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European Journal of Integrative Medicine 7 (2015) 274–286

European Journal of INTEGRATIVE MEDICINE

www.elsevier.com/eujim

Original article

Antioxidant, cytoprotective, anti-inflammatory and anticancer activities of *Pistacia lentiscus* (Anacardiaceae) leaf and fruit extracts

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Received 23 November 2014; received in revised form 26 March 2015; accepted 29 March 2015

Abstract

Introduction: *Pistacia lentiscus* (Anacardiaceae) is used in Algeria for the treatment of inflammation, burns and gastrointestinal complaints. The present work was designed to establish the rational for the medical use of *P. lentiscus*, especially to determine the antioxidant, anti-inflammatory, cytoprotective and anticancer activities of leaf and fruit extracts and their fractions.

Methods: The antioxidant activity was assessed using the ORAC test, and the cytoprotective effect on H_2O_2-induced oxidative stress was also investigated. Anti-inflammatory activity was examined by measuring the secretion of interleukin-1β by macrophages exposed to ATP or H_2O_2. The anticancer potential of the crude extracts against melanoma (B16F10) and mammary (EMT6) cell lines was equally evaluated. UPLC–MS analysis was carried out for compound identification.

Results: The crude extracts of leaf and fruit exhibited strong antioxidant activity in the ORAC assay and showed significant cytoprotective effect with maximum protection at 100 μg/mL increasing cell viability by 108.25 ± 1.73 and 104.13 ± 0.97%, respectively. Fractions obtained from fruit extracts were ineffective, while the hydro-alcoholic-eluted fraction 3 of leaf displayed a significant cytoprotective effect. Leaf extract (100 μg/mL) showed significant anti-inflammatory activity, compared to acetylsalicylic acid (ASA). Moreover, leaf and fruit extracts inhibited the growth of B16F10 cells ($IC_{50} = 56.40$ and 58.04 μg/mL, respectively). Spectral analysis allowed the identification of six flavonol glycosides and five phenolic acids.

Conclusion: Results obtained in this study indicate that *P. lentiscus* extracts exhibited antioxidant, anti-inflammatory and anticancer properties, in accordance with the traditional uses of the plant.

Keywords: *Pistacia lentiscus*; Extract; Fractions; Anti-inflammatory; Myricetin-rhamnoside; Gallic acid

Introduction

Plants have always been used for medicinal purposes and are the primary source of phytochemicals present in conventional medicines. Phenolics are secondary plant metabolites found in the majority of herbs, vegetables and teas. They have more than one phenolic hydroxyl group attached to one or more benzene rings. They have been found to exert diverse biological effects, including antioxidant, anti-inflammatory and anticancer [1–3].

Pistacia lentiscus, commonly known as mastic tree, is part of a large family (Anacardiaceae) consisting of more than eleven species, widely distributed in the Mediterranean basin ecosystems where it grows wild [4]. Previous studies led to the quantification and/or identification of many constituents of different chemical classes such as flavonoids and anthocyanins [5,6], phenolic acids (gallic acid, digallic acid, catechin) [7,8], triterpenoids [9], and tannins [8,9]. These constituents are responsible for antioxidant [7,10] and several pharmacological

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http://dx.doi.org/10.1016/j.eujim.2015.03.009
1876-3820/

properties, including hepatoprotective [11], anti-atherogenic and anticancer [9,12,13] and anti-ulcer and anti-inflammatory [2] activities of *P. lentiscus*.

Previously, we demonstrated the antioxidant characteristics of *P. lentiscus* using several *in vitro* tests. We believe that the strong antioxidant character of this plant lies gives it many healing virtues. In fact, as reactive oxygen species play a key role in signal transduction of cancer cells [14] and in the transcription of pro-inflammatory cytokines such as NF-κB [14,15], their elimination is a crucial step in fighting these diseases. Thus, the objectives of the present study were to further evaluate the antioxidant activity of *P. lentiscus* using the ORAC test complemented with an investigation of its cytoprotective activity against oxidative stress in cell culture. The anticancer and the anti-inflammatory effects of *P. lentiscus* leaf and fruit hydroalcoholic extracts and fractions on cultured cells were equally examined.

Materials and methods

Plant material

Leaves and fruits of *P. lentiscus* reach their maximum maturity in summer and autumn respectively. That is why they were harvested during these seasons from the forest of Azru n Bechar located in the province of Amizour, Department of Bejaia (North eastern Algeria). The plant was identified by Prof. M.S. Benabdelmoumène at the laboratory of botany (University of Bejaia) where a specimen was deposited. The laboratory of botany, University of Bejaia does not have a specific voucher specimen. In fact, only the characteristics of the plant as well as the place and date of harvest and the person who identified the plant are recorded. However, the same plant is recorded by our colleague in the Herbarium of Natural History, Museum of Aix-en-Provence, France under the voucher specimen (D-DH-2013-37-5) [16].

Extraction

The leaves and fruits were dried at room temperature away from the light and ground using an electric mill to obtain fine powder (leaves) and homogeneous paste (fruits). Five hundred grams of the powder and 1 kg of the paste were macerated separately in ethanol (95%) (1:4, w/v) followed by continuous stirring at room temperature for 24 h. The supernatant was collected, centrifuged at 1500 × *g*/10 min and evaporated to obtain the crude hydroalcoholic extracts. Dry weight of the extract was determined and the extraction yield was calculated using the formula: [yield (%) = (weight of dried extract/weight of plant starting material) × 100].

The hydroalcoholic extract was fractionated using silica gel column chromatography (silica gel 0.063–0.2 mm) and eluted as follows: (chloroform/ethyl acetate) (90:10), (ethyl-acetate/methanol) (50:50), (methanol/water) (80:20), (methanol/water) (50:50) and (water/acetic acid) (99.75:0.25) [10]. Extracts and fractions were dissolved in 1% DMSO in phosphate buffer saline to prepare stock solutions and further diluted to the tested concentrations.

Phytochemical analysis

Quantification of total phenolics, flavonoids and tannins

The total phenolics of each extract were determined using *Folin–Ciocalteu* method [17]. Briefly, 0.1 mL of each extract (dissolved in methanol) was mixed with 0.5 mL *Folin–Ciocalteu reagent* (1/10 dilution in distilled water) and 1 mL of distilled water, then incubated at room temperature for 1 min, followed by the addition of 1.5 mL of (20%) (Na_2CO_3) solution. After 2 h of incubation in the dark, absorbance was measured at 760 nm. A standard calibration curve was plotted using catechin (5–20 μg/mL) and the results were expressed as mg catechin equivalent per gram of extract (mgCatE/gE). All measurements were performed in triplicate.

Flavonoid concentration of plant extracts was estimated using the aluminium chloride method [18] and results were expressed as mg rutin equivalent per gram of extract (mgRutE/gE) extrapolated from a standard curve (10–50 μg/mL). Briefly, 1 mL of $AlCl_3$ (10^{-2} M) solution was added to test tubes containing 2 mL of each sample (dissolved in methanol). After 10 min incubation at room temperature, the absorbance of each solution was recorded at 430 nm.

Tannin content in *P. lentiscus* extracts was performed on the basis of protein precipitation using bovin serum albumin (BSA) [19]. One mL of each extract (dissolved in methanol at 100 μg/mL) was mixed with 2 mL of BSA (200 mM acetic acid and 170 mM NaCl adjusted to pH 4.9). After incubation at 4 °C for 24 h, the mixture was centrifuged at 3000 × *g* for 15 min. The supernatant was discarded and the tannin–protein precipitate was solubilized in a mixture of 4 mL of 5% (v/v) triethanolamine and 1% (w/v) sodium dodecyl sulphate (SDS). Finally, a volume of 1 mL of $FeCl_3$ (0.01 M $FeCl_3$ in 0.01 M HCl) was added. The mixture was shaken vigorously, incubated for 15 min at room temperature and absorbance was measured at 510 nm. Results were expressed as mg tannic acid equivalent per gram of extract (mgTAE/gE) extrapolated from a standard curve (50–200 μg/mL).

UPLC–MSn analysis

Phenolic compounds analyses were carried out using a 1290 Infinity Ultra-performance liquid chromatography (UPLC) from Agilent Technologies (Santa Clara, CA, USA) equipped with a binary pump, an autosampler and a DAD detector. The UPLC system was coupled to an Esquire 3000 plus mass spectrometer from Bruker Daltonics (Wissembourg, France) equipped with electrospray ionization (ESI) and Ion Trap analyzer. Column Zorbax SB-C18 (2.1 mm × 100 mm, 1.8 μm) from Agilent Technologies was used. Volume injected was 5 μL. Two different solvents were used as a mobile phase: Solvent A (H_2O/HCOOH 99.9:0.1, v/v) and Solvent B (ACN/HCOOH 99.9:0.1, v/v), at a flow rate of 0.4 mL/min and a gradient as follows: 0 min 1% B, 0.4 min 1% B, 2 min 10% B, 6 min 35% B, 7 min 50% B, 8.8 min 70% B, 10.8 min 92% B, 11 min 100% B, 12 min 100% B, 12.2 min 1% B, 15.2 min 1% B. The MS/MS