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学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

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学位論文題目：
Title of Dissertation

Chemical Constituents, Botanical Origin and Biological Activity of Propolis of *Tetragonula sapiens*, a Stingless Bee, in Southeast Sulawesi, Indonesia
（インドネシア・南東スラウェシ州におけるハリナシミツバチ, *Tetragonula sapiens* のプロポリスの化学成分，基原植物および生物活性）

学位論文要約：
Dissertation Summary

Indonesia, as a country with vast tropical rainforests, has a high biodiversity of various plant species. Many of these plant species have been used as traditional medicine for a long time because they have pharmacological effects (Yanuar et al., 2011). It is estimated that 28,000 plant species exist in the Indonesian forest. Of these, 7,000 species are medicinal plants, which is equal to 90% of medicinal plants in Asia. So far, 1000 species have been known and utilized in traditional medicine (Kusuma et al., 2014).

Several studies have proven that some plant species from Indonesian forests have extractives which contain bioactive compounds that act as antioxidants (Falah et al., 2008; Lelono and Tachibana, 2013; Andrianto et al., 2014), antimicrobial (Kusuma et al., 2014), anti-HIV virus (Wahyuni et al., 2013) and antidiabetic (Andrianto et al., 2014; Suzuki et al, 2017).

Bioactive components may come from various parts of plants such as leaves, stems, roots, fruits, seeds, bark and sap. Some of which have been reported as follows, the new 29-norcucurbitacin was isolated from the seeds of an Indonesian medicinal plant, *Phaleria macrocarpa* (Kurnia et al., 2008), phenylethanoid glycoside from *Gmelina arborea* bark, and Swietenia macrophyllanin from *Swietenia macrophylla* bark (Falah et al., 2008; Falah et al., 2009).

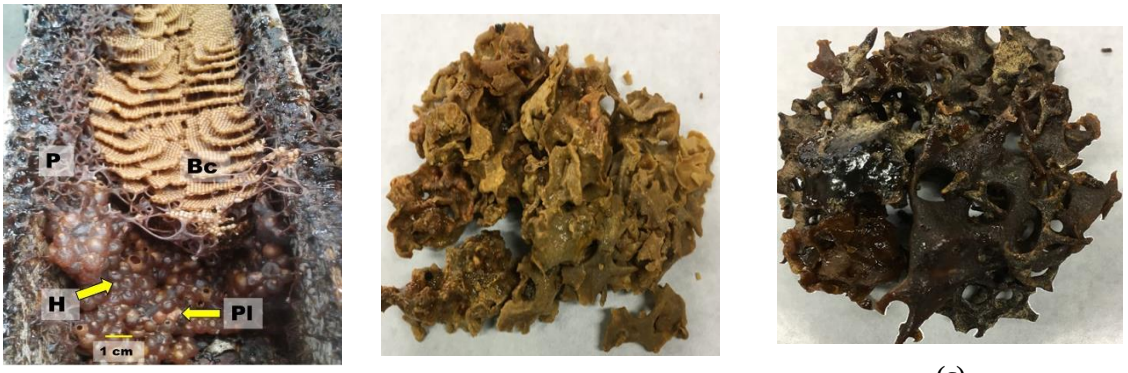
Natural bioactive compounds are not only used by humans as a drug, but also used by animals to protect themselves and their nests. Honeybees are one of the insects that use resin and bud exudates of plants to build and defend a nest from intruders from other animals and microbes. Resin and bud exudates are collected by bees and then mixed with saliva enzymes to convert into material that is now known as propolis.

Propolis is one of the non-timber forest products produced by honeybees. Propolis is a sticky natural substance consists of a mixture of beeswax, gums and bud exudates from some plants gathered by the bees. They collect resin from the crevices of bark and bud exudates, chewed them and then added saliva enzymes and partly digested and mixed with beeswax (Ghisalberti, 1979, Marcucci, 1995, Burdock, 1998). For the bees, propolis is used to build a nest, cover the gap between the nest, prevent animal intruders (Burdock, 1998).

Bee propolis color varies depending on the type and source of plant resin. It can be yellow, brown, red, red-brown or dark brown, green, and aromatic smell. Propolis is an interesting substance; it becomes hard when cold and brittle when frozen, but soft, chewy, and very sticky when warm so it is also called bee glue (Brown, 1989). Some of the chemicals found in propolis are wax, resin, balsam, aromatic oils, pollen and other organic materials. The components in raw propolis come from three sources, namely plant resins collected by bees, substances released from the metabolism of bees and materials developed for the collection of propolis (Ghisalberti, 1979; Marcucci, 1995).

Stingless bees are a group of small-to-medium sized bees with a vestigial (non-functional) sting, and they are found in abundance in warm and humid forests around the world. They are essential for pollinators within

tropical ecosystems (tropical and subtropical forests), (Rasmussen and Cameron, 2009). Stingless bees belong to the family Apidae and tribe Meliponini. The classification of stingless bees has been reported by several researchers. This species has a significant variation in the architecture of the hive. The design of the eggs and pots of storage of honey and pollen are arranging in a horizontal arrangement or cluster (Figure 1). The nests built in the crevices of tree trunks or bamboo that are dead or alive, attach to the branches and twigs of trees, ground, brick walls and active nests of termites, ants or wasps, or traditional hives (Roubik, 2006; Rasmussen and Camargo, 2008).



(a) (b) (c)

Figure 1. (a): *T. sapiens* nest, honey pots (H), pollen pot (Pl), brood cells (Bc) and propolis (P); (b) propolis from South Konawe (P1) and (c) propolis from Kendari (P2) (Pujirahayu et al., 2019[a]).

In Sulawesi Island especially Central Sulawesi several genera of stingless bees were found, namely the genera Geniotrigona (*Trigona incisa* and *Trigona thoracica*) (Sakagami and Inoue, 1989), *Tetragonula fuscobalteata, T. laeviceps, Tetragonula pagdeni* (Schwarz), *Tetragonula fuscobalteata* (Cameron, Lepididata) (Smith), *Geniotrigona incisa* (Sakagami & Inoue) (Rasmussen and Cameron, 2009) and a recent report also found *Tetragonula sapiens* (Cockerell), and *T. biroi* (Suriawanto et al., 2017). But the reports of the stingless bee species from other parts of Sulawesi Island such as South Sulawesi, North Sulawesi, and Southeast Sulawesi are still limited.

Components and properties of propolis depend on the plant source used by bees (Popova et al., 2012). In Europe, North America and non-tropical regions of Asia, the main source of propolis is the resinous exudates of the buds of poplar trees (*Populus nigra, P. alba, P. tremuloides*). In tropical and sub-tropical Asia, *Macaranga tanarius* was reported as a source of propolis from Okinawa, Japan. *Macaranga tanarius* and *Mangifera indica*. are shown as plant sources of Indonesian Apis bee propolis (East Java) (Trusheva et al., 2011) and *Hevea brasiliansis* and *Ceiba pentandra* are plant sources of Apis bee propolis from Batang (Central Java), Lawang (East Java) and Sukabumi (West Java) (Syamsuddin et al., 2009).

Stingless bees produce propolis by collecting a variety of resin of some plants that are around a beehive. In addition to collecting resin, bees sometimes also mix it with bud exudates of particular plant species. Plants used as a source of production of bee propolis varies depending on the type of vegetation around the beehive. Several studies show there is an enormous variation in the chemical composition of propolis, especially propolis from tropical regions (Piccinelli et al., 2011).

Not all plants which have resin are used by a stingless bee to form propolis. Bees have a preference to choose one, or several types of resins that are around the nest and are also the source of the phytochemical component (Isidorov et al., 2014). Based on these, propolis can be characterized according to their plant origin and its main chemical constituents.

Therapeutic properties of propolis are often associated with the presence of polyphenols. However, significant heterogeneity has been found in their chemical composition. As a result, various components, besides phenolic compounds, can have attributed to their biological activity. Comparative studies have revealed that although their chemical composition is different, propolis always shows the presence of biological activity. For this reason, the diversity of propolis chemicals has the potential to provide valuable usage direction (Pujirahayu et al., 2019[b]).

Tetragonula sapiens is one of the stingless bees that are often found around forests and community gardens in the Southeast Sulawesi region, but information about the chemical compounds, plant source and biological activity of propolis of this bees is unknown and has not been identified. For this reason, this study was conducted.

This study consisted of three stages. The objectives of the first stage were to clarify the chemical constituents of *T. sapiens* propolis from two Southeast Sulawesi regions [South Konawe District(P1) and Kendari District (P2)]. Extraction, fractionation, and isolation of the main compounds of propolis are also carried out. The second stage was carried out to find out the botanical origin of propolis by extracting several resin plants that are often visited by bees *T. sapiens*. The third stage is to find antioxidant activity and α-glucosidase inhibitory activity from the isolated compounds, and to study the inhibitory mechanism and the relationship between the activity and their molecular structures.

In chapter 2, the main component of the propolis was found to be cycloartane-triterpenes [mangiferolic acid (**1**), cycloartenol (**2**), ambonic acid (**3**), mangiferonic acid (**4**), and ambolic acid (**5**)] (Figure 2). The constituent of this propolis is very similar to the components of Cameroonian (*A. mellifera*) propolis. All cycloartane-type triterpenes in this propolis were also found in the Cameroonian propolis. Some propolis from other tropical regions were also reported to contain cycloartane-type triterpenes. Myanmar (*A. mellifera*) propolis and Vietnam Trigona propolis are rich in cycloartane-triterpene compounds, both of which contain mangiferonic acid, 27-hydroxymangiferonic acid, and mangiferolic acid as the main constituents. Malaysian propolis (*Trigona itama*) has cycloartenol. Brazilian propolis contains cycloartane-type triterpenes: mangiferolic acid, 3β-hydroxy-24-methylenecycloartane-26-oic acid, ambonic acid and ambolic acid. Thailand propolis contains dammarane-type triterpene: dipterocarpol, ursolic acid, and ocotillone (Pujirahayu et al., 2019[a]).

Propolis at P1 and P2 showed different major compounds. Mangiferolic acid (**1**) and cycloartenol (**2**) are the major compounds in propolis from P1 (South Konawe); more than 80% of the ether fraction of this propolis is dominated by these two compounds. Ambonic acid (**3**), mangiferonic acid (**4**), and ambolic acid (**5**), meanwhile, are that are mostly present in propolis from the Kendari district. However, the five isolated compounds are cycloartane-type triterpenes. This difference may be due to the behavior of bees in collecting resin in a colony, the number of worker bees, plant species, and also the difference in the part and amount of resin taken.

These results also indicate the presence of bee preference traits for plant and resin species available around the nest. Even worker bees are "given" the ability to recognize and select the compounds/types of metabolites their colony must collect. This is how bees use secondary metabolites from plants to build nests as well as to protect nests and colonies from predators and diseases. The results of this study confirm that there are similarities in the types/classes of compounds collected by bees, in this study that are cycloartane-type triterpenes. The results of the comparison of HPLC chromatograms between EEP and EEM showed that the characteristic peaks of both EEP and EEM have the same pattern (Figure 3) which means that the main compounds of *T. sapiens* bee propolis in Southeast Sulawesi was also found in *M. indica* resin (Pujirahayu et al, 2019[a]).

In Chapter 3, several experiments were conducted to determine the inhibition activity of isolated compounds on α-glucosidase from *Saccharomyces cerevisiae* and the rat small intestine. The total phenolic content and antioxidant activity of the isolated compounds by the scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals, were also tested to determine the relationship of antioxidant activity and inhibiting properties of isolated compounds from *T. sapiens* propolis against α-glucosidase. Their inhibitory mechanisms on α-glucosidase and

their structure-activity relationships were also investigated.

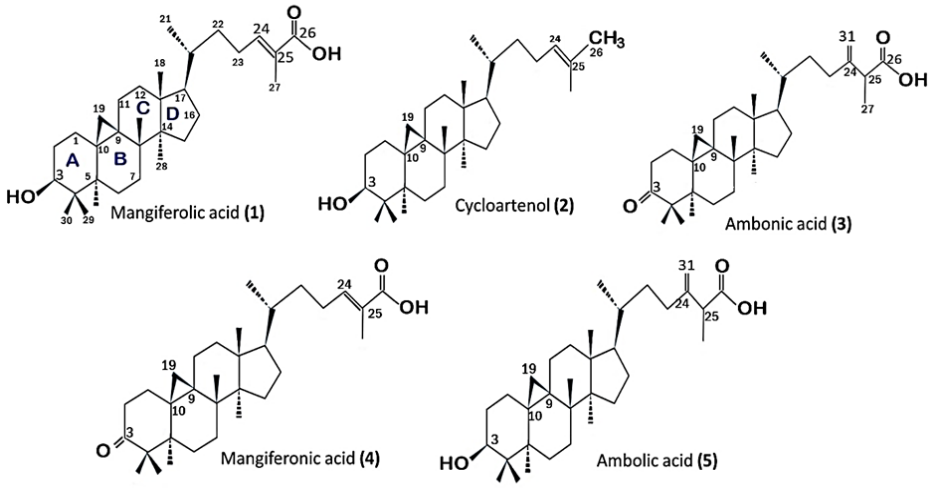


Figure 2. Structure of cycloartane-type triterpenes isolated from *T. sapiens* bee propolis in Southeast Sulawesi. (Pujirahayu et al, 2019[b]).

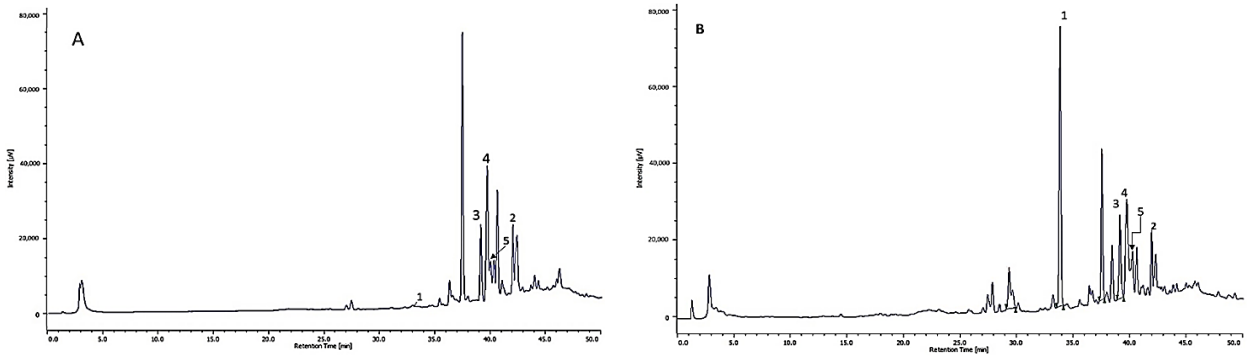


Figure 3. HPLC chromatogram: (A) Ethanol Extract (EE) of *M. indica*, (B) EE of propolis (EEP) of *T. sapiens* P1(South Konawe Propolis) (Pujirahayu et al, 2019[a])

The results in chapter 3 show that propolis from two collection locations (Konawe Selatan and Kendari) had phenolic content with a range between 13.9 and 37.2 μg gallic acid equivalent/mg samples. The ethyl acetate fraction of the propolis from Kendari district (P2) has a higher total phenolic content of 37.2 μg /mg compared to the phenolic content of the other fractions from both P1 and P2, while the lowest content is in the aqueous fraction from P2. The antioxidant activity results showed that mangiferolic acid (**1**) and mangiferonic acid (**4**) had the highest IC_{50} values of 27.11 ± 6.57 μM and 37.74 ± 6.55 μM, respectively compared to Trolox 28.62 ± 0.52 μM. Five cycloartane-type triterpenes, namely mangiferolic acid (**1**), cycloartenol (**2**), ambonic acid (**3**), mangiferonic acid (**4**), and ambolic acid (**5**), isolated from *T. sapiens* propolis were shown to have high inhibition activity against α-glucosidase from *Saccharomyces cerevisiae*, with an IC_{50} range between 2.46 and 10.72 μM. Among the isolated compounds, mangiferonic acid was the strongest α-glucosidase inhibitor followed by ambonic acid with IC_{50} 2.46 ± 0.7 μM and 3.01 ± 1.26 μM, respectively compared to a positive control (−)-epicatechin (1991.1 μM) (Table 1). The IC_{50} of (−)-epicatechin was similar to the previously reported value of 510 μg/mL (Suzuki et al., 2017).

The biological properties of propolis are often associated with the presence of polyphenols and flavonoids. However, it has been reported that propolis has a large heterogeneity in its chemical composition. This means that various components, other than phenolic compounds or flavonoids, can provide biological properties. Several studies have reported that the biological properties of propolis associated with diterpene and triterpenes as

compounds that are responsible for their pharmacological properties (Pujirahayu et al., 2019[b]). In this study, cycloartane-type triterpenes were the main compounds of *T. sapiens* propolis collected from two different regions in Southeast Sulawesi. The results of antioxidant activity showed that the five isolated compounds had moderate - high antioxidant activity. Mangiferolic acid (**1**) and Mangiferonic acid (**4**) have antioxidant activity greater than the original ether-soluble fractions. Therefore it can be assumed that both of these compounds are responsible for antioxidant activity in propolis from P1 and P2. The ideal anti-diabetic compound must show α-glucosidase inhibiting activity and antioxidant properties (Shibano et al., 2008; Dewi et al., 2014). Two of the isolated compounds, namely mangiferonic acid (**4**) and mangiferolic acid (**1**), have strong α-glucose inhibitory activity and antioxidant activity, while ambonic acid (**3**) shows strong inhibiting activity against α-glucosidase and moderate antioxidants activity. These results indicate that the cycloartane-type triterpenes isolated from *T. sapiens* propolis have high potential as an anti-diabetic compound.

According to the results of the enzyme kinetics study, the inhibition types of the isolated compounds were uncompetitive inhibition [mangiferolic acid (**1**) and cycloartenol (**2**)] and mixed inhibition (ambonic acid, mangiferonic acid and ambolic acid) (Figure 4a). The kinetic analysis of compounds **1** and **2** showed that both V_{max} and Km decreased in the presence of an increasing concentration of inhibitors, with Ki values of **1** and **2** being 6.04 and 10.27 μM, respectively. Uncompetitive inhibitors do not bind to the free enzyme site but only to an enzyme–substrate complex. When the inhibitor concentration increases, more enzymes will be converted into unproductive enzyme–substrate inhibitors (E.S.I). Thus, both the Km and the V_{max} values are decreased by the same amount; this is the exact opposite of the competitive case (Ramsay and Tipton, 2017; Pujirahayu et al., 2019[b]).

Table 1. IC_{50} values and inhibition mode of the cycloartane-type triterpenes isolated from Indonesian *T. sapiens* propolis against α-glucosidases.

Compounds	Yield (mg)	IC_{50}		Inhibition Mode
		S. cerevisiae (μM)[a]	Rat Small Intestine (μM)[a]	
Mangiferolic acid (**1**)	20.6	5.52 ± 0.04	ND	Uncompetitive
Cycloartenol (**2**)	5.9	10.72 ± 0.28	ND	Uncompetitive
Ambonic acid (**3**)	42.4	3.01 ± 1.26	ND	Mixed inhibition
Mangiferonic acid (**4**)	68.5	2.46 ± 0.70	ND	Mixed inhibition
Ambolic acid (**5**)	24.8	4.31 ± 0.04	ND	Mixed inhibition
(–)-Epicatechin[c]		1991.1 ± 89.9	ND	
Acarbose[c]		ND [b]	208.95 ± 0.96	
Voglibose[c]		ND	78.57 ± 1.27	

[a] The values are the means ± SEs, $n = 3$; [b] ND: not detected; [c] positive control.
(Pujirahayu et al, 2019[b])

As illustrated in Figure 4b, compound **4** showed the mixed-type inhibition against α-glucosidase. In addition to compound **4**, compounds **3** and **5** also show mixed inhibition types. A compound will behave as a mixture of a competitive and a non-competitive inhibitor, or will show mixed inhibition, when it can bind both to the free sites of enzymes (competitive) and the enzyme–substrate sites (uncompetitive) (Ramsay and Tipton, 2017), which are indicated by increasing Km values and decreased V_{max} values. Furthermore, the values of inhibiting constants (Ki and Ki') of compounds **3**, **4**, and **5** showed that they have a higher affinity for free enzymes than for enzyme–substrate, as the value of Ki was smaller than Ki' in compound **3** (Ki = 2.11 μM and Ki'= 6.42 μM), compound **4** (Ki = 0.58 μM and Ki' = 3.23 μM), and compound **5** (Ki = 1.71 μM and Ki' = 4.58 μM). For mixed inhibition, the inhibitor would be still effective at lower concentrations compared to a competitive inhibitor like

acarbose. It was reported that in competitive inhibitors such as acarbose, higher inhibitor concentrations are needed for higher carbohydrate food intake to show the same inhibitory effect (Pujirahayu et al., 2019[b]).

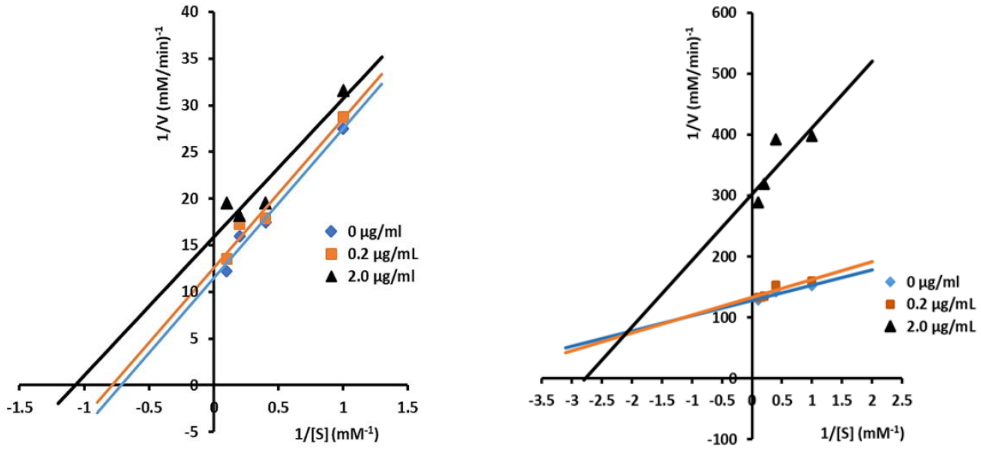


Figure 4. Lineweaver–Burk plots for kinetic analysis of α-glucosidase inhibition by isolated triterpenes: (a) compound **1**, (b) and compound **4**, at varying concentrations (showing uncompetitive and mixed inhibition). The concentration of p-NPG was measured as a substrate in the absence or presence of inhibitors at different concentrations against α-glucosidase. All values are means ± standard errors (n = 3) (Pujirahayu et al, 2019[b]).

The comparison of the structure and the inhibitory activity against α-glucosidase between the five isolated compounds showed that the functional groups of cycloartane-type triterpenes had significant impacts on the inhibition (Figures 5a and b). The ketone group at C-3 and the carboxyl group at C-26 are important to enhance the activity. The IC_{50} of ambonic acid (**3**) is almost same as that of **4**. The substituents at C-3 are important components of the cycloartane skeleton that can strengthen the inhibitory activity of α-glucosidase in the order C=O > β-OH. The substituents at C-26 are also important components that can strengthen it in the order of –COOH > $–CH_3$. Cycloartenol (**2**) has the lowest IC_{50}, where the carboxyl group on C-26 is replaced by a methyl group (Figures 5a and b). If a substituent in C-3 replaces =O with β-OH, the activity will decrease by almost 2.3-fold, (from 2.46 (compound **4**) to 5.52 µM (compound **1**), but if the double bond in C-24–C-25 shifts to C-31, the activity will decreases by 0.3-fold (from 2.46 (compound **4**) to 3.01 µM (compound **3**) (Figure 5 and Table 1)).

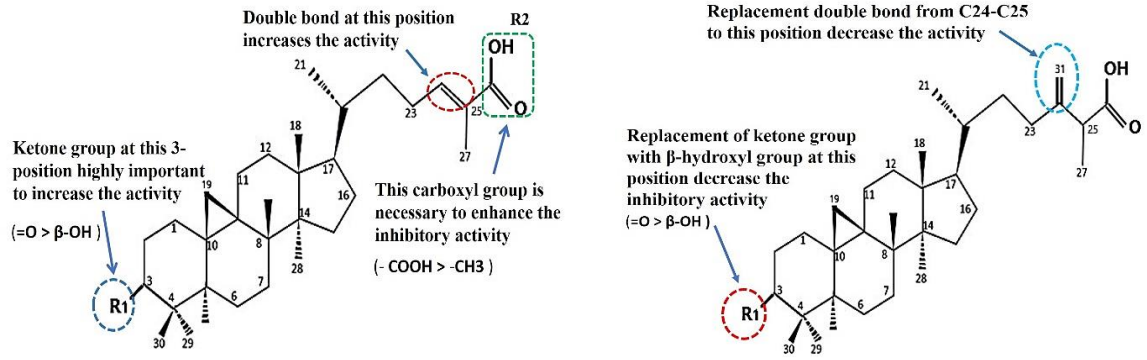


Figure 5. Structure–activity relationship for α-glucosidase inhibition of cycloartane-type triterpenes **1**, **2**, **4** (a) and **3**, **5** (b) from *T. sapiens* propolis (Pujirahayu et al, 2019[b]).

It can conclude that the inhibitory activity of cycloartane-type triterpenes against α-glucosidase depends on the substituents located on C3, the double bonds on C24–C25, and a carboxyl group on C26. This is the first report on inhibitory activity of cycloartane-type triterpenes isolated from propolis against α-glucosidase. This study provides an approach to consider the structural requirements of cycloartane-type triterpenes from propolis as α-glucosidase inhibitors. An understanding of these requirements is needed to be able to find, improve, or design new types of cycloartane-type α-glucosidase inhibitors from triterpene to help in the treatment of diabetes. The isolated compounds also have a moderate-high antioxidant activity where antioxidant activity can help accelerate healing and prevent complications in diabetics, which makes these compounds have ideal potential as an anti-diabetes compound.

In order to reveal more characteristics and study the mechanism of action, future reseach is needed. Information about the inhibitory activity of derivatives of isolated cycloartane-type triterpenes on α-glucosidase is also needed, in order to design a more effective type of an α-glucosidase inhibitor in the future. Clinical studies on animal model and human also need to be done to get more information about the inhibitory activity of isolated compounds on α-glucosidase. Although these five compounds are determined as the main components of *T. sapiens* propolis, still much research needs to be done to find out the other components contained in this propolis. And it is also necessary to test other biological activities for this propolis and its components.

References

Andrianto, D., Nurcholis, W., Katayama, T., Suzuki, T. 2014. Antidiabetic and antioxidant activity of 70% ethanolic extract of *Graptophyllum pictum* leaves improves glucose resistance in rats. *J For. Biomass Util Soc* 9, 31–36.

Burdock, G.A., 1998. Review of the biological properties and toxicity of bee propolis (propolis). *Food Chem. Toxicol*. 36, 347–363. https://doi.org/10.1016/S0278-6915(97)00145-2.

Dewi, R.T., Tachibana, S., Darmawan, A., 2014. Effect on α-glucosidase inhibition and antioxidant activities of butyrolactone derivatives from Aspergillus terreus MC751. *Med. Chem. Res*. 23, 454–460.

Falah, S., Katayama, T., Suzuki, T. 2008. Chemical constituents from *Gmelina arborea* bark and their antioxidant activity. *J. Wood Sci*. 54, 483–489. https://doi.org/10.1007/s10086-008-0983-3

Falah, S., Katayama, T., Suzuki, T. 2009. Isolasi dan Idenftifikasi senyawa fenolik dari kulit kayu Mahoni (*Swietenia machrophylla* King) dan Gmelina (*Gmelina arborea* ROXB) dan aktivitas antioksidannya, in: Bunga Rampai Biofarmaka Kehutanan Indonesia dari Tumbuhan Hutan untuk Keunggulan Bangsa dan Negara. Puslitbang Hutan Tanaman, Litbang, Kehutanan, Kementerian Kehutanan, pp. 165–188.

Ghisalberti, E.L., 1979. Propolis: A Review. *Bee World* 60, 59–84. https://doi.org/10.1080/0005772X.1979.11097738

Isidorov, V.A., Szczepaniak, L., Bakier, S., 2014. Rapid GC/MS determination of botanical precursors of Eurasian propolis. *Food Chem*. 142, 101–106.

Kurnia, D., Aiyama, K., Hayashi, H. 2008. 29-Norcucurbitacin Derivatives Isolated from the Indonesian Medicinal Plant, *Phaleria macrocarpa* (Scheff.) Boerl. *Biosci. Biotechnol. Biochem. advpub*, 0801080707–0801080707. https://doi.org/10.1271/bbb.70627

Kusuma, I.W., Murdiyanto, Arung, E.T., Syafrizal, Kim, Y., 2014. Antimicrobial and antioxidant properties of medicinal plants used by the Bentian tribe from Indonesia. Food Sci. Hum. Wellness 3, 191–196.

Lelono, RAA and Tachibana, S. 2013. Bioassay-guided Isolation and Identification of Antioxidative Compounds from the Bark of *Eugenia polyantha*. Pak. *J. Biol. Sci.* 16, 812–818. https://doi.org/10.3923/pjbs.2013.812.818.

Marcucci, M., C., 1995. Propolis: chemical composition, biological properties and therapeutic activity. *Apidologie* 26, 83–99. https://doi.org/10.1051/apido:19950202.

Piccinelli, A.L., et al., 2011. Cuban and Brazilian Red Propolis: Botanical Origin and Comparative Analysis by HPLC–Photodiode Array Detection/Electrospray Ionization Tandem Mass Spectrometry. *J. Agric. Food*

Chem. 59, 6484–6491.

Popova, M., Trusheva, B., Cutajar, S., et al., 2012. Identification of the Plant Origin of the Botanical Biomarkers of Mediterranean type Propolis. Nat. Prod. Commun. 7, 1934578X120070050.

Pujirahayu, N., Bhattacharjya, K.D., Suzuki, T., Katayama, T., 2019[b]. α-Glucosidase Inhibitory Activity of Cycloartane-Type Triterpenes Isolated from Indonesian Stingless Bee Propolis and Their Structure–Activity Relationship. *Pharmaceuticals* 12, 3, 102. https://doi.org/10.3390/ph12030102.

Pujirahayu, N., Suzuki, T., Katayama, T., 2019[a]. Cycloartane-Type Triterpenes and Botanical Origin of Propolis of Stingless Indonesian Bee *Tetragonula sapiens*. *Plants* 8, 3 ,57. https://doi.org/10.3390/plants8030057.

Ramsay, R.R., Tipton, F.K., 2017. Assessment of Enzyme Inhibition: A Review with Examples from the Development of Monoamine Oxidase and Cholinesterase Inhibitory Drugs. *Molecules* 22, 1–46.

Rasmussen, C., Camargo, J., M.F., 2008. A molecular phylogeny and the evolution of nest architecture and behavior in Trigona s.s. (Hymenoptera: Apidae: Meliponini). *Apidologie* 39, 102–118. https://doi.org/10.1051/apido:2007051

Rasmussen, C., Cameron, S.A., 2009. Global stingless bee phylogeny supports ancient divergence, vicariance, and long distance dispersal. *Biol. J. Linn. Soc*. 99, 206–232. https://doi.org/10.1111/j.1095-8312.2009.01341.x

Roubik, D.W., 2006. Stingless bee nesting biology. *Apidologie* 37, 124–143. https://doi.org/10.1051/apido:2006026

Sakagami, S.F., Inoue, T., 1989. Stingless bees of the genus Trigona (subgen: Geniotrigona) (Hymenoptera: Apidae) with description of T. (G.) incisa sp, nov. from Sulawesi. *Jpn. J Entomol* 57, 605–620.

Shibano, M., Kakutani, K., Taniguchi, M., Yasuda, M., Baba, K., 2008. Antioxidant constituents in the dayflower (*Commelina communis* L.) and their α-glucosidase-inhibitory activity. *J. Nat. Med*. 62, 349.

Suriawanto, N., Atmowidi, T., Kahono, S., 2017. Nesting Sites Characteristics of Stingless Bees (hymenoptera: Apidae) in Central Sulawesi, Indonesia. *J Insect Biodivers* 5, 1–9.

Suzuki, T., Matsuno, F., Katayama, T., and Andrianto, D. 2017. α -Glucosidase Inhibition and DPPH free radical - Scavenging activities of underutilized fruits from Java Island, *Indonesia. J For. Biomass Util Soc* 12, 59–62.

Syamsudin, Wiryowidagdo, S., Simanjuntak, P., Heffen, W.L., 2009. Chemical composition of propolis from different regions in Java and their cytotoxic activity. *Am. J. Biochem. Biotechnol*. 5, 180–183.

Trusheva, B., Popova, M., Koendhori, E.B., Tsvetkova, I., Naydenski, C., Bankova, V., 2011. Indonesian propolis: chemical composition, biological activity and botanical origin. *Nat. Prod. Res.* 25, 606–613. https://doi.org/10.1080/14786419.2010.488235

Wahyuni, T.S., et al. 2013. Antiviral activities of Indonesian medicinal plants in the East Java region against hepatitis C virus. *Virol. J*. 10, 259.

Yanuar, A., et al. 2011. Medicinal Plants Database and Three Dimensional Structure of the Chemical Compounds from Medicinal Plants in Indonesia. Eprint ArXiv11117183. arXiv:1111.7183.

(注)　要約の文量は，学位論文の文量の約１０分の１として下さい。図表や写真を含めても構いません。

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