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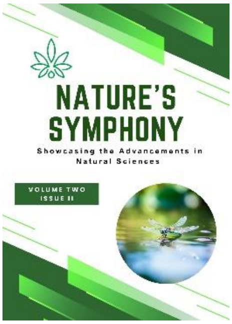

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Research Article

# Strong Cation Exchange Chromatography-based Bioactivity of Cationic Peptides from *Solanum lycopersicum* (Tomato) Under Salt Stress

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***Keywords:***
Cationic peptides; Antibacterial; Saline stress; Mass Spectrometry; Abiotic stress

## ABSTRACT

The rise in antibiotic resistance in pathogens has led to an exponential rise in infectious diseases. It has alarmed the need for new antibiotics with precise actions against pathogens and ability to target multi directionally to encounter disease causing agents. Cationic peptides are also known as natural antibiotics and play a fundamental role in recruiting and promoting agents of innate as well as adaptive immune systems. These are produced constitutively as well as under the influence of biotic and abiotic stresses. In the current study, *Solanum lycopersicum* (tomato) was induced to express cationic proteins/ peptides under salt stress. Proteins up to molecular mass of 10 kDa were isolated and fractionated by cation exchange chromatography followed by antibacterial assays. The results showed that tomato plants could tolerate salt stress of 200 mM, where 100 mM of stress provoked more antibacterial peptides/ proteins. Strong cation exchange fractionation separated enormous positively charged peptides which showed high percentage of antibacterial activity. The prolific antimicrobial peptides authenticate the tomato plant as a promising candidate for pharmaceuticals, nutraceuticals and drug discovery.

## 1. INTRODUCTION

All-embracing use of antibiotics as frontline defense strategy has resulted in multidrug resistant pathogens. Typically, commercialized antibiotics target cellular mechanisms which bacteria efficiently escape from and develop resistance owning to point mutations, anti-antibiotic enzymes synthesis and synchronized efflux pumps (Chiș *et al.*, 2022). Along with the acquired resistance, the formation of bacterial biofilms as a survival strategy exponentiate therapeutic failures (Lee *et al.*, 2011). These biofilms are composed of extracellular matrix of polysaccharides, proteins, teichoic acids and DNA and help protection from antibiotics, antibodies (immune response), biocides and disinfectants (Otto, 2008; Høiby *et al.*, 2010). This rendered resistance has panicked researchers to explore alternatives to fight against increasing infections.

Two disasters, including acquired resistance by manipulated response of cellular agents and extracellular protection via biofilm production has shaken therapeutic industry. The rise in multidrug-resistant strains has significantly increased infectious diseases over the past few decades, highlighting the urgent need to develop new therapies with novel mechanisms to treat and eliminate these infections. (Cantón and Morosini, 2011; Rolain *et al.*, 2012). Ribosomal invented antimicrobial peptides called natural antibiotics are trusted as potent source for the development of multi-dimensional therapeutics in need because they exhibited rapid killing and broad spectrum activities against bacteria, fungi (Schneider *et al.*, 2010), enveloped viruses, parasites and cancerous cells. They are produced fundamentally from all types of living organisms as part of the innate immune system.

Plants' immune systems comprise of local or systemic production of reactive oxygen species, proteins and secondary metabolites with bioactive prospective. They are also dependent on antimicrobial proteins which are produced constitutively or tempted after pathogen attack both locally at infected site and systemically. Plant antimicrobial proteins (PAMPs) are mostly cationic in nature from 12 to 50 amino acids diversified in both primary and secondary structures (Hancock and Diamond, 2000; Hancock, 2001). PAMPs are classified into defensins, thionins, lipid transfer proteins, thaumatins families and have highly assorted mechanisms for efficient killing of bacteria and fungi species. PAMPs interfere with negatively manufactured cell wall (Brogden, 2005), destroy it or use it for cellular targeting of DNA, RNA or protein to inhibit replication, transcription and translation and more destructively onrush bacterial cytokinesis (Hancock and Diamond, 2000; Hancock, 2001). Multidirectional modes of actions made PAMPs reliable candidates for the production of new therapeutics and trusted as competent squaddies against pathogens.

Plants are prone to many biotic and abiotic stresses which are sensed and perceived by external signals and translated into the cellular environment by phytohormones like abscisic acid, ethylene, jasmonic acid and salicylic acid to counter effect the stress by activation, co-activation or de-activation in modulation cross talk between responses to difference biotic and abiotic stimuli (Lorenzo and Solano, 2005; Bari and Jones, 2009; Fan *et al.*, 2009). The growing data has cleared that reactive oxygen species (ROS) played key role in establishment of cross tolerance of plant by triggering responsive genes to abiotic stresses as well as diseases (Apel and Hirt, 2004). A number of biotic stress related proteins and peptides have also been produced as a response to salt stress. These regulated proteins include elicitor peptides, ROS eliminating proteins like cytochrome p450 monooxygenases, peroxiredoxins, and intracellular pathogenesis related proteins, disease related and DR - like proteins, heat shock proteins, and copper homeostasis factor proteins (Nakaminami *et al.*, 2018; Karagianni and Bazopoulou, 2024). A tomato ethylene responsive factor (TERF1) acted as a linker between abiotic and biotic stress responses. TERF1 binds with dehydration responsive GCC element which is responsive to osmoticum and overexpression of TERF1 in tobacco plant activated pathogen related genes (Zhang *et al.*, 2016)

Tomato is a main component of the Mediterranean diet. It has well established that intake of tomato in different forms like raw tomato, tomato sauce, cooked or processed tomatoes, and reduce the risk of lung, prostate, stomach, breast, oral, pancreatic and colorectal cancer incidence (Kapała *et al.*, 2022). It was believed and proved that lycopene and newly isolated bioflavonoids were potent against cancers (Pyo *et al.*, 2024) and lycopene has highest physical quenching power, prevented oxidation of LDL, ultimately diminished formation of plaques in blood arteries (Przybylska and Tokarczyk, 2022). The preliminary work has been done to exhibit the platelet anti-aggregation activity from variety of fruits and vegetables including red grapes, pineapple, kiwi, strawberries, garlic, onion and tomatoes (Geldenhuys *et al.*, 2024) with strong preference of tomato.

The present study was planned to fractionate cationic peptides of less than 10,000 Da from tomato plant by the induction of salt stress. The physical effect of saline stress was also studied, and the extracted proteins/ peptides (< 10 kDa) were fractionated by Strong cation exchange (SCX) chromatography and tested for antibacterial potential. Moreover, the efficiency of salt stress for the induction of antimicrobial proteins was tested by using different salt concentrations and varying day intervals.

## 2. MATERIALS AND METHODS

### 2.1 Germination and Saline Stress

The seeds of *Solanum lycopersicum* (tomato) were purchased from Horizon Herbs LLC, USA. The seeds were washed with distilled water and sowed in damp soil in 4x4 pots. The seeds were transplanted at two leaf stage into individual compartmentalized flats. The selected plants were given salt stress of 0, 100 and 200 mM of NaCl. The stress was given in increments of 50 mM, after each two days to batches of 100- and 200-mM dedicated plant flats. A sub-batch of 200 mM treated plants was harvested after 5 days and 10 days of prolonged stress. Meanwhile, the data for the number of leaves and length of shoots of randomly selected plants (biological replicates) were collected for comparison of growth between control and salt stressed.

### 2.2 Peptidyl Acetic Acid Extraction of Samples

All plant samples were extracted by developed and modified “Acetic acid peptidyl extraction protocol” of Hicks lab (UNC-CH). 100 g of plant material was ground under liquid nitrogen to fine powder and then pulverized powder was extracted in 300 mL of extraction buffer (10% acetic acid, 3% Polyvinylpolypyrrolidone, Roche EDTA-free protease inhibitor cocktail: 2 tablets and Pepstatin (1 mg/ mL): 100 µL) for 4 hours at 4°C. The extracted plant material was centrifuged (Sorvall RC 6+ Centrifuge) for 45 minutes at 4°C and 13000 RCF. After centrifugation, supernatant was passed through vacuum filtration (Millipore, 0.45 µm, HV Durapore membrane) and stored at 4°C.

### 2.3 10 kDa Molecular Weight Cut Off (MWCO)

It was performed by Amicon Ultra-15 Centrifugal filter devices, 10,000 MWCO (Millipore). These devices were prepared to remove glycerin as per manufacturer’s protocol. Added 10 mL of plant material in each device and centrifuged (Eppendorf centrifuge 5810R) for one and half hour at 3220 RCF and 4°C. The filtrate was aliquoted into 6 mL plastic tubes and concentrated down to tenfold in a SpeedVac at 4°C.

### 2.4 Dialysis

Concentrated plant material was dialyzed for the removal of small molecules of less than 1000 Da. Dialyzing membrane devices (Spectra/Por Float-A-Lyzer G2 500-1000 MWCO) were prepared by following manufacturer’s protocol. Two thirds of dialyzing membranes were filled with plant material and air was removed and capped and placed in dialyzing buffer (5 mM ammonium formate, pH: 2.7) at 4°C. After dialysis plant material was concentrated in SpeedVac at 4°C until 1x concentration (100 g of start material was extracted in 300 mL of buffer was concentrated to 4 mL and this concentration was taken as “1x” concentration).

### 2.5 Fractionation by UFLC

The fractionation of samples was performed by UFLC HPLC, Shimadzu by using PolyC-Polysulfoethyl A, strong cation exchanger and UV visible detector (SPD-20A), lamp D2 with positive polarity and cell temperature was 40 °C. The flow rate was 500 µL/ minute and pressure of 4500 of maximum. The spectra were analyzed at two channels 220 and 280 nm. Output intensity signals were calculated as

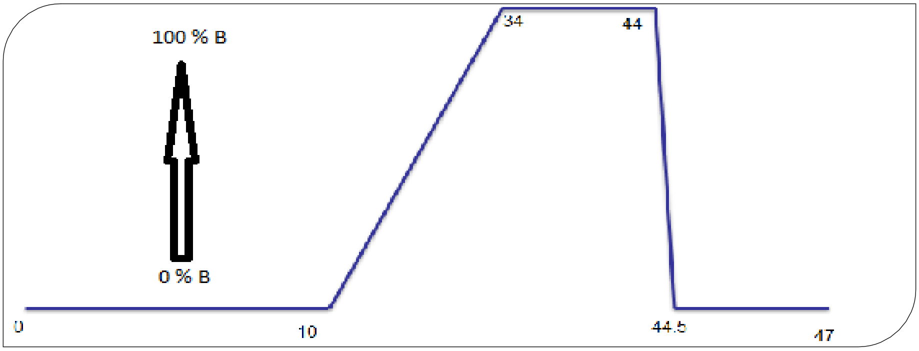

**Figure 1.** Mobile phase gradient ramp over time for UPLC fractionation

volts (V). Two mobile phases, MP-A, 5 mM ammonium formate in 20% acetonitrile, pH 2.7 and MP-B, 500 mM ammonium formate in 20% acetonitrile, pH 3.0 were used. Fractionation study was performed in gradient mode (Figure1) From 0-10 the mobile phase A was 100% and B at 0%, after ten minutes the gradient showed a linear ramp up to 100% B at minute 34. Onwards from minutes 34-44 gradient remained 100% B without any change and then sharply backed down to 0% B/100% A for final wash.

### *2.5.1 Sample Injection and Fractionation*

The instrument was washed with mobile phase A for 30 minutes. Then 400 µL of plant sample was injected at flow rate of 500 µL/minute and fractions were collected in fraction collector in labelled 1.5 mL eppendorfs. After the run was completed, caped all tubes and store at 4°C.

### *2.5.2 Desalting Process*

The presence of salts in fractions produces false positive bioactivity, hence all the fractions were subjected to desalting, done by spinning down to almost dry in SpeedVac at 4°C, then added 1 mL of milliQ (autoclaved) water, spin down, repeated twice. Re-suspended the material in 200 µL of milliQ autoclaved water and stored at 4°C.

## 2.6 Antibacterial Assay

This 96 well plate antibacterial (*Escherichia coli*) assay was developed in Hick's lab. Sterilized LB broth media was inoculated with a single colony from freshly culture plates (maximum two days old) and incubated at 37°C with shaking (325 rpm) overnight. The optical density (0.4) was checked at 600 nm. Then, by using a sterile 96 well microtiter dish, a sterile pipetting reservoir, and a multichannel pipettor, added each of the following elements of the test culture to each test well:

a) 50 µL of 2x media
b) 50 µL of 1x media
c) 50 µL of test solution (Fractions)
d) 50 µL of bacterial culture at $OD_{600}$ =0.4 for a final $OD_{600}$ of 0.1.
e) 50 µL of $H_2O$ as negative control (in place of d)
f) 50 µL of 400 µg/ mL ampicillin stock as positive control (in place of d)
g) 50 µL of 1X LB as blank (in place of d)

Added 4 µL of 50 mM Resazurin (dissolved in water and filter sterilized at 0.22 micron) to every well at 0.1 OD and incubated at 37°C with vigorous shaking (325 rpm) for one hour. The fluorescence was read in spectrophotometer at 544 and 590 nm excitation and emission wavelength respectively.

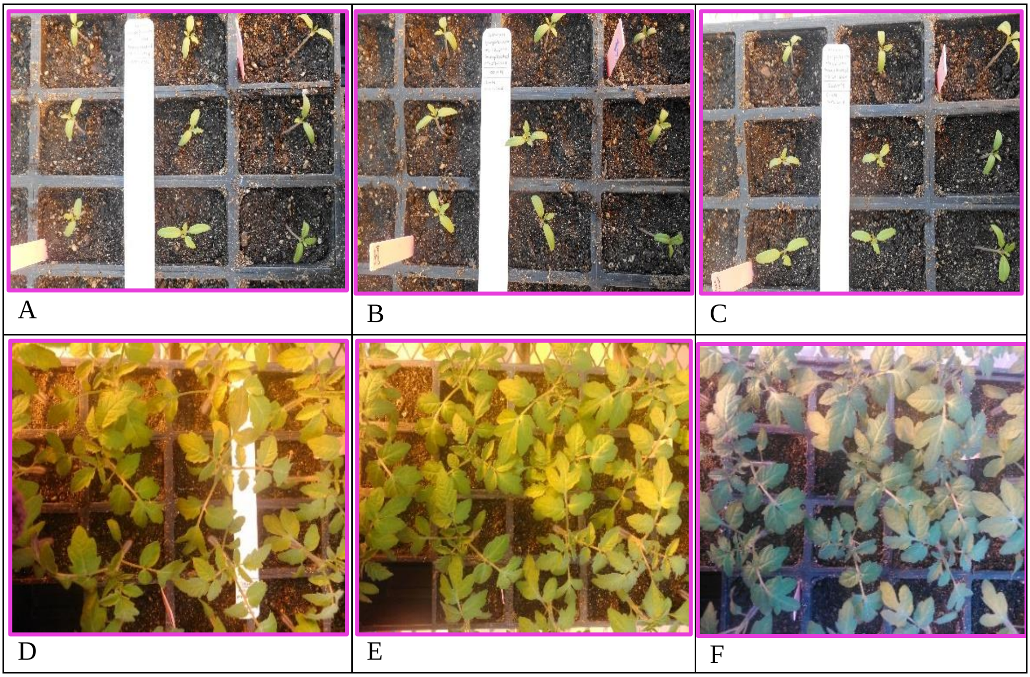

**Figure 2.** Growth stages of tomato plant under control (SLC) and saline stress (SLS100, SLS200); A, B, C: 4 days (after transplantation) under normal and 50 mM of NaCl stress conditions respectively D, E, F: 18 days old (after transplantation) under normal and 100 and 200 mM of NaCl stress for 14 and 10 days respectively.

## 3. RESULTS

### 3.1. Tomato Exhibited Tolerance to NaCl

Tomato plant was subjected to saline stress (100/ 200 mM) to understand the physical response of plant relative to salt concentration and tolerance level. All plants under normal and stressed conditions grew very well and remained healthy throughout the study period. Figure 2 elaborates healthy effects of salt stress on the growth of tomato plants; A, B and C are depicting the growth four days after transplantation under normal and 50 mM NaCl respectively. No retardation or wilting in growth was observed 18 days after transplantation (D, E, F) in response to gradual increase in salt concentration from 0 to 100 and 200 mM for 14 and 10 days respectively except after prolonged stress of 200 mM for 15 days.

### 3.2. Saline Effects on Height of Tomato Plants

The effect of salt was critically observed by measurement of height and number of plant leaves. Figure 3. indicates that tomato grow in height without any significant increase or decrease when treated with 100 mM of NaCl (7 days after transplantation). But when the concentration reached 200 mM (14 days after transplantation), unneglectable decrease in plant height was observed (SLS200 series). It was also noticed that when salt concentration increased from 100-150 (11 days after transplantation) the height of plant was increased relative to both control and 100 mM salt. The analysis of variance showed non-significant variations in heights of plants under control and salt treatments.

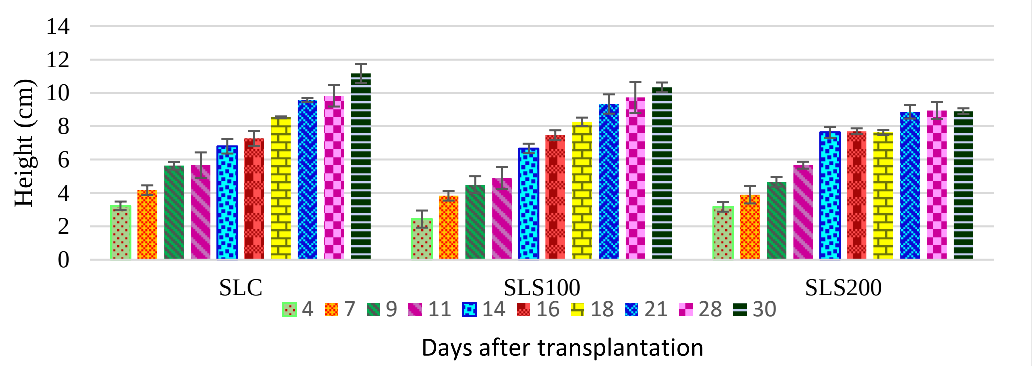

**Figure 3.** A comparative trend of plant height (tomato) irrigated with tap water and salt solutions. The set of series under SLC, SLS100 and SLS200 is showing growth of tomato plant in terms of height (cm) growing under normal and saline stress of 100 and 200 mM of concentration, respectively. The legends (4, 7, 9, 11, 14, 16, 18, 21, 28 and 30) show the number of days after transplantation. The arrows at point 4 and 11 in SLS100 and SLS200 series show NaCl concentrations of 100 and 200 mM, respectively.

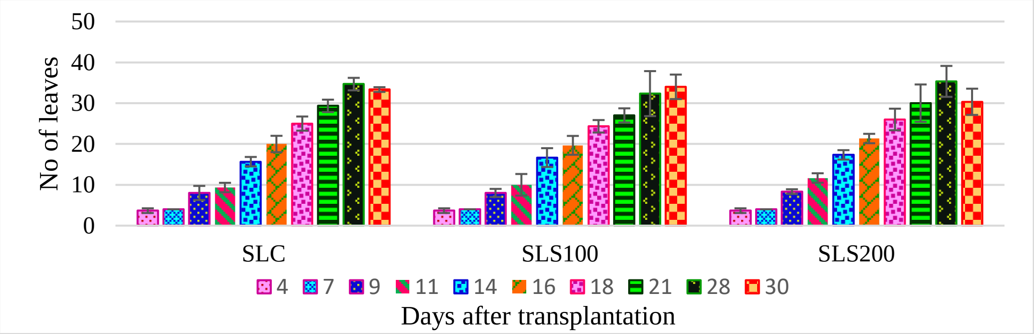

**Figure 4.** Comparative increasing trend of leaf numbers of plant irrigated with tap water and salt solutions. The series under Control, SLS100 and SLS200 shows growth of tomato plant in terms of leaf number growing under normal and saline stress of 100 and 200 mM of concentrations, respectively. The legends (4, 7, 9, 11, 14, 16, 18, 21, 28 and 30) show the number of days after transplantation. The arrows at point 4 and 11 in SLS100 and SLS200 series show NaCl concentrations of 100 and 200 mM, respectively.

### 3.3. Saline Effect on Number of Leaves

Unexpected results were obtained when the number of leaves were counted. Figure 4 declared that gradual increase in salt concentration from low to high has no effect on the number of leaves. The pattern of leaves number shown here is similar under control and salt stress conditions. But after prolonged stress of 200 mM for 15 days resulted in drying of leaves and decrease in leaf number after 30 days of transplantation was due to falling of one to two leaves. The statistical analysis also showed non-significant difference between control and saline treatments.

### 3.4. Strong Cation Exchange (SCX) Chromatography

The concentrated sample was passed through a strong cation exchange column for the fractionation of cationic peptides under high pressure and the fractions were collected at flow rate of 500 µL per minute. The samples were fractionated at two wavelengths, 220 nm for mobile phase and shown in black peaks and other 280 nm specifically for peptidal compounds in plant extracts. A broad, intense and high peak in retention time of 2-6 minutes was clued to belong to colored compounds as fractions collected between 3 to 7 minutes were colored. From 0 to 10 minutes gradient was only 5 mM of ammonium formate (mobile phase A) which eluted low charged stuff. From 10 to 34 minutes, as the gradient ramp reached from 0% B to 100% B (500 mM ammonium formate) the positively charged cations eluted from SCX column; the more the positive charge on compounds, later the retention time (Edelmann, 2011). Many bumps and peaks which can be seen in Figure 5-A in minutes 23-35 correspond to cationic peptides. As the constitutive expression of defensive peptides is well described so these fractions are high potent for the isolation of antimicrobial peptides (Schauber *et al.*, 2006).The heights of the peaks showed the intensity of charged compounds and most of the peptides eluted in minutes 25, 26, 27, and 29 had intensity of 50-100 mV.

### *3.4.1. Alternation of Peptidomics Behavior Under Saline Stress*

In response to salt stress the expression of peptides increased to high intensity (50-250 mV) in all chromatograms except control. These compounds may be responsive to stress or may produce constitutively, and the expression was enhanced due to salt. The broad peak of retention time 26-27 in control sample was split into three sharp peaks at 27-28 minutes with higher intensity. Another a broad peak induced at 18-22 minutes as comparison to control suggested altered protein expression at the cost of 100 mM of NaCl (Figure 5-B). Moreover, highly positive charged peptidal compounds were eluted in the 31-35 minutes as shown by seven peaks.

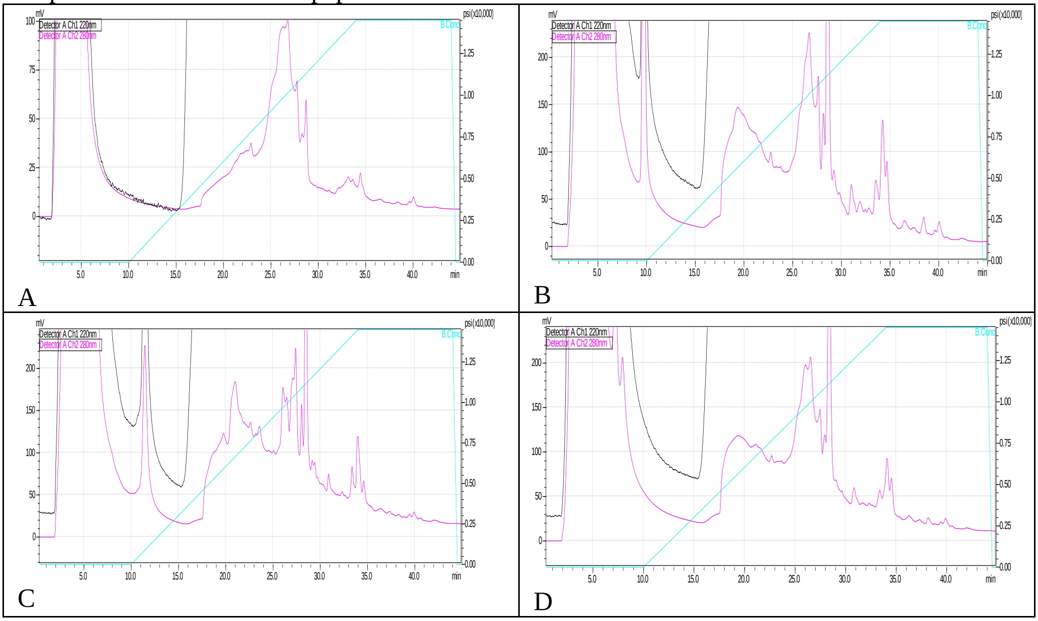

**Figure 5.** Strong cation exchange chromatograms of tomato control and salt treated samples with 100 and 200 mM for 14, 5 and 10 days are represented with A, B, C, D respectively. Fractionation was done by two channels at 220 and 280 nm represented by black and pink peaks respectively. Light blue line depicts gradient ramp. The bumps and peaks of pink are representing absorption at 280 nm of peptides. The intensity of peaks along y-axis increased from 100 to 300 mV under salt stress (B, C, D).

When the concentration of salt increased to 200 mᴍ (SLS200-5), the number of peaks increased to a more splitting pattern between retention times of 26-28 minutes to respond to high stress (Figure 5-C). A less intense but sharp peak was also found at retention time of 34 minutes under salt stress prolonged for five days only. The chromatogram of prolonged stress application of 200 mᴍ stress to 10 days (D) presented no drastic change in peptidal expression when compared to chromatogram of 200 mᴍ stress of 5 days (Figure 5-D).

The differential expression profiling of small proteins and peptides obtained by strong cation exchange chromatography of control and salt induced samples of tomato revealed that plant proteomics was influenced and altered in response to salt stress as reported previously (Kav *et al.*, 2004; Chitteti and Peng, 2007; Wang *et al.*, 2008). These peptides fractionated between minutes 11-34 are highly positively charged as well as strongly potentiated for the discovery of new and novel antimicrobial peptides because of limited range of molecular weight i.e., < 10 kDa.

### 4. Antibacterial Activity of Fractions of Tomato Against *E. coli*

All the SCX fractions were desalted, suspended in deionized water and checked for potential of antibacterial activity against *E. coli*. The percentage activities of all the fractions are shown in Figure 6. Figure 6-A shows that all control fractions of *S. lycopersicum* (tomato) evidenced antibacterial activity except five fractions (1, 2, 3, 24, and 25). The highest percent activity was demonstrated by fraction 4, which was pink colored eluent and represented main pigment of tomato, lycopene. The activity of the later three fractions also suggested the involvement of colored pigments. These compounds have antioxidant potential and reported to increase salt tolerance in plants (Han *et al.*, 2008). The fractions 11 to 32 showed activity in the range of 30 to 50% on average with negative activity depicted by fractions 24 and 25 while all remaining fractions exhibited 80% activity.

The percent bioactivity of 100 mᴍ salt treated (SLS100-14) fractions are displayed in Figure 6-B. The fractions with 1-10 except 4, showed non-significant antibacterial activity in SLS100-14, which were also correlated to SCX spectra of this particular sample. In contrast to control, activity of colored eluents drastically fell down to negative (fraction 5), or less than 10 percent (6 and 7). The activity of the last fractions was due to small cationic compounds having increased positive charge. These organic compounds which may be non-protein in nature exhibited high activities by fractions of all control and induced tomato plants. And the region of 10-35 which was believed to contain peptides showed antibacterial activity. A highly significant potential of antibacterial activity by all other fractions of control and salt stressed (SLS100-14) after 14 days of treatment confirmed constitutive expression of peptides. As the ramp of mobile phases changed linearly with time from 10 to 34 minutes, the antibacterial activity of these fractions was most important. By contrasting both samples in this region, increased antibacterial activity was found in seven (12, 16, 21, 22, 24, 25, 26, 28, 31) and decreased in five fractions (17, 18, 27, 29, 33). This induction of peptides with different charges and nature is related to their activities.

Antibacterial activities of high salt responded fractions (Figure 6-C, 6-D) showed that these peptides might be related to performing other functions to cope with stress. Ignoring the induction time period (5 or 10 days), almost matching pattern was followed. The negative activity by fractions 23-32 displayed that plant shifted to produce peptides related to ionic balance or salt tolerance rather than inducing antimicrobial peptides as cross tolerance. The dramatic contrast was displayed by fractions

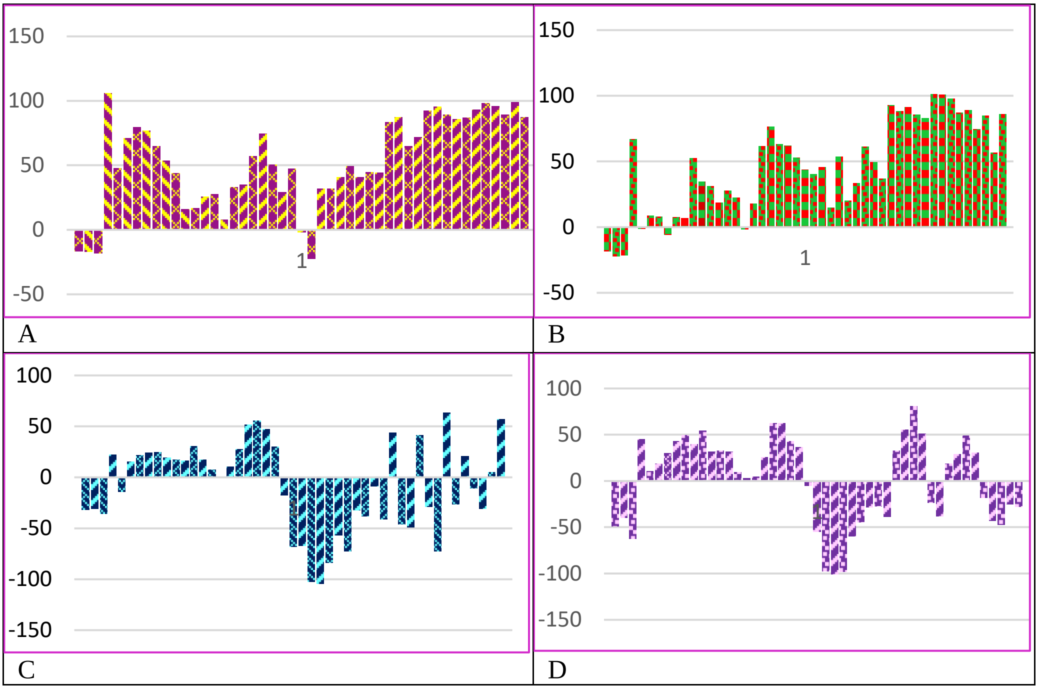

**Figure 6.** Percent activity of tomato fractions against E. coli; X-axis denotes to fractions (1-47); Y-axis shows percent activity in both positive and negative scale; A, B, C, D: percent activity of tomato control, salt treated with 100 mM, 200 mM for five and ten days respectively

(33, 34, 36, 39, 40, and 42) and (35, 38, 43, and 47), the former set showed negative activity after 5 days and changed to a positive response against *E. coli* after 10 days of 200 mM stress. Later fractions were positive after 200 mM stress continued for 5 days and diminished the activity towards negative on prolonged high salt stress for 10 days.

The antibacterial activity of tomato fractions was confirmed by the identification of salt induced thaumatin like protein (NP24) from pericarp of tomato which efficiently killed bacteria (Mohamed *et al.*, 2013). In another study, antimicrobial protein of 10 kDa molecular weight (XSP10) was identified and purified from xylem sap of tomato, structurally similar to lipid transfer proteins (LTPs). A mutant tomato of this particular protein when inoculated with *Fusarium oxysporum*, the functionality of pathogen resistant reporter proteins like PR-1 was also altered which showed that XSP10 is essential for the susceptibility against fungal infections (Krasikov *et al.*, 2011).

These results showed that saline stress is a potential approach for the study of antimicrobial peptides. Number of cationic peptides help in plant innate as well as induced defense system which is elaborated by the peptidal expression of tomato plant in the absence/ presence of saline stress. A very high proportion of these cationic peptides were found to kill bacterial growth. This antibacterial activity suggested that tomato plant constitutively express antibacterial peptides, but the expression increased under moderate saline stress as a result of induced cross tolerance. But, at high salt concentration, induced peptides did not show antibacterial activity, and it is concluded that plant forced systematic approach to balance salt stress for sustainability and produced proteins or peptides with functions other than antimicrobial, which is explained by the present study particularly under 200 mᴍ.

## 5. DISCUSSION

This gradual increase of salt concentration in increments might provoked tolerance capabilities by producing different signals to cope with high $Na^+$ ions and protect plant from osmotic shock (Qados, 2011). Tomato plants under high salt concentrations sucks more $Na^+$ ions from soil through roots and ultimately responsible for the production of reactive oxygen species due to elevated osmotic stress (Dat *et al.*, 2000; Apel and Hirt, 2004). Plants tolerance might be accomplished by balancing low $Na^+$ and high $K^+$ in the cytosol and by the production of phytohormones like abscisic acid and ethylene (Amjad *et al.*, 2014).

The high salt concentration inhibits growth as well as photosynthetic systems I and II and meanwhile, activate defensive system of plants to get rid of ionic imbalance of $Na^+$. Salt stress also induced cross tolerance to other stresses (Pang *et al.*, 2010) with the activation of pathogenic proteins and peptides in which we are interested to evoke (Jiang *et al.*, 2007; Wang, 2008; Zhang *et al.*, 2012).

The results exhibited here with reference to increase in height after 150 mᴍ of salt stress are supported by the reports of Dantas *et al.* (2005) who studied the relationship of salt concentration and height of cowpea (*Vigna unguiculata*), and Memon *et al.* (2010) on their analysis of salt effect on *Brassica*, and showed that height of the plant decreases with the increase of salt stress. The decrease in the heights of tomato plant under high concentration of NaCl i.e. 200 mᴍ was compared with the decreased heights observed in moth beans (*Vigna aconitifolia*), radish (*Raphanus sativus*), cowpea (*Vigna unguiculata*) and *Vigna mungo* (Mathur *et al.*, 2006; Jamil *et al.*, 2007; Taffouo *et al.*, 2009; Kapoor and Srivastava, 2010).

It may confer from the results that plants when treated with low concentration of salt the elongation of the stem might not compromised and plant has adjusted osmotic potential and gradual increase in salt concentration positively adjusted osmotic potential and stem elongated relative to control as well as 100 mᴍ salt treatment. But at high salt concentration plants might compromise photosynthetic process as well as deficiency in protein synthesis which is ultimately responsible for the decrease in plant height.

In contrast to our results, previous studies showed a remarkable decrease in leaf number. In a study decrease in leaf number of beans (*Phaseolus vulgaris*) was observed at 50 and 100 mᴍ of NaCl stress (Gama *et al.*, 2007). But at high salt concentration the dryness of few leaves followed by falling off was explained by the previous reports on the analysis of leaf number in response to salt stress on *Cirer arietinum*, *Phaseolus acutifolius*, *Vigna unguiculata* and *Phaseolus filiformis* plants (Welfare *et al.*, 2002; López-Aguilar *et al.*, 2003).

It may be suggested from the results that tomato plant has potential to cope with high salt concentration effectively. It adjusted osmotic potential which led to making proteins essential for new leaf and it might be concluded that plant evolved new leaves in attempt to stable the photosynthetic process to provide enough energy to remove excess $Na^+$ ions from the plant.

The plant extracts are a very complex mixture of organic dissolvable compounds including a large proportion of proteins and peptides. Most of the bioactive peptides we were interested in fall in the range of 3-7 kDa with the possession of positive charge. Hence, small peptides were screened out with the 10 kDa molecular weight cut off to minimize the complexity of samples. The concentrated plant samples were run through strong cation exchange column of negatively charged resin (PolyC polysulfoethyle A) to fractionate positively charged peptides. The peptides are potentially protonated at carboxyl group at pH 3 of mobile phase B (500 mᴍ ammonium formate in ACN). The charge is based on the presence of positively charged amino acids like arginine, lycine and histidine. Kong *et al.* (2011) designed a variant of reversed phase – reversed phase approach with second cation exchanger to fractionate eluents of first reverse phase – strong cation exchange chromatography to increase the identification of proteins. They confirmed this high-throughput and fully automatic as well as online approach by the identification of proteins from chloroplast of *Arabidopsis thaliana*. In the present study the gradient ramp of mobile phase B was established from retention time 11 to 34, the peptides were attached tightly with the negatively charged resin, but when pH reached at 3 due to mobile phase B (retention time 11 minutes), the elusion of peptides was done followed by the degree of positive charges they had as seen in chromatograms of *S. lycopersicum*. The differential expression profiling obtained by strong cation exchange chromatography of control and salt induced (at different concentrations and day intervals) tomato plants revealed that the plant proteomics was influenced and altered in response to salt stress that is also supported from previous proteomics studies of *Pisum sativum*, *Oryza sativa*, and *Physcomitrella patens* (Kav *et al.*, 2004; Chitteti and Peng, 2007). The chromatographic profiles of the plants, especially tomato, suggested the presence of thousands of peptides fragments that were fractionated to make the sample less complex for downstream peptidomic analysis by LC-MS/MS spectrometry. Zhang *et al.* (2012) reviewed salt responsive proteomics data of 34 plant species and categorized those into thirteen different classes related to defense, energy metabolism, photosynthesis and others. As far as chromatography of halophyte is concerned, although, differential expression was depicted in response to salt stress comparative to control, but expression looked quite calm showing plant tolerance to high salt concentration. These results are correlated with the previous comparative proteomic study of *Thellungiella* and *Arabidopsis*, in which differentially expressed proteins were doubled in the later, and 10% of the expressed proteins were related to defense in *Thellungiella* (Pang *et al.*, 2010).

The antibacterial activity of tomato fractions was confirmed by the identification of salt induced thaumatin like protein (NP24) from pericarp of tomato which efficiently killed bacteria (Mohamed *et al.*, 2013). In another study, antimicrobial protein of 10 kDa molecular weight (XSP10) was identified and purified from xylem sap of tomato, structurally similar to lipid transfer proteins (LTPs). A mutant tomato of this particular protein when inoculated with *Fusarium oxysporum*, the functionality of pathogen resistant reporter proteins like PR-1 was also altered which showed that XSP10 is essential for the susceptibility against fungal infections (Krasikov *et al.*, 2011).

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## 6. CONCLUSION

This antibacterial activity suggested that tomato plant constitutively express antibacterial peptides, but the expression increased under moderate saline stress as a result of induced cross tolerance. But, at high salt concentration, induced peptides did not show antibacterial activity and it is concluded that plant forced systematic approach to balance salt stress for sustainability and produced proteins or peptides with functions other than antimicrobial, which is explained by the present study particularly under 200 mᴍ.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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