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Deep learning based on Molecular Docking for analyzing thePharmacological Properties of Natural substances in treating Hypertension

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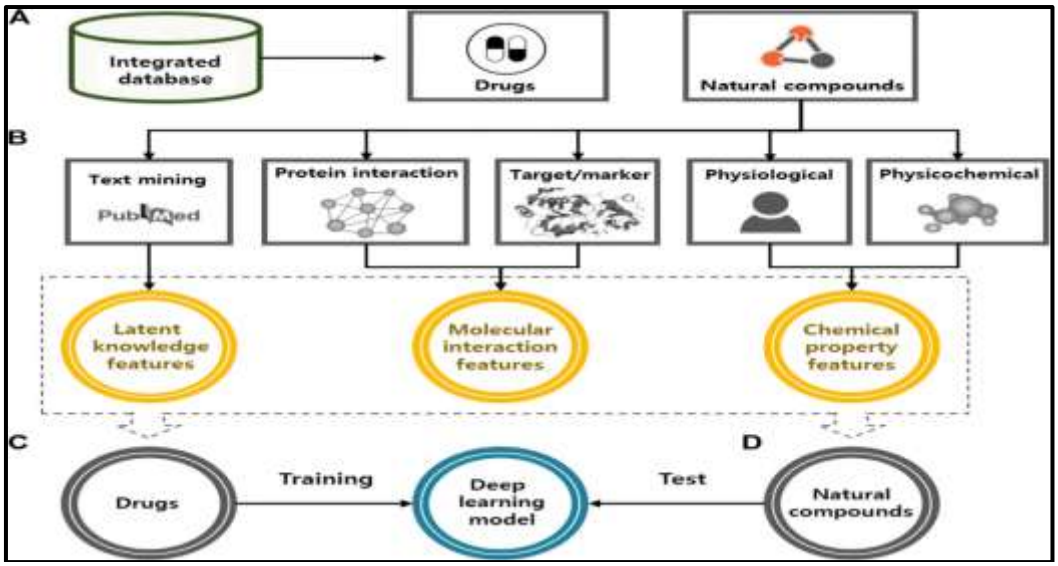
ABSTRACT

Hypertension is widely attributable to genetic, behavioral, and environmental risk factors. Among the genetic reasons, angiotensin II enzyme, produced as a result of abnormal functioning of the renin–angiotensin system, is reported as the foremost cause of hypertension. A cascade of genes, including those encoding for WNK kinases (WNK1 and WNK4), Bp1, Bp2, angiotensinogen, and other enzymes, is involved in the conversion of angiotensin I to angiotensin II. However, the angiotensin-converting enzyme (ACE) plays a crucial role in this pathway. Therefore, ACE could be a potential therapeutic target in regulating the conversion of angiotensin I to angiotensin II and eventually controlling hypertension. In this study, a deep learning molecular docking-based approach was utilized for identifying and evaluating potential inhibitors of ACE present in herbs and other natural sources, on the basis of these compounds' binding affinities and other physicochemical features. In addition, the suitability of these inhibitors as drugs for biological systems, considering their adsorption, distribution, metabolism, and excretion (ADME), was predicted using Lipinski's rule. In conclusion, our study provides a novel and clearer insight into the interaction properties of known putative inhibitors of ACE.

Keywords: angiotensin-converting enzyme; ligands; hypertension; molecular docking; drug design.

Graphical abstract



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I. INTRODUCTION

Hypertensive heart disease mostly known as hypertension refers to a group of disorders including heart failure, ischemic heart disease, and left ventricular hypertrophy and is becoming a major cause of death associated with high blood pressure worldwide [1]. Several genes including WNK1, WNK4, Bp1, Bp2, AGT, and ACE were reported to be involved in hypertension

.[2] Mutation in WNK1 and WNK4 genes could cause disturbances in the homeostasis of K+ , salts, and pH level [3], whereas a mutation in AGT genes present on chromosome 1 results in an imbalance of angiotensinogen production, ultimately leading to hypertension [4-6]. It was revealed that ACE plays a key role in hypertension, its dysfunction being the most frequent cause of hypertension [7]. The most common biological reason behind hypertension is the production of angiotensin II enzyme, which is produced from the conversion of angiotensin I by the action of a series of the enzymes [18-10]. Therefore, regulating the conversion of angiotensin I to angiotensin II could be an effective strategy to control hypertension.

Angiotensin-converting enzyme (ACE) is considered crucial in this pathway and has received considerable attention as a therapeutic target for controlling hypertension. Repressing ACE expression has been proved as an effective strategy in controlling hypertension, as its downregulation will inhibit the conversion of angiotensin I to angiotensin II [11]. A large number of medicinal plants possess diverse natural compounds, contributing to drug development by providing novel candidate therapeutic agents against various diseases. Natural compounds are small molecules synthesized by living organisms, including primary and secondary metabolites [12]. Accumulating evidence has shown that the ingestion of bioactive natural compounds, such as phytochemicals, antioxidants, vitamins, and minerals, through a diet rich in herbs, fruits, vegetables, and spices may promote health via negative immune-regulatory and anti-inflammatory activities [13-15]. Moreover, many natural compounds have been proven to play an important role as modulators of cell signaling andhomeostasis, which enforces the need to identify the medicinal potentials of bioactive natural compounds [16-17].

In this study, the binding affinitiesof various natural and herbal inhibitors for active sites of ACE were predicted using the molecular docking approach, which is becoming an extremely important tool in drug design. Molecular docking is playing a major role in structure-based molecular biology and computer-based drug design. The molecular docking methodology can be utilized to demonstrate the cooperation between a small molecule and a protein at the nanoscale, which empowers us to describe the behavior of small particles in the binding site of the proteins and explain key biochemical processes [18]. Furthermore, drug-likeness and compatibility with gastrointestinal and brain absorption were computed for tested to evaluate their suitability as potential therapeutic agents and orally active drugs for the treatment of hypertension.

II. MATERIALS AND METHODS

2.1. Physiochemical Properties

The physiochemical properties of human ACE were predicted using Protparam [19]. The Protparam tool works on the basis of the Edelhoch method [13], determining the weight value of instability with respect to 400 different dipeptides (DIWV) and the hydropathy values for extinction coefficients, instability index (II), and GRAVY value (grand average of hydropathy value).

2.2. Secondary Structure Predictions

The number of helix turns and coils was calculated using “Psipred” [14]. Psipred used two feed-forward neural networks which perform an analysis of output obtained from PSI–BLAST (Position-Specific Iterated–BLAST) for secondary structure prediction.

2.3. Data Collection

Plant-derived natural compounds and their chemical structure information were collected from KTKP (Portal, TCMID, COCONUT, and FooD, Drug information, including chemical structure and indication, was collected from DrugBank version 5.1.5. The molecular targets of the drugs and natural compounds were collected from the DrugBank, CTD**,** STITCH, and TTD databases. In this study, we used ------ natural compounds and ------approved and investigational drugs that have at least five molecular target information.

2.4. Deep Learning-Based Prediction of the Medicinal Uses of Natural Compounds

In this study, we used a deep learning model to predict the potential medicinal effects of natural compounds. For all natural compounds and drugs, the algorithm works in four steps: 1) collecting various types of natural compound and drug information from public databases; 2)

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generating latent knowledge, molecular interaction, and chemical property features from the collected information via text mining, network analysis, and chemical property analysis; 3) training the deep learning model based on the features of the approved and investigational drugs as inputs and their indication information as outputs; and 4) predicting the medicinal uses of natural compounds based on the trained deep learning model.

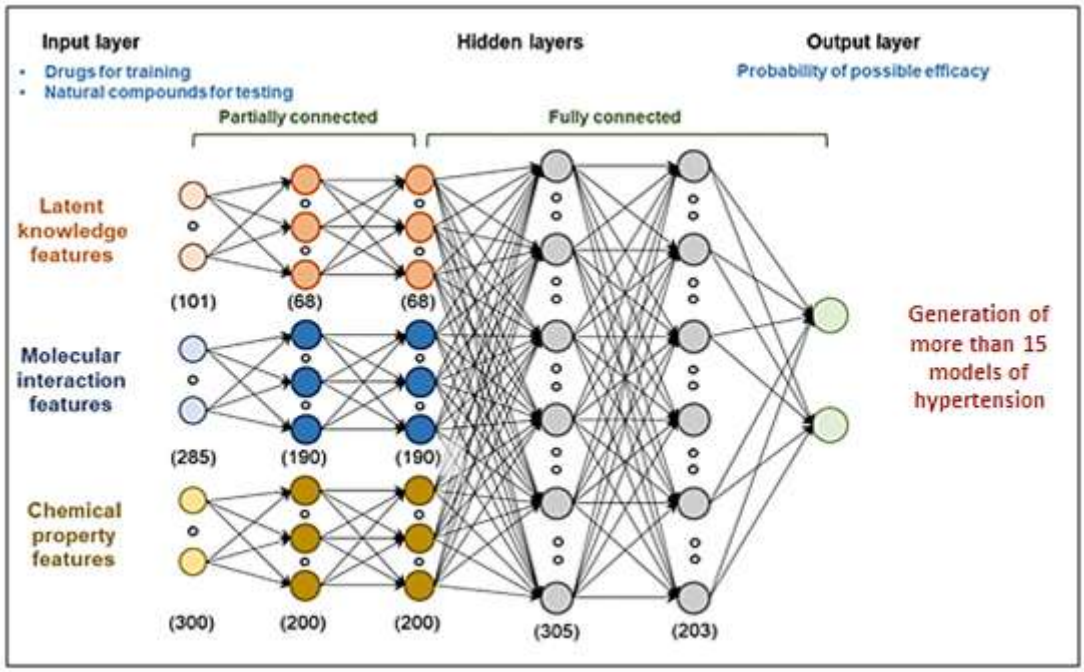

Figure 1:Architecture of the deep learning model for predicting the potential effects of natural compounds.

The proposed model consists of four sequential layers **(Figure 1):** 1) input layer, 2) partially connected hidden layers, 3) fully connected hidden layers, and 4) output layer. The models were generated for 15 diseases, respectively, to predict the effects list from input features. For each drug or natural compound, we generated latent knowledge, molecular interaction, and chemical property features and used them as the inputs of the model. Hidden layers generalized their outputs by providing a high-level representation that was more abstract than the previous layer by discovering nonlinear relationships between the low- and high-level data.Let X_l is the output of the lth hidden layer. The forward propagation of the neural network with lth hidden layer can be represented as follows:

$$X_l = f(W_l X_{l-1} + b_l)$$

whereW_l $[w_{l1}, w_{l2}, \ldots, w_{ln}]$ is the weight matrix of the edge from l-1st layer to lth layer, b_l is the bias of each hidden units, and f (·) is the activation function. In this study, the hidden layers were divided to two parts: the partially connected and fully connected parts.

2.5. Molecular docking

In this study, molecular docking of natural inhibitors of ACE was performed using diverse computational tools, with the aim to discover the optimum inhibitor, which ultimately would provide the basis for designing drugs against hypertension by inhibiting ACE. The structure-based docking method was used because structure-based Computer Aided Drug Designing (CADD) relies on the knowledge of the target protein structure to calculate interaction energies for all compounds tested, whereas ligand-based CADD exploits the knowledge of known active and inactive molecules through chemical similarity searches or construction of predictive, QSAR models. Structure-based CADD is generally preferred where high-resolution structural data of the target protein are available, i.e., for soluble proteins that can readily be crystallized. These advancements in

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research allow computational techniques to analyze all factors involved in drug design and discovery.

2.6. Lipinski's Rule of Five for Drug-Likeness or ADME (Absorption, Distribution, Metabolism, and Excretion) Analysis:

Drug-likeness of our inhibitors, including absorption, distribution, metabolism, and excretion of these inhibitors within the body, was predicted using SwissADME. The Egan BOILED-Egg method available in SwissADME tool was used for the determination of the absorption of the inhibitors in the gastrointestinal tract and brain. BOILED-Egg (Brain or IntestinaLEstimateD permeation predictive model), also called Egan egg, provides a threshold (WLOGP ≤ 5.88 and TPSA ≤ 131.6) and a clear graphical representation of how far a molecular structure is from the ideal one for good absorption [40]. In this 2D graphical representation, the yolk area represents the molecules that can passively permeate through the blood–brain barrier (BBB), whereas the molecules located in the white region are predicted to be passively absorbed by the gastrointestinal (GI) tract.

III. 3. RESULTS AND DISCUSSION

3.1. Physiochemical Properties of ACE

Human ACE was found to exhibit a molecular weight of 67,993.2 Daltons and isoelectric pH 5.82. It is a stable protein with an aliphatic index of 78.86, whereas its instability index was predicted to be 39.46. The prediction of GRAVY value of −0.4441 demonstrates that ACE is a hydrophilic peptide (Table 1).Predicted pharmacological effects of natural compounds in hypertension are Reserpine, Norepinephrine, Octopamine, Digitoxin.

Table1. Physiochemical properties of ACE predicted by ProtParam.

Property	Value
Number of amino acids	589
Total number of atoms	9457
Molecular Weight	67,993.20 Dalton
Theoretical pI	5.82
Instability index	39.46
Aliphatic index	78.86
Grand average of hydropathicity (GRAVY)	−0.441
Chemical Formula	$C_{3076}H_{4656}N_{818}O_{883}S_{24}$
Charge	Negative

3.2. Natural inhibitors of ACE

The 2D structures of reported inhibitors of ACE were downloaded from PubChem in SDF format and are portrayed in Figure 4. Herbal and Natural Inhibitorsinhibitors were in the pocket of the target protein (ACE), exhibiting a possible interaction with ACE. The docking results were manipulated using the GBVI/WSA dG scoring function with the generalized Born solvation model (GBVI). The GBVI/WSA dG is a force fifield-based scoring function, which estimates the free energy of binding of the ligand from a given orientation. Interaction results were evaluated with the S score.

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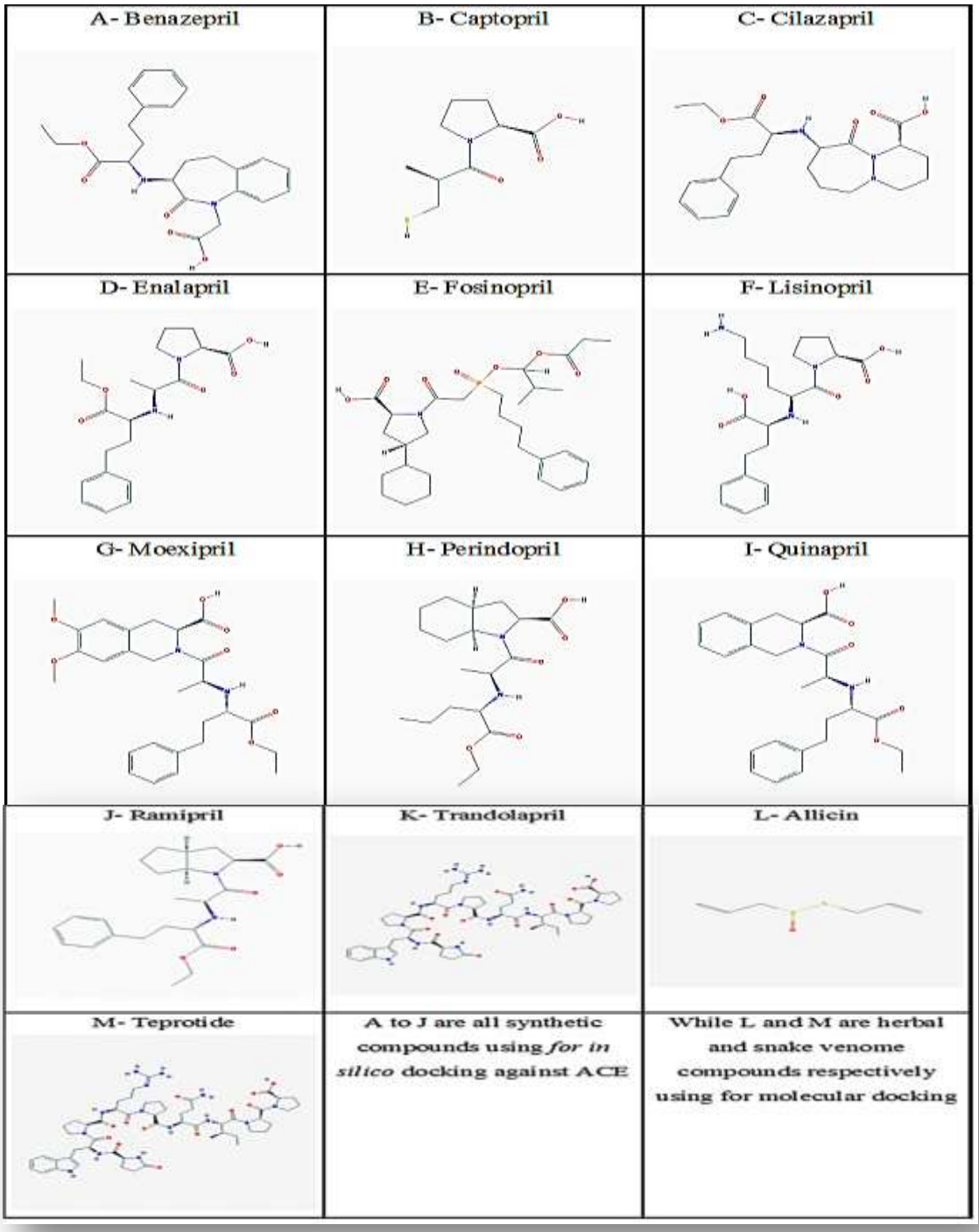
Figure 2. 2D structures of various ACE inhibitors including (A) Benazepril, (B) Captopril, (C) Cilazapril, (D) Enalapril, (E) Fosinopril, (F) Lisinopril, (G) Moexipril, (H) Perindopril, (I) Quinapril, (J) Ramipril, (K) Trandolapril, (L) Allicin, and (M) Teprotide.



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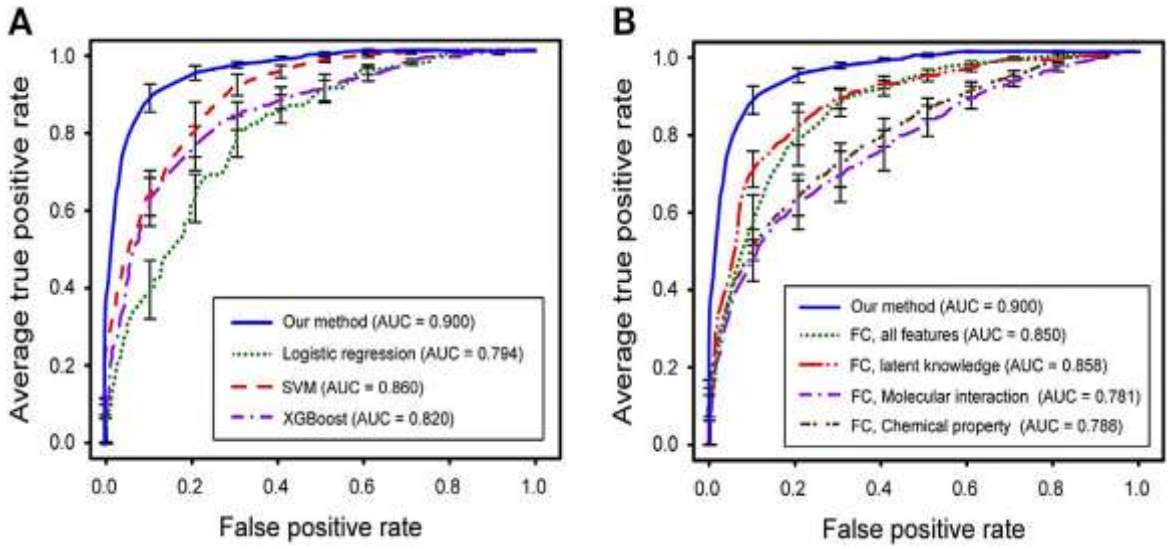

Figure 3:Performance evaluations of predicted medicinal effects of natural compounds.

3.3.Molecular docking

In silico docking of human ACE against selected inhibitors was performed using MOE against all the predicted active sites. The results showed that all selected to establish a strong interaction with ACE on specifific active sites (Table 2). After in silico docking, we identifified a ligand showing the minimum S score among all the inhibitors. Teprotide, which is present in snake venom, showed a minimum S score of −20.1163; therefore, it establishes the strongest interaction with ACE among all the inhibitors discussed in this study. Fosinopril is another widely used and effective drug against hypertension. It was predicted to exhibit a strong binding affinity for ACE, with an S score of −18.9225. Earlier studies demonstrated that fosinopril doses of 10 and 20 mg could inhibit 85% and 93% of ACE activity, respectively, within 24 h of administration [55]. Hayek et al. used ACE as a receptor and fosinopril as an inhibitor to cure hypertension and concluded, after 12 weeks of treatment, that fosinopril remarkably reduces blood pressure in mice [56]. Heart Outcomes Prevention Evaluation Study Investigators evaluated the role of ramipril in reducing the overactivity of ACE and showed that ramiprilsignificantly lessens the rates of myocardial infarction and stroke in a wide range of high-risk patients.

Table 2: Inhibitors ranked on the basis of their S-values.

No.	Name	S-Values
1.	Teprotide	−20.1163
2.	Fosinopril	−18.9225
3.	Moexipril	−16.816
4.	Quinapril	−13.456
5.	Lisinopril	−12.502
6.	Cilazapril	−12.493
7.	Trandolapril	−12.2673
8.	Enalapril	−11.7516
9.	Ramipril	−11.3562
10.	Captopril	−10.8282
11.	Benazepril	−9.3245
12.	Perindopril	−8.105
13.	Allicin	−5.5448

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3.4. Drug likeliness and ADME predictions of our inhibitors

The antagonistic interaction of inhibitors with a receptor protein or enzyme cannot guarantee the suitability of an inhibitor as a drug; therefore, ADME analysis of inhibitors is important in the drug development. ADME is based on Lipinski's rule of fifive [59] and helps to make decisions on the approval of inhibitors for biological systems. Poor ADME characteristics and unfavorable toxicology for a biological system are the major cause of the failure of most medicines in clinical experiments.

All of the inhibitors or ligands discussed herein satisfy the Lipinski's rule, except for teprotide, which signifificantly violates three parameters (MW > 500, number of hydrogen bond donors > 5 and number of hydrogen bond acceptors > 10); furthermore, it also violates the BOILED-egg method. Although teprotide has the highest binding affifinity for human ACE among all the inhibitors, it is not proposed as an orally active drug due to violation of the Lipinski's rule. An Egan's egg graph for the inhibitors was generated using SwissADME. The graph showed that only allicin, a herbal compound, is absorbed by the brain, though in the acceptable range. The remaining inhibitors showed gastrointestinal absorption within an acceptable range, except for teprotide and lisinopril (WLOGP >5.88 and TPSA >131.6) (Figure 3).

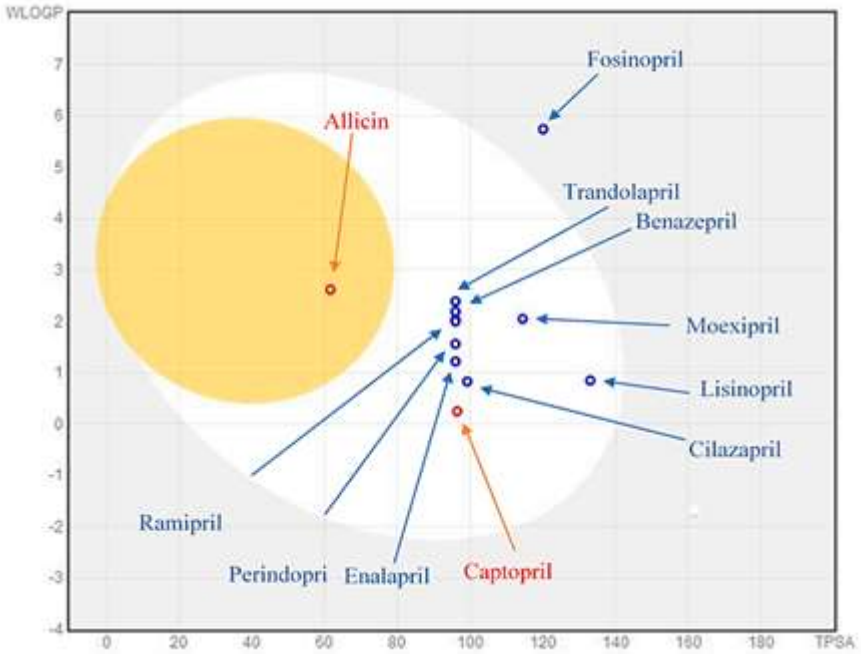

Figure 4:Evaluation of the analyzed ligands by the BOILED-Egg method

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No.	Name	Lipinski's Rule of Five					Drug-Likeness
		Molecular Weight (g/mol)	Lipophilicity (MLog *P*)	Hydrogen Bond Donors	Hydrogen Bond Acceptors	No. of Rule Violations	
		Less than 500 Dalton	Less than 5	Less than 5	Less than 10	Less than 2 Violations	Lipinski's Rule Follows
1.	Teprotide	1101.26	−3.11	10	13	3: MW > 500, NH or OH > 5, N or O > 10, 1: MW > 500	No
2.	Fosinopril	563.66	3.74	1	7	0	Yes
3.	Moexipril	498.57	1.54	2	8	0	Yes
4.	Quinapril	438.52	2.17	2	6	0	Yes
5.	Lisinopril	405.49	−1.46	4	7	0	Yes
6.	Cilizapril	417.50	1.79	2	7	0	Yes
7.	Trandolapril	430.54	2.19	2	6	0	Yes
8.	Enalapril	376.45	1.32	2	6	0	Yes
9.	Ramipril	416.51	1.98	2	6	0	Yes
10.	Captopril	217.29	0.45	1	3	0	Yes
11.	Benzapril	424.49	2.23	2	6	0	Yes
12.	Perindopril	368.47	1.36	2	6	0	Yes
13.	Allicin	162.27	1.18	0	1	0	Yes

Table 3:Lipinski's rule of five for ADME

So, on the basis of Egan's boiled-egg rule threshold values (WLOGP ≤ 5.88 and TPSA ≤ 131.6), only allicin penetrates the blood–brain barrier, though within acceptable limits. The blue dots indicate molecules predicted to be efflfluated from the CNS by P-glycoprotein, and the red dots indicate molecules predicted not to be efflfluated from the CNS by P-glycoprotein.

IV. CONCLUSIONS

In this study, fosinopril was predicted as the best ACE inhibitor (with maximum binding affinity for ACE after teprotide) to be used as a potentially therapeutic orally active drug (on the basis of Lipinski's rule of five and BOILED-egg approach) for the treatment of hypertension. Among the animal inhibitors, teprotide showed the highest binding affinity compared to all other ligands studied here; however, according to Lipinski's rule and BOILED-egg method, it is not recommended as a suitable therapeutic agent. Furthermore, allicin, an herbal ligand, exhibited reasonable binding affinity for ACE and follows Lipinski's rule of five but can only be used as food because of its slight absorption in the brain. In conclusion, our study provides a clearer insight into the interaction properties of known putative synthetic inhibitors of ACE and bioactive inhibitors, including interactions with the blood–brain barrier. In recent years, consumers have paid attention to natural bioactive compounds as potential medicines because of their effectiveness in promoting health, associated with less adverse effects. In future, we will be able to use the knowledge of inhibitors' pharmacological properties, including those of bioactive compounds such as allicin, to make effective therapeutic drugs based on ACE inhibition to cure hypertension.

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