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Chemical composition and biological activity of *Tanacetum audibertii* (Req.) DC. (Asteraceae), an endemic species of Sardinia Island, Italy

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ABSTRACT

The chemical composition and antifungal activity of volatile extracts of *Tanaceutum audibertii* (Req.) DC., an endemic species of Sardinia and Corsica islands, were investigated.

The extract and the essential oil, obtained respectively by supercritical fluid extraction with carbon dioxide (SFE) at pressures of 90 bar and temperature of 40 °C and by hydrodistillation (HD), were analyzed by GC/FID and GC/MS. Artemisia ketone, *trans*-linalyl oxide acetate and 1,8-cineole were the main components.

The HD oil was evaluated against yeasts and dermatophyte strains, being more active against *Cryptococcus neoformans*, with MIC and MLC values of 0.64 μL/mL. Moreover, the oil revealed an important inhibitory effect on germ tube formation in *C. albicans* at sub-inhibitory concentrations. At the concentration of 1/16 MIC the inhibition of filamentation was more than 70% in comparison to untreated control cells.

1. Introduction

The genus *Tanacetum* L., which is an important *taxa* of the Asteraceae family, is widespread in Europe and western Asia and consists of about 200 species. Many species of this genus have traditionally been used as a spicy additive for food, in cosmetics and as herbal remedies due to their biologically active compounds (Rohloff et al., 2004). Especially *Tanacetum parthenium* (L.) Schultz Bip (feverfew) is a known remedy for the treatment of various diseases, including arthritis, fever, vertigo, migraine, menstrual disorders, stomach-ache, toothache, insect bites and psoriasis (Ernst and Pittler, 2000). According to recent studies, essential oils and extracts of *Tanacetum* genus exhibit anti-inflammatory (Brown et al., 1997; Ghantous et al., 2013; Mordujovich-Buschiazzo et al., 1996; Park et al., 2011; Sur et al., 2009), anticancer (Ghantous et al., 2013), antibacterial (Habibi et al., 2009; Hethelyi et al., 1991; Holopainen and Kauppinen, 1989; Mohsenzadeh et al., 2011), antiviral (Onozato et al., 2009), antifungal (Hethelyi et al., 1991), antihelmintic (Godinho et al., 2014), insecticidal (Hough-Golstein and Hahn, 1992; Nottingham et al., 1991; Panasiuk, 1984; Schearer, 1984; Suomi et al., 1986) and antiprotozoal effects (Izumi et al., 2008; Tiuman et al., 2005). Many studies have been reported on the essential oil composition of various *Tanacetum* species (El-Shazly et al., 2002; Goren et al., 2001; Greche et al., 2000; Judzentiene and Mockute, 2005; Kandemir et al., 2008; Majed-Jabari et al., 2002; Marongiu et al., 2009; Mockute and Judzentiene, 2004; Monfared et al., 2002; Piras et al., 2014; Polatoğlu et al., 2011; Rohloff et al., 2004; Weyerstahl et al., 1999) and camphor, 1,8-cineole, α-thujone, carvone, thymol, trans-sabinyl acetate, borneol, caryophyllene oxide, (E)-myroxide, sabinene, bornyl acetate, isopulegone and artemisia ketone were identified as the major constituents. This genus is also found to contain sesquiterpene lactones (Aljancic et al., 2001; Mahmood et al., 2002), a large group of molecules with several biological activities (Chaturvedi, 2011; Ghantous et al., 2010; Kreuger et al., 2012; Lesiak et al., 2010; Merfort, 2011; Wagner et al., 2008; Zhang et al., 2005).

The genus *Tanacetum* is represented in Italy by 9 taxa (Conti et al., 2005) and in Sardinia there is only one spontaneous species, *Tanacetum audibertii* (Req.) DC., used in the local traditional medicine for its digestive, vermifuge, anti-arthritic properties and

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for treatment of menstrual irregularities (Atzei, 2009). *T. audibertii* is an endemism growing in grazing lands of Sardinian and Corsican massifs, above 1300 m of altitude. Endemic *taxa* are very interesting because the geographic isolation has been caused a genetic and metabolic differentiation in these species, as shown by the high number of scientific researches that have been published until now (Fattorusso et al., 2004; Petitto et al., 2009; Ramunno et al., 2005; Serrilli et al., 2005, 2010). Some of Sardinian endemism have also shown very interesting biological and pharmacological activities (Appendino et al., 2005; Calzado et al., 2005; Poli et al., 2005).

Within a project aiming to find new agents with antifungal activity the extract obtained by supercritical fluid extraction (SFE) and the essential oil obtained by hydrodistillation (HD) were analyzed.

Volatile oils represent a small fraction of a plant's composition but confer them important characteristics to be used in the pharmaceutical, food and fragrance industries. They have a complex composition, containing from a few dozen to several hundred constituents, especially hydrocarbons and oxygenated monoterpenes and sesquiterpenes. It is important that the natural proportion of these components is maintained during extraction process. Hydrodistillation has traditionally been applied for essential oils recovery from plant materials. One of the disadvantages of this method is that essential oils undergo chemical alteration and the heat-sensitive compounds can easily be destroyed. The extraction of volatile oils using solvents at high pressure, or supercritical fluids, has received much attention in the past several years, especially in food, pharmaceutical and cosmetic industries, because it presents an alternative to conventional processes such as organic solvent extraction and hydrodistillation. The increasing use of vegetable extracts by several industries can make the extraction of volatile oils by supercritical carbon dioxide an attractive technology compared to conventional processes with respect to the product quality.

Supercritical fluid extraction with CO_2 has several advantages: the problem of toxic residual solvent in the products is eliminated; the lower temperatures involved reduce the deterioration of the thermally labile components in the extract and the organoleptic characteristics of the starting spice materials retained (Marongiu et al., 2003, 2012; Piras et al., 2012).

Thus, the aim of the present work was to compare the chemical composition of the extract obtained by SFE and the essential oil obtained by hydrodistillation. Both the extract and essential oil were analyzed by gas chromatography–mass spectrometry (GC–MS). No studies have been found in the literature concerning the composition of the oil obtained from *T. audibertii.*

2. Materials and methods

2.1. Plant material

Aerial parts of *T. audibertii* (Req.) DC. were collected in August in the Gennargentu massif (Sardinia, Italy) and air dried at 40 °C with forced ventilation for two days. Before utilization, matter was ground with a Malavasi mill (Bologna, Italy) and the particles sizes, were in the range 250–425 μm.

A voucher specimen were deposited at the Herbarium of the Botanical Garden of Cagliari (Herbarium CAG 737/A).

2.2. Volatile fraction isolation and analysis

Hydrodistillation was performed for four hours in a circulatory Clevenger-type apparatus up to exhaustion of the oil contained in the matrix, according to the procedure described in the European Pharmacopoeia (1997).

Supercritical CO_2 (purity 99% – Air Liquide Italia, Cagliari, Italy) extractions (SFE) were performed in a laboratory apparatus equipped with a 400 cm^3 extraction vessel. Waxes and essential oil were recovered in two separator vessels connected in series. Experiments were carried out at 90 bar and 40 °C in the extraction section. In the first separator the temperature was set at −10 °C and the pressure at the same value as the extraction section. The second separator was set at 15 bar and 10 °C. Extractions were carried out in a semi batch mode: batch charging of vegetable matter and continuous flow solvent. About 180 g of material were charged in each run.

Analysis of the volatile extracts were carried out by gas chromatography (GC–FID) and by gas chromatography–mass spectrometry (GC–MS).

GC–MS analysis was carried out using a gas chromatograph (Agilent, Model 6890N, Palo Alto, CA, USA) equipped with a split-splitless injector, an Agilent model 7683 autosampler and an Agilent HP5-MS fused silica column (5% phenyl-methylpolysiloxane, 30 m × 0.25 mm i.d., film thickness 0.25 μm). The GC conditions included programmed heating from 60 °C to 246 °C at 3 °C/min, followed by 20 min under isothermal conditions. The injector was maintained at 250 °C. Helium was the carrier gas, at 1.0 mL/min. Samples were run diluted in hexane with a dilution ratio of 1:100 and (1 μL) were injected in the split mode (1:20). The GC was fitted with a quadrupole mass spectrometer with an Agilent model 5973 detector. The MS conditions were as follows: ionization energy, 70 eV; electronic impact ion source temperature, 200 °C; quadrupole temperature, 150 °C; scan rate, 3.2 scan/s; mass range, 30–480 u. The software that was used to handle and analyze the mass spectra and chromatograms was an Agilent MSD ChemStation E.01.00.237. The linear retention indices (RIs) for all of the compounds were determined by injection of a hexane solution containing the C8–C26 series of n-alkanes (Van Den Dool and Kratz, 1963) were compared with those of authenticated samples from our database. The identification of the constituents was accomplished by comparison of their retention indices and their mass spectra from a home-made library or from with the literature data and the mass spectra databases, including HPCH2205 (Adams, 2007) and W8N05ST (Wiley ver. 8.0 & NIST, ver. 5.0).

Analytical GC was carried out in a gas chromatograph (Agilent, Model 7890A, Palo Alto, CA), equipped with a flame ionization detector (FID), an autosampler (Agilent, Model 7683B), Agilent HP5 fused silica column (5% phenyl-methylpolysiloxane), 30 m × 0.25 mm i.d., film thickness 0.25 μm, and a Agilent ChemStation software system. Oven temperature programme: 60–250 °C (3 °C/min), 250 °C (20 min); injector temperature: 250 °C; carrier gas: helium at 1.0 mL/min; splitting ratio 1:10; detectors temperature: 300 °C.

Percentages of individual components were calculated based on GC peak areas without FID response factor corrections.

2.3. Antifungal activity assay

The antifungal activity of the essential oil was evaluated against yeasts and dermatophytes: two clinical *Candida* strains isolated from recurrent cases of vulvovaginal (*C. krusei* H9, *C. guillermondii* MAT23); three *Candida* type strains from the American Type Culture Collection (*C. albicans* ATCC 10231, *C. tropicalis* ATCC 13803 and *C. parapsilopsis* ATCC 90018); one *Cryptococcus neoformans* type strain from the Colección Espanõla de Cultivos Tipo (*C. neoformans* CECT 1078); three dermatophyte clinical strains isolated from nails and skin (*Epidermophyton floccosum* FF9, *Trichophyton mentagrophytes* FF7 and *Microsporum canis* FF1), and four dermatophyte type strains from the Colección Espanõla de Cultivos Tipo (*T. mentagrophytes* var. *interdigitale* CECT 2958, *T. rubrum* CECT 2794, *T. verrucosum* CECT 2992, and *M. gypseum* CECT 2908). All strains were stored in Sabouraud dextrose broth with 20% glycerol at −80 °C and

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