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A Facile Solution Phase Synthesis of Peptide Assemblies as Functional Moiety for Antimicrobial and Anticancer Peptides

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In recent years, human being are affected by various types of cancer and are highly microbial infected due to changes in environmental conditions and hence, the recovery from this cause of disease using peptide-based drugs as a function of both activities like antimicrobial peptides (AMPs) and anticancer peptides (ACPs). Herein, we report the solution phase peptide synthesis using α-amino acids such as alanine, phenylalanine and lysine. The structural parameters of the polypeptides were investigated by circular dichroism, FT-IR, MALDI-TOF ESI-mass, NMR, *etc.* Water-soluble hydrophilic-based synthesized polypeptides possess various biological applications throughout, especially antibacterial and anticancer activities–short reaction process, less cost of chemicals, minimum glassware and no hazardous chemicals involved. Multiple species have innate immune systems that contain antimicrobial peptides (AMPs). Most of them have been proved to possess dual action, both as antibacterial and anticancer peptides, making them most promising alternative to conventional compounds used today to treat these infections. Present approach offers unique opportunities for developing highly potent and focused antibacterial and anticancer peptide-based drugs.

Keywords: Antimicrobial peptides, Anticancer peptides, Solution phase peptide synthesis, Amino acids.

INTRODUCTION

Peptides are a compatible material in biomedical and drug delivery fields due to their significant applications to the relevant work [1]. In general, peptides synthesized from amino acids are based on the fundamental solution phase peptide synthesis method and BOC (*tert*-butyloxycarbonyl) chemistry protocol. However, the manufacturing of peptides on a large scale [2], often for industrial reasons, is still frequently carried out using solution-phase peptide synthesis. The peptides which are commercially prepared have hydrophilic in nature. Tripeptides containing lysine as monomer is water soluble and thus, promote its biological properties. The applications of various combination of the amino acid tripeptides focused on the drug design, bioimplants and evaluation for the studies, especially microbial applications. Amino acid residue made on the building block polymer-like peptide was synthesized by solution phase peptide synthesis [3] and a short sequence of molecular weight of water-soluble. A higher ratio of lysine amino acid supported the β-sheet conformer of the peptide's secondary structure [4].

This article focuses on the synthesis, characterization, amino acid sequence, optimization and microbial culture used for antimicrobial peptides (AMPs). Antimicrobial peptides are capable of following factors that influence amino acid structure and compositions. The Boc-amino acids in the peptide have an impact on every element. In order to create a polypeptide that would allow researchers to track the beginning of the peptide's obtained monomer's aggregation, amino acids were synthesized using *N*-carboxy anhydrides of protected α-amino acids and initiator-like base nucleophiles [5]. In past, polypeptides with a broad range of uses, such as the engineering of tissues, sensing, drug delivery systems and catalysis, have been prepared using the ring opening polymerization of *N*-carboxy anhydride [6]. The two significant steps involved were ring closure and ring opening. This technique could demonstrate

hydrophilic water-soluble peptide monomers [7], with biological and cancer cell studies applications [8].

The tripeptide, which is over-expressed in cancer cells and bacterial cells, is designed and synthesized based on the findings that are reported earlier [9,10]. Circular dichroism studies shows that peptides having secondary structures from the unordered system. These cationic peptide prefers to interact with the anionic membrane of cancer cells and bacterial cells and assists in determining the selective activity of particular cells.

EXPERIMENTAL

Boc-protected amino acids (alanine, phenylalanine and lysine) were purchased from Sigma-Aldrich and TCI chemicals. Dichloromethane (DCM), trifluoroacetic acid (TFA), ethylacetate, triphosgene, tetrahydrofuran (THF), *N*,*N*-dimethylformamide (DMF), hydrazinehydrate, diethylether, deionized water, acetonitrile HPLC gradient grade were purchased chemicals and solvents from Rankem. The DMSO-d_6 was used NMR spectral analysis. All the chemicals used without purification for synthesis and purification.

Synthesis of peptide: Short tripeptide was synthesized by 7 mmol of three amino acids sequence like AFK was taken 100 mL round bottom flask and added 40 mL of DCM, the reaction mixture was stirred for 2 h at room temperature after the solvent was evaporated. Boc-protective group was removed in this stage, washed with *n*-hexane in several times decanted and concentrated the solution in the flask. Meanwhile, TLC was used to monitor the synthesis of *N*-carboxy anhydride associated with protected amino acids. Then, proceed to next step of reaction with anhydrous ethyl acetate in 50 mL and 1 g triphosgene. The reaction mixture set up under reflux at boiling condition maintain temperature 80-85 ºC on 1 to 5 h followed without reflux only on stirring at 2 h in room temperature. The solvents were evaporated using a rotoevaporator then pale yellow solution obtained. Then THF was added with constant stirring for 3 h followed by the addition of hydrazine hydrate as base (**Scheme-I**). The precipitate formed was again evaporated using roto evaporator and the oily solution obtained ensured that amide carbonyl formation peak using IR spectrum after addition of diethyl ether in ice-cold condition for three times and centrifuged with diethyl ether followed by decanted ether layer washed with acetic acid, peptide was obtained as sticky white solid.

FTIR analysis: Peptide secondary structure was investigated using the FTIR method in the amide I, II and III region used as a small part of a sample ground up finely with KBr and formed into a pellet. A Perkin-Elmer spectrum two UATR FT-IR spectrophotometer was used to record data at the frequency range of 400 to 4000 cm^{-1}.

CD spectrum: Using a Jasco J-715 spectropolarimeter, the circular dichroism (CD) spectra were acquired. Various solvents were employed in a quartz cuvette 0.2 mg/mL solution (path length = 0.1 cm), including ethanol, water, buffer at pH 7.4 and mixtures of buffer with ethanol and buffer with water. The spectra were studied at the secondary structure of the peptide [11,12]. The data were acquired at 25 ºC with a scan rate of 50 nm/min and covered the wavelength range of 197 to 255 nm.

Matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) mass spectrometry: For characterizing tripeptides, matrix-assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI) mass spectrometry were used focusing on their utility in determining molecular weight [13-16]. The preferred matrix for peptides generated by enzymatic digestion is frequently-cyano (CHCA). The ultra flextreme (Bruker Daltonics, Bremen, Germany) reflector type time-of-flight (TOF) mass spectrometer was used for MALDI-TOF analysis. For peptides < 3000 Da, the mode with external calibration is typically 2 to 3 Da. The instrument was externally calibrated before the analysis using Bruker Daltonics reference protein or peptide mixes. The fixed sample concentration was 2 mg/mL and the MALDI-TOF MS recorded ranges from 500 to 1500 *m/z*.

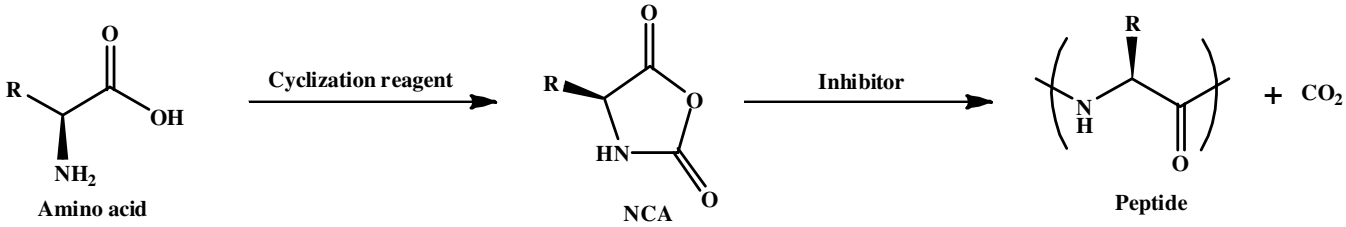
NMR analysis: In order to establish the structure of the peptide using ^{1}H NMR, spectra were collected using a Bruker DMX 400 spectrometer, which would be used to record the NMR spectra at a proton resonance frequency of 400 MHz. The samples were dissolved in deuterated solvent like DMSO-d_6 for recording using the NMR spectra.

Antimicrobial studies

Antibacterial studies: Even Gram-positive and Gram-negative bacteria are resistant to the antibacterial effects of peptide (AFK) [17-19]. The excellent diffusion approach measures the activity of peptides at various concentrations of peptides [20]. Cotton swabs were used to evenly distribute a fresh overnight (24 h) bacterial culture (1×10^5 cells/mL) on top of the nutritional agar. Then, 6 mm holes were punctured using a sterile cork borer. The peptides were loaded with different concentration (25, 50 and 75 µL) into the pits using aseptic techniques. Ampicillin (10 mg/mL) was used as the positive control sample for all tests. The test plates were then incubated for 24 h at 372 ºC to determine the zone of inhibition.

Anticancer studies

Culture of cells: American Type Culture Collection (ATCC), based in the USA, provided the MDA-MB-231 cell line for



Scheme-I: Synthesis of peptide

this study. The antibiotic-antimycotic solution, including streptomycin, amphotericin B and penicillin, was added to 10% heat inactivated fetal bovine serum (FBS) in Dulbecco's modified eagle medium (DMEM), which was used to sustain the cells. The adherent cells were separated during sub-culturing using 0.25% trypsin-ethylene diamine tetraacetic acid (EDTA). Cell culture flasks were kept at 37 ºC in a humidified environment with 5% CO_2.

Cell proliferation assay: The effect of the tripeptide sample on the proliferation of MDA-MB-231 cells was assessed using the water-soluble tetrazolium-1 proliferative assay (WST-1) [9,21]. In a 96-well plate, 100 µL of MDA-MB-231 cells were planted at a density of 2×10^4 cells/wells. The cells were exposed to each substance at specific doses (1 µg and 5 µg) for 24 h after the first 24 h. Cells that were not selected served as control. Subsequent to the treatment, 5 mL of WST-1 reagent was administered to each well, followed by an incubation period of 1 h at 37 ºC. Using a multi-mode plate reader (BioTek, USA), the absorbance was determined at 450 nm. Additionally, a phase contrast microscope (Leica, Germany) was used to view the cells at a 10X magnification and images were taken.

RESULTS AND DISCUSSION

The short peptide synthesis was carried out by solution phase peptide synthesis method. The primary benefit is ability to separate and purify the intermediate molecules after each synthesis step, deprotected and recombined to generate larger peptides of the required sequence. This method involves the synthesize of short peptides made up of only a few amino acid residues like alanine, phenylalanine and lysine. The characterization of the tripeptide (Fig. 1) using various sophisticated instrument techniques was examined, after characterization studies, followed by the biological activity or applications' vital role in peptide synthesis work were also evaluated.

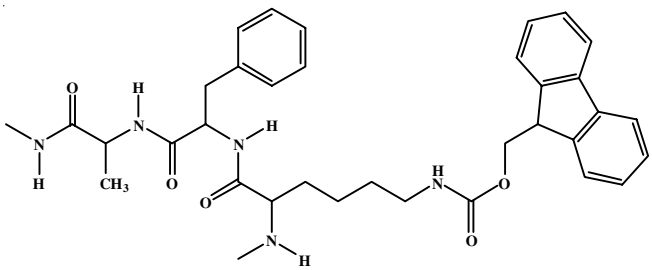


Fig. 1. Primary structure of tripeptide

FT-IR spectral studies: The vibration in amide bonds shows the presence of C=O, C-N and N-H groups in the characteristic peptides. However, the actual data from the spectrum band range at 1686 cm^{-1} was attributed to strands with the β-sheet aggregates (Fig. 2). Polypeptide examined the IR spectrum range of the amide-I band from 1700-1600 cm^{-1}. In contrast, data from the spectrum band region at 1532 cm^{-1} was assigned to the β-sheet, amide II bands from 1580-1480 cm^{-1}. However, data from the spectrum band region at 1247 cm^{-1} was transferred to the β-sheet, amide III bands from 1300-1230 cm^{-1}. The presence of N-H, O-H and C-H in the spectrum region was associated with the appearance of three peaks in the band area at 3037, 3331 and 3426 cm^{-1}, respectively (Fig. 2).

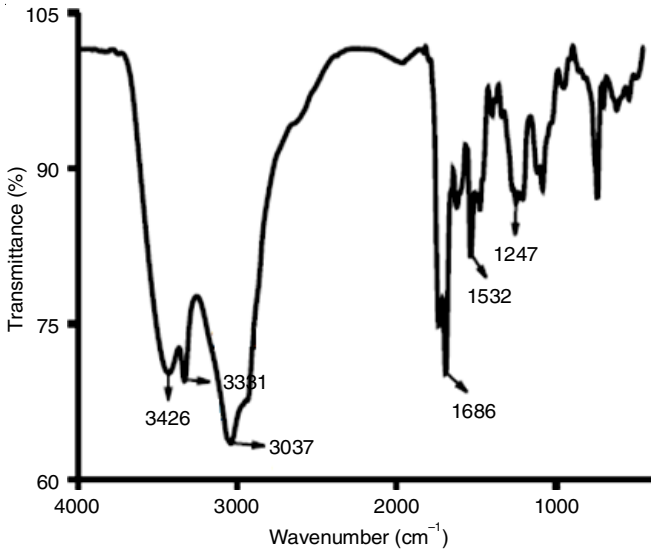


Fig. 2. FTIR spectrum of tripeptide

CD spectral studies: The circular dichroism (CD) spectra were recorded in two prominent bands with centers at ~ 214 nm and ~200 nm. Unusually, there was no negative CD signal seen above 195 nm (Fig. 3). This existence of a negative CD shows that peptide does not have an unordered structure. The presence of two sorts of conformations, presumably including β-sheet and β-turn facilities. This is indicated by the two forms of positive CD bands. The literature shows that a single peptide can have different types of β-sheets and β-turns.

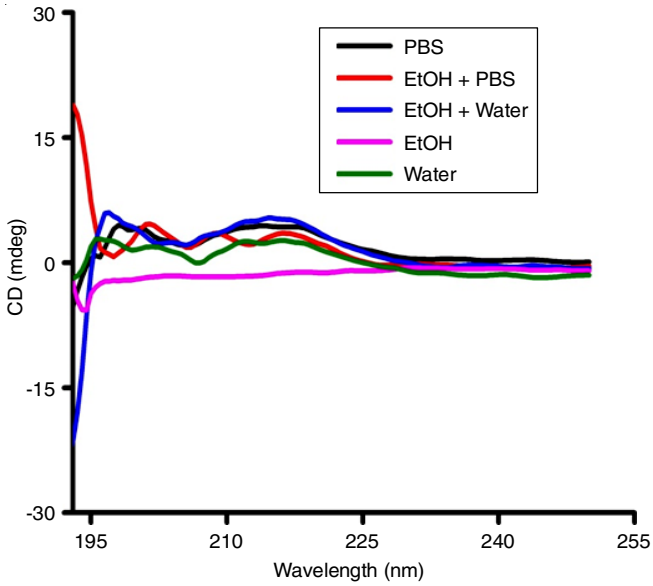


Fig. 3. CD spectrum of peptide

^{1}H NMR spectral studies: The chemical shift values have represented the backbone of the peptide N-H bond and aromatic protons region. Fig. 4 illustrates the peptide's numerous proton signals, including aliphatic, aromatic, OH and amide NH signals. Consequently, the polypeptide that was synthesized exhibited completion and possessed all of its functional groups.

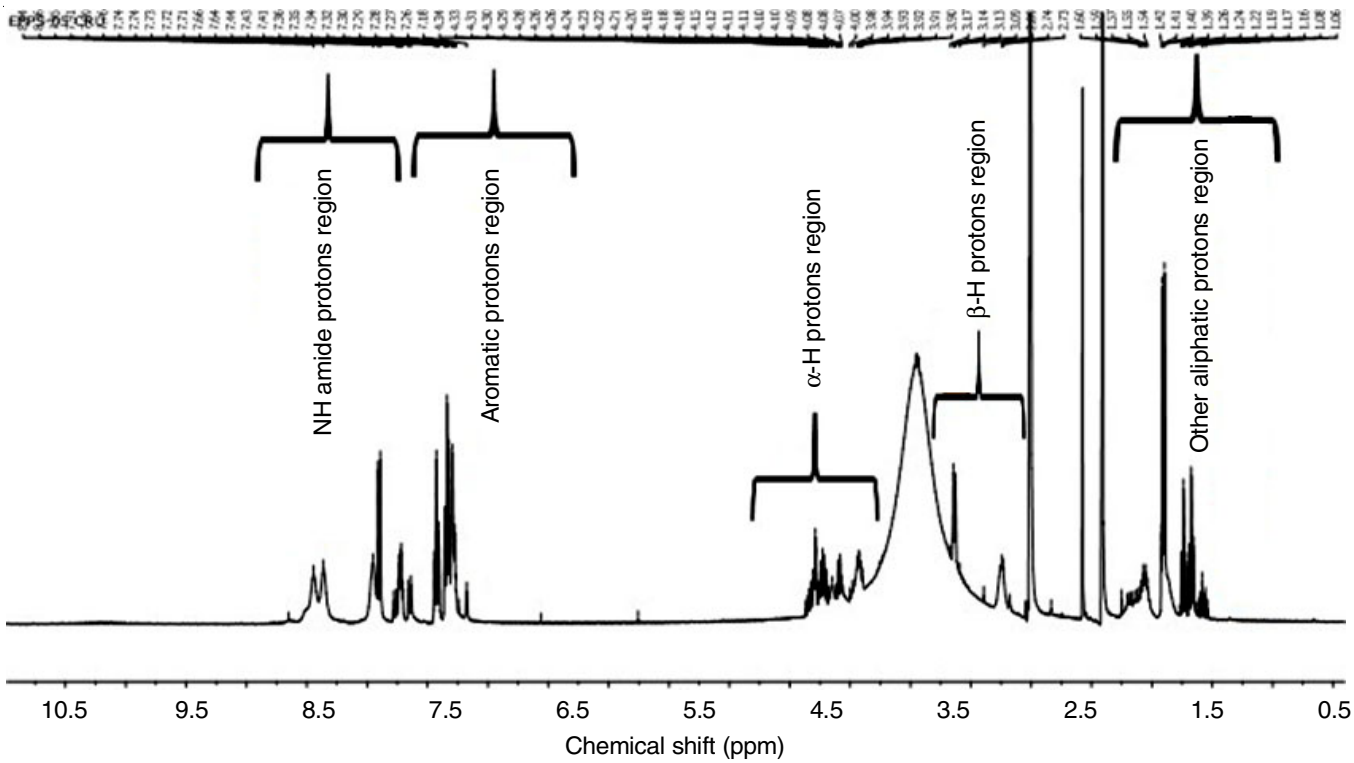


Fig. 4. ^{1}H NMR chemical shifts value for the peptide

MALDI-TOF mass spectral studies: The MALDI-TOF spectrum analyzed crude peptides. The multiply charged ions produced during ionization were analyzed to obtain the mono-isotopic peptide mass (*m/z*). The theoretical mass value is 616.865 and the observed MALDI-TOF mass spectra recorded with water molecule is 634.456 (Fig. 5).

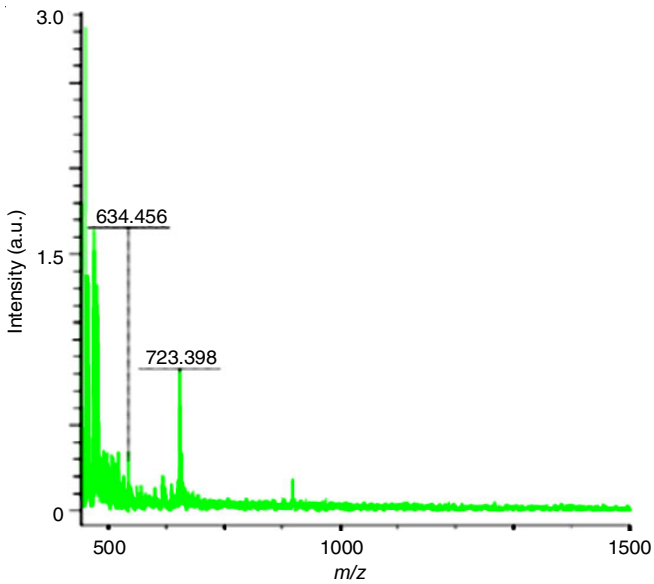


Fig. 5. MALDI-TOF mass spectra of peptide

Antibacterial activity: The peptide was evaluated as antibacterial activity by well diffusion method using nutritional agar medium. Then the antibacterial activity was assessed by measuring the diameter of the zone of inhibition. The results showed that the Gram-positive and Gram-negative bacteria treated with varying peptide concentrations at 25, 50 and 75 µL have better antimicrobial activity (Fig. 6).

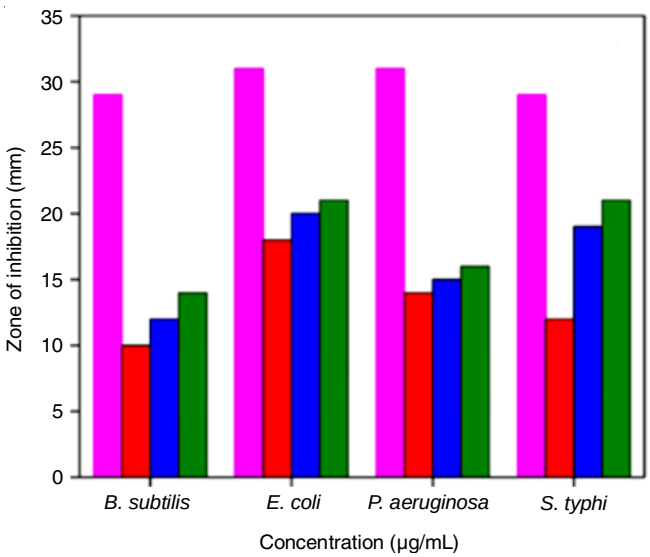


Fig. 6. Tripeptide concentration in 25, 50, 75 and 100 µL

Anticancer activity: Fig. 7 exhibits the effectiveness of the synthesized tripeptide in inhibiting proliferation in human breast cancer (MDA-MB-231) cells. The maximum growth inhibition was observed at 5 µg concentration with 42% inhibition. When the cells were treated with 1 µg concentration, the tripeptide exhibited growth inhibition by15 % (Fig. 7). These data indicated that the tripeptide has potential anticancer properties against breast cancer (MBA-MB-231) cells. The

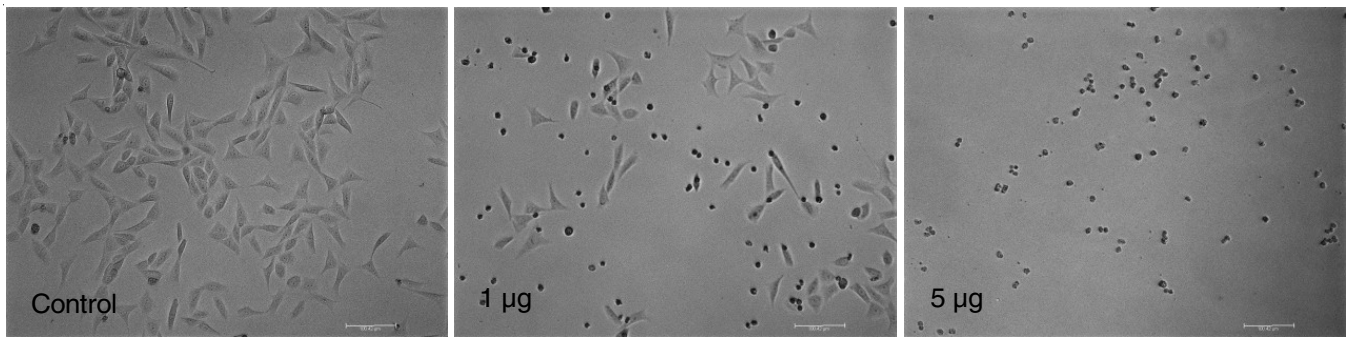


Fig. 8. Tripeptide impact on the growth of human breast cancer (MDA-MB-231) cells

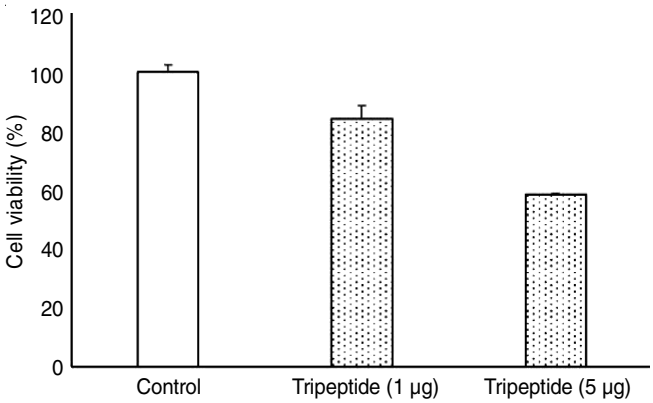


Fig. 7.

microscopic finding supports the results from the cell proliferation assay, which showed that 5 µg peptide-treated cells had decreased cell counts and an increase in dead cells (Fig. 8). Also, a considerable amount of cell death was observed in 1 µg peptide-treated cells.

Conclusion

The synthesis of a simple tripeptide was achieved by solution phase peptide synthesis. The sequence was based on the designed peptide and moderate and polar amino acids like alanine, phenylalanine and lysine. Particularly, lysine has more biologically relevant cationic amino acid characteristics. The analytical techniques were used for characterization and structural investigations to identify short peptides. The synthesized tripeptide was used to assess antimicrobial activity, including the antibacterial and anticancer capabilities. According to the findings of this study, the tripeptide (AFK) must be more effective for biological applications in suitable antimicrobial peptides and anticancer peptides.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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