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# Phytochemical Profile, Antibacterial Activity, and Antioxidant Capacity of *Apis cerana* Honey from Lore Lindu National Park, Indonesia

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**ABSTRACT**

This study aimed to systematically evaluate the phytochemical screening, antibacterial activity, and antioxidant capacity of *Apis cerana* honey collected from Lore Lindu National Park, Central Sulawesi, Indonesia. Qualitative phytochemical screening was conducted to identify major bioactive compounds, while antibacterial activity was assessed using the agar well diffusion method against *Escherichia coli* and *Staphylococcus aureus*. Antioxidant capacity was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay. The phytochemical screening revealed the presence of flavonoids, tannins, and saponins, whereas alkaloids and steroids were not detected. The antibacterial assay demonstrated that *A. cerana* honey exhibited strong inhibitory effects against both test bacteria, producing inhibition zones of 25.73 mm against *E. coli* and 28.51 mm against *S. aureus* at 100% (w/v) concentration. Under the same experimental conditions, the positive control chloramphenicol (30 µg) produced inhibition zones of 22.05 mm (*E. coli*) and 20.95 mm (*S. aureus*). The antioxidant evaluation yielded an $IC_{50}$ value of 171.95 ppm, indicating weak to moderate antioxidant activity. Overall, *A. cerana* honey from Lore Lindu National Park demonstrated remarkable antibacterial activity, with inhibition zones larger than those of the chloramphenicol control used in this assay, and measurable antioxidant capacity. These bioactivities are likely associated with the synergistic effects of its bioactive constituents, highlighting its potential as a natural functional product derived from forest ecosystems.

## 1. INTRODUCTION

Indonesia is globally recognized for its vast tropical forest ecosystems, which harbor exceptional biological diversity and provide a wide range of non-timber forest products (NTFPs) that support local livelihoods [1, 2]. Among these products, forest honey has long played a dual role as both a nutritional resource and a traditional remedy in many Indonesian communities [3]. Beyond its cultural importance, honey has gained increasing scientific attention as a functional food due to its diverse bioactive properties, particularly its antibacterial and antioxidant activities [4, 5].

These biological functions are closely associated with the complex phytochemical composition of honey, which varies according to botanical origin, geographical location, and bee species [6]. Honey is a natural substance produced by bees through the enzymatic processing of plant nectar or honeydew [7]. Its chemical composition includes sugars, organic acids, amino acids, enzymes, vitamins, minerals, and a wide array of secondary metabolites such as phenolic acids, flavonoids, tannins, and saponins [4]. Numerous studies have demonstrated that these phytochemicals play a central role in honey's antimicrobial and antioxidant effects by disrupting microbial cell membranes, inhibiting enzyme activity, and neutralizing reactive oxygen species [8]. Consequently, honey is increasingly explored not only as a traditional medicine but also as a potential alternative or complementary agent to conventional antimicrobial and antioxidant therapies [9]. The bioactivity of honey is not uniform and depends strongly on its floral sources and the species of bees producing it [10]. While most international studies have focused on honey produced by Apis mellifera, relatively less attention has been paid to honey from *A. cerana*, a native Asian honeybee species widely distributed across Southeast Asia, including Indonesia [11]. *A. cerana* honey differs substantially from *A. mellifera* honey in terms of nectar foraging behavior, enzymatic activity, and interaction with local flora, leading to distinctive physicochemical and phytochemical characteristics [12].

Several studies from Southeast Asia have reported that *A. cerana* honey exhibits notable antibacterial activity against both Gram-positive and Gram-negative bacteria, as well as strong antioxidant potential [13]. Research from Malaysia, Thailand, and Vietnam indicates that these properties are often correlated with higher total phenolic and flavonoid contents compared to commercially available honeys. In Indonesia, however, scientific investigations on *A. cerana* honey remain

limited and geographically fragmented. Existing studies primarily focus on basic physicochemical parameters or general antioxidant activity, with few exploring the relationship between phytochemical composition and specific biological functions such as antibacterial potency relative to standard antibiotics [14].

Central Sulawesi, particularly the area surrounding Lore Lindu National Park, represents an ecologically unique landscape characterized by high plant endemism and relatively undisturbed forest ecosystems [15]. Local communities in this region have traditionally used forest honey produced by *A. cerana* for treating wounds, infections, and chronic ailments, suggesting its perceived medicinal value. Given the distinct floral diversity and environmental conditions of Lore Lindu National Park, honey produced in this area is expected to possess a unique phytochemical profile that may influence its bioactivity [16]. However, to date, there is a lack of systematic scientific evaluation of *A. cerana* honey from this region, particularly studies that integrate phytochemical screening with functional bioactivity assays [17].

Understanding the link between phytochemical composition and biological activity is crucial for validating traditional knowledge and supporting the development of honey-based functional foods or natural therapeutic agents. Moreover, comparative evaluation of honey's antibacterial activity against standard antibiotics is essential to assess its potential relevance in the context of rising antimicrobial resistance. Similarly, evaluating free radical scavenging capacity provides insight into honey's potential role in mitigating oxidative stress–related diseases. Therefore, this study aims to systematically evaluate the phytochemical profile of *A. cerana* honey from Lore Lindu National Park and assess its antibacterial efficacy against common pathogenic bacteria in comparison with standard antibiotics, as well as its free radical scavenging ability. By exploring the potential links between phytochemical composition and bioactivity, this research seeks to contribute to the growing body of knowledge on the functional properties of Indonesian forest honey and highlight the scientific value of *A. cerana* honey from ecologically distinctive regions. The findings are expected to support the sustainable utilization of forest honey resources while providing a scientific basis for their potential application in health-related fields.

## 2. RESEARCH METHOD

### 2.1 Research sites and sample collection

Honey samples produced by *A. cerana* were collected from forest areas surrounding Lore Lindu National Park, Central Sulawesi, Indonesia (Figure 1). The sampling location represents a relatively undisturbed tropical forest ecosystem with high floral diversity. All laboratory analyses were conducted at the Chemistry Research Laboratory and the Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Tadulako University, Palu.

### 2.2 Phytochemical screening

Qualitative phytochemical screening was performed to identify major secondary metabolite groups present in *A. cerana* honey, including flavonoids, alkaloids, terpenoids, steroids, saponins, and tannins. The analyses were conducted following standard phytochemical procedures adapted from Harborne [18], with brief methodological descriptions provided below to ensure clarity and reproducibility [15].

Flavonoids: Approximately 2 mL of honey solution was mixed with a small amount of magnesium powder, followed by the addition of concentrated hydrochloric acid (HCl). The formation of a red, orange, or yellow coloration indicated the presence of flavonoids (Shinoda test).

Alkaloids: Honey solution was acidified with diluted HCl and tested using Dragendorff's and Mayer's reagents. The appearance of an orange or creamy precipitate was taken as a positive indication of alkaloids.

Terpenoids and Steroids: The Liebermann–Burchard test was conducted by adding acetic anhydride, followed by concentrated sulfuric acid ($H_2SO_4$). A reddish-brown color indicated terpenoids, while a green or bluish coloration indicated steroids.

Saponins: The foam test was used by vigorously shaking the honey solution with distilled water. The formation of stable foam persisting for at least 10 minutes indicated the presence of saponins.

Tannins: A few drops of 1% ferric chloride ($FeCl_3$) solution were added to the honey extract. The appearance of a blue-black or greenish-black coloration confirmed the presence of tannins.

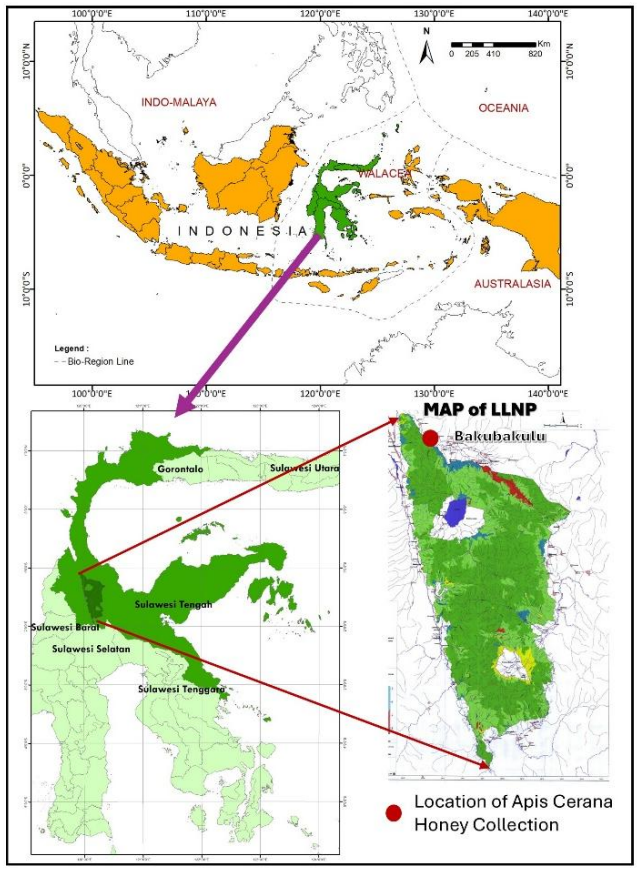


**Figure 1.** *A. cerana* honey harvesting location in Lore Lindu National Park (Babubakulu Village)

### 2.3 Antibacterial activity assay

The antibacterial activity of *A. cerana* honey was evaluated using the agar well diffusion method. Honey solutions were prepared as weight/volume (w/v) concentrations by dissolving honey in sterile distilled water to obtain final concentrations of

100% (w/v), 75% (w/v), 50% (w/v), and 25% (w/v). The use of distilled water as a solvent ensured optimal solubility and avoided antibacterial effects associated with organic solvents. Chloramphenicol was used as the positive control at a standard dose of 30 µg per test unit, in accordance with commonly applied agar diffusion susceptibility testing protocols. To ensure methodological comparability with the agar well diffusion format, an equivalent dose of 30 µg chloramphenicol was applied per well, while sterile distilled water served as a negative control. The concentration of chloramphenicol used is consistent with commonly applied antibacterial diffusion assays and produced inhibition zones within acceptable pharmacopoeial ranges for susceptible bacteria. Bacterial suspensions were standardized to 0.5 McFarland turbidity and evenly spread onto Mueller–Hinton agar plates. Wells of uniform diameter were created, and 50 µL of each test solution was added. Plates were incubated at 37°C for 18–24 hours. Antibacterial activity was indicated by the formation of clear inhibition zones around the wells, which were measured in millimeters using a digital caliper in multiple directions. Antibacterial strength was categorized as very strong (> 20 mm), strong (10–20 mm), moderate (5–10 mm), or weak (< 5 mm). Each treatment was performed in triplicate, resulting in a total of 24 experimental units [1, 19].

### 2.4 Antioxidant activity assay

The antioxidant activity of the honey was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical assay [1]. Antioxidant capacity was assessed using the DPPH free radical scavenging assay. A 100 µM DPPH stock solution was prepared by dissolving DPPH powder in methanol and stored in the dark to prevent photodegradation. Honey extracts were prepared at concentrations of 62.5, 125, 250, and 500 ppm. For each concentration, 3 mL of honey solution was mixed with 1 mL of DPPH solution, incubated in the dark at room temperature for 30 minutes, and the absorbance was measured at 517 nm using a UV–Vis spectrophotometer. Vitamin C was used as a reference antioxidant at concentrations of 2, 4, 6, and 8 ppm. All measurements were conducted in triplicate. The percentage of DPPH radical scavenging activity was calculated using the following equation:

$$Inhibition = \frac{\mathrm{A0} - \mathrm{As}}{A0} \times 100 \quad (1)$$

where, $A0$ is the absorbance of the control and $As$ is the absorbance of the sample.

The $IC_{50}$ value (effective concentration required to scavenge 50% of DPPH radicals) was determined using linear regression analysis based on the relationship between sample concentration and percentage inhibition [15].

### 2.5 Statistical analysis

Data were expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was applied separately for each bacterial species and antioxidant dataset to evaluate the effect of concentration. When significant differences were detected, Duncan's multiple range test was applied as a post hoc analysis using the same significance level ($p < 0.05$). Statistical analyses were performed using Microsoft Excel with appropriate statistical functions [20-22].

## 3. RESULTS AND DISCUSSION

### 3.1 Phytochemical analysis of *A. cerana* honey

The results of the phytochemical analysis of honey from *A. cerana* bees, indicated by the reaction changes in the samples, are presented in Table 1.

**Table 1.** Results of phytochemical analysis of *A. cerana* honey

| Compound Testing | Phytochemical Test | Observation Result |
|---|---|---|
| Alkaloid | No change in the form of yellow coloured precipitate in honey | - |
| Flavonoid | Striking colour change to yellow colour | + |
| Tannin | Green or bluish-black discolouration of honey | + |
| Saponin | Occurrence of stable foam or froth in the honey phytochemical test | + |
| Steroid | No change in the colour layer of honey | - |

Description: +: Positive (Contains compounds/forms colour); -: Negative (No compound contained/no colour formed).

The phytochemical analysis conducted in this study included qualitative tests for alkaloids, flavonoids, tannins, saponins, and steroids. The results presented in Table 1 indicate that *A. cerana* honey tested positive for tannins, flavonoids, and saponins, whereas alkaloids and steroids were not detected, as evidenced by the absence of color changes typically associated with these compounds.

The detection of flavonoids, tannins, and saponins indicates that the bioactivity of *A. cerana* honey is primarily associated with phenolic and glycosidic compounds. These classes of secondary metabolites are widely recognized as key contributors to the biological functions of honey, including antimicrobial and antioxidant activities. The absence of alkaloids and steroids suggests that nitrogen-containing heterocycles and sterol-type compounds do not significantly contribute to the functional properties of the honey examined in this study. The phytochemical profile observed here is consistent with reports on *A. cerana* honey from other Southeast Asian regions, where phenolic compounds dominate the chemical composition. Such consistency supports the reliability of the phytochemical characteristics and provides a foundation for interpreting the antibacterial and antioxidant activities discussed in subsequent sections.

### 3.2 Antibacterial potency of *A. cerana* honey against *Escherichia coli*

The results of the statistical analysis and effectiveness test of honey extract inhibition against *E. coli* are presented in Figure 2.

The results of the study in Figure 2 show that *A. cerana* honey can inhibit the growth of *E. coli* in all concentration treatments and chloramphenicol (30 µg) positive control, recognised by the presence of clear zones around the pits made in each treatment. The clear zone or zone of inhibition was not found in the negative control, indicating that the solvent used could not inhibit the growth of *E. coli*. The inhibition zone at 100% concentration was the highest (25.73 mm) when compared to the zone of inhibition of *A. cerana* honey at other concentrations. Even the inhibition zone is greater when

compared to the positive control inhibition zone or chloramphenicol 30 µg, which is 22.05 mm wide. The inhibition of *A. cerana* honey was categorised into strong inhibition for extracts with a concentration of 25%, and for concentrations of 50%, 75%, and 100% were included in the very strong category.

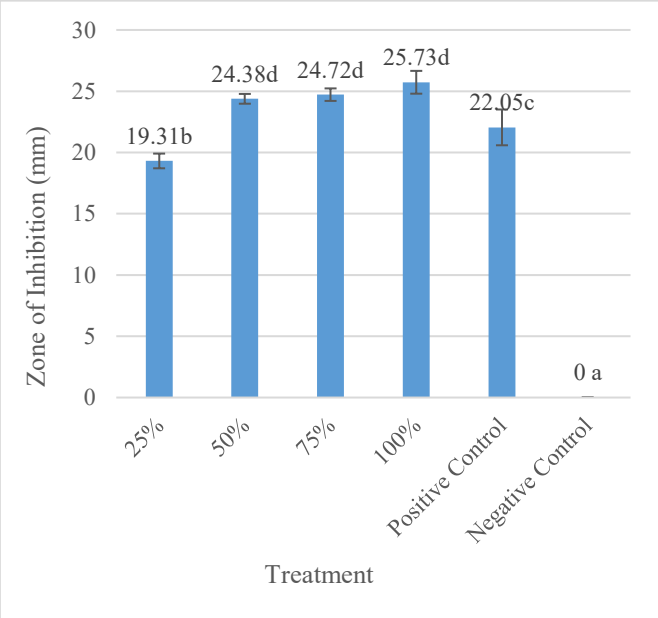


**Figure 2.** Bar graph of the inhibition zone diameter of *E. coli* based on honey concentration

The results of the analysis of variance (ANOVA) demonstrated that the concentration of *A. cerana* honey had a significant effect on the inhibition of *Escherichia coli* growth ($p < 0.05$). Accordingly, Duncan's multiple range test was performed at the 5% significance level to determine differences among treatments. The post hoc analysis revealed that all tested concentrations differed significantly in their inhibitory effectiveness against *E. coli*. Specifically, the 25% honey concentration showed a significantly lower inhibitory effect compared to the 50%, 75%, and 100% concentrations, as well as the positive control. In contrast, the 50% honey concentration did not differ significantly from the 75% and 100% concentrations, indicating comparable antibacterial effectiveness. These results demonstrate that *A. cerana* honey at a concentration of 50% is sufficient to effectively inhibit the growth of *E. coli*. Overall, a positive relationship was observed between honey concentration and the inhibition of *E. coli* growth, whereby higher honey concentrations produced larger inhibition zones. This trend is attributed to the increasing levels of secondary metabolites with antibacterial activity as the honey concentration increases [19, 20, 23].

The high inhibition of *E. coli* in *A. cerana* honey is due to the content of secondary metabolic compounds or active compounds contained in the honey. The results of phytochemical analysis showed that the honey extract detected flavonoids, tannins, and saponins. This is in accordance with the results of research [24, 25], which explains that saponins can inhibit the growth of gram-positive, gram-negative bacteria or act as an anti-fungal agent.

As shown in Figure 2, *A. cerana* honey exhibited strong antibacterial activity against *Escherichia coli* at all tested concentrations. Notably, even at 50% (w/v), the antibacterial effect was comparable to higher concentrations and to the positive control (chloramphenicol 30 µg), indicating substantial inhibitory potential.

This observation suggests that, at a 50% concentration, the antibacterial activity may have reached an optimal level, beyond which increasing the concentration does not significantly enhance the inhibitory effect. The antibacterial activity is likely influenced by a combination of bioactive constituents and physicochemical properties of honey, such as osmotic pressure, acidity, and hydrogen peroxide production, which may act synergistically to suppress bacterial growth. Physicochemical properties of honey, including high osmotic pressure, acidity, and the potential production of hydrogen peroxide, are likely to contribute synergistically to bacterial growth inhibition. Once these mechanisms operate at their maximal level, further increases in honey concentration do not result in statistically significant differences in antibacterial activity.

From an applied perspective, the comparable antibacterial performance observed at lower concentrations highlights the potential of this honey as an efficient natural antibacterial agent. The ability to achieve substantial inhibition at moderate concentrations may offer practical advantages in terms of cost-effectiveness, resource utilization, and reduced risk of adverse effects. This finding is highly relevant for the development of natural antibacterial agents, as the use of lower yet effective concentrations may offer advantages in terms of efficiency, cost-effectiveness, and reduced potential limitations related to material availability or adverse effects.

Moreover, the comparable antibacterial performance between honey and chloramphenicol does not imply that honey is "stronger" than the antibiotic. Rather, it underscores that honey exerts its antibacterial action through multi-target and synergistic mechanisms, in contrast to chloramphenicol, which acts via a single molecular target, namely the inhibition of bacterial protein synthesis. The complexity of honey's mode of action may reduce the likelihood of bacterial resistance development, highlighting the potential of *A. cerana* honey as a complementary or alternative antibacterial agent in specific applications.

### 3.3 Antibacterial potency of *A. cerana* honey against *Staphylococcus aureus* bacteria

The statistical outcomes and the inhibitory performance of *A. cerana* honey against *S. aureus* are presented in Figure 3.

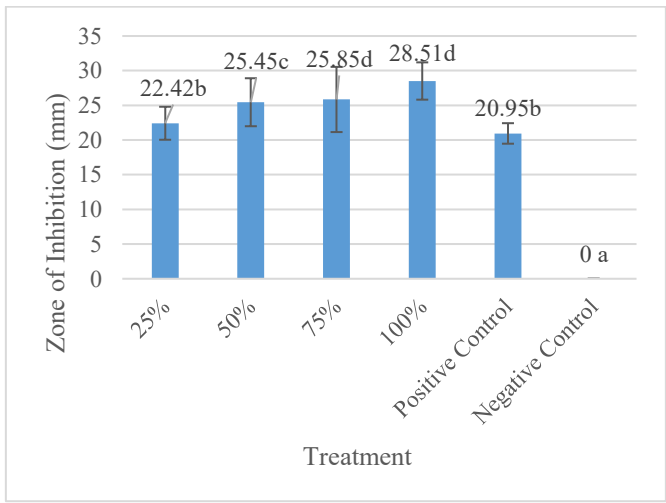


**Figure 3.** Bar graph of the inhibition zone diameter of *S. aureus* based on honey concentration

As shown in Figure 3, *A. cerana* honey effectively inhibited the growth of *S. aureus*, as evidenced by the formation of clear inhibition zones around the wells in all treatments, with inhibition diameters ranging from 22.42 mm (25% concentration) to 28.51 mm (100% concentration), all classified as very strong (> 20 mm). The results of the analysis of variance showed that the concentration of *A. cerana* honey had a significant effect on the inhibition of bacterial growth of *S. aureus,* with a significant $p < 0.05$. To determine the difference in the effect of each concentration of the extract, Duncan's test was conducted at the 5% level. The results of further tests showed that all concentrations were significantly different in their effectiveness in inhibiting the growth of *S. aureus*. The highest inhibition (28.51 mm) was observed at the 100% (w/v) honey concentration. The greater susceptibility of *S. aureus* compared to *E. coli* may be attributed to structural differences in their cell walls. As a Gram-positive bacterium, *S. aureus* possesses a thick peptidoglycan layer without an outer membrane, which may facilitate the penetration of antibacterial agents. In contrast, the outer lipopolysaccharide (LPS) membrane of Gram-negative *E. coli* can act as a protective barrier, reducing the diffusion of antibacterial compounds. These structural differences likely explain the variation in inhibition zones observed between the two bacterial strains. The results of phytochemical analysis showed that *A. cerana* honey contains many flavonoids, tannins, and saponins. The results of several studies show that these compounds are active compounds that are anti-bacterial in nature [26-29].

Based on the results presented in Figure 3, *A. cerana* honey at a 25% concentration demonstrated a greater inhibitory effect against *S. aureus* than the positive control (chloramphenicol 30 µg), as indicated by a larger inhibition zone diameter. This finding is scientifically and practically significant because it suggests that even at relatively low concentrations, *A. cerana* honey contains a sufficient combination of bioactive compounds, including flavonoids, tannins, and saponins, that can effectively inhibit the growth of *S. aureus* [8, 14, 30]. In addition to these phytochemical contributions, the physicochemical properties of honey, such as high osmotic pressure, low pH, and the production of hydrogen peroxide ($H_2O_2$), are known to act synergistically to disrupt bacterial cells and contribute to overall antimicrobial activity [17]. The observation that effective antibacterial activity occurs at a low honey concentration suggests that the activity is not solely dependent on high dosage, but rather on the efficiency of its multi-target mode of action [8]. Honey's antibacterial mechanisms are multifactorial, involving the generation of $H_2O_2$ through glucose oxidase activity, high sugar-induced osmotic stress, low water activity, and the presence of diverse bioactive compounds such as phenolic constituents and bee defensin-1, all of which contribute to inhibiting bacterial growth [14].

The differential response of *S. aureus* to honey compared to a conventional antibiotic may also be explained by differences in cell structure. As a Gram-positive bacterium, *S. aureus* lacks an outer LPS membrane, allowing bioactive components in honey to interact more readily with the bacterial cell wall and membrane [31, 32]. This structural characteristic facilitates the penetration of phenolic compounds and other antimicrobial factors, enabling strong antibacterial effects even at lower concentrations relative to certain synthetic agents. Importantly, the complexity of honey's antibacterial mechanisms offers a potential advantage in reducing the risk of bacterial resistance. Unlike many antibiotics that act on a single molecular target, rendering them susceptible to resistance development, honey employs multiple mechanisms of action simultaneously, making it more difficult for bacteria to adapt or develop resistance. Several studies have highlighted the potential of honey to combat resistant strains and biofilms, further supporting its use as a complementary antimicrobial agent in clinical and non-clinical settings. From a practical perspective, demonstrating strong antibacterial activity at a lower concentration has implications for resource efficiency, cost-effectiveness, and sustainability, especially when considering the use of locally available natural products in low-resource settings. Therefore, these findings reinforce the potential of *A. cerana* honey as a natural antibacterial agent with both scientific relevance and practical applicability, particularly against Gram-positive pathogens such as *S. aureus*.

Based on the data presented in Figures 2 and 3, *A. cerana* honey demonstrated slightly greater inhibitory activity against *S. aureus* than *E. coli* at equivalent concentrations. The inhibition zones ranged from 22.42 to 28.51 mm for *S. aureus* and from 19.31 to 25.73 mm for *E. coli*, indicating higher susceptibility of the Gram-positive strain. This difference indicates that *S. aureus* is more susceptible to the antibacterial activity of *A. cerana* honey than *E. coli*. The observed difference in antibacterial effectiveness is very likely associated with structural differences in the cell walls of Gram-positive and Gram-negative bacteria. *S. aureus*, as a Gram-positive bacterium, possesses a cell wall dominated by a thick peptidoglycan layer and lacks an outer LPS membrane. The absence of this outer membrane facilitates direct interaction of honey's bioactive compounds—such as flavonoids, tannins, and saponins—with the bacterial cell wall and membrane, leading to disruption of cell integrity, protein denaturation, and confirmed leakage of intracellular components.

In contrast, *E. coli*, as a Gram-negative bacterium, contains an additional outer membrane rich in LPS that acts as a selective permeability barrier against antibacterial agents. This structural feature can impede the penetration of phenolic compounds and other secondary metabolites present in honey, rendering *E. coli* relatively more resistant than *S. aureus*. Consequently, although *A. cerana* honey still exhibits strong antibacterial activity against *E. coli*, its inhibitory effect tends to be slightly lower compared to that observed against Gram-positive bacteria. Beyond cell wall architecture, the multi-target and synergistic mechanisms of honey also play a critical role in its antibacterial action. Phytochemical constituents (flavonoids, tannins, and saponins) act in concert with physicochemical factors such as acidity, high osmotic pressure, and the potential generation of hydrogen peroxide, collectively damaging multiple bacterial cellular components. However, the effectiveness of this combination of mechanisms is more readily achieved in Gram-positive bacteria, which present fewer structural barriers. Taken together, the finding that *A. cerana* honey is more effective against *S. aureus* than *E. coli* is consistent with general patterns of antibacterial activity reported for natural products. These results reinforce the understanding that bacterial cell wall structure is a key determinant of susceptibility to natural antibacterial agents and further highlight the potential of *A. cerana* honey as a highly effective antibacterial agent, particularly against Gram-positive bacteria.

To contextualize the bioactivity of *A. cerana* honey from Lore Lindu National Park, comparisons were made with

previously reported honeys from other regions. Studies on *A. cerana* honey from Southeast Asia have reported inhibition zone diameters against *S. aureus* ranging from approximately 18 to 30 mm, and against *E. coli* from 15 to 30 mm, depending on the assay method and sample preparation. In some studies employing modified Oxford cup or agar diffusion techniques with undiluted honey, larger inhibition zones have been reported; however, such differences are strongly influenced by methodological factors, including diffusion dynamics and honey concentration. When compared using standard agar well diffusion assays, the inhibition zones observed in the present study fall within the upper range reported for *A. cerana* honey, indicating strong antibacterial potential.

In comparison with high-activity medicinal honeys such as Manuka honey, previous studies have reported inhibition zones against *S. aureus* of approximately 20–30 mm in agar diffusion assays, values that are comparable to those obtained in this study at moderate to high honey concentrations. Importantly, such comparisons should be interpreted cautiously, as Manuka honey is characterized by unique antibacterial markers (e.g., methylglyoxal), whereas *A. cerana* honey derives its activity primarily from a combination of phenolic compounds, acidity, osmotic effects, and hydrogen peroxide generation.

### 3.4 Antioxidant capacity of *A. cerana* honey

The results of the antioxidant activity assay of *A. cerana* honey are presented in Figure 4.

The $IC_{50}$ value obtained for *A. cerana* honey, measured at 171.95 ppm, categorizes its antioxidant strength as weak to moderate based on established classification systems. While this value is higher (indicating lower antioxidant potency) than purified antioxidant standards such as vitamin C ($IC_{50}$ = 44.95), it remains within a biologically meaningful range for natural products. The finding suggests that although the honey does not exert highly potent radical-scavenging effects, it still contributes measurable antioxidant benefits that may support its traditional medicinal use. The observed antioxidant activity aligns closely with the phytochemical profile identified in the honey sample. The phytochemical screening revealed positive reactions for flavonoids, tannins, and saponins, while alkaloids and steroids were absent. This composition is consistent with patterns found in various monofloral honeys, where phenolic compounds, particularly flavonoids and tannins, are recognized as the primary contributors to antioxidant behavior.

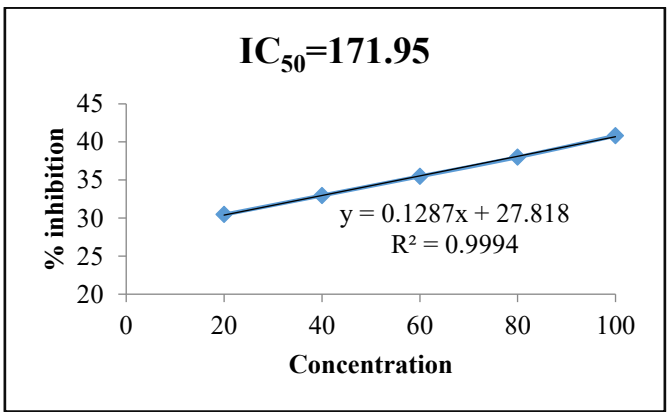


**Figure 4.** Graph of the relationship between the level of inhibition (%) and the concentration and $IC_{50}$ value of *A. cerana* honey

Flavonoids have been widely documented for their strong radical-scavenging potential due to their hydroxyl functionalities and conjugated aromatic ring structures. Their presence in *A. cerana* honey likely plays a central role in shaping the observed inhibition values. These compounds exert antioxidant effects through hydrogen atom transfer (HAT) and single-electron transfer (SET) mechanisms, both of which participate in the reduction of the DPPH radical [33]. Thus, even at moderate concentrations, flavonoids substantially influence the overall antioxidant activity of the honey. Tannins, another phenolic class detected in the sample, may have further enhanced the honey's antioxidant profile. Their high molecular weight and multiple hydroxyl groups allow them to interact strongly with free radicals and metal ions. Such chelating and radical-quenching properties contribute not only to antioxidant action but also to broader antimicrobial and astringent activities often associated with tannin-rich natural extracts. The positive tannin reaction, therefore, complements the flavonoid-mediated antioxidant mechanism.

The detection of saponins adds another dimension to the honey's bioactivity. Although saponins are less potent as direct radical scavengers compared to phenolics, they may exert indirect antioxidant effects by stabilizing cell membranes, inhibiting lipid peroxidation, and modulating oxidative stress pathways. Their amphipathic nature and surface-active properties may also influence the solubility and interaction of other phenolic constituents, subtly enhancing the overall antioxidant performance. The absence of alkaloids and steroids suggests that the antioxidant activity is not contributed by nitrogen-containing heterocycles or sterol-type molecules, which are occasionally present in plant-derived substances. This absence reinforces the conclusion that phenolic groups, particularly flavonoids and tannins, are the dominant drivers of the honey's antioxidant activity. Such clarity in phytochemical composition aids in accurately attributing the mechanisms responsible for the observed $IC_{50}$ value.

Comparatively, the moderate antioxidant levels observed in *A. cerana* honey are consistent with findings from tropical honeys, where floral diversity and nectar composition influence the concentration of bioactive compounds. Honeys with exceptionally high antioxidant activity typically originate from flora with dense polyphenolic profiles. In contrast, the moderate activity in the present sample may arise from environmental variability, nectar source characteristics, and the metabolic processing of nectar by *A. cerana* bees.

Despite its moderate activity, the honey's gradual increase in inhibition percentage across concentrations indicates reliable and reproducible antioxidant behavior. The triplicate measurements at each concentration showed stable absorbance reductions, suggesting that the antioxidant constituents are evenly distributed within the honey matrix. This reproducibility strengthens the validity of the reported $IC_{50}$ value and underscores the honey's potential as a consistent natural antioxidant source. Overall, the antioxidant findings, when integrated with the phytochemical analysis, highlight a synergistic relationship among the phenolic and saponin constituents present in *A. cerana* honey. Although individually moderate in potency, their combined effects result in meaningful radical-scavenging activity. These results support the classification of *A. cerana* honey as a functional natural product with antioxidant potential, complementing its previously demonstrated antibacterial properties. Such multifunctional bioactivity positions this honey as a promising

candidate for applications in nutraceuticals, natural therapeutics, and value-added forest-based products.

Regarding antioxidant activity, comparative studies on A. cerana honey from Thailand and Vietnam have reported DPPH $IC_{50}$ values ranging from approximately 150 to 200 ppm, which are comparable to the $IC_{50}$ value of 171.95 ppm observed in the present study. In contrast, Manuka honey typically exhibits lower $IC_{50}$ values (approximately 50–100 ppm), reflecting its higher phenolic content. These comparisons suggest that although the antioxidant activity of A. cerana honey from Lore Lindu is moderate, it remains biologically meaningful and contributes to its overall bioactivity.

Taken together, these comparisons demonstrate that *A. cerana* honey from Lore Lindu National Park occupies a competitive position among bioactive honeys, exhibiting antibacterial activity comparable to several medicinal honeys and antioxidant activity within the typical range reported for tropical forest honeys. This reinforces its potential value as a functional natural product and supports further exploration of its application in health-related and nutraceutical contexts.

## 4. CONCLUSION

This study demonstrates that honey produced by *A. cerana* from the Lore Lindu National Park exhibits significant antibacterial and antioxidant activities. Phytochemical screening confirmed the presence of key secondary metabolites, namely flavonoids, tannins, and saponins, which play essential roles in contributing to the honey's biological effects. The antibacterial assays revealed that *A. cerana* honey possesses very strong inhibitory activity against *E. coli* and *S. aureus*, with maximum inhibition zones of 25.73 mm and 28.51 mm, respectively, at 100% concentration. These inhibition values exceeded those of the positive control (chloramphenicol 30 µg), underscoring the potential of *A. cerana* honey as an effective natural antibacterial agent against both Gram-positive and Gram-negative bacteria. Moreover, *A. cerana* honey exhibited antioxidant activity with an $IC_{50}$ value of 171.95 ppm, which falls within the weak-to-moderate category. Although its radical-scavenging capacity is lower than that of pure antioxidant compounds such as vitamin C, the presence of bioactive constituents still provides a measurable protective effect against oxidative stress. Overall, the findings highlight the multifunctional bioactivity of *A. cerana* honey and support its potential utilization as a natural source of therapeutic compounds.

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