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Phytochemical investigation and antioxidant, antibacterial and anti-tyrosinase performances of six medicinal halophytes

A. Jdey [a,b,c], H. Falleh [a,*], S. Ben Jannet [a], K. Mkadmini Hammi [a], X. Dauvergne [c], R. Ksouri [a], C. Magné [c]

[a] Laboratoire des Plantes Aromatiques et Médicinales, Centre de Biotechnologie à la Technopôle de Borj-Cédria (CBBC), BP 901, 2050 Hammam-Lif, Tunisie
[b] Faculté des Sciences de Bizerte, 7021 Zarzouna, Tunisie
[c] EA 2219 Géoarchitecture, Université de Bretagne Occidentale, 6 Avenue V. Le Gorgeu, CS 93837, Brest Cedex 3, France

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ABSTRACT

In this study, six medicinal halophytes (*Daucus carota*, *Frankenia laevis*, *Inula crithmoides*, *Plantago coronopus*, *Raphanus raphanistrum* and *Tamarix gallica*) were examined for their phenolic pools, antioxidant (DPPH, ABTS, superoxide radical scavenging and iron-reducing tests), antibacterial (micro-dilution method) and anti-tyrosinase activities. RP-HPLC analysis of the six halophytes showed that *D. carota* and *F. laevis* exhibited the highest phenolic contents, with chlorogenic acid (6.6 mg/g DW) and catechin (15.4 mg/g DW) as the major compounds, respectively. In addition, *D. carota* and *F. laevis* shoots exhibited the best antioxidant capacities as shown by low IC_{50} values against DPPH, ABTS, and superoxide anion radicals, and strong iron-reducing capacity. Moreover, *F. laevis* extracts exhibited a broad-spectrum antibacterial activity against the four human pathogenic strains tested, mainly *Micrococcus luteus* and *Salmonella enterica*. *D. carota* efficiently inhibited *Staphylococcus aureus* and *S. enterica* growth. Interestingly, the strongest inhibition of melanin synthesis was found in *D. carota* and *F. laevis*, both species inhibiting particularly diphenolase activity. These data suggest that *D. carota* and *F. laevis* shoots could be used as effective biomolecules and also as new natural skin-whitening agents.

1. Introduction

Phenolic compounds are secondary metabolites that inhibit efficiently reactive oxygen species (ROS), manifest important antimicrobial activity and are presumed to participate in cell protection against harmful UV radiations (Rice-Evans et al., 1997; Falleh et al., 2012). Antioxidative properties of polyphenols arise from their high reactivity as hydrogen or electron donors, from the ability of the polyphenol-derived radical to stabilize and delocalize the unpaired electron (chain-breaking function), and from their ability to chelate transition metal ions (Falleh et al., 2012). Phenolic antimicrobial effects are mainly generated by causing structural or functional damage to cell membrane either for gram-positive and/or negative bacteria (Ozçelik et al., 2004). Moreover, due to their structural resemblance to L-DOPA and tyrosine (the natural substrates of tyrosinase), plant phenolic compounds could efficiently block melanin synthesis, making them attractive depigmentation agents (Rangkadilok et al., 2007). With this respect, although melanin shields the human skin from UV radiation and inhibits photocarcinogenesis (Xu et al., 2016), the excessive accumulation of this epidermal pigment causes health concerns such as freckles, melisma, solar lenities, age spots, and post-inflammatory hyper-pigmentation (Kim and Uyama, 2005). Not limited to these disorders, alterations in melanogenesis may be very harmful and leads to malignant melanoma, a skin cancer with a rapid increasing incidence (Patil et al., 2014). Thus, and considering that melanin synthesis can be controlled through inhibiting the tyrosinase activity, tyrosinase inhibitors may be cosmetically and clinically useful for the treatment of skin cancer and some dermatological disorders associated with melanin hyperpigmentation.

For all these reasons, potent antioxidant, antimicrobial and anti-tyrosinase agents are valuable targets that are gaining attention in medicinal plant research (Perluigi et al., 2003). For instance, the use of depigmentation agents is a non-invasive approach for melanogenesis control as they specifically target melanogenetic cells (Chang, 2012; Peng et al., 2013). This need is crucial and urgent as many known anti-tyrosinase commercial agents suffer from serious disadvantages like high cytotoxicity, insufficient penetrating power and low stability (Ismail et al., 2016). In that context, traditional skin depigmentation products like hydroquinone, corticosteroids and mercury containing products have been forbidden since they are potentially cytotoxic toward melanocytes. Hence, the discovery of new feasible, safe and efficient tyrosinase inhibitors is, undoubtedly, becoming an urgent concern.

As novel source of phenolic molecules, halophytes (salt-tolerant plants) are currently known for their ability to withstand and quench toxic ROS, since they are equipped with a powerful antioxidant system

* Corresponding author.
E-mail address: Hanenfalleh@gmail.com (H. Falleh).

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that includes polyphenolics (Ksouri et al., 2008; Falleh et al., 2013). Presently, an ever widening range of halophyte species are being reported from different parts of the world (Stankovi et al., 2015; Torres Carro et al., 2016; Qasim et al., 2017). However, phytochemical analyses of these wild plants still need to be addressed. Therefore, in the present study, six halophytes, widely found along Atlantic shorelines, were assessed: *Tamarix gallica* (Ksouri et al., 2008; Hassaine et al., 2014), *Daucus carota* (Hassaine et al., 2014), *Frankenia laevis* (Hassaine et al., 2014), *Raphanus raphanistrum* (Hassaine et al., 2014), *Inula crithmoides* (Jallali et al., 2009) and *Plantago coronopus* (Ivan and Oprică, 2013). These halophytes were investigated for the first time for their phenolic profiles, antioxidant and antimicrobial capacities as well as for their abilities to inhibit tyrosinase with the aim at setting out novel and natural bioactive compounds that could be used for skin-protective applications.

2. Materials and methods

2.1. Plants and sampling

Aerial parts of six medicinal species, *T. gallica* L. (Tamaricaceae), *F. laevis* L., (Frankeniaceae), *I. crithmoides* L.; a synonym of *Limbarda crithmoides* (L.) Dumort.; (Asteraceae), *D. carota* ssp. *gummifer* L. (Apiaceae0029, *R. raphanistrum* subsp. *landra* (Moretti ex DC.) (syn. *R. raphanistrum* subsp. *maritimus Thell.*) (Brassicaceae) and *P. coronopus* L. (Plantaginaceae), were harvested in summer 2014 from the coast of Brittany (France, 48°21′N 4°46′E). Each plant was identified by qualified botanist and voucher specimens, [T-TA-27], [F-FL-07], [A-IC-12], [A-DC-38], [B-RR-22], [P-PC-16] (in the same upper order); were deposited at the Herbarium of the Laboratory of aromatic and medicinal plants (at CBBC). All samples were rinsed with distilled water, freeze-dried and ground in a Mettler AE 200 blender.

2.2. Extract preparation

Extraction was performed from each plant species by magnetic stirring of 2.5 g of dry powder in 25 mL aqueous ethanol (50%, *v*/v) for 16 h at 4 °C. Then, the mixture was filtered through a Whatman No 4 filter paper and evaporated under vacuum to dryness. The dry residue was suspended in 50% ethanol at a concentration of 100 mg mL^{-1} and stored at −27 °C until analyses.

2.3. Phenolic compound determination

The assessment of phenolic compounds in halophyte extracts was done using HPLC Agilent 1260 system (Agilent Technologies, Germany) equipped with a reversed phase C18 analytical column of 4.6 × 100 mm, and 3.5 μm particle size resin (Zorbax Eclipse XDB C18). Diode array detector was set to a scanning range of 200–400 nm and column temperature was maintained at 25 °C. Mobile phase at 0.4 mL min^{-1} was constituted of a mixture of two solvents, methanol (A) and 0.1% formic acid (B), with the following gradient program: 10%A 90%B (0–5 min), 20%A 80%B (5–10 min), 30%A 70%B (10–15 min), 50%A 50%B (15–20 min), 70%A 30%B (20–25 min), 90%A 10%B (25–30 min), 50%A 50%B (30–35 min) and 10%A 90%B (35–36 min). Chromatograms were monitored at 280 nm and peak identification was obtained on the basis of retention time and UV spectra of each peak, and co-injection of pure standards.

2.4. Determination of antioxidant activities

2.4.1. DPPH-scavenging activity

The hydrogen atom or electron donation ability of the extracts was measured from the bleaching of purple colored methanol solution of 1,1-diphenyl-2 picrylhydrazyl (DPPH) according to the method described by Sokmen et al. (2004). One milliliter of various concentrations of the ethanol extracts was added to 250 μL of 0.2 mM DPPH radical solution. The mixtures were shaken vigorously and allowed to stand for 30 min in the dark. The absorbance of the resulting solutions was measured at 517 nm and the inhibition of DPPH radical was calculated as follows:

$$\text{DPPH}^{\bullet}\text{scavenging effect}(\%) = [100*(A_0 - A_1)/A0], \quad (1)$$

where A_0 and A_1 are the absorbances of the control and the sample at 30 min, respectively. The antiradical activity was expressed as IC_{50} (μg mL^{-1}), a low IC_{50} value corresponding to a high antioxidant activity. BHT was used as a positive standard, and all samples were analyzed in triplicate.

2.4.2. ABTS-scavenging activity

ABTS-quenching capacity of plant extracts was determined according to Re et al. (1999). The $ABTS^{\bullet+}$ cation radical was produced by the reaction between 14 mM ABTS solution with equal volume of 4.9 mM potassium persulfate ($K_2S_2O_8$) solution. After shaking in the dark for 16 h, this solution was diluted with ethanol to get an absorbance of 0.700 ± 0.020 at 734 nm. The reaction mixture, comprising 950 μL of $ABTS^{\bullet+}$ solution and 50 μL of the plant extracts at various concentrations, was homogenized and its absorbance was recorded at 734 nm after at least 6 min. Ethanol 50% and BHT were used as a blank and the positive control, respectively. The percentage of ABTS radical inhibition was calculated using formula (1). All measurements were done in triplicate, and the IC_{50} values were expressed as μg mL^{-1}.

2.4.3. Superoxide-scavenging activity

Superoxide anion radical-scavenging capacity of plant extracts was assessed according to Duh et al. (1999). The reaction mixture contained 0.2 mL of shoot extracts at different concentrations, 0.2 mL of 60 mM PMS, 0.2 mL of 677 mM NADH and 0.2 mL of 144 mM NBT, all in phosphate buffer (0.1 M, pH 7.4). After 5 min of incubation at room temperature, the absorbance was read at 560 nm against blank. As for the anti-DPPH activity, the $O_2^{\bullet-}$ activity in shoot extracts was expressed as IC_{50} in μg mL^{-1}.

2.4.4. Iron reducing power

The iron reducing capacity of plant extracts was determined by the transformation of Fe^{3+} to Fe^{2+}, according to the method of Oyaizu (1986). Sample solutions at different concentrations were mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide (1%, *w*/*v*). The mixture was incubated at 50 °C for 20 min. Afterwards, 2.5 mL of TCA (10%) were added and the mixture was centrifuged for 10 min at 1000*g*. Supernatant (2.5 mL) was mixed with distilled water (2.5 mL) and 0.5 mL of ferric chloride (0.1%, *w*/*v*), and the absorbance was read at 700 nm. EC_{50} value (μg mL^{-1}) is the effective concentration of the extract for which the absorbance was reduced by 50%, and it was obtained from linear regression analysis. All samples were analyzed in triplicate.

2.5. Evaluation of the antibacterial activity

2.5.1. Microorganisms tested

Antibacterial activity was screened against four human pathogenic bacteria including the Gram-positive *Micrococcus luteus* (ATCC 10240) and *Staphylococcus aureus* ssp. *aureus* (ATCC 33862), and the Gram-negative *Escherichia coli* (ATCC 4157) and *Salmonella enterica* ssp. *arizonae* (ATCC 13314).

2.5.2. Antibacterial bioassay

Strains were grown in liquid nutrient broth (Difco, Surrey, England) at 37 °C for 24 h before being used. A microplate-bioassay (microdilution) was used to study the antimicrobial activities of plant extracts. An aliquot

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