Food Research International 53 (2013) 417–425

Contents lists available at SciVerse ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Assessment of antioxidant and antiproliferative activities and the identification of phenolic compounds of exotic Brazilian fruits

Luciana Gomes Malta [a,*], Elias Paulo Tessaro [b], Marcos Eberlin [b], Glaucia Maria Pastore [a], Rui Hai Liu [c,**]

[a] Department of Food Science, Faculty of Food Engineering, University of Campinas (UNICAMP), Campinas, SP, Brazil
[b] ThoMSon Mass Spectrometry Laboratory, Institute of Chemistry, University of Campinas (UNICAMP), Campinas, SP, Brazil
[c] Department of Food Science, Cornell University, Stocking Hall, Ithaca, NY, United States

ARTICLE INFO

Article history:
Received 16 July 2012
Accepted 28 April 2013

Keywords:
Fruits
Phenolics
Flavonoids
Antioxidant activity
Antiproliferative activity
ESI–TOF-MS

ABSTRACT

Phenolics including flavonoids are suggested to be the major bioactive compounds contributing to the health benefits of fruits and vegetables. The objective of the study was to determine the total phenolic and flavonoid contents, oxygen radical absorbance capacity (ORAC), peroxyl radical scavenging capacity (PSC), cellular antioxidant activity (CAA) and antiproliferative properties of selected cerrado fruits named gabiroba (*Campomanesia cambessedeana* Berg), murici (*Byrsonoma verbascifolia* Rich) and guapeva (*Pouteria guardneriana* Radlk). Gabiroba fruit showed the highest amount of total phenolics (851.0 ± 40.7 mg/100 g fruit) and the highest antioxidant activity for both the performed assays (ORAC 8027.5 ± 378.6 μmol TE/100 g fruit and PSC 2342.5 ± 48.1 μmol AAE/100 g fruit). Gabiroba fruit and the pulp of guapeva had the highest antiproliferative capacity with the lowest EC_{50} 40.7 ± 4.8 mg/mL and 37.9 ± 2.2 mg/mL, respectively. The results for the analyzed fruits for CAA were not significantly different from each other ($p < 0.05$) (murici 41.3 ± 17.8; gabiroba 33.9 ± 18.8 and the pulp of guapeva 23.3 ± 4.2 μmol quercetin/100 g fruit). More than 30 phenolic compounds in the pulp of guapeva, gabiroba and murici extracts were identified in the present study using *ESI–TOF-MS*. These results characterized for the first time the functional activity of selected Brazilian fruits and demonstrated the importance of these fruits from cerrado biome.

1. Introduction

Cancer is a major public health problem across the world. It has been reported that more than 30% of human cancers could be prevented by an alternative strategy of appropriate dietary modification (Liu, 2003 and Liu, 2004).

The antioxidant activity of fruits is noteworthy, several associations have been made between fruit and vegetable intake and a reduced risk of cancer and incidences of chronic and degenerative diseases (Chu, Sun, Wu, & Liu, 2002; Liu, 2003). Fruits are rich in bioactive phenolic compounds such as flavonoids, phenolic acids, stilbenes, coumarins, and tannins, which have important roles in free radical scavenging (Kim et al., 2011; Roesler, Catharino, Malta, Eberlin, & Pastore, 2008; Wolfe et al., 2008). The antioxidant activity and the anticarcinogenic and antimutagenic effects of phenolic compounds have been widely reported. In fact, phenolics have shown to be capable of inhibiting cell proliferation in vitro (Kim et al., 2010; Meyers, Watkins, Pritts, & Liu, 2003). Over recent years, a large use of different in vitro chemical assays has been employed in order to evaluate the antioxidant properties and the phenolic content of fruits (Goncalves, Genovese, & Fett, 2011; Mertz, Gancel, Gunata, Alter, & Dhuique-Mayer, 2009; Sdiri, Navarroa, Monterdea, Benabdab, & Salvadora, 2012; Serc, Ozgen, Torun, & Ercis, 2010).

Brazil boasts a large number of underexploited native and exotic fruit species which are of a potential interest in the agroindustry and a possible future source of income for the local population (Almeida et al., 2011). However, there are several exotic Brazilian fruit that have not been studied yet for their antioxidant potential, as also other functional capacities such as antiproliferative activity. The native fruits from Brazilian savanna are arousing increasing interest due to their nutritional and functional properties combined with the potential to add value and conserve the biodiversity of this biome (Rocha et al., 2011). The cerrado is the second largest biome in South America, after the Amazon rainforest. In the last 30 years, progressive mechanization with improved techniques for clearing and fertilizing the land has contributed to the accelerated destruction of the natural vegetation, and it is estimated that 40% of the cerrado biome has already been deforested (Roesler, Catharino, Malta, Eberlin, & Pastore, 2007). Searching for an economically viable and environmentally friend application of Brazilian cerrado natural resources is highly important and equally urgent in order to avoid the total destruction of natural vegetation and cerrado biodiversity (Roesler et al., 2008).

* Correspondence to: L.G. Malta, Monteiro Lobato Street, 80, Department of Food Sciences, Faculty of Food Engineering, State University of Campinas, Campinas — SP, Brazil, P.O. Box 13083-862, Campinas, São Paulo, Brazil. Tel.: +55 19 35214090; fax: +55 19 3891513.
** Correspondence to: R.H. Liu, Department of Food Science, Cornell University, Ithaca, NY 14853 Tel.: +1 607 255 6235; fax: +1 607 254 4868.
E-mail addresses: lucianamalta@yahoo.com (L.G. Malta), rl23@cornell.edu (R.H. Liu).

0963-9969/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodres.2013.04.024

The cerrado in Brazil's biodiversity is the source of many plant species that play an important role in folk medicine (Paula, Paula, Barra, Rezende, & Ferreira, 2008). The use of natural medicine for the treatment of many diseases is well disseminated worldwide. Some studies have focused on the history of plant use from an ethnopharmacological perspective. The rich flora of the cerrado biome in Brazil has been poorly studied in order to evaluate the efficacy and therapeutic effects of crude extracts or isolated compounds obtained from a wide range of plant families. It has been demonstrated that some of the extracts or active principles obtained from plants have a broad spectrum of biological activities, including analgesic and anti-inflammatory properties (Junior et al., 2009).

This study analyzed three selected fruits from cerrado biome, gabiroba, murici and guapeva. The objectives were to (i) measure the total phenolic and flavonoid contents; (ii) determine the in vitro antioxidant capacity using Oxygen Radical Absorbance Capacity (ORAC) and Peroxyl Radical Scavenging Capacity (PSC) methods, and Cellular Antioxidant Activity (CAA); (iii) determine the antiproliferative activity on human liver cancer cell growth in vitro and (iv) identify the phenolic compounds (*ESI–TOF-MS*).

2. Material and methods

2.1. Chemicals

All chemicals used in the study, such as methanol, acetone, sodium carbonate, potassium phosphate, hydrochloric acid (HCl), acetic acid were of analytical grade and were purchased from Mallinckrodt Chemicals (Phillipsburg, NJ). Gallic acid was from ICN Biomedical Inc. (Costa Mesa, CA). 2′,7′-Dichlorofluorescin diacetate (DCFH-DA), fluorescein disodium salt, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Folin–Ciocalteu reagent, ascorbic acid, catechin, quercetin dehydrate, ethanol (EtOH, anhydrous, 200 proof), sodium borohydride ($NaBH_4$, reagent grade), chloranil (analytical grade), and vanillin (analytical grade) were purchased from Sigma-Aldrich, Inc. (St. Louis, MO). Tetrahydrofuran (THF, analytical grade), aluminum chloride (AlCl3 ∗ 6H2O, analytical grade) and dimethyl sulfoxide (DMSO) were obtained from Fisher Scientific (Pittsburgh, PA), and 2,2′-azobis (2-amidinopropane) dihydrochloride (ABAP) was purchased from Wako Chemicals USA, Inc. (Richmond, VA). HepG2 liver cancer cells were obtained from the American Type Culture Collection (ATCC) (Rockville, MD). Williams' Medium E (WME) and Hanks' Balanced Salt Solution (HBSS) were purchased from Gibco Life Technologies (Grand Island, NY). Fetal bovine serum (FBS) was obtained from Atlanta Biologicals (Lawrenceville, GA).

2.2. Extract preparation

Ripe fruits, gabiroba (*Campomanesia cambessedeana* Berg), murici (*Byrsonoma verbascifolia* Rich) and guapeva (*Pouteria guardneriana* Radlk), were obtained from the Boa Vista farm in the city of Itapirapuã, Goiás/Brazil. All fruits were washed. While the whole gabiroba fruit and murici fruit were used for the analyses, the guapeva fruit was peeled and its seeds were carefully removed. First, the fruits were lyophilized and then they were homogenized with Polytron homogenizer for 10 min in chilled 80% acetone (1:2, w/v). The homogenates were filtered through Whatman no. 1 paper, and the filtrates were evaporated to dryness under vacuum at 45 °C. The samples were reconstituted in 70% methanol and stored at −40 °C. Before use, the methanol was evaporated under a stream of nitrogen, and the fruit extracts were reconstituted in water. The extractions were done in triplicate.

2.3. Preparation of solutions

A 200 mM stock solution of DCFH-DA in methanol was prepared, aliquoted, and stored at −20 °C. A 200 mM ABAP stock solution in water was prepared, and aliquots were stored at −40 °C. Quercetin solutions were prepared in DMSO before further dilution in treatment medium (WME with 2 mM L-glutamine and 10 mM Hepes).

2.4. Cell culture

HepG2 cells were grown in growth medium (WME supplemented with 5% FBS, 10 mM Hepes, 2 mM L-glutamine, 5 μg/mL insulin, 0.05 μg/mL hydrocortisone, 50 units/mL penicillin, 50 μg/mL streptomycin, and 100 μg/mL gentamicin) and were maintained at 37 °C and 5% CO_2 as described previously by Liu et al. (1994) and Wolfe and Liu (2007). Cells used in this study were between passages 12 and 32.

2.5. Determination of total phenolic content

The total phenolic contents of the investigated fruits were measured using a modified colorimetric Folin–Ciocalteu method (Singleton, Orthofer, Lamuela-Ravento, & Lester, 1999; Wolfe & Liu, 2007). Volumes of 0.5 mL of deionized water and 0.125 mL of diluted fruit extracts (at 25 mg/mL) were added to a test tube. Folin–Ciocalteu reagent (0.125 mL) was added to the solution and allowed to react for 6 min. Then, 1.25 mL of 7% sodium carbonate solution was aliquoted into the test tubes, and the mixture was diluted to 3 mL with deionized water. The color was developed for 90 min, and the absorbance was read at 760 nm using a MRX II Dynex spectrophotometer (Dynex Technologies, Inc., Chantilly, VA). The measurement was compared to a standard curve of gallic acid concentrations and expressed as milligrams of gallic acid equivalents (GAE) per 100 g of fresh fruit ± Standard deviation (SD) for triplicate fruit extracts.

2.6. Determination of total flavonoid content

The total flavonoid content of the fruit extracts was determined using the sodium borohydride/chloranil-based assay developed by our laboratory (He, Liu, & Liu, 2008). Briefly, 4 mL extracts of tested samples were added into test tubes (15 × 150 mm), then dried to dryness under nitrogen gas, and reconstituted in 1 mL of THF/EtOH (1:1, v/v). Catechin standards (0.1–8.0 mM) were prepared fresh each day before use in 1 mL of THF/EtOH (1:1, v/v). To each test tube with 1 mL of sample solution or 1 mL of catechin standard solution, 0.5 mL of 50 mM NaBH4 solution and 0.5 mL of 74.56 mM AlCl3 solution were added. The mixture was shaken in an orbital shaker (Laboratory-Line Instruments, Inc., Melrose Park, IL) at room temperature for 30 min. Then an additional 0.5 mL of $NaBH_4$ solution was added into each test tube with continuing shaking for another 30 min at room temperature. Cold acetic acid solution (2.0 mL of 0.8 M, 4 °C) was added into each test tube, and the solutions were kept in the dark for 15 min after a thorough mix. Thus, chloranil (1.0 mL, 20 mM) was added into each tube, which was heated at 100 °C with shaking for 60 min in a reciprocal shaking bath (Precision Scientific Inc., Chicago, IL). The reaction solutions were cooled using tap water, and the final volume was brought to 4 mL using methanol. Then, 1 mL of 1052 mM vanillin was added into each tube, followed by mixing. Concentrated HCl (2.0 mL, 12 M) was added into each tube, and the reaction solutions were kept in the dark for 15 min after a thorough mix. Aliquots of the final reaction solutions (200 μL) were added into each well of a 96-well plate, and absorbances were measured at 490 nm using a MRX Microplate Reader with Revelation work station (Dynex Technologies, Inc., Chantilly, VA). Total flavonoid content was expressed as milligrams of catechin equivalents per 100 g of fresh fruit ± SD for triplicate fruit extracts.

2.7. Measurement of Oxygen Radical Scavenging Capacity (ORAC)

The peroxyl radical scavenging efficacy of selected fruit extracts was measured using the ORAC assay (Prior et al., 2003). Briefly, 20 μL of blank, Trolox standard, or fruit extracts in 75 mM potassium phosphate