



Bioactive Metabolites in a New Strain of *Streptomyces griseus* RH250 from Marl Clay in Mosul/Iraq

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ABSTRACT

Identification of the bioactive secondary metabolites of a crude butanol extract from a locally isolated strain of *Streptomyces griseus* RH250 isolated from marl clay in Mosul/ Iraq. This new strain possesses the ability to produce various kinds of metabolites, it was screened for it is antibacterial activity against Gram-negative *Alcaligenes faecalis*, *Pseudomonas fluorescens*, *Serratia marcescens*, and *Escherichia coli* and Gram-positive *Staphylococcus aureus*. Screening was performed using the agar-cross streak method, and the growth inhibition line was measured in millimeters. This strain has shown potent *in vitro* antibacterial activities against *Staphylococcus aureus* (25 mm) only. The agar diffusion well technique for crude n-butanol extract was performed. Gas chromatography-mass-mass spectrometry was used to determine bioactive secondary metabolites in crude butanol extract. This analysis showed that twenty-one bioactive metabolite compounds were isolated from this strain, which have different chemical structures and biological activities.

Keywords: Biological activity, cross streak, crude butanol extract, *Streptomyces griseus* RH250.

INTRODUCTION

Biological actives, secondary metabolites produced by Actinobacteria. More than 50% of bioactive secondary metabolites were recognized by these bacteria (Subathra *et al.*, 2022). Actinobacteria, including Streptomyces, are widely spread in ecosystems like soil, water, desert, and volcanic...etc. These bacteria have a common characteristic with fungi and bacteria. They play an important role in the decomposition of complex organic materials by degrading organic waste produced by living organisms, which makes it easier to recycle. The ability of Streptomyces to generate biologically active metabolites with a wide range of biological and chemical structures makes them important bacteria (Anandan *et al.*, 2016). According to Olarewaju and Babalola (2019), streptomyces, the most widespread and significant genus, stands out as a source of bioactive compounds, antibiotics, and extracellular enzymes. These bacteria were classified within the phylum of actinobacteria, are distributed broadly, and recognized by their ability to synthesize a broad spectrum of secondary metabolites, for example, antibiotics (Bennett *et al.*, 2018). *Streptomyces griseus* is a filamentous bacterium that have diverse secondary metabolites with a biological effect such as anti-cancer, biocontrol products (The plant growth-promoting), antibiotics (streptomycin, macrotetrolide: Nonactin) (Řezanka *et al.*, 2010; Ōmura, 2011; Olanrewaju and Babalola, 2019; Westhoff *et al.*, 2020; Zaid *et al.*, 2021; Dow *et al.*, 2023). *Streptomyces griseus* also produces dandamycin and chandrananimycin E, benzoxazines (Barnes *et al.*, 2015).

This study aimed to Identification of the bioactive secondary metabolites of a crude butanol extract from a locally isolated strain of *Streptomyces griseus* RH250 isolated from marl clay in Mosul/ Iraq, and testing their activity against some bacterial strains.

MATERIALS AND METHODS

Bacterial identified

The new Strain of *Streptomyces griseus* RH250 was previously identified in a previous study(Aswad and Al-Tai, 2025), isolated using serial dilution and spread plate techniques(from Marl clay in Mosul city\Iraq (The strain isolated in the microbiology laboratory, College of Science, Biology Department, Mosul University) , and some morphological characteristics of *Streptomyces griseus* RH250 are shown in Figs 1,2, and 3. *Streptomyces griseus* RH250 was identified using 16S rRNA. The 16S rRNA gene revealed that strain this strain belonged to the *Streptomyces griseus* species with 99.95% is confidence. *Streptomyces griseus* RH250 was registered in the National Center for Biotechnology Information NCBI and given the accession number PP384319.1.



Fig. 1: *Streptomyces griseus* RH250 ivory white colonies on nutrient agar after 3 days.

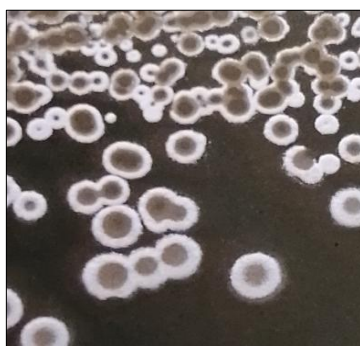


Fig. 2: *Streptomyces griseus* RH250 colonies on nutrient agar after 1 week powdery and velvety colonies, and spore peripheral ring around colonies.

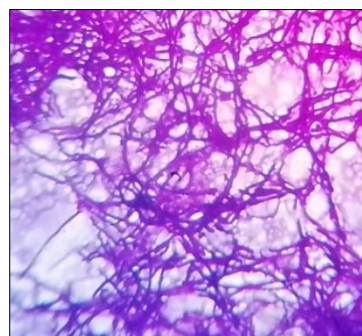


Fig. 3: *Streptomyces griseus* RH250 Branched filamentous aerial mycelium, Positive gram stain under light microscope 100 X magnification oil immersion lens.

Testing bacteria (Primary screening bioactivity)

Streptomyces griseus RH250 streak a single straight of inoculum in the center of the culture (vertical planning (cross streak) method) on a Muller Hinton agar (scharlau, Spain) after 1 week of incubated at 30°C and growth, the plate was streaked with indicator pathogenic bacteria by a single vertical streak at a 90° angle to *Streptomyces griseus* RH250. Screened for antagonistic property against five isolates of pathogenic bacteria indicator included *Alcaligenes faecalis*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas fluorescens*, and *Serrtia marscenes*. The inhibition of bacterial growth was exanimated through the determination of the inhibition zone after 24 hours of vertical streak (Madigan *et al.*, 2021). The pathogenic bacteria were obtained and identified from the microbiology laboratory\ Department biology\ College of Science\ University of Mosul.

Fermentation and extraction of bioactive compounds

The secondary metabolites compounds were taken from the *Streptomyces griseus* RH250 using submerged fermentation, this was done in a liquid made yeast malt extract broth (ISP-2) consist from (4 g yeast extract, 10 g malt extract, 4 g dextrose and 1L of distilled water), after two weeks at 30°C (Kurnianto *et al.*, 2020), bacterial cells were removed by centrifugation at 3000 rpm for 20 minutes (Shrestha *et al.*, 2021). The solvent n-butanol was used to extract external secondary metabolites (Fluka AG, Buchs SG, Switzerland) (Dezfully and Ramanayaka, 2015). N-butanol was added to the supernatant in the ratio of 1ml:1ml (volume/volume) and the mixture was left for one hours then centrifuged at 5000 rpm for 20 minutes (Shrestha *et al.*, 2021). The butanol layer, which held the secondary metabolites, was taken out. The n-butanol was then evaporated in an incubator at 30°C to get a dray extract. finally, 1mL of distilled water was added to this dry crude extract, and it was stored at 4°C (Warsito *et al.*, 2017).

Agar diffusion well for biological activity of crude extracts

The Biological activity of crude extracts was determined by the agar well diffusion method. Cell Concentration of all test microorganisms (*Alcaligenes faecalis*, *Escherichia coli*, *Staphylococcus aureus*, *Serrtia marscenes*, *Pseudomonas fluorescens*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*,) was adjusted at 0.5 McFarland turbidity standards and inoculated on Muller Hinton agar plates by using a sterilized cotton swab. The Wells were drilled using sterilized 1000 µl micro tips (Aswad and Altaii,2020); each was filled with 100 µl of crude extract. The Plates were incubated at 37°C for 1 day (Selvameenal *et al.*, 2009; and Chittepu., 2019).

Gas chromatography–mass spectrophotometry conditions

The GC-MS analysis of crude extract was assessed by employing agelint technologies (7820A) GC mass spectrophotometer (5977E) USA "Environmental and Water Research Center-Environmental Research Department/ University of Baghdad, the Library: NIST20s.lib was used to determine chemical structures in the butanol crude extract".

RESULTS AND DISCUSSION

Test bacteria and agar diffusion well

Streptomyces griseus RH250 inhabits growth of *Staphylococcus aureus*, only Fig. (4). All other bacterial isolates not inhabited by *Streptomyces griseus* RH250. The crude butanol extract inhabited of *Staphylococcus aureus* only at 12 mm only Fig. (5).



Fig. 4: *Streptomyces griseus* RH250 inhibition *Staphylococcus aureus* growth with a diameter of 25 mm. Bacteria arranged from top to bottom *E coli*, *Staphylococcus aureus*, *Serrtia marscenes*, *Alcaligenes faecalis*, and *Pseudomonas florescence*.



Fig. 5: The crude extract of *Streptomyces griseus* RH250 inhibited *Staphylococcus aureus* with a diameter of 12 mm.

Gas chromatography- mass spectrophotometry (GC-MS)

The present study, crude butanol extract of *Streptomyces griseus* RH250 has different peaks in the (Fig. 6) GC-MS spectrum showed, 21 external bioactive compounds listed in (Table 1).

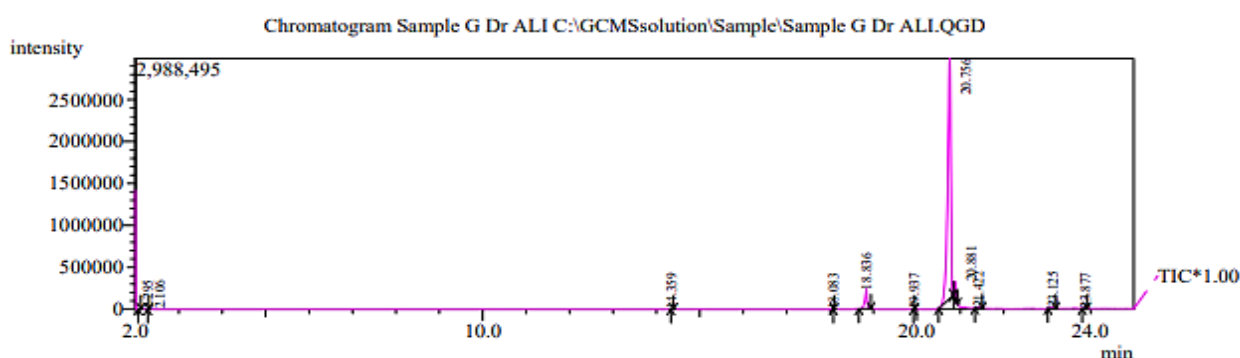
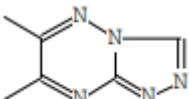
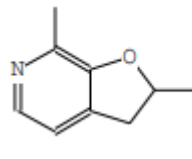
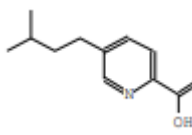
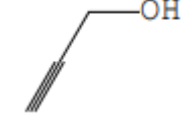
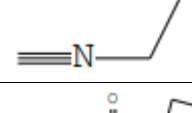
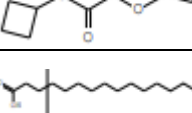
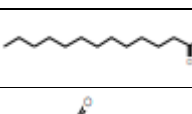
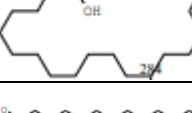
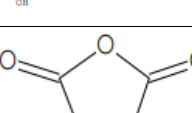
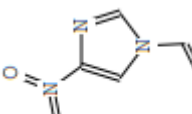
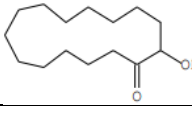
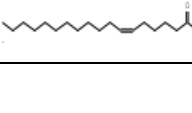
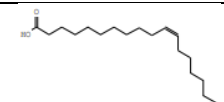
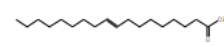
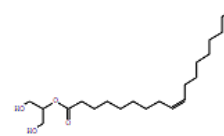
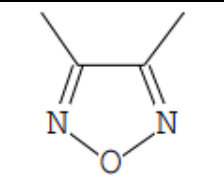
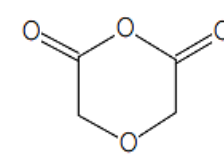


Fig. (6): Chromatogram of butanol crude extracts *Streptomyces griseus* RH250.

Table (1): Secondary metabolites and chemical structures, in butanol crude extract of *Streptomyces griseus* RH250.

S/N	Name Compounds	Retention time RT(min)	Molecular Formulae	Molecular weight	Molecular pattern	The Biological activities
1	Cyclopropane	2.108	C ₃ H ₆	42		Drug design (Chen <i>et al.</i> ,2024)
2	6,7Dimethyl[1,2,4]triazolo[4,3-b][1,2,4]triazine.	14.358	C ₆ H ₇ N ₅	149		Derivative used as anticancer by activation of apoptosis (Gornowicz <i>et al.</i> , 2020), anti-bacterial, anti-viral, anti-fungal ...etc. (Alizadeh and Ebrahimzadeh, 2021) and (Yahya, 2024).. Anti-tubercular, anti-convulsant, analgesic, anti-oxidant, anti-inflammatory, and anti-

						depressant activities (Matin <i>et al.</i> , 2022).
3	2,7-Dimethyl-2,3-dihydrofuro(2,3-c)pyridine.	14.358	C ₉ H ₁₁ NO	149		Alkaloids. Anti-bacterial, anti-viral, anti-fungal, anti-cancer, inhibitors of nicotinic acetylcholine receptors, CLKs, e1F4A, DYRKIA, α-glucosidase and β-glucosidase (Akram <i>et al.</i> , 2024; Rezaeifard <i>et al.</i> , 2024).
4	5-(3-Methylbutyl)-2-pyridinecarboxylic acid	14.358	C ₁₁ H ₁₅ N O ₂	193		To treatment of osteoporosis, anti viral (Duque <i>et al.</i> , 2020; Narayan <i>et al.</i> , 2023)
5	Propargyl alcohol	18.083	C ₃ H ₄ O	56		Used to syntheses anti-microbial nanoparticles (Ghobashy <i>et al.</i> , 2022)
6	Ethyl isocyanide	18.083	C ₃ H ₅ N	55		Derivatives antibacterial, antifungal, antimalarial, antifouling, and anti-tumor. (Massarotti <i>et al.</i> , 2021)
7	Oxalic acid, dicyclobutyl ester	18.083	C ₁₀ H ₁₄ O 4	198		antioxidant capacity (Hasan <i>et al.</i> , 2023)
8	n-Hexadecanoic acid	18.833	C ₁₆ H ₃₂ O 2	256		Antioxidant, anti infilamintry and antibacterial (Ganesan <i>et al.</i> , 2024; Siswadi and Saragih, 2021).
9	Tridecanoic acid	18.833	C ₁₃ H ₂₆ O 2	214		antifungal and antibacterial activities (Chowdhury <i>et al.</i> , 2021)
10	Octadecanoic acid	18.833	C ₁₈ H ₃₆ O 2	284		Antitumor activity (Reza <i>et al.</i> , 2021) antimicrobial, antioxidant, antifungus and antiviral (Linton <i>et al.</i> , 2013).
11	Pentadecanoic acid	18.833	C ₁₅ H ₃₀ O 2	242		Anti-cancer (To <i>et al.</i> , 2020).
12	Maleic anhydride	19.933	C ₄ H ₂ O ₃	98		Antimicrobial and drug carriers (Wal <i>et al.</i> , 2020; Nagaraja <i>et al.</i> , 2019).
13	1-Vinylimidazole, 4-nitro-	19.933	C ₅ H ₅ N ₃ O 2	139		imidazole derivative (Yu and Neborak, 2022) anti-parasitic (Velez <i>et al.</i> , 2022) is alkaloid (Gong <i>et al.</i> , 2016)
14	Oleic Acid	20.758	C ₁₈ H ₃₄ O 2	282		Immunomodulation, protecting from autoimmune diseases and cancer, drug absorption (Sales-Campos <i>et al.</i> , 2013).
15	Cyclopentadecanone, 2-hydroxy-	20.758	C ₁₅ H ₂₈ O 2	240		Macrocyclic lactones used in acosmetics, food, and medicine. (Gane <i>et al.</i> , 2013).
16	6-Octadecenoic acid, (Z)-	20.758	C ₁₈ H ₃₄ O 2	282		Inhibit for COVID19 (Arundina <i>et al.</i> , 2024).

17	Cis-Vaccenic acid	20.758	C ₁₈ H ₃₄ O ₂	282		Anti-carcinogenic effect (Hussein <i>et al.</i> , 2016).
18	9-Octadecenoic acid, (E)-	20.758	C ₁₈ H ₃₄ O ₂	282		anti-inflammatory (Xie <i>et al.</i> , 2022)
19	Beta.-Monoolein	20.758	C ₂₁ H ₄₀ O ₄	356		Anti-microbial, anti-cancer, diuretic and anti-inflammatory (Hussein <i>et al.</i> , 2016).
20	DimethylFurazan.	23.875	C ₄ H ₆ N ₂ O	98		Oxadiazole ring derivatives, Antifungal (Glomb and Świątek., 2021). Non-β-lactam class of antibiotics inhibiting PBP2a (Konai <i>et al.</i> , 2020) Anti-cancer (Gelain <i>et al.</i> , 2019).
21	1,4-Dioxane-2,6-dione	2.108	C ₄ H ₄ O ₄	116		Derivatives used to improve antibacterial activity (Sampaio <i>et al.</i> , 2014)

A total of 21 bioactive metabolites identified were cyclopropane retention time (RT) 2.108.6,7Dimethyl[1,2,4]triazolo[4,3-b][1,2,4]triazine (RT) 14.358, 2,7-Dimethyl-2,3-dihydrofuro(2,3-c)pyridine (RT) 14.358, 5-(3-Methylbutyl)-2-pyridinecarboxylic acid(RT) 14.358, Propargyl alcohol(RT) 18.083, ethyl isocyanide (RT) 18.083, oxalic acid, dicyclobutyl ester (RT) 18.083, n-hexadecanoic acid (RT) 18.833, tridecanoic acid (RT) 18.833, pentadecanoic acid (RT) 18.833, maleic anhydride(RT) 19.933, 1-vinylimidazole, 4-nitro-(RT) 19.933, oleic acid (RT) 20.758, cyclopentadecanone(RT) 20.758, 2-hydroxy-,6-octadecenoic acid, (Z)- (RT) 20.758, cis-vaccenic acid (RT) 20.758, 9-octadecenoic acid, (E)- (RT) 20.758, beta.-monoolein (RT) 20.758, dimethylfurazan (RT) 23.875, and 1,4-dioxane-2,6-dione (RT) 116. The various chemical structures and biological activities of 21 compounds isolated from *Streptomyces griseus* RH250 with diversity biological activity Insecticidal, used as antibiotic, anti-viral, anti-fungal, anti-cancer, anti-oxidant, anti-infilaminty listed in (Table 1), also such compounds contain different chemical structures include imidazole derivative, alkaloids, and oxadiazole ring derivatives listed in (Table 1). The dimethylfurazan responsible for inhibiting *Staphylococcus aureus* this apply with (Konai *et al.*, 2020) furazan, dimethyl-(3, 4-dimethyl-1, 2, 5-oxadiazole) is non-β-lactam class of antibiotics inhibiting PBP2a in *Staphylococcus aureus*. In our study, *Streptomyces griseus* RH250 produces different metabolites under our experimental study compared with previous studies (Sottorff *et al.*, 2019), (Sweetline and Usha, 2018). The metabolites produced by *Streptomyces griseus* RH250 didn't match with any secondary metabolites produced by pervious *Streptomyces griseus*, indicating that it is a new chemical metabolite that may be related to geographical site isolation which affects metabolite production by bacteria.

CONCLUSIONS

The findings of this study demonstrate that *Streptomyces griseus* RH250 exhibits strong and selective antibacterial activity against *Staphylococcus aureus*, as evidenced by a significant inhibition zone in agar diffusion assays. The crude butanol extract of this strain also showed notable inhibitory effects, although to a lesser extent. Through GC-MS analysis, a diverse array of 21 bioactive secondary metabolites was identified in the crude extract, including compounds with known antibacterial, antifungal, antiviral, anticancer, and antioxidant properties.

Importantly, the metabolite profile of *S. griseus* RH250 was distinct from those reported in previous studies, suggesting that geographical factors may influence the production of unique bioactive compounds. Among the identified metabolites, dimethylfurazan was highlighted as a likely contributor to the observed anti-staphylococcal activity, consistent with its known mechanism as a non- β -lactam antibiotic targeting PBP2a in *S. aureus*.

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المستقلبات النشطة حيويًا في السلالة الجديدة *Streptomyces griseus* RH250 المعزولة محليًا من
طين المارل في مدينة الموصل\ العراق

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الملخص

تشخيص المستقلبات الثانوية لمستخلص البيوتانول الخام لعزلة *Streptomyces griseus* RH250 المعزولة محليًا من طين المارل في مدينة الموصل - العراق. تمتلك العزلة الجديدة *Streptomyces griseus* RH250 القدرة على انتاج انواع مختلفة من المسقلبات. تم فحص العزلة لمعرفة نشاطها المضاد ضد البكتيريا سلبية غرام: (*Pseudomonas*, *Alcaligenes faecalis*) والبكتيريا ايجابية غرام (*Staphylococcus aureus*) أُجري الاختبار بطريقة خطوط التقاطع في الأجار وتم قياس خط تثبيط النمو بالمليمترات لهذه السلالة أظهرت فيها أنشطة قوية مضادة للبكتيريا في المختبر ضد المكورات العنقودية الذهبية (25) ملم فقط. تم استخدام كروماتوغرافيا الغاز - الطيف الكتلي لتحديد المستقلبات الثانوية النشطة حيويًا في مستخلص البيوتانول الخام. أظهر هذا التحليل أنه تم عزل واحد وعشرين مركبًا حيويًا نشطًا منها لها بنية كيميائية وأنشطة بيولوجية مختلفة.

الكلمات الدالة: الفعالية الحيوية، الخط المتقاطع، مستخلص البيوتانول الخام، *Streptomyces griseus* RH250.