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Antioxidant Activity, Phenol and Flavonoid Contents of Indonesian Propolis

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In present work, a systematic study of the relative antioxidant activity of Indonesian propolis collected from different regions of Java have been reported. The total phenol varied from 136.2 ± 10.1 to 203.7 ± 9.8 mg g^{-1} in the extracts. Flavones and flavonols were determined using aluminium chloride and expressed as quercetine equivalent, while flavanones were determined using 2,4-dinitrophenylhydrazine and expressed as naringenin. Contents of flavones and flavonols were similar for most samples and ranged from 1.7-7.3 %. The content of flavanones in propolis samples varies from 1.2-5.9 %. 1,1-Diphenyl-2-picrylhydrazine (DPPH) radical scavenging effect of the extracts was determined spectrophotometrically. IC_{50} of the extracts and the standard compounds quercetine was 8.44, 130.35, 19.27, 91.03 and 2.58 μg mL^{-1}.

Key Words: Antioxidant, Phenols, Flavonoids, 1,1-Diphenyl-2-picrylhydrazine, Propolis.

INTRODUCTION

Propolis (bee glue) is a stickly dark, coloured material that honey bees collect from living plants, mix with wax and use in constructions and adaptation of their nests[1]. Propolis is a natural product with a great potential for use in human and veterinary medicine. On the other hand, unlike products derived from medicinal plants, its composition is extraordinary variable, samples from different geographic origin may possess totally different chemical compositions.

Propolis is one of the richest sources of the plant phenolics (flavonoids and phenolic acids), which are generally known as rather strong antioxidants[2-4]. More than 200 constituents of propolis have been identified such as polyphenol (flavonoid aglycones, phenolic acids and their ester), lignans, sesquiterpene, quinones, steroids and amino acids. The composition, however, varies greatly according to geographical and botanical origin[5].

Flavonoids, one of the main groups of phenolic compounds in propolis, are the key compounds for estimation of propolis quality. Flavonoids are a widely distributed group of polyphenolic compounds characterized by a common benzo-γ-pyrone structure. Over 4,000 different flavonoids have been described and they are categorized into

flavonols, flavones, flavanones, isoflavones, catechins and anthocyanidins[6]. Flavonoids in propolis are present as aglycones (without the sugar component), These lipophilic flavonoids are chemically divided into subgroups of flavones, flavonones, flavonols, dihydro-flavonols and chalcones. The concentration of flavonoids in propolis depends on the geographic origin and ecosystem (plant sources). It is known that flavonoid concentration will affect the biological activity of propolis.

Flavonoids have shown potential health benefits arising from the antioxidative effects of these phytochemicals. These properties are attituted to the phenolic hydroxyl groups attached to the flavonoid structure. Scavenging of free radicals seems to play a considerable part in the antioxidant activity of flavonoid compounds. Because of the phytogeographic dependence of the flavonoid content in raw propolis, the aim of present work is to determine the concentration of flavonoids, phenols and antioxidant activity in raw propolis samples collected from four different localities in Indonesia.

EXPERIMENTAL

Propolis origin: Propolis samples were collected from four different localities in Indonesia in April 2007 Sukabumi (West Java), Batang (Central Java) and Lawang (East Java). Hand-collected propolis samples were kept dessicated in the dark up to their processing. Voucher specimens are deposited in the Department of Pharmacology, Faculty of Pharmacy, Pancasila University, Indonesia.

Ethanolic extract of propolis (EEP): The ethanolic extract of propolis for testing was prepared by stirring propolis in ethanol (Synth) 96º GL (50 g in 100 mL ethanol) and submitting to filtration. Final ethanolic extract of propolis yielded a matrix standard concentration of 50 % v/v.

General procedure

Determination of flavones and flavonols: Flavones and flavonols in propolis were expressed as quercetine equivalent. Quercetine (Sigma, Germany) was used to make the calibration curve (standard solutions of 12.5, 25.0, 50.0, 80.0 and 100.0 μg mL^{-1} in 80 % ethanol (v/v). The standard solutions of ethanolic extract of propolis (0.5 mL) were mixed with 1.5 mL 95 % ethanol (v/v), 0.1 mL 10 % aluminium chloride (m/V), 0.1 mL of 1 mol L^{-1} potassium acetate and 2.8 mL water. The volume of 10 % (m/V) aluminium chloride was prepared by the same volume of distilled water in blank. After incubation the solution was kept for 0.5 h, the absorbance of the reaction mixture was measured at 415 nm[7].

Determination of flavanones: Flavanones in propolis were expressed as (±)-naringenin equivalent. (±)-Naringenin (Sigma, Germany) was used to make the calibration curve (standard solution of 0.25, 0.30, 0.50, 1.00 and 2.00 mg mL^{-1} in methanol). One mL of standard solution or ethanolic extract of propolis was separately mixed with 2 mL of 1 % 2,4-dinitrophenylhydrazine (m/V) and 2 mL of methanol at 50 °C for 50 min. After cooling at room temperature the solution was mixed with 5 mL of 1 % potassium hydroxide (m/V) in 70 % ethanol (v/v). Then, 1 mL of the

mixture was taken and centrifuged at 1000 g for 10 min and the supernatant was filtered through Whatman No. 1 filter paper. The filtrate was adjusted to 25 mL. The absorbance of the filtrate was measured at 495 nm[7].

Antioxidant assay: The stable 1,1-diphenyl-2-picrylhydrazine (DPPH) was used for determination of the free radical-scavenging activity of the propolis extracts. A solution of 0.135 mM DPPH in ethanol was prepared and 1.0 mL of this solution was mixed with 1.0 mL of ethanolic extract of propolis containing 0.02-0.1 mg of this ethanolic extract of propolis. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 0.5 h. The absorbance of the mixture was measured spectrophotometrically at 517 nm[8].

Total phenolic content: The procedure used is based on the methods outlined by Folin-Ciocalteu[9]. The methods is based on an oxidation-reduction reaction in alkaline conditions, where the phenolate ion is oxidized while Folin's reagent is reduced, turning the solution blue. Many of the active components in propolis, such as phenolic acids and flavonoids, have a phenolic nucleus and can be evaluated by this method. A calibration curve was built using standard aqueous solutions of phenol containing between 2 and 12 μg/mL. One mL of each solution was added to 250 mL of sodium carbonate-tartrate buffer and 25 mL of the Folin-Ciocalteu reagent in a test tube, homogenized and allowed to react for 0.5 h at a temperature of 20 °C. Absorbance was measured at 700 nm on spectrophotometer and the calibration curve calculated by the minimal squares method. The dry extracts of propolis were dissolved in absolute alcohol to a concentration of 20 % (m/v), one mL of this ethanolic solution was further diluted in 1000 mL of distilled water and homogenized. One mL of this solution was prepared and analyzed in the same way as the standards.

RESULTS AND DISCUSSION

All the samples of propolis analyzed from different regions of Java *i.e.*, Batang (Central Java), Lawang (East Java) and Sukabumi (West Java). Although the three samples were collected during different months, over the period of one year, no variation was observed in the chemical composition. One of the obstacles to standardize propolis is considerable variation of the active content. The variation is due to the locations, climate and local vegetation. Some researchers measure the antioxidant activity of the propolis to determine their quality[10].

The flavones and flavonols contents in the ethanolic extract of propolis from Batang (7.26 ± 0.23) was higher than that in the ethanolic extract of propolis from Lawang (1.76 ± 0.57) and Sukabumi (2.03 ± 0.26). The flavanones contents in the ethanolic extract of propolis from Sukabumi (5.89 ± 0.43) was higher than that in the ethanolic extract of propolis from Batang (1.21 ± 0.12) and Lawang (1.42 ± 0.23). Table-1 also show the contents of total phenols that were measured by Folin Ciocalteu reagent in terms of gallic acid equivalent. The total phenol varied from 136.19 ± 10.1 to 203.66 ± 9.8. The total phenol content in the ethanolic extract of

propolis from Batang (203.66 ± 9.8) was higher than that in the ethanolic extract of propolis from Lawang (136.19 ± 10.1) and Sukabumi (171.16 ± 8.7).

TABLE-1
FLAVONOID AND PHENOLS CONTENT OF PROPOLIS

Propolis samples	Flavones and flavonols (%)	Flavanones (%)	Phenols (mg g^{-1})
Batang	7.26 ± 0.23	1.21 ± 0.12	203.66 ± 9.8
Lawang	1.76 ± 0.57	1.42 ± 0.23	136.19 ± 10.1
Sukabumi	2.03 ± 0.26	5.89 ± 0.43	171.16 ± 8.7

In general, the radical-scavening activity of flavonoids depends on the molecular structure and the substitution pattern of hydroxyl groups[11]. The capacity of the ethanolic extract of propolis to scavenge DPPH was measured and results are shown in Fig. 1. The antioxidant react with DPPH, a purple coloured stable free radical and convert it into a colourless α-α-diphenyl-β-picrylhydrazine. The amount of reduced DPPH could be quantified by measuring the decrease in absorbance at 517 nm. IC_{50} of the standard compound quercetine was 2.58 µg/mL. The highest radical scavenging activity was showed by the ethanolic extract of propolis from Batang with IC_{50} 8.44 µg/mL and Sukabumi with IC_{50} 130.35 µg/mL. The superior antioxidant activity of propolis from Batang might be due to the higher content of flavonols and phenolic than those from other areas. Propolis is one of the natural resources rich in phenolic content. Polyphenolics are the major plant compounds with antioxidant activity. This activity is believed to be mainly due to their redox properties[12], which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. The results from this study strongly suggest that phenolics are important component of these propolis.

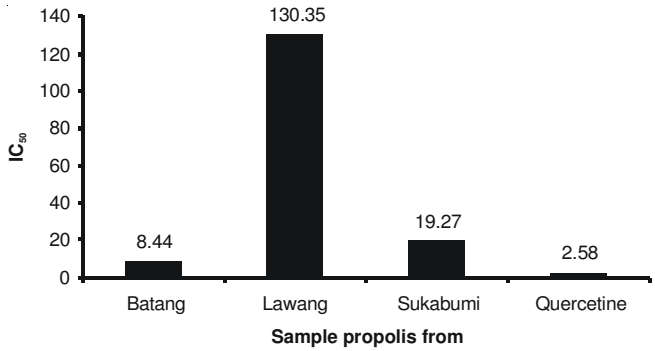

Fig. 1. IC_{50} (µg mL^{-1}) values of propolis extracts for free radical scavenging activity by DPPH radical. Lower IC_{50} value indicates higher antioxidant activity

The presence of *o*-dihydroxy structure on the B ring confers a higher degree of stability on the flavonoid phenoxyl radicals by participating in electron delocalization and is an important feature for the antiradical potential. The high potential of flavonoid

compounds to scavenge free radicals ($R^{\bullet}$) may be explained by their ability to donate a hydrogen atom from their hydroxyl group and thereby scavenge the free radicals:

$$FIOH + R^{\bullet} \rightarrow FIO^{\bullet} + RH$$

Scavenging reaction

This reaction gives the flavonoid phenoxyl radicals ($FIO^{\bullet}$) and a stable molecule (RH). $FIO^{\bullet}$ subsequently undergoes a change to a resonance structure by redistributing the unpaired electron on the aromatic core. Thus flavonoid phenoxyl radicals exhibit a much lower reactivity compared to $R^{\bullet}$, $FIO^{\bullet}$ would react further to form unreactive compounds, probably by radical-radical termination:

$$FIO^{\bullet} + R^{\bullet} \rightarrow FIO - R$$

Radical-radical coupling reaction

$$FIO^{\bullet} + R^{\bullet} \rightarrow FIO + OFI$$

Radical-radical coupling reaction

Combining the above with the obtained results from the modeling procedure, one could suggest the possible mechanism of the free radical scavenge of flavonoids locking OH, on ring B[13]. The results from this study strongly suggest that phenolics and flavonoids are important component of these propolis.

Conclusion

It is shown that the propolis from Batang area has higher content of flavonols and phenolic than those Lawang and Sukabumi areas. The superior content of flavonols and phenolic causes higher antioxidant activity than those from other areas.

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