

Comparason of active components and anti-oxidation ability of four Ajuga Species in Taiwan

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ABSTRACT

Ajuga decumbens Thunb. ex Murray, *A. pygmaea* A. Gray, *A. taiwanensis* Nakai ex Murata and *A. nipponensis* Makino belong to the genus *Ajuga*(Labiatae). These species of *Ajuga* have been used for the treatment of wounds bleeding, abdominal pain, fever, refreshment, lung disease as Taiwanese folk medicines. In this study, ecdysterone, total flavonoids, total polyphenols and DPPH radical scavenging activity of leaves from *A. decumbens* Thunb. ex Murray, *A. pygmaea* A. Gray, *A. taiwanensis* Nakai ex Murata and *A. nipponensis* Makino were analyzed. The results indicated that the highest yield of ecdysterone was 0.031% from *Ajuga taiwanensis* in the 25% of ethanol extraction solution. Under the same condition, the highest yield of total phenols was 1.60% from *Ajuga nipponensis*. However, the best condition of total flavonoids was 75% of ethanol extraction solution, and the highest yield was 18.18% from *Ajuga pygmaea*. The best of scavenging effect on DPPH radical scavenging activity was 90.07% from *Ajuga decumbens* in the 25% of ethanol extraction solution. Moreover, the extraction solutions were also analyzed by the cellular bioassays. The results showed the 75% of ethanol extraction of *Ajuga* species has the best antioxidant activities. The order of antioxidant activities from high to low was: *Ajuga pygmaea*, *Ajuga taiwanensis*, *Ajuga nipponensis*, and *Ajuga decumbens*. All of the extraction solutions had the anti-inflammation effect but the dominated effect was in 50% ethanol extraction solution. The 50% and 25% of ethanol extraction selectively inhibit the growth of HepG2 and Hep3B 2.1-7 liver tumor cell lines and the cytotoxicity rate reached 60%. In the future, we will continue study the bioactivities in animals and develop the *Ajuga* species cultivated in Taiwan as a healthy food as a goal.

Keywords : *Ajuga decumbens*、*Ajuga pygmaea*、*Ajuga taiwanensis*、*Ajuga nipponensis*、ecdysterone、total flavonoids、total polyphenols

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REFERENCES

- 1.尹文清、宋鑫明、陳光英、韓長日、紀明慧、李小麗。2009。青梅葉中多酚含量測定及抗氧化活性研究。食品科技 34(5):282-286。
- 2.王忠合、林泳生。2009。羅勒葉黃酮的提取及其清除自由基的作用。產品加工學刊 3:57-61。
- 3.王艷和張鐵軍。2005。微波萃取技術在中藥有效成分提取中的應用。中草藥 36(3): 470-473。
- 4.白洁、孫海峰、陳翔飛。2007。4種中藥體外抗C核分枝杆菌H37RV的研究。時珍國醫 18(1): 1。
- 5.甘尉棠。2004。現代化工分離技術講座。精細石油化工4:63-65。
- 6.白雪蓮和章華偉。2005。微波萃取技術及在食

品化學中的應用。食品工業科技雜誌11(26):182-185。7.吳立軍。2006。中藥化學。第1-33頁。科技圖書館。台北。台灣。8.吳旭、王武軍、張軍花、熊俊和侯量。2006。植物藥有效成分-蛻皮激素抑制血管內皮細胞的凋亡。中國臨床康10(19)72-73。9.吳德峰。1997。淺析筋骨草的藥用價值。福建畜牧獸醫(1)32-33。10.李岡榮。2005。實用台灣草藥百科。第108頁。漢宇國際文化有限公司。臺北。台灣。11.林進和唐仕榮。2005。論天然黃酮類化合物及其抗氧化作用。徐州工程學院學報20:187-190。12.武繼彪、隋在雲和張玲。2001。-蛻皮甙酮延緩衰老的初步實驗研究。天然產物研究與開發13(5):28-29。13.邱年永和張光雄。2001。原色台灣藥用植物圖鑑(6)。第181及184頁。南天書局有限公司。台北。台灣。14.邱明華和邢其毅。1998。具有生理活性的天然有機化合物。化學進展10(3):265-272。15.金繼曙和都述虎。1994。筋骨草中黃酮類成份的研究。安徽醫學15(2):51-52。16.徐世清、戈志強、戴璇穎、喬洪根、司馬楊虎和鄭必平。2005。20-羥基蛻皮酮的藥理作用和醫學應用研究進展。科技通報21(1):56-62。17.袁珂和俞莉。2006。超聲提取與微波萃取冬凌草甲素的工藝比較。中國中藥雜誌9(31):778-779。18.馬志平和黃榕。2002。筋骨草有效部位黃酮類粗品的保肝試驗。海峽藥學14(5):40-41。19.張英、俞卓裕和吳曉琴。2004。中草藥和天然植物有效成分提取新技術-微波協助萃取。中國中藥雜誌29(2):104-108。20.張英、韋異和粟暉。2002。超聲提取-反相高效液相色譜法測定牛膝中蛻皮甙酮。光譜實驗室19(5):668-671。21.陳小霞、盧偉。2007。福建省三種常用筋骨草屬植物中蛻皮甙酮的含量測定。海?傢謎 19:12。22.陳美璇。2008。台灣產之不同筋骨草屬蛻皮甙酮、總黃酮及抗氧化能力的分析比較。大葉大學碩士論文。23.陳莉莉、吳紅權、李穎和帥琴。2002。漏蘆中蛻皮甙酮提取方法研究。中藥材3(25):195-197。24.單文軍、郭芳齡、王科軍和徐建平。2006。微波技術在天然產物生物活性成份提取中的應用研究。江西化工(4):46-49。25.曾茂貴、賈鋤和吳符火。2003。筋骨草對小鼠S180肉瘤的抑瘤試驗。福建中醫學院學報13(2):30-31。26.馮年平、范廣平、吳春蘭和韓朝陽。2002。微波萃取技術在中藥提取中的應用。世界科學技術-中藥現代化2(4):49-52。27.楊桂福。2009。枇杷葉黃酮對油脂的抗氧化性能研究。黑龍江醫藥 22(2):150-152。28.楊遠波、劉和義、彭鏡毅、施炳霖和呂勝由編著。1999。臺灣維管束植物簡誌第四卷，行政院農業委員會，台北，臺灣。29.褚小蘭和王漢章。1997。筋骨草的本草考証。中藥材20(11):586-587。30.劉斌、石任兵、葛小俠、周瑩和周靜。2001。筋骨草屬植物化學成分與藥理活性。國外醫藥(植物藥分冊)16(3):96-101。31.蔡旻都和陳皓君。2006。蔬果中類黃酮之抗氧化作用與生物活性。化學64(3):315-353。32.蔡寶昌和劉訓紅。2005。常用中藥材HPLC指紋圖譜測定術。第1-33頁。化學工業出版社。北京。中國。33.鄭斌、王存嫻、徐安武。2009。微波輔助提取花生殼黃酮類化合物及其抗氧化性研究。中國油脂 34(3):54-57。34.鄧湘慶和龔盛昭。2006。微波輔助萃取中藥有效成分的研究進展。廣東化工 33(164):89-92。35.黎彧、高虹和歐步青。2004。應用微波技術提取紫荊花色素的 研究。林產化學與工業24(2):80-82。36.蕭久富。2007。不同萃取方法對台灣筋骨草及匍匐筋骨草活性成分之比較研究。大葉大學碩士論文。37.蕭培根。1990。中國本草圖錄。第123頁。台灣商務印書館股份有限公司。台北。台灣。38.龍春、高志強、陳鳳鳴和王林。2006。黃酮類化合物的結構-抗氧化活性關係研究進展。重慶文理學院學報(自然科學版)5(2):13-17。39.薛聰賢。2003。台灣原生景觀植物圖鑑。第149頁。台灣普緣有限公司出版部。彰化。台灣。40.謝宗欣。1998。台灣筋骨草屬植物介紹。自然保育季刊21:21-27。41.謝明勇和陳奕。2006。微波輔助萃取技術研究進展。食品與生物技術學報25(1):105-114。42.鍾錠全。1997。青草世界-彩色圖鑑。第273頁。協聯印書館股份有限公司。台北。台灣。43.顏棟美、姚艾東。2009。金花茶多酚抗氧化性能得研究。河南工業大學學報 30(2):42-45。44.譙斌宗、楊元和高玲。2002。微波萃取在衛生檢驗中的應用。中國衛生檢驗雜誌12(5):632-634。45. Aviram, M., & Fuhrman, V. 2002. Wine Flavonoids Protect against LDL Oxidation and Atherosclerosis. *Annals of the New York Academy of Sciences*, 957:146 – 161. 46. Adhikari, S., Joshi, R., Patro, B. S., Ghanty, T. K., & Chintalwar, G. J., et al. (2003). Antioxidant activity of bakuchiol: experimental evidences and theoretical treatments on the possible involvement of the terpenoid chain. *Chemical Research in Toxicology*, 16:1062 – 1069. 47. Barbieri, S. S., Eligini, S., Brambilla, M., Tremoli, E., & Colli, S. 2003. Reactive oxygen species mediate cyclooxygenase-2 induction during monocyte to macrophage differentiation: critical role of NADPH oxidase. *Cardiovascular Research*, 60:187 – 197. 48. Bhor, V.M., Raghuram, N., Sivakami, S., 2004. Oxidative damage and altered antioxidant enzymes activities in the intestine of streptozotocin-induced diabetic rats. *Int. J. Biochem. Cell. Biol.* 36:89 – 97. 49. Blosi, M.S. 1958. Antioxidant determination by the use of a stable free radical. 50. Bergemdi L, Benes L, Durackova Z, Ferencik M. 1999. Chemistry, physiology and pathology of free radicals. *Life Sci* 65:1865-74. 51. Bredt, D.S. and Snyder, S.H. 1994 Nitric oxide: A physiologic messenger molecule. *Annu. Rev. Biochem.* 63:175 – 95. 52. Chenni, A., Ait Yahia, D., Boukourt, F. O., Prost, J., Lacaille-Dubois, M. A. and Bouchenak, M. 2007. Effect of aqueous extract of *Ajuga iva* supplementation on plasma lipid profile and tissue antioxidant status in rats fed a high-cholesterol diet. *Journal of Ethnopharmacology* 109(2):207-213. 53. Chen, Q., Xia, Y.P. and Qiu, Z.Y. 2006. Effect of ecdysterone on glucose metabolism in vitro. *Life Sciences* 78(10):1108-1113. 54. Chenni, A., Ait Yahia, D., Boukourt, F.O., Prost, J., Lacaille - Dubois, M.A., Bouchenak, M. 2007. Effect of aqueous extract of *Ajuga iva* supplementation on plasma lipid profile and tissue antioxidant status in rats fed a high -cholesterol diet. *Journal of Ethnopharmacology* 109(2):207-213. 55. Fiddler, R.M. 1977. Collaborative study of modified AOAC method of analysis for nitrite in meat and meat products. *J. AOAC* 60:594 – 599. 56. Fishman, M. L., Chau, H. K., Hoagland, P. D. and Hotchkiss, A. T. 2006. Microwave-assisted extraction of lime pectin. *Food Hydrocolloids* 20:1170-1177. 57. Fulzele, D. P. and Satdive, R. K. 2005. Comparison of techniques for the extraction of the anti-cancer drug camptothecin from *Nothapodytes foetida*. *Journal of Chromatography A* 1063:9-13. 58. Ganzler, K., Salg?? A. and Valk?? K. 1986. Microwave extraction: A novel sample preparation method for chromatography. *Journal of Chromatography* 371:299-306. 59. Guo, Z., Jin, Q., Fan, G., Duan, Y., Qin, C. and Wen, M. 2001. Microwave-assisted extraction of effective constituents from a Chinese herbal medicine *Radix puerariae*. *Analytica Chimica Acta* 436:41-47. 60. Hilaly El, J. and Lyoussi, B. 2002. Hypoglycaemic effect of the lyophilised aqueous extract of *Ajuga iva* in normal and streptozotocin diabetic rats. *Journal of Ethnopharmacology* 80(2):109 – 113. 61. Halliwell, B. and Gutteridge, J. M. C. 1985. *Free Radicals in Biology and Medical*. Oxford University Press. 62. Hilaly El, J., Lyoussi, B., Wibo M. and Morel, N. 2004. Vasorelaxant effect of the aqueous extract of *Ajuga iva* in rat aorta. *Journal of Ethnopharmacology* 93(1):69-74. 63. Julkunen-Titto, R. 1985. Phenolic constituents in the levels of northern willows: Methods for

precursors of clarified apple juice sediment. *J. Food Sci.* 33:254-257. 64. Kariba, R. M. 2001. Antifungal activity of *Ajuga remota*. *Fitoterapia* 72:77-178. 65. Konoshima, T., Takasaki, M., Tokuda, H. and Nishino H. 2000. Cancer chemopreventive activity of an iridoid glycoside, 8-acetylharpagide, from *Ajuga decumbens*. *Cancer Letters* 157(1):87-92. 66. Labbozzetta, S., Valvo, L., Bertocchi, P. and Manna, L. 2005. Focused microwave-assisted extraction and LCdetermination of the active ingredient in naproxen-based suppositories. *Journal of Pharmaceutical and Biomedical Analysis* 39:463-468. 67. Lin CC. Practical pharmacognosy (I) Crude Drug and Free Radical. Fu-San Publishing Company, Kaohsiung, Taiwan, R.O.C. 1995. 68. Minhajuddin, M., Beg, Z.H., Iqbal, J., 2005. Hypolipidemic and antioxidant properties of tocotrienol rich fraction isolated from rice bran oil in experimentally induced hyperlipidemic rats. *Food Chem. Toxicol.* 43:747 – 753. 69. Mouming Zhao , Bao Yang , Jinshui Wang , Baozhen Li , Yueming Jiang. 2006. Identification of the major flavonoids from pericarp tissues of lychee fruit in relation to their antioxidant activities. *Food Chemistry* 98:539-544. 70. Naczki, M. and Shahidi, F. 2004. Extraction and analysis of phenolics in food (Review). *Journal of Chromatography A* 1054:95-111. 71. Oyaizu, M. 1986. Studies on products of browning reaction: Antioxidative activities of products of browning reaction Prepared from glucosamine. *Jpn. J. Nutr.* 44: 307-315. 72. Pan, X., Liu, H., Jia, G., and Shu, Y. Y., 2000. Microwave-assisted extraction of glycyrrhizic acid from licorice root. *Biochemical Engineering Journal* 5:173-177. 73. Pi-Jen Tsai , Shiau-Chi Wu , Yu-Kuei Cheng . 2008. Role of polyphenols in antioxidant capacity of napiergrass from different growing seasons. *Food Chemistry* 106:27-32. 74. Scott RB, Collins JM, Matin S, White F, Swerdlow PS . 1990. Simultaneous measurement of neutrophil, lymphocyte, and monocyte glutathione by flow cytometry. *Journal of Clinical Laboratory Analysis* 4:324-327. 75. Shu, Y. Y., Ko, Y. M. and Chang, Y. S. 2003. Microwave-assisted extraction of ginsenosides from ginseng root. *Microchemical Journal* 74:131-139. 76. Shimada, K., Fujikawa, K., Yahara, K. and Nakamura, T. 1992. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *J. Agric. Food Chem.* 40(6): 945-948. 77. Terahara, N., Callebaut A., Ohba R., Nagata T., Ohnishi-Kameyama M. and Suzuki M. 2001. Acylated anthocyanidin 3-sophoroside-5-glucosides from *Ajuga reptans* flowers and the corresponding cell cultures. *Phytochemistry* 58:493-500. 78. Yun Shen, Liang Jin, Peng Xiao, Yan Lu, Jinsong Bao. 2009. Total phenolics, flavonoids, antioxidant capacity in rice grain and their relations to grain color, size and weight. *Journal of Cereal Science* 49:106-111. 79. Zhu, Y.Z., Huang, S.H., Tan, B.K.H., Sun, J., Whiteman, M., Zhu, Y.C., 2004. Antioxidants in Chinese herbal medicines: a biochemical perspective. *Nat. Prod. Rep.* 21:478 – 489.