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Research Article

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Bioactive Compounds of *Bacillus subtilis* strain B237 isolated from the endophytic bacteria in *Houttuynia cordata* Thunb by Gas Chromatography-Mass Spectrometry

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Abstract To identify some compounds by GC-MS from the biologically active compounds have been identified in hexane-acetone, acetone-methanol and ethyl acetate organic solvents. The determination of bioactive chemical compounds is based on peak area, retention time, molecular weight and molecular formula. From the endophytic bacteria of *Houttuynia cordata* Thunb from *Bacillus subtilis* strain B237 having antibacterial activity against *Staphylococcus aureus* from people's furuncles. *Houttuynia cordata* Thunb was collected in Kien Giang province, bacteria were isolated in PDA medium. The investigation of the antibacterial ability of endophytic bacteria of *Houttuynia cordata* Thunb with *Staphylococcus aureus* is carried out by diffusion method by filter paper ring and identified by 16S rRNA gene sequencing method of bacteria strains, then compare the sequence of bacterial strains with sequences of bacterial strains in NCBI data bank. Ten bioactive compounds were identified in the organic solvent ethyl-acetate by GC-MS analysis of *Bacillus subtilis* strain B237 has compounds such as Octadecanoic acid, Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl), Docosanoic acid, ethyl ester, 2,5-Cyclohexadien-1-one, 3,5-dihydroxy-4,4-dimethyl-, 4(1*H*)-Pyrimidinone, 6-amino-2-methyl-5-nitroso-, Benzenemethanol, 3-hydroxy-5-methoxy-, Hexadecanoic acid, ethyl ester, Pentadecanoic acid, ethyl ester, Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-, Diisooctyl phthalate. Especially, that have antibacterial activity against *Staphylococcus aureus*, that was Docosanoic acid- ethyl ester; 4(1H)-Pyrimidinone, 6-amino-2-methyl-5-nitroso-; Hexadecanoic acid-ethyl ester; Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl).

Keywords Antibacterial, bioactive compounds, chemistry of natural compounds, endophytic bacteria, *Houttuyniacor data* Thunb

Introduction

Vietnam has many plants with antibacterial active ingredients and with an extremely rich source of medicinal materials, including many medicinal plants with antibacterial properties that have been medically and traditionally used as medicines for a long time such as *Houttuyniacor data* Thunb, which is a herbaceous plant belonging to the Saururaceae family, perennial, used in medicine, known for its antibacterial, anti-inflammatory, diuretic, and antioxidant effects. In recent years, endophytic bacteria are known for not only supporting plants and developing well but also producing metabolic compounds with natural antibacterial activity. *Houttuynia cordata* Thunb collected in Kien Giang province is used to isolate endophytic bacteria. The investigation of the antibacterial ability of endophytic bacteria of *Houttuyniacor data* Thunb with *Staphylococcus aureus* is carried out by diffusion method

by filter paper ring and identified by 16S rRNA gene sequencing method. The results show that *Bacillus subtilis strain B237* is an endophytic strain of *Houttuynia cordata* Thunb isolated in PDA medium capable of being resistant to *Staphyloccocus aureus* bacteria and then the results identify bioactive compounds from *Bacillus subtilis strain B237* by GC-MS.

The present study is aimed at identifying the chemical components in ethyl acetate extract of *Bacillus subtilis strain B237* bacterium analyzed by GC-MS technique.

2. Methods

2.1. Location of sample collection

Samples of *Houttuynia cordata* Thunb were collected in areas of Kien Giang province.

2.2. Research facilities

Roots, stems, and leaves of *Houttuynia cordata* Thunb were collected in KienGiang province.

Staphylococcus aureus bacterium was isolated from furuncles on a person's face. This person was randomly selected when entering Can Tho Dermatology Hospital to treat furuncles. After being isolated, cultured and stored for the whole experiment process, it was stored and supplied from the Microbiology laboratory Can Tho University of Medicine and Pharmacy Hospital.

2.3. 16S rDNA Gene Amplification and Sequencing

The endophytic bacterial density of *Houttuynia cordata Thunb - Bacillus subtilis* strain B237 for experiment was 10^8CFU/L. The density of *Staphylococcus aureus* bacteria tested was 10^6 CFU/L.

Bacterial DNA was isolated following published protocols; Amplification of 16S rDNA by PCR was carried out using the universal primers 27F and 1492R (Lane, 1991). The 50 µL reaction mixture consisted of 2.5 U Taq Polymerase (Fermentas), 50 µM of each deooxynucleotide triphosphate, 500nM of each primer (Fermentas) and 20 ng DNA. The thermocycling profile was carried out with an initial denaturation at 95^{o}C (5 min) followed by 30 cycles of denaturation at 95^{o}C (30s), annealing at 55^{o}C (30s), extension at 72^{o}C (90s) and a final extension at 72^{o}C (10 min) in C1000 Thermal Cycler (Bio-Rad). Aliquite (10µl) of PCR products were electrophoresed and visualized in 1% agarose gels using standard electrophoresis procedures. Partial 16S rRNA gene of selective isolates in each group were sequenced by PHUSA Company, Vietnam. Finally, 16S rRNA sequence of the isolate was compared with that of other microorganisms by way of BLAST (http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi).

2.4. Fermentation, extraction and isolation

Bacillus subtilis strain B237 was cultured in a 250 ml flask at 30°C for 24 hours with vibration at 150 rpm/minute. The fermentation was carried out in 80L fermentation machine with 40L of BHI medium and 10% of bacteria at 30^{0}C for 48 hours. Neutral pH was maintained automatically by NaOH 1N or HCl 1N. Then, put about 10% *Staphylococcus aureus* bacteria in culture flask, mix in the machine for about 48 hours. After that, centrifuge the solution of *Bacillus subtilis strain B237* with *Staphylococcus aureus* at a rate of 12,000 rpm/minute for 10 minutes and remove the residue. Take the above clear water. Then, take the above clear water to mix with ethyl acetate solvent in a ratio of 1: 1 and then put on the ultrasound machine (to dissolve the above endophytic compounds of *Bacillus subtilis strain B237* and *Staphylococcus aureus* with ethyl acetate), then take that mixture solution to evacuate (on the rotavap). In culture medium (40L), it was extracted with ethyl acetate. The combined organic solutions were then decanted, filtered and concentrated under reduced pressure to yield 2.5 g of crude extract ethyl-acetate organic solvent to afford one fraction with ten bioactive compounds.

2.5. GC-MS analysis

Preparing extract from biomass of *Bacillus subtilis strain B237* with ethyl acetate solvent.

Using methods of extracting natural compounds: Normal phase silicagel column chromatography, reverse phase silicagel column chromatography, Sephadex LH-20 gel chromatography, and thin layer chromatography... to isolate purified compounds.

GC - MS spectroscopy was recorded on Thermo Scientific - MSQ PLUS at the Institute of Chemical Technology, No. 1, Mac Dinh Chi, Ben Nghe Ward, District 1, Ho Chi Minh City.

3. Results and Discussions

GC-MS analysis of compounds from *Bacillus subtilis* strain B237 with *ethyl acetate* organic solvent as shown in (Figure.1)

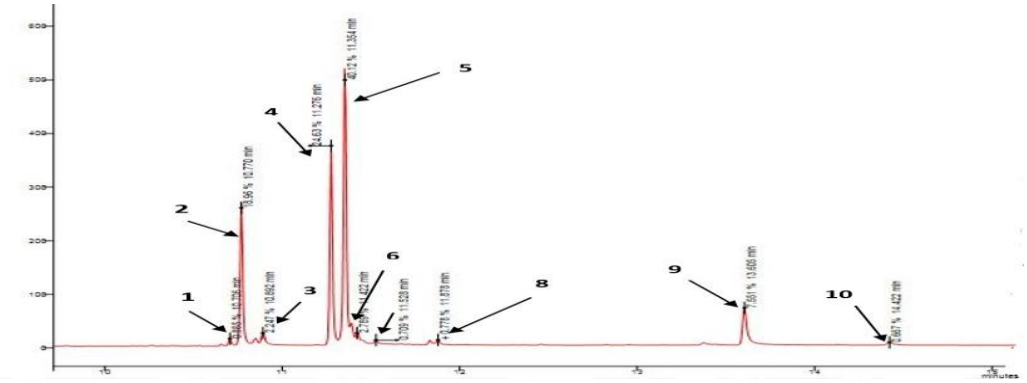

Figure 1: GC-MS chromatogram of extractof Bacillus subtilis strain B237 inorganic solvent ethyl acetate

The first peak of analysis of a sample of *Bacillus subtilis* strain B237 by mass chromatography with RT 10.706 was Octadecanoic acid (Figure 2) [1]

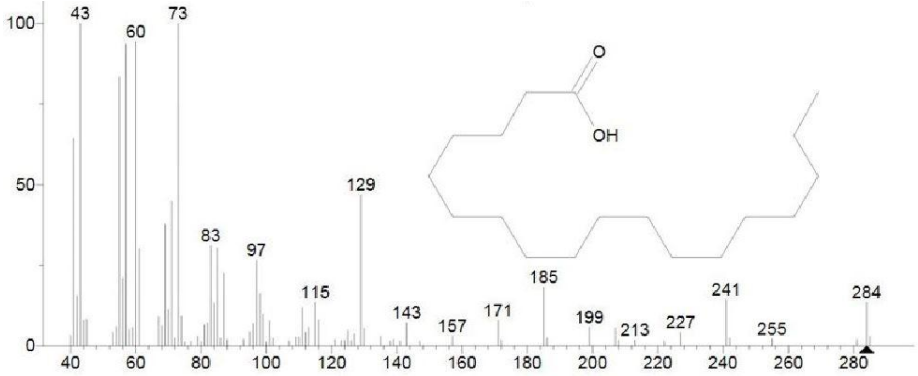

Figure 2: Mass spectrum of Octadecanoic acid with retention time (RT)=10.706

The second peak of analysis of a sample of *Bacillus subtilis* strain B237 by mass chromatography with RT 10.770 was Pyrrolo [1,2-a] pyrazine-1,4-dione, hexahydro-3- (2-methylpropyl) - (Figure.3)

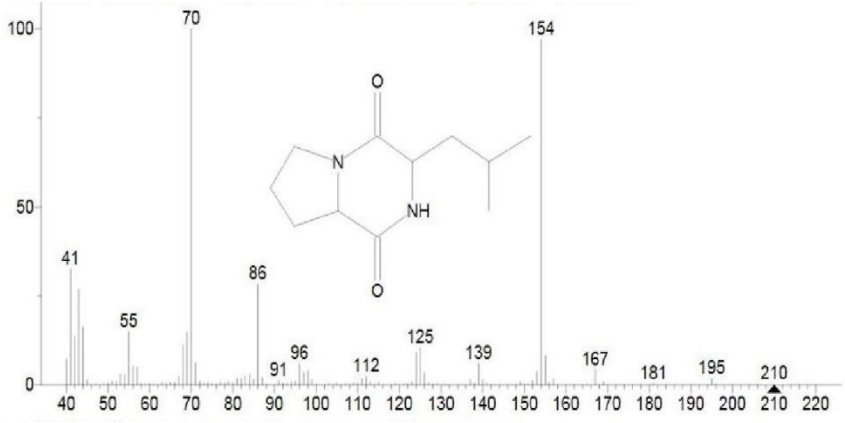

Figure 3: Mass spectrum of Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)- with retention time (RT)=10.770

The third peak of analysis of a sample of *Bacillus subtilis* strain B237 by mass chromatography with RT time of 10.892 was Docosanoic acid, ethyl ester (Figure 4).

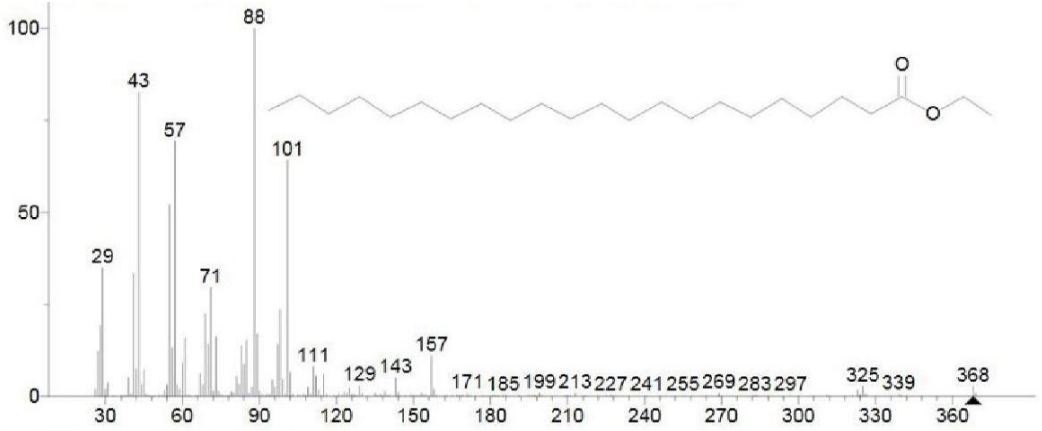

Figure 4: Mass spectrum of Docosanoic acid, ethyl ester with retention time (RT)=10.892

The fourth peak of analysis of the extract sample of *Bacillus subtilis* strain B237 by mass chromatography with RT time 11.276 was 2,5-Cyclohexadien-1-one, 3,5-dihydroxy-4,4-dimethyl- (Figure. 5)

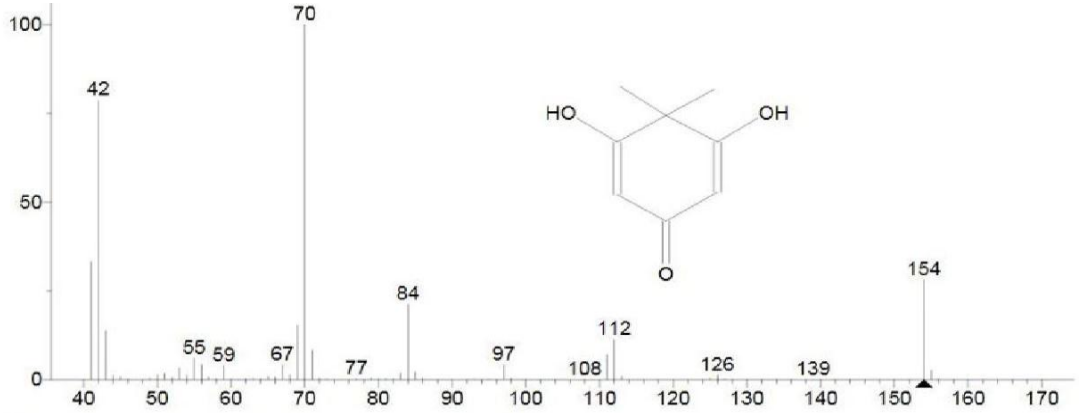

Figure 5: Mass spectrum of 2,5-Cyclohexadien-1-one, 3,5-dihydroxy-4,4-dimethyl- with retention time (RT)=11.276

The fifth peak of the analysis of the extract sample of *Bacillus subtilis* strain B237 by mass chromatography with RT 11.354 was 4 (1H) -Pyrimidinone, 6-amino-2-methyl-5-nitroso- (Figure.6).

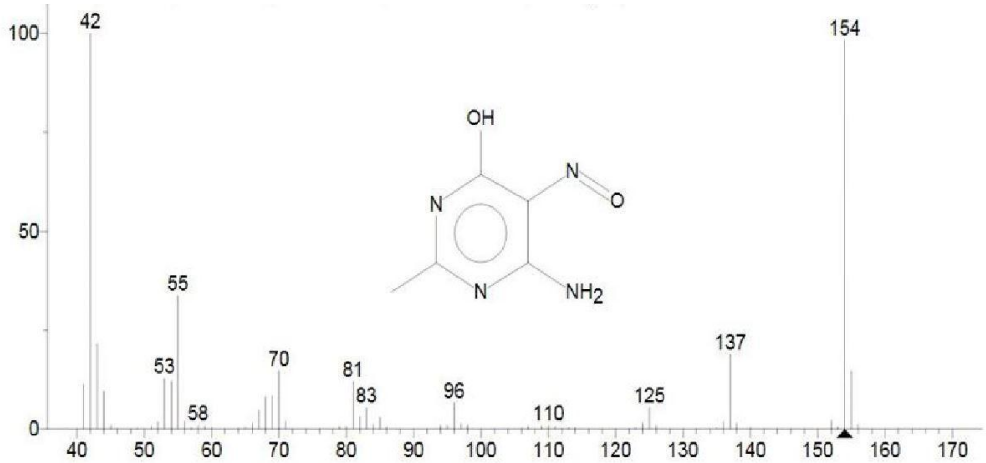

Figure 6: Mass spectrum of 4(1H)-Pyrimidinone, 6-amino-2-methyl-5-nitroso- with retention time(RT)=11.354

The sixth peak of the analysis of the extract sample of *Bacillus subtilis* strain B237 by mass chromatography with RT 11.422 time was Benzenemethanol, 3-hydroxy-5-methoxy- (Figure.7).

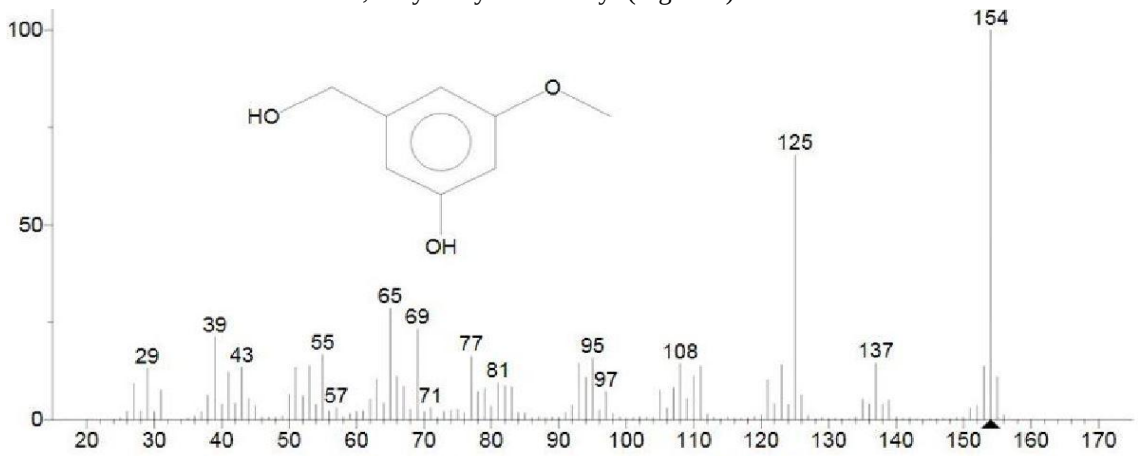

Figure 7: Mass spectrum of Benzene methanol, 3-hydroxy-5-methoxy- with retention time (RT)=11.422

The seventh peak of analyzing the extract sample of *Bacillus subtilis* strain B237 by mass chromatography with RT 11.528 was Hexadecanoic acid, ethyl ester (Figure 8).

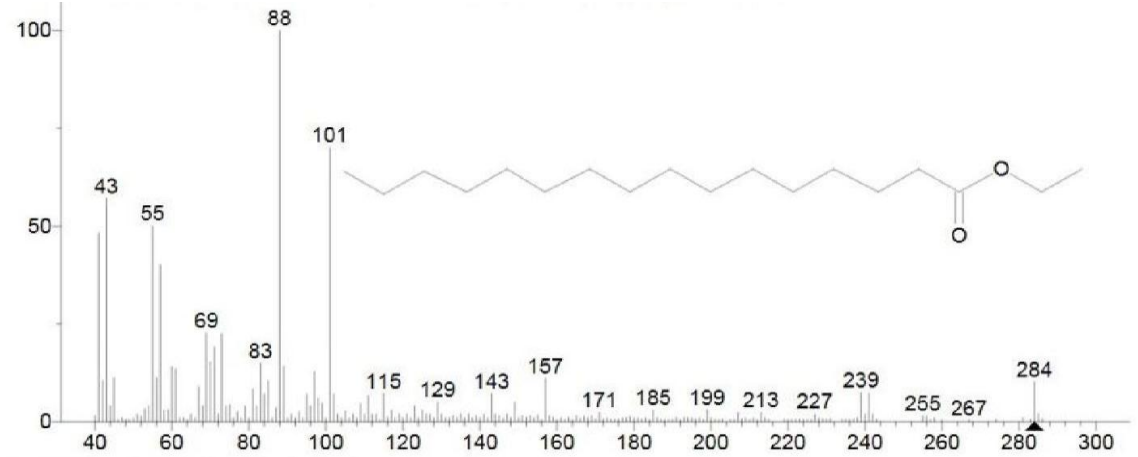

Figure 8: MassspectrumofHexadecanoic acid, ethyl ester with retention time (RT)=11.528

The eighth peak of the analysis of the extract of *Bacillus subtilis* strain B237 by mass chromatography with RT time of 11.878 was Pentadecanoic acid, ethyl ester (Figure.9)

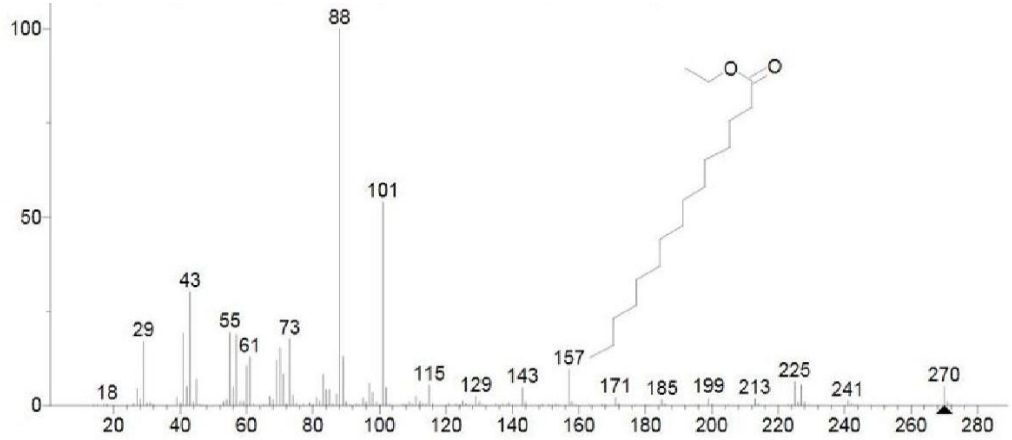

Figure 9: Mass spectrum of Pentadecanoic acid, ethyl ester with retention time (RT) = 11.878

The ninth peak of the analysis of a sample of *Bacillus subtilis* strain B237 by mass chromatography with RT 13.605 was Pyrrolo [1,2-a] pyrazine-1,4-dione, hexahydro-3- (phenylmethyl) - (Figure.10)

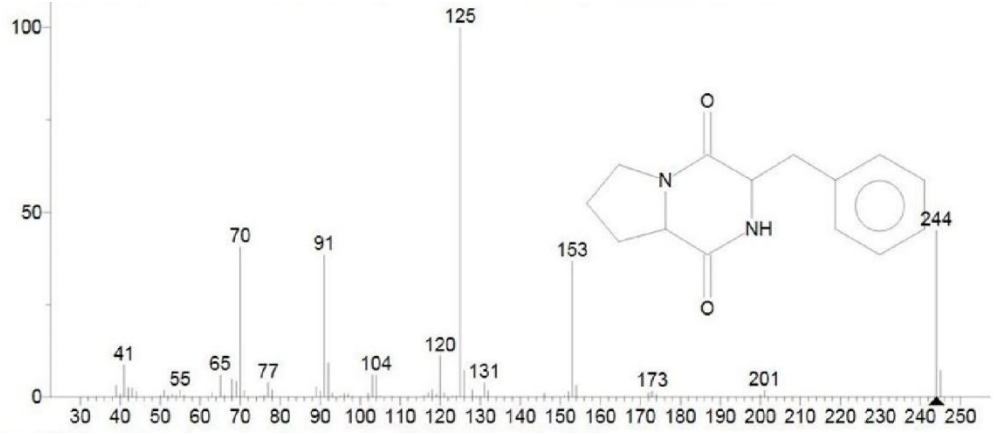

Figure 10: Mass spectrum of Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)- with retention time (RT) = 13.605

The tenth peak of analysis of the extract of *Bacillus subtilis* strain B237 by mass spectrometry with RT time 14.422 was Diisooctyl phthalate (Figure 11).

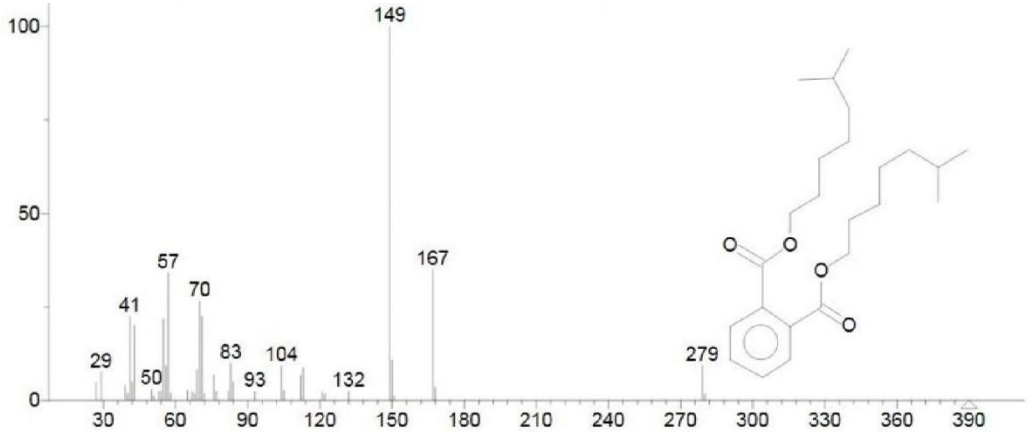

Figure 11: Mass spectrum of Diisooctyl phthalate with retention time (RT)=14.422

Secondary compounds of endophytic *Bacillus subtilis* strain B237 of *Houttuynia cordata* Thunb include:

Table 1: Analysis of chemical components of samples by GC – MS

N°	Retention time (RT) min	Chemical name	Chemical formula	Ratio %	Bioactivity
1	10.706	Octadecanoic acid	$C_{18}H_{36}O_2$	0.885	Antifungal, Antitumor, Antibacterial [1]
2	10.770	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)	$C_{11}H_{18}N_2O_2$	18.96	Antifungal [2]
3	10.892	Docosanoic acid, ethyl ester	$C_{24}H_{48}O_2$	2.247	Antibacterial Activity [3]
4	11.276	2,5-Cyclohexadien-1-one, 3,5-dihydroxy-4,4-dimethyl-	$C_8H_{10}O_3$	24.63	anticancer activity [4]
5	11.354	4(1H)-Pyrimidinone, 6-amino-2-methyl-5-nitroso-	$C_5H_6N_4O_2$	40.12	antibacterial activity [5]
6	11.422	Benzenemethanol, 3-hydroxy-5-methoxy-	$C_8H_{10}O_3$	2.789	anti-fungal and anti-bacterial activity [6]
7	11.528	Hexadecanoic acid, ethyl ester,	$C_{18}H_{36}O_2$	0.709	antioxidant and antimicrobial activity [1, 7, 8]
8	11.878	Pentadecanoic acid-ethyl ester,	$C_{17}H_{34}O_2$	0.778	anticancer activity [4]
9	13.605	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-	$C_{14}H_{16}N_2O_2$	7.551	Antibacterial Activity [9-11]
10	14.422	Diisooctyl phthalate.	$C_{24}H_{38}O_4$	0.667	anticancer activity [12, 13]

4. Conclusion

This study concludes that there are several compounds isolated from *Bacillus subtilis* B237 strain that have antibacterial activity against *Staphylococcus aureus* and were determined by GC-MS method, that was Docosanoic acid- ethyl ester; 4(1H)-Pyrimidinone, 6-amino-2-methyl-5-nitroso-; Hexadecanoic acid-ethyl ester; Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl). Bioactive compounds of identified components range from antimicrobial, antioxidant and antitumoral activities.

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