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Original Article

FORMULATION OF CINNAMON BARK ESSENTIAL OIL GEL AS MOSQUITO REPELLENT

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ABSTRACT

Objective: The purpose of this research is to prepare a cinnamon bark essential oil gel preparation, determines the physical characteristic and physical stability during storage, and examines the activity of mosquito repellent from the best gel preparation.

Methods: The formulations of gel were made with variations in the concentration of Carbopol 940 (0.5%, 1.5%, and 2%) contain 1% of cinnamon bark essential oil. The gel evaluated physical characteristics and physical stability. The parameters of organoleptic, homogeneity, and pH are analyzed descriptive, while the viscosity, the spreadability, and the gel adhesion were analyzed using one-way ANOVA at the level of confidence of 95%. The best gel tested its effectiveness as compared to the negative control and product innovator (which used DEET as an active compound) as a positive control. The activity of repellent was determined by an *Aedes aegypti* mosquito for 6 h with 2 hands respondents. The effectiveness of repellent protection is calculated by the percentage of protection power.

Results: The studies showed that all the gel preparations qualified the organoleptic, homogeneity, and pH parameters. The viscosity, gel adhesion, and spreadability are also stable during storage.

Conclusion: Based on the results, the best gel is the formula I which has mosquito repellent activity effective for 6 h like DEET.

Keywords: Cinnamon *(C. burmanii)* bark, Essential oil, Gel formulation, Repellent, DEET

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INTRODUCTION

Indonesia is a tropical country rich in various types of insects that provide benefits, but some are detrimental [1]. One of the insect groups that harm humans is mosquitoes which are vectors of various diseases, including malaria, dengue hemorrhagic fever (DHF), and chikungunya [2]. To prevent mosquito bites, some efforts are applied such as by using mosquito repellent sprays (spray), mosquito coils (coil), and repellent preparations are generally used [3].

Repellents had been marketed at this time are repellent made of chemical containing the active ingredient N, N-dietil-m-toluamid (DEET) [4]. DEET has good repellency because it can provide a period of protection ranging from 2 to 8 h depending on the concentration in the product [5]. However, the use of DEET potentiates endanger health by having various side effects such as hypersensitivity, irritation, and urticaria and in the long term use can cause cancer [6].

Therefore, to avoid the occurrence of dangers due to the usage of DEET on health, an alternative natural mosquito repellent preparation made from natural ingredients is needed to replace DEET. One of the plants that are known to be effective as mosquito repellent is the cinnamon plant [7]; Cinnamomum genus well known has many biological activities [8]. Cinnamon bark essential oil contains eugenol (17,62%), which is able to resist the bite of *Aedes aegypti* and *Anopheles* mosquitoes [9]. The mechanism of this process is not known for certain, but eugenol has a pungent odor and a very spicy and hot taste; thus the mosquitoes do not like it [10].

The use of essential oil as a direct repellent is less effective because of the volatile property of essential oil, causing discomfort since they are sticky and give an impression of being warm to hot; hence the selection of dosage forms must be considered [11]. Cinnamon bark essential oil will be easier to use if it is made in pharmaceutical preparation such as gels [12]. Gels have soothing, moisturizing, easy to use, easily penetrated into the skin, thus providing a healing effect [13].

Based on that description, it is necessary to develop the formulation of cinnamon bark essential oil gel preparation and evaluate the effectiveness of cinnamon bark essential oil gel preparations.

MATERIALS AND METHODS

Instruments and materials

The instruments used in this research include glassware, digital balance, stir bar, watch glass, pH meter, Brookfield viscometer, measuring pipette, and volume pipette.

The materials used in this study were the main materials, namely cinnamon bark essential oil obtained from Lansida Group, Yogyakarta, Indonesia. The chemicals used in these experiments were carbopol 940, propylene glycol, methylparaben, propylparaben, triethanolamine, aquadest, aluminum foil, and a mosquito repellent product X produced by PT. Johnson as a comparison. The experimental animal used was a four-day-old female *Aedes aegypti* mosquito, which was obtained from the Laboratory of Parasitology Universitas Gadjah Mada Yogyakarta.

Methods

Formulation of cinnamon bark essential oil gel

Formulation of the Cinnamon bark essential oil gel for the experiments could be seen in table 1.

Identification and verification of Cinnamon bark essential oil

Identification and verification of cinnamon bark essential oil are performed by checking the correctness of the data listed on the certificate of analysis of Cinnamon bark essential oil from Lansida Group Yogyakarta Indonesia.

Table 1: Cinnamon bark gel formulations

Gels composition	Formula (% w/v)		
	F1	F2	F3
Cinnamon bark essential oil	1	1	1
Carbopol 940	0,5	1,25	2
Triethanolamine	0,75	1,875	3
Methylparaben	0,18	0,18	0,18
Propylparaben	0,02	0,02	0,02
Propylene glycol	10	10	10
Aquadest	ad 100	ad 100	ad 100

Procedure of preparing Cinnamon bark gel

The gel was prepared by swelling carbopol 940 with distilled water (70 °C) for 24 h until a homogeneous mass was formed. After cooling and forming a homogeneous mass, methylparaben, propylparaben, and propylene glycol were added until well mixed, then Cinnamon bark essential oil was added and add water to a volume of 100 ml and add triethanolamine dropwise while stirring slowly until a clear gel is formed [14].

Physical stability testing

The physical stability test in this research was conducted for 4 w. The parameters of stability testing observed were homogeneity, pH, organoleptic, viscosity, gel spreadability, and gel adhesion.

To determine the spreadability of Cinnamon bark essential oil gel, we weighed 1 g of the gel and placed it at the center of the glass slide of standard dimensions (20x20 cm). The second glass slide was then placed over the first one, on which the gel was put on. A weight of 0.5 kg was allowed to place on the center of the upper glass slide for 5 min. The diameter of gel was measured in cm for triplicate to have the mean value [15].

For the first week, these parameters were observed on day 1 to day 7, continued the second week until the fourth, the evaluation is carried out once a week with 4 repetitions.

Mosquito repellent activity

The mosquito repellent activity test was performed after receiving a certificate no. 265/EP-FKIK-UMY/VI/2015 about ethical eligibility. The mosquito repellent activity test of Cinnamon bark essential oil gel was carried out by preparing mosquitoes to be grouped randomly into 2 cages made of mosquito nets in the form of blocks, each cage contains 25 mosquitoes. The mosquitoes used were female *Aedes aegypti* at the age of 3-5 d and before testing, the mosquitoes fasted for 1 d. The mosquito repellent activity was tested using the respondent hands which had been smeared with gel preparation. The arm was then put into the mosquito cage [16].

In the mosquito repellent activity test, there are 2 groups to be tested; group 1 is the selected formula of cinnamon bark essential oil gel, which is applied to the right hand and the gel preparation without cinnamon bark essential oil (negative control) is applied to the left hand, group 2 is the product innovator as a positive control was applied to the right hand and the selected formula of cinnamon bark essential oil was applied to the left hand.

Data analyses

Organoleptic data, homogeneity, and pH were analyzed descriptively, while data on viscosity, spreadability, and adhesion were analyzed using one-way ANOVA with 95% confidence level. If the data obtained are significantly different, then proceed with the Tukey HSD test. The data of the anti-mosquito test was calculated using the formula for protection power according to the Guideline for Efficacy Testing of Mosquito Repellents for Human Skin from WHO, 2009 [16].

RESULTS AND DISCUSSION

The Cinnamon essential oil used in this study used is part of the bark. At the first, we conducted organoleptic identification includes shape, color, and smell. The results obtained in this process are presented in table 2.

Table 2: Identification of Cinnamon bark essential oil

No.	Organoleptic	Observation	CoA specification
1.	Shape	Watery	Watery
2.	Colour	Dark Yellow	Dark Yellow
3.	Odor	Strong Scent	Strong Scent

The results obtained showed that the shape, color, and smell of Cinnamon bark essential oil indicated on the CoA in accordance with organoleptic observations. The second stage was the verification of the Cinnamon bark essential oil CoA. This verification stage is carried out by comparing the value listed on the CoA of cinnamon bark oil with the theoretical value i. e SNI (2006) [17]. Verification includes data on refractive index, density, solubility in alcohol, solubility in water, and cinnamaldehyde content. The results obtained from this process are showed in table 3.

Table 3: Verification of Cinnamon bark essential oil

No.	Parameter	Theoretical	CoA specification
1.	Refractive index	1.008-1.030 (SNI, 2006)	1.021
2.	Density	1.559-1.599 (SNI, 2006)	1.598
3.	Solubility in alcohol	Soluble (SNI, 2006)	Soluble
4.	Solubility in water	Insoluble (SNI, 2006)	Insoluble
5.	Cinnamaldehyde	65-89% (Kardinan, 2007)	86.73%

The results obtained indicate that the refractive index, density, solubility in alcohol, solubility in water, and the cinnamaldehyde content are in the range or according to the theoretical based on Indonesian National Standard (SNI, 2006) [17] and meet the specifications listed on CoA Lansida Group Yogyakarta.

The organoleptic observations of the gel preparations were conducted by observing the shape, color, and odor of the Cinnamon bark essential oil gel during storage for 4 w. Based on the results of the observations showed that all the gel formulas have a variety of viscosities, milky white color and a characteristic

odor of cinnamon bark essential oil, which did not change shape, color, and odor during observation. The organoleptic observations of the Cinnamon bark essential oil gel could be seen in table 4 and table 5.

Table 4: Organoleptic observation on dosage form changes

Time (Days)	Dosage form			
	F1	F2	F3	C (-)
1	+	++	+++	+
7	+	++	+++	+
14	+	++	+++	+
21	+	++	+++	+
28	+	++	+++	+

Table 5: Observation data on changes in the odor of the preparation

Time (Days)	Odor		
	F1	F2	F3
1	+++	++	++
7	+++	++	++
14	+++	++	++
21	+++	++	++
28	+++	++	++

Annotation: +++: The aroma of essential oils is very sharp, ++:The aroma of essential oils is sharp, +:The aroma of essential oils is less sharp

The various of viscosity can be influenced by variations in carbopol concentrations. The higher the concentration of carbopol, the thicker gel preparation produced. The color of gel preparation is milky white and not transparent like gel preparations commonly; this can occur due to the mixing of essential oils into the water, but according to Cosmetics Formulary, the color of the gel preparation does not have to be transparent, it is still allowed to be opaque [18]. The smell resulted from all formulas of the gel is a distinctive spicy smell of Cinnamon bark essential oil with the sharpest is in formula 1 than formulas 2 and 3.

The results of the homogeneity observations on the Cinnamon bark essential oil gel preparations demonstrated that all the formulas did not present coarse grains when the gels were smeared on a glass plate. This fact shows that all the gel preparations are homogenous. The resulting gel remains homogeneous during the storage period, it can be stated that the gel preparation is stable.

The results of measuring the pH of the Cinnamon bark essential oil gel preparation for 4 w of storage showed that the preparation had a pH between 5.0-6.0. The pH value of the skin ranges from 4.5 to 6.5 [19]. This result displayed that pH of Formula 1 decrease at the 4th week of storage. The pH of Formula 2 and Formula 3 decreased in the 2nd to 4th week of storage. The decreasing of pH is related to the length of storage, the longer the gel storage, the lower the pH of the gel preparation [20]. This condition can be caused by environmental influences such as light and oxygen in acidic contact with the essential oils as active compounds during storage thus, they will undergo an oxidation reaction with air catalyzed by light, will then increase the acid number and form an acidic compound, cause changes in odor and color [21].

The viscosity measurement of the gel preparation for 4 w of storage was performed to determine the magnitude of the changes in viscosity and gel stability. Gel stability parameters can also be seen from the stability of the gel phase that occurs during storage. The viscosity of the gel preparations showed that the higher the concentration of carbopol, the viscosity of the gel preparation increased from 29,160 cp for Formula 1 contain 0.5 grams of carbopol to>1,914,000 cp for Formula 3 containing 2 grams of carbopol on the first day of manufacture. This increasing is due to the swelling property of the base itself and also because an increase in the amount of gelling agent used can strengthen the gel matrix, causing a viscosity increasing [22]. The graph of the results of the measurement of viscosity on storage for 4 w can be seen in fig. 1.

Based on the fig. 1, it was found that Formula 1 had the best and most stable viscosity during storage. We could see only Formula 1 has a viscosity within the range of good viscosity values, which is from 20,000-40,000 cps [23].

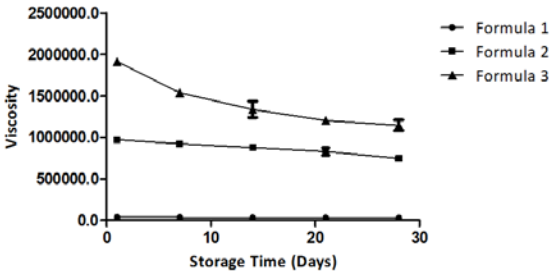

Fig. 1: Profile of relationship between storage time and viscosity, the value resulted from triplicate experiments

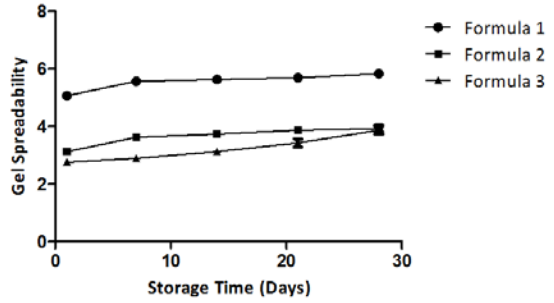

Fig. 2: Profile of relationship between storage time and gel spreadability, the value resulted from triplicate experiments

The gel spreadability test was performed to determine how much the gel's ability to spread on the skin was. One of the factors that affect the spreadability of the gel is the amount and strength of the gel matrix. The more and stronger the gel matrix, the lower the spreadability of the gel [24]. The results of the gel spreadability test displayed that Formula I and Formula II increased slightly since the 1st week to the 4th week of storage, Formula III increased significantly at the 2nd to 4th week of storage. Formula 1, with the lower base concentration and lower viscosity as compared to Formula 2 and 3, had greater spreading. This shows an inverse

relationship between spreadability and viscosity, the greater the viscosity of the preparation, the smaller the spreading. This is because a large viscosity requires greater pressure to flow with the same amount of pressure; the distribution of preparations with a larger viscosity will be smaller. The graph of the results of the measurement of the gel spreadability on storage for 4 w can be seen in fig. 2.

According to the results of the spreadability test of the Cinnamon bark oil gel preparation, we conclude that the best formula is Formula 1 because only formula 1 is included in the range of good gel spreadability, which is between 5 to 7 cm [23].

Adhesion test was performed to determine the strength of the gel attached to the skin. The longer the gel is attached to the skin, the better the action of the drug at the site of action, but if it is attached to the skin for too long it will be difficult to remove and will interfere with the absorption of the drug into the skin [25]. Based on the measurement of gel adhesion, Formula 1 decreased slightly but remained stable. The adhesion of Formula 2 decreased since day 5 in the 1st week to day 28 in the 3rd week. The adhesion of Formula 3 decreased at the 1st to 4th week of storage. Formula 1 has the smallest adhesion; this shows a proportional relationship between the decrease in adhesion and the decrease in viscosity; the smaller the viscosity of the preparation, the smaller the adhesion. The graph of the results of the gel adhesion measurement on storage for 4 w can be seen in fig. 3.

Based on the gel adhesion test, we conclude that the Formula 1 is the best, which is more than 1 second [26]. Formula 1 is also the most stable formula at 4 w of storage due to the relationship between viscosity and adhesion.

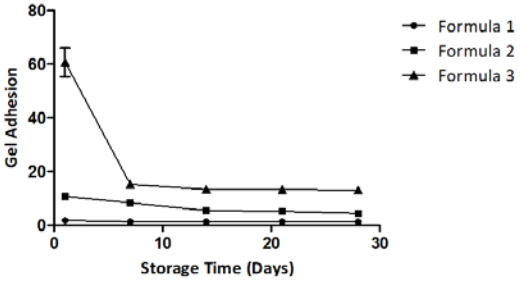

Fig. 3: Profile of relationship between storage time and gel adhesion, the value resulted from triplicate experiments

The results of the mosquito repellent activity of 1% Cinnamon bark essential oil gel at 0 to 6 h were 100%, 96.92%, 83.82%, 91.52%, 92.98%, 91.18%, and 76.81%. These results indicate that the protective power of Cinnamon bark essential oil gel is still below the standard set by WHO where the anti-mosquito protection is effective if at least 90% for 6 h [16]. The results of the mosquito repellent activity test can be seen in table 7.

Table 7: Results of Cinnamon bark oil gel protective power as compared to negative control

Treatment	Times (H)	Average mosquito bite		Protective power (%)
		Control (-)	Sample	
Cinammon bark essential oil gel 1%	0	6.7±1.64	0	100
	1	6.5±0.97	0.2±0.42	96.92
	2	6.8±1.39	1.1±0.74	83.82
	3	5.9±0.99	0.5±0.71	91.52
	4	5.7±0.67	0.4±0.69	92.98
	5	6.8±1.55	0.6±0.52	91.18
	6	6.9±1.66	1.6±1.5	76.81

n = 10, All values represent mean±SD.

The decrease in the protective power of the gel can be caused by the volatile property of the active ingredients of the gel which is at the 5th and 6th hours the amount of essential oil contained in the gel decreases. It can be stated that this Cinnamon bark essential oil gel is effective as a mosquito repellent for 4 h. In addition to the active ingredient of the gel, there is many factors can reduce the protective power of the gel such as smearing technique, environment such as humidity and inner factor such as body temperature [27].

The results of the mosquito repellent activity test for Cinnamon bark essential oil gel preparations as compared to innovator products can be seen in table 8.

Table 8: Results of Cinnamon bark oil gel protective power as compared to innovator product

Treatment	Times (H)	Average mosquito bite		Sig (p<0.05)
		Sample	Control (+)	
Innovator Product	0	0	0	-
	1	0.3±0.48	0.1±0.32	0.267
	2	0.6±0.69	0.3±0.48	0.313
	3	0.4±0.69	0.1±0.32	0.255
	4	0.3±0.67	0.2±0.42	0.914
	5	1.0±1.05	0.6±0.84	0.368
	6	1.5±1.65	1.2±0.92	0.691

n = 10, All values represent mean±SD

In general, for 6 h, the number of mosquitoes landed on the Cinnamon bark oil gel was more than the number of mosquitoes landed on the innovator's product. The difference in the data was then analyzed by the Mann-Whitney test and the significance since the 1st h to the 6th h respectively was 0.267; 0.313; 0.255; 0.914; 0.368; 0.691 ($p<0.05$), which means the selected gel formula containing 1% cinnamon bark essential oil did not provide a significant difference in anti-mosquito protection as compared to the innovator product.

However, the development of gel preparation for repellent should be continued further to provide a pharmaceutical dosage form that comfort to use and is safe for humans and the environment. Currently, some herbs also tested the effectiveness for repellents such as *Duranta plumieri* [28] and *Acalyphia indica* [29].

CONCLUSION

Based on the results of the study, the gel preparation of Cinnamon bark essential oil has mosquito repellent activity. However, if the gel

preparation will be applied to the community, it is necessary to test the irritation to human skin thus, the Cinnamon bark essential oil gel preparation can be well received. The innovator product has a greater protection power than the selected gel. The concentration of 1% Cinnamon bark essential oil is relatively smaller as compared to innovator products with 12.5% DEET content. Hence, it is necessary to do further research on the optimum concentration of Cinnamon bark essential oil as an anti-mosquito preparation.

The selected formula of Cinnamon bark essential oil gel is Formula 1. Formula 1 does not change shape; color; and odor, remained homogeneous during storage, had a pH value of 6 which was still within the skin pH range, had a viscosity value of 39,100-30,137 cp, the spreadability of 5.07-5.83 cm, the adhesion of 1.33-1.86 seconds. The selected formula of Cinnamon bark essential oil gel has activity as an effective mosquito repellent for 4 h.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICTS OF INTERESTS

There is no conflict of interest among the authors.

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