International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

Antiglycemic Activity of Endophytic Fungi from Selected Medicinal Plants by Alpha-Amylase Inhibition Method

Kamana Sahani[1], Deependra Thakur[2], K.P.J. Hemalatha[3], Arkajit Ganguly[4]

[1, 2, 4]Department of Microbiology, Brindavan College, Bangalore University, Banglore-560094.

[3]*Corresponding author*, Department of Microbiology, Andhra University, Visakhapatnam-530003

Abstract: ***The aim of this work was to evaluate the inhibitory activities of ethylacetate extracts of endophytic fungi isolated from tulsi and aloe vera by alpha amylase inhibition method. Diabetes mellitus is a clinical condition characterized by hyperglycemia in which an elevated amount of glucose circulates in the blood plasma. Alpha amylase inhibitors are used to achieve greater control over hyperglycemia in type 2 diabetes mellitus. The present study intends to screen novel alpha amylase inhibitors from natural sources like endophytic fungi from medicinal plants in order to minimize the toxicity and side effects of the inhibitors currently used to control hyperglycemia.***

Keywords: Endophytic fungi, Tulsi, Aloe vera, Ethyl acetate, Secondary metabolites, alpha amylase inhibition.

1. Introduction

Diabetes is a metabolic disorder characterized by high plasma glucose levels that can be classified as either Type 1 or Type 2 (Mitrakou *et al*., 1992[1]). In Type 1 or insulin dependent diabetes, the pancreas fails to secrete insulin. In contrast, Type 2 also called non-insulin dependent diabetes or diabetes mellitus (DM) is caused by an imbalance between blood sugar absorption and insulin secretion, leading to disability due to vascular and neurological complications (Jr. Porte & Kahn, 2001[2]). Controlling plasma glucose level is essential to delay or prevent DM. One possible way to decrease the rate of blood sugar absorption from the small intestine is to slow or interrupt the digestion of dietary starch, the major dietary source of glucose.

The inhibition of enzymes that digest dietary starch, such as α-amylase has been studied as a method to control blood sugar levels (Ali, Houghton, & Soumyanath, 2006[3]). α-Amylase catalyzes the hydrolysis of α-(1,4)-glucosidic linkages to produce maltose and glucose. (Sogaared-Andersen & Valentin-Hansen, 1993[4]). Absorption of glucose into the blood stream can be delayed by inhibiting these enzymes, thereby, ameliorating DM symptoms such as hyperglycemia. There is an on-going interest in evaluating the effects of plants and crude drugs on starch digestive enzymes as potential sources of novel oral hypoglycemic compounds and dietary supplements (Braithwaite *et al*., 2014[5]). Plant food rich in polyphenols have been reported to cause effects similar to insulin in the utilization of glucose and act as good inhibitors of key enzymes like alpha amylase and alpha glucosidase associated with type 2 diabetes and lipid peroxidation in tissues (Reddy *et al*., 2010[6]). Studies have also shown that the bioactivity of polyphenols in plants is linked to their antioxidant activity and many of these plants also possess hypoglycemic properties (Ramkumar K.M. *et al* 2010[7]). Higher plants, animals and microorganisms are found to produce a large number of different protein inhibitors of alpha amylases in order to regulate the activity of these enzymes (Choudhury A, Maeda K, Murayama R, *et al*. 1996[8]). Some of these enzyme inhibitors act by directly blocking the active centre of the enzyme at various local sites (Kavitha Sama *et al*., 2012[9]). In animals alpha amylase inhibitors decrease the high glucose levels that can occur after a meal by slowing the speed with which alpha amylase can convert starch to simple sugars (Boivin M *et al*., 1987[10]). This is of importance in diabetic people where low insulin levels prevent the fast clearing of extracellular glucose from the blood. Hence diabetics tend to have low alpha amylase levels in order to keep their glucose levels under control.

In diabetics the short term effect of these enzyme inhibitor drug therapies is to decrease high blood glucose levels. The presently used synthetic enzyme inhibitors cause gastrointestinal side effects such as diarrhea, flatulence, abdominal bloating etc (Bray GA *et al*., 1999[11]). Therefore natural alpha amylase inhibitors from the dietary plants can be used as an effective therapy for treating post prandial hyperglycemia with minimal side effects. The present study was carried out to investigate the inhibitory potentials of the ethylacetate extracts of endophytic fungi isolated from Tulsi and Aloe vera on alpha amylase, the key enzymes responsible for carbohydrate hydrolysis.

2. Materials and Methods

2.1 Sample Collection

Healthy (showing no visual disease symptom) and mature plants were carefully chosen for sampling. Fresh plant materials (branches, leaves and roots) were collected from ten different sites at Bhoopsandra area, Banglore, India. The plant material was brought to the laboratory in sterile bags and processed within few hours after sampling. Fresh plant materials were used for the isolation work to reduce the chance of contamination.

2.2 Glassware, Chemicals and Media

The glassware used were made up of borosilicate glass obtained from M/s Borosil India Limited. The chemicals used were of analytical grade obtained from M/s Himedia Laboratories Pvt. Limited & M/s Sigma-Aldrich Pvt. Limited, Mumbai, India. The Media used for the experiments were obtained from M/s Himedia Laboratories Pvt. Limited, Mumbai, India.

2.3 Sample Processing

For Tulsi

Isolation of endophytic fungi from *Ocimum sanctum* was carried out by using the protocol described by strobel *et al*., (2003) [12] with slight modification. The plant was washed under running tap water for 10 minutes to remove dust and debris. Highly sterile conditions were maintained for the isolation of endophytes. Before surface sterilization, the leaves, branches and roots were cut into small pieces of about 1cm long and sterilized in series with 70% ethanol for 1min, 1% mercuric chloride for 1 min and further cleaned by passing through two sets of sterile distilled water. All of work needs to be performed in the laminar air flow using sterile glassware and mechanical instruments. The sterile samples were placed on plate containing potato dextrose agar (PDA) media with 200mg/L concentration of streptomycin to suppress the bacterial contamination. The parafilm wrapped petridishes were incubated at 25-27°C till the mycelia start growing from the samples. The endophytic fungi was transferred into new agar slant and stored at 4°C for further studies.

For Aloe vera

The plant was washed under running tap water for 10 minutes to remove dust and debris. Before surface sterilization plant material was cut into small pieces of about 1cm long and sterilized in a series of 70% ethanol for 1-3 minutes, 4% mercuric chloride for 3-5 minutes, and rinse with 70% ethanol for 2-10 seconds further cleansed by passing through two sets of sterile distilled water. All of work needs to be performed in the laminar air flow using sterile glassware and mechanical instruments. The sterile samples were placed on plate containing potato dextrose agar (PDA) with 200mg/L of streptomycin to suppress the bacterial contamination. The parafilm wrapped petridishes were incubated at 25-27°C till the mycelia start growing from the samples. The endophytic fungi was transferred into new agar slant and stored at 4°C for further studies.

Media preparation

Potato Dextrose Agar (PDA) was used for isolation and purification of endophytic fungi. Antibiotic, streptomycin (200mg/L) was added to suppress bacterial growth. The media and antibiotics were purchased from Himedia, India.

Endophyte Fungal identification

The identification procedure of endophytic fungi was based on morphology. The five isolated species were described according to their macroscopic features (i.e. the color, shape and growth of cultured colonies) as well as microscopic characteristics (i.e. the structure of hyphae, conidia and conidiophores). The morphology of fungal culture colony or hyphae and the characteristics of the spore were identified by temporary mounts using lacto phenol cotton blue (LPCB) and viewed under the microscope at 40X. Obtained data were then compared with the descriptions of endophytic fungi species from standard identification manuals and matches were recorded. Analysis of the antiglycemic activity was carried out on all species identified.

Secondary metabolite extraction

Secondary metabolite extraction was carried out by using the protocol Radji *et al*., (2011) [13]. Positive endophytic fungal isolate was inoculated into 1000 ml conical flask containing 500 ml potato dextrose broth and incubated at room temperature for 21 days under stationary condition with intermittent shaking. The broth culture was filtered to separate the mycelia and filtrate. To the filtrate equal volume of ethyl acetate was added, mixed well for 10 min, keep for 5min, till the two clear immiscible layers are formed. The upper layer of ethyl acetate containing extracted compound was separated using separating funnel. The mycelium was grinded properly in pestle and mortar using ethyl acetate as solvent and then it was filtered using cheese cloth. Both mycelia and culture filtrate were pooled together and evaporated to dryness using water bath. The extracted residue was dissolved in dimethyl sulfoxide (DMSO) and stored at 4°C to be used as stock solution for antiglycemic assay.

Screening of antiglycemic activity

Mode of inhibition of crude extract towards α-amylase activity was determined according to the method described by the Ali *et al* [3] with simple modifications. Briefly, 0.5ml of crude extracts obtained from five different fungi isolated form *Ocimum sanctum* and *Aloe vera* were pre-incubated with 1ml of 1 unit/g concentration of amylase for 15 minutes at 37°C in one set of five tubes as test while In the other set of five tubes, pre-heated 0.5ml of crude extracts was incubated with 1ml of 1 unit/g concentration of amylase for 15 minutes at 37°C and considered as control. 1ml of 1% of starch was added to both sets of reaction mixtures to start the reaction. The mixture was then incubated for 20 minutes at 37°C, and then boiled at 100°C for 15 minutes after addition of 2ml of DNS to stop the reaction. The amount of reducing sugars released was determined spectrophotometerically using a glucose standard curve and the percentage inhibition was calculated.

3. Results and Discussion

The inhibitory activity of ethylacetate extract of endophytic fungi isolated from the medicinal plant Tulsi and Aloe vera against alpha amylase was evaluated in the study. Five different endophytic fungi were isolated from the two medicinal plants (Table 1).

In invitro α-amylase inhibition studies demonstrated that the crude extract from *Nigrospora* showed 75.76% inhibitory activity on α-amylase (Table 2, Fig.1) There are several possible mechanism through medicinal plant can act to control the blood glucose level. One such mechanism is that an alteration of activity of enzyme involved in glucose metabolism. The α-amylase inhibitor act as an anti-nutrient that obstruct the digestion and absorption of carbohydrates.

One of the synthetic α-amylase inhibitors is acarbose, complex oligosaccharides that delay the digestion of carbohydrates. Similar antidiabetic activity by endophytic fungi were observed by Edward *et al.*[14]

Among all the fungal extract, *Nigrospora* extract showed promising anti-glycemic novel compound for treatment of diabetes, one of the most prominent and dreadful disease in the world. Further investigations are still required to prove the same.

Table 1: List of endophytic fungi isolated from different parts of medicinal plants

S. No.	Code	Plant	Plant part	Identification
1	BCEF-01*	Tulsi	Root	*Aspergillus* sp.
2	BCEF-02*	Tulsi	Leaf	*Penicillium* sp.
3	BCEF-03*	Aloe vera	Root	*Cladosporium* sp.
4	BCEF-04*	Aloe vera	Leaf	*Nigrospora* sp.
5	BCEF-05*	Tulsi	Stem	*Gliocladium roseum*

Table 2: Antiglycemic activity of fungal extracts

S.No.	*Name of endophytic extract*	*OD Value at 540nm*		*Differences (C-T)*	*Percentage [(C-T)/C*100]*
		Control (C)	*Test (T)*		
1	*Penicillium*	1.78	1.4	0.38	21.35
2	*Gliocladium roseum*	1.67	1.64	0.03	1.8
3	*Cladosporium*	1.3	1.26	0.04	3.08
4	*Aspergillus*	1.38	1.21	0.17	12.32
5	*Nigrospora*	1.98	0.48	1.5	75.76

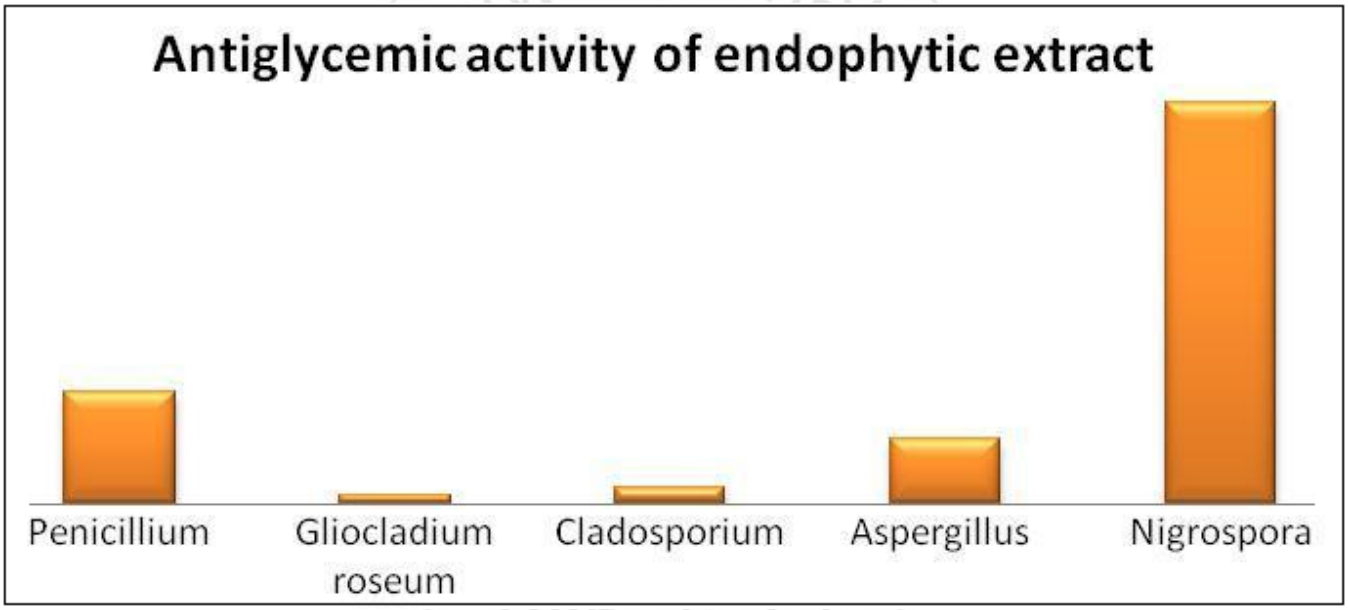

Figure 1: Antiglycemic activity of fungal extracts.

4. Conclusion

The results of the present study indicate ethylacetate extracts of 5 endophytic fungi isolated from Tulsi and Aloe vera showed the alpha amylase inhibitory activity out of which *Nigrospora* showed the maximum alpha amylase inhibitory activity. The plants may essentially contain herbal bioactive compounds inhibiting enzyme activity and hence their endophytic fungi and further structural elucidation and characterization methodologies have to be carried out in order to identify the bioactive constituents. The present study was restricted to the preliminary screening of enzyme inhibitory activities of endophytic fungi from medicinal plants. The expected bioactive components could be flavonols or phenolic acids as literature shows a clear link between polyphenols and antidiabetic activity of herbal extracts KM Maria John *et al*. 2011.[15]). In conclusion, more research is required for developing a potential and valuable antidiabetic therapy using alpha amylase inhibitors of endophytic fungi from medicinal plants.

References

[1] A Mitrakou, D Kelley, M Mokan, T Veneman, T Pangburn, J Reilly, & J Gerich, *New England J. of Med.*,1992, *326*, 22–29
[2] Jr Porte & SE Kahn. *Diabet.*,2001, *50*, 160–163
[3] H Ali, PJ Houghton & A Soumyanath, *J. of Ethnopharmacol.*, 2006, 107, 449–455
[4] L Sogaard-Andersen & P Valentin-Hansen, *Cel.*,1993, *75*, 557–566
[5] MC Braithwaite, C Tyagi, LK Tomar, F Kumar, YE Choonara & V Pillay, *J. of Funct. Foods*., 2014, 6, 82–99
[6] NVLS Reddy, SJ Anarthe, NM Raghavendra, *J. Research. Biomed. Sci.*, 2010,1(2), 72-75

[7] KM Ramkumar, B Thayumanavan, T Palvannan, P Rajaguru, *Med.Chem. Res*., 2010, 19, 948-961
[8] A Choudhury, K Maeda, R Murayama, *Gastroenterol.*, 1996, 111, 1313-20
[9] S Kavitha, M Kamaraj, S Rajeshwari, *As. J. of P. Sci. and Res.*, 2012, 2(4),550-553
[10] M Boivin, AR Zinsmeister, VL Go *et al*, *Mayo. Clin. Proc*.**,** 1987, 62, 249-55
[11] GA Bray, FL Greenway, *Endocr. Rev.*, 1999, 20, 805-79
[12] G. Strobel, Micro. and Infect., 2003, 5, 535-544
[13] M Radji, A Sumiati, R Rachmayani, B Elya, Africa. J. Biotechnol., 2011, 10, 103-07
[14] JD Edward, S Yoice, PS Wahyudi, C Herry and S Partomuan, Asian J. of Biochem., 2011, 6(6),465-471
[15] KM Maria John, J Rajesh, AKA Mandal, Natarajan Sampath, *Eur. J. of Exper.Bio*., 2011,1,145-153