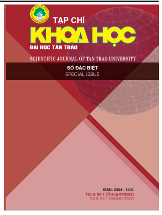
TẠP CHÍ KHOA HỌC ĐẠI HỌC TÂN TRÀO
ISSN: 2354 - 1431
http://tckh.daihoctantrao.edu.vn/



# ESSENTIAL OILS APPLICATION OF SOME SPECIES OF GENUS ELSHOLTZIA IN ANTIBACTERIAL HAND SANITIZER PRODUCTION

*Pham Thi Ngoc Mai*, Nguyen Van Binh, Nguyen Thuy Duong, Luu Hong Son, Nguyen Thi Thom, Do Nhu Quynh, Nguyen Dinh Manh.*

*Thai Nguyen University of Agriculture and Forestry*

*Email address: phamthingocmai@tuaf.edu.vn*

*DOI: 10.51453/2354-1431/2022/867*

**Article info**

*Received:23/9/2022*

*Revised: 18/10/2022*

*Accepted: 30/12/2022*

**Keywords:**

*Elsholtzia, biological activity, steam distillation, agar diffuser, mix ratio*

**Abstract**:

Species of the genus Elsholtzia have great potential for bioactive substances. Although this species has been used a lot in traditional medicines, most of it is only used based on the experience gained or left by forerunners without much knowledge of the species scientific basis. Therefore, the investigation, research, evaluation of chemical composition and screening of potential bioactive substances from plants of the genus Elsholtzia, especially those endemic to Vietnam, will be of great scientific and practical significance, actively contributing to the rational exploitation and use of the country's natural resources, thereby orienting the application of Elsholtzia essential oil in research and develops a number of food, pharmaceutical and cosmetic products. Using the method of extracting essential oils by steam distillation and the evaluation of antibacterial activity by agar diffusion method with the following contents: Results analysis, evaluation and screening of essential oils of some species of the genus Elsholtzia with biological activity obtained in different regions. Research results on mixing ratio to create antibacterial hand sanitizer products, completing the process and calculating the cost of antibacterial hand sanitizer products.

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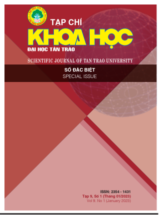


# ỨNG DỤNG TINH DẦU MỘT SỐ LOÀI CHI ELSHOLTZIA TRONG SẢN XUẤT NƯỚC RỬA TAY KHÁNG KHUẨN

*Phạm Thị Ngọc Mai*, Nguyễn Văn Bình, Nguyễn Thùy Dương, Lưu Hồng Sơn, Nguyễn Thị Thơm, Đỗ Như Quỳnh, Nguyễn Đình Mạnh.*

*Đại học Nông Lâm Thái Nguyên*

*Địa chỉ email: phamthingocmai@tuaf.edu.vn*

*DOI: 10.51453/2354-1431/2022/867*

**Thông tin bài viết**

*Ngày nhận bài: 23/9/2022*

*Ngày sửa bài: 18/10/2022*

*Ngày duyệt đăng: 30/12/2022*

**Từ khóa:**

*Elsholtzia, hoạt tính sinh học, chưng cất hơi nước, khuếch tán thạch, tỷ lệ trộn*

**Tóm tắt**

Các loài thuộc chi Elsholtzia có tiềm năng lớn về các chất có hoạt chất sinh học. Mặc dù, loài này đã được sử dụng nhiều trong các bài thuốc cổ truyền, nhưng phần lớn chỉ sử dụng theo kinh nghiệm có được hoặc do kinh nghiệm của người đi trước để lại mà chưa có nhiều hiểu biết về cơ sở khoa học về loài cây này. Do vậy, việc điều tra, nghiên cứu, đánh giá thành phần hóa học và sàng lọc các chất có hoạt tính sinh học tiềm năng từ các loài thực vật thuộc chi Elsholtzia, đặc biệt là những loài đặc hữu của Việt Nam sẽ có ý nghĩa khoa học và ý nghĩa thực tiễn cao, góp phần tích cực vào việc khai thác và sử dụng một cách hợp lý nguồn tài nguyên thiên nhiên của đất nước, từ đó có thể định hướng ứng dụng tinh dầu chi Elsholtzia trong việc nghiên cứu phát triển một số sản phẩm thực phẩm, dược phẩm và mỹ phẩm. Sử dụng phương pháp tách chiết tinh dầu bằng phương pháp chưng cất lôi cuốn hơi nước và phương pháp đánh giá hoạt tính kháng khuẩn bằng phương pháp khuếch tán đĩa thạch để với các nội dung cho kết quả như sau: Kết quả Phân tích, đánh giá, sàng lọc tinh dầu một số loài thuộc chi Elsholtzia có hoạt tính sinh học thu nhận ở các vùng khác nhau. Kết quả nghiên cứu tỉ lệ phối trộn tạo sản phẩm nước rửa tay kháng khuẩn, hoàn thiện quy trình và tính toán giá thành của sản phẩm nước rửa tay kháng khuẩn.

## 1. Introduction

*Elsholtzia* is a genus of at least 33 species in the *Lamiaceae* family, which is distributed in humid tropics such as Southeast Asia, tropical Africa, Northern Australia, Madagascar and Indochina [1]. Plants of the genus *Elsholtzia* are mostly aromatic plants, which have always been utilized as folk medicine, herbal teas, foods, spices, beverages, perfumes, cosmetics, flavorings and as a source of honey production. …

Plants in this genus are a rich source of various bioactive components including phenylpropanoids, terpenoids, phytosterols and cyanogenic glycosides, essential oils. The genus *Elsholtzia* is used in folk medicine in our country and China [2]. Leaves and stems are utilized to treat colds, vomiting, headaches, rashes, and bactericidal. The content of essential oil in fresh plants fluctuates in the range of 0.3-0.9%; Samples obtained from different regions of Vietnam have volatile oil content in the range of 0.3-0.6% [3].

Species of the genus *Elsholtzia* have great potential for bioactive substances. Although this species has been used a lot in traditional medicines, most of it is only used according to the experience gained or left by the forerunners without much knowledge of the scientific basis. Therefore, the investigation, research, evaluation of chemical composition and screening of potential bioactive substances from plants of the genus *Elsholtzia*, especially those endemic to Vietnam, will provide high scientific and practical significance, contributing to the rational exploitation and use of the country's natural resources, thereby orienting the application of *Elsholtzia* essential oil in developing a number of food, pharmaceutical and cosmetic products… Starting from the above problems, we conducted a research on the topic: "Essential oils application of some species of genus *Elsholtzia* in antibacterial hand santinizer production".

## 2. Methods

Chemicals: Distilled water, methanol, meat extract, yeast extract, pepton, alcohol 96˚, glycerin

Research equipment: Analytical balance, Clevenger apparatus.

Titration kit for titration of physicochemical indices: The density of essential oils is determined according to TCVN 8444: 2010, acid index is determined according to TCVN 8450: 2010 [4]

### *2.1. Extraction of essential oils by steam distillation method (Meyer-Warnod, 1984)*

Essential oils of some species of the genus *Elsholtzia* are extracted by steam distillation and specifically as follows:

Weigh 20kg of sample and put in an essential oil distillation pot containing 5 liters of water, raw materials and water are separated by a floating blister. When the water boils, steam rises and carries the essential oil through the cooler. Condensed steam flows down the extraction vessel. Essential oils are lighter than water, thus they float to the top. After 4 hours of extraction, the extraction process was completed and the essential oil was collected. The essential oil is then anhydrous with $Na_2SO_4$ to completely remove the water and stored at a temperature of 18–25$^o$C in the dark.

### *2.2. Evaluation of free radical scavenging ability by DPPH method*

Principle: 1,1-diphenyl-2-picrylhydrazyl (DPPH) is a free radical scavenger used to screen for the antioxidant effect of the investigated substances. DPPH has a deep purple color, with maximum absorption at 517 nm. When adding the test substances to this mixture, if the substance has the ability to neutralize or encapsulate the free radicals, it will reduce the light absorption intensity of DPPH free radicals. The antioxidant activity is shown by reducing the color of DPPH, which is determined by spectrophotometric measurement at λ= 517 nm (the color of the reaction solution will gradually change from purple to light yellow.

Experiment:

- Prepare reagents and test samples:

DPPH solution: Dilute 0.1 mM DPPH solution in methanol by dissolving 3.943 mg DPPH with methanol and make up to 10 ml to get 1 mM DPPH concentration, then dilute 10 times to get 0.1 mm DPPH solution. After mixing, use immediately and store in colored glass bottles.

Sample: Dilute essential oil with methanol at concentrations of 100ppm, 200ppm, 500ppm, 1000ppm. Select ascorbic acid as a positive control to carry out the test procedure:

| Sample | Test solution (ml) | MeOH solution (ml) | DPPH solution (ml) |
|---|---|---|---|
| Blank sample | 0 | 4 | 0 |
| Negative control sample | 0 | 3,5 | 0,5 |
| Sample | 0,5 | 3 | 0,5 |

After mixing, leave in the dark at room temperature for 30 minutes. Then, photometrically measured at 517 nm.

The SC antioxidant activity (%) was calculated according to the formula:

SC (%) = (ODc – ODt) / ODc *100

In there:

SC (%): percentage of free radical inhibition DPPH

ODc: Optical density of DPPH and MeOH . solutions

ODt: Optical density of DPPH solution and sample

SC% activity values >50% at a concentration of 200ppm for the essential oils were considered to be active.

$SC_{50}$:

Definition: SC is a value used to evaluate the strong or weak inhibitory ability of the sample. $SC_{50}$ is defined as the concentration (mg/ml) of a sample that can inhibit 50% of free radicals, cells or enzymes. The more active the sample, the lower the $SC_{50}$ value will be.

* How to determine $SC_{50}$:

From sample concentration and SC (%) using Excel software, prepare a regression equation of the form y = ax + b showing the correlation between SC (%) (y) and concentration (x)

From that deduce the $SC_{50}$ value.

A lower $SC_{50}$ value corresponds to a higher SC and vice versa

***2.3. Evaluation of antibacterial activity by agar diffusion method***

Preparing bacteria: Take *Escherichia coli* gram(-) bacteria that have been stored at deep negative temperature, take out and defrost for about 15 minutes (in ice cold water). Take 100 microliters of bacteria into 10ml of culture medium of grade 1 - *E. coli* at 37 ˚C in shaker for 16-18 hours. After 16-18 hours of culture, take 100 microliters of cultured bacteria into test tubes of 10ml of medium to raise level 2 bacteria, rearing similar to level 1, after 16-18 hours, measure the OD between 0.8-1 and the bacterial density reaches about 109 CFU/g. Dilute the inoculum to 106 CFU/g. *Bacillius subtislis* gram (+) bacteria cultured at 37˚C, suitable pH about 7.0 - 7.4 growing on common culture for 72 hours.

The antibacterial activity of essential oil of *Elsholtzia ciliata* was determined by agar diffusion method. Normal broth medium (g/l): Meat extract (3.0), yeast extract (5.0), peptone (10,0), salt, (5.0), agar (17.0) Dilute in 1000 ml of water, pH = 6.5-6.8. The medium was autoclaved at 121˚C for 15 minutes, cooled to 45-48 ˚C, supplemented with microorganisms (*Escherichia coli* gram(-) and *Bacilus subtislis* gram (+) bacteria) to control the strain to reach 6.5*106 CFU/g at the rate of 1ml of inoculum in 15ml of medium, placed in a magnetic stirrer and poured into a pertri dish with a thickness of 5 mm. Use a button drill to punch holes in the disc (□= 6 mm). Positive control is antibiotic solution (Ampicillin) mixed in distilled water at the concentration of 10mg/ml, negative control is sterile distilled water. Using a micropipette to drip 50µl of *Elsholtzia* essential oil in each hole, drip ampicillin and water into different agar holes, then place the sampled plates in the refrigerator at 4 ˚C, leave for 3 hours to allow diffusion of the solution. Next, transfer the plate to a 37 ˚C incubator with a time of about 16-18 hours for *Escherichia coli* and 72 hours for Bacilus subtislis. The antibacterial ability was determined by measuring the diameter (DK) of the microbial inhibitory ring using the formula:

DK = D - d (mm)

In which:

D: antibacterial ring diameter (mm)

d: diameter of drill hole (mm)

The experiment was repeated three times and the mean radius was measured.

**3. Results and discussions**

***3.1 Evaluation results of the biological activity of essential oils obtained from some species of the genus Elsholtzia***

*3.1.1 Evaluation results of antioxidant activity*

**Table 3.1. Evaluation results of essential oil oxidation activities of some species of the genus *Elsholtzia***

| No. | Name of sample | Initial concentration of test sample (µg/ml) | Ability to neutralize free radicals (SC, %) | $SC_{50}$ (µg/ml) |
|---|---|---|---|---|
| | Positive control [axit ascorbic 5 mM] | 44 | 90,21 ± 0,25 | 11,3 |
| | Negative control [DPPH/MeOH] | - | 0,0 ± 0,0 | - |
| 1 | *Elsholtzia ciliata* | 200 | 7,14 ± 0,25 | - |
| 2 | *Elsholtzia blanda* | 200 | 16,47 ± 0,5 | - |
| 3 | *Elsholtzia winitiana* | 200 | 5,56 ± 0,56 | - |

From the results of Table 3.1, the samples did not show antioxidant activity on the DPPH system with the initial concentration of 200 µg/ml. However, when compared at the same concentration of samples, *Elsholtzia blanda* has a higher ability to neutralize DPPH free radicals (SC = 16.54%) than *Elsholtzia* *ciliata* (SC = 7.14%). and *Elsholtzia winitiana* (SC = 5.56%). Compared with the study of Hoang Dinh Hoa et al., using the DPPH method, the ability to neutralize free radicals of *Elsholtzia winitiana* essential oil collected in Bac Quang, Ha Giang was 40.28 ± 0.25 % with an initial volume of essential oil of 0.1ml (corresponding to a concentration of 17500 µg/ml).

Thus, it can be concluded that the essential oil samples obtained from some species of the genus ***Elsholtzia*** did not show antioxidant activity with the initial concentration of 200 µg/ml, much smaller than the positive control sample, which is vitamin C (ascorbic acid - antioxidant).

*3.1.2 Evaluation results of antibacterial activity by agar diffusion method*

The antibacterial ability of the test sample was determined based on the ability to inhibit the growth of microorganisms expressed through the diameter of the antibacterial ring on the petri dish. Essential oils of some species of the genus *Elsholtzia*, when extracted by steam distillation method, were evaluated for their antibacterial activity against two strains of bacteria, *Escherichia coli* (negative gram bacteria) and *Bacilus subtislis* (positive gram bacteria), negative control is distilled water, positive control is antibiotic Ampicillin. The results are shown in Table 3.2.

**Table 3.2. Evaluation results of the antibacterial activity of essential oils of some species of the genus Elsholtzia**

| *No.* | *Name* | *Antibacterial ring diameter (mm) for Escherichia coli* | *Antibacterial ring diameter (mm) for Bacilus subtislis* |
|---|---|---|---|
| *1* | Elsholtzia ciliata | *13,13 ± 0,32* | *12,00 ± 0,5* |
| *2* | Elsholtzia blanda | *5,33 ± 0,8* | *3,67 ± 0,42* |
| *3* | Elsholtzia winitiana | *16,75 ± 0,38* | *15,85 ± 0,82* |
| *4* | Antibiotic (Ampicilin 10mg/ml) | *20,23 ± 0,68* | *20,4 ± 0,56* |
| *5* | Distilled water | - | - |

Compare the results in Table 3.2 with the publication of Billerbeck (2007) on the classification of antibiotics based on antibacterial ring diameter (d < 0.6cm: resistant; 1.3cm > d > 0.6cm: medium resistance; average; d > 1.3cm: very good resistance) showed that: essential oils of *Elsholtzia ciliata* species collected in Thai Nguyen and *Elsholtzia winitiana* collected in Dong Van rocky plateau, Ha Giang showed very good antibacterial activity on *E. coli*. For the antibacterial activity against *Bacillus subtilis* strain, *Elsholtzia ciliata* species showed at medium level, *Elsholtzia winitiana* species showed at very good level. Particularly, *Elsholtzia banda* species in Lao Cai also showed resistance to both strains of microorganisms tested above, but the antibacterial ability was weaker than the two species collected in Thai Nguyen and Ha Giang. This result is completely consistent with a number of studies on the biological activity of essential oils of some species of the genus *Elsholtzia* collected in the country and around the world.

Based on the research results in **Table** 3.2. Evaluation results of antibacterial activity of essential oils of some species belongging to *Elsholtzia* species, it can be found that essential oil of *Elsholtzia ciliata* collected in Thai Nguyen exhibits good antibacterial activity on strains of E.coli and *Bacillus subtilis*, although the antibacterial ability was less than that of the essential oil of *Elsholtzia winitiana* collected in Dong Van rocky plateau, Ha Giang province. From an economic point of view, in order to study the application of essential oils of some species belonging to genus *Elsholtzia*, it is possible to choose the source of *Elsholtzia ciliata* collected in Thai Nguyen because the source are common, easy to collect, convenient for the extraction of essential oils and product research. From the above reasons, the authors conducted initial research on application of essential oil of *Elsholtzia ciliata* collected in Thai Nguyen in the production of some cosmetic products (with the addition of essential oils of *Elsholtzia ciliata*) [5].

***3.2 Results of mixing ratio to create antibacterial hand sanitizer products***

To evaluate the rate of adding essential oils to antibacterial hand sanitizer, we added the percentages of essential oils, respectively: 0%; 0.50%; 0.75%; 1.00%. The results are shown in Table 3.3 .

**Table 3.3. Investigation results of the mixing ratio between Elsholtzia ciliata essential oil and absolute alcohol in antibacterial hand sanitizer**

| Mixing ratio of essential oils (Essential oils / alcohol) | Sense of smell (test on filter paper) | Scent retention time |
|---|---|---|
| 1,00% | Strong smell of *Elsholtzia ciliata*<br>Bad smell | After 5 minutes: Strong smell<br>After 10 minutes: The smell calms down<br>After 20 minutes: Good smell<br>After 40 minutes: The smell is completely gone |
| 0,75% | Light smell of *Elsholtzia ciliata* | After 5 minutes: Strong smell<br>After 10 minutes: The smell calms down<br>After 20 minutes: Good smell<br>After 40 minutes: The smell is completely gone |
| 0,50% | Medium smell of *Elsholtzia ciliata* | After 5 minutes: The smell is a bit strong<br>After 10 minutes: easy to smell<br>After 20 minutes: a little smelly<br>After 30 minutes: The smell is completely gone |
| 0,00% | Strong alcohol smell | After 5 minutes: Strong alcohol smell<br>After 10 minutes: the smell is completely gone |

**Conclusion**: According to the results of Table 3.3 in the percentage of not adding essential oils that cause strong alcohol smell, when adding 0.75%; 1.00% essential oils, it shows the strong smell of *Elsholtzia ciliata* and causing unpleasant odors which affects the sensory value of the product. Therefore, we choose the rate of 0.5% essential oil with a medium smell to make the product.

**General comment**: All samples of essential oils mixed in alcohol solvent according to the above ratio have created a fragrant product, however, when tested on the skin of the hand, the skin was found to be dry. Thus, in order to prevent the skin from drying out, we added glycerin to the mixture and the results of adding glycerin are shown in Table 3.4.

**Table 3.4. Survey results of Elsholtzia ciliata essential oil with glycerin/alcohol mixing ratios**

| Mix ratio (Glycerin/alcohol) | Sensation (Test on hand skin) |
|---|---|
| 2,00% | Soft, dry, squeaky skin |
| 1,75% | Soft, dry, slightly squeaky skin |
| 1,50% | Soft skin, quick to dry, not sticky |
| 1,00% | Dry skin, quick dry, not sticky |

**Comment**: Through the results of Table 3.4, it is found that the mixing ratio (glycerin/alcohol) is 2% and 1.75%, which softens the skin quickly, but causes it to be hissed when used with a ratio of 1,00% fast drying without squeezing hands but making the skin dry and not soft. Therefore, we have chosen a mixing ratio of 1.5% (glycerin/alcohol) for soft skin, quick drying and no sticky skin when used.

***3.3 Results of completing the production process of hand sanitizer from essential oils of some species of the genus Elsholtzia***

From the results in section 3.1 ; 3.2 and 3.3, we have built the formula and production process of hand sanitizer from essential oils of some species of the genus *Elsholtzia ciliata*

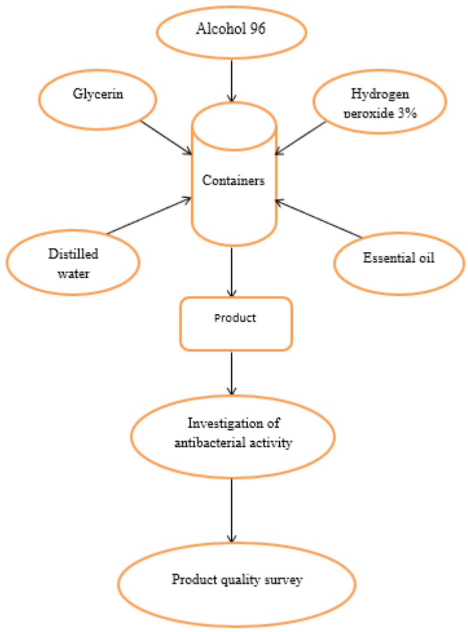


*Figure 3.1: Production process of antibacterial hand sanitizer*

Proceeding steps: It is necessary to prepare all the ingredients for making hand sanitizer with the following ratio:

- Alcohol 96 volume: 415 ml.

- Hydrogen peroxide 3% volume: 20 ml.

- Glycerin volume: 7.5ml.

- *Elsholtzia ciliata* essential oil volume: 2.5 ml.

- Distilled water or boiled water to cool the volume: 55 ml.

First, put 415ml of 96-degree alcohol into a large container. Then use a syringe to take 20ml of hydrogen peroxide and put it into the alcohol container.

Next, continue to use the syringe to take 7.5ml of glycerin and also put it in the same alcohol container as above. Note, because glycerin is very viscous, it will easily stick in the cylinder. Therefore, when cleaning the cylinder, it is necessary to rinse it with cooled boiled water or distilled water before using it to measure the concentration of glycerin.

For the next step, add distilled or boiling water to the alcohol container. Alternatively, you can add about 2.5ml of Elsholtzia ciliata essential oil. This helps reduce the smell of alcohol and makes homemade hand sanitizers smell more pleasant.

Then, immediately close the lid of the bottle after mixing to prevent the solution from volatilization. Pay attention to gently shake hands or stir the solution to be better mixed.

***3.4 Evaluation results of antibacterial activity of hand sanitizer products***

The antibacterial ability of the test sample was determined based on the ability to inhibit the growth of bacteria shown by the diameter of the antibacterial ring on the petri dish.

The product of hand sanitizer evaluated for antibacterial activity on bacteria is *Escherichia coli* gram(-), the negative control is distilled water, the positive control is the antibiotic Ampicillin. The results are shown in Table 3.5 .

**Table 3.5 Evaluation results of antibacterial activity for antibacterial hand sanitizer product supplemented with Elsholtzia ciliata essential oil**

| Name | FM1 (0% essential oil) | FM 2 (0,5% essential oil) | FM 3 (0.75% essential oil) | FM 4 (1% essential oil) | Antibiotic (Ampicilin 10mg/ml) | Distilled water (mm) |
|---|---|---|---|---|---|---|
| Antibacterial ring diameter (mm) | 11 ± 0,1 | 12,5 ± 0,2 | 13 ± 0,1 | 18 ± 0,2 | 21 ± 0,1 | - |

Comment: From the table of results 3.5, it is shown that for *E. coli* strain, antibacterial hand sanitizer was resistant at all specific test concentrations: at 0% concentration, the result gave the lowest antibacterial ring diameter with 11 ± 0.1mm, at 1% concentration, results showed the largest antibacterial ring diameter is 18mm ± 0.2mm.

Results of evaluation of some quality indicators of antibacterial hand sanitizer products

**Table 3.6 Results of analysis of some quality indicators of antibacterial hand sanitizer products**

| | Indicator | Test method | Unit | Result |
|---|---|---|---|---|
| 1 | Status | Feelings | _ | Liquid, homogeneous, not layered |
| 2 | Smell | Feelings | _ | Pleasant smell |
| 3 | Colour | Feelings | _ | No color |
| 4 | pH | ISO 4316 | % | 5,2 - 6,0 |
| 5 | As content | TCVN 6971 : 2001 | mg/kg | < 0, 01 |
| 6 | Pb content | TCVN 6971 : 2001 | mg/kg | < 0, 01 |
| 7 | E. coli | QCVN 6-1:2010 | CFU/g | Not detected |
| 8 | Total coliform | QCVN 6-1:2010 | CFU/g | Not detected |

From the above results, the products have reached the allowable values in the list of national quality standards and these products can be completely distributed in the market as well as the applicability of their production on a large scale industry.

***3.5 Cost estimation results for antibacterial hand sanitizer products***

Cost of producing 1 bottle of 500ml antibacterial hand sanitizer

| No | Item | Unit | Quatity | Cost (VND) |
|---|---|---|---|---|
| 1 | Essential oil | Ml | 2,5 | 15.000 |
| 2 | Distilled water | Ml | 55 | 1.200 |
| 3 | Glycerin | Ml | 7,5 | 2.250 |
| 4 | Alcohol 96 | Ml | 415 | 25.000 |
| 5 | Hydrogen peroxide 3% | Ml | 20 | 1.000 |
| 6 | Labels, packaging | Set | | 10.000 |
| 8 | Other costs | | 1000 | 1000 |
| Total : 55.450 VND | | | | |

Due to the small-scale testing conditions in the laboratory and the use of all natural essential oils, without adding synthetic fragrances, the cost of the products is higher than those available on the market.

## 4. Conclusion

- Marjoram essential oil extracted by steam distillation has a transparent yellow color, a strong aroma, and a slightly spicy taste. All three samples of essential oils showed antibacterial activity against two strains of microorganisms *E. coli* and *B. subtilis*, in which the essential oil samples obtained from the thick marjoram species in the Dong Van rocky plateau, Ha Giang could not be found. exhibits very good antibacterial activity against both strains of microorganisms Antibacterial hand sanitizer formulation

- Alcohol 96 volume: 415 ml.
- Hydrogen peroxide 3% volume: 20 ml.
- Glycerin volume: 7.5ml.
- Essential oil volume: 2.5 ml.
- Distilled water: 55ml.

Improving the production process of antibacterial hand sanitizers from essential oils of some species of the genus *Elsholtzia*. Evaluation of some quality indicators of the obtained products and calculation of product cost.

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