International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304, ISSN(Online): 2455-9563
Vol.9, No.7, pp 140-145, 2016

Formulation of Tobacco Based Mosquito Repellent to Avoid Dengue Fever

Mahdi Jufri[1]*, Evita Irmayanti[1], Misri Gozan[2]

[1]Faculty of Pharmacy, Universitas Indonesia, Depok 16424, Indonesia,
[2]Chemical Engineering Department, Engineering Faculty, Universitas Indonesia, Depok 16424, Indonesia.

Abstract : Commercial mosquito repellents containssynthetic substances such as DEET (N, N-diethyl-3-methylbenzamide), DEPA (N, N-diethyl phenylacetamide), permethrin, and deltamethrin as active component, which can be absorbed to human body and cause some systemic poisoning. This research studiesthe potential of tobacco leaves based repellent which is not only safe for human but also environmentally friendly. Tobacco leaves was extracted using fast pyrolysis at 500°C. The condensed biooil was then made into biomass based repellent. The repellent was tested directly to human to evaluate the effects on the skin and the effectivity as a repellent. The active compounds of repellent found was nicotine, d-Limonene, indole, and pyridine. Nicotine was the highest substance from biooil at 31.1%; 16.7%; and 18.9%, respectively. Biooil was added to repellent mixture as active compound with different concentration (0%; 0.5%; 1.5%; and 3%, all in %wt). Repellent tested showed a desired result, where not only the repellent didn't take any side effect on human skin, the effectivity of each concentration was 38.`%; 45.8%; 46.4%; and 57.1%, respectively.
Keywords : Biooil; Mosquito Repellent; Pesticide; Tobacco Leaves.

1. Introduction

Mosquitoes as vectors of pathogen of some major diseasecausing agents are a global problem that requires all theworking groups to work under an umbrella project so thatrepetition of trials and testing could be avoided and someproductive results achieved in the near future. Comparisonof the repellency data from different bioassays is difficultbecause of the variation in test conditions, lack of uniformityin bioassays, standard materials and the basic assumptionsassociated with each bioassay system. There is strong andurgent need to devise new or improve the current bioassay(1).

Mosquito repellent was an ingredient, which can act locally or from faraway, to prevent mosquitos from coming closer or biting human (2,3). The utilisation of synthetic mosquito repellent has been reduced considerably due to growing concern on the toxic effect to the environment, non-biodegradability, and negative impact on different non-target organisms (4). These commercially predominants contains DEET (N, N-diethyl-3-methylbenzamide), DEPA (N, N-diethyl phenylacetamide), permethrin, and deltamethrin as active component. DEET and DEPA are excellent repellents against mosquitoes and other biting insects,and therefore used in many personal protection formulations. Permethrin and deltame thrinare insecticide with broader spectrum and mostly used to kill mosquitoes on the net. There has been a serious apprehension on the toxicity associated with the use of DEET, DEPA, Permethrin, Deltamethrin and other pyrethroids based formulations. For example, the DEET can be absorbed into the body, and high dosed usage of DEET can cause sensoric and motoric nerve disturbance, neurodegeneration, and systemic poisoning (5,6). Therefore, the idea of using natural anti-mosquito formulations as alternative to the synthetic counterparts could bean amicable solution to scale back the negative impact on human health and environment (7,8, 9, 10)

Some research has examined different essential oil studies and reported the repellent activity of single essential oil based formulations(11,7,12). The optimum mixture of different essential oils was able to provide repellency for a long time. Although the essential oils have promising insect repellent property, but suffer from disadvantage of rapid volatility, which decreases the protection time provided by these oils and requires repeated application after times for achieving a long time efficacy. Besides, the patch formulation did not show any inhalation toxicity in experimental with Wistar rat. The repellent patches developed and evaluated currently, may provide a suitable, eco-friendly, accept-able and safe alternative to the existing synthetic repellent formulations for achieving protection against mosquitoes (13,14).

Characterization of tobacco mostly consists of phenolic compounds, nicotine, and diterpen(15,16,17). This research studiesthe potential of tobacco leaves based repellent which is not only safe for human but also environmentally friendly.

2. Method

2.1 Preparationand Characterization of Biooil

Tobacco leavesfrom Kerinci, Jambi were firtly washed to remove dirts, then dried under the sun before finally put in an oven at 60^{o}C for 2 hours. The dried leaves then was grinded to obtain a homogeneous size ca. 60 μm. At this stage pyrolysis will be done using dried tobacco leaves that had been prepared before to produce biooil. The pyrolysis reactor was loaded with 250 gram dried leaves and then was heated for 3 hours by electric furnace at 500^{o}C. Nitrogen gas was used as carrier and to avoid oxygen.The volatilized ingredients was then condensed by a small chiller containing circulated cooling water. Characterization of biooil was carried out by GC-MS (HP 6890) with a mass selective detector, column model Agilent 19091S-433 with injection volume 1.0 μL.

2.2 Repellent Formulation

The repellent was made by mixing cream base with the biooil. It was made in variouskind of biooil concentration, that is 0%; 0.5%; 1.5%; and 3%. The method used was emulsion of water and oil. The oil phase was a mixture of stearic acid, glyceryl monostearate, isopropyl myristic, dimeticon, and propyl paraben, that was heated at temperature of 70^{o}C. While the water phase was a mixture of methyl paraben, trietanolamine, PEG 400, propylenglycol, and aquadest. The oil phase was mixed into water phase with homogenizer at temperature of 70^{o}C.When the temperature reach 40^{o}C, the citric acid 20% solution was dropped slowly into the mixture together with aquadest, antioxidant, and biooil. Then the stirring continued with a speed of 1000 rpm for another 15 minutes.

2.3 Evaluation of Mosquito Repellent

The evaluation was divided into 3 steps, that is the stability of the repellent lotion, sensitivity of human skin to the repellent, and effectivity of the repellent to prevent mosquito bites.

2.3.1 Repellent Stability Test

This test was done to evaluate the stability of the cream lotion. The lotion was put into centrifugation in a speed of 3750 rpm for 5 hours or 5000 to 10000 rpm for 30 minutes, then observation was done to see if there was a separation of the phase in the lotion.

2.3.2 Sensitivity Test

This test was to observe the effect of repellent on human skin. The repellent used was the one with 3% of biooil concentration. It was smeared into the panelist arm in 5 x 5 cm wide,then a patch was put on it for 24 hours. After 24 hours, the observation was done in the area where the repellent was put. Positive irritation reaction was signified by erythema, itchiness, or edema on the skin.

The data collected was the processed to get index of skin irritation with equation below:

$$\textbf{index of irritation} = \frac{\sum erythema\ score\ + \sum endema\ score}{number\ of\ panelist} \quad (1)$$

2.3.3 Effectivity Test

This test was done to observe the effevtivity of mosquito repellent. The test was done to 10 volunteer for 3 days, using 25 mosquitoes and both hands of the volunteer, which only one of the hands was using the repellent and the other was used as control. The test was carried out for 6 hours and using each concentration to compare the effectiveness. The protection was calculated by the formula below:

$$\frac{\sum Mosquitoes\ used - \sum Mosquitoes\ landed}{\sum mosquitoes\ used}\ x\ 100\% \qquad (2)$$

3. Results and Discussion

3.1 Biooil Composition

The component inside biooil then analyzed by using GC-MS analysis. Each sample was analyzed to get the content in fractions above and below it to determine of the component differences between the top and lower fractions. The results of GC-MS graph was shown in Figure 3.

Tobacco biooil from pyrolysis was analyzed by GC-MS. The top fraction was dominated by heavy hydrocarbons, some aromatics, organic acids, and other oxygenated compounds. While the lower fraction was dominated by organic acids and pyridine derivatives. The most dominant compounds from tobacco biooil was nicotine.

GC-MS result on tobacco biooil show that there were some components that have a function as insect repellent, that were the main component in this experiment. They were:

- **d-limonene**

D-limonene is used as the active and inert ingredients in pesticide products, and as an ingredient in food products, soaps, and perfumes. As the active ingredient, d-limonenen used as an insecticide, insect and animal repellent. While as an inert, these compounds can be used as a solvent and fragrance (18).

- **Indole**

Indole, is an active ingredient found naturally in nature. These compounds are commonly used in the synthesis of perfume or essential oil in low concentrations. . Indole can be used in biochemical pesticides at low concentrations as an attractant, but showed very low levels of toxicity (19)

- **Nicotine**

Nicotine, derived from tobacco, has been used as a pesticide since at least the 15th century. Type the use of nicotine as an insecticide is a pesticide, which is used only on ornamental plants and flowers and is not recommended in food crops (20).

- **Pyridine**

Piridine is a compound that is often used as a solvent and is used to make a variety of products such as medicines, vitamins, food flavorings, pesticides, and other. Its use as an intermediate in the manufacture of various insecticides and herbicides for agricultural use were just start to widely produced since the 20th century (21).

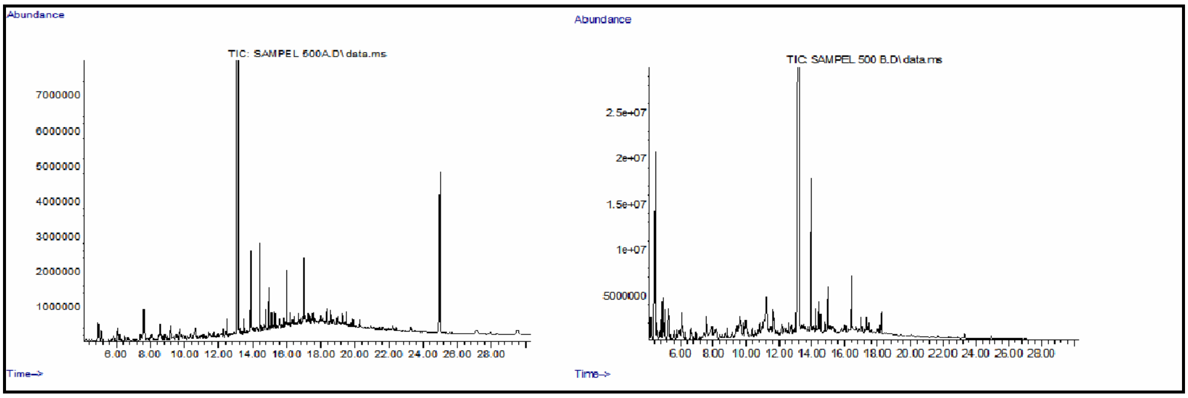

Figure 3. GC-MS of Tobacco Biooil top and bottom phase

3.2 Evaluation of Mosquito Repellent

The lotion was in a form of homogeneous white cream. The three repellent that has biooil in it was white cream lotion with distinctive odor. The higher the concentration of the biooil used, the odor was stronger.

3.2.1 Stability Test

The test was done byusing centrifugation with a speed of 3750 rpm within some radius of centrifugation for 300 minutes. This test could be treated the same as the effect of gravitational force to the lotion for the storage of more or less one year (22). The observation indicate that there were no separation on the repellent after the centrifugation was done.

3.2.2 Sensitivity Test

After 24 hours of test done to 10 panelist, patch was removed and observation was done on the skin where the repellent was applied, and the data were processed according to the erythema and edema score happened to the panelist's skin showedin table 2. Using equation (1), then we got:

$$\text{Skin Irritation Index} = \frac{1+0}{10} \times 1 = 0{,}1$$

Table 2. Sensitivity Test Result

Panelis	Erithema Score	Edema Score
1	0	0
2	0	0
3	0	0
4	1	0
5	0	0
6	0	0
7	0	0
8	0	0
9	0	0
10	0	0

According to respond category then the effect of mosquito repellent to panelist can be categorized as "meaningless".

3.2.3 Effectivity Test

The test was carried out for 6 hours and done with each concentration to compare the effectivity of each concentration. Protection average result of each formula was shown by table 3. As shown, the higher the concentration of biooil in the repellent, the higher the protection.

In general, repellent will manipulate the smell and taste from skin by resisting the lactate acid receptor in mosquito, so it will prevent the mosquito to come near the skin, usually by adding some dominant smell on mosquito repellent such as orange skin or lavender. This principle also applied to tobacco mosquito repellent, where beside it has a potential as pesticide, tobacco biooil also had a dominant smell so it can manipulate mosquito by covering CO_2 smell that was produced by human skin.

3.3 Comparison with Other Biooil

Study to another biooil or essence oil as repellent are also done, one of them was using the mixture of lemongrass and eucalyptus oil to repel Ae. Aegypti (23). The experiment gave a satisfying result,where the mixture of those essence oil could gave a protection in (98,66+11,56) minutes. The protection parameter was the mosquitoes that was landed on the skin is not more than 2 minutes. This time is relative short compares to industrial mosquito repellent, where according Thai Industrial Standards Institute (TISI), the protection given should be more than 120 minutes.

The experiment using repellent containing tobacco biooil show much more better result. Protection given from tobacco mosquito repellent with 3% biooil concentration in it last along the experiment period, that is 6 hours, where to the most panelist, there were just less than 2 mosquitoes land on the volunteer's hand.

Table 3. AverageProtection by Tobacco Mosquito Repellent

Formula	Average Protection
F1 (0%)	38.09 %
F2 (0.5%)	45.82 %
F3 (1.5%)	46.41 %
F4 (3%)	57.07 %

4. Conclusion

From this experiment, it can be concluded that:

1. Nicotine was the most dominant compound on all result of biooil, where it was 31.1% on 500^oC.
2. Mosquito repellent that had biooil in it had a relative hight effectivity, that is 57.07% on the one with 3% biooil concentration.
3. The repellent was proved harmless to human skin. It is proved by the sensitivity test where there are no effect on the volunteer's skin.

Acnowlegment :

The study was funded by CEGs grant 2015 No 2239/UN2R12HKP 05.00 DRPM Universitas Indonesia.

Reference

1. Rehman, Junaid U., Abbas, Ikhlas A. Khan., 2014. Plant based products: Use and development as repellents against mosquitoes: A review. Fitoterapia 95 (2014) 65–74.
2. Choochote, W., Chaithong, U., Kamsuk, K., Jitpakdi, A., Tippawangkosol, P., Tuetun, B., Champakaew, D., and Pitasawat, B. 2007. "Repellent Activity of Selected Essential Oils Against *Aedes aegypti*". *Fitoterapia* 78 (2007) 359–364
3. Nagappan Pappayee ,GomathinayagamSaraswathy2015 Nano-Phytochemicals from the Leaves of Plumbago Zeylanica for Mosquito Control International Journal of Pharm Tech Research 8, 648-652

4. Baril R. H., S. B. 2015, Patil1Room temperature cigarette smoking sensing performance of nanostructured SnO 2 thin films. International Journal of Chem Tech Research 8, 1189-1202
5. Bell, J.W., Veltri, J.C., and Page, B.C. 2002. Human Exposures to N,N-Diethyl-m-Toluamide Insect Repellents. International Journal of Toxicol: 21: 341-352.U.S Environmental Protection Agency. 2009. "D-Limonene: Exposure and Risk Assesment on Bottom Risk Pesticide Chemicals". United State of America
6. Ray Saikat Sinha, Sangeetha D. 2014-2015 Analysis of Metallic Contamination and Toxicity Exposure by Different Branded Cigarettes in India International Journal of Chem Tech Research 7, 2474-2477
7. Dhiman, S., Rabha, B., Chattopadhyay, P., Das, N.G., Hazarika, S., Bhola, R.K., Veer, V.,Singh, L., 2012. Field evaluation of repellency of a polyherbal essential oil against blackflies and its dermal toxicity using rat model. Trop. Biomed. 29, 391–397.
8. Koul, O., Walia, S., Dhaliwal, G.S., 2008. Essential oils as green pesticides: potential and constraints. Biopestic. Int. 4, 63–84.
9. Garud, A., Ganesan, K., Garud, N., and Vijayaraghavan, R. 2013. "Topical Preparation of Newer Safer Analogs of N,N-Diethyl-2-phenylacetamide (DEPA) Against Aedes Aegypti Mosquitoes". Journal of Cosmetics, Dermatological Sciences and Applications. 3, 22-27
10. Al –Younis Fadia, Al -NaserZakaria, Al- Hakim Wassim2015, Chemical composition of Lavandula angustifolia Miller and Rosmarinus officinalis L. essential oils and fumigant toxicity against larvae of Ephestia kuehniella Zeller International Journal of Chem Tech Research 8 , 1382-1390
11. Das, N.G., Dhiman, S., Talukdar, P.K., Rabha, B., Goswami, D., Veer, V., 2015. Synergistic mosquito-repellent activity of *Curcuma longa, Pogostemon heyneanus* and *Zanthoxylum limonella* essential oils. J. Infect. Public Health, http://dx.doi.org/10.1016/j.jiph.2015.01.010
12. Hazarika, S., Dhiman, S., Rabha, B., Bhola, R.K., Singh, L., 2012. Repellent activity ofsome essential oils against simulium species in India. J. Insect Sci. 12, 5.
13. Chattopadhyaya P., Dhiman S., Boraha S., Rabhab B., Chaurasiaa A.K., Veer V., 2015. Essential oil based polymeric patch development and evaluating itsrepellent activity against mosquitoes. Acta Tropica 147 (2015) 45–53.
14. KomansilanAlfritz, Ni Wayan Suriani, 2016, Effectiveness of Seed Extract Hutun (Barringtonia asiatica Kurz), on LarvaAedesaegypti Vector Disease Dengue Fever, International Journal of Chem Tech Research, 9, 617-624
15. Sheng, L. Q.; Ding, L.; Tong, H. W.; Yong, G. P.; Zhou, X. Z.; Liu, S. M. "Determination of nicotine-related alkaloids in tobacco and cigarette smoke by GC-FID". Chromatographia 2005, *62*, 63–68.
16. Shen, J. C.; Shao, X. G." Determination of tobacco alkaloids by gas chromatography-mass spectrometry using cloud point extraction as a preconcentration step". Anal. Chim. Acta 2006, 561, 83–87.
17. Cai, J. B.; Liu, B. Z.; Lin, P.; Su, Q. D. "Fast analysis of nicotine related alkaloids in tobacco and cigarette smoke by megabore capillary gas chromatography". J. Chromatogr., A 2003, 1017, 187–193
18. U.S Environmental Protection Agency. 2009. "D-Limonene: Exposure and Risk Assesment on Bottom Risk Pesticide Chemicals". United State of America.
19. U.S Environmental Protection Agency. 2009. "Indole". Biopesticides Registration Action Document. United State of America
20. U.S Environmental Protection Agency. 2008. "Reregistration Eliglibility Decision (RED) Document for Nicotine". United State of America
21. U.S. Public Health Service. 1992. "Toxological Profile for Pyridine". Agency for Toxic Substances and Disease Registry
22. 22.Lachman, L., H. Lieberman & J. L., Kanig. (1994). "Teori dan Praktek Farmasi Industri. Terjemahan dari The Theory and Practice of Industrial Pharmacy" oleh Siti Suyatmi, J. Kawira, Iis Aisyah. Jakarta: UI Press, 1035-1037, 1051-1052, 1064-1070.
23. Sritabutra, D., Soonwera, M., Waltanachanobon, S., dan Poungjai, S. 2011. "Evaluation of herbal essential oil as repellents against Aedes aegypti (L.) and Anopheles dirus Peyton & Harrion". Asian Pacific Journal of Tropical Biomedicine. S124-S128.

*****