蜂膠中類黃酮物質之測定及其抗菌特性之探討

Determination and antibacterial properties of flavonoids in propolis

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[摘要]

本研究針對由行政院農委會苗栗區農業改良場、怡王蜂業及佳蜂養蜂園有限公司等三處所提供之 9 件原膠及 9 件市售蜂膠產品進行一般成份分析, 並以毛細管電泳分析技術(Capillary Electrophoresis；簡稱 CE)進行蜂膠中類黃酮物質之定性與定量, 並對不同產地來源之原膠探討其抗菌特性。原膠及市售蜂膠之一般成份分析顯示：原膠中固形物佔約 90.64~97.25%, 灰份佔約 1.93~ 10.75%, 乙醚溶出物含量佔約 41.17~99.47%；市售蜂膠產品之固形物佔約 7.41~96.38%, 灰份佔約 0.14~80.57%, 乙醚溶出物含量則約佔 7.15~80.57%。不同來源之原膠於室溫及 40oC 溫度下以 80%乙醇萃取之萃取率, 分別在 35.42~69.63%及 43. 12~69.74%, 顯示原膠樣品間之差異性頗大, 但不同溫度之萃取對萃取率之影響不大。以光 譜法測定原膠及市售蜂膠之總酚類含量, 結果分別為 5.59~11.46 %及 0.25~1.35%；總類黃酮含量分別為 4.72~12.91%及 0.73~6.81%。將 kaempferol 及 luteolin 等 15 種類黃酮標準品混合液, 以 CE 測試其最適分離條件, 發現搭配毛細管區帶電泳(CZE)及膠束動力學毛細管層析(MECC)兩種分離模式, 可完全分離此 15 種 類黃酮標準品。最適分析條件, 以緩衝溶液而言, CZE 模式為 0.1 M H3BO3 (pH9.5)-5%甲 醇液;而對 MECC 而言, 則為 0.03 M Na2B4O7-0.05 M SDS (pH 8.5)。最適溫度為 25oC, 最適偵測波長為 UV 214 nm, 使用電壓分別為 18 KV (CZE)及 15 KV (MECC)。分離效果之決定係數(R2)達 0.99 以上。將上述分離條件應用在 9 件原膠及 9 件市售蜂膠中類黃酮物質之分析, 概括而言, 原膠可被鑑定出的類黃酮物質包括 rutin (12.2~152.2 mg%)、naringin(40. 6~943.5 mg%)、naringenin (25.2~166.7 mg%)、chrysin (30.8~181.1 mg%)、quercetin (8.6~90.9 mg%)、myricetin (22.6~ 50.6 mg%)、kaempferol (12.0~1107.3 mg%) 、hesperetin (10.3~275.3 mg%)、daidzein (3.1~292.6 mg%)、genistein (24.2~224. 8 mg%)、apigenin (54.5~223.5 mg%)、quercitrin (30.5~412.7 mg%) 及 galangin(21. 0~8941.2 mg%)等 13 種, 市售蜂膠可被鑑定出的類黃酮物質包括 rutin (17.9~125.9mg%) 、naringin (14.6~212.0 mg%)、naringenin (4.5~31.3 mg%)、chrysin (6.6~478.0mg%) 、kaempferol (13.1~84.2 mg%)、hesperetin (7.7~55.9 mg%)、daidzein(12.2~77.1 mg%)、genistein (11.1~91.2 mg%)、apigenin (5.7~106.7 mg%)、quercitrin(35.8~ 331.1 mg%) 及 galangin (15.0~136.9 mg%)等 11 種。測試不同產地之原膠對 4 株革蘭氏陽性菌(Sarcina lutea, Staphylococcus aureus, Listeria monocytogenese 及 Bacillus subtilis)、4 株革蘭氏陰性菌(Salmonella typhimurium, Pseudomonas aeruginosa, Escherichia coli 及 Flavobacterium sp.)與 4 株 酵母菌(Debaryomyces hansenii, Kloeckera apiculata, Candida albica 及 Pichia membranaefaciens)之抑菌活性。結果顯示, 不論巴西產、中國大陸產或台灣產之

原膠對革蘭氏陽性菌之抑菌效果皆比對革蘭氏陰性菌為佳, 然而對酵母菌之抑菌試驗顯示，除了大陸產地原膠對 Pichia membranaefaciens 有輕微之抑制作用外, 其餘皆無抑菌效果。9 件不同產地來源之蜂膠萃取液對 G(+)菌及 G(-)菌之 MIC 值, 以抑制 G(+)菌能力而言, 台灣 產之蜂膠抑菌效果最好, MIC 值範圍在 1.2~3.1 mg/mL，其次為大陸產原膠(1.6~25.0mg/ mL)，以巴西產地最差(1.6~50.0 mg/mL)。而對抑制 G(-)菌能力而言，所有的蜂膠萃取液於 200 mg/mL 濃度下, 對 Sal. typhimurium 及 E.coli 仍無抑制能力。9 件原膠薄層層析(TLC)之生物確認試驗，發現不同產地之原膠萃取液的 Rf 值大致在 0.5~0.7 之間的區帶，均呈現有抑菌能力，顯示原膠之抑菌成份屬於極性較小之物質。

關鍵字: 蜂膠 propolis、類黃酮 flavonoid、毛細管電泳分析技術 capillary electrophoresis

[摘要]

The main objective of this study was to determine the flavonoid contentsof 9 raw propolises which provided by Miaoli District Agricultural Improvement Station, Yi Wang Honey Garden Co. and Chia Fong Apiculture Co., and 9 commercial propolis products. Meanwhile, nutritional compositions of propolises and their antibacterial activity were also surveyed. The proximate analysis showed solid, ash, and crude fat contents (ether- soluble) in 9 raw propolises ranged between 90.64~97.25%, 1.93~10.75%, and41. 17~99.47%, respectively, and of those in commercial samples were7.41~96.38%, 0.14~80.57%, and 7.15~80.57%. The extraction yields of raw propolis from different produced area, which extracted by 80% of ethanol at room temperature and 40oC, were rangedbetween 35.42~69.63% and 43.12~69.74%, respectively. The wide range of extraction yields revealed the divergence among the samples, but the differentextraction temperature seemed to have the less effect on the extraction yield. Total phenol contents of raw propolises were ranged between 5.59~11.46% andwhile of those in commercial samples were 0.25~1.35%. Total flavonoid contents determined by spectrophotometry were 4.72~12.91% in raw propolises and0.73~6. 81% in commercial samples. Fifteen flavonoid standard mixture, including kaempferol and luteolin,were analyzed by capillary zone electrophoresis (CZE) and micellarelectrokinetic capillary chromatography (MECC). The optimal separation conditions werefound to be under the 0.1M H3BO3-5% methanol buffer solution at pH 9.5 for CZEand 0. 03 M Na2B4O7-0.05 M SDS buffer solution at pH 8.5 for MECC. Besides, the optimal temperature was at 25oC, detection wavelength was UV 214 nm, and optimal voltage

was 18 KV for CZE and 15 KV for MECC. Correlationcoefficient (R2) were above 0.99 for all the flavonoid standards. Applying the optimal conditions on analyzing nine raw propolises, the flavonoid compounds couldbe identified included rutin (12.2~152.2 mg%)、naringin (40.6~943.5 mg%) 、naringenin (25.2~166.7 mg%)、chrysin (30.8~181.1 mg%)、quercetin(8.6~90.9 mg%)、myricetin (22.6~50.6 mg%)、kaempferol (12.0~1107.3 mg%)、hesperetin(10. 3~275.3 mg%)、daidzein (3.1~ 292.6 mg%)、genistein (24.2~224.8 mg%)、apigenin (54.5~223.5 mg%)、quercitrin (30.5~412.7 mg%) and galangin (21.0~8941.2mg%), and while of those in commercial samples were rutin (17.9~125.9 mg%)、naringin (14.6~ 212.0 mg%)、naringenin (4.5~31.3 mg%)、chrysin (6.6~478.0 mg%) 、kaempferol (13.1~84.2 mg%)、hesperetin (7.7~55.9 mg%)、daidzein(12.2~77.1 mg%)、genistein (11.1~91.2 mg%)、apigenin (5.7~106.7 mg%)、quercitrin(35.8~ 331.1 mg%) and galangin (15.0~136.9 mg%). Strains of G(+) bacteria (Sarcina lutea, Staphylococcus aureus, Listeria monocytogenese and Bacillus subtilis), G(-) bacteria (Salmonellatyphimurium, Pseudomonas aeruginosa, Escherichia coli and Flavobacterium sp.) and yeast(Debaryomyces hansenii, Kloeckera apiculata, Candida albica and Pichia membranaefaciens) were used respectively to detect the antibacterialactivity of the raw propolises. The results showed the antibacterial effect of propolises on G(+), no matter the propolis was from Brazil, China orTaiwan, were all superior to those on G(-). However, all the raw propolis sampleswere found to have no antibacterial effect on yeast. Tests of minimum inhibitory concentration (MIC) of nine raw propolisextracts to G(+) showed Taiwan propolis had the highest inhibitory activity and itsMIC were 1.2~3.1 mg/mL, whereas the MIC of China and Brazil propolis were1.6~25.0 mg/mL and 1.6~50.0 mg/mL respectively. As to G(-), all nine raw propolis extracts showed no inhibition activity to Sal. typhimurium and E.coli even under the concentration up to 200 mg/mL. Bioautographic assays of the TLC profiles indicated that the Rf value ofnine propolises between 0.5~0.7 showed to have antibacterial activity to Sar. lutea, Sta. aureus, Lis. monocytogenese, Bac. subtilis, Fla. sp. and thehigh Rf value revealed the chemical properties of these antibacterial compounds were belonged to the less polar materials.