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Exploring the antioxidant and enzyme inhibitory potential of the *Streptomyces gobitricini* strain: a promising biotechnological resource

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Abstract

Streptomyces strains are renowned for their ability to produce a wide array of secondary metabolites, which play crucial roles in ecological interactions and have significant pharmaceutical applications. The optimization of culture conditions is a key factor in maximizing the production of these bioactive compounds. This study investigated the growth patterns and bioactivity of a newly isolated *Streptomyces gobitricini* cultured on different media: R5, R5E, R2YE, and YEME. Data showed that R5 and R2YE media supported higher biomass accumulation, achieving peak dry weights of 225 mg/L and 175 mg/L, respectively, after 96 h-incubation, compared to R5E (52 mg/L) and YEME (48 mg/L). Growth phases, especially the exponential phases, were longer and more pronounced in nutrient-rich media like R5 and R2YE. Furthermore, the inherent antioxidant activities, enzyme inhibitory properties against α-amylase, α-glucosidase, lipase, and trypsin, as well as secreted phospholipase A2, cyclooxygenases and lipooxygenase, showed significant variations influenced by the growth media, with R5 exhibiting the highest overall bioactivity. Specifically, R2YE extracts demonstrated potent inhibitory effects on α-glucosidase and phospholipases, while YEME showed promising lipase inhibition. These findings emphasized the critical role of media composition in promoting secondary metabolite production in *S. gobitricini*, ultimately enhancing its potential medicinal applications for several human diseases such as obesity and inflammation. Consequently, optimizing bacterial culture conditions could significantly improve yield and efficiency of bioactive compounds.

Keywords: *Streptomyces gobitricini*, Secondary metabolites, Enzyme inhibition, Antioxidant activity.

1. Introduction

Streptomyces genus is among the most prolific producers of secondary metabolites, contributing to a significant portion of naturally derived bioactive compounds used in medicine today [1]. The largest proportion of secondary metabolites was produced by *Streptomyces* strains isolated from a marine environment (39%) flowed by terrestrial soil (27%) and finally unspecific environments [1]. These metabolites are crucial for *Streptomyces* survival in their natural environments, enabling them to compete with other microorganisms and form intricate ecological relationships [2]. Structural identification of *Streptomyces* secondary metabolites showed a wide range of chemical scaffolds including terpenoids, macrolides, polyaromatics, glycosides, and cyclic and linear peptides [1]. Historically, *Streptomyces* strains have been the primary source of the largest number of new antibiotic drugs as secondary metabolites compared to both bacteria and fungi. This genus represents a potent source of antibiotics including tetracycline, chloramphenicol, erythromycin, and aminoglycosides as well as quinine antibiotics [3]. The discovery of new antibiotics produced by *Streptomyces* continues; for example, mediomycins A and B as well as clethramycin were isolated from *Streptomyces mediocidicus* (S. *mediocidicus*) ATCC23936 and *S. malaysiensis* DSM4137, respectively [4].

The production of bioactive compounds in microbes, particularly in *Streptomyces* strains, is significantly affected by culture conditions, including nutrient availability (such as carbon, nitrogen, phosphate, and mineral elements), temperature, pH, and incubation duration [5] In liquid cultures, *Streptomyces* growth is usually limited during the stationary phase, often due to the depletion of essential nutrients, which strongly stimulates the production of secondary metabolites. Consequently, the initial concentrations of nutrients in the medium play a crucial role in determining the yield of these metabolites. The selection of appropriate nutrients and environmental

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conditions can drastically alter the metabolic pathways of *Streptomyces*, leading to the enhanced production of desired metabolites [6]. For instance, the antimicrobial and antioxidant compounds produced by *S. lydicus* A2 grown in half-strength Luria-Bertani broth were found to be more effective than those produced in Yeast Extract Malt Extract (YEME) broth [7]. Moreover, the R2YE medium was specifically designed to support the growth of *S. coelicolor* A3(2) for the production of secondary metabolites [5]. Consequently, optimizing media composition is a crucial step not only in increasing biomass but also in enhancing the bioactivity of secondary metabolites, which can vary significantly depending on the substrate used for growth [6]

Beyond their ecological roles, secondary metabolites produced by *Streptomyces* strains exhibit various biological activities, including antimicrobial, antitumor, antiparasitic and immunosuppressive effects [8]. As resistance to conventional therapies continues to rise, the demand for novel bioactive compounds, particularly from microbial sources like *Streptomyces*, is growing rapidly [9]. Enzyme inhibitors and antioxidant compounds are among the most abundant secondary metabolites produced by *Streptomyces* strains under specific culture conditions [4]. It is worth noting that enzyme inhibitors have gained increasing attention as valuable tools, not only for studying enzyme structures and reaction mechanisms but also for their potential applications in the pharmaceutical industry [4]. Reports indicate that *S. corchorushii* and *S. sp*. CC5 produced specific alpha-amylase inhibitors [10] whereas *S. toxytricini* is known to produce lipstatin, a potent inhibitor of pancreatic lipase [11]. In addition, Acivicin, Amastatin, and Antipain are specific protease inhibitors (PI) produced by *S. sviceus*, *S. sp.* 1.24819 and *S. michigaensis* strains, respectively [12].

Proteases are crucial for major biological processes, however, excessive proteolysis contributes significantly to diseases such as cancer, as well as cardiovascular, inflammatory and neurodegenerative disorders [12]. Consequently, inhibiting proteases can be an effective strategy for developing diagnostic and therapeutic agents for these diseases [12]. Several PIs that inhibit both serine and cysteine proteases, such as antipain and chymostatin, are produced by *S. michigaensis* and S. *hygroscopicus*, respectively. Few PIs are specific to metalloproteases like bestatin which is produced by *S. olivoreticuli* strain [12].

Inhibiting α-glucosidase and α-amylase, key enzymes involved in carbohydrate metabolism, is a recognized therapeutic strategy for managing carbohydrate-related diseases especially diabetes and obesity [13]. Microorganisms are valuable sources of glycosidase inhibitors with potential applications in developing new drugs and dietary supplements. In fact, by suppressing glycosidases' activities, glycosidase inhibitors have potential as weight loss compounds since they decrease carbohydrate absorption, lowering postprandial blood sugar levels and insulin responses to dietary carbohydrates [14]. For instance, acarbose, an α-glucosidase inhibitor derived from Actinoplanes sp., has been effective in controlling type 2 diabetes [13]. However, commercial glycosidase inhibitors exhibit various side effects, hence, developing new inhibitors that are safer, more effective, and more affordable is needed [13]. Likewise, inhibiting pancreatic lipase is also one of the proposed treatments for obesity, which is often associated with diabetes. Pancreatic lipase inhibitors have garnered significant interest from researchers in recent years, particularly those derived from natural products [15]. These inhibitors are valued for their structural diversity, low toxicity, and abundance of sources such as plants and microbes [13]. Indeed, numerous microbial metabolic compounds exhibit strong pancreatic lipase inhibitory activity notably Lipstatin, Panclicins, and Valilactone produced by *Streptomyces* strains [15].

Cyclooxygenase (COX-1/2) and lipooxygenase (LOX) are the key enzymes involved in arachidonic acid (AA) metabolism released by secretory phospholipases A_2 ($sPLA_2$) [16]. They induce inflammation through inflammatory mediators' accumulation, especially eicosanoids, leukotrienes and prostaglandins [16]. Synthetic compounds used as anti-inflammatory drugs act as non-selective inhibitors of COX-1, or of both COX-1 and COX-2, causing significant adverse effects notably gastrointestinal complications and cardiovascular risks [17]. When searching for new drugs that reduce side effects while retaining effective anti-inflammatory properties, the innovative strategy of dual inhibition of COX and 5-LOX as well as $sPLA_2$ has been suggested [17]. Several plant extracts are a potent source of inflammatory enzyme inhibitors while few studies report the presence of these inhibitors as bacterial secondary metabolites.

Oxidative stress, which arises from an imbalance between reactive oxygen species (ROS) and the body's antioxidant defenses, is closely linked to inflammation. Excessive ROS production can harm cellular components and activate inflammatory pathways, creating a continuous cycle of oxidative stress and chronic inflammation. Therefore, effectively managing diseases associated with inflammation requires addressing both oxidative stress and inflammation [18]. Interestingly, secondary metabolites with dual functions acting as both inflammatory enzyme inhibitors and antioxidants hold great promise for developing multifaceted therapeutic agents.

S. gobitricini, a relatively underexplored species, has demonstrated significant potential for producing bioactive metabolites similar to other Streptomyces strains [19]. However, the effects of different growth media on its metabolic pathways are not well understood. This study aimed to examine the growth patterns and bioactivity of *S. gobitricini* cultured on four different media: R5, R5E, R2YE, and YEME. The effect of media on biomass accumulation and metabolite production that inhibit key enzymes such as α-amylase, α-glucosidase, lipase, trypsin, and $sPLA_2$, as well as the antioxidant properties of the extracts have been evaluated. Additionally, the media's effects on the inhibition of COX-1, COX-2, and 5-LOX, which are involved in the inflammatory response have been investigated. By comparing these growth conditions, the objective of the current study was to identify the optimal media for enhancing both biomass production and the bioactive potential of *S. gobitricini*. This investigation contributed to ongoing efforts to discover and develop novel inhibitors from natural sources, particularly microbes, that are effective with minimal side effects.

2. Materials and Methods

2.1. Media preparation and bacterial growth optimization

S. gobitricini strain was isolated from polluted man-

grove soil, identified, and stored in the Botany and Microbiology Department-College of Science at King Saud University (Riyadh, Saudi Arabia). In this study, *S. gobitricini* strain was grown in four different media [20]. The first was R2YE medium, which included sucrose (103 g), glucose (10 g), 5 mL yeast extract (10%), $MgCl_2$ (10.12 g), K_2SO_4 (0.25 g), and Difco Casamino acids (0.1 g) in 800 mL volume with distilled water. Then, 80 mL of the resulting solution was placed in a 250 mL Erlenmeyer flask and 1 mL KH_2PO_4 (0.5%), 8 mL $CaCl_2.2H_2O$ (3.68%), 1.5 mL L-proline (20%), 10 mL TES buffer (5.73%; pH 7.2), 0.5 mL NaOH (1 N), 0.2 mL of trace element ($ZnCl_2$ (80 mg), 400 mg $FeCl_3.6H_2O$), 40 mg each of $CaCl_2.2H2O$, $MnCl_2.4H_2O$, $NaB_4O_7.10H_2O$, and $(NH_4)6(Mo7O2)_4.4H_2O$ were added.

YEME medium contained yeast extract (3 g), peptone (5 g), malt extract (3 g), glucose (10 g), sucrose (340 g), and 2 mL $MgCl_2.6H_2O$ (5 mM). The final volume was adjusted to 1000 mL with the addition of distilled water.

The R5 medium included sucrose (103 g), glucose (10 g), $MgCl_2.6H_2O$ (10.12 g), K_2SO_4 (0.25 g), yeast extract (5 g), Casamino acids (0.1 g), TES buffer (5.73 g), 0.2 mL of trace element ($ZnCl_2$ (80 mg), 400 mg $FeCl_3.6H_2O$), and 40 mg each of $CaCl_2.2H_2O$, $MnCl_2.4H_2O$, $NaB_4O_7.10H_2O$, and $(NH_4)6Mo_7O_{24}.4H_2O$. The final volume was adjusted to 1000 mL with distilled water. To 100 mL of this solution in a 250 mL Erelnmeyer flask, 1 mL KH_2PO_4 (0.5%), 0.4 mL $CaCl_2.2H_2O$ (5 M), 1.5 mL L-proline (20%), and 0.7 mL NaOH (1 N) was added.

The R5 modified (R5E) medium contained glucose (10 g), $MgCl_2.6H_2O$ (10.12 g), K_2SO_4 (0.25 g), yeast extract (5 g), $CaCO_3$ (2 g), Casamino acids (0.1 g), 2 mL of trace element ($ZnCl_2$ (80 mg), $FeCl_3.6H_2O$ (400 mg)), $CaCl_2.2H_2O$ (20 mg), $MnCl_2.4H_2O$ (20 mg), $NaB_4O_7.10H_2O$ (20 mg), and $(NH_4)6Mo_7O_{24}.4H_2O$ (20 mg). The final volume was adjusted to 1000 mL with the distilled water.

2.2. Extract preparation and growth analysis

The growth of *S. gobitricini* was monitored at 12-h intervals across a total period of 168 h in different media. At each interval, 1 mL of culture extract was collected from each medium, dried, and used to measure the bacterial biomass. This process was repeated over seven days to generate growth curves. After the 168-h growth phase, metabolites were extracted using ethyl acetate as the solvent and then dried via vacuum evaporation. These metabolites were subsequently used for antioxidant and enzyme inhibition assays.

2.3. Antioxidant assays

Antioxidant activity of extracted metabolites was assessed following standard for 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and 2, 2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) radical scavenging assays [21]. Bacterial extracts at different concentrations (0 - 200 µg/mL) were mixed in a 1:3 ratio with a 0.1 mM DPPH solution to assess the DPPH free radical scavenging activity. The mixture was then incubated for 30 min at dark. The absorbance was monitored using a UV-visible spectrophotometer at 517 nm (Shimadzu UV-1800).

For the ABTS assay, 1 mL of each bacterial extract at concentrations ranging from 10 to 100 µg/mL was added with 3 mL of ABTS working solution (ABTS and sodium potassium tartrate) and incubated in darkness for 10 min. A UV-visible spectrophotometer was used to quantify the reduction in absorbance at 720 nm. Both DPPH and ABTS were referenced to gallic acid (GA). Additionally, the DPPH or ABTS assays were conducted with methanolic DPPH or ABTS as a control, respectively.

2.4. Enzymes

Dromedary pancreatic lipases (DrPL), dromedary group-IB $sPLA_2$ ($DrPLA_2$-IB) and dromedary group-IIA ($DrPLA_2$IIA) were purified and prepared as previously described [22-24]. The enzymes β-glucosidase, α-amylase and trypsin were purchased from Sigma Aldrich (St. Louis, MO, USA).

2.5. Lipolytic enzyme activity inhibition

The inhibitory effect of tested extracts on DrPL activity was investigated at different concentrations from 0 to 300 µg/ml. For the assay, 12 IU of the enzyme was incubated (50 µL) with 10 µL of extract. A positive control using commercial orlistat (Xenical, Hoffmann-La Roche) was run in parallel in the same conditions serving as positive control. Inhibition of lipase activity was expressed as the percentage of residual activity after incubation with the extract compared to the negative control (no extract added in presence of the enzyme). Lipase activity was measured using tributyrin as substrate under the optimal conditions as previously described [25]. The IC50 values correspond to the half-maximal inhibitory concentration deduced from the obtained curves.

2.6. Phospholipase activity inhibition

The phospholipase activity was measured using the method of De Araújo and Radvanyi [26]. The inhibitory effect of various extracts was determined against $DrPLA_2$-IB and $DrPLA_2$-IIA. A 10 µL of each extract was mixed with 10 µL of each $sPLA_2$ and preincubated for 20 min at room temperature. Then, the mixture was added to 1 mL of the substrate (composed of lecithin (3.5 mM) solubilized in 100 mM NaCl, 3 mM sodium taurodeoxycholate, 10 mM $CaCl_2$, and 0.055 mM red phenol at a pH of 7.6). The phospholipase activity was assessed by measuring spectrophotometrically the absorbance at 558 nm for 5 min. The inhibition percentage was calculated by measuring residual activity compared to the negative control assay (in the absence of extract).

2.7. α-amylase and β-glucosidase activities inhibition

α-amylase activity was measured according to Subranian et al. [27]. A 10 µL-sample of α-amylase (3 IU) was incubated with different concentrations of each extract (0 to 300 µg/mL) at 37 °C for 5 min. Acarbose served as positive control. The residual activity was evaluated by measuring the absorbance at 620 nm after 8 min (A1) and 13 min (A2) followed by an incubation in the presence of the substrate (180 µL of Labtest diluted in water (v/v)). The reaction was carried out, in duplicate, in a microplate (Bio-Tek ELX-800, Winooski, VT, USA). The α-amylase inhibition (i) was calculated as follows: i (%) = 100 – (A2 – A1/ A2 control – A1 control) × 100, where A1 is the absorbance of the initial reading and A2 is the absorbance of the second reading.

The β-glucosidase activity was determined by quantifying the release of 4-nitrophenol from 4-nitrophenyl

α-D-glucopyranoside (4-NPGP). A 180 µL sample of β-glucosidase was preincubated at 37°C for 2 min with 20 µL of each extract or the positive control (acarbose) at concentrations ranging from 0 to 300 µg/mL. The reaction mixture was then incubated with 180 µL of the color reagent (NPGP) for 15 min at 37°C. The assay solution contained 10 mM potassium phosphate buffer, 5 mM 4-NPGP, and 2 IU β-glucosidase at pH 6.9. Absorbance was measured in duplicate at 405 nm using a microplate reader, and the same equation used for α-amylase activity was applied to calculate β-glucosidase activity.

2.8. Protease activity inhibition

The trypsin activity inhibition was performed as described by Kunitz [28]. 1 mL aliquot of each extract was incubated with an equal volume of enzyme at 37°C for 15 min. The reaction was followed by a 30 min incubation with 2 mL of 2% casein as the substrate, also at 37°C. To stop the reaction, 2.5 mL of 5% trichloroacetic acid was added. After centrifuging the mixture at 15,000 rpm for 15 min, the absorbance was measured at 280 nm to determine protease activity. The percentage of protease inhibition was calculated by comparing the results to a control experiment (without extract), with Bowman-Birk Inhibitor serving as a positive control.

2.9. COX-1/2 and LOX inhibition assays

The inhibition of COX-1/2 and 5-LOX by different concentrations (0 - 300 µg/mL) of each extract was assessed in duplicate using the colorimetric COX (Cayman Chemical Company, catalog no. 560131) and LOX inhibitor screening assay kits (Cayman Chemical Company, catalog no. 766700), following the manufacturer's instructions. Diclofenac sodium and nordihydroguaiaretic acid (NDGA) were used as positive controls for COX-1/2 and LOX inhibition assays, respectively.

The assay measured the production of prostaglandin-F2α during the COX reaction, with diclofenac sodium as a positive control for inhibiting both COX-1 and COX-2 enzymes. Each extract (10 µL) was mixed with an equal volume of Tris-HCl buffer (0.1 M, pH 8.0) and preincubated with 100 units of ovine COX-1 and human recombinant COX-2 for 15 min at 37°C. Afterward, 10 µL of arachidonic acid (10 mM) was added, and the reaction was incubated for 2 more min at 37°C. The reaction was halted by adding saturated stannous chloride and 50 µL of 1 N HCl, and the resulting solution was analyzed spectrophotometrically to quantify the prostanoid produced.

The 5-LOX assay measured the production of hydroperoxide by the 5-LOX enzyme acting on AA as the substrate. In a 96-well plate, 90 µL of the 5-LOX enzyme was preincubated with 10 µL of each test extract at concentrations from 50 to 300 µg/mL. Following this, 10 µL of 1 mM AA was added to the wells, and the plate was shaken for 5 min. To stop the reaction, 100 µL of chromogen was added, followed by shaking for another 5 min. The absorbance was measured at 490 nm to assess the results, with nordihydroguaiaretic acid (NDGA) used as a positive control.

2.10. Statistical analysis

All experiments were performed in triplicate, and the results are presented as mean ± standard deviation. IC_{50} values were calculated using dose-response curves fitted with non-linear regression analysis in GraphPad Prism. To assess whether the IC_{50} values differed significantly between the experimental conditions, a one-way ANOVA was performed, followed by Dunnett's multiple comparison test to compare each condition to a positive control.

3. Results

3.1. Growth pattern

The data presented in Figure 1 displayed the dry weight (mg/L) of *S. gobitricini* cultured on four different media (R5, R5E, R2YE, and YEME) over 0-168 h-incubation period. The growth of *S. gobitricini* across the four media revealed distinct differences in biomass accumulation. R5 medium displayed the highest growth, with a peak dry weight of 225 mg/L at 96 h, followed by R2YE, which reached 175 mg/L at the same time. Besides, both media showed rapid growth between 48 and 72 h-culture incubation. However, R5E medium exhibited much slower growth with a maximum dry weight of only 52 mg/L at 84 h, indicating less favorable conditions for *S. gobitricini* growth. The lowest overall growth was obtained with YEME medium peaking at 48 mg/L after 60 h and rapidly declining afterward. Overall, R5 and R2YE provided the best conditions for *S. gobitricini* growth, while R5E and YEME were less effective in producing high biomass (Figure 1).

The duration of the growth phases of *S. gobitricini* varied across the four used media. The lag phase in R5, R5E, and R2YE media lasted approximately 12 h, while for R5, R5E, R2YE, or YEME, the exponential phase varied between 12 and 72 h, 72 and 96 h, 12 and 84 h, or 12 and 60 h, respectively. The decline phase started after a stationary phase that lasted from 72 to 96 h. Overall, R5 and R2YE exhibited longer and more pronounced exponential and stationary phases compared to the slower growth in R5E and YEME media (Figure 1).

3.2. Antioxidant activity

Antioxidant activities of *S. gobitricini* extracts from various culture media R5, R5E, R2YE and YEME were determined by two methods (DPPH and ABTS), compared with the positive control GA, and presented in Figure 2 and Table 1.

The antiradical activities of different extract concentrations using DPPH free radical assay demonstrated a clear difference in antioxidant potential among tested extracts (Figure 2.A). R5 stood out as the most potent antioxidant,

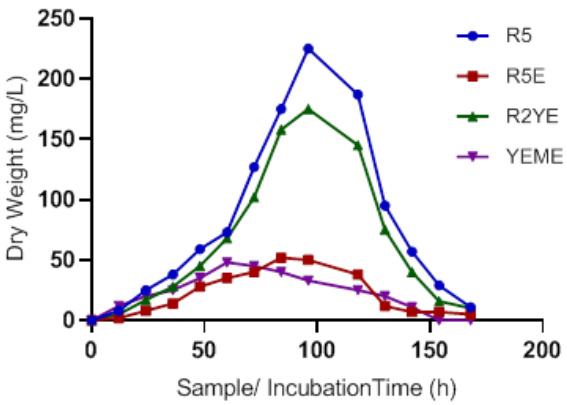

Fig. 1. Growth profile of *S. gobitricini* cultured in R5, R5E, R2YE and YEME media.

followed by R2YE, while R5E and YEME exhibited much lower efficacy. R5 showed the strongest activity, with a steep increase reaching 61.5±2.12% inhibition at 250 µg/mL, indicating potent antioxidant properties. R2YE also exhibited considerable antioxidant capacity, though lower than that of R5, with about 50% inhibition at the highest concentration (300 µg/mL). In contrast, both R5E and YEME displayed significantly weaker antioxidant activities, with maximum scavenging percentages of 13±1.41% and 16.5±2.12% respectively, at 300 µg/mL, suggesting limited effectiveness in neutralizing free radicals (Figure 2A).

Similarly, the R5 extract exhibited the highest scavenging activity measured by ABTS assay, reaching almost 100% at 250 µg/mL, which indicated an excellent antioxidant potential (Figure 1B). R2YE also showed significant scavenging activity of 89±1.41% while R5E demonstrated a moderate antioxidant capacity (69±1.41%), but still higher than that of YEME showing the weakest activity, with only 38±2.82% scavenging power at the highest concentration (300 µg/mL) (Figure 2B).

Table 1 shows that all extracts required higher concentrations to exhibit antioxidant effects compared to the reference, GA, presenting the lowest IC_{50} value (2.77 ± 0.01 µg/mL and 9.21± 2.21 µg/mL for ABTS and DPPH assay respectively). Whereas, R5 and R2YE exhibited comparable scavenging activities with IC_{50} values of 19.50±1.44 µg/mL - 39.88±8.407 µg/mL and 25.26±1.84 µg/mL-43.77±8.06 µg/mL, determined by ABTS and DPPH assays, respectively. However, higher IC_{50} values were recorded for R5E (61.12±7.35 µg/mL) using the ABTS method (Table 1).

3.3. α-Amylase, α-glucosidase and lipase inhibition

The inhibitory potency of various *S. gobitricini* extracts from four media was evaluated against α-amylase, α-glucosidase and DrPL (Figure 3, Table 2).

A clear difference in α-amylase inhibition potency was observed among the studied extracts. The R5 extract, with an IC_{50} of 97.51±1.77 µg/mL, was found the least effective, achieving 77% inhibition at the highest tested concentration (300 µg/mL) (Figure 3A, Table 2). In contrast, the R2YE extract demonstrated the strongest inhibitory effect, with an IC_{50} value 56.32±1.94 µg/mL (Table 2), causing up to 92.5% inhibition at 250 µg/mL (Figure 3A). Similarly, the R5E and YEME extracts displayed comparable IC_{50} values of 36.90±17.36 and 62.42±0.007 µg/mL, respectively (Table 2), and high levels of enzyme suppression up to 80% (Figure 3A).

Notably, *S. gobitricini* extracts grown in different media (R5, R5E, R2YE, and YEME) were also effective in inhibiting α-glucosidase, particularly, the R5 extract with a promising IC_{50} of 72.11±2.71 µg/mL (Table 2). The R2YE and YEME extracts caused up to 87.5% and 75% reduction of the α-glucosidase activity, respectively, at 300 µg/mL (Figure 3B) while R5E extract presented a broader range of IC_{50} value (132.9±29.133 µg/mL) (Table 2). Overall, these data obviously underscored the impact of the growth medium on the bioactivity of *S. gobitricini* extracts. However, acarbose, used as a positive control, remained significantly more potent than the examined extracts showing IC_{50} values of 3.36±0.388 µg/mL and 4.003±0.11µg/mL against α-glucosidase and α-amylase, respectively (Table 2).

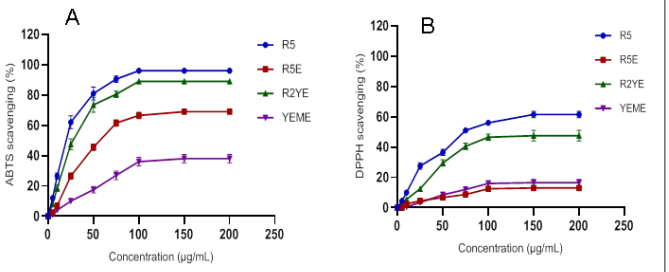

Fig. 2. Antioxidant activities of *S. gobitricini* extracts obtained from various media R5, R5E, R2YE and YEME, evaluated by ABTS **(A)** and DPPH **(B)** methods. Data were collected in triplicate, with the results presented as mean values ± standard deviation.

Table 1. Radical scavenging activities of S. gobitricini extracts were obtained from various cultural media (R5, R5E, R2YE and YEME).

Samples	IC_{50} (µg/mL)	
	ABTS	DPPH
R5	19.50±1.44***	39.88±8.407**
R5E	35.59±1.36****	61.12±7.35***
R2YE	25.26±1.84****	43.77±8.06**
YEME	56.52±0.67****	50.46±2.39**
Gallic acid	2.77 ± 0.01	9.21 ± 2.21

Gallic acid was used as positive control. IC_{50} corresponds to the concentration at which 50% of the activity is inhibited. Data were performed in triplicate and presented as mean values ± standard deviation. (**) indicates $P < 0.01$ (***) indicates $P < 0.001$ (****) indicates $P < 0.0001$.

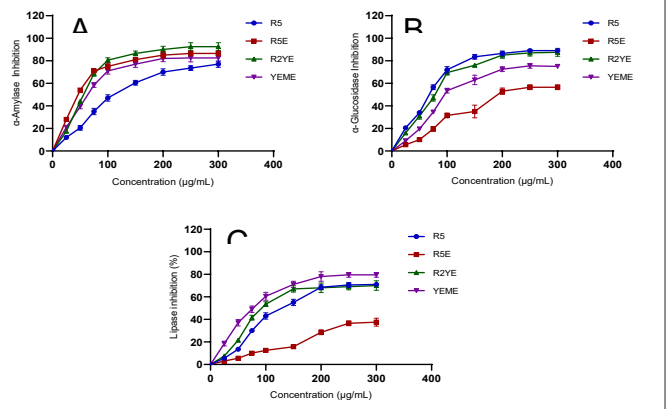

Fig. 3. α-Amylase (A), α-glucosidase (B) and lipase (C) inhibition by *S. gobitricini* extracts obtained from various media R5, R5E, R2YE and YEME. Results were performed in triplicate, and presented as mean values ± standard deviation.

Moreover, the inhibitory potency of *S. gobitricini* extracts against pancreatic lipase varied considerably. As seen in Figure 3C and Table 2, the YEME extract showed the highest inhibitory activity (79.5±2.12%, IC_{50}= 68.84±1.478 µg/mL), followed by R2YE (70±4.24%, IC_{50}= 70.66±2.784 µg/mL) and R5 (70.5±2.12%, IC_{50}= 94.10±1.936 µg/mL). In contrast, the R5E extract showed lower overall effectiveness, with maximal inhibitory activity around 50%, even at the highest concentration. Interestingly, the YEME and R2YE extracts were more effective than the commercial lipase inhibitor, Orlisat (IC_{50} =113.9±20.7 µg/mL) (Table 2).

Table 2. IC_{50} values of the enzymes' inhibition of *S. gobitricini* extracts obtained from various media R5, R5E, R2YE and YEME. Data were performed in triplicate and presented as mean values ± standard deviation. (*) indicates $p < 0.05$ (**) indicates $P < 0.01$ (***) indicates $P < 0.001$ (****) indicates $P < 0.0001$. Acarbose, Orlisat, Oleanolic acid, Bowman-Birk Inhibitor, diclofenac sodium and nordihydroguaiaretic acid (NGDA) served as positive control for α-amylase and α-glucosidase; lipase; phospholipase A_2, COX-1/2, 5-LOX and Trypsin, respectively. -: Not Determined.

Samples	IC_{50} (µg/mL)								
	α-Amylase	**α-glucosidase**	**Lipase**	**Trypsin**	**DrPLA$_2$-GIB**	**DrPLA$_2$-GIIA**	**COX-1**	**COX-2**	**5-LOX**
R5	97.51±1.77***	72.11±2.71*	94.10±5.62	69.65±1.428****	88.67±1.95****	81.75±0.94****	-	-	-
R5E	36.90±17.36*	132.9±29.133 ***	-	81.94±0.66****	108.2±2.12****	174.3±7.49****	-	-	-
R2YE	56.32±1.94**	76.42±0.877**	70.66±0.55	62.58±7.16****	80.41±0.63****	84.64±0.608****	113.3±2.26***	133.4±6.57**	79.210±1.80***
YEME	62.42±0.007**	85.19±5.87**	68.84±1.478	77.36±2.50****	78.67±4.92****	125.6±7.21****	100.7±7.17***	170.60±27.29**	133.9±9.26****
Acarbose	4.003±0.11	3.36±0.388	-		-	-	-	-	-
Orlisat	-	-	113.9±20.7	-	-	-	-	-	-
Oleanolic acid	-	-	-	-	10.00± 0.049	4.125± 0.042	-	-	-
Bowman-Birk Inhibitor	-	-	-	0.269±0.003	-	-	-	-	-
Diclofenac sodium	-	-	-		-	-	1.56± 0.059	0.616± 0.021	-
NGDA	-	-	-	-	-	-	-	-	5.98±0.23

3.4. Trypsin inhibition

The inhibition of trypsin activity was assessed using the four different extracts at various concentrations ranging from 0 to 300 µg/mL. As shown in Figure 4, R5 achieved a maximum inhibition of 91.5±2.12% at the highest concentration of 300 µg/mL, a result that remained consistent across the last three concentrations tested. R2YE and YEME extracts showed a slightly lower trypsin inhibitory activity (86.5±2.12% and 77.5%±3.53%, respectively) while R5E was the least effective among all samples, with a maximal inhibition of 69.5±2.12% at 300 µg/Ml (Figure 4). Comparable IC50 values (~77 µg/mL) were determined for all extracts except R2YE extract showing an IC50 of 62.58±7.16 (Table 2). Overall, R5 consistently exhibited the highest trypsin inhibition across all concentrations tested. These results indicated that all tested media successfully produced the protease inhibitor(s) associated with the S. gobitricini genus [29]. However, the commercial protease inhibitor (Bowman-Birk Inhibitor), serving as a positive control, demonstrated significantly greater potency than all extracts, with an IC50 of 0.269±0.003 µg/mL (Table 2).

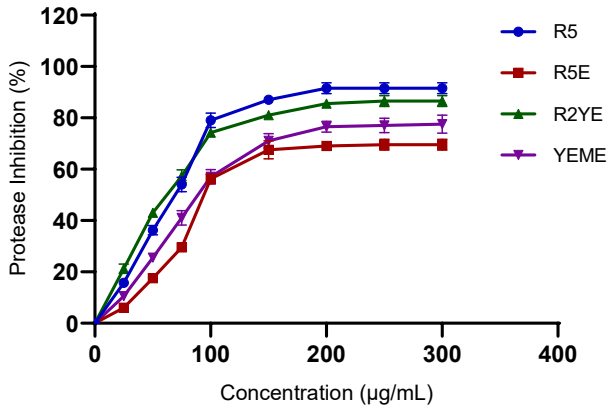

Fig. 4. Trypsin inhibition by various concentrations of *S. gobitricini* extracts obtained from four different media (R5, R5E, R2YE and YEME). Data were collected in triplicate, with the results presented as mean values ± standard deviation.

3.5. Inflammatory enzyme inhibition

3.5.1. Phospholipase inhibition

Since bacterial secondary metabolites have emerged as promising candidates for several diseases as they often exhibit potent enzyme inhibitors, the inhibitory effects of various extracts obtained from *S. gobitricini* grown in different media (R5, R2YE, R5E, and YEME) were evaluated against enzymes involved in inflammation process particularly $sPLA_2$, COX-1 and COX-2 as well as 5-LOX.

As one can see in Figure 5 and Table 2, tested extracts showed varying degrees of effectiveness in inhibiting inflammatory enzymes. Indeed, R2YE was the most effective extract against both tested PLA_2s causing 83.5% and 62.5% inhibition of $DrPLA_2$-IB (IC_{50}= 80.41±0.63 µg/mL) and DrPLA2-IIA (IC_{50}= 84.64±0.608 µg/mL), respectively (Figure 5 A-B, Table 2). A comparable IC_{50} value (88.67±1.95 µg/mL) was recorded for R5 extract against $DrPLA_2$-IB whereas both R5 and YMEM extracts inhibited $DrPLA_2$-IIA activity by 50% at 250 µg/mL (Figure 5B). Data presented in Figure 5 demonstrated that the R5E extract was less effective against the two phospholipases. However, oleanolic acid, used as positive control, displayed much lower IC_{50} values of 10.00± 0.049 µg/mL and 4.125± 0.042 µg/mL against $DrPLA_2$-IB and $DrPLA_2$-IIA, respectively. Overall, the extracts from *S. gobitricini* strain highlighted the potential of using specific media to enhance $sPLA_2$ inhibitory activity, with R5E being particularly promising.

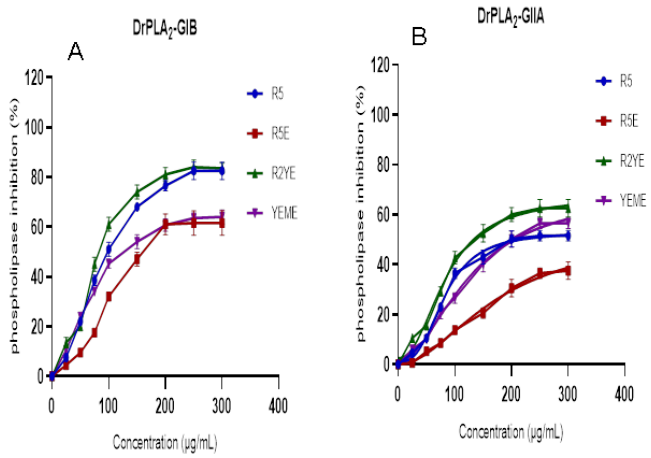

Fig. 5. Effects of *S. gobitricini* extracts from various media (R5, R5E, R2YE and YEME) on $DrPLA_2$-IB (A) and Dr-PLA_2-IIA (B) inhibition. Data were collected in triplicate, with the results presented as mean values ± standard deviation.

3.5.2. COX-1/2 and 5-LOX inhibition

To further investigate the anti-inflammatory effects of *S. gobitricini* extracts grown in various media, their inhibitory activity against key inflammatory enzymes COX-1, COX-2, and 5-LOX was assessed. As illustrated in Figure 6A, the R2Y extract caused the highest inhibition level of both COX-1 (92.5±3.54%) and 5-LOX (93.5±2.12%) enzymes, at a concentration of 300 µg/mL, demonstrating potent enzyme-targeting activity (Figure 6A). The YEME extract was also remarkably effective against COX-1 (67.5±3.54%), COX-2 (91.5±3.54%), and 5-LOX (75.5±2.12%) while the R5E extract exhibited the strongest inhibition of COX-2 (87±2.83%). Conversely, the R5 extract showed the lowest inhibitory effects across all enzymes, with inhibition rates ranging from 35±2.83% to 46.5±2.12%, emphasizing the influence of culture media on enzyme inhibition potential (Figure 6A).

The most potent extracts, R2Y and YEME, were further tested at different concentrations ranging from 0 to 300 µg/mL. The resulting inhibition curves revealed differential sensitivity of the three enzymes to these extracts (Figure 6 B-C). R2Y demonstrated strong dose-dependent inhibition of COX-2 and 5-LOX, reaching 92.5% and 93.5% with IC_{50} values 133.4±6.57 µg/mL and 79.21±1.8 µg/mL, respectively (Figure 6B, Table 2). However, diclofenac sodium, used as a positive control, remained significantly more potent than all the extracts with low IC_{50} values of 1.56± 0.059 µg/mL and 0.616± 0.021 µg/mL for COX-1 and -2 respectively (Table 2).

These findings might indicate the presence of components that selectively target these enzymes. In contrast, COX-1 inhibition by R2Y increased more gradually but reached significant level at higher concentrations. The YEME extract showed a similar inhibition pattern against all three enzymes, particularly COX-2 and 5-LOX (~90%

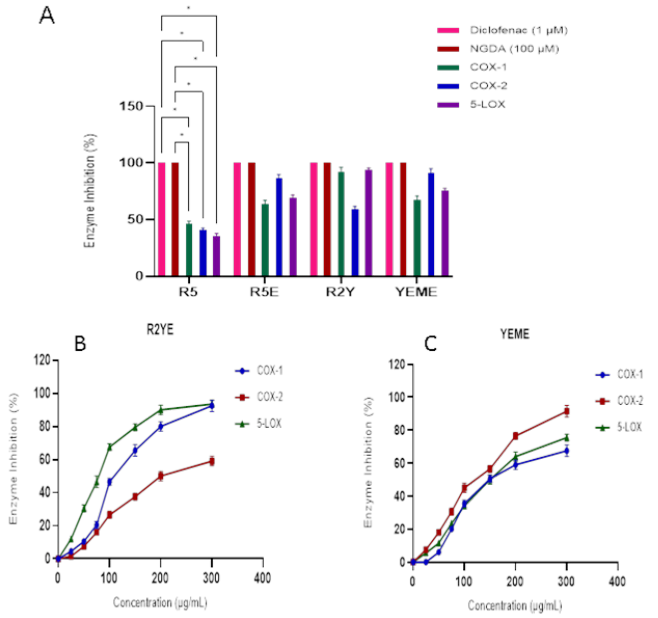

Fig. 6. (A) Inhibitory effects of *S. gobitricini* extracts from various media (R5, R5E, R2YE and YEME) on COX-1, COX-2, and 5-LOX enzymes at 300 µg/mL. Dose-response effect of R2Y (B) and YEME (C) on COX-1, COX-2 and 5-LOX activities. NGDA (100 µM) and diclofenac sodium (1 mM) were used as positive controls for 5-LOX and COX, respectively. The results presented are the mean values obtained from three separate replicates. (*) indicates $p < 0.05$.

inhibition), with IC_{50} values of 170.60±27.29 µg/mL and 133.4±6.75 µg/mL, respectively (Figure 6C, Table 2). These results suggested that R2Y was especially effective in targeting inflammation pathways mediated by 5-LOX and COX-1, making it a promising candidate for inflammation control applications.

4. Discussion

Microbial bioactive compound production is highly influenced by incubation duration, pH, temperature and especially by culture conditions, including nutrient availability in the medium [5]. Specifically, nutrient limitation, particularly primary metabolites, strongly stimulates secondary metabolism in Streptomyces strains [29]. The dry weight (mg/L) of *S. gobitricini* cultured on four different media (R5, R5E, R2YE, and YEME) over 0-168 h-incubation period was investigated. Results showed distinct differences in biomass accumulation across the four media.

These differences in S. gobitricini growth on the various media could be attributed to the distinct nutrient compositions and environmental conditions provided by each medium that, indeed, likely supported growth in unique ways. R5 medium is typically rich in nutrients, especially carbon sources (glucose), nitrogen, and vitamins which are essential for supporting the high growth rate observed. The rapid biomass accumulation and prolonged exponential phase suggested that R5 provided optimal conditions for S. gobitricini leading to the highest peak biomass (225 mg/L). Its composition seemed to favor both primary metabolism (rapid cell growth) and metabolite production [30]. Although based on R5, the R5E medium did not produce as high a growth rate as R5. The slower exponential phase and biomass peak (52 mg/L) could indicate that certain key nutrients needed for optimal growth were limited, leading to reduced metabolic activity and slower biomass accumulation. On the other hand, R2YE nutrient-rich medium often used for Streptomyces, especially for antibiotic production [5], provided a good balance of carbon, nitrogen, and trace elements, which probably explained the robust growth, similar to R5, and the high peak biomass (175 mg/L). However, the slightly slower growth compared to R5 might be due to differences in the composition of specific nutrients or growth factors that favored more gradual cell division and metabolite production. Despite YEME is typically used for general Streptomyces cultivation [3, 31], the observed shorter exponential phase and lower peak biomass (48 mg/L) may indicate that its nutrient composition did not fully offer the high metabolic demands of S. gobitricini, particularly carbon and nitrogen sources. This medium might be better suited for slow or steady growth rather than rapid biomass production.

Similar results were previously described with Streptomyces sp. (G-18) (isolated from the high altitude of Nepal) curve growth, which exhibited maximum dry weight on R2YE (184 mg/L), followed by R5 (144 mg/L), YEME (38 mg/L), and R5M (30 mg/L) media [2]. Likewise, *S. coelicolor* A3(2) reached maximum growth at 72 h in the R2YE medium [5].

Antioxidant compounds produced as secondary metabolites by bacteria, such as *Streptomyces*, play a crucial role in protecting cells from oxidative stress by neutralizing free radicals [32]. These bacterial-derived antioxidants offer a promising alternative to synthetic antioxidants, providing natural and sustainable sources for therapeutic and industrial use [1]. Antioxidant activities of *S. gobitricini* extracts from various culture media were assessed by DPPH and ABTS assays. R5 was the most potent antioxidant, followed by R2YE, while R5E and YEME exhibited much lower efficacy. This highest scavenging activity indicated an excellent antioxidant potential of this extract.

According to the study of Ashok et al. [2], *S. sp*. (G-18) cultivated in R2YE medium exhibited the strongest ABTS radical scavenging activity, achieving 82% inhibition with an IC_{50} value of 61±1.5 µg/mL. In contrast, YEME extracts displayed the weakest ABTS scavenging effect, with only 21% inhibition. Similarly, the highest DPPH radical scavenging activity was found in the R2YE extract, showing 35% inhibition, while no inhibition against DPPH was observed for extracts from the R5M medium [2]. Marine *S. sp*.S2A extract revealed 56.55 ±3.1% and 42.48±3.1% inhibition of radical DPPH and ABTS scavenging activity, respectively, at 1 mg/mL [33]. Likewise, *S. sp*. MUM212 cultured in ISP2 medium exhibited notable antioxidant activity, thanks to its ability to scavenge DPPH and ABTS at a concentration of 4 mg/mL, showing inhibition rates of 22% and 62%, respectively [34].

The ABTS scavenging activity observed in the four samples was likely influenced by the presence and relative concentration of secondary metabolites, which are known to play a crucial role in antioxidant mechanisms [31]. Indeed, several secondary metabolites, such as flavonoids, phenolic acids, tannins, alkaloids, and terpenoids, that are common in plants and microorganisms, are recognized for their ability to neutralize free radicals [35]. Therefore, the variation in antioxidant activities between various extracts (R5, R2YE, R5E, and YEME) suggested differences in the types and quantities of these secondary metabolites. Moreover, R5 which showed the strongest ABTS scavenging activity, may have a higher concentration of polyphenols

or flavonoids, which provide greater radical scavenging capacity [35]. R2YE, with moderate antioxidant activity, likely contained these compounds but in lesser quantities than R5 extract. This result highlighted the importance of secondary metabolites in determining the antioxidant potential of different extracts obtained from various bacterial media.

Obesity has emerged as a significant health issue, contributing to conditions such as diabetes and various other diseases. One strategy for managing obesity involves the use of digestive lipase inhibitors to reduce dietary lipid digestion [36]. Since α-glucosidase and α-amylase are the main glycosidases involved in the metabolism of carbohydrates, their inhibition is crucial for treating diseases involving the absorption of carbohydrates, especially obesity and diabetes. Secondary metabolites generated by various microorganisms offer a rich source of enzyme inhibitors, including lipases or glycosidases, which can be utilized in the development of anti-obesity and anti-diabetic medications [36]. The inhibitory potency of various *S. gobitricini* extracts from four media was evaluated against α-amylase, α-glucosidase and DrPL. Results exhibited that R2YE extract was the strongest inhibitory effect against α-amylase, with an IC50 value of 56.32±1.94 µg/mL

Moreover, *S. gobitricini* extracts were also effective in inhibiting α-glucosidase, particularly, the R5 extract with an IC50 of 72.11±2.71 µg/mL. At 300 µg/mL The R2YE and YEME extracts caused up to 87.5% and 75% inhibition of α-glucosidase, respectively. These inhibitors could have beta- lactone structure like several lipase inhibitors purified and identified from various *Streptomyces* strains including Lipstatin, Panclicins, Valilactone and Ebelactones from *S. toxytricini*; *S. sp*. NR 0619; *S. albolongus* MG147-CF2 and *S. aburaviensis*, respectively [15].

Similar results were previously described with extracts from *S. sp*. G-18 Grown in the same Media. Indeed, R2YE exhibited the strongest α-amylase inhibition, achieving 66% inhibition with an IC_{50} of 130±0.5 µg/mL, while the R5M extract showed the weakest effect, with only 51% inhibition (IC_{50} of 280±1.0 µg/mL). Against lipase, the YEME extract displayed the highest level inhibition (45%, IC_{50} of 157±6.0 µg/mL), followed by R5 and R2YE, while no inhibitory activity against lipase was detected for the R5M extract [2]. The ethyl acetate extract from *S. sp*.S2A cultured in ISP-2 medium, inhibited, in a dose-dependent manner, α-amylase and α-glucosidase with IC_{50} values of 21.17 µg/mL and 20.46 µg/mL, respectively [33]. Significant enzyme inhibitory activity was also demonstrated by the chloroform extract of *S. coelicoflavus* SRBVIT13 against both yeast and mammalian α-glucosidase enzymes [37]. Therefore, the current findings confirmed again that the composition of the media has a considerable impact on the production of secondary metabolites in microorganisms, subsequently affecting their bioactivity.

A large number of inhibitors have been purified from bacterial culture media extracts. For instance, several glycosidase inhibitors were purified from various *Streptomyces* strains such as those from *S. costaricanus* EBL isolated in Vietnam and cultivated in ISP2 medium with IC_{50} 9.59 mg/mL [38]. A specific mammalian α-amylases inhibitor was purified from culture of *S. sp.* strain CC5 isolated from soil [10]. The isobutylhexapeptide TXS-2 purified from the marine *Streptomycetes* SCSIO 40064 presented an IC_{50} value of 18.67±1.27 mM towards α-glucosidase [39]. According to Sun et al. [10], secondary metabolites with amylase/glycosidase inhibitors produced by *Strptomyces* strains are classified as carbohydrate-based compounds (amino sugars and oligostatins) and proteinaceous amylase inhibitors [10]. The cyclic structures of these amylase inhibitors, which are similar to those of amylase substrates, account for the majority of their activity against amylases [10].

Trypsin is a proteolytic enzyme found in the digestive system of many animals, including humans, and plays a crucial role in protein digestion by hydrolyzing into peptides and amino acids [43]. Excessive proteolysis significantly contributes to cancer development as well as cardiovascular, inflammatory and neurodegenerative diseases [12]. Therefore, inhibition of trypsin or proteases is an important tool in several medical applications. Secondary metabolites of plants and, particularly, microbes are a potent source of protease inhibitors that target various proteases with various mechanisms [12]. In this context, the inhibitory effect of various extracts of *S. gobitricini* grown in different media was evaluated, especially since the genus *Streptomyces* is known to produce several protease inhibitors that represent natural inhibitor mechanisms developed by this microorganisms to control the proteolysis by endogenous proteolytic enzymes [44]. R5 was the effective extract against trypsin across all concentrations.

Indeed, it has been reported that several protease inhibitors were isolated from various *Streptomyces* strains among them *Streptomyces subtilisin* inhibitor (SSI) and subtilisin-inhibitor-like (SIL) inhibitor from *S. albogriseolus* S-3253 and *S. antifibrinolyticus*, respectively. Similarly, an alkaline protease inhibitor (API-2c), *S. lividans* protease inhibitor (SLPI) and *Streptomyces* trypsin inhibitor 2 (STI2) were purified from *S. griseoincarnatus* and *S. longisporus*, respectively [44]. Moreover, SMTI, a trypsin inhibitor was purified from *S. misionensis* UMS1 strain isolated in Kuala Lumpur, Malaysia. It showed inhibitory activity against trypsin (74%) and chymotrypsin (41%) with a competitive mechanism and KI value of 5 10-7 M [44].

Regarding the mechanism of trypsin or protease inhibition, protease inhibitors form stoichiometric high-affinity complexes with proteases resulting in suppressing their hydrolytic activity by binding to the active site with a specific interaction with a catalytic key residues [45].

$sPLA_2$, in particular group-IIA and group-IB, are critical enzymes involved in the release of AA from membrane phospholipids. This AA serves as a precursor for the synthesis of potent inflammatory mediators, especially prostaglandins and leukotrienes through activation of COX and LOX pathways. These inflammatory mediators contribute to the development of chronic inflammation as well as the progression of inflammatory diseases like arthritis, asthma, and cardiovascular disorders [16]. Moreover, inhibiting $sPLA_2$ can block the initial step of AA release, while inhibition of COX and LOX pathways further prevents the downstream production of pro-inflammatory molecules. This multi-targeted inhibition is particularly effective in reducing inflammation and preventing tissue damage [40]. Since bacterial secondary metabolites have emerged as promising candidates for several diseases as they often exhibit potent enzyme inhibitors, the inhibitory effects of various extracts obtained from *S. gobitricini* grown in different media (R5, R2YE, R5E, and YEME) were evalu-

ated against enzymes involved in inflammation process particularly $sPLA_2$, COX-1 and COX-2 as well as 5-LOX. R2YE caused 83.5% and 62.5% inhibition of DrPLA2-IB (IC50= 80.41±0.63 µg/mL) and DrPLA2-IIA (IC50= 84.64±0.608 µg/mL). R5 and YMEM extracts inhibited DrPLA2-IIA activity by 50% at 250 µg/mL.

The inhibitory potency of bacterial secondary metabolites is probably due to their chemical structure that allows interaction with the key residues of the active site of the enzyme, thus, preventing substrate fixation [41, 42]. To our knowledge, none of $sPLA_2$ or inflammatory enzyme inhibitors are known to be secondary metabolites of the genus *Streptomyces*.

To further investigate the anti-inflammatory effects of *S. gobitricini* extracts grown in various media, their inhibitory activity against key inflammatory enzymes COX-1, COX-2, and 5-LOX was assessed. The most potent extracts against COX-1 and 2 were R2Y and YEME revealed differential sensitivity of the three enzymes to these extracts. R2Y demonstrated strong dose-dependent inhibition of COX-2 and 5-LOX, reaching 92.5% and 93.5% with IC50 values 133.4±6.57 µg/mL and 79.21±1.8 µg/mL, respectively.

The inhibition of COX-1 and COX-2 may result from the interaction of the extract compounds with key active site residues, specifically Arginine 120 in human COX-1 (Arginine 106 in humanCOX-2) and the phenolic amino acid Tyrosine 355 (Tyrosine 341 in human COX-2). These residues are crucial for binding to the carboxylate group of arachidonic acid via ionic and hydrogen bond interactions, respectively [41]. The inhibition of 5-LOX by the extracts could be attributed to the presence of various types of inhibitors within the extract. These inhibitors may include redox inhibitors or antioxidants that disrupt the enzyme's redox cycle, iron-chelating agents that sequester the iron, and non-redox competitive inhibitors that compete with arachidonic acid for binding to the enzyme's active site [42].

The evaluation of antioxidant and enzyme inhibition activities of *S. gobitricini* extracts from various media revealed valuable insights into their potential health applications. Among the tested media, the R5 extract exhibited the strongest radical scavenging activity, outperforming R5E and YEME. This highlighted the significant influence of media composition on the production of bioactive compounds, such as flavonoids and polyphenols, which likely contribute to its antioxidant effects. The R2YE extract demonstrated strong inhibition of α-glucosidase and lipase enzymes, suggesting potential uses in anti-diabetic and anti-obesity treatments. Additionally, R2YE inhibited effectively the inflammatory enzymes COX-1 and 5-LOX while R5 showed notable inhibition of trypsin, indicating possible therapeutic applications for protease-related conditions as well as inflammation diseases. These variations in bioactivity underscored the intricate relationship between growth conditions and secondary metabolite production. Further research focused on isolating and characterizing the active compounds could lead to the discovery of new therapeutic agents.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by A.A.Z, N. K M.A, E.A, L.A, H.H and A.B.B The first draft of the manuscript was written by N. K and A.B.B. All authors have read and agreed to the published version of the manuscript.

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Data availability statement

The data presented in this study are available on request from the corresponding author.

Conflicts of interest

The authors declare no conflict of interest.

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