ANTI-BACTERIAL ABILITY OF ROSEMARY ESSENTIAL OIL (*ROSMARINUS OFFICINALIS)*

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SUMMARY

Rosemary herb has been widely used in medicine and traditional cosmetics. In addition, it is also used as a flavoring agent in foods. The results of this study show that by steam distillation, the yield of rosemary essential oil is 2.343%. At the same time, the active ingredients in essential oils were identified quite high such as α-Pinene (29.84%), 1,8-cineol (22.456%) and Camphor (2.965%). The anti-bacterial ability of rosemary essential oil was determined with the zone of inhibition for *E. coli* strain of 13.4 mm and *S. aureus* of 9.6 mm. Simultaneously, the MIC and MBC values of both *E. coli* and *S. aureus* strains were 0.032 mg/ml and 0.064 mg/ml, respectively.

Keywords: Anti-bacterial, Component, Essential oil, MIC value, MBC value, Rosemary herb

INTRODUCTION

Rosemary (*Rosmarinus officinalis*) native to the Mediterranean is widely used for ornamental, food and medicinal purposes. In Vietnam, Rosemary is a new ornamental plant and there are not many studies on its nutrition (Pham Thi Minh Tam, Nguyen Thi Bich Phuong, 2018).

The main chemical composition of rosemary essential oil with antibacterial ability has been determined by many previous studies such as the results of Boutekedjiret *et al.,* (2003), analysis by gas chromatography-mass spectrometry method (GC-MS), the composition of substances include α-Pinene (5.2%), Camphene (3.0%), 1.8-1.8-cineol (52.4%), Linalol (1.1%), Camphor (12.6%); Research by Hussain AI *et al.* (2010) has identified the main components of the essential oil as 1,8-cineol (38.5%), camphor (17.1%), α-pinene (12.3%), limonene (6.23%), camphene (6.00%) and linalool (5.70%); Research by Tavassoli *et al.,* (2011) analyzed the composition of Rosemary essential oil including 1,8-1.8-cineole (23.14%), camphor (12.35%), α-pinene (9.87%), β-pinene (6.10%), borneol (5.61%), camphene (5.58%) and α-terpineol (4.30%).

At the same time, Santoyo *et al.,* (2004) tested the antibacterial activity of rosemary essential oil on 6 different microbial species including 2 gram-positive bacteria (*Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633), 2 species of gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 10145, *Escherichia coli* ATCC 11775), yeast (*Candida albicans* ATCC 60193), and mold (*Aspergillus niger* ATCC 16404). Essential oils against all bacteria tested with inhibition rings and MBC values between 17 - 33 mm and 2.25 - 0.25 mg/ml, respectively. *Staphylococcus aureus* is the most sensitive strain, having the zone of inhibition (27 - 33 mm) and the lowest MBC value (0.75 - 0.25 mg/ml).

In 2021, Hezil *et al.,* studied Rosemary essential oil from Hammamet region (Tébessa - Algeria), obtained essential oil content ranging from 1.60 ± 0.004 ml/100g to 2.29 ± 0.041 ml/100g and evaluated antibacterial activity of essential oils. The zone of inhibition for *S. aureus* was 17.1mm (pure essential oil), 10.1mm (dilution ½), 8.2mm (dilution ¼). As for *E. coli*, the zone of inhibition was 17.2mm (pure), 8.1mm (dilution ½), 7.3mm (dilution ¼).

From the above studies, we evaluated the antibacterial ability of the essential oil extracted from *Rosmarinus officinalis* grown in Vietnam.

MATERIALS AND METHODS

Materials

Rosemary leaves (*Rosmarinus officinalis*) are supplied from Viet Phu Company, No. 413/23 Nguyen Tu Luc Street, Da Lat City, Lam Dong Province, Vietnam. Harvest time November, 2022. Age of the plant is 2 years old.

Methods

Samples were washed to remove adhering dust, allowed to dry naturally at room temperature, and then cut into 2 cm pieces. Extraction of essential oils by steam distillation method, the raw materials are placed in a 2000ml spherical glass bottle with 1000ml of distilled water, enough water to cover the raw materials, assemble the complete condenser system, distillation time is about 3 hours at 80^{o}C. Then collect the essential oil layer floating on the liquid surface. Gas chromatography-mass spectrometry (GC-MS) was used to determine the chemical composition of rosemary essential oil, Mass spectrometry probe has high sensitivity of about 10^{-6} – 10^{-9} g (Mahawer *et al.,* 2022).

Evaluation of the antibacterial ability of rosemary essential oil: Determination of antibacterial ability of rosemary essential oil by Disk diffusion test, according to the guidelines M44 – A and M02 – A11 of CLSI (Leber, 2016), based on the zone of inhibition. The test was performed for two strains of bacteria, *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922, which were repeated 5 times. Determine The minimum inhibitory concentration for bacteria (MIC) of Rosemary essential oil by dilution in solid medium (Leber, 2016), which were repeated 5 times. The minimum bactericidal concentration (MBC) of Rosemary essential oil is determined based on MIC-performed agar plates, with different concentrations such as equal to, twice and 4 times the MIC concentration.

RESULTS AND DISCUSSION

Extraction efficiency of Rosemary essential oil

By steam distillation, the yield of essential oil was 2.343% (table 1), much higher than the study of Mathlouthi *et al.,* (2012) with the yield of 0.93% and 0.35%, respectively. While compared with the study of Ojeda-Sana *et al.,* (2013), the efficiency is lower but not significant (2.58%).

Table 1. Extraction efficiency of Rosemary essential oil

Initial sample amount (g)	*Amount of essential oil obtained (g)*	*Efficiency (%)*
101.27 ± 0.041	2.372 ± 0.001	2.343 ± 0.008

Active ingredients in rosemary essential oil

By GC/MS method, 26 compounds in Rosemary essential oil have been identified (table 2), including substances such as α-Pinene (29.084%), Eucalyptol (1.8-cineol) (22.456%) and (+)-2-Bornaone (Camphor) (2.965%) and some others.

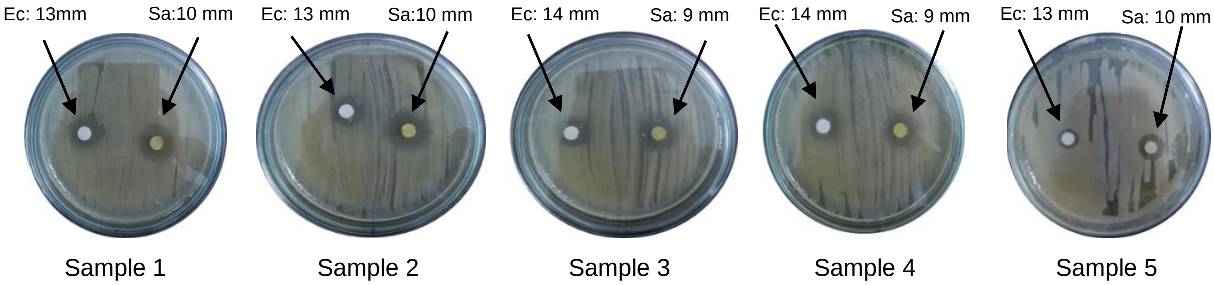
Table 2. Composition of active ingredients in essential oils

Serial	Rt	Substance name	Content (%)	Mass	Mass spectral compatibility
1	5.091	1R-α-Pinene	29.084	136	939
2	5.339	Camphene	3.108	136	939
3	5.415	2,4(10)-Thujadiene	0.575	134	949
4	5.820	β-Pinene	1.923	136	922
5	5.963	β-Myrcene	1.026	136	945
6	6.673	o-Cymene	0.761	134	957
7	6.774	D-Limonene	2.499	136	933
8	6.852	Eucalyptol (cineol)	22.456	154	950
9	7.374	γ-Terpinene	0.802	136	932
10	8.035	α-Terpinolen	0.569	136	926
11	8.211	Linalool	2.842	154	936
12	8.889	Chrysanthenone	0.475	150	914
13	9.395	(+)-2-Bornaone	2.965	152	934
14	9.820	Pinocarvone	0.298	150	831
15	9.897	Camphol	4.674	154	935
16	10.114	Isocamphopinone	0.478	152	884
17	10.151	Terpinen-4-ol	1.241	154	914
18	10.466	Terpineol	2.491	154	935
19	10.630	Myrtenol	0.470	152	893
20	10.757	Isobomeol	0.557	154	788
21	10.986	(-)-Verbenone	11.828	150	951
22	11.958	Geraniol	4.349	154	935

23	12.848	Bomyl acetate	3.102	196	935
24	15.174	2,6-Dimethyl-2,6-octadien-8-yl acetate	0.440	196	911
25	15.717	Methyleugenol	0.307	178	918
26	16.290	Caryophyllene	0.682	204	915

Antibacterial properties of rosemary essential oil

The results showed that rosemary essential oil has the ability to create antibacterial rings for both *E. coli* and *S. aureus* strains when cultured (Figure 1).



Ficture 1. Culture results (*Ec: Escherichia coli* ATCC 25922 and *Sa: Staphylococcus aureus* ATCC29213)

The average diameter of the antibacterial ring of essential oils for *E. coli* is 13.4 mm. This result is 14.3 mm higher than the study result of Mekonnen *et al.,* (2016) at 6 mm. At the same time, the research results on *S. aureus* also had an average antibacterial ring diameter of 9.6 mm, much higher than that of Mathlouthi *et al.,* (2012).

Table 3. Antibacterial ring diameter of rosemary essential oil

The zone of inhibition (mm)	
E. coli	***S. aureus***
13.4 ± 0.054	9.6 ± 0.044

The research results showed that The minimum inhibitory concentration (MIC) of Rosemary essential oil for *E. coli* and *S. aureus* was also negative for in plates with MIC concentrations from 0.032 - 0.256 mg/ml and positive for plates with MIC concentrations of 0.016 mg/ml (table 4). Research results show that the MIC values of *E. coli* and *S. aureus* are lower than some previous studies. However, when compared with the results of Mekonnen *et al.,* (2016), the results were opposite with the MIC value of *S.aureus* at 0.02 mg/ml.

Table 4. MIC value

MIC (mg/ml)	**Positive rate (%)**	
	E. coli	***S. aureus***
0.256	-	-
0.128	-	-
0.064	-	-
0.032	-	-
0.016	100	100

The Minimum Bactericidal Concentration (MBC) of Rosemary essential oil for *E. coli* and *S. aureus* were both 0,064 mg/ml.

Table 5. MBC value

MBC (mg/ml)	**Positive rate (%)**	
	E. coli	***S. aureus***
0.128	-	-
0.064	-	-
0.032	100	100
0.016	100	100

From the above results, rosemary essential oil has a high bactericidal ability with MBC/MIC value < 4. This result is similar to the study results of Gatsing *et al.,* (2009).

CONCLUSION

The extraction efficiency of rosemary essential oil was 2.343%. The identification of active ingredients in essential oils such as α-Pinene (29.084%), 1,8-cineol (22.456%) and Camphor (2.965%) is quite high. The antibacterial ability of essential oils was evaluated on two strains of *E. coli* and *S. aureus* based on the antibacterial ring diameter of 13.4 mm and 9.6 mm, respectively. At the same time, the MIC and MBC values of rosemary essential oil were also determined on *both E. coli* and *S. aureus* strains with the same results of 0.032 mg/ml and 0.064 mg/ml.

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KHẢ NĂNG KHÁNG KHUẨN CỦA TINH DẦU HƯƠNG THẢO (*ROSMARINUS OFFICINALIS*)

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TÓM TẮT

Hương thảo đã được sử dụng rộng rãi trong y học và mỹ phẩm truyền thống. Ngoài ra, nó còn được sử dụng làm chất tạo hương vị trong thực phẩm. Kết quả nghiên cứu này cho thấy bằng phương pháp chưng cất hơi nước, hiệu suất thu được tinh dầu hương thảo là 2,343%. Đồng thời, các hoạt chất trong tinh dầu được xác định khá cao như α-Pinene (29,084%), 1,8-cineol (22,456%) và Long não (2,965%). Khả năng kháng khuẩn của tinh dầu hương thảo được xác định với đường kính vòng kháng khuẩn đối với *E. coli* là 13,4 mm và *S.aeureus* là 9,6 mm. Đồng thời, giá trị MIC và MBC của cả hai chủng *E. coli* và *S. aureus* lần lượt là 0,032 mg/ml và 0,064 mg/ml.

Từ khóa: Kháng khuẩn, thành phần hóa học, tinh dầu, giá trị MIC, giá trị MBC, hương thảo.

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