QUANTIFICATION OF QUERCETIN AND CHLOROGENIC ACID IN PAPAYA SEED ETHANOL EXTRACT

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ABSTRACT

Objective: The objective of the study was to quantify the quercetin and chlorogenic contained in the ethanolic extract of papaya seed.

Methods: Papaya seeds were extracted using the maceration method; then, the qualitative phytochemical analysis was obtained from standard phytochemical screening; furthermore, liquid chromatography-mass spectrometry (LC–MS) examination was used to determine the number of its bioactive compounds.

Results: The quantitative examination using LC–MS showed that the content of chlorogenic acid was greater than the quercetin level. Further studies have to be carried out regarding the papaya seed ethanolic extract in vitro inhibition compare to the antibacterial potential of the commercial antibiotics on the tested bacteria species.

Conclusion: Our results suggest that papaya seed ethanolic extract quantitatively contains chlorogenic acid greater than quercetin.

Keywords: Medicinal plant, Papaya seed extract, Flavonoids, Chlorogenic acid, Quercetin.
It can be conclude from the Table 2 that the concentration average of chlorogenic acid was as much as phenolic derivative found in many plants, one of which is papaya seed [15]. It was found that the average of chlorogenic acid content in papaya seed ethanolic extract was as much as 916.67±32.14 ng/mg. This is consistent with research carried out by Peter et al. [16] and Kumar and Sreeja [9] stated that papaya leaves and seeds contain chlorogenic acid; furthermore, Peter mentioned that chlorogenic acid has antitumor activities. Meanwhile, chlorogenic acid was reported to inhibit osteoclast and bone resorption through the inhibition of cell differentiation mechanism [14]; it was the same as the result given by Pusporini et al. [11] that papaya seed ethanolic extract reduced the number of osteoclasts on the periodontits rat model. It can be concluded that the possible mechanism for papaya seeds decreases the number of osteoclasts through cell differentiation by chlorogenic acid.

It can be seen from the Table 3 that papaya seed ethanolic extract contained quercetin in average 4.58±0.03 ng/ml which is widely known to have antioxidant activity [17] and various other medicinal properties are in accordance with research reported by Xu et al. [18-20] that the application of quercetin can promote fracture healing in smokers by removing free radicals and upregulating the expression of heme oxygenase-1 and superoxide dismutase-1, which protects primary human osteoblasts exposed to cigarette smoke. The previous research conducted by the author found that papaya seed extract can increase the number of osteoblast periodontitis-induced rat cells, possibly derived from the antioxidant and anti-inflammatory activity of quercetin. The prior study by Pusporini et al. [10] mentioned that papaya seed ethanolic extract that papaya seed extract that qualitatively proven contains flavonoids increases the number of osteoblasts on rat-induced periodontitis.

**CONCLUSION**

Our results suggest that papaya seed ethanolic extract quantitatively contains chlorogenic acid greater than quercetin. Besides, there should be another study carried out on other bioactive compounds of papaya seeds extract and regarding the antibacterial properties of papaya seeds extract compared to the commercial antibiotics on the tested bacteria species.

**ACKNOWLEDGMENT**

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**AUTHORS' CONTRIBUTIONS**

This work was carried out in collaboration with three authors. RP designed the study, wrote the protocol, and interpreted the data. HOB and VA anchored the field study, gathered the initial data, and performed preliminary data analysis. HOB and VA managed literature searches and RP produced the draft. All of the authors read and approved the final manuscript.

### Table 2: Liquid chromatography–mass spectrometry result of chlorogenic acid

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sample weight (ng)</th>
<th>Area</th>
<th>Measured concentration (µg/ml)</th>
<th>Dilution factor</th>
<th>Weight (ng)</th>
<th>Counted concentration (ng/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample A</td>
<td>0.2170</td>
<td>8664</td>
<td>0.07</td>
<td>3</td>
<td>220</td>
<td>930</td>
</tr>
<tr>
<td>Sample B</td>
<td>0.2170</td>
<td>8006</td>
<td>0.07</td>
<td>3</td>
<td>200</td>
<td>940</td>
</tr>
<tr>
<td>Sample C</td>
<td>0.2170</td>
<td>7300</td>
<td>0.06</td>
<td>3</td>
<td>190</td>
<td>880</td>
</tr>
</tbody>
</table>

### Table 3: Liquid chromatography–mass spectrometry result of quercetin

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sample weight (ml)</th>
<th>Area</th>
<th>Measured concentration (µg/ml)</th>
<th>Dilution factor (ml)</th>
<th>Counted concentration (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>100</td>
<td>417.00</td>
<td>0.046</td>
<td>1.00</td>
<td>4.5506</td>
</tr>
<tr>
<td>Sample 2</td>
<td>100</td>
<td>437.00</td>
<td>0.046</td>
<td>1.00</td>
<td>4.6081</td>
</tr>
<tr>
<td>Sample 3</td>
<td>100</td>
<td>433.00</td>
<td>0.046</td>
<td>1.00</td>
<td>4.5966</td>
</tr>
</tbody>
</table>

Filterate added 0.1 g of magnesium powder and 1 ml of concentrated hydrochloric acid and 2 ml of amyl alcohol, shaken, and allowed to be separated. Flavonoids are positive if there is a red or yellow or orange color to the amyl alcohol layer [12].

**Saponin**

The test sample was weighed as much as 0.5 g and put into a test tube, then added 10 ml of hot water, let it cool then shaken vigorously for 10 s. If the foam was forming 1–10 cm high, then it is stable and not <10 min and does not disappear with the addition of one drop of hydrochloric acid indicating the presence of saponins [12].

**Triterpenoid**

A total of 1 g of the test sample was macerated for 2 h with 20 ml of n-hexane, then filtered. The filtrate was evaporated in a vaporizer cup. A few drops of Bouchardat’s reagent were added to the remainder. The appearance of blue or green-blue indicates the presence of steroids, while the red, pink, or purple colors indicate the presence of triterpenoids [12].

**Liquid chromatography–mass spectrometry (LC-MS) examination**

Initially, 0.5 g of papaya seed extract was dissolved in 50 ml methanol p.a. Then, the solution was filtered using a 0.22 µ syringe filter and put in a 2 ml vial.

**Data analysis**

Analysis of the data in this study used descriptive analysis which aims to provide a general picture of the data or images obtained and then used to explain the characteristics of the research object.

**RESULTS**

The result of preliminary phytochemical analysis as written in the Table 1 showed the presence of papaya seed ethanol extract qualitatively showed that there were phytochemical compounds in the form of flavonoids, phenols, alkaloids, and saponins, whereas the quantitative examination using LC–MS showed that the content of chlorogenic acid was greater than the quercetin level.

**DISCUSSION**

Papaya seed extracts have some profound medicinal properties such as antioxidant, bactericidal activity, anti-inflammatory, antimicrobial, antidiabetic, antifertility, and its role in the wound healing process [5,6,10-13,14]. The prior study reported by Pusporini et al. [10,11] found that papaya seed ethanolic extract qualitatively contains flavonoids and phenolic acids, but it is not known how much quantitatively. When the bioactive compounds contained in papaya seed extract can be known, this will facilitate further research in determining which compounds possess a therapeutic effect. This study used the LC-MS test to determine the bioactive compounds of papaya seed extract, compared with two standard chlorogenic acid and quercetin.
CONFLICTS OF INTEREST

The authors declared no competing interest.

REFERENCES