# STUDY OF ANTIMICROBIAL ACTIVITY FROM GUAVA (*Psidium guajava* L.) LEAF EXTRACT TOWARDS PATHOGENIC MICROBES

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## ABSTRACT

Guava leaves have been utilized traditionally as medicine and known as an antimicrobial agent as well. In this research, guava leaves were extracted using maceration method. The solvents used in this research were water, ethyl acetate, and hexane. Guava leaves extracts were tested towards *Escherichia coli, Staphylococcus aureus, Bacillus cereus*, and *Penicillium* sp. by the agar diffusion method. The objectives of this research were to (1) determine the MIC and MBC of guava leaves extracts towards tested microbes, (2) determine the active compound in guava leaves 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 cereus* was 0.017% 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, 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.

**Keywords** : *guava leaf, antimicrobial activity*

## INTRODUCTION

Guava plant (*Psidium guajava* L.) is a tropical plant that is easily found in Indonesia. Many parts of this plant are utilized by human, 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 vitamin. Currently, its fruit is also used to treat diabetes mellitus patient and people who have high level blood cholesterol.

Besides its fruit prospective, other part of this plant is utilized for medicinal purpose as well. Its root has potential utilizations, to stop dysentery, its young branch is used to treat leucorrhea patient and its leaf is used to cure diarrhea, stomatitis, and stomach-ache. Leaves of guava are reported to have antibacterial activity. Morton (2006) reported about essential oil found in its leaves have, such as dendrene aromatic, □-selinen, nerolidiol, caryophyllene oxide, triterpenoids and □-sitosterol.

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

**METHODE**

The guava leaves used in this research were obtained from Muara Karang. All the microbes were from PAU, Bogor Agriculture University and most of the chemicals were purchased from Merck. The guava leaves were washed, freeze dried, then blended to become powder. The powder was macerated with three kind of solvent, i.e. : water, ethyl acetate, and hexane. The maceration process took 24 hour at room temperature. The mixture then filtrated, condensed at 45$^{o}$C with oven (for water as the solvent) or vacuum evaporator (for ethyl acetate and hexane as the solvent) to obtained the extracts. The three kind of extracts then analyzed by Harborne method (Harborne, 1996) to determine the active compound.

The antibacterial activities of all the extracts were tested by using 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 were obtained from every solvent were tested in five concentrations 10%, 20%, 30%, 40%, and 50% and the solvent were used as control. The test was done in 37$^{o}$C. After 24 hour the diameter of inhibition zones were measured and the extract that gave the highest diametrical inhibition with minimal concentration were 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 value, 4, 5, 6, 7, and 8. The extract also tested in four kind of sugar concentration : 10%, 20%, 30%, and 40%, four kind of salt concentrations : 1, 2,

3, and 4%, and also in two kind of temperatures : 80$^{o}$C and 100$^{o}$C for 5, 10, and 15 minutes. The extract also tested against the *Bacillus cereus* spore for 24 hours in 37$^{o}$C.

## 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 next analysis *Pencillium* was not used as tested microbes.

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* |
| s o l v e n t | 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 was 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 inhibit the tested bacteria with no 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 shown in Table 3. Base on the result, ethyl – acetate 4% was chosen for 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 compound in guava leaves. The active compounds in guava leaves were alkaloid, saponin, tannin, phenol, flavonoid, triterpenoid, and steroid (Table 4).

Table 4. The Active Compaound Found in Guava Leaves Extract

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

**Influence of pH on Extract Activity**

It was found that ethyl acetate extract was effective under acid condition. 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).

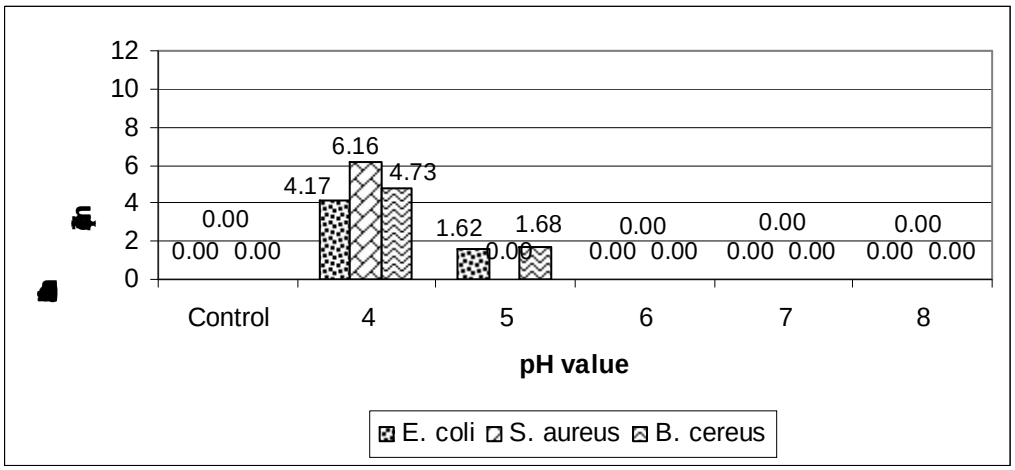


Figure 1. Diameter of Inhibition Zone of Guava Leaves Extract in Several pH Value

Most of the extract components were weak acid. At low pH, weak acids were not dissociated. Non dissociated form weak acid would easy to 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.

**Influence of Sugar on Extract Activity**

The result in Figure 2 shown that there was 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 sugar concentration 10 – 30%, the water activity was 0.978, and at sugar concentration 40%, the water activity was 0.973. Not all water in the solution can be used by the bacterial for its growth. The water that can be used by bacteria is stated as water activity, the water activity restrict the growth of the bacteria.

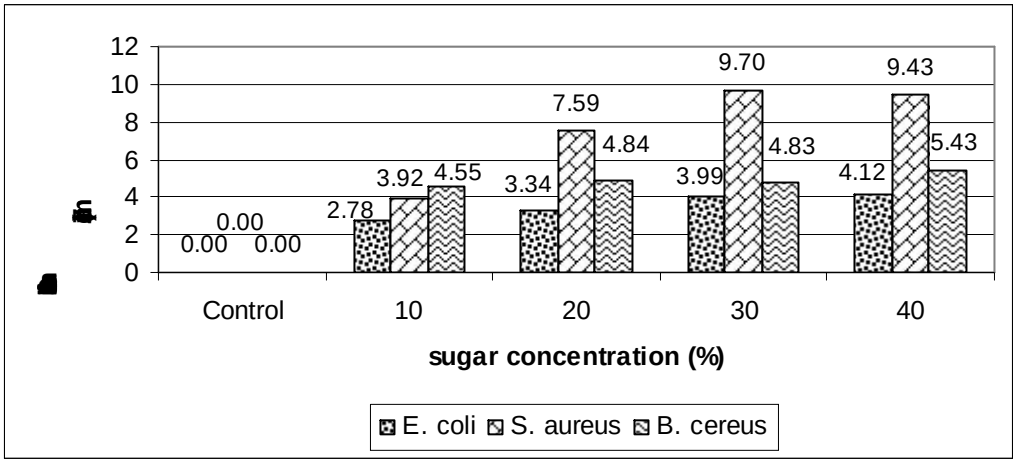


Figure 2. Diameter of Inhibition Zone of Guava Leaves Extract in Several Sugar Concentration

**Influence of Salt on Extract Activity**

The data in Figure 3 shown that different kind of bacteria showed different result. The diameter of inhibition zone were 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 bacterial can be inhibited at Aw (water activity) less than 0.92 that 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 were usually used for food. This salt concentration was not sufficient to inhibit the bacterial growth. This data strengthen that the inhibition was obtained by the extract activity, not by the salt.

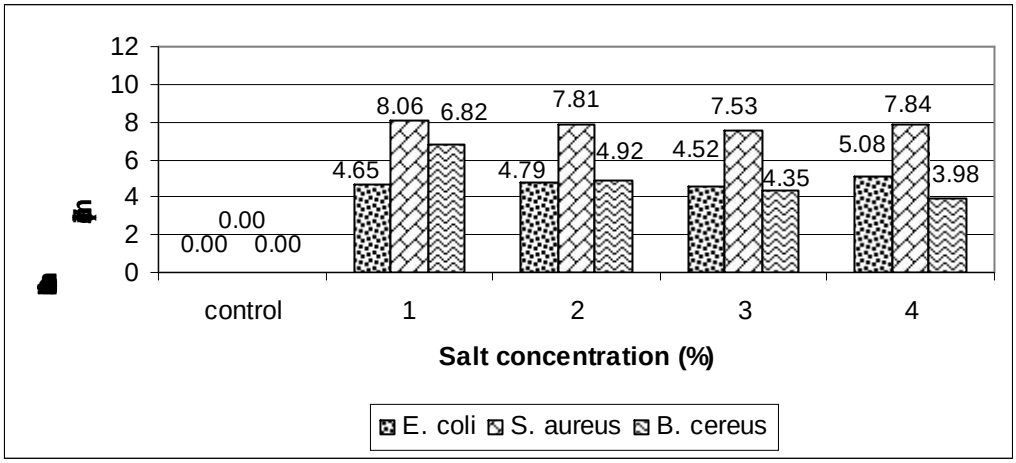


Figure 3. Diameter of Inhibition Zone of Guava Leaves Extract in Several Salt Concentration

**Influence of Heating on Extract Activity**

The data in Figure 4 – 6 shown that the ability of the antibacterial to inhibit the bacterial growth will decrease when the heating temperature and time increase. The diameter of inhibition zones were 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).

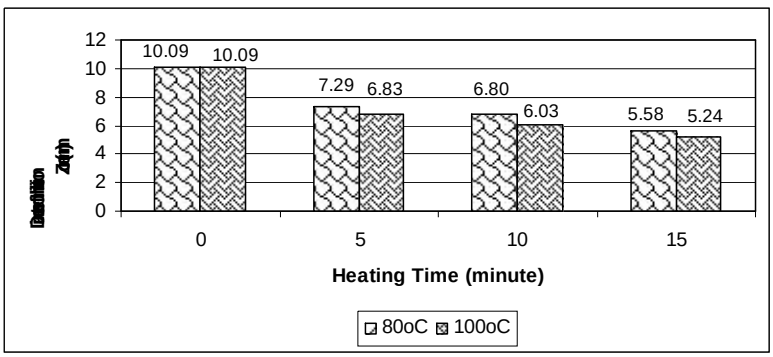


Figure 4. Diameter of Inhibition Zone of Guava Leaves Extract in Several Heating Time towards *E. coli*

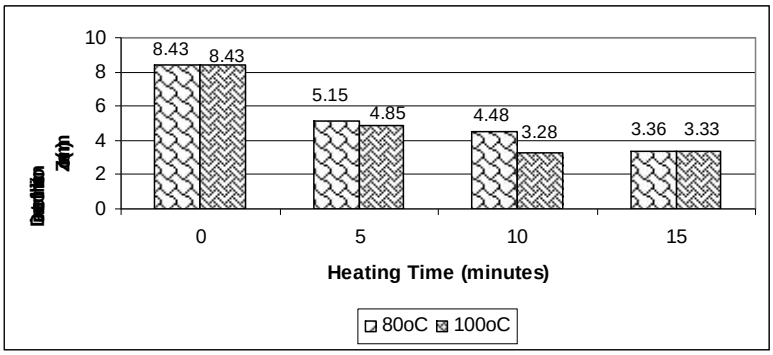


Figure 5. Diameter of Inhibition Zone of Guava Leaves Extract in Several Heating Time towards *S. aureus*

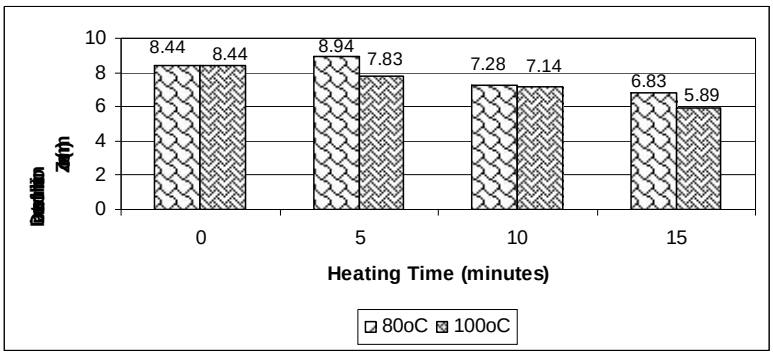


Figure 6. Diameter of Inhibition Zone of Guava Leaves Extract in Several Heating Time towards *B.cereus*

**Extract Activity towards *B. cereus* Spore**

Figure 7 shown that the inhibition zone of 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 cell (Madigan *et al.*, 2006). Bacterial spore is resistant to heat, drying, radiation, acid, and disinfectant. This result showed that the extract could inhibit bacterial spore, even though the spore was more resistant than the vegetative cell.

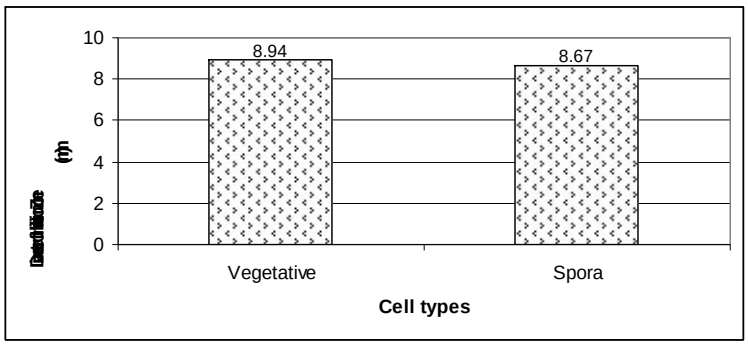


Figure 7. Diameter of Inhibition Zone of Guava Leaves Extract towards *B. cereus* Spore

## CONCLUSION

From the entire experiment, it can be concluded that guava leaves have antibacterial activity. The activity was influenced by pH, sugar, salt, and by heating process. Moreover the antibacterial activity was strong enough to inhibit *B. cereus* spores. This research indicates that guava leaves have potential natural antibacterial compound and can be applied for certain food such as sour food, sugar added food, food with no heating process or short heating process. Further research is suggested to study the application of antibacterial activity of guava leaves.

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