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In vitro antimicrobial and antioxidant activities of bioactive compounds extracted from *Streptomyces africanus* strain E2 isolated from Moroccan soil

Said Rammali[1], Fatima Zahra Kamal[2,3], Mohamed El Aalaoui[4], Bouchaib Bencharki[1], Vasile Burlui[5], Abdelkrim khattabi[1], Aasfar Abderrahim[6], Salhi Saad[7], Laura Romila[8]✉, Bogdan Novac[9], Rokaya Aitlhaj-Mhand[10], Antoneta Dacia Petroaie[9] & Alin Ciobică[11,12,13,14]

This study aimed to isolate *Streptomyces* sp. from Moroccan terrestrial ecosystems and identify bioactive compounds through GC–MS analysis. Antimicrobial activity was assessed against various pathogenic microorganisms including *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 60193, and multi-drug resistant strains comprising *Listeria monocytogenes*, *Klebsiella pneumoniae* 19K 929, *Proteus* sp. 19K1313, *Klebsiella pneumoniae* 20B1572, *Proteus vulgaris* 16C1737, and *Klebsiella pneumoniae* 20B1572. Based on the results of the gene sequencing of gene 16S rRNA and phylogenetic analysis, the E2 isolate belongs to the genus *Streptomyces* with the highest degree of resemblance (97.51%) to the *Streptomyces africanus strain* NBRC 101005 (NR_112600.1). The isolate exhibited broad-spectrum antibacterial activity, with maximum efficacy against *Klebsiella pneumoniae* 20B1572 indicated by an inhibition zone diameter of 22.5 ± 0.71mm and a minimum inhibitory concentration (MIC) of 0.0625 mg/mL. The in vitro antioxidant potential of E2 strain was determined through screening of its ethyl acetate extract against sets of antioxidant assays. The results were indicative of E2 strain displaying strong antioxidant activity against ABTS, DPPH free radicals, and FRAP. Furthermore, there was a high significant correlation ($p < 0.0001$) between the total phenolic and flavonoid content and antioxidant activities. The GC–MS analysis of the extract identified six volatile compounds, with Eugenol (96%) and Maltol (93%) being the most prominent. Additionally, the HPLC–UV/vis analysis revealed six phenolic compounds: gallic acid, chlorogenic acid, vanillic acid, trans-ferulic acid, ellagic acid, and cinnamic acid. Overall, the study highlights *Streptomyces* sp. strain E2 as a potential source of potent antimicrobial and antioxidant metabolites, offering promise in addressing antibiotic resistance and oxidative stress-related conditions.

Keywords *Streptomyces*, Multi-drug resistance, Antimicrobial activity, Antioxidant activity, 16S rRNA. GC–MS analysis, HPLC–UV/vis

[1]Laboratory of Agro-Alimentary and Health, Faculty of Sciences and Techniques, Hassan First University of Settat, B.P. 539, 26000 Settat, Morocco. [2]Higher Institute of Nursing Professions and Health Technical (ISPITS), 40000 Marrakech, Morocco. [3]Laboratory of Physical Chemistry of Processes and Materials, Faculty of Sciences and Techniques, Hassan First University, 26000 Settat, Morocco. [4]Regional Center of Agronomic Research of Settat, Tertiary Road 1406, At 5 Km from Settat, 26400 Settat, Morocco. [5]Department of Biomaterials, Faculty of Dental Medicine, Apollonia University, 700511 Iasi, Romania. [6]Plant and Microbial Biotechnology Center, Moroccan Foundation for Advanced Science, Innovation and Research (MAScIR), Mohammed VI Polytechnic University, Ben Guerir, Morocco. [7]Laboratory of Biochemistry, Neurosciences, Natural Ressources and Environment, Faculty of Sciences and Techniques, Hassan First University of Settat, B.P. 539, 26000 Settat, Morocco. [8]Apollonia University, Păcurari Street 11, 700511 Iasi, Romania. [9]Grigore T. Popa University of Medicine and Pharmacy, 16, Universitatii Street, 700115 Iasi, Romania. [10]Virology Department, National Institute of Hygiene, Rabat, Morocco. [11]Department of Biology, Faculty of Biology, Alexandru Ioan Cuza University of Iasi, 20th Carol I Avenue, 700506 Iasi, Romania. [12]Academy of Romanian Scientists, 3 Ilfov, 050044 Bucharest, Romania. [13]Clinical Department, Apollonia University,

Păcurari Street 11, 700511 Iasi, Romania. [14]CENEMED Platform for Interdisciplinary Research, "Grigore T. Popa" University of Medicine and Pharmacy of Iasi, 16th Universitatii Street, 700115 Iasi, Romania. ✉email: laura.dartu@gmail.com

The constant evolution of microbial resistance to antibiotics today represents a critical challenge to global health security[1]. The alarming figures underscore the scale of this issue and emphasizes the imperative for immediate action. According to data from the World Health Organization (WHO), approximately 700,000 people die every year from drug-resistant diseases, a figure projected to escalate to 10 million by 2050 if substantial measures are not taken[2].

Antimicrobial resistance (AMR) is escalating, posing severe threats to public health. Statistical modeling forecasts estimate that bacterial AMR was responsible for 4.95 million deaths in 2019, with 1.27 million directly attributed to bacterial AMR[3]. The all-age mortality rate attributable to resistance peaked in Western Sub-Saharan Africa, with 27.3 deaths per 100,000 people, while Australasia exhibited the lowest rate at 6.5 deaths per 100,000 people[4]. *Escherichia coli*, followed by *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* Were the primary pathogens contributing to resistance-related deaths, accounting for 929,000 AMR-attributable deaths and 3.57 million AMR-associated deaths in 2019[5]. These alarming figures from statistical models and forecasts underscore the gravity of the situation, with significant consequences for public health[6].

Antibiotics, once considered miracle cures, are increasingly losing their efficacy due to their excessive and misguided use[7]. Studies indicate that 30- 50% of antibiotic prescriptions are unnecessary or do not conform to medical guidelines[8]. Additionally, The extensive application of antibiotics in agriculture exacerbates bacterial resistance[9]. According to the Food and Agriculture Organization of the United Nations (FAO), around 80% of antibiotics manufactured globally are employed in the agricultural sector, accelerating the development of resistant bacterial strains. Given the borderless nature of bacterial resistance, a coordinated global approach is imperative. Governments, healthcare professionals, the pharmaceutical industry, and the general public must collaborate to implement prevention strategies, promote responsible antibiotic use and invest in research into novel therapeutic solutions.

Among promising approaches, the use of *Actinobacteria* is emerging as a compelling avenue for combating bacterial infections. *Actinobacteria*, soil microorganisms, are known for their ability to produce antibacterial compounds, some of which serve as the basis foundation for commonly used antibiotics in medicine[10]. These natural antimicrobial agents, such as streptomycin and chloramphenicol, revolutionized the treatment of infections in the twentieth century[11].

By harnessing the potential of *Actinobacteria*, scientists aim to discover new antibiotics or develop enhanced derivatives to counter bacteria resistant to existing drugs. Extreme environments, such as inhospitable soils, harbor rich reservoirs of *Actinobacteria* diversity, offering a valuable source of new antimicrobial molecules. Our study aims to isolate *Actinobacteria* from two soil samples collected in two distinct areas, followed by the assessment of their antimicrobial properties against various strains, some of which display multiple antibiotic resistance and pathogenicity in humans. Simultaneously, we are undertaking a qualitative and quantitative investigation of the antioxidants produced by these *Actinobacteria* to evaluate their antioxidant activity, thereby contributing to the battle against antimicrobial resistance.

Results

Physico-chemical analysis of soil samples

The soil analysis of the KG site (Kenzi's Garden) revealed alkalinity with a pH of 8.05, and non-salinity with an electrical conductivity (EC) of 0.62 dS/m. According to the texture diagram, this soil is classified as loamy, consisting of 71% silt, with relatively low proportions of clay and sand (14% and 15% respectively). Moreover, X-ray fluorescence analysis of soil minerals identified the presence of exchangeable cations such as potassium (K), magnesium (Mg), aluminum (Al), calcium (Ca), iron (Fe), and manganese (Mn). Some elements like phosphorus (P), calcium (Ca), and iron (Fe) were found in high concentrations, whereas others like aluminum (Al), sulfur (S), chlorine (Cl), copper (Cu), and zinc (Zn) were present in lower quantities (Table 1).

Similarly, the soil from the FG site (FST's Garden) exhibited alkaline properties with a pH of 8.19 and a loamy texture comprising 60% silt. Its electrical conductivity was also low, measured at 0.53 dS/m. Although this soil demonstrated relatively low clay and sand content it stood out for its low content of mineral elements, except for calcium (Ca) and iron (Fe), which were present in moderate concentrations (Table 1).

Isolation of *Actinobacteria* isolates

A total of 6 presumptively phenotypically distinct *Actinobacteria* isolates were recovered from the two types of soil samples, with 4 isolates originating from site A and 2 isolates from site B (Table 2). The *Actinobacteria* isolates were retrieved across all four selective media used (M2, Bennett, GLM, and GA), with the highest number of isolates obtained from M2 medium (n = 4), followed by GLM (n = 2). However, the Bennet and GA media, did not yield any isolates (Table 2).

Phenotypic characteristics and genotypic identification

The E2 Strain, from garden soil, exhibited abundant growth on various culture media such as ISP1, ISP2, ISP7, Bennett, GYEA, as well as *Actinobacteria* isolation media (M2 and GLM) after incubation for 7–14 days at 28 °C. This strain exhibited strong growth on ISP2, Bennett and GYEA media, and moderate growth on ISP1 and ISP7, but no growth on GA agar. Observation of a 10-day culture on ISP2 agar revealed an abundance of well-developed, unfragmented aerial and vegetative hyphae. Growth occurred over a temperature range of 4–37 °C (optimum 28 °C), with tolerance to NaCl concentrations ranging from 1 to 5% (optimum 1–2%)

Physico-chemical parameters	Site kG (Kenzi garden)	Site FG (FST garden)
Textural soil types	Loamy	Loamy
Clay (%	14	20
Sand (%)	15	20
Silt (%)	71	60
pH	8.050	8.190
EC (dS/m)	0.624	0.531
Mg (%)	1.095	0.229
K (%)	1.154	0.518
Ca (%)	9.203	4.478
Cl (%)	0.001	0.0291
S (%)	0.098	0.022
P (%)	16.689	0.102
Fe (%)	7.181	3.448
Mn (%)	0.087	0.044
Cu (%)	0.008	0.004
Zn (%)	0.014	0.012
Al (%)	4.564	1.293

Table 1. Physico-chemical analysis of soil samples. (EC) Electrical conductivity; FST (Faculty of Science and Technology of Settat).

Soil collection site	Number of *Actinobacteria* colonies in different isolation media (10^3 CFU/mL)				Total number of *Actinobacteria* colonies (10^3 CFU/mL)	Number of colonies with morphological characteristics in each medium				Number total of isolates
	M_2	Ben	GLM	GA		M_2	Ben	GLM	GA	
Site A	5	1	10	5	21	2	0	2	0	4
Site B	12	6	6	2	26	1	0	1	0	2
Number total	17	7	16	7	47	3	0	3	0	6

Table 2. Total number of *Actinobacteria* isolates recovered from two sites A and B according to the culture media used. (Site A) Kenzi garden; (Site B) : FST garden.

and pH between 5.0 and 10 (optimum pH 7.8–8.06). The E2 isolate exhibited the ability to assimilate various carbohydrate compounds as a carbon source, including cellobiose, sucrose, mannose, fructose, and glucose. However, it did not assimilate mannitol, xylose, and starch (Table 3, Fig. 1).

A partial sequence of the gene coding for 16S RNA (1407 bp) was determined and deposited in the GenBank database under accession number (PP731514). Analysis of this sequence enabled the isolate E2 to be compared with the *Streptomyces* species, with a similarity rate of 97.51%. The phylogenetic position of this isolate in relation to the closest species in the *Streptomyces* genus is shown in Fig. 2 and Table 4.

Primary and secondary screening of *Actinobacteria* isolates

After identification, the isolates were subjected to primary screening, as illustrated in Figs. 3 and 4. Of the six *Actinobacteria* isolates tested, 4 revealed antimicrobial activity against Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922), Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923), and the fungal strain (*Candida albicans* ATCC 60193). Of the 4 active isolates, E2 and E6 were selected for secondary metabolite extraction because of their high antimicrobial activity compared with the other 2 isolates, E3 and E4. These 2 bioactive isolates were then subjected to secondary screening against various clinically pathogenic and multidrug-resistant bacteria (*Listeria monocytogenes*, *Klebsiella pneumoniae* 20B1572, *Proteus* sp. 19K1313, *Escherichia coli* 19L2418, *Klebsiella pneumoniae* 19K 929, *Proteus vulgaris* 16C1737, and *Escherichia coli* 16D1150) (Table 5 and Fig. 5). This table summarizes the antimicrobial activities extracted using n-hexane, dichloromethane, and ethyl acetate.

Minimum inhibitory concentration

The ethyl acetate extract of isolate E2 was evaluated to determine its minimum inhibitory concentration (MIC) against multidrug-resistant bacterial strains (Fig. 6). The results show that this extract exhibits notable bactericidal activity, with an MIC of 0.0625 mg/mL against *Klebsiella pneumoniae* 20B1572 and an MIC of 0.125 mg/mL against *Klebsiella pneumoniae* 19K929. These results highlight the efficacy of the ethyl acetate extract, particularly against the two Klebsiella pneumoniae strains selected for their most significant inhibition zones and multidrug resistance.

Test	*Actinobacteria* isolates		
	E2	E4	E6
Carbon assimilation			
Manitol	−	−	−
Cellobiose	+	2+	−
Saccharose	+	2+	+
Xylose	−	+	−
Mannose	+	2+	+
Fructose	+	−	+
Amidon	−	2+	−
Glucose	3+	3+	3+
pH tolerance			
4,06	−	−	−
5,02	+	+	3+
6,45	2+	3+	3+
7,28	3+	3+	3+
8,06	3+	3+	3+
9,06	+	+	3+
10,02	2+	2+	3+
NaCl tolerance			
1%	3+	3+	3+
2%	3+	3+	3+
3%	+	3+	3+
4%	+	+	2+
5%	+	+	2+
6%	+	−	2+
7%	+	−	+
8%	+	−	+
9%	+	−	+
10%	+	−	−
Temperature growth on			
4 °C	2+	−	3+
28 °C	3+	3+	3+
37 °C	2+	3+	+
45 °C	−	−	−
Growth on different media			
ISP1	2+	2+	2+
ISP2	3+	3+	3+
ISP7	2+	+	+
Bennett	3+	+	3+
GYEA	3+	3+	3+

Table 3. Biochemical and physiological characteristics of *Actinobacteria* isolates. (–) no growth; (+) low growth; (2+) intermediate growth; (3) good growth.

Determination of phenolic and flavonoid compounds in ethyl acetate extract

The total phenol and flavonoid contents in the ethyl acetate extract of E2 strain were determined at different concentrations (ranging from 0.1 to 1 mg/mL) (Table 6). Absorbance values were measured using the Folin-Ciocalteu reagent for phenols and aluminum chloride reagent for flavonoids. These values were then compared to standard absorbance values of gallic acid ($y = 0.0009x - 0.0201$; $R^2 = 0.9982$) for phenols and quercetin ($y = 0.0005x + 0.0702$; $R^2 = 0.9977$) for flavonoids. Phenol content ranged from 0.472 ± 0.004 to 0.628 ± 0.012 GAE/g of extract, while flavonoid content varied from 0.023 ± 0.010 to 0.249 ± 0.013 mg QE/mg of extract across the tested concentrations.

Fig. 1. Macroscopic aspect of isolate E2 on ISP2 medium.

Antioxidant activity

DPPH and ABTS assays

To assess the antioxidant power of the ethyl acetate extract derived from E2 strain, two assays targeting free radical scavenging, namely DPPH and ABTS, were employed. The scavenging activity was quantified by determining the percentage inhibition of DPPH and ABTS, along with their respective IC_{50} values. Figure 7 illustrates the average percentages of DPPH and ABTS free radical scavenging activities across various concentrations of the ethyl acetate extract of E2 strain.

The results show that the radical scavenging activities of the ethyl acetate extract of E2 strain and the standards (ascorbic acid and Trolox) are directly proportional to their concentration. Ethyl acetate extract showed a moderate inhibition percentage of $43.61 \pm 0.30\%$ at a dose of 1 mg/mL, compared with the standard antioxidant Ascorbic acid (AA), which showed a significant DPPH inhibitory power of $65.32 \pm 1.01\%$ at the same concentration. Similarly, ethyl acetate extract revealed an inhibition rate of $39.21 \pm 2.11\%$ at the same dose for the standard antioxidant Trolox, which showed a significant ABTS inhibitory potency of $63.44 \pm 0.85\%$ at 1 mg/mL. The IC_{50} values calculated for the DPPH and ABTS assays corroborate these results. The ethyl acetate extract of E2 strain shows higher IC_{50} values (1.16 ± 0.01 mg/mL for DPPH and 1.22 ± 0.003 mg/mL for ABTS) than AA (0.58 ± 0.01 mg/mL for DPPH) and Trolox (0.69 ± 0.02 mg/mL for ABTS). A higher IC_{50} indicates lower radical scavenging activity and antioxidant potential. Compared with the synthetic standards AA and Trolox, ethyl acetate extract of E2 strain shows moderate antioxidant capacity.

Ferric reducing antioxidant power (FRAP)

In this experiment, the ferric reducing antioxidant power (FRAP) of ethyl acetate extract of E2 strain and standard Ascorbic acid was shown to be concentration-dependent. An increase in absorbance suggests an increase in ferric reduction capacity. The results revealed a significant ferric reduction activity for ethyl acetate extract between the different concentrations tested ($p < 0.0001$) (Fig. 8). This activity showed values from 0.786 ± 0.007 to 1.164 ± 0.012 mg AAE (ascorbic acid equivalent) per mg extract.

Correlation between total phenol and flavonoid contents and antioxidant activity

A Pearson correlation was established between the phenolic compounds content and the antioxidant activity tested by three DPPH, ABTS and FRAP assays of the ethyl acetate extract of E2 strain (Fig. 9). The results revealed a highly significant positive correlation ($p < 0.0001$) between total phenolic and flavonoid content and antioxidant capacity, analyzed by three different tests (DPPH, ABTS and FRAP). The highest correlation was observed between flavonoid content and DPPH, ABTS and FRAP ($r^2 = 0.97$) (Fig. 9b,d,f), followed by total phenolic content and ABTS ($r^2 = 0.81$), total phenolic content and FRAP ($r^2 = 0.80$), total phenolic content and DPPH ($r^2 = 0.78$) (Fig. 9a,c,e).

GC–MS analysis of E2 ethyl acetate extract

Utilizing gas chromatography-mass spectrometry (GC–MS), an analysis was conducted on the chemical composition of the ethyl acetate extract obtained from E2 strain. This analysis revealed the presence of 6 compounds eluted within the time range of 1.736 to 16.025 min. These compounds include Disulfide, dimethyl

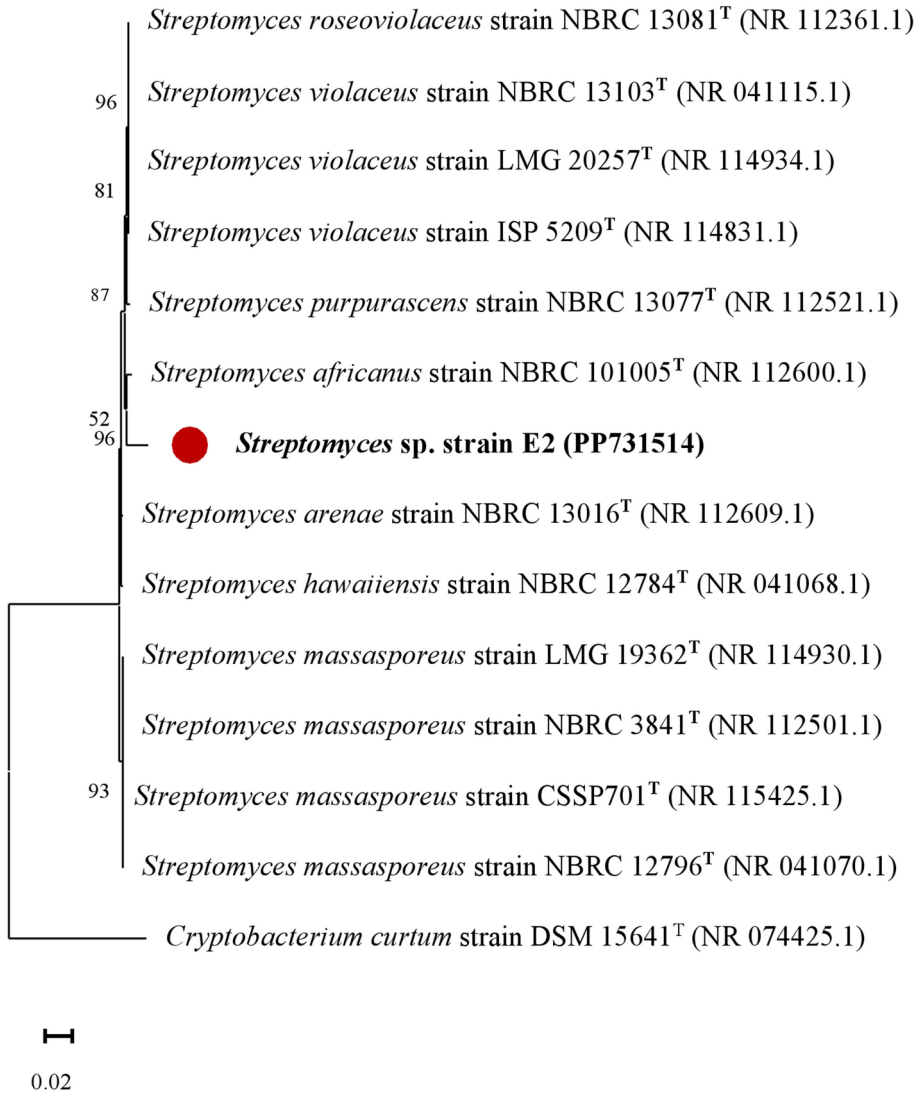

Fig. 2. Phylogenetic tree based on the 16S rRNA gene sequence showing the evolutionary relationship between *Streptomyces* sp. strain E2 and its closest known taxa using MEGA X. The bar 0.02 represents the number of substitutions per nucleotide position (1% divergence between sequences). The tree was generated using 1000 bootstraps and GenBank accession numbers are enclosed in parentheses. Only bootstrap values > 50 are displayed. *Cryptobacterium curtum* was the outgroup in the analysis. T: Type strain.

Isolate name	Query length (bp)	Nearest known species[a]	Percent identity (%)	Identification[b] (scientific name)	NCBI GenBank accession number
E2	1407	*Streptomyces africanus* strain NBRC 101005^T (NR_112600)	97.51	*Streptomyces africanus*	PP731514

Table 4. Molecular identification of the selected E6 *Actinomycete* isolate based on 16S rRNA gene sequencing. [a]NCBI's nucleotide BLAST program was used to find the closest match against the non-redundant reference RNA sequence database (refseqrna). The strain number, strain type (T), and GenBank accession number are all listed after the species name. [b]Isolates with a greater percentage of identity than 98.7% [83] and only one closest match for the same identity characteristics are classified up to the species level. To assess the reliability of a phylogenetic tree, MEGA provides the Bootstrap test. This test uses the bootstrap re-sampling strategy, so you need to enter the number of replicates. For a given data set, applicable tests and the phylogeny inference method are enabled. Neighbor joining has an additional test (Interior Branch), which requires the same input as bootstrap.

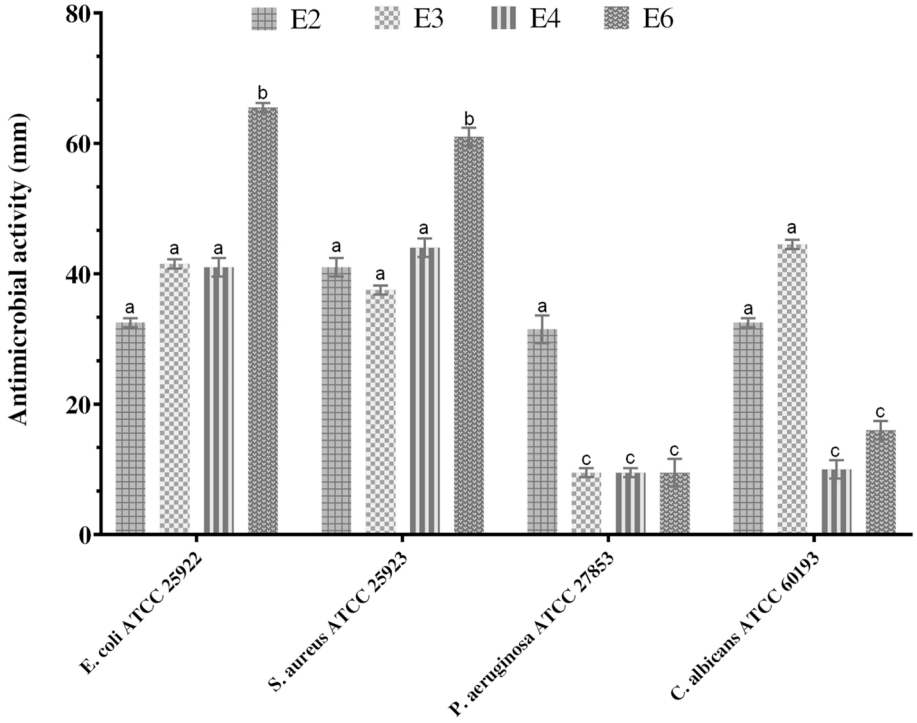

Fig. 3. Primary screening for antimicrobial activity: Double-layer method on ISP2 medium.

(1); S-methyl methanethiosulfonate (2); Dimethylsulfoxonium formylmethylid (3); Maltol (4); 2,4-dithiapentane (5); and Eugenol (6) (Table 7, Fig. 10). These compounds have been noted for their various biological activities such as antimicrobial, antifungal, antioxidant, and antitumor properties (Table 7).

HPLC–UV/vis analysis of E2 ethyl acetate extract

The HPLC–UV/Vis chromatogram (high-performance liquid chromatography using a UV–visible spectrum) revealed the presence of six phenolic compounds in the crude ethyl acetate extract of the *Streptomyces* sp. E2 strain. Each of these compounds exhibited retention times similar to those of the standards used (Fig. 11 and Table 8).

Discussion

Since the discovery of the first antibiotic, penicillin, by Alexander Fleming in 1928[12], antibiotics have been crucial in treating infections caused by pathogenic microorganisms. However, over-the-counter availability and misuse, along with their extensive use in agriculture, have led to the emergence of new pathogens that have acquired resistance. The main concern is the rise of multidrug-resistant bacteria (MDR), which are responsible for a majority of nosocomial infections and can cause severe, difficult-to-treat illnesses, sometimes resulting in prolonged illness and increased risk of death. Given this alarming situation, investment in the research of new bioactive compounds has become essential to combat antibiotic resistance. Recent studies have demonstrated that the *Streptomyces* genus represents the most effective source for discovering powerful and broad-spectrum medications due to the biosynthetic genes responsible for producing unexpressed secondary metabolites carried within the *Streptomyces* genome[13]. In our study, we isolated a strain of *Actinobacteria* E2 from the soil of a terrestrial ecosystem collected from the garden located in the Settat Casablanca region in Morocco.

Previous studies have shown that the physicochemical properties of soil such as texture, pH, salinity, mineral elements, and vegetation can influence the population of *Actinobacteria*[14–16]. *Actinobacteria* are known to be sensitive to the acidic pH of soil[17], except for some genera that are neutrophilic acid-tolerant or strict acidophiles[18]. Indeed, *Actinobacteria* generally thrive in neutral to slightly alkaline conditions (between 7 and 8)[19]. Furthermore, soil pH plays a crucial role in influencing microbial communities and their metabolic activities, and *Actinobacteria* appear to favor slightly alkaline environments[20]. The results of our study, showing an average pH of 8.12, indicate slightly alkaline soil, which is favorable for *Actinobacteria* growth. Soil electrical conductivity reflects salinity levels in the soil and has a direct impact on microbial populations. *Actinobacteria* are sensitive to saline stress, so high electrical conductivity can harm their populations[21]. Our samples are considered non-saline soil since the salinity range is below 6000 µS/cm[22]. Other studies have suggested that *Actinobacteria* are significantly and positively correlated with clay and silt particles. These particles provide a conducive habitat for *Actinobacteria* due to their ability to retain water and nutrients, as well as absorb organic carbon. Soils rich in silt, as observed in the two studied sites (71% and 60% respectively), promote *Actinobacteria* growth by offering a larger surface area for colonization and providing more favorable conditions for their development (M. Annabi, 2005). In contrast, sand particles have lower cation exchange capacity and reduced nutrient content, making

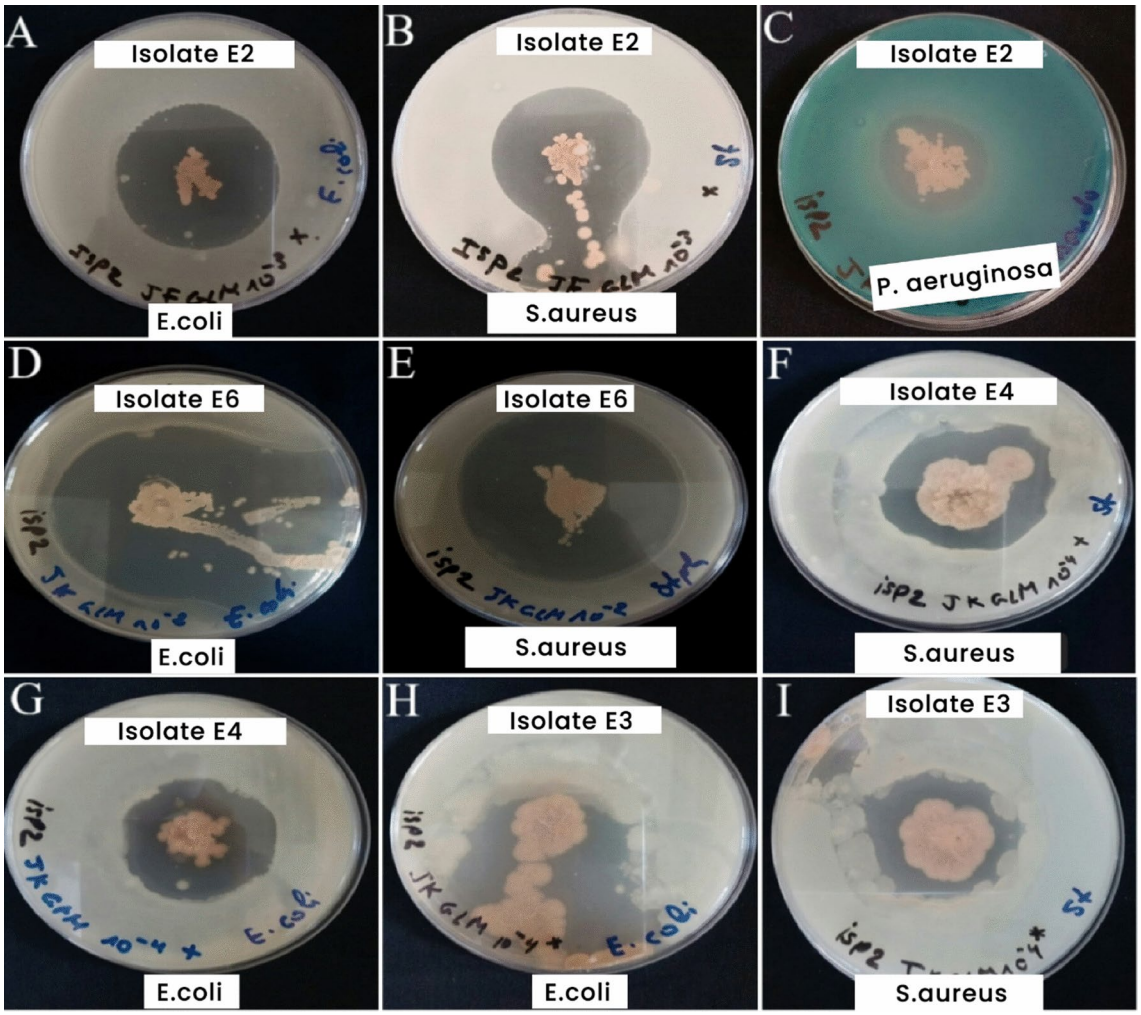

Fig. 4. Evaluation of the antimicrobial activity of pure isolates of *Actinobacteria* using the double layer method on ISP2 medium. (**A**) Antimicrobial activity of E2 isolate against *Escherichia coli* ATCC 25922; (**B**) Antimicrobial activity of E2 isolate against *Staphylococcus aureus* ATCC 25923; (**C**) Antimicrobial activity of E2 isolate against *Pseudomonas aeruginosa* ATCC 27853; (**D**) Antimicrobial activity of E6 isolate against *Escherichia coli* ATCC 25922; (**E**) Antimicrobial activity of E6 isolate against *Staphylococcus aureus* ATCC 25923; (**F**) Antimicrobial activity of E4 isolate against *Staphylococcus aureus* ATCC 25923; (**G**) Antimicrobial activity of E4 isolate against *Escherichia coli* ATCC 25922; (**H**) Antimicrobial activity of E3 isolate against *Escherichia coli* ATCC 25922; and (**I**) Antimicrobial activity of E4 isolate against *Staphylococcus aureus* ATCC 25923.

them less conducive to *Actinobacteria* growth. Several studies have shown that the availability of minerals such as potassium (K), phosphorus (P), and magnesium (Mg) is an important factor for *Actinobacteria* growth[23]. Potassium is an essential macronutrient for microbial growth and metabolism, and its availability in soil can affect microbial community composition[16]. *Actinobacteria* can benefit from potassium availability, making them more abundant in potassium-rich soils. Phosphorus is an essential nutrient for microbial growth, and its availability often limits microbial activities in the soil[16]. However, *Actinobacteria* may have different strategies for phosphorus acquisition or may not be as influenced by available phosphorus levels as other microorganisms[16].

The isolation results on four different culture media indicate that the M2 and GLM media are more conducive to the growth of *Actinobacteria* in both soil samples. This could be explained by the presence of starch (macromolecule) in these two media. This macromolecule is catabolized by most *Actinobacteria*, making the M2 and GLM media favorable for their growth[24]. Additionally, the richness in carbon and nitrogen sources also promotes optimal growth of these bacteria[24]. In contrast, the other two culture media used (Bennett and GYEA) only yielded low to moderate growth, which could be attributed to a lack of essential nutrients for *Actinobacteria* growth.

We isolated a total of 6 *Actinobacteria* isolates from two types of Moroccan soil. One isolate, coded as E2 strain, was selected based on its broad-spectrum antimicrobial activity for further characterization and bioactivity evaluation. The strain was identified as belonging to the genus *Streptomyces* and designated as *Streptomyces* sp. strain E2 with the GenBank accession number. Our results demonstrated that strain E2 produces one or

Extraction solvents			Inhibition zones of isolate E2 in mm				
Multi-drug resistant bacteria (MDR)	Hex	DM	EA	But	RP	C+	C−
Clinical *Listeria monocytogenes*	–	–	12 ± 1.00	–	–	25 ± 0.71	–
Staphylococcus aureus	–	–	10 ± 1.53	–	–	–	–
Clinical *Klebsiella pneumoniae* 20B1572	–	–	22.5 ± 0.71	–	–	–	–
Clinical *Proteus* sp. 19K1313	–	–	9 ± 0.71	–	–	–	–
Clinical *Escherichia coli* 19L2418	–	–	8 ± 1.53	–	–	–	–
Clinical *Klebsiella pneumoniae* 19K 929	–	–	11 ± 0.71	–	–	–	–
Clinical *Proteus vulgaris 16C1737*	–	–	10 ± 0.71	–	–	25.5 ± 0.71	–
*Escherichia coli*16D1150	–	–	–	–	–	30 ± 1	–
Bacillus cereus ATCC 14579	–	8 ± 1	8 ± 1	–	–	23 ± 0.58	–

Table 5. Secondary screening (antimicrobial activity of isolate E2) was performed using the disk diffusion method, varying extraction solvents. Values are 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 inhibition zone, (C−) negative control (DMSO), (C+) positive control (ceftriaxone and cycloheximide), (Hex) Hexane; (DM) dichloromethane; (AE) ethyl acetate; (But) Butanol; (PR) residual phase and disc diameter = 6 mm.

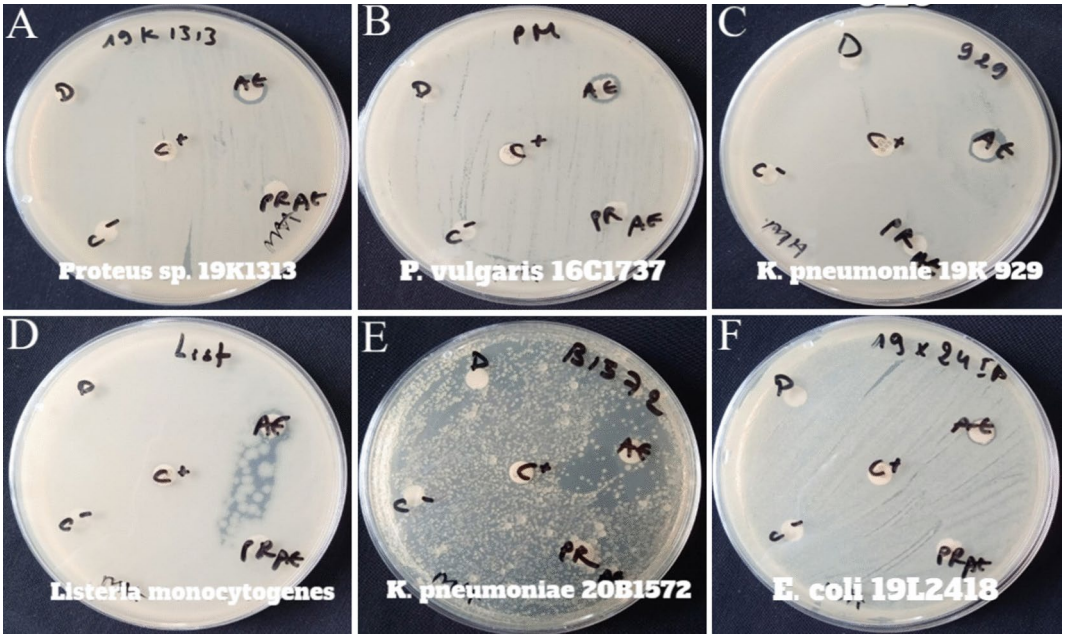

Fig. 5. Evaluation of antimicrobial activity in the ethyl acetate extract from isolate E2 using the disc diffusion method on MH medium. (C+) positive control (nalidixic acid), (C−) negative control (DMSO), (Dich) dichloro-methane; (EA) ethyl acetate; (PR) residual phase.

more secondary metabolites with various bioactivities such as antimicrobial, antioxidant, anticancer, and other biological activities.

Reliable taxonomy of prokaryotes, particularly the genus *Streptomyces*, requires data from both DNA-based methods and phenotypic characterization[25]. Studies have shown that closely related strains of *Streptomyces* can differ in terms of biochemical profiles and carbon source utilization[26,27]. Therefore, to enhance our understanding and complement the phylogenetic analysis of strain E2, we conducted a detailed micro-morphological, biochemical and physiological characteristic. Our data revealed that E2 strain belonged to the genus *Streptomyces* and could thrive across different temperature ranges (4 to 37 °C), pH levels (4 to 10), and NaCl concentrations (1 to 10%, w/v). These findings suggest that E2 strain had the ability to withstand NaCl concentrations of up to 100 g/L (10%). Tian et al.[28] concluded that saline or hypersaline environments warrant special attention as they may offer new avenues for the discovery of natural molecules. *Actinobacteria* capable of growing from 4 to 60 °C have been reported[29]. However, the optimum growth temperature verified for *Actinobacteria* was 28 °C. In addition, some *Actinobacteria* are able to grow in culture media with pH values of 3 and 13[29]. However, the pH range was most often 4 to 10, with the optimum pH for *Actinobacteria* growth being in the neutral region, particularly

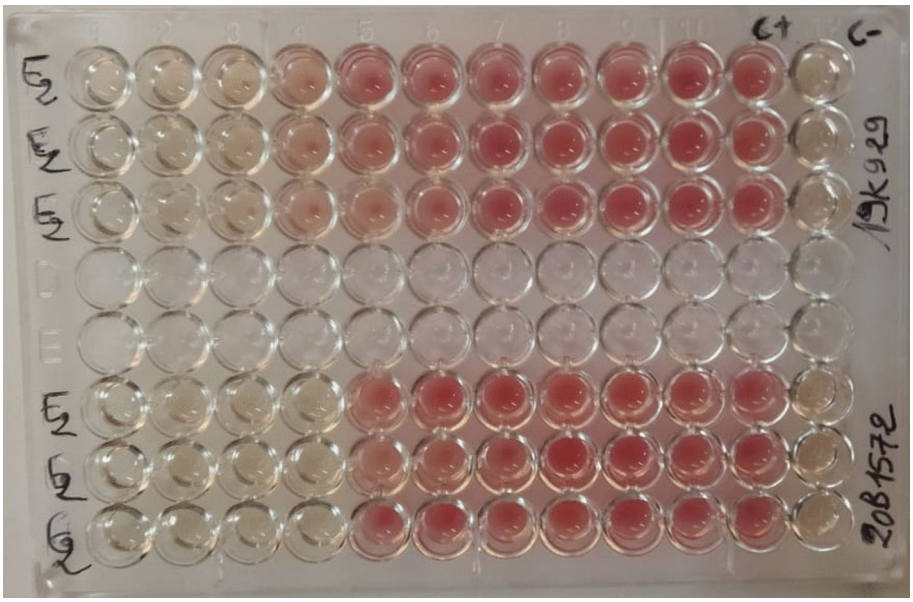

Fig. 6. Minimum Inhibitory Concentration (MIC) of ethyl acetate extract of isolate E2 against multiresistant pathogenic bacteria. 20B1572: clinical *Klebsiella pneumoniae*. 19K 929: clinical *Klebsiella pneumoniae*. E2: ethyl acetate extract of isolate E2.

Concentration of E2 ethyl acetate extract (mg/mL)	Total phenols contents (mg GAE/mg extract)	Total flavonoids contents (mg QE/mg extract)
0.1	0.472 ± 0.004	ND
0.2	0.493 ± 0.006	ND
0.3	0.530 ± 0.007	0.023 ± 0.010
0.4	0.453 ± 0.003	0.048 ± 0.009
0.5	0.473 ± 0.004	0.072 ± 0.004
0.6	0.505 ± 0.004	0.102 ± 0.006
0.7	0.540 ± 0.003	0.128 ± 0.006
0.8	0.569 ± 0.010	0.151 ± 0.006
0.9	0.596 ± 0.008	0.184 ± 0.003
1	0.628 ± 0.012	0.249 ± 0.013

Table 6. Total phenolic and flavonoid contents of ethyl acetate extract of E2 isolate. Values expressed are means ± SD (n = 3). GAE: gallic acid equivalent; QE: quercetin equivalent. (ND): not detected.

8. Microscopic observations in both fresh state and after Gram staining indicated that E2 strain belongs to the group of filamentous Gram-positive bacteria, displaying a filamentous and branched appearance[30].

In primary screening, our results demonstrated significantly higher antimicrobial activity than those of Aouiche et al.[31], who showed lower levels of antimicrobial activity. For example, the *Streptomyces* sp. strain PAL111 isolate showed inhibition zones of 10 mm for Staphylococcus aureus and 20 mm for *E. coli* in the aforementioned study, while our results demonstrated significantly higher antimicrobial activities. Furthermore, our study revealed larger zones of inhibition against *Candida albicans* ATCC 60193 compared to the previous study by Aouiche et al.[31], where zones of inhibition were weaker, ranging from 7 to 13 mm. These results suggest that our primary screening identified *Streptomyces* strains with potentially more potent antimicrobial activities.

The *Streptomyces* genus is widely recognized for its capacity to produce a diverse array of secondary metabolites, which possess a wide range of bioactivities. These bioactivities include but are not limited to antitumor, antiviral, antioxidant, antihypertensive, immunosuppressive, and especially antimicrobial properties. These metabolites serve as essential defense mechanisms against competing microorganisms in the natural environment, allowing *Streptomyces* species to thrive and survive in various ecological niches[32]. Among these bioactive compounds, antimicrobial metabolites produced by *Streptomyces* strains have garnered particular attention due to their potential applications in medicine and agriculture[32]. E2 Strain, in particular, has demonstrated significant antimicrobial activity against both gram-positive and gram-negative bacteria, as well as yeast. This broad-spectrum antimicrobial activity suggests that the metabolites produced by E2 strain may hold promise as potential candidates for the development of novel antimicrobial agents. Understanding the mechanisms behind the antimicrobial activity of *Streptomyces* metabolites, such as those produced by E2 strain,

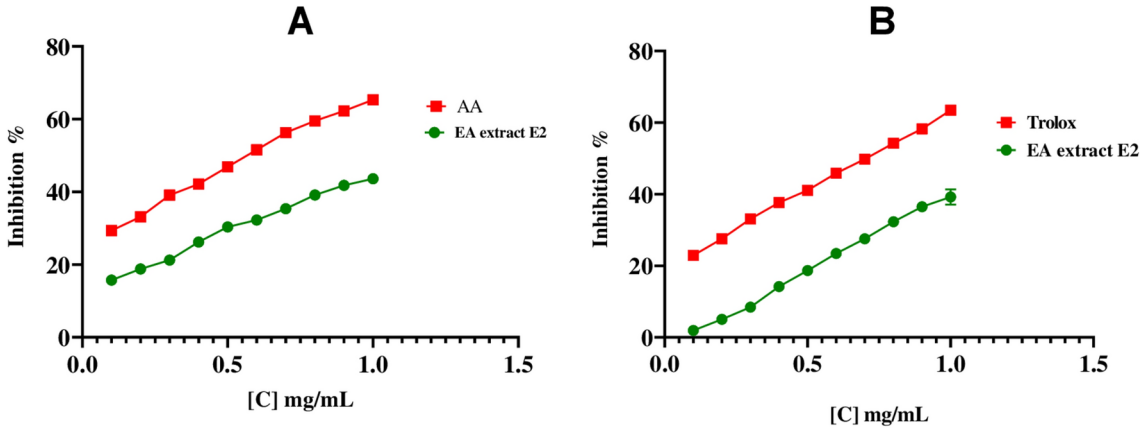

Fig. 7. The inhibition percentages of DPPH (**A**) and ABTS (**B**) by different concentrations of ethyl acetate extract obtained from *Streptomyces* sp. strain E2 and the standards AA and Trolox respectively. Results are expressed as means ± SD of three parallel measurements $p < 0.01$. AA: Ascorbic acid; EA extract E2: Ethyl acetate extract of *Streptomyces* sp. strain E2.

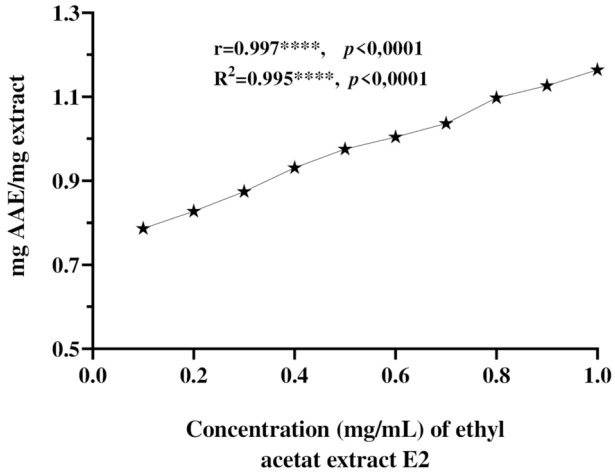

Fig. 8. Ferric reduction antioxidant power (FRAP) of ethyl acetate extract of *Streptomyces* sp. strain E2 at different concentrations. Standard deviation analysis for 10 doses (0.1–1 mg) in triplicate (n = 3). AAE: Ascorbic acid equivalent.

is crucial for harnessing their therapeutic potential. Further research into the bioactive compounds produced by *Streptomyces* strains and their mode of action could lead to the discovery of new antibiotics to combat the growing threat of antibiotic resistance.

Free radicals, like reactive oxygen and nitrogen species, play crucial roles in various biological processes such as cell signaling and immunity. However, excessive free radicals can lead to oxidative stress[33], damaging important molecules and causing various diseases[33]. The body's natural antioxidative mechanisms, involving enzymes like superoxide dismutase and nonenzymatic compounds, usually control free radical levels. But under stress, these mechanisms can become overwhelmed, leading to oxidative stress. Consumption of exogenous antioxidants helps reduce oxidative stress and prevent diseases like cardiovascular disorders and rheumatoid arthritis[34]. Natural antioxidants from microorganisms, particularly *Actinobacteria* like *Streptomyces*, are safer alternatives to synthetic chemicals[35]. *Streptomyces* sp. strain E2 extract has shown promising antioxidant activity in various assays (DPPH, ABTS and FRAP), indicating potential health benefits. To accurately assess antioxidant potential, multiple assays indicating different mechanisms should be employed, and results should be standardized to allow comparison between studies. In this study, we evaluated *Streptomyces* sp. strain E2 extract using various assays and expressed results as equivalents of ascorbic acid, providing valuable insights into its antioxidant capacity.

The application of Gas Chromatography-Mass Spectrometry (GC–MS) analysis has been instrumental in the bioprospecting of natural products derived from *Streptomyces* bacteria[36–38]. Maltol and eugenol are the major compounds present in the extract of the strain *Streptomyces* sp. E2. Maltol has been found in various plants[39,40] and isolated as a bacterial metabolite from *Streptomyces* sp. strain GW3/1538[41] and *Streptomyces* sp. strain

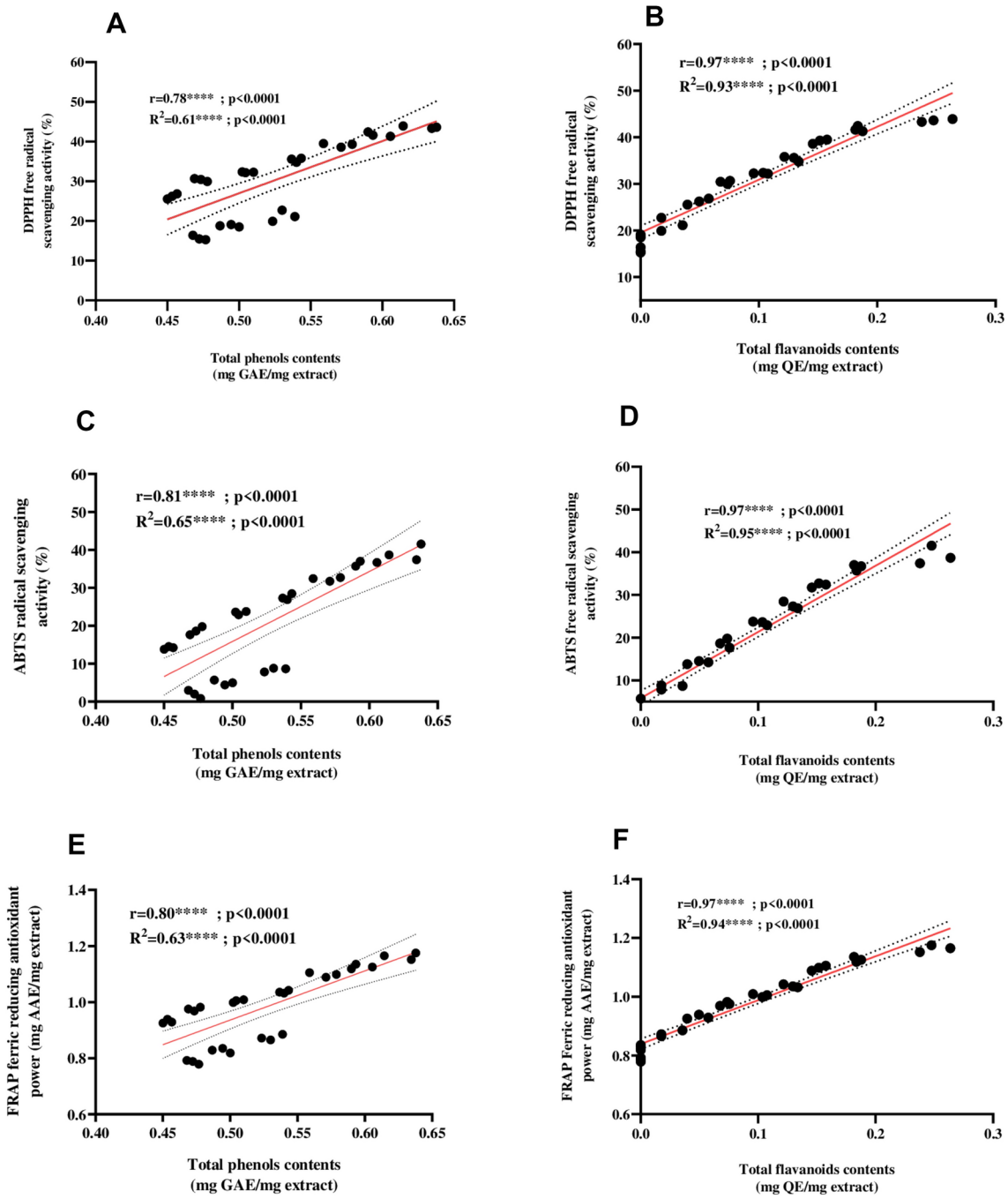

Fig. 9. Visualizing Pearson correlation: (**a**) DPPH and total phenolics contents, (**b**) DPPH and total flavonoids contents, (**c**) ABTS and total phenolics contents, and (**d**) ABTS and total flavonoids contents, (**e**) FRAP and total phenolics contents, and (**f**) FRAP total flavonoids contents.

SBT348 derived from marine sponges[42]. As a chelator of metal ions[43], maltol shows promising applications, particularly in protecting nerve cells against oxidative damage caused by reactive oxygen species (ROS), thereby contributing to the maintenance of normal cellular functions and the reduction of oxidative stress associated with diabetes and irreversible kidney damage[44].

The second main compound identified in the ethyl acetate extract of E2 strain is eugenol. Eugenol exhibits a diverse range of properties, including antimicrobial activity, antioxidant properties, anesthetic potential, anticarcinogenic effects, and anti-inflammatory action, as well as demonstrated efficacy in the treatment of diabetes and the reduction of blood lipid levels[45,46]. The World Health Organization (WHO) generally recognizes eugenol as safe (GRAS) and non-mutagenic. There are several methods for isolating eugenol, including solvent extraction, steam distillation, and hydrodistillation[46]. Eugenol is known for its effects on the cell membrane and cell wall of Gram-negative and Gram-positive bacteria, leading to their lysis and the leakage of their intracellular

RT (time)	Area (%)	M.W. (g/mol)	Quality %	Molecular formula	Compound name	Reported bioactivity
1.736	0.05	94.2	72	$C_2H_6S_2$	Disulfide, dimethyl	Antioxidant, Antifungal, Analgesic effect[84–86]
2.615	0.01	126.20	25	$C_2H_6O_2S_2$	S-Methyl methanethiosulphonate	Antimicrobial activity[87], antibacterial and antioxidant activity[88], antimicrobial potential[89]
3.258	1.06	120.17	38	$C_4H_8O_2S$	Dimethylsulfoxonium formylmethylid	Antioxidant and anticancer activities[90], antimicrobial and antioxidant activities[91], antifungal activity[92]
3.460	4.20	120.17	38	$C_4H_8O_2S$	Dimethylsulfoxonium formylmethylid	Antioxidant and anticancer activities[90], antimicrobial and antioxidant activities[91], antifungal activity[92]
9.410	0.02	126.20	72	$C_2H_6O_2S_2$	S-Methyl methanethiosulphonate	Antimicrobial activity[87], antibacterial and antioxidant activity[88], antimicrobial potential[89]
10.131	0.01	126.11	93	$C_6H_6O_3$	Maltol	Antioxidant, anti-inflammatory, and antitumor[40]
11.450	0.01	101.17	64	C_4H_7NS	2,4-Dithiapentane	Antimicrobial and antioxidant properties[93]
16.025	0.01	164.20	96	$C_{10}H_{12}O_2$	Eugenol	Antimicrobial activity[94], antioxidant, anti-inflammatory, antibacterial and antiviral effects[95], antifungal activity[96], anticancer properties[97], antibiofilm activity[98]

Table 7. Volatile compounds identified by GC–MS from Ethyl acetate crude extract of *Streptomyces* sp. E2 strain. RT. : Retention time ; M.W. : Molecular Weight.

contents, including lipids and proteins[46]. Previous studies, such as that conducted by Gülçin[47], have highlighted eugenol's strong antioxidant properties and its ability to scavenge free radicals. It has exhibited antibacterial effects against various species, including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*[48].

The HPLC–UV/vis analysis of the ethyl acetate extract from *Streptomyces* sp. strain E2 revealed a rich presence of phenolic acids, including gallic acid, chlorogenic acid, vanillic acid, trans-ferulic acid, ellagic acid, and cinnamic acid. These compounds are well-known for their potent antioxidant and antimicrobial properties[49]. Gallic acid and chlorogenic acid are particularly effective at neutralizing free radicals and protecting cells from oxidative stress, which is crucial in preventing diseases such as cardiovascular diseases and cancer[50–52]. Vanillic acid and trans-ferulic acid, on the other hand, have demonstrated significant antibacterial and antifungal activities, indicating the potential of the E2 strain as a natural antimicrobial agent[53,54]. Similarly, the biological properties of ellagic acid and cinnamic acid produced by *Streptomyces* species have been extensively studied. Ellagic acid exhibits anti-carcinogenic properties, inducing apoptosis in cancer cells and inhibiting tumor cell proliferation[55,56]. Additionally, it was reported that cinnamic acid could reduce inflammation, combat microbial infections, and protect against oxidative damage[57,58].

Conclusions

The present study highlights the discovery of a novel actinobacterial isolate, designated as strain E2, originating from the soil of a terrestrial ecosystem in the Settat Casablanca region of Morocco. This strain, identified as belonging to the genus *Streptomyces*, exhibited broad-spectrum antimicrobial activity, along with promising antioxidant properties. The findings of this study underscore the significance of soil microbial biodiversity, particularly actinobacteria, as a potential source of new bioactive molecules. Specifically, *Streptomyces* sp. strain E2 holds particular interest due to its ability to produce secondary metabolites with antimicrobial and antioxidant activities. These results pave the way for further research avenues, including the comprehensive characterization of the mechanisms of action of metabolites produced by *Streptomyces* sp. strain E2, as well as their in vivo evaluation to determine efficacy and safety. Additionally, studies on modulating culture conditions to enhance the production of bioactive metabolites could be explored. Moreover, exploring the potential applications of the compounds identified in the ethyl acetate extract of strain E2, in various fields, including as antimicrobial and antioxidant agents, and even as candidates for the development of novel drugs, warrants investigation. Furthermore, to achieve a more precise taxonomic resolution for strain E2, additional research involving the sequencing of multiple conserved genes and conducting a multi-gene phylogenetic analysis is warranted. Lastly, these findings underscore the importance of preserving natural ecosystems, such as soils, as reservoirs of valuable microbial biodiversity, and the need to continue research efforts to explore their biotechnological potential in the context of combating antibiotic resistance and promoting human and environmental health.

Materials and methods

Soil sample collection

Soil samples were aseptically collected from terrestrial ecosystems in April 2023. The specific locations were the Kenzi garden (GPS: 32° 59′ 15.1″ N, 7° 36′ 16.7″ W) and the Faculty of Science and Technology garden in Settat (GPS: 33° 00′ 37″ N, 7° 61′ 83″ W), located in the Casablanca-Settat region, Morocco. To ensure diversity and avoid duplication in the isolation of *Actinobacteria*, four distinct sampling points were designated for each site. Sampling necessitated the careful removal of a 5-cm layer from the soil surface utilizing a sterile spatula, followed by the extraction of 150 to 200 g of the underlying layer[59]. These meticulously acquired soil samples were then transferred under aseptic conditions to sterile 'Stomacher' bags, where they were blended and standardized to create a uniform soil mixture. Following this, they were conveyed to the Microbiology laboratory for storage at 4 °C until subsequent analysis[59].

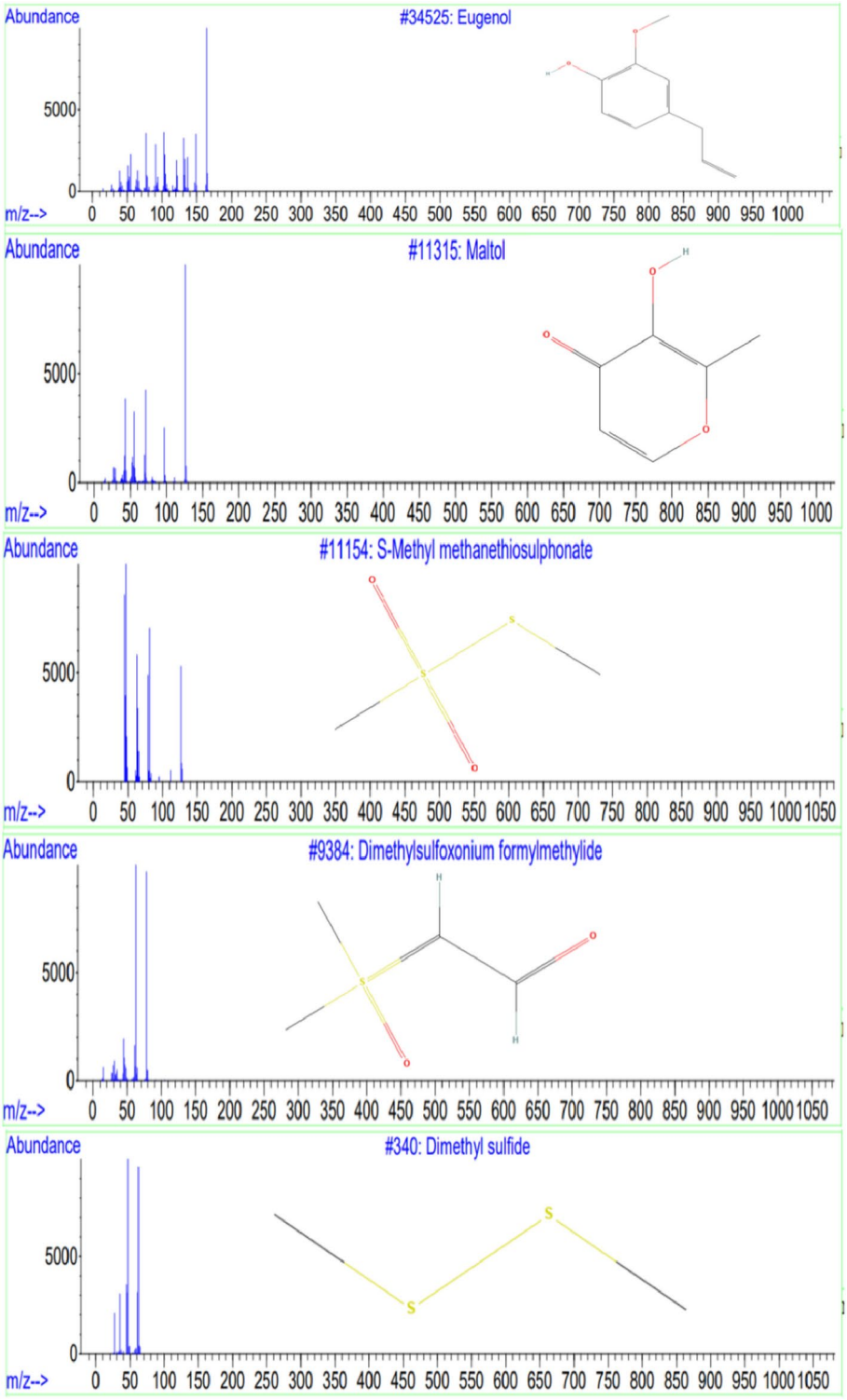

Fig. 10. GC–MS spectra of five major secondary metabolites in ethyl acetate extract of *Streptomyces* sp. Strain E2.

Physical–chemical analysis of the soil samples

The pH, electrical conductivity (EC), mineral content, organic matter (OM), and soil texture of each soil sample were assessed using the methodologies outlined in our previous research[60,61]. Minerals including carbon (C), oxygen (O), magnesium (Mg), silicon (Si), iron (Fe), potassium (K), and calcium (Ca) were examined using a scanning electron microscope (SEM) (JEOL model JSM-IT500HR), as reported by Stefaniak et al.[61]. On the other hand, analysis of zinc (Zn), manganese (Mn), chlorine (Cl), aluminum (Al), phosphorus (P), copper (Cu),

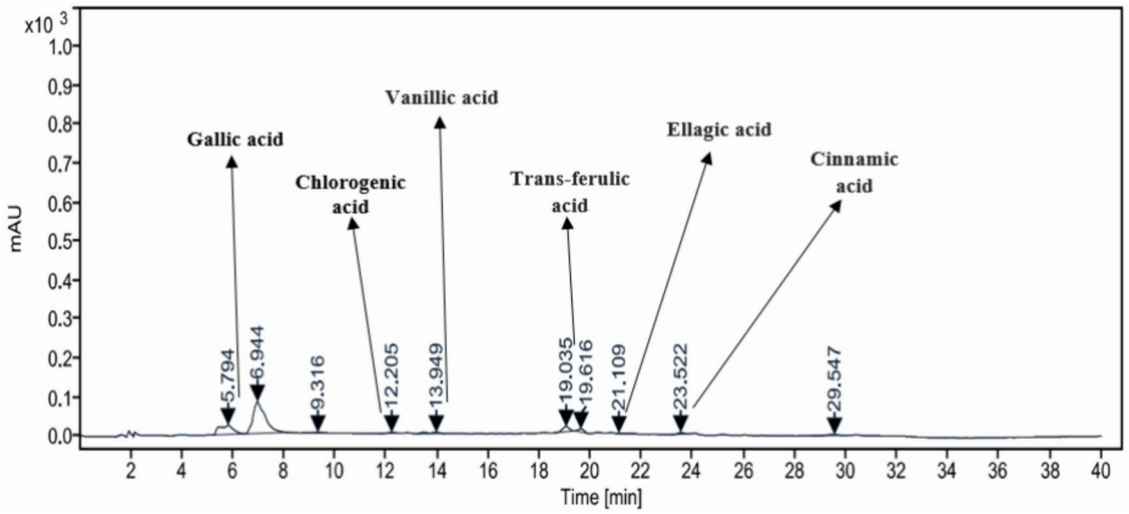

Fig. 11. HPLC chromatogram of crude ethyl acetate extract of *Streptomyces* sp. E2.

Standard number	Standard name	Retention time (min)	E2 strain crude extract
1	**Gallic acid**	**6.4**	**6.9**
2	Resorcinol	8.5	ND
3	**Chlorogenic acid**	**12.3**	**12.2**
4	**Vanillic acid**	**14.1**	**13.9**
5	Caffeic acid	14.7	ND
6	Syringic acid	15.4	ND
7	Vanillin	16.2	ND
8	p-Coumaric acid	17.7	ND
9	Sinapic acid	18.2	ND
10	Ferulic acid	18.3	ND
11	**Trans-ferulic acid**	**18.9**	**19**
12	**Ellagic acid**	**21.9**	**21.1**
13	Epicatechin	22.2	ND
14	**Cinnamic acid**	**23.6**	**23.5**
15	Quercetin	26.6	ND

Table 8. The qualitative analysis of phenolic compounds in the ethyl acetate extract of *Streptomyces* sp. E2 strain using HPLC–UV/visible. ND: not detected. Significant values are in bold.

and sulfur (S) was conducted through the energy-dispersive X-ray fluorescence method, with the Epsilon 3XLE instrument from PANalytical, France, following the methodology described by Thirion-Merle[62].

Pretreatment of soil samples, isolation, purification, and preservation of *Actinobacteria* isolates

To increase the number of *Actinobacteria*, a pre-treatment involving drying the soil for at least a week at room temperature was performed[59]. Subsequently, the dried soil samples underwent grinding with a mortar to eliminate debris and stone particles present in the soil samples. The soil samples were then stored in sterile tubes at 4 °C.

To isolate *Actinobacteria* from the soil samples, 10 g of each soil sample underwent serial dilution to 10^{-4}, and 100 µL of each dilution was spread onto selective culture media (M2, Bennett, GLM, GA) containing 50 mg/L actidone to inhibit fungal growth[10]. Petri dishes were then incubated at 28 °C for 1 week, with daily monitoring. Following the incubation period, colonies exhibiting *Actinobacteria* characteristics based on macroscopic and microscopic observations were sub-cultured on ISP2 medium using the streak method to obtain pure cultures.

For short-term preservation, these cultures were kept in inclined tubes at 4 °C , while longer-term preservation involved storage in 20% glycerol at − 20 °C[63]. This preservation approach maintains the stability and viability of *Actinobacteria* strains.

Assessment of the antimicrobial activity of actinobacterial isolates by in vitro screening

Test microorganisms

The antimicrobial effectiveness of Actinobacteria isolates was assessed against a range of test microorganisms. This panel included *Staphylococcus aureus* ATCC 25923, *Pseudomonas aereginosa* ATCC 27853, *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 60193 (a pathogenic fungus). These test strains were obtained from the Pasteur Institute of Morocco in Casablanca. Additionally, six clinically multi-resistant strains were employed, consisting of *Listeria monocytogenes*, *Klebsiella pneumoniae* 19K 929, *Proteus* sp. 19K1313, *Klebsiella pneumoniae* 20B1572, *Proteus vulgaris* 16C1737, and *Klebsiella pneumoniae* 20B1572. These strains were sourced from the Pasteur Settat Medical Analysis Laboratory in Morocco.

Primary screening

Primary screening of *Actinobacteria* was conducted on ISP2 agar medium (Composition: yeast extract: 4 g, glucose: 4 g, malate extract: 10 g, agar: 16 g, distilled water: 1L, and pH adjusted at 6.51) utilizing the double layer method[64]. On a sterile agar medium, a pure *Actinobacteria* isolate was centrally inoculated onto each plate. Subsequently, the plates were incubated at 28 °C for a duration of 10 days. A second layer, consisting of 5 mL of Muller Hinton medium (MHM) weakly agarized with 0.7% (w/v) agar, was previously inoculated with various microorganisms including non-pathogenic bacteria such as *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, and *Escherichia coli* ATCC 25922, as well as the pathogenic fungus *Candida albicans* ATCC 60193. These microbial strains were sourced from the Pasteur Institute in Casablanca, Morocco. Following an incubation period of 24 h at 37 °C for bacteria and 48 h at 28 °C for fungi, zones of inhibition surrounding the *Actinobacteria* colonies were visually inspected and measured using a caliper.

Secondary screening

Fermentation and extraction of secondary metabolites from actinobacterial isolates

Following the identification of *Actinobacteria* isolates E2 and E6 exhibiting notable antimicrobial properties in the preliminary screening, an extensive investigation into their secondary metabolites was conducted. Fermentation and extraction processes were employed to isolate these bioactive compounds. In this study, 500 mL Erlenmeyer flasks, each containing 100 mL of ISP2 culture medium, were utilized for fermenting the active *Actinobacteria* isolates. The cultures were then subjected to constant agitation at 150 rpm and maintained at 28 °C. Subsequently, the *Actinobacteria* cultures underwent centrifugation at 10,000*g* for 20 min to remove the mycelial mass, and the resulting supernatant was collected. To extract the secondary metabolites, the supernatant was subjected to organic solvent extraction with solvents of increasing polarity. Initially, it was mixed with hexane, followed by dichloromethane, ethyl acetate, and butanol, successively. The organic extracts obtained were then evaporated at 45 °C to remove solvent residues. Finally, the dry extracts, along with residual aqueous phases, were dissolved in dimethyl sulfoxide (DMSO) to facilitate concentration determination, following established protocols[10,65,66].

Evaluation of antimicrobial activity using the disc diffusion method

To assess antimicrobial activity in liquid media, we employed the paper disk technique as described by Badji et al.[64]. Sterile filter paper discs, 6 mm in diameter, were impregnated with 25 µL of each extract, along with DMSO and nalidixic acid serving as negative and positive controls, respectively. After impregnation, the discs were allowed to dry for a brief period near a Bunsen burner before being placed onto the surface of Mueller–Hinton agar (MHA), which had been previously inoculated with test bacteria using the swabbing technique.

Before commencing antimicrobial testing, bacterial cells were harvested and adjusted to an optical density (OD) ranging from 0.08 to 0.13 at 625 nm, roughly equating to 10^6 colony-forming units per milliliter (CFU/mL), using a spectrophotometer (Selectra VR2000, Barcelona, Spain)[67]. Similarly, for assessing antifungal activity, inoculum optical densities were maintained within the range of 0.18 to 0.20 at 623 nm, corresponding to a concentration of approximately 10^6 spores/mL[68]. Subsequently, the plates were refrigerated at + 4 °C for 2 h to allow for the diffusion of molecules before being incubated at 37 °C for 24 h. Following incubation, the diameter of the zones of inhibition was measured in mm.

Determination of minimum inhibitory concentration

The minimum inhibitory concentration (MIC) values were determined using a liquid culture medium dilution method. Briefly, 100 µL of Mueller Hinton broth (MHB) were added to each well of a 96-well plate. Then, 100 µL of each sample's stock solution (1 mg/mL) were mixed in the first column. A series of cascade dilutions were performed up to column 10, resulting in a concentration range from 1 mg/mL to 0.001 mg/mL of the ethyl acetate extract of the E2 isolate. The bacterial culture was adjusted to an absorbance equivalent to 0.5 McFarland, and 10 µL were added to each well except for those in column 12, which served as the negative control (MHB without inoculum). Column 11 served as the positive growth control (tested strain in MHB). Each test was conducted in triplicate. After incubation, 20 µL of a 2, 3, 5-triphenyl-tetrazolium chloride (MTT) aqueous dye (Merck-Germany; CAS No. 298-96-4) were added to the wells and incubated for 3 h. The MIC was determined as the lowest concentration that showed no microbial growth, indicated by a color change from yellow to pink[69].

Characterization of Actinobacteria isolates: cultural, micro-morphological, biochemical and physiological characteristics

Cultural characteristics, including growth intensity, surface pigmentation, colony morphology, and the presence of diffusible pigments in agar, were observed on different media such as Bennett, ISP1, ISP7, and GYEA. The inoculation of these media was performed using the streaking method, and the plates (90 mm in diameter) were then incubated at 28 °C and monitored daily for a duration of 10 days. Microscopic characteristics of pure isolates were examined using light microscopy (Olympus CX43RF), both in fresh samples and after Gram

staining. Additionally, the physiological and biochemical characteristics of the *Actinobacteria* isolates were evaluated using established methodologies as described in previous studies[10,70,71]. These assessments included the evaluation of melanoid pigment production, tolerance to varying concentrations of sodium chloride (NaCl) and pH levels, growth at different temperatures, and carbohydrate assimilation.

Genotypic identification of isolates through 16S rRNA sequencing

Genotypic identification was carried out via 16S rRNA sequencing to verify the species identification of our isolates. DNA extraction from the strain was executed using an automated system, specifically the Mag Purix Bacterial DNA Extraction Kit, in accordance with the manufacturer's instructions. The amplification reaction followed the protocol outlined by[72]. The 16S rRNA gene underwent amplification using universal bacterial primer sequences Fd1 (5′-AGAGTTTGATCATGGCTCAG-3′), and rP2 (5′-ACGGTTACCTTGTTACGACTT-3′), resulting in an amplicon size of 1,500 bp as per established conditions. Subsequently, all amplified products were subjected to sequencing for identity validation. Both strands of the purified amplicons were sequenced using a 3130 × 1 Genetic Analyzer, employing the same primers utilized for PCR amplification. Sequences obtained were assembled into contigs using DNA Baser Assembler software version 5.15.0, saved in FASTA format. Multiple alignment of E2 strain 16S rRNA gene sequence with representative sequences of related *Streptomyces* strains was conducted using MEGA X software[73]. This alignment facilitated construction of a phylogenetic tree via the neighbor-joining method[73]. within the same software. Evolutionary distances were computed using Kimura's two-parameter model, and tree topologies were evaluated through bootstrap analyses employing Felsenstein's 1000 resamples method[73].

Determination of phenolics and flavonoids in ethyl acetate extract

The determination of total phenolics, the Folin-Ciocalteu method, as detailed by Bensadón et al.[74], was employed. This involved combining 3 mL of diluted Folin-Ciocalteu reagent (1:10) with 500 µL of sample or standard (1 mg/mL prepared in methanol), followed by the addition of 3 mL Na_2CO_3 (6%). After one hour's incubation at room temperature, protected from light, absorbance was recorded at 760 nm. Similarly, quantification of flavonoid content was carried out using the aluminum chloride method, according to the protocol described by Bahorun et al.[75]. Briefly, 1 mL of sample or standard (prepared in methanol) was mixed with 1 mL of $AlCl_3$ solution (2% in methanol). After a 10 min incubation period, absorbance was measured at 415 nm.

Antioxidant activity of E2 ethyl acetate extract

The antioxidant capacity of the ethyl acetate extract from *Streptomyces* sp. strain E2 was evaluated using three different assays. Firstly, the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity was determined according to the method described by Blois[76], with absorbance measured at 517 nm using an Elisa microplate reader. Ascorbic acid served as the positive antioxidant control. Secondly, the 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay was conducted following the protocol developed by Re et al.[77]. The resulting absorbance was measured at 743 nm, with a decrease indicating a reduction in radical amount, and Trolox was employed as a positive control. Finally, the ferric reducing antioxidant power (FRAP) of the extracts was assessed as outlined by Oyaizu[78,79], with absorbance measured at 700 nm against a similar blank, where distilled water replaced the extract. Ascorbic acid was utilized as the positive standard in this assay.

Analysis of E2 ethyl acetate extract by GC–MS

Gas chromatography-mass spectrometry (GC–MS) analysis was conducted following the procedure outlined by Chakraborty et al.[80]. The analysis utilized an Agilent 7890A Series gas chromatography (GC) system coupled with mass spectrometry (MS), comprising a multimode injector and an HP-5MS capillary column (30 m × 0.250 mm × 0.25 µm). Solubilized extracts were introduced into the column using helium as the carrier gas (1.7 mL/min) in a 1:4 fractionated injection mode. The ion source and quadrupole temperatures were maintained at 230 and 150 °C, respectively. The temperature program ranged from 60 to 360 °C. Compound identification was achieved by comparing the obtained mass spectra with data available in the NIST MS 2017 library[81].

HPLC–UV/visible analysis

The HPLC–UV/vis analysis was performed using a Shimadzu HPLC system equipped with an SPD-20A UV/absorbance detector. Separation was achieved using a Waters reverse-phase (RP) Symmetry C-18 column (150 × 3.9 mm, 5 µm) at ambient temperature. The mobile phase consisted of deionized water with trifluoroacetic acid (TFA) (pH 2.5) as solvent A and 99.99% methanol as solvent B. A gradient elution method was employed: initially, 100–50% solvent A over 0 to 20 min, followed by 50–40% solvent A from 20 to 30 min, and finally, 40–100% solvent A from 30 to 40 min. The flow rate of the mobile phase was maintained at 1 mL/min, and the detector was set at 280 nm[82]. Phenolic compounds were identified by comparing their retention times and UV–Visible spectra with those of previously injected standards (resorcinol, caffeic acid, syringic acid, vanillin, p-coumaric acid, sinapic acid, ferulic acid, epicatechin, quercetin, gallic acid, chlorogenic acid, vanillic acid, trans-ferulic acid, ellagic acid, and cinnamic acid)[82].

Statistical analysis

The antioxidant and antimicrobial activities, along with the determination of total phenolic and flavonoid compounds, were carried out in triplicate for each test. Data collected were analyzed using GraphPad Prism 8.4.3 software and presented as mean ± standard deviation (SD). Significant differences between groups were determined via one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. A significance level of $p \leq 0.05$ was adopted for all data analyses in this study. Pearson correlation analysis

was conducted using GraphPad Prism 8.4.3 software to evaluate the relationship between total phenolic and flavonoid compounds and antioxidant activity.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request. Genomic sequence of Streptomyces sp. strain E2 has been deposited at the National Centre for Biotechnology Information (NCBI) GenBank (https://www.ncbi.nlm.nih.gov/nucleotide/) under the following accession number PP731514.

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Author contributions

S.R., F.Z.K., and M.E. conceived the experiments. S.R., B.B., F.Z.K., and A.K. conducted the experiments. A.A., S.S., A.R., V.B, S.R., M.E., and B.B. analyzed the results. B.N., A.D.P., L.R., and A.C. conception and visualization. All authors reviewed the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

Correspondence and requests for materials should be addressed to L.R.

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