mL	200 µg/mL	100 µg/mL	**KP**	**KS**
Sampel	**C**	1000 µg/mL	900 µg/mL	800 µg/mL	700 µg/mL	600 µg/mL	500 µg/mL	400 µg/mL	300 µg/mL	200 µg/mL	100 µg/mL	**KP**	**KS**
Sampel	**D**	1000 µg/mL	900 µg/mL	800 µg/mL	700 µg/mL	600 µg/mL	500 µg/mL	400 µg/mL	300 µg/mL	200 µg/mL	100 µg/mL	**KP**	**KS**
Kloram	**E**	256 µg/mL	128 µg/mL	64 µg/mL	32 µg/mL	16 µg/mL	8 µg/mL	4 µg/mL	2 µg/mL	1 µg/mL	0.5 µg/mL	**KP**	**KS**
Kloram	**F**	256 µg/mL	128 µg/mL	64 µg/mL	32 µg/mL	16 µg/mL	8 µg/mL	4 µg/mL	2 µg/mL	1 µg/mL	0.5 µg/mL	**KP**	**KS**
Kloram	**G**	256 µg/mL	128 µg/mL	64 µg/mL	32 µg/mL	16 µg/mL	8 µg/mL	4 µg/mL	2 µg/mL	1 µg/mL	0.5 µg/mL	**KP**	**KS**
DMSO 10%	**H**	2.56%	1.28%	0.64%	0.32%	0.16%	0.08%	0.04%	0.02%	0.01%	0.005%	**KP**	**KS**

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 flavonoida 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^{o}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, 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 thyphi*, 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* Thunb 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 againts *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.

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

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 charactherized 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% $BaCl_2$ and 1% H_2SO_4. 1.175 g 1% $BaCl_2$ solution was mixed with 9.95 ml 1% H_2SO_4 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 $BaCl_2$ 1% and 9.95 mL 1% H_2SO_4 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, 32 µg / mL, 16 µg / mL, 16 µg / mL, 8 µg / mL, 4 µg / mL 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^{o}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 determinated starting from the concentration of 1000-100 µg / mL.

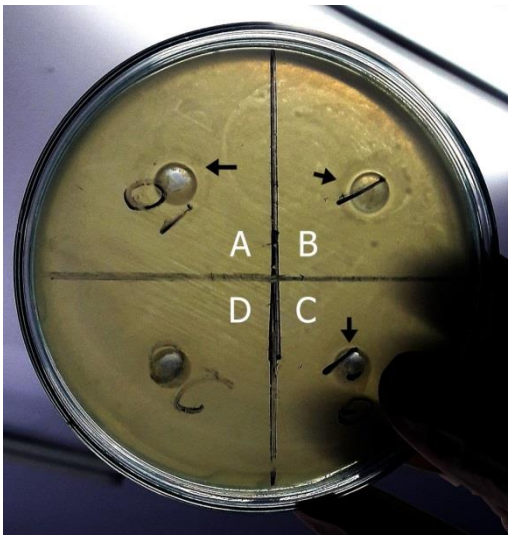

Fig. 1. Results of screening for antibacterial activity of kundur fruit ethanol extract at concentrations of 1000 µg / well (A), 500 µg / well (B), 50 µg / well (C), and 5 µg / well (D) against *S. typhi.*

Table 1. The antibacterial test results of the kundur fruit ethanol extract using the microdilution method

Sampel	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

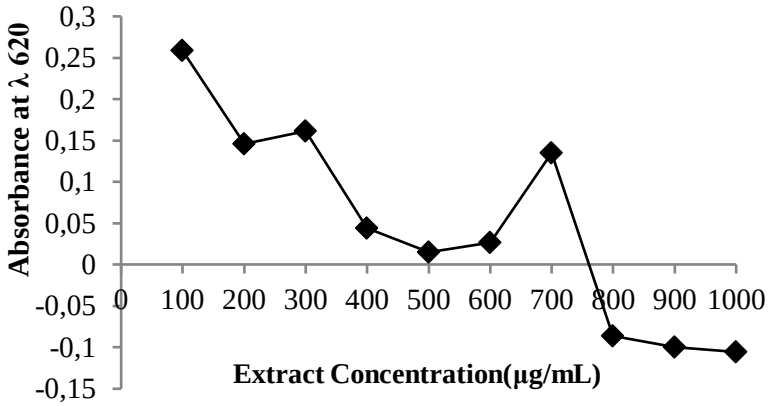

Fig. 2. The effect of the variation in concentration of kundur fruit ethanol extract on *Salmonella typhi* observed by UV-Vis spectroscopic method.

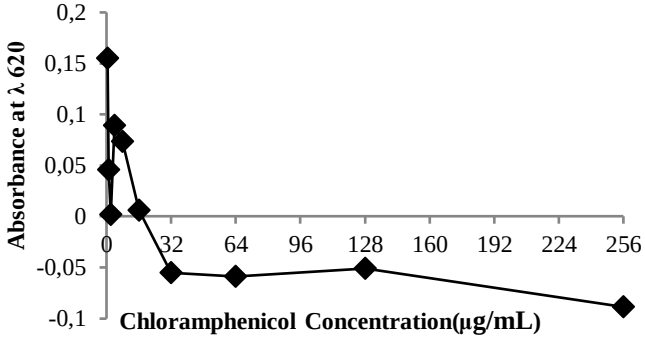

Fig. 3. The effect of the variation in chloramphenicol concentration on *Salmonella typhi* observed by UV-Vis spectroscopic method.

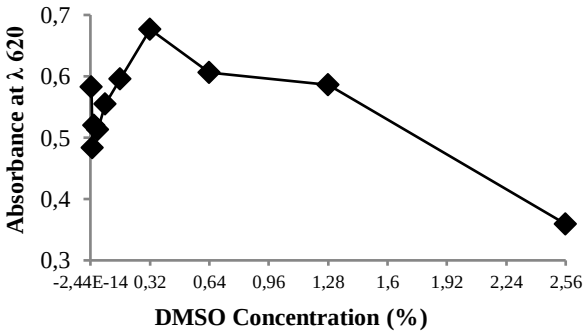

Fig. 4. The effect DMSO (solvent) concentration on *Salmonella typhi* observed by UV-Vis spectroscopic method.

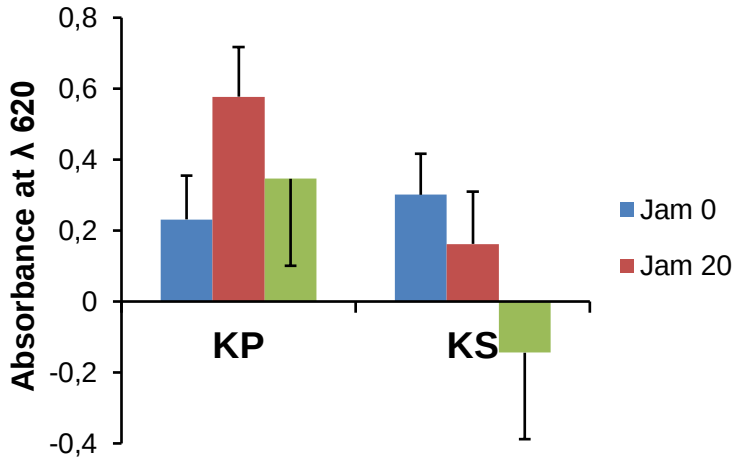

Fig. 5. Control chart of *Salmonella typhi* growth (KP) and sterility control (KS) observed by UV-Vis spectroscopic method.

		1	2	3	4	5	6	7	8	9	10	11	12
Sample	**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
Sample	**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
Sample	**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
Sample	**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	**E**	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	**F**	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	**G**	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%	**H**	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.

		1	2	3	4	5	6	7	8	9	10	11	12
Sampel	**A**	1000 µg/mL	900 µg/mL	800 µg/mL	700 µg/mL	600 µg/mL	500 µg/mL	400 µg/mL	300 µg/mL	200 µg/mL	100 µg/mL	**KP**	**KS**
Sampel	**B**	1000 µg/mL	900 µg/mL	800 µg/mL	700 µg/mL	600 µg/mL	500 µg/mL	400 µg/mL	300 µg/mL	200 µg/mL	100 µg/mL	**KP**	**KS**
Sampel	**C**	1000 µg/mL	900 µg/mL	800 µg/mL	700 µg/mL	600 µg/mL	500 µg/mL	400 µg/mL	300 µg/mL	200 µg/mL	100 µg/mL	**KP**	**KS**
Sampel	**D**	1000 µg/mL	900 µg/mL	800 µg/mL	700 µg/mL	600 µg/mL	500 µg/mL	400 µg/mL	300 µg/mL	200 µg/mL	100 µg/mL	**KP**	**KS**
Kloram	**E**	256 µg/mL	128 µg/mL	64 µg/mL	32 µg/mL	16 µg/mL	8 µg/mL	4 µg/mL	2 µg/mL	1 µg/mL	0.5 µg/mL	**KP**	**KS**
Kloram	**F**	256 µg/mL	128 µg/mL	64 µg/mL	32 µg/mL	16 µg/mL	8 µg/mL	4 µg/mL	2 µg/mL	1 µg/mL	0.5 µg/mL	**KP**	**KS**
Kloram	**G**	256 µg/mL	128 µg/mL	64 µg/mL	32 µg/mL	16 µg/mL	8 µg/mL	4 µg/mL	2 µg/mL	1 µg/mL	0.5 µg/mL	**KP**	**KS**
DMSO 10%	**H**	2.56%	1.28%	0.64%	0.32%	0.16%	0.08%	0.04%	0.02%	0.01%	0.005%	**KP**	**KS**

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 flavonoida 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^{o}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, 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 thyphi*, 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* Thunb 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 againts *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.

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