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Physicochemical profile of the oil from the seed of *Tontelea micrantha* (Celastraceae)

A.R.B. Araujo [a], V.A. Royo [b], M.O. Mercadante-Simões [c,*], F.S.A. Fonseca [d], V.P. Ferraz [e], D.A. Oliveira [f], E.V. Menezes [f], A.F. Melo Júnior [f], M.M. Brandão [f]

[a] Programa de Pós Graduação em Biotecnologia, Universidade Estadual de Montes Claros, Montes Claros, Minas Gerais, Brazil
[b] Laboratório de Produtos Naturais, Universidade Estadual de Montes Claros, Montes Claros, Minas Gerais, Brazil
[c] Laboratório de Anatomia Vegetal, Universidade Estadual de Montes Claros, Montes Claros, Minas Gerais, Brazil
[d] Laboratório de Plantas Medicinas, Instituto de Ciências Agrárias, Universidade Federal de Minas Gerais, Montes Claros, Minas Gerais, Brazil
[e] Laboratório de Cromatografia, Departamento de Química, Universidade Federal de Minas Gerais, Campus Belho Horizonte, MG 31270-901, Brazil
[f] Laboratório de Bioprospecção em Recursos Genéticos, Universidade Estadual de Montes Claros, Montes Claros, Minas Gerais, Brazil

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ABSTRACT

The oil from the seed of *Tontelea micrantha* is utilized in traditional medicine as a topical antiseptic and as an anti-inflammatory for the treatment of respiratory diseases. The dynamic of the oil secretion in seed tissues was analyzed by transmission electron microscopy, classes of secondary compounds were identified by histochemical and phytochemical tests, the quality of the oil was determined by physical and chemical analysis, the fatty acid profile was obtained, and the volatile compounds were extracted by headspace method and identified by gas chromatography coupled to a mass spectrometer. The oil production takes place during the final stages of seed maturation, and large vacuoles are involved in its secretion and accumulation. Flavonoids, tannins and alkaloids, linoleic and oleic acids, α-pinene and junipene are present in the oil. Several of these compounds are known to have biological activity and may support the traditional medicinal use of the species.

1. Introduction

Tontelea micrantha (Mart. ex Schult.) A.C.Sm. is a native species from the Brazilian Savannah. The crude oil of the seeds is used as a wound-healing balm, an anti-inflammatory agent, and for the treatment of respiratory diseases. Major pharmaceutical industries acquire the oil for the manufacture of anti-inflammatory drugs (Dias and Laureano, 2010).

Bioactive compounds protect plants against herbivores and microorganisms (Aguiar et al., 2015; Alvarenga et al., 2015), and the location of these compounds in plants has ecological importance. The presence of volatile compounds in peripheral tissues of plants favor their relationship with the biotic components of the environment, such as pollinators and seed dispersers (Gersbach et al., 2001; Kalachanis and Psaras, 2005).

Knowing the dynamics of secretion of these compounds within the cell allows us to define the best time to collect the plant material. This is important to obtain the highest yield of extraction of the chemical compound in the preparation of herbal medicines (Reis et al., 2012; Mercadante-Simões and Paiva, 2013; Mazzottini-dos-Santos et al., 2015).

Polyunsaturated fatty acids promote beneficial health effects. The olleic and linolenic acids have anti-inflammatory action and control microbial growth (Rodrigues et al., 2012). Some volatile compounds are known to have antimicrobial, analgesic and anti-inflammatory effect. These properties are confirmed for some species of Celastraceae in the treatment of gastric disorders (Yariwake et al., 2005; Mariot and Barbieri, 2007; Pessuto et al., 2009).

Despite the medicinal, ecological, and social importance of the oil from *T. micrantha* seed there is no study on it, and the aim of this work is (1) to identify and locate secondary compounds in the seed tissue, and determine the moment of its accumulation in the secretory cell, (2) assess the quality of the oil extracted from the seed, and (3) detect the presence of chemicals with known bioactivity in the oil.

2. Methodology

2.1. Plant material

The plant material was composed of seeds from 10 individuals in a natural population of *Tontelea micrantha* growing in the Cerrado (savanna) vegetation in the municipality of Montes Claros, state of Minas Gerais, Brazil. The seeds were collected for about a month before the ripening of the fruit (November) and ripe fruit (December). Voucher material was deposited in the BHCB herbarium of the Departamento de

* Corresponding author.
E-mail address: omercadante@hotmail.com (M.O. Mercadante-Simões).

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Botânica of the Instituto de Ciências Biológicas of the Universidade Federal de Minas Gerais (Mercadante-Simões 2; registry number 214463; identified by Dr. Julio Lombardi). The seed oil was extracted by a Cooperative of artisan producers, using a mechanical press at ambient temperature, from seeds collected within a single population.

2.2. Transmission electron microscopy

Seed fragments (1 mm^2 area and 0.5 mm thick) were fixed in Karnovsky solution (Karnovsky, 1965), post-fixed in 1% osmium tetroxide (in 0.1 m L^{-1} sodium phosphate buffer, pH 7.2) for 2 h, dehydrated in an acetone series, and embedded in Araldite resin (Huntsman Advanced Materials, Montreal, Switzerland). Ultrathin sections (50 nm) were stained with uranyl acetate and lead citrate (Roland, 1978) and examined in a Tecnai G2-12 – Spirit Biotwin FEI transmission electron microscope (Philips/FEI Company, Eindhoven, Netherlands) at 120 kV.

2.3. Histochemical analyses

Histochemical tests were performed on hand-cut transversal sections of the seeds using the following reagents: NADI (α-naftol and dimethyl-p-phenylene diamine dihydrochloride) to identify terpenoids (David and Carde, 1964), DMACA (p-dimethyl amino cinnamaldehyde and caffeine) for flavonoids (Feucht et al., 2008), vanillin-hydrochloric acid (Mace and Howell, 1974) for tannins, and Ellram's reagent for alkaloids (Furr and Mahlberg, 1981). Controls were performed simultaneously according to the author recommendations.

2.4. Phytochemical analyses

Phytochemical analyses were performed on crude seed oil by placing a small drop on a glass slide (using a 1 mm diameter glass capillary tube) and freezing it (−10 °C) to adhere to the slide. The same tests for phytochemical analysis were performed (Royo et al., 2015). The digital photographic documentation of the phytochemical and histochemical analyses was performed using a AxioCamMRc camera coupled to a AxioVision LE light microscope (Zeiss, Oberkochen, Germany).

2.5. Physicochemical analyses

Seed humidity was determined using the gravimetric method. Measures of total ashes, pH, density, acidity, refractive index, and saponification and peroxide levels were performed following the recommendations of the AOAC (Association of Official Analytical Chemists) (Helrich, 1990). All of the analyses were performed in triplicate.

2.6. Fatty acid profiles

For the analysis of fatty acids profile, an oil sample (12 mg) was trans-esterified by dissolving it in an ethanol solution of KOH (100 μL, 1 mol L^{-1}) followed by vortexing (10 s), and heating in a microwave oven (Panasonic Piccolo, Manaus, Brazil) (at 80 W for 5 min). After cooling, the oil (1000 μL) was dissolved in a solution containing HCl (400 μL, 20%), $NaCl_{(s)}$ (20 mg), and ethylene acetate (600 μL) was vortexing (10 s), and left standing (5 min). Aliquots (300 μL) of the organic layer were removed and placed separately in micro centrifuge tubes and dried by evaporation. The free fatty acids were methylated in a methanol solution of BF_3 (100 μL, 14%) under heating in a water bath (10 min, 60 °C) (Christie, 1989).

2.6.1. Gas chromatography coupled to a flame ionization detector (CG-FID)

CG-FID chromatographic profiles were obtained using a HP7820A gas chromatograph (Agilent Technologies, Santa Clara, United States) equipped with a SP2560 column, 30 m × 0.25 mm × 0.20 μm (Supelco, Pennsylvania, USA), with injection (1/50 split, 1 μL) at 250 °C with hydrogen as the carrier gas (2 mL min^{-1}). The temperature gradient

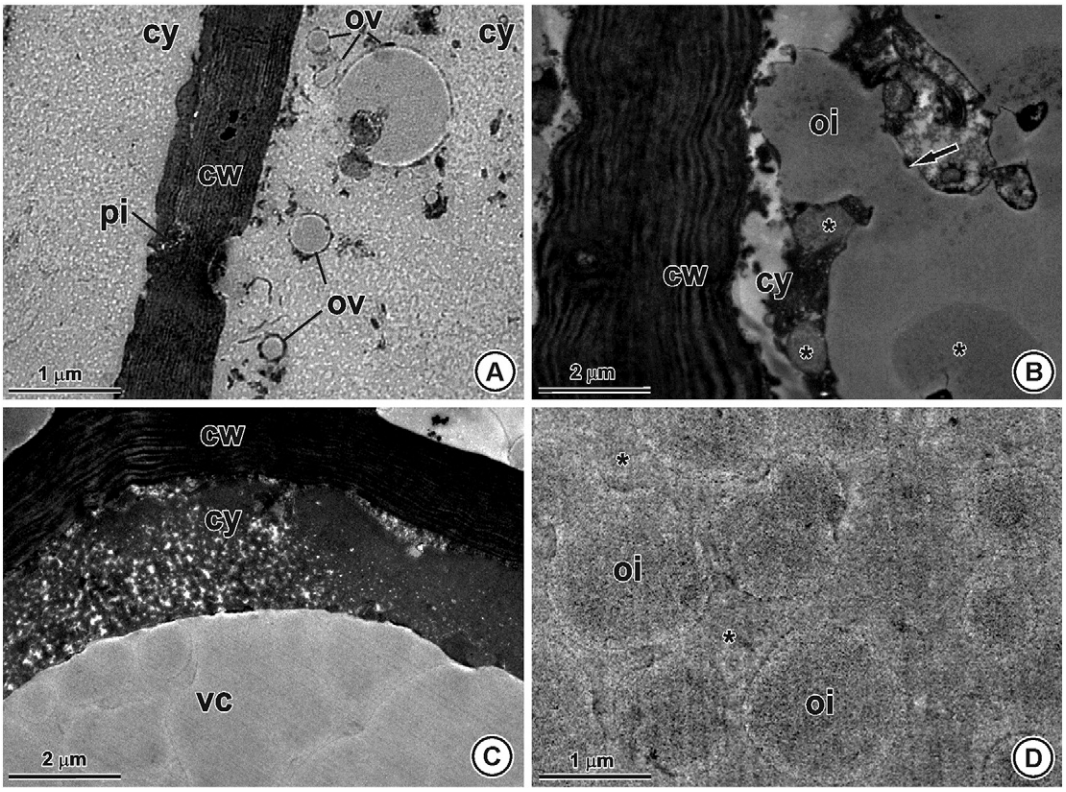

Fig. 1. Ultrastructural aspects of *Tontelea micrantha* seed obtained from immature fruits (30 days before maturation) (A–B) and from mature fruits (B–C). (A) Secretion and oil accumulation in membrane-bound vesicles. (B) Coalescence (arrow) of the vesicles and variable electron-dense material (asterisks). (C) Vacuole occupying a large volume of the cell. (D) Detail of the variable electron-dense material in the vacuole. cy: cytoplasm; cw: cell wall; ov: oil vesicles; oi: oil; ml: middle lamella; pi: pits. Scale: A, D = 1 μm; B–C = 2 μm.