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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

FORMULATION AND ANTIBACTERIAL EVALUATION OF AN EMULGEL CONTAINING METHANOLIC EXTRACT OF PROPOLIS FROM THE STINGLESS BEE (*Homotrigona apicalis)*

İğnesiz Aridan (*Homotrigona apicalis*) Elde Edilen Propolisin Metanolik Ekstresini İçeren Bir Emüljelin Formülasyonu ve Antibakteriyel Değerlendirmesi

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ABSTRACT

Stingless bee propolis (*Homotrigona apicalis*) is known to contain various bioactive compounds, including flavonoids, alkaloids, and terpenoids, which exhibit potential antibacterial properties. This study aimed to evaluate whether the methanolic extract of *H. apicalis* propolis could be formulated into a physically and chemically stable emulgel dosage form, and to assess its antibacterial activity against *Cutibacterium acnes* and *Staphylococcus epidermidis*, the primary causative agents of acne vulgaris. The research was conducted as an experimental laboratory study. The methanolic extract of *H. apicalis* propolis was obtained using the maceration extraction method. Antibacterial activity was determined using standardized methods. The results demonstrated that the emulgel formulations, namely F0 and F1, exhibited good physical and chemical stability. Among them, the formulation containing 10% methanolic extract showed the most significant inhibitory effect, producing inhibition zones of 10.00 mm against *C. acnes* and 10.17 mm against *S. epidermidis*. These findings indicate that the 10% concentration of *H. apicalis* propolis emulgel fulfills the physical and chemical stability requirements and demonstrates strong antibacterial activity. Therefore, *H. apicalis* propolis emulgel has the potential to be developed as an effective topical anti-acne preparation.

Keywords: Evaluation, Topical formulation, Anti-acne, Formulation stability, Stingless bee propolis

ÖZ

İğnesiz arı propolisi (*Homotrigona apicalis*), flavonoidler, alkaloidler ve terpenoidler gibi çeşitli biyoaktif bileşikler içermesiyle bilinir ve bu bileşikler potansiyel antibakteriyel özellikler göstermektedir. Bu çalışma, *H. apicalis* propolisinin metanolik ekstresinin fiziksel ve kimyasal olarak kararlı bir emüljel dozaj formuna dönüştürülüp dönüştürülemeyeceğini ve akne vulgarisin başlıca etkenleri olan *Cutibacterium acnes* ve *Staphylococcus epidermidis*'e karşı antibakteriyel aktivitesini değerlendirmeyi amaçlamıştır. Araştırma deneysel bir laboratuvar çalışması olarak yürütülmüştür. H. apicalis propolisinin metanolik ekstresi maserasyon yöntemiyle elde edilmiştir. Antibakteriyel aktivite

standart yöntemlerle belirlenmiştir. Sonuçlar, F0 ve F1 olarak adlandırılan emüljel formülasyonlarının iyi fiziksel ve kimyasal stabilite sergilediğini göstermiştir. Bunlar arasında, %10 metanolik ekstre içeren formülasyon en yüksek inhibisyon etkisini göstermiştir; *C. acnes'*e karşı 10,00 mm ve *S. epidermidis*'e karşı 10,17 mm'lik inhibisyon zonları oluşturmuştur. Bu bulgular, *H. apicalis* propolis emüljelinin %10 konsantrasyonunun fiziksel ve kimyasal stabilite gereksinimlerini karşıladığını ve güçlü antibakteriyel aktivite gösterdiğini ortaya koymaktadır. Sonuç olarak, *H. apicalis* propolis emüljeli etkili bir topikal anti-akne preparatı olarak geliştirilmeye uygundur.

Anahtar Kelimeler: Değerlendirme, Topikal formülasyon, Anti-akne, Formülasyon stabilitesi, İğnesiz arı propolisi

GENİŞLETİLMİŞ ÖZET

Amaç: Bu çalışmanın amacı, iğnesiz arı propolisi (*H. apicalis*) metanol ekstraktının fiziksel ve kimyasal olarak stabil bir emuljel dozaj formuna dönüştürülüp dönüştürülemeyeceğini değerlendirmek, bu formülasyonların akne vulgarisin başlıca etkenleri olan *Cutibacterium acnes* ve *Staphylococcus* epidermidis'e karşı antibakteriyel aktivitelerini belirlemek ve hangi konsantrasyonların bakteri gelişimini inhibe etmede en etkili olduğunu saptamaktır.

Gereç ve Yöntem: Bu çalışmada kullanılan materyaller *H. apicalis* propolisi, metanol, karbopol, triethanolamin, sıvı parafin, stearik asit, Span 80, Tween 80, nipagin, nipasol, akuadest ve kontrol jel preparatı olup, antibakteriyel aktivite için *Cutibacterium acnes* ve *Staphylococcus epidermidis* kültürleri kullanılmıştır. Propolis örnekleri Doğu Kalimantan'dan toplanarak temizlenmiş ve 3 × 24 saat maserasyon yöntemiyle metanolde ekstrakte edilmiştir; elde edilen makerat süzülmüş, rotary evaporatör ve su banyosunda yoğunlaştırılmıştır. Metanol ekstraktı alkaloid, flavonoid, tanen, fenol, saponin ve kinon varlığı açısından fitokimyasal olarak taranmıştır. Elde edilen ekstrakt dört farklı konsantrasyonda emuljel formülasyonuna (F0: %0, F1: %2,5, F2: %5, F3: %10, K+: K+: Acne Spot Treatment Gel) eklenmiş ve organoleptik özellikler, pH, viskozite, yayılabilirlik, yapışma ve homojenlik açısından değerlendirilmiştir. Antibakteriyel aktivite agar difüzyon yöntemiyle test edilerek inhibisyon zon çapları ölçülmüş, Acne Spot Treatment Gel pozitif kontrol, DMSO (%1) negatif kontrol olarak kullanılmış ve sonuçlar ANOVA ile istatistiksel olarak analiz edilmiştir ($p<0,05$).

Bulgular ve tartışma: Fitokimyasal tarama sonuçları, *H. apicalis* propolisinin metanol ekstraktında alkaloid, flavonoid, tanen, fenol, saponin ve kinonların bulunduğunu göstermiştir; bu bileşikler antibakteriyel aktivitede rol oynayan önemli ikincil metabolitlerdir. Hazırlanan dört farklı emuljel formülasyonu (F0–F3) fiziksel ve kimyasal kalite açısından değerlendirilmiş, tüm formülasyonların homojen, yarı katı kıvamda olduğu, pH değerlerinin 4,5–6,5 arasında kaldığı, viskozite (2000–4000 mPa·s), yayılabilirlik (5–7 cm) ve yapışma sürelerinin farmakopoe standartlarına uygun olduğu belirlenmiştir. En yüksek propolis konsantrasyonunu (%10) içeren F3 formülasyonu depolama sonrası kısmi renk değişimi göstermesine rağmen genel stabilite kriterlerini karşılamıştır. Antibakteriyel aktivite testleri, ekstrakt içermeyen kontrol (F0) formülasyonunun inhibisyon göstermediğini, F1 (%2,5) ve F2 (%5) formüllerinin *C. acnes* ve *S. epidermidis* üzerinde orta düzeyde inhibisyon sağladığını, F3 (%10) formülasyonunun ise en yüksek inhibisyon zon çaplarına sahip olduğunu (sırasıyla 10,17 mm ve 10,0 mm) ortaya koymuştur. Pozitif kontrol klindamisin %0,1'in daha geniş inhibisyon zonu (22,27 mm) oluşturduğu gözlenmiş, bu da propolisin antibakteriyel etkisinin sentetik antibiyotiklere kıyasla daha düşük fakat klinik olarak anlamlı olabileceğini göstermektedir. Bu sonuçlar, flavonoid ve alkaloid gibi fenolik bileşiklerin serbest radikalleri nötralize etme ve bakteri hücre duvarını bozma mekanizmaları ile açıklanabilir. Ayrıca emuljel formülasyonunun fiziksel stabilitesi, topikal uygulama için uygun bir taşıyıcı sistem olduğunu desteklemektedir. Genel olarak, *H. apicalis* propolisi metanol ekstraktının emuljel formülasyonları akneye neden olan bakterilere karşı doza bağımlı bir antibakteriyel aktivite sergilemiş ve doğal bir anti-akne ajanı olarak geliştirilme potansiyeli göstermiştir.

Sonuç: Bu çalışma, iğnesiz arı propolisi (*H. apicalis*) metanol ekstraktının stabil bir emuljel formülasyonuna başarıyla entegre edilebileceğini ve akne vulgarisin etkenleri olan *Cutibacterium acnes* ve Staphylococcus epidermidis'e karşı doza bağlı antibakteriyel aktivite sergilediğini ortaya koymuştur.

%10 propolis içeren emuljel formülasyonu (F3), en yüksek inhibisyon zon çaplarını göstermiş ve fiziksel-kimyasal açıdan kabul edilebilir stabilite özellikleri sunmuştur. Elde edilen bulgular, *H. apicalis* propolis ekstraktının doğal bir anti-akne topikal preparatı olarak geliştirilme potansiyeline sahip olduğunu göstermektedir.

INTRODUCTION

Cutibacterium acnes and *Staphylococcus epidermidis* are among the major commensal bacteria residing on human skin. However, under certain conditions, these microorganisms may behave as opportunistic pathogens (Chessa et al., 2015). *S. epidermidis* is widely distributed across various skin sites, whereas *C. acnes* predominantly inhabits pilosebaceous units. The microbial interplay between these species, mediated by molecules involved in intercellular competition or communication, plays an important role in maintaining the delicate balance of the skin ecosystem. Once this balance is disturbed (dysbiosis), skin health may be impaired, potentially initiating or exacerbating disorders such as acne vulgaris (Christensen et al. 2016). *Cutibacterium acnes,* related significantly to the initial stage of acne, causes increasing lipogenesis originating in sebaceous glands. It induces inflammation and pustules on the skin (Blaskovich et al., 2019, Neves et al. 2015). Meanwhile, *S. epidermidis* could act as an opportunistic pathogen when it enters the bloodstream (Tabri 2019). Skin clinic acne treatment normally uses antibiotics that could overcome inflammation and kill bacteria, such as tetracycline, erythromycin, doxycycline, and clindamycin (Doğan et al. 2017, Nakatsuji et al. 2009). However, these drugs' side effects include irritation, allergic reactions, and, in the case of long-term consumption, resistance, systemic toxicities such as hepatotoxicity and nephrotoxicity, photosensitivity, gastrointestinal irritation, esophagitis, and the risk of *Clostridium difficile*–associated pseudomembranous colitis (Dikicier and Sevimli, 2019, Tan et al. 2018). Antibiotic resistance is defined as the acquired ability of microorganisms to survive or proliferate despite exposure to antibiotic concentrations that would normally inhibit or kill susceptible strains. Therefore, higher concentrations are needed to inhibit the growth of resistant bacteria compared to susceptible strains.

A significant factor that contributes to increasing resistance is the inappropriate or irrational use of antibiotics (Walshet et al. 2016). Antibiotics such as erythromycin and clindamycin are commonly used in the treatment of bacterial acne (Powale et al. 2022). However, the efficacy of this treatment decreases when resistance develops in *Cutibacterium acnes*, and antibiotics may also disrupt the balance of the skin microbiota by killing beneficial microbes (O'Neill et al. 2020, Waranuch et al. 2019). Thus, continued use of antibiotics could cause resistance (Liu et al. 2019, Mera et al. 2019, Zahrah et al. 2019).

The adverse effects of antibiotics have prompted researchers to discover and develop novel antimicrobial agents from natural products. For example, phytochemical compounds extracted from medicinal plants have shown effective antibacterial activity against multi-drug-resistant pathogens, and these compounds could potentially be exploited as antibacterial drugs (Abdallah et al., 2009, Sadeek and Abdallah. 2019). In line with this, alternative approaches using natural ingredients are being explored to treat infections (Utami et al. 2021). The natural ingredient mostly found as an antibacterial is propolis from the stingless bee *H. apicalis*.

H. apicalis is a species of stingless, honey-producing bee belonging to the family Meliponidae (Francoy et al. 2019). Naturally, these insects build nests in tree holes, wall cracks, and bamboo cavities, a source of food for bees in the form of pollen, nectar, and resin. Bees are considered herbivorous insects (Achyani and Wicandra 2019). In addition to honey, *Homotrigona* hives produce royal jelly, bee pollen, and propolis, all of which have recognized health benefits for humans (Syafrizal et al. 2016). These natural bee products have been reported to possess antibacterial, antifungal, anticancer, anti-inflammatory, and anti-asthmatic properties (Campos et al., 2015, Lopes et al. 2019).

Propolis is a natural substance produced by bees consisting of a mixture of bee saliva and plant exudates that they collect (Mardiah 2017). Empirical evidence suggests that propolis is a relatively safe natural product with a wide range of biological activities (Lutpiatina 2015). Its commonly reported applications include use as a medicinal agent or dietary supplement, mouthwash, anti-inflammatory compound, adjuvant in disease therapy, and in accelerating wound healing. The chemical content of propolis has some chemical compounds and differs depending on the environment around the bee farm (Rosyidi et al. 2018). Therefore, differences in

compounds of propolis found in Indonesia. The condition that is often found in the chemical content of propolis consists of amino acids, terpenoids, and polyphenols (phenolic acids, esters, and flavonoids) (Pujirahayu et al. 2014). Flavonoids are among the most important bioactive constituents of propolis, exhibiting antioxidant, anticancer, anti-inflammatory, anti-allergic, antiviral, and antibacterial activities (Grumezescu and Holban 2018, Hermalinda et al. 2019, Rismawati and Ismiyati 2017).

Topical formulations are available in semi-solid and liquid forms and are applied to the skin or mucous membranes for either local or systemic effects. In most cases, those are medicated, and the non-medicated ones are often used as skin protection and moisturizers. The skin provides an effective site for drug delivery, but it also acts as a strong mechanical barrier—mainly through the stratum corneum—limiting the penetration of many drug substances. Emulgels (mixture of pharmaceutical emulsion and gel) as an example of topical formulations, has excellent cutaneous penetration and offer advantages of better stability, controlled release, incorporation of hydrophobic drugs and better loading capacity, in addition to the general advantages of topical formulations such as ease of administration, low cost and fewer toxic effects (Yan et al. 2017). The basic components of emulgels are active ingredient (s), solvents (polar and non-polar), emulsifiers, gelling agents, permeation enhancers, and preservatives. They are prepared in three basic steps: formulation of emulsion (oil-in-water or water-in-oil), formulation of gel base, and incorporation of emulsion into gel base with continuous stirring (Utami et al. 2021).

Emulgels, as emulsions whose outer phase is gelled completed by appropriate gelling agents, represent dual controlled-release systems due to the existence of the gel's network as a structure that provides emulsion stabilization alongside additional control in drug release (Zahrah et al. 2019). Since emulgels have characteristics of both emulsions and gels, they are well accepted by patients (Liu et al. 2019, Mera et al. 2019). Emulgels typically contain, in addition to active ingredients, emulgels typically contain; polar and non-polar solvents, preservatives, emulsifiers, gelling agents, and permeation enhancers, which interact with the skin to increase the rate of absorption of the drug from the site of application (Yadav et al. 2017). A major advantage of emulgels is their ability to incorporate hydrophobic active substances into the oil phase. These hydrophobic compounds, which are otherwise difficult to load into gel matrices, are dispersed within oil globules of an oil-in-water (O/W) emulsion and subsequently incorporated into the gel base (Gaikwad et al. 2024, Milutinov et al. 2023).

Based on previous research, an emulgel formulation was developed from the methanol extract of *H. apicalis* propolis and evaluated for its antibacterial activity against *C. acnes* and *S. epidermidis*, which are known to contribute to acne.

MATERIALS AND METHODS

Equipment and Materials

The equipment used in this study included Pyrex glassware, blender, vortex mixer, hotplate (Ceran®), petri dishes, spirit lamps, autoclaves, incubators, magnetic stirrer, 5–50 µL micropipettes, analytical balance, caliper, water bath, *laminar airflow cabinet* (LAF), maserator, rotary evaporator, spectrophotometer, knife, and cutting board.

The materials employed were methanol, *H. apicalis* propolis (Figure 1), filter paper, sterile cotton, distilled water, Mueller-Hinton Agar (MHA), Acne Spot, DMSO, NaCl 0.9%, carbopol, triethanolamine (TEA), stearic acid, paraffin, Nipagin, Nipasol, Span 80, and Tween 80.

Figure 1. Propolis of *H. apicalis* in Trigona Farm

Works Procedure

Preparation of Simplicia

Propolis was collected from Tani Harapan Loa Janan, Kutai Kartanegara, East Kalimantan, and subsequently wet-sorted to separate propolis from the nest and other impurities.

Extraction Procedure

The amount of methanol used, temperature, and rotation speed (rotary evaporator) are not specified.

The phrase “Evaporated over a water bath until a thick extract is obtained” is somewhat vague; it would be more scientific if the sample weight/density or target concentration were specified.

Phytochemical Screening (Kustiawan et al. 2022, Supomo et al. 2019).

The phytochemical test is one of the screening methods to detect secondary metabolite compounds contained in a plant. In this study, phytochemical tests were carried out on alkaloids, tannins, saponins, flavonoids, phenols, and quinones compounds.

The Formula Plan

Table 1. Plan of Formula Emulgel

Material	Usage	Concentration %				
		F0	F1	F2	F3	K+
Exctract Propolis Homotrigona apicalis	Active	-	2,5	5	10	Acne Spot Treatment Gel
Carbopol	Gelling agent	0,5	0,5	0,5	0,5	
TEA (Triethanolamine)	Humektan	1	1	1	1	
Parafin liquid	Emolient	15	15	15	15	
Span	Emulgator	1,4	1,4	1,4	1,4	
Tween	Emulgator	3,6	3,6	3,6	3,6	
Stearate Acid	Emulgator	5	5	5	5	
Nipasol	Preservatives	0,2	0,2	0,2	0,2	
Nipagin	Preservatives	0,4	0,4	0,4	0,4	
Aquadest	Solvent	Ad 100	Ad 100	Ad 100	Ad 100	

*F0: Antibacterial emulgel preparation without active substances, F1: Antibacterial emulgel preparation containing 2.5% active substance, F2: Antibacterial emulgel preparation containing 5% active substance, F3: Antibacterial emulgel preparation containing 10% active substance, K+: Acne Spot Treatment Gel.

The gel base was formulated by dispersing Carbopol into distilled water preheated to 80–90°C under continuous stirring using a magnetic stirrer. Triethanolamine was gradually added until a clear, homogeneous gel was obtained, and the pH was adjusted to the desired level. Subsequently, paraffin was incorporated into the gel base to form mixture 1. The oil phase was prepared by melting stearic acid and liquid paraffin at 70°C with continuous stirring until a uniform solution was achieved. This oil phase, comprising stearic acid, liquid paraffin, and nipasol, was slowly incorporated into the Carbopol gel base with gentle stirring to minimize air entrapment. To stabilize the oil-in-water (O/W) emulsion, surfactants Tween 80 and Span 80 were added at a weight ratio of 3:1 (Tween: Span) and mixed carefully until a homogeneous emulsion was formed. The emulsion was then gradually combined with the gel base and stirred thoroughly to produce a uniform emulgel. Finally, preservatives (nipagin) and active ingredients (propolis) were incorporated and mixed gently to ensure even distribution throughout the formulation. In this system, the oil phase acts as the dispersed internal phase, while the aqueous gel base serves as the continuous external phase, resulting in a stable O/W emulgel. (Djuwarno et al. 2021).

Evaluation of Emulgels (Suryawanshi and Gawade, 2020)

The evaluation of emulgel formulations includes: organoleptic, pH test, viscosity test, spreadability test, adhesion test, and gel emulsion type.

Physical examination

The optimized emulgel formulations were visually inspected for their color, homogeneity, and

consistency to ensure uniform appearance and texture.

Determination of pH

The pH of emulgel formulations was determined using by digital pH meter. One gram of gel was dispersed in 100 ml of distilled water. The measurement of the pH of each formulation was carried out in triplicate.

Viscosity measurement

Brookfield Synchroelectric viscometer model RVT attached with spindle D was used for the determination of viscosity. Emulgels were filled in a jar, and the spindle was lowered perpendicularly, taking care that the spindle did not touch the bottom of the jar. The spindle was rotated in the gel at increasing shear rates of 0.5, 1, 2.5, and 5rpm. At each speed, the corresponding dial reading was noted. The reverse reading was also noted, and the average was taken for these two readings. The viscosity of the emulgel is calculated by multiplying the average dial reading by the appropriate factor given in the Brookfield viscometer catalogs.

Spreadability study

A modified apparatus consisting of two glass slides with the emulgel formulation placed between them, with the lower slide fixed to a wooden plate and the upper one connected to a balance through a hook, was used to determine spreadability.

Adhesive study

Accurately weighed 1g of emulgel was placed between these two slides containing hairless fresh rat skin pieces, extra weight from the left pan was removed to sandwich the two pieces of glass, and some pressure was applied to remove the presence of air. The balance was kept in the position for 5 min. Weight was added slowly at 200mg/min to the left-hand pan until the two glass slides got detached from each other. The weight required to detach the emulgel from the glass surface gives a measure of adhesive strength by using a formula.

Emulgel Type Test

The type of emulsion in the emulgel was determined using the methylene blue staining method. A small amount of emulgel was placed on a glass slide, and a drop of methylene blue solution was added and gently stirred. If the dye dissolved uniformly upon stirring, the emulgel was identified as an oil-in-water (O/W) type; if the dye remained undissolved, it was classified as a water-in-oil (W/O) type.

Antibacterial Activity Test (Lutpiatina, 2015)

A total of 5 mL of molten Mueller-Hinton Agar (MHA) was poured into a sterile Petri dish and allowed to solidify. After solidification, five wells were made using a sterile cork borer. Separately, 0.1 mL of the bacterial suspension was added to 15 mL of MHA maintained at approximately 40°C, and the mixture was poured over the base layer in the Petri dish to form a uniform inoculated layer. Once the medium had solidified, the borer was removed, and emulgel formulations (F0, F1, F2, F3) and the positive control were carefully introduced into each well. The plates were then incubated at 37°C for 24 hours. After incubation, the inhibition zones were observed and measured.

Data analysis

The antibacterial activity was evaluated by measuring the diameter of the inhibition zones formed around each well. The inhibition zones were measured in millimeters using a vernier caliper, and the results for each formulation were compared with the positive control. The results of measuring the diameter of the inhibition zone were then analyzed statistically by implementing two-way analysis of variance (ANOVA).

RESULT

Phytochemical Screening Test Result of Propolis *H. apicalis*

Table 2. Phytochemical Screening Test Result

Fitokimia Test	Test	Results	Description
Alkaloid	Mayer	White Precipitate	(+)
	Bouchardat	Brown Precipitate	(+)
	Dragendorf	Yellow	(-)
Saponin	HCl 2N	Foam 2 cm	(+)
Flavonoid	Concentrated HCl + Mg powder + Amyl Alcohol	Yellow on Amyl Alcohol coating	(+)
Tannin	$FeCl_3$	Blackish Green	(+)
Phenol	$FeCl_3$ 5%	Dark green	(+)
Quinones	NaOH 1 N	Brownish red	(+)

*(-) = Negative result, and (+) = Positive result

Phytochemical screening of the methanol extract of H. apicalis revealed the presence of alkaloids, tannins, saponins, flavonoids, phenols, and quinones.

Table 3. Results of organoleptic observations

Formula	Organoleptic observations					
	Before cycling			After cycling		
	Form	Color	Smell	Form	Color	Smell
F0	Semi solid	White milk	Special smell	Semi solid	White milk	Special smell
F1	Semi solid	klat palet	Special smell	Semi solid	Light cream	Special smell
F2	Semi solid	Light brown	Special smell	Semi solid	Cream (2 phases are formed)	Special smell
F3	Semi solid	Chocolate	Special smell	Semi solid	Old cream (2 phases are formed)	Special smell

*F0: Antibacterial emulgel preparation without active substances, F1: Antibacterial emulgel preparation containing 2.5% active substance, F2: Antibacterial emulgel preparation containing 5% active substance, F3: Antibacterial emulgel preparation containing 10% active substance

Organoleptic testing aims to identify and evaluate the dosage form with respect to the naked eye in terms of shape, color, and odor. F0, F1, F2, and F3 before and after cycling have a semi-solid consistency, meaning that F0, F1, F2, and F3 remain stable during storage.

Table 4. Result of pH Test

Formula	Use pH		Requirement	Significant
	Before cycling	After cycling		
F0	5,18	6,37	4,5-6,5	*p*>0,05
F1	5,34	6,45		
F2	5,54	5,58		
F3	5,96	5,54		

*F0: Antibacterial emulgel preparation without active substances, F1: Emulgel formulation containing 2.5% of the active ingredient, F2: Emulgel formulation containing 5% of the active ingredient, F3: Emulgel formulation containing 10% of the active ingredient. Differences were considered statistically significant at $p < 0.05$ and not significant at $p > 0.05$. The pH test was conducted using a pH meter. The pH before and after cycling of F0, F1, F2, and F3, was still within the physiological standard of skin pH of 4.5-6.5.

Table 5. The Result of the Homogeneity Test

Formula	Homogeneity Test		Requirement
	Before cycling	After cycling	
F0	Homogeneous	Homogeneous	Homogeneous
F1	Homogeneous	Homogeneous	Homogeneous
F2	Homogeneous	Homogeneous	Homogeneous
F3	Homogeneous	Homogeneous	Homogeneous

*F0: Antibacterial emulgel preparation without active substances, F1: Antibacterial emulgel preparation containing 2.5% active substance, F2: Antibacterial emulgel preparation containing 5% active substance, F3: Antibacterial emulgel preparation containing 10% active substance.

All formulations (F0–F3), both before and after the cycling test, exhibited homogeneity.

Table 6. The Result of Spreadability

Formula	Spreadability test (cm)		Requirement	Significant
	Before cycling	After cycling		
F0	5,7	5,3	5-7 cm	p>0,05
F1	5	5,5		
F2	5,5	6		
F3	6	5,5		

*F0: Antibacterial emulgel preparation without active substances, F1: Antibacterial emulgel preparation containing 2.5% active substance, F2: Antibacterial emulgel preparation containing 5% active substance, F3: Antibacterial emulgel preparation containing 10% active substance. P<0.05: Significant, and p>0.05: Not significant.

The results of the spreadability test for all formulations (F0, F1, F2, and F3) indicated that they still met the acceptable spreadability range for emulgels, which is defined as 5–7 cm.

Table 7. Test Result of Adhesion

Formula	Adhesion test (seconds)		Requirement	Significant
	Before cycling	After cycling		
F0	1	2	More than 1 second	*p*>0,05
F1	17,43	18,19		
F2	18,02	1		
F3	16,07	1		

*F0: Antibacterial emulgel preparation without active substances, F1: Antibacterial emulgel preparation containing 2.5% active substance, F2: Antibacterial emulgel preparation containing 5% active substance, F3: Antibacterial emulgel preparation containing 10% active substance. P<0.05: Significant, and p>0.05: Not significant.

The results of the adhesion test for all formulations (F0, F1, F2, and F3) indicated that they still met the acceptable adhesion standard for emulgels, which is defined as greater than 1 second.

Table 8. The Result of the Viscosity Test

Formula	Viscosity test (mPa's)		Requirement	Sig
	Before cycling	After cycling		
F0	2	2	2000-4000	*p*>0,05
F1	2	2		
F2	2	1		
F3	2	1		

*F0: Antibacterial emulgel preparation without active substances, F1: Antibacterial emulgel preparation containing 2.5% active substance, F2: Antibacterial emulgel preparation containing 5% active substance, F3: Antibacterial emulgel preparation containing 10% active substance. P<0.05: Significant, and p>0.05: Not significan

The viscosity test was conducted using a Brookfield Viscometer; all four formulations showed changes in viscosity values after storage due to the effect of the polymer on temperature changes.

Table 9. The Result of the Determining Emulsion Type Test

Formula	Emulsion type determination test	
	Before cycling	After cycling
F0	O/W	O/W
F1	O/W	O/W
F2	O/W	O/W
F3	O/W	O/W

*If a homogeneous emulsion is obtained, it means that the type of emulsion produced is O/W, and if the opposite is obtained, the type of emulsion produced is O/W. F0: Antibacterial emulgel preparation without active substances, F1: Antibacterial emulgel preparation containing 2.5% active substance, F2: Antibacterial emulgel preparation containing 5% active substance, F3: Antibacterial emulgel preparation containing 10% active substance.

The emulsion type of the emulgel was determined using the methylene blue staining method. The results indicated that all formulations were of the oil-in-water (O/W) type.

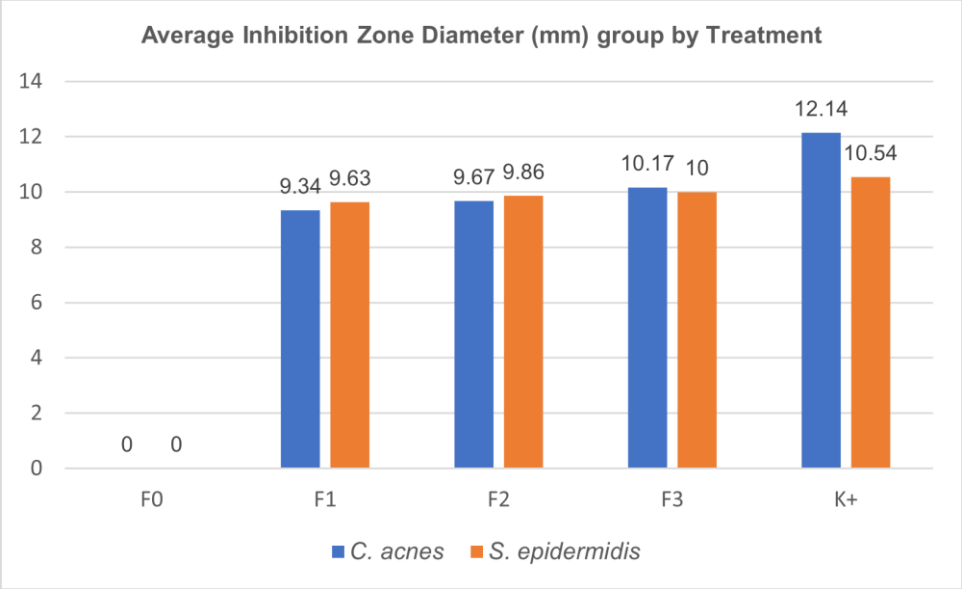

Figure 2. Activity test results for propolis emulgel preparations *H. apicalis*

*Weak resistance: ≤ 5 mm, Moderate resistance: 5-10 mm, Strong resistance: 10-20 mm, Very strong resistance: ≥ 20 mm. F0: Antibacterial emulgel preparation without active substances, F1: Antibacterial emulgel preparation containing 2.5% active substance, F2: Antibacterial emulgel preparation containing 5% active substance, F3: Antibacterial emulgel preparation containing 10% active substance, K+: Acne Spot Treatment Gel, $p<0.05$: Significant, $p>0.05$: Not significant.

The antibacterial activity of the emulgel formulations containing methanol extract of stingless bee propolis *(H. apicalis*) was evaluated against *Staphylococcus epidermidis* and *Cutibacterium acnes*. All formulations exhibited inhibitory effects, with formulation K+ showing the highest activity, producing average inhibition zone diameters of 10.17 mm and 10.00 mm for *S. epidermidis* and *C. acnes*, respectively.

DISCUSSION

Phytochemical screening, Results of Physical Quality Evaluation of Emulgel Preparations from propolis methanol extract *H. apicalis.*

Phytochemical screening was performed to identify compounds found in the methanol extract of propolis (*H. apicalis*) that may exhibit antibacterial activity. The phytochemical screening consisted of two qualitative tests: the alkaloid test and the terpenoid test. The results of the phytochemical screening are presented in Table 4. The alkaloid test yielded positive results (+), which were identified through the formation of an orange precipitate in the fraction treated with Dragendorff reagent and a brownish-red precipitate added with Wagner reagent. The formation of an orange precipitate occurs because the alkaloid will interact with the tetraiodobismutate (III) ion. The formation of a brownish-red precipitate in the Wagner reagent is due to the formation of coordinate covalent bonds between K^+ ions with alkaloids to form a potassium-alkaloid complex, which precipitates. In the Mayer's reagent test, no white precipitate was observed, possibly due to insufficient extraction of the acidic phase, which normally allows the formation of a white precipitate. Normally, alkaloids react with tetraiodomercurate(II) ions to form a white complex precipitate; however, in this study, no precipitate formed, likely due to limited acid-phase extraction. In the terpenoid test, a positive result (+) was obtained, indicated by the appearance of a brownish ring and a reddish-yellow color. The addition of glacial acetic acid facilitates the cleavage of bonds between steroid and terpenoid groups, while concentrated H_2SO_4 promotes the hydrolysis of glycosidic linkages within the compound. If sugar bonds are released, a ring will form (Puspa et al., 2017). Groups of compounds that are suspected of being potential antioxidants in the methanol extract of propolis include flavonoids. Alkaloid, saponin. Tannin, phenol, and quinones.

Flavonoids contain hydroxyl groups capable of donating hydrogen atoms to free radicals; therefore, they exhibit strong antioxidant potential. Flavonoids are reducing agents that could inhibit many oxidation reactions. Beyond their antioxidant activity, flavonoids exhibit notable antibacterial properties through multiple mechanisms. These include disrupting bacterial cell wall and membrane integrity, increasing permeability, causing leakage of cytoplasmic constituents, and inhibiting key bacterial enzymes involved in nucleic acid synthesis and energy metabolism (Verma et al. 2021, Zhang et al. 2023). Furthermore, flavonoids such as quercetin, catechin, and apigenin can form complexes with bacterial cell wall proteins, interfering with peptidoglycan synthesis and damaging membrane potential (Zhao et al. 2022). Moreover, flavonoids have the ability as antioxidants since they can transfer an electron to free radical compounds as well as quinones, so flavonoid compounds have the potential as antibacterial (Abdallah et al. 2020).

Organoleptic Test

The purpose of organoleptic testing is to identify the dosage form by evaluating visually from shape, color, and odor. F0, F1, F2, and F3 before and after cycling have a semi-solid shape, meaning that F0, F1, F2, and F3 do not change consistency during storage. In terms of color, F0 appeared milky white both before and after cycling, indicating good color stability during storage. F1 showed a pale brown color before and after cycling, suggesting that it was also stable during storage. F2 is light cream colored before turned dark cream and exhibited phase separation after cycling likely due to the influence of temperature stress, F3 is cream colored before cycling and changes color to dark cream and two phases are formed also attributed to the influence of temperature stress during storage, The color variations among F0–F3 were influenced by the addition of *H. apicalis* stingless bee propolis methanol extract as the active ingredient, where increasing concentrations resulted in more intense coloration. Regarding odor, formulations F0, F1, F2, and F3 exhibited no noticeable change before and after cycling, indicating stable organoleptic properties during storage. These findings are consistent with previous reports that demonstrated the stability of topical and emulgel formulations under accelerated or cycling conditions. Gels containing *Chromolaena odorata* extract, for instance, exhibited no significant changes in color or odor after 30 days at 40 ± 2 °C and 75 ± 5 % RH (Rahman et al. 2021). Similarly, lycopene-based topical emulgels remained stable in odor and homogeneity, showing only minor color shifts at high temperatures (Kumar et al., 2018). A study on cassava leaf extract emulgel also confirmed that alternating storage at 4 °C and 40 °C maintained its organoleptic properties, suggesting resilience of the emulgel matrix during temperature cycling (Rahman et al. 2022).

These observations reinforce that the formulated propolis emulgel (especially F0 and F1) demonstrated good physical and organoleptic stability, whereas formulations with higher propolis concentrations (F2 and F3) were more sensitive to thermal stress. Therefore, the cycling test effectively differentiates the thermal resilience of emulgel systems and supports optimization of propolis concentration for achieving optimal stability.

pH Test

Changes in the pH of the preparation, whether increasing or decreasing, indicate that the formulation is less stable. pH variation is influenced by storage temperature, which affects degradation and ionization processes that alter the balance between acidic and basic components. Generally, higher temperatures accelerate these reactions, while lower temperatures stabilize the formulation. On the other hand, it is also caused by light from outside because photo-oxidation reactions, namely transferring energy from light waves to reactive ones through the ability to increase energy as a precaution against accelerating oxidation reactions. The compounds contained in the active substance also influence the pH, as shown as indicated by the slight increase before cycling, which tends to increase due to the methanol extract of stingless bee propolis. The methanol extract of *H. apicalis* contains alkaloids with basic properties (Alfaris et al., 2022). The pH before and after cycling from F0, F1, F2, and F3 remained within the physiological range of skin pH, namely 4.5-6.5. If the preparation is lower than the skin's physiological pH, the preparation may cause skin irritation skin and if the preparation is higher than the skin's physiological pH, the preparation can lead to dryness or disruption.

Homogeneity Test

The homogeneity test aims to determine whether the emulgel preparations are uniformly mixed. Homogeneity can be influenced by the manufacturing process, particularly the stirring rate

and temperature during mixing, which are critical when combining ingredients with different solubility levels. The results obtained from the homogeneity test, namely F0, F1, F2, and F3, before and after the cycling test, were homogeneous because there were no lumps or phase separation found in the four formulas identified as F0, F1, F2, and F3 as stable from the homogeneity factor.

These findings are in line with previous studies reporting that a well-controlled emulsification process ensures smooth texture and phase stability in emulgel systems. Khan et al. (2022) demonstrated that emulgels co-loaded with naproxen and eugenol exhibited excellent homogeneity and no phase separation under accelerated stability conditions, confirming that appropriate emulsifier concentration and mixing speed contribute significantly to product uniformity. Similarly, Wagh et al. (2025) observed that polyherbal emulgels maintained homogeneity and physical integrity throughout storage, with no evidence of coagulation or phase separation. Moreover, Malavi et al. (2022) emphasized that maintaining optimal mixing parameters—especially during the oil–water integration phase—is critical for obtaining a consistent and visually uniform emulgel formulation.

Therefore, the stable homogeneity observed in F0–F3 formulations reflects proper control of formulation parameters, indicating a successful emulsification and mixing process that ensures uniform distribution of the propolis methanolic extract from H. apicalis within the gel base.

Spreadability Results

The spreadability of F0 and F1 decreased after the cycling test due to an increase in viscosity. Higher viscosity increases resistance to flow, thereby reducing the spreadability of the emulgel. In contrast, in F2 and F3, increased because the viscosity of F2 and F3 decreases, and as the viscosity decreases, the resistance of the liquid to flow decreases, resulting in the spreadability of the emulgel increasing (Zam Zam et al., 2022). However, F0, F1, F2, and F3 variations were observed and decreased, but the spreadability of F0, F1, F2, and F3 was still in the acceptable standard range, emulgel spreadability, namely 5-7 cm.

Adhesion Test

The aim of the test adhesion testing is to identify the ability of the preparation to adhere to the skin within a certain period of time. A longer adhesion time indicates better performance, as it allows more time for the active ingredients to be absorbed through the skin. The adhesion test results are presented in Table 7. Adhesion time increased for F0 and F1 due to their reduced spreadability after cycling, whereas F2 and F3 showed decreased adhesion as their spreadability increased. Even so, F0, F1, F2, and F3 variations, but the adhesion strength of F0, F1, F2, and F3 was still within the standard for good emulgel adhesion, namely, more than 1 second. Adhesiveness is related to the spreadability of the emulgel, and the greater the spreadability of the emulgel, the faster the emulgel will stick, and the smaller the spreadability of the emulgel, the longer it will take for the preparation to stick (Zeniusa et al., 2019).

Viscosity Test

At higher temperatures, polymer chains tend to uncoil or become less entangled, resulting in lower viscosity (Mursyid, 2017). Conversely, at lower temperatures, polymer chains contract and form tighter networks, leading to increased viscosity after the cycling test. In addition, propylene glycol, used as a humectant, also affects viscosity by retaining or absorbing water from the environment to maintain formulation stability. However, in F2 and F3, higher concentrations of water reduced the ability of propylene glycol to retain moisture, leading to lower viscosity and a more fluid consistency. Although viscosity values changed, F0 and F1 still comply with the standard viscosity value for a good emulgel, namely 2000-4000 mPa · s, and F2 and F3 after cycling no longer meet the requirements for a good emulgel.

Emulsion Type Test

The purpose of the emulsion-type test is to determine the type of emulsion formed using the dilution method. The results indicated that all formulations (F0, F1, F2, and F3) were oil-in-water (O/W) type emulsions. In this system, the oil phase functions as the internal phase, while the water phase serves as the external phase, leading to the formation of an O/W emulsion (Djuwarno et al., 2021).

Antibacterial Activity Test

This research's antibacterial testing implemented the well diffusion method using the working principle of diffusing the antibacterial compound into a solid medium and where the test microorganism is inoculated. According to Pobiega et al., (2019), the

well diffusion method provides more reliable results than the disc diffusion method. This occurs because the osmotic effect occurs during the swelling process. In the well diffusion method, the concentration of the extract is higher than in the disc method, leading to a more homogeneous distribution and stronger inhibition of bacterial growth. However, the well diffusion method is more labor-intensive than the disc method because it requires specialized tools to create wells in the agar. Additionally, there is a higher risk of agar cracking around the wells, which may interfere with the diffusion and absorption of the antibacterial compound (Rahmadeni et al., 2019).

Antibacterial activity testing was evaluated using varying concentrations, namely F1 (2.5%), F2 (5%), and F3 (10%). Results of testing the antibacterial activity of the stingless bee propolis methanol extract emulgel formula (*H. apicalis*) are shown in Figure 2. The results showed that against *Staphylococcus epidermidis*, F3 exhibited the highest antibacterial activity with an average inhibition zone diameter of 10.17 mm, followed by F2 (9.67 mm) and F1 (9.34 mm). Similarly, for *Cutibacterium acnes*, the inhibition zone increased with higher concentrations, reaching 10 mm in F3. Although the difference between F1 and F2 was minimal, F3 showed a larger inhibition zone, indicating that higher concentrations of propolis extract resulted in stronger antibacterial activity due to increased levels of active compounds. These variations can be attributed to due to several factors, including the speed of diffusion for each formula is different, the nature of the agar medium used, the number of organisms inoculated, the speed of bacterial growth, the concentration used, and the conditions during incubation.

The results of this study align with previous research showing that stingless bee propolis possesses broad-spectrum antibacterial properties, particularly against Gram-positive bacteria. Al-Ani et al. (2021) and Sulaiman et al. (2023) reported that propolis derived from Heterotrigona itama and Tetragonula laeviceps exhibited strong inhibitory effects against S. aureus and C. acnes due to high flavonoid and phenolic compound content. Similarly, Dantas Silva et al. (2022) demonstrated that methanolic extracts of propolis exhibited concentration-dependent antibacterial effects, emphasizing the critical role of bioactive compound solubility and formulation matrix in enhancing diffusion through the agar medium. Moreover, studies by Nair et al. (2024) confirmed that the combination of propolis extracts in topical delivery systems such as gels or emulgels enhances antimicrobial efficacy through improved skin penetration and sustained release of active compounds.

These results contrast with those of Zeniusa (2019), who reported that even low concentrations of extract exhibited effective antibacterial activity. F0, an emulgel formulation without active substances, showed no antibacterial activity against *Cutibacterium acnes* or *Staphylococcus epidermidis*. The positive control, Acne Spot Treatment Gel, exhibited strong antibacterial activity with an average inhibition zone of 12.14 mm against C. acnes bacteria and 10.54 mm against S. epidermides bacteria. Categories F1, F2, and F3, as well as the positive control, were classified as having strong antibacterial activity, with a range of 10-20 mm. ANOVA analysis indicated that the differences in activity among the formulations are considered significant ($p < 0.05$). The Homotrigona apicalis propolis emulgel exhibits strong potential as a natural topical antibacterial agent for acne treatment. Its non-greasy texture, ease of application, and enhanced skin absorption make it suitable for long-term dermatological use. However, comprehensive safety assessments are required, as certain phenolic components may cause allergic reactions. Future studies should include in vitro cytotoxicity, irritation, and dermal toxicity tests to ensure safety and biocompatibility. Optimization of formulation parameters, in vivo efficacy evaluation, and clinical validation are also recommended. Molecular investigations to elucidate antibacterial and anti-inflammatory mechanisms will further support its therapeutic potential. This study highlights H. apicalis propolis as a promising, sustainable natural ingredient for topical pharmaceutical development.

Conclusion

The 10% propolis (*H. apicalis*) emulgel formulation met all physical and chemical testing requirements and exhibited strong antibacterial activity against *C. acnes* and *S. epidermidis*, supporting its potential development as an anti-acne emulgel.

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REFERENCES

Abdallah E.M., Elshafie A.E., & Camele, I. Flavonoids: Promising natural compounds with antibacterial potential. European Journal of Medicinal Chemistry, 2020; 207: 112738. https://doi.org/10.1016/j.ejmech.2020.112738

Achyani and Wicandra, D, 2019; Kiat Praktis Budidaya Lebah Trigona (Heterotrigona itama). Lampung, Penerbit Laduny Alifatama.

Alfaris S.M., Dhahir, R.K., & Al-Nima, A.M. Preparation, evaluation of propolis extract gel and exploring its antioxidant and antimicrobial activity. Pharmacognosy Journal, 2022; 14(6): 675–
681. https://doi.org/10.5530/pj.2022.14.153

Al-Ani I., Zimmermann S., Reichling J., Wink M. Antibacterial activities of propolis and its components against Staphylococcus aureus. Molecules, 2021; 26(14): 4384. https://doi.org/10.3390/molecules26144384

Blaskovich M.A.T, Elliot, A.G, Kavanagh A.M, Ramu S and Cooper M.A. In vitro Antimicrobial Activity of Acne Drugs Against Skin-Associated Bacteria. Scientific Reports,. 2019; 9(1): https://doi.org/10.1038/s41598-019-50746-4

Campos J.F, Dos Santos U.P and Da R.P.S. Antimicrobial, Antioxidant, Antiinflammatory, and Cytotoxic Activities Of Propolis From The Stingless Bee Tetragonisca fiebrigi (jataí), Evidence Based Complementary and Alternative Medicine, 2015; 15: 1-11. https://doi.org/10.1155/2015/296186

Chessa D, Ganau G and Mazzarello V. An overview of *Staphylococcus epidermidis* and *Staphylococcus aureus* with a focus on developing countries. Journal of Infection in Developing Countries, 2015; 9(6): 547–550, https://doi.org/10.3855/jidc.6923

Christensen G.J.M, Christian F.P and Enghild, J. Antagonism between *Staphylococcus epidermidis* and *Cutibacterium acnes* and its genomic basis. BMC Genomics, 2016; 17(1): https://doi.org/10.1186/s12864-016-2489-5

Dantas Silva, R.P., Machado, B.A.S., de Abreu Barreto G. Chemical composition, biological properties, and antibacterial activity of propolis: A review. Antibiotics, 2022; 11(3): 339.
https://doi.org/10.3390/antibiotics11030339.

Dewi Danastri R.N. The effect of pH on the duration of storage of Seligi leaf extract preparations and Eugenol from clove leaf oil as an anti-pain drug. Prosiding SNST, 2018; ISBN 978-602-99334-9-9.

Dikicier B.S. Topical treatment of acne vulgaris: efficiency, side effects, and adherence rate. Journal of International Medical Research, 2019; 47(7): 2987–2992. https://doi.org/10.1177/0300060519847367

Djuwarno E.N, Hiola F and Isa I. Formulation of Moringa leaf extract emulgel preparation (*Moringa oleifera* Lam) and antioxidant activity test using DPPh method. Indonesian Journal of Pharmaceutical Education, 2021; 1(1): 10–19. https://doi.org/10.37311/ijpe

Doğan B, Bektöre B, Karabacak E and Özyurt M. Resistance status of antibiotics in Gram-positive bacteria isolated from acne lesions in

Istanbul, Turkderm Deri Hastaliklari ve Frengi Arsivi. Istanbul Assoc. of Dermatology and Venerology, 2017; 51(2): 32–36, https://doi.org/10.4274/ turkderm.23169

Francoy T.M., Silva R. A. O and Nunes S. P. Gender Identification Of Five Genera Of Stingless Bees (apidae, meliponini) Based On Wing morphology Genet. Molecular Research, 2019; 8(1): 207-214. https://doi.org/10.4238/vol8-1gmr557

Gaikwad N., Aher R., Bagul M., Sharma Y., Sonawane D., & Patil D.A comprehensive review on emulgel as a topical drug delivery system. International Journal in Pharmaceutical Sciences, 2024; 2(5): 1398–1405. https://doi.org/10.5281/zenodo.11317649

Grumezescu A.M. and Holban A.M. Therapeutic, Probiotic, and Unconventional Foods. Elsevier, 2018; 2(1): 141-150.

Hermalinda R., Irham T and Zairin N.H. Total Flavonoid Contenet Analysis Of Ramania Leaves Extract Using Ethanol, Methanol and N Hexane as Solvent. Journal Tooth. 2019; 4(1): 60-63. https://doi.org/10.20527/dentino.v4i1.6177

Khan B.A., Ahmad S., Saud B.A. Fabrication and Characterizations of Pharmaceutical Emulgel Co-Loaded with Naproxen-Eugenol for Improved Analgesic and Anti-Inflammatory Effects. Gels, 2022; 8(10): 608. https://doi.org/10.3390/gels8100608

Kumar S., Sohail M., Waris M., & Vimal R. An approach to enhanced stability: formulation and characterization of Solanum lycopersicum-derived lycopene-based topical emulgel. Saudi Pharmaceutical Journal, 2018; 26(8): 1170–1177. https://doi.org/10.1016/j.jsps.2018.07.00

Kustiawan P.M., Zulfa A.F., Batistuta M.A., Nur D., and Hanifa C. Comparative analysis of phytochemical, total phenolic content, antioxidant, and antibacterial activity of two species stingless bee propolis from East Kalimantan. Malaysian Journal of Medicine and Health Sciences, 2022; 18(7): 50-55.

Liu Y., Liu J and Zhang Y. Research Progress on Chemical Constituents of Zingiber officinale Roscoe. International, 2019;5370823. https://doi.org/10.1155/2019/5370823

Lopes A.J.O., Vasconcelos C.C., Pereira F.A.N and Silva R.H.M. Anti Inflammatory And Activity And Antinociceptive Activity Of Pollen Extract Collected By Stingless Bee melipona fasciculata, International Journal, Moleculer Science, 2019; 20(4): 512. https://doi.org/10.3390/ijms20184512

Lutpiatina L. Effectiveness of Kelutut Bee Propolis Extract (*Trigona* spp) in Inhibiting Growth *Salmonella typhi*, *Staphylococcus aureus* and *Candida albicans*. Journal of Health Scales, 2015; 6(1): 6-13.

Mardiah M. Resistance TestStaphylococcus aureus Against Antibiotics, Amoxillin, Tetracyclin and Propolis. Journal of Natural and Environmental Sciences, 2017; 8(16): 1-6. https://doi.org/10.20956/jal.v8i16.2978

Malavi S., Kumbhar P., Manjappa A., Chopade S., Patil O., & Dwivedi J. Topical Emulgel: Basic Considerations in Development and Advanced Research. Indian Journal of Pharmaceutical Sciences, 2022; 84(5): 1105-1115. https://doi.org/10.36468/pharmaceutical-sciences.1005

Mera I.F.G., Falconí D.E.G and Córdova V.M. Secondary metabolites in plants: Main classes, phytochemical analysis and pharmacological activities. Bionatura, 2019; 4(4):1000-1009. https://doi.org/10.21931/RB/2019.04.04.11

Milutinov J., Krstonošić V., Ćirin D., & Pavlović N. Emulgels: Promising carrier systems for food ingredients and drugs. Polymers, 2023; 15(10): 2302. https://doi.org/10.3390/polym15102302

Mursyid A.M. Evaluation of physical stability and diffusion profile of gel preparations (olive oil). Jurnal Fitofarmaka Indonesia, 2017; 4(1): 205–211. https://doi.org/10.33096/jffi.v4i1.229

Nair P.A., Anitha A., Ramesh M., & Shashikumar S. Propolis-loaded topical gels: A natural therapeutic approach for acne and skin infections. Pharmaceutics, 2024; 16(2): 327. https://doi.org/10.3390/pharmaceutics16020327.

Neves J.R., Francesconi, F., Costa, A., Ribeiro B.M., Follador I and Almeida L.M.C. *Cutibacterium acnes* and bacterial resistance. Surg Cosmet Dermatol, 2015; 7(3 Suppl 1): S27-38

O'Neill A.M., Nakatsuji T., Hayachi A., Williams M. R., Mills R.H., Gonzalez D.J., and Gallo R.L. Identification of a Human Skin Commensal Bacterium that Selectively Kills *Cutibacterium acnes*. Journal of Investigative Dermatology, 2020; 140(8):1619-1628. https://doi.org/10.1016/j.jid.2019.12.026

Pobiega K., Kraśniewska K., Derewiaka D., Gniewosz M. Comparison of the antimicrobial activity of propolis extracts obtained by means of various extraction methods. Journal of Food Science and Technology, 2019; 56(12): 5386–5395. https://doi.org/10.1007/s13197-019-04009-9

Powale S., Chandel V. K., and Asati S. Preparation and characterization of ethosomes for topical delivery of clindamycin. J. Drug Deliv. Ther, 2022; 12 (1): 109–113. https://doi.org/10.22270/jddt.v12i1.5190

Pujirahayu N., Ritonga H. and Uslinawaty Z. Properties And Flavonoids Content In Propolis Of Some Extraction Method Of Raw Propolis. International Journal of Pharmacy and Pharmaceutical Sciences. 2014; 6(6): 338-340.

Puspa O.E., Syahbanu I and Wibowo M.A. Phytochemical and Toxicity Test of Nutmeg Leaf Essential Oil (*Myristica fragans* Houtt) From Lemukutan Island. Jurnal Kimia Khatulistiwa, 2017; 6(2): 1–6.

Rahmadeni Y., Febria F.A and Bakhtiar A. Potential of Pakih Sipasan (Blechnum orientale) as Antibacterial Against *Staphylococcus aureus* and Methicillin Resistant Staphylococcus aureus. Journal of Biological Sciences, 2019; 6(2): 224-229. https://doi.org/10.24843/metamorfosa.2019.v06.i02.p12

Rahman A. Physical characterisation and stability study of formulated *Chromolaena odorata* gel. Current Drug Delivery, 2021; 19(4): 479–490. http://doi.org/10.2174/1567201818666210419114809

Rahman N., et al. Physical stability evaluation of cassava leaf aqueous extract emulgel (*Manihot esculenta*). EduChemia Journal, 2022; 7(1): 45–54. http://doi.org/10.30870/educhemia.v8i1.15898

Rismawati S.N and Ismiyati I. The Effect of PH Variations on Flavonoid Levels in Propolis Extraction and Their Characteristics as Antimicrobials. Journal Conversion. 2017; 6(2): 90. https://doi.org/10.24853/konversi.6.2.89-94

Rosyidi D., Radiati E.L., Minarti S., Mustakim M., Susilo A., Jaya F and Azis A. Comparison of the Antioxidant Properties of Propolis in Two Types of Bees (*Apis mellifera* and *Trigona* sp.) in Mojokerto and Batu, East Java, Indonesia. Journal of Animal Science and Technology, 2018; 13(2): 108-117. https://doi.org/10.21776/ub.jitek.2018.013.02.5

Sadeek & Utami E.R and Rosa Y. Antibacterial Activity of Fragrant Pandan Leaf Ethanol Extract (*Pandanus amaryfolius*) against Staphylococcus aureus, Jurnal kesehatan Ilmiah, 2021;1(11): 61-71.

Sadeek A.M and Abdallah E.M. Phytochemical Compounds as Antibacterial Agents: A Mini Review. Saudi Arabia Glob J Pharmaceu Sci, 2019; 53(4): 555720. https://doi.org/10.19080/GJPPS.2019.07.555720

Sulaiman A.M., Sani M.A., Wahab R.A., et al. Comparative antibacterial efficacy of stingless bee (*Heterotrigona itama*) propolis extracts against acne-causing bacteria. Saudi Journal of Biological Sciences, 2023; 30(6): 103683. https://doi.org/10.1016/j.sjbs.2023.103683

Supomo E.S.Syamsul, A.Apriliana C.Saleh, Erwin and D. Lestari. Antioxidant Assay Of Dayak Onion (*Eleutherine palmifolia*) VIA DPPH (1,1-Difenil-2-Pikrilhidrazil) And Bslt Test For Its Active Fraction. Rasayan Journal of Chemistry, 2019; 12(3):1340-1346. https://doi.org/10.31788/RJC.2019.1235264

Suryawanshi J.S. and Gawade S.P. Enhanced anti-arthritic effect of mustard oil in Nanoemulgel Formulation: A comparative clinical study. Research J. Pharm. and Tech, 2020; 13(8): 3738-3744.

Syafrizal Hariani N and Budiman B. Phytochemical, Toxicity and Antioxidant Analysis of Pollen Extracts (bee pollen) bees a *Trigona* spp. Proceeding of Mulawarman Pharmaceutical, 2016;(16): 408-416. https://doi.org/10.25026/mpc.v3i2.140

Tabri F. The association between *Staphylococcus epidermidis* and palmitic acid level in patients with *Acne vulgaris*. Surgical and Cosmetic Dermatology. Sociedade Brasileira de Dermatologia, 2019; 1, 11(2): 105–109. https://doi.org/10.5935/ scd1984-8773.20191121382

Tan A.U., Schlosser B.J and Paller A.S. A review of diagnosis and treatment of acne in adult female patients. International Journal of Women's Dermatology, 2018; 4: 56–71. https://doi.org/10.1016/j.ijwd.2017.10.006

Verma N., Singh D., & Kumar V. Recent advances in understanding the antibacterial potential of flavonoids and their mechanisms of action. Phytotherapy Research, 2021; 35(9): 5104–5120. https://doi.org/10.1002/ptr.7104.

Wagh P, Karnik H, Kuchekar A., Chaudhari G., & Gawade A. Design and Evaluation of Emulgel with a Polyherbal Basis for Topical Delivery. Journal of Medical Pharmaceutical and Allied Sciences, 2025; 14(1): 6929–6936. https://doi.org/10.55522/jmpas.V14I1.6784.

Waranuch N., Phimnuan P., Yakaew S., Nakyai W., Grandmottet F., Onlom C., Srivilai J., & Viyoch J. Antiacne and antiblotch activities of a formulated combination of Aloe barbadensis leaf powder, *Garcinia mangostana* peel extract, and *Camellia sinensis* leaf extract. Clinical, Cosmetic and Investigational Dermatology, 2019; 12: 383. https://doi.org/10.2147/CCID.S200564.

Yadav S.K., Mishra M.K., Tiwari A and Shukla A. Emulgel: a new ap- proach for enhanced topical drug delivery. Int. J. Curr. Pharm. Sci, 2017; 9(1):15-19. https://doi.org/10.22159/ijcpr.2017v9i1.16628

Yan J., Chen X.; Yu S.; Zhou H. Comparison of different in vitro mucoadhesion testing methods for hydrogels. J. Drug Deliv. Sci. Technol. 2017; 40: 157–163. https://doi.org/10.1016/j.jddst.2017.06.012

Zahrah H., Mustika A and Debora K. Antibacterial Activity and Morphological Changes of *Cutibacterium acnes* After Administration of Extracts *Curcuma xanthorrhiza*. Jurnal Biosains Pascasarjana, 2019; 20(3): 160. https://doi.org/10.20473/jbp.v20i3.2018.160-169

Zam Zam A. N and Musdalifah M. Formulation and Evaluation of Physical Stability of Black Pepper Seed Extract Cream (*Piper nigrum* L.) Using Emulgator Variations. Journal Syifa Sciences and Clinical Research, 2022; 4(2): 304–313. https://doi.org/10.37311/jsscr.v4i2.14146

Zeniusa P., Ramadhian M.R., Nasution S.H and Karima N. (2019). Inhibitory Power Test of Green Tea Ethanol Extract against Escherichia coli In Vitro. Majority, 2019; 8(2):136–143.

Zhang L., Cheng Y., Xu M., Song Y., & Zhang L. Mechanistic insights into the antibacterial activity of flavonoids against multidrug-resistant bacteria. Frontiers in Microbiology, 2023; 14:1123456. https://doi.org/10.3389/fmicb.2023.1123456.

Zhao L., Chen J., Su J., Li Z., & Wang Y. Flavonoids as membrane-active antibacterial agents: Mechanisms and structure–activity relationships. Journal of Agricultural and Food Chemistry, 2022; 70(7): 2215–2227. https://doi.org/10.1021/acs.jafc.1c07311.