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Garcinia mangostana Fruit: Determination of the Total Antioxidant Activity in Extracts and Phenolic Compound Characterization using CE-MS(TOF)

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ABSTRACT

Xanthones are an important family of polyphenols present in *Garcinia mangostana* fruit. Products of *G. mangostana* have begun to be commercialized as a dietary supplement because of their potent antioxidant properties. Interest in their beneficial health effects such as antioxidant characteristics, lipid profile and anticarcinogenic activity, etc., has encouraged scientific research to determine compounds responsible for these properties. The antioxidant capacity determination and the phenolic compound characterization in extracts of *G. mangostana* were investigated. High antioxidant capacity was determined in whole fruit dried extract by ORAC, FRAP and $ABTS^{\bullet+}$ assays. The values obtained by FRAP, ORAC and ABTS assays were 1222 and 4088 and 3590 μM TE/g, respectively. Phenolic compound identification in these extracts was carried out by capillary electrophoresis coupled to ionization by electrospray-mass spectrometry (CE-ESI-MS) with a time of flight analyzer (Micro TOF), and a sensible, fast and efficient method was developed. The CE-ESI parameters were optimized to obtain good separation and suitable sensitivity. The CE parameters were: 80 mM ammonium acetate, pH 10.5, 30 kV, 15 s for injection and fused-silica capillary of 50 μm i.d. and 100 cm in length. The ESI-MS parameters were also optimized: drying gas flow of 4L/min, 300°C, nebulization gas pressure at 4 psi, sheath liquid composition and flow (60:40) isopropanol/water + 0.1% triethylamine to 0.18 mL/h. Spectrometric determination was achieved in negative polarity and the mass range was between 100-800 m/z. Considering the scarcity of commercial standards and the scanty bibliography references, some of the most important xanthones from *G. mangostana* fruit have been identified using CE-ESI-MS (micro TOF), such as Garcinine C, Garcinone E, Mangostenone C and β-mangostin.

Keywords: ABTS, capillary electrophoresis, FRAP, mangosteen, ORAC
Abbreviations: AAPH, 2,2'-azobis-(2-amidinopropane)-dihydrochloride; **AUC**, area under the curve; **AWA**, acetone/water/acetic acid; **CE**, capillary electrophoresis; **CUPRAC**, cooper reduction capacity assay; **DPPH**, 2, 2-diphenyl-1-picrylhydrazyl; **ESI**, electrospray ionization; **FC**, Folin-Ciocalteau; **FRAP**, ferric ion reducing antioxidant power; **HAT**, hydrogen transfer mechanism; **MS**, mass spectrometry; **ORAC**, oxygen radical absorbance capacity; **SET**, single electron transfer; **TEAC**, trolox equivalent antioxidant capacity; **TOF-MS**, time of flight-mass spectrometry; **TPTZ**, 2, 4, 6-tripiridil-s-triazine; **TRAP**, total reducing ability of plasma; **TROLOX**, 6-Hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid

INTRODUCTION

Oxidative stress has been associated with the development of many chronic and degenerative diseases, including cancer (Ames *et al*. 1995), heart disease (Diaz *et al.* 1997) and neuronal degeneration (Scalbert *et al*. 2005) and is involved in the process of aging (Ames *et al*. 1993). A diet rich in fruits and vegetables is considered as an excellent source of antioxidants (Block *et al*. 1992; Ness and Powles 1997). Vitamins C and E, polyphenols and carotenoids are considered to be responsible for most of the antioxidant activity in food (Wu *et al*. 2004). But, in terms of disease prevention, a clinical trial with whole fruits and vegetables rather than using vitamin C, E or carotenoid supplements are more likely to give positive (Wu *et al*. 2004).

Garcinia mangostana L. (Clusiaceae), commonly known as mangosteen, is a tropical evergreen tree. The fruit has been used in traditional medicine for the treatment of skin infections, wounds, and diarrhea, is recognized to improve cardiovascular health (Peres *et al*. 2000; Leontowicz *et al*. 2006), and has anti-inflammatory, antitumor, antioxidant and antibacterial activity (Suksamrarn *et al*. 2003; Moongkarndi *et al*. 2004; Suksamrarn *et al*. 2006). The major secondary metabolites of mangosteen have been found to be prenylated xanthone derivatives. Alpha- and gamma-mangostins exhibited a powerful antioxidant activity (Jung *et al.* 2006). Studies on extracts or xanthones obtained from the pericarp of the fruit or isolated from the young fruit have been assayed to determine the anticancer properties (Peres *et al*. 2000; Itoh *et al.* 2008) and these have demonstrated cytotoxic properties against three human cancer cell lines (Ho *et al.* 2002; Suksamrarn *et al.* 2006). Pericarp extract from *G. mangostana* exhibited potent anti-leukemic activity (Chiang *et al.* 2004) and the most abundant xanthone from *G. mangostana*, α-mangostin, showed a possible cancer chemopreventive activity (Chin *et al*. 2008).

Recently, products manufactured from *G. mangostana* have begun to be used as dietary supplements in the United States, because of their potent antioxidant properties. Mangosteen have a high concentration of bioactive compounds, high antioxidant potential and positively affect plasma lipid profile and plasma antioxidant activity in rats fed cholesterol-containing diets (Leontowicz *et al.* 2006). Mangosteen fruit juice has become a major botanical diet-

Received: 1 August, 2008. Accepted: 4 February, 2009.

Original Research Paper

ary supplement, and was ranked as one of the top-selling "botanicals" on the market in 2005 (Nutrition Business Journal 2006).

The antioxidant capacity of mangosteen has been assayed from pericarp or pulp extracts or xanthones isolated from the pericarp of fruit (Jung *et al*. 2006), however little additional information has been reported.

A number of methods have been developed to measure the efficiency of dietary antioxidants, either as pure compounds, botanicals or in foods, food extracts, as well as to determine the antioxidant activity of plasma as an index of the antioxidant status *in vivo*. It is clear that no one antioxidant capacity assay will truly reflect the "total antioxidant capacity" of a particular sample. Total antioxidant capacity needs to reflect both lipophilic and hydrophilic capacity, and at least for physiological activity it needs to reflect and differentiate the mechanisms of the antioxidant defence (Prior *et al*. 2005). Many of the frequently cited assays can be broadly categorised as hydrogen transfer mechanism (HAT) such as ORAC (oxygen radical absorbance capacity; Cao *et al*. 1995) and TRAP (total reducing ability of plasma; Ghiselli *et al*. 1995) assays and mechanisms based on single electron transfer (SET), including FC (Folin-Ciocalteau; George *et al*. 2005), TEAC (Trolox equivalent antioxidant capacity; Miller *et al*. 1993), FRAP (ferric ion reducing antioxidant power; Benzie and Strain 1999), 2,2-diphenyl-1-picrylhydrazyl (DPPH; Sanchez-Moreno *et al.* 1998), and the copper reduction capacity assay (CUPRAC; Apak *et al.* 2004) between others. These methods measure the radical scavenging capacity or the reducing ability, respectively.

Certain methods were suggested to be evaluated for standardization at the "First International Congress on Antioxidant methods" congress in Orlando in 2004; ORAC, the Folin-Ciocalteau assay and TEAC were suggested to be used in the routine quality control and measurement of antioxidant capacity of food, dietary supplements and other botanicals (Prior *et al*. 2005).

Many studies have shown that mangosteen is a rich source of phenolic xanthones and actually more than 40 xanthones have been isolated and identified (Nilar *et al*. 2005; Ee *et al*. 2006; Suksamrarn *et al*. 2006). In this sense, a few chromatographic methods have been proposed for the identification of phenolic compounds in *G. mangostana* (Haruenkit *et al*. 2007; Ji *et al*. 2007). Due to its high efficiency, flexibility, very high resolution and rapidity of the method, capillary electrophoresis (CE) has gained widespread interest as a favourable technique for the analysis of phenolic compounds (Arráez-Román *et al.* 2006; Gómez-Romero *et al*. 2007; Carrasco-Pancorbo *et al.* 2007; Arráez-Román *et al.* 2008). It has become an alternative or complement to chromatographic separations for the analysis of phenolic compounds because it needs no derivatization step, requires only small amounts of sample and buffer and has proved to be a high-resolution technique. The advantages of mass spectrometry (MS) detection include the capacity to determine molecular weights and provide structural information. Also time of flight-mass spectrometry (TOF-MS) provides excellent mass accuracy over a wide dynamic range, if modern detector technology is chosen. The latter, moreover, allows measurements of the isotopic pattern, providing important additional information for the determination of elemental composition. Thus, the on-line coupling of CE with TOF-MS yields a powerful technique for the analysis of phenolic compounds.

The goal of this study was to determine, by different assays, the antioxidant capacity of whole fruit (pericarp and pulp) and describe the first application of CE-ESI-TOF-MS for the identification of phenolic compounds in *G. mangostana*.

MATERIALS AND METHODS

Reagents and materials

The reagents used for antioxidant capacity assays were 2,2'-azobis(2-amidinopropane)-dihydrochloride (AAPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and fluorescein (sodium salt), 2,2'-azinobis (3-etilbenzotiazolin)-6-sulfonic, TPTZ (2,4,6-tripiridil-s-triazine), all obtained from Sigma-Aldrich (Steinheim, Germany). Other reagents (hexane, acetone and dichloromethane, acetic acid, sodium phosphate dihydrate, sodium acetate trihydrate, clorhidric acid, ferric cloride) were purchased from Panreac (Barcelona Spain), and the 96-well polystyrene microplate for fluorescent measures from Biogen Científica (Madrid, Spain).

All chemicals used for characterization of phenolic compounds by CE-ESI-TOF-MS were of analytical reagent grade and used as received. Ammonium hydroxide was purchased from Fluka (Buchs, Switzerland) and ammonium acetate from Merck (Darmstadt, Germany). 2-propanol HPLC grade, used in the sheath flow, methanol, and sodium hydroxide, used for capillary cleaning procedures before each analysis, were obtained from Panreac and triethylamine from Sigma-Aldrich. Distilled water was deionized using a Milli-Q system (Millipore, Bedford, MA). CE buffers were prepared by weighing ammonium acetate at the concentrations indicated and adjusting the pH when necessary by adding 0.5 M ammonium hydroxide. The buffers were stored at 4°C and warmed to room temperature before use. All solutions were filtered through 0.45 μm Millipore (Bedford, MA, USA) membrane filters before injection into the capillary column.

Sample extraction

1. Antioxidant capacity methods

The Prior *et al.* (2003) procedure of sample separation was followed. Mangosteen (pericarp and pulp) dried extract sample (1 g) was initially extracted in a 15-mL screw-cap tube with 10 mL of hexane: dichloromethane (1: 1), followed by centrifugation to 9000 rpm for 15 min and removal of the hexane layer; this extraction was repeated twice. The residue was extracted with 10 mL AWA (acetone/water/acetic acid; 70: 29.5: 0.5, v/v/v). After the addition of solvent, the tube was vortexed for 30 s, followed by sonication at 37°C for 5 min. The tube was kept at room temperature for 10 min, centrifuged at 9000 rpm for 15 min and the supernatant was removed. The samples were extracted one more time with 10 mL AWA using the same procedure, and the supernatants were combined. The supernatants were transferred to a 25 mL volumetric flask, and AWA was added to make up a final volume of 25 ml. The hydrophilic solution from the extracted sample was then diluted as appropriate to be applied to different antioxidant methods. Each sample was extracted in triplicate and assayed in duplicate.

2. Characterization of phenolic compounds

Ten kinds of extraction procedures were used to compare which gave mainly phenolic compounds. The extraction procedure which was selected due to its ability to release a major number of compounds for characterization is as follows: 0.5 g of the dried sample were weighted and extracted with 5 mL methanol: H_2O (50: 50, v/v), followed by centrifugation to 4000 rpm for 4 min and filtered through 0.2 μm Millipore (Bedford, MA, USA) membrane filter. Finally the extract was kept at -4°C until analysis.

Antioxidant capacity methods

1. ORAC assay

The antiradical activity against AAPH was estimated according to a slightly modified procedure reported by Prior *et al.* (2003). Fluorescent measurements were carried out on a spectrofluorimeter Polarstar Optima (BMG Labtechnologies) thermostated and a fluorescence filter with an excitation wavelength of 490 nm and an emission wavelength of 545 nm was used. The hydrophilic solu-

tion from the extracted sample was diluted (1/3500) as appropriate with 75 mM phosphate buffer solution (pH 7.4). The hydrophilic dilution and fluorescein (70 nM) solution were placed in each well of a black 96-well polystyrene microplate. Finally, AAPH solution (36 mM) was added rapidly using the multichannel micropipette, and fluorescence was recorded during 37 cycles, every 210 s, at 37°C. A blank (fluorescein + AAPH) using phosphate buffer (75 mM) instead of the sample solution and eight calibration solutions using Trolox (10, 20, 30, 40, 50, 60, 70, 80 µM) as antioxidant were also carried out in each assay.

The final $ORAC_{FL}$ values were calculated by using a regression equation (y=0.1577X+1.8121, r^2=0.9928) between Trolox concentration (Y) (µM) and net area under the FL decay curve (X).

Area under the curve (AUC) was calculated according to the equation:

$AUC = 1 + F_1/F_0 + F_2/F_0 + \ldots + F_n/F_o$

where F_0 is the initial fluorescence reading at 0 min, F_1 is the fluorescence intensity reading at time 3.5 min and F_n is the fluorescence intensity reading at 129.5 min (37 cycles of 210 s). Finally, the net AUC was calculated by subtracting the AUC of the blank sample from the AUC of each sample. ORAC values were expressed as Trolox equivalents by using the standard curve calculated for each assay and final results were expressed in µmol equivalents of Trolox per g of sample.

2. $ABTS^{\bullet+}$ assay

The antioxidant capacity was estimated in terms of radical scavenging activity following the procedure described by Re *et al*. (1999) and Pulido *et al*. (2003). Absorbance was measured on a spectrophotometer Lambda 3B (Perkin Elmer). Briefly, $ABTS^{\bullet+}$ was produced by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The $ABTS^{\bullet+}$ solution (stable for 2 days) was diluted with 5 mM phosphate-buffered saline (pH 7.4) to an absorbance of 0.70 ± 0.02 at 730 nm. After addition of 40 µL of hydrophilic solution (previously diluted 1/64 for *G. mangostana*), Trolox, or blank (phosphate buffer) to 4 mL of diluted $ABTS^{\bullet+}$ solution, an absorbance reading was taken at 20 min. Eight calibration solutions using Trolox (100-800 mM) as antioxidant were also carried out in each assay. The linear regression equation obtained was (y = -1.8935X+1.1714, r^2=0.9918) between Trolox concentration (X) (mg/ml) and Absorbance (Y) ($A_{Trolox} - A_{blank}$). Results were expressed as µmol equivalents of Trolox per gram.

3. FRAP assay

The ferric reducing ability was estimated according to the procedure described by Pulido *et al.* (2000). The measure of absorbance was performed on a spectrophotometer Lambda 3B (Perkin Elmer) with thermostated bath.

The ferric reducing/antioxidant power (FRAP) reagent contained 2.5 mL of a 10 mM TPTZ solution in 40 mM HCl, plus 2.5 mL of 20 mM $FeCl_3.6H_2O$ and 25 mL of 0.3 M acetate buffer, pH 3.6. Briefly, 900 µL of FRAP reagent, prepared fresh and warmed at 37°C, was mixed with 90 µL of distilled water and 30 µL of hydrophilic solution (previously diluted 1/200), Trolox, or blank (acetate buffer).Temperature was maintained at 37°C an absorbance reading (595 nm) was taken at 30 min. Eight calibration solutions using Trolox (10-80 mM) as antioxidant were also carried out in each assay. The linear regression equation obtained was (y = 6.2485X + 0.1865, r^2 = 0.9915) between Trolox concentration (X) (mg/ml) and Absorbance (Y) ($A_{Trolox} - A_{blank}$). Results were expressed as µmol equivalents of Trolox per gram.

The antioxidant capacity was also determined in a commercial product (dietary supplement), to compare it with *Garcinia* results, extracted and determined by the same procedure. The product is constituted according to labelling information by dehydrated vegetables (carrots, lemon, borage, tomato, alfalfa, red grape, cabbage, garlic, yeast) and propolis, and is recommended as an antioxidant and anti-aging supplement.

Each sample was extracted in triplicate and assayed in triplicate or quadruplicate.

CE-ESI-TOF-MS

CE experiments were performed using a P/ACE™ System MDQ (Beckman Instruments, Fullerton, CA, USA). Fused-silica capillaries of 100 cm in length and 50 µm inner diameter (360 µm outer diameter) were used. After thorough optimization, a running buffer of 40 mM ammonium acetate at pH 9.5 was used. The separation voltage was set to 30 kV at the inlet of the capillary. Injection was performed hydrodynamically at 50 mBar during 15 s, corresponding to about 15 nL injected (0.9% of the capillary).

For CE-MS coupling, a coaxial sheath-liquid sprayer was used (Agilent Technologies). Isopropanol/water (60:40) with 0.1% (*v/v*) TEA was applied as sheath-liquid at a flow rate of 6 µL/min delivered by a 5 mL gas-tight syringe (Hamilton, Reno, NV, USA) using a syringe pump Cole-Parmer (Vernon Hill, IL, USA). The ESI-voltage of the TOF is applied at the end cap of the transfer capillary to the MS with the spray needle being grounded. An electrospray potential of + 4.1 kV was applied at the inlet of the MS (negative mode). A nebulizer gas (N_2) pressure of 0.3 bar was applied to assist the spraying. Dry gas temperature was set to 180ºC at a dry gas flow of 4 L/min.

Before first use, the bare capillaries were conditioned with 0.1 M sodium hydroxide during 20 min followed by a water rinse for another 10 min. between runs the capillary was flushed with water and separation buffer for 5 min. At the end of the day the capillary was flushed with water for 10 min (all rinses during capillary conditioning were performed using N_2 at a pressure of 20 psi).

MS was performed using the micrOTOF™ (Bruker Daltonik, Bremen, Germany), an orthogonal-accelerated TOF mass spectrometer (oaTOF-MS). Transfer parameters were optimized by direct infusion experiments with Tuning Mix (Agilent Technologies) in the range of 50-800 m/z.

The trigger time was set to 50 µs, 49 µs for set transfer time and 1 µs pre-pulse storage time, corresponding to a mass range of 50–800 *m/z*. Spectra were acquired by summarizing 20,000 single spectra, defining the spectra rate to 1 Hz.

The accurate mass data of the molecular ions were processed through the software DataAnalysis 3.3 (Bruker Daltonik GmbH), which provided a list of possible elemental formula by using the GenerateMolecularFormula™ editor. The GenerateFormula™ editor uses the sigmaFit™ algorithm, which provides standard functionalities such as minimum/maximum elemental range, electron configuration and ring-plus double bonds equivalents, as well as a sophisticated comparison of the theoretical with the measured isotope pattern (SigmaValue™) for increased confidence in the suggested molecular formula (Bruker Daltonics Technical Note #008, Molecular formula determination under automation).

During the development of the CE method external calibration was performed using sodium formate cluster by switching the sheath liquid to a solution containing 5 mM sodium hydroxide in the sheath liquid of 0.2% formic acid in water:isopropanol 1:1 v/v at the end of the analysis. Using this method an exact calibration curve based on numerous cluster masses each differing by 68 Da ($NaCHO_2$) was obtained. Due to the compensation of temperature drift in the MicroTOF, this external calibration provided accurate mass values (better at 5 ppm) for a complete run without the need for a dual sprayer setup for internal mass calibration.

RESULTS AND DISCUSSION

Measurement of antioxidant capacity

To evaluate the antioxidant capacities of foods, numerous *in vitro* methods have been developed and reviewed. However, there has not been a consensus as to the preferred method or methods. ORAC, ABTS (TEAC assays) and FRAP are among the more popular methods that have been used (Wu *et al*. 2004; USDA 2007). Reviews of some of the methods, advantages and disadvantages, have been fully discussed in several reviews (Frankel and Meyer 2000; Sánchez-Moreno 2002; Prior *et al.* 2005)

The ORAC assay is considered by some to be a preferable method as a standard tool to measure the antioxidant activity in the nutraceutical, pharmaceutical, and food industries because of its simplicity, biological relevance,

mechanism HAT and the possibility to measure the lipophilic and hydrophilic antioxidant capacity (Prior *et al*. 2003). The $ABTS^{\bullet+}$ (2, 2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) assay has been popularized due to its ease of use and the FRAP assay previously was applied in plasma and in others types of products (Prior *et al*. 2005). In general, a product that has a high value for one measure of antioxidant capacity will also be high for another measure. However, because antioxidant compounds with different chemical structures interact with different radical sources differently, the relation between any two antioxidant capacity methods will be quite low if considered across all products (USDA 2007).

Recently, Jimenez-Alvarez *et al*. (2008) assayed for the measurement of antioxidant capacity of food extracts methods that target different antioxidant mechanisms, that is, radical scavenging capacity (ORAC), reducing capacity (FRAP), and metal chelating properties (ICA) (iron (II) chelating activity) and Folin-Ciocalteu method to phenol total measure. ORAC and FRAP assays provided a comprehensive, precise, and high-throughput assessment of antioxidant capacity in food extracts (Jimenez-Alvarez *et al*. 2008).

ORAC assay. *G. mangostana* extract (pulp and pericarp) showed a high radical scavenger capacity, 4088 µmol of TE/g to hydrophilic extract (**Table 1**), due to the high content of phenols (Haruenkit *et al.* 2007), which has been reported to correlate well with hydrogen-donating capacity (Proteggente *et al*. 2002; Prior *et al*. 2005). *Garcinia* extract exhibited an antioxidant capacity 19.6 times higher than a product (mixed vegetables) commercialized as a dietary supplement (200 µmol of TE/g). Patthamakanokporn *et al*. (2008) reported a value of 122.4 µmol of TE/g of dry matter from pulp of mangosteen. These authors applied the same determination method but used homogenized fresh pulp and acetone solvent to 50% to get the extracts. Interestingly, the peel and seed fractions of some fruits have higher antioxidant activity than the pulp fractions (Jayaprakasha *et al.* 2001).

The antioxidant capacity of ethanolic extract determined by Wu *et al*. (2004) (following the same method applied in this study) of 24 fresh fruit was between 2.97 µmol of TE/g to cantaloupe and 92.56 µmol of TE/g to cranberry; considering dry weight this would be 30.6 and 841.5, respectively. As can be observed the pericarp of mangosteen has a high contribution to antioxidant power of whole tropical fruit.

ABTS assay. The antioxidant capacity (AC) of *Garcinia* by this assay was 3591 µmol TE/g (**Table 1**). The peel of this fruit had recently been reported to have higher antioxidant activity than its pulp and seed (Li *et al*. 2006). Ethanolic extracts of mangosteen fruit peel showed high free radical-scavenging power, 3001 mmol of TE/g (Okonogi *et al*. 2007), approximately 900 times more than our results with whole fruit (pericarp and pulp). On the other hand, antioxidant capacity differences of mangosteen fruit extract can be observed depending on the solvent used from 10 to 50 µmol TE/g when acetone:water or water was applied (Leontowicz *et al*. 2006). Antioxidant capacity of fresh mangosteen determined by Haruenkit (2007) was 20.16 µmol TE/g.

FRAP assay. Antioxidant capacity of *Garcinia* was 1222 µmol TE/g (**Table 1**). As for other methods the value is higher than obtained by Patthamakanokporn *et al.* (2008) for mangosteen edible part, 31.7 µmol TE/g dry weight. The commercial supplement dietary exhibited an antioxidant capacity 35 times less than *Garcinia* (34.4 µmol TE/g).

Higher values of antioxidant activity analysed by ORAC rather than by the FRAP method was found. It is likely that not all ORAC-active antioxidants in the studied fruits are reducing. However, Patthamakanokporn *et al.* (2008) analyzed seven tropical fruits and found than three of them showed less antioxidant capacity by ORAC than FRAP but not for mangosteen fruit. It is not surprising that a different antioxidant capacity among the studied fruit was shown by ORAC and ABTS compared to the FRAP method because in theory the measurement of each method is based on different mechanisms of reaction (Prior and Cao 1999). This confirms that the antioxidant capacity should be investigated by more than one method.

Table 1 Antioxidant capacity (µmol trolox equivalents/g).

Products	FRAP	ABTS	ORAC
Garcinia mangostana [b]	1222 ± 18*	3591 ± 133*	4088 ± 109*
Dietary supplement[a, c]	34 ± 1**	137 ± 7**	200 ± 7**

[a] Dehydrated vegetables labelling as supplement antioxidant and anti-aging
[b] n = 4;
[c] n = 7
*, ** Statistical differences $p<0.05$

Identification of phenolic compounds in mangosteen

The proposed CE-ESI-TOF-MS method was applied for the identification of the phenolic compounds present in the *G. mangostana* extract. The formula, selected ion, m/z experimental and calculated, error (ppm and mDa), sigma value, tolerance, migration time and tentative compound proposed are summarized in **Table 2**.

The present method is able to detect several phenolic compounds previously observed in different studies. These tentative compounds are: (1) Garcinone E ($[M-H]^-_{exp.}$ 463.2126 m/z), (2) Garcinone D ($[M-H]^-_{exp.}$ 427.1762 m/z), (3) Mangostenone C ($[M-H]^-_{exp.}$ 441.1568 m/z), (4) Mangostanol ($[M-H]^-_{exp.}$ 425.1612 m/z), (5) α-Mangostin, 1-Isomangostin, 3-Isomangostin, Cudraxanthone O, F, E, D, C, 8-Hydroxycudraxanthone G ($[M-H]^-_{exp.}$ 409.1667 m/z), (6) Garcinone C ($[M-H]^-_{exp.}$ 413.1621 m/z), (7) Epicatechin ($[M-H]^-_{exp.}$ 289.0717 m/z), (8) Mangostinone, 8-Desoxygartanin, Garcinone A ($[M-H]^-_{exp.}$ 379.1565 m/z), (9) Gartanin, γ-mangosteen, Mangoxanthone, Mangostenone D, Smeathxanthone A, Cudraxanthone P, M, L, J ($[M-H]^-_{exp.}$ 395.1513 m/z), (10) 1, 3, 5, 6-Tetrahydroxyxanthone ($[M-H]^-_{exp.}$ 259.0237 m/z).

As **Table 2** shows, several polar compounds can be found with the same molecular formula and that can be extracted with methanol and described in bibliography related to mangosteen.

As TOF-MS provides excellent mass accuracy over a wide dynamic range and allows measurements of the isotopic pattern, providing important additional information for the determination of the elemental composition. Therefore all detected compounds observed in **Table 2** exhibit good sigma values and mass accuracy (ppm and mDa) as indicated by the error values.

In comparison to the chromatographic methods, the proposed method is a good alternative for simultaneous characterization of phenolic compounds in *G. mangostana* as this technique provides fast and efficient separations and used reduced sample and solvent consumption. Also, the hyphenation of CE to MS combines the advantages of CE with the selectivity, sensitivity and mass accuracy inherent to TOF-MS.

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Table 2 Well-known phenolic compounds determined by CE-ESI-TOF-MS in an extract of *Garcinia mangostana*.

Formula	Selected ion	m/z experimental	m/z calculated	Error		Sigma Value	Tolerance (ppm) in Generate Molecular Formula	Migration time CE (min)	Compounds
				ppm	mDa				
$C_{28}H_{31}O_6$	$[M-H]^-$	463.2132	463.2126	1.4	-0.66	0.0119	5	11.8	*Garcinone E* (Suksamrarn *et al*. 2006)
$C_{24}H_{27}O_7$	$[M-H]^-$	427.1772	427.1762	2.3	-1.00	0.0130	5	11.9	*Garcinone D* (Suksamrarn *et al*. 2006)
$C_{24}H_{25}O_8$	$[M-H]^-$	441.1568	441.1554	3.1	-1.36	0.0333	5	12.0	*Mangostenone C* (Suksamrarn *et al*. 2006)
$C_{24}H_{25}O_7$	$[M-H]^-$	425.1612	425.1600	1.7	-0.72	0.0553	5	12.1	*Mangostanol* (Suksamrarn *et al*. 2003)
$C_{24}H_{25}O_6$	$[M-H]^-$	409.1667	409.1656	2.7	-1.10	0.0145	5	12.2	*α-Mangostin, 1-Isomangostin, 3-Isomangostin, Cudraxanthone O, F, E, D, C 8-Hydroxycudraxanthone G* (Ji *et al*. 2007; Jung *et al*. 2006)
$C_{23}H_{25}O_7$	$[M-H]^-$	413.1621	413.1605	3.9	-1.59	0.0043	5	12.3	*Garcinone C* (Suksamrarn *et al*. 2006)
$C_{15}H_{13}O_6$	$[M-H]^-$	289.0717	289.0717	0.0	0.01	0.0146	5	12.4	*Epicatechin* (Suksamrarn *et al*. 2002)
$C_{23}H_{23}O_5$	$[M-H]^-$	379.1565	379.1550	3.9	-1.48	0.0282	5	12.5	*Mangostinone, 8-desoxygartanin Garcinone A* (Ji *et al*. 2007; Suksamrarn *et al*. 2002)
$C_{23}H_{23}O_6$	$[M-H]^-$	395.1513	395.1500	3.5	-1.38	0.0216	5	12.6	*Gartanin, γ-Mangostin, Mangoxanthone, Mangostenone D, Smeathxanthone A, Cudraxanthone P, M, L, J* (Suksamrarn *et al*. 2003; Nilar *et al*. 2005; Jung *et al*. 2006)
$C_{13}H_7O_6$	$[M-H]^-$	259.0237	259.0248	4.3	1.10	0.0165	5	15.3	*1,3,5,6-Tetrahydroxyxanthone* (Nilar *et al*. 2005)

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