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Original Article

PROPERTIES AND FLAVONOIDS CONTENT IN PROPOLIS OF SOME EXTRACTION METHOD OF RAW PROPOLIS

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ABSTRACT

Objective: This research is aim to determine properties and flavonoids content of some solvent of extraction method of raw propolis *Trigona sp* from Sulawesi, Indonesia and to compare it with the ethanolic and water extracts.

Methods: Propolis samples were extracted with water, ethanol, propylen glycol, olive oil, and Virgin Coconut Oil (VCO). Flavonoids were determined using aluminum chloride and expressed as quercetine equivalent.

Results: The highest yield was Ethanolic Extract of Propolis (18.33%), and the lowest yield was olive oil extract of propolis (14.06 %). The ethanol and water extract were gummy sticky whereas oil extract were gummy oily. The samples studied varied in colour, yellowish-brown to dark brown. The content of flavanoids in propolis ranged from 0.2 % (the olive oil extract) to 0.55% (the propylen extract).

Conclusion: The propolis extract obtained with oil solvent have similar flavonoids content with ethanolic and water extract. So the VCO and Olive Oil can be used to extract raw propolis.

Keywords: Propolis, Flavonoids, Trigona sp, Extraction method.

INTRODUCTION

Propolis is a sticky, gummy, resinous substance collected by honeybees (*Trigona sp*) from various plant sources. Bees collect propolis to seal holes in the hives, smooth out the internal walls and protect the entrance against intruders [1,2]. The composition of propolis varies with the source, generally it is composed by 50% resin and vegetable balsam, 30% wax, 10% essential oil and aromatics, 5% pollen, and 5% other substances [3,4].

Propolis contains a variety of chemical compounds such as polyphenols (flavonoids, phenolic acids and their esters), terpenoids and aminoacids. The composition of Propolis depends on the species of honeybees and the type of vegetation present in that geographical region [5], and by the collection season [6,7]. Propolis contains extremely high in bioflavonoid content that has antioxidant, antibacterial, antifungal, antiviral and anti-inflammatory. Other properties of propolis include acting as a local anaesthetic, reducing spasms, healing gastric ulcers, and strengthening capillaries. Propolis can be used by humans internally or externally [8,5].

Methods of propolis extraction used in biological assays may influence its activity. The common method is solid-liquid extraction, which use ethanol in different concentrations, methanol or water. The extract contains amino acids, flavonoids, terpenes, and cinnamic acid derivatives. The water extract also contains lectin [4]. Chang et al [9] used two complementary colorimetric methods, aluminium chloride method and 2,4-dinitro-phenylhydrazine method to determine the content of total flavonoids in propolis.

The extraction solvent influences the composition and consequently the biological activities. The solvent that mostly used for propolis preparation is aqueous ethanol, followed by others, such as ethyl ether, water, methanol and chloroform [10].

This time, we used water (aqueous) and some oil (virgin coconut oil /VCO), olive oil and propylen glycol) as a solvent to prepare a propolis oily extract with promising preliminary antibacterial. The oily extract presents some advantages against the usually used ethanolic extract [11,10]. Therefore, the object of this study is to determine properties and flavonoids content of some oil solvents for extraction of raw propolis, and to compare them with the ethanolic and aqueous extracts.

MATERIALS AND METHODS

Materials

Raw propolis that was used for extraction was collected from Landono districts, South Konawe, Southeast Sulawesi in 2013. Raw propolis was taken from beehives *Trigona sp* that are widely spread in Landono district, while the solvent used for extraction were ethanol (70%), water, olive oil, Virgin Coconut Oil (VCO), and Propylene glycol. Quersetin standard solution, Aluminium chloride, and Natrium acetate was used to calculate the flavonoid content.

Extraction Procedures

The propolis extract (PE) was prepared according to a method presented by Matienzo and Lamorena [12] and Essam and Raghad [3] with modifications. Fine ground propolis was extracted with different solvent, namely water, ethanol, VCO, olive oil and propylen glycol (same concentrations) at 40 ^{0} C in shaker (Stuart GFL 1086). Thus, 25 gram of propolis (finely grounded in a mixer) was extracted with 250 ml solvents (water, ethanol 70%, VCO, Olive oil and propylene glycol) at 40 ^{0} C in shaker for seven days in dark room. After that, the suspension was filtered (with Whatman filter paper No. 41), and the residue was extracted again. Then for seven days the suspensions was filtered every day. The yield of macerations was further dried in rotary evaporator (Laborota 4002) at 30°C - 40°C. Then Dried extracts were weighed to get yield of extract. All the samples were analyzed in triplicate.

Research Variables

In this study, the observed and calculated variables are:

The yield of extraction, Yield calculation using the formula:

$$\text{Yield} = (Pe/Pm) \times 100\%,$$

Where: Pe is weight of propolis extract (g) and Pm is weight of raw propolis (g)

Physical properties of Propolis

The physical properties of propolis extracts include form, colour and pH.

Total Flavonoids

Total Flavonoids were determined using aluminum chloride and expressed as quercetine equivalent. Quercetine was used make the calibaration curve (standar solutions of 5,10, 15, 20, 25, 75 and 100 µg/ml 90% etanol (V/V). The standard solution were mixed with 0.1 ml AlCl3 10 %; Na Asetat 1 M and 2.8 ml aquades. The solution was shaken well and after incubation at room temperature for 30 minutes, the absorbance of the reaction mixture was measured at 415 nm on a spectrophotometer (Spectronic 20 D). All the samples were analyzed in triplicate. The coefficient of determination was r^2 = 0.943. [4,1] with modification, the content of total flavonoids was expressed as µg of quercetin equivalent per mg of dry weight of the sample, the flavonoid content was calculated with the equation [14]:

(Absorbance = 0.0032 × quercetine (µg) + 0.0089, (R 2 = 0.943)

Data analysis

The results of the calculation of the yield and flavonoid content were analyzed descriptively by comparing the results between the five extraction methods that were used.

RESULTS AND DISCUSSION

Yields and Physical Properties

The study showed that different solvent gave different yields. The yields and physical properties of extract propolis were shown in Table 1.

Table 1: Yield and Physical Properties of Propolis Extract

S. No.	Solvent	Yields (%)[a]	Appearance and form	Colour	pH [b]
1.	Etanol	18.33 ±1.82	Solid sticky	Dark Brown	5.4 ±0.2
2.	Water/Aquades	15.33 ±0.54	Gummy sticky	Dark Brown	5.7 ±0.2
3.	Propylen Glycol	15.88 ±0.48	Gummy oily	Yellowish brown	6.3 ±0.1
4.	Olive Oil	14.06 ±1.07	Gummy oily	Yellowish brown	6.5 ±0.1
5.	VCO	14.22 ±0.22	Gummy oily	Dark Brown	6.8 ±0.1

[a]:Data are mean ± standard deviation (n=3), [b:] Data are mean ± standard deviation (n=3)

Table 1 shows that ethanol produces a higher yield values than other solvents in the amount of 18.33% followed by 15.88% propylene glycol, distilled water was 15.33%, 14.22% for VCO and the lowest yield was olive oil at 14.06%. Ethanol yield value is higher than the yield of propolis from Pandeglang of 17.2 % [15] and 10% [16]. The difference is presumably due to the properties of ethanol as an organic solvent capable of dissolving most of the content of propolis. It also may be due to differences in the origin of propolis, propolis content may be different because of the origin [17], types of bees, food resources and harvest time [6,7]. However, the yield of the other three solvents, namely olive oil, VCO and propylen glycol also does not vary much with solvent distilled water. And approaching the yield of ethanol or distilled water because given the content of propolis which consists of polar and non-polar materials.

The observation of the physical properties of propolis solution of three solvents used oil shows in the form of oil and sediment shaped jelly if stored at low temperature (4^0C), suspected fatty oil content of wax of propolis can be dissolved well in an oil solvent, while the solvent ethanol and water in the form of gummy sticky, likely due to the fat content of wax and propolis has not been dissolved. This is consistent with the statement of Selvan and Prabhu [5] that Propolis dissolves well in ethanol and is easily extracted in 70-80% (v/v) ethanol but beeswax does not dissolve in ethanol and gets settled down as sediment. The 70% ethanol was found to extract most of the active components of propolis but not waxes [18].

The reason is because propolis might contain up to 20-30% of wax, this solvent has been applied in many studies. In fact, water has also been used in many occasions; however, it is important to note that in general water gives the low yield than ethanol because water belongs to the group of polar solvent, so only polar active ingredient that can be dissolved. Polar solvent other than water is glycerol and methanol, while ethanol, propyl alcohol and acetone including less polar solvents [19]. Water dissolves a small part of propolis constituents, about 10% of its weight, where as 70% ethanol may dissolve 50–70% of it, depending on the wax amount [18].

Total flavonoid

Determination of flavonoid in the propolis extract sample at absorbance at wavelength 415 nm shown in Table 2.

Table 2: Flavonoid Content of Propolis Extract with Different Solvent

S. No.	Solvent	Absorbance [c]	Flavonoid content (%) [c]
1.	Propylen glycol	0.536 ±0.010	0.55 ±0.01
2.	Ethanol	0.344 ±0.016	0.33 ±0.02
3.	Aquades	0.215 ±0.009	0.22 ±0.01
4.	Olive Oil	0.206 ±0.038	0.20 ±0.04
5.	VCO	0.233 ±0.011	0.25 ±0.01

[c]: Data are mean ± standard deviation (n = 3).

Table 2 shows that the extract of propolis propylene glycol has the highest flavonoid content of 0.55%, followed by ethanol (0.33%), then the VCO (0.25%) and the lowest was olive oil propolis extract (0.20%). When comparing with studies conducted by Margeretha et al [18] which uses three methods of extraction of propolis (maceration, reflux and microwave-assisted extraction-MAE method) to extract polyphenolic fraction of propolis from Indonesian Trigona spp, the highest levels of flavonoids were obtained from the MAE by 0.4% whereas the levels of flavonoids from maceration method by 0.2%. This value is similar to the extraction of propolis with olive oil amounting to 0.2% and lower than solvent extracts of propolis with ethanol, VCO, water and propopylen glycol.

A flavonoid level of ethanol is lower than propylene glycol solvent probably due to ethanol belongs to the group of less polar solvent. Threfore when used as an solvent, only less polar subtance that can be dissolved, while flavonoids dissolved only in small amounts. Meanwhile, when using the non-polar solvent is non-polar flavonoids can better be extracted from the basic substrate, leading to an increase of their concentration in the final preparation [19] although many studies using 70% ethanol as a solvent because it can dissolve approximately 50-70% of materials [16].

Flavonoids are one of the major groups of phenol components in propolis, which is a key component to assess the quality of propolis. Flavonoids in propolis include aglycones (without sugar component). Chemically flavonoids are divided into sub-groups of flavones, flavanones, flavonols, and chalcones dihydroflavonols [1]. The concentration of flavonoids in propolis depends on the region of origin and ecosystems (plant source of propolis) [1,9] as well as the extraction method used. Solvents for extraction will affect the composition and consequently the biological activities. Solvents are widely used in addition to ethanol, ethyl ether also, water, methanol and chloroform [10] while water and oil are rarely used.

Table 2 shows also the absorbance value and the highest flavonoid content of the extract obtained by solvent propylen glycol. High absorbance value means a higher antioxidant activity [20]. Higher flavonoid from propolis propylen glycol allegedly used contains a lot of class of compounds that enter the oil and grease. It is supported by data from the visible yield Table 2 that shows the yield of extract of propolis propylen quite high (second highest after ethanol).

Propylene flavonoid content of the solvent is higher than other solvents, indicating that propylen glycol can be used to make propolis extract oil that has equivalent or better properties than ethanol extract of propolis. Research Silva et al [10] showed similar results, i.e the oil extract of propolis which uses canola oil has anti-fungal potency against *Aspergillus fumigates* better than ethanol extract and antibacterial equivalent water extract of propolis. Proplis oil extract can be used to produce gelatine capsule, wherein the capsule can be charged directly to extract oil of propolis and solvent removal hidroalkohol avoid like the ethanol extract of propolis, besides these extracts can be used as medicine for external use.

CONCLUSIONS

From the research that has been done can be concluded that:

The highest yield was obtained from solvent ethanol at 18.33%, Properties of propolis produced from solvent extraction with ethanol was solid sticky and distilled water was gummy sticky and dark brown colour while propolis produced from the solvent VCO, olive oil and propylene glycol were gummy oily and yellowish brown colour. Oil solvent (VCO and Olive oil) produces similar flavonoids content with ethanol and water. The highest levels of flavonoids derived from Propylene glycol solvent by 0.55% and the lowest was olive oil solvent by 0.2%. So that VCO and Olive Oil can be used as solvent to extract propolis and are more advantageous because they can be used directly without removing its solvent. Next study is recommended to determine the biologically active compounds and antimicrobial activity from the oil extraction compared with other propolis extract.

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