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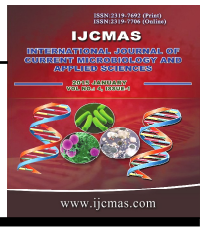

Original Research Article

In vitro anti-diabetic activity of ethanolic and acetone extracts of endophytic fungi – *Syncephalastrum racemosum* isolated from the seaweed *Gracilaria corticata* by alpha-amylase inhibition assay method

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Keywords

Gracilaria corticata, Potato dextrose agar, Alpha amylase, Diabetes,

A B S T R A C T

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 by alpha amylase.The aim was to study the ant diabetic activity of entophytic fungi isolated from Gracilaria corticata . Sea weed was processed, placed on potato dextrose agar (PDA) medium and Sabourds Dextrose Agar (SDA) medium respectively The mycelial growth of S. racemosum was inoculated into Potato Dextrose Broth (PDB) and allowed for fermentation The mycelial mat was extracted with acetone and ethanol. The crude extracts of fungi showed the highest inhibitory activity of 23.7% and 19.4%

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[1]. 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 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[2]. 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[3]. Overweight and obese people have a much higher risk of developing type 2 diabetes compared to those with a healthy body weight. People with a lot of visceral fat, also known as central obesity, belly fat, or abdominal obesity, are especially at risk. Being overweight/obese causes the body to

release chemicals that can destabilize the body's cardiovascular and metabolic systems. Being overweight, physically inactive and eating the wrong foods all contribute to our risk of developing type 2 diabetes. Drinking just one can of (non-diet) soda per day can raise our risk of developing type 2 diabetes by 22%, researchers from Imperial College London reported in the journal Diabetologia. The scientists believe that the impact of sugary soft drinks on diabetes risk may be a direct one, rather than simply an influence on body weight Gestational diabetes affects females during pregnancy. Some women have very high levels of glucose in their blood, and their bodies are unable to produce enough insulin to transport all of the glucose into their cells, resulting in progressively rising levels of glucose. Diagnosis of gestational diabetes is made during pregnancy. The majority of gestational diabetes patients can control their diabetes with exercise and diet. Between 10% to 20% of them will need to take some kind of blood-glucose-controlling medications. Undiagnosed or uncontrolled gestational diabetes can raise the risk of complications during childbirth. The baby may be bigger than he/she should be[4].

Insulin is a hormone that is produced by the pancreas. After eating, the pancreas automatically releases an adequate quantity of insulin to move the glucose present in our blood into the cells, as soon as glucose enters the cells blood-glucose levels drop A person with diabetes has a condition in which the quantity of glucose in the blood is too elevated (hyperglycemia). This is because the body does not produce enough insulin, produces no insulin, or has cells that do not respond properly to the insulin the pancreas produces[5]. This results in too much glucose building up in the blood. This excess blood glucose eventually passes out of the body in urine. So, even though the blood has plenty of glucose, the cells are not getting it for their essential energy and growth requirements

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[6].

Gracilaria corticata belongs to the family Rhodophyceae (Red algae). These are highly evolved multicellular forms with well-developedbranched 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 multiaxial construction. They inhabit intertidal to subtidal zones of coastal areas.

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.[2]

Endophytes have been investigated to be a rich source novel biological active secondary metabolites[7] Novel antibiotics, antimycotics, immunosuppressants and anticancer compounds are only a few examples of which have been found after the isolation and culturing of endophytes followed by purification and characterization of some of their natural products[8.] Many publication were repored that the anti diabetic activity of these sea

weeds and their potential endophytic microbes with capability in producing antidiabetic active compounds are seldom .Endophytic fungi live inside the plant tissue without producing any symptom but provide protection to their host from insect, pest and herbivore and the environmental stress conditions.[9] The proposed research was initiated to investigate metabolic basis of parasitic endophytic fungi for their alpha amylase inhibitory activity in vitro[10]

Materials and Methods

The seaweeds were collected from kovalam fisheries brackish water area near Kovalam beach Chennai. The seaweed was further processed to isolate the endophytic fungi and tested for its inhibition of alpha amylase activity. The collected seaweed were washed with tap water to remove salts and other adhereing particles. The washed sea weeds were immersed in 80% ethanol for 3 mins and rinsed with sterile distilled water three times for 10 seconds and allowed to surface dry under sterile conditions. After drying, the sea weeds was cut into segments approximately 0.5 cm squares and placed on petri plates.

Morphological characterization

The morphological characteristics of the fungal isolates were observed and described according to their standard taxonomic key.

Macroscopic morphology

Colonies of Syncephalastrum grow very rapidly and fill the petri dish or culture tube. Maximum growth temperature is 40°C. The texture is wooly to cotton candy-like. From the front, the color is white initially and turns to dark gray to black in time. Reverse is pale or yellowish-brown Broad (4-8 µm in diameter), nonseptate or sparsely septate hyphae, sporangiophores, merosporangia (finger-shaped, tubular sporangia), (mero) sporangiospores (merospores), and rhizoids are visualized. Septation of the hyphae is mostly observed as the culture gets old. Sporangiophores are frequently branched and rather short. They end up in a vesicle (80 µm in diameter). Around this vesicle are the merosporangia (4-6 x 9-60 µm), which are filled with linear series (chains) of sporangiospores. Each merosporangium contains a single row of 3-18 merosporangiospores.

Merosporangiospores (3-7 µm, may rarely reach 10 µm in diameter) are one-celled and spherical to cylindrical in shape. The obtained pure cfungal cultures were used to perform the fermentation process by the following method.

Fermentation

The endophytic fungus was grown on potato dextrose yeast agar (PDYEA) at 30ºC for 5-7 days depending on growth rate. Loopful of grown culture from the PDA slant were inoculated into 1000 ml Erlenmeyer flasks containing 500 ml potato dextrose yeast extract broth (PDYEB) and incubated at 30ºC for 4 weeks[6].

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 obtained crude extract was stored to perform in vitro ant diabetic activity by alpha amylase inhibition assay method.

Anti diabetic acivity 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^{0}C for 20 mins in water bath. 250µl of substrate solution (0.5% starch in phosphate buffer) and mix well a Incubate at 37^{0}C for 15 mins. 2ml of DNS (Dinitrosalicyclic acid reagent) (40 mM DNS, sodium potassium tararate, 0.4% M NaOH) was added to stop the reaction

Result and Discussion

The percentage of inhibition was calculated by dividing control optical density – Test OD / Control OD X100 in a range 540 nm. The percentage of inhibition at different concentrations of crude acetone extract of Syncephalastrum racemosum were (100µl) 5.8%, (200µl) 10.6%, (300 µl) 13.5 %, (400µl) 17.4%, and (500µl) 19.4%. The percentage of inhibition at different concentrations of crude ethanol extract of Syncephalastrum racemosum were (100µl) 8.8 %,(200µl) 11.4 %(300 µl) 17.8, (400µl) 19.1% and (500µl) 23.7%.

The highest and lowest percentage of alpha amylase inhibition by ethanol crude extract of Syncephalastrum racemosum was found to be 23.7% and 8.7%. The highest and lowest percentage of alpha amylase inhibition by acetone crude extract of Syncephalastrum racemosum was found to be 19.4% and 5.4%.

The present work was focussed on invitro antidiabetic study by alpha amylase inhibition assay using ethanolic and acetone extracts of endophytic fungi that showed similar antidiabetic activity using medicicnal plants

The present study revealed the isolation of endophytic fungi from seaweeds, the isolated endophytic fungi were characterized and identified[11] .The present work was focussed on In vitro ant diabetic study 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 Syncephalastrum racemosum were (100µl) 5.8%, (200µl) 10.6%, (300 µl) 13.5 %, (400µl) 17.4%, (500µl) 19.4%[9]. The lowest and highest amylase inhibition percentage was 5.8% and 19.4%.[13] The percentage of inhibition at different concentrations of crude ethanol extract of Syncephalastrum racemosum were (100µl) 8.8%, (200µl) 11.4%, (300 µl) 17.8%, (400µl) 19.1%, (500µl) 23.7%.[14] The lowest and highest amylase inhibition percentage was 8.8% and 23.7%[15].

Table.1 Acetone extract

ENDOPHYTIC FUNGI	**% of α amylase Inhibition**				
	100µl	**200µl**	**300µl**	**400µl**	**500µl**
Syncephalastrum racemosum	5.8	10.6	13.5	17.4	19. 4

The crude acetone extract of Syncephalastrum racemosum exhibited highest and lowest % of inhibition as 19.% and 5.6%.

Table.2 Ethanol extract

ENDOPHYTIC FUNGI	% of α amylase Inhibition				
	100µl	200µl	300µl	400µl	500µl
Syncephalastrum racemosum	**8.8%,**	**11.4%,**	**17.8%,**	**19.1%,**	**23.7%.**

The crude ethanol extract of Syncephalastrum racemosum exhibited highest and lowest % of inhibition as 23.7.% and 8.8%.

Figure.1 Microscopic appearance of Endophytic fungi

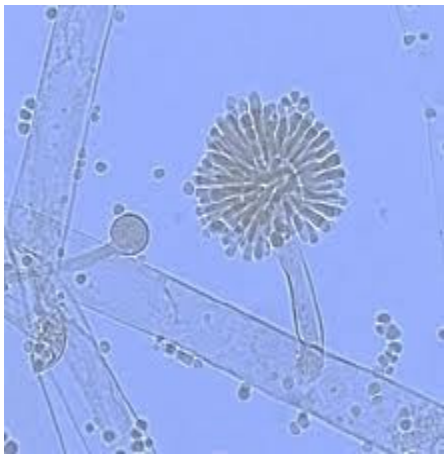
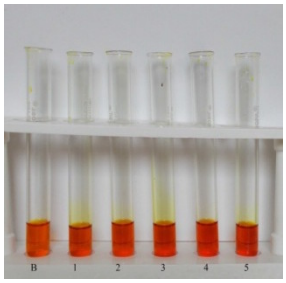


Figure.2 Alpha amylase inhibition assay



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