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Research Article

ENDOPHYTIC *PESTALOTIOPSIS* SPECIES FROM ANDAMAN ISLANDS: A POTENTIAL PANCREATIC LIPASE INHIBITOR

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ABSTRACT

Objective: Obesity is the major cause of deaths worldwide. Inhibition of enzymes involved in breakdown of fats can be a good way for obesity management. Pancreatic lipase (PL) causes 50-70% degradation of ingested fats. The aim of this work is to explore the endophytic fungi from untapped biodiversity of Andaman Islands for PL inhibitors.

Methods: In the current report, culture broths of 39 endophytic fungi from different curative plants of coastal regions of India have been tested for their potential PL inhibitory activity. The bioactive compound was thus isolated, purified, and analyzed using gas chromatography.

Results: It was found that inhibitory concentration of a compound (R_f=0.64) isolated from crude hexane extract of endophytic fungal isolate from *Citrus limon* was 15.46 µg/ml. Gas chromatogram of the extract showed the presence of caryophyllene which might be responsible for the particular activity. The bioactive fungus was microscopically identified as *Pestalotiopsis* species.

Conclusion: As caryophyllene is component of many oils and is non-toxic, so it can be potential source of safe and effective anti-obesity drug.

Keywords: Pancreatic lipase, Endophytic fungi, Caryophyllene, Gas chromatography.

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INTRODUCTION

Discovery of penicillin from *Penicillium notatum* brought a revolution in the age of antibiotics. Since then, fungal domain has been constantly explored for various bioactive metabolites. Over the past two decades, another group of fungal system known as endophytes that reside inside the plant has been constantly explored for its potential pharmaceutical applications. Endophyte produces certain biochemicals that protect the host plant from biotic and abiotic stress [1]. With the course of evolution, these host plant endophytes gain the property to produces similar compounds like their host. In the past decade, over 500 fungal metabolites have been used as potential drugs [2].

Pancreatic lipase (PL) is the main target as anti-obesity agent due to its important role in metabolism of fats. Orlistat is only partly natural PL inhibitor that too has been isolated from a bacterial source *Streptomyces toxytricini* which also serves certain side effects [3]. Shortage of safe and reliable PL inhibitors has motivated researchers to explore new sources for PL inhibitors that might be eukaryotic in origin. Endophytic fungi still remain poorly explored wealth for PL inhibitor.

In the present study, endophytic fungi from medicinal plants of Andaman Islands of India have been explored for their potential to inhibit PL that can further be targeted in obesity management. Culture filtrates of 39 endophytic fungi from three different medicinal plants were tested for their potential to inhibit PL inhibitory under *in vitro* conditions. The current study marks the first report of exploration of flora from untapped Andaman Islands for PL inhibitors. Moreover, this is the first report of endophytic *Pestalotiopsis* sp. isolated from *Citrus limon* producing PL inhibitors.

Different medicinal plants were collected from Andaman Islands (11.7401° N, 92.6586° E) of India. Endophytic fungi were isolated using modified protocol of Schulz *et al.* [4]. The culture filtrates of the isolated endophytes were produced in potato dextrose broth by the procedure described by Raviraja *et al.* [5]. The culture filtrates were then tested for their potential PL inhibitory activity by a quantitative plate assay as described by Kim *et al.* [6]. Endophytes showing inhibition percentage more than 50% were partially purified using chloroform, petroleum ether, and hexane, and these extracts were again tested for same activity. The potential endophytic fungal isolate was examined under the microscope to characterize it on the basis of its microscopic characters and morphology. The culture was grown on potato dextrose agar (PDA) and synthetischer nahrstoffarmer agar and was stained using lactophenol cotton blue. Visualizing certain morphological characteristics such as color, colony size, and texture and microscopic characters such as the conidia and hyphae. The microscopic characters were studied using a polarizing optical microscope (Olympus BX-51 P) coupled with charge-coupled device camera and measurements carried out using image J software. At least 30 observations were made per structure [7,8]. The potential solvent extract was separated on silica plates using thin-layer chromatography using solvent hexane:methanol (70:30). The compounds separated on silica plates were scrapped, and subsequent inhibitory concentration (IC_{50}) was calculated of respective fractions as described by Queiroz *et al.* [9]. The fraction showing highest IC_{50} value was analyzed for compounds present using gas chromatography and mass spectroscopy (GC-MS).

Around 39 endophytic fungal isolates were obtained from which about 38% fungal cultures have been isolated from *Aegle marmelos*, 36% from *C. limon*, and rest from *Azadirachta indica*. From these 39 endophytes, only 5 cultures showed PL inhibitory above 50%. Among these 9CLSTHAI, isolated from stems of *C. limon* showed inhibition percentage of 83% followed by 23AMLPB (75%) from *A. marmelos*. After solvent extraction, there was slight increase in lipase inhibitory activity of all the selected culture filtrates. The hexane extract of 9CLSTHAI inhibited PL by 87%. The hexane extract was then resolved into three bands on silica plates of three different R_f (0.46, 0.64, and 0.85). IC_{50} of compound 2 (R_f=0.64) was better than compound 1 and 3. It inhibited PL with an IC_{50} of 15.46 µg/ml. GC-MS analysis of bioactive compound showed the presence of caryophyllene (Fig. 1).

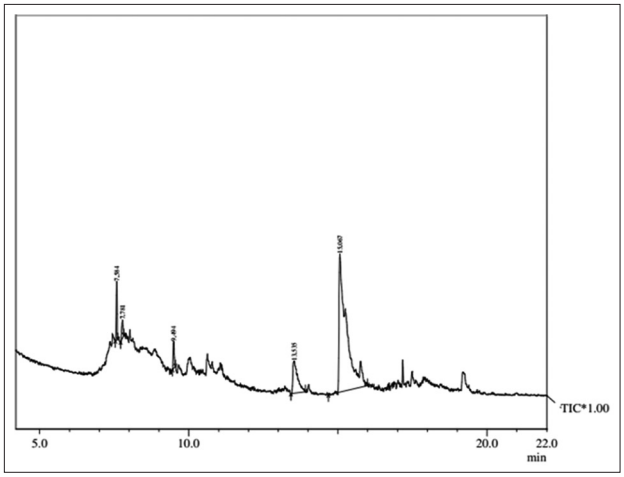

Fig. 1: Gas chromatography and mass spectroscopy analysis of bioactive compound

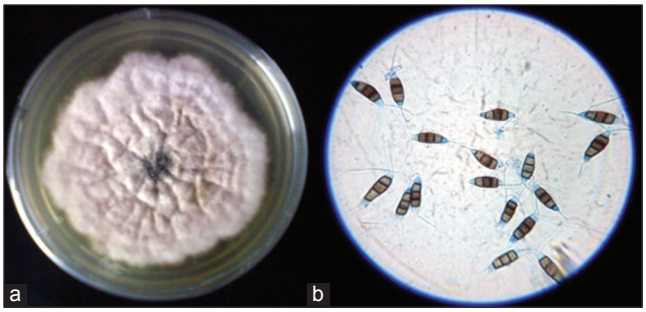

Fig. 2: Morphological and microscopic features of potential endophytic fungi (a) colony of *Pestalotiopsis* on potato dextrose agar (b) conidia of *Pestalotiopsis* sp.

The first peak at retention time 7.584 minutes matches with caryophyllene. Caryophyllene is a sesquiterpene which is a component of oils. Terpenes have been known to possess anti lipase activity [10]. Caryophyllene has been known to possess antioxidant, anti-acne, and anticancer activities [11,12]. Using microscopic tools, the bioactive fungi was identified as *Pestalotiopsis* sp. Colonies on PDA were white, cottony, and raised, and margins were nearly round (Fig. 2).

On maturation, black spores were seen. 4-celled convex spores were seen with 2-3 center cells stained in brown, whereas peripheral two cells were unstained. Two tails on each end are shown in Fig. 2.

Continuous research on anti-obesity drugs has made scientist realize the importance of different ways to treat obesity. Despite the present era, the new drugs in the market are ineffective and are combined with side effects. These anti-obesity drugs are still the combination of old salts with slight modifications. People are now again moving to Ayurveda. Till date, after orlistat, no other natural drug has got the Food and Drug Administration approval, so the present report might confirm it a safe and effective anti-obesity drug.

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