EFFECT OF HYDROXYPROPYL METHYLCELLULOSE (HPMC) AS GELLING AGENT ON PHYSICAL CHARACTERISTICS AND ANTIBACTERIAL POTENTIAL OF TAMOENJU LEAVES EXTRACT GEL (HIBISCUS SURATTENSIS L) AGAINST STAPHYLOCOCCUS AUREUS

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ABSTRACT

Objective: The target of the study was to know the impact of gelling agent in tamoenju (Hibiscus surattensis L) leaves extract gel and evaluate their physical characteristics and antibacterial potential.

Methods: Three gel formulations were prepared using different concentrations (1, 1.5, and 2%) of gelling agents HPMC and were evaluated for physical characteristics such as organoleptic, pH, viscosity, spreadability, adhesiveness and antibacterial potential against Staphylococcus aureus.

Results: The consequences of the organoleptic test for three recipes showed a blackish-green color with a thick texture. The addition of tamoenju leaves extract to the gel base affected the base pH from around 7 to 4.4–4.5 and increase viscosity of the gel. The spreadability test of F2 and F3 met the standard, namely 5-7 cm. The adhesion test of the three formulas met the standard for more than 4 seconds. The inhibition test against S. aureus for the three formulas had an inhibitory activity ranging from 10-11 mm, categorized as a moderate and strong category.

Conclusion: Antibacterial gel formulations can be made using tamoenju leaf extract. The physical stability parameters of all gel preparations containing tamoenju leaf extract show good results on day 1, but they alter after 36 d of storage. F2 (1.5% of HPMC) outperformed the other formulations in terms of physical stability and has the strongest inhibitory effect against the growth of Staphylococcus aureus.

Keywords: Tamoenju leaves (Hibiscus surattensis L), Hydroxypropyl methylcellulose (HPMC), Physical characteristics, Antibacterial activity

INTRODUCTION

Herbal plants are plants that have beneficial functions and uses for society. The current trend in Indonesian society is to use herbal plants to treat diseases or as cosmetics. Herbal plants in Indonesia consist of various types of plants. One of the herbal plants used by the people in Palu is “tamoenju” leaves is one of the traditional medicinal plants commonly used for diabetes treatment in Indonesia, especially in Central Sulawesi [1, 2].

Studies have shown that phenolic and flavonoid compounds can be found in tamoenju leaf ethanol extract [3]. These substances exhibit potent antibacterial activity against Staphylococcus aureus, according to additional research [3]. Acne is one of the illnesses brought on by S. aureus. S. aureus is one of the bacteria that cause acne vulgaris, an infection that is defined by inflammation of the pilosebaceous layer as well as blockage and accumulation of keratin substances. Under normal circumstances, this bacteria is not harmful, but when the skin’s condition changes, it becomes invasive. Bacterial development receives nourishment from the fatty acid, amino acid, urea, water, and salts secreted by sweat and sebaceous glands [4].

The commonness of skin breakout patients in Indonesia goes from 80% to 85% in teenagers, with the peak incidence occurring between the ages of 15 to 18, 12% in women over 25 y old, and 3% in the 35-44 age group [5]. There are two common types of treatments used to treat acne, namely topical treatment applied directly to the acne-prone areas, resulting in a local effect, and oral treatment taken to treat acne systemically [6]. Topical treatment is widely used for acne, and one of the preparations used is an antibacterial gel. Gel is a preparation that contains a lot of water and has drug delivery which is better compared to ointment. The advantage of gel preparations is easy when applied to the skin, give a cool sensation, has good absorption, does not cause scars, and easy to use. Gel preparations require a basis in order to obtain preparations that have high stability and compatibility, low toxicity, and can increase contact time with skin [7].

From the explanation above, the motivation behind this study is to fig out a gel planning from tamoenju leaves extract as an antibacterial gel with various concentrations of HPMC as a gelling agent. The antibacterial preparation was made based on previous research [2] was found phenolic and flavonoid compounds with antibacterial properties in tamoenju leaf extract and antibacterial activity was observed before and after it was formulated into a gel preparation that can treat acne using S. aureus bacteria. The concentration of tamoenju extract was selected based on the results of previous studies. The parameters of this study include physical properties such as: organoleptic test, pH test, spreadability test, viscosity test, stability test, and antibacterial test against S. aureus.

MATERIALS AND METHODS

Materials
Tamoenju leaves, 96% ethanol (onplus), HPMC (Indo Sukses Pratama, Indonesia), propylene glycol (Dow Chemical Pacific, Singapore), methylparaben (IDA Nacharam, India), nutrient agar (NA) (merck), and distilled water. All materials were pharmaceutical grade.

Preparation of extracts
To obtain the ethanol extract (EE), 3 kg of powdered samples were macerated with 96% ethanol for 5 times in 24 h. The mixture was then evaporated using a rotary evaporator. Warm distilled water (1:1) was used to eliminate the chlorophyll content from 300 g of crude extract of tamoenju leaves. The goal was to tie and separate the chlorophyll and produce an ethanol-water remove [3].

Preparation of gel formulation
Following the weighing of each component, the HPMC was thoroughly mixed with CO2-free distilled water to create a clear liquid with a somewhat thick consistency. After being dissolved in propylene glycol, methylparaben was added and homogenised by grinding. In a mortar, the mixture is added gradually and mixed until smooth. Extract from tamoenju leaves was added once the base had formed, and it was then thoroughly mixed. Sealed and enclosed in a tube.
Physical evaluation of tamoenju leaves gel

Organoleptic test

Organoleptic test is a test technique that measures a product’s acceptability primarily through the use of human senses. An essential part of applying quality is organoleptic testing. For four weeks, the gel preparation was physically assessed, with observations made every week to assess its consistency, colour, and odour. Gel preparations that were kept at room temperature were used for this observation [9].

Homogeneity test

Two glass slides or other appropriate transparent materials were coated with a specific quantity of the sample. There should be no discernible coarse grains and a nice arrangement of the preparation [9].

pH test

1 gram of the sample was weighed, and it was then placed in a beaker with thirty millilitres of CO2-free distilled water and stirred until it was dissolved. Analysed with a pH metre by submerging the cathode and anode in the solution viewing the data on the LCD screen until the drift mark [8].

Spreadability test

A one-gram gel sample was positioned in the middle of two glass plates, and a counterbalance weight was added to the upper glass plate to make the total weight of 150 grammes. Up until the gel's spreading diameter stabilises, measurements are made. Spreadability that is good is between 5-7 cm [9].

Adhesiveness test

One object glass was coated with 0.25 g of gel, and another object glass was placed on top of it. Then subjected to a 5-minute 5-kilogram press. The next object, a glass, was fastened to a test apparatus that was subjected to an 80 g load. It was noted how long it took for the two object glasses to separate [10]. A minimum of 4 seconds is required for good adhesiveness [11].

Viscosity test

The Brookfield Viscometer, which is frequently used for semisolid formulations, has a spindle size of 6. It is used to measure viscosity. Room temperature was used for the measurement [8].

Antibacterial activity test

Utilizing the agar dispersion technique, the antibacterial adequacy of the ethanol concentrate of tamoenju leaves gel against S. aureus was explored. The test medium was made up of two layers of agar media. To make the establishment layer, 15 ml of NA was added to each Petri dish and left to solidify. The next layer, 15 ml of NA was added to each petri dish to form a well that will be used in the bacterial test. The antibacterial gel derived from tamoenju leaves underwent a minor modification prior to the freeze-thaw stability test. The gel was sealed in a container and stored at room temperature (25±2°C), hot temperature (40±2°C), and cold temperature (-4±2°C) for a total of 36 d (4 cycles). Throughout this period, the relative humidity (RH) was kept at 75±2%. The stability tests for the formulation included organoleptic, pH, spreadability, viscosity, adhesiveness, and bacterial inhibition [12, 13].

RESULTS AND DISCUSSION

Organoleptic test

An organoleptic test describes the preparation by means of direct observation. For 36 d, organoleptic observations were made on antibacterial gel preparations made from tamoenju leaves. The thick texture, distinct scent of tamoenju leaves, and blackish-green colour of the gel formulae were revealed by the organoleptic visualisation of the gel appearance. The tamoenju leaves gel preparations’ consistency decreased after storage, but their colour and odour remained unchanged (fig. 1).

Table 1: Gel formulation of tamoenju leaves extract

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Function</th>
<th>F1(%)</th>
<th>F2(%)</th>
<th>F3(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tamoenju leaves extract</td>
<td>Active ingredients</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>HPMC</td>
<td>Gelling agent</td>
<td>1</td>
<td>1.5</td>
<td>2</td>
</tr>
<tr>
<td>Propylene Glycol</td>
<td>Thickening agent</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Methyl Paraben</td>
<td>Preservative</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Distilled water</td>
<td>Solvent</td>
<td>ad 100</td>
<td>ad 100</td>
<td>ad 100</td>
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</tbody>
</table>

6 holes using a tube whose diameter is adjusted like a disc, and the distance is adjusted so that the observation area does not overlap. The 0.1 ml bacterial suspension was mixed into the NA culture medium. Then 15 ml of NA was poured into each petri dish, which was placed as a second layer as a backup. After the second layer has solidified, the backing is removed aseptically using tweezers from each petri dish to form a well that will be used in the bacterial test.

Test solution concentration 2.5; 5; 10; and 20% w/v (loading dose 0.025; 0.05; 0.1 and 0.2 mg/µl) control negative (DMSO) and positive control (chloramphenicol) as much as 50 µl put into wells and incubated at 37 °C for 24 h. The clear zone formed was measured using a caliper. The test was carried out in 3 repetitions and incubated at 37 °C for 24 h. The clear zone formed was measured using a caliper. The test was carried out in 3 repetitions and incubated at 37 °C for 24 h. The clear zone formed was measured using a caliper. The test was carried out in 3 repetitions and incubated at 37 °C for 24 h. The clear zone formed was measured using a caliper. The test was carried out in 3 repetitions.
Fig. 1: The organoleptic test results for tamoenju leaves gel before and after storage, from left to right, F1, F2, and F3

Homogeneity test
The goal of homogeneity testing was to observe how the particles were distributed throughout the gel preparation. The absence of coarse grains in the gel preparations during the 36-day observation period on the tamoenju leaves gel suggested that homogeneity had not changed (fig. 2).

pH test
To ascertain the safety level of the gel preparation, a pH test was conducted. The standard (SNI No. 06-2588) states that the pH value is between 4.5 and 6.5 [14]. The preparation’s pH dropped, but it still matched the requirements of normal skin pH, according to observations made on days 1 and 36. The best kind of preparation is one that leaves the skin feeling soothed. The preparation may irritate the skin if it is overly acidic. On the other hand, dry skin may result from an excessively alkaline pH.

Spreadability test
The purpose of the spreadability test was to evaluate the preparations’ capacity to apply to the skin with ease. Greater skin contact surface area and optimal absorption of active ingredients are directly correlated with ease of application. A good gel preparation has a spreading power up to 5-7 cm in diameter. After 36 d of storage, the spreadability of the gel increased in all formulations. F2 and F3 still met the requirements, while F1 exceeded the test parameter value [15].

Fig. 2: The result of homogeneity test of tamoenju leaves gel (a) before and (b) after storage, from left to right F1, F2, F3

Fig. 3: The result of pH test of tamoenju leaves gel before and after storage (mean±SD, n=3)

Fig. 4: The result of the spreadability test of tamoenju leaves gel before and after storage (mean±SD, n=3)
Adhesiveness test

Gel preparations underwent an adhesiveness test to ascertain the amount of time needed for the gel to stick to the skin, as it significantly affects the effectiveness of the preparations in delivering therapeutic effects. The required adhesion time is more than 4 seconds [16]. After 36 d of storage, the adhesion of all gel formulations decreased. However, all formulas still met the requirements.

Viscosity test

Brookfield viscometer, a rotating type viscometer with a cylindrical rotor (spindle) submerged in the gel of tamoenju leaves, was used to perform the viscosity test. The consistency of a gel preparation is ascertained through the use of viscosity testing. The resistance of a fluid to flow is measured by its viscosity. The preparations get thicker with increasing viscosity. For gel formulations, the viscosity range of 2000–4000 cps is ideal [17]. After 36 d of storage, the viscosity of all formulations decreased. However, only F3 still met the requirements. When applied to the skin, F2 and F3 still maintained a comfortable consistency. The decrease in viscosity affects the increased spreadability and decreased adhesion of the gel preparations.

Antibacterial activity test

To conduct the antibacterial activity test, the inhibition zone was measured. Using the inhibition zone measurement, the gel preparations’ antibacterial potential was evaluated. The following criteria are used to determine the strength of an antibacterial agent: 5 millimetres or less is considered weak, 5 to 10 mm, 10 to 20 mm, and 20 mm or more very strong [18]. There was a reduction in the inhibition zone following storage. F3 belongs in the moderate category, whereas F1 and F2 are in the strong category.

The antibacterial activity test results showed that the test bacterium, S. aureus, was unable to grow in the tamoenju leaves gel. The study’s conclusions effectively demonstrated the tamoenju leaf gel’s antibacterial qualities in the management of acne. Table 2 displays the inhibition zone measurement results.

<table>
<thead>
<tr>
<th>Formula</th>
<th>H-1 (mm)</th>
<th>H-36 (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>11.22±0.505</td>
<td>10.02±0.308</td>
</tr>
<tr>
<td>F2</td>
<td>10.76±0.365</td>
<td>10.57±0.508</td>
</tr>
<tr>
<td>F3</td>
<td>10.49±0.069</td>
<td>9.56±0.101</td>
</tr>
<tr>
<td>Benzolac gel (+)</td>
<td>12.96±0.09</td>
<td>-</td>
</tr>
<tr>
<td>Gel base (-)</td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>
Result of this study compared with similar study from Borse [19] using HPMC as a gel base, neem oil and tea tree oil as active agent for acne; there are differences in the viscosity values and inhibition zone for S. aureus bacteria. This difference is due to the higher concentration of HPMC and different active substances to produce different zones of inhibition, while the pH has same value according to the face pH range. Test of spreadability and stickiness compared with the study from Pawestri [20] using HPMC base and ginger extract as an active ingredient are different because the extract uses a different solvent where ginger uses water as solvent while tamaoenju leaves use ethanol, which causes the consistency of the preparation become thicker.

On the first day of preparation, there were no massive contrasts between the recipes according to statistical analysis using one-way ANOVA, with Sig<0.005. The statistical analysis of t students revealed that while there was no massive contrast in the pH test after capacity. In terms of spreadability, there were notable variations between the formulas (Sig<0.005). Only F1 and F3 displayed a statistically significant variation in spreadability, viscosity, and inhibition activity following a 36-day period of storage. F2 revealed absolutely no discernible difference.

CONCLUSION
Tamaoenju leaves extract (Hibiscus surattensis L) can be formulated into gel preparations. Gel preparations containing concentration of 1, 1.5, and 2% HPMC as gelling agent shows good physical stability into gel preparations. Gel preparations containing concentration of variations between the formulas (Sig<0.005). Only F1 and F3 revealed that while there was no massive contrast in the pH test between the recipes according to statistical analysis using one-way ANOVA, with Sig<0.005. The statistical analysis of t students revealed that while there was no massive contrast in the pH test after capacity. In terms of spreadability, there were notable variations between the formulas (Sig<0.005). Only F1 and F3 displayed a statistically significant variation in spreadability, viscosity, and inhibition activity following a 36-day period of storage. F2 revealed absolutely no discernible difference.

CONCLUSION
Tamaoenju leaves extract (Hibiscus surattensis L) can be formulated into gel preparations. Gel preparations containing concentration of 1, 1.5, and 2% HPMC as gelling agent shows good physical stability parameters on day 1 but changes after storage on day 36. After storage, F2 and F3 still met the requirement of test parameters. Staphylococcus aureus growth is most effectively inhibited by tamaoenju leaf extract gel F2. F2 (1.5% of HPMC) demonstrated the best formula among the formulations.

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CONFLICTS OF INTERESTS
Stated no conflict of interests

REFERENCES