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Formulation and Evaluation of Transdermal Dissolving Microneedle Loaded with Ethanol Extract of Cocor Bebek Leaves (*Kalanchoe pinnata*)

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Abstract

Background*: Acne is a chronic skin inflammation caused by blockage of the sebaceous glands in the skin and hypercolonization of the acne-causing bacteria Propionibacterium acnes. Cocor bebek leaves (Kalanchoe pinnata) are known to contain various secondary metabolites, including flavonoids, with antibacterial activity.* ***Objective****: In an effort to prevent side effects from using oral and topical antibiotics to treat acne, an alternative acne treatment that is safer and more effective with a strong drug delivery system is needed: microneedle patch technology containing natural ingredients.* ***Methods****: A microneedle patch formulation of Cocor bebek leaf extract was developed using a combination of HPMC and PVP polymers. The evaluation of microneedle patches included irritation tests with rat test animals and antibacterial activity tests against Propionibacterium acnes. The results showed the formation of yellow microneedle patches with uniform needles. The evaluation results showed that the microneedle patch has an irritation index value classified as non-irritant and has antibacterial activity against Propionibacterium acnes, with the highest inhibitory diameter at an extract concentration of 30% with a moderate inhibition category.* ***Conclusion****: The microneedle patch cocor bebek leaf extract shows potential as an effective drug delivery system for the treatment of acne that is safe for use on the skin.*

Keywords*: acne, antibacterial, cocor bebek leaves, dissolving microneedle, flavonoid*

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INTRODUCTION

The skin is the outermost layer of the tissue that covers the entire surface of the body. Skin protects the body from environmental exposure. Skin has unique characteristics depending on gender, age, race, and climate. The skin has several layers, namely the epidermis, dermis, and subcutaneous tissue. In the epidermis layer, which is the outermost layer, sweat glands secrete waste products through pores, which are called sweat (Heng et al., 2022).

Acne is a skin disease often experienced by teenagers and adults. The appearance of acne is characterized by blackheads, pustules, papules, cysts and nodes on the neck, face, upper arms, back, chest and face (Wahdaningsih et al., 2017). Common bacteria that infect acne include *Propionibacterium acnes*, Staphylococcus aureus, and Staphylococcus epidermidis. Acne treatment usually involves antibiotics such as clindamycin, erythromycin, and doxycycline. Benzoyl peroxide, azelaic acid, and retinoids are also often used. However, these drugs have side effects when used as antibacterial agents that cause acne, namely, antibiotic resistance and irritation, hypersensitivity of the follicular epidermis so that follicle blockage can occur, and excessive sebum production.

One plant with antibacterial activity is the Cocor bebek leaf extract. Cocor bebek leaves (*Kalanchoe pinnata*) have antibacterial, antiviral, antioxidant, antifungal, antiparasitic, and antihypertensive properties. The ompounds present in cocor bebek leaves include steroids, terpenoids, flavonoids, alkaloids, and lipids (Ely et al., 2020).

Based on previous research, the antibacterial activity of the ethanol extract of Cocor bebek leaves was tested against *Propionibacterium acnes*. The results showed that the zone of inhibition was 19 mm (very strong) at a concentration of 15% and 21 mm (very strong) at a concentration of 20%; therefore, it has the potential to be used as a dosage formulation. Microneedles are physical technologies that cause mechanical changes in the epidermal barrier and create micron-sized channels or pores in the skin, thereby allowing delivery of various molecules (Larrañeta et al., 2016). (Larrañeta et al., 2016).

The main problem with transdermal technology is that many drugs are unable to penetrate the skin at the levels necessary to achieve a therapeutic effect. Researchers have developed improved technology using microneedles, which allow hydrophilic compounds with high molecular weights to enter the stratum corneum. Administering medication using a microneedle device allows drug molecules to pass through the stratum corneum, thereby allowing more drug molecules to enter the skin (Waghule et al., 2019).

MATERIALS AND METHODS

Materials

The material used in this study were cocor bebek leaves, ethanol 70%,' Dragendorff's and ' 'Mayer's reagents, ferric chloride (Merck), chloroform (Merck), H_2SO_4 (Merck), CH_3COOH (Merck), and aqua dest. All chemicals used were of analytical grade. Twenty-four male Wistar rats used were 24 male Wistar strain rats.

Tools

The tools used included a laboratory oven (Memmert®), water bath (Memmert®), rotary evaporator (Buchi®), centrifuge (Corona®), analytical balance (Ohaus®), micropipettes (DLab®), vortex (Thermo Scientific), and spectrophotometer UV-VIS (Thermo Fisher Scientific).

Methods

Sample collection and preparation

Cocor bebek leaves (*Kalanchoe pinnata* (Lam) Pers.) were obtained from Bandung, West Java. The plant specimens were identified and authenticated in the Laboratory of Biosystematics and Molecular, Department of Biology, University of Padjadjaran (No. 411/LBM/IT/V/2024). Metabolites were also identified.

Phenol

Dried leaves (50 mg) were boiled in 50 mL of water for 15 min. After cooling, the mixture was filtered to obtain filtrate. The filtrate was treated with $FeCl_3$ reagent. The appearance of a blue or greenish-black color indicated the presence of phenol (Handayani et al., 2020).

Flavonoid

Dried leaves (50 mg) were added to methanol, followed by a few drops of HCl until the solution became acidic. The mixture was then subjected to modified reflux in a water bath for 30 min and subsequently filtered. The resulting filtrate was combined with 10 mL distilled water and placed in a separatory funnel, to which 10 mL hexane was added. The mixture was shaken until two distinct layers formed. The lower layer was collected and evaporated to dryness, and then ethyl acetate was added. Subsequently, Mg powder and concentrated hydrochloric acid were added. Red indicates the presence of flavonoid compounds (Muthmainnah 2019). (Muthmainnah, 2019).

Steroid

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Dried leaves (100 mg) were macerated with 5 mL chloroform for 2 h. The resulting filtrate was evaporated and tested using the Lieberman-Buchard reagent. Observations were made for Color changes were observed: a purple-red hue indicated the presence of triterpenoids, while a blue-green hue signified the presence of steroids (Alwi et al., 2022). (Alwi et al., 2022).

Alkaloid

A total of 15 mL of ethanol was added to the dried leaf powder, followed by the addition of drops of HCl until the solution reached acidic pH. The mixture was then subjected to a modified reflux in a water bath for 30 min and subsequently filtered. The resulting filtrate was collected in a test tube and tested using Dragendorff and Mayer reagents. A positive result was indicated by the development of an orange color in the presence of Dragendorff reagent and a white color with Mayer reagent (Irfansyah et al., 2024).

Saponin

Dried leaf powder (100 mg) was added to hot water (5 mL). After cooling, the mixture was shaken vigorously for 10 s until foam developed. A positive result for saponins was indicated by the formation of foam measuring 1–10 cm in height, which persisted even after the addition of 2 N HCl (Qomaliyah et al., 2023).

Preparation of ethanol extract

Cocor bebek leaves were macerated in 2 L of ethanol 70% for 24 h at room temperature (25 °C) and then filtered through paper filters to obtain the macerate. The residue was macerated. Up to three iterations were performed for these stages. The combined macerate was then thickened using a vacuum rotary evaporator.

Total flavonoid test

Cocor bebek leaf extract 50 mg was dissolved in 50 mL ethanol 96%. The solution was pipetted at 0.5 mL and 2 mL of distilled water and 0.15 mL $NaNO_2$ 5%. Subsequently, 10% AlCl3 was added, and the mixture was left for 6 min. The solution was then added to 2 mL of 4% NaOH and filled with distilled water to 5 mL, and the absorbance was read using a UV-VIS spectrophotometer. The samples were analyzed in triplicate, and the average absorbance was used to calculate the flavonoid concentration using quercetin equivalents.

Antibacterial activity test: cocor bebek leaf extract

The antibacterial activity of the cocor bebek leaf extract was tested using the well method. Nutrient agar was poured into a sterile Petri dish and allowed to solidify. The bacterial suspension of *Propionibacterium acnes* was then poured over the surface of the media, and 20 mL of the media was poured back. Wells were made using a perforator, and the samples and controls were inserted into each well. Incubate in an incubator for 24 h at 37°C, and the zone of inhibition was observed.

Formulation microneedle patch

A transdermal dissolving microneedle patch was fabricated using a printing method. The process involves making silicone molds by pouring a mixture of silicone on a cartridge microneedle and waiting until the mold is formed. The formulation patch was made into four formulations, where each formula consisted of a mixture of 1.5% HPMC polymer and 40% PVP dissolved in ethanol (EtOH) and deionized water (DW) in a ratio of 4:1 and 2:1, respectively. Cocor bebek leaf extract was added at the desired concentration. Each material was combined and centrifuged at 2000 rpm for 3 min. The silicone mold was inserted into the formulation mixture and centrifuged again. After 30 min, the mold was removed, and the excess formulation mixture was cleaned and dried at room temperature for 24 h before being released from the mold (Chanabodeechalermrung et al., 2024). (Chanabodeechalermrung et al., 2024).

Irritation test

The test animals were male Wistar rats aged 6-8 weeks with a minimum body weight range of 170–180 g. The body weight of adult rats reaches 450 grams, with the reported maximum body weight of male Wistar rats being 677.3 ± 9.2 g (Ghasemi et al., 2021; Rejeki et al., 2018). With this body weight and age, male Wistar rats fall into the young adult category, which means they exhibit musculoskeletal maturity and stable physical and mental functioning, as body weight and age influence the results of the study (Ghasemi et al. 2021). (Ghasemi et al., 2021). Test animals were randomized and divided into several groups (n = 6). The test animals were acclimatized for seven days by providing food and drink ad libitum. Test animals were shaved using scissors, smeared with water, and cleaned with distilled water. After the test animals were shaved, no procedures were performed for 24 hours. The skin of the test animal, whose hair had been shaved, was then divided into two parts: the left skin did not receive any treatment except for hair growth, whereas the right skin received treatment from each treatment group. The test material was attached to the skin of the test animals for 4 h. The patch was removed and observed for irritation, such as erythema and edema, at 1, 24, 48, and 72 h. The protocols were approved by the Committee for Animal Experiments at the YPIB University (approval no: 220/KEPK/EC/VII/2024).

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Antibacterial activity test: microneedle patch

The antibacterial activity of the microneedle patch was tested using the agar diffusion technique and the Spread Plate Method. Initially, 15 mL of nutrient agar medium was poured into a sterile petri dish and allowed to solidify. Then, a bacterial suspension of *Propionibacterium acnes* was evenly spread over the surface of the medium at a volume of 0.1 mL. Each Petri dish was divided into five parts for three samples: positive control and negative control. Subsequently, samples of the microneedle patch ethanol extract of cocor bebek leaves, negative control microneedle patch without extract, and positive control 0.5% chloramphenicol were placed on agar media. This process was performed aseptically 2 times in each petri dish. The incubation was carried out for 24 h at 37°C in an incubator, after which the inhibition zone formed was observed using a caliper (Maddeppungeng et al., 2023). (Maddeppungeng et al., 2023).

RESULTS AND DISCUSSION

Sample collection and preparation

Many nutritious plants grow wild without being fully utilized by people, including the leaves of wild plants, such as the cocor bebek. A total of 1095 g of fresh cocor bebek leaves were processed by sorting, washing, drying in an oven, and sun-drying. The dried leaves were stored for further extraction. Phytochemical screening of the dried leaves showed the presence of flavonoids, alkaloids, steroids, and phenolic compounds, which can be seen in Table 1.

The extraction process was conducted using the maceration method chosen for its ability to effectively extract bioactive compounds without damaging the heat-sensitive substances. The macerate obtained was evaporated using a water bath and rotary evaporator, resulting in a thick extract with a yield of 12.8%.

Testing of total flavonoid content

The total flavonoid assay yielded a linear regression equation, $y = 0.0845x + 0.0011$, with an R^2 value of 0.9891. An R^2 value close to 1 indicated a correlation between the concentration of the quercetin standard and the absorption value. The flavonoid content in the cocor bebek leaf extract was determined to be 6.06 ± 0.19 mg QE/g using the aluminium chloride method, indicating the potential for antibacterial activity (Nugraha et al., 2017). Absorbance measurements were conducted at 355 nm, which showed a linear relationship between concentration and absorbance. Flavonoids act as antibacterial agents by denaturing bacterial proteins and potentially damaging cell walls (Nugraha et al., 2017).(Nugraha et al., 2017). The results suggest that cocor bebek leaf extract could be a promising source of antibacterial agents due to its rich flavonoid content.

Antibacterial activity test cocor bebek leaf extract

Antibacterial testing using the well method demonstrated that cocor bebek leaf extract could inhibit the growth of *Propionibacterium acnes*. Chloramphenicol 0.5% served as a positive control for its broad-spectrum antibiotic properties (Cui et al., 2023), whereas 10% DMSO was used as a negative control because it lacks antibacterial activity.. Antibacterial testing of the cocor bebek leaf extract conducted in duplicate showed that the extract has antibacterial properties, as evidenced by the formation of an inhibition zone on the test media against *Propionibacterium acnes*. The strongest inhibitory effect was noted at a 30% extract concentration, which resulted in an inhibition zone diameter of 12.7 ± 0.4 mm, classifying it as strong compared to other concentrations of the extract.

Formulation microneedle patch

Microneedle patches were prepared using a combination of HPMC and PVP polymers. PVP, or polyvinyl pyrrolidone, is a polymer commonly used in formulation development because it is soluble in water; therefore, it can swell and has a gel-like consistency. This causes the formation of layers or pores that facilitate the diffusion of the drug molecules (Daryati et al., 2022). (Daryati et al., 2022). In addition, PVP has the characteristic of being hydrophilic with a low melting point, so it has good melting ability when applied to the skin at body temperature (Zakaria et al., 2021). HPMC, or hydroxypropyl methylcellulose, is a semi-synthetic polymer with mucoadhesive properties, so it can play a role in the film formation process and form a barrier for molecular movement (Daryati et al., 2022).

The formulation results of the microneedle patch cocor bebek leaf extract are shown in Figure 1. The results showed the formation of a microneedle patch with a uniform needle. The results were then carried out by physical evaluation, including diameter and height, using calipers and microneedle patches with a diameter of 8.2 mm and a height of 4 mm. Microneedle patches clear color for those that do not contain active ingredients (F1), whereas microneedle patches with the active ingredient yellow color cocor bebek leaf extract (F2, F3, and F4).

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Table 1. Phytochemical screening results of dried leaves cocor bebek leaves

Identification	Reagent	Result	Information
Flavonoid	Mg powder	+	A red color forms
Alkaloid	Meyer	+	A white precipitate is formed
	Dragendorff	+	An orange color forms
Steroid	Liebermann-Buchard	+	A bluish green color is formed
Phenol	$FeCl_3$	+	A blackish green color forms
Saponin	Shake with hot water	-	No foam is formed

Table 2. Antibacterial test results cocor bebek leaf extract

Sample	Inhibition Zone Diameter (mm)	Inhibitory Power Category
Control + (Chloramphenicol)	25.6 ± 0.0	Very strong
Control - (DMSO 10%)	0.0 ± 0.0	-
Cocor bebek leaf extract 20%	9.4 ± 1.0	Moderate
Cocor bebek leaf extract 30%	12.7 ± 0.4	Strong

Table 3. Formulation microneedle patch

Material	Solvent	Formula (g)			
		F1	F2	F3	F4
Ethanol extract of cocor bebek leaves	DW 1 mL	-	0.2	0.3	0.4
HPMC	EtOH:DW 4:1	0.15	0.15	0.15	0.15
PVP	EtOH:DW 2:1	4	4	4	4

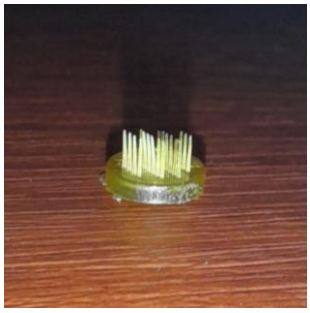

Figure 1. Microneedle patch cocor bebek leaf extract

Irritation test

An in vivo irritation test was performed on male Wistar rats. Test animals were shaved 24 h before treatment to prevent irritation of their skinThe irritation test method in this study was modified from research by Sohail et al. (2020). Each animal was divided into two parts: the left skin did not receive any treatment except for hair growth, while the right skin received treatment from each treatment group (microneedle patch HPMC and PVP based, microneedle patch cocor bebek leaf extract 20%, 30%, and 40%).. This test was performed to determine the irritating effects of the microneedle patch on the skin (Ermawati, 2018).(Ermawati, 2018). Pasting patch was performed behind closed doors (patch test) using a plaster. This was intended to help the patch absorb well and avoid environmental influences. The patch was attached to the right side of the skin and then covered using plaster to keep the test material attached to the skin (Ramli & Fadhila, 2022).

Observations were made after removing the patch at 1, 24, 48, and 72 h based on the Draize method by examining the irritation parameters that arise, namely irritation (reddish reaction) and edema (swelling). Erythema is reddened skin due to blockage of capillaries, whereas edema is an abnormal accumulation of fluid under the skin or in one or more body cavities (Sukirawati, 2019). This test aims to determine whether there is a possibility of a delayed reaction. Observation results are expressed as a score of 0 – 4 based on the severity of erythema and edema, and then accumulated into an irritation index value so that the irritation classification can be known.

The results in Table 3 show that the four treatment groups had irritation index values with a non-irritation classification. In treatment group 1, a microneedle patch blank with a combination of HPMC and PVP showed an irritation score of 0.11 according to the Primary Irritation Index (PII), values within the range of 0 to 0.4 are classified as non-irritating, indicating that the product does not pose a potential risk of causing skin irritation (Han et al., 2021). HPMC is inert, does not irritate the skin, and provides good film strength when it dried(Firmansyah et al., 2022). PVP does not irritate the skin (Kurakula et al., 2020).(Kurakula et al., 2020). When compared with treatment group 4 with a microneedle patch containing the extract, 40% showed an irritation index value of 0. These results are supported by statistical data analysis using the software SPSS in the Kruskal–Wallis test, with results showing that the

Asymp.sig value is > 0.05, so there is no significant difference, so it can be concluded that the preparation is safe to use.

Table 4. Irritation test results microneedle patch

Treatment Group	Irritation Score	Irritation Category
F1 (MN *Blank*)	0.11	Non-irritation
F2 (MN Extract 20%)	0.05	Non-irritation
F3 (MN Extract 30%)	0.14	Non-irritation
F4 (MN Extract 40%)	0.00	Non-irritation

Antibacterial activity test microneedle patch

The purpose of the antibacterial test was to determine the size of the inhibition zone produced by a microneedle patch containing cocor bebek leaf extract, which can inhibit the growth of the acne-causing bacteria *Propionibacterium acnes*. Nutrient agar is used as a medium for bacterial growth, and solid media make it easier to measure the diameter of the inhibition zone (Nurhayati et al., 2020). The results of the antibacterial activity test, conducted in duplicate, indicated that F2 demonstrated moderate antibacterial activity, whereas both F3 and F4 showed strong antibacterial activity. This refers to the inhibition zone criteria by David and Stout (1971) in Mayasari (2022), where the inhibition zone is declared weak if it is <5 mm in diameter, moderate if it is 5–10 mm, strong if it is 10–20 mm in diameter, and very strong if it is >20 mm. The inhibition zone category produced was moderate because, based on the inhibition zone formed, the microneedle patch of the ethanol extract of cocor bebek leaves can inhibit bacterial growth, but its sensitivity is not as high as that of chloramphenicol as a positive control (Suciari et al., 2017).

Based on the results, it can be seen that 20% ethanol extract of cocor bebek leaves at the lowest concentration used can inhibit *Propionibacterium acnes* bacteria. The emergence of the inhibitory zone is attributed to the presence of active compounds, such as steroids, terpenoids, flavonoids, and alkaloids, in the cocor bebek leaf extract. Testing was performed on F1, a blank microneedle patch, to determine the potential antibacterial activity of the combination of HPMC and PVP. The results showed that the combination of HPMC and PVP polymers did not affect the antibacterial activity of the microneedle patch. In the positive control, the antibiotic chloramphenicol 0.5% produced a strong inhibition zone of 16.2 ± 4.4 mm. The data obtained showed that the microneedle patch with ethanol extract from cocor bebek leaves has antibacterial activity, inhibiting the growth of the acne-causing bacteria *Propionibacterium acnes*.

Table 5. Antibacterial test results microneedle patch

Sample	Inhibition Zone Diameter (mm)	Inhibitory Power Category
Control + (Chloramphenicol)	16.2 ± 4.4	Strong
F1 (MN *Blank*)	0.0 ± 0.0	-
F2 (MN Extract 20%)	7.6 ± 0.6	Moderate
F3 (MN Extract 30%)	10.6 ± 1.6	Strong
F4 (MN Extract 40%)	10.2 ± 5.2	Strong

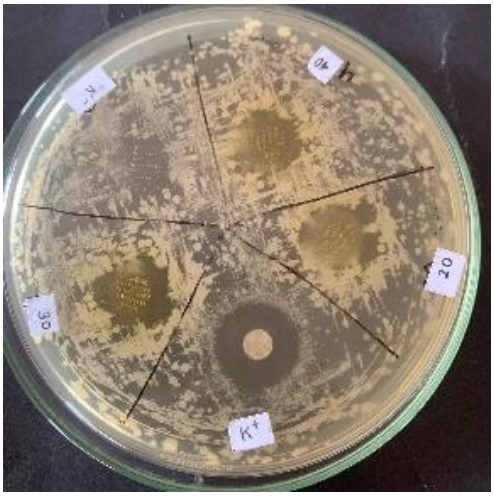

Figure 2. Antibacterial activity of microneedle patch cocor bebek leaf extract

CONCLUSION

The leaves of the cocorbebek plant contain flavonoids that exhibit pharmacological effects including antibacterial properties. This study focused on formulating a microneedle patch using Cocor bebek leaf extract combined with HPMC and PVP polymers dissolved in a suitable solvent to create uniform needles. Evaluation of the microneedle patch revealed that the prepared patch with the cocor bebek leaf extract was effective in inhibiting the growth of acne-causing *Propionibacterium acnes*, with the highest inhibition zone diameter observed at an extract concentration of 30%. Furthermore, the irritation test results classified the microneedle patch as non-irritant, indicating its safety for use on skin.

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AUTHOR CONTRIBUTIONS

Conceptualization, A.A.Z.; Validation, A.D.S.; Formal Analysis, S.R.; Investigation, I.Z.; Resources, S.R.; Writing - Original Draft, R.N.B.; Writing - Review and Editing, M.N.A.; Visualization, A.D.S.; Supervision, A.D.S.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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