



Available online through

www.jbsoweb.com

ISSN 2321 - 6328

Research Article

A COMPARATIVE STUDY ON THE ANTIOXIDANT ACTIVITY OF METHANOL EXTRACTS OF *CARICA PAPAYA* FRUITS PREPARED BY HOT AND COLD MACERATION TECHNIQUES

Seow Lay Jing, Nawfal Akram Bin Morazuki and Gouri Kumar Dash*

Faculty of Pharmacy and Health Sciences, Universiti Kuala Lumpur Royal College of Medicine Perak, 30450 Ipoh, Malaysia

*Corresponding Author Email: gkdash2@gmail.com

Article Received on: 22/05/16 Accepted on: 23/06/16

DOI: 10.7897/2321-6328.04318

ABSTRACT

The present study was aimed to evaluate the antioxidant potential of the methanol extracts of *Carica papaya* fruits using thermal and non thermal methods of extraction. Preliminary phytochemical studies, determination of total phenolic and flavonoid contents and DPPH radical scavenging assay were performed. Preliminary phytochemical screening of the extracts showed presence of alkaloids, flavonoids, tannins and phenolic compounds. The total phenolic and flavonoid contents revealed higher concentration in the extract prepared by cold maceration process than its counterpart, with higher percentage of scavenging DPPH radical. Thus, it may be concluded that application of higher temperature during extraction process affects the phytoconstituents of *C. papaya* fruits.

Keywords: *Carica papaya* L., Antioxidant, preliminary phytochemical studies, DPPH

INTRODUCTION

Carica papaya L. (Family-Caricaceae), a short-lived, soft-stemmed, ever green and small, non-seasonal fruit tree originated from Mexico and Central America, is mainly distributed in tropics all over the world^{1,2}. The delicious papaya fruit, commonly known as 'fruit of the angels', was previously considered to be exotic and rare fruit, but now it is readily available at all times of the year. The fruits are considered to be a powerhouse of essential nutrients and offer protection from cardiovascular diseases and colon cancer. In addition, the fruits are a good source of β -carotene, polyphenols, flavonoids and several antioxidants that protect the body from several deadly diseases^{3,4}. Earlier reports on papaya reveals significant anthelmintic, antimicrobial, antiviral, anti-inflammatory, antidiabetic, hypolipidemic and antihypertensive, free-radical scavenging, antitumor, wound healing, diuretic and neuroprotective activities⁵. There are few reports available in the literature on the antioxidant properties of the fruits^{2,6}. However, there are no reports on the effect of temperature during extraction process on the antioxidant properties. In the present paper, we report the antioxidant activities of the methanol extracts of the fruits prepared by cold and hot maceration process.

MATERIALS AND METHODS

Plant Material

Fresh fruits were collected from the local market in Kedah, Malaysia and authenticated. The samples were washed under running tap water to remove the adherent debris, cut suitably in to small pieces and dried under shade. The dried pieces were subjected to pulverization using a suitable mechanical grinder. The dried coarse powder was used for extraction.

Preparation of Extract

The dried powder (300 g) was extracted with 800 ml methanol at 60°C for 48 h by maceration (hot extraction). Another 300 g of the powder was separately extracted with 800 ml methanol by cold maceration at 25°C for 48 h (cold extraction). After extraction, the methanol extracts were separately concentrated to yield dry solid residue. The extracts prepared by hot and cold extraction methods were labelled as P1 and P2 respectively.

Preliminary Phytochemical Studies

The methanol extracts were subjected to preliminary phytochemical tests to find out presence of different classes of phytochemicals⁷⁻⁹.

Determination of Total Phenolic Content

The test was carried out using Folin-Ciocalteu reagents^{10,11}. Gallic acid (GA) was used as standard. Briefly, 1 ml of Folin-ciocalteu solution was added to 1 ml of the test sample in a test tube, the mixture shaken vigorously and allowed to stand at room temperature for 5 min. Two millilitres of 20% w/v sodium carbonate solution was added to the mixture and kept in darkness for 1 h followed by measuring the absorbance at 750 nm. The same procedure was repeated for the standard gallic acid solutions (20-200 μ g/ml) to obtain the calibration curve. The total phenolic contents were calculated using gallic acid calibration curve. The result is expressed as gallic acid equivalents.

Determination of Total Flavonoid Content

The total flavonoid content was estimated using quercetin as standard¹². Solution of 2% w/v aluminium chloride (1 ml) was added to 0.5 ml of the extract and the mixture was incubated at room temperature for 1 h. The absorbance of the solution was

measured at 420 nm. The total flavonoid contents were calculated using quercetin calibration curve. The result is expressed as quercetin equivalents.

DPPH radical Scavenging Assay

The free radical scavenging activity assay of the methanol extracts (P1 and P2) was evaluated using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) method¹³ with some modifications. Briefly, to a set of clean and dried test tubes, 3 ml of methanol and 150 µl of 0.1% DPPH reagent was added and mixed thoroughly. The solution was allowed to stand for 30 min. To these test tubes, 1 ml of extract was added in increasing concentration (100-500 µg/ml). The solution of ascorbic acid (100-500 µg/ml) was taken as the standard. Two millilitres of methanol with 1 ml DPPH was taken as control. The reaction mixture was left for shaking for 5 min and kept in dark for 30 min. After the reaction time, absorbance of the reaction mixture was measured at 517nm.

The DPPH radicals scavenging activity was calculated using the following formula:

$$\% \text{ scavenging} = (\text{Absorbance of control} - \text{Absorbance of test sample}) / \text{Absorbance of control} \times 100$$

RESULTS

Preliminary Phytochemical Studies

The preliminary phytochemical screening of the methanol extracts revealed presence of alkaloids, flavonoids, tannins and phenolic compounds

Total phenolic content

The total phenolic content in the methanol extracts was estimated by using Folin-Ciocalteu reagent and expressed as gallic acid (GA) equivalent. The standard curve equation represented $y = 0.0081x + 0.0388$, $R^2 = 0.999$ (Figure 1). The concentration of total phenols is expressed as mg of gallic acid/g of extract (Table 1).

Total flavonoid content

The total flavonoid content in the extracts was estimated using quercetin (Q) as the standard. The standard curve equation represented $y = 0.0116x + 0.0402$, $R^2 = 0.9907$ (Figure 2). The values obtained for the concentration of total flavonoids are expressed as mg of quercetin/g of extract (Table 1).

DPPH Radical Scavenging Assay

The antioxidant activity of the methanol extracts was determined using a methanol solution of DPPH reagent. The activity of the extracts is expressed in terms of percentage of inhibition (Figure 3). Parallel to the examination of plant samples, the values for two standard compounds (quercetin and ascorbic acid) were obtained for activity comparison.

The scavenging effect of DPPH radicals assay showed concentration-dependant activity in both the extracts (P1 and P2) with different values. Quercetin and ascorbic acid showed high scavenging percentage of DPPH with the highest scavenging of 58.23% and 40.34% respectively at the concentration of 500µg/ml. Whereas, for both P1 and P2 samples, the scavenging percentage were lower than standard antioxidants, with the inhibition of 11.92% and 13.35% respectively at the concentration of 500µg/ml. The extract P2 (obtained by cold maceration) exhibited higher scavenging percentage than P1 (obtained by hot maceration).

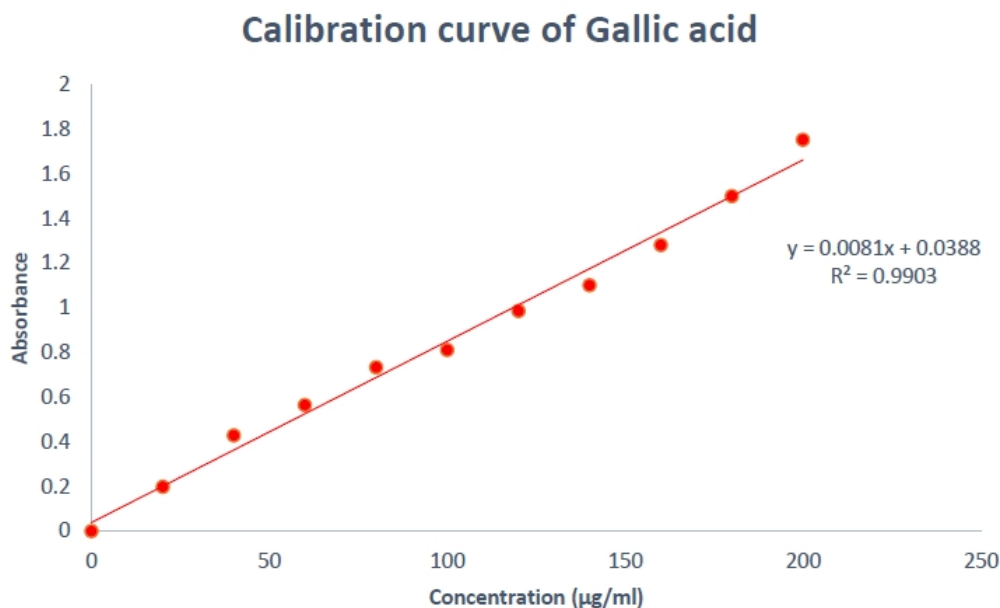


Figure 1: Calibration curve of gallic acid

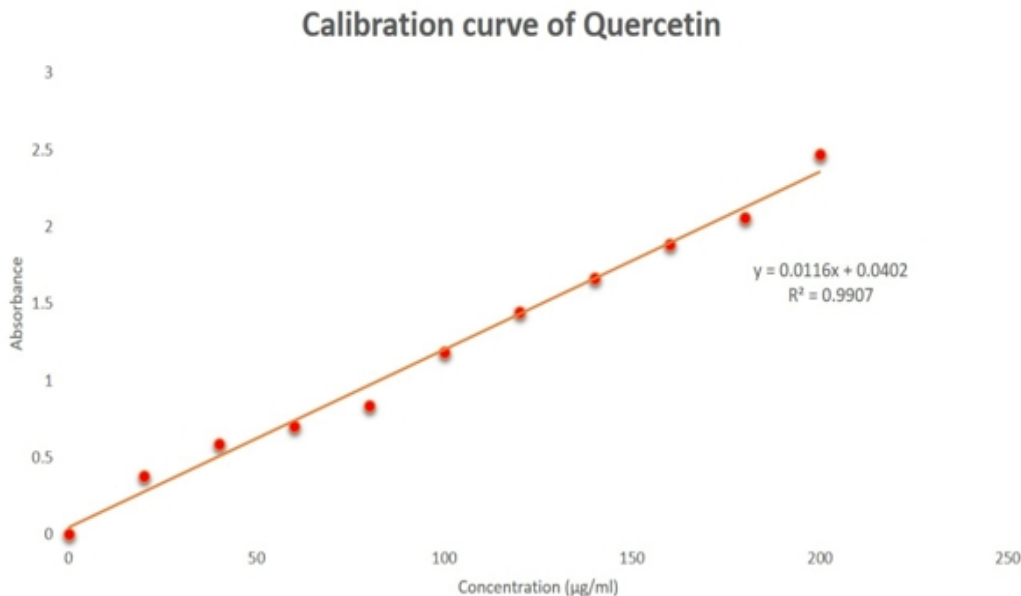


Figure 2: Calibration curve of quercetin

Table 1: Total phenolic and flavonoid contents of methanol extracts of *Carica papaya*

Test sample	Total Phenolic Content (mg of GA/g of extract)	Total Flavonoid Content (mg of Q/g of extract)
P1	108.18 ± 0.93	65.21 ± 1.45
P2	164.72 ± 1.19	93.43 ± 3.49

Results expressed as Mean ± SD from three observations

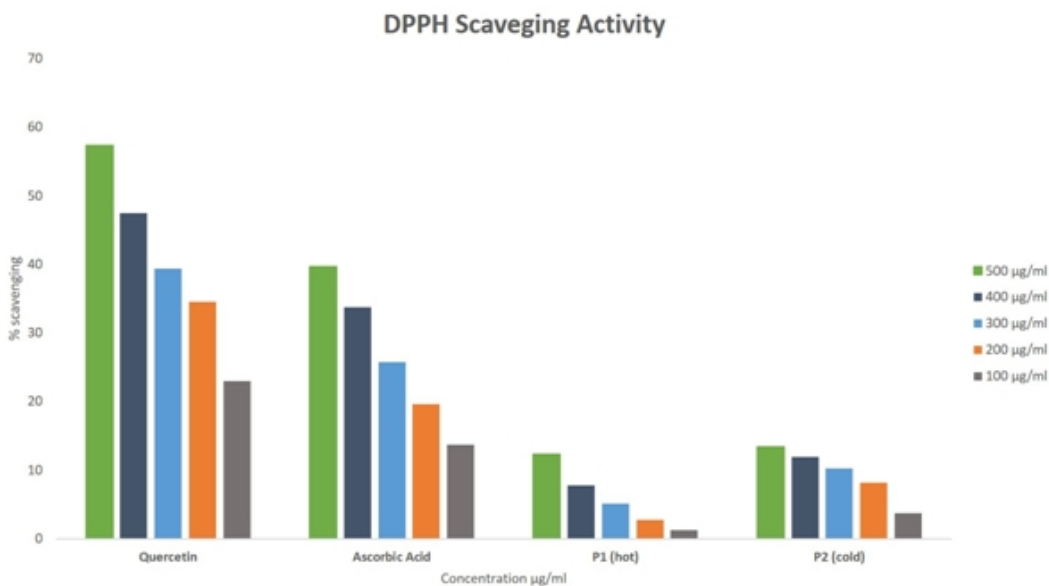


Figure 3: DPPH radical scavenging activity of the methanol extracts of *C. papaya*

DISCUSSION

The total phenolic contents revealed highest concentration in the extract (P2) prepared by cold maceration process (164.72 mg/g). The content of the total phenolics in the extract prepared by hot maceration (P1) techniques was 108.18 mg/g. The total flavonoid content in both extracts (P1 and P2) also showed similar pattern. The total flavonoid content P1 was 65.21 mg/g,

higher than value of total flavonoid content in P2 (93.43 mg/g). The differences in the amount of phenolics and flavonoids in both extracts may be attributed by the effect of temperature during extraction process of the sample. Thus, it may be agreed that the polyphenolic contents in *C. papaya* is influenced by higher temperature during extraction.

DPPH radical scavenging assay is an ideal protocol in estimating antioxidant activities of plant extracts. It is a stable free radical and not affected by enzyme inhibition reactions. When DPPH free radicals becomes paired with hydrogen from a free radical scavenging antioxidant, its purple colour fades rapidly to yellow to form reduced DPPH-H. The resulting decolourization is stoichiometric with respect to number of electrons captured¹⁴. In the present study, the low value in scavenging percentage of DPPH may be due to the presence of antioxidant compounds such as polyphenols that did not react efficiently with DPPH free radicals due to steric resistance. The radicals scavenging activity is not always only due to the phenolic content itself, but with other various antioxidant compounds¹⁵.

CONCLUSION

Based on the results of total phenolic and flavonoid contents of the extracts where the values were higher for P2 compared to P1 and correspondingly, the results of higher scavenging percentage in DPPH assay, it may be concluded that application of higher temperature during extraction process affects the phytoconstituents of *C. papaya* fruit.

ACKNOWLEDGEMENTS

The authors are thankful to Universiti Kuala Lumpur Royal College of Medicine Perak for providing necessary facilities to carry out the research work.

REFERENCES

- Zhou K, Wang H, Mei W, Li X, Luo Y, Dai H. Antioxidant activity of papaya seed extracts. *Molecules* 2011; 16: 6179-6192.
- Maisarah AM, Nurul Amira B, Asmah R, Fauziah O. Antioxidant analysis of different parts of *Carica papaya*. *Int Food Res J* 2013; 20(3): 1043-1048.
- Yogiraj V, Goyal PK, Chauhan CS, Goyal A, Vyas B. *Carica papaya* Linn: An overview. *Int J Herb Med* 2014; 2 (5): 1-8.
- Aravind G, Bhowmik D, Duraivel. S, Harish G. Traditional and medicinal uses of *Carica papaya*. *J Med Plants Studies* 2013; 1(1): 7-15.
- Maisarah AM, Asmah R, Fauziah O. Proximate analysis, antioxidant and antiproliferative activities of different parts of *Carica papaya*. *J Nutr Food Sci* 2014; 4(2): 1-7.
- Sadek KM. Antioxidant and immunostimulant effect of *Carica papaya* Linn. aqueous extract in acrylamide intoxicated rats. *Acta Inform Med* 2012; 20(3): 180-185.
- Evans WC. Trease and Evans Pharmacognosy. 16th ed. London: W.B Saunders; 2009.
- Harborne JB. *Phytochemical Methods - A Guide to Modern Techniques of plant analysis*, London: Chapman and Hall; 1998.
- Nurcholis Waras, Khumaida Nurul, Syukur Muhamad, Bintang Maria, Ardyani I.D.A.A.C. Phytochemical screening, antioxidant and cytotoxic activities in extracts of different rhizome parts from *Curcuma aeruginosa* Roxb. *Int. J. Res. Ayurveda Pharm.* 2015;6(5):634-637 <http://dx.doi.org/10.7897/2277-4343.065118>
- Kim D, Jeond S, Lee Ch. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chem* 2013; 81: 321-326.
- Maraia F. Elmhdwi and Idress Hamad Attitalla. Antioxidant and free radical scavenging activity of essential oil extracted from *Rosmarinus officinalis* L. *J Biol Sci Opin* 2015;3(5):223-229 <http://dx.doi.org/10.7897/2321-6328.03549>
- Ordenez AA, Gomez JG, Vattuone MA, Isla MI. Antioxidant activities of *Sechium edule* (Jacq) Swart extracts. *Food Chem* 2006; 97: 452-458.
- Siddiqua A, Premakumari KB, Rokeya S, Vithya, Savitha. Antioxidant activity and estimation of total phenolic content of *Muntingia calabura* by colorimetry. *Int J ChemTech Res* 2010; 2(1): 205-208.
- Yamagushi T, Takamura H, Matoba T, Terao J. HPLC method for evaluation of the free radical- scavenging activity of foods by using 1, 1,- diphenyl- 2-picrylhydrazyl. *Biosci Biotech Biochem* 1998; 62: 1201-1204.
- Khamsah SM, Akowah G, Zhari I. Antioxidant activity and phenolic content of *Orthosiphon stamineus* benth from different geographical origin. *J Sustainability Sci Mgmt* 2006; 1: 14-20.

Cite this article as:

Seow Lay Jing, Nawfal Akram Bin Morazuki and Gouri Kumar Dash. A comparative study on the antioxidant activity of methanol extracts of *Carica papaya* fruits prepared by hot and cold maceration techniques. *J Biol Sci Opin* 2016;4(3):71-74 <http://dx.doi.org/10.7897/2321-6328.04318>

Source of support: Nil; Conflict of interest: None Declared

Disclaimer: JBSO is solely owned by Moksha Publishing House - A non-profit publishing house, dedicated to publish quality research, while every effort has been taken to verify the accuracy of the contents published in our Journal. JBSO cannot accept any responsibility or liability for the site content and articles published. The views expressed in articles by our contributing authors are not necessarily those of JBSO editor or editorial board members.