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Effect of separation method on chemical composition and insecticidal activity of Lamiaceae isolates

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ABSTRACT

Supercritical fluid extraction was used to isolate volatile compounds from savory (*Satureja hortensis* L.), thyme (*Thymus vulgaris* L.), lavender (*Lavandula angustifolia* L.) and peppermint (*Mentha piperita* L.). Three types of extracts were prepared using the benefit of variable solvent power of supercritical carbon dioxide under different extraction conditions. The composition and toxicity of CO_2 extracts, products of Soxhlet extraction with hexane and ethanol, and essential oils obtained by hydrodistillation were compared. The composition of volatile compounds in the isolates was determined by gas chromatography. The acute toxicity of the isolates was evaluated in *Spodoptera littoralis*, *Musca domestica*, *Culex quinquefasciatus*, and *Leptinotarsa decemlineata*. The highest acute toxicity was exhibited by essentials oils, followed by CO_2 extracts. The lowest LD_{50} value, 22 μg, was measured for savory essential oil against the larvae of *L. decemlineata*.

1. Introduction

Aromatic plants produce biologically active secondary metabolites which can be isolated using various extraction techniques. The products of isolation are used in food, cosmetic, perfume and detergent industries as well as in pharmaceutical industry for their antibacterial, antifungal, antioxidant and anti-carcinogenic properties, and also in agriculture in plant protection for their pesticidal activity.

Disadvantages of long-time application of synthetic pesticides are evident. It was confirmed that the number of insect and mite species resistant to them continues to rise, apart from risks associated with the use of these chemicals (Elhag and Horn, 1983; Roush and McKenzie, 1987). Therefore, there is urgent need for safer, environmentally friendlier and efficient alternatives that have potential to replace synthetic pesticides and are convenient to use. In the search for the alternatives to conventional pesticides, essential oils extracted from aromatic plants have been widely investigated. Their toxicity and repellent effects to stored-product insects and greenhouse pests have been of special interest (Landolt et al., 1999; Mansour et al., 1986). In particular, terpenes and terpenoids belong to active components of essential oils (Klein et al., 2002; Srivastava et al., 2003).

Traditional isolates of aromatic plants are essential oil obtained by steam distillation or hydrodistillation and extracts obtained by extraction with organic solvents. Recently, much attention has been directed to the use of liquid and supercritical carbon dioxide as the solvent, particularly in food, pharmaceutical and perfume industries. The supercritical fluid extraction (SFE) is more selective than the extraction with conventional solvents, which dissolve unwanted compounds as well (Reverchon, 1997). Due to low temperatures used in SFE, no decrease occurs in biological activity of extracted components against harmful organisms such as microbes, viruses, fungi and also pests (El-Ghorab et al., 2004; Marongiu et al., 2003).

Four plant species from Lamiaceae family were chosen for the present study: savory (*Satureja hortensis* L.), thyme (*Thymus vulgaris* L.), lavender (*Lavandula angustifolia* L.) and peppermint (*Mentha piperita* L.). These herbaceous aromatic plants have been traditionally used for their beneficial biological activities (Aslan et al., 2004; Chorianopoulos et al., 2004; Giordani et al., 2004). Some components of their essential oils are responsible for their antifeedant or repellent effects on pests (Borad et al., 2001; Morris et al., 1999; Regnaultroger and Hamraoui, 1993). Supercritical CO_2 extraction of volatile fraction of these herbs was studied and compared in terms of extraction efficiency and extract composition with hydrodistillation (Da Porto et al., 2009; Hawthorne et al., 1993; Oszagyan

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Table 1
Overview of insecticidal tests performed (+).

Insect/plant material	Savory	Thyme	Lavender	Peppermint
Spodoptera littoralis larvae (African cotton leaf worm)	+	+	+	+
Musca domestica larvae (housefly)	+	+	–	+
Musca domestica adults	+	+	+	+
Culex quinquefasciatus larvae (southern house mosquito)	+	+	–	+
Leptinotarsa decemlineata larvae (colorado potato beetle)	+	+	–	–
Leptinotarsa decemlineata adults	–	–	+	+

et al., 1996; Zorca et al., 2006), ultrasonic extraction (Da Porto et al., 2009), and extraction with subcritical water (Ammann et al., 1999; Kubatova et al., 2001). Kinetics of the extraction of volatile oils with supercritical carbon dioxide was simulated using different mathematical models (Akgun et al., 2000; Goto et al., 1993; Kubatova et al., 2002; Walker et al., 1994; Zizovic et al., 2005) and its optimum operating conditions were determined (Dapkevicius et al., 1998; Khajeh, 2011; Reverchon et al., 1995; Roy et al., 1996; Zorca et al., 2007). Biological properties of CO_2 extracts from these plants were tested in terms of antioxidant activity of extracts from savory, thyme and lavender (Dapkevicius et al., 1998; Esquivel et al., 1999; Simandi et al., 2001) and herbicidal activity of thyme extract (Grosso et al., 2010).

The aim of this study was to compare the insecticidal activity and chemical composition of plant isolates obtained using SFE, hydrodistillation and Soxhlet extraction. In our previous papers, two insecticidal effects (acute toxicity and antifeedant effect) of isolates from savory and thyme on the Colorado potato beetle were examined (Pavela et al., 2009), and acute toxicity of savory isolates against four kinds of pests was evaluated (Pavela et al., 2008). The present study is focused on searching for the relationship between the separation method applied, the chemical composition of isolate, and the toxicity of isolate in several kinds of pests in different stages of development.

2. Materials and methods

2.1. Materials

Aerial parts of savory, thyme, lavender and peppermint were obtained from plant culture of the Crop Research Institute, Prague. They were oven-dried at 40 °C for 72 h and stored in dark in closed bottles at room temperature. The water contents were 7.3 wt.%, 6.8 wt.%, 7.1 wt.% and 6.3 wt.% for savory, thyme, lavender and peppermint, respectively. The plant material was ground for 5 min before each experiment using laboratory mixer Blender 80106 (Waring Commercial, Connecticut, USA). Carbon dioxide (>99.9%) was purchased from Linde Technoplyn (Prague, CR). Acetone (pure, Lachema Neratovice, CR) was used as cosolvent. Hexane (Lach-Ner, CR) and technical ethanol (Chemopetrol, Litvinov, CR) were used as solvents for Soxhlet extraction.

n-Heptane p.a. used as solvent of the samples for gas chromatography and hexadecane as internal standard were purchased from Sigma (Sigma–Aldrich, Steinheim, Germany).

2.2. Supercritical fluid extraction

The SFE experiments were carried out using the following set up. Extraction column (150 mL, I.D. 30 mm) was filled with 30–40 g of plant particles placed between layers of glass beads serving as solvent flow distributors. The extractor was immersed in a temperature-controlled water bath. CO_2 was pressurized by compressor NovaSwiss 560.0007 and controlled by pressure regulator unit NovaSwiss 560.0009 to operating pressure. In the experiments with cosolvent, the stream of CO_2 was before entering the extractor mixed with acetone pumped at a constant flow rate by high-pressure pump LCP 4020.3 ECOM, CR. Acetone was selected as cosolvent with respect to its low toxicity in the tested insect, compared to other polar solvents tested in preliminary experiments, methanol and ethanol. The direction of solvent flow in the extractor was selected with respect to the finding that the down-flow of fluid can accelerate the extraction rate, in particular at lower Reynolds numbers and for conditions near the critical point of CO_2, where natural convection is dominant (Stuber et al., 1996). The solution leaving the extractor at its bottom was depressurized to ambient pressure in a heated micrometer valve and the extract was collected in a pre-weighed glass trap cooled in an ethanol-dry ice bath in order to reduce escape of volatile extract components with gaseous CO_2. The flow rate of CO_2 was adjusted to 1.4 g min^{-1} according to the gas meter (Spectrum Skutec, CR) connected to the trap. The total amount of CO_2 was approximately 700 g (400 L of expanded gas) in each extraction run.

Three types of extracts were prepared: SFE1 – oleoresin, SFE2 – the extract rich in volatile compounds, and SFE3 – the extract enriched with polar compounds. The extraction temperature was 50 °C. The extraction pressure was 28 MPa for SFE1 and SFE3 and 12 MPa for SFE2. The solvent for SFE1 and SFE2 was pure CO_2. Water that partially condensed in the cooled trap was separated from other extracted substances by centrifugation and removed using a hypodermic needle. The solvent for SFE3 was CO_2 modified with 4.3 wt.% acetone. To suppress its condensation together with the extract, the trap was not cooled; after the extraction run it was dried to constant weight under a stream of nitrogen.

The extract was weighed and stored in the closed trap in freezer. Before the biological tests, the extract was homogenized by dissolving in acetone (1:1) and a small part of the solution was withdrawn for chromatographic analysis.

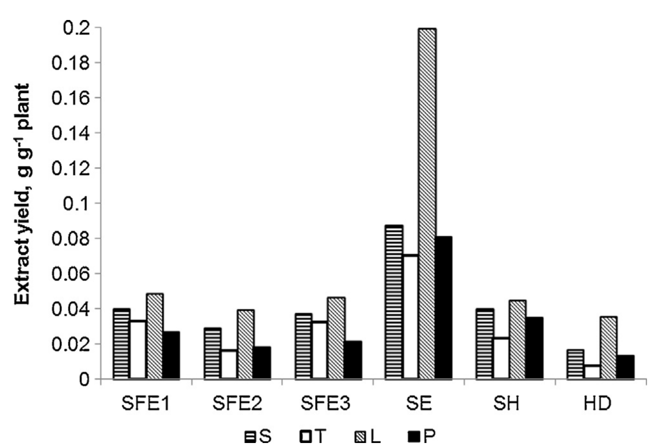

Fig. 1. Yield of isolates (g g^{-1} of plant) from savory (S), thyme (T), lavender (L), and peppermint (P). Separation methods: supercritical fluid extraction at 50 °C and different solvent composition and pressures (SFE1: CO_2 at 28 MPa, SFE2: CO_2 at 12 MPa, SFE3: CO_2 + acetone at 28 MPa), Soxhlet extraction with ethanol (SE) and hexane (SH), and hydrodistillation (HD).