# Multifunctional Actinomycetes: Harnessing Bioactive Metabolites for Innovative Therapeutics and Agricultural Solutions

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**A list of abbreviations**

| | |
|---|---|
| (CFU) | colony-forming units |
| (DPPH) | 2,2-diphenyl-1-picrylhydrazyl |
| (DW) | dry weight |
| (FRAP) | ferric reducing antioxidant power |
| (FW) | fresh weight |
| (GYM) | glucose yeast extract malt extract agar |
| (MTT) | 3-(4,5-dimethylthiazol2-yl)-2,5-diphenyl tetrazolium bromide |
| (NBT) | nitro blue tetrazolium |
| (PCA) | principal component analysis |
| (PGPB) | plant-growth-promoting bacteria |
| (RBC) | red blodd cells |
| (SOS) | superoxide scavenging activity |
| (TPTZ) | 2,4,6–Tris(2–pyridyl)–s–triazine |
| (Trolox) | 6–hydroxy–2,5,7,8–tetramethylchromane–2–carboxylic acid |
| (XOD) | xanthine oxidase |

## Abstract

The global search for novel bioactive metabolites has intensified interest in actinobacteria due to their pharmaceutical and biotechnological potential. This study presents an integrative screening strategy that combines comprehensive biological assays, phytochemical profiling, therapeutic evaluation, and agricultural application. Seventeen actinobacterial isolates were systematically assessed through antioxidant (FRAP, DPPH, SOS, XO inhibition), anti-hemolytic, anti-lipid peroxidation, cytotoxicity, oxidative stress markers, and antimicrobial tests to identify multifunctional strains. Three top-performing isolates were further examined for phenolic acids, flavonoids, and their anticancer, anti-inflammatory, and anti-cholesterol properties. Isolate 17 exhibited the highest activity and was identified as *Plantactinospora endophytica* JTT05 based on 16S rRNA sequencing and phylogenetic analysis. Importantly, we report for the first time that this potent isolate not only displays strong therapeutic bioactivities but also promotes plant development. When applied to *Eruca sativa*, *P. endophytica* JTT05 enhanced fresh weight by 3.14%, dry weight by 24.75%, photosynthetic efficiency by 13.46%, and improved phytochemical contents including phenolics (+12.31%), flavonoids (+0.73%), antioxidants (+4.80%), and glucosinolates (+22.21%). Overall, *P. endophytica* JTT05 emerges as an innovative dual-function microorganism, offering promising antioxidant, anticancer, anti-inflammatory, and anti-cholesterol activities while simultaneously serving as a sustainable plant growth–promoting agent.



## 1. Introduction

Microorganisms are key natural sources of several valuable and commercial metabolites, which currently constitute half of the medicinal products on the global market. Actinomycetes are a leading group of bacteria that possess diverse metabolic and systemic mechanisms, creating a wide range of bioactive metabolites with diverse impacts on human health (El-Sayed et al. 2023). Actinobacteria are aerobic, Gram-positive, and spore-forming filamentous bacteria that exist in terrestrial and aquatic habitats with considerable ability to thrive in adverse conditions. Actinomycetes are featured with a complex growth scheme, highly diverse genetic and metabolic system, and potential productivity of multiple structures of secondary metabolites that include alkaloids, pigments, flavonoids, polyethers, glycosides, lipoproteins, polypeptides, enzymes, and antibiotics. Along with these metabolites, actinobacterial extracts possess antifungal, antiviral, antiprotozoal, antioxidant, anti-cholesterol, anti-inflammatory, anticancer, and cytotoxic activities (Arslan et al. 2025; Krysenko 2023). To realize the great significance of the phylum actinobacteria as potent producers, about 42% of all reported bioactive compounds of

microbial origin have so far been produced by actinobacterial strains (El-Sayed et al. 2023). Moreover, one of the prestigious genera is *Streptomyces* which can synthesize about 75–80% of known antibiotics and more than 100,000 bioactive natural products, which have been grasped in the pharmaceutical and agricultural applications (Anjum et al., n.d.). However, mining for promising strains of actinomycetes that possess astonishing bioactivity remains a challenging approach (Bano et al. 2022).

Given the ascendant concern over the adverse side effects, including carcinogenic properties, toxicity and allergic reactions of synthetic chemicals incorporated in food and pharmaceutical products (Rhenals-Montoya et al. 2024), therefore the replacement of unsafe artificial antioxidants by their extraction from natural origins such as microorganisms is highly prerequisite to cover the daily market demands, because the antioxidants were utilized as efficient food stabilizer and human immunity booster (Hassan et al. 2020). In addition, isolation of pigments from microbial sources to be as natural colorants in food, cosmetics, pharmaceuticals, cloth, painting, and plastics is vastly recommended (Yu et al. 2022), particularly after the ban on numerous synthetic pigments by the World Health Organization (WHO) and others (Rhenals-Montoya et al. 2024; Mohandas et al. 2025). Carotenoids are outstanding family of yellow to orange-red terpenoid pigments which are commonly distributed in plants, algae and microorganisms including actinomycetes. (Rhenals-Montoya et al. 2024; Yu et al. 2022). Exogenous antioxidants, such as α- and β-carotene, and lutein play vital roles in preventing oxidative damages of free radical by scavenging activity. In living organisms, the most important bioactivity of carotenoids is the antioxidant response and the resistance to oxidative stress (Galasso et al. 2017). In humans, carotenoids are not synthesized but are obtained from diets. Carotenoids serve as antioxidants, while some of these pigments were applied as precursors in the biosynthesis of vitamin A (α-carotene, β-carotene, γ-carotene and β-cryptoxanthin), and to protect the macula lutea of the eye (Romero et al. 2012). Moreover, significant side effects including hepatotoxicity and allergies are also linked to synthetic Xanthine oxidase (XOD) inhibitors giving interest in exploring natural compounds as safer substitutes for XOD inhibition (Liu et al. 2024).

Endophytic actinobacteria are prominent microbial symbionts fostering the plant growth by harnessing of plant–microbe interactions. These actinomycetes are recognized as plant-growth-promoting bacteria (PGPB) which act as phytostimulators by facilitating the acquisition of essential minerals, e.g., phosphate, zinc and nitrogen, and regulating plant hormones. Additionally, these bacteria serve as biocontrol agents of plant pathogens and as bioremediators by eliminating unfavorable xenobiotics found in the rhizosphere. In general, PGPB significantly improve physiological and agronomic characteristics of plants, especially those subjected to stress

conditions (Hassan et al. 2024; Santos-Medellín et al. 2021). For instance, *Streptomyces* is reported as the most abundant taxon in endosphere communities during and after drought (Hassan et al. 2024; Santos-Medellín et al. 2021). It is reported that the genus *Plantactinospora* comprises seven defined species that were in association with plants. For instance, *Plantactinospora endophytica* was isolated from the leaves of a medicinal plant so-called *Camptotheca acuminata* (Zhu et al. 2012). *Plantactinospora* members have very close 16S rRNA similarities to the genera *Micromonospora*, *Salinispora*, and *Polymorphospora* (>98.6%); therefore, *Plantactinospora* members have primarily been identified by the morphological and chemotaxonomical traits (Contreras-Castro et al. 2018). The potential applications of *Plantactinospora* species have not been unveiled yet (Contreras-Castro et al. 2018). According to literature, the strain *Plantactinospora endophytica* has not been exploited as PGPB so far.

We are identifying bioactive actinomycetes for medical and health benefits. Our study characterized isolates for their antioxidant, antimicrobial, and antiprotozoal properties, as well as their production of protective compounds. From all isolated actinomycetes, we selected three top isolates for further testing of their phenolics, flavonoids, and their potential anticancer, anti-cholesterol, and anti-inflammatory effects, highlighting their use in food quality and human well-being. The promising results highlight the potential of *Plantactinospora endophytica* in sustainable agriculture. Future studies will explore its anticancer, anti-cholesterol, and anti-inflammatory activities, reinforcing actinomycetes' applications in food stability and human healthcare.

## 2. Methods

### 2.1 Sample collection and bacterial isolation

The fresh leaves of the plant were collected from *Tribulus Terrestris* under study, which consisted of healthy fresh leaves. The leaf samples were collected from the Jazan region. The leaves were washed thoroughly under running tap water and air-dried. The leaf surfaces were sterilized by dipping them in 70% ethanol for 3 minutes, then rinsed in sterilized distilled water five times. The weight of 1 gm of sterilized leaves was taken, cut into small pieces, and then squashed using sterile glass slides. The resulting juice was transferred into tubes with 2 ml of physiological saline solution, then mixed well the contents of the tubes and transferred 100 microliters to Petri dishes containing the Glycerol casein $KNO_3$ agar cultivation medium. The plates were then incubated at 37 °C for seven days, and any bacterial growth was observed. Colonies were counted as colony-forming units (CFU) per

gram. Colonies were selected on differences in shape, size, and color and then purified by repeated subculturing on glucose yeast extract malt extract agar (GYM) and preserved at 4 °C.

### 2.2 Assessment of antimicrobial and antiprotozoal activities

The screening strategy for selection of the most potent isolates was performed by evaluation of their biological activities. For example, the antibacterial activity of all isolates was tested by the plate diffusion method (Bauer et al. 1966) against a variable collection of common pathogenic bacteria, i.e., four Gram-positive strains (*Staphylococcus saprophyticus*, *Staphylococcus epidermidis*, *Enterococcus faecalis* and *Streptococcus salivarius*), as well as six Gram-negative strains (*Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Enterobacter aerogenes* and *Serratia marcescens*). As well the antifungal activity was investigated against a couple of pathogenic yeasts (*Candida albicans* and *Candida glabrata*) and a single fungus *Aspergillus flavus* such that the bacterial isolates under study were grown on the Bennett agar medium for 14 days and three of 10 mm-diameter discs were cut and placed on the surface of Muller–Hinton agar (for bacteria) and Sabouroud agar medium (for yeasts and fungi) which were seeded with a test organism. To allow the diffusion of potential antimicrobial agents, the plates were maintained at 4 °C for 2 h, then were transferred into 28 °C-incubator. The antimicrobial activity was determined by measuring the inhibition zones after 24 h for bacteria and yeast or after 48 h for fungi.

The antimicrobial activity (AA) of each isolate can be expressed as:

$$AA = D_{inhibition} - D_{disc}$$

Where:

- AA = Antimicrobial activity (mm)
- $D_{inhibition}$ = Diameter of the inhibition zone around the disc (mm)
- $D_{disc}$ = Diameter of the disc (usually 10 mm)

The actinobacterial isolates were individually grown in in 250 mL ISP-2 medium broth in 500 mL-flasks which were incubated at 200 rpm at 30°C for 5–10 days with observation of the bacterial proliferation. The fermented medium was filtrated from the bacterial cells and extracted by 250 mL of ethyl acetate solvent, which was evaporated to obtain the desired extracts, and 0.2 mg of each extract was utilized for the antiprotozoal activity that was estimated similar to Cheng et al. (Cheng et al. 2015) by measuring the absorbance of the combination between *Trypanosoma cruzi* ($10^4$ trypanosomes per mL) and each actinobacterial extract (200 µL of test extract at 200 µg/mL in 1% DMSO) at 550 nm. A mixture of the protozoan in 1% DMSO was used as a control. All assays were done in triplicate.

The general formula as:

$$\%\text{Protozoal Inhibition} = \left(1 - \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{control}} - A_{\text{blank}}}\right) \times 100$$

Where

- $A_{\text{sample}}$ = absorbance with protozoa + extract
- $A_{\text{control}}$ = absorbance with protozoa + vehicle (no extract)
- $A_{\text{blank}}$ = absorbance of medium or extract-only blank (no protozoa)

### 2.3 Determination of the overall antioxidant capacity of actinobacteria

The antioxidant power of the actinobacterial isolates 1-17 was investigated by measuring the free radical scavenging activity such as the ferric reducing antioxidant power (FRAP) and the 2,2-diphenyl-1-picrylhydrazyl (DPPH). Besides, the superoxide scavenging activity (SOS) of these isolates was also evaluated. To obtain test extracts from actinomycetes to assay a given activity, 30 mg of the lyophilized actinobacterial cells was smashed in liquid nitrogen and was transferred into 2 mL of chilled ethanol (80%). For FRAP assay, 100 µL of freshly prepared FRAP reagent (0.3 M acetate buffer (pH 3.6), 0.01 mM 2,4,6–Tris(2–pyridyl)–s–triazine (TPTZ) in 0.04 mM HCl and 0.02 M $FeCl_3.6H_2O$) was mixed with 100 µL of the test extract for 30 min, and the liberated ferrous ions in samples caused an increased absorbance that was measured at a wavelength of 590 nm. 6–hydroxy–2,5,7,8–tetramethylchromane–2–carboxylic acid (Trolox) was used as a standard (Schlesier et al. 2002). Similarly for DPPH% assay, 100 µL of freshly prepared DPPH reagent was mixed with 100 µL of the test extract for 30 min in the darkness and the optical density was measured at 517 nm (Cheung et al. 2003). For superoxide-scavenging (SOS) assay, the reaction mixture containing 300 µL of the extract was mixed with 100 µL of 1 mg/mL nitro blue tetrazolium (NBT) in alkaline DMSO (1 mL DMSO containing 5 mM NaOH in 100 µL water), and the absorbance was determined at 560 nm (Srinivasan et al. 2007). For xanthine oxidase (XO) inhibition assay. The enzymatic reaction mixture containing 120 µL of the extract in 50 mM potassium phosphate buffer (pH 7.8) and 60 µL xanthine oxidase (0.025 unit/mL) was incubated at room temperature for 10 min. Then the reaction can be started by adding 100 µL of 0.15 mM xanthine and incubating for 30 min at 37 °C in the darkness. The reaction was terminated by applying 20 µL of 1 N HCl and the absorbance was assessed at 290 nm (Huang et al. 2022). All absorbance values of the actinomycete extracts were measured at least three times using a microplate reader (Synergy Mx, Biotek Instruments Inc., Vermont, USA).

The general equations for assays as :

$$\text{FRAP value (µmol Trolox/g sample)} = \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{Trolox standard}}} \times C_{\text{Trolox}}$$

Where:

- $A_{\text{sample}}$ = Absorbance of test extract at 590 nm
- $A_{\text{blank}}$ = Absorbance of blank (reagent only)
- $A_{\text{Trolox standard}}$ = Absorbance of Trolox standard
- $C_{\text{Trolox}}$ = Concentration of Trolox equivalent (µmol/g)

$$\text{DPPH Scavenging (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Where:

- $A_{\text{control}}$ = Absorbance of DPPH reagent at 517 nm (without sample)
- $A_{\text{sample}}$ = Absorbance of DPPH with test extract

### 2.4 Evaluation of cytotoxicity and hemolytic inhibition

Based on MTT (tetrazolium) assay (Mosmann 1983), the cytotoxic effect of actinobacterial isolates was determined on eukaryotic cells (HL-60) at a density of $10^4$ cells/well. Viable cells were observed using a microplate reader at 490 nm (Yu et al. 2022). The inhibition percentage of erythrocyte hemolysis was investigated as described by Mohammadipanah and Momeniland (2018). 20 µg/mL of the extracts of actinobacterial isolates were mixed with washed RBC suspension and 2.5 mM ferrous sulfate and were incubated at 37 °C for 30 min. After centrifugation of all samples at 2500 rpm for 5 min, the hemolysis was determined by determining the absorbance of supernatants at 540 nm. The samples without glutathione served as a positive control, samples without ferrous sulfate and hemolysis induction served as a negative control, and samples without actinobacterial extracts acted as a blank.

The cell viability (%) or cytotoxicity inhibition (%) can be calculated as:

$$\text{Cell viability (\%)} = \frac{A_{\text{sample}} - A_{\text{blank}}}{A_{\text{control}} - A_{\text{blank}}} \times 100$$

$$\text{Cytotoxicity inhibition (\%)} = 100 - \text{Cell viability (\%)}$$

Where:

- $A_{\text{sample}}$ = Absorbance of treated cells (with extract)
- $A_{\text{control}}$ = Absorbance of untreated (control) cells
- $A_{\text{blank}}$ = Absorbance of media/MTT reagent without cells

The percentage inhibition of erythrocyte hemolysis can be calculated as:

$$\text{Hemolysis Inhibition (\%)} = \frac{\text{Absorbance of positive control} - \text{Absorbance of sample}}{\text{Absorbance of positive control} - \text{Absorbance of negative control}} \times 100$$

Where:

- Positive control = RBCs + ferrous sulfate, no glutathione (max hemolysis)
- Negative control = RBCs only (no ferrous sulfate, no hemolysis)
- Sample = RBCs + ferrous sulfate + actinobacterial extract

## 2.5 Determination of phenolic metabolites

The total phenolic content and total flavonoid content were established according to protocols of Orsavová et al. (Orsavová et al. 2019). The analytical method of determination of pigments such as lutin, α- and β-carotene, β-cryptoxanthin, γ-carotene and lycopene was achieved based on (Biswas et al. 2011).

The TPC was determined using the Folin–Ciocalteu method and expressed as mg gallic acid equivalents (GAE) per g extract:

$$\text{TPC (mg GAE/g extract)} = \frac{C \times V}{m}$$

where:

- C = concentration of gallic acid obtained from the calibration curve (mg/mL)
- V = volume of extract (mL)
- m = mass of the sample extract (g)

## 2.6 Evaluation of oxidative stress markers

The anti-hemolytic activity of the extracts from the seventeen isolates was spectrophotometrically assessed based on the method described by Karim et al. (Karim et al. 2020). The actinomycetes extracts were evaluated for the lipid peroxidation inhibitory activity using a TBARS assay (Rungsang et al. 2023).

## 2.7 The further analysis of the selected most potent actinobacterial isolates

### 2.7.1 The content of phenolic acids and flavonoid compounds

After screening all isolated endophytic actinomycetes for a large set of pharmacological activities and yielding bioactive products, the most potent isolates are subjected to further analysis of their phenolic acids and flavonoid compounds in addition to evaluating their antitumor, anti-cholesterol, and anti-inflammatory characteristics. We detected the individual phenolic acids (caffeic, ferulic, protocatechuic, catechin, gallic, p-coumaric, resorcinol, chlorogenic and syringic), in addition to the individual flavonoid compounds (quercetin, quercetrin, luteolin, apigenin, isoquercetrin, rutin, ellagic acid, velutin, naringenin, genistein, daidzein, fisetin, and *O*-hydroxydaidzein) as described by Orsavová et al. (2019).

The concentration in the sample ($C_s$) can be calculated as:

$$C_s = \frac{A_s}{A_{std}} \times C_{std} \times \frac{V_{final}}{V_{sample}}$$

Where:

- $A_s$ = Peak area of the compound in the sample.

- $A_{std}$ = Peak area of the standard.
- $C_{std}$ = Concentration of the standard.
- $V_{final}$ = Final volume of the sample after extraction.
- $V_{sample}$ = Volume of the sample injected.

### 2.7.2 The anticancer activity

The antitumor activity was routinely measured using the cell growth inhibitory assays of the test samples, which were examined by 3-(4,5-dimethylthiazol2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay described in Lee et al. (Lee et al. 2015). A $5 \times 10^4$ of cancer cells was seeded in 96-well plate, and then the cells were treated with 25-800 µL of the samples, and further incubated for 30 h. The cell viability (%) of cancer cells was determined via ELISA at a wavelength of 540 nm by MTT stock solution (2 mg/mL).

The cell viability (%) is calculated as:

$$\text{Cell Viability (\%)} = \frac{\text{OD}_{\text{treated}}}{\text{OD}_{\text{control}}} \times 100$$

Where:

- $OD_{treated}$ = absorbance of cells treated with your sample
- $OD_{control}$ = absorbance of untreated control cells (100% viable)

### 2.7.3 The anti-inflammatory and anti-cholesterol activity

The anti-inflammatory activity of the extracts obtained from the selected isolates was evaluated by determining both lipoxygenase and cyclooxygenase-2 activity. linoleic acid was served as a substrate for lipoxygenase to assay the anti-lipoxygenase activity. In the darkness, 10 µL (50 mg/mL) of the potent extracts was incubated with 90 µL of lipoxygenase (400 U/mL) for 5 min at 25 °C. Then, 100 µL of linoleic acid (0.4 mM) was mixed into each well, and again the reaction was incubated in the dark for 20 min at 25 °C. A freshly prepared ferrous orange xylenol reagent (100 µL), comprising 90% methanol, 10 µM $FeSO_4$, 100 µM xylenol orange, and 30 mM $H_2SO_4$, were mixed, and the reaction was kept for 30 min at 25 °C. The absorbance was measured at 560 nm and the percentage of inhibition was calculated (Almuhayawi et al. 2020). In the case of measurement of cyclooxygenase-2 activity, the cyclooxygenase assay kit (Cayman Chemical Company, Ann Arbor, MI, USA) was used, where the plate was incubated in the dark for 90 min at 25 °C, and the absorbance was estimated at 420 nm to quantify the percentage of inhibition (Almuhayawi et al. 2020). The anti-cholesterol activity of extracts taken from the most potent isolates of actinomycetes was assayed by considering inhibitory activity of both α-amylase and lipase according to the modified methods reported in Anyanwu et al. (Anyanwu et al. 2019).

The general formula for the anti-inflammatory and anti-cholesterol activity is:

$$\text{Percentage Inhibition (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Where:

- $A_{control}$ = absorbance of the reaction without extract (enzyme + substrate only)
- $A_{sample}$ = absorbance of the reaction with extract

### 2.8 Molecular characterization of the highest potent isolate

Genomic DNA was isolated from the culture by an enzymatic method of Chen et al. (Chen et al. 2016) and subsequently used for amplification of the 16S rRNA gene. Primer pairs were used, 27F-1492R (Farris and Olson 2007). The 25 µL PCR mix consisted of PCR buffer with 1.5 mM $MgCl_2$, each dNTP of 0.2 mM of 0.5 µM, Taq DNA polymerase of 0.625 U, and 5 µL of genomic DNA. Reactions were performed at an initial denaturation temperature of 95°C for 5 min, 35 cycles of denaturation at 95 °C for 45 s, annealing temperatures at 49 °C for 45 s, extension temperature at 72 °C for 90 s, and a final extension of 72 °C for 7 min. PCR products were verified by agarose gel electrophoresis (1.2% w/v agarose added with 1 µL/10 mL v/v LabSafe™ Nucleic Acid Stain, G-Biosciences), followed by purification and sequencing. The obtained sequences were compared with sequences from the NCBI database for genus identification.

### 2.9 Biopriming of arugula seed with the highest potent isolate

For six hours at 30 °C, the sterile arugula seeds (*Eruca sativa*) (Agricultural Research Centre, Egypt) were immersed in a liquid suspension of the highest potent isolate (grown at 30°C, and pH 7.8 for two days yeliding $2.5 \times 10^7$ CFU $mL^{-1}$), while another group of seeds was submerged in sterile distilled water serving as control sample. Both treated and controlled seeds were sown into a sterile clay soil, applying three biological replicates for each group. The cultivation process of treated and untreated seeds was performed as described by Hassan et al. (Hassan et al. 2024; Santos-Medellín et al. 2021). After 5 weeks of plant cultivation, the fresh weight (FW) and dry weight (DW) of the shoots were measured and stored at -80°C for further analysis.

### 2.10 Quantiative analysis of treated arugula seeds

***Determination of photosynthetic rate***

The photosynthetic rate is evaluated utilizing an EGM-4 infrared gas analyzer connected to an Environmental Monitor Sensor Probe Type 3 (PP Systems, Hitchin, UK) (Lichtenthaler 1987; Maruthai et al. 2025).

Equation for assay the photosynthetic rate ($P_n$):

$$P_n = \frac{(C_i - C_o) \cdot F}{A}$$

Where:

- $P_n$ = Net photosynthetic rate (μmol $CO_2$ $m^{-2}$ $s^{-1}$)
- $C_i$ = $CO_2$ concentration entering the chamber (μmol $mol^{-1}$)
- $C_o$ = $CO_2$ concentration leaving the chamber (μmol $mol^{-1}$)
- F = Airflow rate through the leaf chamber (mol $s^{-1}$)
- A = Leaf area enclosed in the chamber ($m^2$)

***Chlorophyll content***

200 mg of plant material in acetone was homogenized by MagNALyser (Roche, Belgium) at 7000 rpm for one minute. Then the homogenate was centrifuged for 20 minutes at 14,000 × g and 4 °C. The supernatant was filtered and analyzed according to Almuhayawi et al. (2021) to determine the level of chlorophyll *a*, *b* and total chlorophyll.

Equation for assay:

$$\text{Chlorophyll } a \text{ (mg/g FW)} = \frac{(12.7 \times A_{663} - 2.69 \times A_{645}) \times V}{1000 \times W}$$

$$\text{Chlorophyll } b \text{ (mg/g FW)} = \frac{(22.9 \times A_{645} - 4.68 \times A_{663}) \times V}{1000 \times W}$$

$$\text{Total chlorophyll } a{+}b \text{ (mg/g FW)} = \frac{(20.2 \times A_{645} + 8.02 \times A_{663}) \times V}{1000 \times W}$$

***Contents of other bioactive metabolites***

Th total phenolics, flavonoids, and antioxidants were detected as mentioned earlier, while the total glucosinolates were determined based on the method of Lazerri et al. (Lazzeri et al. 1993).

## 2.11 Statistical analysis

All statistics were performed using GraphPad Prism version 9.2 (GraphPad Software Inc., La Jolla, CA). One-Way ANOVA (Tukey's post hoc test) was used to determine whether group variance was significant or not. Pearson coefficient r was employed to assess correlation using Origin Pro 2021 (version 9.85). Comprising continuous variables of the dataset were investigated using principal component analysis (PCA). Quantitative

parametric data were subjected to Shapiro-Wilk test to confirm the normal distribution and were expressed as mean ± SD, and statistical differences were defined as * $p < 0.05$ and ** $p < 0.01$.

## 3. Results

### 3.1 Isolation and purification of actinomycetes

In the present study, a total of seventeen endophytic actinobacterial isolates were recovered from *Tribulus terrestris*, an annual plant in the family (Zygophyllaceae) widely distributed worldwide. It is adapted to thrive in dry climate locations. The plant is native to warm temperate and tropical regions from Jazan region, Saudi Arabia, the actinomycetes were distinguished and labelled according to the color of their mature sporulated aerial mycelium; therefore, the bacterial colonies were picked from the mixed cultures based on the colony diversity rather than on the colony abundance, and then were purified under culture conditions and were maintained for future experimentation and analysis.

### 3.2 Antimicrobial and antiprotozoal activities

Both antimicrobial and antiprotozoal activities of the tested isolates were estimated and are shown in **Table 1**. All isolates were more active against the Gram-negative than the Gram-positive bacteria. Among the screened isolates, the three isolates 3, 10 and 17 interestingly exhibited the highest antibacterial activities against all tested pathogenic bacteria (**Table 1**). The most susceptible test organism was *Salmonella typhimurium*, followed by *Serratia marcescens*. Similarly, the highest inhibitions of three examined pathogenic fungi and the parasite *Trypanosoma cruzi* were accomplished by the four isolates 3, 4, 10 and 17 (**Table 2** and **Fig. 1**).

### 3.3 Overall antioxidant capacities

The measurement of FRAP activity showed that isolates 3, 10 and 17 had the top FRAP capacity (145, 130 and 120 µmol Trolox/g cell weight, respectively) (**Table 3**), unlike the other isolates that had lower FRAP activity (less than 100 µmol/g). Similarly, DPPH radical scavenging activity (%) of isolates 3, 10 and 17 was remarkably higher than activity exhibited by other studied isolates. As regard to SOS activity, we observed that isolate 17 had the highest activity (200%), followed by the isolate 10 (125%), and then the isolates 1 and 3 (121% and 186%, respectively). Screening the xanthine oxidase inhibitory activity of all isolates demonstrated that the utmost inhibition was performed by the three isolates 3, 10 and 17 (220%, 340% and 239%, respectively).

### 3.4 Cytotoxicity and hemolytic inhibition

Most studied extracts of actinomycetes exerted a cytotoxic effect on cancer cell lines; moreover, the extracts of the three isolates 3, 10 and 17 possessed notably the highest anticancer effectiveness (172%, 282% and 184%, respectively) (**Fig. 2A**). Of the examined samples, the extracts of isolate 3 (92.1%), isolate 10 (112%) and isolate 17 (106%) had the most inhibitory effect on hemolysis (**Fig. 2B**).

### 3.5 Phenolic metabolites

The extracts of isolate 17, 15 and 12 had the highest contents of the total flavonoids, total phenols and tocopherols as shown in **Fig. 3**. Among the screened extracts of isolates, the best phenolic pigment production in terms of concentration and yield was observed for extracts from isolate 12, 10 and 17, respectively, as shown in **Fig. 3**.

### 3.6 Anti-hemolytic and anti-lipid peroxidation activity

According to the results, the extracts of isolate 3 (104%), isolate 10 (128%) and isolate 17 (119%) exhibited the highest anti-hemolytic activity (**Fig. 2C**). In addition, anti-lipid peroxidation properties have been demonstrated in most extracts of studied isolates. Furthermore, extracts of isolates 3, 10 and 17 inhibited the highest levels of lipid peroxidation by 184%, 190%, and 218%, respectively (**Fig. 2D**).

### 3.7 Detailed screening of the selected isolates

According to our data, the increased levels of biological activities and valuable secondary metabolites exerted by three specific isolates (isolate 3, isolate 10 and isolate 17) and their extracts highlighted the significance of their detailed investigations, particularly in terms of phenolic acids and flavonoid compounds, anticancer, anti-cholesterol, and anti-inflammatory.

***The content of phenolic acids and flavonoid compounds***

We observed that the isolate 17 had relatively the highest contents of caffeic acid (0.31 ± 0.05 $mg.kg^{-1}$), ferulic acid (8.86 ± 1.3 $mg.kg^{-1}$), *p*-coumaric acid (4.43 ± 0.7 $mg.kg^{-1}$), resorcinol (0.045 ± 0.007 $mg.kg^{-1}$) and simultaneously the highest levels of quercetin (3.78 ± 0.57 $mg.kg^{-1}$), quercetrin (0.42 ± 0.06 $mg.kg^{-1}$), isoquercetrin (0.589 ± 0.09 $mg.kg^{-1}$), rutin (2.65 ± 0.40 $mg.kg^{-1}$), ellagic acid (1.10 ± 0.16 $mg.kg^{-1}$), genistein (0.013 ± 0.002 $mg.kg^{-1}$), daidzein (0.008 ± 0.001 $mg.kg^{-1}$), *O*-hydroxydaidzein (0.026 ± 0.004 $mg.kg^{-1}$) as shown in **Table 5.**

***Anticancer properties***

Our investigation firstly demonstrated that actinobacterial isolate 3, isolate 10 and isolate 17 killed the human leukemic cell lines HL-60, K562, and THP-1 (**Table 6** and **Fig. 4**). The susceptibilities to kill the cancerous cells were variable depending on the isolate and the dose of the test samples. All types of examined human leukemic cells were dramatically more susceptible to isolate 17 than the other two isolates, showing lower cell death and higher cell viability as illustrated in **Table 6** and **Fig. 4**. All types of cancer cells are completely killed using 800 µL of isolate 17.

***Anti-inflammatory and anti-cholesterol properties***

The higher percentage of inhibition of both cyclooxygenase-2 and lipoxygenase was detected in the extract of isolate 17 (**Fig. 5**) indicating higher potency of anti-inflammatory effect. Similarly, the percentage of inhibition of both amylase and lipase. The results of α-amylase and lipase inhibitory activities of the extracts are presented in **Fig. 5**. The extract of isolate 17 had the higher inhibitory activities against α-amylase and lipase with $IC_{50}$ values of 2.15 ± 0.25 µg/mL and 1.14 ± 0.14 µg/mL.

### 3.8 Molecular characterization of the highest potent isolate

Based on the data analysis of all pharmacological activities and bioactive metabolites generated by the most potent actinobacterial isolates, the isolate 17 and its related extract had the largest productivity of large set of phenolic acids and flavonoid compounds (**Table 5**), in addition to the highest anticancer (**Fig. 4**), anti-inflammatory (**Fig. 5**), anti-cholesterol activity (**Fig. 5**). Therefore, the actinobacterial isolate 17 was subjected to molecular identification by 16S rRNA gene sequencing, which suggested that the isolate is *Plantactinospora endophytica* JTT05, and the phylogenetic tree was generated to reveal the evolutionary relationships and the structural similarities between the isolate 17 and the previously identified actinomycetes species in the GenBank (**Fig. 6**) where this isolate formed a distinct branch.

### 3.9 *Plantactinospora endophytica* JTT05 improved the growth, physiological, and biological activity of *Eruca sativa*

The application of *Plantactinospora endophytica* JTT05 as a biopriming agent significantly enhances the growth and physiological performance of *Eruca sativa*, as evidenced by improvements in key agronomic and biochemical parameters, as shown in **Table 7**. The bacteria-treated plants exhibited a 3.14% increase in fresh weight (FW) and

a notable 24.75% increase in dry weight (DW), indicating enhanced biomass accumulation. Photosynthetic efficiency improved by 13.46%, with photosynthesis rates rising from 16.2 to 18.4 μmol/m²/s, likely due to increased chlorophyll content, as chlorophyll *a*, chlorophyll *b*, and total chlorophyll levels increased by 9.20%, 10.71%, and 14.31%, respectively. These physiological enhancements suggest that *Plantactinospora endophytica* JTT05 promotes photosynthetic capacity, contributing to robust plant growth. Additionally, the biochemical activity was boosted, with phenolics increasing by 12.3%, flavonoids by 0.73%, total antioxidants by 4.80%, and total glucosinolates by 22.21% (**Table 7**). These increases reflect enhanced antioxidant defenses and secondary metabolite production, which improve plant resilience against environmental stresses. The consistent percentage increases across these parameters highlight the potential of *Plantactinospora endophytica* JTT05 to improve basil growth, physiology, and biochemical activity, supporting its role in sustainable agriculture.

## 4. Discussion

***Actinobacteria as promising sources of bioactive metabolites and pharmacological activities***

This study reports evidence in terms of the potential use of actinomycetes and extracellular metabolites derived thereof. These microbial natural products serve as a fundamental reservoir of several bioactive compounds, including antibiotics. Among microbial sources, actinomycetes represent a particularly important group known for their prolific antibiotic production (Pagmadulam et al. 2020). The screening of seventeen isolated actinomycetes revealed the antimicrobial and antiprotozoal activity of these isolates; moreover, isolate 3, isolate 10 and isolate 17 exhibited the highest activity against various strains of Gram-positive and -negative bacterial strains and infectious fungi. This is in agreement with the ability of actinobacteria to produce broad-spectrum antibiotics (Delgado-Garduño et al. 2025). The microbial secondary metabolites are generally characterized by low molecular weight and exhibit various biological activities (Bano et al. 2023).

We observed that isolate 3, isolate 10 and isolate 17, which exhibited stronger antimicrobial activity than other isolates, also possessed the higher overall antioxidant capacity in terms of DPPH, FRAP, SOS, and XO inhibition than the other isolates. This finding is in accordance with the literature that reported that some microbial metabolites and extracts with antioxidant activity also exhibited effective antimicrobial characteristics. Thus, the future applications of these isolates are substantially considered (Arslan et al. 2025; Dholakiya et al. 2017). The cytotoxicity assay of extracts from all actinobacterial isolates demonstrated that the notable inhibition of the proliferation of cancer cell lines. In addition, inhibiting the hemolysis of erythrocytes induced by oxidant induction is an important feature of the antioxidants (Mohammadipanah and Momenilandi 2018).

In the human body, lipid peroxidation indicates the generation of peroxides, these toxic chemicals are correlated to the aging process. In this study, the anti-lipid peroxidation activity of extracts from isolated actinomycetes was calculated revealing the efficiency of isolate 3, isolate 10 and 17. The flavonoid and phenolic compounds are known as valuable plant secondary metabolites for antioxidation, anti-inflammation, and anticancer impact. These useful compounds are reported to be present in actinobacteria. Therefore, the contents of flavonoids and phenols present in extracts of actinobacterial isolates were characterized in this study.

Our results showed that the highest content of total flavonoid, total phenols, and tocopherols was obtained from isolate 17 and isolate 15. Actinomycetes-derived pigments are vital beyond many industries, particularly the food, cosmetic, textile, paint, coating, and packaging industries (Díez et al. 2025). Furthermore, the results show a strong connection between total phenolic content and anti-hemolytic assay (Karim et al. 2020). A literature survey revealed that bacteria-derived metabolites or extracts with antioxidant activity also exhibit other bioactive properties, such as antimicrobial, antiviral, anti-inflammatory, and anticancer (Arslan et al. 2025). The efficacy and yield of bioactive compounds and biological functions are principal criteria to classify and distinguish the potent actinomycetes.

***The most potent actinobacterial isolates***

Screening the seventeen isolated actinomycetes for their capacity to exert pharmacological activities and to produce bioactive metabolites in this research revealed that only three actinomycetes (isolate 3, isolate 10 and isolate 17) were the most potent strains. Therefore, the further analysis of the bioproductivity and potential biological activity of the three potent isolates was performed to recognize the most powerful strain.

Data evaluating the contribution of individual phenolic acids and flavonoid compounds to the antioxidant activity have been scarcely published, and the contribution of pure phenolic compounds has been studied only (Orsavová et al. 2019). Actinomycetes possess a strong ability to demolish human cancer cells (Simpson et al. 1988). This finding is in agreement with our analysis elucidating that the isolate 17 had the highest target destruction of human leukemic cells. Exposure to 800 µL of the isolate 17 achieved zero% cell viability of all tested tumor cells.

Cyclooxygenase-2 activity is a rate-limiting enzyme for the synthesis of prostaglandins, involved in stimulating cell division of tumor and production of reactive oxygen species in leukemia cells; moreover, lipoxygenase metabolizes arachidonic acid to hydroxyl eicosatetraenoic acids and leukotrienes, which stimulate tumor cell division and suppress apoptosis (Almuhayawi, Mohamed, et al. 2021). Therefore, we studied the inhibitory influence of actinobacterial extracts on cyclooxygenase-2 and lipoxygenase activities (**Figs. 5A** and **5B**). The abnormal accumulation of fats in the human body indicates obesity, which accelerates the development of several

diseases, e.g., diabetes mellitus. It was confirmed that inhibition of lipase decreases the absorption of dietary triacylglycerol and fat accumulation in the body, leading to a low risk of obesity (Anyanwu et al. 2019). Furthermore, the inhibition of α-amylase is used as a strategy to manage the blood glucose levels and reduce the obesity (Anyanwu et al. 2019). Therefore, the inhibitory impact of extracts obtained from actinomycetes on the activity of α-amylase and lipase was investigated, and the extract of isolate 17 was promising (**Figs. 5A** and **5B**). By combining and comparing all data obtained from all tested isolates and extracts, the isolate 17 was the most potent actinobacterium showing the highest biological activity and the highest productivity of metabolites and enzymes, therefore this former isolate was chosen for the molecular identification to the species level using the 16S rRNA gene sequencing, which is widely used as an indispensable gene marker in the bacterial taxonomic analysis (Oberhofer et al. 2019). The isolate 17 was identified as *Plantactinospora endophytica* JTT05. We have also constructed the phylogenetic tree to compare the evolutionary relationships among different species within the phylum Actinobacteria. *Plantactinospora endophytica* JTT05 is closely related to *Plantactinospora endophytica* NR 108607, *Plantactinospora* sp. MN818650 and *Plantactinospora sonchi* NR145927, respectively. A previous study confirmed our phylogenetic analysis, as Zhu et al. (Zhu et al. 2012) showed the close relationship between *P. endophytica* and *Micromonosporus* members. *P. endophytica* was first isolated from the leaves of the medicinal plant *Camptotheca acuminata*, which is a natural source of camptothecin and its derivatives, having several pharmacological properties, such as anticancer, antiviral, antifungal, and anti-obesity (Fan et al. 2022), thereby reexplaining the promising pharmacological results of this isolate in our study, highlighting the potential to exploit it in association with cultivable plants such as arugula (*Eruca sativa*).

***Structure–Activity Relationship (SAR) of Studied Metabolites***

The biological activities of the actinomycete isolates are closely linked are closely linked to their metabolite profiles particularly phenolic acids and flavonoids. *P. endophytica* JTT05 which exhibited the strongest antioxidant, antimicrobial, anticancer, anti-inflammatory, and anti-cholesterol activities, contained the highest levels of structurally diverse phenolic acids (caffeic, ferulic, and *p*-coumaric acids) and flavonoids (quercetin, rutin, quercetrin, isoquercetrin, and ellagic acid). The conjugated aromatic structures and hydroxyl groups in these compounds enhance electron-donating and radical-scavenging, metal-chelating, and lipid peroxidation inhibitory activities, explaining the high antioxidant and anti-hemolytic effects. Additionally, flavonoids and phenolic acids modulate signaling pathways and interact with enzymes such as cyclooxygenase-2 and lipoxygenase, contributing to potent anti-inflammatory, anticancer, and antimicrobial activities. In contrast, Isolates number 3 and 10, though rich in phenolics and flavonoids, had lower concentrations than *P. endophytica* JTT05, corresponding to slightly

weaker antioxidant and antimicrobial activities. This indicates a direct relationship between the degree of hydroxylation, aromatic conjugation, and total phenolic content of the metabolites and their biological potency. Overall, the results reveal that structural features—such as the number and position of hydroxyl groups, conjugated aromatic systems, and glycosylation patterns—play crucial roles in determining the antioxidant, antimicrobial, and cytotoxic activities of the actinomycete-derived metabolites. These findings provide an essential foundation for future structure–activity relationship (SAR) and metabolic engineering studies aimed at optimizing the bioactivity of metabolites, particularly those produced by *P. endophytica* JTT05.

**Plantactinospora endophytica** ***could improve growth and photosynthesis of*** **E. sativa**

Our results confirmed that *E. sativa* plants treated with *Plantactinospora endophytica* JTT05 showed improved growth and yield (fresh and dry weights) compared to the untreated group of the plant. This finding is in line with earlier studies emphasizing the stimulatory impact of PGPB on associated plants (Hassan et al. 2024; Santos-Medellín et al. 2021), as the plant interaction with the environment is substantially affected by functions of microbial secondary metabolites (Bano et al. 2023). The interpretation of the positive influence of this actinobacterium can be attributed to its ability to stimulate the production of growth hormones and mineralize several nutrients for the plants (Hassan et al. 2024; Santos-Medellín et al. 2021). PGPB may supply the associated plant with compounds synthesized only by the bacterium, and these compounds may have nutritional benefit or antagonistic effect against plant pathogens (Beneduzi et al. 2012).

Previous reports demonstrated that $CO_2$ levels can be stabilized by PGPB, yielding high photosystem II efficiency and increasing chlorophyll content. Consequently, the rate of photosynthesis and soluble sugar production are elevated. In addition, the the production of primary (unsaturated fatty acids and essential oils), and secondary bioactive metabolites (phenolics, flavonoids, and antioxidants) is accelerated in treated plants (Hassan et al. 2024; Santos-Medellín et al. 2021). These later findings are consistent with our results obtained from treating *E. sativa* plants with *Plantactinospora endophytica* JTT05.

## 5. Conclusion

Endophytic actinomycetes represent unique producers of bioactive compounds with diverse health-promoting potentials. In this work, seventeen isolates obtained from the leaves of *Tribulus terrestris* exhibited multiple biological activities, including strong antioxidant, antimicrobial, antiprotozoal, and cytotoxic effects. These activities were associated with their ability to scavenge free radicals, inhibit lipid peroxidation, and protect against hemolysis. The isolates also contained high levels of phenolic acids, flavonoids, tocopherols, and various

pigments, indicating their rich secondary metabolite diversity and potential for pharmaceutical and biotechnological applications. Among the studied isolates, *Plantactinospora endophytica* JTT05 showed the most potent and broad-spectrum biological activities, including antioxidant, anticancer, anti-inflammatory, and anti-cholesterol effects. The presence of multiple bioactive mechanisms suggests a complex mixture of metabolites contributing to its activity. Inoculation with *P. endophytica* significantly enhanced the growth, photosynthetic rate, and accumulation of bioactive compounds (phenols, flavonoids, antioxidants, and glucosinolates) in *Eruca sativa*, establishing it as a promising plant growth-promoting bacterium (PGPB) for sustainable agriculture. Future studies will focus on improving metabolite extraction and characterization by applying advanced techniques such as supercritical fluid or microwave-assisted extraction and LC–MS or metabolomics-based analyses. Investigations of *P. endophytica* JTT05 using NMR, MS, and computational modeling will clarify the structural basis of its bioactivities. Additionally, expanding sampling to diverse plant species and ecological environments will facilitate the discovery of novel actinomycetes with unique metabolic and pharmacological potentials, advancing their applications in medicine, biotechnology, and sustainable agriculture.

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**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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• **Tables**

**Table 1** Antibacterial activity of seventeen actinobacterial isolates. Mean ± SD inhibition zone against test organisms in mm. The superscript letters (a, b, c, d) denote significant differences between means at $p < 0.05$

| Isolate No. | Gram-positive strains | | | | | Gram-negative strains | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | ***S. saprophyticus*** | ***S. epidermidis*** | ***E. faecalis*** | ***S. salivarius*** | ***B. cereus*** | ***E. coli*** | ***S. typhimurium*** | ***P. aeruginosa*** | ***P. vulgaris*** | ***E. aerogenes*** | ***S. marcescens*** |
| **Isolate 1** | 16.2±3.3$^{a}$ | 19.7±2.16$^{a}$ | 21.7±2.3$^{a}$ | 20.4±2.3$^{a}$ | 29.2±3.1$^{a}$ | 22.5±2.4$^{a}$ | 25.7±23$^{ac}$ | 28.2±4.5$^{a}$ | 24.4±5.1$^{a}$ | 28.2±2.8$^{a}$ | 28.7±3.7$^{a}$ |
| **Isolate 2** | 13.7±2.89$^{a}$ | 13.9±2.92$^{ac}$ | 13.9±1.1$^{b}$ | 14.0±2.9$^{ac}$ | 19.1±4.0$^{b}$ | 14.1±2.9$^{b}$ | 11.8±2.4$^{be}$ | 11.3±2.2$^{b}$ | 18.8±3.9$^{a}$ | 18.91±2.1$^{b}$ | 19.0±3.9$^{b}$ |
| **Isolate 3** | 16.8±4.11$^{a}$ | 27.3±2.59$^{bd}$ | 23.6±4.2$^{a}$ | 33.3±2.9$^{b}$ | 41.2±3.1$^{c}$ | 27.3±3.7$^{ac}$ | 34.5±6.3$^{c}$ | 30.9±2.6$^{a}$ | 29.9±3.2$^{abc}$ | 34.6±3.9$^{ac}$ | 38.7±1.6$^{c}$ |
| **Isolate 4** | 18.6±3.92$^{a}$ | 22.5±0.53$^{ab}$ | 24.9±5.1$^{a}$ | 24.6±3.1$^{ab}$ | 38.5±3.9$^{ac}$ | 31.9±1.5$^{c}$ | 42.3±4.2$^{d}$ | 19.5±2.5$^{ab}$ | 28.0±2.3$^{ab}$ | 32.3±3.2$^{ac}$ | 33.7±4.7$^{ac}$ |
| **Isolate 5** | 14.3±2.98$^{a}$ | 12.6±2.6$^{c}$ | 11.05±2.35$^{b}$ | 9.1±1.9$^{c}$ | 15.6±3.1$^{b}$ | 13.8±2.8$^{b}$ | 5.81±1.4$^{e}$ | 4.16±1.1$^{c}$ | 18.3±1.9$^{a}$ | 16.1±3.4$^{b}$ | 13.7±2.9$^{b}$ |
| **Isolate 6** | 18.4±3.82$^{a}$ | 19.8±4.12$^{ab}$ | 20.6±4.27$^{a}$ | 21.4±4.4$^{ab}$ | 29.5±3.3$^{a}$ | 21.9±4.1$^{a}$ | 20.9±4.2$^{a}$ | 21.5±4.3$^{ab}$ | 26.0±5.4$^{ab}$ | 27.5±5.7$^{ac}$ | 28.6±5.9$^{ac}$ |
| **Isolate 7** | 18.1±3.76$^{a}$ | 21.5±4.47$^{ab}$ | 23.9±5.0$^{a}$ | 26.5±5.4$^{ab}$ | 35.1±2.7$^{ac}$ | 25.1±4.1$^{ac}$ | 25.0±3.3$^{ac}$ | 23.7±2.5$^{a}$ | 26.9±5.6$^{ab}$ | 30.8±6.4$^{ac}$ | 34.3±7.0$^{ac}$ |
| **Isolate 8** | 23.3±2.13$^{ab}$ | 21.2±1.82$^{ab}$ | 20.3±4.4$^{a}$ | 14.8±3.3$^{ac}$ | 24.2±4.8$^{ab}$ | 20.8±4.2$^{a}$ | 9.7±2.2$^{e}$ | 26.9±4.5$^{a}$ | 30.3±5.0$^{abc}$ | 28.2±2.9$^{a}$ | 23.9±5.2$^{a}$ |
| **Isolate 9** | 20.6±4.37$^{ab}$ | 21.0±4.4$^{ab}$ | 21.7±5.5$^{a}$ | 22.1±4.6$^{ab}$ | 26.6±5.8$^{ab}$ | 17.1±3.9$^{ab}$ | 20.9±4.2$^{a}$ | 28.2±3.9$^{a}$ | 28.3±5.9$^{abc}$ | 29.0±6.1$^{ac}$ | 29.8±6.2$^{ac}$ |
| **Isolate 10** | 23.6±1.312$^{ab}$ | 32.9±2.81$^{d}$ | 26.3±4.43$^{a}$ | 31.9±3.9$^{b}$ | 42.7±3.0$^{c}$ | 30.8±1.9$^{c}$ | 31.6±2.2$^{ac}$ | 34.6±5.1$^{a}$ | 38.1±3.4$^{bc}$ | 40.3±5.4$^{c}$ | 39.6±5.8$^{c}$ |
| **Isolate 11** | 17.3±3.6$^{a}$ | 10.7±2.24$^{c}$ | 7.3±4.3$^{b}$ | 15.6±3.3$^{ac}$ | 24.6±5.4$^{ab}$ | 20.6±2.9$^{a}$ | 19.9±4.0$^{a}$ | 25.6±5.1$^{a}$ | 19.0±3.9$^{a}$ | 12.3±4.4$^{b}$ | 15.6±4.5$^{b}$ |
| **Isolate 12** | 17.8±3.65$^{a}$ | 20.6±4.28$^{ab}$ | 22.1±4.6$^{a}$ | 23.9±4.9$^{ab}$ | 26.4±6.1$^{ab}$ | 14.9±4.3$^{ab}$ | 25.1±5.0$^{ac}$ | 15.9±3.9$^{b}$ | 26.1±5.3$^{a}$ | 29.0±6.01$^{ac}$ | 31.3±6.5$^{ac}$ |
| **Isolate 13** | 19.6±4.0$^{ab}$ | 20.3±4.23$^{ab}$ | 19.9±4.2$^{a}$ | 19.68±4.1$^{a}$ | 30.8±6.2$^{a}$ | 25.7±5.1$^{ac}$ | 18.4±3.8$^{ab}$ | 22.6±4.2$^{a}$ | 27.2±2.7$^{a}$ | 27.4±5.7$^{a}$ | 26.9±5.6$^{a}$ |
| **Isolate 14** | 13.7±2.79$^{a}$ | 21.2±1.91$^{ab}$ | 28.8±2.7$^{a}$ | 29.5±6.2$^{b}$ | 37.3±2.0$^{ac}$ | 25.3±4.3$^{ac}$ | 33.9±4.9$^{cd}$ | 17.9±2.4$^{ab}$ | 23.7±2.2$^{a}$ | 34.0±2.9$^{ac}$ | 39.7±4.3$^{c}$ |
| **Isolate 15** | 15.8±3.18$^{a}$ | 20.1±4.2$^{ab}$ | 22.2±4.6$^{a}$ | 25.1±5.2$^{ab}$ | 35.9±3.1$^{ac}$ | 27.6±2.9$^{ac}$ | 28.4±5.7$^{ac}$ | 22.6±5.9$^{ab}$ | 24.4±5.0$^{a}$ | 28.8±5.9$^{a}$ | 32.2±6.6$^{ac}$ |
| **Isolate 16** | 15.2±3.1$^{a}$ | 15.9±3.3$^{ac}$ | 14.4±1.1$^{b}$ | 14.4±3.0$^{ab}$ | 24.0±4.8$^{ab}$ | 20.9±4.2$^{a}$ | 13.2±2.9$^{abe}$ | 23.3±4.6$^{ab}$ | 21.2±4.3$^{a}$ | 21.1±4.4$^{a}$ | 20.0±4.2$^{b}$ |
| **Isolate 17** | 29.2±2.25$^{b}$ | 30.3±2.05$^{d}$ | 30.9±3.1$^{a}$ | 33.3±3.4$^{b}$ | 43.7±1.7$^{c}$ | 31.0±2.4$^{c}$ | 37.1±3.9$^{cd}$ | 30.9±2.3$^{a}$ | 40.1±4.5$^{c}$ | 41.6±5.2$^{c}$ | 43.6±3.7$^{c}$ |
| ***p*-Value** | 0.0002 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |

**Table 2** The antifungal and antiprotozoal activity of the actinobacterial isolates. Mean ± SD inhibition zone against test organisms in mm. The superscript letters (a, b, ) denote significant differences between means at $p < 0.05$

| Isolate No. | *Candida albicans* | *Candida glabrata* | *Aspergillus flavus* |
|---|---|---|---|
| **Isolate 1** | 5.7±0.5$^{a}$ | 8.33±0.7$^{ac}$ | 9.63±1.2$^{a}$ |
| **Isolate 2** | 3.1±0.6$^{b}$ | 3.57±0.7$^{bc}$ | 5.5±1.1$^{bc}$ |
| **Isolate 3** | 7.3±0.8$^{ac}$ | 10.1±0.7$^{a}$ | 11.1±0.8$^{ad}$ |
| **Isolate 4** | 8.7±0.9$^{c}$ | 9.84±0.8$^{a}$ | 8.7±1.3$^{a}$ |
| **Isolate 5** | 2.3±0.5$^{b}$ | 1.54±0.4$^{c}$ | 4.12±0.9$^{c}$ |
| **Isolate 6** | 5.1±0.5$^{a}$ | 6.56±0.3$^{d}$ | 8.7±0.8$^{a}$ |
| **Isolate 7** | 5.92±0.5$^{a}$ | 7.53±0.6$^{d}$ | 9.3±1.0$^{a}$ |
| **Isolate 8** | 3.6±0.7$^{b}$ | 5.68±0.4$^{d}$ | 10.5±1.4$^{ad}$ |
| **Isolate 9** | 4.48±0.9$^{ab}$ | 7.59±1.2$^{ad}$ | 10.3±1.1$^{ad}$ |
| **Isolate 10** | 7.37±1.3$^{ac}$ | 10.2±1.3$^{a}$ | 13.34±1.4$^{d}$ |
| **Isolate 11** | 4.79±1.0$^{ab}$ | 7.05±1.4$^{d}$ | 8.17±1.6$^{ab}$ |
| **Isolate 12** | 4.73±1.04$^{ab}$ | 6.35±1.3$^{d}$ | 7.69±1.7$^{ab}$ |
| **Isolate 13** | 5.2±1.05$^{ab}$ | 6.35±0.9$^{d}$ | 9.11±1.4$^{a}$ |
| **Isolate 14** | 6.9±0.6$^{ac}$ | 8.01±1.0$^{ad}$ | 7.61±0.3$^{ab}$ |
| **Isolate 15** | 6.6±1.4$^{ac}$ | 7.9±0.9$^{ad}$ | 8.6±1.2$^{ab}$ |
| **Isolate 16** | 4.03±0.8$^{b}$ | 5.64±1.1$^{b}$ | 8.15±1.6$^{ab}$ |
| **Isolate 17** | 8.04±1.1$^{ac}$ | 10.5±1.2$^{ad}$ | 13.1±1.2$^{d}$ |
| *p*-Value | <0.0001 | <0.0001 | <0.0001 |

**Table 3** The overall antioxidant capacity of seventeen actinobacteria isolates in terms of FRAP, DPPH % and superoxide scavenging (SOS)

| Isolate No. | FRAP μmol Trolox/g cell weight | DPPH (%) | SOS (%) | XO inhibition (%) |
|---|---|---|---|---|
| **Isolate 1** | 69.9±4.8 | 113±7.7 | 121±8.4 | 109±7.6 |
| **Isolate 2** | 36.3±2.5 | 57.1±3.9 | 113±7.7 | 127±8.7 |
| **Isolate 3** | 145±9.7 | 196±13.6 | 186±13.2 | 220±15.5 |
| **Isolate 4** | 54.5±3.7 | 43.3±4.3 | 76.6±8.1 | 95.2±9.2 |
| **Isolate 5** | 40.1±2.7 | 45.4±3.1 | 25.6±1.7 | 39.9±2.7 |
| **Isolate 6** | 54.1±3.7 | 79.1±5.4 | 113±7.7 | 131±8.9 |
| **Isolate 7** | 48.1±3.3 | 99.3±6.8 | 22.5±1.6 | 40.6±2.8 |
| **Isolate 8** | 90.8±6.3 | 53.2±3.7 | 21.0±1.5 | 50.9±3.6 |
| **Isolate 9** | 62.8±4.3 | 117±8.1 | 113±7.8 | 134±9.2 |
| **Isolate 10** | 130±9.1 | 264±18.2 | 125±8.6 | 340±23.2 |
| **Isolate 11** | 62.9±4.4 | 39.1±2.7 | 117±7.9 | 134±9.2 |
| **Isolate 12** | 52.4±3.6 | 101±6.9 | 87.1±5.9 | 176±12.0 |
| **Isolate 13** | 76.9±5.4 | 86.0±5.9 | 47.7±3.2 | 67.4±4.5 |
| **Isolate 14** | 57.9±4.1 | 106±7.3 | 60.3±4.1 | 194±13.2 |
| **Isolate 15** | 77.1±5.4 | 100±6.9 | 104±6.9 | 120±7.9 |
| **Isolate 16** | 34.6±2.5 | 39.6±2.7 | 27.4±1.8 | 42.7±2.8 |
| **Isolate 17** | 120±8.4 | 245±16.9 | 200±13.6 | 239±16.3 |

**Table 4** The content of several phenolic acids and flavonoid compounds (mg.kg$^{-1}$) in the extracts obtained from the three potent isolates (isolate 3, isolate 10, isolate 17). The superscript letters (a, b, ) denote significant differences between means at $p < 0.05$

| | Parameter | Isolate 3 | Isolate 10 | Isolate 17 | *p* Value |
|---|---|---|---|---|---|
| **Phenolic acids** | Caffeic acid | 0.23 ± 0.06 $^{a}$ | 0.21 ± 0.01 $^{a}$ | 0.31 ± 0.05 $^{a}$ | 0.0711 |
| | Ferulic acid | 7.27 ± 2.4 $^{a}$ | 2.27 ± 2.3 $^{a}$ | 8.86 ± 1.3 $^{a}$ | 0.5823 |
| | Protocatechuic acid | 3.11 ± 1.0 $^{a}$ | 6.14 ± 1.4 $^{b}$ | 3.72 ± 0.6 $^{ab}$ | 0.0263* |
| | Catechin | 1.34 ± 0.43 $^{a}$ | 2.01 ± 0.67 $^{a}$ | 1.61 ± 0.24 $^{a}$ | 0.3057 |
| | Galic acid | 35.67 ± 11.53 $^{a}$ | 61.47 ± 7.36 $^{b}$ | 42.71 ± 6.43 $^{ab}$ | 0.0269 |
| | *p*-Coumaric acid | 3.70 ± 1.20 $^{a}$ | 2.76 ± 0.9 $^{a}$ | 4.43 ± 0.7 $^{a}$ | 0.1771 |
| | Resorcinol | 0.002 ± 0.0006 $^{a}$ | 0.028 ± 0.009 $^{b}$ | 0.045 ± 0.007 $^{c}$ | 0.0006 |
| | Chlorogenic acid | 0.227 ± 0.073 $^{a}$ | 0.465 ± 0.11 $^{b}$ | 0.002 ± 0.0006 $^{c}$ | 0.0010 |
| | Syringic acid | 0.002 ± 0.0006 $^{a}$ | 2.58 ± 0.85 $^{b}$ | 0.002 ± 0.0006 $^{a}$ | 0.0010 |
| **Flavonoid compounds** | Quercetin | 3.16 ± 1.02 $^{a}$ | 2.36 ± 0.76 $^{a}$ | 3.78 ± 0.57 $^{a}$ | 0.1770 |
| | Quercetrin | 0.35 ± 0.11 $^{a}$ | 0.26 ± 0.08 $^{a}$ | 0.42 ± 0.06 $^{a}$ | 0.1775 |
| | Luteolin | 0.11 ± 0.033 $^{ab}$ | 0.32 ± 0.21 $^{a}$ | 0.002 ± 0.0006 $^{b}$ | 0.0486* |
| | Apigenin | 0.002 ± 0.0006 $^{a}$ | 0.398 ± 0.09 $^{b}$ | 0.002 ± 0.0006 $^{a}$ | 0.0001** |
| | Isoquercetrin | 0.002 ± 0.0006 $^{a}$ | 0.368 ± 0.12 $^{b}$ | 0.589 ± 0.09 $^{c}$ | 0.0005** |
| | Rutin | 1.41 ± 0.55 $^{a}$ | 1.65 ± 0.53 $^{a}$ | 2.65 ± 0.40 $^{b}$ | 0.0486* |
| | Ellagic acid | 0.88 ± 0.29 $^{a}$ | 0.66 ± 0.21 $^{a}$ | 1.10 ± 0.16 $^{a}$ | 0.1769 |
| | Velutin | 0.87 ± 0.38 $^{a}$ | 0.76 ± 0.25 $^{a}$ | 0.002 ± 0.0006 $^{b}$ | 0.0132* |
| | Naringenin | 0.002 ± 0.0006 $^{a}$ | 0.013 ± 0.004 $^{b}$ | 0.02 ± 0.002 $^{b}$ | 0.0017** |
| | Genistein | 0.002 ± 0.0006 $^{a}$ | 0.008 ± 0.003 $^{b}$ | 0.013 ± 0.002 $^{c}$ | 0.0012** |
| | Daidzein | 0.002 ± 0.0006 $^{a}$ | 0.006 ± 0.004 $^{ab}$ | 0.008 ± 0.001 $^{b}$ | 0.0307* |
| | Fisetin | 0.004 ± 0.0002 $^{a}$ | 0.002 ± 0.0006 $^{a}$ | 0.002 ± 0.0006 $^{a}$ | 0.1183 |
| | *O*-hydroxydaidzein | 0.002 ± 0.0006 $^{a}$ | 0.003 ± 0.001 $^{a}$ | 0.026 ± 0.004 $^{b}$ | <0.0001** |

**Table 5** The antitumor activity of isolate 3, isolate 10 and isolate 17 using three different cell lines

| Tumor cell line | Isolate sample (µL) | Isolate 3 | | Isolate 10 | | Isolate 17 | | *p*-Value |
|---|---|---|---|---|---|---|---|---|
| | | Mean | SD | Mean | SD | Mean | SD | |
| HL-60 | **25** | 96.09 | 9.45 | 96.71 | 5.93 | 86.30 | 8.50 | 0.2849 |
| | **50** | 86.59 | 9.10 | 89.70 | 7.04 | 49.42 | 8.33 | 0.0016** |
| | **100** | 72.39 | 5.16 | 62.83 | 9.67 | 39.18 | 7.24 | 0.0045** |
| | **200** | 56.69 | 6.88 | 36.61 | 8.52 | 11.06 | 2.07 | 0.0004** |
| | **300** | 40.78 | 3.95 | 29.77 | 4.43 | 4.55 | 1.10 | <0.0001** |
| | **400** | 18.66 | 2.30 | 18.83 | 2.63 | 0.41 | 0.16 | <0.0001** |
| | **800** | 6.26 | 0.70 | 2.74 | 0.43 | 0.0 | 0.0 | <0.0001** |
| K-562 | **25** | 100.76 | 7.27 | 98.73 | 10.42 | 75.11 | 5.02 | 0.0129* |
| | **50** | 96.35 | 4.45 | 85.39 | 10.12 | 45.63 | 4.07 | 0.0002** |
| | **100** | 88.04 | 5.94 | 69.87 | 9.04 | 14.96 | 3.17 | <0.0001** |
| | **200** | 76.84 | 9.26 | 27.69 | 2.21 | 1.80 | 0.25 | <0.0001** |
| | **300** | 66.85 | 8.93 | 2.75 | 0.76 | 0.87 | 0.13 | <0.0001** |
| | **400** | 47.19 | 5.25 | 1.94 | 0.57 | 0.50 | 0.0 | <0.0001** |
| | **800** | 33.18 | 2.04 | 0.0 | 0.18 | 0.0 | 0.0 | <0.0001** |
| THP-1 | **25** | 92.65 | 11.29 | 91.29 | 7.65 | 79.90 | 9.29 | 0.2736 |
| | **50** | 87.74 | 9.98 | 81.68 | 6.76 | 56.89 | 9.16 | 0.0110* |
| | **100** | 78.00 | 13.31 | 74.80 | 6.77 | 27.75 | 7.00 | 0.0011** |
| | **200** | 64.01 | 9.12 | 41.82 | 3.25 | 7.08 | 0.22 | <0.0001** |
| | **300** | 46.15 | 2.66 | 20.26 | 1.99 | 1.19 | 0.07 | <0.0001** |
| | **400** | 32.39 | 1.26 | 16.29 | 0.72 | 0.88 | 0.20 | <0.0001** |
| | **800** | 25.95 | 1.26 | 2.64 | 0.00 | 0.0 | 0.0 | <0.0001** |

** $p < 0.01$, * $p < 0.05$

**Table 6** The effects of *Plantactinospora endophytica* JTT05 biopriming on *Eruca sativa* compared to a control, measuring fresh weight (FW), dry weight (DW), photosynthesis rate, chlorophyll *a*, *b*, and total chlorophyll, phenolics, flavonoids, total antioxidants, and glucosinolates. Data are mean ± SE, with asterisks (*) indicating significant differences ($p < 0.05$).

| Parameter | Control Mean ± SE | Bacteria Mean ± SE |
|---|---|---|
| FW (g/plant) | 2.73 ± 0.10 | 3.97 ± 0.51* |
| DW (g) | 0.69 ± 0.02 | 0.86 ± 0.06* |
| Photosynthesis (µmol/m²/s) | 13.2 ± 0.35 | 17.4 ± 0.88* |
| Chlorophyll *a* (mg/g) | 2.41 ± 0.15 | 3.03 ± 0.29* |
| Chlorophyll *b* (mg/g) | 0.79 ± 0.05 | 0.87 ± 0.09 |
| Total chlorophyll (mg/g) | 3.21 ± 0.20 | 3.96 ± 0.36* |
| Phenolics (mg/g) | 8.18 ± 1.21 | 9.86 ± 1.49* |
| Flavonoids (mg/g) | 1.51 ± 0.04 | 1.42 ± 0.17 |
| Total antioxidants (mg/g) | 13.4 ± 1.97 | 16.8 ± 1.96* |
| Total glucosinolates (mg/g) | 3.06 ± 0.38 | 4.47 ± 0.46* |

• **Figures**

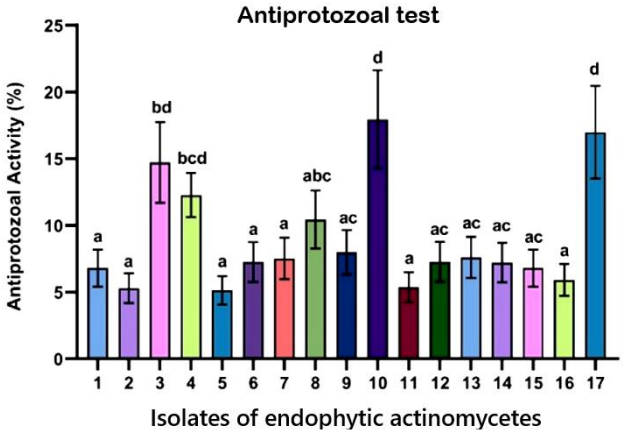


**Fig. 1** The antiprotozoal activity of extracts from the actinobacterial isolates against *Trypanosoma cruzi* ($IC_{50}$ values for in µg/mL). Data are represented by the mean of three replicates ± standard error. The small letters (a, b, c, d) above bars denote significant differences between means at $p < 0.05$

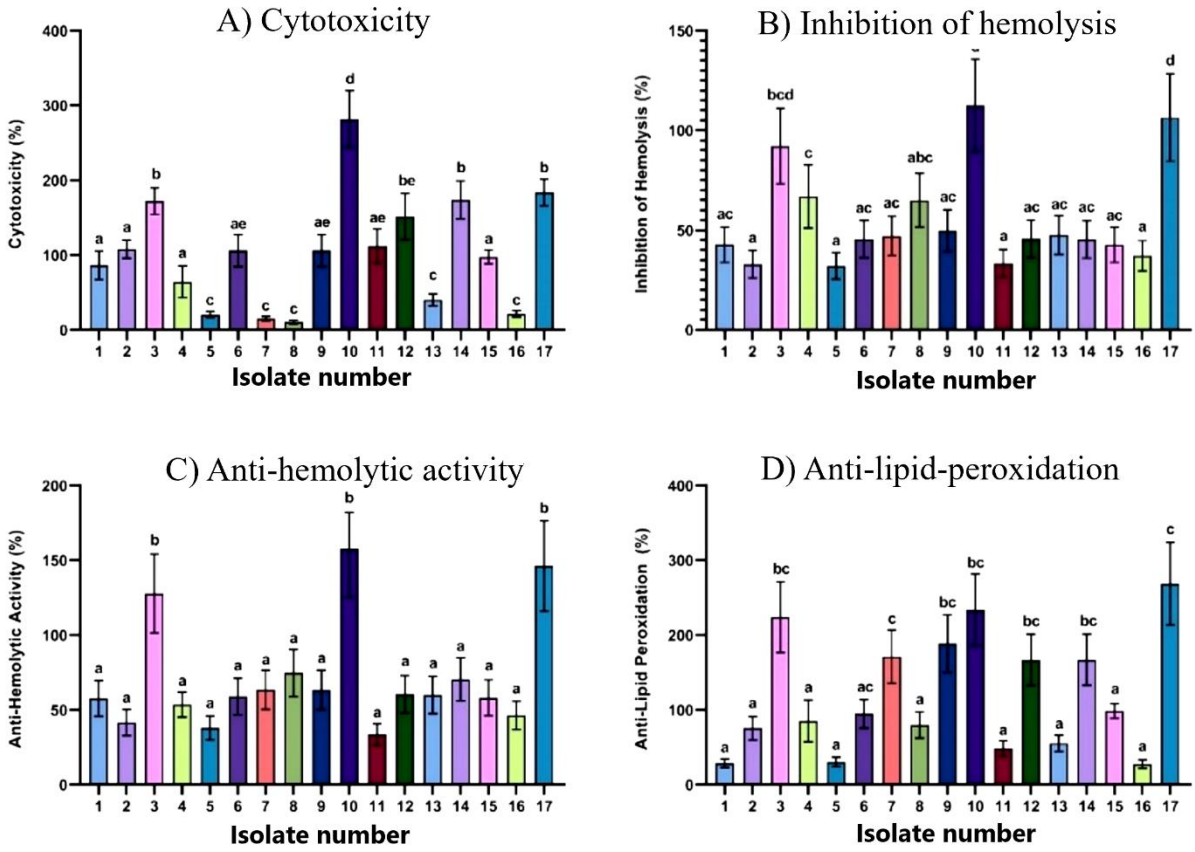


**Fig. 2** Study of selected bioactivity of all extracts of isolated actinomycetes. (**A**) Effect of extracts on cell line HL-60 using MTT assay. The cCells treated with 20 µg/mL of the extracts; (**B**) Inhibition of hemolysis (%) induced by 2.5 mM ferrous sulfate with extracts and glutathione as a positive control at the concentration of 100 µg/mL; (**C**) Anti-hemolytic activity (%) of all studied extracts; (**D**) Anti-lipid-peroxidation activities of extracts from the isolates. Values are presented as mean ± SD

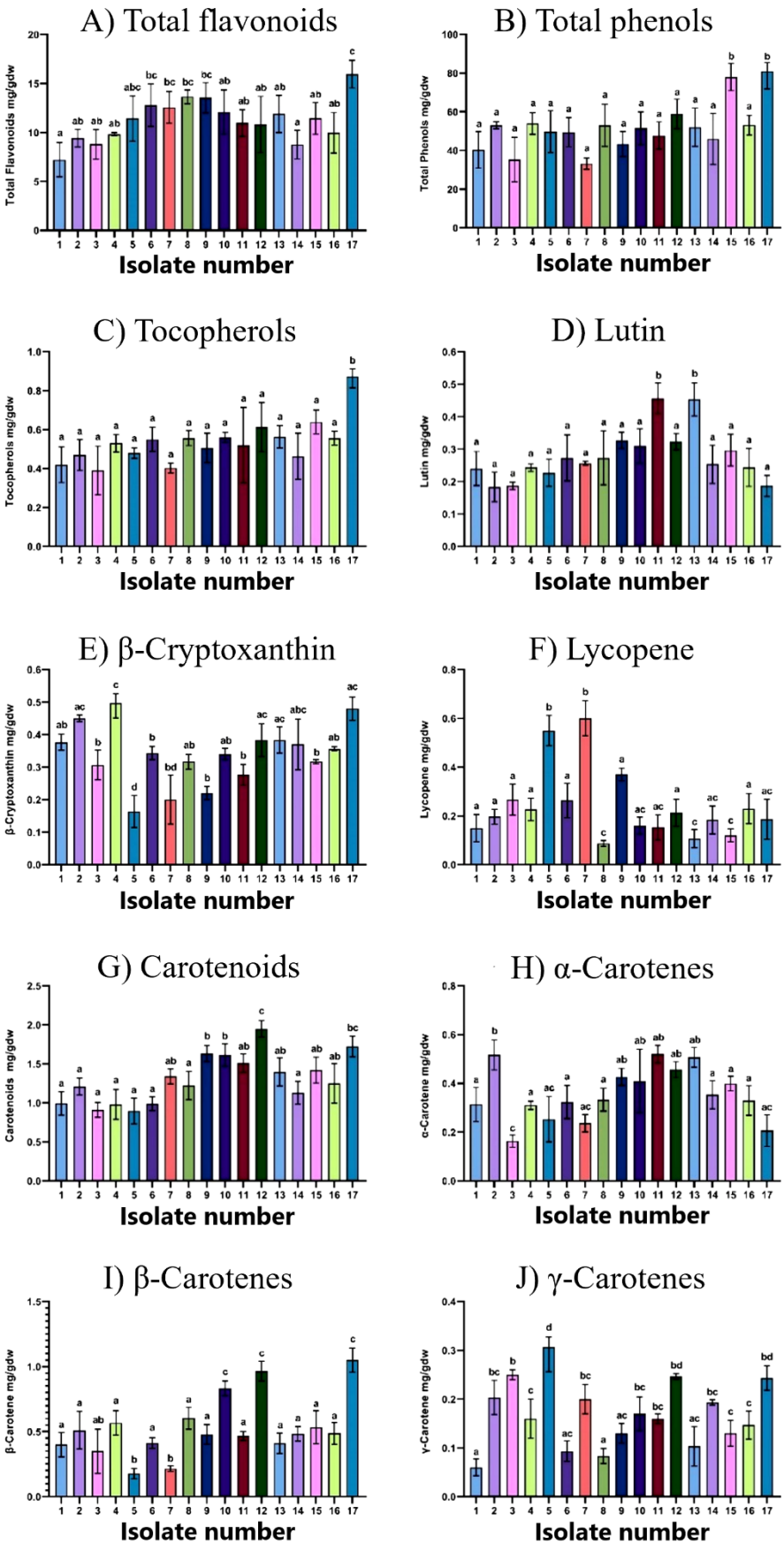
A) Total flavonoids
Total Flavonoids mg/gdw
Isolate number
B) Total phenols
Total Phenols mg/gdw
Isolate number
C) Tocopherols
Tocopherols mg/gdw
Isolate number
D) Lutin
Lutin mg/gdw
Isolate number
E) β-Cryptoxanthin
β-Cryptoxanthin mg/gdw
Isolate number
F) Lycopene
Lycopene mg/gdw
Isolate number
G) Carotenoids
Carotenoids mg/gdw
Isolate number
H) α-Carotenes
α-Carotene mg/gdw
Isolate number
I) β-Carotenes
β-Carotene mg/gdw
Isolate number
J) γ-Carotenes
γ-Carotene mg/gdw
Isolate number

**Fig. 3** The secondary metabolites of the seventeen actinobacterial isolates in terms of total flavonoids (**A**), total phenols (**B**), tocopherols (**C**), and several pigments such as lutin (**D**), β-cryptoxanthin (**E**), lycopene (**F**), carotenoids (**G**), and α-, β-, and γ-carotenes (**H, I** and **J**) per g of bacterial cell weight. Data are represented by the mean of three replicates ± standard error. Different small letters (a, b, c…) above bars indicate significant differences between means at $p < 0.05$

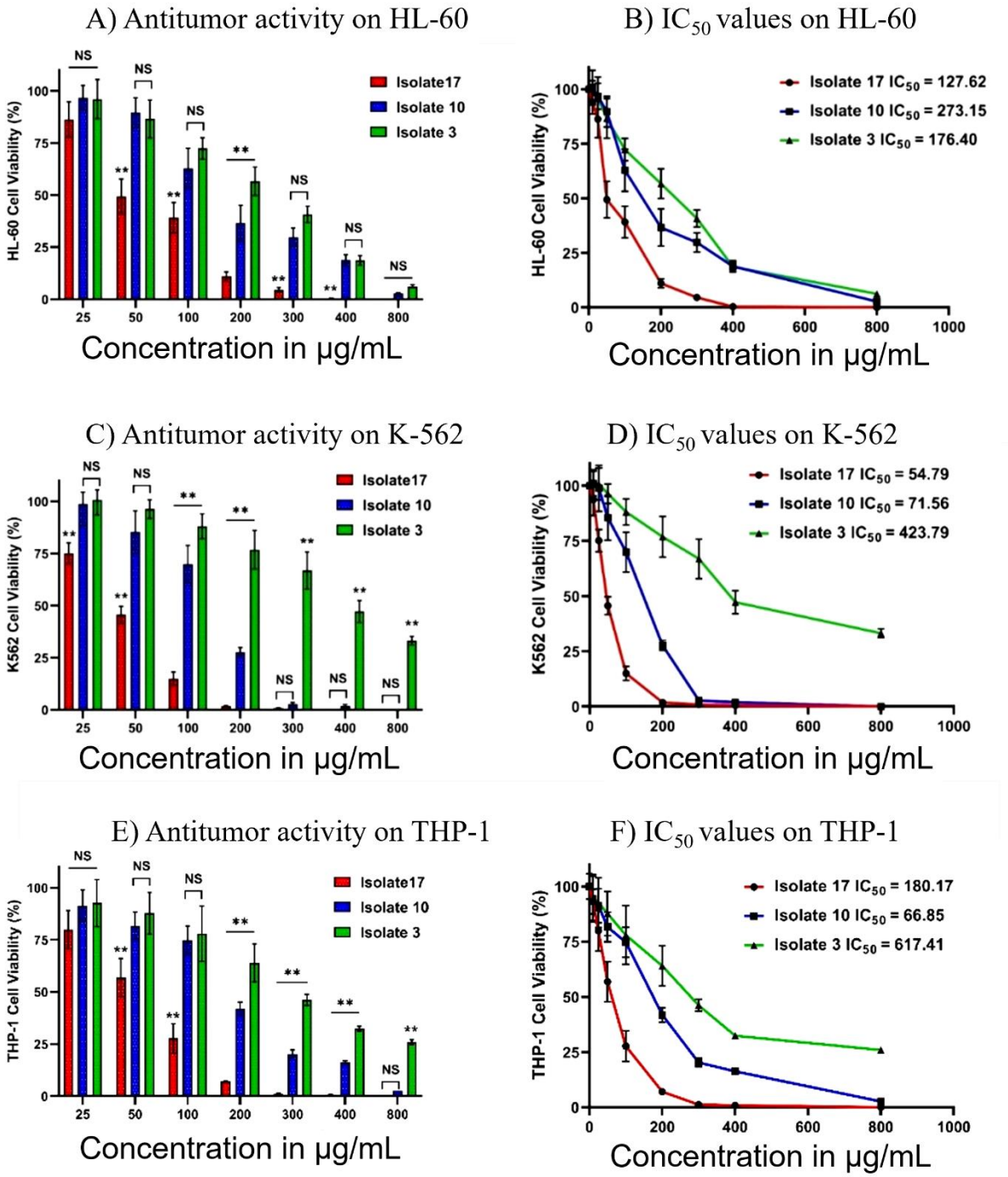


**Fig. 4** The antitumor activity and $IC_{50}$ values of isolate 3, isolate 10 and isolate 17 using three human leukemic cell lines HL-60 **(panel A** and **B)**; K-562 **(panel C** and **D)**; THP-1 **(panel E** and **F)**

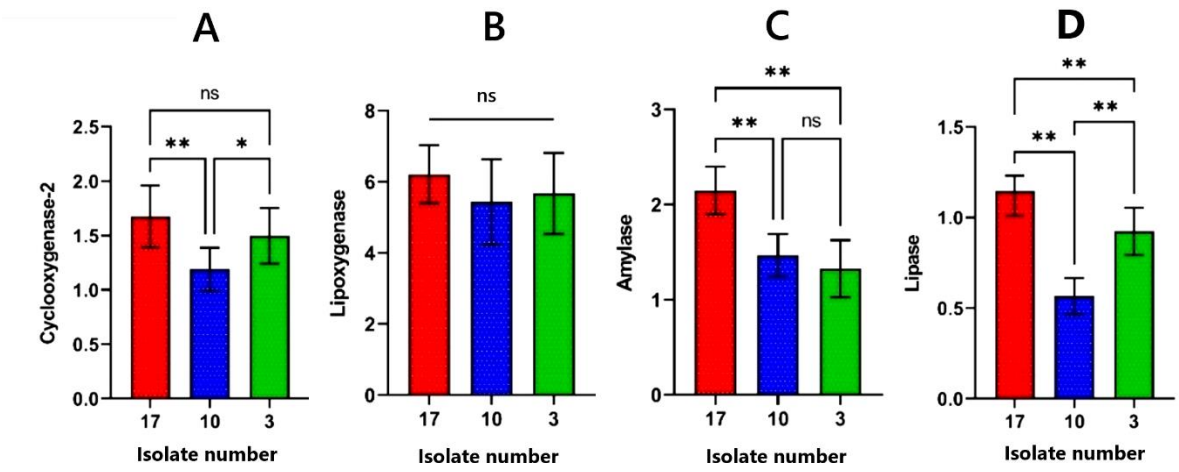


**Fig. 5** The inhibitory effect of the extracts of the most biologically active isolates (isolate 3, isolate 10 and isolate 17) on cyclooxygenase-2 (**A**); lipoxygenase (**B**); α-amylase (**C**); lipase (**D**) activity. Data are represented as $IC_{50}$ values by the means of at least 3 replicates and error bars represent standard deviations. ($p < 0.05$)

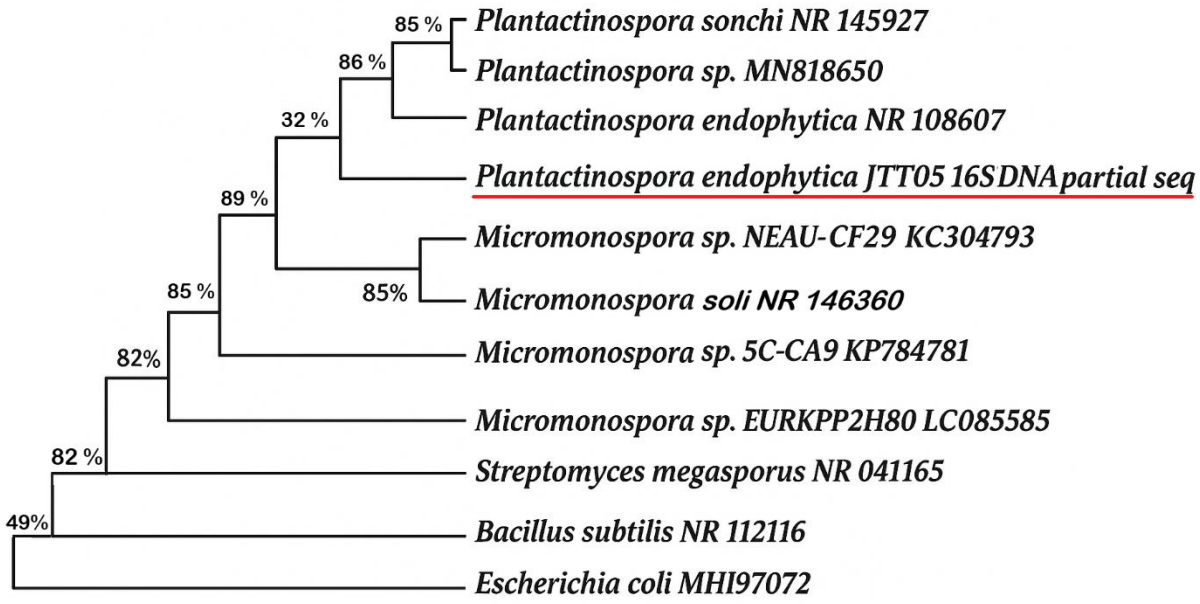


**Fig. 6** Neighbor-joining-based phylogenetic tree of the isolate *Plantactinospora endophytica* **JTT05** based on 16S rRNA sequences. Numbers refer to bootstrap values for each node out of a total of 100 replicate resemblings. The numbers in the brackets are the EMBL accession numbers of the 16S rRNA sequences of reference bacteria. *Streptomyces megasporus* NR041165, *Bacillus subtilis* NR112116, and *Escherichia coli* MH197072 were used as the outgroup