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ULTRASONIC-ASSISTED EXTRACTION, ANALYSIS AND IDENTIFICATION OF WATER EXTRACT OF PROPOLIS

Indha Fitria PANGESTI, Agus SUSILO, and Khothibul Umam AL AWWALY

Faculty of Animal Science, Universitas Brawijaya, Jl. Veteran, Lowokwaru, Malang 65145, Indonesia

Email: agussusilo@ub.ac.id

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ABSTRACT: *Apis mellifera* is one species of bee that produces propolis, a resin-based product. Propolis extraction using ultrasonic assistance is being widely studied. Using water as a solvent is a challenge to capture the bioactive components of propolis. This research aimed to determine the physicochemical quality resulting from the processing of propolis extract from Central Java by ultrasonics using water as a solvent at different temperatures and times. Raw propolis is extracted by the ultrasonic-assisted extraction method at low, medium, and high temperatures. Raw propolis is extracted by the ultrasonic-assisted extraction method at low, medium, and high temperatures. The study used nine treatments with three replications. The extraction time was carried out for 10, 20, and 30 minutes. The study used nine treatments with three replications. The results of the analysis showed that propolis extraction at different temperatures and times had a very significant effect ($P<0.01$) on the yield, total phenolic content (TPC), and total flavonoid content (TFC), with an average of 6.7–13.3%, 1.10–2.21 mg GAE/mL, and 0.07–0.32 mg QE/mL, respectively. Propolis extraction at different temperatures and times had no significant effect on tannin content, pH, and antioxidant activity. Regarding yield, TPC, TFC, and tannin content values, it was determined that extracting at high temperatures for 30 minutes produced the best results. High temperatures and long timespans are used for the best chance of collecting bioactive components.

Keywords: Bee products, Physicochemical, Processing, Propolis extract, Water solvent.

INTRODUCTION

Apis mellifera is Indonesia's most popular type of honey bee because it can adapt to tropical climates (Ustadi et al., 2021). Many of the bioactive compounds found in *Apis mellifera* propolis are polyphenols (flavonoids and tannins), phenols, and terpenoids (Cauich-Kumul and Campos, 2019). They also contain natural enzymes (carotene), antibiotics, vitamins, minerals (Al, V, Fe, Ca, Si, Mn, and Sr), and organic acids (Mammadova and Topchiyeva, 2014; Kolayli and Keskin, 2020). Besides propolis, honey bees produce honey, royal jelly, wax, and pollen products (Nur et al., 2020). The complex content of propolis can provide evidence that propolis has benefits in the food and pharmaceutical fields; Propolis possesses anti-inflammatory, antiviral, antibacterial, antioxidant, and antifungal effects (Pasupuleti et al., 2017). Antioxidant activity operates as an inhibitor, preventing reactive free radicals from oxidizing to become more stable and shielding cells from the damaging effects of free radicals (Wiwekowati et al., 2017).

Propolis can minimize the debilitating effects of heat stress in livestock by increasing intestinal crypt depth, body weight and feed intake, and immunity (Mehaisen et al., 2017; Dantas et al., 2023). Propolis has also been used as a natural supplement that can support body activities without causing adverse effects on animals and the environment (Abu-Seida, 2023). Pure propolis is not allowed to be consumed directly, bearing in mind that there are compositions in propolis that may not be consumed by humans, such as resin and wax. Propolis must be subjected to an extraction process to remove only its bioactive components for consumption.

Solvation, concentration, temperature, time, particle size, and the method utilized all impact the extraction process. Two classes of extraction methods are commonly used, namely conventional methods and modern methods. Extraction using conventional methods, for example, is the maceration method, while an example of a modern method is the UAE method, called ultrasonic-assisted extraction. Since the UAE approach is thought to save time and energy while giving strong selectivity of the targeted compounds, it is regarded as a green extraction method. It has been demonstrated to be effective in extracting several antioxidant chemicals when compared to conventional methods (Oroian et al., 2020). This technique has received much support for the present propolis extraction procedure since it is thought to be more accessible, more successful, and best for extracting propolis in terms of extraction time, extraction outcomes, and cost-effectiveness (Aboulghazi et al., 2022). In addition, compared to the maceration approach and other modern methods like the microwave-assisted extraction (MAE) method, the UAE method is more effective and yields extraction results with

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a greater propolis active component. Currently, several solvents are also used. Water is known to be one extraction method since it may extract more polar propolis components (Suran et al., 2021). However, extraction using water as a solvent still needs to be considered suboptimal in the extraction process using conventional methods. Therefore, many extraction processes are also being developed using water as a solvent, assisted by other technologies such as sonication (Sun, 2019; Dönmez et al., 2020; Contieri et al., 2023). Propolis extraction using a water solvent is also called WEP (water extraction propolis), which is more accessible due to being free of alcohol and ethanol content (Usman et al., 2016).

Based on previous studies (Yuan et al., 2019; Aboulghazi et al., 2022; Kara et al., 2022), propolis using solvents with ultrasonic assistance was able to produce higher total phenolic content (TPC) and total flavonoid content (TFC) values compared to the conventional method, namely 3.449 mg GAE/g and 0.456 mg QE/g, respectively. TPC and TFC results using conventional methods only obtained content values of 2.701 mg GAE/g and 0.336 mg QE/g, respectively (Kara et al., 2022). In addition to the type of solvent that can affect extraction, there are also temperature and time factors. The ultrasonic method can be set for temperature and time. It was stated that excessive temperature and time were also feared to damage the bioactive components. Still, if it was carried out quickly and the temperature needed higher, the compounds could not be captured optimally. The use of ultrasonics for the extraction of propolis with 60% ethanol using a frequency of 50 kHz, a power of 120 W, and a temperature of 35 °C for 15 minutes resulted in the highest TPC and TFC values and the lowest DPPH (2,2-Diphenyl-1-picrylhydrazyl), namely 187.21 mg GAE/g, 38.80 mg QE/g, and 23.70 µg/mL, respectively. Likewise, it also produces a relatively high extract yield, which is 11.25% (Aboulghazi et al., 2022). The efficiency of employing water as a solvent in the ultrasonic method for extracting propolis still needs more investigation. Based on the description above, this study identified the results of *Apis mellifera* propolis extraction obtained from Central Java, Indonesia, to see the physicochemical characteristics produced, including testing for pH, yield, TPC, TFC, tannin content, and antioxidant activity.

MATERIALS AND METHODS

Materials

Samples were prepared from raw propolis obtained from honey beekeepers in Central Java, Indonesia, provided by PT. Kembang Joyo Sriwijaya. There are 27 samples used in this study. Raw propolis samples are round and dark brown and are stored in a laboratory cupboard at room temperature. Before extraction, raw propolis is cut into small pieces to facilitate the extraction process. The solvent used was purely distilled water.

Method and statistical analysis

This study used a laboratory experimental method with a completely randomized design (CRD). Statistical analysis used a two-way ANOVA with 3x3 factorials and three replications. Furthermore, significant results were followed by Duncan's Multiple Range Test (DMRT). The first treatment factor was the use of low (A1), medium (A2), and high (A3) extraction temperatures, and the second treatment factor was the use of extraction times of 10 minutes (B1), 20 minutes (B2), and 30 minutes (B3). The temperature is observed and controlled. The low temperature used starts at room temperature with an estimated temperature of 27-30 °C, medium temperature with an estimated temperature of 40-43°C, and high temperature with an estimated temperature of 60-63 °C. The extraction method used was the ultrasonic-assisted extraction (UAE) method.

Ultrasonic-assisted extraction

The two ingredients (100 mL of aqua distillate and 10 grams of raw propolis, 1:10 ratio) were blended for 3 minutes. After blending, put it in an Erlenmeyer tube and covered with aluminium foil. The ultrasonic system used was an ultrasonic bath system with an ultrasonic frequency specification of 40 kHz and a power of 120 W. 1.5 litres of distilled water were put into the ultrasonic bath. A basket was installed to place the sample. The Erlenmeyer containing the sample was put into the ultrasonic bath and closed. Setting the temperature and time according to the treatment you want to do.

pH

pH was measured using a calibrated pH meter using pH buffers 4 and 7. The pH analysis procedure refers to the AOAC test procedure (2005). 1 mL of propolis extract dissolved in 5 mL of distilled water (1:5) was used for pH testing samples (Primandasari et al., 2021). The pH meter used is a pH meter and an EC meter (2 in 1). One mL of propolis extract was diluted in 5 mL of distilled water (1:5, v/v) and used as a sample for pH testing (Primandasari et al., 2021). The pH meter used was a pH meter and EC meter (2 in 1) (Hidayat et al., 2021). The electrode is dipped in the extract until a stable reading appears on the pH meter. The pH value results are displayed on the pH meter-monitor screen. After measurement, the pH meter was cleaned with distilled water and dried with a dry tissue before being used to collect data from the following samples. The pH electrode is immersed in the propolis extract until the pH reading on the meter stabilizes. The pH value displayed on the meter's monitor screen was then recorded. After each measurement, the pH meter is thoroughly rinsed with distilled water and dried with clean tissue before measuring the pH of the following sample.

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Yield

The percentage yield of the propolis extract was computed by dividing the weight of the freeze-dried extract by the total weight of raw propolis. The results are shown in percentages. The percentage yield is calculated following the equation (Pobiega et al., 2019):

$$\text{Yield} = \frac{\text{dry extract weight}}{\text{raw propolis weight}} 100\%$$

Total phenols content

Phenolic content was measured using UV-Vis spectrophotometry according to Lucas et al. (2022), modified. 1 mL of propolis extract was added to 2 mL of Folin-Ciocalteu reagent (0.2 M). The solution was allowed to stand for 5 minutes, then 4 mL of sodium carbonate (7.5% p/v) was added and homogenized. Until the tera mark, the homogeneous sample was mixed with distilled water. One hour was spent standing the combination at room temperature in the dark before a spectrophotometer was used to measure the absorbance at a wavelength of 760 nm. Gallic acid equivalent (GAE) was used to express the total amount of phenol obtained. Folin-Ciocalteu's Phenol Reagent was used to generate a gallic acid standard curve. Gallic acid solutions in aquadestilate were made at 0, 20, 40, 60, 80, and 100 g/mL concentrations. From each concentration, 15.8 mL of distilled water and 1 mL of Folin-Ciocalteu reagent were added, and the mixture was then homogenized to create a clear, yellowish solution. Eight minutes were given for the solution to stand before 3 mL of a 20% Na_2CO_3 solution was added and homogenized by shaking. Once more, the solution was left to stand at room temperature for 30 minutes until a blue tint developed. A calibration curve was created for the relationship between gallic acid content (mg/L) and absorbance after the solution's absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 760 nm (Hashim et al., 2019).

Total flavonoid content

With the help of the photometric aluminium chloride (AlCl3) method, the total flavonoid content was evaluated. The mixture of 0.5 mL of 2% $AlCl_3$ and 0.5 mL of propolis extract was homogenized and allowed to sit for 10 minutes. At a wavelength of 435 nm, the absorbance was measured using a spectrophotometer (Najafi et al.., 2007). The quercetin standard curve was made by weighing 25 milligrams of quercetin powder and dissolving it in 25 mL of distillate. To get a concentration of 100 ppm, pipette 1 mL of the solution and then add 10 mL of pure water. Then, different concentrations of a standard solution containing 100 ppm of quercetin were created, including six ppm, eight ppm, ten ppm, 12 ppm, and 14 ppm. For each concentration, pipette 1 mL of the quercetin standard solution, followed by 1 ml of the 2% $AlCl_3$ solution and the 120 mM potassium acetate solution. At room temperature, the standard quercetin was incubated for a full hour. At a maximum wavelength of 435 nm, the absorbance was calculated using UV-Vis spectrophotometry (Stankovic et al., 2011).

Tannins content

The tannin content was determined using spectrophotometry analysis. Weigh the sample to a maximum of 0.5 mL and thoroughly mix it with 5 mL of distilled water. Pipette 1.0 mL of the sample and add it to 7.5 mL aquadestilate in a 10 mL container. After adding 0.5 mL of the reagent (Folin-Denis) and letting it sit for 3 minutes, 1.0 mL of the saturated Na_2CO_3 solution was added. The absorbance was measured at a maximum wavelength of 700 nm after 15 minutes of incubation (Padey et al., 2018). Tannic acid measurement is a standard solution used to analyze total tannin content. A standard curve was used to determine the concentration of the measured sample. Standard solutions of concentrations of 10, 15, 20, 25, 30, and 35 ppm were taken in 1 ml each, and then 7.5 mL of distilled water was added. Next, 1 mL of Folin-Ciocalteu reagent was added. 1 mL of saturated Na_2CO_3 was added after the mixture had been allowed to stand for 3 minutes. The solution was kept in a dark place throughout the homogenization procedure for 15 minutes (Diniyah et al., 2023).

Antioxidant activity

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) method examined antioxidant activity. 9 mL of DPPH solution was homogenized with 1 mL of propolis extract. Following homogenization, the mixture was incubated for 30 minutes at room temperature. Absorbance measurements were made using a UV-Vis spectrophotometer with a maximum wavelength of 517 nm (Hidayat et al., 2022). DPPH reagent production process 96 mL of methanol with 4 mL of stock DPPH solution (Padey et al., 2018). The solution is protected with aluminium foil and stored in a dark place. The DPPH solution, which had a concentration of 160 mg/L, was diluted with hexane to create solutions with concentrations of 4, 8, 16, and 32 mg/L. The absorbance of each DPPH solution was measured at its maximum wavelength (517 nm). The linear regression equation resulting from comparing fluctuations in the concentration of the sample against the DPPH solution was used to calculate the IC_{50} of the sample preparation against the DPPH solution. In the linear regression equation, the concentration value of the extract or the comparative antioxidant (BHT) and its inhibition % were plotted on the x and y axes, respectively. y = ax + b is the equation for the discovered linear regression. By specifying the y value of 50 and the x value to be acquired from the IC_{50}, this equation is used to determine the IC_{50} value (50% inhibitor concentration) of each sample. According to Segura-Campos et al. (2014), the IC_{50} value represents the concentration of sample solution (BHT extract or antioxidant comparator) needed to reduce DPPH free radicals by 50%.

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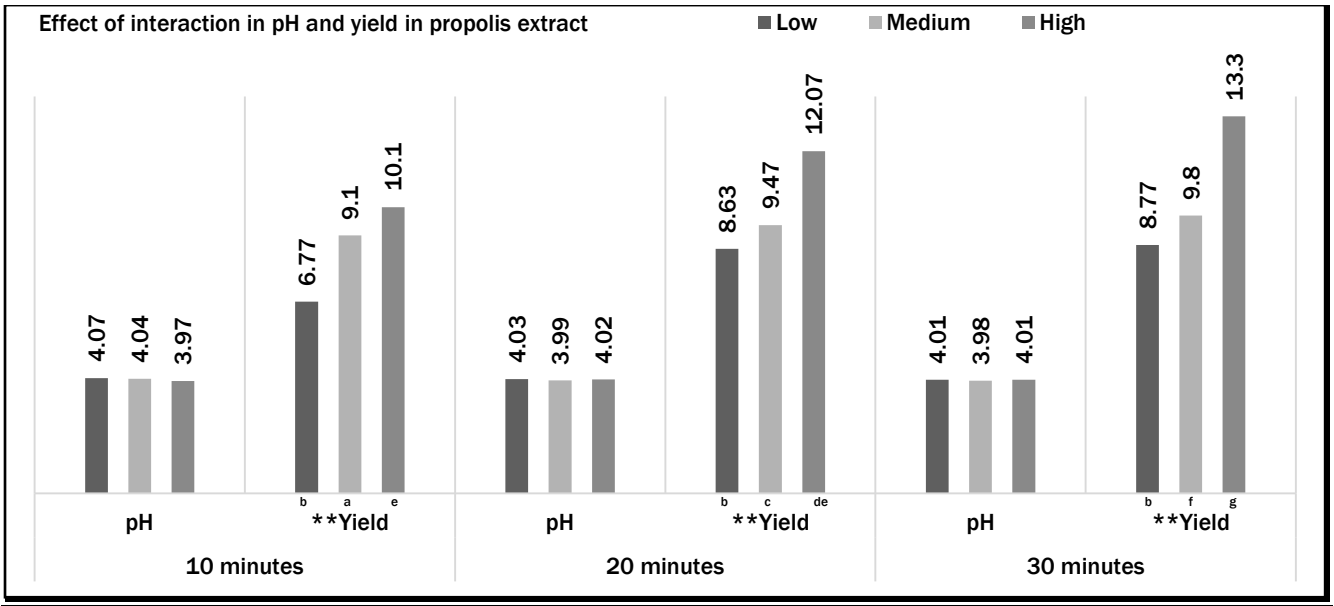
RESULTS AND DISCUSSION

Physical analysis

pH

Graph 1 and Table 2 display the findings of the analysis of the pH level of the propolis extract. The propolis extract was extracted using the UAE method at different temperatures and times, and the interaction between temperature and extraction time had no effect ($p>0.05$) on the pH of the propolis extract. The average pH value based on the interaction of the two factors is 3.97 to 4.07. The pH value shows that the propolis extract has a relatively low acidity level. The pH decreases with increasing temperature and time. Propolis' low pH value is known to prevent the growth of bacteria and fungi. Hence, propolis extract can prolong shelf life. *Apis mellifera* propolis extracted using the UAE method with a water solvent at a temperature of 35–40 °C and carried out for 5–30 minutes is known to produce a pH in the range of 3.44–3.56 (Pangesti et al., 2023). This study produced propolis extract with a slightly higher pH value, but not significantly. The acidic pH value of propolis can also be suspected because propolis contains components of organic acids and vitamin C. Besides that, it is also suspected of the presence of phenolic compounds, quercetin, and calcium. The acidity level in bee products such as propolis and honey is influenced by the plant's organic acid and mineral content, which makes the plant have distinctive characteristics (Hidayat et al., 2023). The extraction results in a lower pH as the temperature and time increase. Therefore, because the pH of the propolis extract in this study produced a low value (acid), it is suspected that there were soluble organic acid compounds. This event is linear with Oroian et al. (2020), who found that different temperature and time treatments in this study could capture organic acids and phenols in propolis, resulting in propolis extract with an acidic pH.



Graph 1 - Interaction of temperature and extraction time of the UAE method on pH and yield. **: superscripted ^{a,b,bc,bcd,cd,de,ef,fg,g} that means columns with superscripts differed significantly ($P<0.01$)

Table 1 – pH and yield with different temperature and time

	Temperature (°C)			Times (minutes)		
	Low (A1)	Medium (A2)	High (A3)	10 (B1)	20 (B2)	30 (B3)
pH	4.04±0.08	4.00±0.05	4.00±0.03	4.03±0.09	4.01±0.03	4.00±0.04
Yield (%)	$8.54±1.52^{p}$	$9.30±0.66^{q}$	$11.49±1.88^{r}$	$8.83^{x}±0.30^{x}$	$9.43±2.34^{y}$	$11.02±1.73^{z}$

p,q,r and x,y,z superscript; Means in columns with superscripts differed significantly ($P<0.01$)

Yield

The yield analysis results on propolis extract are shown in Table 1 and Graph 1. The analysis showed that the propolis extract used the UAE method at different temperatures and times, and the interaction between temperature and extraction time was very significant ($P<0.01$) on propolis extract yield. So, this research shows that the use of temperature and time significantly affects the yield produced. In addition, different temperatures and times interact with each other. This study's average extraction yield values ranged from 6.77% to 13.3%. Low extraction temperatures have not been able to produce maximum yields. However, low-temperature extraction combined with increased extraction time also

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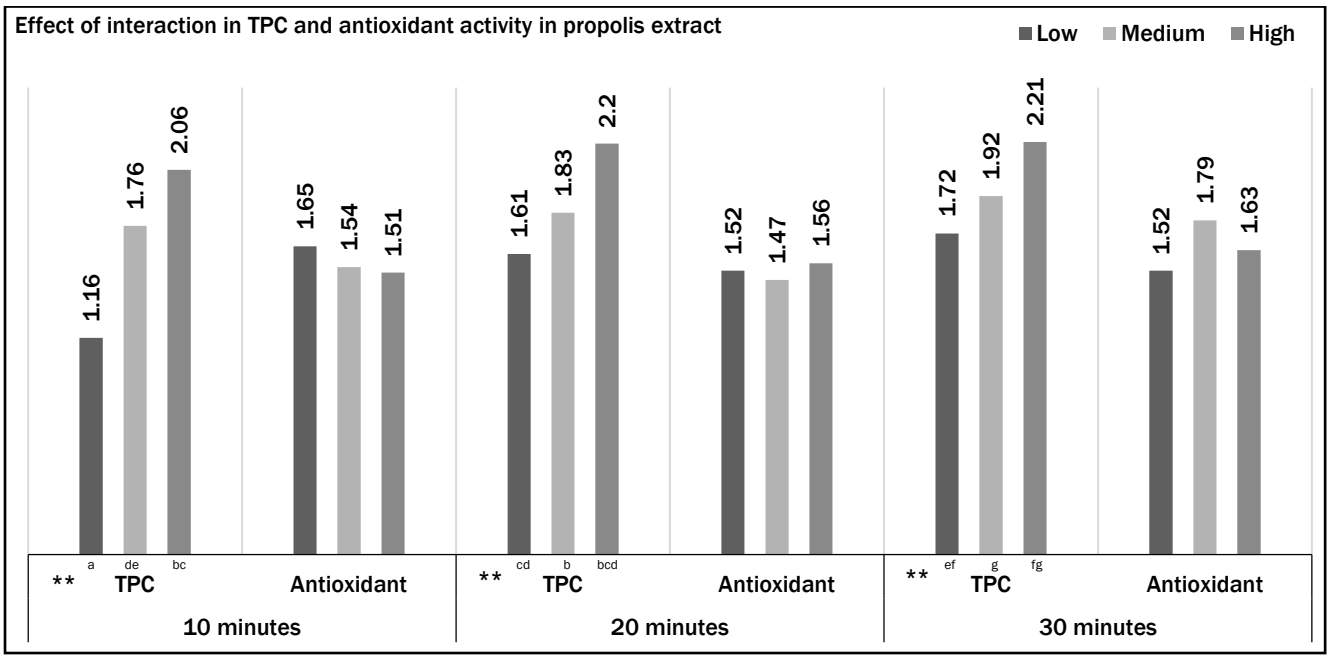
significantly affects the yield value. The increase in temperature and the length of time show an increasing extraction yield. This event shows that an increased temperature and a certain amount of time can capture more solutes. The highest yield was obtained when extraction was performed using ultrasonics at 60–63 °C for 30 minutes.

The yield results in this study tend to be lower than those of propolis extraction using the UAE method with ethanol solvent, which produces an extraction yield of 35.7–42.6% (Chong and Lee, 2020). The yield of propolis extract in this study is known to be higher than the yield of Korean propolis extraction using the UAE method with water as a solvent (Aboulghazi et al., 2022). Apart from that, Moroccan propolis using UAE water and 40% ethanol as a solvent for 30 minutes produced a yield of 8.5%. Ultrasonic-assisted extraction is known to increase yields because the cavitation bubbles produced by ultrasonics cause significant shear forces, resulting in higher extract yields. Longer extraction times can also increase extraction results because sample component degradation will take too long. However, if it is too long, it can also cause a decrease in results (Shen et al., 2023). Various studies show different yields of propolis extract, indicating that the geographical location of the sample greatly influences propolis extract because bees collect different shoots and plant exudates in the surrounding area. It can also be said that the low yield of water extract indicates that there are fewer water-soluble compounds in the propolis (Chong and Lee, 2020).

Chemical analysis

Total phenolic content (TPC)

The findings of the investigation of the total phenolic content (TPC) and IC_{50} antioxidant activity in propolis extract are displayed in Graph 2. When the proper functional derivatives are present, organic molecules called phenols containing aromatic rings are chemically linked to one or more hydrogenated substituents (Pasupuleti et al., 2017). The analysis showed that the propolis extract using UAE at different temperatures and times and the interaction between temperature and extraction time had a very significant effect ($P<0.01$) on the TPC of the propolis extract. TPC on propolis extract using low temperatures gives very different results than medium and high temperatures. The TPC results of propolis extract at different temperatures and times are shown in Table 2. The highest average total phenol was found in propolis extract extracted at high temperature, namely 2.21 mg GAE/mL, and the lowest average was found in propolis extract extracted at low temperature, namely 1.6 mg GAE/mL. Low temperatures (27–30 °C) have not been able to extract the maximum total phenol. High temperatures (60–63 °C) can produce more total phenol. Previous studies stated that using UAE at 60–65 °C for 30 minutes for propolis extraction is the optimal temperature to obtain total phenol and flavonoid content (Oroian et al., 2020). The total phenol in propolis extract is affected by temperature and the length of the extraction time, which has been observed to affect the total phenol. Extraction time for 20 minutes did not differ from extraction time for 30 minutes but was significantly different from extraction time for 10 minutes. Propolis extraction for 20 minutes resulted in a higher TPC of 1.92 mg GAE/mL compared to 30 minutes of extraction, namely 1.89 mg GAE/mL.



Graph 2 - Interaction of temperature and extraction time with the UAE method on TPC (total phenolic content) (mg GAE/mL) and antioxidant activity IC_{50} (µg/mL). **: superscripted ^{a,b,bc,bcd,cd,de,ef,fg,g} that means in columns with superscripts differed significantly ($P<0.01$).

Previous research extracted *Apis mellifera* propolis from Morocco using the UAE method with water and 40% ethanol in a ratio of 1:10 for 30 minutes, producing a TPC of 111.32 mg GAE/g (Aboulghazi et al., 2022). In contrast to the results of previous studies that also extracted Malaysian propolis using UAE at 65 °C for 25 minutes, the highest TPC content was

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0.093 mg GAE/g, while the lowest content was extracted for 55 minutes, which was 0.058 mg GAE/g. This study produced a lower TPC than Moroccan propolis but a higher TPC than Malaysian propolis, namely Central Java propolis, which produced a TPC of 1.16 to 2.21 mg GAE/mL. The phenolic compound content of propolis extract in each country is thought to be because the chemical content of propolis extract is also influenced by differences in regional origin and surrounding plant vegetation, as well as climatic factors (Lim et al., 2023). Water is a more polar solvent than solvents with a mixture of 60% and 80% water and ethanol, which are known to extract more polar compounds. A higher polarity allows the extraction of many binding compounds from the propolis material, which has relatively more polar properties. Phenolic compounds are known to be primarily soluble in polar solvents. Phenolic compounds are phenols and include flavonoids, tannins, and alkaloids. The increasing extraction temperature results in the total phenol value increasing, which can be expected because the high temperature used during extraction can reduce the viscosity of the solvent so that it can increase the penetration ability of the solvent into the propolis mass, which increases extraction efficacy (Suran et al., 2021; Sasongko et al., 2017). The extraction in this research, which utilizes water as a solvent and is supported by ultrasonic methods with increased temperature and time, can produce significantly increased phenolic compounds.

The phenol results are associated with a pH value known to have a low pH (acid), which shows that the extraction process using polar water assisted by ultrasonics can produce propolis extract with a high phenol content. Using a higher sonication temperature will cause changes in vapour pressure, surface tension, viscosity, and solvent, thereby affecting the extraction cavitation process and causing cell wall damage, which can then increase the diffusivity of phenolic compounds. High temperatures can also increase solubility, thereby speeding up the extraction process. High extraction temperatures are only sometimes suitable in the UAE because phenolic compounds are heat-sensitive. It can be concluded that extraction using temperatures up to 100 °C will be expected to reduce the total amount of phenol (depending on the solvent's boiling point). Suppose the heat of sonication exceeds the boiling point of the solvent. In that case, more and more of the solvent will be evaporated, causing the volume to continue to decrease, thereby reducing extraction efficiency (Yusof et al., 2020). Based on the results of the total phenol content in this study, using the highest temperature was considered good because it did not exceed the solvent's boiling point and showed increasing results at temperatures of 60–63 °C.

Antioxidant activity as IC_{50}

The DPPH method, which measures the amount of the reduction in the absorption of free radicals in DPPH solutions at a wavelength of 517 nm, was used to test the antioxidant activity of various substances. The antioxidant activity parameter uses IC_{50} (the initial DPPH concentration by 50%), which is the concentration of the extract (fraction) that contributes 50% antioxidant activity compared to the control through the linear regression line equation (Wiwekowati et al., 2017). There are five classifications of IC_{50} values to determine their strength: a powerful antioxidant group is one with an IC_{50} value of 50 g/mL; a potent antioxidant group is one with an IC_{50} value of 50 to 100 g/mL; a moderate antioxidant group is one with an IC_{50} value of 101 to 150 g/mL; a weak antioxidant group is one with an IC_{50} value of 15 to 200 g/mL; and a frail antioxidant group (Hidayat et al., 2022). The statistical analysis results in this study showed that propolis extract using UAE at different temperatures and times had a significant effect ($p>0.05$) on the antioxidant activity IC_{50}. The average IC_{50} value was obtained in the 1.47-1.79 µg/mL range. Low temperatures produce the lowest IC_{50} value compared to medium and high temperatures. Extraction time for 20 minutes produces the lowest value compared to extraction time for 10 and 30 minutes, as shown in Table 2. The interaction between temperature and time that produces the lowest IC_{50} value is obtained using a medium temperature (40–43 °C) for 20 minutes, as much as 1.47 µg/mL. The DPPH antioxidant activity was observed to have an IC_{50} value <50 µg/mL; this shows that the antioxidant activity in *Apis mellifera* Central Java propolis extract has intense activity. Extraction using different temperatures and times, along with the interaction between the two, does not influence the IC_{50} value, but using a higher temperature with a longer extraction time can potentially increase the IC_{50} value.

Romanian ethanol propolis extracted using UAE produced an average IC_{50} value ranging from 0.0700 to 0.9320 mg/mL. In addition, Malaysian propolis extracted using UAE with water and acid ethanol solvents produced an IC_{50} of 0.1731 mg/mL (Chong and Lee, 2020). This event shows that Central Java propolis extract with distilled water solvent produced a lower IC_{50} value, so it has more robust antioxidant activity than Romanian and Malaysian ethanol propolis. However, this study showed that extraction with temperatures reaching 60 °C and times exceeding 20 minutes reduced antioxidant activity. UAE produced an acoustic cavitation effect that can cause high temperatures to reduce particle size and increase mass transfer. Therefore, the UAE method only requires a short time and low amounts of solvent (Bankova et al., 2021). Based on the research of the relationship between temperature utilization and extraction time, the lowest IC_{50} was obtained from propolis extraction using a moderate temperature (40–43 °C) for 20 minutes. These antioxidant properties are usually directly related to the total phenol content. It was proven in this study that propolis extraction using UAE with water as a solvent could bind phenolic compounds in Central Java propolis, so it could also detect the presence of its antioxidant activity.

The analysis of antioxidant activity in this study used a standard solution of gallic acid as a comparison because gallic acid is known to have strong and stable antioxidant properties. The results of the antioxidant activity study illustrate the ability of total phenols in propolis extract to act as antioxidants. However, based on the statistical analysis results, total phenol is not directly proportional to antioxidant activity. It is suspected that the antioxidant activity in propolis extract comes from total phenols and the interaction of several other phenolic compounds, such as tannins and

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flavonoids, which also have antioxidant activity. Phenolic compounds are known to contribute to antioxidant activity because they can donate hydrogen atoms or electrons to free radicals to bind free radicals and decompose oxidation products (Diniyah and Lee, 2020). Although the antioxidant activity of DPPH in this study did not provide a significant difference, it appeared to have a relationship with total phenols and total flavonoids, so it was able to produce low IC_{50} values (Cottica et al., 2011).

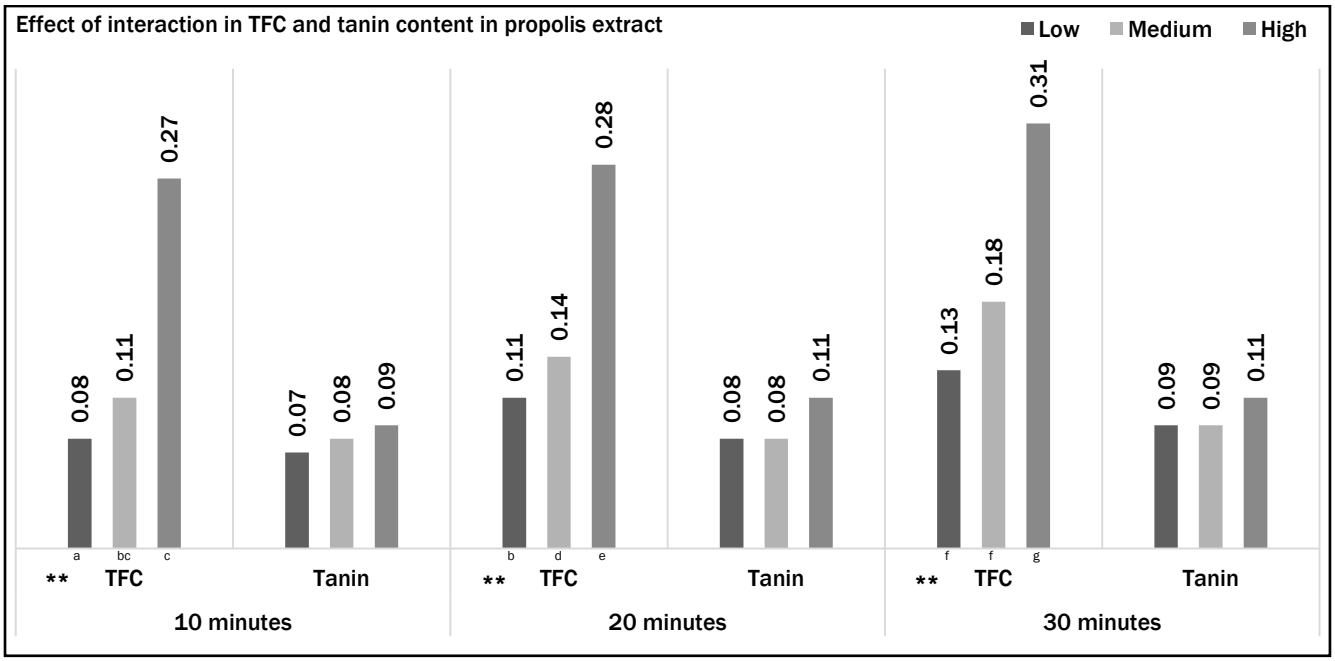
Table 2 –TPC, TFC, tannins content, and antioxidant activity with different temperature and time

Parameters	Temperature (°C)			Times (minutes)		
	Low (A1)	Medium (A2)	High (A3)	10 (B1)	20 (B2)	30 (B3)
TPC (mg GAE/mL)**	1.60±0.35^{p}	1.73±0.11^{q}	2.16±0,10^{r}	1.68±0.41^{x}	1.92±0.27^{y}	1.89±0.23^{y}
TFC (mg QE/mL)*	0.11±0.02^{p}	0.14±0.03^{q}	0.29±0.02^{r}	0.15±0.09^{x}	0.18±0.08^{y}	0.21±0.08^{z}
Tanin content (mg TAE /mL) *	0.08±0.01^{p}	0.09±0.01^{p}	0.10±0.01^{q}	0.08±0.01^{x}	0.09±0.01^{y}	0.10±0.01^{y}
Antioxidant activity IC_{50} (µg/mL)	1.56±0.39	1.60±0.15	1.57±0.08	1.57±0.08	1.52±0.38	1.65±0.13

**p,q,r and x,y,z superscript; Means in columns with superscripts differed significantly (P<0.01). *p,q and x,y superscript; Means in columns with superscripts differed significantly (P<0.05); TPC: Total Phenolic Content; TFC: Total Flavonoid Content; QE= quercetin equivalent; GAE= Gallic acid equivalent.

Total flavonoid content

Graph 3 shows the results of determining the total flavonoid (TFC) and tannin content. Flavonoids are a derivative of phenolic compounds with a conjugated aromatic ring system so that they can absorb UV-vis. The analysis demonstrated that the propolis extract was made using a UAE process with various extraction times and temperatures. As well as the interaction between temperature and time had a very significant effect (P<0.01) on the total flavonoids of the propolis extract. Follow-up tests showed that the total flavonoids of Central Java propolis extract extracted at low temperatures significantly differed from medium and high temperatures. The highest TFC average was obtained in propolis extract that was extracted at a high temperature, which was 0.28 mg QE/mL, and the lowest average was found in extraction that used a low temperature, which was 0.11 mg QE/mL. Low temperatures (27–30 °C) have not been able to extract flavonoids maximally, as shown in the observations in Table 2, as well as the extraction time of 10 minutes. The amount of TFC produced likewise rises as the temperature rises. This condition also holds for the utilization of extraction time; the higher the TFC, the longer the time used. It is demonstrated that the TFC in propolis extract is influenced by both temperature and the duration of extraction. The interaction between temperature and extraction time is also significantly related. Along with the high temperature (60-63 °C) and the long time used, the highest TFC was 0.31 mg QE/mL. The increasing temperature and length of time used show that the total phenol also increases.



Graph 3 - Interaction of temperature and extraction time with the UAE method on TFC (total flavonoid content) (mg GAE/mL and tannins content (mg TAE/mL). **: superscripted ^{a,b,bc,bcd,cd,de,ef,fg,g} that means columns with superscripts differed significantly (P<0.01)

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Ethanol is a solvent commonly used to dissolve flavonoid compounds (Azmir et al., 2013; Pandey and Shalini, 2014). In general, water solvents can only dissolve polyphenols and phenolic compounds (Stalikas, 2007). However, this study proved that extraction using a water solvent with ultrasonic assistance can dissolve flavonoid compounds even at low values, following the statement of Khoddami et al. (2013) that ultrasonic-assisted extraction can help extract phenolic and flavonoid compounds. Ultrasonic waves cause damage to the cell walls, which causes the cell contents in the form of plant metabolites to come out. Ultrasonic-assisted extraction in this study could bind flavonoids even though it only used water as a solvent. Research by Aboulghazi et al. (2022) extracted *Apis mellifera* propolis from Morocco using UAE with 40% water and ethanol as a solvent and the same ratio (1:10) for 30 minutes, producing a total of 34.72 mg QEq/g flavonoids. Based on the results of this research, use of time for 30 minutes showed a lower average total flavonoid result, namely 206.38 µg QE/mL or 0.21 mg QE/mL. The total amount of flavonoids in the study was still below Moroccan propolis. Still, it was relatively higher compared to the results of previous research that used Malaysian propolis, which was extracted using UAE for 30 minutes with 70% distilled water-ethanol solvent, namely only 0.015 mg QE/mL or 150 µg QE/mL (Zainal et al., 2021). The flavonoid content in propolis was higher when extracted using the UAE method (Bankova et al., 2021). The total flavonoids produced in Central Java propolis extract are higher as the extraction time used increases. The longer the UAE extraction time, the more compounds it will produce. It was learned in previous research that increasing the extraction time to more than 30 minutes will reduce total phenolic and total flavonoid compounds because it can cause compound degradation. Cavitation bubbles will burst so that they can damage the substances in the solution. Total flavonoids and total phenols were studied to influence the antioxidant (antiradical) and reduce the activity of propolis (Bouaroura et al., 2019).

Tannins content

Tannins are known as a type of secondary metabolite compound that can be found in plants. In addition, tannins are polyphenols that can react with extracellular enzymes and bacterial cell walls. By blocking the entry of nutrients into cells, this technique can stop the growth of these bacteria. Tannin compounds can dissolve in water solvents (Lim et al., 2023). The analysis showed that the Central Java propolis extract used the UAE method at different temperatures and times, and the interaction between temperature and time had no effect ($p>0.05$) on the tannin content of the propolis extract. A table of the analysis of the effect of using different temperatures and times has been presented in Table 2, and the interaction between temperature and extraction time is shown in Graph 3. The results of further tests showed that the tannin content in propolis extract extracted at low temperatures was not different from that at medium and high temperatures. The average tannin content of Central Java propolis extract ranged from 0.07 to 0.11 mg TAE/mL. Extraction at a low temperature for 20 minutes and 30 minutes produced the same tannin content as extraction at a low temperature for 10 minutes. This condition shows that low temperatures can produce tannin content, and an increase in temperature does not significantly affect the resulting tannin content. However, the results show quite a visible difference as the extraction time increases.

The lowest tannin content was obtained at a low temperature for 10 minutes, while the highest was obtained at a high temperature for 30 minutes. Increasing the temperature and length of time can also result in increased tannin levels, which indicates that the levels of secondary metabolites carried by the solvent increase. The secondary metabolite compounds in propolis are tannin compounds (Chong and Chua, 2020). Previous research studied that extraction using UAE at a temperature of 55 °C produced higher tannin levels than 35 °C. Increasing temperature can increase mass transfer, affecting the observed extraction (Padey et al., 2018). The results of the tannin content of propolis extract in this study accumulated positively with total flavonoids. It is plausible since tannins are part of the total flavonoids. Tannin is also thought to be one element that gives propolis its dark colour (Lim et al., 2023). Propolis tannin levels are rarely discussed in research. Previous studies on conventionally extracted Central Java propolis detected a tannin content of 0.213%, but the concentration of tannins was less than the tannins in South Sulawesi propolis, which was 0.957%. Seven different kinds of plants can produce resin; these include durian, cempaka, cocoa, pine, randu, resak, and cassava. According to Mahani et al. (2021), these tannin compounds are known to have antibacterial and antioxidant effects.

CONCLUSION

This research can evaluate the results of the content of bioactive compounds, such as phenols, flavonoids, and tannins, in *Apis mellifera* Central Java propolis extract. Propolis extraction using a water solvent with ultrasonic assistance produces good physical and chemical quality, although it is still lower than some literature results. Central Java propolis extract (WEP) obtained maximum results with treatment at a temperature of 60–63 °C for 30 minutes in ultrasonics in terms of extract yield (13.3%), TPC (2.21 mg GAE/mL), TFC (0.31 mg QE/mL), and tannin content (0.11 mg TAE/mL). The UAE method is proven to be able to help the extraction process in a shorter time; however, propolis extraction using water as a solvent is too short, and at low temperatures, it is still not optimal for producing bioactive components, especially phenols, flavonoids, and tannins. Based on the research results, use the lowest temperature of 60 °C for 30 minutes to obtain maximum propolis extract.

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DECLARATIONS

Corresponding author

Correspondence and requests for materials should be addressed to Dr. Agus SUSILO; E-mail: agussusilo@ub.ac.id; ORCID: https://orcid.org/0000-0002-4440-5806

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Authors' contribution

IF. Pangesti, A. Susilo, and K.U.A. Awwaly contribute to the research, data analysis, and manuscript writing.

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Consent to publish

The authors agree to the publication of this manuscript.

Competing interests

The authors declare no competing interest in this research and publication.

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