SURVEY OF CHEMICAL COMPOSITION AND ACTIVITY OF ESSENTIAL OILS OF *EUCALYPTUS GLOBULUS* AND *EUCALYPTUS CITRIODORA* COLLECTED IN DONG THAP PROVINCE

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TÓM TẮT

KHẢO SÁT THÀNH PHẦN HÓA HỌC VÀ HOẠT TÍNH CỦA TINH DẦU LÁ BẠCH ĐÀN *(EUCALYPTUS GLOBULUS)* VÀ LÁ BẠCH ĐÀN CHANH *(EUCALYPTUS CITRIODORA)* THU HÁI TẠI TỈNH ĐỒNG THÁP

Cây bạch đàn Eucalyptus globulus và cây bạch đàn chanh *Eucalyptus citriodora* rất phổ biến ở Việt Nam và nhiều quốc gia trên thế giới. Tinh dầu của 2 loài này được sử dụng rộng rãi trong đời sống hàng ngày với nhiều công dụng hữu ích bảo vệ sức khỏe như sát khuẩn, kháng viêm, bảo vệ da, trị mụn, giảm stress,… Mặc dù là 2 loài cùng chi với nhiều đặc điểm tương đồng về cảm quan và công dụng, tuy nhiên kết quả phân tích GC-MS cho thấy chúng hầu như khác biệt về thành phần đơn hương, trong đó thành phần chính của tinh dầu lá *E. globulus* là α–phellandrene (36.9%), cymol (34.2%) trong khi thành phần chính của tinh dầu lá *E. citriodora* là citronellal (67.54%), *iso*pulegol (10.09%), (*R*)–(+)–*β*–citronellol (9.9%). Cả 2 loại tinh dầu đều thể hiện hoạt tính kháng oxy hóa yếu đối với gốc tự do DPPH và $ABTS^{\bullet+}$ trong khi hoạt tính kháng khuẩn tốt trên 5 dòng vi khuẩn *Bacillus cereus, Bacillus subtilis,Staphylococcus aureus, Pseudomonas aeruginosa* và *Escherichia coli.*

Từ khóa: *Eucalyptus globulus***,** *Eucalyptus citriodora, tinh dầu, hoạt tính kháng khuẩn, hoạt tính kháng oxy hóa, DPPH,* $ABTS^{\bullet+}$

1. INTRODUCTION

The *Eucalyptus* genus encompasses over 660 species within the *Myrtaceae* family. These species are predominantly shrubs and tall trees characterized by several shared features. Leaves are typically symmetrical, elliptical, or oblong, with a green hue. Young leaves often exhibit a reddish or purplish coloration. Flowers frequently cluster at branch tips, appearing white or cream-colored. The fruit is a cup-shaped capsule containing numerous small seeds. *Eucalyptus* trees enjoy widespread popularity in Vietnam, with cultivation and natural growth occurring in most provinces and cities across the north-south regions. The genus thrives in hot, humid climates with abundant rainfall [1]. Vietnam have several popular *Eucalyptus* species, including *Eucalyptus globulus*, *Eucalyptus camaldulensis, Eucalyptus citriodora* and *Eucalyptus tereticornis*. Notably, *Eucalyptus* essential oil is a widely used product derived from these trees. According to folk uses, *Eucalyptus* essential oil has many health benefits such as: relieving colds and coughs, alleviating muscle and joint pain, deodorizing, exhibiting antibacterial properties, treating insect bite wounds, and promoting skin health [2]. The ease of harvesting Eucalyptus and its purported health

benefits have driven research efforts worldwide. Numerous studies, both domestic and international, have identified 1,8-cineole, α-terpinyl acetate, α-pinene, β-pinene, o-cymene, limonene, and terpinolene as the principal components of Eucalyptus essential oil [3-5]. Regarding antibacterial activity, previous studies showed that essential oil from *Eucalyptus* leaves has the abiltiy to resist bacterial strains *E. coli*, *S. aureus, B. subtilis* and *P. aeruginosa* [4-6]. In 2015, Harkat-Madouri and colleagues studied the essential oil of *E. globulus* leaves and found its weak antioxidant capacity but significant antibacterial activity against gram-negative bacteria [8]. Chagas et. al., (2002) discovered the insect repellent, analgesic and anti-inflammatory effects of essential oils from *Eucalyptus citriodora* [6]. Singh (2012) studied the antioxidant capacity of *E. citriodora* leaf essential oil and highlighted its potential as a source of antioxidant [7]. Regarding folk uses, essential oil of *E globulus* leaves and *E. citriodora* leaves are both known for their health benefits such as supporting the treatment of respiratory diseases, reducing muscle pain, antibacterial, skin care, hair care, treatment of insect bites,…[1-2]. Extensive research has been conducted on the essential oils derived from *Eucalyptus globulus* and *Eucalyptus citriodora* leaves. However, a comparative analysis of their chemical composition and biological activity remains absent in the existing literature. This study addresses this gap by concurrently investigating the essential oils of both *Eucalyptus* species. Our aim is to generate valuable scientific data that elucidates the relationship between the chemical composition, physicochemical properties, and biological activities of these essential oils. The findings from this research hold the potential to establish a scientific foundation for the development of pharmaceutical-grade raw materials. Additionally, this work can contribute to the informed utilization of *Eucalyptus* essential oil products in practical applications.

(a) (b)

Figure 1: *Eucalyptus globulus (a)* and *Eucalyptus citriodora (b)* leaves

2. MATERIALS AND METHODS

2.1 Materials

Eucalyptus globulus leaves (2.0 kg) and *Eucalyptus citriodora* leaves (2.0 kg) were harvested in April 2023 in Đong Thap province, identified by Dr. Hoang Viet – Department of Bilology, University of Natural Science. The leaves then were washed, removed damaged parts, then pureed with a sufficient amount of water and stored in the refrigerator at 15-20 °C to prepare for essential oil extraction.

2.2 Extracting essential oil

Essential oils from *Eucalyptus globulus* and *Eucalyptus citriodora* leaves were obtained by steam distillation using a Clevenger apparatus. Two kilograms of finely pureed leaves from each *Eucalyptus* species were co-distilled with 3 liters of distilled water in a 5 L flask for two hours. To enhance extraction efficiency, 1 gram of sodium chloride (NaCl) was added to the mixture of essential oils and steam from distillation process. After phase separation in a separatory funnel, the essential oils were collected in a dark glass bottle and stored in a refrigerator for subsequent analyses. These analyses will focus on determining the chemical composition of the oils and investigating their antioxidant and antibacterial activities.

2.3 Antibacterial activity assay

Assessment of antibacterial efficacy using agar disk diffusion method

The test samples consisted of essential oils from *Eucalyptus globulus* and *Eucalyptus citriodora* leaves, diluted in dimethyl sulfoxide (DMSO) to concentrations of 10, 20, 30, 40, and 50 (mg/mL). Bacterial strains were cultivated in Nutrient Broth (NB) medium for 24 hours at 100 revolutions per minute (rpm) on a shaker. Prepare the bacterial suspension to a turbidity of 0.5 McFarland, corresponding to an optical density (OD) value of 600 nm (108 CFU/ml). The bacterial suspension was then spread onto the surface of the agar plate and left to dry for 15 minutes. Make holes of a diameter of 4 mm, add 30 µL of test samples into each well. Incubate the agar plate in an incubator at 35-37 °C. Read results after 18-24 hours. Measure the diameter of the inhibition zone (clear zone) surrounding each well containing a test sample. This zone represents the area of bacterial growth inhibition by the test sample. The negative control used for the test was DMSO.

Assessment of antibacterial efficacy using the MIC method

The bacterial suspensions were standardized to a turbidity equivalent to a 0.5 McFarland standard. The essential oils were serially diluted with a neutral carrier solvent to prepare a concentration range of 10, 20, 30, 40, and 50 mg/mL. In a 96-well plate, combined with 100 µL of each essential oil solution prepared in Nutrient Broth (NB) with 100 µL of the corresponding bacterial suspension. Incubate the plate at 38-40 °C for 24 hours. 20 µL of a 0.1% resazurin solution were added to each well. The minimum inhibitory concentration (MIC) refers to the lowest concentration that demonstrably inhibits bacterial growth [10].

2.4 Antioxidant activity assay

DPPH free radical neutralization method

The antioxidant capacity of essential oil of *E. globulus* leaves and *E. citriodora* leaves were determined by using DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical neutralization method of Sharma et. al. (2009) with modifications [11]. The reaction mixture consisted of 40 µL DPPH (1000 µg/mL) and 960 µL essential oil with different concentrations from 2, 4, 6, 8, 10 mg/mL. The mixture was incubated in the dark at 30 ºC in 30 minutes. Then, measure the spectral absorbance of the reaction mixture at 517 nm. The standard substance used is vitamin C.

$ABTS^{\bullet+}$ free radical neutralization method

The antioxidant capacity of essential oil of *E. globulus* and *E. citriodora* leaves were

determined by using the $ABTS^{\bullet+}$ free radical neutralization method according to Nenadis et. al. (2004) with some modifications [12]. $ABTS^{\bullet+}$ solution was prepared by reacting 2 mL of a 7 mM ABTS solution with 2 mL of a 2.45 mM potassium persulfate ($K_2S_2O_8$) solution. The mixture was incubated in the dark for 16 hours and then diluted 30-fold with ethanol to reach the optical absorbance at wavelength 734 nm is 0.70±0.05. The experiment was surveyed by adding 990 µL $ABTS^{\bullet+}$ to 10 µL of test sample at different concentrations from 2, 4, 6, 8, 10 mg/mL. The reaction mixture was incubated for 6 minutes. Then, measure the spectral absorbance at 734 nm. Vitamin C was used as the standard of the survey.

3. RESULT AND DISCUSSION

3.1 Essential oil extraction efficiency

Table 2: Essential oil distillation efficiency

Samples	(1)	(2)
Volume of material	2.0 kg	2.0 kg
Time	120 minutes	
Volume of essential oil	26.28 g	24.16 g
Efficiency	1.31%	1.21%
Color	Transparent, pale yellow	
Odor	Characteristic strong aroma	

Under the same conditions, including collection area, raw material volume, distillation time and method, the essential oil yield from *Eucalyptus globulus* leaves (1) (H = 1.314%) and *Eucalyptus citriodora* leaves (2) (H = 1.21%) was found to be equivalent.

3.2. Chemical composition

The chemical composition of the obtained essential oil was determined by using gas chromatography-mass spectrometry (GC-MS) (non-targeted) at the Quality Assurance and Testing Centre 3 (Quatest 3), district 1, HCM city.

Chemical composition of *E. globulus* leaves consisted of: *α*–phellandrene (36.9%), cymol (34.2%), *γ*–terpinene (6.1%), *α*–pinene (4.9%), *α*–thujene (2.5%), limonene (2.3%), *β*–phellandrene (2.0%), eucalyptol (4.9%), *β*–eudesmol (2.1%), *β*–caryophyllene (1.8%), terpine–4–ol (1.4%), *α*–terpinene (0.5%),…

Chemical composition of *E. citriodora* leaves consisted of: citronellal (67.54%), isopulegol (10.09%), (*R*)–(+)–*β* –citronellol (9.9%), citronellyl acetate (4.05%), caryophyllene (2.92%), neoIsopulegol (0.84%), neoisopulegol hydrate (1.11%), *β*-pinene (0.64%), *α*-pinene (0.48%), *d*-limonene (0.13%), eucalyptol (0.58%), *γ*–terpinene (0.33%), *α*–humulene (0.17%), caryophyllene oxide (0.22%),…

GC-MS analysis revealed a marked disparity in the major constituents of the essential oils extracted from the leaves of these two Eucalyptus species. Despite belonging to the same genus, the chemical profiles exhibited significant differences. (main ingredient of essential oil of *E. globulus* leaves: *α*–phellandrene (36.9%), cymol (34.2%); main ingredient of essential oil of *E. citriodora* leaves: citronellal (67.54%), isopulegol (10.09%), (*R*)–(+)–*β* –citronellol (9.9%)

3.3 Antibacterial activity by agar disk diffusion method

The antibacterial activity of essential oils from *E. globulus* leaves and *E. citriodora* leaves were evatulated against 5 bacterial strains using the agar disc diffusion method. The tested strains included three gram positive bacteria: *Bacillus cereus, Bacillus subtilis* and *Staphylococcus aureus* and two gram-negative bacteria: *Pseudomonas aeruginosa* and *Escherichia coli*. The essential oils were tested at five concentrations 10, 20, 30, 40 và 50 mg/mL. Gentamicin (1 mg/mL) was used

as the positive control while DMSO served as the negative control. The antibacterial activity was determined by measuring the diameter of the inhibition zone. A larger inhibition zone indicates stronger antibacterial activity. The results revealed that both essential oils exhibited antibacterial activity against 5 strains *B. Cereus,* B. subtilis, P. Aeruginosa, S. aureus and *E. Coli*. However, except for *B. cereus*, this abitily is negligible on the remaining 4 strains at concentration of 10 mg/mL.

mg/mL	Gram-positive						Gram-negative			
	B. cereus		*B. subtilis*		*S. Aureus*		*P. aeruginosa*		*E. coli*	
	(1)	**(2)**	**(1)**	**(2)**	**(1)**	**(2)**	**(1)**	**(2)**	**(1)**	**(2)**
10	4.0±0.4	2.7±0.5	-	-	-	-	-	-	-	-
20	4.5±0.2	3.1±0.3	2.5±0.4	2.5±0.4	2.3±0.4	2.4±0.4	1.0±0.4	2.5±0.4	1.0±0.4	-
30	5.5±0.5	3.5±0.4	3.5±0.4	3.3±0.4	2.8±0.4	2.4±0.4	1.0±0.5	3.2±0.3	3.0±0.4	3.0±0.4
40	6.0±0.5	4.0±0.3	4.5±0.2	3.5±0.5	3.0±0.2	3.3±0.5	2.0±0.4	4.1±0.4	3.5±0.4	4.0±0.3
50	7.0±0.6	5.0±0.4	6.5±0.6	4.2±0.4	3.2±0.6	3.7±0.4	2.0±0.3	5.5±0.5	7.5±0.4	6.8±0.6
Gentamicin	19.5±0.3	21±0.2	27±0.4	32±0.2	20±0.4	19±0.2	20±0.2	19.5±0.3	28±0.5	28±05

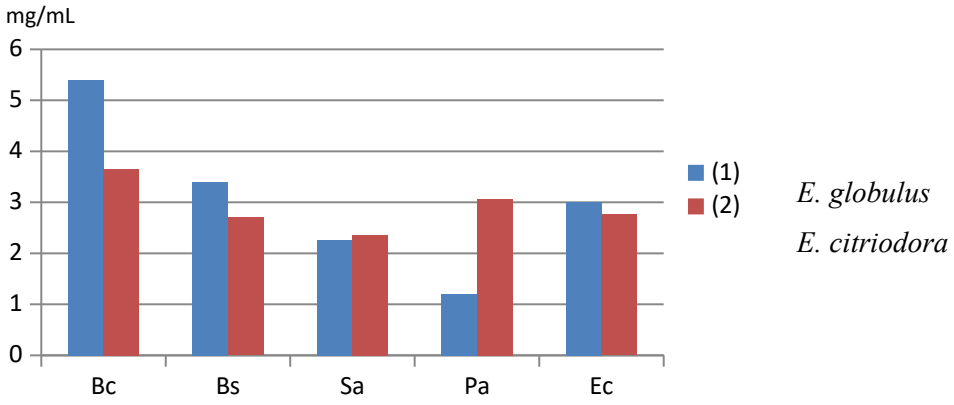

Figure 1: The average antibacterial ability of essential oil of *E. globulus* leaves **(1)** and *E. citriodora* leaves **(2)**

Figure 1 showed that the average antibacterial ability of essential oil of *E. globulus* leaves (1) is stronger than the one of *E. citriodora* leaves on three bacterial strains *B. cereus, B. subtilis* và *E. coli.* This ability is equivalent in *S. aureus* strain.

However, for the *P. aeruginosa* strain, the average antibacterial ability of *E. citriodora* leaf essential oil is significantly stronger. This can be explained by the fact that the outer membrance of *P. aeruginosa* is made up of a water-permeable lipopolysaccharide layer, which acts as a protective layer against harmful agents [8].

3.4 Antibacterial activity using MIC method

The minimum inhibitory concentration (MIC) assay is a highly accurate method for evaluating antibacterial activity. This technique utilizes a standardized environment within a 96-well plate and employs a 0.1% solution of resazurin dye, which is a blue-violet indicator. The presence of viable bacteria leads to the reduction of resazurin, resulting in a color change from purple-blue to pink.

Conversely, wells with no bacterial growth retain the original purple-blue color, signifying the absence of bacterial activity. The amount of resorufin produced corresponds to the number of bacteria present in the well. The assay employed various concentrations (10, 20, 30, 40, and 50 mg/mL) against five bacterial strains: *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli.* The results demonstrated that both essential oils exhibited a minimum inhibitory concentration (MIC) of 10 mg/mL against all five tested bacteria.

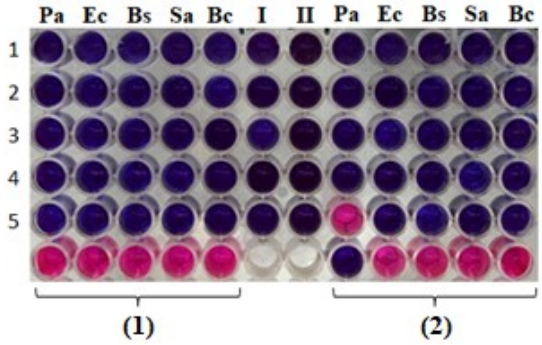

Figure 2: MIC test results of essential oil of *E. globulus* leaves **(1)** and *E. citriodora* leaves **(2)**

3.5 Ability to neutralize DPPH

DPPH is a widely uesd method to test free radical scavenging ability. The unpaired electrons present in the DPPH free radical show the strongest absorption at wavelenght 517 nm. DPPH is purple, when these single electrons pair with electrons of antioxidants, the compound will change from purple to yellow respectively. Therefore, the higher the free radical scavenging ability of a substance, the lower the spectral absorption measured at 517 nm of the DPPH reaction and vice versa.

The ability of essential oil of *E. globulus* leaves **(1)** and *E. citriodora* leaves **(2)** to neutralize DPPH free radicals was evaluated through the optical absorbance of DPPH solution. Survey results showed that free radical neutralization efficiency is proportional to essential oil concentration. From the linear equation, the DHHP free radical removal efficiency of *E. globulus* leaves **(1)**, *E. citriodora* leaves **(2)** and vitamin C are respectively determined through the IC_{50} value presented in the table 5 and 6. The results showed that the IC50 value of (1) (IC_{50} = 2891 µg/ml) is almost equivalent to (2) (IC_{50} = 2562 µg/ml). However, the IC_{50} value of (1) and (2) are 1017 and 903 times lower than those of vitamin C, respectively (table 6).

Table 6: IC_{50} values of (1), (2) and vitamin C

Samples	(1)	(2)	Vitamin C
IC_{50} (µg/ml)	2891	2562	2.84

Table 5: Free radical DPPH neutralization efficiency

mg/ml	IC (%)		µg/ml	IC (%)
	(1)	(2)		Vitamin C
1	29.174	30.944	**1**	16.883
2	37.61	44.406	**2**	31.818
3	49.034	56.556	**3**	54.546
4	66.96	68.269	**4**	69.968
5	73.638	73.863	**5**	92.208
y=ax+b	y=11.83x+15.8	y=10.97x+21.897	y=ax+b	y=18.88x+3,55

3.6 Ability to neutralize ABTS$^{\bullet+}$

The results showed that the free radical ABTS$^{\bullet+}$ neutralization efficiency is proportional to the concentration of the investigated essential oil. The ABTS$^{\bullet+}$ removal efficiency of essential oil of *E. globulus* leaves **(1)**, *E. citriodora* leaves **(2)** and vitamin C were determined through the IC_{50} value presented in tables 7 and 8, respectively. The results showed that the IC_{50} value of essential oil of (1) (IC_{50} = 2882 µg/ml) is 1.36 times stronger than (2) (IC_{50} = 3920 µg/ml). However, the IC_{50} values of (1) and (2) are much lower than the one of vitamin C (1338 and 1820 times, respectively).

Table 7: Free radical ABTS$^{\bullet+}$ neutralization efficiency

mg/ml	IC (%)		µg/ml	IC (%)
	(1)	(2)		Vitamin C
1	29.317	19.757	1	26.404
2	36.622	29.494	2	39.607
3	52.846	40.917	3	53.558
4	65.085	50.187	4	64.513
5	72.96	61.61	5	76.592
y=ax+b	y=11.575x+16.641	y=10.44x+9.073	y=ax+b	y=12.505x+23.074

Table 8: IC_{50} values of (1), (2) và vitamin C

Samples	(1)	(2)	Vitamin C
IC_{50} (µg/ml)	2882	3920	2.15

4. CONCLUSION

Under the same experimental conditions, the distillation efficiency of essential oil of *E. globulus* leaves (1) (H=1.314%) is equivalent to the one of *E. citriodora* leaves (2) (H=1.208%).

The GC-MS results showed that although they come from the same starting material, the leaves of two species of the same genus, both essential oils are completely different in their single-aromatic composition. The main component of *E. globulus* leaf essential oil: *α*–phellandrene (36.9%), cymol (34.2%), *γ*–terpinene (6.1%); while the main component of *E. citriodora* leaf essential oil: citronellal (67.54%), isopulegol (10.09%), (*R*)–(+)–*β* – citronellol (9.9%), citronellyl acetate (4.05%).

The results of the antibacterial activity survey using agar disc diffusion method showed that both essential oil had good inhibitory ability on 5 strains of *P. aeruginosa, S. aureus, B. cereus, B.*

subtilis and E. coli. This result is similar to the MIC test with the minimum inhibition value of both essential oils being 10 mg/mL.

Regarding to antioxidant capacity, both essential oils demonstrated the ability to neutralize DPPH free radicals ((1) (IC_{50} = 2891 μg/ml), (2) (IC_{50} = 2562 μg/mL) and $ABTS^{\bullet+}$ ((1) IC_{50} = 2882 μg/mL; (2) IC_{50} = 3920 μg/ml). However, this ability is much weaker than vitamin C. These results are similar to those of Luís's research [9].

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