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# Red-jambo *(Syzygium malaccense)*: Bioactive compounds in fruits and leaves

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ABSTRACT

*Syzygium malaccense* is poorly studied regarding the bioactive compounds of fruits and leaves. The aim of this study was to determine proximate composition, phenolic compounds, carotenoids, and antioxidant capacity of the fruit parts and leaves of *S. malaccense*. The samples were extracted with different solvents in order to analyze phenolic compounds content (Folin-Ciocalteau and HPLC-DAD/FLD), flavonoids (reaction with $AlCl_3$ and HPLC-DAD/FLD), anthocyanins (differential pH and HPLC-DAD, UPLC-ESI-MS/MS), total carotenoids (colorimetric method) and antioxidant capacity (hydrophilic and lipophilic-ORAC, FRAP, DPPH). The pulp demonstrated high amounts of soluble fibers and reducing sugars. The peel, seeds and leaves of *S. malaccense* showed great contents of phenolic compounds, flavonoids and carotenoids as well as antioxidant capacity. The anthocyanins found in the fruit were cyanidin-3-*O*-glucoside, cyanidin-3,5-*O*-diglucoside, and peonidin-3-*O*-glucoside. Polar bioactive compounds showed strong correlation to hydrophilic antioxidant capacity, while carotenoids did not correlate lipophilic-ORAC.



## 1. Introduction

Several berries have shown great scientific interest due to antioxidant power, antitumoral and anti-inflammatory effects (Nile & Park, 2014; Paredes-López, Cervantes-Ceja, Vigna-Pérez, & Hernández-Pérez, 2010). *Syzygium malaccense* (syn. *Eugenia malaccensis, Jambos malaccensis*) belongs to the *Myrtaceae* family and is an original plant from Malaysia, known as Malay apple. Although, the plant was widespread throughout tropical regions, where was also named as pomerac, mountain-apple or red-jambo (Morton, 1987).

The tree reaches 12–15 m height, shows straight trunk and pyramidal canopy. The red-jambo tree could be used as ornamental plant due to the beauty of its fallen flowers, and offers berry fruits two times per year: February–March and October–November. The fruits are pear-shaped, succulent, containing a single seed, and thin, smooth, waxy peel varying from the rose, crimson, and dark red colors, depending on the stage of maturation or harvest conditions. The pulp is whitish and juicy with acid flavor, similar to apples (Morton, 1987). The aroma of red-jambo is similar to a mixture of roses and herbaceous plants (Pino, Marbot, Rosado, & Vazquez, 2004). The fruits are eaten fresh or in form of handmade products. Although, much of the fruit is wasted during harvest time due to high production, perishability and lack of technological feasibility information for the use by industries.

The scientific literature reports antioxidant capacity of the edible part of red-jambo fruits (Lako et al., 2007; Reynertson, Yang, Jiang, Basile, & Kennelly, 2008), anti-inflammatory and antioxidant effects of leaves use (Andersson Dunstan et al., 1997; Arumugam, Manaharan, Heng, Kuppusamy, & Palanisamy, 2014), cytotoxic power of leaves (Savitha, Padmavathy, & Sundhararajan, 2011), and glycemia/cholesterolemia-lowering effects of the trunk bark

*Abbreviations:* FPP, fresh pulp + peel; PP, pulp + peel; TA, titratable acidity; SS, soluble solids; MetOH, 80% methanol extract; H-MetOH, hydrolyzed 80% methanol extract; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant power; ORAC, oxygen radical absorbance capacity; H-ORAC, hydrophilic ORAC; L-ORAC, lipophilic ORAC; T-ORAC, total ORAC; BHT, 2,6-di-tert-butyl-4-methylphenol; GAE, gallic acid equivalents; CE, catechin equivalents; TE, trolox equivalents; FLD, fluorescence detector; PCA, principal component analysis.

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extract (Bairy, Sharma, & Shalini, 2005). Thus, each tissue of the plant has different chemical characteristics that encourage research to explore their functional effects in separated way, since this data is previously rare in literature.

The aim of this work was to explore different tissues of *S. malaccense* (pulp, peel, seed and leaf) in relation to nutrients composition and phytochemicals with bioactive function, such as phenolic compounds and carotenoids, and to determine antioxidant capacity of the different plant tissues.

## 2. Material and methods

### 2.1. Standards

Benzoic acid standard was purchased from Chem Service (West Chester, USA). Kaempferol-3-*O*-glucoside, (+)-catechin, cyanidin-3,5-*O*-diglucoside-chloride (cyanin chloride), cyanidin-3-*O*-glucoside-chloride (kuromanin chloride), (−)-epicatechin, (−)-epicatechin gallate, isorhamnetin-3-*O*-glucoside, peonidin-3-*O*-glucoside chloride, procyanidin A2, procyanidin B1, procyanidin B2, quercetin (dihydrate), isoquercitrin, and rutin standards were obtained from Extrasynthese (Genay, France). *p*-Coumaric acid was purchased from Sigma (UK).

### 2.2. Samples

Ripe red-jambo fruits were harvested in Araçatuba-SP in February 2013 and purchased from a local market in Campinas-SP, Brazil.

**Physical-chemical determinations:** Ten ripe fresh fruits were randomly chosen (Suppl. 1), cleaned and pulp, peel, pulp + peel (PP) and seeds were separated and weighed. Titratable acidity (TA), soluble solids and pH were determined in the edible part (fresh pulp + peel = FPP). The TA was determined by titration with standardized 0.01 N NaOH and the results were expressed as grams of citric acid. Soluble solids (SS) were measured in a manual refractometer. The pH was evaluated using a digital pH meter (Ion Meter 450).

The leaves were collected in the main campus of the University of Campinas and cleaned. The leaves and the parts of the fruits were dried in a freeze-dryer (LP1010, Liobras, São Carlos, São Paulo, Brazil) at a range from −40 to 25 °C, 300 μm Hg for 95 h, crushed, homogenized and frozen at −18 ± 5 °C.

### 2.3. Macronutrient determinations

Total nitrogen was determined using a NDA 701 Dumas Nitrogen analyzer (VelpScientifica, Usmate, Italy). Moisture and ash were analyzed according to standard methods (AOAC., 2002) and lipids according to Bligh and Dyer (Bligh & Dyer, 1959). Carbohydrates were calculated by difference. Total and insoluble dietary fibers were quantified by the enzymatic–gravimetric method (AOAC., 2002). Soluble fibers were calculated by difference.

In order to determine reducing sugars, a 70% ethanol solution was added to the samples and allowed to react during 60 min at 100 °C. After ethanol evaporation, the extracts were filled with water and then analyzed according to the Somogyi-Nelson method, using a Synergy HT, Biotek microplate reader (Winooski, USA), with readings set at 520 nm (Nelson, 1944).

### 2.4. Bioactive compounds and antioxidant capacity measurements

All the absorbance and fluorescence readings for the analyses were determined in a Synergy HT, Biotek microplate reader (Winooski, USA) with Gen5™ 2.0 data analysis software.

#### 2.4.1. Sample treatments

Extracts (40 g $L^{-1}$ for freeze-dried samples and 120 g $L^{-1}$ for FPP) were made in duplicate as follows:

**80% Methanol extract:** The samples were extracted with 80% methanol (MetOH:$H_2O$, v:v) at 37 °C for 3 h in a shaking water bath, centrifuged at 2000× *g*, for 10 min, and stored in amber flasks at 4–8 °C (Batista, Ferrari, da Cunhada SilvaCazarin, & Correa, 2016).

**Hydrolyzed 80% methanol extract:** A HCl and BHT (2,6-di-tert-butyl-4-methylphenol) solutions were added to the aforementioned methanol extract to result in a final concentration of 1.2 mol $L^{-1}$ HCl, 0.26 g $L^{-1}$ BHT in 50% methanol (MetOH:$H_2O$, v:v) in the extract. In order to complete the hydrolysis, the extracts were allowed to react in water bath at 90 °C for 30 min, with refrigerated reflux condenser.

**Lipophilic extract:** The samples were extracted by successive maceration with hexane at room temperature to obtain a non-polar extract. After solvent evaporation, samples were resuspended with acetone (Prior et al., 2003).

All extracts were stored at 4 °C until analyzed and new extracts were made each 5 days.

#### 2.4.2. Bioactive compounds

**Total phenolic compounds or Folin-Ciocalteau reagent reducing substances method:** Folin-Ciocalteau reagent and sodium carbonate were added to water-diluted extract, and after 2 h in the dark at room temperature, the absorbance of samples and standard curve was read at 725 nm. Gallic acid was used as standard and results were expressed as gallic acid equivalents (mg GAE) (Batista et al., 2016).

**Total flavonoids:** Solutions, such as 5% sodium nitrite, 10% aluminum chloride and 1 mol $L^{-1}$ sodium hydroxide were added to water-diluted extracts (Zhishen, Mengcheng, & Jianming, 1999). A precipitation was observed after the addition of NaOH solution to the leaf extract, and because of that the mix was centrifuged at 2000× *g*, 10 min. A calibration curve was made using (+)-catechin. The samples and standard were read at 510 nm. The results were expressed as catechin equivalents (mg CE).

**Monomeric anthocyanins method:** The extracts were diluted using 0.025 mol $L^{-1}$ potassium chloride buffer (pH 1.0) according to sample absorbance (0.4–0.6), and in 0.4 mol $L^{-1}$ sodium acetate buffer (pH 4.5) using the same proportions (1:2, 1:6 and 1:15 dilution factors for FPP, PP and peel, respectively). After addition of 250 μL in the microplate, the absorbance was read at 520 and 700 nm (Wrolstad, 1976). The absorbance (A) was calculated using Equation (1):

$$A = [(A_{520\ nm} - A_{700\ nm})pH = 1.0] - [(A_{520\ nm} - A_{700\ nm})pH = 4.5] \quad (1)$$

The anthocyanin content (mg 100 $g^{-1}$) was calculated as cyanidin-3-*O*-glucoside (PM = 449.2) using Equation (2):

$$C\left(mgC3G\ 100g^{-1}\right) = A.MW.DF/\xi.L \quad (2)$$

where C = concentration, C3G = cyanidin-3-*O*-glucoside, ξ = molar absorptivity (26,900 mol $L^{-1}$), L = pathlength (cm), MW = molecular weight and DF = dilution factor.

**HPLC analysis:** The analyses of the fruit extracts were performed using a HPLC system Waters e2695 Separation Module Alliance equipped with a quaternary solvent pump and an automatic injector. For the phenolic compounds determination, a diode array detector (DAD) Waters model 2998 and a fluorescence detector (FLD) Waters model 2475 were employed. Acquisition and