

Properties of Water-Soluble Propolis Made with Honey

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Abstract

Propolis is made by bees collecting protective material or essence of plants and mixing with saliva and enzymes produced by the salivary glands. It is used to repair the inside of the honeycomb, keep it sterile, and adjust the temperature and humidity. Propolis is a natural antibiotic substance that it is used to make a clean room by coating the cell before the queen bee lay eggs, and preventing the bacteria from invading by using with wax when sealing the nursery room. Propolis extract is a health functional food with antioxidant and oral antimicrobial effects. In order to use propolis in food, its active ingredients are extracted with ethanol. Water-soluble propolis was prepared by mixing and stirring honey and ethanol extracted propolis (EEP) solution. When 1kg of honey and 100ml of ethanol extracted propolis solution were mixed and stirred, the total flavonoid content of water-soluble propolis was 6.6 ± 1.1 mg/10g, and the free radical scavenging effects of water-soluble propolis were 54 to 74%.

Key words: Antioxidant effects, TFC (Total flavonoid content), Process, Ethanol extracted propolis (EEP)

INTRODUCTION

Propolis is a substance that keeps a bee filled with honeycomb safely. It is a sticky colloidal material with many colors, such as dark brown, which bees collect as a protective material of the growth point of the bees and the liquid and mix it with the saliva. Additionally, it is used for temperature and humidity control. It is also necessary to prevent the bees from decaying debris and the beehive. The most important use of propolis is larvae protection by coating thin layer of this on brood cell for keeping eggs and larvae from microbe before laying eggs of queen bee, and a little amount of propolis was mixed with bee wax for brood cell sealing (Fearnley, 2001).

Propolis contains a variety of constituents depending on the specificity of the flora and collection season, among

which phenolic compounds and flavonoids have strong antioxidant effects (Kurek-Gorecka *et al.*, 2014). Propolis is a composite material consists of various ingredients such as resin and aromatic (45~55%), bees wax (25~35%), volatile essential oil (10%), pollens, minerals (5%), tannins, bee secretions, enzymes (Moreno *et al.*, 2000). The polyphenolic component and flavonoids in propolis show significant anti-oxidant effects. There are several studies referring correlation between anti-oxidation effect and polyphenolic composition in propolis (Bors *et al.*, 1990; Heim *et al.*, 2002; Russo *et al.*, 2002; Kumazawa *et al.*, 2004), and these activities get synergy effect by complex reaction between phenol compound and resin type materials (Burdock, 1998; Markham *et al.*, 1996).

In this study to increase the utilization of propolis extract, we prepared water-soluble propolis by mixing

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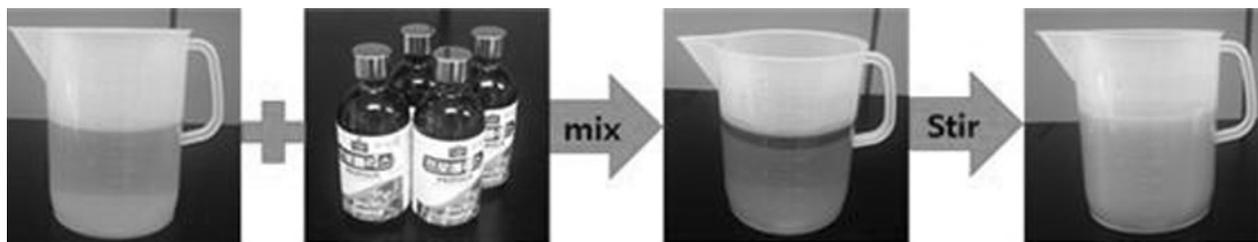


Fig. 1. Water-soluble propolis manufacturing process.

Table 1. Water-soluble propolis recipes

Extracted propolis tincture (ml)	50	100	200	500	1000
Acacia honey (kg)	1	1	1	1	1

honey and ethanol extracted propolis. Additionally, we evaluated total flavonoid contents and antioxidant effects of the water-soluble propolis.

MATERIALS AND METHODS

Ethanol extracted propolis solution

Ethanol extracted propolis (EEP) solution was prepared by extracting Korean propolis. Propolis collected from 10 regions (except Jeju region) in Korea and was mixed with same weights. Mixed propolis (1kg) was extracted with 80% ethanol (3.5L) (Woo *et al.*, 2012).

EEP solution was filtered with Whatman No. 2 filter paper and then concentrated to 18% propolis concentration.

Honey

Hairybetch honey was collected in Seocheon, 2014. Citron honey was purchased from Ivy Farmer's Association. Another variety of honey were purchased from Korean Beekeeping agricultural cooperative. The moisture content was 18.5%.

Water-soluble propolis made with Honey

Water-soluble propolis was made with honey and EEP solution. We prepared 1kg honey in beaker, poured 50 to 1,000ml EEP solution, and stirred with stirrer over 1 hour (Fig. 1, Table 1).

Total flavonoid content (TFC) analysis in propolis

The concentration of the total flavonoid content (TFC) present in propolis was analyzed using the colorimetric method (KFDA, 2010).

0.1g of water-soluble propolis was dissolved with 5ml of 80% ethanol, and then 80% ethanol was added to make 10ml. Then 0.5ml of sample was placed in the test tube, added ethanol 1.5ml, 10% aluminum nitrate (Sigma, USA) solution 0.1ml, water 2.8ml, stirred sufficiently and stationed for 40 min. And another process which aluminum nitrate (Sigma, USA) solution was substituted with 0.1ml water was done absorbance of both sample fluid bed was measured using 10mm cell with 415nm wave length. Using the value by subtracting latter process absorbance value from former one, then total flavonoid content a



Fig. 2. Properties of water-soluble propolis.

Table 2. TFCs of water-soluble propolis and daily intake

Water-soluble propolis	50ml/kg	100ml/kg	200ml/kg
TFC (mg/10g)	2.7 ± 0.7	6.6 ± 1.1	18.0 ± 1.0
Daily intake (g)	59~63	24~26	8.8~9.4

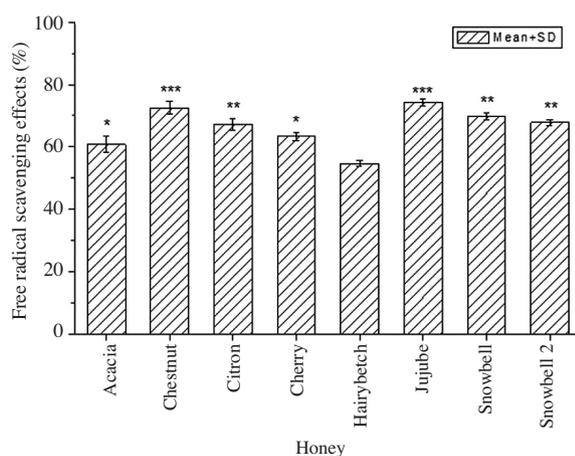


Fig. 3. DPPH free radical scavenge effects (%) of water-soluble propolis made with various honey.

(mg/ml) was calculated using calibration curve acquired by quercetin.

$$\text{TFC (w/w or w/v)} = a \times 10\text{ml}/\text{Sample amount (g or ml)}$$

Free radical scavenging effect by DPPH method

Using water-soluble propolis, samples with $100\mu\text{g}$ concentration were prepared and that of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH, sigma, USA) was 0.2mM . DPPH solution 1ml mixed with 2ml sample, stationed 10min , and absorbance measured at 517nm wavelength using UV-VIS spectrophotometer (Lambda 10, Perkin-Elmer, USA). Free radical scavenging effect calculated using the

following formula.

$$\text{Radical scavenging effects (\%)} = 100 - (A/B \times 100)$$

A: sample absorbance (517nm),

B: blank absorbance (517nm)

RESULTS AND DISCUSSION

Properties of water-soluble propolis

To make a water-soluble propolis, about 50 to 200ml of propolis extract per 1kg of honey is suitable. Water-soluble propolis is easily soluble in water and has mild tastes and aromas. When the amount of EEP solution became large, it was easily separated honey and EEP solution (Table 1).

When the extracted propolis solution was mixed with water, many floating matters were generated, but there are no floating matters when water-soluble propolis is mixed with water (Fig. 2). The taste of water-soluble propolis made with honey, is a good test, but it also has a slightly bitter flavor inherent to propolis.

Total flavonoid contents (TFC)

When 1kg of acacia honey and 50ml of propolis extract were mixed, the TFC of water-soluble propolis was $2.7 \pm 0.7\text{mg}/10\text{g}$, when the propolis extract was 100ml , TFC was $6.6 \pm 1.1\text{mg}/10\text{g}$, when the propolis extract was 200ml , it

was 18.0 ± 1.0 mg/10g. The daily intake of propolis for health functional foods is 16 to 17mg of TFC (KFDA, 2010), water-soluble propolis made of 50ml propolis extract should be taken at 59 to 63g, 24~26g for 100ml propolis extract, 8.8~9.4g for 200ml propolis extract (Table 2).

DPPH free radical scavenge effects of water-soluble propolis made with various honey

Examination of the anti-oxidative effects of water-soluble propolis was used DPPH free radical scavenge methods. DPPH free radical scavenging effects of water-soluble propolis with various honey were 54 to 74%. These results are slightly lower to EEP solution 75~80% (Woo *et al.*, 2013), there are differences depending on the kinds of honey.

One-Way ANOVA on columns selected between Citron, Snowbell and snowbell 2 honey are not significantly different, at the $p < 0.05$ level. Additionally, there is no significant difference between chestnut and jujube honey, between acacia and cherry blossom honey.

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