

# Identification of pancreatic lipase inhibiting endophytic secondary metabolites of *Aspergillus fischeri* VO1R

Tashkhan Gulyamova<sup>1</sup>, Maftuna Yoldosheva<sup>1</sup>, Liliya Abdulmyanova<sup>1</sup>, Dilaram Ruzieva<sup>1</sup>, Uchkun Ishimov<sup>2</sup>, Oybek Mamarakhimov<sup>3</sup>, Iqbol Mukhammedov<sup>1,4\*</sup> and Ulugbek Yusupov<sup>1,3</sup>

<sup>1</sup>Institute of Microbiology, Academy of Sciences the Republic of Uzbekistan, A.Kadyri 7B Street, 100128, Tashkent, Uzbekistan

<sup>2</sup>Institute of Bioorganic Chemistry, Academy of Sciences of the Republic of Uzbekistan, M.Ulugbek Street, 83, 100125, Tashkent, Uzbekistan

<sup>3</sup>National University of Uzbekistan named after Mirzo Ulugbek, University Street,4, 100174, Tashkent, Uzbekistan

<sup>4</sup>Kokand University Andijan branch, 170619, Andijan, Uzbekistan

**Abstract.** Obesity is considered to be a global problem that can contribute to the development of various diseases such as dyslipidemia, metabolic syndrome, hypertension, and an increased risk of cardiovascular mortality. WHO has identified obesity as one of the leading health problems of the 21st century; with more than 1 billion adult world population overweight, at least 300 million of them are obese. A wide range of therapeutic strategies have been proposed to prevent and treat obesity, including pharmacotherapy, diet therapy, surgery, and behavioral therapy. One of the practical therapeutic approaches to the prevention of obesity is to slow down the absorption of fatty acids by inhibiting pancreatic lipase, the primary digestive enzyme that catalyzes the hydrolysis of ester bonds of tri- and diglycerides to monoglycerides and free fatty acids. The escalating prevalence of obesity necessitates the exploration of novel therapeutic avenues. This study presents a unique approach by presenting data on the extraction and identification of inhibitory secondary metabolites from the total ethyl acetate extract of the endophyte *Aspergillus fischeri* VO1R from the root of the plant *Viola odorata*. These metabolites demonstrate a potent ability to suppress the activity of pancreatic lipase by 91.5%. The IC<sub>50</sub> value for pancreatic lipase inhibition by *A.fischeri* VO1R was determined to be 20.5 µg/mL, which is comparable to the reference drug Xenical (Orlistat), exhibiting an IC<sub>50</sub> value of 20.6 µg/mL under the same experimental conditions. The highest inhibitory activity was observed in the butanol fraction (84.9%), while the standard drug Xenical (orlistat) exhibited a pancreatic lipase inhibitory activity of 92%. Further separation of the butanol fraction of *A.fischeri* VO1R by thin-layer chromatography reveals the most active fraction B-2 with Rf 0.75, exhibiting an inhibitory activity of 57.2%. HPLC-MS methods confirm that the inhibitory fraction of B-2 consists of polyphenols identified as cinnamic acid, lariciresinol-sesquiglian, and hydroxyphloretin 2'-O-xylosyl-glucoside.

## 1 Introduction

Obesity is considered to be a global problem that can contribute to the development of various diseases such as dyslipidemia, metabolic syndrome, hypertension, and an increased risk of cardiovascular mortality [1, 2]. WHO has identified obesity as one of the leading health problems of the 21st century; with more than 1 billion adult world population overweight, at least 300 million of them are obese [3]. The World Health Organization (WHO) has recognized the rapid increase in obesity rates as an epidemic of the 21st century and is developing strategies to combat its rise; currently, more than 1 billion adults worldwide are overweight, and at least 300 million of them are obese [1-3].

A wide range of therapeutic strategies have been proposed to prevent and treat obesity, including pharmacotherapy, diet therapy, surgery, and behavioral therapy [4]. One of the practical therapeutic approaches

to the prevention of obesity is to slow down the absorption of fatty acids by inhibiting pancreatic lipase, the primary digestive enzyme that catalyzes the hydrolysis of ester bonds of tri- and diglycerides to monoglycerides and free fatty acids [5]. PL inhibitors are peripheral drugs that mediate a direct decrease in the absorption of calories in the gastrointestinal tract and affect the absorption of lipids. Currently, the only anti-obesity drug of this type approved for long-term use is the lipase inhibitor [6]. It is a saturated derivative of lipstatin, a potential natural inhibitor of PL derived from *Streptomyces toxytricini* [7]. However, prolonged use of Orlistat is accompanied by severe side effects, including hepatotoxicity, gallstones, kidney stones, and acute pancreatitis, which requires the development of new, safe, and effective drugs for the treatment of obesity [8, 9].

Various therapeutic strategies, such as pharmacotherapy, diet therapy, surgery, and behavioral interventions, have been proposed to prevent and treat obesity, with pancreatic lipase inhibition being a

\* Corresponding author: [muxammedov1989@mail.ru](mailto:muxammedov1989@mail.ru)

practical approach to reduce dietary fat absorption. Although Orlistat, a lipase inhibitor derived from lipstatin, is currently the only long-term approved drug of this class, its prolonged use is associated with severe side effects (e.g., hepatotoxicity, gallstones, kidney stones, and acute pancreatitis), highlighting the need for new, safer, and more effective anti-obesity agents [4-9]. As a result, much attention is paid to searching for and developing safe drugs with pharmacokinetic properties that are effective against obesity. In this aspect, natural compounds of plant origin, characterized by structural diversity, low toxicity, and a wide range of sources, have several inherent advantages for treating metabolic disorders [10, 11]. Many reports indicate safety, high efficacy against PL, and a positive effect on the gastrointestinal tract of natural compounds [12]. Natural plant lipase inhibitors of polyphenolic nature are mainly well-studied [13]. Therefore, natural plant-derived compounds, especially polyphenols, are being explored as safe and effective pancreatic lipase inhibitors for obesity management due to their structural diversity, low toxicity, and wide availability [10-13].

Along with many studies on lipase inhibition by natural plant products showing apparent inhibitory effects, there are also reports of microbial products as sources of natural pancreatic lipase inhibitors. Endophytic microorganisms living asymptotically inside plant tissues are recognized as a vast and little-explored resource of known and unique chemical structures exhibiting diverse biological activity [14]. Considering that some endophytic fungi synthesize substances similar to the metabolites of the host plant, they can be considered a new and up-and-coming alternative source of natural medicinal, including lipid-lowering compounds with minimal side effects [15, 16, 17]. In addition to plant-derived lipase inhibitors, microbial products—particularly endophytic microorganisms residing within plant tissues—have emerged as a promising and underexplored source of bioactive compounds. Since some endophytic fungi produce metabolites similar to those of their host plants, they represent a potential alternative source of natural lipid-lowering agents with reduced side effects [14-17]. In particular, fungal endophytes are considered as producers of inhibitors of many therapeutically significant enzymes [18]. It should also be noted that obtaining bioactive compounds from a microbial source is relatively cheaper and environmentally friendly.

The potential prospects of endophytic fungi as possible sources of lipase inhibitors follow from several reports on the PL-inhibitory properties of extracts of secondary metabolites of several endophytes. However, studies still need to be conducted on the chemical nature of endophytic inhibitors. In particular, [19] cultural filtrates of 70 endophytic fungi isolated from *Aegle marmelos* were screened. A high inhibitory potential of PL was found in the 57TBALM isolate with an  $IC_{50}$  value of 3.69 mg/ml, comparable to the  $IC_{50}$  of Orlistat (2.73 mg/ml) as a positive control [20] evaluated the PL-inhibitory activity of 39 endophytic fungi from medicinal plants of the Andaman Islands. The most significant inhibitory potential was found in 2 strains isolated from *C. lemon* and *Aegle marmelos*, with 75%

and 83% activity, respectively. During differential extraction of inhibitory metabolites in various solvents, the hexane extracts 9CLHTAI from *C. lemon* contained a substance with an activity of 87% and an  $IC_{50}$  of 15.46 mcg/ml and identified as terpene caryophyllene. Patil *et al.* (2021) [21] also discovered the inhibitory properties of the pentacyclic triterpenoid lupeol acetate from the endophytic *Colletotrichum gigasporum* isolated from *Withania somnifera*. The authors [22] concluded that the isolated compound could be a potential lead compound in developing a powerful new anti-obesity drug. Purpurolides D–F (1-3), three new polyoxygenated bergamotanes containing a tetracyclic ring system, were isolated from the endophytic fungus *Penicillium purpurogenum* IMM 003. Compounds from 1 to 3 demonstrated a significant inhibitory activity against the enzyme of pancreatic lipase (PL). The authors [23] emphasized that a fragment of 3-hydroxylated decanoic acid in C-14 is essential for increasing the effectiveness of lipase inhibition. These obtained results suggested that rare polyoxygenated bergamot can be viable candidates for a clinical development as inhibitors of PL. #6AMLWLS ( $IC_{50}$  = 2.12  $\mu$ g/mL) exhibited superior pancreatic lipase inhibitory activity compared to the only reported endophytic *Penicillium* species from *Taxus baccata* ( $IC_{50}$  = 3.69  $\mu$ g/mL) [24] and to Orlistat, the sole FDA-approved drug ( $IC_{50}$  = 2.79  $\mu$ g/mL).

In particular, authors [24] and [25] reported a broad spectrum of extrolites produced by species of *Aspergillus* section *Fumigati*, including *A. fischeri*, and highlighted their biological activities. Moreover, [26] demonstrated substantial genomic and chemical diversity in *A. fischeri*, identifying numerous biosynthetic gene clusters with potential pharmacological relevance.

However, to the best of our knowledge, none of these studies specifically reported polyphenolic compounds from *A. fischeri* exhibiting pancreatic lipase inhibitory activity, nor did they experimentally evaluate such activity. In the present study, the identification of polyphenolic constituents with potential pancreatic lipase inhibitory effects was based on LC–MS/MS profiling and *in silico* analysis, which represent a preliminary level of characterization.

Our previous studies have demonstrated the potent inhibitory activity of endophytes isolated from various medicinal plants [27]. Specifically, the ethyl acetate extract of the endophyte *A. fischeri* VOIR, isolated from *Viola odorata*, was found to suppress the activity of pancreatic lipase by 91.5% with an  $IC_{50}$  value of 20.5 mcg/ml, which very close to Xenical ( $IC_{50}$ ). So, presented work aims to determine the major PI inhibitory compounds in the most effective extracts of *A. fischeri* VOIR.

## 2 Materials and methods

### 2.1 Cultivation

In this study, endophytic fungal isolates were initially obtained from medicinal plants, cultivated, and their

secondary metabolites were extracted using standardized established methods. Subsequently, the isolates were screened for pancreatic lipase inhibitory activity, and those exhibiting the highest activity were selected. The optimal cultivation conditions, growth dynamics, nutrient media, and extraction solvents for the *Aspergillus fischeri* VOIR strain were investigated. As a result, the endophytic fungus *Aspergillus fischeri* VOIR was cultivated under submerged conditions in Czapek–Dox medium on an orbital shaker at 180 rpm for 7 days. The fungal biomass was then separated from the culture broth by centrifugation at 6000 rpm for 15 minutes.

## 2.2 Isolation of secondary metabolites

Secondary metabolites were isolated according to Hazalin et al. (2009) [28] 5 g of mycelial mass was homogenized, transferred to conical flasks with 50 ml of ethyl acetate and left for 24 hours on a circular rocker at 180 rpm at room temperature. The mixture was filtered through paper (Whatman No.1). The extracts were evaporated on a vacuum evaporator, and 1 ml of dimethyl sulfoxide (DMSO) was added. The obtained extracts were stored at -4 °C before use.

## 2.3 Fractionation of secondary metabolites

Fractionation of secondary metabolites from an ethyl acetate extract of endophyte biomass was carried out according to Kumar et al. (2013) [29] including sequential extraction with water, butanol, a mixture of methanol, and hexane (1:1). The extracts were dried on a rotary evaporator, and 1 ml of dimethyl sulfoxide was added. The resulting dry extracts were stored at -4° C before use. Differential fractionation was performed in triplicate.

## 2.4 TLC analysis of the extracts

Thin-layer chromatography of the extracts was performed on Silica Gel TLCALfoils plates manufactured by SIGMA-ALDRICH. The size of the plates was 10x10.10x15 cm in the system: benzene: chloroform (20:1) in a standard chromatographic chamber. 25 µl of the extract was applied to the plates manually with a micro-syringe at a distance of 1 cm from the lower edge. The plate with the applied substances was immersed in a chamber pre-saturated with vapors of the mobile phase. Chromatographic strips were scraped and extracted in butanol after separation. The plates were imaged in UV light at a wavelength of 254 nm. TLC was performed in more than three replicates.

## 2.5 Determination of the inhibitory activity

50 mg of lipase (Sigma, 60 u/ml) was suspended in 10 ml tris-HCl buffer containing 2.5 mM tris and 2.5 mM NaCl, pH 7.4. The solution was intensively shaken for 15 minutes and centrifuged at 4000 rpm for 10 minutes, and the supernatant was selected. Initial extracts and the

Xenical standard solutions were prepared in DMSO with linear concentrations in the range of 2-2000 micrograms/ml. The final reaction mixture consisted of 875 µl of buffer, 100 µl of the enzyme, and 20 µl of extract in various initial concentrations, pre-incubated for 5 minutes at 37 °C, followed by the addition of 10 µl of the substrate (4-nitrophenyl palmitate in 10 mM in acetonitrile). The portion of DMSO in the final concentration was not above 2%. The optical mixture was measured on a spectrophotometer (UV-5100 model) after 5 minutes at 405 nm. The percentage of inhibitory activity calculated according to formula below: % inhibition = (Ae-At) /Ae x 100,

Ae is the optical density of the enzyme control (without an inhibitor), and At is the difference between the optical density of the test sample with and without a substrate. Xenical was used as a comparison drug [30]. Experiments were conducted in more than three replicates.

## 2.6 Phytochemical analysis of metabolites

Qualitative composition of the *A. fischeri*VOIR extracts was determined according to Mehta et.al., (2001) [31].

The presence of tannins and phenolic substances was determined by adding 2-3 drops of 1% FeCl<sub>3</sub> solution to 2 ml of the resulting extract. In the presence of iron ions, tannins give a black-blue or black-green color, and phenols are purple.

The presence of saponins was established by dissolving 1 ml of the extract in 5 ml of hot water (60 °C) with further intensive shaking for 5 minutes until a persistent foam was formed. The foam volume was maintained for the next 30 minutes.

The presence of terpenoids was determined by mixing 0.5 ml of the extract with 2 ml of chloroform and 3 ml of H<sub>2</sub>SO<sub>4</sub> (conc.) The formation between the phases of red-brown staining indicated the presence of terpenoids.

The presence of anthraquinones was established by mixing 2 ml of the extract with 4 ml of hexane and subsequent shaking. At the same time, the extract was divided into two layers. The upper layer was separated, 4 ml of diluted 10 % ammonia was treated, and the color of the lower layer was determined. The purple-pink color indicated the presence of anthraquinones.

The presence of cardiac glycosides was determined by mixing 1 ml of the extract with 1 ml of glacial acetic acid and then adding one drop of 3 % ferric chloride in methanol. Then, H<sub>2</sub>SO<sub>4</sub> (conc.) was added along the tube wall, and the color of the lower layer was determined. The blue-green staining indicated the presence of cardiac glycosides.

The presence of alkaloids is based on their ability to form compounds insoluble in water with salts of heavy metals, complex iodides, complex acids, and other acidic compounds. These reactions make it possible to establish the presence of alkaloids even with their insignificant content. Iodine in potassium iodide solution (Wagner reagent, Bouchard reagent) with alkaloids forms brown, hardly soluble in water precipitates. Peptides test: 1 ml of 40 % sodium hydroxide and a few drops of 1% copper sulfate were

added to 2 ml of each extract; the formation of a violet-blue color indicates the presence of peptide bond molecules in the sample extract. Flavonoid test: A few drops of 20 % sodium hydroxide were added to 2 ml of each extract, and an intense yellow color was observed. We added a few drops of 70 % dilute hydrochloric acid to it, and the yellow color disappeared. The presence of flavonoids was detected by the formation and disappearance of yellow color in samples. The total content of polyphenols was determined by the Folin-Ciocalteu (FC) method with minor modifications [32]. 0.5 N FC reagent (diluted with water in a ratio of 1:1 by volume) was added to 1 ml of the extract with a concentration of 1 mg/ml to 100  $\mu$ l and left for 15 minutes. Next, 2.5 ml of sodium carbonate was added and incubated for 30 minutes at room temperature. Post-incubation absorption was measured at 765 nm with butanol as a control. Different gallic acid concentrations (5-100 micrograms/ml) were used to construct the calibration curve. The general content of polyphenols was expressed in micrograms of gallic acid equivalents (GAE) per mg of extract. The total content of flavonoids determined according to Park *et al.* (2008) [33] to 0.3 ml of the extract, 0.15 ml of 0.5 M NaNO<sub>2</sub>, 3.4 ml of 30% methanol, and 0.15 ml of 0.3 M AlCl<sub>3</sub>·6H<sub>2</sub>O were added and thoroughly mixed. After 5 minutes, 1 ml of 1 M NaOH was added. The absorption of the mixture was measured at 506 nm. A standard rutin solution (25 to 250 mg/l) was used to determine the total content of flavonoids expressed in mg of rutin equivalent.

## 2.7 LC-MS/MS analysis

Reverse-phase nano-LC-MS/MS was performed using the Agilent 1200 nano-flow LC system connected to the CHIP-Q-TOF Agilent Technologies 6520B series mass spectrometer. The sample was fractionated using an Agilent Technologies 1200 series chromatograph via a Zorbax SBC18. 5  $\mu$ m, 75 mm x 43 mm CHIP. Mobile phase: A - 0.1% formic acid solution + 5% acetonitrile, B - acetonitrile + 0.1% formic acid + 10% deionized water. The application was carried out on an Agilent Technologies 1260 series CapPump device at a 4  $\mu$ l/min flow rate. The elution was performed on an Agilent Technologies 1260 NanoPump series device at a 0.6  $\mu$ l/min flow rate. The concentration gradient of the solution in minutes: 0% - 3 min, 60% - 12-18 min, 0% - 20 min. The solutions were degassed on an Agilent Technologies 1260  $\mu$ -degasser device. The samples were deposited into the column using an Agilent Technologies MICROWPS device of 2  $\mu$ l. The eluted fractions were analyzed mass spectrometrically under the following conditions: Ionization source: ESI+, drying gas flow: 4 l/min, drying gas temperature: 350°C, voltage on the skimmer cone: 65V, on the fragmenter 175V, mass range: in MS50 - 3000 m/z mode, in MS/MS 50 mode - 2500 m/z, at a voltage at the CAP in the range 1800-2500V. Ionization method: positive. The mass spectra of the fractions were taken on a Q-TOF LC-MS Agilent Technologies 6520V device under the following conditions: ESI+ ion source, positive ion electrospray method, drying gas flow rate five l/min, drying gas temperature 300°C, ion acceleration voltage

on a skimmer 35V, fragmenter 175V, range MS 150 - 1000 m/z, target MS-MS 50 - 1000 m / z, collision energy - 30, 40, 50, 65. Samples were injected into a Zorbax SB C18. 3  $\mu$ m, 150x0. 5 mm column (Agilent Technologies 1200) with a mobile phase: A - 0.1% formic acid, B - acetonitrile + 0.1% formic acid. Elution on the Agilent Technologies 1260 Cap pump at 15  $\mu$ l/min: 5 min - 60%, 15-20 min - 90%, 25 min - 60% of the mobile phase B. All experiments were carried out in triplicate.

### 2.7.1 Determination of total polyphenol content

Total polyphenol content was determined using the Folin-Ciocalteu method. Briefly, 1 mL of the extract (1 mg/mL) was mixed with 100  $\mu$ L of 0.5 N Folin-Ciocalteu reagent, previously diluted with distilled water at a 1:1 (v/v) ratio. The mixture was allowed to stand for 15 min at room temperature. Subsequently, 2.5 mL of 1 N Na<sub>2</sub>CO<sub>3</sub> was added, and the reaction mixture was incubated for 30 min at room temperature. Absorbance was measured at 765 nm using a spectrophotometer.

### 2.7.2 Determination of total flavonoid content

Total flavonoid content was determined by the aluminum chloride colorimetric method. In brief, 0.3 mL of the extract (1 mg/mL) was mixed with 0.15 mL of 0.5 M NaNO<sub>2</sub>, 3.4 mL of 30% methanol, and 0.15 mL of 0.3 M AlCl<sub>3</sub>·6H<sub>2</sub>O. The mixture was thoroughly vortexed, followed by the addition of 1 mL of 1 M NaOH. Absorbance was measured at 506 nm using a spectrophotometer.

## 2.8 Statistical analysis

The reliability of the research findings is substantiated by the use of classical and modern research methods, the consistency of the results obtained through analysis with theoretical data reported in the scientific literature, and statistical processing of the results using Student's t-test and Fisher's analysis of variance (ANOVA). The mean deviation and p-values are presented in the tables and figures.

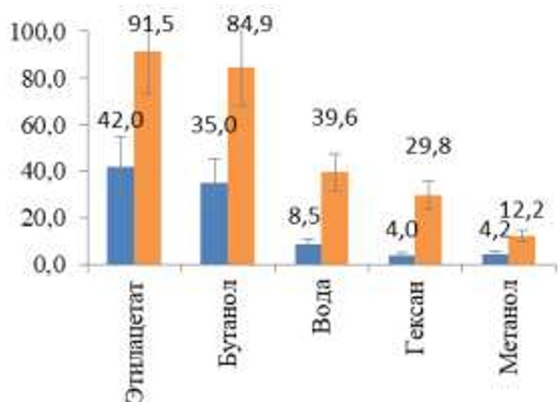
## 2.9 Biosafety considerations

All fungal handling and cultivation procedures were performed in accordance with standard biosafety practices. The work was conducted under Biosafety Level 1 (BSL-1) conditions in a certified microbiology laboratory. All culture media, fungal biomass, and waste materials were sterilized by autoclaving at 121 °C for 20 min prior to disposal. Personal protective equipment (PPE) including lab coat, gloves, and safety goggles was used throughout the study to prevent exposure to spores and metabolites.

### 3 Results and discussion

Previously, we demonstrated that the ethyl acetate extract of the endophytic biomass *A. fischeri* VOIR inhibited bovine pancreatic lipase activity by 91.5%, showing an inhibitory effect closely comparable to that of the standard drug Xenical (orlistat), which exhibited 92% inhibition. [26, 27]. Since endophytes produce various groups of chemical compounds, we extracted active inhibitory metabolites from the total ethyl acetate extract of *A. fischeri* VOIR biomass by stepwise fractionation.

Separation of secondary metabolites included sequential extraction of the initial ethyl acetate extract with water, a mixture of methanol with hexane (1:1), and butanol [29]. As a result, four fractions (aqueous, butanol, methanol, and hexane) were obtained from the initial ethyl acetate extract. As could be seen from the data in Figure.1, the initial content of dry extractives in the total ethyl acetate extract was 65 mg/g of biomass, and the PL inhibitory activity was 91.5%. When fractionating the total ethyl acetate extract with organic solvents with different polarities, 35 mg/g of the metabolites is extracted into the butanol fraction with an inhibitory activity 84.9%. Aqueous fraction contained 8.5 mg/g of biomass with an activity of 39.6%. A low level of extractive substances - 4 mg/g and 4.2 mg/g of biomass with inhibitory activity – 29.8% and 12.2%, is observed in hexane and methanol fractions, respectively.



**Fig. 1.** The yield of secondary metabolites and their inhibitory activity of extracts obtained by fractionation of the initial ethyl acetate extract *A.fischeri* VOIR

Qualitative analysis of the fractions we obtained showed that of the nine identified chemical classes of compounds (terpenoids, saponins, phenols, alkaloids, anthraquinones, cardiac glycosides, tannins, peptides and flavonoids), alkaloids, terpenoids, flavonoids, tannins and peptides were found in the obtained fractions. In the total ethylacetate extract, four classes of compounds (flavonoids, tannins, alkaloids and terpenoids) are present, polyphenols and cardiac glycosides are found in the aqueous fraction, and in the methanol fraction, with the lowest inhibitory activity, only terpenoids were identified. It should be noted that qualitative analysis of the hexane fraction did not show any of the tested compounds. Analysis of the butanol

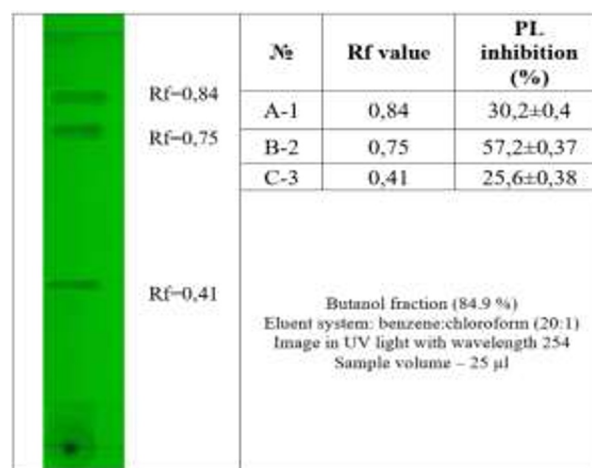
fraction with highest inhibitory activity revealed polyphenols, flavonoids (Table 1).

**Table 1.** Comparison of the phytochemical composition of *A.fischeri* VOIR extracts

№	Phyto-Constituents	Ethylacetate	Methanol	Hexane	Butanol	Water
1	alkaloids	+	-	-	-	-
2	flavonoids	+	-	-	+	-
3	terpenoids	+	+	-	-	-
4	saponines	-	-	-	-	-
5	tannins	+	-	-	-	-
6	polyphenols	-	-	-	+	+
7	cardiac glycosides	-	-	-	-	+
8	anthraquinones	-	-	-	-	-
9	peptides	-	-	-	-	-

"+" - indicates the presence of a substance, "-" - indicates the absence of a substance

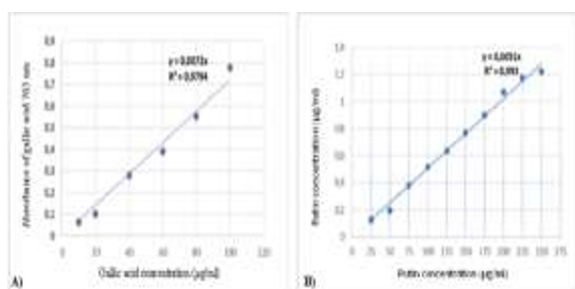
To identify the inhibitory compound, butanol fraction was separated by thin-layer chromatography in the benzene: chloroform system (20:1). The chromatography resulted in separation of the butanol fraction into three bands with different Rf values. Determination of the level of inhibition of PL of each of the fractions showed inhibitory activity of A-1 10.2% and C-3 15.6%, and the highest activity of 57.2%, belonged to the B-2 sample (Figure. 2). All bands applied on the TLC plates were prepared at a concentration of 10 mg/ml. All experiments were conducted in triplicate.



**Fig. 2.** TLC profile of butanol fraction of *A.fischeri* VOIR

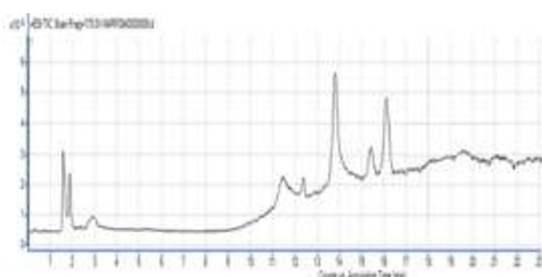
Qualitative analysis of the B-2 fraction revealed a positive reaction to flavonoids and polyphenols, as evidenced by the samples' yellow staining to yellow when 20% NaOH solution is added, and dark green when 10% iron chloride is added. The content of

polyphenols in terms of gallic acid is 24 mcg/ml, and flavonoids in terms of rutin is 4 mcg/ml (Figure 3).



**Fig. 3.** TLC profile of butanol fraction of *A. fischeri* VOIR

Thus, the main inhibitory metabolites of most active butanol fraction are represented by a class of polyphenols. It is well known that due to their potent antioxidant properties, natural polyphenolic compounds, which are secondary metabolites, play an essential role in treating many health-related problems [34]. Moreover, as mentioned above, from natural products of plant origin, the polyphenol class is one of the most critical potential pancreatic lipase inhibitors [13]. It has been shown that plant extracts containing flavonoid and non-flavonoid phenols can reduce body weight, fat mass, and the level of free fatty acids in plasma, and the accumulation of lipids in the liver by inhibiting enzymes, PL, lipoprotein lipase (LPL), and glycerophosphate dehydrogenases (GPDH) [34]. Endophytic fungi also produce medicinal compounds of plant origin, including alkaloids, coumarins, flavonoids, glycosides, lignans, phenylpropanoids, quinones, saponins, terpenoids, and xanthenes. In particular, the prospects for using endophyte-dependent biosynthesis of pharmaceutically active phenolic compounds, including flavonoids, phenolic acids, and lignans, are described in the review by Singh *et al.* (2021) [15]. However, more information is needed about the PL-inhibitory activity of polyphenols from endophytic fungi. Thus, Patil *et al.* (2021) [21] reported that the phenol-rich fraction from endophytic D. orange could competitively inhibit PPL and Orlistat. However, information on the composition of this fraction has yet to be provided. In our experiments, the identification of compounds of the prominent peaks of the bioactive B-2 sample by ultrahigh-efficiency chromatography and mass spectral analysis showed the presence of compounds with a molecular ion (M+H) with m/z 149.8834 at 1.50 minutes, a molecular ion (M+H) with m/z 557.2630 at 13.8 minutes, and a molecular ion (M+H) with m/z 585.3526 at 16.1 minutes (Figure.4).



**Fig. 4.** TIC chromatogram of the endophyte bioactive sample B-2 extract *A. fischeri* VOIR.

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Time (min)	Name	Linear Peak MS (M+H)	Molecular formula	Class of compounds	Source
1.7	Cinnamic acid	149.8834 m/z	C <sub>9</sub> H <sub>8</sub> O <sub>2</sub>	Polyphenol class: Phenolic acids	lign: phenol- explains 20% response
13.8	Lariciresinol- sesquiplignan	557.2630 m/z	C <sub>28</sub> H <sub>36</sub> O <sub>8</sub>	Polyphenol class: Lignans	lign: phenol- explains 20% response
16.1	3-Hydroxyphloretin 2'-O-xylosyl- glucoside	585.3526 m/z	C <sub>28</sub> H <sub>34</sub> O <sub>10</sub>	Polyphenol class: Flavonoids Polyphenol subclass: Diterpenolactones	lign: phenol- explains 20% response

**Fig. 5.** Mass spectral analysis of the bioactive sample B-2 of the butanol fraction of the endophyte *A. fischeri* VOIR

Thus, the most significant inhibitory activity in the butanol extract of *A. fischeri* VOIR is associated with three polyphenolic compounds. The structure–activity relationships between the identified polyphenolic compounds—cinnamic acid, lariciresinol-sesquiplignan, and 3-hydroxyphloretin 2'-O-xylosyl-glucoside—and their inhibitory activities are as follows:

Cinnamic acid is characterized by an aromatic ring conjugated with an unsaturated carboxylic acid moiety. This relatively small yet structurally rigid molecule is capable of interacting with pancreatic lipase at sites other than the catalytic active center. The carboxyl group can form hydrogen bonds with amino acid residues located in allosteric regions of the enzyme, while the aromatic ring contributes to hydrophobic interactions. These interactions may induce conformational changes in the enzyme, leading to reduced catalytic efficiency without directly competing with the substrate.

Lariciresinol-sesquiplignan, a polyphenolic compound with a bulky and complex structure, contains multiple phenolic hydroxyl groups. These functional groups enhance the ability of the molecule to establish strong hydrogen bonding and hydrophobic interactions with allosteric binding sites of pancreatic lipase. Due to its larger molecular size, lariciresinol-sesquiplignan is less likely to access the active site directly; instead, it modulates enzyme activity through conformational alterations, which is consistent with a non-competitive inhibition mechanism.

3-Hydroxyphloretin 2'-O-xylosyl-glucoside, a flavonoid glycoside, possesses both a polyphenolic aglycone and hydrophilic sugar moieties. The presence of multiple hydroxyl groups facilitates extensive hydrogen bonding, while the glycosidic residues increase molecular volume and polarity. These structural characteristics favor binding to peripheral or allosteric regions of pancreatic lipase rather than the substrate-binding site, thereby decreasing enzymatic activity independently of substrate concentration.

Overall, the polyphenolic nature, abundance of hydroxyl groups, and increased molecular size of the identified compounds play a crucial role in their interaction with pancreatic lipase. These features promote binding to allosteric sites and induce conformational changes in the enzyme, ultimately resulting in non-competitive inhibition. The findings highlight a clear structure–activity relationship underlying the lipase inhibitory potential of the active subfraction.

## 4 Conclusion

In this aspect, the results of this study are the first report on the polyphenolic nature of PL inhibitors from the endophytic fungus *A. fischeri* VOIR isolated from *Viola odorata*. Next, it is necessary to evaluate the inhibitory properties of the identified polyphenols, which will determine the prospects of their use as potential leading compounds in the development of new anti-obesity drugs. The extract of *Aspergillus fischeri* VOIR was evaluated in preclinical studies using mice. The results showed a significant reduction in body weight compared to the control group. Blood analysis revealed that the extract exhibited hypolipidemic and hypoglycemic properties.

Although in vitro and preclinical studies have indicated that polyphenolic compounds can effectively inhibit pancreatic lipase, further validation is required to translate these findings into clinical practice.

Therefore, well-designed clinical trials in humans are necessary to determine the therapeutic potential of pancreatic lipase-inhibiting polyphenols. Such studies are essential to evaluate their effectiveness in reducing fat absorption, improving lipid profiles, and supporting weight management, as well as to identify and monitor potential adverse effects. Only through rigorous clinical evaluation can these compounds be considered safe and effective therapeutic agents.

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