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CHEMICAL CONSTITUENTS OF THE METHANOL EXTRACT OF HAIRY ROOTS OF *Physalis ixocarpa*

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Physalis ixocarpa Brot. (synonym *Physalis philadelphica* Lam.) from the family Solanaceae is an annual, edible plant native to Mexico and Central America. Their fruits are a very popular diet component in those countries and southern regions of the United States, where they are used in chili sauce and dressings for tacos and enchiladas [1]. Besides its taste value, *P. ixocarpa* contains biologically active substances like withanolides with cytotoxic activity [2] and calystegines, known as selective inhibitors of glycosidases [3]. Extracts from this plant have been used to cure stomach disorder, for purifying blood, as an antidote against local poison, and in folk medicine to treat sore throat and fever [4]. Chemical studies show that tropane alkaloids and flavonoids are minor secondary compounds isolated from other species of this plants besides withanolides [5]. The chemical composition of the methanol extract of hairy roots of *P. ixocarpa* is reported for the first time in the present study.

The result of GC/MS analysis of all the identified components and their percentages are given in Table 1. The compounds are listed in order of their elution on the HP-5MS column. Twelve components were identified from the methanol extract. The predominant compounds were hexadecanoic acid 21.32%, octadecanoic acid 17.11% and octadecane 8.13%.

A library search was carried out using the NIST 98 and Wiley GC/MS Library. Identification of the compounds was based on comparison of the mass spectra obtained from the samples with those from authentic standards, those in the NIST (National Institute of Standards and Technology, Gaithersburg, MD, USA) libraries, and those in our library. The relative percentage amounts of the separated compounds were calculated from total ion chromatography by a computerized integrator.

The compounds were also identified by comparison of GC retention indices and retention times.

The seeds *of P. ixocarpa* Brot. ex Hornem were a gift from the Botanical Garden of the University of Masarykiana, Brno, Czech Republic (voucher specimen – 823/2002).

The sterilized seeds were germinated in *in vitro* culture. The seedlings were transformed with *Agrobacterium rhizogenes* ATCC 15834 (pRi 15834) to initiate the hairy root cultures taken for experiments [6].

Air-dried hairy roots of *P. ixocarpa* (1 g) were refluxed three times (2 h each time) with 80% methanol. The material was filtered, and the clear supernatant was then concentrated under reduced pressure at 60°C with a vacuum rotary evaporator. The methanol extract (1 mL) was analyzed using solid-phase microextraction (SPME) and analyzed later by GC/MS.

The volatile compounds were analyzed using an HP-5890 series gas chromatograph coupled with a HP-5972 mass selective detector (Hewlett Packard). The column used was an HP5-MS fused silica capillary (30 m × 0.25 mm i.d.) coated with a phenylmethyl silicone phase (film thickness 0.25 μm). The injection was performed in the splitless mode at 250°C, and the injected volume was 1 μL. The flow rate of the helium carrier was 1 mL min^{-1}. The oven temperature was initially set at 100°C for 5 min and then ramped up from 100 to 270°C at a rate of 10°C min^{-1} and maintained at 270°C for 5 min. The mass spectrometer was operated in the electron impact (EI) ionization mode, and full-scan mass spectra were recorded in the range *m*/*z* 35–550 a.m.u.

The HS-SPME (solid phase microextraction) used fibre (from Supelco) precoated with a 100 mm layer of polydimethylsiloxane (PDMS). The extract was in the vial. The fibre was pushed throught the plastic film for exposure to the headspace of the extract for 15 min at 50°C. Next, the fibre was inserted into the injection port of the GC-MS for desorption of the adsorbed volatile compounds for analysis.

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TABLE 1. Composition of the volatile compounds of the methanol extract of hairy roots of *P. ixocarpa* Brot. ex Hornem by HS-SPME-GC-MS

Compound	Retention time, min	Percentage of total identified compounds	Compound	Retention time, min	Percentage of total identified compounds
Acetic acid, phenyl ester	5.34	1.33	Hexadecanoic acid	11.63	21.32
Nadolol	6.34	0.14	Isoborneol	12.27	2.11
Hexadecane	6.82	2.99	1-Tetradecanol	13.57	1.41
Unknown 1	9.78	2.59	Octadecanoic acid	15.77	17.11
Octadecane	9.97	8.13	Hexacosane	18.84	2.10
1-Octen-3-ol	10.06	3.44	Unknown 2	19.29	3.14

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