

The Antioxidativity and Chemical Components of Coleus blumei

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ABSTRACT

In this study, antioxidant activities of extracts from stems and leaves of *Coleus blumei* were examined with different extraction methods. The extraction was associated with either a cold water press or a hot water reflux. In addition, antioxidant activities of extracts were compared using three solvents (n-hexane, ethyl acetate, and water) for extraction of *Coleus blumei*. The antioxidative activities assays, including DPPH radical scavenging ability, Fe^{2+} chelating power, reducing power, superoxide radical anion scavenging ability, ABTS cation scavenging ability, and the inhibition of $\text{Fe}/\text{ascorbate}$ - induced lipid peroxidation in a liposome model system, were measured and compared with those of butylated hydroxyanisole (BHA), ethylene diamine tetraacetic acid (EDTA), α -tocopherol and gallic acid.

The results showed that the extraction rate of leaves (2.23%) is higher than stems (1.75%) when *Coleus blumei* was extracted using the cold water press. The leaf extract had higher contents of total flavonoids and total phenols (1.45 and 4.49 mg/g, respectively) and better antioxidant activities except the Fe^{2+} chelating ability. The IC_{50} of the leaf extract is $0.08 \pm 0.00 \text{ mg/mL}$ for the DPPH radical scavenging ability, $7.55 \pm 0.53 \text{ mg/mL}$ for the relative reducing power, and $0.19 \pm 0.02 \text{ mg/mL}$ for the ABTS cation scavenging ability. The stem extract of *Coleus blumei* had better the Fe^{2+} chelating ability, and its IC_{50} is $0.18 \pm 0.00 \text{ mg/mL}$. In addition, the extracts obtained from the hot water reflux had better antioxidant activities than those from the cold water press.

Extracts from the hot water reflux were further separated by distribution extraction using three solvents including n-hexane, ethyl acetate, and water. The extraction rate of the water extract of leaves (7.36%) is higher than that of stems. The ethyl acetate extract of leaves has the highest DPPH radical scavenging ability, and its IC_{50} is $0.01 \pm 0.00 \text{ mg/mL}$. The ethyl acetate extracts of leaves and stems were analyzed using an HPLC to examine the components in the extracts. The result showed that rosmarinic acid is one of the major components, and its content in leaves (383.7 mg/g) is higher than that in stems. Further analysis by FTIR showed that the ethyl acetate extracts of leaves and stems have absorption of bonds including C—O, C = C, C = O, and O—H, and this fact confirmed that rosmarinic acid indeed exists in the extracts. In the analysis of HPLC/MS/MS, the relative content of m/z 359.1 is the highest, after compared with literature and this fact confirmed the existence of rosmarinic acid.

In summary, the antioxidant activities of extracts from the hot water reflux are higher than those of extracts from the cold water press, and the antioxidant activities of extracts from leaves of *Coleus blumei* are higher than those from stems. The analyses confirmed that the antioxidant components is rosmarinic acid.

Keywords : *Coleus blumei*, antioxidant, rosmarinic acid

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REFERENCES

- 朱惠麗。2004。紫蘇中迭香酸的提取及紫蘇萃取物抗氧化研究。天津科技大學碩士論文。天津。
- 李維鋒。2008。余甘子樹皮中植物多酚的分離純化、鑑定及活性研究。華南理工大學碩士學位論文。廣州。
- 林天坤。2003。光激化學氣象沈積二氧化矽屬於氮化鋁鎵/氮化鎂金氧半異質結構場效電晶體。雲林科技大學電子與資訊工程研究所碩士論文。雲林。
- 易博。2007。丹參迷迭香酸代謝酪氨酸支路重要基因克隆及調控分析。第二軍醫大學碩士學位論文。上海。
- 林富陽。2006。辣木抗氧化特性探討。屏東科技大學碩士論文。屏東。
- 林?。2006。松果菊屬HPLC指紋圖譜和活性成分的研究。四川大學碩士學位論文。四川。
- 耿紅梅。2008。歐洲旋覆花化學成分的研究。時珍國醫國藥。
- 施益民、呂鋒洲。1989。自由基與各種疾病。當代醫學。16:399-407。
- 陳立亞。2007。迷迭香酸的研究概況。中國藥事。21(11):923-929。
- 梁玉香、李海標。2001。Forskolin抗金?地鼠視網膜?細胞凋亡。神經解剖學雜誌。17(3):230-234。
- 許玲玲。2004。毛喉鞘蕊花中二?成分的研究。中國藥科大學碩士學位論文。江蘇。
- 許海棠、徐遠金。2008。HPLC/MS 測定蒲公英顆粒中綠原酸、咖啡酸和阿魏酸的含量。化學通報。71(6):1-6。
- 章偉浩。2006。仙草癒合組織與十種唇形花科植物之迷迭香酸、熊果酸含

量、抗氧化力與絡胺酸抑制能力比較研究。大同大學碩士論文。台北。14.黃龍江。2005. 迷迭香酸衍生物的合成及其抗菌性質研究。青島大學碩士論文。山東。15.楊巧容。2004. 毛喉鞘蕊花有效成分及質量分析研究。湖北中醫學院碩士論文。湖北。16.莊培梃、王鐘毅、吳思敬、曾慶瀛。2006. 明日葉之一般組成及抗氧化性質。臺灣農業化學與食品科學。44(3):181-191。17.莊曉莉、李祥麟、黃檀溪。2003. 蟬蛹草具有顯著之抗氧化性與自由基清除能力。師大學報。48(1,2):13-24。18.翟婷。2008. 迷迭香中迷迭香酸的提取純化及提取物活性測定的研究。廣西大學碩士學位論文。廣西。19.劉立偉。2008. 咸豐草與楓香之抗氧化性及成分分析。大葉大學生物產業科技學系碩士論文。彰化。20.遲秀玲。2007. 迷迭香酸抗支原體源性脂質相關膜蛋白誘導的巨噬細胞凋亡及機制探討。南華大學碩士學位論文。湖南。21.蘇苑菱。2007. 八種藥用植物之抗氧化性研究。大葉大學生物產業科技學系碩士論文。彰化。22.鐘耀慶。2004. 功能性食品。化學工業出版社。北京。23.Arnao, M. B., Cano, A. and Acosta, M. 2001. The hydrophilic and lipophilic contribution to total antioxidant activity. *Food Chem.*, 73: 239-244.24.Argolo, A. C. C., Sant ' Ana, A. E. G., Pletsch, M. and Coelho, L. C. B. B. Antioxidant activity of leaf extracts from *Bauhinia monandra*. *Bioresour Technol.*, 95: 229-233.25.Benzie, I. F. and Strain, J. J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of ' ' antioxidant power ' ' : the FRAP assay. *Anal. Chem.*, 239: 70-76.26.Bonoli, M., Verardo, V., Marconi, E. and Caboni, M. F. 2004. Antioxidant phenols in barley (*Hordeum vulgare L.*) flour: comparative spectrophotometric study among extraction methods of free and bound phenolic compounds. *J. Agric. Food Chem.*, 52: 5195-5200.27.Brand-Williams, W., Cuvelier, M. E. and Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. *Lebenson Wiss Technol.*, 28: 25-30.28.Brenneisen, P., Steinbrenner, H. and Sies, H. 2005. Selenium, oxidative stress, and health aspects. *Mol Aspects Med.*, 26: 256-267.29.Christel, Q. D., Bernard, G., Jacques, V., Thierry, D., Claude, B., Michel, L., Micheline, C., Jean-Claude, C., Francois, B. and Francis, T. 2000. Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum Moench*) hulls and four. *J. Ethnopharmacol.*, 72: 35-42.30.Dorman, H. J. D. and Hiltunen, R. 2004. Fe(III) reductive and free radical-scavenging properties of summer savory (*Satureja hortensis L.*) extract and subfractions. *Food Chem.*, 88: 193-199.31.Gorinstein, S., Zachwieja, Z., Katrich, E., Pawelzik, E., Haruenkit, R., Trakhtenberg, S. and Martin-Belloso, O. 2004. Comparison of the contents of the main antioxidant compounds and the antioxidant activity of white grapefruit and his new hybrid. *Lebenson Wiss Technol.*, 37: 337-343.32.Gris, E. F., Ferreira, E. A., Falca?o, L. D. and Bordignon-Luiz, M. T. 2007. Caffeic acid copigmentation of anthocyanins from Cabernet Sauvignon grape extracts in model systems. *Food Chem.*, 100: 1289-1296.33.Janicz?k, G., M?th?, I., Mikl?ssy-V?ri, V. and Blunden, G. 1999. Comparative studies of the rosmarinic and caffeic acid contents of Lamiaceae species. *Biochem Syst Ecol.*, 27: 733-738.34.Kim, K. H. and Petersen, M. 2002. cDNA-cloning and functional expression of hydroxyphenylpyruvate dioxygenase from cell suspension cultures of *Coleus blumei*. *Plant Sci.*, 163: 1001-1009.35.Kumaran, A. and Joel karunakaran, R. 2006. Antioxidant and free radical scavenging activity of an aqueous extract of *Coleus aromaticus*. *Food Chem.*, 97: 109-114.36.Kumaran, A. and Joel karunakaran, R. 2007. Activity-guided isolation and identi?cation of free radical-scavenging components from an aqueous extract of *Coleus aromaticus*. *Food Chem.*, 100: 356-361.37.Lai, L.-S., Chou, S.-T. and Chao, W.-W. 2001. Studies on the antioxidative activities of Hsian-tsao (*Mesona procumbens Hemsl*) leaf gum. *J. Agric. Food Chem.*, 49(2): 963-968.38.Li, X., Yu , C., Cai, Y., Liu, G., Jia, J. and Wang, Y. 2005. Simultaneous determination of six phenolic constituents of danshen in human serum using liquid chromatography/tandem mass spectrometry. *J. Chromatogr. B.*, 820: 41-47.39.Li, Z. And Wang, J. 2006. A forskolin derivative, FSK88, induces apoptosis in human gastric cancer BGC823 cells through caspase activation involving regulation of Bcl-2 family gene expression, dissipation of mitochondrial membrane potential and cytochrome c release. *Cell Biol Int.*, 30: 940-946.40.Liao, K. L. and Yin, M. C. 2000. Individual and combined antioxidant effects of seven phenolic agents in human erythrocyte membrane ghosts and phosphatidylcholine liposome systems:importance of the partition coefficient. *J. Agric. Food. Chem.*, 48: 2266-2270.41.Makris, D. P., Psarra, E., Kallithraka, S. and Kefalas, P. 2003. The effect of polyphenolic composition as related to antioxidant capacity in white wines. *Food Res. Intl.*, 36: 805-814.42.Parejo, I., Caprai, E., Bastida, J., Viladomat, F., J?uregui, O. and Codina, C. 2004. Investigation of Lepechinia graveolens for its antioxidant activity and phenolic composition. *J Ethnopharmacol.*, 94: 175-184.43.Petersen, M. 1997. Cytochrome P450-dependent hydroxylation in the biosynthesis of rosmarinic acid in coleus. *Phytochemistry.*, 45(6): 1165-1172.44.Petersen, M. and Simmonds, M. S. J. 2003. Rosmarinic acid. *Phytochemistry.*, 62: 121-125.45.Pourmorad, F., HosseiniMehr1, S. J. and Shahabimajd, N. 2006. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *Afr. J. Biotechnol.*, 5(11): 1142-1145.46.Saeedeh, A. S. and Urooj, A. 2007. Antioxidant properties of various solvent extracts of mulberry (*Morus indica L.*) leaves. *Food chemistry.*, 102: 1233-1240.47.Saleem, A. M., Dhasan, P. B. and Rafiullah, M. R. M. 2006. Simple and rapid method for the isolation of forskolin from *Coleus forskohlii* by charcoal column chromatography. *J Chromatogr A.*, 1101: 313-314.48.Sánchez-Moreno, C., Larrauri, J. A. and Saura-Calixto, F. 1998. A Procedure to Measure the Antiradical Efficiency of Polyphenols. *J Sci Food Agric.*, 76: 270-276.49.Scandalios, J. G. 2002. The rise of ROS. *Trends Biochem Sci.*, 27(9): 483-486.50.Tepe, B. 2008. Antioxidant potentials and rosmarinic acid levels of the methanolic extracts of *Salvia virgata* (Jacq), *Salvia staminea* (Montbret & Aucher ex Bentham) and *Salvia verbenaca* (L.) from Turkey. *Bioresour Technol.*, 99: 1584-1588.51.Tepe, B., Daferera, D., Tepe, A. S., Polissiou, M. and Sokmen, A. 2007. Antioxidant activity of the essential oil and various extracts of *Nepeta ?avida* Hub.-Mor. from Turkey. *Food Chem.*, 103: 1358-1364.52.Wang, T. G., Lee, H. I. and Yang, C. C. 2009. Evaluation of in vitro antioxidant and anti-lipid peroxidation activities of Ching-Pien-Taso (*Pteris multifida* Poiret). *J. Taiwan Agric. Res.*, 58(1): 55-60.53.Witzell, J., Gref, R. and N?sholm, T. 2003. Plant-part speci?c and temporal variation in phenolic compounds of boreal bilberry (*Vaccinium myrtillus*) plants. *Biochem Syst Ecol.*, 31: 115-127.54.Yildirim, A., Mavi, A. and Kara, A. A. 2001. Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extracts. *Journal of Agricultural and Food Chemistr.*, 49: 4083-4089.55.Yip, H. K. and To W. M. 2005. An FTIR study of the effects of arti?cial saliva on the physical characteristics of the glass ionomer cements used for art. *Dent Mater.*, 21: 695-703.56.Zhao, G. R., Zhang, H. M., Ye, T. X., Xiang, Z. J., Yuan, Y. J., Guo, Z. X. and Zhao, L. B.

