**In Silico** identification of angiotensin-converting enzyme inhibitory peptides from MRJP1

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Abstract

Hypertension is considered as one of the most common diseases that affect human beings (both male and female) due to its high prevalence and also extending widely to both industrialize and developing countries. Angiotensin-converting enzyme (ACE) has a significant role in the regulation of blood pressure and ACE inhibition with inhibitory peptides is considered as a major target to prevent hypertension. In the current study, a blood pressure regulating honey protein (MRJP1) was examined to identify the ACE inhibitory peptides. The 3D structure of MRJP1 was predicted by utilizing the threading approach and further optimized by performing molecular dynamics simulation for 30 nanoseconds (ns) to improve the quality factor up to 92.43%. Root mean square deviation and root mean square fluctuations were calculated to evaluate the structural features and observed the fluctuations in the timescale of 30 ns. AHTpin server based on scoring vector machine of regression models, proteolysis and structural characterization approaches were implemented to identify the potential inhibitory peptides. The anti-hypertensive peptides were scrutinized based on the QSAR models of anti-hypertensive activity and the molecular docking analyses were performed to explore the binding affinities and potential interacting residues. The peptide “EALPHVPIFDR” showed the strong binding affinity and higher anti-hypertensive activity along with the global energy of -58.29 and docking score of 9590. The aromatic amino acids especially Tyr was observed as the key residue to design the dietary peptides and drugs like ACE inhibitors.

**Introduction**

Pulmonary arterial hypertension (PAH) affects the small pulmonary arterioles, which lead to a progressive disease of the lung vascular system. The progressive narrowing of the blood vessels is a collective effect of increased contractility of the small pulmonary arteries, remodeling, and proliferation of endothelial smooth muscle cells and endothelial dysfunction [1].
PAH is transmitted through an autosomal dominant trait with mitigated trenchancy. The mutations in the bone morphogenetic protein receptor type-II (BMPR2) elucidate 70% of the hereditary cases while 20% of the cases have unknown reasons [2, 3]. BMPR2 belongs to the super-family of TGFb/BMP [4] and its heterozygous alterations occur in the transmissible PAH [5, 6] leads to the illness [7]. The hereditary PAH is localized at chromosome 2q33 [8, 9]. The nonsense, frameshift and missense mutations in BMPR2 lead to change the bone morphogenetic protein and TGF-b1/SMAD signaling pathways, which ultimately cause escalation instead of apoptosis of the vascular cells [6, 7, 10–12]. The system modifications entailed in the cardiovascular attunement are possibly associated with the commencement and conservation of the blood pressure elevation [13].

Angiotensin-converting enzyme (ACE) is a vital constituent of the renin-angiotensin system (RAS), arbitrating various systemic and local effects in the cardiovascular system. The ACE synthesis in somatic tissues endothelium as a transmembrane protein comprising of two active domains which are inhibited by ACE inhibitors [14]. ACE peptides as inhibitors are extensively studied in different bioactive peptides [15–18] for therapeutic purposes. The conversion of ACE transmutes angiotensin I to angiotensin II is a dynamic vasoconstrictor and a vital enzyme in the modulation of blood pressure and body fluids. It is also involved in the anatomiization of bradykinin to dilate the blood vessels [19].

The ACE function could induce the vasoconstriction and progression of hypertension and related pathological manifestations. ACE suppression is considered as an essential approach in regulating hypertension [20]. The synthetic or celluloid ACE inhibitor drugs have side effects including a dry cough, skin rashes or erythema, taste turbulences and the modifications in serum lipid metabolism [21]. The commercially available ACE inhibitor drugs are discouraged and food protein-derived ACE inhibitory peptides are preferred [18, 22, 23] for effective therapies. The amino acid residues determine the inhibitory potency of ACE inhibitory peptides such as the existence of hydrophobic and positively charged amino acids [24, 25]. The purpose to assess the food proteins from primary food products as precursors in producing ACE inhibitory peptides facilitates to develop a principle for proper selection of substrate protein. The high-potential food and the sedentary lifestyle are known to trigger hypertension [26].

The current work demonstrates the in silico identification of potential anti-hypertensive peptides from honey protein MRJP1. Computational approaches have shown considerable success in research methodologies to solve biological problems [27]. After the successful identification of computational drugs and drug targets in neurological disorders [27–32] and cancer [33–36], researchers also utilized the computational approaches to design epitope-based peptide vaccines through immunoinformatic approaches [37]. The 3D model was built by using homology modeling and threading based approaches followed by the Molecular Dynamic (MD) simulations to optimize and analyze the structural features of a model for protein-peptide docking analyses. The screening for ACE inhibitory peptides was performed to identify the potential anti-hypertensive peptides. The observed anti-hypertensive peptide-protein interactions may serve to replace the drugs by dietary peptides and to narrow down the diverse combinatorial search space.

Results and discussion

The objective of the current research was to identify the potential anti-hypertensive peptides derived from MRJP1. The retrieved sequence of MRJP1 was used to identify the appropriate templates but the query coverage and sequence identity against suitable templates were not satisfactory to build a model through a comparative modeling approach. The top-ranked template belongs to Salivary protein having only 25% identity and 61% query coverage was
observed, therefore the threading based approach was utilized through I-Tasser for structure prediction of MRJP1.

The top-ranked five models were predicted by using the templates with higher similarity identified through the threading alignments. It was observed that the template protein (PDB ID: 3q6k) has a resolution of 2.52 Å structure (Salivary protein) and showed the highest confidence score of 0.54. The salivary protein belongs to the MRJP protein family and the first structurally characterized member of the family that is being utilized in MRJP1 structure prediction. The homologous templates for evolutionarily related proteins are identified through the sequence profile analyses and considered as reliable for the prediction of high-resolution structures. The non-homologous proteins may also have the similar structures, and threading approaches have ability to match the query sequences onto the available structures with the aim of identifying the similar folds to the query even though there is no evolutionary relationship among the template protein and the query sequence. The models predicted through homology modeling and threading approaches with the RMSD range of 2–5 Å from distant templates that can be utilized for functional analyses and the identification of the active site residues. MD simulation has been utilized for the ab initio structure prediction to simulate the folding of the protein, while the template-based structure prediction is considered as one of the most reliable approaches.

Numerous models were predicted by utilizing a homology modeling and threading based approach and all the predicted models were evaluated critically. The model showed 78.53% quality factor and further subjected for MD simulations to optimize and extract the structural fluctuations throughout the 30 ns and it was observed that the quality factor was improved up to 92.43% (S1 Fig) and while 98.3% residues appeared in favorable regions.

### 2.1 Molecular dynamics simulation

The predicted structure of MRJP1 was subjected to MD simulations applying ensemble, temperature and appropriate solvent molecules. The constant temperature for 300K, 1atm pressure and heating for 500 ps were applied for simulation experiments in initial equilibration. The steric energy constraints were eliminated or reduced through energy minimization. Newtonian’s dynamics equilibrated the system to locate a thermally bound state, which leads to the production runs and simulations also deliver ensembles of structure to analyze the results. The conformational changes in the MRJP1 structure have been concluded from macroscopic features. The conformational variations of the MRJP1 structure were analogously determined at 0 ns, 10 ns, 20 ns, and 30 ns. Three major physical properties comprising RMSD, RMSF, and B-factor of the simulated system were calculated to analyze the conformational changes in the hydrated environment.

**2.1.1 Root mean square deviation (RMSD).** The atomic position of RMSD was calculated by considering the predicted structure of MRJP1 as a foremost model to find out the sustainability and convergence of the MD simulations. The 30 ns runs of molecular dynamics denoted that the RMSD of Cα-atoms as a function of simulation time (Fig 1). The results indicated that the RMSD values showed minimal fluctuation throughout the simulations studies. The high variation of atoms along with the residues close to NTD and CTD were observed. Overall, the stability of the structure was observed in 30 ns particularly at the end of the simulation, thus the simulated model was utilized for further processes. RMSD analyses of MRJP1 have shown no major fluctuations throughout the 30 ns simulations. The protein showed some higher fluctuations only at the start of the simulation while the stability of the structure was observed at the end of the simulation system.

**2.1.2 Root mean square fluctuation (RMSF).** The RMSF analysis of a protein about their conformations is a significant mark of many biological processes which includes complex
recognition, protein activity and macromolecular recognition [55]. The RMSF graph was computed for each residue of Cα-atoms, while the overall MRJP1 structure exhibited an advanced fluctuation level. The RMSF graph demonstrated the residual fluctuations of the MRJP1 model over 30 ns timeframe (Fig 2) and four major fluctuation peaks were observed. The first major residual fluctuation was observed from 30–67 (37 residues) amino acids, while second, third and fourth were 134–153 (19 residues), 228–256 (28 residues), and 374–405 (29 residues) amino acids respectively.

2.1.3 B-Factor. The applications of computational advances are to anticipate the thermal motion that examines the obscure structure of the proteins with dynamic attributes. The polypeptide backbones and side chains of MRJP1 structure were persistent in motion owing to kinetic energy and thermal motion of atoms. The fluctuations of the atoms regarding their average positioning were reflected by B-factors of protein structure and provided significant evidence about the protein dynamics. RMSD and PMSF plots indicated the stability of the model and only a few structural fluctuations were observed at residues level. It has also been verified through secondary structure analysis that there were few coils (irregular) elements along helices and sheets. Moreover, the observed B-factor analyses were in favor of higher values at corresponding positions anticipating that the MRJP1 structure is reliable for further analyses (Fig 3).

2.2 Structural analyses
The structural analyses were performed of simulated MRJP1 model at varying degrees of MD simulations such as 0 ns, 10 ns, 20 ns, and 30 ns. The structural fluctuations along with

Fig 1. Root mean square deviation graph vs. time; the graph showed the minimal fluctuations throughout the simulation runs and structural stability and optimization were achieved with respect to time.

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The most prominent difference in terms of improvement was the quality factor and the structural stability from 0 ns, 10 ns, 20 ns, and 30 ns structures as 78.53%, 85.37%, 89.14%, and 92.43% respectively. The number of alpha-helices at 0 ns and 30 ns were same as ten (10) helices but vary in residues length from 33 to 39 residues respectively, while the structure at 10 ns and 20 ns contains 14 and 11 helices respectively. On the other hand, 23 beta-sheets were observed at 20 ns and 30 ns while the structure at 0 ns and 10 ns comprise 21 and 24 beta-sheets respectively. The fluctuations in the number and lengths of the secondary structural elements were observed in the simulated model that greatly influenced the structural quality. The terminal directions of the structure have changed during the simulation analyses. The N and C terminals in the unrefined structure were embedded in the structure and projected inwards. The refined structure has terminals projected out of the protein structure with clear ends. It was also seen that the pattern similarity in overall structure and protein model stability incremented with MD simulations.

2.3 Derived peptides

The peptides were manually derived based on the properties of the interacting residues and structural characterization of the amino acids; the peptides considered for the current study were specifically including di-peptides. The criteria for the selection of di-peptides includes both of the amino acids either belong to a hydrophobic group or bulky hydrophobic. The peptides were derived by using the peptide cutter with two enzymes pepsin and trypsin individually, structural characterization and AHTpin server based on support vector machine score (SVM). The peptides derived from the applied techniques are mentioned in Table 1 with the cleavage site, peptide length, and SVM score.
All the derived peptides were evaluated by the regression models of SVM score and the leading peptides were docked with ACE to identify the high binding affinities. SVM regression model was built for di- and tri-peptides, while SVM classification models for peptides have more than three residues. The applied methods were based on the nature of amino acids, atomic composition and chemical descriptors (15,537) while trained by the machine learning techniques to evaluate through regression and classification methods.

Fig 3. The B-factor analysis represents the fluctuations of the atoms regarding their average positioning.

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Fig 4. Structural details of the simulated structure of MRJP1 at 0 ns, 10 ns, 20 ns, and 30 ns.

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Regression analyses were conducted to correlate the chemical descriptors and biological activity (pIC50) of small peptides for the pIC50 prediction of novel peptides.

Di-peptides and tri-peptides belong to a small class of peptides but separate regression models were implemented for each method to predict the biological activity. The classification models predicted the special type of peptides either AHT or non-AHT based on the descriptors of the training set. Mainly, PubChem, CDK-fingerprint, XLogP, electrotopological state atom type, and auto-correlation descriptors were implemented to develop the di-peptide QSAR model while tri-peptide QSAR model was developed primarily by KlekotaRoth fingerprint count, PubChem fingerprint, CDK graph only fingerprint and extended fingerprint descriptors [56]. The biological activity of di-peptides including AV, AI, GL, GM, GV & VL from MRJP1 was validated and evaluated by AHTpin. The reliability and bioactivity of all the derived peptides from MRJP1 were validated by AHTpin.

The lead anti-hypertensive peptides were selected on the basis of SVM scores for molecular docking analyses. The protein-peptide molecular docking analyses were performed and ACE was utilized as receptor against all the derived peptides to determine the binding position and orientation (S1 File). The docking analyses were performed by using the segmentation technique to identify and scrutinize the patches to evaluate the binding conformations and give a score to geometric complementary shapes. The docking complexes were ranked by the observed docking score and top ten ranked peptides having highest binding affinities were selected (Table 2) for further binding interactional studies through PyMol and UCSF Chimera (Fig 5). It was observed that the peptide “EALPHVPIDR” from all the scrutinized peptides showed docking score of 9590 and effective binding affinity. The interesting fact was observed

| Technique | Cleavage Site | Peptide | Peptide Length | SVM Score | Peptide Length | SVM Score |
|-----------|---------------|---------|----------------|-----------|----------------|-----------|
| Proteolysis Pepsin | 110 | LLQYPDPDW | 8 | 1.47 | 265 | LYYSVPASTSLYY | 13 | 1.74 |
| | 294 | QQNDIH | 6 | 0.35 | 264 | NLYYSVPASTSLY | 13 | 1.66 |
| | 135 | AIDKCDRL | 8 | 0.04 | 266 | YSYSVPASTSLYY | 13 | 1.52 |
| | 369 | PHVIPF | 6 | 1.72 | 262 | TNLYYSVPASTS | 13 | 1.49 |
| | 237 | YDPKF | 5 | 0.76 | 102 | PLLQYPDWSFAK | 13 | 1.48 |
| Trypsin | 114 | VGDGGPLLQYPDPDSFAK | 18 | 0.39 | 101 | GPLLQYPDPDSFA | 13 | 1.41 |
| | 62 | QDAILSGEYDK | 12 | 0.65 | 151 | SPKLLTFDLTTSQ | 13 | 1.38 |
| | 166 | LLETDLTTSSLK | 13 | 0.30 | 268 | SPVASTSLYYVNT | 13 | 1.38 |
| | 371 | EALPHVPIDR | 11 | 1.52 | 100 | GGPLLQYPDPDS | 13 | 1.36 |
| Structural Characterization | 175 | AV | 2 | 3.10 (pIC50) | 96 | KVDGGPLLQYPYP | 13 | 1.36 |
| | 53 | AI | 2 | 5.47 (pIC50) | 263 | NNLYYSVPASTSL | 13 | 1.33 |
| | 212 | GL | 2 | 2.60 (pIC50) | 267 | YSPVASTSLYYV | 13 | 1.30 |
| | 255 | GM | 2 | 2.85 (pIC50) | 97 | VGDGGPLLQYPDPDS | 15 | 1.20 |
| | 85 | GV | 2 | 2.34 (pIC50) | 99 | DGGPLLQYPDPDS | 13 | 1.16 |
| | 137 | VL | 2 | 4.89 (pIC50) | 258 | LSPMTNNLYYSVP | 13 | 1.11 |

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that the scrutinized top-ranked peptide was embedded in the receptor surface and engaged the binding domain. The anti-hypertensive peptides EALPHVPIFDR, NLYYSPVASTSLY, PHVPIF, and LYYSPVASTSLYY showed least binding energies may have the potential to behave as ACE inhibitors. The binding interactions of the selected peptides revealed that the Tyr residue is the most common interacting residue that behaved as an ACE inhibitor and has the potential to be a potent drug target.

Majority of the therapeutic agents attain their outcomes by binding and modify the functions of the target proteins. Traditionally, the binding within small cavities and catalytic sites inhibition exhibit the high affinity and successful therapeutics by compounds [57].

Food is considered as a source of nutrients and energy essentials to sustain the appropriate functions of the body. Now, scientists are trying to identify the novel characteristics of food constituents that may assist to overcome the numerous ‘diseases of civilization’. The mutual objectives of nutritionists, food manufacturers and researchers are to focus the proteins that have the origins of ACE inhibitors, to enhance their bioactivity and formulating those as commercial food to improve the human health [58]. In recent years, peptides have gained demanding attention in the pharmaceutical research for being highly efficacious, selective and relatively safe. More than a few hundreds of novel peptide therapeutics are currently being evaluated in pre-clinical and clinical trials while over 60 peptides have reached the market for different therapies [59].

Various side effects such as cough, headache, dizziness, and angioedema of synthetic anti-hypertensive drugs have been reported [60]. Therefore, the identification of potential anti-hypertensive biopeptides from foods gained attention [61]. The peptides of anti-hypertension have been reported in various dietary sources including egg, milk, meat, potato, wheat, soya beans, and vegetables. The synthetic compounds also occur as ACE inhibitors for hypertension therapies, although synthetic drugs contain adverse effects. So, the inclination towards nature-derived anti-hypertensive molecules is highly desired. The in silico identification of ACE inhibitory peptides from honey protein was performed which is considered as a source of anti-hypertension in the form of the ACE inhibitor [17].

The functional and nutritional features of dietary proteins have been studied over decades. The physiological consumption of amino acids after digestion and protein composition exhibit the nutritional characteristics [62]. Glycine was found as a predominant amino acid in AHTs server analysis and possesses two residues, revealed through amino acid composition investigation [56].

The proteolytic processing of food proteins leads to the production of active and bioactive peptides that performs various physiological functions of the body. These bioactive peptides

| Peptide     | SVM Score | PatchDock Score | Global Energy (kcal/mol) | ACE Binding Residues                                   |
|-------------|-----------|-----------------|--------------------------|--------------------------------------------------------|
| EALPHVPIFDR | 1.52      | 9590            | -58.29                   | Tyr62, Ala63, Asn66, Asn70, Ile88, Lys118, Glu123, Met223, Val351, His353, Ala354, Ser355, Ala356, Trp357, Asp358, Tyr360, Lys368, Glu384, Phe391 Arg402, Glu403, Phe512, His513, Ser516, Ser517, Val518, Trp520, Arg522, Tyr523, Zn701 |
| NLYYSPVASTSLY | 1.66    | 11060           | -52.04                   | Trp59, Tyr62, Asn66, Asn70, Leu81, Lys118, Val119, Glu121, Asp121, Glu123, Arg124, Leu139, Leu140, Tyr213, Met223, Val351, His353, Ser355, Trp357, Lys368, Arg402, Glu403, Phe512, Ser516, Ser517, Val518, Pro519, Phe570 |
| PHVPIF     | 1.72      | 6968            | -47.47                   | Trp59, Tyr62, Ile88, Thr92, Lys118, Glu123, Arg124, Tyr360, Arg402, Glu403, Pro519, Arg522 |
| LYYSPVASTSLYY | 1.74  | 10388           | -33.24                   | Trp59, Tyr62, Asn66, Asn70, Lys118, Asp121, Glu123, Arg124, Ser219, Trp220, Ser222, Tyr213, Met223, Ser355, Ala356, Trp357, Tyr360, Glu403, Asn406, Pro407, Ser516, Ser517, Val518, Pro519, Arg522, Phe570, Zn701 |

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may act as an opioid antagonist, agonists, anti-hypertensive agents, and moreover anti-cancer, anti-thrombotic, anti-microbial, immune-modulating and anti-oxidative activity have been reported. The bioactive peptides may be utilized in functional food components due to their therapeutic potentials [63].

The peptides are preferred over the small compounds due to their structural compatibility, small size and ability to interrupt protein-protein interfaces. The rational methods have a key hindrance to design effective peptide ligands for the development of potential drugs. However, numerous computational techniques have evidenced the structural and functional insights into the architecture of protein-peptide interfaces for the rational peptide design approach. These methods help to fulfill the vision of computationally designed peptides for therapies through the high-resolution structures of protein-peptide complexes [64–66].
In vivo studies have found that anti-hypertensive effects can be attained in humans through peptides especially di- and/or tri-peptides [67NR, 68]. Hata et al., [68] demonstrated the effectiveness of Ile-Pro-Pro and Val-Pro-Pro on blood pressure (BP) regulation. They hypothesized that stimulation in aortas along with circulatory ACE inhibition would be the reason for that effect [69]. It has also been reported that the intake of bioactive Val-Tyr di-peptide led to a significant reduction of systolic BP after 1 week on mildly hypertensive subjects [67]. These discoveries strongly recommended that the renin-angiotensin system suppression by bioactive smaller peptides play a significant role in the regulation of BP.

The ACE inhibition was greatly enhanced through gastrointestinal protease hydrolysis of royal jelly by trypsin followed by pepsin and chymotrypsin [70]. These analyses reported that the inactive royal jelly proteins might be an effective ACE inhibitor to regulate the BP and new peptide inhibitors in gut formed through gastrointestinal proteases would be more significant. Uno et al. [71] documented that consumption of royal jelly hydrolysate by trypsin and pepsin amplified the hemoglobin levels and reduced the higher cholesterol levels in human beings. Therefore, the royal jelly is considered as a beneficiary to improve the homeostasis.

Ohashi et al. [72], derived peptides from the royal jelly glycoproteins and demonstrated that most of the isolated peptides have aromatic amino acid residues as Phe and Tyr at C-terminus exhibited the strong inhibitory activity. Cheung et al., [73] confirmed the inhibition potential of these aromatic peptides in their research and observed the additional ACE inhibition for peptides with Trp-Tyr-Phe at the C-terminus. It has also been reported that the peptides having Ile-Val-Tyr residues extracted from the royal jelly hydrolysate with the highest ACE inhibitory contribution rate of 16.9% in addition to wheat germ hydrolysate [74].

Okunishi et al. [75] elaborated the long-term oral therapeutic drug, spirapril that suppresses the ACE activities in blood vessels and induce the extended depressor effects. Their analyses showed that few of the natural inhibitory peptides, specifically royal jelly peptides could gather at the vessels and exert a regulation of secretion for active elements including prostaglandins or endothelin and nitric oxide [76]. The royal jelly protein has the ability to produce plenty of ACE inhibitory peptides throughout the digestion to reduce the depressor effect and it was a latent natural source along with in vivo anti-hypertensive effects.

The development of peptide-based therapeutics is of great interest and has rapid growth [77–79]. Currently, a robust approach has been evolved that incorporates topographical, conformational, dynamic and structural considerations to design the peptides for drugs, drug molecules, and biological tools. Current developments to understand the chemistry of life, specifically molecular biophysics, proteomics, genomics, and molecular biology have described that the macromolecular-peptide interactions establish the key physiochemical processes whereby living mechanisms are modulated and controlled [80]. In this modern era, bioinformatics approaches play a vital role in the discovery of novel peptides [81]. Traditionally, the peptide design utilizes the homology models or structures along with the docking methods to design the peptides with high affinity against the target proteins [80].

The current findings focused to reveal the potent anti-hypertensive peptides as ACE inhibitors from royal jelly protein (MRJP1) through in silico approaches. RMSD and RMSF graphs described the structural stability of MRJP1 in MD simulations at 30 ns along with the quality factor of 92.43%. Peptides were derived by protease hydrolysis, structural characterization/physiological properties, and AHTpin server approaches. The anti-hypertensive potential of scrutinized peptides was measured by QSAR methods of the AHTpin program and the preferable anti-hypertensive candidates with SVM scores ranges from 0.04 to 1.74 were determined. Protein-peptide docking analyses were further carried out to reveal the binding conformations, binding affinities, and potential binding residues. It has also been analyzed that the peptides were embedded with ACE receptor protein and top-ranked 4 peptides were selected
having strong binding affinities \textit{i.e.} 10388 to 9590 docking scores. Top four peptides mainly encompass aromatic amino acid residues including Tyr-Trp-Phe while Tyr was observed as the most abundant amino acid in the selected peptides. Various \textit{in vivo} studies have reported the strong anti-hypertensive activity of aromatic amino acids, particularly Tyr amino acid [82]. It has been suggested that the protease hydrolysis of the royal jelly protein produces many effective ACE inhibitors that would regulate BP.

The molecular docking analyses have the significance of elucidating the interacting residues between the receptor proteins and ligands [83]. Generally, there are three modes of ACE inhibitory peptides as competitive, non-competitive and mixed. The competitive inhibitory peptides possess 2–12 amino acid residues in length and attached at the binding site of ACE. The non-competitive inhibitory peptides showed that the binding other than substrate binding site and affect the ACE enzyme activity. Zn is considered as the significant component of the active site of ACE and ACE activity also depends on Zn [84]. The ACE active site is divided into three binding pockets as $S_1$ (Ala354, Glu384, and Tyr523), $S_1'$ (Glu162) and $S_2$ (Gln281, His353, Lys511, His513, and Tyr520) [85]. The binding stability of peptides at the binding site of the ACE enzyme depends upon hydrogen bonding [86]. Additionally, the involvement of His353, Ala354, Ser355, Glu384, His513, and Pro519 residues are significant for the stability of peptide and enzyme complex while numerous effective peptides have been reported for their interactions at the specified binding sites [87, 88].

The molecular interactional studies of ACE inhibitory peptides are beneficial for the designing and screening of potential novel inhibitory peptides. The reported peptides also present the binding interactions at binding pockets and behave as competitive inhibitory peptides. The top-ranked peptide (EALPHVPIFDR) showed binding interactions in $S_1$ and $S_2$ binding pocket of the ACE enzyme and engaged the significant interacting residues through hydrogen bonding leading to the stability of the complex. The utilized \textit{in silico} approaches provide a novel and potential ACE inhibitors through various distinctive techniques that have the potential to analyze the large-scale conformations through protein-peptide interactions. This leads to an initial step of reducing and eliminating hypertension without drug usage and not to bear their side effects. This could probably be happening only by using those food sources and dietary components, which improves human health and act as preventive measures of these sorts of diseases.

\textbf{Conclusions}

Contemporary research methods including bioinformatics and proteomic tools applied in current research on peptides from honey protein as a food source and identified the potential anti-hypertensive peptides. It has been demonstrated that the scrutinized peptides EALPHVPIFDR, NLYYSPVASTSLY, PHVPIF, and LYYSPVASTSLYY may have the potential to reduce hypertension with minimal side effects. The reported peptides comprise of aromatic amino acids particularly Tyr and its strong anti-hypertensive activity made the selected peptides a better choice after an extensive \textit{in silico} studies. Even if such food and peptides of proteins are not being able to replace drugs in acute hypertension, they still may have the potential to prevent hypertension.

\textbf{Material and methods}

\textbf{3.1 Functional information and canonical sequence}

The present studies demonstrate the identification of ACE inhibitory peptides from MRJP1 honey protein against hypertension by employing the \textit{in silico} approaches comprising computational 3D modeling, MD simulations, peptides designing, molecular docking analyses, and
anti-hypertensive activity predictions. The utilized methodology of the current study is presented in a flow chart (Fig 6). The functional information and canonical sequence of MRJP1 in FASTA format were retrieved from UniProt Knowledgebase (http://www.uniprot.org/) having the accession number O18330. The MRJP1 protein sequence was subjected to the protein-protein basic local alignment search tool (BLASTp) [89] against PDB to identify the possible templates. The homology modeling (Modeller 9.14 [54]) and threading based approaches (I-Tasser [50]) were implemented to build the 3D structure of MRJP1. The 3D crystal structure of human ACE was retrieved from PDB (ID: 1O8A) having a resolution of 2 Å determined by the X-ray diffraction method. Errat [90] and Rampage [91] evaluation tools were utilized to evaluate the predicted structure before and after the simulations analyses.

3.2 MD simulations

The selected MRJP1 model was subjected to MD simulations by employing AMBER v14 [92] with an ff14SB force field. The simulation analyses were executed in explicit solvent and three-point transferable intermolecular potential (TIP3P) water molecules implemented to solvate the initial structures of a modeled system. Pre-equilibrated elementary cubic box of 78.672 Å \(^3\) 84.370 Å \(^*\) 79.589 Å was applied to cover the target protein completely that appended 12,397 water molecules. This system amplified the total mass up to 272215.674 amu accompanied by a density of 0.856 g/cm\(^3\). The system was neutralized by incorporating the 15 Na\(^+\). The comprehensive energy minimization of the solvated protein was carried out for MD simulation experiments. The energy minimization comprising 1500 cycles of conjugate gradient and steepest descent algorithm was executed to eliminate or reduce the energy constraints. SHAKE algorithm was implemented to constrain the hydrogen atoms and bond lengths [93]. A non-bonded cutoff of 10.0 Å with a time step of 0.002 ps was employed by the Berendsen coupling algorithm. Ewald summation method was used to execute the MD simulations for comprehensive electrostatic interactions [94]. The simulation experiments were simulated in initial
equilibration at 1 atm pressure, constant temperature for 300 K and heating time for 500 ps. The simulations for 30 ns were performed and coordinate files were saved after every 5 ns time frame for the structural analyses. PTRAJ module of AMBER generated the output files for the analyses and then visualized by using UCSF Chimera [95]. The obtained results were analyzed by considering various factors including B-Factor, RMSD, and RMSF. The graphs for B-factor, RMSD and RMSF were generated by Microsoft Excel.

### 3.3 Preparation of peptides

The preparation of peptides was performed by three different approaches as structural characterization, proteolysis and AHTpin server [56]. The peptides were manually derived on the basis of anti-hypertensive properties and structural attributes characterizing di-peptides and tri-peptides ACE inhibitors. Di-peptides were composed of amino acids with bulky and hydrophobic side chains, while in tri-peptides, the 1\(^{st}\) residue at N-terminal was aromatic, 2\(^{nd}\) one was positively charged and the 3\(^{rd}\) residue at C-terminal was hydrophobic [25].

#### 3.3.1 Proteolysis

Proteolysis was conducted by employing the Peptide Cutter software (http://www.expasy.ch/tools/peptidecutter/) with pepsin and trypsin enzymes individually.

#### 3.3.2 Peptide derivation

The anti-hypertensive peptide inhibitors (AHTpin), an online server was used to derive the peptides having anti-hypertensive inhibitory activity by submitting the sequence of MRJ1 to the server. The anti-hypertensive peptides extracted from the above-mentioned techniques were prepared for docking experiments with the receptor protein ACE. Protein-peptide docking analyses were carried out through PatchDock [96] with the parameter of clustering RMSD as 4 to identify the binding affinities of securitized peptides. The top-ranked analyzed complexes were further refined by the Fast Interaction REfinement in the molecular DOCKing (FireDock) server [97] and scrutinized the effective complexes on the basis of their global energy. UCSF Chimera visualization tool was implemented to critically analyze and visualize the peptide interactions and binding pockets accompanied by the bond lengths.

### Supporting information

S1 Fig. ERRAT quality factor analyses of the predicted structure. (TIF)

S1 File. Table 1: Manually derived peptides with docking scores. Table 2: Docking Scores for Peptides Generated Using Pepsin Enzyme. Table 3: Docking Scores for Peptides Generated Using Trypsin Enzyme. Table 4: AHTpin peptides docking scores. (DOCX)

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References

1. Lai YC, Potoka KC, Champion HC, Mora AL, Gladwin MT. Pulmonary arterial hypertension: the clinical syndrome. Circulation research. 2014; 115(1):115–30. https://doi.org/10.1161/CIRCRESAHA.115.301146 PMID: 24951762; PubMed Central PMCID: PMC4096686.

2. Aldred MA, Vijayakrishnan J, James V, Soubrier F, Gomez-Sanchez MA, Martensson G, et al. BMPR2 gene rearrangements account for a significant proportion of mutations in familial and idiopathic pulmonary arterial hypertension. Human mutation. 2006; 27(2):212–3. https://doi.org/10.1002/humu.20398 PMID: 16429403.

3. Cogan JD, Pauciulo MW, Batchman AP, Prince MA, Robbins IM, Hedges LK, et al. High frequency of BMPR2 exonic deletions/duplications in familial pulmonary arterial hypertension. American journal of respiratory and critical care medicine. 2006; 174(5):590–8. https://doi.org/10.1164/rcrm.200602-165OC PMID: 16728714; PubMed Central PMCID: PMC2648061.

4. Rigelsky CM, Jennings C, Lehtonen R, Minai OA, Eng C, Aldred MA. BMPR2 mutation in a patient with pulmonary arterial hypertension and suspected hereditary hemorrhagic telangiectasia. American journal of medical genetics Part A. 2008; 146A(19):2551–6. https://doi.org/10.1002/ajmg.a.32468 PMID: 18792970.

5. International PPHC, Lane KB, Machado RD, Pauciulo MW, Thomson JR, Phillips JA 3rd, et al. Heterozygous germline mutations in BMPR2, encoding a TGF-beta receptor, cause familial primary pulmonary hypertension. Nature genetics. 2000; 26(1):81–4. https://doi.org/10.1038/79226 PMID: 10973254.

6. Deng Z, Morse JH, Slager SL, Cuervo N, Moore KJ, Venetos G, et al. Familial primary pulmonary hypertension (Gene PPH1) is caused by mutations in the bone morphogenetic protein receptor–II gene. The American Journal of Human Genetics. 2000; 67(3):737–44. https://doi.org/10.1086/303059 PMID: 10903931.

7. Thomason J, Machado RD, Pauciulo MW, Morgan NV, Humbert M, Elliott GC, Ward K, Yacoub M, Mikhail G, Rogers P, Newman J, Wheeler L, Higenbottam T, Gibbs JS, Egan J, Crozier A, Peacock A, Alcock R, Corris P, Loyd JE, Trembath RC, Nichols WC. Sporadic primary pulmonary hypertension is associated with germline mutations of the gene encoding BMPR-II, a receptor member of the TGF-beta family J Med Genet. 2000; 37:741–5. https://doi.org/10.1136/jmg.37.10.741 PMID: 11015450.

8. Morse JH, Jones AC, Barst RJ, Hodge SE, Wilhelmsen KC, Nygaard TG. Mapping of familial primary pulmonary hypertension locus (PPH1) to chromosome 2q31-32. Nature genetics. 1997; 15(3):277–80. https://doi.org/10.1038/ng0397-277 PMID: 9193425.

9. Nichols WC, Koller DL, Slovis B, Foroud T, Terry VH, Arnold ND, et al. Localization of the gene for familial primary pulmonary hypertension to chromosome 2q31-32. Nature genetics. 1997; 15(3):277–80. https://doi.org/10.1038/ng0397-277 PMID: 9054941.

10. Machado RD, Pauciulo MW, Thomson JR, Lane KB, Morgan NV, Wheeler L, et al. BMPR2 haploinsufficiency as the inherited molecular mechanism for primary pulmonary hypertension. The American Journal of Human Genetics. 2001; 68(1):92–102. https://doi.org/10.1086/316947 PMID: 11115378.

11. Massague J, Chen YG. Controlling TGF-beta signaling. Genes & development. 2000; 14(6):627–44. PMID: 10733523.

12. Morrell NW, Yang X, Upton PD, Jourdan KB, Morgan N, Sheares KK, et al. Altered growth responses of pulmonary artery smooth muscle cells from patients with primary pulmonary hypertension to transforming growth factor-β1 and bone morphogenetic proteins. Circulation. 2001; 104(7):790–5. https://doi.org/10.1161/01.cir.91.12.2603 PMID: 11054941.

13. Mancia G. Introduction to a compendium on hypertension. Circulation research. 2015; 116(6):923–4. https://doi.org/10.1161/CIRCRESAHA.115.307575 PMID: 25767280.

14. Ehlers MR, Riordan JF. Angiotensin-converting enzyme: zinc-and inhibitor-binding stoichiometries of the somatic and testis isozymes. Biochemistry. 1991; 30(29):7118–26. https://doi.org/10.1021/bi00243a012 PMID: 1649623.
15. Korhonen H, Pihlanto A. Food-derived bioactive peptides—opportunities for designing future foods. Current pharmaceutical design. 2003; 9(16):1297–308. https://doi.org/10.2174/1381612033454892 PMID: 12769738

16. FitzGerald RJ, Murray BA, Walsh DJ. Hypotensive peptides from milk proteins. The Journal of nutrition. 2004; 134(4):980S–8S. https://doi.org/10.1093/jn/134.4.980S PMID: 15051858.

17. Vercruysse L, Van Camp J, Smagghe G. ACE inhibitory peptides derived from enzymatic hydrolysates of animal muscle protein: a review. Journal of agricultural and food chemistry. 2005; 53(21):8106–15. https://doi.org/10.1021/jf0508908 PMID: 16218651.

18. Hong F, Ming L, Yi S, Zhanxia L, Yongquan W, Chi L. The antihypertensive effect of peptides: a novel alternative to drugs? Peptides. 2008; 29(6):1062–71. https://doi.org/10.1016/j.peptides.2008.02.005 PMID: 18384915.

19. Yang H, Erdos E, Levin Y. A dipeptidyl carboxypeptidase that converts angiotensin I and inactivates bradykinin. OKLAHOMA UNIV MEDICAL CENTER OKLAHOMA CITY, 1970.

20. Atkinson AB, Robertson JI. Captopril in the treatment of clinical hypertension and cardiac failure. Lancet. 1979; 2(8147):836–9. https://doi.org/10.1016/s0140-6736(79)92186-x PMID: 90928.

21. Israili ZH, Hall WD. Cough and angioneurotic edema associated with angiotensin-converting enzyme inhibitor therapy. A review of the literature and pathophysiology. Annals of internal medicine. 1992; 117(3):234–42. https://doi.org/10.7326/0003-4819-117-3-234 PMID: 1616218.

22. Hartmann R, Meisel H. Food-derived peptides with biological activity: from research to food applications. Current opinion in biotechnology. 2007; 18(2):163–9. https://doi.org/10.1016/j.copbio.2007.01.013 PMID: 17292602.

23. Geleijnse JM, Engberink MF. Lactopeptides and human blood pressure. Current opinion in lipidology. 2010; 21(1):58–63. https://doi.org/10.1097/MOL.0b013e328333813 PMID: 19884823.

24. Wu J, Aluko RE, Nakai S. Structural requirements of Angiotensin I-converting enzyme inhibitory peptides: quantitative structure-activity relationship study of di- and tripeptides. Journal of agricultural and food chemistry. 2006; 54(3):732–8. https://doi.org/10.1021/jf051263l PMID: 16448176.

25. Wu J, Aluko RE, Nakai S. Structural Requirements of Angiotensin I-Converting Enzyme Inhibitory Peptides: Quantitative Structure-Activity Relationship Modeling of Peptides Containing 4-10 Amino Acid Residues. Molecular Informatics. 2006; 25(10):873–80.

26. Iwaniak A, Dziuba J. Animal and plant proteins as precursors of peptides with ACE Inhibitory Activity—An in silico strategy of protein evaluation. Food Technology and Biotechnology. 2009; 31(4):441.

27. Sehgal SA, Khattak NA, Mir A. Structural, phylogenetic and docking studies of D-amino acid oxidase activator (DAOA), a candidate schizophrenia gene. Theoretical Biology and Medical Modelling. 2013; 10(1):3.

28. Sehgal SA, Mannan S, Ali S. Pharmacoinformatic and molecular docking studies reveal potential novel antidepressants against neurodegenerative disorders by targeting HSPB6. Drug design, development and therapy. 2016; 10:1605.

29. Sehgal SA, Mannan S, Kanwal S, Naveed I, Mir A. Adaptive evolution and elucidating the potential inhibitor against schizophrenia to target DAOA (G72) isoforms. Drug design, development and therapy. 2015; 9:3471. https://doi.org/10.2147/DDDT.S63946 PMID: 26170631

30. Sehgal SA. Pharmacoinformatics, adaptive evolution, and elucidation of six novel compounds for schizophrenia treatment by targeting DAOA (G72) isoforms. BioMed research international. 2017;2017.

31. Sehgal SA. Pharmacoinformatics and molecular docking studies reveal potential novel Proline Dehydrogenase (PRODH) compounds for Schizophrenia inhibition. Medicinal Chemistry Research. 2017; 26 (2):314–26.

32. Tahir RA, Sehgal SA. Pharmacoinformatics and Molecular Docking Studies Reveal Potential Novel Compounds Against Schizophrenia by Target SYN II. Combinatorial chemistry & high throughput screening. 2018; 21(3):175–81. https://doi.org/10.2174/1386207321666180213092018 PMID: 29436999.

33. Sehgal S, Tahir R, Shafique S, Hassan M, Rashid S. Molecular modeling and docking analysis of CYP1A1 associated with head and neck cancer to explore its binding regions. J Theor Comput Sci. 2014; 1(112):2.

34. Tahir RA, Sehgal SA, Khattak NA, Khattak JZK, Mir A. Tumor necrosis factor receptor superfamily 10B (TNFRSF10B): an insulin from structure modeling to virtual screening for designing drug against head and neck cancer. Theoretical Biology and Medical Modelling. 2013; 10(1):38.

35. Sehgal SA, Kanwal S, Tahir RA, Khalid Z, Hammad MA. In silico elucidation of potential drug target sites of the Thumb Index Fold Protein, Wnt-8b. Tropical Journal of Pharmaceutical Research. 2018; 17 (3):491–7.
36. Jamil F, Ali A, Sehgal SA. Comparative modeling, molecular docking, and revealing of potential binding pockets of RASSF2; a candidate cancer gene. Interdisciplinary Sciences: Computational Life Sciences. 2017; 9(2):214–23.

37. Tahir RA, Wu H, Rizvan MA, Jafar TH, Saleem S, Sehgal SA. Immunoinformatics and molecular docking studies reveal potential epitope-based peptide vaccine against DENV-NS3 protein. Journal of theoretical biology. 2018; 459:162–70. https://doi.org/10.1016/j.jtbi.2018.10.005 PMID: 30291844

38. The three-dimensional structure of an insec yellow/major royal jelly protein family member and its function in blood feeding by the Leishmania vector Lutzomyia longipalpis. To be published. https://doi.org/10.2210/pdb3q6k/pdb

39. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic acids research. 1997; 25(17):3389–402. https://doi.org/10.1093/nar.25.17.3389 PMID: 9254694

40. Bowie JU, Luthy R, Eisenberg D. A method to identify protein sequences that fold into a known three-dimensional structure. Science. 1991; 253(5016):164–70. https://doi.org/10.1126/science.1853201 PMID: 1853201

41. Jones DT, Taylor W, Thornton JM. A new approach to protein fold recognition. Nature. 1992; 358(6381):86. https://doi.org/10.1038/358086a0 PMID: 1614539

42. Arakaki AK, Zhang Y, Skolnick J. Large-scale assessment of the utility of low-resolution protein structures for biochemical function assignment. Bioinformatics. 2004; 20(7):1087–96. https://doi.org/10.1093/bioinformatics/bth044 PMID: 14764543

43. Yue P, Moult J. Identification and analysis of deleterious human SNPs. Journal of molecular biology. 2006; 356(5):1263–74. https://doi.org/10.1016/j.jmb.2005.12.025 PMID: 16412461

44. Boyd A, Ciuflo LF, Barclay JW, Graham ME, Haynes LP, Doherty MK, et al. A random mutagenesis approach to isolate dominant-negative yeast sec1 mutants reveals a functional role for domain 3a in yeast and mammalian Sec1/Munc18 proteins. Genetics. 2008; 180(1):165–78. https://doi.org/10.1534/genetics.108.090423 PMID: 18757920

45. Ye Y, Li Z, Godzik A. Modeling and analyzing three-dimensional structures of human disease proteins. Bioinformatics. 2006; 22(1):145–55. https://doi.org/10.1093/bioinformatics/btl118

46. Dorn M, e Silva MB, Buriol LS, Lamb LC. Three-dimensional protein structure prediction: Methods and computational strategies. Computational biology and chemistry. 2014; 53:251–76.

47. Lance BK, Deane CM, Wood GR. Exploring the potential of template-based modelling. Bioinformatics. 2010; 26(15):1849–56. https://doi.org/10.1093/bioinformatics/btq294 PMID: 20525820

48. Joo K, Lee J, Lee S, Seo JH, Lee SJ, Lee J. High accuracy template based modeling by global optimization. Proteins: Structure, Function, and Bioinformatics. 2007; 69(8):83–9.

49. Moult J. A decade of CASP: progress, bottlenecks and prognosis in protein structure prediction. Current opinion in structural biology. 2005; 15(3):285–9. https://doi.org/10.1016/j.sbi.2005.05.011 PMID: 15939584

50. Roy A, Kucukural A, Zhang Y. I-TASSER: a unified platform for automated protein structure and function prediction. Nature protocols. 2010; 5(4):725. https://doi.org/10.1038/nprot.2010.5 PMID: 20360767

51. Zhang Y. Protein structure prediction: when is it useful? Current opinion in structural biology. 2009; 19(2):145–55. https://doi.org/10.1016/j.sbi.2008.02.005 PMID: 19327982

52. Ginalska K, Elofsson A, Fischer D, Rychlewski L. 3D-Jury: a simple approach to improve protein structure predictions. Bioinformatics. 2003; 19(6):1015–8. https://doi.org/10.1093/bioinformatics/btg124 PMID: 12761065

53. Misurea K, Baker D. Progress and challenges in high-resolution refinement of protein structure models. Proteins: Structure, Function, and Bioinformatics. 2005; 59(1):15–29.

54. Śali A, Blundell TL. Comparative protein modelling by satisfaction of spatial restraints. Journal of molecular biology. 1993; 234(3):779–815. https://doi.org/10.1006/jmbi.1993.1626 PMID: 8254673

55. Kushwaha SK, Shaky M. Molecular modelling and dynamics studies of Mycobacterium tuberculosis protein RelA (Rv2583c). International Journal of Integrative Biology. 2009; 3(7):135.

56. Kumar R, Chaudhary K, Singh Chauhan J, Nagpal G, Kumar R, Sharma M, et al. An in silico platform for predicting, screening and designing of antihypertensive peptides. Scientific reports. 2015; 5:12512. https://doi.org/10.1038/srep12512 PMID: 26213115; PubMed Central PMCID: PMC4515604.

57. Drews J. Drug discovery: a historical perspective. Science. 2000; 287(5460):1960–4. https://doi.org/10.1126/science.287.5460.1960 PMID: 10720314.

58. Iwaniak A, Minkiewicz P, Darewicz M. Food-originating ACE inhibitors, including antihypertensive peptides, as preventive food components in blood pressure reduction. Comprehensive Reviews in Food Science and Food Safety. 2014; 13(2):114–34.
59. Fosgerau K, Hoffmann T. Peptide therapeutics: current status and future directions. Drug discovery today. 2015; 20(1):122–8. https://doi.org/10.1016/j.drudis.2014.10.003 PMID: 25450771

60. Daliri EB-M, Lee BH, Oh DH. Current perspectives on antihypertensive probiotics. Probiotics and antimicrobial proteins. 2017; 9(2):91–101. https://doi.org/10.1007/s12602-016-9241-y PMID: 27900619

61. Koyama M, Hattori S, Amano Y, Watanabe M, Nakamura K. Blood pressure-lowering peptides from neo-fermented buckwheat sprouts: a new approach to estimating ACE-inhibitory activity. PloS one. 2014; 9(9):e105802. https://doi.org/10.1371/journal.pone.0105802 PMID: 25222748

62. Friedman M. Nutritional value of proteins from different food sources. A review. Journal of agricultural and food chemistry. 1996; 44(1):6–29.

63. Clare D, Swaisgood H. Bioactive milk peptides: a prospectus. Journal of dairy science. 2000; 83(6):1187–95. https://doi.org/10.3168/jds.S0022-0302(00)74983-6 PMID: 10877382

64. Fernández L, Bustos R, Zapata C, Garcia J, Jauregui E, Ashraf G. Immunogenicity in protein and peptide-based therapeutics: an overview. Current Protein and Peptide Science. 2018; 19(10):958–71. https://doi.org/10.2174/1389203718666170828123449 PMID: 28847291

65. Matsui T, Li CH, Osajima Y. Preparation and characterization of novel bioactive peptides responsible for angiotensin-converting enzyme inhibition from wheat germ. Journal of Peptide Science. 1999; 5(7):289–97. https://doi.org/10.1002/(SICI)1099-1387(199907)5:7<289::AID-PSC196>3.0.CO;2-6 PMID: 10442764

66. Takase H, Moreau P, Küng CF, Nava E, Lüscher TF. Antihypertensive therapy prevents endothelial dysfunction in chronic nitric oxide deficiency: effect of verapamil and trandolapril. Hypertension. 1996; 27(1):25–31. https://doi.org/10.1161/01.hyp.27.1.25 PMID: 8591883

67. Vliegher P, Lisowski V, Martinez J, Khrestchatisky M. Synthetic therapeutic peptides: science and market. Drug discovery today. 2010; 15(1–2):40–56. https://doi.org/10.1016/j.drudis.2009.10.009 PMID: 19879957

68. Briand J-P, Muller Sd. Emerging peptide therapeutics for inflammatory autoimmune diseases. Current pharmaceutical design. 2010; 16(9):1136–42. https://doi.org/10.2174/1381612107900683805 PMID: 20030611
79. Bellmann-Sickert K, Beck-Sickinger AG. Peptide drugs to target G protein-coupled receptors. Trends in pharmacological sciences. 2010; 31(9):434–41. https://doi.org/10.1016/j.tips.2010.06.003 PMID: 20655603

80. Hruby VJ. Designing peptide receptor agonists and antagonists. Nature Reviews Drug Discovery. 2002; 1(11):847. https://doi.org/10.1038/nrd939 PMID: 12415245

81. Audie J, Boyd C. The synergistic use of computation, chemistry and biology to discover novel peptide-based drugs: the time is right. Current pharmaceutical design. 2010; 16(5):567–82. https://doi.org/10.2174/138161210790361425 PMID: 19929848

82. Aderinola TA, Fagbeni TN, Enujugha VN, Alashi AM, Aluko RE. Amino acid composition and antioxidant properties of Moringa oleifera seed protein isolate and enzymatic hydrolysates. Heliyon. 2018; 4(10):e00877. https://doi.org/10.1016/j.heliyon.2018.e00877 PMID: 30386828; PubMed Central PMCID: PMC6205298.

83. Tahir RA, Wu H, Javed N, Khalique A, Khan SAF, Mir A, et al. Pharmacoinformatics and molecular docking reveal potential drug candidates against Schizophrenia to target TAAR6. Journal of cellular physiology. 2018. https://doi.org/10.1002/jcp.27999 PMID: 30569503.

84. Pina A, Roque A. Studies on the molecular recognition between bioactive peptides and angiotensin-converting enzyme. Journal of Molecular Recognition: An Interdisciplinary Journal. 2009; 22(2):162–8.

85. Pan D, Cao J, Guo H, Zhao B. Studies on purification and the molecular mechanism of a novel ACE inhibitory peptide from whey protein hydrolysate. Food Chemistry. 2012; 130(1):121–6.

86. Fu Y, Alashi AM, Young JF, Therkildsen M, Aluko RE. Enzyme inhibition kinetics and molecular interactions of patatin peptides with angiotensin I-converting enzyme and renin. International journal of biological macromolecules. 2017; 101:207–13. https://doi.org/10.1016/j.ijbiomac.2017.03.054 PMID: 28300587.

87. Deng Z, Liu Y, Wang J, Wu S, Geng L, Sui Z, et al. Antihypertensive Effects of Two Novel Angiotensin I-Converting Enzyme (ACE) Inhibitory Peptides from Gracilaria lemaneiformis (Rhodophyta) in Spontaneously Hypertensive Rats (SHRs). Marine drugs. 2018; 16(9):299.

88. Ma F-F, Wang H, Wei C-K, Thakur K, Wei Z, Jiang L. Three novel ACE inhibitory peptides isolated from Ginkgo biloba seeds: Purification, inhibitory kinetic and mechanism. Frontiers in pharmacology. 2018; 9:1579. https://doi.org/10.3389/fphar.2018.01579 PMID: 30697161.

89. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. Journal of molecular biology. 1990; 215(3):403–10. https://doi.org/10.1002/jcp.27999 PMID: 20655603.

90. Colovos C, Yeates TO. Verification of protein structures: patterns of nonbonded atomic interactions. Protein science. 1993; 2(9):1511–9. https://doi.org/10.1002/pro.5560020916 PMID: 8401235.

91. Kleywegt GJ, Jones TA. Phi/psi-chology: Ramachandran revisited. Structure. 1996; 4(12):1395–400. https://doi.org/10.1016/s0969-2126(96)00147-5 PMID: 8994966.

92. Case D, Babin V, Berryman J, Betz R, Cai Q, Cerutti D, et al. Amber 14. 2014.

93. Andersen HC. Rattle: A “velocity” version of the shake algorithm for molecular dynamics calculations. Journal of Computational Physics. 1983; 52(1):24–34.

94. Andersen HC. Rattle: A “velocity” version of the shake algorithm for molecular dynamics calculations. Journal of Computational Physics. 1983; 52(1):24–34.

95. Betz R, Cai Q, Cerutti D, et al. Amber 14. 2014.

96. Andersen HC. Rattle: A “velocity” version of the shake algorithm for molecular dynamics calculations. Journal of Computational Physics. 1983; 52(1):24–34.

97. Betz R, Cai Q, Cerutti D, et al. Amber 14. 2014.