INVESTIGATION OF THE ANTIMICROBIAL ACTIVITY OF PEPPERMINT ESSENTIAL OIL *MENTHA ARVENSIS* L. CV. DL97 GROWN ACCORDING TO GACP-WHO GUIDELINES IN HANOI

Tran Van Loc[1], To Minh Tu[1], Nguyen Thi Thuy[1], Nguyen Xuan Nam[1], Ho Khanh Vy[2], Đang Viet Cuong[3]

[1]Institute of Medicinal Materials

[2]Thuy Loi University

[3]Hanoi University of Medicine and Pharmacy, Vietnam National University

Abstract

This study investigates the antibacterial potential of peppermint essential oil against three standard microbial strains: Bacillus subtilis, Staphylococcus aureus, and Escherichia coli using the disc diffusion method. The results showed that all five samples of peppermint essential oil exhibited antibacterial activity against the three tested bacterial strains, with the antibacterial activity ranking as follows: Bh100 < Bh50 < Bh01 < Bh25 < BhGACP-WHO. The essential oil sample BhGACP-WHO showed good effects against all tested bacterial strains. For the two Gram-positive bacteria (B. subtilis and S. aureus), the inhibition zones for the BhGACP-WHO sample were 8.3 mm and 5.3 mm, respectively, and for the Gram-negative bacteria (E. coli), it was 6.7 mm.

Keywords: *Peppermint, Hanoi, menthol, Lamiaceae.*

I. INTRODUCTION

Peppermint (*Mentha arvensis* L.) belongs to the Lamiaceae family. It is a widely used medicinal herb in our country, utilized in both traditional and modern medicine. Peppermint grows wild and is cultivated in many regions of Vietnam. Modern scientific research has shown that peppermint contains a large amount of essential oil with many valuable biological activities. Peppermint essential oil has antioxidant properties [7,2], antibacterial and antifungal activities [5,6], and insecticidal effects [1]. The main components of peppermint essential oil include menthol at a concentration of 40-50%, mentone at 10-20%, along with several other flavonoids. Due to its prominent antibacterial properties and distinctive aroma, peppermint essential oil is widely used in the production of oral health care products such as mouthwash and toothpaste, which are used to treat gum inflammation, mouth ulcers, and toothaches [3,8]. Additionally, peppermint is used as a soothing medicine, to induce sweating, reduce fever, and relieve colds; it helps stimulate digestion, alleviate indigestion; and serves as a treatment for stomach ulcers by reducing gastric secretion and

* Date received: 24/10/2024
* Date accepted: 15/12/2024

relieving pain [9,4]. This paper presents the results of a study on the antibacterial activity of the peppermint variety (Mentha arvensis L. cv. DL97) grown according to GACP-WHO guidelines in Hanoi.

II. METHODS

2.1 Research Subjects

- Peppermint *Mentha arvensis* L. cv. DL97 (BhGACP-WHO) was cultivated and harvested according to GACP-WHO guidelines in June 2024 in Hanoi.

- Other varieties include Sapa peppermint (Bh01), European peppermint (Bh25), Asian peppermint (Bh50), and sw88 peppermint (Bh100).

2.2. Extraction of Essential Oil

Fresh peppermint plants (1 kg) were harvested at flowering stage, chopped, and distilled using steam distillation for 4 hours at normal pressure.

2.3. Determination of Antibacterial Activity of Peppermint Essential Oil

The antibacterial activity of peppermint essential oil was assessed using the disc diffusion method on antibiotic paper (Nguyen et al., 2015; CLSI, 2019) with modifications. The preparation of instruments, chemical solutions, and the experimental procedure are as follows:

Equipment and environment (Solvent): Use Whatman filter paper with a diameter of 1 cm, put it in a fancol tube, Tighten it tightly so that when sterilizing it will not get the paper wet

Making LB liquid environment (solvent) (g/L): Pepton 10, Yeast glue 5, NaCl 5 to conduct bacterial activation culture in a 100 mL Erlenmeyer flask and condensed LB environment: Pepton 10, Yeast glue 5, NaCl 5, Agar 15 to pour on a petri dish to determine the antibacterial ring of the essential oil

Sterilize distilled water to make Kanamysin solution with concentration of 10 pg/mL

Equipment and environment are sterilized at 121oC for 20 minutes using an autoclave (CL 40LDP, ALP, Japam)

Chemical mixing: Weigh exactly 100 mg of Kanamysin and mix it in 10 mL of sterile distilled water

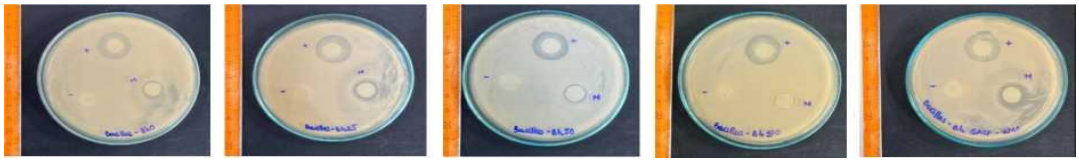
Peppermint essential oil samples have symbols written on the petry dish respectively Bh01, Bh25, Bh50, Bh100, BhGACP-WHO diluted three times with pure methanol

Conduct experiments: Aspirate 150 pL of activated cultured test bacterial strains liquid (24 hours, 37oC, number of shaking cycles is 150 rpm), Spread the inoculation with a stick on solid LB medium until the surface is dry. Place 3 antibiotic paper rings on the agar surface, spacing them evenly. Aspirate 10 pL of sample and drop onto antibiotic paper. The control samples are respectively Positive control Kanamycin 10 pg/mL, diluted Peppermint essential oil sample and negative control pure

methanol. Next, the petri dishes were kept cool for 2-3 hours to allow the essential oils to diffuse onto the agar surface before being incubated at 37ºC for 24 hours. After incubation, the diameter of the sterile zone (D- d) is determined by the difference between the diameter of the outer resistant zone (D, mm) and the diameter of the paper disc (d= 10mm). When D-d > 0mm, the essential oil is considered to have antibacterial properties. The recorded result D is the average of 3 repeated measurements on the same experimental unit.

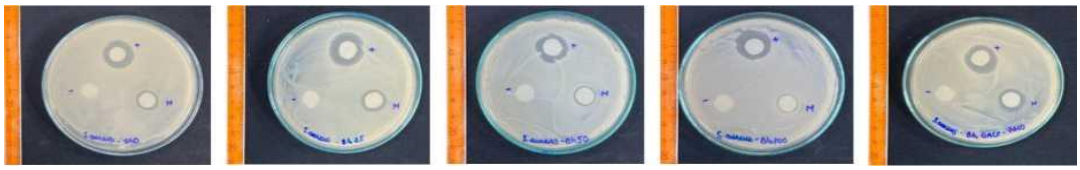
III. RESULTS AND DISCUSSION

The antibacterial ability of Peppermint essential oil is determined based on its ability to inhibit the growth of tested microorganisms. The appearance of a sterile ring around the antibiotic paper disc may be due to the antibacterial active substances in Peppermint essential oil that inhibit the growth of bacteria, as shown by the diameter of the antibacterial ring shown in Picture 1. and Table 1



Bh01 *Bh25* *Bh50* *Bh100* *BhGACP-WHO*

Antibacterial activity of essential oils against Bacillus subtilis bacteria



Bh01 *Bh25* *Bh50* *Bh100* *BhGACP-WHO*

Antibacterial activity of essential oils against Staphylococcus aureus bacteria

Bh01 *Bh25* *Bh50* *Bh100* *BhGACP-WHO*

Antibacterial activity of essential oils against Escherichia coli bacteria

Picture 1. Antibacterial activity of five essential oils against tested microorganisms

Table 1. Sterile circle diameter (D-d, mm) of essential oil for tested microbial strains

Sample	*B. subtilis*	*S. aureus*	*E. coli*
Bh01	2,7±0,05	4 ±0,05	2,8±0,05
Bh25	4±0,1	3,7 ±0,05	4,7±0,05
Bh50	1,7±0,05	2±0,0	2±0,05
Bh100	1±0,0	1,5±0,0	2±0,01
BhGACP-WHO	**8,3 ±0,1**	**5,3 ±0,05**	**6,7±0,05**
Kanamysin (10pg/mL)	9,2±0,02	8,4±0,099	9,8 ±0,18

The study conducted to determine the antibacterial ability of 5 essential oils: Bh01, Bh25, Bh50, Bh100, BhGACP-WHO on 3 bacterial strains: Bacillus subtilis, Escherichia coli, and

Staphylococcus aureus. The results shown in Picture 1 and Table 1 show that BhGACP-WHO essential oil has a good effect on all experimental bacterial strains. In general, all essential oils were tested, and all 5 samples had antibacterial ability. For the two Gram-positive strains of bacteria (*B. subtilis* and *S. aureus),* the antimicrobial rings of the BhGACP-WHO samples were 8.3 mm and 5.3 mm, respectively, for Gram-negative bacteria (*E. coli)* were 6.7 mm, and both were lower than the control of Kanamysin (10 pg/mL). In this study, BhGACP-WHO essential oil showed the best antibacterial properties among the 5 types of Peppermint essential oils that could be related to the quality of essential oils (with high content of Menthol and Trans-(-)-p-Menthan-3-one up to 69.89% and 13.02%). Comparing this result with the publication of Tong Thi Anh Ngoc et al., (2020) on "Antibacterial ability of essential oils against some pathogenic bacteria in food" [2] shows that BhGACP-WHO has higher antibacterial activity, specifically for *E. coli* ATCC 25922 (more than 4.9 ±0.55mm), *E. coli* 92E (more than 5.7 ±0.85mm), *E. coli* 80E (more than 5.7±0.05mm), *E. coli* 74E (more than 4.7±0.05mm) and *S. aureus* (4±1.15mm).

The quality of essential oils greatly affects the antibacterial ability. The antibacterial ability of Peppermint essential oil is one of the important activities, helping to orient the application of this

essential oil in the development of human health care products or food preservation. The product will be even better if the source of raw materials is safe. Therefore, the trend of growing medicinal herbs according to GACP-WHO is inevitable.

IV. CONCLUSION

Investigating the antibacterial ability of 5 samples of Peppermint essential oil for 3 tested microbial strains, namely *B. subtilis*, *S. aureus*, E. *coli* by sterile ring, the results showed that all 5 samples of Peppermint essential oil showed antibacterial activity against 3 experimental bacterial strains, in which the antimicrobial activity is Bh100 < Bh50 < Bh01 < Bh25 < BhGACP - WHO. BhGACP-WHO essential oil has a good effect on all experimental bacterial strains. For the two Gram-positive strains of bacteria (*B. subtilis* and *S. aureus*), the antimicrobial rings of the BhGACP-WHO samples were 8.3 mm and 5.3 mm, respectively, and for Gram-negative bacteria (E. coli) it was 6.7 mm.

The paper is supported by the project "Research and completion of the process of planting, harvesting and preliminary processing of mint varieties (*Mentha arvensis* L. cv. DL97) according to GACP-WHO guidelines in Hanoi"

REFERENCES

1. Ministry of Health. (2015). Chapter IV, Vietnamese Pharmacopoeia, Fourth Edition, Medical Publishing House, Hanoi, pg. 684-685

2. Ministry of Health. (2019). Circular No. 19/2019/TT-BYT issued on July 30, 2019 regulating good practices in cultivation and collection of medicinal herbs and principles and standards for exploitation of natural medicinal herbs.

3. Nguyen Thi Mai Huong, Ho Tuan Anh. (2017). Research on the antibacterial activity of some essential oils. Vietnam Journal of Agricultural Science and Technology (No. 7(80)/2017), pg.57-62

4. Nguyen Van Liem, Nguyen Thi Thanh Van, Tran Thi Thu Huong, Nguyen Thi Thu Hang, Nguyen Thi Thu Trang, Nguyen Thi Thu Ha, Nguyen Thi Thu Nga, Nguyen Thi Thu Ngoc, Nguyen Thi Thu Quyen, Nguyen Thi Thu Quynh, (2022), Research on Selection and Breeding of Mint DL97, Vietnam Journal of Agricultural Science and Technology (No. 10(2)), pg. 152-157

5. Tong Thi Anh Ngoc, Nguyen Cam Tu, Nguyen Cong Ha. (2020). Antibacterial ability of essential oils against certain types of pathogenic bacteria in food.

Journal of Agriculture and Development (No. 9(4)), pg. 64-72.
6. Huynh Xuan Phong, Mai Kim Ngan, Tran Thi Thao Nguyen, Luu Minh Chau, Nguyen Ngoc Thanh, Bach Long Giang, Mai Huynh Cang, Nguyen Tan Dung, Nguyen Van Ai. (2021). Chemical composition and anti-pathogenic microbial activity of essential oil of pomelo peel. Journal of Science of Can Tho University (vol.57, issue of Food Technology (2021)), pg.189-195
7. Tran Bao Tram, Nguyen Thi Hien, Truong Thi Chien, Phan Xuan Binh Minh, Nguyen Thi Thanh Mai, Hoang Quoc Chinh, Vu Xuan Tao. (2021). Evaluation of chemical composition and some biological activities of essential oil of Peppermint (*Mentha arvensis* L.) grown in Vietnam. Vietnam Journal of Science & Technology (No. 63(7) 7/2021), pg. 26-30. DOI: 10.31276/VJST(7).26-30
8. Nguyen Cong Thuy Tram, Ninh The Son, Nguyen Manh Cuong, Nguyen Thi Cuc, Do Thi Thao. (2017). Evaluation of in vitro antioxidant activity of extract segments and compounds isolated from wild pineapple fruit (Pandanus oddoratissimus L. F.). Military Journal of Medicine and Pharmacology (No. 6-2017). pg. 5-12
9. Zhao, S. Ren, H. Yang, S. Tang, C. Guo, M. Liu, Q. Tao, T. Ming & H. Xu. (2022). Peppermint essential oil: its phytochemistry, biological activity, pharmacological effect and application. Biomedicine & Pharmacother (volume 154, 10/2022), 113559. DOI: 10.1016/j.biopha.2022.113559