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Antibacterial Activities of Shallot (*Allium Cepa*) and Garlic (*Allium Sativum*) Skin Extracts

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ABSTRACT

Shallots (Allium cepa) and garlic (Allium sativum) are kind of the plants that are widely planted and distributed in Indonesia. The tropical climate and fertile soil in Indonesia make shallots and garlic easy to grow only by cultivating the seeds or bulbs. Shallots and garlic are type of vegetable forming a small bulb-like shape which can be used to flavor food (spices or seasoning); lower cholesterol; overcome acnes, inflammation; and acting as antioxidants, antimicrobials. Moreover, they can be used to help the growth of the roots plants. This research compared the extraction methods, maceration and soxhletation, for the extraction of secondary metabolites in shallot and garlic skins. the results showed that the extraction yield of shallot skin extract by maceration and soxhletation were 0.96% and 0.70%, respectively. While the extract of garlic skin obtained from maceration and soxhletation method were 0.71% and 0.47%, respectively. Both of the extracts were identified for its secondary metabolite components qualitatively using phytochemical tests. The results claimed that the extracts to contained flavonoids, alkaloids, saponins and tannins. Based on agar diffusion method on disc papers, the antibacterial activity assays of shallot and garlic skin extracts showed that shallot and garlic skin extracts from maceration and soxhletation extraction methods were able to inhibit the growth of Escherichia coli, Staphylococcus aureus, and Propionibacterium acnes bacteria.

KEYWORDS: Allium cepa, Allium sativum, Antibacterial

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INTRODUCTION

Antibacterial agents are compounds used to control the growth of harmful bacteria. Controlling the growth of microorganisms aims to prevent the spread of disease and infection, as an exterminator of microorganisms in infected hosts, and prevent decay and destruction of materials by microorganisms (Marfuah *et al*., 2018) *Escherichia coli*, *Staphylococcus aureus*, *Propionibacterium acnes* are bacteria that are often used as bacteria in antibacterial research.

Escherichia coli bacteria have the ability to infect tissues outside the gut such as in urinary tract infections, sepsis and meningitis. *Escherichia coli* bacteria can be transmitted through contaminated food and drink and enter the body with a low immune system. As in infants, children and the elderly or people who are sick (Sari *et al*., 2020). *Escherichia coli* bacteria can also cause diseases of the food digestive tract such as cholera, typhoid, dysentery, diarrhea and worm diseases (Irawan *et al*., 2022).

Staphylococcus aureus is a major pathogenic bacteria in humans found in various parts of the human body, including the nose, throat, skin, and therefore easily enters food. Almost everyone has experienced various *Staphylococcus aureus* infections during their lifetime, from minor skin infections, to severe food poisoning, to incurable infections (Brooks *et al*., 2005).

Propionibacterium acnes is the most common gram-positive bacteria with no spores, anaerobic stalks, found in clinical specimens *Propionibacterium acnes* generally grows as an obligate anaerobe, mace-shaped or pointed with uneven and beaded staining and sometimes coccus-shaped or round (Rosyad, 2009).

Shallots (*Allium cepa*) contain active flavonoid compounds that are anti-inflammatory or anti-inflammatory, very useful to help heal inflammation due to bruises, burns, or inflammation of internal organs. Shallots function as natural antioxidants that can suppress the carcinogenic effects of free radical compounds. The content of compounds in shallots also plays a role in neutralizing harmful toxin substances and helps remove them from the body (Kurniawati, 2010).

Garlic (*Allium sativum* L.) has antimicrobial activity by enhancing immune system function (Kathi J., 2000). The ability of *Allium sativum* L. as an antimicrobial compound is related to the content of allicin compounds. This allicin

compound plays an antimicrobial role. Initially allicin is formed when there is a garlic defense mechanism against attacks, if garlic is attacked and gets injured then the enzymatic reaction will produce allicin, the allinase enzyme will convert allin into allicin and will make a toxic effect for insects and microorganisms (Ankri and Mirelman, 1999).

Extraction is one way to extract antioxidant compounds contained in Moringa leaves. Extraction can be done by several methods, such as maceration, soxhletation, distillation, fractionation and percolation. The maceration extraction method has many advantages and is the most widely practiced method compared to other extraction methods (Sitepu, 2015). Maceration extraction is a simple extraction process using a solvent with several stirs at room temperature. While the soxhletation extraction method is a solvent extraction that is always new, usually carried out with special equipment in such a way that continuous extraction occurs with a relatively constant amount of solvent under cooling conditions again (Istiqomah, 2013).

The selection of maceration and soxhletation methods is because both methods have many advantages over other extraction methods. The main advantage of the maceration extraction method is that the procedures and equipment used are simple and the maceration extraction method does not require heating, so natural materials are not decomposed. Although many compounds can be extracted by cold extraction, some compounds have limited solubility in the extraction solvent at room temperature. Soxhletation extraction method is the best extraction method to obtain extraction results using a particular solvent, requires a shorter time, the extracted sample becomes maximum because it is done repeatedly (Istiqomah, 2013).

Based on the above background, it is necessary to test the antibacterial activity of shallot and garlic skin extracts against the growth of *Escherichia coli*, *Staphylococcus aureus*, *Propionibacterium acnes* bacteria.

RESEARCH METHODS

The samples used in this study were shallot skin and garlic skin obtained from Jember Regency. Extraction using maceration and soxhletation methods, where the maceration method is carried out using a mixed solvent between ethanol and *n*-hexane (1: 3), while the soxhletation method is carried out using *n*-hexane solvent.

The extraction process in this study used the maceration method with ethanol and *n*-hexane (1:3) as the solvent. Weigh each shallot and garlic powder and put each in an erlenmeyer, then add mixed solvents and macerate for ± 24 hours. Then filtered using a funnel, the filtrate obtained is then concentrated using a rotary evaporator to obtain a thick extract of shallot skin maceration results and thick extract of garlic skin.

Extraction by soxhletation method is done by weighing each 100 grams of shallot and garlic skin simplisia powder wrapped using filter paper, tied with thread at both ends and inserted into the soxhlet tool. The solvent of 96% ethanol is put into the soxhlet flask as much as 300 mL. Perform soxhletation with a heating temperature between 81-96^{o}C until the cycle droplets are no longer colored or ± 7 cycles. The liquid extract obtained was concentrated using a rotary evaporator at 50^{o}C. The remaining solvent is evaporated using a water bath until each thick extract of shallot skin and garlic skin is obtained.

Identification of secondary metabolite compounds is done qualitatively using test tubes. The following is how to test the identification of secondary metabolite compounds:

1. Flavonoid Test

Thick extract was taken as much as 0.5 mL coupled with 5 mL of concentrated H_2SO_4 and 5 mL of dilute ammonia. The sample is observed if it contains flavonoid compounds it will experience a color change from greenish yellow to yellow (Hasanah dkk, 2017).

2. Alkaloid Test

The addition of reagents to determine the presence of alkaloid compounds in the sample can use Wagner reagent (Iodine and KI) positive results contain alkaloid compounds if there is a brown precipitate and Meyer reagent ($HgCl_2$ dissolved in distilled water and KI dissolved in distilled water) positive results contain alkaloids and white-colored deposits (Putra dkk, 2016).

3. Saponin Test

The extract is put into a measuring flask dissolved in 20 mL of distilled water, the sample is shaken for up to 15 minutes. If there is foam as high as 1 cm then the sample indicates the presence of saponin compounds (Prawirodihardjo, 2014).

4. Polyphenol Test

A total of 1 mL of sample was added with 1% $NaCl_3$ reagent mixed until homogeneous. If the solution turns blackish or bluish, the sample is positive for polyphenol (Putra dkk, 2016).

5. Tannin test

The tannin test can be done by adding 1% $FeCl_3$ reagent solution as much as 2 to 3 drops into the sample extract and mixed until homogeneous. If the results show positive, the test solution will turn blue-black or greenish (Yuliani & Dienina, 2015).

Testing the antibacterial activity of shallot and garlic skin extracts was carried out by the agar fusion method using 3 bacteria, namely *Escherichia coli*, *Staphylococcus aureus* and *Propionibacterium acnes*. Sterilize all tools used. Non-glass tools were sterilized using an autoclave for 15 minutes at 121^{o}C and glass tools were sterilized using an oven for 1 hour at 160-170^{o}C. The ose wire and tweezers were sterilized using Bunsen flame.

Making the test media used are *Nutrient Agar* (NA) and *Nutrient Broth* (NB) media. Making NA media by taking the media and weighing it as much as 7 grams and then putting it into an erlenmeyer and adding 250 mL of distilled water. Making NB media by taking the media and weighing as much as 0.32 grams then put into an erlenmeyer and add 25 mL of distilled water. The media is heated on a *hot plate* until the material dissolves. Then the media is sterilized using an

autoclave for 15 minutes at 121ºC. The media that has been sterilized is poured into a sterile Petri dish.

The concentration used in this sample is concentrated extract and 50% concentration. Making negative control using 10% DMSO by means of 10 mL of DMSO put into a measuring cup and added distilled water to a volume of 100 mL. Making positive control using antibiotics by inserting 0.5 mg dissolved in 5 mL DMSO.

Bacterial test preparations were carried out including rejuvenation of *Escherichia coli*, *Staphylococcus aureus* and *Propionibacterium acnes* bacteria which were carried out by taking one ose each and then inoculated by scratching on tilted Nutrient Agar media. After that, it was incubated at 37ºC for 24 hours. After that, one ose of colonies from NA media was taken into NB media and then vortexed to make it homogeneous. After that, it is incubated at 37ºC for 24 hours. If the media is cloudy, there is bacterial growth.

Making a suspension of *Escherichia coli*, *Staphylococcus aureus* and *Propionibacterium acnes* bacteria by taking 1 mL and then putting it into a test tube that already contains 10 mL of 0.9% NaCl then vortexed. Before testing the antibacterial test, turbidity testing was carried out on the suspension with the Mc Farland standard. Mc Farland standard density is checked by measuring the absorbance using a spectrophotometer. Absorbance was measured at a wavelength of 625 nm in the range of 0.08-0.13 (Dalynn, 2014).

The antibacterial activity test was carried out by pouring the media into a petri dish and then waiting for it to solidify, then taking 100 µL of each bacterial suspension and inserting it into the solid media. After that, dip the paper disk into 10% DMSO for negative control. For positive control, dipped in antibiotics and for treatment control dipped in onion and garlic skin extracts with concentrated extract concentration and 50% concentration. After dipping, it is placed on NA media that has been inoculated with test bacteria and then marked. Then incubated at 37ºC for 1x24 hours. Activity was determined based on the presence of a clear zone in the paper disk area. Each antibacterial activity test was carried out 3 times.

RESULT AND DISCUSSION

Simplisia of shallot and garlic skin that has been dried is obtained as much as 1.5 kg. The following is a picture of shallots and garlic that have been made into powdered simplisia:

Figure 1. The samples: shallots and garlic skins

Extraction of shallots and garlic was carried out using maceration and soxhletation methods. The maceration method was carried out using a mixed solvent of ethanol:*n*-hexane (1:3) as much as 1000 mL, while the soxhletation method used *n*-hexane solvent as much as 300 mL. the following is a picture of the maceration and soxhletation extraction methods:

Figure 2. The set up of maceration and soxhletation methods

Identification of secondary metabolite compounds in shallot and garlic extracts was carried out qualitatively using a test tube. In table 1 below is the compound identification data.

Table 1. Phytochemical identification for shallot and garlic skins' extracts

Compound	Results	Description
Favonoids	Positive (+)	Turns yellow in color
Alkaloids	Positive (+)	Wagner reagent: there is a brown precipitate Mayer reagent: there is a white precipitate
Saponins	Positive (+)	There is 1 cm of foam
Polifenol	Negative (-)	No change
Tannin	Positive (+)	Positive for tannin, the test solution turns blue-black or greenish

Figure 3 shows the results of identification of secondary metabolite compounds in shallot and garlic skin extracts

showing that shallot and garlic extracts are positive for flavonoids, alkaloids, saponins and tannins.

Figure 3. Phytochemical assays

Antibacterial activity testing was carried out using *Escherichia coli*, *Staphylococcus aureus*, *Propionibacterium acnes* bacteria. Bacterial testing was carried out using the agar diffusion method, observations were made by measuring the clear zone around the paper disk. The following is a table of antibacterial testing data with maceration and soxhletation extraction methods of shallot and garlic skin samples:

Table 2. Shallot maceration antibacterial testing

Bacteria	Inhibition (mm) Maserasi (Shallot)				
	R	**K +**	**K -**	**Concentrated extract**	**Concentration 50%**
Escherichia coli	1	23.41	0	15.79	10.54
	2	19.68	0	12.07	9.33
	3	20.05	0	14	10.02
	Average	21.05	0	13.95	9.96
	SD	2.06	0	1.86	0.61
Staphylococcus aureus	1	12.38	0	9.19	6.55
	2	11.74	0	9.2	5.81
	3	11.22	0	10	6.02
	Average	11.78	0	9.46	6.13
	SD	0.58	0	0.46	0.38
Propionibacterium acnes	1	17.32	0	13.3	9.5
	2	17.76	0	11.92	7.75
	3	14.21	0	11	8.39
	Average	16.43	0	12.07	8.55
	SD	1.94	0	1.16	0.89

Table 3. Antibacterial testing of garlic maceration

Bacteria	Inhibition (mm) Maceration (Garlic)				
	R	**K +**	**K -**	**Concentrated extract**	**Concentration 50%**
Escherichia coli	1	13.32	0	10.87	9.01
	2	28	0	11.83	9.75
	3	28.09	0	25.77	20.62
	Average	23.14	0	16.16	13.13
	SD	8.50	0	8.34	6.50
Staphylococcus aureus	1	14.98	0	10.02	8.06
	2	10.57	0	9.17	7.8
	3	10.39	0	9.49	7.78
	Average	11.98	0	9.56	7.88
	SD	2.60	0	0.43	0.16
Propionibacterium acnes	1	19.8	0	14.03	11.18
	2	14.23	0	11.25	9.48
	3	13.78	0	11.9	10.55
	Average	15.94	0	12.39	10.40
	SD	3.35	0	1.45	0.86

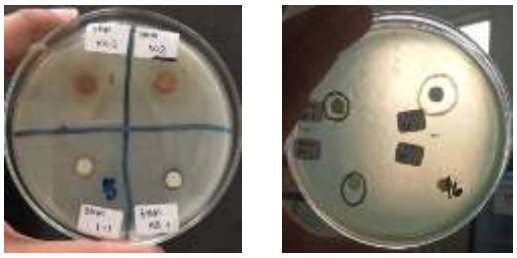
Table 4. Shallot soxhletation antibacterial testing

Bacteria	Inhibition (mm) Soxhletation (Shallot)				
	R	**K +**	**K -**	**Concentrated extract**	**Concentration 50%**
Escherichia coli	1	11.93	0	9.4	5.88
	2	12.76	0	9.88	5.34
	3	11.13	0	9.98	5.49
	Average	11.94	0	9.75	5.57
	SD	0.82	0	0.31	0.28
Staphylococcus aureus	1	10.53	0	9.82	7.27
	2	10.71	0	9.03	7
	3	11.7	0	9.26	6.55
	Average	10.98	0	9.37	6.94
	SD	0.63	0	0.41	0.36
Propionibacterium acnes	1	15.28	0	10.6	8.15
	2	13.84	0	9.78	8.55
	3	15.55	0	10.55	8.02
	Average	14.89	0	10.31	8.24
	SD	0.92	0	0.46	0.28

Table 5. Antibacterial testing of garlic soxhletation

Bacteria	Inhibition (mm) Soxhletation (Garlic)				
	R	K +	K -	Concentrated extract	Concentration 50%
Escherichia coli	1	20.45	0	15.16	11
	2	14.66	0	11.76	10.13
	3	17.29	0	13.09	8.35
	Average	17.47	0	13.34	9.83
	SD	2.90	0	1.71	1.35
Staphylococcus aureus	1	14.98	0	11.06	7.78
	2	16.49	0	11.93	8.24
	3	14.54	0	10.87	7.44
	Average	15.34	0	11.29	7.82
	SD	1.02	0	0.57	0.40
Propionibacterium acnes	1	19.38	0	14.28	11.99
	2	16.49	0	13	11.26
	3	13.72	0	11.26	10.4
	Average	16.53	0	12.85	11.22
	SD	2.83	0	1.52	0.80

The following are images of antibacterial testing of shallot and garlic skin extracts on *Escherichia coli*, *Staphylococcus aureus*, and *Propionibacterium acnes* bacteria.



Maceration **Soxhletation**

Figure 4. Shallot extract testing on *Escherichia coli* bacteria

Maceration **Soxhletation**

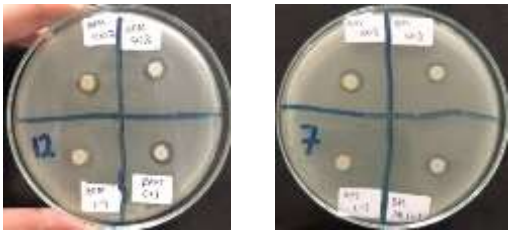
Figure 5. Shallot extract testing on *Staphylococcus aureus* bacteria

Maceration **Soxhletation**

Figure 6. Testing shallot extract of *Propionibacterium acnes* bacteria

Maceration **Soxhletation**

Figure 7. Testing garlic extract *Escherichia coli* bacteria



Maceration **Soxhletation**

Figure 8. Testing of garlic extract for *Staphylococcus aureus* bacteria

Maceration **Soxhletation**

Figure 9. Testing of garlic extract of *Propionibacterium acnes* bacteria

DISCUSSION

Shallots and garlic skins were determined first to prove the correctness of the samples used in this study. Furthermore, making simplisia powder of shallot and garlic skin which will be used for extraction by maceration and soxhletation methods. Shallot and garlic skins in maceration extraction are renadamized with a mixture of ethanol:*n*-hexane solvent (1:3), while the soxhletation method uses *n*-hexan solvent which is extracted for ± 5 hours, until ± 7 cycles or the extraction liquid becomes clear which indicates that most of the sample has been extracted.

The percent of the average extract of shallot skin in the maceration method is obtained 0.96% and soxhletation of

shallot 0.70%, while the percent of garlic skin extract in the maceration method is 0.71% and the garlic soxhletation method is 0.47%. Repetition of the extraction of both methods was carried out as many as 3 (three) replications. The following table 6 shows the percentage of extracts from both extraction methods:

Table 6. Percent yields of the extracts

Sample	Percent of extract	
	Maceration	Soxhletation
Shallot skin	0,96 %	0,70 %
Garlic skin	0,71 %	0,47 %

Note: The percent of extract yield (w/w) is calculated as extract weight/dry sample weight x 100%.

The percent of shallot and garlic skin extracts with the maceration method is greater than the soxhletation method. This can be caused because in the maceration method, the sample is soaked in an organic solvent for 24 hours. The immersion of this sample causes contact between the sample and the organic solvent long enough so that the extracted material is more. This result is in accordance with the statement (Ketaren, 1985) that extraction with organic solvents is more effective because the remaining oil in the material is only 1-2%. Another factor that can cause the maceration method to have a greater percentage is the extraction temperature. The extraction temperature used in the maceration method can avoid degradation of the extract because the maceration method uses room temperature. This is different from the temperature used in the soxhletation method, which is with a heating temperature between 81-96^{o}C carried out until the cycle droplets are no longer colored or ± 7 cycles.

Antibacterial activity testing was carried out to determine whether shallot and garlic skin extracts have antibacterial activity against the growth of *Escherichia coli*, *Staphylococcus aureus*, and *Propionibacterium acnes* bacteria. In this antibacterial test using the agar diffusion method and extracts can be said to inhibit bacterial growth with a clear zone around the paper disk on bacterial growth media. The resulting clear zones can be seen in Figure 4 to Figure 9. The results of the antibacterial activity test show that shallot and garlic skin extracts with maceration and soxhletation methods are able to inhibit the growth of *Escherichia coli*, *Staphylococcus aureus*, and *Propionibacterium acnes*.

CONCLUSION

From the research that has been done, it can be concluded that: Identification of secondary metabolite compounds in shallot and garlic skin extracted using maceration and soxhletation methods showed positive results for the presence of secondary metabolite compounds, namely flavonoids, alkaloids, saponins, and tannins. Antibacterial activity testing on shallot and garlic skin extracts is indicated by the zone of inhibition or clear zone around the paper disk on the growth media of *Escherichia coli*, *Staphylococcus aureus* and *Propionibacterium acnes* bacteria.

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