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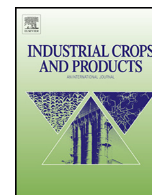

Phenolic contents and biological activities of *Limoniastrum guyonianum* fractions obtained by Centrifugal Partition Chromatography

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ABSTRACT

The ethyl acetate phase of *Limoniastrum guyonianum* Boiss. was used to separate 10 fractions by centrifugal partition chromatography. Phenolic contents and biological capacities were evaluated in all fractions. The qualitative characterization of phenolic compounds was carried out by HPLC. Fraction 8 presented the highest amount of polyphenols (980.9 mg GAE/g DR) and flavonoids (353 mg CE/g DR). However, fraction 4 as well as fraction 5 were the most provided in condensed tannins (763.9 and 755.6 mg CE/g DR) and exhibited the highest capacity to inhibit the β-carotene bleaching (IC_{50} = 36.67 μg/ml). On the other hand, fractions 3 and 4, displayed the greatest anti-amyloidogenic ability with a percentage of inhibition equal to 57 and 54% respectively. Moreover, these two fractions presented a highest and similar efficiency against all microorganisms tested. The strongest biological activities of fractions 3, 4 and 5 are due to the presence of powerful antioxidants such as catechin and phenolic acids derivatives particularly the epigallocatechin-3-O-gallate and the amides of phenolic acids (N-*E*-caffeoyl tyramine and Limoniastramide). These later are identified for the first time in this halophyte species. Results obtained indicated that *L. guyonianum* fractions can be used potentially as a ready accessible and valuable bioactive source of natural antioxidants.

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1. Introduction

Plants have been the basis of traditional medicines throughout the world for thousands of years and continue to provide new remedies to humankind. A great deal of effort has therefore focused on using available experimental techniques to identify natural antioxidants from plants (Krishnaiah et al., 2011). In fact, numerous studies reported the interest in natural substances exhibiting antioxidant properties that can be supplied as food additives or as specific preventive pharmaceuticals (Jennings and Akoh, 2009). Phenolic compounds constitute one of the most numerous and widely distributed groups of antioxidant substances in the plant kingdom with more than 8000 phenolic structures currently known (Paixão et al., 2007). The term "antioxidants" refers to the phenolic molecules that can delay or inhibit the oxidation of lipids or other molecules by inhibition of initiation or propagation of oxidation chain reaction and which can prevent or repair damage into the body's cells by oxygen (Chanwitheesuk et al., 2005). In addition, synthetic antioxidants, such as BHT and BHA, have been widely used as antioxidants in the food industry and may be responsible for liver damage and carcinogenesis (Krishnaiah et al., 2011). For this reason, recently there has been a considerable interest in finding natural antioxidants from plant materials to replace synthetic ones (Jennings and Akoh, 2009; Krishnaiah et al., 2011).

Any antibiotic has a limited effective life and the public is becoming increasingly aware of problems with the over prescription of these antibiotic. Thus, it is reported that one to three antibiotics are launched every year from microorganisms (Karou et al., 2005). Nevertheless, in developing countries where medicines are quite expensive, investigation on antimicrobial activities from traditional medicinal plants may still be needed. In recent years, pharmaceutical companies have spent considerable

Abbreviations: CPC, centrifugal partition chromatography; IC_{50}, inhibition concentration at 50 percent; BHT, butylated hydroxytoluene; BHA, butylated hydroxyanisole; n-hept, n-heptane; EtOAc, ethyl acetate; MeOH, methanol; TFA, trifluoroacetic acid; MeCN, Acetonitrile; GAE, gallic acid equivalents; CE, catechin equivalents; AD, Alzheimer's disease; Aß, amyloid β-peptide.

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time in developing therapeutics based upon natural products extracted from plants (Ben Sassi et al., 2007). In fact, many phenolics extracted from plant are known to possess antimicrobial properties, so they might change the composition of microflora in any environment in which these compounds are applied and/or induced in a proper kind and concentration (Veloz-García et al., 2010). The antimicrobial activity of phenolics is well known and it is related to their ability to denature proteins, being generally classified as surface-active agents. In addition, there is also an increasing interest in exploiting plant extracts that have antimicrobial activities against plant or human pathogens (Veloz-García et al., 2010). Nowadays, Alzheimer's disease (AD) is a neurodegenerative disorder that is claiming in increasing number of victims as the world population ages. AD is a cerebral degeneration with selective neuronal cell death associated with two hallmark pathological lesions: the intracellular neurofibrillary tangles and extracellular amyloid deposits in the form of senile plaques (Ling et al., 2003). Substantial evidence indicates that amyloid β-peptide (Aβ) contributes significantly to the pathological cascades in AD by various mechanisms, such as by generating ROS, elevating intracellular free Ca^{2+} and other cytotoxic stimuli (Henry-Vitrac et al., 2010). Consequently, finding molecules to prevent or reverse the oligomerization and fibrillization of Aβ and determining interactions of these compounds at the molecular level could be of therapeutic value in the treatment of AD. Several studies have shown that phenolic compounds have potent anti-amyloidogenic activities *in vitro* (Ono et al., 2003) and *in vivo* (Bastianetto et al., 2006). In fact, one of the major properties of polyphenols is the important interaction with peptides and proteins particularly in the AD (Henry-Vitrac et al., 2010).

In this way, the aim of the present study was to explore a new source of natural phenolic antioxidants from *Limoniastrum guyonianum* Boiss. In fact, this medicinal halophyte species is an endemic plant from North Africa. Its leaf and gall infusions are used in traditional medicine as anti-dysenteric against infectious diseases or parasites responsible of painful and bloody diarrheal (Chaieb and Boukhris, 1998). This study describes the interesting antioxidant capacities of *L. guyonianum* fractions obtained from the CPC fractionation using the ethyl acetate extract. This investigation was assessed through different biological activities such as antioxidant test (β-carotene-linoleic acid model system), the inhibitory activity of amyloid β-peptide (Aβ) aggregation and the antibacterial activity against some human pathogenic strains. The most active fractions were analyzed by HPLC.

2. Materials and methods

2.1. Plant materials

Areal parts *Limonistrum guyonianum* were collected from El Akarit (Gabes regions) in the vegetative stage (February 2008). El Akarit is located at 35 km from north of Gabes (N 34 06 56; E 09 59 02) and belongs to the arid bioclimatic stage. The harvested plants were identified at the Biotechnology Center at the Technopark of Borj-Cédria, and a voucher specimen [PLM25] was deposited at the Herbarium of the Laboratory.

2.2. Chemicals

Folin–Ciocalteu reagent, sodium carbonate anhydrous (Na_2CO_3), gallic acid and β-carotene were purchased from Fluka (Buchs, Switzerland). Curcumin was obtained by Bachem (Germany) and Thioflavine T (ThT) was purchased from Sigma–Aldrich (Saint-Quentin Fallavier, France). Amyloid β-peptide (Aβ25–35) was purchased from Bachem California (USA). All solvent used were purchased from Sigma–Aldrich (GmbH, Sternheim, Germany).

2.3. Preparation of plant extract

The powdered aerial part of *L. guyonianum* (1 kg) was mixed with 2 L of aqueous acetone (6:4, v/v) at room temperature under agitation; this procedure was repeated two times. After the combination and the concentration of the aqueous acetone extracts at 35 °C under reduced pressure, the residual aqueous phase (1 L) was successively extracted with petroleum ether (3 × 1 L) and ethyl acetate (EtOAc extract) (5 × 1 L). The petroleum ether extract was discarded and the EtOAc extract was reduced in vacuum at 35 °C and redissolved in water to be freeze-dried.

2.4. Fractionation with CPC

2.4.1. CPC apparatus

The laboratory CPC is a FCPC200® apparatus provided by Kromaton Technologies (Angers, France) that is fitted with a rotor made of 20 circular partition disks (1320 partition cells: 0.130 ml per cell; total column capacity of 204 ml; dead volume: 32.3 ml). Distance from the center of the rotor to the center of each cell is 105 mm. Rotation speed can be adjusted from 0 to 2000 rpm, thus producing a centrifugal force field in the partition cell of nearly 1200 ms^{-2} at 1100 rpm and 4200 ms^{-2} at 2000 rpm. The solvents were pumped by a Gilson 321-H1 2-way binary high-pressure gradient pump. The samples were introduced into the CPC column *via* a high pressure injection valve (3725(i) 038 Rheodyne) equipped with a 10 ml sample loop. The effluent was monitored with an ICS UV-Lambda 1010 detector equipped with a preparative flow cell. Fractions were collected by a Gilson FC 204 fraction collector.

2.4.2. CPC procedures

The quaternary biphasic solvent systems were prepared by mixing *n*-Hept, EtOAc, MeOH, and water in the convenient proportion (1:3:1:3; v/v/v/v) for system H. The rotor was entirely filled with the aqueous stationary phase in the ascending mode without rotating. After injection of the EtOAc extract, the organic mobile phase was pumped into the column in ascending mode at a flow-rate of 3 ml min^{-1}. Then, the rotation speed was increased from 0 to 1000 rpm. Fractions of 9 ml were collected every 3 min. The back pressure was 23 bars.

2.5. Total phenolic content of Limoniastrum guyonianum fractions

Total phenolics were determined using the Folin–Ciocalteu procedure as described by Singleton's method slightly modified (Dewanto et al., 2002). Briefly, 0.125 ml of extract solution were mixed with 0.5 ml distilled water and 0.125 ml of Folin–Ciocalteu reagent. After 3 min, 1.25 ml of 7% Na_2CO_3 solution was added to the mixture. The final volume was adjusted to 2.5 ml with distilled water and thoroughly mixed. After 90 min at 23 °C in dark, the absorbance was measured at 760 nm. Total polyphenols were expressed as mg gallic acid equivalents per gram of dry residue (mg GAE/g DR) through the calibration curve with gallic acid. The calibration curve range was 0–400 μg/ml.

2.6. Analysis of flavonoid content

Total flavonoid content was measured by a colorimetric assay developed by Dewanto et al. (2002). An aliquot of suitable diluted samples or standard solution of (+)-catechin was added to a $NaNO_2$ and mixed for 6 min, before adding 0.15 ml of a freshly prepared $AlCl_3$ (10 g/100 ml). After 5 min, 0.5 ml of 1 mol/l NaOH solution was

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