# LC-MS/MS, GG-MS Analysis and *in-silico* approaches in understanding the Mechanism of action of *Aragwadadi yogam* against Diabetic Nephropathy

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**Abstract:**

*Aragwadadi yogam* Sastric Ayurvdic medicine is a potential source of secondary metabolites such as of flavonoids, sterols, terpenoids and tannins and has been traditionally employed to treat Microalbuminurea.The present work attempts to evaluate the activity of *Aragwadadi yogam* extract for glycemic control. Aqueous extract of *Aragwadadi yogam* analyzed by LC-MS/MS have revealed the presence of Caffeic acid hexoside dimer and Propelargonidin B dimer and GC-MS analysis identified Resorcinol and 2-O-Methyl-D-mannopyranosa . Evaluation of hypoglycemic activity through an extensive *in silico* docking approach with Angiotensin converting enzyme as target. This work establishes *Aragwadadi yogam* as a potential Angiotensin converting enzyme inhibitor against diabetic Nephropathy thus enabling a possibility of this plant extract as a new alternative to existing diabetic therapy.

Keyword: *Aragwadadi yogam*, Angiotensin converting enzyme and Diabetic Nephropathy.

## Introduction:

Diabetic nephropathy is the single major cause of end-stage renal disease in the industrialized world. The annual cost of dialyzing diabetic patients in the United States alone exceeds $17 billion.1 Given the increasing prevalence of diabetes mellitus (DM), the burden of diabetic nephropathy (DN) on global health care resources is only expected to increase.2 New therapeutics to treat DN are desperately needed. In addition, the high rate of cardiovascular morbidity and mortality in patients with diabetic nephropathy3,4 mandates these new therapeutics not only slow the renal functional decline but also reduce cardiovascular mortality. Despite this growing public health imperative, substantial hurdles exist to the development of therapies that slow the otherwise inexorable decline in renal function observed in the subset of diabetic patients who are destined to develop end-stage renal disease.

Among the impediments to developing novel therapies for diabetic nephropathy, perhaps the most significant is the lack of a reproducible and dynamic biomarker that is sensitive to the rate of change in kidney function as renal functional impairment slows or accelerates. At present, the biomarker that is closest to meeting the necessary characteristics is proteinuria 5,6 however, this is not accepted as a registration end point for diabetic nephropathy. This gap means that longer and more expensive trials must be run. Another impediment to target identification is the lack of validated animal models that faithfully recapitulate the human disease and for which efficacious therapies also prove efficacious in the clinic. In the past decade, substantial effort has attempted to address this deficiency by better characterizing diabetic nephropathy in the laboratory mouse with diabetic nephropathy. As will be discussed, assessing diabetic kidney disease in the mouse requires attention to species-specific physiological considerations.(1)

Angiotensin converting enzyme (ACE) plays a critical role in the circulating or endocrine renin-angiotensin system (RAS) as well as the local regulation that exists in tissues such as the myocardium and skeletal muscle. ACE inhibitors are widely used to treat cardiovascular diseases, including high blood pressure, heart failure, coronary artery disease, andkidney failure. Due to the critical role of ACE in cardiovascular and diabetic nephropathy, it has been an attractive target for drug design.(2)

## Materials and methods

*In silico* docking studies against Angiotensin converting enzyme, a diabetic target was carried out using the ligands Caffeic acid hexoside dimer and Propelargonidin B dimer identified in the LC-MSMS analysis of *Aragwadadi yogam* and Resorcinol and 2-O-Methyl-D-mannopyranosa identified in GC-MS analysis.

## Protein preparation

AutoDock is a suite of automated docking tool. It is designed to predict how small molecules, such as substrates or drug candidates, bind to a receptor of known 3D structure (3). The 3D structure (PDB ID: 1UZF) of Angiotensin converting enzyme, remove water molecule and add with Kollmann charges were assigned. Through which hydrogens were added, side chains were optimized for hydrogen bonding. The energy minimized protein was then saved in PDB format. Using MGLTools-1.5.6 nonpolar hydrogens were merged,

AutoDock atom type AD4 and Gasteiger charges were assigned and finally saved in protein.pdbqt format.(4)

**Ligand preparation**

Structure of ligands were drawn using ChemSketch (5) , optimized with 3D-geometry and the two-dimensional structures of Caffeic acid hexoside dimer,Propelargonidin B dimer,Resorcinol,2-O-Methyl-D-mannopyranosa bioactive compounds were converted into 3-D structure using the Open Babel format molecule converter (6) and saved in PDB format for AutoDock compatibility. MGLTools-1.5.6 (The Sripps Research Institute) was used to convert ligand.pdb files to ligand.pdbqt files.

**Active site prediction**

The active site of the protein is the binding site or usually a pocket at the surface of the protein that contains residues responsible for substrate specificity which often act as proton donors or acceptors. Identification and characterisation of binding site is the key step in structure based drug design. The binding site has been identified by computational and literature reports. The active site region of the protein is identified by CASTP (7). These servers analytically furnish the area and the volume at the probable active site of each pocket to envisage the binding site.

**Docking protocol**

Grid parameter files (protein.gpf) and docking parameter files (ligand.dpf) have written using MGLTools-1.4.6. Receptor grids were generated using 90x80x50 grid points in xyz with grid spacing of 0.375 Å. Grid box was cantered co crystallized ligand map types were generated using autogrid4. Docking of macromolecule was performed using an empirical free energy function and Lamarckian Genetic Algorithm, with an initial population of 250 randomly placed individuals, a maximum number of 106 energy evaluations, a mutation rate of 0.02, and a crossover rate of 0.80. One hundred independent docking runs were performed for each ligand. Results differing by 2.0 Å in positional root-mean square deviation (RMSD) were clustered together and represented by the result with the most favorable free energy of binding**.**

**Results and Discussion.**

Chronic kidney disease (CKD) has become a worldwide health-care concern that calls for prompt initiatives and firm commitments to find effective treatments. The prevalence of CKD is estimated to be 8% to 16% globally and is steadily rising . It not only causes pain in individuals with the disease, but also has effects on society. CKD patients are exposed to an increased risk of death, comorbidities, and cognitive impairment, which all contribute to poor quality of life. Moreover, the medical expenses covering hospitalizations for both cardiovascular and non-cardiovascular events and all-cause mortality are costly. Unfortunately, the end-stage renal disease (ESRD) population is expanding and the CKD population contains potential ESRD patients. Currently, there are few therapeutic drugs for DN, which mainly consist of antihypertensive and antiproteinuric measures that arise from strict renin-angiotensin-aldosterone system inactivation. However, these traditional therapies are suboptimal and there is a clear, unmet need for treatments that offer effective schemes beyond glucose control. The complexity and heterogeneity of the DN entity, along with ambiguous renal endpoints that may deter accurate appraisal of new drug potency, contribute to a worsening of the situation. To address these issues, current research into original therapies to treat DN is focusing on the intrinsic renal pathways that intervene with intracellular signaling of anti-inflammatory, antifibrotic, and metabolic pathways. Mounting evidence in support of the favorable metabolic effects of these novel agents with respect to the renal aspects of DN supports the likelihood of systemic beneficial effects as well. The use of protein crystal structures solved in complex with specific inhibitors is a valuable tool for rational drug design. The plant compounds docking with Angiotensin converting enzyme using Autodock softer and predict binding affinity of ACE inhibitors. The Caffeic acid compound highly interacts with Angiotensin converting enzyme.

**Best interactions**

The amino acid residues LYS 511 and GLN 281 was involved in interactions with Caffeic acid in the active site of Angiotensin converting enzyme. The length of hydrogen bonds formed 1.819 Å and 1.974Å and also $IC_{50}$ value of this compound have 29 (µm).

The amino acid residues THR282,ASN 277,HIS383,GLU384,ASP377,GLU376 was involved in interactions with Propelargonidin B dimer in the active site of Angiotensin converting enzyme. The length of hydrogen bonds formed 2.082 Å,1.983 Å 2.102 Å,2.042 Å,1.756 Å,1.803 Å and 1.974Å and also $IC_{50}$ value of this compound have 34 (µm).

**Table 1. Molecular docking with Angiotensin converting enzyme (PDB ID: 1UZF)**

| S. No | Compound name | Docking score | $IC_{50}$ value | H-Bond interaction | Distance |
|---|---|---|---|---|---|
| 1 | Caffeic acid hexoside dimer | -7.3 | 29 | LYS 511 N-H...O<br>GLN 281 N-H...O | 1.819<br>1.974 |
| 2 | Propelargonidin B dimer | -7.1 | 34 | THR282 O-H...O<br>ASN 277 N-H...O<br>HIS383 N-H...O<br>GLU384 N-H...O<br>ASP377 O-H...O<br>GLU376 N-H...O | 2.082<br>1.983<br>2.102<br>2.042<br>1.756<br>1.803 |
| 3 | Resorcinol | -6.7 | 46 | GLN281 N-H..O<br>LYS511 O-H...O<br>HIS523 0-H...O<br>HIS353 N-H...O | 1.751<br>1.878<br>2.165<br>2.181 |
| 4 | 2-O-Methyl-D-mannopyranosa | -5.5 | 49 | GLN281 N-H...O<br>HIS513 N-H...O<br>HIS353NN-H...O | 2.07<br>2.113<br>1.99 |

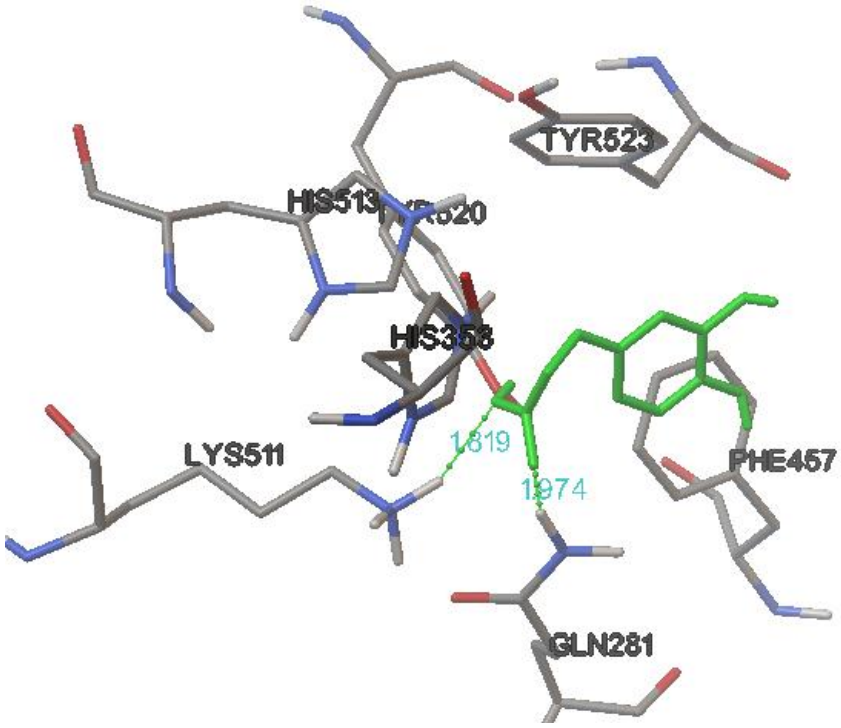


**Figure 1. Angiotensin converting enzyme with Caffeic acid**

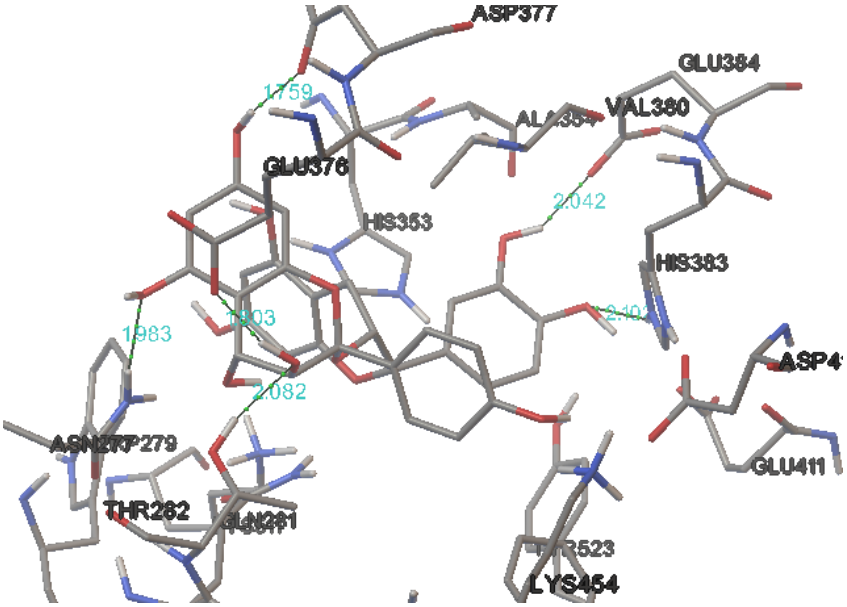


**Figure 2. Angiotensin converting enzyme with Propelargonidin B dimer**

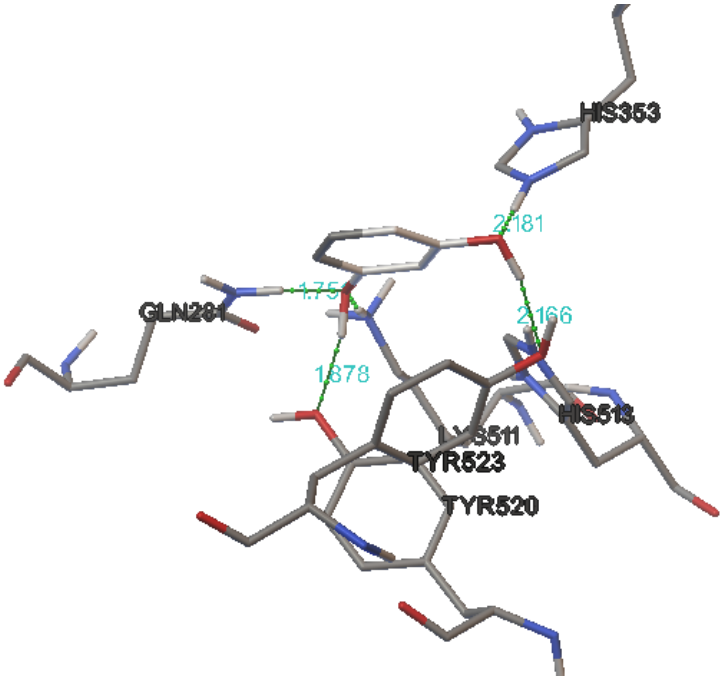


**Figure 3. Angiotensin converting enzyme with Resorcinol**

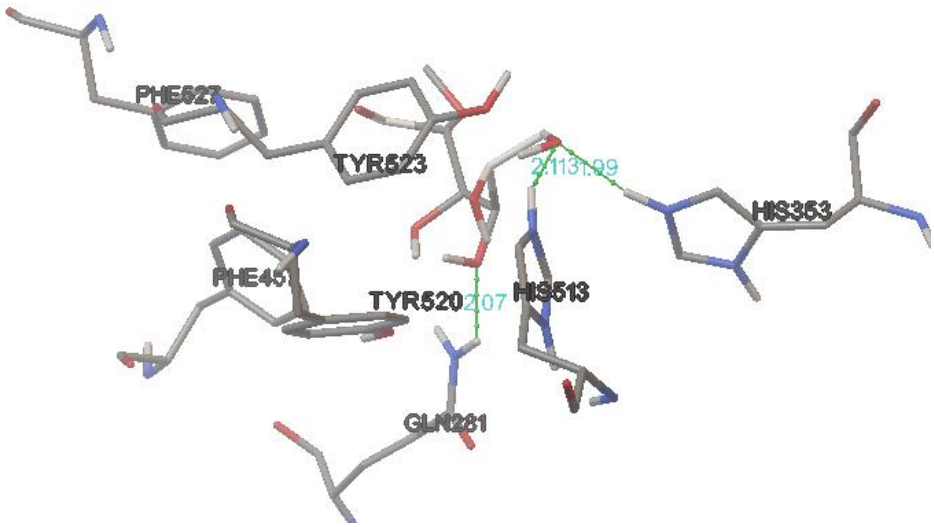


**Figure 4. Angiotensin converting enzyme with 2-O-Methyl-D-mannopyranosa**

The results indicated that Autodock algorithms were valid, as re-docking the native ligand to binding site of Angiotensin converting enzyme showed a RSMD value less than 2.5 Å . The docking result revealed that Propelargonidin B dimer, and Caffeic acid exhibited good binding interaction to catalytic site of Angiotensin converting enzyme.

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