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ORIGINAL ARTICLE

Isolation and characterization of potential food preservative peptide from *Momordica charantia* L.

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KEYWORDS

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Abstract Antimicrobial agents (AMAs) also known as preservatives are being used to keep food safe and unspoiled from microorganism. These preservatives, derived from plant sources or synthesized chemically are mostly non-proteinaceous AMAs. Less attention has been given to proteinaceous AMAs which may be used as preservative in food. Thus, there is a need to explore proteinaceous AMAs having antimicrobial activity with potentials to be used as an alternative to the presently used preservatives which are mostly synthetic. Therefore, the present study was carried out systematically to isolate and characterize peptides having antibacterial activity from different parts of bitter melon (*Momordica charantia* L.). Crude aqueous extracts of seeds, pulp and skin were prepared in phosphate buffer saline (PBS) and antibacterial activity was checked on Luria Bertani (LB) broth agar plates against a number of bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Pseudomonas aeruginosa*. Since antimicrobial activity was observed only in seeds therefore, seed extract was used for peptide(s) precipitation with 75% ammonium sulfate solution and purification by gel filtration chromatography (GFC) using Sephadex G-100. Antimicrobial activity was also checked in dissolved ammonium sulfate precipitate and GFC peak fractions (1–4). Further, homogeneity and molecular mass of GFC pooled fractions along with crude extract and dissolved ammonium sulfate precipitate were determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). One peptide with molecular mass ~10 kDa with antimicrobial activity was obtained from peak 3. The purified peptide was stable at 4–50 °C, active between 5–7 pH and inactivated by trypsin and proteinase K. Based on minimum inhibition concentration (MIC) values of purified peptide *S. aureus* was found to be the most sensitive strain. The use of this strain with minced meat showed significant viable count decreased i.e. from 8.0 to 3.77 log CFU with $P < 0.001$ at concentration of 40 μg peptide. The results of our study suggest

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that antibacterial peptide obtained from seeds of *M. charantia* L. may be used as an alternative natural biopreservative source for minced meat products. However, further studies need to be carried out to check the effect of this peptide on more microorganisms, characterization and its toxicological analysis.

1. Introduction

From ancient times, a large number of plants and herbs have been used in traditional medicines against bacterial infection (Cook et al., 2000; Martin and Ernst, 2003; Okoli and Iroegbu, 2004). The uses of plant and herb extracts as antimicrobial agents in food and soft drinks have also been reported for centuries (Friedman et al., 2002; Gulmez et al., 2006). Due to potential toxicity of chemical food preservatives, there has been increased demand for food preservatives from natural sources. This has led researchers and food processors to come across natural food additives with a wide range of antimicrobial activities. As a result today plant antimicrobial products have acquired importance in food system to retard bacterial and fungal growth (Souza et al., 2005a,b). Consumer interest is also increasing in consumption of food products having natural ingredients. Several studies have reported the use of natural molecules i.e. organic acids, peptides and essential oils in past several years (Barreteau et al., 2004).

Antimicrobial agents used as preservatives, are chemical compounds that protect food from spoilage by inhibiting growth of pathogenic microorganisms and increases shelf life. Naturally occurring antimicrobial compounds isolated from animal, plant and microbial sources can be used alone or in combination with other approved antimicrobial preservatives (Davidson, 2006). Studies, however, carried out by Akinpelu (2001) and Souza et al. (2005a,b), pointed out that resistant microorganisms are appearing because of extensive and random use of chemical preservatives which can lead to an increase in incidences of food borne diseases.

Besides chemical compounds, in nature, antimicrobial peptides (AMPs) are commonly distributed in all life forms and play an important role in non-specific host defense system. Initially peptides from the skin of frog and lymph of insects were depicted to kill bacteria *in vitro*. Now more than 800 AMPs have been described in many living organisms including insects, plants, amphibians and mammals (Rydlo et al., 2006). A number of plant proteins have demonstrated activity against bacteria and fungi, which usually play a key role in plant defense, protecting the plant host from phytopathogens. These proteins have been classified into groups and families according to their function and structural similarities. Main proteins include cyclotides, defensins, lipid transfer proteins (LPTs), lectins, pathogenesis-related proteins, chitin-binding proteins, ribosome-inactivating proteins and digestive enzyme inhibitors (Pelegrini et al., 2008).

Momordica charantia L. commonly known as bitter melon, member of the Cucurbitaceae family is a tropical plant currently distributed across the globe and found to have therapeutic properties like anti diabetic, antioxidant, antiviral and antineoplastic activities (Gover and Yadav, 2004; Beloin et al., 2005; Semiz and Sen, 2007; Paul and Raychaudhuri, 2010). Although numerous constituents of bitter melon have been proposed to have therapeutic properties but a polypeptide (polypeptide p) isolated from seeds has been focused more because of its hypoglycemic activity (Khanna et al., 1981). Another protein MAP30 isolated from bitter melon, recombinant MAP 30 and proteolytic fragments of MAP30 was found to exhibit antiviral, anti HIV and antineoplastic activities in several *in vivo* and *in vitro* studies (Lee-Haung et al., 1990, 1995a,b; Huang et al., 1999; Basch et al., 2003). The extracts of bitter melon also appear to inhibit the growth of numerous gram-negative and gram-positive bacteria including *Escherichia coli*, *Salmonella typhi*, *Shigella dysenteriae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptobacillus moniliformis*, *Streptococcus pneumoniae*, and *Helicobacter pylori*, and parasitic organisms *Entamoeba histolytica* and *Plasmodium falciparum* (Anonymous, 2007). Naturally occurring microbial inhibitors have been recovered from a variety of food materials including onions, garlic, fruits, vegetables, cereals and spices. Many of these antimicrobials contribute to the foodstuff's natural resistance to deteriorations (Kivanc and Kunduhoglu, 1997). A renewed interest in natural preservation appears to be stimulated by present food safety concerns, growing problems with microbial resistance and a rise in production of minimal processed food joined with green image policies of food industries (Suhr and Nielson, 2003). Taking into account, the significance of naturally occurring antimicrobial peptides, the present study was designed to isolate, characterize and determine potentials of peptides having antibacterial activity from different parts of *M. charantia* L. against common food pathogens.

2. Materials and methods

2.1. Collection of plant and microorganisms

M. charantia was collected from the local markets of Rawalpindi and indentified by a plant taxonomist, Department of Botany, PMAS-Arid Agriculture University Rawalpindi, Pakistan. The vegetable was brought to the laboratory and rinsed with water to remove the soil particles. Cultures of food related bacteria were obtained from the Depository of Biotechnology Laboratory, Department of Biochemistry, PMAS-Arid Agriculture University Rawalpindi to be used for determination of antimicrobial activity. These include *E. coli*, *S. aureus*, *S. typhi* and *P. aeruginosa*. Luria Bertani (LB) medium was used for culturing the above mentioned bacteria.

2.2. Protein extraction and partial purification

Protein extraction and partial purification were essentially carried out by the method described by Rehman and Khanum (2011) with some modifications. The seeds, skin and pulp of

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M. charantia; each 20 g were crushed in pestle and then blended with 100 ml phosphate buffer saline (PBS), pH 7.4 in an electric blender. The homogenates were frozen and thawed three times and then centrifuged at 10,000 rpm for 20 min at 4 °C. The supernatants of seeds, skin and pulp were used for determination of antibacterial activity. These supernatants were then precipitated out with 75% ammonium sulfate solution. The precipitated solutions were centrifuged at 10,000 rpm for 30 min at 4 °C. The pellets were dissolved in 1 ml of deionized water by gentle vortexing. The dissolved ammonium sulfate precipitate and supernatant were dialyzed in a dialysis tube MWCO 3500 Da against distilled water with several changes. The protein concentration was estimated in crude extract, dissolved ammonium sulfate precipitate and supernatant.

2.3. Gel filtration chromatography and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE)

The samples of both dissolved ammonium sulfate precipitates and supernatant of seeds, pulp and skin were used for estimation of antimicrobial activity. Since activity was observed only in dissolved ammonium sulfate precipitate of seeds, therefore, dissolved ammonium sulfate precipitate was further purified by gel filtration chromatography using column of Sephadex G-100 with 0.02 M sodium acetate buffer (pH 5). The column was eluted with the same buffer at the flow rate of 1 ml/min. The absorbance of collected fractions was monitored at 280 nm. The homogeneity and molecular mass of pooled fractions along with crude extract and dialyzed ammonium sulfate precipitates were run on 15% SDS-gel (Laemmli, 1970). The separating gel was placed in Coomassie blue R-250 staining solution on a shaker for 3 h. Excess dye was removed by destaining with methanol:acetic acid:water (30:60:10 v/v). The approximate molecular mass of fractions having antimicrobial activity was determined by plotting molecular weight of standard proteins versus Rf.

The protein concentration in samples (crude extract, dissolved ammonium sulfate precipitates and in its supernatant, fractions of gel filtration chromatography) was estimated by the method of Bradford (1976) using bovine serum albumin as a standard. These were then saved for further studies.

2.4. Antimicrobial assay

2.4.1. Sub-culturing of bacterial strains

Isolated bacterial colonies grown of Luria Bertani (LB) broth agar plate were picked up to grow in 3 ml LB medium overnight at 37 °C. From overnight grown culture 100 µl culture was taken, transferred to 50 ml of fresh LB medium and grown at 37 °C under shaking (250 rpm/min). The bacterial growth was monitored by measuring optical density at 600 nm. The size of inoculums was calculated from optical density (0.4 OD_{600nm} is equal to 10^8 colony forming unit (CFU)/ml) (Barreteau et al., 2004).

2.4.2. Disc diffusion method

The antimicrobial activity of extracts of seed of *M. charantia* (crude, ammonium sulfate precipitated sample, and chromatographic pooled fractions) was determined using the disc diffusion method described by Bauer et al. (1996).

2.4.3. Minimum inhibitory concentration in culture media

Minimum inhibitory concentration (MIC) values of peptide isolated from seed was determined with broth dilution assay as described by Wang (2003). The 10^8 CFU/ml *S. aureus* cultures were inoculated into LB broth containing 0–200 µg/ml antimicrobial protein preparation and incubated at 37 °C for 4 h.

2.4.4. Statistical analysis

Mean zone of inhibition in millimeter (mm) and standard deviation of the triplicate incubations were calculated by using Excel statistical software (2007).

2.5. Effect of various temperatures, pH and proteolytic enzymes

The peptide activity was determined at different temperatures; 0 °C, 4 °C, 30 °C, 40 °C, 50 °C, 60 °C ,70 °C, 80 °C and pH; 4, 5, 6, 7 and 8 by heating at different temperatures for 15 min and then bioassayed. Similarly pH effect on isolated peptide was determined by changing pH from 4 to 8. The effect of two enzymes proteinase K (Fermentas® cat #EO0491) and trypsin–EDTA 1X (GIBCO® cat #25300-054) on antimicrobial activity of purified peptide was also checked. Peptide sample of 3–20 µg/10 µl was mixed with 0.02 M Tris–HCl, pH 6.8, 1–2 U/2 µl of enzyme was added into the mixture with total volume of 100 µl and kept for 5 min. Enzyme activity was stopped by heating the solution in a thermo mixer for 5 min. This solution (30 µl) was applied on to the disc to see the effect of different enzymes on bioactivity of the isolated peptides.

2.6. Effect of purified peptide on minced meat inoculated with S. aureus

The antimicrobial activity of purified peptide on minced meat was checked by the method of Wang (2003). Bacterial suspension of *S. aureus* was centrifuged at 13,000 rpm and pellet was resuspended in 10 mM sodium phosphate buffer, pH 7.4. One gram of minced meat was washed with 70% ethanol, rinsed with deionized water and mixed with 1 ml buffer containing different concentrations of antimicrobial peptide i.e. 0–200 µg/g. Minced meat without antimicrobial peptide was taken as control. Culture of *S. aureus* (100 µl) was inoculated in this mixture and incubated overnight at 37 °C. After overnight incubation culture was diluted 10-fold serially and streaked on LB agar plates. Cell counts were determined by counting the colonies after plates were incubated overnight at 37 °C.

3. Results and discussion

3.1. Antimicrobial activity

Antimicrobial activity of crude extract, dissolved ammonium sulfate precipitates of seeds, skin and pulp were investigated on LB agar plates against various food pathogens (*S. aureus*, *E. coli*, *S. typhi*, and *P. aeruginosa*) in triplicate. The results are given in Table 1. Among the tested microorganisms, the crude seed extract showed highest activity against *E. coli* (6.66 ± 1.15) followed by *S. typhi* (4.33 ± 0.577) and *S. aureus* (3.66 ± 0.577) and lowest activity against *P. aeruginosa*

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Table 1 Antibacterial activity of different samples of *M. charantia* seeds against various food related bacteria. Values are the mean ± SEM of triplicate determination from a representative experiment of the three experiments performed. Clear zone is taken in mm excluding 6 mm disc. The sample applied per disc was 20 µg/30 µl. Chloramphenicol (34 mg/ml) used as control.

Section 1.01	Gram positive	Gram negative		
Protein contents 20 µg/30 µl	*S. aureus*	*E. coli*	*S. typhi*	*P. aeruginosa*
Crude seed extract	3.66 ± 0.577	6.66 ± 1.00	4.33 ± 0.577	2.00 ± 0.00
Solubilized $NH_4(SO_4)_2$ precipitate	8.00 ± 1.00	9.33 ± 0.577	6.63 ± 0.577	2.66 ± 0.50
Fraction (Peak 3)	11.66 ± 0.577	12.00 ± 1.15	9.00 ± 0.577	3.33 ± 0.577
Cam^{34} 102 µg/30 µl	25	25	23	20

(2.00 ± 0.00). The dissolved ammonium sulfate precipitates of seeds showed highest activity against *E .coli* (9.33 ± 0.577) followed by *S. aureus* (8.00 ± 1.00), *S. typhi* (6.63 ± 0.577) while lowest activity against *P. aeruginosa* (2.66 ± 0.5). The dissolved ammonium sulfate precipitates have shown better activity as compared to the crude extracts of seeds, thereby indicating that the protein(s)/peptide(s) isolated from the crude extract has been partially purified.

Roopashree et al. (2008) also screened antimicrobial activity of *M charantia* seeds in aqueous, petroleum ether, methanol and ethanol extracts against *S. aureus*, *E. coli* and *P. aeruginosa*. The results of this study showed that aqueous extract has higher antibacterial activity particularly against *S. aureus* i.e. 22 ± 0.64 which is comparable to our study. The antimicrobial activity was also checked in skin and pulp of *M. charantia* L. in this study. However no activity was observed in these parts of vegetable. The results are in contrast to a study carried out by Saeed and Tariq (2005) who observed significant antimicrobial activities in aqueous extracts of skin and pulp of *M. charantia* against a number of microorganisms like *E. coli*, *P. aeruginosa*, *S. typhi*, *K. pneumonia*, *Salmonella paratyphi A*, *Salmonella paratyphi B*, *Proteus vulgaris*, *S. dysenteriae*, *Yersinia enterocolitica*, *Proteus mirabilis*, *Enterobacter aerogenes* with average zone of inhibition of 16.16 mm ± 2.02 mm and 15.88 mm ± 2.24 mm, respectively. The reason could be the use of different varieties of *M. charantia* in our case.

3.2. Chromatographic profile of dissolved and dialyzed ammonium sulfate precipitates of seeds

The dissolved ammonium sulfate precipitates of seeds were then purified by gel filtration column chromatography using Sephadex G-100. The elution profile of various peptide(s) is shown in Fig. 1. Four peaks were obtained ranging from high molecular mass to low molecular mass peptide(s). All peaks were lyophilized and checked for antimicrobial activity, only peak 3 showed activity against *E. coli* (12 ± 1.15), *S. aureus* (11.66 ± 0.577), *S. typhi* (9 ± 0.577) and *P. aeruginosa* (4.3 ± 0.577). Chromatographic fraction showed approximately two and one fold increase in activity than crude and dissolved ammonium sulfate precipitates respectively indicating the further purification of peptide. In several other studies using different plant or animal extracts, peptide(s) were isolated and checked for antimicrobial activity. For example, Dahot (1998) obtained three fractions from leaf extracts of *M. oleifera* on Sephadex G-25 column chromatography. Fraction P1, P2 and P3 showed strong inhibitory activity against *E. coli*, *S. aureus*, *Bacillus subtilis* and *Klebsiella aerogenes*.

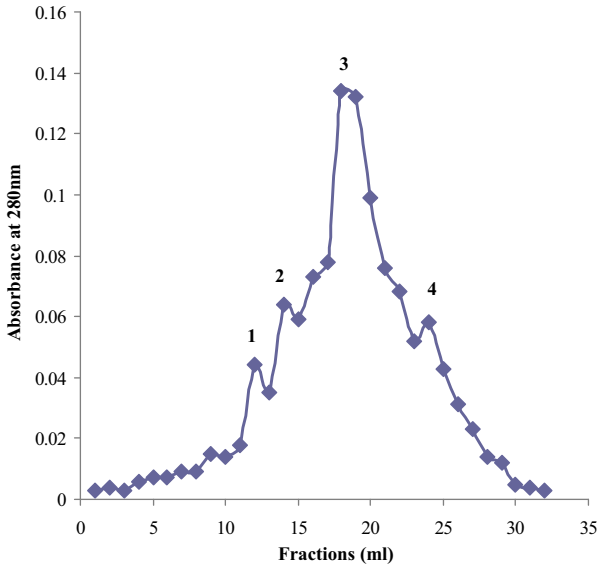

Figure 1 Gel filtration chromatography of dissolved and dialyzed ammonium sulfate precipitates obtained from aqueous crude extract of seeds of *M. charantia* L. Peaks are marked as 1–4.

Fraction P2 showed significant zone of inhibition against *Aspergillus niger*, while fractions P1, P2 and P3 were not found effective against the growth of *Klebsiella pneumoniae*, *Aspergillus fumigatus*, *Aspergillus flavus and Penicillium expansum*. Similarly Hou et al. (2007) purified an antibacterial peptide from housefly larvae by using Sephadex G-15, five peaks were obtained but only peak 1 showed antibacterial activity against *E. coli* and *S. aureus*. Recently, Rehman and Khanum (2011) isolated two peptides from seed and two peptides from pod of *Pisum sativum* L. (garden pea) having molecular weight ~19 kDa, ~22 kDa, ~10 kDa and ~11 kDa, respectively. These peptides have shown strong antimicrobial activity against a number of bacteria.

3.3. SDS–PAGE

The crude extract, dissolved and dialyzed ammonium sulfate precipitates and purified peptide of seeds were subjected to 15% SDS–PAGE along with molecular mass of 14.4–116.0 k Da. The pattern of protein separation on SDS–PAGE was found to be almost same in samples of crude extract and dialyzed ammonium sulfate precipitates while purified peptide obtained by gel filtration chromatography (Peak 3) appeared as a single band with a molecular weight

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of ~10 kDa. The result of SDS–PAGE is shown in Fig. 2. Similar to this study, Parkash et al. (2002) isolated antimicrobial peptide with molecular weight ~9.7 kDa from bitter gourd seeds by affinity chromatography, ion exchange chromatography and gel filtration chromatography. In another study carried out by Diz et al. (2006) they isolated ~10 kDa antimicrobial peptide from chili pepper seeds while Hou et al. (2007) purified an antimicrobial peptide with molecular weight ~8.0 kDa. It was obtained as a single band on SDS–PAGE after purification by Sephadex G-15 and HPLC. Xia and Ng (2005) also isolated antimicrobial protein with molecular weight ~13 kDa from garlic bulbs. All these studies including ours showed that antimicrobial peptides purified by different chromatographic methods and SDS–PAGE have low molecular mass.

3.4. Effect of various temperatures, pH and enzymes on antimicrobial activity of purified peptide

The effect of various temperatures on the antimicrobial activity of purified peptide (Peak 3) of seeds was determined against *S. aureus*, *E. coli* and *S. typhi*. The results are shown in Fig. 3. Peptide exhibited different activities at different temperatures, for example, the activities shown by peptide against *S. aureus* and *E. coli* were approximately constant i.e. 10.33 ± 0.577 and 10.66 ± 0.577 respectively at temperatures 4 °C, 30 °C, 40 °C and 50 °C. A decrease of about 40–54% in activities was observed at temperatures 60 °C and 70 °C against *S. aureus*: 6.66 ± 0.577 and 6.00 ± 0.50 and *E. coli*: 6.00 ± 0.50 and 4.66 ± 0.10 respectively. In case of *S. typhi*, the activity was constant i.e. 8.55 ± 0.70 at 4 °C, 30 °C and 40 °C. This activity was then declined at temperatures 50 °C, 60 °C and 70 °C with 40% (6 ± 0.00), 65% (3.66 ± 0.50) and 70% (3 ± 0.70) respectively. The peptide did not show any activity at 0 °C and complete lose of activity was observed at 80 °C against all tested bacteria. These results showed that the peptide was more effective at low and moderate temperatures i.e. 4 °C or 50 °C.

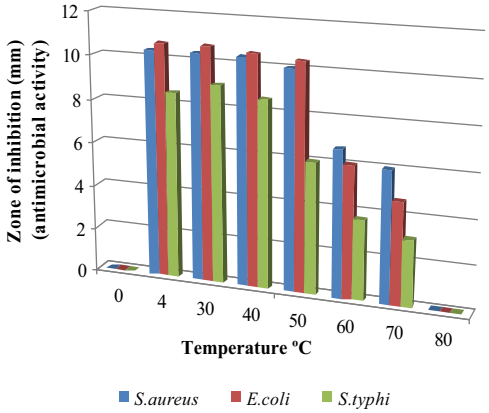

Figure 3 Effect of various temperatures on antibacterial activity of purified peptide against ■ *S. aureus*; ■ *E. coli*; ■ *S. typhi*.

These results support the view that specific conformation of peptide is needed for its function. Any change in conformation leads to the loss of function i.e. antibacterial activity. It is evident from the data that high temperature may have changed the specific configuration of peptide necessary for its antibacterial activity. The data of our study are further supported by Girish et al. (2006) who observed that heating of non-toxic glycoprotein from *Withania somnifera* up to 60 °C for 10 min did not alter the antimicrobial property, whereas the temperature above 70 °C was found to be inhibitory to antimicrobial activity. Similarly, a study carried out by Ngai and Ng (2004) reported that the functional activity of polypeptide isolated from Chinese white cabbage seeds was stable between 10 and 40 °C.

The effect of pH on the antibacterial activity of purified peptide (Peak 3) of seeds was checked against the above mentioned microorganisms. With pH change, the antibacterial activity of peptide has also been changed. At pH 4 and 8 peptide was inactive against all the microbes tested. Peptide showed a sharp increase in activity against *S. aureus* with

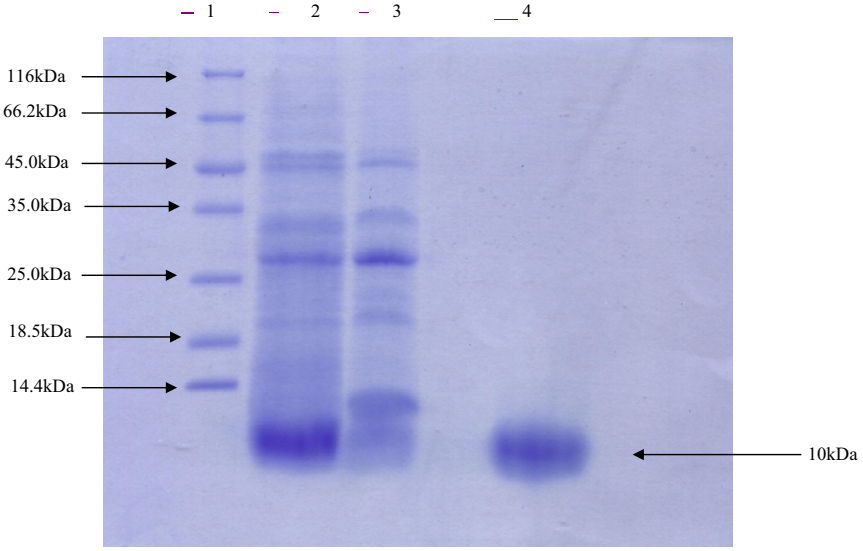

Figure 2 SDS–polyacrylamide gel electrophoresis of various samples of seeds of *M. charantia* L. Lane 1, Molecular weight marker #0431; Lane 2, Crude Extract of seed; Lane 3, Dissolved and dialyzed ammonium sulfate precipitates; Lane 4, Purified peptide.

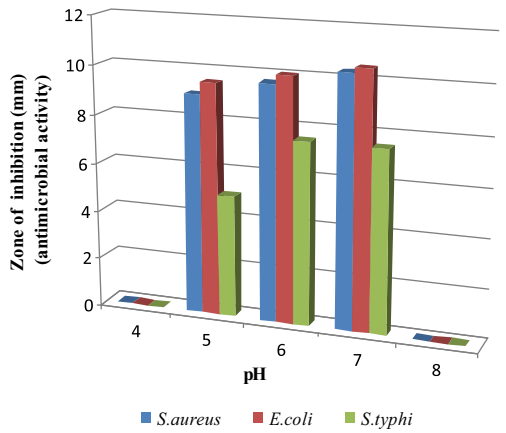

Figure 4 Effect of various pH on antibacterial activity of purified peptide against ■ *S. aureus*; ■ *E. coli*; ■ *S. typhi*.

9 ± 0.00 and *E. coli* with 9.5 ± 0.70 at pH 5 while a slight increase at pH 6 and pH 7 i.e. 9.66 ± 1.57 and 10 ± 2.82. In case of *S. typhi* the pattern of antibacterial activity was different. The activity increased at pH 5 i.e. 5 ± 0.00 then pH 6 i.e. 7.5 ± 0.707 thereafter remained constant at pH 7 (Fig. 4). These results show that activity of purified peptides is pH-dependent and varies for different microbes. The results further revealed that purified peptide is stable between narrow ranges of pH 5–7 which is similar to the results obtained by Rehman and Khanum (2011) for peptides purified from *P. sativum*. However, it is contrast to the broad range antimicrobial substances obtained from *Bacillus cereus* that has high antimicrobial activity in pH range of 2.0–9.0 (Risoen et al., 2004). Similarly the stability of polypeptide isolated from Chinese white cabbage was retained between pH 4–11 as described by Ngai and Ng (2004). The proteinaceous nature of isolated peptide (Peak 3) was confirmed with use of proteolytic enzymes i.e. proteinase K and trypsin against *S. aureus* *E. coli* and *S. typhi* which proved the evidence that proteolytic enzymes did change the specific structure of peptides necessary for their antimicrobial activity by hydrolyzing it. The study of Wang (2003) supports our results by providing evidence that proteolytic enzymes: pepsin, trypsin, and chymotrypsin drastically decline the bactericidal activities of antimicrobial protein.

3.5. Effect of purified peptide on apparent proliferation of bacteria

The active chromatographic fraction was then used for determination of minimum inhibitory concentration (MIC) in culture media. It was observed that the MIC decreases with the increase of concentration of peptide. Therefore, MIC of *S. aureus* decreased by 21% at concentration of 40 µg and 57% decrease occurred at 200 µg (Fig. 5a). In case of *E. coli* bacterial growth dropped down to 25% at 40 µg and 49% at 200 µg (Fig. 5b) while less decrease was observed in case of *S. typhi* and *P. aeruginosa*. *S. typhi* proliferation decreased only by 7% at 40 µg and 29% at 200 µg (Fig. 5c). Similarly *P. aeruginosa* proliferation decreased 8% at 40 µg and 18% at 200 µg

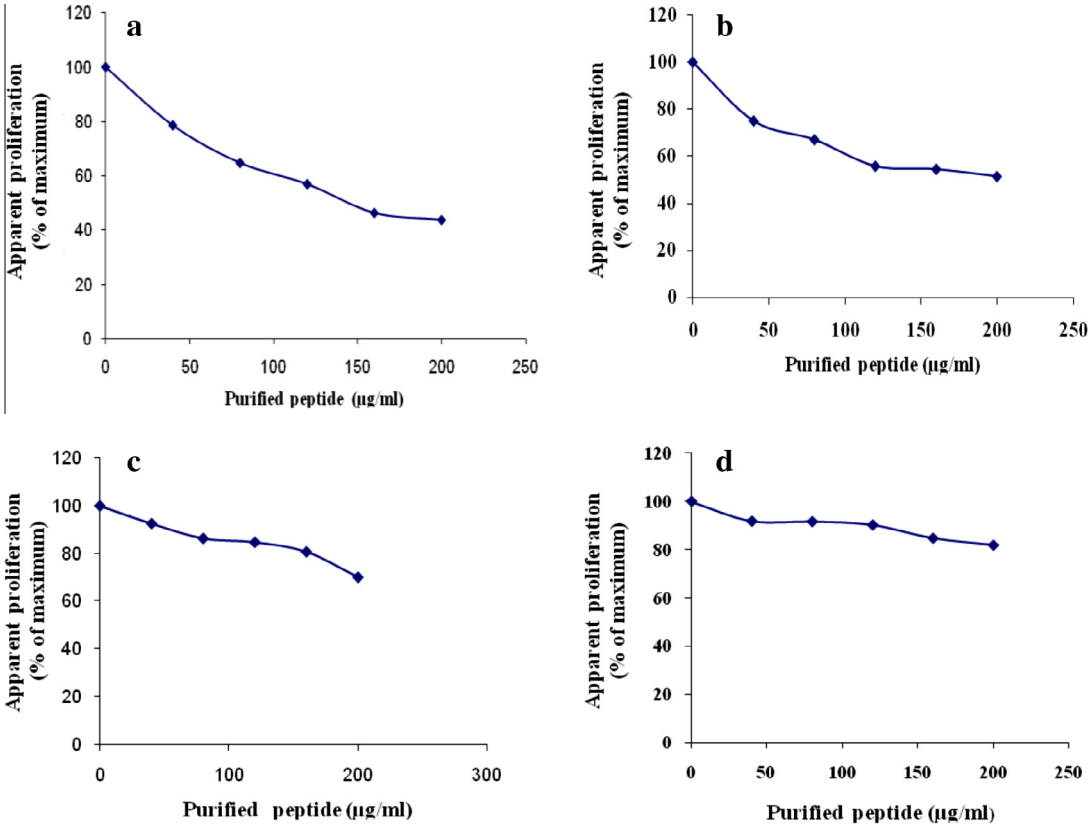

Figure 5 Effect of various concentrations of purified peptide on the apparent proliferation of (a) *S. aureus*, (b) *E. coli*, (c) *S. typhi* (d) *P. aeruginosa*.

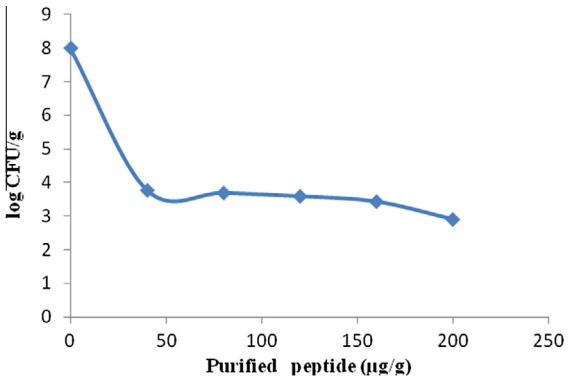

Figure 6 Effect of various concentrations of purified peptide on the apparent proliferation of *S. aureus* inoculated in fresh minced meat.

(Fig. 5d). This shows that purified peptide is more effective against *S. aureus* and *E. coli* as compared to *S. typhi* and *P. aeruginosa*. Effect of various concentrations of antimicrobial protein preparation on the apparent proliferation of *S. aureus* and *E. coli* was also studied by Wang (2003). In which it was noted that bactericidal activity of antimicrobial protein preparation on the test cultures increased with concentration. Accordingly test bacteria exposing to the 40 µg/ml antimicrobial protein preparation in culture media caused a decrease in 40–60% MIC. The MIC of test microorganisms was completely inhibited after exposing to 120 µg/ml antimicrobial protein preparation. In another study by Hou et al. (2007), they showed peptide Hf-1 activity against *E. coli*, *S. dysenteriae* and *S. aureus* with MIC ranging from 18 to 72 µg/ml. *S. aureus* was the most sensitive with the MIC of 18 µg/ml, followed by *Salmonella typhimurium* and *B. subtilis* with MIC of 36 and 72 µg/ml, respectively while *E. coli* Jm109, was inhibited at MIC of 108 µg/ml.

3.6. Effect of purified peptide on viable count formation of S. aureus

In order to check the effectiveness of this peptide as biopreservative agent, minced meat was inoculated with *S. aureus* which was selected based on MIC results. Thus the effect of purified peptide on viable count formation of *S. aureus* culture inoculated with minced meat is shown in Fig. 6. Growth pattern indicated that viable count formation decreases with increasing concentration of peptide. Similarly Fig. 6 shows clearly that viable count sharply decreased at concentration of 40 µg i.e. from 8.00 to 3.77 log CFU ($P = 0.01$). Thereafter a marginal decrease was noted with 160 µg and 200 µg with P values 0.002 and 0.005. When obtained data were compared with other studies it was observed that antimicrobial activity of purified fraction of *M. charantia* was not very high. However, in a study carried by Wang (2003) they noticed that 20 µg/g antimicrobial protein preparation could decrease in 1.3 log CFU of *S. aureus and* 160 µg/g antimicrobial protein preparation completely inhibited the growth of test cultures in 24 h. The cause of less effect of purified peptide of seeds of *M. charantia* L. cannot be explained at this point. More studies are needed to be carried out to address this discrepancy.

4. Conclusion

Based on the results of this study, it is concluded that seeds of *M. charantia* L. contains a low molecular mass peptide having ~10 kDa. This peptide may be used as an alternative bio-preservative agent to control the growth of bacteria in fresh minced meat. However, further study is required to enhance the effectiveness of this peptide.

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