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Research on Endophytic Bacteria in *Houttuynia Cordata* Thunb. With Antibacterial Activity against *Staphylococcus Aureus* from Human Furuncles

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Abstract

Many studies using *Houttuynia cordata* Thunb. for disease treatment are based on the fact that *Houttuynia cordata* Thunb has antibacterial, anti-inflammatory and anti-oxidant activities. However, the study of endophytic bacteria and extracts of endophytic bacteria in *Houttuynia cordata* Thunb with antibacterial activity on *Staphylococcus aureus* from furuncles in humans has not been studied.

The result showed that 231 endophytic bacteria strains that are found in all parts of *Houttuynia cordata* Thunb. such as leaves, stems and roots of *Houttuynia cordata* Thunb. Among 231 strains 65 endophytic bacteria ones in *Houttuynia cordata* Thunb with antibacterial activity on *Staphylococcus aureus* isolated from human furuncles. Thirteen endophytic bacteria strains in *Houttuynia cordata* Thunb which had strongest antibacterial activity on *Staphylococcus aureus* with a sterile ring of 20-33 mm. Endophyic bacteria were identified and belonged to Bacillus genus.

The ethyl acetate extract from selected endophytic bacterial culture (RGT2) determined antibacterial activity on *Staphylococcus aureus* bacteria strain with high concentration of minimal inhibitory extract (MIC) fluctuated from 80 to 160 µg/mL. The minimum bactericidal concentration (MBC) of the extract on the *Staphylococcus aureus* fluctuated from 640 to 1280 µg/mL which are potential in treatment of human diseases of furuncles and skin infections caused by *Staphylococcus aureus*.

Keywords: *Antibacterial, Bacillus sp., endophytic bacteria, furuncle, Houttuynia cordata Thunb.*

1. Introduction

Since the discovery of the first antibiotic, penicillin, by Alexander Flemming, the antibiotic has been widely used in the treatment of human and animal infections. However, the arbitrary use of antibiotics has caused significant harm, including the increase of drug familiarity and resistance. Therefore, it is urgent to discover new antibiotic to destroy pathogenic bacteria, including *Staphylococcus aureus*, a bacteria commonly found on the skin or nasal mucosa with the appearance proportion accounting for 30% of healthy people. When skin is damaged or scratched, *Staphylococcus aureus* gets into the body and may cause a wide range of problems, from mild acne to severe infections, especially in children, the elderly, and those with weak immunity. Most staphylococci are initially sensitive to beta-lactam antibiotics, including penicillin and methicillin. Methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus aureus* have been reported. MRSA outbreaks occurred in Europe in the early 1960s [2].

In nature, there are many plants with antibacterial properties used in medicine to treat diseases so far, including *Houttuynia cordata* Thunb. with its antibacterial, anti-inflammatory, and antioxidant properties, this kind of plant is used as medicine to treat skin diseases, furuncle, impetigo, and bronchitis, etc. As a result, the medical world has recently tended towards antibiotic from medicinal plants and plants. There are currently many studies on endophytic bacteria in medicinal plants and plants, which not only helps plants grow well and produce natural antibacterial compounds [9] but also stimulates the host plant to produce intermediate metabolism compounds [5]. However, the study

of endophytic bacteria in *Houttuynia cordata* Thunb. with antibacterial has not been paid much attention. Therefore, the thesis titled "Isolation of endophytic bacteria in *Houttuynia cordata* Thunb. with antibacterial activity against *Staphylococcus aureus* from human furuncles" is an urge to be studied.

Research objectives

The overall objective of the research is to isolate and identify a number of endophytic bacterial strains in *Houttuynia cordata* Thunb. in ecological regions with antibacterial activity against *Staphylococcus aureus* and determine the antibacterial of the extract from the proliferation culture media of endophytic bacterial strains in *Houttuynia cordata* Thunb. with strongest antibacterial activity against *Staphylococcus aureus*.

2. Materials and methods

2.1 Research instrument

2.1.1. Research subject and scope

Research subject

The research subject mainly focused on Endophytic bacterial strains isolated from *Houttuynia cordata* Thunb. with antibacterial activity against *Staphylococcus aureus*.

Research scope

The scope of the research concentrated on endophytic bacteria strain in *Houttuynia cordata* Thunb. with antibacterial activity against *Staphylococcus aureus* from human furuncles, isolated in Kien Giang, Soc Trang, An Giang, and Can Tho City.

Research time

Research period lasts from December 2015 to December 2019

Research location

The sample of *Houttuynia cordata* Thunb. was collected from vegetable gardens in Phu Quoc, Ha Tien, Rach Gia (Kien Giang Province), Tinh Bien, Phu Tan, and Long Xuyen (An Giang Province), My Xuyen, Chau Thanh, Vinh Chau and Soc Trang City (Soc Trang Province), as well as Binh Thuy, Phong Dien and Ninh Kieu (Can Tho City).

2.1.2. Materials

Houttuynia cordata Thunb. had been identified according to the Vietnamese Pharmacopoeia, and its relevant documents had been recognized by the Department of Pharmacognosy – Botany of the Faculty of Pharmacy, Can Tho University of Medicine and Pharmacy before conducting experiments in the research.

The research used *Staphylococcus aureus* strain isolated from furuncles in humans and stored in the laboratory of the Department of Microbiology of Can Tho University of Medicine and Pharmacy.

2.1.3. Instrument and equipment

Biological safety cabinet CHClab (Korea); Bacteriological incubator Memmert IN110 (Germany); Horizontal shaker Daihan SHR1D (Korea); Centrifuge Hermle Z206A (Germany); Heidolph rotary evaporator (Germany); pH meter Mettler Toledo (Germany); Spectrophotometer Thermo Scientific Multiskan GO, (Finland); Autoclave (Japan), 96-well round bottom plate (Italy) and other instruments.

2.1.4. Chemicals

Chemical for sample processing, chemical for DNA extraction and PCR, Resazurin sodium reagent (Sigma), PDA medium was used to isolate bacteria and test antibacterial activity.

2.2. Research methodology

2.2.1. Methods of collecting and processing samples in the research

Staphylococcus aureus

Staphylococcus aureus was isolated from furuncles provided by the Laboratory of Microbiology Department of Can Tho University of Medicine and Pharmacy.

Collection of *Houttuynia cordata* Thunb. samples

Houttuynia cordata Thunb. was collected at the sampling site, then stored in a cooler. The samples were processed and isolated in the laboratory.

2.2.2. Isolation of endophytic bacterial strains in *Houttuynia cordata* Thunb.

Isolation of endophytic bacteria in *Houttuynia cordata* Thunb.

Diagram of the isolation process of endophytic bacterial strains of *Houttuynia cordata* Thunb.was described in Fig 2.1

The pure culture of bacteria was checked by microscopic observation, and stored in the glycerine tube kept at -80ºC until further experiments of the study were carried out.

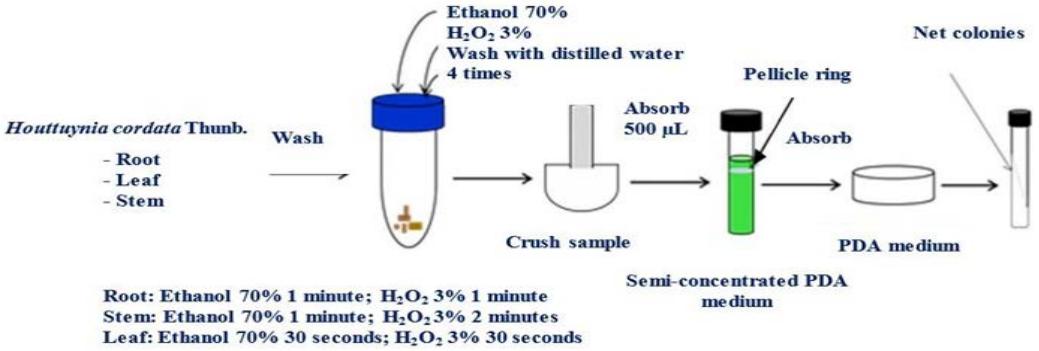


Figure 2. 1. The isolation process of endophytic bacteria in *Houttuynia cordata* Thunb.

Observation of colony characteristics of isolated bacterial strains. Shape, color, elevation, and margin of the colony, motility, Gram staining, and bacteria shape

2.2.3. Determination of antibacterial ability of endophytic bacterial strains isolated from *Houttuynia cordata* Thunb. on *Staphylococcus aureus*.

Evaluation of the antibacterial ability of endophytic bacterial strains of *Houttuynia cordata* Thunb. against *Staphylococcus aureus*

The evaluation process of the antibacterial activity of endophytic bacterial strains was shown in Figure 2.2.

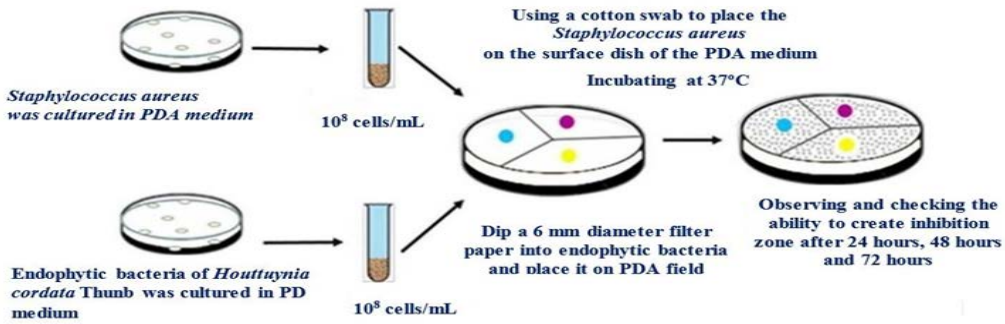


Figure 2.2. The evaluation procedure of antimicrobial activity

Evaluation of the antibacterial ability of endophytic bacterial strains in *Houttuynia cordata* Thunb.. against *Staphylococcus aureus*

Antibacterial diameter = Diameter of inhibition zone - Diameter of filter paper circle for endophytic bacterium absorption

2.3. Identification of endophytic bacterial strains with high antibacterial activity against *Staphylococcus aureus* by 16S rRNA gene sequencing method.

Bacterial DNA was identified by the PCR technique. After DNA purification, PCR reaction was proceeded with 16S RNA primer pair designed according to Lane (1991) with the following sequences.

27F 5' AGAGTTTGATCCTGGCTCAG 3'
1492R 5' GGTTACCTTGTTACGACTT 3'

PCR product was sequenced by the company in Malaysia. These sequences were compared with the sequences found in the NCBI gene bank (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and combined with morphological and cellular characteristics to determine the species of the strains.

2.4. Evaluation of intracellular fluid, extracellular fluid, and proliferation culture media of selected endophytic bacterial strains in *Houttuynia cordata* Thunb. with antibacterial activity on *Staphylococcus aureus*

By taking 100 µL of selected endophytic bacteria with a density of 10^8 cells/mL into 900 µL of liquid PDA medium, bacteria proliferation culture media of 1000 µL was obtained in a test tube with a lid. This process was performed with 03 test tubes. Then, 03 test tubes with bacteria above were prolifically cultured for 24 hours on a horizontal shaker with a capacity of 150 rpm. After 24 hours, proliferation culture media of bacterial strains in 03 test tubes continued to be tested, as follows: The first test tube of proliferation culture media (1000µL) was centrifuged at 3000 rpm within 15 minutes to collect the supernatant fluid, also known as extracellular fluid. Similarly, the second one of the proliferation culture media (1000µL) went through the same steps as above. Simultaneously, the clear supernatant was removed to obtain bacterial cells. After that, bacterial cells were washed with 1000 µL saline three times and centrifuged to remove the supernatant to obtain the clean bacterial cells. Turning to the next step, it was necessary to add 1000 mL of saline to the clean bacterial cell collected and apply ultrasound cleaning step for bacteria cells at 37ºC within 30 minutes to break down bacterial cells and release material inside called intracellular fluid. With the same amount of proliferation culture media (1000 mL), the third test tube kept both the extracellular fluid and the living bacterial cell called the mixed fluids or proliferation culture media of bacterial strains.

After obtaining the extracellular fluid, the intracellular fluid and the mixed fluid, the antibacterial activity was evaluated based on the disk-diffusion agar method as described by [1] by spreading 100 µL of *Staphylococcus aureus* fluid at a density of 10^8 cells/mL over the surface of solid PDA medium. Next, a paper impregnated with extracellular fluid, intracellular fluid and mixed fluid was placed on the surface of a Petri dish with spread *Staphylococcus aureus* bacteria and incubated at 37ºC for 24 hours. After 24 hours of incubation, the diameter of the zone of inhibition (excluding the 6 mm paper disk) was measured.

2.5. Preparation the extract of proliferation culture medium from endophytic bacteria in *Houttuynia cordata* Thunb.

After determing the antibacterial activity of the intracellular fluid, extracellular fluid and the proliferation culture medium of the bacterial strain, the fluid that showed the strongest antibacterial activity were chosen for extraction with ethyl acetate solvents as described below: The endophytic bacteria (40 mL) adjusted at a population density of 10^8 cells/mL was inoculated to 3960 mL of liquid PDA medium. Then, the sample was cultured in the proliferation culture medium for 24 hours on a horizontal shaker at the speed of 150 rpm. Extract from the proliferation culture medium of the endophytic bacterial strain by liquid–liquid extraction with ethyl acetate solvent at the ratio of 1:2, used rotary evaporator for solvent removal to obtain ethyl acetate extract from the endophytic bacteria [12]. The extract was stored and preserved at 4ºC for further evaluations.

2.6. Determination of the antibacterial activity of ethyl acetate extract from endophytic bacteria in *Houttuynia cordata* Thunb.

2.6.1. Qualitative chemical composition of the extract from the proliferation culture medium of the endophytic bacterial bacterial strain

Performation qualitative analysis on the presence of chemical components such as alkaloids, flavonoids, steroids, glycosides, saponins and tannins in the extract from the proliferation culture medium of the bacterial strain as described by [12], as follows:

Evaluation of the presence of Alkaloids

Qualitative analysis by Dragendroff reagent and Wagner's reagent

Evaluation of the presence of flavonoids

Qualitative analysis by concentrated H_2SO_4 and 1% NaOH/ethanol reagent

Evaluation of the presence Steroids - Triterpenoids

Salkowski's reagent and Rosenthaler's reagent

Evaluation of the presence of glycosides

Qualitative analysis by Fehling's reagent and Keller- Killiani's reagent

Evaluation of the presence saponins

Based on the foaming power of saponins: 5 ml distilled water and 3 drops of ethanol solution containing the extract were added to the test tube for testing, sealed the test tube and shake vigorously, let stand for 15 minutes, then observed the foam column in the test tube. If a stable foam column was formed in the test tube, the sample contained saponins.

Evaluation of the presence of tannins

2 ml of extract solution was added to a test tube then added 5 drops of 1% gelatin solution. White flocculation should be observed if tannins was present in the extract.

Evaluation of the presence of polyphenols

Qualitative analysis by 5% $FeCl_3$ reagent

2.6.2. Quantitative chemical composition of the extract from the proliferation culture medium of the endophytic bacterial bacterial strain.

Quantitative determination method of polyphenols

The total polyphenol content was measured using the Folin-Ciocalteu colorimetric method (Singleton et al., 1999). The polyphenol content was expressed as milligram gallic acid equivalent per gram of extract (mg GAE/g extract).

Quantitative determination method of flavonoids

The total flavonoid content was determined based on the procedure described by [11] with some modifications. The content of flavonoid was expressed as milligrams of quercetin equivalent per gram of extract (mg QE/g extract).

2.6.3. Evaluation of the antibacterial activity

Agar well diffusion method by determining the diameter of the zone of inhibition

The agar well diffusion method was used to determine the diameter of the zone of inhibition (Bauer et al., 1966). The bacterial population density was measured at 10^8 cells/mL (OD = 0.08) of bacterial fluid.

Added 100 µL of *Staphylococcus aureus* fluid just mixed onto solid PDA medium that needed to be tested for activity, spreaded the fluid evenly on the surface of the PDA plate and gouge 05 wells on the agar plate with the diameter of 6 mm for testing at the concentrations of 80, 160, 320, 640, 1280 µg/mL of the extract. Then added 50 µL extract of each concentration into the agar wells on the Petri dish of PDA medium with spread *Staphylococcus aureus* fluid and incubate at 37ºC for 24 hours.

The antibacterial activity results were assessed by the diameter of the zone of inhibition (excluded the diameter of the agar wells) after 24 hours of incubation at 37ºC, measured in mm.

Method of determining the Minimum inhibitory concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of the extract from endophytic bacteria was determined by dilution on a 96-well plate and based on the discoloration of resazurin reagent [3]; [14]. The MIC was the lowest in the tested concentration range of the proliferation culture media extracts of selected endophytic bacteria strains in *Houttuynia cordata* Thunb. that could inhibit bacterial growth (the concentration did not change the color of resazurin reagent).

Minimum Bactericidal Concentration (MBC)

The Minimum Bactericidal Concentration (MBC) of the extract from endophytic bacterial culture was determined by the drop plate method as follows: Drip 30 µL of test solution from color-retained wells of resazurin on the surface of a solid PDA medium. After 24 hours, observe the survivor of the bacteria. The MBC was the lowest concentration in the range of extracts that could kill all bacteria in the well, and no colonies appeared on the PDA culture medium [13].

3. Results and Discussions

3.1. Results on colony morphology and characteristics of endophytic bacteria strains in *Houttuynia cordata* Thunb. isolated in the study

3.1.1 Results on colony morphology of endophytic bacteria strains in *Houttuynia cordata* Thunb.

The survey results of 231 isolated endophytic bacteria strains were illustrated in Table 4.1. Colony morphology of endophytic bacteria strains in *Houttuynia cordata* Thunb. in 04 provinces, including Kien Giang, Soc Trang, An Giang, and Can Tho.

Most colonies were opaque white. Moreover, yellow, ivory-white, white, reddish-brown, and light brown colors were also detected, indicating the diversity of colony color in the research locations. Besides, the circular shape and the convex elevation predominated. In addition, the higher proportion of the colonies with entire margin compared to the erose one was indicated in Table 3.1.

Table 3. 1 Colony morphology of bacterial strains isolated from *Houttuynia cordata* Thunb. in Kien Giang, Soc Trang, An Giang, and Can Tho

Colony morphology		Kien Giang	Soc Trang	An Giang	Can Tho	Total
Color	White	6	5	4	5	20
	Opaque white	33	34	28	35	130
	Ivory white	8	9	7	9	33
	Yellow	3	2	2	2	9
	Light yellow	8	8	7	8	31
	Reddish-brown	1	1	1	1	4
	Light brown	1	1	1	1	4
Shape	Circular	40	41	33	41	155
	Irregular	20	19	17	20	76
Elevation	Convex	36	36	30	37	139
	Raised	24	24	20	24	70
Margin	Entire	44	44	36	45	169
	Erose	16	16	14	16	62

In terms of shape, the circular ones constituted a high proportion (168 out of 231). Among 231 colonies, the number of convex ones were 139. In addition, the colonies with entire margin accounted for approximately three-fourth (169 out of 231) colonies.

3.1.2 Results of surveying characteristics of endophytic bacteria strains in *Houttuynia cordata* Thunb.

From Table 3.2, it could be seen that the long rod-shaped cells accounted for 124 out of 231 endophytic bacterial strains in *Houttuynia cordata* Thunb..

Table 3. 2 Characteristics of bacterial strains isolated from *Houttuynia cordata* Thunb. in Kien Giang, Soc Trang, An Giang, and Can Tho

Characteristics			Kien Giang	Soc Trang	An Giang	Can Tho	Total
Bacterial strains	Shape	Short rod-shaped	19	16	15	18	68
		Long rod-shaped	32	34	25	33	124
		Chain	9	10	10	10	39
	Gram-stain	Gram-positive	31	31	23	28	147
		Gram-negative	29	29	27	33	118
	Motility	Motile	37	39	30	38	144
		Non motile	24	21	20	23	88

The number of Gram-negative bacteria was nearly equivalent to the Gram-positive bacteria. In addition, the motility survey showed that among 231 strains of bacteria, 144 strains were motile.

Many studies from different countries on endophytic bacteria demonstrate that the bacteria all appear in the leaves, stems, and roots of plants. This judgment was demonstrated through the study of [6] isolating the endophytic strains of bacteria sampled from the roots, stems, and leaves of mangroves from the mangrove forests of Pichavaram Chidambaram, Tamilnadu in India. The research of [4] also described 28 endophytic bacterial strains isolated from different parts of P. tenuiflorus plants, including roots, stems, and leaves.

3.2 Results of surveying the pH of soil for planting *Houttuynia cordata* Thunb. in the study

The survey on the pH of soil planting *Houttuynia cordata* Thunb. in the research was conducted at the sampling sites in different ecological zones such as Kien Giang, Soc Trang, An Giang, and Can Tho. The result showed that the pH of the *Houttuynia cordata* Thunb. crop soil fell within the range of 6,0 – 6,5 in the sampling areas, even though they were different ecological zones, including saltwater, brackish water, and freshwater.

3.4. Results of identifying 13 strains of endophytic bacteria in *Houttuynia cordata* Thunb. with high antibacterial activity on *Staphylococcus aureus* by 16S rRNA

Table 3. 3 Results of sequencing of endophytic bacterial strains in *Houttuynia cordata* Thunb. selected in Kien Giang, Soc Trang, An Giang, and Can Tho

No.	Endophytic bacterial strains isolated from *Houttuynia cordata* Thunb.	Results of bacterial strain identification from Blast on the NCBI database	Similarity (%)
1	HTT2	*Bacillus amyloliquefaciens* strain CD2901	97
2	PQT4	*Bacillus megaterium* strain 22	97
3	RGT2	*Bacillus subtilis* strain B237	96
4	MXT9	*Bacillus amyloliquefaciens* strain JNL	96
5	STL3	*Bacillus subtilis* strain HB9	96
6	CTL3	*Bacillus pumilus* HB29	97
7	VCT3	*Bacillus velezensis strain* JC-K3	97
8	TBT2	*Bacillus subtilis* JCM 1465	97
9	PTL5	*Bacillus amyloliquefaciens* MPA 1034	96
10	LXT2	*Bacillus megaterium* ATCC 14581	97
11	PDT2	*Bacillus amyloliquefaciens* strain CD2901	98
12	NKT3	*Bacillus subtilis* strain LPB4	98
13	BTT4	*Bacillus megaterium* strain 22	96

Table 3.3 showed that 13 strains of endophytic bacteria in *Houttuynia cordata* Thunb. with high antibacterial activity on *Staphylococcus aureus*. They belonged to Bacillus genus [1].

3.5. Evaluation results of anti-*Staphylococcus aureus* activity of RGT2 bacteria

Evaluation results of antibacterial activity of extracellular fluid, intracellular fluid and bacterial culture suspension of RGT2 strain were presented in Table 3.4.

Table 3. 4 Evaluation results of diameter (mm) of the zone of inhibition of the intracellular fluid, extracellular fluid, and proliferation cululre medium of endophytic bacteria

Bacterial fluid	Diameter of zone of inhibition (mm)
Extracellular fluid	7.67^{b}±0.58
Intracellular fluid	0.00^{c}±0.00
Culture suspension bacterial	32.00^{a}±1.00

Note: Values in the same column followed by the letter are not significantly different at 5%.

The results showed that the proliferation culture media made the zone of inhibition diameter of anti-*Staphylococcus aureus* 32.00 mm, larger than the extracellular fluid significant difference. Based on the results of the survey and the above analysis, the bacterial culture suspension of RGT2 strain was selected for extracting extract with ethyl acetate solvent.

3.6 Results of the preparation, qualitative and quantitative determination of the chemical composition of the extract from the proliferation culture media of RGT2 strain, endophytic bacteria in *Houttuynia cordata* Thunb.

3.6.1 Preparation of ethyl acetate extract from the proliferation culture media of RGT2 strain.

From 4000 mL of the proliferation culture media of RGT2 strain, a liquid extract with 12 liters of ethyl acetate solvent, 0.641 gram ethyl acetate extract was obtained through the solvent evaporation process. The extract was obtained in a thick state with bronze color and characteristic aroma.

3.6.2 Qualitative determination of chemical groups from extracts of RGT2 strain

In this research, the extract from RGT2 strain also contained compounds belonging to the group of polyphenol, flavonoid, alkaloid, tannin, glycoside, and steroid, so it also had a very high potential for biological activity. Among the above groups of compounds, polyphenol and flavonoid were considered to play an important role in the regulation of antibacteria activity in Table 3.5.

Many studies showed that polyphenols and flavonoids play an important role in antibacterial activities and were closely related to other biological activities [8].

Table 3. 5 Evaluation results of the functional groups with antibacteria activity of the extract of and proliferation cululre medium of RGT2

Functional groups	Reagents	Phenomena	Extract results
Flavonoid	$H_2SO_{4\ đđ}$	A dark yellow to orange, red or blue red, or orange to red precipitate appears.	+
	1% NaOH / ethanol	Yellow to orange-red	+
Polyphenol	$FeCl_3$ 5%	Black blue solution	+
Alkaloid	Dragendorff	Red-brown precipitate	-
Steroid	Salkowski	Layer separation solution; the lower layer is dark red	+
	Rosenthaler	Light green color appears	+
Saponin	Shake vigorously for 1 minute	Foaming	-
Tannin	Gelatin	White precipitate	+
Glycoside	Fehling	Brick red precipitate appears.	+
	Keller - killiani	Purple red or brown appears on the interface between the two liquid layers	+

Note: (+) appear, (-) not appear

3.6.3 Quantitative determination of the content of total polyphenol and total flavonoid from extracts of proliferation culture media of RGT2 strain

On the basis of these calibration curves, the results for total polyphenol (TPC) and total flavonoid (TFC) content in the extract were determined to be 39.54 mg GAE/g extract and 330.03 mg QE/g extract respectively were shown in Table 3.6.

Table 3.6 Results of polyphenol and flavonoid content of the extract

Quantitative component	Linear equation	Content
TPC (mg GAE/g extract)	$y = 0.0918x + 0.0487$ ($R^2 = 0.9862$)	39.54±2.50
TFC (mg QE/g extract)	$y = 0.0052x – 0.0087$ ($R^2 = 0.989$)	330.03±11.55

Thus, based on previous research, it could be explained that the antibacteria ability of the extracts from the proliferation culture media of RGT2 strain not only depended on the content of polyphenol and flavonoid, but also depended on the polyphenol and flavonoid component that these extracts possess.

In general, the essential role of flavonoid above was related to the diseases as bacterial infections. Flavonoids affected the inflammatory site by affecting inflammatory cells, releasing ROS, RNS, and proinflammatory cytokines to remove foreign pathogens including bacteria and repaired injured tissues [10].

3.7 Results of evaluating the antibacterial activity of the extracts from proliferation culture media of RGT2 strain

The experiment was conducted to investigate the anti-*Staphylococcus aureus* ability of extract from the proliferation culture media of RGT2 strain at 80, 160, 320, 640, and 1280 µg/mL. Anti-*Staphylococcus aureus* activity of extracts and antibiotics was illustrated in Figure 3.1. For the zone of inhibition diameter on *Staphylococcus aureus* bacteria tested, vancomycin had a larger zone of inhibition than the extract from proliferation culture media of RGT2 strain. It was shown that the extract from proliferation culture media of RGT2 strain had a good antibacterial activity on *Staphylococcus aureus* but not better than vancomycin.

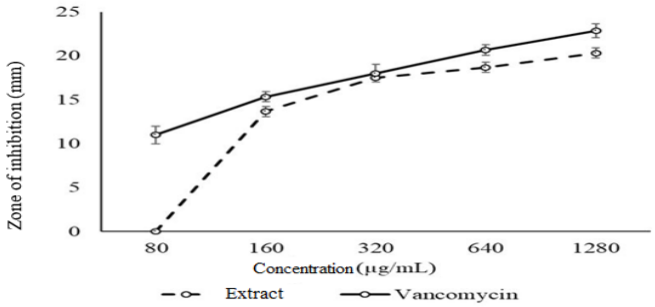


Figure 3. 1 Antibacterial ability of the extract from proliferation culture media of RGT2 strain and vancomycin

3.8 Results of determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the extract from proliferation culture media of RGT2 strain.

The determination was carried out based on the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) [7]. The resazurin color indicator was blue in solution. In the wells, the color of resazurin solution changed from blue to pink, indicating bacterial growth in the well. The results were shown in Table 3.7.

As shown in Figure 3.2, there was no presence of extract or antibiotic vancomycin in the wells, only liquid PDA, saline and 10% DMSO were used as solvents, without the effect of medium in the experiment process, the wells all change from blue to pink.

Table 3.7 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract and vancomycin

Minimum inhibitory concentration (MIC) (µg/mL)		Minimum bactericidal concentration (MBC) (µg/mL)	
Extract	Vancomycin	Extract	Vancomycin
80<MIC≤160	MIC<80	640<MBC≤1280	160<MBC≤320

The extract used at a concentration of 160 µg/mL made the well solution turn purple-blue, which demonstrated that from the concentration of 160 µg/mL, *Staphylococcus aureus* was completely inhibited.

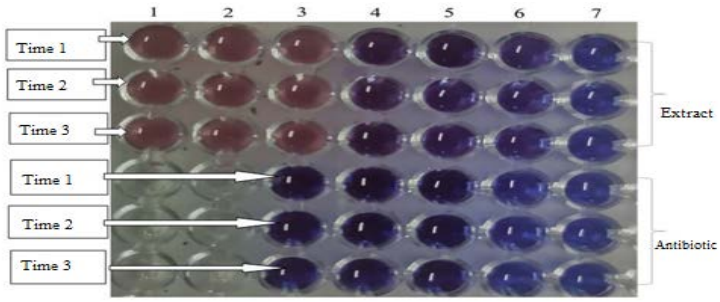


Figure 3. 2 Test of extract and vancomycin with resazurin reagent

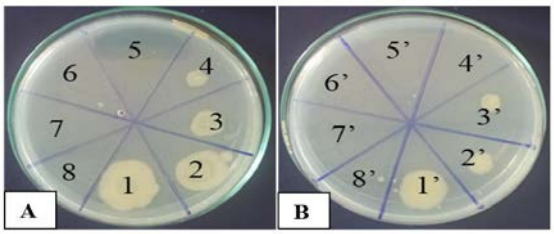
Notes: 1-Well only containing bacteria, DMSO 10% and resazurin; 2-Well only containing bacteria, PDA medium, saline and resazurin; 3-Well only containing bacteria, extract or antibiotic with concentration of 80 µg/L and resazurin; 4-Well only containing bacteria, extract or antibiotic with concentration of 160 µg/Lvà resazurin; 5-Well only containing bacteria, extract or antibiotic with concentration of 320 µg/L and resazurin; 6-Well only containing bacteria, extract or antibiotic with concentration of 640 µg/L and resazurin; 7-Well only containing bacteria, extract or antibiotic with concentration of 1280 µg/L and resazurin.

As a result, the research determined that the minimum inhibitory concentration of the extract from the proliferation culture media of RGT2 strain for *Staphylococcus aureus* ranging from 80 to 160 µg/mL.

Based on the evaluation results of MIC values, the research continued to investigate the minimum bactericidal concentration (MBC) of the extract through testing the regeneration of *Staphylococcus aureus* from a concentration of 160 µg/mL (first well turning blue), 320, 640, 1280, 2560, 5120 and 10240 µg/mL by the drop plate count method.

From the observation of the regeneration of *Staphylococcus aureus* on PDA agar as described in Figure 3.3, it was possible to determine the minimum bactericidal concentration of the extract. Based on Figure 3.3, it could be seen that *Staphylococcus aureus* could still grow on PDA at a high extract concentration of 640 µg/mL (4A). When the high extract concentration increased to 1280 µg/mL (5A), no colony was detected. This proved that at a high extract concentration of 1280 µg/mL, *Staphylococcus aureus* was completely destroyed. Since then, the research determined that the minimum bactericidal concentration of the extract from proliferation culture media of RGT2 strain ranged from 640 to 1280 µg/mL.

Similar to extracts, vancomycin was also investigated for the minimum inhibitory concentration and minimum bactericidal concentration shown in Figure 3.3. Thus, vancomycin had a minimum inhibitory concentration on *Staphylococcus aureus* lower than 80µg/mL.



Firgure 3. 3 Minimum bactericidal concentration (MBC) of the extract from proliferation culture media of RGT2 strain and vancomycin

Note: A-Extract; B- vancomycin. 1-Extract concentration of 0 µg/mL, 2- Extract concentration of 160 µg/mL; 3-Extract concentration of 320 µg/mL; 4- Extract concentration of 640 µg/mL; 5- Extract concentration of 1280 µg/mL; 6-Extract concentration of 2560 µg/mL; 7- Extract concentration of 5120 µg/mL; 8- Extract concentration of 10240 µg/mL. 1'- Antibiotic concentration of 0 µg/mL; 2'- Antibiotic concentration of 80 µg/mL; 3'- Antibiotic concentration of 160 µg/mL; 4'- Antibiotic concentration of 320 µg/mL; 5'- Antibiotic concentration of 640 µg/mL; 6'- Antibiotic concentration of 1280 µg/mL; 7'- Antibiotic concentration of 2560 µg/mL; 8'- Antibiotic concentration of 5120 µg/mL.

As shown in Figure 3.3, vancomycin completely killed *Staphylococcus aureus* bacteria from a concentration of 320 µg/mL (B4'). The minimum bactericidal concentration (MBC) was determined to range from 160 to 320 µg/mL.

4. Conclusions

From the research, 231 endophytic bacterial strains were isolate from leaves, stems and roots of *Houttuynia cordata* Thunb. cultivated in various ecological regious such as saltwater, brackish water

and freshwater areas or islands, high mountains and plains in Kien Giang, Soc Trang, An Giang and Can Tho.

65/231 endophytic bacterial strains in *Houttuynia cordata* Thunb. that had anti-*Staphylococcus aureus* activity from pimples in humans were found. Through surveys 13/65 endophytic bacterial strains in *Houttuynia cordata* Thunb. had the strongest anti-*Staphylococcus aureus* activity with the zones of inhibition of from 20 to 33 mm. All these strains belong to the genus Bacillus.

The minimum inhibitory concentration (MIC) of the extract from the proliferation culture medium (24 hours) of the endophytic bacterial strain (RGT2) in *Houttuynia cordata* Thunb. with anti-*Staphylococcus aureus* activity ranged from 80 to 160 µg/mL. The minimum bactericidal concentration (MBC) of the extract from the proliferation culture medium (24 hours) of the endophytic bacterial strain (RGT2) in *Houttuynia cordata* Thunb. with anti-*Staphylococcus aureus* activity ranged from 640 to 1280 µg/mL.

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