In vitro antioxidant activity of extracts from common legumes

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Abstract

The in vitro antioxidant activity of pinto bean, cowpea, baby lima bean, lentil, chickpea, small red bean, red kidney bean, black kidney bean, navy bean, and mung bean extracts were investigated. Significant differences were observed in the phenolic content and the antioxidant activity amongst the legume extracts. Lentils demonstrated the highest phenolic content (47.6 mg/g), total antioxidant activity (720.68 U/g), DPPH• scavenging activity (38.5%), and total reducing power, whereas baby lima beans and navy beans had the lowest. Amongst the extracts, hydroxyl radicals (•OH) scavenging was higher in black kidney bean (85.68%) and baby lima bean (74.97%) extracts. The total antioxidant activity (r = 0.84), DPPH• scavenging activity (r = 0.83), and total reducing power (r = 0.84) were positively correlated with the total phenolic content. However, •OH scavenging and the phenolic content were not correlated.

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1. Introduction

Grain legumes in the daily diet are of nutritional value, as significant sources of human health food. Legume seeds are consumed extensively by humans in different areas of the world. Legumes are essential sources of macronutrients and micronutrients, as well as rich in flavonoids, proteins, polypeptides, amino acids, vitamins, carotenoids, and phenolics, these compounds are considered to be excellent sources of ingredients for functional foods and other applications (Heimler, Vignolini, Dini, & Romani, 2005; Madhujith, Naczk, & Shahidi, 2004). The highest variety of leguminous plants can mainly be found in tropical and subtropical regions. Legumes have approximately 3 times more protein than cereal grains. The annual production of legumes ranks fifth in the world after wheat, rice, maize, and barley (Hoover, Li, Hynes, & Senanayake, 1997). The global production of edible legumes was about 275 million tons in 2010. China is rich in leguminous plants, and statistical data indicates the cultivation of more than 20 types of legumes in the country.

Leguminous seeds are arable pulse crops that belong to the family of Leguminosae. These crops are largely cultivated for their grains and utilised as valuable ingredients of various products for human consumption, and they are also used for animal feed. The significant role of grain legumes species on nitrogen dynamics has been studied and the main legume species that have been investigated are peas and faba beans (Nemecek, Richthofen, Casta, Charles, & Pahl, 2008). The health benefits of consuming legumes are related to the amount of dietary fibre and polyphenols in legumes. A number of epidemiological studies have correlated the consumption of legumes with high phenolic content to the reduced incidence of diseases such as cancer, ageing, diabetes, and cardiovascular disease (Kris-Etherton et al., 2002). Tannins, phytic acid, saponins, and other factors such as polyphenols in legumes have been hypothesised to prevent chronic diseases (Kabagambe, Baylin, & Ruiz-Narvárez, 2005).

The dominant phenolic compounds present in leguminous seeds are flavonoids, phenolic acids, and procyanidins (Amarowicz & Pegg, 2008). Phenolic compounds act as radical scavengers, reducing agents, and chelators of metal ions (Djordjevic, Šiler-Marinkovic, & Dimitrijevic-Brankovic, 2011). The antioxidant activity and phenolic compounds in various common beans have been demonstrated in previous studies (Xu, Yuan, & Chang, 2007). Similarly, phenolics and antioxidants of grain legume hulls have been studied by several authors (Amarowicz, Troszynska, Barylko-Pikielna, & Shahidi, 2004). The bioactive substances, the antioxidant activity, the radical scavenging capacity of various legumes, and the effect of processing and germination on the bioactivities and antioxidant activity have been reported (Amarowicz & Pegg, 2008). Although a few studies report the in vitro antioxidant activity of various legumes, these studies provide sparse data (Srerramulu, Reddy, & Raghunath, 2009). In the present study, bioactive compounds were extracted from various leguminous seeds by ultrasonic-assisted acidic ethanol treatment. Therefore, the current study is aimed to evaluate the antioxidant potential of ten different common legumes and to explore the relationship between antioxidant activity and phenolic content in the samples. The current study provides useful information on the effective utilisation of legumes in food processing.

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2. Materials and methods

2.1. Materials

Ten commercial legume seeds, pinto bean, baby lima bean, red kidney bean, black kidney bean, navy bean, small red bean, black eye bean, mung bean, lentil, and chickpea were purchased from a local supermarket. All of the seeds were air dried at 25 °C and ground using a kitchen grinder into small sizes that can pass through sieve No. 72 (British Sieve Standards).

Gallic acid, 1,1-diphenyl-2-picryl-hydrazyl (DPPH), and the Folin–Ciocalteu reagent were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The total antioxidant capacity (TAC) test kits were purchased from the Nanjing Institute of Biological Engineering. All other chemicals were of analytical grade, including sodium acetate, anhydrous ethanol, monohydrate gallic acid, and anhydrous sodium carbonate.

2.2. Extract preparation

1 g of the legume flour was mixed with 20 mL of acidified ethanol (60% ethanol with 2% HCl, pH 1.5). The mixture was subjected to ultrasonication for 30 min at 60 °C (300 W). After cooling to room temperature, the mixture was centrifuged at 3000g for 15 min. The supernatant was filtered using filter paper, and the residue was re-extracted twice with ultrasonic oscillation. The pooled extract was concentrated using a rotary evaporator under vacuum at 60 °C. The extract volume was adjusted to 50 mL with deionized water before they were stored in the dark at 4 °C until further use.

2.3. Determination of total phenolic content (TPC)

The total phenolic content of the legume extracts was determined using the Folin–Ciocalteu reagent. For each extract, a 1.0 mL aliquot was placed into a 50 mL volumetric flask, and the extract was mixed with 30 mL deionized water, 2.5 mL of Folin–Ciocalteu reagent, and 7.5 mL of 20% Na2C03 solution. The final volume of the mixture was adjusted to 50 mL using distilled water. After incubation for 30 min at room temperature, absorbance of the mixture was measured at 765 nm using a spectrophotometer, with distilled water as the blank. The total phenolic content of each extract was determined from the standard curve based on gallic acid; the content was expressed in mg of gallic acid equivalents (GAE) per gram of legume (mg/g).

2.4. Determination of total antioxidant capacity (TAC)

The total antioxidant capacity of the legume extracts was measured using a TAC test kit (Nanjing Institute of Biological Engineering, China). The kit is based on the reaction of Fe²⁺ and phenanthroline using a spectrophotometer at 520 nm for determination of total antioxidant capacity of extracts. Results were expressed as U/g extract. U is a unit of total antioxidant capacity, which is defined as the amount of antioxidants required to make absorbance increase 0.01 in 1 mL of reaction liquid at 37 °C. The total antioxidant capacity was calculated using the formula:

\[
TAC = \frac{(OD_U - OD_C)}{0.01 \times 30} \times \frac{V_0}{V_1} \times N
\]

where OD_U and OD_C are the absorbance values of the test sample and the reagent blank, respectively; V_0 is the total volume of reaction liquid (mL); V_1 is the volume of extracts (mL); N is the fold of dilution of sample before tested; 30 is the reaction time (min).

2.5. Estimation of DPPH• scavenging activity

The DPPH• scavenging by each legume extract was evaluated according to the method described by Brand-Williams, Cuvelier, and Berret (1995), with some modifications. The diluted extract at various concentrations was added to the working solution of DPPH• in ethanol, and the volume of each mixture was adjusted to 1.0 mL. Each mixture was shaken vigorously and incubated in the dark at room temperature for 30 min, before it was centrifuged at 3000g for 10 min. The absorbance of each supernatant was measured at 517 nm. The control set-up contained all the reagents except the legume extracts. The percent scavenging of DPPH• was calculated using the formula:

\[
\% \text{scavenging activity} = \frac{1 - (A_{\text{sample}}/A_{\text{control}})}{A_{\text{control}}} \times 100
\]

where A_control and A_sample are the absorbance values at 517 nm of the control and the sample extract, respectively.

2.6. Estimation of •OH scavenging

Each extract (0.5 mL) was mixed with 0.5 mL of 9.1 mmol/L salicylic acid–ethanol solution, 0.5 mL of 9 mmol/L Fe²⁺ solution, and 3.5 mL of distilled water. Subsequently, 5 mL of 88 mmol/L H2O2 was added to start the Fenton reaction. The absorbance A1 of the reaction product was measured at 510 nm. The absorbance A2 was determined with 0.5 mL distilled water instead of the 9 mmol/L Fe²⁺ solution, whereas the absorbance A3 was measured using 0.5 mL of distilled water instead of the extract. The hydroxyl radical (•OH) scavenging activity was calculated using the formula:

\[
\% \text{scavenging activity} = \frac{1 - (A_1 - A_2)/A_3}{A_3} \times 100
\]

2.7. Estimation of total reducing power

The total reducing power was assessed using the Prussian blue method described by Oyaizu (1986), with little modifications. To measure the total reducing power of the tested samples, each extract (2 mL) was mixed in a test tube with 2.0 mL of 2.5 mol/L phosphate buffer (PBS, pH 6.6) containing 2.0 mL of 1% K3Fe(CN)6 solution. The mixtures were incubated at 50 °C for 20 min. After the addition of 2.0 mL 10% trichloroacetic acid, each mixture was centrifuged at 3000g for 10 min. The supernatant (2.5 mL) was collected and mixed with 2.5 mL of deionized water containing 0.5 mL of 0.1% ferric chloride. The absorbance was measured at 700 nm using a spectrophotometer, with distilled water as the blank. Trolox was used to construct the standard curve.

2.8. Statistical analysis

The all tests were performed in triplicate for each independent sample to be analysed. All data were expressed as mean ± standard deviation. Comparison of means was checked by Duncan’s test using the SAS system (version 8.0, SAS Institute, Cary, NC). Correlation between the phenolic compound contents and the antioxidant activity was determined using Pearson’s correlation test. P < 0.05 was considered statistically significant.

3. Results and discussion

3.1. Phenolic content

Phenolic compounds are widely distributed in plant kingdom and are important in the antioxidant capacity in vitro because of their ability to donate hydrogen and form stable radical intermediates (Scalbert, Manach, Morand, Remesy, & Jimenez, 2005).
Significant differences were observed amongst the total phenolic content values of flour from different legumes (Table 1). The total polyphenol content of the different legumes varied from 9.5 mg/g to 47.6 mg/g. Lentils showed the highest phenolic content (47.6 mg/g), which was lower than that of 58.0 mg/g reported by Amarowicz et al. (2009), whereas baby lima bean had the lowest (9.5 mg/g). The phenolic content of small kidney beans (45.7 mg/g) appeared to be similar to that of lentils (47.6 mg/g); this result was higher than that reported by Djordjevic et al. (2011). The phenolic content of pinto bean flour (33.4 mg/g) was similar to that of black kidney bean (32.9 mg/g). The total phenolic content of legumes was significantly higher than that of corn, wheat, millet, and rice (Seeram et al., 2009). The phenolic content of broad beans and peas as reported by Amarowicz et al. (2004) was similar to that of chickpea (21.9 mg/g) in the current study, however, the total phenolic content of other legumes were remarkably high in their studies, as compared with the results of the present study. Previous studies showed that the total phenolic content of lentils is the highest amongst the examined legumes (Djordjevic et al., 2011; Han & Baik, 2008), which is comparable with the current study. The polyphenol variation in different studies may be attributed to genetic factors, variation between cultivars, and the extraction procedure used (Amarowicz & Pegg, 2008). To date, none of the available extraction procedures is completely suitable for whole phenolics from plant materials (Naczka & Shahidi, 2004). The abundant phenolic content of leguminous seeds indicates that legumes are the principal sources of antioxidant activity in food.

3.2. Total antioxidant capacity

The extracts of different legumes have high antioxidant capacity, and the TAC values ranged from 116 U/g to 721 U/g with statistically significant differences (Table 1). The lentil extract had the highest TAC value (721 U/g) followed by chickpea (648 U/g), small red kidney bean (622 U/g), and black kidney bean (602 U/g). By contrast, baby lima bean had the lowest value (116 U/g). The TAC of cowpea (222 U/g) was similar to that of navy bean (215 U/g). The antioxidant capacity of legumes may be influenced by their complex composition like active polysaccharides, proteins, amino acids, vitamins, and microelements. Fernandez-Orozco, Zielinski, and Piskula (2003) reported that low-molecular-weight antioxidants, particularly the phenolic compounds provide the antioxidant capacity of four lentils. A previous report elucidated that the antioxidant activity of lentils is sevenfold higher than that of green pea and chickpea (Han & Baik, 2008). The total antioxidant activity of different legumes differed because of the various processing conditions and the different assays of antioxidant capacity that were used. Given the different possible methods of extraction, estimation, and calculation for studying antioxidant capacity, the results observed in the current study are difficult to compare with those reported in the literature (Djordjevic et al., 2011).

3.3. DPPH** radical scavenging activity

The DPPH** scavenging assay is extensively used to evaluate the free-radical scavenging of plant extracts because of its simple, rapid, sensitive, and reproducible procedure (Mayachiew & Devahastin, 2008). The observed DPPH** scavenging activity of the ethanol-based extracts from different legumes is given in Fig. 1. The DPPH** scavenging activity increased with the increasing quantity of the legume extract. The DPPH** scavenging activity of lentils was significantly higher than that of other legumes. The observed DPPH** scavenging capacity reached up to 11.8% at 20 μL and 38.5% at 100 μL, which agrees with the results of Djordjevic et al. (2011). The baby lima bean and navy bean had the lowest DPPH** scavenging activity, with values less than 15% at 100 μL (Fig. 1). López-Amorós, Hernández, and Estrella (2006) reported that the DPPH** scavenging activity of tannin fraction in red lentil was several times greater than those of the crude extract and the low-molecular-weight phenolics fraction. Cheng, Peng, and Xiang (2009) studied the DPPH** scavenging activity of 14 kinds of beans; the scavenging activity of extracts decreased in the following order: sword bean > black adzuki bean > mixed coloured cowpea > red bean > kidney bean > rice bean > broad bean > black soybean > mung bean > pea > hemp cowpea > soybean > green soybean > chickpea. Their data was significantly different from that of the current study. The DPPH** scavenging activity of cowpea and chickpea were similar to that of some commonly consumed legumes (Madhujith & Shahidi, 2005). The DPPH** scavenging ability of various commonly consumed and under utilised legumes were likewise reported by Siddhuraju (2006). The DPPH** scavenging activity was found to be highest in extracts from raw and dry-heated horse gram seeds (Siddhuraju & Manian, 2007). The plant source of each extract, environmental factors, and the solvent used for extraction may account for the differences in the levels of DPPH** scavenging activity.

3.4. Hydroxyl radical (**OH) scavenging activity

The **OH generated by the Fenton reaction is a highly reactive free radical. This free radical can be formed from hydrogen peroxide and the superoxide anion and may be generated in the human body under certain physiological conditions. Double bonds are formed when **OH reacts with aromatic compounds, thereby producing other oxygen-reactive free radicals, such as the hydroxyclohexadienyl radical (Lee, Koo, & Min, 2004). The scavenging ability of the legume extracts to inhibit **OH is shown in Table 2. The **OH scavenging activity of legume extracts was generally decreased with the reduced extract concentration, with certain differences between legume species. The scavenging rate of the original legume extracts ranged from 58.64% (cowpea) to 85.68% (black kidney bean), whereas the extracts with fivefold and tenfold dilutions had scavenging activity ranging from 19.12% to 25.25% and 9.43% to 13.73%, respectively. The scavenging activity of the black kidney bean extract was reduced by approximately sixfold when extract was diluted by tenfold. The **OH scavenging activity of chickpea (66.22%) was similar to that of red kidney bean (66.15%), whereas the scavenging activity of mung bean (65.92%) was similar to that of lentil (60.09%) (Table 2). A previous study found that chickpea proteins and peptides have a scavenging effect on **OH at concentrations from 0.1 mg/mL to 50 mg/mL, and the

Table 1

<table>
<thead>
<tr>
<th>Material</th>
<th>TPC (mg/g)</th>
<th>TAC (U/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pinto bean</td>
<td>33.4 ± 3.0*</td>
<td>567 ± 9*</td>
</tr>
<tr>
<td>Cowpea</td>
<td>15.2 ± 0.7*</td>
<td>222 ± 6*</td>
</tr>
<tr>
<td>Baby lima bean</td>
<td>9.5 ± 1.0*</td>
<td>116 ± 3*</td>
</tr>
<tr>
<td>Lentil</td>
<td>47.6 ± 5.3*</td>
<td>721 ± 51*</td>
</tr>
<tr>
<td>Chickpea</td>
<td>21.9 ± 2.8</td>
<td>648 ± 18*</td>
</tr>
<tr>
<td>Small red bean</td>
<td>45.7 ± 1.8</td>
<td>622 ± 32*</td>
</tr>
<tr>
<td>Red kidney bean</td>
<td>27.1 ± 3.0*</td>
<td>516 ± 61*</td>
</tr>
<tr>
<td>Black kidney bean</td>
<td>32.0 ± 1.0*</td>
<td>602 ± 12*</td>
</tr>
<tr>
<td>Navy bean</td>
<td>11.6 ± 0.01*</td>
<td>215 ± 29*</td>
</tr>
<tr>
<td>Mung bean</td>
<td>26.7 ± 1.4*</td>
<td>304 ± 23*</td>
</tr>
</tbody>
</table>

* Results are means of three independent samples analysed in triplicate ± standard deviation. Values followed by different letters in a column are significantly different (P < 0.05) by Duncan test.
* Results are expressed as mg of gallic acid equivalents/g legume extract.
* Total antioxidant capacity.
different standard deviation. Values followed by different letters in a column are significantly different (P < 0.05) by Duncan test.

### Table 2
Scavenging effect of legume extract on •OH.\(^A\)

<table>
<thead>
<tr>
<th>Material</th>
<th>Original extract •OH scavenging activity (%)</th>
<th>Dilute 5-fold</th>
<th>Dilute 10-fold</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pinto bean</td>
<td>65.92 ± 0.10(^c)</td>
<td>23.09 ± 0.24(^h)</td>
<td>14.01 ± 0.01(^c)</td>
</tr>
<tr>
<td>Cowpea</td>
<td>58.64 ± 0.33(^d)</td>
<td>19.12 ± 0.69(^e)</td>
<td>9.43 ± 0.81(^b)</td>
</tr>
<tr>
<td>Baby lima bean</td>
<td>74.97 ± 0.93(^b)</td>
<td>24.81 ± 0.15(^a)</td>
<td>11.41 ± 0.19(^bc)</td>
</tr>
<tr>
<td>Lentil</td>
<td>60.09 ± 2.93(^d)</td>
<td>20.15 ± 1.97(^e)</td>
<td>11.30 ± 1.45(^bc)</td>
</tr>
<tr>
<td>Chickpea</td>
<td>66.22 ± 0.09(^a)</td>
<td>22.53 ± 0.22(^cd)</td>
<td>11.23 ± 0.93(^bc)</td>
</tr>
<tr>
<td>Small red bean</td>
<td>65.44 ± 0.85(^b)</td>
<td>20.05 ± 1.10(^f)</td>
<td>11.42 ± 0.64(^e)</td>
</tr>
<tr>
<td>Red kidney bean</td>
<td>66.15 ± 0.01(^f)</td>
<td>20.50 ± 0.28(^ae)</td>
<td>10.24 ± 0.49(^bc)</td>
</tr>
<tr>
<td>Black kidney bean</td>
<td>85.68 ± 2.51(^a)</td>
<td>25.25 ± 0.65(^f)</td>
<td>13.73 ± 0.58(^bc)</td>
</tr>
<tr>
<td>Navy bean</td>
<td>66.82 ± 1.36(^b)</td>
<td>22.46 ± 1.15(^cd)</td>
<td>11.74 ± 1.28(^b)</td>
</tr>
<tr>
<td>Mung bean</td>
<td>59.01 ± 0.76(^d)</td>
<td>19.98 ± 0.69(^f)</td>
<td>10.97 ± 1.21(^bc)</td>
</tr>
</tbody>
</table>

(A) Results are means of three independent samples analysed in triplicate ± standard deviation. Values followed by different letters in a column are significantly different (P < 0.05) by Duncan test.

3.5. Total reducing power

The total reducing power of legume extracts is presented in Table 3. Significant differences in the reducing power were found amongst the various legume extracts. The absorbance values of the original extracts ranged from 0.05 to 1.39. However, absorbance value varied from 0.01 to 0.51 and 0.00 to 0.25 when solutions were diluted fivefold and tenfold, respectively. The absorbance increased with increasing extract concentrations. The higher absorbance values had stronger reducing capacity. The undiluted lentil extract exhibited the highest reducing power (1.39), which was followed by mung bean (0.96). The baby lima bean extract had the least reducing power (0.05), and its absorbance was almost zero after the extract was diluted 5 times. The tannin fraction of red lentil showed a greater reducing power than those of the crude extract and the low-molecular-weight phenolics fraction (Amarowicz et al., 2009). Siddurajju, Mohan, and Becker (2002) reported that the reducing power of bioactive compounds in peanut hulls was related to antioxidant activity, specifically to the free radical scavenging activity. Amarowicz and Troszynska (2004) studied the relationship between the reducing power of extracts from red lentil and their phenolic content, and their study reported a direct relationship between the reducing power and the antioxidant capacity. The higher reducing power of a compound in legumes may be considered an indicator of its potential antioxidant capacity.

Table 3

Total reducing power of legume extract.\(^A\)

<table>
<thead>
<tr>
<th>Material</th>
<th>Total reducing power.(^B)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Original extract</td>
</tr>
<tr>
<td>Pinto bean</td>
<td>0.79 ± 0.10(^c)</td>
</tr>
<tr>
<td>Cowpea</td>
<td>0.35 ± 0.03(^f)</td>
</tr>
<tr>
<td>Baby lima bean</td>
<td>0.05 ± 0.01(^f)</td>
</tr>
<tr>
<td>Lentil</td>
<td>1.39 ± 0.11(^a)</td>
</tr>
<tr>
<td>Chickpea</td>
<td>0.10 ± 0.00(^d)</td>
</tr>
<tr>
<td>Small red bean</td>
<td>0.74 ± 0.01(^d)</td>
</tr>
<tr>
<td>Red kidney bean</td>
<td>0.03 ± 0.07(^d)</td>
</tr>
<tr>
<td>Black kidney bean</td>
<td>0.85 ± 0.07(^c)</td>
</tr>
<tr>
<td>Navy bean</td>
<td>0.17 ± 0.00(^a)</td>
</tr>
<tr>
<td>Mung bean</td>
<td>0.96 ± 0.01(^b)</td>
</tr>
</tbody>
</table>

(A) Results are means of three independent samples analysed in triplicate ± standard deviation. Values followed by different letters in a column are significantly different (P < 0.05) by Duncan test.

3.6. Correlation between TPC and antioxidant activity of legumes

The correlation analysis of TPC, TAC, •OH scavenging activity, DPPH• scavenging activity, and total reducing power of legume extracts are shown in Table 4. The TAC (r = 0.84), DPPH• scavenging activity (r = 0.83), and total reducing power (r = 0.84) of the legume extracts in this study were positively correlated with the phenolic content. No correlation was observed between the •OH scavenging activity and the phenolic content of legume extracts. Velioglu, Mazza, and Gao (1998) reported the positive correlation between TPC and the antioxidant activity of anthocyanins, which is comparable with the current results. Therefore, the different phenolic content of the various legumes exhibited different levels of contribution for the antioxidant activities. Extracts with higher TPC exhibited higher antioxidant ability because phenols contain hydroxyl which could provide more phenolic hydroxyl and hydrogen atoms with higher amount of TPC, thus their scavenging effect on free radicals were stronger.

Fig. 1. Scavenging effect of the legume extracts on the 1,1-diphenyl-2-picryl-hydrazyl radical (DPPH•), as measured by loss in absorbance at 517 nm.
4. Conclusion

The antioxidant activity significantly differed amongst the various legume extracts. Lentils showed the highest phenolic content, total antioxidant activity, DPPH scavenging activity, and total reducing power, whereas baby lima bean and navy bean showed the least value. Significant positive correlation was observed between phenolics and total antioxidant activity, DPPH scavenging activity, total reducing power of the different legume extracts. Further research is needed to conduct on identification of bioactive compounds, determination of the antioxidant activity by in vivo studies, and evaluation of their beneficial health effects in human body.

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