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Study of Antimicrobial Activity from Guava (Psidium Guajava)

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ABSTRACT

Guava leaves have been utilized traditionally as medicine and are known as an antimicrobial agent as well. In this research paper, guava leaves were extracted using the maceration method. The solvents used in this research were water, ethyl acetate, and hexane. Guava leaf extracts were tested towards Escherichia coli, Staphylococcus aureus, Bacillus cereus, and Penicillium sp. by the agar diffusion method [1].

Keywords : Escherichia coli, Staphylococcus aureus, Bacillus cereus, Penicillium sp.

I. INTRODUCTION

The objectives of this research were to (1) determine the MIC and MBC of guava leaf extracts towards tested microbes, (2) determine the active compound in guava leaf extract, (3) observe the influence of certain pH, sugar concentration, salt concentration, and heat treatment on the antimicrobial activity of guava leaves extract. The result showed that ethyl acetate extract could inhibit all the tested bacteria excluding Penicillium sp. The MIC and MBC for Escherichia coli, Staphylococcus aureus, and Bacillus cereuswas0.017% and 0.067%, 1.177% and 4.707%,0.126% and 0.504%, respectively. The active compounds found in guava leaves were alkaloid, saponin, tannin, phenol, flavonoid, triterpenoid, and steroid. The results indicate ethyl acetate extract was influenced by pH and effective at pH 4. Sugar addition could increase the antimicrobial activity. Furthermore, a low concentration of salt could decrease the antimicrobial activity towards B. cereus as well as that by heat. Moreover, the results also indicate ethyl acetate extract could inhibit the growth of B. cereus spores [2].

The Guava plant (Psidium guajava L.) is a tropical plant that is easily found in Indonesia. Many parts of this plant are utilized by humans, especially its fruits and leaves. Particularly, its fruit is commonly consumed as fresh fruit or processed food. Guava fruit contains tryptophan lysine, pectin, calcium, phosphor, minerals and vitamins. Currently, its fruit is also used to treat diabetes mellitus patients and people who have high levels of blood cholesterol.

Besides its fruit perspective, another part of this plant is utilized for medicinal purposes as well. Its root has potential utilization, to stop dysentery, its young branch is used to treat leucorrhea patients and its leaf is used to cure diarrhea, stomatitis, and stomachache. Leaves of guava are reported to have antibacterial activity. Morton

(2006) reported that essential oils found in its leaves, such as dendrite aromatic, β-selinen, nerolidol, caryophyllene oxide, triterpenoids, and β-sitosterol.

Hence, this research was also carried out to observe the antibacterial activity of guava leaf extract against pathogenic microbe and consequently would increase the economical applications of guava leaves.

II. METHODOLOGY

The guava leaves used in this research were obtained from Muzaffarpur. All the microbes were from RAU, Pusha Agriculture University, and most of the chemicals were purchased from Muzaffarpur. The guava leaves were washed, freeze-dried, and then blended to become powder. The powder was macerated with three kinds of solvent, i.e.: water, ethyl acetate, and hexane. The maceration process took 24 hours at room temperature. The mixture was then filtrated, and condensed at 45°C with an oven (for water as the solvent) or vacuum evaporator (for ethyl acetate and hexane as the solvent) to obtain the extracts. The three kinds of extracts were then analyzed by the Harborne method (Harborne, 1996) to determine the active compound [3].

The antibacterial activities of all the extracts were tested by using the agar diffusion method. Four kinds of microbes, *Escherichia coli, Staphylococcus aureus, Bacillus cereus*, and *Penicillium* sp. were used to test the antimicrobial activity of those extracts. Every extract that was obtained from every solvent was tested in five concentrations 10%, 20%, 30%, 40%, and 50% and the solvent was used as a control. The test was done in 37°C. After 24 hours the diameter of inhibition zones was measured and the extract that gave the highest diametrical inhibition with minimal concentration was chosen to be used in the next analysis. Bloomfield method (1991) was used to determine the MIC and MBC of the extracts. To observe the influence of pH, the chosen extracts were tested in five kinds of pH values, 4, 5, 6, 7, and 8. The extract was also tested in four kinds of sugar concentrations: 10%, 20%, 30%, and 40%, four kinds of salt concentrations: 1, 2, 3, and 4%, and also in two kinds of temperatures: 80°C and 100°C for 5, 10, and 15 minutes. The extract was also tested against the *Bacillus cereus* spore for 24 hours at 37°C.

III. RESULT AND DISCUSSION

The water extracts did not inhibit all the microbes tested; in contrast, the ethyl acetate could inhibit all the bacteria tested but not *Penicillium*. The diameter of inhibition of ethyl acetate extracts was between 6.17 mm – 12.95 mm. Furthermore, hexane extract could only inhibit *B. cereus* and the diameter of inhibition was 0.00 mm – 6.79 mm. (Table 1). For the next analysis, *Pencillium* was not used as tested microbes [4].

Table 1. Diameter of Inhibition Zone of Guava Leaves Extract

Diameter of Inhibition Zone (mm)						
			Kind of Bacteria			
			E.coli	*S. aureus*	*B. cereus*	*Penicillium*
solvent	water	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

		50%	0.00	0.00	0.00	0.00
	ethyl-acetate	0%	0.00	0.00	0.00	0.00
		10%	9.34	7.99	6.17	0.00
		20%	9.28	8.58	6.32	0.00
		30%	9.49	9.52	7.17	0.00
		40%	9.73	11.81	7.25	0.00
		50%	10.06	12.95	7.51	0.00
	hexane	0%	0.00	0.00	0.00	0.00
		10%	0.00	0.00	0.00	0.00
		20%	0.00	0.00	5.04	0.00
		30%	0.00	0.00	5.81	0.00
		40%	0.00	0.00	5.79	0.00
		50%	0.00	0.00	6.79	0.00

The MIC and MBC were determined for ethyl-acetate extract only. The Bloomfield method was used and the result is in Table 2. The MIC and MBC for *Escherichia coli, Staphylococcus aureus*, and *Bacillus cereus*were0.017% and 0.067%, 1.177% and 4.707%,0.126% and 0.504% respectively.

Table 2. The MIC and MBC against tested Bacteria

Kind of Bacteria					
E.coli		*S. aureus*		*B. cereus*	
MIC	MBC	MIC	MBC	MIC	MBC
0.017 %	0.067%	1.177%	4.707%	0.126%	0.504%

For ethyl – acetate 10% extract could inhbit the tested bacteria with significant differences with the next higher concentration; the inhibition test was done with the lower concentration, i.e. 2, 4, 6, 8, and 10% and the result is shown in Table 5.3. Based on the result, ethyl–acetate 4% was chosen for the next analysis to inhibit *E. coli* and *S. aureus,* and ethyl acetate 6% was chosen to inhibit *B. cereus.*

Table 3. Diameter of Inhibition Zone of Ethyl – acetate extract

Diameter of Inhibition Zone (mm)				
		Kind of Bacteria		
		E.coli	*S. aureus*	*B. cereus*
Concentration	0 %	0.00	0.00	0.00
	2 %	9.06	7.45	6.49
	4 %	**9.33**	**8.19**	7.29
	6 %	9.59	8.36	**8.44**
	8 %	9.81	8.43	8.49
	10 %	10.84	8.49	9.04

There were a lot of active compounds in guava leaves. The active compounds in guava leaves were alkaloid, saponin, tannin, phenol, flavonoid, triterpenoid, and steroid (Table 4).

Table 4. The Active Compound Found in Guava Leaves Extract

	Kind of Extract		
Active Compound	Water	Ethyl - acetate	Hexane
Alkaloid	+	+	+
Saponin	+	+	-
Tannin	+	+	+
Phenol	+	+	-
Flavonoid	+	+	+
Triterpenoid	-	+	+
Steroid	+	+	+

4.1. INFLUENCE OF pH ON EXTRACT ACTIVITY

It was found that ethyl acetate extract was effective under acid conditions. It could inhibit all the tested bacteria at pH 4, but at pH 5 it could not inhibit *S. aureus*, moreover, it could not inhibit all the tested bacteria at pH 6, 7, and 8 (Figure 1).

Most of the extract components were weak acids. At low pH, weak acids were not dissociated. Non-dissociated form weak acid would easily diffuse inside the cell, then the cell would react to maintain its pH. The cell reaction needs more energy, then the energy to grow would decrease.

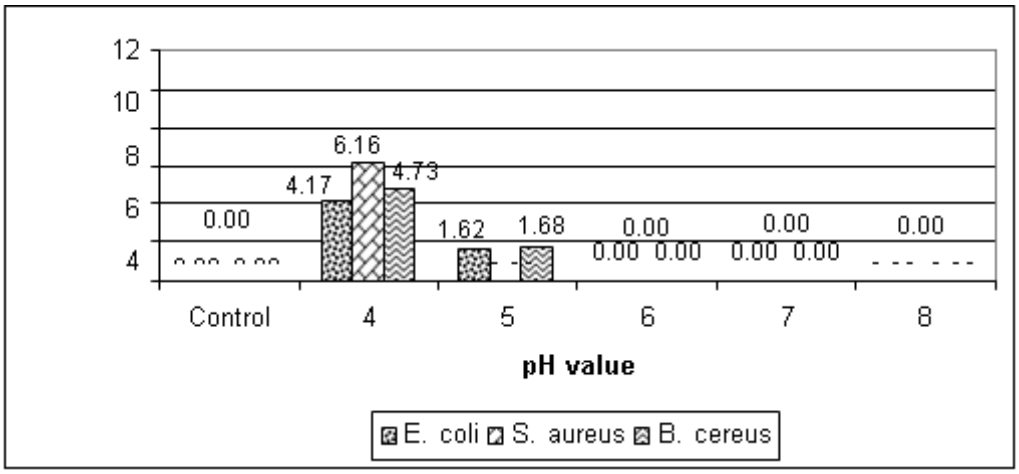

Figure 1. Diameter of Inhibition Zone of Guava Leaves Extract in Several pHValues

4.2. INFLUENCE OF SUGAR ON EXTRACT ACTIVITY

The result in Figure 5.2 shows that there was a sugar concentration influence on the antibacterial activity of the extract. The diameter inhibition range was 2.78 – 9.70 mm. The higher the sugar concentration, the higher the diameter inhibition. The sugar concentration influenced the Aw value (water activity). At a sugar concentration of 10 – 30%, the water activity was 0.978, and at a sugar concentration of 40%, the water activity was 0.973. Not

all water in the solution can be used by the bacteria for its growth. The water that can be used by bacteria is stated as water activity, the water activity restricts the growth of the bacteria.

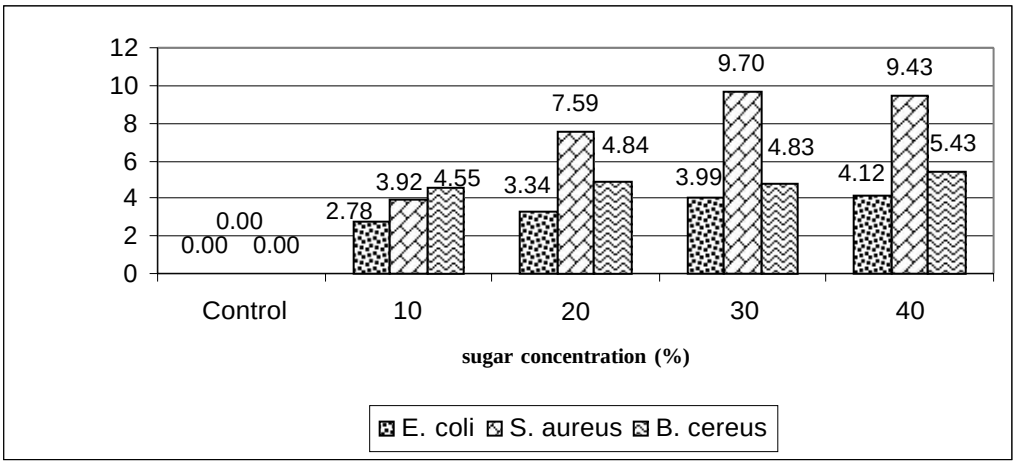

Figure 2. Diameter of Inhibition Zone of Guava Leaves Extract in Several Sugar Concentrations.

4.3. INFLUENCE OF SALT ON EXTRACT ACTIVITY

The data in Figure 3 shows that different kinds of bacteria showed different results. The diameter of the inhibition zone was 4.52 – 5.08 mm for *E. coli* 7.53 – 8.06 mm for *S. aureus*, and 3.98 – 6.82 mm for *B. cereus* The extract activity could be influenced in inhibiting *B. cereus* dissimilar with in inhibiting *E. coli* and *S. aureus.*

The salt will reduce the water activity value (Aw). Generally, pathogen bacteria can be inhibited at Aw (water activity) less than 0.92 which is the same with 13% (w/v) salt concentration. The highest salt solution in this experiment was 4% (w/v). This concentration was chosen for those who were usually used for food. This salt concentration was not sufficient to inhibit bacterial growth. This data strengthens that the inhibition was obtained by the extract activity, not by the salt.

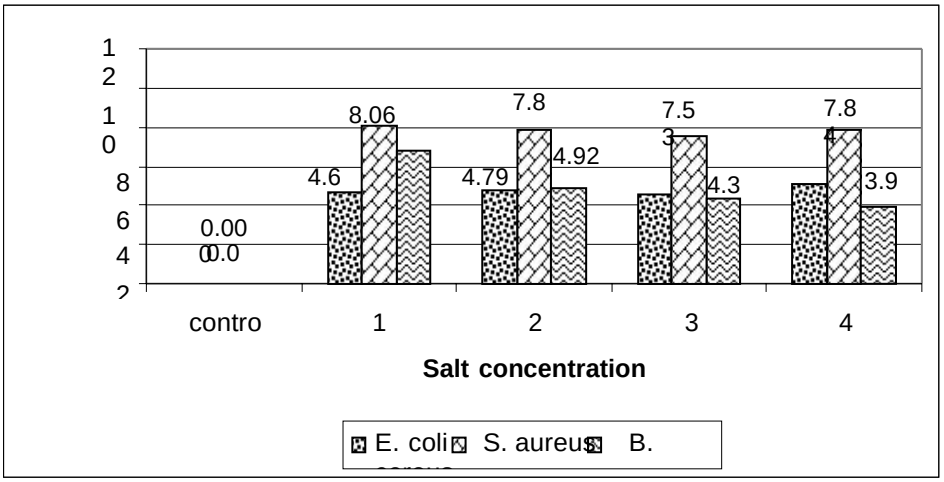

Figure 3. Diameter of Inhibition Zone of Guava Leaves Extract in Several SaltConcentrations

4.4 INFLUENCE OF HEATING ON EXTRACT ACTIVITY

The data in Figure 4 –6 shows that the ability of the antibacterial to inhibit bacterial growth will decrease when the heating temperature and time increase. The diameter of inhibition zones was 5.24 – 7.29 mm for *E. coli* (Fig.

4), 3.28 – 5.15 mm for *S. aureus* (Fig. 5), and 5.89 – 8.04 (Fig. 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 (Ardiansyah, 2002).

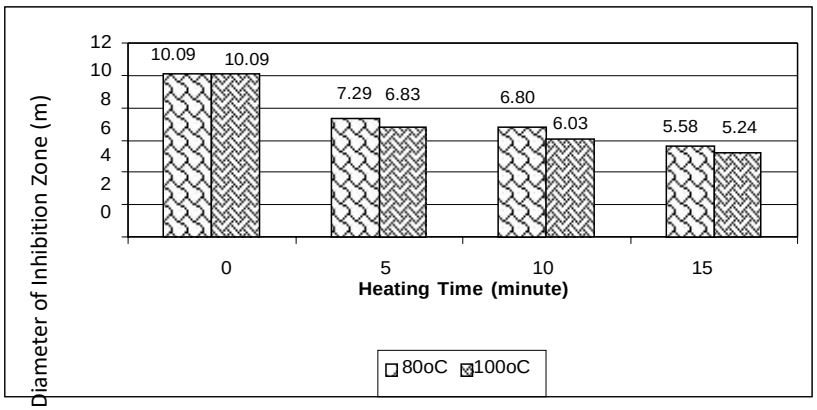

Figure 4. Diameter of Inhibition Zone of Guava Leaves Extract in Several HeatingTimes towards *E. coli*

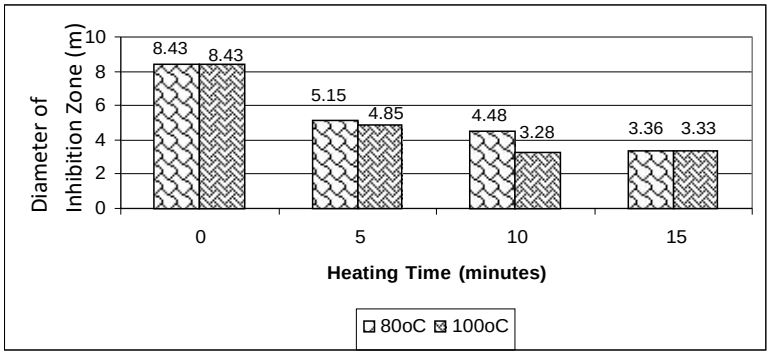

Figure 5. Diameter of Inhibition Zone of Guava Leaves Extract in Several HeatingTimes towards *S. aureus*

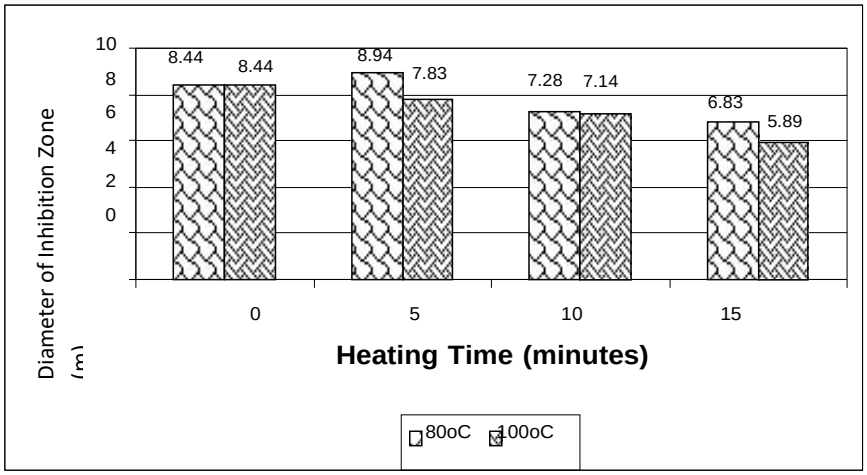

Figure 6. Diameter of Inhibition Zone of Guava Leaves Extract in Several HeatingTimes towards *B.cereus*

5. EXTRACT ACTIVITY TOWARDS *B. CEREUS* SPORE

Figure 7 shows that the inhibition zone of the vegetative cell of *B. cereus was* 8.94 mm in diameter and the inhibition zone of *B. cereus* spore was 8.67 mm in diameter. Bacterial spore is more complex in structure than vegetative cells (Madigan *et al.,* 2006). Bacterial spores are resistant to heat, drying, radiation, acid, and

disinfectant. This result showed that the extract could inhibit bacterial spores, even though the spore was more resistant than the vegetative cell [5].

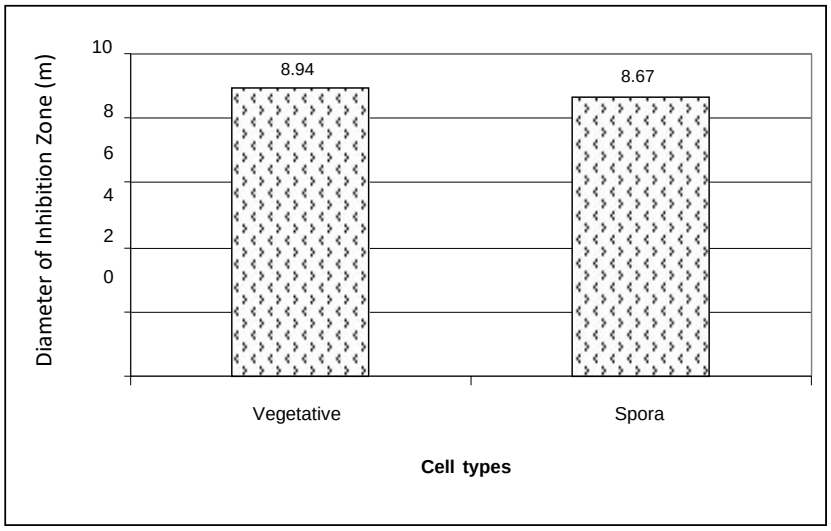

Figure 7. Diameter of Inhibition Zone of Guava Leaves Extract towards *B. cereus* Spore.

IV. CONCLUSIONS

The result showed that ethyl acetate extract could inhibit all the tested bacteria excluding *Penicillium* sp. The MIC and MBC for *Escherichia coli, Staphylococcus aureus*, and *Bacillus cereus*was0.017% and 0.067%, 1.177% and 4.707%,0.126% and 0.504%, respectively. The active compounds found in guava leaves were alkaloid, saponin, tannin, phenol, flavonoid, triterpenoid, and steroid. The results indicate ethyl acetate extract was influenced by pH and effective at pH 4. Sugar addition could increase the antimicrobial activity. Furthermore, a low concentration of salt could decrease the antimicrobial activity towards *B. cereus* as well as that by heat. Moreover, the results also indicate ethyl acetate extract could inhibit the growth of *B. cereus* spores

V. REFERENCES

1. Shrivastava, P. (2007). Clinical and Immunological Study of Some Airborne Allergic Fungi in Poultry Workers' Environment of Jabalpur. *Ph.D. Thesis,* R.D. University, Jabalpur, India.
2. Streets, R.T. (1969). The diagnosis of plant diseases. The University of Arizona, Tucson, Arizona, USA.
3. Naseer, Sumra, Shabbir Hussain, Naureen Naeem, Muhammad Parvaiz and Madiha Rahman (2018). The phytochemistry and medicinal value of *Psidium guajava* (guava). *Clinical phytoscience.*, 4(32): 1-8.
4. Surendranathan, K.K. (2005). Post harvest biotechnology of fruits with special reference to banana-Perspective andScope *Indian J. Biotech.*, 4: 39-46.
5. Tilak, S.T. and R.L. Kulkarni (1970). A new air sampler. *Experentia.,* 25: 443-444.