Jurnal Ilmu dan Teknologi Hasil Ternak (JITEK), July 2022 Vol. 17 No. 2
ISSN : 1978 – 0303 DOI : 10.21776/ub.jitek.2022.017.02.7

OPTIMIZATION OF EAST JAVA PROPOLIS EXTRACTION AS ANTI SARS-Cov-2 BY MOLECULAR DOCKING STUDY

Sofia Aulia Hidayat[1)], Agus Susilo[2)], Khothibul Umam Al Awwaly[2)], Miftakhul Cahyati[3)]
[1)] Postgraduate Student at Department of Animal Product Technology, Faculty of Animal Science, Universitas Brawijaya, Jl. Veteran, Malang City, 65145, East Java, Indonesia
[2)] Lecture at Department of Animal Product Technology, Faculty of Animal Science, Universitas Brawijaya, Jl. Veteran, Malang City, 65145, East Java, Indonesia
[3)] Faculty of Dentistry, Universitas Brawijaya, Universitas Brawijaya, Jl. Veteran, Malang City, 65145, East Java, Indonesia
*Corresponding Email: agussusilo@ub.ac.id

Submitted 21 June 2022; Accepted 25 July 2022

ABSTRACT

The purpose of this research is to obtain the optimum propolis extraction method using microwave assisted extraction so as to produce phytochemical compound as anti-SARS CoV-2. The research method used was experimental with a completely randomized factorial design consisting of 2 factors namely extraction time and power level, 9 treatments and 3 replications. The extraction using microwave assisted extraction was carried out according to treatment factors, namely low power level (A1) producing temperature of 40°C, medium (A2) producing temperature of 58°C, high (A3) producing temperature of 70°C and the length of treatment time was 10 minutes (B1), 20 minutes (B2), and 30 minutes (B3). The results of statistical analysis showed that the interaction had a high significant effect ($p<0.01$) on the alkaloid content which is ranged from 0.665 to 1.452 mg/g and had no effect on color L*a*b* and antioxidant activity which is ranged from 1.533 to 1.553. The percentage of hexadecane in East Java propolis extract was 0.280 %, octadecane was 0.775%, and pentacosane was 6.716%. The results of druglikeness analysis showed that hexadecane, octadecane and pentacosane compounds had potential as antivirals with a probability to be active value of 0.68. The binding affinity value produced by enzalutamide as a native ligand is -6.7 kcal/mol while the highest inhibitory value is octadecane and pentacosane of -5.9 kcal/mol and followed by hexadecane at -5.8 kcal/mol. The conclusion of this research is that the most optimal extraction method using microwave assisted extraction is done with a medium microwave power level for 30 minutes in terms of the alkaloid content and antioxidant activity produced. This method is able to produce extracts with good antiviral bioactive components, although the binding affinity has not been able to exceed the native ligand's ability in terms of the molecular docking approach.

Key words: Alkaloid; antioxidant activity; propolis, SARS-CoV-2; TMPRSS2

INTRODUCTION

Propolis is a mixture of wax with resin material attached to flowers, shoots, and bark. Propolis is widely used because of its bioactivity as antibacterial, anti-inflammatory, antiviral, antioxidant, antiprotozoal, anesthetic, antitumor, anticancer, antiseptic, antifungal, antimutagenic, antihepatotoxic. Geographical location and type of honeybee will affect the content of bioactive compounds in propolis (Chan et al., 2013). Differences in the characteristics of bee species and the bee's ability to fly in search of food will affect the quality of the resulting product. The location of the nest will determine the content of propolis bioactive compounds because the bees will take the resin found in various plants around the grazing area. Indonesian propolis taken from Batu, East Java contains alk(en)ylresorcinol which is proven to have strong antioxidant and cyclooxygenase (COX-1 and COX-2) inhibitory activity (Trusheva et al., 2011).

The bioactive content in propolis can be obtained to the maximum through the extraction process with the right method. The processing of propolis still uses a simple traditional extraction with maceration which takes a long time. Extraction with the right methods and steps can help to optimize the process of dissolving chemical compounds that are the target of extraction. The MAE method is more effective (based on extraction results, extraction time and solvent consumption) in extracting total phenolics and flavonoids than the other methods. The use of microwave heating for a long time makes increased particles collide between solvents and beehives (Hasan et al., 2013). Effective extraction method will save time and energy significantly (Liu et al., 2022). The MAE method showed high selectivity in extracting flavonoid fractions compared to other methods evaluated (Margeretha et al., 2012). Extraction with MAE for 20-30 minutes produces a higher total phenolics and flavonoids than the results of maceration (Hamzah et al., 2015).

The biological ability of natural ingredients as antivirals is very much needed in line with the Covid-19 pandemic. The extraction process produces chemical compounds that have activity as candidate compounds to inhibit SARS-CoV infection. The main components of propolis, such as flavonoids, have been tested against coronaviruses that show some inhibitory effects (Ripari et al., 2021). These components include the flavonoid quercetin help to fight SARS and MERS-CoV infection by modulating the unfolded protein response, preventing the complete viral cycle. CAPE exhibits the property of anti-p21-activated kinases (PAKs), which are important enzymes for entry and replication of several human viruses.

The transmembrane protease, serine 2 (TMPRSS2) is a medium to mediate the entry of SARS-CoV-2 into host cells. TMPRSS2 is an androgen-responsive serine protease that functions to cleave the increased glycoprotein of the S1/S2 protease and facilitate viral entry and activation in the body (Hoffmann et al., 2020). This requires the process of disrupting the mechanism of viral infection by providing inhibition using good antiviral and immunomodulatory compounds in the body. Flavonoids interact with His296 and Ser441 actively and produce TMPRSS2 amino acid residue. Other interacting amino acids with flavonoids are Val280, Lys300, Tyr337, Lys340, Thr341, and Lys342 (Istifli et al.,

*Corresponding author:
Agus Susilo
Email: agussusilo@ub.ac.id
Lecturer of Animal Products Technology, Faculty of Animal Science, Universitas Brawijaya, Jl. Veteran, Malang City, 65145, East Java, Indonesia

How to cite:
Hidayat, S. A., Susilo, A., Al Awwaly, K. U., & Cahyati, M (2022). Optimization of East Java Propolis Extraction as Anti Sars-Cov-2 by Molecular Docking Study. Jurnal Ilmu dan Teknologi Hasil Ternak (JITEK), 17 (2), 123-134

2022). The interaction between bioactive compounds as candidate inhibitors and SARS CoV-2 protein can be done by molecular docking. Molecular docking was conducted to simulate the interaction between propolis compounds which are antiviral and have the potential to become SARS-CoV-2 drugs with proteins or body receptors against SAR CoV-2 infection. Molecular docking is a computational procedure that tries to predict macromolecules (receptors) and small molecules (ligands) that bind noncovalently and conformations are formed, and the binding affinity can be predict by docking score. This research is important to do to determine the optimal interaction of extraction using the microwave assisted extraction method on propolis originating from the East Java region which is a potential animal product as a complementary medicine preparation for Covid-19.

MATERIALS AND METHODS

This research was conducted in February to May 2022 at the Laboratory of Animal Products Technology, Faculty of Animal Science and Laboratorium Sentral Ilmu Hayati, Universitas Brawijaya. The research materials used were raw propolis *Apis mellifera* kept in East Java, ethyl acetate as a solvent, 2N HCl, BC solution, magnetic stirrer, whatman paper number 1, quinine, 0.1 N NaOH, phosphate buffer, DPPH solution and acid error. The research equipment used was a microwave equipped with Soxhlet, 1 ml micro pipette, UV spectrophotometer, Hewlett-Packard gas chromatography, Intel Core i5 computer specifications, 16 GB RAM, Windows 10, 64-bit operating system. The software used includes Discovery Install Studio, PyrX 9.5 vesion and PyMol.

Research Methods

The research method used was experimental with a completely randomized factorial design consisting of 2 factors, 9 treatments and 3 replications. The data obtained were analyzed using Microsoft Excel with Analysis of Variance (ANOVA), if there are differences, it will be continued with Duncan's Multiple Range Test (DMRT). The parameters in this study were color L*a*b*, alkaloid, antioxidant activity, Gas Chromatography-Mass Spectrometry (GCMS) of propolis compound and druglikeness, docking score.

Propolis extract preparation

Propolis extract is made by extraction using microwave assisted extraction. Propolis were cleaned of dirt and cut into small pieces. Weighed 20 grams of propolis and measured 100 ml of ethyl acetate solvent (1:5), then put it in a 250 ml Erlenmeyer (Hamzah and Leo, 2015). Extraction using microwave assisted extraction was carried out according to treatment factors, namely low power level (A1), medium (A2), high (A3) and the length of treatment time was 10 minutes (B1), 20 minutes (B2), and 30 minutes (B3) was repeated three times.

Color L*a*b* Measurement

The procedure for color analysis L*a*b* refers to CIE 2007 L*a*b by attaching the colorider sensor to the sample in the film pot container 3 times and taking the average values of L, a and b. L* is the brightness level (lightness) of the light coordinates which has a value range of 0-100. a* value is the saturation of the red-green axis, a positive value indicates red and a negative a value indicates green. A positive b* value indicates yellow and a negative b value indicates blue (Durmus, 2020).

Alkaloid content

Propolis extract were dissolved in 3 ml of phosphate solution pH 4.5 and into the separatory funnel. Mixed with 3 ml bromocresol 0.03% green solution to 30 minutes. Chloroform about 1, 2, 3, and 4 mL was added and stirred for 2 minutes. The bottom layer is separated after 10 minutes,

and the extracts were collected in 10 ml volumetric flask and diluted to the mark with chloroform, absorbance was measured at 415 nm. Quinine is used as a standard alkaloid (Rinaldi et al., 2017).

Antioxidant Activity

Propolis extract was measured for 50 µL and added to 5 mL of 0.004% methanol solution of DPPH reagent. After 30 minutes incubation in the dark at room temperature, the change in absorbance was measured at 517 nm. The test was carried out in triplicate, and the percentage of inhibition was calculated using the following formula:

% Inhibition = {(A0 A1) |A 0} × 100

A0 and A1 are absorbance at 30 min of control and sample (Aboulghazi, et al., 2022)

Gas Chromatography-Mass Spectrometry Analysis

Propolis extract was measured for 5 ml in each scan. The Hewlett-Packard 6890 series gas chromatograph coupled with a Hewlett-Packard 5973 mass selective detector with a 30 m x 250 m x 0.28 m HP5-MS column was used for GC-MS analysis. The total analysis walk was 36 minutes. With an injector temperature of 110°C, the 110°C program temperature was held for two minutes and then raised to 280°C at a rate of 10°C/min, and a 15-minute hold at 280°C was applied. With helium as the carrier gas, a flow rate of 1.5 mL/min was used. Upon completion, the peaks were identified through their MS spectra using the system database (NIST Mass Spectral Library) (Biladjila, et al., 2018).

Molecular Docking

Ligand Preparation

Propolis compound from the GC-MS result was determine as a ligand, then search for its canonical structure and isomeric SMILE (simplified molecular-input line-entry system) in the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The potency of the compounds in the search results was analyzed using the WAY2DRUG PASS prediction (http://www.pharmaexpert.ru/passonline/predict.php) as an immunomodulatory treatment. Analyzed with ADME on compounds using Swiss ADME (www.swissadme.ch). Analyzed protein targets that can interact with herbs predicted using SEA Target (Similarity Ensemble Approach) (https://sea.bkslab.org/). Docking is done using Autodock on PyRx V.9.5

Protein Preparation

The main protease of the SARS CoV-2 receptor with PDB ID 5CE1 was obtainned from the Protein Data Bank (http://www.rcsb.org). The protein was separate from water molecules and ligands using Discovery Install Studio and save in PDB format.

Molecular Docking Simulation

Docking simulation were analyzed proteins and ligands that have been prepared using PyRx 9.5. The results of the analysis are binding affinity and Lipinski Selection values.

RESULTS AND DISSCUSION

The results of the analysis of the color L*a*b*, alkaloid content and antioxidant activity of IC50 are presented in table1 and figure 1. The results of the GCMS analysis are presented in table 2. The results of the molecular docking simulation are presented in table 3.

Color L*a*b*

The results of the L*a*b* color test can be seen in table 1. The results of statistical analysis showed that the interaction factors had no effect (p>0,05) on the color of the propolis extract. Based on the value of L*, the higher the power level to produce a higher temperature, the color of the propolis extract will be brighter or faded. This can happen because more and more wax had dissolved so the color will fade and more yield will be produced. The extraction

process has a strong correlation with the extract yield and the color produced in the extract (Mendonca et al., 2015). The darker color of propolis extract can be caused by the phenol content. Phenol group compounds in propolis can be in the form of tannins and color pigments in plants, namely carotenes and anthocyanins which give a more reddish or yellowish color. The negative a* value in the extract indicated that the propolis extract was greenish in color. The value of b* ranged from 1.29-2.42 which indicated that the propolis extract was yellowish in color with a positive value.

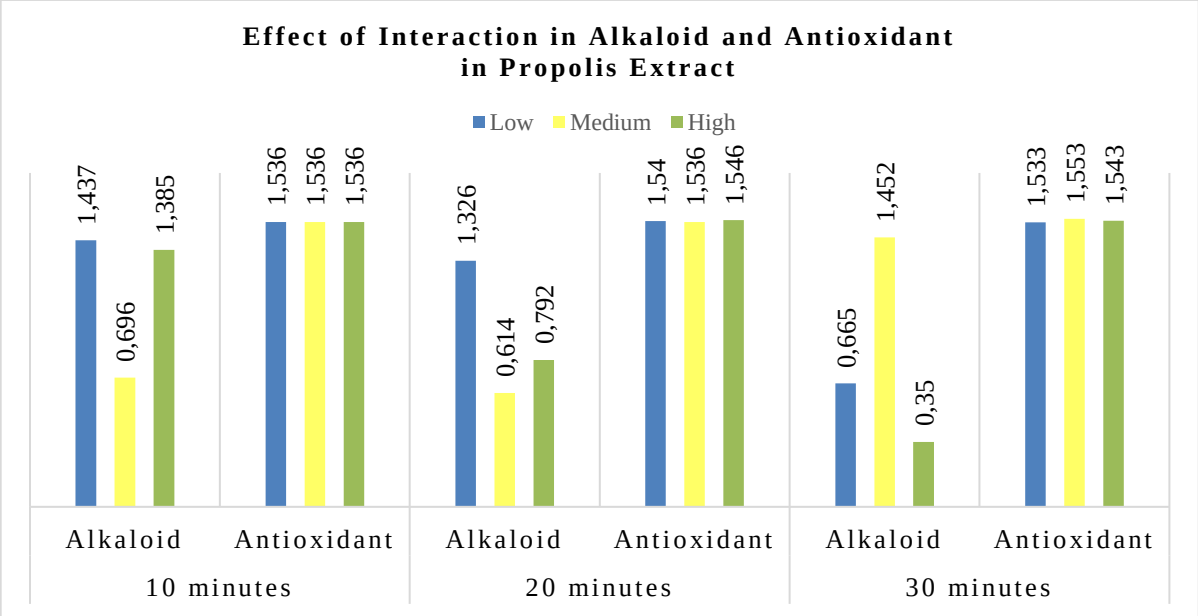

Figure 1. Result of different interaction treatment to the alkaloid content and antioxidant activity of East Java propolis extract

Table 1. The results of the average antioxidant activity of propolis extract based on differences in extraction interactions

Treatments	L*	a*	b*
A1B1	23,801±2,333	-4,90±0,413	1,568±0,257
A2B1	22,953±2,124	-4,85±0,308	1,850±0,201
A3B1	23,466±1,800	-4,97±0,315	1,629±0,153
A1B2	24,813±1,058	-3,70±0,510	1,600±0,426
A2B2	25,078±0,950	-3,73±0,459	1,612±0,181
A3B2	26,821±0,986	-4,11±0,539	1,299±0,452
A1B3	25,561±1,440	-4,01±0,054	2,329±0,045
A2B3	25,938±1,951	-4,18±0,373	2,422±0,168
A3B3	26,717±1,029	-4,22±0,728	2,184±0,237

The propolis ethyl acetate extract in this study produced a blackish brown color. The effect of propolis color can come from the type of plant taken by bees which usually comes from tree bark. There is a significant correlation between the color of propolis and its antioxidant properties (Revilla, et al., 2017).

Alkaloids Content

The results of statistical analysis showed that the interaction factors had a significant effect ($p<0,01$) on the alkaloid content of the propolis extract. The highest propolis content was obtained with medium power level of microwave for 30 minutes. Extraction at a medium power level with ethyl acetate solvent resulted in an

extraction temperature of 58-60°C. Higher temperatures can aid the extraction of high molecular mass compounds due to more favorable transport properties. The extraction process forms a balance curve so that when the equilibrium condition for the extraction time is reached, there will be compounds in that phase dissolved in the extract. The use of ethyl acetate as an extraction solvent is not only to dissolve polar and non-polar compounds in propolis because of its semi-polar nature with a polarity value of 0.215 and a boiling point of 77,1°C, as well as to maintain the extraction temperature so that it is protected from oxidation in phytochemical compounds.

The alkaloid content of propolis extract in propolis extract in this study ranged from 0.665 to 1.452 mg/g. Propolis from Tamil Nadu, Karnataka, Kerala, Haryana, Himachal Pradesh and Uttanchal originating from India, respectively (90µg/mg, 78g/mg, 75g/mg, 72µg/mg, 70µg/mg and 62µg/mg. This shows that the alkaloid content of East Java propolis extract in this study was higher than that of propolis from several parts of India (Ramnath, 2015). Alkaloids are natural chemical compounds that have a wide range of pharmacological activities including antimalarial, antiasthmatic, anticancer, analgesic and antibacterial and are found to be used in both traditional and modern medicine. The alkaloids found in the samples studied were strychane, cyclopropane benzole, papaveroline, chomoerythrinan and neronine (Ramnath, 2015). Pentacyclic guanidinic scaffolds, crambescidins 786 and 826 are the types of alkaloid compounds that have the best binding inhibition and are expected to be anti-COVID-19 candidates (Demerdash, et al., 2021).

Antioxidant Activity (IC50)

The results of statistical analysis showed that the interaction factors had no effect (p>0,05) on antioxidant activity. The average antioxidant activity ranged from 1.533 to 1.553 µg/ml. The extraction interaction treatment had no effect on the antioxidant activity value of IC50 which made this result not in line with the results of the analysis of the alkaloid content which is part of the phytochemical content of propolis. This can be caused by the presence of other components that have different characteristics to the extraction process which have a solubility phase at a certain temperature and time which composes the antioxidant properties of propolis. These components are thought to come from volatile or nonvolatile groups so that they cannot be detected with certainty in this test. Some of the main components of propolis that act as the main constituent of antioxidants are caffeic acid which is a type of compound from the flavonoid group. CAPE exhibits the property of anti-p21-activated kinases (PAKs), which are important enzymes for entry and replication of several human viruses (Ripari, et al., 2021).

ACE The lower the IC50 value, the better the antioxidant activity of the propolis extract sample. The best antioxidant activity value with the lowest value owned by the low power level for 30 minutes extraction. This value is also better than the IC50 value in the propolis extract sample extracted using the microwave assisted extraction (MAE) method using methanol for 10 seconds which produces an IC50 of 1.77 (Charland et al., 2021). IC50 values that are less than 50 ppm are categorized as very strong antioxidant groups, IC50 values of 50-100 ppm are categorized as strong antioxidant groups, IC50 values of 101-150 ppm are categorized as moderate antioxidant groups, IC50 values of 151-200 ppm are categorized as groups. Weak antioxidants and IC50 values that exceed 200 ppm are categorized as very weak antioxidant groups (Leksono, et al., 2013).

The antioxidant activity in this study was classified as better than the content of propolis extract from the Batu area, East Java which was obtained from the maceration process with 70% ethanol solvent, which was 178.79 (Rosyidi et al., 2018). The percentage of inhibition of

propolis extract produced in this study exceeded 50% of the ability to inhibit the free radical. The power to inhibit free radicals is needed so that further reactions that cause oxidative stress can be stopped and avoid cell damage and infection of a disease can be stopped (Parwata, 2016).

Antioxidant activity IC50 is defined as the concentration of antioxidant compounds required to reduce free radicals by 50%. The phenolic content of propolis will affect its antioxidant activity (Mihai et al., 2011). The higher the value of the phenolic content, the lower the IC50 value it has. Flavonoid components (such as chrysin or quercetin) and organic acids (such as firulic acid or caffeic acid) are active compounds that can act as antioxidants so that they have the ability to reduce DPPH free radicals (Hasan et al., 2013).

Gas Chromatography-Mass Spectrometry Propolis Compounds and Druglikeness Analysis

The results of the Gas Chromatography-Mass Spectrometry (GCMS) analysis with Rtx-1 to detect non-polar compounds injected at 110°C are presented in table 2. The percentage of hexadecane in East Java proolis extract was 0.280 %, octadecane was 0.775%, and pentacosane was 6.716%. Hexadecane is a pure compound of aliphatic hydrocarbons belonging to the alkane group. Hexadecane was also found in Venezuelan propolis for the first time at a level of 12.56% (Mohtar et al., 2017). Hexadecane was first identified in Greek propolis. Hexadecane is found in 1.14% Algerian propolis. This shows that the percentage of hexadecane in East Java propolis extract is low.

Table 2. Propolis Extract Compound

Compounds	Percentage (%)	Molecular weight (gram/mol)	Number of H bond acceptor	Number of H bond donor	Rotatable Bond	Lipinski
Hexadecane	0,280	226,44	0	0	13	Yes
Octadecane	0,775	254.5	0	0	15	Yes
Pentacosane	6,716	352,7	0	0	22	Yes

Octadecane in East Java propolis extract in this study was higher than the octadecane component in Iranian propolis extracted using 70% ethanol, which was 0.43% (Hosseini, 2015). Propolis from South Sulawesi which was extracted by maceration method with 70% ethanol solvent contained 11.87% octadecane (Kalsum et al., 2016). Pentacosane in East Java propolis extract was 6.716% lower when compared to Iranian propolis extracted with Soxhlet which was 42.23% (Fayaz, et al., 2017). Hexadecane, octadecane and pentacosane are types of essential oils. Propolis which contains a lot of volatile compounds is called poplar propolis because it provides essential oils derived from poplar. Some of the observed differences may be due to chemical variations of the volatiles of different poplar subspecies and clones, even volatiles of the same species have quantitative variability in chemical composition. This variation in plant sources also has a major influence on the volatile organic matter compounds in the composition of propolis. Essential oil plays an important role in propolis because it can improve properties due to its aroma, biological activity and can provide information about the origin of the sample related to its traceability. An important group of essential oils found in propolis because they play an important role in differentiating premium propolis from counterfeit or lower quality propolis and they exhibit antioxidant, antimicrobial, and other biological activities (Kasiotis et al., 2017). Druglikeness analysis was performed using Way2Drug Pass Server.

The propolis extract components produced have potential as antiviral, anti-inflammatory, free radical scavenger, severe acute respiratory syndrome treatment, Simian immunodeficiency virus proteinase

inhibitor, immunomodulator, immunostimulant and 3C-like protease (Human coronavirus) inhibitor.

The results of druglikeness analysis showed that hexadecane, octadecane and pentacosane compounds had potential as antivirals with a Pa value (probability to be active) of 0.68. Pa value of more than 0.7 indicates that the compound is predicted to have high potential as an antiviral due to its high similarity with the compound in the database that has been proven as a treatment. The Pa value for the severe acute respiratory syndrome (SARS) treatment was low, namely 0.234 for the three compounds, so this indicates that these compounds have low similarity as a treatment for SARS.

Docking Score

The results of the docking analysis are presented in table 3. The binding affinity value produced by enzalutamide as a native ligand is -6.7 kcal/mol while the highest inhibitory value of the East Java propolis extract component is octadecane and pentacosane of -5.9 kcal/mol. hexadecane at -5.8 kcal/mol.

Table 3. Docking Score of Propolis Compound and 5CE1

Compounds	Docking score (kcal/mol)
Enzalutamide (Native ligand)	-6,7
Hexadecane	-5,8
Octadecane	-5,9
Pentacosane	-5,9

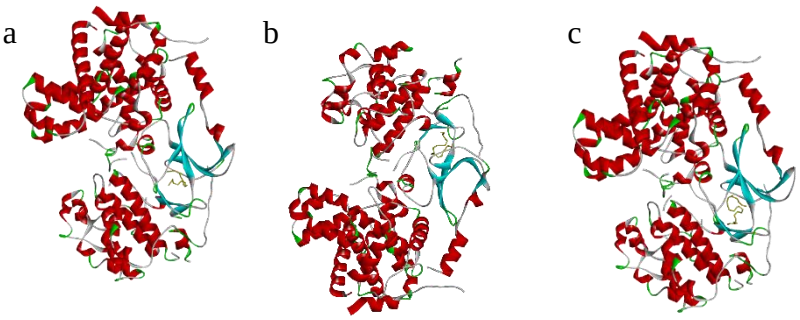

Figure 2. Structural visualization dari TMPRSS2yang berinteraksi dengan hexadecane (a), octadecane (b) dan pentacosane (c).

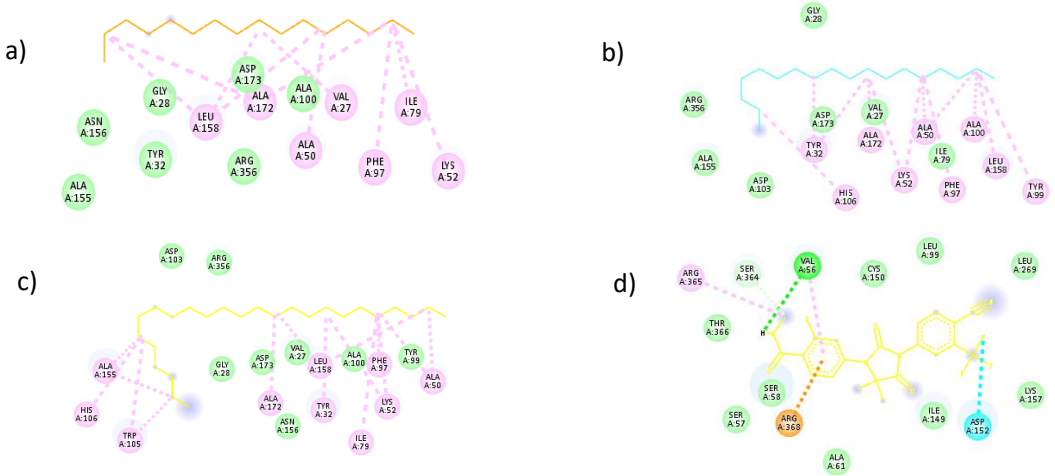

Figure 3. Residu Asam Amino hasil interaksi hexadecane (a), octadecanedane (b) pentacosane (c) enzalutamide (d) dengan reseptor TMPRSS2

The binding affinity value indicates the strength of the bond between the test compound and the receptor. The lower the bond energy, the stronger the bond between the compound and the receptor. This shows that the ability of the propolis component found in this study has not been able to outperform the virus inhibition ability of the native ligand enzalutamide. The interaction between the ligand and the TMPRSS2 receptor is shown in Figure 2. The amino acid residue from the interaction of the ligand and the TMPRSS2 receptor is shown in Figure 3.

The interaction between hexadecane, octadecane and pentacosane produces amino acid residues, namely Ala155, Arg356, Tyr32, Asp173, Asn156, Leu158, Ala172, Lys52, His106. Test ligands with amino acid residues and hydrogen bonds that are close to natural ligands show similar types of interactions in this case describing similar activities. The mechanism of flavonoids in propolis as a pharmacological agent in this study functions by inhibiting the potential of the human protease TMPRSS2, which is one of the potential targets of SARS CoV-2 and helps in preventing virus entry thereby reducing viral load in cells (Varughese, et al., 2022). Melalui docking molekuler, simulasi MD, analisis peta kontak, dan perhitungan MM-PBSA, kami telah mengidentifikasi Amentofavone dan Narirutin sebagaiTMPRSS2 expression was detected in human body tissues in the gastrointestinal tract including stomach, large intestine (transverse), pancreas, small intestine (terminal ileum), minor salivary glands, esophagus (mucosa), liver, and large intestine (sigmoid) (Baughn et al., 2020).

CONCLUSION

The conclusion of this research is that the most optimal extraction method using microwave assisted extraction is done with a medium microwave power level for 30 minutes in terms of the alkaloid content and antioxidant activity produced. This method is able to produce extracts with good antiviral bioactive components, although the binding affinity has not been able to exceed the native ligand's ability in terms of the molecular docking approach.

ACKNOWLEDGMENT

Thank to Dr. Khothibul Umam Al-Awwaly, S.Pt., M.Si. for the financial support of this research.

REFERENCES

Aboulghazi, A., Bakour, M., Fadil, M., & Lyoussi, B. (2022). Simultaneous optimization of extraction yield, phenolic compounds and antioxidant activity of moroccan propolis extracts: improvement of ultrasound-assisted technique using response surface methodology. *Processes*, *10*(2), 297. https://doi.org/10.3390/pr10020297

Baughn, L. B., Sharma, N., Elhaik, E., Sekulic, A., Bryce, A. H., & Fonseca, R. (2020). Targeting TMPRSS2 in SARS-CoV-2 Infection. *Mayo Clinic Proceedings*, *95*(9), 1989–1999. https://doi.org/10.1016/j.mayocp.2020.06.018

Beladjila, K. A., Berrehal, D., Al-Aboudi, A., Semra, Z., Al-Jaber, H., Bachari, K., & Kabouche, Z. (2018). Composition and antioxidant, anticholinesterase, and antibacterial activities of the essential oil of salvia buchananii from Algeria. *Chemistry of Natural Compounds*, *54*(3), 581–583. https://doi.org/10.1007/s10600-018-2414-z

Chan, Q. W. T., Chan, M. Y., Logan, M., Fang, Y., Higo, H., & Foster, L. J. (2013). Honey bee protein atlas at organ-level resolution. *Genome Research*, *23*(11), 1951–1960. https://doi.org/10.1101/gr.155994.113

Durmus, D. (2020). CIELAB color space boundaries under theoretical spectra and 99 test color samples. *Color Research & Application*, *45*(5), 796–

802. https://doi.org/10.1002/col.22521

El-Demerdash, A., Metwaly, A. M., Hassan, A., Abd El-Aziz, T. M., Elkaeed, E. B., Eissa, I. H., Arafa, R. K., & Stockand, J. D. (2021). Comprehensive virtual screening of the antiviral potentialities of marine polycyclic guanidine alkaloids against SARS-CoV-2 (COVID-19). *Biomolecules*, *11*(3), 460. https://doi.org/10.3390/biom11030460

Fayaz, G., Goli, S. A. H., Kadivar, M., Valoppi, F., Barba, L., Balducci, C., Conte, L., Calligaris, S., & Nicoli, M. C. (2017). Pomegranate seed oil organogels structured by propolis wax, beeswax, and their mixture. *European Journal of Lipid Science and Technology*, *119*(10), 1700032. https://doi.org/10.1002/ejlt.201700032

Hamzah, N., & Leo, C. P. (2015). Microwave-assisted extraction of trigona propolis: the effects of processing parameters. *International Journal of Food Engineering*, *11*(6), 861–870. https://doi.org/10.1515/ijfe-2015-0106

Hasan, A. E., 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.

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Krüger, N., Herrler, T., Erichsen, S., Schiergens, T. S., Herrler, G., Wu, N.-H., Nitsche, A., Müller, M. A., Drosten, C., & Pöhlmann, S. (2020). SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell*, *181*(2), 271-280.e8. https://doi.org/10.1016/j.cell.2020.02.052

Hosseini, A. A. (2015). Investigation property of propolis in different areas of Iran and its qualitative and quantitative chemical composition. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, *6*(6).

Istifli, E. S., Netz, P. A., Sihoglu Tepe, A., Husunet, M. T., Sarikurkcu, C., & Tepe, B. (2022). In silico analysis of the interactions of certain flavonoids with the receptor-binding domain of 2019 novel coronavirus and cellular proteases and their pharmacokinetic properties. *Journal of Biomolecular Structure and Dynamics*, *40*(6), 2460–2474. https://doi.org/10.1080/07391102.2020.1840444

Kalsum, N., Sulaeman, A., Setiawan, B., & Wibawan, I. W. T. (2016). Phytochemical profiles of propolis trigona spp. from three regions in Indonesia using GC-MS. *Journal of Biology, Agriculture and Healthcare*, *6*(14).

Kasiotis, K. M., Anastasiadou, P., Papadopoulos, A., & Machera, K. (2017). Revisiting greek propolis: chromatographic analysis and antioxidant activity study. *PLOS ONE*, *12*(1), e0170077. https://doi.org/10.1371/journal.pone.0170077

Leksono, W. B., Pramesti, R., Santosa, G. W., & Setyati, W. A. (2018). Jenis pelarut metanol dan n-heksana terhadap aktivitas antioksidan ekstrak rumput laut gelidium sp. dari pantai drini Gunungkidul – Yogyakarta. *Jurnal Kelautan Tropis*, *21*(1), 9–16. https://doi.org/10.14710/jkt.v21i1.2236

Liu, Y., Sabadash, S., Duan, Z., & Gao, D. (2022). Influence of different microwave-assisted drying methods on the physical properties, bioactive compounds and antioxidant activity of beetroots. *Eastern-European Journal of Enterprise Technologies*, *1*(11(115)), 15–25. https://doi.org/10.15587/1729-4061.2022.251942

Margeretha, I., Fatma Suniarti, D., Herda, E., & Mas'ud, Z. A. (2012). Optimization and comparative study of different extraction methods of biologically active components of

Indonesian propolis Trigona spp. */Journal of Natural Products*, *5*(233).

Mendonça, M. A. A. de, Ribeiro, A. R. S., Lima, A. K. de, Bezerra, G. B., Pinheiro, M. S., Albuquerque-Júnior, R. L. C. de, Gomes, M. Z., Padilha, F. F., Thomazzi, S. M., Novellino, E., Santini, A., Severino, P., B. Souto, E., & Cardoso, J. C. (2020). Red propolis and its dyslipidemic regulator formononetin: evaluation of antioxidant activity and gastroprotective effects in rat model of gastric ulcer. *Nutrients*, *12*(10), 2951. https://doi.org/10.3390/nu12102951

Mihai, C. M., Al Mărghitaş, L., Dezmirean, D. S., & Bărnuţiu, L. (2011). Correlation between Polyphenolic Profile and Antioxidant Activity of Propolis from Transylvania. *Scientific Papers: Animal Science and Biotechnologies*, *44*(2), 100–103.

Mohtar, L. G., Rodríguez, S. A., & Nazareno, M. A. (2018). Comparative analysis of volatile compound profiles of propolis from different provenances. *Journal of the Science of Food and Agriculture*, *98*(9), 3409–3415. https://doi.org/10.1002/jsfa.8852

Mojica, E.-R., Charland, J., Deocaris, C., & Micor, J. (2021). Potential and performance of accelerated solvent extraction (ASE) in obtaining bioactive compounds from bee propolis: comparison with soaking, ultrasonication, and microwave-assisted methods. *Journal of Tropical Life Science*, *11*(2), 187–192. https://doi.org/10.11594/jtls.11.02.08

Parwata, A., Manuaba, P., & Yasa, S. (2018). The potency of flavonoid compounds in water extract gyrinops versteegii leaves as natural antioxidants sources. *Biomedical and Pharmacology Journal*, *11*(3), 1501–1511. https://doi.org/10.13005/bpj/1517

Ramnath, S., Venkataramegowda, S., & Singh, C. (2015). Chemical composition of bee propolis collected from different regions in India by GCMS Analysis. In *International Journal of Pharmacognosy and Phytochemistry* (Vol. 30, Issue 1).

Revilla, I., Vivar-Quintana, A. M., González-Martín, I., Escuredo, O., & Seijo, C. (2017). The potential of near infrared spectroscopy for determining the phenolic, antioxidant, color and bactericide characteristics of raw propolis. *Microchemical Journal*, *134*, 211–217. https://doi.org/10.1016/j.microc.2017.06.006

Rinaldi, M. V. N., Díaz, I. E. C., Suffredini, I. B., & Moreno, P. R. H. (2017). Alkaloids and biological activity of beribá (Annona hypoglauca). *Revista Brasileira de Farmacognosia*, *27*(1), 77–83. https://doi.org/10.1016/j.bjp.2016.08.006

Ripari, N., Sartori, A. A., da Silva Honorio, M., Conte, F. L., Tasca, K. I., Santiago, K. B., & Sforcin, J. M. (2021). Propolis antiviral and immunomodulatory activity: a review and perspectives for COVID-19 treatment. *Journal of Pharmacy and Pharmacology*, *73*(3), 281–299. https://doi.org/10.1093/jpp/rgaa067

Rosyidi, D., Eka Radiati, L., Minarti, S., Mustakim, M., Susilo, A., Jaya, F., & Azis, A. (2018). Perbandingan sifat antioksidan propolis pada dua jenis lebah (Apis mellifera dan Trigona sp.) di Mojokerto dan Batu, Jawa Timur, Indonesia. *Jurnal Ilmu Dan Teknologi Hasil Ternak*, *13*(2), 108–117. https://doi.org/10.21776/ub.jitek.2018.013.02.5

Trusheva, B., Popova, M., Koendhori, E. B., Tsvetkova, I., Naydenski, C., & Bankova, V. (2011). Indonesian propolis: chemical composition, biological activity and botanical origin. *Natural Product Research*, *25*(6), 606–613. https://doi.org/10.1080/14786419.2010.488235

Varughese, J. K., J, K., S, S. K., Francis, D., L, J. L. K., & G, A. T. (2022). Identification of some dietary flavonoids as potential inhibitors of

TMPRSS2 through protein–ligand interaction studies and binding free energy calculations. *Structural Chemistry*, 1–14. https://doi.org/10.1007/s11224-022-01955-7