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Phenolics, organic acids and minerals in the fruit juice of the indigenous African sourplum (*Ximenia caffra*, Olacaceae)

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ABSTRACT

Wild-picked fruits of the indigenous African tree, *Ximenia caffra*, are widely consumed in Southern Africa when in season, yet compositional data on phytochemicals, organic acids and minerals are lacking. The study therefore aimed to characterize juice obtained from ripe *X. caffra* fruits, and to identify individual phenolic compounds using liquid chromatography, high-resolution mass spectrometry (LC-HRMS). The juice had high total phenolic (1030 mg 100 ml^{-1} gallic acid equivalents) and total flavonoid content (852 mg 100 ml^{-1} catechin equivalents), and LC-HRMS analysis identified procyanidin B1 (12.2 mg 100 ml^{-1}), gallic acid (5.56 mg 100 ml^{-1}) and catechin (2.66 mg 100 ml^{-1}) as the most abundant phenolic compounds, while the dominant organic acid was citric acid (8.05 g 100 ml^{-1}), with lesser levels of tartaric, L-malic and L-lactic acids. LC-HRMS further positively identified and quantified a number of other polyphenolic compounds from the juice. The most prevalent minerals were potassium (525 mg 100 ml^{-1}) and phosphorous (24.6 mg 100 ml^{-1}), while heavy metal content was low. Consumption of *X. caffra* fruits can significantly contribute toward dietary phytochemical and mineral intake, and consumption of this fruit should therefore be encouraged.

1. Introduction

Plant foods gathered from the wild provide essential nutrients and potentially health promoting phytochemicals to especially rural populations, and contribute to global food security (Toledo and Burlingame, 2006; Stadlmayr et al., 2013; Ayessou et al., 2014). *Ximenia caffra* Sond. is a seasonal wild fruit that forms part of the diet of people throughout its distribution range and it plays an important ethnobotanical role as medicinal plant; the whole fruit including skins, pulp and seeds are edible (Mabogo, 1990; Nair et al., 2013; Maroyi, 2016). The species are widely distributed and indigenous to central, southern and eastern Africa, including Madagascar, and two varieties of *X. caffra* occur: var. *caffra* and var. *natalensis* Sond. (Coates Palgrave, 2003). The tree is known under a number of different vernacular names and in English it is mainly known as 'sour plum' due to the high acidity of the fruits (Maroyi, 2016). Flowering normally occurs between August and October (Nair et al., 2013), although the exact flowering and fruiting period is heavily influenced by seasonal rainfall (Mr. Dave Rushworth, personal communication).

Comprehensive data on nutritionally relevant compounds and phytochemical aspects of both *X. caffra* varieties are lacking, especially on the fruit pulp and skins. Current nutritional and phytochemical data of the fruit pulp, juice and skin show that the fruits are highly acidic and contain high levels of ascorbic acid, potassium and phenolic compounds, and that methanolic extracts of the fruits and skins exhibit high antioxidant capacity (Wehmeyer, 1966; Ndhlala et al., 2006; 2008; Maroyi, 2016). In contrast to the lack of nutritional data on the fruit, much effort has been spent on analysis of the seeds and the economically valuable seed oil, and data include proximate composition, lipid content and fatty acid profile, protein content and amino acid profile, mineral content, and data on various minor components (Chivandi et al., 2008, 2012; Mitei et al., 2009). These data confirm the high nutritional value of the seeds and the potential contribution that

Abbreviations: AAE, ascorbic acid equivalents; CE, catechin equivalents; DMPD, N,N-Dimethyl-p-phenylenediamine dihydrochloride; GAE, gallic acid equivalents; ICP-AES, inductively coupled plasma atomic emission spectroscopy; ICP-MS, inductively coupled plasma mass spectrometry; LC-HRMS, liquid chromatography, high resolution mass spectrometry.

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consumption of seeds can make to intake of essential and health promoting nutrients.

Despite the knowledge that fruits of *X. caffra* generally contain high levels of organic acids and phenolics, little is known about the nature and levels of the individual compounds. There is further a need to broaden the range and availability of data on compounds of nutritional interest in indigenous fruits in general (Stadlmayr et al., 2013), and for *X. caffra* in particular, since the plant is known to form a part of rural diets when in season. The aim of the current work was therefore to perform an analysis on water-soluble compounds found in *X. caffra* fruit collected from the wild through identification and quantification of phenolic compounds, organic acids and macro- and micro-minerals, as these compounds can all be of nutritional relevance in human diets.

2. Materials and methods

2.1. Raw material collection and preparation

Ripe fruits of *X. caffra* were collected in the Hoedspruit area, Limpopo Province, South Africa on January 10, 2015, at the GPS coordinates: S23°54.567′ E029°48.760′. The variety was tentatively identified as var. *natalensis* by a local botanist, Mr. Dave Rushworth, and fruits were kept on ice until processed. Juice was obtained from the fruits by separating the skin and fleshy portion of the fruit from the seeds through physical means, homogenizing the fruit flesh and skins together in a food processor and filtering the resultant pulp through a cotton cloth. The filtered juice obtained was pooled and mixed to obtain a homogenous sample, and then frozen at −20 °C until analysis. All subsequent analyses were performed on the pooled juice sample.

The weight of 42 randomly selected seeds were determined and expressed as the percentage of total fruit weight. Juice pH and Brix were determined using benchtop instruments, while total moisture (AOAC, 2005), total sugars (AOAC, 2003) and titratable acidity (Mora et al., 2009) were measured using standard analytical methods.

2.2. Juice mineral profile

Major elements Ca, K, Mg, Na, P and Si were analyzed on a Thermo ICap 6200 inductively coupled plasma atomic emission spectrometer (ICP-AES). The instrument was calibrated using NIST (National Institute of Standards and Technology, Gaithersburg MD, USA) traceable standards purchased from Inorganic Ventures (Christiansburg, Virginia, USA) to quantify selected elements. NIST-traceable quality control standards from De Bruyn Spectroscopic Solutions, Bryanston, South Africa, were analyzed to verify the accuracy of the calibration before sample analysis, as well as throughout the analysis to monitor drift.

Trace elements were analyzed on an Agilent 7700 quadrupole inductively coupled plasma mass spectrometer (ICP-MS). Samples are introduced via a 0.4 ml min^{-1} micromist nebulizer into a peltier-cooled spray chamber at a temperature of 2 °C, with a carrier gas flow of 1.05 l min^{-1}. The elements V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se were specifically analyzed under He-collision mode to remove polyatomic interferences. The instrument was calibrated using NIST (National Institute of Standards and Technology, Gaithersburg MD, USA) traceable standards to quantify selected elements. NIST-traceable quality control standards of a separate supplier than the main calibration standards were analyzed to verify the accuracy of the calibration before sample analysis.

2.3. Analysis of phenolics and ascorbic acid

Spectrophotometric methods were employed to measure total phenolic content and total flavonoid content of the fruit juice in triplicate. The spectrophotometer used for all spectrophotometric analyses was from A&E Lab, United Kingdom, model AE-S60-4U. Total phenolics were determined according to the Folin–Ciocalteu method of Singleton and Rossi (1965), using gallic acid as standard. Values were determined by measuring absorbance at 765 nm and expressed as gallic acid equivalents (GAE). Total flavonoids were measured using the method described by Amado et al. (2014), with catechin as standard, and absorbance of samples were measured at 410 nm and expressed as catechin equivalents (CE). Total ascorbic acid levels in the juice were determined by way of UV-HPLC according to the method described in Odriozola-Serrano et al. (2007). The column used was a YMC Pack Pro C18 column, and a total runtime and detection wavelength of 35 min and 250 nm were employed, respectively. Mobile phase A was 0.5% trifluoroacetic acid in water, and mobile phase B was 0.5% trifluoroacetic acid in methanol. Column temperature was 25 °C, mobile phase flow rate was constant at 1.0 ml min^{-1} and sample injection volume was 20 μl.

Phenolics were identified and quantified through a liquid chromatography, high resolution mass spectrometry (LC-HRMS) method, as describe previously (Stander et al., 2017), which utilizes a gradient method specifically focussing on phenolic acids and flavonoids. The only difference to the published method was that a Waters BEH C18, 2.1 × 100 mm, 1.7 μm column was used. A Waters Synapt G2 quadrupole time-of-flight mass spectrometer connected to Waters Ultra pressure liquid chromatograph and photo diode array detection was used for the analysis. In short, a 0.1% formic acid (solvent A) to acetonitrile containing 0.1% formic acid (solvent B) gradient was applied up to 28% solvent B, followed by a wash step.

The instrument was operated using electrospray ionization in negative MS^E mode which consisted of a low collision energy scan (6 V) from m/z 150 to 1500 and a high collision energy scan from m/z 40 to 1500. Positive identification of compounds was based on retention time matching with authentic standards, accurate mass data, UV data as well as MSMS fragmentation data.

The juice was diluted 5- and 10-fold in 50% methanol/water, centrifuged and the supernatant injected directly into the system. A cocktail of the standards were injected into the system at (100, 50, 25, 10, 5 and 0.5 mg l^{-1}) and the application manager Targetlynx 4.1 (Waters, MA, US) was used for the quantifications.

Preliminary antioxidant capacity was estimated using the spectrophotometric N,N-Dimethyl-p-phenylenediamine dihydrochloride (DMPD) assay of Fogliano et al. (1999), at an absorbance wavelength of 505 nm. Ascorbic acid was used as standard and values were reported as ascorbic acid equivalents (AAE).

2.4. Organic acid determination

Individual organic acids and their levels were determined using enzymatic derivatization followed by spectrophotometric detection and quantification. The instrument used was an Arena 20XT Discrete Photometric Analyzer (Thermo Fisher Scientific, Finland). Analysis for each organic acid was performed using a specific analysis kit, and analysis conditions were according to those prescribed by each kit manufacturer. The following organic acids were analyzed for (the specific analytical kit employed is shown in parentheses): acetic acid (Enzytec™ Fluid Acetic acid, catalog number 5226, Thermo Fischer Scientific, Finland), citric acid (R-Biopharm citric acid, Roche catalog number 10139076035, R-Biopharm AG, Darmstadt), D-lactic acid (Enzytec™ Fluid D-Lactic acid catalog number 5240, Thermo Fischer Scientific, Finland), L-lactic acid (Enzytec™ Fluid D-Lactic acid catalog number 5260, Thermo Fischer Scientific, Finland), L-malic acid (Enzytec™ Fluid L-Malic acid catalog number 5280, Thermo Fischer Scientific, Finland), pyruvic acid (Megazyme Pyruvic acid catalog number K-PYRUV 07/12, Megazyme International, Ireland), succinic acid (R-Biopharm succinic acid, Roche catalog number 10176281035, R-Biopharm AG, Darmstadt), and tartaric acid (Enzytec™ Color Tartaric acid catalog number E3100, Thermo Fischer Scientific, Finland).

3. Results

The juice obtained had an attractive red color corresponding to the red color of the fruit, and had a moisture content of 86.4%, a pH of