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SCIENTIFIC NOTE

Phenolic profiles of faveleira (*Cnidoscolus quercifolius* Pohl) seed and press cake extracts: potential for a new trend in functional food

Perfil fenólico dos extratos da torta e da semente de faveleira (Cnidoscolus quercifolius Pohl*): potencial para nova tendência em alimento funcional*

Penha Patrícia Cabral Ribeiro[1], Francisco Canindé de Sousa Júnior[2], Cristiane Fernandes de Assis[2], Bruno Oliveira de Veras[3], Carlos Eduardo de Araújo Padilha[4], Thayza Christina Montenegro Stamford[5], Karla Suzanne Florentino da Silva Chaves Damasceno[6]*

[1]Universidade Federal de Pernambuco (UFPE), Departamento de Nutrição, Recife/PE - Brasil
[2]Universidade Federal do Rio Grande do Norte (UFRN), Departamento de Farmácia, Natal/RN - Brasil
[3]Universidade Federal de Pernambuco, Departamento de Bioquímica, Recife/PE - Brasil
[4]Universidade Federal do Rio Grande do Norte (UFRN), Departamento Engenharia Química, Natal/RN - Brasil
[5]Universidade Federal de Pernambuco (UFPE), Departamento Medicina Tropical, Recife/PE - Brasil
[6]Universidade Federal do Rio Grande do Norte (UFRN), Departamento de Nutrição, Natal/RN - Brasil

***Corresponding Author:** Karla Suzanne Florentino da Silva Chaves Damasceno, Universidade Federal do Rio Grande do Norte (UFRN), Av. Senador Salgado Filho, 3000, Lagoa Nova, CEP: 59078970. Natal/RN - Brasil, e-mail: karlasuzanne@yahoo.com.br

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Abstract

The objective of the present study was to assess the phenolic compounds and antioxidant capacity of faveleira seed and press cake extracts. Phenolic profiles were assessed by Ultra-High Performance Liquid Chromatography (UHPLC). Furthermore, the Total Phenolic Content (TPC) and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging were evaluated. The faveleira seed and press cake extracts are sources of natural phenolic compounds in human diet and have potent antioxidant activity. Gallic acid was the predominant phenolic compound in seed and press cake extracts. The study showed that faveleira seed and press cake extracts can be considered functional foods as well as a potential interest to the food industry.

Keywords: Phenolic compound; Antioxidant activity; Bioactivity; Oilseed; By-product; Waste.

Resumo

O objetivo do presente estudo foi avaliar os compostos fenólicos e a capacidade antioxidante da semente e da torta da semente de faveleira. Os perfis fenólicos foram avaliados por cromatografia líquida de ultra alta eficiência. Além disso, o conteúdo fenólico total e a eliminação do radical DPPH (2,2-diphenyl-1-picrylhydrazyl) foram avaliados.

A torta e a semente de faveleira são fontes de compostos fenólicos naturais para dieta humana e possuem potente atividade antioxidante. O ácido gálico foi o composto fenólico predominante na semente e na torta. O estudo mostrou que a torta e a semente de faveleira podem ser consideradas alimentos funcionais e de interesse potencial para a indústria de alimentos.

Palavras-chave: Composto fenólico; Atividade antioxidante; Bioatividade; Oleaginosa; Subproduto; Resíduo.

1 Introduction

Increasing evidence shows that natural foods containing phenolic compounds can exert protective effects against major diseases. This characteristic is a consequence of the various biological activities of the compounds including their antioxidant properties. Due to safety and restrictions related to the use of synthetic antioxidants, natural antioxidants found in edible sources and also by-products are alternative sources that have increased interest (Shahidi & Ambigaipalan, 2015).

The faveleira (*Cnidoscolus quercifolius* Pohl or *C. phyllacanthus* (Mull. Arg.) Pax & L. Hoffm.) is a xerophile species belonging to the Euphorbiaceae family (Fagg et al., 2015) that occurs in arid and semi-arid environments (Silva et al., 2014), even in periods of prolonged drought, contributing to the balance of the ecosystem (Roberto et al., 2016). The seeds of this plant have been consumed for a long time, but its industrial application and technological potential have not yet been fully explored (Ribeiro et al., 2017). It is already known that faveleira seed has a high nutritional value, with proteins and lipids as major components, being considered as an oilseed (Medeiros et al., 2018). Therefore, the seed can be eaten *in natura*, in other words, in its natural form and added to cakes and cookies (Santos et al., 2017), in addition to being a potential source of edible oil (Moura et al., 2019).

The extraction of oil generates considerable amounts of a by-product known as press cake (Kaur et al., 2018). The use of waste is an opportunity for the development of new food products, improving aspects related to nutritional parameters and food safety, and reducing environmental impacts (Silva et al., 2019). Previous studies have shown that faveleira seeds and press cake extracts contain high antioxidant activity and Total Phenolic Content (TPC) (Ribeiro et al., 2017), however, phenolic compounds have not yet been identified and quantified individually.

To the best of our knowledge, this is the first report in the literature concerning the phenolic profiles of faveleira seed and press cake extracts. Thus, the objective of present study was to evaluate the phenolic profiles, TPC and antioxidant capacity of faveleira seed and press cake extracts.

2 Material and methods

2.1 Sample collection and preparation

The faveleira fruits were harvested from the city of *São José do Seridó* situated in the state of Rio Grande do Norte (RN) in Brazil. A voucher specimen was deposited in the Herbarium of *Universidade Federal do Rio Grande do Norte* (UFRN) (reference number 20064). The fruits were collected between March and April 2017 before dehiscence and were mixed into a single batch. The seeds (0.5 kg total) were manually extracted from the fruits. Seeds were pressed by a hydraulic press (MARCON, MPH-10, Marilia, Brazil) at approximately 20°C.

2.2 Preparation of extracts

The seeds and press cake extracts were ground in a blender (Walita, Sao Paulo, Brazil) and the Seed Extracts (SE) and Press Cake Extracts (PCE) were obtained according to Ribeiro et al. (2017). In 0.5 g of seed, 20 mL of methanol/water solution (50:50, v/v) were added. The mixture was homogenized at room temperature (20–23°C) for 1 h and centrifuged (Fanem, Excelsa 4, 280 R, São Paulo, Brazil) at 2,500 g for 10 min at 20°C. The supernatant was separated and 20 mL of acetone/water solution (70:30, v/v) were added to the residue. The mixture was homogenized and centrifuged under the same conditions described above. The supernatants were mixed and the SE was obtained. The same procedure was used to obtain the PCE and both extracts had a final concentration of 12.5 mg/mL.

2.3 Phenolic profiles

Phenolic profiles of SE and PCE were assessed by Ultra-High Performance Liquid Chromatography (UHPLC) as described by Kim et al. (2013) with some modifications. The phenolic compounds of the extracts were quantified using a Thermo scientific reversed-phase UHPLC (Waltham, USA) equipped with a quaternary pump, an autosampler, and a Diode Array Detector (DAD). The phenolic standards and samples were filtered through a nylon organic membrane (0.22 µm) prior to injection. The data were processed using ChromQuest 5.0 (Thermo Scientific, Waltham, USA). Separation was performed using a Shim-pack CLC-ODS (M) C18 column (250 × 4.6 mm; Shimadzu, Kyoto, Japan) maintained at 30 °C. The mobile phase consisted of 1% acetic acid (A) and acetonitrile (B). The column was eluted with a gradient: 0-30% (B) over 0-10 min, 30-70% (B) over 10-15 min, 70-100% (B) over 15-20 min, and 100% (B) over 20-25 min. The flow rate was 1.0 mL/min and the injection volume was 10 µL. The detection wavelengths were optimized according to the maximum absorption wavelengths of the reference compounds, such as: gallic acid, syringic acid, catechin, vanillin, eugenol and vanillic acid. These compounds were detected at 280 nm; whereas ellagic acid and quercetin were detected at 256 nm. Phenolic compounds were quantified based on the retention time and absorbance spectra of the extracts measured with DAD by comparison with phenolic reference standards. An external calibration curve was constructed for each standard. The values were expressed in microgram of phenolic compound per gram of sample.

2.4 Determination of Total Phenolic Content (TPC)

The TPC was evaluated using the Folin–Ciocalteu method as described by Wong et al. (2006) with some modifications. Briefly, 20 µL of each of the extracts was mixed with 100 µL of freshly prepared Folin–Ciocalteu reagent diluted with water (1:10). After 3 min, 80 µL of sodium carbonate solution (75 g/L) was added. After 2 hours, the absorbance was measured at 735 nm with a spectrophotometer (BioTek µQuant Biospectro, Winooski, USA). The TPC was expressed as milligrams of gallic acid equivalent per 100 gram of sample (mg GAE/100 g) using a standard curve constructed with different concentrations of gallic acid (20-200 µg/mL).

2.5 Antioxidant activity

The antioxidant activities of the seed and press cake extracts were evaluated by DPPH·, known formally as 2,2-diphenyl-1-picrylhydrazyl, used to measure the radical scavenging based on the Blois (1958) method. The results were expressed as a percentage of DPPH• inhibition using the following Equation 1:

$$\%\ inhibition\ of\ DPPH\bullet = \left[\left(Absorbance\ control - Absorbance\ sample\right) / \ Absorbance\ control\right] \times 100 \quad (1)$$

2.6 Statistical analysis

The results were tested for normality using the Shapiro-Wilk test and statistical significance was evaluated by t-test ($p < 0.05$) using GraphPad Prism software version 6 (San Diego, USA).

3 Results and discussion

3.1 Phenolic profiles

The faveleira seed and press cake extracts contained high levels of gallic acid (peak 1 in Figure 1A and B, respectively) that exceeded the levels of other phenolic compounds (Table 1). This phenolic acid is a natural compound with various kinds of biological and pharmacological activities including antioxidant, anti-inflammatory, antimicrobial, anticancer, and antifibrotic (Hsieh et al., 2017). Additionally, gallic acid can be used in the food industry. Roidoung et al. (2016) found that gallic acid can be used in the production of juices in order to preserve endogenous anthocyanins and the red color of a product. The content of gallic acid in the faveleira seed is higher than the levels detected in various types of grape seed (67-91 µg/g) (Farhadi et al., 2016) and that content found in the press cake is higher than the observed by Yang et al. (2019) in the by-product obtained after the extraction of soybean oil (15.81 µg/g).

Table 1. Phenolic profiles of faveleira seed and press cake extracts.

Phenolic compound	SE (µg/g ± SD)	PCE (µg/g ± SD)	p-value SE vs PCE
Gallic acid	76908.74 ± 1778.69[a]	72273.13 ± 1140.26[b]	0.0256
Syringic acid	1.91 ± 0.19[b]	4.16 ± 0.62[a]	0.0206
Ellagic acid	0.32 ± 0.10[a]	0.27 ± 0.01[a]	> 0.9999
Catechin	10.06 ± 1.53[a]	11.88 ± 1.02[a]	0.1789
Quercetin	12.71 ± 2.67[a]	0.40 ± 0.08[b]	0.0152
Vanillin	14.43 ± 1.08[a]	2.09 ± 0.08[b]	0.0025
Eugenol	1.28 ± 0.93[a]	1.91 ± 0.68[a]	0.3959
Vanillic acid	6.41 ± 1.23[a]	6.07 ± 0.46[a]	0.6961

SE: Seed Extract; PCE: Press Cake Extract. [a,b]Different lowercase letters correspond to differences between the SE vs PCE (t test, $p < 0.05$).

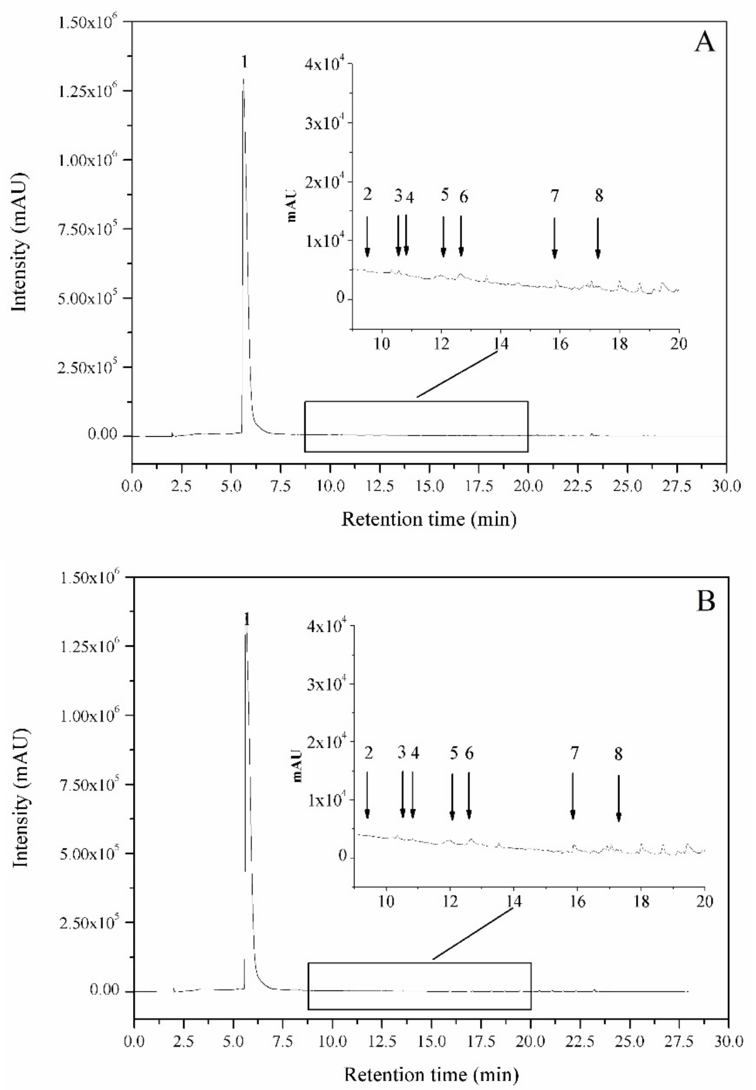

Figure 1. [Chromatogram of the phenolic compounds in SE (A) and PCE (B). Peaks: 1 - Gallic acid, 2 - Catechin, 3 - Vanillic acid, 4 - Syringic acid, 5 - Ellagic acid, 6 - Vanillin, 7 - Quercetin, 8 - Eugenol]

In the SE, concerning the vanillin and quercetin contents, peak 6 and 7 in Figure 1A, respectively, were the most abundant phenolic compounds after gallic acid. The contents of these compounds in the seed were higher than their levels in the press cake. This may result from their higher solubility in the apolar systems (Shakeel et al., 2016). Vanillin is a phenolic compound characterized by an attractive aroma and can be used in aromatherapy. The inhalation of vanillin has muscle relaxant and antinociceptive effects (Ueno et al., 2019). The faveleira seed has a high amount of vanillin when compared to different *Randia monantha* Benth seed extracts (0.0-8.7 µg/g, Juárez-Trujillo et al., 2018).

Quercetin has anti-inflammatory effect and decreases insulin resistance and oxidative stress, and can therefore be used by patients with Type 2 Diabetes (T2D) and metabolic syndrome (Abdelkarem & Fadda, 2017). The faveleira seed presented quercetin contents similar to the average found in *Polygonum equisetiforme* Sm. seeds from different locations (12.38 µg/g, Mahmoudi et al., 2018).

In the PCE, it could also be highlighted the amount of catechin, phenolic compound with anti-inflammatory, anti-oxidant and antibacterial activities (Ma et al., 2019). However, the amount found in faveleira press cake was less than that observed in different cultivars of walnut press cake (38-126 mg/kg, Ojeda-Amador et al., 2018).

3.2 Total phenolic content and antioxidant activity

The TPC for faveleira press cake (456.63 ± 15.86 mg GAE/100 g) was higher ($p = 0.0054$) than the level in the seed (378.14 ± 18.52 mg GAE/100 g). This characteristic is due to the polar structure of several phenolic compounds. Other authors have also observed this behavior when comparing flaxseed (Mannucci et al., 2019) and walnut (Ojeda-Amador et al., 2018) press cake and seed.

The TPCs found in the seed and press cake in this study were slightly higher than levels detected in the previous study (324.92 mg GAE/100 g seed and 398.89 mg GAE/100 g press cake) (Ribeiro et al., 2017) probably due to different harvesting periods and different conditions of analysis.

Compared to other species, the species of faveleira stood out because its seed had a higher TPC than flaxseed (138.6 mg GAE/100 g, Mannucci et al., 2019) and its press cake had a higher TPC than by-product obtained after the extraction of soybean oil (approximately 275 mg GAE/100 g, Chi & Cho, 2016).

Higher total phenolic levels in the press cake corresponded to higher antioxidant activity. The inhibition percentage of DPPH• in the SE and PCE were 81.53 ± 1.80% and 96.63 ± 1.62%, respectively. The DPPH• scavenging activity is routinely used for more rapid evaluation of antioxidant activity compared to other methods (Hossain et al., 2017). The higher inhibition found in PCE may be due to the lower lipid content of the press cake because lipids can interfere in the analysis of antioxidant activity. In addition, most phenolic compounds, which have antioxidant activity, are polar compounds and could be retained in the press cake (Ribeiro et al., 2017).

The antioxidant activity found in the faveleira seed was also higher than that observed in *Cassia absus* L. seed (10-48%) analyzed by Ahmad et al. (2019) and hemp seed (40%, Frassinetti et al., 2018). Moreover, the press cake analyzed in the present study had higher capacity to sequester the DPPH radical than the peanut press cake (approximately 75%, Sadh et al., 2018) and different pecan nut press cake extracts (12.55-74.11%, Maciel et al., 2020).

4 Conclusion

This study showed that PCE and SE of faveleira contained high levels of gallic acid and TPC, in addition to having ability to sequester the DPPH radical. Therefore, the faveleira SE and PCE had the potential to be consumed as bioactive food and used by the food industry. However, further studies that analyze toxicity are needed.

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