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# Identification of Bioactive Constituents in Forest Honey Using GC-MS and Anti-Inflammatory Activity Through Protein Denaturation

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## Abstract
Honey is a natural product rich in bioactive compounds with various pharmacological and anti-inflammatory effects. Bee species and nectar sources influence the composition of bioactive compounds in honey. This study aimed to identify bioactive compounds and evaluate the anti-inflammatory activity of honey from *Apis mellifera* and *Apis dorsata*. Compound identification from both honey types was performed using Gas Chromatography-Mass Spectrometry (GC-MS). At the same time, anti-inflammatory activity was assessed through the human red blood cell (HRBC) membrane stabilization method and protein denaturation inhibition assay, with sodium diclofenac as a positive control. GC-MS analysis revealed various bioactive compounds, notably 2-Propenoic acid, 3-(4-methoxyphenyl)-2-ethylhexyl ester, consistently present in both honey types. In the HRBC assay, *Apis mellifera* honey showed the highest stabilization value of 94.14% at a concentration of 6.25 mg/L honey solution, surpassing sodium diclofenac (87.96%), while *Apis dorsata* honey reached 85.21%. All samples achieved over 95% stabilization at 100 mg/L. In the protein denaturation assay, *Apis dorsata* honey exhibited stronger anti-inflammatory potential with an $IC_{50}$ of 12.85 ± 1.477 mg/L compared to *Apis mellifera* (25.35 ± 0.024 mg/L). In conclusion, both types of honey possess promising natural anti-inflammatory potential through membrane stabilization and protein protection mechanisms.

**Keywords:** anti-inflammatory, bioactive compounds, honey, HRBC, protein denaturation

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# Identifikasi Konstituen Bioaktif dalam Madu Hutan Menggunakan GC-MS dan Aktivitas Antiinflamasi melalui Denaturasi Protein

## Abstrak
Madu merupakan produk alami yang kaya senyawa bioaktif dengan beragam aktivitas farmakologis, salah satunya sebagai antiinflamasi. Kandungan senyawa dalam madu dipengaruhi oleh jenis lebah dan sumber nektarnya. Penelitian ini bertujuan mengidentifikasi senyawa bioaktif serta mengevaluasi aktivitas antiinflamasi madu *Apis mellifera* dan *Apis dorsata*. Identifikasi senyawa dilakukan menggunakan *Gas Chromatography-Mass Spectrometry* (GC-MS), sementara aktivitas antiinflamasi diuji melalui metode stabilisasi membran eritrosit (HRBC) dan penghambatan denaturasi protein, dengan natrium diklofenak sebagai kontrol positif. Hasil GC-MS mengungkap keberadaan berbagai senyawa bioaktif, di antaranya 2-Propenoic acid, 3-(4-methoxyphenyl)-2-ethylhexyl ester yang ditemukan pada kedua jenis madu. Pada uji HRBC, madu *Apis mellifera* menunjukkan stabilitas tertinggi sebesar 94,.14% pada konsentrasi 6,.25 mg/L, melebihi natrium diklofenak (87,96%), sedangkan madu *Apis dorsata* sebesar 85,.21%. Semua sampel mencapai stabilitas di atas 95% pada konsentrasi 100 mg/L. Uji denaturasi protein menunjukkan madu *Apis dorsata* memiliki potensi lebih baik dengan $IC_{50}$ 12,.85 ± 1,.477 mg/L dibandingkan *Apis mellifera* (25,.35 ± 0,.024 mg/L). Kesimulannya, kedua jenis madu berpotensi sebagai agen antiinflamasi alami melalui stabilisasi membran dan proteksi protein.

**Kata Kunci:** antiinflamasi, denaturasi protein, HRBC, madu, senyawa bioaktif

## 1. Introduction

Honey is a natural product produced by various bee species and has long been recognized for its health-promoting properties, particularly its anti-inflammatory effects.[1,2] Two of the most frequently studied species in scientific research are Apis mellifera and Apis dorsata, which produce honey with distinct chemical profiles that can influence their biological activities and therapeutic efficacy. The chemical composition of honey is highly complex. It is determined by the botanical diversity of the nectar sources visited by the bees, resulting in a product rich in bioactive constituents, including flavonoids, phenolic compounds, and terpenoids.[3,4] More than 200 active compounds have been identified in honey, establishing it as a natural source with significant pharmacological and therapeutic potential.[2,5] These bioactive compounds are crucial in mediating various pharmacological effects, particularly in alleviating inflammation associated with chronic diseases such as cardiovascular disorders, diabetes, and autoimmune conditions.[6]

Inflammation is the body's biological response to infection and injury, involving the activation of cells, blood vessels, proteins, and various mediators to eliminate inflammatory agents such as microorganisms, physical trauma, tissue necrosis, immune reactions, and systemic disturbances.[7] This process plays a crucial role in initiating tissue repair mechanisms and is typically characterized by classical signs, including swelling (tumor), elevated temperature (calor), pain (dolor), and redness (rubor). Inflammation can be localized or systemic, depending on its underlying cause and the severity of the condition.[8] Although anti-inflammatory drugs are widely used to manage inflammatory symptoms, prolonged use is associated with potential adverse effects, including gastric ulcers, gastrointestinal disturbances such as constipation, and an increased risk of renal impairment.[7,8]

Chemical constituents were identified using Gas Chromatography-Mass Spectrometry (GC-MS), a widely recognized analytical technique for the accurate and efficient separation and identification of volatile compounds.[9,10] This method is particularly suitable for analyzing the chemical composition of honey, given the unique characteristics of each bee species in producing distinct bioactive constituents. Unlike LC-MS, which is more suitable for analyzing non-volatile and thermolabile compounds such as flavonoids and glycosides, GC-MS was chosen in this study because honey contains a wide variety of volatile and semi-volatile compounds, including terpenes, aldehydes, and esters, that significantly contribute to its biological activities, such as anti-inflammatory potential.[8,11] Two in vitro assays were employed to further assess the anti-inflammatory potential of the honey samples: the human red blood cell (HRBC) membrane stabilization assay and the protein denaturation assay. The HRBC assay evaluates the ability of honey to stabilize red blood cell membranes, which are highly susceptible to damage under inflammatory conditions.[12] At the same time, the protein denaturation assay investigates the capacity of honey to inhibit structural alterations in proteins typically induced by inflammatory processes.[13] The combination of these methods is expected to provide a comprehensive evaluation of the potential of honey as a natural anti-inflammatory agent based on its chemical profile.

A comparative evaluation of the anti-inflammatory activities of Apis mellifera and Apis dorsata honey is essential, as both types are widely utilized in traditional and modern therapeutic practices, yet differ in their chemical compositions. Although the health benefits of honey have been extensively studied, direct comparative investigations focusing on the bioactive constituents and anti-inflammatory potential of these two types of honey remain limited. This study, therefore, offers novel insights in this area. This study aims to identify the bioactive compounds in honey derived from Apis mellifera and Apis dorsata and evaluate their potential anti-inflammatory activities. Applying gas chromatography-mass spectrometry (GC-MS) facilitates the identification of active compounds. At the same time, the HRBC membrane stabilization and protein denaturation assays are employed to assess the effectiveness of honey in mitigating inflammatory responses. Through this integrated approach, a deeper understanding of the mechanisms underlying honey's anti-inflammatory effects can be achieved, potentially supporting the development of honey-based natural therapeutic agents. The findings of this research are expected to contribute valuable information to the existing body of scientific literature and promote the optimized use of Apis mellifera and Apis dorsata honey in health-related applications.

## 2. Materials and Method

### 2.1. Tools

The instruments utilized in this study included an autoclave, an incubator (Bio-Rad®), a microplate reader (Bio-Rad Mark™), a centrifuge (Boeico®, Germany), a UV-Visible spectrophotometer (Techcomp® 2501, Shanghai), and a Gas Chromatography-Mass Spectrometry (GC-MS) system (Agilent 8890 GC coupled with Xevo® TQ-GC, Waters®, UK)

### 2.2. Materials

The materials used in this study included honey

samples from *Apis mellifera* and *Apis dorsata*, sourced from Sidoarjo, East Java, Indonesia. Diclofenac sodium was obtained from Phapros®, Semarang, Indonesia. Other chemicals used were glacial acetic acid, ethyl acetate, sodium, and n-hexane for GC analysis, all obtained from Merck, Germany. Bovine serum albumin and Trisbase were sourced from Himedia®, India.

### 2.3. Methods

#### 2.3.1. Sample Preparation

The honey samples used in this study consisted of honey from *Apis mellifera* and *Apis dorsata*, collected in Sidoarjo, East Java, Indonesia. The identification of bee species was ensured through direct confirmation from local experienced beekeepers, based on morphological characteristics of the bees and the traditional hives used for each species. One gram of honey was dissolved in 10 mL of distilled water and sonicated using a sonicator (Elmazonik®, India) for 30 minutes at room temperature until it was completely homogenized. Following sonication, the solution was filtered through filter paper and transferred into a 100 mL volumetric flask, where it was then diluted with distilled water to the calibration mark to obtain a final concentration of 10,000 mg/L. The prepared solution was subsequently stored at a temperature of 2–4°C until further use for anti-inflammatory activity assays.[14]

#### 2.3.2. Compound Analysis using Gas Chromatography-Mass Spectrometry (GC-MS)

The GC-MS analysis of the honey samples was performed based on a modified procedure adapted from Joshna.[15] A total of 1 gram of each Apis mellifera and Apis dorsata honey sample was weighed and dissolved in 2 mL of distilled water. The mixture was homogenized using a shaker for 10 minutes, until it was fully dissolved. Subsequently, the solution was extracted by adding 10 mL of ethyl acetate, along with 10 grams of anhydrous sodium sulfate (Merck®, Germany), to remove residual water content. To optimize the release of bioactive compounds, the extraction process was accelerated by sonication for 10 minutes. The resulting mixture was filtered through filter paper to separate the liquid extract from solid residues. The filtrate was subjected to further evaporation via sonication until the ethyl acetate solvent was removed entirely. The resulting dry residue was redissolved in 1 mL of n-hexane and transferred into clean, tightly sealed GC vials for subsequent analysis.[16]

The identification of volatile compounds in the honey samples was performed using a Gas Chromatography-Mass Spectrometry (GC-MS) system (Agilent 8890 GC, Xevo® TQ-GC, UK) equipped with a DB-5MS capillary column (30 m × 250 µm × 0.25 µm). Helium was used as the carrier gas at a constant flow rate of 1 mL/min. The oven temperature program was initiated at 110°C with a hold time of 3.5 minutes, followed by a temperature increase (ramp 1) of 10°C per minute up to 200°C (held for 1 minute), then continued (ramp 2) at 5°C per minute to a final temperature of 280°C, maintained for 12 minutes. The total run time for the analysis was 41.5 minutes. The splitless injection mode was applied at an inlet temperature of 280°C, with an injection volume of 1 µL. Detection was carried out in scan mode over a mass range of 50–500 m/z using Electron Ionization (EI+) at an ion source temperature of 200°C and an interface temperature of 250°C.

#### 2.3.3. Red Blood Cell Membrane Stabilization Assay

The anti-inflammatory activity was evaluated using the erythrocyte membrane stabilization assay, based on the method described by Sukmawati *et al*. (2023)[13] with slight modifications. Fresh blood samples were collected from healthy volunteers, supplemented with an anticoagulant. The blood was centrifuged at room temperature at 3,000 rpm for 10 minutes to separate the red blood cells (erythrocytes). The erythrocyte pellet was washed with an isotonic solution (isotonic saline) and then resuspended to a 10% v/v suspension in the same solution. Subsequently, 0.5 mL of the erythrocyte suspension was mixed with 1 mL of phosphate-buffered saline (PBS) at pH 7.4, 2 mL of hypotonic saline, and 1 mL of the honey sample solution at concentrations of 6.25, 12.5, 25, 50, and 100 µg/mL. The mixture was incubated at 56°C for 30 minutes. For the positive control, sodium diclofenac was used at the same concentrations. After incubation, the mixture was centrifuged at 5,000 rpm for 10 minutes. The resulting supernatant was analyzed using a microplate reader at a wavelength of 450 nm.[7] The percentage of hemolysis and the erythrocyte membrane stabilization index were calculated using the following formulas (1) and (2):

$$\% \text{ Hemolysis} = \left[ \left( \frac{\text{Sample absorbance}}{\text{Negative control absorbance}} \right) \times 100 \right] \quad (1)$$

$$\% \text{ Stability} = 100 - \left[ \left( \frac{\text{Sample absorbance}}{\text{Negative control absorbance}} \right) \times 100 \right] \quad (2)$$

#### 2.3.4. Protein Denaturation Assay

The anti-inflammatory activity based on the protein denaturation inhibition mechanism was assessed according to the method outlined by Yodha *et al*. (2024)[12], with the following steps. Tris-buffered saline (TBS) was prepared by dissolving 870 mg of sodium chloride (Merck, Germany) and 120 mg of tris base (Merck, Germany) in 100 mL of distilled water. For the bovine serum albumin (BSA) solution, 200 mg of BSA was dissolved in 100 mL of TBS, and the pH was adjusted to the range of 6.2–6.5 using glacial acetic

acid. The positive control was prepared by dissolving 50 mg of sodium diclofenac in 20 mL of ethanol and then diluting it to a final volume of 100 mL to obtain a 500 ppm stock solution. This stock solution was then serially diluted to prepare concentrations of 100 ppm, 50 ppm, 25 ppm, 12.5 ppm, 6.25 ppm, and 3.125 ppm. Meanwhile, the honey test solution was prepared by dissolving 1 gram of honey in 10 mL of 70% ethanol, then diluting it to a final volume of 100 mL to obtain a 10,000 mg/L stock solution, which was also serially diluted to the same concentrations. The assay was conducted by mixing 2 mL of the sample solution or positive control with 2 mL of 0.2% BSA solution. The mixture was incubated for 30 minutes at 25°C, then heated for 5 minutes at 72°C, and subsequently cooled for 25 minutes at room temperature. The absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 660 nm. The percentage of protein denaturation inhibition was calculated using the following formula (3):

% anti-imflammatory activity inhibition

$$= \frac{Absorbance\ of\ control - Absorbance\ of\ Sample}{Absorbance\ of\ control} \times 100 \quad (3)$$

The inhibition percentage results for each concentration were plotted into a linear regression curve to determine the $IC_{50}$ value (the concentration required for 50% inhibition). Samples exhibiting inhibition greater than 20% were considered to possess anti-inflammatory activity.

2.3.5. Statistical analysis

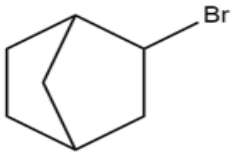
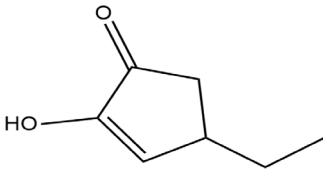
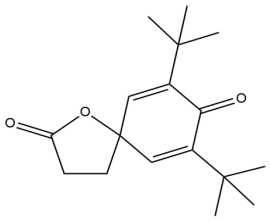
Statistical analysis was performed using SPSS software version 29. The anti-inflammatory activity data were analyzed using One-Way ANOVA to determine significant differences between treatment groups based on the type of honey (*Apis mellifera* and *Apis dorsata*) and the concentration variations (6.25, 12.5, 25, 50, and 100 µg/mL). If the ANOVA results indicated a significant difference ($p < 0.05$), post hoc Tukey's test was conducted to identify which groups showed significant differences. A p-value of less than 0.05 was considered statistically significant.

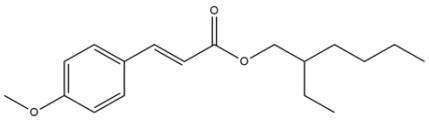
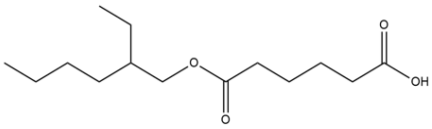
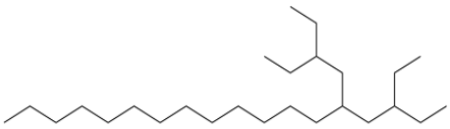
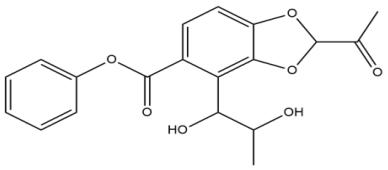
## 3. Result

### 3.1. Compound Analysis using Gas Chromatography-Mass Spectrometry (GC-MS)

The chemical composition of *Apis mellifera* and *Apis dorsata* honey was analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). The volatile compounds detected not only contribute to the aroma and flavor of the honey,[17] but also exhibit important biological activities, such as antioxidant, anti-inflammatory, antimicrobial, and even anticancer properties.[18–20] The chemical structures of the secondary metabolites identified were determined by matching mass spectra with the library database, as shown in Tables 1 and 2. The chromatographic profiles of both honey types revealed distinct retention

**Table 1.** Chemical Composition of Apis mellifera Honey Based on GC-MS Analysis

| Retintion Time | Molecular Weight | Match | Reverse Match | Probability (%) | Formula | Compounds Name | Biological Activity |
|---|---|---|---|---|---|---|---|
| **3.235** | 174 | 714 | 717 | 18.9 | $C_7H_{11}Br$ | 2-Norbornyl bromide  | Anticancer[21] |
| **5.762** | 126 | 596 | 718 | 13.1 | $C_7H_{10}O_2$ | 4-Ethyl-2-hydroxycyclopent-2-en-1-one  | Antioxidant[22] |
| **15.229** | 276 | 722 | 755 | 52.2 | $C_{17}H_{24}O_3$ | 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-di-ene-2,8-dione  | Anticancer[21] |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **21.601** | 290 | 731 | 767 | 34.3 | $C_{18}H_{26}O_3$ | 2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester | Anticancer[21], anti-imflammatory, antioxidant[23] |
| | | | | | |  | |
| **22.700** | 258 | 671 | 694 | 25.0 | $C_{14}H_{26}O_4$ | Hexanedioic acid, mono(2-ethylhexyl) ester | Anti-imflammatory, antioxidant[23] |
| | | | | | |  | |
| **25.956** | 366 | 587 | 592 | 6.47 | $C_{26}H_{54}$ | Octadecane, 3-ethyl-5-(2-ethylbutyl)- | Anticancer[21] |
| | | | | | |  | |
| **28.511** | 358 | 588 | 776 | 4.02 | $C_{12}H_{14}O_3$ | 2-Acetyl-4-(1,2-dihydroxypropyl) phenyl 1,3-benzodioxole-5-carboxylate | Anti-imflammatory, anticancer[24] |
| | | | | | |  | |

time patterns for the volatile compounds, which were clearly recorded in the GC-MS separation results, as illustrated in Figure 1.

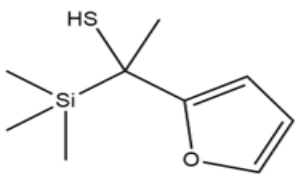
### 3.2. Red Blood Cell Membrane Stabilization Assay

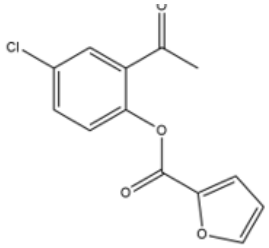
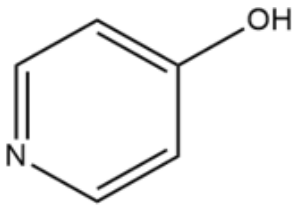
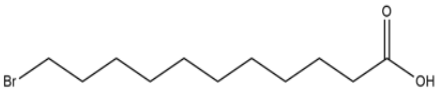
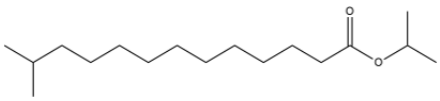
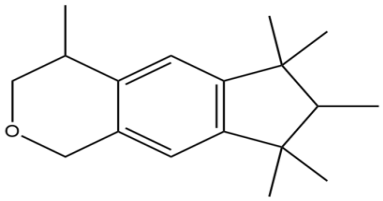
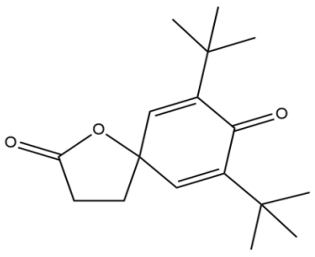
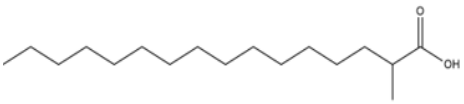
The percentage of red blood cell membrane stability and the degree of hemolysis induced by treatment with various concentrations of honey, along with sodium diclofenac as a positive control, are presented in Figure 2. The *in vitro* anti-inflammatory activity of both honey types compared to sodium diclofenac is visualized through the percentage of membrane stability at various concentrations in this figure.

### 3.3. Protein Denaturation Assay

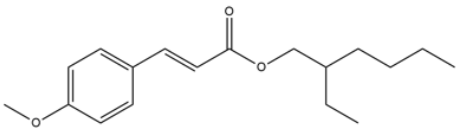
This study demonstrates that both *Apis mellifera* and *Apis dorsata* honey significantly ($p < 0.05$) inhibit protein denaturation. This activity is reflected in the reduction of absorbance values and the increase in inhibition percentage. The effectiveness of honey in inhibiting the inflammatory process is further supported by the $IC_{50}$ values obtained from the protein denaturation assay, as illustrated in Table 3 and Figure 3. Both *Apis mellifera* and *Apis dorsata* honey inhibited protein denaturation, indicating their potential anti-inflammatory activity. Among them, the sample with the lower $IC_{50}$ value demonstrated stronger inhibitory effects.

**Table 2.** Chemical Composition of Apis dorsata Honey Based on GC-MS Analysis

| Retintion Time | Molecular Weight | Match | Reverse Match | Probability (%) | Formula | Compounds Name | Biological Activity |
|---|---|---|---|---|---|---|---|
| **3.230** | 200 | 704 | 709 | 20.0 | $C_9H_{16}OSSi$ | 1-(Furan-2-yl) ethanethiol, TMS | Antioxidant[25] |
| | | | | | |  | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **3.230** | 264 | 684 | 687 | 10.9 | $C_{13}H_9ClO_4$ | 2-Acetyl-4-chlorophenyl 2-furoate | Anti-imflammato-ry,[26] antimicrobial[27] |
| | | | | | |  | |
| **3.230** | 95 | 680 | 690 | 9,76 | $C_5H_5NO$ | 4-Pyridinol | Anti-imflamma-tory[28] |
| | | | | | |  | |
| **13.131** | 264 | 574 | 585 | 6.34 | $C_{11}H_{21}BrO_2$ | 11-Bromoundecanoic acid | Anti-imflamma-tory[29] |
| | | | | | |  | |
| **14.042** | 270 | 678 | 701 | 23.4 | $C_{17}H_{34}O_2$ | Isopropyl 12-methyl-tridecanoate | Anti-imflamma-tory[30] |
| | | | | | |  | |
| **14.428** | 258 | 783 | 800 | 84.8 | $C_{18}H_{26}O$ | Cyclopenta[g]-2-benzopyran, 1,3,4,6,7,8-hex-ahydro-4,6,6,7,8,8-hexamethyl_ | Neuroprotektive[31] |
| | | | | | |  | |
| **15.234** | 276 | 753 | 775 | 68.5 | $C_{17}H_{24}O_3$ | 7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-di-ene-2,8-dione | Anticancer[32] |
| | | | | | |  | |
| **15.500** | 270 | 705 | 714 | 20.0 | $C_{17}H_{34}O_2$ | Hexadecanoic acid, methyl ester | Anti-imflammatory, antibacterial[33] |
| | | | | | |  | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **21.588** | 290 | 730 | 763 | 43.3 | $C_{18}H_{26}O_3$ | 2-Propenoic acid, 3-(4-methoxyphenyl)-, 2-ethylhexyl ester | Anticancer, anti-imflammatory[34] |



## 4. Discussion

The Gas Chromatography-Mass Spectrometry (GC-MS) analysis successfully identified the profiles of bioactive volatile compounds present in *Apis mellifera* and *Apis dorsata* honey. Both honey types were confirmed to contain various compounds associated with pharmacological effects, particularly anti-inflammatory activity. One of the primary compounds consistently detected in both honey samples was 2-Propenoic acid, 3-(4-methoxyphenyl)-2-ethylhexyl ester, though with varying relative abundances (based on peak area %). This finding aligns with previous reports indicating that the chemical composition of honey is primarily influenced by the bee species and the floral sources of nectar, contributing to the unique chemical characteristics of each honey type.[35] The variation in compound content is believed to contribute to the differences in anti-inflammatory potential observed between *Apis mellifera* and *Apis dorsata* honey.

The red blood cell membrane stabilisation (HRBC) assay revealed a significant difference between

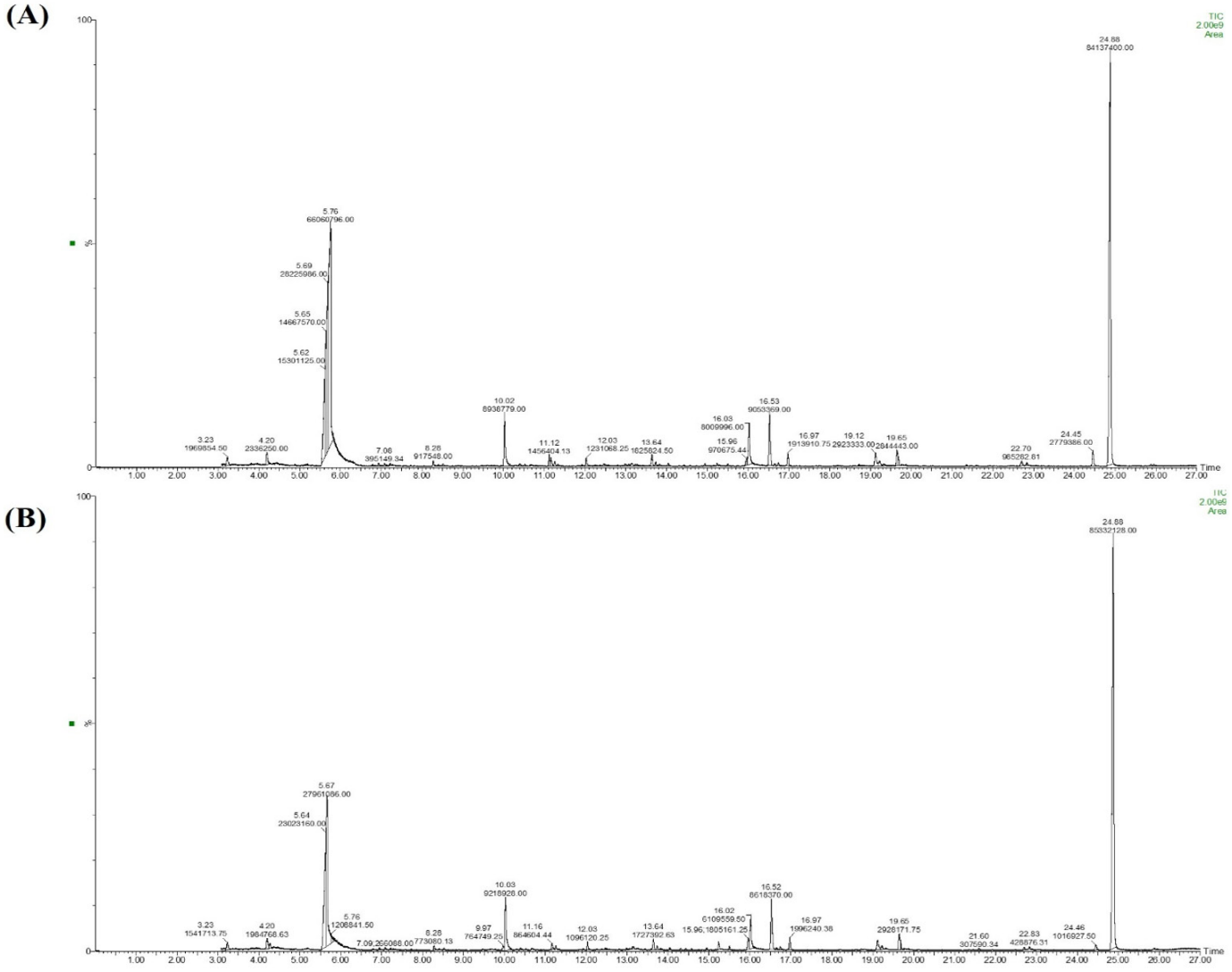



**Figure 1.** Gas chromatogram profiles of *Apis mellifera* (A) and *Apis dorsata* (B) honey obtained using gas chromatography-mass spectrometry (GC-MS).

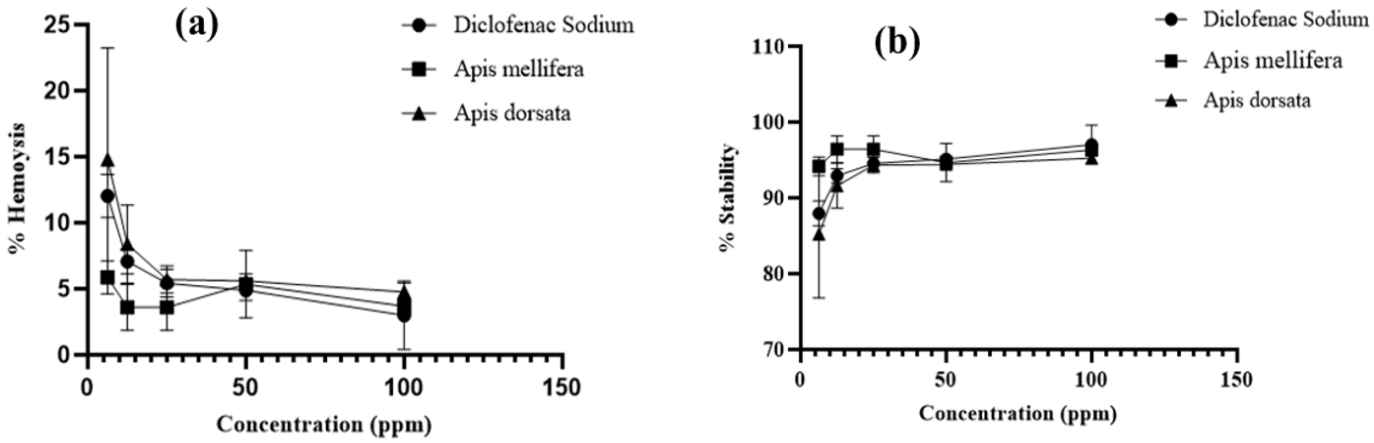



**Figure 2.** In vitro evaluation of the anti-inflammatory activity of Apis mellifera and Apis dorsata honey and sodium diclofenac on red blood cell membrane stabilization method (A) Percentage hemolysis inhibition at various concentrations (6.25, 12.5, 25, 50, and 100 mg/L). (B) Percentage membrane stability at the same concentrations. Data are presented as mean ± SD (n=3).

the two types of honey. At lower concentrations, *Apis mellifera* honey exhibited greater membrane stabilization activity than *Apis dorsata*. This effect is presumably related to the presence of compounds such as hexanedioic acid, mono(2-ethylhexyl) ester, which may play a role in reinforcing lipid membrane structures, although direct pharmacological evidence for this compound is limited. Supporting studies have noted that certain honey-derived compounds contribute to membrane stabilization, a crucial mechanism in anti-inflammatory activity.[36] Conversely, although *Apis dorsata* demonstrated lower membrane stabilization at the initial concentrations, its effectiveness increased in a dose-dependent manner. This pattern suggests that the bioactivity of honey is influenced by a synergistic combination of multiple compounds, where the interaction among these bioactive constituents plays a crucial role in determining the overall pharmacological effect. In contrast, the protein denaturation inhibition assay indicated that Apis dorsata honey exhibited stronger anti-inflammatory potential than *Apis mellifera*, as reflected by its lower $IC_{50}$ value. This result suggests that *Apis dorsata* is more effective in maintaining protein structure integrity under pro-inflammatory conditions. The superior activity of *Apis dorsata* is thought to be associated with the presence of 4-pyridinol, a compound known for its anti-inflammatory properties, which protects proteins from inflammatory stress and suppresses protein denaturation processes.[37,38] Additionally, several fatty acid ester compounds identified in *Apis dorsata* honey may contribute to enhancing this protective mechanism by supporting cell membrane stability and reducing inflammation-induced damage.[35]

These findings further confirm that the anti-inflammatory potential of honey is not solely determined by a single dominant compound, but rather by the combined and synergistic effects of multiple bioactive compounds present in the honey. The interaction between these chemical constituents is believed to produce a more optimal pharmacological response. Accordingly, the observed differences in anti-inflammatory activity between *Apis mellifera* and *Apis dorsata* observed in the protein denaturation assay are likely due to variations in the types and concentrations of bioactive compounds, which are known to modulate inflammatory pathways, which are also influenced by the bee species and nectar sources that shape their distinct chemical profiles. Both types of honey were found to possess similar antioxidant and anti-inflammatory compounds, although their bioactive profiles differ. This compositional variation contributes to differences in their mechanisms of action, with *Apis mellifera* exhibiting a superior membrane stabilization capacity, while *Apis dorsata* demonstrates greater effectiveness in preventing protein denaturation. These differences are closely related to the specific bioactive constituents present in each honey sample and the biological targets they influence.[39]

This study has several limitations, including the

**Table 3.** The $IC_{50}$ value of honey based on anti-inflammatory test. Data are presented as mean±SD (n=3).

| Sample | Mean ±SD |
|---|---|
| IApis mellifera | 25.35±0.024 |
| Apis Dorsata | 12.85±1.477 |
| Diclofenac Sodium | 7.51±1.88 |

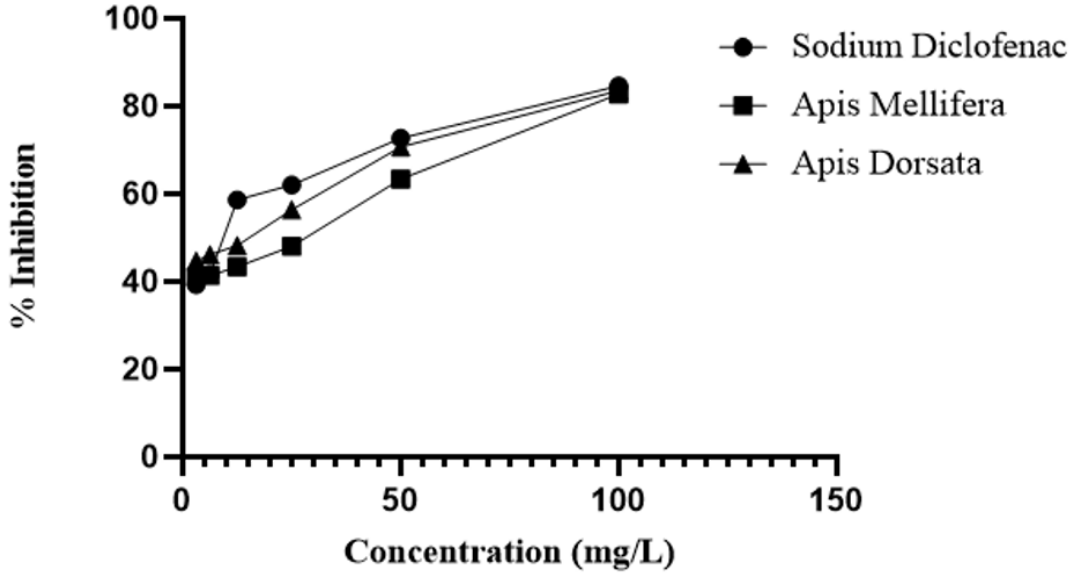



**Figure 3.** Percentage inhibition of protein denaturation at various concentrations, expressed as mean ± SD (n=3).

absence of *in vivo* anti-inflammatory evaluations, a lack of analysis for polar compounds, and the failure to isolate or assess the pharmacological properties of individual bioactive compounds within the honey samples. For future studies, it is recommended to conduct *in vivo* anti-inflammatory activity assays, isolate and identify the dominant active constituents from each honey type, and further explore the potential synergistic effects between these bioactive compounds. These efforts are expected to enhance the scientific foundation for developing forest honey as a safe and effective natural anti-inflammatory agent.

## 5. Conclusion

The study demonstrates that honey from *Apis mellifera* and *Apis dorsata* exhibits significant ($p<0.05$) anti-inflammatory activity, as evidenced by the stabilization of red blood cell membranes and the inhibition of protein denaturation. GC-MS analysis revealed the presence of various bioactive compounds with pharmacological activity, including 2-propenoic acid, 3-(4-methoxyphenyl)-2-ethylhexyl ester, which was consistently found in both types of honey. Comparative analysis revealed that *Apis mellifera* honey showed greater activity in stabilizing the erythrocyte membrane, while *Apis dorsata* honey demonstrated stronger inhibitory effects in the protein denaturation assay. These differences are likely due to variations in the composition and concentration of bioactive compounds. Collectively, the findings provide evidence that both types of honey possess promising anti-inflammatory potential and could serve as effective and safe natural therapeutic agents. This research provides scientific evidence supporting the potential of both types of honey as effective and safe natural anti-inflammatory agents.

## Conflict of Interest

The authors declare no conflicts of interest.

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