African Journal of Biotechnology Vol. 11(19), pp. 4463-4466, 6 March, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.1549
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Three novel antioxidants from *Cinnamomum* plants

Kuo-Chen Cheng[1], Hsi-An Chen [2], Pei-Fang Wu[3], Woei-Ling Yang[4], Hui-Min Wang[3]* and Chung-Yi Chen[4]*

[1]Department of Intensive Care Medicine, Chi-Mei Medical Centre, Tainan 710, Taiwan, ROC.
[2]School of Pharmacy, College of Pharmacy, Kaohsiung Medical University, Kaohsiung 807, Taiwan, ROC.
[3]Department of Fragrance and Cosmetic Science, Kaohsiung Medical University, Kaohsiung 807, Taiwan, ROC.
[4]School of Medical and Heath Science, Fooyin University, Ta-Liao, Kaohsiung 831, Taiwan, ROC.

Accepted 1 December, 2011

In this study, we identified three novel antioxidants, subamolide C (1), subamolide E (2) and isokotomolide A (3) from the constitutes of *Cinnamomum subavenium* and *Cinnamomum kotoense*. The radical scavenging properties and two more antioxidant activities of the three compounds were examined. All compounds presented effective 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging capabilities compared with vitamin C; the metal chelating power evaluated to ethylene diamine tetra-acetic acid (EDTA) and the reducing power judged from 3-tert-butyl-4- hydroxyanisole (BHA). The data proposed that the bio-components from *C. subavenium* and *C. kotoense* act as natural antioxidants and play a potential role in cancer prevention. Meanwhile, the pure constituents will be used as leading compounds in the developments of useful cosmetic ingredients or future human disease therapies.

Key words: *Cinnamomum subavenium*, *Cinnamomum kotoense*, subamolide C, subamolide E, isokotomolide A, antioxidant.

INTRODUCTION

There are many reports related to natural components that have great advantages in the preventions, treatments and therapies of many human diseases, including cancer, neuropathy, inflammation and age-related problems (Surh, 2003; Rahman, 2007; Amin et al., 2009; Chen et al., 2011). Anti-oxidative components are of importance due to their abilities to reduce free radical-mediated degradation of cell tissues and organisms in humans (Mourtzinos et al., 2008; Hsieh et al., 2008). Therefore, antioxidants from plant species which reduce oxidative stress from intrinsic and external sources have huge applications and functions in human health care (Gülçin et al., 2001; Sundararajan et al., 2006; Furiga et al., 2008; Almajano et al., 2008; Pavithra et al., 2009; Wang et al., 2010).

There are many diet sources to decrease oxidative stress, including cereals, fruits, grains, legumes, oats, vegetables and herbal medicines (Rao et al., 2010; Chen et al., 2010; Wang et al., 2011). The *Cinnamomum* species (Lauraceae) have been used in folk medicine for sweating, antipyretic and analgesic effect. *Cinnamomum subavenium* Miq. (Lauraceae) is a medium-sized evergreen tree found in central to southern mainland China, Burma, Cambodia, Taiwan, Malaysia and Indonesia (Liao, 1996). *Cinnamomum kotoense* Kanehira and Sasaki (Lauraceae) is a small evergreen tree, endemic to Lanyu Island of Taiwan, and has recently been cultivated as an ornamental plant (Liao, 1996).

In this study, subamolide C (1), subamolide E (2) and isokotomolide A (3) of a collection of dozens of pure components isolated from these two plants were used for screening their antioxidative activities. Previous studies have shown 1 and 2 as effective in the inhibition of human colorectal cancer SW480 cells (Kuo et al., 2008; Chen et al., 2006). Isokotomolide A (3) is also known to exhibit an anti-proliferative activity in human non-small cell lung cancer A549 cells (Chen et al., 2007). Hence, we evaluated their protective effects of antioxidation which has been reported to be linked with human disease, including

*Corresponding author. E-mail: xx377@mail.fy.edu.tw. Tel: +886-7-7811151-6200. Fax: +886-7-7863667

cardiopathy and cancer.

MATERIALS AND METHODS

Reagents and materials

The leaves and stems of *C. subavenium* were collected from Wulai Hsiang, Taipei County, Taiwan in May, 2005, while the leaves of *C. kotoense* were collected from Fooyin University, Kaohsiung County, Taiwan, in May 2004 (Liao, 1996).

3-*tert*-butyl-4- hydroxyanisole (BHA), dimethyl sulfoxide (DMSO), 1,1-diphenyl-2-picrylhydrazyl (DPPH), ethylene diamine tetra-acetic acid (EDTA), ferrozine, $FeCl_3$, $FeCl_2 \cdot 4H_2O$, MTT, peroxidase type I from horseradish (HRPase), potassium ferricyanide [$K_3Fe(CN)_6$], trichloroacetic acid, vitamin C, were purchased from Sigma Chemical (St. Louis, MO). All buffers and other reagents were of the highest purity commercially available (Lu and Foo., 1997).

Purification of active ingredients from the *C. subavenium* and *C. kotoense*

Subamolide C was isolated from the air-dried stems of *C. subavenium* as previously described (Chen et al., 2007). Briefly, the air-dried stems were extracted with methanol (MeOH) at room temperature, and the MeOH extract was obtained upon concentration under reduced pressure. The MeOH extract, suspended in H_2O, was partitioned with $CHCl_3$ to give fractions soluble in $CHCl_3$ and H_2O. The $CHCl_3$ soluble fraction was subjected to chromatograph over silica gel using *n*-hexane-EtOAc-MeOH as eluent to yield five fractions. Fraction 1-1-2 was re-subjected to silica gel CC and purified by preparative TLC using *n*-hexane-EtOAc to yield subamolide C. The air-dried leaves of *C. subavenium* and *C. kotoense* were extracted with similar methods to obtain subamolide E (Kuo et al., 2008) and isokotomolide A (Chen et al., 2006), respectively. Through the initial screening using 100 µM of each compound, we identified the potential antioxidants.

Determination of DPPH· radical scavenging capacity

DPPH· is a stable free radial with violet color (absorbed at 517 nm) (Wang et al., 2010). DPPH· will change its color to light yellow when free radials are scavenged. The various concentrations of the three compounds were added to 0.1 ml of stable DPPH· (60 µM) solution. When DPPH· reacts with antioxidant compound donating hydrogen, it is reduced, resulting in a decrease in absorbance at 517 nm. The analyzed time interval was 10 min per point, up to 30 min by using UV–vis spectrophotometer (BioTek Co.). Vitamin C was used as a positive control. The DPPH· radical scavenging activity (%) was determined as; $100 \times (A_{control} - A_{sample}) / A_{control}$. In addition, IC_{50} values were evaluated by KaleidaGraph 4.0 statistical software.

Metal chelating activity

The ferrous ion chelating potential of the three compounds from *C. subavenium* and *C. kotoense* were investigated according to a previously described method (Wang et al., 2010). Briefly, various testing concentrations of samples dissolved in DMSO were added to a solution of 2 mM $FeCl_2 \cdot 4H_2O$ (0.01 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.02 ml), and the mixture was vigorously shaken and left standing at room temperature for 10 min. The absorbance of the mixture was then read at 562 nm against a blank. EDTA was used as a positive control.

Reducing power

The reducing powers of our natural pure compounds were determined according to the method of Wang et al. (2010). In brief, testing compounds were mixed with 0.085 ml of 67 mM phosphate buffer (pH 6.8) and 2.5 µL of 20% $K_3Fe(CN)_6$. The mixture was incubated at 50°C for 20 min then 0.16 ml of trichloroacetic acid (10%) was then added to the mixture and centrifuged for 10 min at 3,000 × g. The upper layer of the solution (75 µL) was mixed with 2% $FeCl_3$ (25 µL), and the absorbance was measured with a 96-well plate spectrophotometer at 700 nm. BHA was used as a positive control. A higher absorbance demonstrates a higher reductive capability.

Statistical analysis

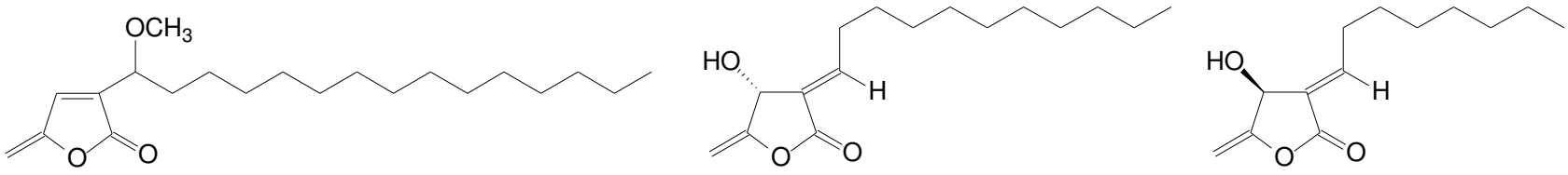
All results were expressed as the mean values. Statistical comparisons were carried out using the Student's t-test for paired values.

RESULTS AND DISCUSSION

To examine the anti-oxidative activities of subamolide C (1), subamolide E (2) and isokotomolide A (3) (Figure 1), we applied multiple antioxidant property assays for identification (Wang et al., 2010). First, we used the antioxidant methodology: DPPH free radical scavenging assays to measure the values of IC_{50} (the inhibitory concentration 50%) of three compounds. The reactions of free proton-radical scavenging are identified as significant mechanisms to reduce excess oxidations. As shown in Table 1, with DPPH· scavenging, IC_{50} values were 105.2 ± 8.3, ≥ 200 and ≥ 200 µM, respectively. The various proton-donating abilities could serve as free radical inhibitors or scavengers. Subamolide C was the most powerful antioxidant of the three in the bioassay.

Furthermore, we verified the ferrous ion chelating abilities of three compounds (Table 1). The reagent used was ferrozine, which formed complexes with Fe(II), and the dark red color was quantified by spectroscopy. EDTA was used as a positive control. All compounds presented chelating properties, thus acting as potential antioxidants. However, subamolide C had the highest chelating ability in this evaluation, with IC_{50} = 125.2 ± 6.8 µM, while the other two IC_{50} values were 162.9 ± 7.1 and 186.5 ± 4.8 µM, respectively.

The reducing capacity of natural compounds may serve as one of the significant indicators because of the potential antioxidant activity. Finally, we investigated the Fe(III)-Fe(II) transition to measure the reducing capacity of these three pure compounds (Table 1). The existence of reductants would result in the reduction of Fe(III)/ferricyanide complex to its ferrous form, which can be monitored spectroscopically. The colors of the solutions changed from their original yellow color to various shades ranging from dark green to blue, depending on the reducing powers of the antioxidants tested. In Table 1, the reducing power of compound 1 exhibited



Subamolide C (1) Subamolide E (2) Isokotomolide A (3)

Figure 1. Chemical Structures of the three compounds from *Cinnamomum* plants (1 to 3).

Table 1. Antioxidant properties of subamolide C, subamolide E and isokotomolide A on DPPH free radical scavenging, ferrous ion chelating and reducing power ability assays.

Sample	DPPH˙ scavenging, IC_{50} (µM)	Chelating inhibition, IC_{50} (µM)	Reducing power, 100 µM (OD_{700})
Subamolide C (1)	105.2 ± 4.3	125.2 ± 6.8	0.40
Subamolide E (2)	≥ 200	162.9 ± 7.1	0.25
Isokotomolide A (3)	≥ 200	186.5 ± 4.8	0.20
Vitamin C	43.1	-	-
EDTA	-	50.6	-
BHA	-	-	0.57

Data were expressed as a mean value of three independent experiments. Vitamin C, positive control on both assays; EDTA, positive control on chelating inhibition; BHA, positive control on reducing power at 100 µM. EDTA, ethylene diamine tetra-acetic acid; BHA, 3-tert-butyl-4-hydroxyanisole.

high level potentials compared to the other two compounds and BHA at the same doses.

Conclusion

In our research, free radical scavenging capacity and antioxidant activities of compounds one to three were assayed effectively by three *in vitro* antioxidant evaluation systems; DPPH radical scavenging activity, ion chelating ability and ferric reducing power capacity. The results presented clearly show that the three compounds from *C. subavenium* and *C. kotoense* efficiently revealed antioxidant activities. These compounds could therefore be used as easily accessible sources of natural antioxidants for cosmetics and food supplements against oxidative deterioration to prevent carcinogenesis or other diseases (Chen et al., 2005; Chen, 2006).

REFERENCES

Almajano MP, Carbo R, Jimenez JAL, Gordon MH (2008). Antioxidant and antimicrobial activities of tea infusions. Food Chem. 108: 55-63.

Amin AR, Kucuk O, Khuri FR, Shin DM (2009). Perspectives for cancer prevention with natural compounds. J. Clin. Oncol. 27: 2712-2725.

Chen BH, Chang HW, Huang HM, Chong IW, Chen JS, Chen CY, Wang HM (2011). (-)-Anonaine induces oxidative stress and DNA damage to inhibit growth and migration of human lung carcinoma H1299 cells. J. Agric. Food Chem. 59: 2284-2290.

Chen CH, Lo WL, Liu YC, Chen CY (2006). Chemical and cytotoxic constituents from the leaves of *Cinnamomum kotoense*. J. Nat. Prod. 69: 927-933.

Chen CY (2006). Butanolides from the stems of *Cinnamomum kotoense*. Nat. Prod. Commun. 1: 453-455.

Chen CY, Chen CH, Wong CH, Liu YW, Lin YS, Wang YD, Hsui YR (2007). Cytotoxic constituents of the stems of *Cinnamomum subavenium*. J. Nat. Prod. 70: 103-106.

Chen CY, Kuo PL, Chen YH, Huang JC, Ho ML, Lin RJ, Chang JS, Wang HM (2010). Tyrosinase inhibition, free radical scavenging, antimicrobial and anticancer proliferation activities of *Sapindus mukorossi* extracts. J. Taiwan Inst. Chem. E. 41: 129-135.

Chen FC, Peng CF, Tsai IL, Chen IS (2005). Antitubercular constituents from the stem wood of *Cinnamomum kotoense*. J. Nat. Prod. 68: 1318-1323.

Furiga A, Funel AL, Badet C (2008). In vitro study of antioxidant capacity and antibacterial activity on oral anaerobes of a grape seed extract. Food Chem. 113: 1037-1040.

Gülçin I, Elmastas M, Aboul-Enein HY. (2001) Determination of antioxidant and radical scavenging activity of Basil (*Ocimum basilicum* L. Family Lamiaceae). Phytother. Res. 21: 354-361.
Hsieh MC, Shen YJ, Kuo YH, Hwang LS (2008). Antioxidative activity and active components of longan (Dimocarpus longan Lour.) flower extracts. J. Agric. Food Chem. 56: 7010-7016.
Kuo SY, Hsieh TJ, Wang YD, Lo WL, Hsui YR, Chen CY (2008). Cytotoxic constituents from the leaves of *Cinnamomum subavenium*. Chem. Pharm. Bull. 56: 97-101.
Liao JC (1996). Lauraceae in Flora of Taiwan, 2nd ed. Editorial Committee of the Flora of Taiwan: Taipei, 2: 443-483.
Lu Y, Foo LY (1997). Identification and quantification of major polyphenols in apple pomace. Food Chem. 59: 187-194.
Mourtzinos I, Konteles S, Kalogeropoulos N, Karathanos VT (2008). Thermal oxidation of vanillin affects its antioxidant and antimicrobial properties. Food Chem. 114: 791-797.
Pavithra PS, Sreevidya N, Verma RS (2009). Antibacterial and antioxidant activity of methanol extract of Evolvulus nummularius. Indian J. Pharmacol. 41: 233-236.
Rahman K (2007). Studies on free radicals, antioxidants, and co-factors. Clin. Interv. Aging, 2: 219-236.
Rao AS, Reddy SG, Babu PP, Reddy AR (2010). The antioxidant and antiproliferative activities of methanolic extracts from Njavara rice bran. BMC Complement Altern. Med. 10: 4.
Sundararajan R, Haja NA, Venkatesan K, Mukherjee K, Saha BP, Bandyopadhyay A, Mukherjee PK (2006). Cytisus scoparius link-A natural antioxidant. BMC Complement Altern. Med. 6: 8.
Surh YJ (2003). Cancer chemoprevention with dietary phytochemicals. Nat. Rev. Cancer, 3: 768-780.
Wang HM, Chen CY, Chen CY, Ho ML, Chou YT, Chang HC, Lee CH, Wang CZ, Chu IM (2010). (-)-*N*-Formylanonaine from *Michelia alba* as human tyrosinase inhibitor and antioxidant. Bioorg. Med. Chem. 18: 5241-5247.
Wang HM, Chou YT, Hong ZL, Chen HA, Chang YC, Yang WL, Chang HC, Mai CT, Chen CY (2011). Bioconstituents from stems of *Synsepalum dulcificum* Daniell (Sapotaceae) inhibit human melanoma proliferation, reduce mushroom tyrosinase activity and to be antioxidants. J. Taiwan Inst. Chem. E. 42: 204-211.
Wang HM, Pan JL, Chiu CC, Chen CY, Yang MH, Chang JS (2010). Identification of anti-lung cancer extract from *Chlorella vulgaris* C-C by antioxidant property using supercritical carbon dioxide extraction. Process Biochem. 45: 1865-1872.