花草茶萃取物中迷迭香酸和咖啡酸的含量分析

方佩珊、吳芳禎

E-mail: 9611988@mail.dyu.edu.tw

摘 要

本研究針對自然界中的酚類化合物－迷迭香酸(rosmarinic acid, RA)和咖啡酸(caffeic acid, CA)二種常見的酚酸，利用高效能液相層析儀，建立其定量分析方法。 本研究採用逆相C18層析管柱，光電二極體陣列偵測器波長330 nm，流速1.0 mL/min，移動相組成為氰甲烷和0.05 M磷酸氫二鈉水溶液，以梯度移動的方式進行沖提。結果顯示迷迭香酸的滯留時間約為30分鐘，而咖啡酸則為9分鐘。當線性濃度範圍在1~ 100 μg/mL內，呈現良好的線性關係(相關係數大於0.99)。迷迭香酸及咖啡酸的偵測極限分別為0.54及0.12 μg/mL，而定量極限分別為1.80及0.40 μg/mL。將此法實際運用於市售之十二種花草茶材料檢測，所得回收率均高於91%，顯示此分析方法，適用於咖啡酸和迷迭香酸的分析。 分別以47.5%乙醇、95%乙醇、70 ℃及50 ℃水等四種不同的溶劑萃取下，迷迭香所含有的RA含量為最高，分別是10.38 ±0.08、7.77 ±0.04、3.21 ±0.05和2.01 ±0.00 mg/g萃取物。而在47.5%乙醇和95%乙醇萃取下，RA含量最低的是菩提葉，分別為0.43 ± 0.01和0.32 ± 0.00 mg/g萃取物。在70 ℃和50 ℃水中，以檸檬馬鞭草所含的RA最低，分別是0.13± 0.00和0.07± 0.00 mg/g萃取物。除薄荷葉在70 ℃及50 ℃水萃取液沒有明顯差異外，其餘花草茶在四種溶劑中均有明顯差異(p＜0.05)。整體而言，在47.5%乙醇萃取下，對迷迭香酸有較佳的萃取效果。 在47.5%乙醇萃取下，CA含量最高的是檸檬草1.88 ± 0.01 mg/g萃取物。而在95%乙醇萃取下，鼠尾草1.64 ± 0.00 mg/g萃取物含量最高。在47.5%乙醇和95%乙醇萃取下，檸檬馬鞭草1.04 ± 0.00 mg/g萃取物最低，且沒有明顯差異。在70 ℃水萃取下，迷迭香0.79 ±0.01 mg/g萃取物含量最高。而菩提葉、檸檬草及矢車菊間無明顯差異，及矢車菊和薄荷葉間亦無明顯差異外，其餘樣品均有顯著差異(p＜0.05)。若以50 ℃水進行萃取，CA含量最高的是鼠尾草0.84 ±0.01 mg/g萃取物。結果顯示，除了菩提葉和香蜂葉、迷迭香和檸檬馬鞭草、及洋甘菊和檸檬草間無明顯差異外，其餘樣品均有顯著差異(p＜0.05)。對咖啡酸而言，在47.5%及95%乙醇比70 ℃及50 ℃水有較佳的萃取效果。

關鍵詞 : 花草茶 ; 迷迭香酸 ; 咖啡酸 ; 高效能液相層析

目錄

參考文獻

1. 李榮貴、騰大為、杜貴彩和王斌。2000。紫蘇癒傷組織迷迭香酸的純化及抗菌活性研究。微生物學通報27(5):324-327。 2. 吳良、袁干軍和蘇秋玲。2006。高效液相色譜法測定迷迭香中迷迭香酸的含量。海南醫學院學報12(2):112-114。 3. 姚興東、聶國梅和Datta-Gupta, N.,。2006。紫錐花種屬中酚類化合物的HPLC分析。分析科學學報22(2):199-201。 4. 徐世霞和晁若冰。2005。RP-HPLC測定海金沙藤中

咖啡酸的含量。華西藥學雜誌20(6):552-553。 5. 楊剛、杜守穎、吳清、郝博和洪燕龍。2004。HPLC法測定忍冬藤中綠原酸和咖啡酸含量。中國藥品標準5(4):47-49。 6. 鄒正午、徐理納和田金英。1993。迷迭香酸抗血栓和抗血小板聚集作用。藥學學報28(4):241-245。 7. 趙春貴、張立傳、王暉和黃登宇。2005。肉桂酸及其衍生物抗氧化活性研究。食品科學26(1):218-222。 8. Ahn, S. C., Oh, W. N., Kim, B. Y., Kang, D. O., Kim, M. S., Heo, G. Y. and 0Ahn, J. S. 2003. Inhibitory effects of rosmarinic acid on Lck SH2 domain 0binding to a synthetic phosphopeptide. Planta Medica 69(7):642-646. 9. Bailly, F. and Cotelle, P. 2005. Anti-HIV activities of natural antioxidant caffeic 0acid derivatives:toward an antiviral supplementation diet. Current Medicinal 0Chemistry 12(15):1811-1818. 10. Bandoniene, D. and Murkovic, M. 2002. The detection of radical scavenging 0compounds in crude extract of borage (Borago officinalis L.) by using an 0on-line HPLC-DPPH method. Journal of Biochemical and Biophysical Methods 53:45-49. 11. Baranowski, J. D., Davidson, P. M., Nagel, C. W. and Branen, A. L. 1980. Inhibition of Saccharomyces cerevisiae by naturally occurring hydroxycinnamates. Journal of Food Science 45:592-594. 12. Boulton, D. W., Walle, U. K. and Walle, T. 1998. Extensive binding of the bioflavonoid quercetin to human plasma proteins. Journal of Pharmacy and Pharmacology 50(2):243-249. 13. Bowles, B. and Miller, A. J. 1994. Caffeic acid activity against Clostridium botulinum spores. Journal of Food Science 59(4):905-908. 14. Caniova, A. and Brandsteterova, E. 2001. HPLC analysis of phenolic acids in Melissa Officinalis. Journal of Liquid Chromatography & Related Technologies 24(17):2647-2659. 15. Chapuis-Lardy, L., Contour-Ansel, D. and Bernhard-Reversat, F. 2002. High performance liquid chromatography of water-soluble phenolics in leaf litter of three Eucalyptus hybrids (Conogo). Plant Science 163:217-222. 16. Chen, J. H. and Ho, C. T. 1997. Antioxidant activities of caffeic acid and its related hydroxycinnamic acid compounds. Journal of Agricultural and Food Chemistry 45(7):2374-2378. 17. Cirico, T. L. and Omaye, S. T. 2006. Additive or synergetic effects of phenolic compounds on human low density lipoprotein oxidation. Food and Chemical Toxicology 44:510-516. 18. Clifford, M. N. 1999. Chlorogenic acids and other cinnamates: nature, occurrence and dietary burden. Journal of the Science of Food and Agriculture 79:362-372. 19. Clifford, M. N. 2000. Chlorogenic acids and other cinnamates-nature, occurrence and dietary burden, absorption and metabolism. Journal of the Science of Food and Agriculture 80:1033-1043. 20. Dangles, O., Dufour, C., Manach, C., Morand, C. and Remesy, C. 2001. Binding of flavonoids to plasma proteins. Methods in Enzymology 335:319-333. 21. Davidson, P. M. and Parish, M. E. 1989. Methods for testing the efficacy of food antimicrobials. Food Technology 43(1):148-155. 22. Ellis, B. E. and Towers, G. H. N. 1970. Biogensis of rosmarinic acid in Mentha. The Biochemical Journal 118:291-297. 23. Exarchou, V., Troganis, A., Gerothanassis, I. P., Tsimidou, M. and Boskou, D. 2001. Identification and quantification of caffeic and rosmarinic acid in complex plant extracts by the use of variable-temperature two-dimensional nuclear magnetic resonance spectroscopy. Journal of Agricultural and Food Chemistry 49(1):2-8. 24. Facino, R. M., Carini, M., Aldini, G., Saibene, L., Pietta, P. and Mauri, P. 1995. Echinacoside and caffeoyl conjugates protect collagen from free radical-induced degradation : a potential use of Echinacea extracts in the prevention of skin photodamage. Planta Medica 61(6):510-514. 25. Farah, A. and Donangelo, C. M. 2006. Phenolic compounds in coffee. Revista Brasileira de Fisiologia Vegetal 18 (1):23-36. 26. Gao, T., Ci, Y., Jian, H. and An, C. 2000. FTIR investigation of the interaction of tumor cells treated with caffeic acid and chlorogenic acid. Vibrational Spectroscopy 24:225-231. 27. Georviev, M., Kovacheva, E., Marcheva, N. and Ilieva, M. 2006. Purification of rosmarinic acid extracts from Lavandula vera MM cell biomass. Food Chemistry 94:111-114. 28. G?lcin, I. 2006. Antioxidant activiy of caffeic acid(3,4-dihydroxycinnamic acid). Toxicology 217(2-3):213-220. 29. He, Y. J., Liu, B. H., Xiang, D. B., Qiao, Z. Y., Fu, T. and He, Y. H. 2006. Inhibitory effect of caffeic acid phenethyl ester on the growth of SW480 colorectal tumor cells involves β-catenin associated signaling pathway down-regulation. World Journal of Gastroenterology 12(31):4981-4985. 30. Ho, P., Hogg, T. A. and Silva, M. C. M. 1999. Application of a liquid chromatographic method for the determination of phenolic compounds and furans in fortified wines. Food Chemistry 64:115-122. 31. Inoue, K. I., Takano, H., Shiga, A., Fujita, Y., Makino, H., Yanagisawa, R., Ichinose, T., Kato, Y., Yamada, T. and Yoshikawa, T., 2005. Effects of volatile constitusents of a rosemary extract on allergic airway inflammation related to house dust mite allergen in mice. International Journal of Molecular Medicine 16:315-319. 32. Janicsak, G., Mathe, I., Miklossy-Vaa, V. and Blunden, G. 1999. Comparative studies of the rosmarinic and caffeic acid contents of Lamiaceae species. Biochemical Systematics and Ecology 27:733-738. 33. Jung, M. Y., Jeon, B. S. and Bock, J. Y. 2002. Free, esterified, and insoluble-bound phenolic acids in white and red Korean ginsengs (Panax ginseng C. A. Meyer). Food Chemistry 79:105-111. 34. Kandaswami, C. and Middleton E. J. 1994. Free radical scavenging and antioxidant antioxidant activity of plant flavondis. Advances in Experimental Medicine & Biology 366:351-376. 35. Kang, M. A., Yun, S. Y. and Won, J. 2003. Rosmarinic acid inhibits Ca2-dependent pathways of T-cell antigen receptor-mediated signaling by inhibiting the PLC- γ 1 and Itk activity. Blood 101(9):3534-3542. 36. Kimura, Y. and Okuda, H. 1987. Studies on the activities of tannins and related compounds, X. effects of caffeetannins and related compounds on arachidonate metabolism in human polymorphonuclear leukocytes. Journal of Natural Products 50(3):329-339. 37. Kwak, W. J., Han, C. K., Kim, H. S., An, J. S. and Kim, T. S. 1999. Process of extracting and purifying biologically effective ingredient from combined medicinal plants and their extract composition. United Status Patent 5910307. 38. Mahmoud, N. N., Carothers, A. M., Grunberger, D., Bilinski, R. T., Churchill, M. R., Martucci, C., Newmark, H. L. and Bertagnollim, M. 2000. Plant phenolics decrease intestinal tumors in an animal model of familial adenomatous polyposis. Carcinogenesis 21(5):921-927. 39. Manach, C., Scalbert, A., Morand, C., Remesy, C., and Jimenez, L . 2004. Polyphenols:food sources and bioavailability. The American Journal of Clinical Nutrition 79(5):727-747. 40. Mazumder, A., Neamati, N. and Sunder, S. 1997. Curcumin analogs with altered potencies against HIV-1 integrase as probes for biochemical mechanisms of drug action. Journal of Medicinal Chemistry 40(19):3057-3063. 41. Milic, B. L., Djilas, S. M. and Canadanovic-Brunet, J. M. 1998. Antioxidative activity of phenolic compounds on the metal-ion breakdown of lipid peroxidation system. Food Chemistry 61(4):443-447. 42. Moure, A., Cruz, J. M., Franco, D., Dominguez, J. M., Sineiro, J., Dominguez, H., Nunez, M. J.and Parajo, J. C. 2001. Natural antioxidants from residual sources. Food Chemistry 72:145-171. 43. O

’ Neil, M. J., Heckelman, P. E., Koch, C. B. and Roman, K. J. (Eds) 2006. The merck index (14th Ed.). p.1636. Merck & Co., Inc. New Jersey, USA. 44. Nomura, M., Kaji, A., Ma, W., Miyamogo, K. and Dong, Z. 2001. Suppression of cell transformation and induction of apoptosis by caffeic acid phenethyl ester. Molecular Carcinogenesis 31(2):83-89. 45. Ohnishi, M., Morishita, H., Iwahashi, H., Toda, S., Shirataki, Y., Kimura, M. and Kido, R. 1994. Inhibitory effects of chlorogenic acids on linoleic acid peroxidation and haemolysis. Phytochemistry 36(3):579-583. 46. Pearson, D. A., Frankel, E. N. and Asechbach, R. 1997. Inhibition of endothelia cell-mediated oxidation of low-density lipoprotein by rosmary and plant phenolics. Journal of Agricultural and Food Chemistry 45(3):578-582. 47. Petersen, M. and Simmonds, M. S. J. 2003. Rosmarinic acid. Phytochemistry 62(2):121-125. 48. Pomponio, R., Gotti, R., Hudaib, M. and Cavrini, V. 2002. Analysis of phenolic acids by micellar electrokinetic chromatography : application to Echinacea purpurea plant extracts. Journal of Chromatography A 945:239-247. 49. Razzaque, A. and Ellis, B. E. 1997. Rosmarinic acid production in Coleus cell cultures. Planta 137(3):287-291. 50. San, R. H. and Chen, R. I. 1987. Inhibitory effect of phenolic compounds on aflatoxin B1 metabolism and induced mutagensis. Mutation Research 177(2):229-239. 51. Sanbongi, C., Takano, H., Osakabe, N., Sasa, N., Natsume, M., Yanagisawa, R., Inoue, K., Kato, Y., Osawa, T. and Yoshikawa, T. 2003. Rosmarinic acid inhibits lung injury by diesel exhaust particles. Free Radical Biology & Medicine 34(8):1060-1069. 52. Scalbert, A., Morand, C., Manach, C. and Remesy, C. 2002 . Absorption and metabolism of polyphenols in the gut and impact on health. Biomedicine Pharmacotherapy 56(6):276-282. 53. Shahrzad, S. and Bitsch, I. 1996. Determination of some pharmacologically active phenolic acids in juices by high-performance liquid chromatography. Journal of Chromatography A 741:223-231. 54. Takeda, H., Tsuji, M., Inazu, M., Egashira, T. and Matsumiya, T. 2002. Rosmarinic acid and caffeic acid produce antidepressive-like effect in the forced swimming test in mice. European Journal of Pharmacology 449(3):261-267. 55. Ueda, H., Yamazaki, C. and Tamazaki, M. 2002. Luteolin as an anti-inflammatory and anti-allergic constituent of Perilla frutescens. Biological & Pharmaceutical Bulletin 25(9):1197-1202. 56. Wang, H., Provan, G. J. and Helliewll K. 2004. Determination of rosmarinic acid and caffeic acid in aromatic herbs by HPLC. Food Chemistry 87:307-311. 57. Wen, D., Li, C., Di, H., Liao, Y. and Liu, H. 2005. A universal HPLC method for the determination phenolic acids in compound herbal medicines. Journal of Agricultural and Food Chemistry 53:6624-6629. 58. Yu, J., Vasanthan, T. and Temelli, F. 2001. Analysis of phenolic acids in barley by high-performance liquid chromatography. Journal of Agricultural Food Chemistry 49(9), 4352-4358. 59. Zenk, M., Etschenberg, E. and Graf, E. 1982. Use of rosmarinic acid in the treatment of inflammations and pharmaceutical products used therein. United States Patent 4329361. 60. Ziakova, A. and Brand?teterova, E. 2003. Validation of HPLC determination of phenolic acids present in some Lamiaceae family plants. Journal of Liquid Chromatography & Related Technologies 26(3):443-453.