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Herbal inhibitors identified for renin and angiotensin converting enzymes by *in silico* structure based methods

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Abstract
The enzymes Renin and Aniotensin Converting Enzymes (ACE's) are key enzymes associated with blood pressure, congestive heart failure and diabetic nephropathy. Renin is a protease, prohormone acting on free floating angiotensinogen. Three kinds of ACE's namely (ACE, ACE2 ACET (Testicular)) in humans, are carboxy peptidases, membrane bound, one of the receptors of insulin, participating in signal transduction convertsf Angiotensin I to Angiotensin II, a potent vaso constrictor raising blood pressure. This study was proposed with the aim to identify herbal inhibitors for these enzymes. The herbals zinger and Pausinystalia yohimbe were selected as herbal inhibitors, contains alkaloids zingeberene and yohimbine. The 3D structure of the enzymes were predicted using Swiss model server and the target sites identified using Thematics server. The chemical structure of the ligands and peptide structure of the target sequences drawn using Argus lab software and then converted into Protein Data Bank (PDB) format. Finally the targets and ligands were docked using Hex software. Docking results on this ligands indicated that the ligands has a potency to bind to the target sites on the enzymes. The proposed, herbal inhibitors has shown all the desirable features of a potent inhibitor and hence it may be a potential lead compound.

Key words: herbal inhibitor, protein engineering, drug discovery, drug design, protein-protein interaction, nano technology, signal transduction

Introduction
The enzymes Renin, and Angiotensin converting enzymes are responsible for elevating the blood pressure, increases salt and water retention in the body, coronary infarction (CHF) and diabetic kidney disease. They act in the formation of Angiotensin I from angiotensinogen and Angiotensin II from Angiotensin I. ACE's are one of the receptors of insulin and take part in the insulin signal transduction also. Till now the so called drugs used for treatment of hypertension are not adequately able to control the blood pressure. So direct inhibition of Renin and Angiotensin converting enzyme may help to solve the problem. According to latest report a direct renin inhibitor called Tekturna (aliskiren), was developed by molecular modelling techniques, it is a potent and specific in vitro inhibitor of human renin (IC50 in the small nanomolar range), with a plasma half-life of ≈24 hours. Tekturna has completely soluble in water and low lipophilicity and is resistive to bio degradation by proteolytic enzymes in the intestine, blood circulation, and the liver. Despite having all properties needed as drug for direct renin inhibition, it produced adverse effects in the clinical trials [1, 2, 3]. Renin inhibitors cause Adverse effects like Angioedema and a adverse event was allergic swelling of the face, lips or tongue and difficulty breathing**.,** Hypotension, diarrhea, rash, increased uric acid, gout, and renal stones [1].
A recent report in International Union of Pure and Applied Chemistry about biological half-life says that the ACE inhibitors used in treatment of hypertension manufactured and promoted by various pharmaceutical companies have their plasma half-life between 0.6 to 17 hrs. But all the key target enzymes have their plasma half-life = 30 hrs (Courtesy: Result of Protparam, a computational tool) the short half-life necessitates 2–3 times daily dosing, which may reduce patient compliance [4, 5].
So this study was proposed to design a better drug, from herbals which casues no side effects with affordable price for all group of people for the treatment of Hypertension using computational tools which direclty acts on the Renin and Angiotensin converting enzymes.

Zingiberene
Research into herbs with actions similar to pharmaceutical ACE inhibitors has found that several traditional medicines yielded promising results. While these ethno pharmacological studies do not deal with herbs commonly used in typical herbal prescribing in the U.S. or Europe they do indicate that plant-based medicines have a significant past and a potentially strong future in treating conditions such as currently treated by drugs such as ACE inhibitors
Zingiberene is a monocyclic sesquiterpene **(**Sesquiterpenes consist of three isoprene units and have the molecular formula $C_{15}H_{24}$.) which is the predominant component of the oil of ginger (Zingiber officinale), from which it gets its name [6, 7, 8].

Ginger
Ginger is the general term for the monocotyledonous perennial plant Zingiber officinale. The term is similarly used to name the edible part of the plant which is frequently used as a flavour in culinary throughout the world. Often mistakenly referred to as "ginger root", the edible component is actually the horizontal underground stem or rhizome of the plant. The ginger plant has a long history of cultivation recognized to come from China and then disseminated to India, Southeast Asia, West Africa, and the Caribbean.
[6, 7, 8].

Medical uses

The therapeutic form of ginger traditionally was called "Jamaica ginger"; it was categorized as a stimulant and carminative, and used normally for dyspepsia and colic. It was also repeatedly engaged to mask the taste of medicines. Ginger is on the FDA's 'normally accepted as safe' list, yet it does interact with some medications, including warfarin. Ginger is contraindicated in people suffering from gallstones as the herb encourages the release of bile from the gallbladder. Ginger may also reduce joint pain from arthritis, though studies on this have been conflicting, and may have blood thinning and cholesterol reducing properties that may make it useful for treating heart disease [6, 7, 8].

The typical odor and flavor of ginger root is produced by a combination of zingerone, shoagoles and gingerols, unstable oils that comprise roughly one to three percent of the mass of fresh ginger. In laboratory animals, the gingerols boost the sensitivity of the gastro intestinal tract and ensure analgesic, sedative, antipyretic and antibacterial characteristics. Ginger has been found powerful by multiple studies for curing nausea triggered by seasickness, morning sickness and chemotherapy, yet ginger was not established superior over a placebo for post-operative nausea. Contemporary research on nausea and motion sickness used roughly 1 gram of ginger powder daily. However there are assertions for efficacy in all causes of nausea, the Physicians' Desk Reference endorses against taking ginger rhizomes for morning sickness ordinarily associated with pregnancy due to possible mutagenic effects, though Chinese women have conventionally used ginger rhizomes during pregnancy to combat morning sickness and the Natural Medicines Comprehensive Database says that it is conceivably safe for use in pregnancy when consumed in food-amounts [6, 7, 8].

Yohimbine

Yohimbine, also known under the ancient terms quebrachin, aphrodin, corynine, yohimvetol and hydroergotocin, is the major alkaloid of the bark of the West-African evergreen Pausinystalia yohimbe Pierre (formerly Corynanthe yohimbe), family Rubiaceae (Madder family).

Alkaloids are normally existing chemical compounds comprising basic nitrogen atoms. The tag originates from the word alkaline and was used to illustrate any nitrogen-containing base. Alkaloids are produced by a broad class of organisms, comprising bacteria, fungi, plants, and animals and are part of the group of natural products (also called secondary metabolites).Around 31 yohimbane alkaloids found in Yohimbe. In Africa, yohimbine has conventionally been used as an aphrodisiac [9, 10, 11].

Yohimbine is a competeting alpha2-adrenergic receptor antagonist that is occasionally used as an alternate medication for erectile defective function. The alpha2 receptor is responsible for detecting adrenaline and noradrenaline and make the body to diminish its production as part of a negative feedback loop. Yohimbine apparently acts as antagonist, or a blocker, by binding to alpha2 receptors but not stimulating them. This in turn upsurges the synthesis and release of adrenaline and noradrenaline from adrenal gland. Yohimbine similarly antagonizes numerous serotonin receptor subtypes: 1A (inhibitory, behaivoral changes), 1B (inhibitory,stenosis), 1D (inhibitory, stenosis), and 2B (smooth muscle emaciation). Meanwhile yohimbine is an antagonist, it will decreases the properties of these receptors, thus triggering stimulation, vasodilation, and smooth muscle loosening. Yohimbine is as well said to increase dopamine and have certain actions as an MAOI, although these mechanisms are unknown [9, 10, 11].

Larger doses of oral yohimbine might generate many side effects such as fast heartbeat, increased blood pressure, and overstimulation. Yohimbine might induce anxiety, and is believed to cause insomnia in some users. Various internet shops sell luxurious preparations of yohimbine for transdermal transport to effect a local reduction of adipose tissue, although there is no indication that it is effective. Request for products of this kind is regularly found in the bodybuilding community [9, 10, 11].

Yohimbine chloride-a established form of yohimbine-- is a recommended medicine and it have been approved to treat erectile dysfunction. Organized studies propose that it is not always an effective cure for impotence, and indication of augmented sex drive (libido) is anecdotal only. It has significant after effects, for example, like anxiety reactions. Mayo Clinic, confirms that, yohimbine can be hazardous if used in excessive amounts. It cannot be excluded that orally administered yohimbine can have a favourable outcome in some patients with ED. The contradictory outcomes available may be credited to alterations in drug design and dosage, patient selection, and definitions of positive response [9, 10, 11].

Materials and Methods

A. Homology modeling by Swiss 3D modeler

The 3D structure of Renin was built by swiss model for the sequence range 70-406 based on the template 1hrnA with 99% sequence identity. The same software server was used for building the other models also. The ACE_2 was built for residue range from 19-615 using 1r42A as template with sequence identity is 100%. And for ACE the model for built for residues from 645-1222 with 1uzeA as template with sequence identity 99%. For ACET the model was built for the residue range from 71-648 with 1uzA as template with 99% sequence identity with E value for all of them E=0.00e-1.[12,13]

Fig 2: gives the 3D structure of Renin and Angiotensin converting enzymes.

B. Active site identification by Thematics computaions

A small region of an enzyme at which the substrate binds and participate in the catalysis. This site is due to the tertiary structure of protein resulting 3D confirmation. It is made of amino acids known as catalytic residues which are from each other in the linear sequence of amino acid. It is regarded as clefts or pockets occupying a small region in a small enzyme molecule. The site is not rigid in structure and shapes. Enzyme specificity of function is due to their activesite [13].

The predicted 3D structures were then submitted to Thematics server a tool which predicts surface binding sites of proteins from their 3D structure. Using a test set of 169 enzymes from the original Catalytic Residue Dataset (CatRes) THEMATICS delivers precise, localized site predictions [12, 13].

THEMATICS computations generally are performed on the biological unit for each enzyme in the dataset. Protein structures were obtained from the Protein Data Bank .If there are missing side chain atoms, the Swiss PDB Viewer (SPDBV) program is used to rebuild the missing atoms. The hydrogen atoms are built into the structure using TINKER and the OPLS-UA force field. Substrates, cofactors, water molecules, and

ions that crystallize with the proteins are not included in the electric field calculations. The values for the dielectric constants are assumed to be 20 for the protein interior and 80 for the solvent. The theoretical titration curve for each ionisable residue is obtained using a Finite Difference Poisson-Boltzmann procedure. The University of Houston Brownian Dynamics program. (UHBD) is used to obtain the electrical potential function. The program HYBRID, calculates the average charge C as a function of pH using a hybrid Monte Carlo procedure [13, 14]. Table1: gives details of target sites in Renin and Angio tensin converting enzymes.

C. Construction of ligands by molecular builder tool of Argus lab

Zingiberene is a monocyclic sesquiterpene consist of *three isoprene* units and have the molecular formula $C_{15}H_{24}$.Using its molecular formula the ligand was constructed by molecular builder software of argus lab. Its valence electrons was 84, and SCF calculated by Hartree-Fock method with starting energy was 39.029 Kcal/mol ending with –62.7618 Kcal/mol.

Yohimbine is the principal alkaloid of the bark of the West-African evergreen Pausinystalia yohimbe Pierre with molecular formula C_{21} H $_{26}$ N_2O_3. Using its molecular formula the ligand was constructed by molecular builder software of argus lab. Its valence electrons was 138, and SCF calculated by Hartree-Fock method with starting energy was 89.433 Kcal/mol ending with –91.0499 Kcal/mol. Both the ligands were converted into PDBformat for docking purpose. The ligand binding sites predicted by Thematics server were drawn into 3D structure with Argus lab and converted into PDB format for docking [14, 15, 16]. Fig3: Shows the argus lab structure of both the ligands.

D. Docking algorithm

Main aim of docking is to predict the structure of a protein–protein complex in atomic detail, starting from the atomic coordinates, determined by X-ray crystallography or MR spectroscopy, of the separate components (‘unbound’).Protein–protein interactions play a central role in biochemistry, because the formation of the complex has a functional consequence.

Hex is an interactive molecular graphics program for calculating and displaying feasible docking modes of pairs of protein and DNA molecules. *Hex* can also calculate small-ligand/protein docking (provided the ligand is rigid), and it can superpose pairs of molecules using only knowledge of their 3D shapesIn *Hex*'s docking calculations, each molecule is modelled using 3D parametric functions which are used to encode both surface shape and electrostatic charge and potential distributions [14, 15, 16].

B. Drug molecules (ligands) usually bind to proteins (receptors) at a cavity of the receptor, which is called binding site. It is usually assumed that the geometric constraints are the main determinants in this process. The energetic factors are also important, since molecules in nature are usually found in their low energy form. Ligands are small molecules, with many degrees of freedom (DOF).Proteins, however, are much bigger molecules, with hundreds of atoms. Given a ligand and a protein, finding whether they will bind to each other and if they will, in which configuration this will happen is a difficult problem to solve, involving many degrees of freedom. For this reason, many approximations are done in order to make the computation feasible [15, 16].

Protein-protein docking basically depends on the calculation of energy minimization and calculation of root mean-square distance (RMSD). For best docking RMSD values must lies between 2.1 to -2.3 A and energy as minimum as possible mostly -1.

Relationship between RMSD and E: RMSD = $\sqrt{E / N}$.

Fig 3 and 4: Shows the docked structure of targets with both the ligands. Table2: shows the duration of docking process of each ligand.

Results & Discussions:

Ligands

Fig.I illustrates the chemical structure of the ligands Zingiberene and Yohimbine as designed by the Argus lab software using the chemical formula and it was then converted into PDB format for docking purpose.

Target sites

An *in silico* structure-based drug designing approach was used to design the herbal inhibitors for our target ACE’s and Renin responsible for causing hypertension. The 3D structure predicted by Swiss model server was submitted to Thematics server for surface binding site identification (ligand binding). The tool identified possible regions where ligand can bind to induce some conformational changes in the enzymes to alter its activity. The regions identified included acidic, basic, and neutral aminoacids. The surface binding sites for Renin was identified as Asp 83 , Tyr 86 , Asp 231 , Arg 387 , Tyr 228 , Asp 104 , Asp 292, and for ACE2 the target sites identified in the region 18,183,199,460,498,187 whose amino acid residues as Tyr, Tyr, Tyr, Arg, Lys In ACE the ligand binding sites are identified in the region of sequences 767, 958, 989, 1116, 1125, 128, 988, 992, 1016, 1015with aminoacids Glu, His, Glu, Lys, Tyr ,Tyr, His, His, Glu, His. In ACET the ligand binding sites are predicted in the region of 193, 384, 415, 542, 551, 554, 414, 418, 442, 441 with residues Gly ,His, Glu, Lys, Tyr , Tyr, His, His, Glu, His

This tool predicted target regions for ACE2 the target region is devoid of Histidine alone. But for ACE and ACET the server identified same set of amino acid residues as ligand binding site. But they are in different locations in the protein sequences. This result indicated difference in the position of ligand binding site and during docking the ligands will behave differently for these enzymes. Fig II: gives the 3D structure of target sites, Table1: gives details of target sites in Renin and Angio tensin converting enzymes, and Table 2 illustrates the time duration of various targets and ligand during the docking process.

Docked complex

Fig. III displays the docked complexes of the target and the ligand by the Hex software

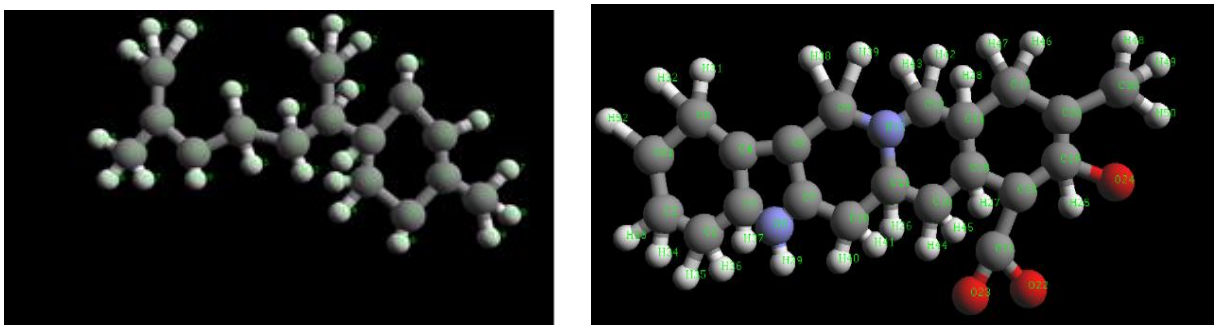
The targets of Angiotensin converting enzymes contains invariably acidic, basic and neutral amino acid residues and it forms a tight complex with any of acidic or basic or any ligands with hydrocarbon chains. The targets and ligands were docked with Hex software and the docking was done from 26-32 minutes for both the ligands .The ligand Zingiberene was a isoprene and the second ligand Yohimbine was a alkaloid and as expected from the nature of target sites they formed a tight target and ligand complex [12-16].

Table 1: Target Sites of All the Enzymes

RENIN		ACE2		ACE		ACET	
Name	Position	Name	Position	Name	Position	Name	Position
Aspartic acid	83	Tyrosine	180	Glutamic acid	767	Glutamic acid	193
Tyrosine	86	Tryosine	183	Histidine	958	Histidine	384
Aspartic acid	231	Tyrosine	199	Glutamic acid	989	Glutamic acid	415
Arginine	387	Arginine	460	Lysine	1116	Lysine	542
Tyrosine	228	Cysteine	498	Tyrosine	1125	Tyrosine	551
Aspartic acid	104	Lysine	187	Trysonie	1128	Tyrosine	554
Aspartic acid	292			Histidine	988	Histidine	414
				Histidine	992	Histidine	418
				Glutamic acid	1016	Glutamic acid	442
				Histidine	1015	Histidine	441

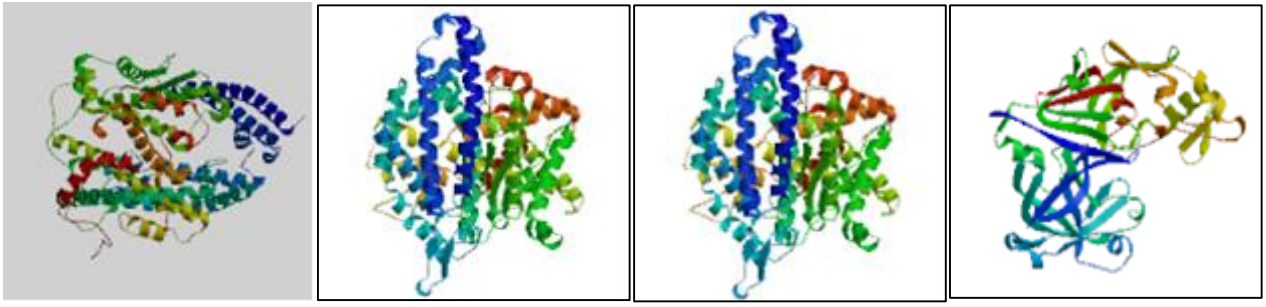
Table 2: duration of docking process

S.No	Name of enzyme	1) Zingiberene	2) Yohimbine
3) 1.	4) ACE_2	5) 26mins 27 sec	6) 27mins 19sec
7) 2.	8) ACE	9) 32min 55sec	10) 29mins 29sec
11) 3.	12) ACET	13) 29mins 06sec	14) 28mins 28sec
15) 4.	16) Renin	17) 31 mins 26sec	18) 28mins 28sec



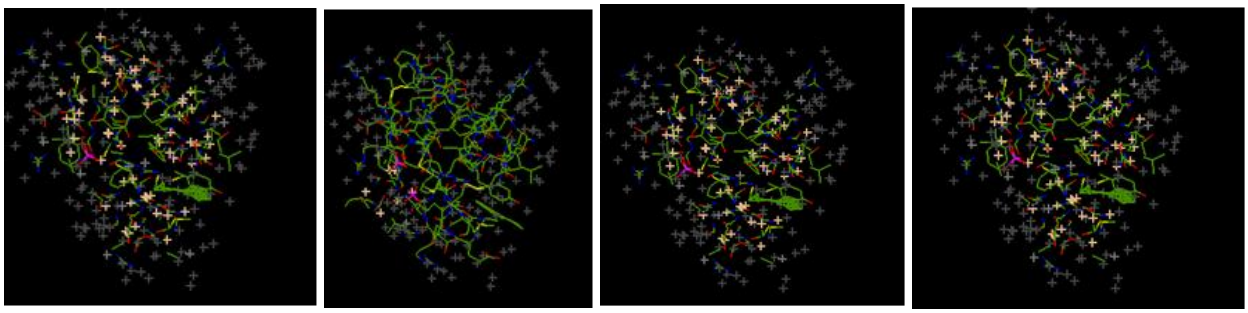
a: Zingiberene: $C_{15}H_{24}$ **b**: Yohimbine: $C_{21}H_{26}N_2O_3$

Fig 1: Chemical Structure of the Herbal Inhibitors (designed using ArgusLab)



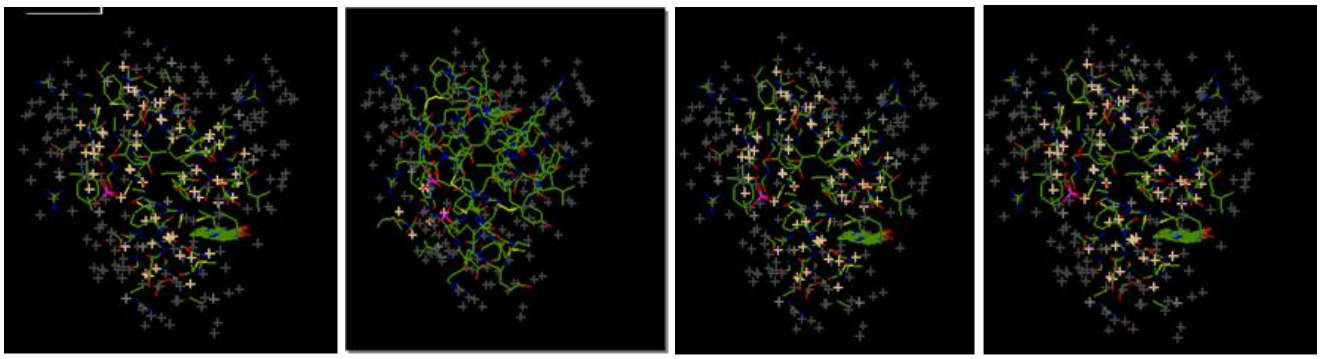
a. ACE2 **b. ACE** **c. ACET** **d. Renin**

Fig 2: 3D Structure of the Enzymes as Predicted by Swiss 3D modeler



a. Renin and zingiberene b. ACE2 and zingiberene c. ACE and zingiberene d . ACET and zingiberene

Fig 3: Docked Complex of Ligand (Zingiberene)



a. Renin and Yohimbine *b* . ACE2 and yohimbine c. ACE and Yohimbine d. ACET and Yohimbine

Fig 4: Docked Complex *of* Ligand: Yohimbine

Conclusion

In the present investigation Targets and ligands were successfully designed and docked to their respective targets using different software tools .This investigation can be further extended to formulations and clinical trials for study of pharmacokinetics of these ligands to produce a effective drugs for the treatment of hypertension and cardiovascular disorders and diabetic nephropathy.

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