African Journal of Biotechnology Vol. 10(46), pp. 9438-9444, 22 August, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.1115
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Antioxidant, antibacterial and *α*-glucosidase inhibitory activities of different extracts of Cortex Moutan

Jinfeng Yang[1#], Ju-Sung Kim[2#], Yeo Jin Sa[1], Myeong Ok Kim[1], Hyun Ju Jeong[1], Chang Yeon Yu[1] and Myong Jo Kim[1]*

[1]Department of Applied Plant Sciences, Kangwon National University, Chuncheon 200-701, Korea.
[2]Majors in Plant Resource Sciences and Environment, College of Applied Life Sciences, Jeju National University, Jeju 690-756, Korea.

Accepted 27 June, 2011

Different extracts of Cortex Moutan (CM) were investigated for their antioxidant, antibacterial and *α*-glucosidase inhibitory activities. The content of paeonol was quantified by high performance liquid chromatography (HPLC). The results show that the yield of acetone extract (57.14%) was significantly higher than those of other solvents. The ethyl-acetate extract exhibited maximum paeonol concentration (60.69 µg/ml), good antibacterial activities (MIC = 100 µg/ml) against *Escherichia coli* and possessed significant α-glucosidase inhibitory activity. In addition, among all of the extracts, ethyl-acetate extract demonstrated a high total phenolic value of 127.12 ± 1.42 mg GAE/g, high DPPH radical scavenging activity with an IC_{50} of 19.88 ± 0.26 µg/ml, and significant reducing power, suggesting that CM is a potential source of natural antioxidants.

Key words: Cortex Moutan*,* antioxidant, 11-diphenyl-2-picrylhydrazyl hydrate (DPPH), reducing power, antibacterial, α-glucosidase

INTRODUCTION

Traditional Chinese medicine (TCM) has a long history dating back to several thousands of years. Due to their high pharmacological activity, low toxicity and rare complications, TCMs play important roles in clinical therapy. Recently, especially in Asia, there has been increased interest in this field (Deng et al., 2006). Cortex Moutan (Chinese name *Danpi*), dried root bark of *Paeonia moutan* Sims. (*Paeonia suffruticosa* Andrews), is an important TCM and is used in many herbal formulations (Yu et al., 2006a). Cortex Moutan (CM) is usually used to reduce body heat, cool the blood (Zhou et al., 2008), increase blood flow (Guo et al., 2003), inhibit blood clotting, drain pus and reduce swelling due to the presence of many bioactive ingredients(Yu et al., 2006b).

Recently, it was found that CM possesses anti-aggregatory (Hirai et al., 1983), radical scavenging (Ha do et al., 2009) and inhibitory activity of phenyl-hydroquinone-induced oxidative DNA cleavage (Yokoyama et al., 2007). Many physiologically active compounds are present in CM, including paeonoside, paeonolide, apiopaeonoside, paeoniflorin and paeonol (Chen, 1997). The composition and quality of herbal medicines are closely related as herbal drugs with different functions contain different active constituents (Chen et al., 2006). 2-Hydroxy-4-methoxy-acetophenone (paeonol) is one of the major active components of CM and has been shown to possess antiinflammatory properties as well as inhibitory effects on angiogenesis and tumor metastasis (Ha do et al., 2009). However, there has been no experimental study on the α-glucosidase inhibitory activity of CM extract. Interest in glucosidase inhibitors has grown recently due to their potential in the management of diabetes mellitus. About 90% of all diabetes cases worldwide are type-2 diabetes

*Corresponding author. E-mail: kimmjo@kangwon.ac.kr Tel: +82 33 250 6413. Fax: +82 33 253 6413.

Abbreviations: CM, Cortex Moutan; **DPPH,** 1,1-diphenyl-2-picrylhydrazyl hydrate; **TCM,** traditional Chinese medicine; **T2DM,** type-2 diabetes.

#These authors contributed equally to this work.

(T2DM). Specifically, T2DM is a major health problem associated with excess morbidity and mortality, resulting in substantial health-care costs. Moreover, the number of patients is estimated to increase to about 300 million by 2025 (Harris, 1998). Therefore, any new scientific discoveries featuring CM would greatly benefit treatment of diabetes and aid drug targeting.

Data from the U.S. Centers for Disease Control and Prevention show that there are 76 million cases of food-borne diseases in the U.S. each year, resulting in about 5,000 deaths (Mead et al., 1999). As a result, there has been increased interest in preservatives. In recent years, polyphenols have received a great deal of attention due to their diverse biological functions. Currently, there is growing interest in the use of natural plant extracts containing antimicrobial active compounds for the preservation of foods (Alzamora et al., 2000).

Recovery and purification of active ingredients from plant materials are typically accomplished through different extraction techniques. Solvent extraction is the most commonly used technique for the isolation of plant compounds. However, extract yields are strongly dependent on the extraction solvent used due to the presence of different compounds with various chemical characteristics and polarities which may or may not be soluble in a particular solvent (Sultana et al., 2009). Solvent polarity plays a key role in increasing the solubility of a compound. Water, along with aqueous mixtures of ethanol, methanol and acetone, are all commonly used extraction solvents. Hexane also has been extensively used to extract non-polar components from various plants and plant-based foods such as strawberry and red beet (Sun and Ho, 2005; Peschel et al., 2006). Other studies have demonstrated the efficacy of acetone and methanol as good solvent systems for the extraction of polar compounds (Luximon-Ramma et al., 2003). Loganayaki et al. (2010) found that the yield of total phenolic compounds is dependent on the choice of solvent. Specifically, the recovery of polyphenols from plant materials is influenced by the solubility of the phenolic compounds in the extraction solvent. Therefore, the choice of an effective solvent system for extraction is important. Here, five concentrations of various solvents were applied to ultrasonic extraction of CM. Thus, this study aimed to determine the effect of solvent concentration on CM extraction yield. In addition, the α-glucosidase inhibitory activity, antimicrobial activity, reducing power and 1,1-diphenyl-2-picrylhydrazyl hydrate (DPPH) radical scavenging ability of each extract were determined.

MATERIALS AND METHODS

1,1-Diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteau reagent, gallic acid, butylated hydroxyannisole (BHA), 2,6-di-*tert*-butyl-4-methylphenol (BHT), 4-nitrophenyl-α-D-glucopyranoside (*p*NPG), sodium phosphate, trichloroacetic acid, ferric chloride, gallic acid, potassium phosphate, acarbose and α-glucosidase were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Methanol, ethanol, acetone and ethyl acetate were obtained from Dae Jung chemicals and metals Co. (Seoul, Korea). All reagents were of analytical grade or better.

Extract preparation

The shade dried whole plant, was powdered and extracted with different solvents (200 ml), including aqueous methanol (60, 80 and 100% methanol are abbreviated as 60, 80 and 100 M, respectively), aqueous ethanol (60, 80 and 100% ethanol as 60, 80 and 100 E, respectively), acetone (A), water (W) and ethyl acetate (E). The extract was filtered, and three replications were extracted under the same conditions with new solvent (200 ml). The filtered extracts were mixed and concentrated using a rotary evaporator. The extract was then stored under refrigeration for further analysis.

Determination of total phenolic content

Total phenolic content was measured by the method of Folin-Ciocalteu (Singleton and Rossi, 1965). Briefly, 0.1 ml each of the samples at different concentrations was mixed with 0.05 ml of Folin-Ciocalteau reagent and then mixed thoroughly. Three minutes later, 0.3 ml of 20% sodium carbonate ($NaCO_3$) was added, followed by mixing with intermittent shaking. The reaction mixture was then incubated for 30 min at room temperature. Finally, reaction mixture before reading the absorbance was at 725 nm using a spectrophotometer (V530, Jasco Co., Japan). Measurements were performed in triplicate, and the data were expressed as gallic acid equivalent (GAE) per mg of extract, based on the calibration curve of gallic acid.

DPPH radical scavenging activity

The DPPH radical scavenging activities of the extracts were determined by the method of Blois (1958) with slight modification. Initially, 4 ml of methanol solution containing 0.1 ml each of the samples at different concentrations was mixed with 1 ml of 0.15 mM DPPH (dissolved in methanol). The reaction mixture was then incubated for 30 min at room temperature. The control contained all reagents without the sample, whereas methanol was used as a blank. All measurements were performed in triplicate. DPPH radical scavenging activity was determined by measuring the absorbance at 517 nm and expressed as the inhibition percentage of free radicals by the sample after calculation using the following formula: (%) inhibition = (absorbance of sample / 1/2 absorbance of control) × 100. The IC_{50} value (mg/ml) represents the concentration at which the scavenging activity is 50%.

Reducing power assay

Reducing power was measured according to the method reported by Oyaizu (1986) with slight modification. An aliquot of each extract (0.1 ml) was mixed with 0.5 ml of sodium phosphate buffer (0.2 M, pH 6.6) and 0.5 ml of 1% potassium ferricyanide, followed by incubation at 50°C for 20 min. After 2.5 ml of 10% trichloroacetic acid (w/v) was added, a 0.5 ml aliquot of the upper layer was mixed with distilled water (0.5 ml) and ferric chloride (0.1 ml, 0.1%), after which the absorbance was measured at 700 nm against a blank containing all of the reagents without the tested sample. A higher absorbance indicated higher reducing power. BHT and BHA were used for comparison purposes.

Table 1. The extract yield and DPPH free radical scavenging activity of Cortex Moutan extracts.

Solvent	Extract yield (%)	Total phenolic (mg GAE/g)	DPPH radical scavenging activity IC_{50} (μg/ml)
W	24.34 ± 0.29$^{d1)}$	54.95 ± 2.95^{i}	23.18 ± 0.15^{i}
E	1.72 ± 0.11^{i}	127.12 ± 1.42^{a}	19.88 ± 0.26^{a}
A	57.14 ± 0.30^{a}	114.17 ± 0.60^{b}	20.14 ± 0.16^{c}
60 E	26.28 ± 2.49^{b}	90.24 ± 4.47^{h}	20.68 ± 0.62^{e}
80 E	24.73 ± 0.39^{c}	97.95 ± 1.53^{f}	20.43 ± 0.97^{d}
100 E	6.03 ± 0.16^{h}	109.78 ± 0.97^{c}	20.13 ± 0.28^{b}
60 M	22.02 ± 3.65^{g}	103.59 ± 2.39^{e}	20.97 ± 0.34^{h}
80 M	22.62 ± 2.76^{f}	96.15 ± 2.24^{g}	20.72 ± 0.63^{f}
100 M	23.27 ± 2.71^{e}	106.01 ± 1.35^{d}	20.92 ± 0.82^{g}
BHA			4.00 ± 0.12
BHT			65.00 ± 0.11
α-Tocopherol			2.00 ± 1.50

W, Water; E, ethyl acetate; A, acetone; M, methanol; E, ethanol; BHA, butylated hydroxyannisole; BHT, 2,6-di-*tert*-butyl-4-methylphenol.
[1)] Values with the same superscript are not significantly different by Duncan's multiple range test at $p<0.05$.

Measurement of α-glucosidase activities

α-Glucosidase inhibitory activities were assayed according to a previously described procedure (Oki et al., 1999) with slight modification. The reaction was initiated with 0.05 ml each of the samples at different concentrations in 0.2 mM phosphate buffer (pH 6.8), followed by incubation at 37°C for 15 min, after which 0.05 ml of enzyme solution was immediately added to the mixture before mixing and incubation at 37°C. Then, 3 mM pNPG (0.1 ml) was added, after which the reaction was stopped by the addition of 0.75 ml of 0.1 M Na_2CO_3. α-Glucosidase inhibitory activity was determined by measuring the release of pNPG at 405 nm. The control (acarbose) contained all reagents without the tested sample. The reactions were conducted in triplicate. The α-glucosidase inhibitory activity was calculated as follows: Inhibitory ratio % = [1-(As- Ab)/ Ac] × 100, where Ac, As and Ab represent the absorbance levels of the control, sample and blank, respectively. The concentration of α-glucosidase inhibitor required to inhibit 50% of α-glucosidase activity under the assay conditions is defined as the IC_{50} value.

Minimum inhibitory concentration (MIC)

Antibacterial activities of CM extracts were tested against *Bacillus subtilis* (KTCT 1021), *Klebsiella pneumonia* (KTCT 2208), *Staphylococus aureus* (KTCT 1916) and *Escherichia coli* (KTCT 1924) using the two-fold dilution method (Ericsson and Sherris, 1971) with some modifications. Each strain was incubated in micrococcus, nutrient medium cultured at 30 and 37°C, respectively. The strains were placed into medium and incubated at each temperature for 24 h. The optical densities were determined at 600 nm using a spectrophotometer. The culturing was stopped when an absorbance of 0.4 was reached. Extracts were then dissolved in methanol to produce 10 mg/ml stock solutions. In a 96-well microassay plate, 20 μl of each stock solution was incorporated into 180 μl of medium containing *B. subtilis*, *K. pneumonia*, *S. aureus* or *E. coli*. Micrococcus medium at a concentration of 1 mg/ml was serially diluted by double technique to achieve a concentration ranging from 1,000 to 8 μg/ml. The culture plates were then incubated at 30 and 37°C for 24 h. Three replicates were performed for each treatment. At the end of each experiment, the growth of the bacteria was checked. The MIC was defined as the lowest concentration of plant extract required to inhibit bacterial growth and the MIC value was determined by eyes.

Apparatus and chromatographic conditions

The standard sample (paeonol) and CM extracts were separately dissolved in methanol and filtered (0.45 μm) for high performance liquid chromatography (HPLC). For HPLC, a Younglin liquid chromatograph (Hogye-dong, Anyang, Korea), which consisted of a pump, a column chamber, a multi-wavelength detector and a Midas for LC system, was used. Chromatographic separation was carried out at room temperature using a j'sphere ODS H80 analytical column (250 x 4.6 mm, 5μl). The mobile phase consisted of methanol (A), 0.2% phosphoric acid in water (B) and acetonitrile (C); A : B : C was as follows: 0 min, 20:80:0; 0.1 min, 20:50:30; 40 min, 100:0:0. The flow rate was 0.8 ml/min. The detector wavelength was set at 350 nm.

Statistical analysis

The data were expressed as the means ± SD of the values. Statistical significance was determined by analysis of variance (ANOVA). Duncan's multiple range tests were used to determine the significance of differences between the groups. A level of $P < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Extract yields with different extraction solvents

In this study, CM was extracted using nine different solvents, aqueous methanol (60, 80 and 100 M), aqueous

ethanol (60, 80 and 100 E), acetone (A), water (W) and ethyl acetate (E), in order to achieve maximum yield. Table 1 shows the yields of CM extraction using different solvents. The lowest extract yield was 1.72% using E significantly higher than the others. These results suggest that A extract of CM achieved maximum yield. In the present analysis, the extract yields were strongly dependent on the extraction solvent used. This is in agreement with a previous investigation by Loganayaki et al. (2010), who reported that the yield of total phenolic compounds is dependent on the solvent. The recovery of polyphenols from plant materials is influenced by the solubility of the phenolic compounds in the extraction solvent used. Other studies have also reported that the activity of extracts varies according to the solvent. It was previously shown that the t/c value of p388 is 178% when the concentration of methylene dichloride extract of *Anabaena variabilis* is 6 mg/kg; however, the t/c value becomes 133% when the concentration of butanol extract is 33 mg/kg (Patterson et al., 1984). Bonoli et al. (2004) obtained maximum yield of phenolic compounds from barley flour using mixtures of ethanol and acetone. Similarly, aqueous methanol was found to be the most effective in recovering highest amounts of phenolic compounds from rice bran.

Total phenolic content

Table 1 shows the content of total phenolic compounds of each CM extract expressed as mg of gallic acid equivalents (GAE) per g of extract. Among the nine extracts, the lowest phenolic concentration was 54.95 ± 2.59 mg of GAE/g using W extract. As expected, E extract showed the highest phenolic content. The amount of phenolic compounds in E extract was 127.12 ± 1.42 mg of GAE/g. The higher content of total phenols in E extract might explain its high reducing power and DPPH radical scavenging activity. Pietta (1998) demonstrated that the antioxidative effect is mainly due to phenolic compounds, including flavonoids, phenolic acids and phenolic diterpenes. Even further, the antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, and decomposing peroxides (Zheng and Wang, 2001). Phenolic compounds have repeatedly been implicated as natural antioxidants in fruits, vegetables and other plants. Daily dietary intake of 1 g of polyphenolic compounds from fruits and vegetables has been shown to have inhibitory effects on mutagenesis and carcinogenesis in humans (Shukla et al., 2009). Therefore, determining the phenolic content of CM will have significant implications in the food industry.

DPPH radical scavenging activity

DPPH, a stable free radical is purple in color, but changes into a stable yellow compound upon reaction with an antioxidant. It is common to measure the concentration of antioxidant required to reduce or "inhibit" 50% of the DPPH radicals present (Ramírez-Mares et al., 2010). The scavenging abilities of various extracts against DPPH radical are illustrated in Table 1, and the results are normalized and expressed as IC_{50} values (mg/ml) for comparison purposes. In our experiments, all of the extracts tested possessed DPPH radical scavenging activity. The E extract was the most active DPPH radical scavenger, whereas the lowest activity was found in W extract. Overall, the DPPH radical scavenging activities of all the extracts were superior to that of the positive control, BHT. Extracts obtained with different solvents varied in their DPPH radical scavenging activities, indicating that the extraction medium significantly affected the types of antioxidants in the extracts. In conclusion, the most active DPPH radical scavenger was E extract, possibly due to a higher content of total phenolic compounds. This observation is consistent with a report that phenolic compounds contribute directly to the anti-oxidative action of CM extract (Duh et al., 1999; Lin and Tang, 2007; Shen et al., 2009).

Reducing power assay

A simple reducing power assay, which measured the conversion of iron (II) to iron (I), was conducted to assess the "antioxidant power" of the extracts (Duan et al., 2007; Wang et al., 2008). All extracts were measured spectro-photometrically by reading the absorbance at 700 nm. A higher absorbance means that the reaction mixture had great reducing power. As shown in Figure 1, the extracts were tested at concentrations of 0.3, 0.5 and 0.7 mg/ml, and all samples showed some degree of reducing power. However, reducing power increased with increasing concentration for all the extracts. In addition, E extract exhibited slightly higher absorbance values as compared to the other extracts. The earlier findings indicate that reducing power was associated with antioxidant activity. This conclusion is in agreement with an early report by Pitotti et al. (1994), who found that the antioxidative effect of Maillard reaction products corresponded with reducing power.

α-Glucosidase activities

Recently, Lee et al. (2008) reported that natural products with wide structural diversity are a good source of α-glucosidase inhibitors. Vetrichelvan and Jegadeesan (2002) noted that certain Sri Lanka plants suppress glucose production from dietary sugars by inhibiting α-glucosidase activity. Thus, it is necessary to determine the α-glucosidase inhibitory activity of CM. In this study, to determine the effects of the CM extracts on α-glucosidase activity, we calculated the IC_{50} values, as

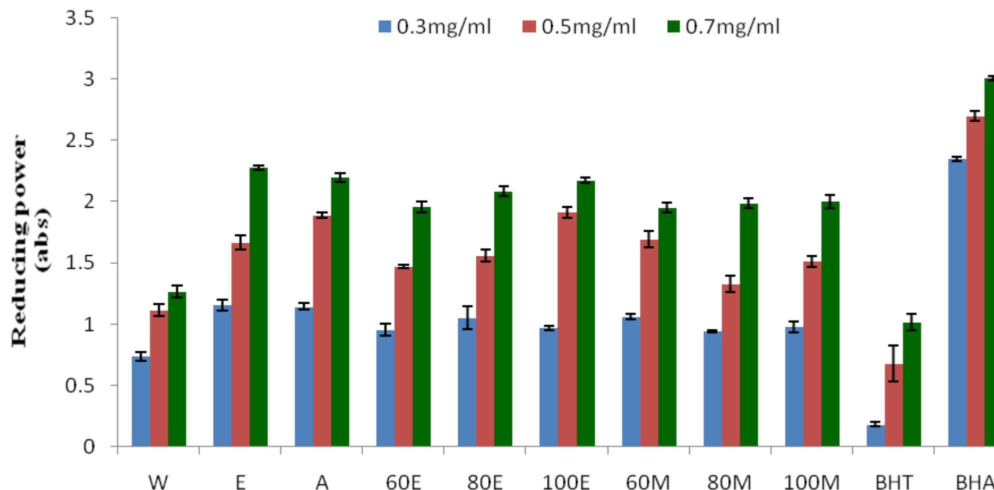

Figure 1. Reducing power activities of Cortex Moutan extracts.

Table 2. *α*-Glucosidase inhibitory activity of Cortex Moutan extracts.

Extract	IC_{50}(μg/ml)
W	10.69 ± 0.91[i1)]
E	6.33 ± 0.18[a]
A	8.56 ± 0.24[g]
60 E	8.37 ± 0.51[f]
80 E	7.38 ± 0.47[c]
100 E	9.04 ± 1.10[h]
60 M	8.22 ± 0.77[e]
80 M	7.02 ± 0.36[b]
100 M	7.95 ± 1.21[d]
Acarbose	3.00 ± 0.21

[1)]Values with the same superscript are not significantly different by Duncan's multiple range test at p<0.05.

shown in Table 2. All of the extracts seemed to have potent inhibitory activities towards α-glucosidase. However, the α-glucosidase inhibitory activities of the different extracts varied greatly. The E extract had a higher IC_{50} value (6.33 μg/ml) as compared to the other extracts. On the other hand, W extract had the lowest IC_{50} value (10.69 μg/ml). This maybe due to the fact that E extract contained more effective α-glucosidase inhibitory compounds, including polyphenols. Matsui et al. (2001) and McDougall et al. (2005) previously found that polyphenol-rich extracts of soft fruits (blueberry and blackcurrant), potato (*Ipomoea batatas* L.) roots and Morning glory (*Pharbitis nil* cv. Scarlett O'Hara) exhibit α-glucosidase activity. Acarbose, which is known to be an effective microbial α-glucosidase inhibitor, is used for diabetes therapy. Therefore, the α-glucosidase inhibitory activity of acarbose (IC_{50} = 3 μg/ml) was better than those of the extracts.

Antibacterial activities

We examined the antibacterial effects of CM extracts. As shown in Table 3, the E extract demonstrated the most effective inhibition against *E. coli* (MIC value of 100 μg/ml) as compared to the other extracts. The MIC value of A extract was 500 μg/ml for *E. coli* and 1000 μg/ml for *S. aureus*. The 60, 80 and 100 E, and 60, 80 and 100 M extracts had MIC values of 1000 μg/ml for *E. coli* and *S. aureus*. On the other hand, the MIC values of W extract for *K. pneumonia*, *E. coli*, *S. aureus* and *B. subtilis*, as well as those of the other extracts for *K. pneumonia* and

Table 3. Anti-microbial activity of Cortex Moutan extracts.

Extract	Bacteria MIC(μg/ml)			
	K.p **(-)**	***E.c*** **(-)**	***S.a*** **(+)**	***B.s*** **(+)**
W	>1000	>1000	>1000	>1000
E	>1000	100	1000	>1000
A	>1000	500	1000	>1000
60E	>1000	1000	1000	>1000
80E	>1000	1000	1000	>1000
100E	>1000	1000	1000	>1000
60M	>1000	1000	1000	>1000
80M	>1000	1000	1000	>1000
100M	>1000	1000	500	>1000
Tetracycline	16	8	8	8

B.s, Bacillus subtilis KTCT 1021; *K.p, Klebsiella pneumonia* KCTC 2208; *S.a, Staphylococus aureus* KCTC 1916*; E.c Escherichia coli* KTCT 1924.

Table 4. Paeonol concentration of Cortex Moutan extracts.

Extract	Paeonol (μg/ml)
W	10.21 ± 0.00[i]
E	60.69 ± 0.00[a]
A	57.79 ± 0.01[b]
60 E	24.58 ± 0.02[e]
80 E	22.48 ± 0.02[g]
100 E	44.07 ± 0.00[d]
60 M	50.82 ± 0.00[c]
80 M	21.62 ± 0.02[h]
100 M	23.63 ± 0.02[f]

B. subtilis only, were all higher than 1000 μg/ml. Among the bacterial strains used in this study, *E. coli* and *S. aureus* were the most sensitive; this maybe due to the solvents containing a particular active compound that inhibited both *E. coli* and *S. aureus*. From ancient to modern times, many cultures have relied on plants and herbs as a main source of drugs to treat human and animal diseases. It is well known that plants produce certain bioactive molecules that react with other organisms and inhibit bacterial or fungal growth (Ogbonnia et al., 2008). Zheng and Zhu (2003) found that the possible mechanisms for antimicrobial activity were: (1) the chitosan on the surface of the cell can form a polymer membrane, which prevents nutrients from entering the cell; (2) the chitosan entered the cell through pervasion. Since it could absorb the electronegative substance in the cell and flocculate them, it disturbs the physiological activities of the bacteria and kill them. The fact that the CM extracts in this study exhibited inhibitory activities against certain bacteria provides scientific basis for the further study of this plant.

HPLC analysis

To obtain a calibration curve, HPLC analysis of the calibration solutions ranged from 0.1 to 100 μg/ml. The retention time of paeonol was 23.426 min, and three replications for each solution were performed. According to the calibration curve, the concentration of paeonol in each CM extract was calculated, and the analytical results are listed in Table 4. The data show that the paeonol concentration in each CM extract was different. The paeonol concentration in E extract was the highest (60.69 μg/ml), followed by A extract (57.79 μg/ml). The W extract had the lowest paeonol concentration in all the extracts (10.21 μg/ml). This maybe due to the variation in the solubility of paeonol according to the solvent used. Paeonol is one of the major active components of CM and plays a key role in the treatment of diseases based on its analgesic (Sun et al., 2000), antipyretic, antiinflammatory (Lee et al., 2008) and antibacterial effects (Yu et al., 2006a). Further, it can be used as pharmaceutical material for the treatment of myalgia, rheumatic pain and neuralgia (Kim et al., 2004; Chou, 2003). Lastly, it was demonstrated in our previous studies that E extract exhibits the effective inhibition of *E. coli* (MIC value of 250 ppm).

Conclusion

In this study, the antioxidative, antibacterial and α-glucosidase inhibitory activities of different CM extracts were investigated. The E extract showed high DPPH radical scavenging activity and reducing power comparable to the positive control, BHT. The α-glucosidase inhibitory activity of E extract was very high, with an IC_{50} value of 6.33 ± 0.18 μg/ml. In addition, E extract exhibited most effective inhibition against *E. coli* (MIC value of 100 μg/ml) as compared to the other extracts and the highest

paeonol concentration. Further work on the individual compounds of E extract would be worthwhile.

REFERENCES

Alzamora SM, López-Malo A, et Tapia de Daza MS (2000). Overview. In Minimally processed fruits and vegetables-fundamental aspects and applications. Eds. Alzamora SM, Tapia MS, López-Malo A. Aspen Publishers, Inc. Gaithersburg, MD, USA.

Blois MS (1958). Antioxidant determinations by the use of a stable free radical. J pn. J. Nature, 181: 1199-1200.

Bonoli M, Verardo V, Marconi E, Caboni MF (2004). Antioxidant phenols in barley (*Hordeum vulgare* L.) flour: comparative spectrophotometric study among extraction methods of free and bound phenolic acids. J. Agric. Food Chem. 52: 5195-5200.

Chen FQ (1997). Determination of the content of the active constituents in commonly used traditional Chinese medicines. PMPH press. Beijing, CHN.

Chen G, Zhang L, Zhu Y (2006). Determination of glycosides and sugars in Moutan Cortex by capillary electrophoresis with electrochemical detection. J. Pharmaceut. Biomed. 41: 129-134.

Chou TC (2003). Anti-inflammatory and analgesic effects of paeonol in carrageenan-evoked thermal hyperalgesia. Br. J. Pharmacol. 139: 1146-1152.

Deng CH, Yao N, Wang B, Zhang XM (2006). Development of microwave-assisted extraction followed by headspace single-drop microextraction for fast determination of paeonol. traditional Chinese med. J. Chromatogr. A. 1103: 15-21.

Duan X, Wu G, Jiang Y (2007). Evaluation of antioxidant properties of phenolic from litchi fruit in relation to pericarp browning prevention. Molecule, 12: 759-771.

Duh PD, Tu YY, Yen GC (1999). Antioxidative activity of water extracts of Hamg jyur (*Chrysanthemum morifolium*). LWT- Food Sci. Technol. 32: 269-277.

Ericsson HM, Sherris JC (1971). Antibiotic sensitivity testing-report of an international collaborative study. Acta Pathol. Microbiol. Scand. 217: 1-90.

Guo XL, Meng Q , Feng YF (2003). Study on quality standard of the extracts in *Paeonia suffruticosa* Andr. by supercritical CO_2 fluid extraction (SFE). Chin. J. Guangdong College Pharm. 11: 81-88.

Ha do T, Tuan DT, Thu NB, Nhiem NX, Ngoc TM, Yim NH, Bae K (2009). Palbinone and triterpenes from Moutan Cortex (*Paeonia suffruticosa*, Paeoniaceae) stimulate glucose uptake and glycogen synthesis via activation of AMPK in sulin-resistant human HepG2 Cells. Bioorg. Med. Chem. Lett. 19: 5556-5559.

Harris MI (1998). Diabetes in America: Epidemiology and scope of the problem.Diabetes Care, 21: 11-14.

Hirai A, Terano T, Hamazaki T, Sajiki J, Saito H, Tahara K, Tamura Y, Kumagai A (1983). Studies on the mechanism of antiaggregatory effect of moutan cortex. Thromb. Res. 31: 29-40.

Kim HK, Tak JH, Ahn YJ (2004). Acaricidal activity of *paeonia suffruticosa* root bark-derived compounds against dermatophagoides farinae and dermatophagoides pteronyssinus. J. Agric. Food Chem. 52: 7857-7861.

Lee B, Shin YW, Bae EA, Han SJ, Kim JS, Kang SS, Kim DH (2008). Antiallergic effect of the root of *Paeonia lactiflora* and its constituents paeoniflorin and paeonol. Arch. Pharmacol. Res. 31: 445-450.

Lin JY, Tang CY (2007). Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. Food Chem. 101: 140-147.

Loganayaki N, Rajendrakumaran D, Manian S (2010). Antioxidant capacity and phenolic content of different solvent extracts from banana (*Musa paradisiaca*) and Mustai (*Rivea hypocrateriformis*). Food Sci. Biotechnol. 19: 1251-1258.

Luximon-Ramma A, Bahorun T, Crozier A (2003). Antioxidant actions and phenolic and vitamin C contents of common Mauritian exotic fruits. J. Sci. Food Agric. 83: 496-502.

Matsui T, Ueda T, Oki T, Sugita K, Terahara N, Matsumoto K (2001). α-Glucosidase inhibitory action of natural acylated anthocyanins. 1. Survey of natural pigments with potent inhibitory activity. J. Agric. Food Chem. 49: 1948-1951.

McDougall GJ, Shpiro F, Dobson P, Smith P, Blake A, Stewart D (2005). Different polyphenolic components of soft fruits inhibit α-amylase and α-glucosidase. J. Agric. Food Chem. 53: 2760-2766.

Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JF, Shapiro C, Griffin PM, Tauxe RV (1999). Food-related illness and death in the United States. Emerg. Infect. Dis. 5: 607-625.

Ogbonnia SO, Enwuru NV, Onyemenem EU, Oyedele GA, Enwuru CA (2008). Phytochemical evaluation and antibacterial profil of *Treculia africana* Decne bark extract on gastrointestinal bacterial pathogens. Afr. J. Biotechnol. 7: 1385-1389.

Oki T, Matsui T, Osajima Y (1999). Inhibitory effect of α-glucosidase inhibitors varies according to its origin. J. Agric. Food Chem. 47: 550-553.

Oyaizu M. (1986). Studies on products of browning reactions: Antioxidative activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr. 44: 307-315.

Patterson GML, Norton TR, Furusawa E, Furusawa S, Kashiwagi M, Moore E (1984). Antineoplastic evaluation of marine algal extracts. J. Bot. Mar. 27: 485-487.

Peschel W, Sanchez-Rabaneda F, Diekmann W, Plescher A, Gartzia I, Jimenez D, Lamuela-Raventos R, Buxaderas S, Condina C (2006). An industrial approach in the search of natural antioxidants from vegetable and fruit wastes. Food Chem. 97: 137-150.

Pietta P (1998) Flavonoids in medicinal plants. In *Flavonoids in health and disease*. Eds. Rice-Evans CA, Packer L, Marcel Dekker, New York, USA.

Pitotti A, Elizalde BE, Anese M (1994). Effect of caramelization and Maillard reaction products on peroxidase activity. J. Food Biochem. 18: 445-457.

Ramirez-Mares MV, Sanchez-Burgos JA, Hernandez-Carlos B (2010). Antioxidant, antimicrobial and antitopoisomerase screening of the stem bark extracts of *ardisia compressa*. Pak. J. Nutr. 9: 307-313.

Shen Y, Jin L, Xiao P, Lu Y, Bao J (2009). Total phenolics, flavonoids, antioxidant capacity in rice grain and their relations to grain color, size and weight. J. Cereal Sci. 49: 106-111.

Shukla S, Mehta A, John J, Singh S, Mehta P, Prasad Vyas S (2009). Antioxidant activity and total phenolic content of ethanolic extract of *Caesalpinia bonducella* seeds. Food Chem. Toxicol. 47: 1848-1851.

Singleton VL, Rossi JAJ (1965). Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagents. Am. J. Enol. Vitic. 16: 144-158.

Sultana B, Anwar F, Ashraf M (2009). Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecule, 14: 2167-2180.

Sun HJ, Wang XQ, Yu LM (2000). The study of paeonol for MDR's reverse action. Chin. J. Prog. Anatomi. Sci. 1: 15-17.

Sun T, Ho C (2005). Antioxidant activities of buckwheat extracts. Food Chem. 90: 743-749.

Vetrichelvan T, Jegadeesan M (2002). Anti-diabetic activity of alcoholic extract of *AerVa lanata* (L.) Juss. Ex Schultes in rats. J. Ethnopharmacol. 80: 103-107.

Wang H, Gao XD, Zhou GC, Cai L, Yao WB (2008). *In vitro* and *in vivo* antioxidant activity of aqueous extract from *Choerospondias axillaris* fruit. Food Chem. 106: 888-895.

Yokoyama Y, Nohara K, Okubo T, Kano I, Akagawa K, Kano K (2007). Generation of reactive oxygen species is an early event in dolichyl phosphate-induced apoptosis. J. Cell Biochem. 23: 199-203.

Yu K, Wang YW, Cheng YY (2006a). Determination of paeonol and paeoniflorin in Chinese medicine Cortex Moutan and ‘Shuangdan’ granule by micellar electrokinetic capillary chromatography. J. Pharm. Biomed. Anal. 40: 1257-1262.

Yu K, Wang YW, Cheng YY (2006b). Determination of the active components in chinese herb cortex moutan by MEKC and LC. Chromatographia, 63: 359-364.

Zheng YL, Zhu JF (2003). Study on antimicrobial activity of chitosan with different molecular weights. Carbohydr. Polym. 54 : 527-530.

Zheng W, Wang SY (2001). Antioxidant activity and phenolic compounds in selected herbs. J. Agric. Food Chem. 49: 5165-5170.

Zhou LY, Wang LS, Wang SM, Wu MX, Chen CY (2008). Studies on the pharmacodynamic fingerprint of Cortex Moutan. Chin. J. Lishizhen Med. Mater. Med. Res. 20: 65-71.