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Estimation of Binding Affinity of Selected Nutmeg Phytochemicals with Human hypocretin-2/Orexin-B by *In Silico* Molecular Docking to Evaluate Their Inhibitory Activity.

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Abstract

Structure based drug design is an important method for the discovery of new drug candidates. A vital part of this method is to find out the binding affinity of the complex formed by docking small molecules in the binding site of the receptor. A few phytochemicals found in nutmeg seeds (Myristica fragrans) were selected for the current in silico docking studies to evaluate their inhibitory activity on human hypocretin-2/Orexin-B which is a hypothalamic neuropeptide.

Ligand preparation and subsequent molecular docking with the target protein were performed using AutoDock Vina software. Binding affinity of the phytochemicals were predicted towards the hypothalamic neuropeptide Orexin-B. Only the lowest energy poses were considered for comparison.

Key words: Nutmeg, phytochemical, Licarin A, Orexin-B, anti-obesity, molecular docking

Introduction

Belonging to the Myristicaceae family, Nutmeg (*Myristica fragrans* Houtt.) is one of the widely used condiments. Though this tropical tree is indigenous to Indonesia, various species of nutmeg are found in other tropical regions such as southern parts of Indian subcontinent, Sri Lanka, Malaysia.[i] The seed (nutmeg) and its outer covering (mace) are used as spices and for flavouring. Traditionally, nutmeg has also been used to treat various stomach disorders as well as other diseases.[ii] Studies have been done to prove the antioxidant property,[iii] antiobesity effect,[iv] and many other diseases.

Obesity has become an global epidemic which is increasing at an alarming rate in both developed and developing nations.[v] Obesity also causes chronic diseases such as type 2 diabetes and cardiovascular diseases. It is can be considered as a risk factor to certain types of cancers. Medicinal plants used in folk remedies contain active phytoconstituents possessing lower side effects.[vi] New drug molecules can be discovered using those phytochemicals and can be helpful in treating various diseases.

Human hypocretin-2/Orexin-B, a hypothalamic neuropeptide.[vii] According to various studies, orexin peptides has role as modulators of acute food intake[viii] which can be linked to energy metabolism and obesity.[ix]

15 to 20% of nutmeg extract constituted of various aromatic ethers such as myristicin, elemicin, safrole, eugenol and its derivatives. Myristicin and elemicin[x] have hallucinogenic effect in high doses. Licarin A has proven anti-allergic effect.[xi] In this communication, an *in silico* study was performed to demonstrate the activity of a few selected nutmeg extract phytochemicals namely, Myristicin, Licarine A, Elemicin and Isoelemicin by molecular docking with Human hypocretin-2/Orexin-B.

Result and Discussion

PubChem database was used as the source for 3D structures of Myristicin, Licarin A, Elemicin and Isoelemicin which are available as .sdf format. The .sdf format of the structures were converted into .pdb format using PyMol software. Ligands were then prepared and saved in the format of .pdbqt using AutoDock Vina. The chemical structures of the compounds/ligands chosen for the study are shown in figure 1.

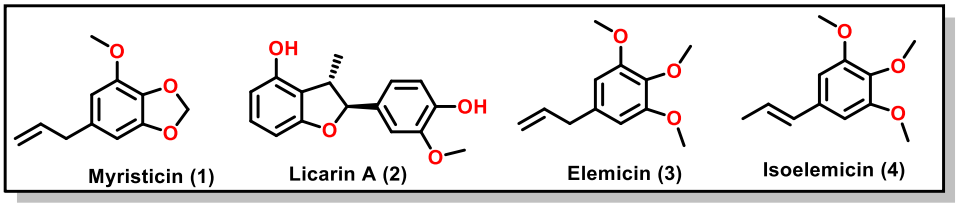

Figure 1: *Chemical structures of the selected compounds Myristicin (1), Licarine A (2), Elemicin (3) and Isoelemicin (4)*

Structure of the target protein 1CQ0 was obtained from Protein Data Bank (https://www.rcsb.org). Water molecules were removed and the protein was prepared in Auto Dock Vina. Docking was performed using AutoDock Vina.[xii] After docking with the protein, nine best docking poses were obtained for each ligand. Ligand-protein interactions were visualized in PyMol software, and poses with minimum binding energy were selected and studied.

Binding energy and number of hydrogen bond interaction of the molecules with each protein are shown in **Table 1**.

	BE and NHB of the 1st pose of each phytochemical with Orexin-B							
	Myristicin		**Licarine A**		**Elemicin**		**Isoelemicin**	
PDB ID	**BE (kcal/mol)**	**NHB**	**BE (kcal/mol)**	**NHB**	**BE (kcal/mol)**	**NHB**	**BE (kcal/mol)**	**NHB**
1CQ0	-4.1	0	-5.2	0	-3.8	3	-4.0	2

Table 1: *Binding energy (BE) and number of hydrogen bonds (NHB) of ligand protein interaction*

The lowest energy poses of myristicin and licarin A did not show any H-bonding with the protein 1cq0. However, the second lowest pose of myristicin exhibits H-bond with ARG 13. There was no H-bond interaction observed for first 3 poses of licarin A and the protein complex. However, it exhibited had the highest binding affinity with the protein with binding energy of -5.2 kcal/mol. 1st pose of elemicin-protein complex shows 3 H-bonds with ARG 13. Isoelemicin exhibited H-bonding interaction with leu14.

Binding affinity of the selected phytochemicals with the protein target and number of hydrogen bond interactions are summarized in **Table 1**.

The best poses of each molecules as visualized in PyMol software are shown in the figures given below.

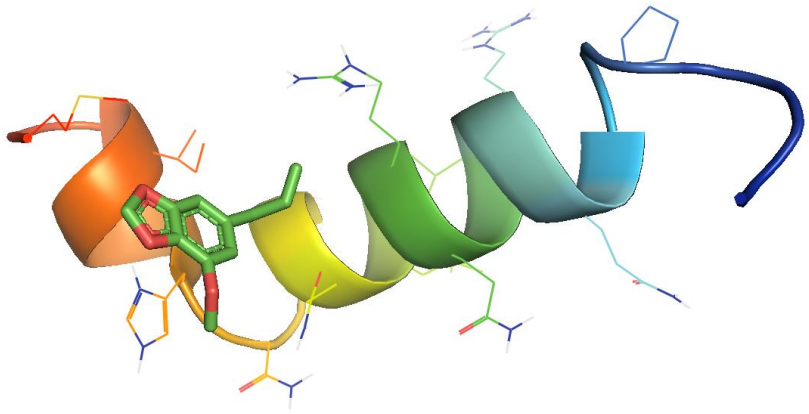

Figure 2: *Lowest energy pose of the Protein-ligand complex between 1CQ0 and Myristicin*

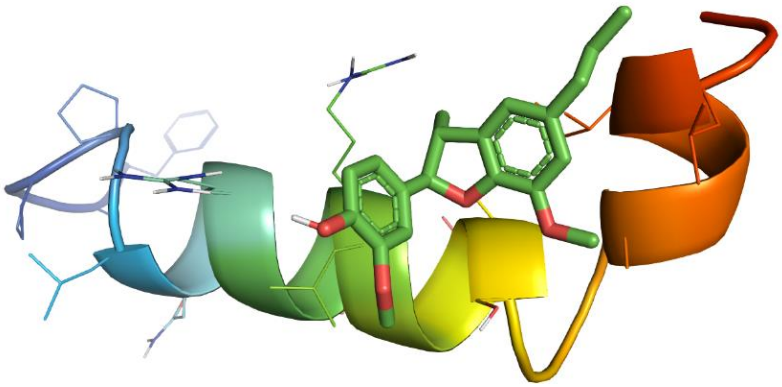

Figure 3: *Lowest energy pose of the Protein-ligand complex between 1CQ0 and Licarine A*

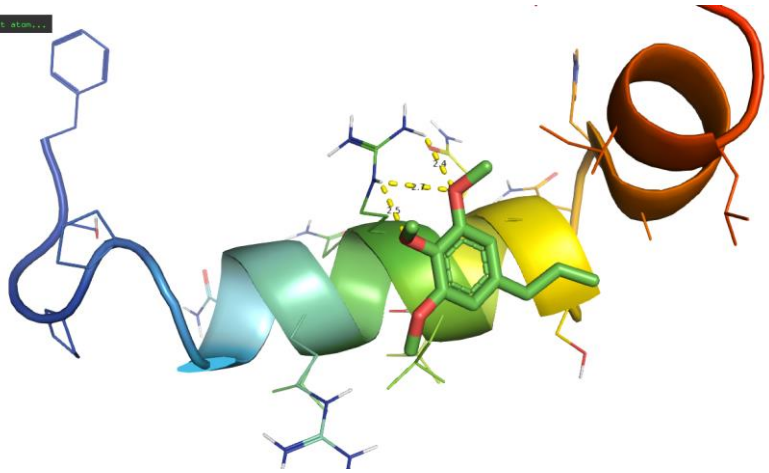

Figure 4: *Lowest energy pose of the Protein-ligand complex between 1CQ0 and Elemicin*

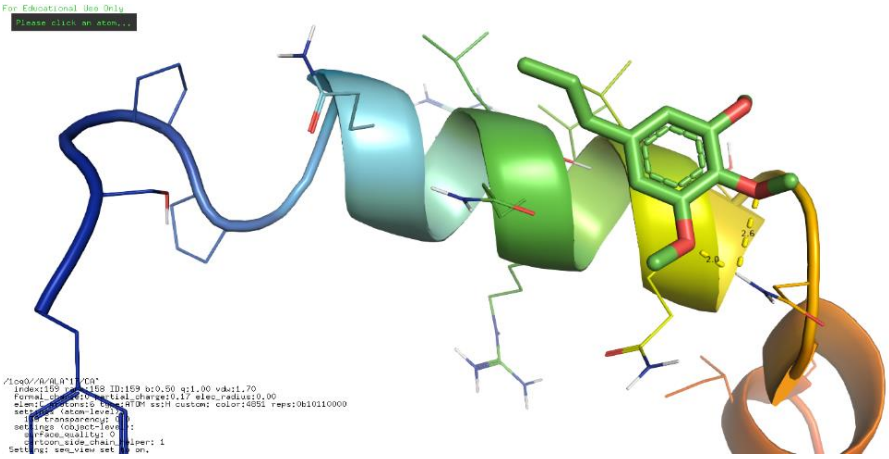

Figure 5: *Lowest energy pose of the Protein-ligand complex between 1CQ0 and Isoelemicin*

Conclusion

Interactions between nutmeg phytoconstituents myristicin, licarin A, elemicin and isoelemicin with the target human hypocretin-2/Orexin-B were studied using molecular docking software. It was observed that the lowest energy poses of myristicin and licarin A did not form any Hydrogen bonding with the active site amino acids of the protein. However, licarine A had the highest binding affinity with the protein. This *in silico* study can give some insight into the protein-ligand interactions of other structurally similar phytochemicals and can further be used to develop analogous molecules with better affinity and activity.

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