

## Effectiveness of Guava Leaves Juice as Antibacterial in Poultry Egg Incubator Disinfection

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### ABSTRACT

The aims of this study was to determine the effectiveness of guava leaves juice as an antibacterial disinfection of poultry egg incubator. The experiment was conducted using completely randomized design (CRD). Three treatments of guava leaf juice concentration were applied, namely: concentration of 25% (T<sub>1</sub>), concentration of 50% (T<sub>2</sub>), and concentration of 75% (T<sub>3</sub>) with 6 replicates per treatment. The parameters tested were inhibition effect on bacteria *in situ*, reduction of the number of total bacteria and fungi on poultry egg incubator. Data obtained were analyzed by analysis of variance and the Duncan test was utilized to determine the difference between treatments. The results showed that: (1) the higher of guava leaves juice concentration resulting a wider inhibition zone, effective both in coccus and bacil bacteria, (2) concentration of guava leaves juice 75% decreased total of bacteria by 89.53 %. It reflects that the guava leaves juice effectively used as a disinfectant on poultry egg incubators.

**Keywords:** Disinfection, Guava leaves, Inhibition zone, Antibacteria, Poultry egg incubators

### INTRODUCTION

Hatching process becomes an important thing to note because this process is an embryo development in eggs to hatch into chicks, ducks, quail, and other poultry. Poultry hatching can be done naturally or artificially through the help of a tool in form of a hatching machine. Utilization of hatching machine has several advantages such as can incubate fertile eggs in large quantities and can be adjusted to the planned time thus more efficient and economical (Rahayuningtyas *et al.*, 2014). In the hatching process there are factors that affect the quality of DOC/DOD, such as egg sanitation and hatching environment (Wells *et al.*, 2008).

Bacteria which often contaminate the hatching machines, namely *Staphylococcus aureus*, *Staphylococcus epidermis*, *Streptococcus pyogenes*, *Bacillus subtilis*, *Bacillus cereus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Salmonella sp.*, and *Escherichia coli* (Gao *et al.*, 1997; Cox *et al.*, 2000; Jones *et al.*, 2002; Fawzia *et al.*, 2013; Karadzhev *et al.*, 2004). Therefore, Several effort to control bacterial contamination of hatching machines, one of which through disinfection of hatching machines using chemical compounds, such as formaldehyde, ozone, ethylene oxide, and ultraviolet light (Sacco *et al.*, 1989; Brake and Sheldon, 1990; Sander and Wilson, 1999; Shahein and Sedeek, 2014). However, there are side-effects in the use of these disinfectants, which are harmful to the respiratory system of the workers (Rodriguez-romo and Yousef, 2005) and are detrimental to the development of chick embryo (Shama, 1992). Alternative use of disinfectants is needed to reduce the negative impacts caused by chemical compounds. Application of natural ingredients derived from plants by utilizing antimicrobial active compounds can be a better option.

The guava leaves (*Psidium guajava L.*) is one of the ingredients that contain the antimicrobial active compound, namely tannin, essential oil (eugenol), fatty oil, resin,

triterpenoid, flavonoid, and malic acid (Begum *et al.*, 2002; Arima and Danno, 2002; Dalimartha, 2003; Biswas, 2013). Antimicrobial compounds contained in guava leaves are able to resist both gram-positive and negative bacteria (Sanches *et al.*, 2005; Biswas *et al.*, 2013) and inhibit the growth of *Staphylococcus aureus* (Vieira *et al.*, 2001). Various methods can be executed in extracting antimicrobial compounds in white guava leaves: macerating with ethanol or using water solvents as juice. This research investigated the effectiveness of white guava leaves juice in reducing the amount of aerobic bacteria in hatching machine.

## MATERIALS AND METHODS

**Sample Preparation.** Guava leaves juice is obtained by extraction using distilled water. The weight ratio of guava leaves and distilled water 1:2 (m/v). Guava leaves are washed, poured by distilled water and blended to get the juice. The mixture filtered to get the guava leaves juice with 100% concentration. Boiled to 98°C while stirring for 15 min. Calculation of the volume of guava leaves solution using the following formula:

$$V_1 \cdot C_1 = V_2 \cdot C_2$$

$V_1$  = Volume of pure guava leaves solution (100%)

$C_1$  = Concentration of pure guava leaves solution (100%)

$V_2$  = The final volume of guava leaves solution

$C_2$  = The final concentration of guava leaves solution

**Total Bacteria Count.** Prepared plates filled with Nutrient Agar. Put the lid off and placed in the poultry incubator for 30 min. Closed the lid and incubated at 37°C for 24 h. Bacterial growth was observed in the plate, and then calculated with density of bacteria in the air formula:

$$\frac{\text{Number of colonies} \times 60 \text{ minutes} \times \text{Incubator area}}{30 \text{ minutes} \times \text{Plates area}}$$

The decreases of total bacteria in the incubator calculated by following formula :

$$\frac{\text{number of initial bacteria} - \text{number of final bacteria}}{\text{number of initial bacteria}} \times 100\%$$

**Anaerob Bacteria Enrichment.** Inoculate dominant bacteria colonies in a hatching machine (from plates) into test tube filled with lactose broth medium. Incubate bacteria for 24h at 37°C. Bacterial growth can be seen from changes in lactose broth becomes turbid.

**Zone of Inhibition Test for Antimicrobial Activity.** Prepare nutrient agar and pour into the petri dish, made a hole and put filter paper which has been dipped in white guava leaves juice. Approximately spread 1 mL anaerob enriched bacteria over agar plate using a sterile swab, then incubated with the presence of antimicrobial object. If the bacterial strain is susceptible to the antimicrobial agent, then a zone of inhibition appears on the plate, otherwise inhibition does not appear if it is resistant to the antimicrobial agent.

**Data analysis.** Data obtained were tabulated using One Way ANOVA and Duncan's Multiple Range Test using SPSS software version 19.0.0.2 (SPSS Inc., 2014).

## RESULTS AND DISCUSSION

**The Decrease of Total Bacteria.** The decrease of total bacteria in hatching machine after disinfection using guava leaves juice ranged between 52.63 to 89.53%. (Table 1). This illustrated the presence of antibacterial activity in white guava leaves juice. The higher

concentrations of guava juice result in a higher decreasing percentage (Bessems, 1998; Chmielewski and Frank, 2003). The antibacterial activity of guava leaves were caused by an antibacterial compound called Phytochemical. Phytochemicals are biologically active compound found in plants which provide human protection against disease (Biswas *et al.*, 2013). Two triterfenoid found in guava leaves are guavanoic acid and guavacoumaric acid. Other antimicrobial compounds in guava leaves are flavonoids. Flavonoids have been shown to be antibacterial, antiviral, anti-inflammatory, antiplatelet, antioxidant, free radical scavenging, and vasodilatory actions (Simonetti *et al.*, 2001).

**Table 1.** The decreasing amount of total bacteria and inhibition zone of guava leaves juice

Variable	T <sub>1</sub> <sup>*</sup>	T <sub>2</sub> <sup>**</sup>	T <sub>3</sub> <sup>***</sup>
Decrease of total bacteria (%)	52,63 ± 9,47 <sup>b</sup>	70,64 ± 6,49 <sup>c</sup>	89,53 ± 3,26 <sup>d</sup>
Inhibitory zone of Bacil Bacteria (mm)	3.41 ± 0.72 <sup>b</sup>	6,15 ± 1.71 <sup>b</sup>	9,25 ± 2.15 <sup>c</sup>
Inhibitory zone of Coccus Bacteria (mm)	3.61 ± 1,21 <sup>b</sup>	5,92 ± 1,41 <sup>b</sup>	8,92 ± 2,98 <sup>c</sup>

\* concentration 25%; \*\* concentration 50%, \*\*\* concentration 75%

Concentration of disinfectant was one of the major factors in biocidal activity (Russell and McDonnell, 2000). The relationship between microbial death and disinfectant concentration was not linear, but usually followed a typical biological sigmoidal death curve (Bessems, 1998). The research by Tuncan (1993), indicated that the effectiveness of quaternary ammonium compound and chlorine on *Listeria* sp. was improved when the concentration was increased from 50 ppm to 100-200 ppm. The concentration of 50% guava leaves juice was quite effective in reducing the total bacteria on the hatching machine. This can be illustrated from the Duncan Test which did not differ between concentrations of 50% and 75% in reducing total bacterial counts.

### **Inhibition of Total Bacteria**

Bacterial sensitivity test is a test to determine and to obtain natural products that have potential as antibacterial material and have the ability to inhibit growth or kill bacteria at low concentration. In this research, bacterial sensitivity test was applied to determine the level of bacterial susceptibility to antibacterial substances contained in guava leaves juice. The size of the clear zone that was visible on the diffusion method demonstrates the ability of bacteria in the poultry incubator (Table 1).

Guava leaves juice was able to inhibit the growth of bacteria both basil and coccus. The study of Sanches *et al.* (2005) suggested that antibacterial activity of guava is able to fight both gram-positive and negative bacteria, but extraction using ethanol was more effective against *Staphylococcus aureus* than with water.

The inhibitory zone formed on bacterial culture of bacilli reached 9.25 mm, whereas in cultured bacteria coccus reached 8.92 mm, with 75% guava leaves juice concentration. Many factors influenced the inhibition effectiveness of a disinfectant, one of which is the type of microorganism and different strains of the same species (Mailard, 2002). Likewise the method of extraction will affect the effectiveness of inhibition of guava leaves juice against bacteria (Sofowora, 1993; Green *et al.*, 2004; Ncube *et al.*, 2008).

The 75% concentration of guava leaves juice produced a 3.41 to 9.25 mm inhibition zone. This illustrated that guava leaves juice had medium category inhibitory power (David and Stout, 1971). The more width the inhibit zone formed, the more effective the disinfectant was in inhibiting bacterial growth.

## CONCLUSIONS

The guava leaves juice is quite effective as a disinfectant because it has adequate ability to reduce the total number of bacteria in the egg poultry incubator and effective to inhibit both of basil and coccus bacteria.

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