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Article

Evaluation of Antioxidant and Anticancer Activities of Guava

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Abstract: Guava is fruit of *Psidium guajava*, and can be consumed with fresh or processed form. In this study, the antioxidant capacities and total phenolic content of guava peel, flesh and seed were evaluated using ferric reducing antioxidant power (FRAP) and Trolox equivalent antioxidant capacity (TEAC) assays as well as the Folin–Ciocalteu method, respectively. In addition, the antiproliferative capacities of guava peel, flesh and seed on four cancer cell lines, A549 (human lung cancer cells), MCF-7 (human breast cancer cells), HepG2 (human hepatoma cells) and HT-29 (human colon cancer cells) were evaluated by the MTT assay. The results showed that guava possesses strong antioxidant and anticancer actions. Furthermore, several compounds, catechin, galangin, homogentisic acid, gallic acid, kaempferol and cyanidin 3-glucoside, were identified as active components of guava, and the contents of these compounds in guava peel and seed were higher than that in guava flesh. The results suggest that guava could be developed to functional food for prevention of some diseases.

Keywords: guava; bioactivity; antioxidant activity; anticancer activity; component.

1. Introduction

Psidium guajava grows in the tropical and subtropical areas of the world, and contains a number of bioactive compounds, such as flavonoids, guajadial, guavanoic acid, guayavolic acid, and

guajaverin (Gutierrez et al., 2008). Different parts of the plant possess many bioactivities, and have been used in traditional medicine for the treatment of various ailments, such as diarrhoea, rheumatism and ulcers (Anas et al., 2008; Goncalves et al., 2008; Hoque et al., 2007; Wang et al., 2014). Guava is fruit of *Psidium guajava*, and can be consumed with fresh or processed form, including beverage, syrup, ice cream and jam (Antonio et al., 2001). It has many nutritional and health effects (Abubakar, 2009; Gutierrez et al., 2008; Sanda et al., 2011; Wang et al., 2014). In this study, antioxidant and anticancer activities of guava were evaluated systematically, and several bioactive compounds of guava were also identified and determined.

2. Materials and Methods

2.1. Chemicals and Samples

2,2'-Azinobis(3-ethylbenothiazoline-6-sulfonic acid) diammonium salt (ABTS), 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), 2,4,6-Tri(2-pyridyl)-S-triazine (TPTZ), Folin–Ciocalteu's phenol reagent, 3-(4,5-dimethylthiazole-2yl)-2,5-diphenyl tetrazolium bromide (MTT) and dimethylsulfoxide (DMSO) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM, pH 7.4), trypsin and fetal bovine serum were bought from Gibco (Invitrogen, NY, USA). Methanol was HPLC grade and bought from Merck (Germany). Tetrahydrofuran, acetic acid, sodium acetate, potassium persulfate, hydrochloric acid, iron (III) chloride 6-hydrate, iron (II) sulfate 7-hydrate and sodium carbonate were analytical grade and bought from Tianjin Chemical Factory (Tianjin, China). All other chemicals and solvents used in this study were of analytical grade. Deionized water was used throughout the experiment.

The standard compounds catechin, galangin, homogentisic acid, gallic acid, kaempferol and cyanidin 3-glucoside were purchased from Sigma–Aldrich and Siyi Biotechnology Company (Chengdu, China). The stock solutions of the standard compounds (10 mg/mL) were prepared in methanol, and stored at 4 °C. The calibration standards (5-100 μg/mL) were prepared from the stock solution by the serial dilution of methanol.

The fruit samples were bought from markets in Guangzhou, China.

2.2. Preparation of Sample

The fresh fruit was cleaned with deionized water and then separated into peel, pulp and seed. Immediately, the separated fruit fractions were ground into fine particles with a special grinder, and hydrophilic and lipophilic components of fruit fractions were extracted as according to the literature with minor modifications (Li et al., 2009). Briefly, 0.5 g precisely weighed sample was extracted with

5 mL of tetrahydrofuran in a shaking water bath (100 rpm, 37 °C) for 30 min, and the mixture was centrifuged at 4200 *g* for 30 min. The supernatant was recovered, and the extraction was repeated once with 5 mL of tetrahydrofuran under the same condition. The two supernatants were combined into fat-soluble fraction. Subsequently, the residue was extracted twice with methanol-acetic acid-water mixture (50:3.7:46.3, v/v) (5 mL each time) in a shaking water bath (100 rpm, 37 °C) for 30 min, and the two supernatants were combined into water-soluble fraction. The extracts were stored at -20 °C and measured within 24 h.

For evaluation of anticancer activity, a precisely weighed amount (3 g) of the ground sample was extracted with 27 mL of a mixture of ethanol–water (50:50, v/v) at room temperature for 24 h in a shaking water bath according to the literature (Fu et al., 2011). The mixture was centrifuged at 4200 *g* for 30 min, and the supernatant was collected. Ethanol in the crude extract was removed under vacuum using a rotary evaporator at 50 ^{o}C. The extract was redissolved in dimethylsulfoxide to obtain 200 mg/mL stock solution. Finally, the extract was filtered through a 0.22 μm Millipore filter and stored at 4 ^{o}C until further use.

2.3. Evaluation of Antioxidant Activity

(1) Trolox equivalent antioxidant capacity (TEAC) assay: The TEAC assay was performed according to the literature with minor modifications (Re et al., 1999). Briefly, the ABTS•+ stock solution was prepared from 7 mmol/L ABTS and 2.45 mmol/L potassium persulfate in a volume ratio of 1:1, and then incubated in the dark at room temperature for 16 h and used within 2 days. A 100 μL of the tested sample was mixed with 3.8 mL ABTS•+ working solution and the absorbance was taken at 734 nm after 6 min of incubation at room temperature. The percent of inhibition of absorbance at 734 nm was calculated and the results were expressed as μmol Trolox/g wet weight of fruit.

(2) Ferric reducing antioxidant power (FRAP) assay: The FRAP assay was performed according to literature with slight modifications (Benzie and Strain, 1996). In this assay, 100 μL of the diluted sample was added to 3 mL of the FRAP reagent and the reaction was monitored after 4 min at 593 nm. The results were expressed as μmol Fe (II)/g wet weight of fruit.

(3) Determination of total phenolic content: Total phenolic contents were determined with Folin–Ciocalteu method (Singleton and Rossi, 1965). Briefly, 0.50 mL extract was mixed with 2.5 mL of 1:10 diluted Folin–Ciocalteu reagent. After 4 min, 2 mL of saturated sodium carbonate solution was added. The mixture was incubated in dark for 2 h at room temperature and its absorbance was detected at 760 nm. Gallic acid was used for calibration, and the results were expressed as mg of gallic acid equivalent (mg GAE) per 100 g wet weight of fruit.

2.4. Evaluation of Anticancer Activity

(1) Cell culture: A549 (human lung cancer cell line), and MCF-7 (human breast cancer cell line) were obtained from the No. 1 hospital affiliated to Sun Yat-Sen University (Zhongshan Road 2, Guangzhou, China). HepG2 (human hepatoma cell line) was provided by the School of Public Health, Sun Yat-Sen University, and HT-29 (human colon cancer cell line) was obtained from the No. 6 hospital affiliated to Sun Yat-Sen University. The four cell lines were grown in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum supplemented with 1% penicillin–streptomycin. All the cell lines were maintained in a 5% CO_2/37 ^oC incubator (Liu et al., 2011; Mou et al., 2011; Yang and Liu, 2009).

(2) Evaluation of antiproliferative effects of the samples on cancer cell lines: The effects of the samples on the viability of various cancer cell lines were determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay. Cancer cells were seeded in 96-well microtiter plates at a density of 1×10^5 cells/mL in 100 μL DMEM complete medium, and allowed 24 h for attachment. Then the culture medium was replaced with 200 μL medium containing 100 mg/mL sample extract. Control wells received the growth medium without sample, and blank wells contained 200 μL of growth medium with no cells, which were incubated for 48 h at 37 ^oC in a humid atmosphere with 5% CO_2. After 48 h, the culture medium was removed and washed with PBS for twice, and 10 μL of sterile filtered MTT solution (5 mg/mL) in phosphate-buffered saline (PBS, pH 7.4) was added to each well, producing a final concentration of 0.5 mg/mL of MTT. After a further incubation of 4 h, the untransformed MTT was carefully removed by pipette and the insoluble formazan crystals were dissolved in 150 μL of DMSO per well, then shaken on a table concentrator for 10 min. The absorbance was measured at 490 nm by an ELX800 microplate spectrophotometer (BIOTEC, Germany) (Liu et al., 2011). Antiproliferative activity of the samples was measured as percent compared to control wells.

2.5. Identification and Determination of Bioactive Compounds

The bioactive compounds in guava samples were analyzed by HPLC-PAD according to the literature with small modification (Sakakibara et al., 2003). In brief, the HPLC system employed a Waters (Milford, MA, USA) 1525 binary HPLC pump separation module equipped with an auto-injector and a Waters 2996 photodiode array detector. Separation was performed with an Agilent Zorbax Extend-C18 column (250 mm × 4.6 mm, 5 μm) at 35 °C with a gradient elution solution A, comprising acetic acid-water solution (0.1% acetic acid) and methanol (9:1; v/v), and solution B, composed of methanol and acetic acid-water solution (0.1% acetic acid) (7:3; v/v), which delivered at a flow rate of 1.0 mL/min as follows: 0 min, 100% A; 15 min, 70% A; 45 min, 65% A; 65 min, 60% A; 70 min, 50% A; and 95 min, 0% A. The UV spectra were recorded between 190 and 700 nm for

peak characterization. Phenolic ingredients were quantified by the peak area of maximum absorption wavelength.

2.6. Statistical Analysis

All the experiments were performed in triplicate, and the results were expressed as mean±SD (standard deviation). Statistical analysis was performed using SPSS 13.0 and Excel 2003.

3. Results and Discussion

3.1. Antioxidant Activity

The antioxidant capacity of a sample might be influenced by several factors, such as test system, and could not be fully described by one single method. Therefore, a reliable antioxidant evaluation protocol requires performing different assessments of antioxidant activity to take into account various mechanisms of antioxidant action (Wong et al., 2006). In this study, the antioxidant capacities and total phenolic content of guava were evaluated using ferric reducing antioxidant power (FRAP) and Trolox equivalent antioxidant capacity (TEAC) assays as well as the Folin–Ciocalteu method, respectively. The results are given in Tables 1-3. In the literature, Chen and Yen (2007) studied the antioxidant activity and free radical-scavenging capacity of extracts from *Psidium guajava* leaves. Budin et al. (2013) found that *Psidium guajava* fruit peel extract could reduce oxidative stress of pancreas in streptozotocin-induced diabetic rats.

Table 1. Trolox equivalent antioxidant capacity (TEAC) values of guava

No.	Sample fraction	TEAC value (μmol Trolox/g)		
		Fat-soluble fraction	**Water-solublefraction**	**Total**
1	Guava peel	34.28 ± 2.34	6.21 ± 0.15	40.49 ± 2.49
2	Guava flesh	9.63 ± 0.85	1.15 ± 0.06	10.78 ± 0.91
3	Guava seed	8.21 ± 0.29	6.26 ± 0.04	14.47 ± 0.33

Table 2. Ferric reducing antioxidant power (FRAP) values of guava

No.	Sample fraction	FRAP value (μmol Fe(II)/g)		
		Fat-soluble fraction	**Water-soluble fraction**	**Total**
1	Guava peel	47.08 ± 3.63	10.65 ± 0.51	57.73 ± 4.14
2	Guava flesh	11.61 ± 1.04	2.12 ± 0.09	13.73 ± 1.13
3	Guava seed	10.15 ± 0.52	6.38 ± 0.41	16.53 ± 0.93

Table 3. Total phenolic contents (TPC) of guava

No.	Sample fraction	TPC value (mg GAE/g)		
		Fat-soluble fraction	Water-soluble fraction	Total
1	Guava peel	7.72 ± 0.57	1.35 ± 0.12	9.07 ± 0.69
2	Guava flesh	0.31 ± 0.02	0.18 ± 0.01	0.49 ± 0.03
3	Guava seed	1.55 ± 0.07	0.47 ± 0.04	2.02 ± 0.11

3.2. Anticancer Activity

Lung, breast, liver and colon cancers are among the most frequent cancer types. In this study, different fractions of guava were evaluated for their abilities to inhibit the growth of human lung (A549), breast (MCF-7), liver (HepG2) and colon (HT-29) cancer cell lines by the MTT assay. The MTT assay is a widely used method for rapid screening of potential anticancer compounds. MTT can be converted to an insoluble purple formazan by a mitochondrial enzyme in living cells, and the amount of formazan produced is directly proportional to the number of viable cells (Barrios et al., 2010; Valente et al., 2012). The results are given in Table 4. In the literature, Hsieh and Peng (2010) studied the action mechanism and signal pathways of *Psidium guajava* aqueous extract in killing prostate cancer LNCaP cells. Lee and Park (2010) reported anticancer activity of *Psidium guajava* branch extracts against HT-29 human colon cancer cells.

Table 4. Antiproliferative capacities (%) of guava on 4 cancer cell lines, A549 (human lung cancer cells), MCF-7 (human breast cancer cells), HepG2 (human hepatoma cells) and HT-29 (human colon cancer cells)

No.	Sample fraction	A549	MCF-7	HepG2	HT-29
1	Guava peel	73.6 ± 1.8	56.3 ± 2.5	71 ± 3.2	83 ± 4.6
2	Guava flesh	92 ± 2.9	66 ± 3.1	89 ± 3.9	91 ± 5.1
3	Guava seed	94 ± 4.3	95 ± 3.7	91 ± 4.1	95 ± 3.3

3.3. Bioactive Components

Polyphenols are widely distributed in fruits, and possess various biological activities, such as antioxidant, antiinflammatory, and anticarcinogenic actions (Li et al., 2014; Mertens-Talcott et al., 2006; Zhang et al., 2008). In this study, several bioactive compounds and their contents in guava sample have been identified and determined according to the method reported in the literature (Fu et al., 2011; Sakakibara et al., 2003). Catechin, galangin, homogentisic acid, gallic acid, kaempferol and cyanidin 3-glucoside have been identified in guava samples. According to the literature, catechin,

galangin, homogentisic acid, gallic acid, kaempferol and cyanidin 3-glucoside all possess antioxidant activities, and gallic acid possesses anticancer activity (Li et al., 2014). Therefore, these compounds could be active components of guava for antioxidant and anticancer actions.

The guava peel contained 29.2±2.1 mg/100 g of catechin, 68.2±3.6 mg/100 g of galangin, and 10.2±0.5 mg/100 g of homogentisic acid. The guava flesh contained 1.7±0.1 mg/100 g of gallic acid, and 7.5±0.4 mg/100 g of homogentisic acid. The guava seed contained 2.8±0.1 mg/100 g of cyanidin 3-glucoside, 6.8±0.5 mg/100 g of gallic acid, and 138±3.6 mg/100 g of kaempferol. In the literature, Chen et al. (2009) reported that the budding leaves of *Psidium guajava* contained 348 mg/g of gallic acid, 102 mg/g of catechin, 60 mg/g of epicatechin, 100 mg/g of rutin, 102 mg/g of quercetin, and 100 mg/g of rutin.

4. Conclusions

The antioxidant and anticancer activities of guava have been evaluated, and the results showed that guava possesses strong antioxidant and anticancer actions. Furthermore, several bioactive compounds of guava have been identified and quantified, and catechin, galangin, homogentisic acid, gallic acid, kaempferol and cyanidin 3-glucoside could be active components of guava for antioxidant and anticancer actions. In addition, the contents of these compounds in guava peel and seed were higher than that in guava flesh. The results suggest that guava could be developed to functional food for prevention of some diseases.

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