ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 13, Issue 1, 2020

Online - 2455-3891
Print - 0974-2441

Research Article

QUANTIFICATION OF QUERCETIN AND CHLOROGENIC ACID IN PAPAYA SEED ETHANOL EXTRACT

RATIH PUSPORINI[1]*, HANAA OMAR BAABDULLAH[2], VERU ANDYKA[2]

[1]Department of Oral Biology, Faculty of Dentistry, Universitas Brawijaya, Indonesia. [2]Herbal Medicine Research Group, Faculty of Dentistry, Universitas Brawijaya, Indonesia. Email: ratih.fk@ub.ac.id

Received: 01 November 2019, Revised and Accepted: 22 November 2019

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.

 DOI: http://dx.doi.org/10.22159/ajpcr.2020.v13i1.36242

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, antidiabetic, and antihelmintic activities and also possess potential in wound healing [4-11]. In a previous study, it was investigated that papaya seed ethanolic extract qualitatively 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 known 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 ethanol 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% solidity (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	–

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	0.07	3	220	930
Sample B	0.2170	8006	0.07	3	200	940
Sample C	0.2170	7380	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	10.00	4.5506
Sample 2	100	437.00	0.046	10.00	4.6081
Sample 3	100	433.00	0.046	10.00	4.5966

filtrate 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 phytocemichal 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, antihelmintic, antidiabetic, antifertility, and its role in the wound healing process [5,6,10-13,14]. The prior study reported by Pusporini [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.

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 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.

ACKNOWLEDGMENT

This research was supported by PNBP grant year 2018 from Faculty of Dentistry Universitas Brawijaya.

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.

REFERENCES

1. Zhang J, Onakpoya IJ, Posadzki P, Eddouks M. The safety of herbal medicine: From prejudice to evidence. Evid Based Complement Alternat Med 2015;2015:316706.
2. Askarinia M, Ganji A, Jadidi-Niaragh F, Hasanzadeh S, Bahram MB. A review on medicinal plant extracts and their active ingredients against methicillin-resistant and methicillin-sensitive *Staphylococcus aureus*. J Herbmed Pharmacol 2019;8:173-84.
3. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: An overview. ScientificWorldJournal 2013;2013:162750.
4. Amazu LU, Azikiwe CC, Njoku CJ, Osuala FN, Nwosu PJ, Ajugwo AO, *et al*. Antiinflammatory activity of the methanolic extract of the seeds of *Carica papaya* in experimental animals. Asian Pac J Trop Med 2010;3:884-6.
5. Zhou K, Wang H, Mei W, Li X, Luo Y, Dai H. Antioxidant activity of papaya seed extracts. Molecules 2011;16:6179-92.
6. Nayak BS, Ramdeen R, Adogwa A, Ramsubhag A, Marshall JR. Wound healing potential of an ethanol extract of *Carica papaya* (*Caricaceae*) seeds. Int Wound J 2012;9:650-5.
7. Napimoga MH, Clemente-Napimoga JT, Macedo CG, Freitas FF, Stipp RN, Pinho-Ribeiro FA, *et al*. Quercetin inhibits inflammatory bone resorption in a mouse periodontitis model. J Nat Prod 2013;76:2316-21.
8. Maver T, Maver U, Stana KK, Smrke DM, Kreft S. A review of herbal medicines in wound healing. Int J Dermatol 2015;54:740-51.
9. Kumar NS, Sreeja DP. The surprising health benefits of papaya seeds: A review. J Pharmacogn Phytochem 2017;6:424-9.
10. Pusporini R, Basori A, Krismariono A. The effect of papaya seed ethanol extract *in vivo* on the number of osteoblasts cells of periodontitis-induced rats. Trad Med J 2019;24:16-21.
11. Pusporini R, Basori A, Krismariono A. Anti-inflammatory role of papaya seed extracts in inhibiting osteoclastogenesis of rats with periodontitis. Majalah Kedokt Gigi Indones 2018;4:95-101.
12. Kwak SC, Lee C, Kim JY, Oh HM, So HS, Lee MS, *et al*. Chlorogenic acid inhibits osteoclast differentiation and bone resorption by down-regulation of receptor activator of nuclear factor kappa-B ligand-induced nuclear factor of activated T cells c1 expression. Biol Pharm Bull 2013;36:1779-86.
13. Mayasari U, Laoli MT. Simplisia characterization and phytochemical screening Lemon Leaves (*Citrus limon* (L.) Burm.f.). KLOROFIL 2018;2:10-6.
14. Zunjar V, Mammen D, Trivedi BM. Antioxidant activities and phenolics profiling of different parts of *Carica papaya* by LCMS-MS. Nat Prod Res 2015;29:2097-9.
15. Kadiri O, Akanbi CT, Olawoye BT, Gbadamosi SO. Characterization and antioxidant evaluation of phenolic compounds extracted from the protein concentrate and protein isolate produced from pawpaw (*Carica papaya* Linn.) seeds. Int J Food Prop 2017;20:2423-36.
16. Peter JK, Kumar Y, Pandey P, Masih H. Antibacterial activity of seed and leaf extract of *Carica papaya* var. *Pusa dwarf* Linn. IOSR J Pharm Biol Sci 2014;9:29-37.
17. Zheng YZ, Deng G, Liang Q, Chen DF, Guo R, Lai RC. Antioxidant activity of quercetin and its glucosides from propolis: A theoretical study. Sci Rep 2017;7:7543.
18. Xu D, Hu MJ, Wang YQ, Cui YL. Antioxidant activities of quercetin and its complexes for medicinal application. Molecules 2019;24:1123.
19. Saeed F, Arshad MU, Pasha I, Naz R, Batool R, Khan AA, *et al*. Nutritional and phyto-therapeutic potential of papaya (*Carica papaya* Linn.): An overview. Int J Food Prop 2019;17:1637-53.
20. Salla S, Sunkara R, Ogutu S, Walker LT, Verghese M. Antioxidant activity of papaya seed extracts against H_2O_2 induced oxidative stress in HepG2 cells. Food Sci Technol 2016;66:293-7.