

# Study on the Antioxidative Properties of Symphytum officinale and Polygonum multiflorum

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## ABSTRACT

In this study, *Symphytum officinale* and *Polygonum multiflorum* were extracted using four different solvents, n-hexane, dichloromethane, methanol-chloroform and methanol-water. The assays of antioxidative activities were included  $\text{Fe}^{2+}$  chelating ability, relative reducing power, superoxide anion scavenging ability, the inhibition of Fe/ascorbate-induced lipid peroxidation in a liposome model system, and ABTS cation scavenging ability. These antioxidative activities of *Symphytum officinale* and *Polygonum multiflorum* were measured and compared with those of butylated hydroxyanisole (BHA), ethylene diamine tetracetic acid (EDTA),  $\alpha$ -tocopherol and gallic acid. The extracts of *Symphytum officinale* and *Polygonum multiflorum* from the methanol-chloroform fraction and the methanol-water fraction had the highest contents of total phenol (39.15 and 96.42 mg/g, respectively). However, the extracts of *Symphytum officinale* and *Polygonum multiflorum* from the methanol-chloroform fraction and dichloromethane had the highest contents of total flavonoids (22.32 and 28 mg/g, respectively). For antioxidant activities, the extracts of *Symphytum officinale* and *Polygonum multiflorum* from the methanol-chloroform fraction and the methanol-water fraction performed the best. These antioxidant activities for the extracts of *Symphytum officinale* and *Polygonum multiflorum* were obtained as follows: the DPPH radical scavenging ability,  $IC_{50} = 0.21 \pm 0.01$  and  $0.01 \pm 0.00$  mg/g, the  $\text{Fe}^{2+}$  chelating ability,  $IC_{50} = 0.17 \pm 0.00$  and  $0.17 \pm 0.01$  mg/g, the relative reducing power,  $k = 0.20 \pm 0.00$  and  $0.17 \pm 0.00$  mL/mg, the superoxide anion scavenging ability, 30.92% and 71.68%, the inhibition of lipid peroxidation, 57.25% and 78.88%, and the ABTS cation scavenging ability,  $IC_{50} = 0.24 \pm 0.05$  and  $0.02 \pm 0.01$  mg/g, respectively. In summary, both *Symphytum officinale* and *Polygonum multiflorum* showed some antioxidant activities. When various solvents were used to extract *Symphytum officinale* and *Polygonum multiflorum*, the extracts from the methanol-chloroform fraction and the methanol-water fraction had the highest antioxidant activities. The findings in this study can help understand the antioxidant activities and processing methods of *Symphytum officinale* and *Polygonum multiflorum*.

Keywords : *Polygonum multiflorum* ; *Symphytum officinale* ; Antioxidant

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## REFERENCES

- 1.丁克祥。1996。SOD生物醫學淺論。藝軒出版社。台北，台灣。2.林玉如。2005。中草藥之生物活性探討及在化粧品之應用。嘉南藥理科技大學化粧品科技研究所碩士論文。台南，台灣。3.林明君。2002。新鮮及乾燥番茄甲醇萃取液之抗氧化性比較。大葉大學食品工程學系碩士論文。彰化，台灣。4.林國華。1998。養生中藥。第268-269頁。好兄弟出版社。台北，台灣。5.林文?。2004。松葉之抗氧化性研究。大葉大學生物產業科技學系碩士論文。彰化，台灣。6.許鴻源。1980。中藥材之研究。新醫藥出版社。台北，台灣。7.拱玉郎。1997。天然抗氧化劑發展近況。食品工業。29(3): 29-37。台北，台灣。8.黃正坤、王國祥、張為憲。1981。台灣產香辛植物之抗氧化性調查研究(二)。中國農業化學會誌。19: 200-207。9.黃喻敏。2004。中藥複方五白散之美白及抗氧化性質評估及美白效能提升研究。南台科技大學化學工程系碩士論文。台南，台灣。10.吳惠萍。2004。三種台灣產迷迭香精油成分及抗氧化力研究。大葉大學生物產業科技學系碩士論文。彰化，台灣。11.高幸子。2004。納豆抗氧化性之研究。屏東科技大學食品科學系碩士論文。屏東，台灣。12.高馥君、李敏雄。1998。食品保存與抗氧化劑。食品工業。30(12): 17-24。13.陳淑茹。2003。石蓮萃取物之抗氧化活性及抗致突變性研究。靜宜大學食品營養學系碩士論文。台中，台灣。14.陳三餘、錢佑、闕錦慧。1999。中藥對口腔病原菌及牙周病治療效果之評估。Chin. Med. Coll. J. 8(1): 47-55。15.楊文清。1983。數種台灣市售藥材誘導干擾素之研究。中國醫藥學院藥學研究所碩士論文。台中，台灣。16.姜淑繡。2001。省產蘿蔔之抗氧化性研究。大葉大學食品工程學系碩士論文。彰化，台灣。17.劉伯康。1997。數種傳統食用植物抗氧化性之研究。國立中興大學食品科技研究所碩士論文。台中，台灣。18.簡錦慈。2004。刺蔥之抗氧化性及安全性探討。靜宜大學食品營養學系碩士論文。台中，台灣。19.莊培挺。2005。明日葉抗氧化性質及降血脂之作用。國立嘉義大學食品科學系碩士論文。嘉義，台灣。20.蘇建。2007。何首烏、夜交藤藥材質量控制研究。河北醫科大學藥物分析專業所碩士論文。河北，中國。21.蘇苑菱。2007。八種藥用植物之精油與萃取物之抗氧化性研究。大葉大學生物產業科技學系碩士論文。彰化，台灣。22.Ames, B. N. 1983. Dietary carcinogens and anticarcinogens: Oxygen radicals and degenerative disease. Sci. 221: 1256-1263. 23.Ames, B. N., Shigenga, M. K. and Hagen, T. M. 1993. Oxidants, antioxidants and degenerative diseases of aging. Sci. 90: 7915-7922. 24.Arnao, M. B., Cano, A. and Acosta, M. 2001. The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chem. 73: 239-244. 25.Arouma, O. I. 1994. Nutrition and health aspects of free radicals and antioxidants. Food Chem. Toxic. 32(7): 671-683. 26.Cook, N. C. and Samman, S. 1996. Flavonoids-chemical, metabolism, cardioprotective effects, and dietary source. Nut Biochem. 7: 66-76. 27.Christel, Q. D., Bernard, G., Jacques, V., Thierry, D., Claude, B., Michel, L., Micheline, C., Cluade, J. C., Francois, B. and Francis, T. 2000. Phenolic compounds and antioxidant activities Ifbuckwheat (Fagopyrum esculentum Moench) hulls and four. J. Ethnopharmacol 72: 35-42. 28.Conforti, F., Silvio, S., Mariangela, M., Fedrica, M., Giancarlo, A. S., Dimitar, U., Aurelia, T., Francesco, M. and Roberto, L. 2007. In vivo anti-inflammatory and in vitro antioxidant activities of Mediterranean dietary plants. Journal of Ethnopharmacology 116: 144-151. 29.Diplock, A. T., Charleux, J. L., Crozier, W. G., Jok, F. J., Rice, E. C., Roberfroid, M., Stahl, W. and Vina, R. J. 1998. Functional food science and 30.Gutteridge, J. M. C. and Halliwell, B. 1990. The measurement and mechanism of defense against reactive oxidative species. British J. Nutr. 80: S77-S112. 31.Hattheill, J., Till, G. and Ward, P. A. 1991. Mechanisms of oxidant-induced changes in erythrocytes. Agents and Actions 32: 351-358. 32.Kim, S., Han, D., Moon, K. D. and Rhee, J. S. 1995. Measurement of superoxide dimutase-like activity of natural antioxidants. Biosci. Biotech. Biochem. 59(5): 822-826. 33.Keli, C. 2000. Antioxidant activities of extracts from five anti-viral medicinal plants, Journal of Ethnopharmacology 96: 201-205. 34.Knekter, P., Jarvinen, R., Seppanen, R., Heliovaara, M., Teppo, L., Pukkala, E. and Aromaa, A. 1997. Dietary flavonoids and the risk of lung cancer and other malignant neoplasms. Am. J. Epidemiol. 146: 223-230. 35.Koll, R., Buhr, M., Dieter, R., Pabst, H., Prede, H. G., Petrowicz, O., Giannetti, B., Klingenberg, S. and Staiger, C. 2004. Efficacy and tolerance of a comfrey root extract (Extr. Rad. Symphyti) in the treatment of ankle distorsions: results of a multicenter, randomized, placebo-controlled, double-blind study. Phytomedicine 11: 470-477. 36.Larson, R. A. 1988. The antioxidants of higher plants. Phytochem. 27: 269-278. 37.Leake, D. S. 2001. Flavonoids and the oxidation of low-density lipoprotein. Nutr. 17: 63-66. 38.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. 39.Lai, L. S., Chou, S. T. And Chao W. W. 2001. Studies on the Antioxidative Activities of Hsian(Mesona procumbens Hemsl)Leaf Gum. J. Agric. Food Chem. 49: 963-968. 40.Meir, S., Kanner, J., Akiri, B. and Philosoph, H. S. 1995. Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. J. Food Chem. 43: 1813-1819. 41.Meyer, A. S. and Isaksen, A. 1995. Application of enzymes as food antioxidants. Trends in Food Science & Technology September 6: 300-304 42.Miller, N. J. and Rice-Evans, C. A. 1997. The relative contributions of ascorbic acid and phenolic antioxidants to the total antioxidant activity of orange and apple fruit juices and blackcurrant drink. Food Chem. 60: 331-337. 43.Moskovitz, J., Yim, M. B. and Chock, P. B. 2002. Free radicals and disease. Arch. Biochem. Biophys. 397(2): 354-359. 44.Nagy, M. and Granca, D. 1996. Colorimetric determination of flavanones in propolis. Pharmazie. 51: 100-101. 45.Niki, E. 1991. Action of ascorbic acid as a scavenger of active and stable oxygen radicals. Am. J. Clin. Nutr. 54: 1119-1124. 46.Okusa, P. N., Penge, O., Devleeschouwer, M. and Duez, P. 2007. Direct and indirect antimicrobial effects and antioxidant activity of Cordia gilletii De Wild (Boraginaceae). Journal of Ethnopharmacology 112: 476-481. 47.Oyaizu, M. 1986. Studies on products of browning reaction: Antioxidative activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutri. 44: 307-314. 48.Ramarathnam, N., Osawa, T., Ochi, H. and Kawakishi, S. 1995. The contribution of plant food antioxidants to human health. Trends in Food Sci. Technol. 6(3): 75-82. 49.Robak, J. and Gryglewski, I. R. 1988. Flavonoids are scavengers of

superoxide anions. Biochem. Pharma. 37: 837-841. 50.Salah, N., Miller, N. J., Paganga, G., Tijburg, L., Bolwell, G. P. and Rice-Evans, C. A. 1995. Polyphenolic flavanols as scavengers of aqueous phase radicals and as chain-breaking antioxidants. Arch. Biochem. Biophys. 322: 339-346. 51.Sato, M., Ramarathnam, N., Suzuki, Y., Ohkubo, T., Takeuchi, M. and Ochi, H. 1996. Varietal differences in the phenolic content and superoxide radical scavenging potential of wines from different sources. J. Agric. Food Chem. 44: 37-41. 52.Stadler, R. H. and Fay, L. B. 1995. Antioxidative reactionsof caffeine: Formation of 8-oxocaffeine (1,3,7-Trimethyuric acid) in coffee subjected to oxidative stress. J. Agric. Food Chem. 43: 1332-1338. 53.Shahidi, F. and Wanasundara, P. K. 1992. Phenolic antioxidants. Crit Rev Food Sci Nutr. 32: 67-103. 54.Simic, M. G. 1988. Mechanisms of inhibition of free-radical processes in mutagenesis and carcinogenesis. Mutat. Res. 202: 377-386. 55.Singketon, V. L. and Rossi, J. A. J. R. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. J. Enol. Vitic. 16: 144-153. 56.Toit, R. Du., Volsteedt, Y. and Apostolides, Z. 2001. Comparison of the antioxidant content of fruits, vegetables and teas measured as vitamin C equivalents. Toxicol. 166: 63-69. 57.Torel, J., Cillard, J. and Chillard, P. 1986. Antioxidant activity of flavonoides and reactivity with peroxy radicals. Phytochem. 25: 383-385. 58.Tseng, H. S., Chien, T. Y., Tzeng, C. F., Lin, Y. H., Wu, C. H. and Wang, C. C. 2006. Prevention of hepatic oxidative injury by Xiao-Chen-Chi-Tang in mice. Journal of Ethnopharmacology 111 : 232-239.