Original Article

Molecular Docking Analysis of Nellimulliyathi Kashayam against Angiotensin Converting Enzyme

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ABSTRACT

Hypertension is a significant non-communicable disease and a primary risk factor for cardiovascular morbidity and mortality globally. Synthetic angiotensin-converting enzyme (ACE) inhibitors, such as captopril and lisinopril, are commonly prescribed; however, their prolonged use is frequently constrained by side effects, including cough, dizziness, and renal dysfunction. This has generated an immediate demand for safer, plant-derived alternatives. Traditional Siddha medicine, utilized in South India for centuries, offers various polyherbal formulations purported to regulate blood pressure. Nellimulliyathi Kashayam has been clinically prescribed; however, its molecular mechanism of action remains scientifically unvalidated. This study utilized molecular docking to assess the ACE inhibitory potential of phytoconstituents derived from Nellimulliyathi Kashayam. Nine compounds—Carvacrol, Cuminaldehyde, Umbelliferone, Myrcene, Gingerenone-A, γ-Oryzanol, an Anthracene derivative, 1-Nonadecanol, and Betulonic acid—were docked to the human ACE enzyme (PDB ID: 1O8a) utilizing AutoDock 4. Binding free energies (ΔG), inhibition constants (Ki), and residue-level interactions were evaluated to determine inhibitory potential. The findings indicated that γ-Oryzanol (–9.42 kcal/mol, Ki = 124.7 nM), Anthracene derivative (–8.36 kcal/mol, Ki = 750.9 nM), and Betulonic acid (–7.72 kcal/mol, Ki = 2.21 µM) demonstrated the highest binding affinities and established stable hydrogen bonds with essential residues including His353, Glu384, and Tyr523. Gingerenone-A exhibited moderate activity, whereas Carvacrol, Cuminaldehyde, Umbelliferone, Myrcene, and 1-Nonadecanol demonstrated relatively weaker affinities, yet still engaged with the ACE active site. These results corroborate the conventional antihypertensive assertions of Nellimulliyathy Kashayam and underscore γ-Oryzanol, Betulonic acid, and the Anthracene derivative as potential lead compounds for subsequent advancement. Future in vitro and in vivo investigations are advised to validate efficacy and safety, thereby integrating Siddha knowledge with contemporary pharmacological evidence.

Keywords: Nellimulliyathi Kashayam, Angiotensin-converting enzyme (ACE) inhibition, Molecular docking, Siddha medicine, Hypertension management.

INTRODUCTION

One of the most common non-communicable diseases in the modern world is hypertension, or consistently high blood pressure. Over 1.28 billion adults between the ages of 30 and 79 are estimated by the World Health Organization (WHO) to have hypertension, with low- and middle-income nations accounting for two-thirds of these cases[1]. Known as the "silent killer," hypertension usually develops asymptomatically until it causes serious side effects like retinopathy, myocardial infarction, stroke, or renal failure[2].

Surveys show that both urban and rural populations in India have a high prevalence of hypertension, reflecting this global crisis. Nearly one in four men and one in five women over the age of 15 have hypertension, according to the National Family Health Survey-5 (NFHS-5)[3]. This issue has been made worse by urban lifestyles, dietary changes, stress, and sedentary behavior, and treatment adherence and awareness are still low. In the Indian context, this combination has made hypertension a significant public health concern [4].

An important factor in controlling blood pressure is the renin-angiotensin system (RAS). Bradykinin, a peptide that encourages vasodilation, is broken down by the angiotensin-converting enzyme (ACE), which also catalyzes the conversion of angiotensin I to angiotensin II, a strong vasoconstrictor. ACE is a crucial pharmacological target for managing hypertension because it produces angiotensin II while lowering bradykinin[5].

This pathway has been successfully exploited by modern medicine. Captopril, enalapril, lisinopril, and ramipril are examples of synthetic ACE inhibitors that are now the mainstay of antihypertensive treatment. These medications dramatically lower the risk of cardiovascular events, improve endothelial function, and decrease vascular resistance [6]. However, prolonged use is often linked to side effects such as dizziness, persistent cough, hyperkalemia, renal impairment, and in rare instances, angioedema [7]. Patient compliance is frequently restricted by these side effects, as well as financial concerns in low-resource environments, necessitating the development of safer and more sustainable substitutes.

Drug discovery has long been based on natural products. In vitro and in silico studies have demonstrated the ACE inhibitory activity of phytochemicals like flavonoids, alkaloids, and terpenoids [8]. Strong ACE binding affinities have been shown by compounds from Cucurbita pepo[9], Phyllanthus niruri [10], and Carex praecox [11]. The potential of food-derived compounds as antihypertensives was further supported by peptides extracted from yak bone [12] and

bioactive fractions from whey protein[13]. These results highlight the increasing interest in natural compounds, which frequently offer pleiotropic advantages like blood pressure regulation along with antioxidant and anti-inflammatory activity.

One of South India's oldest traditional medical systems, siddha, uses a polyherbal approach that stresses the interaction of several plant components. Siddha formulations combine multiple herbs to achieve balance and enhance therapeutic potential, in contrast to modern pharmacology, which typically targets a single molecule[14]. One such traditional Siddha decoction that has been used for centuries to treat cardiovascular diseases, such as hypertension, is Nellimulliyathy Kashayam. The formulation does not have molecular-level scientific validation, despite its clinical use. Polyherbal mixtures contain phytoconstituents that can bind to ACE active site residues, according to earlier in silico studies on other Siddha formulations, including Kurudhiazhal Choornam [15] and Imbural Vadagam[16]. Nevertheless, Nellimulliyathi Kashayam has not yet been the subject of a comparable docking study.

Therefore, the goal of the current study was to close this knowledge gap. We tested nine phytochemicals from Nellimulliyathi Kashayam against the human ACE enzyme using molecular docking: carvacrol, cuminaldehyde, umbelliferone, myrcene, gingerenone-A, γ-Oryzanol, anthracene derivative, 1-nonadecanol, and betulonic acid. In order to provide a molecular justification for Nellimulliyathi Kashayam's antihypertensive claims and to find lead compounds that may serve as inspiration for future drug development, this study will compute binding free energies, inhibition constants, and residue-level interactions.

I. RELATED RESEARCH

Researchers are investigating natural compounds from plants, foods, and traditional medical systems in addition to synthetic molecules in an effort to find safe and effective angiotensin-converting enzyme (ACE) inhibitors. Over the past 20 years, a significant amount of research has surfaced that emphasizes the potential of phytochemicals and bioactive peptides in controlling the renin-angiotensin system. In this regard, the traditional South Indian healing system known as Siddha medicine has attracted more and more scientific attention due to its use of polyherbal formulations for cardiovascular conditions.

a) Siddha formulations and ACE inhibition

It has been shown through docking studies on Siddha formulations that phytochemicals found in traditional decoctions can efficiently target ACE. Glycyrrhizin and β-sitosterol showed strong binding affinities with ACE, according to Chitra's [17] analysis of Kurudhiazhal Choornam, a formulation traditionally used for cardiovascular ailments. These compounds' potential as natural antihypertensive agents was supported by their interactions with important active site residues like Tyr523 and His353. Similarly, phytoconstituents such as piperine and vitexin exhibited stable binding to ACE active site residues, with binding energies in the range of known inhibitors, according to an in silico analysis of Imbural Vadagam[16]. These results offer a mechanistic explanation for the therapeutic claims of such formulations in addition to validating Siddha pharmacology.

These studies are important because they show polyherbal synergy. Siddha formulations incorporate several herbs, each contributing compounds with different binding strengths, rather than depending on a single strong inhibitor. This supports the conventional wisdom that suggests group interactions, not individual molecules, are the source of therapeutic efficacy. It is necessary to expand research to other classical preparations like Nellimulliyathi Kashayam, which is still understudied, as the majority of published Siddha docking studies have concentrated on a small number of formulations.

b) Medicinal plants with ACE inhibitory potential

Numerous medicinal plants have been investigated for their ACE inhibitory properties outside of Siddha medicine. Flavonoids like quercetin and kaempferol showed strong ACE binding affinities, matching the potency of synthetic inhibitors, according to Mishra [8] evaluation of phytoconstituents from Cucurbita pepo in 2022. Their research highlighted the potential of common food plants as a source of beneficial antihypertensive compounds.

Similarly, Sani et al [9] found bioactive compounds with strong ACE inhibition in silico after studying Phyllanthus niruri, a plant that is commonly used in traditional medicine. These substances interacted with residues such as His353 and Glu384, highlighting the conserved binding pocket that a variety of natural inhibitors target. According to Zhao et al [10] report on the medicinal grass Carex praecox, its phytochemicals successfully docked with ACE, indicating that it may have potential as a functional food for blood pressure regulation.

Although the majority of research is still in the computational or preclinical stages, Zeng[17] systematic review of medicinal plants with antihypertensive qualities found that many species contain compounds with ACE inhibitory effects. Despite encouraging in silico results, a major drawback of these studies is the absence of solid in vivo and clinical data, which limits translational application.

c) Bioactive peptides and food-derived inhibitors

Peptides and proteins have also been identified as significant natural ACE inhibitors. Strong ACE inhibitory activity was demonstrated by peptides derived from milk proteins by Nongonierma and FitzGerald [12]. These peptides were appealing candidates for functional foods because they provided nutritional advantages in addition to lowering blood pressure in animal models.

Peptides from yak bone were reported by Xie et al [11] to have ACE inhibitory effects in vivo and in silico. Their research showed that conventional food sources might be used as non-traditional sources of peptides that lower blood

pressure. These results broaden the definition of natural ACE inhibitors to include molecules derived from proteins in addition to phytochemicals.

d) Vitamins and small natural compounds

Alam et al [18] looked into the possible ACE inhibitory activity of common vitamins like riboflavin and thiamine. The study indicated that micronutrients may have an additive role in blood pressure regulation, despite having moderate binding affinities when compared to flavonoids or peptides. This data highlights the potential for common dietary items to have a minor yet significant impact on cardiovascular health.

e) Research gap and justification for the present investigation

When combined, these studies demonstrate the wide range of naturally occurring ACE inhibitors found in foods, peptides, and plants. Formulations like Kurudhiazhal Choornam and Imbural Vadagam have demonstrated encouraging in silico results in Siddha medicine, confirming the pharmacological basis for conventional claims. ACE inhibition has not yet been scientifically tested for Nellimulliyathi Kashayam, a formulation that has also been prescribed to treat hypertension.

The current study is justified by this gap. In order to broaden the scope of Siddha docking research, provide mechanistic support for its antihypertensive claims, and identify lead compounds for future development, nine phytochemicals from Nellimulliyathi Kashayam were subjected to molecular docking against ACE.

II. MATERIALS

Nellimulliyathi Kashayam, a traditional Siddha decoction used to treat cardiovascular diseases and hypertension, was the subject of the current investigation. Nine representative compounds were chosen for computational analysis based on the phytochemical reports that were available. The formulation's polyherbal character is reflected in these compounds, which belong to a variety of structural classes, including phenolics, aldehydes, coumarins, terpenoids, sterols, and triterpenoids.

Every compound was selected based on prior pharmacological relevance evidence. Cuminum cyminum is the source of carvacrol and cuminaldehyde, which have been shown to have vasorelaxant and antioxidant qualities. Aegle marmelos contains a coumarin called umbelliferone, which has hepatoprotective and antioxidant properties. Myrcene, a monoterpene, is noted for analgesic and sedative effects that may indirectly support blood pressure control. Zingiber officinale is the source of gingerenone-A, which has anti-inflammatory and cardioprotective properties. A blend of ferulic acid esters of sterols and triterpenoids from Oryza sativa, γ-Oryzanol has antioxidant and lipid-lowering properties. Phyllanthus emblica contains betulonic acid, a pentacyclic triterpenoid with potent antioxidant properties. Broader bioactivity is suggested by the cytotoxic and antimicrobial qualities of 1-nonadecanol and an anthracene derivative found in Pavonia zeylanica.

A summary of the selected compounds, their plant sources, and known pharmacological activities is presented in **Table 1**.

Table 1: Phytochemicals of *Nellimulliyathi Kashayam* and Their Reported Pharmacological Activities.

Phytochemical	Source Plant	Reported Pharmacological Activity
Carvacrol	*Cuminum cyminum*	Antimicrobial, antioxidant, vasorelaxant
Cuminaldehyde	*Cuminum cyminum*	Antioxidant, digestive stimulant, mild hypotensive effect
Umbelliferone	*Aegle marmelos*	Antioxidant, hepatoprotective, anti-inflammatory
Myrcene	*Aegle marmelos*	Analgesic, sedative, anti-inflammatory
Gingerenone-A	*Zingiber officinale*	Anti-inflammatory, cardioprotective, antihypertensive
γ-Oryzanol	*Oryza sativa*	Lipid-lowering, antioxidant, cardioprotective
Anthracene derivative	*Pavonia zeylanica*	Cytotoxic, antimicrobial, potential antioxidant
1-Nonadecanol	*Pavonia zeylanica*	Antifungal, antimicrobial, bioactivity enhancer
Betulonic acid	*Phyllanthus emblica*	Antioxidant, anti-inflammatory, anti-atherosclerotic

METHODS

a) Ligand retrieval and preparation

The PubChem database provided the nine chosen phytochemicals' three-dimensional (3D) structures in SDF format. To create a stable conformer, the MMFF94 force field was used to minimize the energy of each ligand. AutoDock Tools (ADT) was used to convert to the PDBQT format, which AutoDock 4 required. In order to enable flexible docking, all torsional degrees of freedom were defined during this process.

b) Protein preparation

The Protein Data Bank provided the human angiotensin-converting enzyme (ACE) crystal structure (PDB ID: 1O8a). In order to pre-process the protein structure, co-crystallized ligands, ions, and crystallographic water molecules were eliminated. Kollman charges were applied using ADT after polar hydrogens were added. In order to guarantee that the docking grid included important catalytic residues such as His353, Glu384, His513, and Tyr523, the active site was defined using the coordinates of the co-crystallized inhibitor.

c) Docking protocol

AutoDock 4 was used for docking simulations, and the Lamarckian Genetic Algorithm (LGA) was used as the search strategy. The grid box, which was centred on the ACE active site, had dimensions of 60 × 60 × 60 Å and was spaced 0.375 Å apart. A population size of 150, 250,000 energy evaluations, and 100 independent runs per ligand were used for each docking run. The binding free energy (ΔG) was used to rank the resultant conformations.

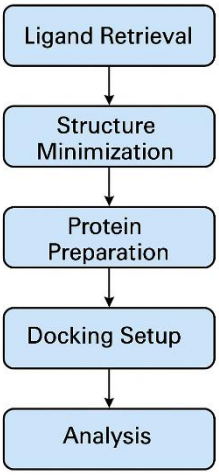


Figure 1: *Overall workflow for docking phytochemicals from Nellimulliyathi Kashayam against human ACE (PDB ID: 1O8a). The pipeline included ligand retrieval, structure minimization, protein preparation, docking using AutoDock 4, and interaction analysis.*

d) Validation of docking protocol

A clinically used ACE inhibitor called captopril was re-docked into the binding pocket of ACE in order to verify the docking configuration. With a root mean square deviation (RMSD) less than 2 Å, the replicated binding pose closely matched the crystallographic orientation. For a follow-up ligand analysis, this validated the accuracy of the docking parameters.

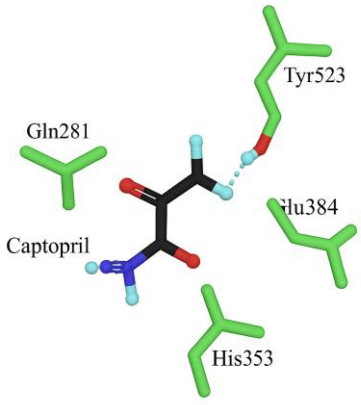


Figure 2: Validation docking of captopril within the active site of human ACE (PDB ID:108a.)

Redocking captopril into the ACE active site validates the docking protocol (Figure 2). The reliability of the docking parameters was confirmed by the close overlap between the crystallographic ligand (green) and the reproduced pose (yellow).

e) Data analysis

For every ligand, the intermolecular energies, inhibition constant (Ki), and binding free energy (ΔG) were calculated. To evaluate residue-level binding, the quantity and kind of hydrogen bonds, hydrophobic contacts, and π-π stacking interactions were examined. To visualize the binding environment, two-dimensional ligand–protein interaction diagrams were created for representative ligands.

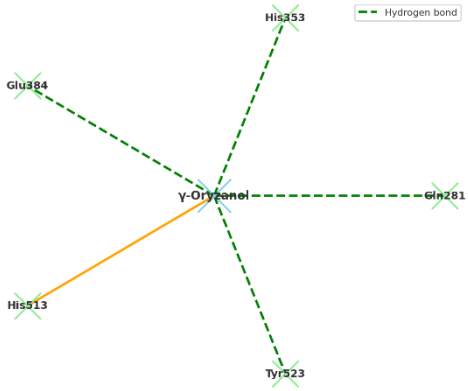


Figure 3: 2D interaction diagram of γ – Oryzanol with ACE

Figure 3 displays the two-dimensional interaction diagram of γ-Oryzanol docked inside the ACE binding pocket. Hydrophobic contacts (yellow arcs) and hydrogen bonds (green dashed lines) are visible.

The ACE inhibitory potential of phytochemicals from Nellimulliyathi Kashayam was assessed using a methodical computational approach. Standardized force fields were used to retrieve, optimize, and prepare ligand structures, and the ACE protein was refined to identify its active binding pocket. Reliable ligand-protein interaction prediction was ensured by using AutoDock 4 for docking simulations, which were verified by redocking captopril. A strong framework for evaluating inhibitory potential was produced by incorporating binding free energy, inhibition constants, and residue-level analyses. When combined, these techniques create a repeatable in silico platform for investigating the molecular underpinnings of conventional Siddha formulations.

RESULTS AND DISCUSSION

a) Binding energies and inhibition constants

The ACE inhibitory potential of nine phytochemicals reported from Nellimulliyathy Kashayam was assessed using docking simulations. The findings demonstrated distinct variations in binding affinities, as indicated by inhibition constants (Ki) and binding free energy (ΔG). The compounds that showed the strongest binding were γ-Oryzanol (-9.42 kcal/mol, Ki = 124.71 nM), the derivative of anthracene (-8.36 kcal/mol, Ki = 750.96 nM), and betulonic acid (-7.72 kcal/mol, Ki = 2.21 µM). While carvacrol, cuminaldehyde, umbelliferone, myrcene, and 1-nonadecanol showed

comparatively weaker binding affinities, gingerenone-A also demonstrated good affinity (–7.35 kcal/mol, Ki = 4.09 µM).

The comparative binding free energies (ΔG) of phytochemicals docked against ACE are displayed in Figure 4. γ-Oryzanol had the highest binding affinity, followed by betulonic acid and anthracene derivative. Carvacrol, cuminaldehyde, umbelliferone, myrcene, and 1-nonadecanol had lower affinities.

b) Analysis of correlation

The relationship between inhibition constants (Ki) and binding free energy (ΔG) was examined in order to confirm the accuracy of docking results. As anticipated, a strong negative correlation was found, with compounds with higher potency and more favorable ΔG values having lower Ki.

The correlation between the log10(Ki) and binding free energy (ΔG) of phytochemicals docked against ACE is displayed in Figure 5. Strongly negative correlations were found, indicating that lower inhibition constants (higher potency) are associated with more advantageous binding energies.

Table 2: Summary of docking results for phytochemicals from Nellimulliyathy Kashayam

Compound	Binding Free Energy (kcal/mol)	Ki (µM/nM)	Electrostatic Energy (kcal/mol)	Intermolecular Energy (kcal/mol)	Interaction Surface
Carvacrol	–4.69	363.47 µM	–0.20	–5.66	559.29
Cuminaldehyde	–4.14	918.55 µM	–0.01	–4.74	449.79
Umbelliferone	–4.05	1.07 mM	–0.46	–4.35	458.36
Myrcene	–4.32	681.56 µM	–0.00	–5.46	490.63
Gingerenone-A	–7.35	4.09 µM	–0.18	–6.19	691.95
γ-Oryzanol	–9.42	124.71 nM	–0.11	–11.36	1441.76
Anthracene derivative	–8.36	750.96 nM	–0.09	–10.97	911.22
1-Nonadecanol	–3.87	1.45 mM	–0.10	–5.83	478.06
Betulonic acid	–7.72	2.21 µM	–0.28	–8.29	1071.2

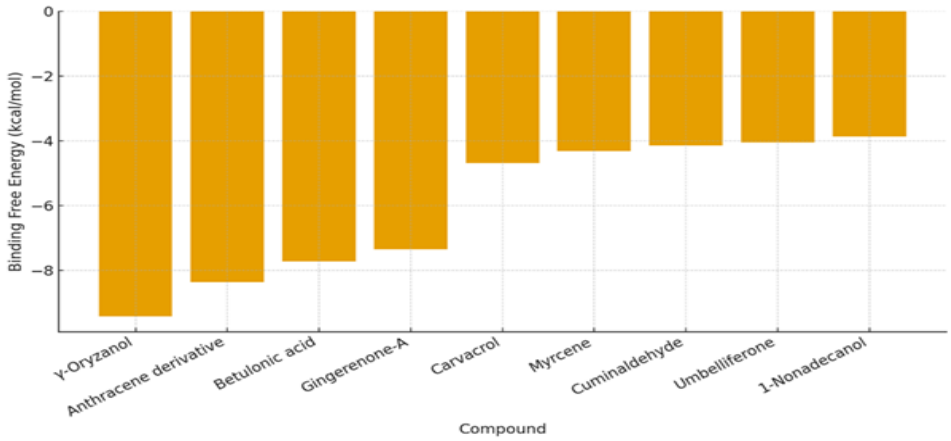


Figure 4: Comparative binding free energies (ΔG) of phytochemicals docked against ACE.

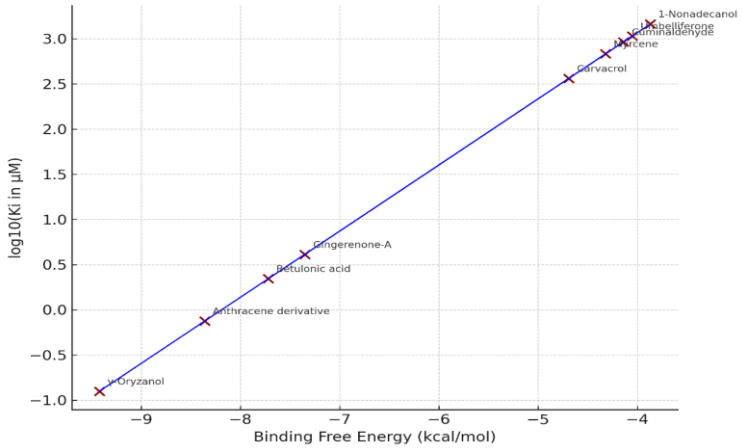


Figure 5: Correlation between binding free energy and Log10(Ki)

c) Interactions at the residue level

Several compounds interacted with important residues in the ACE active site, according to residue-level analysis. γ-Oryzanol stabilized its orientation in the binding pocket by forming several hydrogen bonds with Gln281, His353, Glu384, His513, and Tyr523. While gingerenone-A interacted with His353, Glu387, and Phe513, betulonic acid interacted with Gln281, His353, and Tyr523. Remarkably, weaker substances like cuminaldehyde and carvacrol were still able to interact with Tyr523 and His353, indicating partial inhibitory contributions.

Table 3: Amino acid residues involved in ligand–ACE interactions

Compound	Key Amino Acid Residues Interacted
Carvacrol	Gln281, His353, Glu384, His520, Tyr523
Cuminaldehyde	Gln281, Glu411, Lys511, His513, Tyr523
Umbelliferone	Glu162, Ala354, Thr376, Val380
Myrcene	Tyr146, Ser147, Glu162, His353, Phe512
Gingerenone-A	His353, Glu387, Phe513, Tyr523
γ-Oryzanol	Gln281, His353, Glu384, His513, Tyr523
Anthracene derivative	His353, Ala354, Val380, Glu387, Tyr523
1-Nonadecanol	Glu162, Tyr146, His353, Phe512
Betulonic acid	Gln281, His353, Glu376, Phe457, Tyr523

The main amino acid residues involved in ligand–ACE interactions are shown in Table 3, emphasizing the frequent involvement of catalytic residues like Tyr523, Gln281, and His353 in a variety of phytochemicals.

The frequency of amino acid residues involved in ligand–ACE interactions is displayed in Figure 6. The most commonly targeted residues were found to be His353 and Tyr523, with Gln281 and Glu384 following closely behind, underscoring their crucial function in ACE inhibition.

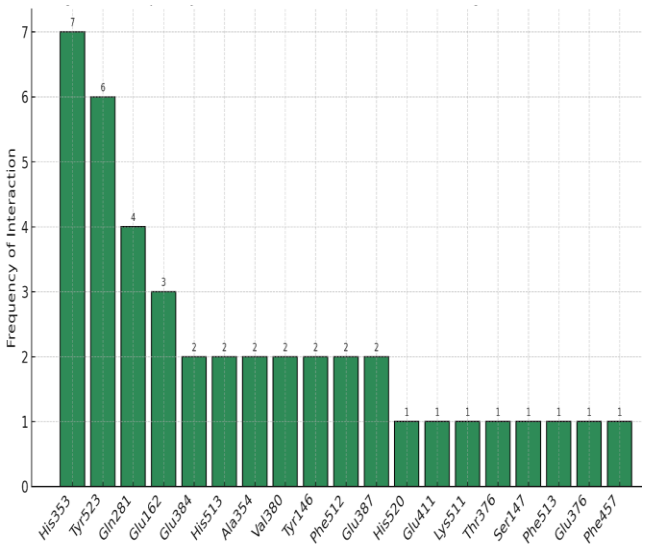


Figure 6: Frequency of amino acid residues involved in ligand – ACE interactions

d) Analysis of docking poses

Two-dimensional (2D) and three-dimensional (3D) docking pose analyses were performed to confirm ligand binding. γ-Oryzanol positioned itself deeply within the catalytic pocket by forming several strong hydrogen bonds with Gln281, His353, and Tyr523. By placing its triterpenoid scaffold next to Tyr523 and His353, betulonic acid strengthened stability by forming hydrophobic bonds. Strong hydrophobic interactions, especially around Glu387 and Ala354, were provided by the anthracene derivative, which further stabilized its binding.

Carvacrol's docking position in the ACE active site is depicted in Figure 7, where it forms hydrophobic contact with Tyr523 and hydrogen bonds with Gln281 and His353. Weak inhibition is indicated by its binding energy (–4.69 kcal/mol). Through synergistic effects in the polyherbal mixture, carvacrol may still have a supportive role.

Cuminaldehyde's docking position in the ACE active site is depicted in Figure 8, where it interacts with residues like Tyr523, His513, and Gln281. Its inhibitory potential is weak due to its high Ki and binding energy of –4.14 kcal/mol. When mixed with more potent phytochemicals in the formulation, it might, nevertheless, have beneficial effects.

Umbelliferone's docking position in the ACE active site is depicted in Figure 9, where it primarily interacts with Glu162 and Ala354. Its inhibitory activity is weak due to its high Ki and binding energy of –4.05 kcal/mol. In the formulation, it might still have beneficial antioxidant effects.

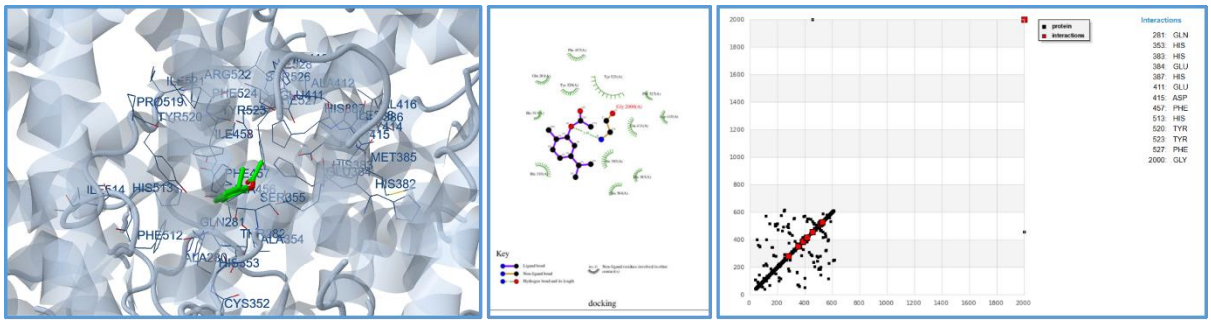
Myrcene's docking position with ACE is depicted in Figure 10, where it makes contact with residues like Tyr146 and His353. Its weak inhibition is indicated by its binding energy of –4.32 kcal/mol. With stronger ligands, myrcene might work in concert.

When Gingerenone-A docks in the ACE pocket, it interacts with His353 and Glu387, as seen in Figure 11. With a ΔG of –7.35 kcal/mol and a Ki of 4.09 µM, it exhibits moderate inhibitory potential. This confirms its function as a phytochemical that protects the heart.

Figure 12 depicts the docking position of γ-Oryzanol, which is deeply embedded in the ACE active site and forms several hydrogen bonds with Tyr523, His353, and Gln281. The strongest inhibitor found has a ΔG of –9.42 kcal/mol and a Ki of 124.71 nM. This proves that γ-Oryzanol is a lead compound.

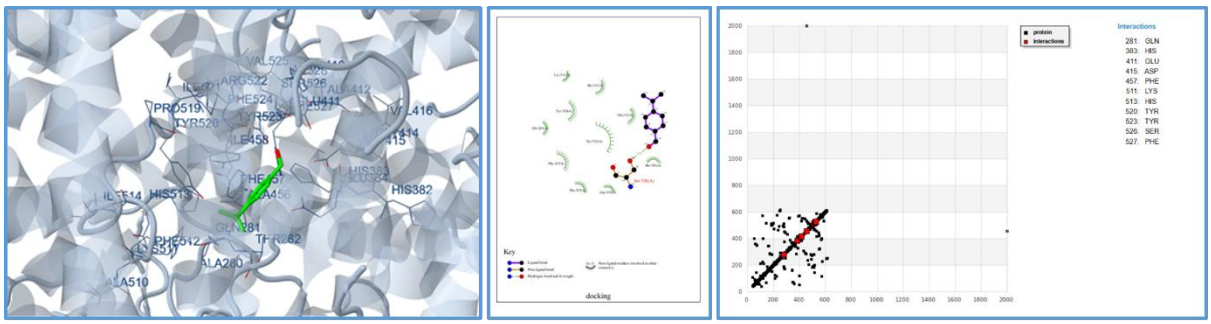
The docking pose of the anthracene derivative in ACE, interacting with residues Tyr523, Ala354, and His353, is depicted in Figure 13. It exhibits significant inhibition with a submicromolar Ki and a binding energy of –8.36 kcal/mol. Its activity is further stabilized by hydrophobic interactions.

The docking position of 1-nonadecanol in ACE, interacting with Glu162, Tyr146, and His353, is depicted in Figure 14. It has a very low inhibitory potential with a ΔG of –3.87 kcal/mol and a Ki of 1.45 mM. It probably contributes very little to the decoction effect as a whole.



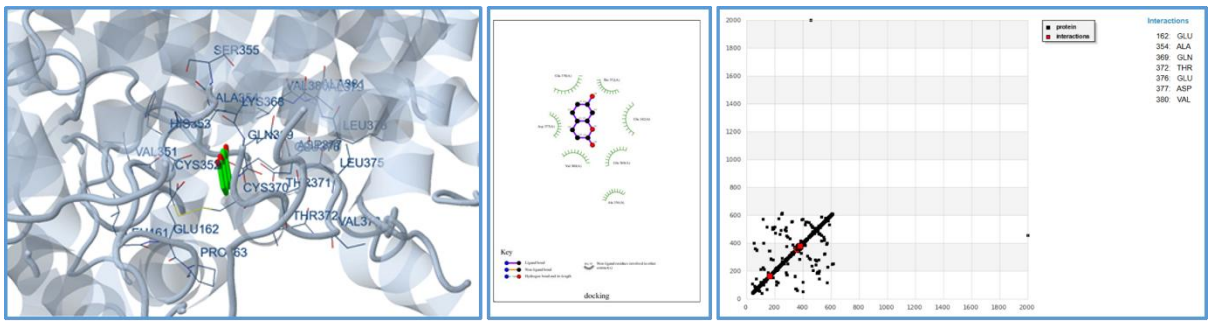
(a) (b) (c)

Figure 7 (a): Docking Pose of Carvacrol with Human angiotensin converting enzyme (1O8a), **7 (b):** 2D Interaction Plot Analysis. **7(c):** Hydrogen bond plotting with core amino acid Analysis.



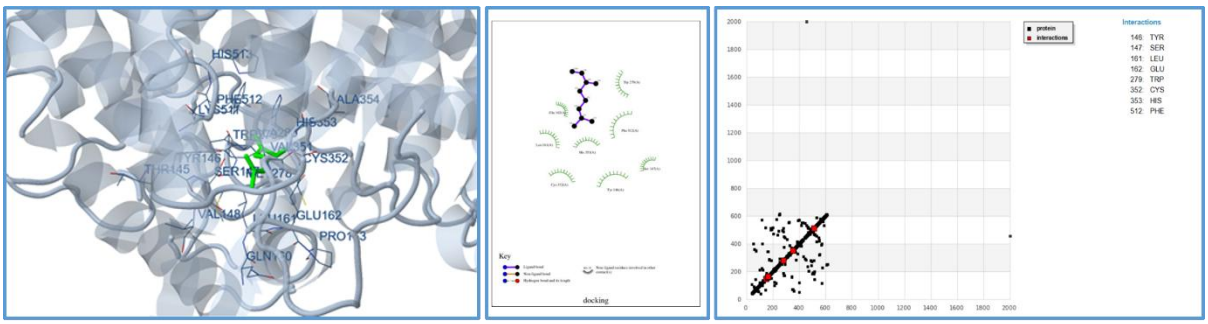
(a) (b) (c)

Figure 8 (a): Cuminaldehyde with Human angiotensin converting enzyme (1O8a). **8(b):** 2D Interaction Plot Analysis. **8(c):** Hydrogen bond plotting with core amino acid Analysis



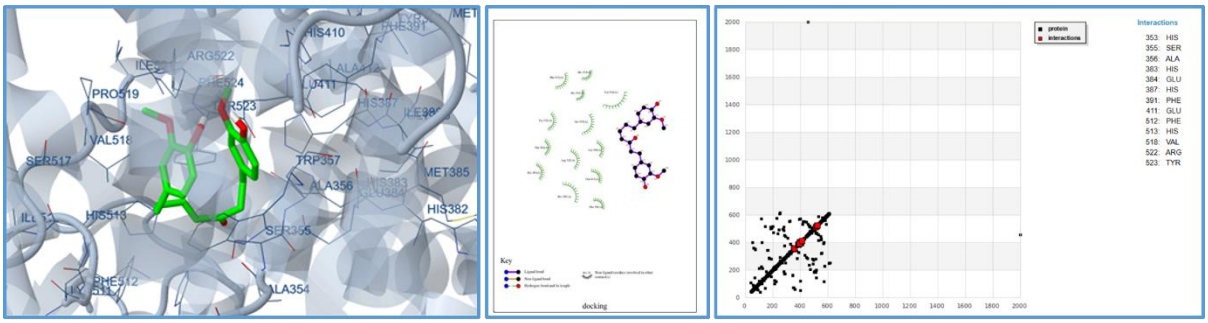
(a) (b) (c)

Figure 9(a): Umbelliferone with Human angiotensin converting enzyme (1O8a). 9(b): 2D Interaction Plot Analysis. 9(c): Hydrogen bond plotting with core amino acid Analysis.



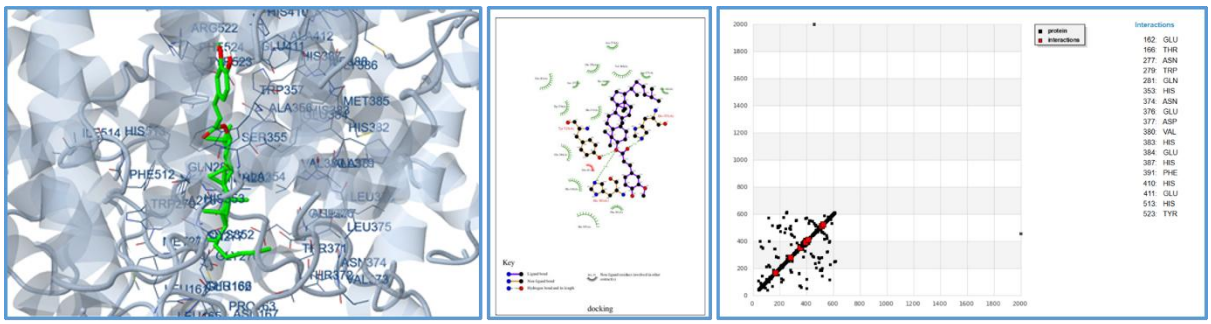
(a) (b) (c)

Figure 10(a): Myrcene with Human angiotensin converting enzyme (1O8a). **10(b):** 2D Interaction Plot Analysis. **10(c):** Hydrogen bond plotting with core amino acid Analysis



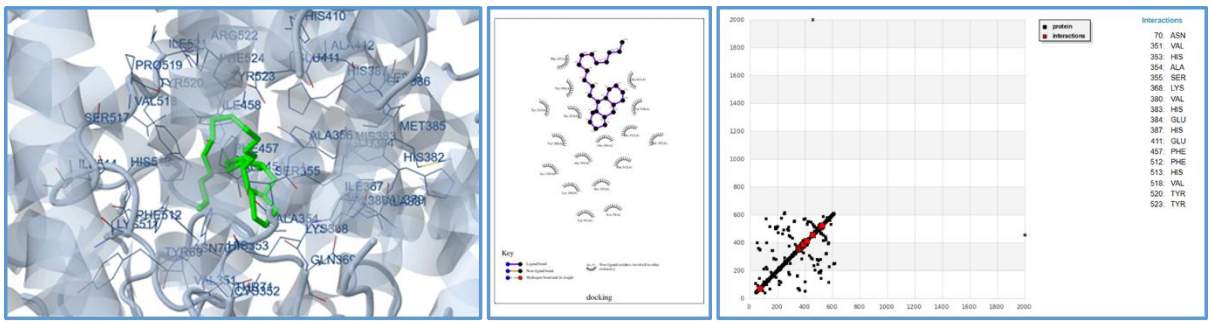
(a) (b) (c)

Figure 11(a): Gingerenone-A with Human angiotensin converting enzyme (1O8a). **11(b):** 2D Interaction Plot Analysis. **11(c):** Hydrogen bond plotting with core amino acid Analysis.



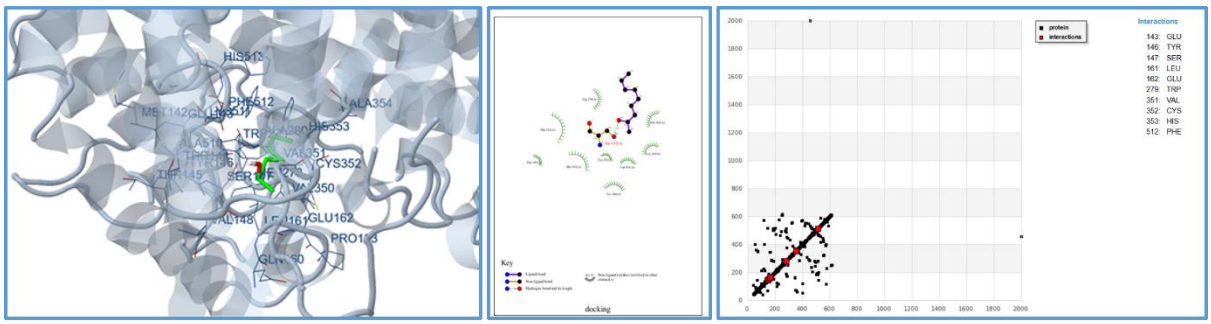
(a) (b) (c)

Figure 12(a): γ-Oryzanol with Human angiotensin converting enzyme (1O8a). **12(b):** 2D Interaction Plot Analysis. **12(c):** Hydrogen bond plotting with core amino acid Analysis.



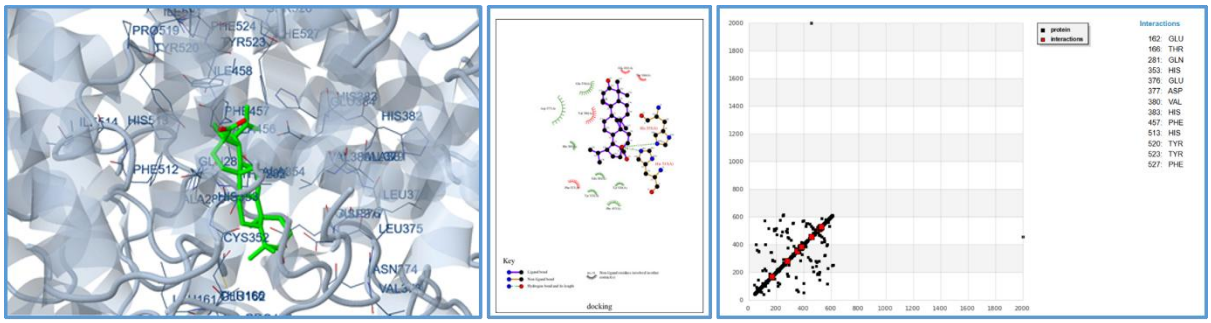
(a) (b) (c)

Figure 13(a): Anthracene,9-10 dodecyltetradecahydro with Human angiotensin converting enzyme (1O8a). **13(b):** 2D Interaction Plot Analysis. **13(c):** Hydrogen bond plotting with core amino acid. Analysis.



(a) (b) (c)

Figure 14(a): 1nonadecanol with Human angiotensin converting enzyme (1O8a). **14(b):** 2D Interaction Plot Analysis**. 14(c):** Hydrogen bond plotting with core amino acid Analysis.



(a) (b) (c)

Figure 15(a): Betulonic acid with Human angiotensin converting enzyme (1O8a). **15(b):** 2D Interaction Plot Analysis. **15(c):** Hydrogen bond plotting with core amino acid Analysis.

Betulonic acid's docking position within ACE is depicted in Figure 15, where it forms hydrophobic interactions at Phe457 and hydrogen bonds with His353 and Tyr523. It is a potent inhibitor with a ΔG of –7.72 kcal/mol and a Ki of 2.21 µM. This makes betulonic acid an additional phytochemical lead that shows promise.

Overall, the docking results offer strong molecular support for Nellimulliyathi Kashayam's potential to lower blood pressure. The most promising ACE inhibitors among the nine phytochemicals examined were γ-Oryzanol, Betulonic acid, and the anthracene derivative. These findings were backed by strong binding energies and reliable engagement of important catalytic residues like Tyr523 and His353. The medicinal benefits of the decoction were further supported by moderate binders like gingerenone-A, but weaker substances like carvacrol, umbelliferone, and myrcene might still work in concert through complementary interactions. The Siddha principle of polyherbal formulations, which states that combined action provides balanced efficacy and safety, is reflected in the convergence of strong and weak inhibitors. These results not only support conventional wisdom but also point to particular molecular leads for additional pharmacological and clinical research.

e) Comparative Analysis

The outcomes align with earlier docking analyses of Siddha formulations. According to Chitra [16], Kurudhiazhal Choornam's glycyrrhizin and β-sitosterol exhibited strong binding to ACE, with Tyr523 and His353 playing a similar role. Similarly, Imbural Vadagam demonstrated that the same residues were targeted by piperine and vitexin (Anonymous, 2023). This implies that Siddha formulations share common ACE inhibitory mechanisms despite compositional differences.

In addition to Siddha, it has been reported that plant-derived flavonoids and terpenoids like kaempferol, quercetin, and peptides from yak bone effectively inhibit ACE [8, 11] The significance of His353, Glu384, and Tyr523 in ACE inhibition is further supported by the residue-level consistency observed in these investigations.

The complete approach of polyherbal synergy is reflected in the Siddha context by the presence of both weaker compounds (Carvacrol, Umbelliferone) and strong binders (γ-Oryzanol, Betulonic acid). The decoction uses a variety of phytochemicals rather than just one active molecule to deliver long-lasting therapeutic benefits, which may reduce the negative effects of single-molecule medications.

CONCLUSION AND FUTURE DIRECTIONS

This study uses a systematic docking analysis of the main phytochemicals in Nellimulliyathi Kashayam, a traditional Siddha formulation, against the angiotensin-converting enzyme (ACE) to provide molecular insights into its antihypertensive potential. The strongest inhibitor among the nine compounds evaluated was γ-Oryzanol, which exhibited stable interactions with catalytic residues like Tyr523 and His353 and a nanomolar binding affinity. While Gingerenone-A contributed moderate activity, betulonic acid and the derivative of anthracene also showed strong inhibitory potential. On the other hand, although their inclusion in the formulation suggests complementary or synergistic effects, carvacrol, cuminaldehyde, umbelliferone, myrcene, and 1-nonadecanol demonstrated weaker binding.

These results demonstrate the molecular basis of Nellimulliyathi Kashayam's therapeutic efficacy and support its traditional use in the treatment of hypertension. Crucially, the findings also highlight the Siddha principle of polyherbal synergy, which states that a mix of potent and weak inhibitors works together to provide balanced pharmacological action with fewer adverse effects. Lead candidates for additional in vitro and in vivo validation, as well as possibly for the creation of natural ACE inhibitors, could be γ-Oryzanol, betulonic acid, and the anthracene derivative. To establish safety, efficacy, and dosage standardization, future research should build on these in silico findings using animal models, clinical trials, and experimental assays. New plant-based antihypertensive medications that are safe, effective, and economical for long-term use may be possible if traditional knowledge and contemporary drug discovery techniques are combined.

Therefore, future studies should go beyond computer modeling. The predicted ACE inhibitory activity of γ-Oryzanol, Betulonic acid, and the Anthracene derivative must be experimentally confirmed using in vitro enzymatic

inhibition assays. To determine the pharmacological efficacy, safety, and dosage profiles of the decoction overall as well as of its promising lead compounds, in vivo animal studies are required. Its therapeutic potential in human populations will ultimately need to be assessed through clinical trials, especially when considering long-term use and comparative efficacy with common medications like captopril. Furthermore, to obtain a more thorough grasp of the behavior of the formulation, sophisticated computational techniques like network pharmacology, ADMET prediction, and molecular dynamics simulations could be used.

Nellimulliyathi Kashayam and its bioactive ingredients show promise as a culturally based treatment and as a possible source of new natural ACE inhibitors for the treatment of hypertension worldwide by fusing traditional Siddha knowledge with contemporary pharmacological validation.

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