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Estimation of Total Flavonoid Content in Propolis by Two Complementary Colorimetric Methods

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ABSTRACT

Flavonoids, with various biological activities, are considered as key compounds in propolis. In this study, quantitative determinations of flavonoids in propolis were conducted by two complementary colorimetric methods, aluminum chloride method and 2,4-dinitrophenylhydrazine method. Results suggested that the sum of flavonoid contents determined by the above two individual methods may represent the real content of total flavonoids. In this work, six raw propolis samples were investigated and the total contents of flavonoids ranged from 10.38 ± 0.14% to 24.91 ± 0.53%. As for the 12 commercial propolis products examined, the levels of total flavonoids in tinctures were all below 7% and those in powdery products varied from 2.97 ± 0.05% to 22.73 ± 0.72%.

Key words: Propolis, flavonoids, flavones, flavonols, flavanones, flavanonols, quantitative determination, colorimetric method, aluminum chloride reaction, 2,4-dinitrophenylhydrazine reaction

INTRODUCTION

Propolis, the material used by bees to protect their hives, is a glue-like substance composed of plant resins, bee waxes and pollens. Since various biological activities of propolis such as antibacterial, antiviral, anti-inflammatory and anaesthetic properties were found[1-3], it is used as a health food. The chemical composition of propolis is quite complicated and over 150 components have been identified[2,4]. Among these compounds flavonoids were suggested to be responsible for the biological activities[5,6]. Therefore, the content of flavonoids is considered as an important index for evaluating propolis quality.

The analysis of flavonoids in propolis has been done by colorimetric methods[7-10], thin layer chromatography[9,11], gas chromatography[12,13], gas chromatography-mass spectrometry[14-16] and high performance liquid chromatography[7,15,17,18]. Although chromatographic techniques in combination with absorption spectrum analysis and mass spectrometry provide definitive information for identification and quantification of flavonoids in propolis, these methods usually require advanced instruments, various authentic standards and are time-consuming. On the other hand, colorimetric methods targeting flavonoids of similar structures are convenient and appropriate for routine analyses. However, none of the colorimetric methods can detect all kinds of flavonoids. For instance, within four major groups of flavonoids in propolis, only flavones and flavonols were found to complex stably with aluminum chloride[19], while flavanones and flavanonols reacted better with 2,4-dinitrophenylhydrazine[8].

Considering the need of criteria for both consumers and government agencies to evaluate numerous commercial propolis products, we propose to determine the content of total flavonoids in propolis complementarily by aluminum chloride and 2,4-dinitrophenylhydrazine reactions. In this work, six raw propolis samples and 12 commercial products were investigated to compare the differences of results from each colorimetric analysis.

MATERIALS AND METHODS

I. *Materials*

Six raw propolis samples named Taiwan-1, Taiwan-2, Taiwan-3, Brazil-1, China-1 and China-2 were provided by Miaoli District Agricultural Improvement Station (Miaoli, Taiwan). Twelve commercial propolis products were obtained from local retailers and the description is shown in Table 1. All samples were stored at room temperature until analysis. Fifteen flavonoid standards including chrysin (catalog number C-3018), apigenin (A-3145), luteolin (L-9283), rutin (R-5143), morin (M-4008), quercetin (Q-0125), myricetin (M-6760), kaempferol (K-0133), quercitrin (Q-3001), galangin (06829HS), naringin (N-1376), (±)-naringenin (N-5893), hesperetin (H-4125), daidzein (D-7802) and genistein (G-6776) were purchased from Sigma-Aldrich (St. Louis, MO) with product numbers in parentheses. The systematic names of 15 flavonoid standards are listed in Table 2. All reagents used were of analytical grade. 2,4-

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