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Original article

Anti-oxidative Activities of Ethanol Extracts from Both Wild Plant and Suspension Cell Cultures of *Rheum franzenbachii*

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ABSTRACT

Objective To evaluate the content of rhaponticin and anti-oxidative activities of the ethanol extracts from both the wild plants and suspension cell cultures of *Rheum franzenbachii*. **Methods** Quantitative analysis of rhaponticin was performed by HPLC. The anti-oxidative activities of the ethanol extracts were evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assays. **Results** The content of rhaponticin in the roots of the wild plant was 4.36 mg/g, while the content was only 1.59 mg/g in the leaves. The content of rhaponticin in suspension cells cultured on Murashige and Skoog (MS) medium supplemented with 1.0 mg/L 6-benzylaminopurine (6-BAP) and 2.0 mg/L 2,4-dicholorophenoxy acetic acid (2,4-D) was 17.64 mg/g, which increased by 4.05 times compared with the content in the roots of the wild plants. The roots of wild plants displayed the strongest anti-oxidative activity, followed by the suspension cells 5 and 6, and the scavenging percent was 91.96%, 91.23%, and 89.27%, respectively, at the concentration of 100 μg/mL. The IC_{50} values were 2.477, 15.644, and 31.415 μg/mL, respectively. In particular, the DPPH scavenging activity of the ethanol extracts from the roots of the wild plant was generally comparable to the control of ascorbic acid (VC), and the IC_{50} value of the extracts was lower than that of VC (2.502 μg/mL). **Conclusion** Rhaponticin production in the cell culture can be modulated and the accumulation can be increased. The roots of the wild plant display the strongest anti-oxidative activity. These results suggest that *R. franzenbachii* could hold a good potential source for human health.

Key words

anti-oxidative activity; rhaponticin; *Rheum franzenbachii*; suspension cells

1. Introduction

Free radicals are known as highly reactive molecules with one or more unpaired electron and play an important role in aging, cardiovascular diseases, cancer, impaired immune system, and inflammatory diseases (Finkel and Holbrook, 2000; Wu and Hansen, 2008; Hsouna et al, 2011). Anti-oxidants are molecules that are capable of neutralising or scavenging the free radicals by hydrogen donation before those free radicals can attack cells and other biological components (Erkana et al, 2011). Therefore, it is necessary to exploit natural anti-oxidants from various sources.

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Many natural phytochemicals extracted from plants exhibit important biological activities and a lot of attention have been gained as these phytochemicals have shown the tremendous advantages to human health (Mehta et al, 2010; Rai et al, 2010; Kirby and Schmidt, 1997). Some phytochemicals may act as anti-oxidant, antimicrobial, and novel source of drugs.

Rheum franzenbachii Munt., a medicinal plant in China, belongs to the Polygonaceae family and distributed in the mountain 1000–1600 m above sea level (Anonymous, 1984). Its leaves and stems are edible and its roots and rhizomes are used as medicine. The properties of *R. franzenbachii* have been described in *Shennong's Herbal Classic* as bitter and cold in nature with the antibacterial, anti-inflammatory, convergence, detumescence, hemostasis, and defaecation functions.

In order to investigate the medicinal properties of *R. franzenbachii*, some chemical analyses have been studied. Six compounds isolated from 95% ethanol extract in the roots and rhizomes of *R. franzenbachii* were identified as des-oxy-rhapontigenin, rhapontigenin, piceatannol, des-oxyrhaponticin, rhaponticin, and piceatannol-3′-*O*-β-*D*-glucopyranoside (Wang et al, 2001). Chrysophanol, physcion, β-sitosterol, emodin, daucosterol, rhein, physcion-8-*O*-β-*D*-glucoside, and other compounds were obtained and identified by spectroscopic analyses and chemical characteristics (Wang et al, 2003a). Recent studies have shown that rhaponticin could be one of the potential compounds with the pharmacological functions of antitumor (Ryu et al, 1994), antithrombosis (Park et al, 2002), anti-oxidation (Ngoc et al, 2008; Wang et al, 2007), inhibiting allergic reactions (Kim et al, 2000), regulating immune defense system, and reducing blood sugar and lipid (Choi et al, 2006).

Owing to its excessive usage, wild resource of *R. franzenbachii* is at the brink of exhaustion in recent years, so there is a need to develop new approaches for sustainable development and utilization. Suspension culture technique may rapidly increase the production of biomass and the accumulation of secondary metabolites. Although much has been learned about the phytochemicals of *R. franzenbachii*, there has been remarkably little research on the bioactivity of *R. franzenbachii*. The aim of the present study is to investigate the anti-oxidative potential of *R. franzenbachii*. Identification of the biologic activities of this plant may be used for human health.

2. Materials and methods

2.1 Plant materials

Wild plants of *Rheum franzenbachii* Munt. were collected from Lingshan Mountain in Beijing, China, 1600 m above sea level, and were used as material in this study (Figure 1A).

2.2 Suspension cell culture

Leaf explants, approximately 0.5 cm × 0.5 cm in size, were sectioned from the sterilized plants for callus induction. Calluses induced from leaf explants incubated on Murashige and Skoog (MS) medium with supplements of 0.5 mg/L *N*-phenyl-*N*′-1,2,3-thiadiazol-5-ylurea (TDZ) in combination with 0.2 mg/L α-naphthalene acetic acid (NAA) under illumination at 30–40 μmol/(m^2·s) and 25–26 ^oC. All media were adjusted pH value to 5.8 with 1 mol/L NaOH, 30 g/L sucrose, and 7 g/L agar (Sigma-Aldrich Chemical Co., USA) were added before being autoclaved at 121 ^oC for 20 min (Wang et al, 2011).

Calluses were suspended in MS medium with appropriate supplements of plant growth regulators. After three subcultures, a good suspension could be obtained. All media were adjusted pH value to 5.8 with 1 mol/L NaOH, and 30 g/L sucrose were added before being autoclaved at 121 ^oC for 20 min. Suspension cells were cultured on a rotator (120 r/min) under illumination at 30–40 μmol/(m^2·s) and 25–26 ^oC.

2.3 Quantitative analysis

For the quantitative analysis of rhaponticin, 0.1 g of each powdered suspension cell cultures and wild plant materials were first mixed with 80% ethanol (25 mL) and extracted under reflux for 2 h. All ethanol in the filtrate (10 mL) was evaporated by decompression in the rotary evaporator, and the remains were dissolved with 10 mL methanol. HPLC analysis (LC–10AVP Plus) was performed on a Hypersil BDS-C_{18} column (250 mm × 4.6 mm, 5 μm) and the effluent was monitored at 320 nm by UV detector. The injection volume was 20 μL. The mobile phase was acetonitrile-H_2O (25:75) at a flow rate of 1.0 mL/min. The reference substance of rhaponticin was purchased from The National Institute for Food and Drug Control.

2.4 Anti-oxidative assays

Free radical scavenging activities of the different samples were determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich Chemical Co., USA) radical, according to the methods of Kirby and Schmidt (1997). Ascorbic acid (Beijing Chemical Works, China) was used as positive control. The percent of DPPH scavenging effect was calculated using the following equation:

$$\text{Scavenging effect of DPPH / \%} = [(A_c - A_t) / A_c] \times 100$$

Where A_c is the absorbance of the control reaction and A_t is the absorbance in the presence of the sample extraction.

The anti-oxidative ability of the fraction was expressed as the IC_{50}. Each value was determined from the regression equation. Experiments were performed in triplicate and the values reported herein were mean values of three experiments.

2.5 Data analysis

All experimental data were statistically analyzed by One-way analysis of variance (ANOVA) using the protected least-significant-difference (LSD) test.

3. Results

3.1 Cell suspension culture

Callus was induced from leaf explants incubated on MS medium with supplements of 0.5 mg/L TDZ in the combination with 0.2 mg/L NAA (Figure 1B). To the cell proliferation, the effects of TDZ, 6-BAP, NAA, and 2,4-D at various concentration were performed on the MS medium. After 3–4 times subculture, suspension cells were obtained (Figure 1C). In the treatment of 6-BAP combined with 2,4-D, the effect on the cell proliferation was very significant (Table 1).

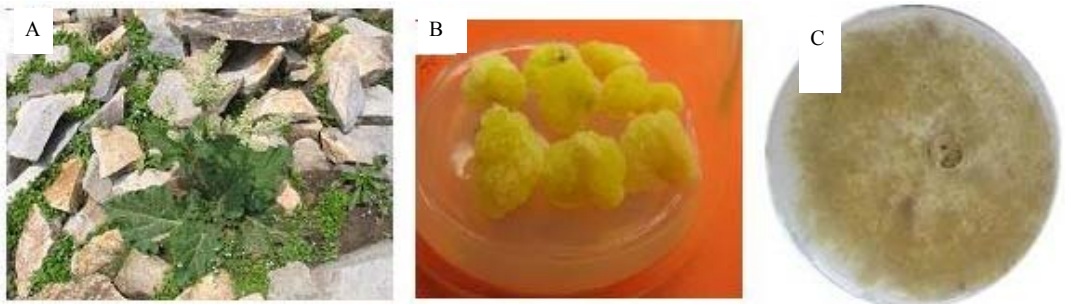

Figure 1 Wild plant (A), callus induced from leaf explants on MS medium (B), and suspension cells cultured on MS medium (C)

Table 1 Effects of plant growth regulators on suspension cell proliferation of *R. franzenbachii* ($\bar{x} \pm s$)

6-BA / (mg·L^{-1})	2,4-D / (mg·L^{-1})	Fresh weight / g	Dry weight / g
0	0	4.50 ± 0.40 a	0.40 ± 0.06 a
	0.5	8.13 ± 0.54 c	0.63 ± 0.06 c
	1.0	8.70 ± 1.73 c	0.60 ± 0.06 c
	2.0	9.43 ± 1.41 c	0.60 ± 0.06 c
	4.0	6.08 ± 0.50 b	0.47 ± 0.05 b
0.5	0	4.93 ± 0.42 a	0.48 ± 0.04 a
	0.5	8.15 ± 1.24 b	0.65 ± 0.05 b
	1.0	8.70 ± 3.75 b	0.68 ± 0.10 b
	2.0	12.05 ± 2.09 d	0.73 ± 0.10 c
	4.0	10.77 ± 0.83 c	0.70 ± 0.28 c
1.0	0	5.40 ± 0.67 a	0.50 ± 0.09 a
	0.5	8.13 ± 1.96 b	0.48 ± 0.01 a
	1.0	9.27 ± 1.57 c	0.60 ± 0.11 b
	2.0	11.10 ± 0.82 d	0.70 ± 0.14 c
	4.0	9.07 ± 2.69 c	0.58 ± 0.08 b
2.0	0	5.15 ± 1.25 b	0.50 ± 0.09 c
	0.5	3.47 ± 0.27 a	0.30 ± 0.63 b
	1.0	3.35 ± 0.10 a	0.28 ± 0.04 b
	2.0	7.83 ± 1.51 c	0.62 ± 0.15 d
	4.0	3.08 ± 0.13 a	0.23 ± 0.05 a
4.0	0	4.13 ± 0.27 a	0.43 ± 0.05 b
	0.5	4.11 ± 1.03 a	0.30 ± 0.06 b
	1.0	5.93 ± 1.59 b	0.35 ± 0.05 b
	2.0	6.95 ± 1.64 c	0.52 ± 0.10 c
	4.0	8.70 ± 1.13 d	0.53 ± 0.12 c

Different letters mean significant difference ($P < 0.05$)

When callus was cultured on MS medium supplemented with 0.5 mg/L 6-BAP in the combination with 2.0 mg/L 2,4-D, fresh and dry weights of cells were 12.05 and 0.73 g, respectively. The proliferation of 6-BAP in combination with NAA was similar to that of 6-BAP in the combination with 2,4-D. However, the proliferation of TDZ in the combination with NAA or indole-3-acetic acid in all experiments was not satisfying.

3.2 Quantitative analysis of rhaponticin

The contents of rhaponticin in wild plants and different suspension cells of *R. franzenbachii* were detected by HPLC analysis (Figure 2). The content of rhaponticin in the roots of the wild plant was 4.36 mg/g (DW), while the content was only 1.59 mg/g (DW) in the leaves of the wild plants (Table 2). Suspension cell 1 was cultured on MS medium supplemented with TDZ 0.5 mg/L and NAA 0.2 mg/L. Suspension cell 2 was cultured on MS medium supplemented with 6-BAP 2.0 mg/L and NAA 1.0 mg/L. Suspension cell 3 was cultured on MS medium supplemented with 2,4-D 2.0 mg/L. Suspension cell 4 was cultured on MS medium supplemented with 6-BAP 0.5 mg/L and 2,4-D 2.0 mg/L. Suspension cell 5 was cultured on MS medium supplemented with 6-BAP 1.0 mg/L and 2,4-D 2.0 mg/L. Suspension cell 6 was cultured on MS medium supplemented in the combination with TDZ 2.0 mg/L and NAA 0.2 mg/L. Suspension cell 7 was cultured on MS medium supplemented in the combination with 6-BAP 4.0 mg/L and NAA 0.2 mg/L. The responses of rhaponticin production were better in MS

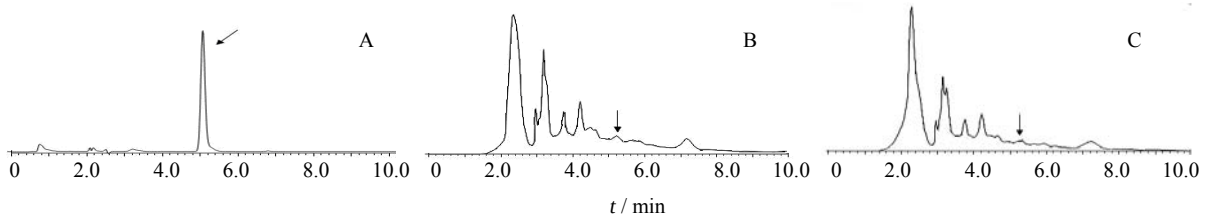

Figure 2 HPLC of rhaponticin reference substance (A), suspension cell 5 (B), and roots of wild plant (C)

Table 2 Contents of rhaponticin in leaves and roots of wild plant and different suspension cell cultures ($\bar{x} \pm s$)

Materials	Contents of rhaponticin / (mg·g^{-1})
suspension cell 1	8.92 ± 0.118 b
suspension cell 2	9.66 ± 0.101 b
suspension cell 3	0.12 ± 0.072 e
suspension cell 4	0.02 ± 0.009 f
suspension cell 5	17.64 ± 0.359 a
suspension cell 6	5.45 ± 0.199 c
suspension cell 7	4.66 ± 0.079 c
leaves of wild plants	1.59 ± 0.077 d
roots of wild plants	4.36 ± 0.135 c

Different letters means significant difference ($P < 0.05$)

media supplemented in the combination with 0.5 mg/L TDZ and 0.2 mg/L NAA, as well as another combination of 2.0 mg/L 6-BAP and 1.0 mg/L NAA, and the contents of rhaponticin in suspension cells 1 and 2 were 8.92 and 9.66 mg/g, respectively. The combination of 1.0 mg/L 6-BAP and 2.0 mg/L 2,4-D gave the best response for the rhaponticin accumulation and the content in cell 5 was about 17.64 mg/g, increased by 4.05 times compared with the content in the roots of wild plants.

3.3 DPPH free radical-scavenging activity

The DPPH radical is a stable organic free radical with an absorption band at 517 nm. This absorption disappeared after accepting an electron or a free radical species, and the result could be observed as the color changes from purple to yellow (Hseu et al, 2008).

Anti-oxidative activities of the ethanol extracts from both the wild plants and different suspension cell cultures of *R. franzenbachii* were evaluated by measuring their ability to scavenge DPPH radicals and VC was used as positive control. The DPPH radical scavenging activities of different suspension cell cultures and wild plants are shown in Figure 3. All tested extracts showed the DPPH radical scavanging activities, and the scavenging percent of suspension cells 1–7, leaves, and roots was 37.59%, 82.65%, 64.24%, 55.14%, 91.23%, 89.27%, 77.63%, 25.03%, and 91.96%, respectively, at the concentration of 100 μg/mL. The roots of wild plant displayed the strongest anti-oxidative activity, followed by suspension cell 5 and then by suspension cell 6. In particular, the DPPH radical scavenging activity of the root of wild plant was generally comparable to the control of VC, and the scavenging percent was 49.90% at the concentration of 5 μg/mL, which was higher than that of VC (44.12%). However, the extract from the leaves showed lower activity than those from cells and roots.

The scavenging activities could be evaluated by the determination of IC_{50} values, which corresponded to the amount of extracts required to scavenge 50% of the DPPH radicals present in the reaction mixture. High IC_{50} values indicated low anti-oxidative activities. The IC_{50} values of different extracts were 2.477–232.779 μg/mL (Table 3). The DPPH free radical scavenging activities of extracts were in the

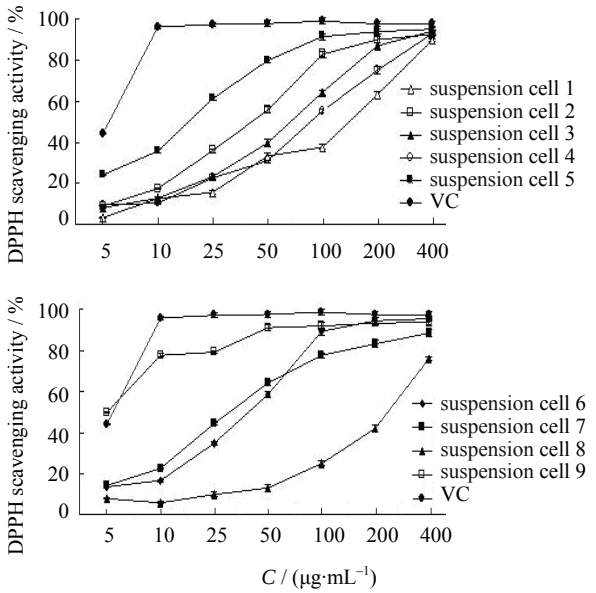

Figure 3 DPPH radical scavenging activities of different extracts from suspension cell cultures ($\bar{x} \pm s$, $n = 3$)

Table 3 IC_{50} of different samples

Materials	IC_{50} / (μg·mL^{-1})
VC	2.502 ± 0.032 a
suspension cell 1	102.793 ± 0.259 f
suspension cell 2	36.994 ± 0.222 c
suspension cell 3	55.296 ± 0.182 d
suspension cell 4	72.195 ± 0.224 e
suspension cell 5	15.644 ± 0.092 b
suspension cell 6	31.415 ± 0.141 c
suspension cell 7	32.455 ± 0.175 c
leaves of wild plant	232.779 ± 0.333 g
roots of wild plant	2.477 ± 0.112 a

Different letters means significant difference ($P < 0.05$)

following order: roots of wild plants (IC_{50} = 2.477 μg/mL) > VC (IC_{50} = 2.502 μg/mL) > suspension cell 5 (IC_{50} = 15.644 μg/mL) > suspension cell 6 (IC_{50} = 31.415 μg/mL) > suspension cell 7 (IC_{50}= 32.455 μg/mL) > suspension cell 2 (IC_{50} = 36.994 μg/mL) > suspension cell 3 (IC_{50} = 55.296 μg/mL) > suspension cell 4 (IC_{50} = 72.195 μg/mL) > suspension cell 1 (IC_{50} = 102.793 μg/mL) > leaves of the wild plants (IC_{50} = 232.779 μg/mL).

4. Discussion

There are several reports in the literature discussing the isolation of the compounds with pharmacological importance or other biological applications. Wang et al (2001) isolated rhaponticin from 95% ethanol extract from the roots and rhizomes of *R. franzenbachii*, while quantitative analysis on rhaponticin has not been conducted.

In the view of increasing demand for *R. franzenbachii*, there is a need to develop new approaches for sustainable development and utilization. Plant cell culture techniques offer a powerful tool to manufacture economically important

secondary metabolites such as flavors and pharmaceuticals within controlled laboratory environments (Wang et al, 2013; 2003b; Bourgaud et al, 2001; Rao and Ravishankar, 2002; Vanisree et al, 2004; Verpoorte et al, 2002). Guo et al (2007) reported that the micropropagated and wild plants of *Saussurea involucrata* showed the same phyochemical profile and comparable content of chlorogenic acid, rutin, and hispidulin.

In vitro culture technique may rapidly increase the production of biomass and the accumulation of secondary metabolites as well as aid the replacement of natural populations. Our result showed that rhaponticin production in cell culture could be modulated, depending on the concentration and combination of the plant growth regulators, and more importantly, rhaponticin accumulation could be increased. The combination of 6-BAP and 2,4-D gave the best response for the rhaponticin accumulation.

The suspension cells and wild plants showed the same phytochemical profile and comparable content of rhaponticin. HPLC analysis could be used as a selection tool for the production of phytochemical-rich clones. We are now in the process of the analysis on other active compounds in *R. franzenbachii*, and the establishment of the biosynthetic route of rhaponticin and development of molecular studies to increase rhaponticin production will be conducted in the future.

Recent studies have shown that rhaponticin is one of potential medicinal compounds with anti-oxidation (Ngoc et al, 2008; Wang et al, 2007). However, the order of rhaponticin contents (cell 5 > cell 2 > cell 1 > cell 6 > cell 7 > roots of wild plants > leaves of wild plants > cell 3 > cell 4) was different from the order of scavenging activities. This result showed that there were some other medicinal compounds except for rhaponticin with anti-oxidative activities in both the suspension cells and wild plants.

5. Conclusion

Rhaponticin production in cell culture can be modulated, and more importantly, rhaponticin accumulation could be increased. All the tested extracts of *R. franzenbachii* could exhibit the anti-oxidative activities to some extents. The roots of wild plant display the strongest anti-oxidative activity, followed by suspension cell 5 and then by cell 6. In particular, the DPPH scavenging activity of the roots of wild plants is generally comparable to the control of VC, and the IC_{50} value is lower than that of VC. These results suggest that *R. franzenbachii* could hold a good potential source for human health and could be used as vegetables or raw material for food processing.

References

Bourgaud F, Gravot A, Milesi S, Gontier E, 2001. Production of plant secondary metabolites: A historical perspective. *Plant Sci* 161: 839-851.

Choi SB, Ko BS, Park SK, 2006. Insulin sensitizing and α-glucoamylase inhibitory action of sennosides, rhein and rhaponticin in *Rhei Rhizoma*. *Life Sci* 78: 934-942.

Erkana N, Cetinb H, Ayranci E, 2011. Antioxidant activities of *Sideritis congesta* Davis et Huber-Morath and *Sideritis arguta* Boiss et Heldr: Identification of free flavonoids and cinnamic acid derivatives. *Food Res International* 44: 297-303.

Finkel T, Holbrook, N J, 2000. Oxidants, oxidative stress and the biology of ageing. *Nature* 408: 239-247.

Flora of China Editorial Committee of Chinese Academy of Sciences, 1984. *Flora of China*. Science Press: Beijing.

Guo B, Gao M, Liu CZ, 2007. *In vitro* propagation of an endangered medicinal plant *Saussurea involucrata* Kar. et Kir. *Plant Cell Rep* 26: 261-265.

Hseu YC, Chen SC, Yechd YJ, Wang L, Yang HL, 2008. Antioxidant activity of *Antrodia camphorate* on free radical-induced endothelial cell damage. *J Ethnopharm* 118: 237-245.

Hsouna AB, Trigui M, Culioli G, Blache Y, Jaoua S, 2011. Anti-oxidant constituents from Lawsonia inermis leaves: Isolation, structure elucidation and antioxidative capacity. *Food Chem* 125: 193-200.

Kim DH, Park EK, Bae EA, 2000. Metabolism of rhaponticin and chrysophanol 8-*O*-β-*D*-glucopyranoside from the rhizome of *Rheum Undulatum* by human intestinal bacteria and their anti-allergic actions. *Biol Pharm Bull* 23: 830-833.

Kirby AJ, Schmidt RJ, 1997. The antioxidant activity of Chinese herbs for eczema and of placebo herbs-I. *J Ethnopharm* 56: 103-108.

Mehta S, Rai PK, Rai DK, Rai NK, Rai AK, Bicanic D, Sharma B, Watal G, 2010. LIBS-based detection of antioxidant elements in seeds of *Emblica officinalis*. *Food Biophysic* 5: 186-192.

Ngoc TM, Minh PTH, Hung TM, 2008. Lipoxygenase inhibitory constituents from Rhubarb. *Arch Pharm Res* 31: 598-605.

Park EK, Choo MK, Moon HK, 2002. Antithrombotic and antiallergic activities of rhaponticin from *Rhei rhizoma* are activated by human intestinal bacteria. *Arch Pharm Res* 25: 528-533.

Rai PK, Jaiswal D, Rai DK, Sharma B, Watal G, 2010. Antioxidant potential of oral feeding of *Cynodon dactylon* extract on diabetes induced oxidative stress. *J Food Biochem* 34: 78-92.

Rao SR, Ravishankar GA, 2002. Plant cell cultures: Chemical factories of secondary metabolites. *Biotechnol Adv* 20: 101-153.

Ryu SY, Choi SU, Lee CO, 1994. Antitumor activity of some phenolic components in plants. *Arch Pharm Res* 17: 42-44.

Vanisree M, Lee CY, Lo SF, Nalawade SM, Lin CY, Tsay HS, 2004. Studies on the production of some important secondary metabolites from medicinal plants by plant tissue cultures. *Bot Bull Acad Sin* 45: 1-22.

Verpoorte R, Contin A, Memelink J, 2002. Biotechnology for the production of plant secondary metabolites. *Phytochem Rev* 1: 13-25.

Wang AQ, Li JL, Wu ZZ, 2001. Studies on stilbenes in *Rheum Franzenbachii*. *Chin Tradit Herb Drugs* 32: 878-880.

Wang AQ, Li JL, Wu ZZ, 2003. Studies on non-stilbenes in *Rheum Franzenbachii*. *Chin Tradit Herb Drugs* 34: 685.

Wang JL, Liao XR, Zhang HM, Du JF, Chen PL, 2003. Accumulation of chlorogenic acid in cell suspension cultures of *Eucommia Ulmoides* Oliv. *Plant Cell Tiss Organ Cult* 74: 193-195.

Wang JL, Liu K, Xu DT, Wang Q, Bi KL, Song YF, Li JF, Zhang L, 2013. Rapid micropropagation system *in vitro* and antioxidant activity of *Scabiosa tschiliensis* Grunning. *Plant Growth Regul* 69: 305-310.

Wang JL, Lu Y, Wang Q, Liu K, Song YF, Bi KL, 2011. An efficient callus proliferation protocol and rhaponticin accumulation of *Rheum franzenbachii* Munt., a medicinal plant. *J Plant Biochem Biotechnol* 20: 252-257.

Wang XD, Bi JJ, Huang LH, 2007. HPLC determination of rhaponticin in *Rheum Hotaoense* C.Y. Cheng et T. C. Kao. *Bull Acad Mil Med Sci* 31: 498-499.

Wu XJ, Hansen C, 2008. Antioxidant capacity, phenolic content, and polysaccharide content of Lentinus edodes grown in whey permeate-based submerged culture. *J Food Sci* 73: 1-8.