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Antibacterial Activity of Acne Patch with the Addition of Sea Grapes (*Caulerpa racemosa*) Extract Against *Propionibacterium acnes*

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ABSTRACT Acne patch preparation is an acne medication that is used topically. Sea grape (*Caulerpa racemosa*) has the potential to contain antibacterial substances that can be applied to acne patch preparations to suppress the growth of acne-causing bacteria, *Propionibacterium acnes*. This research aims to determine the potential of *Caulerpa racemosa* antibacterial substances applied to acne patch preparations in suppressing the growth of bacteria that cause *Propionibacterium* acnes. The treatment used was the addition of different *Caulerpa racemosa* extracts (0%, 15%, 20%, and 25%). Parameters observed were pH, antibacterial, irritation, thickness, hedonic, moisture absorption, water absorption, and adhesion. The analysis showed that adding *Caulerpa racemosa* extract significantly affected antibacterial activity ($P<0.05$). The addition of extract with a concentration of 25% is the best treatment compared to other treatments, with an antibacterial activity value of 5.25 ± 0.35 mm with a weak inhibition zone category, pH 5.92±0.04, non-irritating, thickness 0.54±0.02 mm, hedonic getting somewhat like value (appearance, aroma, and texture), moisture absorption 4.47±0.03%, water absorption 1.16±0.02%, and adhesion 231.24±0.08 seconds.

Keywords: Acne patch; antibacterial; sea grape; *Propionibacterium acne*

INTRODUCTION

Acne sufferers in Indonesia are recorded at approximately 15 million people aged 13-40 (Puspitasari & Riyanto, 2016). The process of acne formation begins with an increase in androgen hormones that make the pilosebaceous unit activity abnormal, which has an impact on increasing the production of keratinocyte proliferation and sebum. The increased output of keratinocyte proliferation and sebum causes the skin to be oilier. Increased sebum production causes the sebaceous units to become clogged with dead skin cells, and sebum buildup occurs. These conditions are favorable for bacteria to colonize due to the lipid-rich and anaerobic conditions. Colonizing bacteria quickly triggers an immune and inflammatory response, resulting in acne (Januario *et al*., 2021). Acne forms when oil glands under the skin accumulate, clog follicles, and produce thick fat (Katarina, 2014). Acne can be caused by various internal and external factors, such as diet, hormonal changes, cosmetics, hygiene, and infection (Nurpriatna *et al*., 2024). Infections in the oil glands that cause acne are due to significantly increased bacterial activity (Soemarie *et al*., 2019). One of the bacterial infections that plays a role in acne formation is *Propionibacterium acnes* (Fitri *et al*., 2023).

Propionibacterium acnes is a typical microorganism found on human skin, especially the facial area, which grows relatively slowly, belongs to Gram-positive bacteria, and grows well in anaerobic conditions (Dewi *et al*., 2020). In acne formation, the pilosebaceous unit experiences anaerobic conditions that stimulate *P. acnes* bacteria to colonize the pilosebaceous unit. Colonization of *P. acnes* bacteria can be inhibited by natural bioactives, one of which is flavonoids (Choi & Chin, 2014). Sea grapes (*Caulerpa racemosa*) have bioactive compounds that can be a source of antibacterial compounds, including phenols, alkaloids, tannins, and flavonoids (Marfuah *et al*., 2018).

Sea grape (*Caulerpa racemosa*) is a macroalgae with green thallus and is widely distributed in the coastal waters of Indonesia. According to Darmawan *et al*. (2022), green seaweed *Caulerpa* sp. is known to be invasive in some areas due to its ability to grow quickly. KEPMEN-KP Number 1 of 2019 determines sea grapes as one of the five superior seaweed species to be cultivated. The utilization and innovation of sea grape commodities must be developed through technology and research (KKP, 2019). In the research of Suleman *et al*. (2023) on the antibacterial activity test of anti-acne ointment preparations of ethanol extract of sea grapes (*C. racemosa*) against *Staphylococcus aureus* bacteria, the best treatment of 20% sea grape extract (*C. racemosa*) produced an inhibition zone diameter of 11.1 ± 0.26 mm in the strong category. Sea grapes have high antibacterial potential because they contain flavonoids, tannins, phenolics, and carotenoids in large enough quantities (Sari *et al*., 2022). According to Hainil *et al*. (2022), sea grapes (*C. racemosa*) have high potential as a source of antibacterial bioactive compounds helpful in inhibiting bacterial growth in patients with infectious diseases.

Acne treatment can be done topically by administering drugs that have antibacterial content. Topical acne medication is usually available in lotions, gels, and creams (Nurpriatna *et al*., 2024). The production of acne patches is the latest development in acne treatment. The use of an acne patch in the treatment of acne in relatively small amounts is more effective because the type of use of acne patch is directly affixed to each area of the appearance of indications of acne and can prevent bacterial contamination due to the use of patches that cover the acne area (Yulianti *et al*., 2021). The advantages of using acne patches include protecting against and preventing the habit of pressing acne, as they are practical. Patch preparations can use transdermal patches, which administer drugs through the skin to cause controlled systemic effects (Wardani & Saryanti, 2021). The potential of sea grape extract (*Caulerpa racemosa*) can be innovated by making acne patch dosage formulations with the addition of sea grape extract (*C. racemosa),* which can be tested for antibacterial activity against *Propionibacterium acnes.*

MATERIALS AND METHODS

Materials

Sea grape (*Caulerpa racemosa*), 70% ethanol, carboxy methyl cellulose, methylparaben, propylene glycol, distilled water, agar count plate, and plaster, analytical balance (USS-DBS15-2), glass jar, sieve, measuring cup, Erlenmeyer, test tube, stirring rod, porcelain cup, oven (Memmert 30-1060), blender (Philips HR2106), rotary evaporator (IKA RV10), desiccator (IKA RW20), hot plate stirrer (MS H280 Pro), micrometer (QST008), autoclave (YX-18 LDJ), bunsen, incubator (Memmert In 55), petri dish, micropipette (Tricle Brand), laminar airflow (Esco LHS 2AG), and pH meter (AC P-2Z).

Methods

Sample processing

Referring to the research of Luhulima *et al.* (2022) with the modification of sea grapes (Caulerpa racemosa) that have been collected and cleaned of dirt, then washed thoroughly with running water, and after being cleaned, chopped, and dried using an oven at 50 °C for 72 hours, [A1] then mashed into simplistic.

Extract preparation

The extraction process referred to the research of Luhulima *et al*. (2022), which was modified using 300 g of sea grape (*Caulerpa racemosa*) simplistic powder, which was then put into a glass jar. It was extracted by the maceration method for 3 x 24 hours using 70% ethanol [A2] [A3] solvent, as much as 900 mL, which was stirred every 6 hours. The extract was filtered using a sieve, and the filtrate obtained was evaporated solvent using a rotary evaporator.

Acne patch preparation

Table 1. Formulation of acne patch preparation (Nurpriatna *et al*., 2024) with modifications.

Composition	Formulation				Usability
	F0 (%)	F1 (%)	F2 (%)	F3 (%)	
Seagrape extract	-	15	20	25	Active substances
Na CMC	3	3	3	3	Polymer/base
Methylparaben	0,3	0,3	0,3	0,3	Preservatives
Propylene glycol	10	10	10	10	Enhancer
Ethanol (70%)	40	40	40	40	Solvent
Lemon oil	1	1	1	1	Fragrance
Aquades	Add 100	Add 100	Add 100	Add 100	Solvent

Making acne patch preparations, as referred to in the research of Nurpriatna *et al*. (2024), starts with preparing varying concentrations of active substances. *Caulerpa racemosa* crude extract [A1] was mixed with 70% ethanol according to the predetermined treatment levels (0%, 15%, 20%, and 25%). Na CMC was then developed with distilled water using a hot plate stirrer at 70 °C until homogeneity was achieved. Methylparaben was dissolved in propylene glycol. Mixed and homogenized in a measuring cup using a stirrer, then added methylparaben solution and homogenized again. Ethanol and lemon oil were added to the mixture, followed by distilled water until the volume reached 100 mL, and then the mixture was homogenized again. The mixture was poured into petri dishes as much as ±10 g and put into the oven at 50 °C for 6 hours. After drying, the mixture was kept in a desiccator for 20 hours and could be molded.

Test methods

pH test

The patchy test sample was developed as much as 3 grams for 2 hours at room temperature in a porcelain cup containing 30 mL of distilled water, and the pH level was measured using a pH meter. Topical preparations such as patches have an average standard pH of 4-8 (Mariadi & Bernardi, 2023).

Antibacterial test

Antibacterial tests were carried out using the diffusion method on PCA (Plate Count Agar). Seven grams of PCA agar media were dissolved in 250 mL of distilled water, boiled in a sealed Erlenmeyer flask, and sterilized in an autoclave at 121 °C for 15 minutes. About 15 mL of PCA agar solution was poured into a petri dish. Propionibacterium acnes test bacteria were taken using a sterile ose needle and suspended in a tube containing 3 mL of physiological solution until a turbidity corresponding to McFarland 0.5 standard solution was obtained. Using a cotton swab, the solidified PCA agar media was then streaked with bacterial suspensions. 6 mm patch samples were then placed on the surface of the PCA agar media aseptically. After that, the media were incubated at 37 °C for 24 hours. The diameter of the resulting inhibition zone was measured vertically and horizontally (Nurpriatna *et al*., 2024).

Irritation test

The irritation test was conducted on the skin of volunteer panelists using the closed patch test method—the number of panelists in the irritation test was four people of different genders and skin types. The patch is affixed to the back of the panelist's ear for approximately 12 hours with repetition, if a positive reaction can be characterized by redness, itching, and swelling on the skin where the test sample is placed (Rahim *et al*., 2016).

Thickness test

Patch preparations are expected to have the same thickness. To ensure this, patches from each formula were measured for thickness on one side using a micrometer. The physical characteristics of the patch significantly affect comfort when used; the thinner the preparation, the easier it is to use (Nurpriatna *et al*., 2024).

Hedonic test

The hedonic test procedure was carried out to determine the level of panelists' liking for the product. Panelists were asked to rate the acne patch preparation, including aroma, appearance, and texture parameters. This test involved 30 non-standard panelists rated on a scale of 1 to 9 (SNI 2346-2015).

Moisture absorption test

Patches were weighed and stored in a desiccator for 24 hours, then oven at 40°C for 24 hours, and weighed to determine the final weight of the patch (Wardani & Saryati, 2021).

$$\text{Moisture absorption (\%)} = \frac{\text{wet weight} - \text{dry weight}}{\text{dry weight}} \times 100\%$$

Water absorption test

Weigh the acne patch preparation as dry weight. The acne patches were soaked in distilled water for 10 seconds, after which the wet weight was weighed (Fadilla *et al*., 2023).

$$\text{Water absorption (\%)} = \frac{\text{wet weight} - \text{dry weight}}{\text{dry weight}} \times 100\%$$

Adhesion test

The adhesion test was carried out by placing a preparation of 1 sample size 6 mm on a glass object, then overlapping it using another glass object to merge the two glasses. A load weighing 1 kg was placed on the top flat glass and allowed to stand for 5 minutes. The weight on top of the flat glass was lifted, and a total weight of 80 g on the bottom flat glass was dropped to pull the flat glass together. The time the two glasses broke apart was recorded (Sukamdi *et al*., 2024).

Data analysis

Data analysis was processed using the IBM SPSS Statistics 2.0 application with a 95% confidence interval. Hedonic test data were analyzed using the Kruskal-Wallis test to determine significant parameter differences. The results will be significantly different and will be continued with the Mann-Whitney test. The pH test, antibacterial test, thickness test, irritation test, adhesion test, moisture absorption test, and water absorption test will be analyzed using the ANOVA test, followed by Duncan's further test if there is a significant difference. The analysis results will be displayed in graphs and tables and explained descriptively.

RESULTS AND DISCUSSION

pH test

pH testing on acne patch preparations is carried out to determine the value of the degree of acidity measured using a pH meter. The pH value of acne patches with the addition of different sea grape extracts can be seen in Figure 1.

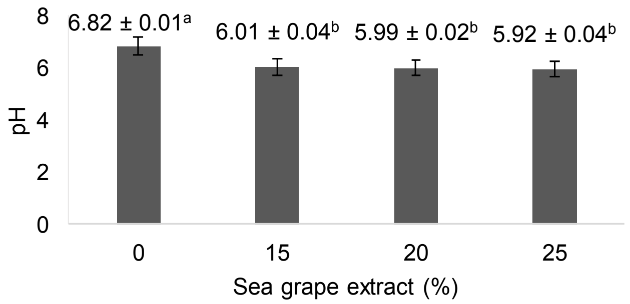

Figure 1. Acne patch pH value.

The highest pH result was in the 0% treatment without adding sea grape extract, with a pH value of 6.82. According to SNI 06-3736-1995, the standard pH value of sodium carboxymethyl cellulose (Na CMC) preparations can indicate an increase in the pH of acne patch preparations. The range of pH values in topical or transdermal preparations is between 4.5 and 8.0 (Budi & Rahmawati, 2019). The highest pH value in acne patch preparations without adding sea grape extract (*C. racemosa*) is included in the standard pH range in topical or transdermal preparations. According to A'yun *et al*. (2020), the pH range in human skin is 4.5 - 6.8, which, if adjusted to the skin's pH, all treatments fall into the normal pH range of the skin. The lowest value is in the treatment of adding 25% sea grape (*C. racemosa*) extract, which has a pH value of 5.92. The pH value of the acne patch preparation added with sea grape extract (*C. racemosa*) can be reduced; the higher the concentration of sea grape extract (*C. racemosa*) given, the lower the acne patch preparation pH value, although the difference is insignificant. The decrease in pH is caused by sea grape extract (*C. racemosa*), which tends to have a pH of around 5-6 or slightly acidic to neutral (Satyantini *et al*., 2024).

Antibacterial test

An antibacterial test on acne patch preparation aims to determine the inhibition zone produced on Propionibacterium acnes bacterial colonies. Table 1 and Figure 2 show the antibacterial value of acne patches with the addition of different sea grape extracts.

Table 1. Zone and inhibition response of acne patch against *P. acnes* bacteria.

Concentration (%)	Inhibition zone diameter (mm)	Growth inhibition response
0%	2.75 ± 0.35[a]	Weak
15%	4.12 ± 0.17[b]	Weak
20%	5.25 ± 0.35[c]	Weak
25%	5.57 ± 0.17[ab]	Weak

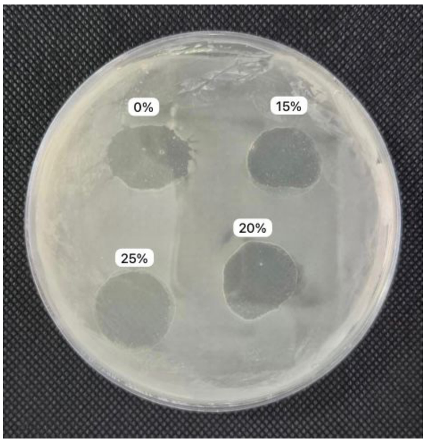

Figure 2. Zone of inhibition of acne patch against *P. acnes* bacteria.

The test was conducted on 0% treatment to determine whether the base formula had antibacterial activity. The 0% treatment obtained the lowest inhibition zone value of 2.75 mm with a weak inhibition zone category. According to Qisti *et al*. (2019), propylene glycol can provide antibacterial activity by providing a dehydrating effect on the bacterial cell membrane; this effect can work well at concentrations of more than 20%. In the acne patch preparation formula, the propylene glycol given is 10% so that it can provide an inhibition zone, even though it has a lower value than the addition of 15%, 20%, and 25% sea grape extract.

Table 1 shows the inhibition zone produced by acne patch preparations by adding sea grape extract (*C. racemosa*). The higher the concentration of extract added to the test sample, the higher the inhibition zone produced against *P. acnes* bacteria. In line with the research results by Luhulima *et al*. (2022), the increase in extract concentration is in line with antibacterial activity. The 25% concentration with the highest value of 5.57 mm is included in the category of weak antibacterial strength. According to Nurfadilah *et al*. (2023), the inhibition zone category in antibacterial tests, when less than five mm, is included in the weak inhibition zone category. The inhibition zone produced at all concentrations (0%, 15%, 20% and 25%) received a weak inhibition zone category. This can be caused by *P. acnes* bacteria that have resistance mechanisms and protect themselves from antibacterial compounds in the form of biofilms, which prevent the penetration of active substances into *P. acne* cells to be inhibited (Coenye & Nelis, 2010). Therefore, acne patch preparations with formulas that include the addition of sea grape extract in the concentration range are not optimal for providing an inhibition zone on *P. acnes* bacterial colonies.

Irritation test

The irritation test of acne patch preparations with the addition of sea grape extract (*Caulerpa racemosa*) was carried out to determine and confirm whether the acne patch preparation formulation was irritant, with reactions such as skin redness, itching, and swelling on the skin of volunteers. The results of the irritation test on volunteers showed different results, which can be seen in Table 2.

Table 2. Irritation test results on acne patches with different additions of sea grape extracts.

Concentration	Results		
	Redness	Swelling	Itching
0%	(-) negative	(-) negative	(-) negative
15%	(-) negative	(-) negative	(-) negative
20%	(-) negative	(-) negative	(-) negative
25%	(-) negative	(-) negative	(-) negative

The reactions caused in volunteers resulted in negative overall results of redness, swelling, and itching. According to Andrini (2023), humans have different skin types. The skin's protective layer is less effective in dry skin, so it is prone to irritation compared to oily or normal skin. Another influence on skin irritation reactions is pH, which can irritate the skin if the topical preparation is too acidic. If it is too alkaline, it can cause dry skin (Yulin, 2015).

Thickness test

The test value of the thickness of acne patch preparations in all treatments (0%, 15%, 20%, and 25%) is less than 1 mm. According to Arifin *et al*. (2019), physical parameters such as the thickness of the acne patch preparation affect the acceptance of its use; if the patch is of the appropriate thickness, its use will be easier. The optimal thickness for an effective acne patch formulation is around 1 mm, since this facilitates the even distribution of the active ingredient and significantly enhances user comfort during application. The thickness of the patch is affected by the base or polymer used, such as Na CMC, which can increase the thickness of the patch if the concentration of its use is higher (Nurpriatna *et al*., 2025).

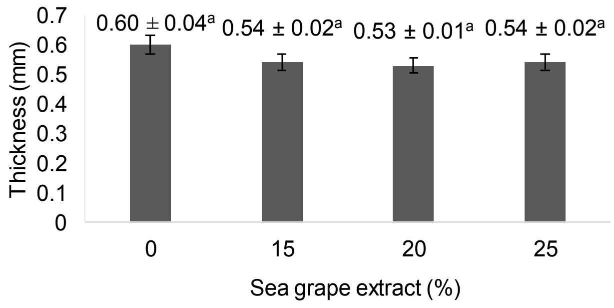

Figure 3. Acne patch thickness value.

Appearance

The appearance value of 0% concentration got the highest score of 7.08, which means that if adjusted to the hedonic test assessment category, it is in the range of liking level. The appearance at 0% concentration has a higher appearance value than 15%, 20%, and 25% concentrations, which tend to have a lighter color. The 0% concentration has a transparent color, which is influenced by the formulation or constituent ingredients used in acne patch preparations.

The addition of sea grape extract (*Caulerpa racemosa*) 25% got the lowest score compared to the concentrations of 0%, 15%, and 20%. 25% concentration has a value of 4.95 in the hedonic assessment category, which is included in the category of neutral level range. The results of the appearance test of the Concentration of the addition of sea grape extract (C. racemosa) at the 25% level received a greater value than the other treatments (0%, 15%, and 20%). Sea grapes (*C. racemosa*) have chlorophyll a (10.08 - 17.4 µg/mL) and chlorophyll b (15.2 - 31.2 µg/mL) green pigments, which affect the color of acne patch preparations (Sarojini *et al*., 2015).

Aroma

The aroma value at 0% concentration is 6.20 (somewhat like), which is the highest compared to other concentrations (15%, 20%, and 25%). The similarity of values in all concentrations (0%, 15%, 20%, and 25%) is due to adding 1% lemon oil as a fragrance agent in this formulation. Using fragrances, such as lemon oil, in topical preparations can induce a sense of comfort through scent. (Fauziah *et al*., 2020).

The 25% concentration gets the lowest average value of 4.75, which means that if categorized in the hedonic assessment, it is included in the neutral level range category. The aroma of acne patch preparations is affected by the aroma of sea grapes (*C. racemosa*), which have a fishy aroma typical of seaweed caused by amino acids contained in sea grapes (*C. racemosa*) (Puspita *et al*., 2019). Incorporating lemon oil is expected to reduce the characteristic odor of sea grapes (*C. racemosa*), enhancing the scent experience of acne patch formulations (Noor *et al*., 2023).

Texture

The texture parameters of acne patch preparations are smooth, flat, and sticky. These results are from the research of Nurpriatna *et al*. (2024), who found that using Na CMC in making acne patch preparations results in an elastic and thin shape. The addition of sea grape extract (*C. racemosa*) affects the elasticity of the preparation. Increased concentrations of sea grape extract (C. racemosa) enhance the elasticity of the acne patch formulation.

The value of the texture parameter of acne patch preparations in the hedonic test falls into the same category in each treatment (0%, 15%, 20%, and 25%), namely in the level range (somewhat like). According to SNI 06-3736-1995, Na CMC functions as a thickener and emulsifier, which can affect the texture of acne patch preparations.

Table 3. Hedonic value of acne patches with different concentrations of sea grape extract.

Parameters	Seagrape extract			
	0%	15%	20%	25%
Appearance	7.08±1.47a	5.48±1.56b	5.35±1.72b	4.95±1.83b
Aroma	5.97±1.40a	6.20±1.20a	5.70±1.18a	4.75±1.39a
Texture	6.28±1.60a	5.97±1.28a	5.75±1.51a	5.89±1.42a

Moisture absorption test

The moisture absorption test on acne preparations aims to determine their resistance to moisture absorption, which affects shelf life. Figure 4 shows the value of the moisture absorption test analysis of acne patch preparations with the addition of different sea grape extracts.

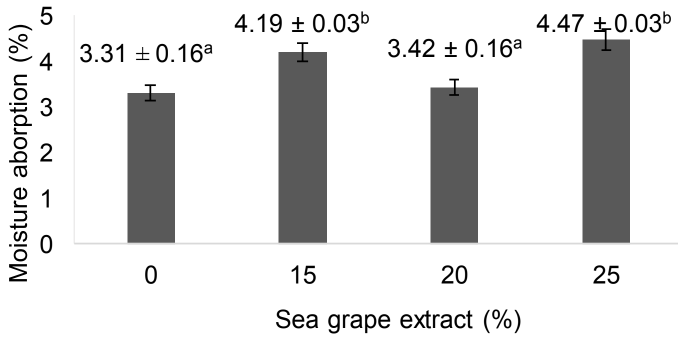

Figure 4. Moisture absorption test results.

According to Novia (2021), a good moisture absorption value for acne patch preparations ranges less than 10%. The results of moisture absorption testing on acne path preparations with the addition of sea grape extract (*Caulerpa racemosa*) at all concentrations of sea grape extract addition (0%, 15%, 20%, and 25%) have values less than 10%. The 0% and 20% concentrations were significantly different from the 15% and 25% concentrations due to the non-uniform weight of the patch. When the patch has a higher weight, the polymer content contained therein is higher than that of the lighter-weight patch. Polymers and enhancers influence the value of moisture absorption in acne patch preparations in the formulation used; the higher the percentage, the higher the moisture absorption value (Nandi & Mondal, 2022). Patches with low moisture absorption values will be more stable and less fragile during long-term storage.

Water absorption test

Water absorption testing is carried out on acne patch preparations to determine the percentage of water absorption from acne patches dipped in distilled water. The value of the results of the water absorption test analysis of acne patch preparations can be seen in Figure 5.

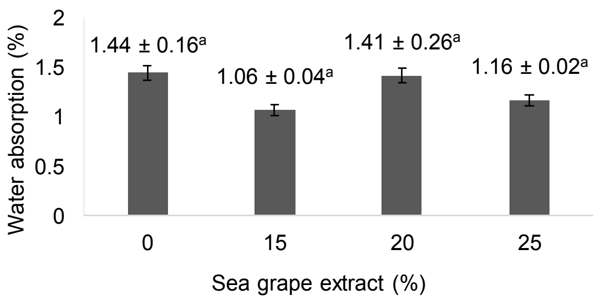

Figure 5. Water absorption test results.

The water absorption value in acne patch preparations is relatively low, at 1.06 - 1.44%. According to Wardani & Saryanti (2021), Excessive water absorption in patches can compromise quality and diminish flexibility, leading to increased susceptibility to tearing. Elasticity and water absorption in acne patch preparations can be influenced by the polymer, Na CMC, which is hygroscopic (Nurpriatna *et al*., 2024). The results of the water absorption value in all treatments showed no differences or changes in the stable period.

Adhesion test

The purpose of the adhesion test on acne patch preparation is to determine the patch's ability to stick and not be easily separated when used. Table 4 shows the value of the analysis of the adhesion test of acne patch preparations.

Table 4. Adhesion test value.

Concentration (%)	Adhesion test value (sec)
0%	3.19±0.63[a]
15%	139.76±0.97[b]
20%	190.37±0.08[c]
25%	231.24±0.07[d]

The adhesion of acne patch preparations with the addition of sea grape extract (*Caulerpa racemosa*) has the highest value at a concentration of 25%, namely, for 231.24 seconds. The value of adhesion sequentially increases with the addition of sea grape extract (*C. racemosa*), which is due to the nature of the active substance produced, which is physically liquid and yellowish green in color, and the use of polymers in the formulation, so that the resulting patch becomes elastic (Nurpriatna *et al*., 2024). The higher the addition of sea grape extract (*C. racemosa*), the more elastic the patch preparation. The more elastic the patch preparation, the stronger the adhesion because it provides higher spreadability on flat glass. The acne patch preparation can adhere well to flat glass and allows it to adhere to human skin so that the active substance can be absorbed into human skin (Anggriani *et al*., 2017).

CONCLUSION AND RECOMMENDATION

Conclusion

Acne patch preparations with sea grape extract (Caulerpa racemosa) can inhibit the growth of Propionibacterium acnes bacterial colonies, one of the bacteria that cause acne. The zone of inhibition produced at a concentration of 0% 2.75 ± 0.35 mm, 15% 4.12 ± 0.17 mm, 20% 5.25 ± 0.35 mm, and 25% 5.57 ± 0.17 mm. The higher the concentration of sea grape extract added, the greater the increase in the zone of inhibition on bacterial colonies P. acnes. The 25% concentration of added sea grape extract (C. racemosa) is the best treatment with an antibacterial activity value of 5.57 ± 0.17mm, including the category of weak inhibition zone. The characteristic test results obtained a thickness value of 0.54 ± 0.02mm, moisture absorption of 4.47 ± 0.03%, water absorption of 1.16 ± 0.02%, and adhesion of 231.24 ± 0.07 seconds. The hedonic value gets a similar value, including appearance, aroma, and texture. The pH value of 5.92 ± 0.04 is safe for human skin and does not irritate, according to SNI.

Recommendation

Uniformity testing of physical parameters and further research are needed on acne patch preparations that add other active substances or combine active substances from sea grape extract with other active substances to obtain better antibacterial value.

AUTHORS' CONTRIBUTIONS

FM as an idea bearer, researcher, writer, data analyst, and script preparation; ANH as writer and script preparation, and GP as a writer and script preparation.

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