# Isolation and study of bioactive properties of secondary metabolites of *Aspergillus fischeri VO1R*, inhibiting pancreatic lipase

**Tashkhan Gulyamova, Dilaram Ruzieva, Maftuna Yoldosheva, Gulchekhra Rasulova, Liliya Abdulmyanova, Iqbol Mukhammedov**
*Institute of Microbiology, Academy of Sciences Republic of Uzbekistan, Tashkent, 100128 Uzbekistan.*
DOI: https://doi.org/10.5281/zenodo.10203202



## Summary

Pancreatic lipase (PL) inhibition is one of the most extensively researched mechanisms for determining the potential effectiveness of natural products as anti-obesity agents. The article discusses the extraction and bioactivity of secondary metabolites inhibiting PL from the endophyte of *Aspergillus fischeri VO1R*, isolated from *Viola odorata*. A comparison of eight solvents revealed that ethyl acetate and methanol extract the most inhibitory metabolites, amounting to 65 and 73 mg/g of biomass with PL-inhibiting activity of 91,5 and 65%, respectively. In addition, a phytochemical analysis of the metabolite composition revealed that flavonoids are abundant in all extracts with high and moderate inhibitory activity. The DPPH(1,1-diphenyl-2-

picrylhydrazyl) and $H_2O_2$ assays demonstrated that ethyl acetate has high antioxidant activity with half maximal inhibitory concentration ($IC_{50}$) values of 301 and 246 µg/ml comparable with $IC_{50}$ of ascorbic acid as the standard. The anti-inflammatory activity of the extract in vitro was 79.3% and exceeded the action of aspirin (74,5%). Therefore, it was concluded that the ethyl acetate extract of *A. fischeri VO1R* contains low-molecular-weight secondary metabolites capable of exhibiting complex PL-inhibitory, antioxidant, and anti-inflammatory effects in vitro and thus can be used to develop a comprehensive obesity treatment.

***Key words***
*obesity, endophytes, secondary metabolites, flavonoids, pancreatic lipase, inhibitory activity, antioxidants, anti-inflammation activity*

***Corresponding author***
***Iqbol Mukhammedov***
***Institute of Microbiology,***
***Academy of Sciences Republic of Uzbekistan,***
***Tashkent, 100128 Uzbekistan***
***E-mail: muxammedov1989@mail.ru***

## Introduction

Obesity is a common chronic lipid metabolism disorder characterized by excessive adipose tissue accumulation.[1,2] Clinical obesity affects approximately 300 million of the world's more than 1 billion overweight adults, according to the World Health Organization.[3,4] Overweight and obesity, on the other hand, are significant risk factors for diabetes, coronary heart disease, hypertension, hyperlipidemia, atherosclerosis, and other chronic diseases.[4] In this regard, one of the tasks of health policy in many countries is to reverse the current trends of rising percentages of overweight and obese individuals.[5,6]

A significant difficulty in determining treatment and prevention of the disease is the multifactorial origin of the etiology, which is associated with oxidative stress and chronic inflammation in obese people.[7,8] Many scientific trials have been conducted to treat obesity.[9] One of the therapeutic approaches to the prevention of obesity is slowing the absorption of fatty acids by inhibiting lipase in the digestive organs.[10,11] Pancreatic lipase (triacylglycerolacylhydrolase, PL), which catalyzes the hydrolysis of triacylglycerides in the gastrointestinal tract, is a crucial enzyme for lipid absorption. It is responsible for 50-70% of all dietary fats hydrolysis. Therefore, PL inhibition is one of the most widely studied mechanisms for determining the potential effectiveness of natural products as anti-obesity agents.[11]

Orlistat, a lipstatin derivative derived from *Streptomyces toxytricini*, is the only PL inhibitor approved by the FDA for treating obesity.[12,13] At the same time, the drug has many undesirable side effects, such as high blood pressure, insomnia, headache, dry mouth and others.[14] Nevertheless, the success of Orlistat stimulated research to identify new inhibitors of PL obtained from natural sources, such as Anthemis palaestina Boiss., Ononis natrix L.,[15] and Sapindus rarak DC[16] and oolong tea.[17]

Endophytes, which live asymptomatically inside plant tissues, have been identified as a rich source of unique chemical structures for pharmaceutical, agricultural and other uses.[18,19] Microbial natural products, particularly secondary metabolites of medicinal plant endophytic fungi, are an alternative to plant sources of enzyme inhibitors.[20] Endophytic fungi produce a wide range of bioactive compounds of various chemical classes, such as alkaloids, terpenoids, flavonoids, quinones, steroids, and phenolic acids, which have antimicrobial, anticancer, and immunomodulatory properties as well as inhibitory activity to several enzymes.[18,21]

Several studies have found that secondary metab-

olites of endophytic fungi can inhibit pancreatic lipase activity.[22-26] In particular, Gupta et al.[23,24] screened the culture filtrates of 70 endophytic fungi isolated from Aegle marmelos. A high PL inhibitory potential was detected in isolate 57TBBALM, 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.[23] Sarkar et al.[25] evaluated the PL-inhibitory activity of 39 endophytic fungi from medicinal plants of the Andaman Islands. The highest inhibitory potential was exposed in 2 strains isolated from Citrus lemon and Aegle marmelos, with an activity of 75% and 83%, respectively. At differential extraction of inhibitory metabolites in various solvents, the hexane extract of the endophyte 9CLHTAI from C. lemon contains a substance with an activity of 87% and an $IC_{50}$ of 15.46 μg/ml.

According to the results of gas-liquid chromatography, the metabolite was identified as caryophyllene.[25] Seven known and one new compounds, named 13-angeloyloxy-diplosporin, were isolated from endophytic Phomopsis sp. 0391 cultured in the presence of a histone deacetylase inhibitor. When assessing their PL-inhibitory activity, it was found for the first time that the compounds cytosporine B and dotiorelon A exhibit significant inhibitory activity with $IC_{50}$ values at 115 and 275 μg /ml, respectively.[26,27]

Previously, we isolated several endophytic fungi from various plants in Uzbekistan that can inhibit pancreatic lipase activity.[28] *Aspergillus sp.VO1R* was isolated from the root of Viola odorata, producing secondary metabolites with at least 70% inhibitory activity during ethyl acetate extraction.The purpose of this study was to determine the nature and bioactivity of secondary metabolites that mediate the strong PL inhibitory effect of *Aspergillus sp. VO1R* extract.

## Materials and methods

### Cultivation of endophytic strain

Endophyte were grown submergely on a Chapek-Dox medium at 28°C for seven days on an orbital shaker at 120 rpm. The biomass was separated by centrifugation at 6000 rpm and stored at +4°C.

### Extraction of secondary metabolites

The isolation of secondary metabolites was carried out according to Hazalin et al.[29] 5 g of mycelial mass was homogenized, transferred into a conical flasks with 50 ml of different solvents (ethyl acetate, ethanol, methanol, acetonitrile, hexane, chloroform, butanol, water) and left for a day on a shaker at room temperature. The mixtures were filtered through a paper (Whatman №1), and $Na_2SO_4$ was added (40 μg/ml) to remove the aqueous layer. Then the extracts were evaporated to dryness, and 1 ml of dimethyl sulfoxide (DMSO) was added. The obtained extracts was used as a stock and stored at +4°C.

### PPL inhibition assay

Porcine pancreatic lipase (PPL) activity was quantified according colorimetrical assay by the release of p-nitrophenol using p-nitrophenyl palmitate (PNPP) as a substrate.[30] 50 mg of lipase (Sigma, 100 U/ml) was suspended in 10 ml in tris-HCl buffer (2.5 mmol, pH 7.4 with 2.5 mmol NaCl). The solution was intensively shaken for 15 min and centrifuged at 4000 rpm for 10 min and the supernatant was recovered. The extracts and Xenical as a positive control were prepared in DMSO with linear concentrations ranging from 1.56–2000 μg/ml and 0.78–1000 μg/mL, respectively. The reaction mixture consisting of 875 μl of buffer, 100 μl of enzyme and 20 μl of extract in various initial concentrations was preincubated for 5 min at 37°C The reaction was then started by adding 10 μL PNPP as a substrate. After incubation at 30°C for 1 hour, the amount of p-nitrophenol released was measured on a spectrophotometer (SPEKOL 1300) at 405 nm, the percentage of inhibition was calculated by the formula: % inhibition = (Ae-At)/Aex100, where 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.

### Phytochemical analysis of extracts of secondary metabolites

Qualitative composition of compounds in extracts of endophytic fungi were determined according to Prabhavathi et al.[31]

The tannins and phenolic substances were determined by adding 2-3 drops of 1% $FeCl_3$ solution to 2 ml of the 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 diluting 1 ml of the extract with 5 ml of hot water (60°C) with intensive shaking for 5 minutes until the formation of a persistent foam. The foam volume was maintained for the next 30 minutes.

The terpenoids were determined by mixing 0.5 ml of the extract with 2 ml chloroform and 3 ml of $H_2SO_4$ (conc.). The formation between the phases of red-brown staining indicates the presence of terpenoids. 2 ml of the extract was mixed with 4 ml of hexane and shaken to determine the terpenoids. At the same time, the separation of the extract into 2 layers was observed. The upper layer was separated, 4 ml of 10%

ammonia was added, and the lower layer's color 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 $H_2SO_4$ (conc.) was added along the tube wall, and the color of the lower layer was determined. Blue-green staining indicated the presence of cardiac glycosides.

To determine flavonoids, a few drops of 20% sodium hydroxide were added to 2 ml of each extract, and the formation of an intense yellow color was observed. Next, a few drops of 70% dilute hydrochloric acid was added, and the yellow color disappeared. The formation and disappearance of yellow color indicate the presence of flavonoids in the sample extract.

The alkaloids was determined by their ability to form compounds insoluble in water with complex iodides, which makes it possible to establish the presence of alkaloids even with their insignificant content. A solution of iodine in potassium iodide (Wagner reagent, Bouchard reagent) with alkaloids form brown, hardly soluble in water precipitates. Five drops of the reagent for precipitation of alkaloids are added to 1 ml of the extract. In the presence of alkaloids, a brown precipitate appears.

**Molecular taxonomy and phylogenetic identification of endophytic fungi**

Genomic DNA extraction of the bioactive isolate of the endophytic fungus *VO1R* was performed by scraping the cultivated mycelium from a 3–4 day old culture using a pre-sterilized inoculation loop and grinding to a very fine powder with liquid nitrogen in a pestle and mortar. Further DNA extraction was performed using the ITS-based genomic DNA purification kit (100). The obtained data were analyzed by alignment in the BLAST program on the international database NCBI.[33]

**DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay**

To check the antioxidant activity through free radical scavenging by the test samples, the change in optical density of DPPH radicals was monitored.[34] Various dilutions of the aqueous extract were prepared (100, 200, 300, 400 and 500 µg /ml). The DPPH solution was prepared in ethanol according to Blois et al.[35] To 1 ml of the extract preparations from each dilution, 1 ml of 0.2 mM DPPH was added and incubated in the dark at room temperature for 30 minutes. A mixture of 1 ml of ethanol and 1 ml of DPPH solution (without extract) was used as a control, and ascorbic acid was used as a standard. The optical density was determined at 517 nm. The formula calculated the percentage of free radical uptake activity: % activity = (Ac – As)/Ac × 100, Where Ac is the absorption of the control; As is the absorption of the extract.

**Hydrogen peroxide scavenging ($H_2O_2$) assay**

The activity of fungal extracts to scavenge hydrogene peroxide was estimated by following the method of Govindappa et al.[34] A solution of hydrogen peroxide (40 mmol/L) was prepared in phosphate buffer (50 mmol/L, pH 7.4). The concentration of hydrogen peroxide was determined by absorption at 230 nm using a spectrophotometer. Endophytic extracts (100, 200, 300, 400 and 500 µg /ml) in distilled water was added to hydrogen peroxide and absorbance at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging was calculated as follows: scavenged $H_2O_2$ (%) = (Ai – At)/Ai × 100, where Ai is the absorbance of control and At is the absorbance of test samples.

**In vitro anti-inflammatory activity**

The anti-inflammatory activity of the extracts was assessed by suppression of albumin denaturation.[36] The test sample consisted of an endophyte extract of a selected concentration (100-500 µg /ml) and a 1% aqueous solution of bovine serum albumin (Sigma). The pH of the reaction mixture was adjusted to 6.5 using 1 n HCl and incubated at 37°C for 20 minutes. The incubation mixture was then heated at 57°C for 10 minutes. The denaturation process stopped by cooling the samples. The turbidity of the obtained solutions was measured on a spectrophotometer at a wavelength of 660 nm. Diclofenac sodium or aspirin in a concentration similar to the experimental extract was used as a standard. Inhibition of protein denaturation (X) was expressed as a percentage and calculated by the formula: X=(A0 – At)/A0 × 100 where A0 – optical control density; At – optical density of the test sample.

All experiments were carried out in triplicate.

**Statistical analysis**

Statistical analysis was performed by carrying out Student's t-test. Values were expressed as mean ± SD. Experiments were conducted in triplicates and the replicates were considered for calculating mean.

## Results and discussion

*Aspergillus sp.VO1R* is the most bioactive endophyte

isolate with high inhibitory activity against pancreatic lipase as identified by subsequent analysis of the ITS using the BLAST program in the international database NCBI. The isolate was found to be 99.12% identical to *Aspergillus fischeri* (MN121343.1). These data were compared with the database, the phylogenetic tree of the stamm was formed by "neighbor-joining" method in the MEGA program, and it was registered in the NCBI data under the name *Aspergillus fischeri VO1R*. (Fig.1).

Isolation and purification of biologically active compounds from endophytes is an essential process determining the suitability of the endophyte for the isolation of desired objects. Various polar and nonpolar solvents are used to extract secondary metabolites, depending on the nature of the extracted compound.[37] Since endophytic fungi produce a variety of bioactive compounds of various chemical classes, including alkaloids, terpenoids, flavonoids, anthraquinones, steroids, phenolic acids, polar and nonpolar solvents are used for extraction separately or in combination based on the solubility of the target metabolite. Ethyl acetate, methanol, dichloromethane, hexane, and ethanol are the most commonly used solvents to extract metabolites from culture broth. When the nature and polarity of the active compound are unknown, it is customary to screen solvents to select the conditions for extracting the substance with the highest desired bioactivity. The most common technique involves liquid-liquid extraction using an organic solvent from liquid media of a fungal culture or mycelial biomass. Many researchers use methanol, which often results in the extraction of large amounts of hydrophilic substances such as sugars, and ethyl acetate and acetone are lipophilic. However, they can also extract a wide range of metabolites.[38]

The degree of secondary metabolites extraction and their inhibitory activity level during the extraction of *A. fischeri VO1R* biomass was studied using eight commonly used solvents - ethyl acetate, methanol, ethanol, acetonitrile, water, hexane, butanol and chloroform.

As seen from the data presented in Table 1, the total yield of secondary metabolites significantly depends on the solvent used. It ranges from 19 to 73 mg/g of wet mycelium, and the level of inhibitory activity of extracts varies from 21% to 91,5%. At the same time, extraction with ethanol and acetonitrile does not lead to the extraction of inhibitory compounds. The most significant number of inhibitory secondary metabolites about 65±0.21 and 73±0.33 mg/g of wet biomass with an activity of 91,5±0.31 and 65±0.25%, was extracted with ethyl acetate and methanol, respectively. It should be noted that during the extraction of Myristica fragrans with petroleum



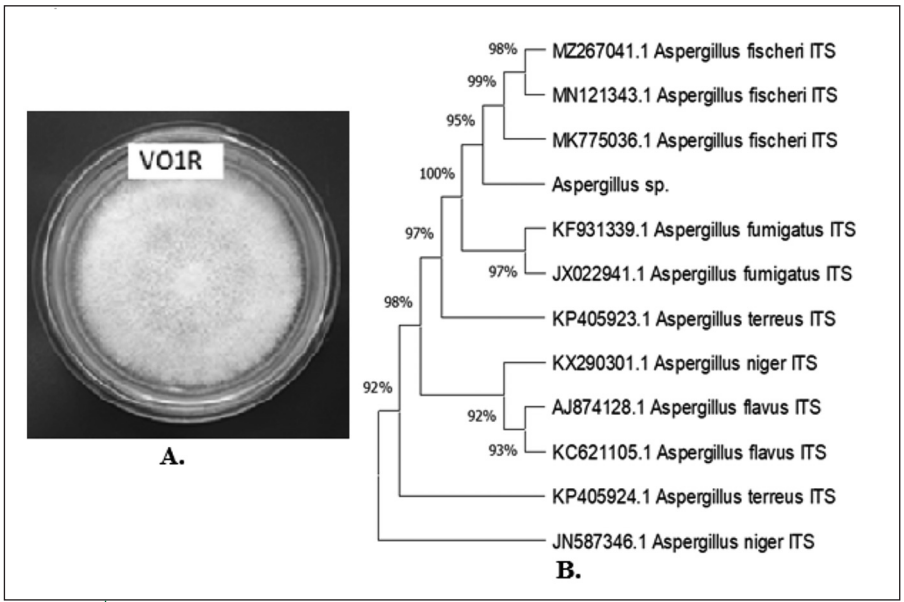


**Figure 1** Macrocolony of *Aspergillus fischeri VO1R* (A), Phylogenetic tree showing rDNA based (ITS region) molecular taxonomy and phylogenetic analysis of bioactive endophyte (B)

**Table 1** Yield and inhibitory activity of *A. fischeri VO1R* extracts during extraction with various solvents.

| № | Solvents | *A.fischeri VO1R* | |
|---|---|---|---|
| | | Extract mg/g | Inhibition % |
| 1 | ethyl acetate | 65±0.21 | 91,5±0.31 |
| 2 | methanol | 73±0.33 | 65±0.25 |
| 3 | ethanol | 26±0.19 | – |
| 4 | acetonitrile | 25±0.29 | – |
| 5 | hexan | 49±0.35 | 36,6±0.28 |
| 6 | butanol | 36,6±0.39 | 47,8±0.30 |
| 7 | water | 19±0.31 | 22,6±0.27 |
| 8 | chloroform | 29±0.26 | 21±0.29 |

ether, chloroform, and ethanol, significant PPL inhibitory (66,24%) activity in vitro compared to other extracts was also shown by ethanol extract at a concentration of 100 µg /ml.[39]

Thus, almost all used solvents, except for ethanol and acetonitrile, extract inhibitory metabolites with varied activity. However, given the nature of the solvents and the degree of their polarity, it is evident that the composition of the metabolites in these extracts is different. Preliminary phytochemical analysis of extracts in various solvents showed that endophytic *A. fischeri VO1R* produces eight chemical compounds, including alkaloids, flavonoids, terpenoids, saponins, tannins, cardiac glycosides, and anthraquinones. All these chemical compounds have different bioactivity and medicinal properties. Nevertheless, in all extracts with high and moderate PL-inhibitory activity, mainly flavonoids are detected. In particular, an ethyl acetate extract with inhibitory activity of 91,5±0.31 %, along with flavonoids contained an insignificant amount of alkaloids and terpenoids. In hexane, butanol and aqueous extracts with activity 36,6±0.28%, 47,8±0.30% and 22,6±0.27%, flavonoids also prevailed. The methanol extract with inhibitory activity of 65% contained terpenoids, glycosides and antraquinones, but not flavonoids. (Table 2).

According to obtained, the inhibitory activity of *A. fischeri VO1R* extracts are mainly associated with flavonoids and terpenoids. It is worth noting that flavonoids and terpenes are the main chemical components responsible for reducing lipid peroxidation.[40]

It is known that obesity, which is a feature of metabolic syndrome, was associated with chronic inflammation in obese people.[7] Due to the prominent role of oxidative stress in the pathogenesis of obesity, there is a growing interest in weakening the pro-oxidant state in obesity since antioxidants contribute to the normalization of the inflammatory response. Acting through inflammatory cascades such as PKC and NF-kb, antioxidants reduce the levels of various inflammatory mediators, including TNF-a, IL-1b, VCAM-1, and IL-6 (182-184). In this regard, antioxidant and anti-inflammatory action agents are of therapeutic importance in the development of anti-obesity drugs. Endophytic fungi are an abundant and reliable source of new antioxidant compounds, evidenced by a sufficient number of reports on the antioxidant properties of endophytic metabolites.[41,43] For example, Yadav et al. demonstrated that metabolites produced by endophytic fungi isolated from Eugenia jambolana can be a potential source of new natural antioxidant compounds using three methods of determining antioxidant activity.[44]

Nakai et al.[18] used the DPPH radical removal method to demonstrate the antioxidant activity of ethyl acetate extracts of 13 isolates of endophytic fungi. The most potent antioxidant capacity was found in *A. minisclerotigens* AKF1 and *A. oryzae* DK7 isolates, with of *Aspergillus sp.VO1R* 0 values of 142.96 µg/ml and 145.01 µg/ml, respectively.

Gautam et al. discovered that ethyl acetate extracts the most significant amount of total phenols and flavonoids from the endophytic *Nigrospora sphaerica* isolated from the pantropical weed Euphorbia hirta L., positively correlating with antioxidant activity. Simultaneously, compounds with inhibitory activity similar to quercetin were discovered in the phenolic compound composition.[45]

In vitro antioxidant activity of an ethyl acetate ex-

**Table 2** Phytochemical screening of PL inhibitory extracts of *A. fischeri VO1R*.

| № | Phyto-Constituents | Solvents | | | | | |
|---|---|---|---|---|---|---|---|
| | | 91,5% | 65% | 36,6% | 47,8% | 22,6% | 21% |
| | | ethylacetate | methanol | hexane | butanol | water | chloroform |
| **1** | alkaloids | + | - | - | - | - | - |
| **2** | flavonoids | ++ | - | ++ | ++ | ++ | - |
| **3** | terpenoids | + | ++ | - | - | - | - |
| **4** | saponines | - | - | + | - | - | + |
| **5** | tannins | - | - | - | - | + | - |
| **6** | phenols | - | - | - | - | + | - |
| **7** | glycosides | - | + | - | + | - | - |
| **8** | antraquinones | - | + | - | - | + | + |

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

**Table 3** DPPH antiradical activity of ethyl acetate extract of *A.fischeri VO1R*.

| № | Concentration µg/ml | antiradical activity (%) | | | |
|---|---|---|---|---|---|
| | | Ascorbic acid,% | $IC_{50}$ µg/ml | Extract, % | $IC_{50}$ µg/ml |
| 1 | 100 | 21,5±0.11 | 288 | 15,6±0.27 | 301 |
| 2 | 200 | 33,4±0.15 | | 29,7±0.29 | |
| 3 | 300 | 44,2±0.09 | | 45,3±0.15 | |
| 4 | 400 | 86±0.11 | | 64,1±0.39 | |
| 5 | 500 | 97,5±0.16 | | 81,3±0.31 | |

tract of *A.fischeri VO1R* containing flavonoids with significant PL-inhibitory effect was determined using two free radical models – DPPH and $H_2O_2$.

The DPPH method measured antioxidant activity by changing the color of DPPH from purple to yellow. At concentrations ranging from 100 to 500 µg/ml, the activity of free radical absorption by ethyl acetate extract increased from 15% to a maximum of 81.3% at 500 µg/ml (Table 3). At the same time, the antiradical activity of the extract 300 µg/ml increased slightly with concentration, reaching 45.3±0.15% compared to 44.2±0.09% for the standard.

In general, antioxidant activity increases with metabolite concentration. The calculated $IC_{50}$ value of ethyl acetate extract was 301 µg/ml, whereas ascorbic acid had an $IC_{50}$ value of 288 µg/ml.

When determining antioxidant activity by $H_2O_2$, the same correlation dependence on extract concentration was observed (Table 4).

This assay shows that the activity of the extract increases from 15.8±0.41% at 100 µg/ml to 78.4±0.33% at 500 µg /ml.

However, the antioxidant activity of ethyl acetate extract is higher than that of ascorbic acid at all concentrations used in this test, with a maximum of 70.5±0.19%.The high antioxidant activity of ethyl acetate extract is confirmed by a significantly lower $IC_{50}$ value of 246 µg /ml compared to the standard's $IC_{50}$ of 300 µg /ml.

This study's findings are consistent with previous research on the antioxidant activity of endophytic fungi. Thus, the total phenol content (TPC) and total flavonoids content (TFC) were highest in the ethyl acetate crude extract of *Nigrospora sphaerica* fermented in potato-dextrose broth (77.74 = 0.046 mg/g and 230.59 = 2.0 mg/g, respectively), with the highest antioxidant activity of 96.80%.[45] Extracts of *A. nidulans* and *A. flavus* isolated from Ocimum basilicum also demonstrated potential antioxidant activity, with $IC_{50}$ of 166.3 g/ml and 347.1 g/ml, respectively, using DPPG.[46]

The anti-inflammatory activity of *A.fischeri VO1R* extract was assessed using an albumin denaturation assay and aspirine as a positive control. The maximum inhibition of albumin denaturation was observed at 500 g/ml aspirin concentration, and the degree of in-

**Table 4** Antioxidant activity of ethyl acetate extract of *A.fischeri VO1R* by $H_20_2$ absorption assay.

| № | Concentration µg/ml | $H_2O_2$ absorption, % | | | |
|---|---|---|---|---|---|
| | | Ascorbic acid,% | $IC_{50}$ µg/ml | Extract, % | $IC_{50}$ µg/ml |
| 1 | 100 | 10,4±0.16 | 300 | 15,8±0.41 | 246 |
| 2 | 200 | 25,8±0.16 | | 30,4±0.37 | |
| 3 | 300 | 30,4±0.20 | | 45,5±0.39 | |
| 4 | 400 | 45,2±0.11 | | 61,5±0.29 | |
| 5 | 500 | 70,5±0.19 | | 78,4±0.33 | |

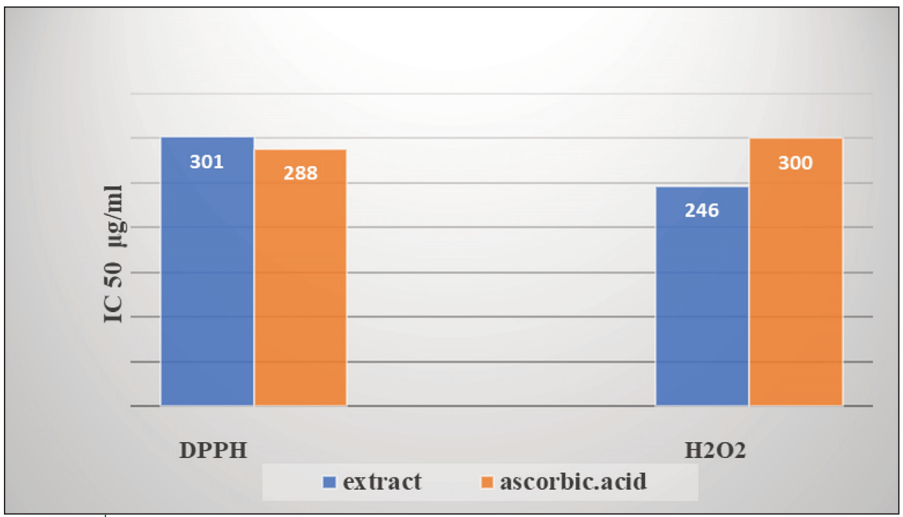


**Figure 2** $IC_{50}$ values of antioxidant activity of *A.fischeri VO1R* extract and ascorbic acid.

**Table 5** Anti-inflammatory activity of *A.fischeri VO1R* by albumin denaturation assay.

| № | Concentration μg/ml | Aspirine, % | Inhibition ,% |
|---|---|---|---|
| 1 | 100 | 25,8±0.20 | 31,2±0.27 |
| 2 | 200 | 36,4±0.15 | 40,2±0.41 |
| 3 | 300 | 49,2±0.18 | 55,2±0.36 |
| 4 | 400 | 60,2±0.24 | 60,5±0.34 |
| 5 | 500 | 74,5±0.1 | 79,3±0.39 |

hibition by the extract at the same concentration exceeded the positive control by 79.3±0.39%. It could be a preliminary indication of the anti-inflammatory properties of *A.fischeri VO1R* ethyl acetate extract.

## Conclusion

This study shows that endophytes associated with V.odorata have tremendous bioactive potential as PL inhibitors. These endophytic extracts can be studied to develop potential drugs for the treatment of obesity by isolating potent molecules.The most active isolate Aspergillus sp.VOR1 was identified as *Aspergillus fischeri VO1R*.

According to the results of the experiments, the ethyl acetate extract of *A.fischeri VO1R* contains low molecular weight secondary metabolites capable of exhibiting a complex PL-inhibitory, antioxidant, and anti-inflammatory effect in vitro. Given that the ethyl acetate extract contains flavonoids, terpenoids, and alkaloids, more research is needed to determine whether the extract's activities are the result of a synergistic effect of these substances or of a single compound with the properties of a multitargeted ligand.

As a result of the data presented, it is possible to speculate that *A.fischeri VO1R* isolated from Viola odorata can be used to develop a new therapeutic agent with multiple actions, allowing for an integrated approach to the treatment of obesity.

***Conflict to interest***

The authors declare that they have no competing financial interests or personal relationships that could influence the research reported in this article.

# Περίληψη

## Isolation and study of bioactive properties of secondary metabolites of *Aspergillus fischeri VO1R*, inhibiting pancreatic lipase

**Tashkhan Gulyamova, Dilaram Ruzieva, Maftuna Yoldosheva, Gulchekhra Rasulova, Liliya Abdulmyanova, Iqbol Mukhammedov***
*Institute of Microbiology, Academy of Sciences Republic of Uzbekistan, Tashkent, 100128 Uzbekistan*
**Corresponding author*

Η αναστολή της παγκρεατικής λιπάσης (PL) είναι ένας από τους πιο εκτενώς ερευνημένους μηχανισμούς για τον προσδιορισμό της πιθανής αποτελεσματικότητας των φυσικών προϊόντων ως παραγόντων κατά της παχυσαρκίας. Η παρούσα μελέτη παρουσιάζει την εκχύλιση και τη βιοδραστικότητα δευτερογενών μεταβολιτών που αναστέλλουν την PL από το ενδόφυτο του *Aspergillus fischeri* VO1R, που απομονώθηκε από το *Viola odorata*. Μια σύγκριση οκτώ διαλυτών αποκάλυψε ότι ο οξικός αιθυλεστέρας και η μεθανόλη αποτελούν τους περισσότερο ανασταλτικούς μεταβολίτες, εκχυλιζόμενοι σε 65 και 73 mg/g βιομάζας με ανασταλτική δράση PL 91,5 και 65%, αντίστοιχα. Επιπλέον, μια φυτοχημική ανάλυση της σύνθεσης του μεταβολίτη αποκάλυψε ότι τα φλαβονοειδή είναι άφθονα σε όλα τα εκχυλίσματα με υψηλή και μέτρια ανασταλτική δράση. Οι αναλύσεις DPPH (1,1-διφαινυλ-2-πικρυλυδραζύλιο) και H2O2 έδειξαν ότι ο οξικός αιθυλεστέρας έχει υψηλή αντιοξειδωτική δράση με τιμές μισής μέγιστης ανασταλτικής συγκέντρωσης (IC50) 301 και 246 μg/ml συγκρίσιμες με το IC50 ασκορβικού οξέος ως πρότυπο. Η αντιφλεγμονώδης δράση του εκχυλίσματος in vitro ήταν 79,3% και ξεπέρασε τη δράση της ασπιρίνης (74,5%). Ως εκ τούτου, συνήχθη το συμπέρασμα ότι το εκχύλισμα οξικού αιθυλεστέρα του *A. fischeri* VO1R περιέχει δευτερογενείς μεταβολίτες χαμηλού μοριακού βάρους ικανούς να επιδεικνύουν σύνθετες ανασταλτικές, αντιοξειδωτικές και αντιφλεγμονώδεις επιδράσεις in vitro και έτσι μπορεί να χρησιμοποιηθεί για την ανάπτυξη μιας ολοκληρωμένης θεραπείας της παχυσαρκίας.



***Λέξεις κλειδιά***
*παχυσαρκία, ενδόφυτα, δευτερογενείς μεταβολίτες, φλαβονοειδή, παγκρεατική λιπάση, ανασταλτική δράση, αντιοξειδωτικά, αντιφλεγμονώδη δράση*

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