SURVEY OF CHEMICAL COMPOSITION AND ACTIVITY OF ESSENTIAL OIL OF LEAVES AND FRUITS OF *EUCALYPTUS GLOBULUS* COLLECTED IN DONG THAP PROVINCE

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TÓM TẮT

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

Cây bạch đàn rất phổ biến ở Việt Nam và nhiều quốc gia trên thế giới. Tinh dầu bạch đàn được sử dụng rộng rãi trong đời sống hàng ngày với nhiều công dụng hữu ích như điều trị cảm lạnh, ho, kháng khuẩn, trị vết thương do côn trùng cắn, bảo vệ da,... Trong bài báo này, chúng tôi nghiên cứu về thành phần hóa học, hoạt tính kháng khuẩn, kháng oxy hóa của tinh dầu lá và quả bạch đàn thu hái tại Đồng Tháp bằng các phương pháp khác nhau. Kết quả cho thấy 2 loại tinh dầu có cùng thành phần một số hợp chất chính như α-phellandrene, p-cymene, γ-terpinene, α-pinene. Tuy nhiên, eucalyptol, một hợp chất có hoạt tính sinh học mạnh chiếm 4.9% trong tinh dầu lá bạch đàn, lại không hiện diện trong tinh dầu quả. Cả 2 loại tinh dầu đều thể hiện khả năng ức chế 4 dòng vi khuẩn B. cereus, B. subtilis, P. aeruginosa and E. coli và hoạt tính kháng oxy hóa yếu trên gốc tự do DPPH và $ABTS^{\bullet+}$.

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

1. INTRODUCTION

The *Eucalyptus* genus is a large genus, consisting of more than 660 species of shrubs and tall trees belonging to the family Myrtaceae with some common characteristics such as leaves that are often symmetrical, elliptical or oblong, green in color, young leaves are red or purple; flowers often grow in clusters at the ends of branches, white or cream in color; fruits is a cup-shaped capsule containing many small seeds. *Eucalyptus* are very popular, planted and growing wild in most provinces and cities from North to South in Viet Nam. This tree grows best in areas with hot and humid climates and abundant rainfall [1]. In Vietnam, there are some popular *Eucalyptus* species such as *Eucalyptus globulus, Eucalyptus camaldulensis, Eucalyptus citriodora* and *Eucalyptus tereticornis.* One of the popular uses of *Eucalyptus* is its essential oil. According to folk uses, *Eucalyptus* essential oil is known for its good health uses such as relieving colds and coughs, reducing muscle and joint pain, deodorizing, antibacterial, treating wounds caused by insect bites, care and protect the skin [2]. With its easy-to-harvest characteristics

and many health benefits, *Eucalyptus* essential oil is of interest in research in many places around the world. Many studies have determined that the main ingredients in *Eucalyptus* essential oil include 1,8-cineol, *α*-terpinyl acetate, *α*-pinene, *β*-pinene, *o*-cymene, limonene, terpinolene [3,5,7-9]. Regarding antibacterial activity, essential oil from *Eucalyptus* leaves has been studied to show its antibacterial ability against *E. coli*, *S. aureus*, *B. subtilis* and *P. aeruginosa* species [3]. In 2015, Madani 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]. In 2016, when researching essential oil of *E. globulus* fruits, Bey-Ould Si Said and his colleagues studied its antibacterial ability against *S. aureus, B. subtilis, L. innocua, E. coli, P. aerugenosa* and weak resistance to DPPH (1,1-diphenyl-2-picrylhydrazyl) free radicals [10]. According to our research, there are many studies on essential oil from *E globulus* leaves, however there is very little about essential oil from the fruit. On the other hand, there have been no study comparing the chemical composition and activity of essential oils from these two parts. In this work, we simultaneously researched essential oil from *E. globulus* leaves and fruits to provide scientific information to help evaluate the relationship between chemical composition and physicochemical characteristics as well as activities. properties of these two essential oils. The results obtained from this study can provide a scientific basis for developing raw materials for the pharmaceutical industry, contributing to the orientation of using products from *E. globulus* essential oil in real life.

Figure 1: Leaves and fruits of Eucalyptus globulus

2. MATERIALS AND METHODS

2.1 Materials

Leaves (1.8 kg) and fruits (1.2 kg) of *E. globulus* were harvested in March 2023 in Dong Thap province, identified by Dr. Hoang Viet - Department of Biology, University of Natural Sciences, Ho Chi Minh city. After

harvesting, *E. globulus* leaves and fruits are washed, damaged parts are removed, then pureed with a sufficient amount of water and stored in the refrigerator to prepare for essential oil extraction.

2.2 Extracting essential oil

Essential oils from leaves and fruits of *E. globulus* were extracted by steam distillation using the Clevenger distillation system. Leaves (1.8 kg) and fruits (1.2 kg) of *E. globulus* after pureeing with 3 liters of distilled water were put into a 5L distillation flask, boil for 2 hours. NaCl (1.0 g) was then added to the mixture of essential oil and steam

after condensation, stir well to increase extraction efficiency. Unlock the separatory funnel. A dark glass bottle was used to collect the essential oils and stored in the refrigerator to prepare for determining the chemical composition and investigating its antioxidant and antibacterial activities.

2.3 Investigation of antibacterial activity using agar disk diffusion method

The test sample are essential oils of *E. globulus* leaves and fruits diluted in ethyl acetate to concentrations of 110, 120, 130, 140, 150 (mg/ml). Bacterial strains were cultured in Nutrient Broth (NB) medium for 24 hours on a shaker (100 rpm). The bacterial suspension was prepared to a turbidity of 0.5 McFarland, corresponding to an 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℃. Read results after 18-24 hours. The antibacterial ability of the test samples are determined by the inhibition ring around the well containing the test sample. The negative control used for the test was ethyl acetate.

Investigation of antibacterial activity using the MIC method

The suspension of bacterial strains were adjusted to McFarland 0.5. Essential oils were diluted with NB to create a series of essential oil solutions with concentrations of 10, 20, 30, 40, 50mg/ml. Mix 100 µl of each essential oil solution in NB with 100 µl of each bacterial suspension in a 96-well plate, incubate at 38–40°C for 24 hours. Add 20 µl of 0.1% resazurin solution to each well in the plate. The MIC of essential oils is defined as the lowest concentration at which no bacteria grow [13].

2.4 Investigation of antioxidant activity

DPPH free radical neutralization method

The antioxidant capacity of essential oil of *E. globulus* leaves and fruits of were determined by using the DPPH free radical neutralization method of Sharma et al (2009) with modifications [14]. The reaction mixture consisted of 40 µL DPPH (1000 µg/mL) and 960 µL essential oil with different concentrations from 30-150 µg/mL. The mixture was incubated in the dark at 30ºC in 30 minutes. Then, measure the spectral absorbance of DPPH 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* leaves and fruits of were determined by using the $ABTS^{\bullet+}$ (2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)) free radical neutralization method according to Nenadis et al. (2004) with modifications [15]. $ABTS^{\bullet+}$ solution was created from the reaction between 2 mL of 7 mM ABTS solution and 2 mL of 2.45 mM $K_2S_2O_8$ solution, incubated in the dark for 16 hours, then diluted with ethanol (30 times) to reach the optical absorbance at wavelength 734 nm is 0.70±0.05. Conduct the survey by adding 990 µL $ABTS^{\bullet+}$ to 10 µL of test sample at different concentrations from 20-100 µg/mL. The reaction mixture was incubated for 6 minutes. Then, measure the spectral absorbance at 734 nm. The standard substance used is vitamin C.

3. RESULTS - DISCUSSION

3.1 Essential oil extraction efficiency

Under the same conditions of collection area, volume of raw materials, time and distillation method, the results showed that the distillation efficiency of essential

oil of *E. globulus* leaves (1) (H=1.314%) is 1.4 times higher than that of fruits (2) (H=0.937%).

Table 1. Essential oil distillation efficiency

Samples	(1)	(2)
Volume of material	1.8 kg	1.4 kg
Time	120 minutes	
Volume of essential oil	23.65 g	13.12 g
Efficiency	1.314%	0.937%
Color	Transparent, pale yellow	
Odor	Characteristic strong aroma	

3.2. Chemical composition

The essential oil obtained after distillation has its chemical composition determined using the GC-MS method (non-targeted) at the Quality Assurance And Testing Center 3 (Quatest 3), district 1, Ho Chi Minh City.

Gas chromatography-mass spectrometry (GC-MS) analysis revealed a high degree of similarity in the chemical composition of the essential oils extracted from Eucalyptus globulus leaves (1) and fruits (2). The main components were present in comparable ratios, with α-phellandrene (36.9% and 39.3%), p-cymene (34.2% and 31.3%), γ-terpinene (6.1% and 3.0%), and α-pinene (4.9% and 3.9%) constituting significant portions of both oils. Notably, eucalyptol, a prominent component in eucalyptus essential oil, was entirely absent in the fruit oil, while present at 4.9% in the leaf oil. Terpinen-4-ol, a compound with documented antibacterial and antifungal properties, was identified in both samples at relatively low concentrations (1.0% and 2.3%). These compositional similarities may explain the applications and sensory characteristics, such as color, aroma, viscosity, and potential biological activity, observed in both leaf and fruit essential oils.

Table 2. Chemical composition of essential oil of E. globulus leaves (1) and fruits (2)

No	**Ingredient**	**Content (%)**		**No**	**Ingredient**	**Content (%)**	
		(1)	**(2)**			**(1)**	**(2)**
	Monoterpene	**89.4**	**83.7**		**Oxygenated monoterpene**	**5.9**	**2.3**
1	*α*–Thujene	2.5	2.4	9	Eucalyptol	4.9	-
2	*α*–Pinene	4.9	3.9	10	Terpinen–4–ol	1.0	2.3
3	*α*–Phellandrene	36.9	39.3	11	Sesquiterpene	1.8	0.0
4	*α*–Terpinene	0.5	-	12	*β*–Caryophyllene	1.8	-
5	Limonene	2.3	1.4	13	Oxygenated Sesquiterpene	2.1	4.8
6	*β*–Phellandrene	2.0	2.4	14	*β*–Eudesmol	2.1	4.8
7	*p*-Cymene	34.2	31.3	15	*α*–Eudesmol	-	2.6
8	*γ*–Terpinene	6.1	3.0				

Table 3. Content of main compounds in essential oil of E. globulus leaves (1) and fruits (2)

Compound	Content (%)				
	(1)			(2)	
	(1)	[6]	[7]	(2)	[9]
α-Pinene	4.9	7.16	4.2	3.9	3.8
Eucalyptol	4.9	85.82	45.4	0	19.8
p-Cymene	34.2	0	9.5	31.3	0.5
α–Phellandrene	36.9	0.55	1.3.0	39.3	1.9.0
Aromadendrene	0	0	0	0	19.7

A comparative analysis of the essential oil composition from *E. globulus* leaves (1) and fruits (2) in Dong Thap province revealed significant differences in content compared to samples from other countries. The eucalyptol content in (1) was remarkably low, representing only 1/17th and 1/9th of the levels found in Montenegro [6] and India [7], respectively. Notably, the eucalyptol content in the fruit samples (2) was entirely undetectable. In contrast, the content of *p*-cymene and *α*-phellandrene in samples (1) and (2) was significantly higher (31-40%), while these constituents were found to be negligible in samples from other countries. This disparity can likely be attributed to variations in climatic and soil conditions, which can significantly influence the chemical composition, physicochemical properties (including color, scent, and viscosity) as well as the biological activity of the essential oil.

3.3 Antibacterial activity by Agar Disk Diffusion Method

The antibacterial activity of essential oils from *Eucalyptus globulus* leaves (1) and fruits (2) was evaluated against five 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: 110 mg/mL, 120 mg/mL, 130 mg/mL, 140 mg/mL, and 150 mg/mL. Gentamicin (1 mg/mL) was used as the positive control, while ethyl acetate 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 *B. cereus, B. subtilis*, *P. aeruginosa* and *E. coli*. However, no activity was observed against *S. aureus* bacteria.

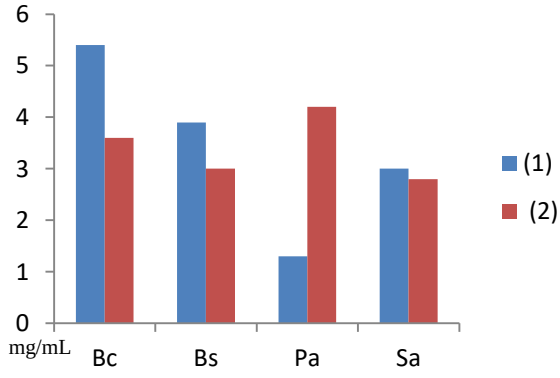

Figure 1. The average antibacterial ability of essential oil of E. globulus leaves (1) and fruits (2)

Table 4. Antibacterial Activity of E. globulus Essential Oil from leaves (1) and fruits (2)

mg/mL	Gram-positive				Gram-negative			
	B. cereus		*B. subtilis*		*P. aeruginosa*		*E. coli*	
	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
110	4.0±0.4	2.5±0.5	2.0±0.4	2.0±0.3	0.5±0.3	3.0±0.2	-	-
120	4.5±0.2	3.0±0.3	2.5±0.4	2.5±0.4	1.0±0.4	3.0±0.4	1.0±0.4	-
130	5.5±0.5	3.5±0.4	3.5±0.4	3.0±0.4	1.0±0.5	3.0±0.3	3.0±0.4	3.0±0.4
140	6.0±0.5	4.5±0.3	4.5±0.2	3.5±0.5	2.0±0.4	4.5±0.4	3.5±0.4	4.0±0.3
150	7.0±0.6	5.5±0.4	6.5±0.6	4.0±0.4	2.0±0.3	7.5±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±0.2	19.5±0.3	28±0.5	28±05

Table 5. Free radical DPPH neutralization efficiency

mg/ml	IC (%)		µg/ml	IC (%)
	(1)	(2)		Vitamin C
1	29.174	24.725	1	16.883
2	37.61	34.615	2	31.818
3	49.034	40.659	3	54.546
4	66.96	53.846	4	69.968
5	73.638	64.286	5	92.208
y=ax +b	y=11.83x +15.8	y=11.54x +20.9	y=ax +b	y=18.88x +3.55

Table 6. IC_{50} values of (1), (2) và vitamin C

Samples	(1)	(2)	Vitamin C
IC_{50} (µg/ml)	2891	3648	2.84

Chart 1 showed that the average antibacterial ability of essential oil from leaves of *E. globulus* is stronger than the one from the fruits on three bacterial strains *B. cereus*, *B. subtilis* and *E. coli*. This can be explained by the fact that although essential oil of *E. globulus* leaves and fruits have the same composition of main compounds, however, the content of eucalyptol, a compound with strong antibacterial properties, accounts for 4.9% in *E. globulus* leaf essential oil while not present in the one from fruits [10]. In the case of *P. aeruginosa*, essential oil from the fruits exhibits superior average antibacterial value compared to the one from the leaves. This is due to the outer membrane structure of *P. aeruginosa* is composed of a water-permeable lipopolysaccharide layer, which acts as a protective layer against harmful agents [9].

3.4 Antibacterial activity using MIC method

MIC (Minimal Inhibition Concentration) is a method to investigate antibacterial ability with high accuracy because it is performed in a synchronized environment. The experiment was performed in a 96-well plate using blue-violet resazurin dye (0.1%). Purple-blue wells indicate the absence of bacteria. Conversely, pink wells signify the presence of bacteria, as they have reduced the resazurin dye to resorufin. The amount of resorufin produced corresponds to the number of bacteria present in the well. The test utilized concentrations of 10, 20, 30, 40 and 50 mg/mL on 5 bacterial strains *B. subtilis*, *B. cereus*, *S. Aureus*, *P. Aeruginosa* and *E. Coli*. The results indicated that for *B. subtilis, B. cereus, P. aeruginosa* and *E. coli*, the essential oil achieved the MIC at a concentration of 10 mg/mL. For *S. aureus*, the essential oil derived from leaves demonstrated an MIC value of 10 mg/mL whereas the fruit-derived essential oil did not exhibit any inhibitory effect.

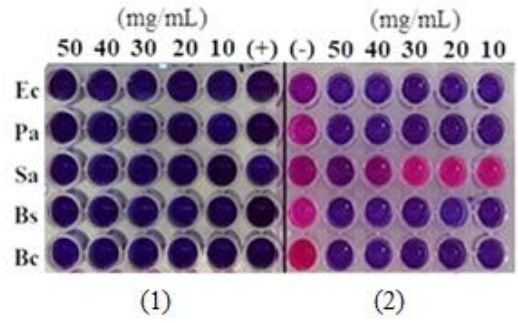

Figure 2. MIC test results of essential oil of E. globulus leaves (1) and fruits (2)

3.5 Ability to neutralize DPPH free radicals

DPPH is a widely used method to test free radical scavenging ability. The unpaired electrons present in the DPPH free radical show the strongest absorption at wavelength 517 nm. DPPH has a purple color, 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 eucalyptus leaf and fruit essential oils to neutralize DPPH free radicals was evaluated through the optical absorbance of DPPH solution. Survey results show that free radical neutralization efficiency is proportional to essential oil concentration. From the linear equation, the DPPH free radical removal efficiency of eucalyptus leaf essential oil (1), eucalyptus fruit (2) and Vitamin C are respectively determined through the IC_{50} value presented in the table. 5 and 6.

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

mg/ml	IC (%)		µg/ml	IC (%)
	(1)	(2)		Vitamin C
1	29.317	14.094	1	26.404
2	36.622	26.211	2	39.607
3	52.846	40.456	3	53.558
4	65.085	54.131	4	64.513
5	72.96	60.114	5	76.592
y=ax+b	y=0.174x+14.797	y=0.1595x+3.38	y=ax+b	y=7.3005x+22.0

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

Samples	(1)	(2)	Vitamin C
IC50 (µg/ml)	2923	3917	2.83

The results show that the IC_{50} value of eucalyptus leaf essential oil (1) (IC_{50} = 2891 µg/ml) is 1.26 times stronger than eucalyptus fruit essential oil (2) (IC_{50} = 3648 µg/ ml). However, the IC_{50} values of (1) and (2) are 1300 and 1017 times lower than those of vitamin C, respectively (table 6).

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

The results showed that the free radical $ABTS^{\bullet+}$ neutralization efficiency is proportional to the concentration of essential oil. The $ABTS^{\bullet+}$ removal efficiency of essential oil of *E. globulus* leaves (1), fruits (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 *E. globulus* leaves (1) (IC_{50} = 2923 µg/ml) is 1.34 times stronger than the one of fruits (2) (IC_{50} = 3917 µg/ml). However, the IC_{50} values of (1) and (2) are much lower than those of vitamin C (1032 and 1384 times, respectively).

4. CONCLUSION

Under the same experimental conditions, the distillation efficiency of *E. globulus*'s essential oil from leaves (1.314%) is 1.4 times higher than the one from fruit (H=0.937%).

GC-MS results showed that both essential oils are equivalent in terms of single-aromatic composition, but the main compounds such as *α*-phellandrene, *p*-cymene, *γ*-terpinene, *α*-pinene, eucalyptol in *E. globulus* leaves essential oil accounts for more content in the essential oil from fruit. This explained the similarity in color, scent as well as some activities such as antiseptic, skin protection and care, insect repellent,...

The results of the antibacterial activity survey showed that *E. globulus* leaves essential oil has better inhibitory ability than the one from fruit on bacterial strains *B. cereus*, *B. subtilis* and *E. coli.* However, for *P. aeruginosa* bacteria, *E. globulus* essential oil of fruits showed superior inhibitory effect compared to the one from leaves.

Regarding antioxidant capacity, both essential oils showed the ability to

neutralize DPPH free radicals ((1) (IC_{50} = 2891 μg/ml), (2) (IC_{50} = 3648 μg/ml) and $ABTS^{\bullet+}$ ((1) IC_{50} = 2923 μg/ml; (2) IC_{50} = 3917 μg/ml). However, this ability is much weaker than that of vitamin C. This result is similar to those of O. S. Said's research [12].

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