Industrial Crops and Products 83 (2016) 113–117

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Tetraclinis articulata (Vahl.) Masters essential oil from Tunisia: Chemical characterization and herbicidal and antifungal activities assessment

Asma Ben Ghnaya[a,b,*], Ismail Amri[b], Mohsen Hanana[c], Samia Gargouri[d], Bassem Jamoussi[e], Abderrahmane Romane[f], Lamia Hamrouni[b]

[a] *Laboratoire des Microorganismes et Biomolécules Actives, Département de Biologie, Faculté des Sciences de Tunis, Campus Universitaire, 2092 Tunis, Tunisia*
[b] *Laboratoire de Gestion et Valorisation des Ressources Forestières, Université de Carthage, Institut National de Recherches en Génie Rural, Eaux et Forêts, PB 10, 2080 Ariana, Tunisia*
[c] *Laboratoire de Physiologie Moléculaire des Plantes, Centre de Biotechnologie de Borj-Cédria (CBBC), BP 901, 2050 Hammam-lif, Tunisia*
[d] *Laboratoire de Protection des Végétaux, Institut National de Recherche Agronomique de Tunisie, PB 10, 2080 Ariana, Tunisia*
[e] *Laboratoire des Matériaux, Institut Supérieur d'Education et de Formation Continue, 43 Rue de la Liberté, 2019 Le Bardo, Tunisia*
[f] *Laboratoire de Chimie Organique Appliquée, Département de Chimie, Equipe Analyse et Contrôle de Qualité, Faculté des Sciences Semlalia, Université Cadi Ayyad, BP 2390 Bd. Prince Moulay Abdallah, Marrakech 40100, Morocco*

ARTICLE INFO

Article history:
Received 24 June 2015
Received in revised form 9 December 2015
Accepted 11 December 2015
Available online 4 January 2016

Keywords:
Antifungal activity
Tetraclinis articulate
Essential oil
Phytotoxicity
Pesticides
Bio-herbicide
Monoterpenes hydrocarbons

ABSTRACT

Our study is in line with the valorization of Tunisian medicinal and aromatic plants in order to discover new bioactive natural products. So the aim was to characterized the physico-chemical properties and yield of the essential oil obtained by hydrodistillation from the leaves of Tunisian *Tetraclinis articulata* (Vahl.) Masters. Thirty one compounds were identified using gas chromatography (GC) and gas chromatography–mass spectrometry (GC/MS): they accounted for 91% of the essential oil composition with a majority of monoterpenes hydrocarbons (62.80%), in particular α-pinene (56.21%) and β-myrcene (3.08%). Further investigations allowed the identification of oxygenated monoterpenes, which represented 18.98%, with 1,8-cineole as the major compound of this fraction. The oil antifungal activity was assessed towards five phytopathogenic fungi. The strongest activity was observed against *Botrytis cinerea* with 71.17% inhibition. The oil herbicidal properties were tested on *Sinapis arvensis* L. and *Phalaris canariensis* L. Results indicated that *T. articulata* essential oil completely inhibited the seed germination of *S. arvensis* L. at high concentration (4 μl/ml), while at low doses (1 and 2 μl/ml), it delayed the germination and reduced the seedling growth of both weeds tested. Indeed, the use of these essential oils causes inhibition of weeds germination by setting their vegetative growth.

1. Introduction

Medicinal and aromatic plants (MAPs) are used as plants themselves or parts of plants or can be processed by extraction of essential oils. They are used in pharmacy, cosmetology, perfumes and the food industry among others. In recent years, their use has increased greatly in the world. In Tunisia, and in other North African countries, the use of MAPs in human and veterinary medicine, in particular against intestinal and respiratory diseases, was reported by Le Folc'h (1983) and Buhagiar et al. (2000). *Tetraclinis articulata* (Vahl.) Masters belongs to the Cupressaceae family and is widespread throughout North Africa. In Tunisia, this coniferous tree is found in the north-east region of the country (Pottier-Alapetite, 1981). The oil composition of some *T. articulata* organs was studied by several authors (Ait et al., 1990; Buhagiar et al., 2000; Tekaya-Karoui et al., 2007) and some of them (Chikhoune et al., 2013) showed that, in Algeria, the major components of volatile oils in leaves were α-pinene (19.8–24.9%) and bornyl acetate (40.2–59.2%). In Tunisia, Tekaya-Karoui et al. (2011) demonstrated that the main compounds of oils prepared from woody terminal branches of *T. articulata* were monoterpenes hydrocarbons, but with no sesquiterpenes detected.

* Corresponding author at: Laboratoire des Microorganismes et Biomolécules Actives, Département de Biologie, Faculté des Sciences de Tunis, Campus Universitaire, 2092 Tunis, Tunisie.
E-mail addresses: benghnaya_asma@yahoo.fr (A. Ben Ghnaya), romane@uca.ma (A. Romane), hamrounilam@yahoo.fr (L. Hamrouni).

http://dx.doi.org/10.1016/j.indcrop.2015.12.026
0926-6690/

While in the literature, the herbicidal effects of essential oils from various plants against weeds had been previously reported (Batish et al., 2008; Dayan et al., 2009; Ens et al., 2009; Amri et al., 2012), no studies describing the herbicidal activity of essential oils of *T. articulata* growing in Tunisia was published yet.

Our study lies therefore within the scope of the valorization of Tunisian medicinal and aromatic plants in order to discover new bioactive natural products. Accordingly, the objectives of this work were, firstly, to characterize the main components of the essential oil obtained from the leaves of *T. articulata*. Secondly, we evaluated their antifungal potential against five phytopathogenic fungi, and their herbicidal effects against germination and seedling growth of two common weeds found in Tunisia, *Sinapis arvensis* L. and *Phalaris canariensis* L.

2. Materials and methods

2.1. Plant material

T. articulata is a traditional plant used in Tunisia, Morocco and Algeria. Leaves from cultivated plants of *T. articulata* were collected during October 2014 at Korbous (Nabeul, Tunisia). Five samples collected from five different trees were harvested, mixed for homogenization, and used in three replicates for essential oil extractions. Each plant specimen was submitted to the herbarium division of the institute and their identification was confirmed in the Laboratory of Forest Ecology according to the flora of Tunisia (Pottier-Alapetite, 1961).

2.2. Isolation of the essential oil

Hydrodistillation (100 g in 500 ml of distilled water) of the fresh material was performed in a clevenger-type apparatus for 3 h according to the standard procedure described in the European Pharmacopoeia (2004). The oil obtained were collected, dried over using anhydrous sodium sulfate (a pinch per 10 ml) and stored in sealed glass vials at 4 °C until analysis. Yield was calculated (w/w%) based on dried weight of the sample (mean of three replications).

2.3. Analysis of the essential oil

The composition of the oil was investigated by gas chromatography (GC) and gas chromatography–mass spectrometry (GC/MS). The analytical GC was carried out on an GC-2014 Shimasu gaz chromatograph (Agilent Technologies, California, USA) equipped with flame ionization detectors (FID) and a fused silica capillary column, apolar HP-5 (30 m × 25 mm ID, film thickness of 0.25 μm). The parameters were set as follows: oven temperature held at 50 °C for 1 min and then programmed at a rate of 5 °C min^{-1} to 240 °C and held isothermal for 4 min. 1.2 ml min^{-1} as carrier gas flow (nitrogen); injector temperature at 250 °C, detector at 280 °C; and the volume injected at 0.1 ml of 1% oil solution (diluted in hexane). The percentages of the constituents were calculated by electronic integration of FID peak areas without the use of response factor correction. GC/MS was performed in a Hewlett Packard 5972 MSD System. Software adopted to handle mass spectra and chromatograms was ChemStation. The identification of the compounds was based on mass spectra (compared with Wiley 275.L, 6th edition mass spectral library). Further confirmation was done from retention index data generated from a series of alkanes retention indices (relatives to C_9–C_{28} on the HP-5 and HP Innowax columns) (Davies, 1990; Adams, 2001).

2.4. Physico-chemical properties of the essential oil

The essential oil refractive index was determined using a refractometer (Euromex Holland). The density was determined using a numeric densimeter. The acid index was calculated by determining the weight in grams of potassium hydroxide needed to neutralize acidic compounds in 1 g of essential oil.

2.5. Seed germination and seedling growth experiments

Seeds of two common weeds in Tunisia, *i.e.*, *S. arvensis* L. and *P. canariensis* L., were used for herbicidal activity tests, they were collected from parent plants growing in cultivated fields.

To avoid possible inhibition of germination due to fungal or bacterial toxins, seeds were surface sterilized with 15% sodium hypochlorite for 20 min, then rinsed with distilled water. Germination was carried out on Petri dishes where seeds were placed on double-layered Whatman No. 1 filter paper moistened with a 8 ml concentration of essential oil diluted with water in a 1% solution of Tween 20 (Tworkoski, 2002). Cultures were incubated under controlled conditions (24 °C, 70% of relative humidity and a 16/8 photoperiod of 1500 lux light). The Petri dishes were closed and sealed with adhesive tape to prevent the volatile oils from escaping. The number of germinated seeds was counted daily and seedling lengths were measured after 15 days. The assays were arranged in a completely randomized design with three replications (10 seeds each) including control. The seed vigor was calculated using the following formula (Agrawal, 1980).

$$\text{Seedvigor} = \frac{\Sigma \text{ Daily counts of number ofgerminated seeds}}{\text{number of days}}$$

2.6. Antifungal activity assays

The five plant pathogenic fungal species used in this study were from the culture collection of the Tunisian National Institute of Agricultural Research (INRAT): *Fusarium avenaceum*, *Fusarium oxysporum*, *Fusarium solani*, *Fusarium culmorum* and *Botrytis cinerea*. Cultures of each fungus were maintained on potato dextrose agar (PDA) and were stored at 4 °C and in 1 ml of glycerol 25% at −20 °C. Antifungal activity, *i.e.*, hyphal growth inhibition, was studied using *in vitro* contact assays (Cakir et al., 2004). The essential oil was dissolved in 1 ml of Tween 20 (0.1% v/v) and then added into 20 ml PDA at 50 °C. A mycelial disk of approximately 5 mm in diameter, cut from the periphery of a 7 days old culture, was inoculated in the center of each PDA plate (90 mm diameter) and then incubated at 25 °C in the dark for 7 days. PDA plates treated with Tween 20 (0.1%) without essential oil were used as negative controls. The percentage of growth inhibition was calculated using the formula,

$$\%\ \text{Inhibition} = \frac{(C - T)}{C} \times 100$$

where "*C*" is the average of three replicates of hyphal extension (mm) of controls, and "*T*" is the average of three replicates of hyphal extension (mm) of plates treated with essential oil.

2.7. Statistical analyses

Data of seed germination (percentage), seedling growth (length in cm), and antifungal activity assays (percentage of inhibition) were subjected to one-way analysis of variance (ANOVA) using the SPSS 13.0 software package. Differences between means were tested through Student–Newman–Keuls and values of $p \leq 0.05$ were considered significantly different.