International Journal of Theoretical & Applied Sciences, 9(2): 92-98(2017)

ISSN No. (Print): 0975-1718
ISSN No. (Online): 2249-3247

Anti-Microbial Effect of Truncated Indolcidin

***Swati Saxena*[*]*, Dipti Bharti*[*] *and Meenakshi Sharma*[**]**
[*]*Department of Applied Sciences, Greater Noida Institute of Technology, Greater Noida*
Plot No. 7, Knowledge Park-II, Greater Noida, (Uttar Pradesh), INDIA
[**]*IEC University Baddi, (Himachal Pradesh), INDIA*

(Corresponding author: Dipti Bharti)
(Received 22 July, 2017 accepted 28 August, 2017)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: Indolicidin is a host defense tri-decapeptide that inhibits catalytic activity of HIV-1 integrase in vitro and it is known to have a broad spectrum for its antimicrobial properties. This is likely due to the partitioning of Indolicidin into membrane lipid bilayer against gram positive and gram negative bacteria. Toxicity of truncated version of indolicidin varied experiments has been done on *Escherichia coli* (*E. coli*) DH5α strain, as the representative gram negative bacteria. Bacteriostatic effect of truncated indolicidin i.e PWWP (proline, tryptophan, tryptophan, proline) was shown in different set of experiments like turbidometry method, colony counting method & agar disc assay. By taking 50 µm 61.7% of growth inhibition found within 9 hr and on 25 µm 36.70 % of inhibition was found to be within 15hr. The number of colonies decreased with increase in the concentration of PWWP. Decrease in the intensity of the bands at a higher dose of PWWP. The investigation of genomic pattern of *E. coli* this shows that there is certain level of DNA degradation in the genomic DNA of *E. coli* of the treated sample have also been carried out in this investigation.

Key words*:* Indolicidin, antimicrobial activity, genomic DNA of *E. coli*, Plasmid DNA of *E. coli*

I. INTRODUCTION

Antimicrobial properties has been studied widely by different researchers [1-4]. Antimicrobial peptides are evolutionarily ancient weapons. Although these models are helpful for defining mechanisms of antimicrobial peptide activity, their relevance to how peptides damage and kill microorganisms still need to be clarified. Recently, there has been speculation that transmembrane pore formation is not only the mechanism of microbial killing infect it suggest that translocated peptides can alter cytoplasmic membrane septum formation, inhibit cell-wall synthesis, inhibit nucleic-acid synthesis, inhibit protein synthesis etc. [5-7].

Peptide and protein self-assembly is a well-studied phenomenon in chemistry and biology, where nanoscopic building blocks exhibit rapid self-association to reveal supramolecular aggregates of defined structural features. These superstructures are stabilized by hydrophobic interactions, hydrogen bonding and a host of other noncovalent interactions. Thus amino acid side chains in the primary structure hold importance in dictating secondary structures and preference for particular conformational signatures in peptide aggregates. Peptide-based self-assembled structures respond to a variety of factors such as pH, ionic strength, concentration, and hydrophobicity and hydrophilicity of amino acid side-chain [8-9].

Indolicidin is one of the member of cathelicidns family. Indolicidin has been isolated from the cytoplasmic granules of bovine neutrophils and belongs to the cathelicidin family of the host defense peptides (HDPs). HDPs play a key role in the innate host defense system and are thought to constitute the first line of defense against invading microorganisms [10].

Indolicidin is a 13-amino acid cationic antimicrobial peptide isolated from the cytoplasmic granules of bovine neutrophils. The shortest known natural-occurring antimicrobial peptide, indolicidin is toxic to both prokaryotes and eukaryotes and acts as an inhibitor/modulator for various enzymes [11-13].

The methyl esterification of indolicidin's carboxyl terminus increased its activity for Gram-negative and Gram-positive bacteria. In Gram-negative bacteria this was associated with an increased binding to lipopolysaccharide and increased permeabilization of the outer membrane.

Antimicrobial peptides have targeted a surprising but clearly fundamental difference in the design of the membranes of microbes and multicellular animals, best understood for bacterial targets. Bacterial membranes are organized in such a way that the outermost leaflet of the bilayer, the surface exposed to the outer world, is heavily populated by lipids with negatively charged phospholipid head groups.

In contrast, the outer leaflet of the membranes of plants and animals is composed principally of lipids with no net charge; most of the lipids with negatively charged headgroups are segregated into the inner leaflet, facing the cytoplasm [15-17].

Because of its broad spectrum of activity, indolicidin may prove a good candidate for therapeutic use as was recently demonstrated in an *in vivo* antifungal study using liposome entrapped peptide. However, because of its small size, unique composition, and potentially different secondary structure,it may have a specific mode of action distinct from the other natural antimicrobial peptides described above [18].

Here we investigated the mechanism of action of indolicidin and the effect of carboxyl-terminal modification of the peptide by examining its ability to bind and permeabilize Gram-negative bacteria membranes and its effect on planar lipid bilayers [18-21].

Indolicidin has been known to have a broad spectrum of antimicrobial activities against Gram negative and positive bacteria. Its eight analogues were chemically synthesized. The analogue design was based on the analysis of sequence to elucidate the role of some residues in the antibacterial mechanism of Indolicidin. Bactericidal activities were assayed against *Escherichia coli* the membrane perturbing abilities of the peptides was assayed using a dye containing liposome. These results suggest that proline and cationic residues are important in the bactericidal activity of Indolicidin. The killing of Gram-negative bacteria due to indolicidin was immediate resulting in several log orders of death within minutes of adding peptide at a concentration equivalent to the MIC [22-26].

II. MATERIAL AND METHODS

Physical Characterization of truncated version of Indolicidin:

Chemical Structure of Truncated Indolicidin: (Fig. 1)

Molar mass and solubility:

Calculated for (M+H) + ($C_{56}H_{77}N_8O_{18}S_2$) =1213.4797

It is completely soluble in methanol, dimethyl formamide and moderately soluble in dimethyl sulphoxide dichloro methane.

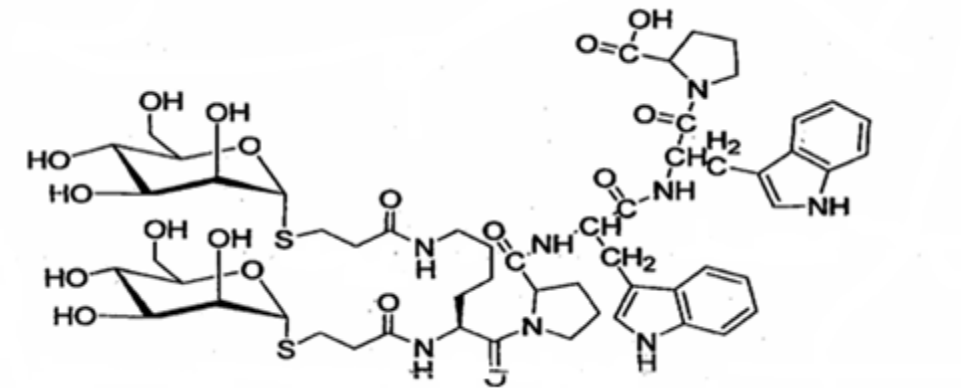


Fig. 1. Chemical Structure of Truncated Indolicidin.
(Mannose mercuptopropionic acid)$_2$ lysine-proline-trytophan-trytophan proline.

Stock sample preparation PWWP: The stock samples were prepared for two different concentrations: 50µm/500µl & 250µl/25µm. For making 50µm/500µl concentrations the amount of 0.00065 gm of PWWP was dissolved in 3.076ml of Phosphate-buffered saline (PBS). For 250µl/25µm concentration, 1/10th dilution of 50µM/500µl concentration was done by Vortex, both the concentrations for uniformly mix and store the samples at 4°C.

Treatment of sample with culture: 20 ml of NB was prepared in three different flasks and autoclaved for 15 mins. Then 100µl overnight culture of *E. coli* was added in each flux under sterile conditions. After this, 100µl of 25µM and 50 µM of stock PWWP samples were added in two flasks respectively. One flask was used as control. The entire flasks were incubated at 37°C for 24 hrs in the incubator.

Measurement of the optical density: Optical density was measured in a spectrophotometer which is used as a measure of the concentration of bacteria in suspension. As visible light passes through a cell suspension the light is scattered. Greater scatter indicates that more bacteria or other material is present. The amount of light scatter is measured in a spectrophotometer.

Typically, the optical density at a particular wavelength (λ=600nm) correlates with the mid log phase of bacterial growth. 2ml of distilled water (blank) was taken into cuvettes for reference. Then, 2ml of nutrient broths taken as control and each culture sample (24 hrs old) were taken into other labeled cuvettes. The cuvettes were placed in the multi sample cuvettes holder. The fixed wavelength λ=600 nm was selected for optical density. Absorbance was recorded, same procedure was followed for treatment of sample and optical density measurement was again taken for 48hrs old sample.

Preparation and serial dilution of sample (24 Hrs. & 48 Hrs)

10 ml of distilled water (DW) were taken into 9 blank test tubes each. Test tubes were plugged using cotton and then autoclaved at 121°C for 15 mins. Dilution blanks were cooled at least 42°C before using 100µl of sample (50µM PWWP) was taken from stock and dispensed into 9 ml of sterile distilled water diluents (1:10 dilution). In a similar manner further dilutions were prepares according to the requirements for same concentrations till 10^6. Same procedure is repeated for 25µM PWWP treated samples (24 hrs & 48 hrs) [31].

Planting and colony counting: 200µl of aliquot was transferred from selected dilutions into sterile petriplate.10-15 ml of nutrient agar was poured into and mixed in clockwise & anticlockwise direction. Plates were then kept at room temperature till the agar solidified. After solidification the plates were incubated at 37 °C for 24 hrs and 48 hrs. Acceptable plates of 10^{-6} and 10^{-7} dilutions were counted for the effect of PWWP for 50µm and 25µm concentrations.

Colonies were counted using the formula by Vincent formula [27].

$$\frac{CCcontrol - CC\ sample}{CC\ control}$$

Agar disc assay: Antibacterial activity of PWWP was determined against *E. coli* using the paper disc assay method [28].

Requirements: Nutrient broth**,** *E. coli* culture**,** Bacterial agar (soft agar), Nutrient agar**,** Sterilized discs, Drug samples of different concentrations: 50 & 100 µg/ml.

Method: Briefly, in the nutrient broth culture was revived by incubating at 37°C for 24 hrs. Nutrient agar plates were surface inoculated & uniformly spread with soft agar containing culture .Wattman no.1 filter paper disc of 6mm were sterilized at 121°C for 15 mins. Sterile discs were impregnated with sample Indolicidin of different concentration. The impregnated discs were place on NA plates along with disc without drug acting as control. Plates were incubated at 37°C for 24 hrs. zone of growth inhibition around each disc was measured in centimeters.

Isolation of genomic DNA from *E. coli*: Genomic DNA is the tota DNA extracted from the cells of the organism.

Requirements

Overnight culture of *E. coli*., Nutrient broth medium, Isopropanol, Bromophenol blue dye, Ethidium bromide, Tae buffer stock, GTE mix, 1% SDS, 10 mM NaCl, Agarose gel (0.8% in tae buffer).

Method: 1.5 ml of overnight grown culture was taken in a microfuge tube capacity 1.5ml. Centrifuged at 10,000 rpm for 5 mins at RT to obtain the pellet. The supernatant was discarded and 200 µl of gte mix was added and then the pellet was broken by knuckles. Incubated for 5 mins. 400ul 1% SDS was added and kept on the ice for 5 mins. Then, 60 ul of 10mM NaCl and 700µl of isopropanol was added. Centrifuged at 10,000 rpm for 20 minutes. Decanted the alcohol and air dry. The samples were mixed by invert mix. 50ul DW (Distilled water) was added to dissolve the pellet obtained. Centrifuged at 10,000 rpm for 5 minutes .The supernatant was taken in a fresh microfuge tube and subject it to gel electrophoresis [29-30].

Preparation of gel: 1.8 gms agarose powder was added in a flask containing 100 ml of 0.5 T.A.E. buffer the mixture was heated in a microwave oven until the agrose dissolved and the solution became clear. Clean gel casting mould was taken, placed it horizontally on the table and leveled it using the equilibrium bubble. The comb 0.5-1mm was positioned above the plate so that a complete well is formed when the agrose is added. The warm agarose gel solution (35-40°C) was poured into the mold. There should not be any air bubble between or under the teeth of the comb. After the gel completely solidified (30-40mins RT) the comb was carefully removed. Then, electrophoresis buffer (0.5X TAE) was added to cover the gel. The samples of DNA (15µl) were mixed with the tracking dye bromophenol blue dye (1.5µl of 10X stock). Slowly the mixture was loaded into slots of the submerged gel using disposable micro tips. Then, the electrical leads were attached so that the DNA could migrate towards the anode (red lead). A voltage of 60-80 volts was applied. The gel was allowed to run until the bromophenol blue dye migrated the appropriate distance ($3/4^{th}$) through the gel. After this the electric current was turned off and the gel was removed from the gel tank. Then the gel was stained with Ethidium bromide (etbr strains), for 100ml stains 5ul stock was added for 20-30 mins. Finally the gel was visualized in a UV illuminator [29, 30].

Isolation of plasmid DNA from *E. coli*: A plasmid is a DNA molecule that is separated and can replicate independently of the chromosomal DNA. Plasmids are considered transferable genetic elements or "replicons", capable of autonomous replication within a suitable host [29].

Requirments: Overnight culture of *E. coli .,* Nutrient Broth medium, Isopropanol, Bromophenol blue, Ethdium bromide, Tae buffer, Gte mix, 1% SDS, NaOH, Potassium acetate, Agarose gel.

Method: Innoculate *E. coli* culture containing plasmid into 5 ml of medium in a test tube containing 150ug/ml ampicillin. Alternatively 50 ml of culture can be raised in a 250ml of conical flask. Take 1.5 ml of overnight grown culture harbouring the plasmid dna in a microfuge tube (of 1.5ml). Centrifuge at 8,000 rpm for minutes at room temperature. Add 200µl of GTE mix and mix gently, incubate for 5min at room temperature. Add 400µl 1 % of SDS and 0.2 NaOH mix well and kept on ice for 5 minutes. Add 200µl 3M KOAC pH 4.8 invert mix. Centrifuge at 10,000 rpm for 20 minutes. Take the supernatant in a microfuge tube. Add equal volume of isopropanol. Keep at room temperature for 5 mins. Centrifuge at 10,000 rpm for 20 minutes. Decant and dry in air. Dissolve in 50µl of distilled water. Load on the gel. In case the isolated plasmid is to be used for digestion with restriction enzyme, it can be further washed with 100µl of 70% of ethanol. For ethanol wash add 100µl of 70% of ethanol and centrifuge at 10,000rpm discard the supernatant air dry the pellet and dissolve in 50µl of distilled water [29-30].

Preparation of gel: The protocol used for the Preparation of gel is same as that of isolation of genomic DNA from *E. coli* [29].

III. RESULTS

Optical density measured in spectrophotometer, used as a measure of the concentration of bacteria in suspension. Greater scatter indicates that more bacteria or other material is present. Typically the optical density at a particular wavelength (λ=600nm) correlates with the mid log phase of bacterial growth. The reduction in the turbidity in the culture containing sample (PWWP) could be seen in the comparison in the pure culture that is without PWWP. This shows growth inhibitory properties of PWWP in different concentration (Table 1,2).

Table 1: Showing O.D. of 24hrs old sample.

	Control	PWWP (50μM)	PWWP(25 µM)
O.D	2.022	1.724	1.528

Table 2: Showing O.D. of 48hrs old sample.

	Control	PWWP (50μM)	PWWP(25μM)
O.D	6.024	2.826	2.306

Antibacterial effect of Indolicidin By measuring optical density: A known volume of the 24 hrs & 48hrs grown *E. coli* culture that was in logarithmic phase was incubated with PWWP (50µM & 35 µM) and its effect on the bacterial growth was monitored. The difference in turbidity of sample showed growth inhibitory property of the sample.

The reduction in the turbidity in the culture containing sample (PWWP) could be seen in the comparison in the pure culture that is without pwwp.

This shows the growth inhibitory properties of the PWWP in different concentration.

As seen from 24hrs data, the number of colonies decreased with increase in the concentration of PWWP. So, the % inhibition of higher dose of PWWP was more.

As seen from 24 hrs data the number of colonies decreased with the number of colonies in control both in *E. coli* & *Pseudomonas.* So, the % inhibition growth of 25µM dose of peptide was much more than 50µM dose of peptide (Table 3,4). Retardation of bacterial growth due to PWWP could be of the two patterns: bacterial (complete killing) or bacteriostatic (inhibition of growth). Since no clear zones were in the number of colonies around the disc proved bacteriostatic effect of PWWP (Fig. 2, 3).

Table 3: Showing No. of colonies of 24 hrs old sample.

	Control	24 hrs.	% Inhibition
PWWP 50 μM	23.5	9	61.702%
PWWP 25 μM	23.5	15	36.702%

Table 4: Showing No. of colonies of 48 hrs old sample.

	Control	48hrs.	% Inhibition
PWWP 50 μM	29.5	18	0.38%
PWWP 25 μM	29.5	7	0.42%

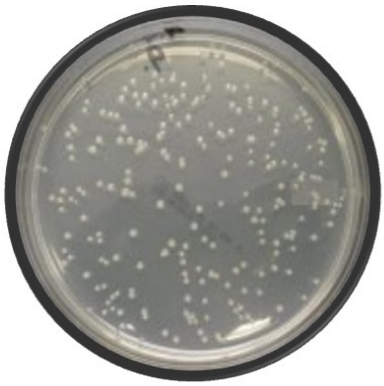


Fig. 2. Colony count plates showing assay for 24 hrs on *E. coli.*

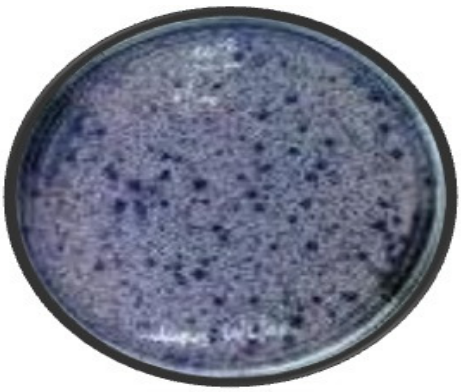
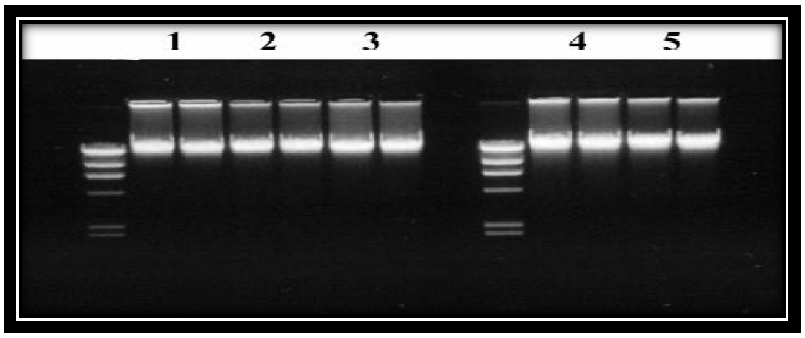
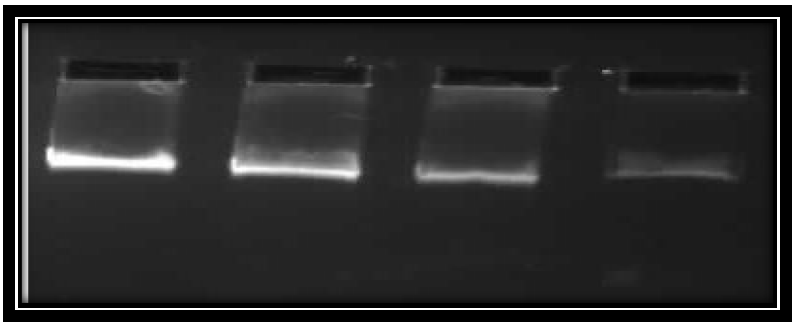


Fig. 3. Colony count plates showing assay for 48 hrs on *E. coli.*

Isolation of genomic DNA from *E. coli*: The genomic pattern of *E. coli* of both 24 hrs & 48 hrs sample showed more or less same intensity in the wells. However, there is a marked decrease in the intensity of the bands at a higher dose of PWWP. This shows that there is certain level of DNA degradation in the genomic DNA of *E. coli* of the treated sample [30] (Fig. 4,5).



5: -Control; Bands 1& 2 :- 25 µM PWWP, Bands 3 & 4:- 50 µM PWWP.
Fig. 4. Plasmid bands of treated sample.



C: -Control; Bands 1 :- 25 µm PWWP, Bands 2 & 3:- 50 µm PWWP.
Fig. 5. Genomics bands of treated sample.

IV. DISCUSSION

As the published literature says that Indolicidin is a host defense tridecapeptide that inhibit catalytic activity of HIV-1 integrase in vitro and is known to have a broad spectrum for its antimicrobial properties which is likely due to the partitioning of Indolicidin into membrane lipid bilayer against gram positive and gram negative bacteria [9-10].

As mentioned in the proceedings sections the, effect of PWWP is mostly cyostatic rather than cytocidal for comparison the bacterial protein component of cell wall was treated with different antibiotics for example chloramphenicol tetracycline which revealed that cell wall ar not typical proteins whose synthesis could be initiated by antibiotics restricted to the level of ribosomes.

The characterization of bacteria was done using nutrient broth which confirms the culture used in the present study. Bacteriostatic effect of PWWP was shown in different set of experiments like turbidometry method, colony counting method & agar disc assay [28]. By measuring the optical density, the inhibitory property of the sample PWWP was established. The time kinetics & their inhibitory effect were elucidated by colony count method in which the number of colonies decreased with the increase in the concentration of PWWP. The agar disc assay revealed that PWWP has no bacteriocidal effect but reduction in number of colonies around the disc proved bacteriostatic effect of PWWP. This result is in correspondence to data discussed earlier. maximun bacteriostatic effect was seen 50µm concentration of PWWP as shown in time kinetics. When antibacterial properties were compared, PWWP was found less toxic to *E. coli.* the high bacteriostatic activity that is of 50µm concentration of PWWP than 25µm concentration can be plausible explained as gompterezian growth pattern as the dose increases PWWP prevents the cell divison or multiplication of *E. coli.* Therefore the biomass decreases but do not show killing.

Further study in the area of this report includes the genomic DNA isolation of *E. coli*. The result revealed that PWWP has certain level of degradative effect at genomic level. In the result the degradation of the DNA can be associated with the level of intensity found in the result. Time kinetics study showed that degradation starts from 24 hrs and continued up to 48hrs. the control being devoid of PWWP shows highest level of intensity in the well. There are no published works available to show comparison between degradation dependent on time kinetics btw 24 hrs and 48 hrs DNA band pattern were not observed the amt of DNA present in the sample containing different concentration of the drug were determined by the spectrophotometric method. The absorption at 260 nm revealed the concentration of nucleic acid was present in the sample to stop to the closer look at the result (O.D) and different literature the sample under study slightly protein contaminated. The sample handling errors contributed a greater extent to the total variation in spectrophotometric readings sample to sample variability was the single largest factor. That contributed to the error in estimated DNA concentration thus, proteins, RNA and salts, all of which are contaminants of DNA extracted would have increased the spectrophotometric estimation of DNA concentratiton. But As per the result, the samples of 48 hrs post treatment with PWWP showed the degradation in the genomic as the concentration of DNA was found less than the control having no drug.

According to the literature showed that PWWP is a protein inhibitory drug but most of these cases were reported in eukaryotes. So all experiments with the pseudomonas showed that when antimicrobial properties were compared PWWP was found less toxic rather showed no effects on pseudomonas [6].

The truncated version of Indolicidin beside having the major amino acid residues does not show any antimicrobial properties which could be due to presence of two conjugated molecules and many more reasons which need to be further investigated. These observations need further investigations to convincingly prove the significance of this inhibition

V. CONCLUSION

As the literature says that Indolicidin is antimicrobial peptide with bactericidal effects on eukaryotes with prokaryotes [6-9]. In depth analysis of literature reveals that truncated version of Indolicidin at varied concentration shows the bacteriostatic activity against *E. coli* at 50µ molar concentration as we have attempted its nucleotide and protein leakage test which revealed that there was no leakage which shows that it does not break the cell wall and permeabilizes within the cell membrane as Indolicidin.

ACKNOWLEDGEMENT

The authors are thankful to Kirori Mal College University of Delhi, New Delhi and IIT Kanpur for providing infrastructure facilities for this work.

REFERENCES

[1]. Sharma G, Srivastava M S and B.P. Nagori (2009). Extraction and evaluation of antimicrobial activity of Cissus quadrangularis Linneaus. *Biological Forum – An International Journal* **1**(2): 92-97.

[2]. Malaviya Anshika, Mishra Neeraj (2011). Antimicrobial activity of tropical fruits. *Biological Forum – An International Journal,* **3**(1): 1-4.

[3]. Najafian Maryam, Hiva Karimi and Nazanin Zand. (2015). Antimicrobial Effect of Spearmint and Dill oils on Yeast *Kluyveromyces marxianus* (*K. marxianus*) in Iranian Doogh. *Biological Forum – An International Journal,* **7**(2): 69-74.

[4]. Dara Dastan and Atousa Aliahmadi (2015). Antioxidant and antibacterial studies on different extracts of Ornithogalum cuspidatum Bertol from Iran. *Biological Forum – An International Journal* **7**(2): 1072-1075.

[5]. Strom, M.B. and Rekdal, O. *et.al.* (2002). Antimicrobial activity of short arginine- and tryptophan-rich peptides. *J Pept. Sci.,* **8**(8): 431-7.

[6]. Lin, M.C. and Hui, C.F. *et.al*. June (2013). Truncated antimicrobial peptides from marine organisms retain anticancer activity and antibacterial activity against multidrug-resistant *Staphylococcus aureu*s. *Peptides,* **44**: 139-48.

[7]. Zasloff, M. (2002). Antimicrobial peptides of multicellular organisms. *Nature*, **415**: 389-395.

[8]. Hancock, R.E.W. and Rozek, A. (2002). Role of membranes in the activities ofantimicrobial cationic peptides. *FEMS Microbiol Lett.,* **206**: 143-149.

[9]. Zhang, L. and Falla, T.J. (2004). Cationic antimicrobial peptides an update. Expert Opin Investig Drugs, **13**: 97-106.

[10]. Kelly, L. B. and Robert, E.W. (2006). Hancock. Cationic host defense (antimicrobial) peptides. *Current Opinion in Immunology*, **18:**24-30.

[11]. Brown, K.L. (2006). Hancock,R.E.Cationic host defense (antimicrobial) peptides. Curr Opin Immunol., **18**: 24-30.

[12]. Jindal, H.M. and Le, C.F *et.al*. (2015). Antimicrobial Activity of Novel Synthetic Peptides Derived from Indolicidin and Ranalexin against Streptococcus pneumoniae. *PLoS One*. **6**: 5-10.

[13]. Strom, M.B. and Rekdal, O. *et. al*. (2002). Antimicrobial activity of short arginine- and tryptophan-rich peptides. *J Pept Sci., 8*(8): 431-7.

[14]. Arias, M. and Nguyen, L.T. *et. al*. (2014). Position-Dependent Influence of the Three Trp Residues on the Membrane Activity of the Antimicrobial Peptide Tritrpticin. *Antibiotics (Basel).*, **4**: 595-616.

[15]. Ladokin, A.S. and Selsted, M.E. *et. al*. (1997). Bilayerinteractions of indolicidin, a small antimicrobial peptide richin tryptophan, proline and basic amino acids. *Biophys. J.,*7**2**:794-805.

[16]. Boman, H.G. and Agerberth, B. *et.al*. (1993). Mechanism of action on *Escherichia coli* of cecropin P1 and PR-39, two antibacterial peptides from pig intestine. *Infect. Immun.,* **61**: 2978-2984.

[17]. Zaslo, M. and Magainins (1987). A class of antimicrobial peptides from Xenopus skin: isolation, characterization of two active forms, and partial cDNA sequence of a precursor. *Proc. Natl. Acad. Sci. USA*., **84**: 5449-5453.

[18]. Nicolos, P. and Mor, A.(1995). Peptides as weapons againstmicroorganisms in the chemical defense system of vertebrates. *Annu. Rev. Microbiol.,* **49**: 277-304.

[19]. Taylor, P.W. (1983). Bactericidal and bacteriolytic activity of serum against gram-negative bacteria. *Microbiol Rev.,* **47**: 46-83.

[20]. Levy, O. (2000). Antimicrobial proteins and peptides of blood: templates for novel antimicrobial agents. *Blood.,* **96**: 2664-2672.

[21]. Chilukuri, S. and Narasimhaiah, S. (1998). Mechanism of antimicrobial action of indolicidin. *FEMS Microbiology Letters,* **160**(1): 91-96.

[22]. Attila Gergely Vegh and Krisztina Nagy *et. al*. (2011). Effect of Antimicrobial Peptide-Amide: Indolicidin on Biological Membranes, *Journal of Biomedicine and Biotechnology*, **6** pages.

[23]. Khandelia, H. and Kaznessis, Y. N. (2007). Cation—π interactionsstabilize the structure of the antimicrobial peptide indolicidin near membranes: molecular dynamics simulations, *Journal of Physical Chemistry*, **111**(1): 242–250.

[24]. Rozek, C.L. and Friedrich, R. E.W. (2000). Hancock, Structure of the bovine antimicrobial peptide indolicidin bound to dodecylphosphocholine and sodium dodecyl sulfate micelles, *Biochemistry*, **39**(51): 15765-15774.

[25]. Shaw, J.E. and Alattia, J. R. *et. at.* (2006). Mechanisms of antimicrobial peptide action: studies of indolicidin assembly at model membrane interfaces by in situ atomic force microscopy. *Journal of Structural Biology***, 154**(1): 42–58.

[26]. Lanyi, J. K. (2004). Bacteriorhodopsin. *Annual Review of Physiology*, **66**: 665–688.

[27]. Vincent, J.M. (1947). Distortion of Fungal Hyphae in the Presence of Certain Inhibitors. *Nature,* **156**: 850-855.

[28]. Perez, C. and Pauli, M. *et. al.* (1990). Anantibiotic assay by agar-welldiffusion method. *Acta Biol Med Exp*., **15**: 113-5.

[29]. Sabine, E. and Dirk, S. (2003). Isolation of Plasmids from *E. coli* by Alkaline Lysis. *E. coli Plasmid Vectors*, 75-78

[30]. Bandyopadhyay, A. and Chatterjee, S. *et. al*. (2011). Sarkar K, Rapid isolation of genomic DNA from *E. coli* XL1 Blue strain approaching bare magnetic nanoparticles. *Current Science*, **101**(2): 210-21.

[31]. Lawrence, J.V. and Maier, S. (1977). Correction for the Inherent Error in Optical Density Readings, *Applied and Environmental Microbiology*, 482-84.