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Chemical composition, antioxidant and anti-inflammatory properties of pistachio hull extracts

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ABSTRACT

Phytochemical and bioactivity analyses of pistachio hulls revealed the presence of anacardic acids (3198 mg/100 g), fatty acids (1500 mg/100 g), and phytosterols (192 mg/100 g) as major components. Carotenoids (4.93 mg/100 g), chlorophylls (10.27 mg/100 g), tocopherols (8.83 mg/100 g), and three triterpene acids (mangiferolic, isomangiferolic and mangiferonic acids) were characterized. A polar (P) extract contained quercetin-3-*O*-glucoside (6.27 mg/g), together with smaller concentrations of quercetin, myricetin and luteolin flavonoids, accounting for 5.53 mg/g. Gallotannins and other phenolic compounds esterified with a gallic acid moiety characterized the P extract. P extract potently inhibited the release of nitric oxide (NO) and reactive oxygen species (ROS) in lipopolysaccharide-stimulated RAW 264.7 macrophage cells. The mRNA expression levels of the anti-inflammatory cytokine COX-2 were significantly inhibited by fractions P2-P5, while IL-6 was only inhibited by fraction P3. Moreover, the P extract significantly decreased the non-mitochondrial oxidative burst associated with inflammatory response in macrophages.

1. Introduction

Pistachio nut (*Pistacia vera* L., family Anacardiaceae) is a high value commodity, widely consumed globally due to its sensory and nutritional characteristics and health-related benefits (Dreher, 2012). The United States is considered the second major worldwide producer of pistachio after Iran. The U.S. pistachio industry, mainly located in California, has experienced phenomenal growth since 1976 (from 1.5 million pounds in 1976 to 415 million pounds in 2007) (American Pistachio Growers, http://www.americanpistachios.org).

A pistachio nut consists of a fleshy hull (mesocarp and epicarp) surrounding the nut shell, which encloses the nutmeat. As the nutmeat grows, it fills the shell and, by virtue of its growth exceeding the shell size, cracks the shell open. This creates the naturally opened nuts characteristic of pistachios. With rare exceptions, the hull remains intact during this entire process, protecting the nutmeat from insects and pathogens. Pistachios are harvested fresh and must be processed rapidly, generally within 24 h, before natural chemical processes and the release of tannins from the fleshy hull results in shell staining, which would preclude their sale as an in-shell product. Hulls are one of the well-known by-products of pistachio production, and are accumulated in high volumes during industrial post-harvest processing.

In some cases, plant food processing results in accumulation of by-products that can be attractive sources for natural antioxidants and bioactive compounds. These by-products can be used in food technology applications to prevent oxidation of lipids or to reduce the effects of radical oxygen degradation (Balasundram, Sundram, & Samman, 2006). Plant hulls or husks are agro-industrial by-products and typically contain a greater concentration of antioxidant polyphenolic compounds, as expected due to their role as the protective outer layer around the fruit or nut. Bioactive compounds with antioxidant activity have been identified in hulls from peanut (Yen & Duh, 1993), mung bean (Duh, Yen, Du, & Yen, 1997), buckwheat (Watanabe, Ohshita, & Tsushida, 1997), rice (Ramarathnam, Osawa, Namiki, & Kawakishi, 1989), almond (Takeoka & Dao, 2003) and others.

Aqueous and methanolic extracts of pistachio hull are rich in phenolics, which effectively retarded the deterioration of soybean oil at 60 °C, with efficiency comparable to the synthetic antioxidant BHA (butylated hydroxyanisole) (Goli, Barzegar, & Sahari, 2005). Pistachio hull's aqueous crude and purified extracts inhibited the growth of Gram positive bacteria (*Bacillus cereus*) with MIC of 1.0

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and 0.5 mg/mL, respectively. The same extracts showed anti-mutagenicity against a direct mutagen of 2-nitrofluorene (Rajaei, Barzegar, Mobarez, Sahari, & Esfahani, 2010). However, there is insufficient information on the phytochemical composition of the hulls. A related species, *Pistachia atlantica* subsp. Kurdica, has been recently investigated to evaluate the antioxidant activity and phenolic profile of its hull, shell and kernel tissues. Hull extract showed significantly higher levels of total phenolics, flavonoid content, and antioxidant capacity, compared to extracts from shell or kernel, which suggested that it could be an important and cost-effective source of compounds with health protective potential (Hatamniaa, Abbaspoura, & Darvishzadeh, 2014).

Inflammation is an essential protective process that helps to preserve the integrity of an organism against physical, chemical and infective insults. Reactive oxygen species (ROS) are formed as a natural by-product of regular oxidative cell function. Although ROS have been classically known for their damaging effects, increasing evidence of their use in regulating and maintaining normal processes in living organisms (homeostasis) has been accumulating. Therefore the term redox regulation seems to better describe the redox status and its consequences (Alfadda & Sallam, 2012). During environmental stress, ROS increase dramatically and may then result in damage to cell structure. Nitric oxide (NO) is a reactive nitrogen species (RNS) produced via the activity of inducible nitric oxide synthase (iNOS) and is expressed primarily in macrophages after induction by lipopolysaccharide (LPS). The human body counteracts reactive radical accumulation via regulation of the expression or function of antioxidant enzymes such as superoxide dismutase, catalase, and the glutathione system, and the use of exogenous phytochemicals such as carotenoids, vitamins, and polyphenols from dietary sources to maintain metabolic homeostasis (Lei et al., 2016; Scalbert, Manach, Morand, Remesy, & Jimenez, 2005). Macrophages play a central role in managing many different immune-pathological phenomena, such as the overproduction of the pro-inflammatory cytokine, tumor necrosis factor (TNF)-α and inflammatory mediators [reactive oxygen species (ROS) and nitric oxide (NO)] generated by activated inducible nitric oxide synthase (iNOS) (Lundberg, 2000).

In this study, the composition of pistachio hull was explored through a comprehensive phytochemical screening of its polar and non-polar components. The antioxidant and anti-inflammatory properties of pistachio hull extracts and semi-purified fractions were tested in vitro in lipopolysaccharide-stimulated murine RAW 264.7 macrophages, and compared against the determined chemical composition. Real-time measurements of oxygen consumption rate (OCR) and extracellular acidification rate (ECAR) were made to determine the influence of the extracts on cellular bioenergetics associated with inflammatory status.

2. Material and methods

2.1. Chemicals

Catechin, epicatechin, quercetin, quercetin-3-*O*-glucoside and luteolin were purchased from Chromadex (Irvine, CA). Anacardic acid (15:0), linoleic acid (18:2), oleic acid (18:1), gallic acid, α-carotene, β-carotene, lutein, zeaxanthin, chlorophyll (a and b), and tocopherols (α, γ and δ) were purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals and solvents were obtained from Fisher Scientific (Pittsburg, PA).

2.2. Plant material

Fresh raw un-hulled pistachios (*Pistacia vera* L., family Anacardiaceae), harvested in September 2013 in California, were provided by the American Pistachio Growers. The hulls were removed within 24 h of harvest, and freeze-dried. The dried hulls were finely ground using an analytical mill (IKA A11 basic, Wilmington, NC) and stored at −80 °C until extracted.

2.3. Preparation of non-polar and polar extracts

Finely ground hull material (500 g) was extracted at room temperature by shaking with dichloromethane (1L × 4 times). The combined CH_2Cl_2 extract was evaporated using a rotary evaporator to afford the non-polar (NP) extract (45 g). The plant material was air-dried in the fume hood, and then extracted (1L × 3 times) with 80% acidified methanol (0.05% trifluoroacetic acid). The combined methanol extract was evaporated and remaining aqueous extract was freeze-dried to afford the polar (P) extract (155 g). Both NP and P extracts were fractionated and subjected to an anti-inflammatory cell culture assay, and a complete phytochemical analysis for bioactive components.

2.4. Analysis of non-polar (NP) extract

2.4.1. Fatty acid analysis

2.4.1.1. Preparation of fatty acid methyl esters (FAME). FAME were prepared as described (Slover & Lanza, 1979), with some modifications. Approximately 60 mg of NP extract was treated with 3 mL of 1 M KOH in 95% EtOH, and sonicated until dissolved. The tube was heated at 40 °C for 2 h with continuous shaking. One mL of water and 0.6 mL of 6 M HCl were added along with 2 mL of hexane and mixed. After layers separated, 1 mL of the organic layer was removed and evaporated to dryness under vacuum. The dry extract was then re-dissolved in 1 mL of 14% boron trifluoride in methanol, of which 100 μL was taken into a vial and made up to 1 mL with BF_3 solution before being heated at 40 °C for a further 20 min. Upon removal from heat, 1 mL of water and 1 mL of hexane were added and mixed thoroughly, before 100 μL of the organic layer was taken and 10 μL of hexanoic acid methyl ester was added as an internal standard and made up to 1 mL with hexane. Three replicates were prepared.

2.4.1.2. Gas chromatography-mass spectrometry analysis. GC–MS analysis was carried out on an Agilent 7890A GC system attached to an Agilent 5975C MS detector. An Agilent HP-5 ms (30 m × 0.25 mm ID 0.25 μm film thickness) was used for separation using helium as a carrier gas at a flow rate of 1.2 mL/min, and linear temperature gradient (0–2 min 60 °C, 44–64 min 270 °C). GC–MS parameters at electron impact (EI) mode include ionization voltage 70 eV injector temperature of 250 °C with injector port set to standard splitless mode and a mass scan range of 100–800 *m/z*. The GC retention time and mass fragmentation pattern of each component were compared with that of authentic compound for identification. The peak areas were normalized using unity response factors. The total amount of palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acids was calculated at the level of a hundredth of a percent. Measurements were carried out in triplicate and means were calculated.

2.4.2. Phytosterol analysis

Analysis of NP extract for phytosterols was performed by Medallion Laboratories (Minneapolis, MN) in accordance with the official AOAC method 2007.03 for determination of campesterol, stigmasterol and beta-sitosterol (Sorenson & Sullivan, 2007). Results were expressed as mg/g dry extract.

2.4.3. Carotenoid, tocopherol and chlorophyll analyses

The extraction of compounds from NP extract was conducted according to a previously reported protocol (Kurilich et al., 1999)