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ANTIBACTERIAL ACTIVITIES OF ETHANOL EXTRACTS OF DURIAN FRUIT SKIN (Durio zibethinus Murr.) ON Salmonella BACTERIA in ATCC 14028 and Bacillus cereus ATCC 11778 CAUSE OF DIARRHEA

Kurnia Putri Jamal[1], Muhaimin[1], Fitrianingsih[1*]

1. Department of Pharmacy, Faculty of Science and Technology, Universitas Jambi.
Contact Address : Jl. Jambi-Ma. Bulian KM 15 Mendalo Darat Jambi 36361

Abstract

Diarrhea is a condition where the frequency of defecation occurs more than 3 times a day. One of the causes of diarrhea is a bacterial infection, so an antibacterial agent is needed to overcome the problem. One of the natural ingredients that can be utilized is Durian. Based on phytochemical screening, durian fruit skin meat contains alkaloid compounds, saponins, flavonoids and tannins which can inhibit bacterial growth. This study aimed to determine the antibacterial activity of ethanol extract of durian fruit skin flesh (Durio zibethinus Murr) against Salmonella bacteria on ATCC 14028 and Bacillus cereus ATCC 11778. This study used an experimental design, extract was obtained by maceration method. Testing of the antibacterial activity of ethanol extract using the disc diffusion method. The treatment used 15%, 20%, 25%, 30% and 35% concentrations, negative controls using distilled water and positive controls using tetracycline solution. The results showed that testing on Salmonella bacteria had good antibacterial activity up to a concentration of 30% with a diameter of 11 mm inhibition zone, whereas in Bacillus cereus bacteria at a concentration of 20% gave antibacterial activity which included a weak category with a diameter of inhibitory zone 15 mm. Based on the results of phytochemical screening of durian fruit peel extract containing secondary metabolites, namely flavonoids, alkaloids, saponins and tannins and the results of testing data on the activity it can be concluded that durian fruit flesh extract still has potential as an antibacterial agent. So it is necessary to do the next step with fractionation and isolation of other active compounds. antibacterial activity data.

Keywords: *Antibacterial Activity, Bacillus cereus, Salmonella typhi, Durian Skin, Inhibitory Zone*

Introduction

Infectious disease is one of the health problems in human digestion due to the presence of foreign antigens that attack the body, causing a high rate of morbidity and mortality in Indonesia (Darmadi, 2008). In developed countries, diseases caused by infections are still a high problem (Noer, 2012). In 2013 the incidence rate in Indonesia reached 4,128,526 patients, increasing every year from 2006 to the highest in 2013. Bacterial infections are pathogenic conditions caused by microorganisms that develop in the body (Subandi, 2010). Some types of microorganisms that cause infection are Escherichia coli, Salmonella, Shigella, and Yersinia enterocolitica (Radji, 2010).

One of the infectious diseases caused by microorganisms is diarrhea. Diarrhea is a condition where there is an unusual frequency of defecation (more than 3 times a day), changes in the amount and consistency (liquid stool) (Baughman, 1996). The prevalence of diarrhea in Indonesia is still fluctuating. Based on data from the Basic Health Research (Riskesdas) in 2007, the prevalence of clinical diarrhea was 9.0% (range: 4.2% - 18.9%), the highest in the Province of NAD (18.9%) and the lowest in D> I Yogyakarta (4.2%). Some provinces have clinical diarrhea prevalence> 9% (NAD, West Sumatra, Riau, Jambi, West Java, Central Java, Banten, West Nusa Tenggara, East Nusa Tenggara, South

Kalimantan, Central Sulawesi, Southeast Sulawesi, Gorontalo, West Papua, and Papua).

According to the 2013 Riskesdas data the prevalence decreased by (3.5%) for all age groups. The high prevalence is caused by the emergence of causes of diarrhea such as infections, allergies, malabsorption, poisoning, immunodeficiency and other causes. According to Hikmawati (2012) the bacteria that often cause diarrhea are E. coli so that it is necessary to eradicate infectious bacteria as an antibacterial agent for diarrhea. One of the natural ingredients that can be used as an antibacterial agent is durian plants (D. zibethinus Murr).

Durian fruit (D. zibethinus Murr) is a tropical plant that grows well in Indonesia with mature durian fruit reaching 30-45 cm wide by 20-25 cm and weighing between 1.5-2.5 kg containing 5 juring in it 15 seeds covered in white, cream, yellow or dark yellow flesh (Nazaruddin, 1994). The abundance of durian in Indonesia, especially in Jambi Province reached 7,037 tons in 2010 (data on durian cultivation). The skin of durian fruit can be used as a mosquito repellent and durian skin ash can be used as a medicine for skin rashes, durian leaves can be used as vegetables, and the seeds can be used as chips (Yahyono, 2012).

Salmonella Thypi and Escherichia coli bacteria cause diarrheal diseases, Shigella dysenteriae bacteria cause dysentery (Gibson, 1996). Salmonella Thypi can cause diarrhea, because Salmonella Thypi produces poisons called cytotoxin and enterotoxins (Dharmojono, 2001).

Previous research showed that the ethanol extract of durian fruit peel meat had antibacterial activity against Pseudomonas aeruginosa with a minimum inhibitory content of 4% and a minimum killing rate (KBM) of 6% (Azhari, 2015). Then durian bark extract was also used to test the antibacterial activity of Stovylococcus aureus and Salmonella enteria Serovar Typhi by using 95% ethanol extract at concentrations of 6% with inhibitory zones formed 742 mm and 2.1 mm (Muhsin et al, 2016). The skin of durian fruit contains chemical compounds including durian skin containing tannin, saponins (DePadua, 1978), alkaloids, flavonoids, triterpenoids (Nurliani, 2007).

The urgency of the research will be carried out because there is no research that tests the antibacterial activity of ethanol extracts of durian fruit skin flesh against Salmonella typhi bacteria and Bacillus cereus causing diarrhea. This study wanted to see whether durian skin produced in Jambi Province had antibacterial agents and could be useful for treating diarrhea caused by looking at the inhibition zones formed on bacterial media.

Material and Methods

Material

The skin of durian fruit taken from the Strait, Muaro Jambi Regency, Salmonella thypi and Bacillus cereus obtained from the Microbiology Laboratory, Pharmacy School, ITB. 96% ethanol, aquades, 70% alcohol, 2% dilute HCL, concentrated H2SO4, Mayer reagent, mayer reagent, bouchardat reagent, NaOH, methanol, Mg powder, concentrated HCl, 2N hydrochloric acid, 1% FeCL3, acetic acid, CHCL3, Concentrated H2SO4, aluminum foil, label paper, weigh paper, filter paper, paper discs, matches, NA (Nutrient Agar).

The tools used are rotary evaporator, analytical scale, electric stove, spatula, 2 L glass alarm, filter cloth, test tube, measuring cup, drop pipette, maceration bottle, incubator, autoclave, oven, stirring rod, petri dish, measuring flask, test tube, erlenmeyer, knife, sprite lamp, tweezers, horn spoon, stirrer, BSC (Biology Safety Cabinet)

Methods

Phytochemical Test

This test was conducted to determine the qualitative test on ethanol extract of fruit peels durian where this test was carried out to see what secondary metabolites contained in the ethanol extract of durian fruit peels.

The phytochemical test was carried out by testing the content of alkaloid compounds, flavonoids, terpenoids, saponins and tannins and repeated three times.

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*Corresponding author: fitrianingsih@unja.ac.id

Antibacterial Activity Test of Disc Diffusion Method

This test uses the disc diffusion method by means of the prepared bacterial suspension which is applied evenly on NA media using sterile stick cotton, then prepared paper discs with Tetracycline drops as a negative control, aquabidest as a positive control for Salmonella thypi and Bacilus cereus. Other disc paper was dripped with ethanol extract of durian fruit peel which had been concentrated series of 15%, 20%, 25%, 30%, 35% and negative controls, repeated four times and then incubated for 24 hours at 37oC. The inhibition zone is marked by the clear zone around the disc paper and then the diameter is measured. Data collection techniques used in this study are experimental methods, literature, and documentation. Observations carried out include qualitative testing and diameter of the inhibition zone. Data analysis was carried out in a qualitative descriptive manner.

RESULT AND DISCUSSION

Phytochemical Test

The phytochemical test is a qualitative test of the content of the active compound in a sample. Analysis of chemical content was carried out in the Agro-Industrial Laboratory of the Medicinal Plants of the Faculty of Science and Technology, University of Jambi, by looking at the presence or absence of color change reactions that occurred in the tube test. The results of phytochemical screening of ethanol extract of durian fruit peel (D. zibethinus Murr) showed the presence of alkaloid compounds, saponins, flavonoids and tannins. Screening results can be seen in Table 1.

Table 1. Phytochemical Screening Results Ethanol Extract of Durian Fruit Skin Meat

Phytochemical Screening	Reagent	Results	Information
Alkaloid	Mayer	Forms white deposits	Positive
	Bouchardat	Forms blackish brown color	Positive
	Dragendroff	Brown orange is formed	Positive
Flavonoid	Mg + HCl pekat	Concentrated Orange red color	Positive
Saponin	Air + HCl	Forms Foam	Positive
Terpenoid	Anhidrat asetat+ sulfat pekat	Concentrated sulfate Red-green-violet-blue	Negative
Tanin	FeCl3 1%	Formed violet green	Positive

The content of flavonoid compounds shows that the ethanol extract of durian fruit skin (D. zibethinus Murr) has antimicrobial activity. Flavonoids work on bacteria by damaging the cytoplasmic membrane. The bacterial cytoplasm membrane itself regulates the entry of food ingredients or nutrients, if the cytoplasmic membrane is damaged then important metabolites in the bacteria will come out and food ingredients to produce energy cannot enter so that the inability of bacterial cells to grow and occureventually death (Dzen, 2003).

Saponins are active compounds that are strong and foamy when shaken in water so they are like soap (Robinson, 1995). Saponin can increase permeability of bacterial cell

membranes so that it can change the structure and function of membranes, causing denaturation of membrane proteins so that cell membranes will be damaged and lysed. According to Dwidjoseputro (1994) states that saponins have molecules that can attract water or hydrophilic and molecules that can dissolve fat or lipophilic so that it can reduce the cell surface tension which ultimately causes the destruction of germs.

According to Akiyama et al., (2001) Tanin has antibacterial activity, in broad outline the mechanism is to damage bacterial cell membranes, tannin adstringent compounds can induce the formation of complex compound bonds to enzymes or microbial substrates and the formation of a complex bond of tannins to metal ions that can increase the toxicity of the tannin itself. The antibacterial activity of tannin compounds is by shrinking the cell wall or cell membrane, thus disrupting the permeability of the cell itself. As a result of disruption of per-

meability, cells cannot carry out living activities so that growth is stunted or even dead (Ajizah, 2004).

The mechanism of action of alkaloids as antibacterial is by disturbing the constituent components of peptidoglycan in bacterial cells, so that the cell wall layer is not formed intact and causes cell death (Darsana, 2012). Another mechanism for antibacterial alkaloids is the alkaloid component known as DNA intercellator and inhibits topoisomerase enzymes of bacterial cells (Karou, 2005). Generally the secondary metabolites obtained are polar so that it is found in the solvent used, namely 96% ethanol. Previous research conducted by Setyowati (2014) showed that methanol extract of durian skin (D. zibethinus Murr) positive petruk varieties contained secondary metabolites, namely alkaloids, flavonoids, tannins, saponins, steroids and terpenoids. Based on this, most of the results obtained are in accordance with previous research.

Antibacterial Activity Test of Disc Diffusion Method

Determining the diameter of the inhibitory zone is done by using the disc diffusion method, namely by looking at the clear zone and measuring the diameter of the clear zone. Based on the results of the study it can be seen that ethanol 96% extract of durian fruit skin flesh (D. zibethinus Murr.) Has antibacterial activity characterized by the presence of clear zones on the agar medium in determining the diameter of the inhibitory zone. The concentration of the test used in this study is 15%, 20%, 25%, 30% and 35%, this is because in Hanif's research (2015) testing has been carried out using the smallest concentration of 3.125% to 25% has provided inhibitory power in testing of antibacterial activity. The choice of concentration used in this study is based on previous research and also based on literature which says that the extract is said to have the potential to be antimicrobial if at the giving level of ≤ 1000 µg / mL it can inhibit the growth of antimicrobials (Mitscher et al., 1992).

This test is carried out starting from a concentration of 15%, 20%, 25%, 30%, 35% and repeated four times. In S. thypidia with four repetitions at a concentration of 15% giving the first inhibition zone 5 mm, the second 5 mm third 5 mm and the fourth 6 mm with an average of 5.25 mm. At a concentration of 20% the first inhibition zone is 7 mm, second is 7 mm, third is 8 mm and fourth is 7 mm with an average of 7.25 mm. At a concentration of 25% it gives the first zone of inhibition 9 mm, second 6 mm, third 8 mm and fourth9 mm with an average of 8 mm. At a concentration of 30% it gives the first zone of inhibition 12 mm, second 10 mm, third 10 mm and fourth 12 mm with an average of 11 mm. At a concentration of 35% it gives the first inhibition zone of 18 mm, second 18 mm, third 12 mm and fourth 11 mm with an average of 14.75 mm. This shows that the results of the bacterial growth inhibition zone response according to Greenwood (1995) small than 10 mm did not respond to inhibition of bacterial growth, whereas 10-15 mm response to resistance to bacterial growth was said to be weak can be seen in Table 2.

The B. cereus bacteria were tested starting from 15%, 20%, 25%, 30% and 35% concentrations and repeated four times. At a concentration of 15% the first inhibition zone is 7 mm, the second is 10 mm, the third is 10 mm and the fourth is 8 mm with an average of 8.75 mm. At a concentration of 20% the first inhibition zone is 16 mm, second is 15 mm, third is 14 mm and fourth is 15 mm with an average of 15 mm. At a concentration of 25% the first inhibition zone is 16 mm, the second is 16 mm, the third is 20 mm and the fourth is 20 mm with an average of 18 mm. At a concentration of 30% the first inhibition zone is 24 mm, the second is 25 mm, the third is 20 mm and the fourth is 20 mm with an average of 22.25 mm. At a concentration of 35% it gives the first inhibition zone of 24 mm, second 25 mm, third 25 mm and fourth 26 mm with an average of 25 mm.

Table 2. Antibacterial Activity Test for *Salmonella typhi*

Concentration	Meter of Inhibition Zone (mm)				Average	S. deviation
	I	II	III	IV		
15%	5	5	5	6	5.25	0.5
20%	7	7	8	7	7.25	0.5
25%	9	6	8	9	8	1.414
30%	12	10	10	12	11	1.155
35%	18	18	12	11	14.75	3.775
K (-)	0	0	0	0	0	0
K (+)	30	30	-	-	30	0

This shows that the result of a bacterial growth inhibition zone response according to Greenwood (1995) small than 10 mm does not respond to bacterial growth barriers, 10-15 mm response inhi

bition of bacterial growth is said to be weak, 16-20 mm response inhibition of bacterial growth is said to be medium and large A 20 mm response to resistance to bacterial growth is said to be strong can be seen in Table 3.

Table 3. Antibacterial Activity Test for *Bacillus cereus*

Concentration	Meter of Inhibition Zone (mm)				Average	S. deviation
	I	II	III	IV		
15%	7	10	10	8	8.75	1.5
20%	16	15	14	15	15	0.816
25%	16	16	20	20	18	2.309
30%	24	25	20	20	22.25	2.629
35%	24	25	25	26	25	0.816
K (-)	0	0	0	0	0	0
K (+)	27	27	-	-	27	0

Aquadest as a control (-) provides a inhibitory zone (0 mm) in S. thypi and B. cereus bacteria, so it does not provide antibacterial activity in both bacteria. Aquadest is a negative control used in this study, in the control there is no inhibition zone because there is no clear zone around the disc paper. This shows that aquadest does not have antibacterial properties because it does not affect the growth of S. thypi and B. cereus bacteria. It can inhibit the growth of gram positive bacteria and gram negative bacteria (Brooks, 2005). The workings of tetracycline by blocking Tetracycline as a positive (+) control provide a large inhibition zone for S. thypi and B. cereus bacteria which are 30 mm and 27 mm. Tetracycline has a very strong antibacterial activity because tetracycline has a broad-spectrum bacteriostatic property, namely the binding of RNA (aminoacilic RNA) to a specific cycle in the ribosome

That is, in the 30S ribosome unit during elongation of the peptide chain, protein synthesis is hampered (Pelczar and chan, 2012). Tetracyclines provide a larger measured inhibition zone, but the results given to the ethanol extract of durian fruit skin meat on the use of B. cereus bacteria obtained results comparable to controls (+) Ethanol extract 96% durian fruit skin flesh (D.zibethinus Murr.) Is active as an antibacterial because of the chemical components contained in extracts. Based on the results of phytochemical tests, the extract contains flavonoids, alkaloids, saponins and tannins which are suspected of being compounds that have the potential to have antibacterial activity. This is in accordance with the literature which states that chemical compounds that have the potential as antibacterial are flavo-

noids and saponins (Noorhamdani et, al., 2009).

Based on the results of this study, the inhibitory zone for gram-negative bacteria, S. thypi, did not show the best inhibitory zone results, because at a diameter of 10-15 mm the response to inhibition of bacterial growth showed weak inhibition (Greenwood, 1995). Whereas B. cereus at a concentration of 20% has shown the results of a moderate inhibition zone and at a concentration of 35% shows a strong inhibitory power. Thus it can be stated that durian peel extract has the potential as an antibacterial agent.

CONCLUSION

Based on the results of phytochemical screening of durian fruit peel extract containing secondary metabolites, namely flavonoids, alkaloids, saponins and tannins and the results of testing data on the activity it can be concluded that durian fruit flesh extract still has potential as an antibacterial agent. So it is necessary to do the next step with fractionation and isolation of other active compounds. antibacterial activity data.

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REFERENCES

Ajizah, A. Sensitivitas Salmonella Typhimurium terhadap Ekstrak Daun Psidium Guajava L. *Bioscientiae* Vol.1 No.1. pp: 8-31; 2004

Akiyama H., Kazuyasu F., Osamu Y., Takashi O., Keiji I. Antibacterial action of several tannins against Staphylococcus aureus. *Journal ofantimocrobial Chemotheraph* (2001) 48:487-491.http://www.jac.oupjournals.org/cgi. May, 5th 2005; 2001

Darmadi. *Infeksi nosokomial : Problematika dan Pengendaliannya.* Jakarta: Salemba Medika; 2008

Dharmojono. *Kapita Selekta Kedokteran Veteriner*. Jakarta: Pustaka Populer Obor; 2001.

Dwidjoseputro, D. *Dasar-Dasar Mikrobiologi.* Jakarta: Djambatan. Dzen, S.M. 2003. Bakteriologi Medik Edisi 1. Malang: Bayumedia Publishing; 1994.

Gibson, J.M. *Mikrobiologi dan Patologi Modern.* Jakarta : EGC; 1999

Karou, D., Dicko, M. H., Simpore, J., dan Traore, A. S.Antioxidant and Antibacterial Activities of Polyphenol From Ethnomedicinal Plant of Burkina Faso, *African Journal of Biotechnology,* 4 (8), 823-828; 2005.

Mitscher, L.A., Leu R.P., Bathala M.S., Wu W., Beal J.L. Lloydia 35: 157; 1972

Radji, M. *Buku Ajar Mikrobiologi Panduan Mahasiswa Farmasi & Kedokteran*. Jakarta : EGC; 2010.

Robinson, T. *Kandungan Organik Tumbuhan Tinggi*. Bandung : ITB Press. Subandi. 2010. Mikrobiologi. Rosda: Jakarta; 1995.