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RESEARCH ARTICLE

# Isolation, identification and *in silico* characterisation of antimicrobial peptides from mangrove plants, *Suaeda nigra* and *Suaeda maritima*

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## Abstract

Infectious diseases continue to be the primary cause of death, representing a significant public health issue globally. While antibiotics are an effective remedy, bacterial infections have developed resistance to these drugs, resulting in antibiotic treatment failure. Hence there is a quest for novel antibacterial agents from natural sources, including plants and other creatures. Plants possess the tendency to synthesise substances to defend themselves from challenging surroundings. This has prompted the investigation of antimicrobial peptides that are commonly derived and possess strong efficacy against pathogens. Moreover, the utilisation of mangroves in traditional medicine is garnering significant attention due to its reputation as an exceptional reservoir of bioactive substances for the treatment of cancer, diabetes and others. Therefore, the current study concentrated on identifying the antimicrobial peptides from *Suaeda nigra* and *Suaeda maritima.* The analysis of leaf proteomes in *S. nigra* and *S. maritima* showed that the 15 and 20 KDa peptides exhibited strong antimicrobial activity, which were identified as Alpha-2-purothionin precursor and Vicin-like antimicrobial peptides-2-2-like proteins. The physicochemical characterization of Alpha-2-purothionin and Vicin-like antimicrobial peptides shows respective molecular weights of 14557.8 and 16353.78 Da. As well as their pI values were calculated as 5.13 and 6.11. The 3D structural analysis revealed that the Alpha-2-purothionin precursor and Vicin-like antimicrobial peptides showed an accurate model more similar to the templates PDB ID: 1nbl and PDB ID: 1fxzA. This study concluded that the identified proteins have significant antimicrobial potential against bacterial and fungal species and their predicted structures were reliable.

## Keywords

Mangroves; protein; antimicrobial peptide; SDS-PAGE; MALDI-TOF; I-TASSER

## Introduction

Infectious diseases continue to be the primary cause of death globally, posing a significant public health challenge worldwide (1). Bacterial infections provide a significant risk among all infectious diseases, causing 14 million fatalities worldwide each year (2). The World Health Organisation has officially declared that the significant rise in resistance to traditional antimicrobials poses a substantial and grave threat to public health (3). Even though antibiotics or chemicals are the only viable solution to this issue, bacterial pathogens have developed resistance to antibiotics, resulting in a growing failure of medication and antibiotic treatment (4). This is due to the rise in infectious diseases, the toxic effects of prolonged use of existing antimicrobial drugs and the development of microbial drug resistance (5). Presently, it is essential to search for novel antimicrobial medications from alternative sources, such as plants, which are rich in unique antimicrobial

chemotherapeutic substances. In addition, extracts from plants and plant-based products are utilised in the management of pathogenic diseases, thereby playing a significant role in the development of drugs (6). In recent decades, the discovery and analysis of new antimicrobial substances synthesised from natural sources have played a significant role in the creation of powerful antimicrobials and their subsequent testing in clinical trials. However, only a small number of these substances have demonstrated encouraging results (7). AMPs, or antimicrobial peptides, are considered promising treatments for fungal and bacterial infections due to their possible biological activity in both *in vitro* and *in vivo* conditions (8). In addition, they demonstrate bacteriostatic, microbicidal and cytolytic capabilities (9). They belong to a diverse group of peptides that are extremely stable and commonly found in the natural world. Natural antimicrobial peptides (AMPs) exhibit strong and wide-ranging effectiveness against several types of bacteria, yeast and parasites (3). Plants can synthesise chemicals as a means of self-defense in extreme conditions. This has prompted the investigation of plant antimicrobial peptides (AMPs) that are commonly derived to assess their effectiveness in averse to plant, animal and human diseases (10).

Plants contain chemicals that possess disease-fighting antibiotic, antiviral and antifungal properties, as stated by the World Health Organisation (11). The medicinal properties of plants have been utilised for ages as cures for human maladies and disorders due to their bioactive components (12). As a result, there is a growing interest in the application of mangroves in folk medicine compared to other plant species. Mangroves are known to be a great supplier of antibacterial and antiviral substances and have demonstrated various activities against human, animal and plant pathogens (13). Furthermore, mangrove companions are used as a powerful reservoir of natural antioxidants and antimicrobial substances in diverse herbal remedies for the management of ailments like diabetes, HIV, cancer and others (14). Nevertheless, the literature on Indian mangrove companions has provided a small amount of data on antibacterial, antioxidant and several other biological capabilities.

*Suaeda maritima* (L) Dumort. is an herbaceous plant that grows in salt marshes and mangroves. It belongs to the Chenopodiaceae family, as well as the Suaedoideae sub-family. It is sometimes referred to as seep weeds or sea blites since it is found in coastal salt flats and tidal wetlands (15). It has a broad distribution at the inland edge of mangrove environments (16). *S. maritima* is used as an ethnomedicine to address various ailments such as bacterial infections, chronic inflammation, hepatitis and conditions associated with oxidative stress (17). *Suaeda nigra* (Raf.) J.F.Macbr., a member of the Amaranthaceae family, is a halophyte that thrives in saline and alkaline environments and people commonly refer to it as bush seep weed or Mojave sea blite. *S. nigra* demonstrates remarkable antibacterial, antioxidant and anticancer properties (18). The primary objective of the present investigation is the identification and characterisation of antimicrobial peptides derived from *S. maritima and S. nigra.*

## Materials and Methods

### Sample collection

Healthy and young leaves of *Suaeda maritima* and *Suaeda nigra* were collected from the estuarine mangrove areas of Coringa Wildlife Sanctuary, which is situated (Long: 18°33' 52"to 18°32' 11"; N; Lat: 84°21' 26" E to 84°18' 22" E) in the northeast of Andhra Pradesh, adjoining the Bay of Bengal, Kakinada, India. The collected leaf samples were aseptically transferred to zip bags and taken to the lab. The plant species was authenticated by Dr. K. S. Pydal, Taxonomist, Department of Botany, Andhra University, Visakhapatnam and the voucher specimen (AU/BH/2024-102) was deposited at the herbarium, Department of Botany, Andhra University. The collected leaves were washed with tap water and then with distilled water. For future investigation, the leaves were preserved at -20°C.

### Extraction and estimation of proteins

Ferreira *et al.* (19) method was employed to extract the total protein, with a few modifications. 1 g of leaf tissue from *S. nigra* and *S. maritima* was weighed and ground into a fine powder using liquid nitrogen. The powder was then homogenised with a homogenisation buffer at a ratio of 1:3 (weight/volume) of sample weight to sample buffer. The homogenisation buffer was prepared by dissolving 50 mM Tris-HCl (pH 6.8), 0.5 M sucrose, 5 mM EDTA, 0.1 M NaCl, 1% SDS and 2% beta-mercaptoethanol. Subsequently, the homogenates were subjected to centrifugation at a speed of 6000 rpm at 4°C for 20 min. The supernatant was collected and preserved at -20°C for further studies. The total protein concentration in the leaf tissue of *S. nigra* and *S. maritima* was estimated using Lowry *et al.* (20) method.

### Antimicrobial activity with crude protein extracts

The well diffusion technique was used to assess the antibacterial activity of crude protein extracts from *S. maritima* and *S. nigra.* The antibacterial efficacy was evaluated using gram-positive bacteria including *Staphylococcus aureus, Bacillus subtilis* and *Micrococcus luteus*, as well as gram-negative bacteria such as *Esherichia coli, Salmonella typhi* and *Pseudomonas aeruginosa*. The antifungal activity was evaluated using *Candida albicans, Aspergillus niger* and *Saccharomyces cerevisiae* as test fungi. Erythromycin and nystatin were employed as positive controls for evaluating antibacterial and antifungal activities. Whereas for both antibacterial and antifungal activity, protein extraction buffer was used as a negative control. Antimicrobial activity was conducted by the preparation of nutrient agar media plates using the pour plate technique. 0.5 mm wells were punctured and filled with erythromycin, protein extraction buffer and protein extracts from *S. maritima* and *S. nigra.* Plates were sealed with paraffin and incubated at 37°C for 24 h. After incubation, the zones of inhibition were measured in millimetres using an antibiotic zone scale.

### Proteome profiling by SDS-PAGE

SDS-PAGE was used to separate the proteins from the crude protein extracts of *S. maritima* and *S. nigra.* To perform SDS-PAGE, the crude protein extracts of *S. maritima* and *S. nigra* were mixed with the sample loading buffer in a 1:1 ratio. The sample loading buffer consists of 0.5 M Tris-HCl (pH 6.8), 20.2% glycerol, 0.0001% bromophenol blue, 0.04% SDS, 0.03% DTT and 0.04% 2-mercaptoethanol. Subsequently, the protein extract and sample loading buffer were heated at 95°C for 10 min and then cooled. A 15% resolving gel and 5% stacking gel were prepared according to the previously described method (21). The cooled protein samples were loaded in the wells and the electrophoresis was

accomplished at 100 V for 1 h using a vertical electrophoresis unit with running buffer consisting of 25 mM Tris-base, 192 mM glycine and 3 mM SDS (pH 8.9). Following electrophoresis, the gel was stained with a staining solution containing 0.5% Coomassie Brilliant Blue R-250 in a mixture of 50% methanol, 40% distilled water and 10% acetic acid overnight and subsequently destained using a solution composed of 40% methanol, 50% distilled water and 10% acetic acid until the background was visibly clear. Finally, the gel was observed and photographed with Geldoc.

### *Antimicrobial activity from separated peptide gel bands*

Following the SDS-PAGE, the low molecular weight gel bands from the species *S. maritima* and *S. nigra* within the 25-10 KDa range were extracted from the gels and subjected to protein elution. The proteins were extracted from the gel by employing a gel elution buffer (pH 7.5). To extract peptides from gels, the gel fragments were suspended in an elution buffer and carefully crushed, following that, the solution was centrifuged at 10,000 rpm for 10 min at 4°C. The supernatant containing the eluted peptide fractions was collected and tested for antibacterial activity against selected gram-positive, gram-negative and fungal species.

### *Peptide mass fingerprinting*

Eluted protein fractions of *S. maritima* and *S. nigra* that exhibited antimicrobial activity were identified by peptide mass fingerprinting (PMF) using MALDI-TOF MS. To perform PMF, the eluted peptide gel fractions, which have shown antimicrobial activity, were digested with 1.0 mg of trypsin at 37°C overnight. Following digestion, the gel slices were thoroughly washed using HPLC grade water, 50% acetonitrile and 5% triflouroacetic acid three times at room temperature. Following that, a small amount of the peptide sample that was digested was placed on the matrix containing saturated α-cyano-4-hydroxycinnamic acid prepared in a mixture of 50% acetonitrile and 10% triflouroacetic acid. Mass spectrometric data was collected using an ABI 4800 MALDI TOF/TOF (Applied Biosystems, CA). The data was obtained in reflector mode, covering a mass range of 600-9000 Da. GPS explorer was used to generate a list of peaks from the raw data obtained from the ABI 4800. The obtained mass spectrum was subjected to sequence database searches using MASCOT software.

### *In silico characterisation of identified antimicrobial peptide*

### *Physicochemical characterisation*

The FASTA sequences of identified proteins were retrieved from NCBI (22). The physicochemical properties such as isoelectric point (pI), molecular weight, total number of positive and negative residues, extinction coefficient, instability index, aliphatic index and grand average hydropathy (GRAVY) for the identified protein sequences were computed using Expasy's ProtParam server (23).

### *Molecular modelling and model validation*

The secondary structure of the identified protein was predicted using the SOPMA server (24). The 3D structures and functional annotations, such as ligand-binding sites, enzyme commission numbers and gene ontology terms of proteins, were predicted by the I-TASSER (iterative threading assembly refinement algorithm) server (25). The predicted structural 3D models were validated by the Ramachandran plot statistics using the PROCHECK server.

### *Statistical analysis*

The results of present studies were given as mean ± standard deviation (SD) obtained from three independent experiments and the data was assessed by one-way analysis of variance by using SPSS 10.0 software.

## Results and Discussion

### *Protein estimation*

The results were given as the mean ± standard deviation (SD) obtained from three independent experiments. Significant variation was found in the total leaf protein content of *Suaeda maritima* and *Suaeda nigra*. The total leaf protein content in both *S. maritima* and *S. nigra* was found to be 7.95±0.9 and 9.25±1.2 mg/gm. The results of leaf protein contents are shown in Fig. 1. These results revealed that *S. nigra* had a higher protein content.

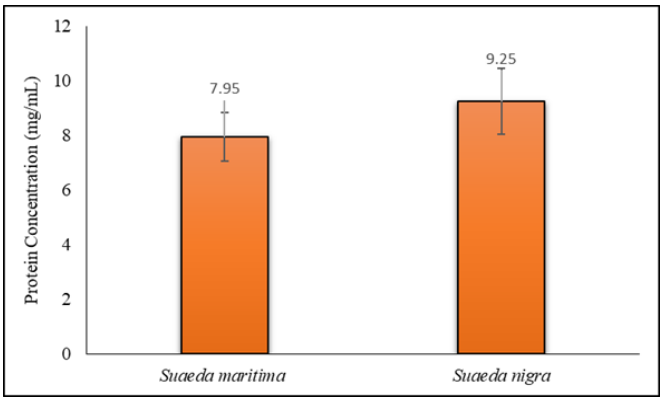


**Fig. 1.** Total protein content from the leaves of *Suaeda maritima* and *Suaeda nigra*.

The pharmacogenetic assessment of crude protein extracts is critical for discovering new bioactive peptides. Organisms from diverse environments serve as a valuable reservoir of medicinal compounds. Understanding the bioactive potential of organisms is crucial as it directly relates to their therapeutic value, which is determined by their biochemical contents (26). Mangroves are renowned for their abundant reservoir of potential proteins. The therapeutic efficacy of proteins is primarily determined by their sufficient content of essential amino acids. The protein concentrations in plants exhibit varying outcomes, which might be contingent upon the species of the plant. Protein accumulation under stressful conditions has a crucial function in enhancing stress tolerance in plants. It serves both as an energy reserve and a regulator of osmotic potential (27). Doganlar *et al.* (28) found that the increased production of stress proteins causes the fluctuation in protein content. These compounds can be produced by a de novo pathway in response to stress or may already be present at a low concentration (29).

### *Antimicrobial activity with crude protein*

The crude protein extract from the *S. maritima* and *S. nigra* leaves was tested for primary screening of antimicrobial activity against six bacterial species: *Staphylococcus aureus*, *Bacillus subtilis, Micrococcus luteus, Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa* and three fungal species *viz. Candida albicans, Aspergillus niger* and *Saccharomyces cerevisiae*. The antimicrobial activities of crude protein extracts from *S. maritima* and *S. nigra* leaves are given in Table 1. These results revealed that *S. nigra* leaf protein extracts demonstrated antimicrobial activity against all tested microbial species except *S. cerevisiae.*

**Table 1.** Antimicrobial activity of *Suaeda nigra* and *Suaeda maritima* leaf crude protein extracts

| Type of the organism | Name of the organism | Diameter of the inhibition zone (mm) | | | |
|---|---|---|---|---|---|
| | | ***Suaeda nigra*** | ***Suaeda maritima*** | **+Ve control** | **-Ve control** |
| Gram positive bacteria | *Staphylococcus aureus* | 11 | - | 34 | - |
| | *Bacillus subtilis* | 12 | - | 21 | - |
| | *Micrococcus luteus* | 10 | 10 | 32 | - |
| Gram negative bacteria | *Escherichia coli* | 14 | - | 35 | - |
| | *Pseudomonas aeruginosa* | 12 | - | 32 | - |
| | *Salmonella typhi* | 10 | 11 | 27 | - |
| Fungi | *Candida albicans* | 13 | 18 | 30 | - |
| | *Aspergillus niger* | 19 | 18 | 36 | - |
| | *Saccharomyces cerevisiae* | - | - | 39 | - |

The crude protein extract of *S. nigra* showed maximum antimicrobial activity against *A. niger* (17 mm), followed by *E. coli* (14 mm), *C. albicans* (13 mm), *B. subtilis* (12 mm), *P. aeruginosa* (12 mm), *S. aureus* (11 mm), *M. luteus* (10 mm) and *S. typhi* (10 mm). The protein extract of *S. nigra* did not exhibit microbicidal activity in *S. cerevisiae.* The crude leaf protein extract of *S. maritima* showed maximum antimicrobial activity against *A. niger* (18 mm) and *C. albicans* (18 mm), followed by *S. typhi* (11 mm) and *M. luteus* (10 mm). The protein extract of *S. maritima* did not exhibit microbicidal activity against *S. aureus, B. subtilis, E. coli, P. aeruginosa* and *S. cerevisiae*. Fig. 2 illustrates the antimicrobial activity of *S. nigra* and *S. maritima* leaf protein extracts against bacterial and fungal species. According to these results, the leaf protein extracts of both *S. maritima* and *S. nigra* exhibited greater inhibitory activity against fungal species than bacterial species.

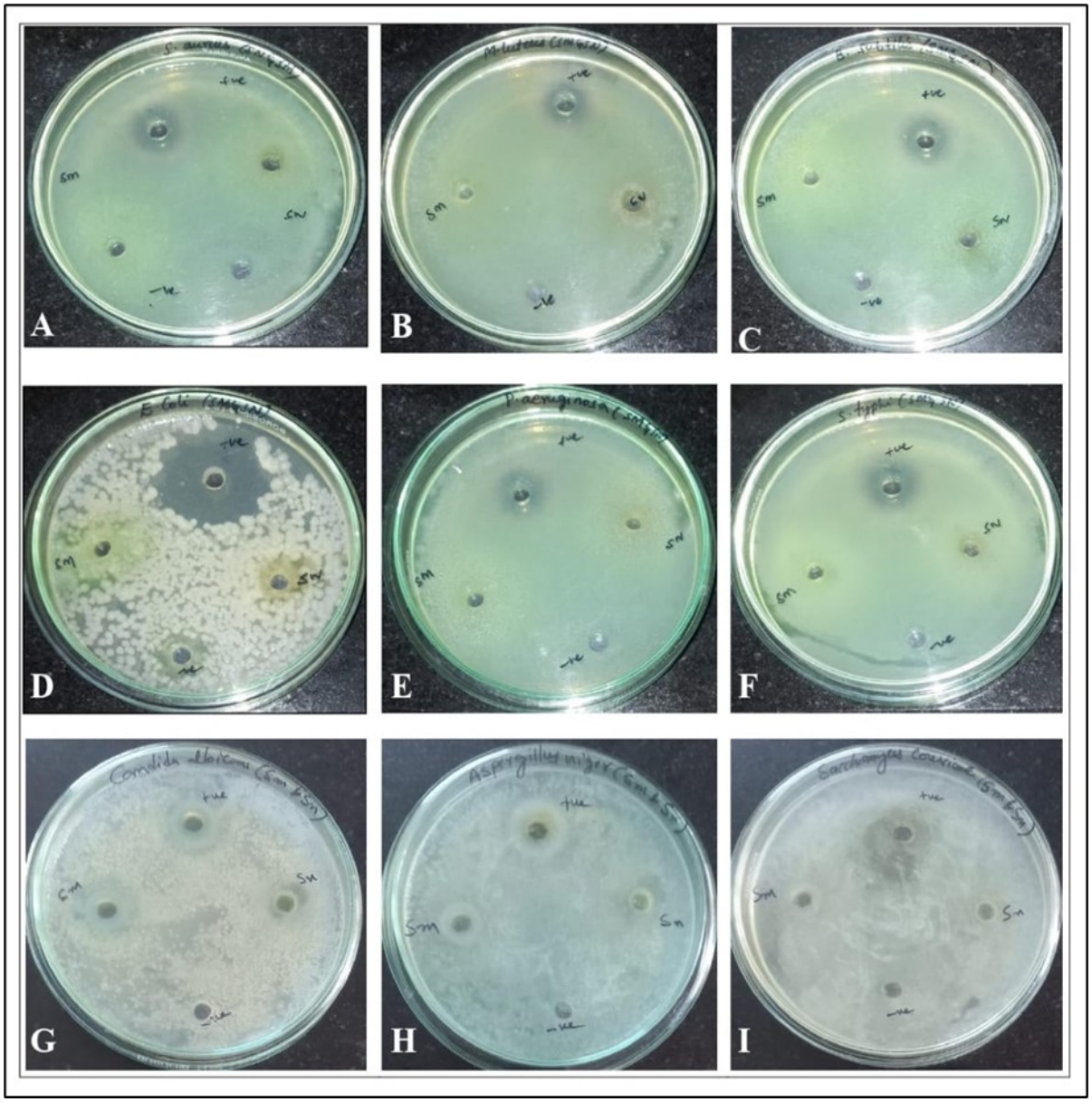


**Fig. 2.** Antimicrobial activity of *Suaeda nigra* (S.n) and *Suaeda maritima* (S.m) leaf protein extracts. Inhibitory zones against A) *Staphylococcus aureus* B) *Micrococcus luteus* C) *Bacillus subtilis* D) *Escherichia coli* E) *Pseudomonas aeruginosa* F) *Salmonella typhi* G) *Candida albicans* H) *Aspergillus niger* I) *Saccharomyces cerevisiae.*

### Protein profiling by SDS PAGE

SDS-PAGE is a highly effective technique for separating and analysing expressed proteins according to their molecular weight, allowing for both quantitative and qualitative examination. Fig. 3 illustrates the protein separation using SDS-PAGE from the leaves of *S. nigra* and *S. maritima.* The leaf proteome of *S. nigra* and *S. maritima* consists of proteins with molecular weights ranging from 10 to 200 KDa. Furthermore, the proteomes of *S. nigra* and *S. maritima* exhibited the highest proportion of proteins within the molecular weight range between 200 and 32 KDa. However, the antimicrobial peptides have a small molecular weight. Hence, in this study, darkly stained low molecular weight peptide bands were selected for further evaluation of their antimicrobial properties. Among the low molecular weight proteins, the prominently expressed peptide bands having an approximate molecular weight of 25 and 15 KDa were selected from the gel of *S. nigra.* While from the *S. maritima* darkly stained low molecular weight peptide bands having an approximate molecular weight of 35 and 20 KDa were selected. Several researchers successfully extracted the antibacterial peptides from marine algae. The current findings align with prior research byRouxel *et al.* (30) who observed that the SDS gels of *Ulva rotundata* include seven bands ranging from 69.9 to 15.5 KDa and three bands having molecular weights of 29.5, 26.3 and 22.9 KDa exhibited significant antimicrobial properties.

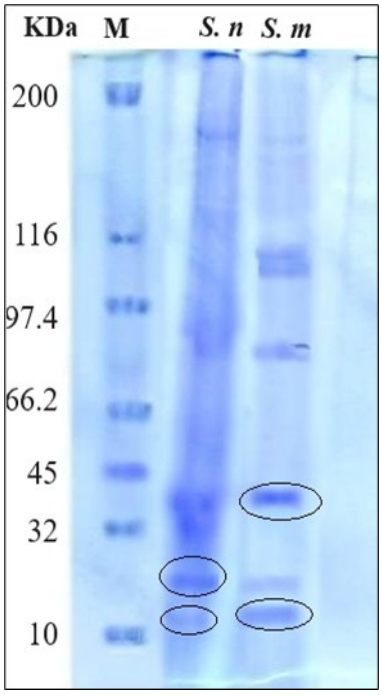


**Fig. 3.** Protein profiles of *Suaeda nigra* and *Suaeda maritima* leaves by SDS polyacrylamide gel.

### Antimicrobial activity from separated peptide gel bands

The low molecular weight peptide gel bands were extracted from the SDS polyacrylamide gel of *S. nigra* and *S. maritima* leaf proteome using optimized conditions for their antimicrobial activity. The peptide bands with molecular weight cut-offs of 25 KDa and 15 KDa from *S. nigra* and 35 KDa and 20 KDa peptide gel bands from *S. maritima* were extracted for antimicrobial screening. The prepared protein extracts were tested for antimicrobial activity from crude protein extracts.

Table 2 presents the antimicrobial activities of the extracted low molecular weight peptides from *S. maritima and S. nigra*. From these results, it was observed that among two tested peptide fractions from both *S. nigra* and *S. maritima,* the low molecular weight peptides, i.e., 15 and 20 KDa fractions, respectively, exhibited significant antimicrobial activity against the tested organisms. The 15 KDa peptide fraction of *S. nigra* showed maximum antimicrobial activity against *B. subtilis* (28 mm), followed by *E. coli* (22 mm), *S. typhi* (19 mm), *P. aeruginosa* (13 mm) and *M. luteus* (12 mm). Whereas, the 20 KDa peptide fractions of *S. maritima* showed maximum antimicrobial activity against *B. subtilis* (27 mm), followed by *S. typhi* (19 mm), *P.* aeruginosa (19 mm), *E. coli* (18 mm), *M. luteus* (13 mm) and *S. aureus* (13 mm). All the tested fungal species exhibited resistance against both 15 and 20 KDa peptide fractions of *S. nigra* and *S. maritima.* Fig. 4 illustrates the antimicrobial activity of extracted peptide gel fractions from *S. nigra* and *S. maritima.*

### Peptide mass fingerprinting

Peptide mass fingerprinting is the term used to describe the systematic method of identifying unknown proteins by analysing their peptide masses. The PMF method is a simple and efficient strategy that is ideal for automation. However, its success relies heavily on the availability of high-quality protein databases to get accurate assignments (31). Low molecular weight protein gel bands of 15 and 20 KDa, respectively from the leaf proteomes of *S. nigra* and *S. maritima* were subjected to in-gel digestion and analysed by matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS). The resulting peptide mass fingerprints were used to search the National Centre for Biotechnology Information databases with Mascot. The identified proteins from the SDS gel bands of *S. nigra* and *S. maritima* leaf were given in Table 3 and the MALDI-TOF MS spectra of 15 and 20 KDa protein bands of *S. nigra* and *S. maritima* leaf were given in Fig. 5 and 6. The protein spots of 15 and 20 KDa from the *S. nigra* and *S. maritima* leaf proteomes were identified as Alpha-2-purothionin precursor and Vicin-like

**Table 2.** Antimicrobial activity of extracted peptide gel fractions from *Suaeda nigra* and *Suaeda maritima*

| Type of the organism | Name of the organism | Diameter of the inhibition zone (mm) | | | | | |
|---|---|---|---|---|---|---|---|
| | | *Suaeda nigra* | | *Suaeda maritima* | | +Ve control | -Ve control |
| | | 25 KDa | 15 KDa | 35 KDa | 20 KDa | | |
| Gram positive bacteria | *Staphylococcus aureus* | - | - | - | 13 | 34 | - |
| | *Bacillus subtilis* | - | 28 | - | 27 | 21 | - |
| | *Micrococcus luteus* | 6 | 12 | 12 | 13 | 32 | - |
| Gram negative bacteria | *Escherichia coli* | 10 | 22 | 18 | 18 | 35 | - |
| | *Pseudomonas aeruginosa* | - | 13 | 15 | 19 | 32 | - |
| | *Salmonella typhi* | - | 19 | - | 19 | 27 | - |
| Fungi | *Candida albicans* | - | - | - | - | 30 | - |
| | *Aspergillus niger* | - | - | - | - | 36 | - |
| | *Saccharomyces cerevisiae* | - | - | - | - | 39 | - |

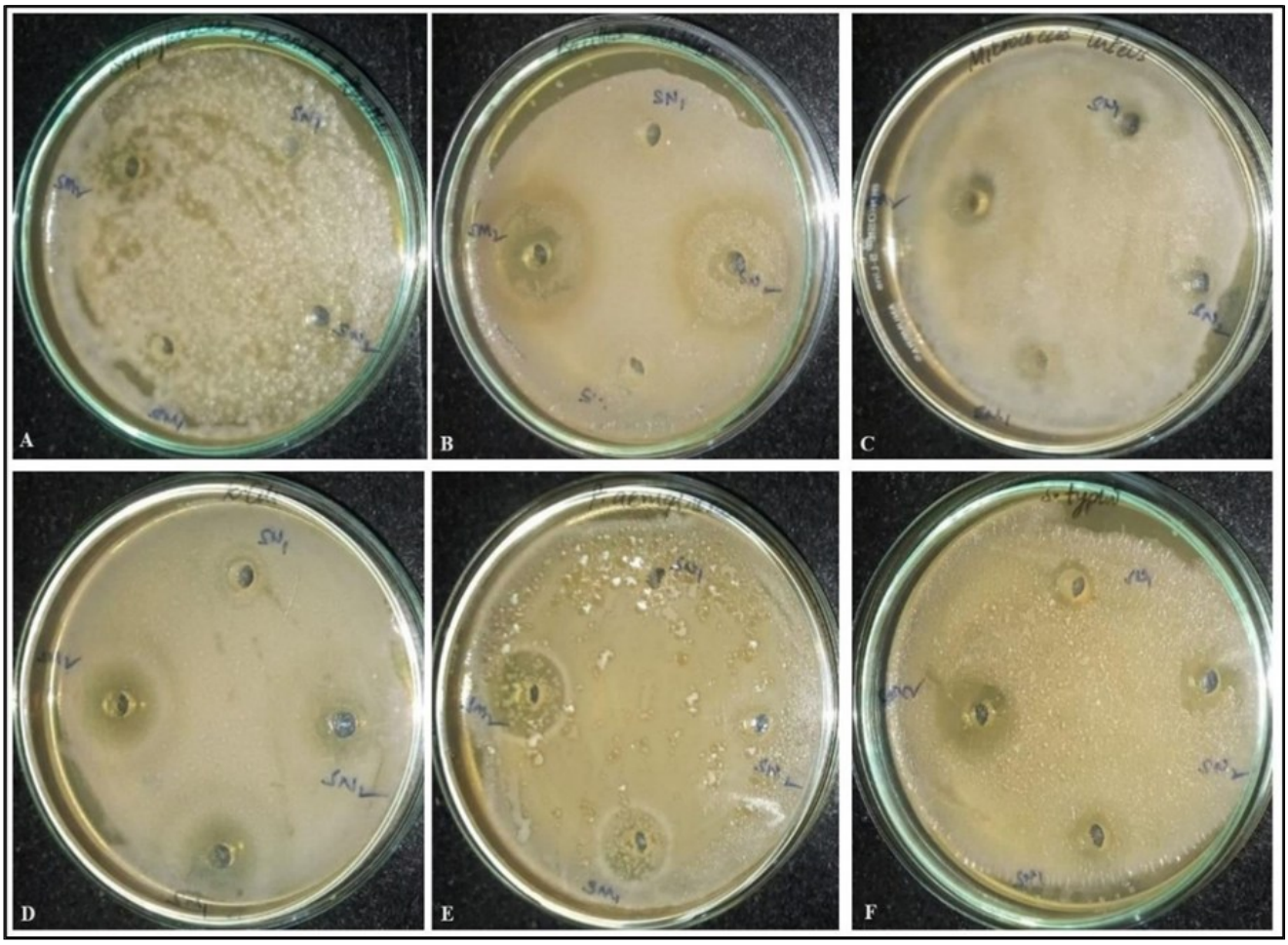


**Fig. 4.** Antimicrobial activity of extracted peptide gel fractions from *Suaeda nigra* (Sn1-25 KDa and Sn2-15 KDa) and *Suaeda maritima* (Sm1-35 KDa and Sm2-20 KDa). Inhibitory zones against A) *Staphylococcus aureus* B) *Bacillus subtilis* C) *Micrococcus luteus* D) *Escherichia coli* E) *Pseudomonas aeruginosa* F) *Salmonella typhi*.

**Table 3.** Proteins identified from the SDS gel bands of *Suaeda nigra* and *Suaeda maritima* leaf.

| Source organism | Protein gel band | Max. homology (protein name) | Best protein match organism | Expt/Theor.Mw (KD) | Score (MS/MS) | Accession no. |
|---|---|---|---|---|---|---|
| *Suaeda nigra* | 15 KDa | Alpha-2-purothionin precursor | *Triticum aestivum* | 15/14.9 | 69% | NP_001392710 |
| *Suaeda maritima* | 20 KDa | Vicin-like antimicrobial peptides-2-2like protein. | *Triticum pratense* | 20/16.5 | 78% | PNY16735 |

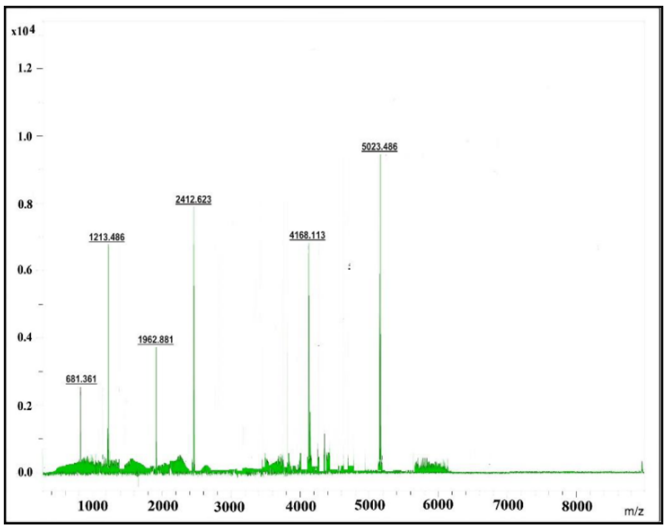


**Fig. 5.** MALDI-TOF MS spectra of 15 KDa protein band of *Suaeda nigra* leaf. Protein band was subjected to in-gel digestion by trypsin and after that analyzed by MALDI -TOF MS in reflector mode over the mass range of 500-8000 Da.

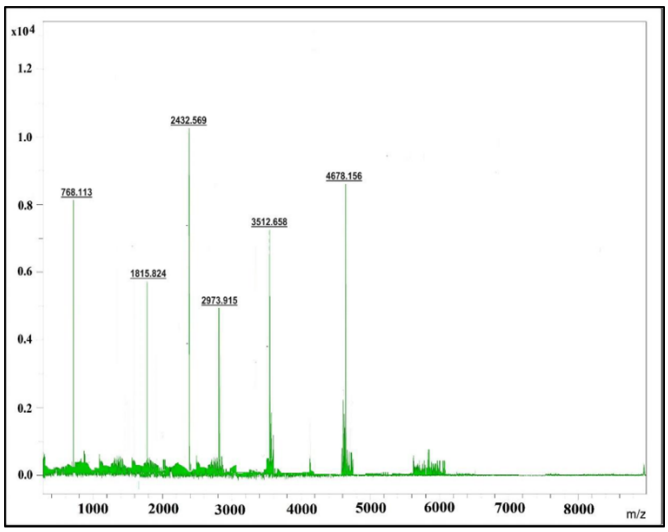


**Fig. 6.** MALDI-TOF MS spectra of 20 KDa protein band of *Suaeda maritima* leaf. Protein band was subjected to in-gel digestion by trypsin and after that analyzed by MALDI-TOF MS in reflector mode over the mass range of 500-8000 Da.

antimicrobial peptide, respectively. The PMF result of the 15 KDa peptide band from *S. nigra* shows the greatest score, 69% with *Triticum aestivum* (Accession number NP_001392710). The PMF results for the 20 KDa peptide band from *S. maritima* show the highest score, 78% with *Triticum pratense* (Accession number PNY16735).

### *In silico characterisation of identified protein*

### *Physicochemical characterisation*

The primary sequences of the protein Alpha-2-purothionin precursor and Vicin-like antimicrobial peptide of *S. nigra* and *S. maritima* were retrieved from GenBank for homology using the BLASTP suite by choosing the protein database. The physicochemical characterisation of the identified proteins demonstrated that the Alpha-2-purothionin precursor protein consists of 136 amino acids and its molecular weight was predicted to be 14557.8 Da. The Vicin-like antimicrobial peptide consists of 148 amino acids and its molecular weight was 16353.78 Da. The physicochemical characteristics of the Alpha-2-purothionin precursor and Vicin-like antimicrobial peptide are presented in Table 4. The computed pI values of the Alpha-2-purothionin precursor and Vicin-like antimicrobial peptide were 5.13 and 6.11, respectively. Less than 7 indicates that the protein is acidic. The isoelectric point (pI) is the value at which the molecule carries no charge or the negative and positive charges are equal. Due to the least mobility (i.e., zero) of the protein at pI, the isoelectric point will be useful for developing buffer systems for purification by isoelectric focusing. The extinction coefficient of the Alpha-2-purothionin precursor protein is 6835 $M^{-1}$ $cm^{-1}$when all pairs of Cys residues form cystines. Similarly, the Vicin-like antimicrobial peptide has an extinction coefficient of 2980 $M^{-1}$ $cm^{-1}$. If all the cysteine residues are decreased, the extinction coefficient of the Alpha-2-purothionin precursor protein is 6835 $M^{-1}$ $cm^{-1}$and Vicin-like antimicrobial peptide is 2980 $M^{-1}$ $cm^{-1}$. The highest extinction coefficient suggests the existence of a substantial concentration of Cys, Trp and Tyr. The EC value provides a measure of the concentrations of tryptophan and tyrosine. The computed ECs are used to quantitatively investigate interactions between proteins and ligands in solution.

**Table 4**. Physicochemical characterisation of Alpha-2-purothionin and Vicin-like antimicrobial peptide.

| Parameter | Alpha-2-purothionin precursor | Vicin-like antimicrobial peptide |
|---|---|---|
| Total no. of amino acids | 136 | 148 |
| Molecular weight | 14557.8 Da | 16353.78 Da |
| Theoretical pI | 5.13 | 6.11 |
| Negatively charged residues | 16 | 15 |
| Positively charged residues | 14 | 14 |
| Extinction coefficient | 6835 $M^{-1}$ $cm^{-1}$ | 2980 $M^{-1}$ $cm^{-1}$ |
| Instability index | 34.89 | 19.86 |
| Aliphatic index | 81.69 | 80.34 |
| GRAVY | -0.001 | -0.116 |
| Formula | $C_{608}H_{999}N_{173}O_{201}S_{19}$ | $C_{731}H_{1157}N_{189}O_{221}S_7$ |

The instability indices of the Alpha-2-purothionin precursor protein and Vicin-like antimicrobial peptide were predicted to be 34.89 and 19.86, respectively. The protein instability index offers insights into its stability when tested in a controlled laboratory setting. A protein with an instability index below 40 is considered stable, whereas a number above 40 indicates potential protein instability (32). Zaccaria *et al.* (33) discovered that minor factors located at the N-terminus have an impact on the stability of a protein, thereby affecting its lifespan. Rogers *et al.* (34) found that proteins with a half-life of less than 5 h have an instability index greater than 40, while proteins with a half-life of more than 16 h have an instability index of less than 40. The Alpha-2-purothionin precursor protein has an estimated half-life of 30 h in mammalian reticulocytes when tested in *in vitro* conditions and a half-life of more than 20 h in yeast. The Vicin-like antimicrobial peptide has an estimated half-life of 30 h in human reticulocytes when tested in *in vitro* conditions and more than 20 min when tested in yeast. The aliphatic index of the protein sequences of the Alpha-2-purothionin precursor and Vicin-like antimicrobial peptide were determined to be 81.69 and 80.34, respectively. The aliphatic index of these proteins suggests that they are thermally stable across a wide temperature range. The aliphatic index quantifies the percentage of a protein's volume occupied by aliphatic side chains (A, V, I and L). It is considered to be a positive factor in improving the thermal stability of globular proteins. Protein sequences with a high aliphatic index are likely to remain stable across a wide range of temperatures. The decreased thermal stability suggests a more pliable structure. The indices of the Alpha-2-purothionin precursor and Vicin-like antimicrobial peptide in GRAVY were evaluated as -0.001 and -0.116, respectively. The negative grand average of hydropathicity signifies that proteins possess a polar, hydrophilic nature, resulting in enhanced interaction between the protein and water. These data suggest that the presence of ionisable amino acids on the protein surface, which are accessible to water, is the primary factor that affects the isoelectric points (pIs) of proteins and protein complexes.

### *Secondary structure*

The secondary structure of the Alpha-2-purothionin precursor consists of 38.97% alpha helices, 38.97% random coils, 14.71% extended strands and 7.35% beta turns. The Vicin-like antimicrobial peptide exhibits a secondary structure consisting of 35.81% random coils, 29.73% alpha helix, 27.03% extended strands and 7.34% beta turns. The secondary structural components of the Alpha-2-purothionin precursor and Vicin-like antimicrobial peptide are presented in Table 5 and Fig. 7. The secondary structure provides information on the conformation of a specific amino acid, indicating whether it is located in a helix, strand, or coil. The findings suggest that alpha helices and random coils are the most prevalent secondary structure elements, followed by prolonged strands and beta turns in all sequences.

Determining a protein's secondary structural configuration aids in comprehending the hydrogen bonds that exist within the protein, providing insights into its structural and functional efficacy. Buxbaum (35) stated that random coils play crucial roles in proteins by providing flexibility and facilitating conformational changes. The greater coil percentage is due to the abundance of highly pliable glycine and hydrophobic proline amino acids. Proline possesses the distinctive characteristics of inducing bends in polypeptide chains and disturbing organised secondary structures (36). Neelamathi *et al.* (37) reported that the prevalence of the coiled sections suggests a significant degree of preservation and robustness of the protein structure. The presence of hydrophobic residues in a composition leads to favourable interactions with the hydrophobic lipid bilayer (38). Shelar *et al.* (39) proposed that the structures of α-helical

**Table 5.** Secondary structural elements of Alpha-2-purothionin precursor and Vicin-like antimicrobial peptide

| Structural elements | Alpha-2-purothionin precursor | | Vicin-like antimicrobial peptide | |
|---|---|---|---|---|
| | No. of residues | % of residues | No. of residues | % of residues |
| Alpha helix (Hh) | 53 | 38.97% | 44 | 29.73% |
| 310 helix (Gg) | 0 | 0.00% | 0 | 0.00% |
| Pi helix (Ii) | 0 | 0.00% | 0 | 0.00% |
| Beta bridge (Bb) | 0 | 0.00% | 0 | 0.00% |
| Extended strand (Ee) | 20 | 14.71% | 40 | 27.03% |
| Beta turn (Tt) | 10 | 7.35% | 11 | 7.43% |
| Bend region (Ss) | 0 | 0.00% | 0 | 0.00% |
| Random coil (Cc) | 53 | 38.97% | 53 | 35.81% |
| Ambiguous states | 0 | 0.00% | 0 | 0.00% |
| Other states | 0 | 0.00% | 0 | 0.00% |

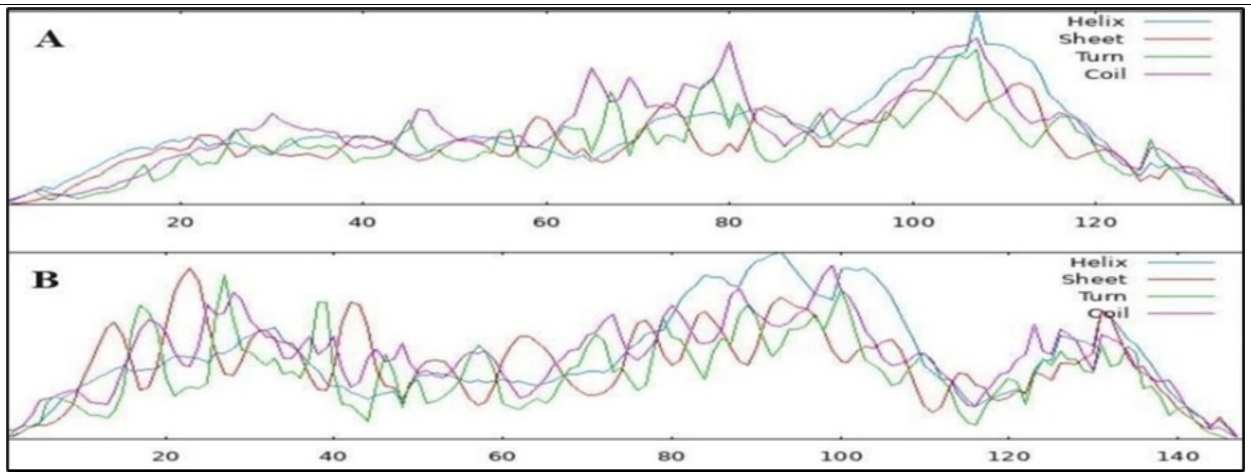


**Fig. 7.** Spectra of secondary structural elements. A) Alpha-2-purothionin precursor B) Vicin-like antimicrobial peptide.

proteins determine their diverse functions. These functions include signal recognition, receptor activity, transport of ions and molecules across membranes, energy transfer and preservation. The extended structure of α-helical proteins may also contribute to sliding motion and dynamic behavior, both of which are essential for their proper functioning.

### *3D structure*

The identified protein sequences were used to construct 3D models using the I-TASSER server. The predicted 3D model of Alpha-2-purothionin precursor with the best C-Score (-3.17) has an estimated accuracy of 0.36±0.12 (TM Scora) and 11.8±4.5A° (RMSD). Furthermore, the 3D model of Alpha-2-purothionin precursor protein has the best Z-score with the PDB ID:1nbl. The 3D model of Vicin-like antimicrobial peptide exhibited the highest Z-score with PDB ID: 1fxzA and the model with the most favourable C-score (-1.22) was selected as a suitable model, which was projected to have an accuracy of 0.56±0.15 (TM Score) and a deviation of 7.3±4.2A° (RMSD). The 3D models of the Alpha-2-purothionin precursor and Vicin-like antimicrobial peptide are displayed in Fig. 8. The structural alignment programme indicates that the protein sequence of Alpha-2-purothionin and Vicin-like antimicrobial peptide shows substantial structural homology to 2hnhA and 5cadA2. In addition, due to their structural analogy, they exhibit a comparable function to 2hnhA and 5cadA2. Fig. 9 displays the projected structural alignment of the Alpha-2-purothionin precursor protein and Vicin-like antimicrobial peptide with their respective templates.

Threading enhances the accuracy of sequence alignment by incorporating structural data that pertains to the secondary or tertiary structural characteristics of proteins. Employing ab initio

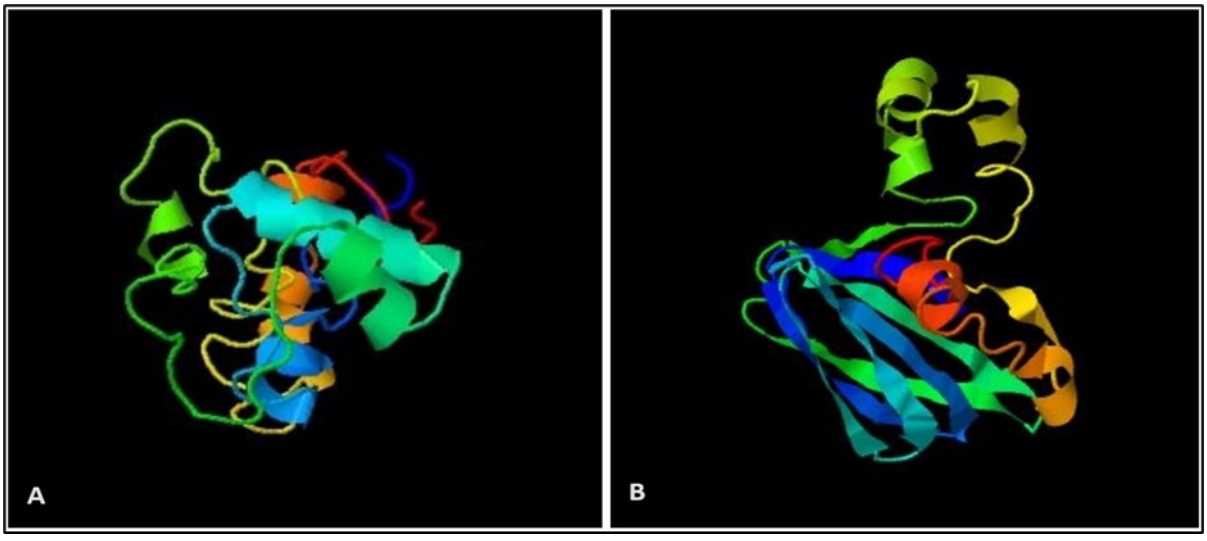


**Fig. 8.** I-TASSER predicted 3D models. A) Alpha-2-purothionin precursor B) Vicin-like antimicrobial peptide.

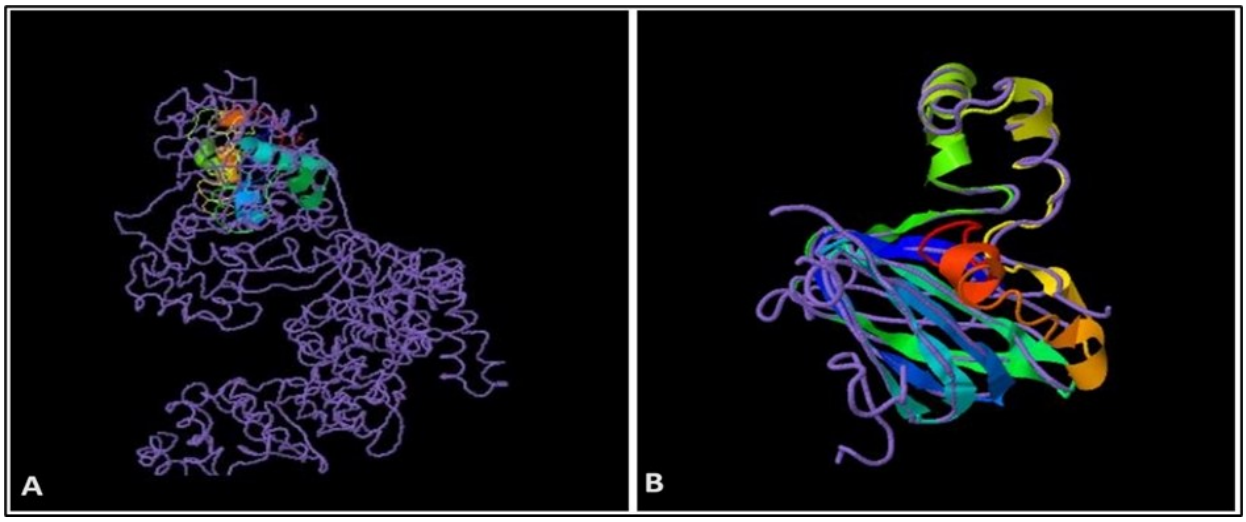


**Fig. 9.** Structural alignment with the template. A) Alpha-2-purothionin precursor B) Vicin-like antimicrobial peptide.

techniques to systematically investigate an extensive dynamic domain of protein molecules, aiming to determine the paths that guide proteins towards their native conformations. These approaches rely on the premise that the native structure of a protein molecule represents the state of minimum free energy compared to all other potential conformations. Arshad *et al.* (40) employed I-TASSER and PROCHECK to forecast and authenticate the three-dimensional configuration of silicon transporters. They also anticipated the probable functions based on gene ontology (GO) terms in the I-TASSER system.

### *Model validation*

The structural validation of the models, which includes analysing the geometrical features of the backbone conformations, was performed using Ramachandran plot calculations with the PROCHECK programme. Ramachandran plot of the Alpha-2-purothionin precursor shows (Fig. 10) 48.3% of residues are in the most favoured regions, 38.1% residues are in the additional allowed regions, 6.8% residues are in the generously allowed regions and 6.8% are in the disallowed regions. The Ramachandran plot of the Vicin-like antimicrobial peptide shows (Fig. 11) 68% of residues are in the most favoured regions, 28.1% are in the additional allowed regions, 3.1% are in the generously allowed regions and 0.8% are in the disallowed regions. The findings indicate that most of the amino acid phi-psi distributions

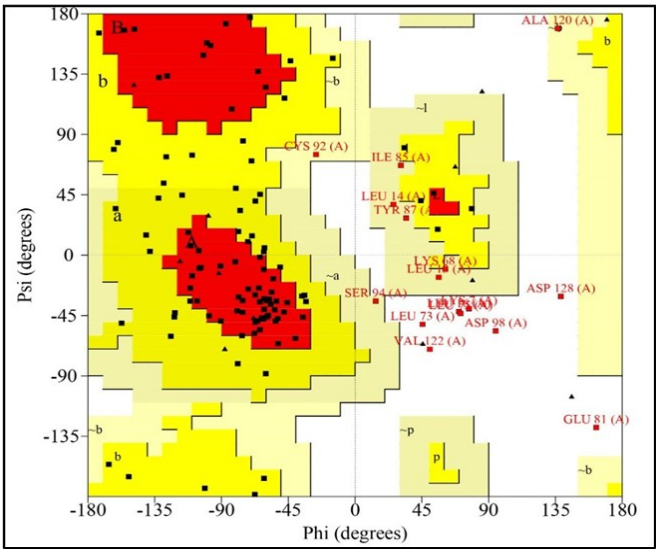


**Fig. 10.** Structural validation of Alpha-2-purothionin precursor protein model by Ramachandran plot using PROCHECK server.

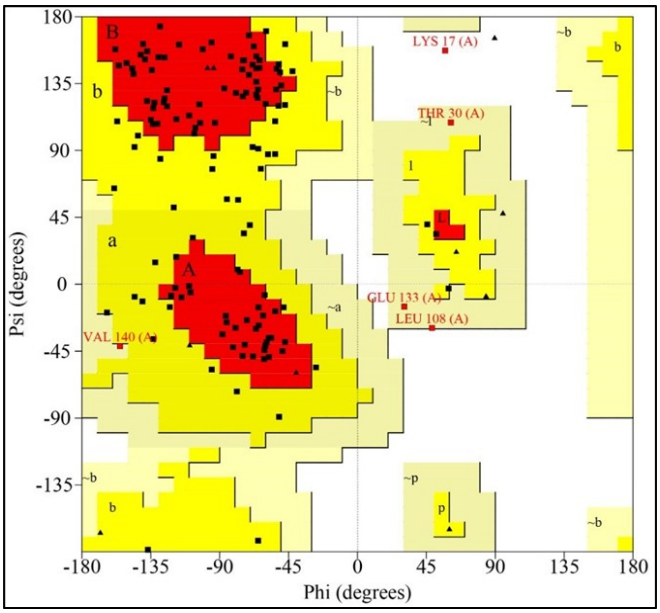


**Fig. 11.** Structural validation of Vicin-like antimicrobial peptide model by Ramachandran plot using PROCHECK server.

conform to a right-handed α-helix and the models exhibited high reliability and stability. The results show that most of the amino-acid phi-psi distributions fit a right-handed α- helix and the models were very reliable and stable.

### *Functional annotation*

The projected function of the query protein was determined using functional analogues of the predicted 3D model and its confidence score. The GO term provides insight into the hypothetical function of the modelled structure. The 3D model of Alpha-2-purothionin was associated with GO:0043167 with a score of 0.32, which has the molecular function of binding to charged ions or groups of atoms. The Alpha-2-purothionin protein is engaged in the biological process associated with GO:0009060, which has a score of 0.09 and is specifically related to vesicle docking in exocytosis. The cellular component of the Alpha-2-purothionin is linked to the GO:0016021 term with a score of 0.09, which indicates that the protein is membrane-attached or embedded. The 3D model of Vicin-like antimicrobial peptide exhibited a strong association with the GO term GO:0045735 with a score of 0.85, which indicated that this peptide is involved in functions related to the storage of nutrients. The GO for biological processes and cellular components was not predicted for Vicin-like antimicrobial peptide.

Furthermore, the modelled Alpha-2-purothionin protein represents EC 2.8.4.1, which is responsible for catalysing the last step in methanogenesis. The potential residues for ligand binding site in the Alpha-2-purothionin protein are found to be $L^{42}$, $C^{43}$, $R^{46}$, $G^{47}$, $A^{48}$, $T^{54}$ and $V^{55}$(Fig. 12A). Modelled Vicin-like antimicrobial peptide representing EC 4.1.1.2, which exhibits oxalate carboxy-lyase function. The potential residues for ligand binding in the Vicin-like antimicrobial peptide were identified as $H^{4}$, $N^{6}$, $E^{11}$, $F^{51}$ and $G^{65}$ (Fig. 12B).

Liu *et al.* (41) proposed a technique that uses the resemblance of protein surface proteins to deduce GO terms associated with the protein. The terms are categorised into three distinct ontologies: molecular function (MF), biological process (BP) and cellular component (CC). These ontologies do not overlap with each other. Functional prediction relies on three distinct components: EC numbers, GO keywords and ligand-binding sites. The minimum value for the EC number of the anticipated functional analogues should be more than 1.1 (42). The GO can serve as a repository for retrieving genes that exhibit comparable functionality or are situated in the same cellular region (43). The GO is commonly employed to analyse the outcomes of high throughput studies. An option is to deduce the location or role of genes that exhibit either increased or decreased expression (44). Functional profiling uses the GO to identify the specific processes that vary between gene sets. The process involves employing a likelihood ratio test to ascertain whether there are significant differences in the representation of GO keywords between the two sets of genes (45).

## Conclusion

AMPs are a group of small proteins that have a wide range of antibacterial activities and play an important role in the defence system of plants. The findings of this study demonstrated that the antimicrobial peptides extracted from *Suaeda nigra* showed superior antibacterial efficacy against all the studied microbial species in comparison to *Suaeda maritima.* Furthermore, the study revealed that AMPs derived from *S. nigra* and *S. maritima* exhibited stronger inhibitory effects on fungal species compared to bacterial species. The PMF results revealed that AMPs isolated from *S. nigra* and *S. maritima* are Alpha-2-purothionin proteins and Vicin-like antimicrobial peptides. In addition, their structural modelling determined that they bear a strong resemblance to 1nbl and 1fxzA. The functional prediction determined that the modelled Alpha-2-purothionin protein corresponds to EC 2.8.4.1, which is responsible for catalysing the last step in methanogenesis. The Vicin-like antimicrobial peptide was represented by EC 4.1.1.2, which exhibits oxalate carboxy-lyase function.

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## Authors' contributions

RPG is working as a research scholar in the Department of Zoology, Andhra University, Visakhapatnam, Andhra Pradesh, India. She is the corresponding author for the paper manuscript entitled “Isolation, identification and *in silico* characterisation of antimicrobial peptides from mangrove plants *Suaeda nigra* and *Suaeda maritima*”. She carried out the protein isolation, screening, identification, insilico characterisation and drafting of manuscripts. MG is working as a Professor in Department of Zoology, Andhra University, Visakhapatnam. She acted as GRP’s research guide and participated in the design and planning of research work. She helped to write the paper by correcting the manuscript. YU is working as a research scholar in the Department of Zoology, Andhra University, Visakhapatnam. She contributed in performing the laboratory work. The final manuscript was read and approved by all the authors.

## Compliance with ethical standards

**Conflict of interest:** The authors declare that there is no conflict of interest.

**Ethical issues:** None

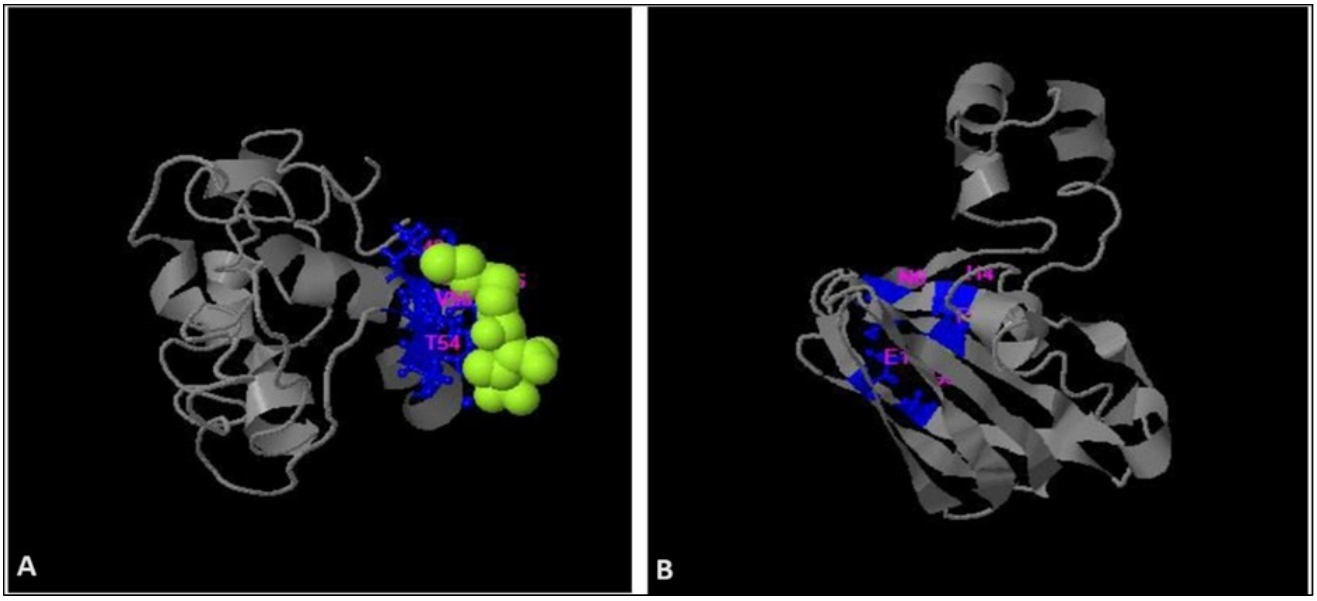


**Fig. 12.** Predicted ligand binding site residues. A) Alpha-2-purothionin B**)** Vicin-like antimicrobial peptide.

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