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Artigo

Aroeira (*Schinus terebinthifolius* Raddi) Fruit: Chemical Composition and Antioxidant Capacity

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Fruto da Aroeira (*Schinus terebinthifolius* Raddi): Composição Química e Capacidade Antioxidante

Resumo: A inserção de fontes naturais de compostos bioativos na dieta humana é essencial para a prevenção de diversas doenças degenerativas e crônicas. Desta forma, este estudo caracterizou a composição química e determinou a capacidade antioxidante do fruto da *aroeira* (*Schinus terebinthifolius* Raddi). Os ácidos graxos mais representativos foram os ácidos oleico (34,51 ± 0,50 g/100 g), palmítico (24,53 ± 0,38 g/100 g) e linoleico (17,58 ± 0,09 g/100 g). Fitosteróis como o β-sitosterol, estigmasterol e campesterol foram identificados, apresentando maiores conteúdos de β-sitosterol (166,70 ± 8,44 mg/100 g). O teor de fenólicos totais foi 13,06 ± 0,76 mg AGE/g dry sample. Além disso, o extrato da *aroeira* apresentou potencial antioxidante para os ensaios de DPPH, FRAP e ABTS. Assim, estes resultados destacam os frutos de *Schinus terebinthifolius* Raddi como fonte promovedora de saúde, apresentando componentes funcionais e propriedades antioxidantes.

Palavras-chave: *Schinus terebinthifolius* Raddi; ácidos graxos; fitosteróis; capacidade antioxidante.

Abstract

The insertion of natural sources of bioactive compounds in the human diet is crucial for the prevention of several degenerative and chronic diseases. Thus, this study characterized the chemical composition and determined the antioxidant capacity of *aroeira* (*Schinus terebinthifolius* Raddi) fruit. The most representative fatty acids found were oleic (34.51 ± 0.50 g/100 g), palmitic (24.53 ± 0.38 g/100 g) and linoleic acid (17.58 ± 0. g/100 g). Phytosterols such as β-sitosterol, stigmasterol, and campesterol were identified, presenting greater contents of β-sitosterol (166.70 ± 8.44 mg/100 g). The total phenolics level was 13.06 ± 0.76 mg GAE/g. In addition, *aroeira* extract showed antioxidant potential in DPPH, FRAP, and ABTS assays. Thus, these results suggest *Schinus terebinthifolius* Raddi fruit as a health-promoting source that presents functional components and antioxidant properties.

Keywords: *Schinus terebinthifolius* Raddi; fatty acids; phytosterols; antioxidant capacity.

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Aroeira (*Schinus terebinthifolius* Raddi) Fruit: Chemical Composition and Antioxidant Capacity

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1. Introduction

Recently consumers' demand for natural and nutritive food has increased since they are more concerned about their healthy lifestyle habits. Bioactive compounds found in plants play an important role in human health, being associated to the prevention of several diseases.[1,2] Thus, health-promoting components, which are widely available in the native Brazilian flora, must be studied and exploited.[3]

Aroeira (*Schinus terebinthifolius* Raddi) is a member of the Anacardiaceae family and native to South America, being extensively found in Brazil, where it is popularly known as "*aroeira*", "*pimenta rosa*", "*aroeira pimenteira*", "*pimenta brasileira*" or "*aroeira da praia*". *Aroeira* tree presents a great potential for exploration and use; while its fruits are commonly used as spice in culinary dishes, leaves and bark are traditionally applied by the folk medicine.[4,5]

Antioxidant, antimicrobial, anti-inflammatory, antihypertensive, and anticancer potentials are attributed to *aroeira*, and its rich composition in bioactive compounds justifies its nutritional and pharmacological properties.[6] Although the phytochemical composition depends on various factors, the presence of secondary metabolites, especially phenolic compounds, flavonoids and terpenoids have been described in the literature, highlighting *aroeira* antioxidant capacity.[4,7-9] These antioxidant components may provide protection against harmful free radicals and have been associated with lower incidence and mortality rates of cancer, heart and other degenerative diseases.[10-12]

In addition, the consumption of fatty acids and phytosterols naturally occurring in food of vegetal origin has also been demonstrating benefits to human health. Phytosterols may influence cholesterol absorption and reduce its level in blood serum, exhibiting a hypocholesterolemic function.[13] Fatty acids regulate a variety of physiological and biological functions, such lipids may present a cholesterol-lowering effect and have a structural role in the brain, improving the cognitive development, for example.[14,15]

In this context, it is important to characterize the composition and antioxidant capacity of *aroeira* fruit. Thus, this study aimed to perform a detailed characterization of *Schinus terebinthifolius* Raddi fruit by means of proximate composition and functional lipids content. The total phenolics and antioxidant capacity were also determined.

2. Material and Methods

2.1. Chemicals

Gallic acid, ((±)-6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid) (Trolox), 2,4,6-tris-2,4,6-tripyridyl-2-triazine (TPTZ) and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) were obtained from Alfa Aesar (Ward Hill, MA, USA). 2,2'-azinobis (3-ethyl-benzo-thiazolione-6-sulphonate) (ABTS) was purchased from Fluka Chemie (Buchs, Switzerland). Tri-Sill reagent was acquired from Pierce (Rockford, IL, U.S.A.). The standard mixtures of fatty acids were obtained from Supelco TM 37 (FAME Mix 18919, Bellefonte, Pa., U.S.A.). Phytosterol standards, including Brassicasterol, Campesterol, Stigmasterol and β-Sitosterol, and undecanoic methyl ester were acquired from Sigma-Aldrich (St. Louis, MO, USA), as well as Sodium methoxide. HPLC grade n-hexane was purchased from Mscience (Darmstadt, Germany), and all other analytical grade solvents were obtained from Vetec (Sigma, São Paulo, Brazil). The purities of the standards ranged from 95 % to 99 %.

2.2. Samples

Aroeira fruits (*Schinus terebinthifolius* Raddi) were obtained from a local farm located in Seropédica, Rio de Janeiro, Brazil (22º44'38'' S, 43°42'27'' W, and 26 m), in May 2013. The identification of plant material was authenticated by the staff of the Department of Botany, Institute of Biology, and a voucher specimen was deposited in the Herbarium of the UFRRJ under the number RBR 15557.

The fruits were manually collected at the ripe stage, free from any physical and microbial damage. Then, they were dried in a ventilated oven (Solab, SP, Brazil) at 30 °C for 24 hours and ground in a domestic processor (Walita, Brazil). Subsequently, the samples were stored in polyethylene bags at room temperature in the dark until analyses.

2.3. Proximate composition

The protein content was accessed by the macro-Kjeldahl method, using the conversion factor of 6.25. The total fat level was determined by extracting a known weight of powdered sample with petroleum ether, using a Soxhlet apparatus. The ash content was obtained by incinerating the samples in a muffle furnace at 600 °C. Total carbohydrate was calculated "by difference", using the formula: 100 – (% moisture + % lipids + % protein + % ash). Total energy was estimated by the following formula: Energy (Kcal) = 4 x (g protein + g carbohydrate) + 9 x (g lipid).[16] The total fiber content was determined according to the procedures described by Adolfo Lutz Institute.[17]

2.4. Fatty acids composition

The samples (25 mg) were converted into methyl esters by transesterification.[18] The fatty acids were determined using a gas chromatograph (Shimadzu GC 2010, Tokyo, Japan), equipped with a split injector (1:50), flame ionization detector and a workstation. The chromatographic separation was achieved in a fused silica CP-SIL 88 capillary column (50 m × 0.25 mm i.d., 0.20 µm film thickness) (Chrompack, Middelburg, The Netherlands). The chromatographic conditions were: initial temperature 100 °C (5 minute) followed by 5 °C/minute up to 160 °C (zero minute), 8 °C/minute up to 230 °C (12 minute). The injector and detector temperatures were 250°C and 280 °C, respectively. The equipment used hydrogen as the carrier gas at a flow rate of 1 mL/minute. In order to identify the chromatographic peaks of the sample, the retention times of FAME standards were used, and the quantification was performed by internal standardization, with undecanoic methyl ester as the internal standard.

2.5. Phytosterols

Phytosterols were obtained from direct saponification of samples (1 g of sample, 4 mL of a 50 % ethanol solution of KOH and 6 mL of ethanol) at 20 °C for 22 h in the dark and the nonsaponifiable matter was extract 4 times with hexane.[19] The samples and standards were derivatized to thrimethylsilyl (TMS) ethers.[20] The TMS derivates were diluted with 1 mL of hexane, filtered through a 22 µm filter (Millipore, Maryland, MD, USA), and injected into a gas chromatograph (Shimadzu GC 2010, Tokyo, Japan), equipped with a split injector (1:20), a flame ionization detector, and a workstation. The TMS-ether derivates of sterols were separated in a capillary column Rtx-5-MS (30m x 0.25mm x 0.25 µm, *Restek,* Bellefonte, USA). The oven program was: initial temperature, 230 °C (0 min); a heating rate of 2 °C/min to a temperature of 264 °C (5 min); and then a heating rate of 1 °C/min to a final temperature of 275 °C (2 min). The injector temperature was 290 °C and detector 350 °C. The carrier gas was hydrogen at a flow rate of 1 mL/min. Identification was done by comparison with the retention times of phytosterols standards. Quantification was done by external standardization with concentrations ranging from 0.05 to 1.0 mg/mL.

2.6. Preparation of *aroeira* fruit extract

The extract of *Schinus terebinthifolius* Raddi fruits was prepared at a concentration of 10 mg/mL in acetone: ethanol: water solution (40:30:30). The samples were submitted to agitation for one hour in the dark.[21]

2.7. Determination of total phenolics

The total phenolic contents were obtained with the Folin-Ciocalteu reagent.[22] Aliquots of

7 mL of deionized water were mixed with 0.5 mL of the extract and 0.5 mL of Folin-Ciocalteu reagent. After three minutes, 2 mL of 20 % sodium carbonate solution was added to the mixture. Then, the mixture was heated in a water bath at 100 °C for one minute. The absorbance was measured at 685 nm using a spectrophotometer (Model NOVA 2000 UV). A calibration curve of standard gallic acid (from 5 to 50 µg/mL, r>0.99) was prepared and the results were expressed as mg gallic acid equivalent (GAE) per g of sample (dry basis).

2.8. DPPH free radical scavenging assay

Antioxidant capacity was determined by scavenging the radical 2,2-diphenyl-1-picryhydrazyl (DPPH) as described by Rufino *et al*.[23] A methanol solution containing 0.06 mM DPPH was prepared. Then, 0.1 mL of extract was added to 3.9 mL of DPPH solution. The mixture was homogenized and after 60 min the absorbance was measured at 517 nm using a spectrophotometer (Model NOVA 2000 UV). The quantification was performed by external standardization with a calibration curve of Trolox ranging from 100 to 2000 µM/mL (r>0.98). Antioxidant capacity was expressed as the percentage of DPPH radical-scavenging activity (% DPPH), which was calculated as follows: %DPPH = (A_0- A) / A_0) x 100, where A_0 is the absorbance of DPPH (control) and A is the absorbance of each sample added with DPPH.

2.9. ABTS assay

Aliquots of 30 µL of extracts were added to 3 mL of radical cation ABTS+ solution, formed by the chemical reaction with potassium persulfate. The absorbance was determined at 734 nm after 6 min at 30 °C using a spectrophotometer (Model NOVA 2000 UV).[23] The free radical scavenging ability was expressed as µmol of Trolox Equivalent (TE) /g of sample. The quantification was performed by external standardization with a calibration curve of Trolox ranging from 100 to 2000 µM/mL.

2.10. FRAP assay

Frap reagent was prepared by diluting an aqueous solution of 20 mM TPTZ (2,4,6-tripyridyl-s-triazine) and 20 mM ferric chloride and 0.3 M sodium acetate buffer (pH 3.6) at a ratio of 1:1:10 (v:v:v). The reaction was started by adding 90 µL of the extract to 270 µL of distilled water and 2.7 mL of the FRAP reagent. The mixture was heated at 30 °C for 30 min.[23] The absorbance was measured at 595 nm using a spectrophotometer (Model NOVA 2000 UV). The free radical scavenging ability was expressed as µmol of Fe^{+2}/g of the sample. The quantification was performed by external standardization using a calibration curve with concentrations ranging from 300 to 2000 µM/mL (r>0.99).

2.11. Statistical analysis

The analyses were done in triplicate and the results were expressed as the mean ± standard deviation.

3. Results and Discussion

3.1. Proximate composition

The proximate composition of *aroeira* is presented in Table 1. The moisture level determined was 13.54 ± 0.02 g/100 g of sample (fresh weight). Previous studies found values ranging from 12.09 to 29.04 %.[24-27]

Lipid and protein amounts were 15.49 ± 0.76 and 8.06 ± 0.26 g/100 g, respectively. A lower protein content of 7.1 g/100 g was determined[24], while a level of 8.4 g/100 g was found for lipids.[28] Carbohydrates showed quantities of 59.20 ± 0.58 g/100 g, being the

highest calorie contributor. A content of 17.96 ± 0.04 g/100 g was found for fibers. The ash level was 3.71 ± 0.15 g/100 g, it agrees with values previously obtained.[24,27.] A total energy of 391 ± 2.26 kcal/ 100 g dry basis was observed. The differences in the proximate composition previously reported for *aroeira* may be due the use of different cultivars in analysis, as well as growing condition.

3.2. Fatty acids composition

A total of 10 main fatty acids were identified and quantified in samples (Table 1). Oleic acid (C18:1*n*9*c*), palmitic acid (C16:0) and linoleic acid (C18:2*n*6*c*) were predominant, with contents as follows: 34.51 ± 0.50 g/100 g (oleic acid), 24.53 ± 0.38 g/100 g (palmitic acid) and 17.58 ± 0.09 g/100 g (linoleic acid), respectively.

Same fatty acids were determined as principal in *Schinus terebinthifolius* Raddi fruit; however, linoleic acid was found to be the most representative one.[29] Saturated fatty acids such as palmitic, stearic, eicosanoic, heneicosanoic, and docosanoic acids were identified in ethanolic extract of *aroeira* fruit, with higher contents for stearic and palmitic acids, as it was observed in this study.[30]

The fatty acid percentages decreased in the order of saturated (SFA, 36.77 g/100 g) > monounsaturated (MUFA, 36.52 g/100 g) > polyunsaturated (PUFA, 19.16 g/100 g). Thus, *aroeira* fruit presented a PUFA/SFA ratio of 0.52.

Fatty acids are crucial elements on human health. The main fatty acid of *aroeira* samples, oleic acid, reduces the levels of total cholesterol, triglycerides, low density proteins (LDL), and high density proteins (HDL).[31] Linoleic acid, the main *n*-6 PUFA identified in this study, presents potential anti-inflammatory, anticarcinogenic, antiadipogenic, antidiabetic, and antihypertensive functions.[32]

Representing the *n*-3 group, eicosapentaenoic (C20:5*n*3, 0.92 ± 0.03 g/100 g) and α-linolenic (C18:3*n*3, 0.13 ± 0.04 g/100 g) were found in *aroeira*. Besides the cardiovascular protective effect of eicosapentaenoic acid, it exerts a relevant impact on arthritis, hypertension, cancer and other inflammatory and autoimmune disorders.[14,15] α-linolenic, occurring primarily in plants, also provides potential cardiovascular benefits when ingested.[33]

3.3. Phytosterols

Phytosterols such as β-sitosterol, stigmasterol, and campesterol were identified and quantified in *Schinus terebinthifolius* Raddi samples (Table 2). The main phytosterol found was β-sitosterol, with a level of 166.70 ± 4.44 mg/100 g. The amounts of stigmasterol and campesterol were 26.09 ± 0.36 and 3.58 ± 0.44 mg/100 g, respectively. Thus, *aroeira* samples presented a total phytosterol content of 196.60 ± 5.00 mg/100 g.

To our knowledge, no studies were found available in the literature regarding *aroeira* phytosterols profile for comparison. Wang *et al.* studied the phytosterol profile and found total contents varying from 150.4 to 1230.9, 129.6 to 275.6, 18.9 to 255.2 and 11.9 to 93.8 mg/100 g in vegetable oil, legumes, nuts and cereals, respectively.[34] Considering theses values, *aroeira* may be considered a good source of such components.

β-sitosterol, stigmasterol, and campesterol were also determined in red pepper (*Capsicum annuum* L.) fruits. In comparison with *aroeira*, lower quantities of β-sitosterol were determined (from to 9.85 to 10.7 mg/100 g), while campesterol presented results in the same range (from 2.49 to 2.70 mg/100 g).[35] Kim *et al*. performed a study to evaluate the phytosterol content of 5 pepper fruit cultivars and observed stigmasterol levels of 10.44, 4.52 and 10.76 mg/100 g for green pepper (Put pepper), red pepper (Hong

pepper) and violet pepper (Gaji pepper), respectively.[36]

Phytosterols naturally occurring in food from vegetal origin has been demonstrating several benefits to human health. They may influence cholesterol absorption and reduce cholesterol levels in blood. Rich phytosterol diets may also contribute for the reduction of cancers development in 20 %.[13] Thus, due to the difficulty of obtaining phytosterols in diets based on low consumption of foods from vegetal origin, natural sources as *aroeira* may be applied as active ingredients in many food products and dishes, improving their nutritious value.

3.4. Determination of total phenolic contents and antioxidant properties

The results obtained for the total phenolics and the antioxidant capacities determined by DPPH, ABTS and FRAP assays are shown in Table 3. The total phenolic content was 13.06 ± 0.09 mg GAE/g of dry sample.

Previous studies found higher contents for *aroeira* fruit, with values of 20.13 mg GAE/g[28] and about 270 mg GAE/g.[29] Other authors demonstrated that experimental conditions applied during extraction direct affect results of total phenolics using the Folin-Ciocalteu reagent. *Aroeira* extracts obtained by Soxhlet showed an amount of 5.44 mg GAE/g of extract, while the same sample presented a level nearly 20 times higher when the extract was obtained by maceration.[37] By evaluating different methods (ultrasound-assisted extraction, soxhlet, supercritical fluid extraction) and solvents (ethyl acetate, ethanol, hexane), contents ranging from 2.9 ± 0.4 to 60 ± 1.0 mg GAE/g extract were observed.[9]

Besides the methodology, solvent and experimental condition applied to obtain a natural extract, several factors such as cultivar, origin and geographical growing location, growing conditions, seasonal variations, and storage conditions may influence plants composition, resulting in the great diversity of results available in the literature.

Regarding the antioxidant capacity, *Schinus terebinthifolius* Raddi extract was evaluated by 3 distinct methods, DPPH, FRAP and ABTS (Table 3). Due to the complexity of natural extracts composition, when a single assay is employed to determine the antioxidant capacity it is not possible to achieve a consistent result that considers the action of all groups of antioxidant compounds present in the matrix. Thus, different methods should be applied to obtain more accurate results, since different methods present different experimental conditions and principles.[38]

The DPPH assay exhibited inhibition of 42.93 ± 3.40 %. In other scientific studies, *aroeira* ethanolic extracts were analyzed showing inhibition percentage of 35.58[26], 53[39] and 60 %,[37] while methanolic extracts presented antioxidant activity of 95.6 %.[8] Results in the same range were found for red pepper (*Capsicum annuum* L.), with DPPH percentages varying from approximately 43 to 48 %.[40]

Aroeira fruit also exhibited antioxidant capacity by ABTS and FRAP assays, presenting values of 348.20 ± 3.07 µmol TE/g dry sample and 189.58 ± 2.39 µmol Fe^{2+}/g dry sample, respectively. Other authors evaluated *aroeira* by FRAP and ABTS assays, demonstrating its antioxidant potential by these methods. FRAP was employed to analyze ethanolic extracts of *aroeira* leaves.[41] Da Silva *et al*. found percentages of inhibition varying from 63.21 to 90.41 % in methanolic extracts of leaves analyzed by the ABTS assay.[42] Essential oil of *aroeira* fruit also exhibited antioxidant potential when ABTS assay was applied by Bendaoud *et al*.[43]

Considering other species, Rufino *et al*. evaluated 18 Brazilian tropical fruits using FRAP assay and found values hanging from 16.1 to 2502 µmol Fe^{2+}/g (dry basis). In comparison with the results obtained in our study, puçá-preto (*Mouriri pusa*), camu-camu (*Myrciaria dubia*) and acerola (*Malpighia*

emarginata) presented higher results, while lower ones were observed for cajá (*Spondias mombin*), umbu (*Spondias tuberosa*) and carnaúba (*Copernicia prunifera*). When evaluated by the ABTS method, the fruits ranged from 16.4 to 1237 µmol TE/g (dry matter).[23]

Lower values were observed by Kim *et al*. when applying ABTS assay for *Capsicum annuum* L.extracts with results varying from 24.34 to 66.42 µmol TE/g dry sample.[44] Different genotypes of pepper (*Capsicum sp.)* also showed lower ABTS values, hanging from 46.79 to 113.08 µmol TE/g.[45] Jabuticaba (*Myrciaria jaboticaba*) and jussara (*Euterpe edulis*) fruits presented lower values when evaluated by FRAP.[46]

4. Conclusion

The presence of phytosterols (campesterol, stigmasterol, and β-sitosterol) and fatty acids in *Schinus terebinthifolius* Raddi samples offers established beneficial health effects. Overall, *aroeira* fruit exhibited an antioxidant capacity in DPPH, FRAP and ABTS assays, as well as phenolic compounds content. Thus, the findings of the present study support the valorization and exploitation of *Schinus terebinthifolius* Raddi fruit, suggesting its use as a natural source of natural antioxidants and other health-promoting compounds by the food industry, since it may improve the nutritional quality of products and provide good advantages for consumer health.

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