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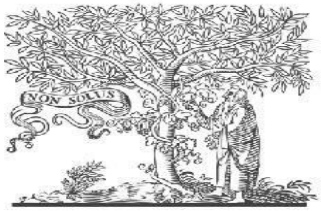

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Insilico studies of antiviral peptides, Blocks the viral entry by impeding the binding of spike protein SARS-COV-2 to ACE-2

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Abstract:

The recent outbreak of corona virus disease 2019 has become a global challenge for scientific community globally and there is a need of prevention and treating disease. Viral entry is accommodated by the fusion of glycoprotein with ACE2 human receptor. Therefore, spike glycoprotein of SARS Cov-2 is considered to be the potential target for the diagnosis, vaccine development, and antibodies. In present study we retraived sequences of 216 antiviral peptides from DRAMP in that only 49 peptides and bacteriocin peptides are modeled using Swiss modeling and pep-FOLD 3.5 and docked against spike protein by using peptide protein docking software. It was discovered that some peptides have exhibited affinity towards protein spike. In that only 10 peptides have been selected, these are selected on the basis of lowest binding energy and lowest Z-score. DRAMP02563-2.7, DRAMP02546-1398.4 is the peptides have more binding affinity with lowest z-score and binding energy. These best complex models are again docked with ACE2 (protein-protein binding). Interestingly it was noticed that the ACE2 complex binds to the B-chain of spike protein instead of A chain .Where peptide is bind to the spike A chain. This interaction suggests that the peptides may be employed in inhibiting the viral entry into human ACE2 cells to stop the spread of covid19 pandemic or it may be used in vaccine preparation.

Keywords: Bacterial defensins, spike protein, sars cov-2, ACE 2, Peptides, molecular docking.

Introduction:

COVID-19 virus first identified in December 2019 in Wuhan, Hubei Province, China (Huang *et al.,*2020; Wu et al., 2020b; Zu et al., 2020) and it has spread rapidly across the world, causing severe acute respiratory syndrome 2 (SARS-CoV-2). It has spread over 200 countries (Naqvi et al., 2020) the most effected being USA, Brazil, India, Russia, Colombia, Spain, the UK, Peru,

Argentina, Mexico, France, and Italy. In India, the first case was reported on 30th January 2020 and it is identified to be imported from china. The Indian council of medical research (ICMR) and Ministry of Health and family welfare has confirmed that a total of 719 cases were reported out of which 45 cases got recovered and 16 deaths in India as of 23rd April 2020 (https //www.icmr.gov.in). Interesting fact is, a targeted specific medicine for the treatment of COVID 19 has not been developed till date. As per the latest update published by WHO (https://covid19.who.int/) **235,426,111**confirmed cases are reported out of which **4,811,697** are death cases worldwide. The higher mortality rate has been reported in individuals having delicate immune systems or effected with chronic diseases viz renal diseases, diabetes, malignant tumors, liver diseases and cardiovascular disease etc., rate (Emergency and Team, 2020; Gao et al., 2020; Guan et al., 2020; Li et al., 2020; Report, 2020; Uddin et al., 2020).

The viral entry in human is facilitated by spike glycoprotein fusion with Angiotensin Converting Enzyme 2 (ACE2) receptor. Therefore, spike glycoprotein has become the potential target in the process of diagnostics antibodies and vaccines. Viral entry ceasing by blocking ACE2 can be a promising technique in therapeutics. Not that the vaccines have been developed and are used in prevention of COVID 19. As a part of primary treatment, the antiviral, antimalarial drugs and convalescent plasma therapy are being used (Duan et al. 2020; Mitjà and Clotet 2020; Vincent et al. 2005). As a part of research, the researchers have studied the extracts from natural products, natural compounds from marine eco system, viral inhibitors that have already shown efficacy against SARS-CoV-2 proteins (Khan et al. 2020a,b; Khan *et al.* 2020c; Maurya et al. 2020; Quimque et al. 2020) besides some repurposed drugs.

Spike glycoprotein facilitates the viral entry and recognizes the human cells receptor Angiotensin-Converting Enzyme 2 (ACE2) by fusing with host cell membrane (Gallagher and Buchmeier 2001; Simmons *et al*. 2013). The glycoprotein is composed of S1 and S2 subunits making it a trimeric protein. The ACE2 receptor is binded by S1 domain which has a receptor binding domain (RBD) while membrane fusion is facilitated by S2 domain (Belouzard et al. 2009). The enzyme ACE2 in the cell membrane is expressed in kidney, heart, intestines, lungs and arteries (Donoghue *et al.*2000; Hamming *et al*. 2004). In human, the ACE2 is known as hACE2 (Bolles et al. 2011) and it mediates viral entry into human cells (Nicholls and Peiris 2005). In the recent study it is evident that RBD of S1 subunit in SARS-CoV-2 strongly binds to ACE2 when compared to RBD of SARS-CoV (Wrap *et al.*2020). To develop an effective treatment methodology, the researchers identified the viral protein targets. Some of the references are Papain-Like Protease 2 (PLP2), RNA-dependent RNA polymerase (RdRp), 3C-Like Protease (3CL), Nucleoside Triphosphatase (NTPase) or Helicase, Spike Glycoprotein (S Protein), Hemagglutinin Esterase (HE), Envelop Protein (E Protein), Nucleocapsid Protein (N Protein), Membrane Protein (M Protein) and Endoribonuclease Non-structural Protein 15 (NSP15)) (Kim et al. 2020; Prajapat et al. 2020; Wu et al. 2020). The evolution of peptide therapeutics has started in 1920s with insulin for diabetic patients. Peptides are different from molecules and proteins by means of their biochemical and therapeutic activity. More than 150

peptides underwent human trials for the treatment of various diseases like diabetes etc., (Tong 2009) but with limited short half-life in plasma and low bioavailability. synthesis of a radiolabelled peptide analog of somatostatin (SST) was a significant development and was used for targeting endocrine tumors which express SST receptor (receptor (Andy Chi-Lung Lee *et al* 2019).

Using peptides as targeting moiety in cancer diagnosis and treatment has let to current development in peptide drug discovery both in industry and academia. Besides the cancer treatment, it opens up lot of therapeutic opportunities, like peptides that mimic natural peptide hormones.

Peptides target intracellular molecules like tyrosine kinases receptor and are basically meant to be developed as drug candidates by disrupting protein –protein interaction (Birk, A.V *et al* 2013; Chang, Y.S.; Graves, B. *et al* 2013). These efforts and strategies have turned therapeutics into a leading research industry with almost 20 latest peptide-based clinical trials per year. Currently there are more than 400 peptide drugs that are contributing to the global clinical developments out of which 60 have already got approval for clinical use in United States. Europe and Japan (Andy Chi-Lung Lee *et al* 2019). The small molecules and peptides that interfere with PPIs are therefore in high demand as therapeutic agents in the pharma industry because of their capacity to modulate and interact with the protein associated with disease. The increasing research results has suggested that identification of targetable disease related PPIs and optimizing the binding between the peptide and drug characteristics will be the key for the future research (Thomas, D.2013). The current paper illustrates the docking of different peptides against SARS COV-2. The main objectives of the study are: To screen the peptide molecules from peptide databases reported to have antiviral activity. Docking of different peptide molecules against SARS COV2 spike protein and ACE2 receptor.

Material and Methods

Databases and software's

DRAMP (Drug repository of antimicrobial peptides), PDB (Protein Data Bank), SWISS modeling, UCSF chimera, CASTP (ACTIVE-SITE-PREDICTION-SERVER), CLUS-PRO, HAD DOCK and Biovia Discovery studio were different databases and software's were used in this study.

Macro molecule preparation

The Crystallographic structure of protein has been retrieved from protein data bank (PDB) with specific resolution of 2.16 A˚. Active sites of these proteins has been identified by using auto ligand fallowed by visualization by using the molecular graphics program PyMol® software (Michel F. Sanner, 1999; Harris et al, 2008).

The downloaded Macromolecule in the PDB format has been energy minimized by UCSF Chimera (Land and Humble, 2018).

The macro molecule was prepared for docking before preparation of protein, using Discovery Studio (San Diego: Accelrys Software Inc., 2012), and water molecules are deleted and saved it as PDB format.

Macromolecule were added with Kollman, Polar hydrogens and gasteiger charges and saved it in in a PDBQT format for the purpose of docking (Berman, 2002).

Peptide collection and screening

Peptides with effective antiviral potential were retrieved from DRAMP (Data Repository of Antimicrobial Peptides (Kang et al., 2019). The structures of all proteins were generated in PDB format and then the respective 3-D structures were generated by using PyMol® software. The peptides were prepared for docking purpose by selecting root and torsions.

Molecular docking

Cluspro docking was used to dock peptides with spike protein RBD which can predict the best 10 to 30 modules. Therefore screening of 45 peptide samples with spike (protein) generates 1350 models among which a peptide model which binds the surface of RDB spike protein is selected based on the binding energy.

On the other hand, protein-protein dock is conducted by using HADDOCK (**H**igh **A**mbiguity **D**riven protein-protein **DOCK**ing), while the score is not a true binding energy but it provides sources of various peptide models related. Interactions between the proteins were investigated by using PDBePISA web tool (https://www.ebi.ac.uk/msd-srv/prot_int/cgi-bin/piserver).

Results

Three-dimensional structures of spike proteins (PDB ID: 6XM5) and ACE-2 (PDB ID: 7JVO) retrieved from protein data bank (PDB) **Fig 1.** And based on antiviral activity, a total of 216 antiviral peptides from 5891 peptides were screened. Out of 216 peptides, 49 potential peptides were selected based on microbial origin. Ten peptides (DRAMP02563, DRAMP02524, DRAMP02558, LATEROSPORULIN, MERSACIDIN, DRAMP02546, DRAMP02549, DRAMP02528, DURAMYCIN and STREPTOLYSIN) with potential antiviral activity was selected, visualized by using PyMol and were depicted in **Fig 2.**

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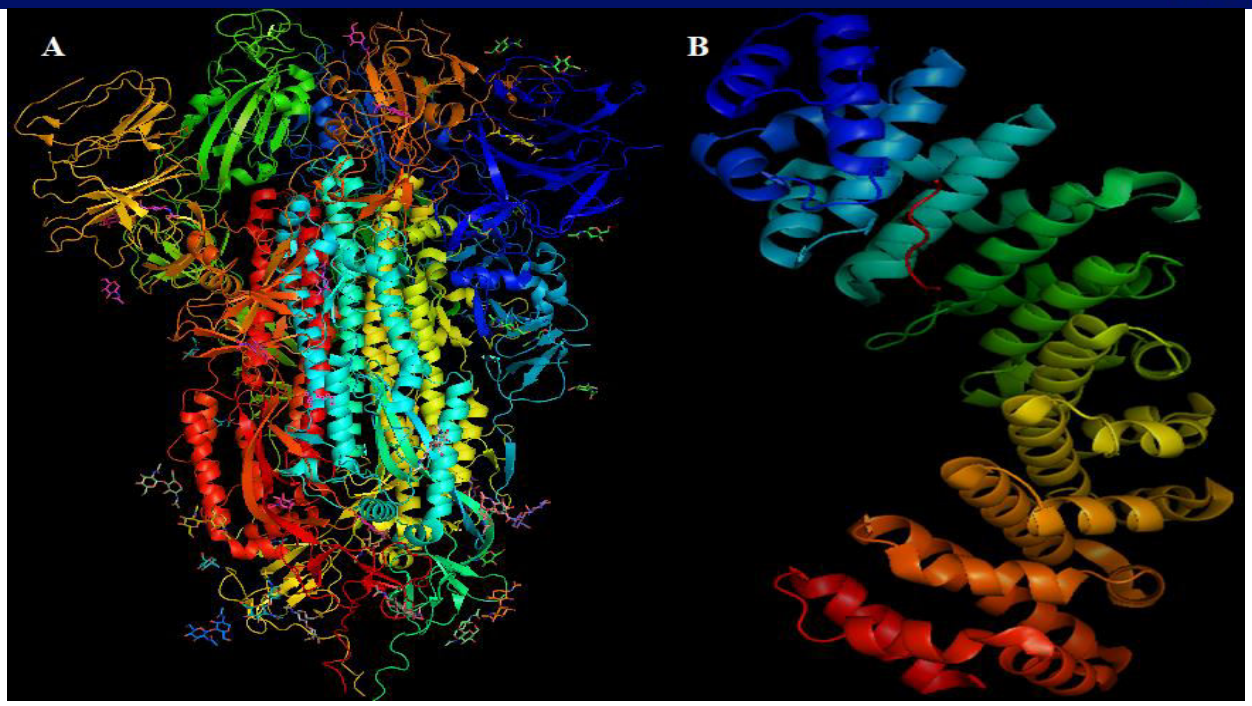

Fig 1.3D structures of proteins A) spike protein (PDB ID: 6XM5); B) ACE-2 (PDB ID: 7JVO)

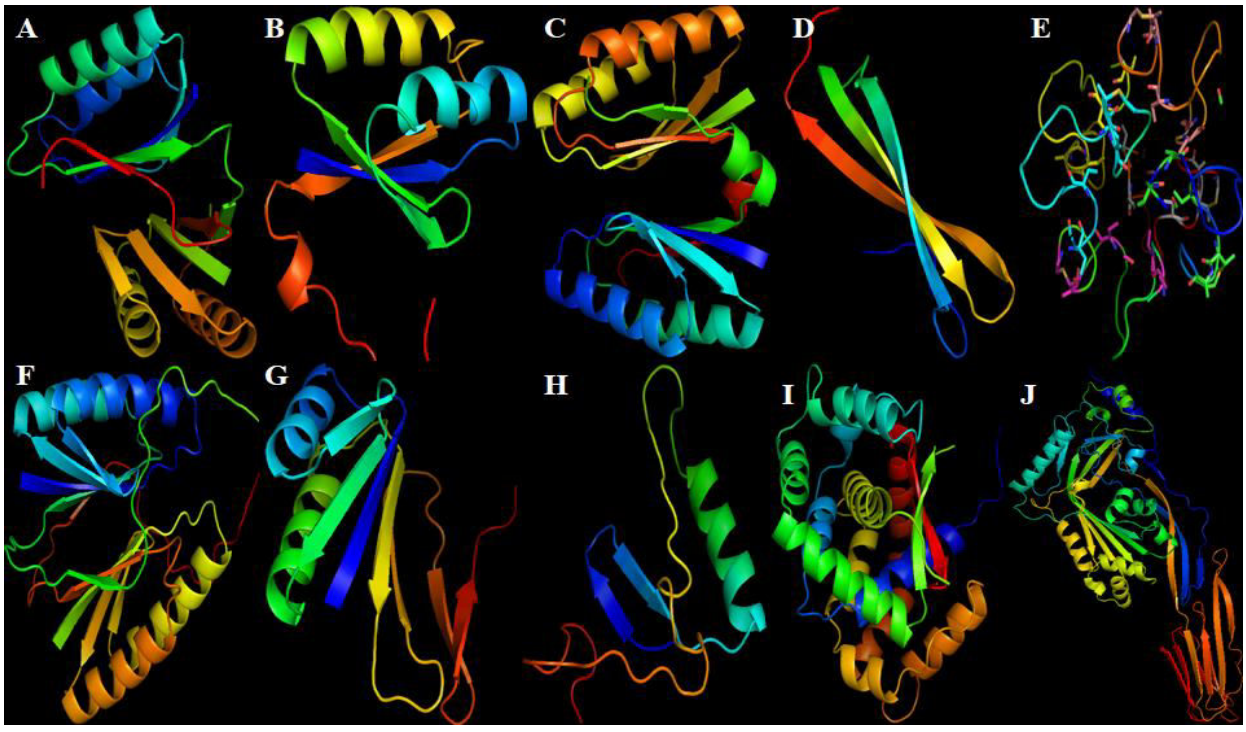

Fig 2.3D structures of peptides A) DRAMP02563; B) DRAMP02524; C) DRAMP02558; D) Laterosporulin; E) Mersacidin; F) DRAMP02546; G) DRAMP02549; H) DRAMP02528; I) Duramycin; J) Streptolysin

Peptide-protein (Spike and Ace2) interaction

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Peptides with antiviral properties from microbial (bacterial) origin were docked against Spike protein and ACE 2 protein using Swiss modeling, which can precisely forecast the peptide structure longer than 5 - 50 amino acid lenght by PEP-FOLD-3.Molecular docking of peptides with spike protein and ACE receptor are performed upon completion of the run the results will be displayed with a graphical structure along with some parameters like Z Score, Binding energy, Vanderwaals energy, Hydrogen bonds, hydrophobic energy etc. Upon analyzing, the results of docked proteins are as follows.

Interaction of DRAMP02563 with spike and ace 2

DRAMP02563 is a peptide originated from *Pyrococcuskodakaraensis* has both antimicrobial and antiviral activity which binds to chain A of spike protein with the Z score of -2.7 and binding energy 1139.6kj which inhibits binding of ACE2 protein **Table 1 & Fig 3A.**

Interaction of DRAMP02524 with spike and ace 2

DRAMP02524 is a peptide originated from *Chloroflexusaurantiacus* has both antimicrobial and antiviral activity which binds to chain A of spike protein with the Z score of -2.5 and binding energy 1359.4kj which inhibits binding of ACE2 protein **Table 1 & Fig 3B.**

Interaction of DRAMP02558 with spike and ace 2

DRAMP0258 is a peptide originated from *Methanobrevibacterruminantium* has both antimicrobial and antiviral activity which binds to chain A and B of spike protein and ACE2 protein with the Z score of -2.4 and binding energy -1279kj **Table 1 & Fig 3C.**

Interaction of leptosporulin with spike and ace 2

Leptosoprulin is a peptide originated from *S. aureus, Mycobacterium tuberculosis* has both antimicrobial and antiviral activity which binds to chain A of spike protein with the Z score of -2.3 and binding energy -119.5kj which inhibits binding of ACE2 protein **Table 1 & Fig 3D.**

Interaction of Mersacidin with spike and ace 2

Mersacidinis a peptide originated from *B. amyloliquefaciens* and *Methicillin-resistant S. aureus* has both antimicrobial and antiviral activity which binds to chain A of spike protein with the Z score of -2.3 and binding energy -1298.1kj which inhibits binding of ACE 2 protein **Table 1 & Fig 3E.**

Interaction of DRAMP02546 with spike and ace 2

DRAMP02546 is a peptide collected from DRAMP database having antiviral activity. It is a defensing originated from leptospira having two chains one binds to A chain and another to chain B of spike protein with Z score of -2.1 and -1398.7kj. This complex inhibits the binding of ACE2 to A chain and binds to chain B besides to the peptide chain **Table 1 & Fig 3F.**

Interaction of DRAMP02549 with spike and ace 2

DRAMP02549 is a peptide originated from *E-Coli* k12 has both antimicrobial and antiviral activity collected from DRAMP database. It binds to chain of spike due to this ACE2 molecule binds to B and C chains instead of Awith Z score of -2.5 and binding energy -1255.7kj **Table 1 & Fig 3G.**

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Interaction of DRAMP02528 with spike and ace 2

DRAMP02528is a peptide originated from *Methicillin-resistant S. aureus* has both antimicrobial and antiviral activity which binds to chain B of spike protein with the Z score of -1.9and binding energy -1247.7.1kj which inhibits binding of ACE2 protein **Table 1 & Fig 3H.**

Interaction of Duramycin with spike and ace 2

Duramycin is originated by *Streptomyces* and *B.subtilis*which binds to chain A of spike protein with the Z score of -2.2 and binding energy -1257.1kj which inhibit the binding of ACE 2 to chain A and it binds to chain B instead of A **Table 1 & Fig 3I.**

Interaction of TOMM Streptolycin with spike and ace 2

It binds to chain A of 6XM5 spike protein with the Z score of -2.1 and binding energy 1251.2kj which inhibits the binding of ACE2 to chain A of spike protein instead of bind to chain A it binds to chain B. And further evaluation to study the effect of this interaction either in inhibiting the viral entry/replication or it may progress the disease mechanism **Table 1 & Fig 3J.**

Table 1. Molecular docking interactions of Peptides with spike and ace 2 proteins

S.no	Mol. ID	DRAMP ID	Peptide name	Peptide source	Binding	Bonds	Z score	Binding energy
1	KSS21077	DRAMP02563	CRISPR-associated endoribonuclease Cas2	*Pyrococcuskodakaraensis*	A chain	Hydrogen, Hydrophobic, electrostatic	-2.7	-1139.6
2	KSS21084	DRAMP02524	CRISPR-associated endoribonuclease Cas21	Chloroflexusauraantiacus	A chain	Hydrogen, Hydrophobic, electrostatic	-2.5	-1359.4
3	KSS21093	DRAMP02558	CRISPR-associated endoribonuclease Cas2	*Methanobrevibacterruminantium*	A chain	Hydrogen, Hydrophobic, electrostatic	-2.4	-1279.1
4	KSS21188	LATEROSPORULIN	Brevibacillus sp. strain SKDU10	*S. aureus, Mycobacterium tuberculosis*	A chain	Hydrogen, Hydrophobic, electrostatic	-2.3	-119.5
5	KSS21147	MERSACIDIN	B. amyloliquefaciens	*Methicillin-resistant S. aureus*	A chain	Hydrogen, Hydrophobic, electrostatic	-2.3	-1298.1
6	KSS21194	DRAMP02546	CRISPR-associated endonuclease Cas2	*Bacillus halodurans*	B chain	Hydrogen, Hydrophobic, electrostatic	-2.1	-1398.4

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7	KSS21226	DRAMP02549	CRISPR-associated endoribonuclease Cas2	*Escherichia coli*	A chain	Hydrogen, Hydrophobic, electrostatic	-2.5	-1255.7
8	KSS21264	DRAMP02528	CRISPR-associated endoribonuclease Cas2 1	*Thermusthermophilus*	B chain	Hydrogen, Hydrophobic, electrostatic	-1.9	-1247.7
9	KSS21308	DURAMYCIN	Streptomyces	*B.subtilis*	B chain	Hydrogen, Hydrophobic, electrostatic	-2.2	-1275.1
10	KSS21420	STREPTOLYSIN	S.pyogenes	*Clostridium sp., Listeria sp.*	A & B chains	Hydrogen, Hydrophobic, electrostatic	-2.1	-1251.2

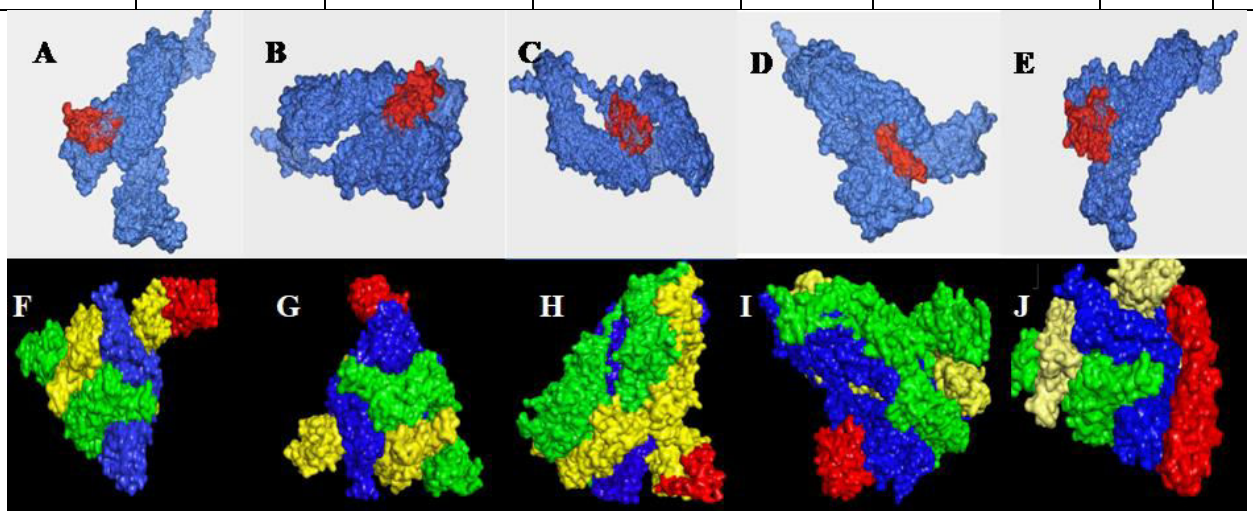

Fig 3. Interactions of peptides with Spike protein: A chain – Blue; B Chain – Yellow; C Chain – Green; Peptide – Red; A) Interaction of DRAMP02563 with spike protein; B) Interaction of DRAMP02524 with spike protein; C) Interaction of DRAMP02558 with spike protein; D) Interaction of leptosporulin with spike protein; E) Interaction of Mersacidin with spike protein; F) Interaction of DRAMP02546 with spike protein; G) Interaction of DRAMP02549 with spike protein; H) Interaction of DRAMP02528 with spike protein; I) Interaction of Duramycin with spike protein; J) Interaction of TOMM Streptolycin with spike protein.

Discussion:

From the above results, it can conclude that the peptides screened and selected have the affinity to bind with the spike protein at different chains A, B, C respectively. And it is there evidence that the region where peptide molecule binds to the spike protein either of the chains is similar to that of the ACE 2 receptor and it has been evaluated with multiple sequence alignment and molecular similarity study that is with the amino acids interactions. The binding of the ACE 2

receptor and the selected peptides can be hypothetically explained; as the sequence and amino acids are similar, the peptide may also bind to the ACE receptor. When the ace 2 receptor is bound to the peptide molecule, the open or binding site of the ACE 2 receptor is closed, due to this the spike protein has no site of binding in ACE2 receptor the spike remains unbound to the receptor. These peptides can be either used as prophylactic and therapeutics. As they have capability to restrict the spike protein binding to ACE 2 receptor.

CONCLUSION

Antimicrobial peptides have an effective feature in therapeutics. In present study the peptides are targeted against spike and ACE2 receptor, these results in the inhibition of binding ACE receptor to spike protein to the actual binding site. As the peptides bind to the ACE 2 receptor and it is known that the molecular binding results in change of physical or chemical structure of the parent molecule. It is a very need to further study the weather a change in the ACE 2 receptor is seen, or is there any cross reactions or impairments seen in ACE 2 and associated organs.

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Conflict of interest: All the authors state no conflict of interest among the authors.

Author's Contribution: Sirisha, Prabhu, Seema, Vasundhara, Akshita Raj, Gayatri, Laluram, Ravikiran, Vivek, and Meghana have executed and did the project as part of Msc project dissertation. Krishnaveni R has designed, written the manuscript and supervised the research project. Sadam DV Satyanarayana helped in proof reading and plagiarism of the manuscript and Sairam M have helped in organizing the images and tables in the manuscript.

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Data Availability: Data has been collected from the open source public library PDB (Protein database)

Ethics Statement: No animals, birds or human live samples are used in this project and had not breached any ethical regulations. No clinical trials are involved.

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