

Comparison of Antioxidant Properties in Juiced and Brewed *Carica Papaya* Leaves Extracts

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ABSTRACT

Carica papaya is widely cultivated not only for its delicious fruit, but also for its medicinal properties. In Malaysia, papaya leaf is perceived to cure many ailments. Recently, papaya leaves extract was reported to possess anti-dengue properties. Some researchers suggested that the antioxidants in papaya leaf contributing to its anti-dengue effects. This study compared two common household methods to prepare papaya leaves extracts as medicinal supplement, namely juicing and brewing, in terms of total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity based on ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical scavenging assays. The results obtained are as follows: TPC (in mg GAE/g) were 6.05 ± 0.05 and 4.17 ± 0.05 ; and TFC (in mg QUE/g) 1.38 ± 0.02 and 0.30 ± 0.07 for brewing and juicing, respectively. Meanwhile, for the antioxidant activities: 6.29 ± 0.25 mg TE/g on FRAP and 52% scavenging activity with IC_{50} of 926.31 ± 2.21 mg TE/g on DPPH for brewing; and 8.59 ± 0.22 mg TE/g on FRAP and 60% scavenging activity with IC_{50} of 758.02 ± 9.32 mg TE/g for juicing. Pearson correlation analysis showed a strong, positive correlation between TPC and TFC and their antioxidant activities, with $r^2 > 0.900$ for all of the analyses. Overall, papaya leaves extract prepared by brewing contained higher ($p < 0.05$) TPC, TFC and activities compared to juicing. The results suggest that antioxidant contents in papaya leaves extracts are readily influenced by the preparation methods used.

KEYWORDS: TPC; TFC; FRAP; DPPH scavenging assay; *Carica papaya* leaves

Data With Detail Description - Food and Beverages

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INTRODUCTION

Carica papaya (papaya), or locally known as “betik”, is cultivated widely in tropical and subtropical countries for its edible fruit. In Malaysia, different parts of papaya tree such as latex, seeds, leaves, unripe fruits and roots have been consumed as traditional medicine for various ailments (Subenthiran *et al.*, 2013; Ahmad *et al.*, 2011; Kathiresan *et al.*, 2009; Ahmad and Ismail, 2003). Recently, papaya leaves have been reported to possess anti-dengue properties where intake of its extracts improved blood platelet count in dengue patients (Kumar *et al.*, 2015; Subenthiran *et al.* 2015). While researchers have not identified the specific bioactive compounds contributing to its dengue-selective effect, it is suggested that the synergy of the plant phytochemicals, especially its abundant antioxidant bioactive compounds play a vital role in revitalizing and reduce oxidative stress experienced by the body due to the virus infection (Kumar *et al.*, 2015).

Currently there is no standardized preparation of papaya leaves extract for dengue treatment (Ansari, 2016). Thus, this paper compared two traditional methods commonly practiced to prepare papaya leaves extract for medicinal purpose, namely juicing and brewing (i.e. boiling). The comparison is based on the TPC, TFC and antioxidant activity (FRAP and DPPH assay) of the methanol extracts of young papaya leaves.

METHODOLOGY

Sample collection

Papaya leaves were harvested from papaya trees planted at the vicinity of Analytical Chemistry Laboratory in Universiti Malaysia Sabah (UMS). Throughout the entire study, young (i.e. third leaves from the uppermost shoot of papaya trees) and healthy leaves were freshly harvested from the same papaya trees. The plant species was authenticated by Mr. Johnny Gisil, a botanist from Biology Tropical and Conservation Institute, UMS.

Chemicals and reagents

Folin–Ciocalteu’s phenol reagent, DPPH, (\pm)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox) and potassium acetate ($\text{CH}_3\text{CO}_2\text{K}$) were purchased from Sigma-Aldrich (USA). Gallic acid and anhydrous sodium carbonate (Na_2CO_3) were obtained from Fluka (USA). Thermo Fisher Scientific supplied 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ), ferric chloride, hydrochloric acid and quercetin hydrate with $\geq 95\%$ purity while sodium acetate trihydrate ($\text{CH}_3\text{COONa}\cdot 3\text{H}_2\text{O}$) were supplied by Merck (Germany). Methanol AR grade was supplied by Qrec (Malaysia).

Sample preparation

The sample preparation employed in this study were based on the common household practices when preparing herbal medicine which is either by pounding the fresh leaves to obtain the juice or brewing them in hot water.

a. Juicing

Fresh papaya leaves weighing 10.0 mg was washed gently, cut into small pieces and then pounded using pestle and mortar until the leaves turned into paste. Then, 10ml of deionized water was added to aide in the maceration process. After about 5 min of maceration, the papaya leaves extract (“juice”) were collected into a beaker by straining the paste using cotton fabric as filter. Four milliliters of the juice was then added with 10 ml of methanol and sonicated using ultrasonic bath for 20 minutes. The extract was further filtered using 0.45 μm micro-filter to remove fine solid particles and immediately analyzed for TPC, TFC and antioxidant scavenging assays.

b. Brewing

Fresh papaya leaves weighing 10.0 g was washed, cut into small pieces, briefly pounded using pestle and mortar and then added with 10 ml deionized water to aid the maceration process. The extract was then transferred to a beaker, made up to 100ml with deionized water and then heated at medium heating rate on a hotplate until it reached the boiling point. After boiling, the brewed papaya leaves was left to cool down at room temperature. The “papaya tea” was filtered to remove any solid particles and then followed by extraction with methanol as described in (a).

The difference in the preparation procedures for juicing and brewing are summarized in Table 1.

Table 1. Variables in juicing and brewing methods of *Carica papaya* leaves.

	Juicing	Brewing
Temperature (°C)	Room temperature	100°C
Time length (min)	5 min (maceration)	30 min (until boiling point is reached)
Water-to-leaf ratio (ml/g)	1:1	10:1

Analysis of Antioxidant Contents and Activities

a. Total phenolics content (TPC)

The TPC was determined as described by Ainsworth & Gillespie (2007) with a slight modification. Exactly 100 µl of the sample extract was added with 200 µL of 10% (v/v) Folin-Ciocalteu reagent and then sonicated for 5 minutes in an ultrasonic bath. Addition of 800 µL of 7.5% (w/v) Na₂CO₃ was done before the mixture was once again sonicated for 5 minutes and then incubated in the dark for 2 hours at room temperature. Then, 200 µl of the mixture was loaded into the 96-well microplate and the absorbance was measured at 765 nm using a Multiskan™ Go 1510 microplate spectrophotometer (ThermoFisher Scientific, USA). Gallic acid was used as the standard for a calibration curve and the results were expressed as mg of gallic acid equivalents per g of sample (mg GAE/g FW).

b. Total flavonoid content (TFC)

TFC was determined according to the method described by Chang *et al.* (2002) with a slight modification. To a 120 µl of the sample extract, 360 µL methanol, 24 µl AlCl₃, 24 µl CH₃COOK and 680 mL of deionized water were added. This mixture was sonicated for 5 minutes using ultrasonic bath before undergoing incubation at room temperature in the dark for 30 minutes. Similar procedures as in TPC were then carried out but the absorbance was measured at 415 nm. Quercetin was used as the standard for a calibration curve and the results were expressed as mg of quercetin equivalents per gram of sample (mg QUE/g).

c. FRAP Assay

The FRAP assay was carried out as reported by Russo *et al.* (2013) with slight modifications. Preparation of FRAP reagent was carried out by mixing 38 mM sodium acetate anhydrous buffer in deionized water (pH 3.6), with 10 mM of TPTZ in 40 mM HCl and 20 mM ferric chloride solution in deionized water at 10:1:1 ratio. Then, 20 µl of sample extract was mixed in 180 µl of the FRAP reagent in a 96-well microplate followed by incubated in the dark at 37°C for 40 minutes. The absorbance of the mixture was measured at 593 nm using the microplate spectrophotometer. Trolox was used as a standard and the results were expressed as mg of trolox equivalents per gram of sample (mg TE/g).

d. DPPH radical scavenging assay

For this assay, the method described by Chan *et al.* (2012) with slight modification was employed. A series of 50 µl of sample extract at differing concentration (0, 125, 250, 500, 1000, 2000 and 4000 µg/ml) was each reacted with 195 µl of 0.1 mM DPPH methanolic solution in a 96-well microplate. After one minute of gentle swirling, the mixture was allowed to stand for one hour. Following the allocated incubation, the absorbance of the mixture was measured at 540 nm using the microplate spectrophotometer. Trolox was used as the standard for a calibration curve and the results were expressed as mg of trolox equivalents per g of sample (mg TE/g).

Statistical analysis

Statistical analysis using independent sample T-test was conducted using SPSS statistical software version 22. Pearson correlation test was also performed using the same software.

RESULTS AND DISCUSSION

In this study, antioxidant compounds in the papaya leaves juice and brew ("tea") were extracted with methanol by sonication for 20 min at room temperature. Variations in the results during the solvent extraction were considered not significant (i.e. < 20 % RSD in recovery). The extraction parameters were not optimized (e.g. time, temperature, solvent polarity) because the scope of this study is to compare these two common methods of preparing papaya leaves extract for dengue patients. Methanol was used for the extraction instead of water because generally flavonoids have lower solubility in water.

Calculated based on their respective calibration curves (correlation coefficient, r^2 , are 0.9937 and 0.9940 for GA and QUE, respectively), the results are shown in Table 2. The Pearson correlation analysis shows strong coefficient correlation between the phenolics and flavonoids contents with their antioxidant activities (FRAP and DPPH free radical scavenging assays) with r^2 values more than 0.900 for all of the analyses. Strong correlation between polyphenols content and their activity assays were also reported by previous researchers (Maisarah *et al.*, 2014; Butsat and Siriamornpun, 2016; Vuong *et al.*, 2013).

Table 2. TPC, TFC and antioxidant activities of juiced and brewed *Carica papaya* leaves extracts.

	Juicing	Brewing
TPC (mg GAE/g)	4.17 ± 0.05 ^a	6.05 ± 0.05 ^b
TFC (mg QUE/g)	0.3 ± 0.07 ^a	1.38 ± 0.02 ^b
FRAP (mg TE/g)	6.29 ± 0.25 ^a	8.59 ± 0.22 ^b
DPPH (mg TE/g)	926.31 ± 2.21 ^a	758.02 ± 9.32 ^b

The values shown are mean ± standard deviations for triplicate extractions and those in the same row not sharing the same superscript letters are significantly different from each other ($p < 0.05$).

The results show higher antioxidant contents and activities in the brewed compared to in juiced papaya leaves extracts ($p < 0.05$). As expected, heating has facilitated (i.e. provide higher energy) an increase in mass transfer rate during the brewing process which allows higher amount of phenolic and flavonoid compounds to leach out into the surrounding liquid. Previous researchers have reported similar observations where higher extraction temperature improved the phytochemical contents in natural products (Okoduwa *et al.*, 2016; Vuong *et al.*, 2013; Gertenbach, 2001). Beside higher temperature, the longer duration (30 min) is another advantage in brewing. The longer time has provided ample time for the antioxidant compounds to leach out from the plant tissues which subsequently extracted out by methanol. Another point to note is that the water-to-leaf ratio used in the two methods are different. The lower ratio in brewing (10:1 vs. 1:1 in juicing) provides substantial concentration gradient between the polyphenols on the surface and those trapped inside the leaf tissues, leading to accelerated extraction kinetics (Gertenbach, 2001).

It is worthy to highlight here that although antioxidant properties in the papaya leaves juice are essentially lower than in brewed papaya, however, the method is simpler, faster and cheaper because heating is not required. Beside, even though a brief pounding and maceration, the TPC, FRAP and DPPH of the papaya leave juice are still more than half of those in the papaya tea. The

TFC in the papaya juice is much lower possibly due to insufficient time for the low solubility flavonoids to be extracted out. Moreover, high temperature promotes the risk of decomposition and epimerization in the polyphenols and other plants phytochemicals (Sultana *et al.*, 2009; Vuong *et al.*, 2010) which potentially reducing the antioxidant properties of the plant extracts. More importantly, juicing preserves the thermal-sensitive phytochemicals and nutrients in papaya leaves such as glucosinolates, vitamins and proteins. For instance, benzyl glucosinolate i.e. a precursor to a potent natural anticancer called benzyl isothiocyanate (abundant in papaya leaf) is degraded by high temperature (Ahmad, 2015).

CONCLUSION

Based on the results obtained, brewing fresh papaya leaves to the boil for about 30 minutes produced significantly higher TPC, TFC and antioxidant activity compared to 5 minutes juicing. Perhaps with optimized method (e.g. longer maceration plus stirring or shaking), juicing may potentially a better method when taking the other thermal-sensitive phytochemicals and nutrients into consideration. This should be studied further.

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