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# Insilico Docking Studies of Benzoic Acid from *Indigofera aspalathoides* Against Liver Cancer

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## Abstract
Civanarvembu/ Iraivanvembu in Tamil is botanically equated as *Indigofera aspalathoides* Vahl.ex.DC. Various phytochemical compounds such as Tannin, Flavonoid, Saponin, Sterol and Phenol were found in the plant extract. *Indigofera aspalathoides (I.aspalathoides)* concern to the family Fabaceae and it grows in Southern India. This plant has been used in traditional medicine for the treatment of tumors, gastric hyperacidity, ulcers, and toothache. The stem was conventional used for various skin disorders and cancer. Hepatocellular carcinoma (HCC) is a major health burden and most common cause of cancer-related death in India and other countries. MAP kinases are activated within protein kinase cascades that regulate cell proliferation, differentiation, and cell death. The potential ligand candidate was identified from Pubchem database. *I.aspalathoides* derived compounds such as Phenanthrenol, Benzoic acid, cyclohexanol, Tetradecanoic acid, 2-Methoxy-4α-methylandrost-2-en-17ol-l-one 5β and n-hexadecanoic acid. Lipinski rule was employed to check the ligand likeliness of the compound. The 3D crystallographic structure of MAPK Tumour Suppressor Protein (ID.1CM8) fetched from the PDB (Protein Data Bank) and protein target sites of the ligands were identified. Molecular docking studies was executed using Autodock Vina (Autodock 4.2). Hence it has been concluded Benzoic acid as a novel inhibitor for MAPK protein in Liver Cancer.

**Keywords**
Civanarvembu, *Indigofera aspalathoides,* Benzoic acid**,** Liver Cancer and Autodock 4.0.

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## INTRODUCTION
Civanarvembu/ Iraivanvembu in Tamil is botanically equated as *Indigofera aspalathoides* Vahl.ex.DC belonging to the family Fabaceae. In Sanskrit it is Patakohomba/ Sivanimba [1].

**Classification:**
Kingdom: Plantae
Order: Fabales
Family: Fabaceae
Sub family: Faboideae
Tribe: Indigofereae
Genus: Indigofera. L
Species: *Indigofera aspalathoides* Vahl [2]

Civanar vembu Tailam and Civanarvembuk kulit Tailam are two popular Siddha preparations used for various types of skin diseases including leprosy. It is

used along with camphor for different kinds of wounds. This plant is regarded as one used in Kayakalpa drugs and in the discovery of anticancer [3,4]. The plant was investigated for different pharmacological activities but still more has to be explored. It was widely investigated for anti-tumour activity and antioxidant activity [2]. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins, phenolic compounds, steroids, resins, fatty acids and gums which are capable of producing definite physiological action on body. Medicinal plants are relied upon by 80% of the world's population and in India there is a rich tradition of using herbal medicine for the treatment of various infectious diseases, inflammations, injuries and other diseases. Many of the plant materials used in traditional medicine are generally proved more effective and relatively cheaper than modern medicine [5]. *I.aspalathoides* is derived compounds such as Phenanthrenol, Benzoic acid, cyclohexanol, Tetradecanoic acid, 2-Methoxy-4α-methylandrost-2-en-17ol-l-one 5β and n-hexadecanoic acid [6].

Cancers of the liver are one of the most common types of cancer worldwide, and hepatocellular carcinoma (HCC) accounts for the majority (70% – 90%) of them. HCC

is a major health burden with a steadily increasing incidence globally [7]. According to GLOBOCAN 2012 data, liver cancer is largely a problem of the less developed regions where 83% (50% in China alone) of the estimated 782,000 new cancer cases worldwide occurred in 2012. It is the fifth most common cancer in men (554,000 cases, 7.5% of the total) and the ninth in women (228,000 cases, 3.4%). HCC is the second most common cause of cancer-related death worldwide, which is estimated to be responsible for nearly 746,000 deaths in 2012 (9.1% of the total). It is more common in men than in women (male: female ratio of 2.4:1). The prognosis for liver cancer is very poor with an overall ratio of mortality to incidence of 0.95; and as such, the geographical patterns in incidence and mortality are similar [8].

The mitogen-activated protein kinase (MAPK) cascade is a highly conserved module that is involved in various cellular functions, including cell proliferation, differentiation and migration. Mammals express at least four distinctly regulated groups of MAPKs, extracellular signal-related kinases (ERK)-1/2, Jun amino-terminal kinases (JNK1/2/3), p38 proteins (p38alpha/beta/gamma/delta) and ERK5, that are activated by specific MAPKs: MEK1/2 for ERK1/2, MKK3/6 for the p38, MKK4/7 (JNKK1/2) for the JNKs, and MEK5 for ERK5. Each MAPK, can be activated by more than one MAPK, increasing the complexity and diversity of MAPK signalling. Presumably each MAPKKK confers responsiveness to distinct stimuli. For example, activation of ERK1/2 by growth factors depends on the MAPKKK c-Raf, but other MAPKKKs may activate ERK1/2 in response to pro-inflammatory stimuli [9].

## DATABASE AND METHODOLOGY

### PREPARATION OF RECEPTOR STRUCTURE

The Protein Data Bank (PDB) is a crystallographic database for three dimensional structure data of large biological molecules such as proteins and nucleic acids. Crystal structure of MAPK Protein was obtained from Protein Data Bank (http://www.rcsb .org) with PDB ID 1CM8 [10]. The protein consists of two chains and 367 residues length with resolution 2.4 Å. structural and active site studies of the protein were done by using Pymol molecular visualization software [11].

### PUBCHEM

It is a product of NCBI data base. Useful for collecting the information about the specified chemicals. It is an online archive containing the information of all the known chemicals their properties and their biological importance. The user can use these chemicals based on their function and download their structures for other analysis. The 2D structure of the *I.aspatholoides* compounds of Phenanthrenol, Benzoic acid, cyclohexanol, Tetradecanoic acid, 2-Methoxy-4α-methylandrost-2-en-17ol-l-one 5β and n-hexadecanoic acid were obtained from the PubChem database [11].

### ACD CHEMSKETCH

ACD/Chemsketch is the powerful chemical drawing and graphics package from ACD/Labs software. Which will draw molecular structures, reactions and calculate chemical properties very quickly and easily [11]. The three dimensional structures Phenanthrenol, Benzoic acid, cyclohexanol, Tetradecanoic acid, 2-Methoxy-4α-methylandrost-2-en-17ol-l-one 5β and n-hexadecanoic acid were drawn by chemsketch.

### LIGAND STRUCTURE PREPARATION

In this study, for the ligand Phenanthrenol, Benzoic acid, cyclohexanol, Tetradecanoic acid, 2-Methoxy-4α-methylandrost-2-en-17ol-l-one 5β and n-hexadecanoic acid 2D structure and chemical formula were obtained from PubChem. The structure of Phenantheronol, Benzoic acid, cyclohexanol, Tetradecanoic acid, 2-Methoxy-4α-methylandrost-2-en-17ol-l-one 5β and n-hexadecanoic acid was computed by drawing using chemsketch software (chemically intelligent drawing interface freeware). This was followed by ligand construction using

chemsketch draw mode 3D structure optimization were compute and finally ligand as" MOL" file format and then mol file was converted and save as "PDB" using open babel molecular editor software. The protein and ligand files which are prepared were then taken for docking.

**DOCKING METHODOLOGY**

AutoDock Vina is very popular, and highly cited, open source docking program. The software is used for modeling flexible small molecules such as drug molecules and it's binding to receptor proteins of known three dimensional structures [11].

**VISUALIZATION AND ANALYSING DOCKING RESULTS**

PyMol is an open source tool to visualize molecules available from (www.pymol.org) PyMol has excellent capabilities in creating high quality images from 3D structures; it has well developed functions for manipulating structures and some basic functions to analyze their chemical properties. The possibilities to write scripts and plug in as well as to incorporate PyMol in custom software are fast and superior to most other programs. Once the target ligand was docked against all protein of interest, the result was visualized for their interactions, binding energy, H-bond formation and few other parameters using the PyMol software [11].

**RESULT AND DISCUSSION**

**STRUCTURE RETRIEVAL:**

The 3D crystal structure of MAPK Protein (ID.1CM8), was retrieved from the Protein Data Bank (PDB).

**PREPARATION OF LIGAND:**

For further docking analysis of the compounds Phenanthrenol, Benzoic acid, cyclohexanol, Tetradecanoic acid, 2-Methoxy-4α-methylandrost-2-en-17ol-l-one 5β and n-hexadecanoic acid from *I.aspatholoides* are taken. The two-dimensional structures of the ligand were generated using the ACD/ Chemsketch tool. This tool contains for 2D cleaning, 3D optimization and viewing. These data are saved as a molecular format file (MDL, MOL format). The molecular format converter tool (Arguslab) is used to convert this file into the PDB format and is used during docking analysis.

According to Lipinski's rule of a compound having not more than 5 hydrogen bond donors (OH an NH groups), not more than 10 hydrogen bond acceptors (notably N and O), molecular weight under 500 g/mol, partition coefficient log P of less than 5 was shown in Table 1.

**Table 1. Lipinski's rule of compounds**

| COMPOUND NAME | MOLECULAR FORMULA | MOLECULAR WEIGHT (g/mol) | DONOR | ACCEPTOR | Log P Value |
|---|---|---|---|---|---|
| Phenanthrenol | $C_{20}H_{14}O$ | 270.3 g/mol | 1 | 1 | 5.7 |
| Benzoic acid | $C_7H_6O_2$ | 122.12 g/mol | 1 | 2 | 1.9 |
| Cyclohexanol | $C_6H_{12}O$ | 100.16 g/mol | 1 | 1 | 1.2 |
| Tetradecanoic acid | $C_{14}H_{28}O_2$ | 228.37 g/mol | 1 | 2 | 5.3 |
| 2-Methoxy-4α-methylandrost-2-en-17ol-l-one 5β | $C_{21}H_{32}O_3$ | 332.5 g/mol | 1 | 3 | 4.5 |
| n-hexadecanoic acid | $C_{16}H_{32}O_2$ | 256.43 g/mol | 1 | 2 | 6.4 |

**DOCKING STUDY:**

The inhibitors docked with MAPK Protein using Auto Dock Vina software (Version 4.2). The Graphical User Interface program "Auto-Dock Tools" was used to prepare, run, and analyse the docking simulations. Kollman united atom charges, solvation parameters and polar hydrogens were added into the receptor PDB file for the preparation of protein in docking simulation. Grid generated for X axis, Y axis, Z axis. Auto dock vina results were analysed to study the interactions and the binding energy of the docked structure. The best ligand-receptor structure from the docked structures was chosen based on the lowest energy and minimal solvent accessibility of the ligand. The docking results were visualized using the PyMol visualizer tool. A bond is formed between two atoms by overlapping the atomic orbitals. This overlap of atomic orbitals to form molecular orbitals occurs only at certain distances between the atom. When the amino acid residues of the active site are closer, then the interactions are much higher than the other sites [11].

**Table 2. Binding energy between MAPK Protein and Phenanthrenol**

| mode | affinity (kcal/mol) | dist from best mode rmsd l.b. | rmsd u.b. |
|---|---|---|---|
| 1 | -7.5 | 0.000 | 0.000 |
| 2 | -7.4 | 1.818 | 2.978 |
| 3 | -7.1 | 26.590 | 30.540 |
| 4 | -7.0 | 25.981 | 29.694 |
| 5 | -7.0 | 26.512 | 29.697 |
| 6 | -6.9 | 3.417 | 5.511 |
| 7 | -6.9 | 1.812 | 6.229 |
| 8 | -6.8 | 25.099 | 29.818 |
| 9 | -6.6 | 26.094 | 30.087 |

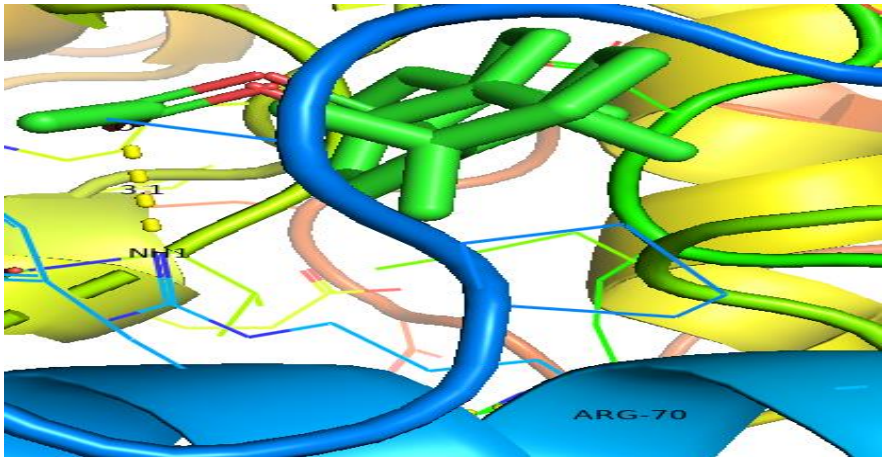


**Figure 1. Docking of MAPK Protein and Phenanthrenol is visualized using PYMOL**

**Table 3. Binding energy between MAPK Protein and Benzoic acid**

| mode | affinity (kcal/mol) | dist from best mode rmsd l.b. | rmsd u.b. |
|---|---|---|---|
| 1 | -4.4 | 0.000 | 0.000 |
| 2 | -4.1 | 20.433 | 23.325 |
| 3 | -3.9 | 2.045 | 3.264 |
| 4 | -3.7 | 2.567 | 4.963 |
| 5 | -3.6 | 21.346 | 24.265 |
| 6 | -3.5 | 1.825 | 4.015 |
| 7 | -3.4 | 33.264 | 35.351 |
| 8 | -3.3 | 21.284 | 23.874 |
| 9 | -3.3 | 32.843 | 35.215 |

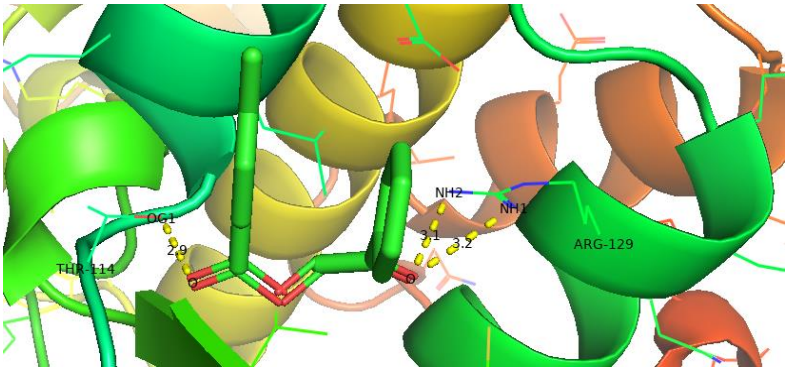


**Figure 2. Docking of MAPK Protein and Benzoic acid is visualized using PYMOL**

**Table 4. Binding energy between MAPK Protein and Cyclohexanol**

| mode | affinity (kcal/mol) | dist from best mode rmsd l.b. | rmsd u.b. |
|---|---|---|---|
| 1 | -5.4 | 0.000 | 0.000 |
| 2 | -5.4 | 30.382 | 31.439 |
| 3 | -5.2 | 30.398 | 31.539 |
| 4 | -5.2 | 15.070 | 15.819 |
| 5 | -4.8 | 31.866 | 32.931 |
| 6 | -4.4 | 34.876 | 35.704 |
| 7 | -4.4 | 14.850 | 16.038 |
| 8 | -4.3 | 34.862 | 35.682 |
| 9 | -4.1 | 23.508 | 24.929 |

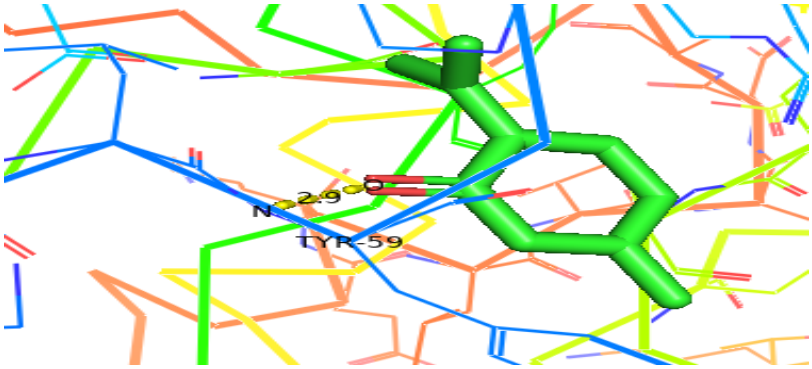


**Figure 3. Docking of MAPK Protein and Cyclohexanol is visualized using PYMOL**

**Table 5. Binding energy between MAPK Protein and Tetradecanoic acid**

| mode | affinity (kcal/mol) | dist from best mode rmsd l.b. | rmsd u.b. |
|---|---|---|---|
| 1 | -4.3 | 0.000 | 0.000 |
| 2 | -4.1 | 1.721 | 2.388 |
| 3 | -4.0 | 27.637 | 29.628 |
| 4 | -3.8 | 27.494 | 30.007 |
| 5 | -3.7 | 3.248 | 4.926 |
| 6 | -3.7 | 1.856 | 4.022 |
| 7 | -3.6 | 2.508 | 4.751 |
| 8 | -3.5 | 3.925 | 6.232 |
| 9 | -3.5 | 3.468 | 5.920 |

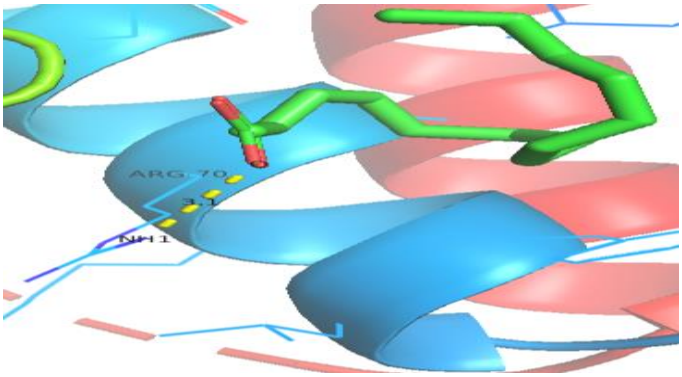


**Figure 4. Docking of MAPK Protein and Tetradecanoic acid is visualized using PYMOL**

**Table 6. Binding energy between MAPK Protein and 2-Methoxy-4α-methylandrost-2-en-17ol-l-one 5β**

| mode | affinity (kcal/mol) | dist from best mode rmsd l.b. | rmsd u.b. |
|---|---|---|---|
| 1 | -8.1 | 0.000 | 0.000 |
| 2 | -7.8 | 21.998 | 24.487 |
| 3 | -7.7 | 27.300 | 29.526 |
| 4 | -7.6 | 8.725 | 10.557 |
| 5 | -7.4 | 26.360 | 29.579 |
| 6 | -7.3 | 25.963 | 27.793 |
| 7 | -7.2 | 8.306 | 11.084 |
| 8 | -7.2 | 27.266 | 29.031 |
| 9 | -7.0 | 35.490 | 37.091 |

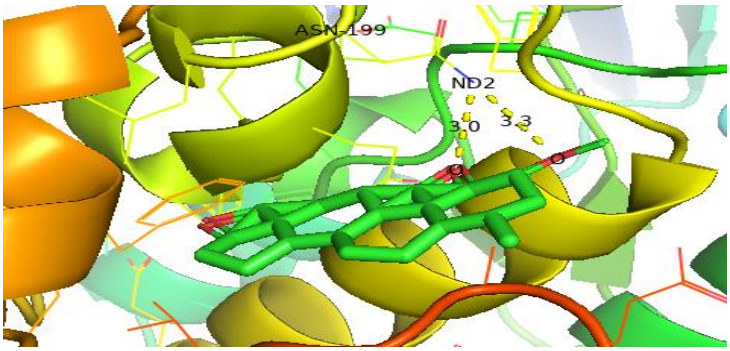


**Figure 5. Docking of MAPK Protein and 2-Methoxy-4α-methylandrost-2-en-17ol-l-one 5β is visualized using PYMOL**

**Table 7. Binding energy between MAPK Protein and n-hexadecanoic acid**

| mode | affinity (kcal/mol) | dist from best mode rmsd l.b. | rmsd u.b. |
|---|---|---|---|
| 1 | -4.1 | 0.000 | 0.000 |
| 2 | -3.9 | 3.369 | 5.853 |
| 3 | -3.7 | 22.626 | 25.484 |
| 4 | -3.7 | 3.596 | 5.428 |
| 5 | -3.7 | 2.998 | 4.654 |
| 6 | -3.7 | 47.876 | 49.765 |
| 7 | -3.6 | 12.466 | 15.273 |
| 8 | -3.6 | 40.724 | 42.101 |
| 9 | -3.6 | 40.591 | 42.605 |

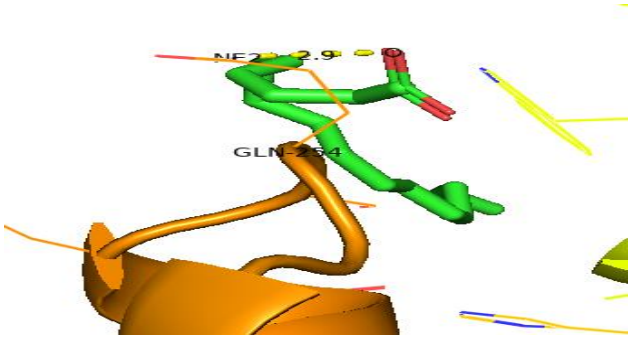

**Figure 6. Docking of MAPK Protein and n-hexadecanoic acid is visualized using PYMOL**

## CONCLUSION

The recent study aimed to analyse Target biomarker protein and Ligand interaction plays a meaningful role in structural based studies. In this present study, we have taken the human MAPK Protein and identified a novel inhibitor. The receptor (MAPK Protein) was docked with Benzoic acid, the binding energy value of -4.1kcal/mol using Autodock Vina (Figure 2, Table 3). Phenanthrenol, Cyclohexanol, Tetradecanoic acid, 2-Methoxy-4α-methylandrost-2-en-17ol-l-one 5β and n-hexadecanoic acid was docked against the same receptor and a binding energy values of -7.4kcal/mol, -5.4 kcal/mol, 4.1 kcal/mol, 7.8 kcal/mol, 3.9 kcal/mol was obtained (Figure 1,3,4,5,6, Table 2,4,5,6,7). Hence it has been concluded Benzoic acid as a novel inhibitor for MAPK protein in Liver Cancer.

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