

ESSENTIAL OILS OBTAINED FROM *EUCALYPTUS* AND *CYMBOPOGON* LEAVES BY STEAM DISTILLATION FOR MAKING NATURAL MIXTURE HAVING EFFECT AS A MOSQUITO REPELLENT

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Abstract. Taking the essential oils out of plant has not been unfamiliar with people all over the world including Viet Nam because of their useful applications especially in taking insects away. Therefore, the study was conducted to aim of extracting essential oils from *Eucalyptus* and *Cymbopogon* leaves as well as examining their effect on mosquito repellent. The factors affecting the amount of extracted oils are the time to wither materials (0 h; 2.5 h; 5 h), the distillation time (2 h; 2.5 h; 3 h) the amount of the distilled water (400 mL, 500 mL, 600 mL) and how to process the materials (crushing or not). Mixing ratio of *Cymbopogon* oil: *Eucalyptus* oil: coconut oil, one of the important criteria to be investigated for evaluating the ability to repel mosquitoes is in turn (40:10:50), (35:15:50), (30:20:50). The contents of mixing formula of the *Cymbopogon* essential oil: *Eucalyptus* essential oil: coconut oil (40: 10: 50) is the most effective with the ability to falling mosquitoes only after 5 minutes and killing 84.67 % mosquitoes after 24 hours. It can be concluded that *Cymbopogon* oil, *Eucalyptus* oil, and coconut oil are a promising natural repellent due to their safety advantage over chemical repellents.

Keywords: essential oil, *Eucalyptus*, *Cymbopogon*, mosquito repellent, steam distillation.

Classification numbers: 1.4.6.

1. INTRODUCTION

Mosquitoes have an important role in the transmission of diseases like malaria, dengue, chikungunya, Japanese encephalitis and filariasis, and cause millions of deaths annually [1]. Repellents are an important part of these preventive actions [2]. Among developed synthetic chemicals, DEET (N,N-diethyl-3-methyl benzamide) has a wide range of repellency to keep humans far from mosquito bites with most persistence and effectiveness on the body [3], yet other evidence shows that use of DEET is associated with both systemic and local adverse effects, known to permeate the skin, and also cause allergic and toxic reactions on application, with odor that is unpleasant to some people [4, 5]. Essential oil repellents have short duration of

action because of their volatility. As possible sources of repellents and insecticides, there are many preparations from natural origins that are repellent to certain insects [6]. Essential oils are potentially good repellents and insecticides because of their selectivity, safety, and negligible adverse effects on the environment and non-target organisms [7]. The major component of *Eucalyptus globulus* is 1,8-Cineole (Eucalyptol) and has been recognized as a high ovipositional repellent and mild mosquito feeding repellent [8]. Chemical instability, volatility, propensity for oxidation and poor water solubility of essential oils make them inconvenient for extensive use [9]. *Cymbopogon winterianus*, commonly known as citronella, is native to the tropical and subtropical areas, consisting of citronellol, citronellal, and geraniol as its major compounds [10]. *Cymbopogon* oil has been proven to exhibit low acute toxicity in laboratory animals [11]. Various studies have evaluated the mosquito repellent activity of citronella oil extracted from *Cymbopogon*. Consequently, the incorporation of essential oils from *Eucalyptus* and *Cymbopogon* leaves have not been extensively investigated. In this study, we aim to extract the essential oil from *Eucalyptus* and *Cymbopogon* leaves, grown in southern Viet Nam, and examine the repellent activities of the essential oil against mosquitoes.

2. MATERIALS AND METHODS

2.1. Materials

The fresh leaves of the *Eucalyptus citriodora* were collected during autumn from area 3 Thuan An, Long My Town, Hau Giang Province. The fresh leaves of the *Cymbopogon citratus* were collected during autumn from the area of An Binh Ward, Ninh Kieu District, Can Tho City, expanded to the laboratory Department of Food Technology, Tay Do University.

Sodium sulfate 99 % sigma – Aldrich, distilled water at Tay Do university, coconut oil at Cai Rang market were used.

Mosquitoes (Culicidae) grown from mosquito larvae were collected from nests right the Tay Do University using for laboratory repellent bioassays with essential oil mixture in evaporated breeder cages having dimension of 40 cm × 40 cm × 40 cm by paraffin candle burner.

2.2. Methods

2.2.1. Experiment 1: investigating the effects of wilting time of *Eucalyptus* and *Cymbopogon* leaves during extraction for essential oil content

Eucalyptus and *Cymbopogon* leaves were cleaned, weighed exactly of 300 g for each treatment, conducted to naturally wilt respectively in 3 levels specified times of 0 h, 2.5 h, 5 h; and then distilled by the steam distillation method, taking the anhydrous essential oil layer with Na₂SO₄ to obtain pure essential oils, each treatment repeated 3 times.

2.2.2. Experiment 2: investigating the effects of distillation time, additional water and *Eucalyptus* and *Cymbopogon* leaves crushed or without crushed during extraction for essential oil content

The fresh leaves of the *Eucalyptus* and *Cymbopogon*, after processing as above were crushed or without crushing, adding the amount of water, respectively, of 400 mL, 500 mL, 600 mL. The steam distillation method was applied in each specified time interval is 2 h, 2.5 h, 3 h

for collecting crude essential oil, then taking the anhydrous essential oil layer with Na₂SO₄ to obtain pure essential oils, each treatment repeated 3 times.

2.2.3. Experiment 3: laboratory repellent bioassays based on and innovated by WHO protocol using essential oil mixtures

Preparing 2 mosquito cages of size of 40 cm × 40 cm × 40 cm, one cage was used to test mosquito repellency, the another was the control treatment. Two mosquito trap seach was placed in a cage having 50 mosquitoes. Leaving the mosquitoes rested and stabilized for 30 minutes. Then blend essential oils including *Eucalyptus*, *Cymbopogon* and coconut oil (oil background), mixed in 3 ratios, respectively, of *Eucalyptus*, *Cymbopogon* and coconut oil (40:10:50), *Eucalyptus*, *Cymbopogon* and coconut oil (35:15:50), *Eucalyptus*, *Cymbopogon* and coconut oil (30:20:50) of three treatments. This rate was selected in a preliminary survey. Coconut oil was used because of safety and friendly environment. Taking 5 mL of oil mixture of each dish above of parafin oil burner and placed in the bottom of mosquito cage, the distance of isolating was 8.5 cm. It was observed and recorded for the time mosquito begins abnormal moving for shelter until the mosquitoes stops moving since the mosquito begins to expose essential oils blend in each concentration. Observing, counting and recording the number of mosquitoes fallen were conducted at 5 minutes, 10 minutes, 15 minutes, 20 minutes, 30 minutes, 45 minutes and 60 minutes from each treatment. Immediately after the end of the exposure time, the mosquito cage was kept for 24 hours in a cool place with a temperature not exceeding 30 °C. Numbering the dead mosquitoes and calculating the mosquito death ratio were done after 24 hours [12].

2.3. Statistical analysis

Data were analyzed using oneway ANOVA with Post Hoc Duncan test by statistical software SPSS 16.0. All the experiments were repeated three times and data were expressed as mean value ± SD.

3. RESULTS AND DISCUSSION

3.1. The effects of wilting time of *Eucalyptus* leaves to essential oil content during extraction

The results of the essential oil content obtained depending on 0 hours, 2.5 hours and 5 hours wilting of *Eucalyptus* leaves, and statistical analysis the data were presented in Table 1 as follows:

Table 1. The effects of the wilting time of *Eucalyptus* leaves to essential oil content during extraction.

Wilting time (hours)	<i>Eucalyptus</i> essential oil content (g)
0	0.886 ± 0.003 ^c
2.5	0.557 ± 0.008 ^b
5	0.473 ± 0.001 ^a

Note: different letters (a, b or c) above the columns indicate statistically significant difference at $P \leq 0.05$.

The statistical results in Table 1 showed that the fresh leaves of the *Eucalyptus* (not to wilt, 0 hours), after extraction, given the most essential oil content (0.886 g), statistically significant difference at 95 % interval in comparison with other treatments. The simple reason is that in the longer wilting time, the water and essential oil inside the leaves partially loss, so that reduced the amount of essential oil getting.

3.2. The effects of wilting time of *Cymbopogon* leaves to essential oil content during extraction

Effecting of the wilting time of 0 hours, 2.5 hours and 5 hours of *Cymbopogon* leaves for extraction of the essential oil content, the results are statistically analyzed and presented in Table 2.

Table 2. The effects of wilting time of *Cymbopogon* leaves to essential oil content during extraction.

Wilting time (hours)	<i>Cymbopogon</i> essential oil content (g)
0	1.017 ± 0.001 ^c
2.5	0.600 ± 0.001 ^b
5	0.437 ± 0.002 ^a

Note: different letters (a, b or c) above the columns indicate statistically significant difference at P ≤ 0.05

The results in Table 2 showed that the fresh *Cymbopogon* leaves (without wilting) having the highest yield of essential oil 1.017 ± 0.001 where as at the time of withering 5 hours for the lowest number of essential oil 0.437 ± 0.002 statistically significant difference at 95 % confidence interval in comparison with the other treatments, because of the process of drying the materials, the amount of water in *Cymbopogon* leaves evaporated, contains the amount of essential oil. Therefore, the fresh *Cymbopogon* leaves are suitable for extracting *Cymbopogon* essential oil.

3.3. The effects of distillation time, additional water and *Eucalyptus*, crushed or without crushing during extraction

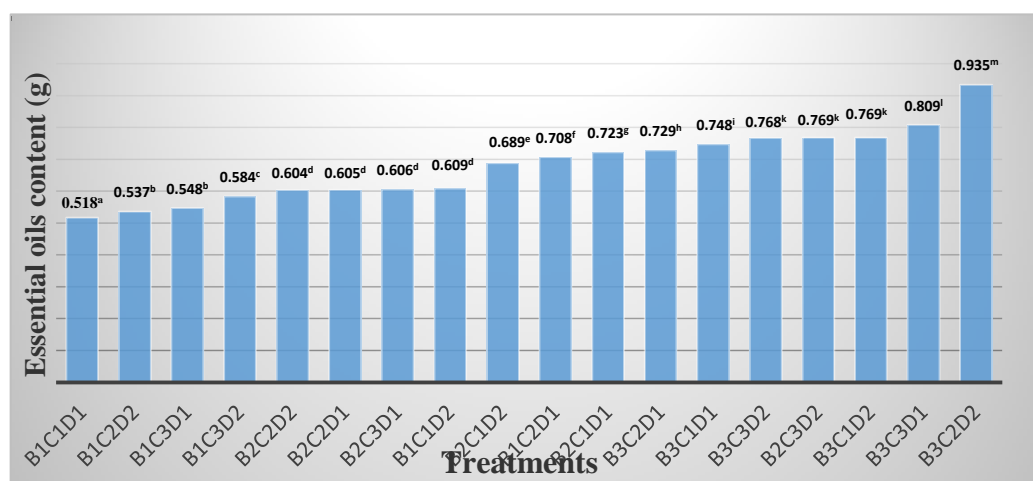


Figure 1. Chart showing the effect of distillation time, additional water and crushed *Eucalyptus* leaves or without crushing during extractions.

B1C1D1: 2 h, 400 mL, crushed
 B1C2D1: 2 h, 500 mL, crushed
 B1C3D1: 2 h, 600 mL, crushed,
 B2C1D1: 2.5 h, 400 mL, crushed
 B2C2D1: 2.5 h, 500 mL, crushed
 B2C3D1: 2.5 h, 600 mL, crushed
 B3C1D1: 3 h, 400 mL, crushed
 B3C2D1: 3 h, 500 mL, crushing
 B3C3D1: 3 h, 600 mL, crushing

B1C1D2: 2 h, 400 mL, without crushing
 B1C2D2: 2 h, 500 mL, without crushing
 B1C3D2: 2 h, 600 mL, without crushing
 B2C1D2: 2.5 h, 400 mL, without crushed
 B2C2D2: 2.5 h, 500 mL, without crushing
 B2C3D2: 2.5 h, 600 mL, without crushing
 B3C1D2: 3 h, 400 mL, without crushing
 B3C2D2: 3 h, 500 mL, without crushing
 B3C3D2: 3h, 600 mL, without crushing

Different letters (a, b, c) above the bars indicate statistical significance ($p \leq 0.05$).

The effects of other factors on *Eucalyptus* essential oil content extracted including distillation time, additional water and *Eucalyptus* crushed or without crushing are shown in Figure1. In the treatment (B3C2D2), the distillation time of 3 hours, additional water 500 mL, *Eucalyptus* leaves without crushing, the essential oil content obtained as high as 0.935 g, statically significant difference at 95 % confidence in comparison with the other treatments, can explain the reason as follows:

When distillation time is longer than 1 hour (2 hours, 3 hours), the water is evaporated more carrying more essential oil, this result is also consistent with the results of the studies [13].

3.4. The effects of distillation time, additional water and *Cymbopogon*, crushed or without crushing during extraction

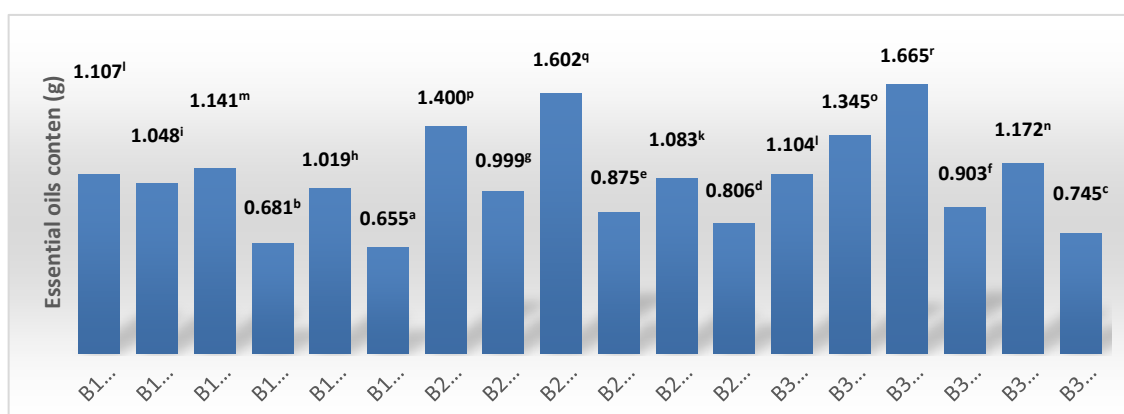


Figure 2. Chart showing the effect of distillation time, distilled water added and crushed *Cymbopogon* leaves or without crushing after extraction.

B1C1D1: 2 h, 400 mL, crushed
 B1C2D1: 2 h, 500 mL, crushed
 B1C3D1: 2 h, 600 mL, crushed,
 B2C1D1: 2.5 h, 400 mL, crushed
 B2C2D1: 2.5 h, 500 mL, crushed
 B2C3D1: 2.5 h, 600 mL, crushed
 B3C1D1: 3 h, 400 mL, crushed
 B3C2D1: 3 h, 500 mL, crushing
 B3C3D1: 3 h, 600 mL, crushing

B1C1D2: 2 h, 400 mL, without crushing
 B1C2D2: 2 h, 500 mL, without crushing
 B1C3D2: 2 h, 600 mL, without crushing
 B2C1D2: 2.5 h, 400 mL, without crushed
 B2C2D2: 2.5 h, 500 mL, without crushing
 B2C3D2: 2.5 h, 600 mL, without crushing
 B3C1D2: 3 h, 400 mL, without crushing
 B3C2D2: 3 h, 500 mL, without crushing
 B3C3D2: 3h, 600 mL, without crushing

Different letters (a, b, c) above the bars indicate statistical significance ($p \leq 0.05$).

The effects of other factors on *Cymbopogon* essential oil content extracted including distillation time, additional water and *Cymbopogon* crushed or without crushing are shown in Figure 2.

The results in Figure 2 showed that the *Cymbopogon* essential oil extracted has been also affected by distillation time, the amount of distilled water added and fresh *Cymbopogon* leaves crushed or without crushing. The essential oil content extracted with fresh *Cymbopogon* leaves crushed for 3 hours distilled, 500 mL additional water was the highest at 1.665 g (B3C2D1), whereas essential oil content with fresh *Cymbopogon* leaves were not crushed, distilled for 2 hours, distilled water 600 mL for essential oil content as low as 0.655 g, statistically significant difference at 95 % confidence level in comparison with other treatments.

In extracting essential oil by the steam distillation method, water vapor penetrates the cell layers, expanding and breaking the cell walls of *Cymbopogon* leaves and attracting *Cymbopogon* essential oil according to water vapor. In terms of size, materials crush the contact area between materials and solvents are greater, water vapor easily penetrates the cell layers, quickly attracting *Cymbopogon* essential oil according to the water vapor.

3.5. Investigating the time of mixing *Cymbopogon* essential oil, *Eucalyptus* essential oil and coconut oils

The results in Table 3 showed that the concentration of 40:10:50 oil mixture had the fastest effecting time, after 5 minutes of testing, 100 % of mosquitoes moved continuously, eluded to contact the essential oil vapor and after 10 minutes the mosquitoes should stop moving, some of them fell to the bottom of the test cage, statistically significant difference in comparison with other treatments in 95 % confidence level with other treatments. In the control treatment, the mosquitoes had been normal.

3.6. Evaluating the effectiveness of expelling mosquitoes of the essential oil mixture

Table 3. Investigating the time of mixing essential oil begin to work at 3 concentrations of *Cymbopogon*, *Eucalyptus* and coconut oils.

Time	Rate of the mosquito move / 50 mosquito (%)			Mosquito Control
	Concentration 30:20:50	Concentration 35:15:50	Concentration 40:10:50	
5 minutes	Mosquitoes abnormal moving 10 %	Mosquitoes abnormal moving 30 %	Mosquitoes abnormal moving 100 %	normal
10 minutes	Mosquitoes abnormal moving 40 %	Mosquitoes abnormal moving 100 %	Mosquitoes stop moving	normal
15 minutes	Mosquitoes abnormal moving 100 %	Mosquitoes stop moving		normal
20 minutes	Mosquitoes stop moving			normal

Table 4. Evaluating the effectiveness of expelling mosquitoes of the essential oil mixture.

Concentration	Mosquitoes	Ratio of mosquitoes fallen after steamed with essential oils (%)					Mortality rate (%) after 24 hours
		15 mins	20 mins	30 mins	45 mins	60 mins	
30:20:50	50	0	0	22.6 ± 1.3	42 ± 2.3	54.7 ± 2.4	40 ± 1.1
35:15:50	50	0	43.3 ± 4.7	52 ± 2	66 ± 1.2	72.7 ± 1.8	62.7 ± 2.9
40:10:50	50	34.6 ± 5.8	52.6 ± 1.8	72 ± 1.15	78.7 ± 1.3	86 ± 2.3	84.6 ± 2.9

The concentration of *Cymbopogon* essential oil + *Eucalyptus* essential oil + coconut oil at a ratio of 40:10:50 has been likely to cause high fall for mosquitoes, after 20 minutes the average fall 52.6 % of mosquitoes, after 24 hours the mosquito mortality (%) is 84.67 %, so essential oil mixture in this concentration has a good effectiveness on mosquito repellent, statistically significant difference in comparison in 95 % confidence level with other treatments. It is recommended that a natural mixture using for mosquito repellent should be prepared as: *Cymbopogon* essential oil: *Eucalyptus* essential oil : coconut oil as 40:10:50, because at this concentration ratio, the effectiveness on mosquitoes proved to be the highest.

4. CONCLUSION

The factors affecting on essential oils obtained from *Eucalyptus* leaves and *Cymbopogon* leaves show that the fresh *Eucalyptus* leaves without crushing, the fresh *Cymbopogon* leaves crushed, additional water 500 mL, distillation time 3 hours were extracted of high essential oil content. The essential oil mixture of *Cymbopogon* essential oil: *Eucalyptus* essential oil: coconut oils on 40:10:50 is most suitable for creating product as mosquito repellent of safety for human health and more friendly to the environment.

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Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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