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Research Article

Chemical and physicochemical characterization and selected biological activities of *Apis dorsata* honey from Buon Ho, Dak Lak, Vietnam

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Abstract

Apis dorsata honey collected from Buon Ho, Dak Lak, Vietnam, was characterized for its physicochemical quality, chemical profile, and selected biological activities. The evaluated quality parameters, including sensory characteristics, pH, moisture, sugar content, HMF, and diastase activity, complied with the requirements of TCVN 12605:2019. GC-MS analysis of the honey extract tentatively detected 20 constituents, with fatty acid esters and long-chain hydrocarbons as the predominant chemical groups. The compounds showing the highest relative abundance were 9,12,15-octadecatrienoic acid ethyl ester, hexadecanoic acid ethyl ester, and heptadecane. Total polyphenol content was measured at 90.32 ± 1.88 mg GAE/100 g. In biological assays, the honey showed inhibitory activity against protein denaturation (IC_{50} = 74.11 µg/mL) and inhibited the growth of both *Escherichia coli* and *Staphylococcus aureus*. Overall, the results provide evidence that *A. dorsata* honey from Buon Ho, Dak Lak has acceptable physicochemical characteristics and measurable biological activity. These findings support further investigation of its potential value as a natural ingredient for food-related and pharmaceutical applications.

Keywords: *Apis dorsata, honey characterization, chemical profile, GC-MS, protein denaturation, antibacterial activity.*

1. INTRODUCTION

The properties of honey vary according to the biological and environmental conditions under which it is produced. Differences in floral resources, local ecology, and geographical origin can lead to substantial variation in honey composition and, consequently, in its quality characteristics and biological properties. For this reason, information obtained for honey from one geographical area cannot necessarily be generalized to products collected from another region. Within this context, *Apis dorsata* honey represents a valuable natural resource in the Central Highlands of Vietnam. In addition to its nutritional importance, this type of honey has economic value because it is obtained from wild bee colonies that occur naturally in the region, including Buon Ho, Dak Lak province. However, despite the local availability of this honey, its chemical characteristics and biological properties under the specific conditions of Buon Ho have not yet been comprehensively documented. A clearer understanding of the chemical profile and biological potential of locally collected *A. dorsata* honey is therefore needed. Such information may contribute to evaluating the characteristics of this natural product and provide a basis for its future use. Accordingly, the present study aimed to determine the chemical composition and investigate selected biological activities of *A. dorsata* honey collected from Buon Ho, Dak Lak. The findings are expected to provide scientific evidence supporting further assessment and development of this honey as a potential functional food resource.

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2. MATERIALS AND METHODS

2.1. Research materials

In April 2025, five honey samples were obtained independently from five wild *A. dorsata* colonies in Buon Ho, Dak Lak province. Each sample was treated as a separate experimental sample. After collection, visible impurities were removed by coarse filtration, and the honey was transferred to sealed, labeled glass bottles. The samples were maintained at room temperature until the analyses were carried out. The collected honey was used for the assessment of quality-related parameters and biological activities. Antibacterial experiments were performed using *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213. Both reference strains were supplied by the Institute of Hygiene and Epidemiology of the Central Highlands.

2.2. Chemicals and culture media

Analytical-grade reagents and chemicals, or materials of higher purity, were used throughout the study without additional purification. These reagents were obtained from Merck (Germany). For the microbiological assays, Luria-Bertani (LB) broth and Tryptic Soy Agar (TSA) were used as culture media; both were purchased from Sigma-Aldrich (Merck, Germany).

2.3. Research methods

2.3.1. Physicochemical analysis of honey

The physicochemical properties of the honey samples were evaluated in accordance with TCVN 12605:2019 [1]. The assessed variables included pH, reducing sugar content, sucrose content, water-insoluble solids, hydroxymethylfurfural (HMF), and diastase activity.

2.3.2. GC-MS analysis of chemical constituents

In the extraction process, 10 g of honey was weighed accurately and placed in a glass beaker. The sample was first mixed with 20 mL of distilled water and shaken mechanically for 10 min. Extraction was then performed using 100 mL of ethyl acetate. To remove moisture, 50 g of anhydrous sodium sulfate was added, and the mixture was vigorously vortexed to improve extraction efficiency. Following phase separation, the organic fraction was collected, filtered, and concentrated to dryness under reduced pressure using a rotary evaporator. The resulting residue was subsequently subjected to GC-MS analysis. Chemical profiling was carried out using a Hewlett Packard 7890 gas chromatograph coupled to a 5973 Mass Selective Detector (MSD). High-purity helium (99.999%) was employed as the carrier gas in constant-flow mode at 1.0 mL/min. Samples were introduced in splitless mode, with the injector maintained at 250 °C and a purge time of 0.5 – 1 min to enhance analytical sensitivity.

The oven program began at 60 °C and was maintained for 2 min. The temperature was then increased at 10 °C/min until 200 °C, followed by a second ramp of 5 °C/min to 280 °C. The final temperature was maintained for 10 min, depending on sample complexity.Mass spectrometric detection was performed in electron ionization mode at 70 eV. The temperatures of the ion source, quadrupole, and interface were 230 °C, 150 °C, and 280 °C, respectively. Mass spectra were acquired over an m/z range of 40 – 550. A solvent delay of 3 – 5 min was applied to minimize solvent-related signals. The detected compounds were assigned by comparison of the obtained mass spectra with the NIST 20 reference library [2].

2.3.3. Determination of total flavonoid content

Total flavonoids were quantified using the $AlCl_3$ colorimetric procedure. The reaction was initiated by combining 100 µL of the diluted honey sample with 100 µL of distilled water. Next, 20 µL of 5% $NaNO_2$ was added, and the mixture was allowed to react for 5 min. This was followed by the addition of 20 µL of 10% $AlCl_3$ and a further incubation period of 6 min. Subsequently, 200 µL of 1 M NaOH was added, followed by 60 µL of distilled water. After thorough mixing, the absorbance of the reaction mixture was measured at 510 nm using a spectrophotometer. Total flavonoid content was calculated from the calibration curve prepared with quercetin as the standard [3]

2.3.4. Determination of total polyphenol content

Total polyphenols were measured using the Folin-Ciocalteu assay. The honey was initially diluted to an appropriate concentration. 0.5 mL aliquot of the diluted sample was transferred into a test tube, after which 2.5 mL of Folin-Ciocalteu reagent, previously diluted tenfold, was added. The mixture was homogenized with a vortex mixer and left to react for 4 min. Subsequently, 2.0 mL of 7.5% Na_2CO_3 was added and mixed thoroughly. The reaction mixture was then kept in the dark at room temperature for 2 h. After incubation, absorbance was recorded at 760 nm using a spectrophotometer. Gallic acid was used as the reference standard, and the total polyphenol content was expressed as milligrams of gallic acid equivalents per sample (mg GAE/sample) [4].

2.3.5. Evaluation of the anti-inflammatory activity by the inhibition of heat-induced albumin denaturation

The anti-inflammatory potential of the honey was assessed by its ability to inhibit heat-induced denaturation of bovine serum albumin (BSA). The procedure followed the method described in the original study protocol. Honey samples were tested at concentrations of 4, 20, 50, and 100 µg/mL. Diclofenac was used as the positive control [5].

The percentage of inhibition was determined from the absorbance values of the blank and test samples using the following equation

$$\text{Percentage inhibition (\%)} = \frac{(OD_{blank} - OD_{sample}) \times 100}{OD_{blank}}$$

where OD_{blank} represents the absorbance of the blank sample, in which the extract was replaced with buffer; and OD_{sample} represents the absorbance measured for the test sample.

2.3.6. Antibacterial activity was determined using the agar disc diffusion method

Antibacterial activity was investigated using the agar disk diffusion technique [6, 7]. *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213 were cultured in Luria-Bertani broth until the cell density reached approximately 10^6 CFU/mL. Subsequently, 0.1 mL of each bacterial suspension was spread uniformly over the surface of Tryptic Soy Agar plates.

Sterile paper discs (6 mm in diameter) were loaded with 30 µL of the test sample and placed on the inoculated agar. The plates were kept at 4 °C for 4 h to allow the active compounds to diffuse through the medium, then incubated at 35 °C for 24 h [6]. Paper discs containing 30 µg of ampicillin served as the positive control, while sterile distilled water was used as the negative control. Antibacterial activity was determined by measuring the inhibition zone and was expressed as D − d, where D is the diameter of the inhibition zone in millimeters and d is the diameter of the paper disc (6 mm) [7].

2.3.7. Statistical analysis

Each analysis was conducted in triplicate. Data were processed using SPSS version 17.5 and presented as mean values with corresponding standard deviations. Comparisons among groups were performed using one-way analysis of variance (ANOVA), followed by Tukey's HSD test. A difference was considered statistically significant when $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Chemical composition of *A. dorsata* honey

The chemical constituents detected in the ethyl acetate extract of *A. dorsata* honey were examined by GC-MS, and the resulting chromatogram and identified compounds are presented in **Table 1** and **Figure 1**.

The detected constituents were mainly lipid-related compounds, including fatty acid esters, unsaturated fatty acid derivatives, long-chain hydrocarbons, and several minor terpenoid compounds. The most prominent components of the ethyl acetate fraction were ethyl 9,12,15-octadecatrienoate (18.23%), hexadecanoic acid ethyl ester (10.06%), heptadecane (8.59%), tricosane (5.86%), heneicosane (4.75%), and tetracosane (4.50%). The occurrence of these compounds indicates that the extract contained a complex mixture of lipid-derived substances associated with the honey matrix.

Table 1. *Chemical composition of the honey* of *A. dorsata*

Peak	Name	t_R (min)	Area (%)
1	Tetradecanoic acid, ethyl ester	19.230	0.80
2	Heptadecane	20.609	1.52
3	Ethyl 9-hexadecenoate	21.137	0.34
4	Hexadecanoic acid, ethyl ester (Ethyl palmitate)	21.414	10.06
5	Heneicosane	22.466	4.75
6	9, 12, 15-Octadecatrienoic acid, ethyl ester (Z, Z, Z)	22.864	18.23
7	Ethyl oleate	22.914	0.72
8	Octadecanoic acid, ethyl ester	23.089	1.66
9	Tricosane	24.019	5.86
10	9, 12-Octadecadienoic acid (Z,Z)	24.299	0.32
11	11, 14, 17-Eicosatrienoic acid, methyl ester	24.335	0.64
12	Eicosanoic acid, ethyl ester	24.557	0.57

Peak	Name	t_R (min)	Area (%)
13	Docosanoic acid, ethyl ester	25.883	1.92
14	Heptadecane	26.665	8.59
15	Ethyl tetracosanoate	27.103	0.77
16	Squalene	27.297	0.40
17	9-Nonadecene	27.648	2.41
18	Tetracosane	27.810	4.50
19	17-Pentatriacontene	28.982	2.21
20	Triacontyl acetate	30.808	3.52

Among the relatively abundant compounds, hexadecanoic acid ethyl ester, also known as ethyl palmitate, is of particular interest because previous studies have reported its anti-inflammatory effects in experimental models. Saeed *et al.* showed that this compound reduced edema and suppressed inflammatory mediators, including nitric oxide (NO) and prostaglandin E_2 (PGE_2), under both subacute and acute inflammatory conditions [8]. Therefore, the presence of ethyl palmitate in the analyzed honey may be one of the factors contributing to its observed anti-inflammatory potential, although the activity of the honey cannot be attributed to a single constituent alone.

The GC-MS profile also revealed several unsaturated fatty acid derivatives, including 9,12-octadecadienoic acid and 9,12,15-octadecatrienoic acid, ethyl ester (Z, Z, Z). These lipid components have previously been associated with biological functions such as antioxidant and anti-inflammatory activity. According to Shahidi and Ambigaipalan, polyunsaturated fatty acids with omega-3-related structural characteristics may help regulate inflammatory responses and reduce oxidative damage in biological systems [9]. Thus, the detected unsaturated lipid derivatives could contribute to the overall biological properties of the honey; however, their individual contributions require further investigation.

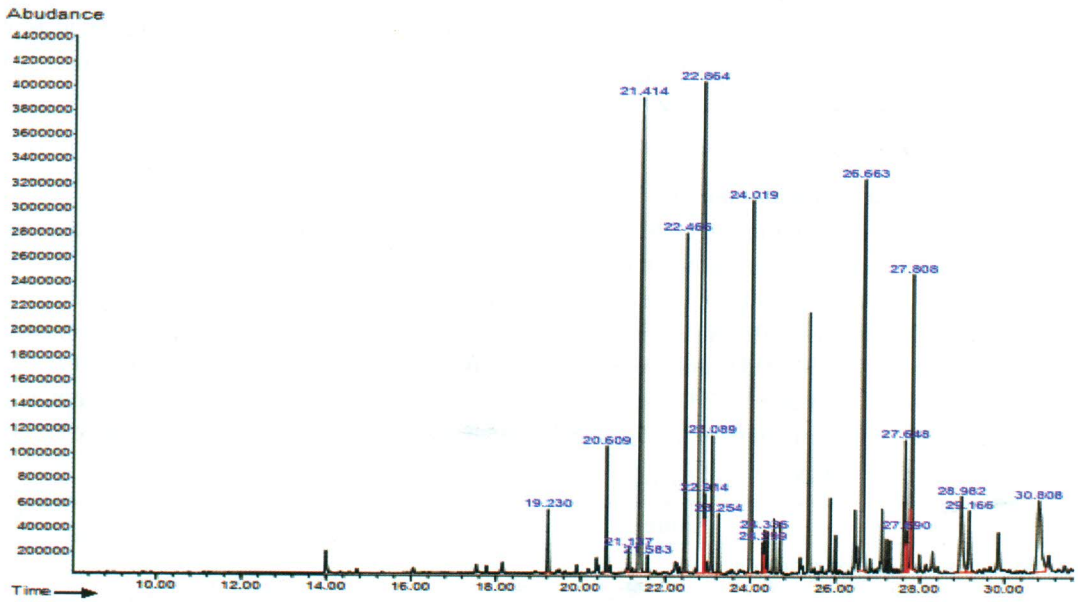


Figure 1. *GC-MS chromatogram of A. dorsata honey*

In addition to fatty acid derivatives, several long-chain hydrocarbons were identified, including heptadecane, heneicosane, tricosane, tetracosane and other very-long-chain hydrocarbons. Such compounds are commonly associated with natural beeswax and may serve as useful chemical indicators of the origin and composition of bee products. Da Silva *et al.* emphasized the importance of chemical constituents, including hydrocarbon profiles, in evaluating honey authenticity and stability [10]. Although these hydrocarbons are not necessarily responsible for direct biological activity, their presence may provide valuable information for authenticity assessment, quality evaluation, and product traceability.

Squalene was also detected, although its relative abundance was low (0.40%). Despite its minor proportion in the extract, this triterpenoid has received considerable attention because of its reported antioxidant and cytoprotective properties. The available literature therefore provides a basis for considering squalene as a potentially relevant component when interpreting the biological characteristics of natural products containing this compound [11]. Nevertheless, because

of its low abundance in the present sample, its contribution to the overall activity of *A. dorsata* honey cannot be determined from the current data alone.

Another important limitation to consider when interpreting the GC-MS results is the following. Many of the identified constituents were detected as ethyl esters, while ethyl acetate was used during the extraction procedure. Since solvent blanks, procedural blanks, and confirmation with authentic standards were not included in the present analysis, the possibility that the extraction solvent or sample preparation process affected the formation or detection of certain ethyl ester derivatives cannot be completely excluded. For this reason, the natural occurrence of these compounds in the honey should be interpreted cautiously and should be confirmed in future studies using appropriate controls and complementary analytical approaches.

Overall, the ethyl acetate fraction of *A. dorsata* honey was characterized primarily by fatty acid esters and long-chain hydrocarbons, with several unsaturated lipid derivatives and squalene also being detected. This chemical profile may be relevant to the observed biological activities of the honey and may also provide useful markers for quality control, authenticity evaluation, and traceability. Further work using confirmatory standards and additional analytical techniques is needed to establish the identity and origin of the detected compounds more definitively.

3.2. Biological activities of *A. dorsata* honey

The composition and functional characteristics of honey can vary substantially among samples because they are influenced by geographical origin, botanical source, and local environmental conditions. In the present study, the physicochemical and biochemical characteristics of *A. dorsata* honey are summarized in **Table 2**. These parameters were evaluated to provide an overall assessment of honey quality and to support the interpretation of its potential biological properties.

Regarding sensory characteristics, the *A. dorsata* honey exhibited a light amber color on the Pfund scale, together with a natural aroma, pronounced sweetness, and the characteristic flavor of wild forest honey. These sensory properties are consistent with the general characteristics of natural honey collected from the Central Highlands of Vietnam. Nguyen Minh Trung *et al.* reported similar observations for coffee blossom honey collected in Dak Lak, although differences in floral origin may cause variation among honey types [12].

Table 2. *Selected biochemical components of the A. dorsata honey*

Parameter	Analysis results	TCVN 12605:2019
Water content (%)	20.04 ± 0.12	≤ 23%
pH	3.97 ± 0.01	–
Insoluble solids (%)	0.142 ± 0.01	< 0.5
Free reducing sugar (%)	63.26 ± 1.53	> 60
HMF (mg/kg)	20.76 ± 0.08	≤ 40
Diastase activity (Schade)	9.20 ± 0.01	≥ 8
Flavonoid (mgQE/100 g)	1.07 ± 0.01	–
Polyphenol (mgGAE/100 g)	90.32 ± 1.88	–

Note: (–) not present in TCVN 12605:2019 on honey quality.

The moisture content of the analyzed honey was 20.04 ± 0.12%, which was below the maximum limit of 23% specified in TCVN 12605:2019. This result indicates that the sample satisfied the relevant quality requirement and suggests that the honey was harvested at an appropriate stage of maturity. A moisture level within the accepted range may also contribute to improved storage stability. The pH value was 3.97 ± 0.01, which is within the acidic range commonly observed in natural honey and may contribute to the inhibition of microbial growth and maintenance of product stability.

The reducing sugar content was 63.26 ± 1.53%, exceeding the minimum requirement of 60% established by TCVN 12605:2019. Although this value fulfilled the standard, it was lower than the 65.72% reported for coffee blossom honey from Dak Lak by Nguyen Minh Trung *et al.* [12]. Such variation between samples may be associated with differences in botanical origin, environmental conditions, and other factors related to honey production.

The hydroxymethylfurfural (HMF) concentration was 20.76 ± 0.08 mg/kg, which was below the maximum allowable value of 40 mg/kg. This finding is also comparable with the range of 7.67 – 36.13 mg/kg reported by Tran Thi Hong Hanh and Tran Van Nguyen for Spanish needle, white flower, and coffee blossom honeys [13]. The HMF level obtained in this

study therefore suggests that the analyzed honey retained acceptable quality and had not been substantially affected by excessive heating or prolonged storage.

The diastase activity was 9.20 ± 0.01 Schade units, meeting the minimum requirement of 8 established by TCVN 12605:2019. However, this activity was lower than the range of 17.037 – 19.807 DN reported for coffee blossom honey by Nguyen Minh Trung *et al.* [12]. Differences in diastase activity among honey samples may be related to variation in floral source, environmental conditions, temperature exposure, and storage duration.

In addition to the standard physicochemical parameters, the honey contained 90.32 ± 1.88 mg GAE/100 g of total polyphenols and 1.07 ± 0.01 mg QE/100 g of total flavonoids. The total polyphenol content was higher than the 35.27 mg GAE/100 g reported for Malaysian Tualang honey (*A. dorsata*) by Moniruzzaman *et al.* [14]. In contrast, the flavonoid content in the present study was lower than the 6.57 mg CE/100 g previously reported for Tualang honey. These findings suggest that, although both samples were produced by *A. dorsata*, their phenolic profiles may differ considerably. Such differences may be influenced by floral origin, seasonal variation, geographical conditions, and post-harvest handling.

It should be noted that only total polyphenol and total flavonoid contents were measured in the present study. Therefore, the results do not provide direct evidence of the antioxidant capacity of the honey. Additional assays, such as DPPH, ABTS, or FRAP, would be required to evaluate antioxidant activity more directly.

Overall, the *A. dorsata* honey collected in Buon Ho showed physicochemical properties that complied with the relevant requirements of TCVN 12605:2019 and were generally comparable with results reported for honey from the Central Highlands and other regions where the same bee species is found [12], [14]. Variations between studies likely reflect differences in floral origin, ecological conditions, and harvesting season. The relatively high total polyphenol content, together with the acceptable physicochemical parameters, indicates that the honey had good overall quality and nutritional value. Its biological potential is promising; however, further studies using additional functional assays are required to characterize this potential more comprehensively.

3.3. *In vitro* anti-inflammatory potential of honey

The anti-inflammatory potential of *A. dorsata* honey was preliminarily evaluated using the heat-induced albumin denaturation assay. This *in vitro* method was used to assess the ability of the test sample to reduce protein denaturation under heat stress. The results, presented in **Table 3**, showed that the inhibitory effect of the honey increased as its concentration increased.

At 4 µg/mL, *A. dorsata* honey produced an inhibition rate of 8.91%. The inhibitory activity increased to 18.84% and 32.47% at concentrations of 20 and 50 µg/mL, respectively. At the highest tested concentration of 100 µg/mL, the inhibition reached 52.81%. These results indicate a concentration-dependent response, with higher honey concentrations providing greater protection against heat-induced albumin denaturation under the experimental conditions used.

Table 3. *Inhibitory effect of honey on albumin denaturation (n = 5)*

Criteria	Concentration (µg/mL)	*A. dorsata* honey	Control (Diclofenac)
Albumin denaturation inhibition efficiency (%)	4	8.91	13.42
	20	18.84	21.31
	50	32.47	42.33
	100	52.81	71.25
IC_{50}		74.11	32.43

Diclofenac, the positive control, showed stronger inhibitory activity than honey at all tested concentrations. Its inhibition values increased from 13.42% at 4 µg/mL to 21.31%, 42.33%, and 71.25% at concentrations of 20, 50, and 100 µg/mL, respectively. The calculated IC50 for *A. dorsata* honey was 74.11 µg/mL, whereas Diclofenac had an IC_{50} of 32.43 µg/mL. Therefore, although the honey demonstrated the ability to inhibit albumin denaturation, its activity was lower than that of the standard anti-inflammatory drug under the same test conditions.

The observed inhibitory effect may be associated with the ability of honey constituents to stabilize proteins. Wong *et al.* reported that honey could improve the thermal and chemical stability of bovine serum albumin (BSA), thereby reducing the extent of protein denaturation caused by heat and other denaturing factors [15]. This previous finding is consistent with the protein-protective effect observed for the *A. dorsata* honey evaluated in the present study.

Comparable anti-inflammatory activity has also been reported for other forest honeys. Hariana *et al.* investigated Indonesian forest honey and reported albumin-denaturation inhibitory activity, with IC_{50} values of 12.85 mg/L for

A. dorsata honey and 25.35 mg/L for *A. mellifera* honey [16]. The differences between those results and the present study may reflect variation in the chemical composition of the honey samples. As indicated in the cited study, biological activity may differ according to bee species and the chemical characteristics of each honey type.

Overall, the present results demonstrate that *A. dorsata* honey from Buon Ho inhibited heat-induced albumin denaturation in a concentration-dependent manner. However, its inhibitory activity was weaker than that of Diclofenac. Since this evaluation was based on a single preliminary in vitro model, the results should be interpreted as an initial indication of anti-inflammatory potential rather than definitive evidence of anti-inflammatory efficacy. Additional experimental approaches are needed to further characterize the biological activity and underlying mechanisms of this honey.

3.4. Antibacterial activity of *A. dorsata* honey

The antibacterial activity of *A. dorsata* honey was evaluated against two reference bacterial strains, *S. aureus* ATCC 29213 and *E. coli* ATCC 25922. As shown in **Table 4** and **Figure 2**, the inhibitory effect of the honey varied according to its concentration.

Table 4. *Antibacterial activity of honey at different dilution concentrations (n = 5)*

Experiment	Concentration	Difference in sterile zones D – d (mm)	
		***S. aureus* ATCC 29213**	***E. coli* ATCC 25922**
Control	Ampicillin (30µg)	24.53 ± 0.13	25.23 ± 0.12
A. dorsata honey	100%	22.81^{a} ± 0.11	23.45^{a} ± 0.12
	75%	18.94^{b} ± 0.10	19.16^{b} ± 0.11
	50%	13.15^{c} ± 0.09	14.43^{c} ± 0.08
	25%	10.21^{d} ± 0.05	11.49^{d} ± 0.06

Note: Mean values within the same column followed by different letters are significantly different (P < 0.05).

For both bacterial strains, the inhibition zones became larger as the concentration of honey increased, indicating a concentration-dependent antibacterial response under the conditions of the present experiment. At the highest concentration (100%), *A. dorsata* honey produced inhibition zones of 22.81 ± 0.11 mm against *S. aureus* and 23.45 ± 0.12 mm against *E. coli.* At 75%, the corresponding inhibition zones were 18.94 ± 0.10 mm and 19.16 ± 0.11 mm, respectively. Further dilution of the honey to 50% and 25% resulted in smaller inhibition zones, with values of 13.15 ± 0.09 mm and 10.21 ± 0.05 mm against *S. aureus* and 14.43 ± 0.08 mm and 11.49 ± 0.06 mm against *E. coli*, respectively. These results demonstrate a progressive reduction in antibacterial activity as the honey concentration decreased.

The positive control, ampicillin (30 µg), produced inhibition zones of 24.53 ± 0.13 mm for *S. aureus* and 25.23 ± 0.12 mm for *E. coli*. Although the inhibition zones produced by pure honey were smaller than those of ampicillin, the differences were relatively limited under the conditions of this assay. Nevertheless, direct comparisons between honey and an antibiotic should be interpreted carefully because their antimicrobial mechanisms and diffusion behavior in agar differ [18].



S. aureus ATCC 29213 *E. coli* ATCC 25922

Figure 2. *Antibacterial activity of A. dorsata honey against bacterial strains*

Among the two tested strains, *E. coli* showed slightly larger inhibition zones than *S. aureus* at all honey concentrations. This finding indicates that, in the present experiment, *E. coli* was somewhat more susceptible to the inhibitory effect of *A. dorsata* honey. Mohapatra *et al.* previously reported a similar antibacterial effect of honey against bacterial growth [17].

However, the relative susceptibility of bacterial species may vary depending on the honey type and experimental conditions.

Several physicochemical and chemical factors may influence the antibacterial properties of honey. The present study did not separately evaluate the contribution of individual factors to the observed inhibition. Therefore, although honey-related factors such as high osmotic pressure, low pH, hydrogen peroxide production, and phenolic compounds have been discussed in relation to antibacterial activity [19], the current results do not allow identification of the primary mechanism responsible for the inhibition.

Overall, *A. dorsata* honey exhibited concentration-dependent inhibitory effects against both *S. aureus* and *E. coli* under the experimental conditions used. However, because the study did not include corresponding osmotic and pH controls, the respective contributions of sugar-related osmotic effects, acidity, and specific antimicrobial constituents remain unclear. Further studies incorporating appropriate controls and additional microbiological approaches are needed to clarify the mechanisms underlying the antibacterial activity observed in this study

The observed antibacterial activity helps explain why honey has long been used in traditional medicine to support wound and minor burn care. Numerous studies have shown that honey can inhibit microbial growth, promote wound healing, and facilitate tissue regeneration. However, its clinical effectiveness depends on various factors, including the source of the honey, the condition of the wound, and the method of application. At all tested concentrations, *E. coli* exhibited larger inhibition zones than *S. aureus*, indicating that this bacterial strain was more susceptible to honey under the experimental conditions. This finding is consistent with the study by Mohapatra *et al.*, which reported that honey is capable of inhibiting both Gram-positive and Gram-negative bacteria [17].

4. CONCLUSION

The present study provides a characterization of *A. dorsata* honey collected from Buon Ho, Dak Lak, Vietnam, based on its physicochemical properties, chemical composition, and selected biological activities. The evaluated quality parameters were in accordance with the requirements of TCVN 12605:2019, indicating acceptable physicochemical quality of the investigated honey. GC-MS analysis tentatively identified 20 compounds, with fatty acid esters and long-chain hydrocarbons representing the main chemical groups. Among the detected constituents, 9,12,15-octadecatrienoic acid ethyl ester, hexadecanoic acid ethyl ester, and heptadecane showed the highest relative abundances. The honey also contained a measurable amount of total polyphenols, reaching 90.32 ± 1.88 mg GAE/100 g. In the biological assays, *A. dorsata* honey showed concentration-dependent inhibition of protein denaturation, with an IC_{50} value of 74.11 µg/mL, and inhibited the growth of both *E. coli* and *S. aureus*. These findings demonstrate that the honey possesses measurable biological activity under the experimental conditions applied in this study.

Overall, the results provide a scientific basis for further characterization of *A. dorsata* honey from Buon Ho. Future studies should include additional experiments to clarify the antioxidant potential, confirm the identity of key chemical constituents, and investigate the mechanisms responsible for the observed anti-inflammatory and antibacterial effects. Such work would provide a more comprehensive understanding of the potential value and applications of this natural honey.

REFERENCES

[1] Ministry of Science and Technology, "Honey," *Vietnam National Standards TCVN 12605:2019*, 2019. (in Vietnamese).

[2] K. Joshna, V. Gopal, and B. Kavitha, "Analysis of bitter honey using gas chromatography and tandem mass spectrometry," *Bioinformation*, vol. 18, no. 3, pp. 196 – 199, 2022.

[3] C. C. Chang, M. H. Yang, H. M. Wen, and J. C. Chern, "Estimation of flavonoid total content in propolis by two complementary colorimetric methods," *Journal of Food and Drug Analysis*, vol. 10, no. 3, 2002.

[4] L. Fu *et al.*, "Antioxidant capacities and total phenolic contents of 62 fruits," *Food Chemistry*, vol. 129, no. 2, pp. 345 – 350, 2011.

[5] T. Q. Tuan *et al.*, "Standardization of an in vitro screening model for anti-inflammatory compounds based on the inhibition of heat-induced bovine albumin denaturation," *Journal of Science and Technology*, vol. 52, no. 5B, pp. 532 – 538, 2014 (in Vietnamese).

[6] P. T. N. Lan, *Microbiology Laboratory Manual*, Hue: Hue University Press, 2012 (in Vietnamese).

[7] N. Celikel and G. Kavas, "Antimicrobial properties of some essential oils against some pathogenic microorganisms," *Czech Journal of Food Sciences*, vol. 26, no. 3, pp. 174 – 181, 2008.

[8] N. M. Saeed *et al.*, "Anti-inflammatory activity of methyl palmitate and ethyl palmitate in different experimental rat models," *Toxicology and Applied Pharmacology*, vol. 264, no. 1, pp. 84 – 93, 2012.

[9] F. Shahidi and P. Ambigaipalan, "Omega-3 polyunsaturated fatty acids and their health benefits," *Annual Review of Food Science and Technology*, vol. 9, pp. 345 – 381, 2018.

[10] P. M. Da Silva *et al.*, "Honey: Chemical composition, stability and authenticity," *Food Chemistry*, vol. 196, pp. 309 – 323, 2016.

[11] C. Gabás-Rivera *et al.*, "Dietary squalene induces cytochromes Cyp2b10 and Cyp2c55 independently of sex, dose, and diet in several mouse models," *Molecular Nutrition & Food Research*, vol. 64, no. 20, p. e2000354, 2020.

[12] N. M. Trung *et al.*, "Physicochemical properties, biological activities, and bioactive compound composition of coffee blossom honey collected in Cu Kuin District and Ea Kao Commune, Buon Ma Thuot city, in 2023," *Proceedings of the National Biotechnology Conference 2024, 2024*, pp. 318 – 325, 2024 (in Vietnamese).

[13] T. T. H. Hanh and T. V. Nguyen, "Study of the physicochemical profile and antioxidant capacity of bidens, blong song, and coffee honey originating from Vietnam," *Journal of Agriculture and Environment*, no. 21, pp. 42 – 50, 2024 (in Vietnamese).

[14] Moniruzzaman *et al.*, "Physicochemical and antioxidant properties of Malaysian honeys produced by *Apis cerana*, *Apis dorsata* and *Apis mellifera*," *BMC Complementary and Alternative Medicine*, vol. 13, p. 43, 2013.

[15] Y. H. Wong, H. Abdul Kadir, and S. Tayyab, "Honey-induced protein stabilization as studied by fluorescein isothiocyanate fluorescence," *The Scientific World Journal*, vol. 2013, p. 981902, 2013.

[16] H. Hariana *et al.*, "Identification of bioactive constituents in forest honey using GC-MS and anti-inflammatory activity through protein denaturation," *Indonesian Journal of Pharmaceutical Science and Technology*, vol. 12, no. 3, pp. 238 – 248, 2025.

[17] D. P. Mohapatra, V. Thakur, and S. K. Brar, "Antibacterial efficacy of raw and processed honey," *Biotechnology Research International*, vol. 2011, p. 917505, 2011.

[18] M. L. Hossain *et al.*, "A review of commonly used methodologies for assessing the antibacterial activity of honey and honey products," *Antibiotics*, vol. 11, no. 7, p. 975, 2022.

[19] M. I. Zainol, K. M. Yusoff, and M. Y. M. Yusof, "Antibacterial activity of selected Malaysian honey," *BMC Complementary and Alternative Medicine*, vol. 13, p. 129, 2013.

