ANTIMICROBIO ACTIVITY OF ESENTIAL OILS FROM LEAF OF SYZYGIUM TSOONGII (MERR.) MERR. & PERRY, SYZYGIUM BULLOCKII (HANCE) MERR. & PERRY AND SYZYGIUM ZEYLANICUM (L.) DC. IN HA TINH PROVINCE

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ABSTRACT

Research on antimicrobial activity of essential oils from leaf of *Syzygium tsoongii*, *Syzygium bullockii* and *Syzygium zeylanicum* in Ha Tinh Province showed that essential oils from leaves of three species had antibacterial activity against three strains on the Gram (+): (*Enterococus faecalis* (ATCC 299212), *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 14579) and against *Candida albicans* (ATCC 10231). The essential oil from the leaves of *Syzygium tsoongii* and *Syzygium zeylanicum* had not antibacterial activity against Gram (-), while the essential oil from the leaves of *Syzygium bullockii* could be against 2 strains of Gram (-): *Escherichia coli* and *Salmonella enterica* strains.

Keywords: Antimicrobial activity; Ha Tinh; *Syzygium bullockii; Syzygium tsoongii; Syzygium zeylanicum.*

1. Introduction

In the world, there are about 1,200 species of genus *Syzygium* distributed in tropical and subtropical countries [1], [3]. In the Indochina region, 27 species have been recorded in Cambodia, 30 species in Laos and 49 species in Vietnam [4]. In Vietnam, the genus *Syzygium* has been recorded with 60 species and 1 variety [5]. There are many species in this genus that have been used medicinally [6], [8]. In the world, a number of species in the genus such as *Syzygium polyanthum* and *Syzygium aromaticum* have been studied for their chemical composition and antimicrobial activity of essential oils. The main components in the essential oil from the leaves of *S. polyanthum* are cis-4-decanal (43.4%), 1-decyl aldehyde (19.7%), capryl aldehyde (14.9%). Meanwhile, the main ingredients in essential oil from *S. aromaticum* leaves are p-eugenol (75.1%) and β-caryophyllene (18.3%) [9]. In Vietnam, the chemical composition of essential oils and the uses of some species have also been studied such as Clove *(Syzygium aromaticum)* whose essential oil from flower buds contains the compounds eugenol (70-95%), eugenyl acetate (1-5%) and β-caryophyllene (4-12%), and essential oil from Gioi

leaves *(Syzygium jambos)* has main active ingredients including α-pinene (26.8%) and L-limonene (23.8%) [10]. The main chemical components in the leaf essential oil of *Syzygium tsoongii* are β-caryophyllene (23.40%), bicyclogermacrene (21.23%), (Z)-β-ocimene (10.61%), α-humulene (6.33%) [11]; Leaf essential oil of the species *Syzygium bullockii* are β-caryophyllene (49.65%), spathulenol (4.29%), caryophyllene oxide (4.14%), bicyclogermacrene (3.35%), 2-tridecanone (3.25%), *α*-humulene (2.78%) [11], while the leaf essential oil of *Syzygium zeylanicum* is bicyclogermacrene (25%), β-caryophyllene (20.14%), *α*-pinene (7.66%) [12]. Currently in Vietnam, as to our knowledge, there is no published research on the antibacterial activity of essential oils from the leaves of *Syzygium tsoongii* (Merr.) Merr. & Perry, *Syzygium bullockii* (Hance) Merr. & Perry and *Syzygium zeylanicum* (L.) DC.

2. Research materials and methods

2.1. Materials

Essential oils from the leaves of *Syzygium bullockii*, *Syzygium tsoongii* and *Syzygium zeylanicum* were collected in Ha Tinh Province.

Gram (+) bacterial strains were used: *Enterococus faecalis* (ATCC 299212), *Staphylococcus aureus* (ATCC 25923) and *Bacillus cereus* (ATCC 14579);

Gram (-) bacterial strains were used: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Salmonella enterica* (ATCC 13076);

Candida albicans strain (ATCC 10231) was used.

2.2. Research methods

Antimicrobial activity of essential oils on a number of Gram (+) bacterial strains has been tested, including: *Enterococus faecalis* (ATCC 299212), *Staphylococcus aureus* (ATCC 25923) and *Bacillus cereus* (ATCC 14579); Same with Gram (-) bacterial strains: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella enterica* (ATCC 13076) and *Candida albicans* strain (ATCC 10231).

Antimicrobial activity was tested based on the multi-concentration dilution method. This method allows testing the antibacterial and antifungal activity to evaluate the strength and weakness of the test samples against microorganisms through values representing activity as MIC (minimum inhibitory concentration). The initial sample was diluted in DMSO (dimethylsulfoside) at decreasing concentration ranges: 256 µg/ml, 128 µg/ml, 64 µg/ml, 32 µg/ml, 16 µg/ml, 8 µg/ml, 4 µg/ml and 2 µg/ml with the number of repeated experiments N = 3. Bacterial or fungal solutions are prepared with a concentration of 2×10^5 CFU/ml [14], [15].

2.3. Testing sequence

Took 5.12 µl of sample solution with a concentration of 10 mg/ml into the first row containing 100 µl of Luria-Bertani broth (LB broth) medium. Then two-fold dilutions of the sample solution into rows containing 50 µl until a concentration of 2 µg/ml was reached (1, 2, 4, 8, 16, 32, 64, 128 and 256 µg/ml), added each well was inoculated with 50 µl microbial inoculum at 10^5 CFU/mL, incubation at 37^{o}C.

After incubation for 24 hours at 37^{o}C, minimum inhibitory concentrations (MIC) were determined by observation. MIC was determined as the lowest test compound concentration completely inhibiting spore germination after 24 hours and MIC was determined accurately based on cell turbidity measurement data using a Bioteck spectrophotometer and Raw data software. The streptomycin was the control for bacterial strains and cyclohexamide was the control for fungi [13], [14].

The IC_{50} value is determined at the lowest concentration of test substance that inhibits 50% of microbial growth after 24 hours of culture and is determined accurately based on cell turbidity measurement data using a Bioteck spectrometer and Raw data software. The percentage inhibition of bacterial growth in the presence of the test substance will be determined through the following formula [13], [14]:

% Inhibition = 100% - [OD (sample) - OD (bacteria without test substance)]/[OD (DMSO) - OD (bacteria without test substance)].

Note: Take positive numbers in the ratio: [OD (sample) - OD (bacteria without test substance)]/[OD (DMSO) - OD (bacteria without test substance)].

3. Results and discussions

3.1. Antibacterial activity against Gram (+) bacterial strains

The data in Table 1 shows that essential oils from the leaves of the 3 studied species have the same ability to resist 3 strains of Gram (+) bacteria, including *Enterococus faecalis* (ATCC 299212), *Staphylococcus aureus* (ATCC 25923) and *Bacillus cereus* (ATCC 14579). Among them, the essential oil of the leaves of *Syzygium bullockii* has stronger resistance than the two others and reaches the MIC value 32 μg/ml (IC_{50} = 10.23 μg/ml) for strain *Enterococus faecalis*, MIC = 32 μg/ml (IC_{50} = 6.78 μg/ml) for *Staphylococcus aureus* and MIC = 64 μg/ml (IC_{50} = 29.78 μg/ml) for *Bacillus cereus* (Figure 1). This result explains why local people often use the leaves of the above plants to treat some common diseases such as toothache, diarrhea, dysentery, worm treatment, ringworm, etc. [6], [8].

Table 1: *Determination of MIC và IC_{50} of leaf essential oil against Gram (+) bacterial strains*

No	**Test sample (Essential oil)**	**Minimum inhibitory concentration MIC (μg/ml)**		
		E. faecalis	*S. aureus*	*B. cereus*
1	*Syzygium tsoongii*	64	64	64
2	*Syzygium bullockii*	32	32	64
3	*Syzygium zeylanicum*	32	128	128
IC_{50} (μg/ml)				
4	*Syzygium tsoongii*	19.10	17.89	19.87
5	*Syzygium bullockii*	10.23	6.78	29.78
6	*Syzygium zeylanicum*	10.34	45.78	48.87

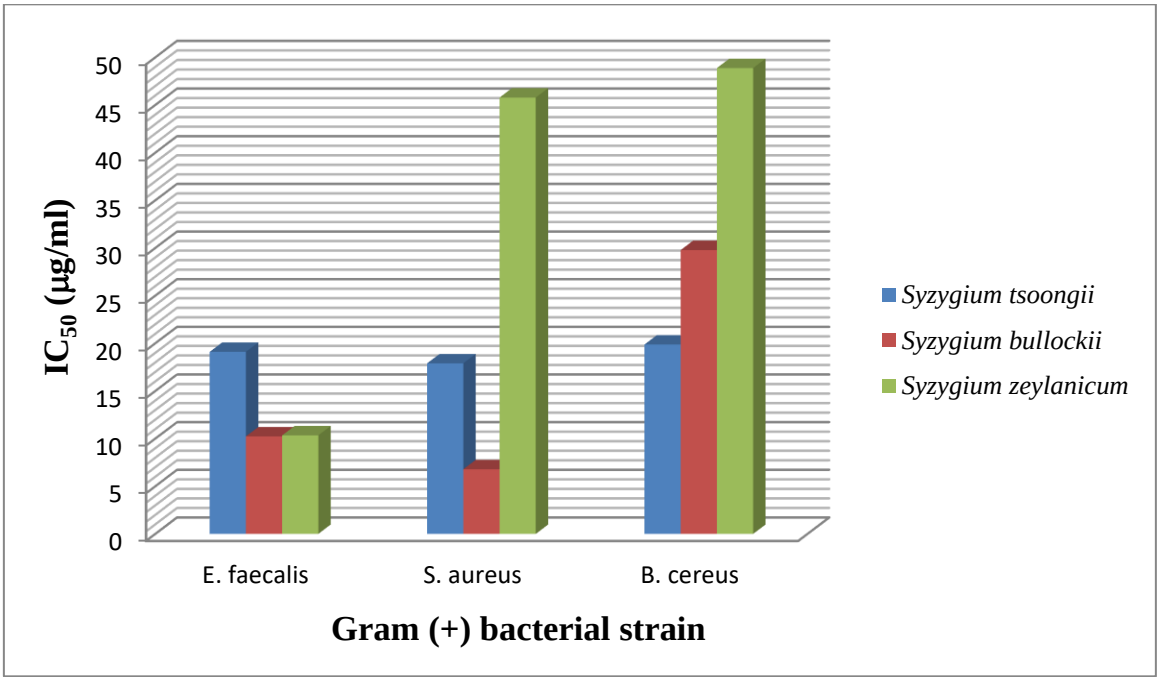

Figure 1: *Antibacterial activity against Gram (+) bacterial*

3.2. Antibacterial activity against Gram (-) bacterial strains

The results in Table 2 show that the essential oil from the leaves of *Syzygium bullockii* has the ability to resist *Escherichia coli* strains at MIC = 128 µg/ml and IC50 = 46.67 mg/ml, and is also resistant to *Salmonella enterica* (ATCC 13076) at MIC = 256 µg/ml and IC_{50} = 120.78 µg/ml. The remaining two species, including *Syzygium tsoongii* and *Syzygium zeylanicum*, are not resistant to Gram (-) strains, including *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and *Salmonella enterica* (ATCC 13076) (Figure 2).

Table 2: *Determination of MIC và IC_{50} of leaf essential oil against Gram (-) bacterial strains*

No	Test sample (Essential oil)	Minimum inhibitory concentration MIC (µg/ml)		
		E. coli	*P. aeruginosa*	*S. enterica*
1	*Syzygium tsoongii*	(-)	(-)	(-)
2	*Syzygium bullockii*	128	(-)	256
3	*Syzygium zeylanicum*	(-)	(-)	(-)
IC_{50} (µg/ml)				
4	*Syzygium tsoongii*	(-)	(-)	(-)
5	*Syzygium bullockii*	46.67	(-)	120.78
6	*Syzygium zeylanicum*	(-)	(-)	(-)

Note: (-): Not determined

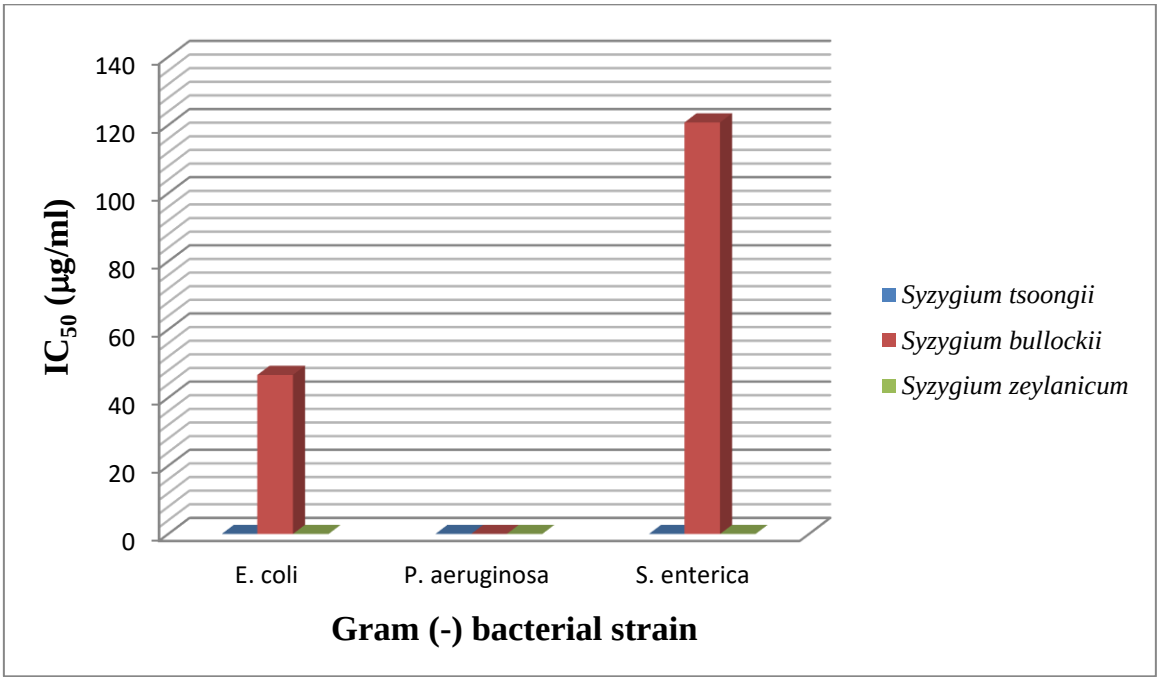

Figure 2: *Antibacterial activity against Gram (-) bacterial*

3.3. Antibacterial activity against Candida albicans (ATCC 10231)

The data in Table 3 shows that the essential oils from the leaves of the three studied species have the same ability to resist the yeast strain *Candida albicans* (ATCC 10231). Among them, essential oil from the leaves of *Syzygium bullockii* (Hance) Merr. & Perry has the strongest resistance and reaches MIC value 64 µg/ml (IC_{50} = 28.78 µg/ml). Next is the essential oil from the leaves of *Syzygium tsoongii* (Merr.) Merr. & Perry, reaching MIC = 128 µg/ml (IC_{50} = 45,67 µg/ml) and finally the essential oil from the leaves of *Syzygium zeylanicum* (L.) DC., eaching MIC value 256 µg/ml (IC_{50} = 78.78 µg/ml) (Figure 3). The results of this study show the potential for bioactive essential oils from the leaves of the three studied species to be used to treat some diseases related to the pathogens tested above.

Table 3: *Determination of MIC và IC_{50} of leaf essential oil against Candida albicans (ATCC 10231)*

No.	**Test sample (Essential oil)**	**Minimum inhibitory concentration MIC (µg/ml)**
		Candida albicans
1	*Syzygium tsoongii*	128
2	*Syzygium bullockii*	64
3	*Syzygium zeylanicum*	256
IC_{50} (µg/ml)		
4	*Syzygium tsoongii*	45.67
5	*Syzygium bullockii*	28.78
6	*Syzygium zeylanicum*	78.78

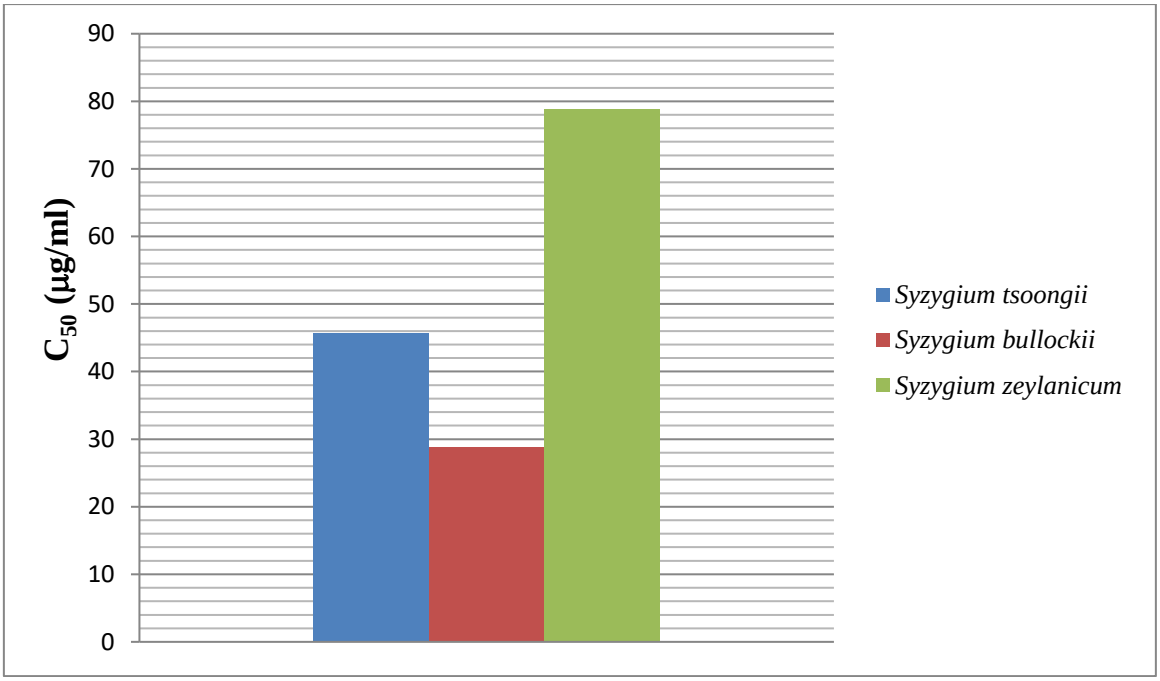

Figure 3: *Antibacterial activity against Candida albicans (ATCC 10231)*

The above research results show promising potential when investing in exploiting the medicinal properties of essential oil from the leaves of *Syzygium bullockii* in therapeutic effects with compounds contained in the essential oil of the leaves of this species such as: *β*-caryophyllene (49.65%), spathulenol (4.29%), caryophyllene oxide (4.14%), bicyclogermacrene (3.35%), 2-tridecanone (3.25%), *α*-humulene (2.78%) [12]. This is the initial result of testing the biological activity of essential oils from the leaves of the species *Syzygium tsoongii*, *Syzygium bullockii* and *Syzygium zeylanicum*. Future studies can evaluate antioxidant activities, cytotoxic activities, tumor inhibition activities, etc. to confirm the beneficial effects of essential oils from the leaves of the above species based on the results obtained in this study. From the essential oils of the leaves of these three species, it is possible to prepare a number of common medicinal drugs related to the pathogens tested above, especially from the leaf essential oil of *Syzygium bullockii* (Hance) Merr. & Perry, which has greater potential than the other two species.

4. Conclusion

Research results show that essential oils from the leaves of all three species have the ability to resist Gram (+) bacterial strains including: *Enterococus faecalis* (ATCC 299212), *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 14579) and is resistant to *Candida albicans* (ATCC 10231). Besides, the essential oil from the leaves of *Syzygium bullockii* species, in addition to the above abilities, also has the ability to resist 2 additional Gram (-) strains including: *Escherichia coli* (ATCC 25922) at MIC value 128 µg/ml and IC_{50} = 46.67 µg/ml; and *Salmonella enterica* (ATCC 13076) at MIC value 256 µg/ml and IC_{50} = 120.78 µg/ml.

The research results serve as a scientific basis for orienting further research on chemistry and provide additional information about the bioactivities of the essential oils of the leaves of *Syzygium tsoongii*, *Syzygium bullockii* and *Syzygium zeylanicum* in Ha Tinh Province.

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TÓM TẮT

HOẠT TÍNH KHÁNG VI SINH VẬT CỦA TINH DẦU TỪ LÁ LOÀI TRÂM TRÁI TRẮNG (*SYZYGIUM TSOONGII* (MERR.) MERR. & PERRY), TRÂM BULLOCK (*SYZYGIUM BULLOCKII* (HANCE) MERR. & PERRY) VÀ TRÂM TÍCH LAN (*SYZYGIUM ZEYLANICUM* (L.) DC.) Ở HÀ TĨNH

Trần Hậu Khanh
Sở Khoa học và Công nghệ Hà Tĩnh, Việt Nam
Ngày nhận bài 11/8/2023, ngày nhận đăng 25/8/2023

Nghiên cứu hoạt tính kháng vi sinh vật của tinh dầu từ lá loài Trâm trái trắng (*Syzygium tsoongii*), Trâm bullock (*Syzygium bullockii*) và Trâm tích lan (*Syzygium zeylanicum*) ở Hà Tĩnh cho thấy tinh dầu từ lá của 3 loài nghiên cứu cùng có khả năng kháng 3 chủng vi khuẩn Gram (+): (*Enterococus faecalis* (ATCC 299212), *Staphylococcus aureus* (ATCC 25923) và *Bacillus cereus* (ATCC 14579) và kháng chủng *Candida albicans* (ATCC 10231). Tinh dầu từ lá của loài Trâm trái trắng và Trâm tích lan không có khả năng kháng các chủng vi khuẩn Gram (-), trong khi đó tinh dầu lá của loài Trâm bullock có khả năng kháng 2 chủng vi khuẩn Gram (-), cụ thể: kháng chủng *Escherichia coli* và chủng *Salmonella enterica*.

Từ khóa: Hà Tĩnh; hoạt tính kháng vi sinh vật; Trâm bullock; Trâm tích lan; Trâm trái trắng.