Utilization of Avocado Leaves (*Persea americana* Mill) Ethanol Extract in Acne Spot Gel Formulation

Nurul Hidayati[1*], Putri Fadillah Amanda[1], Arif Setiawansyah[1,2]
[1]Pharmacy Diploma Program, Akademi Farmasi Cendikia Farma Husada, Pulau Enggano street No. 100, Sukabumi, Bandar Lampung, Indonesia 351534
2Faculty of Pharmacy, Universitas Kader Bangsa, Jl. HM Ryacudu No. 88, Palembang, Indonesia 30253
*Corresponding Email: nurulhidayati.cefada@gmail.com

ABSTRACT
Avocado leaves (*Persea americana* Mill) are classified as a type of herbal plant that has many benefits and can be used as traditional medicine. The components in avocado leaves that have antibacterial properties are flavonoids, saponins, and tannins. To increase public interest in utilizing avocado leaves, the development of a formulation in the form of acne spot gel from avocado leaf ethanol extract can be undertaken. The base used in this study is carbopol 934, the base that commonly used in gel production. The purpose of this study is to determine the effect of variations in the concentration of avocado leaf ethanol extract on the physical properties of acne spot gel. The extract was obtained by macerated 50.06 grams of avocado leaves (yield 25.03%). The formulation was produced in gel form with four formulas that had variations in the concentration of ethanol extract, those are formula 0 (0%), formula 1 (5%), formula 2 (10%), and formula 3 (15%). The acne spot gel was subjected to physical property tests including organoleptic, pH, spreadability, and adhesion. All tested formulas met the quality requirements of the acne spot gel formulation. The evaluation data were analyzed using One-Way ANOVA to determine significant differences. Based on the evaluation results, the variation in ethanol extract concentration had a significant effect ($p < 0.05$) on adhesion, where higher concentrations of ethanol extract could increase the adhesion of the formulation.
Key words: Avocado leaves, Acne spot gel, Extract.

INTRODUCTION
Acne vulgaris, a chronic inflammatory skin condition, affects millions of people worldwide, causing significant physical and psychological distress. While various treatment options exist, many are associated with adverse effects and high costs, prompting a growing interest in exploring natural and cost-effective alternatives. Avocado leaves (*Persea americana* Mill) have been traditionally used in folk medicine for their medicinal properties, and recent studies have revealed their potential antimicrobial and anti-inflammatory activities, making them a promising candidate for acne treatment.

The primary objective of this research is to evaluate the potential of avocado leaf ethanol extract as an active ingredient in an acne spot gel formulation. Specifically, the study aims to determine the extract's antimicrobial activity against *Propionibacterium acnes*, the primary bacteria implicated in acne

pathogenesis. Additionally, the research will investigate the extract's anti-inflammatory properties and assess its efficacy in reducing the appearance of acne lesions when incorporated into a topical gel formulation.

Previous studies have explored the medicinal properties of avocado leaves. Olufunmi Akpomie et al. (2021) demonstrated the antimicrobial activity of avocado leaf extracts against various bacterial strains, including *Staphylococcus aureus* and *Escherichia coli*. Yasir et al. (2010) reported the anti-inflammatory potential of avocado leaf extracts, attributing this effect to the presence of flavonoids and terpenoids. Furthermore, Yulistina (2022) investigated the efficacy of a gel formulation containing avocado leaf extract, observing promising results in reducing the severity of acne lesions.

The development of an effective and natural acne spot gel formulation utilizing avocado leaf extract could offer a promising alternative to conventional treatments, potentially benefiting individuals seeking safe and affordable options for managing acne. The findings of this research may contribute to the growing body of knowledge on the therapeutic potential of plant-based extracts and pave the way for further investigations into their application in dermatological products.

MATERIAL AND METHODS

Material

Avocado (*Persea americana* Mill) leaves, ethanol 96%, carbopol, glycerin, aquadest, triethanolamine, corrigen odoris (lemon), NaOH, $FeCl_3$, adhesive strength testing instrument, spreadability testing instrument, and other laboratory glasswares.

Methods

Extraction

The extract was obtained by macerating 200 grams of avocado leaf simplicia, soaked using 96% ethanol for 24 hours. After the first extraction process was completed, they were re-macerated with a new 96% ethanol solution. The obtained extract was then concentrated using a water bath until a viscous extract was obtained (Merwanta et al., 2019).

The formulation used in this study was adapted from research by Mustawa, 2011) with modifications. F0 is the base and F1-F3 have differences in the concentration of avocado leaf ethanol extract. The desired weight of the acne spot gel is 110 grams per formula with detailed composition listed in Table 1. The lowest concentration is 5% and the highest is 15%, with a 5% difference range between formulas referring to Merwanta et al. (2019).

Formulation

Carbopol was dispersed in hot water, then stirred. Avocado leaf extract was added to the carbopol and a small amount of water was added, then TEA was added drop by drop while stirring slowly until the desired pH was obtained, then glycerin was added little by little and aquadest was added up to 110 grams. After that, evaluations were carried out including organoleptic, pH, adhesion, and spreadability tests.

The data on weight uniformity, pH and stiffness test obtained were analyzed using One-Way ANOVA test ($p<0.05$) with 95% confidence level. Previously, normality test and homogeneity of variance test were carried out to compare data between acne spot gel preparation formulas.

Table 1. Formulas of acne spot gel from avocado leaves extract

Materials	Concentration (%)			
	F0	**F1**	**F2**	**F3**
Avocado leaves extract	0	5	10	15
Carbopol	1	1	1	1
Triethanolamine	0,5	0,5	0,5	0,5
Glycerin	5	5	5	5
Corrigen odoris (lemon)	0,1	0,1	0,1	0,1
Aquadest	ad 100	ad 100	ad 100	ad 100

RESULT AND DISCUSSION

Avocado (Persea americana Mill) Leaves Extract

A quantity of 1 kg of avocado (*Persea americana* Mill) leaves were collected and processed into dried leaf simplicia, yielding 200 grams. Maceration extraction was performed on this simplicia using 800 mL of 96% ethanol as the solvent. The extraction process produced 50.06 grams of a viscous avocado leaf extract. Calculating the yield percentage, this amounts to 25.03% of the initial dried leaf weight. According to the Indonesian Herbal Pharmacopoeia (2017) standards, a yield of over 10% for a viscous extract is considered satisfactory. Therefore, the 25.03% yield obtained meets the acceptable criteria. Subsequently, qualitative phytochemical screening tests were conducted on the viscous avocado leaf extract to detect the presence of flavonoid, saponin and tannin compounds, as summarized in Table 2.

To test for the presence of flavonoids, 10% NaOH was added to the avocado leaves extract. A color change from light green to yellow, red, brown, or green indicates flavonoid content (Maryati & Fidrianny, 2015). The extract turning a reddish-brown hue after adding NaOH, compared to the extract with just distilled water added, confirming the presence of flavonoids.

The saponin test involved adding distilled water to the avocado leaf extract and vigorously shaking for 30 seconds. Formation of a stable 1cm foam for 30 seconds indicates saponins are present (Maryati & Fidrianny, 2015).

Stable foam was produced when water was added and shaken, unlike the extract with just water added, proving saponin compounds exist in the extract.

To detect tannins, 1% $FeCl_3$ solution was mixed with the extract. A blackish-green or dark blue color developing signifies tannin presence (Maryati & Fidrianny, 2015). The extract turning blackish green after adding $FeCl_3$, whereas the extract with just water remained unchanged. This result confirms the avocado leaves extract contains tannin compounds.

This found is in line with previous findings. Studies have shown that avocado leaves are an abundant natural source of flavonoid compounds like quercetin, kaempferol and their glycoside derivatives (Rahmah et al., 2023). They also contain significant quantities of tannin phytochemicals, including condensed tannins as well as hydrolyzable tannins such as gallic acid and ellagic acid derivatives (Castro-López et al., 2019). Additionally, avocado leaves have been found to be rich in triterpenoid saponins including α-amyrin acetate and lupeol acetate (Castro-López et al., 2019; Wijaya, 2020). The presence of these bioactive phytochemical constituents like flavonoids, tannins and saponins likely contributes to the medicinal properties attributed to avocado leaves in traditional medicine practices.

Table 2. Qualitative phytochemical screening of avocado leaves extract.

Compound	Result
Flavonoids	Positive
Saponins	Positive
Tannins	Positive

Avocado (Persea americana Mill) Leaves Acne Spot Gel

The acne spot gel preparation obtained for each formula weighed 110 grams, and physical evaluation tests were conducted, including organoleptic testing, pH testing, spreadability testing, and adhesion testing.

The organoleptic evaluation revealed clear visual distinctions in color among the different acne spot gel formulations, despite their physical forms appearing consistent as semi-solid gels (Table 3). While all the gels had a similar semi-solid consistency, their colors differed depending on the amount of avocado leaf extract added. The base formula (F0) with no extract was completely clear. As the extract concentration increased (F1: 5%, F2: 10%, F3: 15%), the gel turned progressively greener, going from a brownish-green tint to a moss-green and finally a blackish-green shade. This color change directly reflects the amount of extract used, with higher concentrations leading to deeper and more intense green colors. Importantly, despite these color variations, the gels all maintained a uniform, semi-solid consistency.

Table 3. Organoleptic result of acne spot gel from avocado leaves extract

Formula	Physical Form	Color	Aroma
F0	Homogeneous semi-solid gel consistency	Transparent	Lemon
F1	Homogeneous semi-solid gel consistency	Brownish-green	Lemon
F2	Homogeneous semi-solid gel consistency	Moss-green	Lemon
F3	Homogeneous semi-solid gel consistency	Blackish-green	Lemon

Adding avocado leaf extract to the acne spot gel did not change the formulation's pH. Both the base formula and the one with extract had a pH of 5, indicating that the avocado leaf extract acne spot gel is safe for topical use according to SNI 16-4380-1996. Maintaining the right pH level in skin cosmetics is crucial for ensuring compatibility with the skin's natural acidic environment and supporting its overall health and barrier function. The skin's surface is slightly acidic, with a pH typically ranging from 4.1 to 5.8 (Lukic et al., 2021). This acidic pH plays a vital role in several aspects of skin physiology. One of the primary reasons for formulating cosmetics within the pH range of 4.1-5.8 is to preserve the skin's acid mantle (Sehgal & Singh, 2022). The acid mantle is a thin, protective film composed of sweat, sebum, and natural skin components, which helps maintain the skin's acidic pH and prevents the overgrowth of harmful microorganisms (Li et al., 2023). Cosmetic products with an alkaline pH can disrupt this crucial barrier, leading to increased susceptibility to infections, impaired skin barrier function, and enhanced penetration of irritants (Lukic et al., 2021).

The spreadability of the acne spot gel formulations was determined by placing 0.5 grams of the formulation on a 20 x 20 cm glass plate. Another glass plate of the same size was placed on top, and a weight of 125 grams was applied. The diameter of the spread area was measured after one minute. All four formulations (F0 to F3) exhibited spreadability values below 7 cm, as depicted in Figure 1, meeting the quality standards established by Garg et al. (2002).

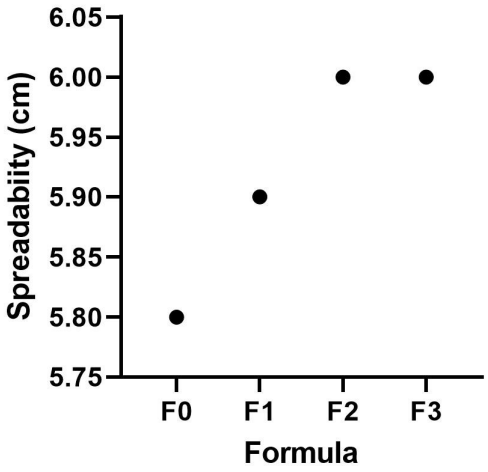

Figure 1. Spreadability of the acne spot gel formulations

The adhesion of the acne spot gel formulations was determined by placing 0.5 grams of the formulation on a loaded glass slide and letting it sit for 5 minutes. After 5 minutes, a weight of 80 grams was attached to the slide, and a stopwatch was started to record the time it took for the two glass slides to separate. All four formulations (F0 to F3) exhibited adhesion times of at least 4 seconds, meeting the quality standards established by Ratnapuri et al. (2019). Surprisingly, the adhesion test showed the opposite effect of the extract. Formula 0 (no extract) stuck less ($p<0.05$) compared to formulas with extract (1-3). While Figure 2 suggests higher extract concentration leads to better adhesion, further investigation is needed to explain this contradiction.

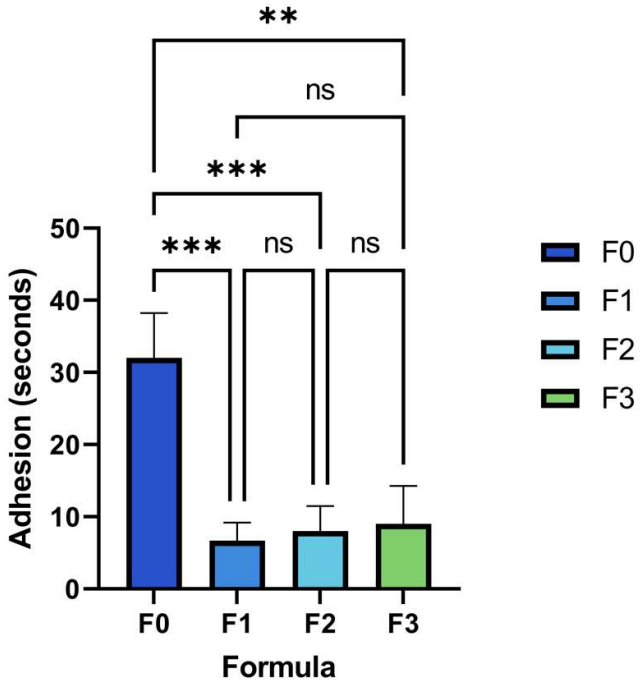

Figure 2. Adhesion of the acne spot gel formulations; ** significantly different with p-value <0,05; *** significantly different with p-value <0,0004.

CONCLUSION

Based on the research results, it can be concluded that the optimum formula for the avocado (*Persea americana* Mill) leaves ethanol extract acne spot gel preparation is formula 1 with an extract concentration of 5%. All formulas of the avocado (*Persea americana* Mill) leaves ethanol extract acne spot gel preparation meet the quality requirements including organoleptic, pH, spreadability and adhesion. The avocado (*Persea americana* Mill) leaves ethanol extract only effected the physical property of adhesion, where the higher the avocado leaf ethanol extract, the higher the obtained adhesion.

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