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RESEARCH ARTICLE

Acetone Extract of Almond Hulls Provides Protection against Oxidative Damage and Membrane Protein Degradation

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Abstract
Several studies have revealed that among foods, the consumption of edible nuts has beneficial effects on health which are attributed to their high content of potent antioxidants. Among nuts, the whole seed of the almond (*Prunus dulcis*) has been demonstrated to possess potent free radical scavenging activity, which is related to the presence of phenolic compounds. The aim of the current study is to evaluate the polyphenol content and the antioxidant ability of almond hull, which is an agriculture solid waste. The present results revealed that among different extraction methods, the acetone extract of almond hulls has a high content of phenolic and flavonoid compounds and a high antioxidant ability, which were determined by using the phosphomolybdenum method and by measuring the potency of the antioxidant, respectively. Moreover, the experimental data disclosed that the acetone extract of almond hulls provides protection against the oxidative damage and the membrane protein degradation that are caused in human erythrocytes by hydrogen peroxide. These phenomena may likely be due to the recruitment of antioxidants by cell membranes and/or translocation to cytosol. Overall, almond hull extract could be considered as a natural source of antioxidants, and its consumption could have a positive effect on human health.

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1. Introduction

Phenolic compounds are aromatic secondary metabolites in plants which are found largely in fruits, vegetables, cereals, and beverages, and constitute a main part of the human diet. These compounds are categorized into non-soluble compounds such as condensed tannins, lignins, and cell-wall bound hydroxycinnamic acids and soluble compounds such as phenolic acids, phenylpropanoids, flavonoids, and quinones. All of these groups have received more attention due to their multiple roles in plants and their impact on human health [1].

In fact, there are several pathological conditions which are correlated with perturbation of intracellular redox status such as cancers, cardiovascular diseases, diabetes, osteoporosis, and neurodegenerative diseases. An overproduction of oxidants or free radicals to an extent that overcomes the endogenous antioxidant system brings about a period of oxidative stress which results in a disturbance of signal transductions and consequently biological processes [2,3]. Antioxidants are beneficial compounds that protect cells against oxidative damage by controlling free radical formation. When availability of antioxidants is limited, oxidative damage such as lipid peroxidation, DNA degradation, protein modification, and inflammation become cumulative and threaten human health. Therefore, antioxidants that scavenge reactive oxygen species or chelate metal transition ions have great value in preventing the onset and propagation of oxidative diseases [4]. In that respect, epidemiological studies have revealed that long term consumption of diets, rich in plant polyphenols, prevents development of oxidative stress associated diseases by reducing reactive oxygen species production and inhibition of macromolecules oxidation [5,6]. In addition to antioxidant activity of phenolic compounds, several studies have demonstrated that polyphenolic extract also has antimicrobial activity, which has led to it being considered as a good alternative for the conventional antibiotics. Because of these virtues, and also due to their low toxicity and minor microbial resistance to these natural compounds, phenolic compounds are used as natural antimicrobials in the food industry instead of chemical preservatives [7]. Moreover, it has been shown that injection of natural herbs or biologic substances into acupuncture points promotes, maintains, or restores health, and prevents disease. So, phenolic-rich plant extract can also be used in herbal acupuncture [8,9]. Based on these properties, identification and development of such agents has become a major area of experimental studies for diseases.

Almond, scientifically known as *Prunus dulcis*, belongs to the family Rosaceae, is cultivated in a variety of growing conditions and climates. Although the United States, especially California, is the leading producer of almonds in the world, it is actually native to the Mediterranean climate region of the Middle East, from where then it was dispersed by humans in ancient times to other regions of the world. Almond is an important crop which produces fruits with high commercial value and is currently used worldwide in bakery and confectionery [10,11]. So far, $>$ 30 wild almond species have been recognized which have a bitter taste and eating even a relatively small number of these nuts can be fatal due to the presence of glycoside amygdalin. Indeed, following mechanical damage to the kernel, glycoside amygdalin is transformed into deadly prussic acid (hydrogen cyanide).

It has previously been shown that almond is high in monounsaturated fats, the same type of health-promoting fats which have low-density lipoprotein-lowering effects and reduce risk of heart diseases [12]. Moreover, it has been revealed that almond seed, its brown skin, and green cover (hull) possess radical scavenging potential because of a wide variety of phenolic acids and flavonoids that are predominantly conjugated with sugars or other polyols via O-glycosidic or ester bonds [13,14]. High performance liquid chromatography and gas chromatography-mass spectrometry analyses have been shown that among different parts of almond, the skin and hull are phenolic-rich relative to the kernel and shell. Chlorogenic acid and its isomers are the major phenolic compounds in almond hull, however, quercetin, morin, stigmasterol, β-sitosterol, kaempferol, isorhamnetin, and *p*-coumaric acid were also identified. Quantitative analyses also revealed trace amounts of phenolic compounds in the kernel and shell of almond such as ferulic acid, sinapinic acid, caffeic acid, *p*-coumaric acid, kaempferol, quercetin, and isorhamnetin [15]. The antioxidant activity of these phenolic compounds in almond, which is mainly due to their redox properties, makes them appropriate candidates in medicine, since oxidative stress eventually leads to many chronic diseases such as arteriosclerosis, cancer, and inflammation [16]. In that respect, according to literature reports, several studies have focused on antioxidant and antiradical characteristics of almond seed and its byproducts in wild or domesticated species from different countries [17—21]. However, there is a lack of information relevant to the antioxidant capacity of Iranian domesticated almond hull in literature. In the present study, the effect of extraction conditions on total phenolic content and free radical scavenging capacity of domesticated almond hull which grows in northeastern Iran was assessed. Moreover, the effect of phenolic-rich extract of almond hull on human erythrocyte membrane integrity was also evaluated.

2. Material and methods

2.1. Materials

Methanol, acetone, ethanol, Folin-Ciocalteu reagent, and sodium carbonate were purchased from Merck (Darmstadt, Germany). Gallic acid, catechin, sodium nitrate, sodium phosphate, and ammonium molybdate were obtained from Sigma Chemical Co. (St Louis, MO, USA).

2.2. Extraction preparation

Almonds were collected from suburbs of Nishabur (Razavi Khorasan province, Iran) in August 2013 and September 2013. The green cover of samples was separated and dried at room temperature away from sunlight. Before the extraction process, almond hulls were ground in a mill. For the extraction with methanol/water (v/v, Me/Wa, 70/30), ethanol/water (v/v, Et/Wa, 70/30), or acetone/water (v/v, Ac/Wa,