



The insecticidal activity of *Tanacetum parthenium* (L.) Schultz Bip. extracts obtained by supercritical fluid extraction and hydrodistillation

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ABSTRACT

CO₂ extracts and essential oil obtained by hydrodistillation from feverfew aerial parts were applied to *Spodoptera littoralis* (Boisduval) larvae to examine their effects on mortality, antifeedancy and growth inhibition, and their composition of volatile substances was determined using GC. The mortality strongly correlates with the content of terpenoids in the samples, the lowest LD₅₀ being for essential oil (0.05 µl/g) and SFE2 (0.11 µl/g). The extracts obtained with CO₂, pure or modified by acetone, at 280 bar and 50 °C were more efficient antifeedants and growth inhibitors than the essential oil alone, showing the combined effect of essential oil and non-volatile bioactive substances.

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1. Introduction

Apart from risks associated with the use of chemicals, such as the negative environmental and health impacts of synthetic insecticides, problems associated with long-term use of toxic insecticides include pest resistance and a negative impact on natural enemies. These problems lead to increasingly stringent environmental regulation of pesticides (Isman, 2006; Pavela, 2007a).

There is therefore an urgent need to develop safer, more environmentally friendly and efficient alternatives that have the potential to replace synthetic pesticides and are convenient to use. In the search for alternatives to conventional pesticides, essential oils extracted from aromatic plants have been widely investigated. Their toxicities as well as arresting and repellent effects on pests were of special interest during the last decade. Feverfew, *Tanacetum parthenium* (L.) Schultz Bip., family Asteraceae, is one of the medicinal and aromatic herbs widely distributed in the northern hemisphere (Hulten, 1968; Hussey, 1974; Heywood, 1976). Feverfew has been historically used as an anti-helminthic for migraine, neuralgia, rheumatism and loss of appetite (Blumenthal, 1998) as well as for the treatment of headache, menstrual irregularity,

stomach ache and fever by Greek and European herbalists (Grieve, 1984).

Feverfew extracts contain volatile oil, sterols (Rateb et al., 2007), a range of sesquiterpene lactones (Kaplan et al., 2002), flavonoids such as luteolin and apigenin (Wu et al., 2006), different flavone glycosides (Williams et al., 1999), and tannins (Marete et al., 2009). Many of these components possess antioxidant properties. Parthenolide, the most abundant sesquiterpene lactone in this herb, is reported to be its main bioactive ingredient. The content of essential oil in aerial parts of cultivated feverfew plants ranges between 0.23 and 0.55% on a dry weight basis (Akpulat et al., 2005; Mirjalili et al., 2007; Omidbaigi et al., 2007; Stevanovic et al., 2009). Its major components are the oxygenated monoterpenes camphor and (Z)-chrysanthenyl acetate, their content in the oil varying around 49% and 26%, respectively (Kery et al., 1999; Akpulat et al., 2005; Besharati-Seidani et al., 2006; Mirjalili et al., 2007; Rateb et al., 2007; Shafaghat et al., 2009; Stevanovic et al., 2009).

Other studies were devoted to the extraction and chromatographic determination of parthenolide (e.g. Zhou et al., 1999; Abourashed and Khan, 2001; El-Shamy et al., 2007; Marete et al., 2009). It should be noted that parthenolide is relatively unstable and degrades upon storage (Smith and Burford, 1992; Kaplan et al., 2002; Jin et al., 2007). To protect the substance during its isolation from the plant, extraction with supercritical carbon dioxide (SFE)

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was performed, being an environmentally benign and mild method which reduces thermal degradation.

Smith and Burford (1992) conducted the SFE on an analytical scale, examined the effect of modifier added to CO₂ on the extraction yield, and processed the extract further to increase parthenolide purity. Kaplan et al. (2002) compared the parthenolide yields obtained with CO₂ and with conventional solvents, and used the tunability of supercritical fluid to extract a different group of compounds at successively increasing pressure. Kery et al. (1999) optimised the extraction conditions, in larger scale equipment and with respect to total extraction yield and the yield of parthenolide, and compared the composition of essential oil obtained by hydrodistillation with that of the extract obtained with supercritical CO₂.

Cretnik et al. (2005) conducted the SFE in laboratory- and pilot-scale equipment and compared the results with extracts obtained using conventional extraction. Two separators were used to partially separate the extracted parthenolide from waxy substances. The dependence of parthenolide yield on extraction pressure was found to pass a minimum at pressures near 300 bar.

Although the insecticidal properties of feverfew extract and essential oil are well known (Hough-Golstein and Hahn, 1992; Nottingham et al., 1991; Panasiuk, 1984; Scheerer, 1984; Suomi et al., 1986; Kordali et al., 2006), no literature exists, to our knowledge, comparing insecticidal effects and the methods of obtaining extracts.

The objective was to compare the insect control effectiveness of the essential oil extracted through the standard method, i.e. distillation, to that of the extracts obtained under varied SFE conditions, thus determining whether SFE would be a convenient method of gaining extracts that could be potentially used to produce botanical insecticides. Keeping in mind the premise that terpenic substances contained in essential oils are the major effective components (Hummelbrunner and Isman, 2001; Pavela et al., 2008b, 2009), a GC analysis of extract and distillate composition was conducted. Carbon dioxide as a solvent was either pure or modified by acetone in order to dissolve also polar components that should have some biological activity or synergistic effect with terpenic substances.

2. Methods

2.1. Plant material

Aerial parts (flowering) of *T. parthenium* (L.) Schultz Bip. (Asteraceae) were obtained from a plant culture of the Crop Research Institute, Prague. They were oven dried at 40 °C for 72 h, stored at room temperature and milled prior to extraction experiments.

2.2. Insects

Spodoptera littoralis (Boisduval) (Lepidoptera: Noctuidae) larvae were selected from established laboratory colonies (at the Crop Research Institute, Dept. of Entomology). Larvae were reared on an artificial diet (Stonefly Industries, Bryan, TX, USA). All colonies and experiments were reared in climatic chambers at 25 °C, 75% RH and a 16:8 (L:D) light:dark photoperiod.

2.3. Supercritical fluid extraction

Carbon dioxide from a pressure bottle was pumped by a compressor to a heated extractor with a volume of 150 ml and ID of 30 mm, filled with 50 g of savory. The CO₂ flow rate was adjusted to 1.4 g min⁻¹. The solution flowing out of the extractor expanded in a valve to atmospheric pressure, and the extract was collected in a glass trap. The trap was cooled in a mixture of dry ice and ethanol. In the experiments with entrainer, it was pumped by

a high-pressure pump and mixed with the CO₂ stream before it entered the extractor. Acetone was selected as polar entrainer after preliminary experiments confirming that it was biologically inactive, contrary to ethanol. To reduce condensation of acetone in the trap, the separator was not cooled. Trace amount of acetone in the extract was evaporated off-line under a stream of nitrogen. These separation conditions were preferred to cooling the trap and collecting acetone in it, which would require a frequent change of traps and following evaporation of large amounts of acetone from the extract, connected with a higher loss of volatile compounds than the applied separation method.

Details of the equipment are given elsewhere (Sajfrtova et al., 2005).

Three extracts were prepared under different operational conditions using the variable solvent power benefits of supercritical CO₂:

- SFE1, oleoresin extracted at 28 MPa and 50 °C with pure CO₂.
- SFE2, essential oil-rich extract obtained at 12 MPa and 50 °C with pure CO₂.
- SFE3, extract enriched with polar components, extracted at 28 MPa and 50 °C with CO₂ modified by 4.3% acetone.

2.4. Hydrodistillation

The distillation was conducted in a Clevenger-type apparatus where the essential oil (HD) is collected in a side arm. Dry plant material (30 g) was immersed in 300 ml water and distilled for 2 h, which was found to be sufficient for completing the process. The density of the essential oil, 0.93 g/ml, was calculated from the weight of its volume in a calibrated vessel at 23 °C.

2.5. Bioassays

To assess biological effectiveness using the specific extraction methods, three basic biological assays were selected:

Mortality – With respect to the nature of the extracts, acute toxicity for topical application was determined. The issue to be addressed by this type of test was to determine whether there was any significant difference among the specific extracts in terms of toxicity in topical application, which has been regarded as the most important effect of insect control agents.

Antifeedancy – To determine the antifeedant effect, a no-choice test was selected, as this type of test is closest to the methods applied in the field; in addition, such a testing type can answer the question of whether damage to plants caused by phytophagous larvae feeding on them could be potentially reduced by substances contained in the extracts.

Growth inhibition – Since healthy larval growth and development present the most critical fitness factors for any adults to survive, and therefore for the next generation to evolve successfully, a relative larval growth rate was determined within the test series. This type of test has successfully resolved the question of the extracts' potential to depress larval growth and thus decrease the vitality of the larvae.

2.5.1. Insecticidal activity

Acute toxicity was determined as mortality after topical application of the extract or distillate to early third instars larvae *S. littoralis* (wt. 25 ± 5 mg).

The isolates were dissolved in acetone as a carrier, and each larva received 1 µl of the solution per treatment, with acetone alone as the control. The range of five doses that were used to establish the lethal doses was determined by preliminary screening. Four replications of 20 larvae were tested per dose. The doses were applied to the dorsum using a repeating topical dispenser attached to 100 µl

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