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Formulation Cream of *Patikan Kebo* Weeds (*Euphorbia hirta* L.) Extract as an In Vitro Anti-Acne

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Abstract

Background: *Acne is a skin inflammation that is prevalent in 80-100% of the population, particularly during adolescence. The Patikan Kebo (Euphorbia hirta L.) weed plant is employed in a cream formulation to treat acne. Therefore, this study aims to determine the formula of the Patikan Kebo (E. hirta L.) weed cream preparation that is as effective as an in vitro anti-acne.* **Methodology:** *the research method was carried out experimentally with the stages of sample preparation, phytochemical screening, extraction, formulation, evaluation, and antibacterial activity test that caused acne using the well diffusion method.* **Findings:** *The results showed the Patikan Kebo (E. hirta L.) weed has secondary metabolites consisting of alkaloids, flavonoids, saponins, tannins, and steroids/triterpenoids. The evaluation of the cream preparation of each formula has met cosmetic standards. The diameter of the F4 inhibition zone has antibacterial activity against acne-causing Propionibacterium acnes (12.03 ± 0.81 mm), Staphylococcus epidermidis (11.48 ± 1.10 mm), S. aureus (12.07 ± 0.06 mm), and the F5 inhibition zone P. acnes (13.53 ± 0.15 mm), S. epidermidis (12.54 ± 0.75 mm), and S. aureus (13.03 ± 0.61 mm). Formulas F4 and F5 are more effective as anti-acne with a strong inhibition zone diameter.* **Contribution:** *These findings indicate that formulas F4 and F5 can be further developed as alternative natural active ingredients in cosmetic or pharmaceutical products for acne therapy, thus contributing to the development of safer, more economical, and more sustainable local herbal medicine.*

Keywords: Anti-Acne, Cream, *Euphorbia hirta* L., In Vitro, Weed

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INTRODUCTION

A variety of factors, including genetics, hormones, psychology, climate, diet, stress, and bacterial infections, can induce *Acne vulgaris* (acne), a skin inflammation. These factors include *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Propionibacterium acnes* (Ginting et al., 2023). Skin conditions facilitate the rapid growth of acne-infecting bacteria, which in turn triggers acne. Many things help bacteria grow well, such as the nutrients they need on the skin, which can be increased by hormonal changes or puberty, and the warm and humid weather in Indonesia, which has high humidity and low temperatures (Barus et al., 2023).

Propionibacterium acnes is a bacterium that induces opportunistic skin infections by metabolizing glycerol as a source of nutrition. The lipase enzyme from *Propionibacterium acnes* is responsible for the breakdown of sebum, which is the source of glycerol. Androgen hormones (puberty) influence the growth of sebaceous glands in the skin and the increase in sebum production (Pariury et al., 2021). The *stratum corneum* layer of the skin can be damaged by an increase in the population of *P. acnes* bacteria, which in turn damages the pores and causes inflammation (Zulfa, 2023).

Staphylococcus epidermidis is non-pathogenic on human skin; however, it can become infectious when the host's immune system is deficient. Exopolysaccharide intercellular adhesins are secreted by *S. epidermidis* bacteria, which are responsible for the formation of biofilms. These biofilms protect the bacteria from the human innate immune system. Biofilms generate anaerobic conditions that are conducive to the proliferation of *P. acnes* (Kumar et al., 2016). This condition has the potential to cause skin irritation and swelling, which may ultimately lead to inflammation and rupture (Azmalah, 2023).

Staphylococcus aureus contributes to acne lesions by producing extracellular matrix, serum-binding proteins, and various extracellular enzymes, such as *proteases*, *lipases*, *hyaluronidases*, *collagenases*, *staphylokinases*, and *toxins*. These enzymes cause tissue damage and facilitate the pathogen's spread to deeper tissues (Kumar et al., 2016).

Acne is the most prevalent skin condition, affecting 80-100% of the population, particularly adolescents (between the ages of 16 and 19 for men and 14 and 17 for women) during puberty. Some of them can result in long-term symptoms, such as the formation of scar tissue on the skin, which has a psychological impact on the individuals who experience it (Ruchiatan et al., 2020). Cream preparations that contain antibiotics, including *macrolides*, *lincosamides*, *fluoroquinolones*, *disulone*, and *benzoyl peroxide* (BPO), are employed in the treatment of acne. Side effects, including erythema, desquamation, burning skin, itching, scaly and dry skin, and irritation, may result from the continuous use of antibiotics (Leccia et al., 2015). Additionally, resistance may accumulate. Consequently, it may induce new skin health issues in the long term. Therefore, it is necessary to explore alternative methods for the development of natural biological resources, such as the *Patikan Kebo* weed plant (*Euphorbia hirta* L.), which has the potential to be used in the formulation of an anti-acne cream.

The *Euphorbiaceae* family, which includes 334 genera, 52 tribes, and 5 subfamilies, is the sixth-largest flowering plant family (Khan et al., 2024). The *Patikan Kebo* weed plant (*Euphorbia hirta* L.), also known as the asthma plant, is a member of this family. *Euphorbia hirta*, an annual plant, grows wild in any location. It has been traditionally used to treat various conditions, including women's diseases, respiratory diseases (cough, colds, bronchitis, asthma), worm infections, dysentery, jaundice, acne, gonorrhea, digestive problems, and tumors (Barus et al., 2023). The pharmacological bioactivity of patikan kebo weeds is influenced by the content of secondary metabolites, including flavonoids, phenolic compounds, and triterpenoids (Praveen et al., 2017). The development of the *Patikan Kebo* weeds as an anti-acne cream preparation is based on their efficacy as traditional medicine and their accessibility as plants. One of the anti-acne cosmetic products available for application is a cream. Not only is the cream easy to apply to the skin, but it also absorbs easily, is safe, and is easy to clean. Additionally, it is non-sticky. Kurniadi et al., (2024) aim to enhance the effectiveness and efficiency of cream preparations as anti-acne cosmetics compared to other preparations. Consequently, it is imperative to develop innovative anti-acne cosmetic products that are derived from the *Patikan Kebo* weed plant. This study aims to determine the formula of the *Patikan Kebo* (*Euphorbia hirta* L.) weed cream preparation that is as effective as an in vitro anti-acne.

METHOD

Tools and materials

The following tools were employed: a mortar and stamper, a 100 ml measuring cup, a 1000 ml beaker glass, a spatula, parchment paper, a binocular microscope, a stirring rod, a porcelain cup, an ose needle, a spirit lamp, a glass object, a refrigerator, an analytical balance (0.1 mg), a pH meter, an oven, an autoclave, a spatula, a dropper pipette, a test tube, sterile gauze, a watch glass, a vernier caliper, a petri dish, a cork borer, a viscometer, and a vortex.

The used materials were extract of the *Patikan Kebo* weed (*Euphorbia hirta* L.), test bacteria (*Propionebacterium acnes*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*), 96% ethanol, nutrient agar, Mueller Hinton Agar (MHA), Mc. Farland standard, sterile distilled water, physiological NaCl, stearic acid, cetyl alcohol, TEA (*triethanolamine*), methyl paraben, propyl paraben, glycerin, dragendroff reagent, wagner reagent, mayer reagent, Mg, concentrated HCl, iron (III) chloride reagent, n-hexane, and Lieberman-Bouchard reagent.

Sample Preparation

All of the plants utilized were components of the *Patikan Kebo* weed. The plants were identified at the Herbarium Medanense (MEDA) of the University of North Sumatra under the species name of *Euphorbia hirta* L. and the identification number 2770/MEDA/2024. Samples weighing up to 5 kg were collected, washed, sorted, and dried in a *simplicia* dryer at a temperature of ± 50°C for one week. After the sample had dried, it was ground using a blender to create *simplicia* powder.

Making of Patikan Kebo Weed Extract

The *Patikan Kebo* weed extract was prepared by weighing 500 grams of the powdered *Patikan Kebo* weed and transferring it to a container. The powdered *simplicia* was extracted using the maceration method. In order to conduct maceration, 1.5 L of ethanol solvent was soaked (pro analysis) to ensure that the *simplicia* powder had completely submerged in the ethanol solvent, every day for approximately three days, the macerate undergoes intermittent stirring. The macerate was filtered using Whatman filter paper no. 1. The macerate results were evaporated using a rotary evaporator to produce a thick extract of the *Patikan Kebo* weed (Simanjuntak & Rahmiati, 2021).

Phytochemical Screening

Qualitative phytochemical screening tests were conducted on simple drugs and extracts to determine the secondary metabolite groups present in the *Patikan Kebo* weed plants. Alkaloid tests (Dragendorff, Wagner, and Mayer reagents), flavonoids (Mg powder and concentrated HCl), saponins, tannins (iron (III) chloride reagent), and steroids/triterpenoids (Lieberman-Bouchard reagent) were employed to identify several secondary metabolites (Johnson et al., 2018).

Procedures and Formulations of Patikan Kebo Weed Extract Cream

The oil phase (stearic acid, cetyl alcohol) is melted over a water bath (mass 1) to produce the cream. A water bath (mass 2) is used to heat the water phase, which consists of TEA, methyl paraben, propyl paraben, and glycerin dissolved in water. Gradually combine masses 1 and 2, and then grind until a cream mass is achieved. The extract is incorporated into the grinding process until it is homogeneous and smooth. It is then placed in a container. Table 1 illustrates the test formula utilized, which is a modification of Safitri et al., (2016).

Table 1. Formulation of *Euphorbia hirta* L. (*Patikan Kebo*) Weed Extract Cream

Material	Concentration %					Utility
	F1	F2	F3	F4	F5	
Patikan Kebo Weed Extract	0	1	2	3	4	Active substance
Stearic acid	12	12	12	12	12	Emulsifier
Cetyl alcohol	0.5	0.5	0.5	0.5	0.5	Thickener
TEA	1	1	1	1	1	Emulsifier
Methyl paraben	0.1	0.1	0.1	0.1	0.1	Preservative
Propyl paraben	0.5	0.5	0.5	0.5	0.5	Preservative
Glycerin	2	2	2	2	2	Humectant
Distilled water	100	100	100	100	100	Solvent

Stability Test of Cream Preparations

Stability testing of cream preparations aimed to determine the quality of cream preparations to be applied to the skin. Some of the tests carried out were:

a. Organoleptic Test

Organoleptic determination was carried out by conducting visual observations of cream preparations such as color, texture and aroma/smell (Dudhe et al., 2023).

b. Homogeneity Test
Homogeneity test determination was done visually and by touch. Weighed 1 gram of cream was applied to a glass object then observed under a microscope to see the uniformity of particles, the absence of grains, lumps and the distribution of cream is more even or homogeneous (Evelia et al., 2024).

c. pH test
The acidity level (pH) determination in the cream aimed to be applied to the skin. The ideal pH of the cream was 4.5-8.0 by weighing 5 grams of cream and then dissolving it in 50 ml of distilled water. Then the cream suspension was measured with a pH meter (Prajakta & Shahu, 2020).

d. Spreadability Power Test
The spreadability test determination with the area of spread applied topically to the skin. The effectiveness of formulation therapy depended on its spreadability value (Prajakta & Shahu, 1970). We weighed 1 gram of cream placed on a watch glass, then covered it with a watch glass and gave it a load. The diameter of the spread area was calculated (Evelia et al., 2024). Spreadability was between 5 and 7 cm (Roosevelt et al., 2019).

e. Viscosity Test
The viscosity test determination was related to the spreadability of the preparation and played an important role in determining the quality of the emulsion. The viscosity was done using a Brookfield viscometer at a speed of 50 rpm using a spindle tool number 03 at a speed of 50 rpm (Tungadi et al., 2023).

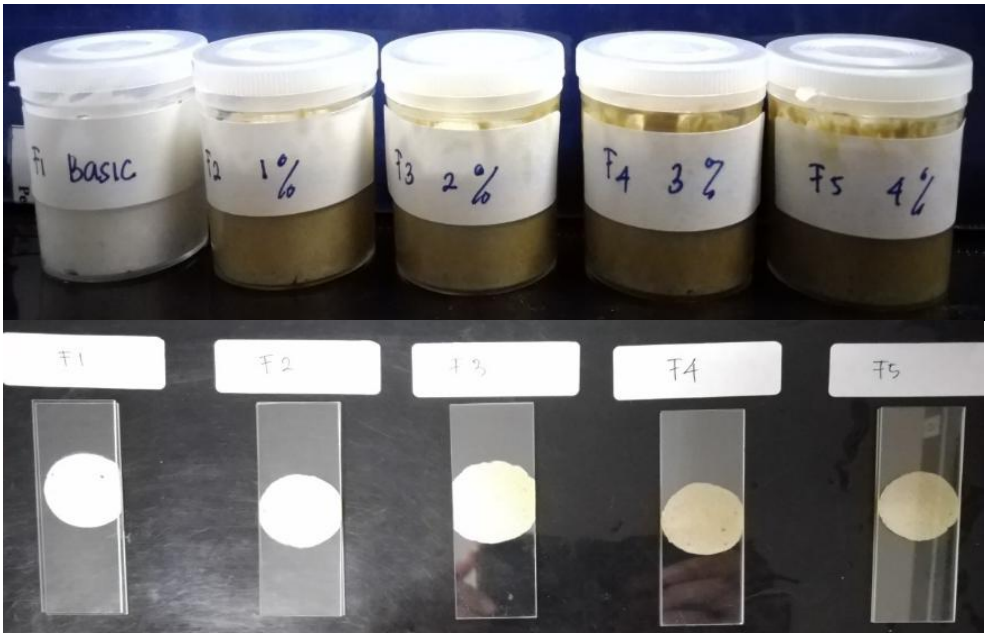

Figure 1. Formulation of Cream Preparation of *Patikan Kebo* Weed Extract

In Vitro Anti-Acne Activity Test

The determination of anti-acne activity in vitro was carried out by testing the antibacterial activity of acne-causing bacteria, namely *Propionibacterium acnes*, *Staphylococcus aureus*, and *Staphylococcus epidermidis*, using the well diffusion method (cup plate technique). The MHA media was weighed at 38 grams and then dissolved in 1000 ml of distilled water. Then, it was heated until homogeneous and sterilized in an autoclave. The media was cooled to 40 - 50°C, then put into a petri dish. We waited until it solidified and dipped a cotton swab into the bacterial suspension. It then spread evenly on the media. The well holes in the agar media were made using a cork borer, then each well hole was marked (F1, F2, F3, F4, F5) and repeated three times. Incubated in an incubator at 37 °C for 24 hours. The diameter of the clear zone formed around the formula was measured (Evelia et al., 2024).

Data analysis

The data analysis was carried out descriptively, namely by measuring the diameter of the clear zone formed around each formula according to Davis & Stout (1971) categories in Table 2.

Table 2. Inhibition Zone Diameter Category

Inhibition Zone Diameter (mm)	Category
< 5	Low
5 - 10	Medium
10 - 20	Strong
> 20	Very strong

RESULT AND DISCUSSION

Phytochemical Screening Results

Secondary metabolite testing was carried out by phytochemical screening tests on *simplicia* powder and the *Patikan Kebo* weed extract. Phytochemical screening aims to identify the secondary metabolite compounds that contribute to the antibacterial activity responsible for causing acne. Table 3 displays the results of the phytochemical screening.

Table 3. Results of Phytochemical Screening of Simplex and Ethanol Extract of *Patikan Kebo* Weed

Number	Secondary Metabolite Groups	Results	
		Simple	Extract
1.	Alkaloid	+	+
2.	Flavonoid	+	+
3.	Saponins	+	+
4.	Tannin	+	+
5.	Steroids/Tritepnoids	+	+

Description: + = secondary metabolite groups found

The secondary metabolite contents of the *Patikan Kebo* weed plant (*Euphorbia hirta* L.) are classified into the alkaloid, flavonoid, saponin, tannin, and steroid/triterpenoid groups as a result of phytochemical screening tests conducted on the simplex and extracts of all parts of the plant. The medicinal properties of the secondary metabolite groups found in plants are responsible for the provision of pharmacological effects, including antimicrobial, antioxidant, and anti-inflammatory properties. At a concentration of 75 %, the *Patikan Kebo* extract contains compounds that include alkaloids, flavonoids, saponins, tannins, and steroids/triterpenoids. These compounds have the potential to act as antimicrobials against *Staphylococcus epidermidis* bacteria (18.78 mm), *Escherichia coli* (18.47 mm), and *Candida albicans* fungus (14.17 mm) (Simanjuntak & Rahmiati, 2021).

In the aerial ethyl acetate extract of the *Euphorbia hirta* plant, flavonoids, alkaloids, and phenolics are found and have antioxidant activity with an IC_{50} of 32.23 µg/mL and anti-inflammatory activity with a maximum inhibition of 68.20 % at a concentration of 1000 µg/mL (Basyal et al., 2021). The methanol extract of all parts of the *Patikan Kebo* plant was found to contain reducing sugars, terpenoids, alkaloids, steroids, tannins, flavonoids, and phenolics, which have the potential as antioxidants (Basma et al., 2011). Meanwhile, the chloroform and hexane extracts of the leaves and flowers ofcthe *Patikan Kebo* plant are found to contain alkaloids, flavonoids, terpenoids, tannins, and carbohydrates in chloroform solvents (leaves, flowers), while the hexane solvent (leaves, flowers) was found to contain flavonoids. The extract of the *Patikan Kebo* herb with an ethanol solvent has an antibacterial activity of 15 mm and a water solvent of 13 mm against *Staphylococcus aureus* bacteria (Ahmad et al., 2017).

Evaluation Results of Ethanol Extract Cream Preparation of *Patikan Kebo* Weed

The goal of the evaluation test for natural cream preparations made from the *Patikan Kebo* weed extract is to ascertain the quality of these cream preparations for topical application. Table 4 presents the evaluation results of the cream preparation.

Table 4. Cream Preparation Evaluation Results

Formula	Organoleptic			Homogeneity	pH	Spread Power (cm)	Viscosity (cPs)
	Color	Texture	Aroma				
F1	White	Semisolid	There isn't any	No grains/Homogeneous	6	5.5	2120
F2	Greenish brown	Semisolid	Typical	No grains/Homogeneous	7	5.6	2350
F3	Greenish brown	Semisolid	Typical	No grains/Homogeneous	6	5.6	2880
F4	Greenish brown	Semisolid	Typical	No grains/Homogeneous	6	5.6	3110
F5	Greenish brown	Semisolid	Typical	No grains/Homogeneous	6	5.6	3220

Numerous tests, including homogeneity, pH, spreadability, viscosity, and organoleptic evaluation, are employed to standardize cream preparations.

Organoleptic results indicate that the cream formulation is white in F1 and greenish brown in F2-F5. All formulations are semisolid, and formulas F2-F5 possess a unique aroma. The success of a cosmetic product in the form of a cream preparation that consists of color, shape, and aroma characters can be determined using sensory organoleptic profile parameters (Adejokun & Dodou, 2020). The cream preparation's homogeneity is determined by the absence of lumps and granules and the even distribution of the cream composition on the skin. The uniformity of particle size in the cream preparation is demonstrated by its homogeneity. The study's findings suggested that all formulations were homogeneous. The physical quality of the cream preparation is influenced by the homogeneity test, which ensures that it is evenly dispersed and does not clump (Tungadi et al., 2023).

The formulation's pH value determines the quality of the cream preparation during topical application. Skin health issues may result from creams that have an excessively alkaline or acidic pH. Scaly skin can result from an alkaline pH, while skin irritation can be caused by an acidic pH (Satpute & Kalyankar, 2019). The findings indicated that all formulations were still regarded as suitable for the skin and had a pH range of 6-7. The optimal cream pH for the skin is 4.5-8.0, as stated in SNI 16-4399-1996.

The efficacy of topical therapy and the standard dosing on the skin are significantly influenced by the spreadability of the cream formulation. The cream's spreadability is determined by the diameter of the cream spread (Tan et al., 2022). The cream will be of superior quality as the diameter of the spread increases. This parameter pertains to the cream preparation's capacity to absorb and diffuse when applied to the skin. The study's findings indicated that the spreadability's diameter fell within the range of 5.5 to 5.6 cm. The ideal spreadability of cream is between 5 and 7 cm (Murdiana et al., 2022), ensuring that all formulations have the desired spreadability. Physical properties, chemical properties, consistency, polarity, and viscosity are among the numerous factors that influence spreadability (Franco-Gil et al., 2024).

Viscosity aims to determine the amount of flow resistance in a liquid. Viscosity plays an important role in determining the quality of the emulsion, which depends on the room temperature. The results indicated that the viscosity of the cream preparation ranged from 2120 to 3220 cPs. The viscosity value of 4000-40,000 cPs is a good viscosity in semisolid preparations so that all appropriate formulations have met the physical-chemical standards of cream preparations (Rafique & Shah, 2019).

In Vitro Anti-Acne Activity Test Results

The ability of the formulation of the cream preparation of the *Patikan Kebo* weed extract as an anti-acne can be seen from the antibacterial activity of acne-causing agents with the parameter of the diameter of the clear zone formed. Table 5 presents the formulations that have antibacterial activity. Based on table 5, it shows that the formulation of ethanol extract cream of the *Patikan Kebo* weed has antibacterial activity against acne-causing bacteria along with the increase in extract concentration in the cream formulation. This indicates that the extract content in the formulation influences its antibacterial activity against *Propionibacterium acnes, Staphylococcus epidermidis, and*

Staphylococcus aureus bacteria. The ability of the *Patikan Kebo* extract cream formulation as an anti-acne can be associated with the presence of chemical components that have the potential to be antibacterial.

Table 5. In Vitro Anti-Acne Activity Test Results

Formula	Average Diameter of Inhibition Zone (mm)		
	Propionibacterium acnes	***Staphylococcus epidermidis***	***Staphylococcus aureus***
F1	0	0	0
F2	7.43±0.81	6.83±0.29	6.83±0.29
F3	9.70±0.40	9.82±0.49	9.63±2.00
F4	12.03±0.81	11.48±1.10	12.07±0.06
F5	13.53±0.15	12.54±0.75	13.03±0.61

Based on the criteria for the diameter of the inhibition zone of Davis & Stout (1971), F2-F3 showed a moderate category against all test bacteria with criteria of 5-10 mm, and F4-F5 showed a strong category against all test bacteria with criteria of 10-20 mm. So that F4-F5 is more effective as an anti-acne in vitro. Previous research has been conducted by Gani et al., (2020), explaining that the o/w cream of water extract of the *Patikan Kebo* herb has antioxidant activity so that it shows potential in cosmetic use. The methanol extract of *Euphorbia hirta* cream is able to inhibit dermatophytosis infection fungi (Gi et al., 2022). The combination of nanocellulose with 1.5% *Euphorbia hirta* extract showed antibacterial activity against *Staphylococcus epidermidis* with the parallel streak method (Barus et al., 2023).

Based on research by Pramod et al., (2020), it is explained that *Euphorbia hirta* serum at a concentration of 1500 mg/ml has a *Propionibacterium acnes* bacterial inhibition zone diameter of 14 mm. The formulation of Euphorbia hirta leaf ethanol extract ointment at a concentration of 15% has antibacterial activity against acne-causing bacteria such as *Staphylococcus epidermidis* with an inhibition zone diameter of 13.60 mm (Ningtias & Aniqoh, 2024). The ability of *Euphorbia hirta* as an acne-causing antibacterial formulated in cosmetic preparations is closely related to the presence of phytochemical compounds contained in it. So that the formulated the *Patikan Kebo* extract still shows its pharmacological activity. The F4-F5 cream formula effectively stops the growth of acne-causing bacteria like *Propionibacterium acnes, Staphylococcus epidermidis,* and *Staphylococcus aureus*, and it meets the necessary quality standards. The F4-F5 cream formula can be used as an anti-acne cosmetic with further research.

CONCLUSION

Based on the results of the research that has been done, it can be concluded that the formulation of *Euphorbia hirta* L. The *Patikan Kebo* weed extract cream has activity in inhibiting the growth of acne-causing bacteria, especially formulas F4 and F5. The diameter of the F4 inhibition zone has antibacterial activity against acne-causing *Propionibacterium acnes* (12.03 ± 0.81 mm), *Staphylococcus epidermidis* (11.48 ± 1.10 mm), *Staphylococcus aureus* (12.07 ± 0.06 mm), and F5 *Propionibacterium acnes* (13.53 ± 0.15 mm), *Staphylococcus epidermidis* (12.54 ± 0.75 mm), and *Staphylococcus aureus* (13.03 ± 0.61 mm), so that F4 and F5 are more effective as anti-

acne with a strong inhibition zone diameter. These findings provide a scientific basis that *Euphorbia hirta* L. extract has the potential to be developed as a natural active ingredient in topical anti-acne formulations. Further research is needed in the form of preparation stability tests, in vivo tests, and clinical trials to ensure the effectiveness and safety of its use for commercial applications.

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