In Vitro Antidiabetic Activity And Bioactive Ingredients Of Apis mellifera and Apis dorsata Binghami Nest Extracts

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Abstract

Indonesia ranks 7th out of ten countries with diabetes mellitus, which continues to increase yearly. Honeycomb is rich in secondary metabolites formed from plant resins (propolis) which contain alkaloids, flavonoids, saponins, tannins, steroids and triterpenes. The research aimed to analyze the comparison of flavonoid content and antidiabetic activity with the enzyme α-glucosidase In Vitro nest extract of A. mellifera and A. dorsata Binghami. The samples used were nests of A. mellifera from northern Toraja, southern Sulawesi and A. dorsata Binghami from Southeast Minahasa, North Sulawesi. Using 96% ethanol, HPLC method of flavonoid testing and in vitro antidiabetic activity testing using α-glucosidase enzymes. The flavonoid content results were based on the number of peaks produced and the retention time of A. mellifera produced 26 compounds, and A. dorsata Bingham produced 21 compounds. The results of in vitro antidiabetic tests with α-glucoside enzyme inhibitors obtained IC50 values from the nest extract of A. mellifera of 3.605 mg/L and the nest extract of A. dorsata Binghami of 4.992 mg/L. The extract from A. mellifera had better antidiabetic potential than that of A. dorsata Binghami, although the difference is insignificant

Keywords: Antidiabetic; Nest Extract, Apis dorsata Binghami, Apis mellifera L

INTRODUCTION

The prevalence of people with diabetes mellitus in the world in 2019 was 9.3% of the world population and continues to increase yearly (Pangestika et al., 2022). Indonesia is ranked seventh worldwide after China, India, USA, Pakistan, Brazil, and Mexico (Ministry of Health, 2020; Wa et al., 2019). Diabetes mellitus (DM) is a metabolic disease characterized by increased blood sugar levels caused by abnormalities in
insulin secretion, insulin action, or both in the pancreas (Laily et al., 2022). Apart from increased blood sugar, DM is also caused by oxidative stress (Maulana et al., 2022).

Sulawesi is the largest island in the Wallace Bio-Region which plays an essential role in biodiversity preservation (Mustari, 2020). Sulawesi is an island that was not formed from the Asian continent and the Australian continent, so it has a high level of endemic flora and fauna compared to other regions of Indonesia. The island of Sulawesi was discovered by A. R. Wallace in the 18th century (Mokosuli et al., 2019). Sulawesi honey bees were first collected by A. R. Wallace, which was reported by Smith in 1859 (Semuel, et al., 2019). Apis dorsata Binghami is one of the endemic species of Sulawesi, known as a giant bee that naturally lives in the forest and is more aggressive with a variety of food sources compared to *Apis mellifera* (Mokosuli, 2021). *A. mellifera* is the western honey bee (Mokosuli et al., 2019). *A. mellifera* is a more docile bee species with an excellent ability to adapt to different climates, making it easy to cultivate (Rosyidi et al., 2018). *A. mellifera* is one of the most common flower visitors to natural environments worldwide and plays an essential role in the wild pollination of crop plants, with substantial implications for the global economy and natural ecosystems, thus providing a critical link in food and fruit production (Nowak et al., 2021).

Hives produce honey, propolis, royal jelly, bee pollen, and bee venom (Ririmasse et al., 2022). Honey bee products that are pharmacological are honey, propolis, royal jelly, bee pollen, and bee venom, which are produced by almost all types of bees (Cornara et al., 2017 Kocot et al., 2018). Honeycomb contains phenolic compounds, flavonoids, terpenoids, beta-steroids, aromatic aldehydes, vitamins (B1, B2, B6, C, E), minerals, amino acids, lactones, quinones, polysaccharides, essential oils (Pasupuleti et al., 2017). The composition of the secondary metabolites of the hives varies according to the geographical area, the type of species of bee, the climate (season), the diversity of the plants which are at the origin of the raw materials for the manufacture of the hives from resins plants or propolis (Rivera-Yañez et al., 2018; Da Cruz et al. al., 2022). Since ancient times, the Egyptians, Greeks, and Romans have used hives for wound healing, disinfectants, and antiseptics (Balica et al., 2021).

Minahasa Utara Community have long known honey bee products as traditional food and medicine (De Lima et al., 2020). Sulawesi uses honey as a stamina booster, cough medicine, canker sores, wound medicine, cancer, and antidiabetic. The primary key to medical therapy is the availability of plants rich in secondary metabolites, low cost, and fewer side effects, especially in rural areas (Mokosuli et al., 2019).

Extracts are viscous preparations obtained by extracting the active compounds from plant simplicia using a suitable solvent; then, the solvent is evaporated to get a thick solution. Extraction filters nutrients or active substances from parts of medicinal plants, animals, and several types of fish, including marine biota. Plant and animal cells have different thicknesses, thus requiring extraction techniques with certain solvents to extract them (Ririmasse et al., 2022). Research results on the ethanolic extract of Chihuahua propolis significantly inhibited the increase in blood sugar and decreased body weight in diabetic rats (Rivera-Yañez et al., 2018). The study's results aimed to determine the antihyperlipidemic activity of *A. dorsata* Binghami nest infusion extract that can reduce rats' total cholesterol, LDL, and blood triglyceride levels.
North Sulawesi has the potential to be developed as a source of antioxidant and anticancer bioactive (Mokosuli, 2021). This study is based on several previous research findings regarding the pharmacological potential of *A. dorsata* Binghami hives, especially for degenerative diseases such as cancer and diabetes mellitus. Based on the above background, there is a need for comparative research on the flavonoid content and in vitro antidiabetic activity of extracts from the nests of *A. mellifera* and *A. dorsata* Binghami.

**METHOD**

**Sample**

The extract samples were nests of *A. mellifera* from the coffee plantation PT. Toarco Jaya Factory Pedamaran, Bokin Village, Rantebua Toraja Utara District, South Sulawesi, and nests of *A. dorsata* Binghami obtained from the Ratahan Forest of Southeast Minahasa, North Sulawesi.

![Sampling locations of hives of *A. mellifera* and *A. dorsata* Binghami](image)

**Research Procedure**

This research was carried out in several stages consisting of the first stage of making simplicia, the second stage of extracting the nests of *A. mellifera* and *A. dorsata* Binghami, the third step of flavonoid content analysis, and the fourth step of testing antidiabetic activity in vitro.

**Simplicia Preparation and extraction**

Honeycomb samples are collected, cleaned, and air-dried to avoid bacteria and fungi that can cause decay. The extraction used the maceration method (Harborne, 1996; Samuel, et al., 2019). Fresh nests of *A. mellifera* and *A. dorsata* Binghami were weighed and crushed using a blender, 100 grams, and 250 grams, respectively. Each was poured into a glass jar filled with 96% ethanol, Core med 400ml, and 1000ml (1: 1 ratio: 4) for 3 x 24 hours at room temperature. Shake every 6 hours using an orbital shaker for 30 minutes. The filtrate was filtered using DR Watts filter paper. The extracted filtrate was then evaporated using a Heidolph rotary evaporator at 50° C.
and 55 rpm. The resulting solvent evaporation is then referred to as the Ethanolic Extract Honeycomb (EESL) of *A. mellifera* and *A. dorsata* Binghami.

**Analysis of bioactive content**

The analysis of the flavonoids EESL *A. mellifera* and EESL *A. dorsata* Binghami was carried out by the High Performance Liquid Chromatography (HPLC) method. Two grams of each EESL sample were added to 14 ml of 70% acetonitrile and left for 24 hours. Samples were filtered using filter paper (Whatmann #41) and Millipore Polyvinylidene fluoride (PVDF) filters. Measurement of flavonoid levels was performed by HPLC (Samuel et al., 2019).

**In Vitro Antidiabetic Test.**

Test the activity of α-glucosidase inhibitors (Mayur et al., 2010). The reagent mix used in this test contained 50 μL of 0.1 M phosphate buffer (pH 7.0), 25 μL of 0.5 mM 4-nitrophenyl α-D-glucopyranose, ten μL of EESL samples at various concentrations (50–1000 ppm) and 25 μL of α - glucosidase solutions (0.2 units/mL). This reaction mixture was incubated at 37°C for 30 minutes. The reaction was stopped by adding 100 μL of 0.2 M sodium carbonate solution. Enzymatic hydrolysis of the substrate was monitored by the amount of p-nitrophenol released into the reaction mixture at 410 nm using a Shimadzu UV-160 spectrophotometer. Individual blanks are prepared to correct background absorbance, where the enzyme is replaced with buffer. The control uses a solvent (water or ethanol) to replace the sample. Acarbose was used as a positive control. All experiments were performed in triple. The following formula (Febrinda et al., 2013) obtains the percentage inhibition of α-glucosidase:

\[
\text{% inhibition} = \frac{1 - (\text{Abs sample})}{\text{Abs control}} \times 100
\]

The IC50 value is determined by plotting the percentage inhibition against the concentration until the regression equation is obtained. From the regression equation, it can be determined that the concentration of the extract, which can inhibit the activity of the α-glucosidase enzyme is 50%.

**Research Data Analysis**

Data from the HPLC method of flavonoid analysis were analyzed descriptively. Data from in vitro antidiabetic activity test results were analyzed by linear regression. The IC50 value is the concentration of extract required for 50% inhibition of the α-glucosidase enzyme.

**RESULT AND DISCUSSION**

**Extraction**

Samples of nests of *A. dorsata* Bingham were obtained directly from natural nests in Ratahan Forest, southeast of Minasa. Samples of *A. mellifera* were collected from coffee plantations in PT. Toarco Jaya Factory Pedamaran, Bokin Village, Rantebua District, North Toraja, South Sulawesi. The nest sample of *A. dorsata* Bingham is golden yellow, and the well where the eggs are laid is hexagonal but more significant than that of *A. mellifera*. The nest sample of *A. mellifera* is pale yellow, and
the hole where the eggs are laid is smaller. The nests of *A. dorsata* Bingham were harvested at three months, while the nests of *A. mellifera* were harvested at three months. Fresh samples were kept in sample boxes from locations with an average temperature of 25 °C.

Fresh samples were prepared before being extracted by the maceration method. The nest samples used were nest samples where the hexagonal wells where the eggs were not yet contained bee eggs. When macerated with a solvent, the sample was crushed with a 0.1-0.5 mm size to increase the surface area. The extraction was performed with a 95% ethanol solvent aiming for the efficiency of removal of the bioactive from the simplicia hive. The ratio of solvent and simplicia 1:4 (w/v) is based on previous research, where the extraction efficiency of the nests of *A. dorsata* Bingham was obtained at a ratio of 1:4 and 95% ethanol solvent (Mokosuli et al., 2019). The filtrate of *A. dorsata* Bingham was blackish brown, while the filtrate of *A. mellifera* was light yellow. The aroma of the nest extract of *A. dorsata* Bingham has a more pronounced honey smell than that of *A. mellifera*. After evaporation of the solvent, the crude extract of *A. dorsata* Bingham was dark brown, while the crude extract of *A. mellifera* was yellow.

The percentage yield of the extract of *A. mellifera* was 60%, while that of data was 23.6%. The percentage yield of *A. mellifera* was superior to that of *A. dorsata* Bingham (Table 1). The yield is the product's dry weight ratio to the raw material's weight. Although the weights of *A. mellifera* and *A. dorsata* Bingham simplicia used are different, the ratios of solvent and simplicia were the same. The difference in the total weight of simplicial is due to simplistic availability. Simple *A. mellifera* was obtained from North Toraja, South Sulawesi, while Simplification *A. dorsata* Bingham was obtained from Southeast Minahasa, North Sulawesi. The nests of *A. dorsata* Bingham are more significant than those of *A. mellifera*. The yield of the extract was calculated based on the ratio of the final weight (weight of the extract produced) to the initial weight (weight of the cell biomass used) multiplied by 100% (Sani et al., 2014). The yield value is also related to the extract's bioactive content. Based on the percentage yield obtained, it is assumed that the bioactive content of *A. mellifera* is superior to that of *A. dorsata* Bingham. Ethanol is a polar solvent, so in theory, it is a polar bioactive compound that will significantly interest the extract (Harborne, 1996).

### Table 1. Extract yield

<table>
<thead>
<tr>
<th>Simplisia</th>
<th>Solvent Volume Etanol 96% (ml)</th>
<th>Weight Simplicia (g)</th>
<th>Weight Extract (g)</th>
<th>Rendemen (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. mellifera</em> nest</td>
<td>400</td>
<td>100</td>
<td>60</td>
<td>60</td>
</tr>
<tr>
<td><em>A. dorsata</em> Bingham nest</td>
<td>2000</td>
<td>500</td>
<td>118</td>
<td>23,6</td>
</tr>
</tbody>
</table>

The retention time is required for the compounds of the sample components to pass through the column to the detector. The retention time is calculated from when the sample is injected until the peak of the maximum reading on the detector. Different compounds will have different times so that each concentration can be calculated. The chromatogram represents the sample components seen from the number of peaks.
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**Figure 2.** Chromatogram Retention Time of High Performance Liquid Chromatography Results: (a). *Apis mellifera*  (b). *Apis dorsata* Binghami (c). Quersetin standard
(peaks) produced. The concentration of the component to be analyzed can be calculated by comparing the peak area of the component with the peak area and the standard concentration. Based on the number of peaks produced and the retention time, *A. dorsata* Binghami produced 21 types of compounds, while *A. mellifera* produced 26 types of compounds.

Based on the retention time, the extract of *A. mellifera* and *A. dorsata* Binghami contain quercetin. The standard retention time of quercetin is 11.410. The extract from *A. dorsata* Binghami obtained a retention time of 11,453. In the extract of *A. mellifera*, four peak retention times were obtained close to quercetin, namely 11,020, 11,233, 11,450, and 11,690 (Figure 2). Thus, it is suspected that four types of quercetin derivatives are detected in the extract of *A. mellifera*.

The in vitro antidiabetic test by α-glucoside enzyme inhibition works by inhibiting glucose absorption in the intestine, thus preventing the increase in postprandial glycemia. Therefore, the α-glucosidase enzyme is one of the targets for drug development for type II diabetes mellitus. Based on the study's results using five test concentrations and three replicates, the regression equation for the nest extract of *A. dorsata* Binghami was obtained: $Y = 1.092 + 0.078x$ (Figure 3).

![Normal P-P Plot of Regression Standardized Residual](image)

**Figure 3.** Regression analysis curve of α-glucosidase enzyme inhibition of *A. dorsata* Binghami

Based on the study's results using five test concentrations and three replicates, the regression equation for the nest extract of *A. mellifera* was obtained: $Y = 1.055 + 0.051x$ (Figure 4).
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Apis mellifera, which also showed a yield percentage of over 50% (Puspitasari & Pramono, 2015). In addition, the water content of simplicia hives during maceration affects the percentage yield produced (Pobiega et al., 2019). The best hive maceration time to obtain maximum bioactive content is less than ten days (Pobiega et al., 2019). This study only extracted nests within three days, so the bioactive content was always optimal. Ethanol is the best solvent to extract the hives' bioactivity (Kara et al., 2022).

**Bioactive Contents**

The colour of the extract can indicate the bioactive content. The brownish-yellow colour indicates a higher content of polyphenolic compounds (Pujirahayu et al., 2014; Šuran et al., 2021). According to the extraction results, the nest extract of *A. dorsata* Binghami showed a brownish yellow compared to the extract of *A. mellifera* of egg yolk colour. This shows that the content of bioactive polyphenols in the nests of *A. dorsata* Binghami is superior to that of *A. mellifera*. Based on the results of bioactive content analysis by HPLC, the content of the quercetin group of flavonoid compounds in *A. mellifera* was higher than that of *A. dorsata* Binghami. *A. dorsata* Binghami produced 21 types of compounds while *A. mellifera* had 26 types of compounds. Analysis of quercetin flavonoid content in *A. dorsata* Binghami was reported by Mokosuli et al. and showed a substantial variation in flavonoid compounds (Mokosuli et al., 2019).

Propolis from different places in Turkey showed very different flavonoid content (Özkök et al., 2021). The same was also reported on Malaysian stingless flavonoid content, which differed from various locations (Awang et al., 2018; Asem et al., 2020). Time of harvest, geographic location, forage plants, and extraction methods influence the flavonoid content of hive extract. Additionally, samples of nests of *A. dorsata* Binghami were collected during the rainy season, while those of *A. mellifera* were collected during the summer. Rainy seasons and summer affect the content of flavonoids in honey bee hives (Rodiahwati et al., 2019). Bees will collect more propolis with more significant plant variations in summer compared to the rainy season. During the rainy season, the hives of *A. dorsata* Binghami will contain high water content, and the variety of propolis harvested by the worker bees will be less.

**Antidiabetic activity**

The honeycomb inhibition activity against the α-glucosidase enzyme used in this study is the in vitro method. The α-glucosidase is an enzyme that plays a role in the hydrolysis of carbohydrates into glucose (Yuniarto & Selifiana, 2018). The in vitro antidiabetic test results showed a difference in inhibition between the nests of *A. mellifera* and the nests of *A. dorsata* Binghami against the α-glucosidase enzyme. Differences in the inhibition of α-glucosidase enzymes indicate differences in the antidiabetic activity of each extract (Hardoko et al., 2015). The IC$_{50}$ value indicates that the lower the value, the higher the potential. An extract is said to have active capacity as an α-glucosidase enzyme inhibitor if it has an IC$_{50}$ value≤100 µg/mL (Lee & Lee, 2001).

The nest extract of *A. mellifera* had better inhibitory activity than the nest extract of *A. dorsata* Binghami with an IC50 value of *A. mellifera* of 3.605 mg/L and the nest extract of *A. dorsata* Binghami of 4.992 mg/L. These results are in agreement with the results of the analysis of the bioactive content of *A. mellifera* and *A. dorsata* Binghami,
namely the content of quercetin group flavonoid compounds *A. mellifera* produced 26 kinds of compounds, and *A. dorsata* Binghami produced 21 kinds of compounds. Thus, the content of flavonoid compounds in the nests of *A. mellifera* is more potent in inhibiting α-glucosidase enzyme than *A. dorsata* Binghami. Hence, the nest extract of *A. mellifera* has better potential as a drug for type II diabetes mellitus (Mokosuli et al., 2019). Propolis can inhibit the action of the α-glucosidase enzyme and therefore has the potential to cure diabetes mellitus (Pujirahayu et al., 2019). Differences in the activity of secondary metabolites, i.e., flavonoids, can be caused by different bee species, food sources, harvesting time, and habitat so that they can be characteristic honey (Mokosuli et al., 2019).

**CONCLUSION**

Comparative analysis of flavonoid content in extracts from the nests of *A. mellifera* and *A. dorsata* Binghami using quercetin class of flavonoid compounds, namely *A. mellifera* produced 26 kinds of compounds, and *A. dorsata* Binghami had 21 types of compounds.

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