

咸豐草與楓香之抗氧化性及成分分析 = Study on the antioxidativity and chemical components of bidens pilosa and ...

劉立偉、吳淑姿；?瑞澤

E-mail: 9708287@mail.dyu.edu.tw

摘要

本研究以咸豐草經不同烘乾溫度(40、60、80和100)處理後，再以50%乙醇為溶劑進行熱回流萃取，探討烘乾溫度對抗氧化活性之影響，咸豐草與楓香以不同溶劑(正己烷、二氯甲烷、甲醇-水及甲醇-氯仿)依序進行熱回流萃取，量測不同溶劑萃取物之抗氧化活性。抗氧化成分含量測試包括總多酚化合物及類黃酮化合物含量分析，抗氧化活性測試包括清除DPPH自由基能力、螯合亞鐵離子能力、相對還原力、清除超氧陰離子能力、抑制微脂粒氧化作用及清除ABTS陽離子等，並與BHA、EDTA、 α -tocopherol及gallic acid之抗氧化性做比較。結果顯示，以不同溫度處理咸豐草莖部與葉部樣品，再經50%乙醇進行熱回流萃取，所得萃取物之萃取率，葉高於莖。於各試驗皆以40 烘乾之葉部抗氧化活性最佳，其類黃酮及總多酚含量最多，分別為10.89與63.96 mg/g；在清除DPPH自由基試驗中，其IC₅₀為0.03 ± 0.00 mg/g；在螯合亞鐵離子能力試驗中，其IC₅₀為0.08 ± 0.01 mg/g；在相對還原力試驗中，k值為1.13 ± 0.01 mL/mg；在清除超氧陰離子能力試驗，其清除率為60.70%；在抑制微脂粒氧化作用試驗，其抑制率為70.99%；在清除ABTS陽離子試驗，其IC₅₀為0.03 ± 0.01 mg/g。另外，以不同溶劑依序萃取咸豐草與楓香，萃取物之總多酚含量皆以甲醇萃取物之水層萃取物含量最高，分別為63.60與49.41 mg/g，類黃酮含量以甲醇萃取物之氯仿層萃取物含量最高，分別為49.91與26.81 mg/g，於抗氧化活性試驗中，皆以甲醇萃取物之水層萃取物表現之抗氧化活性最佳，在清除DPPH自由基試驗中，其IC₅₀分別為0.03 ± 0.00與0.06 ± 0.00 mg/g；在螯合亞鐵離子能力試驗中，其IC₅₀分別為0.06 ± 0.01與0.23 ± 0.00 mg/g；在相對還原力試驗中，k值分別為0.88 ± 0.05與0.40 ± 0.01 mL/mg；在清除超氧陰離子能力試驗，其清除率分別為64.14與72.58%；在抑制微脂粒氧化作用試驗，其抑制率分別為57.75與69.67%；在清除ABTS陽離子試驗，其IC₅₀分別為0.05 ± 0.01與0.02 ± 0.00 mg/g。綜合上述研究結果：咸豐草與楓香萃取物皆具有抗氧化性。咸豐草經不同烘乾處理，以40 烘乾過所得之葉部萃取物所表現之抗氧化活性最高，不同溶劑萃取則以甲醇萃取物之水層萃取物所表現之活性最高，且具有較高含量的總多酚，此顯示，抗氧化活性與總多酚含量間具有相關聯性。此結果有助於了解咸豐草與楓香之抗氧化性及加工方式之選擇。

關鍵詞：咸豐草；楓香；抗氧化；總酚；類黃酮

目錄

目錄 封面內頁 簽名頁 授權書iii 中文摘要iv 英文摘要vi 誌謝viii 目錄ix 圖目錄xii 表目錄xiv 1. 緒論1 2. 文獻回顧2 2.1 植物簡介2 2.1.1 咸豐草2 2.1.2 楓香4 2.2 天然物6 2.2.1 天然物之療效6 2.2.2 天然物有效成分之萃取6 2.3 自由基之介紹8 2.3.1 自由基定義8 2.3.2 自由基分類10 2.3.3 人體內自由基來源10 2.4 生物體內抗氧化之防禦系統10 2.5 抗氧化劑13 2.5.1 抗氧化劑之介紹13 2.5.2 天然抗氧化劑介?17 2.6 抗氧化活性測定原理22 2.6.1 清除DPPH自由基能力測定22 2.6.2 亞鐵離子螯合能力24 2.6.3 還原力24 2.6.4 超氧?離子清除能力25 2.6.5 微脂粒氧化作用之抑制25 2.6.6 清除ABTS氧離子能力試驗25 3. 材料與方法27 3.1 植物材料27 3.2 藥品27 3.3 儀器28 3.4 實驗方法29 3.4.1 植物萃取物製備29 3.4.2 抗氧化活性測定32 4. 結果與討論37 4.1 咸豐草不同部位之含水率及萃取率37 4.2 不同烘乾處理對咸豐草抗氧化性質之影響37 4.2.1 抗氧化成分含量37 4.2.2 抗氧化活性40 4.3 不同溶劑萃取對咸豐草抗氧化性質之影響53 4.3.1 抗氧化成分含量54 4.3.2 抗氧化活性56 4.4 不同溶劑萃取楓香之萃取物率67 4.5 不同溶劑萃取對楓香抗氧化性質之影響67 4.5.1 抗氧化成分含量67 4.5.2 抗氧化活性69 5. 結論81 5.1 結論81 5.2 未來展望82 參考文獻83 圖目錄 圖2.1 咸豐草3 圖2.2 楓香5 圖2.3 生物體內的酵素性抗氧化防禦系統12 圖2.4 生物體內非酵素性抗氧化防禦系統14 圖2.5 類黃酮之基本結構20 圖2.6 α -tocopherol之化學式21 圖2.7 抗氧化劑與DPPH自由基反應之機制23 圖3.1 萃取流程30 圖3.2 不同溶劑之萃取流程31 圖3.3 抗氧化分析方法33 圖4.1 不同烘乾溫度對咸豐草萃取物之DPPH自由基清除能力之影響，(a)莖 (b)葉41 圖4.2 不同烘乾溫度對咸豐草萃取物之螯合亞鐵離子能力之影響，(a)莖 (b)葉45 圖4.3 不同烘乾溫度對咸豐草萃取物之相對還原力之影響，(a) 莖 (b)葉47 圖4.4 不同烘乾溫度對咸豐草萃取物之清除超氧陰離子能力之影響，(a)莖 (b)葉49 圖4.5 不同烘乾溫度對咸豐草萃取物之抑制微脂粒氧化作用之影響，(a)莖 (b)葉51 圖4.6 不同烘乾溫度對咸豐草萃取物之清除ABTS陽離子能力之影響，(a)莖 (b)葉52 圖4.7 不同溶劑萃取咸豐草對清除DPPH自由基能力之影響57 圖4.8 不同溶劑萃取咸豐草對螯合亞鐵離子能力之影響60 圖4.9 不同溶劑萃取咸豐草對相對還原力之影響61 圖4.10 不同溶劑萃取咸豐草對清除超氧陰離子能力之影響63 圖4.11 不同溶劑萃取咸豐草對抑制微脂粒氧化之影響64 圖4.12 不同溶劑萃取咸豐草對清除ABTS能力之影響66 圖4.13 不同溶劑萃取楓香對清除DPPH自由基能力之影響70 圖4.14 不同溶劑萃取楓香對螯合亞鐵離子能力之影響73 圖4.15 不同溶劑萃取楓香對相對還原力之影響75 圖4.16 不同溶劑萃取楓香對清除超氧陰離子能力之影響77 圖4.17 不同溶劑萃取楓香對抑制微脂粒氧化之影響78 圖4.18 不同溶劑萃取楓香對清除ABTS能

力之影響80 表目錄 表2.1 菊科植物之清除自由基之IC50比較7 表2.2 cROS 及RNS 之分類 11 表2.3 人體血液中之抗氧化劑15 表2.4 抗氧化劑依作用原理分類16 表2.5 天然抗氧化劑之來源18 表4.1 咸豐草之含水率及萃取率38 表4.2 不同烘乾處理之咸豐草總多酚與總類黃酮含量39 表4.3 烘乾處理之咸豐草的比率常數(k)及半清除濃度(IC50)43 表4.4 不同溶劑萃取之咸豐草總多酚類與總類黃酮含量之影響55 表4.5 不同溶劑萃取對咸豐草之比率常數(k)及半清除濃度(IC50)58 表4.6 不同溶劑萃取對楓香總多酚類化合物與總類黃酮化合物含量之影響68 表4.7不同溶劑萃取對楓香之比率常數(k)及半清除濃度(IC50)72

參考文獻

- 1.王振瀾、許富蘭、李鴻麟。2006。細葉山茶樹種抽出物之抗氧化性質探討。台灣林業科學。21(4):559-65
- 2.朱亮、郭??。1991。中草藥。22(9):404-404
- 3.余灼?。1998。青莖疏醋治療惡性瘧疾122例。中西??合?用?床急救。5(6):284-285
- 4.李春。2001。中藥路路通化學成分的研究。中國中醫研究院碩士論文。北京。
- 5.林佩伶、王正新、賴麗旭。2006。不同乾燥處理對小麥苗粉水萃取物抗氧化性之影響。台灣農業化學與食品科學。44(1):7-15。
- 6.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。
- 7.洪熒環。2003。不同加工處理對小麥草抗氧化性質之影響。靜宜大學食品營養學系碩士論文。台中
- 8.晏文潔、李家璞、杜平愨。2000。類黃酮抗氧化力與結構之關係。台灣農業化學與食品科學。38(1):80-88
- 9.高馥君、李敏雄。1998。食品保存與抗氧化劑。食品工業。30:(12):17-24。
- 10.許曉茹、莊大永、楊文欽、賴邦嶽。2007。咸豐草的功效。科學發展。417:40-43
- 11.楊洋、韋小英。2002。柚皮黃酮類化合物提取方法和抗氧化性的研究。食品與發酵工業。28(6):9-12
- 12.盧祉彤。2003。牛樟芝菌絲體對腫瘤細胞的影響。南台科技大學生物科技研究所碩士論文。台南。
- 13.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班。台中。
- 14.顏幸達。1996。楓香之草本考察及果實路路通之陣痛藥理研究。中國醫藥學院中國藥學研究所碩士論文。台中。
- 15.蘇正德、陳正雄。2001。新編實品化學。華格納企業。台中。
- 16.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。
- 17.鐘耀慶。2004。功能性食品。化學工業出版社。北京。
- 18.Arnau, M. B., Cano, A. and Acosta, M. 2001. The hydrophilic and lipophilic contribution to total antioxidant activity. Food Chem. 73:239-244.
- 19.Arouma, O. I. 1994. Nutrition and health aspects of free radicals and antioxidants. Food Chem. Toxic. 32(7): 671-683.
- 20.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.
- 21.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.
- 22.Cai, Y. And Ruan, J. 2005. Studies on the chemical constituents from the leaf of *Liquidambar formosana* Hance. J. Chin. Med. Mater. 28:294-295.
- 23.Chang, J. S., Chiang, L. C., Chen, C. C., Liu, L. T., Wang, K. C. and Lin, C. C. 2001a. Antileukemic activity of *Bidens pilosa* L. var. *minor* (Blume) Sherff and *Houttuynia cordata* Thunb. Am. J. Chin. Med 29 : 303 – 312.
- 24.Chiang, Y. M., Chuang, D. Y., Wang, S. Y., Kuo, Y. H., Tsai, P. W. and Shyur, L. F. 2004. Metabolite profiling and chemopreventive bioactivity of plant extracts from *Bidens pilosa*. J. Ethnopharmacol. 95: 409 – 419.
- 25.Choundhary, D. and Kale, R. K. 2002. Antioxidant and non-toxic properties of piper betle leaf extract: in vitro and in vivo studies. Phytother Res. 16(5): 461-466.
- 26.Christel, Q. D., Bernard, G., Jacques, V., Thierry, D., Claude, B., Michel, L., Micheline, C., Jean-Cluade, C., Francois, B. and Francis, T. 2000. Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. J. Ethnopharmacol. 72:35-42.
- 27.Cohen, L. A. 2002. A review of animal model studies of tomato carotenoids, lycopene, and cancer chemoprevention. Exp. Biol. Med. 227(10):864-868.
- 28.Deba, F., Tran, D. X., Masaaki, Y. and Shinkichi, T. 2008. Chemical composition and antioxidant, antibacterial and antifungal activities of the essential oils from *Bidens pilosa* Linn. Var. *Radiata*. Food Control. 19: 346-352.
- 29.Dziezak, J. D. 1986. Preservatives:antioxidant. Food Technol 40: 94-102.
- 30.Fang, Y. Z., Yang, S. and Wu, G. 2002. Free radical, antioxidant, and nutrition. Nutri. 18: 872-879.
- 31.Dastmalchi, K., Damiem, H. J., Oinonen, D. P., Darwis, Y., Laakso, I. and Hiltunen, R. 2008. Chemical composition and in vitro antioxidative activity of a lemon balm (*Melissa officinalis* L.) extract. LWT 41 : 391 – 400.
- 32.Dreosti, I. E. 2000. Antioxidant polyphenols in tea, cocoa and wine. Nutri. 16: 692-694.
- 33.Ferda, C., Unlu, M., Tepe, B., Daferera, D., Polissiou, M., Sokmen, A. and Akpulat, H. A. 2003. Antioxidant and antimicrobial activity of the essential oil and methanol extracts of *Achillea millefolium* subsp. *Millefolium* Afan. (Asteraceae). J. Ethnopharmacology, 87:215-220
- 34.Ferreira, E. C., Nogueira, A. R. A., Souza, G. B. and Batista, L. A. R. 2004. Effect of drying method and length of storage on tannin and total phenol concentrations in Pigeon pea seeds. Food Chem. 86: 17-23.
- 35.Groff, J. L. and Gropper S. S. 1999. Advanced nutrition and human metabolism (3rd ed). Belmont, CA: Wadsworth/Thomson Learning.
- 36.Halliwell, B., Murcia H. A., Chirico, S. and Aruoma O. I. 1995. Free radicals and antioxidants in food and in vivo: what they do and how they work. Crit Rev Food Sci Nutr 35 (1-2) :7-20.
- 37.Ivonne, M. C. M.R., Marelle, G. B., Laura, D. H., Bert, S., Hanem, M. A., Nicole, H. P. C., Jelmer, J. V. Z., Hester, V. D. W., Gerrit, M. A. and Jan, H. K. 2002. The pro-oxidant chemistry of the natural antioxidant vitamin C, vitamin E, carotenoids and flavonoids. Environ. Toxicol. Pharmacol. 11: 321-33.
- 38.Jacob, H. A. 1995. The integrated antioxidant system. Nutr. Res. 15 : 755-766.
- 39.Jenkins, D. J. A., Kendall, C. W. C., D ' Costa, M. A., Jackson, C. J. A., Vidgen, E., Singer, W., Silverman, J.A., Koumbriidis, G., Honey, J., Rao, A. V., Fleshner, N. and Klotz, L. 2003. Soy consumption and phytoestrogens: effect on serum prostate specific antigen when blood lipids and oxidized low-density lipoprotein are reduced in hyperlipidemic men. J. Urol. 169: 507 – 511.
- 40.Kannol, C. 1988. Plant Med. 54(5): 417-419.
- 41.Keaney, J. R. and Frei B. 1994. Antioxidant protection of low-density lipoprotein and its role in the prevention of atherosclerotic vascular disease. In " Nature Antioxidants in Human Health and Disease. " ed. by B Frei, p.303-351. Academic Press:San Diego, CA.
- 42.Khan, M. R., Kihara, M. and Omoloso, A. D. 2001. Anti-microbial activity of *Bidens pilosa*, *Bischofia javanica*, *Elmerillia papuana* and *Sigesbekia orientalis*. Fitoterapia. 72: 662 – 665.
- 43.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. 44.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. 45.Larson R. A. 1988. The antioxidants of higher plants. *Phytochem.* 27 (4) :969-978. 46.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. 47.Liu, J., Chang, S. K. and Wiesenborn, D. 2005. Antioxidant properties of soybean isoflavone extract and tofu in vitro and in vivo. *J. Agric. Food Chem.* 53(6): 2333-2340. 48.Mak, O. D., Huang, D. D. and Law, C. S. 1990. Rock Anoctochilus formosanus Hay. Contains substances that affect arachidonic acid metabolism. *Phytotherapy Res.* 4 : 45-48. 49.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. 50.Martinez-Cayuela, M. 1995. Oxygen free radical and human disease. *Biochimie.* 77: 147-161. 51.Miguel, G., Simões, M., Figueiredo, A. C., Barroso, J. G., Pedro, L.G. and Carvalho, L. 2004. Composition and antioxidant activities of the essential oils of Thymus caespitius, Thymus camphoratus and Thymus mastichina. *Food Chem.* 86 183 – 188. 52.Meir, S., Kanner, B., Akiri B., and Philosoph-Hadas. 1995. Determination and involvement of aqueous reducing compounds in oxidative defense system of various senescencing leaves. *J Agric. Food Chem.* 43:1813-1819. 53.Namiki, M. 1990. Antioxidants/antimutagens in food. *Crit. Rev. Food Sci. Nutr.* 29:213-300. 54.Neuza, P., Perestrelo, R., Camara, J. S. and Marques, J. C. 2007. Relationship between antioxidant capacity and total phenolic content of red, rose and white wines. *Food chem.* 105:204-214. 55.Njenga, E. W. and Viljoen, A. M. 2006. In vitro 5-lipoxygenase inhibition and anti-oxidant activity of Eriocephalus L.(Asteraceae) species. *South African Journal of Botany*, 72:637-641. 56.Packer L. 1992. Vitamin E in Health and Disease, Marcel Dekker, Inc. New York, Basel, Hong Kong. 57.York, Basel, Hong Kong Pannala, A. S., Rice-Evans, C. In: Flavonoids and Other Polyphenols (Methods in Enzymology Vol. 335); Packer, L. Ed. Academic Press: San Diego, 2001. p 266-72. 58.Porasuphatana, S., Tsai, P. and Rosen, G.. M. 2003. The generation of free radicals by nitric oxide synthase. *Comp. Biochem. Physiol.* 134(3): 281-289. 59.Pyo, Y. H., Lee, T. C., Logendra, L. and Rosen, R. T. 2004. Antioxidant activity and phenolic compounds of Swiss chard (Beta vulgaris subspecies cyclo) extracts. *Food chem.* 85 ; 19-26. 60.Rabe, T., VanStaden, J. 1997. Antibacterial activity of South African plants used for medicinal purposes. *J. Environ.* 56: 81 – 87. 61.Reiter R. J. 1998. Oxidative damage in the central nervous system: protection by melatonin. *Prog Neurobiol.* 56: 359-384. 62.Rice-Evans, C., Miller, N., and Paganga, G. 1997. Antioxidants properties of phenolic compound. *Trends in Plant Sci.* 2(4): 152-159. 63.Irenen, P., Bastida, J., Vuladomat, F. and Codina, C. 2005. Acylated quercetagenin glycosides with antioxidant activity from Tagetes maxima. *Phytochem.* 66:2356-2362. 64.Rouseff, R. and Nagy, S. 1994. Health and nutritional benefits of citrus fruit component. *Article.* 48:125-126. 65.Sarker, S. D., Bartholomew, B., Nash, R. J. and Robinson, N. 2000. 5-OMethylhoslundin: an unusual flavonoid from Bidens pilosa (Asteraceae). *Biochem. Syst. Ecol.* 28: 591 – 593. 66.Saeedeh, A. S. and Urooj, A. 2007. Antioxidant properties of various solvent extracts of mulberry (Morus indica L.) leaves. *Food Chem.* 102:1233-1240. 67.Siddhuraju, P., Mohan, P. S. and Becker, K. 2002 Studies on the antioxidant activity of Indian Laburnum (Cassia fistula L.): a preliminary assessment of crude extracts from stem bark, leaves, flowers and fruit pulp. *Food Chem.* 79(1):61-67. 68.Sofija, D., Petrovic, S., Dobric, S., Milenkovic, M., Vucicevic, D., Zizic, S. and Kukic, J. 2007. Antimicrobial, anti-inflammatory, anti-ulcer and antioxidant activities of Carlina acanthifolia root essential oil. *J. Ethnopharmacology*, 109: 458-463. 69.Temple, N. J. 2000.: Antioxidants and disease: more questions than answers. *Nutr Res* 20: 449-459. 70.Tepe, B., D. Dimitra, A. S. Tepe, P. Moschos, S. Atalay. 2007. Antioxidant activity of the essential oil and various extracts of Nepeta flavida Hub.-Mor. From Turkey. *Food chem.* 103: 1358-1364. 71.Ubillas, R. P., Mendez, C. D., Jolad, S.D., Luo, J., King, S.R., Carlson, T.J. and Fort, D.M. 2000. Antihyperglycemic acetylenic glucosides from Bidens pilosa. *Planta Medica.* 66: 82 – 83. 72.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 Chem.* 49: 4083 – 4089. 73.Zhang, Z., Chang, Q., Zhu, M., Hung, Y., Ho, W. K. K. and Chen, Z. Y. 2001. Characterization of antioxidants present in hawthorn fruits. *J. Nutr. Biochem.* 12:144-152. 74.Zoran. M. 2008. In vitro antioxidant activity of ragweed (Ambrosia artemisiifolia L., Asteraceae) herb. *ELSEVIER.* XXX:XXX-XXX. 75.Zulueta, C. A., Zulueta, A., Tada, M. and Ragasa, C.Y. 1995. A diterpene from Bidens pilosa. *Phytochem.* 38: 1449 – 1450.