Tropical Journal of Pharmaceutical Research August 2012; 11 (4): 545-551

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v11i4.4

Research Article

Characterization and Antioxidant Activity of the Complex of Phloridzin and Hydroxypropyl-β-cyclodextrin

Erdong Yuan[1], Benguo Liu[2], Wei Li[3] and Qiong Li[4*]

[1]College of Light Industry and Food Science, South China University of Technology, Guangzhou 510640, [2]School of Food Science, Henan Institute of Science and Technology, Xinxiang 453003, [3]College of Biological Engineering, Hubei University of Technology, Wuhan 430068, [4]School of Chemistry and Chemical Engineering, South China University of Technology, Guangzhou 510640, China.

Abstract

Purpose: *To improve the aqueous solubility of phloridzin by complexing it with hydroxypropyl-β-cyclodextrin (HP-β-CD).*
Methods*: The complex of phloridzin with HP-β-CD was prepared by freeze-drying method. The physicochemical properties of the complex were investigated by ultraviolet-visible spectrometry (UV), infrared spectrometry (IR), differential scanning calorimetry (DSC) and x-ray diffractometry (XRD). The antioxidant activity was examined by DPPH and ABTS radical-scavenging activities.*
Results*: Phloridzin in the complex was molecularly dispersed in HP-β-CD matrix. The complex was an effective scavenger of DPPH and ABTS radicals. At a concentration of 0.8 mg/mL and 30 μg/mL, DPPH and ABTS radical scavenging activities of the complex were 83.7 and 74.9 %, respectively.*
Conclusion*: By forming inclusion complex with HP-β-CD, the solubility of phloridzin in water was significantly enhanced. The complex showed strong DPPH and ABTS radical scavenging activities.*

Keywords: *Phloridzin, Hydroxypropyl-β-cyclodextrin, Complex, Antioxidant*

Received: 11 June 2012 *Revised accepted: 21 July 2012*

Corresponding author:* ***Email:* *qiongli@scut.edu.cn,* ***Tel:*** *+86 20 87111884;* ***Fax:*** *+86 20 22236337*

INTRODUCTION

Phloridzin is a nutraceutical with good prospects in food and pharmaceutical industries, and can exhibit antioxidant, anti-inflammatory, immunosuppressive effect, antitumor, antimutagenic, antidiabete, antiobesity and membrane permeability properties [1-4]. However, its poor aqueous solubility limits its application.

Hydroxylpropyl-β-cyclodextrin (HP-β-CD), a hydroxyalkyl derivative of β-cyclodextrin, is an alternative to α-, β- and γ-cyclodextrin (CD), and can impart improve aqueous solubility considerably(> 500 g/L, 20 °C) [5-7]. The objective of this study was to prepare an inclusion complex of phloridzin with HP-β-CD to enhance the aqueous solubility of the former as well as evaluate the physicochemical properties and antioxidant activity of the complex.

EXPERIMENTAL

Materials and chemicals

Phloridzin (purity, 98 %) was obtained from Yangling Dongke Maidisen Pharmaceutical Co Ltd (Yangling, China) while HP-β-CD (purity, 97 %) was purchased from Aladdin (Shanghai, China). Other reagents including 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and 2, 2′-azinobis (3-ethylbenzthiazoline-6-sulfonate) (ABTS) and linoleic acid were purchased from Sigma Chemicals Co (USA). Other chemicals used were of analytical grade.

Preparation of the inclusion complex of phloridzin and HP-β-CD

Phloridzin (0.16 g) and HP-β-CD (0.48 g) were dissolved in 15 mL of ethanol and stirred for 6 h. After ethanol was removed, the residue was dissolved in 100 mL water and filtered. The filtrate was frozen at -40 °C for 24 h and then lyophilized and collected. The resultant podwer was collected as the inclusion complex of phloridzin with HP-β-CD.

Preparation of physical mixture of phloridzin and HP-β-CD

Phloridzin (0.16 g) and HP-β-CD (0.48 g) were mixed and stirred in a small beaker at room temperature. This was used as the physical mixture of phloridzin and HP-β-CD.

Ultraviolet-visible spectrometry (UV) and infrared spectrometry (IR)

UV analysis was performed on a UV-6100A spectrophotometer (Shanghai Metash Instruments Co., Ltd., China) while IR analysis was performed on a SP2000 infrared spectrophotometer (Pye Unicam Ltd, UK) using compressed discs of the material mixed in KBr.

Differential scanning calorimetry (DSC)

DSC was performed on a differential calorimeter (model DSC204F1, Netzsch, Germany). The samples were sealed in crimped aluminum pans and heated at rate of 10 °C/min from 20 to 300°C in nitrogen atmosphere.

X-ray diffractometry (XRD)

XRD analysis was performed on a D8 Advance X-ray diffractometer (Bruker, Germany). The powders were packed tightly in a rectangular aluminum cell and exposed to the X-ray beam. The scanning region of the diffraction angle, 2θ, was 3 - 80˚.

DPPH radical scavenging assay

DPPH radical scavenging assay was done according to the method of Heo et al [8]. Briefly, 2 mL of DPPH solution (0.2 mmol/L, in ethanol) was incubated with different concentrations of the samples. The reaction mixture was shaken and incubated in the dark for 30 min at room temperature. The

absorbance was read at 517 nm against ethanol. Controls containing ethanol instead of the antioxidant solution, and blanks containing ethanol instead of DPPH solution were also made. The inhibition of the DPPH radical by the sample was calculated according to Eq 1.

DPPH scavenging activity (%) =
{(Dc – Ds)/Dc}100 …….. (1)

where Dc is the absorbance of control and Ds is the absorbance of the test sample.

ABTS radical scavenging assay

The scavenging activity of ABTS was measured according to the method of Lo et al [9] with some modifications. A stock solution of ABTS was prepared by mixing 5 mL of 7 mM ABTS with 88 μL of 140 mM potassium persulfate. The mixture was maintained in the dark at room temperature for 12 - 16 h to allow for completion of radical generation. The working ABTS solution was produced by diluting the stock solution with ethanol until the absorbance reached 0.7 ± 0.05 at 734 nm. In the assay, 1 mL working ABTS solution was mixed with 1 mL sample or blank. Absorbance was measured at 734 nm after reaction for 30 min in the dark. The inhibition of the ABTS radical by the sample was calculated as in Eq 2.

ABTS scavenging activity (%) =
{(Ac – As)/Ac}100 …… (2)

where Ac is the absorbance of control and As is the absorbance of the test sample.

Statistical analysis

The data obtained in this study are expressed as mean ± standard deviation (SD). Statistical comparisons were made using Student's t-test. *P* values of < 0.05 were considered significant.

RESULTS

UV and IR analysis

The UV and IR spectra of HP-β-CD, phloridzin, their physical mixture and the complex are shown in Figures 1 and 2. There was no UV absorbance of HP-β-CD in the range of 200 to 500 nm. There was also no difference between the UV spectra of the physical mixture and the complex. The characteristic absorption peaks of phloridzin, the physical mixture and the complex were still present at 224 and 285 nm. For the infrared results, the spectra of the physical mixture and the complex showed an additive effect of HP-β-CD and phloridzin. However, no new peaks were observed in the mixture and complex.

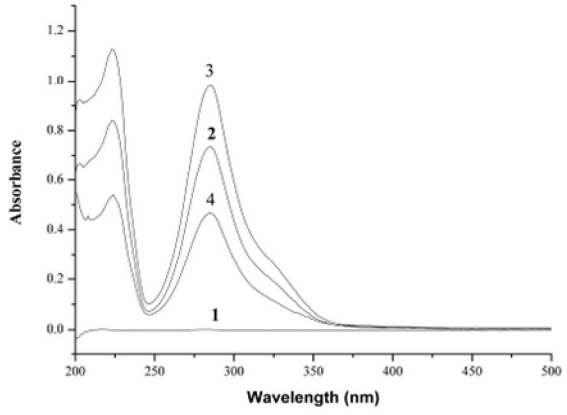

Figure 1: UV spectra of (1) HP-β-CD, (2) phloridzin, (3) their physical mixture and (4) complex

DSC analysis

Figure 3 shows the DSC thermograms of HP-β-CD, phloridzin, their physical mixture and the complex. The DSC thermogram of phloridzin showed an endothermal peak with onset temperature at 105.3 °C, which was attributed to the melting of phloridzin. The DSC curve of the physical mixture mainly showed the individual characteristics of phloridzin and HP-β-CD but the thermogram of the complex was different.

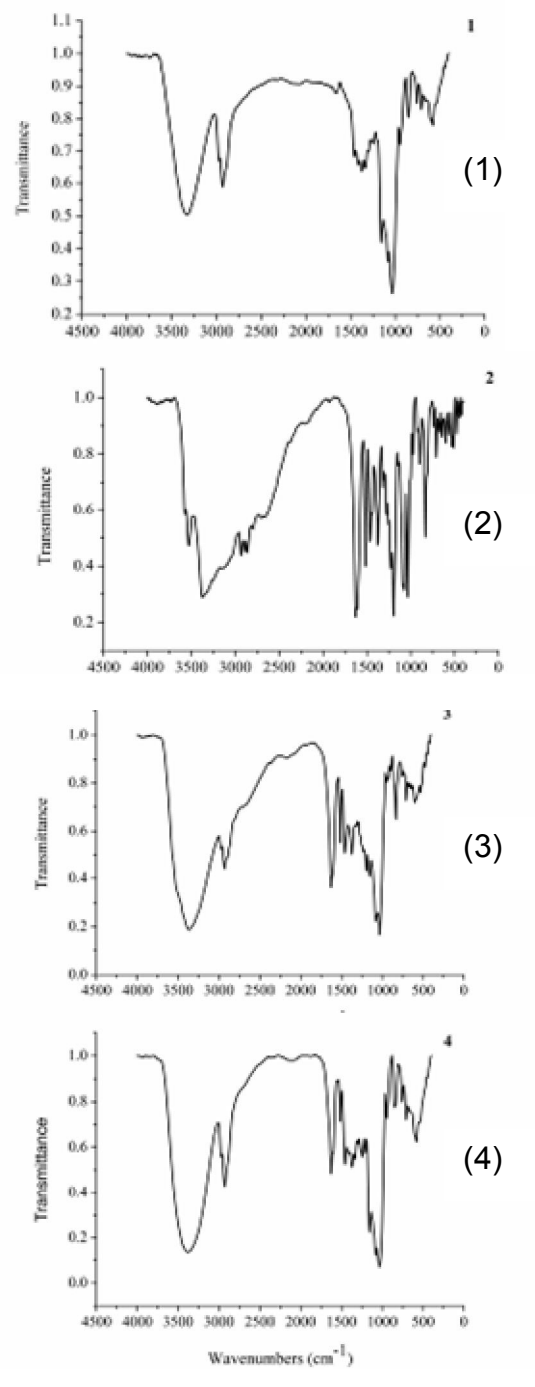

Figure 2: IR spectra of (1) HP-β-CD, (2) phloridzin, (3) their physical mixture and (4) complex

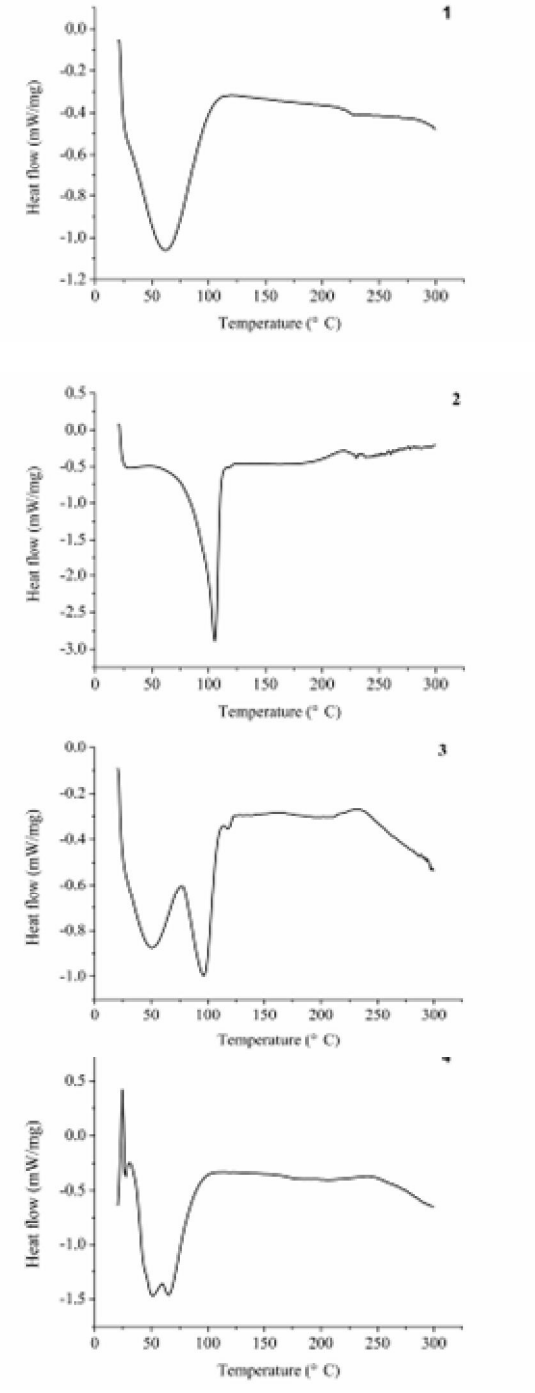

Figure 3: DSC thermogram of (1) HP-β-CD, (2) phloridzin, (3) their physical mixture and (4) complex

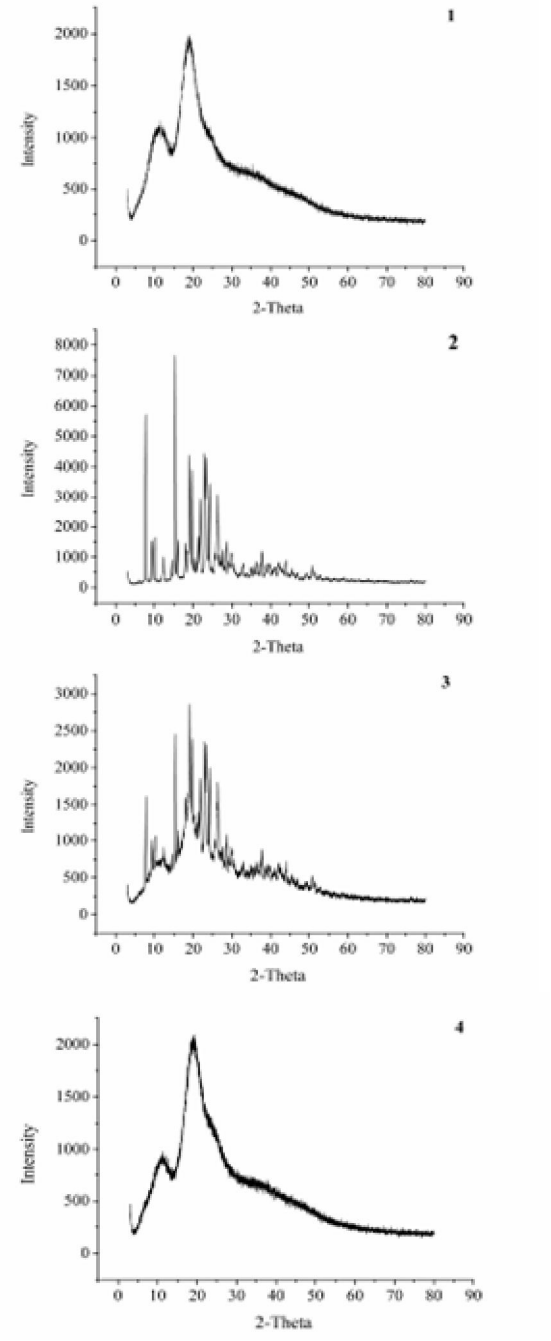

Figure 4: X-ray diffractogram of (1) HP-β-CD, (2) phloridzin, (3) their physical mixture and (4) complex

XRD analysis

The powder X-ray diffraction patterns of HP-β-CD, phloridzin, their physical mixture and the complex are shown in Figure 4. The diffractogram of phloridzin displayed sharp crystalline peaks. In contrast, HP-β-CD was amorphous as it lacked crystalline peaks. Furthermore, compared with the diffractogram of the physical mixture, the crystalline peaks had disappeared in the diffractogram of the complex.

DPPH radical scavenging activity

The DPPH scavenging activity of phloridzin and its corresponding complex at the same concentration of phloridzin are shown in Figure 5. There was no significant difference in their DPPH scavenging activities. At the concentration of 0.8 mg/mL, the DPPH scavenging activities of phloridzin and its complex were 84.7 and 83.7 %, respectively.

ABTS radical scavenging activity

The ABTS radical scavenging activities of phloridzin and its corresponding complex, at the same concentration of phloridzin, are shown in Figure 5. There was no significant difference in their ABTS radical scavenging activities. At a concentration of 30 µg/mL, ABTS scavenging activity of phloridzin alone and its complex was 77.4 and 74.9 %, respectively.

DISCUSSION

Phloridzin is a useful flavonoid in food and pharmaceutical production whose poor aqueous solubility often limits its application. At present, the aqueous solubility of flavonoids is generally enhanced by glycosidation using enzyme or chemical method. However, glycosidation reduces the number of conjugated hydroxyl groups, and thus may potentially weaken the bioactivities of flavonoids. Furthermore, glycosidation could create a group of new compounds with unknown safety which might adversely affect their application in food or medicine.

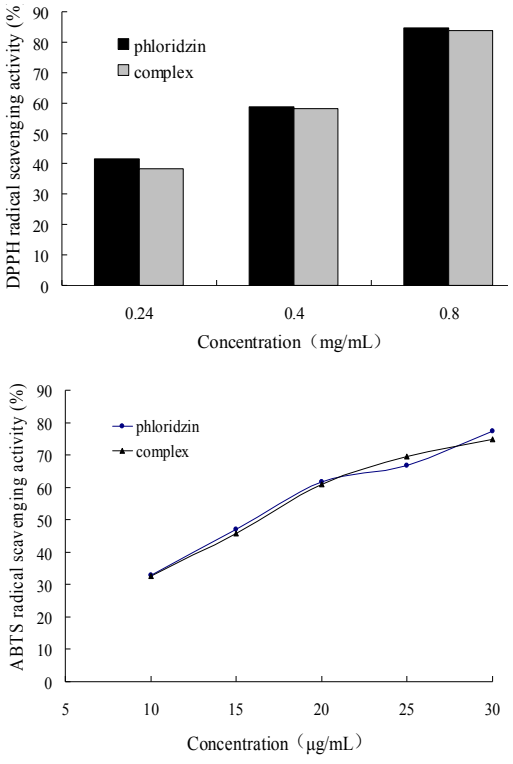

Figure 5: DPPH (a) and ABTS (b) free radical scavenging activities of phloridzin and its complex.

Generally, there are several methods for preparing inclusion complexes, and these include coprecipitation, neutralisation, kneading, spray drying, freeze-drying, solvent evaporation, ball-milling and sealed-heating. In the present study, the freeze-drying method was adopted to prepare the inclusion complex of phloridzin and HP-β-CD. This method would likely minimize chemical decomposition and loss of bioactivity. To the best of our knowledge, this is the first time the method has been used to prepare phloridzin complexed with HP-β-CD.

UV and IR data indicate that there was no difference between the physical mixture and the complex, which suggests that weak physical interactions between phloridzin and HP-β-CD are involved during the formation of the complex. On the other hand, DSC results mainly showed the presence of HP-β-CD in the complex with the endotherm of the flavonoid virtually absent, unlike the physical mixture which manifested clearly the presence of the two substances. It is believed that phloridzin was completely dispersed in HP-β-CD and that some interactions, probably involving the combination of hydrogen bonds and van der Waals force [10]. This appears to be buttressed by the x-ray diffraction (XRD) data. XRD is a useful method for the detection of CD complexation in powder or microcrystalline states. Therefore, XRD should be able to clearly distinguish an inclusion complex from that of the physical mixture of the components, if a true inclusion complex has been formed [11]. The disappearance of the crystalline peaks of phloridzin in the complex suggests that phloridzin was molecularly dispersed in the HP-β-CD matrix.

It is significant that the fact that the antioxidant activity of the complex was not significantly different from that of phloridzin alone demonstrate that the formation of inclusion complex has no effect on the antioxidant activity of phloridzin.

CONCLUSION

Formation of the inclusion complex of phloridzin with HP-β-CD considerably enhanced the solubility of phloridzin in water. In the complex formation process, phloridzin is molecularly dispersed in the HP-β-CD matrix. The complex showed strong DPPH and ABTS radical scavenging activities. The findings indicate that the freeze-drying technique used could expand the potential application of phloridzin/HP-β-CD complex in the food and pharmaceutical industries.

ACKNOWLEDGEMENT

The financial support provided by the National Natural Science Foundation of China (31101232), the Fundamental Research Funds for the Central Universities, SCUT (2011ZM0104), is greatly appreciated

REFERENCES

1. Puel C, Quintin A, Mathey J, Obled C, Davicco MJ, Lebecque P, Kati- Coulibaly S, Horcajada MN, Coxam V. Prevention of bone loss by phloridzin, an apple polyphenol, in ovariectomized rats under inflammation condition. Calcif Tissue Int 2005; 77: 311-318.
2. Ehrenkranz JRL, Lewis NG, Kahn CR, Roth J. Phlorizin: a review. Diabetes Metab Res Rev 2005; 21: 31-38.
3. Gosch C, Halbwirth H, Stich K. Phloridzin: Biosynthesis, distribution and physiological relevance in plants. Phytochem 2010; 71: 838-843.
4. Xü K, Lu H, Qu B, Shan H, Song J. High-speed counter-current chromatography preparative separation and purification of phloretin from apple tree bark. Separation and Purification Technology 2010; 72: 406-409.
5. Gould S, Scott RC. 2-Hydroxypropyl-β-cyclodextrin (HP-β-CD): a toxicology review. Food and Chemical Toxicology 2005; 43: 1451-1459.
6. Talegaonkar S, Yakoob KA. Development and characterization of paracetamol complexes with hydroxyl-β-cyclodextrin. Iran J Pharm Res 2007; 6: 95-99.
7. Wang L, Jiang X, Xu W. Complexation of tanshinone II A with 2-hydroxyproply-β-cyclodextrin: Effect on aqueous solubility, dissolution rate, and intestinal absorption behavior in rats. Int J Pharm 2007; 341: 58-67.
8. Heo SJ, Park EJ, Lee KW, Jeon, YJ. Antioxidant activities of enzymatic extracts from brown seaweeds. Bioresour Technol 2005; 96: 1613-1623.
9. Lo KM, Cheung P. Antioxidant activity of extracts from the fruiting bodies of Agrocybe aegerita var. alba. Food Chem 2005; 89: 533-539.
10. Lasonder E, Weringa, WD. An NMR and DSC study of the interaction of phospholipid vesicles with some anti-inflammatory agents. J Colloid Interface Sci 1990; 139: 469-478.
11. Wang J, Cao Y, Sun B, Wang C. Physicochemical and release characterisation of garlic oil-β-cyclodextrin inclusion complexes. Food Chemistry 2011; 127: 1680-1685.