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Short Communication

Free radical scavenging, anti-glycation and tyrosinase inhibition properties of a polysaccharide fraction isolated from the rind from *Punica granatum*

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Abstract

The present investigation deals with the isolation of a polysaccharide fraction from Pomegranate (PFP), which was found to inhibit 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-Azinobis[3-ethylbenzothiazoline-6-sulfonate] $ABTS^{\cdot+}$ radical activities by 69% and 88%, respectively with 4 μg/ml concentration. The activity of PFP for free radical scavenging was also evaluated by electron spin resonance (ESR) Spectrophotometer and DPPH dot blot test. Anti-glycation ability of PFP was tested using BSA, which inhibited the formation of advanced glycation end-products (AGEs) by 28% and also inhibited the formation of fructosamine in the BSA/Glucose system. The inhibition of mushroom tyrosinase by 43% at 10 μg/ml concentration of PFP strongly suggested its efficacy as a possible skin whitener.

Keywords: Pomegranate; DPPH; $ABTS^{\cdot+}$; Reducing power; Fructosamine; Tyrosinase

1. Introduction

Antioxidant properties elicited by plant species have a full range of perspective applications in human health care. In recent years, the prevention of cancer and cardiovascular diseases has been associated with the ingestion of fresh fruits, vegetables or teas rich in natural antioxidants (Wang et al., 2000). Proteins are modified by glucose through the glycation reaction, resulting in the formation of advanced glycation end-products (AGEs). The contribution of AGEs to diabetes, aging and Alzheimer's disease has received considerable attention in recent years and free radicals have been shown to participate in AGEs formation (Halliwell, 2001). It has been reported that antioxidants and radical scavengers inhibit these processes (Nakagawa et al., 2002). Free radicals also upregulate the m-RNA level for tyrosinase, the rate-limiting enzyme in melanin biosynthesis. Increased productions of melanin possess a serious aesthetic problem. Tyrosinase inhibitors may therefore be clinically useful for the treatment of some dermatological disorders associated with melanin hyperpigmentation and important in cosmetics for depigmentation (Shiino et al., 2001).

The phenolic components extracted from pomegranate (*Punica granatum* L.) have been shown to possess antioxidant property in several instances (Noda et al., 2002; Negi et al., 2003; Kulkarni et al., 2004; Li et al., 2006; Sudheesh and Vijayalakshmi, 2005). To evaluate the effect of water soluble high molecular weight compounds, a polysaccharide fraction from pomegranate (PFP) was extracted using gel permeation chromatography (GPC). The aim of the present work was to study the antioxidant, antiglycation and tyrosinase inhibition properties with an objective to propose the various bioactive roles of a new class of compound from pomegranate.

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2. Methods

2.1. Chemicals

Bovine Serum Albumin (BSA), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-Azinobis[3-ethylbenzothiazoline-6-sulfonate] (ABTS) were purchased from Sigma–Aldrich, USA.

2.2. Isolation of PFP

Rind (100 g fresh weight) was crushed with 100 ml of water and the extract after centrifugation at 10,000 rpm for 10 min at 4 °C was sequentially precipitated twice with 3 times and 2 times volume of ethanol, respectively to remove the lipid soluble compounds. The precipitate obtained was freeze dried to obtain 160 mg of dry powder, which was dissolved in water and its polysaccharide and protein content were measured by phenol-sulphuric acid method (Dubois et al., 1956) and Lowry method (Lowry et al., 1951) respectively. The crude polysaccharide was subjected to GPC to isolate PFP on a column (1.5 × 60 cm) packed with Sephadex G-100, eluted with distilled water and monitored using the phenol-sulfuric acid method. The flow rate was 0.3 ml/min.

2.3. Evaluation of total antioxidant potential

The free radical scavenging activity of PFP was measured by the DPPH and $ABTS^{\cdot+}$ scavenging methods proposed by Brand-Williams et al. (1995) and Re et al. (1999) respectively. DPPH radical scavenging activity was also measured by electron spin resonance (ESR) spectrometer. DPPH was dissolved in methanol to give a 0.4 mM solution and then mixed with different concentrations (0, 16.66 and 33.32 μg/ml respectively) of PFP. After mixing vigorously for 10 s, the solutions were transferred into a 100 μl teflon capillary tube, fitted into the cavity of the ESR spectrometer and the spin adduct was measured after 2 min. Measurement conditions were central field 3475 G, modulation frequency 100 kHz, modulation amplitude 2 G, microwave power 5 mW and gain 6.3×10^5.

For rapid evaluation of antioxidant activity by DPPH dot blot test, an aliquot 1 μl of PFP and vitamin C in different concentrations were loaded on a TLC layer (silica gel 60 F_{254}; Merck) and allowed to dry. The sheet was dipped for 10 s in a 0.4 mM DPPH solution in methanol. Stained silica layer revealed a purple background with yellow spots at the location of the drops, which showed radical scavenger capacity.

2.4. Measurement of reducing power, metal chelation and superoxide anion scavenging property

The reducing power of PFP was quantified by the method described earlier by Yen and Chen (1995). The ferrous ion-chelating potential of PFP was investigated according to the method of Decker and Welch (1990), wherein the Fe^{2+}-chelating ability of PFP was monitored by measuring the ferrous iron–ferrozine complex at 562 nm. The method used by Banerjee et al. (2005) for determination of superoxide anion scavenging was followed to evaluate the scavenging effect of PFP. The percentage inhibition of superoxide generation was measured by comparing the absorbance values of the control and those of the reaction mixture containing sample solution.

2.5. Evaluation of anti-glycation property

Experiments were performed as described by Tang et al. (2004). BSA (10 mg/ml) in phosphate buffer (50 mM, pH 7.4) containing 0.02% (w/v) sodium azide was preincubated with PFP and vitamin C at various concentrations for 30 min at room temperature (25 °C). 25 mM each of glucose and fructose solutions were added to the reaction mixture and incubated at 37 °C for 2 weeks. Fluorescence was determined using a Fluorescence spectrophotometer (Perkin Elmer) with an excitation wavelength of 350 nm and an emission wavelength of 450 nm. Anti-glycation property was also evaluated by determining the fructosamine (the early glycation product) content by nitroblue tetrazolium (NBT) reduction method (Johnson et al., 1982).

2.6. Tyrosinase inhibition property

Tyrosinase inhibitory activity test was performed according to the method of Khanom et al. (2003) with minor modifications. Briefly, the reaction mixture contains 0.5 ml of 5 mM L-tyrosine, 0.4 ml of 25 mM phosphate buffer pH 6.8 and 0.05 ml of different concentrations of PFP. After 10 min of incubation, 0.05 ml of 400 U/ml mushroom tyrosinase was added. The OD was taken at 475 nm after 30 min of incubation.

3. Results and discussion

3.1. Isolation of PFP

The rind of pomegranate contained 94% carbohydrate. Chromatography of crude polysaccharides on sephadex G-100 gave a sharp peak at fraction # 31 (PFP) containing 99% carbohydrate and less than 1% protein (data not shown).

3.2. Evaluation of total antioxidant potential

In order to investigate the antioxidant activity of PFP, different free radical scavenging methods were tested. PFP of rind showed a higher inhibitory effect of 69% for DPPH radical and 88% for $ABTS^{\cdot+}$ radical in 4 μg/ml concentration. Vitamin C (4 μg/ml) was able to inhibit DPPH and $ABTS^{\cdot+}$ radical by 9% and 66% respectively (data not shown). The effectiveness of PFP was also studied for the