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Screening of small peptides from various germinating seeds having antimicrobial activity

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Abstract: *Antibiotics administered against bacterial infections develop resistant strains which are an alarming trend that impacts great significance in human health perspective. Research indicates that antimicrobial peptides (AMP) can be used as potential alternatives to antibiotics, without inducing early antimicrobial resistance. Most of AMPs reported in the data base were of animal origin while very few were reported from plant sources. In light of this, 50 types of germinating seeds were systematically investigated for the presence of small peptides having AMPs. Selected seeds were germinated on brown sheet at different time intervals and proteins were extracted with both liquid nitrogen and phosphate buffer (PBS) treatments. Small peptides of <10kDa were obtained by 5kDa flow through and the same was confirmed by SDS-PAGE. Extracted small peptides were checked for antimicrobial activity against four clinical isolates i.e., Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa. Results indicate that Soya, Barley, Maize, Jowar and Wheat germinating seeds were found to have more antimicrobial peptides acting on gram positive and gram negative bacteria.*

Key words:*Small peptides, germinating seeds, antimicrobial activity.*

INTRODUCTION

Antimicrobial peptides (AMPs) are small and evolutionarily conserved components of the innate immune response, in majority of living organisms. They are found among all classes of life ranging from prokaryotes to humans [1,2]. The major reported AMPs were of animal origin comprising mainly Cecropin, Dermaseptins, Magainin, Opistoporin, Cathelicidin, Proline rich, Glycine /arginine rich peptides, Brevinin peptides, Tachyplesin, Alpha defensins, Beta defensins, θ defensins and Insect defensins [3]. In plants, however few antimicrobial peptides have been isolated from roots, seeds, flowers, stems, and leave from a wide variety of species and have demonstrated activities against pathogenic organisms like viruses, bacteria, fungi, protozoa, and parasites. Thionins, Defensins, Lipid transfer proteins, Puroindolines, and Snakins were different groups of AMPs reported in plants [4].

The antimicrobial peptides were gaining more attention because of the importance of combating with new generations of antibiotics against drug resistant bacteria [5]. One of the most important advantages of antimicrobial peptides is that in contrast to conventional antibiotics, they have several targets and several modes of actions. So resistance against such antibacterial peptides is apparently more difficult to be emerged in comparison with existing antibiotics [5]. However, some human pathogenic bacteria had been able to develop resistance against human antimicrobial peptides during evolution [6,7]. Plant antimicrobial peptides could be better than human because they have rare contact with human pathogens to induce such resistance mechanisms in them.

Seeds are generally rich in proteins which would be fragmented by number of proteases during germination. Proteases cleave storage protein to give small peptides. Major AMPs reported in plants were 5- 13 kDa[4]. Such small AMPs were shown to demonstrate good antibacterial activity even at extreme conditions. Research is warranted on screening of antimicrobial peptides from plant sources specially germinating seeds that can significantly contribute to the development of new antimicrobial peptides. In the present study, we made an attempt to extract and isolate small peptides of < 10kDa from germinating seeds and evaluate their antimicrobial activity against selected bacteria like Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa.

MATERIALS AND METHODS

Chemicals

All the media and chemicals employed in this study were from Hi-Media Chemicals of A.R grade, and all the solvents used were from Qualigens of A.R grade.

Bacterial cultures

The reference /standard organisms used in this study viz., *Staphylococcus aureus* (MTCC 9542),*Escherichia coli* (MTCC 1698), *Klebsiella pneumoniae* (MTCC 10309), and *Pseudomonas aeruginosa* (MTCC6458) were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. All cultures were sub-cultured at regular intervals on nutrient agar and stored at 4° C as well as at -20°C by making their suspension in 10% glycerol.

Seeds

Soya, Barley Vijay, Maize, Jowar, Paddy, Millets, Foxtail-Millets, Red gram, Green gram, Black gram, Ground nut, Pea, Field Bean and Wheat seeds were procured from Faculty of agriculture, Agriculture University and some of them were purchased from local markets (Table 1).

Table: 1. Seed varieties collected from different Agriculture Universities

S.No	Name	Variety	Place
1	Red gram	LRG41	Lam Guntur
2	Green gram	LGG460	Lam Guntur
3	Black gram	LBG 752	Lam Guntur
4	Paddy	BPT5204	Bapatla
5	Millets	ABH1	Anantapur
6	Ground nut	K6	Anantapur
7	Foxtail Millets	Surayanandi	Nandyala
8	Jowar	NTJ2	Nandyala
9	Soya	JS335	Adelabad
10	Barley Vijay	M130	Dharwad
11	Wheat	DWR 195	Dharwad
12	Maize	DHM117	Hyderabad
13	Pea	Arkel	New Delhi
14	Field Bean	TFB5	Tirupathi

Seed Germination

Seeds were surface sterilized using 1% mercuric chloride (HgCl2).The seeds were soaked in distilled water for 6-12 hours, and germinated on sterile brown paper in the dark at 26°C for 2, 4, 6, 8, 10, 12, 14 and16 days respectively based on the seed variety.

Extraction of proteins from plant materials for AMPs

Ungerminated and germinated seeds were dried to reduce 10% moisture at 600C. These samples were kept at 40C in a tightly closed polyethylene bags [8] before use. Dried seeds were ground with liquid nitrogen using pre chilled motor and pestle [9, 10] and Phosphate buffer pH 7.5 [11].The supernatant was further salt precipitated, cutoff separated and used for the estimation of total protein, antimicrobial activity and subjected to SDS-PAGE [12]. The sample was centrifuged at 10000 rpm for 15 min and stored at -20 in a freezer.

Partial purification of small peptides

Ammonium sulfate precipitation

PBS and liquid nitrogen processed samples containing soluble portions were treated with 75% ammonium sulfate maintained at 4^0C for 20 h [13, 14] and centrifuged at 10K rpm for 20 minutes. Precipitated proteins were further purified by buffer exchanged with 0.1M phosphate buffer by American stirrer. Protein concentration was estimated after ammonium salt precipitation [15].

Cut off separation

The dialyzed protein samples were passed through 5 kDa cutoff spin column (Thermo Scientific Protein Concentrators PES, 5K MWCO)[16]. The flow through obtained after centrifugation at 5000 rpm for 10 min was used protein for estimations and antimicrobial activity.

Estimation of Total Proteins by Lowry's method

Germinated and ungerminated seeds extracted mixture, ammonium sulfate precipitated samples and 5Kd cutoff flow throughwere used for estimation of Protein content by Lowry (1951)[19] method using bovine serum albumin (BSA) as standard. The experiments were repeated in triplicates.

Antimicrobial assay

The antimicrobial activity was determined using agar well diffusion assay (ADA) [17] against MTCC standard cultures (*Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*). Inoculum was prepared by adding 0.1 ml of overnight cultures of each organism into 20 ml of sterile LB broth and incubated for 5 – 8 h to standardize the culture of 108cfu/ml. Wells of 8 mm diameter were bored on Mueller-Hinton agar plates to which 100µl of culture were spread and 100µg protein containing each cutoff flow through samples were added to the wells. The plates were incubated for 24 hours at 37^0C [18] and zone of inhibition was measured. For each combination of extract and the bacterial strain, the experiment was performed in duplicate and repeated thrice.

The bacteria with a clear zone of inhibition for more than 12 mm were considered to be sensitive. The antibacterial activity of different germinating seed extracts was compared with five commonly employed antibiotics viz. Ampicillin, Chloramphenicol, Streptomycin, Ciprofloxacin and Kanamycin of specific concentrations used.

SDS-PAGE

The fractions having antimicrobial activity were run on sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) using 15% gel, by method described by Laemmli (1970). After completion of the electrophoresis the gel was sensitized using 0.02% sodium thiosulfate ($Na_2S_2O_3$) solution followed by staining with 0.1% silver nitrate ($AgNO_3$) at room temperature for 2 hours. 3% (w/v) sodium carbonate (Na_2CO_3, pH 11.4) containing 0.05% formalin (v/v) in water was used as developer, finally the gel placed in the solution of 5% (v/v) acetic acid in water to stop the staining process.

RESULTS

Mature seeds of different varieties (Table 1) were collected and stored in room temperature. 100gm seed sample of each variety was used for following experiments.

Partial purification of small peptides

Ammonium sulfate precipitation

Ammonium sulfate precipitated samples were dissolved in 0.1M phosphate buffer and were used for protein estimation. The concentrations of protein ranging from 0.3±0.01 – 270.87±0.28 mg/g wt. Germinated Glycine max seeds liquid nitrogen treated sample got maximum of proteins through ammonium sulfate precipitation (270.87±0.28 mg/gmwt) and lower concentration of proteins resulted in Cocosnucifera L (0.3±0.01 mg/gmwt). Moderate concentrations (175.87±0.28-224.63±0.42 mg/gmwt) were observed in Phaseolus vulgaris and Citrullus lanatus.

Cutoff separation

After salt precipitation and dialysis, sample solutions were passed through 5MWCO column and the filtrates were again subjected to protein estimation. The estimated protein content ranged from 0.05±0.03-18.6±0.1 mg/gm.

Estimation of Total Proteins by Lowry's method

50 germinated and ungerminated seeds were processed with PBS and liquid nitrogen, samples were used for total protein estimation. The estimated proteins were ranging from 0.5±0.02 to 370.53±0.78 mg/gm seed weight (table 4). The extracted proteins were high in germinated seeds treated with liquid nitrogen Glycine max, (370.53±0.78mg/gm seed wt) whereas total protein content was found to be very less in Cocosnucifera L 0.5±0.02 mg/gm seed wt.

Table: 2.Antibiotics concentrations: Ampicillin (A-10 µg), Chloramphenicol (C-30 µg), Ciprofloxacin (Cf-5 µg), Kanamycin (K-30 µg), Streptomycin (S-10 µg), and Tetracycline (T-30 µg).

S.No	Standard antibiotics	Inhibition Zone in mm			
		S.aureus	**P.aeruginosa**	**E.coli**	**K.pneumoniae**
1	**Ampicillin**	18.1 ± 0.5	R	19.9 ± 0.4	7.3 ± 0.1
2	**Streptomycin**	22.1 ± 0.2	10.1 ± 0.3	9.1 ± 0.3	14.7 ± 0.2
3	**Ciprofloxacin**	24.8 ± 0.3	26.7 ± 0.1	23.2 ± 0.3	-
4	**Chloramphenicol**	17.2 ± 0.1	20.8 ± 0.1	12.8 ± 0.4	-
5	**Kanamycin**	10.5 ± 0.3	20.4 ± 0.4	21.5 ± 0.1	9.1 ± 0.3
6	**Tetracycline**	16.2 ± 0.2	17.5 ± 0.1	13.8 ± 0.3	16.9 ± 0.1

Table: 3, protein concentration (mg/gmwt) with different treatments; TP: total proteins; NS: ammonium sulphate buffer; CS: cutoff separation; PBS: Phosphate buffer; LiqN2: liquid nitrogen.

S.No	Name/(mg/gm wt)	Ungerminated seeds						Germinated seeds					
		PBS			Liq N2			PBS			Liq N2		
		TP	NS	CS	TP	NS	CS	TP	NS	CS	TP	NS	CS
1	Glycine max	249.6 ± 0.71	170.53 ± 0.35	10.57 ± 0.28	280.27 ± 0.78	196.43 ± 0.21	11.28 ± 0.11	320. ± 0.71	230.8 ± 0.57	12.85 ± 0.05	370.53 ± 0.78	270.87 ± 0.28	18.6 ± 0.1
2	Arachis hypogaea	150.17 ± 0.28	90.57 ± 0.42	7.26 ± 0.05	170.77 ± 0.28	107.63 ± 0.64	7.17 ± 0.02	200.87 ± 0.71	130.7 ± 0.78	8.24 ± 0.04	250.57 ± 0.71	175.87 ± 0.28	10.1 ± 0.1
3	Sorghum vulgare	55.87 ± 0.35	36.47 ± 0.07	2.41 ± 0.03	73.27 ± 0.71	49.87 ± 0.21	3.29 ± 0.14	85.5 ± 0.28	58.8 ± 0.57	3.77 ± 0.16	91.7 ± 0.85	65.8 ± 0.21	5.51 ± 0.05
4	Pennisetum glaucum	68.93 ± 0.21	46.8 ± 0.35	2.3 ± 0.04	82.87 ± 0.57	57.9 ± 0.35	3.75 ± 0.05	90.87 ± 0.99	63.7 ± 0.28	4.53 ± 0.04	120.53 ± 0.78	87.63 ± 0.35	6.17 ± 0.04
5	Eleusine coracana	38.23 ± 0.64	24.43 ± 0.07	1.72 ± 0.08	43.3 ± 0.85	28.3 ± 0.42	1.93 ± 0.02	51.4 ± 0.57	34.67 ± 0.49	2.19 ± 0.05	69.18 ± 0.07	48.27 ± 0.42	3.79 ± 0.06
6	Trigonella foenum-graecum L	130.2 ± 0.64	89.63 ± 0.42	4.7 ± 0.02	150.57 ± 0.78	105.57 ± 0.64	6.6 ± 0.07	170.17 ± 0.28	120.87 ± 0.28	7.72 ± 0.08	210.8 ± 0.35	151. ± 0.35	13.7 ± 0.04
7	Vigna radiata	139.83 ± 0.21	98.5 ± 0.71	5.29 ± 0.04	161.73 ± 0.64	115.8 ± 0.57	7.44 ± 0.04	178.77 ± 0.35	130.89 ± 0.37	7.71 ± 0.09	220.63 ± 0.78	162.1 ± 0.28	12.99 ± 0.1
8	Vigna mungo	149.17 ± 0.07	108.33 ± 0.64	4.97 ± 0.11	182.63 ± 0.42	136.7 ± 0.07	8.64 ± 0.17	193.87 ± 0.42	143.17 ± 0.42	10.1 ± 0.02	231.57 ± 0.71	175.23 ± 0.07	14.28 ± 0.2
9	Triticum aestivum	70.23 ± 0.07	46.4 ± 0.71	3.21 ± 0.07	90.67 ± 0.64	63.5 ± 0.21	3.9 ± 0.04	110.3 ± 0.07	76.13 ± 0.14	5.87 ± 0.04	130.77 ± 0.28	91.8 ± 0.42	8.1 ± 0.04
10	Zea mays	40.2 ± 0.07	25.9 ± 0.57	1.71 ± 0.03	56.57 ± 0.42	38.7 ± 0.57	2.45 ± 0.05	67.5 ± 0.21	45.87 ± 0.21	4.39 ± 0.02	86.6 ± 0.42	61.37 ± 0.14	4.86 ± 0.05
11	Hordeum vulgare	76. ± 0.14	49.17 ± 0.35	3.29 ± 0.19	81.9 ± 0.28	54.77 ± 0.78	3.69 ± 0.01	97.67 ± 0.49	64.14 ± 0.06	4.3 ± 0.15	110.7 ± 0.07	77.1 ± 0.49	6.4 ± 0.02
12	Avena sativa	98.13 ± 0.14	66.2 ± 0.49	4.26 ± 0.03	127.57 ± 0.28	89.13 ± 0.21	5.15 ± 0.06	145.8 ± 0.78	100.31 ± 0.04	7.31 ± 0.06	157.7 ± 0.64	113.33 ± 0.42	10.27 ± 0.04
13	Oryza sativa	7.9 ± 0.14	4.03 ± 0.07	0.38 ± 0.04	11.6 ± 0.49	6.53 ± 0.28	0.49 ± 0.02	13. ± 0.07	8.12 ± 0.04	0.69 ± 0.02	20.8 ± 0.42	13.13 ± 0.21	0.96 ± 0.04
14	Phaseolus vulgaris	174.1 ± 0.14	121.4 ± 0.64	7.81 ± 0.08	190.77 ± 0.92	136.77 ± 0.28	9.15 ± 0.03	211.93 ± 0.49	154.9 ± 0.14	8.74 ± 0.05	240.73 ± 0.42	186.7 ± 0.49	14.9 ± 0.02
15	Cicer arietinum	124.93 ± 0.21	86.07 ± 0.21	5.11 ± 0.07	134.67 ± 0.64	96.8 ± 0.42	6.11 ± 0.05	156.13 ± 0.21	111.03 ± 0.64	6.47 ± 0.06	181.2 ± 0.78	135.2 ± 0.42	10.39 ± 0.04
16	Brassica juncea	3.97 ± 0.14	2.08 ± 0.01	0.17 ± 0.02	7.93 ± 0.07	4.6 ± 0.07	0.44 ± 0.04	13.57 ± 0.07	7.93 ± 0.07	0.72 ± 0.03	21.43 ± 0.78	13.33 ± 0.07	1.2 ± 0.04
17	Cajanus cajan	132.17 ± 0.49	82.03 ± 0.14	4.6 ± 0.08	153.87 ± 0.21	92.6 ± 0.28	5.88 ± 0.03	180.67 ± 0.71	110.03 ± 0.64	11.75 ± 0.05	212.33 ± 0.99	136.37 ± 0.28	13.69 ± 0.03
18	Punica granatum	4.37 ± 0.42	2.6 ± 0.07	0.15 ± 0.03	7.17 ± 0.21	4.5 ± 0.21	0.29 ± 0.02	10.67 ± 0.71	7.67 ± 0.49	0.48 ± 0.03	13.47 ± 0.21	8.62 ± 0.11	0.58 ± 0.03
19	Zingiber officinale	5.03 ± 0.14	2.7 ± 0.14	0.25 ± 0.04	9.14 ± 0.01	4.93 ± 0.14	0.39 ± 0.02	12.67 ± 0.64	7.63 ± 0.42	0.45 ± 0.02	14.37 ± 0.49	9.07 ± 0.14	0.81 ± 0.04
20	Ocimum tenuiflorum	14.9 ± 0.28	9.45 ± 0.11	0.7 ± 0.06	25.37 ± 0.28	16.33 ± 0.35	1.1 ± 0.05	31.8 ± 0.42	21. ± 0.49	1.45 ± 0.05	37.93 ± 0.64	25.8 ± 0.14	2.29 ± 0.02
21	Curcuma longa	41.97 ± 0.07	26.97 ± 0.57	1.83 ± 0.06	51.7 ± 0.42	34.47 ± 0.21	2.21 ± 0.03	65.7 ± 0.42	44.87 ± 0.64	2.15 ± 0.03	71.67 ± 0.71	50.73 ± 0.49	4.09 ± 0.03
22	Aloe vera	23.1 ± 0.28	8.73 ± 0.07	1.14 ± 0.13	31.7 ± 0.85	14.6 ± 0.14	1.29 ± 0.01	42.77 ± 0.28	22. ± 0.71	1.11 ± 0.06	51.77 ± 0.28	26.83 ± 0.14	2.53 ± 0.04
23	Cocos nucifera L.	0.5 ± 0.02	0.3 ± 0.01	0.05 ± 0.03	1.24 ± 0.04	0.85 ± 0.04	0.07 ± 0.01	3.37 ± 0.49	2.2 ± 0.07	0.19 ± 0.02	5.43 ± 0.21	3.37 ± 0.21	0.28 ± 0.02
24	Carica papaya L	132.23 ± 0.14	87.67 ± 0.49	5.82 ± 0.07	171.8 ± 0.64	118.37 ± 0.64	6.87 ± 0.03	201.83 ± 0.92	142.9 ± 0.14	11.89 ± 0.02	250.63 ± 0.78	182.37 ± 0.28	12.22 ± 0.26
25	Sapindus saponaria	15.03 ± 0.07	7.52 ± 0.04	0.68 ± 0.03	23.37 ± 0.71	12.65 ± 0.04	1.1 ± 0.06	42.4 ± 0.78	24.93 ± 0.57	1.92 ± 0.05	61.67 ± 0.49	36.2 ± 0.49	3.65 ± 0.1
26	Olea europaea	1.2 ± 0.21	0.81 ± 0.02	0.06 ± 0.01	3.25 ± 0.07	16.72 ± 0.01	0.15 ± 0.03	5.24 ± 0.07	3.5 ± 0.28	0.28 ± 0.03	7.37 ± 0.42	5.37 ± 0.21	0.35 ± 0.03
27	Pisum sativum	44.8 ± 0.35	29.7 ± 0.64	2.1 ± 0.05	54.53 ± 0.49	36.33 ± 0.49	2.58 ± 0.05	67. ± 0.14	45.83 ± 0.28	4.1 ± 0.02	82.73 ± 0.49	57.13 ± 0.35	3.4 ± 0.02
28	Magifera indica	6.24 ± 0.08	4.57 ± 0.28	0.25 ± 0.02	9.3 ± 0.07	6.43 ± 0.07	0.46 ± 0.03	20.3 ± 0.07	14.57 ± 0.42	1.08 ± 0.03	38.2 ± 0.42	26.37 ± 0.35	1.62 ± 0.05
29	Prunus armeniaca	1. ± 0.14	0.55 ± 0.05	0.06 ± 0.02	4.5 ± 0.35	2.33 ± 0.07	0.21 ± 0.02	8.05 ± 0.06	5.23 ± 0.21	0.39 ± 0.01	13. ± 0.35	7.7 ± 0.07	0.57 ± 0.06
30	Secale cereale	21.27 ± 0.64	12.87 ± 0.35	1.02 ± 0.07	51.07 ± 0.78	31.43 ± 0.78	2.1 ± 0.04	71.7 ± 0.64	46.83 ± 0.28	3.63 ± 0.06	90.57 ± 0.78	61.7 ± 0.42	4.12 ± 0.06
31	Solanum lycopersicum	43.47 ± 0.71	28.17 ± 0.14	2.07 ± 0.06	91.8 ± 0.99	60.77 ± 0.64	4.19 ± 0.05	178.13 ± 0.35	121.47 ± 0.35	8.03 ± 0.02	210.77 ± 0.92	147.57 ± 0.71	12.46 ± 0.06
32	Citrus sinensis	209.2 ± 1.41	116.03 ± 0.42	8.89 ± 0.09	247.27 ± 0.78	144.4 ± 0.71	12.1 ± 0.01	281. ± 0.78	174.03 ± 0.35	11.56 ± 0.11	391.07 ± 0.49	257.3 ± 0.49	18.39 ± 0.04
33	Psidium guajava	8.1 ± 0.35	4.93 ± 0.07	0.37 ± 0.02	20.3 ± 1.13	13.87 ± 0.21	1.02 ± 0.07	31.1 ± 0.92	19.8 ± 0.21	1.46 ± 0.08	41.23 ± 0.99	27.27 ± 0.14	2.1 ± 0.05
34	Areca catechu	9.17 ± 0.14	4.8 ± 0.21	0.45 ± 0.04	18.73 ± 0.21	10.5 ± 0.07	0.66 ± 0.04	27.8 ± 0.49	15.6 ± 0.07	1.43 ± 0.05	43.07 ± 0.42	25.47 ± 0.07	2.55 ± 0.05
35	Cucurbita maxima	49.83 ± 0.49	29.33 ± 0.14	2.28 ± 0.02	90.53 ± 0.21	54.7 ± 0.07	3.15 ± 0.03	121.1 ± 0.78	78.5 ± 0.28	6.49 ± 0.04	171.03 ± 0.92	118.6 ± 0.49	9.4 ± 0.04
36	Citrullus lanatus	220. ± 0.14	138.7 ± 0.28	10.18 ± 0.02	24.6 ± 0.07	158.57 ± 0.28	1.07 ± 0.06	281.17 ± 0.07	191.13 ± 0.99	14.67 ± 0.05	311. ± 0.85	224.27 ± 0.42	18.09 ± 0.07
37	Helianthus annuus	119.77 ± 0.35	76.93 ± 0.21	5.27 ± 0.05	150.5 ± 0.28	97.67 ± 0.42	6.68 ± 0.1	173.7 ± 0.64	117.63 ± 0.35	7.3 ± 0.03	202. ± 0.14	142. ± 0.64	9.5 ± 0.02
38	Ficus carica	3.03 ± 0.14	1.77 ± 0.07	0.18 ± 0.04	9.23 ± 0.07	5.7 ± 0.07	0.48 ± 0.03	18.47 ± 0.35	12.7 ± 0.57	0.88 ± 0.03	31. ± 0.71	21.47 ± 0.28	1.41 ± 0.06
39	Amaranthus cruentus	79.77 ± 0.57	53.87 ± 0.21	3.52 ± 0.08	100.53 ± 0.92	65.6 ± 0.78	4.61 ± 0.04	121.33 ± 0.78	82.07 ± 0.35	5.48 ± 0.05	150.8 ± 0.57	10.83 ± 0.14	6.2 ± 0.04
40	Phaseolus vulgaris	179.93 ± 0.07	120.4 ± 0.57	7.65 ± 0.02	220.63 ± 0.78	149.8 ± 0.07	9.28 ± 0.02	281.4 ± 0.78	187.7 ± 0.71	12.78 ± 0.24	320.77 ± 0.49	224.63 ± 0.42	14.22 ± 0.13
41	Raphanus sativus	5.5 ± 0.07	3.77 ± 0.28	0.28 ± 0.04	8.2 ± 0.07	5.7 ± 0.07	0.36 ± 0.03	15.37 ± 0.21	10.6 ± 0.28	0.77 ± 0.06	20.87 ± 0.57	13.8 ± 0.21	0.97 ± 0.04
42	Phyllanthus emblica	4.9 ± 0.07	2.93 ± 0.07	0.28 ± 0.04	7.12 ± 0.06	4.57 ± 0.28	0.32 ± 0.03	9.14 ± 0.06	5.9 ± 0.14	0.4 ± 0.02	12.5 ± 0.07	8.67 ± 0.35	0.68 ± 0.04
43	Capsicum annuum ayenne	16.9 ± 0.14	11.67 ± 0.71	0.78 ± 0.03	19.4 ± 0.28	12.83 ± 0.28	0.87 ± 0.02	19.47 ± 0.49	13.8 ± 0.35	0.84 ± 0.03	23.23 ± 0.14	16.67 ± 0.49	1.12 ± 0.05
44	Cuminum cyminum	49.77 ± 0.42	32.97 ± 0.14	2.27 ± 0.02	90.5 ± 0.21	62. ± 0.49	3.93 ± 0.04	110.73 ± 0.78	77.6 ± 0.99	4.81 ± 0.08	150.57 ± 0.78	108.5 ± 0.28	7.02 ± 0.1
45	Coriandrum sativum	14.93 ± 0.21	9.73 ± 0.04	0.69 ± 0.02	16.73 ± 0.49	10.4 ± 0.28	0.78 ± 0.03	17.17 ± 0.14	12.07 ± 0.42	0.71 ± 0.04	21.73 ± 0.49	14.6 ± 0.14	1.06 ± 0.04
46	Medicago sativa	199.9 ± 0.92	119.97 ± 0.14	9.45 ± 0.05	250.87 ± 0.71	160.7 ± 0.57	10.56 ± 0.05	270.7 ± 0.71	175.8 ± 0.42	10.3 ± 0.04	350.53 ± 0.71	237.57 ± 0.71	14.39 ± 0.04
47	Canavalia ensiformis	49.9 ± 0.28	29.6 ± 0.21	2.13 ± 0.02	86.73 ± 0.21	57.67 ± 0.35	4.47 ± 0.05	90.8 ± 0.49	58.43 ± 0.28	3.81 ± 0.03	151. ± 0.64	105.67 ± 0.35	7.6 ± 0.04
48	Nigella sativa	47.97 ± 0.14	28.53 ± 0.28	2.04 ± 0.04	72.4 ± 0.14	44.27 ± 0.14	2.99 ± 0.1	81. ± 0.78	55.7 ± 0.64	3.83 ± 0.06	120.7 ± 0.64	85.7 ± 0.71	6.17 ± 0.04
49	Vigna unguiculata	15.03 ± 0.07	9.68 ± 0.18	0.64 ± 0.01	24.77 ± 0.14	15.73 ± 0.35	1.08 ± 0.05	31.63 ± 0.64	20.9 ± 0.28	1.21 ± 0.03	50.77 ± 0.92	37.67 ± 0.42	2.66 ± 0.05
50	Cucurbita pepo	60. ± 0.78	35.77 ± 0.28	2.59 ± 0.02	90.8 ± 0.71	55.27 ± 1.06	3.78 ± 0.03	120.6 ± 0.71	70.7 ± 0.28	4.59 ± 0.03	150.4 ± 0.28	97.67 ± 0.42	7.57 ± 0.07

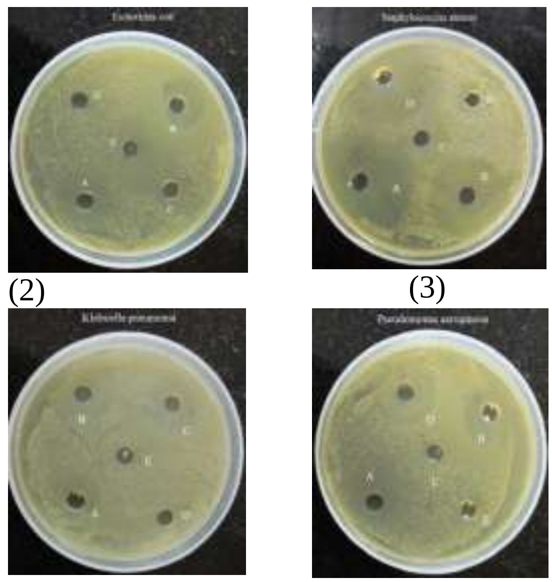
Antimicrobial assay

Total of 50 germinating and un-germinated seeds extracted peptides were screened for antimicrobial activity against Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli and Staphylococcus aureus species.

In agar well diffusion method using extracted AMPs, there were clear and noticeable zone of inhibition resulted, against gram positive and gram negative bacteria. 5Kd column flow through of each germinating and ungerminating seed sample with constant concentration 100 µg of small peptides used for antibacterial activities. The results of the antimicrobial activity are shown in the Tables 3a and 3b.

Forty four seed extracts have shown antimicrobial activity against at least one of the test micro-organisms with inhibition zones ranging from 0.52±0.04mm to 22.16±0.04 mm. Both the germinating and ungerminating protein extracts of 20 seed samples exhibited antimicrobial activity against 4 different pathogenic gram positive and negative bacterial species. The broad spectrum activity was shown by both PBS and liquid N2 extracts of Vignamungo, Triticumaestivum, Zea mays, Hordeumvulgare, Brassica juncea, Cajanuscajan, Punicagranatum, Pisumsativum, Magiferaindica kernel, Prunusarmeniaca, Helianthus annuus, Amaranthuscruentus, Phyllanthusemblica, Coriandrumsativum**.** Among these, both active extracts of Zea mays, Cajanuscajan, Punicagranatum, Pisumsativum, Mangiferaindica kernel and Coriandrumsativum exhibited a strong activity against the 4 bacterial species studied with diameter of inhibition zones ranging from 16.44±0.04mm - 22.16±0.04 mm. The highest activity was observed with liq N2 treated germinating seed extracts of Pisumsativum, showing 22.16±0.04 mm diameter of zone against Staphylococcus aureus, 18.58±0.03 mm against Escherichia coli, 9.35±0.05 mm against Klebsiella pneumoniae, and 8.23±0.05 mm against Pseudomonas aeruginosa. The PBS extract of ungerminated seeds of Avena sativa show minimum antimicrobial activity against Staphylococcus aureus (0.84±0.03mm), Curcuma longa- Escherichia coli (0.61±0.03mm),Solanumlycopersicum - Klebsiella pneumoniae (0.49±0.03mm), andAmaranthuscruentusPseudomonas aeruginosa (0.51±0.01mm).. The moderate activity against at least one of the four strains was shown by Coriandrumsativam, Glycine max, Avena sativa, Cicerarietinum, Zingiberofficinale, Ocimumtenuiflorum, Cocosnucifera L, Carica papaya L, Oleaeuropaea, Solanumlycopersicum, Citrus sinensis, Psidiumguajava, Areca catechu, Raphanussativus,Cuminumcyminum, Medicago sativa, Canavaliaensiformis, Nigella sativa Vignaunguiculata and Prunusarmeniaca(5.11±0.02– 19.61±0.02 mm)(Table 4).

Both PBS and liq N2 extracts of *Aloe Vera, Sapindussaponaria, Secalecereale, Ficuscarica, Capsicum annuum 'Cayenne* and *Cucurbitapepo* showed no antimicrobial activity. PBS, which served as negative control, produced no zone of inhibition. The activity of PBS and liquid N2 extracts of *Pisumsativum* against *Staphylococcus aureus* was found to be similar to that of (S-10 µg) Streptomycin, more than that of (A-10 µg) Ampicillin, (C-30 µg), Chloramphenicol, (K-30 µg) Kanamycin, (T-30 µg) Tetracycline and less than (Cf-5µg) Ciprofloxacin (Table 2).

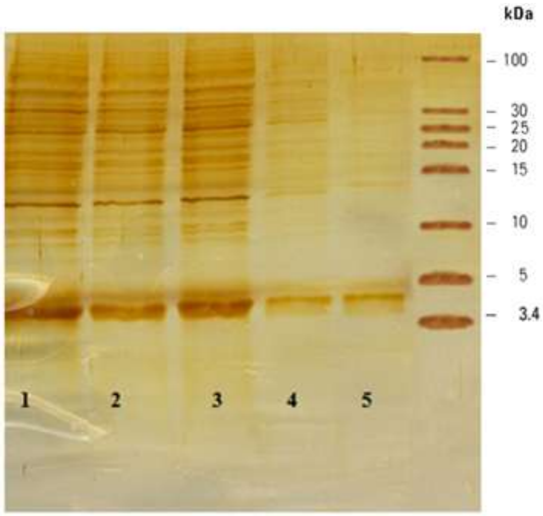


(2) (3) (4) (5)

Fig 2-5: Small peptides activity against 4 different pathogenic organisms (*Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) A –*Pisumsativum*; B –*Punicagranatum*; C-*Cocosnucifera* L; D –*Zea mays*; E-*Phaseolus vulgaris.*

SDS–PAGE

Proteins extracted from germinating and ungerminated seeds were precipitated with 75% ammonium sulfate, while cutoff separated samples were subjected to antimicrobial activity. Samples showing maximum zone of inhibition were separated through 15% SDS-PAGE. Different bands of less than 10kDa resulted after germination and liquid nitrogen treatment as seen in Fig: 1



(A)

Fig: 1 (A): *Pisumsativum*(1) Phosphate buffer extraction sample, (2) Liquid nitrogen extraction sample, (3)75% ammonia precipitation sample, (4) > 5Kd cutoff flow through sample, Molecular weight markers

Statistical analysis

All the experiments were carried out in triplicate thrice (n=9). The statistical analysis of the data was carried out by analysis of the variance (ANOVA). Results were considered significant when $p < 0.05$.

DISCUSSION

Plants store proteins in seeds. Deposition and reactivation of storage protein in seeds through proteolytic cleavage plays an important role in germination processes [20]. In germinating seeds special endopeptidases trigger storage protein breakdown. So germinating seeds contains abundantly small proteins and peptides. These small peptides show antimicrobial activity against surrounding microbial environment to control diseases and enhance the growth of the early plant lets [21]. Hence an attempt is made to explore the possible antimicrobial peptides generated through different extraction processes in germinating and ungerminated seeds.

The degradation of proteins in germinating barley seeds was initiated by proteinases, which converts insoluble from (high molecular weight) to soluble form (low molecular weight) of proteins [22]. Cysteine proteinase [23] and endopeptidases were increased to mobilized and degraded storage proteins in germinating seeds of Vignamungo[24].Under liquid nitrogen, proteins pulverized to a powder with a mortar and pestle for extraction of fragmented peptides [25].

We investigated the yield of antimicrobial peptides with different extraction methods, as 0.1M phosphate buffer reported in M. sativa L. seeds (2) and liquid nitrogen extraction for M. truncatula seeds protein extraction [26,

27. Compared the yields of peptides obtained by both methods for germinating and ungerminated seeds.

Protein extraction from different kinds of plant materials like leaves fruits and seeds using liquid nitrogen [10] in Tobacco [28] were reported.

Initial small peptides purification was achieved by ammonium sulfate precipitation. 75% ammonium sulfate saturation was used for precipitation of proteins and peptides [22]. Precipitated proteins were dissolved in 0.1M phosphate buffer pH 7 (15). This solution was further filtered through <5Kd cutoff column (16). In the present work, Pisumsativum showed maximum activity against Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa using disc diffusion method. Saima and Azra [29] reported antimicrobial peptide(s) from seed/pod of *PisumsativumL*. (garden pea). Their study assessed antibacterial activity against a number of bacteria: Micrococcus luteus, Staphylococcusaureus, Staphylococcus epidermidis, Escherichia coli, Klebsiella pneumonia, Salmonella typhi, Proteus vulgaris,Pasterurellamultocida, and Pseudomonas aeruginosa.

Plant antimicrobial peptides are very similar to other antimicrobial peptides regarding their greater activities against Gram positive bacteria [30, 7, and 31]. Different kinds of antimicrobial substances were reported like saponins [32], canavanine [33] and some important antifungal defensins [34].

Antimicrobial activity of AMPs was reported in wheat endosperm against bacteria *Staphylococcus aureus*, *Micrococcus lutius*, *Klebsiella* and *Bacillus* [35]. Constant protein concentration samples were used for antimicrobial activity with agar well diffusion method [36] with standard reference antibiotics. Antimicrobial activity showing samples were separated through 12%SDS-PAGE with constant voltage 100V (12). It revealed that germinating seeds were containing more number of small peptides. Most of them were having antimicrobial activity and present in the water soluble fraction of seeds.

Concluded that, extracts of antimicrobial peptides from different germinating and ungerminated seeds of *Pisumsativum*, *Punicagranatum*, *Cocosnucifera L*, *Zea mays*, *Phaseolus vulgaris* showed broad spectrum of antibacterial activity. 5kDa MWCO eluted peptide samples were active against number of bacteria.

Table: 4 Cutoff separated samples (100µg/ml) zone of inhibition; *Escherichia coli* (E.c), *Klebsiella pneumoniae* (K.p), *Pseudomonas aeruginosa* (P.a) and *Staphylococcus aureus*(S.a).

S.No	cutoff separation Name	zone of inhibition (100µl)(mm) — ungerminated seeds							
		PBS				Liq N2			
		E.c	K.p	P. a	S. a	E. c	K.p	P. a	S. a
1	Glycine max	3.27 ± 0.17	2.71 ± 0.03	2.58 ± 0.03	2.19 ± 0.03	6.8 ± 0.01	6.12 ± 0.04	5.8 ± 0.01	4.9 ± 0.04
2	Arachis hypogaea	NA	NA	NA	NA	NA	NA	NA	NA
3	Sorghum bicolor	NA	NA	NA	NA	NA	NA	NA	NA
4	Pennisetum glaucum	1.52 ± 0.06	1.4 ± 0.01	1.53 ± 0.02	1.42 ± 0.02	1.6 ± 0.01	1.7 ± 0.01	1.81 ± 0.03	1.54 ± 0.03
5	Eleusine coracana	1.18 ± 0.06	1.79 ± 0.02	1.5 ± 0.01	NA	4.52 ± 0.02	4.69 ± 0.03	4.94 ± 0.05	NA
6	Trigonella foenum-graecum L	0.62 ± 0.02	0.52 ± 0.04	0.72 ± 0.02	0.9 ± 0.01	1.8 ± 0.01	1.32 ± 0.02	1.69 ± 0.03	1.22 ± 0.02
7	Vigna radiata	1.93 ± 0.12	2.12 ± 0.02	1.24 ± 0.03	NA	3.7 ± 0.01	4.22 ± 0.02	2.34 ± 0.03	NA
8	Vigna mungo	1.6 ± 0.14	1.8 ± 0.04	NA	1.89 ± 0.02	3.41 ± 0.02	3.59 ± 0.02	NA	3.67 ± 0.02
9	Triticum aestivum	2.61 ± 0.07	2.16 ± 0.05	2.34 ± 0.03	1.62 ± 0.02	5.13 ± 0.03	4.36 ± 0.04	4.72 ± 0.02	3.82 ± 0.02
10	Zea mays	NA	2.33 ± 0.03	2.14 ± 0.03	2.42 ± 0.02	NA	4.09 ± 0.01	4.32 ± 0.05	4.87 ± 0.04
11	Hordeum vulgare	0.74 ± 0.05	1.14 ± 0.04	1.22 ± 0.02	1.8 ± 0.01	1.22 ± 0.02	2.26 ± 0.06	2.73 ± 0.04	3.43 ± 0.02
12	Avena sativa	1.1 ± 0.09	NA	0.93 ± 0.03	0.84 ± 0.03	2.97 ± 0.05	NA	1.85 ± 0.04	1.5 ± 0.01
13	Oryza sativa	NA	NA	NA	NA	3.08 ± 0.02	NA	2.39 ± 0.03	NA
14	Phaseolus vulgaris	2.59 ± 0.09	NA	NA	NA	3.5 ± 0.01	NA	NA	NA
15	Cicer arietinum	1.82 ± 0.02	NA	NA	1.72 ± 0.02	3.62 ± 0.02	NA	NA	3.46 ± 0.03
16	Brassica juncea	1.82 ± 0.01	1.9 ± 0.01	2.14 ± 0.03	2.2 ± 0.01	3.71 ± 0.03	4.12 ± 0.02	3.88 ± 0.03	4.1 ± 0.01
17	Cajanus cajan	1.72 ± 0.02	NA	1.82 ± 0.02	2.31 ± 0.02	3.42 ± 0.02	NA	3.79 ± 0.02	4.48 ± 0.05
18	Punica granatum	1.82 ± 0.02	1.49 ± 0.03	2.2 ± 0.01	2.62 ± 0.02	3.1 ± 0.01	3.79 ± 0.03	4.21 ± 0.02	5.28 ± 0.02
19	Zingiber officinale	2.26 ± 0.07	0.89 ± 0.26	2.52 ± 0.02	1.85 ± 0.07	3.51 ± 0.02	2.18 ± 0.02	4.82 ± 0.02	3.79 ± 0.04
20	Ocimum tenuiflorum	NA	NA	NA	NA	0.49 ± 0.02	0.84 ± 0.03	1.12 ± 0.02	1.5 ± 0.01
21	Curcuma longa	0.61 ± 0.03	NA	0.54 ± 0.03	0.83 ± 0.05	2.11 ± 0.03	NA	2.23 ± 0.02	1.48 ± 0.02
22	Aloe Vera	NA	NA	NA	NA	NA	NA	NA	NA
23	Cocos nucifera L	1.39 ± 0.13	NA	NA	1.12 ± 0.02	2.3 ± 0.01	NA	NA	2.49 ± 0.03
24	Carica papaya L	1.1 ± 0.01	NA	1.71 ± 0.03	1.48 ± 0.02	2.42 ± 0.02	NA	2.7 ± 0.01	3.18 ± 0.02
25	Sapindus saponaria	NA	NA	NA	NA	NA	NA	NA	NA
26	Olea europaea	1.42 ± 0.02	1.34 ± 0.03	1.53 ± 0.02	1.14 ± 0.05	3.39 ± 0.03	2.81 ± 0.01	2.42 ± 0.02	2.59 ± 0.02
27	Pisum sativum	3.25 ± 0.12	1.22 ± 0.02	1.9 ± 0.04	2.48 ± 0.05	5.77 ± 0.05	3.19 ± 0.03	3.14 ± 0.03	5.53 ± 0.09
28	Magifera indica	1.99 ± 0.08	NA	2.61 ± 0.04	3.41 ± 0.01	3.5 ± 0.01	NA	5.18 ± 0.06	6.28 ± 0.03
29	Prunus armeniaca	2.14 ± 0.05	NA	NA	2.72 ± 0.04	4.81 ± 0.03	NA	NA	5.11 ± 0.02
30	Secale cereale	NA	NA	NA	NA	NA	NA	NA	NA
31	Solanum lycopersicu	0.81 ± 0.07	0.49 ± 0.03	1.49 ± 0.03	1.62 ± 0.02	2.12 ± 0.02	2.19 ± 0.01	2.73 ± 0.02	2.55 ± 0.04
32	Citrus sinensis	NA	NA	1.15 ± 0.04	2.99 ± 0.06	NA	NA	2.85 ± 0.05	3.08 ± 0.02
33	Psidium guajava	1.75 ± 0.04	NA	NA	1.79 ± 0.03	3.49 ± 0.02	NA	NA	3.17 ± 0.02
34	Areca catechu	0.88 ± 0.02	1.21 ± 0.01	1.62 ± 0.02	2.07 ± 0.02	4.2 ± 0.01	3.16 ± 0.05	2.8 ± 0.04	4.31 ± 0.03
35	Cucurbita maxima	1.98 ± 0.06	2.71 ± 0.02	2.31 ± 0.07	1.45 ± 0.05	4.2 ± 0.01	4.5 ± 0.01	4.15 ± 0.04	3.24 ± 0.03
36	Citrullus lanatus	1.2 ± 0.01	1.75 ± 0.04	2.41 ± 0.03	1.51 ± 0.01	2.5 ± 0.01	3.41 ± 0.03	4.13 ± 0.05	3.42 ± 0.04
37	Helianthus annuus	1.72 ± 0.02	2.15 ± 0.04	NA	2.64 ± 0.04	3.3 ± 0.01	3.55 ± 0.05	NA	4.1 ± 0.01
38	Ficus carica	NA	NA	NA	NA	NA	NA	NA	NA
39	Amaranthus cruentus	1.31 ± 0.02	0.8 ± 0.04	0.51 ± 0.01	1.22 ± 0.02	2.64 ± 0.5	2.72 ± 0.02	2.52 ± 0.02	4.17 ± 0.06
40	Phaseolus vulgaris	1.8 ± 0.01	NA	NA	NA	3.42 ± 0.02	NA	NA	NA
41	Raphanus sativus	1.14 ± 0.04	1.42 ± 0.02	1.5 ± 0.01	2.4 ± 0.01	3.72 ± 0.04	3.59 ± 0.03	3.22 ± 0.02	4.14 ± 0.03
42	Phyllanthus emblica	2.41 ± 0.03	2.48 ± 0.02	1.93 ± 0.06	2.22 ± 0.02	4.15 ± 0.04	5.12 ± 0.02	3.81 ± 0.03	4.59 ± 0.06
43	Capsicum annuum 'C	NA	NA	NA	NA	NA	NA	NA	NA
44	Cuminum cyminum	0.75 ± 0.04	1.3 ± 0.01	NA	1.55 ± 0.07	2.1 ± 0.01	3.39 ± 0.02	NA	3.59 ± 0.05
45	Coriandrum sativum	2.19 ± 0.09	1.5 ± 0.01	1.7 ± 0.01	2.08 ± 0.03	4.11 ± 0.03	3.88 ± 0.04	3.45 ± 0.04	4.31 ± 0.03
46	Medicago sativa	1.99 ± 0.08	1.71 ± 0.02	1.62 ± 0.02	2.41 ± 0.03	3.82 ± 0.02	3.5 ± 0.04	2.74 ± 0.05	4.61 ± 0.03
47	Canavalia ensiformis	2.13 ± 0.05	1.48 ± 0.02	1.79 ± 0.03	1.64 ± 0.06	4.52 ± 0.02	3.58 ± 0.03	3.84 ± 0.03	3.58 ± 0.02
48	Nigella sativa	NA	NA	NA	2.09 ± 0.01	NA	NA	NA	4.12 ± 0.02
49	Vigna unguiculata	1.83 ± 0.05	1.63 ± 0.03	1.62 ± 0.02	2.6 ± 0.03	3.89 ± 0.02	3.47 ± 0.03	3.78 ± 0.02	4.14 ± 0.05
50	*Cucurbita pepo*	NA	NA	NA	NA	NA	NA	NA	NA

S.No	cutoff separation Name	zone of inhibition (100µl)(mm) — Germinating seeds							
		PBS				Liq N2			
		E. c	K.p	P. a	S. a	E. c	K.p	P. a	S. a
1	Glycine max	6.11 ± 0.03	5.39 ± 0.03	5.26 ± 0.03	4.5 ± 0.02	11.24 ± 0.05	10.59 ± 0.06	10.23 ± 0.05	8.13 ± 0.03
2	Arachis hypogaea	2.47 ± 0.03	1.5 ± 0.02	1.11 ± 0.03	1.26 ± 0.04	5.2 ± 0.05	2.23 ± 0.05	2.14 ± 0.04	2.49 ± 0.03
3	Sorghum bicolor	2.37 ± 0.02	2.25 ± 0.04	1.24 ± 0.03	1.16 ± 0.04	5.92 ± 0.02	2.67 ± 0.15	2.54 ± 0.05	3.45 ± 0.06
4	Pennisetum glaucum	1.4 ± 0.01	1.23 ± 0.02	1.57 ± 0.11	1.37 ± 0.29	1.5 ± 0.05	1.47 ± 0.02	1.45 ± 0.04	1.72 ± 0.02
5	Eleusine coracana	2.5 ± 0.01	2.8 ± 0.04	2.88 ± 0.1	NA	6.74 ± 0.03	6.78 ± 0.02	6.38 ± 0.06	NA
6	Trigonella foenum-graecum L	3.71 ± 0.02	2.61 ± 0.05	3.51 ± 0.06	2.52 ± 0.04	7.47 ± 0.06	5.44 ± 0.03	7.56 ± 0.03	4.85 ± 0.04
7	Vigna radiata	7.25 ± 0.04	8.38 ± 0.02	4.14 ± 0.15	NA	13.21 ± 0.45	16.24 ± 0.11	8.49 ± 0.02	NA
8	Vigna mungo	6.7 ± 0.02	7.38 ± 0.08	NA	7.52 ± 0.02	13.45 ± 0.04	14.58 ± 0.03	NA	15.45 ± 0.04
9	Triticum aestivum	10.24 ± 0.03	9.71 ± 0.03	9.59 ± 0.02	7.76 ± 0.06	20.28 ± 0.09	19.58 ± 0.09	19.24 ± 0.04	15.84 ± 0.04
10	Zea mays	NA	8.47 ± 0.06	8.72 ± 0.07	9.23 ± 0.04	NA	16.86 ± 0.04	17.35 ± 0.04	18.55 ± 0.04
11	Hordeum vulgare	2.3 ± 0.03	4.7 ± 0.01	5.18 ± 0.07	6.37 ± 0.02	5.21 ± 0.02	9.5 ± 0.07	10.24 ± 0.06	12.79 ± 0.02
12	Avena sativa	5.39 ± 0.02	NA	3.68 ± 0.09	3.82 ± 0.07	10.54 ± 0.05	NA	7.77 ± 0.05	8.31 ± 0.08
13	Oryza sativa	4.79 ± 0.03	NA	5.47 ± 0.06	NA	8.19 ± 0.03	NA	10.83 ± 0.05	NA
14	Phaseolus vulgaris	7.44 ± 0.05	NA	NA	NA	14.58 ± 0.06	NA	NA	NA
15	Cicer arietinum	6.45 ± 0.04	NA	NA	7.11 ± 0.03	12.49 ± 0.02	NA	NA	13.52 ± 0.29
16	Brassica juncea	7.6 ± 0.04	6.46 ± 1.38	6.86 ± 0.06	7.47 ± 0.03	15.79 ± 0.02	14.55 ± 0.07	12.31 ± 0.07	15.31 ± 0.09
17	Cajanus cajan	6.78 ± 0.17	NA	7.22 ± 0.04	8.29 ± 0.02	13.2 ± 0.02	NA	15.3 ± 0.08	16.24 ± 0.05
18	Punica granatum	5.78 ± 0.03	6.91 ± 0.03	7.48 ± 0.08	8.9 ± 0.01	11.22 ± 0.04	13.52 ± 0.1	12.14 ± 0.04	17.42 ± 0.02
19	Zingiber officinale	7.59 ± 0.03	4.46 ± 0.05	8.39 ± 0.08	6.41 ± 0.03	14.19 ± 0.03	8.61 ± 0.04	14.86 ± 0.04	12.83 ± 0.02
20	Ocimum tenuiflorum	2.39 ± 0.01	2.66 ± 0.06	3.23 ± 0.04	4.19 ± 0.03	4.79 ± 0.03	4.53 ± 0.02	6.56 ± 0.06	8.39 ± 0.04
21	Curcuma longa	4.11 ± 0.03	NA	4.41 ± 0.06	3.39 ± 0.01	8.19 ± 0.03	NA	8.77 ± 0.05	6.82 ± 0.02
22	Aloe Vera	NA	NA	NA	NA	NA	NA	NA	NA
23	Cocos nucifera L	4.78 ± 0.02	NA	NA	5.96 ± 0.07	8.59 ± 0.02	NA	NA	10.35 ± 0.04
24	Carica papaya L	4.81 ± 0.03	NA	5.74 ± 0.05	6.39 ± 0.02	8.6 ± 0.02	NA	10.72 ± 0.06	12.39 ± 0.03
25	Sapindus saponaria	NA	NA	NA	NA	NA	NA	NA	NA
26	Olea europaea	6.11 ± 0.02	5.21 ± 0.02	4.49 ± 0.06	5.37 ± 0.02	11.45 ± 0.04	10.24 ± 0.04	9.43 ± 0.05	10.45 ± 0.04
27	Pisum sativum	9.37 ± 0.02	5.42 ± 0.02	4.11 ± 0.03	10.17 ± 0.03	18.58 ± 0.03	9.35 ± 0.05	8.23 ± 0.05	22.16 ± 0.04
28	Magifera indica	6.38 ± 0.02	NA	8.81 ± 0.06	11.19 ± 0.02	13.89 ± 0.03	NA	16.3 ± 0.08	19.61 ± 0.02
29	Prunus armeniaca	7.91 ± 0.03	NA	NA	8.63 ± 0.03	14.82 ± 0.02	NA	NA	16.45 ± 0.05
30	Secale cereale	NA	NA	NA	NA	NA	NA	NA	NA
31	Solanum lycopersicu	3.98 ± 0.06	4.12 ± 0.05	4.88 ± 0.05	5.15 ± 0.04	6.49 ± 0.03	6.84 ± 0.04	8.63 ± 0.05	9.82 ± 0.02
32	Citrus sinensis	NA	NA	4.85 ± 0.04	5.47 ± 0.03	NA	NA	8.23 ± 0.05	10.45 ± 0.04
33	Psidium guajava	7.21 ± 0.03	NA	NA	6.15 ± 0.05	13.55 ± 0.04	NA	NA	12.36 ± 0.05
34	Areca catechu	7.83 ± 0.02	5.79 ± 0.03	4.29 ± 0.03	8.21 ± 0.03	15.36 ± 0.03	10.44 ± 0.04	9.23 ± 0.05	14.81 ± 0.01
35	Cucurbita maxima	7.11 ± 0.02	8.11 ± 0.03	7.59 ± 0.02	6.39 ± 0.02	13.82 ± 0.02	14.54 ± 0.04	12.63 ± 0.05	11.84 ± 0.03
36	Citrullus lanatus	4.88 ± 0.02	5.72 ± 0.18	6.51 ± 0.05	5.56 ± 0.04	9.19 ± 0.02	11.24 ± 0.04	13.53 ± 0.05	10.16 ± 0.04
37	Helianthus annuus	5.68 ± 0.03	7.09 ± 0.03	NA	7.77 ± 0.02	10.4 ± 0.01	12.55 ± 0.04	NA	15.24 ± 0.04
38	Ficus carica	NA	NA	NA	NA	NA	NA	NA	NA
39	Amaranthus cruentus	6.25 ± 0.04	5.11 ± 0.05	4.89 ± 0.02	7.76 ± 0.03	11.48 ± 0.02	9.13 ± 0.05	8.4 ± 0.08	15.74 ± 0.04
40	Phaseolus vulgaris	7.25 ± 0.05	NA	NA	NA	14.35 ± 0.24	NA	NA	NA
41	Raphanus sativus	7.1 ± 0.08	5.85 ± 0.07	5.31 ± 0.02	7.8 ± 0.04	12.38 ± 0.05	10.43 ± 0.05	10.27 ± 0.05	14.44 ± 0.04
42	Phyllanthus emblica	7.23 ± 0.08	8.95 ± 0.05	7.38 ± 0.09	8.14 ± 0.03	15.12 ± 0.03	14.24 ± 0.04	11.59 ± 0.07	15.15 ± 0.04
43	Capsicum annuum 'C	NA	NA	NA	NA	NA	NA	NA	NA
44	Cuminum cyminum	7.6 ± 0.01	5.7 ± 0.03	NA	6.75 ± 0.04	14.15 ± 0.05	9.8 ± 0.08	NA	12.42 ± 0.02
45	Coriandrum sativum	7.51 ± 0.05	6.57 ± 0.47	6.18 ± 0.06	8.65 ± 0.06	14.67 ± 0.02	11.33 ± 0.05	10.53 ± 0.05	16.44 ± 0.04
46	Medicago sativa	7.14 ± 0.03	6.81 ± 0.03	5.56 ± 0.04	8.1 ± 0.01	13.2 ± 0.01	12.35 ± 0.03	10.15 ± 0.04	14.54 ± 0.05
47	Canavalia ensiformis	8.23 ± 0.02	6.83 ± 0.06	7.88 ± 0.03	7.11 ± 0.03	15.34 ± 0.04	13.61 ± 0.07	14.47 ± 0.05	13.52 ± 0.07
48	Nigella sativa	NA	NA	NA	6.78 ± 0.02	NA	NA	NA	12.43 ± 0.03
49	Vigna unguiculata	6.7 ± 0.01	7.17 ± 0.06	6.84 ± 0.05	6.12 ± 0.05	12.59 ± 0.02	13.55 ± 0.04	12.48 ± 0.03	10.43 ± 0.03
50	*Cucurbita pepo*	NA	NA	NA	NA	NA	NA	NA	NA

REFERENCES

[1] Andrea G, Giovanna P, Silvia FN, Antimicrobial peptides: an overview of a promising class of therapeutics, *Central European Journal of Biology*, (2007), DOI: 10.2478/s11535-007-0010-5.

[2] Atousa A, Rasoul R, Giti E, Fatemeh M, Alireza G Identification and primary characterization of a plant antimicrobial peptide with remarkable inhibitory effects against antibiotic resistant bacteria; *African Journal of Biotechnology Vol. 11(40)*, (2012), pp. 9672-9676.

[3] Wojciech K, Are antimicrobial peptides an alternative for conventional antibiotics?,*Nuclear Medicine Review, Vol. 8*,(2005), No. 1, pp. 78–86.

[4] Robert N, JakubB, GrzegorzN, JustynaB, Waldemar B and Anna GJ, Plant antimicrobial peptides, Folia Microbiol, 59, (2014),181–196.

[5] Hancock REW, Sahl HG, Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. *Nat. Biotechnol.* (2006), 24(12): 1551-1557.

[6] Fernanda G, Nathália V, Patrícia A, Lorena da S. Derengowski, Ildinete Silva-Pereira and Cynthia M. K, Antibiotic development challenges: the various mechanisms of action of antimicrobial peptides and of bacterial resistance,*Frontiers in Microbiology | Antimicrobials, Resistance and Chemotherapy Volume 4, (2013)*, Article 353.

[7] Peschel, A., and Sahl, H. G, The co-evolution of host cationic antimicrobial peptides and microbial resistance. *Nat. Rev. Microbiol*. 4, (2006), 529–536. doi: 10.1038/nrmicro1441

[8] BienvenidoOJ,Varner JE, Enzymic Degradation of Starch Granules in the Cotyledons of Germinating Peas. *Plant Physiol. 44*, (1969), 886-892.

[9] Tayebeh A, Abbas A, Seyed AK, Wheat yield and grain protein response to nitrogen amount and timing, *Astralian journal of crop science AJCS 5(3)*, (2011), 330-336.

[10] Daniel Martínez-Maqueda , Blanca Hernández-Ledesma , Lourdes Amigo , Beatriz Miralles , and José Ángel Gómez-Ruiz (2013) Proteomics in Foods: Principles and Applications Chapter 2 Extraction/Fractionation Techniques for Proteins and Peptides and Protein Digestion Food Microbiology and Food Safety 2. DOI 10.1007/978-1-4614-5626-1_2, © Springer Science+Business Media New York.

[11] Yong-Su Song, Dong-Jun Seo, Woo-Jin Jung, Expression Patterns of PR proteins with Different Extract Methods during Germination of Rape Seed (Brassica napus L.). J. Plant Biol 54, (2011), 49–56.

[12] Atousa A, Roghanian R, Emtiazi G, Ghassempour A, A simple method for primary screening of antibacterial peptides in plant seeds. Iran. J. Microbiol. 3 (2), (2011), 104-108.

[13] Bhavith K P, NarayanaSwamy M, RamachandraSwamy N and Chandrashekharaiah K S, Purification and Characterization of esterase from the seeds ofCaesalpiniamimosoides, *Journal of Experimental Biology and Agricultural Sciences*, Volume – 2(6), (2014).

[14] Shazia K, Muhammad ZQ, Abida I, Zahid N, Amina S, Muhammad I, Production and purification of horseradish peroxidase in Pakistan. *International Journal of the Physical Sciences Vol. 7*(19), (2012), pp. 2706-2712.

[15] Amna KK, Sanaa BA, Purification, characterization of thermostableAmylopullulanase from Bacillus licheniformis (BS18) by using solid state fermentation (SSF), *J. Baghdad for Sci. Vol.11 (2)*, (2014).

[16] Valery B,Vladimir S, Ivan D, Obtaining Bacteriocins by Chromatographic Methods, *Advances in Bioscience and Biotechnology*, 446-451 (2014).

[17] Gurinder JK, Daljit SA, Antibacterial and phytochemical screening of Anethumgraveolens, Foeniculumvulgare and Trachyspermumammi. *BMC Complementary and Alternative Medicine*, (2009), doi:10.1186/1472-6882-9-30. -9-30.

[18] Selvamohan T, Ramadas V, ShibilaS, Kishore S, Antimicrobial activity of selected medicinal plants against some selected human pathogenic bacteria. *Adv. Appl. Sci. Res* 3(5), (2012), 3374-3381.

[19] Lowry OH, Nira JR, Lewis Farr A, Rose JR , Protein Measurement with the Folin Phenol Reagent, .*J. Biol. Chem*.193(1951) ,265-275.

[20] Miintz K, Proteases and proteolytic cleavage of storage proteins in developing and germinating dicotyledonous seeds, *Journal of Experimental Botany, Vol. 47*, (1996), No. 298, pp. 605-622.

[21] SaadSD,MirNaiman A, Hajera, Tabassum, Mazharuddin K, Studies on Antibacterial and Antifungal Activity of Pomegranate (Punicagranatum L.). *American-Eurasian J. Agric. & Environ. Sci 9 (3)*, (2010), 273-281.

[22] Poulle M, Berne J, A Proteinase from Germinating Barley, I. purification and some physical properties of a 30 kd cysteine endoproteinase from green malt, .*Plant Physiol*. (1988),1454-1460

[23] Kiminori T, Takashi O, and Takao M,Mass Transport of Proform of a KDEL-tailed Cysteine Proteinase (SH-EP)to Protein Storage Vacuoles by Endoplasmic Reticulum–derived Vesicle Is Involved in Protein Mobilization in Germinating Seeds. The Journal of Cell Biology, Volume 148,(2000), 453–463.

[24] Mitsuhashi W, Koshiba T, Minamikawa T, Separation and Characterization of Two Endopeptidases from Cotyledons of Germinating Vignamungo Seeds, *Plant Physiol* 80, (1986), 628-634

[25] A. Joseph D'ercole, Alan D. Stiles, And Louis E. Underwood, Medical Sciences Tissue concentrations of somatomedin C: Further evidence for multiple sites of synthesis and paracrine or autocrine mechanisms of action (insulin-like growth factor I/growth factor/growth hormone) *Proc. Natl. Acad. Sci. Vol. 81*, (1984),pp. 935-939,

[26] Satish N (2007), Medicagotruncatula Handbook version.Proteomics of Medicagotruncatula.

[27] Gallardo K, Le SignorC, VandekerckhoveJ, ThompsonRD, Burstin J. ,Proteomics of Medicagotruncatula seed development establishes the time frame of diverse metabolic processes related to reserve accumulation. Plant Physiol. 133, (2003) 664-82.

[28] W. Jordi, A. Schapendonk, E. Davelaar, G. M. Stoopen, C. S. Pot, R. De Visser, J. A. Van Rhijn, S. Gan, and R. M. Amasino, Increased cytokinin levels in transgenic PSAG12–IPT tobacco plants have large direct and indirect effects on leaf senescence, photosynthesis and N partitioning, .*Plant, Cell and Environment*,Volume23,Issue3,(2000),Pages 279–289

[29] Saima R and Azra K Isolation and characterization of peptide(s) from *pisumsativum*having antimicrobial activity against various bacteria.,*Pak. J. Bot* 43(6), (2011), 2971-2978.

[30] Bart P.H.J. Thomma, Bruno P.A. Cammue and Karin Thevissen, Plant defensins, *Planta* (2002) 216: 193–202, DOI 10.1007/s00425-002-0902-6.

[31] Portieles R, Ayra C, Borras O, Basic insight on plant defensins. Biotechnol. 23, (2006), 75–8.

[32] Avato P, Bucci R, Tava A, Vitali C, Rosato A, Bialy Z, Jurzysta M, Antimicrobial activity of saponins from Medicago sp.: structure–activity relationship. Phytoth Res 20, (2006) ,454–457.

[33] Elizabeth A. B. Emmert, Jocelyn L. Milner, Julie C. Lee, Kristie L. Pulvermacher, Heidi A. Olivares, Jon Clardy And Jo Handelsman, *Applied And Environmental Microbiology*, Vol. 64, (1998), p. 4683–4688

[34] Gao, A.-G., Hakimi, S. M., Mittanck, C. A.,Wu, Y.,Woerner, B. M., Stark, D. M., et al. (2000). Fungal pathogen protection in potato by expression of a plant defensin peptide. Nat. Biotechnol. 18, 1307–1310. doi: 10.1038/82436

[35] Vinod Kumar D, O.P. Sati, M.K. Tripathi and Ashok Kumar, Isolation, Characterization and Antimicrobial Activity at Diverse Dilution of Wheat Puroindoline Protein ,*World Journal of Agricultural Sciences 5 (3)*, (2009), 297-300.

[36] Naseer U, Hajera T, Mir naiman A, Kritika P, Evaluation of antibacterial activity of five selected fruits on bacterial wound isolates. *Int J Pharm Bio Sci*, (2012), (P) 531 – 546.