



## Original article

# Streptomyces isolate SOM013, a potential agent against microbial resistance and gastric ulcers

Kizito Eneye Bello<sup>a</sup>, Ahmad Adebayo Irekeola<sup>b,c</sup>, Ahmad A. Alshehri<sup>d,e,\*</sup>

<sup>a</sup> Department of Microbiology, Faculty of Natural Science, Kogi State (Prince Abubakar Audu) University, Anyigba, PMB 1008, Anyigba, Kogi State, Nigeria

<sup>b</sup> Department of Medical Microbiology and Parasitology, School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian, Kelantan 16150, Malaysia

<sup>c</sup> Microbiology Unit, Department of Biological Sciences, College of Natural and Applied Sciences, Summit University Offa, Offa PMB 4412, Kwara, Nigeria

<sup>d</sup> Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Najran University, P.O. Box 1988, Najran, Saudi Arabia

<sup>e</sup> Health research center, Najran University, P.O. Box 1988, Najran, Saudi Arabia

## ARTICLE INFO

## Keywords:

Streptomyces  
Antimicrobial  
Antiulcer  
Resistant bacteria  
Bioactive secondary metabolites

## ABSTRACT

The menace of microbial resistance and re-emerging disease is still a problem for healthcare givers globally, and the need for newer sources of potent antibiotics has become paramount. This study investigated the antimicrobial and antiulcer activities of Streptomyces isolate SOM013. Streptomyces isolates were cultivated and purified following standard microbiological protocols. Secondary metabolites were recovered and characterized from Streptomyces isolate SOM013 via broth fermentation and extraction. Varying concentrations (0.5 mg/mL, 0.025 mg/mL and 0.0125 mg/mL) of the SOM013 extract were used for antimicrobial screening against resistant bacteria and medically important fungi (methicillin-resistant *Escherichia coli*, Oxacillin resistant *Helicobacter pylori*, *Shigella spp.*, extended broad-spectrum resistant *Pseudomonas aeruginosa*, *Streptococcus spp.*, *Campylobacter spp.*, *Candida albicans*, *Aspergillus niger*, and *Aspergillus flavus*). The antiulcer activity of the SOM013 was also examined in a methanol-induced gastric ulcer animal model. A total of 23 Streptomyces spp were recovered from the study. Methanolic extract of the SOM013 isolates was more potent across the clinical test microorganisms compared to water extract. The antimicrobial activity was dose dependent, with methanolic extract at 0.05 g/mL displaying the highest zone of inhibition ( $18.8 \pm 0.3$  mm) when tested against extended broad-spectrum resistant *Pseudomonas aeruginosa*. Further, the extract's ulcer index and protection efficacy were significant as the concentration increased ( $P < 0.01$ ). SOM013 isolate has a moderate antimicrobial and high antiulcer activity worthy of pharmacological exploration.

## 1. Introduction

As highlighted and discussed in various research articles (Kim et al., 2022; Procópio et al., 2012; Salwan and Sharma, 2020), there has been a growing interest in examining marine sources to discover novel natural compounds with enormous medicinal potential. Microorganisms inhabiting maritime habitats have been recognized as prolific producers of secondary metabolites with significant pharmacological properties. These compounds have been extensively investigated in drug discovery projects. Actinobacteria have proven to be productive in this area, producing a wide range of bioactive compounds of significant industrial and medical use (Naine and Devi, 2014; Sathya et al., 2016). Notably, Actinobacteria is a classic repository of fresh bioactive substances, such

as antibiotics and enzymes (Quinn et al., 2020). They also produce diverse enzymes of medical importance (Nawani et al., 2013).

Marine actinobacteria are particularly attractive due to the diverse biological functions exhibited by their compounds. These compounds showcase a wide array of activities, encompassing antibacterial, antifungal, cytotoxic, neurotoxic, antimetabolic, antiviral, and antineoplastic properties (Kizito and Nwankwo, 2013; Quinn et al., 2020). However, certain marine actinobacteria strains are shrouded in mystery, as with many encouraging discoveries, making it difficult to determine their classification. Consequently, binomial identification presents several difficulties and frequently defies classification (Shirling and Gottlieb, 1966).

Over 55 % of antibiotics come from the main genus of actinobacteria,

\* Corresponding author at: Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Najran University, P.O. Box 1988, Najran, Saudi Arabia.

E-mail address: [aaalshehri@nu.edu.sa](mailto:aaalshehri@nu.edu.sa) (A.A. Alshehri).

<https://doi.org/10.1016/j.jsps.2024.102101>

Received 16 February 2024; Accepted 12 May 2024

Available online 13 May 2024

1319-0164/© 2024 The Authors. Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Streptomyces, which contains an astounding repertoire of more than 5000 compounds (Fouda et al., 2020). Notably, Streptomyces have often been recovered from marine sources, including decaying materials, sediments, and animals. This has sparked intensive research to understand its qualities. The findings regarding the Streptomyces species, *S. parvulus*, which thrives in both terrestrial and marine habitats and possesses a plethora of potentially bioactive compounds, including Actinomycin D and Manumycin A, some of which have already been successfully commercialized, are encouraging (Prashith and Onkarappa, 2015). Moreover, ongoing research efforts have resulted in the identification of bioactive metabolites, such as that from *Streptomyces coeruleorubidus* that exhibits notable antibacterial potency, thereby advancing clinical medicine and therapeutic strategies (Abdelaziz et al., 2024).

Certain compounds, particularly those produced by Actinobacteria such as Streptomyces, may possess both antimicrobial and antiulcer activity. This is pertinent, as identification of such bioactive compounds will not only combat infections but also improve gastrointestinal health. The organisms implicated in gastric ulcer have become resistant to known therapeutic strategies over the past few decades (Vu et al., 2022). The need for newer potent bioactive compounds is therefore crucial to resolve the antimicrobial menace and offer antiulcer protection to tissues (Sadigh-Eteghad et al., 2013; Vu et al., 2022).

Given that agricultural and nonagricultural soils are rich sources of Actinobacteria, and that these resources are still mostly unexplored, we investigated the antimicrobial and antiulcer activities of a Streptomyces isolate (SOM013) derived from soil, with the view to further highlighting the potentials and medical importance of this antibiotic producing genus (Streptomyces). Moreover, the problem of antimicrobial resistance underscores the urgent need to explore alternative sources of antimicrobial compounds. Further, the epidemiological burden of peptic and gastric ulcer (Ren et al., 2022) calls for proactive health interventions. Thus, finding effective treatments for ulcers, especially those with anti-inflammatory and mucosal protective properties, is essential for improving patient outcomes and reducing healthcare costs. Streptomyces and their bioactive compounds present a promising avenue for discovering antiulcer agents that can alleviate symptoms and promote ulcer healing (Ambarwati et al., 2020; Hassan and Shaikh, 2017).

## 2. Materials and methods

The protocols used in this study were approved by the Institutional Ethical Committee Board of Prince Abubakar University, Anyigba (approval ID: PAU/ethics/1023).

### 2.1. Soil sample collection

Soil samples were collected from multiple sites across Okene, Kogi State, Nigeria, during the period of January to March 2022. Streptomyces strains were identified in both agricultural and nonagricultural soil types originating from diverse locations, including farmlands, a garbage dump, a seashore, and a grassland. The samples were gathered from a depth of 20 cm after removing approximately 3 cm of the soil surface using a drill. They were then put in sterile polythene bags and labelled appropriately (Kizito and Nwankwo, 2013). Physical characteristics of the soil samples, like pH, were measured on the spot using a soil pH meter (OrionStar, Thermo-scientific, USA). The test terminal was dipped into the soil sample that was moistened with deionized water, and the pH was read and recorded appropriately. Ten soil samples were collected from all the monitoring sites throughout the survey. After air-drying for a period of ten days away from direct sunlight, the collected soil samples were transported to the laboratory for microbiological screening and further investigations (Tantithanagornkul et al., 2011). The dried soil samples were stored in sterile airtight sample collection container, away from direct sunlight at room temperature (27 °C – 32 °C). The samples were further screened in the laboratory.

### 2.2. Isolation of Streptomyces pure culture

Using standard microbiological methods, Streptomyces species were isolated utilizing the pour plate technique in a modified Czepadox agar medium. Out of the recovered isolates, isolate SOM013 which, in our preliminary study, exhibited better inhibitory activity against *Escherichia coli* and displayed evidence of diffusible pigmentation was selected for further screening. The isolation process began with the combination of 20 g of dry soil sample with 180 mL of distilled water, with the mixture swirled for a duration of 30 s.

The soil samples underwent pretreatment before being serially diluted up to a dilution factor of  $10^{-5}$ . Each dilution tube was vortexed to produce a consistent suspension. Each dilution tube was divided into duplicate 100  $\mu$ L aliquots, which were then plated and covered with a modified Czepadox agar (containing 200  $\mu$ g/mL of streptomycin and 100  $\mu$ g/mL of cycloheximide). Subsequently, the isolation media were incubated at ambient temperature (28–30 °C) for a week, simulating their normal environment. According to previous descriptions (Kizito and Nwankwo, 2013), the distinct colonies were counted in colony-forming units. Isolates were purified by streaking colonies on fresh plates of modified Czepadox agar using sterile wire loops. Pure cultures of isolated Streptomyces species were then transferred onto Czepadox slants and stored at 4 °C until further use.

In yeast malt extract and Czepadox agar, the morphological characteristic of the isolate such as color of the isolates on varying medium, the presence and shape of spores, substrate mycelium, elevation and edges were examined and recorded appropriately according to previous method (Kizito and Nwankwo, 2013; Shirling and Gottlieb, 1966). Spore-carrying hyphae and spore chains were analyzed by looking directly at the cultures under a microscope with an oil immersion objective. Additionally, the isolate underwent biochemical characterization utilizing Shirling and Gottlieb's techniques (Shirling and Gottlieb, 1966). For catalase test, the isolate was introduced into a drop of hydrogen peroxide and examined for bubbles. The isolate was also screened by employing Gram staining, coagulase, oxidase, citrate, nitrate, methyl red, lipid utilization, gelatin utilization, and sugar fermentation tests following standard procedure as previously described (Kizito and Nwankwo, 2013; Shirling and Gottlieb, 1966).

### 2.3. Molecular characterization

Chromosomal DNA was extracted, and polymerase chain reaction (PCR) was conducted. The template DNA was amplified in an Eppendorf PCR thermal cycler by using a set of published primers of 1  $\mu$ L of forward (5'-CGCGGCCTATCAGCTTGTG-3') and reverse primer (5'-CCGTACTCCCCAGGCGGG-3') to target the 16S rRNA gene of Streptomyces (Oloumi et al., 2023). The amplification was carried out in 30 cycles, denaturation for a minute at 91 °C, primer annealing for 1 min at 56 °C and extension for 5mins at 72 °C. The resulting amplicons were subjected to analysis via 1.5 % agarose gel electrophoresis (Quinn et al., 2020; Saygin et al., 2020). The amplicon with evidence of electrophoretic bands was sequenced and compared with other pools of organisms using the NCBI BLAST tool for ancestral genomic similarity pairing. A phylogenetic tree was constructed using the neighbor-joining method at a bootstrap of 1000 replicates in MEGA 11 software (Kumar et al., 2016).

### 2.4. Extraction of secondary metabolites

In this study, we aimed to extract secondary metabolites and identify potential bioactive compounds from Streptomyces spp, a promising source of natural antibiotics. A modified solid state fermentation process was used to extract the active biocides as previously described (Ganesan, 2018). The fermentation process involved inoculating Streptomyces spp. on Czepadox agar for five days at 28 °C. Subsequently, three 60 mL centrifuge tubes containing 20 mL of Czepadox broth medium were inoculated with Streptomyces colonies and incubated at 28 °C in a rotary

shaker incubator set at 130 rpm for a duration of 10 to 14 days. After that, six 500 mL conical flasks with cotton wool plugs containing 300 mL of Czepadox broth media were inoculated with the seed cultures. The fermentation process was conducted at 28 °C for two weeks at 130 rpm in a rotary incubator. The culture underwent Soxhlet extraction using sterile distilled water to extract the secondary metabolites. The resulting extract was then stored at – 20 °C until needed. Fresh stock solutions were prepared prior to each bioassay.

A 0.5 g powder was dissolved in 10 mL of distilled water to make a 0.05 g/mL concentration. Two-fold dilutions was performed up to a factor of 0.025 g/mL and 0.0125 g/mL by adding 10 mL of distilled water to the initial concentration (Log<sub>2</sub>) (Ambarwati et al., 2020).

## 2.5. GC–MS analysis

To accomplish the GC–MS analysis of the ethyl acetate extract, an electron ionization apparatus with ionizing energy of 70 eV was used. With a split ratio 10:1 and injection volumes of 3 L, helium gas (99.9 % purity) was used as the carrier gas at a constant flow rate of 1 mL/min at ion source temperatures of 280 °C. The oven temperature was programmed as follows: starting from 110 °C, it remained isothermal for 2 min. Then, it increased at a rate of 10 °C per minute until reaching 200 °C, without any hold. Subsequently, the temperature was raised at a rate of 5 °C per minute until reaching 280 °C. Finally, a 9-minute isothermal period at 280 °C concluded the process (Kathirvel and Sujatha, 2016). The Turbomass software was utilized to compare the average peak area of each component to the total areas, thereby determining the relative percentage amount of each component. Additionally, the mass spectrum of the GC–MS was interpreted using the National Institute of Standards and Technology's database (NIST) (Wu and Yates, 2003).

## 2.6. Antimicrobial spectrum of isolates

The antimicrobial activity of the isolates was tested using medically important bacteria, including those implicated in ulcer (*Helicobacter pylori*, *Escherichia coli*, *Campylobacter spp* and *Streptococcus spp*) and some fungi (*Candida albicans*, *Aspergillus niger* and *Aspergillus flavus*). The test microorganisms were retrieved from the type culture collection of the Department of Microbiology, Kogi State (Prince Abubakar Audu) University, Anyigba, Nigeria. The antimicrobial screening of the test microorganism was the primary screening. In the primary screening, the test microorganism was tested (streaked) perpendicularly against the *Streptomyces* isolate (five days old culture) on nutrient agar and incubated for a day at room temperature (30 °C) according to previous methods (Kizito and Nwankwo, 2013). The above method was repeated for fungal test microorganism in a Potato Dextrose Agar and incubated at 28 °C. The antimicrobial activity of the *Streptomyces* isolates was measured to the nearest millimeters and recorded appropriately.

## 2.7. Antiulcer activity of the streptomyces secondary metabolite extract

This study involved fifteen male albino rats, each six weeks old and weighing between 215 and 270 g. The rats were housed in the animal facility located within the Natural Science Faculty at Kogi State University, Anyigba, Nigeria. They were subjected to a 12-hour light–dark cycle throughout the experiment and were maintained at a room temperature ranging from 25 °C to 30 °C. The rats were kept in an environment with a relative humidity varying from 55 % to 70 %. Adequate provisions of food and water were continuously made available to the animals throughout the duration of the study. For the actual experiment, the rats were randomly placed into five groups (three rats per group), fasted for 24 h, and given a vehicle (physiological saline) containing the aqueous extract concentrate (secondary metabolites). The secondary metabolites from *Streptomyces* isolates were given orally to three groups of rats at 100 mg/kg, 200 mg/kg, and 300 mg/kg, respectively.

The administered dosage is as recommended by the findings of others (Gupta and Rao, 2014; Rujjanawate et al., 2005) and is within the range of reference drug dosages for the treatment of peptic and gastric ulcer. The negative control group received physiological saline while the positive control group received ranitidine at 20 mg/kg (Yeomans et al., 2006). The intervention was administered for 4 weeks. Thereafter, animals were killed by cervical dislocation one hour after receiving 1 mL of 80 per cent ethanol orally. One hour after the ethanol administration, the stomachs were isolated, sliced open along the greater curvature, and pinned on a soft board. Each gastric lesion's length was measured, and the lesion index was calculated as the sum of those lengths in millimeters (Veraldi et al., 2021).

## 2.8. Data analysis

Descriptive statistics and measures of central tendency were used to present and analyze the data. The Chi-square tool was used to assess the measurement of the association between antiulcer activity and the concentration of secondary metabolites. A confidence level was 95 % with a probability value of 0.05.

## 3. Results

### 3.1. Characteristics of *Streptomyces* isolate SOM013

A total of twenty-three (23) *Streptomyces*-like isolates were recovered from the varying sample sources. There were more *Streptomyces* isolates from Ihima than the other locations (47.8 % [n = 11]) as represented in Fig. 1. Out of the 23 recovered *Streptomyces* isolates, SOM013 was selected for further screening based on the preliminary findings on SOM013, as it exhibited better inhibitory activity against *Escherichia coli* and displayed evidence of diffusible pigmentation which indicates high bioactive compound secretion. This study presents the antimicrobial activity of the *Streptomyces* isolate (SOM013) and the antiulcer activity of the extract on methanol-induced gastric ulcer animal model. The morphological characteristics of the isolates varied across different media. The aerial substrate mycelium ranged from white to grey and cream to yellow, respectively (Table 1).

SOM013 was non-reactive to coagulase test, oxidase and nitrate utilization but was reactive to catalase, Gram reaction, urease, and citrate, as represented in Table 2. There were disparities in the utilization of sugar, protein, and lipid (Table 2).

The recovered blast results from the NCBI database blast revealed that the *Streptomyces* isolate SOM013 sequence aligned largely with *Streptomyces anulatus*, with up to 99.81 % identity (Supplementary File S1). Further, phylogenetic analysis of the sequence revealed that SOM013 belong to the strain *Streptomyces anulatus* (Fig. 2).

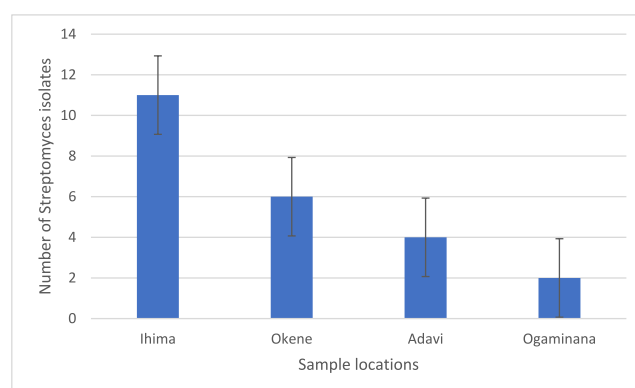


Fig. 1. Number of isolated *Streptomyces* in relation to sample location.

**Table 1**  
Morphological characteristics of *Streptomyces* isolate SOM013 on two media.

| Agar medium             | Appearance     | Elevation | Edge   | Arial mycelium | Substrate mycelium | Diffusible pigment |
|-------------------------|----------------|-----------|--------|----------------|--------------------|--------------------|
| Czepadex agar           | dry and smooth | convex    | entire | white grey     | yellow             | oxblood            |
| Yeast Malt Extract Agar | dry and smooth | convex    | entire | white          | cream              | brown              |

**Table 2**  
Biochemical and sugar utilization of *Streptomyces* isolate SOM013.

| S/N | Biochemical and sugar utilization tests | Isolate |
|-----|---|---------|
| 1   | Catalase                                | +ve     |
| 2   | Gram reaction                           | +ve     |
| 3   | Coagulase                               | -ve     |
| 4   | Urease                                  | +ve     |
| 5   | Oxidase                                 | -ve     |
| 6   | Citrate                                 | +ve     |
| 7   | Nitrate utilization                     | -ve     |
| 8   | Methyl red                              | +ve     |
| 9   | Starch utilization                      | +ve     |
| 10  | Xylose utilization                      | -ve     |
| 11  | Lipid utilization                       | +ve     |
| 12  | Gelatin utilization                     | +ve     |
| 13  | Arabinose utilization                   | -ve     |

3.2. Metabolites of *Streptomyces* isolate SOM013

The potential bioactive compounds in the extracted secondary metabolites were recovered after a series of extraction and fermentation processes. GC-MS analysis revealed the presence of steroids, alkaloids, and phenol moiety. The recovered compounds were pure. Details of the compounds present in the metabolites are represented in Table 3.

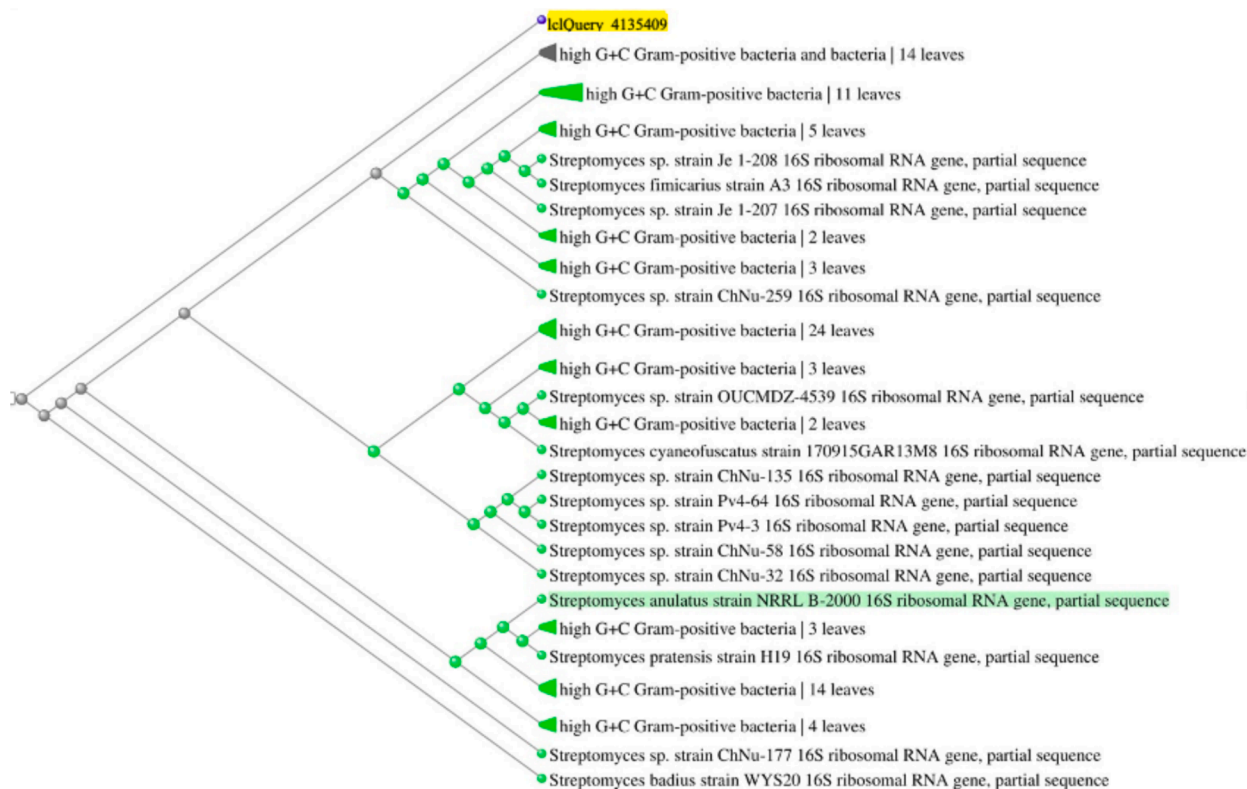
3.3. Antimicrobial properties of *Streptomyces* isolate SOM013

The antimicrobial spectra of SOM013 were evaluated using clinically resistant bacteria and medically important fungi across three different extract concentrations. The zone of inhibition, a measure of antimicrobial activity, was assessed and interpreted.

The inhibitory effects of the extract against the test microorganisms were generally found to be dose-dependent, with the methanolic extract displaying higher zones of inhibition compared to the water extract (Table 4). Moderate zone of inhibition was observed when the highest concentration of the methanolic extract (0.05 g/mL) was tested against methicillin-resistant *Escherichia coli* (15.8 ± 0.4 mm) and *Streptococcus spp* (15.7 ± 0.4 mm). Additionally, moderate zones were observed for more than one concentration of the investigated methanolic extract

**Table 3**  
Identified active secondary metabolite of *Streptomyces* isolate SOM013.

| S/ N | Name of compound   | Molecular mass | Molecular formula   | Nature of compound |
|------|--|----------------|---|--------------------|
| 1    | Ethyl cholate  | 436            | C <sub>26</sub> H <sub>44</sub> O <sub>5</sub>                | Steroid            |
| 2    | 3-(2-methyl propyl)-2,3,6,7,8,8a-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione | 210            | C <sub>11</sub> H <sub>15</sub> N <sub>2</sub> O <sub>2</sub> | Alkaloids          |
| 3    | Docosyl ferulate   | 503            | C <sub>32</sub> H <sub>54</sub> O <sub>4</sub>                | Steroid            |
| 4    | 2,4-Di-tert butyl phenol   | 206            | C <sub>14</sub> H <sub>22</sub> O                             | Phenol             |



**Fig. 2.** A Neighbor joining a slightly branched phylogenetic tree of SOM013 at bootstrap 1000 replicate. Query 4135409, highlighted in yellow, represents SOM013, and *Streptomyces anulatus*, highlighted in green, shows the highest similarity index.

**Table 4**  
Antimicrobial spectrum of *Streptomyces* isolate SOM013.

| S/N | Test microorganism  | Zone of inhibition (in mm) |            |             |            |             |           |
|-----|---|----------------------------|------------|-------------|------------|-------------|-----------|
|     |   | 0.05 g/mL                  |            | 0.025 g/mL  |            | 0.0125 g/mL |           |
|     |   | Methanol                   | Water      | Methanol    | Water      | Methanol    | Water     |
| 1   | Methicillin-resistant <i>Escherichia coli</i>                   | 15.8 ± 0.4*                | 11.2 ± 0.3 | 13.2 ± 0.2  | 10.5 ± 0.4 | 9.4 ± 0.5   | 5.3 ± 0.2 |
| 2   | Oxacillin-resistant <i>Helicobacter pylori</i>                  | 17.4 ± 0.3*                | 11.9 ± 0.2 | 14.9 ± 0.1* | 9.6 ± 0.2  | 10.4 ± 0.3  | 6.2 ± 0.6 |
| 3   | <i>Shigella</i> spp   | 14.4 ± 0.2                 | 9.3 ± 0.4  | 12.7 ± 0.3  | 8.1 ± 0.1  | 12.1 ± 0.1  | 7.7 ± 0.2 |
| 4   | Extended broad-spectrum resistant <i>Pseudomonas aeruginosa</i> | 18.8 ± 0.3*                | 12.5 ± 0.3 | 15.3 ± 0.6* | 10.7 ± 0.4 | 15.8 ± 0.2* | 9.4 ± 0.3 |
| 5   | <i>Streptococcus</i> spp  | 15.7 ± 0.4*                | 10.7 ± 0.2 | 12.6 ± 0.5  | 7.9 ± 0.1  | 10.7 ± 0.6  | 7.2 ± 0.2 |
| 6   | <i>Campylobacter</i> spp  | 13.6 ± 0.3                 | 10.2 ± 0.1 | 11.1 ± 0.3  | 8.3 ± 0.2  | 10.9 ± 0.4  | 6.8 ± 0.4 |
| 7   | <i>Candida albicans</i>   | 17.8 ± 0.3                 | 9.7 ± 0.4  | 14.7 ± 0.3  | 8.8 ± 0.4  | 12.9 ± 0.2  | 7.1 ± 0.2 |
| 8   | <i>Aspergillus niger</i>  | 13.8 ± 0.3                 | 10.7 ± 0.4 | 11.2 ± 0.3  | 8.2 ± 0.3  | 10.1 ± 0.4  | 7.4 ± 0.1 |
| 9   | <i>Aspergillus flavus</i>                                       | 14.2 ± 0.5                 | 11.4 ± 0.3 | 10.8 ± 0.1  | 7.1 ± 0.2  | 9.5 ± 0.5   | 5.4 ± 0.2 |

Antimicrobial spectra values were expressed as mean ± SEM, \* Intermediate activity.

when tested against oxacillin-resistant *Helicobacter pylori* and extended broad-spectrum resistant *Pseudomonas aeruginosa* and (Table 4). There was low to no antifungal activity.

### 3.4. Antiulcer properties of *Streptomyces* isolate SOM013

The antiulcer test results outline the effects of different treatments on ulcer index and protection percentage in the experimental groups (Fig. 3). The ulcer index serves as a measure of ulcer severity, while the protection percentage indicates the level of therapeutic effectiveness in mitigating ulcers. In the control group where physiological saline was administered, the ulcer index was recorded at  $4.24 \pm 0.19$ . This baseline value sets the context for evaluating the efficacy of the other treatments. Ranitidine, administered at a dosage of 20 mg/kg, exhibited a remarkable reduction in ulcer index to  $0.39 \pm 0.05$ , reflecting a substantial 90.80 % protection against ulcers. The significant reduction in ulcer severity following administration of ranitidine in comparison with control ( $P < 0.01$ ) underscores the potency of ranitidine as a gastro-protective agent (Fig. 3).

The experimental groups receiving the SOM013 extract were also compared with control. Group 1, treated with 100 mg/kg of the extract, yielded an ulcer index of  $1.95 \pm 0.15$ , corresponding to a protection rate

of 54.01 %. This result suggests a moderate protective effect. Group 2, administered with 200 mg/kg of the extract, displayed a significant decreased ulcer index of  $1.66 \pm 0.18$  ( $P < 0.01$ ), translating to a protection rate of 60.85 % (Fig. 3). The most substantial protective effect was observed in group 3, treated with 300 mg/kg of the extract. The ulcer index dropped to  $1.27 \pm 0.16$  ( $P < 0.01$ ), signifying a notable 70.05 % protection against ulcers. This outcome underscores the potential of higher dosages to enhance gastroprotective effects (Fig. 3).

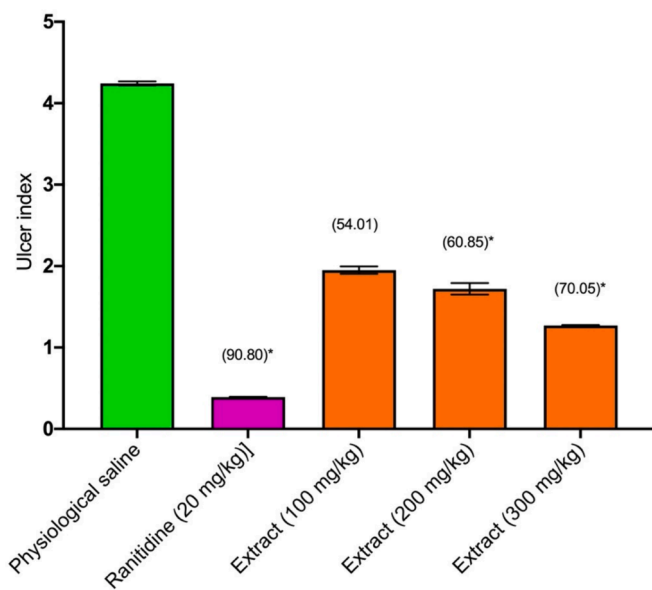
## 4. Discussion

The search for alternative and effective agents against pathogens of medical importance is a non-ending voyage. In this study, the antimicrobial and antiulcer potentials of extracts from *Streptomyces* spp were explored. The biochemical spectra of the isolate revealed a positive catalase test and a positive reaction to Gram staining test. This adaptation suggests an aerobic metabolism, allowing the isolate to tolerate oxidative stress and thrive in oxygen-rich environments. A positive Gram reaction suggests a thick peptidoglycan layer in the cell wall, characteristic of Gram-positive bacteria. This structural feature can influence cell permeability and nutrient acquisition. The sugar utilization pattern reveals that the *Streptomyces* isolates could utilize starch. The findings of this study are consistent with the report of others (Kim et al., 2022; Procópio et al., 2012).

The identification of active secondary metabolites within natural sources is of paramount importance for various scientific disciplines, including pharmacology, ecology, and biochemistry (Kim et al., 2022; Yamin et al., 2023). *Streptomyces* are known bioactive antibiotic producers, and our study reveals the presence of active biomolecules of different moieties, ranging from steroids to alkaloids. The presence of ethyl cholate in our extracted secondary metabolite is consistent with the report of others (Kim et al., 2022; Rajendran et al., 2023; Shirling and Gottlieb, 1966; Singh and Prakash, 2012). Steroids have been well-recognized for their versatile roles in numerous physiological processes such as cell signaling, membrane stability, and hormonal regulation.

The presence of an alkaloid (3-(2-Methylpropyl)-2,3,6,7,8,8a-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione) unveils alkaloid complexity and biological activity. The structural complexity of this compound hints at a rich potential for bioactivity, underscoring the importance of investigating its effects and potential applications. The findings of this study is in line with the reports of others (Hu et al., 1994; Singh and Prakash, 2012; Weisberg et al., 2021). Further, the presence of 2,4-Di-tert Butylphenol in our extract requires investigating its potential contribution to antioxidant defenses and other biological functions.

In this study, we examined the zone of inhibition exhibited by different test microorganisms at varying concentrations of methanol and water extracts. The inhibition zones were measured at three concentrations: 0.05 g/mL, 0.025 g/mL, and 0.0125 g/mL. Our analysis aimed to explore the potential antimicrobial activity of the extracts against a panel of resistant bacterial and fungal strains.



**Fig. 3.** Antiulcer activity of *Streptomyces* isolate SOM013. Five groups of albino rats (3 per group) were administered physiological saline (control group), ranitidine, or extracts of different concentrations. Ulcer-indexed values are expressed as mean ± SEM. Values in parenthesis represent percentage protection, which were calculated as compared to the control group. \* $p < 0.01$  indicates statistically significant protection.

Overall, the methanolic extract demonstrates greater inhibitory effects than the water extract, although the inhibitory activities were only moderate. This could be attributed to the higher solubility of bioactive compounds in methanol, leading to enhanced bioavailability and subsequently stronger antimicrobial activity. This study's findings are consistent with the report of others (Raguvaran et al., 2022; Sumithra et al., 2023). The zones of inhibition were dose-dependent responses, with higher concentrations generally resulting in larger inhibition zones. This aligns with the concentration-dependent antimicrobial effect, wherein higher concentrations of extract lead to increased inhibitory action. The findings of this study are in compliance with the reports of others (Fouda et al., 2020; Raguvaran et al., 2022; Sumithra et al., 2023).

Furthermore, among the microorganisms tested, our results indicate a contrast between bacteria and fungi. Among the bacterial strains, methicillin-resistant *Escherichia coli*, oxacillin-resistant *Helicobacter pylori*, extended broad-spectrum resistant *Pseudomonas aeruginosa* and *Streptococcus spp* were moderately inhibited at the highest concentration of methanolic extract. However, at lower concentrations, moderate inhibitory activity was observed in fewer test microorganism. This could imply the potential effectiveness of the extracts against these drug-resistant pathogens, especially at a higher concentration. The findings of this report are consistent with previous studies that documented the effectiveness of *Streptomyces* isolates against clinical isolates (Kizito and Nwankwo, 2013).

The antiulcer properties of the examined extract were remarkable, particularly at higher concentrations. Although the extract exhibited lower efficacy compared to ranitidine, it still demonstrated significant antiulcer effects at concentrations of 200 mg/kg and higher. The observed dose-dependent response suggest enhanced protective influence against ulcer formation (Brito et al., 2018; de Carvalho et al., 2011). Additionally, it emphasizes the significance of appropriate dosage selection in achieving optimal protective effects.

Our study does have limitations. While the results provide valuable insights into the inhibitory potentials of the extracts, further investigations are warranted to identify the specific mechanism of action of the active compounds responsible for these effects. Moreover, we did not investigate the antibacterial activity of the pure compounds using an approach such as the microdilution assay. Additionally, we assigned three animals per group in the antiulcer experiments. Larger size would help strengthen the confidence in the results. We, however, think our preliminary data would support future research. Lastly, the ecological conditions underlying the observed variations in antimicrobial activity warrant deeper exploration.

## 5. Conclusion

Considering the need for alternative and novel medicines, this research explored the antimicrobial and antiulcer properties of *Streptomyces* isolate SOM013. It was found that the methanolic extract from SOM013 displayed greater potency against clinical test microorganisms compared to the water extract. Notably, the methanolic extract of the isolate exhibited moderate antimicrobial activity against medically important pathogens, including those implicated in ulcer such as *Helicobacter pylori*, *Escherichia coli*, and *Streptococcus spp*. Additionally, the extract demonstrated significant reduction in ulcer index and protection efficacy, with notable improvements as the concentration increased. The SOM013 isolate exhibits robust antimicrobial and antiulcer activities, warranting further exploration in pharmacological studies. More studies, especially on the antiulcer potentials of *Streptomyces* are required to further validate the findings of our work.

## CRedit authorship contribution statement

**Kizito Eneye Bello:** Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis, Data curation,

Conceptualization. **Ahmad Adebayo Irekeola:** Writing – review & editing, Writing – original draft, Methodology, Conceptualization. **Ahmad A. Alshehri:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization.

## Declaration of competing interest

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.

## Acknowledgement

The authors are thankful to Dr. Zakari David for providing the samples for this study. The authors are also grateful for the invaluable support provided by Mr. Godwin Audu Amoka from the Department of Biochemistry, Kogi State University, Anyigba, for his assistance in conducting the animal sacrifices. The authors are thankful to the Deanship of Graduate Studies and Scientific Research at Najran University for funding this work under the Najran Research Funding Program grand code (NU/GP/MRC/13/193-1).

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jpsps.2024.102101>.

## References

- Abdelaziz, R., Elsheshtawy, H.M., El-Houseiny, W., Aloufi, A.S., Alwutayd, K.M., Mansour, A.T., Hadad, G., Arisha, A.H., El-Murr, A.E., Yassin, A.M., 2024. A novel metabolite of *Streptomyces coeruleorubidus* exhibits antibacterial activity against *Streptococcus agalactiae* through modulation of physiological performance, inflammatory cytokines, apoptosis, and oxidative stress-correlated gene expressions in Nil. Fish Shellfish Immunol. 148, 109496 <https://doi.org/10.1016/j.fsi.2024.109496>.
- Ambarwati, A., Wahyuono, S., Moeljopawiro, S., Yuwono, T., 2020. Antimicrobial activity of ethyl acetate extracts of *Streptomyces* sp. CRB46 and the prediction of their bioactive compounds chemical structure. Biodiversitas 21, 3380–3390. <https://doi.org/10.13057/biodiv/d210763>.
- Brito, S.A., de Almeida, C.L.F., de Santana, T.I., da Silva Oliveira, A.R., do Nascimento Figueiredo, J.C.B., Souza, I.T., de Almeida, L.L., da Silva, M.V., Borges, A.S., de Medeiros, J.W., da Costa Silva Neto, J., de Cássia Ribeiro Gonçalves, R., Kitagawa, R. R., Sant'Ana, A.E.G., Rolim, L.A.de, Menezes, I.R.A., da Silva, T.G., Caldas, G.F.R., Wanderley, A.G., 2018. Antiulcer Activity and Potential Mechanism of Action of the Leaves of *Spondias mombin* L. Oxid. Med. Cell. Longev. 2018 <https://doi.org/10.1155/2018/1731459>.
- de Carvalho, C.A., Fernandes, K.M., Matta, S.L.P., da Silva, M.B., de Oliveira, L.L., Fonseca, C.C., 2011. Evaluation of antiulcerogenic activity of aqueous extract of *Brassica oleracea* var. capitata (cabbage) on Wistar rat gastric ulceration. Arq. Gastroenterol. 48, 276–282.
- Fouda, A., Hassan, S.E.D., Abdo, A.M., El-Gamal, M.S., 2020. Antimicrobial, antioxidant and larvicidal activities of spherical silver nanoparticles synthesized by endophytic streptomycetes spp. Biol. Trace Elem. Res. 195, 707–724. <https://doi.org/10.1007/s12011-019-01883-4>.
- Ganesan, P., 2018. Larvicidal, ovicidal and repellent activities of *Streptomyces enissocaesilis* (S12–17) isolated from Western Ghats of Tamil Nadu India. J. Entomol. Zool. Stud. 6, 1828–1835.
- Gupta, P.C., Rao, C.V., 2014. Gastroprotective effect of standardized leaf extract from *Careya arborea* on experimental gastric ulcers in rats. Pharm. Biol. 52, 1003–1008. <https://doi.org/10.3109/13880209.2013.876052>.
- Hassan, S.S.U., Shaikh, A.L., 2017. Marine actinobacteria as a drug treasure house. Biomed. Pharmacother. 87, 46–57. <https://doi.org/10.1016/j.biopha.2016.12.086>.
- Hu, Z., Bao, K., Zhou, X., Zhou, Q., Hopwood, D.A., Kieser, T., Deng, Z., 1994. Repeated polyketide synthase modules involved in the biosynthesis of a heptaene macrolide by *Streptomyces* sp. FR-008. Mol. Microbiol. 14, 163–172. <https://doi.org/10.1111/j.1365-2958.1994.tb01276.x>.
- Kathirvel, A., Sujatha, V., 2016. Phytochemical studies, antioxidant activities and identification of active compounds using GC–MS of *Dryopteris cochleata* leaves. Arab. J. Chem. 9, S1435–S1442. <https://doi.org/10.1016/j.arabjc.2012.03.018>.
- Kim, S.J., Cantrell, C.L., Avula, B., Chen, J., Schrader, K.K., Santo, S.N., Ali, A., Khan, I. A., 2022. *Streptomyces distallicus*, a potential microbial biolarvicide. J. Agric. Food Chem. 70, 11274–11280. <https://doi.org/10.1021/acs.jafc.2c03537>.
- Kizito, B.E., Nwankwo, E., 2013. Antibiotic activity of *Streptomyces* isolates collected from soil of Kogi Central. Nigeria. IOSR J. Pharm. Biol. Sci. 8, 78–84.

- Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* 33, 1870–1874. <https://doi.org/10.1093/MOLBEV/MSW054>.
- Naine, S.J., Devi, C.S., 2014. Larvicidal and repellent properties of streptomyces sp. VITJS4 crude extract against anopheles stephensi, aedes aegypti and culex quinquefasciatus (Diptera: Culicidae). *Polish J. Microbiol.* 63, 341–348. <https://doi.org/10.33073/pjm-2014-045>.
- Nawani N, Aigle B, Mandal A, Bodas M, Ghorbel S, Prakash D. (2013). Actinomycetes: role in biotechnology and medicine. *Biomed Res. Int.* doi: 10.1155/2013/687190.
- Oloumi, H., Khaleghi, M., Dalvand, A., 2023. Isolation and identification of endophytic actinobacteria from *Iris persica* and *Echium amoenum* plants and investigation of their effects on germination and growth of wheat plant. *Food Sci. Nutr.* 11, 5296–5303. <https://doi.org/10.1002/fsn3.3488>.
- Prashith, K.T.R., Onkarappa, R., 2015. Bioactivities of streptomyces species from soils of western ghats of Karnataka. *India. J. Chem. Pharm. Res.* 7, 181–189.
- Procópio, R.E. de L., Silva, I.R. da, Martins, M.K., Azevedo, J.L.de, Araújo, J.M. de, 2012. Antibiotics produced by *Streptomyces*. *Brazilian J. Infect. Dis.* 16, 466–471.
- Quinn, G.A., Banat, A.M., Abdelhameed, A.M., Banat, I.M., 2020. Streptomyces from traditional medicine: sources of new innovations in antibiotic discovery. *J. Med. Microbiol.* 69, 1040–1048. <https://doi.org/10.1099/jmm.0.001232>.
- Raguvaran, K., Kalpana, M., Manimegalai, T., Maheswaran, R., 2022. Larvicidal, antibacterial, antibiofilm, and anti-quorum sensing activities of silver nanoparticles biosynthesized from streptomyces sclerotialus culture filtrate. *Mater. Lett.* 316 <https://doi.org/10.1016/j.matlet.2022.132000>.
- Rajendran, K., Krishnamoorthy, M., Karupiah, K., Ethiraj, K., Sekar, S., 2023. Chitinase from streptomyces mutabilis as an effective eco-friendly biocontrol agent. *Appl. Biochem. Biotechnol.* <https://doi.org/10.1007/s12010-023-04489-8>.
- Ren, J., Jin, X., Li, J., Li, R., Gao, Y., Zhang, J., Wang, X., Wang, G., 2022. The global burden of peptic ulcer disease in 204 countries and territories from 1990 to 2019: a systematic analysis for the global burden of disease study 2019. *Int. J. Epidemiol.* 51, 1666–1676. <https://doi.org/10.1093/ije/dyab033>.
- Rujjanawate, C., Kanjanapothi, D., Amornlerdpison, D., Pojanagaroon, S., 2005. Anti-gastric ulcer effect of *Kaempferia parviflora*. *J. Ethnopharmacol.* 102, 120–122. <https://doi.org/10.1016/J.JEP.2005.03.035>.
- Sadigh-Eteghad, S., Dehnad, A., Mahmodi, J., Hoseyni, H., Khalili, I., Razmaray 2nd, N., 2013. Healing potential of a *Streptomyces* sp. secondary metabolite, SEM-1-111, on experimental full-thickness excision cutaneous wounds in Wistar rats. *Clin. Exp. Dermatol.* 38, 178–184. <https://doi.org/10.1111/ced.12026>.
- Salwan R., Sharma V., (2020). Chapter 15 - Bioactive compounds of Streptomyces: Biosynthesis to applications, in: Atta-Ur-Rahman, B.T.-S. in N.P.C. (Ed.), *Bioactive Natural Products*. Elsevier, pp. 467–491. doi: 10.1016/B978-0-12-817903-1.00015-2.
- Sathya, A., Vijayabharathi, R., Kumari, B.R., Srinivas, V., Sharma, H.C., Sathyadevi, P., Gopalakrishnan, S., 2016. Assessment of a diketopiperazine, cyclo(Trp-Phe) from *Streptomyces griseoplanus* SAI-25 against cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae). *Appl. Entomol. Zool.* 51, 11–20. <https://doi.org/10.1007/s13355-015-0366-3>.
- Saygin, H., Ay, H., Guven, K., Cetin, D., Sahin, N., 2020. *Streptomyces cahuitamycinicus* sp. nov., isolated from desert soil and reclassification of *Streptomyces galilaeus* as a later heterotypic synonym of *Streptomyces bobili*. *Int. J. Syst. Evol. Microbiol.* 70, 2750–2759. <https://doi.org/10.1099/IJSEM.0.004103>.
- Shirling, E.B., Gottlieb, D., 1966. Methods for characterization of streptomyces species. *Int. J. Syst. Bacteriol.* 16, 313–340. <https://doi.org/10.1099/00207713-16-3-313>.
- Singh, G., Prakash, S., 2012. Lethal effect of *Streptomyces citreofluorescens* against larvae of malaria, filaria and dengue vectors. *Asian Pac. J. Trop. Med.* 5, 594–597. [https://doi.org/10.1016/s1995-7645\(12\)60123-0](https://doi.org/10.1016/s1995-7645(12)60123-0).
- Sumithra, D., Bharathi, S., Kaviyaran, P., Suresh, G., 2023. Biofabrication of selenium nanoparticles using marine streptomyces sp. and assessment of its antibacterial, antibiofilm, antioxidant, and in Vivo cytotoxic potential. *Geomicrobiol. J.* <https://doi.org/10.1080/01490451.2023.2196280>.
- Tantithanagonggul, W., Sujitwanit, A., Piluk, J., Tolieng, V., Petsom, A., Sangvanich, P., Palaga, T., Puthong, S., Thamchaipenet, A., Pinphanichakarn, P., 2011. Screening for brine shrimp larvicidal activity of streptomyces isolated from soil and anti-tumor activity of the active isolates. *Aust. J. Basic Appl. Sci.* 5, 15–22.
- Veraldi, S., Faraci, A.G., Valentini, D., Bottini, S., 2021. Tropical ulcers: the first imported cases and review of the literature. *Eur. J. Dermatology* 31, 75–80. <https://doi.org/10.1684/EJD.2021.3968>.
- Vu, T.B., Tran, T.N.Q., Tran, T.Q.A., Vu, D.L., Hoang, V.T., 2022. Antibiotic resistance of helicobacter pylori in patients with peptic ulcer. *Medicina (kaunas)*. 59 <https://doi.org/10.3390/medicina59010006>.
- Weisberg, A.J., Kramer, C.G., Kotha, R.R., Luthria, D.L., Chang, J.H., Clarke, C.R., 2021. A novel species-level group of streptomyces exhibits variation in phytopathogenicity despite conservation of virulence loci. *Mol. Plant-Microbe Interact.* 34, 39–48. <https://doi.org/10.1094/MPMI-06-20-0164-R>.
- Wu, C.C., Yates, J.R., 2003. The application of mass spectrometry to membrane proteomics. *Nat Biotechnol* 21, 262–267. <https://doi.org/10.1038/nbt0303-262>.
- Yamin, D., Uskoković, V., Wakil, A.M., Goni, M.D., Shamsuddin, S.H., Mustafa, F.H., Alfouzan, W.A., Alissa, M., Alshengeti, A., Almaghribi, R.H., Fares, M.A.A., Garout, M., Al Kaabi, N.A., Alshehri, A.A., Ali, H.M., Rabaan, A.A., Aldubisi, F.A., Yean, C.Y., Yusof, N.Y., 2023. Current and future technologies for the detection of antibiotic-resistant bacteria. *Diagnostics (basel, Switzerland)* 13. <https://doi.org/10.3390/diagnostics13203246>.
- Yeomans, N.D., Svedberg, L.-E., Naesdal, J., 2006. Is ranitidine therapy sufficient for healing peptic ulcers associated with non-steroidal anti-inflammatory drug use? *Int. J. Clin. Pract.* 60, 1401–1407. <https://doi.org/10.1111/j.1742-1241.2006.01147.x>.