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Evaluation of nutritional composition, bioactive compounds and antimicrobial activity of *Elaeocarpus serratus* fruit extract

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The species *Elaeocarpus serratus* is widely used as an ornamental tree and their fruits are still little explored as food. Thus, the objectives of this research were to determine the physical, chemical and antimicrobial properties of *E. serratus* fruit in order to evaluate its food potential. Therefore, this study evaluated the physical and chemical composition and the antimicrobial activity of *E. serratus* fruit. The biometry revealed an average pulp yield of 82.16%. The physical and chemical characteristics of the *E. serratus* fruit showed that the pulp has pH 2.84 and has a higher content of moisture and crude fiber. The determination of bioactive compounds showed that *E. serratus* fruit presented a good source of flavonoids, condensed tannins, carotenoids and Vitamin C. In the chromatographic analyses, the presence of β-amirin was observed as the major compound by gas chromatography. Among the main phenolic compounds, the presence of kaempferol and quercetin in the liquid chromatography method was evidenced. Additionally, ethanolic extract from *E. serratus* fruit showed antimicrobial activity against *Bacillus cereus*, *Escherichia coli*, *Salmonella cholaresuis*, *Staphylococcus aureus* and *Xanthomonas campestris.*

Key words: Antimicrobial, ceylon olive, food science, functional food, phytochemistry.

INTRODUCTION

The consumption of fruits has become increasingly important due to their potential beneficial health effects related to their nutrient composition (Albuquerque et al., 2016), such as the presence of vitamins, phenolic,

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anthocyanins, flavonoids, tannins, among others (Dimitrios, 2006). Most of these compounds have the ability to prevent cancer, cardiovascular diseases, diabetes, neurodegenerative diseases and osteoporosis (Scalbert et al., 2005). In this context, studies concerning the evaluation of bioactive compounds, especially from unconventional fruit and vegetables, may provide important data concerning their use as food or medicinal product. Besides that, the evaluation of the content of nutrients and bioactive compounds from unconventional crops may be an alternative to their enhancement, providing information concerning the discovery of significant or high levels for specific nutrients or bioactive compounds that can improve the market demand (Herraiz et al., 2016).

Elaeocarpus serratus (Elaeocarpaceae) is an ornamental tree of Asiatic origin. Furthermore, it is a medium size tree with simple leaves, commonly called by Ceylon-olive, being found at East Africa, subtropical and tropical Asia either tropical Australia (Ghani, 2003). Their fruits are considered as drupes and are used to prepare juices in order to increase the appetite of patients by stimulating secretions from taste buds (Biswas et al., 2012). The main characteristic of *E. serratus* fruit is the astringent taste when consumed *in natura*, which are related with the higher amount of alkaloids and tannins present in its composition (Sharker and Shahid, 2010).

From the literature survey, it was found that many works have been conducted to determine the bioactivity of the *E. serratus* leaves. Extracts of *E. serratus* leaves and stem bark have shown antioxidant, antibacterial, antifungal and insecticide activities (Parvin et al., 2009; Sharker and Shahid, 2010; Indhiramuthu et al., 2014). Geetha et al. (2013) have found thirty substances in the ethanolic extract of leaves of *E. serratus* with potential biological activities, which means that this plant can be considered as a good source of bioactive compounds. However, there are still few studies concerning the chemical characterization of *E. serratus* fruit, as well as their biological activities.

As it is an under-utilized fruit tree, the information about physical, chemical and pharmacological characteristics of the *E. serratus* fruit are important to improve its applicability on the food and pharmaceutical industry, contributing to the development of new products. Thus, this study is aimed to evaluate the physical and chemical composition and the antimicrobial activity of *E. serratus* fruit.

MATERIALS AND METHODS

E. serratus fruit

E. serratus fruit were collected in Dourados, Mato Grosso do Sul, Brazil (Latitude: 22°07'09.04", Longitude: 54°47'55.05"). The fruits were selected to obtain a uniform batch regarding maturity stage, size and absence of injuries, washed with tap water and sanitized with a solution of 0.66% sodium dichloroisocyanurate dihydrate (content of active chlorine 3%).

Fruits biometry and yield

The longitudinal and transversal diameters of 120 fruits were determined with the aid of a digital caliper (Mitutoyo). The mass of the whole fruit, pulp (mesocarp), endocarp and seed was determined in an analytical balance (Shimadzu-AUY220).

Physical and chemical analyses of *E. serratus* pulp

Fresh pulp was characterized as to its chemical composition according to AOAC (1975, 1984, 1997, 2000) methods: moisture content, determined using a gravimetric method in an oven with air circulation, adapted for 70°C and 24 h; total sugars, ash, total lipids, and protein using Micro-Kjedahl method and crude fiber content.

Titratable acidity (TA) was determined according to AOAC (1997), pH by direct reading on a digital pH-meter (L*abmeter*) and water activity (a_w) by direct measurement in a hygrometer Aqualab Series 3.0 (Decagon Devices Inc.). Soluble solids were determined using a manual refractometer (Tecnal).

Phytochemical composition by spectrophotometry

Flavonoids

The flavonoids content was determined in the acetonic extracts of *E. serratus* using the colorimetric method involving the reaction with aluminium chloride (Sigma, St. Louis, USA), as described by Chang et al. (2002). Extracts were prepared with 100 g of sample added in 500 mL of acetone (50% w/v) (Sigma Aldrich, Duque de Caxias, Brazil), and they were kept under constant agitation (150 rpm) for seven days. The sample was filtered and the filtrate was considered the flavonoid extract for analysis.

The extract was reacted with aluminium chloride and the readings were performed in a spectrophotometer (Biochrom – *Libra S60*) adjusted at 415 nm. Quercetin solutions at nine concentrations (0.01 to 0.2 $\mu g.\mu L^{-1}$) were reacted with sodium aluminium chloride in order to construct a standard curve. The results were expressed as milligrams of quercetin equivalent (QE mg.100 g^{-1} sample) using the quercetin (Sigma Aldrich, São Paulo, Brazil) standard curve.

Condensed tannins

The content in tannins was determined in the acetonic extracts of *E. serratus* through the colorimetric method described by Maxson and Rooney (1972). Extracts were prepared with 4 g of sample added in 20 mL of acetone (50% w/v), and they were kept under constant agitation (150 rpm) for seven days. The samples were filtered and the filtrate was considered tannins extract for analysis.

The reaction mixture consisted of 1 mL of the extract with 4 mL of vanillin (Dinâmica, Diadema, Brazil) solution (v/v) (concentrated HCl (Vetec, Duque de Caxias, Brazil)) in methanol (Vetec, Duque de Caxias, Brazil) and 8% vanillin in methanol (4%). The absorbance readings were performed after 20 min at a wavelength of 500 nm, using a spectrophotometer (Biochrom – *Libra S60*). Vanillin solution was used as the blank. The results were expressed as milligrams of catechin equivalent (CE mg.100 g^{-1} of sample) using the catechin (Sigma, St. Louis, USA) standard curve.

Carotenoids content

The fruit samples were weighed (2.5 g), macerated with the aid of Hyflosupercel (0.5 g), and then acetone at 10°C was added until the

extraction of all the pigment; the mixture was vacuum filtered and the extract was collected and transferred to a separating funnel containing 40 mL of petroleum ether (Vetec, Duque de Caxias, Brazil). The mixture was slowly washed with distilled water until complete removal of the acetone. The material was transferred into a volumetric flask and the volume completed with petroleum ether (50 mL). The absorbance readings were performed at 450 nm (Rodriguez-Amaya, 2010) using the conversion factor for β-carotene (2592), the most predominant carotenoid with provitamin A function found in olives. The results are expressed as mg.100 g^{-1} of sample.

Vitamin C content

Vitamin C content was determined by volumetric method with a solution of 2.6-dichlorophenolindophenol (Vetec, Duque de Caxias, Brazil), according to the methodology proposed by AOAC (2000). The results are expressed as mg.100 g^{-1} of sample.

Phytochemical composition chromatography

Preparation of the samples

The samples (2 mg) were fractionated with hexane:ethyl acetate (7:3 v:v) and water in proportion (1:1 v:v). The fraction soluble in hexane:ethyl acetate was analyzed by GC-MS and fraction in water by HPLC.

Gas chromatography analysis (GC-MS)

The GC-MS analysis was performed on a gas chromatograph equipped with a mass spectrometer detector (GC-MS Ultra 2010, Shimadzu Kyoto Japan). The identifications were completed by comparing the mass spectra obtained in the NIST21 and WILEY229 libraries. The compound was confirmed by comparison of standards. Standards of the stigmasterol, β-amyrin, α-amyrin, and β-amyrin acetate (Sigma-Aldrich with purity ≥97%) were prepared in the initial concentration of 500 µg/mL. The concentrations of compounds were determined by extern calibration after dilutions appropriated in the range of 0.1 to 50 µg/mL. The analysis was performed in triplicate.

Liquid chromatography analysis (HPLC)

The HPLC analysis was performed in LC (LC-6AD, Shimadzu, Kyoto, Japan) system with a diode array detector (DAD) monitored at λ = 200-800 nm. Standards of the vanilic acid, ferulic acid, p-coumaric acid, quercetin and kaempferol (Sigma, ≥97%) were prepared in the concentration initial of 1000 µg/mL. The concentrations of compounds were determined by extern calibration after dilutions appropriated in the range of 0.01 to 10 µg/mL. The analysis was performed in triplicate.

Antimicrobial activity

Extract preparation

The ethanolic extract was obtained from mixing 10 g of dried powder material with 500 mL absolute ethanol (LabSynth, Diadema, Brazil), followed by agitation at 200 rpm in shaker at 25°C during 3 h. After this period, the extracts were filtered and the remaining residue of the plant material was extracted as previously. Finally, the residue was washed with 250 mL of ethanol and the filtered extracts combined and evaporated under vacuum at 30°C, resulting in dry extract.

An amount of 40 mg of *E. serratus* dry extract was dissolved in 0.016 g.mL^{-1} ethanolic solution of polyvinylpyrrolidone (PVP) (LabSynth, Diadema, Brazil), according to methodology described by Breda et al. (2016). After dissolution, extract in PVP was evaporated under vacuum at 30°C and an aliquot of 10 mL of Mueller Hinton broth (MHB; HiMedia Laboratories, Mumbai, India) was added to the flask containing dry extract in PVP, resulting in a 4 mg.mL^{-1} solution.

Microorganisms

E. serratus extract was evaluated against the following bacterial strains: *Bacillus cereus* ATCC 10876, *Bacillus subtilis* ATCC6051, *Escherichia coli* ATCC 11775, *Listeria innocua* ATCC 33090, *Pseudomonas aeruginosa* ATCC 13388, *Rhodococcus equi* ATCC6939, *Salmonella cholaresuis* ATCC 10708, *Serratiam arcescens* ATCC 1953, *Staphylococcus aureus* ATCC 6538 and *Xanthomonas campestris* ATCC 13951. All the strains were obtained from the American Type Culture Collection, Manassas, VA, USA and cultivated in Nutrient Agar (NA; HiMedia Laboratories, Mumbai, India) at 37°C for 24 h and maintained at 4°C.

Minimal inhibitory concentration (MIC)

Bacteria strains were grown at 37°C for 24 h in Nutrient Agar (NA) plates. Inoculum for antibacterial assays were prepared by diluting the scraped cell mass in sodium chloride solution 0.9% and adjusted in spectrophotometer Shimadzu UV-Mini 1240 (Shimadzu Co., Kyoto, Japan) to an absorbance between 0.08 and 0.10 at 625 nm (corresponding to 1.5×10^8 UFC.mL^{-1}). These suspensions were diluted to 10^5 UFC.mL^{-1} in MHB.

MICs were performed in tissue test plates (96 wells) containing MHB. Diluted extract was transferred into the first wells (second column) and serial dilutions were performed up to 12th column to obtain concentrations ranging between 1.95 and 2000 µg.mL^{-1}. Then, the bacterial suspensions were transferred into the plates and incubated at 37°C for 24 h. After incubation, 50 µL of 0.5% triphenyltetrazolium chloride (TTC; Vetec, Duque de Caxias, Rio de Janeiro, Brazil) (w/v) solution was added into each well and re-incubated for 2 h. MIC was determined as the lowest concentration of the *E. serratus* extractable to inhibit the development of red color in wells (NCCLS 2003). Chloramphenicol (Sigma-Aldrich, St. Louis, USA) was used as positive control.

Minimal bactericide concentration (MBC)

In order to determine the MBC, an aliquot of 10 µL of each incubated well of MIC and higher concentrations was sub cultured on Petri dishes containing NA and incubated at 37°C for 24 h. MBC was defined as the lowest concentration of extract that allowed no visible growth on the specific solid medium.

To determine the nature of the bactericidal effect of extracts, the MBC: MIC ratio was calculated according to Donlan and Costerton (2002). The extract was considered bactericidal when MBC: MIC ratio was between 1:1 and 2:1 and bacteriostatic when the ratio was higher than 2:1.

Statistical analysis

The results were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by the Tukey test was performed using GraphPad Prism (Version 6.0 – GraphPad Software) in order to evaluate possible differences between groups.

Table 1. Biometry and yields of *E. serratus* fruit.

Evaluated parameter	Mean ± Standard deviation
Longitudinal diameter (mm)	41.55 ± 2.31
Transversal diameter (mm)	27.26 ± 1.47
Whole fruit weight (g)	19.45 ± 2.80
Pulp weight (g)	15.98 ± 0.70
Seed weight (g)	3.48 ± 2.33

RESULTS AND DISCUSSION

Biometry and yield

Biometric features and fruit yield are reported in Table 1. The results showed an average longitudinal diameter of 41.55 mm, average cross-sectional diameter of 27.26 mm and the total weight average 19.45 g, the pulp represent the equivalent of 82.16% of the whole fruit and 17.89% of the weight is a seed. The amount of pulp is an important feature, reflecting in the appreciation of the fruit extractivism. Thus, the higher pulp yield found in this work shows a promising potential for industrial use of *E. serratus* fruits.

Olives fruits produced by *Olea europaea* L. tree are often used for production of oil and canned olives. Their fruits generally show biometric values for longitudinal diameter between 14.17 and 18.15 mm and for transversal diameter between 10.66 and 13.99 mm and an average pulp yield around 83.25% (Nogueira, 2012). Knowledge of biometric values and pulp yield of *E. serratus* allows its use for technological development and use on an industrial scale. According to Machado et al. (2015), biometric characterization enables to evaluate the genetic variation between populations of the same species and their relationships with environmental factors.

Physical and chemical characterization

Physical and chemical evaluations of the olive fruits are presented in Table 2. The physical and chemical evaluation shows acidity value of 1.78 lactic acid.100 g^{-1}, pH of 2.84, water activity of 0.98 and soluble solids with a value of 2.33°Brix. According to Lehkoživová et al. (2009), the found value of pH defines the pulp of *E. serratus* fruit as an acid food (pH < 4.5), which is confirmed by the acidity value.

In chemical assessment (Table 2), the moisture content was found to be 84.62 g.100 g^{-1}, indicating high moisture content characteristic of tropical fruit, which is corroborated by the high value of a_w. The high value of water activity characterizes it as a highly perishable food (Fontana, 1998). The ash content was of 6.1 g.100 g^{-1}, which is consistent with the values of ash of the most land vegetables (5 to 10%, dry weight) (United States Department of Agriculture, 2001). The low total sugar content (3.15 g.100 g^{-1}) was expected, being characteristic of the olive tree fruit, while the lipid constituents (1.10 g.100 g^{-1}) were below those different fruits of the olive tree (International Olive Council, 2015).

The values of protein and crude fiber were 4.92 and 17.50 g.100 g^{-1}, respectively. The protein content is in accordance with other olive species which constituted 2.9 to 5.3 g.100 $g^{-1}$of protein (Nogueira, 2012). Concerning the crude fiber content, *E. serratus* fruit may be considered a good source of fibers. The dietary fiber intake recommendation for adults is >25 g/day (Nishida et al., 2004). In other words, the consumption of 100 g of the pulp from *E. serratus* fruit (approximately 6 fruit) could provide 70% of the necessary fiber's amount for an adult.

Phenolic compounds, carotenoids and Vitamin C

Besides the basic nutrition, the fruit presents in their composition some bioactive compounds that exerts an important role in biological functions for humans, such as chronic diseases prevention and maintenance of immune system (Liu, 2004, 2013). Thus, the quantification of these compounds is of utmost importance and the results of bioactive compounds content found in *E. serratus* fruits are shown in Tables 3, 4 and 5.

According to McClements and Decker (2010), phenolic compounds may be found in plants as simple phenolic, phenolic acids, anthocyanins, cinnamic acid derivatives, flavonoids and tannins, whose structures allows free radicals scavenging activity. In plants, these compounds are believed to be related to protection against phytopathogens or insects (Chen et al., 2013), as well as tannins, which can act as a natural antimicrobial agent, increasing the plant resistance against pathogens (Scalbert, 1991).

As shown in Table 3, *E. serratus* fruits show high amount of flavonoids and condensed tannins, whose values were 120.49 QE mg.100 g^{-1} and 16142.40 CE mg.100 g^{-1}, respectively. This value is in accordance with the values of phenolic compounds found by Machado et al. (2013) in olives cv. Cobrançosa under three irrigation regimes and on three different picking dates.

The carotenoids and vitamin C contents (Table 3) were

Table 2. Physical and chemical characteristics of *E. serratus* pulp.

Evaluated parameter	Mean ± Standard deviation
Titratable acidity (lactic acid.100 g^{-1})	1.78 ± 0.01
pH	2.84 ± 0.01
Water activity	0,98 ± 0.00
Soluble solids (°Brix)	2.33 ± 0.11
Moisture content (g.100g^{-1})	84.62 ± 0.00
Ash (g.100g^{-1})	6.01 ± 1.06
Total Sugars (g.100g^{-1})	3.15 ± 0.05
Lipids (g.100g^{-1})	1.10 ± 0.00
Protein (g.100g^{-1})	4.92 ± 0.17
Crude fiber (g.100g^{-1})	17,50 ±0,65

Table 3. Flavonoids, condensed tannins, carotenoids and vitamin C contents of *E. serratus* pulp.

Evaluated parameter	Mean ± Standard deviation
Flavonoids (mg of QE.100 g^{-1})	120.49 ± 0.01
Condensed tannins (mg of CE.100g^{-1})	16142.40 ± 0.00
Carotenoids (mg.100 g^{-1})	4.97 ± 0.90
Vitamin C (mg.100 g^{-1})	5.93 ± 0.00

Table 4. Compounds identified in *E. serratus* pulp by GC-MS.

Retention time (min)	Compounds	Molecular mass	mg/g ± SD
17.02	Stigmasterol	412	3.6 ± 0.1^{a}
17.93	β-amyrin	426	12.5 ± 0.6^{c}
18.45	α-amyrin	426	4.0 ± 0.2^{b}
19.65	β-amyrin acetate	468	4.3 ± 0.2^{b}

Medium results ± standard deviation. Mean values followed by different letters indicate significant differences ($p < 0.05$).

4.97 mg.100 $g^{-1}$and 5.93 mg.100 g^{-1}, respectively. The presence of carotenoids and vitamin C in fruits is often associated with antioxidant activity. Carotenoids are precursors of vitamin A and can prevent the development of chronic diseases (Singh et al., 2012), whereas the consumption of fruit rich in vitamin C is associated with the prevention of cardiovascular diseases and obesity (González-Molina et al., 2010; Ramful et al., 2011). Thus, the supplement of these nutrients from dietary intake of fruits and vegetables is vital, since the human body is unable to synthesize them (Leong and Oey, 2012).

The gas and liquid chromatography phytochemical composition results are shown in Tables 4 and 5, respectively. The presence of β-amyrin (12.5 mg.g^{-1}), β-amyrin acetate (4.3 mg.g^{-1}), α-amyrin (4.0 mg.g^{-1}) and Stigmasterol (3.6 mg.g^{-1}) were identified and quantified by gas chromatography. The component found in greater quantity was the β-amyrin and α-amyrin. Studies with α-amyrin and β-amyrin indicate its potential as a medicinal agent with hepatoprotective and anti-inflammatory activity (Oliveira et al., 2005; Holanda Pinto et al., 2008).

The high-performance liquid chromatography method in the *E. serratus* pulp extract revealed kaempferol (13.4 mg.g^{-1}) and quercetin (12.9 mg.g^{-1}) as the compounds expressed in greater amounts, followed by vanillic acid, ferulic acid and ρ-coumaric acid. Studies with isolated kaempferol and quercetin demonstrated anti-inflammatory activity in acute and chronic inflammatory processes in experimental models *in vivo*, however, they present lower antimicrobial activity than those of the other phenolics (Guardia et al., 2001; Morikawa et al., 2003; Fattouch et al., 2007).

Antimicrobial activity

Antimicrobial activity of *E. serratus* extract evaluated in the concentration range between 1.95 and 2000 µg.mL^{-1}

Table 5. Compounds identified in *E. serratus* pulp by HPLC.

Retention time (min)	Compounds	Molecular mass	mg/g± SD
7.95	Vanillic acid	168	4.9 ± 0.1^{b}
13.48	p-coumaric acid	164	3.3 ± 0.2^{a}
17.28	Ferulic acid	194	3.6 ± 0.1^{a}
35.33	Quercetin	302	12.9 ± 0.3^{c}
41.43	Kaempferol	286	13.4 ± 0.3^{c}

Medium results ± standard deviation. Mean values followed by different letters indicate significant differences ($p < 0.05$).

Table 6. Antimicrobial activity of ethanolic extract from *E. serratus* pulp.

Microorganism	MIC ($\mu g.mL^{-1}$)	MBC ($\mu g.mL^{-1}$)	MBC:MIC ratio
B. cereus	1600	2000	1,25:1
E. coli	1300	2000	1,5:1
S. cholaresuis	1000	1000	1:1
S. aureus	2000	2000	1:1
X. campestris	500	1000	2:1

was only observed for *B. cereus*, *E. coli*, *S. cholaresuis*, *S. aureus* and *X. campestris*, with MIC values between 500 and 2000 $\mu g.mL^{-1}$ (Table 6). According to Duarte et al. (2005), *E. serratus* pulp extract presented elevated inhibition against *X. campestris*, moderate inhibition for *E. coli* and *S. cholaresuis* and weak inhibition against *B. cereus* and *S. aureus*. Regarding to MBC, the extract showed bactericidal effect for all inhibited microorganisms (Donlan and Costerton, 2002).

The antimicrobial activities of *E. serratus* fruit may be explained by higher content of phenolic compounds, flavonoids and tannins in its composition. Flavonoids are common polyphenolic compounds widely found in edible plants, especially fruit, vegetables, tea and wine and are categorized into several subgroups (Puupponen-Pimiä et al., 2001). According to Ammar et al. (2013), the probable mechanism of flavonoids on antimicrobial activity is due to its properties of complexation with soluble extracellular proteins, resulting in microorganism cell wall break, allowing the inhibition of important enzymatic pathways as P450 oxidases dependents, with specific action in blocking steroid hydroxylases dependents.

Condensed tannins are compounds constituted by oligomeric or polymeric various flavonoid units that consists of two phenolic rings with different vicinities (Çakar et al., 2016). These compounds are responsible for defending plants against insects and pathogens attacks (Haslam, 1988). According to Scalbert (1991), the action mechanism of tannins includes action on membranes, enzyme inhibition, substrate or metal ions deprivation. Some hydrolysable tannins have demonstrated antimicrobial activity.

In conclusion, *E. serratus* fruit has high pulp yield and high moisture content, which justify the feasibility and necessity to obtain a processed product. Moreover, the fruit exhibits in their composition high concentrations of bioactive compounds such as flavonoids, condensed tannins and carotenoids. The chromatographic analysis (GC and HPLC) identified β-amyrin, kaempferol and quercetin as majoritary compounds found in *E. serratus* fruits. Ethanolic extract of *E. serratus* fruit shows antimicrobial activity against microorganisms of importance mainly in food, which is possibly related to the presence of flavonoids, hydrolysable tannins and phenolic compounds.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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