Antibacterial and antioxidant activities of essential oil from *plectranthus amboinicus* collected in Vietnam

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Abstract
From leaves of Plectranthus *amboinicus* (Lour.) Spreng collected in Buon Ma Thuot city, Dak Lak province were used to extract essential oil. The antibacterial activity of the essential oil was tested on *E. coli bacteria*, showing a strong antibacterial ability of 98.26%. The antioxidant capacity of basil essential oil was determined by the DPPH method. The highest antioxidant efficiency of basil essential oil was 92.15%, IC_{50} = 2.34 µL/mL Preliminary research results show that the essential oil from this plant has potential as a source of natural antibacterial and antioxidant.

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Keywords: Plectranthus amboinicus (Lour.) Spreng, antibacterial, E.coli, essential oils, antioxidants.

1. Introduction

Plectranthus *amboinicus (Lour*. Spreng), a member of *the* mint family Lamiaceae, has more than 200 genera and 3500 species distributed in tropical regions. They are widely cultivated in Vietnam, where they have more than 40 genera and about 145 species [1-2].

This plant is also known as "rau tan day la", "rau thom lun". In food processing, Lemon Basil leaves are used as a spice with a spicy, aromatic, non-toxic taste. In folk medicine, they are used to treat coughs, fevers, diarrhea, or to apply on insect bites [3]. In addition, Lemon Basil is one of the plants that contain essential oils [4]. Several articles related to Lemon Basil have been discovered, including Lemon Basil essential oil in Cu Chi by Lu Thi Mong My, physical composition and antibacterial activity of Lemon Basil in Can Tho by Nguyen Thi My Huyen [5, 6]. They are also of interest to international scientists [7-13]. Long-standing medicinal recipes made from Lemon Basil have been partially explained in research articles. However, preparations and health care products from Lemon Basil are still few and have received little attention for use.

According to our research, up to now, there has been no research project related to Lemon Basil conducted in Dak Lak. From there, we research to expand knowledge about Lemon Basil. We conducted research to determine the biological activity and composition of Lemon Basil to support effective exploitation and use.

2. Materials and methods

Plant materials

Leaves of *Plectranthus amboinicus* collected in Hoa Thang commune, Buon Ma Thuot city, Dak Lak province, Vietnam in dry season in March of 2023 year. The specimen was identified by Dr. Nguyen Quoc Binh (Vietnam Museum of Nature, Vietnam Academy of Science and Technology). The specimen certificate, HC-BMT-03, is kept at the Faculty of Natural Sciences and Technology, Tay Nguyen University, Buon Ma Thuot city, Dak Lak province, Vietnam.

Essential oil extraction

Fresh leaves of *Plectranthus amboinicus* was cleaned, chopped and steam distilled in a Clevenger-type apparatus for 3 h. The

obtained essential oil was dried over anhydrous sodium sulfate and stored in a sealed bottle at 10°C in the dark prior to analysis.
Collected parts: leaves.

Method
Distillation method
After collection, the samples were pre-selected, washed and dried for 3 hours, cut into small pieces. Place 150 g of chopped basil leaves with 500 ml of water in a Clevenger distillation flask. Distill for 3 hours. The mixture is heated by an electric stove, when the mixture boils, the steam produced will draw the essential oil up and into the condensing system. After condensing to obtain an insoluble mixture of water and essential oil, the essential oil is extracted from the mixture with diethyl ether, the extracted solution is dried with anhydrous Na_2SO_4 salt and collected. get the finished essential oil. The yield of essential oil is 0.18–0.3%, light yellow in color and light in aroma.

Method of testing biological activity
Antioxidant activity test method
The experiment uses the DPPH method. This method is to determine the antioxidant capacity of the mixture. DPPH (1,1-diphenyl-2-picrylhydrazyl) can generate free radicals inside methanol and reaches a maximum absorption at a wavelength of 517 mm. By adding the sample to this mixture, if the chemical is able to neutralize or surround the free radicals, DPPH will change from purple to yellow. This signal is measured by an Elisa reader. The antioxidant activity of the test substance is measured by the percentage of light absorption reduction in the samples compared to the control.
IC_{50} the value, which reports the antioxidant activity, is the concentration of extract that reduces DPPH free radicals by 50% under appropriate conditions. The lower the IC_{50} value, the higher the DPPH free radical scavenging activity.
The DPPH inhibition rate was calculated according to the following formula:

$$\%IC = \frac{OD_C - OD_S}{OD_C - OD_b} x100\%$$

In which: OD control: absorbance of control sample (when there is no sample)
Sample OD: absorbance of the sample
OD blank: absorbance of blank sample (methanol)
Based on the linear relationship between their concentrations and percentage of free radical scavenging activity, the IC_{50} values of the samples and controls were determined as follows: $IC_{50} = (50-b)/a$.
In which: IC_{50}: 50% inhibitory concentration
A,b: respectively the slope and opacity of the linear equations between their concentrations and percentage of free radical scavenging activity

Antibacterial activity testing method
Antibacterial activity was tested at the Institute of
Method: Disc diffusion, using Mueller-Hinton agar (MHA) to test antibacterial activity.
Bacterial strains tested: *Escherichia coli*
Test steps:
Preparation of microbial strains: culture in Tryptic Soy Broth (TSB) medium for 16-18 hours at 37° C and shake 100 times/minute before use. Bacterial density after culture in TSB medium is determined by optical density (OD) measured at 610mm wavelength.
Preparation of essential oil solution: Dissolve essential oil in DMSO (2%), using Tween 80 (0.2%) as emulsifier. Standard solution consists of DMSO (2%), using 0.2% emulsifier (Tween 80 in distilled water).
Use a pipette to aspirate 100µl of bacteria (with a cell density of 108 CFU /ml), then pour evenly into the stable dried medium. MHA surface and wait for it to dry.

3. Results and discussion
Biological activity of Plectranthus essential oil
Antioxidant activity
The results are presented in Table 1 below:

Table 1: Antioxidant activity of *Plectranthus amboinicus* essential oil

Model name	Essential oil concentration (µL/mL)	% Inhibition	IC $_{50}$ (µL/mL)	Analytical method
Lemon Basil Essential Oil Sample	5	92.15	2.34	Spectral method
	4	75.44		
	3	58.05		
	2	44.38		
	1	30.18		
	0.5	22.19		

The test results showed that when the essential oil concentration ranged from 0.5 µL/mL to 5 µL/mL, the percentage of free radical inhibition activity gradually increased from 22.19 % to 92.15 % (Table 1). The best antioxidant capacity was observed in the sixth sample, with the percentage of DPPH free radical inhibition activity being 92.15 %.
In addition, IC_{50} of Lemon Basil essential oil is 2.34 µL/mL, Lemon Basil essential oil shows remarkable antioxidant capacity under experimental conditions using spectrophotometric method. Compared with the study of Ngu Truong Nhan and colleagues on Lemon Basil essential oil in the rainy season, the antioxidant capacity of Lemon Basil essential oil in the dry season is much stronger [2].

When compared with another study by Bezerra, R. de C *et al*., the antioxidant capacity of Lemon Basil essential oil was also much stronger [7]. According to another report by A. Manjamalai *et al*., it was also observed that Lemon Basil essential oil had weaker performance than the essential oil in Buon Ma Thuot, with antioxidant levels of about 5 - 100µg/ml [12].
A study from India by Jena *et al*. reported the IC_{50} value for the DPPH free radical scavenging ability of Lemon Basil essential oil to be 18.64 ppm [17]. This value is also much lower than the results of the present study.
These findings demonstrate that antioxidant capacity will vary depending on geographic location, soil type, and environmental conditions between seasons.

Antibacterial activity

The antibacterial ability of the essential oil (*Plectranthus amboinicus* (Lour.) Spreng) was determined based on its ability to inhibit bacterial growth, expressed by the diameter of the bacterial rings formed on a petri dish. The results of the antibacterial activity evaluation are shown in Table 2 and Table 3. Hereafter:

Table 2: Positive control (Ampicillin)

Bacterial density	Antibiotic concentration (mg/ml)	Diameter of sterile ring (mm)	Resistance
10^7 CFU	2.00	54mm; 54mm; 54mm	100% (bacteria do not grow on the entire agar plate)
10^7 CFU	1.75	54mm; 54mm; 54mm	100% (bacteria do not grow on the entire agar plate)
10^7 CFU	1.50	54mm; 54mm; 54mm	100% (bacteria do not grow on the entire agar plate))
10^7 CFU	1.00	54mm; 54mm; 54mm	100% (bacteria do not grow on the entire agar plate)
10^7 CFU	0.75	52mm; 52.5mm; 52.5mm	96.91%
10^7 CFU	0.50	50.5mm; 50mm; 50mm	92.90%

Table 3: Antibacterial activity of Plectranthus amboinicus essential oil

Bacterial density	Essential oil concentration (µg/mL)	*E. coli* resistance (%)	Analytical method
10^7 CFU	100	98.26%	CLSI M26 – A
10^7 CFU	80	78.91%	
10^7 CFU	60	62.15%	
10^7 CFU	20	28.65 %	
10^7 CFU	10	17.25 %	
10^7 CFU	5	10.11 %	

The results of the antibacterial activity test of *Plectranthus amboinicus essential oil on E. coli* bacteria as presented in Table 3 clearly show the correlation between the concentration of essential oil and the ability to inhibit bacterial growth. As the concentration of essential oil increased, the antibacterial ability expressed as the percentage of *E. coli inhibition* also increased significantly. At the lowest tested concentration of 5 µg/mL, the essential oil showed an inhibitory ability of 10.11%. This ability gradually increased and reached 98.26% at a concentration of 100 µg/mL. This reinforces the finding of a dose-dependent antibacterial activity of Lemon Basil essential oil against *E. coli*. Compared with the study on the antibacterial ability of lemon basil *essential* oil (*Plectranthus amboinicus* (Lour.) Spreng) by Lu Thi Mong My in Ho Chi Minh City and the study by Nguyen Thi Bich Thuyen in Can Tho, there are similarities [2].

Compared with another study in Thua Thien Hue conducted by Do Thi Binh Thuy and colleagues, Lemon Basil essential oil showed the best ability to resist *E. coli* bacteria at 100% concentration [1].

According to several international reports, Lemon Basil has strong antibacterial properties against several tested bacterial strains [10-14]. Notably, Carvacrol, the main component of Lemon Basil essential oil, is a phenolic with strong antibacterial activity [15-18]. This explains why Lemon Basil essential oil has the potential to be a natural antibacterial agent.

Comment: Different geographical locations and soil types affect the biological activity of essential oils in different ways.

4. Conclusion

Basil essential oil (*Plectranthus amboinicus* (Lour.) Spreng) was collected in Buon Ma Thuot, Dak Lak, containing 0.18-0.3%, the essential oil is light yellow, has a light aroma. The results of biological activity testing showed that Lemon Basil essential oil (*Plectranthus amboinicus* (Lour.) Spreng) has very high antioxidant capacity, with the ability to inhibit DPPH free radicals of 92.15 %, IC_{50} = 2.34 µL/mL and the ability to inhibit *E.coli* bacteria of 98.26 %. These studies have similarities with previous studies. In-depth research will support the effective exploitation and use of Lemon Basil (*Plectranthus amboinicus* (Lour.) Spreng) to produce medicines and functional foods.

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