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Evaluation of Antioxidant Activity and Preventing DNA Damage Effect of Pomegranate Extracts by Chemiluminescence Method

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The antioxidant activities of three parts (peel, juice, and seed) and extracts of three pomegranate varieties in China were investigated by using a chemiluminescence (CL) method in vitro. The scavenging ability of pomegranate extracts (PEs) on superoxide anion, hydroxide radical, and hydrogen peroxide was determined by the pyrogallol−luminol system, the $CuSO_4$−Phen−Vc−H_2O_2 system, and the luminol−H_2O_2 system, respectively. DNA damage preventing the effect of PE was determined by the $CuSO_4$−Phen−Vc−H_2O_2−DNA CL system. The results showed that the peel extract of red pomegranate had the best effect on the scavenging ability of superoxide anion because its IC_{50} value (4.01 ± 0.09 μg/mL) was the lowest in all PEs. The seed extract of white pomegranate could scavenge hydroxide radical most effectively of the nine extracts (the IC_{50} value was 1.69 ± 0.03 μg/mL). The peel extract of white pomegranate had the best scavenging ability on hydrogen peroxide, which had the lowest IC_{50} value (0.032 ± 0.003 μg/mL) in the nine extracts. The seed extract of white pomegranate (the IC_{50} value was 3.67 ± 0.03 μg/mL) was the most powerful on the DNA damage-preventing effect in all of the PEs. Also, the statistical analysis indicated that there were significant differences (at $P < 0.05$) among the extracts of the different varieties and parts in each system.

KEYWORDS: Pomegranate; *Punica granatum* L.; chemiluminescence; antioxidant activity in vitro; DNA damage

1. INTRODUCTION

Chemiluminescence (CL) has been widely used as a sensitive assay for monitoring free radicals and reactive metabolites from enzyme, cell, or organ systems (*1*−*3*). The generation of reactive oxygen species (ROS) and metabolites will emit light that can be monitored by a variety of luminometers (*4*, *5*). Becasue of its high sensitivity and rapidity, the chemiluminescent method has been widely used in antioxidation effect evaluation.

Punica granatum L., commonly known as pomegranate, native to Persia, is an edible fruit cultivated in Mediterranean countries, Afghanistan, India, China, Japan, Russia, and some parts of the United States, and is a large deciduous shrub or small tree, belonging to the family Punicaceae (*6*). Pomegranate is of significant economic importance since the fruits are either consumed fresh or used commercially in the juice, jam, and wine industries (*7*, *8*). The edible part of the fruit is called the aril and constitutes 52% of the total fruit (w/w), comprising 78% juice and 22% seeds (*9*).

Pomegranate has been extensively used as a folk medicine in many cultures. Pomegranate preparations, especially of the dried pericarp, but also of the roots, barks of the tree and roots, and the juice of the fruit, are employed as per orum medication in the treatment of colic, colitis, diarrhea, dysentery, leucorrhea, menorrhagia, oxyuriasis, paralysis, and rectocele and as external applications to caked breast (*10*) and to the nape of the neck in mumps (*11*) and headache (*12*). Previous studies have shown that flower extracts reduced blood sugar levels in rodents (*13*). Strong antioxidant properties of the fermented juice have been reported, while oil polyphenols were found to inhibit the eicosanoid enzymes cyclooxygenase and lipoxygenase (*14*). Fresh juice was shown to inhibit low-density lipoprotein oxidation and the formation of atheromatous plaque, which could be related to its potent antioxidative characteristics in rodents and humans (*15*). As some antioxidants were recently shown to reduce blood pressure, pomegranate juice can offer a wide protection against cardiovascular diseases, which could be related to its inhibitory effect on oxidative stress and on serum angiotensin converting enzyme activity (*16*). Furthermore, the chemopreventive and adjuvant therapeutic applications of pomegranate to human breast cancer have been warranted recently (*17*).

Pomegranate is rich in polyphenols and anthocyanidins that are powerful free-radical scavengers even more effective than those found in red wine and green tea (*18*). Extracts from many parts of this plant such as juices, seed oil, and peel have been reported to exhibit strong antioxidant activities. The pomegranate fermented juice and cold-pressed seed oil showed strong

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antioxidant activities close to those of butylated hydroxyanisole and green tea and significantly greater than that of red wine (*13*, *19*).

In this study, we evaluated the ROS scavenging ability and protective effect on DNA damage of nine extracts by utilizing a chemiluminescent method in vitro, which was achieved from three pomegranate varieties in China (sour pomegranate, red pomegranate, and white pomegranate) and their individual peels, juices, and seeds. We found that all nine extracts could scavenge ROS and prevent DNA against damage. The data also indicated that both the varieties of pomegranate and the parts could significantly influence the antioxidant activity and prevent DNA damage effect ($P < 0.05$).

2. MATERIALS AND METHODS

2.1. Plant Materials. Three varieties of pomegranate, sour pomegranate, red pomegranate, and white pomegranate, were all obtained from an orchard in Lintong, Shanxi, China. All of the fruits were harvested during October 2006. Preventing oxidation, pomegranates were packed in polyethylene bags and stored at 4 °C.

2.2. Reagents and Instrument. Chemicals and regents were obtained from the following commercial sources: calf thymus DNA (Sigma, United States); Amberlite ADS-17 resins from Nankai Hecheng Science & Technology Co., Ltd (Tianjin, People's Republic of China), BPCL-4 Ultra-weak Luminescence Analyzer (Institute of Biophysics, Academia Sinica, China), and 755B UV−visible spectrophotometer (Cany Precision Instruments Co., Ltd., Shanghai, China).

2.3. Preparation of Pomegranate Peel Extracts. Pomegranate (*P. granatum* L.) fruits were washed in cold tap water, and the outer skins were hand-peeled. The peel (200 g, fresh weight) was put into boiling water (1 L) for 10 min and then was heated for 30 min at 90 °C and for 1 h at 80 °C. Then, the thick husk puree was filtered through cheesecloth to yield a dark yellow aqueous extract.

The ADS-17 resin column was prepared for chromatography by pre-equilibrating it in water for 12 h. The aqueous extract was adsorbed onto the column of ADS-17 resin (200 g, ø3 cm × 16 cm) and then eluted with copious amounts of distilled water (2 L) until the sugary pale yellow eluate was clear in color. The adsorbed tannins were then eluted with EtOH (70%) from the resin to yield a dark brown solution. Ethanol was removed by a Rota-vap (Buchi) in vacuo at low temperature (<40 °C) and freeze-dried to yield pomegranate peel extract as a dark brown powder.

2.4. Preparation of Pomegranate Juice Extracts. Following peeling out, the edible portion was squeezed. The red juice was filtered through cheesecloth, and the filtrate was centrifuged at 4000 rpm for 20 min. The juice had a deep-red color. After the same process in section 2.3, the pomegranate juice extract was obtained.

2.5. Preparation of Pomegranate Seed Extracts. After they were separated manually from the pericarps, following juice extraction, the remaining pomegranate seeds were washed in running water and dried for 48 h at 50 °C. The dried, clean seeds were then crushed up in a pilot plant disintegrator. Degreased with petroleum ether, dried pomegranate seeds were extracted with 70% aqueous acetone. The acetone was removed under reduced pressure at 35 °C, and the residue was chromatographed on the column of ADS-17 resin. The column was washed with a great deal of distilled water, and then, the tannins were eluted with 70% aqueous ethanol. The eluent was concentrated and freeze-dried to obtain pomegranate seed extracts. To prevent oxidation, all experimental pomegranate extracts (PEs) were stored frozen (−20 °C) until analyzed.

2.6. Analysis of Phenol and Total Tannin of PE. Total phenols were estimated by reduction of the Folin−Ciocalteu reagent (*20*), and results were expressed as gallagic acid equivalents. Ten milligrams of each dried PE was dissolved with 100 mL of distilled water. Samples (0.2 mL) were diluted with distilled water (12 mL). Folin−Ciocalteu reagent (1 mL) and 20% (w/v) sodium carbonate solution (3 mL) were added, and the contents were mixed thoroughly. After the mixture stood for 2 h at room temperature, the absorbance was measured at 765 nm with a 755B UV−visible spectrophotometer (Cany Precision Instruments Co., Ltd.). The estimation of phenolic compounds in the extracts was carried out in triplicate.

Analysis of total tannin was based on the titration method (*21*). Excessive zinc ion reacted with tannin compounds in alkali to form complexes. The zinc of residual was titrated by ethylenediaminetetraacetic acid (EDTA). According to the consumption of EDTA and the total content of zinc, the tannin that was bound with zinc was calculated. The extracts (10 mg) were dissolved with 200 mL of distilled water, and then, beakers were placed in a 35 ± 2 °C water bath for 5 min. After 1 mol/L ZnAc (10 mL) and ammonia (7 mL) mixed, 10 mL of extract solution was added after pretreatment. They were placed in a 35 ± 2 °C water bath for 30 min. Some distilled water was added in order to make the final volume 250 mL. After it was carefully filtered, the sample solution was obtained. Twenty milliliters of sample was diluted with 130 mL of distilled water, and then, 12.5 mL of ammonia−NH_4Cl was added. Finally, the mixture was titrated by 0.05 mol/L EDTA.

2.7. Determination of Superoxide Anion Scavenging Ability of PE (*22*). The superoxide anion scavenging ability of PEs was determined by a CL method in the pyrogallol−luminol system on a BPCL Ultra-weak luminescence analyzer (Institute of Biophysics, Academia Sinica, China). Ten microliters of PEs of different concentrations and 50 μL of pyrogallol (6.25×10^{-4} mol/L) were mixed in a glass vial. The light emission from the reactive vial was recorded soon after 0.94 mL of a mixture containing luminol (0.05 mol/L) and carbonate buffer (pH 10.2) was added to the glass vial and mixed. The test condition: Hi-V, 800; Kv, −1; the spectral range of CL, 180−800 nm; and 30 °C. The emission light intensity was recorded every 2 s, and the total integral of the light intensity of 300 s was determined. (The control was performed in the same manner in the mixture without the sample solution, and the background was detected without pyrogallol addition.)

2.8. Determination of Scavenging Ability on Hydroxide Radicals (*23*, *24*). The scavenging ability of PE on hydroxide radical was measured in a $CuSO_4$−Phen−Vc−H_2O_2 CL system. It was composed of 50 μL of sample solution, 50 μL of a 1.0 mmol/L $CuSO_4$ solution, 50 μL of a 1 mmol/L 1,10-phenanthroline solution, 700 μL of a 0.05 mol/L borate buffer (pH 9.0), 100 μL of a 1 mmol/L ascorbate solution, and 50 μL of a 0.15% H_2O_2 solution. The volume of the reaction was 1.0 mL. Then, the reaction was initiated immediately, and kinetic curves were obtained at 3 s intervals over a period of 400 s. (The control was performed in the same manner in the mixture without the sample solution, and the background was detected without H_2O_2 addition.)

2.9. Determination of Scavenging Effect on Hydrogen Peroxide (*25*). The luminol−H_2O_2 system was used to measure the scavenging effect on hydrogen peroxide. The luminescent reaction was initiated by manually adding 1 mL of a solution containing 0.15 mol/L hydrogen peroxide and 0.1mol/L luminol per liter of carbonate buffer (0.05 mol/L, pH 9.4). Light emission vs time was recorded for 3 min at 2 s intervals. (The control was performed in the same manner in the mixture without the sample solution, and the background was detected without H_2O_2 addition.)

2.10. Determination of Preventing DNA Damage Effect (*26*). Preventing the DNA damage effect of PE was determined by a $CuSO_4$−Phen−Vc−H_2O_2−DNA CL system. Copper and 1,10-phenanthroline were premixed in 0.1 mol/L NaOAc/HOAc (pH 5.5) buffer, and 3 μg/mL DNA was incubated with a phen−Cu solution. Following this, 800 μL of phen−Cu/DNA solution, 100 μL of 4.2×10^{-3} mol/L ascorbate, and 200 μL of 6% H_2O_2 were added without interval to a 100 μL sample solution to give a final volume of 1.2 mL. The kinetic curve of CL produced in the phen/Cu/H_2O_2/ascorbate system was immediately recorded. (The control was performed in the same manner in the mixture without the sample solution, and the background was detected without H_2O_2 addition.)

2.11. Data Statistics. All experiments were performed three times. The integrated area of the curve expressed the relative luminescent

Table 1. Polyphenol and Total Tannin Content of PE (Weight %)[a]

varieties	parts	total phenol content (w/w, %)	total tannin content (w/w, %)
sour pomegranate	peel extract	83.45 ± 2.1	39.61 ± 2.4
	juice extract	44.91 ± 2.3	37.70 ± 2.0
	seed extract	51.06 ± 1.5	13.50 ± 0.8
red pomegranate	peel extract	91.54 ± 2.6	40.74 ± 2.5
	juice extract	55.10 ± 1.4	14.84 ± 1.5
	seed extract	29.39 ± 1.3	13.85 ± 1.0
white pomegranate	peel extract	88.50 ± 3.4	34.37 ± 2.2
	juice extract	54.54 ± 2.6	14.84 ± 1.2
	seed extract	30.57 ± 1.8	13.70 ± 1.0

[a] Values are the means of three replicates ± SD.

intensity. The scavenging activity was represented by the following equation.

$$\text{scavenging activity} = \frac{(CL_{control} - CL_0) - (CL_{sample} - CL_0)}{CL_{control} - CL_0}$$

where $CL_{control}$ is the relative luminescent intensity of the control group, CL_0 is the relative luminescent intensity of the background group, and CL_{sample} is the relative luminescent intensity of the experimental group.

Statistical analysis was performed by Origin 7.0 Version soft. CL values were expressed as means ± SD. Standard differences were considered significant at $P < 0.05$.

3. RESULTS

3.1. Analysis of Phenol and Total Tannin of PE. The average contents of phenol and total tannin in PEs are given in **Table 1**. Values are only indicative because they varied widely according to varieties and parts.

3.2. Scavenging Ability of Superoxide Anion. Pyrogallol was autoxidized in alkaline conditions to generate superoxide anion. In this reaction, luminol was excited by superoxide anion, and the decay from the excited state back to the ground state is accompanied by emission of light (luminescence). The results show that light emission in the pyrogallol−luminol system increased for the first 10 s after the luminescent measurement described above was initiated and then declined. All kinetic curves of CL were superposed at around 200 s.

The scavenging effect of superoxide anion was observed in the concentration range of 0.25−250 μg/mL when nine PEs were added. Our results indicated that by addition of PE, the peak CL was reduced, and the integral area of the curve was also decreased, which showed a dose-dependent relation. PE can availably scavenge superoxide anion. Red pomegranate peel extract (RP) led to an almost complete inhibition (98.63%) at the 83 μg/mL concentration, and the half-inhibition concentration (IC_{50}) of RP was 4.01 ± 0.09 μg/mL (**Figure 1**).

3.3. Effect of Scavenging Hydroxide Radical. In the copper/phenanthroline/ascorbate/H_2O_2 system, Cu(II) strongly mediated the oxidation of VC, producing Cu(I) through a Cu(II)/Cu(I) redox mechanism. The generation of hydroxyl radical (•OH) required the presence of Cu(I) and H_2O_2 in the Fenton reaction. Phenanthroline was excited through the oxidation of Phen by •OH. The decay from the excited state back to the ground state is accompanied by CL.

The kinetics of the reaction showed maximal luminescence in the first 50 s after the reactants were mixed and then declined. All kinetic curves of CL were superposed at around 300 s.

The effects of PEs were tested between the concentrations of 0.25 and 25 μg/mL, and marked inhibitions of •OH were obtained (**Figures 3** and **4**).

Red pomegranate peel:

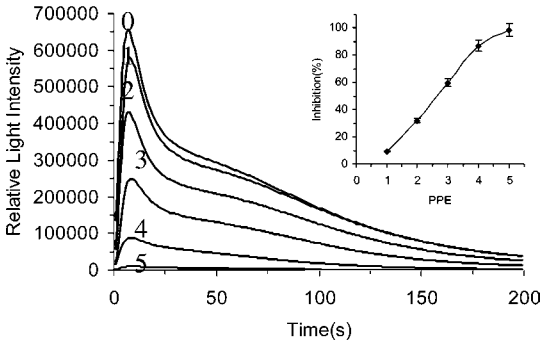

Figure 1. Effect of RP on luminescence of the pyragollic acid–luminol system. Inset: The concentration-dependent inhibitory effect of RP on superoxide anion ($\bar{X}$ ± SD, $n = 3$); 0, 0 μg/mL; 1, 0.25 μg/mL; 2, 1.25 μg/mL; 3, 6.25 μg/mL; 4, 25 μg/mL; and 5, 83 mg/mL. IC_{50} = 4.01 ± 0.09 μg/mL.

Red pomegranate seed:

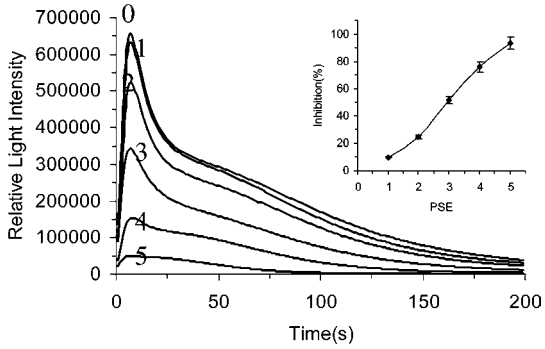

Figure 2. Effect of RS on luminescence of the pyragollic acid–luminol system. Inset: The concentration-dependent inhibitory effect of RS on superoxide anion ($\bar{X}$ ± SD, $n = 3$); 0, 0 μg/mL; 1, 2.5 μg/mL; 2, 6.25 μg/mL; 3, 25 μg/mL; 4, 83 μg/mL; and 5, 250 μg/mL. IC_{50} = 23.28 ± 0.18 μg/mL.

Red pomegranate juice:

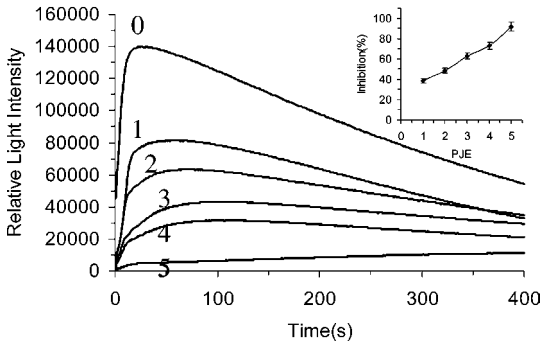

Figure 3. Effect of RJ on luminescence of the $CuSO_4$–Phen–Vc–H_2O_2 system. Inset: The concentration-dependent inhibitory effect of RJ on •OH ($\bar{X}$ ± SD, $n = 3$); 0, 0 μg/mL; 1, 0.25 μg/mL; 2, 2.5 μg/mL; 3, 3.125 μg/mL; 4, 6.25 μg/mL; and 5, 12.5 μg/mL. IC_{50} = 5.56 ± 0.06 μg/mL.

3.4. Scavenging Ability of Hydrogen Peroxide. In the oxygen and alkaline condition, H_2O_2 can oxidize luminol to produce luminescence. The light emission intensity in the H_2O_2−luminol system increased to the maximum in the first 3

White pomegranate seed:

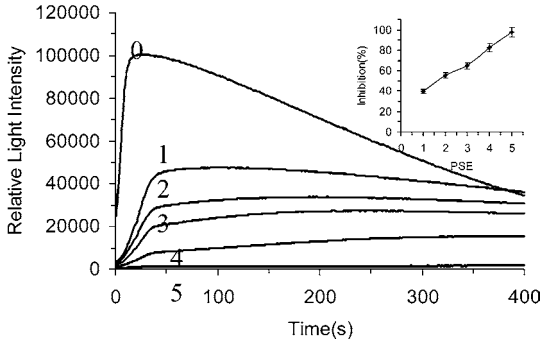

Figure 4. Effect of WS on luminescence of the $CuSO_4$–Phen–Vc–H_2O_2 system. Inset: The concentration-dependent inhibitory effect of WS on •OH ($\bar{X} \pm$ SD, $n = 3$); 0, 0 μg/mL; 1, 0.25 μg/mL; 2, 1.25 μg/mL; 3, 2.5 μg/mL; 4, 6.25 μg/mL; and 5, 12.5 μg/mL. $IC_{50} = 0.83 \pm 0.04$ μg/mL.

White pomegranate peel:

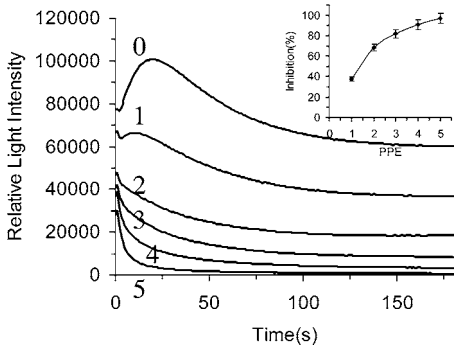

Figure 5. Effect of WP on luminescence of the H_2O_2–luminol system. Inset: The concentration-dependent inhibitory effect of WP on H_2O_2 ($\bar{X} \pm$ SD, $n = 3$); 0, 0 μg/mL; 1, 0.025 μg/mL; 2, 0.0625 μg/mL; 3, 0.125 μg/mL; 4, 2.5 μg/mL; and 5, 25 μg/mL. $IC_{50} = 0.033 \pm 0.003$ μg/mL.

White pomegranate seed:

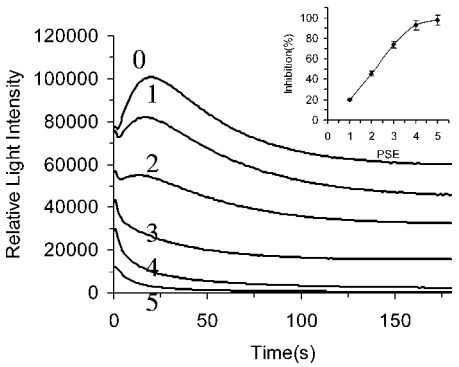

Figure 6. Effect of WS on luminescence of the H_2O_2–luminol system. Inset: The concentration-dependent inhibitory effect of SE on H_2O_2 ($\bar{X} \pm$ SD, $n = 3$); 0, 0 μg/mL; 1, 0.125 μg/mL; 2, 0.25 μg/mL; 3, 0.625 μg/mL; 4, 2.5 μg/mL; and 5, 250 μg/mL. $IC_{50} = 0.29 \pm 0.02$ μg/mL.

s and then declined. At about 180 s, the relative light intensity was minimum.

We observed the CL signal of H_2O_2 in the presence of the luminol. H_2O_2-induced luminol CL was significantly inhibited in a concentration-dependent manner by PEs. The results are shown in **Figures 5** and **6**.

Red pomegranate seed:

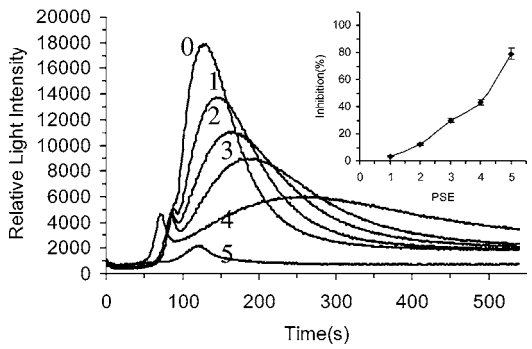

Figure 7. Effect of RS on luminescence of the $CuSO_4$–Phen–Vc–H_2O_2–DNA system. Inset: The concentration-dependent inhibitory effect of RS on DNA ($\bar{X} \pm$ SD, $n = 3$); 0, 0 μg/mL; 1, 1.25 μg/mL; 2, 2.5 μg/mL; 3, 6.25 μg/mL; 4, 12.5 μg/mL; and 5, 25 μg/mL. $IC_{50} = 61.47 \pm 2.12$ μg/mL.

White pomegranate seed:

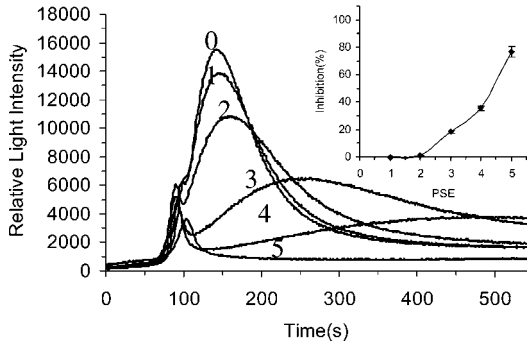

Figure 8. Effect of WS on luminescence of the $CuSO_4$–Phen–Vc–H_2O_2–DNA system. Inset: The concentration-dependent inhibitory effect of WS on DNA ($\bar{X} \pm$ SD, $n = 3$); 0, 0 μg/mL; 1, 1.25 μg/mL; 2, 6.25 μg/mL; 3, 25 μg/mL; 4, 62.5 μg/mL; and 5, 250 μg/mL. $IC_{50} = 3.67 \pm 0.03$ μg/mL.

3.5. Effect of Preventing DNA Damage. In the $CuSO_4$−Phen−Vc−H_2O_2−DNA system, when the time-course of CL emission was studied, an initial small peak was found within 3 min and a second peak appeared around 3.5 min after H_2O_2 addition. •OH produced in the Fenton reaction attacked Phen to generate the initial peak. The second was a lag peak that had DNA damage by hydroxide radical.

In this system, the reduction of the tow peak values and integral areas was observed with increasing PEs concentration. In addition, the two peaks appeared displaced (**Figures 7** and **8**).

4. DISCUSSION

Because of its high sensitivity and rapidity, the chemiluminescent method had been widely used in ROS detection, such as superoxide anion and hydroxide radical, as well as hydrogen peroxide. In this paper, a series of simple, steady, sensitive, and characteristic systems of CL were chosen to measure ROS.

Superoxide anion generation was detected via pyrogallol−luminol-induced CL. The integral of the CL peak reflected the formation of $O_2^{\bullet -}$. As shown in **Figures 1** and **2**, the peak values of CL showed good $O_2^{\bullet -}$ scavenging activity with the PE added. The nine PEs at 25 μg/mL had inhibition rates of 84.41, 69.33, 79.80, 86.91, 63.49, 51.64, 85.35, 74.20, and 55.61%, respectively. In **Table 2**, the half-inhibition concentration (IC_{50}) of

Table 2. IC_{50} of PEs[a]

extracts from the parts of pomegranate		half-inhibition concentration (IC_{50}) (μg/mL) pomegranate sour	red	white
$O_2^{-\bullet}$	peel extract	6.28 ± 0.13	4.01 ± 0.09	4.87 ± 0.01
	juice extract	12.29 ± 0.11	16.60 ± 0.11	8.84 ± 0.07
	seed extract	14.38 ± 0.13	23.28 ± 0.18	21.6 ± 0.85
•OH	peel extract	2.75 ± 0.05	2.37 ± 0.08	1.69 ± 0.03
	juice extract	2.32 ± 0.04	5.56 ± 0.06	2.03 ± 0.06
	seed extract	1.88 ± 0.11	4.10 ± 0.02	0.83 ± 0.04
H_2O_2	peel extract	0.068 ± 0.004	0.047 ± 0.006	0.033 ± 0.003
	juice extract	0.146 ± 0.005	0.075 ± 0.002	0.051 ± 0.002
	seed extract	0.061 ± 0.003	0.194 ± 0.006	0.29 ± 0.02
DNA	peel extract	11.67 ± 0.14	9.64 ± 0.04	10.35 ± 0.09
	juice extract	10.34 ± 0.08	16.15 ± 0.22	9.57 ± 0.20
	seed extract	11.26 ± 0.11	61.47 ± 2.12	3.67 ± 0.03

[a] Values are the means of three replicates ± SD.

peel, juice, and seed extracts of three pomegranate cultivars in different systems was summarized. The peel extract of red pomegranate (**Figure 1**) had the best scavenging effect in nine PEs, but the scavenging effect of red pomegranate seed extract (RS) (**Figure 2**) on $O_2^{\bullet-}$ was the worst (**Figure 9A**). Among all pomegranate peel extracts tested, RP had the lowest IC_{50} value (4.01 ± 0.09μg/mL), followed by white pomegranate peel extract (WP) and sour pomegranate peel extract (SP). That is, the $O_2^{\bullet-}$ scavenging activity of RP was higher than WP and SP. There were some differences between $O_2^{\bullet-}$ scavenging activities in various pomegranate peel extracts. Of the pomegranate juice extracts analyzed, white pomegranate juice extract (WJ) displayed the lowest IC_{50} value (8.84 ± 0.07 μg/mL), followed by sour pomegranate juice extract (SJ) and red pomegranate juice extract (RJ). The $O_2^{\bullet-}$ scavenging activity of WJ was higher than SJ and RJ. For the pomegranate seed extracts, sour pomegranate seed extract (SS) showed the lowest IC_{50} value (14.38 ± 0.13 μg/mL) and the best $O_2^{\bullet-}$ scavenging activity, followed by white pomegranate seed extract (WS) and RS. In the comparison among all pomegranate peel, juice, and seed fraction extracts tested, the $O_2^{\bullet-}$ scavenging activity was as follows: peel extracts > juice extracts > seed extracts. The statistic analysis showed that there were significant differences among the extracts of different varieties and parts ($P < 0.05$). The difference of parts was a more important factor as compared with the varieties in this system.

$CuSO_4$−Phen−Vc−H_2O_2-induced CL was depressed by all of the extracts (0.25−25 μg/mL) in a concentration-dependent manner (**Figures 3** and **4**). While each extract at 12.5 μg/mL, respectively, generated 96.41, 90.53, 88.76, 97.45, 91.64, 70.15, 99.55, 91.70, and 97.85% inhibition, the most marked scavenging activity of •OH was observed with WS added (**Figure 4**). The IC_{50} of WS was 0.83 ± 0.04 μg/mL (**Table 2**). Among all pomegranate peel extracts tested, WP had the lowest IC_{50} value (1.69 ± 0.03 μg/mL), followed by RP and SP. The •OH scavenging activity of WP was higher than RP and SP. Of the pomegranate juice extracts and seed extracts analyzed, white varieties exhibited a higher scavenging activity of •OH than sour varieties and red varieties. In the comparison among three parts of pomegranate, in sour varieties, seed extracts > juice extracts > peel extracts; in red varieties, peel extracts > juice extracts > seed extracts; and in white varieties, seed extracts > peel extracts > juice extracts. By the statistical analysis, the significant differences were found among the extracts of different varieties and parts ($P < 0.05$), and the difference of varieties was the most influenced factor (**Figure 9B**).

The H_2O_2 scavenging ability of the PEs was assayed in the luminol−H_2O_2 system by measuring the peak values of CL produced in the reaction of H_2O_2 with luminol. **Figures 5** and **6** showed that all of the PEs were effective to scavenge hydrogen peroxide. Their IC_{50} values are shown in **Table 2**; WP (**Figure 5**) had the minimum IC_{50} of 0.033 ± 0.003 μg/mL. Except for seed extracts, in all pomegranate peel extracts and juice extracts tested, both of their scavenging activities on H_2O_2 were white

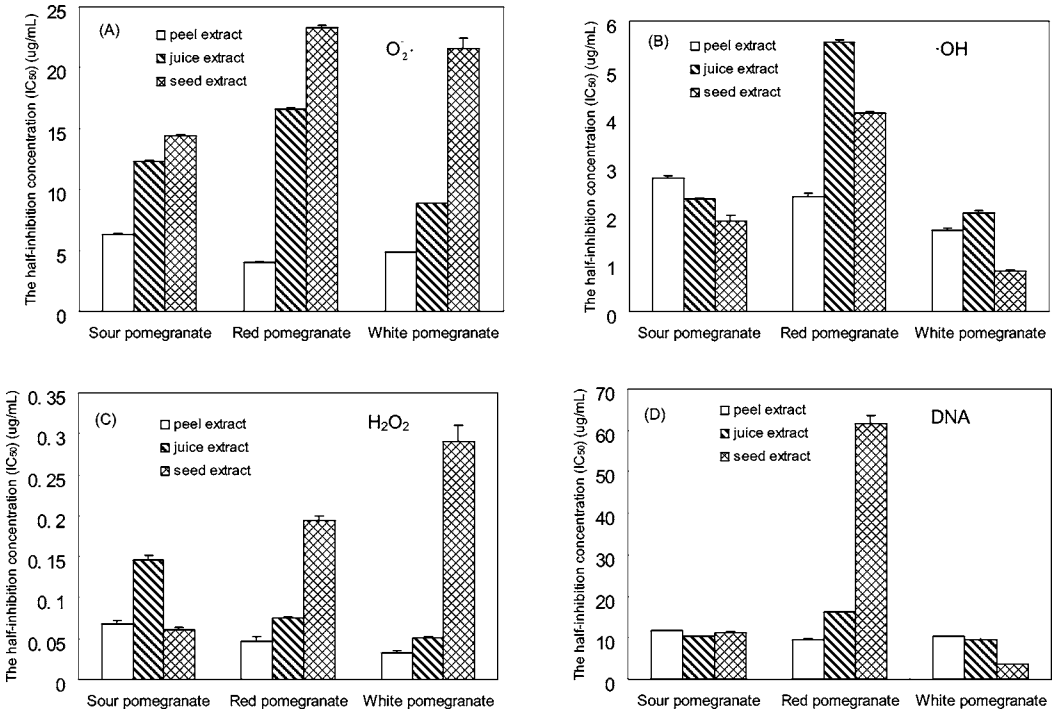

Figure 9. IC_{50} of PEs. Data shown are the means of three replicates; the vertical bar represents ±SD. (**A**) The IC_{50} of nine PEs on luminescence of the pyragollic acid–luminol system. (**B**) The IC_{50} of nine PEs on luminescence of the $CuSO_4$–Phen–Vc–H_2O_2 system. (**C**) The IC_{50} of nine PEs on luminescence of the H_2O_2–luminol system. (**D**) The IC_{50} of nine PEs on luminescence of the $CuSO_4$–Phen–Vc–H_2O_2–DNA system.

varieties > red varieties > sour varieties. Of the pomegranate seed extracts analyzed, SS showed a higher scavenging activity on H_2O_2 than RS and WS (**Figure 6**). In the comparison among three parts of pomegranate, in sour varieties, seed extracts > peel extracts > juice extracts; both in red varieties and white varieties, peel extracts > juice extracts > seed extracts (**Figure 9C**). By the statistical analysis, there were significant differences among the extracts of different varieties and parts ($P < 0.05$). The difference of parts was the most important factor.

In addition, according to their differences in affecting the luminescence, antioxidants were sorted to three types in different CL systems. These were inhibiting, delaying, and mixed type, respectively. The CL yield was decreased by increasing the antioxidant concentration. This was called the inhibiting type. The luminescence peak was delayed, and the interval became longer and longer with an increasing antioxidant concentration. This was called the delaying type. The mixed type showed both inhibiting and delaying activities. Thus, PEs belonged to the inhibiting type in the above three systems.

There was a considerable amount of evidence revealing an association between DNA damage and some human problems, such as cancer and aging. In our experiment, we reported the CL method in the $CuSO_4$−phenanthroline−Vc−H_2O_2−DNA system for DNA damage detection and the effect of some PEs on the luminescence. By increasing the extract concentration, the emission intensity gradually reduced in this system. As shown in **Table 2**, parts of different pomegranates extracts, e.g., white pomegranate seed, red pomegranate juice, and sour pomegranate peel, at a low concentration range (IC_{50}, 3.67 ± 0.03−16.15 ± 0.22 μg/mL), remarkably inhibited the CL and delayed the two peaks (mixed type). We compared the IC_{50} values of all PEs in this system and found that WS (**Figure 8**) exhibited the best effect in preventing against DNA damage. Among all peel extracts analyzed, the ability of preventing against DNA damage of RP was greater than WP and SP. However, for juice extracts and seed extracts, white varieties > sour varieties > red varieties. The comparisons among three parts of pomegranate are as follows: in sour varieties, juice extracts > seed extracts > peel extracts; in red varieties, peel extracts > juice extracts > seed extracts; and in white varieties, seed extracts > juice extracts > peel extracts (**Figure 9D**). The statistical analysis results revealed that there were significant differences among the extracts of different varieties and parts ($P < 0.05$). Our data demonstrated that PEs possess a powerful ability of preventing against DNA damage.

As indicated in **Table 2**, the IC_{50} of the four CL system varied considerably from pomegranate to pomegranate and by statistical analysis, intervarietal differences in antioxidant activity and the prevention of DNA damage effects of pomegranate varieties were found to be significant within the confidence interval of 95% and the extracts of different parts of same varieties also had significant differences (at $P < 0.05$). In conclusion, PEs had a powerful free radical-scavenging activity and DNA damage prevention ability in different experimental systems. As compared with DNA and $O_2^{\bullet -}$, the scavenging ability of PE on $^{\bullet}OH$ and H_2O_2 was better. DNA damage was a complex process; many factors can affect it. So, it is extremely difficult to explain in existence study. These results showed that all of the extracts had high antioxidant activities. Thus, PEs can be exploited as natural antioxidants. Nasr et al. (*27*) reported that pomegranate contains ellagic acid, ellagitannins, and gallic acids. Otherwise, according to former work in our lab, liquid chromatography/mass spectrometry analyses revealed that PE also contains proanthocyanidin monomers and dimers. In **Table 1**, we found that there was an amount of phenol and tannin in PE; moreover, their phenol and tannin contents were higher, and their scavenging ability was better. The presence of these polyphenols in the pomegranate may be responsible for the antioxidant activity of PEs (*7*). Also, our results raised a number of issues for further investigation: the isolation and characterization of individual phenolic compounds present in various extracts and the determination of the mechanisms involved in the antioxidant abilities of PEs.

ABBREVIATIONS USED

PE, pomegranate extract; SP, sour pomegranate peel extract; SJ, sour pomegranate juice extract; SS, sour pomegranate seed extract; RP, red pomegranate peel extract; RJ, red pomegranate juice extract; RS, red pomegranate seed extract; WP, white pomegranate peel extract; WJ, white pomegranate juice extract; WS, white pomegranate seed extract.

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