Molekul, Vol. 21. No. 2, July 2026: 280 – 296

MOLEKUL
eISSN: 2503-0310

Articles
https://doi.org/10.20884/1.jm.2026.21.2.18327

Multifunctional Bioactivities of *Apis dorsata* Binghami Nest Extract-Fortified Tea: Antioxidant, Cytotoxic, and α-Glucosidase Inhibitory Potential

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Received November 11, 2025; **Accepted** June 04, 2026; **Available online** July 20, 2026

ABSTRACT. Bee-derived products are increasingly acknowledged as valuable functional ingredients because of their abundant phenolic and flavonoid content; yet, the biological potential of honeycomb or nest extracts is still inadequately investigated. The current research examined the bioactive characteristics and multifunctional properties of a tea formulation enhanced with *Apis dorsata* Binghami nest extract. The total phenolic and flavonoid concentrations were measured with Folin–Ciocalteu and $AlCl_3$ colorimetric tests, respectively, with results reported as equivalents of gallic acid and quercetin. The antioxidant capacity was examined by DPPH radical scavenging, anticancer activity was determined by cytotoxicity against human breast adenocarcinoma (MCF-7) cells through the Presto Blue™ assay, and antidiabetic potential was investigated via α-glucosidase inhibition. The formulation demonstrated significant quantities of phenolic and flavonoid compounds, which were associated with pronounced free radical scavenging activity. Cytotoxicity experiments demonstrated a concentration-dependent decrease in MCF-7 viability, with IC_{50} values reflecting moderate potency relative to cisplatin, although above the potency of crude extracts noted in previous investigations. The extract exhibited notable α-glucosidase inhibitory actions, indicating its promise in glycemic management. Collectively, our data underscore the potential synergistic interaction of tea polyphenols and nest-derived bioactives in augmenting antioxidant, anticancer, and antidiabetic effects. This is, to our knowledge, the inaugural integrated study assessing the multifunctional bioactivities of *Apis dorsata* Binghami nest extract prepared as tea. The findings highlight its promise as an innovative functional beverage and nutraceutical option, offering scientific validation for the enhancement of underutilized bee resources in combating oxidative stress–related ailments.

Keywords: *Apis dorsata* Binghami, anticancer, antidiabetic, antioxidant, Multifunctional Bioactivities, phenolic content

INTRODUCTION

Natural products produced by honeybees have been acknowledged as sources of bioactive compounds exhibiting diverse pharmacological activities (Ratajczak et al., 2021; Bava et al., 2024). Propolis, royal jelly, honey, and bee bread are extensively studied bee products that exhibit antioxidant, anticancer, antidiabetic, and antimicrobial properties, primarily due to their phenolic and flavonoid constituents (Matilenello et al., 2021; Nainu et al., 2021; Hashemirad et al., 2024; Tlak Gajger & Vlainić., 2025). Recent advancements in functional food research underscore the significance of honeycomb-related matrices, including comb or hive extracts, which comprise metabolites from nectar along with lipid and wax fractions. This combination yields a distinctive chemical profile when compared to traditional bee products (Guo et al., 2022; Anjum et al., 2024). This bioactive matrix demonstrates considerable promise for advancement as a nutraceutical and therapeutic candidate, particularly in the context of oxidative stress, metabolic disorders, and cancer (Sanyal et al., 2023; Kacemi and Campos, 2024; Alcalá-Orozco et al., 2024).

Apis dorsata Binghami, a subspecies endemic to the Wallacea Region, remains a relatively under-researched species among Asian forest bees, despite its substantial ecological and ethnomedicinal significance. The composition of the nest is a complex mixture of nectar residue, pollen-derived compounds, wax, and resin, which are thought to synergistically enhance its biological activity. Research on bee products indicates that the phenolic and flavonoid content of these products varies based on

geographical origin, floral source, and extraction method. These factors, in turn, influence the antioxidant potential and pharmacological activity of the products (Becerril-Sánchez et al., 2021; Sawicki et al., 2022; Semuel et al., 2024). Research specifically targeting the formulation of tea enriched with *Apis dorsata* Binghami nest extract remains limited, presenting novel opportunities that have not been extensively investigated in the development of functional beverages.

The increasing popularity of tea-based formulations as a natural bioactive delivery system is indicative of a growing focus in nutraceutical research (Sahukat et al., 2023). Tea polyphenols have been demonstrated to possess substantial antioxidant and chemopreventive characteristics. The honeycombs of *A. dorsata* Binghami have been found to contain a high polyphenol content, encompassing a range of derivative compounds. The combination of extracts from beehives has been shown to exhibit suggests potential synergistic interaction that may enhance the bioactivity profile (Bava et al., 2024; Aboulghazi et al., 2024). The incorporation of nest extract into tea formulations provides two primary advantages: it stabilizes the bioactive compounds within the tea matrix and presents them in a culturally acceptable and consumer-friendly manner. This combination of types has the potential to enhance the functional food landscape by providing antioxidant alternatives and synthetic drugs, which may result in a reduction of adverse effects.

The pharmacological impact of local natural extracts in Indonesia indicates that the variation in secondary metabolites significantly influences their antidiabetic, anticancer, and antioxidant efficacy (Mokosuli et al., 2023). This study investigates the bioactivity of *A. dorsata* Binghami nest extract formulated as tea, emphasizing its antioxidant, anticancer, and antidiabetic properties. The objective of this study is to provide a comprehensive functional profile of the innovative formulation by evaluating phenolic and flavonoid content, as well as conducting assays to assess toxicity and enzyme inhibition.

Unlike previous studies that focus on isolated bee products, this study integrates honeycomb-derived materials into a tea-based delivery system, providing a novel approach to functional beverage development. This research provides a novel integrated evaluation of the multifunctional bioactivity of the tea–honeycomb *A. dorsata* Binghami formula. This report is the first to systematically characterize the antioxidant potential of the extract via DPPH radical scavenging, anticancer effects against MCF-7 breast cancer cells, and α-glucosidase inhibitory activity as an antidiabetic mechanism. The findings of this study correlate these effects with the phenolic and flavonoid content of the extract. This finding enhances scientific knowledge of bee-related biological resources and identifies the *A. dorsata* Binghami nest as a viable candidate for developing functional foods and nutraceuticals targeting oxidative stress, cancer, and metabolic diseases.

Despite the increasing interest in bee-derived products and tea-based functional beverages, the integration of *A. dorsata* Binghami nest extract into a tea formulation remains largely unexplored. Previous studies have predominantly focused on isolated bee products such as propolis, honey, or bee pollen, either as crude extracts or encapsulated forms, without incorporating the complex matrix of honeycomb-derived materials into a consumable beverage system.

The formulation developed in this study was designed as a novel combination of dried *Apis dorsata* Binghami comb extract and plant-derived components (*Myristica fragrans* leaf extract), processed into a tea bag format to enhance stability, bioavailability, and consumer acceptability. To the best of our knowledge, no prior study has systematically evaluated such a formulation integrating honeycomb-derived bioactive compounds into a tea matrix.

This approach is based on the hypothesis that combining tea polyphenols with bee nest-derived metabolites may result in synergistic enhancement of biological activities, particularly antioxidant, anticancer, and antidiabetic effects. Therefore, this study aims not only to evaluate the bioactivity of the formulation but also to establish a scientific basis for the development of novel functional beverages derived from underutilized bee resources. The selection of these components was based on previous studies demonstrating that bee-derived products are rich in phenolic compounds, while *M. fragrans* contains bioactive terpenoids and flavonoids that may enhance synergistic biological effects.

EXPERIMENTAL SECTION

Sample

Collections of honeycombs from *Apis dorsata* Binghami were gathered from wild colonies residing in four different natural forest environments in North Sulawesi, Indonesia, between March and June 2025. Sampling occurred at four geographically distinct locations: Gunung Klabat Forest (1°30′12″ N, 125°00′04″ E), Manembo Forest (1°10′03″ N, 124°18′10″ E), the slopes of Mount Soputan (1°11′03″ N, 124°40′19″ E), and the slopes of Mount Ambang (0°44′03″ N, 124°23′19″ E). These locations exemplify biologically diverse environments, offering a comprehensive spatial framework for evaluating the biochemical properties of *A. dorsata* honeycombs.

Preparation of Tea Formulations

The combs of *A. dorsata* Binghami were first air-dried at room temperature for three days to facilitate gradual desiccation and minimize thermal degradation of bioactive compounds. The material was subsequently oven-dried at 75°C for approximately 45 minutes to achieve complete dehydration while preserving antioxidant integrity. The

dried combs were then ground to a fine powder using a laboratory grinder and passed through a 10-mesh sieve to ensure uniform particle size distribution.

Precisely 2 g of the powdered comb extract was weighed and combined with *M. fragrans* leaf extract at specified compositional ratios. Two formulations were prepared: Formula A, designed with a higher proportion of Apis dorsata Binghami comb extract, and Formula B, incorporating a relatively greater proportion of *M. fragrans* leaf extract. This compositional variation was intended to enable a comparative assessment of bioactivity profiles. The mixtures were homogenized under controlled conditions and subsequently enclosed in food-grade filter tea bags to protect against moisture, air, and microbial contamination, ensuring the preservation of bioactive compounds and compliance with food-contact safety standards (**Figure 1**). The detailed formulation ratios are presented in Supplementary Material (**Supplementary 1**).

DPPH Free Radical Scavenging Assay

The antioxidant effectiveness of the *A. dorsata* nest extract was evaluated utilizing the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. Serial dilutions of the extract were formulated in methanol at final concentrations of 10, 50, 100, 200, and 250 ppm. Each sample (500 µL) was combined with 500 µL of freshly made 1 mM DPPH solution in methanol, and the total volume was adjusted to 5 mL using methanol. The mixtures were incubated in darkness at 37 °C for 30 minutes, after which absorbance was measured at 515 nm using a PerkinElmer UV–Vis spectrophotometer. Butylated hydroxytoluene (BHT; Merck) served as a positive control at concentrations of 2, 4, 6, and 8 ppm. All experiments were conducted in duplicate. The proportion of radical scavenging activity was determined using the following equation:

$$\%\,\text{Inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

A control refers to the absorbance of the DPPH solution devoid of extract, while A sample denotes the absorbance in the presence of extract or standard. The extract concentration necessary to inhibit 50% of DPPH radicals (IC_{50}) was ascertained from the dose–response curve by linear regression analysis (Kurek-Górecka et al., 2022; Mokosuli et al., 2023).

Antidiabetic Assay

The tea–beehive (*A. dorsata* Binghami) combined dry extract was used as the test sample, dissolved in phosphate buffer (0.1 M, pH 7.0) to prepare stock solutions, which were then serially diluted to achieve final concentrations of 50, 100, 250, 500, and 1000 µg/mL for inhibition testing. The α-glucosidase inhibiting capacity of the extract was assessed following the procedure of Mayur et al. (2010) with modifications. The reaction mixture comprised phosphate buffer (0.1 M, pH 7.0), 0.5 mM 4-nitrophenyl-α-D-glucopyranoside (pNPG) as the substrate, varying quantities of the extract (50–1000 µg/mL), and α-glucosidase enzyme (0.2 U/mL). Following a 30-minute incubation at 37 °C, the reaction was halted with 0.2 M sodium carbonate, and the release of p-nitrophenol was measured at 410 nm using a Shimadzu UV-160 spectrophotometer. Blanks devoid of enzyme were utilized to adjust for background absorbance, whereas acarbose functioned as the positive control. The % inhibition was determined in relation to solvent-treated controls utilizing the formula:

$$\%\,\text{Inhibition} = \left(1 - \frac{A_{\text{sample}}}{A_{\text{control}}}\right) \times 100$$

IC_{50} values were calculated by four-parameter logistic (4PL) nonlinear regression of inhibition curves, defined as the extract concentration required to inhibit 50% of α-glucosidase activity, using GraphPad Prism software (version 9.0). All studies were conducted in triplicate (Uddin et al., 2022; Hernández-Martínez et al., 2024).

Cytotoxicity Assay on MCF-7 cells

Human breast cancer MCF-7 cells were cultivated in Dulbecco's Modified Eagle Medium (DMEM, high glucose) enriched with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. The cells were cultivated at 37°C in a humidified incubator containing 5% CO_2 (Thermo Scientific 8000DH Series) under aseptic conditions within a Class II biosafety cabinet (Thermo Scientific 1300 Series A2). Cell harvesting was conducted with Trypsin-EDTA (0.25%) and centrifugation was performed with a Micro CL17 centrifuge. The viability of the cells was evaluated through trypan blue exclusion. Absorbance measurements were conducted using a multimode plate reader (Tecan Infinite M200 PRO). Cisplatin (43.18 µM) was utilized as the positive control, while dimethyl sulfoxide (DMSO) was employed as the vehicle control (≤0.5% v/v).

The tea–beehive (*A. dorsata*) extract was prepared in a dry state, then diluted in DMSO (100 mg/mL), vortexed, and subsequently filtered through a 0.22 µm membrane. Serial two-fold dilutions were conducted in complete medium to achieve final concentrations ranging from 7.8125 to 1,000 µg/mL. The cells were inoculated at a density of 1.7 × 104 cells per well in 96-well plates. The plates were then incubated for 24 hours and subsequently subjected to extract dilutions in triplicate, along with cisplatin, vehicle, and medium-only controls. In an effort to mitigate edge effects, exterior wells were filled with PBS or medium, and controls comprising extract and Presto Blue™ without cells were incorporated to account for optical interference. Subsequent to a 48-hour exposure, the media were substituted with a Presto Blue™ working solution (10% reagent in full medium), and the plates were incubated for 1 to 2 hours in the dark at 37 °C. The quantification of resazurin reduction to resorufin by metabolically active cells was conducted at 570 nm, using 600 nm as a reference wavelength. Cell viability

was determined in relation to vehicle controls following background correction, whereas the calculation of cell viability as a percentage was performed by determining 100 minus the viability percentage.

The IC_{50} values were determined by fitting the sigmoidal dose–response data to a four-parameter logistic (4PL) regression model of the form: Y = Bottom + (Top − Bottom) / [1 + (IC_{50}/X)^HillSlope], where Y is the percent cell viability, X is the extract concentration, and the IC_{50} represents the concentration producing 50% inhibition of cell viability. All experiments were conducted in a minimum of three separate biological replicates, each including technical triplicates. The data are expressed as the mean ± standard deviation (SD). The statistical analyses employed one-way ANOVA followed by Tukey's Honest Significant Difference (HSD) post hoc test, with a significance level of $p < 0.05$ (Hermansyah et al., 2022).

Determination of Total Phenolic Content (TPC)

The total phenolic content (TPC) of the tea–beehive (*A. dorsata*) extract was quantified using the Folin–Ciocalteu colorimetric method, with some modifications to the standard method. Gallic acid was utilized as the calibration reference. For both standards and samples, 1.0 mL of solution was combined with 5.0 mL of 10% Folin–Ciocalteu reagent 4.0 mL of 7% (w/v) Na_2CO_3 solution to achieve a final volume of 10.0 mL. The reaction mixtures were vortexed and subsequently incubated in a water bath maintained at 40°C for a duration of 30 minutes. Following this, the absorbances were measured at a wavelength of 760 nm in comparison to a reagent blank, utilizing a UV-Vis spectrophotometer. All measures were conducted in triplicate, and the findings are expressed as the mean ± standard deviation (SD).

A calibration curve for gallic acid was established utilizing standard solutions at concentrations of 10, 30, 50, 70, and 100 milligrams per liter. The linear regression analysis of the calibration data yielded the equation A=0.0077C−0.0324 (where A represents the measured value of the sample's light absorption and C denotes the concentration of the sample in milligrams per liter), with an R^2 value of 0.9965, signifying exceptional linearity over the evaluated range. A series of sample extracts were prepared at a concentration of 25 mg·mL^{-1}, which was achieved by thoroughly dissolving 20.8 milligrams of dry extract in 25 milliliters of a suitable solvent. These extracts were then subjected to identical reaction and measurement conditions as the standards. The concentration of gallic acid equivalents in each sample solution (c, mg·L^{-1}) was derived by rearranging the calibration equation:

$$c = \frac{A - b}{m}$$

A represents the measured absorbance of the sample, b denotes the calibration intercept (−0.0324), and m signifies the slope (0.0077). For the depicted sample (absorbance = 0.016), c = (0.016 - (-0.0324)) / 0.0077 ≈ 6.29 mg·L^{-1}. TPC was quantified in milligrams of gallic acid equivalents per gram of dry extract (mg GAE·g^{-1}) and determined using:

$$\mathrm{TPC} = \frac{C \times V}{M}$$

where V represents the extract volume in milliliters (25.0 mL) and M denotes the mass of the dry extract in grams (0.0208 g). The sample TPC is approximately 7.56 mg GAE·pg^{-1}. All published TPC values denote the average of three independent measurements ± standard deviation; calibration and sample concentrations were derived using the aforementioned linear regression equation (Oses et al., 2020; Yayinie et al., 2022; Mokosuli et al., 2023).

Determination of Total Flavonoid Content (TFC)

The aluminum chloride colorimetric method was used to measure total flavonoids, and quercetin was used as a reference (Oses et al., 2020; Mokosuli et al., 2023). Quercetin solutions (1–20 μg·mL^{-1}) were formulated and subsequently treated with $AlCl_3$ reagent. Following a 30-minute incubation at room temperature, absorbance was recorded at 415 nm. The calibration curve exhibited linearity (y = 0.0488x – 0.0664; R^2 = 0.9915). The concentration of flavonoids in samples (c, mg·L^{-1}) was determined using the formula (A−b)/m, where A represents absorbance, m denotes the slope, and b indicates the intercept. TFC was quantified as mg quercetin equivalents per gram of dried extract (mg QE·g^{-1}) utilizing:

$$\mathrm{TFC} = \frac{c \times V \times f}{1000 \times m}$$

where V = extract volume (mL), f = dilution factor, and m = sample mass (g). Results were reported as mean ± SD of triplicates.

GC–MS Analysis

The chemical constituents of Formula A and Formula B were identified by gas chromatography–mass spectrometry (GC–MS). Dried extracts were derivatized using N,O-bis(trimethylsilyl)trifluoro acetamide (BSTFA) with 1% trimethyl chlorosilane (TMCS) prior to analysis. GC–MS analysis was performed using an Agilent 7890A/5975C instrument equipped with an HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm). Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The oven temperature was programmed from 60°C (held for 2 min) to 280°C at 10°C/min (held for 10 min). The injector temperature was set at 250°C. The mass spectrometer was operated in electron ionization (EI) mode at 70 eV. Compounds were identified by comparison with the NIST/EPA/NIH Mass Spectral Library (NIST 17) with a minimum match quality of 80%.

RESULTS AND DISCUSSION

Tea Bag Formulation of *Apis dorsata* Binghami Comb Extract

The tea bag formulation incorporating Apis dorsata Binghami nest extract was successfully developed as a novel functional beverage prototype (**Figure 1**). Two formulations (Formula A and Formula B) were prepared with different compositional ratios of comb extract and Myristica fragrans leaf extract, as described in the Materials and Methods section. The tea bag format was selected to provide a consumer-friendly delivery system that stabilizes bioactive compounds against oxidative degradation and ensures dosage consistency. The distinct compositional ratios of Formula A and Formula B enabled a comparative evaluation of how bee-derived phenolic fractions versus plant-derived phytochemicals influence the overall bioactivity profile of the formulation. Two formulations (Formula A and Formula B) were prepared with different ratios of *Apis dorsata* Binghami comb extract and *M. fragrans* leaf extract to evaluate the effect of compositional variation on biological activity. Formula A was designed with a higher proportion of Apis dorsata Binghami comb extract, while Formula B incorporated a relatively greater proportion of Myristica fragrans extract. This compositional variation enabled a comparative assessment of the bioactivity profiles associated with bee-derived phenolic-rich fractions and plant-derived synergistic phytochemicals. The combinations were enclosed in food-grade tea bags to safeguard against air, moisture, and microbial contamination. This technique ensured the preservation of bioactive substances and adherence to food-contact safety regulations (**Figure 1**).

Antioxidant Activity

According to the results of the DPPH assay, Formula A exhibited superior antioxidant activity in comparison to Formula B when both were administered at equivalent concentrations. At a concentration of 2,000 ppm, Formula A demonstrated an average inhibition of 33.44%, while Formula B exhibited an inhibition of only 23.44%. A consistent trend was observed at lower concentrations, with Formula A maintaining detectable activity (62.5 ppm: 8.75–8.13%), whereas Formula B exhibited lower inhibition (10.68–11.57%). Overall inhibition values for Formula B remained lower than those of Formula A at higher concentrations. The findings suggest that Formula A demonstrates higher antioxidant potential compared to Formula B. However, it should be noted that both formulas exhibit significantly lower effectiveness compared to pure vitamin C, which is used as the standard reference (see Appendix 1 for further details). The results demonstrated that vitamin C exhibited significantly enhanced radical scavenging capacity, achieving inhibition levels of 88.65–87.76% at 20 ppm and maintaining notable activity of 73.13–72.23% at 10 ppm. The substantial activity of vitamin C at low concentrations suggests its effective capacity for radical scavenging. The findings indicate that while Formula A surpassed Formula B in performance, both extracts demonstrated inferior antioxidant effectiveness compared to vitamin C. This suggests a necessity for optimizing the composition or processing methods of the extracts to improve their bioactive potential (see **Figure 2**).

IC_{50} Determination

According to the standard calibration curve, Formula A exhibited an IC_{50} value of 256.03 ppm, while Formula B demonstrated a lower IC_{50} of 119.48 ppm, indicating comparatively enhanced antioxidant activity. In comparison, vitamin C, serving as the positive control, exhibited a significantly higher potency, with an IC_{50} of merely 3.95 ppm. The findings indicate that while Formula B exhibited greater activity than Formula A, both extracts were significantly less effective than vitamin C, which effectively reduced DPPH radicals at minimal concentrations. The observed differences can be attributed to variations in phenolic and flavonoid content, as elevated levels of these bioactive compounds are closely associated with radical scavenging capacity, in line with previous studies on propolis and other bee-derived products documented in Food & Function (Fathi Hafshejani et al., 2023; Oren et al., 2024) and Antioxidants (Rendueles et al., 2023; Kardilag, 2025). The existing body of literature supports the hypothesis that bee comb extracts may serve as natural antioxidant sources. However, further optimization is necessary to achieve the same efficacy as the standard compound, vitamin C. To enhance the bioactivity of phenolic fractions, it is essential to optimize extraction methods and purification processes.

Figure 1. (**a**). *A. dorsata* Binghami nest. (**b**). Tea formula. (**c**). *Apis dorsata* nest extract tea packaging

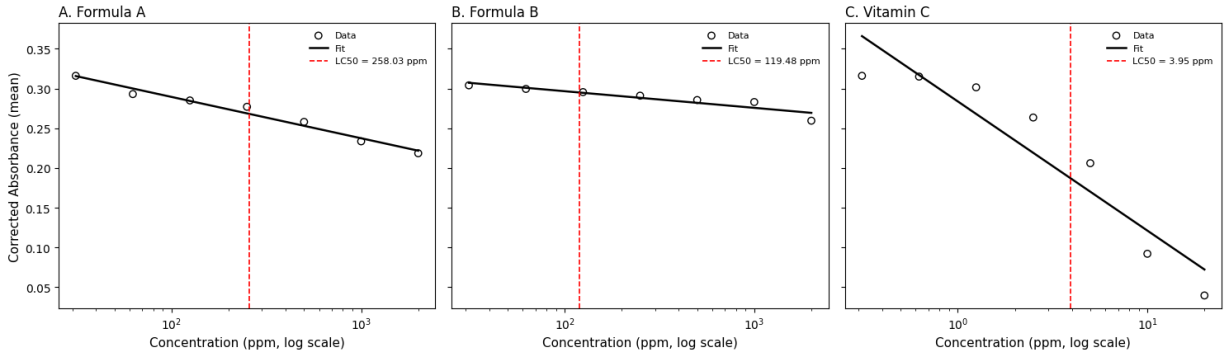


Figure 2. Regression analysis and IC_{50} of *A. dorsata* Binghami nest tea formula. (**a**). Formula A, (**b**). Formula B and (**c**). Vitamin C (ascorbic acid)

DPPH Radical Scavenging Capacity

As demonstrated in the experimental results, Formula A exhibited a higher degree of DPPH radical scavenging activity in comparison with Formula B across all of the tested concentrations. At a concentration of 2000 parts per million (ppm), Formula A exhibited approximately 33.4% inhibition, while Formula B demonstrated only 22.6% inhibition. However, both values were found to be significantly lower than those of vitamin C, which achieved over 70% inhibition at concentrations that were much lower (10–20 ppm). This pattern corresponds with recent findings that bee-derived products, including propolis, bee bread, royal jelly, and honey, exhibit significant variability in antioxidant activity based on their phenolic and flavonoid content as well as the extraction method employed. Recent research indicates that propolis exhibits the most potent DPPH activity, whereas honey and royal jelly show lesser effects, necessitating higher concentrations to reach levels comparable to vitamin C (Pobiega et al., 2023; Nakajima et al., 2009). The findings indicate that Formula A possesses a higher potential for development compared to Formula B. Moreover, the significance of optimizing extraction techniques or purifying phenolic fractions is emphasized, as recent research indicates that DPPH IC_{50} values of propolis extracts have been enhanced to levels comparable to those of ascorbic acid (Amin et al., 2023). It is noted that the inhibition of Formula A at the tested concentration range did not reach 50%, and thus the IC_{50} value of 256.03 ppm was obtained by linear extrapolation from the regression equation (% inhibition = a·C + b, where C is concentration in ppm). This approach, while an accepted approximation, yields an extrapolated IC_{50} value with inherent uncertainty. Future studies should employ higher concentration ranges or fractionated extracts to obtain sigmoidal dose-response curves suitable for robust IC_{50} determinationThe IC_{50} values for antioxidant activity were determined from dose–response regression equations. Formula A yielded an IC_{50} of 256.03 ppm (regression: $y = 0.1015x + 24.01$; $R^2 = 0.9823$), obtained by linear extrapolation since maximum inhibition did not reach 50% within the tested range. Formula B demonstrated an IC_{50} of 119.48 ppm (regression: $y = 0.2053x + 25.45$; $R^2 = 0.9764$), indicating comparatively greater antioxidant potency. Vitamin C (positive control) exhibited an IC_{50} of 3.95 ppm, confirming its superior radical scavenging capacity. The marked difference between the formulas and vitamin C reflects the lower concentration of pure phenolic antioxidants in the complex extract matrix.

Anticancer Activity

Cytotoxicity assays conducted on MCF-7 breast cancer cells demonstrated notable differences in antiproliferative efficacy between Formula A and Formula B. Formula A demonstrated a modest cytotoxic effect, as indicated by an IC_{50} value of 467.04 μg/mL. A substantial decline in cell viability was observed exclusively at elevated concentrations (e.g., 22.94% viability at 1,000 μg/mL), while viability exhibited comparatively high levels at intermediate concentrations (91.55% at 62.5 μg/mL and 73.78% at 250 μg/mL). In contrast, Formula B demonstrated a substantial increase in activity, exhibiting an IC_{50} value of 79.43 μg/mL. A substantial decline in viability was observed at 62.5 μg/mL, exhibiting a 50.53% reduction, while nearly complete cell death (0.25%) was recorded at 1000 μg/mL. This comparison demonstrates that Formula B exhibits a greater antiproliferative capacity compared to Formula A, although both are less effective than cisplatin (positive control), which decreased viability by approximately 44% at a concentration of 43.18 μM (**Figure 3**).

Interpretation of Cytotoxic Potential

The observed discrepancies in their cytotoxic potential can be attributed to the presence of varying bioactive metabolite content, particularly flavonoids, polyphenols, and other phenolic compounds, which have been identified as crucial contributors to anticancer activity (Ali et al., 2024; Wali et al., 2025). According to the extant literature, the extraction techniques and bee feeding sources employed significantly impact the phytochemical composition and cytotoxic potency of natural extracts (Mokosuli et al., 2023). The IC_{50} value of Formula B (<100 μg/mL) is indicative of moderate-to-strong activity based on

established criteria for plant extract activity, while Formula A (IC_{50} >400 μg/mL) is classified as weak. It is evident that Formula B exhibits promise as a natural extract-based anticancer candidate. Nevertheless, its efficacy is contingent upon enhancement through the optimization of fractionation, the purification of active compounds, or the potential combination with standard chemotherapeutic agents to achieve synergistic effects and minimize toxicity.

These findings suggest that Formula A exhibits limited cytotoxic potential; however, the observed biological activity at elevated concentrations indicates the presence of significant bioactive compounds that necessitate further investigation. Formula B, which demonstrated heightened toxicity, should be accorded priority for further investigation, thereby establishing both formulations as substantial contributors to the advancement of natural product–derived anticancer candidates. This development aligns with the global trend towards the exploration of safer alternatives to conventional chemotherapy.

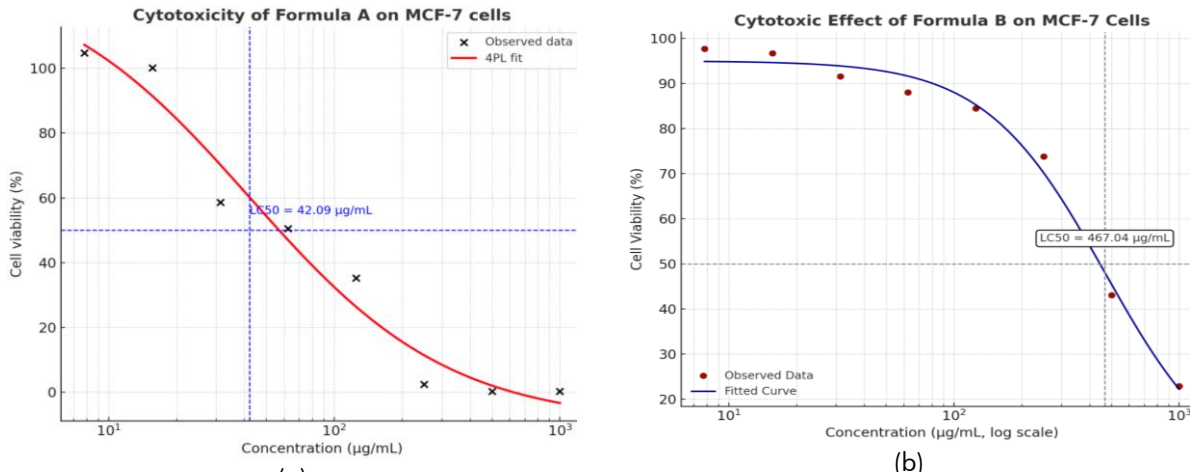
Morphological Observations on MCF-7 Cells

The morphological assessment of MCF-7 cells indicated that Formula A demonstrated greater cytotoxic effects compared to Formula B at medium to high concentrations (1,000–125 μg/mL), as evidenced by cell shrinkage, detachment from the substrate, and an increased number of dead cells. It was demonstrated that Formula B exhibited cytotoxic activity; however, its effects were less pronounced and more variable, indicating a comparatively lower anticancer potential (**Figure 4**). The observed differences can be attributed to variations in bioactive metabolites, including flavonoids, phenolic acids, and long-chain alcohols, in *A. dorsata* comb extracts. These metabolites have been identified as playing a critical role in antiproliferative mechanisms, particularly through the induction of apoptosis and cell-cycle arrest (Karundeng et al., 2024). This finding is consistent with the findings of recent studies indicating that bee-derived products, such as propolis, beeswax, and comb extracts, demonstrate notable anticancer effects on MCF-7 cells through the enhancement of ROS generation, promotion of DNA fragmentation, and modulation of apoptosis-related proteins like Bax and Bcl-2 (Alasmari, 2024). The findings suggest that Formula A has the potential to be a more promising candidate for development as an anticancer nutraceutical herbal tea. However, further in vivo and mechanistic studies are necessary to validate these results.

The IC_{50} value for Formula A (467.04 μg/mL) was obtained through 4PL curve extrapolation, as the observed cell viability at the maximum tested concentration (22.94% at 1,000 μg/mL) did not fully reach the lower asymptote of the sigmoidal curve. This extrapolated IC_{50} should be interpreted with caution and warrants confirmation through testing at higher concentrations or with fractionated extracts in future studies.

Antidiabetic Activity

In accordance with α-glucosidase inhibition assays, Formula A demonstrated average inhibitory activity ranging from 61.33% to 68.86%, while Formula B exhibited higher activity, ranging from 72.18% to 78.14%. The inhibitory effect of Formula B approached that of the positive control, Acarbose (96.40%), suggesting notable antidiabetic potential via the inhibition of carbohydrate hydrolysis (Supplementary Data 2). The observed discrepancy in activity can be attributed to variations in the composition of bioactive metabolites, particularly flavonoids, phenolics, and phenolic acids, which have been identified as capable of interfering with α-glucosidase activity (Nan et al., 2026; Shaker et al., 2023). These findings are consistent with the conclusions of earlier studies indicating that bee comb extracts and propolis can diminish digestive enzyme activity, consequently lowering postprandial glucose spikes (Hernández-Martínez et al., 2024; Gercek et al., 2024). Consequently, Formula B exhibits augmented potential as a natural adjuvant antidiabetic candidate derived from *Apis dorsata* Binghami comb extracts.



(a) (b)

Figure 3. LC_{50} curve of formula A for MCF-7 cells (**a**). Formula B. and (**b**). Formula A

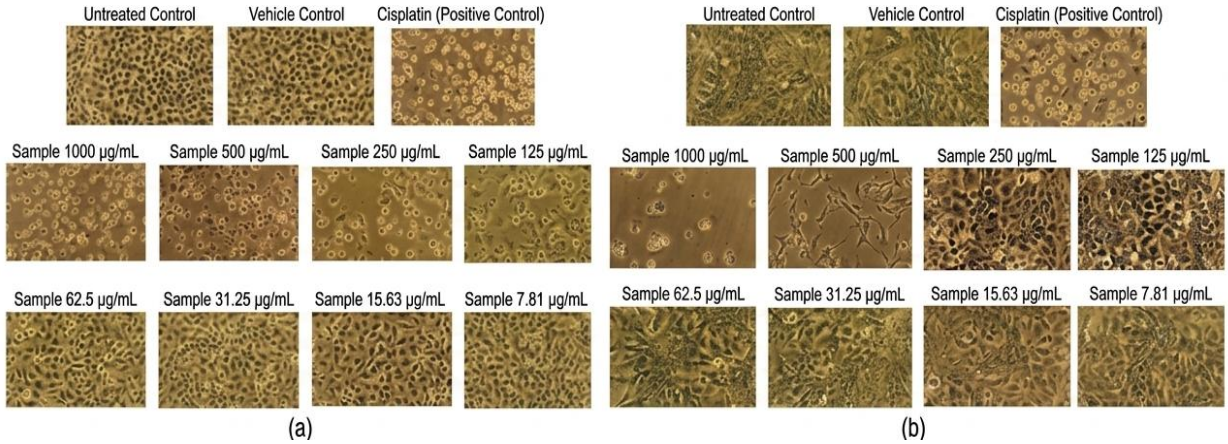


Figure 4. Formula A Morphological changes and cytotoxicity assessment of [cell type: MCF-7] cells treated with different formulations: (a) Formula B and (b) Formula A, at various concentrations 7.81 – 1000 µg/mL after 24 h. Untreated cells, 2% DMSO, and Cisplatin served as untreated, vehicle, and positive controls, respectively.

The IC_{50} values determined by 4PL nonlinear regression were approximately 2,828.44 ppm for Formula A and 476.82 ppm for Formula B, demonstrating a significantly stronger inhibitory effect for Formula B in comparison to Formula A, albeit still less potent than the synthetic control Acarbose (approximately 0.85 µM) (**Figure 5**). The observed discrepancy can be ascribed to the distinctive bioactive compounds present in the extracts. Phenolic compounds, flavonoids, and organic acids derived from bee-derived products have exhibited the capacity to impede the action of carbohydrate-digesting enzymes (Gercekl et al., 2024). Mokosuli et al. (2023) discovered that the presence of variability in secondary metabolites within local natural extracts has the potential to significantly enhance their antidiabetic properties by means of enzymatic inhibition mechanisms. From a pharmacological perspective, while Formula B exhibits lower potency compared to synthetic inhibitors, its bioactive profile underscores its potential as a safer natural α-glucosidase inhibitor, aligning with recent findings regarding the blood glucose-regulating effects of bee comb extracts (Bakour et al., 2021). The findings suggest that bee comb–based formulations have the potential to function as alternative adjuvant therapies for type 2 diabetes, with their effectiveness contingent upon the composition of secondary metabolites (Koodathil et al., 2023). It is acknowledged that the inhibition data for Formula B at lower concentrations were insufficient to fully define the lower asymptote of the sigmoidal curve, which may have introduced uncertainty in the IC_{50} determination. Future studies should include additional concentration data points in the lower inhibition range (e.g., 10–50 µg/mL) to obtain a complete sigmoidal dose–response profile and improve IC_{50} accuracy.

Bioactive Constituents

The GC–MS chromatogram exhibited several prominent peaks at retention times of approximately 5.3, 6.6, 8.0, 16.9, 19.2, 25.5, 35.5, 45.6, and 49.7 minutes, suggesting the presence of distinct compounds in the sample. The observed peak intensities indicate variations in relative concentrations, with prominent peaks occurring at 5–8 minutes, suggesting the presence of polar compounds with high solubility in the mobile phase (**Figure 6**). The presence of additional peaks at longer retention times (25–50 min) indicates the existence of more hydrophobic constituents that necessitate extended elution. The chromatographic profile indicates the chemical complexity of the extract and is consistent with recent studies that report a variety of natural bioactive metabolites, including flavonoids, phenolics, and organic acids, in bee-derived products (Anjos and Miguel, 2025; Mokosuli et al., 2023). The gas chromatography–mass spectrometry (GC–MS) data provide a foundational basis for subsequent structural elucidation. This is achieved through the comparison of the data with pure standards or the execution of complementary liquid chromatography–mass spectrometry (LC–MS) analyses. These approaches are employed to verify the identity of bioactive compounds associated with the observed biological activities.

The inclusion of *M. fragrans* leaf extract in the formulation may have contributed to the observed biological activities due to its known phytochemical composition, including flavonoids, terpenoids, and phenolic compounds. Previous studies have reported that Myristica fragrans exhibits antioxidant, anti-inflammatory, and anticancer properties, which could enhance the overall bioactivity of the formulation. However, it should be noted that the present study did not include a control formulation without *M. fragrans* extract. Therefore, the specific contribution of this component to the observed effects cannot be conclusively determined, and further studies are required to isolate its individual impact.

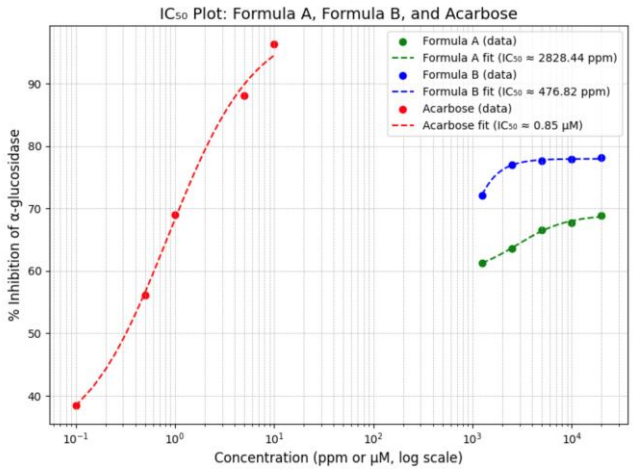


Figure 5. Comparison of IC_{50} formula A, formula B and positive control acarbose

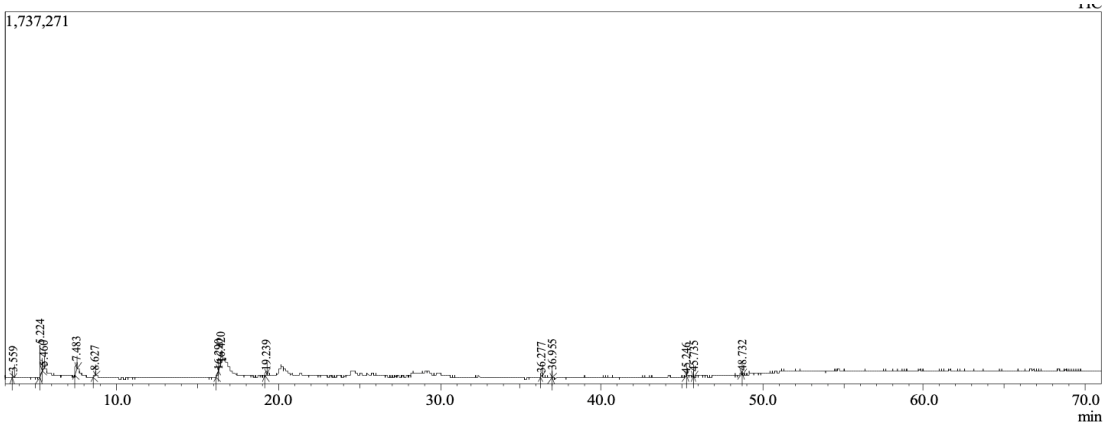


Figure 6. GC–MS chromatogram of formula A

GC–MS Analysis of Formula A

The GC–MS profile of Formula A (*A. dorsata* Binghami comb extract) exhibited a predominance of polar carbohydrate-related compounds. Notable peaks included glyceraldehyde, accounting for 50.08% and 3.36% area, and dihydroxyacetone (DHA) at 12.29%. Furthermore, the presence of glycerol, denoted as "V," and 1,2,3-propane-triol 1-acetate was also observed. The findings are consistent with the conclusions of earlier studies indicating that honey and comb matrices contain measurable sugar intermediates and polyols following derivatization, with DHA recognized as a precursor of methylglyoxal, which contributes to the antimicrobial properties of specific honeys. Furthermore, 1,2-cyclopentanedione (5.43% area) has been identified as an early Maillard reaction or degradation product of sugars. This compound has been frequently documented in GC–MS analyses of sugar-rich matrices exposed to heat or storage conditions. The non-polar fraction included fatty acids and wax-derived esters, notably n-hexadecanoate/palmitate (3.08% area) and ethyl palmitate (5.65%), as well as 1,7-hexadecadiene and the long-chain hydrocarbon dotriacontane (2.10–3.06% area). These compounds have been identified as characteristic markers of beeswax composition. This chemical pattern corresponds with the established constituents of beeswax, which primarily consist of fatty acid esters, hydrocarbons (particularly n-alkanes C20–C35 like dotriacontane), and free fatty acids. Palmitate is commonly identified as an indicator of ester hydrolysis. The combination of oxygenated carbohydrate derivatives (DHA, glyceraldehyde, glycerol), early Maillard intermediates (1,2-cyclopentanedione), and lipid wax markers (palmitate, ethyl palmitate, C>30 alkanes) collectively suggests that Formula A encompasses both nectar/honey-derived and wax-derived phytochemistry. This finding has the potential to impact bioactivity and source authentication, in line with recent evidence from gas chromatography–mass spectrometry (GC–MS) analysis of honey and beeswax matrices (see **Table 1**).

The identified compounds, including glyceraldehyde, dihydroxyacetone (DHA), and long-chain hydrocarbons, are consistent with previously reported chemical profiles of honeycomb and bee-derived products. For instance, glyceraldehyde and DHA are commonly associated with sugar metabolism in honey matrices and have been detected in various Apis species, reflecting nectar-derived biochemical pathways. Similarly, long-chain hydrocarbons such as dotriacontane and fatty acid esters like hexadecanoic acid are well-established constituents of beeswax, serving as structural and protective components of the

comb matrix. Previous studies have demonstrated that these compounds are widely distributed in honeycomb and propolis samples across different geographical regions, indicating their role as chemical markers of bee nest materials. The presence of these compounds in the current study confirms that the chemical profile of *A. dorsata* Binghami nest extract aligns with known bee-derived metabolite patterns, while also suggesting potential contributions to the observed biological activities, particularly through antioxidant and cytoprotective mechanisms.

GC–MS Analysis of Formula B

The gas chromatography–mass spectrometry (GC–MS) chromatogram of Formula B exhibited multiple prominent peaks, with a substantial signal detected at a retention time of approximately 45.3 minutes. This was accompanied by smaller peaks within the 34–54 minute range, while only minor compounds were identified at earlier retention times (less than 10 minutes) (**Figure 7**). This distribution indicates a preponderance of semi-volatile to non-volatile constituents of high molecular weight, which are commonly associated with lipids, esters, long-chain hydrocarbons, and wax derivatives derived from bee comb matrices. The prominent peak at approximately 45 minutes indicates the presence of long-chain fatty acids or their derivatives, which are commonly recognized as characteristic constituents of beeswax and propolis (Mazurek et al., 2019). The peaks identified between 48–52 minutes likely indicate a combination of long-chain hydrocarbons (C27–C33) or complex esters, which are commonly recognized as chemical markers for the authentication of honeybee products (Montaser et al., 2023). The chromatographic profile underscores the chemical intricacy of the extract, predominantly comprising lipophilic compounds that contribute to its structural wax properties and potentially augment its biological activities, consistent with recent GC–MS studies of beeswax and propolis metabolites.

Table 1. GC–MS analysis of chemical constituents in formula A.

Peak #	R.Time (min)	I.Time(min)	F.Time(min)	Area	Area (%)	Height	Height (%)	A/H	Mark	Compound name
1	3.559	3.52	3.61	47,900	2.73	25,844	6.40	1.85		2,2-Dimethoxylbutane
2	5.224	5.2	5.395	879,590	50.08	150,902	37.34	5.83	V	Glyceraldehyde
3	5.46	5.395	5.475	58,933	3.36	7,885	1.95	7.47		Glyceraldehyde
4	7.483	7.445	7.575	215,916	12.29	52,682	13.04	4.10		Dihydroxyacetone
5	8.627	8.585	8.71	95,401	5.43	25,930	6.42	3.68		1,2-Cyclopentanedione N-(1-Methoxycarbonyl-1-methylethyl)
6	16.29	16.195	16.315	51,366	2.92	8,000	1.98	6.42		1,2-Cyclopentanedione N-(1-Methoxycarbonyl-1-methylethyl)
7	16.42	16.315	16.44	47,350	2.70	5,695	1.41	8.31	V	Glycerin
8	19.239	19.2	19.33	89,343	5.33	23,300	5.77	3.83		1,2,3-Propanetriol, 1-acetate
9	36.277	36.245	36.355	54,071	3.08	16,873	4.18	3.21		n-Hexadecanoic acid
10	36.955	36.91	37.02	99,212	5.65	39,825	9.84	2.49		Hexadecanoic acid, ethyl ester
11	45.246	45.215	45.295	22,396	1.28	11,110	2.75	2.02		1,7-Hexadecadiene
12	45.735	45.695	45.79	53,790	3.06	20,614	5.10	2.61		Dotriacontane
13	48.732	48.69	48.78	36,935	2.10	15,414	3.81	2.40		Dotriacontane

Note: R.Time = retention time; I.Time = initial time; F.Time = final time; A/H = area-to-height ratio; V = verified match; GC–MS = gas chromatography–mass spectrometry.

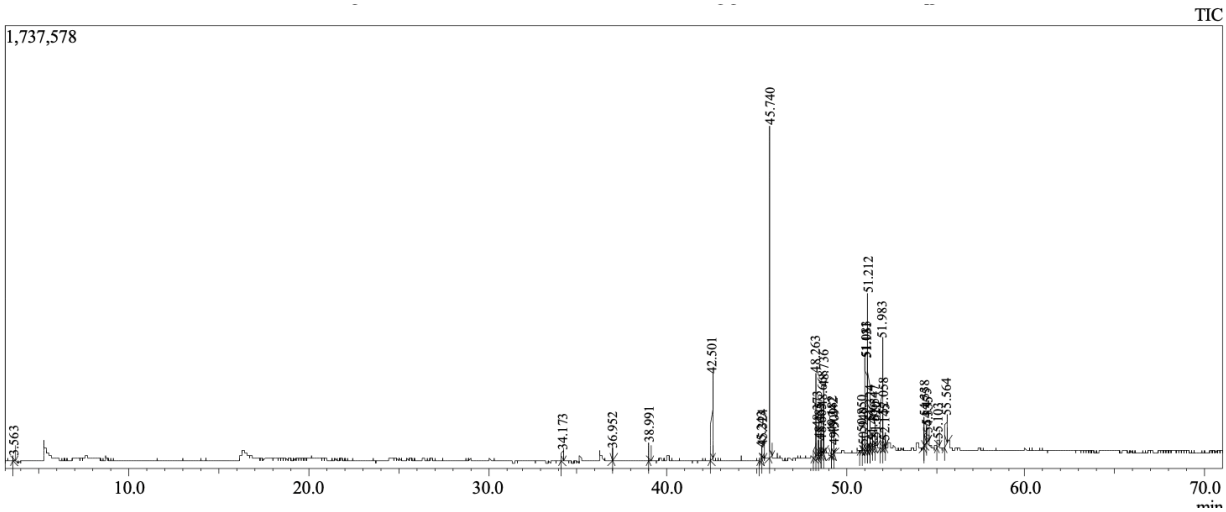


Figure 7. GC–MS chromatogram of formula B

The predominance of long-chain aliphatic alcohols such as hexacosanol and triacontanol in Formula B is consistent with previous reports on beeswax composition, where such compounds are known to contribute to lipid metabolism modulation and bioactive functionality. These compounds have also been reported to exhibit antioxidant and anti-inflammatory properties, which may partly explain the enhanced biological activity observed in this formulation.

The GC–MS analysis of Formula B revealed a predominance of long-chain aliphatic alcohols, specifically 1-heptacosanol, 1-hexacosanol, 1-octacosanol, and 1-triacontanol, which constituted 7.30–19.42% of the area. This was accompanied by esters such as hexadecanoic acid ethyl ester, saturated hydrocarbons including pentacosane, dotriacontane, and tetratetracontane, as well as unsaturated fatty acid derivatives like 8,11,14-eicosatrienoic acid (**Table 2**). Long-chain alcohols are essential components of beeswax, influencing its physicochemical properties and demonstrating bioactivities, including antioxidant and antimicrobial effects, as previously documented for propolis (Bankova et al., 2014; Huang et al., 2025). The identification of heavy n-alkanes (C27–C34) and fatty acid esters reinforces the chemical profile of beeswax, commonly employed as an authenticity biomarker for propolis (Martinotti et al., 2025). 1-Triacontanol has been recognized for its pharmacological potential, especially in the modulation of lipid metabolism and its cytoprotective activity (Sawaya et al., 2011). The findings indicate that the lipophilic fraction significantly contributes to the chemical profile of Formula B, aligning with the wax-derived composition of *A. dorsata* propolis and corroborating comparative studies on bee species.

The chemical constituents identified in both formulas show remarkable similarity to previously reported GC–MS profiles of Apis dorsata and Apis mellifera honeycomb matrices. Specifically, the dominance of glyceraldehyde and dihydroxyacetone in Formula A is consistent with findings by Guo et al. (2022), who reported high proportions of sugar-derived volatile compounds in Apis cerana honeycomb. Similarly, the long-chain aliphatic alcohols (1-hexacosanol, 1-heptacosanol) and n-alkanes (C_{25}–C_{34}) identified in Formula B are in close agreement with the established beeswax composition described by Bankova et al. (2014) and Martinotti et al. (2025). These chemical similarities confirm the authenticity of the Apis dorsata Binghami nest material and support the classification of the two formulas as representing distinct chemical fractions: a nectar/honey-derived polar phase (Formula A) and a wax-derived lipophilic phase (Formula B).

The observed differences in chemical composition between Formula A and Formula B, despite originating from the same Apis dorsata Binghami comb material, can be attributed to variations in formulation ratios and the differential extraction behavior of bioactive compounds. Formula A, which contained a higher proportion of polar components, was enriched in hydrophilic metabolites such as glyceraldehyde and dihydroxyacetone, reflecting nectar- and honey-derived fractions. In contrast, Formula B exhibited a higher abundance of lipophilic compounds, including long-chain alcohols, hydrocarbons, and fatty acid esters, which are characteristic of beeswax-derived components.

These differences may also be influenced by the interaction between the comb extract and *M. fragrans* leaf extract, which can alter the solubility and partitioning of phytochemicals during extraction and formulation. Similar variations in chemical profiles have been reported in previous studies on bee products, where extraction conditions and matrix composition significantly affect metabolite distribution. Therefore, the compositional differences observed in this study do not reflect inconsistencies in the raw material, but rather highlight the influence of formulation design and physicochemical interactions on the resulting bioactive profile.

Table 2. GC–MS analysis of chemical constituents in formula B.

Peak #	R.Time (min)	I.Time (min)	F.Time (min)	Area	Area (%)	Height	Height (%)	A/H	Mark	Compound name
1	3.563	3.535	3.595	29,291	0.17	17,199	0.30	1.70		2,2-Dimethoxybutane
2	34.173	34.13	34.24	109,579	0.63	41,696	0.73	2.63		8,11,14-Eicosatrienoic acid, (Z,Z,Z)-
3	36.952	36.91	37	100,079	0.58	42,865	0.75	2.33		Hexadecanoic acid, ethyl ester
4	38.991	38.945	39.06	165,994	0.96	68,070	1.19	2.44		Heneicosane
5	42.501	42.445	42.58	832,245	4.85	336,282	5.88	2.47		Pentacosane
6	45.243	45.19	45.285	142,500	0.83	48,228	0.84	2.95		1-Heptacosanol
7	45.324	45.285	45.375	123,122	0.71	49,010	0.86	2.51	V	1-Heptacosanol
8	45.74	45.675	45.83	3,353,077	19.42	1,306,776	22.85	2.57		Pentacosane
9	48.263	48.195	48.34	1,260,812	7.30	331,409	5.79	3.80	V	1-Hexacosanol
10	48.373	48.34	48.435	328,130	1.90	109,372	1.91	3.00	V	1-Hexacosanol
11	48.485	48.435	48.535	189,801	1.10	59,963	1.05	3.17	V	1-Hexacosanol
12	48.605	48.555	48.63	134,798	0.78	52,774	0.92	2.55	V	4-Hexadecanone
13	48.668	48.63	48.695	473,635	2.74	180,774	3.16	2.62		Octacosanol
14	48.736	48.695	48.805	751,384	4.35	258,653	4.52	2.90	V	Dotriacontane
15	49.182	49.13	49.215	265,882	1.54	88,264	1.54	3.01		13-Methylheptacosane
16	49.242	49.215	49.28	201,057	1.16	77,717	1.36	2.59	V	Dotriacontane
17	49.309	49.28	49.35	80,553	0.47	34,666	0.61	2.32	V	Triacontane, 1-iodo-
18	50.85	50.795	50.9	199,468	1.16	71,289	1.25	2.80	V	13-Docosen-1-ol, (Z)-
19	50.94	50.9	50.98	60,753	0.35	22,275	0.39	2.73		13-Docosen-1-ol, (Z)-
20	51.083	51.02	51.105	936,776	5.43	39,500	6.28	2.61		1-Hexacosanol
21	51.131	51.105	51.17	1,004,539	5.82	356,290	6.23	2.82	V	1-Hexacosanol
22	51.212	51.17	51.305	2,193,153	12.70	609,702	10.66	3.60	V	1-Hexacosanol
23	51.324	51.305	51.38	277,278	1.61	103,862	1.82	2.67	V	1-Hexacosanol
24	51.466	51.38	51.495	80,538	0.47	24,776	0.43	3.25		4-Tridecanone
25	51.547	51.495	51.585	278,455	1.61	98,210	1.72	2.84	V	Dotriacontane
26	51.62	51.585	51.675	101,325	0.59	29,453	0.51	3.44	V	10-Chloro-1-decanol, TBDMS derivati
27	51.983	51.915	52.04	1,565,833	9.07	429,630	7.51	3.64		13-Methylheptacosane
28	52.058	52.04	52.11	336,069	1.95	129,641	2.27	2.59	V	Tetrapentacontane
29	52.145	52.11	52.18	79,291	0.46	27,129	0.47	2.92	V	Tetrapentacontane
30	54.338	54.27	54.395	494,040	2.86	116,505	2.04	4.24	V	1-Hexacosanol
31	54.475	54.395	54.525	406,721	2.36	69,503	1.22	5.85	V	1-Hexacosanol

Peak #	R.Time (min)	I.Time (min)	F.Time (min)	Area	Area (%)	Height	Height (%)	A/H	Mark	Compound name
32	54.535	54.525	54.575	47,945	0.28	31,207	0.55	1.54		Octatriacontyl trifluoroacetate
33	55.103	55.045	55.145	86,827	0.50	23,678	0.41	3.67	V	Octacosanol
34	55.564	55.485	55.67	572,468	3.32	114,293	2.00	5.01		Tetrapentacontane

Note: R.Time = retention time; I.Time = initial time; F.Time = final time; A/H = area-to-height ratio; V = verified match; GC–MS = gas chromatography–mass spectrometry; TBDMS = tert-butyldimethylsilyl.

Total Phenolic Content

The analysis of total phenolic content indicated that Formula A exhibited greater levels of phenolics compared to Formula B, consistent with the polar chemical profile of Formula A as revealed by GC–MS analysis. It should be noted that the carbohydrate-derived compounds identified by GC–MS (glyceraldehyde, dihydroxyacetone, glycerol) are not phenolic compounds per se; rather, they serve as chemical markers of the nectar-derived and honey matrix components. The observed phenolic content is more likely attributable to phenolic acids and flavonoid-class compounds co-present in the polar fraction, consistent with previous reports on hive-derived materials (**Table 3**; **Figure 8**). This suggests that components derived from nectar and honey play a more significant role in phenolic accumulation in Formula A, while Formula B, characterized by lipophilic alcohols and hydrocarbons from beeswax, displayed reduced phenolic levels. The findings are consistent with earlier studies indicating that polar fractions of propolis and hive materials contain elevated levels of flavonoids and phenolic acids, known for their antioxidant, anti-inflammatory, and antimicrobial properties (Karlıdağ et al., 2025; Tiak Gajger and Vlainic, 2025). The reduced phenolic content in Formula B indicates its wax-rich composition, wherein long-chain alcohols and hydrocarbons serve structural and biological roles but are classified as non-phenolic components. The phenolic profile indicates that *A. dorsata* Binghami combs serve as a multifunctional source of secondary metabolites, with Formula A demonstrating enhanced potential as a reservoir of bioactive phenolic compounds. All TPC measurements were performed in triplicate (n=3), and results are expressed as mean ± SD

Total Flavonoid Content

The analysis of total flavonoid content indicated that Formula A had a concentration of 0.286% w/w, which is marginally greater than Formula B's concentration of 0.271% w/w (**Table 4**; **Figure 9**). The observed levels, while relatively low, confirm the presence of flavonoid-type secondary metabolites that contribute to the biological activity of the extracts. The values align with earlier research indicating flavonoid contents ranging from 0.2% to 1.0% w/w in hive materials, influenced by bee species, botanical sources, and environmental conditions (Gámbaro et al., 2025; Martinello et al., 2022). The slight variation between the two formulas may indicate differences in the composition of wax, resin, and honey, consistent with findings of chemical diversity among Apis species and their geographic origins (Mokosuli et al., 2023). These findings underscore the significance of *Apis dorsata* Binghami combs as a natural source of bioactive flavonoids, though at lower concentrations than those found in pure propolis extracts.

The contrasting study of *A. dorsata* Binghami nest extracts demonstrated significant variations in secondary metabolite composition between Formula A and Formula B. Formula A exhibited a higher flavonoid content (0.286% w/w compared to 0.271% w/w in Formula B), indicating its enrichment in polar metabolites such as glyceraldehyde, dihydroxyacetone, and glycerol, as identified by GC–MS, which are commonly linked to honey and nectar fractions. Conversely, Formula B was characterized by lipophilic components, such as long-chain alcohols (1-hexacosanol, 1-heptacosanol, 1-triacontanol), n-alkanes (C_{27}–C_{34}), and fatty acid esters, which serve as distinctive indicators of beeswax and propolis (Bankova et al., 2014). The chemical differences resulted in distinct functional outcomes, with phenolic- and flavonoid-rich fractions (Formula A) exhibiting a greater contribution to antioxidant capacity, whereas the lipophilic fraction (Formula B) offered structural relevance and additional bioactivities, including antimicrobial and lipid-modulating effects.

The tea formulation containing *A. dorsata* Binghami nest extract exhibited various bioactivities, such as increased antioxidant capacity, moderate cytotoxic effects on MCF-7 breast cancer cells, and notable inhibition of α-glucosidase. The antioxidant and cytoprotective effects are associated with the accumulation of phenolic and flavonoid compounds, known for their role in alleviating oxidative stress-related disorders, such as cancer and metabolic diseases (Ayad et al., 2025; Xu et al., 2025). The cytotoxic effect, while less potent than that of cisplatin, supports the hypothesis that synergistic polyphenols from both bee-derived and plant-derived sources can inhibit cancer cell proliferation while maintaining a favorable safety profile. The α-glucosidase inhibitory

Table 3. Total phenol content of *A. dorsata* Binghami nest extract tea formula

Sample Absorbance	Sample Concentration (mg/L)	Extraction Volume (mL)	Sample Weight (mg)	Dilution Factor (FP)	Sample Content (mg/g)	Sample Name
0.1123	10.3766	25	20.8	1	12.4719	Formula 1
0.1032	9.1948	25	20.8	1	11.0514	Formula 2

Note : FP (Dilution Factor) = 1 indicates that the sample solution was applied directly without additional dilution prior to the Folin–Ciocalteu colorimetric reaction. The sample stock was prepared at 25 mg/mL by dissolving 20.8 mg of dry extract in 25 mL solvent, and 1.0 mL of this solution was used directly in the assay.

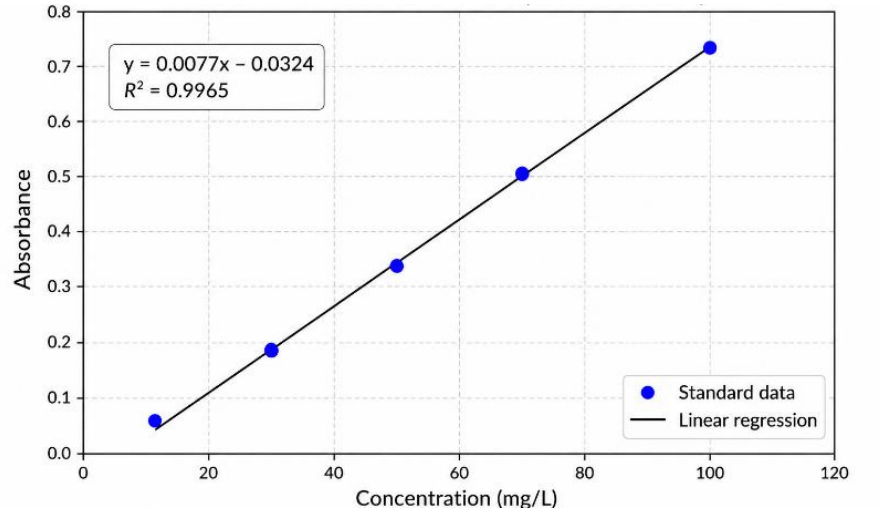


Figure 8. Calibration curve of gallic acid standard

Table 4. Total flavonoid content of *A. dorsata* Binghami nest extract tea formula

Sample Weight (g)	Absorbance	Slope (a)	Intercept (b)	Flavonoid Content (ppm)	FP	Volume (mL)	Flavonoid Content (% b/b)	Sample Name
0.3054	0.157	0.0488	-0.0664	4.577868852	6.25	100	0.286116803	Formula 1
0.3105	0.1454	0.0488	-0.0664	4.340163934	6.25	100	0.271260246	Formula 2

FP (Dilution Factor) indicates the dilution applied prior to flavonoid analysis. Flavonoid content was calculated based on the regression equation obtained from the quercetin calibration curve.

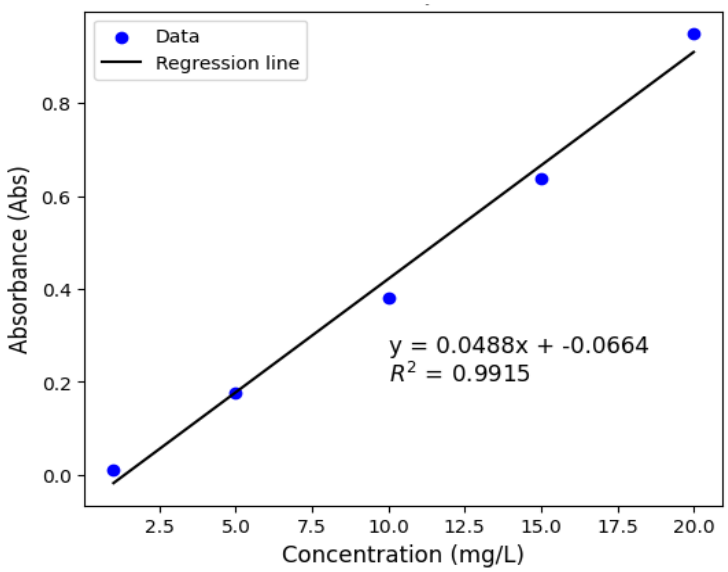


Figure 9. Quercetin calibration curve

activity underscores its potential role in regulating postprandial hyperglycemia, thereby enhancing its significance in diabetes management. This study represents the inaugural systematic evaluation of *A. dorsata* Binghami nest extract integrated into a tea matrix, highlighting its uniqueness as a culturally recognized and consumer-friendly formulation with potential applications in functional food and nutraceutical development.

One limitation of this study is the absence of a control group consisting of comb extract without the addition of *M. fragrans*. This limits the ability to definitively attribute the observed biological effects to synergistic interactions between the components. Future studies should incorporate comparative formulations to better elucidate the individual and combined effects of each component.

CONCLUSIONS

This study demonstrates that tea formulations enriched with *A. dorsata* Binghami nest extract exhibit measurable antioxidant, cytotoxic, and α-glucosidase inhibitory activities. These effects are associated with phenolic and flavonoid content and are influenced by formulation composition. The findings highlight the potential of integrating honeycomb-derived materials into functional beverages; however, further studies are required to elucidate the individual contributions of each component and to validate these effects *in vivo*.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Mokosuli Yermia Semuel: Writing – original draft, Software, Methodology, Formal analysis, Conceptualization. Alva Sahiri Supit: Writing – review & editing. Herry Maurits Sumampouw: Writing – review & editing, Supervision, Project administration, Funding acquisition. Mokosuli Yermia Semuel and Herry Maurits Sumampouw: Writing – review & editing, Software, Investigation.

AKNOWLEDGMENTS

The author would like to thank the Directorate of Research and Community Service, Ministry of Higher Education, Science and Technology, Republic of Indonesia for funding this research through the Regular Fundamental Research scheme in 2025 with Contract Number: 882/UN41.9/TU/2025. The author would also like to thank the leadership and staff of the Bioactivity and Molecular Biology Laboratory, Faculty of Mathematics and Earth Sciences, Manado State University for permitting the use of the research laboratory.

CONFLICT OF INTEREST

All authors hereby declare that there is no conflict of interest in the research, data processing and preparation of this research article.

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