Physical Quality of Halal Propolis Extract Using the Ultrasonic as an Active Drug Ingredients

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Abstract. Propolis is currently widely used as an active ingredient in natural anti-inflammatory and antimicrobial drugs on the skin and mouth. The needs of the times, the extraction process requires the latest method with a shorter time with optimal results. The purpose of this study was to obtain an optimal propolis extraction method using water-based ultrasonic assisted extraction (UAE) that can produce propolis extracts in a short time with good physical quality. The research method used was experimental using a completely randomized design with sonication time factor, 6 treatments, and 3 replications by observing the physical qualities consisting of color intensity, pH, electrical conductivity, and total dissolved solids. *Apis mellifera* bee propolis is taken from Central Java. The solvent used is distilled water. The results of statistical analysis showed that ultrasonic propolis extraction had a very significant effect ($p < 0.01$) on pH, electrical conductivity, and total dissolved solids, each ranging from 3.48 to 3.55, 0.83 to 1.10 m/s, and 257 to 306 ppm and had a significant effect ($p < 0.05$) on the color intensity of L*a*b*. The conclusion of this study is that the optimal ultrasonic assisted extraction uses a sonication time of 5 min in terms of color intensity, pH, electrical conductivity, and total dissolved solids produced. This method is able to make propolis extract in a short time by providing an optimal product, so that the extract produced can be used as an active drug ingredient in good and halal.

Keywords: Physical Quality · Propolis Extract · Ultrasonic

1 Introduction

Propolis is a resin and wax material that has been collected from plants, such as from flowers, shoots, and bark [1]. Propolis contains active compounds such as phenols, flavonoids, terpenes, triterpenes, aldehydes, aromatics, alcohols, fatty acids, and others

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[2]. In addition, it also contains organic acids, antibiotics, vitamins, minerals (Al, V, Fe, Ca, Si, Mn, Sr), and from natural enzymes (carotene) [3]. Propolis is currently widely used as an active ingredient in natural anti-inflammatory and antimicrobial drugs on the skin and oral [4]. Previous studies have shown that propolis is a natural ingredient with great potential and promise as a medicinal raw material, because it contains many benefits for human health. So, there needs to be technological development in obtaining propolis extraction. Currently, propolis has been used as a raw material for medicine and marketed in the form of wound medicine, toothpaste, and food supplements [5].

The extraction of bioactive compounds from propolis extraction is influenced by the solvent, solvent concentration, temperature, time, particle dimensions and the procedure used. The extraction method consists of conventional extraction methods, namely maceration and modern extraction methods, one of which is Ultrasound-Assisted Extraction (UAE). UAE is considered a green extraction method and has proven to be efficient in extracting different antioxidant compounds compared to conventional methods, because this method is considered to save time and energy while providing good selectivity of the targeted compound [2]. This method is recommended as the current method of propolis extraction because this method is considered simple, efficient, and optimal for propolis extraction based on extraction time, extraction yield, and cost effectiveness [6]. It was reported that the use of UAE is considered to be more efficient and provides higher extraction yields containing active propolis substances compared to maceration and MAE [7]. Water-based propolis extraction (WEP) is an alcohol free, because extraction using water solvent, having the advantage of being "the greenest solvent" [8].

As an extraction technology advances, it is possible to use water as a solvent in single extraction. Water was stated to be a more polar solvent than ethanol and water and it was known that water would extract the more polar propolis compound. One way to optimize the use of water as a solvent is using ultrasonic assistance. It is known that water-based propolis extraction using ultrasonic can increase the total phenolic content (TPC) significantly compared to without ultrasonic [7]. Previous research, it was found that the extraction of rapeseed oil by the UAE method gave optimum results at an extraction time of 5 min by producing higher total carotenoids than the reflux and maceration methods [9]. It has been found that ultrasonic-assisted propolis extraction uses a sonication time of 15 min to achieve maximum extraction efficiency and the solvent penetrates cell membranes by producing high TPC and TFC [2].

Therefore, water-based propolis extraction using ultrasonic assistance has the potential to produce a good and safe propolis extract to be used as an active drug ingredient. This study aims to make halal propolis extract using ultrasonic-assisted extraction methods with variations in sonication time to obtain the most optimal results in terms of L*a*b* color intensity, pH, electrical conductivity, and total dissolved solids.

2 Materials and Methods

2.1 Locations

The research was conducted at the Laboratory of Animal Products Technology, Faculty of Animal Sciences, Universitas Brawijaya and samples of propolis were taken from PT. Kembang Joyo Sriwijaya. The research was conducted in September 2022.

2.2 Materials

Pure propolis is taken from the *Apis mellifera* bees which is cultivated in Central Java, Indonesia. The place of cultivation has a tropical climate and its surroundings are mostly overgrown with kapok trees. The solvent used is distilled water with a pH value of 7.1. The equipment used is a measuring cup, 50 ml erlenmayer, blender (Philips®), knife, film pot, filter paper Whatman No. 1, buchner fuller, and ultrasonic cleaner "bau" brand with ultrasonic frequency of 40 kHz, ultrasonic power of 120 W, heating power of 100 W, and ultrasonic temperature approaching 35–40 °C. The sample testing equipment consists of a colorimeter CS-10 (CHN Spec®), pH and EC meter (pH/EC-9853), and TDS meter (TDS-3).

2.3 Method

The method used is a laboratory experimental using a ANOVA one way followed by Duncan's Multiple Range Test (DMRT). The factors used were variations in the length of sonication time with 6 treatments and 3 replications, consisting of:

P1 = UAE with sonication time of 5 min.
P2 = UAE with sonication time of 10 min.
P3 = UAE with sonication time of 15 min.
P4 = UAE with sonication time of 20 min.
P5 = UAE with sonication time of 25 min.
P6 = UAE with sonication time of 30 min.

2.4 Preparation Sample

Prepared tools and materials used for extraction, then pure propolis was weighed as much as 5 g and cut into small pieces. Propolis is put into a blender and then added with 50 ml of distilled water or 1:10 (w/v). The sample was crushed at a power of 1 for 5 min. Then, the sample was transferred to an Erlenmayer and covered with aluminum foil. The sample is ready for sonication.

2.5 Extraction with Ultrasound

The bath of the ultrasonic cleaner is filled with 1.5 L of distilled water, then the tool is turned on and ultrasonically adjusted using a temperature of 35 °C and time according to the treatment. Then, the sample is put into a basket in the bath. Next, the ultrasonic and degassing buttons are turned on. The extraction results were taken by filtering using filter paper Whatman No. 1. Propolis extract was stored in a refrigerator at 8 °C. Samples continued with quality testing [6, 10].

2.6 Variable Analysis

Color Intensity L*a*b*. The color intensity test L*a*b* uses a calibrated colorimeter and the procedure refers to the CIE 2007 L*a*b* is by attaching the dye sensor to the sample provided in the film pot container 3 times, then taking the average value L, a,

and b. the value of L* is to determine the level of brightness (lightness) of the light coordinates which have a value range of 0–100. The a* value is the saturation of the red green axis, a positive a* value indicates red and a negative a* value indicates green. A positive b* value indicates a yellow color and a negative b* value indicates a blue color [1].

pH. The pH test was carried out using a pH meter which refers to the AOAC (2005) test procedure, namely by calibrating the pH electrode first with buffer solutions 4 and 7. Next, dry the electrode using a dry tissue and then rinse with distilled water and dry with a dry tissue again. The electrode was dipped into the extract until the pH meter showed a stable number. Then, note the results of the numbers that are read on the pH meter monitor screen. After taking measurements, the pH meter is rinsed with distilled water and dried with a dry tissue, then it is ready to be used for the next sample [11].

Electrical Conductivity (EC). Electrical conductivity measurements are carried out using an EC meter by first cleaning the electrodes using distilled water. Next, the electrode is dipped into the sample and the electrical conductivity value is recorded [12].

Total Dissolved Solids (TDS). The measurement of total dissolved solids is carried out using a TDS meter by immersing the TDS meter electrode into a propolis extract sample solution [13].

3 Results and Discussion

3.1 Result

The results of statistical analysis showed that different sonication times had a significantly different effect ($p < 0.05$) on the value of L*a*b*, while giving a very significant effect ($p < 0.01$) on pH, electrical conductivity, and total dissolved solids. The results of the analysis of color intensity are in Table 1 and the results of the analysis of pH, electrical conductivity, and total dissolved solids are in Table 2.

Table 1. The effect of sonication time on color intensity L*a*b* ($p < 0{,}05$)

Treatments	Color Intensity		
	L*	a*	b*
P1	26.29 ± 0.17^{f}	-4.75 ± 0.02^{b}	2.39 ± 0.07^{d}
P2	21.77 ± 0.08^{c}	-3.92 ± 0.01^{d}	2.27 ± 0.02^{d}
P3	20.67 ± 0.02^{b}	-3.50 ± 0.03^{e}	1.85 ± 0.08^{a}
P4	20.36 ± 0.41^{a}	-3.94 ± 0.11^{d}	1.92 ± 0.10^{b}
P5	21.88 ± 0.01^{d}	-4.14 ± 0.02^{c}	2.35 ± 0.01^{d}
P6	26.02 ± 0.03^{e}	-5.09 ± 0.01^{a}	1.84 ± 0.01^{a}

Table 2. The effect of sonication time on pH, EC, and TDS ($p < 0{,}01$)

Treatments	Average ± SD (pH)	Average ± SD (EC)	Average ± SD (TDS)
P1	3.55 ± 0.0016^{d}	1.10 ± 0.0001^{d}	306 ± 7.0^{f}
P2	3.50 ± 0.0001^{b}	1.01 ± 0.0001^{c}	302 ± 7.0^{e}
P3	3.48 ± 0.0001^{a}	0.83 ± 0.0000^{a}	257 ± 1.3^{a}
P4	3.48 ± 0.0000^{a}	0.84 ± 0.0000^{a}	264 ± 1.3^{b}
P5	3.52 ± 0.0001^{c}	0.91 ± 0.0000^{b}	284 ± 5.3^{d}
P6	3.50 ± 0.0000^{b}	0.90 ± 0.0014^{b}	282 ± 3.0^{c}

Fig. 1. Color of propolis extract with different sonication time

Table 1 shows that the average value of color intensity L*a*b* is 20.36 to 26.29, −4.75 to −3.50, and 1.84 to 2.39, respectively. In general, the color of the propolis extract produced is dark brown, as shown in Fig. 1. Table 2 shows that the average values for pH, electrical conductivity, and total dissolved solids are 3.48 to 3.55, 0.83 to 1.10 m/s, and 257 to 306 ppm, respectively. Based on Table 1 and Table 2, most of the parameters indicate the highest value is in P1 which is extraction using a sonication time of 5 min. This, as in the test results of pH, electrical conductivity, and total dissolved solids were 3.55, 1.10 m/s, and 306 ppm, respectively. Based on the color intensity value, the P1 values are 26.29, −4.75, and 2.39, respectively. The sonication time of 15 min (P3) and 20 min (P4) resulted in the lowest values in each parameter. Almost all parameters occur that the initial sonication time shows the highest value, then it decreases and then at 25–30 min it increases again.

3.2 Discussion

Color Intensity L*a*b*

A high L* value or a brighter extract color indicates that the more wax is dissolved and the resulting yield is higher. A negative a* value indicates that the results of the propolis extract are greenish and a positive b* value indicates that the results of the propolis extract are yellowish. Phenol compounds, namely tannins and plant color pigments in the form of carotenes and anthocyanins, give a reddish or yellowish color [1]. Based on the chart in Fig. 2, the highest L* value was found in P1 which was 26.29, so P1 was the brightest propolis extract. The highest a* value was found in P6, which was −5.09, then P6 produced propolis extract with a higher greenish color. The highest b* value is

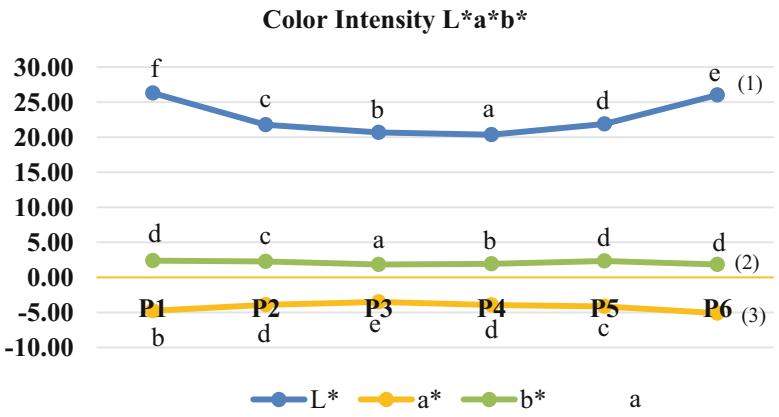

Fig. 2. A figure caption is illustration the graph of the effect of different sonication times on color intensity L*a*b*. (1) the blue line shows the value of L*, (2) the green line shows the value of a*, and (3) the yellow line shows the value of b*.

found in P1 which is 2.39, then P1 produces propolis extract with a higher yellowish greenish color.

Water-based extraction can capture anthocyanins, starches, tannins, saponins, terpenoids, polypeptides, and lectins compounds. In addition, it was found that water-based propolis extract had a higher intensity of antioxidant activity, due to its higher polyphenol content [14]. Polyphenols are antioxidant compounds that function as free radical scavengers because they are able to radical oxygen species (ROS) and are able to increase the expression of antioxidant enzymes such as superoxide dismutase and kata-lase [8]. The propolis extract in this study resulted in the appearance of a dark brown color (see Fig. 1), because the color produced was influenced by tree bark and plant species taken by bees [1]. So, this research proves that sonication for 5 min produces propolis extract which contains higher carotenoids and anthocyanins (polyphenol), so that it can be used in the prevention of diseases caused by free radicals.

pH

The highest pH value is at P1 which is 3.55, while the lowest value is at P4 which is 3.48. The pH of water-based propolis extract has a pH ranging from 3.48 to 3.55. The pH of the propolis extract increased at the beginning and end of the diagram, while in the middle of the diagram it decreased (see Fig. 3).

Low pH can inhibit the growth of fungi and bacteria, so it has a longer shelf life [7]. Propolis has an acidic pH because it contains organic acids and vitamins [3]. Therefore, the results of Central Java propolis extraction resulted in a low pH value. It was found that propolis honey has a low pH that is 4 with a high content of total phenol, quercetin, vitamin C, and calcium [15]. So, the closest pH is P1 which is 3.56. This is related to the positive b* value at P1 which produces a yellowish color which means that propolis extract contains higher phenolic compounds. It is known that the extraction of propolis using ultrasound with a higher concentration of ethanol will produce a large amount of lipid constituents, thus limiting the extraction of phenolic from propolis. In addition, the

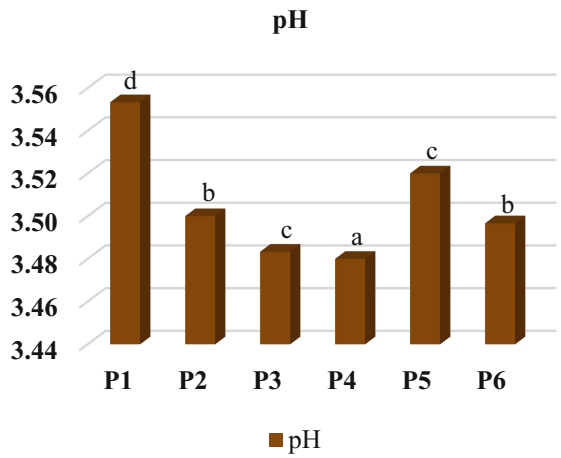

Fig. 3. A figure caption is illustration the graph of the effect of different sonication times on pH value.

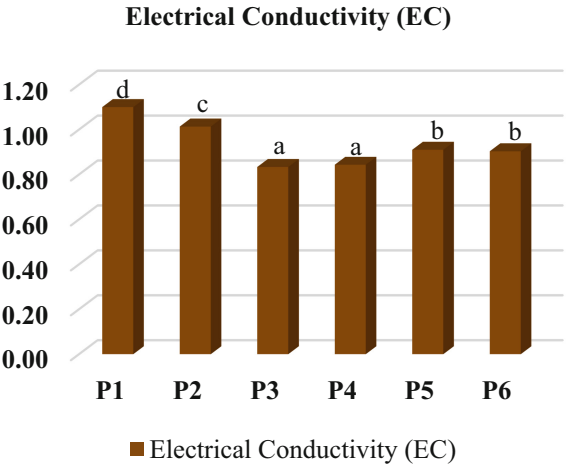

Fig. 4. A figure caption is illustration the graph of the effect of different sonication times on electrical conductivity (EC).

longer extraction process allows the oxidation of phenolic compounds, so as to reduce the amount of phenolic compounds produced [10].

Electrical Conductivity (EC)

The highest value of electrical conductivity is at P1 which is 1.10 m/s, while the lowest value is at P3 which is 0.83 m/s. The electrical conductivity of water-based propolis extract has an electrical conductivity ranging from 0.83 m/s to 1.10 m/s. The electrical conductivity of the propolis extract increased at the beginning and then decreased and increased at the end of the diagram (see Fig. 4).

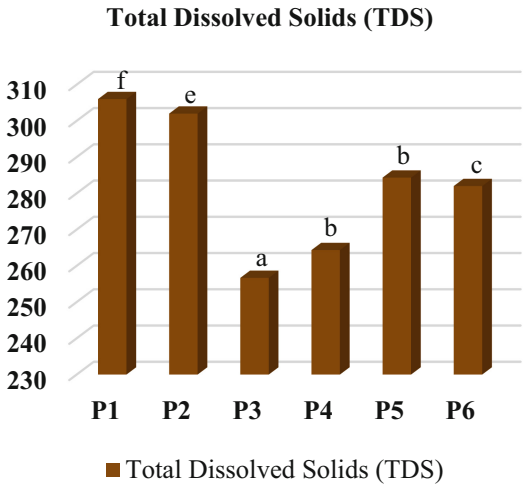

Fig. 5. A figure caption is illustration the graph of the effect of different sonication times on total dissolved solids (TDS).

Electrical conductivity is influenced by the content of dissolved organic acids and minerals [16]. Propolis contains organic materials (liquid and solid) and is classified as a semiconductor because it can conduct electricity depending on the given temperature [3]. The content of minerals and organic matter carried depends on the types of plants that are around beekeeping. This is related to the resulting pH, namely P1 has a low pH value so it is suspected that it contains calcium minerals that are higher than the others, this condition affects the EC which also experiences an increase in the graph at P1. Electrical conductivity is influenced by ash content and acidity, the higher the ash content and acidity, the higher the electrical conductivity produced [16]. The high soluble organic components indicate that the water solvent can dissolve organic components and bactericidal components in Central Java propolis [14].

Total Dissolved Solids (TDS)

Total dissolved solids are influenced by the content of organic and inorganic compounds [12]. The highest value of total dissolved solids is at P1 which is 306 ppm, while the lowest value is at P3 which is 257 ppm. The total dissolved solids of the propolis extract increased at the beginning and then decreased and increased at the end of the diagram (see Fig. 5).

The total dissolved solids in the solution has a maximum limit of 1000 ppm. Solutions that have total dissolved solids below 1000 ppm are considered safe for consumption. If it shows the number 1, then the dissolved substance in the solution is 1 ppm which indicates that there is 1 g of solid in 1000 L of water [17]. The results showed that the highest total dissolved solids values occurred at P1 and was below the maximum limit. An increasing total dissolved solids value indicates that an increase in the concentration of sulfate and other ions in the solution [9]. The increasing value of Total dissolved solids is followed by an increase in the value of electrical conductivity. The total dissolved solids value can be known by looking at the results of the electrical conductivity value in water [9].

Therefore, increasing the acidity of the pH of the solution will increase the electrical conductivity and also increase the content of total dissolved solids.

4 Conclusions

Propolis extraction using ultrasonic solvents with distilled water can bind the desired propolis compounds, especially phenol compounds, organic acids, vitamins, and minerals. Giving a sonication time of 5 min in a temperature condition between 35–40 °C has given the most optimal results in terms of L*a*b* color intensity, pH, electrical conductivity, and total dissolved solids. Therefore, halal propolis extract products have the potential to be used as active ingredients for skin and oral drugs, which can then be developed for chemical and sensitivity testing.

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