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Chemical composition, antioxidant activity and thermal analysis of oil extracted from favela (*Cnidoscolus quercifolius*) seeds

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ABSTRACT

Favela (*Cnidoscolus quercifolius*) is a plant native to the Brazilian Caatinga biome that is extremely tolerant to drought. Although favela has seeds that are rich in oil, being considered as a potential alternative for edible oil production, little information has been found regarding its characterization. In this context, this study aimed to determine the fatty acid profile of the oil extracted from favela seeds and its tocopherol, phytosterol and total phenolic contents. Moreover, its antioxidant potential and thermal and oxidative stability were also determined. The results showed that the seeds had 35.20% lipid, consisting mainly of unsaturated fatty acids, notably linoleic acid (54.39%) and oleic acid (20.13%). The α-, δ- and γ-tocopherols were present at 0.87, 3.15 and 15.09 mg 100 g_{oil}^{-1}, respectively. The phytosterol, β-sitosterol, was found at 127.98 mg 100 g_{oil}^{-1}, while phenolic compounds were detected at 23.88 mg gallic acid equivalent 100 g_{oil}^{-1}. The oil presented high antioxidant activity, decreasing DPPH radicals by 76.68%, which was equivalent to 3.83 mmol Trolox kg_{oil}^{-1} by the ABTS assay. Thermogravimetric analysis showed that the triglycerides started degrading at 188 °C. The oil showed high oxidative stability and an induction time of 265.3 min at 110 °C.

1. Introduction

Favela (*Cnidoscolus quercifolius* Pohl or *Cnidoscolus phyllacanthus*) belongs to the family Euphorbiaceae (Fagg et al., 2015). This plant occurs throughout the semi-arid Caatinga regions of Brazil (Oliveira et al., 2008) and is well recognized for its high drought tolerance. The tree has a height of 2–12 m (Melo and Sales, 2008) with stinging trichomes distributed over the plant. White sap (latex) is produced throughout its length. Its leaves are simple and thick, ranging from 8 to 16 cm (Maia, 2004). The inflorescences are composed of small, white flowers (Maia-Silva et al., 2012), and its fruits are capsules that contain oilseeds measuring 1.5–2.0 cm in length, covered with stinging hairs. Favela seeds are gray-brown, ovoid, rigid and smooth, similar to the fruits of castor bean (*Ricinus communis* L.), which belongs to the same family (Drumond et al., 2007).

Due to its many uses, favela is a species of great importance for the development of the Brazilian semi-arid region. Its dissemination is high, and the plant shows full adaptation to the conditions of the area (Neto et al., 2009). According to Ribeiro and Brito (2010), favela trees can be used for reforestation, restoration of degraded areas, animal and human consumption, medicine, wood and energy production. For instance, animals feed on the leaves, branches, fruits, roots, buds and bark of favela (Cavalcanti and Bora, 2010; Oliveira et al., 2008), while the fresh seeds, flour and oil are consumed by humans. Medeiros et al. (2013) assessed the effects of favela, sesame (*Sesamum indicum* L.) and castor oils on the nutritional and sensory quality of goat milk and cheese. The authors noted that the inclusion of favela oil had no effect on the cheese composition but significantly increased the unsaturated fatty acids. As a medication, favela is used in semi-arid areas of Paraiba, Brazil. In particular, its bark is used to heal human and animal wounds, and its latex is used against toothache (Oliveira et al., 2011).

Albuquerque et al. (2007) state that the plants of the Brazilian Caatinga, such as the favela, have potential phytochemical and pharmacological activities. The authors report that favela leaves and flowers are therapeutically indicated for cancer, hepatic problems, tumors and uterine inflammation. Its stem, bark, roots and latex are suggested for hemorrhoids, renal problems, ophthalmic diseases, blow, injury, fractures, warts, skin problems, eye cleansing, urinary infection and inflammation of the uterus, ovaries

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and prostate. More recently, Paredes et al. (2016) reported the extracts of favela leaves, roots and root bark displayed antimicrobial potential, antioxidant effects and inhibitory action against acetylcholinesterase comparable with the reference agent.

Favela seeds are rich in edible oil and protein. The oil extracted from the seeds contains high levels (approximately 41%) of unsaturated fatty acids, mainly linoleic acid (C18:2n6) (Santos et al., 2005). Thus, favela oil could be an alternative to similar edible oils, such as sunflower (*Heliantus annuus* L.) oil, corn (*Zea mays* L.) oil (Cavalcanti et al., 2012) and soybean (*Glycine max* L.) oil (Silva et al., 2014). In addition to its oil, favela bran, which is rich in minerals and proteins, can also be used after its extraction (Arriel et al., 2005).

Even though favela is identified as a species with oil production potential (Harand et al., 2016; Silva et al., 2014), a complete characterization of its oil has not been studied, since the works found in the literature basically report the oil content of the seeds and its fatty acid composition. Thus, this study aimed to determine the fatty acid profile of the oil extracted from favela seeds, as well as the composition of tocopherols, phytosterols and total phenolic content. Moreover, this study aimed to assess the oil for its antioxidant capacity and thermal and oxidative stabilities.

2. Material and methods

2.1. Favela seeds (Cnidoscolus quercifolius)

The seeds of favela (thorned variety) were collected in a rural property located in Juazeiro, Brazil. The naturally dried seeds were ground in a domestic blender, and their particle size was classified by sieving (16–48 Tyler mesh size). The material used in the experiments consisted of 59, 19 and 22% of the mass retained in the 20–28, 28–32 and 32–48 mesh sieves, respectively.

2.2. Centesimal composition

The moisture, lipid, ash and protein contents of the ground favela seeds were determined according to the Official Methods of Analysis (AOAC, 2012). The determinations were performed in triplicate and expressed as mean values and standard deviation.

The lipid amount was determined by Soxhlet extraction, using 2 g of sample and petroleum ether. The moisture content was obtained by drying 2 g of sample at 105 °C to constant weight, while the ash content was determined by heating the sample dried in the oven at 550 °C for 4 h. Crude protein was determined by the Kjeldahl method using a factor of 6.25 for total nitrogen conversion into protein.

2.3. Oil extraction

The oil from 10 g of the ground material was extracted with *n*-hexane using a Soxhlet apparatus for 6 h. The solvent was then removed from the oil using a rotary evaporator (IKA, RV 10 Control). Finally, the oil was placed in an amber glass vessel and oven-dried at 60 °C to completely remove residual solvent. The oil was stored under refrigeration at approximately 4 °C.

2.4. Oil characterization

2.4.1. Total fatty acids

The total fatty acids were analyzed by gas chromatography (Thermo Scientific, Trace 1310) using a capillary column (Thermo Scientific, TR-BD (F)), 30 m × 0.25 mm × 0.25 μm). An initial temperature of 50 °C was maintained for 2 min, then increased to 180 °C at 15 °C min^{-1}, remaining at this temperature for 5 min and then increased up to 240 °C at 5 °C min^{-1}. The flow of carrier gas (N_2) was 1.2 mL min^{-1}. The injector and detector temperature were 220 °C and 260 °C, respectively, and the injection volume of the sample was 1 μL, in split mode (1:40). The methyl esters were prepared according to the AOCS Ce 2-66 method (2009). The fatty acids were identification by comparing their retention times with those of authentic standards (C4 – C24 Sigma – Aldrich) analyzed under the same chromatographic conditions.

2.4.2. Tocopherols

The α-, γ- and δ-tocopherols in favela oil (20 mg mL^{-1} in isopropanol) were analyzed in a high-performance liquid chromatograph (LC-20AT, with UV–vis SPD-20A detector, both by Shimadzu) equipped with a Phenomenex Kinetex C18 column (4.6 mm × 250 mm × 5 μm). A 20 μL loop was used. The mobile phase (methanol:water 96:4) flow rate was 1 mL min^{-1} in isocratic mode, and the wavelength was 292 nm, according to the methodology employed by Santos et al. (2015).

Calibration curves of α-, γ- and δ-tocopherol standards (Sigma–Aldrich) were quantified at 0.25–10 mg L^{-1}.

2.4.3. Phytosterols

Free glycerol compounds (free fatty acids and phytosterols) were derivatized with *N,O*-bis(trimethylsilyl)trifluoroacetamide and trimethylchlorosilane (BSTFA + TMCS, Sigma–Aldrich), as described by Garcia et al. (2012) and Santos et al. (2015). Twenty μL of BSTFA + TMCS was added to 20 mg of oil, and the mixture was kept at 60 °C for 30 min. Then, 80 μL (3470 mg L^{-1}) of 5α-cholestane (Sigma–Aldrich), was added as the internal standard, and the solution made up to 1 mL with heptane.

The analyzis were performed on a gas chromatograph coupled to a mass spectrometer (GCMS, model QP2010SE, Shimadzu) using a Shimadzu RTx-5MS capillary column (30 m × 0.25 mm × 0.25 μm). The initial temperature was set at 100 °C, which was maintained for 6 min, then increased to 230 °C at 5 °C min^{-1} and finally, to 280 °C at 15 °C min^{-1}, where it remained for 15 min. The flow rate of the carrier gas (helium) was 1 mL min^{-1}. The temperature of the injector and detector was 280 °C, and 1.0 μL of sample was injected in split mode (1:10). The compounds were identified by comparing their mass spectra with those in the National Institute of Standards and Technology (NIST) library.

2.4.4. Total phenolic content (TPC)

The total phenolic content was determined according to the method proposed by a Szydłowska-Czerniak et al. (2008), with modifications. Approximately 500 mg of oil was dissolved in 1.5 mL of *n*-hexane and then extracted with methanol (3 × 1 mL with stirring for 2 min). The mixture was incubated for 16 h in the dark. The methanolic extract was washed with 2.5 mL of *n*-hexane, and 1 mL was then transferred to a test-tube for reaction with the Folin-Ciocalteu reagent (0.5 mL), according to the method of Haiyan et al. (2007). The TPC was calculated as gallic acid equivalents (GAE) from the gallic acid (Vetec) standard curve (5–60 mg L^{-1}). The assay was done in triplicate, and the results expressed as mg GAE 100 g^{-1} of oil.

2.4.5. Antioxidant activity

According to the methodology used by Rezig et al. (2012), 4 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma–Aldrich) in ethyl acetate (10^{-4} M) was added to 1 mL of the oil in ethyl acetate (0.1 mg mL^{-1}). After vortexing (Kasvi, K45-2810), the mixture was kept in the dark for 30 min and its absorbance was then measured (515 nm) using ethyl acetate as a blank. The control sample comprised 1 mL of ethyl acetate and 4 mL of the DPPH solution. The