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The phytochemical and bioactivity profiles of wild *Asparagus albus* L. plant

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ABSTRACT

The ethanolic extracts from the leaves, pericarps and rhizomes of *Asparagus albus* L. were investigated for their phytochemical composition, antioxidant (DPPH and FRAP assays), anti-microbial against human pathogenic isolates and cytotoxic (human colon carcinoma HCT-116 cells) activities. The highest flavonoid content was obtained in the leaf extract followed by the pericarp but there were no flavonoids detected in the rhizome. However, the rhizome had a high concentration of saponins. Flavonoid and saponin profiles were similar to those previously described for the *triguero* Huetor Tajar asparagus landrace. It was found that the pericarp ethanolic extract exhibited higher antioxidant activity than rhizome and leaf extracts. Moreover, the rhizome possessed more evident cytotoxic activity against HCT-116 cells in comparison to leaf and pericarp. All extracts showed varying degrees of antimicrobial activity against most of the human pathogenic isolates. In addition, the leaves showed more powerful inhibitory activities against the maximum number of bacteria and all the fungai isolated and the highest activity was in the pericarp extract against multidrug resistant *Pseudomonas aeruginosa* (MDR) and Erythromycin resistant *Streptococcus agalactiae* (ER) with an inhibition zone of 21 mm and 19 mm, respectively. The results show that *A. albus* could be a new crop with pharmaceutical interest because its richness in bioactive compounds provides considerable benefits for human health.

1. Introduction

Since the middle of the twentieth century, a continuous and significant increase in the incidence and prevalence of opportunistic infections has been observed, not only in immuno-compromised and hospitalized patients, but also in the general population. The indiscriminate and disseminate use of antimicrobial agents has contributed to an increase in microbiological resistance (Kon & Rai, 2012). It has been established that plant secondary metabolites are responsible for preservative effects, which are generally correlated with the presence of high amounts of antioxidant, antimicrobial and anticancer constituents in their tissues (Kammoun El Euch, Bouajila, & Bouzouita, 2015). Based on recent research, it is clear that several bioactive secondary metabolites extracted from plants were found to show biological activities (Bouraoui et al., 2011; Falleh et al., 2012; Meot-Duros et al., 2010). Among them, flavonoids possess many biochemical properties, but the best described property of almost every group of flavonoids is their capacity to act as antioxidants. The antioxidant activity of flavonoids depends on the arrangement of functional groups around the nuclear structure. The configuration, substitution, and total number of hydroxyl groups substantially influence several mechanisms of antioxidant activity such as radical scavenging and metal ion chelation ability (Kelly, Anthony, & Dennis, 2002; Pandey, Mishra, & Mishra, 2012). In addition to their role as antioxidants, these compounds exhibit a wide spectrum of medicinal properties such as hepato-protective (Kim et al., 2011), antimicrobial (Cushnie & Lamb, 2005), anti-inflammatory (Tunon, Garcia-Mediavilla, Sanchez-Campos, & Gonzalez-Gallego, 2009) and antiviral activities (Zandi et al., 2011). In this respect, they have been shown to inhibit cancer cell proliferation by scavenging reactive oxygen species, which prevents potential damage to cell components such as DNA, proteins, and lipids (Ren, Qiao, Wang, Zhu, & Zhang, 2003).

Saponins are naturally-occurring structurally and functionally diverse phytochemicals that are widely distributed in plants. They are a complex and chemically varied group of compounds consisting of triterpenoid or steroidal aglycones linked to oligosaccharide moieties. The combination of a hydrophobic aglycone backbone and hydrophilic

Abbreviations: TFC, total flavonoids content; *triguero* HT, *triguero* Huetor Tajar asparagus landrace; HTSAP, Huétor-Tájar saponin; WSAP, wild saponin; ALBSAP, Albus saponin

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sugar molecules makes the saponins highly amphipathic and confers foaming and emulsifying properties. These surface-active molecules play important roles in plant ecology and are also exploited for a wide range of commercial applications in the food, cosmetic and pharmaceutical sectors. A number of therapeutic properties have been ascribed to saponins (Francis, Kerem, Makkar, & Becker, 2002). These molecules are potent membrane permeabilizing agents. They are also immunostimulatory, hypo-cholesterolemic, anti-carcinogenic, anti-inflammatory, anti-microbial, and have anti-oxidant properties. In general, the saponins and their biosynthetic intermediates display an array of properties that can have positive or negative effects on different animal hosts (Francis et al., 2002; Sparg, Light & Van Staden, 2004).

In spite of its small area, Tunisia has a large plant biodiversity (Le Houerou, 1995). Its flora accounts for > 2150 species (Pottier-Alapetite, 1979) growing on various bioclimatic zones from humid to saharian regions and giving particular landscapes and agro-ecosystems such as oases and Jessours in the southern part of the country. Many spontaneous plants are considered as multi-purpose and are of multiple interests in the therapeutic and cosmetic industries. These aspects were recently reported in many species such as *Diplotaxis harra* and *Diplotaxis simplex* (Falleh et al., 2013), *Cynara cardunculus* (Falleh et al., 2008), and *Nigella sativa* (Bourgou et al., 2008). These works highlighted that these Tunisian species used for traditional medicine displayed high bioactive secondary metabolites levels, powerful scavenging capacity against free radicals, and strong anti-bacterial activity. *Asparagus* genus (Liliaceae family), native to the Mediterranean region, is widespread in Tunisia. Precisely, it grows in many sandy areas in North and South Tunisia. Many previous studies refer to *Asparagus* as a traditionally used plant with therapeutic properties (Al-Snafi, 2015; Thakur & Sharma, 2015) and as an alimentary plant usually consumed in salads.

In our experience, *A. albus* is a perennial plant that grows very well under different ecological conditions and environmental stresses. This plant can be propagated by seed and by vegetative methods as well. Therefore, it could easily be cultivated and used for the extraction and preparation of therapeutic substances such as active pharmaceutical ingredients. In addition, it could also be an alternative to traditional crops with species belonging to the same family, in high demand at the current international market such as *A. racemosus* and *A. officinalis*. As a consequence, this plant not only has a key ecological function in stabilizing highly-disturbed ecosystems, but may be a very interesting, and still not fully explored, source of metabolites with potential use in human health care.

The aim of this work is to characterize the profile of phytochemicals and their bioactivities in different plant parts of *A. albus*.

2. Materials and methods

2.1. Chemicals

Authentic standards of rutin (quercetin-3-*O*-rutinoside) and 2,2-diphenyl-1-picrylhydrazyl (DPPH free radical) were purchased from Sigma-Aldrich Quimica (Madrid, Spain); kaempferol-3-*O*-rutinoside (nicotiflorin) and isorhamnetin-3-*O*-rutinoside (narcisin), were purchased from Extrasynthese (Genay, France). Protodioscin (97%) and shatavarin (98.6%), whose purity was checked by NMR, were purchased from Chromadex Chemical Co. (Barcelona, Spain). All solvents were of HPLC grade purity (Romyl and Teknokroma, Barcelona, Spain). Ethanol, formic acid (96%), and acetonitrile, high-performance liquid chromatography (HPLC) grade, were purchased from Sigma Chemical Co. (St. Louis, MO). The C-18 cartridges (500 mg) were purchased from Varian Incorporated (Lake Forest, CA). Pure de-ionized water was obtained from a Milli-Q50 system (Millipore Corporation, Bedford, MA).

2.2. Plant material

The leaves, pericarp (fruits without seeds) and rhizomes (minimum 1 Kg fresh weight each) of the *A. albus* L. plants were collected in February 2013 from Borj Touil (20 km north Tunis; superior semi-arid bioclimatic stages, mean annual rainfall, MAR: 476 mm). The plant materials were identified and authenticated by Professor Smaoui Abderrazek and voucher specimens [PLM 53] have been deposited at the Herbarium of the Department of Biology, Faculty of Science of Tunis, Tunisia.

The samples of leaves, pericarps and rhizomes of *A. albus* L. were cleaned and washed thoroughly with distilled water. Each organ was then left at room temperature for 7 days in the dark, oven-dried for 2 h at 60 °C, and then ground to fine powder in a Mettler AE 200 (Dangoumau type) grinder. All samples were stored at − 20 °C until analysis and treatment.

2.3. Preparation of ethanolic extract

Ethanolic extraction was performed using the plant material (1 g) with 40 mL of ethanol:water (80:20, v/v) in an Ultraturrax (Ultra-Turrax T25, Janke & Kunkel/IKA Labortechnik) for 1 min at maximum speed and filtered through filter paper. The residue was extracted again in the same conditions. Ethanolic extracts were stored at − 20 °C until analysis by HPLC. All extractions were made in duplicate.

2.4. Analysis and quantification of flavonoids by HPLC–DAD

The analyses of flavonoids were carried out using a Jasco-LC-Net II ADC liquid chromatograph system equipped with a diode array detector (DAD). Flavonoid compounds were separated using a MEDITERRANEA SEA C_{18} reverse-phase analytical column (25 cm length × 4.6 mm i.d., 5 µm particle size; Teknokroma, Barcelona, Spain). The gradient profile for the separation of flavonoids was formed using solvent A (water with 1% formic acid) and solvent B (acetonitrile with 1% formic acid) in the following program: the proportion of B was increased from 0% B to 20% B for the first 20 min, then to 21% B over the next 8 min, maintained at 21% B for 2 min, then to 30% B over the next 10 min, and to 100% over the next 5 min, maintained at 100% B for 5 min and finally returned to the initial conditions over the next 5 min. The flow rate was 1 mL/min and the column temperature was 30 °C. Spectra from all peaks were recorded in the 200–600 nm range and the chromatograms were acquired at 360 nm for flavonoid glycosides and 370 nm for their aglycones.

A quantitative evaluation of flavonoid content was carried out as described by Fuentes-Alventosa et al. (2007). The identification of individual flavonoid glycosides was carried out using their retention times and both spectroscopic and mass spectrometric data were collected on a quadrupole mass analyzer (ZMD4, Micromass, Waters Inc., Manchester, U.K.). Electrospray ionization (ESI) mass spectra were obtained at ionization energies of 50 and 100 eV (negative mode) and 50 eV (positive mode), with MS scans from *m/z* 100 to 1000. Capillary voltage was 3 kV, dissolving temperature was 200 °C, source temperature was 100 °C, and extractor voltage was 12 V. The flow was maintained at 1 mL/min. The quantification of individual flavonoid monoglycosides and flavonoid diglycosides was directly performed by HPLC-DAD using an eight-point regression curve in the range of 0–250 µg on the basis of standards. The results were calculated from the mean of three replicates.

2.5. Saponin analysis and quantification by HPLC-DAD–MS.

The evaluation of saponin content was carried out as described by Vázquez-Castilla et al. (2013a). An HPLC Waters Alliance (Manchester, U.K.) system fitted to a MEDITERRANEA SEA C_{18} reverse-phase analytical column (25 cm length × 4.6 mm i.d., 5 µm particle size; Teknokroma, Barcelona) was used. An elution gradient was used with solvents A (water with 1% formic acid) and B (acetonitrile with 1% formic acid): 0–30 min, 20% B; 30–60 min, linear gradient to 30% B; 60