JURNAL ILMU KEFARMASIAN INDONESIA
Indonesian Journal of Pharmaceutical Sciences
ISSN p-ISSN 1693-1831 (Print) e-ISSN 2614-6495 (Online) PUBLISHER : Faculty of Pharmacy Universitas Pancasila

Formulation and test of mosquito repellent lotion from the extract of kecombrang (*Etlingera elatior* (Jack)) and beluntas (*Pluchea indica*) leaves extract

Endah[1], Shelly Taurhesia[1*], Witono Basuki[1], Ratna Djamil[2]

[1]*Masters of Pharmaceutical Science, Faculty of Pharmacy, Universitas Pancasila, Jakarta, 12640, Indonesia*
[2]*Department of Phytochemistry, Faculty of Pharmacy, Universitas Pancasila, Jakarta, 12640, Indonesia*

*Corresponding Author: shellytaurhesia@univpancasila.ac.id

Received: 12 December 2023 / Accepted: 29 April 2024

ABSTRACT: Dengue fever is an infectious disease that is transmitted by *Aedes aegypti* mosquitoes and commonly occurs in Indonesia. The use of natural ingredients for mosquito repellent is becoming a trend. Kecombrang (*Etlingera elatior* (Jack)) is known as a medicinal plant that contains alkaloids, saponins, tannins, phenolics, flavonoids, triterpenoids, steroids, and glycosides. Beluntas (*Pluchea indica*) contains tannins, alkaloids, flavonoids, phenols, saponins, and essential oils. This study aims to develop mosquito repellent lotion using combined leaf extracts of *E. elatior* and *P. indica* at different ratios (1:1; 1:2; 2:1). The materials used in this study were *E. elatior* and *P. indica* leaves, *Aedes aegypti* mosquitoes, 96% ethanol, stearic acid, triethanolamine, white oil, methyl paraben, cetyl alcohol, and distilled water. The repellent activity is tested using a standard method of WHO, comparing the control group and treatment group, repeating four times, and then the protection level was calculated. The three formulas of lotion fulfilled both physical and chemical requirements and had a repellent activity of above 80%. The formulae of F3, a lotion containing both *E. elatior* and *P. indica* leaf extracts of 2:1, gave the highest protection of 88.1%.

KEYWORDS: *Aedes aegypti; Etlingera elatior;* mosquito repellent; *Pluchea indica.*

INTRODUCTION

Dengue hemorrhagic fever (DHF), commonly called dengue, is an infectious disease that commonly occurs in tropical countries. It has become a health problem worldwide, especially in developing countries [1]. In Indonesia, this disease has emerged since 1968 in Surabaya [2]. The Indonesian Ministry of Health reported 143,266 DHF cases in 2022 and a mortality of 1,237, mostly found in the cities of Depok, Medan, Bekasi, and Bandung [3]. One way to prevent the spread of dengue is to find protective measures from mosquito bites. One of the most practical protections is to apply mosquito repellent.

A good repellent does not disturb the user, and it is not sticky and toxic. It has a pleasant smell and does not cause skin irritation [4]. However, most of the repellent products processed in Indonesia contain diethyl toluamide (DEET) [5]. In this study, we used a commercial product containing 13% DEET as a control positive. DEET is a synthetic chemical which could be toxic in a concentration of 10-15%, can cause erythema (redness of the skin), urticaria (itching or hives), irritation, and may pose a hazard to health if used for a long time [6].

These warnings and dangers are a clear reason to explore alternative repellents derived from natural ingredients as Indonesia has various types of herbal medicinal plants, such as kecombrang *(Etlingera elatior)* and beluntas *(Pluchea indica)* [7]. Kecombrang or *Etlingera elatior* is one of the *Zingberaceae* family which is often found in Southeast Asia, especially Indonesia [8]. This plant is known by various names including "kencong" or "kincung" in North Sumatra, "kecombrang" in Java, and "honje" in Banten. Kecombrang is a spice that has been known and used as medicine since a long time ago as an antibacterial, anticancer, and antioxidant [9]. Kecombrang contains carbohydrates, dietary fiber, fat, protein, water, iron, calcium, magnesium, and secondary metabolites such as flavonoids, polyphenols, steroids, saponins, essential oils, and alkaloids; therefore, it can be used as a source of antioxidants [10]. Beluntas *(Pluchea indica)* belongs to the *Asteraceae* family, *Asteraceae* is a plant family that is easy to find in Indonesia [11].

It generally grows wild in dry areas on hard and rocky soil, or it is planted as a hedge. This plant smells aromatic and tastes bitter. This plant has other benefits, such as high antioxidant, antibacterial,

How to cite this article: Endah E, Taurhesia S, Witono Basuki W, Djamil R. Formulation and test of mosquito repellent lotion from the extract of kecombrang (Etlingera elatior (Jack)) and beluntas (Pluchea indica) leaves. JIFI 2024; 22(1): 54-60.

ISSN: 2614-6495
https://doi.org/10.35814/jifi.v22i1.1521

anticancer and anti-inflammatory activity [12]. *P. indica* is commonly found in coastal areas close to the sea up to an altitude of 1,000 meters above sea level [13]. A previous study reported that combined leaf extracts from kecombrang *(E. elatior*) and beluntas (*P. indica*) have the potential as a bio larvicidal with an LC_{50} value of 910 ppm [10].

However, none of the previous studies used a combination of beluntas and kecombrang leaf extracts to create a repellent lotion and to test its repellency to *Aedes agypti* bites. The lotion of mosquito repellent can manipulate the smell and taste of human skin so that mosquitoes cannot approach the skin and inhibit the lactic acid receptors on mosquito antennae by blocking the receptors on mosquito antennae so that mosquitoes lose contact with humans.

▪ MATERIALS AND METHODS

Material

Kecombrang and beluntas leaves were collected from Kp. Pabuaran, Kadomas Village, Pandeglang District, Pandeglang Regency, Banten Province, Indonesia. *Aedes aegypti* mosquitoes were taken from the Entomology Laboratory, IPB, Bandung Indonesia, with reagents, and 96% ethanol.

Equipment

This study used a rotary evaporator (Eyela® R-1001VN, Japan), oven (Memmert®, Germany), laboratory glassware (Pyrex, US), pH meter (Amtast® AMT20, USA), test tubes, vaporizer cups, petri dishes, measuring cups (Pyrex®, US), blender (Maspion®, Indonesia), hotplate (National®, Indonesia), and analytical scales (Fujitsu®, Japan), Viscometer (NDJ®-8S, China).

Methods

This study was conducted from April – August 2023 at the Integrated Pharmacy Laboratory of STIKES Salsabila in Serang. Mosquito repellent testing was performed at the Entomology Laboratory, IPB, Bogor. The scopes of this experimental study include determination, preparation of simplicia, preparation of kecombrang and beluntas leaf extracts, quality testing of both extracts including phytochemical screening, mosquito repellent test of the extracts, preparation of mosquito repellent lotion, and evaluation of lotion quality, efficacy, and irritation test.

Preparation of the lotion

Table 1 shows the lotion products formulated in this study. The lotion contains *E. elatior* (EDK) and *P. indica* (EDB) leaf extracts at the ratios of 1:1; 1:2; 2:1. The previous research conducted by Shobah (2021) showed that a combination of kecombrang leaf extract and beluntas extract in a ratio of 1:1 had an LC_{50} value in larvae of 910 ppm. The combination do not show good larvicide, therefore the researcher is using the same combination for mosquito repellent test. The evaluation of repellent lotion was conducted based on the lotion's organoleptic, pH, diameter for spreading, viscosity and mosquito repellency test, and irritation test.

Table 1. Formulas of mosquito repellent lotion at different ratios of *E. elatior* and *P. indica* leaf extracts.

No	Ingredients	Function	F0	F1	F2	F3
1	*E. elatior* leaf extracts	Active	-	1	1	2
2	*P. indica* leaf extracts	Active	-	1	2	1
3	Glycerin	Humectant	5	5	5	5
4	Stearic acid	Emulsifier	4	4	4	4
5	Triethanolamine		1	1	1	1
6	Acetyl alcohol	Emollient	1	1	1	1
7	White oil	Oil	6.5	6.5	6.5	6.5
8	Methylparaben	Preservative	0.1	0.1	0.1	0.1
9	Distilled water	Solvent	Ad 100	Ad 100	Ad 100	Ad 100

Mosquito repellent testing

The mosquito repellent test was conducted at the Entomology Lab, IPB using laboratory repellent bioassay methods [14]. Further details of the test are shown in Figure 1. The ethical clearance has been

granted from the health research ethics committee of Aisyiyah University, Yogyakarta under the letter No. 3232/KEP.UNISA/X/2023. A 25 cm area of a subject's forearm was given an ethanolic solution of repellent (treatment).

Figure 1. Mosquito-repellent tests using laboratory-repellent bioassay methods.

The same-sized area on the adjacent forearm of repellent (treatment). The same-sized area on the adjacent forearm was treated with alcohol (negative control). Both arms were simultaneously introduced into a cage filled with 25 female mosquitoes. The number of mosquitoes biting each arm was recorded for five minutes. The percentage of protection was calculated by comparing biting rates on the treatment and control arms.

$$\text{Protection (\%)} = \frac{\Sigma C - \Sigma T}{\Sigma C} \times 100$$

Note:

C is the number of mosquitos landed for five minutes on the arms of the control group

T is the number of mosquitos landed for five minutes on the arms of the treatment group

Irritation test of the repellent lotion

The irritation test was using 3 (three) rabbits which have been conditioned. A total of 0.5 ml of each formula was applied to rabbit's skin on the back. Then, the presence or absence of erythema and edema was observed. Observations were made within 1, 24, 48 and 72 hours [15]. This test has been granted ethical clearance from the health research ethics committee of Muhammadiyah University, Prof. Dr. Hamka Jakarta with No. 02/23.09/02891.

▪ RESULTS AND DISCUSSION

Determination

The research was begun with determination to ensure simplicia. The determination was conducted

at Herbarium Bogor – BRIN, and it was confirmed by the letter of B-1095/II.6.2/IR.01.02/5/2023 concerning *Etlingera elatior* (Jack) and *Pluchea indica.*

Phytochemical screening of both *E. elatior* and *P. indica* leaf extracts

The results of the phytochemical screening shown in Table 2 follow the previous study by Shobah [10]. The combination of extracts for mosquito repellent may have potential efficacy to protect humans from mosquito bites.

Table 2. Results of phytochemical screening of *E. elatior* and *P. indica* leaf extracts.

Tests	Reagents	Results	
		EDK	EDB
Alkaloids	Mayer	+	+
	Dragendorff	+	+
Flavonoids	Mg + HCl	+	+
Tannins	$FeCl_3$	+	+
Steroids and Triterpenoids	CH_3COOH anhidrat + H2SO4	+	+
Saponins	H_2O + HCl	+	+

Mosquito repellent testing of the extract

Both extracts are checked on the protection level of mosquito repellent. As can be seen in Table 3 that 1% kecombrang extract showed a protection level of 90.37% while 1% beluntas extract gave 87.01%. This data is quite different to the previous study on bio larvicidal activity with LC_{50} of 910 ppm (low activity of bio larvicidal). This indicated that there is no correlation between biolarvicidal activity and the protection level of mosquito repellent. Each extract showed protection level is higher than 80%, The next study is preparing lotions can proceed. Started combining 1% of *E. elatior* (EDK) and 1% *P. indica* (EDB) leaf extracts in the lotion's formulae, and then preparing another ratio of 1:2; and 2:1 to check the protection level of mosquito repellent.

Table 3. The result of mosquito repellent on the extracts and protection power (%)

No.	Testing Group	No of mosquitoes	Number of mosquitoes landed on the arms observed per hour							Average	Protection level (%)
			0	1	2	3	4	5	6		
1.	Kecombrang extract (1%)	25	19	23.75	24.5	27.5	36.75	48	53.75	33.32	**90.37%**
	Control	25	257	295.5	340	375.8	380.5	375.5	400	346.32	
2.	Beluntas extract (1%)	25	29.5	31.25	29.5	40.5	49.75	58.75	54	41.89	**87.01%**
	Control	25	243	257.5	253	299.8	357.5	394.3	387.5	322.50	

Evaluation of the repellent lotion quality

Table 4 shows an evaluation of both physical and chemical parameters of base lotion (F0) and lotion products containing various ratios of combined leaf extracts (F1, F2, and F3). The addition of extracts changed the color of the base lotion from white to the color of the leaf extracts. For example, F2 was greenish, and it was influenced by the color of *P. indica* extracts. Meanwhile, F1 and F3 were brownish, and it was influenced by the color of *E. elatior* extracts.

All formulas fulfilled the physical and chemical requirements for good repellent lotion. The pH of lotions is in the range of 7-8 which is slightly higher than skin pH but can still be tolerable [16], as shown on the irritation test all formulas have an irritation index of 0 which means do not irritate.

Table 4. Evaluation results of the repellent lotion.

Evaluation	F0 (base)	F1 (1:1)	F2 (1:2)	F3 (2:1)
Organoleptic: Texture, Color	Semi-solid, white	Semi-solid, brownish	Semi-solid greenish	Semi-solid brownish
Homogeneity	Homogeneous	Homogeneous	Homogeneous	Homogeneous
pH	8	7	7	7
Spreadability (cm)	5	5	5	5
Viscosity (mPa.s)	3.170	3.170	3.571	3.570

Mosquito repellent testing of lotions

The result of three formulas of mosquito repellent lotions can be seen in Table 5. prepared at various ratios of EDK:EDB, 1:1; 1:2; 2:1 (F1, F2, and F3) showed a protection level higher than 80%. The protection levels (%) of F1, F2, and F3 were 81.14%, 86.58%, and 88.1%, respectively, whereas that of a commercial repellent product containing 13% DEET as a positive control was 92.21%.

Using ANOVA to check any differences in protection level between F3 and commercial repellent products, and found the difference was 0.288 higher than 0.05. This means that the repellency levels of F3 and the commercial repellent product were not significantly different. The formula of lotion is suitable as it is still enabling the combination of extracts to manipulate the smell and taste of human skin so that mosquitoes cannot approach the skin and inhibit the lactic acid receptors.

Table 5. Observation results of mosquito repellent lotions and protection power (%).

Testing group	Number of mosquitoes	Number of mosquitoes landed on the arms observed per hour								Protection levels (%)
		0	1	2	3	4	5	6	Average	
F0	25	115	125.5	160.8	115.3	122	123	200	137.4 ± 31.3	
Control	25	145.8	267	367.3	321	432.5	534	300	338.2 ± 84.5	**59.39**
F1 (1:1)	25	25.8	138	45.8	56.5	67	74.8	84	70.3 ± 14	
Control	25	317.3	238	298.3	338.8	440	437.5	429.8	372.5 ± 93.1	**81.14**
F2 (1:2)	25	29.5	31.3	29.5	40.5	49.8	58.8	54	41.9 ± 8.4	
Control	25	162.3	243.3	253	340.5	404.5	394.3	387.8	3122 ± 78	**86.58**
F3 (2:1)	25	16.3	28.5	29	36.5	46.8	50.8	53.5	37.3 ± 7.5	
Control	25	207.8	208.5	275.8	343.8	380	385.5	408	315.6 ± 65.1	**88.17**
F4	25	21.5	29.5	24.75	29.8	30.5	38.8	41.3	30.9 ± 8.2	
Control	25	238	298.3	338.8	440	437.5	429.8	435	396.1 ± 73.7	**92.21**

Note: F4 is a commercial mosquito repellent lotion product with 13% DEET as an active ingredient.

Irritation test of the repellent lotion

The primary irritation tests were carried out on Albino rabbits and the purpose of this test is to determine the irritating effect on the skin by observing erythema and edema. As seen in Table 6, all formulas provide an irritation index of 0, which means they do not irritate. The observation on the back skin of rabbits for 72 hours indicated that all formulas showed no irritation effects on the skin as shown in Table 6.

Table 6. Calculation of irritation index of lotion formulas.

Testing Group	Irritation Index
F0 (Base)	0
F1 (A combination of EDK:EDB, 1:1)	0
F2 (A combination of EDK:EDB, 1:2)	0
F3 (A combination of EDK:EDB, 2:1)	0

▪ CONCLUSION

It is concluded that a combination of extracts from *E. elatior* and *P. indica* has a potential mosquito

repellent. All developed lotions fulfill physical and chemical parameters and show mosquito repellent, but F3 had the same level of protection as commercial products containing 13% DEET.

Acknowledgements: The authors would like to thank the Entomology Laboratory, IPB – Bogor for providing *Aedes aegypti*'s eggs and also facilities to perform repellency tests

Funding: This study was funded by the Higher Education Service Agency (LLDIKTI) through the 2023 grant.

Author contributions: Concept – E., S.T., ; Design – E., S.T., R.D.; Supervision – S.T.; Resources – E., S.T., W.B., R.D.; Materials – E.; Data Collection and/or Processing – E., S.T.; Analysis and/or Interpretation – S.T., W.B., R.D.; Literature Search – E., S.T., R.D.; Writing – E., S.T.; Critical Reviews – S.T., R.D.

Conflict of interest statement: "The authors declared no conflict of interest" in the manuscript.

REFERENCES

[1] W. H. Wang *et al.*, "Dengue hemorrhagic fever – A systemic literature review of current perspectives on pathogenesis, prevention and control," *J. Microbiol. Immunol. Infect.*, vol. 53, no. 6, pp. 963–978, 2020, doi: 10.1016/j.jmii.2020.03.007.

[2] A. Kardinan, "POTENSI SELASIH SEBAGAI REPELLENT TERHADAP NYAMUK Aedes aegypti," *J. Penelit. Tanam. Ind.*, vol. 13, no. 2, p. 39, 2007, doi: 10.21082/jlittri.v13n2.2007.39-42.

[3] K. RI Kesehatan, "Kasus DBD di Indonesia sampai minggu ke 33 tahun 2023." 2023.

[4] Rashman, L. Taha, and Juhaerah, "Ability of Papaya Leaf Extract (Carica papaya) in Controlling Aedes Aegypti Mosquito (Experimental Study)," *Int. J. Sci. Basic Appl. Res.*, vol. 24, no. 3, pp. 164–172, 2015.

[5] D. Champakaew *et al.*, "Repellent effect of local indigenous knowledge-based repellent in Nakhon Si Thammarat, Thailand, against Aedes aegypti mosquito," *Heliyon*, vol. 9, no. 11, p. e21589, 2023, doi: 10.1016/j.heliyon.2023.e21589.

[6] A. Y. Fitriana, R. Wahyuningrum, and Sudarso, "Daya Repelan Dan Uji Iritasi Formula Lotion Ekstrak Etanol Daun Sirih (Piper Betle Linn) Dengan Variasi Basis Lanolin Terhadap Nyamuk Aedes aegypti," *Pharmacy*, vol. 09, no. 02, pp. 1–9, 2012.

[7] M. E. Koraag, H. Anastasia, R. Isnawati, and O. Octaviani, "Efikasi Ekstrak Daun dan Bunga Kecombrang (Etlingera elatior) terhadap Larva Aedes aegypti," *ASPIRATOR - J. Vector-borne Dis. Stud.*, vol. 8, no. 2, pp. 63–68, 2016, doi: 10.22435/aspirator.v8i2.4615.63-68.

[8] A. Maimulyanti and A. R. Prihadi, "Chemical composition , phytochemical and antioxidant activity from extract of Etlingera elatior flower from Indonesia," *J. Pharmacogn. Phytochem.*, vol. 3, no. 6, pp. 233–238, 2015.

[9] K. Nisrina Effendi, N. Fauziah, R. Wicaksono, Erminawati, P. Arsil, and R. Naufalin, "Analysis of bioactive components and phytochemical of powders stem and leaves of kecombrang (etlingera elatior)," *IOP Conf. Ser. Earth Environ. Sci.*, vol. 406, no. 1, 2019, doi: 10.1088/1755-1315/406/1/012003.

[10] A. N. Shobah, F. Noviyanto, and N. M. Kurnia, "Kombinasi Ekstrak Daun Kecombrang (Etlingera elatior) dan Daun Beluntas (Pluchea indica) sebagai Biolarvasida," *J. Kesehat. PERINTIS (Perintis's Heal. Journal)*, vol. 8, no. 2, pp. 100–109, 2021, doi: 10.33653/jkp.v8i2.675.

[11] E. Susetyarini, P. Wahyono, R. Latifa, and E. Nurrohman, "The Identification of Morphological and Anatomical Structures of Pluchea indica," *J. Phys. Conf. Ser.*, vol. 1539, no. 1, pp. 0–13, 2020, doi: 10.1088/1742-6596/1539/1/012001.

[12] E. W. C. Chan, Y. K. Ng, S. K. Wong, and H. T. Chan, "Pluchea indica: An updated review of its botany, uses, bioactive compounds and pharmacological properties," *Pharm. Sci. Asia*, vol. 49, no. 1, pp. 77–85, 2022, doi: 10.29090/PSA.2022.01.21.113.

[13] D. Sukandar, N. Radiastuti, I. Jayanegara, and A. Hudaya, "Karakterisasi Senyawa Aktif Antibakteri Ekstrak Air Bunga Kecombrang (Etlingera elatior) Sebagai Bahan Pangan Fungsional," *J. Kim. Val.*, vol. 2, no. 1, 2010, doi: 10.15408/jkv.v2i1.232.

[14] D. Barnard, U. Bernier, R. Xue, and M. Debboun, "Standard Methods for Testing Mosquito Repellents," *Insect Repellents*, no. January, pp. 103–110, 2007, doi: 10.1201/9781420006650.ch5.

[15] BPOM, "Peraturan Badan Pengawas Obat Dan Makanan Nomor 10 Tahun 2022 Tentang Pedoman Uji Toksisitas Praklinik Secara In Vivo," *Badan Pengawas Obat dan Makanan Republik Indones.*, pp. 1–220, 2022.

[16] Syaiful, A. Zaeni, R. Suryani, and J. Hamsidi, "Formulasi Losio Antinyamuk Dengan Zat Aktif Minyak Atsiri Lantana

camara Linn.," *J. Farm. dan Ilmu Kefarmasian Indones.*, vol. 2, no. 1, pp. 2–5, 2015.