Tropical Biomedicine 34(3): 540–549 (2017)

Three repellent gels that contain essential oils from local Malaysian plants against dengue vector

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Received 12 October 2016; received in revised form 26 January 2017; accepted 28 January 2017

Abstract. The essential oils of *Litsea elliptica*, *Piper aduncum*, and *Piper sarmentosum* were prepared as repellents in gel formulation, and their repellent properties against *Aedes aegypti* were experimentally investigated. The lowest effective doses against adult mosquitoes were 0.8%, 0.5%, and 0.4% for *Lit. elliptica*, *P. sarmentosum* and *P. aduncum*, respectively. In laboratory testing with human subjects, all three gels provided over 90.0% repellency at one hour after application and over 80.0% repellency at four hours, compared with 100% and 95.8% protection after one and four hours, respectively, by DEET. In the field, gels with ED_{95} concentrations of *Lit. elliptica*, *P. aduncum*, and *P. sarmentosum* essential oils provided 99.3%, 97.5%, and 100% protection, respectively, at two hours. The physical properties and biological stability of the three repellents after storage in hot and cold conditions were also compared. In conclusion, all three gels have the potential for development as repellents against *Ae. aegypti*.

INTRODUCTION

The incidence of dengue fever and dengue hemorrhagic fever has increased dramatically in recent decades. It has become endemic in more than 100 countries, mainly in Africa, the Americas, the eastern Mediterranean, South and Southeast Asia, and the western Pacific. A study on prevalence of dengue estimates that 3.9 billion people in 128 countries are at risk of infection. There is currently no specific treatment for dengue. So one of the effective ways to hinder dengue transmission is through vector control methods such as use of insecticides and mosquito habitat removal (WHO, 2016).

A variety of methods can be used to control dengue vectors. Chemical insecticides are commonly used, but although they are effective, they have negative impacts on the environment, including the killing of non-target organisms. They also lead to resistance when used excessively. *Aedes spp.* mosquitoes can also be controlled via elimination of breeding places and use of repellents to prevent mosquito bites (Hidayatulfathi *et al.*, 2005). These methods can be quite effective, but they require human behavior modification. While elimination of breeding places relies largely on education of the population, repellent use entails a number of other factors, including perceived safety and comfort, as well as a positive public opinion based on factors such as environmental friendliness and positive local economic impact.

Repellents are chemicals that cause insects to avoid the source of the repelling agent. An effective repellent displays optimal evaporation; volatile enough to be perceived by the insect, but persistent on the surface of the skin (Fradin, 1998). N, N-diethyl-3-

methylbenzamide (DEET), is the repellent of choice for consumers. However, recent studies have reported toxic effects on infants, children, and some adults (Fradin & Day, 2002). The study of repellents has been enhanced by the recent discovery of natural ingredients, including plant-based ones, with insect repellent properties. Malaysia is rich in natural vegetation that has not been fully explored with respect to its potential commercial value as a source of repellent.

Repellents have historically been formulated into a variety of delivery devices, including lotions, creams, aerosols, patches, and wrist bands. However, surprisingly, no gel formulation could be found on the market. Gels, topical preparations for application to the skin, consist of a semi-solid two-component system that is rich in liquid. The gel manufacturing process is relatively economical. Crucially to its use as an effective repellent, it fulfills the fundamental criterion of not being easily absorbed into the skin (Sri, 2005). Compared with pharmaceutical creams, which tend to be easily absorbed into the skin, aqueous gels are poorly absorbed (Lund, 1994). Thus, aqueous gel formulations are more suitable as repellents.

The results of the present study are needed in order to facilitate the development of essential oil-based gel repellents as commercial products comparable to DEET. Such products would be beneficial to Malaysians, in terms of both economic impact and increased accessibility for local people. Further, this type of natural repellent would serve to offer consumers an alternative choice that may be more environmentally friendly and aesthetically pleasing than synthetic chemicals. These properties may persuade people who will not or cannot use DEET to use other, natural repellents, a key behavior in reducing transmission of dengue.

MATERIALS AND METHODS

Plant collection

Leaves from three plant species were collected from three different locations in Malaysia. *P. aduncum* leaves were collected from Batu 13, Gombak Selangor; *Lit. elliptica* leaves were collected from Pekan, in Pahang state; and *P. sarmentosum* leaves were collected from the Agricultural Park in Pahang. Identification of the plants was confirmed by botanists at the Forest Research Institute of Malaysia.

Extraction of essential oil

The essential oils (100% concentration) were obtained by hydro-distillation in a Clevenger-type apparatus for 8 hours. The distillate was dried over anhydrous magnesium sulfate to extract the oil. The essential oils were then formulated into Carbopol 934 hydrogels.

Gel formulations

Gel formulation was done by added 1.5 g of Carbopol 934 into 100 ml distilled water and left on magnetic stirrer for 24 hours. Triethanolamine was then added until pH reached 4.5 to 5.5. An amount of essential oil was added into the gel based on the value of 95% Effective Dose (ED_{95}) obtained.

Colonization of mosquitoes

A colony of *Ae. aegypti* (WHO susceptible strain), originally from the Institute of Medical Research and established at the insectariums of the Department of Biomedical Science, Universiti Kebangsaan Malaysia (UKM), was used. Nulliparous 4-7 day-old adult female *Ae. aegypti* were used to test the repellent effects.

Evaluation of repellents on human volunteers

The method of Buescher *et al.* (1982) was modified and conducted as follows. Testing was conducted between 08.00 h and 16.00 h, at 25-30°C, 60-80% relative humidity. Five circles (29 mm in diameter) were drawn on the flexor of each volunteer's forearm using a plastic template and permanent marker, and 0.025 g each of plain gel (control) and four gels containing repellent at different concentrations (1%, 1.5%, 2.5%, and 5%) were applied randomly to these marked areas. After air drying for 5 minutes, a plastic cage (4 x 5 x 18 cm) with matching cut outs in its floor was secured over the treated areas by rubber bands. Each plastic cage contained

15 blood-starved 5- to 7-day-old *Ae. aegypti* females. The number of mosquitoes biting at each test site was recorded after 90 seconds' exposure. The experiment was replicated four times on each human volunteer. The same experiment was repeated using DEET (0.01%, 0.1%, 0.25%, and 0.5%) as a positive control. Percentage repellency was determined using the formula:

$$\%\ repellency = 100 - \left[\frac{BT}{BC} \times 100\right]$$

where BT is the number of bites on the circles treated with the experimental repellent, and BC is the number of bites on untreated areas.

Evaluation of repellents on human volunteers

Repellency was also evaluated by the SIRIM method (MS 1497:2000), the Malaysian standard protocol for the evaluation of biological efficacy of personal mosquito repellents on human skin in a designated laboratory environment. This standard method is applicable to any commercial formulation intended to be used to repel mosquitoes when applied on human skin. The study was conducted using a 60 cm x 60 cm x 60 cm cage with two 15-cm-diameter circular openings fitted with cloth sleeves. The cage was divided by a polyethylene Perspex partition into two compartments. A fresh batch of 25 4- to 7-day-old female *Ae. aegypti* mosquitoes were introduced into each compartment through a circular opening. A square area, 3.1 cm x 8 cm (about 25 cm^2), was drawn on one forearm of each volunteer, and 0.4 g of repellent gel was applied evenly to this designated area and left to dry for 10 minutes.

A rubber sleeve with an opening corresponding to this ~25 cm^2 area was fitted on both arms of the volunteer, and both hands were covered with thick rubber gloves up to the wrists to confine the bites to only the designated exposed area. The volunteer was treated with a test sample on one arm and the other arm was left untreated (as the control). Both hands were inserted through the circular opening into the screen cage containing the mosquitoes and were exposed simultaneously for a period of 3 minutes, and the number of mosquitoes landing or biting was recorded. Assessment time-points were 1, 2, 4, 6, and 8 hours post-application. The experiment was repeated with DEET (5.0%) as a positive control. The effectiveness of each essential oil was assessed through calculation of % repellency of the treated arm, compared with the untreated arm, according to the following equation:

$$\%\ repellency = \frac{MU - MT}{MU} \times 100$$

where MU is the number of mosquitoes on the untreated arm, and MT is the number of mosquitoes on the treated arm.

Field trial

The protocol for the field trial was modified from the method of Tuetun *et al.* (2009). Gels with 95% Effective Dose (ED_{95}) concentrations of repellent were tested at a suburban area located in Kampung Sungai Lang, Banting, Selangor. This location was selected as a test site because of the large mosquito population and rarity of mosquito-borne diseases in the area. Two teams of volunteers, each comprising two women and two men (three treated subjects and one untreated control), were employed as bait. Three of the volunteers in each team applied 2 g of repellent sample to their skin, as uniformly as possible, on both lower legs, from the base of the knee to the ankle. The negative control volunteer was treated with plain gel. Untreated areas on the volunteers were covered with protective material to ensure that blood-seeking mosquitoes had access only to the lower-leg test areas. During the study, use of soap when washing or application of any cosmetics, including perfume, cologne, and lotion, was also avoided.

The two teams of volunteers were situated at least 20 m apart. Three repellent treatment subjects and one negative control subject from each team sat in a row 5 m apart, with both legs exposed for 10 min. Any mosquitoes that landed on the exposed lower

legs were collected by the volunteer before commencement of feeding. After each 10-min period, the volunteers moved to a new site at least 10 m from the previous one. The collections were performed for a total of 120 min, split into twelve 10-min periods, so that twelve separate collections were conducted by each volunteer. The collected mosquitoes were kept separately in cups labelled with the volunteer (treatment condition) and collection site, and were counted and analyzed later.

Throughout the study, each subject was tested in triplicate for each test sample and volunteer's positions were randomly rotated to minimize errors that affect repellent efficacy. Data from the field assessments were analyzed to determine which mosquito species were biting, the total number of bites during the exposure period, and the percentage of repellency provided by the test samples. Percentage repellency for this field assay was calculated according to a formula based on previous study (Naucke *et al*., 2007):

$$\%\, repellency = \frac{K - R}{K} \times 100$$

where K is total mosquitoes biting or landing on control and R is total mosquitoes biting or landing on the treated subject. Exposure times were 10 min for treated and control subjects.

Research ethics

The human volunteers and methods of this study conformed to Ethical and Principle and had been approved by Universiti Kebangsaan Malaysia Research Ethics Committee (UKM 1.5.3.5/244/NN-143-2011).

Assessment of Biological Stability

To assess qualities of the repellent gels relevant to commercial development, the stability of the repellent gels was investigated. Appearance (color), odor, and pH values were determined after keeping samples under two conditions: a heating and cooling cycle and varying temperature storage (4ºC, 25ºC, and 40ºC, for 3 months). After 3 months, these gels were then compared with the fresh preparation. Color and odor were assessed subjectively by 3 subjects, while pH values were measured by a pH meter (Mettler Toledo, Switzerland). In addition, the protraction of repellent activity was also determined for the gel with 1.6% *P. sarmentosum* after 3 months of storage at 25-30ºC, by using the SIRIM protocol.

Statistical Analysis

The data were analyzed using software developed by Raymond (1985) to determine ED_{50} and ED_{95} values. The effective dose was determined by analysis of variance (ANOVA) while the percentages of repellency for the bioassays and field trial were determined by split-plot analysis of variance (SPANOVA) to compare controls and treated subjects with respect to time after application.

RESULTS

Table 1 shows the median effective doses for four gel formulations. The ED_{50} values were 0. 8%, 0.1%, and 0.4% for the *Lit. elliptica*, *P. sarmentosum*, and *P. aduncum* gels, respectively. There were no significant differences in ED_{50} values between the standard repellent, DEET 25.0%, and any of the three experimental repellants ($p<0.05$;

Table 1. The ED_{50} and ED_{95} for repellent gels against *Ae. aegypti*

Repellent gels	ED_{50} (95% CI)	ED_{95} (95% CI)	Regression coefficient (slope) ± standard error
P. sarmentosum	0.1 (0.02–0.1)	1.6 (1.0–3.0)	1.1 ± 0.2
P. aduncum	0.4 (0.1–0.6)	1.7 (1.4–2.3)	2.5 ± 0.7
Lit. elliptica	0.8 (0.5–1.1)	4.3 (3.4–5.9)	1.8 ± 0.2
DEET	0.01 (0.01–0.02)	0.2 (0.1–0.3)	1.4 ± 0.2

F (3,8)), as assessed by one-way ANOVA. Whereas *Lit. elliptica* was the most effective repellent in terms of ED_{50}, *P. sarmentosum* was the most effective in terms of ED_{95}. The ED_{95} values for the three gels were 1.6%, 1.7%, and 4.3% for *P. sarmentosum*, *P. aduncum*, and *Lit. elliptica*, respectively. One-way ANOVA indicated that there were no significant differences ($p>0.05$; F (3,8)) in ED_{95} between DEET 25.0% and *P. sarmentosum*, *P. aduncum*, and *Lit. elliptica*.

Table 2 shows the percentage repellency against *Ae. aegypti* biting or landing based on the SIRIM standard procedure. All four gels provided 100% repellency at the time of application. One hour after application, 5.0% *P. sarmentosum*, 5.0% *P. aduncum*, and 5.0% DEET still provided 100% repellency, while 5.0% *Lit. elliptica* provided 91.3% protection. At four hours, the three experimental gels all still showed over 80.0% repellency, compared with 95.8% for DEET, and at eight hours, the three gels actually exceeded DEET; *P. sarmentosum*, *P. aduncum*, and *Lit. elliptica* showed repellencies of 69.0%, 78.4%, and 65.0%, compared with 33.3% for DEET. Split-plot ANOVA indicated that there were no significant differences in percentage repellency between these three gels and DEET 5.0% F (1,3), ($p>0.05$) at all time intervals.

The field evaluation is summarized in Table 3. The repellencies of the experimental gels, 1.7% *P. aduncum*, 1.6% *P. sarmentosum*, and 4.3% *Lit. elliptica* (the ED_{95} concentrations for the three repellents, as determined in the laboratory, see Table 1) were evaluated in comparison with the standard repellent, DEET, against mosquitoes in the field. In this trial, 4.3% *Lit. elliptica* provided the highest repellency, followed by 1.6% *P. sarmentosum* and 1.7% *P. aduncum*, with repellencies of 99.1%, 95.5%, and 95.4%, respectively, at 60 min. At 120 min, the 1.6% *P. sarmentosum* gel provided 100% protection, followed by the 4.3% *Lit. elliptica* gel and the 1.7% *P. aduncum* gel, with 99.3% and 97.5% repellency, respectively. The species of mosquitoes collected in the field were: *Armigeres* spp. (92.3%), *Aedes* spp. (4.6%), *Culex* spp. (2.7%), *Anopheles* spp. (0.2%), and *Mansonia* spp. (0.2%).

The appearance and physical properties of the gel preparations after storage, heating, and cooling are shown in Table 4. After a single cycle of heating and cooling, all heated and cooled gels were creamy white in color

Table 2. Repellency percentage over time for *P. sarmentosum*, *P. aduncum*, *Lit. elliptica*, and 5% DEET repellent gel against *Ae. aegypti* biting or landing, as determined by the SIRIM[b] standard method

		Time after treatment (hours)					
		0	1	2	4	6	8
5% *P. sarmentosum*	NMBL[a], treated	0	0	0.7±0.3	7.0±1.7	11.3±5.8	13.0±7.0
	NMBL, untreated	3.1±2.8	6.6±1.9	11.9±4.6	12.7±5.6	15.4±3.5	13.9±6.9
	% repellency	100	100	98.0	82.0	76.0	69.0
5% *P. aduncum*	NMBL, treated	0	0	1.0±0.2	2.2±0.2	6.8±0.9	2.3±0.3
	NMBL, untreated	10.4±0.6	9.8±0.6	7.5±0.5	8.7±0.4	7.8±1.5	10.5±0.7
	% repellency	100	100	87.0	85.8	83.9	78.4
5% *Lit. elliptica*	NMBL, treated	0	0.7±0.3	1.3±0.7	2.0±1.0	3.0±2.1	3.7±1.8
	NMBL, untreated	1.3±0.3	8.0±4.6	4.7±2.3	16.0±4.7	12.0±1.2	13.7±4.3
	% repellency	100	91.3	76.0	81.7	77.7	65.0
5% DEET	NMBL, treated	0±0.0	0±0.0	0±0.0	2±1.0	14±5.8	22.7±8.5
	NMBL, untreated	35.0±5.8	43.4±6.2	43.0±6.2	47.7±1.5	46.3±3.7	44.0±6.0
	% repellency	100	100	100	95.8	69.8	33.3

[a]NMBL, number of mosquitoes biting or landing (mean ± standard deviation);
[b]SIRIM, Standard and Industrial Research Institute of Malaysia. Repellents were applied on human subjects in a laboratory setting and repellency was assayed using the SIRIM standard method, described in detail in the Methods section.

Table 3. Repellency of three botanical gels and DEET (ED_{95} doses) against a field mosquito population

Repellent gels	Repellency (%) (±SD)			
	30 min	60 min	90 min	120 min
1.6% *P. sarmentosum*	97.2±0.8	95.5±1.1	97.5±0.9	100±0.0
1.7% *P. aduncum*	97.1±0.6	95.4±1.1	97.3±0.7	97.5±0.6
4.3% *Lit. elliptica*	99.0±0.4	99.1±0.2	99.2±0.4	99.3±0.1
0.17% DEET	98.7±0.4	98.0±0.5	97.2±0.6	96.8±1.0

Table 4. Appearance, odor, and pH range of *Lit. elliptica*, *P. aduncum*, and *P. sarmentosum* gels. Comparison of fresh preparations with gels after storage under four conditions: a single heating and cooling cycle (heating at 40ºC for 24 hours and cooling at 4ºC for 24 hours), or storage for three months at 4ºC, ambient temperature (AT : 25-30ºC), or 40ºC

Gel preparation	Storage condition	Appearance and physical characteristic		pH range
		Color	Odor	
Lit. elliptica	Fresh preparation	Creamy white	Aromatic	pH (4.5–5.5)
	Single heating/cooling cycle	Creamy white	Aromatic	
	Three-month storage:			
	4ºC	Creamy white	Aromatic	
	25ºC–30ºC	Creamy white	Aromatic	
	40ºC	Yellowish	Less aromatic	
P. aduncum	Fresh preparation	Creamy white	Aromatic	pH (4.5–5.5)
	Single heating/cooling cycle	Creamy white	Aromatic	
	Three-month storage:			
	4ºC	Creamy white	Aromatic	
	25ºC–30ºC	Creamy white	Aromatic	
	40ºC	Yellowish	Less aromatic	
P. sarmentosum	Fresh preparation	Creamy white	Fresh	pH (4.5–5.5)
	Single heating/cooling cycle	Creamy white	Fresh	
	Three-month storage:			
	4ºC	Creamy white	Spicy	
	25ºC–30ºC	Grayish	Less spicy	
	40ºC	Dark grey	Less spicy	

and had a pleasant and fresh aroma similar to the fresh preparation. After three months, all formulae kept at 4ºC or ambient temperature (25-30ºC) were still creamy white in color with a pleasant, fresh aroma, except for the *P. sarmentosum* gel, which had changed to a grayish color and spicy odor. When kept at 40ºC, clearer changes were evident: the *Lit. elliptica* gel had turned yellowish, and the *P. aduncum* and *P. sarmentosum* gels had changed to dark grey. The pH values for all of the prepared gels were still within normal pH ranges for application to the skin (pH 4.5- 5.5).

After considering the lowest ED_{95} and repellent gel that display good repellent activity, *P. sarmentosum* gel was chosen for further testing to access the effectiveness

Table 5. The comparison of repellent activity between freshly formulated gel and after 3 months of storage *P. sarmentosum* gel

Treatment		Mean numbers of mosquitoes biting or landing after various treatment times (hours)					
		0	1	2	4	6	8
1.6% *P. sarmentosum*	Treated	0.3±0.3	2.7±0.3	6.3±2.3	12.3±3.8	15±2.3	9.7±1.9
	Control (untreated)	7.8±2.9	10.4±0.8	11.2±2.1	11.3±2.0	10.9±1.7	9.6±2.1
	% repellency	98.6	91.5	81.2	63.7	55.1	66.3
1.6% *P. sarmentosum* (after 3 months storage)	Treated	0.4±0.2	2.1±0.5	5.9±1.4	14.8±1.9	18.8±2.1	21.6±1.9
	Control (untreated)	20.6±1.5	23.8±0.7	24.4±0.4	24.9±0.1	25.0±0.0	25.0±0.0
	% repellency	97.8	91.5	80.4	55.2	28.0	15.5

after 3 months of storage. Table 5 shows the protraction of repellent activity against *Ae. aegypti* mosquitoes as determined by the SIRIM protocol after storage. The efficacy of the fresh gel and the gel that had been stored for 3 months at 25°C were compared. The fresh and stored gels showed 81.2% and 80.4% repellency, respectively, at two hours after application. Split-plot ANOVA indicated that there were no significant differences in percentage repellency between fresh and stored gels [F(1,1), p>0.05].

DISCUSSION

This study investigated gel formulations of natural repellents. A laboratory study of *Syzygium aromaticum* (clove) and *Zanthoxylum limonella* extracts conducted by Trongtokit *et al.* (2005) investigated the efficacy of these natural repellents in gel and cream formulations. They found that, under laboratory conditions, the repellents in gel formulation provided much longer-lasting protection against *Ae. aegypti*, *Culex quinquefasciatus*, and *Anopheles dirus* than the repellents in cream formulation.

Many factors can influence the results of laboratory repellent tests, including abiotic factors such as evaporation, perspiration, and fabric contact (Rueda *et al.*, 1998; Barnard, 2005). Light intensity, temperature, humidity, and air quality in the laboratory are the most important factors for repellent assays (Frances *et al.*, 1996). Cage size also plays an important role; repellency time has been inversely related to cage size. However, such repellency results may be species-specific; repellency was not influenced by density for *Ae. aegypti* mosquitoes, but was influenced by density for *An. Quadrimaculatus* (Barnard, 2005).

In the field trial, the 4.3% *Lit. elliptica* gel provided the highest repellency, followed by the 1.6% *P. sarmentosum* gel and the 1.7% *P. aduncum* gel, with repellencies of 99.1%, 95.5%, and 95.4%, respectively, at 60 min. At 120 min, 1.6% *P. sarmentosum* provided 100% protection, followed by 4.3% *Lit. elliptica* and 1.7% *P. aduncum*, with 99.3% and 97.5% repellency. For comparison, 0.17% DEET provided 98.0% repellency at 60 min and 96.8% at 120 min. This result is similar to that of Tuetun *et al.* (2009) who used 5% *Apium graveolens* hexane extract (celery) as a topical repellent in a field trial test. They also showed that this repellent provided 100% protection at two hours post-application, compared with 99.68% protection for DEET. Kim *et al.* (2004) reported that a repellent containing *Foeniculum vulgare* (fennel) in 5% aerosol and 8% cream formulations provided 84.0% and 70.0% repellency, respectively, at 90 min post-application.

In our field study, *Armigeres* spp. was the dominant species that bit or landed on the control and treatment volunteers. Tuetun *et al.* (2008) tested topical repellents in the field, and found that the *Armigeres* spp. mosquitoes were tolerant towards DEET. The results of repellent bioassays in the field

may be influenced by season, geographical location, and duration of observation (Barnard, 2005). Tuetun *et al.* (2008) reported that a limitation when using botanical-based repellents is unstable physical and biological properties; in the long term, they are susceptible to be influenced by temperature, which may impact the biting bioactivity of mosquitoes.

The present study supported the results of Naucke *et al.* (2007), who reported that the effectiveness of repellents in the field depends on biting pressure and also on other mosquito activities at the field site. Other factors that can influence the potency of repellents, causing them to decrease over time, include evaporation of the essential oil and changes in mosquito activities (Trongtokit *et al.*, 2005).

It is the vapor phase of essential-oil repellents that affects mosquito behavior, and therefore the duration of effectiveness is directly related to vaporization (Barnard, 2000). Essential oils have high vaporization rates, resulting in short time periods of effectiveness, but this can be ameliorated by developing repellent formulations that prevent release of the active compound, slowing the rate of vaporization and increasing protection time after application on the skin (Nerio *et al.*, 2010). Another strategy is the addition of vanillin to gel preparations. In previous studies, this has increased repellency, an effect thought to be due to the decreased evaporation rate of the repellent from the skin surface (Tawatsin *et al.*, 2001; Tuetun *et al.*, 2005).

Extraction method and stability test might also play a role in repellency effectiveness. This study using hydro distillation method provided the best repellency effect (ED_{50} = 0.005 µgcm^{-2}) compared to ethanol extract of *P. aduncum* (ED_{50} = 1.24 µgcm^{-2}) (Norashiqin *et al.*, 2008) and hexane fractionation extract of *P. aduncum* (ED_{50} = 0.03 µgcm^{-2}) (Hidayatulfathi *et al.*, 2004). The stability test is a quantitative analytical method based on chemical and biological characteristics of an active compound (Carstensen & Rhodes, 2000). During the three-month stability test, the pH values for all gels remained within 4.5 to 5.5, which is the optimum pH for normal skin (Casagrande *et al.*, 2009). Thus, the results showed that the prepared gels could safely be applied to skin.

Carbopol 934 is a hydrogel that is usually used in pharmaceutical products due to its high stability, skin compatibility, and low toxicity on the skin (Lu & Jun, 1998). Hydrogel characteristics are influenced by environmental factors such as pH, temperature, the strength of the ionic bonds in the gel itself, and light intensity (Jagur-Grodzinski, 2009). The impact of temperature and pH can cause the gel structure to become unstable (Nam *et al.*, 2004).

A long-term stability test conducted on our gel preparations, stored under various temperatures for three months, revealed color and odor changes in all three gels that had been kept at high temperature (40ºC). However, only *P. sarmentosum* gel showed changes in color and odor at ambient temperature (25-30ºC). By taking account of repellent activity and dosage in term of economical need, *P. sarmentosum* gel is the most appropriate gel to proceed with stability test. Therefore, only *P. sarmentosum* gel was tested for repellency after storage. Repellency for the first two hours after application was equivalent in the fresh and stored gels. However, the stored gel lost effectiveness over time much more rapidly than the fresh gel, with effectiveness rates of 66.3% for the fresh gel vs. 15.5% for the stored gel at 8 hours after application.

We identified the value from computerized Log Probit which extrapolated from a line indicating 8% as the ED_{95}. We believe that *Lit. elliptica* essential oil extract did not exhibit effectiveness at a higher dosages.

Acknowledgments. This work received financial support from DPP-2013-094 and GUP-2012-064, Fund of Research Development and Faculty of Health Science, Universiti Kebangsaan Malaysia, Malaysia. The authors are grateful to individuals who volunteered as subjects for this project. We also wish to thank the Institute for Medical Research for providing mosquito eggs for repellent testing.

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