Advances in Engineering Research, volume 197
Proceedings of the 2020 9th International Conference on Applied Science, Engineering and Technology (ICASET 2020)

Evaluation of Free Radical Scavenging and Antioxidation Capacity of Resveratrol and Polydatin

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ABSTRACT
Cellular damage induced by free-radicals like reactive oxygen species(ROS) has been implicated in several diseases. The ROS radicals like alkoxy radical(RO-) and peroxy radical (ROO-) are similar to those that are physiologically active and thus might initiate a cascade of intracellular toxic events leading to DNA damage and cell death. Hence naturally anti-oxidant play a vital role in combating these conditions.Resveratrol (RES) and polydatin (PD) are two kinds of natural phytoalexin with excellent antimicrobial and anti-inflammatory activity. In this study aim to evaluation of free radical scavenging and antioxidation capacity of Res and PD to provide a basis for the potential application and development in the future. The free radical scavenging and antioxidant ability were comprehensively evaluated by different systems, including the scavenging rates of DPPH, ABTS radical, hydrogen peroxide scavenging, lipid peroxidation inhibition abilities. The results showed that PD is significantly stronger than RES in DPPH$^{\bullet}$ scavenging, hydrogen peroxide scavenging and lipid peroxidation ($P < 0.05$), but RES is significantly stronger than PD in ABTS radical scavenging activity($P < 0.05$). RES and PD show their respective antioxidant activity advantages under different indicators, which means we should choose RES or PD according to the different uses.
Keywords: *free radical, scavenging, antioxidant, polydatin, Resveratrol.*

1. INTRODUCTION

Reactive oxygen species (ROS) are believed to be one of the most dangerous factors to threaten human body. It can be increased by improper diet to induce cellular damage, which lead to aging, metabolic diseases, arteriosclerosis and different types of cancer[1]. Thus the demands for free radical scavenging have drawn increasing attention in recent years.

Resveratrol(RES, Fig.1a), Trans-3,4,5-trihydroxystilbene, is a major symbol ingredient in red grapes and peanuts[2, 3]. Res was first isolated from the roots of white hellebore in 1940 in Japan, and later found in traditional Chinese medicine[4]. It was initially characterized as a phytoalexin (substance produced by higher plants in response to attack by pathogens such as bacteria and fungi, or stress), and achieved notoriety in the scientific literature in 1992, when it was postulated as being responsible for the cardiac protective effects of wine (effect called "French paradox")[5]. Since then, Res has been shown to exert a variety of pharmacological effects such as antioxidant, antidiabetes, anti-inflammatory and anti-cancer activities. Res is a natural compound currently under investigation due to its important biological anti-cancer properties, including effects on leukemia, skin, breast, lung gastric, colorectal, neuroblastoma, pancreatic and hepatoma cancers[6-10].

Polydatin or piceid (PD, Fig.1b), resveratrol-3-O-β-glucopyranoside, is a precursor of resveratrol isolated from Polygonum cuspidatum Sieb. et Zucc.(Chinese name: Huzhang), and is commonly used as a quality control marker. Pol has obtained permission for clinical trials from the China Food and Drug Administration. PD, the most abundant form of resveratrol in nature, glycosylation of resveratrol protects it from enzymic oxidation[11]. The content of PD was found higher than that of resveratrol in grape seed, red wine and red sorghum grains[12]. PD has been reported to exhibit promising pharmacological activities including anticarcinogenic[13], antiplatelet aggregation[14], anti-inflammatory[15], antihemorrhagic shock[16], protect against carbon tetrachloride-induced liver Injury[17], ameliorates oxidative stress-related inflammatory responses resulting in renal injury[18], anti-aging[19], ameliorates insulin resistance and hepatic steatosis[20], and anti-oxidation activity[21]. Recently, it is found that Pol promotes Nrf2-ARE anti-oxidative pathway through activating Sirt1 to resist AGEs-induced up-regulation of fibronetin and transforming growth factor-β1 in rat glomerular messangial cells[22].

However, up to now, it is still unclear whether Res and PD has DPPH, FRAP, ABTS free radical scavenging and anti-oxidative damage capacities, or possesses potential the oxidative diseases treatment. In this study aim to evaluation of free radical scavenging and antioxidation capacity. The free radical scavenging and antioxidant ability were comprehensively evaluated by different systems, including the scavenging rates of DPPH, ABTS radical, hydrogen peroxide scavenging, lipid peroxidation inhibition abilities.

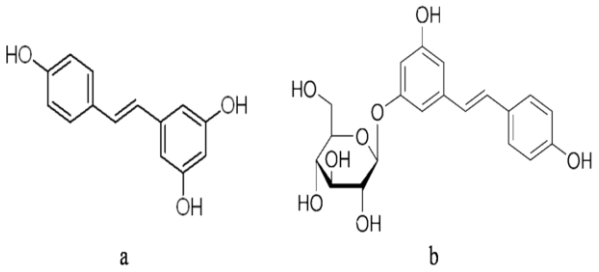

Fig.1 Chemical structure of RES (a) and PD (b)

2. MATERIALS AND METHODS

All chemicals used were of analytical grade. 2, 2'-azino-bis-3-ethylbenzothiazoline-6- sulphonic acid (ABTS), 1,1-diphenyl-2-picryl-hydrazil (DPPH), 2,4,6-tripyridyl-s-triazine (TPTZ), potassium persulfate, iron (III) chloride, Res and polydatin (≥98% by HPLC) were purchased from Aladdin Industrial Inc.(Shanghai, China).

2.1. Free radical scavenging capacity determination

2.1.1. DPPH• free radical scavenging activity

The DPPH• assay was used to measure radical scavenging activity as reported[21], but with slight modifications. All samples were tested individually at different concentrations by addition to an ethanolic solution of DPPH radical (40μmol/L). For each measure 100μl appropriately diluted standards or the sample was added to 3.0 ml of DPPH reagent, the mixtures were stirred and allowed to stand in the dark at room temperature. The same volume ethanol instead of the diluted sample was used as control solution. The absorbance of the resulting solutions was measured at 515nm after 30min. From this, the radical scavenging activity was determined by the following equation:

$$\text{DPPH}^{\bullet}\ \text{scavenging effect}\ (\%) = \left[\frac{A_0 - A_1}{A_0}\right] \times 100\%$$

where A0 is the absorbance of control and A1 is the absorbance of samples.

2.1.2. ABTS • free radical scavenging activity.

The ABTS• method was carried out as reported[21], but with some modifications. Briefly, ABTS•+ was prepared by mixing an ABTS stock solution (7 mmol/L in water) with 2.45 mmol/L potassium persulfate solutions and then incubated in the dark at room temperature for 12 h. This reagent was stable for 2-3 days when stored at 4 °C in the dark. On the day of analysis, the ABTS•+ solution was diluted with ethanol to an absorbance of 0.70 (±0.02) at 734 nm. After the addition of 1.0 ml ABTS•+ solution to 0.10 ml of sample, the mixture was stirred for 30 s and the absorbance reading was started after another 30 s and finished after 6 min. The readings were performed at 734 nm and 25°C. The inhibition rate was calculated with the following equation:

$$\text{ABTS}^{\bullet+}\ \text{radical scavenging activity}\ (\%) = \left[\frac{A_0 - A_1}{A_0}\right] \times 100\%$$

where A0 is the absorbance of control and A1 is the absorbance of samples.

2.2. Hydrogen peroxide scavenging activity.

The hydrogen peroxide scavenging assay were carried out according to the method[23]. The principle of this method is a decrease in absorbance of Hydrogen peroxide (H_2O_2) upon oxidation of H_2O_2. 43 mM H2O2 solution was prepared in 0.1 M phosphate buffer (pH 7.4). Different concentrations of RES and PD (50.0-500.0 nM) in 3.4 mL ethanol were added to 0.6 mL H_2O_2 solution and the absorbance of the reaction mixture was recorded at 230 nm. A blank solution contained the sodium phosphate buffer without H_2O_2. The percentage of H_2O_2 scavenging by RES, PD or ascorbic acid was calculated with the following equation:

$$H_2O_2\ \text{scavenging effect}(\%) = \left[\frac{A_0 - A_1}{A_0}\right] \times 100\%$$

where A0 is the absorbance of control and A1 is the absorbance of samples.

2.3. Lipid peroxidation inhibition ability.

The thiobarbituric acid reactive species method were carried out according to the method [24], and modified according to our situation. Briefly, this method was carried out using yolk homogenate (10%) in Tris buffer (pH 8.0) as lipid rich media. A stock solution of each sample in methanol was prepared at different concentrations (20.0-80.0 nM), 1mL of each solution were transferred into different test tubes, adding 1.0 mL 10% egg yolk emulsion, 100 μL concentration 0.07 mol/L AAPH solution, 3.0 mL 20% acetic acid solution and 3.0 mL 0.8% thiobarbituric acid solution. The reaction mixture was boiled for 60 min, after cooled, adding 4.0 mL butanol and fully extracting, and centrifuged at 2000 g for 10 min, extracting the organic layer, A blank was prepared with

the same reagents without sample, and using ascorbic acid as a positive control. The absorbance was measured at 532 nm and the decrease of absorbance indicates an increase of antioxidant activity. The values of antioxidant activity were expressed as the percentage inhibition of lipid peroxidation in yolk homogenate as follows equation:

$$\text{The inhibition of lipid peroxidation}(\%) = \left[\frac{A_0 - A_1}{A_0}\right] \times 100\%$$

where A0 is the absorbance of control and A1 is the absorbance of samples.

3. RESULTS AND DISCUSSION

3.1. Free radical scavenging capacity determination

3.1.1.DPPH• free radical scavenging activity

The results of the DPPH• free radical scavenging activity of RES and PD are showed in Fig.2a, The IC_{50} values of ascorbic acid, RES and PD are 20.4±0.6, 353.3±33.8, 61.9±2.8 nM respectively, indicating that PD is significantly stronger than RES ($P < 0.05$), and both RES and PD are significantly weaker than ascorbic acid ($P < 0.05$).

3.1.2. ABTS • free radical scavenging activity.

By means of assessment of ABTS•+ radical scavenging activity, the IC_{50} values of ascorbic acid, RES and PD are 14.7±0.7, 7.2±0.4, 8.9±0.5 nM respectively (Fig.2b). The results suggest that the ABTS•+ radical scavenging active of RES and PD is significantly stronger than that of ascorbic acid ($P < 0.05$), and RES is significantly stronger than PD ($P < 0.05$).

3.2. Hydrogen peroxide scavenging activity.

The results (Fig.2c) shows that the hydrogen peroxide scavenging activity of RES and PD were significantly stronger ascorbic acid ($P < 0.05$), and the hydrogen peroxide scavenging activity of PD is significantly stronger than RES ($P < 0.05$). The IC_{50} values of ascorbic acid, RES and PD are 450.5±32.6, 125.1±11.3 and 76.5±5.2 nM respectively. In addition, we observed a concentration-dependent activity of RES.

3.3. Lipid peroxidation inhibition ability.

The lipid peroxidation inhibition ability was shown in Fig.2d, the IC_{50} values of ascorbic acid, RES, PD are 37.9±3.6, 38.7±3.4 and 24.5±1.7 nM respectively, indication that ascorbic acid and RES's lipid peroxidation inhibition ability are no significantly different ($P > 0.05$), but PD's lipid peroxidation inhibition ability is significantly stronger than that of both RES and ascorbic acid ($P < 0.05$).

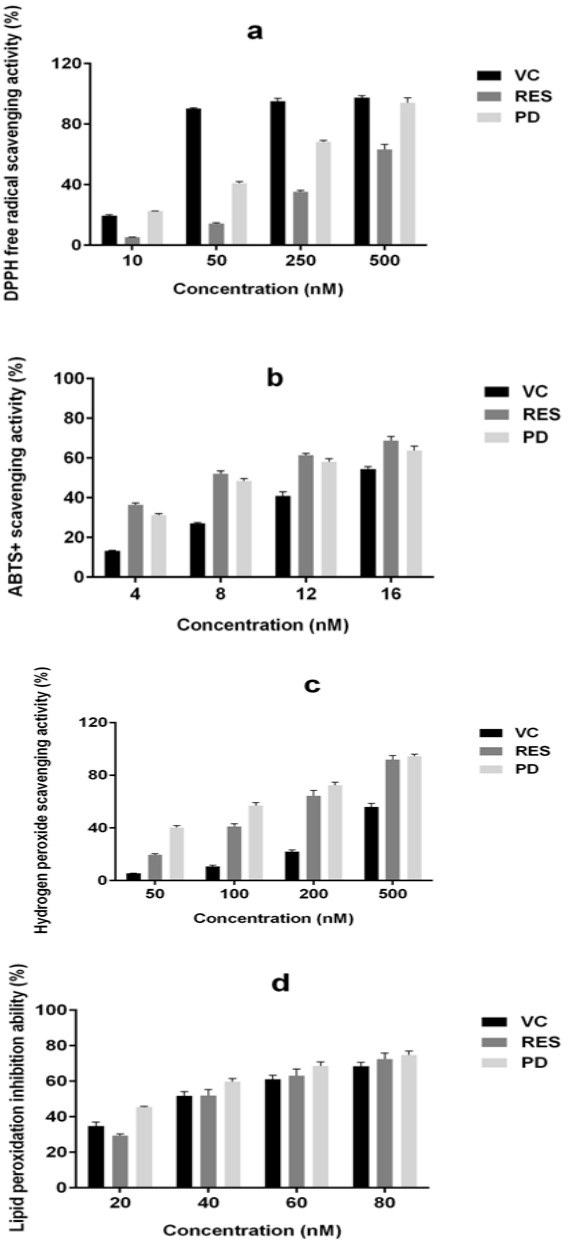

Fig.2 DPPH• (a), ABTS•+ (b) free radical scavenging activity, hydrogen peroxide scavenging activity(c) and lipid peroxidation inhibition ability(d) of RES and PD

3.4. Discussion

PD is the glycoside of RES. Previous research had demonstrated that both of RES and PD could be found in the same sources and have lots of similar biological activities, such as antioxidant. Although there are many studies on the antioxidant capacity of RES or PD, the

directly comparisons of RES and PD using the same method have not been described yet. In order to evaluate directly the antioxidant capacity between RES and PD, we first compared the differences between RES and PD in DPPH• method in vitro. Ascorbic acid, which is known as one of the most powerful anti-oxidants, was used as positive control.

In the DPPH• method, the antioxidant capacity was analyzed by detecting the changes in the absorbance of DPPH• solution at 517 nm in a spectrophotometer. Lower absorbance level implies the stronger antioxidant activity. The DPPH• method is fast and simple that only requires an UV spectrophotometer. However, its disadvantage is that the results are easily disturbed especially when the analyte is overlapped with the UV absorption of DPPH•. In order to overcome the defects above, lots of methods are used to comprehensive evaluate the antioxidant activity between PD and RES. The ABTS•+ radical elimination as well as the DPPH• method are common antioxidant methods to evaluate the antioxidant capacity of chemical compound. The ABTS•+ radical with blue/green coloration is produced by the oxidation of the ABTS•+ radical solution by potassium persulphate and the decrease of absorbance at 734 nm shows the enhancement of antioxidant capacity. ABTS method is fast and simple, suitable for the detection of a large number of samples, but it can react with any hydroxylated aromatic compound, which also involves oxidation of hydroxyl groups that do not react with oxidation. Hydrogen peroxide is a potentially harmful free radical which can get cross the cell membranes rapidly and may cause toxic effects. PD and RES have the ability to scavenge hydrogen peroxide, which must be removed from cells to maintain normal bodily activity and healthy metabolic function. Lipid peroxidation can change the fluidity and permeability of cell membrane, and damage the structure and functions of cells. Lipid peroxidation inhibition ability method can simulate the oxidation process in vivo to a certain extent, but there are many interfering substances in the biological system and conventional colorimetric method is non specificity.

4. CONCLUSION

In present study, the free radical scavenging and antioxidant ability were comprehensively evaluated by different systems, including the scavenging rates of DPPH, ABTS radical, hydrogen peroxide scavenging, lipid peroxidation inhibition abilities. The The results showed that PD is significantly stronger than RES in DPPH• scavenging, hydrogen peroxide scavenging and lipid peroxidation ($P < 0.05$), but RES is significantly stronger than PD in ABTS radical scavenging activity($P < 0.05$). RES and PD show their respective antioxidant activity advantages under different indicators, which means we should choose RES or PD according to the different uses.

ACKNOWLEDGMENT

This achievement is supported by the talent training project of guangdong province joint training demonstration base for graduate students,
the innovation strong school project of department of education of guangdong province, study on the optimization of the curriculum system for master of pharmacy, degree and graduate education reform research program, guangdong postgraduate education innovation plan project(2017JGXM-MS39),
the innovation and entrepreneurship training program for university students, guangdong pharmaceutical university(S202010573044).

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