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Antagonistic Test of Several Bacterial Isolates Against *Fusarium* Sp. Causing Fusarium Wilt in Purple Eggplant (*Solanum Melongena* L.)

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ABSTRACT: *Fusarium* sp. is one of the pathogens that causes fusarium wilt disease in purple eggplant (*Solanum melongena* L.). The control measures commonly used to date involve the application of chemical fungicides, which have negative impacts on the environment and health. Therefore, alternative control measures using safe and environmentally friendly antagonistic bacteria are needed. This study aims to determine the ability of *Lactobacillus bulgaricus*, *Lactobacillus plantarum*, *Paenibacillus polymyxa*, and *Pseudomonas fluorescens* to inhibit the growth of *Fusarium* sp. fungi and to identify the antifungal compounds produced by these four bacteria. *Fusarium* sp. was isolated from purple eggplant plants exhibiting fusarium wilt symptoms. The fungal isolates were purified and identified macroscopically and microscopically. Antagonism tests were conducted in vitro using the disk diffusion method, while the identification of active compounds was performed using Gas Chromatography-Mass Spectrometry (GC-MS). The results of the disc diffusion assay showed that *L. bulgaricus* exhibited the highest inhibition zone of 4.50 mm, followed by *L. plantarum* with 4.00 mm. In contrast, *P. polymyxa* and *P. fluorescens* showed no inhibitory activity 0 mm. GC-MS analysis showed that the filtrates of the four bacteria contained active antifungal compounds, namely cinnamaldehyde (2-propenal, 3-phenyl), hexadecanoic acid methyl ester, 2-benzenedicarboxylic acid bis(2-ethylhexyl), 8-octadecenoic acid methyl ester, and methyl tetradecanoate.

KEYWORDS: Purple eggplant, *Fusarium* sp., antagonistic bacteria, and antifungal compounds.

I. INTRODUCTION

Purple Eggplant (*Solanum melongena* L.) is a type of vegetable that belongs to the Solanaceae family. Eggplant is rich in nutrients, particularly vitamin A and phosphorus. Every 100 g of eggplant contains 24 calories, 1.1 g of protein, 0.2 g of fat, 5.5 g of carbohydrates, 15.0 mg of calcium, 37.0 mg of phosphorus, 0.4 mg of iron, 4.0 IU of vitamin A, 5 mg of vitamin C, 0.04 mg of vitamin B, and 92.7 g of water (Aulia *et al*., 2024). Eggplant also has medicinal properties due to its alkaloid, solanine, and solasodine content, which serve as the base ingredients in the production of oral contraceptives (Huruna *et al*., 2015). Additionally, purple eggplant contains antioxidant compounds such as anthocyanins, which play a role in preventing hypertension, and trypsin (protease) enzymes that depend on inhibitors to combat cancer-causing substances (Muldiana *et al*., 2017; Rosmiah *et al*., 2024).

Demand for eggplant continues to increase along with population growth and increased public awareness of the benefits of eggplant. Eggplant consumption increased by 9.7% in 2022 compared to the previous year (Ministry of Agriculture, 2022). However, there are several obstacles in the process of cultivating eggplants in the field, one of which is fusarium wilt disease caused by Fusarium sp. fungus. Fusarium sp. infects the roots, especially through wounds on the plant roots. This pathogen settles and multiplies within the vascular bundles. This disrupts the transport of water and nutrients, ultimately leading to pathological wilting and eventual plant death (Suanda., 2019).

Fusarium wilt disease can cause a decline in eggplant production if control measures are inadequate. Chemical control using fungicides is the most commonly employed strategy, leading to pathogen resistance, killing beneficial soil microorganisms, causing damage to the natural soil structure, polluting the environment, leaving residues on products, and impacting human health (Flori *et al*., 2020). Alternative methods need to be explored to suppress pathogen development and support the environment.

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Utilizing antagonistic bacteria is one of the control measures that can be implemented. The use of antagonistic bacteria can also enhance plant growth through various mechanisms, thereby providing protection against pathogen attacks. The ability of these bacteria can be utilized to prevent and reduce damage caused by plant pathogens (Arimbawa *et al*., 2019). Based on the above discussion, this study aims to explore antagonistic bacteria that have the ability to suppress the growth of Fusarium wilt-causing pathogens, thereby providing an environmentally friendly alternative control method

II. RESEARCH METHODS

1. Time and Location

The research was conducted from September 2024 until completion at the Microbiology Laboratory and Integrated Research Laboratory of the Faculty of Mathematics and Natural Sciences, Udayana University.

2. Tools and Materials

The materials used in this study were *P. flourescens, P. polymyxa, L. bulgarius*, and *L. plantarum* bacteria from the Plant Disease Laboratory collection at the Faculty of Agriculture, Udayana University, *Fusarium* sp. fungi, Potato Dextrose Agar (PDA) (200 g potato, 15 g dextrose, 8 g agar, 1000 ml distilled water), and Potato Dextrose Broth (PDB) (200 g potato, 15 g dextrose, 1000 ml distilled water).

The tools used in this study were measuring cups, Petri dishes, test tubes, micropipettes, object glasses, deck glasses, microscopes, autoclaves, stirring spoons, gas stoves, Bunsen burners, pots, digital scales, Ose needles, knives, scissors, laminar flow cabinet, clamps, filters, aluminum foil, cotton, masks, rulers, measuring tapes, digital cameras, tracing paper, sticker paper, markers, Gas Chromatography–Mass Spectrometry (GC-MS).

3. Research Implementation

3.1 Isolation of Pathogens

The root parts of the plant showing symptoms of disease were washed with running water, then sprayed with alcohol. The inner part of the root was cut using a cutter measuring approximately 1 x 1 cm^2. The root parts were then placed on sterile PDA media and incubated for 7 days at room temperature.

3.2 Macroscopic and Microscopic Identification of Fungi

Morphological identification of pathogenic fungi is performed macroscopically and microscopically. Macroscopically, fungal colonies growing on PDA medium in Petri dishes are observed, including colony shape, surface color, and underside color. Microscopic identification is observed under a microscope, including hyphal structure (septate or non-septate, hyaline or colored), and the shape of macroconidia and microconidia. The morphological characteristics observed are compared with references in the GMS book and images from the internet to determine the type of pathogen.

3.3 Production of Antagonistic Bacterial Filtrate

The preparation of bacterial filtrate begins with the preparation of PDB medium as a growth medium for antagonistic bacteria. Next, an antagonistic bacterial suspension is made by adding bacteria to 40 ml of sterile water in a test tube, then vortexing for 3 minutes or until the suspension is evenly mixed. Then, 4 ml of the antagonistic bacterial suspension is added to an Erlenmeyer flask containing 1000 ml of PDB medium that has been cooled beforehand. Next, the antagonistic bacterial culture is shaken using a shaker at a speed of 100 rpm and incubated for 14 days. After that, the supernatant is filtered using a 0.45 µm Millipore membrane filter paper. The filtered bacteria are then stored in a freezer.

3.4 In vitro testing of the inhibitory power of antagonistic bacterial filtrate against Fusarium sp fungi

The antifungal activity of the antagonistic bacterial filtrate against Fusarium sp. was tested using the paper disc diffusion method. The initial stage involved preparing the medium by adding 1 ml of Fusarium sp. suspension to a Petri dish, followed by the addition of 10 ml of PDA medium. After the medium had solidified, 6 mm diameter discs that had been soaked in the antagonistic bacterial filtrate were inoculated. The effect of the antagonistic bacterial filtrate on the growth of Fusarium sp. can be determined by measuring the clear zone. The diameter of the clear zone is measured vertically and horizontally using a ruler. To calculate the inhibitory power of the antagonistic bacterial filtrate against the growth of Fusarium sp., the following formula can be used (Surjowardojo *et al*., 2016)

$$\text{inhibitory activity} = \frac{D1-D2}{D1}$$

Keterangan :

D1 = Vertical diameter of the clear zone (mm)

D2 = Vertical diameter of the clear zone (mm)

3.5 Identification of Compounds in Antagonistic Bacterial Cultures Using GC-MS

100 ml of antagonistic bacterial filtrate was dissolved in 50 ml of methanol and 50 ml of ethyl acetate. It was then analyzed using GC-MS. The analysis process used liquid nitrogen as the eluent, a Wakosil ODS/5C18-200 column with dimensions of 4.6 x 200 mm, an eluent flow rate of 1 ml/minute, a temperature of 250°C, and detection was performed using UV light at a wavelength of 254 nm. The detection results were evaluated by comparing the molecular weight and fragmentation patterns of the isolated compounds with those in the GC-MS library.

III.RESULTS AND DISCUSSION

1. Macroscopic and Microscopic Identification of Fungi

Pure cultures of Fusarium sp. fungi grown on PDA medium exhibit several macroscopic characteristics, including white-colored fungal colonies with a lower surface appearing white-yellowish, circular colonies spreading in all directions, and a thick, smooth texture resembling cotton. Meanwhile, microscopic observation of Fusarium sp. revealed characteristics such as hyaline hyphae that are septate and slender. Microconidia are oval in shape and have 0 to 1 septum. One of the distinctive characteristics of *Fusarium* sp. is the shape of its macroconidia, which resemble a crescent moon, are hyaline, and have 3-4 septa (Figure 1).

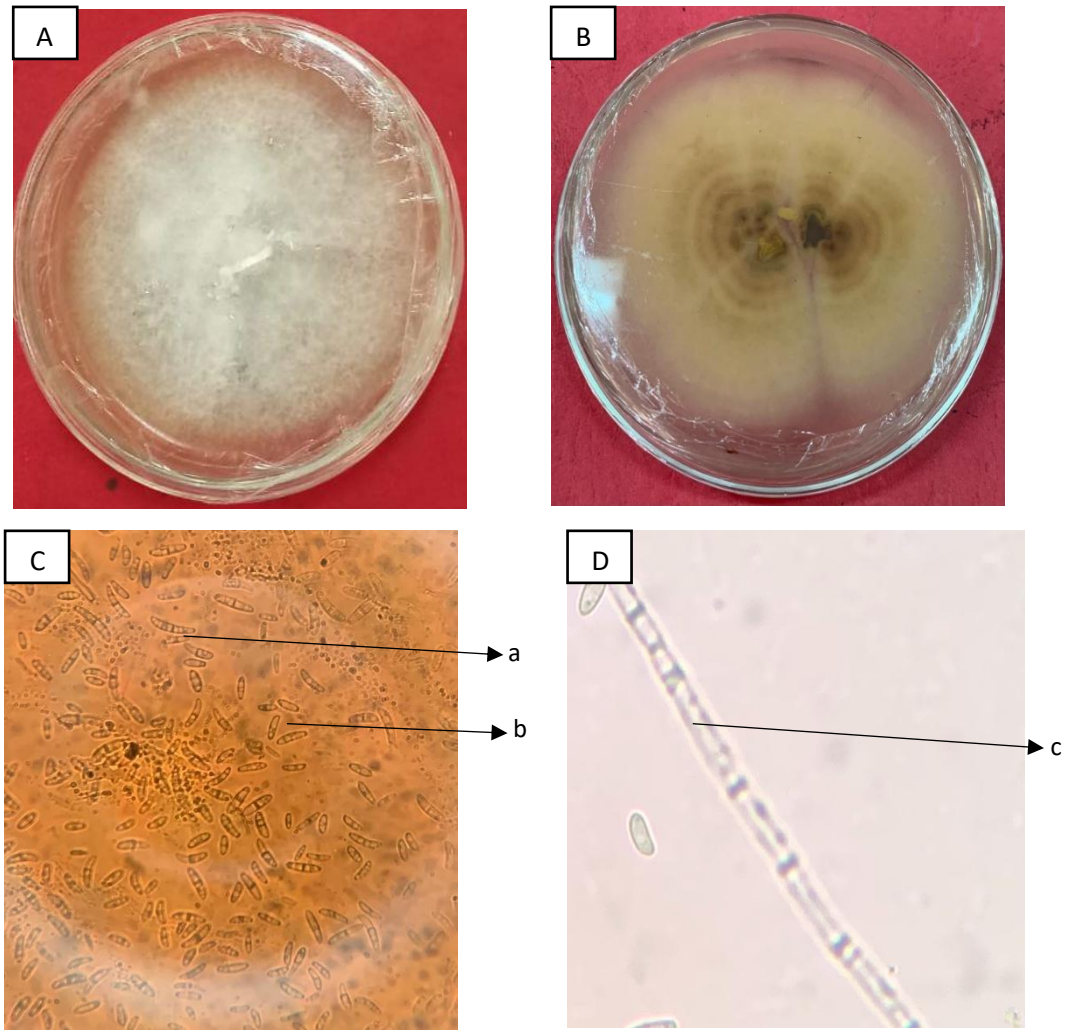

Figure 1 Macroscopic and microscopic images of Fusarium Sp. fungi. Figures A and B: Macroscopic images of Fusarium Sp. fungi. Figures C and D: Microscopic images of Fusarium Sp. fungi. (a: macroconidia, b: microconidia, c: hyphae)

2. In Vitro Testing of the Inhibitory Power of Antagonistic Bacterial Filtrate Against *Fusarium* Sp.

The testing of the inhibitory power of the filtrate that has been carried out for 7 HSI against Fusarium Sp. shows that each

bacterium shows different results (Table 1).

Table 1. Inhibitory power of antagonistic bacterial filtrate against *Fusarium* sp. at 7 HSI observation

No	Treatment	Average (mm)
1	*Lactobacillus Plantarum*	4,50
2	*Lactobacillus Bulgaricus*	4,00
3	*Pseodomonas Flourescens*	0
4	*Paenibacillus Polymyxa*	0

Based on the results of the filtrate inhibition test, the bacterial isolates tested showed different abilities to inhibit the growth of Fusarium sp. fungi. The test results showed that *L. bulgaricus* had the highest average inhibition of 4.50 mm, followed by *L. plantarum* with an average of 4.00 mm. Meanwhile, the isolates *P. polymyxa* and *P. fluorescens* showed no inhibitory activity at all, with a value of 0 mm. The differences in inhibition percentage observed in each treatment are likely due to the varying ability of bacterial isolates to produce inhibitory compounds. This aligns with the research by Pitasari and Ali (2018), which states that differences in inhibition caused by bacteria can occur due to variations in the types and quantities of inhibitory secondary metabolites produced by each isolate. The clear zone formed between the antagonistic bacterial colonies and the Fusarium sp. fungus serves as an indicator of the inhibitory activity of each treatment (Figure 2). The *P. polymyxa* and *P. fluorescens* isolates did not show the formation of a clear zone or inhibitory activity against the growth of *Fusarium* sp. when tested. The absence of an inhibition zone diameter may be due to several factors: the bacterial isolate produces antifungal compounds but these are not active against the test fungus; the antagonistic bacteria produce intracellular antifungal compounds which are not excreted and accumulate within the cell, thus not directly affecting the target fungus; antagonistic bacteria possess genes encoding the formation of metabolites but these are not expressed under normal conditions and require induction by specific compounds, and bacterial isolates produce secondary metabolites that are too small to inhibit fungal growth (Wahyuni & Purba., 2016; Setyati et al., 2022).

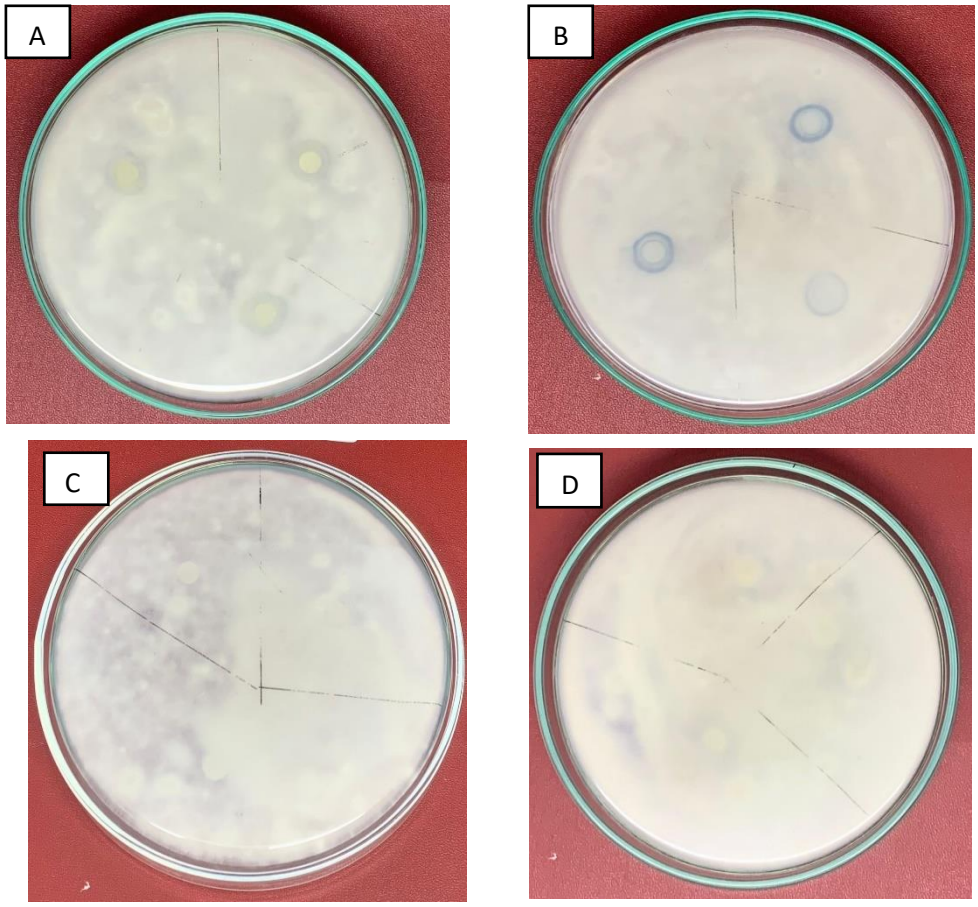

Figure 2. Inhibitory effect of antagonistic bacterial filtrates against *Fusarium* sp. fungi (A) *L. Bulgaricus,* (B) *L. plantarum*, (C) *P. flouresces*, (D) *P. polymyxa*.

Identification of Antifungal Compounds in Filtrate Bacteria Antagonistic to *Fusarium* sp.

3.1 Identification of *L. Bulgaricus* bacterial filtrate compounds using Gas Chromatography Mass Spectrometry (GC-MS).

Table 2. Data on compounds identified from *L. bulgaricus* bacterial filtrate

Peak	Retention Time (minutes)	Area (%)	Name of the compound	Biological Activity	Reference
1	4,450	4,80	Decane (CAS) n-Decane ($C_{10}H_{22}$)	Antimicroba	Prasetyaningsih *et al*. (2018)
2	6,125	2,40	Benzoic acid, methyl ester (CAS) Methyl benzoate ($C_8H_8O_2$)	Insecticide	Mostafiz *et al*. (2022)
3	6,725	5,58	Octanoic acid, methyl ester (CAS) Methyl octanoate ($C_9H_{18}O_2$)	Anticancer and anti inflamantory	Krishnappa *et al*. (2024)
4	9,070	4,99	2-Propenal, 3-phenyl (C_9H_8O)	Antifungal	Sudirga *et al*. (2024)
5	14,654	7,06	Oxalic acid, cyclohexymethyl tridecyl ester ($C_{22}H_{40}O_4$)	Antibacterial	Igwe & Mgbemena. (2014)
6	17,244	39,73	Hexadecanoic acid, methyl ester (CAS) ($C_{17}H_{34}0_2$)	Antifungal, antibacterial, antioxidant, anti-inflammatory, anticancer	Gupta *et al*. (2023)
7	17,687	5,19	4-t-Butyl-2-(1-methyl-2-nitroethyl)cyclohexanone ($C_{13}H_{23}NO_3$)	Antibacterial, anti inflamantory	Koziol *et al*. (2019)
8	17,780	4,02	Sulfurous acid, cyclohexymethyl hexyl ester ($C_{14}H_{28}O_3S$)	Antibacterial	Igwe & Nnaji (2014)
9	18,174	8,30	4-t-Butyl-2-(1-methyl-2-nitroethyl)cyclohexanone ($C_{13}H_{23}NO_3$)	Antibacterial, anti inflamantory	Koziol *et al*. (2019)
10	18,291	0,76	Tetrapentacontan, 1,54-Dibromo ($C_{54}H_{108}Br_2$)	Antioxidant antimicrobial	Bensaad *et al*. (2022)
11	19,044	2,46	8-Octadecenoic acid (Z), methyl ester ($C_{19}H_{36}O_2$)	Antifungal	Akpuaka *et al*. (2012)
12	19,295	8,90	Octanoic acid, methyl ester ($C_9H_{18}O_2$)	Anticancer, antimicrobial	Krishnappa *et al*. (2024)
13	20,908	2,89	Oxiraneotanoic acid, 3-octyl-, methyl ester,cis- ($C_{19}H_{36}O_3$)	Antibacterial	Nahar *et al*. (2016)
14	23,013	1,39	1,2-Benzenedicarboxylic acid, bis(2ethylhexyl) ($C_{24}H_{38}O_4$)	Antifungal, antidiabetic, anticancer, and antitumor	Akpuaka *et al*. (2012)

Based on GC-MS analysis, L. bulgaricus filtrate contains 4 main compounds that are antifungal, namely 2-Propenal, 3-phenyl; Hexadecanoic acid, methyl ester; 8-Octadecenoic acid (Z), methyl ester and 1,2-Benzenedicarboxylic acid, bis(2ethylhexyl). The compound 2-Propenal, 3-phenyl, was detected at peak 5 with a retention time of 9.070 minutes with a percentage area of

4.99%. Hexadecanoic acid, methyl ester compound was detected at peak 7 with a retention time of 17.244 with a percentage area of 39.73%. The compound 8-Octadecenoic acid (Z), methyl ester was detected at peak 12 with a retention time of 19.044 with a percentage area of 2.46%. The compound 1,2-Benzenedicarboxylic acid, bis(2ethylhexyl) was detected at peak 15 with a retention time of 23.013 with a percentage area of 1.39%.

3.2 Identification of *P. flourescens Bulgaricus* bacterial filtrate compounds using Gas Chromatography Mass Spectrometry (GC-MS).

Table 3. Data on compounds identified in *P. fluorescens* filtrate

Peak	Retention Time (minutes)	Area (%)	Name of the compound	Biological Activity	Reference
1	4,445	7,15	Decane (CAS) n-Decane ($C_{10}H_{22}$)	Antimicroba	Prasetyaningsih *et al*. (2018)
2	6,195	4,41	Benzoic acid, methyl ester (CAS) Methyl benzoate ($C_8H_8O_2$)	Insecticide	Mostafiz *et al.* (2022)
3	6,675	15,14	Octanoic acid, methyl ester (CAS) Methyl octanoate ($C_9H_{18}O_2$)	Anticancer and anti inflamantory	Krishnappa *et al.* (2024)
4	9,047	28,22	Cinnamaldehyde, (E)- (C_9H_8O)	Antifungal	Ouyang *et al.* (2019)
5	14,658	9,63	Oxalic acid, cyclohexylmethyl tridecyl ester ($C_{22}H_{40}O_4$)	Antibacterial	Igwe & Mgbemena. (2014)
6	17,272	10,08	Hexadecanoic acid, methyl ester ($C_{17}H_{34}O_2$)	Antifungal, antibacterial, antioxidant	Sukamto *et al*., 2019
7	17,691	6,42	4-t-Butyl-2-(1-methyl-2-nitroethyl)cyclohexanone ($C_{13}H_{23}NO_3$)	Antibacterial	Koziol *et al.* (2019)
8	17,784	5,35	Sulfurous acid, cyclohexylmethyl heptyl ester ($C_{13}H_{26}O_3S$)	Disinfectant	Amerol *et al.* (2020)
9	18,178	8,68	4-t-Butyl-2-(1-methyl-2-nitroethyl)cyclohexanone ($C_{13}H_{23}NO_3$)	Antibacterial, anti-inflammatory	Martha *et al.* (2020)
10	23,029	4,93	1,2-Benzenedicarboxylic acid, bis(2ethylhexyl) ($C_{24}H_{38}O_4$)	Antifungal, antidiabetic, anticancer, and antitumor .	Akpuaka *et al.* (2012)

Based on GC-MS analysis, the filtrate of *P. fluorescens* contains three main antifungal compounds, namely Cinnamaldehyde,(E)-; Hexadecanoic acid, methyl ester; and 1,2-Benzenedicarboxylic acid, bis(2ethylhexyl). The compound Cinnamaldehyde, (E)- was detected at peak 4 with a retention time of 9.047 minutes and an area percentage of 28.22%. The compound Hexadecanoic acid, methyl ester was detected at peak 6 with a retention time of 17.272 minutes and an area percentage of 10.08%. The compound 1,2-benzenedicarboxylic acid, bis(2-ethylhexyl) was detected at peak 10 with a retention time of 23.029 minutes and an area percentage of 4.93%.

3.3 Identification of *L. plantarum* bacterial filtrate compounds using gas chromatography mass spectrometry (GC-MS)

Table 4. Data on compounds identified in *L. plantarum* filtrate

Peak	Retention Time (minutes)	Area (%)	Name of the compound	Biological Activity	Reference
1	4,457	25,39	Decane (CAS) n-Decane ($C_{10}H_{22}$)	Antimicroba	Prasetyaningsih *et al*. (2018)
2	6,893	0,70	Octanoic acid, methyl ester (CAS) ($C_9H_{18}O_2$)	Antioxidants	Mungali *et al*. (2019)
3	9,072	8,46	2-Propenal, 3-phenyl- (CAS) (C_9H_8O)	Antifungal	Sudirga *et al*. (2024)
4	14,655	14,95	Sulfurous acid, cyclohexylmethyl heptyl ester ($C_{14}H_{28}O_3S$)	Disinfectant	Amerol *et al*. (2020)
5	17,250	8,96	Hexadecanoic acid, methyl ester ($C_{17}H_{34}0_2$)	Antifungal, antibacterial, antioxidant, anti-inflammatory, anticancer	Gupta *et al*. (2023)
6	17,690	10,46	4-tert-Butyl-2-(1-methyl-2-nitroethyl)cyclohexanone ($C_{13}H_{23}NO_3$)	Antibacterial, anti-inflammatory	Koziol *et al*. (2019)
7	17,781	8,09	Sulfurous acid, cyclohexylmethyl heptyl ester ($C_{13}H_{26}O_3S$)	Disinfectant	Amerol *et al*. (2020
8	18,176	16,11	4-t-Butyl-2-(1-methyl-2-nitroethyl)cyclohexanone ($C_{13}H_{23}NO_3$)	Antibacterial, anti-inflammatory	Koziol *et al*. (2019)
9	23,026	6,88	1,2-Benzenedicarboxylic acid, bis(2ethylhexyl) ($C_{24}H_{38}O_4$)	Antifungal, antidiabetic, anticancer, and antitumor .	Akpuaka *et al*. (2012)

Based on GC-MS analysis, the *L. plantarum* filtrate contains three main antifungal compounds, namely Hexadecanoic acid, methyl ester; 2-Propenal, 3-phenyl- (CAS) Cinnamaldehyde; and 1,2-Benzenedicarboxylic acid, bis(2ethylhexyl). Hexadecanoic acid, methyl ester was detected at peak 5 with a retention time of 17.250 minutes and an area percentage of 9.96%. 2-Propenal, 3-phenyl- (CAS) Cinnamaldehyde was detected at peak 3 with a retention time of 9.072 minutes and an area percentage of 8.47%. The compound 1,2-benzenedicarboxylic acid, bis(2-ethylhexyl) was detected at peak 9 with a retention time of 23.026 minutes and an area percentage of 6.88%.

3.4 Identification of P. *polymyxa* bacterial filtrate compounds using gas chromatography mass spectrometry (GC-MS).

Table 5. Data on compounds identified in *P. polymyxa* filtrates

Peak	Waktu Retensi (menit)	Area (%)	Senyawa yang Terindetifikasi	Aktivitas Biologi	Referensi
1	9.030	61,40	Cinnamaldehyde, (E)- (C_9H_8O)	Antifungal	Prasetyaningsih *et al*. (2018)
2	9,710	3,30	Decanoic acid, methyl ester (CAS) ($C_{11}H_{22}O_2$)	Antibacterial	Mostafiz *et al*. (2022)
3	12,546	6,92	Dodecanoic acid, methyl ester (CAS) ($C_{13}H_{26}O_2$)	Antimicrobial	Krishnappa *et al*. (2024)

4	14,655	1,72	Sulfurous acid, cyclohexylmethyl heptyl ester ($C_{14}H_{28}O_3S$)	Disinfectant	Ouyang *et al.* (2019)
5	15,007	3,39	Methyl tetradecanoate ($C_{15}H_{30}O_2$)	Antifungal	Igwe & Mgbemena (2014)
6	17,233	18,52	Hexadecanoic acid, methyl ester ($C_{17}H_{34}0_2$)	Antifungal, antibacterial, antioxidant, anti-inflammatory, anticancer	Gupta *et al.* (2023)
7	17,690	1,20	4-t-Butyl-2-(1-methyl-2-nitroethyl)cyclohexanone ($C_{13}H_{23}NO_3$)	Antibacterial	Koziol *et al.* (2019)
8	17,781	0,88	Phosphit, Tris(2,4-Dimethylpent-3-en-2-ol	Antimicrobial	Amerol *et al.* (2020)
9	18,177	1,82	4-t-Butyl-2-(1-methyl-2-nitroethyl)cyclohexanone ($C_{13}H_{23}NO_3$)	Antibacterial, anti-inflammatory	Martha *et al.* (2020)
10	19,047	0,97	8-Octadecenoic acid, methyl ester ($C_{19}H_{36}O_2$)	Anifungal	Akpuaka *et al.* (2012)

Based on GC-MS analysis, the filtrate of P. polymyxa contains four main antifungal compounds, namely Cinnamaldehyde, (E)-; Methyl tetradecanoate; Hexadecanoic acid, methyl ester; and 8-Octadecenoic acid, methyl ester. Cinnamaldehyde, (E)- was detected at peak 1 with a retention time of 9.030 minutes and an area percentage of 61.40%. Methyl tetradecanoate was detected at peak 5 with a retention time of 15.007 minutes and an area percentage of 3.39%. Hexadecanoic acid, methyl ester was detected at peak 6 with a retention time of 17.233 minutes and an area percentage of 18.52%, and 8-Octadecenoic acid, methyl ester was detected at peak 10 with a retention time of 19.047 minutes and an area percentage of 0.97%.

Based on the results of GC-MS analysis of *L. bulgaricus, P. fluorescens, L. plantarum,* and *P. polymyxa*, there are five compounds that exhibit antifungal activity against Fusarium sp., namely cinnamaldehyde, (E)- or (2-propenal, 3-phenyl); hexadecanoic acid, methyl ester; 2-benzenedicarboxylic acid, bis (2-ethylhexyl); 8-octadecenoic acid, methyl ester; and methyl tetradecanoate.

Cinnamaldehyde, (E)-, belongs to the phenylpropanoid group and is a derivative of phenolic compounds (Antasionasti *et al.*, 2020). This compound is known by various names, including 3-beta-phenylacrolein, cinnamic aldehyde, and 2-Propenal, 3-phenyl. This compound effectively inhibits various pathogenic microorganisms, including bacteria, yeast, and fungi, and prevents the production of microbial toxins (Doyle & Stephens, 2019). Its antifungal activity has been demonstrated through the inhibition of the growth of Geotrichum citri-aurantii (Ouyang *et al*., 2019), Fusarium verticillioides (Xing *et al*., 2014), Candida albicans (*Pootong et al*., 2017), and Fusarium sambucinum (Wei *et al*., 2019). Cinnamaldehyde acts as an antifungal agent through several mechanisms, including inhibiting ergosterol biosynthesis, inducing the accumulation of Reactive Oxygen Species (ROS), inhibiting enzymes involved in cytokine interactions, reducing microbial biofilm formation, and most significantly, disrupting the integrity of fungal cell membranes (Wei *et al*., 2019; Ouyang *et al*., 2019; Sudirga *et al*., 2024).

According to the Human Metabolome Database (2014), the compound known as hexadecanoic acid, methyl ester is also called methyl palmitate or methyl hexadecanoate. This compound belongs to the category of organic compounds in the fatty acid methyl ester group. Fatty acid methyl esters consist of compounds formed when fatty acids are esterified with methyl groups. Several studies have shown that hexadecanoic acid, methyl ester has the ability to inhibit various types of pathogenic fungi, including Candida albicans ATCC 18804, C. gatti ATCC 24065, C. neofarmans ATCC 24047, and Paracoccidioides brasiliensis Pb18 (Pinto *et al*., 2017), Fusarium oxysporum f.sp. vanillae, Fusarium solani, and Sclerotium rolfsii in vitro (Sukamto *et al*., 2019), and Fusarium solani (Mart.) Sacc., which causes stem wilt and root rot in banana plants (Wahyudi *et al*., 2019). The mechanism of hexadecanoic acid, methyl ester in inhibiting fungal growth involves inhibiting biofilm formation, inducing apoptosis, causing DNA and mitochondrial damage, and inhibiting ergosterol biosynthesis (Prasath *et al*., 2020).

Compound 1, 2-benzenedicarboxylic acid, bis (2-ethylhexyl) is a compound belonging to the dibutyl phthalate group. Dibutyl phthalate is an ester derivative of benzoic acid. Benzoic acid has been shown to reduce the growth rate of Alternaria solani in tomato plants (Nehela *et al*., 2021) and has an inhibitory effect on several soil fungi, such as Fusarium sp., as well as seed pathogens like Aspergillus flavus, Penicillium citrinum, and Alternaria alternata (Liu *et al*., 2017; Heflish *et al*., 2020). The primary mechanism by which benzoic acid inhibits fungal growth is through the blocking of cell septation during cell division (Nehela *et a*l., 2021).

8-Octadecenoic acid, methyl ester is also known as methyl 8-octadecenoic or methyl trans-8-octadecenoate. This compound belongs to the oleic acid group. Oleic acid is an unsaturated fatty acid. Oleic acid inhibits the growth of Candida spp. filaments (Muthamil *et al*., 2020), Rhizoctonia solani, Pythium ultimum, Pyrenophora avenae, and Candida perniciosa (Walters *et al*., 2004). The mechanism by which unsaturated fatty acids inhibit fungal growth is by damaging the permeability of fungal cell membranes (Thibane *et al*., 2010).

Methyl tetradecanoate is an ester derivative of myristic acid (tetradecanoic acid). Myristic acid inhibits the growth of Candida albicans (Prasath *et al*., 2019), Aspergillus spp, and Penicillium spp. (Altierti *et al*., 2007). The mechanism of myristic acid's antifungal activity involves inhibiting hyphal formation and disrupting biofilms (Kim *et al*., 2021).

IV.CONCLUSIONS

1. *L. bulgaricus* and *L. plantarum* showed antagonistic activity against *Fusarium* sp. with inhibition zones of 4.50 mm and 4.00 mm, while *P. polymyxa* and *P. fluorescens* showed no inhibition.
2. GC-MS analysis showed that the fourth bacterial filtrate contained active antifungal compounds, namely cinnamaldehyde (2-propenal, 3-phenyl), hexadecanoic acid methyl ester, 2-benzenedicarboxylic acid bis(2-ethylhexyl), 8-octadecenoic acid methyl ester, and methyl tetradecanoate.

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