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Formulasi dan Evaluasi Aktivitas Antibakteri Krim Daun Impatiens Balsamina terhadap Propionibacterium acnes

Formulation and Evaluation of Antibacterial Activity of Impatiens balsamina Leaf Cream Against Propionibacterium acnes

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Abstrak

Penanganan jerawat dapat dilakukan dengan pemberian antibiotik secara oral maupun topikal. Sediaan topikal memiliki keunggulan waktu kontak yang lebih lama dengan permukaan kulit, sehingga memungkinkan zat aktif terserap secara optimal. Salah satu alternatif yang dapat digunakan untuk mencegah resistensi bakteri adalah dengan memanfaatkan senyawa metabolit sekunder yang terdapat pada tumbuhan. Henna air (Impatiens balsamina) merupakan salah satu tumbuhan yang memiliki aktivitas antibakteri terhadap Propionibacterium acnes. Penelitian ini bertujuan untuk menentukan formulasi, evaluasi dan uji aktivitas antibakteri sediaan krim ekstrak etanol daun pacar air. Penelitian ini diawali dengan preparasi ekstrak menggunakan metode maserasi, kemudian dilakukan pengujian aktivitas antibakteri ekstrak dan optimasi basis. Basis dievaluasi meliputi uji organoleptik, homogenitas, pH, daya sebar, daya lekat, viskositas. Basis terbaik dipilih untuk memformulasi ekstrak daun pacar air menjadi bentuk sediaan krim. Krim diformulasikan dengan variasi konsentrasi ekstrak 20%, 25% dan 30%. Uji aktivitas antibakteri dilakukan dengan metode difusi cakram (Kirby-Bauer). Hasil penelitian menunjukkan bahwa semua formula memiliki aktivitas antibakteri dengan kategori kuat terhadap Propionibacterium acnes, dengan daya hambat berturut-turut sebesar F1 (11,07 mm), F2 (11,67 mm), dan F3 (12,05 mm). Berdasarkan evaluasi fisik, semua formula secara umum memenuhi persyaratan, meskipun F3 tidak homogen dan daya sebarnya belum memenuhi persyaratan.

Kata Kunci: *Antibakteri, Krim, Impatiens balsamina, Propionibacterium acnes*

Abstract

Treatment of acne can be done by administering antibiotics orally or topically. Topical preparations have the advantage of longer contact time with the skin surface, allowing the active substance to be absorbed optimally. One alternative that can be used to prevent bacterial resistance is to utilize secondary metabolite compounds found in plants. Water henna (Impatiens balsamina) is one of the plants that has antibacterial activity against Propionibacterium acnes. This study aims to determine the formulation, evaluation and test the antibacterial activity of cream preparations of ethanol extract of water henna leaves. This research begins with the preparation of extracts using the maceration meth od, after which testing the antibacterial activity of extracts and base optimization is carried out. The base was evaluated including organoleptic test, homogeneity, pH, spreadability, adhesiveness, viscosity. The best base was selected to formulate the water henna leaf extract into a cream dosage form. The cream was formulated with variations of 20%, 25% and 30% extract concentrations. Antibacterial activity test was conducted using disc diffusion method (Kirby-Bauer). The results showed that all formulas had antibacterial activity with a strong category against Propionibacterium acnes, with inhibition power successively equal to F1 (11.07 mm), F2 (11.67 mm) and F3 (12.05 mm). Based on physical evaluation, all formulas generally meet the requirements, although F3 is not homogeneous and its spreadability has not met the requirements...

Keywords: *Antibacterial, Cream, Impatiens balsamina, Propionibacterium acnes*

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BACKGROUND

One of the most common skin problems is acne (acne vulgaris), which can occur in all people but generally affects adolescents to young adults. The prevalence of acne in adolescents in Indonesia reaches around 80-85%, with a peak incidence at the age of 15-18 years. The main causes of acne can be caused by several factors such as excess sebum production, clogged pores due to dead skin cells, hormonal changes, environmental factors, and bacterial infection caused by Propionibacterium acnes. Propionibacterium acnes causes acne by producing lipase enzymes that convert unsaturated fatty acids into saturated fatty acids, resulting in dense sebum. Increased sebum production will trigger the growth of these bacteria in the sebaceous glands. To inhibit its growth, antibacterial compounds can be used, which are substances capable of killing or inhibiting the growth of pathogenic bacteria. However, excessive and long-term use of antibacterials can cause bacterial resistance (Widiastuti et al., 2023).

Based on the statement of Sintya et al (2023), acne treatment can be done through the administration of antibiotics both orally and topically, such as clindamycin, tetracycline, and erythromycin. However, the use of these antibiotics can cause side effects such as skin irritation and the risk of causing bacterial resistance if not used properly. One alternative to prevent and inhibit bacterial resistance is to utilize secondary metabolite compounds found in plants. Impatiens balsamina is one of the plants known to have antibacterial activity against Propionibacterium acnes. In a study conducted by Octora and Waruwu (2022), ethanol extract of water henna leaves showed strong antibacterial activity against P. acnes at concentrations of 0.25 mg/mL, 0.5 mg/mL, and 0.75 mg/mL, with inhibition zone diameters of 11.46 mm, 12.06 mm, and 17.96 mm, respectively. This activity shows the potential of water henna as an anti-acne agent, which is caused by the content of active compounds such as flavonoids and quinones. Flavonoids that are thought to play a role include quercetin and kaempferol, where quercetin inhibits the enzymes DNA gyrase and ATPase in the process of DNA replication, while kaempferol is thought to have a similar mechanism due to the similarity in structure with quercetin. The good antibacterial activity of this water henna plant shows. it is potential to be used as an anti acne. One of the many dosage forms on the market as an antacne is in the form of a cream. Cream is a semi-solid dosage form containing one or more medicinal ingredients dissolved or dispersed in a suitable base material.

Cream is one of the most commonly used topical dosage forms in the pharmaceutical and cosmetic world. Cream has a semi-solid texture consisting of an oil-in water (M/A) or water-in-oil (A/M) emulsion, which serves to deliver active ingredients to the skin surface (Depkes RI, 2020). This study aims to determine the formulation, evaluation and test the antibacterial activity of cream preparations of ethanol extract of water henna leaves.

METHODS

Tools and Materials

The tools used in this study include an analytical balance (Osuka), ohaus balance, Brookfield viscometer (DV-ETM), centrifuge (Centurion Scientific), autoclave (ES-315), stirring rod, porcelain cup, maceration container, blender, rotary evaporator (RV 10 digital V), measuring cup, beaker, pipette, test tube, petri dish, ose needle and spatula. The materials used in this study include distilled water, ethanol extract of water henna leaves (Impatiens balsamina), 96% ethanol, 70% ethanol, liquid paraffin, stearic acid, cetyl alcohol, glycerin, TEA, a-tocopherol, methyl paraben and propyl paraben, nutrient agar (Himedia, India), nutrient broth (Himedia, India), paper disc (Macherey Narey, Germany), NaCl 0.9%, erythromycin cream (PT. Surya Dermato Medica Laboratories, Indonesia), sudip, and spatula.

Procedur

Sample Processing

Henna leaves (Impatiens balsamina) obtained from Mongiilo Village were wet sorted, washed using running water to remove dirt. Cleaned samples were cut into small pieces and then dried covered with a black cloth under direct sunlight. Samples that have been dried are sorted dry and then continued with the process of making powder using a blender.

Preparation of Impatiens balsamina Leaf Extract

Samples of water henna leaf powder were weighed as much as 450 grams, then extracted using 96% ethanol solvent by maceration method. Total maceration was carried out for three days, then filtering was carried out. The filtrate obtained was then evaporated using a rotary evaporator to obtain a thick extract (Dermawan and Liza, 2015).

Flavonoid Test of Impatiens balsamina Leaf Extract

Flavonoid testing is done by dissolving 1 mg of extract with 10 drops of ethanol, then adding Mg powder and 4 drops of concentrated HCl. The appearance of yellow, orange, or red color indicates positive flavonoids (Oktavia and Sutoyo, 2021).

Ethanol Free Test

This test is carried out by means of 1 ml of thick extract plus 2 drops of H2SO4 and 2 drops of acetic acid, then heated. The extract is declared ethanol-free if there is no distinctive ester odor indicating that it is ethanol-free (Lilyawati et al., 2019).).

Antibacterial Activity Test of Impatiens balsamina Leaf Extract

Antibacterial activity testing was carried out on ethanol extracts of water henna leaves with various concentrations (20%, 25% and 30%). This test uses the agar diffusion method with disc paper. An inoculum of 0.1 ml was placed into a sterile petri dish, then 20 ml of nutriet agar media was added at 45-50°C. The dish is then shaken on a table so that the media and bacterial suspension are evenly mixed. After the media hardened, several paper disks were placed on it, then 0.1 ml of ethanol extract solution of water henna leaves in various concentrations as well as positive control (erythromycin) and negative control (aquadest) was pipetted on each paper disk. Incubation was carried out in an incubator at 35±2°C for 18-24 hours, the inhibition zone (clear area) formed was measured using a caliper (Dermawan and Liza, 2015).

Cream Formulation of Impatiens balsamina Leaf Extract

Formulation begins with separating the oil phase and water phase. The oil phase consisting of stearic acid, liquid paraffin, cetyl alcohol, propyl paraben and a-tocopherol was dissolved into a porcelain cup on a water bath at 70°C and stirred until homogeneous. The aqueous phase was prepared by putting extracts with various concentrations of 20%, 25% and 30%, TEA, glycerin, methyl paraben and distilled water into a cup at the same temperature. After melting the oil phase was transferred into a mortar that had previously been heated. Then the water phase is slowly added and stirred until homogeneous. The complete formula design can be seen in table 1.

Tabel 1. Cream Preparation of Impatiens balsamina Extract

Ingredients	Formula(%)		
	F1	F2	F3
Oilphase			
Liquid paraffin	20	20	20
Stearic acid	12	12	12
Cetyl alcohol	5	5	5
α-tocopherol	0,05	0,05	0,05
Propylparaben	0,02	0,02	0,02
Water phase			
Impatiens balsamina extract	20	25	30
Glycerin	10	10	10
TEA	3	3	3
Ascorbic acid	0,05	0,05	0,05
Methyl paraben	0,18	0,18	0,18
Aquadest	Ad100		

Physical Characterization of Impatiens balsamina Cream

1. Organoleptic Test Organoleptic testing includes examination of textures, color and odor which are observed visually (Dermawan and Liza, 2015)
2. Homogeneity Test Homogeneity testing is done by applying the cream that has been made on a glass object, then clenched with another glass object and then seen whether the base applied on the glass object is homogeneous and whether the surface is smooth homogeneous and whether the surface is smooth and evenly distributed (Tari and Indriani, 2023) .
3. Spreadability Test 0.5 g of cream was placed in the center of a round glass, then covered with another glass and left for 60 seconds. The spread diameter is measured from the average of several sides. A load of 50 g was added, allowed to stand for 60 seconds, then measured the diameter formed (Dermawan and Liza, 2015).
4. Adhesion Test 0.25 g of cream was placed on an object glass. Another object glass was placed on top of the cream, then pressed with a load of 1 kg for 5 minutes. Then the 80 g weight is released and the time is recorded until the two glasses are released (Dermawan and Liza, 2015).
5. pH Test Determination of the pH of the preparation was carried out using a soil tester pH meter. The pH meter was dipped directly into the cream preparation (Dermawan and Liza, 2015)
6. Viscosity Test This test is carried out to determine the viscosity level of the preparation. This viscosity test was carried out using a Brookfield viscometer with spindle No. 7 and a speed of 100 rpm (Thomas et al., 2024).
7. Antibacterial Activity Test of Impatiens balsamina Leaf Extract Cream Preparation The antibacterial activity test of water henna leaf extract cream was carried out using the Kirby-Bauer disc diffusion method. Paper disks were dipped in cream preparations with variations in extract concentrations of 20%, 25% and 30%.

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Then placed on the surface of the media that has grown bacteria. Petri dishes were incubated at 37 °C for 24 hours then observed the inhibition zone formed. In this test, the negative control used was cream base (without extract) and the positive control used was erythromycin cream.

RESULTS AND DISCUSSION

In this study, the maceration method was used with ethanol solvent for 3x24 hours. According to Oktavia and Sutoyo (2021), the maceration method is easy to do and uses fairly simple tools. Another advantage of this method is that the process is very effective in extracting compounds that are not resistant to heat so that the metabolite compounds to be analyzed are not damaged. The longer the extraction time, the quantity of extracted material will also increase due to the greater opportunity for contact between the material and the solvent so that the results will increase to the optimum point (Widodo et al., 2021)..

Table 2. Extraction yield

Sample Weight(g)	Solvent (mL)	Extract (g)	Yield(%)
450	2000	47,2	10,4

From the extraction process that has been carried out, it can be seen in table 2, where from as much as 450 g of water pacar (Impatiens balsamina) leaf powder, a thick extract of 47.2 g is obtained with the percent yield obtained, which is 10.4%. The yield is the ratio between the extract obtained and the initial simplisia. Yield uses units of percent (%), the more high value of the resulting yield indicates the value of the extract produced more and more. The results obtained in this study indicate that the water henna leaf extract meets the requirements of a good % yield which is not less than 10% (Indonesian Herbal Pharmacopoeia, 2017).

Flavonoid Test

This flavonoid test aims as a preliminary test carried out to determine the presence of secondary metabolites in an extract (Qomaliyah et al., 2023). This identification is carried out using Mg and added HCl, this flavonoid qualitative test method is called the Wilstater cyaniding method.

Table 3. Flavonoid Test Results

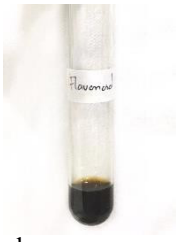
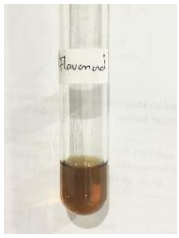
Test	Reagent	Parameter	Color Change		TestResult
			Before	After	
Flavonoid	Mg powder and 4 drops of concentrated HCl	Yellow,orangeor red	 Dark green	 Orange	(+) Positive

Table 3. shows the positive results of the flavonoid compound test on the water pacar (Impatiens balsamina) leaf extract, indicated by the color changes that occur, this is in accordance with the statement of Oktavia and Sutoyo (2021), the onset of yellow, orange or red color indicates a positive result of flavonoid compounds.

Ethanol-free

Test Ethanol-free testing is carried out to determine whether or not ethanol is still contained in the extract. An extract is said to be ethanol-free if there is no characteristic ester odor from ethanol (Lilyawati et al., 2019)

Table 4. Ethanol-free Test

Test	Reagent	Parameter	HasilUji
Solvent free (Ethanol)	2 drops of H_2SO_4 and 2 drops of acetic acid then heated	Absence of distinctive Eter odor	No ester odor

Based on the test results that have been carried out, there is no distinctive ester odor from the extract. This indicates that the water henna (Impatiens balsamina) leaf extract no longer contains ethanol. This test aims to ensure that the antibacterial testing carried out is not disturbed by the presence of ethanol. This is according to Nur et al (2022), that alcohol can denature proteins contained in bacteria and viruses, causing metabolic disorders that cause the death of bacterial and viral cells.

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Evaluation of Physical Characterization of Impatiens balsamina Cream

Before formulating the cream of water henna leaf extract (Impatiens balsamina), base optimization was first carried out. Base optimization has the aim of obtaining the optimal formula in formulating water henna leaf extract into a cream preparation to match the pH requirements of the skin, homogeneous, has good spreadability and adhesion, and a qualified viscosity. base optimization was carried out by varying the concentration of cetyl alcohol, namely 3%, 5%, 7% and 9%, this variation was carried out to determine the consistency of a good cream. According to Nining et al (2019), the higher the concentration of cetyl alcohol used, the higher the viscosity, this is because cetyl alcohol has a function as a consistency enhancer as well as a stability enhancer for cream preparations. From the results of base optimization that have been carried out, 5% cetyl alcohol concentration shows good physical evaluation results. The results of the water henna leaf extract cream preparation can be seen in Figure 1.

Figure 1. Cream of Impatiens balsamina Leaf Extract

Table 5. Results Physical Evaluation of the Characterization Impatiens balsamina Cream

Parameter	Formula		
	F1	F2	F3
Organoleptic	Specific odor, brown, semi-solid	Specific odor,brown, semi-solid	Specific odor,brown, semi-solid
Homogeneity	Homogeneous	Homogeneous	Not homogeneous
Spreadability	6 cm	5,9 cm	4,5 cm
Adhesiveness	4 seconds	8 seconds	10 seconds
pH	4	4	4,1
Viscosity	4080 cPs	4880 cPs	12,700 cPs

Organoleptic Test

Organoleptic evaluation of water henna leaf extract cream was carried out by looking at several parameters such as aroma, color and texture of the preparation. The results of the organoleptic evaluation can be seen in table 5. Where the three preparations show that the three formulas have the same aroma and texture, which is typical of water henna leaf extract and has a semi-solid texture, indicating that the cream is easy to apply. And the color observation of the three is the same, namely dark brown. The color of the three formulas is thought to occur due to the results of the extract used as the active substance in this formulation. From the observation of the color of the water henna leaf extract cream, further modification is still needed. Where according to Qamariah et al (2022), color has an important role in the acceptance of a product ranging from food to medicinal preparations.

Homogeneity Test

This test is carried out to determine whether the cream preparation applied is homogeneous and whether the surface is smooth and evenly distributed or there are particles that are not well distributed. From the test results, it can be seen in table 5, the observation of F1 and F2 cream preparations shows that both formulas are homogeneous. However, it is different from F3 which is not homogeneous, characterized by the presence of uneven particles. According to PPermatasari (2021), this may occur due to the mixing process occurring at a temperature lower than the melting point of the oil phase, causing crystallization or solidification. Therefore, F3 does not meet the requirements of the cream, namely that the preparation must be homogeneous and the active substance is evenly distributed in the cream base.

Spreadability Test

Spreadability testing is carried out to determine the ability of semi-solid preparations to spread and spread evenly on the skin or area applied. The results of the spreadability evaluation can be seen in table 5, from the table it shows that the results of the measurement of the spreadability of the F3 cream preparation do not meet the requirements, which is only 4.5 cm. Where according to Tungadi et al (2023), the predetermined requirements are around 5-7 cm.

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Adhesive Test

Adhesive power testing is carried out with the aim of seeing how well and how long a preparation can adhere to the skin surface. Based on table 5, it can be seen that the three formulas are in accordance with the requirements where according to Muthoharoh and Ratna (2020), the requirement for good cream adhesion is >1 second. pH Test This test was conducted using a pH meter by diluting the test solution with distilled water. The results of the pH evaluation can be seen in table 5, which shows that three formulas meet the pH requirements of facial skin. According to Nana et al (2021), the pH of the skin ranges from 4-6.5. This is in line with the statement of Tungadi et al (2023) that the pH of the preparation should be adjusted to the pH of the skin because if the pH of the cream is too acidic it can irritate the skin and if the pH of the cream is too alkaline it can cause dry and flaky skin.

Viscosity Test

Viscosity is a statement of the resistance of a liquid to flow. Where the higher the viscosity of a liquid, the greater the resistance of the liquid to flow. Based on table 5, it can be seen that the viscosity measurement results of the three formulas meet the requirements for good cream viscosity. This is based on SNI 16-4399-1996 regarding the quality standards of cream preparations, the three cream formulations of water henna leaf extract (Impatiens balsamina) meet the requirements for good viscosity of cream preparations because they are still in the range of 2000-50,000 cPs.

Antibacterial Activity Test

Testing the antibacterial effectiveness of water henna leaf extract against Propionibacterium acnes was carried out by the disc diffusion method (Kirby Bauer). The principle of this method is the inhibition of the growth of microorganisms, namely the inhibition zone will be seen as a clear zone around the area containing the antibacterial substance (Prasetia et al., 2019).

Table 6. Antibacterial Activity Test Results

Description	Concentration/Formula	InhibitionZone (mm)	Category
Extract	20%	21,41	Very strong
	25%	23,46	Very strong
	30%	25,58	Very strong
C(+)		40	Very strong
C(-)		0	Inactive
Cream	F1	11,07	Strong
	F2	11,67	Strong
	F3	12,05	Strong
C(+)		33,9	Very strong
C(-)		0	Inactive

The results of the effectiveness test of water henna leaf extract (Impatiens balsamina) are shown in table 6, where it can be seen that the diameter of the inhibition zone produced with an extract concentration of 20% is 21.41 mm. At 25% extract concentration, it is 23.46 mm, and at 30% concentration, the inhibition zone formed is 25.58 mm. This shows that the three variations of extract concentration have a very strong inhibition. In line with the statement of Datta et al (2019) antimicrobial inhibition zone activity is grouped into four categories, namely: weak activity (10-20 mm), and very strong (>20-30 mm).

The results of the antibacterial activity test of the cream preparation of water henna leaf extract (Impatiens balsamina) can be seen in table 5. Where from the test results it was found that the inhibition zone of the cream was sequentially F1 (11.07 mm), F2 (11, 67 mm) and F3 (12.05 mm). From the results of the three, it can be said that the cream preparation of water henna leaf extract has antibacterial activity against Propionibacterium acnes with a strong category. However, these results cannot match the results of the inhibition zone formed by erythromycin cream used as a positive control, which amounted to 31.6 mm. According to Leonita et al (2024), the use of a positive control aims to observe the zone of inhibition formed as an illustration of the killing of test bacteria and to verify whether the procedure performed is appropriate. In addition, the use of base as a negative control does not show any antibacterial activity characterized by the formation of no zone of inhibition. Leonita et al., (2024), in their research said that the purpose of using the base as a negative control is to show that the base does not affect the tested bacteria.

The results of the antibacterial activity test of water henna (Impatiens balsamina) leaf extract cream showed that there was a decrease in the diameter of the inhibition zone formed when compared to the inhibition zone of the extract. This is thought to occur due to the addition of excipients in the cream formulation that can reduce the effectiveness of the extract in inhibiting bacterial growth. This is as explained by Febriyanti et al (2024), which states that the decrease in inhibition can be caused by the difficult diffusion of the base so that the active substance cannot be separated properly from the cream base.

CONCLUSION

Water henna leaf extract (Impatiens balsamina) can be formulated into a cream dosage form. The three cream formulations of water henna leaf extract have antibacterial activity with a strong category against Propionibacterium acnes, with inhibition of F1 (11.07 mm), F2 (11.67 mm) and F3 (12.05 mm) respectively.

RECOMMENDATIONS

Researchers suggest in the future to develop a crean formula of leaf extracts water henna into the form of a nanoparticle delivery system or adding a type of penetration enhancer to improve the delivery of the active substance. In addition, the authors suggest improving the appearance of the preparation so that it can increase public acceptability.

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