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Bioactivity of chitosan microparticles loaded with Apus bamboo (*Gigantochloa apus*) shoot extract: Emphasis on characterization and in vitro antibacterial properties for acne treatment.

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Abstract

Acne vulgaris is characterized by the production of comedones, papules, pustules, nodules, and cysts due to pilosebaceous unit expansion and inflammation. The presence of bacteria and accompanying inflammation increase this process even more. This study aimed to assess the anti-acne effects of chitosan microparticles (CMPs) loaded with the methanol extract of Apus bamboo (*Gigantochloa apus*) shoots (CMPs/Ab). The morphology of the CMPs/Ab appears to be spherical, with a particle size of 838.9 ± 33.4 nm and a zeta potential of +29.06 ± 1.22 mV, suggesting that the particles are micro-sized and stable. Moreover, the formulation of CMPs/Ab has an encapsulation effectiveness of 46.4 ± 0.91%. The antibacterial property of CMPs/Ab inhibits the growth of pathogenic bacteria caused by acne vulgaris, with an average inhibition zone of 41.67 ± 1.53 mm, 42.67 ± 2.52 mm, and 22.33 ± 1.53 mm for *Staphylococcus aureus*, *S. epidermidis*, and *Cutibacterium acne*, respectively. The CMPs/Abs formulated into anti-acne spray gel showed stable physical features for 28 days. It is a safe and potent anti-acne candidate that can disrupt the membranes of acne-causing bacteria, *S. epidermidis*, due to their natural composition, reducing the risk of adverse reactions and antibiotic resistance.

Keywords: acne vulgaris, Apus bamboo shoots, chitosan, microparticles, spray gel

1. Introduction

Acne vulgaris is an inflammatory skin condition often occurring during adolescence, with the face and back being the most commonly affected areas [1]. In Indonesia, the prevalence of acne has grown, reaching up to 80–85% at the peak incidence age 15–18 years. Acne prevalence increases in the adolescent age group due to psychological and hormonal development; thus, it requires attention to avoid further psychosocial difficulties and mental health disorders [1]. Several bacteria, such as *Staphylococcus epidermidis, S. aureus,* and *Cutibacterium acne,* may cause bacterial infection in the pathogenesis of acne [2]. It should also be noted that *S. aureus* is a pathogenic bacterium capable of causing localized infections and consequences such as endocarditis and renal disease. Antibiotics known to kill bacteria and reduce inflammation, such as erythromycin, doxycycline, and clindamycin, are commonly used to treat back acne [1]. However, these medications can cause discomfort, organ damage, immunological hypersensitivity, and resistance to long-term antibiotic use [3]. As a result, it is critical to find an effective alternative acne treatment.

Natural products are gaining popularity for treating bacterial infections since they have fewer adverse effects and are less expensive. Indonesia is declared the country with the highest biodiversity in the world, with 31,750 species, and 15,000 can be utilized as materials for herbal medicine [4]. Apus bamboo *(Gigantochloa apus*), belonging to the Poaceae family, has been known to have anti-inflammatory properties due to its antioxidant

properties that act as free radical scavengers [5]. Flavonoids in Apus bamboo extract are also known to have anti-inflammatory activities, as they inhibit the proliferation and exudation stages of the inflammatory process and release arachidonic acid and lysosomal enzyme secretion [6]. Apus bamboo shoot exceeds gentamicin in antibacterial activity against *S. aureus* and *Escherichia coli* [7]. n-Hexadecanoic acid, which dominates Apus bamboo shoots and the concentration of phenolic compounds, has significant antibacterial, anti-inflammatory, and antioxidant action [8].

Referring to these benefits, the bioactive compounds found in Apus bamboo shoots have great potential for development as therapeutic agents. However, bioactive chemicals derived from bamboo shoots, like those derived from other plants, are highly hydrophobic, easily oxidized, and volatile [9]. Thus, encapsulating Apus bamboo shoot extract into chitosan-based particles is needed to improve functional properties and provide stability in delivering active compounds. Implementing nanotechnology to plant extracts proved to be a practical approach for herbal medicinal products by taking into account all of the advantages to be obtained from nanostructured systems, such as increased solubility, pharmacological efficacy, bioavailability, toxicity safety, controlled delivery, and safety from physical and chemical degradation [10]. Chitosan is a hydrophilic polymer formed from the deacetylation of chitin that contains D-glucosamine and N-acetylglucosamine repeating units [10]. Because of their beneficial properties, chitosan particles are the subject of extensive investigation. Chitosan particles are frequently used in biomedical applications, including drug delivery carriers, vaccine administration, antibacterial agent administration, and wound healing [10]. Chitosan particles cannot only be used to deliver antibiotics, but they can also be used to adsorb inorganic and organic compounds from aqueous solutions and eradicate a variety of microorganisms from water, such as *S. aureus, Pseudomonas aeruginosa, E. coli*, and *Candida albicans* [11]. Previously, 154-188 nm chitosan particles were utilized to encapsulate ginger root extract and exhibited increased antibacterial activity against *S. aureus, P. aeruginosa,* and *E. coli* compared to free extract [12].

The current study used chitosan microparticles (CMPs) as carriers to develop techniques for increasing the therapeutic potential of Apus bamboo shoot extract (Ab) to produce Apus bamboo-loaded chitosan microparticles (CMPs/Ab). This encapsulation strategy could improve the stability of physical and functional features in releasing active chemicals [13]. Furthermore, the successful encapsulation of CMPs/Ab was followed by the formulation of the spray gel. We chose a gel form for our formulation due to the use of chitosan. This polymer is a structural material in the gel network, composed of inorganic particles or organic macromolecules, primarily polymers. [14] A spray gel preparation is considered more effective for ease of application because it reduces contact with lesions and contamination and is more practical considering the various locations of acne. This research aimed to synthesize and characterize CMPs/Ab as an antibacterial agent for treating acne vulgaris. These CMPs/Ab were formed into a spray gel before being tested for antibacterial activity against the acne-causing bacteria *S. epidermidis, S. aureus,* and *C. acne*. To the best of our knowledge, the encapsulation of Apus bamboo shoot extract into chitosan particles has never been done before. As a result, this will be the first study on an encapsulating technique to improve the efficacy of Apus bamboo shoot extract, particularly for anti-acne treatment. Finally, this preparation is expected to provide an alternative for optimizing the antibacterial activity of the acne spray gel application.

2. Materials and methods

2.1 Extraction of Apus bamboo shoots

The Apus bamboo shoots were collected from Ngadong village, Yogyakarta, Central Java, Indonesia. The dried Apus bamboo shoot was ground to a fine powder. The extraction process utilized the maceration method with methanol as a solvent with a ratio of extract to methanol of 1:10 at room temperature for 3 × 24 hours. The obtained extract was filtered using Whatman No. 1 filter paper and evaporated using a rotary evaporator (Heidolph Rotary Evaporator Hei-VAP). The extract was stored in the refrigerator for further use.

2.2 Phytochemical analysis

Gas chromatography-mass spectrometry (GC-MS) analysis of the Apus bamboo shoot extract was performed using a GCMS (Shimadzu GCMS-QP 2010 Ultra) instrument with a FAMEWAX (polyethylene glycol) stationary phase with a column length of 30 m and a diameter of 0.25 mm. The Helium was used as carrier gas with a pressure of 30 kPa. The injector was operated at250°C with an iron source temperature of 300°C and interface temperature of 250°C, under split mode 10:1. The column temperature was programmed from 100°C to 280°C with an increase rate of 10°C/min, held for 2 minutes. The obtained component was identified based on NIST Database 11.

2.3 Synthesis of CMPs/Ab

The CMPs/Ab were synthesized using an ionic gelation method [15]. The chitosan solution (0.2% w/v) was prepared by dissolving it in glacial acetic acid, and the sodium tripolyphosphate (STPP) solution (0.1 % v/v) was prepared in distilled water. The bamboo shoot extract (5% w/v) was added to the mixture solution to obtain a concentration ratio of chitosan: STPP: extract (0.2:0.1:5%). The stirring process was repeated at 500 rpm for 60 minutes at room temperature. The prepared particles were coated with maltodextrin casein (M: C, 4:6) at 20% (w/v,) and stirring was continued for 30 minutes. Homogenization was performed with a homogenizer at 10,000 rpm for 5 minutes, followed by hydration at 4°C for 18 hours. After hydration, the mixture was homogenized for 30 seconds before freeze-drying.

2.4 Synthesis of CMPs/Ab

2.4.1 Particle size, PDI, and Zeta potential

The prepared CMPs/Ab particle size, polydispersity index (PDI), and zeta potential were determined using a dynamic light scattering (DLS) technique-based particle size analyzer (Zeta sizer instrument, Malvern). The sample was diluted in ultrapure water at a ratio of 1:1 before analysis. The measurement was performed in triplicate [16].

2.4.2 Shape and surface morphology analysis

The shape and morphology of prepared CMPs/Ab were analyzed using a scanning electron microscope (SEM, Quattro S, Thermo Fisher Scientific) and transmission electron microscope (TEM, Talos F200X G2, Thermo Scientific). For SEM analysis, the microparticle samples were lyophilized using a freeze-drying machine for 48 hours. The powder sample was placed onto a carbon tape. For TEM analysis, the liquid sample of CMPs/Ab was diluted in ultrapure water at a ratio of 2:3, dropped on a copper grid, and dried at room temperature. The CMPs/Ab grid was scanned for TEM images [16].

2.4.3 Spectroscopic analysis

Fourier transform infrared (FTIR) spectroscopy analysis was used to determine the interaction between each component. The FTIR analysis was performed on Apus bamboo shoot extract (Ab) and CMPs/Ab. The Ab and CMPs/Ab were dried using a freeze dryer (BUCHI Lyovapor L200) for 72 h before the analysis. The spectra of the samples were recorded in the range of 400 to 4000 cm using the Perkin Elmer Spectrum Two FT-IR Spectrometer [17].

2.4.4 Encapsulation Efficiency (E.E.)

A UV-visible spectrophotometer (SHIMADZU UV-Vis Spectrophotometer UV-1280) was used to measure the encapsulation efficiency of the developed CMPs/Ab. The extract in the supernatant was measured at 340 nm after centrifuging particles at 1000 rpm for 30 minutes, and the amount of extract in the suspension was measured. The equation was used to compute the encapsulation efficiency (E.E%):

$$E.E.\% = \frac{(mass\ of\ initially\ added\ extract\ -\ mass\ of\ free\ extract)}{mass\ of\ initial\ added\ extract}\ x\ 100$$

2.5 Anti-acne activity assay

Anti-acne activity properties of CMPs/Ab were carried out using an agar well diffusion method against *S. aureus*, *S. epidermidis,* and *C. acnes*. The 0.5 McFarland bacterial suspension was inoculated on the Nutrient Agar medium. The petri dish was divided into four parts, and a well with a diameter of 6 mm was made in each part. The CMPs/Ab (100 µL) were loaded into the well, and distilled water and 100 µg clindamycin were used as negative control (-) and positive control (+), respectively. The samples were incubated for 24 hours in an incubator at 37 °C, and the clear zone around the well was measured. The test was carried out with three repetitions.

2.6 Microparticle gel spray preparations

The spray gel was prepared by grinding 0.1% Hydroxyethyl cellulose (HEC) and 0.1% Hydroxypropyl Methyl Cellulose (HPMC) as gelling agents. The 0.18% methylparaben, CMPs/Ab (10% v/v), and 15% propylene glycol were dissolved using a magnetic stirrer at 1,200 rpm for 10 minutes. The material was left in HEC and HPMC containers at 30°C with constant stirring until a thick and uniform gel mass formed [18]. The formulations were subjected to physical characterization, including organoleptic observations by examining the color, aroma, and

shape of the preparation as well as viscosity (Brookfield, DV1 viscometer), homogeneity, and pH (Mettler Toledo, SevenCompact S220). The spray gel was characterized from day 0 to day 28.

2.7 Statistical analysis

Results were given as means ± standard deviations and compared using ANOVA and Tukey test. Significant differences were indicated by *p*-values < 0.05.

3. Results and Discussion

3.1 Phytochemical analysis

In this study, GCMS analysis was performed to identify chemical components contained in the Apus bamboo shoot extract. The NIST 11 database was utilized to determine the compounds in Apus bamboo shoot extract. Ten compounds were discovered, as illustrated in Figure 1 and Table 1, its dominated by antibacterial compounds, namely n-Hexadecanoic acid (23.69%), cis-9-Hexadecenal (17.61%), Benzaldehyde, 4-hydroxy- (14.78%), Octadecanoic acid, 2-(2 -hydroxyethoxy) ethyl ester (3.44%), Bis(2-ethylhexyl) phthalate (3.38%), and phenolic group compounds of Benzenemethanol, 3-hydroxy, and Benzeneacetonitrile, 4-hydroxy.

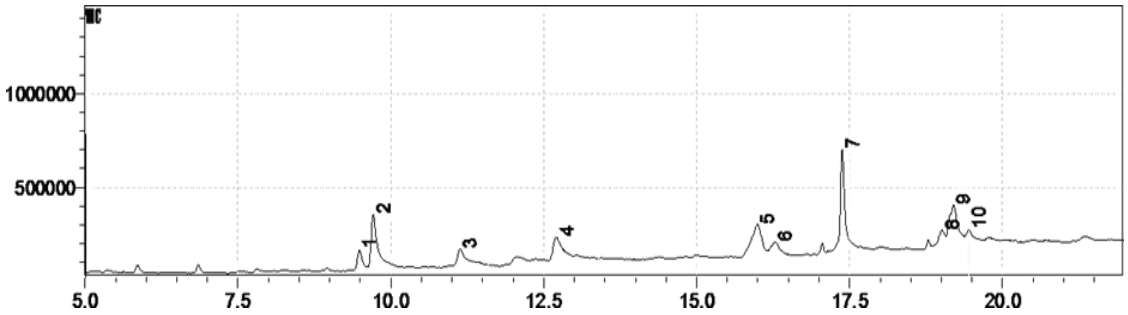

Figure 1 GCMS chromatogram of Apus bamboo shoot extract.

Table 1 Identified compound from GCMS identification of Apus bamboo shoot extract.

Peak	Ret. Time	Area (%)	Compound	Activity
1	9.485	4.57	Benzenemethanol, 3-hydroxy-	Phenolic, antioxidant [19]
2	9.714	14.78	Benzaldehyde, 4-hydroxy-	Phenolic, antioxidant [19]
3	11.129	3.67	Benzeneacetonitrile, 4-hydroxy-	Phenolic, antioxidant [19]
4	12.707	7.77	1,2,3,5-Cyclohexanetriol, (1. alpha.,2. beta.,3. alpha.,5. beta)	-
5	15.994	15.53	Tetratriacontane	-
6	16.274	3.38	Bis(2-ethylhexyl) phthalate	Cytotoxic, antimicrobial [20]
7	17.380	23.69	n-Hexadecanoic acid	Antibacterial, antivirus [20]
8	19.017	5.56	Triacontane	-
9	19.202	17.61	cis-9-Hexadecenal	Antifungal, antibacterial, anti-melanogenic [21]
10	19.452	3.44	Octadecanoic acid, 2-(2-hydroxyethoxy) ethyl ester	Antioxidant, antibacterial [19]

Apus bamboo is a famous bamboo in Indonesia, and its shoots can be processed into various health foods and pharmaceuticals. In Bali, the Apus bamboo branch was used to treat coughs, hepatitis, hypertension, breast cancer, and diabetes [22]. According to Radiastuti et al., Benzenemethanol, 3-hydroxy, Benzaldehyde, 4-hydroxy, Benzeneacetonitrile, and 4-hydroxy are phenolic compounds that serve as antioxidants [19]. Moreover, Apus

bamboo shoots contain phenol, which has powerful antibacterial, anti-inflammatory, and antioxidant properties [5]. Phenol is an antibacterial drug that affects bacterial biological processes by either inhibiting growth (bacteriostasis) or killing the bacteria (bactericidal impact) [23]. The most abundant component in Apus bamboo shoot extract is N-hexadecanoic acid. These chemicals include fatty acid groups that serve as antibacterial action by altering the structure of cell walls and membranes synergistically, thereby increasing the effect of antibacterial activity. Other compounds, such as Bis(2-ethylhexyl) phthalate, cis-9-Hexadecenal and Octadecanoic acid, 2-(2-hydroxyethyl) ethyl ester, act as antioxidants and are classed as cytotoxic antimicrobials [21].

3.2 Particle size, PDI, and zeta potential of microparticles

The synthesized CMPs and CMPs/Ab were subjected to particle size and zeta potential analysis using the Zetasizer instrument. The results show that CMPs exhibit a particle size and PDI of 782.9 ± 23.9 nm and 0.56 ± 0.0, respectively (Table 2). The encapsulation of Ab extract significantly increases the particle size to 838.9 ± 33.4 nm with a PDI value of 0.68 ± 0.06 (Table 2). The particle size of the chitosan particles is influenced by several conditions, such as stirring speed, volume, pH, and storage. The PDI was used to determine particle homogeneity and stability, an essential aspect of the synthesized microparticles. Polydispersity is reported to be excellent and homogeneous in samples with a homogeneity value of 0.01 - 0.7 [24]. Based on this research, the synthesized sample meets the criteria in terms of homogeneity, thus indicating that the sample has a narrow size distribution.

Chitosan is a nontoxic and biocompatible polymer that has been intensively researched for various biomedical applications, including developing small-scale drug delivery systems. This polymer can be destroyed enzymatically. For example, in humans, chitosan can be destroyed by lysozymes and bacterial chitosanolytic enzymes found in the gastrointestinal tract and lung [25]. Therefore, chitosan-based particles were chosen as an Apus bamboo shoot extract carrier material based on their physiochemical qualities. Chitosan provides good protection for the core and can bind active compounds such as phenol, while maltodextrin has high solubility and protects flavors from oxidation [26].

Table 2 Particle size, PDI, and zeta potential of CMPs/Ab and CMPs.

Parameters	CMPs/Ab	CMPs
Particle size (nm)	838.9 ± 33.4	782.9 ± 23.9
PDI	0.68 ± 0.0	0.56 ± 0.0
Zeta potential (mV)	+29.06 ± 1.22	+28.12 ± 2.19

Moreover, referring to the zeta potential results, the CMPs and CMPs/Ab sample exhibit a zeta potential value of +28.12 ± 2.19 and +29.06 ± 1.22 mV, respectively (Table 2), indicating that both microparticles are stable. The role of chitosan is very influential in these positive results because its presence creates a potential difference between the positive charge medium and the electrical double layer. The more positive the zeta potential value, the greater the repulsive force compared to the attractive force between particles and supports the stability of the dispersion system [17].

3.3 Shape and Morphological Characteristics of Microparticles

SEM and TEM were used to examine the shape and morphology of CMPs/Ab. Figure 2(A) shows SEM images of CMPs/Ab with a 5 µm scale bar, revealing a spherical shape. In contrast, the TEM image depicts the CMP's irregular form shape, as shown in Figure 2(B). The particles tend to be distributed amorphously, and the core-shell particle structure is visible. The darker layer within the light layer is thought to be the interaction between the fibre-shaped chitosan particles and the spherical CMPs/Ab. It can be concluded from the results that the encapsulation process of Apus bamboo shoot extract has worked optimally. Similar research with different samples has also been carried out. The results of this research show the formation of a spherical shape with flat edges, indicating a perfect and homogeneous distribution of the chitosan matrix [27]. These findings confirm the validity of our microparticle formula.

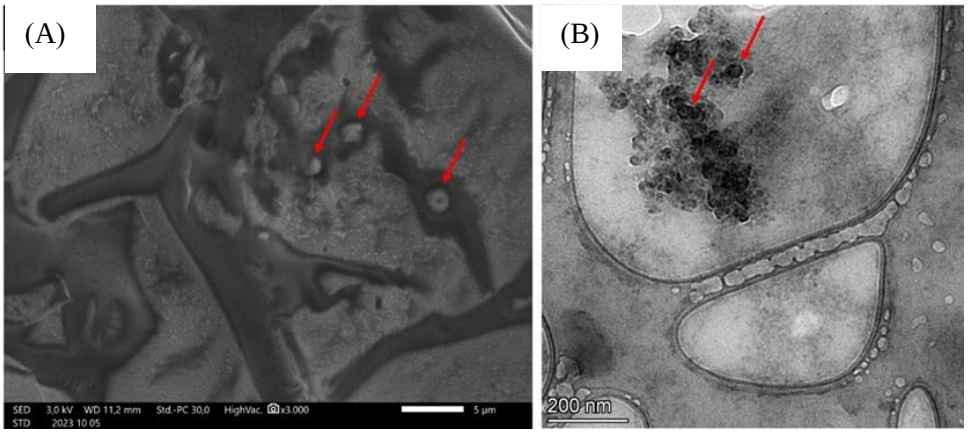

Figure 2 Shape and morphology of CMPs/Ab (A) SEM Image (scale bar = 5µm) and (B) TEM image (scale bar = 200 nm). Red arrows indicate CMPs/Ab.

3.4 FTIR Characterization of Microparticles

FTIR was used to characterize the interaction between Ab extract and CMPs on the synthesized CMPs/Ab, which were synthesized through the ionic gelation method. As shown in Figure 3, Ab extract and CMPs/Ab showed a characteristic peak at 3202 and 3195 cm, corresponding to the stretching vibration of N-H groups. The distinct spectra at 2921 and 2930 cm on the Ab extract and CMPs/Ab corresponded to the C-H bond vibration in alkanes [28]. Peaks of 1740-1720 cm were discovered in the spectra of CMPs/Ab and Ab extract that corresponded to the C=O stretching carbonyl functional group from palmitic acid (n-Hexadecanoic acid). The alkene C=C stretching functional group, which appears at 1648-1638 cm was found in the spectra of CMPs/Ab samples from an Ab extract, which is thought to originate from Tetratriacontane. Moreover, the spectra of 1149 cm of CMPs/Ab corresponded to the –COOH group of STPP [28]. According to the FTIR results, incorporating Ab extract into the CMP technique does not modify the structure of the extract, as indicated by the spectra profile, demonstrating that the goal of encapsulating as a coating was attained.

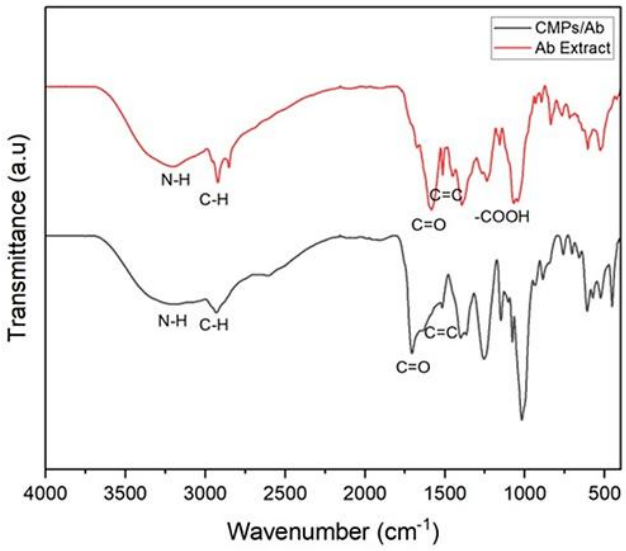

Figure 3 Comparative pattern of FTIR spectra of CMPs/Ab and Ab extract.

3.5 Encapsulation efficiency (EE)

The encapsulation efficiency was utilized to calculate the amount of Ab extract encapsulated in CMPs/Ab. In this study, the encapsulation efficiency was determined using UV-vis spectrophotometry technique. The encapsulation efficiency results show the ability of the microparticles to absorb Apus bamboo shoot extract with an encapsulation efficiency percentage of 46.4 ± 0.91 %.

Encapsulation is a method of entrapping the core material in a coating material that aims to protect the active compound components in the material [29]. Based on previous research, chitosan-STPP microparticles have a folic acid encapsulation efficiency of 37.60 ± 2.81% [30]. The protonated amine groups (NH^{3+}) interactions are formed, resulting in increased cross-linking interactions between the protonated amine groups on chitosan and the opposing groups of STPP in forming chitosan-STPP microparticle complexes. The more chitosan-STPP microparticle complexes were produced, the better the ability of the microparticles to absorb active chemicals [26]. In this case, it is related to the encapsulation efficiency value.

Encapsulation of Ab extract in chitosan microparticles aims to provide increased solubility due to its large surface area, control the release of active compounds, and protect it from interactions with other substances in its application as a spray gel. The aim of using combined coating materials is to obtain optimal products as a controlled delivery system for active compounds. Maltodextrin has safe and nontoxic properties and was chosen as an encapsulant from the polysaccharide group [26]. The choice of a combination of casein and protein-type coating material can also increase optimality in controlling the release of active substances with a mechanism that can form a gel, making it possible as an encapsulant material for lipophilic or hydrophilic active compounds [29]. Chitosan particle systems can be classified as nanogels that combine nanomaterials' characteristics with hydrogels' properties. The definition of hydrogel is a hydrophilic polymer cross-linked network that can absorb large amounts of water and swell [29]. According to the FTIR results, the prominent peaks of Ab extract were detected in the CMPs/Ab, indicating that the Apus bamboo shoot extract was loaded in the core of chitosan particles.

The encapsulation efficiency of 46% is moderate, and the study would benefit from a comparative analysis with other encapsulation methods or materials, as this would provide valuable insights into the effectiveness of the chitosan encapsulation technique in retaining bioactive compounds. Improvements in encapsulation efficiency could be achieved by adjusting the chitosan-STPP ratio or exploring alternative encapsulation methods, as a higher efficiency would likely enhance the bioavailability and stability of the active compounds.

3.6 In vitro anti-acne activity

The anti-acne activities of CMP/Ab were investigated using an agar-well diffusion antibacterial activity assay against acne-causing bacteria *S. aureus, S. epidermidis,* and *C. acnes.* According to the results of the antibacterial activity assay, CMPs/Ab produced an inhibition zone that can be seen in Table 3, with an average diameter of inhibition zone of 41.67 ± 1.53, 42.67 ± 2.52 and 22.33 ± 1.53 mm for *S, aureus, S. epidermidis*, and *C. acnes*, respectively.

Table 3 Antibacterial activity of the CMPs/Ab and antibiotic clindamycin.

Test bacteria	Inhibition zone (mm)	
	CMPs/Ab	Clindamycin
S. aureus	41.67 ± 1.53[a]	16.00 ± 3.46[b]
S. epidermidis	42.67 ± 2.52[a]	12.00 ± 2.65[b]
C. acnes	22.33 ± 1.53[a]	12.33 ± 2.52[b]

The antibacterial efficacy of CMPs/Ab is related to several mechanisms of action, which relate to microorganisms in multiple structures at once and provide them with the ability to kill various types of bacteria [31]. Research indicates that chitosan exhibits antimicrobial properties through interactions with bacterial cell membranes, disrupting their integrity and inhibiting nutrient intake, which is crucial for bacterial survival. [32] Additionally, incorporating bioactive compounds from Apus bamboo may synergistically enhance these effects, making it essential to investigate how these components interact at the molecular level to optimize their application in antibacterial treatments. These findings correspond with a prior study by Shaaban (2021), which revealed that n-hexadecanoic acid damages proteins and interacts with lipids in bacterial cell membranes. It causes changes in membrane permeability, obstructs cellular energy generation, lowers enzyme function, and even causes cell death. This discovery confirms that n-hexadecanoic acid is an antibacterial chemical [33].

Apart from that, phenolic compounds, including flavonoids, saponins, and alkaloids, also act as antibacterial compounds. This toxic compound can bind to bacterial lipid membranes and block bacterial enzymes through oxidized molecules, generating reactions with sulphihydryl groups or non-specific interactions with acne-causing bacteria proteins [34]. The phenol compounds bound in the microparticle extract can denature bacterial proteins through an adsorption process using hydrogen bonds [12]. Through an adsorption mechanism based on hydrogen bonding, the phenol chemicals bound in the microparticle extract can denature bacterial proteins. When the peptidoglycan component of the bacterial cell wall is inhibited, the phenolic chemicals become active. Bacteria

lose their cell walls, leaving the cell membranes open to injury and leaking. Thus, the content of Apus bamboo shoot extract influences its antibacterial activity in inhibiting bacteria.

3.7 Physical characteristics of CMPs/Ab spray gel

The organoleptic characteristics of the prepared spray gel were examined, including smell, color, and form. The preparation has a distinctive extract odor, a clear-yellow color, and a slightly thick form. The preparation appears homogeneous, and the results change on the 21st day, indicating that the preparation has stable physical features (Figure 4). In the viscosity and pH results, there was a decrease, as seen in Table 4 The acne spray gel preparation's viscosity still meets the standard range of 500 - 5000 cPs.

Table 4 Physical attributes of spray gel of CMPs/Ab and Ab extract.

Day	pH (Mean ± S.D.)		*P*	Viscosity (Mean ± S.D.)		*P*
	Spray of CMPs/Ab	Spray of Ab extract		Spray of CMPs/Ab	Spray of Ab extract	
0	5.93 ± 0.11	5.86 ± 0.11	0.642*	1000 ± 13.4	1000 ± 13.42	0.925*
7	6.03 ± 0.25	5.93 ± 0.11		1001 ± 9.64	997 ± 15.37	
14	6.00 ± 0.00	5.70 ± 0,17		996 ± 14.43	927 ± 60.38	
28	5.83 ± 0.28	5.66 ± 0.76		995 ± 13.23	997 ± 15.37	

Description: *One way ANOVA test; p (p-value)

According to the pH results, it is in the 5-6 range, which fits the pH requirements for spray gel preparations [35]. The spray gel preparations that have been made are evaluated, including organoleptic tests, homogeneity tests, pH tests, and viscosity tests. Angelia et al. (2022) stated that a good spray gel preparation is in liquid form, has no phase separation, is colored according to the active ingredient, and does not have a strong odor [18]. The microparticle extract data was homogeneous; no lumps were visible under a 40x magnification microscope, and no coarse grains were found in the preparation. The homogeneity results of Ab extract without the microparticle synthesis process are shown in Fig. 5. There is a picture of lumps of extract or tiny particles that need better mixed. An excellent topical preparation is homogeneous because it does not cause skin irritation.

The results of measuring the pH of the preparation that has been produced show that the pH of the spray gel meets the range because it ranges from 4.5 to 6.5. The pH of topical preparations suitable for normal human skin is 4.5-6.5 [35]. The results of pH measurements for 28 days showed a decrease but were still within the pH range, so the resulting spray gel preparation had good stability. The viscosity of the CMPs/Ab gel spray preparation showed good results because it was in the range of 500 - 5000 cPs. The viscosity suits this range because the preparation can be sprayed easily using a spray bottle, producing good spreadability [18].

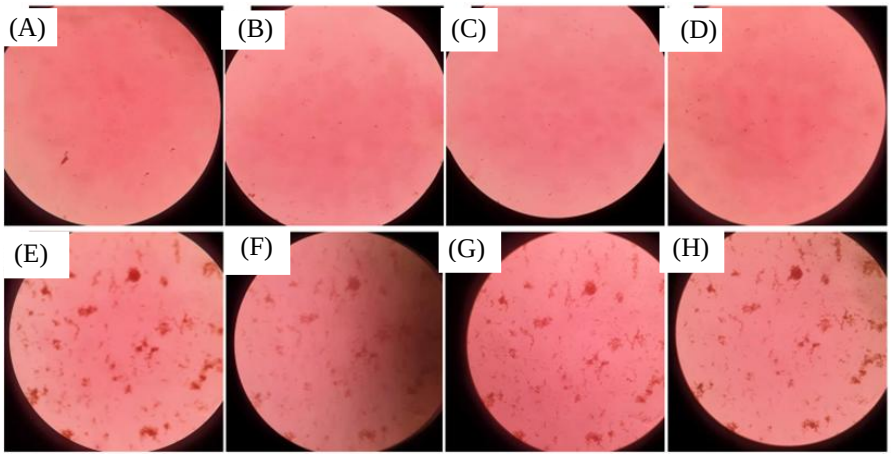

Figure 4 Microscopic homogeneity test of spray CMPs/Ab (A) day-0, (B) day-7, (C) day-14, (D) day-28; and Ab extract without the microparticles synthesis process (E) day-0, (F) day-7, (G) day-14, (H) day-28.

4. Conclusions

The ionic gelation technique successfully produced CMPs/Ab with good stability, effectively increasing antibacterial activity against S. aureus, S. epidermidis, and C. acnes and damaging the membranes of acne-causing microbes. The characteristics of the extract formulated into a spray gel show that CMPs/Ab has the potential for safety and are more effective in acne treatment. However, the study has some limitations, such as a moderate % encapsulation efficiency of 46%, which may affect the bioavailability and stability of active compounds. Also, the absence of comparative analysis with established treatments limits the understanding of the effectiveness of CMPs/Ab. Therefore, future studies, including the optimization of the chitosan-STPP ratio and in vivo antibacterial activity testing, are needed to evaluate the further potential effects.

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