RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Macroporous adsorbent resin-based wheat bran polyphenols extracts inhibition effects on H_2O_2-induced oxidative damage in HEK293 cells

Yuan-Yuan Bian, Jia Guo, Ke-Xue Zhu*, Xiao-Na Guo, Wei Peng, Hamid Majeed, Hui-Ming Zhou*

State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, 1800 Lihu Avenue, Wuxi-214122, Jiangsu Province, PR China.

* Corresponding author

Prof. Ke-Xue Zhu

Fax: +86 510 85329037 Tel: +86 510 85329037

E-mail: kxzhu@jiangnan.edu.cn

Prof. Hui-Ming Zhou

Fax: +86 510 85809610 Tel: +86 510 85809610

E-mail: hmzhou@jiangnan.edu.cn

Abstract:

In the present study, polyphenol-rich extracts of wheat bran (PEWB) were prepared via macroporous adsorption resins and desorpted by ethanol. Extraction was performed using aqueous ethanol and four different types of macroporous adsorbent resins for isolation. Specially, properties of various macroporous resin were investigated by adsorption and desorption tests. Total polyphenolic content of PEWB was determined using the Folin–Ciocalteau method, and its resistance effects against hydrogen peroxide (H_2O_2)-induced oxidation on HEK293 cells were assessed by cell viability and reactive oxygen species (ROS) assay. The results indicated that resin NKA-9 displayed excellent adsorption and separation ability, as well as provided insight in the generation of PEWB from the wheat bran extracts. In addition, these results suggested that pretreating HEK293 cells with PEWB prior to H_2O_2 exposure exhibited significantly increased survival ratio and reduced the ROS levels. Further investigation involving phenolic content of PEWB identification and quantification demonstrated that that ferulic acid was the most abundant phenolic compound in a number of extracts, which was also confirmed with MTT and ROS assays. Our study revealed that PEWB can prevent HEK293 cells of H_2O_2-induced oxidative damage.

Keywords: Polyphenol-rich extracts, Macroporous resin, UPLC/TQD, Oxidative damage, HEK293 cells, ROS

1. Introduction

Cereals are important part of human nutrition which provide a wide range of nutrients and biologically active compounds. Cereal bran, separated in the milling process during the production of refined flours [1], are rich in phenolic acids [2] and have been widely used for providing health benefits to consumers in addition to general nutrition. Specially, phenolic acids and flavonoids are existed in cereals in both free and conjugated forms, reaching highest concentrations in the aleurone layer of creal grains [3]. They have excellent antioxidant activity [4] such as displaying anti-inflammatory responses, prevention of low-density lipoprotein oxidation, antithrombic, antihypertensive, and carcinostatic actions [5]. Wheat bran, which is produced in enormous quantities worldwide as an important byproduct of the cereal industry, serves as a good source of dietary fibre and antioxidants [6, 7]. Previous studies reported that wheat bran extracts (WBEs) possess various physiological activities, such as anticancer capacity [8], indicating that antioxidant ability of wheat in the diet is associated with its antitumor activity [9].

Several approaches of enriching and separating active constituents from the fermentation broth are reported, such as liquid–liquid extraction [10, 11], solid–liquid extraction [12-15], solid–phase extraction [16], membrane filtration [17], ion exchange, and adsorption [18]. Among all these methods, adsorption attracted great most attention, due to its low cost, high efficiency, and simple operation procedure [1]. Macroporous resins are durable polar, nonpolar, or slightly hydrophilic polymers that display high adsorption capacity, high recovery, with low cost [19]. Macroporous resin adsorption technology has gained popularity in pharmaceutical recently, and it has also been used for polyphenol and flavonoids purification [20-22]. Effects of different macroporous adsorbent resins (DA201-C, NKA-II, NKA-9, and H1020) on the purification of

WBEs were evaluated. Static, dynamic adsorption, and desorption tests were applied in our experiements. Further investigation on phenolic content of polyphenol-rich extracts of wheat bran (PEWB) was also performed. The resistance effects of WBEs against H_2O_2-induced oxidative damage had been reported previously. However, to the best of our knowledge, there are very few studies involve PEWB resistance effects on cell against oxidative damage. Therefore, to have deep understanding about PEWB ability on preventing H_2O_2-indcued damaged on cells is of significance.

2. Materials and Methods

2.1 Chemicals and reagents

Raw wheat bran was provided from Yihai Kerry Food Industry Co, LTD (Kunshan, China). HEK 293 cells were obtained from American Type Culture Collection. Ferulic acid, o-coumaric acid, p-coumaric acid, and gallic acid (Sigma-Aldrich, St.Louis, MO, USA), HG-DMEM (Gibco BRL, Life Technologies, USA), trypsin-EDTA solution (Beyotime, Jiangsu, China), fetal bovine serum (FBS; Sijiqing, Zhejiang, China), MTT (Sigma, St. Louis, MO, USA). ROS assay kit was purchased from Beyotime Institute of Biotechnology (Haimen, China). SH-1000 Lab microplate reader (Corona Electric Co. Ltd., Ibaragi, Japan), SpectraMax M5 Multifunctional microplate reader (Molecular Devices, USA), Acquity UPLC-TQD system (Waters, Milford, MA) were used in the experiments.

2.2 Adsorbents

Three types of macroporous resins—NKA-II, NKA-9 and H1020—were purchased from Nankai Hecheng S & T Co., Ltd. (Tianjing, China), while DA201-C resin was purchased from Jiangsu Suqing Company (Jiangsu, China). All resins were pretreated with 100% ethanol for 24 h, then washed with 5% HCl and 5% NaOH solution followed by distilled water. Other chemicals used in this study were analytical grade.

87 **2.3 Preparation of PEWB**

88 The wheat bran was washed three times to remove starch and then heated at 50 ^{o}C
89 for 12 h. The dried wheat bran was ground into powders by hammer mill and filter
90 through 100 mesh. The bran was extracted twice with 80% ethanol at 8:1 ratio (v/w)
91 for 15 h at room temperature. The mixture was centrifuged for 20 min at 9000×g and
92 evaporator at 40 ^{o}C. The ethanol extract was further freeze-dried and stored in the
93 dark environment in a sealed container at 4 ^{o}C for further analysis.

94 Then WBEs were added to the column (2.6 cm × 80 cm) containing macroporous
95 resin, the outflow liquid was collected and added to the column containing four
96 macroporous resins. The WBEs were subjected to DA201-C, NKA-II, NKA-9 and
97 H1020 macroporous resin column chromatography eluting with a gradient of ethanol–
98 H_2O (20:80, 40:60, 60:40, and 80:20, respectively) for fractions. The fractions were
99 detected using a UV detector at wavelength of 310 nm. The products were collected,
100 concentrated, and lyophilized for the subsequent experiments. PEWB was obtained
101 after freeze-dried process. Various kinds of eluotropic fractions were dissolved in
102 sterile 1% ethanol, filtered with sterile 0.2 μm filter, attenuated with a DMEM-based
103 culture medium then added for cell culture.

104 **2.4 Total phenolic contents**

105 The total phenolic contents in WBEs were determined using the Folin–Ciocalteu
106 reagent [23]. The mixture contained 100 μL of WBEs in dimethyl sulfoxide (DMSO),
107 500 μL of the Folin–Ciocalteu reagent, and 1.5 mL of 20% sodium carbonate was
108 used for reaction. The final volume was made up to 10 mL with pure water. After 2 h
109 of reaction at ambient temperature, phenolic contents was calculated the using a
110 standard curve prepared with gallic acid at wavelength of 765 nm. The reactions were
111 repeated in triplicate.

2.5 Static adsorption and desorption tests

2.5.1 Adsorptive properties of resins

All four macroporous resins were screened through static adsorption tests. Firstly, crude extract (30 mL) was added into pretreated resin (1 g dry weight basis each) in Erlenmeyer flasks and shaken at 200 rpm for 24 h in the temperature of 25 ^{o}C to reach adsorption equilibrium [24]. Then, the resins were washed by 30 mL double-distilled water and then desorbed with 30 mL 95% ethanol v/v and shaken at 200 rpm for 4 h at 25 °C. These experiments were repeated in triplicate. Following equations were used to quantify the adsorption capacity as well as the ratios of adsorption and desorption.

Adsorption capacity:

$$Q_e = \frac{V_0(C_0 - C_e)}{W}$$

Adsorption ratio:

$$E = \frac{C_0 - C_e}{C_0} \times 100\%$$

Where Q_e is the adsorption capacity, representing the mass of adsorbate adsorbed on 1 g dry resin at adsorption equilibrium, E is the adsorption ratio, representing the precentage of total adsorbate being adsorbed at adsorption equilibrium; C_0 and C_e are initial and equilibrium concentrations of sample solutions, respectively; V_0 is the initial volume added into the flask; W is the mass of the dry resin.

Desorption ratio:

$$D = \frac{C_d V_d}{V_0(C_0 - C_e)} \times 100\%$$

Where D is the desorption ratio (%); C_d is the concentration of the solution in the desorption solutions (mg/mL); V_d is the volume of the desorption solution; C_0, C_e, and V_0 are the same as those defined above.

2.5.2 Static adsorption experiment

The adsorption kinetics of the NKA-II, NKA-9, H1020 and DA201-C resins were evaluated by adding crude extracts (30 mL each) into the pretreated resin (1 g dry weight basis each) in Erlenmeyer flasks and shaken at 200 rpm at 25 °C. The flasks were shaken using a thermal shaker for 10 h. One hundred microliter (100 μL) of the solutions was taken out to be analyzed using Folin–Ciocalteu reagent method. Total phenol concentrations in liquid phase at different time intervals were monitored using the Folin–Ciocalteu reagent method.

2.6 Dynamic adsorption and desorption tests

The dynamic adsorption and desorption experiments were performed using a column (26 × 800 mm, Shanghai Qite Analytical Instrumental, Shanghai, China) packed with the four hydrated macroporous resins. The bed volume (BV) was 350 mL and the flow rate was 2 mL/min. For the dynamic desorption experiment, both gradient and isocratic elution were carried out, with a total loading of 400 mL of crude extract on the NKA-9 column mentioned above. For the gradient elution, the column was eluted with 3–6 BV of each ethanol aqueous solutions (20%, 40%, 60%, and 80%) successively. The eluting solvent was changed when the adsorbance of eluate at 310 nm showed little alteration. For the isocratic elution, after the column was washed with 3 BV of double-distilled water, all the ethanol eluents were collected and concentrated to dryness under reduced pressure at 40 °C. The resin-refined sample was stored and frozen for further experiment.

2.7 Cell Viability Assay

Cell viability was determined using the MTT assay. The MTT cytotoxicity assay was performed as previously described [25, 26] with minor modifications. Briefly, HEK 293 cells were seeded at a density of 3×10^4 cells per well in 96-well plate (Costar 3599; Corning, NY) for 12 h attchment. The PEWB and ferulic acid (positive control) were dissolved in ethanol, diluted with HG-DMEM. The cells were treated with PEWB or ferulic acid for 1 h, then exposure to 1 mM H_2O_2 for 2 h. After being replaced with fresh medium, 20 μL MTT solution (5 mg/mL in PBS) was added and cells were incubated at 37 °C for 4 h with 5% CO_2. The medium was then carefully removed, and colored formazan were dissolved in 150 μL DMSO. The plate was shaken for 10 min, and the absorbance was measured at 570 nm using a microplate reader (SH-1000 Lab microplate reader). The cell viability expressed as % = [(MTT OD value of treated cells/MTT OD value of control cells)]. All assays were performed in at least three individual experiments, at least six replicates were repeated.

2.8 ROS assay

HEK293 cells were seeded at 3×10^4 cells per well in clear-bottom, black-walled, 96-well plates (Costar 3606; Corning, NY) for 12 h attachment. Then, the cells were incubated with carboxy-2',7'-dichloro-dihydro-fluorescein diacetate probe for 20 min and washed twice with PBS. Then, the cells were exposed to WBEs, PEWB and ferulic acid for 1 h and H_2O_2 for 2 h. Fluorescence was recored at 488 nm (excitation) and 525 nm (emission) wavelengths using a SpectraMax M5 microplate reader. All assays were performed in at least three individual experiments, each experiement was repeated six replicates.

2.9 Chromatographic system and conditions

All samples were analyzed using the Acquity UPLC-TQD system (Waters, Milford, MA), including an autosampler, photodiode array detector and an MS pump

equipped with an electrospray ionization (ESI) probe as the interface. The samples were separated by ultra-high performance liquid chromatography using an Acquity UPLC BEH C18 column (2.1×50 mm, 1.7 μm) with a mobile phase consisting of acetonitrile solution (A) and 0.1% (v/v) formic acid water solution (B) at a flow rate of 0.3 mL/min and injection volume of 1 μL.

2.10 Identification of phenolic compounds by UPLC-MS

The analysis performed is used to identify phenolic compounds in different extracts which may have antioxidant activity. Specially, extracts were dissolved in methanol (1 mg/mL), diluted with 50% acetonitrile, centrifuged at 10,000 rpm and syringe filtered using 0.22 μm polyvinylidene diflouride filters. The starting condition for each experiment was 5:95 mobile phase A (acetonitrile):mobile phase B (water+0.1%(v/v) formic acid) held for 0.8 min, with a ramp-up to 10:90 (A:B) by 1.2 min, then to 15:85 by 2.4 min, and held for 1.3 min. Further gradient increases were carried out to 21:79 by 4.0 min and to 50:50 by 7.8 min, with a change to 100:0 (A:B) by 8.8 min and held for 0.5 min, and finally, reconditioned to initial starting conditions. The mass spectrometry was operated in negative mode ESI. Detection of the four phenolics was conducted in the multiple reaction monitoring (MRM) mode. Individual compounds were identified using MRM with a specific precursor–production transition: *m/z* 163.08>119.64 for o-coumaric acid; *m/z* 163.08>119.15 for p-coumaric acid; *m/z* 193.00>134.10 for ferulic acid; *m/z* 169.05>125.00 for gallic acid. Detected phenolic compounds were quantified against standard curves generated with commecial phenolic standards. Results were expressed as milligrams compound per gram of extract (mg compound/g extract).

2.11 Statistical Analysis

All the experiments were performed in triplicate, and data were expressed as mean ± SD based on three separate experiments. Statistical analysis [27] was performed using Student's t-test and one-way SPSS (16.0 software) analysis. Probability values of $p<0.05$ were considered as significant.

3. Results and Discussion

3.1 Macroporous resin purification of PEWB

Macroprous adsorbent resins have been applied successfully in the separation and isolation of effective components from many natural products as an efficient apporach with a high absorption capacity, low operating costs, low solvent consumption, moderate purification effect, and easy regeneration [18, 28, 29]. Macroporous adsorbent resins have typically been selected based on the chemical nature of the phenolic acid and physical properities such as polarity, surface areas and average pore diameter. The adsorption capacity of polymeric adsorbent is proportional to its specific surface area; higher specific surface area promotes enhanced adsorption capacity. In the present study, four types of macroporous resins were analyzed and their physical properties are summarized in Table 1. Generally, resins that are weakly polar structure having strong affinity with weak polar phenolic acid, and resins with higher polarity exhibit stronger adsorption abilities toward polar substances. Among the four resins tested, H1020 and DA201-C showed significantly higher adsorption capacities compared to others. Adsorption capacity is usually determined by the degree of compatibility and similitude between sorbent and adsorbate. Specially, NKA-II displayed higher adsorption/desorption ratio than other resins ($p<0.05$) (Table 2). The non-polar resins (DA201-C and H1020) and polar resin (NKA-II) all exhibited high adsorption capabilities. However, NKA-II exhibited relative lower desorption capability for PEWB. It could be attributed that NKA-II possessed a strong affinity

for the solute. Similar results were also observed on the glycyrrhetinic acid mono-glucuronide separation from the crude extracts of fermentation broths by macroporous resins [1].

3.2 Adsorption kinetics of macroporous resins

In general, the selection of proper resins should be in accordance with their physical and chemical properties. Basically, polarity, surface area, average pore diameter, etc. are important factors for selecting a proper resin. The adsorption kinetics curves of the four resins over time (600 min) for PEWB are shown in Fig.1. The adsorption capacities increased dramaticly in the first 60 min, then slowly in the following time and eventually reached equilibrium at approximately 420 min. The fast initial rate may be due to the occurrence of adsorption in the easily accessible mesopores of the particles. The later slower uptake can be attribute to high mass transfer resistance inside the particle during the process. Similar results were also observed in the flavonoids purification from *Houttuynia cordata* Thunb by macroporous resins [20]. The results showed indicated that the adsorption capacity of NKA-9 was lower compared with others. Based on the adsorption kinetics curves, 60 min was sufficient to achieve adsorption capacities of PWBE for these four resins.

3.3 Dynamic adsorption and desorption experiments

Dynamic adsorption and desorption results are affected by several factors, such as feed rate, flow rate of the eluting solvent, feed pH value, temperature, initial concentration, ratio of column height to diameter. The desorption solvent of macroporous resin is usually ethanol solution because it can be recycled easily and has lower cost, little toxicity to the samples. Different concentrations of ethanol solutions were used to perform desorption tests in order to find most suitable desorption solution. In this study, WBEs were subjected to DA201-C, NKA-II, NKA-

9 and H1020 macroporous resins column chromatography eluting with a gradient of ethanol-H_2O (20:80, 40:60, 60:40, and 80:20, respectively for generation of fractions. In the experiments, the feed rate of 3 mL/min and eluting solvent flow rate of 2 mL/min were fixed. Sixteen samples were obtained after using the four macroporous resin: PEWB (D-2, D-4, D-6, D-8), PEWB (H-2, H-4, H-6, H-8), PEWB (NII-2, NII-4, NII-6, NII-8) and PEWB (N9-2, N9-4, N9-6, N9-8), respectively (Fig. 2).

3.4 Effects of PEWB on H_2O_2 injured cell growth and viability in HEK293 cells

MTT and related assay are widely used for cell viability evaluation when exposure to toxicial chemical or materials [30, 31]. In our experiment, cell viability, demonstrated by MTT assay, showed a time- and dose-dependent decrease manner. Cell viability was significantly decreased when HEK293 cells were incubated for 2 h, suggesting that HEK293 cells were damaged in presence of H_2O_2, and the IC_{50} value of H_2O_2 was 0.98 ± 0.02 mM. Therefore, 1 mM H_2O_2 was used to induce injury for further experiments. In addition, cytotoxic and resistence effects of PEWB on HEK293 cells were performed before subsequent assay. The samples did not show significant cytotoxicity in cells proliferation over 12 h and 24 h (data not shown).

The resistence effects of various PEWB prepared using different macroporous resins were further investigated and there was considerable variation in cell viability corresponding with increased ethanol concentration (Fig. 4). The highest cell viability obtained with PEWB (N9-4) separated using NKA-9 macroporous adsorbent resin at 40:60 ethanol–water ratio. HEK293 cells pretreated with PEWB prior to exposure to H_2O_2- exhibited increased cell viability. However, the other macroporous adsorbent resins showed better cell viability at an ethanol–water ratio of 40:60. N9-4 displayed greater protection effect on HEK293 cells against oxidative damage and then was selected for further investigation.

3.5 Effect of PEWB on intracellular ROS accumulation

It is well demonstrated that treatment HEK293 cells with H_2O_2 resulted in nuclear damage, loss of mitochondrial membrane potential, and elevated ROS levels. Specially, ROS generation is regards as indicator for cell under abnormal physiological condition [32]. As shown in Fig. 5a, ROS levels increased greatly when the HEK293 cells were exposed to 1 mM H_2O_2. However, when the HEK293 cells were pretreated with ferulic acid and PEWB, the ROS levels were significantly lower. Thus, it is reasonale to assume that PEWB plays important role in preventing oxidative damage in HEK293 cells upon exposure to H_2O_2.

The formulations that were separated at ethanol–water ratio of 40:60 through macroporous adsorbent resins showed decreased ROS levels (Fig. 5b, c, d, e). N9-4 is proved to be the best formulation of PEWB which could reduce ROS levels of cell to the same level as ferulic acid. These results indicated that PEWB (N9-4) had the strongest ability to decreasing the ROS level in cell (Fig. 5e).

3.6 UPLC-TDQ analysis

UPLC-TDQ analysis of each extract was performed and total ion chromatograms of the standard solutions of the four phenolic compounds were shown in Fig. 3. The results are shown in Table 3. The phenolic compounds identified in each sample are as follows: PWEB (D-2, D-4, D-6, D-8; H-2, H-4, H-6, H-8; NII-2, NII-4, NII-6, NII-8; N9-2, N9-4, N9-6, N9-8). For each sample, the total number of phenolics quantified in the 40% ethanol extract were greater than that in the corresponding 60% ethanol extract. This result further comfirmed with the results observed in the MTT and ROS assays. Ferulic acid was the most abundant phenolic compound in a number of extracts, including N9-4 (35.49 ± 2.7 mg/g), D-4 (27.46 ± 1.03 mg/g), H-4 (24.71 ± 2.17 mg/g), NII-4(24.07 ± 1.76 mg/g). Similar results were also observed in phenolic

acid concentrations in spring and winter wheat [33]. P-coumaric acid was indentified in a number of extracts, with the exception of 60% and 80% ethanol extracts. These extracts also showed increased antioxidant activity. Ferulic acid is antioxidant chemical [34] and it may play important role in the antioxidant activities of these extracts.

Conclusions

Preparative separation of PEWB with macroporous resins was successfully achieved. Among the four resins investigated, NKA-9 resin exhibited the best separation performance for PEWB on the oxidative ability as compared to the others. The adsorption–desorption method was shown to be more efficient than other conventional methods due to high efficiency, lower cost as well as procedural simplicity. Our work also demonstrated that pretreatment of PEWB was highly capable of inhibiting H_2O_2-induced oxidative damage in HEK293 cells. The resistance effects can be attributed to the ability of phenolic acids components to neutralize radicals and other ROS. Further investigations on the presence of phenolics using UPLC-TQD analysis revealed that some 40% ethanol extracts with high antioxidant activity also contained large amounts of ferulic acid, compared with 60% ethanol extracts. The results indicated that NKA-9 had good adsorption and separation ability, which can be used for preparation of PEWB from the WBEs.

Acknowledgments

This work was sponsored by Qing Lan Project, China Postdoctoral Science Foundation (Grant No. 2014M560396), Jiangsu Planned Projects for Postdoctoral Research Funds (Grant No.1402072C), and the National Key Technology R&D Program (Grant No. 2013AA102201).

References

1. S. P. Zou, J. J. Zhou, I. Kaleem, L. P. Xie, G. Y. Liu and C. Li, *Sep. Sci. Technol.*, 2012, **47**, 1055-1062.
2. S. Senter, R. Horvat and W. Forbus, *J. Food Sci.*, 1983, **48**, 798-799.
3. M. Naczk and F. Shahidi, *J. Pharm. Biomed. Anal.*, 2006, **41**, 1523-1542.
4. Y. P. Zou, Y. H. Lu and D. Z. Wei, *J. Agric. Food Chem.*, 2004, **52**, 5032-5039.
5. S. Kaviarasan, K. Vijayalakshmi and C. Anuradha, *Plant Foods Hum. Nutr.*, 2004, **59**, 143-147.
6. X. P. Yuan, J. Wang and H. Y. Yao, *Food Chem.*, 2005, **90**, 759-764.
7. L. Yu, *Food Chem.*, 2005, **90**, 311-316.
8. L. Liu, K. M. Winter, L. Stevenson, C. Morris and D. N. Leach, *Food Chem.*, 2012, **130**, 156-164.
9. J. W. Carter, R. Madl and F. Padula, *Nutri. Res.*, 2006, **26**, 33-38.
10. J. Liu, X. X. Wang and Z. Zhao, *J. Sci. Food Agric.*, 2014, **94**, 126-130.
11. H. Wu, G. Li, S. Liu, Z. Ji, Q. Zhang, N. Hu, Y. Suo and J. You, *Food Anal. Methods*, 2014.
12. Q. Xu, M. Li, L. Zhang, J. Niu and Z. Xia, *Langmuir*, 2014, **30**, 11103-11109.
13. Q. Xu, M. Li, J. Niu and Z. Xia, *Langmuir*, 2013, **29**, 13743-13749.
14. G. Li, S. Liu, Z. Sun, L. Xia, G. Chen and J. You, *Food Chem.*, 2015, **170**, 123-130.
15. C. Gu and C. Shannon, *J. Mol. Catal. A: Chem.*, 2007, **262**, 185-189.
16. H. Guo, P. Zhang, J. Wang and J. Zheng, *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, 2014, **951-952**, 89-95.
17. X. Gong, *Phys. Chem. Chem. Phys.*, 2013, **15**, 10459-10465.
18. Y. Zhao, B. Chen and S. Yao, *Sep. Purif. Technol.*, 2007, **52**, 533-538.

19. J. K. Zhang, X. Y. Zhu, F. L. Luo, C. D. Sun, J. Z. Huang, X. Li and K. S. Chen, *J. Sep. Sci.*, 2012, **35**, 128-136.
20. Y. Zhang, S. F. Li, X. W. Wu and X. Zhao, *Chin. J. Chem. Eng.*, 2007, **15**, 872-876.
21. F. J. Yang, L. Yang, W. J. Wang, Y. Liu, C. J. Zhao and Y. G. Zu, *Int. J. Mol. Sci.*, 2012, **13**, 8970-8986.
22. M. Murzakhmetova, S. Moldakarimov, L. Tancheva, S. Abarova and J. Serkedjieva, *Phytother. Res.*, 2008, **22**, 746-751.
23. L. L. Yu, S. Haley, J. Perret, M. Harris, J. Wilson and M. Qian, *J. Agric. Food Chem.*, 2002, **50**, 1619-1624.
24. R. Yang, D. M. Meng, Y. Song, J. Li, Y. Y. Zhang, X. S. Hu, Y. Y. Ni and Q. H. Li, *J. Agric. Food Chem.*, 2012, **60**, 8450-8456.
25. Y. Liu, J. Fang, Y. J. Kim, M. K. Wong and P. Wang, *Mol. Pharm.*, 2014, **11**, 1651-1661.
26. B. R. Schroeder, M. I. Ghare, C. Bhattacharya, R. Paul, Z. Yu, P. A. Zaleski, T. C. Bozeman, M. J. Rishel and S. M. Hecht, *J. Am. Chem. Soc.*, 2014, **136**, 13641-13656.
27. Z. Qiao, C. Xia, S. Shen, F. D. Corwin, M. Liu, R. Guan, J. R. Grider and L. Y. Qiao, *PLoS One*, 2014, **9**, e114536.
28. S. Zhao, J. Y. Liu, S. Y. Chen, L. L. Shi, Y. J. Liu and C. Ma, *Molecules*, 2011, **16**, 8590-8600.
29. E. Silva, D. Pompeu, Y. Larondelle and H. Rogez, *Sep. Purif. Technol.*, 2007, **53**, 274-280.
30. Y. C. Wang, Y. Y. Ma, Y. Zheng, J. Song, X. Yang, C. Bi, D. R. Zhang and Q. Zhang, *Int. J. Pharm.*, 2013, **441**, 728-735.

31. R. E. Chenbo Dong, Linda Sargent, Michael L Kashon, David Lowry, Yon Rojanasakul, Cerasela Zoica Dinu, *Envir. Sci. Nano* 2014
32. A. Manke, S. Luanpitpong, C. B. Dong, L. Y. Wang, X. Q. He, L. Battelli, R. Derk, T. A. Stueckle, D. W. Porter, T. Sager, H. L. Gou, C. Z. Dinu, N. Q. Wu, R. R. Mercer and Y. Rojanasakul, *Int. J. Mol. Sci.*, 2014, **15**, 7444-7461.
33. J. Zuchowski, K. Jonczyk, L. Pecio and W. Oleszek, *J. Sci. Food Agric.*, 2011, **91**, 1089-1095.
34. M. Srinivasan, A. R. Sudheer and V. P. Menon, *J. Clin. Biochem. Nutr.*, 2007, **40**, 92.

Figure Captions

Fig. 1. Static adsorption tests of the four macroporous resins.

Fig. 2. Dynamic adsotption and desorption tests of the four macroporous resins. (a) DA201-C (b) H1020 (c) NKA-II (d) NKA-9 (D-2, H-2, NII-2, N9-2, 20% ethanol eluents; D-4, H-4, NII-4, N9-4, 40% ethanol eluents; D-6, H-6, NII-6, N9-6, 60% ethanol eluents; D-8, H-8, NII-8, N9-8, 80% ethanol eluents).

Fig. 3. TIC chromatogram from standard solution of gallic acid (A), p-coumaric acid (B), ferulic acid (C), o-coumaric acid (D) in methanol, TIC tatol ion chromatogram.

Fig. 4. Effects of PEWB on cell viability in H_2O_2-injuried HEK293 cells. HEK293 cells were pre-incubated with extracts for 2 h prior to treatment with 1 mM H_2O_2 for 2 h. After the treatment, cell viability was determined by MTT analysis (n=6). Data were shown as means ± S.D.

Fig. 5. Effect of WBEs and PEWB on H_2O_2 induced intracellular ROS. (a) WBEs (1 mg/mL) and ferulic acid (b) DA201-C (c) H1020 (d) NKA-II (e) NKA-9 (D-2, H-2, NII-2, N9-2, 20% ethanol eluents; D-4, H-4, NII-4, N9-4, 40% ethanol eluents; D-6, H-6, NII-6, N9-6, 60% ethanol eluents; D-8, H-8, NII-8, N9-8, 80% ethanol eluents).

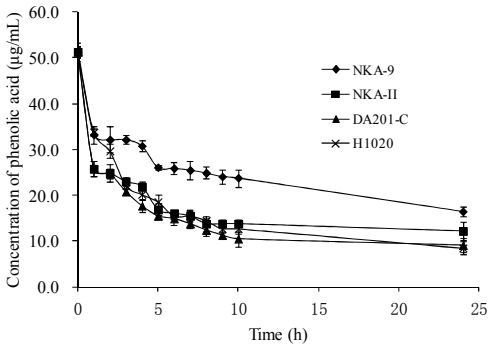

Fig. 1

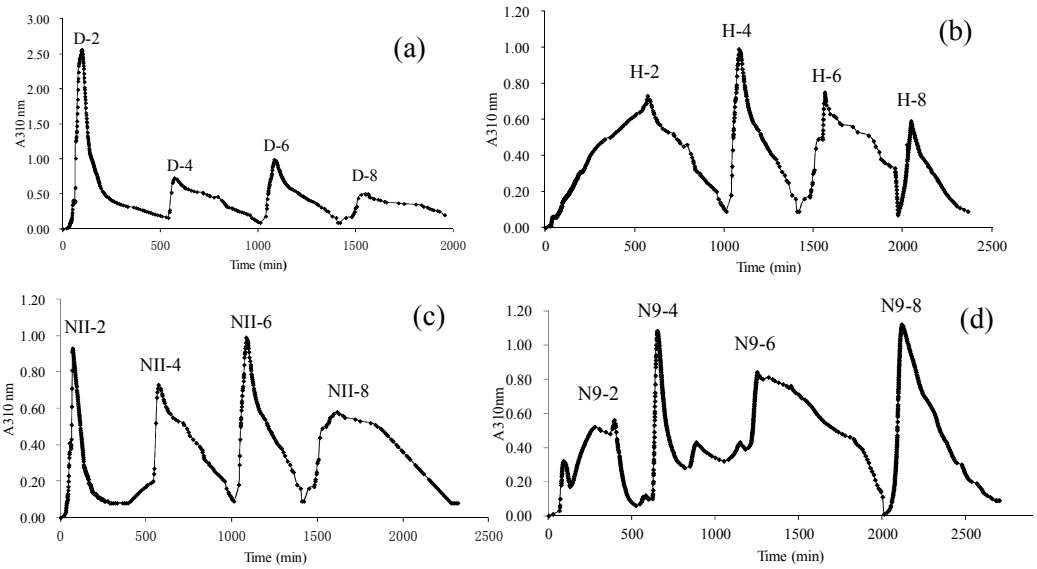

Fig. 2

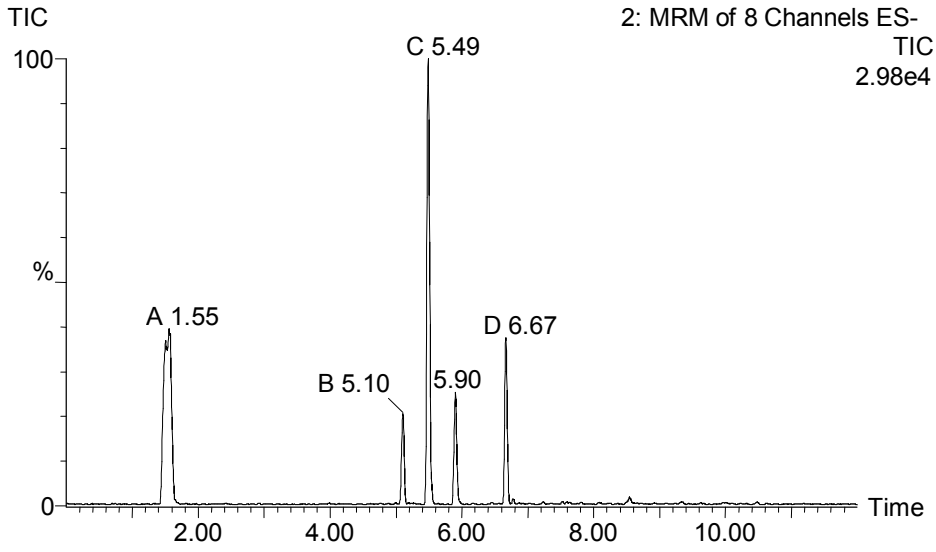

Fig. 3

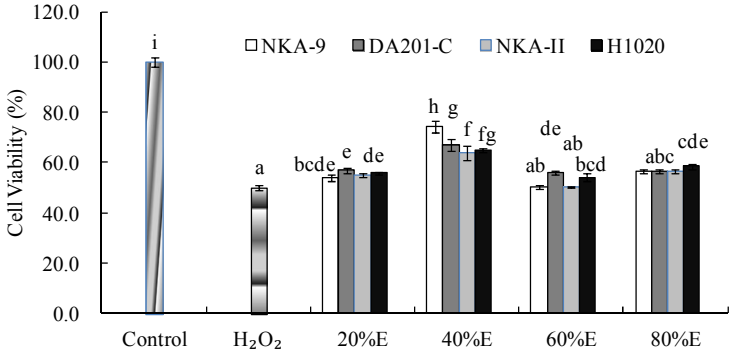

Fig. 4

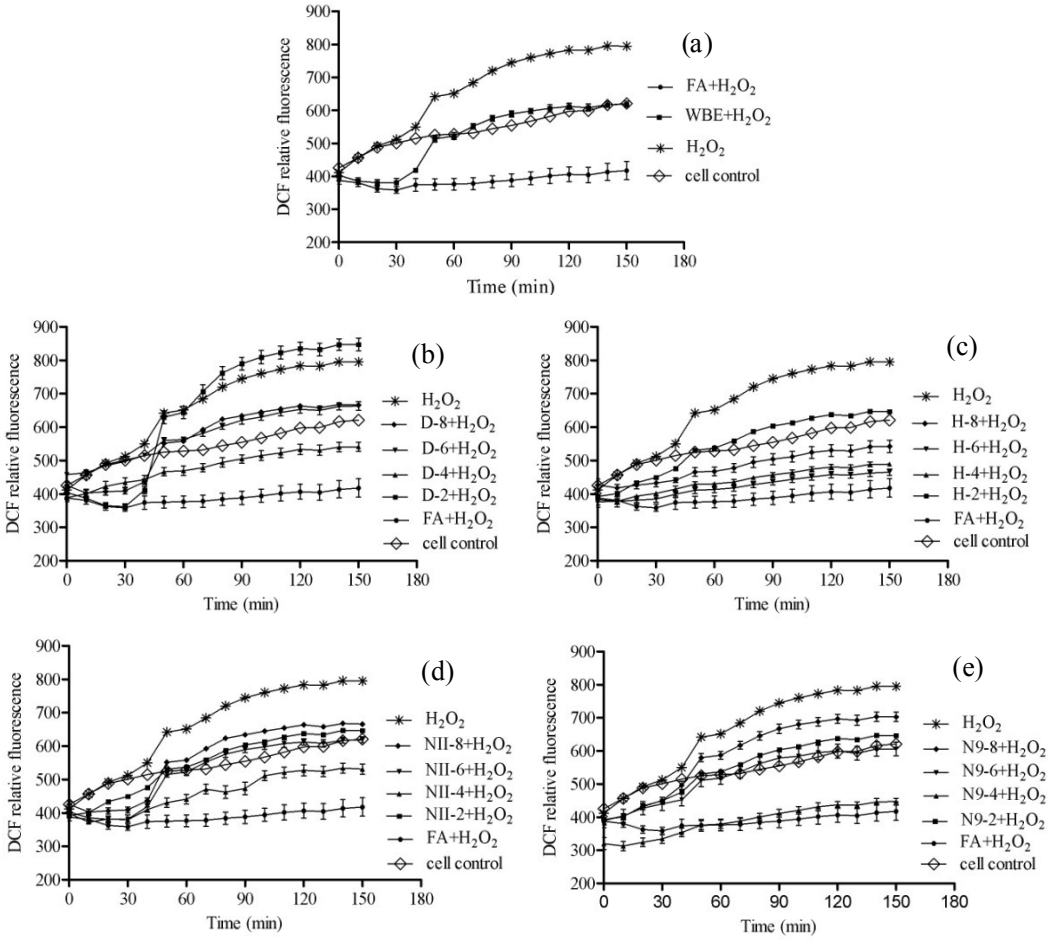

Fig. 5

Table 1 Physical properties of macroporous resins used

Resins	Polarity	Moisture content (%)	particle diameter	Surface area (m^2/g)	Average pore dimaeter (nm)
DA201-C	Non	50-60	0.40-1.25	1000-1300	3.0-4.0
H1020	Non	50-60	0.30-1.25	700-1000	12.0-17.0
NKA-II	polarity	42-52	0.30-1.25	160-200	14.5-15.5
NKA-9	polarity	67-73	0.30-1.25	250-290	15.5-16.5

Table 2 Adsorption capacity, adsorption, and desorption ratios of phenol acid on different macroporous resins

Resins	Adsorption capacity (mg/g)	Adsorption ratio (%)	Desorption ratio (%)
DA201-C	1.25±0.03^{b}	80.6±0.9^{b}	68.8±6.9^{b}
H1020	1.27±0.06^{b}	81.7±3.4^{b}	63.7±7.5^{b}
NKA-II	1.20±0.05^{b}	77.9±3.2^{b}	47.3±2.0^{a}
NKA-9	1.03±0.01^{a}	67.1±0.9^{a}	87.2±3.0^{c}

Results are mean±SD (n=6). Numbers followed by different letters are significantly different at the level of $p<0.05$ according to Duncan test.

Table 3 Quantification of phenolic compounds in PWBE using UPLC-TDQ. Values are expressed as mg compound/g extract (mg/g), where the annotation 'n/d' represents compounds not detected.

Samples	o-coumaric acid	p-coumaric acid	Ferulic acid	Gallic acid
D-2	0.30±0.03^{b}	13.19±0.02^{e}	20.34±1.53^{de}	1.83±0.06e
D-4	0.92±0.03^{e}	12.33±0.21^{d}	27.46±1.03^{g}	1.80±0.08^{e}
D-6	n/d	0.74±0.06^{a}	0.31±0.02^{a}	n/d
D-8	n/d	1.32±0.05^{a}	0.62±0.03^{a}	0.21±0.02^{b}
H-2	n/d	11.83±0.14^{d}	18.93±0.53^{cd}	2.00±0.04^{f}
H-4	1.51±0.02^{f}	13.01±0.41^{e}	24.71±2.17^{f}	2.44±0.05^{h}
H-6	n/d	0.76±0.11^{a}	0.21±0.02^{a}	0.04±0.01^{a}
H-8	0.41±0.02^{c}	9.05±0.19^{b}	21.23±1.78^{e}	1.49±0.02^{c}
NII-2	n/d	10.27±0.87^{c}	17.11±1.51^{bc}	1.63±0.03^{d}
NII-4	0.85±0.06^{d}	12.34±0.22^{d}	24.07±1.76^{f}	2.10±0.06^{g}
NII-6	n/d	n/d	0.13±0.06^{a}	n/d
NII-8	n/d	0.91±0.03^{a}	0.84±0.07^{a}	0.20±0.01^{b}
N9-2	n/d	10.33±0.51^{c}	16.20±1.01^{b}	1.70±0.03^{d}
N9-4	0.92±0.05^{e}	16.79±0.68^{f}	35.49±2.7^{h}	2.08±0.09^{fg}
N9-6	n/d	1.10±0.20^{a}	0.18±0.01^{a}	n/d
N9-8	0.02±0.01^{a}	0.86±0.09^{a}	0.55±0.07^{a}	0.18±0.01^{b}