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Exploring the Antimicrobial and Antioxidant Activities of *Streptomyces* sp. EIZ2 Isolated from Moroccan Agricultural Soil

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Abstract: Antibiotics play a crucial role in preserving and improving public health, saving millions of lives every year. However, their effectiveness is currently under threat due to the ability of bacteria to adapt and develop resistance to these treatments. Therefore, this study was carried out on two soil samples collected in two areas of Arba Aounate, Sidi Bennour province, Morocco, to identify natural antibiotic-producing *Actinobacteria* capable of combating multi-drug-resistant (MDR) bacteria. A primary screening revealed that of the 50 isolates, 16 exhibited antimicrobial activity against *Pseudomonas aeruginosa* ATCC 27,853, *Staphylococcus aureus* ATCC 25,923, *Escherichia coli* ATCC 25,922, and *Candida albicans* ATTC 60193. A secondary screening showed that of the 16 isolates, only EIZ1 and EIZ2 isolates displayed outstanding antimicrobial and antifungal activity against 6 MDR bacteria, including *Escherichia coli* 19L2418, *Listeria monocytogenes*, *Proteus* sp. 19K1313, *Klebsiella pneumoniae* 19K 929, *Proteus vulgaris* 16C1737, and *Klebsiella pneumoniae* 20B1572. These two isolates were also characterized culturally, morphologically, physiologically, and biochemically. Afterward, the amplification of 16S rRNA revealed that isolate EIZ2 was 99.06% strongly related to the genus *Streptomyces*. Furthermore, this extract exhibits strong antioxidant activity against DPPH and ABTS free radicals and elevated ferric-reducing antioxidant power. A significant ($p < 0.0001$) positive correlation was observed between antioxidant activities and total phenolic and flavonoid contents. A GC–MS analysis of the ethyl acetate extract revealed the presence of 10 compounds, mainly diethyl phthalate (97%) and benzeneacetic acid (94%). This research demonstrates that *Streptomyces* sp. strain EIZ2 represents a potential source of antimicrobial and antioxidant compounds. These compounds could offer considerable potential as therapeutic agents, paving the way for future developments in medical applications.

Keywords: *Streptomyces*; multi-drug resistance; antimicrobial activity; antioxidant activity; 16S rRNA; GC–MS analysis

1. Introduction

The emergence and widespread dissemination of multi-drug-resistant (MDR) pathogens represent a serious threat to global public health [1,2]. Antibiotic resistance, a consequence of bacterial acquisition of genetic mutations or resistance genes [3,4], makes standard antibiotics ineffective [5]. Recent statistics indicate that antibiotic-resistant infections contribute to over 35,000 deaths annually in the United States alone [6], while globally, they are responsible for approximately 700,000 deaths per year [7], resulting in heightened rates of illness and mortality. Simultaneously, the disruption of cellular equilibrium by the imbalance in reactive oxygen species (ROS) production establishes oxidative stress as a significant mechanism, with considerable implications for human health [8]. Uncontrolled oxidative stress can initiate a cascade of adverse effects, significantly elevating the risk of chronic conditions such as neurological disorders, cardiovascular diseases, cancer, and kidney disorders [8–10]. It was reported that ROS play a multifaceted role in bacterial survival, adaptation, and the development of MDR [11]. For instance, ROS can cause direct DNA damage, leading to genetic mutations that facilitate the emergence of MDR [12,13]. On the other hand, certain non-antibiotic drugs, including non-steroidal anti-inflammatory drugs (NSAIDs) and lipid-lowering medications, can increase ROS levels, which in turn promotes the transfer of antibiotic-resistance genes [14].

This pressing challenge underscores the urgent need for innovative antimicrobial strategies to combat evolving resistance mechanisms [2,15]. Therefore, to expand the array of treatments available for antibiotic-resistant infections, numerous studies have focused on investigating microorganisms, particularly *Streptomyces*, across diverse environments [16–18] to develop new bioactive compounds from microbial sources [19–21]. *Actinobacteria*, diverse Gram-positive bacteria, play a crucial role in addressing global multi-drug resistance [22]. Renowned for their ability to produce potent secondary metabolites with antimicrobial, antifungal, and antioxidant properties, these microorganisms are ubiquitous in various environments, including soil, marine habitats, and human microbiota. Belonging to the phylum *Actinobacteria*, notable genera include *Streptomyces*, *Micromonospora*, *Nocardia*, and *Actinomyces* [23].

Given the significance of *Actinobacteria* in addressing global multi-drug resistance, this research contributes to the understanding of *Actinobacteria*-derived bioactive compounds' antimicrobial and antioxidant activities and their potential in combating multi-drug resistance. To the best of our knowledge, there is no available information on the biological activities of *Streptomyces* strains isolated from agricultural soils in Arba Aounate, Sidi Bennour province, Morocco. Specifically, this study aimed to: (1) isolate *Actinobacteria* strains from Arba Aounate in Sidi Bennour, Morocco, and assess soil physicochemical parameters; (2) conduct a primary screening of *Actinobacteria* isolates for their antimicrobial activity; (3) perform morphological, biochemical, and physiological characterizations of the isolated *Actinobacteria*, accompanied by 16S rRNA gene sequencing analyses; (4) evaluate the antimicrobial efficacy of selected isolates exhibiting high activity against *Escherichia coli* ATCC 25,922, *Pseudomonas aeruginosa* ATCC 27,853, *Staphylococcus aureus* ATCC 25,923, *Candida albicans* ATCC 60,193 and six multi-drug-resistant pathogens, including *Escherichia coli* 19L2418, *Listeria monocytogenes*, *Proteus* sp. 19K1313, *Klebsiella pneumoniae* 19K 929, *Proteus vulgaris* 16C1737, and *Klebsiella pneumoniae* 20B1572; (5) determine the antioxidant activity of the selected isolates and analyze the polyphenol and flavonoid contents in their extracts; (6) assess the toxicity of the selected isolates using UV-visible and hemolytic activity assays; and (7) identify the profile of the main compounds of the most active isolate (determined based on high antimicrobial and antioxidant activities) using gas chromatography–mass spectrometry (GC–MS) analysis. This work aimed to not only identify potent antimicrobial and antioxidant agents from *Actinobacteria* but also assess their safety and explore their chemical composition, paving the way for potential therapeutic applications in combating multi-drug resistance and oxidative stress-related disorders.

2. Materials and Methods

2.1. Collecting Soil Samples

The soil samples used in this study were collected in April 2022 from two locations in Arba Aounate, Sidi Bennour province, Morocco (GPS: 32°44′24″ N, 8°11′24″ W). In order to avoid species similarity between *Actinobacteria*, soil samples were taken at five different points in a 400 m^2 area for each site [19,24]. After removing the top few centimeters of soil, each sample was taken to a depth of 10 cm from the top layer of soil. Approximately 200 g of soil was carefully placed into a sterile bottle using a sterile spatula, with the reference duly noted. The collected soil samples were then transported to the laboratory and conserved at 4 °C until use [19].

2.2. Physico-Chemical Soil Analysis

The pH of each soil sample was determined using a calibrated pH meter (OCRISON, micro-pH 2000). The electrode was immersed in a suspension at a ratio (soil/distilled water) of 2:5 (g/mL). Electrical conductivity (EC) was determined using a conductivity meter (Seven GoT M). Texture characterization was performed using the sedimentation technique. Briefly, 25 g of soil were placed in a glass beaker containing one-third distilled water. The mixture was then stirred and left to stand for 2 days to allow the three soil phases to emerge: clay, silt, and sand. Chemical elements such as Mg, Si, Fe, K, Zn, Mn, Cl, Al, P, Cu, and S were analyzed by energy-dispersive X-ray fluorescence (Epsilon 3XLE from PANalytical, France) [25].

2.3. Pre-Treatment of Soil Samples and Isolation of Actinobacteria Strains

The pre-treatment process is essential for the selective isolation of *Actinobacteria*, which are known to grow more slowly than other microorganisms. Typically, this process involves drying soil samples at room temperature (around 26 °C) in the laboratory for 7 days, thereby inhibiting or eliminating unwanted Gram-negative bacteria [26]. The isolation was carried out using the serial dilution technique [27]. Briefly, 10 g of each soil sample were ground and mixed with 90 mL of distilled water in an Erlenmeyer flask while noting the reference of each sample. The resulting suspension (10^{-1}) was agitated at maximum speed for 30 min. After settling, the supernatants were collected, and a series of decimal dilutions (10^{-1} to 10^{-4}) were prepared. Four different culture media (Bennett, GLM, M2, and GA) were employed for *Actinobacteria* isolation. The detailed compositions of these media are provided in the supplementary data [28–31], and these media were inoculated with a volume of 100 µL per dilution (10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}) per Petri dish (4 dishes for each medium) [19]. Plates were incubated at 28 °C and checked daily for one week.

2.4. Preliminary Identification of Actinobacteria Isolates

Colonies displaying typical macroscopic characteristics of *Actinobacteria*, such as being solid, pigmented or whitish, strongly adherent to the agar surface, and featuring a compact, dry, smooth, or rough texture, were cultured in ISP2 medium. These colonies were then examined under a light microscope at 40× magnification and after Gram staining at 100× magnification. Purified isolates underwent a 7-day incubation at 28 °C, were subsequently stored at 4 °C, and were suspended in glycerol before being preserved for the long term at −20 °C [32].

2.5. Genotypic Identification and Phylogenetic Tree Construction

Genotypic identification involved 16S rDNA sequencing to confirm isolate species. DNA extraction used the Mag Purix Bacterial DNA Extraction Kit, following the manufacturer's instructions. The DNA extracted from a specific *Actinobacteria* isolate was quantified using a NanoDrop 8000 spectrophotometer (Thermo Scientific, Waltham, MA, USA) and then stored at −20 °C until use. The concentration of the extracted DNA from this isolate was adjusted to 100 ng/µL for the PCR reaction. The 16S rRNA gene was amplified with the universal primers Fd1 (5′-AGAGTTTGATCATGGCTCAG-3′) and rP2

(5′-ACGGTTACCTTGTTACGACTT-3′) in a Verity DNA thermal cycler [33]. The cycling program included initial denaturation at 95 °C for 2 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 52 °C for 30 s, extension at 72 °C for 30 s, and a final extension at 72 °C for 3 min. Sequencing of successful PCR products was conducted on a 3130x1 genetic analyzer. The National Center for Scientific and Technical Research (CNRST) in Rabat, Morocco, conducted extraction, amplification, and sequencing. Nucleotide sequences were analyzed using the BLAST program on the NCBI website. Assembled sequences used DNA Baser Assembler software, and MEGA X software aligned sequences with the refseq_rna database archived in the Genomic Data Bank, which is accessible on the Internet at the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov), accessed on 31 October 2023 [34]. The phylogenetic tree employed the maximum likelihood method [34].

2.6. Evaluation of Antimicrobial Activity

2.6.1. Test Bacteria Used

The study employed three bacterial strains (*Escherichia coli* ATCC 25,922, *Pseudomonas aeruginosa* ATCC 27,853, and *Staphylococcus aureus* ATCC 25,923) and *Candida albicans* ATCC 60,193, a pathogenic fungus, sourced from the Pasteur Institute in Casablanca, Morocco. In addition to these strains, 6 clinically pathogenic multi-drug-resistant (MDR) strains, namely *Escherichia coli* 19L2418, *Listeria monocytogenes*, *Proteus* sp. 19K1313, *Klebsiella pneumoniae* 19K 929, *Proteus vulgaris* 16C1737, and *Klebsiella pneumoniae* 20B1572 were also included in the study. The antibiotic resistance profile of the clinically tested MDR strains was confirmed using 16 different antibiotics (Supplementary Table S1).

2.6.2. Primary Screening of Actinobacteria Isolates for Antimicrobial Activity

Antimicrobial activity was evaluated using the double-layer technique described by Badji et al. [35] against *Pseudomonas aeruginosa* ATCC 27,853, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25,922, and a pathogenic fungus (*Candida albicans* ATCC 60,193). *Actinobacteria* isolates were seeded in 10 mm diameter keys, either at the edge [36] or in the center [35] of a 90 mm diameter Petri dish containing ISP2 medium. After a 10-day incubation in an oven at 28 °C, the cultures were covered with 5 mL of Muller–Hinton medium, weakly agarized (7 g/L agar) and previously seeded with a test bacterium. Antimicrobial activity was assessed by measuring the zones of inhibition in mm.

2.6.3. Fermentation and Extraction of Secondary Metabolites

Erlenmeyer flasks at a size of 250 mL were used for this experiment. In each flask, 100 mL of agar-free ISP2 liquid medium was inoculated with active *Actinobacteria* isolates (EIZ1 and EIZ2). The Erlenmeyer flasks were then incubated for 3 days under constant agitation at 120 rpm on a shaker at room temperature [27].

Liquid–liquid extraction involves transferring solutes between immiscible solvents. Despite facilitating substance transfer, vigorous mixing during this process can form emulsions, complicating phase separation [37]. In this study, the objective was to recover secondary metabolites, specifically fermentation products. After fermenting, the mycelial mass is removed via centrifugation at 8000× g for 20 min [38]. Supernatant from each medium is mixed with dichloromethane in a separating funnel. The resulting aqueous phase is then combined with an equal volume of ethyl acetate. Organic extracts are obtained and evaporated at 45 °C using a rotary evaporator (Buchi B-491 Heating Bath). Dry residues were subsequently solubilized in DMSO [39].

2.6.4. Evaluation of the Antimicrobial Activity of Organic Extracts

To assess antimicrobial activity in liquid medium, the disc technique was used [35] against 6 clinical MDR strains (*Proteus* sp. 19K1313, *Proteus vulgaris* 16C1737, *Klebsiella pneumoniae* 19K 929, *Listeria monocytogenes*, *Escherichia coli* 19L2418, and *Klebsiella pneumoniae* 20B1572), and *Candida albicans* ATCC 60,193. For the assay, sterile 6 mm diameter filter

paper discs were impregnated with 20 µL of each extract. As a negative control, a disc loaded with an equivalent volume of dimethyl sulfoxide (DMSO) to that of the extract was used. Nalidixic acid and cycloheximide served as positive controls for antibacterial and antifungal activities, respectively. The impregnated discs were left to dry for a few min using a Bunsen burner, then placed with sterile forceps on the surface of Muller–Hinton medium previously inoculated with test bacteria using the swabbing technique. The plates were then placed at 4 °C for 2 h to allow for diffusion of the molecules, then incubated at 37 °C for 24 h. Afterward, the diameter of inhibition zones was measured in mm after 24 h of incubation at 37 °C for bacteria and 48 h at 28 °C for fungi.

2.6.5. Cultural, Physiological, and Biochemical Characteristics of Actinobacteria Isolates

The cultural characteristics, such as extent of growth, pigmentation of the surface and reverse (the back of the colony), colony diameter, colony appearance, and presence of diffusible pigments in the agar of two isolates EIZ1 and EIZ2, were determined on the following culture media (ISP1, ISP2, ISP7, GYEA, and Bennett) by inoculating each *Actinobacteria* isolate on all 5 media using the tight streak method. The plates were then incubated at 28 °C for 10 days [35]. *Actinobacteria* isolates were inoculated in ISP2 solid culture medium buffered to different pH values (4.02; 5.02; 6.45; 7.28; 8.02; 9.06; and 10.02) with NaOH (sodium hydroxide) or HCL (hydrochloric acid) solutions [40]. The growth of two isolates was assessed visually after 7 days incubation at 28 °C.

The tolerance of *Actinobacteria* to NaCl was tested on a yeast extract agar medium (Supplementary Materials) supplemented with a graduated series of NaCl concentrations (1, 2, 3, 4, 5, 6, 7, 8, 9, and 10%). Then, we inoculated our *Actinobacteria* isolates into YEA-NaCl medium using the tight streak method at each NaCl concentration. After inoculation, plates were incubated at 30 °C for 4 days [41]. The medium used to test carbohydrate uptake by purified *Actinobacteria* isolates was ISP9 (Supplementary Materials), which is rich in mineral components. We added simple sugars and other complexes as carbon sources (starch, mannitol, cellobiose, sucrose, xylose, mannose, fructose, and glucose (positive control)). After inoculation of our *Actinobacteria* isolates by the tight streak method, plates were incubated at 28 °C for one week.

2.7. Determination of Total Phenolic and Flavonoid Contents

The determination of total polyphenols contained in the ethyl acetate extract of *Actinobacteria* isolate EIZ2 was conducted using the Folin–Ciocalteu method described by Kumazawa et al. [42], whereas the total flavonoid content in the same extract was quantified according to Bahorun et al. [43].

2.8. Evaluation of the In Vitro Antioxidant Activities

The free radical scavenging activity of the ethyl acetate (EA) extract from *Streptomyces* sp. EIZ2 was assessed using the DPPH assay, following the protocol outlined by Blois [44]. Absorbance measurements were performed at 517 nm with an ELISA microplate reader (2100-C, Optic Ivymen Systems, Barcelona, Spain). As a positive control, ascorbic acid was used. Additionally, the free radical scavenging activity (ABTS) was determined based on the method developed by Re et al. [45], with Trolox serving as the positive control. Concerning the ferric-reducing antioxidant power (FRAP), it was evaluated according to the procedure described by Oyaizu [46]. Measurements were conducted in triplicate, and ascorbic acid was employed as a positive antioxidant control.

2.9. UV-Visible Analysis

The absorption spectra of *Streptomyces* sp. EZ2 ethyl acetate extract were measured at wavelengths ranging from 190 to 850 nm using a HACH Lange DR6000 scanning UV-visible spectrophotometer [47].

2.10. Evaluation of Hemolytic Activity

The hemolytic activity of the ethyl acetate of EIZ2 isolate was evaluated using a hemolysis assay with human red blood cells (RBCs), following the methods reported by Rajendran et al. [48] and Zhu et al. [49] with some modifications. Human erythrocytes (RBCs) were collected from healthy individuals in tubes with purple caps containing EDTA as an anticoagulant. An amount of 2 mL of whole blood was combined with 4 mL of phosphate-buffered saline (PBS) and subjected to centrifugation at 9000× *g* for 5 min at 24 °C. The resulting supernatant was discarded, and the pellet was washed twice with 10 mL of PBS, then ultimately diluted to 20 mL using PBS. An amount of 400 µL of the diluted RBC suspension was mixed with 1.6 mL of each extract prepared in PBS, spanning concentrations of 0.125, 1.25, and 3.12 mg/mL for the test group. Additionally, PBS served as the negative control, while sodium dodecyl sulfate (SDS) was the positive control. The experimental setup was duplicated. Tubes were incubated at 37 °C for 1 h and subsequently centrifuged for 5 min at 5000× *g*.

2.11. GC–MS Analysis of EIZ2 Strain Crude Ethyl Acetate Extract

Analysis of the profile of compounds present in the crude ethyl acetate extract of strain EIZ2 was carried out by gas chromatography coupled with mass spectrometry (GC–MS) on an Agilent 7890A Series instrument. The system was equipped with a multimode injector and an HP-5MS column with the dimensions 30 m × 0.250 mm × 0.25 µm at the Moroccan Foundation for Advanced Science, Innovation and Research (MAScIR) Institute. Briefly, a volume of the extract solubilized in chloroform was injected into the column (split mode 1:4) with helium as a carrier gas at a flow rate of 1.7 mL/min. The ion source and quadrupoles were maintained at temperatures of 230 and 150 °C, respectively. The oven temperature program started at 60 °C and ended at 360 °C. Compound identification was carried out by comparing the mass spectra obtained with reference data from the NIST MS 2017 library.

2.12. Statistical Analysis

The experiments were conducted in triplicate, and the results were presented as the mean value along with the standard deviation (SD). To assess differences between groups in phenolic compound, flavonoid compound, and antioxidant activity assays, GraphPad Prism 8.4.3 software was used. Specifically, we utilized a standard two-way ANOVA test followed by Tukey's multiple comparisons test. Statistical significance was set at $p < 0.05$. Pearson correlation analysis was conducted using GraphPad Prism 8.4.3 software to assess the relationship between total phenolic and flavonoid compounds and antioxidant activity.

3. Results

3.1. Physico-Chemical Analysis of Soil Samples

The pH, electrical conductivity, texture, and minerals measured for the two soil samples are shown in Table 1. Analysis of the soil at Site A indicated that it possesses alkaline characteristics with a pH level of 8.08 and is not saline, as evidenced by its low electrical conductivity (EC = 0.004 ds/m). According to the textural diagram, it falls into the clay-loam category and has a relatively low sand content of 28%. Our observations also unveiled the presence of various exchangeable cations, including K, Mg, Al, Ca, Fe, Zn, Cu, and Si. Notably, mineral elements like Si, Fe, Al, K, and Ca were found in higher concentrations (Table 1). In contrast, the soil at Site B leans slightly towards alkalinity, is predominantly sandy (75%), and displays not-salty characteristics (EC = 0.003 ds/m). This soil type has a lower proportion of clay and silt and generally lower concentrations of mineral elements, except for Si, which is specifically abundant at 21.22%. Comparing the pH values of the soils at both sites, we observed variations among different soil textures. The lowest pH value (7.79) was recorded in the Argilo-loamy texture at Site A, while the highest pH value (8.08) was documented in the sandy texture at the same location.

Table 1. Physico-chemical analysis of soil samples.

Physico-Chemical Parameters	Site A	Site B
Textural soil types	Silty clay	Sandy
Clay (%)	33	9
Sand (%)	28	75
Silt (%)	39	14
pH	8.08	7.79
Electrical conductivity (ds/m)	0.004	0.003
Mg (%)	0.448	0.327
Al (%)	5.088	4.653
Si (%)	16.769	21.229
P (%)	0.265	0.360
S (%)	0.068	0.138
Cl (%)	0.013	0.038
K (%)	3.233	2.103
Ca (%)	1.220	0.901
Mn (%)	0.087	0.039
Fe (%)	6.501	3.129
Cu (%)	0.008	0.004
Zn (%)	0.021	0.006
Ga (%)	0.004	0.002
Rb (%)	0.028	0.013
Sr (%)	0.018	0.007
Zr (%)	0.045	0.063
Ag (%)	0.095	0.084

Site A (garden soil); Site B (agriculture soil); EC: electrical conductivity.

3.2. Isolation of Actinobacteria

For the isolation of Actinobacteria, the following media were selected: Bennett, GLM, M2, and GA were chosen as selective culture media suitable for Actinobacteria to grow. Numerous colonies, distinguished by their macroscopic aspect, appeared after one week of incubation. These were isolated and then subcultured in ISP2 medium. Colony counts for both soil samples in all media were shown in Table 2. In our study, we isolated a total of 50 distinct phenotypically presumed Actinobacteria isolates from soil samples obtained from the two sampling locations. These isolates were distributed as follows: 58% from Site A and 42% from Site B (Table 2). M2 medium produced the highest number of isolates (n = 27), followed by Bennett (n = 16), GLM (n = 7), and GA (n = 0) (Table 2). According to the results, only the M2 and Bennett media had significant numbers of Actinobacteria colonies, then the GLM medium with low numbers in the two soil samples; however, no colonies appeared in the GA medium.

Table 2. Total number of *Actinobacteria* colonies at Sites A and B using four different culture media.

Sampling Site for Soil Samples	The Count of *Actinobacteria* Colonies across Various Isolation Media (Expressed as $\times 10^2$ CFU/mL)				The Total Number of *Actinobacteria* (Expressed $\times 10^2$ CFU/mL)	Count of Colonies with Specific Morphological Characteristics in Each Medium				Total Number of Isolates
	GA	GLM	M2	Bennett		GA	GLM	M2	Bennett	
Site A	0	3	20	15	38	0	4	15	10	29
Site B	0	4	19	12	34	0	3	12	6	21
Total	0	7	39	27	72	0	7	27	16	50

Site A (garden soil); Site B (agriculture soil).

3.3. Primary Screening of Actinobacteria Isolates

Among the 50 *Actinobacteria* isolates, only 16 (32%) showed important antimicrobial activity against at least one test microorganism, including Gram-positive bacteria such as *Staphylococcus aureus* ATCC 25,923, Gram-negative bacteria such as *Escherichia coli* ATCC 25 922, and *Pseudomonas aeruginosa* ATCC 27,853 and *Candida albicans* ATCC 60,193 (Table 3, Figure 1).

Table 3. Evaluation of the antimicrobial activity of *Actinobacteria* isolated by the double-layer method in ISP2 medium.

***Actinobacteria* Isolates**	**Antimicrobial Activity (mm)**			
	***Pseudomonas aeruginosa* ATCC 27,853**	***Staphylococcus aureus* ATCC 25,923**	***Escherichia coli* ATCC 25,922**	***Candida albicans* ATCC 60,193**
EIZ1	30	20	31	-
EIZ2	32	40	-	41
EIZ3	-	57	30	-
EIZ4	-	-	-	-
EIZ5	-	-	-	-
EIZ6	-	-	-	-
EIZ7	-	-	-	-
EIZ8	-	-	-	-
EIZ9	-	30	20	-
EIZ10	-	-	-	-
EIZ11	-	-	-	-
EIZ12	-	-	-	-
EIZ13	-	-	-	-
EIZ14	-	-	30	-
EIZ15	-	-	-	24
EIZ16	-	-	-	-
EIZ17	20	20	20	25
EIZ18	-	-	-	-
EIZ19	45	40	20	-
EIZ20	-	-	-	-
EIZ21	-	-	-	-
EIZ22	-	-	-	-
EIZ23	-	-	-	-
EIZ24	-	-	-	-
EIZ25	-	-	-	-
EIZ26	-	-	-	-
EIZ27	-	-	-	-
EIZ28	-	-	-	-
EIZ29	15	40	15	-
EIZ30	-	-	-	-
EIZ31	-	-	-	-
EIZ32	-	-	-	-

Table 3. *Cont.*

Actinobacteria Isolates	Antimicrobial Activity (mm)			
	Pseudomonas aeruginosa **ATCC 27,853**	***Staphylococcus aureus*** **ATCC 25,923**	***Escherichia coli*** **ATCC 25,922**	***Candida albicans*** **ATCC 60,193**
EIZ33	-	-	-	-
EIZ34	-	-	-	-
EIZ35	-	-	-	-
EIZ36	-	-	-	-
EIZ37	-	-		28
EIZ38	-	40	-	-
EIZ39	-	23	-	-
EIZ40	-	40	-	-
EIZ41	-	25	-	-
EIZ42	20	30	20	18
EIZ43	-	-	-	-
EIZ44	-	-	-	-
EIZ45	45	13	-	20
EIZ46	-	-	-	-
EIZ47	-	-	-	-
EIZ48	-	-	-	-
EIZ49	-	-	-	-
EIZ50	-	-	-	-

(-) No inhibition zones.

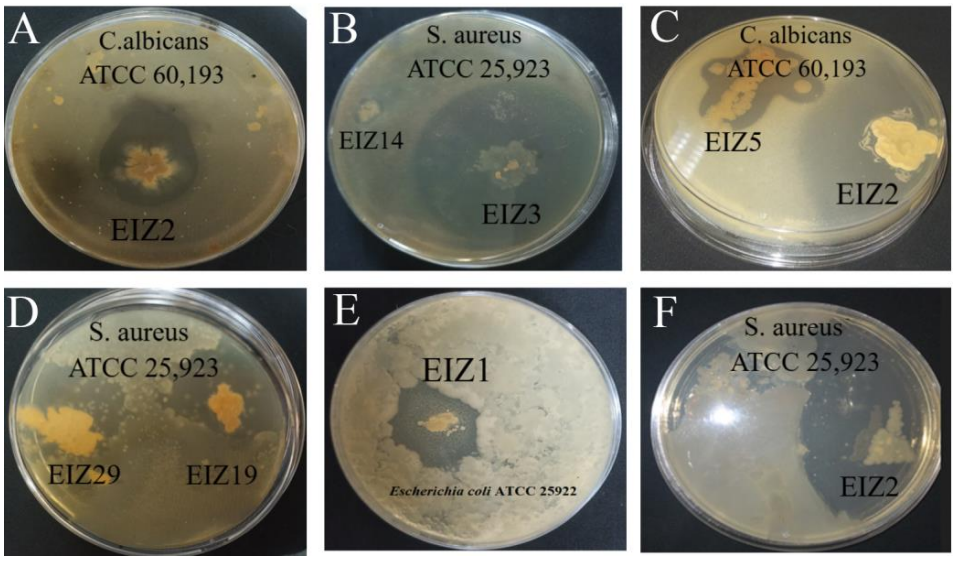

Figure 1. Evaluation of the antimicrobial activity of pure isolates of *Actinobacteria* using the double layer method in ISP2 medium. (**A**) Antimicrobial activity of EIZ2 isolate against *Candida albicans* ATCC 60,193; (**B**) antimicrobial activity of EIZ3 and EIZ14 isolates against *Staphylococcus aureus* ATCC 25,923; (**C**) antimicrobial activity of EIZ2 and EIZ5 isolates against *Candida albicans* ATCC 60,193; (**D**) antimicrobial activity of EIZ19 and EIZ29 isolates against *Staphylococcus aureus* ATCC 25,923; (**E**) antimicrobial activity of EIZ1 isolate against *Escherichia coli* ATCC 25,922; (**F**) antimicrobial activity of EIZ2 isolate against *Staphylococcus aureus* ATCC 25,923.

3.4. Secondary Screening of Actinobacteria Isolates

The 16 bioactive isolates that showed antimicrobial activity against at least one test microorganism in the primary screening were subjected to a secondary screening against certain multi-drug-resistant (MDR) pathogenic bacteria (Table 4, Figure 2), as well as against *Candida albicans* ATCC 60,193. The greatest zones of inhibition were observed with ethyl acetate and dichloromethane extracts. It should be noted that EIZ1, tested in liquid medium, showed no observable antimicrobial or antifungal activity. In contrast, EIZ2 showed significant activity against all six multi-drug-resistant clinical bacterial strains tested, indicating its broad-spectrum antimicrobial ($p < 0.05$) potential. Of the extracts tested, the ethyl acetate of EIZ2 consistently showed the largest and most pronounced areas of antimicrobial activity.

Table 4. Secondary screening (antimicrobial activity of isolate EIZ2) was performed using the disc diffusion method, varying extraction solvents.

Multi-Drug-Resistant Bacteria (MDR)	PC ^{BC}	NC ^{D}	DM ^{BA}	EA ^{A}	RP ^{D}
Clinical *Proteus* sp. 19K1313	-	-	19.67 ± 1.53	20 ± 1.00	-
Clinical *Proteus vulgaris* 16C1737	25.5 ± 0.71	-	20 ± 1.00	15 ± 1.00	-
Clinical *Klebsiella pneumonie* 19K 929	-	-	16 ± 0.58	22.66 ± 1.53	-
Clinical *Listeria monocytogenes*	25 ± 0.71	-	-	30 ± 2.08	-
Clinical *Escherichia coli* 19L2418	-	-	-	15.67 ± 1.15	-
Clinical *Klebsiella pneumoniae* 20B1572	-	-	-	40.67 ± 0.58	-
Candida albicans ATCC 60,193	27 ± 2.83	-	-	40.33 ± 0.57	-

Values are the means ± SD ($n = 2$). Columns with different letters A, B, C, and D are significantly different (ordinary one-way ANOVA; Tukey's multiple comparisons test; p Tukey's multiple comparisons test, $p < 0.05$). (-) No zone of inhibition; (PC) positive control (nalidixic acid and cycloheximide); (NC) negative control (DMSO); (DM) dichloromethane; (EA) ethyl acetate; (PR) residual phase and disc diameter = 6 mm.

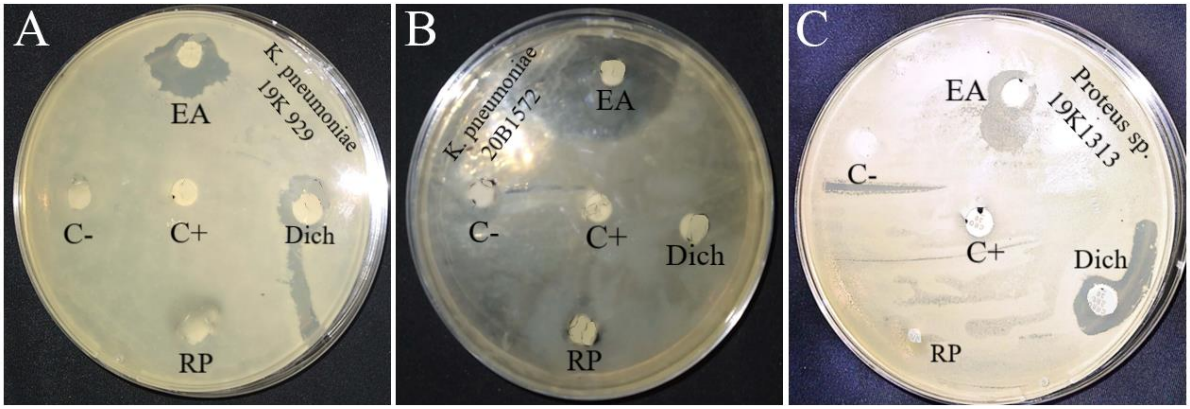

Figure 2. Evaluation of antimicrobial activity in the ethyl acetate extract from isolate EIZ2 using the disc diffusion method on MH medium. (**A**) Antimicrobial activity of EIZ2 isolate against clinical *Klebsiella pneumonie* 19K 929; (**B**) Antimicrobial activity of EIZ2 isolate against clinical *Klebsiella pneumoniae* 20B1572; (**C**) Antimicrobial activity of EIZ2 isolate against clinical *Proteus* sp. 19K1313; (C+) positive control (nalidixic acid); (C−) negative control (DMSO); (Dich) dichloromethane; (EA) ethyl acetate; (PR) residual phase.

3.5. Micro-Morphological, Biochemical, and Physiological Characteristics of Isolates

Among the 16 bioactive isolates tested, only 2 showed notable antimicrobial activity (EIZ1 and EIZ2), which led to their selection for taxonomic, physiological, and biochemical investigations (Figure 3, Table 5). The macroscopic characteristics of the two isolates are as follows: EIZ1 exhibits a round, wrinkled surface with a powdery texture, appearing whitish towards the ends and firmly adhered to the agar surface. In contrast, EIZ2 displays a smooth, powdery surface, also firmly attached to the agar. Both *Actinobacteria* isolates

grow over a wide pH range. EIZ1 was able to grow in pH values ranging from 5.02 to 10.02, and EIZ2 in a wider range from 4.02 to 10.02. Additionally, both isolates tolerated NaCl concentrations ranging from 1% to 7%, while EIZ2 was able to grow in concentrations of up to 10%. Furthermore, when assessing their growth preferences on different culture media, both isolates exhibited strong growth in ISP1, ISP2, ISP7, GYEA, and Bennett media. In particular, macroscopic observation of the two *Actinobacteria* isolates showed the production of melanoid pigments by isolate EIZ2 after one week of incubation at 28 °C: a brown exogenous pigmentation diffused in the agar around the colonies. No pigmentation was produced by isolate EIZ1. Regarding temperature tolerance, both isolates showed high temperature tolerance, growing efficiently in the range between 4 °C and 37 °C. Notably, EIZ2 stood out by even tolerating a high temperature of 45 °C. Under microscopic examination, both isolates, with and without Gram staining, were presented as filamentous and Gram-positive.

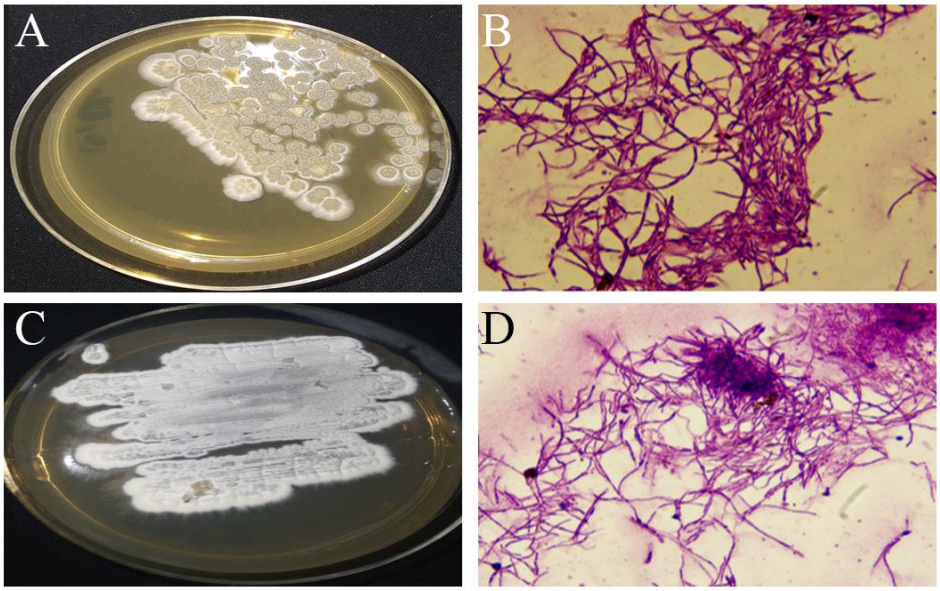

Figure 3. Morphological (**A**,**C**) and microscopic (**B**,**D**) characteristics of 2 active *Actinobacteria* isolates. (**A**,**B**) EIZ1 isolate; (**C**,**D**) EIZ2 isolate.

Table 5. Cultural, physiological, and biochemical characteristics of *Actinobacteria* isolates.

Test	EIZ1	EIZ2
	Carbon assimilation	
Amidon	-	2+
Mannitol	+	3+
Cellobiose	3+	3+
Sucrose	+	+
Xylose	+	2+
Mannose	3+	3+
Fructose	3+	3+
Positive control (Glucose)	3+	3+
	pH tolerance	
4.02	-	+
5.02	3+	3+
6.45	3+	3+
7.28	3+	3+
8.02	3+	3+
9.06	3+	3+
10.02	2+	2+

Table 5. *Cont.*

Test	EIZ1	EIZ2
	NaCl tolerance	
1%	3+	3+
2%	3+	3+
3%	3+	3+
4%	3+	2+
5%	3+	2+
7%	2+	2+
10%	-	+
	Temperature growth	
4 °C	3+	3+
28 °C	3+	3+
37 °C	3+	3+
45 °C	-	2+
Growth on different ISP media		
ISP1	3+	3+
ISP2	3+	3+
ISP7	2+	2+
GYEA	3+	3+
Bennett	2+	3+

(-) No growth; (+) low growth; (2+) intermediate growth; (3+) good growth.

3.6. Genotypic Characterization of EIZ2 Isolate and Construction of Phylogenetic Tree

The partial sequencing of the 16S rRNA gene revealed a remarkable 99.06% similarity to *Streptomyces bacillaris*, as reported in the NCBI GenBank database (Supplementary Table S2). The resulting sequence was aligned with the sequences of 19 closely related *Streptomyces* sp. obtained from the NCBI GenBank database. These sequences were then assembled in MEGA7 software for phylogenetic analysis using the maximum likelihood method, and evolutionary distances were calculated using Kimura's two-parameter method. The resulting phylogenetic tree (Figure 4) confirmed the similarity of isolate EIZ2 to *Streptomyces bacillaris*, with a confidence level represented by the bootstrap value of the similarity matrix reaching 89. The GenBank accession number for the partial 16S rRNA gene sequence of strain EIZ2 is OR731196.

3.7. Total Phenolic Content of Crude Extracts

The results concerning the total polyphenol and flavonoid contents of the ethyl acetate extract of EIZ2 were summarized in Table 6. The data show an increase in both phenolic and flavonoid contents with increasing extract concentration, ranging from 0.261 ± 0.003 to 0.470 ± 0.013 mg GAE/mg extract for phenolics and from 0.006 ± 0.005 to 0.114 ± 0.007 mg QE/mg extract for flavonoid contents. Notably, the concentration of flavonoids in the extract is lower compared with phenolics.

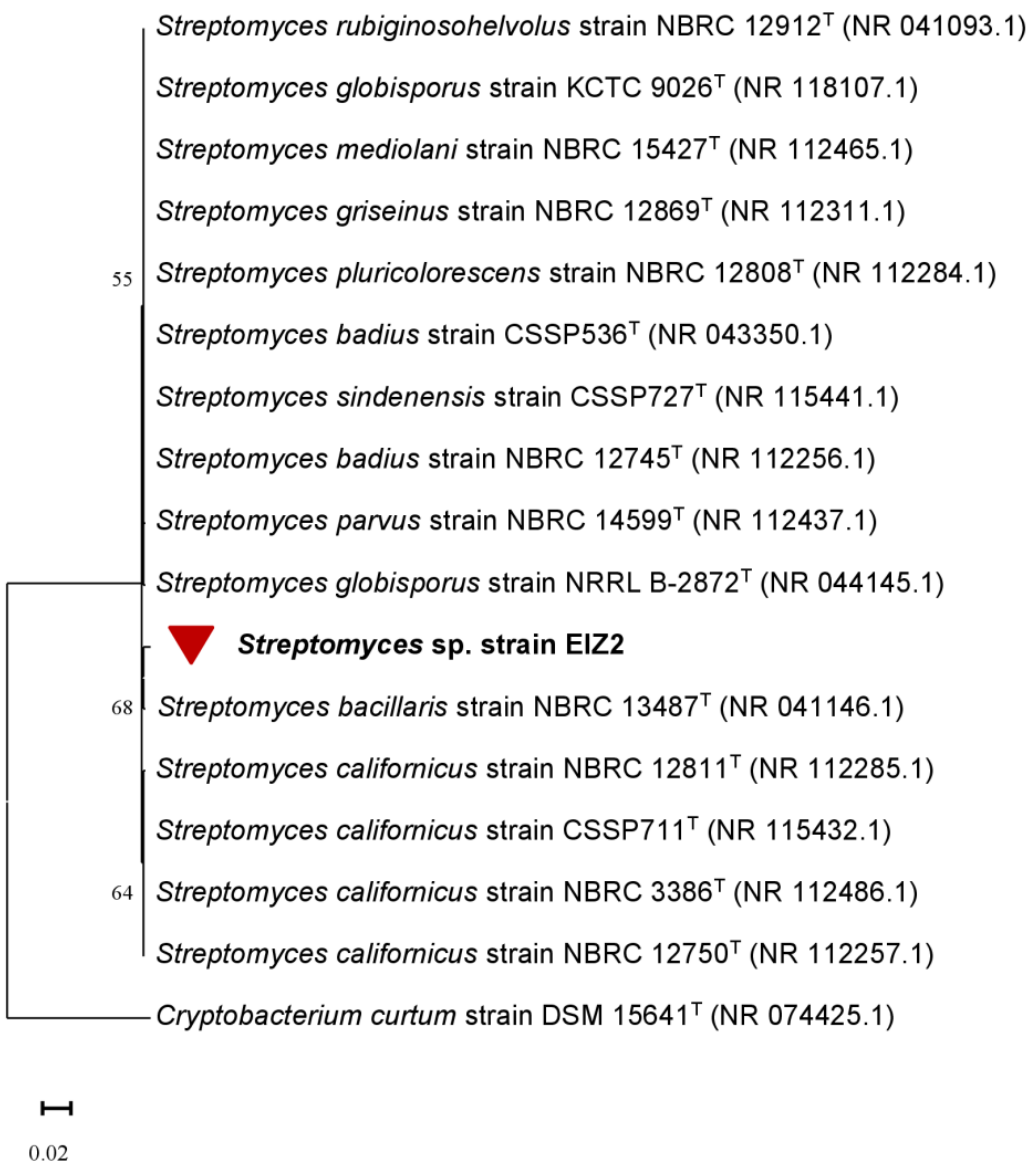

Figure 4. Phylogenetic tree constructed via the maximum likelihood tree test using the 16S rRNA gene, illustrating the evolutionary relationships between *Streptomyces* sp. EIZ2 (highlighted with a red triangle) and closely related taxa. The bar (0.02) denotes substitutions per nucleotide position (1% sequence divergence). GenBank accession numbers are indicated in brackets. *Cryptobacterium curtum* was the outgroup in the analysis. T: Type strain.

Table 6. Total phenolic and flavonoid contents of ethyl acetate extract of EIZ2 isolate.

Concentration of EIZ2 Ethyl Acetate Extract (mg/mL)	Total Phenolic Contents (mg GAE/mg Extract)	Total Flavonoids Contents (mg QE/mg Extract)
0.2	0.261 ± 0.003	ND
0.3	0.309 ± 0.010	ND
0.4	0.361 ± 0.005	0.006 ± 0.005
0.5	0.395 ± 0.002	0.024 ± 0.003
0.6	0.430 ± 0.004	0.053 ± 0.006
0.7	0.464 ± 0.002	0.080 ± 0.006
0.8	0.470 ± 0.013	0.114 ± 0.007
0.9	0.492 ± 0.019	0.156 ± 0.005
1	0.546 ± 0.013	0.1929 ± 0.02

Values expressed are the means ± SD (n = 3). GAE: Gallic acid equivalent; QE: quercetin equivalent; ND: not detected.

3.8. In Vitro Antioxidant Activities

The results presented in the Table 7 indicate significant DPPH radical scavenging activity ($p < 0.0001$), with inhibition percentages ranging from 2.91 ± 0.92 to 37.78 ± 1.93% at concentrations ranging from 0.2 to 1 mg/mL. Furthermore, the extract also showed significant ABTS radical scavenging activity ($p < 0.0001$), with inhibition percentages ranging from 1.32 ± 1.96 to 30.58 ± 1.78% at concentrations ranging from 0.2 to 1 mg/mL. Regarding the antioxidant reducing power of iron (FRAP), the extract demonstrated significant iron-reducing activity ($p < 0.0001$), measured from 0.43 ± 0.01 to 0.89 ± 0.02 mg AAE/mg extract at concentrations of 0.2 to 1 mg/mL.

Table 7. Antioxidant activities of ethyl acetate extract of EIZ2 isolate assessed using different antioxidant assays (DPPH, ABTS, and FRAP).

Concentrations (mg/mL)	Antioxidant Activities				
	EIZ2 Ethyl Acetate Extract			Trolox (PC)	Ascorbic Acid (PC)
	DPPH Free Radical Scavenging Activity (%)	ABTS Free Radical Scavenging Activity (%)	FRAP (mg AAE/mg Extract)	ABTS Free Radical Scavenging Activity (%)	DPPH Free Radical Scavenging Activity (%)
0.2	2.91 ± 0.92 ****	ND	0.43 ± 0.01	27.54 ± 0.94	33.13 ± 1.12
0.3	7.10 ± 0.83 ****	1.32 ± 1.96 ****	0.49 ± 0.01	33.09 ± 1.18	39.09 ± 0.70
0.4	11.12 ± 0.86 ****	2.56 ± 0.42 ****	0.55 ± 0.01	37.64 ± 0.86	42.14 ± 0.61
0.5	18.19 ± 0.26 ****	6.92 ± 0.35 ****	0.61 ± 0.01	41.06 ± 0.71	46.86 ± 0.58
0.6	22.04 ± 0.35 ****	10.47 ± 0.80 ****	0.66 ± 0.01	45.85 ± 1.29	51.55 ± 1.31
0.7	25.92 ± 0.26 ****	15.41 ± 1.00 ****	0.72 ± 0.01	49.78 ± 0.56	56.28 ± 0.53
0.8	30.45 ± 0.46 ****	19.67 ± 2.91 ****	0.76 ± 0.01	54.19 ± 0.65	59.49 ± 0.75
0.9	34.63 ± 0.90 ****	25.69 ± 0.99 ****	0.81 ± 0.01	58.22 ± 0.71	62.24 ± 0.76
1	37.78 ± 1.93 ****	30.58 ± 1.78 ****	0.89 ± 0.02	63.44 ± 0.85	65.32 ± 1.01

Values expressed are the means ± SD ($n = 3$). Symbol (****) indicates a $p < 0.0001$ significant difference between ethyl acetate extract. EIZ2 and controls according to a one-way analysis of variance (ANOVA) using Tukey's multiple comparisons test. ND: Not detected; AAE: ascorbic acid equivalent; PC: positive control.

3.9. Correlation between Antioxidant Activities and Total Phenolic and Flavonoid Contents

The Pearson correlation coefficients between the variables are presented in Figure 5A–F. The results showed a highly significant positive correlation between antioxidant activity assessed by DPPH, FRAP, and ABTS assays and the levels of polyphenols and flavonoids present in ethyl acetate extract of *Streptomyces* sp. isolate EIZ2.

3.10. Analysis of Ethyl Acetate Extract of EIZ2 Isolate Using UV-Visible Spectroscopy

The absorption spectrum of ethyl acetate extract of EIZ2 isolate does not exhibit the three absorption peaks characteristic of polyene molecules. Instead, only one absorption peak was observed at 295 nm (Figure 6).

3.11. Evaluation of the Toxicity of EIZ2 Isolate Ethyl Acetate Extract by Hemolysis Test

The findings of Figure 7 revealed that ethyl acetate extract of EIZ2 isolate induced no damage to red blood cells at the tested doses (0.125, 1.25, and 3.12 mg/mL).

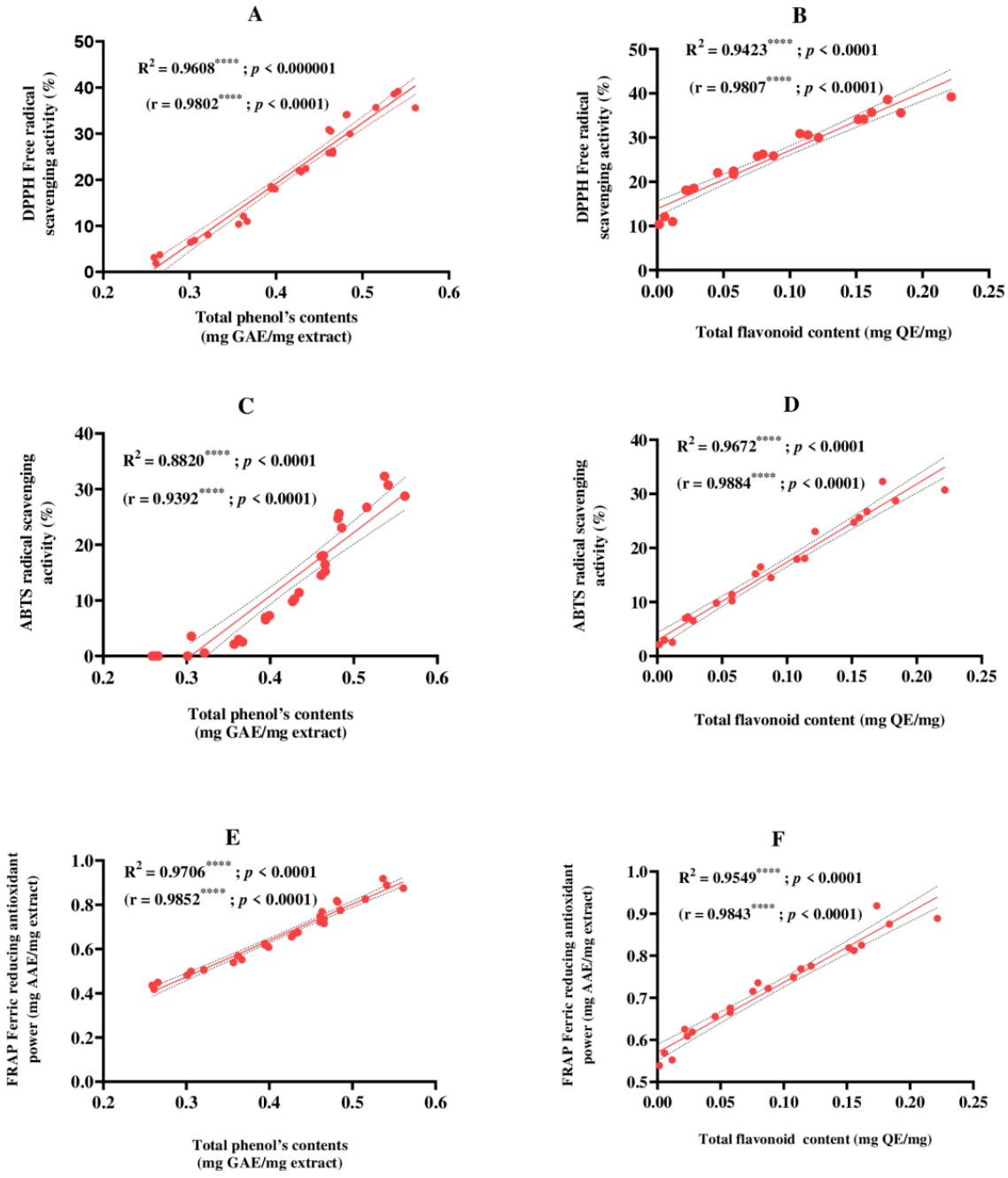

Figure 5. Pearson correlation analysis graphics. (**A**) Pearson correlation between DPPH and total phenolic content, (**B**) Pearson correlation between DPPH and flavonoids content, (**C**) Pearson correlation between ABTS and total phenolic content, (**D**) Pearson correlation between ABTS and flavonoids content, (**E**) Pearson correlation between FRAP and total phenolic content, and (**F**) Pearson correlation between FRAP and flavonoids content. **** ($p < 0.0001$) means highly significant between tests.

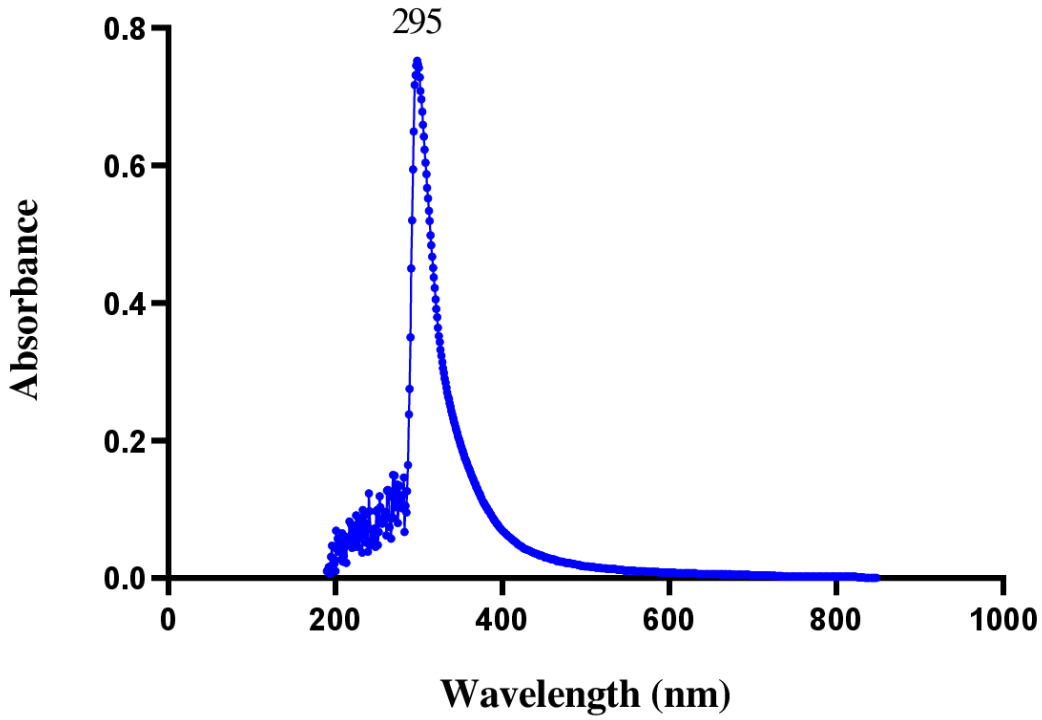

Figure 6. UV-visible spectrum of the ethyl acetate-active crude extract of EIZ2 isolate.

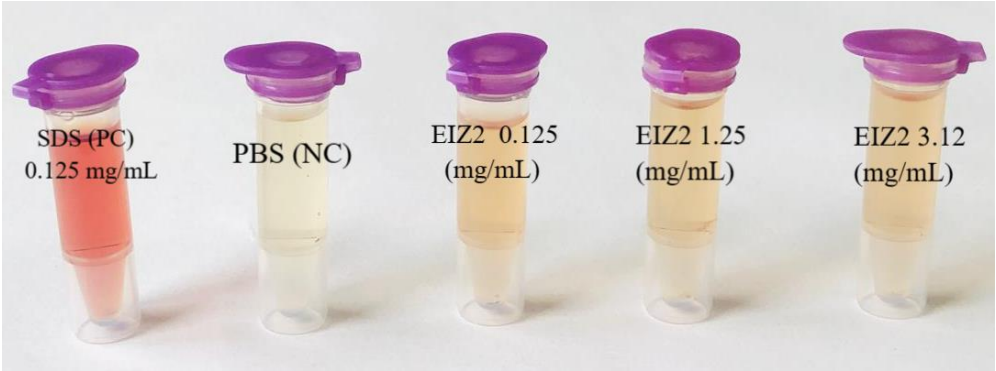

Figure 7. Evaluation of the toxicity of ethyl acetate extract of EIZ2 isolate using the hemolysis test on human red blood cells. SDS: positive control; PBS (NC): negative control.

3.12. Identification of Bioactive Compounds Using GC–MS

The result of the GC–MS analysis of the ethyl acetate extract derived from strain EIZ2 revealed 10 bioactive compounds, eluting within the time range of 1533 to 21,963 min (Figure 8). Among these identified compounds, eight have been acknowledged for their various biological activities, including antimicrobial, antioxidant, antifungal, anticancer, and others (Table 8). The primary identified compounds include 2,3-diazabicyclo [2.2.1] hept-2-ene,1,4-dimethyl-; hydroxylamine, O-pentyl-; 1,2-ethanediol, monoacetate; ethanol, 2,2′-oxybis-; butanoic acid, 3-hydroxy-3-methyl-; benzeneacetic acid; and diethyl phthalate (Table 8, Supplementary Figure S1).

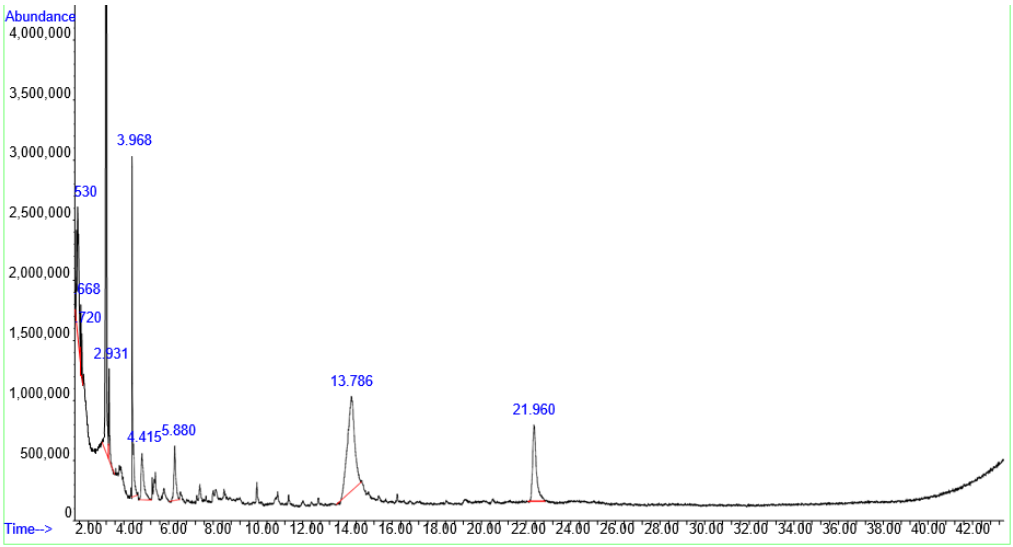

Figure 8. GC–MS of ethyl acetate crude extract of EIZ2, with peaks indicating the presence of bioactive compounds (according to the NIST database).

Table 8. Compounds identified by GC–MS from ethyl acetate crude extract of EIZ2 strain.

RT (Time)	Area (%)	M.W. (g/mol)	Molecular Formula	Compound Name	NIST Database Similarity (%)	Reported Bioactivities
1.669	1.08	124.18	$C_7H_{12}N_2$	2,3-Diazabicyclo [2.2.1] hept-2-ene,1,4-dimethyl-	43	Antifungal activity, anti-inflammation, anti-bacterial, antioxidants, anticonvulsant, antiallergic, herbicidal activity, and anticancer [50]
1.725	0.52	103.16	$C_5H_{13}NO$	Hydroxylamine, O-pentyl-	9	Antimicrobial activity [51]
2.795	37.30	60.05	$C_2H_4O_2$	Acetic acid	91	Antimicrobial activity [52]
2.931	1.81	104.10	$C_4H_8O_3$	1,2-Ethanediol, monoacetate	45	Antioxidant activities [53]
4.418	4.15	106.12	$C_4H_{10}O_3$	Ethanol, 2,2′-oxybis-	40	Antimicrobial and antioxidant [54]
5.883	3.53	118.13	$C_5H_{10}O_3$	Butanoic acid, 3-hydroxy-3-methyl-	72	Anti-inflammatory, antioxidant [55]
13.782	22.94	136.14	$C_8H_8O_2$	Benzeneacetic acid	94	Antifungal, Antimicrobial, and Antioxidant [56–58]
21.963	9.69	222.24	$C_{12}H_{14}O_4$	Diethyl phthalate	97	Antimicrobial, Antifungal [59]

RT: Retention time; M.W.: molecular weight; NIST: National Institute of Standards and Technology

4. Discussion

The pH range favorable to the growth of *Actinobacteria* is typically between 6 and 9; this pH range supports the proliferation of *Actinobacteria* in the soil samples [2]. Certainly, *Actinobacteria* exhibit heightened sensitivity to acidic conditions [60]. As a result, the average pH of our samples was 7.94, indicating that the pH of the two soils tested was slightly alkaline and did not hinder the growth of *Actinobacteria*. Soil conductivity plays a crucial role in influencing the growth of *Actinobacteria*, both directly and indirectly. This conductivity is closely linked to soil moisture levels and water availability. Similar to other microorganisms, *Actinobacteria* rely on water for their metabolic activities and overall growth. Therefore, lower soil conductivity values are generally preferred as they indicate conditions that are conducive to the water requirements essential for the optimal function-

ing and growth of *Actinobacteria* [61]. Elevated soil conductivity may signify the presence of salts or minerals, creating osmotic stress and potentially limiting water availability essential for microorganisms, including *Actinobacteria*, thereby impeding their growth [61]. Additionally, soil conductivity can impact the availability of crucial nutrients for *Actinobacteria* [62]. Higher conductivity levels are often associated with increased concentrations of specific ions such as sodium, potassium, and calcium. These ions can disrupt the equilibrium and accessibility of vital nutrients like nitrogen, phosphorus, and micronutrients, crucial for the growth of *Actinobacteria* [62]. Consequently, imbalances induced by high soil conductivity can compromise the growth capabilities of *Actinobacteria* [62].

In this study, the higher abundance of *Actinobacteria* colonies in M2 medium can be explained by the presence of macromolecules (starch and casein) in this medium (M2), which are catabolized by the majority of *Actinobacteria*, making the medium (M2) favorable to their growth [17]. In addition, this medium contains trace elements essential for bacterial growth [17]. Consequently, the specific factor contributing to the limited growth support of *Actinobacteria* by GA medium depends on its composition of L-asparagine, which may not provide an adequate nitrogen source for *Actinobacteria* isolated from the studies region. For subculture, ISP2 medium was chosen because of its balanced combination of nutrients adapted to the needs of *Actinobacteria*, including those of the *Streptomyces* genus, which are reputed producers of antibiotics.

In the current study, the macroscopic characteristics of the two isolates align with the characteristics commonly associated with identified *Actinobacteria* [26]. While *Actinobacteria* can exhibit tolerance to a wide pH range, their growth rate, metabolic activity, and specific requirements may vary under different pH conditions. Optimal growth for various *Actinobacteria* species typically occurs within a narrower pH range, usually in neutral or slightly alkaline conditions (pH 6–8) [63]. However, it is worth noting that some *Actinobacteria* are known to exhibit broader optimum pH values, depending on their ecological niche and adaptation. The optimal salt concentration for the growth of *Actinobacteria* can vary from nearly zero (0% NaCl) to approximately 5% NaCl, depending on the specific strain and environmental niche of the microorganism [64]. For instance, EIZ2 has the ability to tolerate NaCl concentrations of up to 10%, which can be explained by the unique characteristics of this species. Some *Actinobacteria* have developed mechanisms to cope with osmotic stress caused by salt, allowing them to maintain their growth and survival. However, it is important to note that the growth of *Actinobacteria*, like that of other microorganisms, can be inhibited by high salt concentrations (10%) [61,65]. Excessive amounts of NaCl can create osmotic stress, interfere with cellular processes, and disrupt the water balance inside the cells [61]. This can result in reduced growth or even cell death, as observed in the case of the EIZ1 isolate.

The findings of the current study revealed that EIZ1 and EIZ2 exhibit the capability to assimilate a wide range of carbohydrates. In this context, it was reported that *Actinobacteria* regulate sugar assimilation at the transcriptional level, with gene expression influenced by the presence or absence of specific carbohydrates [66,67]. Various regulatory mechanisms control genes encoding carbohydrate-degrading enzymes, transporters, and other metabolic enzymes [67]. Additionally, *Actinobacteria* employ diverse carbohydrate transport systems to absorb hydrolyzed molecules into their cells [67,68]. These transport systems can be specific to certain sugars or exhibit a broad substrate specificity, allowing the bacteria to use a wide range of carbohydrate derivatives present in their environment [69]. Additionally, *Actinobacteria* have the capacity to use alternative carbohydrates, such as pentoses (xylose), disaccharides (sucrose), and other sugar derivatives. Specific transporters and enzymes are involved in the absorption and metabolism of these alternative carbohydrate sources [69].

For most *Actinobacteria*, the optimal growth temperature falls within the mesophilic range, typically between 20 °C and 40 °C [70]. Nevertheless, exceptions exist, with some *Actinobacteria* thriving optimally at lower temperatures (psychrophilic) or higher temperatures (thermophilic) [71]. The growth rate of *Actinobacteria* is generally temperature-sensitive. The growth rate of *Actinobacteria* is sensitive to temperature variations [2]. Unlike

some bacterial groups, *Actinobacteria* generally demonstrate a slower growth pace, with their growth rates diminishing both at lower and higher temperatures outside their optimal range [19,72]. This temperature sensitivity is characteristic, leading to a modulation in growth rates depending on the temperature conditions. Barka et al. [2] have highlighted these temperature-dependent characteristics of *Actinobacteria* in their research. *Actinobacteria* produce melanoid pigments through complex enzymatic pathways, starting with the conversion of amino acids or carbohydrates into intermediate compounds, which are further transformed to yield melanin or melanoid pigments [73]. Furthermore, in many cases, tyrosine serves as a precursor for the production of melanoid pigments [74]. *Actinobacteria* possess enzymes like tyrosinases, which convert tyrosine into reactive intermediates. These intermediates subsequently undergo polymerization and oxidation processes to form melanin or melanoid pigments [75]. Environmental conditions and culture media composition significantly influence the expression of genes related to pigment biosynthesis in *Actinobacteria*, thereby affecting pigment production levels [75,76]. Melanoid pigments produced by *Streptomyces* often serve as protective agents against UV irradiation and adaptive functions [76,77]. They can act as antioxidants, effectively trapping harmful ROS and thus providing protection to the bacteria against oxidative damage [75].

Out of the 16 isolates tested against multi-drug-resistant bacteria, only isolate EIZ2 exhibited important antimicrobial activity. The lack of activity in the other isolates can be attributed to the fact that antimicrobial compounds produced by microorganisms on solid substrates may have limited mobility in liquid media. In solid media, these compounds can diffuse and disperse, making contact with bacteria over a larger surface area [78]. Conversely, in liquid media, the diffusion of these compounds may be restricted, diminishing their range and inhibitory effect. Moreover, the antimicrobial activity of certain compounds may require a longer incubation time to manifest itself in liquid media than in solid media [79]. In the case of EIZ2, ethyl acetate proved to be the most effective extraction solvent. Ethyl acetate is a moderately polar solvent capable of attracting both polar and apolar molecules, making it more likely to extract the desired bioactive molecules [80].

Polyphenols have been associated with various bioactive properties, including antioxidant, anti-inflammatory, anticancer, and antimicrobial activities [19,81–83]. Statistical analysis of this study supports this hypothesis, as an excellent positive correlation was found between antioxidant activity and total phenol and flavonoid content. Similarly, Tan et al. [84] reported that the in vitro antioxidant capacity of the MUM212 extract of the *Streptomyces* sp. strain is mainly due to the presence of phenolic compounds. Furthermore, Yogeswari et al. [85] reported that phenolic compounds have strong antioxidant activity, possessing the ability to donate hydrogen-reducing free radicals.

Our study confirms that phenolic compounds are responsible for the antioxidant activity observed against DPPH, ABTS, and FRAP. This suggests that the EIZ2 isolate tested is capable of producing one or more antioxidants, which could have significant implications for the prevention against oxidative stress. This is a biological process implicated in premature aging and the development of numerous chronic issues, including cardiovascular disease, cancer, and neurodegenerative disorders [86–88]. The capacity of the EIZ2 isolate to produce antioxidants may help attenuate the effects of ROS by neutralizing free radicals and reducing cellular damage caused by oxidation. Furthermore, this opens up the possibility of further exploring the antioxidant compounds produced by this isolate, both in terms of basic research and practical applications. For example, these antioxidants could be used in foods or dietary supplements to promote health and prevent disease. In addition, a better understanding of the mechanisms by which these isolates produce antioxidants could lead to advances in biotechnology and the production of natural antioxidants.

The absence of 3 absorption peaks characteristic of polyene molecules in the absorption spectrum of ethyl acetate extract of EIZ2 isolate indicates that this extract likely contains no polyene molecules. The structural similarity between polyene antifungal molecules and cholesterol, in particular ergosterol, the sterol predominant in fungal cells, is responsible for their toxicity [89]. This toxicity has been proven by previous studies [19,89]. Understanding

this mechanism is essential for selecting effective, non-toxic antifungal compounds for therapeutic applications. Interestingly, the ethyl acetate extract of EIZ2 isolate did not exhibit hemolysis of red blood cells at the tested concentrations. This result emphasizes the potential therapeutic or biomedical importance of this extract in the prevention of cell damage.

The GC–MS analysis was performed to identify the compounds that could explain the bioactivities of the ethyl acetate extract of the EIZ2 strain. Among the compounds detected were phenolic compounds comprising an aromatic ring bearing one or more hydroxyl groups. These molecules are also recognized for their antioxidant properties [81]. The phenolic compounds detected in strain EIZ2 were benzene acetic acid and diethyl phthalate. They have been previously detected in *Streptomyces* sp. E23-4 [19]. Moreover, these phenolic compounds were associated with the antimicrobial and antioxidant activities exhibited by this *Streptomyces* sp. E23-4 strain [19].

The extract from strain EIZ2 revealed the presence of heterocyclic compounds, including 2,3-diazabicyclo [2.2.1] hept-2-ene,1,4-dimethyl. These compounds have been associated with antimicrobial, antioxidant, anticancer, anti-inflammatory, antiallergic, anticonvulsant, herbicidal, and antiseptic activities [50]. In addition, hydroxylamine, O-pentyl- has also been identified in the ethyl acetate extract of strain EIZ2, which showed antimicrobial activity [51]. Moreover, phenolic glycosides like butanoic acid, 3-hydroxy-3-methyl- have been associated with various biological activities, including antioxidant, antimicrobial [55], and anti-herbivore activity [90]. Furthermore, the presence of the ethanol, 2,2′-oxybis- identified in our extract has been previously documented for its diverse biological properties, including antioxidant and antimicrobial activities [54]. Finally, the presence of acetic acid and 1,2-Ethanediol, monoacetate in the extract has been previously documented for their reported antimicrobial and antioxidant properties [52,53]. The wide range of biological activities exhibited by the ethyl acetate extract from strain EIZ2 may potentially be attributed to the collective presence of the identified compounds. Additionally, the elevated antimicrobial and antioxidant activities observed in the EIZ2 strain might be attributed to the synergistic impact of the secondary metabolites existing in the ethyl acetate extract from EIZ2. The outcomes from the GC–MS analysis enable us to deduce that a significant portion of the chemical compounds identified in the ethyl acetate extract of strain EIZ2 are acknowledged for their antimicrobial and antioxidant abilities. Consequently, these identified compounds may be implicated as contributors to the observed antimicrobial and antioxidant activities exhibited by the ethyl acetate extract of strain EIZ2 in the experimental setting. Therefore, further investigations involving the isolation and characterization of individual bioactive compounds, as well as the assessment of their bioactivities using purified forms, are warranted to provide a clearer understanding of their roles in mediating the observed biological effects.

5. Conclusions

In conclusion, the isolation of the EIZ2 strain from garden soil has revealed its remarkable antimicrobial properties against multi-drug-resistant (MDR) bacterial strains and its strong antioxidant activities. Our investigation into *Streptomyces* sp. EIZ2, derived from previously unexplored garden soil, suggests its potential for large-scale production of antimicrobial and antioxidant agents. The various bioactive compounds identified through gas chromatography–mass spectrometry (GC–MS) in the ethyl acetate extract of strain EIZ2 exhibit a broad range of biological activities. These substances can be considered as potential agents for treating multi-drug-resistant (MDR) bacterial infections and managing conditions associated with oxidative stress, including cancer, cardiovascular diseases, renal disorders, and other pathologies. These findings hold significant implications for the development of therapeutic drugs aimed at addressing multidrug-resistant bacterial infections and diseases associated with oxidative stress. It is crucial to highlight that our conclusions are solely derived from the results of our experiments. Therefore, additional research is necessary to thoroughly characterize and ascertain the chemical structures of

the compounds present in the EIZ2 extract, utilizing diverse analytical techniques such as Fourier-transform infrared spectroscopy (FTIR), high-performance liquid chromatography (HPLC), high-resolution mass spectrometry (HRMS), and nuclear magnetic resonance (NMR). Furthermore, further explorations into the intracellular molecular mechanisms of action of the compounds within the EIZ2 strain are needed.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/microbiolres15020050/s1, Figure S1: GC–MS spectra of 5 important secondary metabolites present in ethyl acetate extract of the EIZ2 strain; Table S1: Resistance profile of multi-drug-resistant (MDR) clinical bacteria; Table S2: Molecular identification of the selected EIZ2 *Actinobacteria* isolate based on 16S rRNA gene sequencing.

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