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α- AMYLASE AND α-GLUCOSIDASE INHIBITORY ACTIVITY OF ETHYL ACETATE EXTRACT OF ASPERGILLUS TAMARII ISOLATED FROM JUSTICIA BEDDOMEI

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ABSTRACT

Fungal endophytes attain importance due to the innumerable diverse and potent activity of their secondary metabolites. The present study was carried out to screen the anti diabetic activity of the fungal endophyte *Aspergillus tamarii* isolated from *Justicia beddomei*. Bioactive metabolites were extracted with ethyl acetate and the qualitative phytochemical analysis established the presence of phenol, flavonoids and alkaloids. α amylase and α glucosidase inhibitory activity of the ethyl acetate extract was assessed by biochemical assays with Acarbose as standard. α – amylase inhibitory activity was found to be 83.26% at a concentration of 1000µg/ml. A maximum inhibition of 91.79%was achieved at a concentration of 1000µg/ml while standard acarbose inhibition was about 97% for α – glucosidase inhibitory activity. The inhibitory activity can be attributed to the phytochemicals present in the ethyl acetate extract of the endophyte. Further studies need the purification of the compound and the study on cell lines.

KEYWORDS: ***Endophytic Aspergillus, antidiabetic, GOD-POD method, Acarbose, α – amylase inhibitory activity***

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INTRODUCTION

Diabetes mellitus is a metabolic disorder caused due to the deficiency in insulin secretion or the cellular resistance to the uptake of insulin. The characteristic features of diabetes are exhibited as an increased blood glucose level and disturbances in carbohydrate, protein and fat metabolisms.[1] Conventional methods of treatment include supplementation of insulin along with proper diet and exercise. The alternative therapeutic method for diabetes is based on the administration of enzyme inhibitors of α amylase and α glucosidase. These inhibitors decrease the glucose uptake with an evident hypoglycemic effect. They exert their action by delaying carbohydrate digestion leading to a marked decrease in the rate of glucose absorption thereby blunting the post prandial glucose rise.[2] Acarbose, voglibose are such inhibitors in current use. Conversely due to their side effects on the gastrointestinal tract, researchers have turned the focus on search for potent natural enzyme inhibitors.[3] Endophytes are gaining enormous importance because of their potential of production of a wide range of bioactive metabolites. Endophytes produce a wide range of compounds with a broad spectrum of activity such as antimicrobial, anticancer, antioxidant, antiviral, antiparasitic, immunosuppressant, agrochemicals. Bioprospecting of endophytic fungi is preferred over the other sources due to the rapid growth, ease of cultivation and recovery of the metabolite.[4] Several works have reported the antidiabetic activity of the endophytes.[5-6] *Justicia beddomei* is a glabrous shrub belonging to the family Acanthaceae which has been traditionally used for various ailments. In the present study, we report α amylase and α glucosidase inhibitory activity of the endophytic fungi isolated from *Justicia beddomei*.

MATERIALS & METHODS

Isolation and identification of endophyte

The isolation of endophytic fungi from *Justicia beddomei* was done by modified method described by Petrini, 1986.[7] The plant specimens were identified by Prof. V. Chelladurai, Research Officer-Botany, C.C.R.A.S. Government of India (Retired). Healthy leave samples were surface sterilized and explants were placed on potato dextrose agar supplemented with chloramphenicol(50µg/ml). The plates were incubated at 27ºC for a period of 4 weeks and checked for growth of fungi periodically. Fungal colonies appeared were subcultured repeatedly to obtain pure cultures. The endophytic fungi were identified by both morphological and molecular characterization. Fungal rDNA-ITS sequences was analyzed for homology by using nucleotide-nucleotide "BLAST" search feature located on NCBI. Identification of the endophyte was based on the BLAST search.

Extraction of bioactive metabolites

The fungal endophyte was cultivated on the Potato Dextrose Broth (PDB) at 28ºC for 7 days at120 rpm in a shaker incubator. The bioactive metabolites were extracted from the culture filtrate and biomass with ethyl acetate. The extract residues were dissolved in DMSO (1mg/ml) and stored at 4ºC until further use.

Preliminary phytochemical analysis

The fungal extract was subjected to the preliminary phytochemical analysis following standard methods of Harborne 1973.[8] These methods were performed to screen the presence of the various active principles in a qualitative way.

In – vitro α- amylase inhibitory assay

In vitro amylase inhibition was studied by the method of Bernfeld.[9] 100µL of the different concentrations of the ethyl acetate extract (1 – 1000µg/ml) was allowed to react with 200µl of α-amylase enzyme (Hi media RM 638) and 100µL of 2mM of phosphate buffer (pH-6.9). After 20-minute incubation, 100µl of 1% starch solution was added. The procedure was repeated for the controls where 200µl of the enzyme was replaced by buffer. After incubation for 5 minutes, 500µl of dinitrosalicylic acid reagent was added to both control and test samples, kept in boiling water bath for 5 min. The absorbance was recorded at 540 nm and the percentage inhibition of α-amylase enzyme was calculated using the formula

% inhibition = [(Control – Test)/Control]*100

Suitable reagent blank and inhibitor controls were simultaneously carried out.

In vitro α-glucosidase inhibitory activity

In vitro α-glucosidase inhibitory activity was evaluated by Li et al.[10] α-glucosidase inhibitory assay is based on the breakdown of maltose to glucose. 200µl of α - glucosidase solution was pre-incubated with the different concentrations of the ethyl acetate extract (Concentration 1 – 1000µg/ml) and control sample for 5 min. The reaction was started by adding 200µl of sucrose and it was terminated after 30 min incubation at 37^0C by heating at 90–100^0C. The liberated glucose was determined. The enzyme activity is directly proportional to the liberated glucose and the liberated glucose is measured by GOD-POD method at 546nm using semi auto analyser. The inhibitory activity of the extract was calculated as follows

% Inhibition= [(control-test)/control)]*100

RESULTS AND DISCUSSION

Upon isolation of the endophyte from *Justicia beddomei*, LPCB staining revealed the microscopic morphology of the fungi (Fig 1). BLAST search was done with the ITS followed by, phylogenetic tree was constructed using Clustal program (Fig 2). Based on the morphological and molecular characterization, the isolated endophyte was identified closely related to *Aspergillus tamarii.* Very often endophytic *Aspergillus sp* has been isolated and reported to produce bioactive compounds with novel structures.[11-12]

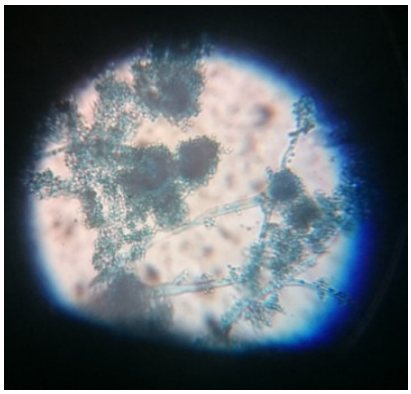

Figure 1
LPCB staining and Microscopic morphology of the isolated endophytic fungi observed under low objective

Figure 2
Phlyogenetic tree construct for the isolated endophytic fungi

The endophytic *Aspergillus tamarii* was found to produce the phytochemicals phenols, flavonoids, tannins and alkaloids predominantly (Table 1). The bioactivity of the secondary metabolites has been attributed to the phytochemicals. The phytochemical synthesis has been either *de novo* or acquired from plant due to the long co-evolution. The presence of phytochemicals in endophytes is an indicator of its use as precursors in the development of synthetic drugs.[13-14]

Table 1
Standard Phytochemical Qualitative Analysis of ethyl acetate extract of the endophytic Aspergillus tamari

Phytochemical	Presence/Absence
Phenol	+++
Triterpenoids	-
Flavonoids	+++
Alkaloids	++
Reducing sugars	+
Glycosides	+
Saponins	+
Quinones	-
Proteins	++
Tannins	+++
Anthraquinones	+
Steroids	-

+ = Mild Presence; ++ = Moderate Presence; +++ = High; - = Absence

The in vitro α – amylase inhibitory activity of the ethyl acetate extract was investigated. The extract showed a potent α – amylase inhibitory activity against α - amylase with a maximum inhibition percentage of 83.26% at 1000µg/ml which was compared with standard Acarbose (Table 2). The inhibition of amylase activity in the human digestive tract has been known as the effective method to control diabetes by reducing the absorption of glucose.[15]

Table 2
***α - amylase inhibitory activity of the ethyl acetate extract of the endophytic Aspergillus tamarii* in comparison with standard Acarbose.**

Concentration (µg/ml)	%Inhibition of Amylase activity	
	Ethyl acetate extract	Standard Acarbose
1	11.24±0.20	13.73±0.63
3	23.25±0.05	23.84±0.09
10	25.43±0.13	39.03±0.19
30	34.35±0.07	50.26±0.09
100	52.18±0.21	69.25±0.06
300	63.51±0.11	76.51±1.51
1000	83.26±0.27	93.32±1.08

The results also showed strong α – glucosidase inhibitory activity of ethyl acetate extract of endophyte which is compared with standard acarbose. A maximum inhibition of 91.79%was achieved at a concentration of 1000µg/ml while standard acarbose inhibition was about 97% (Graph1). Similar results have been observed by other workers in endophytes isolated from other plants.[16-17] A dose dependent inhibitory action was observed. The inhibitory action of the ethyl acetate extracts can be attributed to phytochemicals solubilised in the polar solvent ethyl acetate. Phenols, flavonoids, tannins have been known as antidiabetic constituents in phytochemicals of various plants.[18] The inhibitory action was comparatively less than the standard acarbose in both the assays. The difference might arise due a difference in source, as acarbose is isolated from mammalian source. Further the synergistic action of other components in the ethyl acetate extract might alter the activity

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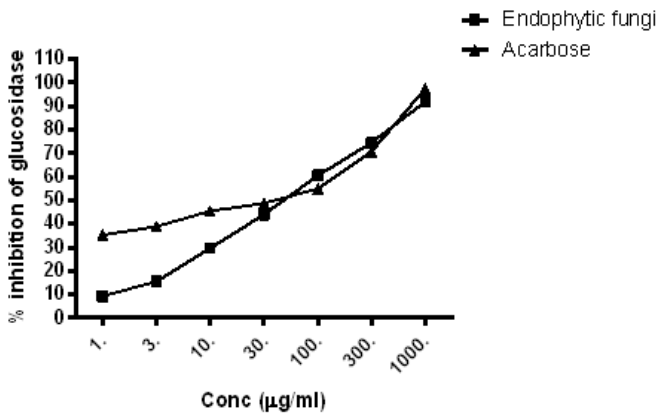
Graph 1
Graphical representation of α – glucosidase inhibitory activity of the ethyl acetate extract



CONCLUSION

The present study demonstrated the anti diabetic activity of the ethyl acetate extract of the isolated endophytic *Aspergillus tamarii* isolated from *Justicia beddomei*. Our preliminary screening indicates a correlation between the phytochemicals identified and the enzyme inhibitory action. Further studies on cell lines and phytochemical charcterisation would lead to the use of the extract as an alternative therapeutic method for diabetes treatment.

CONFLICT OF INTEREST

Conflict of Interest declared none.

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