Research Article

Exploration of ACE-Inhibiting Peptides Encrypted in Artemisia annua Using In Silico Approach

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The renin-angiotensin system (RAS) is involved in body fluid regulation, but one of its enzymes, angiotensin-converting enzyme (ACE), indirectly increases blood pressure [4]. Angiotensin-converting enzymes in the RAS system convert angiotensin I to angiotensin II, which narrows the blood vessels and causes hypertension [5]. From different natural resources, many ACE-inhibiting peptides have been studied to stabilize blood pressure [6].

1. Introduction

Most cardiovascular diseases caused by hypertension have a high death ratio, and approximately 66% of hypertension cases are found, especially in developing countries [1]. Autoimmune illnesses, such as systemic lupus erythematosus and rheumatoid arthritis, are linked to an increased risk of hypertension and cardiovascular disease [2]. A major community-based research, for example, discovered a higher prevalence of hypertension among RA patients (31%), compared to the general population (23%) [3]. A hormone system renin-angiotensin system (RAS) is involved in body fluid regulation but indirectly increases blood pressure [4]. Angiotensin-converting enzymes in the RAS system convert angiotensin I to angiotensin II, which narrows the blood vessels and causes hypertension [5]. From different natural resources, many ACE-inhibiting peptides have been studied to stabilize blood pressure [6].

As the frequency of hypertension increased day by day, antihypertensive activity of most of the bioactive peptides was studied and gained much attention [7]. For the inhibition of ACE, many antihypertensive drugs have been discovered, such as captopril, lisinopril, and aliskiren. On the one hand, these drugs are beneficial, but at the same time, they cause serious side effects, such as disturbing the potassium level, loss of taste, and dizziness [8]. Therefore, antihypertensive peptides were studied from different sources, such as chia seeds, sesame seeds, and flaxseeds.
By hydrolysis of mung bean proteins, five ACE-inhibiting peptides (LPRL, YADLVE, LRLESF, HLNVVHEN, and PGSGCAGTDL) were released, and their effect was studied when given to hypertensive rats. The results showed that YADLVE was more effective than others [9]. Banana pulp was purified into three protein extracts as purified, partially purified, and crude. Upon hydrolysis with proteolytic enzymes, the crude extract released more ACE-inhibiting peptides (85.20%) [10].

Liang et al. [11] identified a peptide IAF from pumpkin seeds using in silico approaches. By molecular docking, a strong interaction was found between IAF and ACE, which shows hydrogen bonding between two residues of ACE, His513 and Glu162, with IAF. Similarly, many antihypertensive peptides are released from different plant sources, such as bitter melon seeds [12], peach seeds [13], cottonseed [14], hemp seeds [15], sesame seeds [16], and date seeds [17].

Four novel ACE-inhibiting peptides (MAF, NMF, HPF, and MCG) were identified from quinoa proteins. Hydrolysis of proteins was performed by an in silico method using plant proteolytic enzymes, ficin, papain, and stem bromelain [18]. *Artemisia annua* is a short-day plant containing a high protein content and several essential amino acids. Due to high antimalarial activity, the Nobel Prize was awarded to the species in 2015 [19].

In this research, antihypertensive peptides were studied from *A. annua* proteins using bioinformatics tools. A molecular docking study revealed the stability of the peptide and ACE complex. These peptides act as inhibitors of angiotensin-converting enzyme (ACE). The released peptides are then incorporated into food products to make functional foods.

## 2. Materials and Methods

**2.1. Hydrolysis of Proteins by Proteolytic Enzymes.** Proteins were selected on the basis of secondary metabolite synthesis, and for sequence retrieval, the UniProt database (https://www.uniprot.org/) was used. The BIOPEP-UWM database [20] (http://www.uwm.edu.pl/Biochemia/Index.Php/En/Biopep) was used to hydrolyze the proteins by different proteolytic enzymes. Nine types of proteases from three sources were used: plant proteases (papain, ficin, and stem bromelain), digestive enzymes (pancreatic elastase II, pepsin, and trypsin), and microbial enzymes (subtilisin, thermolysin, and proteinase P1). After hydrolysis, ACE-inhibiting peptides were selected using the BIOPEP-UWM “search for active fragment” feature.

**2.2. Physiochemical Parameters of ACE Inhibitory Peptides Released by Proteolytic Enzymes.** Using the “peptides” package in RStudio [21], the physicochemical properties of the released peptides were studied. The properties include molecular weight, net charge, isoelectric point, hydrophobicity, and Boman index.

**2.3. Molecular Docking of Antihypertensive Peptides with ACE Receptor.** From released ACE inhibitory peptides, only

### Table 1: List of selected proteins and their attributes.

| S. no. | Accession no. | Protein          | Function                        | Residue length | MW (kDa) |
|-------|---------------|-----------------|---------------------------------|----------------|----------|
| 1     | Q9LLR9        | Epi-cedrol synthase | Terpenoid biosynthesis         | 547            | 63.57    |
| 2     | Q9SPN0        | R-linalool synthase QH1, chloroplastic | Terpenoid biosynthesis         | 567            | 65.71    |
| 3     | Q8SA63        | Beta-caryophyllene synthase | Sesquiterpene biosynthesis     | 548            | 63.75    |
| 4     | Q94G53        | (-)-beta-Pine synthesize, chloroplastic | Monoterpene biosynthesis      | 582            | 67.52    |
| 5     | Q1PS23        | Amorpha-4,11-diene 12-monoxygenase | Antimalarial endoperoxide artemisinin biosynthesis | 495            | 55.72    |
| 6     | Q9AR04        | Amorpha-4,11-diene synthase | Antimalarial endoperoxide artemisinin biosynthesis | 546            | 63.94    |
| 7     | Q43319        | 3-Hydroxy-3-methylglutaryl coenzyme A reductase | Isoprenoid biosynthesis        | 560            | 60.34    |
| 8     | Q9SWQ3        | Hydroxymethylglutaryl-CoA reductase (NADPH) | Isoprene biosynthesis         | 567            | 61.7     |
| 9     | C5H429        | Artemisinic aldehyde delta(11(13)) reductase | Antimalarial endoperoxide artemisinin biosynthesis | 388            | 42.59    |
| 10    | C5I9X1        | Aldehyde dehydrogenase 1 | Sesquiterpene biosynthesis     | 499            | 53.8     |
| 11    | P49350        | Farnesyl pyrophosphate synthase | Sesquiterpene biosynthesis     | 343            | 39.41    |

### Table 2: The source of the enzyme, type of enzyme, and released ACE inhibitory peptides by each enzyme are listed.

| Enzyme source | Enzyme type | Total no. of ACE-inhibiting peptides |
|---------------|-------------|--------------------------------------|
| Plant         | Papain      | 204                                  |
|               | Ficin       | 252                                  |
| Microbial     | Subtilisin  | 164                                  |
|               | Proteinase P1 | 83                                  |
| Digestive     | Pepsin      | 51                                   |
|               | Pancreatic elastase II | 69                                  |
| Total         |              | 1160                                 |
16 peptides were selected for molecular docking using the PeptideRanker tool (http://distilldeep.ucd.ie/PeptideRanker/). These 16 peptides with the inhibitory drug captopril were used as ligands, and their structures were generated using Discovery Studio 2020 (https://discover.3ds.com/discovery-studio-visualizer-download). Human ACE structure was used as receptor for docking. AutoDock Vina [22] was used to prepare the receptor by removing the water molecules and adding charges to the protein. For ligand binding, a site was constructed (radius 13 Å; coordinates $x$: 38.7154, $y$: 35.4135, and $z$: 41.6065).

For docking result visualization, Discovery Studio 2020 was used, and hydrogen bonding and electrostatic and hydrophobic interactions were studied between the ligand and receptor residues.

2.4. Evaluation of Drug-Like Properties of Peptides. The drug-like properties of peptides were evaluated in silico using SwissADME (http://www.swissadme.ch). This tool follows the ADME rule (absorption, distribution, metabolism, and excretion) to check the pharmacokinetics and drug-likeness of compounds. ToxinPred (http://crdd.osdd.net/raghava/toxinpred/) was used to predict the toxicity of compounds.

3. Results

3.1. Proteolytic Enzyme Sources and Effect on Antihypertensive Peptides. A total of 11 proteins of *Artemisia annua* were selected, and their characteristics are shown in Table 1. On hydrolysis, most of the released peptides were di- and tripeptides. The number of released peptides depends on the enzyme source and type (Table 2). A total of 1160 ACE inhibitory peptides were released, from which 631, 141, and 388 were released by plant, digestive, and microbial proteases, respectively. Approximately 54.3% of
peptides were released by plants, of which 32.3%, 28%, and 40% were released by enzymes, papain, stem bromelain, and ficin, respectively. Microbial proteases release 33.4% of ACE inhibitory peptides with a high degree of hydrolysis by thermolysin (36.3%), proteinase P1 (21.3%), and subtilisin (42.2%). However, fewer peptides were released by digestive enzymes (12.2%) than by the other two types. For trypsin, pepsin, and pancreatic elastase II, the degree of hydrolysis was 14.8%, 36%, and 49%, respectively. The number of peptides revealed that plant proteases were superior to microbial and digestive enzymes.

3.2. Physiochemical Parameters of ACE Inhibitory Peptides Released by Proteolytic Enzymes. The physiochemical parameters of ACE-inhibiting peptides that have been released by hydrolysis were demonstrated (Figure 1). The molecular weight varies between 170 and 410 Da. The molecular weights of dipeptides ranged from 170 to 350 Da, and they were abundantly released from proteins. The MW of the majority of the dipeptides ranged between 250 and 300 Da. Triptides ranging in size from 300 to 410 Da were also found (Figure 1(a)). The isoelectric point of peptides ranged from 3.8 to 12.5, and approximately 212 peptides had a pI less than 5 with a net charge of -1, which indicates the presence of amino acids with negative charges in most of the peptides. Approximately 790 peptides had pI ≤ 8 with a net charge of zero, while 180 peptides had pI ≤ 11 with a net charge of 1. These peptides mostly contain amino acids with positive charges (Figures 1(b) and 1(c)). The hydrophobicity of the 1160 peptides ranged from -3.50 to 5.00. Approximately 494, 196, and 410 peptides were neutral, hydrophilic, and hydrophobic, respectively (Figure 1(d)). The ACE inhibitory peptide Boman index ranged from -3.62 to 14.92, and most of the peptides had BI less than 2 (Figure 1(e)).

3.3. Molecular Docking of Antihypertensive Peptides with ACE Receptor. The probability of peptides’ bioactivity was predicted by PeptideRanker using score values ranging from 0.021 to 0.99. The first 16 peptides with a probability value close to 1 were selected for docking. The binding energies of the ligand-receptor complex ranged from -31.81 to -20.09 (Table 3). According to the results, most of the peptides showed strong hydrogen bonding as well as electrostatic and hydrophobic interactions with ACE residues (Asn70, Val518, His513, Thr140, and Phe512), which showed the ACE-inhibiting properties of peptides (Figure 2). RF and RW interacted with ACE active site pockets as S1 and S2, respectively.

GW interacted with Asp141, Val148, and Ile73 via hydrogen bonding and hydrophobic interactions. Hydrophobic amino acids of peptides, present near the C-termi

nus, strongly interact with active site residues. Hydrogen bonds were displayed (Table 4) that are found in ligand-receptor complexes. His348, Glu372, and His344 coordinates interact with the zinc ion present in the ACE structure, showing the importance of Zn in ACE inhibition. As none of the peptides interacted with Zn ions, peptides showed low inhibitory activity compared to captopril (Table 3).

3.4. Drug-Likeness Evaluation. The peptide drug-likeness profile was demonstrated (Table 5). The results revealed numerous similarities of peptides when compared to the inhibitory drug captopril. As the number of ROTB and TPSA of RF, RW, and FP peptides were not according to the required value, they were present outside the estimated range (see the shaded region in Figure 3).

All of the other peptides had the same bioavailability as captopril (0.55). None of the peptides showed CYP3A4 inhibition except for RF, RW, and FR, and all the peptides also had a high GIA. Except for MW and WL, all other peptides acted as P-glycoprotein substrates and had high bioavailability and GIA.

4. Discussion

For the breakdown of peptide links in proteins, proteolytic enzymes (also known as proteases or proteinases) were used. Because of their critical roles in biological processes, they are vital in medicine, pharmaceuticals, biotechnology, and a variety of research applications, such as protein digestion, peptide synthesis, cell culture, and peptide sequencing [23]. The amino acid specificity at both terminals determines the type of protease used for peptide synthesis [24]. As most ACE inhibitory peptides consist of 2-12 amino acids, the binding of peptides with ACE residues becomes very easy [25].

This is due to the wide range of specificity of amino acids, such as papain, which primarily cleaves hydrophobic and basic amino acids [23]. The peptide’s affinity for ACE was increased when positively charged amino acids and basic amino acids were present at the C and N termini, respectively. As a result, antihypertensive activity also increased [26].

Table 3: Evaluated binding energies and Zn II coordination distances of ligand-receptor complexes.

| Ligand | Affinity energy (kJ/mol) | Zn coordination |
|--------|--------------------------|-----------------|
| AF     | -26.98                   | No zinc coordination |
| FG     | -26.79                   | No zinc coordination |
| FP     | -23.02                   | No zinc coordination |
| FY     | -31.81                   | No zinc coordination |
| FR     | -25.12                   | No zinc coordination |
| GW     | -22.19                   | No zinc coordination |
| LW     | -28.47                   | No zinc coordination |
| MW     | -23.02                   | No zinc coordination |
| RF     | -30.98                   | No zinc coordination |
| RW     | -27.44                   | No zinc coordination |
| WG     | -28.88                   | No zinc coordination |
| WL     | -24.28                   | No zinc coordination |
| YF     | -28.28                   | No zinc coordination |
| CF     | -27.95                   | No zinc coordination |
| GF     | -20.09                   | No zinc coordination |
| MF     | -21.35                   | No zinc coordination |

Captopril -26.78 2.76 Sulphhydryl group of captopril
The hydrophobicity of amino acid side chains typically depends on the molecular weight of the peptides [27]. Amino acid hydrophobicity at the C-terminus influences ACE inhibition activity, as higher hydrophobicity directly increases the inhibition action [28]. Most of the dipeptides had high hydrophobicity at the C-terminus; therefore, the inhibition action of peptides increased [29]. Hydrogen bonding plays an important role in the structure of ligand-receptor complexes [30]. Coordination with other residues, on the other hand, caused distortion of Zn ions, due to which ACE lost its inhibitory action [31]. The optimized cutoff values for

![Figure 2: The best pose of ligand docked with receptor showing hydrogen bonding as well as hydrophobic and electrostatic interaction.](image-url)
Table 4: In the best docking pose of the ligand-receptor complex, hydrogen bonds and their distances (Å) with ACE residues are shown.

| ACE residues in H-bonding | FG   | FP   | AF   | FR   | FY   | GW   | LW   | RF   | RW   | WG   | MW   | WL   | YF   | CF   | GF   | MF   | Captopril |
|---------------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|---------|
| THR144:OG1                | 1 (2.7) | 1 (2.1) |     |     | 1 (2.6) | 1 (3.7) | 1 (1.9) | 1 (2.0) | 1 (2.0) |     |     |     |     |     |     |     |     |         |
| TYR2:O                    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 1 (2.8) |
| ASN70:OD1                 | 1 (3.3) | 1 (1.9) | 1 (2.6) | 1 (1.9) |     |     |     |     |     |     |     |     |     |     |     |     |         |
| LEU140:O                  | 1 (3.0) |     |     | 2 (2.9, 2.5) | 1 (2.7) | 1 (2.6) | 1 (3.0) | 1 (3.7) |     |     |     |     |     |     |     |     |         |
| ILE73:O                   | 1 (2.6) |     | 1 (2.3) |     |       |       |       |       |       |       |       |       |       |       |       |       | 1 (2.5) |
| SER516:O                  | 2 (2.7, 2.9) |     | 1 (2.3) |     |       |       |       |       |       |       |       |       |       |       |       |       |         |
| PHE1:O                    | 1 (2.1) | 1 (3.0) | 1 (3.6) |     |       | 1 (2.2) | 1 (3.3) | 1 (2.9) |     |     |     |     |     |     |     |     |         |
| TRP2:O                    |     |     |     |     | 1 (3.8) |       | 2 (3.4) |     |     |     |     |     |     |     |     |     |         |
| ARG1:O                    |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 1 (2.8) |
| PRO515:O                  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 1 (2.4) |
| GLU143:OE1                | 1 (2.5) | 1 (2.1) | 1 (2.7) |     |     |     |     |     |     |     |     |     |     |     |     |     | 1 (3.6) |
| LEU1:O                    |     |     |     |     | 1 (2.4) |       |       |       |       |       |       |       |       |       |       |       |         |
| THR75:O                   |     |     |     |     | 1 (2.6) |       |     |       |       |       |       |       |       |       |       |       | 1 (3.6) |
| TYR523:OH                 | 1 (2.0) |     |     | 2 (2.4, 2.0) |       |       |       |       |       |       |       |       |       |       |       |       |         |
| ASN66:OD1                 | 1 (3.2) |     | 1 (2.5) |     |       |       |       |       |       |       |       |       |       |       |       |       | 1 (2.2) |
| SER78:OG                  |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 2 (2.3, 2.4) |
| TRP2:OXT                  |     |     |     |     | 1 (3.8) |     | 1 (3.0) |     | 1 (2.3) |     |     |     |     |     |     |     |     |         |
| HIS513:NE2                | 1 (2.4) | 2 (2.5, 3.0) |     |     | 1 (2.1) |     |     |     |     |     |     |     |     |     |     |     |         |
| ASP141:OD1                |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | 1 (2.5) |
| Total                     | 6 | 4 | 5 | 6 | 4 | 3 | 4 | 5 | 5 | 3 | 3 | 6 | 3 | 4 | 4 | 5 | 5 |
Table 5: *In silico* drug-likeness assessment illustrating the antihypertensive peptide ADMET profile.

| Rule | Mol. wt. (g/mol) | ROTB (n) | HBA (n) | HBD (n) | ESOL log S | Toxicity | Lipophilicity | Drug-likeness | Pharmacokinetics | GIA | P-glycoprotein | CYP3A4 |
|------|-----------------|----------|---------|---------|-------------|----------|---------------|---------------|-----------------|-----|---------------|--------|
|      | Rule | <500 | <10 | <10 | <5 | <140 | <5 |  | Bioavailability | Lipinski filer | GIA | Substrate | Inhibitor |
| AF   | 236.27 | 6  | 4  | 3  | 0.39 (HS) | Nontoxic | 92.42 | 0.05 | 0.55 | Yes (0) | High | No | No |
| FG   | 222.24 | 6  | 4  | 3  | 0.48 (HS) | Nontoxic | 92.42 | -0.16 | 0.55 | Yes (0) | High | No | No |
| FP   | 262.3 | 5  | 4  | 3  | -0.51 (VS) | Nontoxic | 83.63 | 0.32 | 0.55 | Yes (0) | High | No | No |
| FR   | 321.37 | 11 | 5  | 6  | 0.79 (HS) | Nontoxic | 154.32 | -0.66 | 0.55 | Yes (1) | Low | No | No |
| FY   | 328.36 | 8  | 5  | 4  | -0.66 (VS) | Nontoxic | 112.65 | 0.83 | 0.55 | Yes (0) | High | No | No |
| GW   | 261.28 | 6  | 4  | 4  | 0.23 (HS) | Nontoxic | 108.21 | -0.18 | 0.55 | Yes (0) | High | No | No |
| LW   | 317.38 | 8  | 4  | 4  | -0.18 (VS) | Nontoxic | 108.21 | 1.09 | 0.55 | Yes (0) | High | No | No |
| MW   | 335.42 | 9  | 4  | 4  | -0.67 (VS) | Nontoxic | 133.51 | 0.86 | 0.55 | Yes (0) | High | Yes | No |
| RF   | 321.37 | 11 | 5  | 6  | 0.77 (HS) | Nontoxic | 154.32 | -0.59 | 0.55 | Yes (1) | Low | No | No |
| RW   | 360.41 | 11 | 5  | 7  | 0.26 (HS) | Nontoxic | 170.11 | -0.44 | 0.55 | Yes (1) | Low | No | No |
| WG   | 261.28 | 6  | 4  | 4  | -0.11 (VS) | Nontoxic | 108.21 | 0.08 | 0.55 | Yes (0) | High | No | No |
| WL   | 317.38 | 8  | 4  | 4  | -1.11 (VS) | Nontoxic | 108.21 | 1.13 | 0.55 | Yes (0) | High | Yes | No |
| YF   | 328.36 | 8  | 5  | 4  | -0.66 (VS) | Nontoxic | 112.65 | 0.78 | 0.55 | Yes (0) | High | No | No |
| CF   | 268.33 | 7  | 4  | 3  | 0.32 (HS) | Nontoxic | 131.22 | -0.02 | 0.55 | Yes (0) | High | No | No |
| GF   | 222.24 | 6  | 4  | 3  | 0.32 (HS) | Nontoxic | 92.42 | -0.23 | 0.55 | Yes (0) | High | No | No |
| MF   | 296.39 | 9  | 4  | 3  | -0.25 (VS) | Nontoxic | 117.72 | 0.72 | 0.55 | Yes (0) | High | No | No |
| Captopril | 217.29 | 4  | 3  | 1  | -1.14 (VS) | 96.41 | 0.62 | 0.56 | Yes (0) | High | No | No |
molecules being permeable have been proposed, which include PSA < 140, ClogP < 5, HBA < 10, HBD < 5, and MW < 350 [32]. By following these optimized values, the oral administration properties of molecules increased [33]. The flexibility and polarity of the drugs affect their oral bioavailability. The number of ROTB and TPSA represents the flexibility and polarity of a compound. The oral bioavailability of a compound becomes low and high due to the presence of more rotatable bonds and small topological surface areas, respectively [34].

ACE inhibitors are partially metabolized by CYP3A4 because they have little effect on cytochrome interactions [35, 36]. The CYP3A5 enzyme family is important in drug metabolism [37]. Interactions between drug-active compounds and any of the CYP isozymes can result in drug bioaccumulation (when a CYP isozyme is activated) or rapid
metabolism (when a CYP isozyme is inhibited) in the body. Both scenarios are undesirable because the first can result in overdosing and the second in toxicity [38].

ACE inhibitors are routinely given for the treatment of hypertension and renal dysfunction in systemic lupus erythematosus (SLE) patients, despite the fact that no randomised controlled studies have been conducted [39]. The use of ACE inhibitors during SLE is generally well tolerated and associated with a delay in the onset of renal involvement and a decrease in the risk of disease relapse in SLE patients, which is likely due to a decrease in angiotensin II as well as the immunomodulatory effect of renin-angiotensin system blockade [40]. As a result, in individuals with autoimmune illness, RAS blockade may have a dual impact in controlling the autoimmune disease and its accompanying hypertension [41].

5. Conclusion

Artemisia annua proteolytic enzymes (papain, ficin, and stem bromelain) produced more antihypertensive peptides than microbial (thermolysin, proteinase P1, and subtilisin) and digestive (trypsin, pepsin, and pancreatic elastase I) enzymes. In molecular docking, a stable interaction between ligands and receptors by hydrogen bonding was studied. In addition, in silico drug-likeness evaluation of the ACE-inhibiting peptides revealed that all peptides followed at least four of the five rules of Lipinski filters, but FR, RW, and RF violated one of the rules. As peptides are released from proteins of medicinal plants through proteolytic enzyme hydrolysis, therefore they are used in therapeutic settings and have the ability to improve food products by being used as nutraceuticals.

Data Availability

All data is available in the main manuscript.

Conflicts of Interest

The authors declare no conflicts of interest.

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