Antioxidant Activity and Total Flavonoid of Propolis Stingless Bee

Aktivitas Antioksidan dan Total Flavonoid Propolis Trigona sp.

Akhmad Endang Zainal Hasan[1], Ukhradiya Maharaniq Safira[1], Anita Purnamasari[2], Sri Wardatun[2]

[1]Departemen Biokimia, FMIPA, Institut Pertanian Bogor
[2]Program Studi Farmasi, FMIPA, Universitas Pakuan
[a]Korespondensi : Akhmad Endang Zainal Hasan, E-mail: zainalhasan@apps.ipb.ac.id

Diterima: 08 – 10 – 2022, Disetujui: 31 – 08 - 2023

ABSTRACT

Propolis from Indonesia contains secondary metabolites which include flavonoids and other organic compounds. The ethanol extract of 70% propolis Trigona spp from Pandeglang can be used as an antibacterial compound. The aim of this study was to measure the toxicity of the propolis and nanopropolis with an LC50 value, test the antioxidant activity, and determine the total flavonoid content. The Brine Shrimp Lethality Test (BLST) method was used to determine LC50 using Artemia salina Leach larvae. The DPPH method (1,1 diphenyl-2-picrylhydrazil) used for the antioxidant activity test, and the determination of flavonoid content was carried out with aluminum chloride. The results showed that the LC50 value of propolis was 16.010 ppm, and for 20% nanopropolis was 18.689 ppm. Antioxidant activity obtained IC50 value for propolis is 95.54593 ppm, for 20% nanopropolis is 527.7939 ppm. The Propolis extract contains 2.1123% of flavonoids and 1.5293% of nanopropolis.

Keywords: antioxidant activity, flavonoid, nano propolis, propolis, toxicities

ABSTRAK

Propolis dari Indonesia mengandung metabolit sekunder yang meliputi flavonoid dan senyawa organik lainnya. Ekstrak etanol propolis Trigona sp. 70% asal Pandeglang dapat dimanfaatkan sebagai senyawa antibakteri. Tujuan dari penelitian ini adalah mengukur toksisitas propolis dan nanopropolis dengan nilai LC50, menguji aktivitas antioksidan, dan menentukan kandungan total flavonoid. Metode BLST digunakan untuk menentukan LC50 menggunakan larva Artemia salina Leach. Metode DPPH (1,1 diphenyl-2-picrylhydrazil) digunakan untuk uji aktivitas antioksidan, dan penentuan kandungan flavonoid dilakukan dengan aluminium klorida. Hasil penelitian menunjukkan nilai LC50 propolis sebesar 16.010 ppm, dan untuk nanopropolis 20% sebesar 18.689 ppm. Aktivitas antioksidan yang diperoleh nilai IC50 untuk propolis adalah 95,54593 ppm, untuk nanopropolis 20% adalah 527,7939 ppm. Ekstrak propolis mengandung 2,1123% flavonoid, dan 1,5293% nanopropolis.

Kata kunci: propolis, nanopropolis, toksisitas, aktivitas antioksidan, flavonoid

Hasan, A. E. Z., Safira, U. M., Purnamasari, A., & Wardatun, S. (2023). Antioxidant Activity and Total Flavonoid of Propolis Stingless Bee. *Jurnal Agroindustri Halal,* 9(2), 149 – 157.

INTRODUCTION

Propolis is a kind of resin that because of its sticky shape like glue, is called bee glue. Propolis is produced by bees by collecting resins from various kinds of plants (Anjum *et al.*, 2019). One type of bee that is able to produce propolis in large quantities is the type of Trigona sp (Anjum *et al.* (2019); Wahyuni & Riendriasari (2021)).

Nanotechnology is an attempt to change the composition or engineering of structural materials at the nanoscale so that the desired function of the material is obtained. Nano itself is a size that is 10^{-9} m or 10^{-3} micro. Nanotechnology is a science that studies particles in the size range of 1-1000 nm (Bayda et al., 2019). The shape and size or the particle size are very influential in the process of drug absorption, solubility, and distribution of drugs, thus increasing the effectiveness of the efficacy of propolis, propolis is made in nanoparticle size. For example, in a study using 70% ethanol extract of propolis which was made into nanoparticles, it was shown to have higher antibacterial activity compared to 70% ethanol extract of propolis against E. coli bacteria in vitro (Hasan *et al.*, 2016).

A toxicity test method widely used in the search for bioactive compounds guided by fractionation of natural ingredients because it is fast, easy, inexpensive, and quite reproducible is Brine Shrimp Lethality Test (BSLT). The method is good correlation with anticancer test. Toxicity is determined by looking at the LC50 price which is calculated based on probit analysis (Kumar *et al.*, 2020).

A compound that donates electrons (electron donor) or is a reducing agent is known as an antioxidant. Even though it has a small molecular weight, it is capable of deactivating oxidation reactions by preventing the formation of radicals. These antioxidant compounds can inhibit oxidation reactions by binding to reactive molecules from free radicals. So that cell damage does not occur or is said to be inhibited (Martemucci *et al.*, 2022).

Antioxidant activity can be measured by DPPH method (1,1-diphenyl-2-picrylhydrazyl). The mechanism of DPPH radical scavenging is by donating H atoms from antioxidant compounds which causes the color of the purple picrylhydrazyl radicals to be reduced to yellow, non-radical picrylhydrazil. The parameter used in the DPPH method is IC50 that causes a 50% loss of DPPH radical activity (Rivero-Cruz *et al.*, 2020).

Flavonoids are the largest group of polyphenolic compounds, therefore extract solutions containing flavonoid components will change color when given an alkaline solution or ammonia. Flavonoids are grouped into 9 classes, namely anthocyanins, proanthocyanins, flavonols, flavones, glyco flavones, bioflavonoids, chalcones and aurones, flavanones, and isoflavones. Flavonoids in plants bind to sugars as glycosides and some are in aglycones (Dias *et al.*, 2021).

MATERIALS AND METHODS

Materials

The materials used were crude propolis *Trigona* sp., 70% ethanol, distilled water, maltodextrin, magnesium stearate, plastic wrap, aluminum foil, *Artemia salina* L. eggs, DPPH reagent, methanol, AlCl3, sodium acetate, vitamin C.

Extraction of Propolis Trigona spp

Propolis was extracted by maceration using the Trusheva *et al.* (2007) method. Put a 60 g of raw propolis into a 500 mL Erlenmeyer containing 360 mL of 70% ethanol, then macerated for 72 hours using a shaker at a speed of 125 rpm. The extract was separated by filtering, then the filtrate formed was evaporated to form a thick extract. The yield was obtained by calculating the percent weight of the extract against the weight of the simplicia.

Production of 20% Nanopropolis

The manufacture of nanopropolis was carried out by a modified method (Akhmad Endang Zainal Hasan *et al.*, 2016) as much as half of the formula. Put into Erlenmeyer a 11.25 g of propolis ethanol extract and added 50 mL of 70% ethanol. Dissolved 42.5 g of maltodextrin as a coating material with 40 mL distilled water and 2.5 g of magnesium stearate is added then stirred with a homogenizer until well mixed, then the propolis ethanol extract which has been dissolved in 70% ethanol is mixed and the mixture is quickly homogenized again at speed of 22,000 rpm for 1 minute x 30 with a rest time of 3 minutes. After that, used vacuum dryer at a temperature of 50^0C to dried solution. The powder formed is nano-sized particles and the size identification is carried out using Scanning Electron Microscopy (SEM). The yield was obtained by calculating the percent weight of the extract and the simplicia.

Extract and Nanopropolis Characterization

a) Determination of Water Content

By using a moisture balance tool the water content is determined (BPOM, 2014). The sample used is 1 g. Performed with two repetitions.

b) Determination of Ash Content

Weigh 2-3 g of the sample and then store it in the furnace ±600°C until it becomes charcoal. Calculation of ash content is carried out according to what is stated in BPOM (2014).

c) Phytochemical Test of Extracts and Nanopropolis

Flavonoid Test

Phytochemical test parameter (flavonoid, saponin, alkaloid, dan tannin) measurements were carried out according to the procedure from (BPOM, 2014).

Toxicity Test

Hatching Eggs *Artemia salina* Leach

Hatching of *Artemia salina* Leach eggs was carried out in clear containers such as beakers or jars that were given plastic, negative film, or glass by using salt water media with a salt content (NaCl) of 15 g/L. Illuminated with 40-60 watt incandescent or fluorescent lamps of 25-30˚C is maintained and oxygen is supplied with an airator. Active *nauplii* that have aged 48 hours were used as test animals in the study (Filha *et al.*, 2011).

Toxicity Test Procedure with Brine Shrimp Lethality Test (BSLT) Method (Filha *et al.*, 2011)

The 70% ethanol extract of propolis and nanopropolis were weighed as much as 50 mg each and diluted with seawater and then put into a 100 mL volumetric flask, and added with seawater to the volume limit. The mother liquor of 500 ppm was obtained, then the mother liquor was pipetted 4; 2; 1; 0.5; 0.4; 0.2; and 0.1 mL was put into a 10mL vial. The control solution contained only seawater without the addition of extracts and nanopropolis. 10 shrimp larvae of *Artemia salina* L were added and seawater was added to the limit so that the concentrations in each vial were 200, 100, 50, 25, 20, 10, and 5 ppm. Each vial was added with 1 drop of 0.6 mg/mL yeast suspension as a food for shrimp larvae. Toxicity test was carried out on the test solution and control solution that had been made, the toxicity test treatment was carried out 3 times for each sample. Observations were made for 24 hours on the mortality of shrimp larvae.

$$\textbf{\% Larvae death} = \frac{\textbf{Number of death larvae} - \textbf{Number of control deaths}}{\textbf{Number of test larvae } (\mathbf{10})} \; \boldsymbol{x}\; \mathbf{100}\% \qquad (1)$$

Perform a Probit calculation of the mortality of *Artemia salina* Leach larvae, then use the following equation.:

$$\mathbf{Y = Bx + A} \qquad (2)$$

Where:

X = log concentration, and

Y = Number of probit

Total Flavonoid Test

Total flavonoid content was determined spectrophotometrically with aluminum chloride reagent. Weighed as much as 200 mg of 70% propolis extract and nanopropolis powder, dissolved with methanol. 5 mL of 70% propolis extract solution was pipetted and 20 mL of nanopropolis solution was pipetted, then added 10 mL of aquadest, 1 mL of 10% $AlCl_3$, 1 mL of 1 M sodium acetate. Then calculated total flavonoids using the formula:

$$\% \text{ percentage of flavonoid} = \frac{ppm\ x\ ml\ x\ fp\ x\ 10^{-6}}{g\ weight\ ekstract} \text{ x } 100\% \quad (3)$$

Antioxidant Activity Test

The variation of the test solution was made by first making 2000 ppm mother liquor by dissolving 200 mg of 70% propolis ethanol extract and nanopropolis powder. Dissolved with methanol. Standard series were 25, 50, 100, 200, 400, 600, and 800 ppm in a 10 mL volumetric flask by pipetting 0.125; 0.25; 0.5; 1; 2; 3; and 4 mL of mother liquor in a 10 mL and adjusted to the limit using methanol p.a. Each row flask was added with 1 mL of 1mM DPPH solution then diluted using methanol and homogenized. The absorbance was measured at the maximum wavelength. The test solution series, the vitamin C positive control solution series and the blank for control. The percentage value of DPPH is calculated using the following formula:

$$\%\text{inhibition} = \frac{\text{blank absorbance} - \text{sample absorbance}}{\text{blank absorbance}} \text{x } 100\% \quad (4)$$

The IC50 determined with a linear equation (y=bx+a), where y = 50 and x indicates IC50 (Molyneux, 2004).

RESULTS AND DISCUSSION

Ethanol Extract 70% Propolis

The propolis used was obtained from Trigona spp beehives originating from the Pandeglang area. The propolis extraction process is carried out by cold extraction method by maceration using organic solvents. The use of 70% ethanol is better than ethanol with other concentrations because 70% ethanol can reduce the amount of beeswax that is also extracted and can produce more extract (Wieczorek *et al.*, 2022).

Propolis was extracted by maceration method with a ratio of 1:5 where every 60 grams of crude propolis was extracted with 300 mL of 70% ethanol. Propolis was macerated for 72 hours with a shaker and had passed the filtration stage in the form of a reddish-brown filtrate. Furthermore, the resulting filtrate is evaporated with a rotary evaporator or the solvent is evaporated until a thick brown extract is obtained and has a very sticky consistency. The yield of the extract obtained is 2.376% in this study, which is smaller than that obtained in Hasan *et al.* (2013)'s research, which is 8.20%. Differences in the yield of extracts can be caused by the method of maceration, the length of time of maceration, the process of taking propolis also affects the yield of the extract. The process of taking propolis includes the place of taking propolis, time, and geographical conditions at the time of taking crude propolis (Hasan *et al.*, 2014).

20% Nanopropolis Powder

Nanopropolis powder is made with the aim of increasing the effectiveness of the efficacy of propolis. The process of making nanopropolis; begins with dissolving 70% propolis ethanol extract which has a very sticky consistency and is difficult to dissolve in water and ethanol by heating so that the 70% propolis ethanol extract has a liquid consistency so that it can dissolve

in water and ethanol. The extract was coated with maltodextrin and given magnesium stearate as a lubricant, lubricant and anti-sticky. The mixture of extracts with maltodextrin and magnesium stearate could not blend perfectly, using a homogenizer at a speed of 22,000 rpm for 30 minutes so that the active substances contained in the extract could be coated properly. This homogenization process also makes the particle size of the active substance from the coated extract smaller. According to Artika *et al.* (2011) and Marinho *et al.* (2018) the higher the stirring speed the smaller the particle size produced.

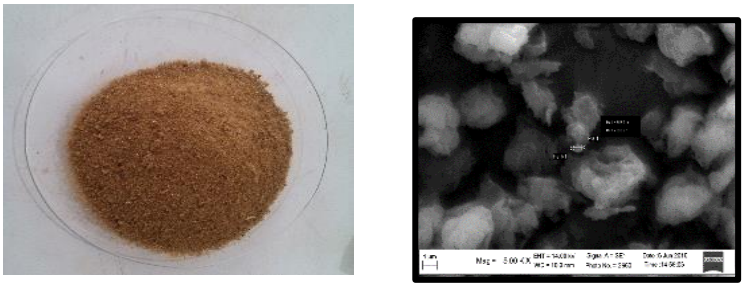

Figure 1. Nanopropolis and SEM result

The resulting nanopropolis powder is a very dry and light brown powder. The yield of nanopropolis powder obtained is 80.84% where the yield obtained is greater than the results of Wahyumiranti (2015) research which is 79.31%. The results of SEM observations with a magnification of 5000x for nanopropolis powder is 806.7 nm, where these results are consistent with Prabha *et al.* (2016) literature that the nano-size does not exceed 1000 nm.

Nanopropolis SEM

Nanopropolis Powder

a. Water Content Ethanol Extract 70% Propolis and Nanopropolis

The average water content of propolis thick extract is 6.44%, this indicates that the extract meets the requirements for water content in general, which should not be more than 10%. The average water content of nanopropolis powder is 4.36%, which also shows that the nanopropolis powder meets the requirements for the powder in general, which should not be more than 5%. The water content of nanopropolis is smaller than that of the extract, it can be caused because the nanopropolis powder has a very small particle size compared to the extract so that it can affect the evaporation process by heating (Hasan *et al.*, 2016).

b. Ash Content of Ethanol Extract 70% Propolis and Nanopropolis

The average yield of ash content obtained from the ethanol extract of 70% propolis is 10.3905%. The average result of the ash content of nanopropolis powder is 1.19135% where this result is not much different from the study of (Hasan *et al.*, 2016). The difference in the ash content of the extract and the nanopropolis powder can be caused by the difference in the amount of inorganic and mineral substances in the extract with the nanopropolis powder. According to (Batista *et al.*, 2012) the content of minerals and other vitamins contained in propolis reaches 5%. Meanwhile, nanopropolis powder only contains 20% propolis extract.

c. Phytochemical Test Results

Table 1. Results of Phytochemical Test of Ethanol Extract 70% Propolis and Nanopropolis

Sample	Test Parameters			
	Flavonoid	Alkaloid	Saponin	Tanin
Extract Ethanol 70 % Propolis	+	+	+	+
Nanopropolis Powder	+	+	+	+

From Table 1, it is known that the ethanol extract of 70% propolis and the positive nanopropolis powder contain secondary metabolites of alkaloid, flavonoids, tannins, and saponins.

Toxicity Test Results

Table 2. Toxicity Test Results of 70% Propolis and Nanopropolis Ethanol Extracts

Sample	Repetition	LC_{50} (ppm)	average LC_{50} (ppm)
Extract Ethanol 70 % Propolis	1	14.361	16.010
	2	15.118	
	3	18.552	
Nanopropolis Powder	1	14.733	18.689
	2	16.221	
	3	25.113	

The test results show that nanopropolis preparations in the form of nanoparticles have a better potential for toxicity than 70% propolis ethanol extract because nanopropolis containing 20% propolis extract concentration and smaller particle size produces LC50 values which are not much different from 70 ethanol extracts. % propolis. The results obtained are 70% ethanol extract of propolis and nanopropolis have an average value of LC50 below 1000 ppm which means that both have potential toxicity to *Artemia salina* L according to the BSLT method because they have an LC50 of less than 1000 ppm so that it can be developed into further research to isolate plant cytotoxic compounds as an effort to develop alternative anti-cancer drugs because they have a positive correlation as anti-cancer.

Results of Determination of Total Flavonoid Levels thanol Extract 70% Propolis and Nanopropolis

Determination of flavonoid content from ethanol extract of propolis and nanopropolis was carried out by complementary colorimetry which has a measurement principle based on the formation of yellow color by aluminum chloride. The maximum wavelength resulting from the measurement of quercetin is 430 nm and shows a stable absorbance at 20 minutes. The results of a linear regression equation Y = 0, 0824x + 0,0336 and the correlation coefficient (R2) is 0,9992.

Table 3. Test Results of Total Flavonoid Levels Ethanol Extract 70% Propolis and Nanopropolis

Sample	Flavonoid I Percentage (%)	Flavonoid II Percentage (%)	Average Flavonoid Percentage (%)
Extract Ethanol 70 % Propolis	2.1220	2.1026	2.1123
Nanopropolis	1.6273	1.6313	1.6293

The flavonoid content of the 70% propolis ethanol extract were 2.1123% and the total flavonoid content for the nanopropolis extract that had been equalized was 1.6293%. The results of total flavonoid content of 70% propolis ethanol extract and nanopropolis powder have a relationship with antioxidant activity, because the total flavonoid content higher, the better the activity of antioxidant.

Results of Determination of Antioxidant Activity of 70% Propolis and Nanopropolis Ethanol Extracts

The result of determining the maximum wavelength of DPPH is 512 nm. The optimum incubation time obtained was at 30 minutes, where a stable absorbance was obtained at that minute. Furthermore, a standard series of vitamin C was made with several concentrations,

the equation y = 6.419x + 24,256 with R^2 = 0.999 was obtained. The absorbance results obtained were entered into the linear regression equation of the standard vitamin C solution so that the % inhibition value of each sample concentration was obtained. Based on the table, it can be seen that the antioxidant activity of the ethanol extract of 70% propolis is greater than that of the nanopropolis powder, which is 95.54593 ppm which is an active antioxidant. This difference can be caused by the manufacturing process which can damage the antioxidants and the coating of the active substances in nanopropolis. The antioxidant activity IC_{50} of 70% propolis ethanol extract did not differ much from research by Hasan et al (2013), which was 75.43 ppm which is *Trigona* sp. honey bee propolis from Pandeglang, Banten Indonesia. The greater its ability as an antioxidant is a smaller the IC_{50} value, and more active propolis is as an antiproliferative agent for cancer cells by inhibiting the rapid growth of cancer cells (Hasan et al, 2013). In the presence of organic acid compounds, polyphenols and flavonoids in propolis play a role in inhibiting the proliferation of cancer cells. This is because flavonoids and caffeic acid are able to inhibit protein kinases used for cell proliferation, resulting in inhibition of the cell formation process resulting in apoptosis (Batra & Sharma, 2013).

Table 4. Results of Antioxidant Activity

Sample	**Antioxidant Activity (*ppm*)**
Vitamin C Standard	4.0100
Extract Ethanol 70% Propolis	95.5459
Nanopropolis Powder	527.7939

CONCLUSION

The LC50 value for 70% propolis ethanol extract was 16.010 ppm and the LC50 value for nanopropolis powder was 18.689 ppm. The results of antioxidant activity obtained the IC50 value for the ethanol extract of 70% propolis was 95.54593 ppm and the IC50 value for the nanopropolis powder was 527.7939 ppm. The Propolis extract contains 2.1123% of flavonoids and 1.5293% of nanopropolis.

ACKNOWLEDGMENTS

The authors are grateful to the Department of Biochemistry, Faculty of Mathematics and Natural Sciences, IPB University and Study Program Pharmacy, Faculty of Mathematics and Natural Sciences, Pakuan University Bogor.

CONFLICT OF INTEREST

The authors declare that there are no competing interests. This current study does not involve experiments on animals or human subjects and the data are available from the authors.

REFERENCE

Anjum, S. I., Ullah, A., Khan, K. A., Attaullah, M., Khan, H., Ali, H., Bashir, M. A., Tahir, M., Ansari, M. J., Ghramh, H. A., Adgaba, N., & Dash, C. K. (2019). Composition and functional properties of propolis (bee glue): A review. *Saudi Journal of Biological Sciences*, *26*(7), 1695–1703.

https://doi.org/https://doi.org/10.1016/j.sjbs.2018.08.013

Batista, L. L. V., Campesatto, E. A., Assis, M. L. B. De, Barbosa, A. P. F., Grillo, L. A. M., & Dornelas, C. B. (2012). Comparative study of topical green and red propolis in the repair of wounds induced in rats. *Revista Do Colégio Brasileiro de Cirurgiões*, *39*(6), 515–520.

Batra, P., & Sharma, A. K. (2013). Anti-cancer potential of flavonoids: recent trends and future perspectives. *3 Biotech*, *3*(6), 439–459. https://doi.org/10.1007/s13205-013-0117-5

Bayda, S., Adeel, M., Tuccinardi, T., Cordani, M., & Rizzolio, F. (2019). The History of Nanoscience and Nanotechnology: From Chemical-Physical Applications to Nanomedicine. *Molecules (Basel, Switzerland)*, *25*(1). https://doi.org/10.3390/molecules25010112

BPOM. (2014). Persyaratan Mutu Obat Tradisional. *Badan Pengawas Obat Dan Makanan*, 1–25.

Dias, M. C., Pinto, D. C. G. A., & Silva, A. M. S. (2021). Plant flavonoids: Chemical characteristics and biological activity. *Molecules*, *26*(17), 1–16. https://doi.org/10.3390/molecules26175377

Filha, Z. S., Lombardi, J., Guzzo, L. S., & Saúde-Guimarães, D. (2011). Brine shrimp (A*rtemia salina* Leach) bioassay of extracts from different Lychnophora species. *Revista Brasileira de Plantas Medicinais*, *14*, 358–361. https://doi.org/10.1590/S1516-05722012000200016

Hasan, A. E., Hasan, Mangunwidjaja, D., Sunarti, T. C., Suparno, O., & Setiyono, A., (2013). Kstraksi Propolis Menggunakan Cara Maserasi Dengan Pelarut Etanol 70% Dan Pemanasan Gelombang Mikro Serta Karakterisasinya Sebagai Bahan Antikanker Payudara. *Jurnal Teknologi Industri Pertanian*, *23*(1), 13–21.

Hasan, A. E.Z., Mangunwidjaja, D., Sunarti, T. C., Suparno, O., & Setiyono, A. (2014). Investigating the antioxidant and anticytotoxic activities of propolis collected from five regions of Indonesia and their abilities to induce apoptosis. *Emirates Journal of Food and Agriculture*, *26*(5), 390–398. https://doi.org/10.9755/ejfa.v26i5.16549

Hasan, Akhmad Endang Zainal, Mangunwidjaja, D., Sunarti, T. C., Suparno, O., & Setiyono, A. (2016). Antibreast cancer activity of nanopropolis Indonesia on induced mammary gland tumor by dmba in virgin sprague-dawley rats. *Biotropia*, *23*(1), 35–41. https://doi.org/10.11598/btb.2016.23.1.473

Kumar, V., Sheoran, O. P., Rani, S., & Malik, K. (2020). Development of a web-based tool for probit analysis to compute lc50/ld50/gr50 for its use in toxicology studies. *Journal of Applied and Natural Science*, *12*(4), 641–646. https://doi.org/10.31018/jans.v12i4.2408

Made Artika, I., Susilo, H., Virginia Dwi Setyo, A., & Endang Zainal Hasan, A. (2011). Antibacterial Activity of Propolis Supplemented-Chewing Candy Against Streptococcus mutans. *Microbiology Indonesia*, *5*(2), 99–102. https://doi.org/10.5454/mi.5.3.1

Marinho, R., Horiuchi, L., & Pires, C. A. (2018). Effect of stirring speed on conversion and time to particle stabilization of poly (vinyl chloride) produced by suspension polymerization process at the beginning of reaction. *Brazilian Journal of Chemical Engineering*, *35*(2), 631–639. https://doi.org/10.1590/0104-6632.20180352s20160453

Martemucci, G., Costagliola, C., Mariano, M., D'andrea, L., Napolitano, P., & D'Alessandro, A. G. (2022). Free Radical Properties, Source and Targets, Antioxidant Consumption and Health. *Oxygen*, *2*(2), 48–78. https://doi.org/10.3390/oxygen2020006

Prabha, S., Arya, G., Chandra, R., Ahmed, B., & Nimesh, S. (2016). Effect of size on biological properties of nanoparticles employed in gene delivery. *Artificial Cells, Nanomedicine and Biotechnology*, *44*(1), 83–91. https://doi.org/10.3109/21691401.2014.913054

Rivero-Cruz, J. F., Granados-Pineda, J., Pedraza-Chaverri, J., Pérez-Rojas, J. M., Kumar-Passari, A., Diaz-Ruiz, G., & Rivero-Cruz, B. E. (2020). Phytochemical constituents, antioxidant, cytotoxic, and antimicrobial activities of the ethanolic extract of mexican brown propolis. *Antioxidants*, *9*(1), 1–11. https://doi.org/10.3390/antiox9010070

Trusheva, B., Trunkova, D., & Bankova, V. (2007). Different extraction methods of biologically active components from propolis; a preliminary study. *Chemistry Central Journal*, *1*(1), 1–4. https://doi.org/10.1186/1752-153X-1-13

Wahyumiranti. (2015). *Studi Formulasi Granul Instan Nano Propolis Sebagai Obat Kumur*. Pakuan University, Indonesia.

Wahyuni, N., & Riendriasari, S. D. (2021). The content of propolis's flavonoid from two species of stingless bee in Lombok. *IOP Conference Series: Earth and Environmental Science*, *914*(1). https://doi.org/10.1088/1755-1315/914/1/012064

Wieczorek, piotr pawel, Hudz, N., Yezerka, O., Horcinova-sedlackova, V., Shanaida, M., Korytniuk, O., & Jasicka-misiak, I. (2022). Chemical Variability and Pharmacological Potential of Propolis Pharmaceutical Products. *Molecules*, *27*(1600), 1–28.