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Phenolic content, antioxidant, anti-inflammatory and anticancer activities of the edible halophyte *Suaeda fruticosa* Forssk

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ABSTRACT

Suaeda fruticosa is an edible and medicinal halophyte known for its hypoglycaemic and hypolipidaemic activities. In this study, novel biological activities of the shoot extracts related to their phenolics were investigated. Results showed an appreciable total phenolic (31.8 mg GAE/g DW) in shoot extracts. The estimation of antioxidant capacities using oxygen radical absorbance capacity (ORAC method) and a cell based-assay (WS1) of four extracts (hexane, dichloromethane, methanol and water) showed that shoot methanol extract exhibit the highest antioxidant activities. The same extract displayed the utmost anti-inflammatory activity, inhibiting nitric oxide (NO) release, by 66.4% at 160 μg/ml in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages. Besides, the dichloromethane extract showed the highest anticancer activity against human lung carcinoma (A-549) and colon adenocarcinoma cell lines (DLD-1, Caco-2 and HT-29) with specificity against DLD-1 (IC_{50} = 10 ± 1 μg/ml). These findings demonstrate the remarkable potentiality of this edible halophyte as valuable source of antioxidants which exhibit original and interesting anti-inflammatory and anticancer capacities.

1. Introduction

Halophytes are known for their ability to withstand and quench toxic reactive oxygen species (ROS), since they are equipped with a powerful antioxidant system that includes enzymatic and non-enzymatic components (Ksouri et al., 2008). A large flora of medicinal halophytes exhibit higher content of bioactive compounds as stress metabolites mainly polyphenols which have potentially useful for remedial purposes such as *Mesembryanthemum edule* known as traditional remedy against fungal and bacterial infections and as treatment of sinusitis, diarrhoea, infantile eczema and tuberculosis (Van Der Watt & Pretorius, 2001), and *Tamarix gallica* as astringent, detergent, diuretic, expectorant, and laxative (Ksouri et al., 2009). Traditionally *Suaeda fruticosa* aqueous extract contains two compounds responsible for hypoglycaemic and hypolipidaemic activities (Bennani-Kabchi, El Bouayadi, Kehel, Fdhil, & Marquie, 1999; Benwahhoud, Jouad, Eddouks, & Lyoussi, 2001), furthermore methanolic extracts of *Suaeda pruinosa* were used to detect antimicrobial activity (El-Hagrasi, El-Desouky, El Ansari, & Rabie, 2005). The excellent medicinal properties of these halophytes are mainly attributed to their antioxidant constituents. Among the various kinds of natural antioxidants, polyphenols constitute the main powerful compounds, owing to their multiple applications in food industry as additives or functional food, cosmetic, pharmaceutical and medicinal materials (Maisuthisakul, Suttajit, & Pongsawatmanit, 2007). Besides, polyphenolic constituents of various plants have been reported to contain several biological properties. In fact, these natural polyphenols may exert a chemopreventive role towards cardiovascular and degenerative diseases (Halpern et al., 1998) including neurodegenerative pathologies and cancer (Esposito et al., 2002). For centuries, traditional medicine used plants for their remedial and defensive abilities, some of widely used plants have been selected for their investigation of their chemical constituents and biological activities such as anti-inflammatory, antibacterial, anticancer in an attempt to establish a scientific basis for their ethnomedical uses (Ojewole, 2005). The search for anticancer agents from plant sources started in the 1950s (Gordon & David, 2005). Based on the recent researches, it is clear that several bioactive compounds from plants were found to possess antioxidant activities and anticarcinogenic (Dufour et al., 2007). Among them, flavonoids, carotenoids and triterpenes have been shown to inhibit cancer cell proliferation *in vitro* and have antioxidant activity by scavenging reactive oxygen species which prevent potential damage to cellular components such as DNA, proteins, and lipids (Dufour et al., 2007).

The halophyte *S. fruticosa* Forssk belonging to the Chenopodiaceae family, and to the order Caryophyllales, is an annual herbaceous plant particularly abundant in Mediterranean salt marshes. Hypoglycaemic and hypolipidaemic activities have been reported in *S.*

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fruticosa, however, no studies have been conducted on the other biological capacities. Thus, the aim of the present work was to quantify phenolic compounds of *S. fruticosa* and to evaluate the antioxidant capacity of extracts from this plant using ORAC and cell based assay as well as to screen anti-inflammatory and anticancer activities.

2. Materials and methods

2.1. Chemical and reagents

Folin–Ciocalteu reagent, sodium carbonate anhydrous (Na_2CO_3), gallic acid, sodium nitrite solution ($NaNO_2$), aluminium chloride hexahydrate solution ($AlCl_3$, $6H_2O$), and vanillin were purchased from Fluka (Buchs, Switzerland). Sulphuric acid (H_2SO_4) was obtained from Merck (Darmstadt, Germany). Fluorescein sodium salt (FL), 2′,7′-dichlorofluorescin-diacetate (DCFH-DA), 2′,7′-dichlorofluorescin (DCFH), 2′,7′-dichlorofluorescein (DCF), *tert*-butyl hydroperoxide (*t*-BuOOH), 6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (Trolox), quercetin, gallic acid and 2,2′-azobis(2-amidino-propane) dihydrochloride (AAPH) were all purchased from Sigma–Aldrich (Oakville, ON). The solvents were purchased from EMD (Canada). All other chemical reagents were purchased from Sigma–Aldrich Co., Canada or Alfa Aesar Co. and were used as received.

2.2. Plant material and extract preparation

Shoots of *S. fruticosa* were collected in August 2007 from salt flat (sebkha) of Kairouan city (35°48′ N, 10°09′ E, 133 km from Tunis, semi arid climate). This halophyte was identified at the Biotechnology Centre (Technopark of Borj-Cédria), and a voucher specimen was deposited at the Herbarium of the Laboratory of Extremophile Plants at the Biotechnology Centre. For quantification the air-dried and finely ground shoots (2.5 g) were extracted by magnetic stirring with 25 ml of acetone/water (80:20) for 30 min. Extracts were kept for 24 h at 4 °C, and then filtered through a Whatman N°4 filter paper. Then powdered samples were stored at 4 °C until analysis.

To assess biological activities, powdered shoots (30 g) were extracted in a soxhlet apparatus using several solvents with increasing polarity (hexane, dichloromethane, methanol and water). Afterwards, the extracts were filtered, and solvent was evaporated under reduced pressure using rotary vacuum evaporator. At last, shoot extracts were freeze-dried and the residue was reconstituted in Dimethyl sulphoxide (DMSO) before testing.

2.3. Phenolic content analysis

2.3.1. Analysis of total phenolic content

Total phenolic content of the shoot extracts (acetone/water (80:20)) was determined using Folin–Ciocalteu reagent slightly modified by Dewanto, Wu, Adom, and Liu (2002) using gallic acid as a standard. An aliquot of diluted sample extract was added to 0.5 ml of distilled water and 0.125 ml of the Folin–Ciocalteu reagent. The mixture was shaken and allowed to stand for 6 min, before addition of 1.25 ml of 7% Na_2CO_3. The solution was then adjusted with distilled water to a final volume of 3 ml and mixed thoroughly, and held for 90 min at ambient temperature. After incubation in dark, the absorbance at 760 nm was recorded. Total phenolic content of plant parts was expressed as milligrammes of gallic acid equivalents per gramme of dry weight (mg GAE/g DW) through the calibration curve with gallic acid. All samples were analysed in triplicates.

2.3.2. Analysis of flavonoïd content

Total flavonoid content was measured using a colorimetric assay developed by Dewanto et al. (2002). An aliquot of diluted sample or standard solution of (+)-catechin was added to 75 μl of $NaNO_2$ solution (7%), and mixed for 6 min, before adding 0.15 ml $AlCl_3$ (10%). After 5 min, 0.5 ml of 1 M NaOH solution was added. The final volume was adjusted to 2.5 ml, thoroughly mixed, and the absorbance of the mixture was determined at 510 nm. Total flavonoid content was expressed as mg (+)-catechin equivalent per gramme of dry weight (mg CE/g^{-1} DW), through the calibration curve of (+)-catechin (0–400 μg ml^{-1} range). All samples were analysed in triplicates.

2.3.3. Assessment of total condensed tannin content

The proanthocyanidins were determined by spectrophotometer method (Sun & Richardo-da-Silvia, 1998). Three millilitres of 4% methanol vanillin solution and 1.5 ml of concentrated H_2SO_4 were added to 50 μl of suitably diluted sample. The mixture was allowed to stand for 15 min, and the absorbance was measured at 500 nm against solvent as a blank. All samples were analysed in triplicates.

2.4. Assessment of antioxidant activities

2.4.1. $ORAC_{FL}$ assay

The procedure was modified from the method described by Ou, Hampsch-Woodill, and Prior (2001). Briefly, Trolox was used as a control standard. Briefly, the ORAC assay was carried out in black round bottom 96-well microplates (Costar) on a Fluoroskan Ascent FL™ plate reader (Labsystems) equipped with an automated injector. Four concentrations of Trolox (the control standard) were used (1.56; 3.13; 6.25 and 12.5 μM) in quadruplicate, and a gradient of 16 concentrations of the samples was prepared without replication. The experiment was conducted at 37.5 °C and in pH 7.4 phosphate buffer, with a blank sample in parallel. The fluorimeter was programmed to record the fluorescence (λ ex.: 485 nm/em.: 530 nm) of fluorescein every minute after addition of 375 mM of 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), for a total of 35 min. The final results were calculated using the net area under the curves of the sample concentrations for which decrease of at least 95% of fluorescence was observed at 35 min and which also presented a linear dose–response pattern. ORAC values were expressed in micromoles of Trolox equivalents (TE) per gramme (μmol TE/g).

2.4.2. Antioxidant cell assay using 2′,7′-dichlorofluorescin-diacetate (DCFH-DA)

Antioxidant activity was evaluated using the DCFH-DA assay as described by Legault, Dahl, Debiton, Pichette, and Madelmont (2003), with some modifications. Briefly, human skin fibroblast cells were plated in 96 microwell plates at 10,000 cells per well and incubated for 48 h at 37 °C and 5% CO_2. The cells were washed with 150 μl Hank's balanced salt solution (HBSS) at pH 7.4 and incubated for 30 min with 100 μl HBSS (pH 7.4) containing 5 μM DCFH-DA (Sigma–Aldrich). The cells were then washed again with 150 μl HBSS. To assess antioxidant activity, the cells were incubated either with a growing concentration of extract from *S. fruticosa*, Trolox or quercetin, in the absence or presence of 200 μM *tert*-butylhydroperoxide (*t*BH). Fluorescence was measured after 1 and 4 h on the automated 96-well plate reader (Fluoroskan Ascent FL™, Labsystems) using an excitation wavelength of 485 nm and an emission wavelength of 530 nm.

2.5. Measurement of anti-inflammatory activity by nitrite quantification

Exponentially growing cells were plated in 24-well microplates (BD Falcon) at a density of 2×10^5 cells per well in 400 μl of culture medium and were allowed to adhere overnight. Cells were then treated with or without positive control N(G)-nitro-L-arginine