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Editor-in-Chief: Dr. dr. Dewa Putu Wisnu Wardhana, S.Ked, MHPE, Sp.BS, Susp.TB(K), FICS, FINSS, FACS — Faculty of Medicine, Universitas Udayana

Section: **Biomedicine, Surgery, Medicine, Neurosurgery, Spine surgery** • **Open Access**

Original Article

CAVERA: Hydrogel Spray Combining Sea Lettuce (Ulva lactuca) and Aloe Vera (Aloe vera L) Extracts as an Anti-inflammatory and Antibacterial Agent for Atopic Dermatitis

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Received: 01 July 2026
Accepted: 20 July 2026
Published: 12 August 2026

Article Type
Literature Review

ABSTRACT

Atopic dermatitis is a skin condition commonly experienced by people of all ages, with a prevalence of 15–20% worldwide. The standard treatment for this condition is Topical Corticosteroids (TCS). However, the use of corticosteroids can lead to non-adherence due to discomfort and local and systemic side effects. Given these limitations, a hydrogel spray formulation could be an appropriate solution. A combination of sea lettuce and aloe vera extracts was formulated into a hydrogel spray named CAVERA. In this study, three treatments were formulated based on varying concentrations of the extract combination. Phytochemical and FT- IR analysis of the extract revealed the presence of flavonoids (quercetin, kaempferol), tocopherols, tannins, saponins, triterpenoids, quinones (aloin and emodin), and polysaccharides (alpha-glycosides). Antibacterial testing of the extract combination against *Staphylococcus aureus* at a 100% concentration showed the highest inhibition zone, with a diameter of 19.57 mm. Homogeneous results were obtained in the CAVERA homogeneity test, and the results showed that C(-) and P1 were stable, while P2 and P3 were unstable in the stability test. pH values of 5.7, 5.3, and 5.2 were obtained, and only P1 fell within the viscosity range. In tests of spray patterns and formulation weight, the results showed that as viscosity decreased, so did the weight. In the organoleptic test, P1 was found to be the most preferred in terms of color, texture, and aroma. The effectiveness of CAVERA as a hydrogel spray was tested using an in vivo atopic dermatitis model in mice, and the results showed that P3 significantly reduced the thickness of the dorsal skin. In the scratching behavior test, the results showed a significant reduction in scratching behavior with P3 compared to CN. In the histological test, P3 showed a significant reduction in epidermal thickness and skin keratin on the back compared to C(-).

Keywords: *CAVERA, Atopic dermatitis, Sea Lettuce, Aloe Vera, Skin thickness.*

ABSTRAK

Dermatitis atopik merupakan penyakit kulit yang kerap dialami segala usia dengan prevalensi 15-20% di seluruh dunia. Medikasi standar yang digunakan dalam penanganannya adalah kortikosteroid topikal (TCS). Namun, penggunaan kortikosteroid dapat menimbulkan ketidakpatuhan akibat ketidaknyamanan dan efek samping pada bagian lokal dan sistemik tubuh. Dengan kekurangan tersebut, sediaan *hydrogel spray* dapat menjadi solusi tepat. Dilakukan kombinasi ekstrak selada laut dan aloe vera dalam *hydrogel spray* yang diberi nama CAVERA. Dalam penelitian ini dibuat 3 perlakuan berdasarkan perbedaan konsentrasi kombinasi ekstrak. Didapat hasil uji fitokimia dan FT-IR ekstrak, yaitu senyawa flavonoid (quercetin, kaempferol), tokoferol, tanin, saponin, triterpenoid, kuinon (aloin dan emodin), dan polisakarida (alfa glikosida). Pengujian antibakteri kombinasi ekstrak terhadap *Staphylococcus aureus* pada konsentrasi 100% memiliki zona hambat tertinggi, yaitu diameter 19,57 mm. Didapatkan hasil homogen pada uji homogenitas CAVERA serta didapat hasil K(-) dan P1stabil, serta P2 dan P3 tidak stabil pada uji stabilitas. Didapat pH sebesar 5,7; 5,3; dan 5,2 serta hanya P1 yang masuk rentang viskositas. Pada uji pola penyemprotan dan bobot sediaan, didapat hasil semakin rendah viskositas maka semakin rendah bobotnya. Pada uji organoleptis didapat hasil P1 paling disukai pada parameter warna, tekstur, dan aroma. Dilakukan pengujian efektivitas CAVERA sebagai *hydrogel spray*, berdasarkan uji In Vivo model dermatitits atopik pada mencit dan didapat hasil P3 memiliki penurunan ketebalan kulit punggung yang berkurang signifikan. Pada pengujian perilaku menggaruk, didapat hasil penurunan perilaku menggaruk yang signifikan pada P3 jika dibandingkan dengan KN. Pada uji histologi, P3 memiliki penurunan ketebalan epidermis dan keratin kulit punggung yang signifikan dibandingkan dengan K(-).

Keywords: *CAVERA, Atopic dermatitis, Sea Lettuce, Aloe Vera, Skin thickness.*

INTRODUCTION

Background

Atopic dermatitis (eczema) is a skin condition commonly experienced by people of all ages worldwide. This is supported by data from the International Study of Asthma and Allergies in Childhood (ISAAC), which indicates that atopic dermatitis can affect children in their first decade of life with a prevalence of 15–20% worldwide and adults with a prevalence of 10% (Susanto, 2022; IEC, 2022). Atopic dermatitis has complex causes, it can be influenced by genetic and environmental factors, leading to abnormalities in the epidermis and the immune system. Complications can also easily occur in patients with atopic dermatitis, such as skin that is prone to colonization and infection by *Staphylococcus aureus*. MRSA is a form of *S. aureus* resistant to methicillin and can cause complications such as skin and soft tissue infections (SSTIs) (Wang *et al*., 2021). The complexity of atopic dermatitis manifests in five main pillars of its management, some of which include strengthening the skin barrier function, reducing inflammation, and breaking the itch-scratch cycle (Susanto, 2022).

The standard medication used after moisturizers in the management of atopic dermatitis is topical corticosteroids (TCS), which help repair the skin barrier and reduce *Staphylococcus aureus* colonization on atopic dermatitis lesions (Wang et al., 2021). However, the use of TCS and general moisturizers can lead to non-adherence to treatment due to time-consuming application, occlusive moisturizers that are too oily and messy to use, and concerns about side effects and "steroid phobia" (Sivyer et al., 2022). TCS can cause various local and systemic side effects due to suppression of the hypothalamic-pituitary-adrenal axis (HPA axis) (Susanto, 2022). Some local side effects, such as skin atrophy, perioral dermatitis, rosacea, and others, may occur with long-term use. Systemic effects include increased strain on the adrenal glands, particularly in infants and children, and hyperglycemia due to enhanced alanine transport (Susanto, 2022; Sue and Milanesi, 2019). Therefore, given the side effects and limitations of TCS therapy, a hydrogel spray formulation containing herbal medicine may serve as an appropriate solution to optimize medication use in patients with atopic dermatitis.

Herbal medicines can serve as complementary treatments because they cause minimal side effects compared to conventional drugs. The use of herbal medicines is supported by the availability of easily accessible plants (Dewi et al., 2023). In the prevention and treatment of atopic dermatitis, it is necessary to strengthen the skin barrier function, reduce inflammation, and reduce *Staphylococcus aureus* colonization. Sea lettuce (*Ulva lactuca*) is highly nutritious and represents one of the marine commodities that has not yet been fully utilized. In Indonesia, sea lettuce is found along the eastern coastal regions, including several locations in Bali such as Sanur Beach, Serangan Beach, Sawangan Beach, Nusa Penida,

and Nusa Dua (Yunita et al., 2018). Several studies indicate that sea lettuce contains saponins, flavonoids, and triterpenoids that function as antibacterial agents (Dewi et al., 2023). Quercetin and kaempferol, which belong to the flavonoid group, have also been detected in sea lettuce and exhibit anti-inflammatory effects (Putra et al., 2024). Sea lettuce also contains alpha tocopherol, a form of vitamin E that can stabilize the skin barrier, protect the skin from irritants, and prevent excessive water loss (Yunita et al., 2018). Thus, the compounds in sea lettuce can be utilized as a herbal remedy for atopic dermatitis.

In addition to sea lettuce, aloe vera also acts as a moisturizer that can strengthen the skin barrier, thereby reducing the risk of atopic dermatitis (Rosita, 2022). Aloe vera contains mucopolysaccharides that help retain moisture in the skin and provide a soothing, cooling sensation, thereby reducing the risk of patients scratching the resulting lesions. Aloe vera contains anthraquinone, campesterol, sitosterol, and lupeol compounds that act as anti-inflammatory and antibacterial agents (Puteri and Milanda, 2016). One development involving aloe vera in pharmaceutical products is the topical M/A cream formulation, which has been shown to reduce TEWL (trans epidermal water loss) (Kurnia and Ratnaputri, 2019). The compounds found in aloe vera also hold potential for use as a herbal treatment for atopic dermatitis.1Based on the issues outlined above, the researchers are interested in conducting research on the production of a hydrogel spray utilizing sea lettuce and aloe vera based on alginate hydrogel.

PROBLEM STATEMENT

Atopic dermatitis (eczema) is a condition commonly experienced by people of all ages worldwide. Complications can easily arise in patients with atopic dermatitis, such as skin that is prone to colonization and infection by *Staphylococcus aureus*. Furthermore, corticosteroids remain the standard therapy for this condition; however, their use carries side effects including skin atrophy, which can increase skin sensitivity to allergens and risk exacerbating infections. To optimize drug use and minimize the side effects of corticosteroids, the use of herbal preparations as an adjunct to conventional drugs in the treatment of atopic dermatitis is necessary. A hydrogel spray formulation combining sea lettuce and aloe vera extracts, named CAVERA, can be an appropriate solution for optimizing drug use in patients with atopic dermatitis. Thus, the research question arises:

1. How effective is the combined extract of sea lettuce and aloe vera as an antibacterial agent in vitro against *Staphylococcus aureus*?
2. What are the characteristics of CAVERA as a hydrogel spray based on characterization and organoleptic tests?
3. What is the efficacy of CAVERA as a hydrogel spray based on an in vivo test using anatopic dermatitis model in mice (*Mus musculus*)?

RESEARCH OBJECTIVES

The objectives of this study are as follows:

1. To determine the in vitro antibacterial efficacy of a combined extract of sea lettuce and aloe vera against *Staphylococcus aureus.*
2. To determine the characteristics of CAVERA as a hydrogel spray based on physical and organoleptic tests.
3. To determine the efficacy of CAVERA as a hydrogel spray based on an in vivo test using an atopic dermatitis model in mice (*Mus musculus*).

RESEARCH HYPOTHESIS

1. Hypotesis h (0): The concentration of the extract from CAVERA Hydrogel Spray has no effect on in vitro antibacterial activity, in vivo anti-inflammatory activity, and physical characteristics.
2. Hypotesis h (1): The concentration of the extract from CAVERA Hydrogel Spray has an effect on in vitro antibacterial activity, in vivo anti-inflammatory activity, and physical characteristics.

RESEARCH BENEFITS

1. To explore the potential of active compounds found in sea lettuce and aloe vera as a treatment for atopic dermatitis.
2. For the public, this research offers the opportunity to utilize sea lettuce and aloe vera as plants that can be used to treat atopic dermatitis.
3. For the government, this helps reduce the incidence of atopic dermatitis in Indonesia through prevention and treatment.

LITERATURE REVIEW

Dermatitis Atopik

Atopic dermatitis is a chronic, recurrent inflammatory skin condition characterized by severe itching. In the chronic phase of atopic dermatitis, a characteristic feature is lichenification caused by the disease atopic themselves or their families (Yudho, 2020). Atopic dermatitis can occur due to abnormalities in the skin's epithelial cells, which impair the skin's natural protective function. This impairment is partly caused by a decrease in ceramide levels, which results in a reduced ability of the skin to prevent transepidermal water loss (Logan, 2023).

A decrease in the skin's ability to prevent transepidermal water loss (TEWL) can lead to xerosis (Yudho, 2020). As a result, irritants and allergens can penetrate the skin, triggering inflammation through an overactive Th2 immune response in the acute phase and a Th1 response in the chronic phase (Logan, 2023).

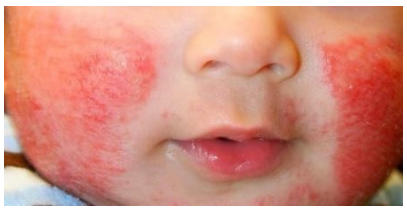

Figure 1. *Atopic dermatitis (Iskandar, 2019)*

Sea Lettuce (Ulva lactuca)

Sea lettuce can be found in shallow, rocky waters. Sea lettuce has thin, sheet-like thalli that appear thickened at the base, its texture is smooth and sheet-like, ranging in color from light to dark green and sometimes appearing translucent. Sea lettuce grows attached using its short stalks connected to thin leaves or using disk-like structures that act as adhesives on rocks (Anggreni, 2020). Sea lettuce contains saponins, flavonoids, and triterpenoids that prevent bacterial cell wall formation by damaging the peptidoglycan components of bacterial cells, thereby altering cell membrane permeability. This leads to an imbalance in water influx into bacterial cells, resulting in bacterial death. Quercetin, as an antiinflammatory agent, inhibits the production of endogenous pro-inflammatory cytokines, thereby reducing the inflammatory response (Setiawan., 2024). Sea lettuce also contains alpha-tocopherol, a form of vitamin E that can stabilize the skin barrier, protect the skin from irritants, and prevent excessive water loss (Yunita et al., 2018). The taxonomy of sea lettuce is as follows:

Kingdom : Plantae
Divisi : Thallophyta
Kelas : Chlorophyceae
Ordo : Ulvales
Famili : Ulvaceae
Genus : Ulva
Spesies : Ulva lactuca (Dewi et al., 2023)

Figure 2. *Selada laut (Dewi et al., 2023)*

Aloe Vera (Aloe vera L)

Aloe vera typically grows as a wild plant or is cultivated in home gardens as an ornamental plant. This plant is drought-tolerant in arid regions, making it well-suited for cultivation in Indonesia (Sikumbang, 2020). The aloe vera stem is short and spear-shaped and its leaves are upright with relatively blunt spines along the edges. Aloe vera leaves are green and thick due to the presence of a clear, gel-like substance within the leaf, which is used as a raw material for medicine (Kurnia and Ratnapuri, 2019). Anthraquinones, sterols, and other natural substances, including polysaccharides in aloe vera, work synergistically to produce anti-inflammatory effects. Aloe vera can inhibit the growth of Staphylococcus aureus bacteria, a complication of atopic dermatitis. This antibacterial mechanism is attributed to saponins and anthraquinones, which stimulate leukocyte phagocytosis to destroy bacteria. Aloe vera also contains mucopolysaccharides that help maintain skin moisture and provide a soothing cooling sensation, thereby reducing the risk of patients scratching the resulting wounds (Kurnia and Ratnapuri, 2019). As for the taxonomy of aloe vera, it is as follows:

Kingdom : Plantae
Divisi : Magnoliopyhta (Tumbuhan berbunga)
Kelas : Liliopsida
Ordo : Asparagales
Famili : Asphodelaceae
Genus : Aloe
Spesies : Aloe vera L (Itrat, 2023: Sikumbang, 2020)

Figure 3. *Lidah buaya (Alina, 2021)*

Hydrogel Spray

Hydrogel spray is a gel with an aqueous dispersion phase that uses a suitable hydrophilic gelling agent. A hydrogel is a three dimensional network of cross-linked polymer chains in a hydrophilic polymer, enabling it to absorb large amounts of water. Suitable gelling agents include hydrophilic polymers such as carbopol, sodium alginate, hydroxypropyl methylcellulose (HPMC), and others (Dewi et al., 2021). Hydrogel release occurs via diffusion, which involves the bulk movement of the polymer matrix. Through chemical stimulation such as changes in pH, temperature, or enzymatic action, the gel swells and can open its pores to release the entrapped drug (Dewi et al., 2021). Many patients exhibit poor adherence to topical therapy for atopic dermatitis. This may occur due to occlusive moisturizers being too greasy, causing a messy application (Mayba and Gooderham, 2017), time-consuming medication application, children's refusal, and concerns about TCS side effects or "steroid phobia" (Sivyer et al., 2022). Given the limited effectiveness of these topical medications, hydrogel sprays may offer a suitable solution. The benefits of using this formulation include higher absorption rates, a spray format that facilitates application and makes it more practical, relatively lower microbial contamination since no applicator is used, and reduced irritation from topical application (Dewi et al., 2021).

Staphylococcus Aureus

More than 90% of patients with atopic dermatitis have Staphylococcus aureus colonies (Logan, 2023). The presence of Staphylococcus aureus can potentially disrupt the skin's protective barrier, reduce lipid levels, increase skin pH, and impair the immune response (Yudho, 2020). Staphylococcus aureus can produce polysaccharide and protein biofilms, enabling the bacteria to adhere to the stratum corneum of the epidermis with the assistance of fibronectin and fibrinogen (Yudho, 2020). Staphylococcus aureus fibronectin has a specific affinity for type 2 inflammation. Additionally, enterotoxins (superantigens) produced by Staphylococcus aureus can disrupt the skin barrier and exacerbate type 2 inflammation (Wang et al., 2021).

Domain : Bacteria
Kingdom : Eubacteria
Ordo : Eubacteriales
Famili : Staphylococcaceae
Genus : Staphylococcus
Spesies : Staphylococcus aureus (Tamam, 2016)

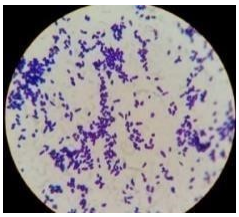

Figure 4. *Bakteri Staphylococcus aureus (Wijaya, 2019)*

RESEARCH METHODS

Time and Location of the Research

This research was conducted at the Chemistry Laboratory of Denpasar State High School 3, the Biomedical Laboratory of the Faculty of Medicine at Udayana University, the Pharmaceutical Laboratory of the Faculty of Pharmacy at Mahasaraswati University, the Bali Provincial Health Laboratory, the Laboratory of the Faculty of Agriculture at Warmadewa University, and the Histopathology Laboratory of the Denpasar Veterinary Research Institute. The research was conducted from May to September 2024.

Tabel 1. *Research Period*

No	Activity	Month				
		1	2	3	4	5
1	Preparation of crude drugs					
2	Submission of ethical clearence, preparation for in vivo testing					
3	Extraction of sea lettuce and aloe vera					
4	Antibacterial testing using the solid diffusion method					
5	Formulation of the product					
6	Testing of product characteristics, in vivo anti-inflammatory testing					
7	Data collection, processing, and analysis					
8	Preparation and completion of the research report					

Materials and Equipment

Data source

This study is an experimental study. The data sources used in this study include primary and secondary data. Primary data were obtained through experiments at each research site, while secondary data were obtained from literature reviews. The complete research design is presented in Figure 5 below:

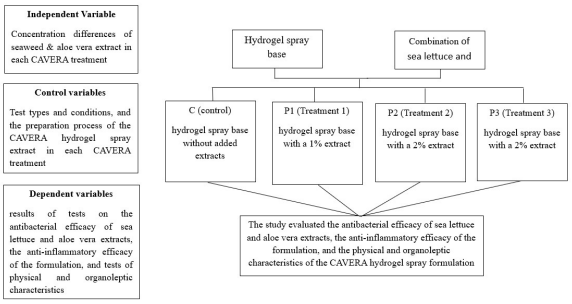

Figure 5. *Research design*

Research Equipment and Materials

The equipment used in this study included a blender, filter paper, a knife, a digital scale, a cutting board, a measuring cup, a stirring rod, spatula, separatory funnel, glass jar, sample bottle, incubator, petri dish, micropipette, filter paper, syringe, gloves, magnetic stirrer, vernier caliper, universal pH paper, animal cage, microscope slide, plastic, Brookfield viscometer, and ULA stirrer,hot plate, stopwatch, mortar. The materials used included sea lettuce, aloe vera, 70% ethanol, DNCB, sodium diclofenac, Carbopol, topical corticosteroids (TCS), distilled water, rice husks, rat chow, glycerin, methylparaben, propylparaben, and 70% alcohol.

Indicators, Parameters, and Research Workflow

The parameters in this study include the test parameters for the combined extract of sea lettuce and aloe vera, such as the antibacterial activity inhibition zone; the test parameters for the formulation characteristics, such as homogeneity, stability, pH, viscosity, spray pattern, formulation weight, spreadability, and organoleptic properties; and the anti-inflammatory efficacy test parameters were measured based on scratching activity, the thickness of the mouse back skin, and histological examination of the mouse back skin. The research flowchart is illustrated in Figure 6, as follows:

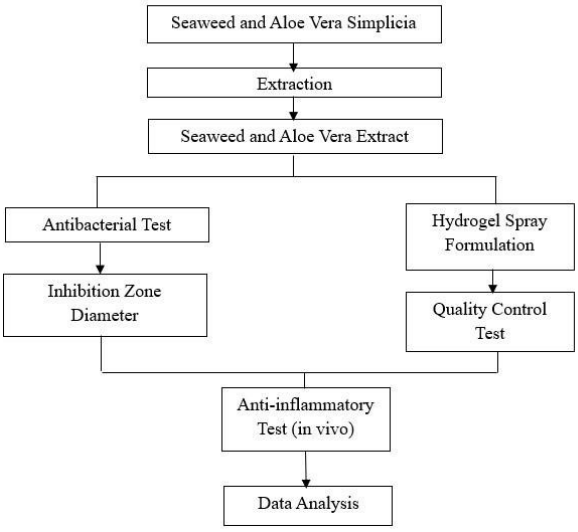

Figure 6. *Research Flowchart*

Research Procedure

The Process of Producing Sea Lettuce (Ulva lactuca) and Aloe Vera (Aloe vera L) Extracts (Makhunah and Rini, 2020).

The crude drug material was cleaned by washing to remove any adhering impurities. Once clean, the material was air dried for several days. The dried material was then ground using a blender. The ground crude drug is weighed and mixed with 70% ethanol as a solvent in a 1:10 ratio, then macerated for 3 days. The resulting extract is filtered using filter paper to separate the extract from the residue. The extract is then evaporated until it thickens. Finally, the extract is stored in an airtight container.

Testing Procedure for the Antibacterial Efficacu of Extratcs

The method used in this study was the agar diffusion method (Kirby and Bauer disc diffusion). 100 µL of cultured microorganisms were pipetted and inoculated into 100 mL of agar medium and stirred until the agar medium solidified. Test solutions were spotted onto each disk using a micropipette. After the agar solidified, the disks were spotted with a combination of Ulva lactuca and Aloe vera L extracts, C (+) Bioplacenton, and C (-) 70% ethanol, which were placed in Petri dishes using tweezers. The petri dishes were then labeled and incubated in an incubator at 37°C. Clear zone observations were made after a 24-hour incubation period. Clear zones were measured and categorized according to the guidelines of Davis and Stoud (1971) (Keintjem et al., 2019). This test was repeated three times.

Process for Preparing Hydrogel Spray (Puspita et al., 2020)

The combination of sea lettuce (Ulva lactuca) and aloe vera (Aloe vera L) extracts used was at concentrations P1 (1%), P2 (2%), and P3 (3%). The composition for preparing the hydrogel spray is shown in the following table:

Tabel 2. *Composition of the hydrogel spray Base Ingredients*

Base Ingredients	Formulation (%)			
	C(-)	P1	P2	P3

Combination extract of sea lettuce and aloe vera	-	1	2	3
Karbopol 940	0,5	0,5	0,5	0,5
Trietanolamine	0,5	0,5	0,5	0,5
Propylene glycol	10	10	10	10
Glycerin	10	10	10	10
Methylparaben	0,18	0,18	0,18	0,18
Propylparaben	0,02	0,02	0,02	0,02
Aquades ad	100	100	100	100

Gradually mix Carbopol 940, methylparaben, and propylparaben into warm distilled water while stirring with a magnetic stirrer until the mixture foams, then blend until homogeneous. The next step is to mix the Carbopol 940 base with triethanolamine and stir until thickened. Once thickened, propylene glycol and glycerin are added. The Carbopol 940 base mixture is combined with a blend of sea lettuce and aloe vera extracts according to their respective concentrations, then stirred until homogeneous to form a gel mass. The final CAVERA hydrogel spray is dispensed into a spray bottle.

Testing Procedure for the Physical Characteristics of Hydrogel Spray.

Homogeneity Testing Procedure

This test is designed to observe particles or substances that are not yet homogeneous. The test is conducted by spraying the hydrogel onto a transparent glass slide (Cendana et al., 2021).

pH Test Procedure

This test is performed using a universal pH indicator. It is conducted to measure the pH and assess whether the topical formulation meets the pH requirements (4.5–7) (Cendana et al., 2021).

Spray Pattern Test Procedure

This test is performed by spraying the formulation at distances of 3 cm, 5 cm, and 10 cm onto a plastic sheet. The sprayed plastic is then weighed, and the weight of the formulation adhering to the plastic is calculated as the amount of formulation dispensed (grams) per spray. The spray pattern and diameter formed are observed (Cendana et al., 2021).

Adhesive Spread Test Procedure

This test is conducted by spraying the formulation from 3 cm onto the skin surface of the upper arm. After spraying, the adhesion of the formulation to the skin surface is observed for 10 seconds to ensure it forms a strong, adherent layer on the skin and does not run (Cendana et al., 2021).

Viscosity Testing Procedure

This test is conducted to determine a liquid's resistance to flow. Hydrogel spray has a viscosity standard of 25–500 cPs to facilitate spraying. This test uses a Brookfield viscometer and a ULA stirrer (Estikoma et al., 2021).

Organoleptic Testing Procedure

The test was conducted by 30 panelists to visually assess the physical appearance of the hydrogel spray. The evaluation criteria for this test included color, aroma, texture, consistency, clarity, and the presence of separation. In Vivo Anti-Inflammatory Activity Testing in Mice (Mus musculus) with Atopic Dermatitis (Cendana et al., 2021).

Animal Testing Procedure for Mice (Mus musculus)

This study used 30 male mice, 6 weeks old, weighing approximately 20 grams. The mice used must be healthy, exhibit normal behavior, and show no significant abnormalities. The test animals were acclimated to the environment for one week in clean cages to minimize stress, they were fed a standard diet and provided with drinking water routinely and regularly, with bedding replaced every 3 days (Suryandari et al., 2021). The treatment groups were divided into 6 groups,as follows: C (normal control), C (-) (DNCB model treatment + hydrogel spray base), C (+) (2,4-dinitrochlorobenzene (DNCB) + Clobetasol 0.25%), P1 (DNCB + Clobetasol 0.25% + CAVERA hydrogel spray (1%)), P2 (DNCB + Clobetasol 0.25% + CAVERA hydrogel spray (2%)), P3 (DNCB + Clobetasol 0.25% + CAVERA hydrogel spray (3%)).
Next, the fur on the dorsal region of the mice was shaved using an electric razor to an area of approximately 2 cm × 3 cm, and the fur was removed with depilatory cream on day 0. On days 1 through 3, 0.2 ml of a 0.5% DNCB solution was mixed with a 3:1 mixture of acetone and olive oil in a separate container. This mixture was then applied to the dorsal skin on days 1 through 3 in each treatment group, except for the normal control group. From days 10 through 29, 0.2 ml of a 1% DNCB solution was applied to the dorsal region every 3 days. The DNCB induction treatment and in vivo testing are illustrated in Figure 7 as follows:

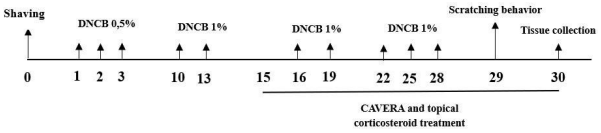

Figure 7. *In Vivo testing workflow*

On day 15, the groups began receiving CAVERA and 0.25% clobetasol propionate once daily for 15 consecutive days. Body weight and dorsal skin thickness measurements were recorded on days 22 and 29. Mice were sacrificed on day 30 by cervical dislocation under xylazine anesthesia, and dorsal skin samples were collected for histopathological examination (Wang et al., 2021).

Procedures for Measuring Body Weight and Skin Thickness of the Back and Ears

Body weight and skin thickness of the back and ears of the mice were recorded on days 22 and 29. A digital caliper was used to measure the thickness along the midline of the mice's back, and the thickness was recorded automatically (Wang et al., 2021).

Procedure for Measuring Scratching Behavior

Scratching behavior was observed on day 29, scratching is a characteristic symptom of atopic dermatitis. Mice were placed individually into transparent plastic cages for 60 minutes. Mouse behavior was recorded for 20 minutes, and the video clips were then reviewed by researchers blinded to the animal groupings. A mouse was scratching if it scratched with its hind paws. The number of scratching behaviors was calculated using a scoring system, where: 0 for no scratching; 2 for scratching for less than 1.5 seconds; 4 for scratching for more than 1.5 seconds (Wang et al., 2021).

Histological Examination Procedure on the Back Skin of DNCB-Treated Mice

The collected back skin samples were fixed in 10% neutral buffered formalin for 24 hours. The tissue was embedded in paraffin, then sectioned, deparaffinized, and rehydrated according to standard histological techniques. Sections 5 µm thick were then stained with hematoxylin and eosin (H&E) to identify general skin components, namely the epidermis, hyperplasia, hyperkeratosis, and inflammatory cell infiltration (Wang et al., 2021).

Data Processing and Analysis

Extraction Process (Metungun et al., 2023)

The analysis of extract yield data in this study involved descriptive analysis and was used to determine the percentage of extract produced from 100 grams of crude drug. It was calculated using the following formula:

$$\%Yield = \frac{\text{Extraxt weight (final)}}{\text{Extract weight (initial)}} \text{ x } 100\%$$

Statistical Analysis of the Antibacterial Test Using the Solid Diffusion Method (Keintjem et al., 2019)

Final data analysis of the activity of sea lettuce (Ulva lactuca) and aloe vera (Aloe vera L) extracts using a descriptive-qualitative method based on medium turbidity and bacterial growth inhibition activity, as well as the diameter of the clear zone or kill zone against Staphylococcus aureus bacteria for each concentration combination of sea lettuce and aloe vera extracts. The results of the inhibitory activity test were subjected to normality and homogeneity tests, the data were found to be non-normally distributed in group C (-). Therefore, a comparison test was conducted using the Kruskal- Wallis test, followed by the Mann-Whitney test to determine differences among the groups.

Analysis of Formulation Characteristics

The data obtained were summarized and analyzed using descriptive and analytical methods

Statistical Analysis of Anti-Inflammatory Tests (Suryandari et al., 2021)

The data obtained were statistically analyzed using computer software. First, a normality test was performed using the Shapiro-Wilk test, the data were considered normally distributed if the p-value was greater than 0.05 ($p > 0.05$). A homogeneity test was also performed using the Levene Test; the data were considered homogeneous if the p-value was greater than 0.05 ($p > 0.05$). In this study, the data were normally distributed and homogeneous, therefore, the effectiveness of the extract as an anti-inflammatory agent was assessed using a one-way ANOVA. The extract was considered effective as an anti-inflammatory agent if the p-value was <0.05. To determine the effectiveness between groups, the analysis was followed by a post hoc test.

RESULTS AND DISCUSSION

Research Results

Results of the Extract Effectiveness Test

Extract Yield Results for Sea Lettuce and Aloe Vera

Yield calculations were performed on each extract to determine the total potential mass of the extract dissolved

in a solvent (Metungun et al., 2023). The results of the yield calculations are presented in Table 4.1 as follows:

Table 3. *Table of Extraction Yields for Sea Lettuce and Aloe Vera*

Maceration no-	Crude Drug	Initial weight of the crude drug (gr)	Weight of the extract (gr)	Extract yield (%)
1	Sea lettuce	250	26	
2	Aloe vera	250	32	

Table 3 shows that the yield of the sea lettuce extract was 10.4%. Meanwhile, the aloe vera extract yielded 12.8%.

Results of Phytochemical Analysis of Sea Lettuce and Aloe Vera Extracts

Phytochemical analysis was conducted using the Harborne method (1987) to determine the content of secondary metabolites in sea lettuce and aloe vera extracts. The results of the phytochemical analysis are presented in Table 4 below:

Table 4. *Results of Phytochemical Testing of Sea Lettuce and Aloe Vera Extracts*

No	Chemical group	Observation of the reaction	Identification results	
			Sea lettuce	Aloe vera
1	Alkaloids (Dragendorff)	Brown sediment	-	-
2	Flavonoids (Wilstater)	Red or orange color	+	-
3	Saponins (Froth)	Stable foam forms	+	+
4	Tannins / Phenolics	Greenish-violet color	+	-
5	Triterpenoids / Steroids (Lieberman Burchard)	Red-orange/purple color	+	+
6	Quinones	Red color	-	-

Based on Table 4, it can be seen that sea lettuce extract contains secondary metabolites such as flavonoids of the quercetin and kaempferol groups, saponins, tannins, and triterpenoids. In comparison, aloe vera extract contains only saponins and triterpenoids as secondary metabolites.

FTIR Analysis Results of Sea Lettuce and Aloe Vera Extracts

TIR analysis was conducted at the Faculty of Mathematics and Natural Sciences' Integrated Laboratory at Udayana University. The analysis was performed on samples of sea lettuce and aloe vera extracts. The purpose of this analysis was to identify the functional groups present in the samples. The FTIR spectra of the samples are shown in Figure 8, as follows:

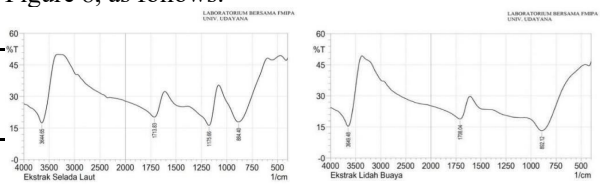

Figure 8. *FTIR Spectra of Sea Lettuce and Aloe Vera Extracts*

The interpretations of the bond types listed in Tables 5 and 6 are as follows:

Table 5. *Interpretation of FTIR Test*

Peak	Functional group
884.00	=C-H bend
1175.66	Alcohol (C-O stretch)
1713.83	Carboxylic acid (C=O)
3644.65	Hidroxyl (-OH)

Table 6. *Interpretation of FTIR Test Results for Aloe Vera Extract*

Peak	Functional group
892.12	=C-H bend
1708.04	Carboxylic acid (C=O)
3649.48	Hidroxyl (-OH)

In the FTIR spectrum of the sea lettuce extract sample, a C=O bond (1713.83) was detected, which is the primary functional group of fatty acids. In addition, an -OH functional group (3644.65) was identified, which is a hydroxyl group that constitutes phenolic components such as alpha- tocopherol. Alkene (=C-H bend) and alcohol (C-O stretch) functional groups are components of the compounds that make up the sea lettuce extract. Meanwhile, in the aloe vera extract, bonds were found indicating that the sample contains polysaccharide functional groups, marked by the presence of β- glycosidic bonds (Li, K et al., 2023) at a wavenumber of 892.12 cm^{-1} and C=O functional groups (1708.04 cm^{-1}). Additionally, an -OH functional group (3649.48) was identified, which is a hydroxyl group constituting phenolic components such as emodin and aloin (Chismirina, S., 2024).

Results of In Vitro Testing of a Combined Extract of Sea Lettuce and Aloe Vera Against Staphylococcus aureus

The in vitro test was conducted using the agar diffusion method at the laboratory of the Faculty of Agriculture, Warmadewa University. The in vitro test was performed to analyze bacterial growth inhibition activity and the diameter of the clear zone against Staphylococcus aureus. To assess differences in inhibitory activity, a statistical test using the Kruskal-Wallis test was performed. The test results are presented in Table 7 as follows:

Table 7. *Results of the Inhibitory Activity Test of Sea Lettuce and Aloe Vera Extracts against Staphylococcus aureus*

Treatment	Mean inhibition zone of Staphylococcus aureus (mm)			Grand mean (mm)	P-Value
	RU1	RU2	RU3		
C(-)	0	0	0	0	
C(+)	25,98	25,78	25,75	25,84	0,015*
75%	9,38	9,13	11,77	10,09	
100%	16,83	18,91	22,99	19,57	

Based on the results of the antibacterial activity test of the combined extract of sea lettuce and aloe vera, it was found that the extract combination at a 75% concentration had an average inhibition zone diameter of 10.09 mm, classified as having moderate antibacterial activity. The extract combination at a 100% concentration had an average inhibition zone diameter of 19.57 mm, classified as having strong antibacterial activity. Higher concentrations indicate a greater ability of the extract combination to inhibit the growth of Staphylococcus aureus. Statistical analysis using the Kruskal- Wallis test revealed significant differences between groups (p-value < 0.05). To determine the differences in inhibitory power among the groups, the analysis was continued using the Mann-Whitney test, as shown in Table 8 below:

Table 8. *Results of the Test on Differences in Bacterial Inhibitory Activity*

	P-Value	
Treatment	C (+)	C (-)
75%	0.05*	0.037*
100%	0.05*	0.037*

Based on Table 8 above, there are significant differences in bacterial inhibitory activity among the groups, as indicated by p-values ≤ 0.05. There is a significant difference in inhibitory activity between the 75% extract combination group and the C(+) and C(-) groups, with a p-value of 0.05. Meanwhile, the 100% extract combination group compared to the C(+) and C(-) groups has a p-value of 0.037.

Results of the CAVERA Hydrogel Spray Characterization Test

The characterization testing of the CAVERA hydrogel spray was conducted at the Pharmaceutical Laboratory of the Faculty of Pharmacy, Mahasaraswati University. The purpose of this testing was to determine the physical characteristics of the CAVERA hydrogel spray, including homogeneity testing, formulation pH, spray pattern, adhesion, formulation weight, viscosity, and stability.

Results of Homogeneity and Stability Tests for Hydrogel Spray

The homogeneity test was conducted to determine whether the active ingredient in the formulation is evenly distributed, thereby revealing whether the base and active ingredient are truly mixed uniformly. Stability testing was performed to determine whether phase separation occurs in the formulation. Stability refers to a formulation's ability to maintain its properties and characteristics over a specific period, remaining consistent with the formulation at the time of initial production (Cendana et al., 2021)

Table 9. *Results of homogeneity and stability for CAVERA*

Formulation	**Homogeneity**		**Stability**
	Transparency	Clumps	Identification results
C(-)	Clear	No clumps	No phase separation
P1	Clear	No clumps	No phase separation
P2	Clear	No clumps	Phase separation present
P3	Clear	No clumps	Phase separation present

Table 9 shows that all CAVERA hydrogel spray formulations are homogeneous. This indicates that the active ingredients used are compatible with the base, as they mix and blend homogeneously across all three concentration variations of the formulations. Stability test results show that groups C(-) and P1 did not exhibit phase separation, as indicated by the absence of liquid leaching out of the gel to form a layer on top of the preparation. This indicates that groups C(-) and P1 are stable. Meanwhile, groups P2 and P3 showed phase separation, characterized by liquid leaking from the gel and forming a layer on top of it. This indicates that groups P2 and P3 are unstable, a condition known as syneresis. Syneresis is a state in which

the formulation naturally shrinks when left undisturbed for a certain period (Cendana et al., 2021).

Test Results for pH, Viscosity, and Adhesion of Hydrogel Spray

A pH test was conducted to determine whether the pH value of CAVERA falls within the pH range required for topical formulations (4.5–6). A viscosity test was conducted to determine the consistency of a formulation, which affects comfort during application. A good hydrogel spray formulation should not be too runny but should be easy to spray. An ideal viscosity range is between 25–500 cps (Estikomah et al., 2021). The duration for which the formulation adheres indicates better drug efficacy. A gel spray formulation is considered to adhere if it does not drip after 10 seconds.

Table 10. *Results of pH, viscosity, and setting time tests for CAVERA*

Formulation	Average ± SD (pH)	Average ± SD (viscosity)	Setting time
C (-)	5.5 ± 0	-	>10 second
P1	5.71 ± 0.01	137 ± 2,8 cps	>10 second
P2	5.3 ± 0.02	6,3 ± 0 cps	>10 second
P3	5.22 ± 0.01	4,3 ± 0 cps	>10 second

The average pH values obtained from the three concentration variations of the formulations were 5.7, 5.3, and 5.2, respectively. Thus, all formulations met the pH requirements for topical preparations, which are 4.5–6. Based on the viscosity test results, it was found that only group P1 fell within the required viscosity range. Meanwhile, none of the C(-), P2, and P3 groups met the viscosity requirements. This is because in the C(-) formulation, only the hydrogel spray base was tested, containing 0.5 grams of carbopol, resulting in a thick formulation with a high viscosity value. Meanwhile, in groups P2 and P3, the formulations contained a concentration of a combination of sea lettuce and aloe vera extracts, which are acidic, thereby affecting the characteristics of the carbopol and causing a decrease in viscosity. All formulations adhered for more than 10 seconds without dripping. Thus, it can be concluded that the preparations have good adhesion properties, lasting over 10 seconds.

Results of the Spray Pattern and Hydrogel Spray Weight Tests

A spray pattern test was conducted to determine the droplet size distribution of the hydrogel spray formulation after it was sprayed from the applicator. The spread is greatly influenced by its viscosity; the larger the droplet diameter, the greater the effectiveness, and the smaller the sprayed particles, the faster the absorption into the skin. This also illustrates the distribution of the hydrogel spray when applied to the skin (Estikomah et al., 2021).

Table 11. *Results of the CAVERA Spray Pattern Test*

Formulation	Distance	Mean Diameter ± SD	Mean weight ± SD
C(-)	-	-	-
P1	3 cm	4,2 ± 0,3	115 ± 8
	5 cm	4,5 ± 0,5	105 ± 12,5
	10 cm	8,7 ± 1	104 ± 11,6
P2	3 cm	4,7 ± 0,6	112 ± 3
	5 cm	7,5 ± 0,5	97 ± 8
	10 cm	13,3 ± 0,8	43,7 ± 5,2
P3	3 cm	4,8 ± 0,3	105 ± 14
	5 cm	6,2 ± 0,6	104 ± 4,4
	10 cm	11 ± 0,9	45,5 ± 3,1

Images of the spray pattern test results can be found in the appendix. Variations in the diameter of the spray pattern are influenced by viscosity and spray distance. The diameter is directly proportional to the spray distance. The larger the diameter, the greater the spray pattern distance. Formulation C (-) cannot be sprayed from the applicator because it has high viscosity, making it difficult to spray. Meanwhile, formulations P1, P2, and P3 exhibit spray patterns forming elongated, spreading circles but with varying diameters. Specifically, the diameter range is 4.2–8.7 cm for P1, 4.7–13.3 cm for P2, and 4.8–11 cm for P3. Groups P2 and P3 have a wider dispersion range, consistent with their lower viscosity values. Based on the CAVERA hydrogel spray weight test results, each formulation has a different weight. Formulation P1 has a higher weight compared to formulations P2 and P3. This is consistent with its viscosity, where the lower the viscosity, the lower its density. However, as viscosity increases, so does its density. Meanwhile, the density of the C(-) formulation cannot be calculated because it cannot be sprayed

Organoleptic Test Results for Hydrogel Spray

The organoleptic test results, including the color, texture, and aroma of the hydrogel spray formulation, are shown in Figure 9 as follows:

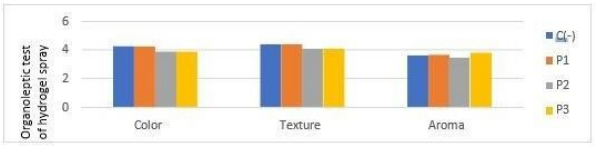

Figure 9. *Organoleptic Test of Hydrogel Spray*

Based on the data from the organoleptic test, the CAVERA formulation in P1 showed a higher level of acceptability than the other formulations. The diagram shows that the rating scale for the color parameter ranged from 3.86 to 4.20, with P1 receiving the highest score, indicating that the panelists rated the color parameter within the neutral to favorable range. Based on the texture parameter, P1 received the highest score of 4.34. The diagram shows that the rating scale for the texture parameter ranged from 4.06 to 4.34. The texture of CAVERA is related to the ratio used in hydrogel production and the addition of sea lettuce and aloe vera extracts, which resulted in a texture preferred by the panelists.

Regarding the aroma parameter, P3 received the highest score and was preferred by the panelists. The aroma of CAVERA is closely related to the ratio of sea lettuce and aloe vera extracts added during hydrogel production. The aroma of sea lettuce was more dominant than that of aloe vera, so P1, P2, and P3 had aromas dominated by sea lettuce, which was subsequently preferred by the panelists. Based on the average scores from the organoleptic test, the scores ranged from 3.44 to 3.79, indicating that the panelists' evaluations of CAVERA regarding the aroma parameter fell within a range from neutral to favorable.

Results of In Vivo Testing in an Atopic Dermatitis Model

The in vivo testing in this study was conducted using white mice with an atopic dermatitis model. The parameters used in this study included a scratching behavior test, measurement of skin thickness on the mice's backs, and histological examination of the skin on the mice's backs. These tests were conducted to assess the anti-inflammatory and antihistamine effects of CAVERA as an adjunctive therapy.

Results of the CAVERA Effect Test on Mouse Scratching Behavior

Test of mice scratching behavior was conducted to determine the effect of CAVERA as an adjunct to corticosteroids in reducing itching induced by DNCB in mice. The parameter in this test was scratching behavior observed over a 20-minute period and is shown in Figure 10 as follow:

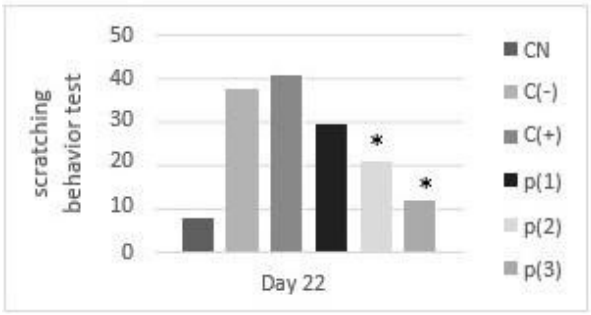

Figure 10. *Results of the Mouse Scratching Behavior Test on Day 22*

Scratching behavior is a characteristic of atopic dermatitis; this is due to a correlation between increased scratching behavior and the extent of mast cell degranulation, which is a hallmark of atopic dermatitis (Pareek et al., 2024; Wang et al., 2022). This study compared the mean scores of scratching behavior across 6 different groups: CN (normal control), C (-) (DNCB model treatment + hydrogel spray base), C (+) (2,4-dinitrochlorobenzene (DNCB) + Clobetasol 0.25%), P1 (DNCB + Clobetasol 0.25% + CAVERA hydrogel spray (1%)), P2 (DNCB + Clobetasol 0.25% + CAVERA hydrogel spray (2%)), P3 (DNCB + Clobetasol 0.25% + CAVERA hydrogel spray (3%)). Before statistical analysis
using One-Way ANOVA, the data were first tested for normality and homogeneity using the Shapiro- Wilk and Levene tests. The data were normally distributed ($p > 0.05$) but not homogeneous ($p < 0.05$); they were then analyzed using the Kruskal-Wallis test, which revealed significant differences between groups ($p < 0.05$). The analysis continued with the Mann-Whitney test to determine differences in scratching behavior between groups. The Mann-Whitney test results showed that there was a significant difference between groups P2 and P3 compared to group C(-) ($p < 0.05$), but there was no significant difference between groups C(+) and P3 compared to group C(-) ($p > 0.05$). The data results can be seen in the Appendix.

Results of the Test on the Effects of CAVERA on Thickness of the Back of Mice

Tests on the thickness of the back of mice were conducted on days 22 and 29 by measuring the thickness of the back skin using a caliper. The results of the tests on the thickness of the back skin of mice are shown in Figure 4.4 as follows:

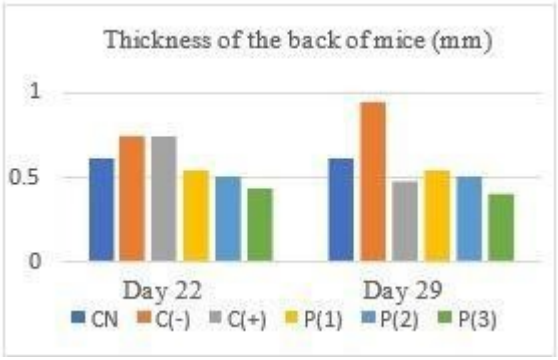

Figure 11. *Graph of mice back skin thickness on days 22 and 29 using a Vernier caliper*

Based on Figure 11, the highest skin thickness was found in the C(-) group on both days 22 and 29. The thinnest back skin thickness on days 22 and 29 was observed in the P3 group. Based on the t- test, there was no significant difference between the C(-) and C(+) groups on day 22 ($p>0.05$), but there was a significant difference on day 29 ($p<0.05$).

Results of the CAVERA Effect Test on Epidermal Thickness and Keratin

Measurements of epidermal thickness and keratin in the skin of the mice's backs were performed via histological examination, with the results illustrated in Figure 12 as follows:

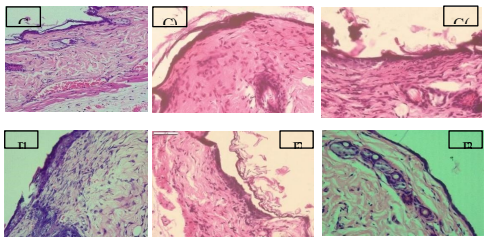

Figure 12. *Histological image of a specimen stained with hematoxylin-eosin at 400x magnification. The arrow indicates an area of epidermal thickening on the back of a mice.*

Measurements of epidermal and keratin thickness are summarized in Figure 13 as follows

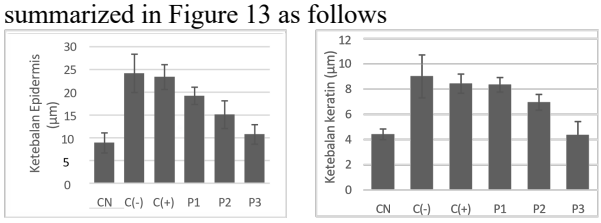

Figure 13. *Graph of Epidermis and Keratin Test Results*

Based on Figures 4.5 and 4.6, it can be seen that the highest epidermal thickness was found in the C(-) group, with an average thickness of 24.1 µm, and the lowest thickness in the CN group, with an average thickness of 8.86 µm. The highest keratin thickness was found in the C(-) group, with an average thickness of 9.006 µm, and the lowest thickness was found in the P3 group, with an average thickness of 4.346 µm.

Thickening of the epidermis and keratin is a symptom of atopic dermatitis (Kim et al., 2018). Thickening of the mouse back is a characteristic of atopic dermatitis, specifically lichenification caused by repeated scratching (Wang et al., 2021). This study compared the mean scores of scratching behavior across 6 different groups: C (normal control), C (-) (DNCB model treatment + hydrogel spray base), C (+) (2,4-dinitrochlorobenzene (DNCB) + Clobetasol 0.25%), P1 (DNCB + Clobetasol 0.25% + CAVERA hydrogel spray (1%)), P2 (DNCB + Clobetasol 0.25% + CAVERA hydrogel spray (2%)), P3 (DNCB + Clobetasol 0.25% + CAVERA hydrogel spray (3%)). Before statistical analysis using One- Way ANOVA, the data must first be tested for normality and homogeneity using the Shapiro-Wilk and Levene tests. Data with a p-value > 0.05 were subsequently analyzed using a one-way ANOVA, and the thickness measurements of the mice were found to differ significantly, as they had a p-value < 0.05. The data found to be significant were further analyzed using an LSD post hoc test to determine the significance between each group. The results of this analysis can be seen in the table.

A one-way ANOVA was conducted to compare the epidermal thickness and skin keratin levels on the backs of mice, revealing significant differences between groups ($p < 0.05$). A post hoc test was then performed to identify differences within each study group. The results of the post hoc test are presented in Table 4.11 as follows:

Table 12. *Results of the Post Hoc Test for Epidermal Thickness and Keratin*

Variabel	Epidermal thickness		Keratin thickness	
	Mean	P value	Mean	Nilai P value
CN vs C(+)	14.47000*	.000	-4.01800*	.000
CN vs P1	10.34200*	.000	-3.92800*	.000
CN vs P2	6.20800*	.002	-2.54200*	.001
CN vs P3	187.200	.303	.04800	.944
C(-) vs C(+)	.81800	.650	.57600	.406
C(-) vs P1	4.94600*	.010	.66600	.337
C(-) vs P2	9.08000*	.000	2.05200*	.006
C(-) vs P3	13.41600*	.000	4.64200*	.000
C(+) vs P1	4.12800*	.029	.09000	.896
C(+) vs P2	8.26200*	.000	1.47600*	.040
C(+) vs P3	12.59800*	.000	4.06600*	.000
P1 vs P2	4.13400*	.029	138.600	.053

P1 vs P3	8.47000*	.000	3.97600*	.000
P2 vs P3	4.33600*	.023	2.59000*	.001

The results of the post hoc test showed that there were significant differences between C(-) and C(+) and P1, P2, and P3, as indicated by $P < 0.05$. Among the three treatments, P3 showed the highest significance when compared to the epidermal and keratin thicknesses of C(-) and C(+), as evidenced by the mean values and $P < 0.001$. The epidermal and keratin thickness of P3 yielded results closest to CN.

Discussion

Effectiveness of the Extract

Atopic dermatitis (AD) is a disease with multiple pathogenic factors, including immune response disorders, skin colonization by microorganisms, impaired epidermal barrier function, and certain psychological factors. Common symptoms observed in AD patients include skin thickening and damage initiated by Th1 and Th2 interactions. Severe inflammation is also a hallmark of AD lesions, characterized by lichenification and fibrosis. The key pathomechanisms underlying the development of atopic dermatitis are disruption of the epidermal barrier function and changes in the immune response. Disruption of epidermal function is associated with the proteins filaggrin and keratin (Lugović-Mihić, L., et al., 2023).

Patients with atopic dermatitis have a compromised skin barrier, which can lead to xerosis that makes the skin susceptible to irritants and allergens, resulting in inflammation and pruritus. Skin barrier dysfunction can be caused by reduced levels of ceramides, filaggrin, and keratin—the primary proteins in the epidermal layer that play a role in maintaining skin integrity and preventing transepidermal water loss (TEWL) (Flohr et al., 2010). A damaged skin barrier allows irritants and allergens to penetrate the skin and cause inflammation through increased Th2 response activity, which releases the cytokine IL- 4 as an IgE stimulator, and IL-5 as an inducer of eosinophil differentiation and infiltration toward DA lesions in the acute phase, and a Th1 response that releases IFN-γ and IL-12 in the chronic phase (Kim et al., 2019). Scratching the skin also stimulates keratinocytes to release inflammatory cytokines such as TNF-α, IL-1, and IL-6. Decreased antimicrobial peptides (human beta-defensins, cathelicidins) in the epidermal layer. When the skin barrier is compromised due to a lack of filaggrin, S. aureus colonization can easily proliferate within the skin microbiome. S. aureus colonization prompts keratinocytes to produce proteases and release harmful substances such as δ-toxin and α-toxin, which can worsen skin conditions. δ-toxin on the skin triggers mast cells to release their contents without killing the S. aureus colonization, especially when IgE is present. This is what continues to worsen the skin condition in atopic dermatitis due to the itching and scratching caused by histamine released from mast cells, and the damage to the skin barrier resulting from the scratching, which initiates inflammation all over again.

CAVERA is an innovative topical medication designed to assist in the management of atopic dermatitis. The anti-inflammatory, antibacterial, and antihistamine effects of CAVERA are derived from a combination of secondary metabolites found in sea lettuce and aloe vera extracts, formulated as a hydrogel spray. Based on antibacterial testing of CAVERA, results indicate that the combination of Sea Lettuce and Aloe Vera extracts is effective in inhibiting the growth of S. aureus bacteria. This ability to inhibit bacterial growth stems from the effects of the secondary metabolites in the extract combination used in CAVERA.

Based on FT-IR analysis, the components of the Cavera extract were identified as containing phenolic compounds, some of which are flavonoids (quercetin, kaempferol), quinones (aloin and emodin), tocopherols, tannins, and saponins. Triterpenoids, which belong to the terpenoid group, and polysaccharides (alpha-glycosides) were also identified. The anti-inflammatory activity observed in the extract combination plays a role in the recovery from atopic dermatitis. Kaempferol, a derivative of the flavonoid secondary metabolite, exhibits anti-inflammatory activity by inhibiting NF-κB signaling (Lee, H. S. et al., 2021) and reduces the production of inflammatory cytokines such as IL-4, while also influencing the Th2 response, which leads to a decrease in filaggrin expression in keratinocytes, thereby improving epidermal barrier function in mice (Zhang, Yu et al., 2022). Quercetin, as an anti- inflammatory agent, also acts to suppress the release of pro-inflammatory cytokines and inhibits the release of histamine from mast cells and basophils (Rakha et al., 2022). The tocopherol present in sea lettuce extract exhibits anti-inflammatory activity by suppressing IgE production, thereby reducing the production of mast cells capable of releasing histamine and cytokines, and achieving an anti- inflammatory effect. Quercetin and tocopherol are flavonoid compounds that also act as antihistamines by stabilizing mast cell membranes; thus, quercetin functions as an inhibitor of histamine production and release in mouse skin. Quercetin inhibits mast cell activation by blocking the influx of Ca2+, histamine, and leukotrienes, as well as the release of prostaglandins and the activation of protein kinases (Mlcek et al., 2016). This antihistamine activity also plays a crucial role in breaking the itch-scratch cycle, which can cause further damage to the skin barrier. Breaking this itch-scratch cycle accelerates the healing of DA lesions and simultaneously acts as an agent that inhibits further inflammation in the skin.

Preventing Staphylococcus aureus colonization, which can worsen skin lesions in patients with atopic dermatitis, requires active compounds that inhibit bacterial growth. Phytochemical and FTIR analysis revealed that the extract contains secondary metabolites, including flavonoids, tannins, saponins, and triterpenoids that can inhibit the growth of S. aureus.

Based on the results of the antibacterial test, the combined extract of sea lettuce and aloe vera exhibited a minimum inhibition zone of 10.09 mm at a 75% concentration,

which is classified as having moderate antibacterial activity, and a maximum inhibition zone of 19.57 mm at a 100% concentration, which is classified as having strong antibacterial activity. The mechanism of action of these compounds in inhibiting bacteria is shown in Figure 4.7. Thus, the compounds comprising the Cavera extract are considered to have great potential for alleviating the symptoms of atopic dermatitis while also serving as a complementary herbal remedy.

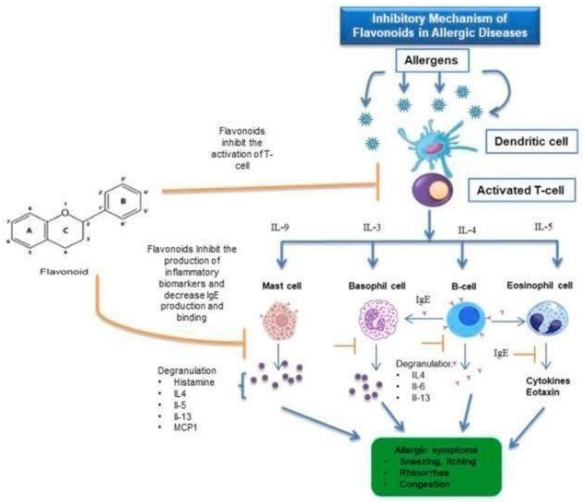

Figure 14. *Mechanism of Action of Secondary Metabolites in CAVERA in Anti-Inflammatory Effects (Rakha, A., et al., 2022)*

The Effectiveness of CAVERA on Scratching Behavior, Back Skin Thickness, and Epidermal Thickness of the Back Skin in Mice

In this study, an in vivo test was conducted using a mice (Mus musculus) model of atopic dermatitis induced by the hapten 2,4-dinitrochlorobenzene (DNCB). The hapten was applied topically, and the inducing chemical compound bound to extracellular and intracellular proteins, thereby triggering an inflammatory response in the mice's skin. After induction, the mice also
were treated with CAVERA. Efficacy parameters were measured by assessing scratching
behavior, back skin thickness, and back epidermal thickness.
Scratching behavior was assessed on day 29 of the study. Based on the data presented in the graph, the C(-) group exhibited the highest scratching behavior, which was attributed to the absence of medication capable of reducing itching in the mice. However, in the C(+) group, there was no significant difference in scratching behavior compared to the C(-) group. The lack of significance in the C(+) group may be due to the relatively low percentage of topical corticosteroid medication. Regarding the frequency of scratching behavior, P2 and P3 showed a significant decrease, approaching the behavior of the normal group and moving away from the negative control frequency in a dose-dependent manner. These results are significant due to the mechanism of quercetin (a flavonol) in inhibiting allergic mediators to reduce itching in an atopic dermatitis mice model (Mlcek et al., 2016).
Skin thickness in mice following DNCB induction and treatment was assessed on day 29 by measuring skin thickness with a caliper and performing histological examination of skin tissue. The results showed an increase in skin thickness in the C(-) group compared to the normal control (CN) group. This may be because in the C(-) group, the mice were only induced with DNCB without being given any medication. DNCB induction triggers an allergic inflammatory response that results in skin hardening and increased thickness (Kim, J., et al., 2019). In the C(+) group, there was a decrease in skin thickness compared to the normal control group (CN). This reduction in skin thickness is attributed to the side effects of corticosteroid therapy in the C(+) group (Susanto, 2022; Sue and Milanesi, 2019). The treatment groups (P1 and P2) appeared to have thicker skin than the C(+) group but thinner skin compared to the C(–) group. This demonstrates that the administration of a combination of 1% and 2% Sea Lettuce and Aloe Vera (CAVERA) extracts is capable of reducing the side effects of corticosteroid use in the treatment of atopic dermatitis.
Based on the results of the back skin thickness test in mice, thickening was observed in the C(-
) group, likely because the mice were not administering any medication; these findings are consistent with the histological examination of the mice skin. In the C (+) group, there was a decrease in skin thickness because of corticosteroids. Meanwhile, in the P1 and P2 treatment groups, there was no decrease in thickness; instead, the mice showed an increase in thickness from days 22 to 29. Comparing the C(-) and C(+) groups on day 22, there was no significant difference in back skin thickness; however, by day 29, the C(-) group showed an increase due to receiving no medication, while the C(+) group decreased due to the suppression of back skin thickening factors by the corticosteroid effect. Meanwhile, in groups P1 and P2, there was no decrease in back thickness on day 29 compared to P3; however, the back skin was maintained to prevent thickening, unlike the C(-) group, which continued to thicken on day 29. The graph for P3 shows a decrease in mouse back thickness on day 29. The results obtained depend on the differences in extract concentrations. The reduction and prevention of back skin thickening serve as indicators of the mice's successful recovery from atopic dermatitis.

CONCLUSION AND RECOMMENDATIONS

Conclusion

1. Based on the results of phytochemical and FT-IR analyses of the hydrogel spray extract, the sea lettuce extract had a yield of 10.4% and contained phenolic compounds, including flavonoids (quercetin, kaempferol), tocopherols, tannins, and saponins. Based on phytochemical and FT-

IR testing, the aloe vera extract had a yield of 12.8% and contained saponins, triterpenoids, quinones (aloin and emodin), and polysaccharides (alpha-glycosides). In an antibacterial test combining sea lettuce and aloe vera extracts against Staphylococcus aureus, it was found that the 100% extract concentration exhibited the highest inhibition zone with a diameter of 19.57 mm.

2. Based on the results of the homogeneity test, it can be concluded that all CAVERA hydrogel spray formulations are homogeneous. Based on the stability test results, no phase separation was observed in groups C (-) and P1. However, phase separation was observed in groups P2 and P3. pH and viscosity tests were conducted. The average pH values obtained from the three concentration variations of the formulations were 5.7, 5.3, and 5.2, respectively. Thus, all formulations met the pH requirements for topical preparations, which are 4.5–6. Based on the viscosity test results, it was found that only group P1 fell within the required viscosity range. Meanwhile, none of the C (-), P2, and P3 groups met the viscosity requirements. Based on the results of the spray pattern and formulation weight tests, it was found that the lower the viscosity, the lower the weight; however, this could not be measured for C (-) because it could not be sprayed. Based on the organoleptic results, P1 was the most preferred formulation in terms of color, texture, and aroma.
3. The efficacy of CAVERA as a hydrogel spray was tested using an in vivo model of atopic dermatitis in mice. Results regarding the thickness of the mice's dorsal skin on days 22 and 29 showed that the P3 group exhibited a significantly reduced thickness of the dorsal skin. Testing of scratching behavior showed a significant reduction in scratching behavior in the P3 group compared to the CN group. Histological testing was conducted to measure the thickness of the epidermis and skin keratin on the backs of the mice; P3 showed a significant reduction in epidermal and keratin thickness compared to C (-), and the thickness was approaching that of the CN group.

Recommendation

1. It is hoped that the public will be able to utilize underutilized natural resources with minimal economic value as a complementary treatment for atopic dermatitis through an innovative hydrogel spray.
2. For other researchers, further studies are needed to refine this research regarding the development of hydrogel spray, as well as in vivo testing and the characterization of other parameters.

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