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Variability of antioxidant and antibacterial effects of essential oils and acetonic extracts of two edible halophytes: *Crithmum maritimum* L. and *Inula crithmoïdes* L.

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ABSTRACT

This work aimed to assess the richness of the food halophytes *Crithmum maritimum* and *Inula crithmoïdes* on phenolics and essential oils (EOs) and to evaluate the antioxidant and antibacterial potential of these metabolites. Results displayed that extract of *I. crithmoïdes* possesses considerable contents of phenolic compounds (14.1 mg GAE.g^{-1} DW) related to important antioxidant activities (IC_{50} = 13 μg ml^{-1} for the DPPH test) as compared to *C. maritimum*. *C. maritimum* EOs composition is dominated by oxygenated monoterpenes, while *I. crithmoïdes* one is mainly consisted by monoterpene hydrocarbons. EOs have low antioxidant activity as compared to acetone extracts; nevertheless, they show best antimicrobial activity. A significant variability is also depicted between the provenances of each species and depended on the chemical nature of antioxidant and antibacterial molecules as well as the used tests.

1. Introduction

Synthetic antioxidants have been used in the food industry since the 1940s, but trends in many health-related industries tend to shift preferences to natural sources of natural antioxidants and antimicrobial that can be employed as food additives (Gourine, Bombarda, Nadjemi, Stocker, & Gaydou, 2010). The EOs and extracts of many plant species have become popular in recent years and attempts to characterise their antioxidant activity and their use in food processing are advised (Gourine et al., 2010; Ksouri et al., 2012Ksouri, Megdiche Ksouri, Jallali, Debez, Magné & Abdelly, 2011). However, plants of importance in modern agriculture and trade are not restricted to traditional food, but increasingly include species with secondary metabolites providing source material for food preservation (Ksouri et al., 2009, 2012). In this context, halophyte species are promising candidates with important nutritional, medicinal and economic potentials (Ksouri et al., 2012). In fact, in arid and semi-arid regions, plants are often subjected to severe environmental conditions (salinity, drought, extreme temperatures, etc.) that strongly influence phenolics and EOs biosynthesis and distribution given that these secondary metabolites are involved in stress defense mechanism in these plants (Jallali et al., 2012; Karray-Bouraoui et al., 2011).

Crithmum maritimum (Apiaceae), or sea fennel, is a food halophyte found on rocky shores of Mediterranean sea and Atlantic ocean (Głoniak et al., 2006). This plant has ethnopharmacological utilisation in folk medicine (essentially as antiscorbutic and diuretic) due to its richness on vitamin C, iodine, carotenoids, and phenolics (Ben Amor, Ben hamed, Debez, Grignon, Abdelly, 2005). Moreover, *C. maritimum* represents a part of human diet. The whole plant is "of a spicy taste with certain saltiness" justifying its use as ingredient in salads (Hedrick, 1972). Its seeds contain also appreciable amounts of oil, potentially edible due to its fatty acid composition, close to olive-oil (Ben Amor, Ben hamed, Debez, Grignon, & Abdelly, 2005).

The genus *Inula*, from Asteraceae family, exists as more than 100 species and is mainly found in Europe, Africa, and Asia. *Inula crithmoïdes* constitute a part of human diet. Young leaves are eaten raw or cooked (Facciola, 1990). The fleshy leaves and young shoots are pickled and used as a relish in salads (Facciola, 1990; Hedrick, 1972). The flowering branches of *Inula* species are used in traditional medicine for treatment of bronchitis, tuberculosis, anemia, as astringent, for malaria and diseases of urinary system (Abdel-Wahhab, Abdel-Azim, El-Nekeety, 2008).

Several studies demonstrated that plant secondary metabolites differ significantly between species. Even extracts of the same species may vary, depending on plant origin, climate, soil quality,

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harvest season, and extraction procedures (Jallali et al., 2012). Therefore, the aim of this work was to study the interspecies (*C. maritimum* and *I. crithmoïdes*) and intra-species (two different localities for each species) variability of phenolics and EOs contents and their antioxidant and antibacterial activities.

2. Materials and methods

2.1. Chemical and reagents

All chemicals and reagents were purchased from Sigma–Aldrich (GmbH, Sternheim, Germany).

2.2. Plant sampling

Crithmum maritimum and *I. crithmoïdes* were harvested in their native salty ecosystems, from two different Tunisian regions each. *C. maritimum* aerial parts were collected from Kelibia costs (CM_K) (Sub-humid), and in Monastir diff (CM_M) (Lower semi-arid). *I. crithmoïdes* aerial parts were collected in Soliman estuary (IC_S) (Upper semi-arid), and in El Kalbia marsh (IC_K) (Upper arid) (Table 1). *C. maritimum* was harvested at the end of August 2009 when umbels started the fructification; while *I. crithmoïdes* was collected in October 2009 corresponding to the flowering period.

2.3. Extraction of phenolic compounds

Crithmum maritimum and *I. crithmoïdes* aerial parts were air-dried in shadow at room temperature then reduced to a fine powder. Extracts were obtained by mixing 2.5 g of the powder of each sample with 25 ml of 80% aqueous acetone (v/v). Maceration was performed at ambient temperature in a plug container with frequent agitation for 30 min. The extracts were then kept for 24 h at 4 °C, filtered through Whatman filter paper N°4, and evaporated under vacuum to dryness. The extracts were then stored in the darkness at 4 °C until analysis.

2.4. Determination of phenolic compound contents

2.4.1. Determination of total polyphenol content

Colorimetric quantification of total polyphenol was determined as described by Dewanto, Wu, Adom, and Liu (2002). Briefly, 125 μL of suitable diluted sample extract was dissolved in 500 μl of distilled water and 125 μl of the Folin–Ciocalteu reagent. The mixture was shaken before adding 1250 μl sodium carbonate anhydrous (Na_2CO_3) (70 g l^{-1}), then adjusted with distilled water to a final volume of 3 ml, and mixed thoroughly. After incubation for 90 min at 23 °C in the dark, the absorbance versus prepared blank was read at 760 nm (LABOMED, INC. UV/Vis apparatus). Total phenolic content of plant aerial parts was expressed as mg gallic acid equivalents per gram of dry weight (mg GAE g^{-1} DW) through the calibration curve with gallic acid ranging from 0 to 400 μg ml^{-1} (r^2 = 0.99). All samples were analysed in three replications.

2.4.2. Estimation of total flavonoid content

Total flavonoids were measured by colorimetric assay according to Dewanto et al. (2002). An aliquot of diluted sample or standard solution of (+)-catechin was added to a 75 μl of sodium nitrite solution ($NaNO_2$), and mixed for 6 min before adding 0.15 ml of aluminum chloride hexahydrate solution ($AlCl_3$, $6H_2O$) (100 g l^{-1}). After 5 min, 0.5 ml of sodium hydroxide NaOH (1*M*) was added. The final volume was adjusted to 2.5 ml with distilled water and thoroughly mixed. Absorbance was determined at 510 nm against the same mixture, without the sample, as a blank. Total flavonoid content was expressed as milligram catechin equivalent per gram dry weight (mg CE.g^{-1} DW), through the calibration curve of (+)-catechin ranging from 0 to 400 μg ml^{-1} (r^2 = 0.99). All samples were analysed in three replications.

2.4.3. Quantification of total condensed tannins

Proanthocyanidins were measured using the modified vanillin assay described by Sun, Richardo-da-Silvia, and Spranger (1998). To 50 μl of properly diluted sample, 3 ml of methanol vanillin solution and 2.5 ml of sulfuric acid (H_2SO_4) were added. The absorption was measured at 500 nm against extract solvent as a blank. The amount of total condensed tannins is expressed as mg CE.g^{-1} DW. The calibration curve range 0–400 μg ml^{-1} (r^2 = 0.99). All samples were analysed in three replications.

2.5. Antioxidant activities of C. maritimum and I. crithmoïdes extracts

2.5.1. Evaluation of total antioxidant capacity

The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH. An aliquot (0.1 ml) of plant extract was combined to 1 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate), then incubated in a thermal block at 95 °C for 90 min. After the mixture had cooled to room temperature, the absorbance of each solution was measured at 695 nm against a blank. The antioxidant capacity was expressed as mg GAE.g^{-1} DW (Koleva, Teris, Jozef, Linssen, & Lyuba, 2002). The calibration curve range was 0–500 μg ml^{-1}. All samples were analysed in triplicate.

2.5.2. DPPH assay

DPPH (2,2-Diphenyl-1-picrylhydrazyl) quenching ability of plant extracts and EOs was measured according to Hanato, Kagawa, Yasuhara, and Okuda (1988). 1 ml of the samples was added to 250 μl of 0.2 mM solution of DPPH. After 30 min of incubation at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of DPPH free radical in percent (I%) was calculated as follows:

$$I(\%) = [(A_0 - A_1)/A_0] \times 100 \quad (1)$$

Table 1
Location and main ecological traits of the analysed provenances of *C. maritimum* L. and *I. crithmoïdes* L.

Species	Locality	Bio-climatic zone	Latitude	Longitude	Altitude (m)	Q_2[a]	Rainfall (mm/year)
CM	Kélibia	Sh	N 36°57'47	E08°45'23	2	162	710
	Monastir	Lsa	N35°46'38	E10°50'01	4	68	350
IC	Soliman	Usa	N36°71'80	E10°42'90	2	80	432
	El Kalbia	Ua	N35°48'53	E10°09'07	29	46	300

CM: *C. maritimum*.
IC: *I. crithmoïdes*.
Sh: sub-humid, Usa: upper semi-arid; Lsa: Lower semi-arid; Ua: Upper arid.
$Q_2 = 2000P/(M^2-m^2)$ where P is the mean annual rainfall (mm), M is the average maximum temperatures (K) in the warmest month (June) and m (K) is the mean minimum temperatures in the coldest month (February).
[a] Bioclimatic zones were defined according to Emberger's (1966) pluviometric coefficient.