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ScienceDirect
J. of Supercritical Fluids 42 (2007) 334–341
THE JOURNAL OF Supercritical Fluids
www.elsevier.com/locate/supflu

Extraction and composition of volatiles from *Zanthoxylum rhesta*: Comparison of subcritical CO_2 and traditional processes

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Received 28 July 2006; received in revised form 27 December 2006; accepted 4 January 2007

Abstract

The dried fruits of *Zanthoxylum rhesta* DC syn *Z. budrungawall* syn. *Z. limonella* (Dennst) are used as condiments and have spice value. While the essential oil is concentrated in the pericarp, the seeds have fatty oil. The extraction of fragrance/flavour components is carried out from the pericarp by subcritical CO_2, modified methanol–subcritical CO_2, hydrodistillation and traditional solvent extraction processes and the composition of these extracts are compared. The components are identified by GC–MS and the composition is determined by GC–FID. The principal components such as sabinene, terpinen-4-ol, α-terpineol are present in different amounts in the extracts. The traditional solvent extraction processes provide more amounts of waxy components along with the principal components. Though hydrodistillation process provides a wax free essential oil, the yield is low. The extract obtained by the subcritical CO_2 method is superior in comparison to traditional processes, but it contains higher percentage of monoterpenes in comparison to oxygenated monoterpenes. On the other hand, extraction of all the desired components is possible through pre-treatment of pericarp by small amounts of methanol. These details are described and discussed.

Keywords: *Zanthoxylum rhesta* DC; Subcritical CO_2 extract; Essential oil; Concrete; Absolute; Methanol extract; Sabinene; Terpinen-4-ol

1. Introduction

The genus *Zanthoxylum* L. comprises over 200 trees, shrubs and lianes [1] and is included in the tribe Zanthoxyleae of the Rutaceae [2]. Species of *Zanthoxylum* are primarily pan-tropical in distribution. About 13 species are reported from India [3]. *Zanthoxylum* species are used for medicinal and culinary purposes [4].

Zanthoxylum armatum (Syn *Z. alatum* DC, *Z. planispinium* Sieb) is an evergreen shrub or a small tree growing up to 12 m height. It is widely distributed in hot valleys of Himalayas along the foothills up to a height of 120 m and in the Eastern ghats of Orissa and Andhra Pradesh. The powdered seeds are taken as an aromatic tonic, stomachic and for fever, dyspepsia, cholera, etc., [5]. The essential oil from the fruits and fruit pericarp has been the subject of study by several investigators. Jain et al. [5], Ramidi et al. [6], Dubey and Purohit [7] and Weyerstahl et al. [8] have reported linalool as the major constituent in the essential oil obtained by hydrodistillation method. Linalool content varies in the range of 34.5–87.7%.

Zanthoxylum rhesta DC, Syn *Z. budrugawall* Syn. *Z. limonella* (Dennst) Alston [4,9] is found in the evergreen forests of Assam, Meghalaya and the Eastern and Western ghats of Peninsular India. The dried fruits are used as condiments and have spice value especially for fish preparations. The fruits are digestive and appetizing [4]. The essential oil obtained from the dried fruits is called Mullilam oil [10] and has been investigated by several workers. While the essential oil is concentrated in the pericarp, the seeds have fatty oil [11]. The structure of Mullilam diol isolated as a crystalline solid from the essential oil has been revised [10] as *p*-mentha-1α,2β,4β-triol from the previously assigned structures [12,13]. The major constituent of the essential oil has been reported [9,14,15] as sabinene 35.7–67.7%. The essential oil from the fruits is reported to have anti-inflammatory, anesthetic and hypotensive activities [16].

The supercritical CO_2 extraction is commonly used for extraction of natural materials because of the non-toxic, non-flammable characteristics of CO_2 and its availability in high purity with low cost. Reverchon and Marco [17] reviewed the applications of supercritical carbon dioxide extraction in food

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doi:10.1016/j.supflu.2007.01.001

processing, pharmaceuticals and nutraceuticals. Some compounds show very low and moderate solubilities in SC–CO_2. Addition of a co-solvent in the supercritical CO_2 extraction medium has been proposed to improve solubility of polar components. Naik et al. [18] and Tuan and Iiangantileke [19] have studied the advantages of liquid CO_2 over the supercritical CO_2 extraction of aroma compounds. Liquid CO_2 is the most interesting solvent for fragrance and flavor compounds with medium molecular weight. The main advantages lie in the low extraction temperature; inert extraction atmosphere is an advantage for recovery of volatile and thermally labile components.

2. Experimental

The dried *Z. rhesta* fruits (*Tirphal*) used is a commercial sample purchased from Mumbai (18.96°N, 72.82°E), India. The solvents hexane and methanol are of reagent grade and are distilled before use. All the yields reported in the paper are simple arithmetic average of two experiments with the deviation mentioned. The pericarp is manually separated from the fruit before extraction. Fifty grams of pericarp is distilled with water in a Clevenger type apparatus for 3 h and the essential oil separated as upper light yellow layer weighed 0.9 ± 0.05 g. The pericarp is ground in a domestic mixer grinder to obtain particles of 0.5–1.1 mm size. Extraction of the ground pericarp (100 g) at room temperature (25 °C) with distilled hexane (200 ml, contact time 10 h, two successive extractions) followed by a quick wash (50 ml). The combined extracts on solvent removal in a rotary evaporator under vacuo below 40 °C afforded a reddish waxy residue, the so called concrete (5.9 ± 0.3 g). Addition of 50 ml of methanol to the waxy residue, warming to 50 °C for 5 min to get a homogeneous mixture, followed by refrigeration for 12 h at −12 °C, precipitated most of the waxes. Filtration through a sintered funnel followed by evaporation of methanol below 40 °C afforded the dewaxed absolute as a yellow semi solid (2.2 ± 0.05 g). Cold extraction of the ground pericarp (100 g) with distilled methanol (200 ml, contact time 10 h, two successive extractions) followed by a quick wash (50 ml). The combined extracts on careful removal of solvent afforded a deep red viscous residue (20.1 ± 0.7 g). It is triturated with diethyl ether (50 ml, two successive treatments) and the ether layer decanted. On removal of the solvent from the combined ether layer under vacuo, *a* light reddish residue 6.4 ± 0.4 g, designated ether soluble methanol extract is obtained.

For obtaining the CO_2 extract, the ground pericarp (50 g) is taken in the special glass soxhlet type apparatus in the high-pressure stainless steel extraction vessel for subcritical CO_2 extraction. It consists of an upper compartment for placing the material to be extracted and is provided with a siphon for the periodical removal of the extract into a separate glass vessel, which serves as a reservoir, for evaporating CO_2 from the siphoned extract and for the storage of the extract. This glass vessel has a volume larger than the volume of the extract delivered periodically by the siphon. The details of the experimental set up (Fig. 1) and the procedure of extraction has appeared in earlier publications [18–20]. The extraction is carried out at room temperature maintained at 20–22 °C and CO_2 pressure of 65–70 bar is used.

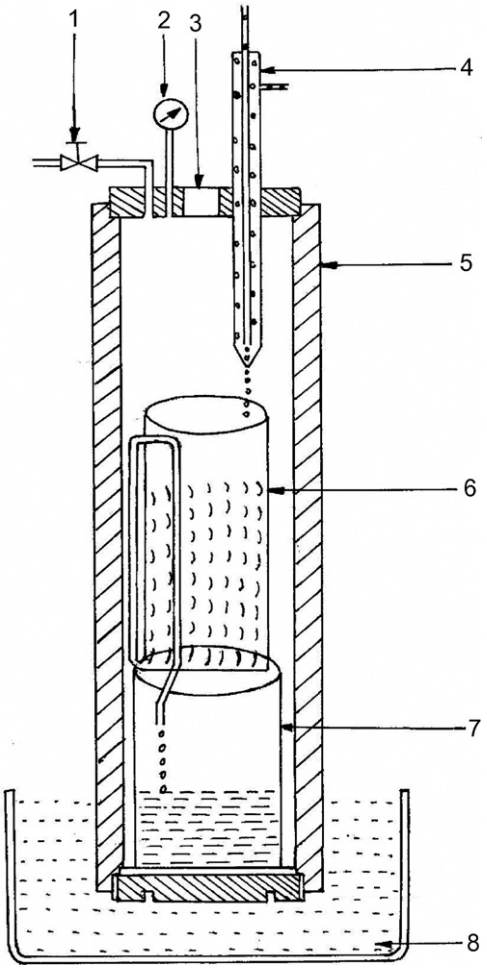

Fig. 1. Subcritical CO_2 extraction apparatus. (1) High pressure valve, (2) pressure gauge, (3) sapphire window, (4) cooling finger, (5) cylinder, (6) glass extractor with thimble, (7) glass vessel serving as reservoir and (8) water bath.

The CO_2 content in the apparatus is around 260 g. Chilled water of 5 °C from a thermostatic bath is circulated through the cooling finger of the apparatus. The liquid CO_2 extraction is carried out for 3 h. Then CO_2 is released from the extractor slowly through a Teflon tube connected through a glass bottle, placed in an ice bath. Yield of the light yellow residue is 2.55 ± 0.05 g.

In the modified liquid CO_2 extraction experiments, ground pericarp (50 g) in the upper compartment is treated with 3 and 10 ml methanol, respectively, before extraction with CO_2. The extractions are carried out for 3 h under similar conditions described. The extracts are collected after releasing the CO_2 from the high-pressure extraction vessel. The yield of the extracts is 2.6 ± 0.05, 2.7 ± 0.05 g (average of two experiments) for first and second experiments, respectively. The experimental data on extraction using different processes are tabulated in Table 1.

GC analysis of each sample is carried out twice on a Shimadzu GC 17A Gas chromatograph equipped with a flame ionization detector and a 30 mm × 0.25 mm WCOT column coated with 0.25 μ 5% diphenyl dimethyl siloxane supplied by J&W (DB-5). Helium is used as the carrier gas at a flow rate of 1.2 ml/min at a column pressure of 42 kPa. 0.2 μl of each sample is injected