

## Optimization and bioactive compounds production by *Streptomyces griseus* to resist *Staphylococcus aureus* isolated from human semen

Ayat Saied<sup>1\*</sup>, Eman A. Hassan<sup>1</sup>, Hany Sabry<sup>1</sup>, Zeinab K. Abd El-Aziz<sup>2</sup>

<sup>1</sup>International Islamic Center for Population Studies and Research, Al-Azhar University.

<sup>2</sup>Botany and Microbiology Department, Faculty of Science (Girls), Al-Azhar University, Cairo, Egypt.

### ARTICLE INFO

Received:17/8/2025

Revised: 29/10/2025

Accepted:16/11/2025

Corresponding author:

Ayat Saied, Ph.D

E-mail: [ayatsaied1990@gmail.com](mailto:ayatsaied1990@gmail.com)

Mobile: +20 11 58680014

P-ISSN: 2974-4334

E-ISSN: 2974-4324

DOI:

10.21608/bbj.2025.414736.1127

### ABSTRACT

The purpose of this work was to optimize the conditions under which *Streptomyces griseus* might effectively develop its antibacterial capabilities against resistant *Staphylococcus aureus*. In this work, *S. griseus* was grown at various incubation periods, different pH levels, various incubation temperatures, different agitation rates, different media, and various nitrogen sources. The yield in each case was extracted and tested versus previously isolated resistant *S. aureus*, which was isolated from semen and had a correlation with infertility. The optimal conditions for the cultivation of *S. griseus* to produce extract with maximal antibacterial efficacy were recorded. The chemical composition of the extract was tested using GC-MS. The antioxidant capability of the extract was determined using DPPH. Furthermore, the extract cytotoxicity versus Vero cells was examined via MTT assay. The results revealed that cultivation of *S. griseus* on starch nitrate at 3 days for incubation, pH 7.0, 30°C, 150 rpm, and addition of soybean as a nitrogen source resulted in maximal production of antimicrobial extract of *S. griseus* with a zone of inhibition of  $4.62 \pm 0.42$  cm, and MIC was detected at  $125 \pm 0.35$  µg/ml. The extract had 39 various compounds and antioxidant effect ( $IC_{50}$   $2.45 \pm 0.21$  µg/ml), and minimal cytotoxicity with  $IC_{50}$   $192.97 \pm 2.64$  µg/ml. Thus, *S. griseus* is an intriguing supplier of novel antimicrobials, according to this study. In order to find powerful antibacterial agents against resistant infections, the synthesis of bioactive chemicals was successfully increased by combining statistical optimization, antimicrobial evaluation, and culture condition improvement.

**Keywords:** Actinomycetes, Antioxidant, GC-MS, Resistant bacteria *Streptomyces griseus*, *Staphylococcus aureus*.

## 1. Introduction

Exploring and discovering new microbes that produce novel metabolites may be necessary to maintain a competitive edge against emerging illnesses and other infections (Li et al., 2023; Kamel et al., 2024). In both natural and artificial environments, actinomycetes are widely spread and play a crucial part in the breakdown of organic waste. They are also noteworthy because they are abundant in bioactive secondary metabolic products (De Simeis and Serra, 2021; Chen et al., 2021). Secondary metabolites are organic substances that are not directly linked to

an organism's normal development, enhancement, or reproduction (Elhalik et al., 2024). Actinomycetes have a prominent place because of their broad range and ability to synthesize new compounds. Of the bioactive secondary metabolites investigated, they are responsible for producing almost half of them (Mohan and Rajamanickam, 2018; Ngamcharungchit et al., 2023). Over the last fifty years, a great deal of success has been focused on the successful isolation of novel actinomycetes from diverse sources for pharmaceutical surveillance initiatives, given the remarkable notoriety of actinomycetes (Takahashi and

Nakashima, 2018; Ezeobiora et al., 2021). While basic soil-derived microorganisms generated an enormous amount of formerly recognized secondary metabolites, the discovery of new important chemicals from these organisms has decreased during the past 20 years (Radwan et al., 2024; Helmi, 2025). As a result, there is an increase in the discovery of novel actinomycete species from unusual settings, leading to the creation of a new generation of drug experts (Kim et al., 2021; Pan et al., 2025).

Numerous physiologically active substances, including enzymes, antibiotics, and enzyme inhibitors, are produced by actinomycetes (Dhanasekaran et al., 2005; Salwana and Sharmab, 2020). Recently, the proportion of re-isolation of existing molecules has increased, whereas the frequency of new substance production from naturally occurring actinomycetes has decreased. Therefore, it is noteworthy that emerging taxa of actinomycetes from unknown or underexploited habitats are regarded as crucial suppliers of novel bioactive chemicals (Karuppiah and Mustaffa, 2013; Al-Ansari et al., 2020).

Studying intricate biosynthetic methods, boosting manufacturing, and guaranteeing metabolite integrity and bioavailability are some of the difficulties in enhancing the manufacturing of metabolites for therapeutic use. Innovations in delivery techniques, fermentation improvement, and metabolic technology are necessary to overcome these obstacles (Rusu et al., 2023). The generation of metabolites is greatly impacted by variables such as pH, temperature, and supply of nutrients, necessitating an optimal setting of fermentation processes. Before being used therapeutically, the security characteristics of certain microbial metabolites must be carefully evaluated because they can be hazardous. Additionally, exploitation depends on striking a balance between possible therapeutic benefit and production costs (Meenakshi et al., 2024).

Male infertility may result from infections due to bacteria, in addition to other treatments that tackle inflammation and sperm destruction. Treatment usually consists of antibiotics to eradicate the infection (Ramadan et al., 2024; Potiris et al., 2025). To increase the likelihood of conception, these infections must be identified

and treated as soon as possible. Certain bacteria have the capacity to directly harm sperm cells, affecting their form (shape) and motility (swimming capability). Herbal therapy and other complementary medicines may occasionally be implemented in conjunction with traditional medical care (Khedr et al., 2023; Henkel, 2024). The study aims to determine the best ways to develop a bioactive substance of actinomycetes that has antibacterial properties against bacteria isolated from human semen and to characterize this bioactive compound.

## 2. Materials and methods

### Collection of samples

Patients were referred to or received visits from the urology department at El-Hussien Hospital. A total of 100 participants were enrolled between May and June of 2022. The participants' age range was 20-45 years. Semen cultures were done on all 100 research participants. Out of the 100 patients, only 60 had a positive semen culture. Of the 60 individuals with bacteriospermia, 40 (66.6 %) had organisms with Gram positive infection, while only 20 (33.3 %) had organisms with Gram negative infection. The study received ethical approval from the International Islamic Center for Population Studies and Research, Al-Azhar University (ethical no. and consent AZF22820222).

### The rationale for using the *Streptomyces griseus*

A resistant *Staphylococcus aureus* Gram-positive isolate, which has been commonly isolated, identified from semen samples in the International Islamic Center for Population Studies and Research, Al-Azhar University, was involved in this work. Besides, *S. griseus* strain ZN1 with accession number OP782088.1, which was previously isolated and molecularly identified by the current research group, was used in this study. *S. griseus* was cultured on Liquid Starch nitrate medium to produce metabolites to be used in the upcoming experimental work.

### Optimization of environmental and nutritional conditions for metabolite production

To find the ideal settings influencing the yield of the intended metabolites with antimicrobial impact, *S. griseus* strain ZN1 was grown under various growth conditions as follows: 1. For one, two, three, four, five, six, and seven days, *S. griseus* strain ZN1 was incubated at 45°C. Every day, specimens were collected to evaluate the productivity of the active metabolites (Meenakshi et al., 2024). 2. Before sterilization, the media's initial pH values were altered to fall between 4 and 10 (Usha et al., 2011). *S. griseus* strain ZN1 was then allowed to grow at a different pH value appropriate to produce metabolites (Xu et al., 2024). 3. Different temperatures were used to incubate *S. griseus* strain ZN1, including 20, 25, 30, 35, 40, and 45°C. For every incubation temperature, the efficacy of the metabolites was evaluated (Zhao et al., 2021). 4. The impact of agitation rate on the generation of metabolites was examined. A 250 ml Erlenmeyer flask with 25–100 ml manufacturing media was used to develop *S. griseus* strain ZN1, which was then placed on a rotary shaker set to 150, 200, 250, and 300 rpm (Lee et al., 2023). 5. Additionally: to identify which nutritional media—starch-nitrate, inorganic-salts starch, yeast extract-malt extract, and Czapek's medium—are necessary for *S. griseus* strain ZN1 to produce the greatest number of metabolites (Kronheim et al., 2023). 6. Finally, to determine the optimum productivity of the metabolites, the *S. griseus* strain ZN1 was allowed to grow independently on each nitrogen source, including sodium nitrate, ammonium nitrate, soybean meal, ammonium chloride, urea, casein, yeast extract, and peptone (Chen et al., 2025).

### Antibacterial properties screening

To evaluate the antibacterial qualities of each of the produced metabolites during optimization against *S. aureus* isolated from semen, 100 µl of *Streptomyces griseus* strain filtrate extract ZN1 and commonly used antibiotics were employed to fill the holes employing the agar diffusion procedure. At the end of the incubation time, the areas of inhibition were identified, and each group was compared to the reference drug. Serial dilutions of the effective dose (minimal inhibitory level) were produced and assessed versus *S. aureus* (Mohamed et al., 2025).

### GC-Mass characterization and extraction for the *Streptomyces griseus* potential metabolites

After being blended and mashed for three days at room temperature, 2.0 grams of crushed dry *S. griseus* strain ZN1 were placed in a fresh jar and mixed with 0.3 liters of methanol. In a traditional extraction method, the extract was placed in a sonicator set to 45°C for 65 minutes. The extract was subsequently filtered and condensed at 45°C in a vacuum using a rotatory separator to create crude extract (Aamer et al., 2024). *S. griseus* strain ZN1's chemical composition was determined using an obvious terminal column TI-5MI (33 m x 0.29 mm x 0.29 m film width) and a Trace GC12400-ISI mass scanner (Waters, USA). Initially maintained at 42° C, the temperature of the column oven increased by 5°C each minute to 210° C and maintained there for 5 minutes. Last but not least, it was heated by 5°C per minute to 295°C and held there for 15 minutes. While helium was utilized as a transport gas at an ongoing rate of flow of 1.1 ml/min, the needle and MS conversion line heaters were maintained at 275°C and 295°C, subsequently. The automatic selector AS1300 in divided arrangement and a GC with a 6-minute fluid pause were used to automatically inject a diluted quantity of 1 µl. Over the 40–1000 m/z range, a full scanning EI mass spectrum was obtained at 70 eV ionization voltages. The temperature of the ion generator was fixed at 230 °C. By comparing each component's mass spectrum and retention time to those found in the WILEY 07 and NIST 11.1 substance spectrum databases, the specific components of the metabolites were determined (Sehim et al., 2023).

### Assessment of the best extract of *Streptomyces griseus* for the antioxidant capacity

The DPPH method was used to evaluate the actinomycete extract's antioxidant capacity under ideal conditions. A range of extract concentrations (50, 100, 150, 200, 250, and 300 µg/mL) were produced by dispersing in water of high purity. After that, one milliliter of the resulting solution was placed in a test tube with 1 ml of DPPH (made in methanol) and 400.0 small amounts of Tris-HCl buffer (pH 7.6, 50.0 mM). The tube was thoroughly mixed and then allowed to stand at 38°C with 150 rpm stirring for 35 minutes in a dark environment. The same

conditions and dosages were used for a second set of ascorbic acid (positive standard) experiments. Additionally, DPPH and Tris-HCl buffer free of ascorbic acid and the material under investigation were used in the same incubation conditions as the control group's negative condition (Sherif et al., 2023).

### Evaluation of the best extract for cytotoxic properties of *Streptomyces griseus* strain ZN1

The collected specimen had been suspended in DMSO, the cytotoxic effect on Vero cells which are indicative of normal cells was assessed using the MTT test. The blue hue created by using typical levels represents the quantity of live cells. The value of absorbance at 560 nm was measured using an automated microplate scanner (Thermo-Fisher, USA). After adding samples with contents ranging from 1000 to 31.25 µg/mL and allowing them to adhere for 24 hours, the cells were cultivated for an additional 24 hours at 38°C. After incorporating the new medium, it was left at 38°C for four hours prior to 100 µL of MTT solution (5.1 mg/mL) was added. A CCD camera which placed on a microscope (Olympus, Japan) is used to observe cells (Abou El-Enain et al., 2023).

### Examining statistical data

The findings of the quantitative examination of the information collected were presented employing Graph Pad Prism V58 USA and the means findings of three different repeats. To assess the variance among characteristics and find any significant variations, t-test or ANOVA was implemented with the Tukey test at  $p < 0.05$ .

## 3. Results

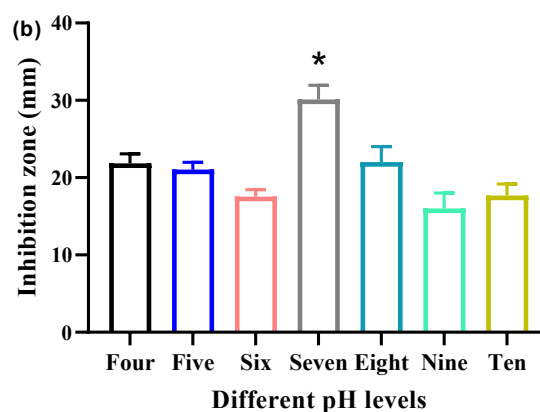
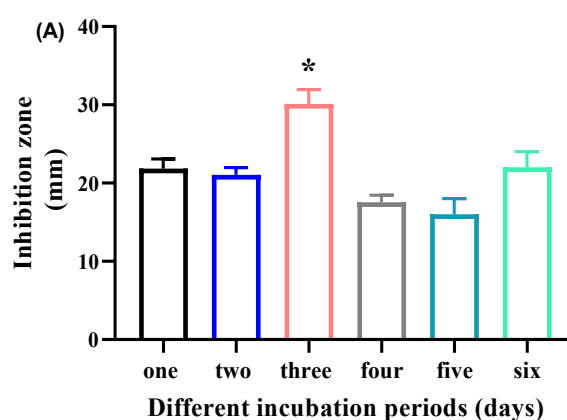
### Optimization outcomes

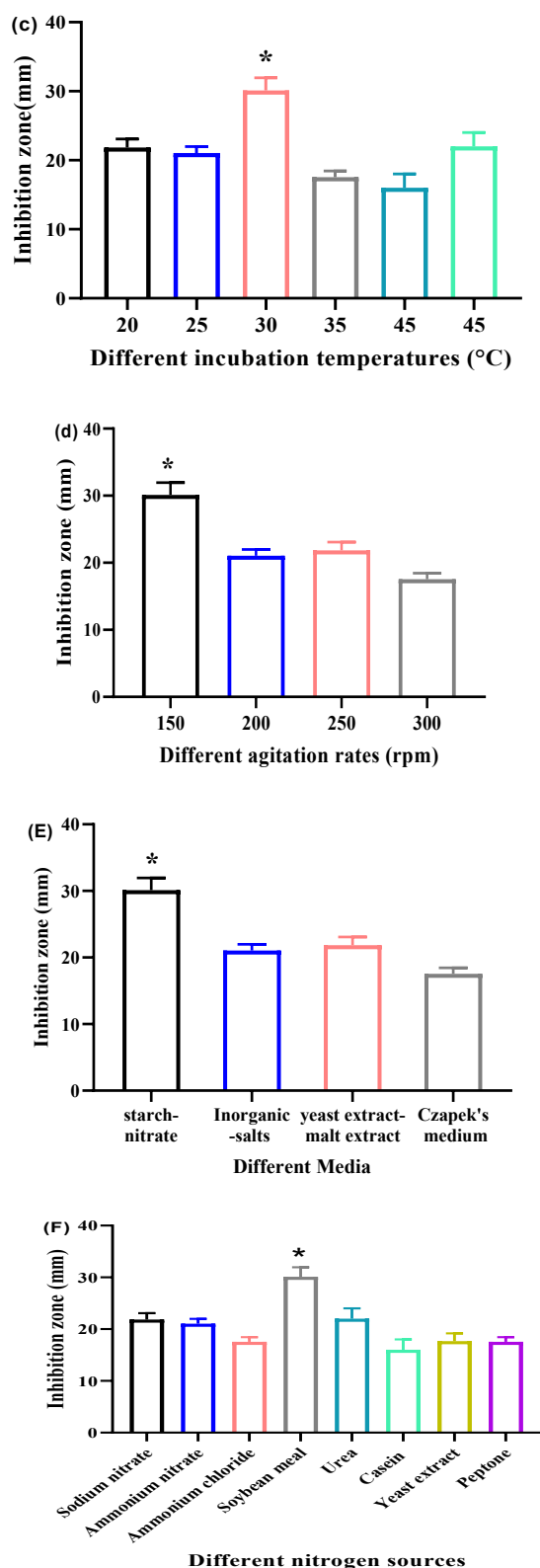
Cultivation of *S. griseus* at different incubation periods revealed that the best impact could be seen at 3 days of incubation (Fig. 1A). While, cultivation of *S. griseus* at different pH levels of media showed that pH= 7.0 had the best result relative to other pH level (Fig. 1B). Furthermore, culture of *S. griseus* at different incubation temperatures reflect that 30°C gives the best outcome (Fig. 1C). Besides, culture of *S. griseus* at various agitation rates showed that culture at 150 rpm give the best yield (Fig. 1D). Moreover, cultivation of *S. griseus* at different media

revealed that starch nitrate is the best media (Fig. 1E). Finally, cultivation of *S. griseus* at upon using different nitrogen sources reflects that soybean meal had the best outcome (Fig. 1F).

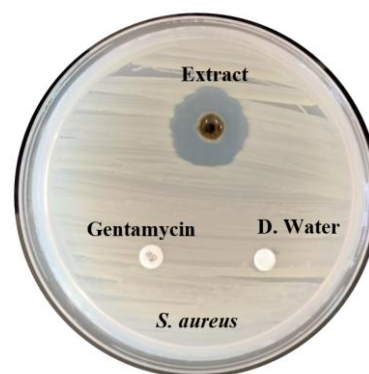
### Antibacterial activity for *Streptomyces griseus* at optimized conditions

Cultivation of *S. griseus* under all of the optimum conditions results in the production of an actinomycete extract which had an effective antibacterial effect against *S. aureus* isolated from semen, with an inhibition diameter of  $4.62 \pm 0.42$  Cm and MIC was detected at  $125 \pm 0.35$  µg/ml (Fig. 2).





**Fig. 1.** Optimization of growth and production of antimicrobial extract from *S. griseus* versus resistant *S. aureus* from semen upon using (A) different incubation periods (B) different pH levels (C) different incubation temperatures (D) different agitation rates (E) different media, and (F) different nitrogen sources. \*Refers to the optimal level.



**Fig. 2.** Agar diffusion assay upon using optimum conditions for production of antimicrobial extract of *S. griseus* versus resistant *S. aureus* isolated from semen.

### Outcome for GC-MS examination

Thirty nine different volatile molecules could be seen upon testing *S. griseus* extract which were: Cyclobutanol; Phenol, 2,4-bis(1,1-dimethyl ethyl); Cyclohexane, butyl-, 3-Methoxymethoxy-3,7,16,20 tetrameth yl-heneicosa- 1,7,11,15,19-pentaene; Nerolidol-epoxyacetate; n-Hexadecanoic acid; 9-Octadecenoic acid (Z)-; [1,1'-Bicyclopropyl]-2- Octadecatrienoic acid, 2'-hexyl-, methyl ester; 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (z,z,z)- ; 9,12-Octadecadienoic acid (z,z)-; Isosolanidine ; E-2-Decenal; Tetradecane; Cyclohexane, 1,1'-(1,2-dimethyl); Oxiranemethanol; 2-Undecanone; 5-Eicosene; 3-Tetradecane; Ethyl iso-allocholate; 1,2-Benzenedicarboxylic acid, di-isooctyl ester; 1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester; Dodecane, 1,1-difluoro; 2, 4, 6-Cycloheptatrien-1-one; 2, 2, 5, 5, 6-Pentamethyl-4, 7, 9-trioxabicyclo [4.2.1] nonane; 2,4-Bis(α,α-dimethylbenzyl)phenol; Dotriacontane; Octacosane; 5-Eicosene; Bis-(3-oxo-6'-diethylamino-spi); Bis-(3,5,5-trimethylhexyl) ether; 1,2-Benzenedicarboxylic acid; 1, 1, 1, 2-Tetrafluoro-2-tridecene; Acrylic acid hexadecanyl ester; 8-Pentadecanone; Hexadecane; Bis(3,5,5-trimethylhexyl) ether; 1,1-Dichloro-2-dodecanol; Cyclohexane, octyl- ; and 3-Methoxymethoxy-3,7,16,20 tetrameth yl-heneicosa- 1,7,11,15,19-pentaene. All of the molecules had peak areas in the same range (Table 1, Fig. 3).

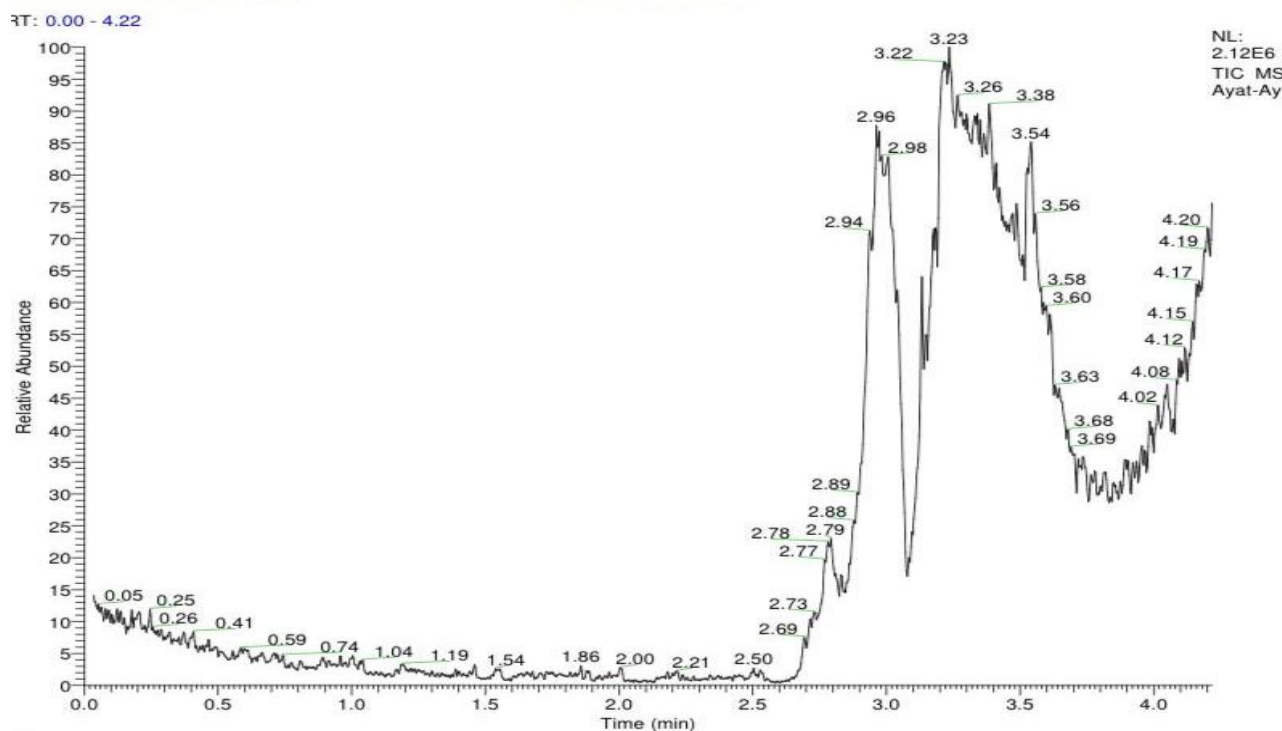


Fig. 3. GC-MS output showing various molecules in *S. griseus* extract.

#### Antioxidant impact of *S. griseus* extract

*S. griseus* extract was examined at various levels and showed a good antioxidant effect with  $IC_{50}$   $8.47 \pm 0.42$   $\mu\text{g/ml}$ , whereas ascorbic acid (norm) had  $IC_{50}$   $2.45 \pm 0.21$   $\mu\text{g/ml}$  (Fig. 4).

The cytotoxicity level of *S. griseus* extract towards normal cells was examined at various concentrations versus normal cells (Vero cells) at  $2.64$   $\mu\text{g/ml}$  (Table 2, Fig. 5).

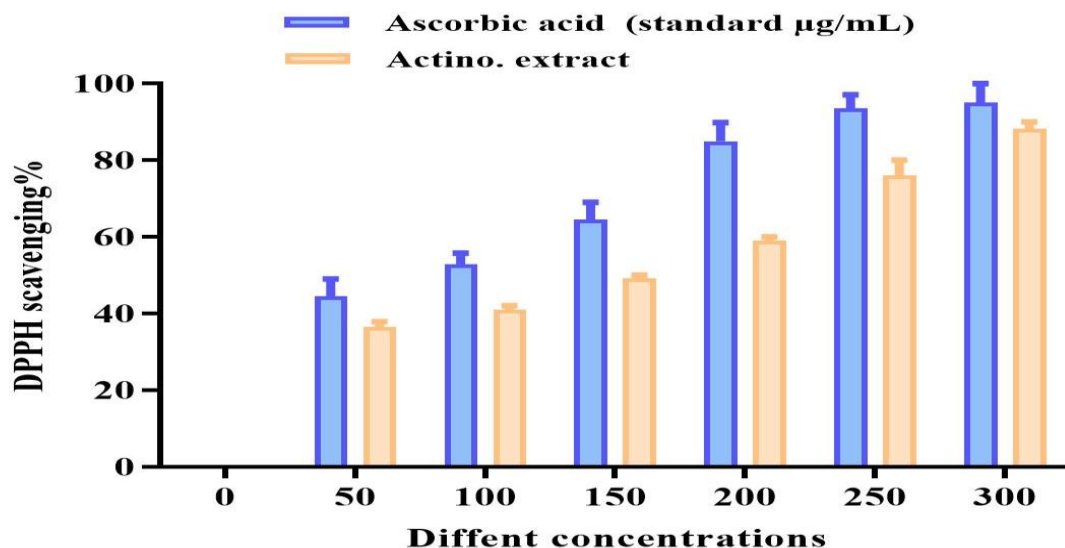


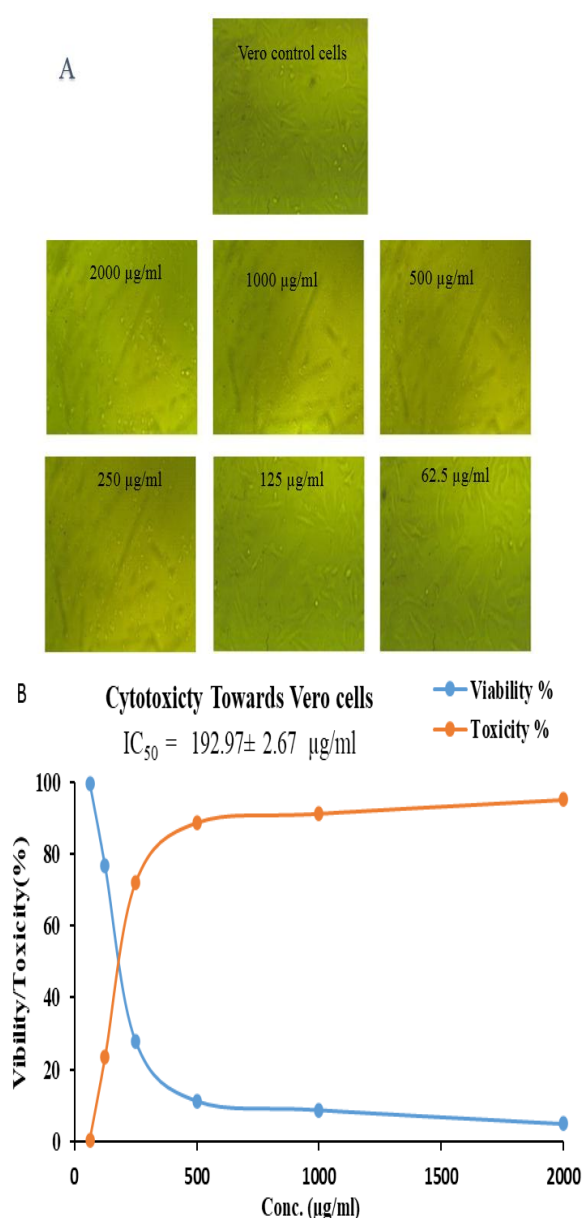
Fig. 4. Antioxidant capacity of *S. griseus* extract (actino. Extract) versus ascorbic acid standard

**Table 1.** GC-Mass outcomes for various molecules in extract of *S. griseus* extract

| RT    | Compound name                                                           | Peak Area% | Molecular Formula                                             | Molecular Weight |
|-------|-------------------------------------------------------------------------|------------|---------------------------------------------------------------|------------------|
| 4.77  | Cyclobutanol                                                            | 2.5        | C <sub>4</sub> H <sub>8</sub> O                               | 72.11            |
| 5.72  | Phenol, 2,4-bis(1,1-dimethyl ethyl)                                     | 2.25       | C <sub>14</sub> H <sub>22</sub> O                             | 206              |
| 7.82  | Cyclohexane, butyl-                                                     | 1.26       | C <sub>10</sub> H <sub>20</sub>                               | 140.2            |
| 9.02  | 3-Methoxymethoxy-3,7,16,20 tetrameth yl-heneicosa-1,7,11,15,19-pentaene | 2.41       | C <sub>27</sub> H <sub>46</sub> O <sub>2</sub>                | 402              |
| 25.35 | Nerolidol-epoxyacetate                                                  | 2.59       | C <sub>17</sub> H <sub>28</sub> O <sub>4</sub>                | 296              |
| 29.90 | n-Hexadecanoic acid                                                     | 2.74       | C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>                | 256              |
| 31.21 | 9-Octadecenoic acid (Z)-                                                | 2.04       | C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>                | 282              |
| 32.3  | [1,1'-Bicyclopropyl]-2- Octadecatrienoic acid, 2'-hexyl-, methyl ester  | 2.19       | C <sub>21</sub> H <sub>38</sub> O <sub>2</sub>                | 322              |
| 32.43 | 9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester, (z,z,z)-      | 2.54       | C <sub>21</sub> H <sub>36</sub> O <sub>4</sub>                | 352              |
| 33.25 | 9,12-Octadecadienoic acid (z,z)-                                        | 2.86       | C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>                | 280              |
| 39.43 | Isosolanidine                                                           | 2.00       | C <sub>27</sub> H <sub>43</sub> NO                            | 397              |
| 2.21  | E-2-Decenal                                                             | 2.21       | C <sub>10</sub> H <sub>18</sub> O                             | 154.2            |
| 2.50  | Tetradecane                                                             | 2.50       | C <sub>14</sub> H <sub>30</sub>                               | 198.3            |
| 2.69  | Cyclohexane, 1,1'-(1,2-dimethyl)                                        | 2.69       | C <sub>16</sub> H <sub>30</sub>                               | 222.4            |
| 2.73  | Oxiranemethanol                                                         | 2.73       | C <sub>9</sub> H <sub>10</sub> O <sub>2</sub>                 | 150.1            |
| 2.77  | 2-Undecanone                                                            | 2.77       | C <sub>11</sub> H <sub>22</sub> O                             | 170.2            |
| 2.79  | 5-Eicosene                                                              | 2.79       | C <sub>20</sub> H <sub>40</sub>                               | 280.5            |
| 2.89  | 3-Tetradecane                                                           | 2.89       | C <sub>14</sub> H <sub>28</sub>                               | 196.3            |
| 2.94  | Ethyl iso-allocholate                                                   | 2.94       | C <sub>26</sub> H <sub>44</sub> O <sub>5</sub>                | 436              |
| 2.96  | 1,2-Benzenedicarboxylic acid, di-isooctyl ester                         | 2.96       | C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>                | 390              |
| 2.98  | 1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester                   | 2.98       | C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>                | 390              |
| 3.22  | Dodecane, 1,1-difluoro                                                  | 2.22       | C <sub>12</sub> H <sub>24</sub> F <sub>2</sub>                | 206.3            |
| 3.23  | 2, 4, 6-Cycloheptatrien-1-one                                           | 2.23       | C <sub>7</sub> H <sub>6</sub> O                               | 106.1            |
| 3.26  | 2, 2, 5, 5, 6-Pentamethyl-4, 7, 9-trioxabicyclo [4.2.1] nonane          | 2.02       | C <sub>11</sub> H <sub>20</sub> O <sub>3</sub>                | 200.2            |
| 3.28  | 2,4-Bis(α,α-dimethylbenzyl)phenol                                       | 2.28       | C <sub>24</sub> H <sub>26</sub> O                             | 330.5            |
| 3.54  | Dotriacontane                                                           | 2.54       | C <sub>32</sub> H <sub>66</sub>                               | 450.9            |
| 3.56  | Octacosane                                                              | 2.56       | C <sub>28</sub> H <sub>58</sub>                               | 394.8            |
| 3.58  | 5-Eicosene                                                              | 2.58       | C <sub>20</sub> H <sub>40</sub>                               | 280.5            |
| 3.60  | Bis-(3-oxo-6'-diethylamino-spi)                                         | 2.60       | C <sub>48</sub> H <sub>40</sub> N <sub>2</sub> O <sub>7</sub> | 756.9            |
| 3.63  | Bis-(3,5,5-trimethylhexyl) ether                                        | 2.63       | C <sub>18</sub> H <sub>38</sub> O                             | 270.5            |
| 3.68  | 1,2-Benzenedicarboxylic acid                                            | 2.68       | C <sub>8</sub> H <sub>6</sub> O <sub>4</sub>                  | 166.1            |
| 3.69  | 1, 1, 1, 2-Tetrafluoro-2-tridecene                                      | 2.69       | C <sub>13</sub> H <sub>22</sub> F <sub>4</sub>                | 254.3            |
| 4.02  | Acrylic acid hexadecanyl ester                                          | 2.62       | C <sub>19</sub> H <sub>36</sub> O <sub>2</sub>                | 296.2            |
| 4.08  | 8-Pentadecanone                                                         | 2.88       | C <sub>15</sub> H <sub>30</sub> O                             | 226.4            |
| 4.12  | Hexadecane                                                              | 2.92       | C <sub>16</sub> H <sub>34</sub>                               | 226.4            |
| 4.15  | Bis(3,5,5-trimethylhexyl) ether                                         | 2.95       | C <sub>18</sub> H <sub>38</sub> O                             | 270.5            |
| 4.17  | 1,1-Dichloro-2-dodecanol                                                | 2.97       | C <sub>12</sub> H <sub>24</sub> Cl <sub>2</sub> O             | 255.2            |
| 4.19  | Cyclohexane, octyl-                                                     | 2.99       | C <sub>14</sub> H <sub>28</sub>                               | 196.3            |
| 4.20  | 3-Methoxymethoxy-3,7,16,20 tetrameth yl-heneicosa-1,7,11,15,19-pentaene | 2.80       | C <sub>27</sub> H <sub>46</sub> O <sub>2</sub>                | 402              |

**Table 2.** Viability and toxicity testing of *S. griseus* extract versus Vero cells (Outcomes were recoded as means  $\pm$  SE).

| ID                           | $\mu\text{g/ml}$ | O. D |      |      | O. D<br>Mean $\pm$ SE | Viability<br>% | Toxicity % | IC <sub>50</sub><br>$\pm$ SD<br>$\mu\text{g/ml}$ |
|------------------------------|------------------|------|------|------|-----------------------|----------------|------------|--------------------------------------------------|
| Vero                         | -----            | 0.61 | 0.60 | 0.59 | $0.6 \pm 0.007$       | 100            | 0.00       |                                                  |
| <i>S. griseus</i><br>extract | 2000             | 0.02 | 0.03 | 0.03 | $0.03 \pm 0.002$      | 5.01           | 94.99      | 192.97<br>$\pm 2.64$                             |
|                              | 1000             | 0.06 | 0.04 | 0.05 | $0.054 \pm 0.004$     | 8.86           | 91.14      |                                                  |
|                              | 500              | 0.06 | 0.07 | 0.06 | $0.069 \pm 0.002$     | 11.39          | 88.61      |                                                  |
|                              | 250              | 0.18 | 0.15 | 0.16 | $0.169 \pm 0.008$     | 27.94          | 72.06      |                                                  |
|                              | 125              | 0.47 | 0.45 | 0.46 | $0.464 \pm 0.004$     | 76.62          | 23.38      |                                                  |
|                              | 62.5             | 0.60 | 0.70 | 0.60 | $0.603 \pm 0.003$     | 99.66          | 0.44       |                                                  |

**Fig. 5.** Cytotoxicity testing (A) Microscopic examinations at various concentrations of *S. griseus* extract for cytotoxicity, (B) Statistical analysis for viability and toxicity percentage of *S. griseus* extract towards Vero cells.

#### 4. Discussion

This study focuses on finding optimal conditions for *S. griseus* to produce yield with maximal antibacterial efficiency versus *S. aureus* isolated from semen. In the same direction, other researchers have used starch casein agar as a cultivation medium to cultivate other *Streptomyces* sp. strains (AL-Ghazali and Omran 2017). According to a previous publication, *Streptomyces* can create metabolites that are important from a pharmacological standpoint. *Streptomyces* sp., which was isolated from nature, was found to have created novel chemicals with antibacterial qualities (Ahsan et al., 2017). Primary assessment of actinomycetes' detrimental behaviors was conducted using the cross-streak approach, which was consistent with previous studies (Kalyani et al., 2019). Furthermore, it rarely evaluates the effects of several variables and their interactions simultaneously, which is problematic when there are significant interactions across parameters (Latha et al., 2017).

In this work culture of *S. griseus* on starch nitrate for 3 days of incubation, pH 7.0, 30°C, 150 rpm, and the addition of soybean as a nitrogen source resulted in maximal production of antimicrobial extract of *S. griseus* with a zone of inhibition =  $4.62 \pm 0.42$  cm and MIC was detected at  $125 \pm 0.35$   $\mu\text{g/ml}$ . The extract had been tested via GC-MS, and 39 different volatile compounds could be seen in the extract produced at optimal conditions, and had maximal antibacterial action versus *S. aureus*.

N-hexadecanoic acid, pentadecanoic acid, and oleic acid, as identified by GC-MS analysis, exhibit the typical characteristics of fatty acids with a carboxyl group (-COOH) and a methyl

group (-CH<sub>3</sub>) in each of the ends of an aliphatic hydrocarbon (Tonisi et al., 2020). It has been shown that the main ingredients of the biological activity in herbal and ethnic remedies are fatty acids (Nisha et al., 2025; Aldalin et al., 2024). The current results revealed that optimization of cultivation conditions of *S. griseus* yields an extract with promising antioxidant potential. According to Law et al. (2017), soil-derived *Streptomyces* may generate antioxidant-active, physiologically active compounds. As demonstrated by the DPPH and free radical elimination assays, *Streptomyces* sp. (Fahmy and Abdel-Tawab, 2021; Djebbah et al., 2022) ethyl acetate extracts had potent antioxidant properties. *Streptomyces* sp. ethyl acetate extract's antioxidant properties were assessed using DPPH radical scavenging capacity, metal binding activity, and ABTS activity to neutralize radicals (Siddharth and Vittal, 2018). GC-MS study revealed that *Nocardiopsis dassonvillei*'s hexadecanoic acid and hexadecanoic acid methyl ester had antioxidant qualities (Faja et al., 2017; Mekky et al., 2024). *Streptomyces* sp. ethyl acetate extract showed antibacterial properties in both types of octadecenoic acid and hexadecanoic acid (Mothana et al., 2022). Octadecenoic acid from Actinomycetes had antifungal characteristics (Elsayed et al., 2020). Therefore, it is anticipated that the fatty acids identified in this study may also be responsible for the antioxidant as well as antibacterial qualities. The present results revealed the *S. griseus* extract had a safe impact on normal cells which facilitate its usage for various applications. Safety has a significant impact on the many uses of natural substances in biotechnological and medical fields (Mekky et al., 2025).

## Conclusion

The antibacterial property of *S. griseus* isolate is thoroughly examined in this work, along with the adjustment of cultivation conditions for the generation of more potent bioactive substances with antioxidant capacity. The results lay the groundwork for further studies targeted at battling antibiotic resistance by advancing our knowledge of *Streptomyces* species as a promising source of new antibiotics. The techniques used and knowledge acquired from

this research highlight these microbial strains' likelihood of use in biotechnology-related fields.

## Funding

Non

## Conflict of interest

The authors have no conflict of interest to declare.

## Data availability

Data available on request / reasonable request

## Authors' contribution

All authors participated in all practical experiments, scientific writing, review, and data collection, and there is no conflict of interest between them.

## 5. Reference

- Abou El-Enain IM, Abed N, Helal E, Abdelkhalek SE, Suleiman W, Safwat AN, Yosri M, 2023. Eco-friendly biosynthesis of Ag-NPs by *Streptomyces griseus* with anti-*Candida albicans* and antitumor activity. Recent Advances in Antiinfective Drug Discovery. 19(1), 73-87.
- Ahsan T, Chen J, Zhao X, Irfan M, Wu Y, 2017. Extraction and identification of bioactive compounds (eicosane and dibutyl phthalate) produced by *Streptomyces* strain KX852460 for the biological control of *Rhizoctonia solani* AG-3 strain KX852461 to control target spot disease in tobacco leaf. AMB Express, 7:1–9.
- Al-Ansari M, Kalaiyarasi M, Almalki MA, Vijayaraghavan P, 2020. Optimization of medium components for the production of antimicrobial and anticancer secondary metabolites from *Streptomyces* sp. AS11 isolated from the marine environment. Journal of King Saud University – Science, 32(3):1993–1998.
- Aldalin HK, Alharbi NK, Hadi AM, Sharaf M, Mekky AE, Ragab SM, Abdelnour SA, 2024. Bioactivity screening and molecular identification of *Anchusa milleri* L. sunflower crud extract for antioxidant, antiviral, antimicrobial, and anticancer properties. Natural Product Research, 1–14.

- Al-Ghazali LH, Omran RABAB, 2017. Optimization of production conditions of antibacterial metabolite from *Streptomyces* sp. Asian Journal of Pharmaceutical and Clinical Research, 10:386–391.
- Chen F, Xuan H, Ziyang H, Jing D, Sha Z, Jie C, Dan W, Haiping L, 2025. Screening, identification, and fermentation optimization of the antagonistic actinomycete strain TCS21-117 against *Botrytis cinerea*. Microorganisms, 13(2):379.
- Chen J, Xu L, Zhou Y, Han B, 2021. Natural products from actinomycetes associated with marine organisms. Marine Drugs, 19:629.
- De Simeis D, Serra S, 2021. Actinomycetes: A never-ending source of bioactive compounds—an overview on antibiotics production. Antibiotics (Basel), 10(5):483.
- Dhanasekaran D, Rajkumar G, Sivamani P, Selvamani S, Panneerselvam A, Thajuddin N, 2005. Screening of salt pans actinomycetes for antibacterial agents. International Journal of Microbiology, 2:62–66.
- Djebbah FZ, Al-Dhabi NA, Arasu MV, Belyagoubi L, Kherbouche F, Abdelouahid DE, 2022. Isolation and characterization of *Streptomyces* sp. strain GLD25 with antimicrobial and antioxidant effects from Gueldaman cave (GLD1) Akbou-Algeria. Journal of King Saud University Science, 34:101719.
- Eini F, Kutenaei MA, Zareei F, et al., 2021. Effect of bacterial infection on sperm quality and DNA fragmentation in subfertile men with leukocytospermia. BMC Molecular and Cell Biology, 22:42.
- Elhalik MA, Mekky AE, Khedr M, Suleiman WB, 2024. Antineoplastic with DNA fragmentation assay and antioxidant, anti-inflammatory with gene expression activity of *Lactobacillus plantarum* isolated from local Egyptian milk products. BMC Microbiology, 24(1):443.
- Elsayed TR, Galil DF, Sedik MZ, Hassan HM, Sadik MW, 2020. Antimicrobial and anticancer activities of actinomycetes isolated from Egyptian soils. International Journal of Current Microbiology and Applied Sciences, 9:2020.
- Esmailkhani A, Akhi MT, Sadeghi J, Niknafs B, Zahedi Bialvaei A, Farzadi L, Safadel N, 2018. Assessing the prevalence of *Staphylococcus aureus* in infertile male patients in Tabriz, northwest Iran. International Journal of Reproductive Biomedicine, 16(7):469–474.
- Ezeobiora CE, Igbokwe NH, Amin DH, et al., 2022. Uncovering the biodiversity and biosynthetic potentials of rare actinomycetes. Future Journal of Pharmaceutical Sciences, 8:23.
- Fahmy NM, Abdel-Tawab AM, 2021. Isolation and characterization of marine sponge-associated *Streptomyces* sp. NMF6 strain producing secondary metabolite(s) possessing antimicrobial, antioxidant, anticancer, and antiviral activities. Journal of Genetic Engineering and Biotechnology, 19:102.
- Faja O, Sharad AA, Younis KM, Usup G, Ahmad A, 2017. Isolation, screening and antibiotic profiling of marine actinomycetes extracts from the coastal of Peninsular Malaysia. International Journal of ChemTech Research, 10:212–224.
- Henkel R, 2024. Leukocytospermia and/or bacteriospermia: Impact on male infertility. Journal of Clinical Medicine, 13(10):2841.
- Kalyani BS, Krishna PS, Sreenivasulu K, 2019. Screening and identification of novel isolate *Streptomyces* sp. NLKPB45 from Nellore coastal region for its biomedical applications. Saudi Journal of Biological Sciences, 26:1655–1660.
- Kamel M, Aleya S, Alsubih M, Aleya L, 2024. Microbiome dynamics: A paradigm shift in combatting infectious diseases. Journal of Personalized Medicine, 14(2):217.
- Karuppiyah P, Mustaffa M, 2013. Antibacterial and antioxidant activities of *Musa* sp. leaf extracts against multidrug resistant clinical pathogens causing nosocomial infection. Asian Pacific Journal of Tropical Biomedicine, 3(9):737–742.
- Khedr M, Youssef FS, El-Kattan N, Abozahra MS, Selim MN, Youssef A, Mekky AE, 2023. *FolE* gene expression for folic acid productivity from optimized and characterized probiotic *Lactobacillus*

- delbrueckii. Journal of Genetic Engineering and Biotechnology, 21(1):169.
- Kim JH, Lee N, Hwang S, Kim W, Lee Y, Cho S, Palsson BO, Cho BK, 2021. Discovery of novel secondary metabolites encoded in actinomycete genomes through coculture. Journal of Industrial Microbiology and Biotechnology, 48(3–4): kuaa001.
- Kronheim S, Solomon E, Ho L, Glossop M, Davidson AR, Maxwell KL, 2023. Complete genomes and comparative analyses of *Streptomyces* phages that influence secondary metabolism and sporulation. Scientific Reports, 13:9820.
- Latha S, Sivaranjani G, Dhanasekaran D, 2017. Response surface methodology: A non-conventional statistical tool to maximize the throughput of *Streptomyces* species biomass and their bioactive metabolites. Critical Reviews in Microbiology, 43:567–582.
- Law JWF, Ser HL, Duangjai A, 2017. *Streptomyces colonosanans* sp. nov., a novel actinobacterium isolated from Malaysian mangrove soil exhibiting antioxidative activity and cytotoxic potential against human colon cancer cell lines. Frontiers in Microbiology, 8:877.
- Lee JA, Kim HU, Na JG, Ko YS, Cho JS, Lee SY, 2023. Factors affecting the competitiveness of bacterial fermentation. Trends in Biotechnology, 41(6):798–816.
- Li X, Xu H, Li Y, Liao S, Liu Y, 2023. Exploring diverse bioactive secondary metabolites from marine microorganisms using co-culture strategy. Molecules, 28:6371.
- Meenakshi S, Hiremath J, Meenakshi MH, Shivaveerakumar S, 2024. Actinomycetes: Isolation, cultivation and its active biomolecules. Journal of Pure and Applied Microbiology, 18:118–143.
- Mekky AE, El-Barkey NM, Abd El Halim HM, Nasser SA, Mahmoud NN, Zahra AA, Nasr-Eldin MA, 2025. Exploring the potential of hydro alcoholic crude extract of beeswax as antibacterial, antifungal, antiviral, anti-inflammatory and antioxidant agent. Scientific Reports, 15(1):32512.
- Mekky AE, Saied E, Abdelmouty ES, Haggag MI, Khedr M, Khalel AF, Mahmoud NN, 2024. Phytochemical analysis of *Centaurea calcitropa* L. aerial flowering parts serial solvent extracts and its antibacterial and antioxidant activities. Life, 14(7):900.
- Mohamed AN, Mohamed AKS, Zahran AM, Gad AM, Mekky AE, 2025. Antimicrobial, anti-inflammatory, anticancer and antiviral activity of bioactive compounds from *Pseudomonas aeruginosa* isolated from Mediterranean Sea, Alexandria, Egypt. Microbial Biosystems, 10(1):123–134.
- Mohan KD, Rajamanickam U, 2018. Biodiversity of actinomycetes and secondary metabolites. Innovative Origin International Journal of Science, 5(1):21–27.
- Mothana AA, Al-Shamahy HA, Mothana RA, Khaled JM, Al-Rehaily AJ, Al-Mahdi AY, Lindequist U, 2022. *Streptomyces* sp. 1S1 isolated from southern coast of the Red Sea as a renewable natural resource of several bioactive compounds. Saudi Pharmaceutical Journal, 30:162–171.
- Ngamcharungchit C, Chaimusik N, Panbangred W, Euanorasetr J, Intra B, 2023. Bioactive metabolites from terrestrial and marine actinomycetes. Molecules, 28(15):5915.
- Nisha SJ, Uma G, Sathishkumar R, et al., 2025. Optimization and characterization of bioactive secondary metabolites from *Streptomyces* sp. CMSTAAHL-4 isolated from mangrove sediment. BMC Microbiology, 25:57.
- Pan C, Hassan SSU, Ishaq M, Yan S, Jin H, 2025. Marine actinomycetes: A hidden treasure trove for antibacterial discovery. Frontiers in Marine Science, 12:1558320.
- Radwan M, Moussa MA, Manaa EA, El-Sharkawy MA, Darweesh KF, Elraey SM, Mekky AE, 2024. Synergistic effect of green synthesis magnesium oxide nanoparticles and seaweed extract on improving water quality, health benefits, and disease resistance in Nile tilapia. Ecotoxicology and Environmental Safety, 280:116522.
- Ramadan A, Abdel-Monem MO, El-DougDoug NK, Mekky AE, Elaskary SA, Al-Askar AA, Khedr M, 2024. Fully characterized effective bacteriophages specific against antibiotic-

- resistant *Enterococcus faecalis*, the causative agent of dental abscess. *Medicina*, 60(3):501.
- Rusu AV, Trif M, Rocha JM, 2023. Microbial secondary metabolites via fermentation approaches for dietary supplementation formulations. *Molecules*, 28(16):6020.
- Salwana R, Sharma V, 2020. Molecular and biotechnological aspects of secondary metabolites in actinobacteria. *Microbiological Research*, 231:126374.
- Sehim AE, Amin BH, Yosri M, Salama HM, Alkhalifah DH, Alwaili MA, Abd Elghaffar RY, 2023. GC–MS analysis, antibacterial, and anticancer activities of *Hibiscus sabdariffa* L. methanolic extract: In vitro and in silico studies. *Microorganisms*, 11(6):1601.
- Sherif MM, Elshikh HH, Abdel-Aziz MM, Elaasser MM, Yosri M, 2023. In vitro antibacterial and phytochemical screening of *Hypericum perforatum* extract as potential antimicrobial agents against multi-drug-resistant (MDR) strains of clinical origin. *BioMed Research International*, 6934398.
- Siddharth S, Vittal R, 2018. Evaluation of antimicrobial, enzyme inhibitory, antioxidant and cytotoxic activities of partially purified volatile metabolites of marine *Streptomyces* sp. S2A. *Microorganisms*, 6(3):72.
- Takahashi Y, Nakashima T, 2018. Actinomycetes, an inexhaustible source of naturally occurring antibiotics. *Antibiotics (Basel)*, 7(2):45.
- Tonisi S, Okaiyeto K, Hoppe H, Mabinya LV, Nwodo UU, Okoh AI, 2020. Chemical constituents, antioxidant and cytotoxicity properties of *Leonotis leonurus* used in the folklore management of neurological disorders in the Eastern Cape, South Africa. *3 Biotech*, 10:1–41.
- Usha KM, Sudhakar P, Sreenivasulu K, Vijayalakshmi M, 2011. Optimization of culturing conditions for improved production of bioactive metabolites by *Pseudonocardia* sp. VUK-10. *Mycobiology*, 39(3):174–181.
- Xu ZY, Lu HL, Shi WB, Zhou XM, Ren JX, Zhang YL, Ma R, 2024. Optimization of fermentation and biocontrol efficacy of *Bacillus atrophaeus* XHG-1-3m2. *Microorganisms*, 12:2134.
- Zhao S, Cheng M, Lin C, Liu H, Wang Z, Zhang K, Song S, Yang Q, 2021. *Streptomyces luteolifulvus* sp. nov., a novel actinomycete isolated from soil in Nanjing, China. *Antonie van Leeuwenhoek*, 114(11):1829–1839.