**SUPPLEMENTARY MATERIAL**

Antioxidant and antifungal activity of different extracts obtained from aerial parts of *Inula crithmoides* L.

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The total phenolic content, antioxidant and antifungal activities of three *Inula crithmoides* extracts (n-hexane, DCM and MeOH) were investigated. The methanolic extract showed the highest total phenolic content. In the DPPH assay the methanolic and hexane extracts exhibited the highest DPPH-radical scavenging activity; in the 5-lipoxygenase assay the hexane extract showed greater inhibitory effect with an IC50 similar to that of Trolox and Ascorbic acid. The antifungal activity of the methanolic extract revealed a higher activity against *Phytophtora cryptogea* and *Alternaria solani*.

**Keywords**: *Inula crithmoides*;phenolics;antioxidant activity; antifungal activity

**Experimental**

***Plant material***

Aerial parts of *I. crithmoides* were collected during the flowering period (August) at Fano (PU), in the area restricted to coastal habitats, and authenticated by Doctor Laura Giamperi, Botanical Institute, University of Urbino. Voucher specimens of these plants have been deposited at the Herbarium of the Botanical Garden of the University of Urbino (# GS 200). The aerial parts of *I. crithmoides* were dried in a drying chamber with circulation and continuous renewal of air for 6, 12, 24, 48, 72, 96 and 120 h at a temperature of 35 ± 5 °C. After drying, the material obtained was pulverized with Moulinex A5052HF apparatus.

***Sequential extraction***

The ground aerial parts (13 g) were extracted at room temperature (r.t.) for 24 h with 250 mL of *n*-hexane (H). Then, the suspension was filtered under vacuum, the solid residue pressed and the solvent removed under reduced pressure leading to the H extract. The residue was sequentially extracted at r.t. for 72 h with 250 mL of dichloromethane (DCM). After this period it was filtered, pressed and the filtrate was evaporated under reduced pressure, leading to the DCM extract. Finally, the residue was extracted with 250 ml of methanol (MeOH) at r.t. for 24 h. After the usual processing procedure, leading to the MeOH extract. All the extractions were triplicated.

***Total phenolic determination***

Total content of polyphenolic compounds in *I. crithmoides* extracts was determined by the Prussian Blue method described by Giamperi et al. (2012). Quercetin (SIGMA Chemical Co., St.Louis, MO) was used as standard. All tests were repeated three times.

***DPPH free-radical scavenging activity***

Radical scavenging activity was determined by a spectrophotometric method based on the reduction of an ethanol solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) according to a previously described procedure (Giamperi et al., 2012). Tests were carried out in triplicate. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), BHT (butylated hydroxytoluene) and Ascorbic acid were used as positive controls and purchased from SIGMA. The EC50 values, defined as the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%, were calculated from the results previously plotted and analyzed. The results are the mean±standard deviation (SD) of three experiments.

***5- Lipoxygenase test***

Inhibition of lipid peroxides formation was evaluated by the 5-lipoxygenase test in samples and in positive controls. The activity of the enzyme was assayed spectrophotometrically according Giamperi et al. (2012). The IC50 values, defined as the amount of antioxidant necessary to inhibit lipid peroxidation by 50%, were calculated from the results. All tests were repeated three times.

***Antifungal activity***

Fungal plant pathogens used in these tests were *Fusarium poae* (Peck) Wollenweber, *Fusarium graminearum* Schwabe, *Fusarium semitectum* Berkeley et Ravenel, *Fusarium oxysporum* Schl., *Fusarium culmorum* (Smith) Saccardo, *Fusarium avenaceum* (Corda: Fries), *Alternaria solani* and *Phytophtora cryptogea* kindly supplied by the DI.PRO.VAL. (Dipartimento di Protezione e Valorizzazione Agroalimentare, Università degli Studi di Bologna). All the microorganisms used were maintained in potato dextrose agar (PDA), Sigma, and subcultured every 30 days. The phytopathogenic fungi were tested by an agar dilution method (Bucchini et al., 2013) and extracts concentrations of 100, 200, 400, 800, 1600, 3200 and 6400 ppm were tested. The values were expressed in terms of percent inhibition of growth compared to control = 100. The fungicidal activity of the extracts was evaluated using the method described in our previous work (Bucchini et al 2013): the mycelia discs were transferred from Petri dishes in which no growth was observed (total inhibition = 100) onto fresh plates of PDA, in order to verify, after three days, either fungistatic or fungicidal activity of such inhibition. All tests were repeated three times.

***Statistical analysis***

All data are the average of triplicate analyses. The data were recorded as the mean ± standard deviation and analysed by SPSS (version 9.0 for Windows 98, SPSS Inc.). One-way analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by Duncan’s multiple range test. P values of < 0.05 were regarded as significant and p values of < 0.01 very significant.

**Table S1**. Extraction yields, total phenolic content and antioxidant activities of the studied extracts.a

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Extract | Extraction yielda | Total phenolic contentb | DPPHc  EC50 (mg DW/mL) | 5-LOX IC50c  (µg DW/mL) |
| H | 1.39 ± 0.04 | 6.64 ± 0.67 | 0.57 ± 0.03 | 13.48 ± 1.02 |
| DCM | 0.67 ± 0.03 | 3.85 ± 0.35 | 1.48 ± 0.09 | 951.38 ± 89.42 |
| MeOH | 0.62 ± 0.03 | 15.52 ± 1.76 | 0.59 ± 0.02 | 97.45 ± 9.20 |
| BHT |  |  | 0.087 ± 0.012 | 3.86 ± 0.25 |
| Ascorbic acid |  |  | 0.110 ± 0.007 | 18.63 ± 1.31 |
| Trolox |  |  | 0.007 ± 0.001 | 11.89 ± 1.22 |
| a yield %, w/w is the mean of three extractions±standard deviation (SD); b mg/g DW of starting material; cValues are the mean of three determinations±standard deviation (SD). | | | | |

**Table S2.** Fungal growth inhibition of methanol extract (%)

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Phytopathogenic fungi | Nystatin  (ppm)a | Tested doses (ppm) of MeOH extract  Inhibition (%) | | | | | | |
| 50 | 100 | 200 | 400 | 800 | 1600 | 3200 | 6400 |
| ***F. oxysporum*** | 100 | 41.2±3.5 | 45.3±4.1 | 100b | 100a | 100a | 100a | 100a |
| ***A. solani*** | 100 | 42.0±3.8 | 50.5±5.0 | 100b | 100a | 100a | 100a | 100a |
| ***P. cryptogea*** | 100 | 45.0±4.0 | 58.0±5.3 | 100b | 100a | 100a | 100a | 100a |

afungicidal; bfungistatic

**Table S3.** Fungal growth inhibition of hexane extract (%)

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Phytopathogenic fungi | Nystatin  (ppm)a | | Tested doses (ppm) of H extract  Inhibition (%) | | | | | | | | |
| 50 | | 100 | | 200 | | 400 | 800 | 1600 | 3200 | 6400 |
| ***F. oxysporum*** | | 100 | | 40.6±3.5 | | 45.3±4.3 | 100b | 100a | 100a | 100a | 100a |
| ***A. solani*** | | 100 | | 40.0±3.8 | | 49.5±4.0 | 100b | 100a | 100a | 100a | 100a |
| ***P. cryptogea*** | | 100 | | 44.0±4.0 | | 57.8±5.2 | 100c | 100a | 100a | 100a | 100a |

afungicidal; bfungistatic

**Table S4.** Minimal inhibitory concentration (MIC) and minimal fungicidal concentration (MFC) of methanolic and hexane extracts

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | MIC MeOH | MIC H | MFC MeOH | MFC H |
| ***F. oxysporum*** | 250 | 250 | 600 | 600 |
| ***A. solani*** | 250 | 250 | 600 | 600 |
| ***P. cryptogea*** | 250 | 250 | 600 | 600 |
|  |  |  |  |  |