ISOLATION OF ASPERGILLUS FUMIGATUS FROM THE SEAWEED GRACILARIA CORTICATA AND ITS ANTIDIABETIC ACTIVITY BY ALPHA-AMYLASE INHIBITION ASSAY

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ABSTRACT
Diabetes mellitus (DM) is a metabolic disorder resulting from deficiency in insulin secretion, insulin action, or both promoting disturbance of carbohydrate, fat and protein metabolism. Inhibition of alpha amylase, enzyme that plays a role in digestion of starch and glycogen. The aim was to study the ant diabetic activity of endophytic fungi isolated from Gracilaria corticata sea weed. Sea weed was processed and placed on potato dextrose agar (PDA) medium and Sabourds Dextrose Agar (SDA) medium respectively. The plates were incubated at room temperature. Fungal growth was characterized and identified. The mycelial growth of Aspergillus fumigatus was inoculated into Potato Dextrose Broth (PDB) and allowed for fermentation. The mycelial mat was separated and extracted with acetone and ethanol the amylase inhibitory assay method was done using the spectrometry method. The crude ethanol and acetone extract of fungi showed the highest inhibitory activity of 33% and 21%. 

KEYWORDS: Gracilaria corticata, potato dextrose agar, Alpha amylase, Diabetes, entophytic fungi, DNS.

INTRODUCTION
Diabetes, often referred to by doctors as diabetes mellitus, describes a group of metabolic diseases in which the person has high blood glucose (blood sugar), either because insulin production is inadequate, or because the body's cells do not respond properly to insulin, or both. Patients with high blood sugar will typically experience polyuria (frequent urination), they will become increasingly thirsty (polydipsia) and hungry (polyphagia). The body does not produce insulin. Some people may refer to this type as insulin-dependent diabetes, juvenile diabetes, or early-onset diabetes. People usually develop type 1 diabetes before their 40th year, often in early adulthood or teenage years. Type 1 diabetes is nowhere near as common as type 2 diabetes. Approximately 10% of all diabetes cases are type 1. Patients with type 1 diabetes will need to take insulin injections for the rest of their life. They must also ensure proper blood-glucose levels by carrying out regular blood tests and following a special diet. Between 2001 and 2009, the prevalence of type 1 diabetes among the under 20s in the USA rose 23%, according to SEARCH for Diabetes in Youth data issued by the CDC (Centers for Disease Control and Prevention). The body does not produce enough insulin for proper function, or the cells in the body do not react to insulin (insulin resistance).

Approximately 90% of all cases of diabetes worldwide are of this type. Some people may be able to control their type 2 diabetes symptoms by losing weight, following a healthy diet, doing plenty of exercise, and monitoring their blood glucose levels. However, type 2 diabetes is typically a progressive disease - it gradually gets worse and the patient will probably end up have to take insulin, usually in tablet form.

Inhibition of α-amylase, enzyme that plays a role in digestion of starch and glycogen, is considered a strategy for the treatment of disorders in carbohydrate uptake, such as diabetes and obesity, as well as, dental caries and periodontal diseases. Plants are an important source of chemical constituents with potential for inhibition of α-amylase and can be used as therapeutic or functional food sources. These secondary metabolites offer avenues for developing cost-effective, safe and potent drugs. Nearly 50 lakhs species available in the sea are virtually untapped sources of secondary metabolites. Those compounds already isolated from seaweeds are providing valuable ideas for the development of new drugs against cancer, microbial infections and inflammation apart from their potential ecological significances such as controlling reproduction, sottement and feeding deterrent.
Gracilaria corticata belongs to the family Rhodophyceae (Red algae). These are highly evolved multicellular forms with well-developed branched thalli. Except for few species they are exclusively marine and vary in size and shape. They are epiphytes, growing as crust on the rocks or shells as a large fleshy, branched or blade like thalli. The thallus is basically filamentous, simple or branched, free or compacted to form pseudoparenchyma with uni or multialxial construction. The present study was aimed to screen the pharmacological activity of Gracilaria corticata solvent extracts against insulin level secreted in human for Type 2 Diabetes.

ENDOPHYTIC FUNGI

Endophytic fungi is one of the potential natural resources for new antidiabetic compound sources. Endophytic microbes are bacteria including Actinomycetes, or fungi which spend part or whole of its life span inside intra or intercellular tissue of its healthy host without giving any symptom. Endophyte and its host can build symbiosis mutalism to latent phytopathogen relationship involving numerous secondary metabolites produced by both endophytes and its host. Endophytes increase adaptation capability of its host and host’s indurance against disease caused by pathogen. Endophytes also have potency in medicinal, agricultural and industrial development. These groups of fungi are widely recognised as prolific sources of bioactive secondary metabolites that might represent useful leads for the development of new pharmaceutical bioagents. Since more than 1.5x 10^6 endophytic fungi are thought to thrive within the estimated 270,000 species of vascular plants, the prospects for additional discoveries of metabolites from these fungi are promising.

An endophytic Phomopsis sp., isolated from the stem of the filtrate formation of ectopic vessels in the subintestinal vessel plexus (SIV), whereas homopsis-H76 C (Fig 20) was found to inhibit blood vessel formation. Reported isolation of pullularin A, B and C from culture of endophytic fungus Pullularia sp.[7] These compounds showed strong anti-malarial activity as they inhibit activity of Plasmodium falciparum K1 (K1, refer to multi-drug resistant strain) with IC50 3.6, 3.3, and 9.8μg/ml values respectively.[8] Taxol is very potent anticancer agent, first isolated from the bark of the pacific yew. It stabilizes microtubule formation due to its specific binding site on the microtubule polymer.[9] Endophytic fungi have been studied as a source of anticancer agent since taxol was isolated from endophytic fungi Taxomyces andreanae.[10] It stabilizes microtubule formation due to its specific binding site on the microtubule polymer. The FDA have approved taxol for the treatment of ovarian and breast cancer.

Studies have shown that the production of secondary metabolites is under light regulatory control. As mentioned above secondary metabolites typically are produced towards the end of the exponential growth phase in batch culture when growth is limited by a critical nutrients but when other nutrients are still available. In continuos culture, secondary metabolites can be produced throughout the exponential growth phase. The critical factor in the continuos culture conditions for the production of secondary metabolites is that the gene encoding secondary metabolites can be repressed by high level of a particular nutrient or can be acivated by other metabolites.

MATERIALS AND METHODS

(I). Collection of sea weeds
The seaweeds were collected from kovalam fisheries brackish water area near Kovalam beach chennai.

(II) Cultured on Sabourds dextrose agar

Hyphae are septate and hyaline. Conidial heads are strongly columnar in an undisturbed culture. Conidiophores are smooth-walled, uncolored, up to 300 μm long, and terminate in a dome-shaped vesicle that is 20- 30 μm in diameter. This species is unisieriate with closely compacted phialides (5-10 x 2-3 μm) occurring only on the upper portion of the vesicle. Conidia are smooth to finely roughened, subglobe, 2-3.5μm in diameter. (Figure 1).

(III) Fermentation
The endophytic fungus was grown on potato dextrose yeast agar (PDYEA) at 30°C for 5-7 days depending on growth rate. Colonies on Potaro dextrose agar at 25°C
are smoky gray-green with a slight yellow reverse. Some isolates may display a lavender diffusible pigment. Loopful of grown culture from the PDA slant were inoculated into 1000 ml Erlenmeyer flasks containing 500ml potato dextrose yeast extract broth and incubated at 30°C for 4 weeks.

(IV) Extractions with Acetone and Ethanol
After incubation period, the fungal cultures were harvested and filtered through two layers of cheese cloth. The dried mycelium was extracted three times with ethanol and acetone. The solvent was evaporated to dryness under reduced pressure to obtain a crude extract. The dried mycelium was extracted three times with ethanol and acetone. The solvent was evaporated to dryness under reduced pressure to obtain a crude extract. The dried mycelium was extracted three times with ethanol and acetone. The solvent was evaporated to dryness under reduced pressure to obtain a crude extract. The obtained crude extract were stored to perform invitro authentication of Entophytic fungi from seaweed.

The isolated endophytic fungi were characterized and identified in similar to the isolation work done.[11] The present work was based on review of literature Invitro ant diabetic study was done by alpha amylase inhibition assay using ethanol and acetone extracts of endophytic fungi that showed similar ant diabetic activity by alpha amylase inhibition assay method.

(V) Anti Diabetic Activity By Alpha Amylase Inhibition Assay Method
250µl of α amylase solution (1mg/ml phosphate buffer) to this 100µl of sample was added except blank and mixed well. Pre incubate the prepared mix at 37°C for 20 mins in water bath. 2ml of DNS (Dinitrosalicyclic acid reagent): (40 mM DNS, sodium potassium tartrate, 0.4% M NaOH) was added to stop the reaction 0.4% Mix well and boil at 100°C for 10mins. Cool the mixture and measure the absorbance in spectrophotometre at 540 nm.10.

RESULTS
The acetone crude extract of Aspergillus fumigates showed highest alpha amylase inhibitory concentration of 21.3% and lowest alpha amylase inhibitory concentration of 21.3%. (Table 1)

Table 1: Acetone Extract.

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<th>ENDOPHYTIC FUNGI</th>
<th>% of α amylase Inhibition</th>
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<td>Aspergillus fumigatus</td>
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The ethanol crude extracts of Aspergillus fumigates showed highest alpha amylase inhibitory concentration of 33.4% and lowest concentration of 4.8%. (Table 2).

Table 2: Ethanol Extract.

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<td>4.8</td>
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</tbody>
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DISCUSSION
The present study revealed the isolation of endophytic fungi from seaweeds the isolated endophytic fungi were characterized and identified in similar to the isolation work done.[11] The present work was based on review of literature Invitro ant diabetic study was done by alpha amylase inhibition assay using ethanol and acetone extracts of endophytic fungi that showed similar ant diabetic activity using medicinal plants.[12]

The percentage of inhibition at different concentrations of crude acetone extract of Aspergillus fumigatus were (100µl) 8.7%, (200µl) 14.5%, (300 µl) 17.4%, (400µl) 19.4%, (500µl) 21.3%.[13] The lowest and highest amylase inhibition percentage was 8.7% and 21.3%.[14] The percentage of inhibition at different concentrations of crude ethanol extract of Aspergillus fumigatus were (100µl) 4.8%, (200µl) 14.6%, (300 µl) 20.2%, (400µl) 27.9%, (500µl) 33.4%. The lowest and highest amylase inhibition percentage was 4.8% and 33.4%.[15]

CONCLUSION
The final conclusion was made based on the result obtained the crude acetone extract of Aspergillus fumigatus showed the highest percentage of inhibition as 21% and highest percentage of inhibition of crude ethanol extract of Aspergillus fumigates was 33.4%. It was concluded that the percentage of inhibition of crude ethanol extract was found to be higher than the acetone extract.

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