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RESEARCH NOTES

Antioxidant Activity of Iranian Pomegranate (*Punica granatum* L.) Seed Extracts

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ABSTRACT

Pomegranate, a small tree with potential human health benefits, is grown mainly in Iran, India and USA as well as in most Near and Far East countries. It has been used extensively in folk medicine for a number of therapeutic purposes. In the present study, the ferric reducing/antioxidant power assay (FRAP) was employed and the FRAP value of the seed fraction of six different cultivars of pomegranate in Iran was determined in an attempt to compare their differing antioxidant acitivity. The antioxidant activity of seed fraction of six different cultivars of pomegranate in water extracts showed that *the Sour white peel* cultivar has the highest FRAP value (3.45±0.85 μM) and *the Agha Mohamad Ali* cultivar has the lowest value (2.76±0.76 μM); ethanolic extract of the seeds showed that *Sour white peel* and *Black peel* cultivars have the highest (3.88±1.31 μM) and lowest (1.62±0.47 μM) antioxidant activity, respectively. Results indicated that the extracts obtained from pomegranate seeds using various solvents exhibited various degrees of antioxidant activity. Further, it was cleared that *Sour white peel* had the highest potent antioxidant activity among different pomegranate seed cultivars and, so might be useful for its health benefits.

Keywords: Antioxidant activity, FRAP, Pomegranate seeds.

INTRODUCTION

Pomegranate, a small tree with potential human health benefits, is grown mainly in Iran, India and USA as well as in most near and Far East countries [11]. Edible parts of pomegranate fruit comprise 78% juice and 22% seed [5]. Pomeganate seeds are rich in sugers, vitamins, polysaccharides, polyphenols and minerals. They have low oil content but are rich in polyunsaturated fatty acids [7].

Pomegranate has been used extensively in folk medicine for a number of therapeutic purposes [10]. It is also used as part of antifungal preparations and for its antiviral activity against the genital herpes virus [16]. The pericarp of pomegranate as well as its roots, bark and juice are used in the treatment of colic, colitis-diarrhia, dysentery, leucorrhia, menorrhagia, oxyariasis, paralysis, rectocele and headaches in traditional medicine [11].

A common denominator in the pathogenesis of most chronic diseases is the involvement

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of oxidative stress related to the production by all aerobic organisms of reactive oxygen and nitrogen species including free radicals and antioxidants in dietary plants [3].

Epidemiological studies suggest that a reduced risk of cancer is associated with higher consumption of a phytochemical-rich diet that includes fruits and vegetables [12]. Consumption of polyphenoles and flavonoids is benefitial for the perevention of cardiovascular, inflammatory and other diseases [9] by preventing oxidative stress that is lipid peroxidation in arterial macrophage and in lipoproteins [8].

The presence of antioxidants has been reported from pomegranate juice [9, 13]. Pomegranate contains some species of flavonoids and anthocyanidins in its seed oil and juice and shows antioxidant activity three times greater than green tea extract [10]. Pomegrante juice contains tannins, ellagic tannis, anthocyanins, catechins, gallic and ellagic acid as antioxidant chemicals.

Pomegrante seeds are known to contain esterogenic compounds. One of the most remarkable characteristics of pomegranate fruit is that its seeds are the richest plant source of esterogen [10]. Furthermore, it inhibits breast cancer cell proliferation and invasion and promotes breast cancer cell apoptosis [6].

In the present study, the ferric reducing/antioxidant power assay (FRAP) was employed, and the FRAP value of the seed fraction for six different cultivars of pomegranate in Iran was determined in an attempt to compare their antioxidant activity.

MATERIALS AND METHODS

Reagents: 2, 4, 6-tri-pyridyl-s-triazine (TpTz), $FeSO_4 \times 7H_2O$, $FeCl_3 \times 6H_2O$, acetate buffers (sodium acetate trihydrate) and Hcl 37%, all chemicals were of analytical grade and the highest purity available and obtained from Merck (Darmstadt, Germany).

Sample Preparation

A total of six different cultivars of pomegranate including *Sweet white peel, Agha Mohamad Ali, White peel* and *No seed of north, Sour white peel, Sour summer* and *Black peel* were prepared at the Saveh Farmer Investigation Center at Saveh, Iran.

Extraction

The seed fraction of the pomegranate was manually separated and washed with excess water for the removal of sugars and any adhering materials, and then dried. A portion of 0.5 gram was grounded in grinder and extracted by distilled water (50 ml) or Ethanol (50 ml). The extracts were filtered and used directly for FRAP assay.

Method

The FRAP assay was used to measure the total concentration of antioxidants. The principle of this method is based on the reduction of a ferric-tripyridyl triazine complex to its ferrous colored form in the presence of antioxidants. Briefly, the FRAP reagent contained 5 ml of a 10 mmol L^{-1} TpTz (2, 4, 6- tripyridyl-s-trizaine) soloution in 40 mmol L^{-1} HCl plus 5 ml of 20 mmol L^{-1} $FeCl_3$ and 50 ml of 0.3 mol L^{-1} acetate buffer, pH 3.6, and was prepared freshly and warmed at 37°C for 5 minutes. Aliquots of 50 µl of the sample supernatant were mixed with 1.5 ml FRAP agent and the absorbance of the reaction mixture at 593 nm was measured spectrophotometrically after incubation at 37°C for 10 minutes. The 5 mmol L^{-1} $FeSO_4$ was used as the standard soloution. The final result was expressed as the concentration of antioxidants having a ferric reducing ability equivalent to that of 5 mmol L^{-1} $FeSO_4$. Adequate dilution was needed if the FRAP values measured were over the linear range of the standard curve.

Table1. FRAP values of the seed fraction of six different cultivars in water extract**.**

Clutivar	N [a]	FRAP value (μM)
Sweet white peel	27	3.13 ± 0.85
Agha Mohamad Ali [b]	27	2.76 ± 0.76
White peel and no seed of north	27	3.31 ± 0.64
Sour white peel [b]	27	3.45 ± 0.85
Sour summer	27	3.20 ± 0.76
Black peel	27	2.79 ± 1.16
Mean (water extract)	162	3.10±0.87

[a] Sample number of each cultivar.
[b] Significant difference (P< 0.05).

Statistical Analysis

Experimental data was analysed using analysis of variance (ANOVA) and unpaired student's *t*-test using the SPSS software.

RESULTS AND DISCUSSION

Antioxidant activities of the seed fraction of six different cultivars of pomegranate in water exratct are shown in Table1. The results show that *Sour white peel* cultivar has the highest FRAP value (3.45±0.85 μM) and *Agha Mohamad Ali* cultivar has the lowest value (2.76±0.76 μM). Antoxidant activity of the seed fraction of six different cultivars in ethanolic extract are shown in Table 2, which shows *Sour white peel* and *Black peel* cultivars have the highest (3.88±1.31 μM) and lowest (1.62±0.47 μM) antioxidant activity, respectively.

The total antioxidant activity of the seed of the *Agha Mohammad Ali* cultivar and *Sour white peel* exracted with water cultivar had significant differences (P< 0.05), such that antioxidant activity of *Sour white peel* cultivar was much greater than in *Agha Mohamad Ali*. In ethanolic extract, there are some significant differences (P< 0.01) in antioxidant acitivity among the cultivars mentioned in Table 2, amongst which the antioxidant activity of *Sour white peel* cultivar was more than the others.

Comparison with the results of a study on the pulp and peel of these pomegranates [2, 3] shows that peel of the fruit have a greater FRAP value than pulp and seed (P< 0.01). In a study by Surveswaran, total antioxidant capacities of 133 Indian medicinal palnt species sampled from 64 families were assessed by FRAP assays. The pericarp of *Punica granatum* showed very high levels of hydrolysable tannins and total antioxidant capacity, but its seed had lower capacity [14]. Guo determined the antioxidant activities of peel, pulp and seed fractions of 28 fruits commonly consumed in china using the ferric

Table2. FRAP values of the seed fraction of six different cultivars in ethanolic extract.

Clutivar	N [a]	FRAP value (μM)
Sweet white peel	18	3.18 ± 0.87
Agha Mohamad Ali [b]	18	2.18 ± 0.47
White peel and no seed of north [c]	18	2.22 ± 0.36
Sour white peel [1,2,3,4]	18	3.88 ± 1.31
Sour summer [d]	18	2.26 ± 0.55
Black peel [e]	18	1.62 ± 0.47
Mean (ethanolic extract)	108	2.55±1.04

[a] Sample number of each cultivar.
[b, d, c, e] Significant differences (P< 0.01).

reducing/antioxidant power assay (FRAP assay). In the comparison between all fruit peel, pulp and seed fractions tested, pomegranate peels had the highest FRAP value [1]. At a 0.05% level of pomegranate peel extract, its antioxidant activity was greater than 0.02% of the two synthetic antioxidants butylated hydroxylanisole and butylated hydroxyltoluene [15].Halverson systematically assessed total antioxidants in a variety of dietary plants used worldwide. Analysis of the fruits demonstrated that pomegranate contained very high concentration of antioxidants [4]. Schubert investigated the antioxidant and eicosanoid enzyme inhibition properties of pomegranate seed oil and its fermented juice flavonoids. The results obtained showed that fermented pomegranate juice and cold pressed seed oil had strong antioxidant activity close to that of butylated hydroxyanisole and green tea [11].

In the present study, the extracts obtained from pomegranate seeds using various solvents exhibited various degrees of antioxidant activity, too. The antoxidant activity of water extracts (3.10±0.87μM) was significantly more than that of ethanolic (2.55±1.04μM) ($P< 0.01$). Singh studied the antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using various solvents; methanol (MeOH), ethyl acetate (EtOAC) and water. From the peel, the phenolic content of the MeOH extract was maximum, while it was very low in the water extract, while in the case of seeds the water extract possessed high phenolic content followed by the MeOH and EtOAc extracts. Pomegranate peel and seed extracts prepared by different solvents exhibited various degrees of antioxidant activity as measured using the β-carotene-linoleat model system (the bleaching of β-carotene in the absence of an antioxidant is a free radical mediated phenomenone resulting from linoleic acid). At 50 ppm concentration, EtOAc, MeOH and water extracts of peel were shown to exhibit 53, 82 and 64% antioxidant activity, respectively, while the seed exhibited 19, 11 and 28%. The results indicated the presence of various compounds possessing antioxidant activity in various solvents, of which peel is known as an enriched source of the antioxidants exhibiting higher activitiy compared to the seeds. The difference in the antioxidant acitivity of the peel and seeds may be attributed to their different phenolic compositions [13]. However, further studies are needed on the isolation and characterization of individual phenolic compounds to elucidate their different antioxidant mechanisms and the existence of possible synergism, if any, among the compounds.

In the present study, we found that the extracts obtained from pomegranate seeds using various solvents exhibited various degrees of antioxidant activity. Further, in this study, it was made clear that the highest potent antioxidant activity in different pomegranate seed cultivars was in *Sour white peel* cultivars, so it can be used in health protective or therapeutic agents.

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فعالیت آنتی اکسیدانی عصاره هسته انار ایران

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چکیده

انار درختی کوچک با فوائد بسیار برای سلامتی بشر، بیشتردر ایران ، هندوستان و آمریکا و همچنین در اکثر کشورهای شرق نزدیک و دور می روید. انار از سالها قبل در طب سنتی برای اهداف درمانی بیشماری به کار گرفته شده است. جهت بررسی فعالیت آنتی اکسیدان تام هسته انار در این مطالعه، روش بررسی قدرت احیاء کنندگی آهن فریک، (Ferric Reducing Ability Of Plasma) FRAP انتخاب شد. در این طرح اثرات آنتی اکسیدانی بذر ۶ رقم انار مختلف که در منطقه ساوه کشت می شوند(انار پوست سفید شیرین، انار آقامحمدعلی، انار پوست سفید بی هسته شمال، انار پوست سفید ترش، انار تابستانی ترش و انار پوست سیاه یا انار دارویی) بررسی می گردد. در بررسی و تحقیقی که بر روی استخراج های آبی و الکلی هسته انجام شد، توان آنتی اکسیدانی هسته انار در استخراج آبی در بین ۶ رقم مورد بررسی در محدوده بین (انار پوست سفید ترش) ۳/۴۵±۰/۸۴ µm و (انار آقای محمدعلی) ۲/۷۵±۰/۷۵ µm قرار دارد. توان آنتی اکسیدانی هسته انار در حلال الکلی در محدوده بین ۳/۸۸±۱/۳۰ µm (انار پوست سفید ترش) و ۱/۶۱ ±۰/۴۷ µm (انار پوست سیاه) قرار دارد. میانگین کل فعالیت آنتی اکسیدانی(value of FRAP) استخراج های آبی برابر با (µm) ۳/۱۰±۰/۸۷ و میانگین کل فعالیت آنتی اکسیدانی استخراج های الکلی برابر با(µm)۲/۵۵±۱/۰۴ بود که تفاوت معنی داری با هم دارند($p<0/01$) نتایج به دست آمده نشان داد که هسته انار پوست سفید ترش هم در استخراج آبی و هم استخراج الکلی بیشترین اثرات آنتی اکسیدانی را دارد و ممکن است جهت استفاده های درمانی مفید واقع شود.