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Formulation and Antibacterial Activity of Gel Mask Based on Wild Honeybees Honey Nanoparticles Againts Propionibacterium acnes

Original Article

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Abstract:
Acne is a common skin condition associated with *Propionibacterium acnes*. Wild forest honey from the Asian honeybee (*Apis dorsata*) has antibacterial properties that may be enhanced through nanoparticle technology. This study aimed to determine the antibacterial activity of wild forest honey and its nanoparticles against *Propionibacterium acnes*, characterize the nanoparticles, and evaluate the antibacterial activity of the resulting gel mask. Honey nanoparticles were prepared using the ionic gelation method with chitosan and sodium tripolyphosphate (Na-TPP). Nanoparticles were characterized by particle size, polydispersity index (PDI), and zeta potential. Gel masks containing 25%, 35%, and 45% nanoparticles were evaluated for physical properties and antibacterial activity. MIC and MBC were also determined. Wild forest honey showed an MIC of 25% and MBC of 50%, while its nanoparticles showed an MIC of 6.25% and MBC of 12.5%. The nanoparticles had a particle size of 137.37 nm, PDI of 0.329, and zeta potential of +32.73 mV. All formulations met the physical requirements, with F3 (45%) showing the highest antibacterial activity. Honey nanoparticles showed greater antibacterial activity than wild forest honey. The 45% nanoparticle gel mask has potential as a topical antibacterial preparation against *Propionibacterium acnes*.

INTRODUCTION

Acne vulgaris is one of the most common skin disorders among adolescents and young adults. The condition is characterized by inflammation of the pilosebaceous unit and is associated with increased sebum production, follicular hyperkeratinization, bacterial colonization, and inflammatory responses. Among these factors, *Propionibacterium acnes* plays an important role in acne pathogenesis by producing lipase enzymes that hydrolyze sebum triglycerides into free fatty acids, thereby triggering skin inflammation (Alouw et al., 2022).

The management of acne commonly involves topical or oral antibiotics, such as clindamycin, erythromycin, and tetracycline. However, prolonged use of antibiotics may lead to bacterial resistance, reducing treatment effectiveness and increasing the risk of therapeutic failure. Therefore, the development of alternative antibacterial agents from natural sources has become an important area of research (Murphy et al., 2024).

Honey is a natural product known for its antioxidant, anti-inflammatory, and antibacterial properties. Wild forest honey (*Apis dorsata*), which is widely found in Indonesia, contains various bioactive compounds, including flavonoids, alkaloids, tannins, and phenolic compounds. These compounds have been reported to inhibit bacterial growth through various mechanisms, such as disrupting cell membrane integrity, inhibiting nucleic acid synthesis, and interfering with bacterial metabolism (Guge et

al., 2024).

Despite its antibacterial potential, the direct use of honey has several limitations, including low stability and limited penetration of active compounds into target tissues. Nanoparticle technology offers a promising approach to overcome these limitations by increasing surface area, improving stability, enhancing penetration, and facilitating the delivery of active compounds. In this study, honey nanoparticles were prepared using the ionic gelation method employing chitosan and sodium tripolyphosphate (Na-TPP). Chitosan was selected because of its biocompatibility, biodegradability, and intrinsic antibacterial activity (Rahmah et al., 2023).

Furthermore, the incorporation of honey nanoparticles into a gel mask dosage form may improve topical application and prolong contact between active compounds and the skin surface. Although several studies have reported the antibacterial activity of *A. dorsata* honey, studies investigating honey nanoparticles formulated as gel masks against *P. acnes* remain limited. Therefore, this study aimed to evaluate the antibacterial activity of wild forest honey and honey nanoparticles against *P. acnes*, characterize the resulting nanoparticles, and determine the effectiveness of honey nanoparticle gel masks as a potential anti-acne formulation.

METHODS

Materials

Wild forest honey (*A. dorsata*), chitosan, sodium tripolyphosphate (Na-TPP), acetic acid, Tween 80, Mueller Hinton Broth (MHB), Mueller Hinton Agar (MHA), clindamycin, dimethyl sulfoxide (DMSO), and other analytical-grade reagents were used in this study.

Determination of Wild Forest Honey (*A. dorsata*)

The bees to be studied were first identified. Identification was conducted to verify the species of the animals to be studied and to avoid errors in specimen collection. The identification was carried out at the Biology Laboratory of Ahmad Dahlan University.

Total Flavonoid Content

Total flavonoid content was determined using the aluminum chloride ($AlCl_3$) colorimetric method, with quercetin as the reference standard. This method is widely used because it offers good sensitivity in measuring flavonoid content based on the formation of a complex between aluminum ions and the hydroxyl groups of flavonoids, which produces a yellow color that can be measured using a UV-Vis spectrophotometer.

Preparation of Honey Nanoparticles

Honey nanoparticles were prepared using the ionic gelation method. Chitosan solution was combined with honey and Tween 80, followed by the gradual addition of Na-TPP solution. Nanoparticles were formed through electrostatic interactions between positively charged amino groups of chitosan and negatively charged phosphate groups of Na-TPP. The resulting suspension was sonicated and characterized using Particle Size Analyzer (PSA) to determine particle size, polydispersity index (PDI), and zeta potential.

Determination of MIC and MBC

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined using the liquid dilution method against *P. acnes*. Honey and honey nanoparticles were tested at various concentrations. MIC was identified as the lowest concentration inhibiting visible bacterial growth, whereas MBC was determined as the lowest concentration showing no bacterial growth after subculturing.

Gel Mask Formulation

Honey nanoparticles were formulated into gel masks at three concentrations: F1 = 25%, F2 = 35%, F3 = 45%. The formulations were evaluated for organoleptic properties, homogeneity, pH, spreadability, adhesiveness, viscosity, and drying time.

Antibacterial Activity Test

Antibacterial activity against *P. acnes* was evaluated using the agar well diffusion method. The inhibition zone diameter formed around the wells was measured after incubation. Clindamycin served as the positive control, whereas the gel base served as the negative control.

Statistical Analysis

Data were analyzed using the Kruskal-Wallis test with a significance level of 95% ($p < 0.05$).

RESULT AND DISCUSSION

Determination of Wild Forest Honey (*A. dorsata*)

The identification was conducted to confirm the identity of the material used in the study so that the authenticity and validity of the samples could be scientifically substantiated. The results of the identification

Table 1. Results of Phytochemical Screening of Wild Honey

No.	Screening	Reagents	Results
1.	Alkaloid	Mayer	+
		Wagner	+
2.	Flavonoid	Mg + HCl conc.	+
3.	Saponin	Aquadest	-
4.	Tanin	$FeCl_3$	+
5.	Terpenoid	Chloroform	-
6.	Steroid	Chloroform	+

Table 2. Standard Absorbance Results for Quercetin at a Wavelength of 416 nm

Conc. (ppm)	R1	R2	R3	$\bar{x} \pm SD$	Deviation Range
2	0,072	0,069	0,069	0,070 ± 0,002	0,069–0,072
4	0,150	0,151	0,150	0,150 ± 0,001	0,150–0,151
6	0,244	0,244	0,243	0,244 ± 0,001	0,243–0,244
8	0,403	0,403	0,405	0,404 ± 0,001	0,403–0,405
10	0,500	0,503	0,503	0,502 ± 0,002	0,500–0,503

showed that the sample used was wild forest honey from the species *A. dorsata*. *A. dorsata* is a species of honey-producing bee commonly found in the tropical forests of Asia, including Indonesia. This species is known as a wild forest bee that builds nests in tall trees and obtains nectar from various types of plants that grow naturally in the forest environment.

The characteristics of the *A. dorsata* bee's habitat result in honey with a more complex diversity of bioactive compounds compared to farmed honey. The diversity of nectar sources influences the content of phenolic compounds, flavonoids, alkaloids, and other secondary metabolites that play a role in the biological activity of honey. Therefore, the sample characterization process is a crucial step to ensure that the samples used are appropriate for the research objective, namely evaluating the antibacterial potential of wild honeybee (*A. dorsata*) honey against *P. acnes*.

Phytochemical Screening

Phytochemical screening of wild honey was conducted to identify and determine the classes of secondary metabolites it contains. Phytochemical screening results indicate that wild bee (*A. dorsata*) honey contains flavonoids, alkaloids, tannins, and steroids. The presence of these compounds is believed to contribute to the honey's antibacterial activity. Flavonoids are known to inhibit nucleic acid synthesis and damage bacterial cell membranes, while alkaloids can disrupt peptidoglycan formation in bacterial cell walls. Tannins play a role in inactivating microbial enzymes and increasing cell membrane permeability, while steroids can disrupt the integrity of bacterial membranes. The combination of these various bioactive compounds is thought to produce a synergistic effect in inhibiting the growth of *P. acnes*, which was subsequently observed in the results of antibacterial activity tests on both honey and honey nanoparticles (Kaligis et al., 2022).

Total Flavonoid Content

The maximum wavelength was determined using a quercetin standard solution in the 400–800 nm range after incubation for 30 minutes as the operating time (Mahani et al., 2022). The measurement results showed a maximum wavelength of 416 nm, which was subsequently used to measure the absorbance of the quercetin standard solution and the wild honey sample (*A. dorsata*).

In this study, to determine the total flavonoid content in the samples, quercetin was used as a standard solution at concentrations of 2, 4, 6, 8, and 10 ppm, which

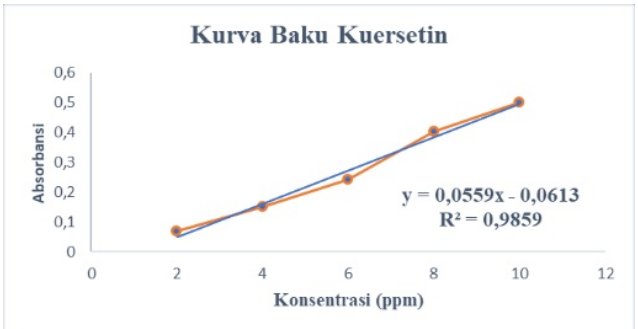

Figure 1. Calibration curve for quercetin at a wavelength of 416 nm

Table 3. Total Flavonoid Content in Wild Honey

Replication	Absorbance	Total Flavonoid Content (mg QE/g)	x̄ Total Flavonoid Content (mg QE/g) ± SD
1	0,481	9,701	
2	0,482	9,719	9,719 ±
3	0,483	9,737	0,018

Tabel 4. Nanoparticle Formulation

Materials	Formula 1	Formula 2	Formula 3
Kitosan 0,1%	50 mL	-	50 mL
Kitosan 0,2%	-	60 mL	-
NaTPP 0,1%	10 mL	10 mL	10 mL
Tween 80	0,5 mL	0,5 mL	3 mL
Honey	0,75 gr	0,75 gr	0,75 gr

was reacted with AlCl3 to produce a linear relationship as shown in Figure 1. The measurement results indicate that as the concentration of quercetin increases, so does its absorbance value.

The measurement results show that the absorbance of quercetin increases with increasing concentration, in accordance with Lambert-Beer's Law, which states that absorbance is directly proportional to concentration. The calibration curve obtained has a coefficient of determination (R^2) of 0.9859, indicating a good linear relationship between concentration and absorbance, making it suitable for determining total flavonoid content (Krisyanella et al., 2021).

The total flavonoid content of wild forest honey (*A. dorsata*) was determined to be 9.719 mg QE/g. The presence of flavonoids in honey may contribute to its biological activities, including antibacterial and antioxidant effects. The flavonoid content obtained in this study was higher than that reported by Fitriana et al. (2023), who found total flavonoid contents of 0.42 mg QE/g in *A. dorsata* honey and 0.90 mg QE/g in *A.* mellifera honey. Kaligis and Mokosuli (2022) also reported a lower value of 1.6 mg QE/g for *A. dorsata* honey collected from the Manembo forest. However, the result obtained in this study was lower than that reported by Mahani et al. (2022), who found total flavonoid contents of 17.24 mg QE/g and 29.48 mg QE/g in *A. dorsata* honey from East Nusa Tenggara and Bangka Belitung, respectively. These differences may be attributed to variations in botanical origin, geographical conditions, environmental factors, and analytical methods used in each study.

Honey Nanoparticle Formulation

Honey nanoparticles were prepared using the ionic gelation method involving chitosan and sodium tripolyphosphate (Na-TPP). In this system, chitosan acts as a cationic polymer, while Na-TPP functions as a crosslinking agent through electrostatic interactions with the protonated amino groups of chitosan. The interaction between both components results in the formation of nanoparticle structures capable of encapsulating wild forest honey (*A. dorsata*) (Safitri, 2022).

Three formulations were developed by varying the concentration of chitosan and Tween 80. Formula 1 contained 0.1% chitosan, Formula 2 contained 0.2% chitosan, while Formula 3 contained 0.1% chitosan with a higher concentration of Tween 80. The variation of formulation components was intended to evaluate their influence on nanoparticle characteristics, particularly particle size, particle distribution, and colloidal stability (Syarmalina et al., 2019).

Chitosan concentration is known to affect nanoparticle formation because increasing polymer concentration can increase the viscosity of the system and promote particle aggregation, resulting in larger particle sizes. Tween 80 was incorporated as a surfactant to improve particle dispersion and reduce interfacial tension during nanoparticle formation. Therefore, the balance between chitosan concentration and surfactant level plays an important role in obtaining nanoparticles with desirable physicochemical characteristics (Marwah et al., 2022)

Characterization of Honey Nanoparticles

Nanoparticle characterization was performed to determine particle size, polydispersity index (PDI), and zeta potential, which are important parameters in evaluating the quality and stability of nanoparticle systems. The characterization results of the three formulations are presented in Table 5.

The particle size analysis showed that Formula 1 (F1) had an average particle size of

Tabel 5. Results of Particle Size and PDI Characterization of Wild Honeybee (*Apis dorsata*)

Replication	Particle Size (nm)			Polydispersity Index			Requirements
	F1	F2	F3	F1	F2	F3	
R1	126,9	756	260,9	0,322	0,463	0,314	Particle Size: 1-200 nm PDI: <0,5
R2	148,9	855,2	94,82	0,346	0,101	0,821	
R3	136,3	2.975	546	0,319	1.000	0,604	
$\bar{x} \pm SD$	137,37 ± 11,02	1.528,7 ± 1.252,34	300 ± 227	0,32 ± 0,015	0,521 ± 0,452	0,58 ± 0,256	
Range	126,9 – 148,9	756 – 2.975	94,82 – 546	0,31 – 0,346	0,101 – 1,000	0,31 – 0,821	

137.37 ± 11.02 nm, Formula 2 (F2) had a particle size of 1528.73 ± 1252.34 nm, and Formula 3 (F3) had a particle size of 300.57 ± 227.01 nm. Based on these results, only F1 met the nanoparticle size criterion of 1–200 nm. The larger particle size observed in F2 may be attributed to the higher concentration of chitosan (0.2%), which increased the viscosity of the system and promoted particle aggregation during nanoparticle formation. Meanwhile, F3 exhibited a smaller particle size than F2 but still exceeded the nanoparticle size range, indicating that the higher concentration of Tween 80 was not sufficient to maintain particle size within the desired range (Prihantini et al., 2019).

The PDI values obtained were 0.329 ± 0.015 for F1, 0.521 ± 0.452 for F2, and 0.580 ± 0.256 for F3. The PDI value of F1 was below 0.5, indicating a relatively homogeneous particle size distribution. In contrast, F2 and F3 showed PDI values above 0.5, suggesting a broader particle size distribution and lower uniformity. A low PDI value is desirable because it indicates better formulation consistency and reduced particle aggregation.

The zeta potential analysis revealed values of +32.73 ± 1.99 mV for F1, +23.03 ± 0.97 mV for F2, and +13.67 ± 0.90 mV for F3. Among the three formulations, only F1 fulfilled the stability criterion of zeta potential values greater than ±30 mV. The higher zeta potential value indicates stronger electrostatic repulsion between particles, which helps prevent aggregation and improves colloidal stability. The positive charge observed in all formulations originated from the protonated amino groups of chitosan. However, the lower zeta potential values observed in F2 and F3 suggest reduced electrostatic stabilization, making these formulations more susceptible to aggregation (Fitriana et al., 2023).

The smaller particle size, lower PDI value, and higher zeta potential observed in F1 indicate that this formulation possessed the most favorable nanoparticle characteristics. The nanoscale particle size increases the surface area available for interaction with bacterial cells, thereby enhancing the delivery and effectiveness of bioactive compounds. In addition, the positive surface charge may facilitate electrostatic interactions with negatively charged bacterial membranes, contributing to antibacterial activity. Therefore, F1 was selected as the optimum nanoparticle formulation and used for subsequent gel mask formulation studies (Rahmah et al., 2023).

Comparison of Antibacterial Activity Between Honey and Honey Nanoparticles

The antibacterial activity assay demonstrated that nanoparticle formulation enhanced the antibacterial effectiveness of wild forest honey against *P. acnes*. Wild forest honey exhibited MIC and MBC values of 25% and 50%, respectively, whereas honey nanoparticles showed MIC and MBC values of 6.25% and 12.5%, respectively.

The reduction in MIC and MBC values indicates that nanoparticle honey required lower concentrations to inhibit and kill bacterial growth compared with unmodified honey. This enhancement may be attributed to the nanoscale particle size obtained in Formula 1, which increased the surface area available for interaction with bacterial cells and facilitated the delivery of bioactive compounds (Rahmah et al., 2023).

In addition, the positive zeta potential (+32.73 mV) generated by chitosan contributes to antibacterial activity through electrostatic interactions with negatively charged bacterial cell membranes. These interactions can increase membrane permeability, promote leakage of intracellular components, and ultimately cause bacterial cell death. Therefore, the superior antibacterial activity of honey nanoparticles is likely the result of both improved physicochemical properties and the combined antibacterial effects of honey

Tabel 6. Results of the Characterization of the Zeta Potential of Wild Honeybee (Apis dorsata) Nanoparticles

Parameters	R1 (mV)	R2 (mV)	R3 (mV)	$\bar{x} \pm SD$ (mV)	Deviation Range (mV)
F1	+31,3	+31,9	+35,0	32,73 ± 1,99	31,3 – 35,0
F2	+23,2	+23,9	+22,0	23,03 ± 0,96	22,0 – 23,9
F3	+13,2	+13,1	+14,7	13,67 ± 0,90	13,1 – 14,7
Req.: >+30 mV or <-30mV					

bioactive compounds and chitosan (Safitri, 2022).

Gel Mask Formulation

The optimum honey nanoparticle formulation (F1) was subsequently incorporated into a gel mask dosage form at concentrations of 25%, 35%, and 45%, designated as F1, F2, and F3, respectively. The gel base consisted of hydroxypropyl methylcellulose (HPMC) as the gelling agent, glycerin as the humectant, triethanolamine (TEA) as the pH adjuster, and preservatives to maintain formulation stability.

The incorporation of honey nanoparticles into the gel system aimed to enhance the practicality of topical application while maintaining the antibacterial activity of the active ingredient. A gel mask dosage form offers several advantages, including ease of application, prolonged contact time with the skin, good patient acceptability, and the ability to facilitate the release of active compounds onto the skin surface. Variations in nanoparticle concentration were expected to influence the physical characteristics and antibacterial activity of the resulting formulations (Windy et al., 2022)

Physical Evaluation of Honey Nanoparticle Gel Mask Organoleptic and Homogeneity Evaluation

Organoleptic evaluation showed that all formulations had a semi-solid consistency with a characteristic honey aroma. An increase in nanoparticle concentration caused the gel to become slightly cloudy. Homogeneity testing showed that all formulations were evenly mixed with no visible coarse particles, indicating the successful incorporation of nanoparticles into the gel matrix. Homogeneous nanoparticle distribution is crucial because it ensures even dispersion of the active ingredient throughout the formulation, which can contribute to consistent antibacterial activity and product quality.

pH Evaluation

The pH values of all formulations ranged from 5.27 to 5.40 and complied with the acceptable pH range for topical preparations (4.5–6.5). The results indicate that the incorporation of honey nanoparticles did not cause substantial changes in the acidity of the gel system. Appropriate pH values are important to minimize the risk of skin irritation and maintain skin compatibility. Furthermore, pH stability contributes to the overall quality and acceptability of topical formulations.

Spreadability Evaluation

The spreadability values of the formulations ranged from 5.37 to 6.33 cm, meeting the recommended spreadability range for gel preparations. Formula F0 exhibited the highest spreadability, whereas Formula F3 showed the lowest value. The decrease in spreadability observed with increasing nanoparticle concentration may be related to the increased viscosity of the gel system. Higher concentrations of dispersed particles can strengthen the internal structure of the gel, reducing its ability to spread on the skin surface. Nevertheless, all formulations remained within the acceptable range, indicating good applicability.

Adhesiveness Evaluation

The adhesiveness values ranged from 4.51 to 6.20 seconds, with all formulations fulfilling the minimum requirement of more than 4 seconds. Formula F3 demonstrated the highest adhesiveness value. The increase in adhesiveness with increasing nanoparticle concentration may be attributed to stronger interactions within the gel matrix, resulting in improved retention on the skin surface. Adequate adhesiveness is desirable because it prolongs the contact time between the formulation and the skin, thereby potentially enhancing the effectiveness of active compound delivery.

Viscosity Evaluation

The viscosity values ranged from 5,573.67 to 8,290.33 cPs and complied with the recommended viscosity range for topical gel preparations. Formula F3 exhibited the highest

viscosity, whereas Formula F0 showed the lowest viscosity. The increase in viscosity observed with increasing nanoparticle concentration may be due to the greater amount of dispersed material within the gel system, which increases resistance to flow. Viscosity is an important parameter because it influences spreadability, adhesiveness, stability, and overall user acceptability. The results also demonstrated an inverse relationship between viscosity and spreadability, where formulations with higher viscosity tended to exhibit lower spreadability values.

Overall, all formulations met the required physical evaluation parameters. However, Formula F3 exhibited the most favorable characteristics, showing adequate pH, spreadability, adhesiveness, and viscosity while providing the highest antibacterial activity against *P. acnes*

Antibacterial Activity of Honey Nanoparticle Gel Mask

The antibacterial activity of honey nanoparticle gel mask was evaluated using the agar well diffusion method against *P. acnes*. This method was selected because it is suitable for semi-solid preparations and allows the active compounds to diffuse into the agar medium. Antibacterial activity was determined by measuring the diameter of the inhibition zone formed around the wells. A larger inhibition zone indicates stronger antibacterial activity.

The results showed that all formulations containing honey nanoparticles were able to inhibit the growth of P. *acnes*. Formula F1 (25%), F2 (35%), and F3 (45%) produced inhibition zones of 15.50 ± 0.50 mm, 18.50 ± 0.87 mm, and 20.33 ± 0.58 mm, respectively. The positive control (clindamycin) showed an inhibition zone of 18.17 ± 0.58 mm, whereas the negative control (gel base) did not produce any inhibition zone. These findings indicate that the antibacterial activity increased with increasing concentrations of honey nanoparticles in the gel formulation.

Based on the classification proposed by Davis and Stout, inhibition zones of 10–20 mm are categorized as strong, while inhibition zones greater than 20 mm are classified as very strong. Accordingly, F1 and F2 exhibited strong antibacterial activity, whereas F3 demonstrated very strong antibacterial activity against P. *acnes*. The superior performance of F3 suggests that increasing the concentration of honey nanoparticles enhanced the antibacterial effectiveness of the gel mask.

The enhanced antibacterial activity observed in F3 may be attributed to the higher concentration of bioactive compounds present in the formulation. Phytochemical screening confirmed the presence of flavonoids, alkaloids, tannins, and steroids in wild forest honey. These compounds are known to possess antibacterial properties through different mechanisms, including disruption of bacterial cell membranes, inhibition of nucleic acid synthesis, interference with enzyme activity, and inhibition of cell wall formation. The combined action of these bioactive compounds may contribute to the strong antibacterial effect observed in the present study.

In addition to the contribution of honey-derived bioactive compounds, the nanoparticle system itself may have enhanced antibacterial performance. The optimized nanoparticle formulation exhibited a particle size of 137.37 nm and a zeta potential of +32.73 mV. The small particle size increases the surface area available for interaction with bacterial cells, thereby facilitating more effective delivery of active compounds. Furthermore, the positively charged chitosan matrix can interact electrostatically with negatively charged bacterial cell surfaces, resulting in membrane disruption and increased permeability. These mechanisms may enhance the antibacterial efficacy of honey nanoparticles compared with conventional honey.

Interestingly, Formula F3 produced a slightly larger inhibition zone than the positive control clindamycin. However, the antibacterial mechanisms of the two agents are different. Clindamycin acts by inhibiting bacterial protein synthesis through binding to the 50S ribosomal subunit, whereas honey nanoparticles exert antibacterial effects through multiple mechanisms involving honey bioactive compounds and the antibacterial properties of chitosan. The presence of several antibacterial mechanisms may provide a synergistic effect that contributes to the high

Tabel 7. Antibacterial Activity of Gel Mask Formulations

Formula	Inhibition Zone (mm)
F1 (25%)	**15.50 ± 0.50**
F2 (35%)	**18.50 ± 0.87**
F3 (45%)	**20.33 ± 0.58**
Clindamycin	**18.17 ± 0.58**
Negative Control	**0.00**

inhibitory activity observed in Formula F3.

It should be noted that the inhibition zone produced by the positive control was not completely clear. This observation may be related to the bacterial inoculation technique used in the study. The bacterial suspension was distributed using the cotton swab method, which may result in less homogeneous bacterial growth on the agar surface compared with pour plate or spread plate methods. Consequently, slight bacterial growth may still be observed within the inhibition area despite the antibacterial effect of clindamycin.

Statistical analysis using the Kruskal–Wallis test demonstrated a significant difference among the tested formulations ($p = 0.015$; $p < 0.05$). These results indicate that variations in honey nanoparticle concentration significantly affected the antibacterial activity of the gel mask. Overall, Formula F3 (45%) was identified as the optimum formulation because it exhibited the highest antibacterial activity while still meeting all physical evaluation requirements.

CONCLUSION

Wild forest honey (*A. dorsata*) was successfully formulated into nanoparticles using the ionic gelation method with chitosan and Na-TPP. The optimum nanoparticle formulation (Formula 1) exhibited a particle size of 137.37 nm, a PDI of 0.329, and a zeta potential of +32.73 mV, indicating good particle uniformity and stability. Nanoparticle formulation enhanced the antibacterial activity of honey against *P. acnes*, as demonstrated by lower MIC and MBC values compared to conventional honey. The honey nanoparticle gel masks fulfilled all physical evaluation requirements, including organoleptic properties, homogeneity, pH, spreadability, adhesiveness, and viscosity. Among the tested formulations, Formula F3 containing 45% honey nanoparticles showed the highest antibacterial activity with an inhibition zone of 20.33 ± 0.58 mm and was categorized as having very strong antibacterial activity. These findings suggest that honey nanoparticle gel masks have potential as a natural anti-acne topical formulation against *P. acnes*.

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CONFLICT OF INTEREST

The authors declare no conflict of interest regarding this study.

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