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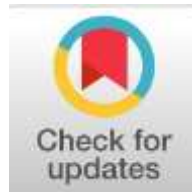
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Effectiveness Test Of Betel Leaf Extract Gel Repellent Against Anopheles Mosquitoes Using The Whopes Method

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Abstract

General Background Malaria remains a critical global health challenge directly linked to mosquito vectors. **Specific Background** Commercial repellents heavily rely on synthetic DEET, which frequently causes hypersensitivity and skin irritation during prolonged use. **Knowledge Gap** While betel leaf exhibits insect-repellent potential, previous studies lack comprehensive formulation stability evaluations and standardized bioassays. **Aims** This experimental study evaluates the physical stability and repellent efficacy of betel leaf essential oil gel against Anopheles mosquitoes following WHOPEs guidelines. **Results** Physicochemical tests confirmed that formulations with 17%, 20%, and 23% essential oil achieved excellent homogeneity, ideal viscosity, and physiological skin compatibility. Adult mosquito bioassays demonstrated that 20% and 23% formulations provided six hours of continuous protection, matching commercial synthetic products. **Novelty** Integrating rigorous pharmaceutical stability parameters with international WHOPEs standards establishes an optimal dosage for a standardized natural vector repellent. **Implications** Public health programs should utilize locally sourced botanical gels to provide safer, environmentally sustainable alternatives for malaria prevention.

Highlights

- Synthetic DEET repellents trigger skin hypersensitivity during prolonged topical application.
- Gel matrices ensure optimal skin adhesion for volatile botanical compounds.
- Twenty percent essential oil formulations guarantee six hours of vector protection.

Keywords

Betel Leaf Essential Oil; Gel Repellent; Anopheles Mosquitoes; Malaria Prevention; Bioassay Standards

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INTRODUCTION

Malaria remains a real global health challenge, especially in tropical countries like Indonesia.(1)According to the Indonesian Ministry of Health (2023), malaria elimination at the district/city level has been achieved. Of the 514 districts/cities, 372 have achieved malaria elimination, or approximately 72%, by 2022. Indonesia's target is to eliminate 90% of malaria by 2025.(2). According to the 2023 report, there were 418,546 positive malaria cases in Indonesia. This number decreased slightly by 5.6% from 443,530 cases in 2022. It was recorded that 92% of national malaria cases throughout last year originated in Papua. Specifically, there were 163,962 cases in Papua; 150,225 cases in Central Papua; 43,862 cases in South Papua; 11,070 cases in Highland Papua; 10,170 cases in West Papua; and 7,702 cases in Southwest Papua.(3) (4)Malaria is an infectious disease caused by parasites of the genus *Plasmodium* and transmitted by the *Anopheles* mosquito. Malaria can re-emerge if prevention programs are not consistently implemented.(5) (6).

Nowadays, efforts to eradicate malaria are carried out by eradicating the vector that causes malaria (*Anopheles* mosquitoes) and continuing with providing treatment to those suspected of suffering from malaria or treatment is also very necessary to be given to malaria sufferers who are proven positive in the laboratory.(7)In addition to direct treatment, malaria eradication often involves spraying homes and the surrounding area with insecticide to kill adult mosquitoes. Other efforts are also made to eradicate mosquito larvae. Another individual effort to prevent *Anopheles* mosquito bites is the use of insect repellents.(8) (9). Most repellents circulating in the community contain chemicals, namely DEET (N, N-diethyl-m-toluidine) 12.5%.(10). DEET has excellent repellent power, but its use can cause hypersensitivity and irritation(11)Therefore, to limit the use of DEET as a repellent, it is necessary to find an alternative repellent that is safe, comfortable, and effective in preventing mosquito bites.(10).

One way is to utilize natural ingredients, such as betel leaves. Betel leaves are widely used as mosquito repellent due to their active ingredient.(12). In a number of studies, betel leaves contain 1-4.2% essential oil, hydroxychavicol, 7.2-16.7% chavicol, 2.7-6.2% chavibetol, 0-9.6% allylpyrocatechol; 2.2-5.6% carvacol; 26.8-42.5% eugenol; 4.2-15.8% eugenol methyl ether; 1.2-2.5% p-cymene; 2.4-4.8% cineole; 3-9.8% caryophyllene; and 2.4-15.8% cadinene, in addition to containing estragol, terpenene, sesquiterpene, phenyl propane, tannin, diastase, sugar, and starch.(13) (14).

Previous studies have primarily focused on evaluating the repellent activity of essential oils or extracts from betel leaf in simple formulations, such as lotions, sprays, or crude preparations. While these studies reported varying degrees of effectiveness, many lacked comprehensive evaluation of formulation stability, physicochemical properties, and standardized efficacy testing methods. Furthermore, only a limited number of studies have applied internationally recognized evaluation protocols, such as the World Health Organization Pesticide Evaluation Scheme (WHOPES), particularly against *Anopheles* mosquitoes, which are the primary malaria vectors. This constraint limits the ability to analyze and depend on current findings and slows down the more general use of plant-based repellents in malaria management initiatives. Considering these factors, this study intends to fill the present gap by creating a gel-type mosquito repellent using essential oil taken from betel leaf and checking its physical stability and efficacy against *Anopheles* mosquitoes following the WHOPES guidelines. The special quality of this study is that it combines thorough pharmaceutical testing with the creation of natural repellents and conformity to worldwide bioassay standards. Looking at various levels of betel leaf essential oil in gels, this study provides relative knowledge of the ideal dose levels balancing safety, stability, and effectiveness in gel forms.

The physical stability of betel leaf extract gel when administered on the skin as well as its effectiveness as an mosquito repellent for *Anopheles* are the primary goals of this study. Particularly in regions where the disease is common, the findings of this study are expected to significantly support malaria control initiatives and scientifically forward the development of safer, plant-derived mosquito repellents. Moreover, the study aims to promote use of local natural resources as sustainable substitute for synthetic repellents, so enabling environmentally friendly and community-led approaches of malaria management.

METHODS

This study examined the physical stability and repellent properties of a gel formulation generated from essential oil of betel leaves (*Piper betle* Linn) against *Anopheles* mosquitoes using a lab-based experimental study design. The research progressed in two phases. Using steam distillation first to get the essential oil from betel leaves, then making repellent gel compositions with three different oil amounts: 17%, 20%, and 23%. Following the World Health Organization Pesticide Evaluation Scheme (WHOPES), the next phase concentrated on assessing the physical stability and repelling effectiveness of these gel formulations. By means of maintenance of controlled conditions, the experimental approach chosen sought to offer exact measurements of the formulation's quality and biological effectiveness (WHO, 2013; 16).

Several trials, including organoleptic evaluations, homogeneity checks, pH measurements, viscosity readings, spreadability, adhesion, and cycling stability tests, were covered in the review of the physical stability of the gel formulations. Visual observations of organoleptic characteristics—color, smell, texture—were carried out; homogeneity was evaluated by examining the constant look of the gel on glass slides. To ensure their conformity with skin physiology, pH levels were ascertained with a calibrated digital pH meter. To assess the gel's performance on the skin, spreadability and adhesion tests were conducted and a Brookfield viscometer was employed to establish viscosity readings. To identify any possible physical changes over time, stability tests used a cycling test approach that exposed samples to alternate storage temperatures of 4 °C and 40 °C. Triple-time conduct of these studies sought to enhance data consistency and dependability (18) (22).

The WHOPES guidelines for adult *Anopheles* mosquitoes in a regulated environment (27 ± 2 °C and 60–80% relative

humidity) were applied to assess the repelling ability of the formulations. Five qualified fit human volunteers were testing targets in everything. The study investigated how many mosquitoes landed on untreated skin (negative control), store-bought repellent-applied skin (positive control), and skin that got the betel leaf gel formulations. Observations were noted at 1, 3, and 6 hourly intervals after application. The physical stability test results were analyzed descriptively by contrasting the measured counts with accepted standards for topical preparations; the repellent's effectiveness was then evaluated from the reduction in mosquito landings relative to the control groups. This analytical approach allowed for an integrated assessment of formulation quality and biological effectiveness of the betel leaf repellent gel (20)(WHO, 2013).

RESULTS AND DISCUSSION

This research was conducted at the Pharmaceutical Technology Laboratory of Jayapura University (Isolation Stage of betel leaf essential oil, gel formulation stage and physical stability test of the preparation) and then for testing the effectiveness of the preparation using the WHOPES (World Health Organization Pesticide Evaluation Scheme) method was conducted at the Vector Control and Pest Control and Parasitology Laboratory of Jayapura Health Polytechnic. The process of presenting the results of this research includes:

1. The initial stage of this research was carried out by isolating betel leaf essential oil (Distillation Method)

The procedure for isolating betel leaf essential oil begins with washing 15 kg of fresh betel leaves, then chopping them (cutting them into small pieces) and drying them (not in direct sunlight) for 7 days until dry, until a dry weight of 6.18 kg is obtained. The dried betel leaves are put into a distillation vessel that has been assembled and is ready to be turned on (turned ON) the distillation device, so that the water vapor and oil components are obtained, then put into a closed container. The next process is the separation of the water vapor and oil components in a separating funnel, so that the betel leaf essential oil is obtained.

Figure 1. *Distillation apparatus circuit*



Figure 2. *Results of Isolation of Betel Leaf Essential Oil*



2. Gel Repellent Preparation Formulation Stage

The gel formulation is made in 3 variations of betel leaf essential oil concentration or betel leaf extract, where Formula 1 (17% betel leaf extract), Formula 2 ((20% betel leaf extract) and Formula 3 (23% betel leaf extract). The gel formulation begins with weighing the ingredients (betel leaf essential oil or betel leaf extract, HPMC, Triethanolamine, Propylene glycol, methyl paraben and distilled water), the betel leaf essential oil is dissolved with a little distilled water and then homogenized. Then mix propyl paraben, TEA and glycerin (called Mixture A), then develop HPMC with distilled water that has been heated at a temperature of 80o C (called Mixture B), then mix and homogenize Mixture A and Mixture B and add the extract and the remaining distilled water

Table 1. Formula for Repellent Gel with Betel Leaf Essential Oil Extract

Material	Formula (%)			Function
	F1	F2	F3	
Betel leaf extract (Essential oil)	17	20	23	Active Ingredients
HPMC	1.5	1.5	1.5	Gelling agent
Triethanolamine	0.12	0.12	0.12	pH Buffer
Propylene glycol	15	15	15	Humectant
Methyl Paraben	0.18	0.18	0.18	Preservative
Aquadest	Ad 100	Ad100	Ad 100	solvent

Figure 3. Gel Formula Making Process



3. Physical Stability Test Stage of Gel Preparation

Physical stability tests of the preparation were carried out to determine the stability of the betel leaf extract gel repellent, which includes:

a) Organoleptic Test

Organoleptic tests include observing the color, smell, and shape of the gel preparation visually (15)

Table 2. Organoleptic Test Results of Betel Leaf Essential Oil Gel Repellent

Formula	Organoleptic Test		
	Color	Smell	Form
Formula 1 (17%)	Yellowish White	Typical betel leaf	Thick
Formula 2 (20%)	Yellowish White	Typical betel leaf	Thick
Formula 3 (23%)	Yellowish White	Typical betel leaf	Thick

Organoleptic tests include visual observations of the color, odor, and shape of the gel preparation.

b) Homogeneity Test

The gel homogeneity test is carried out by applying some of the gel using two glass objects, then observing the composition

of the gel preparation to see whether there are coarse grains or not (15)

Table 3. Results of the Homogeneity Test of the Gel Repellent from Betel Leaf Essential Oil

Formula	Homogeneity Test
Formula 1 (17%)	Homogeneous
Formula 2 (20%)	Homogeneous
Formula 3 (23%)	Homogeneous

Figure 3. Homogeneity Test Results



c) pH test

The pH test is performed by weighing 0.5g of the gel preparation and dissolving it in 50 mL of distilled water. Next, calibrate the pH meter with a buffer solution before each measurement. Clean the electrode and dip it into the sample. The pH value will then be displayed on the pH meter scale, and the reading should be recorded.(16).

The pH value obtained from the betel leaf essential oil gel repellent preparation ranges from 5.37 to 5.57. The ideal pH value for topical preparations is in the range of 4.5 to 6.5. Preparations that are too acidic can cause skin irritation, while conditions that are too alkaline can cause scaling. Therefore, it is important to ensure that the gel preparation has an appropriate pH value to meet health and safety requirements (17).

Table 4. Results of pH Test of Betel Leaf Essential Oil Gel Repellent

Formula	pH value			Average pH Value (pH requirement = 4.5-6.5)
	Replication 1	Replication 2	Replication 3	
Formula 1 (17%)	5.5	5.4	5.5	5.47
Formula 2 (20%)	5.3	5.4	5.4	5.37
Formula 3 (23%)	5.6	5.6	5.5	5.57

Figure 4. pH Test Results



d) Spreadability Test

To test the spreadability of the gel, place 0.5g of gel in the center of a glass scale, cover it with another glass, and let it sit for 5 minutes. Then, measure the diameter of the spread.(16).

The results of the spreadability test of the gel repellent preparation, the diameter ranges from 5.12 - 5.45 cm, while the ideal requirement is 5 - 7 cm. The greater the spreadability value, the lower the viscosity of the gel. Gels that have a low viscosity (thinner) will produce a spread diameter of (18)

Table 5. Results of the Spreading Power Test of Betel Leaf Essential Oil Gel Repellent

Formula	Diameter of Spread Power Test (cm)				Average Diameter (Requirements= 5-7 cm)
	Diameter 1 (cm)	Diameter 2 (cm)	Diameter 3 (cm)	Diameter 4 (cm)	
Formula 1 (17%)	5.4	5.5	5.5	5.4	5.45
Formula 2 (20%)	5.1	5.3	5.2	5.2	5.20
Formula 3 (23%)	5.0	5.2	5.1	5.2	5.12

Figure 5. Results of the Spread Power Test



e) Adhesion Test

The adhesive strength test was carried out by weighing 0.25 g of gel and then placing it on the first glass object, then the second glass object was placed on top of the first glass object with half of the body of each glass coming out of the body of the other glass / not touching the other glass completely. The time required for the two glass objects to be detached was recorded. This test was replicated 3 times.

The results of the adhesive strength test for this formula show that the time from when the two glass objects joined until they were separated was 8.96 – 20.61 seconds. This is in accordance with the adhesive strength test requirements, which is no less than 4 seconds (19).

Table 6. Results of the Adhesion Test of the Gel Repellent from Betel Leaf Essential Oil

Formula	Time it takes for the object glass to come off			Average Time (Requirement >4 seconds)
	Replication 1 (second)	Replication 2 (second)	Replication 3 (Second)	
Formula 1 (17%)	20.11	21.28	20, 43	20.61
Formula 2 (20%)	14.44	14.56	14, 25	14.47
Formula 3 (23%)	8.97	8.72	9.19	8.96

Figure 6. Adhesion Test Results



f) Viscosity Test

Measuring the viscosity of the gel preparation using a Brookfield viscometer. Prepare 100 mL of gel, set spindle no. 6 to a rotation speed of 60 rpm, lower the spindle until it is immersed in the gel preparation. Move the switch to the on position and run the power and record the gel viscosity results. According to SNI 16-4380-1996, the viscosity value of the gel preparation is 3000-5000 cp or 500-10,000 mPa.s.(16). The viscosity test result for this formula is 4995 mPa.s. This is in accordance with the Viscosity test requirements of 500-10,000 mPas (21)

Table 7. Viscosity Test Results of Betel Leaf Essential Oil Gel Repellent

Formula	Viscosity Test			Average Viscosity (Requirements= 500- 10,000 mPa.s)
	Replication 1 (m.Pa.s)	Replication 2 (m.Pa.s)	Replication 3 (m.Pa.s)	
Formula 1 (17%)	4,995	4,995	4,995	4,995
Formula 2 (20%)	4,995	4,995	4,995	4,995
Formula 3 (23%)	4,995	4,995	4,995	4,995

Figure 7. Viscosity Test Results



g) Stability test

The stability test of the gel preparation was conducted using the Cycling Test method. The test was conducted by storing the betel leaf extract gel preparation with various gelling agents in a refrigerator at 4°C for 24 hours, then transferred to a drying cabinet at 40°C for 24 hours (one cycle).(15).

Table 6. Results of the Stability Test of the Gel Repellent of Betel Leaf Essential Oil

Formula	Homogeneity	p H	Adhesive Power (seconds)	Spread Power (cm)	Viscosity (mPa.s)
Formula 1	Homogeneous	5.	20.61	5.45	4,995

(17%)	s	47			
Formula 2 (20%)	Homogeneous	5.37	14.47	5.20	4,995
Formula 3 (23%)	Homogeneous	5.57	8.96	5.12	4,995

4. Effectiveness Test Stage of Preparation (Gel Formulation)

The effectiveness test of the gel repellent preparation was conducted at the Vector Control and Pest Control Laboratory & Parasitology of the Jayapura Health Polytechnic using the WHOPES (World Health Organization Pesticide Evaluation Scheme) method, namely using Anopheles mosquitoes placed in a container and conditioned at a temperature of $27 \pm 2^\circ\text{C}$, relative humidity of 60-80%. The subject numbered 5 people who met the inclusion criteria, namely healthy, aged 17-44 years and had no history of allergies.

Repellent activity testing consists of negative control treatment, positive control (using commercial repellent) and treatment with gel repellent preparations (Formula 1, Formula 2 and Formula 3).

Catching these mosquitoes took quite a long time, because it encountered several obstacles because for every 30-50 mosquitoes caught, only about 5 of them were Anopheles mosquitoes for further testing of the gel repellent activity against Anopheles mosquitoes.

Table 7. Results of the Effectiveness Test using the Whopes method

Formula	Number of mosquitoes that land		
	1 hour	3 hours	6 hours
Formula 1 (17%)	-	1 Tail	4 Tails
Formula 2 (20%)	-	-	1 Tail
Formula 3 (23%)	-	-	1 Tail
Control (+)	1 Tail	1 Tail	3 Tails
Control (-)	5 Tails	4 Tails	8 Tails

Figure 7. Results of the Gel Repellent Effectiveness Test



In this study, the research sample in the form of Betel leaf extract was obtained from Doyo Baru, Waibhu District, Jayapura Regency. The procedure for isolating betel leaf essential oil begins with washing 15 kg of fresh betel leaves, then chopping them (cutting them into small pieces) and drying them (not in direct sunlight) for 7 days until dry, until a dry weight of 6.18 kg is obtained. The dried betel leaves are put into a distillation vessel that has been assembled and is ready to be turned on (ON) the Distillation apparatus, so that the water vapor and oil components are obtained, then put into a closed container. The next process is the separation of the water vapor and oil components in a separating funnel, so that the betel leaf essential oil is obtained.

In this study, a gel repellent preparation containing betel leaf extract was formulated, with the addition of several supporting ingredients to produce a high-quality and stable gel. The ideal gel has a base that does not react with other components in the formula and is safe to use. The gel mask was made using a gelling agent, humectant, and preservative, which were added according to established formulation procedures (23).

In gel formulations, the gelling agent component functions as a gel structural network former. In this gel formulation, HPMC

is used as a gelling agent in varying amounts to obtain the optimal gel form. HPMC has advantages, including: a more stable pH value, a higher spreadability value, and gels with HPMC extract do not affect spreadability (24).

In some topical formulations, the addition of triethanolamine (TEA) is required as a pH neutralizer to maintain stability and reduce irritation. Preservatives such as methylparaben are used to prevent the growth of microorganisms during storage. Moreover, propylene glycol functions as a moisture-retaining agent, helping to sustain the hydration levels of the formulation while also enhancing the dissolvability of the active component within the gel base. Additionally, propylene glycol may engage with the polymers that form the gel, lessening the rigidity of the gel network. This interaction helps create a gel that is more stable and flexible, therefore enabling the active component's effective release.

Then, the physical stability and performance of every formulation were evaluated. Physical stability tests included evaluations of consistency in thickness, spreadability, stickiness, sensory qualities, uniformity, and acidity levels. Using the Whopes approach, which was implemented right on the subjects, the performance evaluation was carried out.

The findings of the sensory evaluations showed that every formulation had a different smell linked to the extract, which pointed to the fact that the volatile components from betel leaves were still present in the product. Because the three formulations varied in active component concentration by just 3%: 17% in formulation one, 20% in formulation two, and 23% in formulation three, there were no observable color differences among them. Different extract concentrations show a relationship between the extract and the gel base (HPMC), whereby more extract can change the gel structure and influence its viscosity. The physical characteristics of a formulation including color, smell, and texture can change with the amount of active ingredients in it.

Homogeneity testing guarantees that the preparation's active components and other ingredients are uniformly mixed. As seen in Table 2, the homogeneity test findings on the preparation indicated by the lack of coarse grains, lumps, or phase separation show that all compositions seem homogenous. This demonstrates that the mixing process is going on properly.

Viscosity testing seeks to find out how consistent the gel preparation is. Differences in viscosity values between formulas are influenced by differences in the concentration of Chinese betel leaf extract added. A viscosity value that is too low can cause the gel to be less stable and easily spread excessively, while a viscosity that is too high can make it difficult to take and apply the preparation to the skin. Thus, the viscosity values obtained in this study indicate that the three betel leaf extract gel formulas have good viscosity and meet the requirements for topical preparations (23).

Based on the pH measurement results in Table 3, the pH value obtained ranged from 5.37 to 5.57. This value is in accordance with the ideal pH requirements for topical preparations, namely 4.5–6.5 (24). In addition to extracts, the use of other additives such as polymers or solvents can also affect the final pH of the preparation. A pH that is too high risks causing skin irritation because it does not match the skin's physiological pH (around 4.5–6.5). Therefore, it is necessary to adjust the pH by adding a neutralizing agent or pH regulator so that the preparation meets the requirements and is safe to use. The results of this study are in line with the findings of which stated that the presence of active compounds in herbal extracts can affect the pH stability of the preparation (25).

The effectiveness of mosquito repellent gel preparations was tested using the Whopes method, which involves direct intervention or testing on the subject. The results showed that the repellent's effectiveness was equal to, and even exceeded, that of the positive control. This concludes that all three formulas are effective and can be used as mosquito repellents.

Anopheles mosquitoes suck blood to help their eggs develop. Mosquitoes suck human blood because they are attracted to the presence of carbon dioxide, excretion products, and lactic acid in the human body, which are recognized as odor molecules by mosquito receptors. The mechanism of action of betel leaf essential oil as a mosquito repellent is that the aroma of the essential oil blocks the human body odor molecules from reaching the mosquito odor receptors, which causes failure to detect the presence of the human body due to interference in odor recognition by the mosquito's brain (19). The odor molecules bind to odorant-binding proteins (OBPs) which are then carried to the olfactory receptor neurons (ORNs). The odor molecules are transmitted to the mosquito's antennal lobe, triggering a response in the form of mosquito behavior to avoid the smell of the essential oil (20).

The observed sustained repellent activity of the betel-leaf (*Piper betle*) gel particularly at 20%–23% essential-oil concentrations aligns with a growing body of literature showing that plant essential oils can provide meaningful protection against mosquito landings, but that effect size and duration are highly formulation-dependent. Plant-based oils have variable repellent effects depending on species and testing circumstances, recent research and real-world tests indicate: some essential oils offer significant short protection, while others may require more doses or better techniques to increase duration (26). Earlier *Piper betle* research showing concentration-based protection (27) correspond with our results which show equal or better efficacy over a 6-hour period on the WHOPES test for the greater amounts. However, 28 shows that they improve on those results by demonstrating long-lasting efficacy in a gel formulation instead than a simple lotion or basic extract. This supports the theoretical premise that eugenol and hydroxychavicol—volatile natural chemicals—disrupt mosquito smell sensing (by interfering with OBPs and ORNs), but that their adherence to the skin and controlled release via the gel formulation are critical for obtaining long-lasting repellent effects (20) (29).

Conventional topical-delivery methods help to explain the results on formulation and stability: semi-solid compounds including HPMC gels reduce volatility, lengthen the time on the skin, and enable a regulated release of active volatile components, hence offering continuous protection—unlike unformulated essential oil (22) (18). The pH, viscosity, and adhesion values we discovered fall within the acceptable range for topical applications, and the cycling test showed physical resilience—these physicochemical features likely help to explain the variations in efficacy seen among the 17%, 20%, and 23% formulations. Recent research on controlled-release systems show greatly increasing the longevity of botanical repellents that the matrix and adhesive strategies such polymer gels, encapsulation, or fixatives strengthen this relationship

between the carrier qualities and bioactivity (30) (31). Reviews further warn that the efficacy of essential-oil repellents might vary depending on mosquito species and environmental factors, hence emphasizing that the most reliable way to get practical, ready-to-use products in the field is through reviewing formulations rather than just increasing active concentrations (26)

CONCLUSION

The primary issue of finding a safe, effective, and environmentally friendly substitute for synthetic mosquito repellents in regions where malaria is prevalent is discussed in this research. Under WHOPES laboratory conditions, gel-based solutions including betel leaf (*Piper betle* Linn) essential oil show excellent physical stability and significant repellent action against *Anopheles* mosquitoes. In particular, the formulations with 20% and 23% essential oil concentrations offered extended protection equivalent to, occasionally even better than, a market accessible repellent. These findings strengthen the hypothesis that suitably prepared local plant materials following medicinal norms provide excellent personal protection alternatives against vectors carrying malaria. By demonstrating that naturally derived repellents can attain stability and efficacy independent of potentially hazardous synthetic substances, the study therefore relieves the investigators' first worry.

Of all the formulas tried, Formula II which contains 20% betel leaf essential oil stands out as the best choice since it has great repellent properties and the most sought-after and consistent physical features during testing. This version presents a natural repellent option that is safe and effective and allows for development.

These good results notwithstanding, some drawbacks have to be mentioned. The study, carried out in a tiny group of human subjects and mosquitoes under controlled lab settings, might not accurately reflect the variety of real-life scenarios or consumers' prolonged exposure. Furthermore, the review mostly neglects possible skin irritation or consumer happiness, and the product's long-term storage robustness addresses the immediate physical stability and short-term repellent effect. Therefore, upcoming studies ought to seek to cover longer testing periods, bigger semi-field or field tests, and skin safety evaluations. More study might also look at ways to maximize formulas, like using encapsulation methods or combining with other natural oils, to lengthen the repellent's effectiveness against several mosquito species and so improve its duration. These developments might help betel leaf-derived repellents' practical use be enhanced as component parts of all-encompassing malaria prevention plans.

More study is so needed to fully assess the formulation's safety and effectiveness including long-term stability tests to ascertain quality retention over storage, field trials to measure the repellent's performance in real conditions, and skin irritation assessments to verify safe use. The findings of these future investigations should enable Formula II (20%) to naturally repel suitable for public use.

References

- [1] Nay DNSU, Winarti E. Perilaku Pencegahan Malaria Di Wilayah Endemis Malaria: Literature Review. *J Kesehat Tambusai*. 2024;5(1):11286–2754.
- [2] Marina R, Manalu HSP, Letelay AM, Rokhmad MF, Isnani T. Inovasi program pengendalian malaria menuju eliminasi malaria di Kabupaten Fakfak, Papua Barat: Malaria Control Program Innovation Towards Malaria Elimination in Fakfak District, West Papua. *ASPIRATOR-Journal Vector-Borne Dis Stud*. 2024;15(1):9–22.
- [3] Kapitan M, Beta MO, Selasa P, Mulhaeria M, Meme MY. Artikel e-Health Method “Malaria and Pregnancy” Early Detection and Prevention of Malaria in Pregnancy. *Wind Heal J Kesehat*. 2023;190–8.
- [4] Putri D. Evaluasi Program Eliminasi Malaria Di Kota Samarinda. 2024;
- [5] Agustin E, Resnhaleksmana E, Getas IW. Hubungan Kepadatan Parasit Terhadap Kadar Hemoglobin Pada Penderita Malaria Asimtomatik Di Gunung Sari. *J Anal Med Biosains*. 2022;9(1):60–5.
- [6] Gunawan D, Kurniaty R. Pemanfaatan minyak atsiri daun sirih (*Piper betle* Linn) sebagai anti nyamuk. *J Pharm Heal Res*. 2021;2(2):46–9.
- [7] Aesum LM, Hidayati L, Pratiwi M, Margasira NS, Visthian NKD. Teknik Surveilens Epidemiologi Dan Kejadian Luar Biasa (KLB) Penyakit Malaria. *HARAPAN J Ilmu Kesehat dan Psikol*. 2024;1(1):43–52.
- [8] Natanel A, Pareta DN, Ginting AR, Tulandi SS. Uji Aktivitas Repelan Minyak Atsiri Daun Jeruk Purut (*Citrus hystrix* Dc) Terhadap Nyamuk. *Maj INFO Sains*. 2024;5(2):9–13.
- [9] Soenjono SJ, Sambuaga J, Kalonio DE. Lotion Minyak Atsiri Daun dan Kulit Buah Lemon Cui (*Citrus microcarpa*) Sebagai Repelen Terhadap Nyamuk *Anopheles* sp. *J Kesehat Lingkung*. 2022;12(1):103–9.
- [10] Sari DA, Ambari Y, Ningsih AW, Rahmawati D. Uji Stabilitas Fisik Lotion Anti Nyamuk Kombinasi Minyak Lavender (*Lavandula Angustifolia*) Dan Minyak Rosemary (*Romarinus officinalis*). *Jiis (Jurnal Ilm Ibnu Sina) Ilmu Farm dan Kesehat*. 2023;98–107.
- [11] Palapessy VED. Penyuluhan Pencegahan Penularan Penyakit Malaria kepada Masyarakat di Desa Kampung Baru Kelurahan Galang Baru Kota Batam. *J Masy Mengabdikan Nusan*. 2024;3(1):55–62.
- [12] Gunawan D, Kurniaty R. Pemanfaatan Minyak Atsiri Daun Sirih (*Piper Bette* Linn) Sebagai Anti Nyamuk. *J Pharm Heal Res*. 2021;2(2):46–9.
- [13] Hutagalung DK. Pemanfaatan bunga lavender untuk pencegahan malaria pada ibu hamil. *J Innov Res Knowl*. 2022;1(11):1587–90.
- [14] Triyani MA, Pengestuti D, Khotijah SL, Susilaningrum DF, Ujilestari T. Aktivitas Antibakteri Hand Sanitizer Berbahan Ekstrak Daun Sirih dan Ekstrak Jeruk Nipis. *NECTAR J Pendidik Biol*. 2021;2(1):16–23.
- [15] Sulistyowati, M. A. (2023). *Formulasi Dan Evaluasi Sediaan Gel Antioksidan Ekstrak Buah Strawberry (Fragaria X ananassa (Weston) Duchesne ex Rozier) (Doctoral dissertation, Universitas Islam Sultan Agung Semarang)*.
- [16] Irianto IDK, Purwanto P, Mardan MT. Aktivitas antibakteri dan uji sifat fisik sediaan gel dekoka sirih hijau (*Piper*

- betle L.) sebagai alternatif pengobatan mastitis sapi. Maj Farm. 2020;16(2):202–10.
- [17] Yusuf, A. L., Nugraha, D., Wahlanto, P., Indriastuti, M., Ismail, R., & Himah, F. A. (2022). Formulasi Dan Evaluasi Sediaan Gel Ekstrak Buah Pare (*Momordica Charantia* L.) Dengan Variasi Konsentrasi Carbopol 940. *Pharmacy Genius*, 1(1), 50-61
- [18] Anggun, B. D., & Pambudi, D. B. (2020). Uji stabilitas fisik formula sediaan gel ekstrak daun kelor (*Moringa oleifera* Lamk.). *Jurnal Ilmiah Kesehatan*, 13(2), 115-122.
- [19] Elissa AH, Nicole FA, Laurence J, John R. Olfaction: Mosquito receptor for human-sweat odorant. *Nature*. 2004: 212–213
- [20] Paluch G, Bartholomay L, Coats J. osquito Repellents: A Review Of Chemical Structure DiversityAnd Olfaction. *Pest Management Science*. 2009; 66: 925– 935.
- [21] Erwiyani, A. R., Destiani, D., & Kabelen, S. A. (2018). Pengaruh lama penyimpanan terhadap sediaan fisik krim daun alpukat (*Persea americana* mill) dan daun sirih hijau (*Piper betle* Linn). *Indonesian Journal of Pharmacy and Natural Product*, 1(1).
- [22] Nurlely, N., Rahmah, A., Ratnapuri, P. H., Srikartika, V. M., & Anwar, K. (2021). Uji karakteristik fisik sediaan gel ekstrak daun kirinyuh (*Chromolaena odorata* L.) dengan variasi karbopol dan HPMC. *Jurnal Pharmascience*, 8(2), 79-89.
- [23] Samsul, E., Sinala Jurusan Farmasi, S., & Kemenkes Makassar, P. (2022). Formulasi Masker Gel Peel Off Ekstrak Kulit Buah Langsat (*Lansium domesticum* L) dengan Variasi PVA (Polivinil Alkohol). *Jurnal Mandala Pharmacon Indonesia*, 8(2), 151–164. <https://doi.org/10.35311/JMPI.V8I2.203>
- [24] Hidayati, N., & Widhiastuti, N. (2019). Optimasi Formula Masker Gel Peel Off Ekstrak Buah Mahkota Dewa (*Phaleria macrocarpa* (Scheff.) Boerl) Dengan Variasi PVA Dan HPMC Menggunakan Metode Simplex Lattice Design. In *Jurnal Ilmu Farmasi* (Vol. 10, Issue 1).
- [25] Lestari, U., Muhaimin, M., Yuhana1, Y., & Yuliawati1, Y. (2023). Formulation And Test Of Antioxidant Activity Of Gel Mask Peel Off From Surian (*Toona Sinensis*) LEAF ETHANOL EXTRACT IN VITRO. *Indonesian Journal of Pharmaceutical Science and Technology*, 0(0), 90–99. <https://doi.org/10.24198/IJPST.VoIo.46456>
- [26] Corzo-Gómez, J. C., Espinosa-Juárez, J. V., Ovando-Zambrano, J. C., Briones-Aranda, A., Cruz-Salomón, A., & Esquinca-Avilés, H. A. (2024). A review of botanical extracts with repellent and insecticidal activity and their suitability for managing mosquito-borne disease risk in Mexico. *Pathogens*, 13(9), 737.
- [27] Khomsatun, K., Widianto, A., & Susiyanti, S. (2013). Effectiveness of betel leaf extract (*Piper betle*, Linn) as *Anopheles* spp mosquito repellent. *Jurnal Riset Kesehatan*, 2(3), 392-396.
- [28] Nazmin, F., Barek, M. A., Miah, A., Hossen, M. S., Islam, M. S., & Ahmed, J. (2025). Mosquito repellent and larvicidal activity of essential oils of aromatic plant growing in Bangladesh: a review. *Clinical Phytoscience*, 11(1), 7.
- [29] Rants'o, T. A., Koekemoer, L. L., & van Zyl, R. L. (2023). In vitro and in silico analysis of the *Anopheles* anticholinesterase activity of terpenoids. *Parasitology International*, 93, 102713.
- [30] Amodizaj, Z. G., Akbari, S., Haghighatkish, M., & Vatandoost, H. (2025). Controlled Release of Mosquito-Repellent Essential Oils by Simultaneous Encapsulation into Electrospun Fiber/Dendritic Materials. *Journal of Natural Fibers*, 22(1), 2583024.
- [31] Farina, P., & Conti, B. (2024). Liabilities of essential oils as insect repellents. *Current Opinion in Environmental Science & Health*, 40, 100564.