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学 位 論 文 要 旨
Dissertation Abstract

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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）

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This research has been conducted to explore chemical constituents, botanical origin and biological activity of propolis of *Tetragonula sapiens* Cockerell, a stingless bee in Southeast Sulawesi, Indonesia. Southeast Sulawesi is one of a region that has unique plants and animal species because it is located on the Wallacea area with the Maluku Island and islands in the Nusa Tenggara sub-region. Propolis has been known as one of the natural products that has extensive biological activity and has various benefits such as health food and beverages, medicines, cosmetics and packaging of good food (fruits, livestock products, etc). Propolis from stingless bees is collected by the bees from plant resin. Therefore, by knowing the main components of propolis, its botanical sources can be identified. Because the composition of propolis consists of more than 50% of plants resin, the nature of secondary metabolites found in propolis is also influenced by the species of plant and of course influenced by the species of bees.

This study consisted of three stages. The objective 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)]. Propolis samples were extracted with 99% ethanol to obtain an ethanol extract of propolis (EEP), which was then partitioned between diethyl ether and water. The aqueous layer was extracted with ethyl acetate. The yield of the ether fraction was the highest. Column chromatography, thin-layer chromatography, and high-performance liquid chromatography (HPLC) were used for the separation and isolation of compounds from the ether-soluble fraction. The structure of the isolated compounds was determined by nuclear magnetic resonance spectroscopy and gas chromatography–mass spectrometry. Five compounds were isolated from the EEP, and their structures were determined as mangiferolic acid, cycloartenol, ambonic acid, mangiferonic acid, and ambolic acid, which are cycloartane-type triterpenes.

The second stage was carried out to find out the botanical origin of propolis by extracting several resin plants that are often visited by *T. sapiens*. The *Mangifera indica* resin sample was ground into a fine powder, which was then extracted three times with 99% ethanol, using the same method as the EEP of P1 to give an ethanol extract from *M. indica* resin (EEM). The EEM was then applied to HPLC (UV 254 nm) and the profiles were compared with those of the EEP of P1 and P2. The characteristic peaks of the HPLC chromatograms of the EEP and EEM showed a similar pattern, that is, the main components of propolis were also found in the *M. indica* resin. These results suggested that the propolis from Southeast Sulawesi was rich in the five cycloartane-type triterpenes, and the plant source of the propolis could be *M. indica* (mango).

The third stage is to find antioxidant activity and α-glucosidase inhibitory activity of the five isolated compounds from the propolis and to study the inhibitory mechanism and the relationship between the activity and their molecular structures. The antioxidative activity of the fractions was assayed by the scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals. Inhibition activity of the isolated compounds against α-glucosidase from *Saccharomyces cerevisiae* and rat small intestine was measured. The inhibitory test results of all isolated triterpenes against α-glucosidase from *S. cerevisiae* showed a high potential for inhibitory activity with an IC_{50} range between 2.46 and 10.72 μM. Among the compounds tested, mangiferonic acid was the strongest α-glucosidase inhibitor with IC_{50} 2.46 μM compared to the standard (–)-epicatechin (1991.1 μM), and also had antioxidant activities with IC_{50} values of 37.74 ± 6.55 μM. The study on the structure-activity relationships among the compounds showed that the ketone group at C-3 and the double bonds at C-24 and C-25 are needed to increase the α-glucosidase inhibitory activity. The carboxylic group at C-26 is also more important for increasing the inhibitory activity compared with the methyl group. This is the first report of inhibitory activity of cycloartane-type triterpenes isolated from propolis against α-glucosidase. This study provides an approach to help consider the structural requirements of cycloartane-type triterpenes from propolis as α-glucosidase inhibitors. An understanding of these requirements is deemed necessary to find a new type of α-glucosidase inhibitor from the cycloartane-type triterpenes or to improve those inhibitors that are known to help in the treatment of diabetes. Some compounds [mangiferolic acid (IC_{50}: 27.11 ± 6.575 μM), mangiferonic acid, and ambonic acid (IC_{50}: 90.57 ± 9.60 μM)] also have a moderate-high antioxidant activity where antioxidant activity can help accelerate healing and prevent complications in diabetics, which makes this compound have ideal potential as an anti-diabetes compound.