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Formulation of an Antibacterial Peel-Off Gel Mask from Kombucha Fermented with Gerga Orange Peel Infusion (*Citrus x aurantium* L.)

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ABSTRACT

Kombucha fermentation transforms complex organic compounds into beneficial substances such as polyphenols, vitamins, minerals, fiber, and probiotics. Gerga orange (*Citrus x aurantium* L.) peel, rich in bioactive compounds with antibacterial potential, is a promising substrate for kombucha fermentation. This study aimed to develop a fermented kombucha infusion from Gerga orange peel for use in an antibacterial peel-off gel mask and to assess the impact of hydroxypropyl methylcellulose (HPMC) concentrations (2%, 3%, and 4%) on the mask's properties. Extract preparation, organoleptic evaluation, total phenolic content (TPC) analysis, and gel mask formulation were conducted. Antibacterial activity was evaluated against *Propionibacterium acnes* using the well-diffusion method. Kombucha fermentation increased the TPC of Gerga orange peel infusion (26.53 mgGAE/g vs 25.77 mgGAE/g), imparted a fermentation aroma, and reduced pH from 4 to 2.7. HPMC concentration significantly influenced gel texture, spreadability, drying time, and pH, while color, aroma, and homogeneity remained unaffected. Antibacterial tests showed moderate activity for Formula 1 (2% HPMC; inhibition zone: 9.25 ± 0.35 mm). Stronger antibacterial effects were observed in Formulas 2 (3% HPMC; 13.93 ± 0.49 mm) and 3 (4% HPMC; 14.25 ± 0.71 mm). Kombucha fermentation of Gerga orange peel offers an innovative method for creating antibacterial gel masks. Higher HPMC concentrations improve antibacterial efficacy and physical properties, demonstrating potential against *Propionibacterium acnes*.



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ABSTRAK

Fermentasi kombucha dapat mengubah senyawa organik kompleks menjadi senyawa sederhana yang menghasilkan polifenol, vitamin, mineral, serat, dan probiotik. Kulit jeruk Gerga (*Citrus x aurantium L.*) berpotensi sebagai substrat fermentasi kombucha karena kandungan senyawa bioaktifnya yang memiliki aktivitas antibakteri. Penelitian ini bertujuan mengembangkan infus kombucha dari kulit jeruk Gerga sebagai masker gel peel-off antibakteri dan mengevaluasi pengaruh variasi konsentrasi hydroxypropyl methylcellulose (HPMC) (2%, 3%, dan 4%) terhadap sifat masker. Metode yang digunakan meliputi ekstraksi, evaluasi organoleptik, pengukuran total fenol (TPC), serta formulasi masker gel peel-off. Aktivitas antibakteri diuji terhadap *Propionibacterium acnes* menggunakan metode *well-diffusion*. Hasil penelitian menunjukkan bahwa TPC infusa kombucha kulit jeruk Gerga meningkat dibandingkan infus biasa (26,53 mgGAE/g vs 25,77 mgGAE/g), dengan penurunan pH dari 4 menjadi 2,7 dan aroma fermentasi yang khas. Variasi konsentrasi HPMC mempengaruhi tekstur gel, daya sebar, waktu pengeringan, dan pH masker, sementara warna, aroma, dan homogenitas tidak terpengaruh. Uji antibakteri menunjukkan bahwa formula 1 (2% HPMC) memiliki aktivitas antibakteri sedang (zona hambat: $9,25 \pm 0,35$ mm), sedangkan formula 2 dan 3 (3% dan 4% HPMC) menunjukkan aktivitas antibakteri kuat dengan zona hambat $13,93 \pm 0,49$ mm dan $14,25 \pm 0,71$ mm. Kombucha substrat kulit jeruk Gerga dapat dikembangkan sebagai masker gel antibakteri. Konsentrasi HPMC yang lebih tinggi meningkatkan efikasi antibakteri dan sifat fisik masker, menunjukkan potensi terhadap *Propionibacterium acnes*.

Kata Kunci: *Citrus x aurantium L.*; Kombucha; Peel-off; *Propionibacterium acnes*

1. Introduction

Kombucha is a fermented beverage traditionally prepared using green or black tea leaves, sucrose (5-10%), and a Symbiotic Culture of Bacteria and Yeasts (SCOBY), with a fermentation period ranging from 7 to 21 days [1,2]. This beverage is rich in polyphenolic compounds, organic acids, vitamins, minerals, and sugars [3]. Numerous studies have highlighted kombucha's potent antioxidant properties [4], along with various bioactivities, including antibacterial, antihyperglycemic, antiproliferative, immunomodulatory, hypocholesterolemic, and antihypertensive effects [5]. Recent studies highlight the increasing diversification of kombucha compounds for a range of applications, particularly in cosmetic products [6].

Another research has focused on the production of kombucha using alternative substrates, which has generated considerable interest within the scientific community. Alternative substrates explored for kombucha production include herbal infusions, fruits, and vegetables [7]. Studies have shown that kombucha derived from these alternative substrates contains unique bioactive compounds and exhibits distinct pharmacological properties. The production of beverages fermented with SCOBY from non-traditional raw materials is often referred to as kombucha analogs [8].

Gerga orange (*Citrus x aurantium L.*) is a variety of orange native to Kerinci Regency, Jambi, Indonesia. It is known for its bitter-sour taste and thick peel that encloses the fruit. Gerga orange is a prominent fruit commodity with significant economic value, showing consistent annual growth. Currently, its primary use is focused on the nutritional and vitamin content of the fruit pulp, while the peel, which constitutes approximately 40-50% of the total fruit weight, remains largely underutilized [9]. Peel of

C. aurantium contains a rich array of polyphenols. Flavonoids, the major bioactive compounds, are categorized into flavanones, flavones, and flavonols. Other compounds such as limonoids, including limonin and nomilin, as well as alkaloids like p-synephrine, are also present. The most abundant phenolic compounds in the peel of *C. aurantium* are p-coumaric acid (25%) and ferulic acid (24%) [10]. Additionally, the yield of pectin from *C. aurantium* peel is approximately 28%, with a total phenolic content (TPC) of 40 ± 3 mg GAE/g of pectin, composed of 65% galacturonic acid [11].

C. aurantium is a well-established source of natural antioxidants [10]. The total antioxidant activity of *C. aurantium* peel extracts have been shown to be higher than that of the pulp extracts [12]. This effect has been attributed to the chemical composition of the peel, which is rich in phenolic acids and their derivatives [9-11]. Orange peel extract exhibits antibacterial activity against a range of gram-negative and gram-positive bacterial strains, including *Propionibacterium acnes*, demonstrating moderate to strong efficacy in inhibiting bacterial growth [13],[14]. As a result, it holds promise for use in cosmeceutical applications as an antibacterial agent.

This study explores the potential of Gerga orange (*C. x aurantium* L.) peel infusion as a kombucha analog for use in cosmeceutical products, with a particular focus on peel-off gel masks. These masks were chosen for their user-friendly application and proven efficacy in both anti-aging and anti-acne treatments. The formulation of peel-off gel masks is largely determined by the composition of their ingredients, with hydroxypropyl methylcellulose (HPMC) serving as a key gelling agent. HPMC plays a critical role in enhancing the physical texture of the gel mask, providing effective microbial resistance, ensuring optimal spreadability, and maintaining high gel viscosity stability [15]. Accordingly, this study aims to evaluate Gerga orange peel kombucha with varying concentrations of HPMC to develop an effective peel-off gel mask with antibacterial properties against *Propionibacterium acnes*.

2. Methodology

Materials

The materials used in the research include Gerga orange (*C. x aurantium* L.) peel, SCOBY, water, sugar, folin ciocalteu, distilled water, NaOH, gallic acid, methanol pa, PVA, HPMC, methyl paraben, propyl paraben, Propylene glycol, Nutrient agar, test bacteria (*Propionibacterium acnes*).

Collection, Identification, and Preparation of Simplicia

A total of 20 kg of Gerga orange peel was collected from Kerinci Regency in Jambi Province, Indonesia. The samples were verified and identified at the Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Andalas, with Identification Number 53/K-ID/ANANDA/I/2023. The Gerga orange peels were then dried in an oven set at 50 °C for approximately three days, yielding dried Gerga orange peel simplicia.

Preparation of Gerga orange peel Infusion and Kombucha

This study used a 5% concentration of Gerga orange peel infusion as a substrate for Kombucha fermentation. The infusion was prepared by heating a mixture containing 5 grams of Gerga orange peel powder and 10 grams of sugar in 100 mL of distilled water for 30 minutes at a temperature of 70°C. The mixture was then filtered to separate the solid residues from the infusion [16]. To prepare fermented kombucha from Gerga

orange peel, 100 mL of the prepared infusion was added with 3 grams of SCOBY in a glass container. The container was then covered with a cloth and left to ferment for 14 days at room temperature, away from direct sunlight [17].

Organoleptic Evaluation and Total Phenol Content

The organoleptic evaluation of the Gerga orange peel infusion and its fermented kombucha included an assessment of several parameters: color, aroma, and taste [18]. Additionally, pH measurements were conducted on both the infusion and the fermented kombucha using a pH meter to determine any changes in acidity that occurred during the fermentation process.

To evaluate the total phenol content, 5 mL of Folin-Ciocalteu reagent (7.5% in water) was added to each 1 mL of the test solution, which included either Gerga orange peel infusion or its fermented kombucha infusion. This was followed by adding 4 mL of 1% NaOH solution, and the mixture was allowed to incubate for 1 hour. The absorbance of each solution was measured at a maximum wavelength of around 730 nm. The total phenol content was then determined using the regression equation generated from the gallic acid calibration curve [18]

Peel-off Gel Mask Formulation

The formulation of the peel-off gel mask using Gerga orange peel kombucha was prepared according to the formula presented in table 1. The process involved dissolving polyvinyl alcohol (PVA) and hydroxypropyl methylcellulose (HPMC) in separate containers in a water bath. HPMC was then gradually mixed into PVA until the mixture became homogeneous. In another container, methylparaben and propylparaben were dissolved uniformly in propylene glycol and then combined with the PVA-HPMC mixture [19]. Finally, Gerga orange peel kombucha was added slowly while stirring the mixture thoroughly.

Table 1. Peel-off gel mask formulation

Material	Formulation (%)		
	F 1	F 2	F 3
Polyvinyl alcohol (PVA)	12	12	12
Hydroxypropyl methylcellulose (HPMC)	2	3	4
Methylparaben	0,18	0,18	0,18
Propylparaben	0,2	0,2	0,2
Propylene glycol	10	10	10
TEA	0.3	0.3	0.3
Gerga orange peel kombucha	Ad 100	Ad 100	Ad 100

Peel-off Gel Mask Evaluation

The evaluation of the peel-off gel mask formulation using Gerga orange peel kombucha included assessments of organoleptic properties, homogeneity, pH, spreadability, and drying time according to Miksusanti et al [20]

Evaluation of Antibacterial Activity

The antibacterial activity of the three formulations, F1, F2, and F3, was evaluated using the well diffusion method against human pathogenic bacteria, *Propionibacterium acnes*. Clindamycin gel was used as the positive control, while the negative control consisted of a placebo gel without the active ingredient. *P. acnes* bacterial suspensions were prepared at a concentration of 1.5×10^8 CFU/mL, standardized with a 0.5

McFarland solution. The bacterial suspensions were inoculated onto the surface of the agar medium and allowed to rest for 15 minutes to ensure uniform distribution. Wells were then created in the agar [21].

Each test formulation was placed into a separate well, and were incubated at 37°C for 24 hours. Following incubation, antibacterial activity was evaluated by measuring the diameter of the inhibition zones. Larger inhibition zones indicated stronger antibacterial activity [22]. Results from the test formulations were compared with the positive control (clindamycin gel) and the negative control (placebo gel) to assess the relative antibacterial efficacy.

Data Analysis

The results are presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to determine statistical significance between groups, followed by the Duncan Multiple Range Test. Differences were considered statistically significant at $p < 0.05$.

3. Result and Discussion

Organoleptic evaluation and Total Phenolic Content (TPC)

The characteristics of Gerga orange peel infusion and the fermented kombucha infusion of Gerga orange peel after a 14-day fermentation period were evaluated based on several parameters, including color, aroma, taste, and pH. The observed data for these characteristics are presented in table 2. This assessment aimed to highlight the changes that occur during fermentation, offering insights into the transformation of sensory and chemical properties through the kombucha fermentation process.

Table 2. Evaluation of infusion and kombucha fermentation

characteristics	Gerga orange peel infusion	Gerga orange peel kombucha
color	yellowish	yellowish
aroma	sour	distinctive of fermented product (sour)
flavor	bitter	sour
pH	4	2.7

From the characteristics observed, it is evident that kombucha fermentation with SCOBY significantly alters the aroma, taste, and pH of the Gerga orange peel infusion substrate. The fermentation process transforms the initial bitter taste of the Gerga orange peel infusion into a sour flavor. This change is attributed to the production of various organic acids during fermentation, including acetic acid, gluconic acid, citric acid, lactic acid, glucuronic acid, malic acid, succinic acid, pyruvic acid, ascorbic acid, and gallic acid [14]. The presence of acetic acid bacteria plays a pivotal role by converting ethanol – produced from sugar metabolism by the yeast in the SCOBY – into these organic acids [23]. The accumulation of these acids not only influences the flavor profile but also decreases pH, from an initial pH of 4 to 2.7 after 14 days of fermentation. This pH reduction is a typical indicator of successful kombucha fermentation, reflecting the acidic transformation of the substrate.

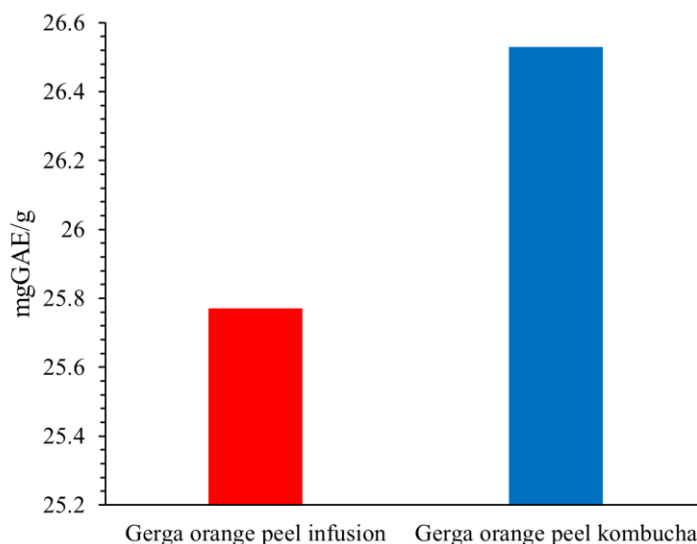


Figure 1. TPC of Gerga orange peel infusion and kombucha

Total phenol content was determined at a wavelength of 730 nm. A concentration range of 25-60 ppm was utilized to establish the gallic acid calibration curve. Upon measuring the absorbance values for each concentration, a calibration curve was constructed, yielding a linear regression equation of $y=0.0158x-0.1997$ with an R^2 value of 0.9931, indicating strong linearity and reliability of the calibration model. Using this equation, the total phenol content in the Gerga orange peel infusion and its kombucha was determined. This analysis provides insight into the phenolic compound concentration, which is indicative of antioxidant potential and is a key metric for evaluating the bioactive properties of the samples.

Based on the results, there was a significant increase in total phenol levels after 14 days of fermentation (figure 1), with kombucha showing of Total Phenolic Content 26.53 mg/g and the infusion showing 25.77 mg/g as Gallic acid equivalents. This increase is attributed to the enzymatic activity of the bacteria and yeast present in the SCOBY, which break down complex polyphenolic compounds into smaller molecular forms, thereby enhancing the compound content during the fermentation process [24]. This result aligns with previous studies showing that *Citrus aurantium* has a thick peel with higher levels of pectin compared to sweet orange peels, featuring a TPC of 40 ± 3 mg GAE/g of pectin [11].

Peel-off Gel Mask Evaluation

The organoleptic evaluation demonstrated that the variation in HPMC concentration did not affect the color, aroma, or homogeneity of the formulations. All formulations yielded homogeneous preparations with a yellowish color and a distinct fermentation aroma. However, differences were observed in texture, where F1, containing 2% HPMC, had a more liquid consistency compared to F2 (3% HPMC) and F3 (4% HPMC). This finding is consistent with previous studies, which show that gel viscosity increases with higher HPMC concentrations. This is attributed to the ability of HPMC to enhance polymer fiber formation, which helps retain and bind liquids [25].

Table 3. Peel-off gel mask evaluation

Parameter Evaluation	Formulation		
	F1	F2	F3
organoleptic properties			
Texture	liquid consistency	thick	thick
Color	yellowish	yellowish	yellowish
Aroma	typical fermentation	typical fermentation	typical fermentation
homogeneity	homogeneous	homogeneous	homogeneous
pH	4.70±0.300	4.90±0.2646	5.07±0.3786
spreadability (cm)	7.57 ±0.1528	6.88 ±0.8145	5.73 ±0.4933
drying time (min)	35.67±1.15	35.00±1.00	32.00±2.00

The pH evaluation showed that the peel-off gel mask containing fermented kombucha infusion of Gerga orange peel had an acidic pH ranging from 4.7 to 5,07, with no significant differences between the formulations ($p = 0.363$; $p > 0.05$). This result is consistent with the findings of Widayanti [26], which indicated no effect of HPMC variation on the pH of the peel-off gel mask. The pH values of the three formulations using Gerga orange peel kombucha were within the optimal pH range for topical preparations, which is typically between 4.5 and 6.5. A pH that is too acidic is not recommended for topical use, particularly on facial skin, as it may cause irritation [26].

The spreadability evaluation aimed to assess the ease of applying the peel-off gel mask to the face, with the ideal range for spreadability being between 5 and 7 cm. The results showed that F2 and F3 met the spreadability requirements, while F1 exceeded this range (table 2). Spreadability values greater than 7 cm or less than 5 cm would make the mask difficult to apply on the face. The higher spreadability of the F1 formulation can be attributed to its more liquid consistency. This finding is consistent with the research by Silvia and Dewi [27], which demonstrated that a lower concentration of HPMC increases spreadability.

The drying time evaluation was conducted to determine the time required for the peel-off gel mask to dry after application. An ideal drying time falls within the range of 15 to 30 minutes. Among the three formulations, F3 required the shortest drying time. Statistical analysis using one-way ANOVA yielded a p-value of 0.045 ($p < 0.05$), indicating a significant difference between the formulations. Following the Duncan test, it was found that F3 significantly differed from both F1 and F2. This finding aligns with the research by Silvia and Dewi [27], which reported that higher HPMC concentrations result in shorter drying times. Drying time can also be influenced by the type and concentration of humectants used, as these substances function as moisturizers that help retain water content in the formulation, thereby affecting the drying time of the peel-off gel mask [28]. Furthermore, the addition of ethanol or other drying accelerants can help speed up the formation of a film layer, facilitating faster drying [27].

Evaluation of antibacterial activity

An antibacterial activity test against *Propionibacterium acnes*, a bacterium associated with acne, was conducted using the well diffusion method. The results of the antibacterial activity test demonstrated that the peel-off gel mask containing fermented kombucha infusion of Gerga orange peel exhibited antibacterial activity, as indicated by the formation of an inhibition zone around the well.

As shown in table 5 and figure 2, the three formulations of the peel-off gel mask containing fermented Gerga orange peel infusion displayed antibacterial activity against *P. acnes*, with inhibition zones ranging from 9.25 to 14.25 mm. According to Harun et al [22], the antibacterial strength is categorized based on the diameter of the inhibition zone: ≤ 5 mm is considered weak, 5-10 mm is moderate, 10-20 mm is strong, and ≥ 20 mm is very strong. Based on these categories, the peel-off gel mask with fermented kombucha infusion of Gerga orange peel falls into the moderate to strong category. In comparison, the positive control, clindamycin gel, exhibited a very strong inhibition zone, while the negative control showed no inhibition, confirming the absence of any effect on bacterial growth.

Table 4. Diameter of the inhibition zone of the peel-off gel mask against *Propionibacterium acnes*

Formula	Diameter of the inhibition zone (mm)
Positive control	40.920±0.00 ^c
Negative control	-
F1 (2% HPMC)	9.250±0.353 ^a
F2 (3% HPMC)	13.925±0.485 ^b
F3 (4% HPMC)	14.250±0.707 ^b

Values are presented in mean ± SEM; values with different letters in the columns indicate statistically significant differences ($p < 0.05$) based on the Duncan Multiple Range Test

The results revealed significant statistical differences among the formulations, with the higher concentration of HPMC yielding better outcomes than the lower concentration. HPMC not only serves as a gelling agent but also plays a crucial role in various antibacterial applications. It enhances the stability and antibacterial properties of formulations, thereby increasing their efficacy [29].



Figure 2. Antibacterial activity evaluation of peel-off gel mask formulation using Gerga orange peel kombucha against *Propionibacterium acnes*

The antibacterial activity observed in the Gerga orange peel kombucha fermentation peel-off gel mask is closely related to the presence of hesperidin, a flavonoid polyphenol. Flavonoids, including hesperidin, exert antibacterial activity by inhibiting nucleic acid synthesis, disrupting membrane function, altering membrane

permeability, downregulate metalloenzymes, while simultaneously enhancing the host's defense mechanisms, and weakening pathogenicity. Furthermore, flavonoids exhibit antibacterial properties by forming complexes with hyaluronidase through electrostatic and hydrophobic interactions [30], [31].

Although the peel-off gel mask formulated with Gerga orange peel kombucha was successfully performed, the several limitations were noted in this study. Firstly, the pH of the peel-off gel mask formulated with Gerga orange peel kombucha was found to be in the range of 4.70-5.07, which is within the ideal pH range (4.5-6.5) for topical cosmetic products. The lower pH may lead to potential skin irritation with prolonged use, limiting its broader application in skincare. Secondly, while the antibacterial activity was observed against *Propionibacterium acnes*, the study only tested a single bacterial strain, which restricts the generalizability of the results to other skin pathogens. The formulation also lacked long-term stability testing to assess whether the mask would maintain its properties (texture, spreadability, drying time) over time. Furthermore, while the antibacterial efficacy was demonstrated *in vitro*, the safety and effectiveness of the product on human skin were not evaluated, which is crucial for confirming its practical use. Finally, the study found that higher concentrations of HPMC resulted in better antibacterial activity, but the optimal concentration for balancing antibacterial effects with desirable physical properties like spreadability and drying time has not yet been fully explored.

Future research could address these limitations by focusing on optimizing the pH levels of the gel mask to fall within the ideal range for topical use. Additionally, expanding the antibacterial testing to include a wider range of bacterial strains, especially other acne-related pathogens, would provide a clearer understanding of the gel's potential. Clinical trials should be conducted to assess the safety, efficacy, and skin compatibility of the gel mask on human volunteers, ensuring its practical application. Stability studies should also be a priority, as determining the shelf-life and long-term stability of the gel mask under various storage conditions would ensure its commercial viability. Furthermore, exploring alternatives to HPMC, or combining it with other biopolymers, could improve the formulation's physical properties without compromising its antibacterial efficacy. Finally, future research could examine the synergistic effects of combining Gerga orange peel kombucha with other bioactive ingredients to enhance its multifunctional benefits, such as improving skin health and overall product performance.

4. Conclusion

This study concluded that varying the concentration of HPMC in the peel-off gel mask formula containing kombucha fermentation from Gerga orange peel did not significantly affect the color, aroma, homogeneity, or pH of the mask. However, higher concentrations of HPMC led to a thicker gel consistency, reduced spreadability, and shorter drying times, with the 4% HPMC formulation yielding the best overall results. While some evaluations did not meet standard requirements, the antibacterial test revealed moderate to strong activity against *Propionibacterium acnes*. Overall, the Gerga orange peel kombucha peel-off gel mask demonstrated promising potential as a cosmeceutical product for acne treatment, harnessing the antibacterial properties of bioactive compounds such as hesperidin. This suggests potential for further development and application in skincare products.

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