以 HPLC 法及光譜法測定蜂膠中之類黃酮物質

Flavonoids determination of propolis measured by HPLC and spectrophotometry

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[摘要]

本研究採用苗栗區農業改良場所提供之 6 件原膠，及市售之 12 件蜂膠產品，進行其一般成分分析，並以以 HPLC 法及光譜法進行蜂膠中類黃酮物質之定性及定量研究。原膠及市售蜂膠之一般成分分析顯示：原膠中固形物佔約 91.09~96.09%，灰分佔約 1.39~5.64%，粗脂肪含量佔約 38.24~99.87%；市售蜂膠之固形物佔約 8.49~96.72%，灰分佔約 0.51~33.36%，粗脂肪含量則約為 6.98~92.58%。以 HPLC 分析醣類成分，原膠中含有少量的果糖(12.17~29. 68mg%)及葡萄糖(13.62~64.45mg%)，部分市售蜂膠則僅含葡萄糖或蔗糖，多數市售蜂膠甚至完全偵測不到任何糖分。脂肪酸之分析結果發現，原膠或市售蜂膠均僅含微量的脂肪酸，總含量不及 2%，顯示高含量的粗脂肪(即乙醚溶出物)非來自油脂成分。原膠以不同濃度之酒精溶液萃取，結果顯示在 60%以上之酒精溶液中即具有較佳之萃取效果。各原膠之最大萃取率的差異性很大，範圍在 47.5~99.2%，其中巴西或中國產地的蜂膠萃取率較低，而台灣產地的則較高。以 HPLC 法鑑定蜂膠中類黃酮物質，以 15 種類黃酮標準品進行測試，尋得之最佳分離條件為 ：RP-18 之分離管柱；動相為水：甲醇：醋酸(80：75：5)；流速為 1ml/min；偵測器則為 UV 275nm。以上述條件分離 Chrysin 等 15 種類黃酮標準品，可得相當良好的分離效果，每一標準品之線性關係(R2)均達 0.99 以上。將此條件應用在原膠及市售蜂膠之分析上，結果顯示，即使來源相同之原膠，所含的類黃酮種類及含量亦不盡相同，而來自不同產地的蜂膠，則差異性更大。概括而言，原膠中可被鑑定出來的類黃酮物質包括 chrysin (8~3917mg%)、apigenin (3~219mg%)、Rutin (22~1257mg%)、Morin (48~339mg%)、Quercetin (8~728mg%)、Myricetin(72~1543mg%) 、Kaempferol (2~200mg%)、Galangin (102~1282mg%)、Naringin (147~922mg%)、(±) Naringenin (52~442mg%)、Daidzein (2~60mg%)及 Genistein (0.7~3.6mg%)等；而市售蜂膠中可被鑑定的類黃酮物質則包括 Chrysin (3~1727mg%)、Apigenin (2~9mg%)、Quercetin (36~439mg%)、Myricetin (31~439%mg)、Kaempferol (1~38mg%)、Galangin(3~489mg%) 、Daidzein (0.7~1mg%)及 Genistein (2~8mg%)等。總類黃酮含量之測定以光譜法行之，分別採用 AlCl3 及 2,4-Dinitrophenylhydrazine 為不同的呈色劑。由測試 15 種標準品之結果顯示，AlCl3 法對 flavone 及 flavonol 等物質具較佳之反應性，而 2,4-Dinitrophenylhydrazine 法則對 flavanone 較佳。上述二法應用於原膠之酒 精萃取液及市售蜂膠之分析上，結果發現兩者的差異性相當大，6 件原膠中總類黃酮含量，以 AlCl3 法測得之範圍在 0.03~7.73%之間，而以 2,4-Dinitrophenylhydrazine 法則在 0.19~ 21.48%之間。另外，12 件市售蜂膠中的

總類黃酮含量，以 AlCl3 法測得之含量為 0.55~3.95% ，而以 2,4-Dinitrophenylhydrazine 法測得的含量為 0.29~21.30%。由於 AlCl3 法及 2,4-D 法分別針對不同的類黃酮物質具有不同的專一性，故本研究建議將兩 種方法測得之總類黃酮含量相加之後所得之數值可能更為接近樣品之真正總類黃酮的含量 。

關鍵字: 蜂膠 Propolis、類黃酮 Flavonoid

[摘要]

The objectives of this study were to determine the flavonoid contents of 6raw propolises provided by Miaoli District Agricultural Improvement Stationand 12 commercial propolis products, analyzed the nutritional compositions and measured the flavonoids by HPLC and spectrophotometry. The proximateanalysis showed solid, ash and crude fat contents (ether-soluble) in 6 rawpropolises were 91.09~96.09%, 1.39~5.64%, 38.24~99.87%, respectively, and of thosewere 8. 49~96.72%, 0.51~33.36%, and 6.98~92.58% in commercial samples. Saccharides determination showed there were mainly fructose (12.17~29.68mg%) andglucose (13.62~64.45mg%) in raw propolis, and only trace of glucose or sucrose were found in commercial propolis. The fatty acids analysis showed both raw and commercial propolises contained only a few different kind of fatty acidsand their total amounts were less than 2%. The raw propolises were extracted by different concentration of alcohol solution. The results showed the extraction yields could be relativelybetter when alcohol concentration was over 60%, and the yield varied from47.5~99.2% among the raw propolis from different produced area. Propolis was extracted and filtered before determined by HPLC. The optimal conditions for flavonoid identification were found as follows: RP-18 was applied as separation column, dH2O: methanol: acetic acid (80:75:5, v/v)was used as mobile phase, flow rate was adjusted to 1.0ml/min, and UV (275nm)was as a detector. Good linear relationship(R2＞ 0.9984) could be reached when using the conditions above to determine the 15 flavonoid standards. The variances of types and contents of flavonoids were both existed in rawsamples even from the same source, and their variances became much significantamong samples from different production area. Summarized speaking, the flavonoid compounds identified in raw samples included Chrysin (8~3917mg%), Apigenin(3~ 219mg%), Rutin (22~1257mg%), Morin (48~339mg%), Quercetin (8~728mg%), Myricetin (72~1543mg%), Kaempferol (2~200mg%), Galangin

(102~1282mg%), Naringin (147~922mg%), (±)Naringenin (52~442mg%), Daidzein(2~60mg%), and Genistein(0.7~3.6mg%), and while of those in commercial propolis sampleswere Chrysin(3~1727mg%), Apigenin(2~9mg%), Quercetin (36~439mg%), Myricetin(31~439% mg), Kaempferol (1~38mg%), Galangin (3~489mg%), Daidzein (0.7~1mg%), and Genistein (2~8mg%). Total flavonoid contents were determined by two colorimertric methods, and using AlCl3 and 2,4-Dinitrophenylhydrazine as different colorific reagent, respectively. Data from the 15 flavonoid standards tested showed AlCl3method was more sensitive to flavone and flavonol compounds, while 2,4- Dinitrophenylhydrazine method were found to be more suitable to flavanone compounds. Significantly difference existed in total flavonoid contentswhen applying the two different colorimetric methods on raw and commercialpropolis samples. In raw propolis, it ranged from 0.03~7.73% by AlCl3 method, and0.19~ 21.48% by 2,4-Dinitrophenylhydrazine method. As to the 12 commercialproducts, the total flavonoid contents were 0.55~3.95% and 0.29~21.30% when measuredby the two methods, respectively. Due to the different specificity of AlCl3and 2, 4-Dinitrophenylhydrazine reacted with various flavonoids, it is suggestedthat total sum of the data adding up from these two methods might be moreclosed to the real value of total flavonoid contents.