

Antioxidant properties of several tropical fruits: A comparative study

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Abstract

Nine tropical fruits were analyzed for total phenol contents, ascorbic acid contents and antioxidant activities. The antioxidant activities were evaluated based on the ability of the fruit extracts to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH), reduce iron(III) to iron (II) and to bind to iron(II) ions. The results were compared to those of orange. It was found that guava, papaya and star fruit have higher primary antioxidant potential, as measured by scavenging DPPH and iron(III) reducing assays. Banana, star fruit, water apple, langsat and papaya have higher secondary antioxidant potential as measured by the iron(II) chelating experiment.

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1. Introduction

Fruits are rich with antioxidants that help in lowering incidence of degenerative diseases such as cancer, arthritis, arteriosclerosis, heart disease, inflammation, brain dysfunction and acceleration of the ageing process (Feskanich et al., 2000; Gordon, 1996; Halliwell, 1996). Antioxidants are substances that can prevent or delay oxidative damage of lipids, proteins and nucleic acids by reactive oxygen species, which include reactive free radicals such as superoxide, hydroxyl, peroxy, alkoxy and non-radicals such as hydrogen peroxide, hypochlorous, etc. They scavenge radicals by inhibiting initiation and breaking chain propagation or suppressing formation of free radicals by binding to the metal ions, reducing hydrogen peroxide, and quenching superoxide and singlet oxygen (Shi, Noguchi, & Niki, 2001). The most abundant antioxidants in fruits are polyphenols and Vitamin C, Vitamins A, B and E and carotenoids are present to a lesser extent in some fruits. These polyphenols, most of which are flavonoids, are present mainly in ester and glycoside forms (Fleuriet & Macheix, 2003).

Malaysia is a tropical country with a large diversity of fruits. The antioxidant properties of a number of tropical fruits have been investigated (Jimenez-Escrig, Rincon, Pulido, & Saura-Calixto, 2001; Leong & Shui, 2002; Someya, Yoshiki, & Okubo, 2002) on an individual basis using different analytical methods. It is therefore difficult to rank the antioxidant capabilities of these tropical fruits based on the existing literature reports, although study to compare the antioxidant capabilities has been reported for temperate fruits (Garcia-Alonso, Pascual-Teresa, Santos-Buelga, & Rivas-Gonzalo, 2004). It is the aim of this paper to measure the antioxidant properties of some common fruits available in tropical regions by using three common standard methods: 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging assay, ferric reducing assay using ferricyanide ion and ferrous ion chelating assay. Each of the above assays measures different aspects of the antioxidant activity of the fruit extracts.

2. Materials and methods

2.1. Fruits

Nine types of tropical fruits and one imported fruit (orange) used as reference were studied. They were banana

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(*Musa sapientum*, pisang mas cultivar), dragon fruit (*Hylocereus undatus*), guava (*Psidium guajava*; Kampuchea cultivar GU8 – with seed), and guava (seedless), kedondong (*Spondias cytherea*), langsung (*Lansium domesticum* – longkong manis cultivar), mangosteen (*Gardinia mangostana*), papaya (*Carica papaya* L., solo cultivar), star fruit (*Averrhoa carambola*), and water apple (*Syzygium aqueum*). The orange was a seedless variety from Australia (Valencia). Guava (Kampuchea – with seed) and kedondong were harvested from the garden trees and the rest were obtained from local markets. All the fruits were analyzed within two days after acquisition.

2.2. Chemicals and equipment

The following chemicals were used for the following experiments. Total phenol determination: Folin-Ciocalteu's phenol reagent (Fluka, 2N), gallic acid (Fluka, 98.0%), anhydrous sodium carbonate (Fluka, 99.0%). Ascorbic acid determination: potassium iodide (Hamburg, 99%), arsenic trioxide (Fluka, 99.5%), iodine (Fisher, 99.9%), sodium hydrogen carbonate (Fisher, 99.8%), sodium hydroxide (Hamburg, 98%), sulphuric and hydrochloric acids (Fisher). DPPH scavenging activity: 1,1-diphenyl-2-picrylhydrazyl (Sigma, 90%), L(+)-ascorbic acid (Merck, 99.7%), methanol (Mallinckrodt, 100%). Ferric reducing power: iron(III) chloride 6-hydrate (Fisher, 100%), potassium ferricyanide (Unilab, 99%), trichloroacetic acid (Fisher, 99.8%), potassium dihydrogen phosphate (Bendosen, 99.5%), dipotassium hydrogen phosphate (Merck, 99%). Ferrous ion chelating activity: Iron(II) sulphate 7-hydrate (Hamburg), ferrozine iron reagent (Acros Organics, 98+%).

Water used was purified by an Elga deionizer and ethanol for extraction was from HmbG Chemicals (95 V%).

Optical absorbance was measured with an Anthelie Advanced 5 Secoman or GBC Cintra 5 UV–vis spectrophotometer. pH was measured with a Hanna pH211 meter. HPLC determination of ascorbic acid was measured with a Waters Associate (2487) instrument.

2.3. Sample preparation

The edible portions (weight varying between 10 and 30 g depending on the type of fruits) were either crushed to a paste-like state for approximately 1 min (with intermittent stops to minimize heating) using a blender or crushed and homogenized by the pestle-and-mortar method. Guava, star fruit and water apple were homogenized without peeling off the skin and the other seven fruits were crushed with the skin removed. The homogenized sample was transferred into a 100-ml volumetric flask and 50% ethanol was added up to the mark. The mixture was shaken manually or with a vibrator for 10 min and then filtered under suction. In situation where the filtrate appeared to be very cloudy, the filtrate was centrifuged to obtain a clear supernatant liquid, which was subsequently used for the various

assays. All tests were performed within a week. The extracts were stored at $-20\text{ }^{\circ}\text{C}$.

2.3.1. Ascorbic acid content (AAC)

The AAC was determined by the iodine titration method (Suntornsuk, Kritsanapun, Nilkamhank, & Paochom, 2002) or the RP-HPLC method: Waters C-18 column ($3.9 \times 150\text{ mm}$, $5\text{ }\mu\text{m}$ particle size), mobile phase 5% acetic acid, flow-rate 0.5 ml/min and 254 nm detection wavelength. Both methods gave similar results to within 5%.

2.3.2. Total phenol content (TPC)

TPC was determined using the Folin-Ciocalteu's reagent (Singleton & Rossi, 1965). Samples (0.3 ml, triplicate) were introduced into test tubes followed by 1.5 ml of Folin-Ciocalteu's reagent (diluted 10 times with water) and 1.2 ml of sodium carbonate (7.5% w/v). The tubes were vortexed, covered with parafilm and allowed to stand for 30 min. Absorption at 765 nm was measured. If the sample absorbance exceeded 1, the sample was appropriately diluted to give reading less than 1. Total phenol contents were expressed in gallic acid equivalents (mg per 100 g fresh fruit).

Since ascorbic acid also contributes to the formation of the blue molybdenum–tungsten complex, it is important to correct for the absorbance originating from it. An ascorbic acid calibration curve was therefore prepared. The TPCs reported in this paper have all been corrected for ascorbic acid.

2.4. DPPH assay

The free radical scavenging activity of the fruit extracts was measured by measuring the decrease in absorbance of methanolic DPPH solution at 517 nm in the presence of the extract (Krings & Berger, 2001). The initial concentration of DPPH was 0.1 mM and the reading was taken after allowing the solution to stand for 30 min. In cases where the absorbance decreased too much (when the solution turned yellow) before the 30 min period, the sample was appropriately diluted. The antioxidant activity was expressed as

$$\% \text{ disappearance} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100\%$$

IC_{50} , the amount of sample extracted into 1 ml solution necessary to decrease by 50% the initial DPPH concentration was derived from the % disappearance vs. concentration plot. (Concentration here means mg of fruit extracted into 1 ml solution.) The results are also expressed as ascorbic acid equivalent antioxidant capacity (AEAC) (Leong & Shui, 2002) using either one of the following equations where

$$\begin{aligned} \text{AEAC (mg AA/100g)} &= (A_{\text{control}} - A_{\text{sample}}) / (A_{\text{control}} - A_{\text{AA}}) \\ &\quad \times \text{conc. AA (mg/ml)} \times \text{vol extract (ml)} \\ &\quad \times 100/\text{g sample} \end{aligned}$$

or

$$\text{AEAC} = (\text{IC}_{50(\text{AA})} / \text{IC}_{50(\text{sample})}) \times 10^5$$

IC_{50(AA)} value used was determined to be $(3.72 \pm 0.24) \times 10^{-3}$ mg/ml based on 18 repeated measurements.

2.5. Ferric reducing power

The ferric reducing power of the fruit extracts was determined by using potassium ferricyanide–ferric chloride method (Oyaizu, 1986). Different dilutions of extracts amounting to 1 ml were added to 2.5 ml 0.2 M phosphate buffer (pH 6.6) and 2.5 ml potassium ferricyanide (1%). The mixtures were incubated at 50 °C for 20 min, after which 2.5 ml trichloroacetic acid (10%) was added. Two and one half milliliters of the mixture was taken and mixed with 2.5 ml water and 0.5 ml 1% FeCl₃. The absorbance at 700 nm was measured after allowing the solution to stand for 30 min. A graph of absorbance vs. fruit extract concentration was plotted to observe the reducing power.

2.6. Ferrous ion chelating activity

The ferrous ion chelating (FIC) activity was measured by the decrease in the absorbance at 562 nm of the iron (II)-ferrozine complex (Carter, 1971; Dinis, Madeira, & Almeida, 1994). One milliliter 0.125 mM FeSO₄, and 1.0 ml 0.3125 mM ferrozine were mixed with 1.0 ml sample (with different dilutions). The mixture was allowed to equilibrate for 10 min before measuring the absorbance. Sample solutions with appropriate dilutions were used as blanks as the fruit extracts may also absorb at this wavelength. The ability of the sample to chelate ferrous ion was calculated relative to the control (consisting of iron and ferrozine only) using the formula

$$\text{Chelating effect \%} = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100$$

3. Results and discussion

3.1. TPC and AAC

The TPC values summarized in Table 1 were obtained from Folin-Ciocalteu's reagent with correction for the presence of ascorbic acid. The calibration equation for ascorbic

acid was determined to be $y = 0.0072x - 0.0176$ ($R^2 = 0.9900$) where y is absorbance at 765 nm and x is concentration of ascorbic acid in mg/l. The gallic acid standard line has the equation $y = 0.0111x - 0.0148$ ($R^2 = 0.9998$).

Among the nine tropical fruits studied, guava (both seeded and seedless), star fruit and langsat have the largest TPC values, which are higher than that of orange (75 ± 10 mg/100 g). The wide range of values among the various samplings is mainly caused by the different maturation stage of the fruit. For example, it has been reported that TPC of guava decreased throughout the immature stage to the full-ripe stage (Bashir & Abu-Goukh, 2003). Besides, banana, mango and date have also been reported to have decreasing TPC on fruit ripening (Ibrahim, Abu-Goukh, & Yusuf, 1994; Abu-Goukh & Abu-Sarra, 1993; Al-Ogaidi & Mutlak, 1986).

Fruits with relatively low TPC (lower than orange) are mangosteen, banana, water apple, kedondong, papaya and dragon fruit. Dragon fruit has been only commercially available in Malaysian markets recently and therefore its antioxidant activity has not been reported. Kedondong is also another tropical fruit that has not been studied much.

Guava and papaya are the only two fruits that have ascorbic acid content higher than that of orange ($= 67 \pm 9$ mg/100 g). Kedondong has moderate amount of ascorbic acid. The remaining fruits have AAC less than 10 mg/100 g.

Based on the limited number of sampling done on the seeded ($n = 8$) and seedless ($n = 6$) guava, no significant difference was found between them in TPC and AAC. Table 2 shows that the antioxidant contents are always higher in guava fruit with skin than fruit with the skin peeled off. This result is consistent with the results reported earlier (Bashir & Abu-Goukh, 2003; Jimenez-Escrig et al., 2001).

3.2. DPPH assay and reducing power

The bleaching of the DPPH solution increases regularly with increasing amount of fruit in a given volume of solution. The bleaching action is mainly attributed to the presence of polyphenols and ascorbic acid extracted into the solution. The amount of fruit required to scavenge 50%

Table 1
The total phenol and ascorbic acid contents, IC₅₀ and AEAC of tropical fruits (orange is included for comparison)

Fruits	TPC (mg/100 g)	AAC (mg/100 g)	IC ₅₀ (mg/ml)	AEAC (mg/100 g)
Guava (seeded) ⁸	138 ± 31	144 ± 60	1.71 ± 0.61a	218 ± 79
Guava (seedless) ⁶	179 ± 44	132 ± 46	2.11 ± 0.63a,b	176 ± 54
Banana (mas) ⁶	51 ± 7	4.9 ± 0.6	13.4 ± 2.5e	27.8 ± 5.5
Dragon fruit ³	21 ± 6	8.0 ± 1.6	27.5 ± 3.9f	13.5 ± 2.1
Kedondong ⁷	33 ± 5	30 ± 5	9.9 ± 1.9d	37.6 ± 7.6
Langsat ³	100 ± 29	3.9 ± 1.0	25.4 ± 7.3f	14.6 ± 4.3
Mangosteen ⁵	54 ± 7	5.8 ± 0.8	11.5 ± 3.6d	32.3 ± 10.3
Papaya (Solo) ³	28 ± 6	108 ± 16	3.5 ± 0.9c	106 ± 28
Star fruit ⁷	131 ± 54	5.2 ± 1.9	3.8 ± 2.1b,c	98 ± 55
Water apple ⁴	35 ± 4	4.1 ± 2.1	12.0 ± 3.8d,e	31 ± 10
Orange ⁵	75 ± 10	67 ± 9	5.4 ± 1.3c	69 ± 17

The superscript numerals in the fruits column are the number of samples studied. The values in IC₅₀ column with similar letters are not significantly different ($p = 0.05$).

Table 2
Antioxidant contents of peeled and non-peeled guava fruits

Sample	TPC (mg/100 g)		AAC (mg/100 g)	
	Non-peeled	Peeled	Non-peeled	Peeled
1	169	129	92	82
2	140	119	193	177
3	161	152	217	180
4	227	176	109	103
5	134	118	95	69

of DPPH, IC_{50} , and the ascorbic acid equivalent antioxidant capacity (AEAC) are summarized in Table 1. The ferric reducing power also increases regularly with increasing amount of fruit (Fig. 1a and b) in the range 1–20 mg/ml. For a given amount of fruit, the higher the absorbance, the better is the reducing power.

Correlation of IC_{50} with reducing power: DPPH assay measures the ability of the extract to donate hydrogen to the radical and the reducing power measures the ability of the extract to donate electron to Fe(III). In DPPH assay the lower the IC_{50} the better it is able to scavenge the radicals, particularly peroxy radicals which are the propagators of the autoxidation of lipid molecules and thereby break the free radical chain reaction (Frankel, 1991). It is observed that guava (seeded and seedless), star fruit and papaya, having low IC_{50} , are very potent radical scavengers, but langsat and dragon fruit, which are not distinguishable in terms of IC_{50} values, rank low in scavenging property. In terms of reducing power, guava, star fruit and papaya also rank highest but dragon fruit is significantly lower than that of langsat. In this study, the reducing power of langsat is no different from that of water apple, mangosteen and banana

(Fig. 1b). The high antioxidant potential (as characterized by low IC_{50} and high reducing power) of guava is attributed to its high TPC and AAC. The high antioxidant potential of star fruit ($IC_{50} = 3.8$ mg/ml) is attributed to its high TPC, but for papaya ($IC_{50} = 3.5$ mg/ml) it is due to its high AAC. The low antioxidant potential of dragon fruit ($IC_{50} = 27.5$ mg/ml) is due its low TPC and AAC. Langsat in spite of its relatively high TPC has low antioxidant potential ($IC_{50} = 25.4$ mg/ml). Three possible reasons may be able to account for this: First, it has been reported that (Bondet, Brand-Williams, & Berset, 1997) reaction of DPPH with certain phenols such as eugenol and its derivatives is reversible, resulting in low readings for antioxidant activity (% disappearance). The second possible reason could be due to the slow rate of the reaction between DPPH and the substrate molecules (Huang, Ou, & Prior, 2005). The third possible explanation (for the relatively low reducing power) could be that certain phenols in the langsat extract have a higher redox potential than that of other fruit extracts. To clarify this anomaly further work is necessary. Finally, it is also observed that the antioxidant potential correlates well with AEAC.

3.3. Chelating power

The chelating ability of the extract measures how effective the compounds in it can compete with ferrozine for ferrous ion. The iron–ferrozine complex has maximum absorbance at 562 nm and a large decrease in absorbance indicates strong chelating power. By forming a stable iron(II) chelate, an extract with high chelating power reduces the free ferrous ion concentration thus decreasing the extent of Fenton reaction which is implicated in many diseases (Halliwell & Gut-

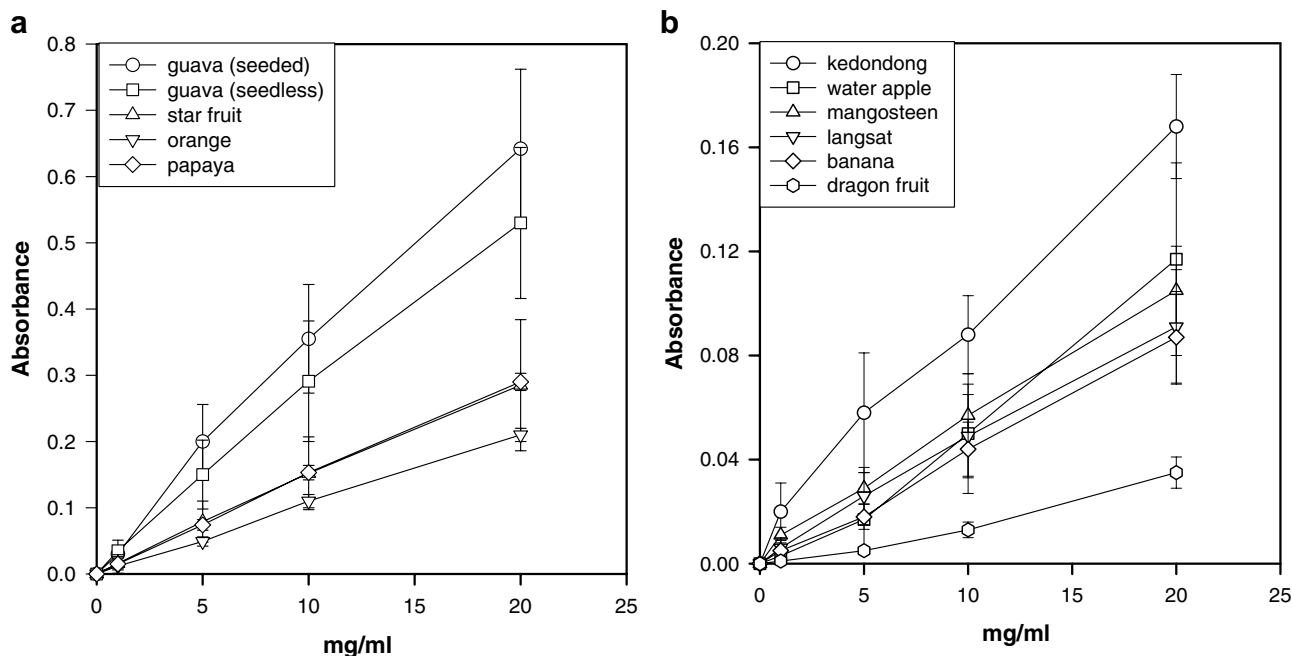


Fig. 1. Iron(III) reducing power of fruit extracts.

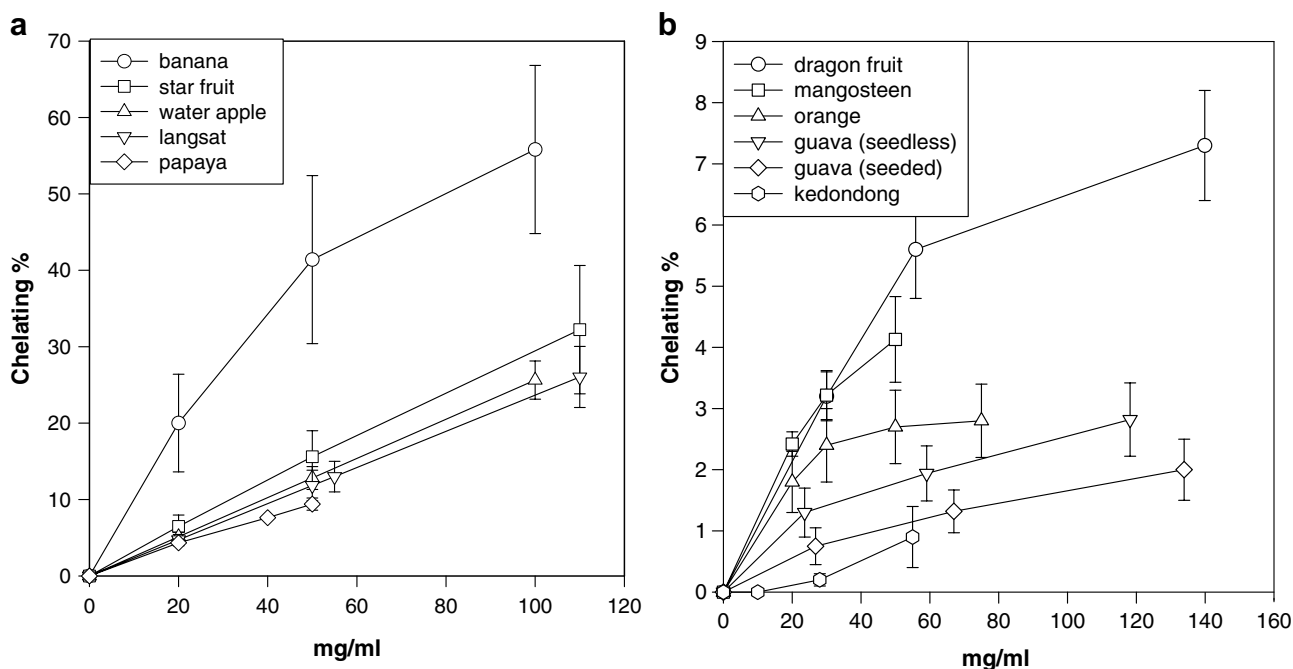


Fig. 2. Chelating power of fruit extracts.

teridge, 1990). The plot of chelating % vs. mg fruit/ml is shown in Fig. 2a and b. It is found that banana has the best chelating power; star fruit, water apple, langsung and papaya have moderate chelating power and fruits such as dragon fruit, mangosteen, guava, kedondong have very low chelating power. For comparison, orange is also found to have low chelating power under the same conditions.

Several striking observations are noted. Guava has very potent primary antioxidant property but its function as secondary or preventive antioxidant is poor. Primary antioxidants scavenge radicals to inhibit chain initiation and break chain propagation. Secondary antioxidants suppress the formation of radicals and protect against oxidative damage. Though banana does not have high TPC and AAC values, it is a potent secondary antioxidant which contains active components that bind to metal ions strongly. Langsung though acts as a weak primary antioxidant, can act as a moderate secondary antioxidant.

4. Conclusion

The present study shows that tropical fruits such as guava, star fruit and papaya have high primary antioxidant potential when compared to orange. Banana though weaker than orange as a primary antioxidant is, however, a powerful secondary antioxidant.

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