Molecular identification and comparative studies of cinnamomum osmophloeum and C. burmannii based on

莊凱扉、李世傑

E-mail: 345439@mail.dyu.edu.tw

ABSTRACT

Cinnamomoum osmophloeum Kanehira is not only an indigenous species but also a very valuable forest resources in Taiwan. Recent years, Cinnamomum burmannii is often mistaken and sale by the name of C. osmophloeum Due to the similarity in leaf and stem, especially in the seedling stage, these two plants are not able to tell each other morphologically.In present research, comparative authentication studies of C. osmophloeum and Cinnamomum burmannii were performed based on chemical and molecular methodologies. C. osmophloeum and C. burmannii of samples collected from the Hualien Mega Farm , Chiayi Shekou and Kaohsiung Okayama were used in present studies. Experimental conditions were developed for simultaneously determining of 11 major components in these two species by gas chromatographyThe results clearly showed that thecomponents in leaves essential oils of C. osmophloeum and C. burmannii different significantly. The GC chromatogram for the main components of C. osmophloeum were eluted between 30 to 55 minutes, it contains Linalool, Terpineol and cinnamaldehyde. Whereas the retention times for C. burmannii major components was between 15 minutes to 30 minutes with pinene, cymene, limonen and cineole. Generally, C. osmophloeum essential oil has much superior DPPH antioxidant capacity than that of C. burmannii ’ s. DNA marker by analyzing three non-coding region of ribosomal DNA and chloroplast DNA sequence of pITS2 and the trnL-trnF sequence were used to explore the suitabilities in molecular authentication of C. osmophloeum and C. burmannii. Our results have showed thatpITS2 has much higher nucleotide polymorphisms among three marker regions. Phylogenetic trees constructed using maximum parsimony and neighbor-joining methods, being successful in grouping the right species together based on pITS2 for Cinnamomoum spp. We believe that by molecular identification of C. osmophloeum and C. burmannii wull be successful and reproducible based on pITS2 nucleotide sequence polymorphism. The determination of these two species chemically using GC chromatograms of major components in essential oil.

Keywords : Cinnamomoum osmophloeum、Cinnamomum burmannii、gas chromatography、essential oil、molecular marker

Table of Contents

REFERENCES

1.王振瀾、尹華文。1991。栽培地區及生長季節對土肉桂葉子精油含量成分之影響。林業試驗所研究報告季刊 6: 313-328。 2.亓勇。2002。引火歸元藥物應用考辨。中醫藥學刊 22(2)278。 3.尹華文、陳正豐、呂勝由。2007。土肉桂與陰香形態特徵及精油特性之研究。中華林學季刊 40(4):535－546。 4.朱紹洪。1993。天然實用香料。食品工業發展研究所。 5.李漢中、鄭森松、劉如芸、張上鎮。2003。不同地理品系土肉桂葉子精油之化學多態性。中華林學季刊 36(4): 411-422。 6.李俊億、謝幸媚、蔡麗琴。2009。DNA分析於刑事鑑識之應用。生物醫學 2（2）:106-120。 7.李雅婷。2009。魚針草基原鑑定、指標成分純化分析及系列化學成分生理活性綜論。朝陽科技大學生物科技研究所碩士論文。 8.余勇建。2002。葉綠體trnL intron與trnL-trnF IGS序列於刑事鑑識應用之研究。中央警察大學鑑識科學研究所碩士論文。 9.吳瑞鈺、劉祖惠、賴宗賢、鐘玉山、張嘉銘、陳博勳、錢瑤珍、何士慶。2005。生物技術在中草藥之研究應用。生物產業 16（1）:10-22。 10.林金和。2001。肉桂-高效價中藥。中醫藥研究論叢 4(1)183-185。 11.易光輝、王曉芬、李依蒨。2011。精油之化學基礎與實務應用。華杏出版。第58-66 頁。 12.柯季宏、張惠婷、蘇裕昌、鍾振德、郭幸榮、張上鎮。2006。台灣肖楠葉子精油之成分與化學多態性分析。中華林學季刊 39(2): 247-256。 13.高馥君、李敏雄。1998。食品保存與抗氧化劑。食品工業30(12):17-24。 14.胡大維、林耀堂、何政坤。1985。台灣土肉桂葉子精油化學成分之天然變異。台灣省林業試驗所試驗報告 No.78。 15.許盟宗。2008。台灣土肉桂抑菌性及抗氧化性之研究。亞洲大學生保健營養生技學系研究所碩士論文。 16.張上鎮、王升陽。1998。來自台灣森林之芳香維他命。台灣林業 24: 33-37。 17.張上鎮、陳品方。2000。精油之抗細菌與抗真菌活性。林產工業 19 (2): 275-284。 18.張杰。2002。肉桂及其偽品的鑑別。時珍國醫國藥 13(4):216。 19.邱淑嬌。2003。中藥材DNA鑑定資料庫應用。工研院生醫中心中藥材基原DNA鑑定技術推廣訓練班。P3-55。 20.黃思霖，曾美珍，黃介宏，呂車鳳。2007。以核內的內轉錄區間序列探討台灣馬兜鈴屬之親緣關係。特有生物研究9(2):19-27。 21.程必強、喻學儉、丁靖塏、孫漢董。1997。中國樟屬植物資源及其芳香成分。雲南科技出版社。第68-71 頁。 22.程必強、喻學儉、丁靖塏、孫漢董。1997。土肉桂與陰香形態特徵及精油特性之研究中國樟屬植物資源及其芳香成分。雲南科技出版社。第 68-1 頁。 23.楊開聰。1992。肉桂類在食品上的利用。土肉桂專欄-林業叢刊第38號。第 85-90 頁。 24.楊政川，李世傑，何坤益，林敏宜。2010。臺灣森林特產物－土肉桂。科學發展月刊 2(446):p28-33。 25.歐秋明、游銅錫、林麗雲。2009。精油化學。華杏出版。第58-67 頁。 26.劉業經、呂福原、歐辰雄。1988。臺灣樹木誌。第136-137頁。 27.林讚標編。1992。土肉桂專論。台灣省林業試驗所林業叢刊第38 號。 28.劉業經、呂福原、歐辰雄。1988。臺灣樹木誌。第136-137 頁。 29.劉棠瑞、廖日京。1980。樹木學。台灣商務印書館。第 314 頁。 30.陳品方、張上鎮、吳懷慧。2002。土肉桂葉子精油及其成分之抗?活性。中華林學季刊 35(4): 397-403。 31.陳哲民。1996。植物油抑制植物病源真菌孢子發芽之效果。花蓮區農業改良場研究彙報。12:71-92。 32.陳師瑩、陳靜慧、王櫻娟、江靜欣、鍾玉玲、葉東柏。2003。海巴戟天之葉萃取物的抗氧化活性分析。嘉南學報。29: 87-96。 33.謝瑞忠。2006。肉桂類天然香料的成分與應用。林業研究專訊 13(4):14-16。 34.蔡麗琴。2007。植物DNA分析於鑑識科學應用之研究。國立師範大學生命科學研究所博士論文。 35.蔡麗琴。2007。植物DNA分析於鑑識科學應用之研究。國立師範大學生命科學研究所博士論文。 36.韓俊生。2004。肉桂"引火歸元"之我見。上海中醫藥雜誌 38(8):47-48。 37.薛士緯。2009。以ITS序列探討台灣土肉桂和其相似種之親緣關係。國立嘉義大學森林暨自然資源研究所碩士論文。 38.蘇正德、陳正雄。2001。新編實品化學。華格納企業。 39.Aeschbach, R., J. Lolliger, B.C. Scott, A. Murcia, J. Butler, B. Halli-well, O. I. Aruoma. 1994. Antioxidant action of thymol, carvacrol, 6-gingerol, zingerone and hydroxytyrosol. Food Chem. Toxico. 32: 31-36. 40.Adams, R. P. 2001. Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy. Allured, Illinois. 456pp. 41.Alvarez, I.; Wendel, J. F. Ribosomal ITS sequences and plant phylogenetic inference. Mol. Phylogenet. Evol. 2003, 29, 417-434. 42.Blatt, C.R., R. Ciola. 1991. Analysis of vetiver essential oil by supercritical fluid extraction and online capillary gas-chromatography. Hrc-journal of high resolution chromatography. 14:775-777. 43.Barrata, M.T., H.J.D. Dorman, S. G. Deans, A.C. Figueiredo, J.G. Barroso, G.

Ruberto. 1998. Antimicrobial and Antioxidant properties of some commercial essential oils. Flavour Frag J. 13: 235-244. 44.Baratta, M.T., H.J.D. Dorman, S.G. Deans, A.C. Figueiredo, J.G. Barroso, G. Ruberto. 1998. Antimicrobial and antioxidant properties of some commercial oils. Flavour Fragr. J. 13: 235-244. 45.Chen, Q.Y., H. Shi, C.T. Ho. 1992. Effects of rosemary extracts and major constituents on lipid oxidation and soybean lipoxygenase activity. J. Amer. Oil Chem. Soc. 69 (10): 999-1002. 46.Chester, T.L., J.D. Pinkston, D.E. Raynie. 1996. Supercritical fluid chromatography and extraction. Anal. Chem. 68 (12):487-514. 47.Chiou, S. J.; Yen, J. H.; Fang, C. L.; Chen, H. L.; Lin, T. Y. 2007. Authentication of medicinal herbs using PCRamplified ITS2 with specific primers. Planta Med. 73, 1421-1426. 48.Cheng, S. S., J. Y. Liu, C. Y. Lin, Y. R. Hsui, M. C. Ju, W. J. Wu and S. T. Chang 2008a. Terminating red imported fire ants using Cinnamomum osmophloeum leaf essential oil. Bioresour. Technol. 99(4): 889-893. 49.Cheng, S. S., J. Y. Liu, E. H. Chang and S. T. Chang. 2008b. Antifungal activity of cinnamaldehyde and eugenol congeners against wood-rot fungi. Bioresour. Technol. 99(10): 5145-5149. 50.Cheng, S. S., J. Y. Liu, C. G. Huang, Y. R. Hsu, W. J. Chen and S. T. Chang. 2009. Insecticidal activities of leaf essential oils from Cinnamomum osmophloeum against three mosquito species. Bioresour. Technol. 100(1):457-464. 51.Chang, S.-T. and S.-S. Cheng. 2002. Antitermitic activity of leaf essential oils and components from Cinnamomum osmophloeum. J. Agric. Food Chem. 50(6):1389-1392. 52.Chang, S. T., P. F. Chen and S. C. Chang 2001. Antibacterial activity of leaf essential oils and components from Cinnamomum osmophloeum. J. Ethnopharmacol. 77:123-127. 53.Chang, S. T. and S. S. Cheng. 2002. Antitermitic activity of leaf essential oils and components from Cinnamomum osmophloeum. J. Agric. Food Chem. 50:1389-1392. 54.Chang, C. W., W. L. Chang, S. T. Chang and S. S. Cheng. 2008. Antibacterial activities of plant essential oils against Legionella pneumophila. Water Research. 42(1):278-286. 55.Chao, L. K., K. F. Hua, H. Y. Hsu, S. S. Cheng, J. Y. Liu and S. T. Chang. 2005. Study on the anti-inflammatory activity of essential oil from leaves of Cinnamomum osmophloeum. J. Agric. Food Chem. 53:7274-7278. 56.Chao, L. K., K. F. Hua, H. Y. Hsu, S. S. Cheng, I. F. Lin, R. Y. Tsai, S. T. Chen and S. T. Chang. 2008. Cinnamaldehyde inhibits pro-inflammatory cytokines secretion from monocytes / macrophages through suppression of intracellular signaling. Food Chem. Toxicol. 46(1):220-231. 57.Cheng, S. S., J. Y. Liu, K. H. Tsai, W. J. Chen and S. T. Chang. 2004. Chemical composition and mosquito larvicidal activity of essential oils from leaves of different Cinnamomum osmophloeum provenances. J. Agric. Food Chem. 52: 4395-4400. 58.Cheng, S. S., J. Y. Liu, Y. R. Hsui and S. T. Chang. 2006. Chemical polymorphism of essential oils and their antifungal activities from different provenances of indigenous cinnamon (Cinnamomum osmophloeum) leaves. Bioresour. Technol. 97: 306-312. 59.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. 60.Deans, S. G. and Richie, G. 1987. Antibacterial properties of plant essential oils. Int. J. Food Micobiol. 5: 165. 61.Deans, S.G., R.C. Noble, R. Hiltunen, W. Wuryani, L.G. Penzes. 1995. Antimicrobial and antioxidant properties of Syzygium aromaticum (L.) Merr. and Perry: Impact upon bacteria, fungi and fatty acid levels in ageing mice. Flavour Fragr. J. 10(5): 323-328. 62.Dziezak, J. D. 1986. Preservatives: antioxidant. Food Technol. 40: 94-102. 63.Deighton, N., S.M. Glidewell, S. G. Deans, B.A. Goodman. 1993. Identification by EPR spectroscopy of carvacrol and thymol as the major source of free radicals in the oxidation of plant essential oils. J. Sci. Food Agric. 63: 221-225. 64.Dey, P. M. and J. B. Harborne. 1991. Terpenoids. Methods in plant biochemistry Vol.7. (Charlwood, B.V. and D.V. Banthorpe Eds.). 17:1-185. Academic Press. New York 65.Fritsch, P. W., and Rieseberg, L. H. 1996. The use of random amplified polymorphic DNA（RAPD）in conservation genetics. In Smith T. and Wayne B. （eds.）, Molecular Genetic Approaches in Conservation, pp. 54-73.Oxford Univ. Press, New York. 66.Fang, S. H., Y. K. Rao and Y. M. Tzeng. 2004. Cytotoxic Effect of trans-cinnamaldehyde from Cinnamomum osmophloeum leaves on human cancer cell lines. Int. J. Appl. Sci. Eng. 2(2):136-147. 67.Fukuda, T.; Yokoyama, J.; Ohashi, H. 2001. Phylogeny and biogeography of the genus Lycium (Solanaceae): inferences from chloroplast DNA sequences. Mol. Phylogenet. Evol. 19, 246 – 258. 68.Harborne, J.B. 1973. Phytochemical methods. Academic press. London. 89-105. 69.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. 70.Huang, T. C., H. Y. Fu, C. T. Ho, D. Tan, Y. T. Huang and M. H. Pan. 2007. Induction of apoptosis by cinnamaldehyde from indigenous cinnamon Cinnamomum osmophloeum Kaneh through reactive oxygen species production, glutathione depletion, and caspase activation in human leukemia K562 cells. Food Chem. 103:434 – 443. 71.Hussain, R. A., J. Kim, T. W. Hu, J. M. Pezzuto, D. D. Soejarto and A. D. Kinghorn. 1986. Isolation of a highly sweet constituent from Cinnaamomum osmophloeum leaves. Planta Med. 52: 403-404. 72.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. Envirion. Toxicol. Pharmacol. 11: 321-33. 73.Jenkins, D. J. A., Kendall, C. W. C., D ' Costa, M. A., Jackson, C. J. A., Vidgen, E., Singer, W., Silverman, J.A., Koumbridis, 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 hyperlipidemicmen. J. Urol., 169: 507 – 511. 74.Kim, H. K., J. R. Kim and Y. J. Ahn. 2004. Acaricidal activity of cinnamaldehyde and its congeners against Tyrophagus putrescentiae (Acari: Acaridae). J. Stored Prod. Res. 40:55-63. 75.Kojoma, M.; Kurihara, K.; Yamada, K.; Sekita, S.; Satake, M.; Iida, O. 2002. Genetic identification of cinnamon (Cinnamomum spp.) based on the trnLtrnF chloroplast DNA. Planta Med. 68, 94 – 96. 76.Lee, S.M., N.A. Garas, A.C. Waiss. 1986. High-performance liquid chromatographic determination of sesquiterpenoid stress metabolites in Verticillium dahlia binfected cotton stele. J. Agric. Food Chem. 34: 490-493. 77.Lau, D. T.; Shaw, P. C.; Wang, J.; But, P. P. 2001. Authentication of medicinal Dendrobium species by the internal transcribed spacer of ribosomal DNA. Planta Med. 67, 456-460. 78.Lee, H. C., S. S. Cheng and S. T. Chang. 2005. Antifungal property of the essential oils and their constituents from Cinnamomum osmophloeum leaf against tree pathogenic fungi. J. Sci. Food Agric. 85 (12): 2047-2053. 79.Lee, C. C., Wa, S. J., Chang, C. H.,and Ng, L. T. 2003 Antitermitic activity of Cinnamomum cassia. Phytotherapy Research 80.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. 81.Larson R. A. 1988. The antioxidants of higher plants. Phytochem. 27(4): 969-978. 82.Mario, C.F., K.U. Ingold. 2003. Mechanism of inhibition of lipid peroxidation by γ-Terpinene, are unusual and potentially useful hydrocarbon antioxidant. J. Agric. Food Chem. 51: 2758-2765. 83.Madsen, H.L., G. Bertelsen.1995. Species as antioxidants. Food Sci & Tech. 6(8): 271-277. 84.Mareshige Kojoma, Kogo Kurihara, Kazuya Yamada, Setsuko Sekita,Motoyoshi Satake, Osamu Iida. 2002. Genetic Identification of Cinnamon(Cinnamomum spp.) Based on the trnL-trnF Chloroplast DNA. 85.Niki, E. 1992. Active oxygens and free radicals in biology. J. Jap. Oil Chem. Soc. 41: 768-773. 86.Namiki, M. 1990. Antioxidants/antimutagens in food. Crit. Rev. Food Sci. Nutr. 29: 213-300. 87.Ruberto, G., M.T. Baratta. 2000. Antioxidant activity of selected essential oil components in two lipid model systems.Food Chem. 69 (2): 167-174. 88.Rouseff, R. and Nagy, S. 1994. Health and nutritional benefits of citrus fruit component. Article., 48:125-126. 89.Sung, H. K., Hyun, S. H., and Choung, S. Y. 2006. Anti-diabetic effect of cinnamon extract on blood glucose in db/db mice. Journal of Ethnopharmacology. 104:119 – 123. 90.Slatkin, M. 1995. A measure of population subdivision based on microsatellite allele frequencies. Genetics. 139:457-462 91.Shu-Jiau Chiou, Jui-Hung Yen, Cheni-Li Fang, Hui-Ling Chen, and Tsai-YunLin. 2007. Authentication of Medicinal Herbs using PCR-Amplified ITS2 withSpecific Primers. 92.Shimada, K., K. Fujikawa, K. Yahara, and T. Nakamura. 1992. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. Journal of Agricultural and Food Chemistry. 40: 945-948. 93.Saini, A.; Reddy, S. K.; Jawali, N. 2008. Intra-individual and intra-species heterogeneity in nuclear rDNA ITS region of Vigna species from subgenus Ceratotropis. Genet Res (Camb). 90, 299-316. 94.Simic, M.G. 1988. Mechanisms of inhibition of free radical processes in mutagenesis and carcinogenesis. Mutat Res. 202: 377-386. 95.Stashenko E.E., M.A. Puertas, M.Y. Combariza. 1996. Volatile secondary metabolites from Spilanthes americana obtained by simultaneous steam distillation solvent extraction and supercritical fluid extraction. J. Chromatogr. 752: 223-232. 96.Tsai, L. C.; Yu, Y. C.; Hsieh, H. M.; Wang, J. C.; Linacre, A.; Lee, J. C. 2006. Species identification using sequences of the trnL intron and the trnL-trnF IGS of chloroplast genome among popular plants in Taiwan. Forensic Sci Int. 164, 193-200. 97.Ude, G., and Pillay, M., Nwakanma, D., and Tenkouano, A. 2002. Genetic diversity in Musa acuminate Colla and Musa balbisiana Colla and some of their natural hybrids using AFLP markers. Theoretical and Applied Genetic. 104（8）:1246-1252. 98.White, T. J.; Bruns, T.; Lee, S.; Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In “ PCR protocols: a guide to methods and applications. ”, p.315-322; Innis, M. A.; Gelfand, D. H.; Sninsky, J. J.; White, T. J. eds.; Academic Press, New York, USA. 99.Wang, S. Y., P. F. Chen and S. T. Chang. 2005. Antifungal activities of essential oils and their constituents from indigenous cinnamon (Cinnamomum osmophloeum) leaves against wood decay fungi. Bioresour. Technol. 96:813-818. 100.Wang, S. Y., C. W. Yang, J. W. Liao, W. W. Zhen, F. H. Chu and S. T. Chang. 2008. Essential oil from leaves of Cinnamomum osmophloeum acts as a xanthine oxidase inhibitor and reduces the serum uric acid levels in oxonate-induced mice. Phytomedicine 15:940-945. 101.Williams, W. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A., and Tingey, S. V. 1990. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucleic acid research. 18:6531-6535. 102.Zietkiewicz, E., Rafalski, A., and Labuda, D. 1994. Genome Fingerprinting by Simple Sequence Repeat （SSR）-Anchored Polymerase Chain Reastion Amplifcation. Genomic. 20（1994）:176-183 103.Yen, T. B. and S. T. Chang. 2008. Synergistic effects of cinnamaldehyde in combination with eugenol against wood decay fungi. Bioresour. Technol. 99:232-236. 104.Yi, T.; Miller, A. J.; Wen, J. 2004. Phylogenetic and biogeographic diversification of Rhus (Anacardiaceae) in the Northern Hemisphere. Mol Phylogenet. Evol. 33, 861 – 879. 105.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.