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ANTIBACTERIAL ACTIVITY OF PAPAYA LEAF EXTRACTS AGAINST PATHOGENIC BACTERIA

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Abstract

It was reported that the extracts of papaya leaves could inhibit the growth of *Rhizopus stolonifer*. Antibacterial activity of *Carica papaya* leaf extracts on pathogenic bacteria was observed in this study. Papaya leaves were extracted by using maceration method and three kinds of solvents: ethanol, ethyl acetate, and hexane. Papaya leaf extracts were tested against *Bacillus stearothermophilus*, *Listeria monocytogenes, Pseudomonas* sp., and *Escherichia coli* by agar diffusion method. The objectives of this study were to determine extract ability against pathogenic bacteria, to observe the influence of pH, NaCl, and heat on extracts ability, and to observe extract ability against *B. stearothermophilus* spores. The data showed that ethyl acetate extract could inhibit *B. stearothermophilus, L. monocytogenes, Pseudomonas* sp., and *E. coli*. The extract activity was influenced by pH, and it was more effective in low pH. The extract activity was influenced by NaCl against *B. stearothermophillus* and *E. coli*. However, it was not influenced by NaCl in bioassay against *L. monocytogenes* and *Pseudomonas* sp. The extract activity was influenced by heating process against all the bacteria tested. The extracts inhibited *B. stearothermophilus* spores as well. Papaya leaves are potential natural anti-bacteria, which might be used in certain kinds of food.

Keywords: antibacterial activity, heat, NaCl solution, papaya leaves, pH

1. Introduction

Papaya plant (*Carica papaya* L.) is widely found in Indonesia. Almost all parts of the plant can be utilized by humans for food or for medicinal purposes [1-6]. Its fruits, leaves, and flowers are edible. Its roots can be used as medicine for renal and urinary bladder problem, and its seeds have anthelmintic activity [4-7]. Papaya is also known as the source of papain enzyme, a kind of enzyme that is utilized as meat tenderizer [7]. Papaya leaf extracts have phenolic compounds, such as protocatechuic acid, p-coumaric acid, 5,7-dimethoxycoumarin, caffeic acid, kaempferol, quercetin, chlorogenic acid [8-11]. These compounds have antimicrobial activity and have been proven to be able to inhibit the growth of *Rhizopus stolonifer* [3-13]. This research was done to observe the antibacterial activity of papaya leaf extracts against pathogenic bacteria.

2. Methods

The chemicals that were used in this research were purchased from Merck and Brataco (tween-80). The papaya leaves used in this research were 20-25 cm in length. The papaya leaves were washed, dried with oven, blended to obtain leaf powder. The leaf powder was then macerated with shaker incubator for 24 hours in 37 ^{o}C, 250 rpm with three kinds of different solvents: ethanol, ethyl acetate, and hexane. The mixture was then filtrated, condensed with rotary evaporator to obtain three kinds of different extracts [16].

The antibacterial activities of all the extracts were tested by using agar diffusion method [16]. Four kinds of bacteria, *Bacillus stearothermophilus*, *Listeria monocytogenes*, *Escherichia coli*, and *Pseudomonas* sp. were used to test the antibacterial activity of those extracts. Every extract that was obtained from every solvent was tested in four concentrations 10%, 20%, 30%, and 40%, and control. The test was done in 37 ^{o}C for every kind of bacterium, except for *Bacillus stearothermophilus* that was done in 55 ^{o}C. After 24 hours, the diameters of inhibition zones were measured and the extracts that gave the highest diametrical inhibition with minimal concentration were chosen to be used in the next analysis. To observe the influence of pH, the extracts were tested in five kinds of pH value, 4, 5, 6, 7, and 8. The extracts were also tested in four kinds of NaCl concentrations: 1, 2, 3, and 4%, and in two kinds of temperatures: 80 ^{o}C and 100 ^{o}C for 5, 10, and 15 minutes. The extracts were also tested against the *Bacillus stearothermophilus* spore for 24 hours in 55 ^{o}C.

3. Results and Discussion

Choosing Extracts. All hexane extracts could not inhibit all bacteria used for the test. *B. stearothermophillus* and *L. monocytogenes* could be inhibited by ethanol extract and by ethyl acetate extract (Table 1). From the statistic test, it could be seen that ethanol extract significantly different from ethyl-acetate extract for both bacteria. The ethyl-acetate extract had higher diametrical inhibition than ethanol extract; it was 5.65–10.55 mm against *B. stearothermophilus* and 3.02–6.00 mm against *L. monocytogenes*. The chosen extract for inhibiting both *B. stearothermophilus* and *L. monocytogenes* was ethyl-acetate 30% extract.

In this research *Pseudomonas* sp. and *E. coli* could be inhibited by ethyl acetate extract only. The diameter of inhibition zone was 2.33–4.78 mm against *Pseudomonas* sp. and 1.60–3.00 mm against *E. coli*. The chosen extracts for *Pseudomonas* sp. and *E. coli* were ethyl-acetate 30% and ethyl acetate 40% respectively.

Influence of pH on Extract Activity. The result of this research showed that the extract activity was influenced by pH. The data showed that the highest diameters of inhibition zone against *B. stearothermophilus*, *L. monocytogenes*, *Pseudomonas* sp., and *E. coli* were 12.08 mm, 5.68 mm, 5.95 mm, and 5.93 mm respectively. All of the highest results were obtained at pH 4. The extract activity shows that at pH value from 4 to 8, the higher the pH value, the smaller the inhibition zone is, the lower the antibacterial activity (Fig. 1). No inhibition zone is at pH 7 and 8 for all kinds of bacteria.

Most of antibacterial activities are more effective in acidic condition than in basic condition [13,17]. Bacteria cells will keep the pH value constant inside the cell [18-20]. If the pH value outside the cell is lower than inside the cell, the acid ion will spontaneously move inside the cell until the equilibrium acid ion concentration inside the cell and outside the cell is achieved [18-20]. The bacteria cell will react to that condition. The bacterial cell will pump out the acid ion out of the cell, and this effort needs energy [17]. Bacterial cell will be more inhibited when they meet antibacterial activities in acid condition because the bacterial cells utilize their energy to keep their pH value inside the cells and to face the antibacterial activity [21].

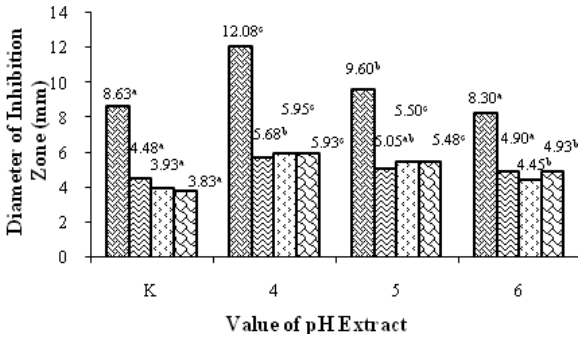

Figure 1. Diameter of Inhibition zone of Papaya Leaf Extract in Several Different pH Values with Different Indicator Strains (*B. stearothermophilus* ( ), *L. monocytogenes* ( ), *Pseudomonas sp.* ( ), and *E. coli* ( )). Different Notations at Each Kind of Bacteria Indicate the Value Has a Significant Difference at α = 0.05.

Table 1. Diameter of Inhibition Zone of Papaya Leaf Extract against Pathogen Bacteria

Solvent	%	Diameter of Inhibition Zone (mm)			
		Indicator strain			
		B. stearotermophilus	*L. monocytogenes*	*Pseudomonas* sp.	*E. coli*
Ethanol	0	0.00	0.00	0.00	0.00
	10	5.27^{a}	1.98^{a}	0.00	0.00
	20	6.22^{ab}	2.72^{ab}	0.00	0.00
	30	7.42^{b}	3.00^{b}	0.00	0.00
	40	9.30^{c}	3.50^{c}	0.00	0.00
Ethyl-acetate	0	0.00	0.00	0.00	0.00
	10	5.65^{a}	3.02^{a}	2.33^{a}	1.60^{a}
	20	7.38^{b}	3.60^{a}	3.37^{ab}	2.15^{b}
	30	9.38^{c}	5.15^{b}	4.28^{bc}	2.23^{b}
	40	10.40^{c}	6.00^{b}	4.78^{c}	3.00^{c}
Hexane	0	0.00	0.00	0.00	0.00
	10	0.00	0.00	0.00	0.00
	20	0.00	0.00	0.00	0.00
	30	0.00	0.00	0.00	0.00
	40	0.00	0.00	0.00	0.00

Different notation at each kind of extract and extract concentration indicate the value has a significant difference at α = 0.05

Influence of NaCl on Extract Activity. The average of the diameter of inhibition zone of extract activity against tested bacteria can be seen in Figure 2. The data show that the diameters of inhibition zone were 9.78–12.08 mm for *B. stearothermophilus,* 5.65–6.16 mm for *L. monocytogenes*, 4.78–5.33 mm for *Pseudomonas* sp., and 3.53–4.75 mm for *E. coli.* Different kinds of bacteria show different results. The extract activity could be influenced in inhibiting *L. monocytogenes* and *Pseudomonas* sp. dissimilar with inhibiting *B. stearothermophilus* and *E. coli.*

According to Ardiansyah [22], NaCl concentration will reduce antibacterial activitiy of *Plucea indica* extract. Ardiansyah [22] reported that antimicrobial activity can be influenced by NaCl concentration. The increase of NaCl concentration results in the decrease of inhibition zone and antibacterial activity.

The NaCl solution will reduce the water activity value (Aw). NaCl ties the water molecule from the environment and also from the inside of the bacterial cells; therefore, the water molecule inside the cell will move outside. For the osmosis occurrence, the cell volume will reduce, and the plasmolisis occurs. The plasmolisis will inhibit the cell reproduction [17-20].

Generally pathogenic bacteria can be inhibited at Aw (water activity) less than 0.92 that is the same with 13% (w/v) NaCl concentration [22]. The highest NaCl solution in this experiment was only 4% (w/v). This concentration was chosen for those which were usually used for food. This NaCl concentration was not sufficient to inhibit the bacterial growth [23-24]. The data support the fact that the inhibition was obtained by the extract activity, not by the NaCl. The data also showed that NaCl concentrations that were used in this experiment could not reduce the antibacterial activity. The antibacterial activity was stable in low NaCl concentration.

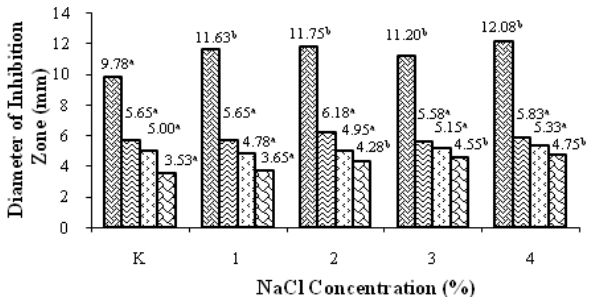

Figure 2. Diameter of Inhibition Zone of Papaya Leaf Extract in Several Different NaCl Solution Concentrations with Different Indicator Strains (*B. stearothermophilus* (▨), *L. monocytogenes* (▨), *Pseudomonas* sp. (▨), and *E. coli* (▨). Different Notations at Each Kind of Bacteria Indicate the Value Has a Significant Difference at α = 0.05

Influence of Heating on Extract Activity. The influence of heating on extract activity can be seen in Figures 3–6. The higher the heating temperature and the longer the heating time, the less the active compound and the less the volatile component of the extract [17,20] are. The ability of the antibacterial activity to inhibit the bacterial growth will decrease when the heating temperature and time increase [17,23]. The result was obtained by using *L. monocytogenes* and *Pseudomonas* sp. as the tested bacteria strengthen this statement. The diameters of inhibition zones were 5.45–6.13 mm for *L. monocytogenes* (Fig. 4) and 4.20–5.58 mm for *Pseudomonas* sp. (Fig. 5). On the contrary, *B. stearothermophilus* and *E. coli* showed different results. The diameters of inhibition zones were 8.98–10.88 mm for *B. stearothermophillus* (Fig. 3) and 4.10–4.53 mm for *E. coli* (Fig. 6) The heating temperatures and times that were used in this research might not be sufficient to influence the antimicrobial activity [24]. The extract showed stability in inhibiting *B. stearothermophillus* and *E. coli*.

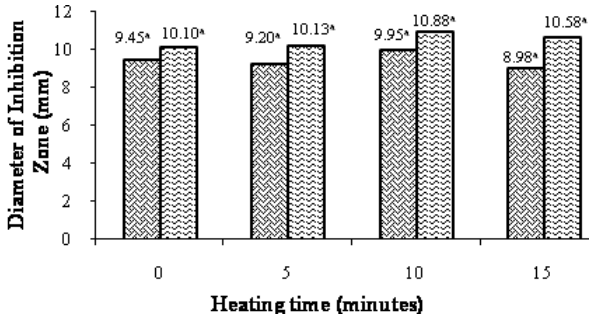

Figure 3. Diameters of Inhibition Zone of Papaya Leaf Extracts in Several Heating Temperatures, 80 °C (▨) and 100 °C (▨) and Time against *B. stearothermophillus.* Different Notations at Each Heating Temperature Indicate the Value Has Significant Difference at α = 0.05

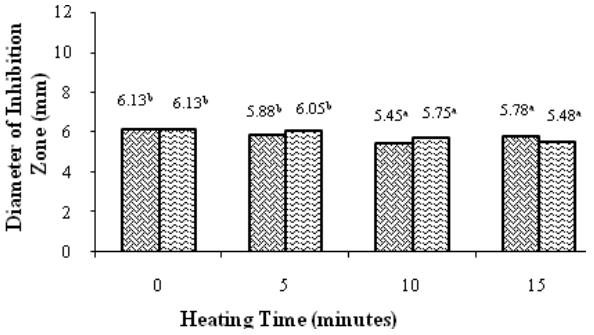

Figure 4. Diameters of Inhibition Zone of Papaya Leaf Extracts in Several Heating Temperatures, 80 °C (▨) and 100 °C (▨) and Time against *L. monocytogenes.* Different Notations at Each Kind of Bacteria Indicate the Value Has a Significant Difference at α = 0.05

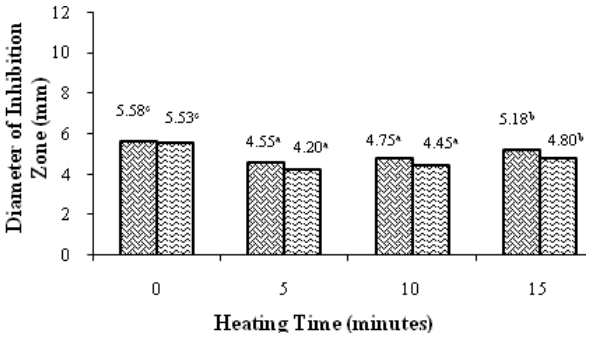

Figure 5. Diameters of Inhibition Zone of Papaya Leaf Extracts In Several Heating Temperatures, 80 °C (▨) and 100 °C (▨) and Time against *Pseudomonas* sp. Different Notations at Each Heating Temperature Indicate the Value Has a Significant Difference at α = 0.05

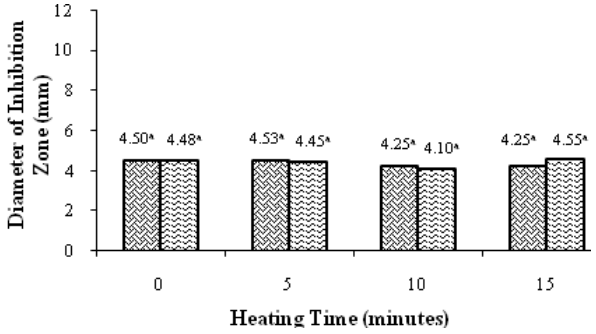

Figure 6. Diameters of Inhibition Zone of Papaya Leaf Extracts in Several Heating Temperatures, 80 °C (▨) and 100 °C (▨) and Time against *E. coli* Different Notations at Each Heating Temperature Indicate the Value Has a Significant Difference at α = 0.05

Analysis of Antibacterial Activities of Extract Against *B. stearothermophilus* Spores. Extracts could inhibit the growth of *B. stearothermophilus.* The inhibition zone of vegetative cell of *B. stearothermophilus* was not wider than the inhibition zone of *B. stearothermophilus* spore. The inhibition zone was 10.58 mm in diameter for vegetative cell, and 10.25 mm in diameter for spore (Fig. 7).

Bacterial spore is more complex in structure than vegetative cell [17-20]. Spore consists of exosposrium, spore coat, cortex, spore wall, and spore protoplast. Cortex contains a keratin like protein and numerous disulfide bonds that cause spore to be resistant to the antimicrobes compound [19]. Dipicolinic acid of spore can react with calcium ion to form dipicolinic calcium.

The water content of spore cell wall is only 10%-30%. It leads the spore cell wall to having gelling characteristics. The action of characteristics and dipicolinic calcium makes the spore more resistant to heat

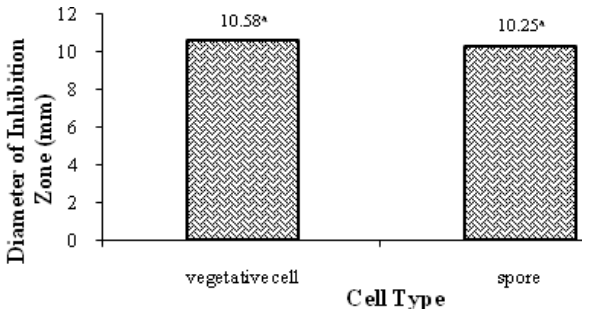

Figure 7. Diameters of Inhibition Zone of Papaya Leaf Extracts against *B. stearothermophillus* Spore. Different Notations Indicate the Value Has a Significant Difference at α = 0.05

than the vegetative cell [19]. The complex structure of bacterial spores also makes spores resistant to the environmental changing. Bacterial spores is resistant to heat, drying, radiation, acid, and disinfectant. This result showed that the extract could inhibit bacterial spores, even though the spores were more resistant than the vegetative cell.

4. Conclusion

From the entire experiment, it can be concluded that papaya leaves have antibacterial activity. The activity was influenced by pH and it was more effective in low pH. The activity could be influenced by NaCl solution against certain bacteria. The activity could be influenced by heating process. The activity could inhibit *B. stearothermophilus* spores as well. This research indicates that papaya leaves have potential natural antibacterial compounds and can be applied for certain food. Further research is suggested to study the application of antibacterial activity of papaya leaves.

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