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A Green Approach to Hand Hygiene: Formulation and Evaluation of Tangerine (*Citrus reticulata*) Peel Bio-Enzyme with Iota-Carrageenan (*Eucheuma spinosum*)

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Abstract— *This study aimed to develop a tangerine (Citrus reticulata) peel bio-enzyme through a three-month fermentation process and evaluate its potential as the active component in a hand sanitizer gel formulated with iota-carrageenan (Eucheuma spinosum) as a gelling agent. Three concentrations of bio-enzyme—low (5 mL), medium (10 mL), and high (15 mL)—were prepared in triplicate, along with a negative control, resulting in ten samples. The formulations were evaluated for microbiological activity, physical properties, and potential acceptability. Antibacterial testing against Staphylococcus aureus and Escherichia coli showed 0 mm zones of inhibition across all samples. Physical evaluation based on SNI 06-2588-1992 standards for hand sanitizer preparations revealed non-homogeneous gels with watery consistency, clumping, and phase separation, although spreadability remained consistent at 5.0 cm. The pH values ranged from 2.96 to 3.40, which were below the acceptable range specified in SNI 06-2588-1992 for skin application. Alcohol content analysis showed values ranging from 0.01 to 0.05 g/100 mL, confirming that the formulations were non-alcohol based. Due to the acidic pH of the formulations, acceptability testing was not conducted. In contrast, the formulated bio-enzyme hand sanitizer gel exhibited limited antibacterial activity compared to the commercial hand sanitizer, indicating the need for further improvement in its formulation and overall effectiveness. It is recommended that future studies focus on optimizing formulation parameters, including pH adjustment, fermentation conditions, and the use of alternative gelling agents. Additionally, the antibacterial potential of the bio-enzyme should be further evaluated independently, and its application may be explored in non-topical products.*

Keywords— *Tangerine peel bio-enzyme; Citrus reticulata; hand sanitizer gel; iota-carrageenan; Eucheuma spinosum; antibacterial activity; non-alcohol-based sanitizer; fermentation*

I. INTRODUCTION

Hand hygiene is one of the most reliable methods for preventing the spread of infectious diseases [1]. Hand sanitizers are widely used because they are convenient alternatives to soap and water. However, alcohol-based sanitizers may cause skin irritation, while some commercial products have been reported to contain harmful residues or fail to meet quality standards [2] [3].

Food waste remains an environmental concern in the Philippines, where households generate approximately 2.95 million tons of food waste annually [4]. Fruit residues contain bioactive compounds with functional value and may be converted into useful industrial products [5] [6]. One potential product is bio-enzyme, also known as eco-enzyme or garbage enzyme, which may serve as a natural alternative to synthetic cleaning agents [7] [8].

Citrus peels are commonly used in bio-enzyme production because they contain antioxidants and flavonoids with potential antibacterial properties [9]. Tangerine (*Citrus reticulata*) peel contains vitamins A and C, as well as flavonoids such as naringin, hesperidin, tangeretin, and nobiletin [10]. Previous research showed that eco-enzyme derived from mixed fruit peel waste and fermented for three months demonstrated improved antibacterial activity and acceptable organoleptic properties when used in a hand sanitizer spray [11].

Iota-carrageenan from *Eucheuma spinosum* is another natural material that may be used in hand sanitizer formulation. It is widely used as a thickener, stabilizer, and gelling agent due to its ability to produce soft and clear gels [12] [13] [14]. Although citrus-derived bio-enzymes and carrageenan have been studied separately, limited research has examined their combined use in hand sanitizer gels.

Therefore, this study developed a tangerine (*Citrus reticulata*) peel bio-enzyme and evaluated its potential as an active component in a hand sanitizer gel formulated with iota-carrageenan (*Eucheuma spinosum*) as a gelling agent. The hand sanitizer gels were assessed for antibacterial activity, physical properties, and compared with commercial hand sanitizer gels.

II. METHODOLOGY

A. Study Design and Sample Preparation

This experimental laboratory-based study developed tangerine (*Citrus reticulata*) peel bio-enzyme and evaluated its potential as an active ingredient in a hand sanitizer gel formulated with iota-carrageenan from *Eucheuma spinosum*. The study included three bio-enzyme concentrations, namely low (5 mL), medium (10 mL), and high (15 mL), each prepared in triplicate, along with one negative control containing no bio-enzyme.

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Tangerine peel bio-enzyme was prepared using a 3:1:10 ratio of tangerine peels, brown sugar, and distilled water. A total of 450 g of chopped tangerine peels, 150 g of brown sugar, and 1.5 L of distilled water were mixed and placed in recycled polyethylene terephthalate bottles. The mixture was stored in a cool, dark, and well-ventilated area for three months. After fermentation, the bio-enzyme solution was filtered through muslin cheesecloth before use in gel formulation. The prepared tangerine peel bio-enzyme is shown in Fig.1.

Fig. 1. Prepared Tangerine Peel Bio-Enzyme After 3 Months

The gel base was prepared by dispersing 1.2 g of iota-carrageenan powder in 80 mL of hot distilled water at approximately 80°C. The mixture was continuously stirred using a magnetic stirrer until a viscous gel was formed. After cooling to room temperature, glycerin and the appropriate volume of filtered bio-enzyme were incorporated into the gel base through gentle mixing.

Each 100 mL formulation contained 80 mL of iota-carrageenan gel base and 5 mL of glycerin. The low-, medium-, and high-concentration formulations contained 5 mL, 10 mL, and 15 mL of bio-enzyme, respectively. Distilled water was adjusted to complete the final volume of 100 mL. The control formulation contained no bio-enzyme. All prepared gels were stored in opaque bottles before testing. Table I shows the composition of the formulated hand sanitizer gels.

TABLE I. Composition of Hand Sanitizer Gel Formulations in 100 mL

Materials	Description	Formulations concentration			
		Low	Medium	High	Control
Filtered bio-enzyme	Active ingredient	5 mL	10 mL	15 mL	0 mL
Eucheuma spinosum iota-carrageenan gel base	Gelling agent	80 mL	80 mL	80 mL	80 mL
Glycerin	Humectant	5 mL	5 mL	5 mL	5 mL
Distilled water	Solvent	10 mL	5 mL	0 mL	15 mL

B. Evaluation of Formulations

The formulated hand sanitizer gels were evaluated during the first week after preparation for antibacterial activity and physical properties. Antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, as well as ethanol content, was analyzed at the Department of Science and Technology - Regional Standards and Testing Laboratory, Pettit Barracks, Zamboanga City, Region IX, Philippines.

Antibacterial activity of the formulated samples and the negative control were submitted in 10 mL containers and was determined using the Kirby-Bauer disk diffusion method, in which zones of inhibition were measured in millimeters. A larger zone of inhibition indicated greater antibacterial activity. A commercial hand sanitizer gel was included as a comparison sample also submitted in 10 mL container.

Ethanol content of the formulated hand sanitizer gels was measured to confirm that the formulation were non-alcohol based. Samples were submitted in 50 mL containers and analyzed using the Dichromate Oxidation Method following AOAC Official Method 969.12 as described in the Official Methods of Analysis of AOAC INTERNATIONAL, 22nd Edition. Ethanol content was expressed in g/100 mL.

The physical properties of the formulations, including homogeneity, spreadability, and pH, were evaluated in accordance with SNI 06-2588-1992 standards for hand sanitizer preparations. Homogeneity was assessed visually by spreading each sample on a transparent glass surface and checking for the presence of lumps or uneven particles. Spreadability was measured by placing 0.5 mL of gel inside a 2 cm-diameter circle on a glass plate. A second glass plate and a 0.5 kg weight were placed over the sample for 5 minutes, after which the final spread diameter was measured using (1).

$$\% \text{ spread by diameter} = D1/D2 \times 100 \quad (1)$$

where D_1 (2cm) is the initial diameter and D_2 is the final diameter after spreading.

The pH of each formulation was measured using a calibrated digital pH meter and compared with the acceptable pH range specified in SNI 06-2588-1992.

C. Statistical Analysis

Data from the physical and microbiological tests were summarized using descriptive statistics, including mean and standard deviation. One-way analysis of variance was used to determine differences in pH and ethanol content among the control, low-, medium-, and high-concentration formulations at a significance level of $p < 0.05$. Inferential analysis was not performed for antibacterial activity because all formulated samples showed zero zones of inhibition.

III. Results and Discussion

A. Physical Appearance

The formulated hand sanitizer gels were yellowish and turbid, while the control formulation was white and turbid. This appearance may be due to pigments and natural bioactive compounds, such as flavonoids and carotenoids, present in the tangerine peel bio-enzyme [15].

All formulations also showed visible clumping and poor homogeneity, which may be caused by incomplete dispersion of the bio-enzyme and its acidic nature affecting the hydration and gel-forming ability of iota-carrageenan [16]. The instability observed may also be influenced by particle interactions, pH, ionic strength, and the incorporation of natural compounds into carrageenan-based gels [17] [18].

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The physical appearance of the samples is presented in Fig. 2, Fig. 3, Fig. 4, and Fig. 5.

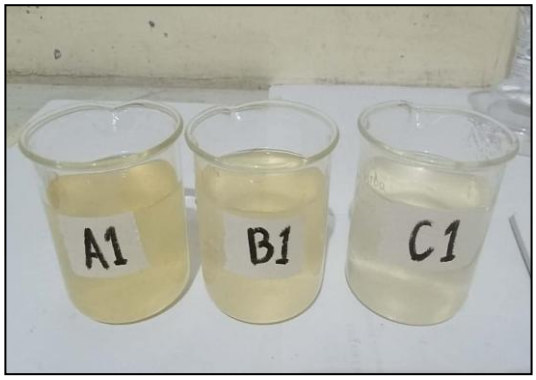

Fig. 2. Physical Appearance of Formulated Hand Sanitizer Gel in Trial 1

Fig. 3. Physical Appearance of Formulated Hand Sanitizer Gel in Trial 2

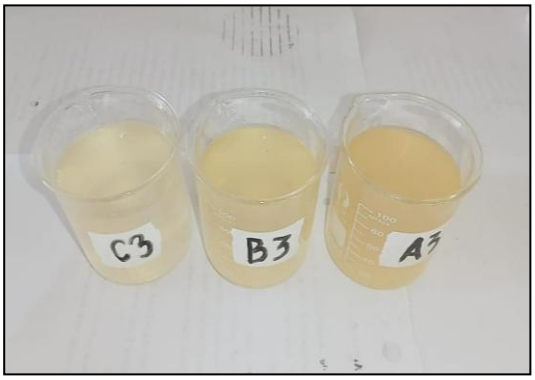

Fig. 4. Physical Appearance of Formulated Hand Sanitizer Gel in Trial 3

Fig. 5. Physical Appearance of the Control (No Bio-Enzyme)

B. Antibacterial Activity

Antibacterial activity was determined by measuring the diameter of the inhibition zone in millimeters (mm), and the results are summarized in Table II. All formulated gels, including the control, showed a 0 mm zone of inhibition against both test organisms, indicating no detectable antibacterial activity. In contrast, the commercial hand sanitizer produced measurable inhibition zones of 20 mm against *S. aureus* and 17 mm against *E. coli*, demonstrating its antibacterial effectiveness.

TABLE II. Zone of Inhibition of Hand Sanitizer Gels Against Test Organisms

Samples	***S. aureus*** **(mm)**	***E. coli*** **(mm)**
High (A)	0	0
Medium (B)	0	0
Low (C)	0	0
Control (N)	0	0
Commercial (O)	20	17

The lack of antibacterial activity observed in the formulated gels may be attributed to the absence of natural alcohol, which is the primary active antimicrobial component in effective hand sanitizers [19], and to the limited antimicrobial efficacy of tangerine peel bio-enzyme at low concentrations. It may also be due to chemical interactions between the enzyme and carrageenan during the mixing process, as hydrocolloids such as carrageenan are known to bind enzymes and bioactive compounds, potentially reducing their biological activity [20].

C. Physical Properties of Hand Sanitizer Gel

The physical properties of the formulated hand sanitizer gels were evaluated through homogeneity, spreadability, pH, and alcohol content tests to assess formulation stability, consistency, safety, and suitability for topical applications.

1. Homogeneity Test Results

Based on Table III, all formulations, including those with high (A), medium (B), and low (C) bio-enzyme concentrations, appeared watery and contained visible clumps, indicating a lack of homogeneity. The negative control (N) likewise showed uneven gel formation. According to SNI 06-2588-1992, a proper hand sanitizer should exhibit a homogeneous consistency [21].

TABLE III. Homogeneity Test Results

Samples	**Observation**	**Remarks**
High (A)	Watery, visible clumps	Not homogeneous
Medium (B)	Watery, visible clumps	Not homogeneous
Low (C)	Watery, visible clumps	Not homogeneous
Control (N)	Gel not consistent	Not homogeneous

The lack of homogeneity suggests incompatibility between the bio-enzyme solution and the gelling agent, which may affect product quality and consistency. Proper gel formation requires complete hydration of the gelling polymer and even distribution of all components [22]. If particles (e.g., bio-enzymes) remain clumped or unhydrated, the gel network is disrupted, resulting in poor texture, syneresis, or phase separation.

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2. Spreadability Test Results

All formulations reached a final spread diameter of 5.0 cm, corresponding to the maximum diameter of the glass plates used. Descriptive statistics are presented in Table IV.

TABLE IV. Spreadability Results of Formulated Hand Sanitizer Gels

Formulation	Trial 1 (cm)	Trial 2 (cm)	Trial 3 (cm)	Mean ± SD (cm)	% Spread
High (A)	5.0	5.0	5.0	5.0 ± 0.0	40%
Medium (B)	5.0	5.0	5.0	5.0 ± 0.0	40%
Low (C)	5.0	5.0	5.0	5.0 ± 0.0	40%
Control (N)	5.0	0	0	5.0 ± 0.0	40%

According to the SNI 06-2588-1992 standard, acceptable spreadability ranges from 5 to 7 cm [21]. The results show that all formulations exhibited similar spread behavior, suggesting comparable flow properties. However, the use of a 5 cm glass plate limited the ability to distinguish differences among the samples. Even with this limitation, the findings indicate that all formulations were able to adequately spread beyond the initial application area.

3. pH Test Results

The pH of each formulation was measured to ensure compatibility with the skin, as topical products are generally recommended to have a pH between 4 and 6. Additionally, the pH standard was referenced from SNI No-2588-1992, which specifies a safe range of 4.5–8.0 for cosmetic products.

Table V presents the observed pH values of the formulated hand sanitizer gels across three trials for each bio-enzyme concentration. The results show consistent acidic values for all bio-enzyme formulations, likely due to the organic acids produced during the 3-month fermentation process of the tangerine peel bio-enzyme, such as acetic acid and other short-chain organic acids formed through microbial breakdown of sugars and plant materials.

TABLE V. Observed pH Values of Formulated Hand Sanitizer Gels

Samples	Trial 1	Trial 2	Trial 3	Mean ± SD
High (A)	2.94	2.94	2.99	2.96 ± 0.03
Medium (B)	3.09	3.01	3.17	3.09 ± 0.08
Low ©	3.30	3.36	3.53	3.40 ± 0.12
Control (N)	4.81	–	–	4.81 ± –

This prolonged fermentation period allows continuous microbial activity, which increases acid accumulation and stabilizes the mixture at a low pH range [23]. As a result, the high concentration (A) exhibited the lowest mean pH (2.96 ± 0.03), followed by the medium (B) at 3.09 ± 0.08, and the low concentration (C) at 3.40 ± 0.12. The negative control (N) recorded a higher pH value of 4.81, indicating comparatively less acidity than the bio-enzyme-based formulations. Fig. 6 shows the mean pH value of the formulations.

Studies have shown that long-term fermentation of fruit peels under sugar-based anaerobic conditions promotes the production of organic acids, particularly acetic, lactic, and citric acids, which significantly contribute to acidic pH stability in the final product [24].

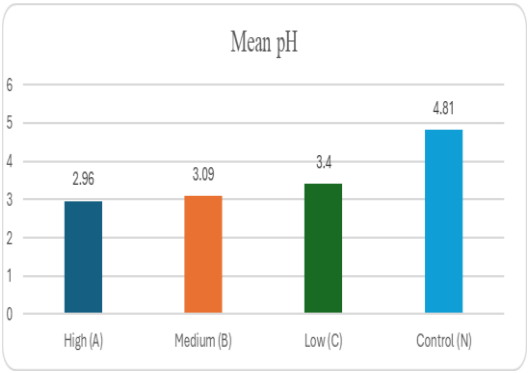

Fig. 6. Shows the mean pH value of the formulated hand sanitizer gels and the control

The one-way ANOVA results indicate a statistically significant difference in mean pH among the bio-enzyme formulations ($p<0.05$). This implies that varying concentrations of tangerine peel bio-enzyme significantly influence the acidity of the hand sanitizer gels. The Tukey's HSD post-hoc test further showed that the low concentration © had a significantly higher pH than both the medium (B) and high (A) concentrations, while no significant difference was observed between the medium and high formulations.

TABLE VI. One-Way ANOVA Table for pH Values of Hand Sanitizer Formulations

Source of variation	Sum of squares	DF	Mean square	F statistic	P-value
Between groups	0.3054	2	0.1527	21.3415	0.001872
Within groups	0.04293	6	0.007156		
Total	0.3484	8	0.04354		

Table VI presents the One-Way ANOVA results for the pH values of the formulated hand sanitizer gels. The ANOVA results confirm the statistical significance of the observed differences among groups, as indicated by a high F-statistic (21.3415) and a low p-value (0.001872). The between-group variation is substantially greater than the within-group variation, reinforcing that bio-enzyme concentration is a significant factor affecting the pH of the formulations rather than random experimental variation.

All bio-enzyme formulations were below the recommended pH range for topical application, likely due to organic acids produced during tangerine peel fermentation. A study by Lambers et al. [25], healthy human skin maintains an acidic surface pH typically between 4.0 and 6.0, and topical formulations are generally designed to remain within or close to this range to avoid irritation and barrier disruption. The reduced pH is likely attributable to organic acids generated during citrus peel fermentation, including citric and acetic acids [26]. Because topical products with excessively low pH may compromise skin compatibility, these formulations were considered unsuitable for direct application [27].

4. Ethanol Content Test Results

Table VII presents the ethanol content results of the formulated hand sanitizer gels across three trials for each bio-enzyme concentration. The data shows consistently low ethanol values for all formulations, with mean concentrations

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ranging from 0.02 to 0.05 g/100 mL. Both the high (A) and low (C) formulations recorded similar mean values (0.05 ± 0.02 and 0.05 ± 0.03, respectively), while the medium formulation (B) exhibited the lowest mean ethanol content (0.02 ± 0.02). The negative control (N) recorded the lowest value at 0.01 g/100 mL.

TABLE VII. Alcohol Content Test Results

Formulation	Trial 1 (g/100 mL)	Trial 2 (g/100 mL)	Trial 3 (g/100 mL)	Mean ± SD
High (A)	0.07	0.04	0.04	0.05 ± 0.02
Medium (B)	0.04	0.01	0.01	0.02 ± 0.02
Low (C)	0.03	0.03	0.08	0.05 ± 0.03
Control (N)	0.01	–	–	0.01 ± –

All formulations exhibited very low ethanol content, confirming that the gels are non-alcohol based. Hand sanitizers are classified as alcohol-based only when they contain ethanol or isopropanol at concentrations of approximately 60–95%, which are required for effective antimicrobial action. Formulations containing alcohol far below this threshold are considered non-alcohol based [28].

D. Comparison with Commercial Hand Sanitizer Gel

A comparison of the zone of inhibition produced by the formulated hand sanitizer gels and a commercial hand sanitizer gel is presented in Table VIII.

TABLE VIII. Mean Zone of Inhibition of Formulated Hand Sanitizer Gels and Commercial Hand Sanitizer

Sample Category	Sample Code	Mean Zone of Inhibition against *S. aureus* (mm)	Mean Zone of Inhibition against *E. coli* (mm)
Formulated hand sanitizer gels	A	0.00	0.00
	B	0.00	0.00
	C	0.00	0.00
Negative control	N	0.00	0.00
Commercial hand sanitizer	O	20.00	17.00

Antibacterial activity was evaluated against *Staphylococcus aureus* and *Escherichia coli* using the agar diffusion (Kirby–Bauer) method, a standardized technique for assessing antimicrobial efficacy [29]. The commercial hand sanitizer (O) produced measurable zones of inhibition against both test organisms, indicating effective antibacterial activity. In contrast, all formulated samples exhibited a 0 mm zone of inhibition, signifying the absence of detectable antibacterial action under the conditions of the assay [30].

Because all formulated samples showed no inhibition, resulting in no variability in the data, inferential statistical analysis was not applicable. The comparison between the formulated gels and the commercial product was therefore assessed descriptively based on the presence or absence of inhibition. This result emphasizes the importance of formulation optimization, pH control, and inclusion of effective antimicrobial agents in hand sanitizer development.

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