BIOACTIVE ENDOPHYTIC MICROORGANISMS OF UZBEKISTAN: A PROMISING SOURCE OF PANCREATIC LIPASE INHIBITORS

Yoldosheva Maftuna Mengbutayevna

PhD, Acting Associate Professor, University of Business and Science, Tashkent, Uzbekistan

Alimova Sevara Alimovna

Student, Faculty of Medicine, University of Business and Science, Tashkent, Uzbekistan

Abstract. According to the latest statistical reports of the World Health Organization (WHO), 13% of the total world population suffers from obesity. The most effective therapeutic approach for treating obesity is the inhibition of pancreatic lipase (PL). In the process of searching for new PL inhibitors, we screened the anti-lipase activity of intracellular metabolites of 122 endophytic fungi isolated from medicinal plants growing in Uzbekistan. The highest number of endophytes with a high degree of PL inhibitory activity was found in 18 extracts (70–87%). The data obtained showed that metabolites capable of inhibiting pancreatic lipase at the level of the Xenical standard (more than 70%) are synthesized by endophytes isolated from the medicinal plants *Ginkgo biloba, Aloe vera, Viola odorata, Taraxacum officinale, Achillea millefolium, Achillea tomentosa, Peganum harmala.* Based on the results, we conclude that endophytic fungi represent a potential source of PL inhibitors, which may lead to the development of new valuable drugs for the treatment of obesity.

Keywords: pancreatic lipase, inhibitory activity, obesity, Xenical, orlistat, endophytic fungi.

Introduction: Obesity is the most widespread disease worldwide. Targeting lipid metabolism for the development of anti-obesity drugs is the best therapeutic option. The primary drug target is pancreatic lipase, which is responsible for the breakdown of 50–70% of dietary fats [1].

One therapeutic approach to preventing obesity is to slow the absorption of fatty acids by inhibiting lipase in the digestive tract [2]. Pancreatic lipase (PL) is the primary digestive enzyme that catalyzes the hydrolysis of ester bonds in tri- and diglycerides to produce monoglycerides and free fatty acids. PL inhibitors disrupt the activity of pancreatic lipase. They belong to the class of peripherally acting drugs that directly reduce caloric absorption in the gastrointestinal tract and affect lipid absorption [3, 4]. Currently, the only approved long-term anti-obesity drug of this type is the lipase inhibitor Orlistat [5]. It is a saturated derivative of lipstatin, a potential natural PL inhibitor obtained from *Streptomyces toxytricini*. However, it has recently been reported that prolonged use of Orlistat is associated with serious side effects, including hepatotoxicity, gallstones, kidney stones, and acute pancreatitis, highlighting the need to develop new safe and effective drugs for the treatment of obesity [6].

Research based on endophytic natural products may be key to developing drugs against such health problems [7, 8]. Endophytes contribute greatly to the process of drug discovery and development, as they produce natural products with a wide variety of novel chemical structures and biological activities [9].

In this regard, the aim of the present work was to isolate and study endophytes from certain medicinal plants as potential producers of pancreatic lipase inhibitors.

MATERIALS AND METHODS

Isolation of endophytic fungi: Isolation of endophytic fungi was carried out according to Hazalin et al. [10] from roots, stems, leaves, and inflorescences of the collected plants. After surface sterilization with 70% ethanol for 1 minute, followed by 0.1% NaOCl for 3 minutes, then 30% ethanol for 30 seconds, the plant material was rinsed with sterile water. Each plant segment was aseptically cut into pieces no larger than 0.5 cm and placed on Petri dishes containing Czapek-Dox agar supplemented with chlortetracycline at a concentration of 50 mg/ml and streptomycin sulfate at 250 mg/ml to suppress bacterial growth.

Plates were incubated for 7–14 days at 28°C. Individual endophyte colonies that emerged after incubation were picked with a fine needle, transferred to agar slants, and incubated at 28°C for seven days.

Czapek-Dox medium with antibiotics was used as a control. Endophytic fungal cultures were maintained by periodic subculturing onto Czapek-Dox agar slants. All isolates are stored in a refrigerator at +4°C.

Cultivation of endophytes: To accumulate biomass for subsequent extraction and assessment of biological activity, endophytes were grown by submerged fermentation in flasks on an orbital shaker at 120 rpm for 7 days at 28°C. At the end of the cultivation period, the biomass was separated from the fermentation broth by centrifugation at 4,000 rpm for 15 minutes.

Extraction of secondary metabolites from endophytic fungal biomass.

Extraction of secondary metabolites from the endophytic fungal biomass was carried out according to Lang et al. with modifications by Hazalin et al. [10]. For this purpose, 5 g of biomass was homogenized in a Potter homogenizer, transferred to a conical flask, and extracted twice with ethyl acetate (25 ml per 5 mg of wet homogenized biomass) for 24 hours on a rotary shaker at room temperature. The mixture was then filtered through filter paper (Whatman No. 1) and Na_2SO_4 was added at 40 µg/ml to remove the aqueous layer. The mixture was then evaporated to dryness on a rotary evaporator and 1 ml of dimethyl sulfoxide (DMSO) was added. The resulting extract was used as a stock solution and stored at +4°C.

Spectrophotometric determination of PL inhibition.

50 mg of porcine pancreatic lipase was suspended in 10 ml of Tris-NaCl buffer (containing 2.5 mmol Tris and 2.5 mmol NaCl, pH adjusted to 7.4 with HCl). The solution was vigorously shaken for 15 min followed by centrifugation (4,000 rpm for 10 min). The supernatant was collected and used as the enzyme solution. Stock solutions of extracts and Xenical were prepared in DMSO at

concentrations of 10 mg/ml. The final reaction mixture consisted of 875 µl buffer, 100 µl enzyme, and 20 µl extract, pre-incubated for 5 min at 37°C, followed by the addition of 10 µl substrate (4-nitrophenyl palmitate, 10 mM in acetonitrile). The absorbance of the final mixture was measured on a SPECOL-1300 spectrophotometer at 405 nm after 5 minutes. The assay was performed in triplicate, and the percentage of inhibition was calculated using the formula:

$$\% \text{ PL Inhibition} = [(Ae - At) / Ae] \times 100$$

where, Ae is the absorbance of the enzyme control (without inhibitor), and At is the difference between the absorbance of the test sample with substrate and without it.

RESULTS AND DISCUSSION

A total of 122 endophytic fungal isolates were obtained from roots, stems, leaves, and inflorescences of 15 previously unstudied plants growing in Uzbekistan, as presented in Table 1.

Table 1. Endophytic fungi of medicinal plants of Uzbekistan

No.	Plant name (latin)	Inflorescence	Leaf	Stem	Root	Total isolates
1.	*Aloe vera*	—	6	—	9	15
2.	*Caléndula officinális*	—	—	1	2	3
3.	*Matricaria chamomilla*	—	—	2	—	2
4.	*Armorácia rusticána*	—	—	—	1	1
5.	*Viola*	2	2	—	4	8

	odorata					
6.	*Taraxacum officinale*	7	6	—	6	19
7.	*Artemisia officinalis*	—	2	1	5	8
8.	*Achillea millefolium*	2	3	—	2	7
9.	*Achillea tomentosa*	—	5	9	2	16
10.	*Hypericum*	4	2	—	2	8
11.	*Salvia officinalis*	—	—	5	3	8
12.	*Peganum harmala*	—	—	2	2	4
13.	*Tanacetum vulgare*	3	—	2	2	7
14.	*Cuscuta L.*	—	—	6	—	6
15.	*Ginkgo biloba*	—	6	4	—	10
Total		18	32	32	40	122

Different quantitative distributions of endophytic fungi were observed across different plant parts. Of all the plant parts studied, the highest number of endophytic fungi was isolated from roots — 32.7%, followed by leaves and stems at 26.2% each, and inflorescences at 14.7%.

The isolated endophytic fungal cultures were maintained on Czapek-Dox agar slants with monthly subculturing. Submerged cultivation in liquid Czapek-Dox medium was used to accumulate endophytic fungal biomass for subsequent extraction of secondary metabolites and study of their properties.

The inhibitory properties of the isolated endophytes were studied in an in vitro system using pancreatic lipase and p-nitrophenyl palmitate as a substrate. As can be seen from the data presented in Table 2, screening of the PL inhibitory activity of 122 endophytic fungal isolates revealed inhibitory activity in nearly all newly isolated endophytes.

Table 2. Screening of PL inhibitory activity of extracts from 122 newly isolated endophytes

No.	Plant name (latin)	PL inhibition (%)
1.	*Aloe vera*	10–70
2.	*Caléndula officinális*	26–33
3.	*Matricaria chamomilla*	10–15
4.	*Armorácia rusticána*	11.2
5.	*Viola odorata*	28–75
6.	*Taraxacum officinale*	20–73
7.	*Artemisia officinalis*	14–33
8.	*Achillea millefolium*	44–84
9.	*Achillea tomentosa*	32–79
10.	*Hypericum*	19–32
11.	*Salvia officinalis*	35–40

12.	*Peganum harmala*	64–73
13.	*Tanacetum vulgare*	24–50
14.	*Cuscuta L.*	25–40
15.	*Ginkgo biloba*	53–87
Xenical standart		72

CONCLUSION

The highest number of endophytes with a high degree of PL inhibitory activity was found in 18 extracts (70–87%). A cumulative analysis of the data shows that metabolites capable of inhibiting pancreatic lipase at the level of the Xenical standard (more than 70%) are synthesized by endophytes isolated from the medicinal plants *Ginkgo biloba, Aloe vera, Viola odorata, Taraxacum officinale, Achillea millefolium, Achillea tomentosa, and Peganum harmala.*

Thus, the results of the study serve as a basis for investigating the metabolites of these endophytic fungal strains as a promising source of novel native pancreatic lipase inhibitors and may be used in the development of potential agents for the treatment of obesity.

REFERENCES

1. Mahiti Gupta, Sanjay Saxena, Dinesh Goyal. Lipase inhibitory activity of endophytic fungal species of Aegle marmelos: A bioresource of potential pancreatic lipase inhibitors. // Symbiosis, 2015. pp. 149–157.

2. Elangbam K.S. Current strategies in the development of anti-obesity drugs and their safety concerns. // American College of Veterinary Pathologists, 2009. pp. 1–10.

3. Colon-Gonzalez F., Kim G.W., Lin J.E., Valentino M.A., Waldman S.A. Pharmacotherapy of obesity: What lies ahead? // Molecular Aspects of Medicine, 2013. pp. 71–83.

4. Information on orlistat (marketed as Alli and Xenical).

5. Weibel E.K., Gadwary P., Hocheli E., Kupfer E., Lengsfeld N. Lipstatin, an inhibitor of pancreatic lipase, produced by Streptomyces toxytricini. I. Producing organism, fermentation, isolation and biological activity. // The Journal of Antibiotics, 1987. pp. 1081–1085.

6. Dias D.A., Urban S., Roessner U. A historical overview of natural products in drug discovery. // Metabolites, 2012. No. 2, pp. 303–336.

7. Ali A.H., Debbab A., Kjer J., Proksch P. Fungal endophytes of higher plants: An abundant source of phytochemicals and other bioactive natural products. // Fungal Diversity, 2010. No. 1, pp. 1–16.

8. Samantha S., Ghosh S., Mandal N.K. Endophytic fungi: A source of novel pharmaceutical compounds. // In: Endophytes, 2021. pp. 103–151. Springer, Singapore.

9. Gupta S., Chaturvedi P., Kulkarni M., van Staden J. A critical review of the pharmaceutical potential of plant endophytic fungi. // In: Fungal Endophytes of Medicinal Plants, 2019.

10. Hazalin N.A., Ramasamy K., Lim S.M., Wahab I.A., Cole A.L.J., Majid A.A. Cytotoxic and antibacterial activities of endophytic fungi isolated from plants of a National Park. // BMC Complementary and Alternative Medicine, 2009. Pahang, Malaysia. pp. 9–46.