Research Article

Identification of Total Bioflavonoid Compound of Propolis Extract from Wild Honey Bee Hives *Apis dorsata* in Sumbawa Region, Indonesia

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Abstract
Propolis is one of potential products derived from bee hives that contained chemical compounds such as flavonoids and polyphenols. This research aims to extract propolis from Sumbawa bee hives *Apis dorsata* and identify its total bioflavonoid compound. The method used in this research is experimental studies with two-steps extraction. First, propolis was extracted from the bee hives in maceration process with three different solvents of propylene glycol, which are 10, 20, and 30%. Second, the extraction was optimised using microwave-assisted extraction for 30 s. The Total Flavonoid Equivalent Quercetin (TFEQ) were then investigated using UV-Vis spectrophotometer. The results showed that the highest propolis yield was achieved in the propolis extracted in 20% of propylene glycol with microwave-assisted extraction. On the other hand, the highest Total Flavonoid Equivalent Quercetin (TFEQ) was resulted in the propolis extracted in 30% of propylene glycol continued by microwave-assisted extraction, which is 519.05 μg/mL.

Keywords: *Apis dorsata*, Propolis, Flavonoid, Bee hives, Extraction

1 Introduction

Wild honey bee (*Apis dorsata*) is one of local wisdom resources of Sumbawa and it has become one of main products of Sumbawa Regency, Indonesia. The forest area of Sumbawa Regency reaches 516,242 ha and as much as 45% of the forest area is a protected forest area that is very closely related to forest honey production in the Sumbawa region [1]. In addition to honey production, various products can be derived from honey bees, such as lip balm, food and beverages, bees wax, soap and propolis. *Apis dorsata* has a widespread distribution throughout southern countries of Asia, including Pakistan, India, Malaysia, Philippines and Indonesia. In Indonesia, *Apis dorsata* was found in Sumatera, Kalimantan, Sulawesi, Papua and Nusa Tenggara islands [2]. *Apis dorsata* builds a single large open nest (up to 150 cm in length and 70 cm tall) that hang under thick tree branches or under cliffs, as shown in Figure 1. The comb is permanently covered by a curtain of up to 100,000 worker bees [3].

Propolis is one of the potential products that can be derived from forest honeycomb. Basically, propolis is a resin produced by honey bees to build, repair and protect nests from microorganisms because propolis has a complex content consisting of several chemical compounds including bioflavonoids and polyphenols. As Al Firman *et al*. [4] and Scully [5] said that propolis is a dark brown plant resin, bitter and has a unique flavour, which is used as an insulation material by honey bees. In the last fifty years, various studies have revealed the versatile biological activities of propolis, namely as anti-bacterial, anti-fungal, anti-viral, anti-oxidant, anti-inflammatory and immunomodulatory

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Figure 1: *Apis dorsata* (right) and its honeycomb (left) [10].

[6]–[8]. Propolis contents depend on the bee species and its breeding type [9].

So far, honey farmers in Batu Dulang Village, Sumbawa Regency only took forest honey by draining and filtering it directly from the nest. After that, the honeycomb is not used anymore and become a waste. This is due to the lack of knowledge of the Sumbawa people about the potential of honeycomb. In fact, honey bee hives can be utilized for various products, such as propolis. Moreover, Sumbawa honey is a native forest honey originating from a typical plant in the Sumbawa plateau, so the quality and type of bioflavonoid in the propolis will have a uniqueness that is interesting to be studied [11].

Studies of propolis on various type of bee have been investigated. However, there is no publication on *Apis dorsata* propolis using a combination of maceration method and microwave assisted extraction in Sumbawa regency so far. Therefore, this research was conducted to determine the total bioflavonoid compound of *Apis dorsata* propolis by using both maceration and microwave-assisted methods with propylene glycol solvent on propolis extraction.

2 Materials and Methods

2.1 *Materials*

Study was carried out in Sumbawa Regency, West Nusa Tenggara Province of Indonesia. The materials are *Apis dorsata* hives, propylene glycol, aquades, H_2SO_4, quercetin standard, $AlCl_3$, beaker glass, hotplate stirrer, microwave oven, filter paper, centrifuged tube and spectrophotometry UV-VIS.

2.2 *Extraction of propolis*

Apis dorsata hives was collected from Batu Dulang forest located in Batulanteh District, Sumbawa Regency, Indonesia. The hives then cut into small pieces, grounded and extracted. Propolis extraction was conducted by using maceration method in propylene glycol solvent (10, 20, and 30%) (1 : 3 w/v) at room temperature in an incubation shaker (100 rpm) for 24 h. The propylene glycol extract solution then heated to the microwave for 30 s before filtered and centrifuged. The flowchart of this research was shown in Figure 2.

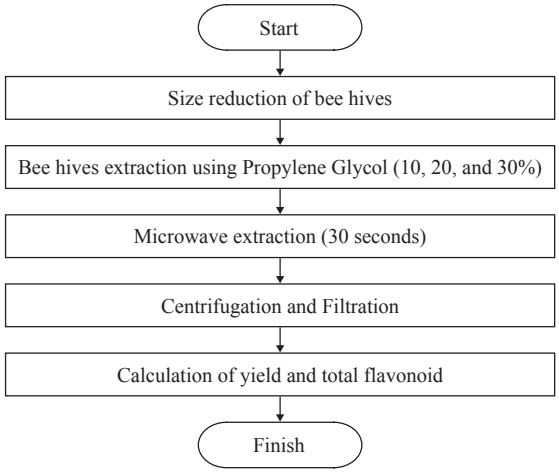

Figure 2: Flowchart of the research.

2.3 *Determination of bioflavonoid content*

The bioflavonoid content was evaluated by spectrophotometric method based on reaction between $AlCl_3$ and bioflavonoid (colour reaction) then measured at 510 nm according to the methodologies proposed by Chanda and Dave. Bioflavonoid was measured on quercetin standard. In this case, the bioflavonoid content is in Total Flavonoid Equivalent Quercetin (TFEQ). Quercetin is chosen due to its appreciable amount in the flavonoid of beehives compared to the other compounds (Figure 3) [12].

The results were then analysed using analysis of variance (ANOVA) and Duncan test by SPSS 16.0.

3 Results and Discussion

3.1 *Propolis extracts*

The propolis has been successfully collected and extracted from *Apis dorsata* hives by combining

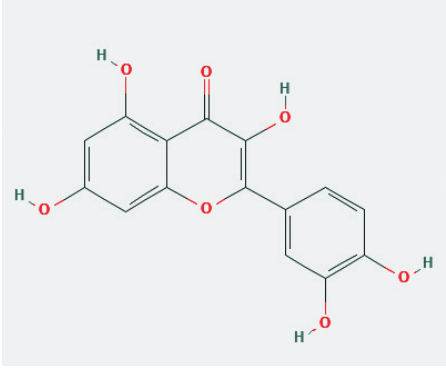

Figure 3: Molecular structure of quercetin [12].

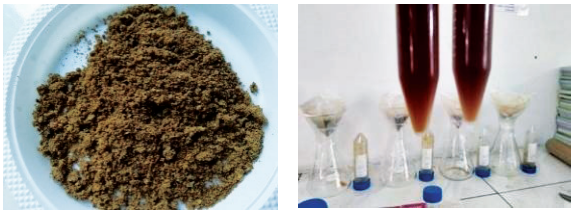

Figure 4: Grounded (left) *Apis dorsata* hives and Propolis extract (right).

maceration method and microwave assisted extraction. The preparation of *Apis dorsata* hives including draining and grounding into small pieces (Figure 4).

The *Apis dorsata* hives has a characteristic that is quite sticky, brownish yellow, but still quite easily destroyed at room temperature (25ºC). But at a higher temperature (> 50ºC), the shape of the hives becomes increasingly fluid because the wax contained in it is melted, but the nest structure will be stickier if it has been cooled. Therefore, to reduce the wax content of propolis, the sticky part (wax) is separated from the part of propolis which is not sticky.

Propolis yield then calculated by comparing its final mass with its initial mass before extraction process. The highest propolis yield was obtained from 20% propylene glycol solvent with 30 s microwave heating treatment and the lowest yield was obtained from 10% propylene glycol solvent without microwave heating treatment.

The results showed that the average yield of propolis extract produced was not significantly different between the treatment of concentration and with or without microwave-assisted extraction. With microwave heating, the propolis yield is slightly

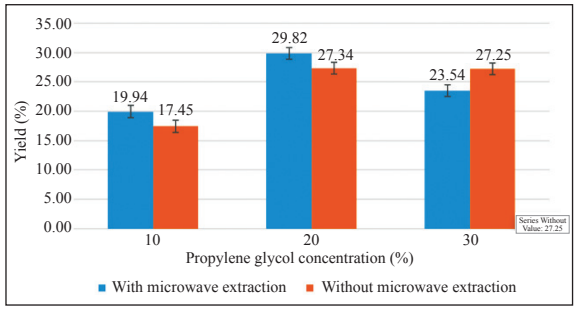

Figure 5: Propolis yield.

higher, which is 24.43% compared to the yield without microwave treatment, which is 24%. However, the combination of propylene glycol concentration and microwave-assisted extraction had an effect on yield. The effect of the best concentration of propylene glycol by microwave heating on yield is in concentration of 20%, which produce 29.13% propolis extract (Figure 5).

The increase of solvent concentration and microwave-assisted extraction were directly proportional to the increasing of propolis yield from 10% to 20%. However, the higher solvent concentration (30%) resulted to the lower propolis yield. Whereas without microwave heating, the yield of propolis at a concentration of 20% and 30% did not provide a significant difference.

These results above can be described by the following discussion. At 10% propylene glycol concentration, the yield produced was lower than the yield which is due to lower ability of solvent in binding process. As Sun *et al*. pointed out, propolis extracted by water solvent produced lower yield than extracted by ethanol solvent [13]. This is because of water solvent has higher polarity (80.00) as of extract only bioactive compound with equal polarity. On the other hand, ethanol is semi polar solvent (25.00) which was able to extract semi polar and weak polarity compound and extract even non-polar compound. High water concentration within solvent might increase the solvent polarity and its ability to extract other than flavonoid compound but also protein and carbohydrate compound [14]. In this study, water concentration was reduced along with the increasing of propylene glycol concentration. This treatment was used to keep the semi polarity of propylene glycol solvent. However, at the concentration of propylene glycol 30%, the yield is decrease that could be caused by the extraction process

has reached the maximum equilibrium phase which is at a concentration of 20%, so that the increase in propylene glycol does not necessarily follow an increase in yield on the results of propolis extract.

On the other hand, microwave heating showed a slightly higher results compared to non-microwave heating. This is because microwave heating could break down the plant cell walls through energy transfer into the solvent [15]. High heating temperature treatment leading to molecules interaction diminished within solvent so that the molecule movement are increased and affect the increasing of solvent solubility [16]. The increasing of temperature lead to the thermostable compound to extract faster as of increasing the propolis yield [17]. In general, the solvent contains high dielectric constants. The higher its dielectric constant, the higher its ability to absorb microwave energy [16]. Zhang *et al*. studied that *epimedin B* extraction from *Epimodium koreanum* by using microwave assisted extraction resulted in the diminish of its chloroplast and indicate that microwave heating demolished plant tissues and cell walls so that increased the yield [18].

Analysis of Variance and Duncan test of propolis yield then performed with 5% degree of freedom. The results showed the concentration of propylene glycol affects the yield significantly, while microwave heating does not have a significant effect.

3.2 *Bioflavonoid content*

Flavonoid content was analysed by Chanda and Dave method in which flavonoid content is indicated by colour reaction of complex formation between $AlCl_3$ and bioflavonoid (quercetin). Quercetin standard was used as parameter of bioflavonoid content calculation due to its large availability in propolis up to 60–75% [12], [19]. Therefore, the bioflavonoid content was measured in Total Flavonoid Equivalent Quercetin (TFEQ).

TFEQ analysis is done by making a standard curve first. The analysis of flavonoids was carried out by weighing the standard of quercetin 10.0 mg, adding 0.3 mL of 5% sodium nitrite. The dilution was then carried out and the solution was transferred to cuvette to be analysed on a 510 nm UV Vis wave. The following is the standard curve produced (Figure 6).

The result showed that the average TFEQ in the propolis extract from the treatment of variations in

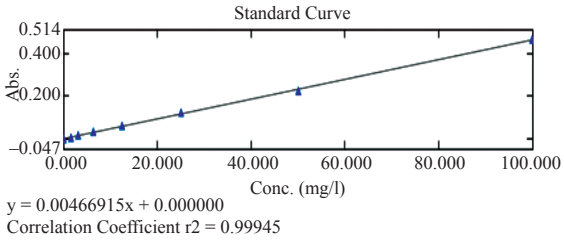

Figure 6: Standard curve of TFEQ using UV Vis.

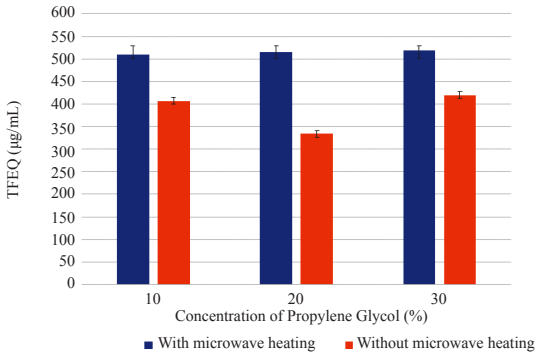

Figure 7: TFEQ content of *Apis dorsata* propolis.

concentration and microwave heating were 513.51 µg/mL, whereas without microwave heating had an average TFEQ level of 382, 98 µg/mL. The highest flavonoid level was measured at the concentration of 30% propylene glycol by microwave heating which was as much as 519.05 µg/mL, while the lowest level was measured at concentration of 20% propylene glycol without microwave heating, which is 333.75 µg/mL (Figure 7).

All treatments using propylene glycol and microwave heating on propolis extract produced higher levels of TFEQ compared to the treatment without microwave solvents. This could be due to the heating by microwaves may break down the cell wall through the energy transferred into the solvent [15]. The use of a solvent in the form of propylene glycol can also affect the differences in the levels of flavonoids produced. This is because propylene glycol and quercetin from the flavonoid group have similar properties which are semi-polar so that propylene glycol can dissolve flavonoids better than polar water [18].

Quercetin (flavonol) is a semi polar compound which is soluble in semi polar solvent such us propylene glycol solvent. The higher concentration of propylene glycol, the more semi polar the solvent become as if

the increasing of flavonoid content. This is because some solvent would only extract some compound with equal polarity [18].

Flavonoid content of *Apis dorsata* propolis in this study was lower compared to other study as shown in Table 1. These differences might be affected by the diversity of bee species, bees food sources and harvesting time [20]. Tables 1 and 2 showed the differences of food sources between *Apis dorsata* species in Batulanteh and *Apis mellifera* in other regions or other countries. *Apis dorsata* food sources in Batulanteh are mainly *Mirabilis jalapa l*, *Solanum melongen and Coffea Arabica* flowers [21], which contain less flavonoid than Populus plant which is mainly food sources of *Apis mellifera* in other countries especially in Europe.

Low propolis content also affected by harvesting time and condition. These research samples were collected on rainy season. Honey bees adjust themselves into the climate changes such as not to step out their hives on hot weather or on heavy rainy days quantity [22]. Climate changes also change honey bee's behaviour and floral environment quality which is increase or decrease its quantity [22].

Climate changes affect flower nectars and pollens development and production as if influence honey bees foraging activity. For example, honey bees are not interested into Acacias nectar (Acacia denticulos) when its exposed to rain because water would dilute Acacias nectar [22].

Table 1: Flavonoid content of *Apis dorsata* nectars sources in Batulanteh

Plant Species	Flavonoid Content	References
Mirabilis jalapal	0,19%	Walker *et al*. [23]
Rosa villosa	98 mg/100 g	Adamczak *et al*. [24]
Blumea balsamifera	0,2940 mg	Toralba [25]
Cucumis sativus	62,43 mg	Olajire & Azees [26]
Solanum melongena	14 mg/100g	Kandoliya *et al*. [27]
Echium vulgare	46,43 mg/g	Eruygur *et al*. [28]
Coffea Arabica	60 mg/100 g	Kreicbergs *et al*. [29]
Astragalus onobrychis	25 mg	Benbassat & Nikolov [30]

Each honey bee species use different level of propolis. Some honey bee species use propolis only slightly, while some other honey bee species used propolis (resin) extensively [32]–[34]. (Therefore, the propolis use could be replaced by massive wax utilization [33]. *Apis dorsata* colony use propolis occasionally when they want to stick their hives onto tree branches. Apis cerana does not use propolis at all [33], [35]. Meanwhile, *Apis mellifera* colony use propolis extensively. However, each of honey bee species use propolis on different level [34]–[36].

Table 2: Flavonoid content of *Apis mellifera* nectars sources in Europe

Plant	Flavonoid Content	Region	References
Populus	43% (Birtas *et al*., 2010)	Europe, north America, non tropic of Asia, New Zealand, Africa.	Bankova *et al.* (2000) Hegazi dan El Hady (2002)
Clusia flower	13,93% (Da Silva dan Paiva, 2012)	Venezuela, Amazon, Cuba.	De Castro Ishida *et al.* (2011) Trusheva *et al.* (2004)
Cupressus	9,5 mg/g (Al Snafi, 2016)	Sisilia, Malta, Mediterania	Kumazawa *et al.* (2008) Popova *et al*. (2009) Popova *et al.* (2011)
Pinus	740 mg/g (Maimoona *et al*., 2011)	Greek	Melliou dan Chinou (2004)
Acacia	26,65 mg/100 mg (Amoussa *et al*., 2015)	Australia	Tran *et al.* (2012)
Dalbergia	100 µg/ml (Laksmi *et al*., 2014)	Brazil and Nepal.	Alencar *et al.* (2007) Awale *et al.* (2005)
Eucalyptus	35,76 mg/g (Dezsi *et al*., 2015)	Australia, Turkey, Egypt, dan Brazil.	Silici *et al.* (2007) El Hady dan Hegazi (2000)

Sources: (Huang *et al*., 2014) [31]

Analysis of Variance and Duncan test of flavonoid content of propolis then performed with degree of freedom 5%. The results showed that *p*-value is lower than α (0,05), which mean there was an interaction between propylene glycol solvent and microwave heating treatment that affect flavonoid content of propolis. Narrow significances (*p*-value) between the two factors means that the interaction that occurred between them was just a small-scale interaction.

4 Conclusions

In conclusion, the bee hives of *Apis dorsata* from Batu Dulang Village, Sumbawa Regency has the potential to be used as raw material for propolis. The treatment of various concentrations of propylene glycol and microwave heating in the honeycomb extraction process has an effect on the yield of propolis and the levels of bioflavonoids (TFEQ) of propolis produced. Based on the measurements of flavonoid levels in *Apis dorsata* propolis extract, it was concluded that the best treatment was the concentration of 30% solvent and microwave heating that resulted in 519.05 µg/mL TFEQ. Further studies are required to determine the chemical characterization and its antioxidant activity of *Apis dorsata* propolis.

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