Identification of Secondary Metabolites and Antibacterial Activity of Non Polar Fraction from *Heterotrigona itama* Propolis

Abdul Aziz; Veggy Nadya Yuliawan; Paula Mariana Kustiawan*

Department of Pharmacy, Faculty of Pharmacy, Universitas Muhammadiyah Kalimantan Timur, Jl. Ir. H. Juanda No.15, Samarinda, Kalimantan Timur 75124.

**Abstract**

Propolis is one of the natural products produced by kelulut bees and is still not widely used. The type of stingless bee that is the prima donna in the community is *Heterotrigona itama*. This study aims to determine the phytochemical content of the n-hexane fraction of *Heterotrigona itama* bee propolis collected from Kutai Kartanegara, East Kalimantan. The n-hexane fraction was obtained from the methanol extract of *H. itama* propolis by the liquid-liquid partition method. After obtaining the n-hexane fraction, the research continued with a qualitative phytochemical test to identify the compound and total phenolic content. Antibacterial activity was determined by the agar well diffusion method with a serial concentration in *Escherichia coli* bacteria. Qualitative phytochemical analysis in the form of color changes showed that the n-hexane fraction of *H. itama* propolis contained flavonoids, alkaloids, saponins, and tannins. Based on the results, the total phenolic content of the n-hexane fraction sample was 490 mgGAE/100 g. It caused the n-hexane fraction to have lower phenolic content than the methanol extract (792 mg GAE/100 g). Furthermore, this result indicated that the non-polar fraction was not substantial enough to extract phenolic compounds. It correlated to the antibacterial activity of the n-hexane fraction, which was very weak (2 mm ± 1.5) at 200µg/mL concentration.

**Keywords:** heterotrigona itama; n-hexane; phenolic content; propolis

**INTRODUCTION**

Indonesia has incredible biodiversity, but non-timber forest products' potential is still limited and not managed properly. Bee product is one of the non-timber forest products that have a potential effect on health. Nowadays, in the pandemic era, stingless bee (*kelulut* bee) products have more attention for maintaining our health than utilized as a biodiversity-based economic potential. This honey is even more expensive than honey from *Apis* spp bees. However, the potential use of another product, such as propolis, is still limited. If this potential is appropriately managed, it can contribute to the community's economy, especially the villagers. According to FAO (Food and Agriculture Organization), beekeeping is...
one of the best economic opportunities for people living in forest areas. These benefits are directly obtained in the form of beekeeping products. Generally, propolis was generated by different resinous substances with antimicrobial activities from plants near the hive and collected to protect the hive. Bee colonies make a barrier immunity in the hive against pathogens and parasites, while propolis has prospective as a natural antibacterial.

Escherichia coli, one of the anaerobic facultative gram-negative bacteria, was abnormal flora in the large intestine of humans. Most of the strains of this bacterium are harmless, but some strains have acquired the DNA encoding for plasmid bacteriophage or enterotoxin or invasion factor and become pathogenic. Strains that contain this virulence factor are responsible for the incidence of global diarrhea, neonatal meningitis, sepsis, and urinary tract infection. If the bacteria are located outside the intestine or in their abnormal habitat, E. coli can become pathogenic bacteria. Furthermore, E. coli with certain strains usually cause acute diarrhea. This bacteria can be transmitted through water, food, or people who have bad sanitation. To overcome this, natural ingredients are used, such as propolis which is usually naturally used by bees to protect their hives from bacteria and fungi.

Heterotrigona itama is the most cultivated species of kelulut bee in Indonesia. In East Kalimantan, people are starting to be interested in cultivating kelulut bees. The community around PLTGU (Pembangkit Listrik Tenaga Gas dan Uap) in Kutai Kartanegara region try to develop kelulut bee farms as additional income in this pandemic era. Previous research showed that the ethyl acetate fraction of propolis from that location contains an alkaloid, saponin, and terpenoid. This study was conducted to identify the secondary metabolite of n-hexane fraction and antibacterial activity against E. coli. The n-hexane fraction from methanolic extract of plants source indicated has inhibition to multidrug resistance human pathogenic strains. Propolis is collected by a bee from plant resin to prevent bacteria and fungi from their hive. Based on the background above, this study aims to evaluate the antibacterial activity of the n-hexane fraction of stingless bee propolis from that area.

METHOD

This study used Heterotrigona itama propolis obtained from Bukit Biru, Kutai Kartanegara, East Kalimantan. Furthermore, the used materials also included methanol, Folin Ciocalteu reagent (Merck), sodium carbonate (Na₂CO₃) (Merck), n-hexane (CH₃(CH₂)₄CH₃), distilled water, gallic acid (Sigma-Aldrich), ethanol pro analysis (Merck), 70% ethanol (CV. Mulawarman Medika), filter paper, Quercetin (Sigma Aldrich), aqua dest (CV. Mulawarman Medika), and aluminum foil. The Genesys 10S UV-Vis Spectrophotometer was used as instrument analysis.

Sample preparation

Fresh propolis was collected from kelulut bee apiary, then kept at -4°C until used. The dirt, bees, and larva were removed out from propolis, then cut into pieces. The 100 grams of propolis were kept in containers, and methanol solvent was added and mixed. The Propolis mixture that has been left for 24 hours was filtered, and the solvent was changed 3 times. The filtrate was evaporated at low pressure at a temperature of 60-70°C using an evaporator to obtain a thick extract.
Specimen of stingless bee was determined at Laboratory of Faculty of Forestry, Mulawarman University and identify as *H. Itama*.

**Fractionation**
The 20 g of methanol extract was put into a container then added 400 ml of hot water. The extract was mixed, added in a separating funnel, and 400 ml of n-hexane solvent was added in a ratio (1:1). It was mixed by shaking for one minute then allowed to stand until the solution separated and formed two layers. Furthermore, n-hexane fraction filtrate was obtained and evaporated until a thick extract was obtained, and the yield was calculated.

**Phytochemical Assay**

**Flavonoid Test Identification**
The extract was pipetted as much as three drops into the drip plate. The extract was then added with 1 drop of H2SO4 p.a. Positive samples contain flavonoids if the solution undergoes a very striking color change to yellow, red, or brown.

**Tannin Test Identification**
The 1 mL methanol extract of propolis was added into a test tube and added 2-3 drops of 1% FeCl3. Samples were contained tannins when there was a color change from light green to blackish green.

**Alkaloid Test Identification**
A sample of 1 ml was put in a test tube, then 1 drop of HCl was added and homogenized. Next, 3 drops of Meyer's reagent were added. The test results were considered positive for containing alkaloids if the precipitate was yellowish-white.

**Terpenoid Test Identification**
The 1 ml of ethyl acetate fraction was taken and put into a test tube, and 0.5 ml of chloroform was added. A few drops of H2SO4 concentrated were later added to the side of the tube. If it showed reddish-brown color between the surfaces, it indicated the presence of triterpenoid compounds.

**Saponins Test Identification**
A sample of 1 ml was put in a test tube. The 1 ml of distilled water was added, then shake, and let stand for 30 seconds. Furthermore, the 5 drops of 1% HCl were mixed into the solution. The presence of saponin compounded with the formation of foam.

**Total Phenolic Content Assay**
First, The 10 % Na2CO3 solution was prepared 24 hours before use. On the next day, the n-hexane fraction of propolis was dissolved with 10 % methanol and distilled water to make a 10 mg/mL concentration. The gallic acid was used as a standard curve with a 3-50 µg/mL concentration. The phenolic content of the standard curve was determined with a 10 mL solution of 500 µL gallic acid, 500 µL of Folin-Ciocalteu reagent, 3 mL of 10% Na2CO3 solution distilled water. It was then incubated for 2 hours in a dark condition and measured on a UV-Vis spectrophotometer at a wavelength of 765 nm. The total phenolic content was calculated as natural compound (gallic acid) equivalent (GAE) by the following equation: T= C XV/M. T is the total phenolic content in mg/g of the extracts as GAE, while C is the concentration of gallic acid established from the calibration curve in mg/ml. V is the volume of the extract solution in ml, and M is the weight of the extract in g. The total phenolic content analysis results were expressed in units of g Gallic Acid Equivalent (GAE)/
100g samples. The n-hexane fraction was carried out in the same step of total phenolic determination.\textsuperscript{15}

**Antibacterial Assay**

The antibacterial test used the agar well disk of diffusion method with some modification\textsuperscript{16}. First, the media agar was prepared to culture bacteria and sterilized, then 20mL Nutrient Agar solution was poured into Petri dishes and wait until solid. After that, the 20 µL *E.coli* bacteria solution was added and spread on the surface of the media on the plates. Media agar plates were divided into five holes with a size four-mm diameter. The 20 µL of 25-200 µg extracts in acetone solution was added into the hole. A concentration of 30 µg/20 µL of positive control (Chloramphenicol) was added to each Petri disk. Acetone was used as a negative control and then incubated in the dark at 32°C for 24 h. Inhibition of bacteria was measured (mm) by calipers. Antibacterial activity was calculated as the mean inhibition zone for the test sample divided by the mean inhibition zone for the standard drug.

**RESULTS AND DISCUSSION**

**Phytochemical Screening**

The phytochemical screening process is one of the preliminary stages in providing an overview of the class of compounds contained in *H. itama* propolis to overview the sample. The results of phytochemical screening of stingless bee propolis extract contained secondary metabolite compounds, which can be seen in Table 1.

| Compounds   | Result | Indicator                                      |
|-------------|--------|-----------------------------------------------|
| Flavonoid   | +      | Color change to yellow, red to brown          |
| Alkaloid    | +      | Yellowish-white precipitate                   |
| Terpenoid   | -      | No color change between the surfaces          |
| Tannin      | +      | Color change from light green to blackish green|
| Saponin     | +      | Changes in the initial solution and showed foam|

Based on the data in Table 1, stingless bee propolis extract contains flavonoids, alkaloids, tannins, and saponins. These compounds are secondary metabolites produced from plants. This condition is related to the food source of the bees\textsuperscript{17,18}. Stingless bees visit the several flowers of plants for nectar and pollen\textsuperscript{19}. In addition, stingless bees take plant sap to build their nests\textsuperscript{20,21,22}. The n-hexane fraction was obtained for the phenolic determination. Compounds of phenol are described by the presence of a light green or slightly blue color, and the sample shows a blackish green stain so that it is suspected to be positive for phenolic\textsuperscript{23}. Phenolic responds with 1% FeCl\textsubscript{3} to frame intense red, blue, purple, or dark colors as FeCl\textsubscript{3} will respond with –OH. Aromatics identification of tannins can also be carried out with ferric chloride expansion. One of them is because tannins are polyphenolic compounds, which means they have a place with a group of phenolic compounds. Both examples show positive tannins by showing a blackish green stain\textsuperscript{24}.

Furthermore, a few drops of concentrated HCl and magnesium were added to the sample during the flavonoid test. Flavonoids were indicated by the appearance of maroon or pink color within 3 minutes. Both samples showed a red
color suspected to be positive for flavonoids. Besides, in the saponin test, distilled water is added to the sample, then bubbled using a water shower and shaken to form a stable foam. A fixed foam formation indicates the presence of saponins. Two examples showed positive results. Saponin compounds have a glycosyl group as a polar group and a steroid or triterpenoid group as a nonpolar group. Thus, they surfaced dynamically and formed micelles when it is shaken with water. In micellar structure, the polar groups face outward while the nonpolar groups face inwards, which looks like foam.

**Total phenolic content**

The total phenolic content results showed phenolic compounds from the n-hexane fraction of *H. itama* propolis with absorbance values (Table 2). The data generated from the total phenolic test content was processed in absorbance tables and standard curves (Figure 1).

![Figure 1. Standard curve of gallic acid to determine phenolic content](image)

The results of the measurement of the phenolic content of propolis extract depend on the solvent as the solvent can affect the total phenolic content. When mixed in food, the phenolic compounds contained in propolis extract will be absorbed. However, it does not change in structure to increase the function of the food as an antioxidant. The phenol content of the *kelulut* bee *H. itama* propolis was determined by UV-Vis spectrophotometry, with the standard used gallic acid as gallic acid is a derivative of hydroxybenzoic acid indicating a simple phenolic acid. The researchers used gallic acid as a standard based on the

| Absorbance | Mean of Absorbance | Gallic Acid Equivalent (μgGAE/mL) | Total Phenolic Content (mgGAE/100 g) |
|------------|--------------------|----------------------------------|-------------------------------------|
| 0.896      | 0.3375             | 2.45                             | 490                                 |
| 0.889      |                    |                                  |                                     |
availability of a stable and pure substance. Another factor is that gallic acid is cheaper than other standard compounds. The total phenol content was calculated by putting the sample absorption value at a wavelength of 767 nm into the linear equation $y = ax + b$ obtained from the gallic acid calibration curve. The yield is expressed in mg gallic acid equivalent per 100 grams (mg GAE/100 grams). Standard curves were made with a concentration of 0.5; 1; 2; 4; 8; 16 g/mL. The calibration curve was made from the level of gallic acid done in duplicate, and the $R^2$ value was searched. The $R^2$ or coefficient of determination value is a number whose value ranges from 0 to 1, indicating how close the estimated value for regression analysis represents the actual data. Regression analysis will be the closest and most reliable one if the $R^2$ value is equal to or close to 1. Based on the calibration curve measurement results, the $R^2$ value was 0.8482. The tested phenolic content had the highest concentration in the n-hexane fraction, which was 490 mgGAE/g, while the phenolic content in the methanol extract was 792 mgGAE/g. The total phenolic content test results were influenced by the high methanol content and could also affect the withdrawal process of phenolic compounds. It means the higher the methanol content is, the more the absorbed phenolic compounds will be. It occurs because H. itama propolis has a short hydrocarbon chain so that the non-polar solvent ability of n-hexane in absorbing extracts from propolis is still lacking. Based on the total phenolic test results, the total phenolic content in the n-hexane fraction sample was lower than the methanol extract (Table 3).

| Sample          | Total Phenolic Content |
|-----------------|------------------------|
| n-Hexane Fraction | 490 mgGAE/100 g        |
| Methanol Extract | 792 mgGAE/100 g        |

This result aligns with the research of²⁶,²⁷, stating that the total phenolic content of methanol extract was more significant than the n-hexane fraction. It could be due to the polar nature of methanol, which could attract phenolic compounds well in the extraction process. Moreover, it also aligns with the results of previous studies revealing that methanol was a polar solvent that did not have a high boiling point so that compounds susceptible to high temperatures would not be damaged.²⁸ Furthermore, the chemical composition of propolis varies qualitatively and quantitatively due to the diversity of plant resins, in addition to the various geographic and climatic characteristics of the environment around the beehive.²⁹,³⁰

**Antibacterial activity**

Antibacterial activity of non-polar fraction from H. itama propolis was determined with agar well diffusion. Acetone was used as a negative control, and broad-spectrum antibacterial drug (Chloramphenicol) was used as a positive control. E. coli is a gram-negative bacterium and is well known for its resistance to many antibiotics due to the permeability barrier provided by its outer membrane³¹. The result of antibacterial activity of n-hexane fraction of H. itama propolis against E. Coli showed a weak level of inhibition (Table 4).
The antibacterial activity was categorized by diameter inhibition. If the inhibition diameter regions on 5 mm or less, antibacterial activities are categorized as weak. 5-10 mm is categorized as medium; 10-19 mm is categorized as strong, while 20 mm or more is categorized as very strong. The maximum concentration of 200 µg/mL only showed inhibition of 2 mm width. Table 4 shows the inhibition in the weak category as it is around 1-2 mm. The non-polar fraction of propolis was had weak activity against E.coli. This gram-negative bacteria activity was caused by differences in the structure of the outer membrane of bacteria and the production of hydrolytic enzymes that caused degradation of the active components of propolis. Most of the polyphenol compound was detected in polar solvent rather than non-polar solvent. Propolis was collected by a bee from plant resin and correlated to a specific compound from that plant. Bioactivities of plants were related to total phenolic compounds, such as flavonoids and lignin as an antioxidant, alkaloid as antibacteria. The low content of phenolic components in the n-hexane fraction of H.itama propolis was correlated with antibacterial activity against E.coli. Another research about the antibacterial activity of the n-hexane fraction of plant extract showed relatively low phenolic content. The n-hexane fraction also indicates it had good gram-positive activity rather than gram-negative bacteria. That present of secondary metabolite in n-hexane fraction and phenolic content also had a contribution to antibacterial activity. It is was influenced by the time of sample collected, climate, and plants near the sample collected. The chemical composition of propolis was determined by the composition of the plant source. Most of the activity was not directly affected by the presence of PLTGU.

CONCLUSION

The n-hexane fraction of H. itama propolis contained alkaloid, flavonoid, saponin, and tannin, indicating potential compounds. This non-polar fraction was insufficient to extract phenolic compounds (490 mg GAE/100 g). The total phenolic content of the non-polar fraction, which was less than the crude extract, indicated that some phenolic compounds were present in polar fractions. It correlated to the weak antibacterial activity of n-hexane fraction (2 mm ± 1.5) at 200µg/mL concentration. Furthermore, the results of this study are preliminary studies. Further research is suggested to carry out the bioactivity of the kelulut bee propolis H.itama from Kutai Kartanegara to benefit the community for the use of kelulut bee propolis.

ACKNOWLEDGMENT

The authors would like to thank the research institute and community service.
Universitas Muhammadiyah Kalimantan Timur (KDM grant) for their support in this study.

CONFLICT OF INTEREST

All authors declare no potential for conflict of interest with the research, authorship, and article publication.

REFERENCES

1. Adanan, N. A., Rosmidi, F. H., & Abdullah, M. T. (2017). Establishing a Link Between Bee Conservation and Ecotourism. Ecotourism Potentials in Malaysia. Universitas Putra Malaysia, p. 48.
2. Bradbear, N. (2009). Bees and their role in forest livelihoods. Food and Agricultural Organization. FAO: Rome, pp. 1-194.
3. Simone-Finstrom, M. D., Evans, J. D., & Spivak, M. (2009). Resin collection and social immunity in honey bees. Evolution (N Y), 63(11), pp. 3016–3022. https://doi.org/10.1111/j.1558-5646.2009.00772.x
4. Makvana, S., & Krilov, L. R. (2015) Escherichia coli infections. Pediatrics in review. Pediatr Rev, 36(4), pp. 167–170.
5. Gomes, T. A., Elias, W. P., Scaletsky, I. C., Guth, B. E., Rodrigues, J. F., Piazza, R. M., et al. (2016). Diarrheagenic Escherichia coli. Brazilian J Microbiol, 47(1), pp. 3–30. https://doi.org/10.1016/j.bjm.2016.10.015
6. Hoff, C. (2021). Notes from the Field: An Outbreak of Escherichia coli 0157: H7 Infections Linked to Romaine Lettuce Exposure—United States, 2019.
7. Yuliawan, V. N., Aziz, A., & Kustiawan, P. M. (2021). Uji Fitokimia Fraksi Etil Asetat Dari Propolis Lebah Kelulut Heterotrigona Itama Asal Kutai Kartanegara Pengumpulan sampel, 2(2), pp. 131–137.
8. Mukherjee, S., Dey, A., Das, T. (2012). In vitro antibacterial activity of n-hexane fraction of methanolic extract of Alstonia scholaris LR Br. stem bark against some multidrug resistant human pathogenic bacteria. European J Med Plants, 1(1). https://doi.org/10.9734/EJMP/2012/794
9. Sibero MT, Zilda DS, Haryanti D, Igarashi Y. (2021). Astrogorgia sp. from Saparua, Maluku: Phytochemical Content, Antimicrobial, Antioxidant, and Cytotoxicity Properties. IOP Conference Series: Earth and Environmental Science, 75, p. 012062. https://doi.org/10.1088/1755-1315/75/1/012062
10. Suwendar, S., Muljie, L., Choeraina, R., & Mardliyani, D. (2020) Antibacterial effect potentiation of n-hexane fraction of rose apple leaves. Journal of Physics: Conference Series, 1469(1), p. 012023. https://doi.org/10.1088/1742-6596/1469/1/012023
11. Yuliana R, Santoso HB, Riendrasari SD. (2015). Daya Antimikrobia Sarang Lebah Madu Trigona spp terhadap Mikrobia Patogen. Bioedukasi J Pendidik Biol. 8(1), pp. 67-72. https://doi.org/10.20961/bioedukasi-uns.v8i1.3546
12. Munte L, Runtuwene MR, Citraningtyas G. (2015). Aktivitas Antioksidan dari Ekstrak Daun Prasman (Eupatorium triplinerve Vahl.). Phamacon, 4(3), pp. 41-50. https://doi.org/10.35799/pha.4.2015.8836
13. Kadji, M. H., Runtuwene, M. R., & Citraningtyas, G. (2013). Uji fitokimia
dan aktivitas antioksidan dari ekstrak etanol daun soyogik (Saurauia bracteosa DC). *Pharmacogn.* 2(2), pp. 13-17. https://doi.org/10.3579g/pha.2.2013.1

14. Khairstunnisa, B., Rosamah, E., Kuspradini, H., Kusuma, I. W., Tandirogang, N., & Arung, E. T. (2020). Uji Fitokimia dan Antioksiidan Ekstrak Etanol Propolis Lebah Kelulut (Tetragonula iridipennis) Dari Samarinda. *J Ilm manunung*, 6(1), pp. 65–9. https://doi.org/10.51352/jim.v6i1.309

15. Hairunisa I, Mentari IA, Julianti T, Wikantyasning ER, Kuspradini H, Kedokt Bandung Resistant Staphylococcus aureus. *Assessment of Antimicrobial and Natural Antioxidant Activities in Different Parts of Pulasan (Nephelium mutabile Blume) from East Borneo*. *IOP Conf Ser Earth Environ Sci.* 736(1).

16. Egra S, Kusuma, Irawan Wijaya, Arung ET, Kuspradini H. (2019). The potential of white-oyster mushroom (Pleurotus ostreatus) as antimicrobial and natural antioxidant. *Biofarmasi J Nat Prod Biochem*, 17(1), pp. 17–23. https://doi.org/10.13057/biofar/f37010

17. Nursidika, P., Saptarini, O., & Rafiqua, N. (2014). *A*ktivitas Antimikro Fraksi Ekstrak Etanol Buah Pinang (Areca catechu L) pada Bakteri Methicillin Resistant Staphylococcus aureus. *Mag Kedokt Bandung*, 46(2), pp. 94–9. https://doi.org/10.15395/mkb.v46n2.2

18. Abduh, M. Y., Adam, A., Fadhullullah, M., Putra, R. E., & Manurung, R. (2020). Production of propolis and honey from Tetragonula laeviceps cultivated in Modular Tetragonula Hives. *Heliyon*, 6(11). https://doi.org/10.1016/j.heliyon.2020.e05405

19. A’yunin, Q., Rauf, A., Harahap, I. S. (2019). Foraging Behaviour and Pollination Efficiency of Heterotrigona itama (Cockerell) and Tetragonula laeviceps (Smith) (Hymenoptera: Apidae) on Chayote. *J Ilmu Pertan Indones*, 24(3), pp. 247–57.

20. Mujetahid, A. (2008). Teknik Pemanenan Madu Lebah Hutan Oleh Masyarakat Sekitar Hutan Di Kecamatan Mallawa Kabupaten Maros. *PERENNIAL*, 4(1), pp. 36–40. https://doi.org/10.24259/perennial.v4 i1.181

21. Khairstunnisa K, Mardawati E, Putri SH. Karakteristik Fitokimia dan Aktivitas Antioksiidan Ekstrak Propolis Lebah Trigona Sp. *J Ind Pertan*. 2020;2(1), pp. 124–9.

22. Hirmarizqi, A. A. N., Sari, E., Fembriyanto, R. K., Hidayati, N. A., & Hertati, R. (2019). Identifikasi lebah kelulut asal Bangka dan pendataan jenis tumbuhan penghasil resin bahan baku pembuatan propolis. *EKOTONIA J Penelit Biol Bot Zool dan Mikrobiol*, 4(2), pp. 37–42. https://doi.org/10.33019/ekotonia.v4i 2.1667

23. Haryanti, P., Supriyadi, S., Marseno, D. W., & Santoso, U. (2018). Effects of Different Weather Conditions and Addition of Mangosteen Peel Powder on Chemical Properties and Antioxidant Activity of Coconut Sap. *Agritech*, 38(3), pp. 295-303. https://doi.org/10.22146/agritech.298

24. Kumajas, J., & Howan, D. H. O. (2018). Studi Kandungan Kimia Ekstrak Buah Pakoba Merah (Syzygium sp). *Fuller J Chem*, 3(2), p. 58.

25. Sangi, M., Runtuwene, M. R. J., & Simbala, H. E. I. (2008). Analisis Fitokimia Tumbuhan Obat Di Kabupaten Minahasa Utara. *Chem Prog*, 1(1), pp. 47–53.
26. Yulis, P. A. R., & Sari, Y. (2020). Aktivitas Antioksidan dari Limbah Kulit Pisang Muli (Musa acuminata Linn) dan Kulit Pisang Kepok (Musa paradisiaca formatypica). Al Kim [Internet]. pp. 189–200. Available from: http://journal.uin-alauddin.ac.id/index.php/al-kimia%0AAktivitas

27. Elisya, Y., Cartika, H., & Rizkiana, A. (2017). Antioxidant Activity and Total Phenolic Content of Date Palms Syrup (Phoenix dactylifera L.). SANITAS J Teknol dan Seni Kesehat, 8(1), pp. 63–71. https://doi.org/10.36525/sanitas.2017.10

28. Adawiyah, R., & Riani, A. (2005). Ekstraksi Flavonoid Metode Soxhletasi dari Batang Pisang Ambon (Musa Paradisiaca Var. Sapientum) dengan Berbagai Jenis Pelarut. In: Prosiding Simposium Nasional Inovasi dan Pembelajaran Sains. pp. 625–8.

29. Reis, J. H. D. O., Barreto, G. D. A., Cerqueira, J. C., Anjos, J. P. D., Andrade, L. N., Padilha, F. F., ..., et al. (2019). Evaluation of the antioxidant profile and cytotoxic activity of red propolis extracts from different regions of northeastern Brazil obtained by conventional and ultrasound-assisted extraction. PLoS One. 14(7), p.e0219063. https://doi.org/10.1371/journal.pone.0219063

30. Xu, X., Pu, R., Li, Y., Wu, Z., Li, C., Miao, X., et al. (2019). Chemical compositions of propolis from China and the United States and their antimicrobial activities against Penicillium notatum. Molecules, 24(19), p. 3576. https://doi.org/10.3390/molecules24193576

31. Viveiros, M., Dupont, M., Rodrigues, L., Couto, I., Davin-Regli, A., Martins M., et al. (2007). Antibiotic stress, genetic response and altered permeability of E. coli. PLoS One, 2(4), p. E365. https://doi.org/10.1371/journal.pone.000365

32. Davis, W. W., & Stout, T. R. (1971). Disc plate method of microbiological antibiotic assay: I. Factors influencing variability and error. Appl Microbiol, 22(4), pp. 659–65.

33. Sforcin, J. M. (2007). Propolis and the immune system: A review. J Ethnopharmacol, 113(1), pp. 1–14. https://doi.org/10.1016/j.jep.2007.05.012

34. Herrera-pool, E., Ramos-díaz, A. L., Lizardi-jím, M. A., García-cruz, U., & Pacheco, N. (2021). Of solvent polarity on the Ultrasound Assisted extraction and antioxidant activity of phenolic compounds from habanero pepper leaves (Capsicum chinense) and its identification by UPLC-ESI-MS/MS. Ultrason Sonochem, 76 https://doi.org/10.1016/j.ultsonch.2021.105658

35. Verrillo, M., Cozzolino, V., Spaccini, R., & Piccolo, A. (2021). Humic substances from green compost increase bioactivity and antibacterial properties of essential oils in Basil leaves. Chem Biol Technol Agric. 8(28), pp. 1–14. https://doi.org/10.1186/s40538-021-00226-7

36. Saddique, Z., Naeem, I., Hellio, C., Patel, A. V., & Abbas, G. (2020). Phytochemical profile, antioxidant and antibacterial activity of four Hypericum species from the UK. South African J Bot. 133, pp. 45–53. https://doi.org/10.1016/j.sajb.2020.05.018
37. Khotimah, T. K., Krisridwany, A., Orbayinah, S., & Harimurti, S. (2021) Antibacterial Activity of Fractions from Extract Ethanolic of Hylocereus Polyrhizus Peel Against E. Coli and S. aureus. *J Fundam Appl Pharm Sci*, 1(2), pp. 65–71. https://doi.org/10.18196/jfaps.v1i2.11001

38. Dezmirean, D. S., Pașca, C., Moise, A. R., & Bobiș, O. (2021). Plant sources responsible for the chemical composition and main bioactive properties of poplar-type propolis. *Plants*, 10(1), pp. 1–20. https://doi.org/10.3390/plants10010022

39. Vardar-Ünlü, G., Silici, S., & Ünlü, M. (2008). Composition and in vitro antimicrobial activity of Populus buds and poplar-type propolis. *World J Microbiol Biotechnol*, 24(27), pp. 1011–1017. https://doi.org/10.1007/s11274-007-9566-5