

## Determination of the constituents, inhibition microorganisms tested and microorganisms in pork of the essential oil from *Eucalyptus citriodora* Hook leaves

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### Abstract

The objective of this study is to determine all the constituents, inhibition microorganisms tested and microorganisms in pork of the essential oil from *Eucalyptus citriodora* Hook leaves. The essential oil from *Eucalyptus citriodora* Hook leaves in Vinh Phuc, was obtained by steam distillation and dried with Na<sub>2</sub>SO<sub>4</sub>. By Gas chromatography-mass spectrometry (GC-MS) method, seventeen components in the essential oil of *Eucalyptus citriodora* Hook leaves were predicted by comparing their retention times and molecular weights with the standards. In particular, among thirteen hydrocarbons, monoterpene accounts for 1.74%, and sesquiterpene accounts for 22.30%; Among the 14 oxidized hydrocarbon components: 24.72% alcohols, 5.95% ketones, 29.84% aldehydes, and 14.26% esters. The results showed that the essential oil of *Eucalyptus citriodora* Hook leaves possessed antibacterial activity against all of the five microorganisms tested. Among them, the antibacterial activity against *Bacillus subtilis*, *Salmonella enterica*, and *Lactobacillus fermentum* was the highest. Therefore, based on the microbiological criteria and to reduce the cost during the preservation process, the content of essential oil of *Eucalyptus citriodora* Hook leaves of 0.4% per pork weight was selected as the basis for building a process to preserve pork with essential oil of *Eucalyptus citriodora* Hook leaves at 6°C. This is the scientific basis for the application of essential oil *Eucalyptus citriodora* Hook leaves in food processing and preservation, which is very necessary with high scientific and practical significance.

## 1. Introduction

Essential oils play an important role in daily life, especially in food processing and preservation. The chemical profile of the volatile oils is complicated because in most cases they contain a complex mixture of ingredients like; monoterpenes, sesquiterpenes, and diterpenes as well as their oxygenated derivatives. Furthermore, they possess a wide spectrum of biological and pharmacological activities such as; antioxidant, anticancer, antimicrobial, and insecticidal (Mosad *et al.*, 2016). When adding essential oils to foods it also gives flavoring and preserves food. Vietnam is a country with a diverse and rich source of raw materials for the production of essential oils, including *Eucalyptus citriodora* Hook leaves. The *Eucalyptus citriodora* Hook leaves are planted in areas of the Tam Dao, Binh Xuyen, Lap Thach, Tam Duong, Song Lo, and Yen Lac districts of the Vinh Phuc province. The essential oil *Eucalyptus*

*citriodora* Hook leaves possess medicinal properties. It has been used to weld increase digestion, and reduce swelling pain, and fever (Moi *et al.*, 2002). In particular, in Vietnam and China, essential oil *Eucalyptus citriodora* Hook leaves is used to treat skin diseases, dyspepsia, some digestive tract symptoms, flu, malaria, rheumatoid arthritis, and other kinds of infections. The essential oil *Eucalyptus citriodora* Hook leaves have also been used to produce medicines to treat fever, malaria, colic, and lousy breath (Moi *et al.*, 2002). The components of different varieties of this plant have shown variability (Mosad *et al.*, 2016). The features and bioactivities of some essential oil *Eucalyptus citriodora* Hook leaves have been reported (Manika *et al.*, 2012). However, the constituents and microbial inhibition properties of essential oil *Eucalyptus citriodora* Hook leaves in Vinh Phuc have not been evaluated yet. Therefore, this study was aimed at primarily analyzing

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the constituents, and inhibition of tested microorganisms and microorganisms in pork of the essential oil of *Eucalyptus citriodora* Hook leaves in Vinh Phuc, as a basis for applying this essential oil in food processing and preservation. which is very necessary with high scientific and practical significance.

## 2. Materials and methods

### 2.1 Materials

The *Eucalyptus citriodora* Hook leaves were harvested from the Tam Dao district of the Vinh Phuc province, Vietnam in 2021. The essential oil was obtained by steam distillation after drying with Na<sub>2</sub>SO<sub>4</sub>. The sample was stored in the Department of Biotechnology and Food Processing, Hanoi University of Industry.

The control tested bacterial strains (*Bacillus subtilis*, *Lactobacillus fermentum*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella enterica*) were obtained from the Institute for Quality Testing and Inspection, which is located at number 7, niche 168/21, line Nguyen Xien, Ha Dinh, Thanh Xuan, Hanoi, Vietnam

#### 2.2.1 Gas chromatography-mass spectrometry

The sample and standards were run parallelly in the GC-MS experiment. Gas chromatography (GC) analysis was performed by using Agilent Technologies HP 6890 Plus Gas chromatograph system equipped with Flame Ionization Detector (FID) and fitted with HP-5MS columns (30 m × 0.25 mm, film thickness 0.25 μm). The temperature was programmed as follows, the column temperature was programmed from 80 to 150°C in 23.5 mins at a rate of 3°C/mins and then from 150 to 220°C in 8.85 mins at a rate of 8°C/mins. The used injector temperature was 230°C. The mass spectrometry conditions were as follows: ionization voltage was 70 eV, transfer temperature was 250°C, the carrier gas was helium used at a flow rate of 0.5 mL/mins, and the split ratio of the injector was 1:5 (Teresita et al., 2000; Choi and Sawamura, 2002; Wang et al., 2014). The MS fragmentation patterns were compared with known patterns of other essential oils and those in the literature by using Wiley (Wiley 9<sup>th</sup> Version), and NIST 08Libraries (on ChemStation HP). The content of the components is calculated as a percentage of the chromatographic peak area.

#### 2.2.2 Determination of antibacterial activity using agar diffusion method

Antibacterial activity was roughly determined by the agar diffusion method. A volume of 50 μL of the

essential oil was put into wells on the plates containing tested bacterial strains (*B. subtilis*, *L. fermentum*, *E. coli*, *P. aeruginosa*, and *S. enterica*). The activity was roughly estimated by the diameter of the antibacterial round (mm), which was calculated by the formula  $D-d$ (mm), wherein D was the diameter of the antibacterial round (mm) and d was the hole diameter (mm) (Cui et al., 2017).

#### 2.2.3 Experimental setup

Based on the results of exploratory research, to determine the effect of essential oil of *Eucalyptus citriodora* Hook leaves on the preservation of pork, they carried the experiment out according to 4 formulas: CT-1: 3 kg pork; 0% essential oil per pork weight (control, do not use essential oils); CT-2: 3 kg pork; 0.2% essential oil per pork weight; CT-3: 3 kg pork; 0.4% essential oil per pork weight; CT-4: 3 kg pork; 0.6% essential oil per pork weight. These recipes are all made at 6°C. Experiment: Pork, after slaughtering and cutting into equal pieces, with dimensions: 6cm in length, 4cm in width and 2 cm in thickness), using a syringe to inject essential oil of *Eucalyptus citriodora* Hook leaves into the pieces, pork and spray this oil on the surface of the pork pieces (with the content of essential oil of *Eucalyptus citriodora* Hook leaves used for injection is 60% and the content of essential oil of *Eucalyptus citriodora* Hook leaves used for spraying is 40%), and then sealed with plastic bags polyethylene packaging and put in the refrigerator, adjust the temperature to about 6°C. Periodically to take the pork sample after 1, 2 and 3 days, analyze and evaluate the microbiological indicators of the test pork. Experiment with these formulas and find the right formula to build a preservation procedure (Nguyen, 2022).

#### 2.2.4 Microbial resistance in pork of essential oils

Coliform strains were quantified according to Vietnamese standards TCVN 6848: 2007 (Vietnamese National Standards, 2007). This method uses the technique of counting colonies on solid media after incubation at 30°C or 37°C. Petrifilm™ counting plates were used to quantify *E. coli* (Vietnamese National Standards, 2013). Standard TCVN 4991:2005 was used to determine *C. perfringens*. This standard specified a method for quantifying *C. perfringens* capable of growing on agar plates (Vietnamese National standards, 2005a). *Staphylococcus aureus* was enumerated according to Vietnamese standards TCVN 4830-1: 2005 (Vietnamese National Standards, 2005b). The method specified for the quantification of coagulase-positive staphylococci on agar plates present in products for human consumption or animal feed by counting the number of colonies obtained on solid medium (Baird-

Parker medium) after aerobic incubation at 35°C to 37°C. Vietnamese standard TCVN 4829:2005 was referred to detect *S. enterica* (Vietnamese National Standards, 2005c).

### 3. Results and discussion

#### 3.1 Constituents of the essential oil of *Eucalyptus citriodora* Hook leaves

By GC-MS methods were to determine the constituents of the essential oil of *Eucalyptus citriodora* Hook leaves. The analytical results of essential oils are shown in Table 1 and Figure 1, which are indicated in APPENDIX 1 and 4.

Table 1. The constituents of the essential oil of *Eucalyptus citriodora* Hook leaves

No.	Constituents	Retention time (min)	Proportion <sup>a</sup> (%)
Monoterpenes			1.74
1	$\alpha$ -pyronene	20.25	1.74
Sesquiterpenes			22.3
2	longifolene	18.94	20.54
3	$\alpha$ -copaene	21.06	1.76
Alcohols			24.72
4	isopulegol	13.84	2.07
5	pulegol	15.10	12.54
6	neomenthol	16.34	2.43
7	citronellol	16.68	1.85
8	trans-myrtanol	17.58	1.91
9	hydroxy citronellol	17.66	1.53
10	$\alpha$ -acorenil	19.37	2.39
Ketones			5.95
11	thujone	13.73	1.19
12	menthone	14.80	1.73
13	cis-3-pinanone	15.47	1.05
14	isomenthone	16.06	1.98
Aldehydes			29.84
15	citronellal	14.15	27.82
16	cinnamaldehyde	19.05	2.02
Esters			14.26
17	citronellyl acetate	18.60	14.26
Total			98.81

<sup>a</sup> Calculated by chromatographic peak area

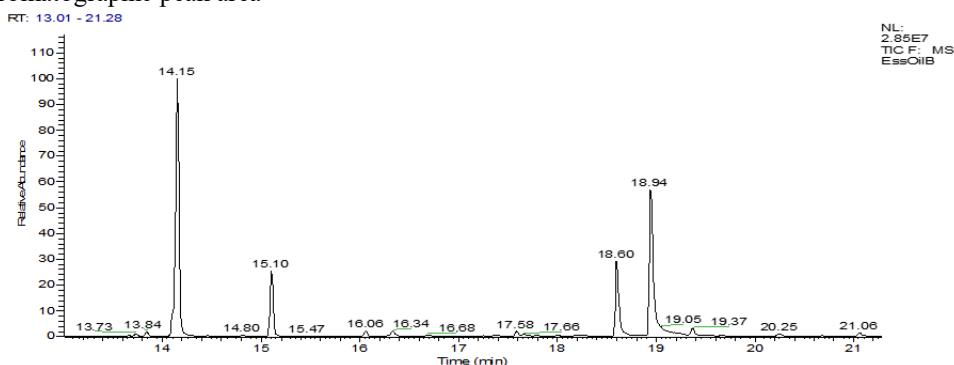


Figure 1. The content of the constituents is calculated as a percentage of the chromatographic peak area.

Based on the retention times and molecular weights of the sample and the standards, seventeen constituents and their percentages in the essential oil were evaluated and shown in Table 1. Table 1 shows that seventeen constituents were predicted in the essential oil of *Eucalyptus citriodora* Hook leaves. Three out of seventeen were hydrocarbons (such as monoterpenes 1.74% and sesquiterpenes 22.30%) and the rest were oxygenated hydrocarbons (alcohols 24.72%, ketones 5.95%, aldehydes 29.84% and esters 14.26%). The results provided additional evidence to show varied percentages of the components of the essential oils of *Eucalyptus citriodora* Hook leaves. Notably, the amounts of aldehydes in the essential oil were higher than those of the oil in Egypt (Mosad *et al.*, 2016). Compared to Mosad's research results, the essential oil of *Eucalyptus citriodora* Hook leaves in Vinh Phuc province, Vietnam, has four more constituents than the essential oil of *Eucalyptus citriodora* Hook in Egypt (Mosad *et al.*, 2016). That shows that the factors of soil, climate, and geographical location also affect the components of the essential oil of *Eucalyptus citriodora* Hook leaves. Compared with the research results of Manika, the citronellal content of essential oil of *Eucalyptus citriodora* Hook leaves in Vinh Phuc province, Vietnam, is equivalent to the citronellal content of essential oil of *Eucalyptus citriodora* Hook leaves in India (Manika *et al.*, 2012). This result is similar to the research result of Manika, citronellal is the component with the highest concentration and characteristic aroma in the essential oil of *Eucalyptus citriodora* Hook leaves.

#### 3.2 The ability of essential oil of *Eucalyptus citriodora* Hook leaves to inhibit microorganisms tested

The agar diffusion method was used to estimate the antibacterial potentials of the essential oil of *Eucalyptus citriodora* Hook leaves. The diameters of antibacterial activity rounds of the essential oil against these bacteria are shown in Table 2, which is indicated in APPENDIX 2. The results showed that the essential oil of *Eucalyptus citriodora* Hook leaves., possessed antibacterial activity against all the four microorganisms tested. Among them,

Table 2. The ability of essential oil of *Eucalyptus citriodora* Hook leaves to inhibit microorganisms tested.

No.	Microorganisms tested	Diameter of antibacterial round (mm)	Antibacterial activity (%)
1	<i>Bacillus subtilis</i>	43.17±0.12	62.67±0.23
2	<i>Lactobacillus fermentum</i>	41.08±0.17	54.41±0.32
3	<i>Escherichia coli</i>	40.31±0.24	47.64±0.45
4	<i>Pseudomonas aeruginosa</i>	39.42±0.38	41.59±0.54
5	<i>Salmonella enterica</i>	42.13±0.14	59.76±0.36

Table 3. The ability of essential oil of *Eucalyptus citriodora* Hook leaves to inhibit microorganisms in pork.

Microorganisms in pork (CFU/g)	Storage time (days)	Limit of infection	Experimental formulas			
			CT-1 (0% essential oil)	CT-2 (0.2% essential oil)	CT-3 (0.4% essential oil)	CT-4 (0.6% essential oil)
Coliform	1	50	12	not detected	not detected	not detected
	2	50	25	6	not detected	not detected
	3	50	-	20	not detected	not detected
<i>Escherichia coli</i>	1	3	10	not detected	not detected	not detected
	2	3	15	5	not detected	not detected
	3	3	-	8	not detected	not detected
<i>Clostridium perfringens</i>	1	10	6	not detected	not detected	not detected
	2	10	10	10	not detected	not detected
	3	10	-	15	not detected	not detected
<i>Staphylococcus aureus</i>	1	10	4	not detected	not detected	not detected
	2	10	8	8	not detected	not detected
	3	10	-	12	not detected	not detected
<i>Salmonella enterica</i>	1	0	not detected	not detected	not detected	not detected
	2	0	4	4	not detected	not detected
	3	0	-	6	not detected	not detected

the antibacterial activity against *B. subtilis*, *S. enterica* and *L. fermentum* was the highest. The research results on *Eucalyptus citriodora* Hook essential oil's activity are similar to the results of Mosad *et al.* (2016). These essential oils were found to have antibacterial activity against all five microorganisms tested (Mosad *et al.*, 2016). Compared with the essential oil of *Perilla frutescens* Var. *Crispa*, *Eucalyptus citriodora* Hook leaves has similar antibacterial properties (Vu *et al.*, 2021).

### 3.3 The ability of essential oil of *Eucalyptus citriodora* Hook leaves to inhibit microorganisms in pork

Microorganisms cause spoilage of pork during storage. Therefore, all preservation methods are aimed at inhibiting microbial activity to prolong the shelf life of pork. The results of determining the effect of essential oil of *Eucalyptus citriodora* Hook leaves on the change of microbial parameters of pork during storage are presented in Table 3, which is indicated in APPENDIX 3.

Based on Table 3, it shows that pork preserved in CT-1 formula, on the 1 day of storage, was contaminated with microorganisms coliform, *E. coli*, *C. perfringens*, *S. aureus* and *S. enterica*. By the end of the 2 days of storage, all the pork pieces in the CT-1 formula were completely rotten. For pork preserved in formula CT-2,

by the 2 days of storage, some microorganisms were infected and by the 3 days, it exceeded the permissible limit, typically strains *E. coli*, *S. enterica* and coliform. This shows that with the content of essential oil of *Eucalyptus citriodora* Hook leaves 0.2%, it is not enough to inhibit the growth of these microbial strains. The research results showed that pork preserved in the formulas CT-3 and CT-4 did not detect microbial strains coliform, *E. coli*, *C. perfringens*, *S. aureus* and *S. enterica*. It was concluded that the essential oil of *Eucalyptus citriodora* Hook leaves content from 0.4-0.6% per pork weight, had an effective concentration in inhibiting the growth of microorganisms that adversely affect the quality of pork fresh anise. This result is also consistent with the research results of Mosad *et al.* (2016) and Manika *et al.* (2012) on the ability to inhibit the activity of essential oil of *Eucalyptus citriodora* Hook leaves. Therefore, based on the microbiological criteria and to reduce the cost during the preservation process, the content of the essential oil of *Eucalyptus citriodora* Hook leaves of 0.4% per pork weight, was selected as the basis for building a process to preserve pork with essential oil of *Eucalyptus citriodora* Hook leaves at 6°C.

## 4. Conclusion

The results of the above study show that, by the GC-

MS method, seventeen components in the essential oil of *Eucalyptus citriodora* Hook leaves were predicted by comparing their retention times and molecular weights with the standards. In particular, there were thirteen hydrocarbons some of them such as (monoterpenes 1.74% and sesquiterpenes 22.30%) and fourteen constituents of oxygenated hydrocarbons and some of them such as and some of them such as (alcohols 24.72%, ketones 5.95%, aldehydes 29.84% and esters 14.26%). The results showed that the essential oil of *Eucalyptus citriodora* Hook leaves possessed antibacterial activity against all the five microorganisms tested. Among them, the antibacterial activity against *B. subtilis*, *S. enterica* and *L. fermentum* was the highest. Therefore, based on the microbiological criteria and to reduce the cost during the preservation process, the content of essential oil of *Eucalyptus citriodora* Hook leaves of 0.4% per pork weight was selected as the basis for building a process to preserve pork with essential oil of *Eucalyptus citriodora* Hook leaves at 6°C.

### Conflict of interest

The author declares the research results in this article to be completely honest. The data has never been used or rotated from other research projects in any form.

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