CHEMICAL COMPOSITION, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITITES OF ESSENTIAL OIL FROM (*OCIMUM BASILICUM* L.) COLLECTED IN THANH HOA PROVINCE

Ngo Xuan Luong[1], Ngu Truong Nhan[2], Nguyen Thi Thuy[3], Trinh Xuan Vinh[4]

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Abstract: *The leaves of Ocimum basilicum* L. *were collected in Thanh Hoa province. Chemical composition of (Ocimum basilicum* L.*) essential oil has been examined by GC-MS. The identified components constitute 0.5-0.6%. There are 23 compounds and the main compounds are estragole (92.16%), 1.8-Cineole (1,69%), 3-Carene (0.99%), and trans-α-Bergamotene (0.52%). Estragole (92.16%) was higher than the previously published. In addition, we also tested the antibacterial activity of (Ocimum basilicum* L.*) essential oil on* E. coli. *The results showed that the essential oil had strong antibacterial activity of about 89.20% on* E. coli. *The antioxidant capacity of basil essential oil was determined by DPPH method. The highest antioxidant efficiency of basil essential oil was 96.40% (IC_{50} = 1,08 μg/mL) at 2.5 mg/mL. The results of this research show that the of Ocimum basilicum* L. *might be a natural potential source of antibacterial and antioxidants.*

Keywords: *Ocimum basilicum L, antibacterial, E.coli, essentialoil, antioxidant.*

1. Introduction

Ocimum basilicum L. is also known as basil and purple, which is grown very popularly in our country to make spices. Basil has a warm spicy taste, so it is used in medicine to treat colds, flu, indigestion, etc. In addition, basil contains 0.4-0.8% essential oils [1]. Since 1975, a number of provinces have grown on a large scale to store essential oils for domestic and foreign aromatics industry. According to the report of author Tran Thanh Quynh Anh and colleagues [2], analysis by gas chromatography coupled mass spectrometry (GC-MS) showed that basil essential oil (*Ocimum basilicum* L.) in Thua Thien Hue has the main chemical components such as: p-allylanisole (49.09%), aromadendrene (8.27%) and trans-ocimene (5.71%). The study also investigated the antioxidant and antibacterial activities of essential oils. The results indicated that basil essential oil had low antioxidant capacity (IC_{50}=35.89 μg/mL) and was able to inhibit two strains of *E. coli*, *Salmonellasp*. There have been studies showing that materials grown in different geographical locations and soils lead to different chemical compositions and biological activities of essential oils. However, there is still no research on the chemical

[1] Faculty of Science and Technology, Hong Duc University
[2] Faculty of Science and Technology, Tay Nguyen Universiy
[3] Master student K13 Organic Chemistry, Hong Duc University; Email: thuyhoaqx1@gmail.com
[4] Tho Xuan 4 High School, Tho Xuan District, Thanh Hoa Province

composition and biological activity of basil essential oil in Thanh Hoa. Therefore, we conducted this study to create a database of *Ocimum basilicum* L. in Thanh Hoa, thereby contributing to the efficient exploitation and use of the species.

Figure 1. *Ocimum basilicum* L. *- Thanh Hoa*

2. Experiment

2.1. Equipment: Light Clevenger Distillation Kit, Thermo Trace GC Ultra GC-MS analyzer system- ITQ900.

2.2. Chemistry: Anhydrous Na_2SO_4, diethyl ether.

2.3. Material

Ocimum basilicum L. was collected in Quang Xuong district, Thanh Hoa province, Vietnam in June 2021.

Parts Used: Leaves

2.4. Method

2.4.1. Distillation method

After collection, samples were preliminarily selected, washed and dried for 2 hours, cut into small pieces. Place in the Clevenger distillation flask 150 g chopped basil leaves with 500 ml of water. Distill for 2.5 hours, the mixture is heated by an electric stove, when the mixture boils, the steam formed will attract the essential oil up and into the condenser system. After condensing to obtain an insoluble mixture of water and essential oils, extracting the essential oil from the mixture with diethyl ether, anhydrous the extracted solution with anhydrous Na_2SO_4 salt, and obtain the finished essential oil. The yield of essential oil was 0.6 %. The oil is light yellow in color and has a mild fragrance and a density of 0.953 g/mL

2.4.2. Method for determining chemical composition

The chemical composition of essential oils was determined by gas chromatography-mass spectrometry (GC-MS), measured at the Institute of Biotechnology and Environment, Tay Nguyen University. Using Thermo Trace GC Ultra - ITQ900 GC/MS machine, TG-SQC

chromatographic column with the length of 30 m, inner diameter (ID) = 0.25 mm, thin film of 0.25 nm. Heli carrier gas. Sample injection chamber temperature (Temperature Program Technique-PTV) of 250°C, Detector temperature of 260°C. The thermostatic chamber temperature program: 60°C (2min), increasing 4°C/min until 200°C, stopping at this temperature for 5 minutes, increasing 10°C/min until 260°C, stopping at this temperature for 10 minutes.

2.4.3. Test method for antibacterial activity

Antibacterial activity was tested at the Institute of Biotechnology and Environment, Tay Nguyen University, by using diffusion on agar plate with MHA medium.

Tested bacteria strain: *Escherichia coli*. Active test steps:

Preparation of test microorganisms: strains before use are grown on TSB medium for 16-18 hours at 37°C, shaken at 100 rpm. Bacterial density after culture in TSB medium was determined by optical densitometry (OD) at 610 nm.

Prepare essential oil solution: essential oil is dissolved in DMSO 2%, using emulsifier is Tween 80 - 0.2%. The control solution consisted of 2% DMSO, using 0.2% emulsifier tween 80 in distilled water.

Use a pipette to suck up 100 µl of bacteria (cell density 10^8 CFU/ml), then spread evenly on the surface of the stable dried MHA agar, wait for the surface to dry. Use sterile 6mm paper plates saturated with essential oil solutions at different concentrations and control solutions, wait to dry and then place on the infested agar surface, gently press the paper plate to fix on the agar surface. Transfer the petri dishes to the refrigerator (10°C) for about 4 - 8 hours for the essential oils to diffuse into the agar. Then rear at 37°C for 16 - 20 hours. Read the results and record the diameter of the sterile ring.

The diameter of the sterile ring (D-d) is determined by the diameter of the outer ring minus the diameter of the paper plate.

2.4.4. Antioxidant activity test

Antioxidant activity of basil essential oil was carried out at the Institute of Biotechnology and Environment of Tay Nguyen University. The test process is carried out according to the DPPH free radical scavenging method: A method to determine the antioxidant capacity of a compound based on its free radical scavenging ability. DPPH (1,1-diphenyl-2-picrylhydrazyl) is capable of generating stable free radicals in methanol and has a maximum absorbance at 517 nm. When the test samples are added to this mixture, if the substance has the ability to neutralize or encapsulate the free radicals, the DPPH will change from purple to yellow. This signal is measured with an ELISA reader. The antioxidant activity of the test substance was evaluated through the percentage reduction in the light absorption value of the test sample compared to the control. The antioxidant activity test results are reported by the IC50 value as the concentration of the extract that can reduce 50% of DPPH free radicals under specified conditions. The lower the IC50 value is the higher the DPPH free radical scavenging activity becomes. The inhibition of DPPH was calculated according to the following formula:

$$\% \text{ IC} = \frac{\text{OD}_{froaf} - \text{OD}_{\text{try on}}}{\text{OD}_{\text{froaf}} - \text{OD}_{\text{white}}} \text{ x } 100\%$$

Where: OD: absorbance of control sample (no sample);

Test OD: absorbance of sample;

OD blank: absorbance of the blank (methanol).

The IC50 value of the test sample and the control sample is based on a linear equation between their concentration and % free radical scavenging activity, calculated by the formula: IC50= (50-b)/a.

In there: IC50: is the concentration of the sample that can capture 50% of the free radical DPPH

a, b are the slope and intercept of the linear equation between concentration and % free radical capture, respectively.

3. Results and Discussion

3.1. Chemical composition results

The essential oil of *Ocimum basilicum* L. obtained is light yellow in color and has a mild fragrance. The GC-MS chromatogram of basil essential oil is shown in Figure 2. The chemical composition of the essential oil is shown in Table 1.

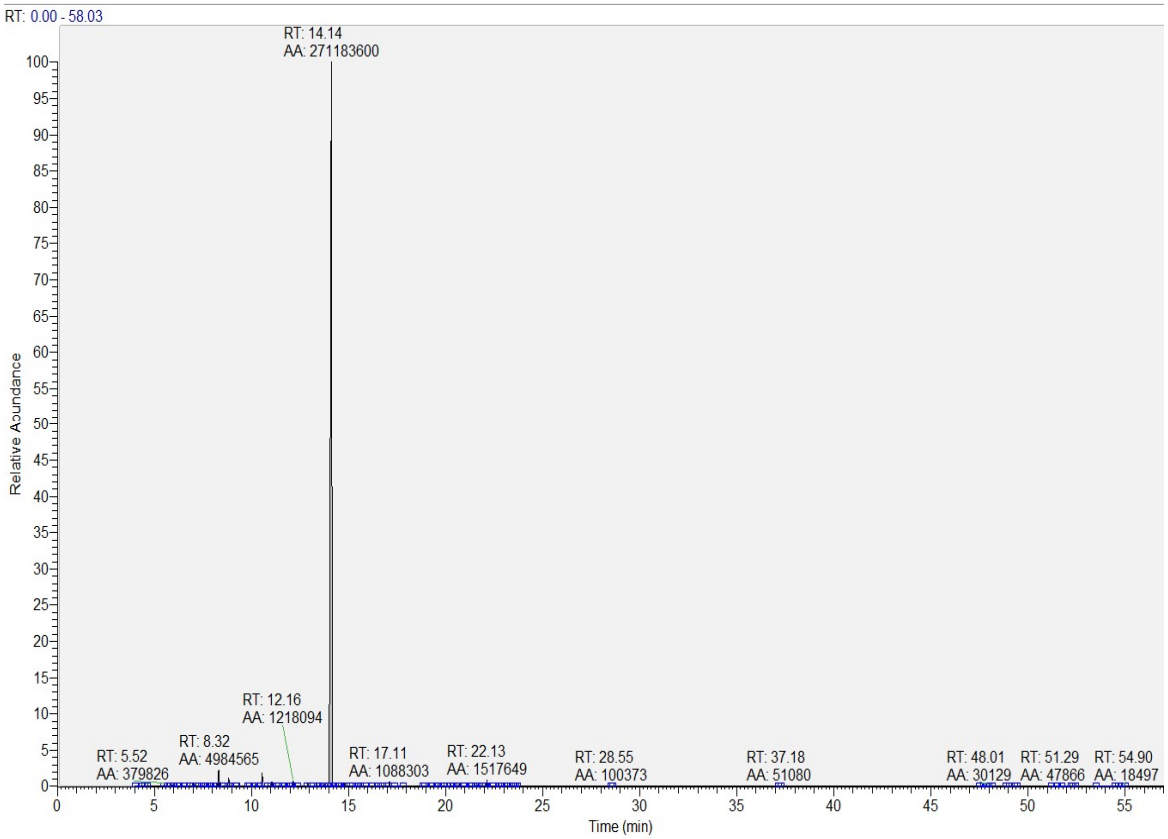
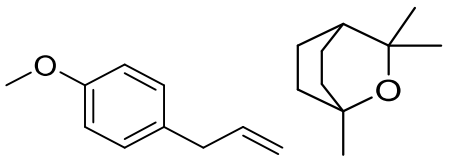


Figure 2. *GC-MS chromatogram of essential oil Ocimum basilicum L.*

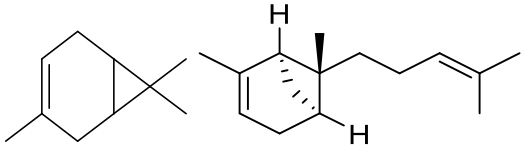
Table 1. *Chemical composition of essential oil Ocimum basilicum* L.

TT	RT	Compounds	kind	AI^r	Area(%)
1	5.52	α-Thujene	mh	**924**	0,13
2	5.92	β-Phellandrene	mh	1025	0,09
3	6.69	Sabinene	mh	969	0,17
4	7.04	β-Terpinene	mh	-	0,28
5	8.09	*p*-Cymene	mh	1020	0,07
6	**8.32**	**1,8-Cineole**	**mh**	**1026**	**1,69**
7	8.83	γ-Terpinene	mh	1054	0,62
8	10.21	3-ρ-Menthen-7-al	mo	-	0,17
9	**10.55**	**3-Carene**	**mh**	**-**	**0,99**
10	11.06	2-β-Pinene	mh	974	0,36
11	12.16	(+)-2-Bornanone	mh	1141	0,41
12	12.91	Camphene	mh	946	0,27
13	13.31	ι-Phellandrene	mh	-	0,07
14	13.78	δ-3-Carene	mh	1008	0,22
15	**14.14**	**Estragole**	**mh**	**1195**	**92,16**
16	14.37	ρ-Mentha-1,8-dien-7-ol	mo	1003	0,06
17	14.81	Z-Citral	mo	1235	0,23
18	16	unknow	-	-	0,05
19	17.11	Endobornyl acetate	mo	-	0,37
20	20.19	α-Cubebene	sh	1348	0,05
21	20.72	(-)-Sinularene	sh	-	0,17
22	21.12	cis-Methyl isoeugenol	mo	1451	0,2
23	21.65	trans-Caryophyllene	sh	1417	0,12
24	**22.13**	**trans-α-Bergamotene**	**mh**	**1432**	**0,52**
25	-	Other compounds	-	-	0,53
Total					100
Hydrocarbons					98,39
Derivatives containing oxyge					1,03

Chemical structures of the main compounds:



Estragole **1,8-Cineole**



3-Carene **trans-α-Bergamotene**

mh: monoterpene hydrocarbons; mo: oxygenated monoterpenes; sh: sesquiterpene hydrocarbons; nt: non-terpenes; AI^r: Reported Arithmetic Index.

Through analysis, the chemical composition of *Ocimum basilicum* L.essential oil has 23 identified components (accounting for 99.42%). Of which, hydrocarbons accounted for 98.39% and oxygen derivatives accounted for 1.03%. The main components in the essential oil are: Estragole (92.16%), 1,8-Cineole (1.69%), 3-Carene (0.99%) and trans-α-Bergamotene (0.52%). Compared with the national standard TCVN 11887:2017, the Estragole content is higher, the 1,8-Cineole content is similar. This proves that the quality of basil essential oil obtained is of good quality.

The results of our study on the chemical composition of essential oil of *Ocimum basilicum* L. collected in Thanh Hoa have many similarities with those published by Vo Thi Thanh Tuyen et al [3]. Research has found 25 constituents in essential oils extracted from plants grown in Quy Nhon City, Binh Dinh Province. The main components of the essential oil include: Estragole (85.92%), (E)-β-ocimen (1.95%), 1,8-Cineole (1.66%) and trans-α-Bergamotene (1.65%). The two components Estragole and 1,8-Cineole in our study have higher concentrations. However, the trans-α-Bergamotene conjugate was found in lower concentrations. The conjugate (E)-β-ocimen in this report was not found in our study. Another report by author Thien Hien Tran et. al. [4], also indicated that the main component of essential oil *Ocimum basilicum* L. collected in Ho Chi Minh City is Estragole (87.90%). Compared with the estragole content in essential oil collected in Thanh Hoa (92.16%), it is lower.

More interestingly, when comparing the research results by author Tran Thanh Quynh Anh et. al. It is shown that the essential oil content of *Ocimum basilicum* L. collected in Thua Thien Hue province has some compounds. The main components such as p- Estragole (49.09%) were lower than that of Estragole (92.16%) of in Thanh Hoa and the remaining main components Aromadendrene (8.27%), trans-Ocimene (5.71%) was completely absent in our study [2].

Along with the domestic studies, foreign studies were also reported. Accordingly, the authors Chalchat, J.-C and colleagues [5] investigated the chemical composition of essential oils from the leaves of *Ocimum basilicum* L. species in Mersin province. The results showed that essential oils have the main components: Estragole (58.60%), Limonene (13.54%), Exo-fenchyle acetate (10.99%) and Fenchone (5.7%). Compared with our study and the two studies mentioned above, the estragole content in this study was much lower. Notably, the remaining components were not present in our study. In addition, many other constituents were also found in both studies: α-Thujene, Sabinene, Sabinene and p-Cymene.

This result is higher than the study of Rajesh et. al. (2014) with estragole accounting for 38.3% and much lower estragole content in essential oil of *Ocimum basilicum* L. collected in Thanh Hoa. (92.16%). In addition, the content of 1.8-Cineole accounting for 1.66% in the essential oil from *Ocimum basilicum* L.in Thanh Hoa was much lower than the study of Ismail (2006) when researching essential oil. cinnamon in India with 1.8-Cineole content of (13.65%) [6].

Another study by author Al Abbasy, D. W and colleagues [1] showed that the main chemical composition of the essential oil of Omen species *(Ocimum basilicum* L.) is: linalool (69.87%), geraniol (9.75%), p-allylanisole (6.02%), 1.8-cineole (4.90%), trans-α-bergamotene (2.36%) and neryl acetate (1.24%)). The components linalool, geraniol, p-allylanisole and neryl acetate were not present in our study. However, two components,

1.8-cineole and trans-α-bergamotene, appeared in our study but at higher concentrations than in our study. The above analysis results show that the composition and chemical content of essential oil *Ocimum basilicum* L. is different depending on factors of geography, climate and soil conditions.

3.2. Results of the assessment of biological activity

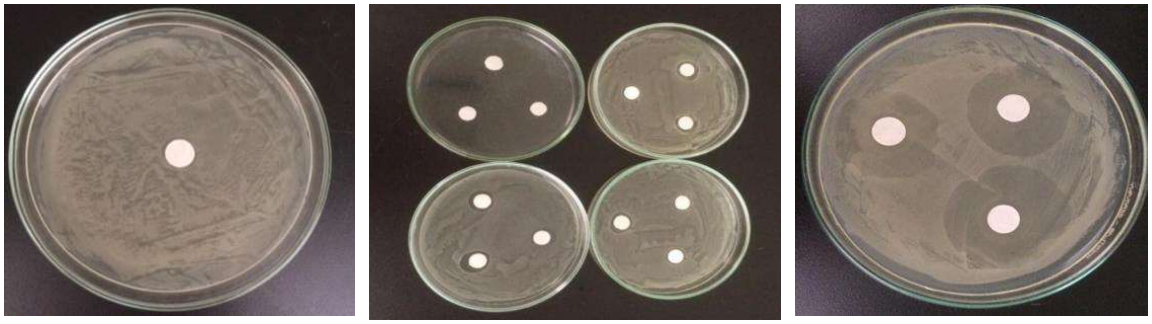
The antibacterial activity of the essential oil of *Ocimum basilicum* L. was determined based on its ability to inhibit the growth of bacteria, shown by the diameter of the antibacterial-halo zonering. The results of antibacterial activity evaluation are presented in Tables 2 and 3 below:

Table 2. *Positive test Ampicillin*

Bacterial density	**Essentialoil concentration (mg/ml)**	**Diameter of sterile ring (mm)**	**Resistance**
10^7 CFU	2,00	54mm, 54mm, 54mm	100% (b*acteria* do not grow all over the agar plate)
10^7 CFU	1,75	54mm, 54mm, 54mm	100% (*bacteria* do not grow all over the agar plate)
10^7 CFU	1,50	54mm, 54mm, 54mm	100% (*bacteria* do not grow all over the agar plate)
10^7 CFU	1,00	54mm, 54mm, 54mm	100% (bacteria do not grow all over the 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 essential oil Ocimum basilicum L.*

Bacterial density	**Essentialoil concentration (mg/ml)**	**Diameter of sterile ring (mm)**	**Resistance**
10^7 CFU	14	54mm, 54mm, 54mm	100% (bacteria do not grow all over the agar plate)
10^7 CFU	12	48 mm, 48mm, 48.5mm	89.20%
10^7 CFU	10	41.5mm, 41.5mm, 41mm	76.54%
10^7 CFU	8	32.5 mm, 32.5mm, 32.5mm	60.19%
10^7 CFU	6	24.5mm, 24mm, 24mm	44.75%
10^7 CFU	4	14mm; 14mm, 14.5mm	26.23%
10^7 CFU	2	6.5mm, 6mm, 6mm	11.42%



control (-) (DMSO 2%)

Figure 3. *Antibacterial ability at different concentrations of essential oil*

With a dilution concentration of 2.0 mg/ml, the antibacterial circle with the diameter measured against the resistance ring of *E. coli* is 6.2 mm, the inhibitory ability is 11.42%. The ability to inhibit *E. coli* bacteria increased gradually from the concentration of 2.0 mg/ml to 12.0 mg/ml and to the concentration of 14 mg/ml, the bacteria did not grow on the whole plate. At the concentration of 12.0 mg/ml, the antibacterial circle was 48.2 mm and the ability to inhibit *E. coli* was 89.20%. Compared with the study of author Tran Thanh Quynh Anh and colleagues, the antibacterial ability of Basil essential oil (*Ocimum basilicum* L.) in Hue City (*E. coli* antibacterial circle is 5.08 mm) and author Vo Thi Thanh Tuyen in Binh Dinh (16.0 mm *E.coli* antibacterial circle), the results showed the ability of basil essential oil to inhibit *E. coli* bacteria (*Ocimum basilicum* L.) in Thanh Hoa is stronger [2] [3]. This result is consistent with the study by Lu et al (2011) that basil essential oil is a potential natural antibacterial agent. Therefore, the combination of basil essential oil with other essential oils will increase its antibacterial ability. The results of antioxidant activity evaluation are presented in Tables 4 and 5 below:

Table 4. *Positive test: Acid ascorbic*

Acid ascorbic	**% Inhibition**			IC_{50} (µg /ml)
µg/ml	1	2	3	
50	70.87	70.85	70.89	34.99
40	56.11	56.02	56.14	
30	41.95	41.98	42.09	
20	30.17	30.14	30.07	
10	18.17	18.09	18.16	

Table 5. *Antioxidant activity of essential oil Ocimum basilicum L.*

Essential oil	**% Inhibition**			IC_{50} (mg/ml)
mg/ml	1	2	3	
2.5	96.93	96.11	96.16	1.08
2.0	80.28	80.76	80,45	
1.5	62.24	62.15	62,17	
1.0	47.15	47.18	47,23	
0.5	32.17	31.98	32,06	
0.25	22.76	22.78	22,86	

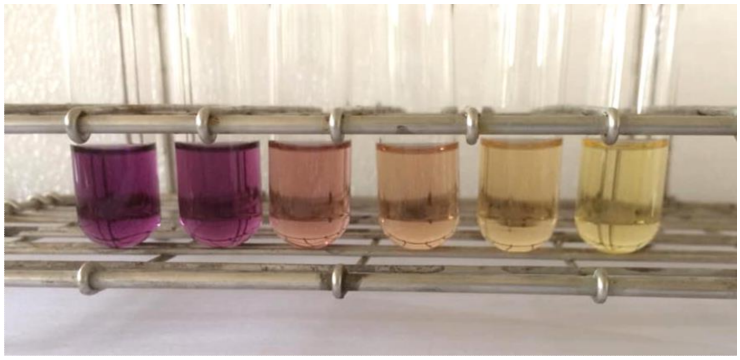


Figure 4. *Antioxidant capacity at different concentrations of essential oil*

The test showed that when the concentration of essential oil was from 0.25 mg/mL to 2.5 mg/mL, the percentage of DPPH free radical inhibitory activity of basil essential oil (*Ocimum basilicum* L.) gradually increased from 22, 80% to 96.40% (Table 5). The best antioxidant activity was demonstrated in sample 6 with the percentage of DPPH free radical scavenging activity of 94.40%. In addition, compared with the positive control ascorbic acid with IC50 = 34.99 µg/mL while basil essential oil with IC50 = 1.08 µg/mL, it can be concluded that the antioxidant capacity of essential oil is 32.5 times stronger than ascorbic acid. Compared with the study of author Tran Thanh Quynh Anh and colleagues, the antioxidant capacity of essential oil *Ocimum basilicum* L. in Hue City with IC50 = 35.89 µg/mL, the antioxidant capacity of essential oil of *Ocimum basilicum* L. in Thanh Hoa is much stronger [2].

This result also shows that the antioxidant activity of *Ocimum basilicum* L.essential oil in Thanh Hoa is much higher than that of Hadj-Khelifa et al. (2012), essential oil. *O. basilicum* from Algeria exhibited lower antioxidant activity (IC50 = 83.4 mg/mL) [7].

Comment: Through the test table, it was found that basil essential oil has a very strong antioxidant capacity. With IC50 = 1.08 mg/ml at a concentration of 0.25-2.5 mg/ml compared to the control substance Ascorbic Acid (IC50 (mg/ml) = 34.99) is 32.5 times higher. This result opens up the potential for the antioxidant capacity of *Ocimum basilicum* L.in the future.

4. Conclusion and Proposal

The essential oil of *Ocimum basilicum* L. was collected in Quang Xuong district, Thanh Hoa province. The survey contained from 0.5-0.6%, the essential oil was light yellow in color and had a slight fragrance. The main chemical constituents of essential oil are Estragole (92.16%), 1,8-Cineole (1.69%), 3-Carene (0.99%) and trans-α-Bergamotene (0.52%).

Essential oil has very strong antioxidant capacity, DPPH free radical inhibition rate is 94.40% with IC50 value = 1.08 mg/ml and ability to inhibit *E. coli* is 89.20% with an antibacterial diameter of 48.2 mm. Further studies will contribute to opening up the potential of *Ocimum basilicum* L. into functional foods and pharmaceuticals, contributing to the effective exploitation and use of this plant.

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