

# Study on optimal conditions of lemongrass extraction

Jaruwan Chutrtong<sup>1,\*</sup>, and Kunyanuth Kularbphetong<sup>1</sup>

<sup>1</sup>Faculty of Science and Technology, Suan Sunandha Rajabhat University, 10300 Bangkok, Thailand

**Abstract.** This research was conducted to find the optimal conditions to extract active ingredients from lemongrass for using to inhibit microorganisms. The solvents selected for testing are safe chemicals such as water, ethanol and methanol. We also studied the ratio of the lemongrass and the extract solvent and extract time which make the most extraction. Using water as an extract solvent, whether it is heating or not, the extract cannot inhibit the microbes. While using ethanol and methanol as extract solvent, no heat, the extract showed effectiveness in microorganisms inhibition at lemongrass extract concentration 50 mg/ml. Ethanol and methanol used in the extraction has been evaporated to prevent ethanol from being microorganisms deterrent. Therefore, it can be concluded that water cannot used as solvent for extraction of microbial inhibitors from lemongrass. Ethanol and Methanol are suitable solutions because of the low price, safety and the method is not complicated.

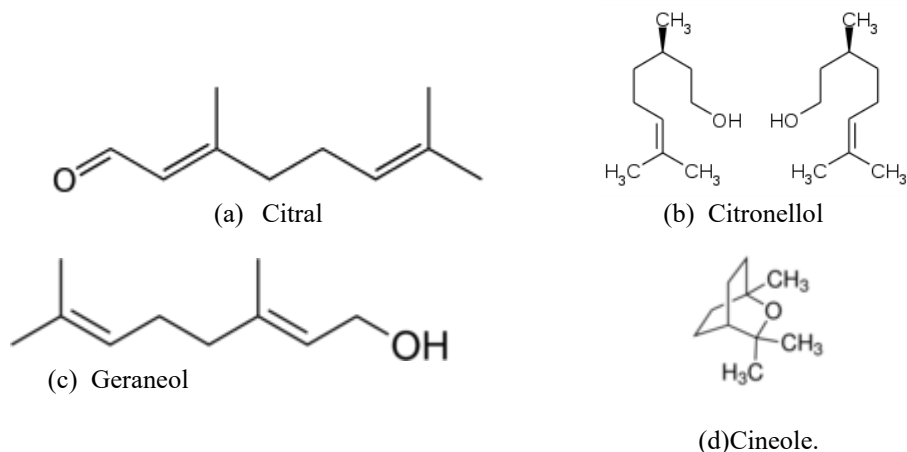
## 1 Introduction

Microbial contamination in the environment may affect more or less to humans health. Those germs microbes can infect human body through many way such as breathing, wound area, eating contaminated food or water. Moreover, being stuck on the surface of various appliances which exposed or infected by germs such as *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli* and *Pseudomonas aeruginosa* can also cause infection and illness. Therefore preventing infection by cleaning on these exposed surfaces is another way to prevent infection. To control germ on the surface of the contact area or equipment, disinfect chemical substances (such as alcohol, glutaraldehyde, chlorine compounds, Iodophor, phenol) are used. These substances can cause irritation of various mucous membranes such as membranes in the mouth, pharynx, esophagus and intestinal system. They are also residues in the soil and water. The effects and dangers of chemicals using cause interest in using natural substances to replace chemicals. It was found that many herbal extracts have the ability to inhibit microorganisms. Extracts from plants are complex organic compound. They accumulate in leaves, flowers and stems. Herbal substances are easily decomposed which make them safe for users and the environment. Lemongrass (Scientific name *Cymbopogon citratus* (DC.) Stapf) is one of the most popular Thai herbs used for cooking. This herb originated in India, Indonesia, Myanmar, Sri Lanka and Thailand [1]. It was found that

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\* Corresponding author: [Kunyanuth.ku@ssru.ac.th](mailto:Kunyanuth.ku@ssru.ac.th)

chemicals in lemongrass essential oils can inhibit *E. coli* bacteria. Those active chemicals include Citral (3,7-dimethyl-2,6-octadienal), Citronellol (acyclic monoterpene alcohol), Geraneol (monoterpene alcohol) and Cineole. Therefore, using lemongrass extracts to inhibit bacteria is a way that can reduce chemicals using. And it is also another way to use herbs as raw materials in industrial products which will encourage farmers for increasing income and causes sustainability in their profession [2].



**Fig. 1.** Chemical structure of Citral, Citronellol, Geraneol and Cineole.

Citral, or or lemonal, is either a pair, or a mixture of terpenoids with the molecular formula  $C_{10}H_{16}O$ . The two compounds are double bond isomers. The E-isomer is known as geranial or citral A. The Z-isomer is known as neral or citral B.

This research objective is to find suitable conditions for lemongrass extraction. The extract from lemongrass which has the effect on microorganisms inhibiting will be developed to use as an ingredient in many product for destroying pathogenic germ without using chemicals.

## 2 Procedure

### 2.1 Crude extraction from lemongrass

#### 2.1.1 Extraction using water as solvent

Wash the lemongrass thoroughly, dry, and cut into small pieces. Ground thoroughly. Add 20 gram of ground lemongrass to 30 ml water (ratio of lemongrass and water is 2: 3). Shake with shaking machine at 100 rpm for 3, 5 and 7 days in room temperature. Another flask of blended lemongrass with water is boiled at 100 degrees Celsius for 3 hours [3, 4]. Extracted by reflux. Filter with a thin white cloth, then filter with Whatman filter paper no.4. Collect crude extract and dry by freeze dryer. Keep the residue at 4 degrees Celsius in the refrigerator.

### 2.1.2 Extraction using ethanol and methanol as solvent

Wash the lemongrass thoroughly, let it dry and cut into small pieces. Ground thoroughly. Soak 500 grams of ground lemongrass in 1,000 ml ethanol and methanol (lemongrass and ethanol/methanol ratio is 1: 2). Shake with shaking machine at 100 rpm for 7 days in room temperature. Filter with Whatman Filter paper No. 4 to separate the plant parts and then concentrated all the solutions with an evaporator under vacuum condition. Get viscous substances and dry with freeze dryer. Keep the residue at 4 degrees Celsius in the refrigerator.

### 2.2 Determination of microbial inhibiting lowest concentration by agar dilution method

Prepare the dilution of the test bacteria expected to contain  $1.5 \times 10^8$  CFU/ml (Colony forming unit per milliliter). Add crude extract from 2.1 in bacteria dilution and mix well. Spread 0.01 ml. of bacteria dilution on nutrient agar (NA). Incubate at 35 degrees Celsius for 24 hours. Check bacterial growth on NA.

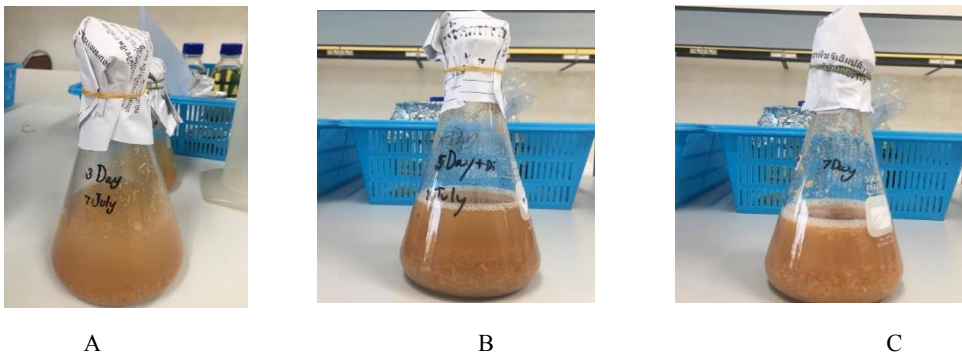
### 2.3 Statistical data analysis

The data from the bacterial inhibition test of the extract are analysed for variance (ANOVA) set by DMRT method.

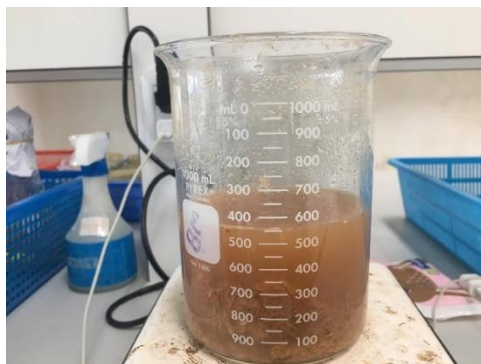
## 3 Result

### 3.1 Extraction using water as solvent

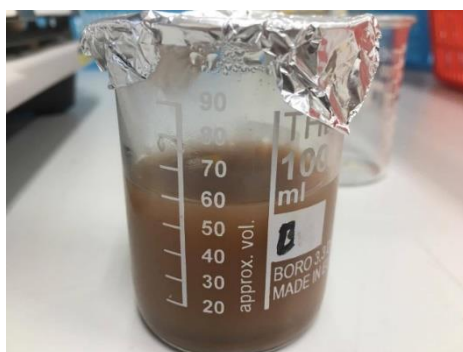
When applying the finely ground lemongrass with water to the specified ratio, 3 set left at room temperature.



**Fig. 2.** Water extraction at 3 (A), 5 (B) and 7 (C) days.



**Fig. 3.** Water extraction by using heat



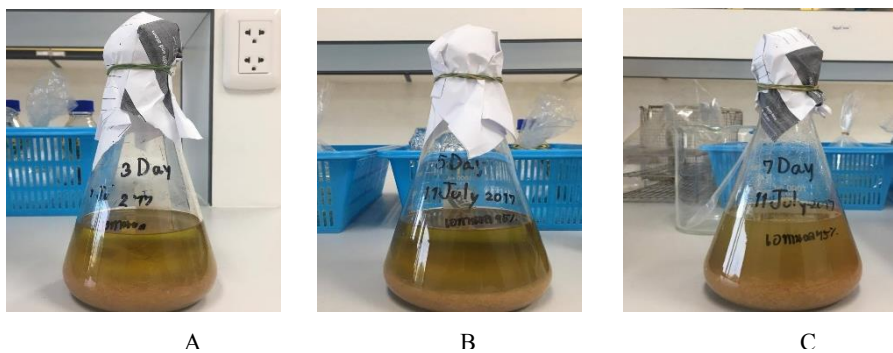
**Fig. 4.** Liquid separation filtrate.



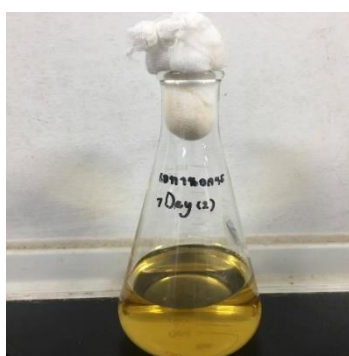
**Fig. 5.** Evaporated crude extract.

### **3.2 Extraction using ethanol and methanol as solvent**

When applying the finely ground lemongrass with ethanol and methanol to the specified ratio, left at room temperature. After that, filter with Whatman filter paper No. 4 and concentrate the filtrate with evaporator.



**Fig. 6.** Ethanol extraction at 3 (A), 5 (B) and 7 (C) days.



**Fig. 7.** Clear filtrate of 7 days' soak sample.



**Fig. 8.** Evaporate filtrate with rotary evaporator.

### 3.3 Determination of microbial inhibiting lowest concentration by agar dilution method

After add different amount of prepared crude extract to the dilution of the test bacteria, set aside 5–180 minute before spread 0.01 ml. of bacteria dilution on nutrient agar (NA) and incubate at 35 degrees Celsius for 24 hours. The result of bacterial growth is show in table 1–3.

**Table 1.** Growth of *Escherichia coli* on NA food after adding lemongrass extract.

solvent	concentration (mg/ml)	variable time before planting on NA (min)						
		5	10	15	30	60	120	180
water	200	+	+	+	+	+	+	+
	100	+	+	+	+	+	+	+
	50	+	+	+	+	+	+	+
95% ethanol	200	+	-	-	-	-	-	-
	100	+	-	-	-	-	-	-
	50	+	-	-	-	-	-	-
methanol	200	+	-	-	-	-	-	-
	100	+	-	-	-	-	-	-
	50	+	-	-	-	-	-	-

+ = GROWTH    - = NO GROWTH

**Table 2.** Growth of *Staphylococcus aureus* on NA food after adding lemongrass extract.

solvent	concentration (mg/ml)	variable time before planting on NA (min)						
		5	10	15	30	60	120	180
water	200	+	+	+	+	+	+	+
	100	+	+	+	+	+	+	+
	50	+	+	+	+	+	+	+
95% ethanol	200	-	-	-	-	-	-	-
	100	-	-	-	-	-	-	-
	50	-	-	-	-	-	-	-
methanol	200	-	-	-	-	-	-	-
	100	-	-	-	-	-	-	-
	50	-	-	-	-	-	-	-

+ = GROWTH    - = NO GROWTH

**Table 3.** Growth of *Bacillus spp* on NA food after adding lemongrass extract.

solvent	concentration (mg/ml)	variable time before planting on NA (min)						
		5	10	15	30	60	120	180
water	200	+	+	+	+	+	+	+
	100	+	+	+	+	+	+	+
	50	+	+	+	+	+	+	+
95% ethanol	200	-	-	-	-	-	-	-
	100	-	-	-	-	-	-	-
	50	-	-	-	-	-	-	-
methanol	200	-	-	-	-	-	-	-
	100	-	-	-	-	-	-	-
	50	-	-	-	-	-	-	-

+ = GROWTH      - = NO GROWTH

## 4 Conclusion

This research study the efficacy of bacterial inhibition of lemongrass extract by different solvents. The 3 testing pathogenic bacteria are *Bacillus*, *Staph. aureus* and *E. coli*. The result showed that lemongrass extract by water could not inhibit all testing bacteria but lemongrass extract by ethanol and methanol could inhibit all 3 testing bacteria. The efficacy of extract by methanol and ethanol are similar. Both of them can inhibit *Bacillus* and *Staph. aureus* from the lowest concentration (50 mg/ml). The period of time which left substance to react with bacteria is effective for *E. coli*. The extract can inhibit *E. coli* after 10 minute left. From this result, it may be due to different gram of testing bacteria. Gram negative bacteria have an outer membrane and periplasmic space, which are not found in gram-positive bacteria, and lipoprotein-based polysaccharides that form the outer membrane. This property are good barrier to permeability. Various substances are easier to penetrate into the cell membrane of gram-positive bacteria than gram-negative. Information from this research can use as guideline to use lemongrass extract as a component in many product such as cosmetic products or food products to reduce the amount of pathogenic bacteria or extend shelf life. In addition, lemongrass is an easily obtainable raw material in the local area of Thailand, so large amount of lemongrass extract usage will be the benefit to agricultural products. Further study is searching appropriate procedure for using lemongrass extract in products.

## References

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