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Antioxidant Activities of Different Fractions of Peanut Testa Extracts

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Abstract. Antioxidant activities of crude extract (CE) performed by aqueous ethanol from red peanut testae, fractions of n-butanol/water and ethyl acetate/water of crude extract were investigated. The antioxidant activities were measured by reducing power, DPPH free radical scavenging, and oxidation stability tests. The results show that the portion of the n-butanol fraction (BF) has the maximum reducing power, followed by the ethyl acetate fraction (EAF), crude extract (CE), the water-soluble fraction of ethyl acetate (WEA) and the water-soluble fraction of n-butanol (WBF). The IC_{50} value of BF has shown the highest inhibition on DPPH radical, followed by EAF, CE, WEAF and WBF. The accelerated oxidation test shows the order of anti-oil oxidant activity is EAF, WBF, BF and WEAF. The antioxidants in the peanut testae are mainly some fat-soluble substances.

Keywords: Peanut testa, antioxidant, extract.

1. Introduction

Peanut is an important food crop with many health benefits of their consumption realized by consumers and constitute as a multimillion-dollar crop worldwide. With the development of peanut industry, the process and comprehensive utilization of peanut by-products becomes more and more important. Peanut testae, accounting for 1% up to 3% of the total peanut, are the principal by-product of the peanut industry [1],[2]. Peanut testae, separated from the kernels during the oil process of peanuts, are used to prevent from bleeding as a traditional medicine in China. It contains proanthocyanidins, resveratrol, vitamin K and other bioactive substances [3]-[6]. The clinical application showed that peanut testae are with the beneficial effects on a wide range of hemorrhagic disease, such as hemophilia, types of hemophilia, primary and secondary thrombocytopenic purpura, liver hemorrhagic disease, postoperative hemorrhage, tumor hemorrhage, stomach, intestine, lung and uterus bleeding. A recent study by Feldman, 1999 [7] found that peanut testae might have an inhibitory effect on the prevention against cardiovascular diseases. However, little information has been done on different fractions of different solvents.

The aim of this work was to investigate the antioxidant activities of the different fractions extracted by n-butanol /water and ethyl acetate/ water from the crude extract performed by aqueous ethanol from peanut testae.

2. Materials and Methods

2.1. Materials and Chemicals

Peanut testae were provided by Limited company of Shandong Luhua Group. 1, 1-diphenyl-2-picrylhydrazyl (DPPH) was purchased from SIGMA-ALDRICH. Inc. Anhydrous ethanol was from Laiyang Shuangshuang Chemical Co., Ltd. (Shandong, China). Potassium ferricyanide was from Sinopharm Chemical Reagent Co., Ltd (Ningbo Road 52, Shanghai, China). Disodium hydrogen phosphate,

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sodium dihydrogen phosphate, acetic acid, ferric chloride and all chemicals and reagents were analytical grade.

2.2. Preparation of Extracts

Peanut testae in red colour were ground in a grinder to reach 40-mesh, degreased by petroleum ether and stored in refrigerated at 4℃ before use. A certain quantity of defatted red peanut testa powder extracted by an aqueous 75% ethanol assisted by ultrasonic, power 320W, 10min, ratio of solvent to raw material 16. The extracts were centrifuged using a desktop centrifuge (TDL-5-A, Shanghai Anting Scientific Instrument Factory, China) at 4000×g for 10 min. The supernatant were evaporated by a rotary vacuum evaporator (RE52-86A, Shanghai Yarong Biochemical Instrument Factory, China) at 45℃ to remove the ethanol, and residues were divided into three groups. One group was freeze-dried using a freeze dryer (FD-1-55, Beijing Boyikang experimental apparatus Co., Ltd., China), named as a crude extract (CE). The other two groups were extracted successively with equal volumes of n-butanol/water and ethyl acetate /water, respectively. Each fraction was separated by the separatory funnel, evaporated in vacuum and freeze-dried to obtain the n-butanol fraction (BF), the water soluble fraction of n-butanol (WBF), the ethyl acetate fraction (EAF), and the water soluble fraction of ethyl acetate (WEAF). All 5 samples were sealed and stored in a freezer at dark and 4℃ until used.

2.3. The Reducing Power

The reducing power of the extracts was determined by the following procedure proposed by Jang et al., 2008 [8] with a little modification. A 2.5 mL of sample solutions of different concentrations was placed in the test tubes, respectively, and 2.5 mL of 0.2 mol/L phosphate buffer (pH = 6.6), 2.5 mL of 1% $K_3[Fe(CN)_6]$ was added to each tube, mixed thoroughly. Then the mixture was incubated in a water bath (HH-4 Digital constant temperature water bath, Beijing Guohua Electric Appliance Co., Ltd. China) at 50℃ for 20 min, and then cooled to room temperature rapidly. To this end, 10 mL of distilled water and 2 mL of 0.1% $FeCl_3$ was added to the test tubes. Absorbance of the mixtures was measured at 700 nm after 10min in a UV-vis spectrophotometer (Carry50, Varian, American). Using distilled water as the blank. The reducing power varied directly with absorbance.

2.4. DPPH Free Radical Scavenging Activities

The free radical scavenging activities of the extracts were evaluated as described by the method with a few modifications in the presence of DPPH stable radical [9]-[12].

Briefly, a full wavelength scan was detected for 0.025 mg/mL of DPPH prepared with anhydrous ethanol at 440-600 nm, anhydrous ethanol as a control, to determine the maximum absorption wavelength.

A 2.5 mL aliquot of each extract (0.04-0.2 mg/mL) was mixed with an equal volume of 0.025 mg/mL of DPPH ethanolic solution in the test tubes, and the absorbance was measured at maximum absorption wavelength after mixing in 30 min. Anhydrous ethanol was used as blank. The results were expressed as a percentage inhibition (RSA) according to the following equation:

$$RSA\ (\%) = [1-(A_i-A_j)/A_0] \times 100\%$$

Where A_i= absorbance of 2.5 mL of DPPH radical + 2.5 mL of sample

A_j= absorbance of 2.5 mL of sample + 2.5 mL of anhydrous ethanol

A_0= absorbance of 2.5 mL of DPPH radical + 2.5 mL of anhydrous ethanol

IC_{50} the inhibitory concentration of 50%, was also expressed this activity, means the concentration of the test solution required to give a 50% decrease in the absorbance of the test solution compared to that of a blank solution [13].

2.5. The Oxidative Stability of Extracts

Determination of oxidation stability was by the method of accelerated oxidation test [14]. The oxidative stability index of lard, represented as induction time in hours, was measured with an automated Metrohm Rancimat apparatus model 743 (Metrohm, Switzerland) by using a flow of air and high temperatures to accelerate oxidation. Eight oil samples were analyzed in the equipment at the same time. For each sample, 3.00 g of lard, and then 3 mL of distilled water, CE, BF, EAF, WBF and WEAF, respectively. And 3 drops of

Tween 20 were added into reaction tube and mixed, respectively. The conductimetry cells were filled with deionized water up to 90 mL. The air was flown through the heated oil at a temperature of 110 ±0.1℃, with an air flow of 20 L/h. The time taken until there is a sharp increase of conductivity measured by the instrument is termed as the induction time [15]-[18]. Oil samples without any antioxidants (control) were also analyzed under the same conditions.

2.6. Statistical Analysis

Multiple comparison test was applied for detecting the significance of difference between different groups.

3. Results and Discussions

3.1. Reducing Power of Different Fractions of Peanut Testae

The ferricyanide complex is converted to the ferrous form and is accompanied by a color change from yellow to green via reducing power [11], which could be measured at 700nm. As shown in **Fig.1**, all fractions of the extracts had the activities of reducing power. Each fraction exhibited an increase in their activities of reducing power with the increased concentration of extracts. The absorbance was proportional to the concentration of the extracts. Namely, as the concentration of extracts increased, so did the reducing power. It was determined that CE, BF, EAF, WBF and WEAF had differences in reducing power at the same concentrations (0.04-0.2mg/mL). At 0.12 mg/mL, the absorbance of CE, BF, EAF, WBF and WEAF were 0.52, 1.152, 0.842, 0.272 and 0.53, respectively. And at 0.2 mg/mL, the absorbance of CE, BF, EAF, WBF and WEAF were 1.39, 1.99, 1.75, 0.88, and 1.26, respectively. The reducing powers of the extracts were in the order of: BF > EAF> CE >WEAF >WBF. That indicated BF had the highest reducing power among the all of the fractions. The reducing power of fractions of the extracts of organic solvents is higher than that of the fractions of water-soluble fractions. It also showed that the higher the polarity of extract solvent was, the lower the reducing power of the extract was. From Figure 1, it can be seen that the 1/2 of the sum of the reducing power of EAF and WEAF nearly equals to that of CE, so did BF and WBF (Table 1). And according to the results of the reducing power of the extracts, the regression equations of the reducing power of extracts of different fractions could be got. Y = 0.9052x - 0.0343, r = 0.9928; CE=(EAF+WEFA)/2. Y = 0.9607x - 0.0428, r = 0.9857;CE=(BF+WBF)/2. The reducing power of (EAF+WEFA) /2 agreed very well with a real value of CE, and so did that of (BF+WBF) /2.

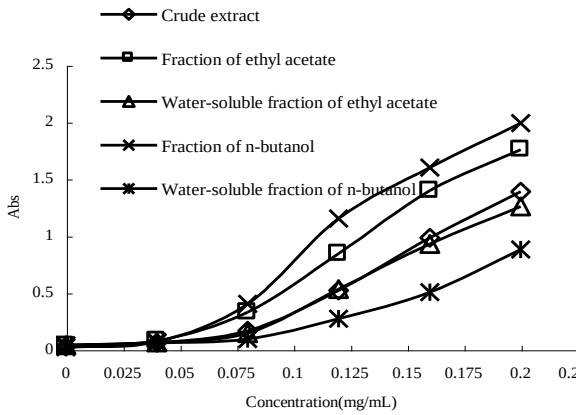

Fig. 1: The reducing power of the different fractions of ethanol extract of peanut testae

3.2. DPPH Free Radical Scavenging Activities of Extracts

The 1-diphenyl-2-picrylhydrazyl (DPPH) is a stable radical substance widely used to evaluate the antioxidant activity [19]-[22]. The DPPH radical scavenging method was used to determine antioxidant activity of the different fractions of extracts with ethanol, n-butanol/water and ethyl acetate /water in this study. The maximum absorption wavelength of 0.025 mg/mL of DPPH ethanol solution through the spectrum of the full wavelength scanning was 517 nm.

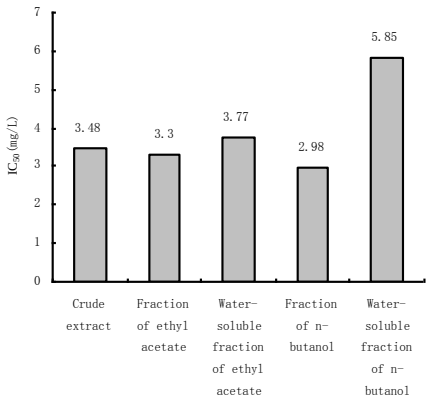

Fig. 2: The IC50 values of DPPH radical scavenging activities of the extracts of peanut testae

Table 1: The relationship of reducing power between different fractions of extracts

Concentration (mg/mL)	CE[a]	M (EAF[b]+WEAF[c])	M (BF[d]+WBF[e])
0	0.0248	0.0344	0.0271
0.04	0.0587	0.069	0.0619
0.08	0.1649	0.23455	0.24535
0.12	0.5171	0.68545	0.7104
0.16	0.9812	1.1601	1.05
0.2	1.3867	1.50585	1.43415

a CE ethanolic extracts from peanut testae; b EAF fraction of ethyl acetate from CE;
c WEAF water-soluble fraction of ethyl acetate from CE; d BF fraction of n-butanol from CE;
e WBF water-soluble fraction of n-butanol from CE; all values are mean of 3 replicates.

As shown in Table 2 and Fig.2, the IC_{50} values of DPPH scavenging activities of the extracts of peanut testae were obtained by the DPPH method. The scavenging activities between samples could be compared with the IC_{50} value. The higher DPPH radical scavenging activity is, the lower IC_{50} value is. The highest one was detected in BF, followed by EAF, CE, WEA and WBF, respectively.

Table 2: The DPPH scavenging activities and the reducing power of peanut testae

Extracts	IC_{50}[a] (mg/L)	EC_{50}[b] (mg/mL)
Crude extract (CE)	3.48	0.09687
Ethyl acetate fraction (EAF)	3.30	0.07451
Water soluble fraction of ethyl acetate (WEA)	3.77	0.10129
n-butanol fraction (BF)	2.98	0.05892
Water-soluble fraction of n-butanol (WBF)	5.85	0.12343

a IC_{50} (mg/L), concentration for scavenging 50% of DPPH.
b EC_{50} (mg/mL), concentration for increasing 0.5 value in optical density

3.3. Effects of the Extracts on Oxidative Stability of Fat

As shown in **Fig. 3**, the results of the test of oxidative stability of fat of the extracts were obviously different. The antioxidant activity ranks according to the induction time as the following order: EAF > CE >WBF > BF >WEA.

3.4. Deference of antioxidant activity between different fractions

As Table 2 displays, the difference between the fractions of organic and water extracted with n-butanol is obviously greater than that of ethyl acetate. And this suggests that, as far as antioxidants of peanut testae are concerned, n-butanol is superior to ethyl acetate for extraction.

4. Conclusions

This experiment had determined and compared the antioxidant activity in vitro of the extracts of peanut testae with different solvents. On the basis of the results, it came to a conclusion that the reducing power positively correlates to the DPPH radical scavenging ability, while the oxidative stability of lipid capacity showed a different pattern. It is possible that the extract with n-butanol did not work for the anti-oil oxidation stability while the ethyl acetate extracts had an obvious effect.

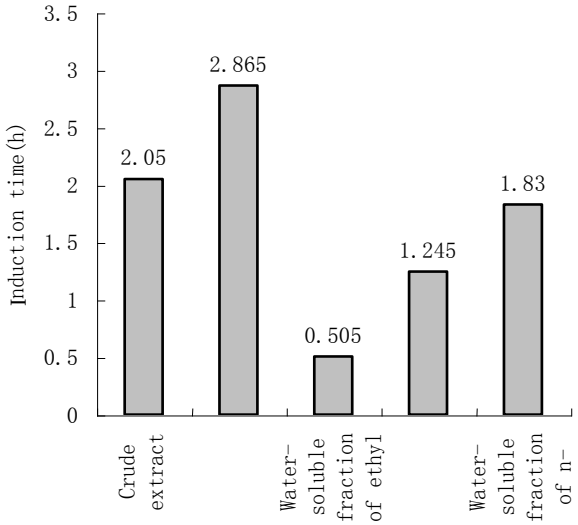

Fig. 3: The anti-oil oxidation activity of the extracts of peanut testae

Besides, from the results of the reducing power and DPPH radical scavenging ability, it could find that the IC_{50} value of CE is in the middle. Ahead of it is the fractions of organic solvents, and behind is the water-soluble fractions of organic solvent extracts, respectively, which is a symmetrical distribution. The reducing power of the CE is approximately equal to the mean value of all the crude extract fractions extracted by organic solvents. The antioxidant activity of the organic fraction is higher than that of CE and water-soluble fraction. It shows that the antioxidants in the peanut testae are mainly some fat-soluble substances, and their mechanism of antioxidant activity needs further research.

5. Acknowledgements

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