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Propolis Bioactive Compounds of Stingless Bees (*Tetragonula laeviceps*) from Mount Merapi Slope, Sleman, Yogyakarta

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Abstract. The emergence of many new diseases due to viruses and bacteria demands for an increase in the discovery of natural alternative medicines. One example of sources of natural medicine is the honey and the propolis of *Tetragonula laeviceps* bee. The efficacy, physical and chemical properties of honey are known to be influenced by the type of pollen and the environment whilst the composition of propolis is influenced by the geological factors. Therefore, the purpose of this research was to study the composition of propolis' bioactive compounds of T.*laeviceps* bee from Mount Merapi Slope, Sleman, Yogyakarta. The bioactive compound composition of the propolis sample were analysed by Gas Chromatograph-Mass Spectrometry (GC-MS). The analysis showed that the propolis has various type of compounds containing up to 20 bioactive compounds which can be classified in three different groups namely alkaloids (70%), phenolics (20%), and terpenoids (5%). Most of these compounds were found to be biologically important. However, further research is still required to confirm the potential antimicrobial properties of the propolis' bioactive compounds.

Introduction

Many new diseases caused by viruses and bacteria generally attack the human immune system. A solution to prevent this is to take an alternative medicine to strengthen the body immunity. Indonesia has many potential of natural resources for medicinal products, such as the beekeeping products. *Tetragonula laeviceps* is classified as a stingless bee that is suitable for tropical areas. *T. laeviceps* produces many products such as honey, bee pollen, propolis, royal jelly, and beeswax [1].

Propolis is a complex resin compound produced by bees using the plant exudates. Propolis has a function to strengthen the structural stability of the hive. The composition of the chemical compounds in propolis can be efficacious as antibacterial, antiviral, and antitumor. The different characteristics of propolis are due to the differences in the composition of chemical compounds. The geological factors such as the place and the type of the plant-producing resin are the main factors that cause these differences in the composition of chemical compounds in propolis [2].

The area of Mount Merapi Slope, Sleman, Yogyakarta has a distinctive topography, and its vegetation is dominated by *Salacca* plantations. Therefore, this research was aimed to explore the composition of bioactive compounds found in *T. laeviceps* bee propolis as a source of antimicrobial medicine from Mount Merapi Slope in Sleman, Yogyakarta.

Experimental Methods

Sample collection of raw propolis. The raw propolis of *T. laeviceps* sample was collected from their beehives in the Meliponiculture practice conducted in Mount Merapi Slope, Sleman, Yogyakarta.

Analysis of the bioactive compounds of propolis. This research was conducted at the Integrated Research and Testing Laboratory (LPPT) UGM. The extraction steps were generally done using the maceration method followed by an analysis using the Gas Chromatograph-Mass Spectrometry (GC-MS) method [3]. First, the sample was heated in distilled water to remove the remaining honey. Next, the sample was cut into small pieces and left to air-dry. The dried sample was further grounded into smaller pieces and weighed to about 0.5 g. Then, it was mixed with MeOH solution with a ratio of 1:1 followed by a centrifugation at 900 rpm at room temperature for 5 min. About 3 µL of the solution was then injected into Gas Chromatography (Shimadzu GCMS-QP2010S) set with an initial temperature of 50°C with a final temperature of 240°C. The type of column used was the Agilent HP-5MS UI and the type of detector used was the FID set at 300 °C temperature using helium gas as the carrier gas.

Data Analysis, the resulting spectra were then compared with databases in WILEY229.LIB and NIST62 Mass spectra to identify the possible compounds contained in the sample.

Results and Discussion

Stinggless Bee Species. *T. laeviceps* is one of many stingless bee species that is widely cultivated in most tropical regions including Mount Merapi Slope, Sleman, Yogyakarta. Through their foraging activities, *T. laeviceps* produces honey, bee pollen, and propolis which are high in nutritional values and beneficial for human health. One apparent morphological characterization of their worker bee is its generally glossy black body colour (Fig. 1).

Fig. 1. The Worker Stingless bee (*T. laeviceps*)

The body of the worker bee of *T. laeviceps* is predominantly shiny black. The abdomen is blackish brown. Head (caput) is black, and is sparsely covered with white fine hairs. Compound eyes are reddish, ocelli are blackish and large. Clypeus is blackhead and fully covered with fine white hairs. Antennal has 11 flagellomeres, scape is yellowish-brown, socket is grey, pedicel brown, first flagellomere is brown, second to tenth flagellomeres are brown and slightly black. Mandible has two teeth, brown and slightly black at basal. Mesoscutum is black and is fully covered with yellowish setae, scutellum is black entirely, and is covered with yellowish setae at posterior. Tegula is brown and forewings coloration uniform. Hindwings are semi-transparent and wing venation is dark brown. Hind tibiae is short, corbicula is pear shape sparsely is covered with long setae at apical but short at basal, entirely black and basitarsi is wholly black. First to sixth gastral tergites are fully brown and dark brown at dorsal part [4].

Bioactive compounds of propolis. In this study, the chromatogram of the sample in the form of GC-MS spectra were analyzed and compared with the databases in WILEY229.LIB and NIST62 to identify the possible compounds contained in the sample.

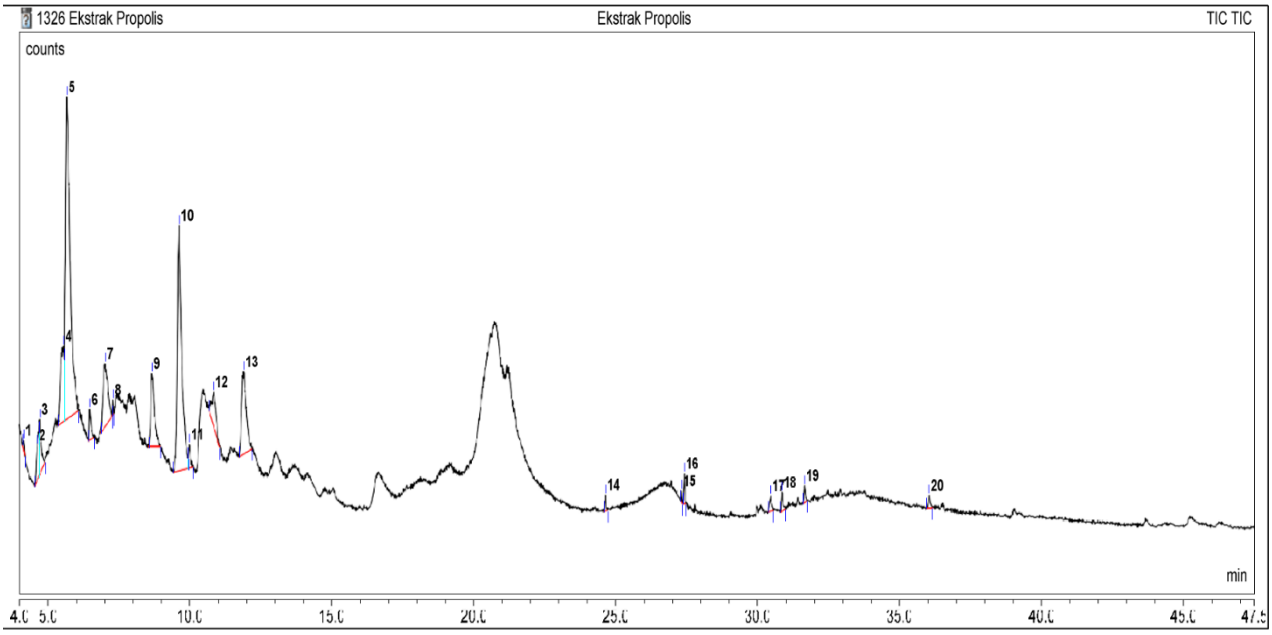

Fig. 2. Chromatogram Stingless bee's propolis from Mount Merapi Slope, Sleman

Fig. 2 showed that there are 60 peaks detected in the chromatogram profile of the propolis sample. The compounds that represent each peak were listed in Table 1.

Table 1. Bioactive compounds in Stingless bee's propolis from Mount Merapi Slope, Sleman

Number.	Compound	Chemical Formula	Area (%)	Description
Group: **Alkaloids** (70%)				
1	(*R*)-Oxiran-2-ylmethanol	$C_3H_6O_2$	0,19	Volatile compounds
2	Isobutane	C_4H_{10}	1,92	Cosmetic raw materials
3	(2*R*,3*R*)-2,3-dimethyloxirane	C_4H_8O	2,89	Anti inflammatory, anticancer
4	Ethanimidic acid, ethyl ester	C_4H_9NO	5,92	Antioxidant, anticancer
5	**α-Fluoro-ß-alanine (3-amino-2-fluoropropanoic acid)**	**$C_3H_6FNO_2$**	**33,55**	Antifungi
6	Glycidol	$C_3H_6O_2$	1,20	*In vivo* induction, genotoxicity, and carcinogenicity
7	Carbamic acid, methylnitroso-,ethyl ester	$C_4H_8N_2O_3$	6,89	Aromatic compound
8	Methyl 3,6 anhydrohexopyranoside	$C_7H_{12}O_5$	0,25	Antioxidant
9	1,2,3-Butanetriol	$C_4H_{10}O_3$	6,27	Food substitute for oils and fats
10	**[(2*S*)-oxiran-2-yl]methanol**	**$C_3H_6O_2$**	**24,07**	Volatile compounds
11	Tetrahydrofuran-3,4-diol	$C_4H_8O_3$	0,97	Antioxidant
12	Tetrahydrofuran-3,4-diol	$C_4H_8O_3$	3,28	Antioxidant
13	**Ethanethioic S-acid**	**C_2H_4OS**	**8,79**	Fine chemical raw materials
14	2,3-Epoxyhexanol	$C_6H_{12}O_2$	0,44	Antibacterial, Antioxidant
15	2,3-Epoxyhexanol	$C_6H_{12}O_2$	0,51	Antibacterial, Antioxidant
Group: **Phenolics** (20%)				
16	Hydroperoxide, heptyl	$C_7H_{16}O_2$	0,22	Antioxidant
17	Hydroperoxide, heptyl	$C_7H_{16}O_2$	0,68	Antioxidant
18	Hydroperoxide, heptyl	$C_7H_{16}O_2$	0,68	Antioxidant
19	Cyclopenta[c]furo[3',2':4,5]furo[2,3-h][1]benzopyran-11(1*H*)-one	$C_{17}H_{14}O_7$	0,65	Antioxidant
Group: **Terpenoids** (5%)				
20	3-Trifluoroacetoxydodecane	$C_{14}H_{25}F_3O_2$	0,64	Antiviral

Based on the data in Table 1, there are 20 different bioactive compounds identified from the propolis sample. The profile of the propolis' bioactive compounds shows the highly active foraging activity of *T. laeviceps* which produces propolis dominated by a group of bioactive alkaloids compounds (70%) with alpha-Fluoro-ß-alanine (3-amino-2-fluoropropanoic acid) ($C_3H_6FNO_2$) (33,55%) as the largest percentage within the group. This specific compound includes the organo-oxygen compounds and the organo-nitrogen compounds derived from ß-amino acids and belongs to an alkaloid group that functions as a substrate for bile acid conjugate enzymes in cancer patients and has antifungal activity. The 2nd largest percentage is the [(2*S*)-oxiran-2-yl] methanol ($C_3H_6O_2$) (24,07%). This compound belongs to an alkaloid group that functions as an ingredient in making cosmetics. The next specific important compound is ethanethioic *S*-acid (C_2H_4OS) (8,79%). This compound is a combination of anhydride and H_2S with clear yellow coloured liquid, a strong odour, and toxicity effect if swallowed, inhaled, and absorbed by the skin. This compound includes organosulfur compounds which are generally used as chemical raw materials for synthetic drugs [5]. The reactivity of ethanethioic *S*-acid is generally in selective amide or peptide bonds synthesized by the reaction between active carboxylic acids, such as hydrochloric acid, or esters with Na_2S, H_2S, or NaSH [6].

The GC-MS analysis (Table 1) showed that propolis' chromatogram has various types of compounds consisting of up to 20 bioactive compounds which can be classified in 3 (three) different groups namely alkaloids (70%), phenolics (20%), and terpenoids (5%). The high number of alkaloid-producing plants may indicate a need for chemical protection against herbivores under the harsh conditions of the mountain environment [7]. Meanwhile, the high portion of phenolic compounds could be due to the large constituent of chemicals found in plants that are natural antioxidants and the phenolic compounds are secondary metabolites produced and accumulated by plants during their defence mechanism due to stress [8]. The phenolic compounds with high bioactive properties have the ability to develop into functional foods, nutraceuticals and drugs due to the presence of bioactive compounds can possess anti-microbial, anti-cancer, antioxidant, chemoprotective, anti-cholesterol, and immunomodulatory properties. [9,10].

In general, propolis contains alkaloids, phenolics, terpenoids and their esters, sugars, alcohols, ketones, hydrocarbons, amino acids,vitamins and minerals [11]. Nearly 300 chemical compounds have been identified. These compounds are not necessarily present in all propolis, because their presence depends mainly on the botanical and geographical origin, the species of bee and the harvest season, as well as of various environmental factors [12,13].

Summary

The propolis' bioactive compounds of *T. laeviceps* bees from the meliponiculture practice in Mount Merapi Slope, Sleman, Yogyakarta consists of three groups which are alkaloids (70%), flavonoids (20%), and terpenoids (5%) with alpha-Fluoro-ß-alanine or 3-amino-2-fluoropropanoic acid (33,55%) as the most dominant compound in the alkaloid group. Further research is still required to explore the potential bioactivity in various medicinal treatments as well as its antimicrobial activity.

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References

[1] S.N.A.S. Salleh, W.L.W. Johari, N.A.M. Hanapiah, A comprehensive review on chemical compounds, biological actions and potential health benefits of stingless bee propolis, Sains Malays. 51 (2022) 733-745.

[2] A. Sulaeman, A.M. Fikri, N. Kalsum, M. Mahani, Chapter 25 - Trigona propolis and its potency for health and healing process, in: R.B. Singh, R.R. Watson, T. Takahashi (Eds), The role of functional food security in global health, Academic Press, Cambridge, Massachusetts, United States, 2019, pp. 425-448.

[3] J.A.I. Devi, A.K. Muthu, Gas chromatography mass spectrometry analysis of bioactive constituents in the ethanolic extract of *Saccharum spontaneum* Linn., Int. J. Pharm. Pharm. Sci. 6 (2014) 1-23.

[4] M. Trianto, H. Purwanto, Morphological characteristics and morphometrics of Stingless Bees (Hymenoptera: Meliponini) in Yogyakarta Indonesia, Biodiversitas 21 (2020) 2619-2628.

[5] C. Ignea, E. Ioannou, P. Georgantea, S. Loupassaki, F.A. Trikka, A.K. Kanelis, A.M. Makris, V. Roussi, S.C. Kamprani, Reconstructing the chemical diversity of labdane-type diterpene biosynthesis in yeast, Metab. Eng. 28 (2015) 91-103.

[6] M.M. Sachitanand, N.G. Hosahudya, Thioacetic acid/NASH-mediated synthesis of *N*-protected amino thioacids and their utility in peptide synthesis, J. Org. Chem. 79 (2014) 2377-2383.

[7] K. Tayjanov, N.Z. Mamadalieva, M. Wink, Diversity of the mountain flora of central asia with emphasis on alkaloid-producing plants, Diversity 9 (2017) 11.

[8] A.T. Getachew, C. Jacobsen, S.L. Holdt, Emerging technologies for the extraction of marine phenolics: opportunities and challenges, Mar. Drugs. 18(8) (2020) 389.

[9] L. Paiva, E. Lima, A.I. Neto, M. Marcone,J. Baptista, Nutritional and functional bioactivity value of selected azorean macroalgae: *Ulva Compressa*, *Ulva Rigida*, *Gelidium Microdon*, and *Pterocladiella Capillacea*, J. Food Sci. 82 (2017) 1757-1764.

[10] J. Cotas, D. Pacheco, A.M. Gonçalves, P. Silva, L.G. Carvalho, L. Pereira, Seaweeds' nutraceutical and biomedical potential in cancer therapy: a concise review, J. Cancer Metastasis Treat. 7 (2021) 13.

[11] S.I. Anjum, A. Ullah, K.A. Khan, M. Attaullah, H. Khan, H. Ali, M.A. Bashir, M. Tahir, M.J. Ansari, H.A. Ghramh, Composition and functional properties of propolis (bee glue): A review, Saudi J. Biol. Sci. 7 (2018) 1695–1703.

[12] T.G. do Nascimento, R.E. dos Santos Arruda, E.T. da Cruz Almeida, J.M. dos Santos Oliveira, I.D. Basílio-Júnior, I.C.C. de MoraesPorto, A.R. Rodriges Sabino, J. Tonholo, A. Gray, R.E. Edrada Ebel, Comprehensive multivariate correlations between climatic effect, metabolite-profile, antioxidant capacity and antibacterial activity of Brazilian red propolis metabolitesduring seasonal study, Sci. Rep. 9 (2019) 18293.

[13] S. Gardini, D. Bertelli, L. Marchetti, R. Graziosi, D. Pinetti, M. Plessi, G.L. Marcazzan, Chemical composition of Italian propolisof different ecoregional origin, J. Apic. Res. 57 (2018) 639-647.