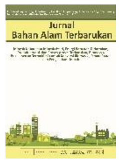
JBAT 10(2) (2021) 75 – 80

Jurnal Bahan Alam Terbarukan

p-ISSN 2303 0623
e-ISSN 2407 2370

Accredited SINTA 2 by Ministry of Research and Technology/National Research and Innovation Agency
SK No. 200/M/KPT/2020
http://journal.unnes.ac.id/nju/index.php/jbat



Analysis of Molecular Stability on Waste Extracts of *Trigona spp*. Bees Haves. Ethanolically

Andi Muhamad Iqbal Akbar Asfar[1,✉], **Andi Muhammad Irfan Taufan Asfar**[2]

DOI: https://doi.org/10.15294/jbat.v10i2.33471

[1]Chemical Engineering, Politeknik Negeri Ujung Pandang, Makassar, Indonesia
[2]Mathematic Education, Universitas Muhammadiyah Bone, Watampone, Indonesia

Article Info

Article history:
Received
October 2021
Accepted
November 2021
Published
December 2021

Keywords:
Trigona spp;
Stingless Bee;
Antioxidant Capacity;
GCMS;
Terpenoid

Abstract

One of the local honey of South Sulawesi is Trigona honey which comes from *Trigona spp*. There are three types of trigona bees in South Sulawesi with each colony consisting of 300-80,000 individuals. Trigona bees are found nesting in hollow places such as logs, tree holes, and cracks in the walls of houses. The nest entrance is made of plant resin mixed with soil and mud, with different shapes and colors depending on the species. This type of bee produces a lot of propolis compared to honey and the price of honey produced is much higher because it has a chemical content (health benefits) that is much better than ordinary honey from the *Apis mellifera* group. This study was designed by extracting in ethanolic process at a concentration of 70%. The choice of concentration was based on previous research with an extraction temperature of 60 °C. The treatment of this research is to vary the time, namely 10 minutes, 20 minutes, and 30 minutes as a setting for the extraction time. Each treatment will be carried out in duplicate. This shows that in the honeycomb waste *Trigona spp.* contains terpenoids which are classified as antioxidants. Therefore, beehive waste *Trigona spp.* potential to be used as a source of antioxidants. In addition, several compounds are also relatively large antibacterial and anti-microbial compounds contained in the waste of *Trigona spp* beehives. The optimum solvent used by comparing the percentage of abundance (abundance/relative) through the percentage of the area and molecular stability after fragmentation is through the ethanolic process using ethanol solvent with percent abundance at 218 m/z and 189 m/z with the largest compound contained is Methyl Commate A and Alpha-Amyrin

INTRODUCTION

Honey is a natural product derived from insects and is formed from flower nectar which has many health benefits including antioxidants and anti-inflammatory properties. Honey is one of the traditional medicines used by the community and has been known since 10,000 years ago and has the ability as an antimicrobial (Mamada et al., 2018). One of the local honey of South Sulawesi is Trigona honey which comes from *Trigona spp*. There are three types of trigona bees in South Sulawesi, each colony consisting of 300-80,000 individuals. Trigona bees are found nesting in hollow places such as logs, tree holes, and cracks in the walls of houses. The nest entrance is made of plant resin mixed with soil and mud, with different shapes and colors depending on the species. A hive is a place for a bee colony to shelter, store food and reproduce. The structure of the Trigona honeycomb is different from that of the Apis honey bee, wherein the Trigona hive the storage pot for pollen and

✉ Corresponding author:
E-mail: andiifalasfar@gmail.com

honey is separated from the brood chamber. The daughter cell is where the queen lays eggs and where the daughter develops from the egg phase to the imago.

The amount of honey produced by the Trigona species is less than that of Apis honey-producing bees and it is more difficult to harvest from the hive (Nur et al., 2019). The honey produced by *Trigona spp.* has a special aroma, a mixture of sweet and sour tastes like lemon and has high antioxidant activity (Salampe et al., 2018; Kafaween et al., 2019; Al-Kafaween et al., 2020).

In addition to honey, in the beehive of *Trigona spp*. propolis was also found which serves to strengthen the nest and is used for the defense system of *Trigona spp*. Honey and propolis produced can be used as a source of probiotics because they contain lactic acid bacteria and can help maintain the health of human digestive organs through the synthesis of exopolysaccharides that can inhibit the growth of pathogenic bacteria (Manguntungi et al., 2020; Renita et al., 2015). Propolis is one of the ingredients that contain natural antioxidants from secondary metabolite compounds (Lutpiatina, 2015), where this propolis extract contains compounds in the form of flavonoids, phenols, triterpenoids, mono and sesquiterpenes, tannins, and alkaloids. However, flavonoids are one of the most important constituents representing about 50% of the content of propolis. Flavonoid compounds are compounds that are soluble in water and have antioxidant activity, so that propolis can be used as antibacterial, antiviral and antifungal substances (Rismawati & Ismiyati, 2017). Meanwhile, antioxidants are nutritional and non-nutritive substances contained in food ingredients and are able to prevent or slow down the occurrence of oxidation reactions in the body (Pratama et al., 2018). Antioxidants are very beneficial for health and cosmetics and play an important role in maintaining the quality of food products. According to Thamrin et al. (2016) the ability of propolis as an antioxidant can capture hydroxy radicals and superoxide then neutralize free radicals, thereby protecting cells and maintaining the integrity of cell and tissue structures and can protect lipid membranes against damaging reactions. Flavonoid compounds, polyphenols, and tannins are bioactive compounds in propolis that act as antimicrobial compounds and are polar (Yarlina et al., 2020). Flavonoids and polyphenols have the potential to reduce free radicals and prevent the formation of oxygen species (Asfar & Asfar, 2021; Asfar & Yasser, 2018) and flavonoids function as antioxidants in the human body (Asfar & Asfar, 2020).

MATERIALS AND METHODS

Materials

The main material used in this research is honeycomb waste Trigona spp. from *Trigona spp*. beekeepers in Bontocani District, Bone Regency. The chemical used for extraction is Ethanol. Chemicals used for analysis are pyridine HCl, Aquadest, and helium gas.

The tools for chemical analysis are analytical balance, blender, 500 ml beaker, 100 ml volumetric flask, 100 ml measuring cup, aluminum cup, porcelain cup, erlenmeyer, dropper, desiccator, oven, ultrasonic bath (Merck Elma, S. 30Hz), stopwatch, tupperware, filter paper, aluminum foil, glass, cotton, biurette, and rotary vacuum evaporator.

Methods

Analysis

This study was designed by extracting using solvent namely Ethanol at a concentration of 70%. The choice of concentration was based on previous research with an extraction temperature of 60^0C. The treatment of this research is to vary the time, namely 10 minutes, 20 minutes and 30 minutes as a setting for the extraction time. Each treatment will be carried out in duplicate.

Antioxidant Analysis by GCMS Method

Antioxidant analysis consist of two steps were sample preparation and GCMS analysis. sample preparation is done by first that the honeycomb waste of *Trigona spp* is weighed as much as 10 grams, and add a solvent with a concentration of 70% as much as 220 ml (ratio of material to solvent = 1 gr: 22 ml). Extraction with ultrasonic assistance at a temperature of 60^oC for the appropriate treatment, namely 10 minutes, 20 minutes and 30 minutes. time variation was carried out to determine the increase in antioxidant capacity in honey bee hive waste *Trigona spp.* This variation is based on previous research. The obtained suspension is filtered through filter paper. Then it is concentrated with a rotary vacuum evaporator until it reaches a concentration of 30 ml.

The next step is GCMS analysis. This analysis was carried out by taking a sample 1 ml of the extract was reacted with 50 μl of pyridine + 100 μl of bis-(trimethylsilyl) trifluoroacetamide (BSTFA) including 1% trimethylchlorosilane (TMCS) in a sealed glass tube for 30 min at 100 °C to prepare samples for gas chromatography. A sample volume of 1 μl was injected and analyzed by GC-MS. The GCMS results are identified through a computer search in commercial reference libraries and GCMS will adjust the level of similarity between the target and the data in the library to determine the tendency of the compounds or molecules identified. The results of the GCMS analysis will provide spectrogram and chromatogram data (Kartal, Kaya & Kurucu, 2002).

RESULTS AND DISCUSSION

The results obtained through the ethanolic process with the results of the GCMS analysis showed the characterization of ionic stability for each treatment was different. The ethanolic process through the extraction of beehive waste *Trigona spp.* showed the presence of terpenoids, steroids, and phenols. The largest compounds in ethanolic extraction are Methyl Commate A, Beta-Amyrin, Ethyl Oleate, Beta Amyernyl, 24 - Methylenecycloartan – 3-One, Alpha-Amyrenone, Lanosterol, Betulin and Methyl Commate D. There are five largest compounds in the waste extract of *Trigona spp.* honeycomb, namely Beta-Amyrin, Methyl Commate A, Alpha Amyrenone, Lanosterol, and Alpha Amyrin. The spectrogram results showed that there were 3 molecules at a molecular stability of 218 m/z with an abundance percentage of 100% (abundance/relative). The five molecules are Beta-Amyrin, Beta-Amyron, and Methyl Commate A. In addition, 9- Octadecenoic Acid, (E) has a base peak at 327 m/z. Stability at 109 m/z consists of 2 molecules, namely 3-Pentadecylphenol and Adrenic Acid.

The results of the comparison of bioactive components in Table 2 show that the content of -Amyrin and -Amyrin in *Trigona spp*. bee nest waste. originating from Bontocani, Bone Regency, is still relatively high with area percentages of 18.09% and 6.71%, respectively. However, the content of Cyclolanost in *Trigona spp.* propolis from Wonosobo (Indonesian Propolis) was 15.75% higher. Lupenone compounds, namely Lup-20(29)-En-3-Ol, (3.Beta) are only found in beehive waste *Trigona spp.* Bontocani by 9.85%. One of the largest content in beehive waste *Trigona spp.* from Bontocani which is not owned by propolis from Wonosobo and from Brazil is the content of Methyl Commate A and Methyl Commate D with percentages of 19.98% and 3.06%, respectively, including pyrimidine compounds of 10.97%.

Several important compounds were identified from the extraction process of *Trigona spp*. bee nest waste. are The content of -Amyrin, -Amyrin and Lupeol were identified as anti-inflammatory (Okoye et al., 2014). Lupeol is reported to exhibit anticarcinogenic and antitumor activity (Santos et al., 2012). -Amyrin and -Amyrin have hypoglycemic activity in treating diabetes which were tested in mice and have anti-inflammatory and antioxidant benefits (Santos et al, 2012). Ethyl oleate is one of the fatty acid ethyl ester (FAEE) group which is a pheromone found in bees formed from the digestive process containing ethanol. Also included in this group are Hexadecanoic acid, ethyl ester which is an antioxidant (Sativa & Agustin, 2018; Kim et al., 2020)

Lanosterol which is an eye drop for cataract sufferers, Betulin is an antimicrobial and Beta-Himachalenoxide and its derivatives have antifungal and insecticidal effects. Caryophyllene oxide, an oxygenated terpenoid, known as a preservative in food, medicine and cosmetics, has been tested in vitro as an antifungal against dermatophytes. Its antifungal activity has been compared with ciclopiroxolamine and sulconazole, which are commonly used in the treatment of onychomycosis and were chosen because of their very different chemical structures.

This shows that in the honeycomb waste *Trigona spp.* contains terpenoids which are classified as antioxidants. Therefore, beehive waste *Trigona spp.* potential to be used as a source of antioxidants. In addition, several compounds are also relatively large antibacterial and anti-microbial compounds contained in the waste of Trigona spp beehives. The optimum solvent used by comparing the percentage of abundance (abundace/relative) through the percentage of area and molecular stability after fragmentation is through the ethanolic process using ethanol as a solvent.

Table 1. Identification of Honeycomb Waste Content of *Trigona spp*. with Ethanolic Proces

Molecule	Molecule Weight g/mol	Retention Time Minute	Base Peak m/z
Methyl Commate A	500	31.790	218
Beta-Amyrin	426	29.132	218
Ethyl Oleat	310	22.432	218
24 -Methylenecycloartan – 3- one	438	33.218	95
Beta-Amyrenyl	468	35.756	109
Alpha-Amyrin	426	31.526	218
Betulin	442	31.767	189
Methyl Commate D	486	36.047	218

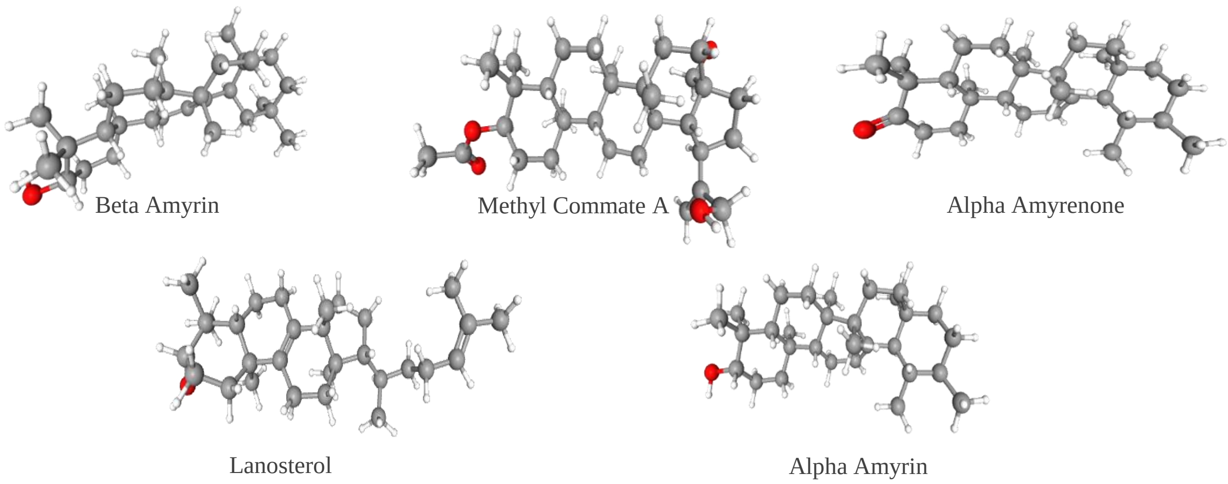


Figure 1. Molecules with the highest stability based on the base peak.

Table 2. Comparison of Chemical Compound Components of Ethanolic Extract of Propolis *Trigona spp.* with *Trigona spp.* Honeycomb Waste Extract.

Bioactive Component	Indonesian Propolis (Renita et al., 2015) (%)	Brazilian Propolis (Renita et al., 2015) (%)	Propolis From Honeycomb Waste Extract (Bontocani District) (%)
α-Amyrin	4.32	2.87	18.09
β-Amyrin	-	1.40	6.71
Cyclolanost	15.75	1.81	0.35
Lupeol Compound	0.68	-	4.28
Lupenon Compound	-		9.85
Hexadecanoic Acid, Ethyl Ester	-	-	0.37
Ethyl Oleate	-	-	1.57
Methyl Commate A	-	-	19.98
Methyl Commate D	-	-	3.06
Senyawa Pirimidin	0.81	0.40	10.97

CONCLUSION

The extraction treatment using ethanol and ethyl acetate solvents gave different extract results and the content of secondary metabolites that were able to extract well. The ethanolic process is highly recommended in extracting the honeycomb waste of *Trigona spp*. which is able to extract terpenoids and steroids which are antioxidants. The results of the analysis through GCMS with the identification of chromatogram and spectrogram results showed that the largest antioxidant capacity obtained was Methyl Commate A and Alpha-Amyrin which almost dominated the chemical compounds in *Trigona spp.* honeycomb waste.

ACKNOWLEDGEMENT

Thank you to the Ujung Pandang State Polytechnic through the 2021 DIPA Routine for funding this research and all analysts and staff of the Chemical Engineering Department of the Ujung Pandang State Polytechnic.

REFERENCES

Al-Kafaween, M. A., Hilmi, A. B. M., Jaffar, N., Al-Jamal, H. A. N., Zahri, M. K., Jibril, F. I. 2020. Antibacterial and Antibiofilm Activities of Malaysian Trigona Honey Against Pseudomonas Aeruginosa ATCC 10145 and Streptococcus Pyogenes ATCC 19615. Jordan Journal of Biological Sciences. 13(1): 69–76.

Asfar, A.M.I.A., Asfar, A.M.I.T. 2020. Efektifitas Ekstrak Kayu Sepang Sebagai Pengawet Alami Daging Olaha. Jurnal Biosains. 6(3): 98-102.

Asfar, A.M.I.A., Asfar. A.M.I.T. 2021. Antioxidant Activity in Sappan Wood (*Caesalpinia sappan L.*) Extract Based on pH of the Water. Jurnal Ilmiah Farmako Bahari. 12(1): 39-44.

Asfar, A.M.I.A., Yasser. M. 2018. Analisis Kualitatif Fitokimia Kandungan Flavonoid Estrak Kayu Sepang (*Caesalpinia sappan L.*) dari Ekstraksi Metode Ultrasonic Assisted Solvent Extraction. Jurnal Chemical. 9(2): 15-25.

Kafaween, M. A. Al, Hilmi, A. B., Khan, R. S., Bouacha, M., Amonov, M. 2019. Effect of Trigona Honey on Escherichia Coli Cell Culture Growth: In Vitro Study. Journal of Apitherapy. 5(2): 10–17.

Kartal, M., Kaya, S., Kurucu, S. 2002. GC-MS Analysis of Propolis Samples from Different Regions of Turkey. Faculty of Pharmacy, Department of Pharmacognosy. Ankara University (Published).

Kim, B.R., Kim, H.M., Jin, C.H., Kang, C.H., Kang, S.Y., Kim, J.B., Jeon, Y.G., Park, K.Y., Lee, I.K., Han, A.R. 2020. Composition and Antioxidant Activities of Volatile Organic Compounds in Radiation-Bred Coreopsis Cultivars. Plants. 9(717): 1-9.

Lutpiatina, L. 2015. Efektivitas Ekstrak Propolis Lebah Kelut (*Trigona spp*) dalam Menghambat Pertumbuhan Salmonella typhi, Staphylococcus aureus dan Candida albicans. Jurnal Skala Kesehatan. 6(1): 1–8.

Mallick, S.S., Dighe, V.V. 2014. Detection and Estimation of Alpha-Amyrin, Beta-Sitosterol, Lupeol, and n-Triacontane in Two Medicinal Plants by High Performance Thin Layer Chromatography. 2014:1-7.

Mamada, S. S., Usmar, Aliyah, Aminullah, Rahayu, A. I., Hidayat, K., Salampe, M. 2018. Pengaruh Suplementasi Madu Trigona terhadap Parameter Fungsi Hati dan Ginjal Tikus Albino (Rattus norvegicus) yang Diberikan Simvastatin. *Jurnal Farmasi Galenika (Galenika Journal of Pharmacy).* 4(1): 36–43.

Manguntungi, B., Sari, A. P., Chaidir, R. R. A., Islam, I., Vanggy, L. R., Sufiyanti, N., Kusuma, W. D. 2020. Isolasi, Karakterisasi dan Aktivitas Antibakteri BAL Indigenous dari Sarang Lebah *Trigona spp.* Asal Kabupaten Sumbawa. Biotropika: Journal of Tropical Biology. 8(1): 13–18.

Nur, A., Noor, A., Sirajuddin, S. 2019. Aktivitas Antibakteri Madu Trigona terhadap Bakteri Gram Positif (Stapylococcus Aures) dan Bakteri Gram Negatif (Escherichia Coli). Jurnal Kesehatan. 12(1): 134–140.

Okoye, N.N., Ajaghaku, D.L., Okeke, H.N., Ilodigwe, E.E., Nworu. C.S., Okeye, F.B.C. 2014. Beta-Amyrin and Alpha-Amyrin acetate Isolated from the Stem Bark of Alstonia Boonei Display Profound Anti-Inflammatory Activity. Pharmaceutical Biology. 52(11): 1478-1486.

Pratama, M., Muflihunna, A., Octaviani, N. 2018. Analisis Aktivitas Antioksidan Sediaan Propolis yang Beredar di Kota Makassar dengan Metode FRAP (Ferric Reducing

Antioxidant Power). As-Syifaa. 10(01): 11–18.

Renita, Y., Sutarningsih, E., Santoso, H. B., Hendarto, K. A., Riendrasari, S. D. 2015. Daya Antimikrobia Sarang Lebah Madu *Trigona spp* terhadap Mikrobia Patogen. Bioedukasi: Jurnal Pendidikan Biologi. 8(1): 67–72.

Rismawati, S. N., Ismiyati. 2017. Pengaruh Variasi Ph terhadap Kadar Flavonoid pada Ekstraksi Propolis dan Karakteristiknya sebagai Antimikroba. Jurnal Konversi. 6(2): 89–94.

Salampe, M., Kabo, P., Djabir, Y. Y. 2018. Pengaruh Madu Trigona terhadap Stress Oksidatif padda Tikus Putih (Rattus norvegicus) yang Diinduksi Statin untuk Mencegah Miotoksisitas. Majalah Farmasi dan Farmakologi. 22(2): 35–39.

Santos, F.A., Frota, J.T., Arruda, B.R., de Melo, T.S., Silva, A.A.D.C.A.D., Britto, G.A.D.C.B., Chaves, M.H., Rao, V.S. 2012. Antihyperglycemic and Hypolipidemic Effects of α, β-Amyrin, A Triterpenoid Mixture From Protium Heptaphyllum in Mice. 11(98): 1-8.

Sativa, N., Agustin, R. 2018. Analisis Uji Kadar Senyawa dan Uji Antioksidan Ekstrak Propolis Coklat Dari Lebah *Trigona sp*. JAGROS. 2(2): 61-68.

Thamrin, A., Erwin, Syafrizal. 2016. Uji Fitokimia, Toksisitas serta Antioksidan Ekstrak Propolis Pembungkus Madu Lebah *Trigona Incisa* dengan Metode 2,2-diphenyl-1-picrylhidrazyl (DPPH). Jurnal Kimia Mulawarman. 14(1): 54–60.

Yarlina, V.P., Sumanti, D.M., Sofiah, B., Mahani. 2020. Kajian Konsentrasi Etanol, Metode Ekstraksi Propolis Dan Karakteristik Ekstrak Propolis Lebah *Trigona Sp.* Terhadap Aktivitas Antimikroba *Escherichia Coli.* Jurnal Teknologi & Industri Hasil Pertanian. 25(1): 27-34.