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Original Article

THE EFFECT OF DIFFERENT STORAGE TEMPERATURES ON ANTISEPTIC GEL STABILITY CONTAINING GREEN TEA EXTRACT FORMULATED WITH ALOE VERA GEL

SRI AGUNG FITRI KUSUMA[1*], DEFU YUSUF[2], ARDIAN BAITARIZA[2]

[1]Department of Biology Pharmacy, Faculty of Pharmacy, Padjadjaran University, Sumedang, West Java, Indonesia 45363, [2]Departement of Pharmacy, Faculty of Mathematics and Natural Sciences, Al-Ghifari University, Bandung, West Java, Indonesia
Email: s.a.f.kusuma@unpad.ac.id

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ABSTRACT

Objective: The aim of this study was to analyze the stability of antiseptic gel products containing green tea extract combined with aloe vera gel by observing the physical properties and the antibacterial effectivity during storage time with different temperature.

Methods: The gel of *Aloe vera* were separated from the leaves using a sterile knife. While the dried green tea leaves were extracted using a maceration method. Both of active agents were combined with different concentration and formulated into an antiseptic gel product. The carbopol in a fixed concentration was used as a gelling agent. The antiseptic gel in different formulas (F1-F9) were evaluated for 56 d in different temperature storage of 18 and 25 °C. The physical properties (color, pH and viscosity) were observed and the antibacterial effectivity of each formula was analyzed using the agar diffusion method against *Staphylococcus aureus*.

Results: As a result, the green tea extract and aloe vera gel were successfully incorporated into the carbopol formulation. Each formula represented a slight difference in the pH and viscosity value of each temperature during the storage time. But statistically, both temperatures did not give a significant difference to the pH and viscosity of each gel product. Therefore, the different effect given by the storage temperature was determined by analyzing the antibacterial effectivity results. The storage temperature of 18 °C gave a significant difference in the diameter of inhibition zones against *S. aureus*, compared to that of 25 °C. Formula number 9 containing the combination of 5% green tea extract and 6.24% aloe vera gel performed the highest antibacterial effectivity with the optimum storage temperature at 18 °C.

Conclusion: The storage temperatures used in this study gave significant effect to physical properties stability and its antibacterial effectivity.

Keywords: Green tea, Aloe vera, Gel, Stability, Temperature, Influence

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INTRODUCTION

Herbal drugs have seen tremendous predominates not only in traditional medicine but also as an alternative medical treatment, especially in the developed countries. The natural antimicrobial can be derived from plants that contain certain secondary metabolites. Green tea extract and aloe vera gel are kinds of herbal drugs which have been reported having potent natural antibacterial. Green tea (*Camellia sinensis*) is attributed with high production of polyphenolic compounds, which z agent [2]. Among those herbal drugs application, the treatment related to skin disease is ranked top. Some studies have reported that each of green tea extract and aloe vera gel potentially inhibits oral and skin pathogens, including bacteria and fungus [3-8]. Furthermore, this research information reinforces the reason for applying these herbs in the polyherbal formulation as natural antiseptics.

The most practical form of antiseptic for daily use is the topical form. The topical drug delivery is a practical method to treat local and systemic disease, but it is commonly used for inflammatory conditions such as dermatological diseases or musculoskeletal injuries [9, 10]. The topical treatments are chosen because of several advantages such as: action occur directly at the action site because of the drug bioavailability increased at the local site, prevents the inconveniences and risk than using intravenous therapy and can avoid the systemic side effect which could be happen in oral treatment. In the topical treatment, the drugs do not pass the gastrointestinal route and not metabolized in the liver. Therefore, it can avoid the risks associated with absorption conditions, such as pH changes, gastric emptying time and enzymes presence [8, 11, 12]. The increasing demand of herbal medicinal products has an impact on the manufacturing of these products in the large scale production. Especially, the common use of herbal drugs prepared in the form of gel antiseptic. Thereby, it must also be considered that large quantities of production can encourage the storage of products with a longer period. Large scale production lead to longer storage periods which could lead to product deterioration. The improper storage and length of time for storing herbal products could lead to degradation processes which causing active substances decreasing, resulted in metabolites with no activity, and the most extreme is the forming of toxic metabolites [13]. The formulation in this study used one of the plants that were Aloe vera gel which reported has a critical component instability. Besides a synergic action between different components in aloe vera gel, some polysaccharides characteristic seem to play roles in its pharmacological and physiological [14, 15]. Among those of polysaccharides in aloe vera gel, acemannan are the important active compounds, but a rapid degradation of aloe vera gel's active compounds in different conditions was also reported [16, 17]. Improper storage conditions, especially the temperature effect can lead to the degradation of active compounds [18]. In fact, this information has been proven from other study, which reported that leaves of Aloe vera lose their biological activity at room temperature after since 6 h from harvest [14].

Therefore, regarding to the stability of β-polysaccharides in Aloe vera gel, a potent influence of the storage temperature was observed to ensure the stability of antiseptic gel antibacterial effectivity in combining with green tea extract. The evaluation of the physical and chemical stability of a final product during the storage period is an important step in controlling herbal drug preparation quality. The product quality must be ensured to be harmless because it has consequences for patient safety [19]. Therefore, stability studies on products containing herbal have become very important to determine the shelf-life of products and ensure product quality at storage periods and during usage. Thus, the aim of this study was to analyze the stability of antiseptic gel products containing green tea extract combined with aloe vera gel by observing the physical properties and the antibacterial effectivity during storage time with different temperature.

MATERIALS AND METHODS

Materials

The mature, fresh and healthy leaves of Aloe vera were obtained from Subang city, West Java, Indonesia. Meanwhile, the dried green tea leaves were sourced from tea plantation center at Gambung Ciwidey, West Java, Indonesia. All the herbal used in this study have been authenticated (No. 2626/Ko1.14.2/PP/2010) by the plant determination institute of biology department, Faculty of mathematics and natural sciences, Padjadjaran University, Jatinangor, Indonesia. The *Staphylococcus aureus* bacteria used in this study was obtained from the culture collection in Microbiology Laboratory, Faculty of pharmacy, Padjadjaran University, Indonesia. Mueller Hinton Agar (MHA-OXOID) and Mueller Hinton Broth (MHB-OXOID) were used as the growth medium for bacteria. The chemicals used were 2N hydrochloric acid (Merck), amyl alcohol (Merck), chloroform (Merck), 10% ammonia (Merck), ether (Brataco), iron (III) chloride (Merck), anhydrous acetic acid (Merck), H_2SO_4 (Merck), 1% gelatin (Brataco), Mayer reagents (potassium mercury iodide solution), reagent Dragendorff (potassium bismuth iodide solution), 10% vanillin solution in concentrated H_2SO_4, 1N sodium hydroxide, potassium permanganate powder (Merck), magnesium powder (Merck), ethanol (Brataco), sterile physiological sodium chloride (Brataco), demineralized water, Carbopol (Brataco), glycerine (Brataco), sodium metabisulfite (Merck), and triethanolamine (Merck).

Herbal preparation

Mature, healthy and fresh leaves of Aloe vera were washed in the running tap water and rinsed using sterile distilled water. The leaves then sliced and the gel scraped out using a sterile knife. The obtained gel, then washed by a sterile distilled water to remove the yellow sap. After that, gel slices were homogenized using a blender. The gel homogenats were subjected to a freeze dryer instrument to achieve the dried powder of Aloe vera gel. Meanwhile, the green tea extracts were prepared by a maceration method using 70% ethanol as the solvent. The dried green tea leaves were sliced into small pieces using scissors then powdered coarsely. The weight of 1 Kg leaf powder was macerated successively in 7 L of 70% ethanol for 3 d. The liquid extracts were collected and then subsequently concentrated with a rotary evaporator at 40 °C to get a thick extract.

Phytochemical screening analysis

Phytochemical screening was analyzed according to standard methods. The purpose of this step was to determine the metabolite content such as alkaloids, tannins, polyphenols, flavonoids, saponins, triterpenoids, quinones, monoterpenoids, and sesquiterpenoids [20].

Preparation of antiseptic gel

The antiseptic gel was formulated with a combination of Aloe vera gel and green tea extract as active agents with a carbopol gel base. The gel was prepared using carbopol, glycerin, sodium metabisulfite, triethanolamine (TEA) and distilled water in sufficient amount to obtain 100 g gel. Carbopol was dispersed in 50 ml of distilled water and stirred continuously in a magnetic stirrer at 200 rpm for 2 h. Then the mixture was neutralized by the addition of 0.5% triethanolamine and then 0.2% sodium metabisulfite was added. The mixing process was continued to achieve a transparent gel. Each of the green tea ethanolic extract and the dried powder of aloe vera gel in various concentrations was dissolved in distilled water, then incorporated into the gel base and followed by stirring for 5 min or until a homogenized gel was formed. The corigen odoris was also added to the gel mixture while stirring process. The detail formulas of herbal antiseptic gel were presented in table 1.

Table 1: Formula of antiseptic gel

Composition (%)	Formula									
	Gel basis	1	2	3	4	5	6	7	8	9
Green tea extract	0	1.25	1.25	1.25	2.50	2.50	2.50	5.00	5.00	5.00
Aloe vera gel	0	1.56	3.12	6.24	1.56	3.12	6.24	1.56	3.12	6.24
Carbopol	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
TEA	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
glycerin	10	10	10	10	10	10	10	10	10	10
sodium metabisulfite	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Corigen odoris (drops)	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00	8.00
demineralized water (ml)	100	100	100	100	100	100	100	100	100	100

Stability test and the physical evaluation

All gel formulas were submitted to stability tests at different storage temperature (18 and 25 °C) for 56 d. The gel was evaluated on physical properties (color, odor, pH and viscosity) and its antibacterial effectivity. During storage, each test was performed in triplicate with 30 g samples of each. Physical parameters such as color and odor were observed visually. Meanwhile, the pH of the gel was measured using pH meter [21]. The viscosity of the gel was measured using the RION Viscometer tool. The gel was rotated at 300 rpm using R5 spindle.

Preparation of the bacterial suspension

From slant agar, a loop full Ose of *S. aureus* colony was suspended in sterile physiological NaCl aseptically. The final *S. aureus* inoculum suspension was adjusted to 0.5 Mc Farland ($1.5x10^8$ cfu/ml). The 0.5 McFarland solution was made from 0.05 ml of 1% $BaCl_2$ and 9.95 ml of 1% H_2SO_4. The turbidity of the bacterial suspension and McFarland solution was measured at a wavelength of 530 nm by using distilled water as a blank [22].

Antibacterial test against *S. aureus*

Antibacterial activity of each formula against S. aureus was evaluated by an agar well diffusion method. The S. aureus bacteria used in this study was obtained from the culture collection in Microbiology Laboratory, Faculty of pharmacy, Padjadjaran University, Indonesia. A volume of 0.2 ml bacterial suspension was poured and homogenized into a sterile Petri disk containing 20 ml of liquid MHA (at a temperature of 40 °C). After the medium solidified, the wells were made by a sterile perforator. Each of these wells was filled with gel preparations with different formulas from storage with different temperatures at certain storage times. Plates were allowed for 30 min for pre-diffusion. After that, the plates were incubated at at 37 °C for 24 h and the diameter of the clear zones was measured by a calliper [23].

RESULTS AND DISCUSSION

Extraction result

Extraction is an important process to discover bioactive constituents from plant materials. The different technique of herbal preparation in this study may cause the different yield level of the rendement. From 1 kg of the dried green tea leaves, a weight of 20.31% rendemen extract was obtained. Meanwhile, the rendemen yield from 8.5 Kg of the fresh aloe vera gel was 1.65%.

Phytochemical screening

Green tea leaves extract and aloe vera gel contains natural phytochemicals that have been used as alternative drug to treat many infection diseases. The following secondary metabolites were found to present in the powdered of aloe vera gel and the ethanolic extract of green tea leaves. The metabolites were summarized in table 2.

Table 2: Phytochemical composition of the herbal

Metabolites	Result	
	Green tea extract	Aloe vera gel powder
Alkaloids	+	-
Flavonoids	+	+
Polyphenols	+	+
Tannins	+	-
Monoterpenoids	+	-
Sesquiterpenoids	+	-
Steroids and triterpenoids	-	-
Quinones	+	-
Saponins	-	+

Notes: (+) presence; (-) absence

Based on the data in the table 2, it can be concluded that green tea ethanolic extract was more potent as an antiseptic agent than aloe vera gel. Green tea extract possessed more antibacterial than aloe vera gel. The saponin presence solely in aloe vera gel could improve the antibacterial effect of antiseptic gel, since its absence in green tea extract. Herbal products containing various secondary metabolites with pharmacological activities often showed broad-spectrum antibacterial activity. Green tea leaf extract is a well-known plant with high polyphenolic components as it had been reported that it can inhibit the growth of Gram-positive and negative bacteria with moderate potency [6]. The polyphenols mechanism as an antibacterial is by coagulating the bacterial protein and disrupting the cell membrane resulted in the bacterial lysis [24]. The presence of saponins in the aloe vera gel content also increasing the bacterial membrane permeability and reducing the cell walls surface tension by interacting with lipopolysaccharide in the bacterial cell wall [25]. Besides saponin, tannins also can damage bacterial cell membrane the antibacterial potency in this antiseptic gel also must be mounted up because both herbal in combination contain of flavonoids, which had been known as a potent antimicrobial agent. Flavonoid play an important role as antibacterial because of its mechanism on the DNA bacteria. From the phytochemical screening result, it informed that the compound herbal in antiseptic gel preparation in this study was more effective as antibacterial while single herbal preparation against bacterial pathogen due to its combined effects of green tea extract and aloe vera gel [26].

Evaluation results on organoleptic stability

Gels are a semisolid preparation that is used for skin with easier application than creams and lotions. Gel application capable to release drugs immediately, due to solubility characteristic of the active agent in the water. Gel preparations are very biocompatible with a lower risk of adverse reactions or inflammation, easy to apply and do not need to be removed [27]. Aloe vera gel and green tea in extract form will not stable in a long periode of storage, moreover, thus extract could result the degradation process which harmful to the skin. Therefore, this *in vitro* study was conducted to formulate the green tea extract and aloe vera gel in various concentrations incorporated in carbopol gel. Carbopol was selected as a gelling agent due to its better gelling characteristics than other polymers [28]. The other advantage of carbopol are its irritation free and a promising carrier active phytoconstituents with controlled release from the gel formulation. In this gel design formula, triethanolamine was added to neutralize the anionic characteristic of carbopol in nature [29]. But, the main consideration that must be kept in mind is the stability of the active substance in the gel preparation. Therefore, this study concerned about the stability of antiseptic gel products containing herbal that has become very important to ensure product quality at storage periods and during usage.

The resulted formula was assessed for its stability on the organoleptic characteristics such as color and odor of the gels during the storage period at different temperature of storage. Each formula with the same green tea extract concentration produces the same color and gel odor. The transparent color of aloe vera gel did not affect the color of the gel in all formulas. Likewise, gel odor, even though the aloe vera gel concentration used was increasing at the same concentration of green tea extract. The tea odor of each formula increasing as the higher green tea extract concentration used in the formula. For the color, all gel formula performed the black color with different density color level. F1-F3 resulted in the light black gel; F4-F6 resulted in brownish black gel and F6-F9 resulted in solid black gel.

From the stability observations during the storage period, the different temperature showed no effect to the organoleptic characteristic of all gel formulas. All formula remains stable in the odor and color until the end of storage periods.

Table 3: The effect of storage temperature at 18 °C on pH of the gel formulas

Storage period	pH Formula									
(day)	F1	F2	F3	F4	F5	F6	F7	F8	F9	base
0	5.2±0.000	5.1±0.000	5.8±0.000	5.7±0.000	5.9±0.000	5.3±0.000	5.4±0.000	5.9±0.000	5.9±0.000	5.5±0.000
3	5.2±0.000	5.1±0.000	5.8±0.000	5.7±0.000	5.9±0.000	5.3±0.000	5.4±0.000	5.9±0.000	5.9±0.000	5.5±0.000
7	5.2±0.000	5.1±0.000	5.8±0.000	5.7±0.000	5.9±0.000	5.3±0.000	5.4±0.000	5.9±0.000	5.9±0.000	5.5±0.000
14	5.2±0.000	5.1±0.000	5.8±0.000	5.7±0.000	5.9±0.000	5.3±0.000	5.4±0.000	5.9±0.000	6±0.000	5.5±0.000
21	5.2±0.001	5.1±0.000	5.8±0.000	5.7±0.000	5.9±0.000	5.7±0.000	5.5±0.000	5.9±0.000	6±0.000	5.5±0.000
28	5.2±0.001	5.1±0.000	5.8±0.000	5.7±0.000	5.8±0.000	5.7±0.000	5.6±0.000	5.9±0.000	6±0.000	5.5±0.000
35	5.2±0.001	5.1±0.000	5.8±0.000	5.7±0.000	5.8±0.000	5.7±0.000	5.5±0.000	5.9±0.000	6±0.000	5.5±0.000
42	5.2±0.000	5.1±0.000	5.8±0.000	5.9±0.000	5.9±0.000	5.7±0.000	5.7±0.000	6.1±0.000	6±0.000	5.5±0.000
49	5.2±0.000	5.1±0.000	5.9±0.000	5.9±0.000	6.2±0.000	5.7±0.000	5.7±0.000	6.1±0.000	6±0.000	5.5±0.000
56	5.2±0.001	5.3±0.000	5.9±0.000	5.9±0.000	6.4±0.001	5.8±0.000	5.7±0.000	6.4±0.000	6±0.000	5.5±0.000

Evaluation results on pH stability

From the result in table 3, all pH gel formulas relatively stable with a range of 5.1-6.4 at 18 °C during the storage period. Storage at 25 °C also showed the stability on the pH gel of all formulas with the same range of 5.2-6.0, presented in table 5. The pH values of gel preparations were in the normal skin pH range of 4.0-6.0. Those pH range has been stated as the skin acid mantle which is important for the function of skin and its resistance to the airborne pathogen [30]. Thus, all developed gel formulas might not irritate the skin and can maintain the balance of the skin normal flora. The presence of active agents in gel formulas resulted in different pH value compared to the

gel base. The smaller concentration combination of the extract and aloe vera gel used, then the smaller of pH values were produced. In contrast, on F9 with the highest concentrations combination of tea extract and aloe vera gel, the pH was much higher than the gel base. This data showed the effect of the different combination on an active agent's concentration to the gel pH. Based on statistical analysis using a random block complete design ANOVA with 95% confidence level or alpha 0.05, obtained that the p-value was smaller than alpha value, which means Ho was rejected this shows that there is an effect of storage time at difference temperature i.e. 18 °C and 25 °C on the pH stability of the gel preparation, presented in table 4 and 6. The pH values of F1 showed the pH stability at 18 °C.

Likewise, the pH changes of the other gels were more stable at a storage temperature of 18 °C than 25 °C. However, the change in pH of the gel did not exceed the normal pH range of the skin.

Table 4: ANOVA test of pH changes at 18 °C during storage periods

Source	Sum of squares	Df	Mean square	F	P-value
Corrected Model	3190.388	19	167.915	18580.776	0.00
Formula	8.844	9	0.983	108.738	0.00
Block	0.584	9	0.065	7.180	0.00
Error	0.732	81	0.009		
Total	3191.120	100			

Fig. 1: The pH stability of antiseptic gel at 18 °C

Table 5: The effect of storage temperature at 25 °C on pH of the gel formulas

Storage period	pH Formula									
(day)	F1	F2	F3	F4	F5	F6	F7	F8	F9	Base
0	5.2±0.000	5.1±0.000	5.8±0.000	5.7±0.000	5.9±0.000	5.3±0.000	5.4±0.000	5.8±0.000	5.9±0.000	5.5±0.000
3	5.2±0.000	5.1±0.000	5.8±0.000	5.7±0.000	5.9±0.000	5.3±0.000	5.4±0.000	5.8±0.000	5.9±0.000	5.5±0.000
7	5.2±0.000	5.1±0.000	5.8±0.000	5.7±0.000	5.9±0.000	5.3±0.000	5.4±0.000	5.8±0.000	5.9±0.000	5.5±0.000
14	5.2±0.000	5.2±0.000	5.8±0.000	5.7±0.000	5.9±0.000	5.3±0.000	5.4±0.000	5.8±0.000	6±0.000	5.5±0.000
21	5.2±0.000	5.2±0.000	5.8±0.000	5.7±0.000	5.9±0.000	5.7±0.000	5.5±0.000	5.8±0.000	6±0.000	5.5±0.000
28	5.2±0.000	5.3±0.000	5.8±0.000	5.9±0.000	5.8±0.000	5.7±0.000	5.6±0.000	5.8±0.000	6±0.000	5.5±0.000
35	5.2±0.000	5.4±0.000	5.±0.0008	5.9±0.000	5.8±0.000	5.7±0.000	5.5±0.000	5.9±0.000	6±0.000	5.5±0.000
42	5.4±0.000	5.5±0.000	5.9±0.000	5.9±0.000	5.9±0.000	5.7±0.000	5.7±0.000	5.9±0.000	6±0.000	5.5±0.000
49	5.4±0.000	5.6±0.000	5.9±0.000	5.9±0.000	5.9±0.000	5.8±0.000	5.7±0.000	5.9±0.000	6±0.000	5.5±0.000
56	5.4±0.000	5.6±0.000	5.9±0.000	5.9±0.000	5.9±0.000	5.8±0.000	5.7±0.000	5.9±0.000	6±0.000	5.5±0.000

Fig. 2: The pH stability of antiseptic gel at 25 °C

Table 6: ANOVA test of pH changes at 25 °C during storage periodes

Source	Sum of squares	Df	Mean square	F	P-value
Corrected Model	3235.90	19	170.31	17028.95	0.00
Formula	7.16	9	0.80	79.56	0.00
Block	1.36	9	0.15	15.14	0.00
Error	0.81	81	0.01		
Total	3236.71	100			

Evaluation results on viscosity stability

The pH values of the formulated gels might play a role in supporting the carbopol in the gel-forming because it was reported in another study that pH values between 5 and 5.5 are sufficient to achieve a good viscosity and gel clarity [31]. This fact was important information for antiseptic gel effectivity related to its viscosity. Because the viscosity values can reflect the gel consistency which determines the active substances permeation to the thin layers of the skin. Table 7-8 presented the viscosity of all formulated gel during storage in different temperature were ranged from 26-29 poise. Among those gel formulas, F6 was the most unstable viscosity gel product during the storage period at both temperatures. This was presumably due to pH instability in formula 6 during the storage period, which increased sharply. This finding showed an association between pH and viscosity of the gel. The relationship also strengthened the assumption that the viscosity stability in F1 was related to the stability of F1 pH value during the storage period at 18 °C. The F1 showed the most stable viscosity at 18 °C, but unstable at 25 °C.

Table 7: The effect of storage temperature at 18 °C on the viscosity of the gel formulas

Storage period	Viscosity of the gel formulas (poise)									
(day)	F1	F2	F3	F4	F5	F6	F7	F8	F9	Base
0	29±0.000	29±0.000	28±0.000	28±0.000	27±0.000	28±0.000	28±0.000	29±0.000	29±0.000	30±0.000
3	29±0.000	29±0.000	28±0.000	28±0.000	27±0.000	28±0.000	28±0.000	29±0.000	29±0.000	30±0.000
7	29±0.000	29±0.000	28±0.000	28±0.000	27±0.000	28±0.000	28±0.000	29±0.000	29±0.000	30±0.000
14	29±0.000	29±0.000	28±0.000	28±0.000	27±0.000	28±0.000	28±0.000	29±0.000	29±0.000	30±0.000
21	29±0.000	29±0.000	28±0.000	28±0.000	27±0.000	28±0.000	28±0.000	29±0.000	29±0.000	30±0.000
28	29±0.000	29±0.000	28±0.000	28±0.000	27±0.000	28±0.000	28±0.000	28±0.000	29±0.000	30±0.000
35	29±0.000	29±0.000	28±0.000	27±0.000	27±0.000	28±0.000	28±0.000	28±0.000	28±0.000	30±0.000
42	29±0.000	29±0.000	28±0.000	26±0.000	26±0.000	27±0.000	28±0.000	28±0.000	28±0.000	30±0.000
49	29±0.000	29±0.000	28±0.000	26±0.000	26±0.000	27±0.000	28±0.000	28±0.000	28±0.000	30±0.000
56	29±0.000	29±0.000	28±0.000	26±0.000	26±0.000	26±0.000	28±0.000	28±0.000	28±0.000	30±0.000

Fig. 3: The viscosity stability of gel formulas stored at 18 °C

Table 8: The effect of storage temperature at 25 °C on the viscosity of the gel formulas

Storage period (day)	Viscosity of the gel formulas (poise)									
	F1	F2	F3	F4	F5	F6	F7	F8	F9	Base
0	29±0.000	29±0.000	28±0.000	28±0.000	27±0.000	28±0.000	28±0.000	29±0.000	29±0.000	30±0.000
3	29±0.000	29±0.000	28±0.000	28±0.000	27±0.000	28±0.000	28±0.000	29±0.000	29±0.000	30±0.000
7	29±0.000	29±0.000	27±0.000	28±0.000	27±0.000	28±0.000	28±0.000	29±0.000	29±0.000	30±0.000
14	29±0.000	29±0.000	27±0.000	27±0.000	27±0.000	28±0.000	28±0.000	29±0.000	29±0.000	30±0.000
21	28±0.000	29±0.000	27±0.000	27±0.000	27±0.000	28±0.000	27±0.000	29±0.000	28±0.000	30±0.000
28	28±0.000	28±0.000	27±0.000	27±0.000	25±0.000	27±0.000	27±0.000	28±0.000	28±0.000	30±0.000
35	27±0.000	28±0.000	26±0.000	27±0.000	25±0.000	27±0.000	27±0.000	28±0.000	27±0.000	30±0.000
42	27±0.000	27±0.000	26±0.000	26±0.000	25±0.000	27±0.000	27±0.000	28±0.000	27±0.000	30±0.000
49	27±0.000	27±0.000	26±0.000	26±0.000	25±0.000	27±0.000	27±0.000	27±0.000	27±0.000	30±0.000
56	27±0.000	27±0.000	26±0.000	26±0.000	25±0.000	27±0.000	27±0.000	27±0.000	27±0.000	30±0.000

The viscosity stability of gel formulas stored at 25 °C

Evaluation results on antibacterial effectivity stability

The antibacterial effectivity of different storage temperatures of the gel preparations was evaluated against *S. aureus* after 56 d of storage time. The results were presented in table 9. It was found after the storage period that the all antiseptic gel formulas exhibited a maximum diameter of inhibition against *S. aureus*. But from the results, all formulas at a storage temperature of 18 °C produced a higher diameter of inhibition than at 25 °C. The highest inhibition performed by F9 at 18 °C. The more increasing combination of active agent concentration showed the more inhibition diameter against *S. aureus*. Green tea leaf extracts with high polyphenolic components contributed to the optimal inhibition of the antiseptic gels against *S. aureus*. The polyphenolic metabolites can inhibit the growth of Gram-positive and negative bacteria with moderate potency [6]. Moreover, in these gel formulas were combined with another antibacterial herbal such as Aloe vera gel. Besides a synergic action between different components in aloe vera gel, some polysaccharides characteristic seem to play roles in its pharmacological and physiological [14, 15]. But, the improper storage and length of time for storing herbal products could lead to degradation processes which causing active substances decreasing, resulted in metabolites with no activity, and the most extreme is the forming of toxic metabolites [13]. The formulation in this study used one of the plants that were Aloe vera gel which reported has critical component instability. In addition, the release of the active agent strongly influenced by some physical characters, especially the gel viscosity. All formulated gel storage at 18 °C had a higher final gel viscosity than at 25 °C. The difference in viscosity due to the effect of storage at different temperatures was thought to affect the difference in antibacterial effect of the gel stored at a temperature and play a role in the releasement of active agent that improving its bacterial inhibition.

Table 9: The effect of storage different temperature of antibacterial effectivity result after 56 d of the storage period

Formula	Inhibitory diameter (mm)	
	18 °C	25 °C
F1	15.23±0.022	14.74±0.026
F2	16.27±0.022	15.29±0.026
F3	16.75±0.022	16.45±0.026
F4	17.22±0.022	16.65±0.026
F5	17.90±0.022	17.73±0.026
F6	17.57±0.022	16.63±0.026
F7	18.66±0.022	17.30±0.026
F8	18.69±0.022	18.41±0.020
F9	20.53±0.022	20.35±0.024

CONCLUSION

It can be concluded that the storage temperatures used in this study gave significant effect to physical properties stability and its antibacterial effectivity. The best storage temperature for that stability of antiseptic gel containing a combination of green tea extract and aloe vera gel was at 18 °C.

AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

Declared none

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