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Anti-hypertensive effect of Siddha formulation *Imbural Vadagam* against Human angiotensin converting enzyme using In-Silico model

Research Article

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Abstract

Background: *Siddha* Medicine is emerging as the treatment of choice for various diseases. Hypertension is one among the comorbidities which plays a major role leading to stroke, cardiovascular diseases and renal failure. Objective: To perform the In Silico computational studies of phytocomponents of *Imbural vadagam* and to evaluate its anti-hypertensive potential. Methods: Auto dock program was used for the molecular docking studies against Angiotensin Converting Enzyme(ACE). Results: Asperulosidic acid, 6α-Hydroxygeniposide, Piperic acid, Piperine, Apigenin, Orientin and Vitexin that exist in the siddha drug *Imbural vadagam* exhibit remarkable binding against the target Angiotensin converting enzyme. Conclusion: It can be that these phytocomponents exhibit remarkable antihypertensive activity.

Key Words: *Siddha Medicine, Imbural vadagam,* Hypertension, Angiotensin converting enzyme inhibitor, Docking study, ACE.

Introduction

Hypertension is one among the comorbidities which plays a major role leading to stroke, cardiovascular diseases and renal failure. The definition of hypertension is that persistent blood pressure of about 140/90mmHg or more should take medication with the usual therapeutic target of 130/80mmHg or less (1). Hypertension is defined as systolic BP of 140 mm Hg or greater, and diastolic BP of 90 mm Hg or greater. Isolated systolic hypertension is defined as systolic BP of 140 mm Hg or greater and diastolic BP below 90 mm Hg (2). Like diabetes, ischemic heart disease and strokes, hypertension is another major public health problem for India. It is estimated that 30% of the adult population is having hypertension. Essential Hypertension is defined as hypertension without an identifiable cause and is an important reason for hypertension, 10% of patients are suffering from secondary hypertension (3). In the past four decades Global mean blood pressure has remained constant or decreased slightly. "But in contrast", it is -estimated that the prevalence of hypertension has increased, especially in low and middle-income countries (LMICs). The adult prevalence of hypertension was more in LMICs (31.5%, 1.04 billion people) than in high-income countries (28.5%, 349 million people) (4).

ACE converts angiotensin I to II. Angiotensin II narrows or constricts blood vessels and cause increase in blood pressure. Hence, when amino acid residues are occupied, enzymatic action of ACE will be inhibited thereby decreasing the blood pressure due to reduction in release of Angiotensin II.

Lead molecules/ Phytocomponents which inhibit the enzyme ACE will have promising effect in lowering higher blood pressure and thereby aid in management of hypertension. *Imbural vadagam* is made from *Oldenlandia umbellata* L., *Saccharum officinarum* L. and *Piper nigrum* L. *Oldenlandia umbellata* L. contains phytochemicals like Scandoside, Asperulosidic acid, 6α-Hydroxygeniposide (5) and *Piper nigrum* L. contains Piperidine, Piperic acid and Piperine (6) (7) and *Saccharum officinarum* L. contains Apigenin, Luteoline, Orientin, Vitexin (8). *Imbural vadagam* has indication for the treatment and management of hypertension. It also aids in treating cough, bronchial asthma, hematemesis (9). Hence it is chosen for evaluation of Angiotensin Converting Enzyme inhibition.

Objective

To identify the efficacy of the phytocomponents to bind with the bio active amino acid residues GLU162, GLN281, HIS353, ALA354, HIS383, GLU384, HIS387, GLU411, LYS511, HIS513, TYR520, TYR523 which are the mediators of enzymatic action of Angiotensin-converting enzyme (ACE) and aids in potential management of blood pressure.

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Materials And Methods

Protein preparation

3D shape of the target protein Human angiotensin converting enzyme (PDB) 1O86 were taken from the online repository of Protein Data Bank and put through protein clean before docking simulation.

Ligand Preparation

Phytochemical subjected to the investigation were taken from the herbs listed in the table based on the literature review and 3D structures were made with Chem Draw prof online tool version 12.0. Geometry optimization method (MMFF94) was used for ligand preparation.

Crystalline structure of the target protein Human angiotensin converting enzyme with PDB 1O86 was retrieved from protein data bank and clean-up process was done for protein. Essential missing hydrogen atoms were added. Different orientation of the lead molecules with respect to the target protein was evaluated by Auto dock version 4 program and the finest dock pose was selected on the basis of interaction study analysis.

Figure 1: Human angiotensin converting enzyme

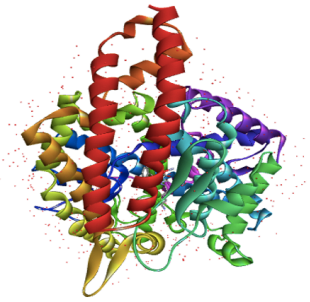
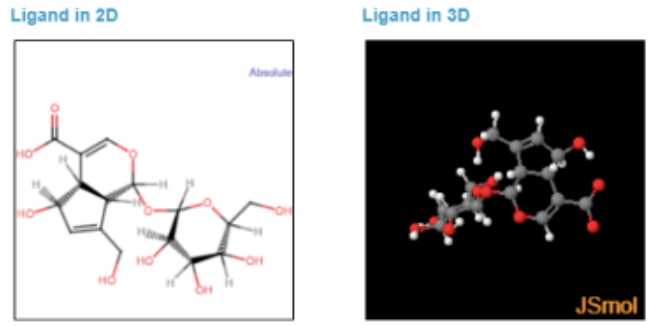
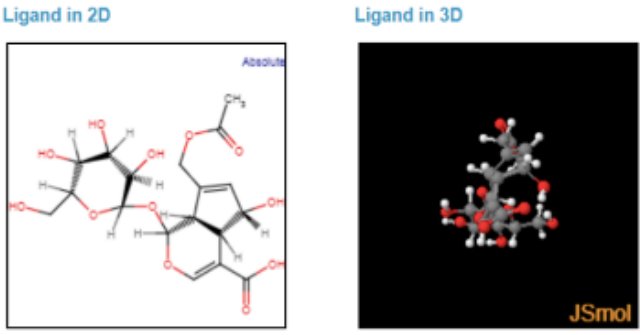
Receptor structure	
PDB	**Name of the Target**
1O86	**Crystalline Structure of Human angiotensin converting enzyme**

Figure 2: 2D and 3D Structure of Selected Ligands

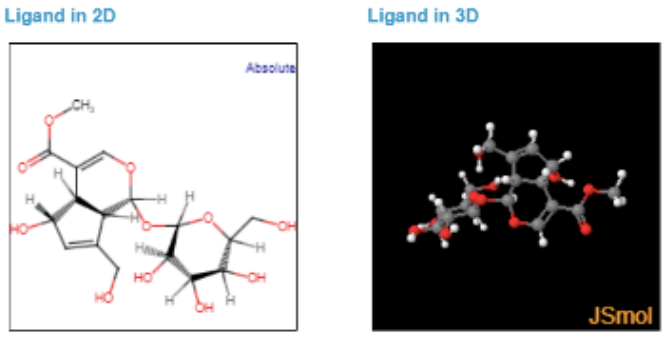
Scandoside



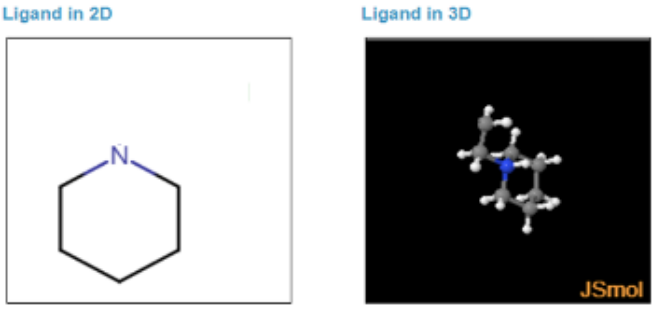
Asperulosidic acid



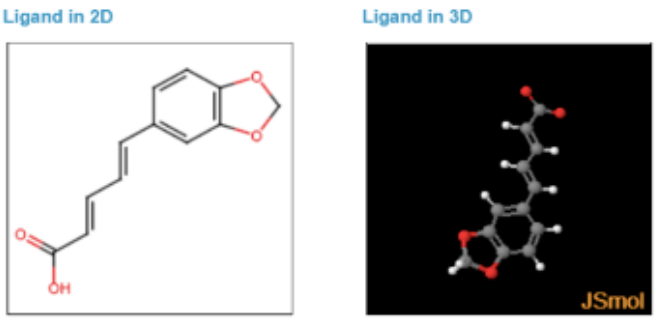
6α-Hydroxygeniposide



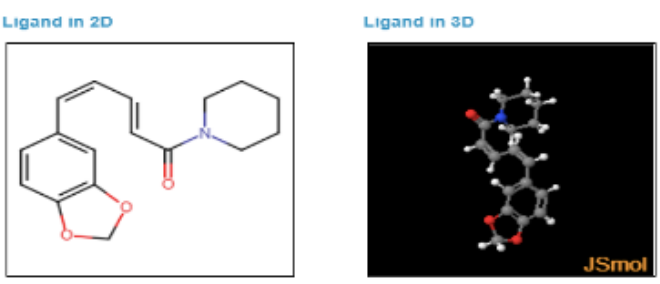
Piperidine



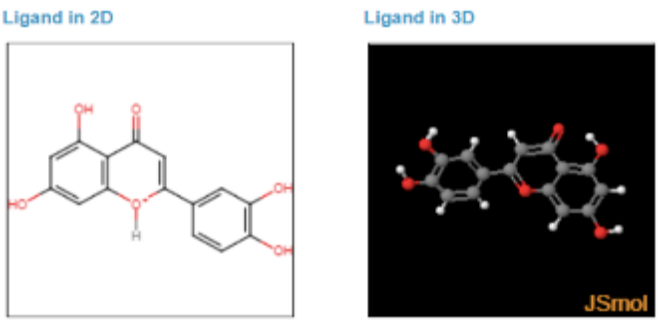
Piperic acid



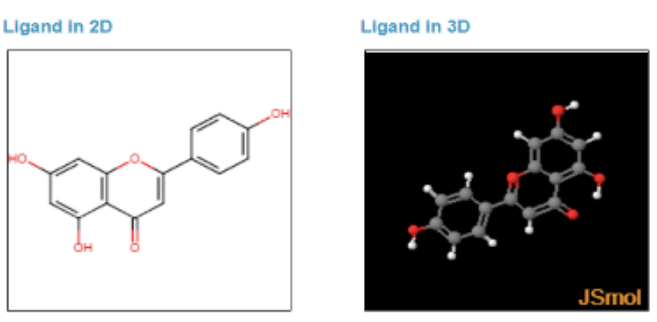
Piperine



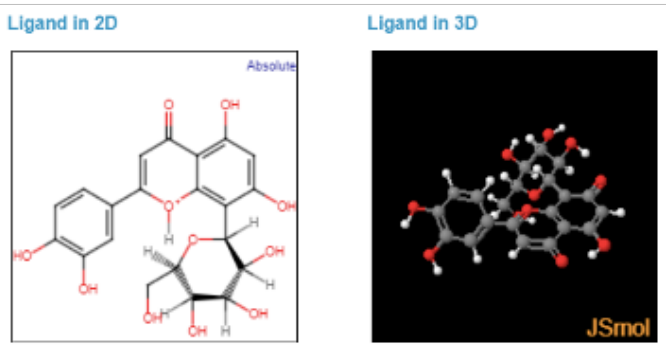
Luteolin



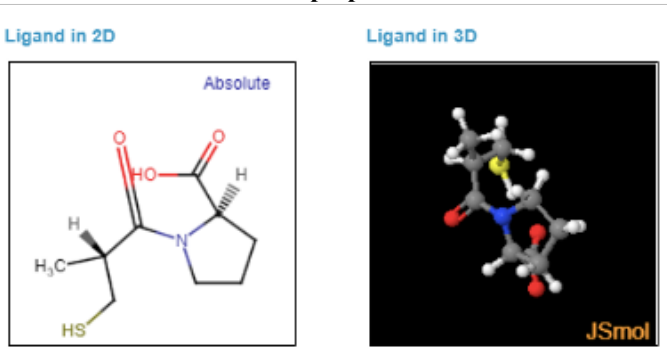
Apigenin



Orientin



Captopril



Docking Methodology

Docking calculations were done against target protein ACE for the retrieved phytocomponents. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the help of Auto dock -4 virtual screening tools.Affinity (grid) maps of 60×60×60 Å grid points and 0.375 Å spacing were generated using the Autogrid program.Auto dock 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 done using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method.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 2 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. A translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied during a search. Auto Dock 4 was used for docking calculations. ligand atoms were added with Gasteiger partial charges. Non-polar hydrogen atoms were fused , and rotatable bonds were determined. (10-12).

Table 1 : Ligand Properties of the Compounds

Compound	Molar weight g/mol	Molecular Formula	H Bond Donor	H Bond Acceptor	Rotatable bonds
Scandoside	390.34 g/mol	$C_{16}H_{22}O_{11}$	7	11	5
Asperulosidic acid	432.4 g/mol	$C_{18}H_{24}O_{12}$	6	12	7
6α-Hydroxygeniposide	404.4 g/mol	$C_{17}H_{24}O_{11}$	6	11	6
Piperidine	85.15 g/mol	$C_5H_{11}N$	1	1	0
Piperic acid	218.2 g/mol	$C_{12}H_{10}O_4$	1	4	3
Piperine	85.15 g/mol	$C_5H_{11}N$	1	1	0
Apigenin	270.24 g/mol	$C_{15}H_{10}O_5$	3	5	1
Luteolin	286.24g/mol	$C_{15}H_{10}O_6$	4	6	1
Orientin	448.4 g/mol	$C_{21}H_{20}O_{11}$	8	11	3
Vitexin	432.4 g/mol	$C_{21}H_{20}O_{10}$	7	10	3
Captopril	217.29 g/mol	$C_9H_{15}NO_3S$	2	4	3

Table 2 : Molecular docking studies of 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
Scandoside	-7.72 kcal/mol	2.19 uM	-0.63 kcal/mol	-5.82 kcal/mol	918.185
Asperulosidic acid	-7.57 kcal/mol	2.81 uM	-0.78 kcal/mol	-7.97 kcal/mol	906.487
6α-Hydroxygeniposide	-7.62 kcal/mol	2.61 uM	-0.19 kcal/mol	-6.41 kcal/mol	873.094
Piperidine	-5.60 kcal/mol	78.42 uM	-2.25 kcal/mol	-5.60 kcal/mol	297.238
Piperic acid	-4.90 kcal/mol	255.36 uM	-0.69 kcal/mol	-5.77 kcal/mol	575.11
Piperine	-6.67 kcal/mol	12.90 uM	-0.17 kcal/mol	-7.31 kcal/mol	719.673
Apigenin	-4.13 kcal/mol	931.50 uM	-0.18 kcal/mol	-5.72 kcal/mol	613.656
Luteolin	-6.73 kcal/mol	11.57 uM	-0.43 kcal/mol	-6.47 kcal/mol	606.174
Orientin	-8.29 kcal/mol	841.27 nM	-0.40 kcal/mol	-6.65 kcal/mol	897.989
Vitexin	-7.96 kcal/mol	1.47 uM	-0.08 kcal/mol	-6.79 kcal/mol	869
Captopril	-7.13 kcal/mol	5.97 uM	-0.64 kcal/mol	-5.73 kcal/mol	880.11

Table 3: Interaction of Lead and Standard against Human angiotensin converting enzyme (1O86)

Compounds	Interactions	Amino acid Residues														
Scandosde	3	162 GLU	166 THR	277 ASN	281 GLN	282 THR	354 ALA	372 THR	376 GLU	377 ASP	383 HIS	2000 GLY				
Asperulosidic acid	8	162 GLU	277 ASN	281 GLN	353 HIS	354 ALA	376 GLU	377 ASP	383 HIS	384 GLU	411 GLU	523 TYR	527 PHE			
6α-Hydroxygeniposide	10	162 GLU	281 GLN	353 HIS	354 ALA	376 GLU	377 ASP	380 VAL	383 HIS	384 GLU	387 HIS	411 GLU	513 HIS	523 TYR		
Piperidine	2	162 GLU	354 ALA	369 GLN	370 CYS	377 ASP										
Piperic acid	9	281 GLN	353 HIS	355 SER	383 HIS	384 GLU	387 HIS	457 PHE	511 LYS	513 HIS	520 TYR	523 TYR				
Piperine	8	281 GLN	353 HIS	355 SER	383 HIS	384 GLU	387 HIS	457 PHE	511 LYS	512 PHE	513 HIS	523 TYR	527 PHE			
Apigenin	7	353 HIS	354 ALA	355 SER	380 VAL	383 HIS	384 GLU	387 HIS	391 PHE	411 GLU	512 PHE	518 VAL	523 TYR	2000 GLY		
Luteolin	5	162 GLU	353 HIS	354 ALA	369 GLN	372 THR	376 GLU	377 ASP	380 VAL	383 HIS	384 GLU	2000 GLY				
Orientin	9	162 GLU	281 GLN	353 HIS	354 ALA	355 SER	383 HIS	384 GLU	387 HIS	457 PHE	513 HIS	523 TYR	527 PHE			
Vitexin	7	162 GLU	277 ASN	281 GLN	282 THR	353 HIS	354 ALA	355 SER	376 GLU	380 VAL	383 HIS	384 GLU	457 PHE	513 HIS	523 TYR	527 PHE
Captopril	8	281 GLN	353 HIS	383 HIS	384 GLU	411 GLU	457 PHE	513 HIS	520 TYR	523 TYR	2000 GLY					

Results and Discussion

Renin angiotensin aldosterone system (RAAS) is vital in controlling systemic blood pressure. Numerous ACE inhibitors are being synthesized and are in clinical practice. Renin is produced and stored in the granules of the juxtaglomerular cells surrounding the afferent arterioles of glomerulus. Renin release is stimulated by the RAAS, renal ischaemia, sympathetic nervous system stimulation, depressed sodium concentration, fluid depletion and decreased potassium intake. Released renin is transported through blood stream to the liver where it acts upon substrate angiotensinogen, an a-globulin synthesised in the liver, to produce angiotensin I, a decapeptide. Angiotensin I is converted into angiotensin II by the action of convertase in the lungs. Angiotensin II is a potent naturally- occurring vasoconstrictor substance and its pressor action is mainly attributed to peripheral arteriolar vasoconstriction (13). ACE inhibitors block the activity of angiotensin-converting enzymes, thus it prevents the angiotensin I to angiotensin II conversion.

Molecular docking studies were conducted for the compounds Scandoside, Asperulosidic acid, 6α-Hydroxygeniposide, Piperidine, Piperic acid, Piperine, Apigenin, Luteolin, Orientin, Vitexin, Captopril which are there in *Imbural vadagam* against Angiotensin converting enzyme to identify molecular interactions. All the ingredients *Imbural vadagam* were docked with Angiotensin converting enzyme using Auto dock programme.

A total of 10 bioactive phytocomponents were taken from the herbs indicated in *siddha* literature. On comparing binding affinities of the compounds, it was found that Orientin showed the highest binding affinity of -8.29 Kcal/mol. Vitexin showed the second-highest binding affinity with the binding free energy of -7.96 Kcal/mol followed by Scandoside and 6α-Hydroxygeniposide with -7.72Kcal/mol and -7.62Kcal/mol. Asperulosidic acid had -7.57Kcal/mol, Captopril

had -7.13Kcal/mol, Luteolin had -6.73Kcal/mol, Piperine had-6.67Kcal/mol , Piperidine had -5.60Kcal/mol, Piperic acid had -4.90Kcal/mol, Apigenin had-4.13Kcal/mol binding free energies. The phytocomponents such as Asperulosidic acid, 6α-Hydroxygeniposide, Piperic acid, Piperine, Apigenin, Orientin and Vitexin reveals maximum of 7 to 10 interactions with the amino acid residues of target ACE. Next, the phytocomponents Scandoside and Luteolin ranked second with the maximum of 3-5 interactions with the target enzyme. Captopril has 8 interactions with the target enzyme. Hence from the above results, it is found that the compounds Asperulosidic acid, 6α-Hydroxygeniposide, Piperic acid, Piperine, Apigenin, Orientin and Vitexin present in *Imbural vadagam* act as Angiotensin converting enzyme inhibitors and help in treating and managing hypertension.

Conclusion

On the basis of computational results, the bio-active compounds like Asperulosidic acid, 6α-Hydroxygeniposide, Piperic acid, Piperine, Apigenin, Orientin and Vitexin present in the herbal ingredients that exist in the Siddha Medicine *Imbural vadagam* exhibit notable binding against the target protein Angiotensin converting enzyme. It is due to the interaction with active amino acid present on the active site. thereby it can be concluded that that these compounds may exerts remarkable anti-hypertensive activity. It can be concluded that the phytochemicals present in the *siddha* drug *Imbural vadagam* possess significant anti-hypertensive activity. Further clinical trials need to be performed for identifying the efficacy and effectiveness of *Imbural vadagam* in the treatment and management of hypertension.

Competing interests

There is no conflict of interest among the authors.

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