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ANTIFUNGAL ACTIVITY OF TRYPSIN INHIBITORS FROM THE SEEDS OF *ABELMOSCHUS MOSCHATUS*

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ABSTRACT: The aim of the present study was to investigate the antifungal potential of trypsin inhibitors (AMTI-I and AMTI-II) isolated from the seeds of *Abelmoschus moschatus* on selected pathogenic fungal strains. The purified inhibitors have been found to be homogenous by the criteria of native PAGE and gel filtration with apparent molecular weights of 22.4kDa and 21.2 kDa as judged by SDS-PAGE. While both the inhibitors were strongly active against bovine trypsin, they showed moderate effect on porcine elastase. AMTI-I and AMTI-II significantly affected the growth of *Candida albicans, Candida tropicalis, Asperigillus flavus, Saccharomyces cerevisiae, Candida glabrata and Asperigillus niger* with notable zones of inhibition. The inhibitors, however, did not show any inhibitory effect on the growth of other fungal strains- *Fusarium oxysporum, Alternaria alternate, Mucor indicus and Penicillium chrysogenum.* The fungicides, Flucanazole and Ketoconazole were used as positive controls in this study. Results obtained suggest that AMTI-I and AMTI-II may be regarded as excellent candidates for the development of novel antimicrobial agents against human pathogenic diseases.

INTRODUCTION: Plants actively react to pathogen and insect attack by producing various classes of proteins such as thaumatin-like proteins, lectins, thionins, ribosome inactivating proteins, chitin binding proteins, protease inhibitors etc. as defensive agents[1]. Protease inhibitors are proteins capable of inhibiting the catalytic activity of proteolytic enzymes and are widely distributed in plants, animals and microorganisms. These inhibitors play essential roles in blood coagulation system, compliment cascade, apoptosis, cell cycle and hormone processing pathways [2]. They are also involved in the treatment of human pathologies such as inflammation, hemorrhage [3] and cancer [4]. In plants, they are abundant in storage organs such as seeds and tubers.

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In addition to regulating endogenous proteinase activities, the inhibitors are also involved in plant defense mechanisms against insects, fungi and other pathogenic microorganisms [5-8].

In recent years, appearance of new mutant strains of microorganisms resistant to commonly used antibiotics have stimulated a systematic analysis of natural products for fungicidal properties having therapeutic applications. Of late, protease inhibitors are chosen as new drugs in highly active antiretroviral combination therapy, increasing life expectancy in HIV-positive patients. Many phytopathogenic fungi are known to produce extracellular proteinases [9] which may play an active role in the development of diseases [10]. In response to such attack by proteinases, plants synthesize inhibitory polypeptides that can suppress the enzyme activities. This phenomenon was first recorded in tomatoes infected with *Phytophthora infestans* [11]. In this case, increased levels of trypsin and chymotrypsin inhibitors correlated with the plants resistance to the pathogen.

Some of the serpins, cystatins, pepstatins and metallo protease inhibitors have been reported to possess antimicrobial activities [12]. Double-headed inhibitors from broad beans and potato tubers showed antifungal activity [13, 14]. Proteinase inhibitors, Mungoin from mung bean and Potide G from potato tubers exhibited both antifungal and antibacterial activities [15, 16].

Abelmoschus moschatus (L.) Medic, family *Malvaceae,* is an aromatic and medicinal plant popularly known as Mushkdana / Kasturi bhendi. The seeds are added to coffee and unripe pods, leaves and new shoots are eaten as vegetables and check excessive thirst, cure for stomatitis, dyspepsia, urinary discharge, gonorrhea, leucoderma and itchiness.

Even though AMTI-I and AMTI-II were found to be very active against trypsin, their influence on the growth of fungi is not yet examined. In view of the importance of protease inhibitors as defensive agents and seeds of *Abelmoschus moschatus* being a potential source of protease inhibitors, the present investigation was undertaken to study the antifungal properties of trypsin inhibitors isolated from the seeds of *Abelmoschus moschatus* on selected fungal strains.

MATERIALS AND METHODS:

Source:

Abelmoschus moschatus plants bearing pods of uniform size were selected in and around Visakhapatnam district. Pods were collected at the ripening stage and seeds removed from the pods were used for the isolation and purification of trypsin inhibitor.

Chemicals:

Bovine pancreatic trypsin (1 x crystallized, DCC-treated, type xi), bovine serum albumin (BSA), chymotrypsinogen A, ovalbumin, lysozyme, phosphorylase b, soybean trypsin inhibitor (type I-S) were purchased from Sigma Chemical Company, St. Louis, Missouri, U.S.A. α-N-benzoyl-DL-arginine-p-nitoanilide HCl (BAPNA), DEAE-cellulose were also from Sigma Chemical company, St. Louis, Missouri, U.S.A. Sephadex G-100 and G-200 were purchased from Pharmacia Fine Chemicals, Uppsala, Sweden. Potato dextrose agar (PDA) was purchased from Himedia Pvt Ltd, Mumbai, India.

All other chemicals used were of analytical grade.

Test organisms:

The fungal strains, *Asperigellus niger* (MTCC 2723), *Asperigillus flavus* (MTCC 4633), *Fusarium oxysporum* (MTCC 1755), *Alternaria alternata* **(**MTCC 1362), *Candida albicans* (MTCC 227), *Candida glabrata* (MTCC 3016), *Candida tropicalis* (MTCC 184), *Mucor indicus* (MTCC 6333), *Penicillium chrysogenum* (MTCC 161) and *Saccharomyces cerevisiae* (MTCC 2918) were collected from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh.

Purification of *Abelmoschus moschatus* trypsin inhibitors (AMTI & AMTI-II):

A procedure has been established for the purification of proteinase inhibitors from the seeds of *Abelmoschus moschatus*. 25 g of the seeds were homogenized with 150 ml of 0.1 M sodium phosphate buffer, pH 7.6 and then made up to 250 ml with the same buffer. The extract was then centrifuged at 5,600 rpm for 15 min at 4°C. The supernatant treated with 4 volumes of ice cold acetone for 1 h was centrifuged at 2,500 rpm for 15 min at 4°C. The precipitate was resuspended in buffer, and the extract was subjected to heat treatment for 10 min at 70°C, then quickly cooled in ice, then centrifuged at 5,600 rpm for 15 min at 4°C and solid ammonium sulfate was added gradually to the supernatant with constant stirring at 4°C to obtain 60% saturation and after overnight standing at 4°C, the precipitate collected after centrifugation at 3,000 rpm for 10 min at 4-6°C was dissolved in the 0.1 M sodium phosphate buffer, pH 7.6 dialyzed against the same buffer.

The dialyzed sample was loaded on a DEAE-cellulose column and the elution was performed with 0.1- 1.0 M NaCl in the buffer. Fractions of 8 ml were collected at a flow rate of 60 ml/h and were assayed for protein by measuring their absorbance at 280 nm as well as the inhibitory activity against trypsin using BAPNA as the substrate.

Protein from the previous step was loaded on Sephadex G-100 column and eluted with the same buffer. Fractions (2 ml) were collected at a flow rate of 12 ml/ h and the protein was monitored by measuring the absorbance at 280 nm. The trypsin inhibitory activities of the fractions were assayed using BAPNA as the substrate. Fractions containing the trypsin inhibitory activities were pooled, dialyzed against distilled water at 4-6°C and then lyophilized.

Protein estimation:
Protein was estimated by the method of Lowry[17] using bovine serum albumin as the standard.

Determination of molecular weight:
Molecular weight of the inhibitor was determined by SDS-PAGE using the method of Laemmli [18] and also by gel filtration on Sephadex G-200 column.

Measurement of trypsin and trypsin inhibitory activity:
The inhibition of inhibitor was established by first assaying the proteinase activity of the enzyme on an appropriate substrate and then incubating a fixed amount of the enzyme with various amounts of the inhibitor and assaying the residual enzyme activity. Trypsin activity was assayed by the method of Kakade[19] using BAPNA as the substrate. Trypsin (30µg) in 2 ml water was incubated with 7 ml of substrate solution at 37°C for 10 min. The reaction was stopped by adding 1 ml of 30%(v/v) acetic acid. The absorbance of the solution was measured at 410 nm against an incubated blank containing 2 ml of water instead of trypsin solution.

To determine the inhibitory activities, suitable aliquots of the inhibitor solutions were included in the assay medium to obtain 30-70% inhibition. One enzyme unit is defined as an increase in 0.01 absorbance unit at 410 nm for trypsin under the assay conditions. One enzyme inhibitory unit is defined as the number of enzyme units inhibited under these conditions.

Determination of antifungal activity:
Active cultures were generated by inoculating a loopful of culture in separate 100 ml potato dextrose broths and incubating on a shaker at 37^{o}C for 48h. The cells were harvested by centrifuging at 4000 rpm for 5 min, washed with normal saline, spun at 4000 rpm for 5 min again and diluted in normal saline.

Antifungal activity:
Antifungal activity of AMTI-I and AMTI-II was performed using the agar well diffusion method [20]. The cultures of 48 h old grown on potato dextrose agar (PDA) were used for inoculation of fungal strains on PDA plates. An aliquot (0.2 ml) of inoculum was introduced to molten PDA and poured into a petridish by pour plate technique. After solidification, the appropriate wells were made and they were filled with the buffer containing 50 - 100 µg of each of the inhibitor and allowed for diffusion of inhibitors for 45 min. The plates were incubated at 25°C for 48 h. The fungicides, Flucanazole and Ketoconazole replaced the inhibitors in the positive control. The inhibition zones were measured with antibiotic zone scale in mm and the experiment was carried out in triplicates.

Minimum inhibitory concentration (MIC) assay:
Minimum Inhibitory Concentrations (MIC) of both the inhibitors were determined according to the method of Elizabeth [21]. A series of two fold dilution of each inhibitor, ranging from 500-2000 µg/ml, was prepared. After sterilization, the medium was inoculated with the aliquots of culture containing spores / slant cultures and incubating for 48 h in aseptic condition and transferred into sterile 6 inch diameter petri dishes and allowed to set at room temperature for about 10 min and then kept in a refrigerator for 30 min.

After the media was solidified, wells were made and different concentrations of each inhibitor ranging from 25-2000 µg/ml were added to the wells of each petri dish. The blank plates were without inhibitors. Inhibition of the growth of the organism in the plates containing inhibitor was judged by comparison with the growth in the control plates. The MICs were determined as the lowest concentration of the AMTI inhibiting visible growth of each organism on the agar plate.

RESULTS AND DISCUSSION: Two trypsin inhibitors from *Abelmoschus moschatus* seeds were purified to homogeneity following conventional

methods of protein purification such as thermal denaturation, ammonium sulphate fractionation and ion exchange chromatography on DEAE-cellulose and gel filtration on Sephadex G-100. When the ammonium sulphate fraction was subjected to DEAE-cellulose column chromatography, trypsin inhibitory activity was found to be associated not only with protein present in the void volume, but also with the proteins bound to the matrix.

A weakly bound protein eluted by 0.1 M NaCl showed trypsin inhibitory activity. These fractions were assayed for the inhibitory activity against trypsin using BAPNA as the substrate. These two inhibitors were designated as *Abelmoschus moschatus* trypsin inhibitors, AMTI-I and AMTI-II, in the order of their elution from DEAE-cellulose column. Both the inhibitors eluted out as a single protein when subjected to gel filtration on Sephadex G-100. The purification of these inhibitors is summarized in **Table 1**. Yields of AMTI-I and AMTI-II were 11.21% and 16.81%, respectively.

The molecular weights of AMTI-I and AMTI-II, as determined by SDS-PAGE were found to be 22.4kDa and 21.2 kDa, respectively. These values were close to those obtained with gel filtration on Sephadex G-200 (**Fig.1**). Trypsin inhibitors gave a single sharp band on SDS-PAGE even in the presence of 2-mercaptoethanol supporting the monomeric nature of the protein.

TABLE 1: SUMMARY OF PURIFICATION OF TRYPSIN INHIBITORS FROM *ABELMOSCHUS MOSCHATUS* SEEDS

Preparation	Vol. (ml)	Total protein(mg)	Total activity units TIU×10^3	Specific activity Units/mg protein TIA×10^2	Yield%	Fold purification
Crude extract	250	2087.5	788.4	3.77	100	1.00
Acetone Treatment	230	1988.2	771.5	3.89	97.86	1.03
Heat treatment	215	1016.4	626.4	6.16	79.45	1.63
$(NH_4)_2SO_4$ (60%) Fractionation	60	424.8	482.8	11.36	61.24	3.01
DEAE-Cellulose Unbound Fraction, AMTI-I	248	55.2	104.4	18.91	13.24	5.01
DEAE-Cellulose 0.1MNaCl elution, AMTI-II	216	58.8	136.8	23.26	17.35	6.17
Sephadex-G-100, AMTI-I	46	40.8	88.4	21.67	11.21	5.75
Sephadex G-100 AMTI- II	50	52.4	132.6	25.30	16.81	6.71

*Yield and fold purification were calculated on the basis of TIU and TIA respectively.
TIU – Trypsin inhibitory unit, TIA – Trypsin inhibitory activity

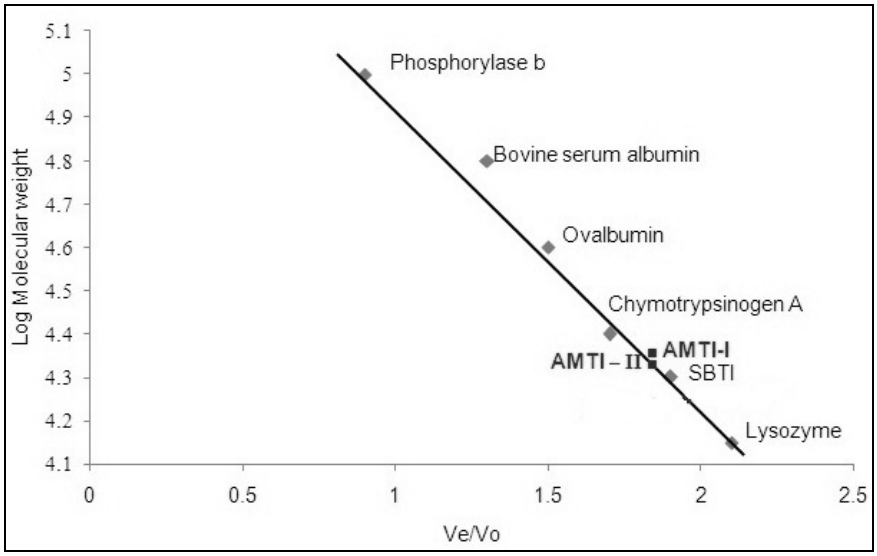

FIG.1: MOLECULAR WEIGHT DETERMINATION OF TRYPSIN INHIBITORS BY GEL FILTRATION ON SEPHADEX G-200
Plot of elution volume against log molecular weight of standard proteins (♦) and AMTI (■). - AMTI-I, AMTI-II.

The purified trypsin inhibitors were tested for their antifungal activity against *Asperigellus niger, Asperigillus flavus, Fusarium oxysporum, Alternaria alternate, Candida albicans, Candida glabrata, Candida tropicalis, Mucor indicus, Penicillium chrysogenum and Saccharomyces cerevisiae* in the range 500-2000 µg/ml along with the positive control containing the fungicides, Flucanazole and Ketoconazole.

Table 2 shows the effect of AMTI-I and AMTI-II on the growth of fungal strains. Both the inhibitors significantly affected the growth of *Candida albicans, Candida tropicalis, Asperigillus flavus, Saccharomyces cerevisiae, Candida glabrata and Asperigellus niger* with zones of inhibition recorded as 19 mm, 20 mm, 17 mm, 17 mm, 18 mm and 20 mm for AMTI-I and 21 mm, 21 mm, 19 mm, 19 mm, 20 mm and 21 mm for AMTI-II respectively. The inhibitors did not exhibit any inhibitory effect on the growth of other fungal strains tested. The fungicides, Flucanazole (20µg) and Ketoconazole (20µg), on the other hand, produced an inhibition zone of 32 – 34 mm in the control (**Fig 2**).

Minimum inhibitory concentrations of both inhibitors for antifungal activity were presented in **Table 3**. Except for *Saccharomyces cerevisiae*, the MIC of AMTI-I and AMTI-II for other fungal strains were found to be 250µg/ml. The trypsin inhibitors were found to be active against selected fungal strains with varying efficiencies and this property may be explored for their use in combating various fungal infections.

TABLE 2: EFFECT OF AMTI-I AND AMTI-II ON FUNGAL GROWTH

Name of the fungal strain	Zone of Inhibition (Diameter in mm)					
	AMTI-I		AMTI-II		Positive controls	
	50 µg	100 µg	50 µg	100 µg	Flucanazole (20 µg)	Ketoconazole (20 µg)
Asperigillus niger	12	20	12	21	34	33
Asperigillus flavus	10	17	11	19	34	32
Fusarium oxysporum	-		-		32	31
Alternaria alternate	-		-		31	34
Candida albicans	12	19	13	21	34	33
Candida glabrata	12	18	12	20	32	34
Candida tropicalis	11	20	13	21	34	32
Mucor indicus	-	-	-	-	32	31
Penicillium chrysogenum	-	-	-	-	31	32
Saccharomyces cerevisiae	10	17	10	19	32	34

Fungal strains were spread on potato dextrose agar plates. Different amounts of the inhibitors (50 µg and 100 µg) were placed in the wells and allowed for diffusion. Controls contained Flucanazole (20 µg) and Ketoconazole (20µg) in place of inhibitors. The incubation period was 48 h at 25^{0}C. Zone of inhibition was measured and minimum inhibitory concentration of each inhibitor was determined.

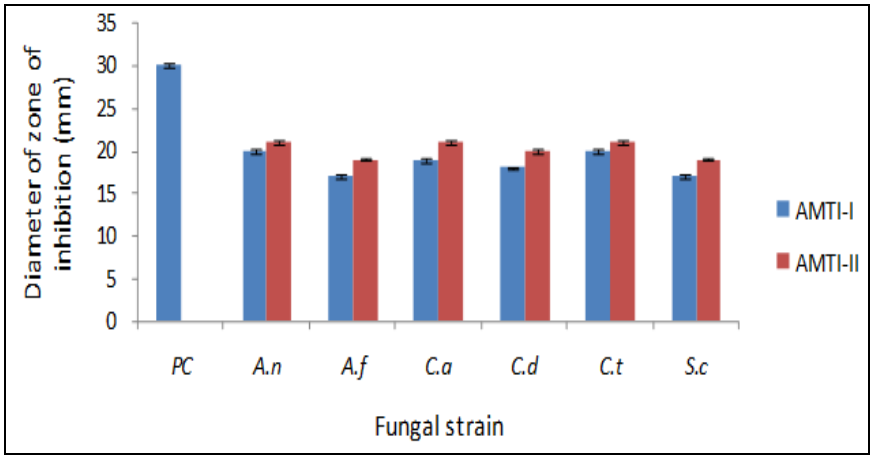

FIG. 2: EFFECT OF AMTI-I AND AMTI-II ON FUNGAL GROWTH

P.C- Flucanazole / Ketoconazole. *A.n - Asperigillus niger, A.f- Asperigillus flavus, C.a- Candida albicans, C.g- Candida glabrata, C.t- Candida tropicalis, S.c- Saccharomyces cerevisiae.*

Fungal strains were spread on potato dextrose agar plates. 100 µg of each inhibitor was placed in the wells and allowed for diffusion. Controls contained Flucanazole (20 µg) and Ketoconazole (20 µg) in place of inhibitors. The incubation period was 48h at 25^0C. Zone of inhibition was measured and minimum inhibitory concentration of each inhibitor was determined.

TABLE 3: MINIMUM INHIBITORY CONCENTRATIONS (MIC) OF AMTI ON FUNGAL GROWTH

Name of the fungal strain	Minimum Inhibitory Concentration(µg/ml)	
	AMTI-I	AMTI – II
Asperigillus niger	250	250
Asperigillus flavus	250	250
Fusarium oxysporum	-	-
Alternaria alternate	-	-
Candida albicans	250	250
Candida glabrata	250	250
Candida tropicalis	250	250
Mucor indicus	-	-
Penicillium chrysogenum	-	-
Saccharomyces cerevisiae	500	500

Fungal strains were spread on potato dextrose agar plates Different amounts of the inhibitor (50-2000 µg/ml) were placed in the wells and allowed for diffusion. Controls contained Flucanazole (20 µg) and Ketoconazole (20µg) in place of inhibitor. The incubation period was 48h at 25^0C. Zone of inhibition was measured and minimum inhibitory concentration of each inhibitor was determined

Fungal strains were spread on potato dextrose agar plates Different amounts of the inhibitor (50-2000 µg/ml) were placed in the wells and allowed for diffusion. Controls contained Flucanazole (20 µg) and Ketoconazole (20 µg) in place of inhibitor. The incubation period was 48 h at 25^0C. Zone of inhibition was measured and minimum inhibitory concentration of each inhibitor was determined.

It is well known that some plant proteinase inhibitors possessed *in vitro* antifungal activity. The inhibitors, AMTI-I and AMTI-II have antifungal activity with varying degrees against pathogenic fungal strains tested. AMTI-I and AMTI-II inhibited the growth of fungal strains in a dose dependent manner. The two inhibitors have no inhibitory effect on the growth of fungi- *Fusarium oxysporum, Alternaria alternata, Mucor indicus and Penicillium chrysogenum* tested. These inhibitors are similar to proteinase inhibitors from broad beans (*Vicia faba*) and buckwheat (*Fagopyrum esculentum*) seeds in their antifungal activity [22, 23].

Trypsin inhibitors exhibiting antifungal activity include those from seeds of the pearl millet [24], seeds of *Eucalyptus urophylla* affecting the mycelial growth of *Pisolithus tinctorius* [25], malaytea scurf pea (*Psoralea corylifolia*) active against *Alternari brassicae, Aspergillus niger, Fusarium oxysporum* and *Rhizoctonia cerealis* [26], *Acacia plumosa* inhibiting the growth profiles of *Aspergillus niger, Thielaviopsis paradoxa* and *Colletotrichum sp.* P10 [27], limenin, large lima beans (*Phaseolus limensis*) suppressing the growth of *Botrytis cinerea, Alternaria alternata* and *Pythium aphanidermatum* [28] and seeds of *Mucuna pruriens* active against *Aspergillus niger* and *Trichoderma viridae* [29].

A trypsin inhibitor from soap nut seeds (SNTI) have been reported to exert potent antifungal activity against dermatophytic fungi, *Trichophyton rubrum* and *Malassezia fur fur* in addition to its antibacterial activity against *Staphylococcus aureus, Bacillus subtilis, Proteus vulgaris and Escherichia coli* [30]. Protein isolated with sequence homology to protease inhibitors from seeds of the chilli pepper, *Capsicum annuum* inhibited *Saccharomyces cerevisiae* [31].

Trypsin inhibitor isolated from *Clausena lansium* seeds showed antifungal activity toward *Physalospora piricola* but was ineffective towards *Mycosphaerella arachidicola, Botrytis cinerea, Fusarium oxysporum* or *Coprinus comatus* [32]. A 14.3 kDa protease inhibitor isolated and purified from the leaves of *Coccinia grandis* exhibited antifungal activity *towards Candida albicans,*

Mucor indicus, Penicillium notatum, Aspergillus flavus and *Cryptococcus neoformans* [33]. *NtKTI1*, a Kunitz trypsin inhibitor from *Nicotiana tabacum* exerted prominent antifungal activity towards *Rhizoctoni solani*, moderate antifungal activity against *Rhizopus nigricans* and Phytophthora *parasitica* var. *nictianae* [34]. Proteinase inhibitors from *Capsicum chinense* Jacq. seeds also exhibited strong antifungal activity against different yeasts - *C. albicans, P. membranifaciens, S. cerevisiae, C. tropicalis and K. marxiannus* with morphological changes, including cellular agglomeration and formation of pseudohyphae [35].

In our previous study, we have reported that the four purified trypsin inhibitors purified from the seeds of *Abelmoschus moschatus* possessed antibacterial activity against pathogenic bacterial strains with varying efficiencies[36]. In this paper, we report the antifungal potential of trypsin inhibitors (AMTI-I and AMTI-II) against some pathogenic fungal strains.

The growth of inhibition of fungi cannot be fully explained by trypsin inhibition alone. The antifungal role of trypsin inhibitors has also been attributed to their ability to interfere with chitin biosynthetic process during fungal cell wall development by inhibiting the proteolytic activation of chitin synthase zymogen [37]. Fungal hyphae may penetrate the plant cell wall by secreting lytic enzymes and then ramify throughout the leaves to absorb nutrients. Protease inhibitors inhibit the fungal proteases and thus increase the resistance of plants to fungal pathogens.

CONCLUSION: The results of the present investigation clearly demonstrate that the trypsin inhibitors, AMTI-I and AMTI-II, isolated and purified from seeds of *Abelmoschus moschatus* may serve as potential antifungal agents. These inhibitors can be explored in the agricultural front for developing transgenics after carrying out extensive *in vitro* studies and they can also find application in the medical front as therapeutic agents for infections caused by specific pathogenic fungal strains.

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REFERENCES:

1. Van Loon LC, Rep M and Pietersel CMJ: Significance of inducible defense-related proteins in infected plants. Annu Rev Phytopathol.2006; 44:135–162.
2. Lingaraju M.H and Gowda LR: A Kunitz trypsin inhibitor of *Entada scandens* seeds: another member with single disulfide bridge. Biochim. Biophys. Acta. (BBA) - Proteins and Proteomics. 2008; 1784(5): 850-855.
3. Oliva M LV, Souza-Pinto JC, Batista IF, Araujo M S, Silveria VF, Auerswald E A, Menrele R, Eckerskorn C, Sampaio MU and Sampaio CAM: *Leucena leucocephala* serine protease performance and feed utilization. Aquaculture.2000; 196: 105-123.
4. Kennedy RC: The Bowman-Birk inhibitor from soybeans as an anticarcinogenic agent. Am. J. Clin. Nutr.1998; 68: 1406 -1412.
5. Valueva TA and Mosolov VV: Protein inhibitors of proteinases in seeds: Classification, distribution, structure and properties. Russ. J. Plant Physiol.1999; 46: 307–321.
6. Carlini CR and Grossi de sa M.F: Plant toxic proteins with insecticidal properties. A review on their potentialities as bioinsecticides. Toxicon. 2002; 40: 1515-1539.
7. Kim JY, Park SC, Kim MH, Lim HT, Park Y, Hahm KS: Antimicrobial activity studies on a trypsin – chymotrypsin protease inhibitor obtained from potato. Biochem. Bioph. Res. Co. 2005; 330(3): 921 – 927.
8. Breiteneder H and Radauer C: A classification of plant food allergens. J. Allergy Cln. Immun, 2004; 113(5): 821-830.
9. Kalashnikova EE, Chernyshova MP and Ignatov VV: The extracellular proteases of the phytopathogenic bacterium *Xanthomonas campestris*, Mikrobiologiia.2003; 72: 498–502.
10. Sara M and Heale JB: The roles of aspartic proteinase and endopectin lyase enzymes in the primary stages of infection and pathogenesis of various host tissues by different isolates of *Botrytis cinerea* Pers ex. Pers. Physiol. Mol. Plant Pathol. 1990; 36:303–324.
11. Woloshuk CP, Meulenhoff JS, Sela-Buurlage M, van den Elzen PJ and Cornelissen BJ: Pathogen-induced proteins with inhibitory activity toward *Phytophthora infestans*. Plant Cell. 1991; 3: 619–628.
12. Kim Jin-Young, Seong -Cheol Park, Indeok Hwang, Hyeonsook Cheong, Jae-Woon Nah, Kyung-Soo Hahm and Yoonkyung Park: Protease inhibitors from plants with antimicrobial activity. Int.J.Mol.Sci. 2009; 10(6): 2860-2872.
13. Ye XY, Ng TB and Rao PF: A Bowman-Birk-type trypsin chymotrypsin inhibitor from broad beans. Biochem. Biophy. Res. Commun. 2001; 289 (1): 91-96.
14. Kim JY, Park SC, Kim MH, Lim HT, Park Y and Hahm KS: Antimicrobial activity studies on a trypsin - chymotrypsin protease inhibitor obtained from potato. Biochem. Biophys. Res. Commun. 2005; 330 (3): 921–927.
15. Wang HX and Ng TB: Concurrent isolation of a Kunitz-type trypsin inhibitor with antifungal activity and a novel lectin from *Pseudostellaria heterophylla* roots. Biochem. Biophys. Res. Commun. 2006; 342(1): 349-353.
16. Kim MH, Park SC, Kim JY, Lee SY, Lim HT, Cheong H, Hahm KS and Park Y: Purification and characterization of a heat-stable serine protease inhibitor from the tubers of

new potato variety "Golden Valley". Biochem. Biophys. Res. Commun. 2006; 346(3): 681-686.
17. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ: Protein measurement with Folin phenol reagent. J.Biol.Chem. 1951; 193, 265-275.
18. Laemmli UK : Cleavage of structure proteins during the assembly of the head of bacteriophage T4. Nature. 1970 ; 227: 680-685.
19. Kakade ML, Simons NR and Liener IE: An evolution of natural vs synthetic substrates for measuring the antitryptic activity of soybean substrates. Cereal Chem. 1969; 46: 518-526.
20. Perez C, Pauli M and Bazerque P: An antibiotic assay by the well agar method. Acta Biologiae et Medicine Experimentalis. 1990; 15: 113-115.
21. Elizabeth M, Adrien Szekely Johnson and David W. Warnock: Comparison of e-test and broth microdilution methods for antifungal drug susceptibility testing of molds. Journal of Clinical Microbiology. 1999; 37: 1480-1483.
22. Ye XY and Ng TB: "A new peptidic protease inhibitor from *Vicia faba* seeds exhibits antifungal, HIV-1 reverse transcriptase inhibiting and mitogenic activities." Journal of Peptide Science. 2002; 8 (12): 656-662.
23. Dunaevsky Yakov E and Galina Bellakova A and Mikhail Belozersky A: The proceeding of the 8th ISB. 2001; 46.
24. Joshi BN, Sainani MN, Bastawade KB, Gupta VS and Ranjekar PK: Cysteine protease inhibitor from pearl millet: a new class of antifungal protein. Biochem. Biophys. Res. Commun.1998; 19, 246(2): 382-387.
25. Tremacoldi Celia Regina and Pascholati Sergio Florentino: Detection of trypsin inhibitor in seeds of *Eucalyptus urophylla* and its influence on the in vitro growth of the fungi *Pisolithus tinctorius* and *Rhizoctonia solani*. Braz. J. Micro. 2002; 33(4): 281-286.
26. Yang X, Li J, Wang X, Fang W, Bidochka MJ, She R, Xiao Y and Pei Y: Psc-AFP, an antifungal protein with trypsin inhibitor activity from *Psoralea corylifolia* seeds. Peptides.2006; 27(7): 1726-1731.
27. Lopes LS, Valadares NF, Moraes DI, Rosa JC, Araújo HSS and Beltramini LM: Physico-chemical and antifungal properties of protease inhibitors from *Acacia plumosa*. Phytochemistry. 2009; 70:871–879.
28. Wang Shaoyun and Rao Pingfan: A leguminous trypsin-chymotrypsin inhibitor Limenin with antifungal activity from *Phaseolus limensis*. Eur Food Res Technol. 2010; 231(2): 331-338.
29. Chandrashekharaiah KS: Physico-chemical and antifungal properties of prypsin inhibitor from the seeds of *Mucuna pruriens*. Oriental Journal of Chemistry. 2013; 29(3): 1061-1070.
30. Rachel Vijaya K, Vimala Y and Chaitanya Apta D: A trypsin inhibitor- SNTI with antidandruff activity from *Sapindus trifoliatus*. Ind. Journ. Appl. Res. 2013; 3: 3-5.
31. Ribeiro SFF, Carvalho AO, Cunha MD, Rodrigues R, Cruz LP, Melo VMM, André O. Carvalho, Cunha MD, Rodrigues R, Cruz LP, Melo VMM, Vasconcelos IM, Melo EJT and Gomes VM: Isolation and characterization of novel peptides from chilli pepper seeds: antimicrobial activities against pathogenic yeasts. Toxicon. 2007; 50: 600-611.
32. Ng TB, Lam SK and Fong WP: A homodimeric sporamin-type trypsin inhibitor with antiproliferative, HIV reverse transcriptase-inhibitory and antifungal activities from wampee (*Clausena lansium*) seeds. Biological Chemistry. 2003; 384(2): 289-293.
33. Satheesh L Shilpa and Murugan K: Antimicrobial activity of protease inhibitor from leaves of *Coccinia grandis* (L.) Voigt. Ind. J. Exp. Biol.2011; 49: 366-374.
34. Hao Huang, Sheng-Dong Qi, Fang Qi, Chang-Ai Wu, Guo-Dong Yang and Cheng-Chao Zheng: *NtKTI1*, a Kunitz trypsin inhibitor with antifungal activity from *Nicotiana tabacum*, plays an important role in tobacco's defense response. FEBS Journal. 2010; 277(9): 4076–4088.
35. Germana Bueno Dias, Valdirene Moreira Gomes, Umberto Zottich Pereira *et al*., Isolation, characterization and antifungal activity of proteinase inhibitors from *Capsicum chinense* Jacq. seeds. Protein J. 2013; 32:15–26.
36. Muni Kumar D and Siva Prasad D: A Comparative study on the antibacterial activity of Trypsin Inhibitors from the seeds of *Abelmoschus Moschatus* L. Int. J. of Sci. and Res. 2014; 3(10):114-119.
37. Adams DJBE, Causier KJ, Mellor V, Keer R, Milling J and Dada: Regulation of chitin synthase and chitinase in fungi. In Muzzarelli R.A.A (ed.,) Chitin Enzy.1993; 15-25.

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