

Extract of Polyphenols from Pomegranate Seed and its Antioxidant Activity in Vitro

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Abstract: *In this study, the pomegranate seeds were treated by micro-cutting assisted interaction technique. The effective components were extracted from pomegranate seeds with 95% ethanol at room temperature, and their antioxidant capacity in vitro was determined. The results showed that the scavenging rates of DPPH radical, superoxide anion radical, hydroxyl radical and lipid peroxidation were 70.97, 51.95, 52.85, and 80.62%, respectively. The antioxidation ability of alcohol extract of pomegranate seed was studied in order to provide theoretical basis for developing more value of pomegranate seed in the future.*

Keywords: *Microshearing assisted interaction technique; Pomegranate seed; ascorbic acid*

1. Introduction

Pomegranate (*Punicagranatum L*), a perennial deciduous fruit tree, belongs to the pomegranate family of pomegranate, mainly grown in subtropical and temperate regions, and is widely cultivated in China. With the study of the medicinal effects of various parts of pomegranate, pomegranate has various effects such as anti-inflammatory [1, 2], anti-oxidation [3], anti-cancer [4] and improvement of cardiovascular system. Pomegranate seeds have strong anti-cancer and anti-oxidation ability [5], which can effectively prevent and treat various diseases and promote physical health. Pomegranate seeds are one of the main by-products produced during the processing of pomegranate and are usually discarded directly as waste [6, 7]. Research on pomegranate seeds to develop the potential role of pomegranate seeds in order to enable waste utilization and reduce production costs. Antioxidants are widely used in food, beauty and other fields due to their unique effects. However, synthetic antioxidants currently do not guarantee their absolute safety, while natural antioxidants have natural properties compared with synthetic antioxidants. Low toxicity, positive effects on physical health, etc [8]. Therefore, in some industries, “natural” has become a sales selling point.

In recent years, domestic scholars have done a lot of experiments on the extraction of pomegranate seed active substances (such as pomegranate seed polyphenols), chemical composition analysis, anti-cancer and anti-oxidation [9, 10]. At present, in many experiments, the pretreatment of pomegranate seeds is generally carried out by drying and pulverizing [11, 12]. Direct drying and pulverization may cause the sample to be incompletely pulverized and affect the dissolution rate of the effective substance. Therefore, for the pretreatment of pomegranate seeds, the experiment used micro-cut assisted interaction technology combined with ultra-fine pulverization to treat pomegranate seeds. Micro-cut assisted interaction technology refers to the use of mechanical effects between samples and chemical additives to break the cell wall during the grinding process, increasing the contact area between cells and chemical additives, causing a certain reaction, thus changing the effective The solubility of the ingredients makes them soluble in water [13]. This technology has been applied to the extraction of active ingredients from various substances such as andrographis paniculata, orange peel essential oil, orange peel pectin and pepper. It has been found that the extraction rate of active ingredients has improved compared with traditional methods [13-15]. In this study, pomegranate seeds were used as materials, and the samples were treated by micro-cutting and mutual-assisted technology.

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The active ingredients were extracted with 95% ethanol at room temperature, and their antioxidant capacity in vitro was determined, which provided a theoretical basis for further development of pomegranate seeds in the future.

2. Materials and methods

2.1. Main equipment

JFM-12A (B) vibrating superfine pulverizer, Jinan Jianchen Machinery Co., Ltd.; H2-16K high speed refrigerated centrifuge, Chongqing Songlang Electronic Instrument Co., Ltd.; RE-5200 rotary evaporator, Gongyi City to China Instrument Co., Ltd.; SZM-20 superfine pulverizer, Taian Zhengxin Technology Co., Ltd.; UV-1780 UV spectrophotometer, Shimadzu Corporation, Japan.

2.2. Raw materials and reagents

2.2.1. Test materials

Pomegranate seed crude powder, Yishan Chinese herbal medicine in Anguo City, Hebei Province.

2.2.2. Primary reagent

Anhydrous ethanol, Chengdu Jinshan Chemical Reagent Co., Ltd.; anhydrous calcium chloride, Tianjin Damao Chemical Reagent Factory; 1,1-dinitro-2-trinitrophenylhydrazine (DPPH), Hefei Bomei Biological Co., Ltd.; Ascorbic Acid (Vitamin C, Vc), Xiqiao Chemical; Tris, Shanghai Ruji Biotech Co., Ltd.; pyrogallol, Shanghai Ika Biotechnology Co., Ltd.; o-phenanthroline, Tianjin Damao Chemical Reagent Factory; 2-thiobarbituric acid (TBA), Hefei BASF Biotechnology Company; Trichloroacetic acid (TCA), Tianjin Guangfu Fine Chemical Research Institute.

2.3. Pomegranate seed alcohol extract preparation

(1) The pomegranate seeds are washed and dried, and then super finely pulverized and passed through a 120 mesh sieve. Then, take an appropriate amount of pomegranate seed crude powder and add 3% anhydrous calcium chloride, mix and grind in the ultrafine pulverizeer for 15 min, and store for later use.

(2) Weigh 10 g of pretreated pomegranate seed powder, add 95% ethanol solution at a ratio of 1:12, shake well, let stand overnight, take the supernatant the next day, then press 1:12 It is more than ethanol extraction, mixed and allowed to stand for 30 min, the supernatant is taken, the appeal step is repeated, and then the supernatant is filtered to finally obtain a pale yellow transparent sample solution. The round bottom flask was washed and repeatedly dried to constant weight, poured into a sample liquid, and concentrated by evaporation using a rotary evaporator. The temperature was controlled to be constant weight at 55 °C, weighed, and the data was recorded. The sample was diluted to a solution of 2, 4, 6, 8, and 10 mg/mL and sealed for storage.

2.4. Determination of DPPH scavenging ability by pomegranate seed alcohol extract

DPPH was formulated into a solution of 0.04 mg/mL with absolute ethanol. 2 mL of the sample solution and DPPH were placed in the tube, shaken, and allowed to stand for half an hour. The wavelength was adjusted to 517 nm and the absorbance was measured [16].

The calculation formula is:

$$\text{Clearance rate(\%)} = [1 - (A_1 - A_2)] / A_0 \times 100\% \quad (1)$$

Note: A_0 : (2 mL absolute ethanol + 2 mL DPPH); A_1 : (2 mL sample + 2 mL DPPH); A_2 : (2 mL sample + 2 mL absolute ethanol).

2.5. Determination of superoxide anion radical scavenging ability by ethanol extract of pomegranate seed

The experimental reagent (except concentrated HCl) was bathed at 25 °C for 20 min. Add 4 mL of Tris-HCl buffer (concentration: 0.05 mol/L, pH 8.2) to the tube, add 1 mL of sample solution, and add 1 mL (prepared with 0.05 mol/L HCl) 0.2 mmol/L phenylene The phenol solution was shaken, placed in a water bath at 25 °C, reacted for 4 min, and immediately stopped by dropping 2 d of concentrated HCl. Adjust the wavelength to 325 nm and then measure the absorbance A. Three replicates were taken for each concentration of sample and the average was calculated. The original sample solution was replaced with 1 mL of water, the others are the same as above [17].

$$\text{Result calculation: Clearance rate(\%)} = (A_0 - A_s) / A_0 \times 100\% \quad (2)$$

Note: A_s reference: pomegranate seed alcohol extract 1 mL + buffer 4 mL + 1 mL HCl (0.05 mol/L); A_0 reference: water 1 mL + buffer 4 mL + 1 mL HCl (0.05 mol/L).

2.6. Determination of Hydroxyl Radical Scavenging Capacity of Pomegranate Seed Extracts

Take 2 mL of 0.75 mmol/L phenanthroline solution, add 2 mL of 150 mmol/L Phosphate buffer saline (PBS) solution (adjust pH to 7.4), add 2 mL of 0.75 mmol/L FeSO_4 , mix well, add 1 mL of 0.01% H_2O_2 , make up to 10 mL, and react at 37 °C for 1 h. Adjust the wavelength to 536 nm and measure the absorbance A_d . In the undamaged tube, replace 1 H_2O_2 with 1 mL of distilled water, and absorb the light value of A_{nd} . The sample tube was replaced with 1 mL of the above sample with 1 mL of sample solution, and the absorbance was A_s [18].

$$\text{Result calculation: Clearance rate(\%)} = [(A_s - A_d) / (A_{nd} - A_d)] \times 100\% \quad (3)$$

2.7. Determination of anti-lipid inhibition ability of pomegranate seed alcohol extract

Fresh eggs are taken from egg yolks. Prepare 0.1 mol/L PBS solution, adjust pH 7.4, take appropriate amount of egg yolk, add PBS solution in a ratio of 1:1 to make a suspension, stir the suspension at 37 °C for 10 min, and add PBS solution at 1:25. Dilute, stir well, and spare at low temperature. Take 0.2 mL of egg yolk suspension in a test tube, add 1 mL of sample solution, add 0.2 mL of FeSO_4 (25 mmol/L) solution, dilute to 2 mL with PBS solution, shake well, and put in 37 °C water bath for 30 min. Take 2 mL of 10% TCA solution into the tube, mix and place for 10 min, add 2 mL of TBA solution with mass fraction of 0.67 %, shake well and react in boiling water for 15 min. After cooling, shake vigorously. After 1 min. Pour into a centrifuge tube, centrifuge at 4000 r/min for 10 min, and take the supernatant. Adjust the wavelength to 532 nm and measure the absorbance A_e . Model tube (A_m) plus the same volume of suspension, replace the drug with the same volume of distilled water; Blank tube (A_b) does not add FeSO_4 [19].

$$\text{Result calculation: Inhibition rate(\%)} = [(A_m - A_e) / (A_m - A_k)] \times 100\% \quad (4)$$

3. Results and discussions

3.1. Changes in appearance during pomegranate seed treatment

The Changes in appearance during pomegranate seed treatment shown in Table 1.

Table 1. Appearance change of Pomegranate seed during treatment

Sample	Appearance characteristics
Pomegranate seeds	The color is slightly yellow, the shape is slightly elliptical, no smell.
Pomegranate seeds crushed	The powder is fine, slightly yellow, with the peculiar smell of pomegranate seeds.
Micro-cutting assisted interaction technology	After adding anhydrous calcium chloride, the color of the powder is slightly whiter than before, and there is no smell.

Resting for one night	The liquid is yellowish, turbid and precipitated.
Filtered supernatant	The filtrate is clear and transparent, showing a yellowish.
Drying	Solid state is darker yellow, close to brown.
Dissolve again	The liquid is yellow, a bit turbid, no odor.
Dilution	The liquid is yellowish and lightens as the concentration decreases

3.2. Determination of DPPH free radical scavenging ability by ethanol extract of pomegranate seed

It can be seen from Figure 1 that the scavenging ability of the Vc and pomegranate seed alcohol extracts on the radicals increases substantially as the concentration increases within the experimentally set concentration range. In the whole tested concentration range, the scavenging ability of the extract of pomegranate seed on free radicals showed an increasing trend, reaching up to 70.97%, and the scavenging effect was better. This indicates that some antioxidant active substances may be present in the pomegranate seed alcohol extract. The clearance rate of DPPH free radicals by Vc was maintained at a high level throughout the measured concentration range, and was significantly higher than that of pomegranate seed alcohol extracts.

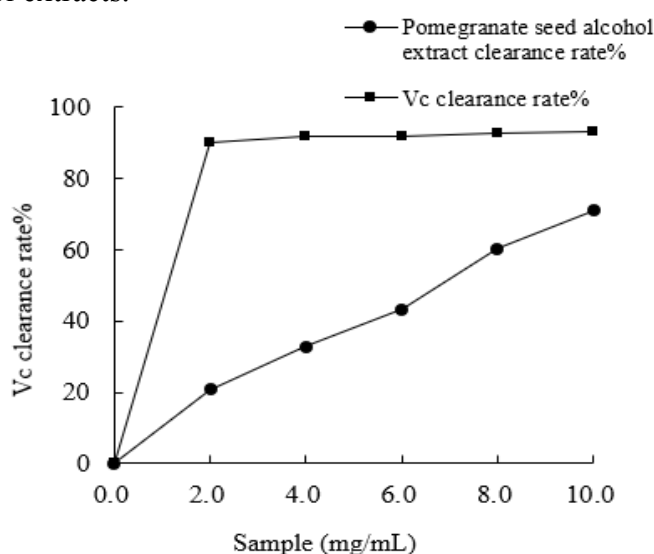


Figure 1. Scavenging rate of DPPH radical by ethanol extract from Pomegranate seed

3.3. Determination of superoxide anion radical scavenging ability by ethanol extract of pomegranate seed

It can be seen from Figure 2 that in the experimentally set concentration range, the pomegranate seed alcohol extract and Vc have a certain scavenging effect on the free radicals, and the scavenging ability basically increases as the sample concentration increases. In the range of 2 to 4 mg/mL, the extraction ability of pomegranate seed alcohol extracts for free radicals increased gently; while in the range of 6 to 10 mg/mL, the removal ability of pomegranate seed alcohol extracts increased. The speed is obviously faster. The higher the concentration measured, the stronger the removal ability of the pomegranate seed alcohol extract. Within the measured concentration range, the pomegranate seed alcohol extract removal rate can reach up to 51.95%. Figure 2 illustrates that the pomegranate seed alcohol extract has good scavenging ability to superoxide anion radicals and has excellent traits in antioxidants, which is worthy of research and development in this respect.

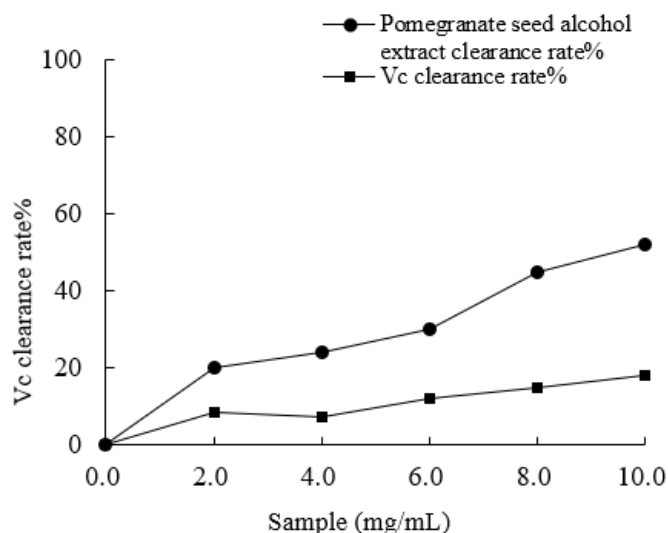


Figure 2. Removal rate of superoxide anion by alcohol extract from pomegranate seed

3.4. Determination of hydroxyl radical scavenging ability of ethanol extract of pomegranate seed

According to Figure 3, in the measured concentration of 0 to 10 mg/mL, the scavenging ability of pomegranate seed alcohol extract and Vc on hydroxyl radicals increased with the increase of sample concentration. Ascorbic acid has a high level of scavenging ability to hydroxyl radicals over the entire experimental concentration range. In the concentration range of 2 to 4 mg/mL, the extraction ability of pomegranate seed extract was relatively gentle, ranging from 4 mg/mL to 10 mg/mL. With the increase of pomegranate seed alcohol extract concentration, The scavenging ability of hydroxyl radicals shows a rapid upward trend. Within the tested concentration range, the pomegranate seed alcohol extract removal rate is up to 51.85%.

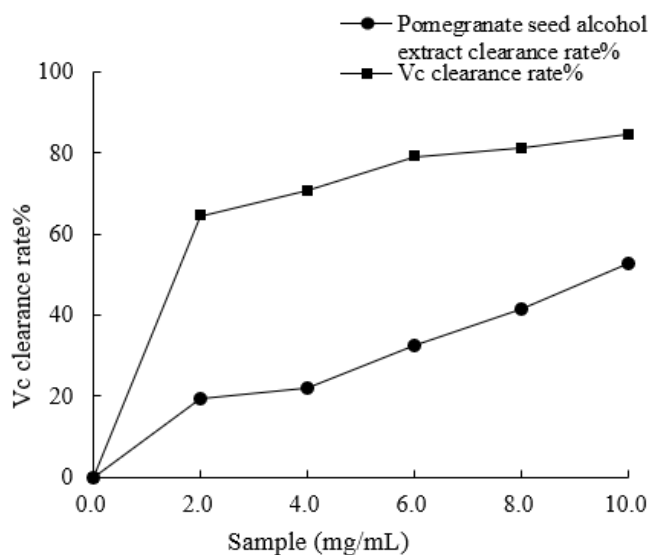


Figure 3. Scavenging rate of hydroxyl radical from ethanol extract of pomegranate seed

3.5. Determination of anti-lipid peroxidation inhibition ability of pomegranate seed alcohol extract

It can be seen from Figure 4 that the pomegranate seed alcohol extract and Vc have a certain inhibitory ability against lipid peroxidation inhibition ability within the experimentally determined

concentration range. The inhibition rate of pomegranate seed alcohol extract against lipid peroxidation increased with the increase of sample concentration, and the increase trend was faster. The inhibition rate of lipid peroxidation of pomegranate seed alcohol extract was up to 80.62% and the inhibition ability was better in the whole tested concentration range. Figure 4 also shows that the pomegranate seed alcohol extract has a good inhibition rate and has excellent traits in anti-lipid peroxidation, which is worthy of research and development in this respect.

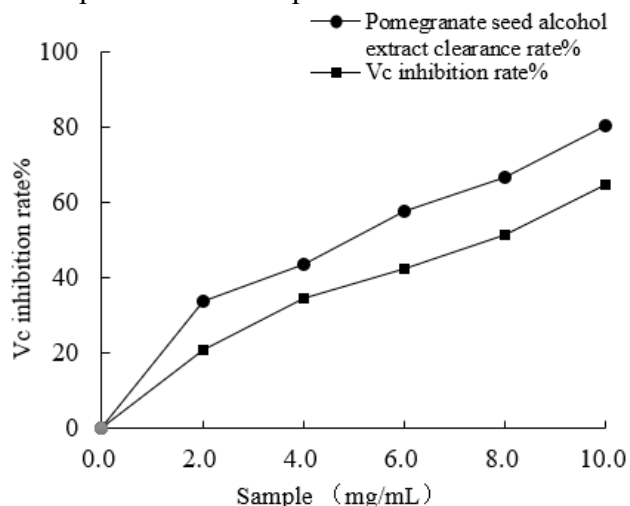


Figure 4. Inhibition rate of anti-lipid peroxidation of alcohol extract from pomegranate seed

4. Conclusions

For the pretreatment of pomegranate seeds, the experiment used micro-cutting and mutual-assisting technology to treat pomegranate seeds, increasing the solubility of active ingredients and making them soluble in solvents. The clearance capacity of the pomegranate seed alcohol extract increased with increasing concentration within the experimentally set concentration range. In the DPPH free radical scavenging assay, Vc has a strong ability to scavenge free radicals, while the pomegranate seed alcohol extract has a good scavenging ability, and the scavenging rate is up to 70.97%, while the two are compared at the same concentration. The scavenging ability of Vc was significantly higher than that of pomegranate seed extract; in the superoxide radical scavenging test, the scavenging ability of pomegranate seed extract for free radicals increased in the range of 2 to 4 mg/mL. In the range of 6 to 10 mg/mL, the removal rate of the extract of pomegranate seed alcohol extract was significantly accelerated, and the clearance rate was up to 51.95%. In the hydroxyl radical scavenging test, the ascorbic acid clearance rate increased. It is relatively gradual, while the ethanol extract of pomegranate seeds is in the range of 2 to 4 mg/mL, and the scavenging ability is relatively gentle, in the range of 4 to 10 mg/mL, with the concentration of alcohol extract of pomegranate seeds [15-19].

The increase of hydroxyl radical scavenging ability shows a rapid rising trend, the highest scavenging rate can reach 51.85%, and at the same concentration, the scavenging ability of Vc is higher than that of pomegranate seed extract; in the anti-lipid peroxidation test Medium, pomegranate seed alcohol extract and ascorbic acid inhibition rate High trends are faster, and the inhibition rate of alcohol extract from pomegranate seed up to 80.62%. Experiments show that the removal rates of DPPH free radicals, superoxide anion free radicals, hydroxyl free radicals and lipid peroxidation were 70.97, 51.95, 52.85, and 80.62%, respectively, in the range of pomegranate seed alcohol extract concentration of 2 to 10 mg/mL [19].

The above experimental results indicate that antioxidant active substances are present in pomegranate seeds. Therefore, antioxidant active substances can be extracted from pomegranate seeds to prepare natural and excellent antioxidants, which can play an important role in many aspects such as beauty and beauty prevention and treatment.

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