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Constituents, biological activities and quality control parameters of the crude extract and essential oil from *Arracacia tolucensis* var. *multifida*☆

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Abstract

Bioassay guided fractionation of an antimycobacterial extract of *Arracacia tolucensis* var. *multifida* (Umbelliferae) led to the isolation of isoimperatorin (**1**), osthol (**2**), suberosin (**3**), 8-methoxypsoralen (8-MOP) (**4**), herniarin (**5**), scoparone (**6**), umbelliferone (**7**), dihydroxypeucedanin (**8**), 5-methoxypsoralen (5-MOP) (**9**), isoscopoletin (**10**) and scopoletin (**11**). The isolates were tested against *Mycobacterium tuberculosis* and only **1**–**4** showed significant activity with MIC values of 64, 32, 16 and 128 μg/mL, respectively. The essential oil showed moderate in vitro antibacterial activity against representative Gram-positive and Gram-negative bacteria. The volatile oil of *Arracacia tolucensis* var. *multifida* was analyzed by GC–MS and found to be composed mainly by **2** and **3**. The essential oil ($IC_{50} = 116.4 \pm 23.2$ μg/mL) and the extract ($IC_{50} = 1153.1 \pm 53.2$ μg/mL) of the plant provoked concentration dependent inhibition of the tone and amplitude of the guinea-pig ileum spontaneous contractions; the latter activity was related with the high coumarin content of this species. A suitable (novel and rapid) HPLC method to quantify the major active coumarins of the plant was developed. The method provides also a reproducible fingerprint useful for identity tests of this plant.

Keywords: *Arracacia tolucensis* var. *multifida*; Spasmolytic activity; Antimicrobial activity; Coumarins; Antimycobacterial; Umbelliferae

1. Introduction

Arracacia tolucensis var. *multifida* Hemsley (S. Wats.) Mathias & Constance (Umbelliferae) is one of 38 species of the genus *Arracacia* found in the Sierra Madre mountains of Mexico down through the Andes in South America. The plant is a tall perennial herb that grows commonly in the dry grasslands and oak-juniper woodlands of Mexico ranging in altitude from 2250 to 2950 m over the sea level, from the States of Durango to Hidalgo south through Oaxaca. Local population refer to this species by its Nahuatl name, "acocotli", or the common Spanish names of "comino rústico", "hierba del oso" and "neldo". The fruits and aerial parts of *Arracacia tolucensis* var. *multifida* have been used as carminative and digestive stimulant agent along with *Arracacia atropurpurea* (Lehm.) Benth. & Hook (Martínez, 1989). It has also been employed for treating gonorrhea, fevers and anger (Argueta, 1994). The fruits have been employed in the past as a condiment and the plant eaten as food (Bois, 1904). Although *Arracacia tolucensis* var. *multifida* has not been previously investigated from the chemical point of view, studies on the related species *Arracacia vaginata* and *Arracacia nelsonii* led to isolation of several pyranocoumarins, phenylpropanoids and monoterpenoids (Calderon and Ríos, 1975; Delgado and Garduño, 1987). Recently we demonstrated that the crude extract of this species was not toxic for mice or mutagenic when tested by the Lorke and Ames procedures, respectively (Déciga et al., 2007).

Despite the continued use of *Arracacia tolucensis* var. *multifida* its composition and pharmacological properties as well as the quality control procedures for the crude drug have not been

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established yet. Therefore, the present study was undertaken (i) to determine the potential antispasmodic action, chemical composition and active principles of the extract and essential oil of *Arracacia tolucensis* var. *multifida* and (ii) to develop an analytical method using HPLC to quantify the most important active principles of the plant. Altogether, the results of these studies will be useful for establishing quality control and preclinical pharmacological parameters for the elaboration of scientific and pharmacopoeic monographs of this Mexican medicinal plant.

2. Materials and methods

2.1. General experimental procedures

IR spectra were obtained on a Perkin-Elmer 599-B spectrophotometer. ^{1}H NMR (300 and 400 MHz) and ^{13}C NMR (75 and 100 MHz) spectra were recorded in a Varian VXR-300S or Varian Unit Inova spectrometer in $CDCl_3$ using tetramethylsilane (TMS) as an standard internal. EI mass spectra (ionization energy of 70 eV) were obtained on a HP 5890 spectrometer. HPLC was carried out with a Waters HPLC instrument equipped with Waters Dual 2487 detector. Control of equipment, data acquisition, processing and management of chromatographic information were performed by the Millenium 2000 software program (Waters). The analyses were carried out on a Nova-Pak® column (300 mm × 3.9 mm, 6 μm particle size, Waters). The mobile phase was an isocratic Hex–CH_2Cl_2–MeOH (80:7:13) system. The flow rate was kept constant at 0.6 mL/min for 30 min. All solvents and reagents were analytical grade *n*-hexane, CH_2Cl_2, MeOH and EtOAc were obtained from Merck (Darmstadt, Germany) and Burdick & Jackson (Muskegon, USA). GHP® membrane filters (0.45 μm) for the mobile phase were supplied by Pall Corporation (New York, USA) and PVDF® membranes (0.45 μm) for the preparation of samples before HPLC injection were from Whatman (Germany). GC–MS analysis was carried out in a JEOL JMS-AXOCCHA gas chromatograph interfaced to a Hewlett Packard 5890 mass spectrometer equipped with a 30 m long × 0.32 mm i.d. × 0.30 μm film thickness composed of 5% phenylmethylsilicon HP column, connected to an ion trap detector operating in the electron impact mode at 70 eV; carrier gas was He, flow rate 1 mL/min and injection volume of 20 μL (in CH_2Cl_2). The oven temperature was programmed from 150 to 300 °C with increase of 10 °C/min. Column chromatography (CC) was carried out using silica gel 60 (Merck, 70–230 μm; ASTM, 0.063–0.200 nm). Thin layer chromatography (TLC) was performed on plates with silica gel 60 F_{254} (Merck, 0.25 mm); visualization of plates was carried out using a ceric sulphate (10%) solution in H_2SO_4.

2.2. Plant material

The aerial parts of *Arracacia tolucensis* var. *multifida* were collected southern Mexico City, Mexico on September 1999 (batch 1) and January 2004 (batches 2 and 3). The species was identified by Dr. Robert Bye from the Biology Institute of UNAM. Voucher specimens (Bye & Morales 27040 and Bye 33821, respectively) have been deposited at the National Herbarium of Mexico (MEXU), UNAM, Mexico City.

2.3. Extraction and Isolation

2.3.1. Essential oil

The essential oil (4 g) was obtained by hydrodistillation from the aerial parts (batch 1, 1.0 kg). Major constituents of the essential oil were identified by matching their 70 eV mass spectra with those of the reference library. In the case of **1**, **2**, **4** and **9** the identities were established by comparison with authentic samples.

2.3.2. Extraction and isolation

The air-dried aerial parts (batch 1, 2.0 kg) was ground into powder and extracted by maceration with CH_2Cl_2–MeOH (1:1, 10 L) at room temperature. After filtration, the extract was evaporated under reduced pressure to yield 160 g of residue.

The organic extract was subjected to open column chromatography on silica gel (1.0 kg) and eluted with a gradient of Hex–EtOAc (1:0 → 0:1) and EtOAc–MeOH (1:0 → 0:1). Fractions of 200 mL each were collected and then combined according their TLC patterns to yield seven primary fractions (F_1–F_7). Each primary fraction was tested for its antimycobacterial activity. From fraction F_1 (GI of *Mycobacterium tuberculosis* = 99%) eluted with Hex–EtOAc (9:1) crystallized 500 mg (0.025%) of compound **1**. HPLC purification of the mother liquors from fraction F_1 [0.5 mL/min, Hex–CH_2Cl_2–EtOAc (70:25:5)] yielded **2** (100 mg, 0.005%) and **3** (30 mg, 0.0015%). The retention times were 12.61 and 19.50 min, respectively.

Fraction F_2 (GI of *Mycobacterium tuberculosis* = 100%), eluted with Hex–EtOAc (8:2), was subjected to open column chromatography on silica gel (170 g) and eluted with a gradient of increasing polarity of CH_2Cl_2–MeOH (1:0 → 0:1), afforded five secondary fractions ($F_{2\text{-I}}$–$F_{2\text{-V}}$). Preparative TLC of $F_{2\text{-II}}$ using CH_2Cl_2–MeOH (9:1) yielded **6** (17 mg, 0.00085%, pf 143–145 °C). HPLC purification of $F_{2\text{-III}}$ [0.35 mL/min, CH_2Cl_2–MeOH (99:1)] afforded the known coumarins **1** (50 mg, 0.0025%), **4** (70 mg, 0.0035%) and **5** (10 mg, 0.0005%); retention times: 18.76, 20.48 and 21.94 min, respectively.

Fraction F_4 (GI of *Mycobacterium tuberculosis* = 96%) eluted with Hex–EtOAc (1:1) was rechromatographed on a Si gel (300 g) open column eluting with a gradient CH_2Cl_2–MeOH (1:0 → 0:1). Eight secondary fractions were obtained ($F_{4\text{-I}}$–$F_{4\text{-VIII}}$). Extensive TLC [CH_2Cl_2–MeOH (9:1)] of $F_{4\text{-IV}}$ yielded **7** (35 mg, 0.00175%, pf 230–232 °C). Further purification of secondary fraction $F_{4\text{-V}}$ by preparative TLC [CH_2Cl_2–MeOH (95:5)] led to isolation of **8** (25 mg, 0.00125%, pf 134–135 °C).

Primary fraction F_5 (GI of *Mycobacterium tuberculosis* = 99%) eluted with Hex–EtOAc (3:7) was rechromatographed on a Si gel column using a gradient system of CH_2Cl_2–MeOH (1:0 → 0:1). Seven secondary fractions were obtained ($F_{5\text{-I}}$–$F_{5\text{-VII}}$). HPLC purification [Hex–CH_2Cl_2–EtOAc (40:30:30)] of $F_{5\text{-II}}$ afforded **11** (12 mg, 0.0006%, Rt 9.49, pf 230–232 °C). Finally, preparative TLC of