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.

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INTRODUCTION

Herbal medicines embrace a wide range of practices and therapies outside the realm of traditional Western medicine. Although herbal medicines are not risk free, they can still be safer than synthetic drugs. The potential benefits of herbal medicines include the high acceptance by patients, effectiveness, relatively safe, and relatively low costs [1,2].

Flavonoids are of a large group of secondary metabolites in plants, many studies have reported that the secondary metabolites of phenolic nature including flavonoids are responsible for the variety of pharmacological activities. Flavonoids have antioxidant capacity since they can bind metal ions such as iron and copper serving to catalyze the formation of free radicals, limiting the formation of free radicals. Moreover, the flavonoid is thought to have health-promoting properties both in vivo and in vitro systems due to its medicinal properties [3].

One of the plants that are also rich in flavonoids is papaya. According to the previous studies, it is known that papaya seeds have better antioxidant activity than other plant parts and have several antioxidants, anti-inflammatory, antibacterial, anti-diabetic, and anthelmintic activities and also possess potential in wound healing [4-11]. In a previous study, it was investigated that papaya seed ethanolic extract quantitatively contains flavonoids and phenol, but the contents are not known quantitatively [10,11]. The rationale of this study is whether the bioactive compounds contained in papaya seed extract contain quantitatively, it will facilitate further research in determining which compounds contain a therapeutic effect. The objective of this study is to quantify the quercetin and chlorogenic contained in ethanolic extract papaya seed.

METHODS

Papaya seed extraction
Papaya seeds are extracted using the maceration method to modify the study reported by Pusporini [10]. This study modifies the drying method time, 2 weeks shorter than described. The papaya seed ethanolic extract was made from the dark brown seed of ripe Carica papaya Linn., dried in the room temperature where it is safe from direct sun rays, then processed in Materia Medika, Batu-Malang. The damp papaya seed was washed using water then blended to shrink the size, as much as 500 g of the papaya seed powder was macerated with 70% alcohol solution for 3 days and then filtered using white cotton fabric and Buchner funnel. The filtration was then evaporated until it reached the optimum level of dryness on the temperature of 40°C until the thick extract form of 24.0% solility (w/w) was gained. The extraction process was repeated 4 times and the solid powder produced was weighted afterward then inserted to a refrigerator at a temperature of 4°C.

Qualitative phytochemical analysis

Alkaloids
Samples were weighed as much as 0.5 g then mixed into 10 ml of 96% ethanol. Then, the filtrate obtained was used for the alkaloids test, three test tubes were taken; then, 0.5 ml of the filtrate was added. Each test tube added a different reagent.
1. Test tube 1: Added two drops of Mayer reagent
2. Test tube 2: Added two drops of Bouchardat’s reagent
3. Test tube 3: Added two drops of Dragendorff’s reagent

Alkaloids are positive when there is sedimentation or turbidity in at least two of the three experiments above [12].

Flavonoid
The sample was weighed as much as 10 g and then added to 10 ml of hot water, boiled for 5 min and filtered under heat, into 5 ml of

Table 1: Phytochemistry screening result of papaya seed extract

| S. No. | Compound   | Contain |
|--------|------------|---------|
| 1.     | Alkaloid   | +       |
| 2.     | Flavonoid  | +       |
| 3.     | Saponin    | +       |
| 4.     | Triterpenoid | −      |
flavonoids, phenols, alkaloids, and saponins, whereas the quantitative showed that there were phytochemical compounds in the form of n-hexane, then filtered. The filtrate was evaporated in a vaporizer cup. A total of 1 g of the test sample was macerated for 2 h with 20 ml of methanol p.a. Then, the solution was filtered using a 0.22 μm 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 the preliminary phytoc hemical 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 test 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, anthelmintic, antidiabetic, antifertility, and its role in the wound healing process as antioxidant, bactericidal activity, anti-inflammatory, antihelmintic, and neuroprotective activity [5,6,10-13,14]. The prior study reported by Pusporini et al [10,11] found that papaya seed ethanol extract quantitatively 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.

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

| Sample  | Sample weight (mg) | Area  | Measured concentration (µg/g) | Dilution factor | Weight (ng) | Counted concentration (ng/mg) |
|---------|--------------------|-------|-------------------------------|----------------|-------------|-------------------------------|
| Sample A | 0.2170             | 8664.00 | 0.07                          | 3              | 220         | 930                           |
| Sample B | 0.2170             | 8006.00 | 0.07                          | 3              | 200         | 940                           |
| Sample C | 0.2170             | 7300.00 | 0.06                          | 3              | 190         | 880                           |

Table 3: Liquid chromatography–mass spectrometry result of quercetin

| Sample  | Sample weight (ml) | Area  | Measured concentration (µg/ml) | Dilution factor (ml) | Counted concentration (ng/ml) |
|---------|--------------------|-------|-------------------------------|---------------------|-------------------------------|
| Sample 1 | 100                | 417.00 | 0.046                         | 1.00                | 4.5506                       |
| Sample 2 | 100                | 437.00 | 0.046                         | 1.00                | 4.6081                       |
| Sample 3 | 100                | 433.00 | 0.046                         | 1.00                | 4.9666                       |

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 9.16±0.32 µg/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 anticancer 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 periodontitis 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.

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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.
CONFLICTS OF INTEREST
The authors declared no competing interest.

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