Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Issue 3, 2013

Research Article

ANTIMICROBIAL AND ANTIDIABETIC ACTIVITY OF AN ENDOPHYTIC FUNGI ISOLATED FROM *ADATHODA BEDDOMEI*

PRABAVATHY D* AND VALLI NACHIYAR C

Department of Biotechnology, Sathyabama University, Jeppiaar Nagar, Chennai, India. Email: prabagrp@yahoo.co.in

Received: 30 Apr 2013, Revised and Accepted: 02 Jun 2013

ABSTRACT

Introduction: Endophytes are mutualistic symbionts harboring the living tissues of healthy plants.

Objective: The study was carried out to isolate and screen the bioactivity of endophytic fungi isolated from Adathoda *beddomei*.

Methods: An endophytic *Syncephalastrum sp* was isolated from *Adathoda beddomei*. The crude ethyl acetate extract of the fungi was screened for antimicrobial activity against few representative organisms by agar well diffusion assay.

Results: MIC was determined to be 40µg\ml. GC-MS analysis of the crude extract identified known antimicrobial compounds. The extract also exhibited antidiabetic activity by inhibition of α-amylase activity. The potency was found to be similar to that of Acarbose. The Line Weaver – Burk plot indicated that the structure of the compounds present in the crude extract might mimic acarbose, thus acting as a competitive inhibitor for α-amylase.

Conclusion: These results suggest the use of endophytic fungi for medicinal activity as an alternate to the host.

Keywords: Endophytic fungi, Agar well diffusion assay, Acarbose, Antidiabetic activity, α-amylase inhibition

INTRODUCTION

Microbial drug resistance and the growing need for useful therapeutic compounds with high potency and low toxicity has attracted interest towards the endophytic fungi research [1]. Endophytes are microorganisms that inhabit the healthy tissues of living plants without causing any apparent symptoms of disease [2]. A majority of endophytes are fungi [3]. Endophytic fungi have a mutualistic relationship with the host, protecting the host against pathogen and in some cases may be an opportunistic pathogen [4]. Most of the endopytes are known to possess biosynthetic capabilities greater than the host plant due to their long co evolution and genetic recombination [5]. Endophytic fungi are thus a rich source of novel organic compounds with interesting biological activities and a high level of biodiversity [6]. Novel antibiotics, antimycotics, immunosuppressants, anticancer, antidiabetic compounds are few natural products obtained from endophytic fungi [7].

Medicinal plants have been recognized as a repository of fungal endophytes with novel metabolites of pharmaceutical importance [8]. Distinctly from plants, endophytes can be cultured quickly and the biomass can be accumulated by large scale fermentation. Production of bioactive compounds can be increased by biotechnology of endophytic fungi in order to meet demands while keeping biodiversity and sustainable ecosystem [9].

In view of the above considerations, *Adathoda beddomei* was selected for this study. It is a well known medicinal plant that has been used as an effective drug for asthma and cough. The root, leaves and flowers are used in the form of juice and decoction to treat fever, intrinsic hemorrhage, cough, asthma, skin diseases, obesity, edema, skin diseases, leucorrhoea, vomiting, piles, pox, retention of urine, diseases of mouth and as a rejuvinative In this paper, we report the isolation of endophytic fungi from *Adathoda beddomei* and the antimicrobial, antidiabetic activity of the crude ethyl acetate extract.

MATERIALS AND METHODS

The plant *Adathoda beddomei* was obtained from Siddha Institute, Chennai. The fresh leaf samples were used for the study.

Isolation of endophytic fungi

The healthy leaves were surface sterilized by modified method of Strobel *et al* as sequential washings with a solution of 5.3% of hypochlorite and 70% ethanol [10]. Before surface sterilization, the leaves were cut into pieces, 1-cm long with sterile blade. The surface sterilized leaves were inoculated on PDA (potato dextrose agar) supplemented with chloramphenicol (50µg/ml) at room temperature for 7 -12 days. The endophytic fungi grown were isolated and maintained on PDA slants. The identification was carried out by LPCB mount and 18s rRNA analysis. The bioactive compounds of the culture filtrate were extracted with ethyl acetate.

Screening of antimicrobial activity

Antimicrobial evaluation was carried out by agar well diffusion method. The test cultures used were *Candida albicans, Escherichia Coli*, Staphylococcus *aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*. The minimum inhibitory concentration was found out by following the standard tube dilution method.

Screening of antidiabetic activity

Mode of inhibition of crude extract towards α-amylase activity was determined according to the method described by the Ali *et al* 2006[11]. Briefly, 100µl of crude extract was pre-incubated with 200µl of standard amylase for 15 minutes at 37°C in one set of tubes. In the other set of tubes human urinary amylase was pre-incubated with 100µl of phosphate buffer, pH 6.9. 400µl of potato starch at increasing concentration (0.15-5.0mg/ml) 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 maltose standard curve and converted to reaction velocities. A double reciprocal plot (1/v versus 1/[s]) where v is reaction velocity and [s] is substrate concentration was plotted. The type (mode) of inhibition of the crude extract on α-amylase activity was determined by analysis the double reciprocal (Line Weaver – Burk) plot using Michaelis- Menten kinetics.

Percentage inhibition of crude mycelial extract on α-amylase activity

The crude mycelial extract on alpha amylase activity was determined according to the method described by Kim *et al* 2005 with some modification [12]. Briefly 0.25 µl of alpha amylase was preincubated with 0.20µl of 20 mg/ml of crude extract solution for 15 minutes at 37°Cwater bath. The reaction was started by addition of 0.2µl of 0.5% potato starch dissolved in 20 mM phosphate buffer, pH 6.9.The reaction mixture was incubated at 37°C for 20 minutes

and terminated by addition of 2ml of DNS reagent (1 % 3, 5-dinitro salicylic acid, 12% of sodium phosphate tartarate in 0.4M NaOH.The reaction mixture was heated for 15 minutes at 100°C.Amylase activity was determined by measuring the observance at 540nm and expressed as percentage of blank control without the extract.

RESULTS AND DISCUSSION

The fungi isolated from the plant *Adhathoda beddomei has* been identified by subjecting the fungi to LPCB mount tests. Fungal identification based on conventional methods is laborious and time consuming. Genetic methods present high sensitivity and specificity is used for classifying microbial strains in diverse hierarchical taxonomic levels [13]. The fungal DNA sequence was analyzed by NCBI nucleotide BLAST. A maximum sequence identity of 97% was found with *Syncephalastrum racemosum*. The phylogenetic tree is represented in Figure 1. The fungi isolated was identified to be *Syncephalastrum sp,* by morphological characteristics and 18s r RNA analysis.

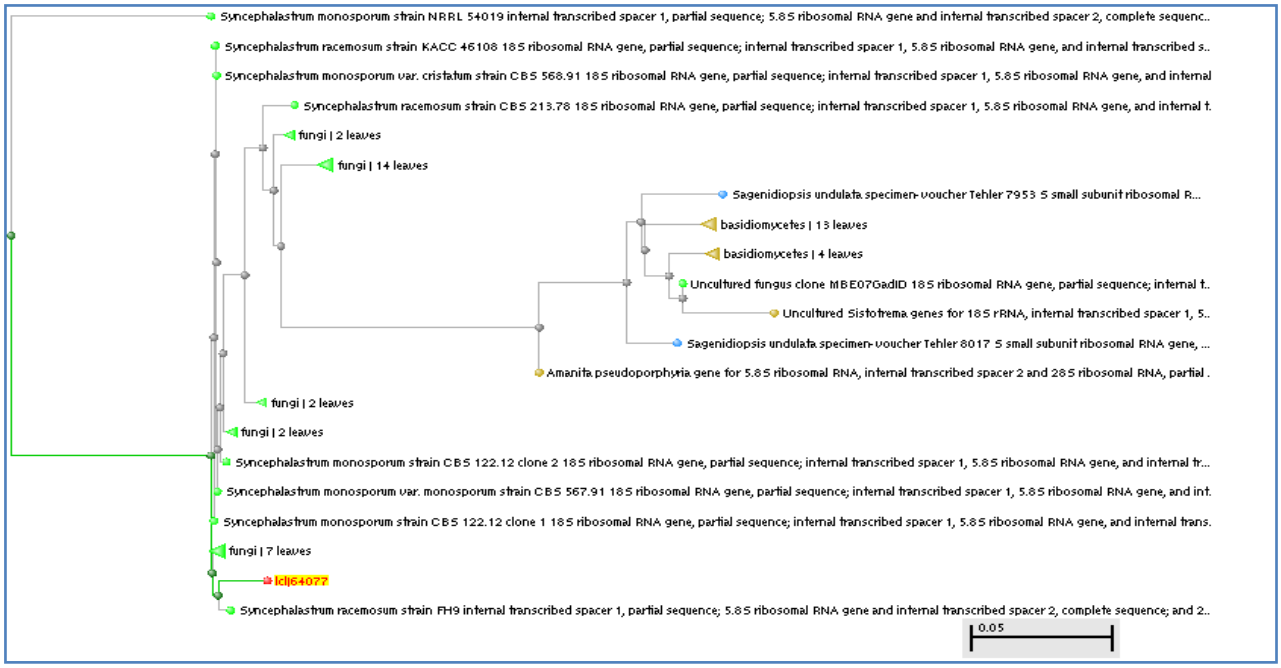

Fig. 1: Phylogenetic tree of the isolated endophytic fungi

The results of the present study show that *Syncephalastrum sp* has antimicrobial activity against both bacteria and fungi. The diameters of inhibition of the filtrate extract of Syncephalastrum sp are presented in Table 1. The maximum zone of inhibition was found against *Klebsiella pneumonia.* The MIC is the lowest concentration of the agent that completely inhibits visible growth and it was determined to be 40µg/ml (Figure 2).

Table 1: Zone Of Inhibition of Crude Mycelial Extract

Organism	Zone of Inhibition (mm)
Candida albicans	11±0.32
Escherichia Coli	13±0.5
Staphylococcus aureus	11±0.24
Pseudomonas aeruginosa	11±0.76
Klebsiella pneumoniae	12±0.2

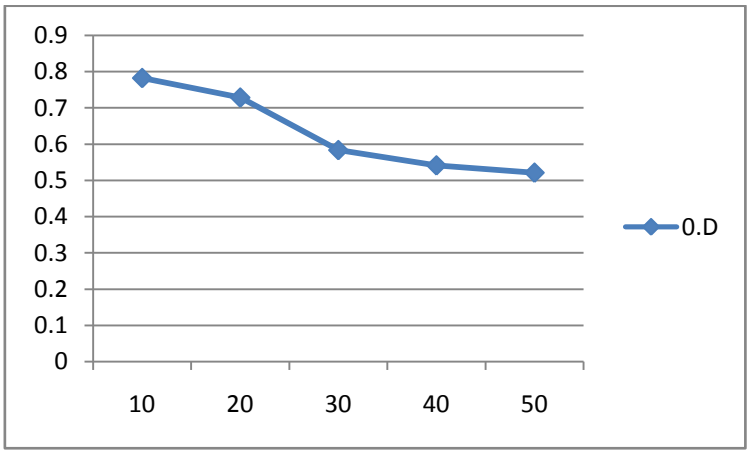

Fig. 2: Determination of minimum inhibitory concentration.

The concentration of the mycelial extract (inhibitor) required for 50% of inhibition (IC_{50}) were determined from corresponding dose-response curves. The percentage inhibition versus inhibitor concentration was compared for both crude mycelial extract as well as acarbose, a known inhibitor of α-amylase shown in Figure 3.

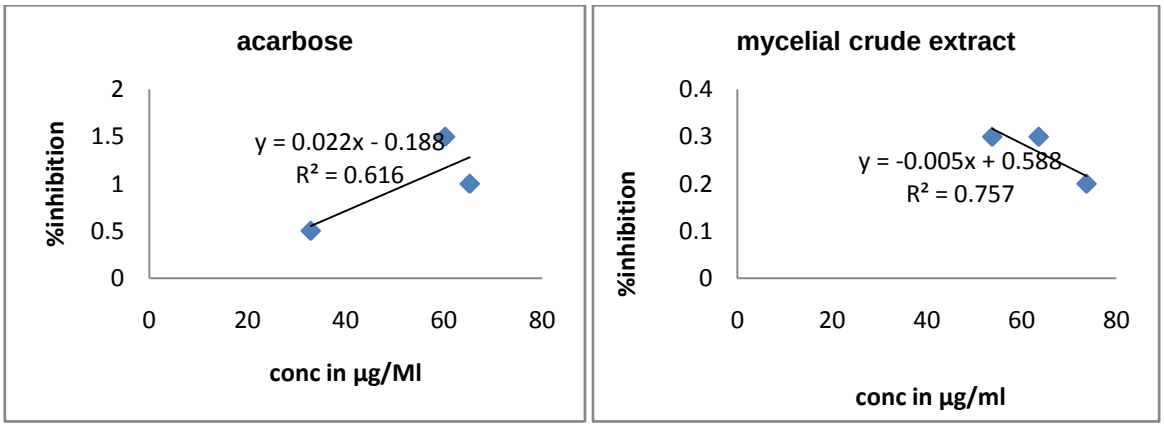

Fig. 3: IC_{50} values of mycelial crude extract (a) Acarbose against α-amylase (b)mycelial crude extract against α-amylase

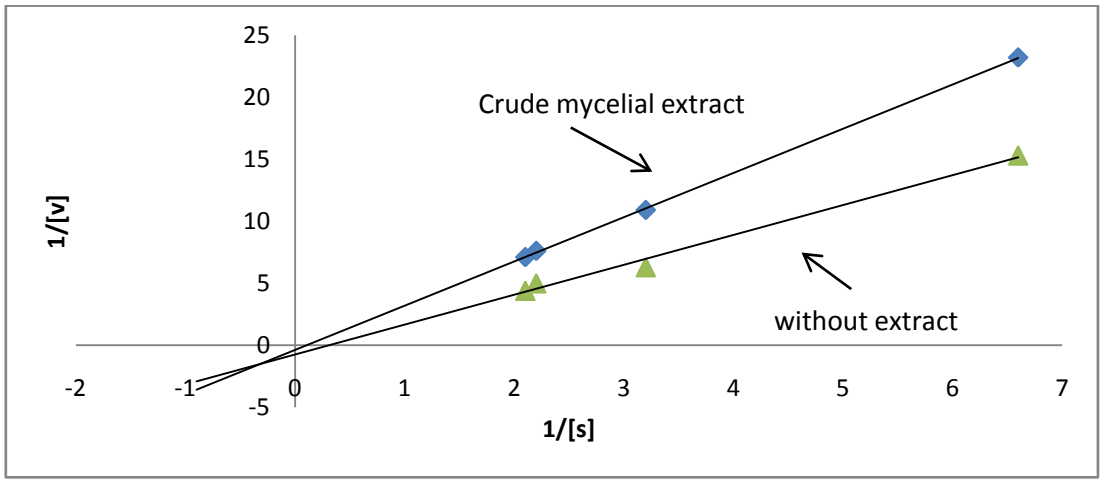

Fig. 4: Lineweaver-Burk plot of the activity of α-amylase in the absence or presence of crude mycelial extract.

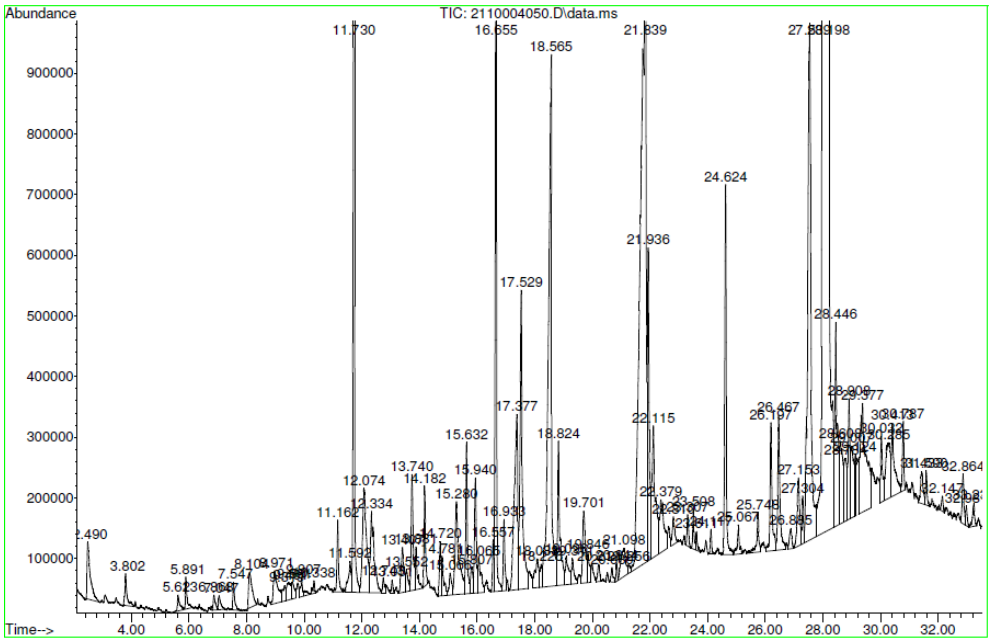

Fig. 5: GC- MS analysis of the crude extract

In invitro α-amylase inhibitory studies demonstrated that the mycelial crude extract showed 75.2% of inhibitory activity on α-amylase. There are several possible mechanisms through which medicinal plants can act to control the blood glucose level [14]. One such mechanism is that an alteration of the activity of enzymes involved in glucose metabolism. The α-amylase inhibitors act as an anti-nutrient that obstructs the digestion and absorption of carbohydrates [15]. One of the Synthetic α-amylase inhibitors is acarbose is a complex oligosaccharides that delay the digestion of carbohydrates. It inhibits the action of pancreatic amylase in breakdown of starch. Synthetic inhibitor causes side effect such as abdominal Pain, diarrhea and soft feces in the colon. The reference drug acarbose was not a potent inhibitor of α-amylase under the current study assay conditions. This is consistent with other reports that either described a very weak inhibitory activity of acarbose (IC50 of about 1 mg/ml) or no inhibition of α-amylase [16]. The mycelial crude extract inhibited α-amylase with IC_{50} value of 0.25 µg/ml similar principle to that of acarbose with IC_{50} value 0.75µg/ml.

Similar antidiabetic activity by endophytic fungi were observed by Edward et al and Ramdanis *et al* [7, 17], however the mode of action was α-glucosidase inhibition. Mode of inhibition of mycelial crude extract on α-amylase activity was determined by means Lineweaver-Burk plot (double reciprocal) analysis of data according to Michaelis-Menten kinetics shown in Figure 4. The mode of inhibition of mycelial crude extract on α-amylase appeared to be competitive (K_m is Increases whereas V_{max} remains the same)

The crude extract was subjected to GC- MS analysis and represented in Figure 5. Since the non purified extract was used, nearly 87 compounds were identified. Among them few compounds like furandione, phenyl esters, anthracenemethanol are identified antimicrobial compounds, whereas compounds like diethyl phthalate, pentadecanoic acid exert their antimicrobial activity through their breakdown products. Further bioassay guided purification would lead to the identification of the antimicrobial compound.

CONCLUSION

The discovery and production of secondary metabolites from endophytic fungi has begun as an exciting field in Biotechnology. Various compounds of pharmaceutical importance are being identified and isolated. The endophytic Syncephalastrum *sp* of Adathoda beddomei promises to be one such candidate for isolation of antimicrobial and antidiabetic compounds.

REFERENCES

1. Gary Strobel, Bryn Daisy and Uvidello Castillo. The Biological Promise of Microbial Endophytes and their Natural Products. Plant Pathology Journal 2005;4 (2): 161-176.
2. Strobel G,Daisy B.Bioprosecting for microbial endphytes and their natural products. Microbial Mol Biol Rev (2003);67: 491-502.
3. Ravindra N Kharwar, Vijay C Verma , Gary Strobel and David Ezra. The endophyic fungal complex of Catharanthus roseus(L) G Don. Current Science 2008; Vol.95,No 2: 228-232.
4. Sadia Sultan,Syed Adnan Ali Shah,Lin Sun, Kalavathy Ramasami,Anthony Cole,John Blunte *et al*. Bioactive Fungal metabolites of 9PR2 isolated from roots of Callophyllum ferrugineum. International Journal of Pharmacy and Pharmaceutical Science 2011; Vol 3, Suppl 1: 7-9.
5. Maurette dos Reis Vieira Fernandes, TalesAlexandre Costa e Silva, Ludwig Heinrich Pfenning, Claudio Miguel da Costa – Neto, Tassiela AndreaHeinrich, Severino Matias de Alencar *et al*. Biological activities of the fermentation extract of the endophytic fungus Alternaria alternata isolated from Coffea Arabica L.Brazilian Journal Of Pharmaceutical Sciences 2009;vol 45,no 4:677-685.
6. Hidayat Hussain Karsten Krohn, Siegfried Draeger, Kathrin Meier and Barbara Schulz. Bioactive chemical constituents of a sterile endophytic fungus from Meliotus dentatus. Rec.Nat.Prod 2009;3:2:114-117.
7. Edward J Dompeipen, Yoice Srikandace,Wahyudi Priyono Suharso, Herry Cahdyana and Partomuan Simanjuntak. Potential endophytic microbes selection for antidiabetic bioactive compounds production. Asian Journal of Biochemistry 2011;6(6):465-471.
8. Strobel, G. , Daisy, B., Castillo, U. and Harper.Natural products from endophytic microorganisms. Journal of *Natural Prod*uct. 2004;67: 257-268.
9. Onifade, A.K. Research trends: Bioactive metabolites of fungal origin. Res. J. Biol. Sci 2007; 2: 81-84.
10. G.Strobel,R.Sidhu,W.M.Hess, and EL Ford. Endophytic taxol-production fungi from bald cypress,*Taxodium distichum.* Microbiology 1996;vol.142, no.8:2223-2226.
11. Ali MM, Roe SM, Vaughan CK, Meyer P, Panaretou B, Piper PW, Prodromou C, Pearl LH. Crystal structure of an Hsp90-nucleotide-p23/Sba1 closed chaperone complex. Nature 2006 ;440: 1013–1017.
12. Kim Y.M, Y.K. Jeong, M.H. Wang, W.Y. Lee, H.I. Rhee. Inhibitory effect of pine extract on alpha-glucosidase activity and postprandial hyperglycemia.Nutrition 2005;21: 756–761.
13. LDSette,M.R.ZPassarini,C.Delarmerina,F.Salati,M.C.T.Duarte.Molecular characterisation and antimicrobial activity of endophytic fungi from coffee plants. World J Microbiol Biotechnol 2006;22:1185-1195.
14. Tanira, M.O.M., Antidiabetic medicinal plants: a review of the present status and future directions. Int. J. Diabetes 1994; 2(1):15-22.
15. Narkhede M.B, P. V. Ajimire, A. E. Wagh, Manoj Mohan and AT. Shivashanmugam: In vitro antidiabetic activity of Caesalpina digyna (R.) methanol root extract. Asian Journal of Plant Science and Research 2011; 1 (2): 101-106.
16. Subramanian R, Asmawi MZ, Sadikun A In vitro α-glucosidase and α-amylase enzyme inhibitory effects of Andrographis paniculata extract and andrographolide. Acta Biochimica Polonica 2008; 55:91-398.
17. Rahmani Ramdanis, Ateik Soemiati, Abdul Munim.Isolation of α-glucosidase inhibitory activity of fungi from mahogany(*Swietenia macrophylla* King) seeds. Int. J. Med.Arom. Plants 2012;Vol 2,No 3:447-452.