Study of the antimicrobial effect of the protein fraction isolated from germinated *Vigna radiata* (Mung bean) seeds

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Abstract- Antimicrobial proteins (AMPs) are widely distributed in nature. In higher eukaryotes, AMPs provide the host with an important defense mechanism against invading pathogens. AMPs of lower eukaryotes and prokaryotes may support successful competition for nutrients with other microorganisms of the same ecological niche. AMPs show a vast variety in structure, function, antimicrobial spectrum and mechanism of action. Most interestingly, there is growing evidence that AMPs also fulfill important biological functions other than antimicrobial activity. The present study aims at isolation and determination of antimicrobial properties of common leguminous plant, *Vigna radiata* (Mung bean) which will develop some novel aspects for their future use in medicine, agriculture and biotechnology. In the present study, the proteins were isolated from *Vigna radiata* (Mung bean) germinating seeds by homogenization in alkaline phosphate buffer followed by centrifugation. The protein isolated was evaluated for its concentration which was found to be 32% in comparison to non-germinating seeds via Folin-Ciocalteau's reagent. The protein isolated was further dissolved in N- saline to prepare the concentrations viz. 0.5. 1.0, 1.5, 2.0 and 2.5 mg/ml to determine the antimicrobial potential of the protein extract. The antimicrobial activity was performed against *Bacillus subtilis*, *Bacillus licheniformis*, *Pseudomonas aeruginosa* and *Lactobacillus* sp., *Aspergillus niger* and *Cladosporium cladosporioides.* It was found that the leguminous protein had significant antibacterial potential pattern at 0.5, 1.0 and 1.5 mg/ml in comparison to erythromycin (1 mg/ml) and antifungal activity against at 2.5 mg/ml in comparison to Flucanazole (1 mg/ml). The characterization of the protein isolated is in progress.

Index Terms- Vigna radiata, germinating seeds, antimicrobial protein, defense mechanism

I. INTRODUCTION

Antimicrobial proteins (AMPs) are widely distributed in nature. In higher eukaryotes, AMPs provide the host with an important defianse mechanism against invading pathogens. AMPs of lower eukaryotes and prokaryotes may support successful competition for nutrients with other microorganisms of the same ecological niche. AMPs show a vast variety in structure, function, antimicrobial spectrum and mechanism of action. Most interestingly, there is growing evidence that AMPs also fulfill important biological functions other than antimicrobial activity [1-5].The plant derived/origin peptides has important significance in defense mechanism within the plants and Fabaceae family is comprising several genera which are having pharmacological importance [6-7]. Plant defensins are a major component of the chemical defense system of plants. The *de novo* synthesis of these peptides can be constitutive, resulting in the formation of protective barriers around specific plant organs, or between different tissue types within a plant organ. The best evidence of a role for plant defensins in plant defense is the ability of these peptides to confer resistance against pathogens in susceptible plant hosts. Since Leguminous plants (Family: Fabaceae) proteins have strong pharmacological impacts, thus , the study was emphasized on *Vigna radiata* (Mung bean) in order to isolate and purify the protein and to evaluate the antimicrobial activity of the same against dreadful pathogens.

II. MATERIALS AND METHODS

Protein extraction

The leguminous seeds of *Vigna radiata* were collected from the local markets of Uttarakhand. The modified strategy used for protein extraction [8] is described in the following steps-

- Seeds were washed with HgCl2 and further taken for the study (10g-100 g)
- Crushed and blended with 150 ml of PBS at 4^0C.
- Frozen and thawn 3-4 times.
- Centrifuged at 10, 000 rpm for 10 minutes at 4^0C.
- After centrifugation, pellet and supernatant fractions were obtained.
- Supernatant fractions were subjected to 75% ammonium sulphate precipitation while pellet fractions were discarded.
- Further centrifugation (10, 000 rpm for 1 hour at 25^0C) of the ammonium sulphate precipitated fractions was done to obtain the pellet.
- The pellet fraction was solubilized in deionized water and will be followed for dialysis and SDS PAGE.
- Further Gel-column chromatography of the pellet fractions was performed in order to obtain the pure crude fractions.
- Furthermore SDS-PAGE analysis of the pure fractions eluted was performed.

Column Gel Chromatography of eluted protein fractions

About 2 ml having ~500 µg/ml protein concentration of this sample was applied onto the 1.5 x 70 cm column of

Sephadex G-100 pre-equilibrated with 0.02M Sodium acetate buffer (pH 4.5). The column was eluted with the same buffer at the rate of 1ml/1min. The elution pattern was monitored by taking absorbance of collected fractions at 280 nm [9].

SDS-PAGE analysis of eluted protein fractions & Quantitative estimation by Folin- Ciocalteau's reagent

The fractions along with crude extract and solublized ammonium sulphate precipitated protein were run on sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) using 12% - 14% gel by method described [10]. The gels were stained with coomassie brilliant blue R-250 and distained with methanol: acetic acid: water (30:60:10 v/v). The approximate molecular weight of fractions activity was determined by plotting Rf versus molecular weight of known standard proteins. Protein fractions isolated and purified from seeds was determined [11]. Absorbance was measured at 750 nm in UV-VIS spectrophotometer.

Determination of Antimicrobial activity

Culture Media

For antibacterial test, Soyabean Casein Digest agar/broth and Sabouraud's dextrose agar/broth of Hi Media Pvt. Bombay, India was used for antifungal test.

Inoculum

The bacteria viz. *Bacillus subtilis*, *Bacillus licheniformis*, *Pseudomonas aeruginosa* and *Lactobacillus* sp. were inoculated into Soyabean Casein Digest broth and incubated at 37 ^{0}C for 18 h and suspension was checked to provide approximately, 10^5 CFU/ml. The same procedure was done for fungal strains viz. *Aspergillus niger* and *Cladosporium cladosporioides* and these strains were inoculated into Sabouraud's dextrose broth but the fungal broth cultures were incubated at 48-72 h.

Determination of diameter of zone of inhibition by well diffusion method

The agar well diffusion method [12] was modified. Soyabean casein digest agar medium (SCDM) was used for bacterial cultures. The culture medium was inoculated with the bacteria separately suspended in nutrient broth. Sabouraud's dextrose agar/broth will be used for fungal cultures. The culture medium was inoculated with the fungus separately suspended in Sabouraud's dextrose broth. A total of 8 mm diameter wells were punched into the agar and filled with protein extracts prepared in N-saline at different concentrations viz. 0.5. 1.0, 1.5, 2.0 and 2.5 mg/ml. Standard antibiotic (Erythromycin, 1 mg/ml) was simultaneously used as the positive control. The plates were incubated at 37 ^{0}C for 18 h. The antibacterial activity was evaluated by measuring the diameter of zone of inhibition observed. For assaying, antifungal activity of plant extracts, Sabouraud's dextrose agar/ broth medium plates was used. The same procedure as that for determination of antibacterial property was adopted and then after the diameter of zone of inhibition will be observed after 48-72 h. Flucanazole (1mg/ml) was used as standard for determination of antifungal activity. The procedure for assaying antibacterial and antifungal activity was performed in triplicates to confirm the readings of diameter of zone of inhibition observed for each of the test organism.

III. RESULTS AND DISCUSSION

The protein fractions determined after ammonium sulphate precipitation showed pH of 6.23 and showed complete solubility in water. The protein fractions after ammonium sulphate precipitation and gel chromatography at different time periods were processed for SDS- PAGE. There were about 6-7 bands observed after electrophoresis. The protein fraction (s) eluted from gel chromatography showed molecular weight of 32 KDa after staining with Coomassie brilliant blue (R-250) in SDS-PAGE. The protein isolated was evaluated for its concentration which was found to be 32% in comparison to non-germinating seeds via Folin-Ciocalteau's reagent. The protein isolated was further dissolved in N- saline to prepare the concentrations viz. 0.5. 1.0, 1.5, 2.0 and 2.5 mg/ml to determine the antimicrobial potential of the protein extract. The antimicrobial activity was performed against *Bacillus subtilis*, *Bacillus licheniformis*, *Pseudomonas aeruginosa* and *Lactobacillus* sp., *Aspergillus niger* and *Cladosporium cladosporioides*. It was found that the leguminous protein had significant antibacterial potential pattern at 0.5, 1.0 and 1.5 mg/ml in comparison to erythromycin (1 mg/ml) and antifungal activity against at 2.5 mg/ml in comparison to Flucanazole (1 mg/ml). The results are shown in **Figures 1-4** and **Table 1**.

Different studies were reported previously in order to determine the protein content and corresponding molecular weight of protein in *Vigna radiata* (mung bean) seeds. The results of the present study are almost correlated with the previous studies. In one of the previous study, it is reported that, mung bean protein isolate is composed of 16 protein bands with the molecular weights of 11.1-127.4 KDa [13]. Although the antimicrobial nature of the protein as per the current study performed was also confirmed and reported in previous studies [14-16].

IV. CONCLUSION

The protein isolated from *Vigna radiata* (Mung bean) showed the molecular weight of 32 Kilo Daltons (KDa) and having the pH optima of 6.23 value which confirms its slight acidic nature. The present study suggests that, protein isolated from germinating seeds of *Vigna radiata* is potent antimicrobial agent against human and plant pathogens. The study may thus lead to the isolation and identification of significant antimicrobial peptide (s) from such leguminous plants. These antimicrobial peptides can thus be utilized as a potent antimicrobial agent against such dreadful human and phyto-pathogens. Further studies are however needed to evaluate the antimicrobial profile against drug resistant pathogens and to elucidate the structure and sequence the protein fraction for establishing structure-activity relationships.

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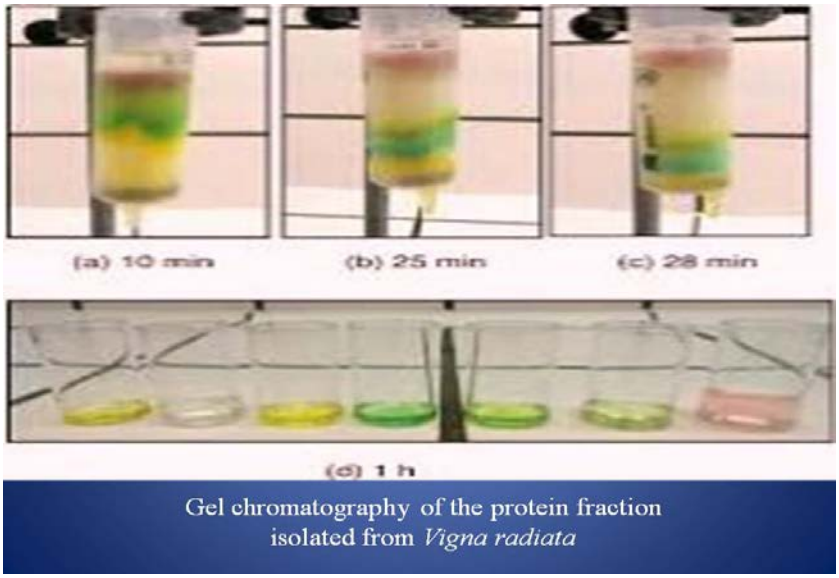

Figure 1: Protein fractions purified from *Vigna radiata* (Mung bean) seeds by Gel chromatography

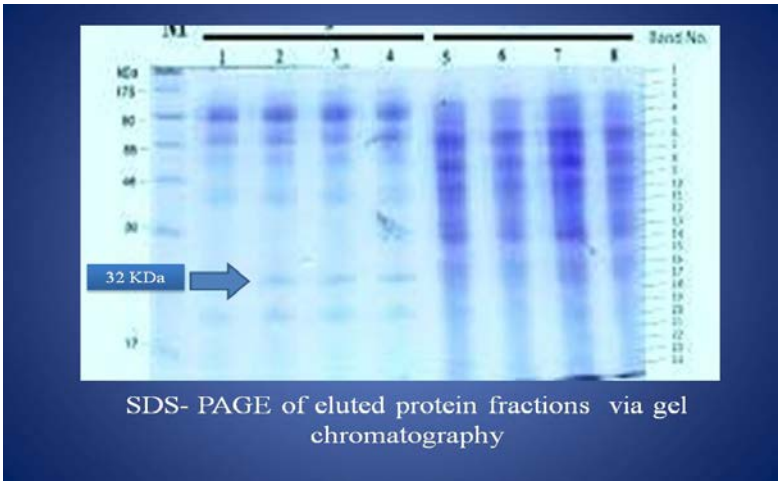

Figure 2: SDS PAGE of the protein fraction purified from *Vigna radiata* (Mung bean) seeds

Table 1: Antimicrobial activity of the eluted protein fraction against selected pathogens

Eluted protein fraction	Conc. of protein/positive control (mg/ml)	Diameter of zone of inhibition (mm)					
		Bacillus subtilis	*Bacillus licheniformis*	*Lactobacillus sp.*	*Pseudomonas aeruginosa*	*Aspergillus niger*	*Cladosporium cladosporioides*
	0.1	15	10	7	10	NT	NT
	0.2	18	12	10	12	NT	NT
	0.3	25	18	15	15	NT	NT
	0.5	28	23	18	20	20	18
	1	32	26	25	30	25	22
	1.5	35	29	28	35	28	27
	2	NT	NT	NT	NT	30	28
	2.5	NT	NT	NT	NT	37	35
Erythromycin (positive control)	1.0	26	22	25	22	NT	NT
Flucanazole (positive control)	1.0	NT	NT	NT	NT	18	25

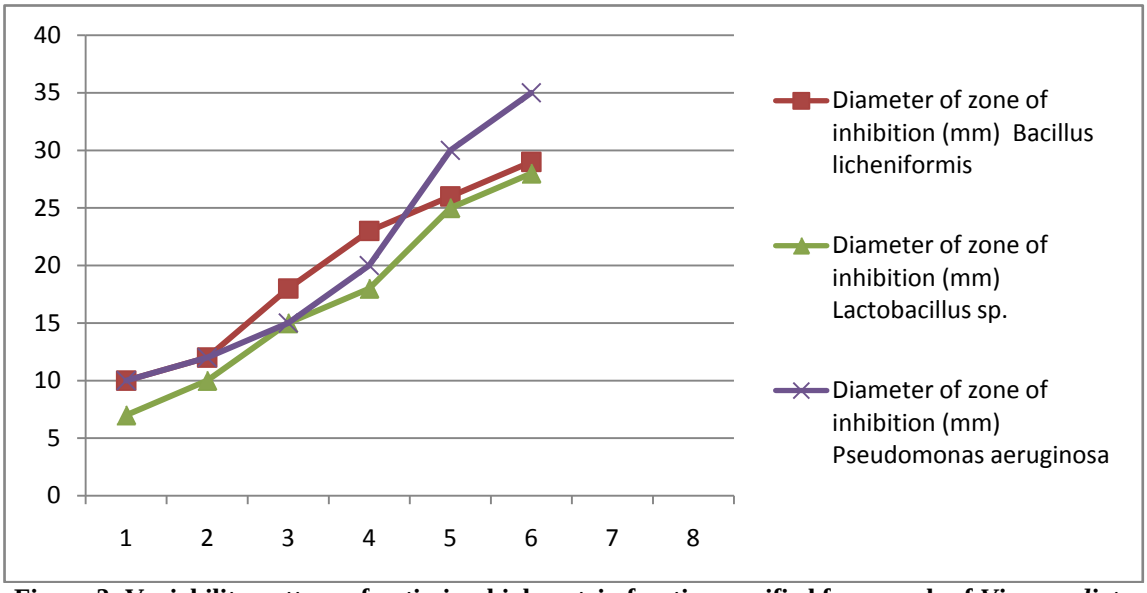

Figure 3: Variability pattern of antimicrobial protein fraction purified from seeds of *Vigna radiata*

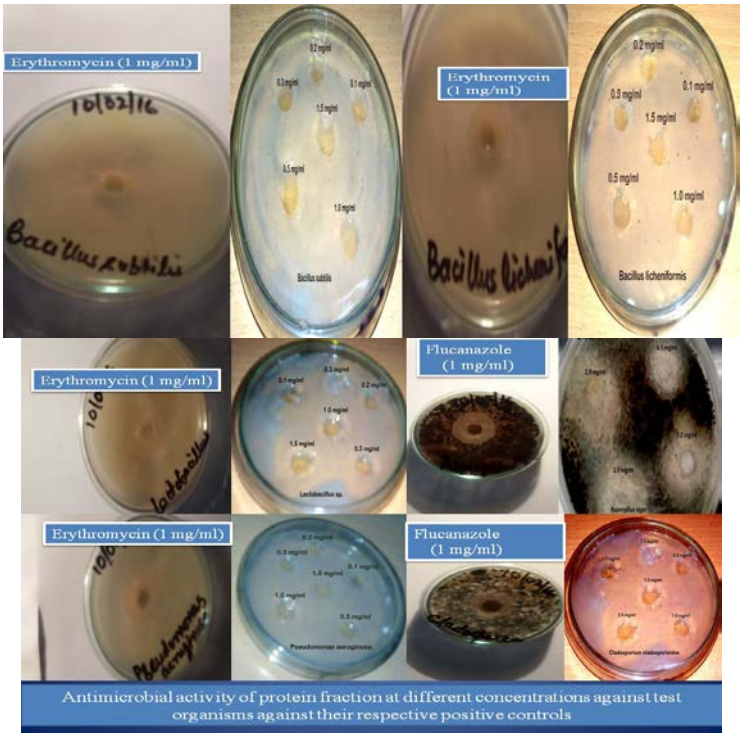

Figure 4: Zone of inhibition measured of the isolated protein and positive controls against different pathogens