IBJ
Indonesian Biodiversity Journal
http://ejurnal.unima.ac.id/index.php/ibj

indonesian.biodivers.j.
Vol. 6, No. 1. April 2025
ISSN : 2722-2659

Anticancer Activity Of *Apis Dorsata* Binghami Nest Extract From West Uluway, Tana Toraja District

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Received: May 14th, 2025 *Accepted: June 10th, 2025*

Abstract

Cancer is the leading cause of death in the world, with a high prevalence in Indonesia, especially breast cancer. Conventional therapies such as chemotherapy and radiotherapy have various limitations, including severe side effects and cancer cell resistance. This study aims to explore the anticancer potential of the ethanol extract of *Apis dorsata* Binghami nests originating from Uluway Barat Village, Tana Toraja Regency. Extraction was carried out using the maceration method with 96% ethanol solvent, with a yield of 51.42%, and bioactive compounds were identified through GC-MS analysis. Anticancer activity tests were carried out *in vitro* on MCF-7 breast cancer cells using the *resazurin assay method* . The results showed that the extract contained various bioactive compounds such as flavonoids, furanones, and aromatic aldehydes which are known to have antioxidant and anticancer activities. The IC50 value $_{obtained}$ was 434.30 µg/mL, indicating weak cytotoxic activity. Nevertheless, this extract showed significant ability to kill cancer cells at certain concentrations, especially 15.63 µg/mL which reduced cell viability by 44.39%. These results indicate that *A. dorsata* Binghami nests contain compounds that have the potential as natural anticancer agents, but require further research for fractionation and identification of more specific active compounds and further testing *in vivo* .

Keywords: *Apis dorsata* Binghami, anticancer, MCF-7, GC-MS, Tana Toraja

INTRODUCTION

Breast cancer is one of the most common and deadly types of cancer for women worldwide. Based on data from *the World Health Organization* (WHO) in 2020, breast cancer contributed to more than 2.3 million new cases, with a death rate reaching around 685,000 cases. This high incidence rate makes breast cancer a global health threat that requires serious treatment. (WHO, 2021). In Indonesia, breast cancer is also the type of cancer with the highest prevalence in women. According to Globocan data in 2020, there were 68,858 new cases of breast cancer in Indonesia, with a death rate reaching 22,430 people. This condition shows that breast cancer is not only a global problem, but also a major challenge in the national health system (Globocan, 2020).

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Conventional therapies such as chemotherapy, radiotherapy, and surgery are still the mainstay of cancer treatment, although these approaches have many limitations. Side effects such as nausea, vomiting, hair loss, chronic fatigue, and even organ damage are common. In addition, some types of cancer show resistance to treatment, thus decreasing the effectiveness of therapy. This resistance can come from genetic changes in cancer cells or cellular defense mechanisms against drugs. (Khalid et al., 2021).

This research focuses on the development of anticancer drugs from natural sources to treat the above problems. Natural materials have advantages in terms of the diversity of active compounds and the potential for milder side effects compared to synthetic drugs. Various treatment methods, such as chemotherapy and radiotherapy, often have significant side effects, so the search for safer and more effective alternative treatments is very important. Among the many sources of natural medicine, bee products, including nests and honey, have received widespread attention. *Apis dorsata* Binghami nests are known to have various health benefits. Previous studies have shown that extracts from this honeycomb contain bioactive compounds that have the potential to have anticancer activity (Newman & Cragg, 2020).

Honeycomb contains active compounds such as flavonoids (*quercetin, chrysin*), phenolic acids (*caffeic acid*), and wax esters that have pharmacological activity. These compounds play a role in inhibiting free radicals, suppressing inflammation, and potentially suppressing the growth of cancer cells. Beeswax products also contain resins from plants that have the potential as *chemopreventive agents* . Bioactive compounds in honeycomb, such as flavonoids and polyphenols, have been shown to have antioxidant and anti-inflammatory properties. These properties are very important in cancer prevention, because oxidative stress and chronic inflammation are major risk factors for tumor development.

There have been many studies on bee products, but those that specifically examine the anticancer potential of *A. dorsata* Binghami nests are still very limited. Exploration and utilization of beehives for anticancer products not only provide health benefits, but can also be a new source of economy for local communities, through sustainable management of forest bees. This will create a symbiotic relationship between nature conservation and community welfare. Based on this background, this study will examine the anticancer activity of *A. dorsata* Binghami nest extract from Uluway Barat, Tana Toraja Regency, which is expected to provide valuable information for the development of alternative cancer treatments in the future.

RESEARCH METHODS

Time and Place

Apis dorsata Binghami beehives were obtained from Uluway Barat Village, Toraja Regency, South Sulawesi in March 2025. The extraction process using the maceration method was carried out at the Integrated Forestry and Environment Laboratory, Faculty of Forestry, Hasanuddin University in March 2025. Meanwhile, the analysis of the characteristics and content of bioactive compounds and anticancer

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ISSN : 2722-2659

tests on MCF-7 cells at the Central Laboratory of Padjajaran University in April-May 2025.

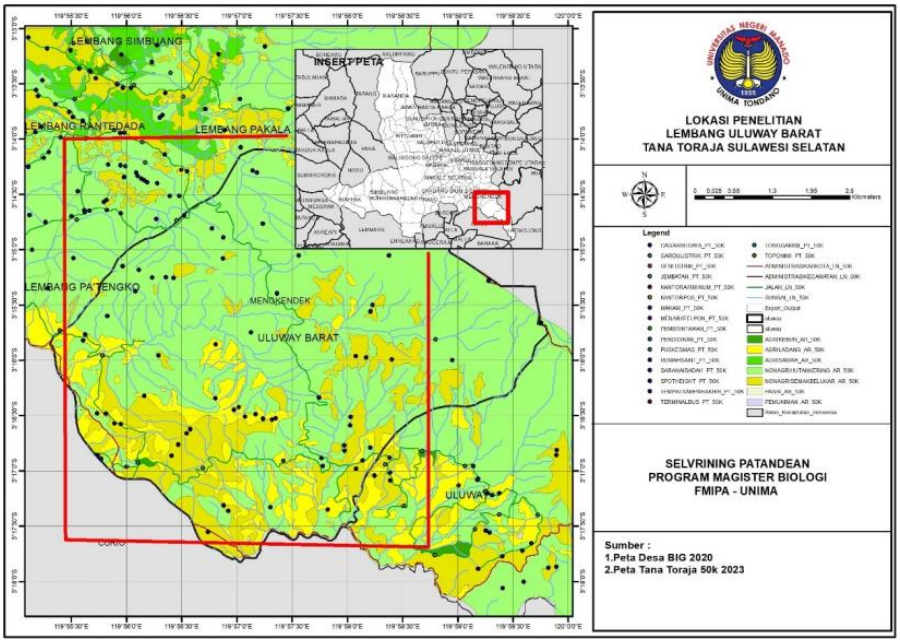

Figure 1. Sampling Location

Tools and materials

Apis dorsata Binghami nest samples were obtained from Uluway Barat Village, Tana Toraja Regency, South Sulawesi Province. The beehives were cleaned of dirt before extraction. The beehives used for extraction were those that had not been occupied or had eggs or larvae found.

Extraction. Tools and materials used: Ethanol proanalys 96%, water, label paper, filter paper, analytical balance, aluminum foil, Erlenmeyer flask, glass funnel, rotary evaporator, spatula, measuring cup, pipette, Erlenmeyer flask, extract storage bottle, round flask, stirrer.

Cytotoxicity of MCF-7 Cells. Equipment used: Biosafety Cabinet (BSC)(Thermo scientific 1300 series a2), Centrifuge (Thermo scientific microCL17), CO2 Incubator (Thermo scientific series 8000DH), Microscope (Thermo scientific EVOS XL Core), Multimode Reader (Tecan Infinite M200 PRO). Materials used: 1.5 mL microtube (Gene follower Brand MCTB015), 15 mL tube (Biologix Brand 109151), 75 ml T-flask (NEST Brand 708001), 96 well plate (NEST Brand 701001), Cisplatin (EDQM C2210000), Antibiotics (Sigma Aldrich P4333), Dimethyl sulfoxide (DMSO) (Merck D1435), Phosphate buffered saline (PBS) (Gibco 18912-014), Resazurin Sodium Salt-Powder, BioReagent (Sigma Aldrich R7017), Roswell Park Memorial Institute Medium (RPMI) (Gibco 11875-093), Fetal Bovine Serum (FBS) (Gibco 10270-106), Trypsin-EDTA (Gibco 25200-056), Trypan Blue (Sigma Aldrich T-8154).

Analysis of bioactive content using the GCMS method. The materials used are methanol, Apis dorsata Binghami nest extract. The tools used are Measuring pipette, Measuring flask, Gas Chromatography

Research Procedures

Apis dorsata Binghami beehives were collected from their habitat (Sinaji dead-end forest) in Uluway Barat Village, Mengkendek District, Tana Toraja Regency, South Sulawesi Province. Honeycomb

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extraction was carried out using the maceration method (Harborne, 1996, Mokosuli, 2008). Apis dorsata Binghami honeycomb was weighed as much as 35 grams, ground using a blender and then mixed with 160 ml of 96% ethanol. Furthermore, it was incubated for 3x24 hours at room temperature, every 3 hours it was shaken manually. Filtration of the filtrate was carried out using Whatman filter paper. The extracted filtrate was then evaporated into a solvent using a Heidolp rotary evaporator, at a temperature of 80C and 120 rpm. The results of the solvent evaporation are then called ethanol extract of A. dorsata Binghami honeycomb (Mokosuli et. al, 2023).

Phytochemical tests were carried out qualitatively to determine the presence of flavonoid, phenolic, alkaloid, terpenoid, and saponin compounds that have the potential as anticancer agents using chromatography techniques (GC-MS). Data were obtained in the form of total ion chromatograms (TIC) and mass spectra. Compound identification was carried out based on the Robert P. Adams spectra library.

Cytotoxicity test was carried out on MCF-7 cancer cells using the resazurin assay method.

a) The cell culture to be used is placed in a 96 well plate and then incubated (at a temperature of 37°C and 5% CO2 gas until the cell growth percentage reaches 70%).
b) Cells were treated with samples and then incubated (for 48 hours at 37°C and 5% CO2 gas).
c) Add the working reagent resazurin to the cells.
d) Absorbance measurement using Multimode Reader.

Research Data Analysis Techniques

The research data were analyzed:

a) Toxicity of the extract using the resazurin assay method.

   The use of the Resazurin Sodium Salt reagent, which undergoes a color change from blue (resazurin) to pink (resorufin) by the activity of living cells, allows estimation of cell viability based on metabolic activity.

b) Activity of bioactive compounds using the GCMS method.

   The IC50 value is the concentration of extract that is able to inhibit cell growth by 50%. This value is calculated based on the dose-response curve (% viability vs concentration) generated from absorbance data.

c) Anticancer activity. The IC50 value is the concentration of extract required for 50% inhibition of MCF-7 cancer cell growth.

RESULTS AND DISCUSSION

Apis dorsata Binghami Nest

Apis dorsata Binghami nest sample was taken in the Buntu Sinaji hill area, a natural forested area in Uluway Barat Village, Mengkendek District, Tana Toraja Regency. The location is at an altitude of around 1,350 meters above sea level, with cool environmental conditions and relatively high humidity throughout

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the year. The nest is located on the upper branches of local banyan trees (Ficus spp.) or Pinus merkusii growing on the edge of a small ravine and surrounded by secondary forest vegetation.

The size of the observed beehive is about 50 cm long, 35 cm wide, and 25 cm high, with a typical elongated hanging shape of A. dorsata bees. The nest is classified as active with dense bee in-and-out activity, indicating a healthy colony population.

Figure 2. Apis dorsata Binghami nest

The diversity of food plants around the nest is very high, which is an advantage of the Buntu Sinaji ecosystem. Vegetation around the nest consists of a combination of natural forest plants, flowering shrubs, and several local fruit trees. The food plants identified include: Food plants *Austroeupatorium inulifolium* , *Chromolaena odorata* , *Ficus sp* ., *Pinus merkusii* , *Mimosa pudica* . *Ageratum conyzoides* .

Apis dorsata Binghami Nest Extract

The beehives used in this study came from the Apis dorsata Binghami bee species found naturally in the Uluway area, Mengkendek District, Tana Toraja Regency, South Sulawesi. These nests were collected manually by local residents from their natural habitat, namely tall trees in the Uluway hills. The collected nests consisted of bee combs that still contained remaining honey, beeswax, and natural propolis. The nests were then cleaned of physical dirt such as wood or leaves, squeezed, and stored at room temperature before the extraction process.

After that, the sample was ground by cutting it into small pieces using a clean knife. Extraction of honeycomb was carried out using the maceration method. The ratio of solvent and simplicia (1:4 w/v). A total of 35 grams of honeycomb simplicia was macerated with 160 ml of 96% ethanol. After 72 hours, the solution was cloudy yellow. The filtrate produced after being filtered was greenish yellow. Evaporation of the solvent with a rotary evaporator produced a yellow extract with a distinctive honeycomb aroma. The

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96% ethanol extract was gel-like. The results of the extraction of honeycomb simplicia obtained a total extract weight of 18 grams. So that the yield percentage was 51.42%.

Figure 3. Results of Apis dorsata Binghami Nest Extract

Analysis of Bioactive Compound Content of *Apis dorsata* Binghami Nest Extract

Apis dorsata Binghami nest extract was analyzed using GCMS. GCMS can read compounds with low concentrations so that secondary metabolites in an extract can be identified with output in the form of chromatograms and mass spectra. The results of GCMS analysis obtained ten main peaks. This indicates that ten main compound groups were detected in the honeycomb extract. The largest area presentation is at the ninth peak while the smallest is at the sixth peak.

Table 1. Bioactive Compound Content of Apis dorsata Binghami Nest Extract

No.	Start	RT	End	Height	Area	Area %	Compound
1.	8436	38582	38722	934368	4520912	4480	n-Hexadecanoid acid
2.	46016	46092	46199	1006494	3247505	3218	Docosa
3.	50073	50160	50300	2403467	8011471	7938	Pentacosane
4.	53203	53310	53407	1018820	5849021	5795	Heptacos-1-ene:
5.	53731	53785	53871	664411	2369348	2348	3-Ethyl-2-pentadecanone
6.	56794	56860	56882	660803	1998996	1981	Nonacos-1-ene:
7.	56882	56925	56968	796939	3130481	3102	Nonacos-1-ene:
8.	56968	57033	57141	1030031	5499830	5449	Nonacos-1-ene:
9.	57853	57950	58155	1777213	10092391	10000	Heptadecane, 2,3-dimethyl
10.	61208	61370	61478	1091800	7121535	7056	Heptyl octadecyl ether

Discussion

Extraction by maceration method of *Apis dorsata* Binghami nest produced 11.82%. This shows

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that ethanol solvent is effective in attracting active compounds contained in *Apis dorsata nest. The color and aroma of the crude extract still show the characteristics of the origin of the Apis dorsata Binghami* beehive used. Extraction of the maceration method and microwave heating on *Apis dorsata nests* , obtained an average yield of 24.43%, with the highest value reaching 30% (Rodiahwati *et al* . (2019). The yield of ethanol extract of Apis dorsata Binghami nests was 11.82% using the maceration method (Pratasik *et al* . (2022). The yield of 13.3% of Kalimantan *Apis dorsata propolis* using 96% ethanol solvent with the maceration method (Fitriani *et al* . (2021). The yield of 20–30% of South Sulawesi Apis dorsata propolis using ethanol solvent (Mokosuli *et al* . 2021).

The diversity of forage plants in the Uluway Barat Village area, Mengkendek District, Tana Toraja Regency can greatly affect the bioactive content of the ethanol extract of *Apis dorsata nests* , because the chemical composition of bee products (including nests, honey, propolis, and wax) is greatly influenced by the source of forage or nectar and resin collected by bees. Forage plants can produce secondary metabolite compounds such as flavonoids, alkaloids, tannins, terpenoids, phenolics, and others. These compounds can accumulate in beehives, especially if bees utilize the sap, nectar, and pollen from these plants. In areas with high plant diversity, nests tend to contain a variety of bioactive compounds with broad pharmacological activities, including anticancer, antibacterial, antioxidants. Differences in the bioactive content of nests harvested at different times even in the same location. This is because the composition of flowering plants will change throughout the year and the flowering season affects the types of food available.

Based on the GC-MS results, there were 39 compounds successfully identified from the honeycomb extract. The large number of compounds detected was due to several factors such as the chemical complexity of the honeycomb extract. Honeycombs contain a mixture of wax, resin, propolis, and environmental contamination (from feed plants). Extraction method with ethanol. Ethanol as a solvent extracts a broad spectrum of compounds (polar and moderate non-polar), resulting in a more diverse compound profile than other solvents. The diversity of feed plants in West Uluway results in a variety of secondary metabolites carried in the nest. The high resolution of GC-MS allows the separation of compounds in large quantities even in small concentrations.

in the nest extract of *Apis dorsata* Binghami from North Sulawesi was lower, ranging from 10–25 compounds (Mokosuli *et. al* . 2023). Differences in the content of compounds detected in honeycomb extracts are influenced by bee species, food sources, season, geographical location, extraction methods and solvents, harvest time or nest age, and GC-MS resolution and parameters (Mokosuli *et. al.* 2023, Mokosuli *et.al.,* 2024).

The compounds found in the *Apis dorsata* Binghami nest extract obtained by researchers are Furaneol (4-Hydroxy-2,5-dimethyl-3(2H)-furanone), 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, Neral (citral isomer), Geranial (2,6-Octadienal, 3,7-dimethyl-, (E)-), 2-Furanmethanol (Furfuryl alcohol), 2(3H)-Furanone, dihydro-4-hydroxy-, 1,6:2,3-Dianhydro-4-O-acetyl-β-D-allopyranose, Glycidol, (3S,3aR,6R,8aS)-7,7-Dimethyl-8-methyleneoctahydro-1H-3a,6-methanoazulene-3-carboxylic acid,

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Propanediamide, 2(3H)-Furanone, 5-acetyldihydro-.

Apis dorsata Binghami honeycomb extract contains various bioactive compounds such as flavonoids (e.g. chrysin, pinocembrin), phenolic acids (e.g. caffeic acid phenethyl ester/CAPE), and aromatic aldehydes. These compounds have been shown to have cytotoxic activity against various types of cancer cells, including MCF-7 breast cancer cells. The mechanisms of cytotoxicity induced by these compounds involve several major molecular pathways.

A study by Adnan *et al* . (2024) evaluated the cytotoxic effect of *Apis dorsata forest honey* on T47D breast cancer cells using the MTT assay method. The results showed that forest honey did not have significant cytotoxic activity against T47D cells at various concentrations tested. In comparison, doxorubicin, a standard chemotherapy drug, showed an IC50 value of 3.746 µg/mL. Although forest honey contains compounds such as flavonoids, saponins, alkaloids, and tannins which are known to have antioxidant activity, this study did not find any inhibitory effect on the growth of T47D breast cancer cells.

Another study showed that giving *Apis dorsata honey* as an additional therapy in post-chemotherapy breast cancer patients can significantly increase the number of T lymphocytes, although it does not significantly reduce interleukin-6 (IL-6) levels. Increased T lymphocytes can help improve the body's immune response to cancer cells. Based on the Cytotoxic Test Results Report on MCF-7 breast cancer cells from the ethanol extract of Apis dorsata Binghami honeycomb, the IC_{50} value obtained was 434.30 µg/mL.

Apis dorsata Binghami honeycomb against MCF-7 cancer cells is categorized as having weak cytotoxic activity. This indicates that although there are bioactive compounds in the extract (such as flavonoids, aromatic aldehydes, and furanones), the concentration is not high enough to show a strong anticancer effect against MCF-7. A combination of active compounds or further fractionation may be needed to increase anticancer potential.

The concentration with the most dead cells was 15.63 µg/mL where Viability was only 44.39%. This means: about 55.61% of cells experienced death or damage. This is the lowest viability value, indicating the highest cytotoxic activity among other concentrations. While the concentration with the least dead cells was 7.81 µg/mL where Viability was 99.81%. This shows that almost all cells remain alive at this concentration, so it does not show significant cytotoxic effects.

The highest decrease occurred at 15.63 µg/mL, possibly caused by several factors such as the non-linear response of cells to the extract, the presence of active compounds in optimal amounts that trigger cell death at medium concentrations, at higher concentrations, non-active components or interfering agents in the extract can actually neutralize the toxic effects (this phenomenon is known as the *hormesis effect or self-neutralizing mixture*), it could also be caused by reagent interference, interactions between compounds, or decreased compound stability at high concentrations.

CONCLUSION

This study shows that the ethanol extract of *Apis dorsata* Binghami nest from Toraja has unique

physical-chemical characteristics and contains various bioactive compounds, such as furaneol, glycidol, and furanone derivatives, which are known to have biological activity. The results of GC-MS analysis identified the presence of compounds that have the potential to support pharmacological effects, including anticancer effects. In vitro anticancer activity test against MCF-7 cancer cells showed that the extract has cytotoxic activity with an IC50 value of 434.30 µg/mL, indicating its potential as a natural anticancer agent with a low level of activity.

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