

Antimicrobial and antioxidative activities of polygonum multiflorum thunb

蘇鵬翰、涂瑞澤、吳淑姿

E-mail: 343891@mail.dyu.edu.tw

ABSTRACT

The prepared rhizome of Polygonum multiflorum Thunb (HE SHOU WU in Chinese), a perennial herb botanical belonged to Polygonaceae, widely distributed around the southern area of Yangtze River, was harvested during fall and winter after the leaves become wilted. It has been used over centuries for wellness, circulation nourishment, and has some bioactivities: anti-ageing, anti-microbial, and anti-virus. In this study, the rhizome of P. multiflorum was extracted by various solvents (distilled water, methanol, and 50% ethanol) via hot reflux methodology, and both antioxidative and antimicrobial efficacy of P. multiflorum extracts were assayed for evaluating the development of natural, non-toxic, and low-dosage preservation. Results of antioxidative activities showed that the methanolic extract of P. multiflorum has the highest DPPH scavenging activity (95.9%) at a concentration of 0.08 mg/mL; the ethanolic extract of P. multiflorum has the highest efficacy among assays of reducing power, ferrous ion-chelating ability, ABTS cation scavenging capacity, and the inhibitory activity of thiobarbituric reactive species (TBARS). When compared with butylated hydroxyanisole (BHA) in reducing power assay, the ethanolic extract of P. multiflorum has the highest efficacy at a concentration of 4.0 mg/mL and is greater than that of BHA for 50%. However, emodin, one of the anthraquinone constituents of P. multiflorum, dissolves in ddH₂O and exerts an auto-oxidation phenomenon which produces more superoxide anions that affect the SOD-like activity and has reversed the concentration-dependent tendency. With the formation of inhibition ring, the methanolic extract of P. multiflorum possessed antimicrobial activities on the micro-organism cultures of Salmonella typhimurium, Staphylococcus aureus, Escherichia coli, and Candida tropicalis. In summary, P. multiflorum extracts have been demonstrated to be a high effective antioxidant and might be used as a natural and non-toxic food additive in the future.

Keywords : Polygonum multiflorum Thunb, Polygonaceae, DPPH scavenging activity, reducing power assay, antimicrobial

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REFERENCES

- 王浴生。1986。何首烏。中藥藥理與運用。10:305-307。
- 王靜修。1996。何首烏。傳統醫學雜誌。9(1):64-67。
- 李建北、林茂。1993。何首烏化學成分的研究。中草藥。24:115-118。
- 許家津。2003。何首烏萃取物抗氧化性與致突變性之研究。靜宜大學食品營養研究所碩士論文。
- 尚志鈞。1999。開寶本草。基層中藥雜誌。第一期。
- 金國琴、趙佛康。1994。首烏製劑對老年大鼠胸腺、肝臟蛋白質和核酸含量的影響。中草藥。25(11):587-589。
- 唐慎微著, 那琦編。1976。證類本草。國立編譯館。台北。
- 陳建霖。2010。Ergothioneine 對ci splat in 誘導小鼠神經細胞傷害的影響及其作用機制探討。大葉大學生物產業科技研究所碩士論文。
- 陳曉光、崔志勇、常一丁、王本祥。1991。何首烏對老年小鼠衰老指標的影響。中草藥。22(8):357-358。
- 陳雅菁。2001。不同濃度何首烏萃取物對腦神經傳導物質及抗氧化能力之影響。靜宜大學食品營養系碩士論文。
- 郭悅維。1995。自由基、活性氧與抗氧化劑。台灣科學 48(2):164-177。
- 張志鵬。2008。刀豆素A誘導細胞自噬在肝癌治療與急性肝炎的作用。國立成功大學基礎醫學研究所博士論文。
- 羅宇強。1992。中草藥對大白鼠心臟粒線體脂質過氧化之效果。國立陽明大學傳統醫藥學研究所碩士論文。
- 趙學敏。1765。本草綱目拾遺。國立編譯館。台北。
- 應久皓、周學優、朱秀琴。1992。何首烏炮制品藥理臨床研究。中國中藥雜誌。17(12):722-724。
- 童承福。2007。首烏類中藥有三種。中國醫訊 十月號:63-67。
- 戴友平、唐國華、郭衍坤。1998。何首烏提取液對犬心肌缺血再灌流損傷的預防作用實驗研究。中國生化藥物雜誌。19(2):79-80。
- 錢汝紅、丁鏞發、宋宇紅。1994。首烏對大鼠外周淋巴細胞DNA 損傷修復能力的影響。上海中醫藥雜誌。4:41-42。
- 韓志芬、?文聰。1995。不同首烏提取液對老年大鼠心、肝、腦自由基代謝及腦內MAO-B 的影響。中國中醫藥科技 2(1):36-37。
- 劉易修。2008。夜交藤與康復力之抗氧化性研究。大葉大學生物產業科技學系碩士論文。
- 蔡瑞齡。2009。彩葉草之抗氧化活性及成分分析。大葉大學生物產業科技學系碩士論文。
- Aebi, H. 1983. Catalase. In " Methods of Enzymatic Analysis. " p.673-686. Bergmeyer, H. U. Weinhein Deerfield beach.
- Ahamd, S. 1995. Antioxidant mechanisms of enzymes and proteins. In " Oxidative Stress and Antioxidant Defenses in Biology. " p.240-272. Ahmad, S. Chapman & Hall. International

Thomaon Publishing Inc. New York. 24. Amin, N. 1988. Maize production, distribution policy, and the problem of food security in Zimbabwe's communal areas. Development Policy and Practice Research Group, Milton, U.K. 25. Andrads, R. G., Ginani, J. S., Lopes, G. K., Dutra, F., Alonso, A., and Germes-Lima, M. 2006. Tannic acid inhibits in vitro iron-depebdment free radical formation. *Biochimie*. 88: 1287-1296. 26. Aruoma, O.I., Spencer, J.P., and Mahmood, N., 1999. Protection against oxidative damage and cell death by the natural antioxidant ergothioneine. *Food Chem. Toxicol.* 37: 1043-1053. 27. Aruoma, O.I., Whiteman, M., England, T.G., and Halliwell, B. 1997. Antioxidant action of ergothioneine: assessment of its ability to scavenge peroxynitrite. *Biochem. Biophys. Res. Commun.* 231: 389-391. 28. Asmus, K. D., Bensasson, R. V., Bernier, J. L., Houssin, R. and Land, E. J. 1996. One electron oxidation of ergothioneine and analogues investigated by pulse radiolysis: redox reactions involving ergothioneine and vitamin C. *Biochem. J.* 315: 635-639. 29. Babbs, C. F. and Steiner, M. G. 1990. Simulation of free radical reactions in biology and medicine: a new two-compartment kinetic model of intracellular lipid peroxidation. *Free Radic. Biol. Med.* 8: 471-485. 30. Badr, F. M., El-Habit, O. H. M. and Harraz, M. M. 1999. Radioprotective effect of melatonin assessed by measuring chromosomal damage in mitotic and meiotic cells. *Mutation Research* 444: 367-372. 31. Bandopadhyay, U., Dipak, D., and Banerjee, R. K. 1999. Reactive oxygen species: oxidative damage and pathogenesis. *Curr. Sci.* 77: 658-666. 32. Bhunia, A. K., Johnson, M. C., Ray, B. and Belden, E. L. 1990. Antigenic property of pediocin ACH produced by *Pediococcus acidilactici* H. *J. Appl. Microbiol.* 69: 221-215. 33. Bonorden, W. R. and Pariza, M. W. 1994. Antioxidant nutrients and protection from free radicals. In: *Nutr. Toxicol.* Kostsonis, F. N., Mackey, M. and Hjelle, J. ed. Raven press. New York. p.19-48. 34. Brodo, D., Djnjovic, K. and Bolognesi, M. 1994. Conserved patterns in the Cu,Zn superoxide dismutase family. *J. Mol. Biol.* 283: 366-386. 35. Bureau, G., Longpre, F., and Martinoli, M. G. 2008. Resveratrol and quercetin, two natural polyphenols, reduce apoptotic neuronal cell death induced by neuroinflammation. *J. Neurosci. Res.* 86(2): 403-440. 36. Byung, P. Y. 1993. Oxidative damage by free radicals and lipid peroxidation in aging. In "Free Radicals in Aging." p.59. CRC press. Florida. 37. Cadenas, E. 1995. Mechanisms of oxygen activation and reactive oxygen species detoxification. In "Oxidative Stress and Antioxidant Defenses in Biology." p.1-25. Ahmad, S. Chapman & Hall. International Thomaon Publishing Inc. New York. 38. Cai, K., Hagerman, A. E., Minto, R. E., and Bennick, A. 2006. Decreased polyphenol transport across cultured intestinal cells by a salivary proline-rich protein. *Biochem. Pharmacol.* 71: 1570-1580. 39. Candelario-Jalil, E., de Oliveira, A. C., Graf, S., Bhatia, H.S., Hull, M., Munoz, E., and Fiebich, B. L. 2007. Resveratrol potently reduces prostaglandin E2 production and free radical formation in lipopolysaccharide-activated primary rat microglia. *J. Neuroinflammation.* 4: 25. 40. Chan, Y. C., Cheng, F. C., and Wang, M. F. 2002. Beneficial effects of different *Polygonum multiflorum* Thunb. extracts on memory and hippocampus morphology. *J. Nu. Sci. Vita.* 48: 491-497. 41. Chance, B., Sies, H., and Boveris, A. 1979. Hydroperoxide metabolism in mammalian organs. *Physiol. Rev.* 59: 527-605. 42. Chander, V., and Chopra, K. 2005. Role of nitric oxide in resveratrol-induced renal protective effects of ischemic preconditioning. *J. Vasc. Surg.* 42(6):1198-1205. 43. Chander, V., and Chopra, K. 2006a. Protective effect of resveratrol, a polyphenolic phytoalexin on glycerol-induced acute renal failure in rat kidney. *Ren. Fail.* 28(2): 161-169. 44. Chander, V., and Chopra, K. 2006b. Protective effect of nitric oxide pathway in resveratrol renal ischemia-reperfusion injury in rats. *Arch. Med. Res.* 37(1):19-26. 45. Chaudiere, J. and Freeari-Iliou, R. 1999. Intracellular antioxidants: from chemical to biochemical mechanisms. *Food Chem. Toxicol.* 37: 949-962. 46. Chauhan, V. and Chauhan, A. 2006. Oxidative stress in Alzheimer's disease. *Pathophysiology* 13: 195-208. 47. Chen, Y., Wang, M., Rosen, R. T., and Ho, C. T. 1999. 2,2-Diphenyl-1-picrylhydrazyl radical-scavenging active components from *Polygonum multiflorum* Thunb. *J. Agric. Food Chem.* 47: 2226-2228. 48. Cheng, K. C., Cahill, D. S., Kasai, H., Nishimura, S. and Loeb, L. A. 1992. 8-hydroxyguanine, an abundant form of oxidative DNA damage, causes GT and AC substitution. *J. Biol. Chem.* 267: 166-172. 49. Chu, Y. H., Chang, C. L., and Hsu, H. F. 2000. Flavonoid content of several vegetables and their antioxidant activity. *J. Sci. Food Agric.* 80: 561-566. 50. Coursin, D. B. Cihla, H. P., Will, J. A. and McCreary, J. L. 1985. Adaptation to chronic hyperoxia. Biochemical effects and the response to subsequent lethal hyperoxia. *Am. J. Physiol.* 226: 1401-1407. 51. Csiszar, A., Smith, K., Labinsky, N., Orosz, Z., Rivera, A., and Ungvari, Z. (2006) Resveratrol attenuates TNF-alpha-induced activation of coronary arterial endothelial cells: role of NF-kappaB inhibition. *Am. J. Physiol. Heart Circ. Physiol.* 291(4): 1694-1699. 52. Cybulsky, M. I. and Gimbrone, M. A. (1991) Endothelial expression of a mononuclear leukocyte adhesion molecule during atherosclerosis. *Science.* 251:788. 53. Dai, Z., Li, Y., Quarles, L.D., Song, T., Pan, W., Zhou, H., Xiao, Z. 2007. Resveratrol enhances proliferation and osteoblastic differentiation in human mesenchymal stem cells via ER-dependent ERK1/2 activation. *Phytomedicine.* 14(12): 806-814. 54. De Almeida, L.M., Pineiro, C.C., Leite, M.C., Brolese, G., Leal, R.B., Gottfried, C., Goncalves, C.A. 2008. Protective effects of resveratrol on hydrogen peroxide induced toxicity in primary cortical astrocyte cultures. *Neurochem. Res.* 33(1):8-15. 55. De Jesus Soares, T., Volpini, R. A., Francescato, H. D., Costa, R.S., da Silva, C.G., and Coimbra, T. M. 2007. Effects of resveratrol on glycerol-induced renal injury. *Life Sci.* 81(8): 647-656. 56. Do Amaral, C. L., Francescato, H. D., Coimbra, T. M., Costa, R. S., Darin, J. D., Antunes, L. M., and Bianchi, M. D. 2007. Resveratrol attenuates cisplatin-induced nephrotoxicity in rats. *Arch. Toxicol.* 82(6): 363-370. 57. Dong, W., Gao, D., Lin, H, Zhang, X., Li, N., and Li, F. 2008. New insights into mechanism for the effect of resveratrol preconditioning against cerebral ischemic stroke: Possible role of matrix metalloproteinase-9. *Med. Hypotheses.* 70(1): 52-55. 58. Duh, P.D., Yen, G.C., Yen, W.J., Wang, B.S., Chang, L.W. 2004. Effects of pu-erh tea on oxidative damage and nitric oxide scavenging. *J. Agric. Food Chem.* 52: 8169-8176. 59. Dunn, E. T., Grandmasion, E. W., and Goosen, M. F. A. 1997. Application and properties of chitosan. Applications of chitin and chitosan, Technomic Publishing, Lancaster, USA, p.3-29. 60. Ekshyyan, V.P., Hebert, V.Y., Khandelwal, A., and Dugas, T.R. 2007. Resveratrol inhibits rat aortic vascular smooth muscle cell proliferation via estrogen receptor dependent nitric oxide production. *Cardiovasc Pharmacol.* 50(1): 83-93. 61. Encinas, M. V., Lissi, E. A. and Olea, A. F. 1985. Quenching of triplet benzophenone by vitamins E and C and by sulfur containing amino acids and peptides. *Photochem. Photobiol.* 42: 347-352. 62. Fita and Rossman 1985. The NADPH binding site on beef liver catalase.

Proc.Natl. Acad. Sci. U S A. 82(6): 1604-1608. 63. Frankel, E. N. 1991. Recent advances in lipid oxidation. *J. Sci. Food Agric.* 54:495-511. 64. Gao, Z.B., Chen, X.Q., and Hu, G.Y. 2006. Inhibition of excitatory synaptic transmission by trans-resveratrol in rat hippocampus. *Brain Res.* 1111(1): 41-47. 65. Gerrity, R. G. 1981. The role of the monocyte in atherogenesis: transition of blood-borne monocytes into foam cells in fatty lesions. *Am. J. Pathol.* 103: 181. 66. Gledhill, J.R., Montgomery, M.G., Leslie, A.G., Walker, J.E. 2007. Mechanism of inhibition of bovine F1-ATPase by resveratrol and related polyphenols. *Proc.Natl. Acad. Sci. USA.* 104(34): 13632-13637. 67. Goosen, M. F. A. 1997. Applications of chitin and chitosan, Technomic Publishing, Lancaster, USA, p.117-143. 68. Gould, D. G. W. 1996. Natural antibacterials from plants. *New Method of Food Preservation.* Springer. 40: 57. 69. Green, M. J. and Hill, H. A. 1984. Chemistry of dioxygen. *Methods Enzymol.* 105: 3-22. 70. Hagerman, D., Goodisman, J., Dabrowiak, J.C., Souid, A.K., 2003. Kinetic study on the reaction of cisplatin with metallothionein. *Drug Metab. Dispos.* 31:916-923. 71. Halliwell, B. 1987. Oxidants and human disease: some new concepts. *Faseb. J.* 1: 358-364. 72. Halliwell, B. and Aruoma, O. I. 1992. DNA damage by oxygen-derived species. In "Molecular Biology of Free Radicals Scavenger System." Scandalios, J. G. p.47-67. Cold Spring Harbor Laboratory Press. New York. 73. Halliwell, B. and Gutteridge, J. M. C. 1998. Free radicals in biology and medicine. Oxford University Press. New York, p.548-549. 74. Harman, D. 1957. Atherosclerosis : a hypothesis concerning initiating steps in pathogenesis. *J. Gerontol.* 12: 199. 75. Harman, D. 1986. Free radical theory of aging. Role of free radicals in the origination and evolution of life, aging, and disease processes. "In Free radicals, Aging, and Degenerative Diseases." p.3. Johnson, J. E., Jr., Walford, R., Harman, D. and Miqued, J. Alan R. Liss. New York. 76. Hartman, P. E. 1990. Ergothioneine as an antioxidant. *Meth. Enzymol.* 259:310-318. 77. Hata, K., Kozawa, M. & Baba, K. 1975. New stilbene glucoside from Chinese crude drug Heshouwu, roots of *Polygonum multiflorum*. *Yakugaku Zasshi* 95:211-213. 78. Hong, C. Y., Lo, Y. C., Tan, F. C., Wei, Y. H. and Chen, C. F. 1994. *Astragalus membranaceus* and *Polygonum multiflorum* protect rat heart mitochondria against lipid peroxidation. *Am. J. Chin. Med.* 22: 63-70. 79. Hsu, C.Y. 2006. Antioxidant activity of extract from *Polygonum aviculare*. *Biol.Res.* 39: 281-288. 80. Hsu, C. Y., Chan, Y. P. and Chang, J. 2007. Antioxidant activity of extract from *Polygonum cuspidatum*. *Biol. Res.* 40: 13-21. 81. Hwang, I.K., Yoo, K. Y., Kim, D. W., Jeong, S.J., Won, C. K., Moon, W. K., Kim, Y. S., Kwon, D. Y., Won, M. H. and Kim D. W. 2006. An extract of *Polygonum multiflorum* protects against free radical damage induced by ultra violet B irradiation of the skin. *Braz. J. Med. Biol. Res.* 39: 1181-1188. 82. Igarashi, M., Ishibashi, T., Nishihira, J., and Tachikawa, H. 2003. Energetics of Catalytic Reaction of Acetylcholinesterase (AChE) with Acetylcholine (ACh): Role of the Oxyanion Hole. *Int. Elec. J. Molecular Design.* 2: 712-722. 83. Imlay, J. A. and Linn, S. 1988. DNA damage and oxygen radical toxicity. *Science.* 240: 1302. 84. Jocelyn, P. C. 1971. Biochemistry of the SH Group. Academic Press, New York. 85. Johansson, J., Cederlund, E., Moodbidri, S. B., Sheth, A., Jornvall, H. 1986. Superoxide dismutase in human testis preparations. *Biosci. Rep.* 6(6):535-541. 86. Jonas, N. and Elias, S. J. 2001. Reactive oxygen species, antioxidants, and the mammalian thioredoxin system. *Free Radical Biol.* 31(11): 1287-1312. 87. Kaur, P. and Arona, S. 2010. Polyphenols of caesalpiniaceae. *J. Chi. Med.* 5(5):282-290. 88. 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. 89. Kim, M. S., Park, M. J., Kim, S. J., Lee, C. H., Yoo, H., Shin, S. H., Song, E. S., and Lee, S. H. 2005. Emodin suppresses hyaluronic acid-induced MMP-9 secretion and invasion of glioma cells. *Int. J. Oncol.* 27(3): 839-846. 90. Kim, Y. A., Kim, G. Y., Park, K. Y., and Choi, Y. H. 2007. Resveratrol inhibits nitric oxide and prostaglandin E2 production by lipopolysaccharide-activated C6 microglia. *J. Med. Food.* 10(2): 218-224. 91. Kim, Y. A., Rhee, S. H., Park, K. Y., and Choi, Y. H. 2003. Antiproliferative effect of resveratrol in human prostate carcinoma cells. *J. Med. Food.* 6(4): 273-280. 92. Kumar, P., Padi, S.S., Naidu, P.S., and Kumar, A. 2006. Effect of resveratrol on 3-nitropropionic acid-induced biochemical and behavioural changes: possible neuroprotective mechanisms. *Behav. Pharmacol.* 17(5-6): 485-492. 93. Kuo, T. C., Yang, J. S., Lin, M. W., Hsu, S. C., Lin, J. J., Lin, H. J. Hsia, T. C., Liao, C. L., Yang, M. D., Fan, M. J., Wood, W. G., and Chung, J. G. 2009. Emodin has cytotoxic and protective effects in rat C6 glioma cells: Roles of mdr1a and nuclear factor B in Cell Survival. *J. pharmacology and experimental therapeutics* 330(3): 736-744. 94. Li, X., Matsumoto, K., Murakami, Y., Tezuka, Y., Wu, Y. L., and Kadota, S. 2005. Neuroprotective effects of *Polygonum multiflorum* on nigrostriatal dopaminergic degeneration induced by paraquat and maneb in mice. *Pharm. Biochem. Beh.* 82: 345-352. 95. Li, Z., Li, L. J., Sun, Y., Li, J. 2007. Identification of natural compounds with anti-hepatitis B virus activity from *Rheun plamatum* L. ethanol extract. *Chemotherapy.* 53: 320-326. 96. Lisbeth, T. H., John, W. A., and Tom, A. G. 2001. Antibacterial effect of protamine in combination with EDTA and refrigeration. *Int. J. Food Microbiol.* 66: 149-161. 97. Liu, Q. L., Xiao, J. H., Ma, R., Ban, Y., and Wang, J. L. 2007. Effect of 2,3,5,4'-tetrahydroxystilbene-2-O-beta-d-glucoside on lipoprotein oxidation and proliferation of coronary arterial smooth cells. *J. Asian Nat. Prod. Res.* 9(5):421-429. 98. Liu, Z. P., Li, W. X., Yu, B., Huang, J., Sun, J., Huo, J. S., and Liu, C. X. 2005. Effects of trans-resveratrol from *Polygonum cuspidatum* on bone loss using the ovariectomized rat model. *J. Med. Food.* 8(1): 14-19. 99. Lu, C. C., Yang, J. S., Huang, A. C., Hsia, T. C, Chou, S. T., Kuo, C. L., Lu, H.F., Lee, T. H., Wood, W. G., and Chung, J. G. 2010. Chrysophanol induces necrosis through the production of ROS and alteration of ATP levels in J5 human liver cancer cells. *Mol. Nutr. Food Res.* 54: 1-10. 100. Lu, K.T., Chiou, R.Y., Chen, L.G., Chen, M.H., Tseng, W.T., Hsieh, H.T., Yang, Y.L. 2006. Neuroprotective effects of resveratrol on cerebral ischemia-induced neuron loss mediated by free radical scavenging and cerebral blood flow elevation. *J. Agric. Food Chem.* 54(8): 3126-3131. 101. Ma, X., Tian, X., Huang, X., Yan, F., and Qiao, D. 2007. Resveratrol-induced mitochondrial dysfunction and apoptosis are associated with Ca²⁺ and mCICR-mediated MPT activation in HepG2 cells. *Mol. Cell Biochem.* 302(1-2):99-109. 102. Melville, D. B. 1959. Ergothioneine. *Vitamin Horm. XVII:* 155-204. 103. Mencherini, T., Picerno, P., Scesa, C., and Aquino, R. 2007. Triterpene, antioxidant, and antimicrobial compounds from *Caesalpinia officinalis*. *J. Nat. Prod.* 70: 189-1894. 104. Mendonca, L.M., Dos Santos, G.C., Antonucci, G.A., Dos Santos, A.C., Bianchi Mde, L., and Antunes, L.M. 2009. Evaluation of the cytotoxicity and

genotoxicity of curcumin in PC12 cells. *Mutat. Res.* 675: 29-34. 105. Menedez-Palaez, A., Poeggeler, B., Reiter, R. J., Walden, L. R. B., Pablos, M. I. and Tan, D. X. 1993. Nuclear localization of melatonin in different mammalian tissues : immunocytochemical and radioimmunoassay evidence. *J. Cell Biolchem.* 53: 372-382. 106. Mijatovic, S., Maksimovic-Ivanic, D., Radovic, J., Miljkovic, D. J., Harhaji, L.J., Vuckovic, O., Stosic-Grujicic, S., Mostarica Stojkovic, M., and Trajkovic, V. 2005. Anti-glioma action of aloe emodin: the role of ERK inhibition. *Cell Mol. Life Sci.* 62(5): 589-98. 107. Morrissey, P. A. and O'Brien, N. M. 1998. Dietary antioxidants in health and disease. *Int. Dairy J.* 8: 463-472. 108. Nakatani, N. 1997. Antioxidants from spices and herbs. In " Natural Antioxidants Chemistry, Health Effects, Applications. " Shahidi, F. Ed., New Foundland, Canada. p. 64-75. 109. Niki, E. 1992. Active oxygens and free radicals in biology. *J. Jpn. Oil. Chem. Soc.* 41: 768-773. 110. Niwa, Y. and Miyachi, Y. 1986. Antioxidant action of natural health products and chinese herbs. *Inflammation.* 10: 79-91. 111. Nobuhiko, T., Tadayoshi, S., Koji, M., Hisao K. 1995. Molecular cloning of the defense factor in the albumen gland of the sea hare *Aplysia kurodai*. *Febs let.* 377: 373-376. 112. Okatani, Y., Wakatsuki, A., Morioka, N., and Watanabe, K. 1999. Melatonin inhibits the vasorelaxant action of peroxynitrite in human umbilical artery. *J. Pineal. Res.* 27: 111-115. 113. Peng, X., Cheng, K. W., Ma, J., Chen, B. Ho, C. T., Lo, C., Chen, F., and Wang, M. 2008. Cinnamon bark proanthocyanidins as reactive carbonyl scavengers to prevent the formation of advanced glycation endproducts. *J. Agric. Food Chem.* 56: 1907-1911. 114. Pierrefiche, G. and Laborit, H. 1995. Oxygen free radicals, melatonin and aging. *Exp. Gerontol.* 30: 213-227. 115. Ramarathnam, N., Osawa, T., Namiki, M., and Kawakishi, S. 1989. Chemical studies on novel rice hull antioxidants. 2. Identification of isovitexin, a c-glycosyl flavonoid. *J. Agric. Food Chem.* 37: 316-319. 116. Raval, A. P., Dave, K. R., and Perez-Pinzon, M. A. 2006. Resveratrol mimics ischemic preconditioning in the brain. *Cereb Blood Flow Metab.* 26(9):1141-1147. 117. Reiter, R. J. 1993. Interactions of the pineal hormone melatonin with oxygen-centered free radicals : a brief review. *Braz. J. Med. Biol. Res.* 26(1):1141-1155. 118. Reiter, R. J. 1995a. Functions pleiotropy of the neurohormone melatonin: antioxidant protection and neuroendocrine regulation. *Front. Neuroendocrinol.* 16(4): 383-415. 119. Reiter, R. J. 1995b. Functions pleiotropy of the neurohormone melatonin: antioxidant protection and neuroendocrine regulation. *Front. Neuroendocrinol.* 16: 383. 120. Reiter, R. J. 1998. Oxidative damage in the central nervous system : by melatonin. *Prog. Neurobiol.* 56: 359-384. 121. Rhoades, J. and Roller, S. 2000. Antimicrobial actions of degraded and native chitosan against spoilage organisms in laboratory media and foods. *Appl. Environ. Microbiol.* 66(1): 80-86. 122. Ripa, F. A., Haque, M and Bulbul, I. J. 2010. In vitro Antibacterial, Cytotoxic and Antioxidant Activities of *Plant. Nephelium longan*. *Pak. J. Biol. Sci.* 13(1):22-27. 123. Sano, M., Ernesto, C., Thomas, R. G., Klauber, M. R., Schafer, K., Grundman, M., Woodbury, P., Growdon, J., Cotman, C. W., Pfeiffer, E., Schneider, L. S., and Thal, L. J., 1997. A controlled trial of selegiline, alpha-tocopherol, or both as treatment for Alzheimer's disease. The Alzheimer's Disease Cooperative Study. *New Eng. J. Med.* 336: 1216-1222. 124. 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. 125. Sengottuvelan, M., Senthilkumar, R., and Nalini, N. 2006. Modulatory influence of dietary resveratrol during different phases of 1,2-dimethylhydrazine induced mucosal lipid-peroxidation, antioxidant status and aberrant crypt foci development in rat colon carcinogenesis. *Biochim. Biophys. Acta.* 1760(8): 1175-1183. 126. Sharma, S., Kulkarni, S.K., and Chopra, K. 2007. Effect of resveratrol, a polyphenolic phytoalexin, on thermal hyperalgesia in a mouse model of diabetic neuropathic pain. *Fundam. Clin. Pharmacol.* 21(1): 89-94. 127. Shires, T. K., Brummel, M. C., Pulido, J. S., and Stegink, L. D. 1997. Ergothioneine distribution in bovine and porcine ocular tissues. *Comp. Biochem. Physiol. C Pharmacol. Toxicol. Endocrinol.* 117: 117-120. 128. Simic, M. G. 1988. Mechanisms of inhibition of free-radical processes in mutagenesis and carcinogenesis. *Mutat. Res.* 202: 377-386. 129. Skjak, B. G., Anthonsen, T., and Sandford, P. 1989. Chitin and chitosan. Elsevier Applied Science, London, p.560. 130. Song, L. H., Pan, W., Yu, Y. H., Quarles, L. D., Zhou, H. H., and Xiao, Z. S. 2006. Resveratrol prevents CsA inhibition of proliferation and osteoblastic differentiation of mouse bone marrow-derived mesenchymal stem cells through an ER/NO/cGMP pathway. *Toxicol In Vitro.* 20(6): 915-922. 131. Song, T. Y., Chen, C. L., Liao, J. W., Ou, H. C., and Tsai, M. S. 2010. Ergothioneine protects against neuronal injury induced by cisplatin both in vitro and in vivo. *Food chemtox.* 48: 3492-3499. 132. Srivastava, A. K., Bhargava, P., Thapar, R., Rai, L. C. 2009. Differential response of antioxidative defense system of *Anabaena doliolum* under arsenite and arsenate stress. *J. Basic Microbiol.* 49(1): 63-72. 133. Stadtman, E. R. 1992. Protein oxidation and aging. *Science.* 257(28): 1220-1224. 134. Stutz, J. 2004. " Radical chemistry in the nocturnal boundary layer: Observations and modeling studies " , Telluride Workshop on Atmospheric Radicals. 135. Stutz, J. R., Ackermann, J. D. Fast, A., and Barrie, L. A. Atmospheric reactive chlorine and bromine at the Great Salt Lake, Utah, *Geophys. Res. Lett.* 29(10):2002. 136. Tan, D. X., Chen, D., Poeggeler, B., Manchester, L. C., and Reiter, R. J. 1993. Melatonin: a potent endogenous hydroxyl radical scavenger. *Endocr. J.* 1: 57-60. 137. Tang, T., Yin, L., Yang, J., and Shan, G. 2007. Emodin, an anthraquinone derivative from *Rheum officinale* Baill, enhances cutaneous wound healing in rats. *Eur. J. Pharmacol.* 567(3): 177-185. 138. Terman, A. and Brunk, U. T. 2004. Lipofuscin. *Int J Biochem Cell Biol* 36(8):1400-1404. 139. Tsai, S., Hung, L. M., Fu, Y. T., Cheng, H., Nien, M. W., Liu, H. Y., Zhang, F.B., and Huang, S. S. 2007. Resveratrol neuroprotective effects during focal cerebral ischemia injury via nitric oxide mechanism in rats. *J. Vasc. Surg.* 46(2): 346-353. 140. Um, M. Y., Choi, W. H., Aan, J. Y., Kim, S. R., and Ha, T. Y. 2006. Protective effect of *Polygonum multiflorum* Thunb on amyloid-peptide 25-35 induced cognitive deficits in mice. *J. Ethnopharm.* 104: 144-148. 141. Van den Broeke, L. T. and Beyersbergen van Henegouwen, G. M. 1993. Thiols as potential UV radiation protectors : an in vitro study. *J. Photochem. Photobiol. Biol.* 17: 279-286. 142. Wang, C. H., Gao, Z. Q., Ye, B., Cai, J.T., Xie, C. G., Qian, K. D., and Du, Q. 2007. Effect of emodin on pancreatic fibrosis in rats. *World J Gastroenterol.* 13(3): 378-82. 143. Xia, X. M., Wang, F.Y., Wang, Z. K., Wan, H. J., Xu, W.A., and Lu, H. 2010. Emodin enhances alveolar epithelial barrier function in rats with experimental acute pancreatitis. *World J. Gastroenterol.* 16(24): 2994-3001. 144. Yang, J., He, R., Gao, F., Sang, H., Tang, X., and Ye, R. D. 2004. Emodin enhances arsenic trioxide-induced apoptosis via generation of

reactive oxygen species and inhibition of survival signaling. *Cancer Res.* 64(1): 108-116. 145. Yan, J. J., Cho, J. Y., Kim, H. S., Kim, K. L., Jung, J. S., Huh, S. O., Suh, H. W., Kim, Y. H., and Song, D. K. 2001. Protection against β -amyloid peptide toxicity in vivo with long-term administration of ferulic acid. *British Journal of Pharmacology* 133: 89-96. 146. Yim, T. K., Wu, W.K., Mak, D. H., and Ko, K. M. 1998. Myocardial protective effect of an anthraquinone-containing extract of *Polygonum multiflorum* ex vivo. *Planta. Med.* 64: 607-611. 147. Yu, H., Pan, C., Zhao, S., Wang, Z., Zhang, H., and Wu, W. 2007. Resveratrol inhibits tumor necrosis factor- α -mediated matrix metalloproteinase-9 expression and invasion of human hepatocellular carcinoma cells. *Biomed. Pharmacother.* 1(4): 285-290. 148. Yun, K. J., Min, B. S., Kim, J.Y., and Lee, K. T. 2007. A isolated from the stem bark of *Styax japonica* inhibits lipopolysaccharide-induced expression of inducible nitric oxide synthase and cyclooxygenase-2 in RAW 264. 7 cells by suppressing nuclear factor-kappa B activation. *Biol. Pharm. Bull.* 30: 139-144. 149. Zeashana, H., Amresha, G., Singhb, S., and Rao, C. V. 2009. Hepatoprotective and antioxidant activity of *Amaranthus spinosus* against CCl₄ induced toxicity. *J. Ethnopharm.* 125: 364-366. 150. Zhang, C., Teng, L., Shi, Y., Jin J, Xue, Y., Shang, K., and Gu, J. 2002. Effect of emodin on proliferation and differentiation of 3T3-L1 preadipocyte and FAS activity. *Chin. Med. J.* 115(7): 1035-1038. 151. Zhang, H., Jeong, B. S., and Ma, T. H. 1999. Antimutagenic property of an herbal medicine, *Polygonum multiflorum* Thunb. detected by the *Tradescantia* micronucleus assay. *J. Environ. Pathol. Toxicol. Oncol.* 18(2): 127-130. 152. Zhang, L., Lau, Y. K., Xi, L., Hong, R. L., Kim, D. S., Chen, C. F., Hortobagyi, G. N., Chang, C., and Hung, M. C. 1998. Tyrosine kinase inhibitors, emodin and its derivative repress HER-2/neu-induced cellular transformation and metastasis-associated properties. *Oncogene.* 16(22): 2855-2863. 153. Zhang, Y. Z., Shen, J. F., Xu, J. Y., Xiao, J. H., Wang, J. L. 2007. Inhibitory effects of 2,3,5,4'-tetrahydroxystilbene-2-O- β -D-glucoside on experimental inflammation and cyclooxygenase 2 activity. *J. Asian Nat. Prod. Res.* 9(4):355-363. 154. Zhang, Z., Liao, L., Moore, J., Wu, T., and Wang, Z. 2009. Antioxidant phenolic compounds from walnut kernels (*Juglans regia* L.) *Food Chem.* 113: 160-165.