Short Communication

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Comparison of Anti-oxidant Activity of Ethyl Acetate Extracts of Different Floral Honeys from Korea

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Abstract

This study was done to compare anti-oxidant capacity of honeys from different floral sources (acacia, chestnut, linden, citrus and styrax). The ethyl acetate extracts of individual honey samples were taken to measure the anti-oxidant activity and total phenolic content (TPC) by 2,2-diphenyl-1-picryhydrazyl (DPPH) free radical scavenging assay, and Folin-Ciocalteu's assay respectively. The ethyl acetate extract of chestnut honey at the concentration of 1mg/mL showed the highest free radical scavenging activity of 45%. Its TPC which was found to be 17μg GAE/mg, was also the highest value compared to that of other honey extracts. The study results indicate that phenolic compounds in honey are the major constituents to contribute for their antioxidant activity.

Key words: Korean honeys, Anti-oxidant activity, Total phenolic content

INTRODUCTION

Floral honey, a natural sweetener collected by bees from nectar, is a complex mixture of sucrose, fructose, glucose, water, proteins, amino acids, vitamins, minerals and organic acids, and widely used as supplementary diets, tonics, cosmetics and so on (Chang *et al*., 1988; Burlando and Cornara, 2013). It also has been reported to have various biological activities as anti-oxidant, anti-inflammatory and anti-bacterial agents (Yaghoobi *et al*., 2013). Since, the classification of honey is based on their floral sources such as acacia, chestnut, astragalus, heather, clover, lavender, oak etc and there has been hundreds of types of honey found globally (Can *et al*., 2015). Monofloral honeys show significant difference of chemical composition, taste, scent, color and purpose of use in accordance with floral origin (Ozcan and Olmez, 2014). Typically, manuka (*Leptospermum scoparium*) honey produced in New Zealand is used as material for wound dressing, and is sold as pharmaceuticals in USA and Europe, and is known as an effective therapeutic agent for severe burns. (Lusby *et al*., 2002). Heather (*Calluna vulgaris*) and lavender (*Lavandula stoeclias*) honeys, which contained constituents such as phenolic compounds from nectar, are known to have anti-oxidant activity (Can *et al*., 2015). Thus, honey possesses different biological activities which are affected greatly by secondary metabolites and enzymes from floral origin. In present study, we determined the antioxidant activity and phenolic content of five different monofloral honeys (acacia, chestnut, linden, citrus and styrax honeys) from Korea and analyzed the relationship between total phenolic contents and the antioxidant activity of each type of honey.

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MATERIALS AND METHODS

Honey samples

Acacia honey from *Robinia pseudoacacia*, chestnut honey from *Castanea crenata*, linden honey from Tilia amurensis, citrus honey from *Citrus unshiu* and styrax honey from *Styrax japonica* produced in 2014 were obtained from Korea Apicultural Agriculture Cooperative (Seoul, Korea).

Extraction

500g of each five honey sample was extracted with ethyl acetate (EA, SK chemicals, Seongnam, Korea) using hand mixer in water bath of 60°C for 5 min and then supernatant was evaporated *in vacuo* to give the ethyl acetate extract. Yield of 669, 960, 519, 960, and 800mg were obtained from ethyl acetate extracts of each honey sample.

DPPH assay

The antioxidant activities of samples were carried out by free radical scavenging activity test using 2,2-diphenyl-1-picryhydrazyl (DPPH, Sigma, St. Louis, MO) reagent (Hazra *et al*., 2010). 20μL of the samples at different concentrations (100, 500 and 1000μg/mL) were added to 180μL of a DPPH methanolic solution (200μM) and left it at room temperature in the dark. The optical density of reactive solutions was measured at 517 nm using ELISA reader (Molecular device spectramax M2^e, Sunnyvale, CA). The radical scavenging activity was calculated from the formula given below.

Radical scavenged (%) = $[(Abs_0\text{-}Abs_1)/Abs_0] \times 100$

Abs_0: the absorbance of blank, Abs_1: the absorbance of samples at different concentrations.

Total phenolic content

Total phenolic content (TPC) of samples was evaluated by Folin-Ciocalteu's (Sigma, St. Louis, MO) assay according to the modified method of Chun *et al*. (2003). Sample (1mg/mL) was vortexed with 1N Folin-Ciocalteau's reagent (1mL) and distilled water (5mL). After incubation at room temperature for 5 min, 10% Na_2CO_3 (1mL) solution was added. The absorbance was measured at 725 nm using ELISA reader. The TPC was presented as μg of gallic acid equivalents (GAE) in mg of sample.

Thin layer chromatography (TLC) analysis

Ethyl acetate extracts of five different honeys was subjected to TLC on SiO_2 plate (Merck 5715, NJ, USA) and C_{18} plate (Merck 15683) using mobile phase CH_2Cl_2 : MeOH : H_2O = 9 : 1.5 : 1 and MeCN : H_2O : CH_2O_2 = 45 : 55 : 0.01, respectively. 10% H_2SO_4 and 1% $FeCl_3$ were used for the detection of spots.

RESULTS AND DISCUSSION

Oxidative stress has been one of the major causes of different pathological conditions like Parkinson's disease, Alzheimer's disease, vascular disease and aging (Mariani *et al*., 2005; Okusaga, 2014). It occurs due to the production of excessive reactive oxygen species (ROS) which oxidize the intracellular constituents like lipids and proteins (Haraguchi *et al*., 2000). So, the antioxidants which fight against the oxidative stress are very essential for a healthy body. Many researches have been conducted on development of antioxidants from natural sources available as food. Among them honey is also an important natural food having various medicinal values. In this study we evaluated the antioxidant property of the ethyl acetate extracts of five monofloral honeys: acacia (AH), chestnut (CH), linden (LH), *Citrus* (CIH) and *Styrax* honeys (SH). Organic solvents such as methanol, ethanol, acetone and ethyl acetate are widely used for extraction of secondary metabolites of natural products like plant sources (Kim *et al*., 2009). Since, ethyl acetate has higher ability to effectively elute the phenolic portion than other solvents, it was used for the extraction of honeys derived from floral origin. The ethyl acetate extracts of five honeys scavenged DPPH free radicals in a dose dependent manner. The ethyl acetate extract of CH at the concentration of 1mg/mL exhibited the

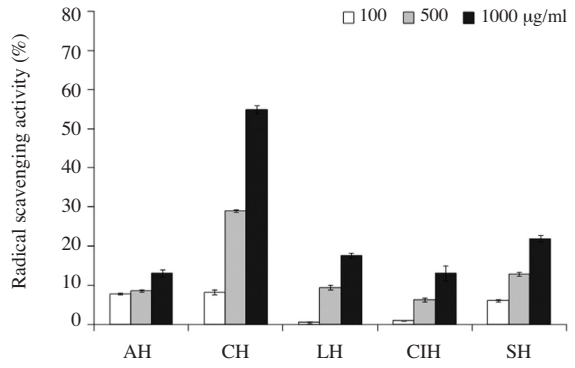

Fig. 1. DPPH free radical scavenging activity of ethyl acetate extracts of five different honeys from Korea. AH: acacia honey, CH: chestnut honey, LH: linden honey, CIH: citrus honey and SH: styrax honey.

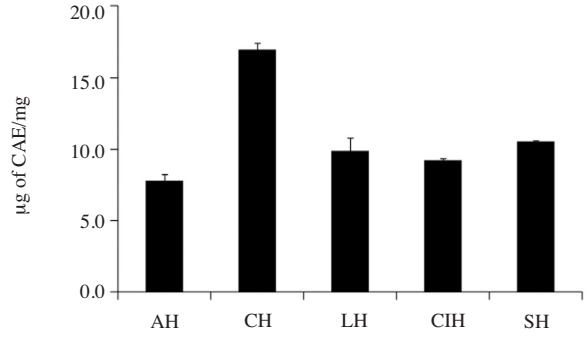

Fig. 2. Total phenolic content of ethyl acetate extracts of five different honeys from Korea. AH: acacia honey, CH: chestnut honey, LH: linden honey, CIH: citrus honey and SH: styrax honey.

highest radical scavenging activity (Fig. 1).

Kim *et al*. (2010) have been reported that content of phenolic compounds in honeys has a correlation with antioxidant activity and our result followed the similar pattern. Phenolic compounds have been recognized as a natural antioxidants present in plant kingdom (Goufo and Trindade, 2014). Results in Fig. 2 showed that TPC in honey samples was highest in CH (17μg GAE/mg), and then followed by SH (10.5μg GAE/mg), CIH (9.2μg GAE/mg), LH (9.9μg GAE/mg) and AH (7.8μg GAE/mg).

Pearson correlation coefficient (R^2) between antioxidant activity (at the concentrations of 100, 500 and 1,000μg/mL) and total phonolic content in honeys, measured by DPPH and Folin-Ciocalteu's assays was more than 0.97 and there

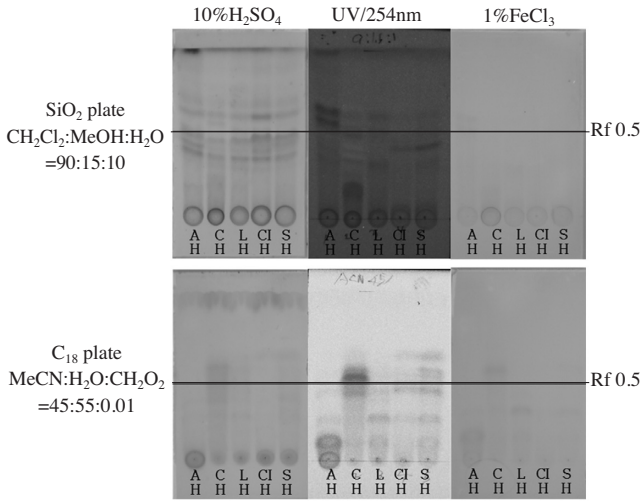

Fig. 3. TLC chromatograms of ethyl acetate extracts of five different honeys from Korea. Rf: retardation factor, AH: acacia honey, CH: chestnut honey, LH: linden honey, CIH: citrus honey and SH: styrax honey.

was a noteworthy correlation with each other, showing that phenolic compounds were responsible for dominant antioxidant activity. TLC analysis of five different honey extracts on SiO_2 and C18 TLC plates showed similar chromatogram suggesting similar chemical constituents in all of them. When TLC plates were sprayed with 10% H_2SO_4, several spots were detected at R_f value around 0.2-0.7. SiO_2 and C18 TLC plates treated with 1% $FeCl_3$ revealed that AH and CH had different pattern compared to other samples. Most components existed in honey samples were detected at 254 nm of UV wavelength (Fig. 3). We found that ethyl acetate extract of CH displayed a higher antioxidant activity and TPC than other honey extracts. These findings suggest that TPC in honeys may be responsible for the antioxidant property.

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