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A COMPARATIVE STUDY ON ANTI DIABETIC ACTIVITY OF CRUDE EXTRACTS OF ENDOPHYTIC FUNGI FROM SEA WEED GRACILARIA CORTICATA

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ABSTRACT
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).Insulin is a hormone that is produced by the pancreas.. 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. Gracilaria corticata have been used since ancient times as food, fodder, and fertiliser and as source of médicine. Gracilaria corticata. Endophytic fungi are one of the potential natural resources for new ant diabetic compound sources. The aim of the study was to compare anti diabetic activity of crude extracts of Endophytic fungi from Gracilaria corticata against diabetes. Endophytic fungi were isolated from seaweed Gracilaria coricata, characterized and identified based on morphological and biochemical characters. The identified endophytic fungi were tested for invitro antidiabetic activity by alpha amylase inhibition assay, method the higehest percentage of alpha amylase inhibition at the concentration of 500µl of ethanolic extract of A. niger that showed 41.4% followed by penicillium (39%) A.fumigatus (33.4%) Phoma spp (28.9%) and Syncephalastrum racemosum (23.7%) in comparison with acetone crude extracts of Penicillium (25.2%), Phoma spp (24.2%), A.fumigatus (21.3%), Syncephalastrum racemosum (19.4%), A.niger (17.4%).

KEYWORDS: Endo phytic fungi, DNS, Gracilaria corticata, Diabetes, Alpha amylase.

INTRODUCTION
Diabetes (Diabetes mellitus) is classed as a metabolism disorder. Metabolism refers to the way our bodies use digested food for energy and growth. Most of what we eat is broken down into glucose. Glucose is a form of sugar in the blood - it is the principal source of fuel for our bodies. 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. 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. 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

ROLE OF ALPHA AMYLASE IN DIABETES
Amylase plays an important role in digestion of starch in to glucose in Blood of Human body. When starch is consumed amylase is secreted and converts in to Sugar. Insulin is the hormone produced by Pancreas to inhibit alpha amylase. In certain cases insulin is not enough to inhibit alpha amylase activity hence leads to increase in blood glucose levels. The main aim of the study was to inhibit alpha amylase by using crude extracts of Endophytic fungi isolated from Gracilaria corticata.

SEA WEED – GRACILARIA CORTICATA
Gracilaria corticata is a sea weed which belongs and haposses well branched thalli and are multicellular. They survive in marine environments on rocks andappears fleshy. Gracilaria species accomodate medically and commercially important metabolites.

ENDOPHYTIC FUNGI

Endophytic fungi are the excellent resources for metabolites against Diabetes. Endophytic fungi are organisms found in plants, sea weeds etc.They grow within the tissues producing secondary metabolites exhibiting antidiabetic activities without affecting the host cells.

MATERIALS AND METHODS

The Gracilaria corticata, a sea Weed was collected from Kovalam Beach, Chennai and processed by washing 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.

ISOLATION OF ENDOPHYTIC FUNGI

The cut sea weeds were placed on to PDA and SDA plates incubated at 30^0C for 2 weeks. They were monitored everyday for the growth of endophytic fungal colonies.Fungi growing out from the samples were subsequently transfered to fresh PDA plates.The procedure of tranferring to fresh PDA plates and finally to slant for several times inorder to isolate pure colonies.

FERMENTATION

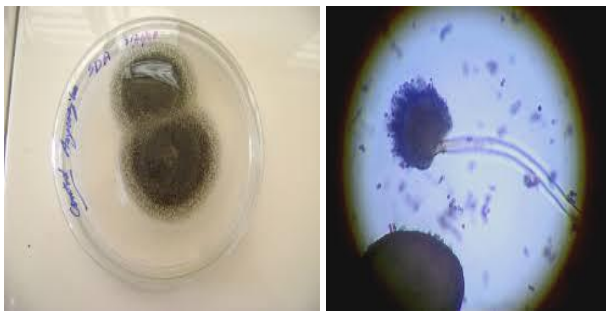
The endophytic fungus was grown on potato dextrose yeast agar (PDYEA) at 30ºC for 5-7 days depending on growth rate. Loopful of grown culture from the PDA slant were inoculated into 1000 ml Erlenmeyer flasks containing 500 ml potato dextrose yeast extract broth (PDYEB) and incubated at 30ºC for 4 weeks.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

ANTI DIABETIC ACIVITY BY ALPHA AMYLASE INHIBITION ASSAY METHOD

250µl of α amylase solution (1mg/ml phosphate buffer) was added to 100µl of sample using blank solution and mixed well. The tubes were incubated at 37^0C for 20 mins in water bath.250µl of substrate solution (0.5% starch in phosphate buffer) was added and mixed well followed by Incubation at 37^0C 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.Mix well and boil at 100^0C for 10mins. Cool the mixture and measure the absorbance in spectrophitometer at 540 nm.

RESULTS

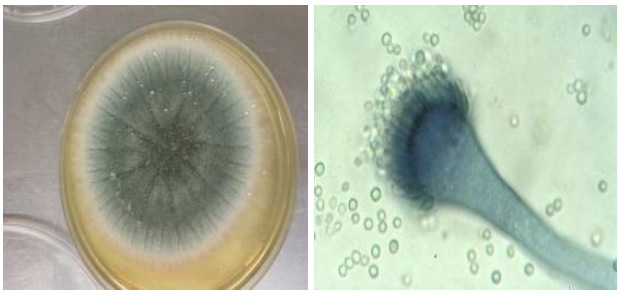
The morphological characeristics of the fungal isolates were observed and described according to their standard taxonomic key included



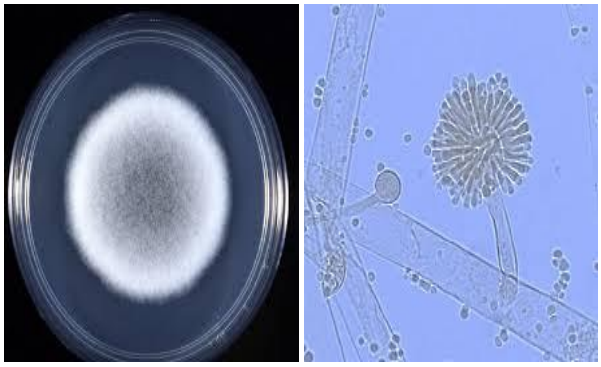
Mcroscopic and Microscopic view of A. niger.

Macroscopic and microscopic view of Pencillium sps.

Macroscopic and microscopic view of Phoma sps.



Macroscopic and microscopic view of A. fumigates.



Macroscopic and microscopic view of Syncephalastrum racemosum.

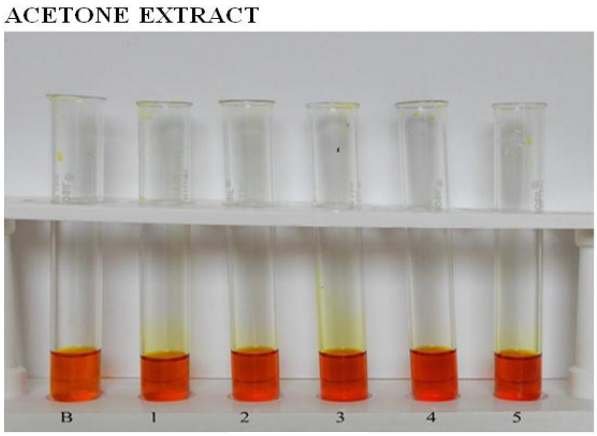
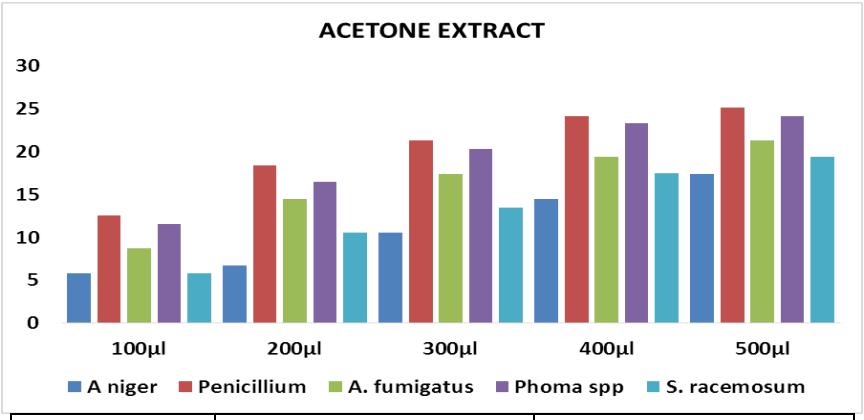


FIGURE: 1.

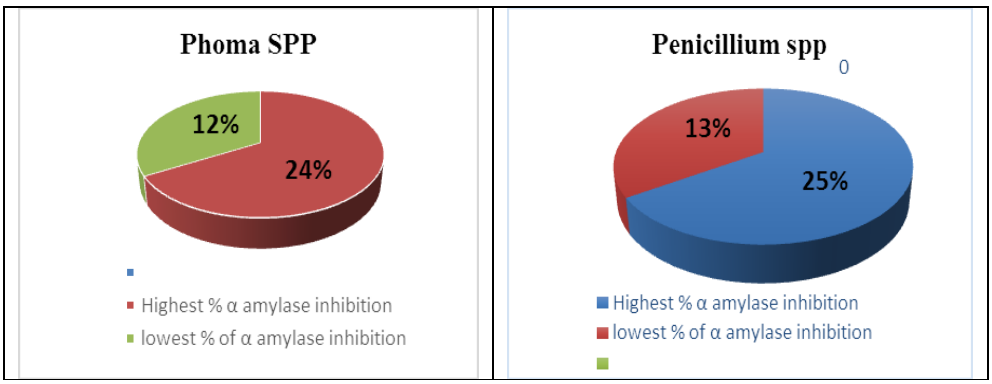
TABLE: 1.

ENDOPHYTIC FUNGI	% of α amylase Inhibition				
	100µl	200µl	300µl	400µl	500µl
Aspergillus niger	5.8	6.7	10.6	14.5	17.4
Penicillium spp	**12.6**	18.4	21.3	24.2	**25.2**
Aspergillus fumigatus	8.7	14.5	17.4	19.4	21.3
Phoma spp	11.6	16.5	20.3	23.3	24.2
Syncephalastrum racemosum	5.8	10.6	13.5	17.4	19.4



FUNGI	HIGHEST % OF α AMYLASEINHIBITION	LOWEST % OF α AMYLASEINHIBITION
Penicillium spp	**25%**	**13%**
Phoma spp	**24%**	**12%**

PIE CHART



The above table and chart showed the highest and lowest percentage of inhibition of alpha amylase by crude acetone extract of Penicllium and Phoma spp.

ETHANOL EXTRACT

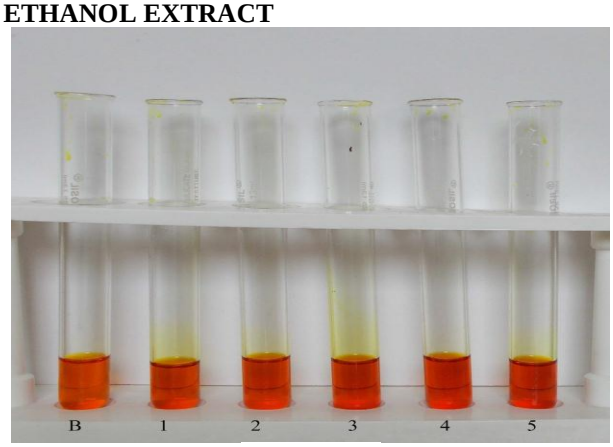
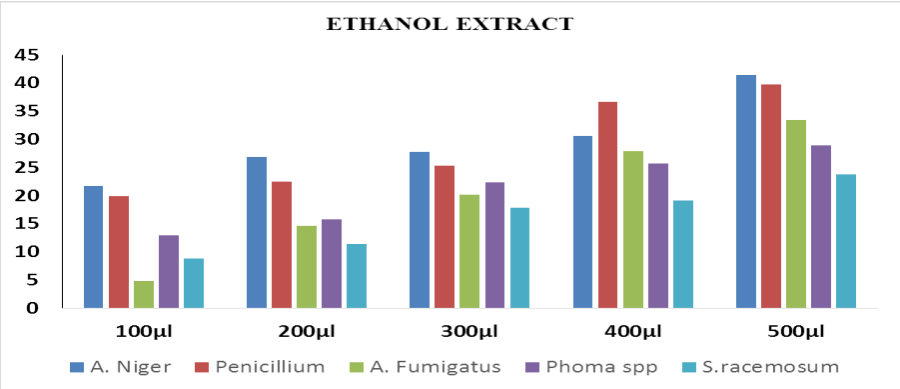


FIGURE: 2.

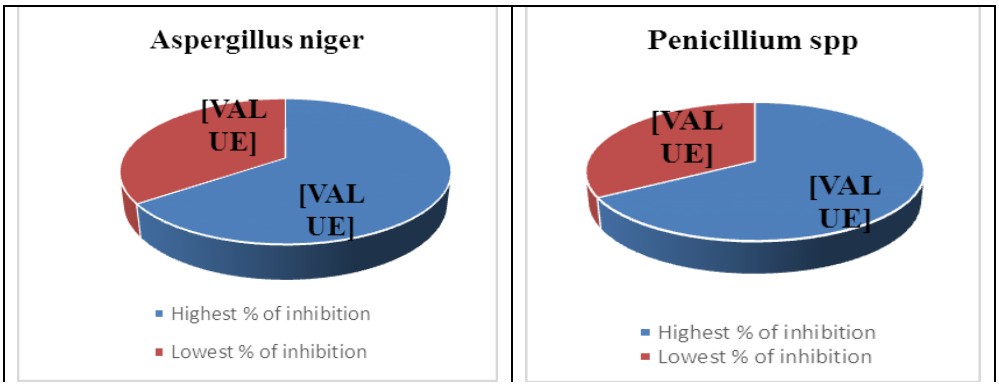
TABLE: 2.

ENDOPHYTIC FUNGI	% of α amylase Inhibition				
	100µl	200µl	300µl	400µl	500µl
Aspergillus niger	**21.7**	26.8	27.8	30.6	**41.4**
Penicillium spp	19.9	22.5	25.3	36.7	39.8
Aspergillus fumigatus	4.8	14.6	20.2	27.9	33.4
Phoma spp	12.9	15.8	16.3	17.7	19.1
Syncephalastrum racemosum	8.8	11.4	17.8	19.1	23.7



FUNGI	HIGHEST % OF α AMYLASEINHIBITION	LOWEST % OF α AMYLASEINHIBITION
Aspergillus niger	**41%**	**22%**
Penicillium	**40%**	**20%**

PIE CHART

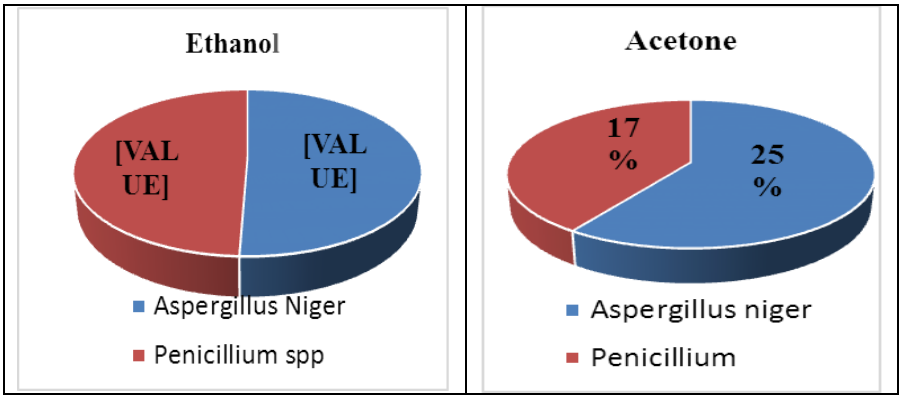


The above table and chart showed the highest and lowest percentage of inhibition of alpha amylase by crude ethanolic extract of Aspergillus niger and Penicillium.

COMPARISON OF ALPHA AMYLASE INHIBITORY ACTIVITY BY CRUDE ETHANOL AND ACETONE EXTRACTS OF ENDOPHYTC FUNGI

FUNGI	ETHANOL EXTRACT	ACETONE EXTRACT
Aspergillus niger	**41%**	**17%**
Penicillium	**40%**	**25%**

PIE CHART



DISCUSSION

The present work resulted in exhibiting the higehest percentage of alpha amylase inhibition at the concentration of 500µl of ethanolic extract of A. niger that showed 41.4% followed by penicillium (39%) A.fumigatus (33.4%) Phoma spp (28.9%) and Syncephalastrum racemosum (23.7%) in comparison with acetone crude extracts of Penicillium (25.2%), Phoma spp (24.2%), A.fumigatus (21.3%), Syncephalastrum racemosum (19.4%), A.niger (17.4%).

CONCLUSION

Based on alpha amylase inhibition assay by different endophytic fungi isolated from sea weed Gracilaria corticata it was found that crude ethanolic extract of Aspergillus niger showed highest percentage of inhibition follwed by Penicillium spp, the crude acetone extracts of Penicillium showed highest percentage follwed by Phoma spp. The crude ethanolic extract of Aspergillus niger showed maximum percentage of alpha amylase inhibition compared to crude acetone extract of penicillium Sps.

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