Industrial Crops and Products 87 (2016) 315–323

Contents lists available at ScienceDirect

Industrial Crops and Products

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

Sub-lethal concentrations of Colombian *Austroeupatorium inulifolium* (H.B.K.) essential oil and its effect on fungal growth and the production of enzymes

Carlos David Grande-Tovar[a], Clemencia Chaves-Lopez[b,*], Manuel Viuda-Martos[c], Annalisa Serio[b], Johannes Delgado-Ospina[a], Josè Angel Perez-Alvarez[c], Nilson Ospina[a], Salvatore la Tora[b], Silvia Palmieri[b], Antonello Paparella[b]

[a] *Grupo de Biotecnología, University of San Buenaventura Sede Cali, Avenida 10 de Mayo, La Umbría, Vía a Pance/PBX: 488 22 22 - 318 2200, Cali, Colombia*
[b] *Faculty of Bioscience and Technology for Food, Agricolture and Environment, University of Teramo, Via C.R. Lerici 1, 64023 Mosciano Sant'Angelo (TE), Italy, Italy*
[c] *IPOA Research Group (UMH-1 and REVIV-Generalitat Valenciana), Agro-Food Technology Department, Escuela Politécnica Superior de Orihuela, Miguel Hernández University, Orihuela, Alicante, Spain*

ARTICLE INFO

Article history:
Received 18 November 2015
Received in revised form 21 March 2016
Accepted 24 April 2016
Available online 2 May 2016

Keywords:
Austroeupatorium inulifolium
Essential oils
Enzymatic activities
Antifungal activity
Penicillium

ABSTRACT

In this work, we studied the effects of *Austroeupatorium inulifolium* (H.B.K.) essential oil on the growth and the enzyme production of phyto-pathogens species such as *Aspergillus* spp., *Penicillium* spp. and *Fusarium* spp. *"in vitro"*.

Fungal growth was inhibited up to 70%, with *P. brevicompactum* and *F. oxysporum* being the most sensitive strains, while *Aspergillus parasiticus* and *Penicillium nalgiovense* were the most resistant. Sub-lethal concentrations of *A. inulifolium* essential oil increased pectinase, cellulase, protease and amylase activities in *P. brevicompactum*, *A. flavus* and *F. oxysporum*, with a concentration-dependent induction.

Among the 71 compounds revealed by GC–MS analysis, *trans* β –caryophyllene and ledene oxide (II) were the most abundant molecules. The antioxidant potential was measured using four *in vitro* assays, revealing a good antioxidant activity.

The obtained results suggested the importance of the assessment of enzyme production involved in plant wall cell degradation, when the antifungal activity is evaluated. In addition, the use of sub-lethal concentrations of *A. inulifolium* essential oil to increase enzymatic activities in fungi can have great potential in the field of bioprospecting and biotechnology.

1. Introduction

In order to prevent the fungal growth, several synthetic antifungal compounds have been used; however, there is some concern due to their effects related to environmental issues, residual toxicity, safety, development of resistant races of the pests, resurgence of secondary pests, and ecological imbalance (Shukla et al., 2009). In the last decades, a variety of natural compounds derived from microorganisms such as bacteriocins, peptides, lipopeptides, volatile compounds (Chaves-López et al., 2015; De Lucca and Walsh, 1999; Lu et al., 2009) or derived from plants such as aqueous extracts or essential oils (Cowan, 1999; Ramasamy and Charles, 2009) have been demonstrated to possess antifungal action and may provide an alternative to chemical compounds.

In particular, the antifungal activities of the natural essential oils have been widely recognized, and currently several studies are underway to develop plant-based essential oils antifungal agents to control phytopathogenic fungi that cause severe damage to crops.

"Salvia amarga", whose scientific name is *Austroeupatorium inulifolium* Kunth *(Austroeupatorium inulifolium* (H.B.K.), R. M. King & H. Rob. *Eupatorium inulifolium* Kunth) is an environmental weed native to South America. It can be found from Panama to Argentina. It belongs to the Asteraceae family. It grows well in savannas, swamps, forest borders, disturbed areas, from 100 to 2100 m and is listed as an "agricultural and environmental weed" in the Global Compendium of Weeds (Randall, 2012). *A. inulifolium* provides continuous soil cover after crop harvest, suppresses invasive grasses, and generates large quantities of leguminous biomass.

* Corresponding author.
E-mail address: cchaveslopez@unite.it (C. Chaves-Lopez).

http://dx.doi.org/10.1016/j.indcrop.2016.04.066
0926-6690/

A. inulifolium is among the ten plants most used empirically as medicine in the rural areas of the Colombian Andes. Some studies report that essential oil and extracts obtained from this species have shown biological activities including insecticidal (Arango, 2004; Lancelle et al., 2009), antibacterial against *Staphylococcus aureus, Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* (Álvarez et al., 2005; Ferraro et al., 1977; Sanabria-Galindo et al., 1998), and anti-inflammatory (Gorzalczany et al., 1996).

No previous work on the antifungal effect of the essential oil of *A. inulifolium* has been published. Therefore, considering the traditional use of *A. inulifolium* and its biological properties, the aim of the present study was to examine the chemical composition of the essential oil, as well as to determine its antifungal activity against some phyto-pathogens species. Since hemi-cellulase, protease, amylase, lipase, and other enzymes have an important role in fungal infestation and deterioration of host plants (Subramoni et al., 2009), we also studied the influence of *A. inulifolium* essential oil on some fungal enzymatic activities.

2. Materials and methods

2.1. Plant material

The aerial parts of *Austroeupatorium inulifolium* Kunth were collected from Dapa (Valle del Cauca – Colombia) geographical position 3° 32′ 57.9″N and 76° 34′ 29.3″, altitude 1800 m, in July 2014. The leaves were separated from the other parts. All collected fresh samples were dried at room temperature for 6 h. A sample of this specimen was deposited in the Department of Biology, Faculty of Science, Universidad del Valle, Cali, Colombia.

2.2. Essential oil preparation

The leaves of *Austroeupatorium inulifolium* were dried at room conditions for two days after harvest (approximately 12% humidity), afterwards 40.0 g were placed in the Clevenger type extraction apparatus and stripping was performed with steam for 4 h, following the European Pharmacopoeia (2004). The essential oil obtained was then dried with anhydrous sodium sulfate (Sigma Aldrich, United States) and kept in a sealed dark glass bottle at 4° C until chemicals and antifungal activities analyses were carried out.

2.3. GC–MS analysis

The composition of essential oil was determined by gas chromatography-mass spectrometry (GC–MS) in a gas chromatograph spectrometer AT 6890 Series plus (Agilent Technologies, Palo Alto, California, USA), with a mass selective detector (Agilent Technologies, MSD 5975, Inert XL) (full scan). Analysis was carried out using DB-5MS fused silica capillary column (60 m, 0.25 mm; 0.25 μM, J&W Scientific Inc., Folsom, CA, USA). The temperature program used was 10 min at 60 °C, then to 250 °C at 5 °C/min, held for 10 min. Other operating conditions were as follows: carrier gas, helium (99.99%), with a flow rate of 1.1 mL/min; injection volume of 2:1 and split ratio 1:30; 0.1 μL of samples were injected manually in split mode. The identification of the main components of the essential oil was carried out using electron ionization (EI, 70 eV). Mass range was from *m*/*z* 50–550. The constituents were identified by comparison of their RI (retention index) relative to C_5–C_{24} *n*-alkanes obtained on a nonpolar HP-5MS column, with the RI provided in the literature, and by comparison of the mass spectra with those recorded by Adams database (Wiley, 138 and NIST05).

2.4. Antioxidant activity

2.4.1. DPPH radical scavenging assay

The free radical scavenging activity of the sample (20 mg/mL) was measured according to the methodology described by Brand-Williams et al., 1995 using the stable radical DPPH. Results were expressed in μg Trolox equivalent/g sample.

2.4.2. Ferric reducing antioxidant power

The ferric reducing antioxidant power (FRAP) of the sample (20 mg/mL) was determined by using the potassium ferricyanide-ferric chloride method (Oyaizu, 1986). Results were expressed in μg Trolox equivalent/g sample.

2.4.3. Ferrous ion-chelating ability assay

Ferrous ions (Fe^{2+}) chelating activity (FIC) of sample (20 mg/mL) was measured by inhibiting the formation of Fe^{2+}- ferrozine complex after treatment of test material with Fe^{2+}, following the method of Carter (1971). Results were expressed in μg EDTA equivalent/g sample.

2.4.4. ABTS radical cation ($ABTS^{\bullet+}$) scavenging activity assay

The $ABTS^{\bullet+}$ scavenging activity assay of sample (20 mg/mL) was determined as described by Mazzarrino et al., 2015. Absorbance values were measured on a spectrophotometer at 734 nm. The results were calculated based on a calibration curve of Trolox, and results were expressed as μg Trolox equivalent (TE)/g of sample.

2.5. Antifungal activity assays

2.5.1. Fungal species

Eight phytopathogenic fungi, *Aspergillus flavus* ATCC® 32612™, *Aspergillus flavus* DSMZ 62065, *Aspergillus parasiticus* DSMZ 2038, *Fusarium culmorum* ATCC® 60362™, *Fusarium oxysporum* ATCC® 62506™, *Penicillium brevicompactum* DSMZ 3825, *Penicillium expansum* DSMZ 1282, *Penicillium nalgiovense* MS01, were used in this study. The last strain, that belonged to the Faculty of Bioscience and Technology for Food, Agricolture and Environment University of Teramo, was isolated from Italian salami (Chaves-López et al., 2015) and identified on the bases of morphological characteristics according to Domsch et al., (1980) and Samson et al. (1995).

2.5.2. Antifungal activity assays

Antifungal analysis was based on hyphal growth inhibition. 20 μg/mL of the pure essential oil and its dilutions (50, 25, 12.5, 6.25, 3.13 and 1.56%) prepared by dispersing the oil in sterile PBS (Phosphate Buffer Saline) 50 mM pH 7.0 and Tween 80 (1%), were spread on the plate surface containing potato dextrose agar (PDA Oxoid Thermofisher, Rodano, Italy). Petri dishes were inoculated with 8 mm plugs from 5 day old cultures and incubated at 25 °C. The test was performed in triplicate. The diameter of the radial growth of the fungi was measured daily for 7 days using a Caliper.

The antifungal index was calculated as follows:

$$\text{Antifungalindex}(\%) = 100(C - T)/C$$

where C is the diameter of fungal growth on the control and T is the diameter of the fungal growth on the test plate.

Data obtained were fitted with the Gompertz equation modified by Zwietering et al. (1990) to estimate the main growth parameters: λ (lag time), μmax (the maximum exponential growth rate), and A (maximum growth value) as follow:

$$\text{Ln}(Dt/Do) = A\exp\{-\exp[\mu m.e/A)(\lambda - t) + 1]\}$$

where Dt (cm) is the average colony diameter at each of the time of the experiment, Do (cm) is the average colony diameter at the