

Evaluation of different solvents for extraction of total phenolic compounds in flour of *Byrsonima coccolobifolia* Kunth. fruit

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Abstract

The present work aimed to evaluate different solvents for the extraction of total phenolic compounds from murici-bravo fruit flours. Flours from the husk, pulp and pyrene with seeds were produced. The extracting solvents were: water, 100, 80 and 70% ethanol and 100, 80 and 70% methanol. To determine the amount of total phenolics, *Folin-Ciocalteu* reagent was used. The total phenolic contents for husk flour were 19.19 mg GAE 100 g⁻¹ for 70% ethanol solution, followed by 18.91 80% ethanol solution and 18.72 mg GAE 100 g⁻¹ for 100% methanol; the farinaceous extracts of the pulp showed better yields in 100% ethanol 19.08 and water 19.04 mg EAG 100 g⁻¹ followed by 80% ethanol and 70% methanol solutions equal to 18.80 and 18.70 mg GAE 100 g⁻¹, and for the farinaceous extracts of pyrene and seeds with 17.54 for 80% methanolic solution and 17.10 mg EAG 100 g⁻¹ for water, followed by 16.70 for 70% ethanolic solution and 16.56 mg GAE 100 g⁻¹ for 100% methanol. The solvents applied in this study showed satisfactory results in the extraction of phenolic compounds. However, the use of less aggressive solvents such as water and ethanol proved to be excellent options for use, since methanol presents toxicity in the environment.

Keywords: *Byrsonima*, *Cerradão*, Pyrene, *Folin-Ciocalteu*

Resumo

O presente trabalho teve por objetivo avaliar diferentes solventes visando à extração de compostos fenólicos totais das farinhas do fruto de murici-bravo. Farinhas da casca, polpa e pirênio com sementes foram produzidas. Os solventes extratores foram: água, etanol 100, 80 e 70% e metanol 100, 80 e 70%. Para a determinação dos quantitativos de fenólicos totais, foi utilizado reagente de *Folin-Ciocalteu*. Os teores de fenólicos totais para farinha da casca foram 19,19 mg EAG 100 g⁻¹ para solução etanol 70%, seguido de 18,91 solução de etanol 80% e 18,72 mg EAG 100 g⁻¹ para metanol 100%; os extratos farináceos da polpa apresentou melhores rendimentos em etanol 100% 19,08 e água 19,04 mg EAG 100 g⁻¹ seguidos pelas soluções de etanol 80% e metanol 70% iguais a 18,80 e 18,70 mg EAG 100 g⁻¹, e para os extratos farináceos do pirênio e sementes com 17,54 para solução metanólica 80% e 17,10 mg EAG 100 g⁻¹ para água, seguidas de 16,70 para solução etanólica 70% e de 16,56 mg EAG 100 g⁻¹ para metanol 100%. Os solventes aplicados neste estudo apresentaram resultados satisfatórios na extração dos compostos fenólicos. Contudo, o uso de solventes menos agressivos como a água e o etanol apresentou ser excelentes opções de uso, visto que, o metanol apresenta toxicidade no ambiente.

Palavras-chave: *Byrsonima*, *Cerradão*, Pirênio, *Folin-Ciocalteu*

Resumen

El presente trabajo tuvo como objetivo evaluar diferentes solventes para la extracción de compuestos fenólicos totales a partir de harinas de frutos de murici-bravo. Se produjeron harinas a partir de la cáscara, pulpa y pireno con semillas. Los solventes de extracción fueron: agua, etanol al 100, 80 y 70% y metanol al 100, 80 y 70%.

Para determinar la cantidad de fenoles totales se utilizó el reactivo de *Folin-Ciocalteu*. Los contenidos fenólicos totales para la harina de cáscara fueron 19,19 mg EAG 100 g⁻¹ para solución de etanol al 70%, seguido de 18,91 solución de etanol al 80% y 18,72 mg EAG 100 g⁻¹ para metanol al 100%; los extractos farináceos de pulpa presentaron mejores rendimientos en etanol 100% 19,08 y agua 19,04 mg EAG 100 g⁻¹ seguido de soluciones en etanol 80% y metanol 70% igual a 18,80 y 18,70 mg EAG 100 g⁻¹, y para los extractos farináceos de pireno y semillas con 17,54 para solución metanólica al 80% y 17,10 mg EAG 100 g⁻¹ para agua, seguido de 16,70 para solución etanólica al 70% y 16,56 mg EAG 100 g⁻¹ para metanol al 100%. Los solventes aplicados en este estudio mostraron resultados satisfactorios en la extracción de compuestos fenólicos. Sin embargo, el uso de solventes menos agresivos como el agua y el etanol resultaron ser excelentes opciones de uso, ya que el metanol presenta toxicidad en el medio ambiente.

Palabras clave: *Byrsonima*, *Cerradão*, Pireno, *Folin-Ciocalteu*

1. Introduction

The “murici-bravo” (*Byrsonima coccolobifolia*) belongs to the Malpighiaceae family, which is found in the American continent, where it has about 63 genera and around 1,100 species. The genus *Byrsonima* is popularly known as muricis, murici-pequeno, muricizinho and murici-rasteiro, being widely found in some variants of the Cerrado domain, such as in Campos Cerrado, Cerradão, Cerrado Ralo and tropical savannas (Neves et al., 2015; Benezar; Pessoni, 2006), and some species of muricis are also found in the Amazon biome (Cavalcante, 1991).

The species *Byrsonima coccolobifolia* can also be found cohabiting other regions of Brazil, as well as in Bolivia, Venezuela and Guyana (Pott & Pott, 1994). In the Cerrado domain, this species has an average size and can reach about 5 meters in height, the flowering period comprises between the months of September to October and the end of the months of November to December. It is considered a woody plant species (Nezes et al., 2015; Barros, 1992).

In Goiás state, this species of murici is still little used, possibly due to the strongly astringent taste that the fruits provide or due to the lack of knowledge of the population, being only appreciated by birds and rodents. In a study carried out by Resende-Moreira et al. (2017) on the dispersion of the species of *B. coccolobifolia*, individuals with identical genetic characteristics in distant areas were observed in several areas. In the state of Roraima, however, some indigenous tribes and local communities use the fruits of *B. coccolobifolia* in the artisanal preparation of juices, liqueurs, jams and preserves (Sannomiya et al., 2004).

The fruits of *B. coccolobifolia* have compounds rich in carbohydrates, organic acids, antioxidant and total phenolic activities in considerable levels, being a thriving plant species for numerous researches aimed at the use of the fruits in various food and pharmaceutical processes in the production of new foods and herbal medicines. with bioactive characteristics in topical lines, and for internal use (Menezes; Castro, 2018).

Today, the food industry seeks to produce new formulations based on natural products, aiming to satisfy the taste of consumers who are looking for natural products that have significant amounts of minerals, vitamins, sugars, compounds with antifungal and antibacterial activity, as well as compounds that fight free radicals (antioxidants), this in a functional food (Martínez et al., 2012). The use of flour from fruits, vegetables, vegetables and waste has been gaining market not only in Brazil, but in the world, flour products can be incorporated in the production of breads, snacks and nutritious bars.

In bakery, the usual wheat flour has been replaced in significant percentages by flour from products that would previously be discarded without the correct reuse these flours, are called "mixed", being also used in the food industry in the production of the infant to adult food line. Fernández-López et al. (2008), complements on the exploration of the use of exotic and native fruits being incorporated in food formulations, thus producing products with desirable characteristics presenting different nutritional and technological properties.

This study aimed to investigate different solvents for a better extraction of the total phenolic compounds in the murici-bravo (*Byrsonima coccolobifolia*) fruit flour in order to improve the extraction process and correct and effective targeting to obtain maximum production quantities.

2. Materials and Methods

B. coccolobifolia fruits were collected in the permanent preservation area located at the University of Rio Verde, Goiás state, Brazil, with the following geographic coordinate: 17°47'12.2"S and 50°58'00.0' W. This species was classified by the first author of this study, and an exsiccate was herborized and deposited in the Herbarium of the

laboratory of Plant Systematics at the Goiano Federal Institute with the following Voucher number. HRV 10040.

The fruits of *B. coccolobifolia* were collected and taken to the Technological Chemistry laboratory, where they were washed in running water and left to dry on paper towels. Soon after, the fruits with sometype of attack by phytopathogens were discarded. To separate the peel, pulp and pyrene with seeds, a semi-industrial pulper from the Fruits and Vegetables laboratory was used.

After pulping, the material was packed in high-density polyethylene (HDPE) molds and taken to an oven with forced air circulation at 60 °C for 48 hours. The material after drying was ground in a cyclonetype knife mill with an internal 32 mesh sieve. The flours produced received the following names: Bark Flour (BF), Pulp Flour (PF) and Pyrene with Seed Flour (PSF), as described by Menezes Filho et al.(2018) adapted.

The procedure for extracting the total phenolic compounds was determined according to the methodology proposed by Filho et al. (2017) adapted. About 100 mg of flour was added with 150 mL of an extractor solution. Soon after, the solution was homogenized on an orbital shaker table at 170 rpm for 1 hour. After this time, the solution was filtered through qualitative filter paper (weight 80 g, 110 mm, diameter 18.5 cm, thickness 0.16 mm), with a filtration speed between 20 and 25 s. Then the supernatant was centrifuged at 3000 rpm for 20 min. The supernatant was collected and stored in amber flasks kept in a refrigerator at 8 °C until analysis.

Different types of extracting solvents were evaluated: water, ethanol (P.A-ACS), 80% aqueous ethanol solution (v/v) (Filho et al., 2017; Vieira et al., 2011), 70% aqueous ethanol solution (v/v) (Filho et al., 2017; Koolen et al., 2013); methanol (P.A-ACS), 80% methanol solution (v/v) (Filho et al., 2017; Barreto et al., 2009), 70% methanol solution (Filho et al., 2017; Koolen et al., 2013).

The content of total phenolic compounds was determined in different extracting solvents, as described by Filho et al. (2017) and Cândido et al. (2015) with adaptations. An aliquot of 250 µL of extract was added to a test tube with 250 µL of an aqueous solution of *Folin-Ciocalteu* reagent in the proportion (1:9). Immediately afterwards about 2.5 mL of distilled water was added to the tube. The sample was homogenized in Vortex-type equipment for 1 min. and left to rest for 5 minutes. Then an aliquot of 250 µL of an aqueous solution of anhydrous sodium carbonate 7.5% (prepared one day before use). Thesolution was left to rest in a dark place for 1 h.

Readings were performed in a UV-Vis spectrophotometer at wavelengths at 725 nm. A standard curve of gallic acid was prepared at concentrations starting from 20, 80, 120, 160, 200, 240, 280, 320, 360, 400 to 420 mg L⁻¹. For the instrumental blank, distilled water was used as described by Menezes Filho et al. (2018) adapted. Results were determined and expressed as mg GAE 100 g⁻¹ of flour.

Analyzes were performed in triplicate with ± SD, by means of analysis of variance (ANOVA) and comparison between means by Tukey's test ($p < 0.05$) of significance. Statistical data were evaluated by thePAST 3 Software (free version, 3.21, 2018).

3. Results and Discussion

Table 1 describes the results of the quantitative contents of total phenolic compounds extracted from *B. coccolobifolia* fruit flours in different extracting solvents.

Table 1. Determination of the total phenolic compounds content of *Byrsonima coccolobifolia* fruit flours.

Solvent	Content of total phenolic compounds (mg GAE 100 g ⁻¹)*		
	BF	PF	PSF
Water	17.96 ± 0.54c	19.04 ± 0.40a	15.20 ± 0.18c
Ethanol 100%	18.65 ± 0.45bc	19.08 ± 0.17a	16.38 ± 0.22b
Ethanol 80%	18.91 ± 0.12b	18.80 ± 0.34b	17.10 ± 0.18a
Ethanol 70%	19.19 ± 0.04a	18.19 ± 0.08c	16.70 ± 0.31ba
Methanol 100%	18.72 ± 0.27b	18.12 ± 0.11c	16.56 ± 0.15ba
Methanol 80%	18.15 ± 0.48cb	17.37 ± 0.41d	17.54 ± 0.39a
Methanol 70%	16.83 ± 0.28dc	18.70 ± 0.33ba	16.06 ± 0.25b

Note: BF = Bark Flour. PF = Pulp Flour. PSF = Pyrene with Seed Flour. * mg of GAE 100 g⁻¹ expressed in gallic acid. * F = Flour. Equal letters in the same column show no statistical difference according to Tukey's test ($p <$

0.05).

Phenolic compounds act as free radical scavengers in the body, these reactive radical species include groups such as, O_2^- , OH, NO., as well as neutral intermediates or with charges H_2O , ONOO⁻ and among others such as 1O_2 , O_3 , Fe and Cu (Oliveira et al., 2009). For the BF the results with the highest amounts of total phenolics extracted were, in the ethanolic extract 70% showing better extraction results with 19.19 mg GAE 100 g⁻¹, showing a significant difference a ($p < 0.05$), followed by 80% ethanolic solutions with 18.91 and 100% methanolic solutions with 18.72 mg GAE 100 g⁻¹, which showed no significant difference a ($p < 0.05$). For the farinaceous extracts of the PF the best extraction results were for the solvents, water and 100% ethanol of 19.04 and 19.08 mg GAE 100 g⁻¹, with no significant difference at ($p < 0.05$), followed by 80% ethanol and 70% methanol with 18.80 and 18.70 mg Gae 100 g⁻¹, which showed no significant difference between them and a significant difference between the first group a ($p < 0.05$).

In the farinaceous extracts of PSF, the extracting solutions 80% methanol and 80% ethanol showed extractives equal to 17.54 and 17.10 mg GAE 100 g⁻¹ of phenolic compounds, respectively, with no significant difference in both. Then, extracting solutions composed of 100% ethanol and 70% methanol, which showed extractives equal to 16.38 and 16.06 mg GAE 100 g⁻¹, did not show significant difference between them a ($p < 0.05$).

Among the extracting solvents, 70% ethanol and 100% methanol for PSF showed amounts of phenolics equal to 16.70 and 16.56 mg GAE 100 g⁻¹ where there was no significant difference between group (b), and significant difference between group (a) to ($p < 0.05$).

The amounts of total phenolics, even being below those verified comparing flours from other fruits, still present satisfactory results when compared to the results obtained by Neves et al. (2015) using the in natura pulp of *B. coccolobifolia* where they obtained a result equal to 2,308.84 mg GAE 100 g⁻¹. Studies carried out by Resende et al. (2019), evaluated the flours of the fruit of *Mauritia flexuosa* (buriti), in extracting solutions composed of 50% methanol and 70% acetone, where they obtained for rind, unbleached rind, bleached endocarp, manually produced bran and lipid-free pulp bran results of 934.6; 785.1; 114.9; 93.2; 676.8 and 740.1 mg GAE 100 g⁻¹ respectively.

Menezes Filho et al. (2019) found a higher extraction efficiency for *Muntingia calabura* flour using a 70% ethanolic solution with a total phenol content equal to 18.13 mg GAE 100 g⁻¹. Leão et al. (2017) found results similar to those of this study for flours, from the epicarp and mesocarp of the pequi fruit (*Caryocar brasiliense*) equal to 17.42 and 15.49 mg GAE 100 g⁻¹. Castro et al. (2017) found levels varying between 5.60; 4.55 and 4.42 mg GAE 100 g⁻¹ in aqueous extracts of taro flour obtained by differential heat treatment at 70, 80 and 90 °C, being very low when compared to those observed in this study for murici-bravo. Arbos et al. (2013) found 80% of ethanol extracts from mango peel and almond flour extracts equal to 6,644.68 and 3,123.13 mg expressed in catechin 100 g⁻¹. Costa et al. (2012) found for the methanolic extracts 50% of the jatobá-do-cerrado (*Hymenaea stigonocarpa*) and pequi (*C. brasiliense*) flours had levels of 108.91 and 167.66 mg expressed in 100 g⁻¹ tannic acid.

The flours showed considerable amounts of total phenolic compounds, even after the thermal convection process where some compounds reduce their contents. However, it can still be observed that the levels obtained in this study are satisfactory even after the farinaceous manufacturing process (Martínez et al., 2012). Some authors discuss the lack of methodologies in the process of drying fruits, vegetables, vegetables and residues for the production of flour, complementing that at high temperatures the levels of chemical compounds such as antioxidants and phenolics are lost, mainly in the bleaching process (Resende et al., 2019; Abu-Ghannam; Jaiswal, 2015).

4. Conclusions

The results obtained in this study for the quantitative contents of total phenolic compounds in flours from the fruit of *Byrsonima coccolobifolia*, evaluating different extracting solvents, are unprecedented for this species so little studied. The bark, pulp and pyrene with seed flours from *B. coccolobifolia*, showed satisfactory amounts of important phenolic compounds as anti-free radical agents.

The solvents used in the extraction showed good efficiency, especially for the ethanol solvent. Methanol also proved to be a good extracting solvent, however due to its toxicity, this solvent has restrictions mainly for human and animal use, not being an option for these purposes.

It is worth mentioning that the importance of evaluating phytochemical compounds in fruit species from the Cerrado domain is of great relevance to Science since the results obtained can compose important data for the use of these compounds by the food and pharmaceutical industries in the elaboration of new products, which aim to use natural compounds extracted from plant species, thus guaranteeing the preservation and genetic maintenance of this domain.

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