Inhibition Strength of Free Radicals of DPPH and Staphylococcus aureus by Ethanol Extract of Papaya Leaves (Carica papaya L)

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ABSTRACT

The DPPH and Staphylococcus aureus inhibition by ethanol extract of papaya leaves has been evaluated. This research focused on the use of papaya leaves extract as one potential herbal to be applied in cosmetics as an ingredient. Papaya leaves were extracted using the maceration method with 70% and 96% ethanol for 24 hours. Both ethanol extracts of papaya leaves showed that they could inhibit the free radicals of DPPH and S. aureus bacteria. Their activity related to its active compounds in phenolic compound form. The total phenolic content of 70% and 96% ethanol extract of papaya was 65.27 ± 1.21% and 12.64 ± 0.27% respectively. This total phenolic content was significantly different. The 70% ethanol extract of papaya has higher total phenolic content than 96% ethanol extract. Therefore, both 70% and 90% ethanol extract of papaya also resulted in different activity in S. aureus bacteria and DPPH inhibition. Antioxidant activity of both extracts possessed low ability to inhibit free radicals of DPPH. In contrast, their bacteria activity showed that 70% and 96% ethanol extract of papaya on the concentration of 8 mg/mL could inhibit the S. aureus bacteria growth around 1.47 ± 0.05 mm and 1.37 ± 0.05 mm, respectively. Based on these properties, papaya leaves extract can be applied for cosmetic ingredients in high doses to maximize its activity.

INTRODUCTION

Indonesia is a country that is producing herbal plant sources, including papaya (Carica papaya L.). The part of papaya used as traditional medicine is the leaves of papaya. The papaya leaves were widely used as a traditional medicine because its leaves contain flavonoids types such as quercetin 3-(2G-rhamnosylrutinoside), kaem ferol 3-(2G-rhamnosylrutinosid), quercetin 3-rutinoside, myrisetin 3-rhamnoside, kaem ferol 3-rutinoside, quercetin and kaem ferol (Nugroho et al., 2017). The papaya leaves were used as an antibacterial, antioxidant, anti-cancer, etc. The antioxidant activity of ethanol extract of papaya leaves and fruit were showed by inhibiting and capturing the free radicals of DPPH (2,2-diphenyl-1-picrylhydrazyl) (Zahra et al., 2017; El-Nekeety et al., 2017). The bacterial activity of ethanol extract of papaya leaves about 25 mg/mL with inhibiting the bacterial growth of Pseudomonas aeruginosa and Staphylococcus aureus, which is around 4 mm and 3 mm, respectively. In contrast, the water extract of papaya leaves had no inhibit the bacterial (Awah et al., 2017).

In this study, we evaluate the inhibition strength of ethanol extract of papaya leaves of free radicals of DPPH and Staphylococcus aureus bacteria. Both 70% and 96% ethanol extract of papaya...
were studied to find the effectiveness of papaya leaves extract as antioxidant or antibacterial. Ngumah et al. (2016) reported that 70% ethanol could take out the secondary metabolites from papaya leaves such as alkaloids, anthraquinones, flavonoids, saponins, steroids, tannins, terpenoids, and glycosides. Whereas, 96% ethanol concentration gains secondary metabolites such as flavonoids, tannins, glycosides (Alorkpa et al., 2016).

Consequently, the difference of metabolites obtained can give different antioxidant activities. The research from Yim et al. (2012) reported that water extract of papaya seeds has the highest activity to capture the free radical of DPPH and has the highest of total phenolic content compared with methanol extract of papaya seeds. Furthermore, Junka et al. (2017) also informed that 70% ethanol concentration could be used as a solvent to extract the polar bioactive compounds including antioxidant hydrophilic, even it has greater antioxidant activity than 50% and 95% ethanol concentrations. Besides, Chew et al. (2011) reported that the highest inhibition activities of DPPH and 2,2’-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) can be extracted using 60% and 80% ethanol concentration, respectively.

**MATERIALS AND METHODS**

**Sample preparation and Psidium guajava leaves extraction**

Samples of papaya leaves were collected from the Jatianom Klaten Plantation area from December to January. The sample was cleaned and dried before used and made into powder. 250 gram of papaya leaf powder is macerated using 96% and 70% ethanol with a powder: ethanol ratio of 1: 4 for 24 hours. The maceration process was repeated to maximize the active compounds extracted. The solutions were evaporated using a vacuum rotary evaporator at a temperature of 60 °C. The concentrated extract obtained can be stored in the closed box under 20 °C.

**Determination of Phenol Total Content**

Determination of the total phenol content in the extract of papaya following the work reported by Javanmardi (2003) with a slight modification. The gallic acid solution of 1 mg/mL was used as solution standard with concentration variation of 0.010; 0.0125; 0.015; 0.0175; 0.020; 0.0225 mg/mL. Each standard solution was pipetted 1 mL and then added 1 mL Folin-Ciocalteu reagent and vortexed until homogeneous for 1 minute. After 8 min, the solution was added 0.8 mL of 7.5% Na$_2$CO$_3$ solution and vortexed for 40 seconds and waited to reach optimum time and measured at 750 nm. The sample also were prepared similarly to the gallic acid standard solution. The total phenol content was written with an equivalent mass of gallic acid (GAE).

**Inhibition Strength of DPPH**

Inhibition strength of DPPH was determined using agar well diffusion following by Liu et al. (2014) with modification. The ethanol extract of papaya leaves was dissolved with ethanol by ranging of concentrations of papaya leave extracts of 500-2000 μg/mL. Each solution was pipetted 1 mL and added by 1 Ml of DPPH 1 Mm. The solutions were measured using a UV-Vis spectrophotometer at 517 nm. The inhibition equation of DPPH is written by:

\[
IC_{50} (%) = \frac{(\text{Abs of control} - \text{Abs of samples})}{\text{Abs of control}} \times 100%
\]

**Inhibition strength against Staphylococcus aureus**

Determination of antibacterial activity that was tested using the well diffusion method was carried out on MHA (Mueller Hilton Agar) media. A total of 20 μL was inserted into the hole (5 mm) of the MHA media. Inhibition strength will be determined after the incubation process for 24 hours at 37°C. The measurement of inhibition zone as reported by Peter et al. (2014) which inhibition of the zone.
Table 1: Total phenol content on papaya leaves extracted using 70% and 96% ethanol

| Sample            | Total phenol content (%, wt/wt) (x̄ ± sd)* | Sig |
|-------------------|-------------------------------------------|-----|
| 70% Ethanol Extract | 65.27±1.21                                 | 0.00|
| 96% Ethanol Extract | 12.64±0.27                                 |     |

*6 replicates

Table 2: Inhibition zone diameter of Staphylococcus aureus inhibition

| Samples               | Zone Diameter Inhibition (mm), x̄ ± SD* |
|-----------------------|----------------------------------------|
|                       | 8 mg/mL                                |
| 70% Ethanol Extract    | 1.47±0.05                              |
| 96% Ethanol Extract    | 1.37±0.05                              |
|                       | 4 mg/mL                                |
| 70% Ethanol Extract    | 1.17±0.05                              |
| 96% Ethanol Extract    | 1.07±0.05                              |
|                       | 1 mg/mL                                |
| 70% Ethanol Extract    | 1.00±0.00                              |
| 96% Ethanol Extract    | 1.00±0.00                              |

*3 replicates

of bacterial is defined by the clear zone that is measured in mm scale, around the well after incubation.

RESULTS AND DISCUSSION

Total phenolic content of ethanol extract of papaya leaves

The linear regression of gallic acid reacted with Folin Ciocalteu shown in Figure 1, and the total phenolic content on papaya leaves extracted using 70% and 96% ethanol was written in Table 1. Determination of total phenol content was carried out using a gallic acid solution with a concentration range of 10-23 μg/mL which was reacted with Folin-Ciocalteu reagent in alkaline conditions and measured at a wavelength of 750 nm with operating time range for 129 to 135. The calibration curve has a linear regression equation y = 30.934x - 0.112 with a determination coefficient (R²) of 0.9949 Figure 1. Table 1 showed that 70% ethanol extract has higher total phenolic content than 96% ethanol extract, which is 65.27 ± 1.21% and 12.64 ± 0.27% respectively. T-test illustrated that both the 70% ethanol extracts and 96% ethanol extract had a difference of total phenol content with a significance value of 0.00 <0.05. Whereas, 96% ethanol extract papaya fruit contains total phenol of 296.85 ± 14.25 g / kg or 29.685 ± 1.425% (El-Nekeety et al., 2017). This shows that 70% ethanol extract of papaya leaves are greater total phenol content than those 96% ethanol extract of papaya fruit, but 96% ethanol extract of papaya leaves has smaller than 96% ethanol extract of papaya fruit.

Determination of inhibition strength against DPPH and Staphylococcus aureus

Antioxidant activity of DPPH illustrated in Figure 2. Ethanol extract of papaya resulted in the antioxidant activity of DPPH lower than synthetic antioxidant as butylated hydroxytoluene (BHT). Furthermore, inhibition strength against S. aureus was reported in Table 2. This study showed operating time on the DPPH inhibition process of 70% and 96% ethanol extract of papaya leaves which is 76-80 min and 62-71 min respectively. Besides, the Butylated hydroxytoluene (BHT) can inhibit DPPH free radicals with operating time ranging from 50-81 min. Therefore, the DPPH inhibition process is carried out on the same stable condition, which is ranging from 63-70 min and measured at a maximum lambda of 517 nm. Determination of inhibitory strength of a herb against DPPH can be seen the IC₅₀ value resulted. This research showed that positive control (BHT) had a greater free radical scavenging activity than the others. BHT has smaller IC₅₀ value than ethanol extract of papaya leaves Figure 2. IC₅₀ values produced by BHT, 70% ethanol extract and 96% papaya leaves were 52.73 ± 1.53 μg/mL, 1276.63 ± 23.61 μg/mL and 1570.00 ± 37.09 μg/mL, respectively. Therefore, BHT has strong antioxidant strength, while ethanol extract of papaya leaves has a weak ability to inhibit DPPH. According to Molyneux (2003), states that the IC₅₀ value obtained> 200 μg / mL showed that the substance is less active, but it still has the potential as an antioxidant. The scavenging of DPPH free radicals from other studies such as Zahra et al. (2017) and El-Nekeety et al. (2017) who reported that the ethanol extract of papaya leaves had an IC₅₀ of 95.26 ± 5.26% or 950.6 ± 50.26 mg/mL and also ethanol extract of papaya fruit showed IC₅₀ of 20.75 ± 3.26% or 207.5 ± 32.6 mg / mL respectively. This research showed that the ethanol extract of papaya has better antioxidant activity than previous stud-
ies from El-Nekeety et al. (2017) and Zahra et al. (2017). While the inhibition of S. aureus bacteria growth by 70% and 90% ethanol extract shown in Table 2. The S. aureus inhibition by differences concentration of extract also did not show a significant difference, because all concentration of papaya extracts have small inhibition activity of S. aureus bacteria. This research has reported that its activity to inhibit Staphylococcus aureus bacteria growth from both 70% and 90% ethanol extract of papaya leaves is very small. Another research has reported that ethanol extract of papaya leaves with a concentration of 25 mg/mL only inhibit Staphylococcus aureus growth as big as 3 mm. In contrast, a concentration of 100 mg/mL of papaya extract can inhibit the same bacteria reach 16 mm (Awah et al., 2017). Based on the other research and literature showed that the concentrations of papaya extract commonly affect the inhibition ability against bacterial growth.

CONCLUSION

Both 70% and 96% ethanol extract of papaya leaves resulted in different total phenol content which is 65.27 ± 1.21% and 12.64 ± 0.27% respectively. While both extracts also produced the antioxidant activity as week as S. Aureus inhibition in low concentration. Based on this result, the use of ethanol extract of papaya leaves should be used in high concentrations.

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Conflict of Interest

All authors do not have a conflict of interest for this study.

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