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Supercritical extraction of essential oils of *Piper auritum* and *Porophyllum ruderale*

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A R T I C L E I N F O

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A B S T R A C T

Essential oils (EO) of *Piper auritum* ("hoja santa") and *Porophyllum ruderale* ("pápalo" or "pápalo quelite"), previously dried, were obtained by supercritical extraction (SCE) with CO_2. Oil extraction was performed at two temperatures (40 and 50 °C) and two pressures (10.34 and 17.24 MPa). The steam distillation (SDE) method was used also for extraction of EO. Oils were analyzed in antioxidant activity (ABTS method). The chemical composition of oils, by gas chromatography–mass spectroscopy (GC–MS). *Piper auritum* oil yields were in the range 2.37 ± 0.05–3.09 ± 0.12 g oil/100 g dry material and in *Porophyllum ruderale* the yields were 0.82 ± 0.01–1.35 ± 0.09 g oil/100 g dry material. The antioxidant activity, for plants, were in the range 1.30–11.65 mg equivalent of Trolox/g of oil and 1.09–10.16 mg of ascorbic acid equivalent/g of oil. The main components identified by GC–MS in *Piper auritum* oil were safrole and phytol and in *Porophyllum ruderale* oil were isosafrole, α-copaene and phytol.

1. Introduction

At present, the technology of supercritical solvent extraction is applied for extraction of components that are thermally labile or solvent sensitive. According to Rizvi et al. [1], when a gas is compressed isothermally to its critical temperature at pressures above its critical pressure, the gas acts as a solvent: supercritical fluid. Carbon dioxide (CO_2) is a non-oxidizing gas and has a critical temperature of 31.1 °C, this makes it ideal for the extraction of natural chemicals labile to heat. The extracts obtained using this technology have been recognized for their superior quality compared to those obtained by hydrodistillation or liquid-solid extraction [2]. Thus, high quality products, without containing solvents, could be obtained [3]. In the traditional extraction processes, there are practical limitations that involve the removal of toxic solvents [4]. However, the reason for the limited use of the supercritical solvent extraction technology is the high initial investment in comparison with the liquid solvent extraction [3].

Essential oils extracted from a variety of parts of plant have been traditionally used in the manufacture of cosmetics, fragrances, herbicides, cleansers, insecticides and in the traditional medicine. Also, some of these plants have been used in the traditional medicine in many countries since ancient times (as digestives, diuretics, expectorants, sedatives, among many other ailments). Currently, some plants or extracts from them are commercially available as infusions, tablets or extracts. Recently, "fragrant" essential oils have become very popular in aromatherapy, a branch of the alternative medicine that involves essential oils and other aromatic compounds that have benefits. In the last decade, scientists have reported many beneficial biological properties (antioxidant, anti-inflammatory, antiviral, antibacterial, etc.) of some plants due to certain chemical compounds found in the essential oils of the cells of these plants [5–9].

In general, essential oils represent less than 5% of the dried plant material. Although all parts of plants may contain essential oil, its composition may vary with the type of plant. Furthermore, climatic factors, soil type, harvest time, among other factors, can determine the composition and quality of essential oils [10,11].

Piper auritum ("hoja santa") is a small shrub belonging to the Piperacea family Mexico and Central America. Depending on the culture and the region where it grows, *Piper auritum* is commonly named as "hoja santa", "yerba santa", "anisillo", "acuyo", "star leaf" or "momo" [12,13]. In the United States, this herb is named "pepperleaf", "eared pepper" or "root beer plant" [14]. The essential oil of *Piper auritum* includes safrole as the main component (70%); it has more than 40 chemical constituents in small amounts from which many are mono- and sesquiterpenes [14]. *Piper auritum* extracts have been used as antimicrobial agents to inhibit *Colletotrichum gloeosporioides* (mold) on papaya [15]. According to Jiménez-Merino [16] *Piper auritum* tea can be used to stimulate digestive functions, release colic and bronchial disorders as well as a diuretic. Shredded leaves are used against erysipelas (an infection inside the skin). *Piper auritum* is available all

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year since it grows in any type of weather. However, there are few (or perhaps none) reports on performance and antioxidant activity of essential oil of *Piper auritum* obtained by supercritical extraction. The essential oil and leaves could be used in the food industry as a seasoning.

Porophyllum ruderale ("pápalo" or "pápalo quelite") is an herb native to Mexico, known as "papalo", "papalo quelite", "tepelcasho", "deer grass", "tepegua" [13,17,18] among others. This herb has a strong aroma and unique flavor, its leaves and stems are commonly used for foods dressings [19]. *Porophyllum ruderale* has been used in the folk medicine as diaphoretic and sedative [20]. The aerial parts of *Porophyllum ruderale* have already been analyzed to elucidate their volatile compounds. Bohlmann and Zdero [21] and Bohlmann et al. [22] found thiophene derivatives with unsaturated chains and a derivative of thymol in petroleum ether extracts. The essential oil can also be used as a pesticide [23].

The aim of this study was to evaluate the antioxidant properties and compassion of essential oils and *Piper auritum* and *Porophyllum ruderale*, grown in Mexico, obtained by supercritical extraction and steam distillation.

2. Materials and methods

2.1. Materials

Plant material. Fresh *Piper auritum* and *Porophyllum ruderale* were acquired in the local market of the city of Cholula, Puebla, Mexico.

Samples preparation. Leaves of *Piper auritum* and *Porophyllum ruderale* were air dried at room temperature (20° C) for one week. The dried leaves of *Piper auritum* and *Porophyllum ruderale* were ground with a mortar/pestle. The dried powders were placed into plastic bags, sealed under vacuum, protected from light and stored at room temperature until use or analysis.

2.2. Supercritical (SCE) extraction

The supercritical extraction equipment (Fig. 1) was assembled at the Universidad de las Americas Puebla and is a modification based on the equipment located at the Laboratory of Thermodynamics in the Thermophysics Research Area devoted to Solubility of Hydrocarbons [24] and Recovery of Hydrocarbons [25] at the Mexican Petroleum Institute (Instituto Mexican del Petróleo, IMP).

The extraction equipment consists of three main sections: inlet, extraction and outlet sections.

Inlet section. In this section, carbon dioxide gas enters to the system and is allowed to reach the required pressure conditions in the extraction section. It is made up of a 99% CO_2 tank (1), valves (2, 4, 7) (Worcester, Mexico City) coupled to a gauge (5), a laboratory compressor (3) (Swagelok Co.), a CO_2 storage reservoir (6), a Supercritical Fluid Technologies Inc. model SFT-10 high pressure pump (8), coils (9) and a CO_2 inlet valve (10) headed to the extraction cell.

Extraction section. In this section, the removal of components takes place at selected conditions of temperature and pressure. It consists of the following parts inside of a box made of acrylic: a cell for extraction (11), made of 316 stainless steel (6.1 cm in diameter and 18 cm in height, having an effective volume of 0.526 L), a pressure gauge (12) coupled to the extraction cell, a heating system made up with a simple 200 W light bulb (13) and two fans (14) for homogenizing temperature, and an ASL F200 Precision Thermometer (Burlington, VT, USA), coupled to a temperature controller (Digi-Sense temperature Controller R/S) (Cole Parmer, Vernon, IL, USA) (15), for measuring temperature.

Outlet section. This section separates the extract. It consists of an exit valve of the extraction cell (16), an oil collector (17), a thermal bath (18) and a flowmeter (19).

Extraction of sample. Twenty five grams of dried pulverized (400 ± 25 μm) leaves of *Piper auritum* ("hoja santa") and *Porophyllum ruderale* ("papalo") were placed in the supercritical extraction cell. The oil was extracted at two temperatures (40 and 50 °C) and two pressures (10.34–17.24 MPa). Each batch for extraction was carried out for 180 min. The CO_2 flow rate was 126.24 ± 20.83 mL/min. The cell for collecting extracts was immersed in an ice-water bath (8 ± 0.5 °C). All extractions were performed in duplicate.

2.3. Steam distillation (SDE) extraction

The extraction of essential oils was carried out in a Clevenger type distillation apparatus. The apparatus is made up of a heating source, a 2 L pear shaped flask, a 2 L ball shaped flask, a thermometer, a condenser, and a glass collector for separating essential oil from water. 100 g of sample was extracted for 2 h. The yield essential oil was calculated as percentage (w/w).

2.4. Antioxidant activity

The antioxidant activity was evaluated by the ABTS (2,2′-azinobis (3-ethyl-acid benzotiazolino-6-sulfonic acid) (Sigma-Aldrich, Louis, USA) method according to the methodology developed by [26]. The radical ABTS$\cdot^+$ was formed after the reaction of 7 mM ABTS with 2.45 mM potassium persulfate (final concentration) (Sigma-Aldrich, Louis, USA) for 16 h of incubation at room temperature. Once the radical ABTS$\cdot^+$ was formed, dilutions with ethanol (ABTS-radical-ethanol) were done to obtain an absorbance value of about 0.700 ± 0.020 (*Ai*) at 754 nm. To measure the antioxidant activity,

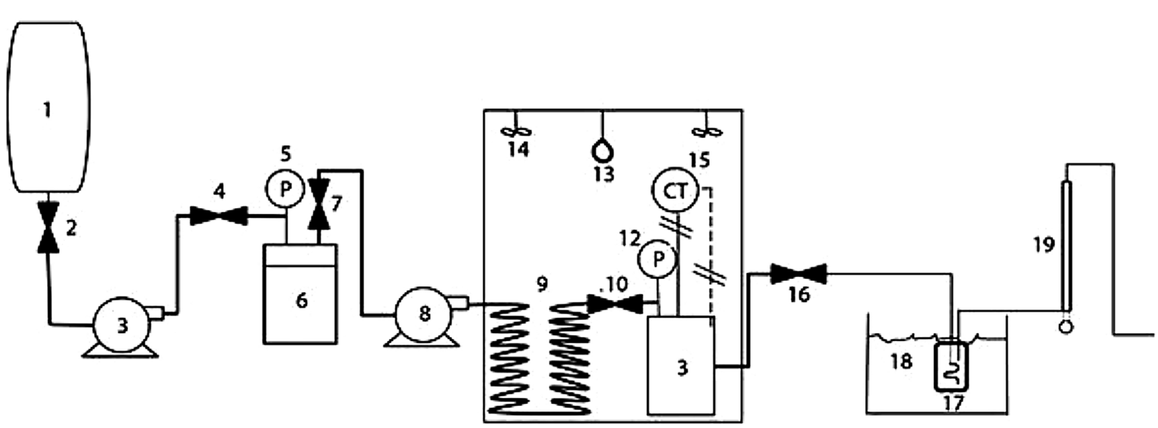

Fig. 1. Flow diagram of a supercritical fluid extraction system.
(Adapted from Hernández-Conde, 2013)