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Antioxidant properties of pulp and peel of yellow mangosteen fruits

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Abstract: The aim of this work was to compare the antioxidant activity of the peel and pulp extracts of *Garcinia tinctoria* (yellow mangosteen) fruits. Total phenolic content (TPC) assay showed that the peels contained higher phenolic content than the pulps. Ferric reducing antioxidant power (FRAP) test indicated that the peel extract of *G. tinctoria* fruits showed a highest antioxidant capacity, and reached a maximum value of 2,7 µM at 10 µg/mL. Peel extract of *G. tinctoria* fruits exhibited higher scavenging activity of DPPH radical in comparison with the pulp extract, due to the lowest value of IC_{50} (48,8 µg/mL). The TPC results showed a good relationship with the radical scavenging activity obtained for the pulps and peels extracts.

Key words: Antioxidant activity, *Garcinia tinctoria*, peel, pulp, DPPH, FRAP

خصائص مضادة الأكسدة في لب وقشور فاكهةالمانغوستين الصفراء

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الملخص : الهدف من هذه الدراسة مقارنة نشاط مضادات الأكسدة فى مستخلصات قشور ولب *Garcinia tinctoria* (المانغوستين الأصفر). عند قياس المحتوى الفينولي الكلى(TPC) تبين أن للقشور محتوى فينولى أعلى من اللب. اختبار القوة المضادة للأكسدة للحديديك المختزل (FRAP) أشارت إلى أن مستخلص قشور الفواكه G tinctoria. يحتوى على أعلى قدرة مضادة للأكسدة حيث ووصلت قيمة الحد الأقصى إلى ٢،٧ ميكرومتر في ١٠ ميكروغرام/مل. يحتوى مستخلص قشور فاكهة G. tinctoria على نشاط تنظيفى عالي من DPPH الجذري مقارنة بمستخلص اللب، ويرجع ذلك إلى القيمة المنخفضة لIC_{50} (٤٨،٨ ميكروغرام/مل). أظهرت نتائج الTPC علاقة جيدة مع النشاط التنظيفى الجذرى المتحصل عليه من مستخلصات اللب والقشور.

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Introduction

Garcinia is the largest genus of the tropical family Guttiferae that contains about 400 species of polygamous trees or shrubs, distributed in the tropical Asia, Africa and Polynesia, (Waterman and Hussain, 1883; Chattopadhyay and Kumar, 2006). The fruit of several species are edible, being quite widely cultivated throughout the tropics. *Garcinia* species are also characterized by the production of a yellow or occasionally white latex in the endocarp of the fruit, in the bark and perhaps also in the wood (Negi et al., 2008).

The genus *Garcinia* has demonstrated to be an interesting source of active compounds with a great biological versatility. It is well known to be a rich source of oxygenated and prenylated xanthones (Mbwambo et al., 2006; Chen et al., 2010). Numerous investigations have demonstrated their antioxidant, antibacterial and antitumoral activities (Xing-Cong et al., 2004; Verdi et al., 2004; Rui-Min et al., 2009; Jawed et al., 2010).

The antioxidant activity in fruits is notable since fruits are rich in compounds that have an important role in free radical scavenging activity. Those compounds are vitamins and polyphenols such as flavonoids, tannins, and catechins. Interestingly, the peel and seed fractions of some fruits possess higher antioxidant activity than the pulp fractions (Jayaprakasha et al., 2001). For example, pomegranate peel has a higher antioxidant activity than its pulp (Li et al., 2006). Grape seed is higher than its pulp in antioxidant capacity and is a rich source of proanthocyanidin, which is very effective in scavenging various reactive oxygen free radical species (Guo et al., 2003).

In the present study the antioxidant activity of the peel and pulp extracts of *Garcinia tinctoria* (yellow mangosteen) fruits were compared. To achieve this purpose total phenolic content (TPC), the ferric reducing antioxidant power (FRAP) and the radical scavenging activity (DPPH assay) of the extracts were determined.

Materials and Methods

Sample collection

Mature fruits of *Garcinia tinctoria* were collected in the Jardín Botánico Nacional (Habana, Cuba) in January-April 2011 and identified by Dr. Cristina Panfet. A voucher specimen has been deposited at HAJB Herbarium (Havana, Cuba) under number 700.

Sample preparation and extraction

Fresh fruits were washed and peeled in order to separate the peels from the pulps for further tests. Pulps and peel (each 5g) were separately macerated with 80% ethanol (10mL) at room temperature (25-28°C) until 24 hours. Later, samples were shaken in ultrasonic water bath for 1 hour before filtered using Whatman filter paper. The filtrate was concentrated under reduced pressure at 45°C using a rotary vacuum evaporator. The extraction of each sample was done five times and the yield of ethanolic extracts was reported as mean ± SD.

TPC assay

The total phenolic content were quantified according to the method described by Singleton et al. (1999) using the Folin-Ciocalteu's reagent. A volume of 100 µL of each extract was mixed with 5mL of Folin-Ciocalteu's reagent (diluted 1:10) and 1,8 mL of distilled water in a test tube. Mixture was shaken and waited five minutes to add 4 mL of sodium carbonate (7.5% w/v). The tube was shaken again and was incubated for 2 hours at room temperature. After that, absorbance readings were taken by measuring the sample using Perkin Elmer Lambda 25 UV/Vis spectrophotometer at of 760 nm. Gallic acid was used as standard with concentrations of 100 to 900 mg/L prepared by dissolving in 80% ethanol. Samples and gallic acid solutions were measured against 80% ethanol which was used as blank. All samples and readings were measured in triplicate. Results of TPC of the peel and pulp extracts of *Garcinia tinctoria* fruit were expressed in mg of gallic acid/100 mL of the extracts.

FRAP assay

The procedure described by Benzie and Strain (1996) was followed. The assay

consisted on measuring the capacity of sample to reduce the ferric iron to its ferrous form. The complex Fe^{3+}-TPTZ in the presence of reductor agents decreases to Fe^{2+}-TPTZ that develops an intense blue color with a maximum of absorption at 593 nm. Different concentrations of the peel and pulp extracts were prepared (2; 10, 20, 30 and 40 g/mL).

Ascorbic acid was used at different concentrations (100, 200, 400, 800 and 1 000 µM) as standards to obtain the calibration curve. Readings for the standard and the extracts were carried out for triplicate until 4 minutes.

DPPH assay

The radical scavenging activity of each sample was measured using a method described by Brand-Williams et al. (1995). The assay consists on the determination of the reduction of the free radical 2,2-difenil-1-picril hidrazilo (DPPH) to 517 nm. The stable radical has an intense violet color that diminishes in presence of an antioxidant (that is able to capture a free electron) or another radical, what allows quantifying the bleaching effect caused by certain compounds.

Each sample was prepared in a series of dilution (5; 13; 25; 37 y 50 µg/mL) with final volume of 10 mL in 80% ethanol. Trolox at 20 µM was used as standard to be compared with the samples. The radical scavenging activity of Trolox at that concentration is 50% aproximately. Each sample was measured in triplicate until 30 minutes (every 5 min) to compare the reactions kinetic.

The radical scavenging activity was calculated accordingly**:**

$$\%\,\mathbf{Inhibition} = \frac{(A_{control} - A_{sample})}{A_{control}} \times 100$$

$A_{control}$ = Absorbance of control.

A_{sample} = Absorbance of the samples.

The amount of sample (µg) extracted in 1 mL solution necessary to decrease by 50% the initial DPPH concentration was calculated (IC_{50}).

Statistical analysis

Results are expressed as the means ±SD of three replicates. One-way analysis of variance (ANOVA) was used to determine the statistical difference, using PASW statistics software package (version 18.0, 2009). Statistical significance (α) was 0.05.

Results and Discussion

The yield of extracts

The yields of peel and pulp extracts of *G. tinctoria* fruit was shown in Table 1. The highest yield of extraction was obtained from the peel of *G. tinctoria* fruit.

Table 1. Percent yield of peel and pulp extracts of *Garcinia tinctoria* fruits.

G. tinctoria fruit portion	% Yield of extracts*
Peel	9.21 ± 0.30
Pulp	8.65 ± 0.42

*Expressed as mean ± SD (n=5)

TPC assay

TPC of each sample was calculated from calibration curve of gallic acid (not shown) where the calibration equation was determined to be y=9,750x + 0,007 (R^2=0,996), whereby y=absorbance at 760nm and x=concentration of total phenolic compounds in mg per 1 ml of the extract.

It was observed that the reaction mixture with peel extract of *G. tinctoria* fruits was dark blue in colour, that indicated a high phenolic content. On the other hand, reaction mixture with pulp extract of *G. tinctoria* fruits was light blue in colour indicating low phenolic content. In general, TPC results for peel and pulp extracts of *G. tinctoria* showed that the peels contained higher phenolic content than the pulps (Table 2).

Table 2. Total phenolic content of fruits extracts.

G. tinctoria fruit portion	Phenolic content* (mg/100mL)
Peel	474,53 ± 11.60
Pulp	6,54 ± 0,62

*Expressed as mean ± SD (n=3)

This result suggests that the content of secondary metabolites xanthones, flavonoids, coumarins, chromenes and benzophenones) it's much higher in this portion of the fruit. Usually, the flavonoid compounds are located in the peels, seeds and stems (Paixão et al., 2007; Nurliyana et al., 2010). Plant phenolics have multiple biological effects as they constitute one of the major groups of compounds acting as primary antioxidant or free radical terminator. Several studies have demonstrated that the antioxidant activity is strongly correlated with the total content of phenolic compounds (Lim et al., 2007; Nurliyana et al., 2010; Sim Choo and Khing Yong, 2011).

FRAP assay

The results indicated that pulp and peel extracts of *G. tintoria* fruits acted as weak or moderate redactors. However, the peel extract of the fruits of *G. tinctoria* showed a higher antioxidant property in comparison with the peel extract, and reached a maximum value of 2,7 µM at 10 µg/mL (Figure 1). This could be explained through the principle of this method based on the reduction of a ferric-tripyridyltriazine complex to its ferrous, colored form in the presence of antioxidants. In this case, a second reaction could be taking place in reverse sense. This chemical process that it involves to organic compounds is described by Leach and Gillet (2007).

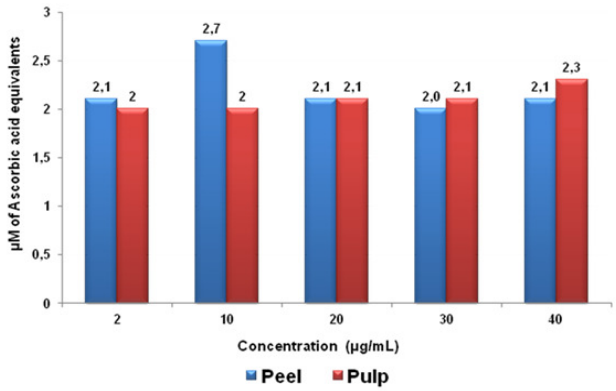

Figure 1. Ferric reducing antioxidant power for peel and pulp extracts of *G. tinctoria* fruits.

The reducing power property indicates that the antioxidant compounds are electron donors and can reduce the oxidized intermediates of the lipid peroxidation process (Tachakittirungrod et al., 2007; Sulaiman and Udaya, 2009).

Even though peel extract of *G. tinctoria* fruits showed higher phenolic content than the pulp extract the reductor power was rather high at low concentartion. The peel extract of *G. tinctoria* fruits showed strongest antioxidant property, because increased the intensity of the blue colour indicating the formation Fe^{2+}/TPTZ from its colourless form (Fe^{3+}). Thus, a higher absorbance indicated higher activity.

DPPH assay

During visual examination, it could appreciate the quick change of purple color to yellow provoked by Trolox and the peel extract of *G. tinctoria* in the mixture of the reaction (Figure 2). This is only due to the presence of antirradical substances that reduced the radical 2,2-difenil-1-picrilhidracilo (DPPH) with the concomitant absorbancia loss in the solution. The extract of the pulp didn't cause an appreciable bleaching effect in the solution of the DPPH.

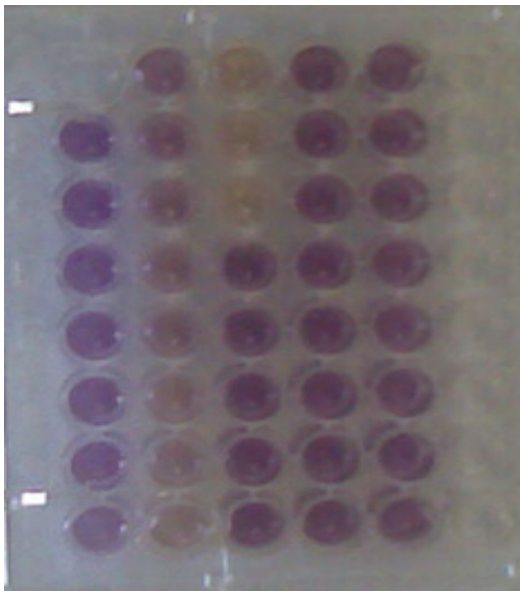

Figure 2. Bleaching of DPPH radical provoked by Trolox and the peel and pulp extracts of *G. tinctoria* in the mixture of the reaction.

The bleaching kinetic of DPPH radical at different concentrations of the peel extract of *G. tinctoria* fruits showed a behavior dependent on the extract concentration during

the 30 minutes of reaction (Figure 3). The highest concentration of the peel extract (50 µg/mL) reached to the 30 minutes the maximum value of DPPH radical scavenging activity (50%), coinciding with the maximum value of bleaching by Trolox at that same time.

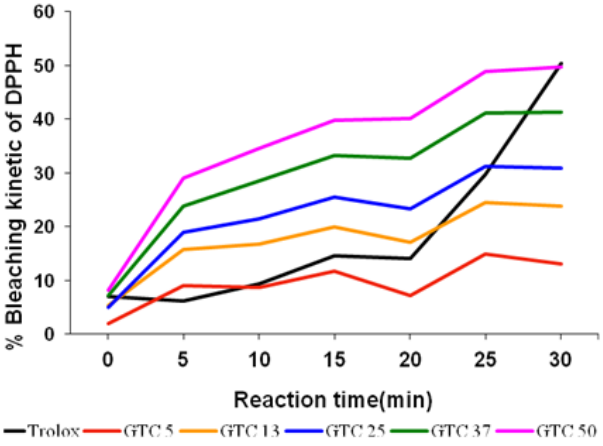

Figure 3. Bleaching kinetic of DPPH radical at different concentrations of the peel extract of *G. tinctoria* fruits.

On the other hand, the bleaching kinetic of DPPH radical for the pulp extract was uniform but very slow in comparison with Trolox (Figure 4). The highest activity value reached for the pulp extract was 18% at maximum concentration of 50 µg/mL.

For both extracts, the inhibition of DPPH radical was plotted as a function of concentration in order to determine the IC_{50} value (Figure 5), which is defined as the necessary sample concentration to reduce 50%

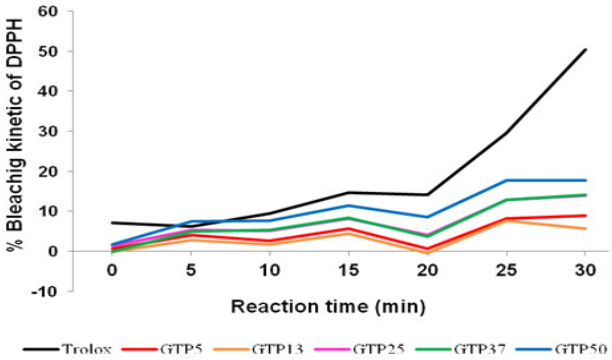

Figure 4. Bleaching kinetic of DPPH radical at different concentrations of the pulp extract of *G. tinctoria* fruits.

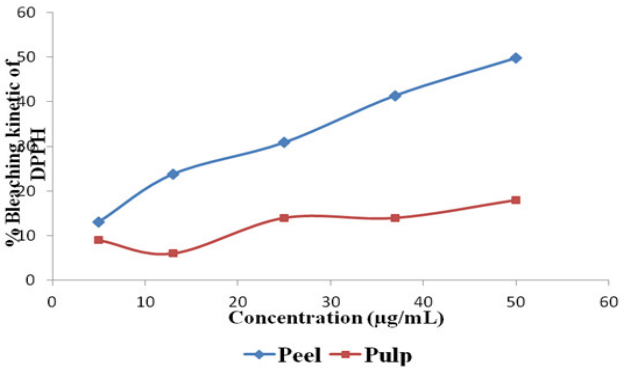

Figure 5. Radical scavenging activity for the peel and pulp extracts of *G. tinctoria* at different concentrations.

Table 3. IC_{50} values for the extracts of *G. tinctoria* fruits

IC_{50} values	
Peel extract	48,8 µg/mL
Pulp extract	153,2 µg/mL

In Table 3 was observed that peel extract of *G. tinctoria* fruits exhibited higher scavenging activity of DPPH radical in comparison with the pulp extract, due to the lowest value of IC_{50} (48,8 µg/mL). The fruit peel contains a high quantity of bioactive compounds able to capture free radicals like DPPH, and this is expressed in a quick decrease of the absorbance in the reaction mixture. Guo et al. (2003) reported a major antioxidant activity for fruit peel that for the pulp. In some cases, it refers to values that are from 2 to 27 time superiors.

Okonogi et al. (2007) evaluated through the ABTS and DPPH assays the antioxidant potential of ethanolic extracts (95%) of *G. mangostana* peel and other consumed fruits commonly in Thailand. The IC_{50} value for G.

mangostana fruit peel was 0.023 mg/mL (23 µg/mL), being inferior to the value obtained in this study. G. mangostana and G. tinctoria don't contain the same chemical composition. This factor could has contributed to the different level of phenolic compounds in peels of the two *Garcinia* species even though they are of the same genus (Verzelloni et al., 2007).

DPPH radical is reduced to the corresponding hydrazine when it reacts with hydrogen donors that can be phenolic compounds. The ability to capture free radicals is characteristic of primary antioxidants (Wang et al., 2008). Plant phenolics constitute one of the major groups of antioxidants acting as free radical terminators (Samarth et al., 2008). This results showed a good relationship with the TPC obtained for the pulps and peels extracts. Many studies reported that high polyphenols content contributes towards high radical scavenging activity (Garcia- Alonso et al., 2004; Lim et al., 2007).

Nevertheless, antioxidant capacity cannot only be related to the phenolics content because is the result of multiple factors. Natural antioxidants are multifunctional and their activity should be estimated through several methods that keep in mind the different mechanisms of action.

Conclusions

This study, demonstrated that peel ethanolic extract of *G. tinctoria* fruits had the major antioxidant activity in comparison to pulp. Peel extract showed a highest content of total phenolics. The radical scavenging activity of peel extract was higher than pulp with a IC_{50} value of 48,8 µg/mL and 153,2 µg/mL respectively. The FRAP assay results revealed that the peel extract of *G. tinctoria* fruit showed the strongest reductor power. The peel of this fruits can be a good source of antioxidant agents.

In the future, investigation of the activity associated with further purification, identification and quantification of each phenolic compound are necessary to provide useful comparative information on the antioxidant level and activities in *Garcinia tinctoria*.

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