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Original Article

Antibacterial activity of propolins from Taiwanese green propolis

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ABSTRACT

Taiwanese green propolis is a prenylated flavonoid rich honeybee product and propolins isolated from Taiwanese green propolis exert a broad spectrum of biological activities, such as anti-cancer and anti-oxidant. However, the anti-bacterial effects of Taiwanese green propolis or propolins are still poorly understood. In the current study, the antibacterial effects of Taiwanese green propolis and propolins were evaluated. Results show that the maximum dry matter yields of Taiwanese green propolis were observed in the 95% and 99.5% ethanol extracts compared to other extraction methods. Consistently, the highest concentration of propolins C, D, F and G from Taiwanese green propolis was obtained in 95% and 99.5% ethanol extracts. Propolins inhibited the growth of gram-positive bacterial strains (*Staphylococcus aureus*, *Bacillus subtilis*, *Listeria monocytogenes* and *Paenibacillus larvae*). The average minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of propolins from ethanol extracts were 20 μg/ml. Among the propolins, propolin C had the highest antibacterial activity. Furthermore, Taiwanese green propolis also showed antibacterial activity against methicillin-resistant *S. aureus* (MRSA). In conclusion, these results demonstrate that Taiwanese green propolis and propolins have significant antibacterial activity, particularly against gram-positive bacterial strains.

1. Introduction

Propolis is a natural and resinous product collected by honeybees (*Apis mellifera*) from buds and leaves of trees and then mixed with beeswax. Propolis is used by bees for the construction, repair and protection of beehives due to its mechanical properties and biological activity. In humans, propolis has been widely used as a folk medicine worldwide from ancient times. It has been demonstrated that propolis from Europe and China contains high levels of flavonoids and phenolic acid esters [1]. In addition, several studies have reported that the major compounds with biological activity of Brazilian propolis are prenylated *p*-coumaric acids and diterpenic acids [2]. Currently, ten prenylated flavanone derivatives, propolins A–J, have been isolated from Taiwanese green propolis and characterized [3–6]. Propolins C, D, F and G

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are the most abundant propolins in Taiwanese green propolis [4,6]. Furthermore, Taiwanese green propolis has been reported to have a broad spectrum of biological activities, including anticancer [5] and antioxidant [4].

Over the past few years, several studies have examined the antibacterial activity of propolis. The antimicrobial activity of propolis may differ depending on its geographic region and the season [7,8]. Previous study identified season as a critical factor for determining the total propolin levels in Taiwanese green propolis [9]. Overall, the propolis collected from different regions showed activity against gram-positive bacteria, but showed limited activity against gram-negative bacteria [8,10–14]. Taiwanese green propolis has been shown to have antimicrobial activity against gram-positive bacteria [15–17].

The active compounds in propolis vary depending on area and season. Brazilian green propolis is rich in prenylated derivatives including coumaric acid and diterpenic acids [18]. The main biologically activity compounds in propolis from European are flavonoid aglycones and phenolic acids [1,19,20]. Flavonoids and esters of phenolic acids contribute to the antimicrobial activity of propolis [8,21]. However, tropical propolis still exhibited similar antibacterial activity, despite these substances being undetectable. These findings indicate that different substance combinations in different types of propolis are essential for the biological activity [11]. Furthermore, a synergistic effect of antibacterial activity was observed between the flavonoids in propolis [22]. However, the effect of propolins isolated from Taiwanese green propolis, including propolins C, D, F and G and any interaction between propolins on antibacterial activity have not been studied.

The purpose of this study is to evaluate the antibacterial activity of propolins from Taiwanese green propolis.

2. Methods

2.1. *Preparation of ethanol extracts*

Taiwanese green propolis was purchased from a local commercial company. The propolis was originally collected from beehives located at different regions in Taiwan from May to July 2015 using propolis collectors. Propolis from the collectors at each location was gathered every month and kept at −20 °C until processed. The ground propolis (10 g) was extracted with 100 ml of 99.5%, 95%, 80%, 70%, 60% of ethanol, methanol and diethyl ether for extraction by shaking (250 rpm) at 25 °C for 48 h. For water extraction, 10 g of ground propolis was extracted with 100 ml H_2O by shaking (250 rpm) at 50 °C for 48 h. The extracts were then filtered through a Whatman no. 4 filter paper and reconstituted to its original volume (100 ml) with original solvents.

2.2. *Analysis of propolins*

The extract was concentrated by vacuum evaporation, reconstituted and then filtered by syringe filter with a 0.22 μm membrane. The extracts were then tested for propolin (C, D, F and G, chemical structures shown in Fig. 1) content by high performance liquid chromatography (HPLC). In brief, an Agilent 1200 HPLC system (Santa Clara, CA, USA) fitted with a programmable UV detector was used in this study. A reverse phase RP-18 column (ZORBAX SB-C18, 4.6 × 250 mm; Agilent, USA) protected with a guard was used throughout the experiments. Twenty microliters of the samples were injected into the HPLC column heated to 30 °C. The mobile phase consisted

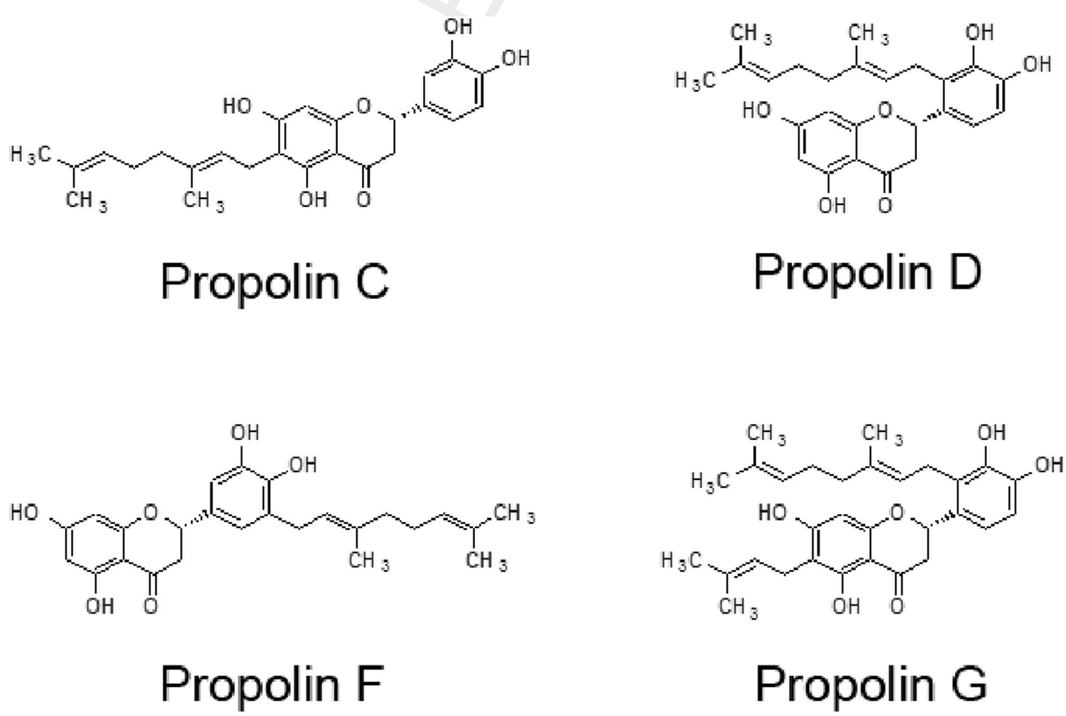

Fig. 1 – Structure of propolins identified in Taiwanese green propolis.