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RESEARCH ARTICLE

# *In Silico* Computational Analysis of *Citrus aurantium* of *Thurunji Manapagu* in Inhibiting the Receptor Target of Angiotensin-converting Enzyme Against *Uratha Pitha Vatham* (Hypertension)

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## Abstract

*Citrus aurantium* (*Thurunji*) is a tree cultivated in various parts of the world. The fruit pulp is used to make juices, marmalade and pickles. It is commonly used by tribes to treat digestive disorders, constipation, heaviness in the chest, prolapse of the uterus, anorexia, chest pain, cold, and cough. In the Siddha system of medicine, *Citrus aurantium* fruit pulp is used to prepare a formulation, syrup in consistency named *Thurunji manapaagu* indicated for diseases due to deranged *pitha* humor. According to the Siddha system of medicine Hypertension termed *Uratha Pitha Vatham* is an ailment that occurs due to an elevated *pitham*. Considering the facts an attempt of *in silico* docking study was done with the selected phytochemicals such as Linalool, Apigenin, Ichangin, Kaempferol, Luteolin, Limonin, Limonene, Myrcene, Diosmetin and Tangeretin of *Citrus aurantium* against Angiotensin Converting Enzyme (ACE) and the results are detailed in this article.

**Keywords:** Antihypertensive, *Citrus aurantium,* Molecular Docking, Siddha Medicine, *Uratha Pitha Vatham*

## 1. Introduction

*Citrus aurantium* (Bitter orange), also called Seville orange or sour orange, is an evergreen tree cultivated all over the world. It bears spherical fruits with a thick, orange or sometimes green peel. The juicy pulp tastes sweet or sour, is orange in colour used in the treatment of gastrointestinal problems, abdominal distension heaviness in the chest, uterine prolapse, treatment of anorexia, chest pains, colds, coughs, etc. The fruit and other parts of the plant are being used in traditional systems of medicines for the cure of different ailments. The phytochemical composition of *C. aurantium* includes vitamins, minerals, phenolic compounds, and terpenoids play a major role in its health-promoting effects[1-4]. The fruit pulp is rich in phytocomponents (Limonoid glucosides) such as limonin, nomilinin, nomilin, obacunone, ichangin, and flavanols kaempferol, quercetin, free flavones such as apigenin, luteolin, tangeretin and diosmetin[5-8].

The pharmacological profile of the fruit indicates limonene, protective against lesions induced by ethanol and nonsteroidal anti-inflammatory drugs in rats by increasing the production of gastric mucus[9].

*In vitro* assays had proved the antimicrobial activity of *C. aurantium* juice against *Salmonella species* and *Listeria monocytogenes.* The antioxidant, cytotoxic, anxiolytic, and antidiabetic effects of *C.*

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*aurantium* juice were established by various studies. It has been also used for weight loss and to enhance sports performance[10-16]. A drink called Zhi-Zi-Hou-Po (ZZHPD) prepared with *C. aurantium, Gardenia jasminiodes* and *Magnolia officinalis* has been used to treat depression-like symptoms[17].

### 1.1 *Uratha Pitha Vaatham*

The symptoms of Hypertension are more similar to those of *Uratha Pitha Vatham* elaborated in Siddha literature *Yugi vaithiya Cinthamani* – 800. *Uratha pitha vaatham* is one among the 42 types of *azhal noi* that arise due to the increased azhal kutram caused by the heat generated in the abdomen and moved to the neck region, which affects the healthiness of blood. The *azhal kutram* is affected due to increased intake of sour, spicy and salty foods, frequent anger, mental stress, insomnia, eating of undercooked food and exposure to heat like flame and sunlight.

According to Siddha's basic principles, human beings are incorporated with 96 principles *(thathuvangal)*, which include physical, functional, psychological and intellectual components and the diagnosis is based on Seven physical constituents (*Ezhu udal thathukal),* Eight investigatory tools *(Envagai thervu)*, Three vital forces *(Mukkuttram)*. In *Uratha pitha vaatham* - the *azhal kuttram (Anar pitham, Saadhaga pitha, Prasaga pitham, Aalosaga pitham)* gets affected which continuously tends to affect the *vali kuttram (Uyirkaal, Paravukaal, Nadukaal)* and as a result will increase the blood pressure[18].

### 1.2 Hypertension

Hypertension is the increase in Blood pressure, known to end in cardiovascular and renal complications[19]. It was estimated that 1.28 billion adults aged 30-79 have hypertension globally. Considering this fact, the World Health Organization (WHO) released a new guideline regarding the pharmacological management of hypertension in adults during 2021[20].

*Thurinji manapaagu* is a Siddha formulation cited in the traditional literature book *Siddha vaithiya thirattu* used to treat the *Pitha thodam* and diseases preceding the *Pitha thodam*[21]. *Thurunji* (*Citrus aurantianum*) belongs to the family Rutaceae. Besides vitamin C, flavonoids and volatile oil, the other phytoconstituents of citrus fruits are phenethylamine alkaloids like octopamine, synephrine, tyramine, etc. Fruit extract of *Citrus aurantium* has astringent, antioxidant, carminative and spasmolytic activity. It is used in gastrointestinal disorders, sleep disturbances, cardiovascular diseases and cancer treatments[22].

The Renin Angiotensin System (RAS) cascade is responsible for the regulation of blood pressure[23]. Therapeutic agents that can block RAS initiation through inhibition of ACE, angiotensin receptor inhibitors and renin inhibitors are commonly used in Hypertension management[24].

Molecular docking or ligand-protein docking is usually performed between a small molecule and a target macromolecule. It has a variety of applications in drug discovery, including structural activity study, lead optimization, discovery of potential leads to facilitate predictions in mutagenesis studies, assisting X-ray crystallography, and designing chemical mechanisms[25]. Considering the facts mentioned, selected phytochemicals of *Thurunji* (*Citrus aurantium*) were subjected to molecular docking and the outcome is discussed in this article.

## 2 Materials and Methods

### 2.1 Prepation of *Thurunji Manapaagu*

The investigational drug *Thurunji Manapaagu* is an herbal formulation prepared using fresh fruit juice of *Citrus aurantium* and sugar obtained from *Saccharum officianlae*. The juice is extracted from *Citrus aurantium* fruit, filtered and sugar added in the ratio1:2 boiled to *Manapaagu* (syrup) consistency[21].

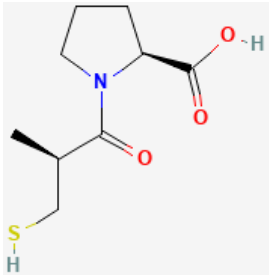
### 2.2 *In silico* Method - Docking Study

Molecular docking was done computationally to identify the architecture of compounds generated by two or more distinct molecules. It plays a critical role in rational drug design by anticipating the alignment of small molecules of therapeutic compounds, their affinity and activity[26]. Preferring with minimal side effects, use of phytochemicals has emerged as one of the holistic alternative approaches[27].

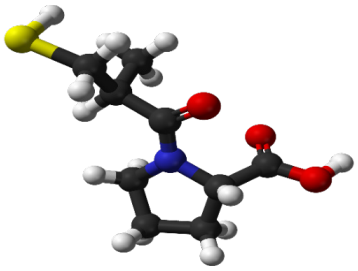
Screening molecules with potential bioactivity is quite costly and may consume time, which could be overcome by Computer-Aided Drug Design (CADD). In recent years, chemoinformatics, molecular docking, and artificial intelligence have considerably increased in designing a drug, its development and discovery[28,29]. *In silico* molecular docking virtually predicts the binding efficacy and the structure-based drug design[30]. It also

provides details on structure-activity relationships, mode of action, and analysis of interaction between protein and ligand[30]. Therefore, *in silico* analysis would result in providing better chances of discovering suitable drug candidates in considerably short duration and less expense. Nowadays, several molecular docking approaches based on molecular structure and ligands are available to facilitate drug discovery[28,30].

### 2.2.1 Retrieval of Drug Compound-Captopril




**2D**



**3D**

**Figure 1.** 2D and 3D structure models of drug compound - Captopril.

Captopril (C9H15NO3S) (structure given in Figures 1 and 2) is an analogue of proline that possesses antihypertensive and antineoplastic activity by competitively inhibiting ACE and reduces the level of Angiotensin II by promoting plasma renin activity and suppressing aldosterone secretion.

### 2.2.2 Retrieval of Phytochemical Data

Phytochemical data of components such as Linalool, Limonene, Myrcene, Apigenin, Ichangin, Kaempferols, Luteolin, Diosmetin Limonin, Tangeretin are retrieved from published articles[31] using search engines, IMPPAT (Indian Medicinal Plants, Phytochemistry and Therapeutics). IMPPAT is a database that contains the 2D and 3D structures of phytochemical constituents present in Indian medicinal plants.

**Captopril**

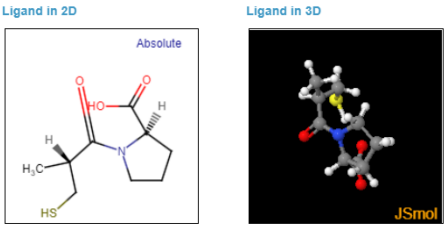



**Figure 2.** 2D, 3D pictures of standard Captopril.

### 2.2.3 Ligand Preparation

A literature survey conducted using the search engines PubMed and Google Scholar to retrieve the phytochemicals present in the drug to be subjected to docking, and ten lead phytocomponents were identified and retrieved (Table 1). The 3D structure of the ligands was built using ChemDraw prof online tool version 12.0. The ligands were prepared with 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 of the standard drug captopril are shown in Table 1.

Table 2 represents 2D and 3D pictures of selected ligands of *Cirtus aurantium*.

### 2.2.4 Protein Receptor Preparation

Three dimensional protein structure of target protein ACE( PDB)1086 given in Figure 3 was retrieved from the online repository of Protein Data Bank (www.rcsb.org/pdb) and subjected to a protein cleanup process before docking simulation. Essential missing hydrogen atoms Kollman charges were added using the MGL AutoDock Tools 1.5.7, and the statistical distribution of the combinations of the amino acid backbone dihedral Ø (Phi) angles and ψ (Psi) angle were identified by subjecting to Ramachandran plot analysis using Rampage. Before the docking simulation, the water molecules were removed, and the protein clean geometry procedure was done. Orientation of the lead molecules concerning the target protein and their best dock pose was selected based on the interaction study analysis using the Auto dock version 4 program[32].

### 2.2.5 Molecular Docking

To define the rotatable bonds, Gasteiger partial charges were added, and non-polar hydrogen atoms were merged

**Table 1.** Ligand properties of selected phytochemicals for docking against ACE (1O86) and captopril

| S. No. | Compound | Molar weight g/mol | Molecular Formula | H Bond Donor | H Bond Acceptor | Rotatable bonds |
|---|---|---|---|---|---|---|
| 1. | Linalool | 154.25 g/mol | C10H18O | 1 | 1 | 4 |
| 2. | Limonene | 136.23 g/mol | C10H16 | 0 | 0 | 1 |
| 3. | Myrcene | 136.238 g/mol | $C_{10}H_{16}$ | 0 | 0 | 4 |
| 4. | Ichangin | 488.5 g/mol | C26H32O9 | 2 | 9 | 2 |
| 5. | Kaempferol | 286.239 g/mol | $C_{15}H_{10}O_6$ | 4 | 6 | 1 |
| 6. | Luteolin | 286.24g/mol | $C_{15}H_{10}O_6$ | 4 | 6 | 1 |
| 7. | Diosmetin | 300.26 g/mol | C16H12O6 | 3 | 6 | 2 |
| 8. | Limonin | 470.5 g/mol | C26H30O8 | 0 | 8 | 1 |
| 9. | Tangeretin | 372.4 g/mol | C20H20O7 | 0 | 7 | 6 |
| 10. | Captopril | 217.29 g/mol | C9H15NO3S | 2 | 4 | 3 |

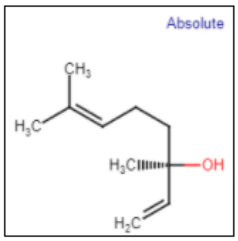
Ligand Properties of the Compounds selected for docking against Acetyl Cholinesterase (1B41)

**Table 2.** 2D and 3D structure of selected ligands of *Citrus aurantium* compounds

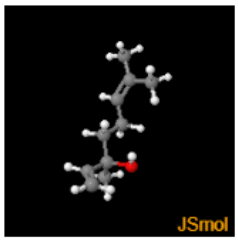
**2D and 3D Structure of selected phytocomponents**

**Linalool**

Ligand in 2D

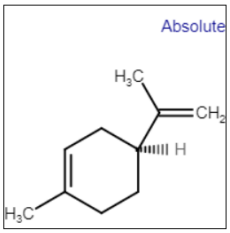



Ligand in 3D

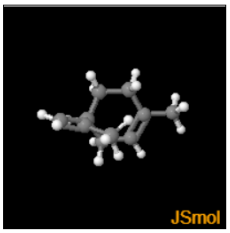



**Limonene**

Ligand in 2D

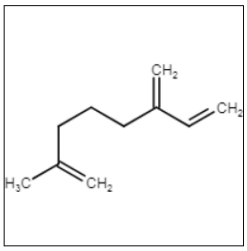



Ligand in 3D

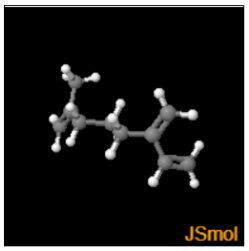



**Myrce**

Ligand in 2D

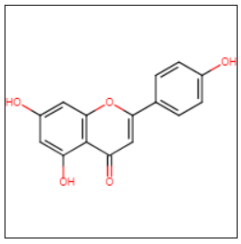



Ligand in 3D




**Apigenin**

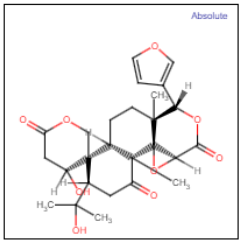
Ligand in 2D



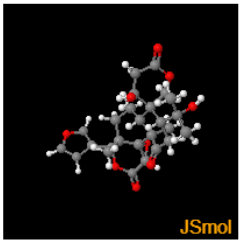

Ligand in 3D


**Ichangin**

Ligand in 2D

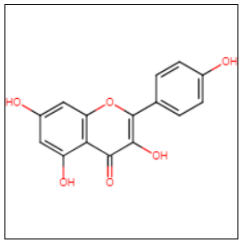



Ligand in 3D




**Kaempferols**

Ligand in 2D




Ligand in 3D

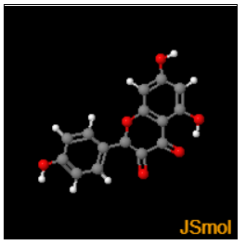
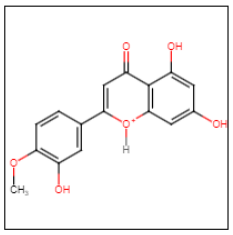
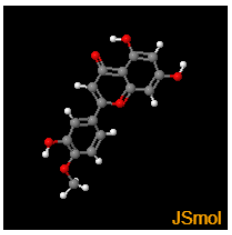
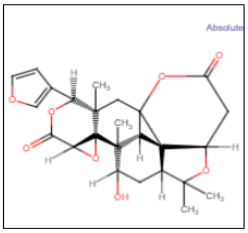
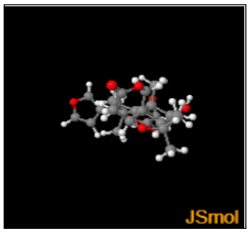
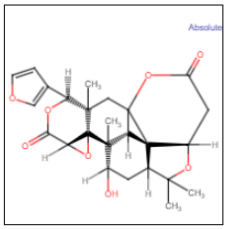
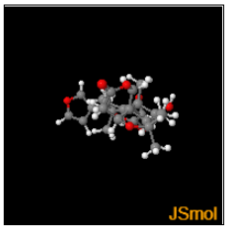
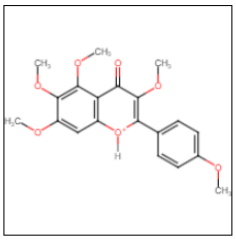
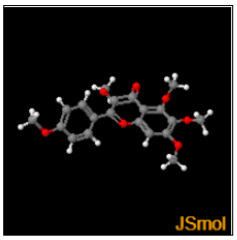


**Table 2.** *to be continued...*

| | |
|---|---|
| **Luteolin**<br>Ligand in 2D<br>  <br>Ligand in 3D<br>  | **Diosmetin**<br>Ligand in 2D<br>  <br>Ligand in 3D<br>  |
| **Limonin**<br>Ligand in 2D<br>  <br>Ligand in 3D<br>  | **Tangeretin**<br>Ligand in 2D<br>  <br>Ligand in 3D<br>  |

**Human Angiotensin Converting Enzyme (1O86)**

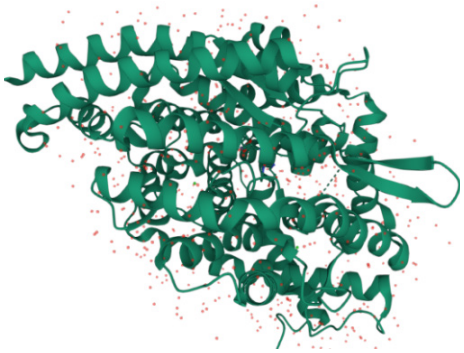


**Figure 3.** 3D protein structure of Human angiotensin converting enzyme (1O86).

with ligand atoms. The retrieved phytocomponents Linalool, Apigenin, Ichangin, Kaempferol, Luteolin, Limonin, Limonene, Myrcene, Diosmetin and Tangeretin were subjected to docking calculations against ACE using AutoDock 4. With the aid of the AutoDock 4 virtual screening tool, required parameters were added, and with the help of the Autogrid program, Affinity (grid) maps with 60 × 60 × 60 Å grid points, 0.375 Å spacing were generated. To calculate the van der Waals and the electrostatic terms, the AutoDock parameter set- and distance-dependent dielectric functions were used. The Lamarckian genetic algorithm and the Solis and Wets local search method were used for docking simulations and Torsions were released, setting the Initial position, orientation, and torsions of the ligand molecules randomly. Each docking experiment was subjected to two different runs that were fixed to terminate after energy evaluations of 250,000 with a population size of 150 and applying a translational step of 0.2 Å, and quaternion and torsion steps of 5[33].

### 2.2.6 Rigid Docking

To get a group of permissible orientations and conformations for the ligand at the binding site. Computational docking was carried out at the active site of spike protein of the selected phytocomponents withMGL AutoDock Tool-Autodock Vina 1.1.2.

### 2.2.7 Visualization

The Discovery Studio Visualizer program version 2016 was used to analyse the interaction of hydrogen bonds and aminoacids.

## 3. Results

Ten bioactive lead compounds of *Citrus aurantium* fruit, such as Linalool, Apigenin, Iehangin, Kaempferol, Luteolin, Limonin and Tangeretin were retrieved. Their interactions with the receptor site of amino acid residues present on ACE was determined. The details are tabulated in Tables 3-5.

**Table 3.** Summary of *in silico* analysis of phytocompounds against ACE (1O86)

| Compounds | Binding Free energy Kcal/mol | Inhibition constant Ki µM (*mM)(**nM) | Electrostatic energy Kcal/mol | Intermolecular energy Kcal/mol | Total Interaction Surface |
|---|---|---|---|---|---|
| Linalool | -4.04 kcal/mol | 1.10 mM | -0.16 kcal/mol | -5.49 kcal/mol | 534.936 |
| Limonene | -4.45 kcal/mol | 546.45 µM | -0.02 kcal/mol | -4.75 kcal/mol | 436.552 |
| Myrcene | -4.38 kcal/mol | 616.81 µM | -0.01 kcal/mol | -5.52 kcal/mol | 480.415 |
| Apigenin | -5.10 kcal/mol | 182.65 µM | -0.74 kcal/mol | -6.60 kcal/mol | 731.524 |
| Ichangin | -8.45 kcal/mol | 639.78 µM | -0.11 kcal/mol | -7.62 kcal/mol | 1049.019 |
| Kaempferol | -5.34 kcal/mol | 121.82 µM | -0.10 kcal/mol | -5.71 kcal/mol | 704.237 |
| Luteolin | -6.66 kcal/mol | 13.17 µM | -0.60 kcal/mol | -6.29 kcal/mol | 707.988 |
| Diosmetin | -6.30 kcal/mol | 24.25 µM | -0.18 kcal/mol | -6.61 kcal/mol | 650.991 |
| Limonin | -7.23 kcal/mol | 5.01 µM | -0.13 kcal/mol | -7.47 kcal/mol | 1022.766 |
| Tangeretin | -5.00 kcal/mol | 216.20 µM | -0.17 kcal/mol | -6.53 kcal/mol | 829.921 |
| Captopril | -5.09 kcal/mol | 186.95 µM | -0.61 kcal/mol | -5.13 kcal/mol | 504.357 |

**Table 4.** Interaction of amino acid residue of phytochemicals and captopril ACE

| S. No. | Protein | Phyto chemical | Affinity | RMSD (I. B.) | RMSB (U. B.) | No. of Hydrogen Bonds | Ligand – Allele Interaction (Non-bound) | Distance | Category |
|---|---|---|---|---|---|---|---|---|---|
| a | ACE2 | Linalool | -5.3 | 9.227 | 11.55 | 1 | :UNL1:H - A:GLN102:O | 1.99989 | Hydrogen Bond |
| | | | | | | | :UNL1 - A:LEU73 | 4.88994 | Hydrophobic |
| | | | | | | | :UNL1 - A:LEU100 | 5.49351 | Hydrophobic |
| | | | | | | | :UNL1:C - A:ALA99 | 4.48489 | Hydrophobic |
| | | | | | | | :UNL1:C - A:LEU100 | 4.68179 | Hydrophobic |
| | | | | | | | :UNL1:C - A:LEU391 | 4.93524 | Hydrophobic |
| b | ACE2 | Limonene | -5.3 | 37.54 | 38.868 | 0 | :UNL1:C - A:LEU95 | 4.65071 | Hydrophobic |
| | | | | | | | :UNL1:C - A:VAL209 | 5.16234 | Hydrophobic |
| | | | | | | | :UNL1:C - A:VAL212 | 4.44133 | Hydrophobic |
| | | | | | | | :UNL1:C - A:PRO565 | 4.6178 | Hydrophobic |
| | | | | | | | :UNL1:C - A:VAL209 | 4.81661 | Hydrophobic |
| | | | | | | | A:LEU95 - :UNL1 | 4.34903 | Hydrophobic |
| | | | | | | | A:VAL209 - :UNL1 | 4.05884 | Hydrophobic |
| | | | | | | | A:PRO565 - :UNL1 | 4.4849 | Hydrophobic |
| | | | | | | | A:TRP566 - :UNL1:C | 4.92793 | Hydrophobic |
| c | ACE2 | Myrcene | -5.1 | 50.354 | 51.875 | 0 | :UNL1 - A:LEU95 | 4.86109 | Hydrophobic |
| | | | | | | | :UNL1:C - A:LEU95 | 4.13041 | Hydrophobic |
| | | | | | | | :UNL1:C - A:VAL209 | 4.48599 | Hydrophobic |
| | | | | | | | :UNL1:C - A:PRO565 | 3.93989 | Hydrophobic |
| | | | | | | | A:LYS562 - :UNL1 | 4.96802 | Hydrophobic |

**Table 4.** *to be continued...*

| S. No. | Protein | Phyto chemical | Affinity | RMSD (I. B.) | RMSB (U. B.) | No. of Hydrogen Bonds | Ligand – Allele Interaction (Non-bound) | Distance | Category |
|---|---|---|---|---|---|---|---|---|---|
| d | ACE2 | Apigenin | -7.4 | 34.271 | 37.007 | 4 | :UNK0:H - A:ASP509:OD2 | 2.76684 | Hydrogen Bond |
| | | | | | | | A:SER511:HN - :UNK0:O | 1.95281 | Hydrogen Bond |
| | | | | | | | A:SER511:HG - :UNK0:O | 2.47835 | Hydrogen Bond |
| | | | | | | | A:ARG514:HH12 - :UNK0:O | 2.68592 | Hydrogen Bond |
| | | | | | | | :UNK0 - A:TRP203 | 5.54802 | Hydrophobic |
| | | | | | | | A:TRP203 - :UNK0 | 4.6941 | Hydrophobic |
| | | | | | | | A:TRP203 - :UNK0 | 4.29446 | Hydrophobic |
| | | | | | | | A:GLY205:C,O; ASP206:N - :UNK0 | 4.43928 | Hydrophobic |
| e | ACE2 | Ichangin | -9.2 | 0 | 0 | 6 | :UNL1:H - A:ASP382:OD1 | 2.42224 | Hydrogen Bond |
| | | | | | | | :UNL1:H - A:ASP382:OD1 | 2.83962 | Hydrogen Bond |
| | | | | | | | :UNL1:H - A:TYR385:OH | 3.09578 | Hydrogen Bond |
| | | | | | | | A:TYR385:HH - :UNL1:O | 2.83545 | Hydrogen Bond |
| | | | | | | | A:ASN394:HD21 - :UNL1:O | 2.66288 | Hydrogen Bond |
| | | | | | | | A:HIS401:HE2 - :UNL1:O | 2.48791 | Hydrogen Bond |
| | | | | | | | :UNL1:C - A:HIS401 | 3.97227 | Hydrophobic |
| | | | | | | | :UNL1:C - A:HIS401 | 3.83153 | Hydrophobic |
| f | ACE2 | Kaempferols | -8.9 | 8.524 | 13.03 | 9 | :UNK0:H - A:ASP206:OD1 | 2.43929 | Hydrogen Bond |
| | | | | | | | :UNK0:H - :UNK0:O | 1.7923 | Hydrogen Bond |
| | | | | | | | :UNK0:H - A:SER511:OG | 2.71878 | Hydrogen Bond |
| | | | | | | | :UNK0:H - A:ASP509:OD2 | 2.55348 | Hydrogen Bond |
| | | | | | | | A:TYR199:HH - :UNK0:O | 2.61501 | Hydrogen Bond |
| | | | | | | | A:SER511:HN - :UNK0:O | 1.8683 | Hydrogen Bond |
| | | | | | | | A:SER511:HG - :UNK0:O | 2.59959 | Hydrogen Bond |
| | | | | | | | A:ARG514:HH12 - :UNK0:O | 2.59917 | Hydrogen Bond |
| | | | | | | | A:ASP206:OD1 - :UNK0 | 4.42701 | Electrostatic |
| | | | | | | | :UNK0 - A:TRP203 | 5.56745 | Hydrophobic |
| | | | | | | | A:TRP203 - :UNK0 | 4.85631 | Hydrophobic |
| | | | | | | | A:TRP203 - :UNK0 | 4.33686 | Hydrophobic |
| g | ACE2 | Luteolin | -8.2 | 15.289 | 17.927 | 5 | :UNL1:H - :UNL1:O | 1.80092 | Hydrogen Bond |
| | | | | | | | :UNL1:H - A:GLU398:O | 2.29594 | Hydrogen Bond |
| | | | | | | | :UNL1:H - A:ALA348:O | 2.69512 | Hydrogen Bond |
| | | | | | | | :UNL1:H - A:ASP382:OD2 | 2.80261 | Hydrogen Bond |
| | | | | | | | A:ARG514:HE - :UNL1:O | 2.13238 | Hydrogen Bond |
| | | | | | | | A:HIS401:NE2 - :UNL1 | 3.6884 | Electrostatic |
| | | | | | | | A:HIS401 - :UNL1 | 5.2828 | Hydrophobic |
| | | | | | | | A:HIS401 - :UNL1 | 3.79672 | Hydrophobic |

**Table 4.** *to be continued...*

| S. No. | Protein | Phyto chemical | Affinity | RMSD (I. B.) | RMSB (U. B.) | No. of Hydrogen Bonds | Ligand – Allele Interaction (Non-bound) | Distance | Category |
|---|---|---|---|---|---|---|---|---|---|
| h | ACE2 | Diosmetin | -8.2 | 13.366 | 15.315 | 7 | :UNL1:H - :UNL1:O | 1.80115 | Hydrogen Bond |
| | | | | | | | :UNL1:H - A:ASN394:OD1 | 2.7819 | Hydrogen Bond |
| | | | | | | | :UNL1:H - A:ASP206:OD1 | 2.67978 | Hydrogen Bond |
| | | | | | | | A:GLY395:HN - :UNL1:O | 2.63704 | Hydrogen Bond |
| | | | | | | | A:ARG514:HE - :UNL1:O | 2.93816 | Hydrogen Bond |
| | | | | | | | A:ARG514:HH21 - :UNL1:O | 2.26149 | Hydrogen Bond |
| | | | | | | | A:HIS401:CD2 - :UNL1:O | 3.46778 | Hydrogen Bond |
| | | | | | | | A:ASN397:C,O;GLU398: N - :UNL1 | 4.62598 | Hydrophobic |
| | | | | | | | A:ASN397:C,O;GLU398: N - :UNL1 | 4.16251 | Hydrophobic |
| i | ACE2 | Limonin | -8.9 | 1.8 | 7.878 | 0 | :UNL1:C - A:PRO415 | 4.95867 | Hydrophobic |
| | | | | | | | :UNL1:C - A:ILE291 | 5.03567 | Hydrophobic |
| | | | | | | | A:ILE291 - :UNL1 | 4.1403 | Hydrophobic |
| | | | | | | | A:PRO415 - :UNL1 | 4.97759 | Hydrophobic |
| | | | | | | | A:PHE438 - :UNL1 | 4.50987 | Hydrophobic |
| | | | | | | | A:PHE438 - :UNL1:C | 5.03793 | Hydrophobic |
| j | ACE2 | Tangeretin | -8.3 | 3.225 | 8.494 | 6 | A:GLN102:HE22 - :UNL1:O | 2.19866 | Hydrogen bond |
| | | | | | | | A:TYR196:HH - :UNL1:O | 2.411 | Hydrogen bond |
| | | | | | | | A:LYS562:HZ1 - :UNL1:O | 2.2789 | Hydrogen bond |
| | | | | | | | :UNL1:C - A:ASP206:O | 3.5189 | Hydrogen bond |
| | | | | | | | :UNL1:C - A:GLN102:OE1 | 3.47682 | Hydrogen bond |
| | | | | | | | A:ASN210:HN - :UNL1 | 3.23243 | Hydrogen bond |
| | | | | | | | :UNL1:C - A:LEU95 | 5.12456 | Hydrophobic |
| | | | | | | | :UNL1:C - A:VAL209 | 3.73609 | Hydrophobic |
| | | | | | | | :UNL1:C - A:VAL212 | 4.66831 | Hydrophobic |
| | | | | | | | :UNL1:C - A:PRO565 | 3.659 | Hydrophobic |
| | | | | | | | :UNL1 - A:LEU95 | 4.88785 | Hydrophobic |
| | | | | | | | :UNL1 - A:VAL209 | 4.93861 | Hydrophobic |

Table 4 shows the amino acid residue interaction of (a) Linalool, b) Limonene, c) Myrcene, d) Apigenin, e) Ichangin, f) Kaempferol, g) Luteolin, h) Diosmetin, i) Limonin, j) Taneretin and captopril against the structure of ACE in crystal form.

## 4. Discussion

Hypertension is one of the non-communicable diseases in high prevalence in our country, causing complications of the cardiovascular system and also a

**Table 5.** 2D interaction plot analysis and docking pose of selected phytochemicals present in *Citrus aurantium* with ACE

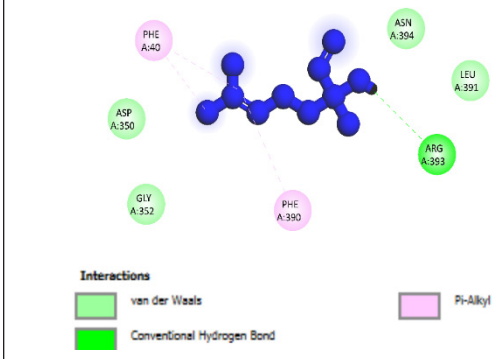
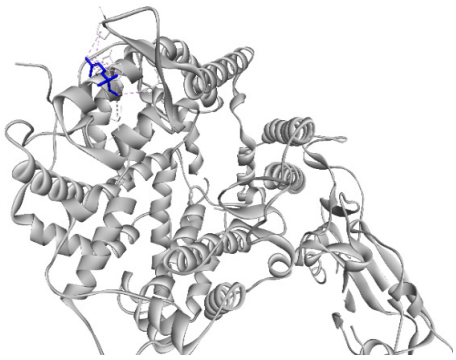
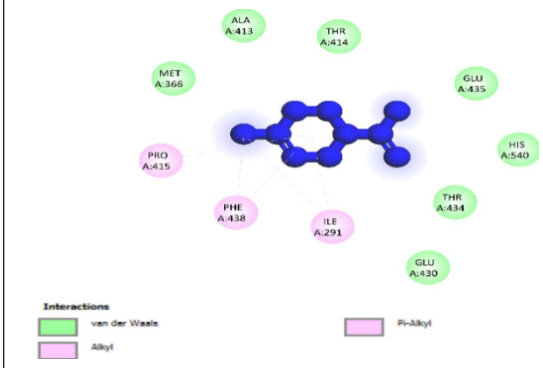
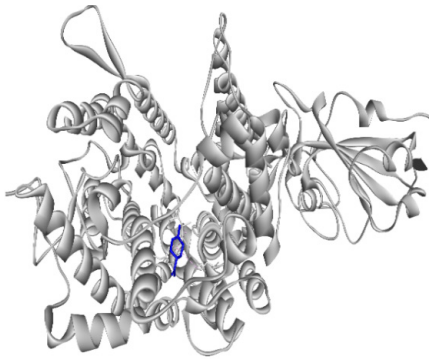
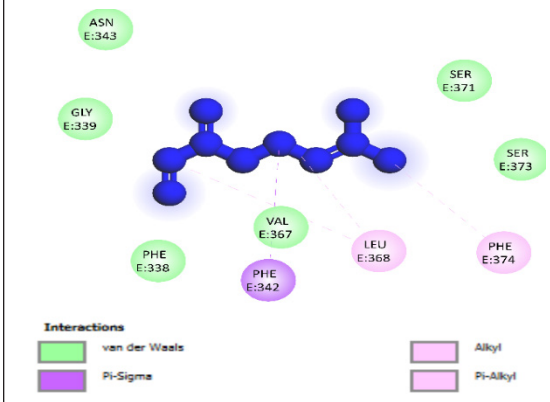
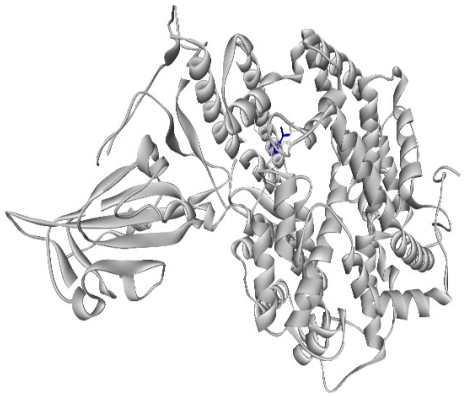
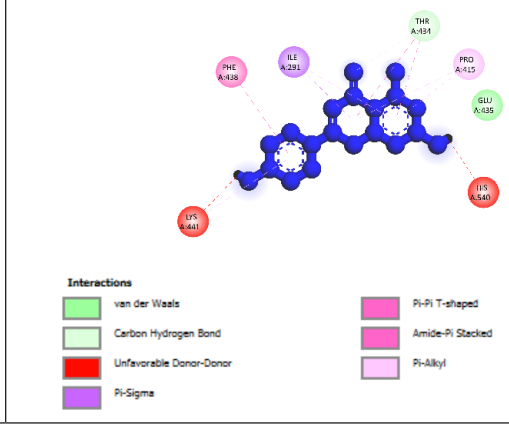
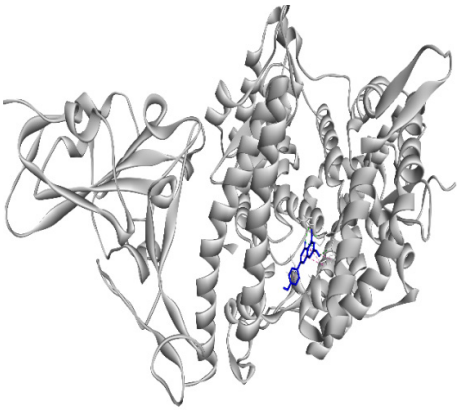
| Phytochemicals of *Citrus aurantianum* | 2D Interaction plot analysis | Docking pose phytochemicals with ACE |
|---|---|---|
| Linalool |  |  |
| Limonene |  |  |
| Myrcene |  |  |
| d) Apigenin |  |  |

**Table 5.** *to be continued...*

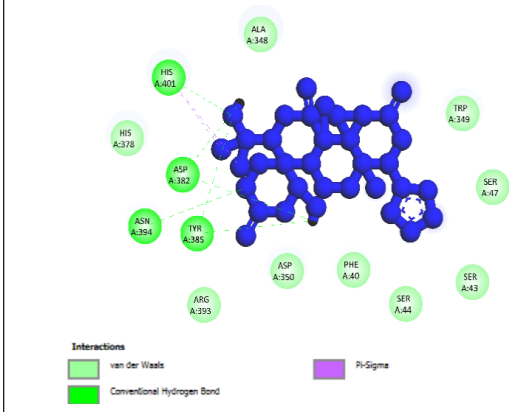
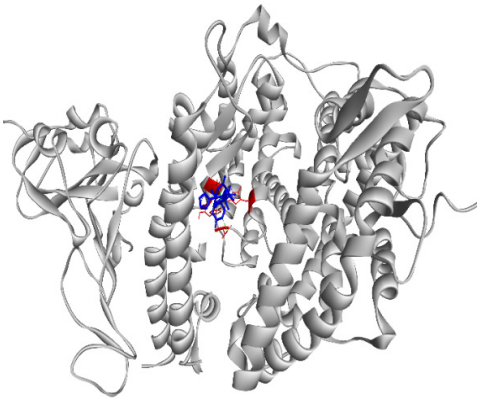
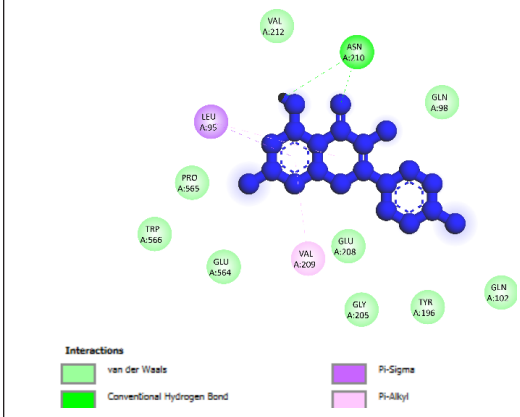
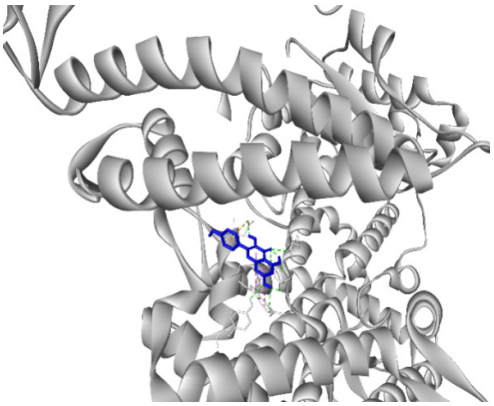
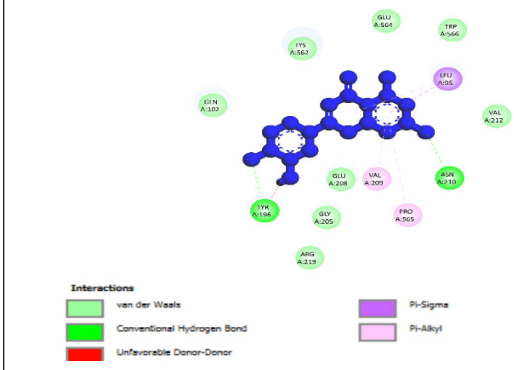
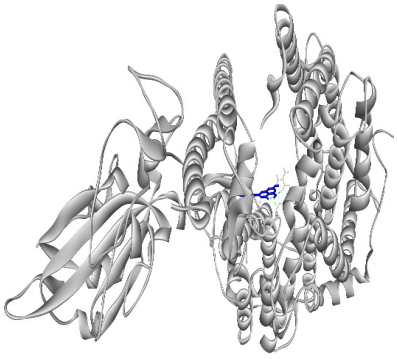
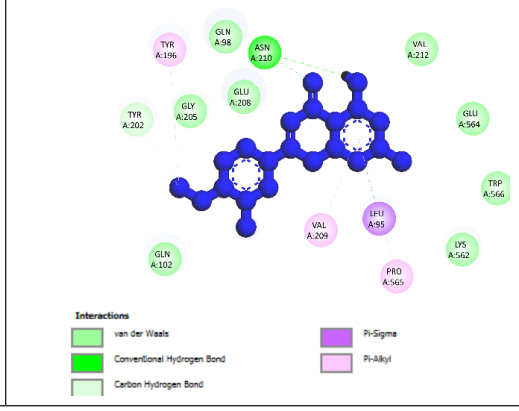
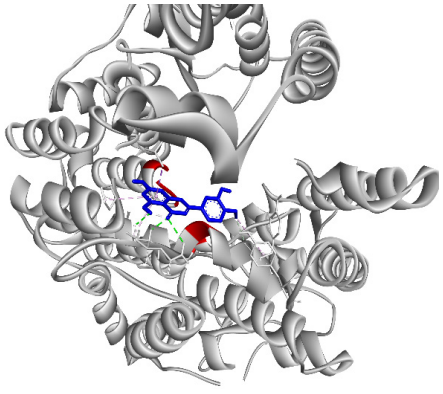
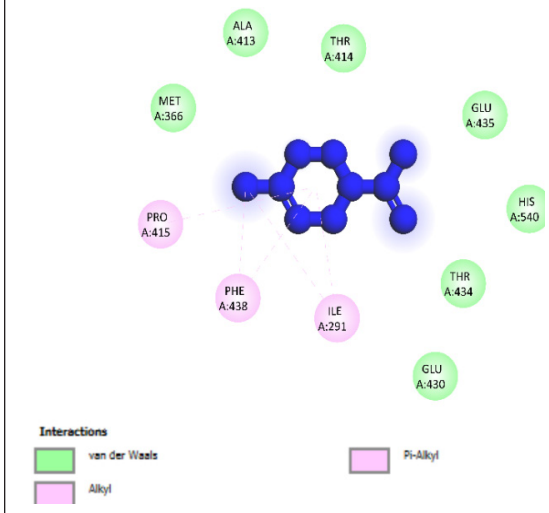
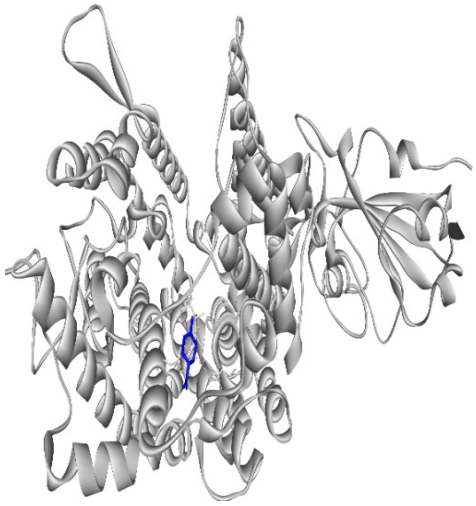
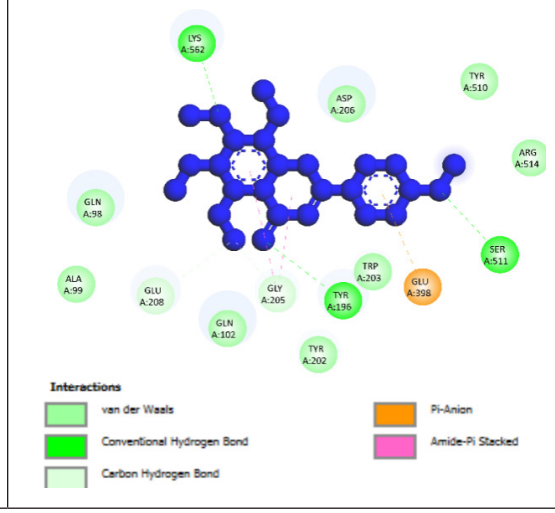
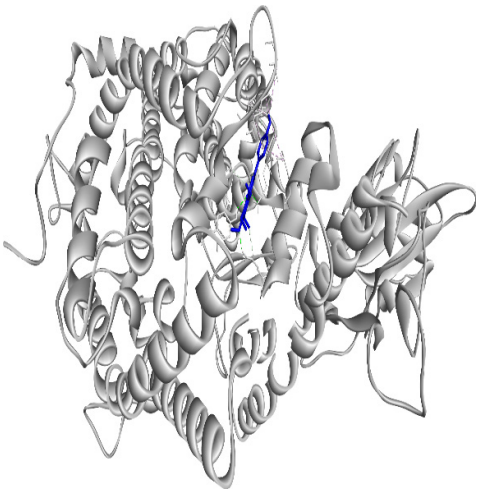
| Phytochemicals of *Citrus aurantianum* | 2D Interaction plot analysis | Docking pose phytochemicals with ACE |
|---|---|---|
| e) Ichangin |  |  |
| f) Kaempferols |  |  |
| g) Luteolin |  |  |
| h) Diosmetin |  |  |

**Table 5.** *to be continued...*

| Phytochemicals of *Citrus aurantianum* | 2D Interaction plot analysis | Docking pose phytochemicals with ACE |
|---|---|---|
| i) Limonin |   |  |
| j) Tangeretin |   |  |

high mortality rate among the middle aged as well as elderly population. The study was carried out to find the efficacy of the molecules with lead activity to bring out the core bioactive amino acid residues, GLU162, LYS511, HIS513, TYR520, and TYR523, which mediates the enzymatic action of the ACE. Since ACE is involved in converting Angiotensin I to II, which constricts blood vessels and raises blood pressure, reducing the enzyme concentration of ACE will inhibit the conversion and in turn could be effective in controlling the elevation of blood pressure[34].

The enzymatic action of ACE could be reduced by occupying the receptor sites. Phytochemicals that have the potency to inhibit the enzyme ACE could lower the high blood pressure. Captopril is a synthetic antihypertensive drug developed by structural modification of venom of Brazilian viper acts as an ACE inhibitor. Long-term use of ACE inhibitors were known to produce serious irreversible side effects, such as lightheadedness, cough, and angio edema. Such effect could be overcome by replace synthetic drugs with natural medicine. Natural ACE inhibitors of plant origin were known to inhibit ACE. The mechanism of such biocomponents resemble synthetic ACE inhibitors[35].

In Siddha literature single herbs such as *Cuminum cyminum, Glyzrrhiza glabra, Citrus limon, Vetiveria zizanoides, Eletaria cardamum, Terminalia bellarica,*

*Oldenlandia umbellate, Alllium cepa* and prepared medicines like *Thiratchadi kudineer, Nerunjil kudineer, Seeraga chooranam, Maruthampattai chooranam, Inji ilagam, Kesari ilagam* are also recommended for the hypertension management. The same has been emphasized in the Siddha treatment guidelines for Hypertension published by Siddha Clinical Research Institute, Chennai[18]. The selected fruit is used in the treatment of hypertension by the people of Edo state, Enderta, Northern Ethiopia and Nigeria[36,37]. Other than the suggested pharmacological activity, the phyto components of *Citrus aurantium* are said to have protective activity against gastrointestinal lesions induced by alcohol and NSAIDS in rats[9] and antimicrobial activity against pathogens of *Salmonella species. Also, its* antidiabetic effects were well established by various studies. Extract of *C. aurantium* has been commonly used in dietary supplements for weight loss and to enhance sports performance[10-16].

Among 32 phytochemicals, components such as Linalool, Limonene, Myrcene, Apigenin, Ichangin, Kaempferols, Luteolin, Diosmetin, Limonin, Tangeretin were selected based on their activity from published articles[32]. Phytochemical data, including the molecular structure of the selected compounds were retrieved using search engines, IMPPAT (Indian Medicinal Plants, Phytochemistry and Therapeutics). Since pharmacological screening of all the phytochemicals is time-consuming and costly, molecular docking of selected phytochemicals was done with computer-aided drug design, the results are mentioned in Tables 4 and 5.

The hydrogen bond counts present in the phytoconstituent is said to have a favourable contribution to affinity towards the target protein. Of the ten bioactive lead compounds retrieved from the herb *Citrus aurantium,* Kaempferol has the highest number, i.e. 9 interactions with the active site of the target enzyme ACE when compared to Captopril which reveals 8 interactions over the target enzyme. Diosemetin is ranked second with 7 interactions, whereas Tangeretin and Ichangin are with 6 interactions. Luteolin Apigenin and Linalool have 5,4 and 1hydrogen bonds, respectively. Limonin, Limonene and Myrcene possess only hydrophobic bonds that are said to be unfavourable for contribution towards affinity. Kaempferol has a binding affinity of -8.9, Limonin -8.9, Ichangin- 9.2, Tangeretin -8.3, Diosemetin and Luteolin – 8.2, Apigenin -7.4, Limonene and Linalool -5.3 Mycrene -5.1.

## 5. Conclusion

The docking study results prove that the phytochemical Kaempferol shows about 9 interactions against the active site of ACE. Diosmetin, Tangeretin and Ichangin also have more than 5 hydrogen bonds for active interaction. Results of the *in silico* analysis suggest that the phytochemical Kaempferol, Diosmetin, Tangeretin and Ichangin present in *Citrus aurantium,* the prime ingredient of *Thurunji Manapaagu* reveals effective binding to the active receptor site of ACE that supports the conclusion that *Thurunji Manapaagu* could have a promising anti-hypertensive activity.

## 6. Acknowledgement

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