ORIGINAL ARTICLE

In silico Computational Analysis of Siddha Formulation Veppampoo mathirai in Inhibition of Angiotensin-Converting Enzyme Receptor Target against Hypertension

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Abstract

Introduction: Hypertension (HTN) is a leading non-communicable disease in the world which affects most people. Even though, modern medicines are available widely, people show their interest to take traditional system of medicines like Siddha to overcome some untoward side effects. In this regard, Veppampoomathirai, a polyherbal siddha formulation prescribed for HTN was studied by *in silico* computational analysis for its potential action against HTN in inhibition of Angiotensin-converting enzyme receptor target compared with standard captopril drug of modern medicine. **Materials and Methods:** The phytochemicals present in the formulation were identified, selected, and retrieved through literature survey. Molecular docking analysis was performed by AutoDock tool version 4. The lead molecules were identified by their binding affinity toward target enzyme angiotensin-converting enzyme (ACE) and compared with standard captopril. **Results:** The phytoprinciples such as Ascorbic acid, Elemene, Cinnamaldehyde, Gallic Acid, Nimbiol, Piperic acid, Piperine, Eugenol, Nimbolide, Rutin, and Solasodine had 7–10 interactions with more interaction surface area. Nimbiol and Gallic acid had ten interactions, whereas Piperic acid, Peperine, and Nimbolide had nine interactions which are higher than the standard Captopril with the active amino acids at the target ACE enzyme. **Conclusion:** The study concludes that the formulation Veppampoomathirai has potential binding action against ACE target enzyme that helps in reducing blood pressure.

Key words: Hypertension, *In silico* computational analysis, Siddha, Veppampoomathirai

INTRODUCTION

Hypertension (HTN) due to its high prevalence is considered as a major public health problem in the world. A blood pressure (BP) reading of an individual having systolic >140 mm Hg and diastolic >90 mm Hg is termed as HTN. Around 7.5 million deaths or 12.8% of the total of all annual deaths worldwide occur due to high BP.[1] It is predicted to be increased to 1.56 billion adults with HTN in 2025.[2] Globally, 3.5 billion adults now have non-optimal systolic BP levels (that is, >110–115 mmHg) and 874 million adults have systolic BP ≥140 mmHg. Thus, approximately one in four adults has HTN.[3] An increased BP is a major risk factor for stroke, chronic heart disease, and coronary heart disease. Other than coronary heart disease and stroke, its complications include heart failure, peripheral vascular disease, renal impairment, retinal hemorrhage, and visual impairment.[1]

In modern medicine, the treatment for Anti-HTN starts with first-line anti-hypertensive medications such as monotherapy or in combination.[4] First-line anti-hypertensive medications include angiotensin-converting enzyme inhibitors (ACE),

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angiotensin II receptor blockers (also known as sartans), dihydropyridine, calcium channel blockers, thiazide diuretics, and beta-blockers.[5] The choice of the drug recommended by the physician for an individual is based on their symptoms and tolerability.

The renewed interest in the search for new drugs from natural sources, especially from plant sources, has gained global attention during the past two decades. Thus, natural plants and herbs can be our source of drugs, with fewer side effects and better bioavailability for treatment of HTN in future. Evidently, the rationale for the use of herbal and plant remedies is definitely not surprising, considering the fact that they contain thousands of bioactive components that have known therapeutic applications. Indeed, plants and herbs have actually provided a starting point for synthesis of over 50% of currently used pharmaceutical drugs.[6]

Herbal plant-based formulations or drugs are pivotal to Traditional practices such as Chinese, Ayurvedic, and Unani, Tibb medicine, which is practiced worldwide.[7] Siddha is one of the oldest traditional medicines practiced mostly in Southern India. It had so many formulations for all the ailments since centuries which had benefited the people till now. The formulation Veppampoomathirai, a polyherbal, prescribed for HTN was effective in reducing the BP. Unlike modern medicine, the mechanism of pathway in reducing the BP was unpredictable. Among the classes of anti-hypertensive drugs, ACE inhibitor is the main one which works by causing relaxation of blood vessels as well as decrease in blood volume that leads to lower BP and decreased oxygen demand from heart. Therefore, the formulation, Veppampoomathirai consisted of bioactive phytoprinciples were aimed to do an *in silico* computational analysis to found, whether it has any inhibitory action against HTN by binding with the ACE enzyme target site compared with standard captopril drug of modern medicine.

The main objective of the study was to find the lead molecules by screening the phytoprinciples for its efficacy to bind with core bio active amino acid residues, GLU162, GLN281,HIS353, ALA354,HIS383, GLU 384, HIS 387, GLU 411, LYS 511, HIS 513, TYR 520, and TYR523 located in the active binding sites of ACE which mediates the enzymatic action that has higher level of significance in the management of BP in comparison with standard captopril. ACE involved in the conversion of angiotensin I to II. Angiotensin II tends to increase BP by constriction of blood vessels. Hence, occupying these amino acid residues inhibit the enzymatic action of ACE, thereby reduce the BP by depleting the release of Angiotensin II. Phytochemicals that inhibit the enzyme ACE will considerably have higher therapeutic potential in lowering higher BP and in the management of HTN.

MATERIALS AND METHODS

The polyherbal formulation was described in classical Siddha text, Noigaluku Siddha parigaaram, part I, by author Shanmugavelu.[8] It consisted of 15 herbs-*Azadirachta indica, Solanum trilobatum, Phyllanthus niruri, Eclipta prostrate, Zingiber officinale, Piper nigrum, Piper longum,Terminalia chebula, Terminalia bellerica, Emblica officinalis, Eugenia caryophyllata, Cinnamom zeylanicum, Elatteria cardamomum, Coeus vettiveroides, and Citrus lemon* that were purified and processed to tablet formulation indicated for regulating BP.[9]

Ligand preparation

The herbs present in the formulation Veppampoomathirai were investigated for phytochemicals selection. Based on the literature survey through PubMed and Google Scholar, 15 lead phytocomponents are identified, retrieved, and listed in Table 1.[10-27] ChemDraw prof online tool version 12.0. was used to build the 3D structure of the phytochemicals. The ligands were prepared through geometry optimization method Merck Molecular Force Field 94 (MMFF94). The ligand properties of the selected compounds, molecular weight, molecular formula, H bond donor, receptor, rotatable bonds for docking against ACE (1O86), and standard drug captopril are depicted in Table 2.

Protein preparation

Figure 1 shows the three-dimensional protein structure of the target protein, Human ACE 1O86. Crystalline structure of the target protein was retrieved from the online repository of Protein Data Bank (www.rcsb.org/pdb). Protein clean-up process was done and essential missing hydrogen atoms were added. After that, they were subjected to Ramchandran plot analysis using Rampage to identify the statistical distribution of the combinations of the amino acid backbone dihedral Ø (Phi) angles and ψ (Psi) angle and subjected to removal of water molecule, protein clean geometry procedure, before docking simulation. Different orientation of the lead molecules with respect to the target protein was evaluated by AutoDock version 4 program and the best dock pose was selected based on the interaction study analysis.[28,29] 3D pictures of the selected phytocomponents and standard captopril are shown in Figure 2.

Docking methodology

Docking calculations were carried out using AutoDock 4. Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined. Docking calculations were carried out for the retrieved phytocomponents against the target protein

Table 1: List of phytocomponents selected for docking

S. No	Botanical name	Family	Phytocomponents selected for docking	Literature survey reference
1	*Azadirachta indica*	Meliaceae	Nimbolide Nimbiol Nimbin	[10,11]
2	*Solanum trilobatum*	Solanaceae	Solasodine	[12]
3	*Zingiber officinale*	Zingiberaceae	Gingerol	[13,14]
4	*Piper nigrum*	Piperaceae	Piperic acid	[15]
5	*Piper longum*	Piperaceae	Piperine	[16,17]
6	*Terminaliachebula*	Combretaceae	Gallic acid	[18,19]
7	*Emblica officinalis*	Euphorbiaceae	Rutin, Quercetin	[20,21]
8	*Eugenia caryophyllata*	Myrtaceae	Eugenol	[22,23]
9	*Cinnamomum zeylanicum*	Lauraceae	Cinnamaldehyde	[24]
10	*Elettaria cardamomum*	Zingiberaceae	Gama-Elemene	[25]
11	*Coleus vettiveroides*	Lamiaceae	Vetiverol	[26]
12	*Citrus lemon*	Rutaceae	Ascorbic acid	[27]

Table 2: Ligand properties of the compounds selected for docking against angiotensin-converting enzyme (1O86)

Compound	Molar weight g/mol	Molecular Formula	H Bond Donor	H Bond Acceptor	Rotatable bonds
Nimbolide	466.5 g/mol	$C_{27}H_{30}O_7$	0	7	4
Nimbiol	272.4g/mol	$C_{18}H_{24}O_2$	1	2	0
Nimbin	540.6 g/mol	$C_{30}H_{36}O_9$	0	9	8
Solasodine	413.6 g/mol	$C_{27}H_{43}NO_2$	2	3	0
Gingerol	294.4 g/mol	$C_{17}H_{26}O_4$	2	4	10
Piperic acid	218.2 g/mol	$C_{12}H_{10}O_4$	1	4	3
Piperine	285.34 g/mol	$C_{17}H_{19}NO_3$	0	3	3
Gallic acid	170.12g/mol	$C_7H_6O_5$	4	5	1
Rutin	610.5 g/mol	$C_{27}H_{30}O_{16}$	10	16	6
Quercetin	302.23 g/mol	$C_{15}H_{10}O_7$	5	7	1
Eugenol	164.2 g/mol	$C_{10}H_{12}O_2$	1	2	3
Cinnamaldehyde	132.162 g/mol	C_9H_8O	0	1	2
Gamma Elemene	204.35 g/mol	$C_{15}H_{24}$	0	0	3
Vetiverol	220.35 g/mol	$C_{15}H_{24}O$	1	1	0
Ascorbic acid	176.12 g/mol	$C_6H_8O_6$	4	6	2
Captopril	217.29 g/mol	$C_9H_{15}NO_3S$	2	4	3

g/mol-gram per mole

ACE. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock 4 virtual screening tool. Affinity (grid) maps of 60 × 60 × 60 Å grid points and 0.375 Å spacing were generated using the Autogrid program. AutoDock parameter set-and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm and the Solis and Wets local search method. Torsions were released during docking. Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from two different runs that were set to terminate after a maximum of 250,000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied.[30] Table 3 shows the Binding Free energy, Inhibition constant, Electrostatic energy, Intermolecular energy and total interaction surface of the phytocompounds present in Veppampoo mathirai and standard captopril against ACE (1O86).

Table 3: Summary of the molecular docking studies of investigational compounds against Angiotensin-converting enzyme (1O86)

Compounds	Binding Free energy Kcal/mol	Inhibition constant Ki μM (*mM)(**nM)	Electrostatic energy Kcal/mol	Intermolecular energy Kcal/mol	Total Interaction Surface
Nimbolide	−6.47 kcal/mol	17.95 uM	−0.32 kcal/mol	−7.45 kcal/mol	915.12
Nimbiol	−6.29 kcal/mol	24.34 uM	−0.12 kcal/mol	−6.59 kcal/mol	672.098
Nimbin	−7.73 kcal/mol	2.14 uM	−0.23 kcal/mol	−8.39 kcal/mol	981.974
Solasodine	−9.03 kcal/mol	238.73 nM	−0.75 kcal/mol	−9.33 kcal/mol	880.868
Gallic Acid	−4.59 kcal/mol	435.26 uM	−0.96 kcal/mol	−4.09 kcal/mol	433.666
Gingerol	−5.90 kcal/mol	47.19 uM	−0.02 kcal/mol	−7.60 kcal/mol	648.18
Piperic acid	−4.91 kcal/mol	252.85 uM	−0.70 kcal/mol	−5.78 kcal/mol	575.676
Piperine	−6.64 kcal/mol	13.51 uM	−0.18 kcal/mol	−7.30 kcal/mol	717.549
Quercetin	−6.54 kcal/mol	16.16 uM	−0.17 kcal/mol	−5.94 kcal/mol	613.884
Rutin	−10.46 kcal/mol	21.44 nM	−0.59 kcal/mol	−6.72 kcal/mol	1084.195
Eugenol	−4.42 kcal/mol	577.07 uM	−0.17 kcal/mol	−4.82 kcal/mol	512.289
Cinnamaldehyde	−4.96 kcal/mol	233.07 uM	−0.17 kcal/mol	−5.53 kcal/mol	430.404
Gamma Elemene	−5.44 kcal/mol	102.68 uM	−0.02 kcal/mol	−6.02 kcal/mol	585.466
Vetiverol	−6.60 kcal/mol	14.42 uM	−0.01 kcal/mol	−6.90 kcal/mol	597.26
Ascorbic acid	−4.04 kcal/mol	1.10 mM	−0.16 kcal/mol	−5.49 kcal/mol	534.936
Captopril	−5.09 kcal/mol	186.95 uM	−0.61 kcal/mol	−5.13 kcal/mol	504.357

Kcal/mol-kilocalories per mole, μM-micro moles

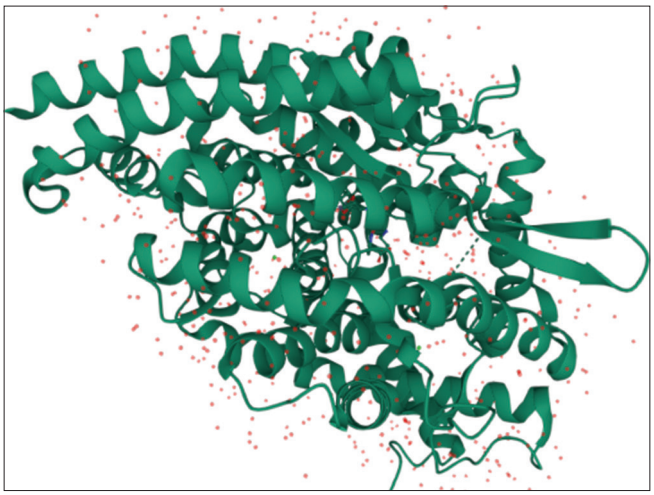

Figure 1: Receptor structure of Human angiotensin-converting enzyme (1O86)

RESULTS

Table 4 shows the amino acid residue interaction of the phytocomponents present in the formulation Veppampoomathirai and standard drug captopril against the crystal structure of ACE. Figure 3 illustrates the Docking Pose of the selected phytocomponents and standard captopril with core amino acid with ACE (PDB-1O86).

Based on the number of active amino acids in ACE active binding site with which hydrogen bond formation occurred, the phytoprinciples such as Ascorbic acid, Elemene, Cinnamaldehyde, Gallic Acid, Nimbiol, Piperic acid, Piperine, Eugenol, Nimbolide, Rutin, and Solasodine had a range of 7–10 interactions with lesser free binding energy and relatively more interaction surface area. The principles such as Piperic acid, Peperine, and Nimbolide had nine interactions and Nimbiol and Gallic acid had ten interactions which is higher than the standard Captopril which revealed only eight interactions with the active amino acids at the target ACE enzyme. Followed by this, the compounds such as Quercetin and Nimbin ranked second with a maximum of five interactions with the active amino acids at the target site of the target enzyme ACE in comparison with standard drug Captopril.

DISCUSSION

Most of the people had turned toward consuming herbal medicines for their ailments due to their acceptability nature toward herbs and low incidence of side effects. Approximately 47.5% of hypertensive patients simultaneously use herbal medicines and anti-hypertensive drugs.[31] A recent study by Xingxing Lai *et al*. reported, traditional Chinese medicine was found to be non-inferior when compared with Losartan in mild systemic HTN patients.[32] Among the classes of drugs for HTN in modern medicine, ACE is considered crucial and had received considerable attention as a therapeutic target for

Table 4: Amino acid residue interaction of the lead phytocomponents and standard drug captopril against crystal structure of Angiotensin-converting enzyme

Compound	Interactions	Amino acid residues															
Ascorbic acid	8	281 GLN	353 HIS	380 VAL	383 HIS	384 GLU	457 PHE	511 LYS	513 HIS	520 TYR	523 TYR						
Elemene	6	353 HIS	355 SER	383 HIS	384 GLU	387 HIS	411 GLU	512 PHE	513 HIS	518 VAL	522 ARG						
Cinnamaldehyde	6	281 GLN	353 HIS	411 GLU	415 ASP	511 LYS	513 HIS	520 TYR	523 TYR	526 SER	527 PHE						
Gallic Acid	10	281 GLN	353 HIS	354 ALA	380 VAL	383 HIS	384 GLU	511 LYS	513 HIS	520 TYR	523 TYR						
Nimbiol	10	281 GLN	353 HIS	354 ALA	355 SER	383 HIS	384 GLU	387 GLU	411 GLU	513 HIS	520 TYR	523 TYR					
Piperic acid	9	281 GLN	353 HIS	355 SER	383 HIS	384 GLU	387 GLU	457 PHE	511 LYS	513 HIS	520 TYR	523 TYR					
Piperine	9	281 GLN	353 HIS	355 SER	383 HIS	384 GLU	387 GLU	457 PHE	511 LYS	512 PHE	513 HIS	520 TYR	523 TYR	527 PHE			
Quercetin	5	70 ASN	351 VAL	353 HIS	355 SER	357 TRP	368 LYS	384 GLU	387 GLU	391 PHE	512 PHE	513 HIS	518 VAL	523 TYR			
Eugenol	8	281 GLN	353 HIS	380 VAL	383 HIS	384 GLU	387 GLU	457 PHE	513 HIS	520 TYR	523 TYR	527 PHE					
Nimbin	5	70 ASN	353 HIS	355 SER	357 TRP	383 HIS	384 GLU	387 GLU	411 GLU	512 PHE	516 SER	518 VAL	522 ARG	523 TYR			
Nimbolide	9	162 GLU	281 GLN	282 THR	353 HIS	354 ALA	377 ASP	380 VAL	384 GLU	453 ASP	457 PHE	511 LYS	513 HIS	520 TYR	523 TYR	527 PHE	2000 GLY
Rutin	8	162 GLU	353 HIS	354 ALA	355 SER	369 GLN	377 ASP	380 VAL	383 HIS	384 GLU	387 GLU	411 GLU	512 PHE	513 HIS	518 VAL	523 TYR	
Solasodine	7	353 HIS	354 ALA	380 VAL	383 HIS	384 GLU	387 GLU	391 PHE	403 GLU	411 GLU	512 PHE	513 HIS	523 TYR				
Vetiverol	0	123 GLU	124 ARG	135 TYE	204 ILE	207 ALA	208 ALA	219 SER	220 TRP	517 SER							
Gingerol	0	121 ASP	123 GLU	124 ARG	135 TYE	139 LEU	204 ILE	207 ALA	208 ALA	211 ASN	219 SER	220 TRP	517 SER				
Captopril	8	281 GLN	353 HIS	380 VAL	383 HIS	384 GLU	457 PHE	511 LYS	513 HIS	520 TYR	523 TYR	2000 GLY					

Glu: Glutamic acid, His: Histidine, Tyr: Tyrosine, Gln: Glutamine, Gly: Glycine, Val: Valine, Lys: Lysine, Phe: Phenyl alanine, Arg: Arginine, Asp: Aspartic acid, Leu: Leucine, Ile: Isoleucine, Ala: Alanine, Asn: Asparagine, Trp: Tryptophan, Ser: Serine. Yellow color indicates binding of the phytochemicals and standard captopril with core Amino acid residues of Angiotensin-converting enzyme (1O86)

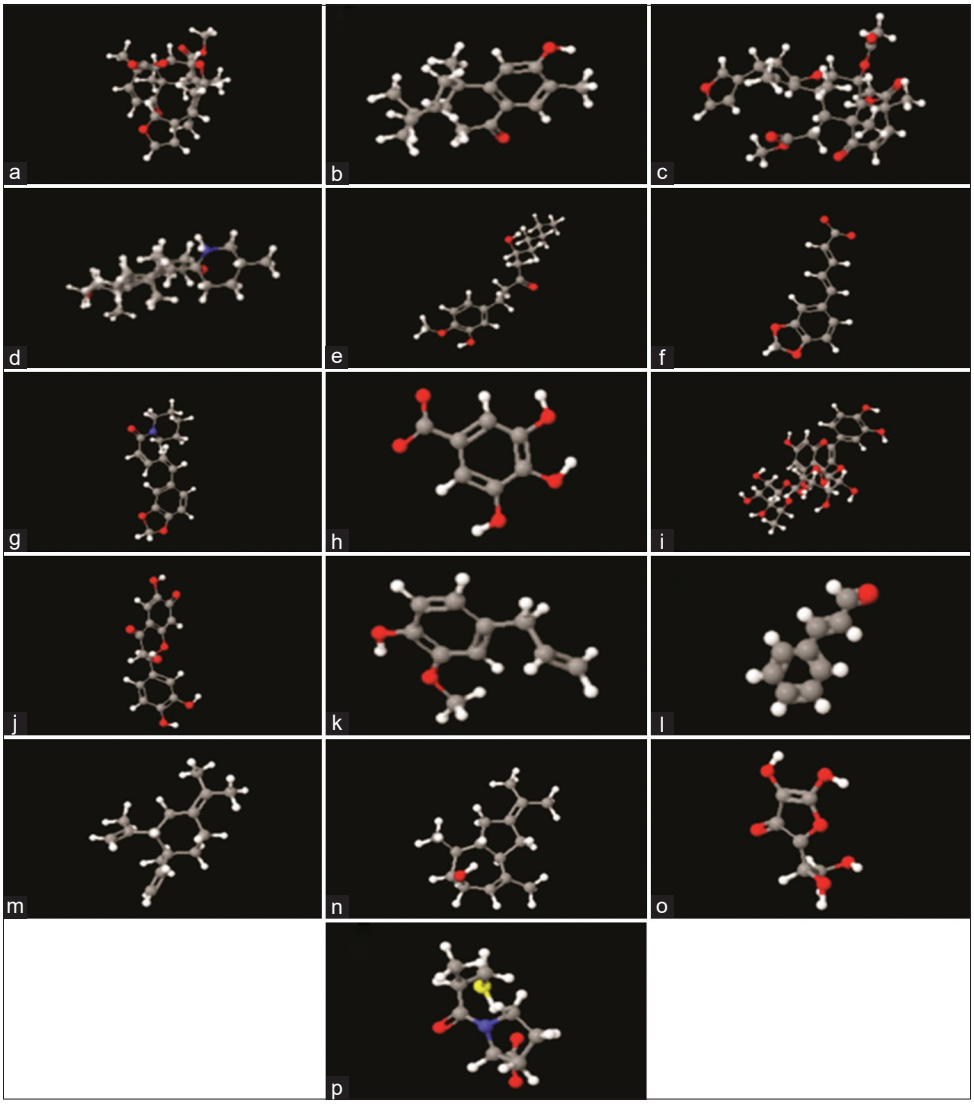

Figure 2: 3D Images of the selected phytocomponents and standard. (a) Nimbolide, (b) Nimbiol, (c) Nimbin, (d) Solasodine, (e) Gingerol, (f) Piperic acid, (g) Piperine, (h) Gallic acid, (i) Rutin, (j) Quercetin, (k) Eugenol, (l) Cinnamaldehde, (m) Gamma Elemene, (n) Vettiverol, (o) Ascorbic acid, and (p) Captopril

controlling HTN. Repressing ACE expression has been proved as an effective strategy in controlling HTN, as its down regulation will inhibit the conversion of angiotensin I to angiotensin II.[33]

Natural products could be important sources of ACE inhibitors such as captopril, a synthetic antihypertensive drug, which is developed by changing and optimizing the structure of the venom of the Brazilian viper. Active substances derived from medicinal plants can also be a source of new ACE inhibitors. Natural ACE inhibitors from plant sources have been shown to inhibit ACE through competitive, noncompetitive, and uncompetitive binding. ACE inhibitors from plant origin have shown high potency in regulating HTN. ACE-inhibitory plant metabolites mostly belong to the groups of peptides, protein hydrolysates, phenolics, flavonoids, terpenoids, and alkaloids. The mechanisms of action of these bioactive phytocompounds appear to resemble those of synthetic ACE inhibitors.[34]

Similar molecular docking studies had been done before on herbs against ACE inhibitors.[35] In 2009, Charles *et al*. reported naturally occurring glycosides as ACE inhibitors.[36] Anthocyanins delphinidin-and cyanidin-3-O-sambubiosides constituents from *Hibiscus sabdariffa* was studied for ACE inhibition and recorded positive by Ojeda *et al*., 2010.[37] A study by Attique *et al*., in 2019, concluded, allicin a herbal ligand exhibited reasonable binding affinity for ACE so that it can be used to make effective therapeutic drugs based on ACE inhibition to cure HTN.[38] The herbs of the formulation Veppampoomathirai had scientifically proven antihypertensive and other related anti-inflammatory, anti-oxidant pharmacological properties. Hence, as a consequence, the phytocomponents of the herbs present in

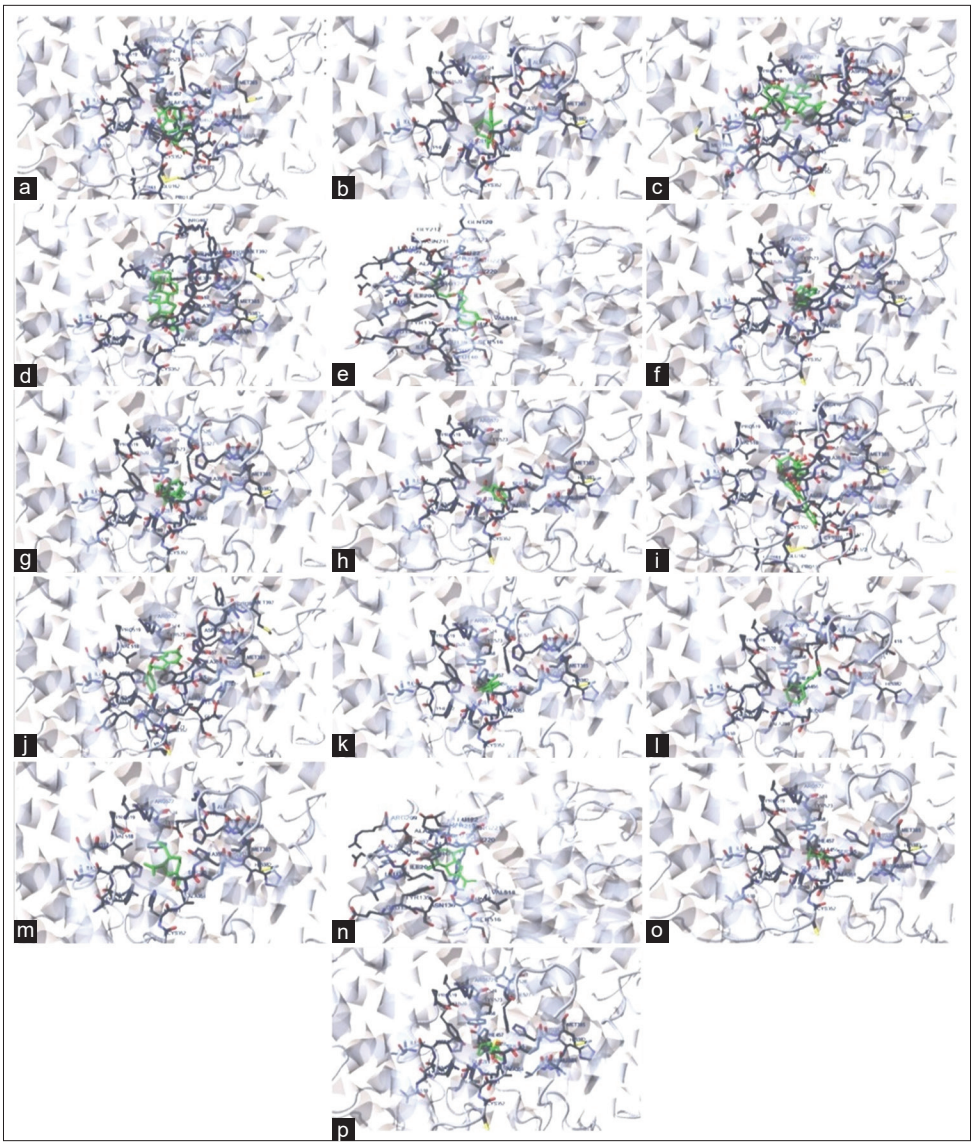

Figure 3: Docking Pose of the selected phytocomponents from the formulation Veppampoomathirai and standard with ACE (PDB-1O86), (a) Nimbolide, (b) Nimbiol, (c) Nimbin, (d) Solasodine, (e) Gingerol, (f) Piperic acid, (g) Piperine, (h) Gallic acid, (i) Rutin, (j) Quercetin, (k) Eugenol, (l) Cinnamaldehde, (m) Gamma Elemene, (n) Vettiverol, (o) Ascorbic acid, and (p) Captopril

the formulation were studied for inhibition activity against ACE with standard Captopril drug and found positive with encouraging findings.

CONCLUSION

The computational analysis of the present study concludes that, the bioactive compounds Ascorbic acid, Elemene, Cinnamaldehyde, Gallic Acid, Nimbiol, Piperic acid, Piperine, Eugenol, Nimbolide, Rutin, Solasodine, Quercetin, and Nimbin present in the siddha formulation Veppampoomathirai showed a significant binding against the target protein ACE. Hence, it was confirmed that these compounds may exerts promising anti-hypertensive activity and has considerable therapeutic potential in lowering higher BP in the management of HTN. Further, *in vitro* and *in vivo* studies are needed to establish the results.

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CONTRIBUTION OF AUTHORS

S. M. Chitra conceptualized, executed the study and written the manuscript. K. S. Uma edited and N. Anbu, approved the study.

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