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RESEARCH ARTICLE

DOCKING AND GENOMIC STUDIES FOR SWINE FLU BASED ON PHYTOCHEMICAL COMPOUNDS OF OCIMUM SANCTUM

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ABSTRACT

This study investigates that detailed analysis of phytochemical compounds from *ocimum sanctum* by using GC-MS. The phytochemical compounds are to be docked with the receptor PB1F2 by Hex tool and calculate the docking scores. We can also focus on Genomic studies to identify the Coding regions and predict the structure**.** The Restriction sites present in the sequence are to be identified by NEB Cutter tool. This detailed study will focus on *ocimum sanctum* can act as herbal drug for swine flu.

INTRODUCTION

The swine flu is an infectious disease caused by any virus belonging to the family Orthomyxoviridae, which is endemic in population pig. These strain viruses, known as swine influenza virus. Although the swine flu does not affect regular human population, there are sporadic cases of infectious in human. Generally, these cases occur in those working with poultry and pigs, especially those individuals who are heavily exposed to this type of animal, and are at higher risk of infection, if they carry any viral strain that is also capable to infect humans. This is because the virus can mutate and additionally, through a process called re-acquire characteristics. That allows transmission between people. Geographical and temporal diffusion patterns of a human pandemic due to Swine Origin Influenza Virus (S-OIV) remain uncertain. The extent to which national and international pandemic preparedness plants and control strategies can slow or stop the process is not known. (Ref 1)

H1N1 VIRUS

A molecular model of the swine influenza A/*H1N1* type-I neuraminidase was built using the pathogenic avian *H5N1* type-I neuraminidase as a basis, due to the higher sequence identity between A/*H1N1* and *H5N1* (91.47%) compared to Spanish *H1N1* (88.37%) neuraminidase. All-atom molecular dynamics (MD) simulations of all three neuraminidases were performed, either as apo-structures or with commercial antiviral drugs Tamiflu or Relenza separately bound; the simulations allowed for the identification of both conserved and unique drug-protein interactions across all three proteins. (Ref 2).

The rapid spread of influenza virus subtype *H1N1* poses a great threat to million lives worldwide. To search for new anti-influenza compounds, we performed molecular docking and molecular dynamics simulation to identify potential traditional Chinese medicine (TCM) constituents that could block influenza M2 channel activity. (Ref 3). The interaction between hemagglutinin (HA) and receptors is a kernel in the study of evolution and host adaptation of *H1N1* influenza A viruses. The notion that the avian HA is associated with preferential specificity for receptors with Siaalpha2, 3Gal glycosidic linkage over those with Siaalpha2, 6Gal linkage is not all consistent with the available data on *H1N1* viruses. By x-ray crystallography, the HA structure of an avian *H1N1* influenza A virus, as well as its complexes with the receptor analogs, was determined.(Ref 4). In view of the widespread emergence of resistant isolates, an attempt was made to isolate and characterize the component(s) of *Ocimum sanctum* with activity against Neisseria gonorrhoeae. (Ref 5).

PB1-F2 PROTEIN- RESPONSIBLE FOR SWINE FLU

PB1-F2 is a proapoptotic influenza A virus protein of approximately 90 amino acids in length that is located in the nucleus, cytosol and in the mitochondria membrane of infected cells. Previous studies indicated that the molecule destabilizes planar lipid bilayers and has a strong inherent tendency for multimerization. This may be correlate with its capacity to induce mitochondrial membrane depolarization. The enhanced response to one of the subdominant

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determinants PB1F2 correlates with increased generation by LMP2 virus-infected cells. (Ref 6)

Ocimum sanctum

Ocimum sanctum, the Indian holy basil, has significant ability to scavenge highly reactive free radicals. Shade dried leaf powder of the plant was extracted with water and alcohol, and then fractionated with different solvents. Both extracts and their fractions have in vitro anti-lipidperoxidative activity at very low concentrations. In vivo, hypercholesterolemia-induced erythrocyte lipid peroxidation activity was inhibited by aqueous extracts of *Ocimum* in a dose-dependent manner in male albino rabbits. Aqueous extract feeding also provided significant liver and aortic tissue protection from hypercholesterolemia-induced peroxidative damage. (Ref 7). The effects of *Ocimum sanctum* leaf extract on the changes in the concentrations of serum triiodothyronine (T3), thyroxine (T4) and serum cholesterol; in the activities of hepatic glucose-6-phosphatase (G-6-P), superoxide dismutase (SOD) and catalase (CAT); hepatic lipid peroxidation (LPO) and on the changes in the weight of the sex organs were investigated. It appears that *Ocimum sanctum* leaf extract is antithyroidic as well as antioxidative in nature. (Ref 8). *Ocimum sanctum*, a well known herb in Indian medicine, possesses various therapeutic properties including healing properties and cytokine induction. Wound healing activity of cold aqueous extract of *O. sanctum* leaves along with its effect on tumor necrosis factor-alpha (TNF-alpha) was assessed using excision model of wound repair in Wistar albino rats. (Ref 9). A hydro alcoholic extract of ocimum sanctum leaves has been investigated for its antioxidant activity in animal models of peptic ulcer with the aim of exploring a possible correlation between its antioxidant and antiulcer activities. (Ref 10)

DRUG DESIGNING AND DOCKING

A combination of protein-ligand docking and ligand-based QSAR approaches has been elaborated, aiming to speed-up the process of virtual screening. In particular, this approach utilizes docking scores generated for already processed compounds to build predictive QSAR models that in turn assess hypothetical target binding affinities for yet undocked entries. This progressive-docking procedure therefore substantially accelerates high throughput screening, especially when using high accuracy (slower) docking approaches and large-sized datasets, and has allowed us to identify several novel potent nonsteroidal SHBG ligands. (Ref 11)

METHODOLOGY

Ocimum sanctum were collected from Thuraiyur. The leaves are then peeled out and air dried at room temperature for one week. Then dried the leaves into uniform powder by using a mixer. The sample was prepared by soaking five grams of dried powdered in 75ml of alcohol and with the help of shaker shake well until the colors change into dark green; Incubate the sample for over night at room temperature and after that kept the extract in water bath at 50 to 60 temperature until the liquid form change into powder form. Protein sequences for Swine flu were collected from NCBI Database. The sequences were saved in notepad and submitted in to PROSITE Tool. The active site of the receptor was identified by PROSITE tool. The structure of the receptor and phytochemical compounds were retrieved and subjected to docking studies by Hex tool. The docking scores can be calculated from Hex tool. Finally, docked structures were compared and identify the best drug for Swine flu. The coding and non coding regions were identified by Gen Scan Tool. The Restriction enzyme sites and regions were identified by NEB Cutter Tool.In GC-MS Analysis, Ocimum Sanctum having variety of phytochemical compounds. This table shows that the no of phytochemical compounds present in Ocimum Sanctum. These Phytochemical compositions can be identified by Gas Chromatography Mass Spectrometry Technique.

CHROMATOGRAM

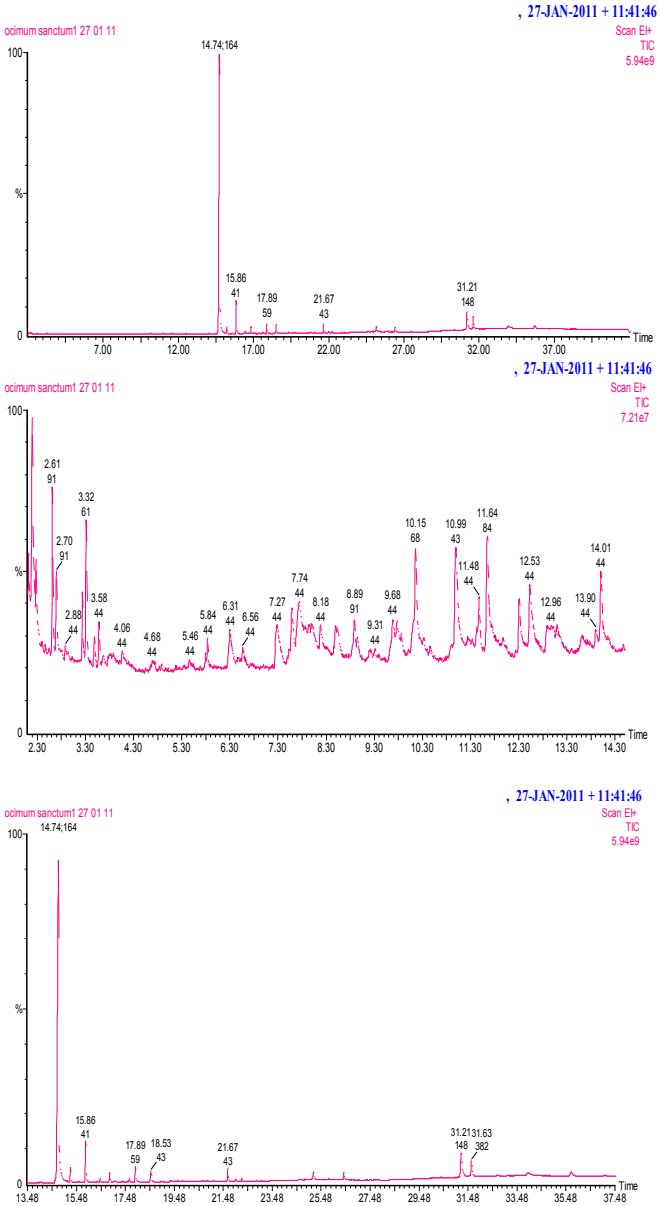


Fig 1: Geometrical Representation of Phytochemicals identified by GC-MS Technique

This graph shows that the graphical representation of the Phytochemical compounds present in Ocimum Sanctum identified by Gas Chromatography and Mass Spectrometry.

These graphs are based on the peak values for the photochemical compounds.

Table 1: Phytochemicals identified from oc*imum Sanctum* by GC-MS Technique

S.No.	Peak Name	Retention time	Peak area	% Peak area
1.	Butyrolactone Formula: $C_4H_6O_2$ MW: 86	6.31	742934	0.1238
2.	Glycerin Formula: $C_3H_8O_3$ MW: 92	7.74	2153155	0.3587
3.	3-Aminopyrazole Formula: $C_3H_5N_3$ MW: 83	8.18	618599	0.1031
4.	Benzeneacetaldehyde Formula: C_8H_8O MW: 120	8.89	449811	0.0749
5.	2,3-Hexanedione Formula: $C_6H_{10}O_2$ MW: 114	8.95	230139	0.0383
4	Thymine Formula: $C_5H_6N_2O_2$ MW: 126	9.68	674299	0.1123
6.	Phenol, 2-methoxy- Formula: $C_7H_8O_2$ MW: 124	9.78	805427	0.1342
7.	1H-Pyrrole, 2,5-dihydro- Formula: C_4H_7N MW: 69	10.15	1954782	0.3257
8.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- Formula: $C_6H_8O_4$ MW: 144	10.99	1818361	0.3029
9.	Borneol Formula: $C_{10}H_{18}O$ MW: 154	11.48	610430	0.1017
10.	5-Methoxypyrrolidin-2-one Formula: $C_5H_9NO_2$ MW: 115	11.64	2201992	0.3668
11.	L-Proline, 1-acetyl- Formula: $C_7H_{11}NO_3$ MW: 157	12.31	647514	0.1079
12.	Benzofuran, 2,3-dihydro- Formula: C_8H_8O MW: 120	12.53	1116154	0.1859
13.	Phenol, 2-methoxy-3-(2-propenyl)- Formula: $C_{10}H_{12}O_2$ MW: 164	14.74	390702176	65.0889
14.	á-Elemene, (-)- Formula: $C_{15}H_{24}$ MW: 204	15.11	1018737	0.1697
15.	á-Elemene, (-)- Formula: $C_{15}H_{24}$ MW: 204	15.23	10915870	1.8185
16.	Vanillin Formula: $C_8H_8O_3$ MW: 152	15.58	1400229	0.2333
17.	Caryophyllene Formula: $C_{15}H_{24}$ MW: 204	15.86	30402254	5.0649
18.	Phenol, 2-methoxy-4-(1-propenyl)- Formula: $C_{10}H_{12}O_2$ MW: 164	16.32	1139380	0.1898
19.	à-Caryophyllene Formula: $C_{15}H_{24}$ MW: 204	16.45	2799542	0.4664
20.	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [s-(E,E)]- Formula: $C_{15}H_{24}$ MW: 204(Germacrene D)	16.84	6455574	1.0755
21.	4-Chromanol Formula: $C_9H_{10}O_2$ MW: 150	17.00	1333112	0.2221
22.	Cadina-1(10),4-diene Formula: $C_{15}H_{24}$ MW: 204	17.35	1080571	0.1800
23.	Germacrene D-4-ol Formula: $C_{15}H_{26}O$ MW: 222	17.64	3788620	0.6312
24.	Elemol Formula: $C_{15}H_{26}O$ MW: 222	17.89	13171072	2.1942
25.	Caryophyllene oxide Formula: $C_{15}H_{24}O$ MW: 220	18.53	10882110	1.8129
26.	trans-Z-à-Bisabolene epoxide Formula: $C_{15}H_{24}O$ MW: 220	18.94	704483	0.1174
27.	Megastigmatrienone Formula: $C_{13}H_{18}O$ MW: 190	19.06	219360	0.0365
28.	à-Eudesmol Formula: $C_{15}H_{26}O$ MW: 222	19.35	2274356	0.3789
29.	2-Naphthalenemethanol, decahydro-à,à,4a-trimethyl-8-methylene-, [2R-(2à,4aà,8aá)]- Formula: $C_{15}H_{26}O$ MW: 222(Eudesm-4(14)-en-11-ol)	19.63	1362367	0.2270
30.	Lanceol, cis Formula: $C_{15}H_{24}O$ MW: 220	20.35	787015	0.1311
31.	Aromadendrene oxide-(2) Formula: $C_{15}H_{24}O$ MW: 220	20.64	1126816	0.1877
32.	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol Formula: $C_{10}H_{12}O_3$ MW: 180	20.81	961235	0.1601
33.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol Formula: $C_{20}H_{40}O$ MW: 296	21.67	8270434	1.3778
34.	Undecanoic acid, 10-methyl-, methyl ester Formula: $C_{13}H_{26}O_2$ MW: 214	22.84	422241	0.0703
35.	Isophytol Formula: $C_{20}H_{40}O$ MW: 296	23.14	187661	0.0313
36.	Linolenic acid, methyl ester Formula: $C_{19}H_{32}O_2$ MW: 292	25.04	233691	0.0389
37.	Phytol Formula: $C_{20}H_{40}O$ MW: 296	25.17	6375676	1.0622
38.	3,7,11,15-Tetramethyl-2-hexadecen-1-ol Formula: $C_{20}H_{40}O$ MW: 296	26.42	7161676	1.1931
39.	Pregn-5-en-20-one, 3-(acetyloxy)-6,16-dimethyl-, (3á,16à)- Formula: $C_{25}H_{38}O_3$ MW: 386	31.21	31092902	5.1799
40.	4-Chlorocholest-4-en-3-one Formula: $C_{27}H_{43}ClO$ MW: 418	31.63	18437502	3.0716
41.	Pregn-5-en-20-one, 3-(acetyloxy)-16,17-epoxy-6-methyl-, (3á,16à)- Formula: $C_{24}H_{34}O_4$ MW: 386	33.93	19066188	3.1763
42.	Squalene Formula: $C_{30}H_{50}$ MW: 410	35.70	8982577	1.4965
43.	(-)-Coreximine Formula: $C_{19}H_{21}NO_4$ MW: 327	37.08	3479861	0.5797

The PB1F2 Protein sequence was submitted in to PROSITE tool and identifies the binding sites involved in it. PB1F2 Protein is responsible for swine flu and the protein sequence can be retrieved from NCBI Database.

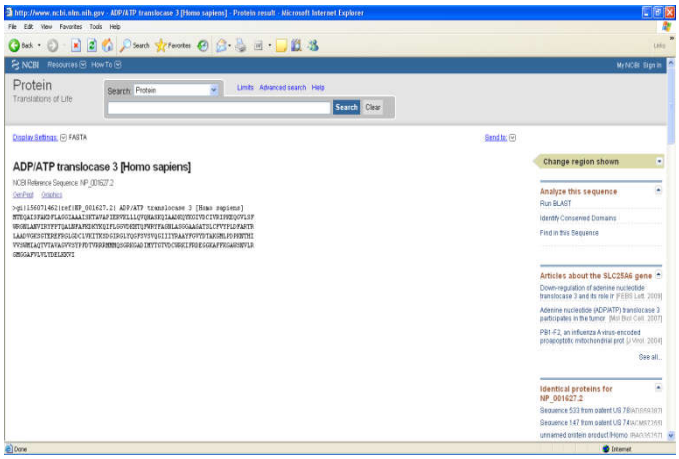


Fig 2:PB1F2 Protein Sequence retrieved from NCBI database

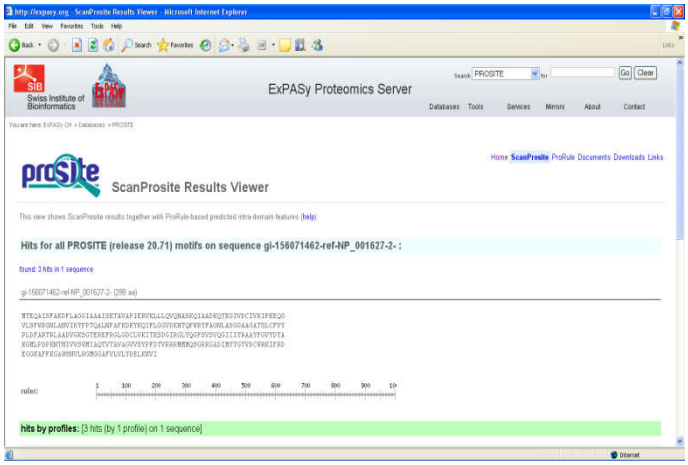


Fig 3: Identification of Binding sites by PROSITE Tool

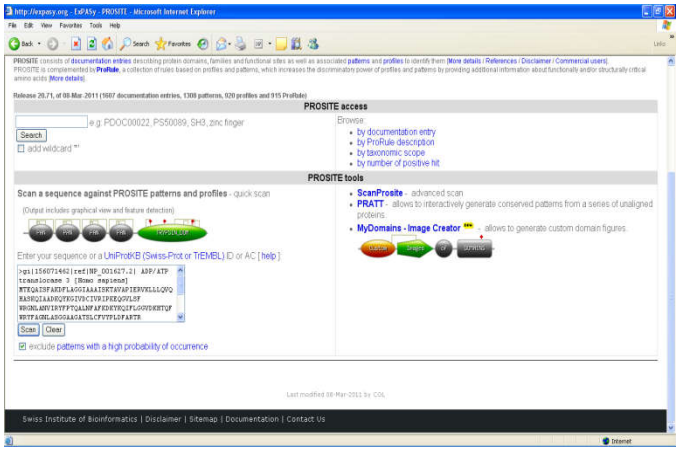


Fig 4: PROSITE Tool shows only one Binding Site

This fig shows that the PB1F2 Protein has only one binding site and it also shows the positions. Fig 5 shows that the receptor PB1F2 docked with the ligand 3LGGZ by Hex tool. The docked structure was also visualized by Rasmol tool. The PB1F2 receptor was also docked with the ligand 3LEE and the docked structure was shown by Rasmol tool.

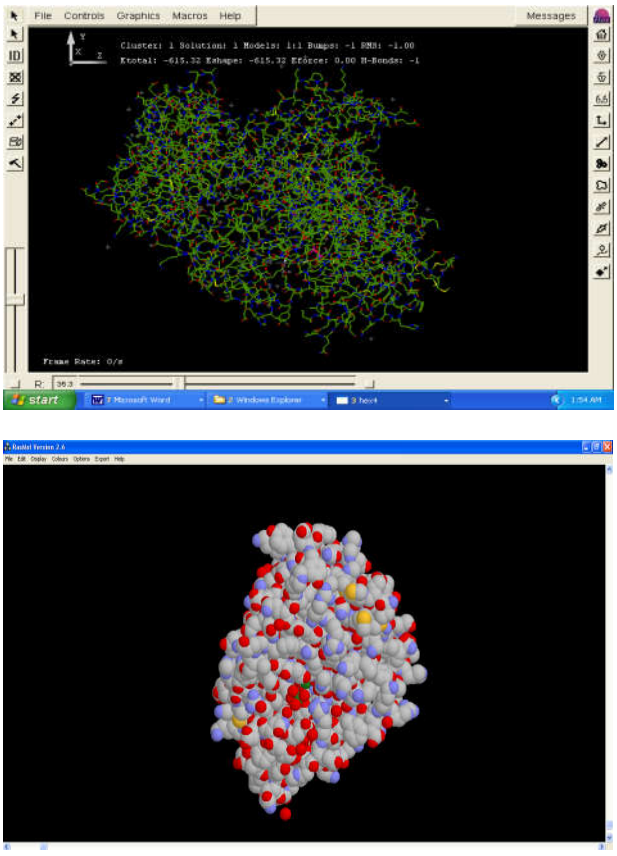


Fig 5: RECEPTOR PB1F2 DOCKED WITH the LIGAND 3LGGZ

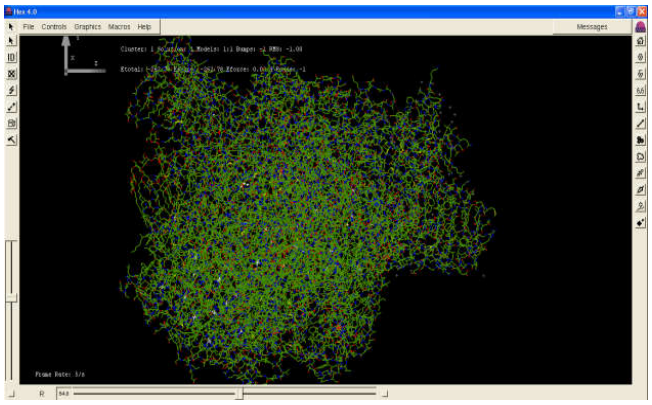


Fig 6: RECEPTOR PB1F2 DOCKED WITH the LIGAND 3PRQ

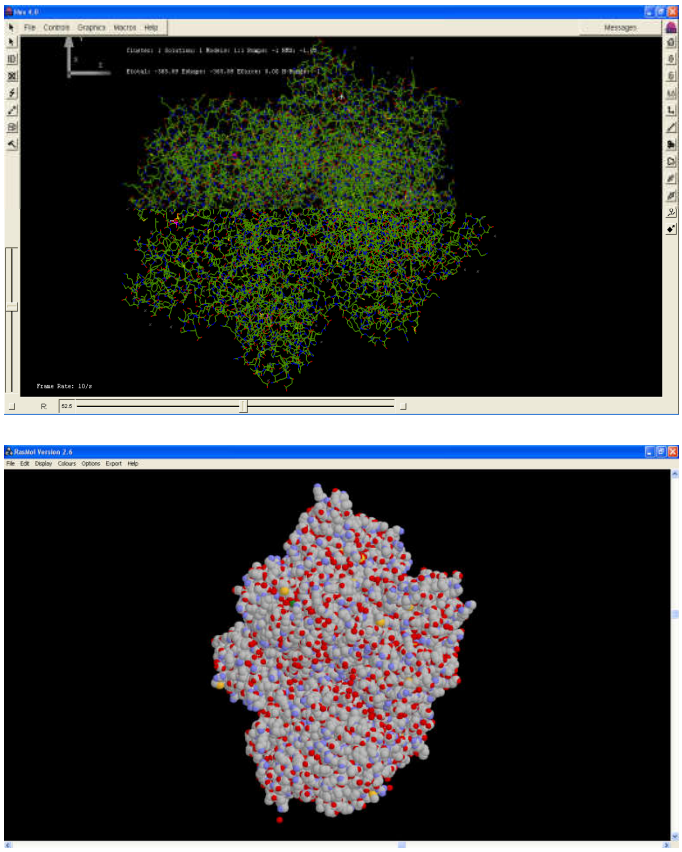


Fig 7: Receptor PB1F2 docked with the ligand 3lee

The PB1F2 receptor was also docked with the ligand 3LEE and the docked structure was shown by Rasmol tool.

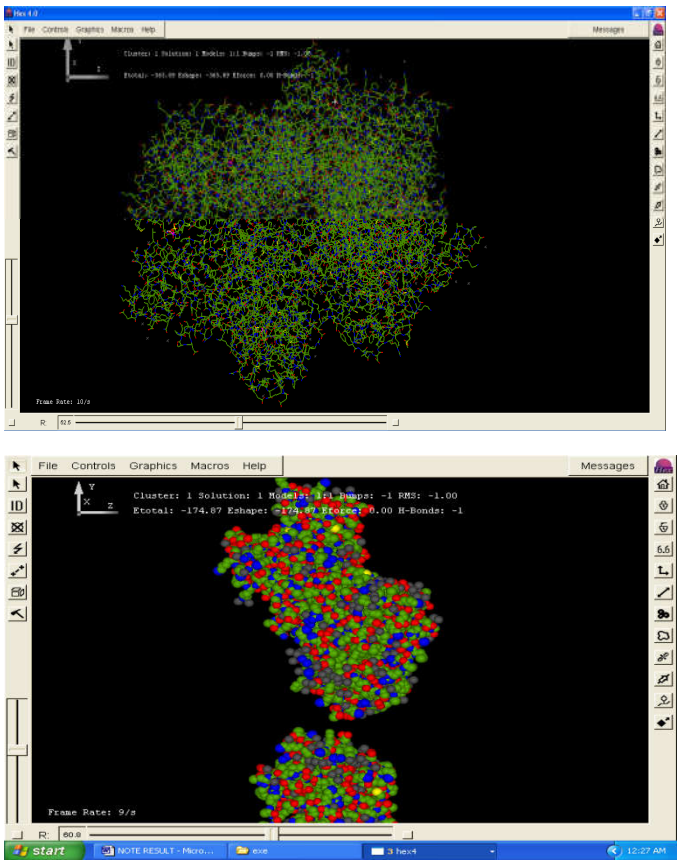


Fig 8: Receptor PB1F2 docked with the ligand 1DI1

The receptor which is responsible for swine flu was docked with the ligand 1DI1 and the docked structure was visualized..

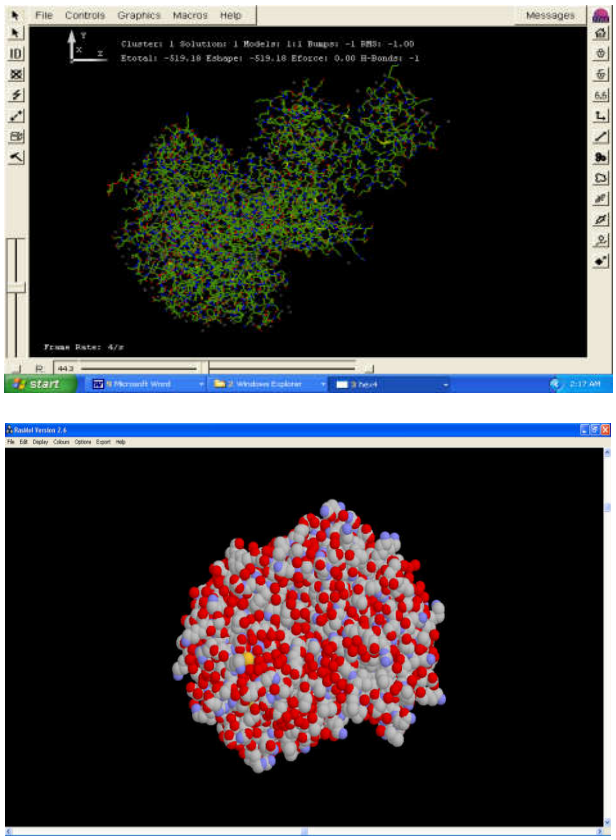


Fig 9: Receptor PB1F2 Docked with Ligand 2ADD

The receptor PB1F2 was docked with the ligand 2ADD and the docked structure was visualized by Rasmol tool.

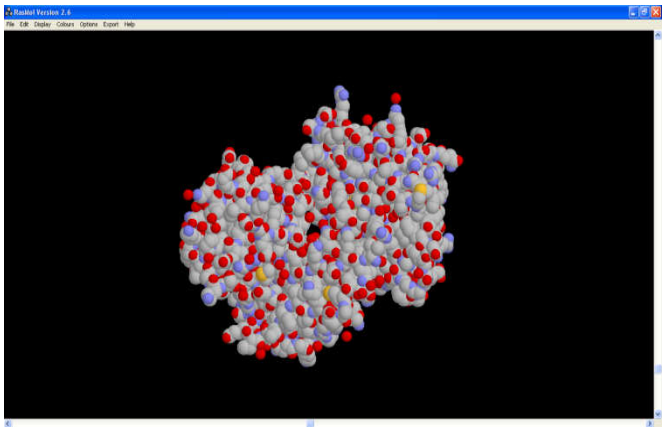


Fig 10: 3D Structure Visualization of PB1-F2 protein by rasmol Tool

Table 2: Docking Score values for all the Receptor-Ligand Interaction

S.No	NAME OF THE DRUG	DOCKING SCORE
1	CARYOPHYLLENE (3LGGZ)	86.17
2	4-CHLOROCHOLESE-4en-3-one (3PRQ)	81.02
3	SQUALENE (3LEE)	78.26
4	GERMACRENE D-4-OL (1DI1)	71.82
5	PHYTOL (2ADD)	88.4

The table 2 shows that the docking score values for the above receptor – ligand interaction. Among the above interactions, PB1F2 and 3LGGZ docking shows the highest score value 86.17.These score values can be calculated by Hex tool during the process of docking.

GENOMIC STUDY

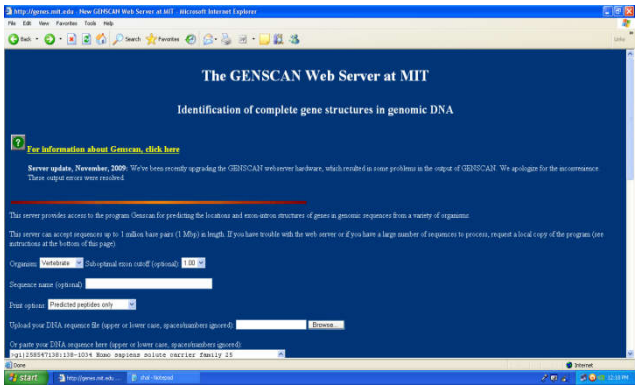
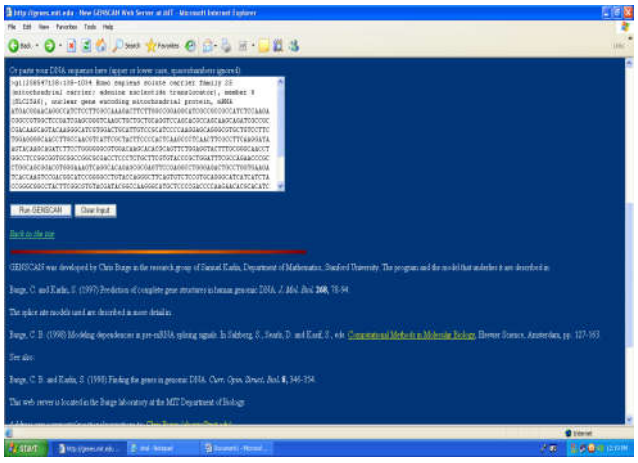


Fig 11: Submission of sequence in Genscan tool

The PB1F2 Protein coded gene sequence was retrieved from NCBI CDS and submitted in to Gen Scan tool for the identification of coding and non coding regions.



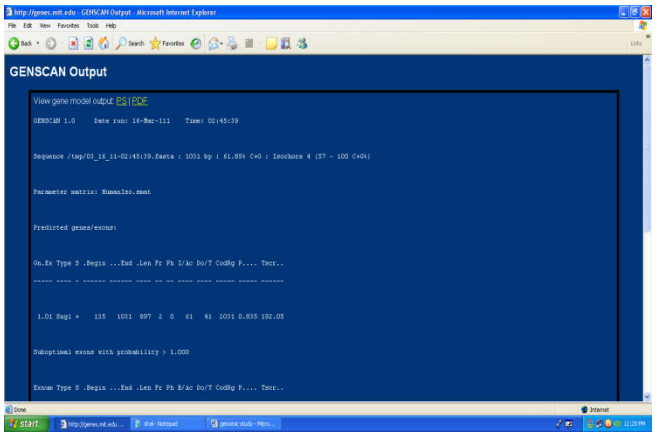


Fig 12: Identification of Exons and Introns using GENSCAN

This fig shows that the identification of coding regions (Exons) and Non coding Regions (Introns).

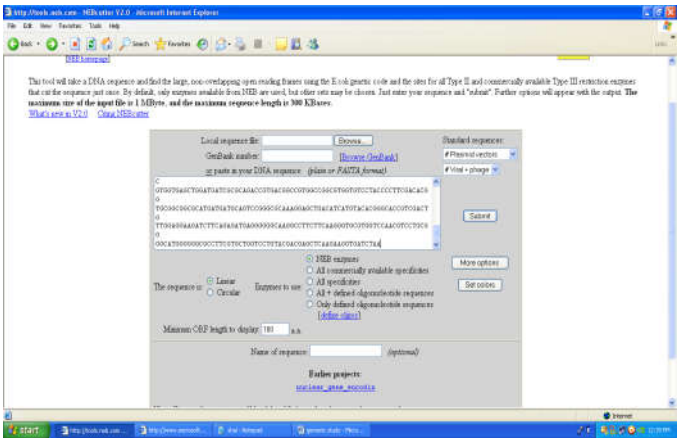


Fig 13: Submission of Sequence to NEB CUTTER

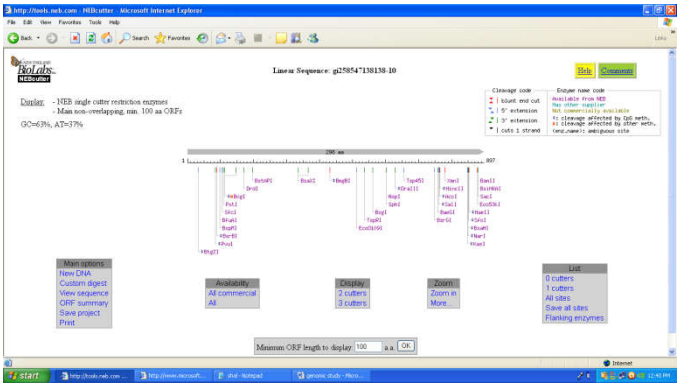
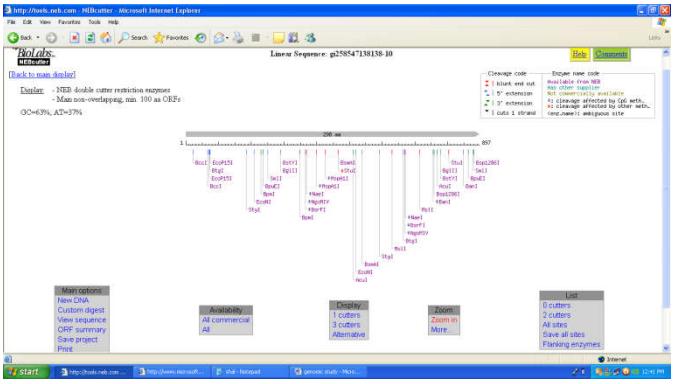


Fig 14: Identification of Restriction Enzymes by NEB CUTTER

It shows the restriction enzymes that are present in the gene sequence identified by NEB Cutter tool.



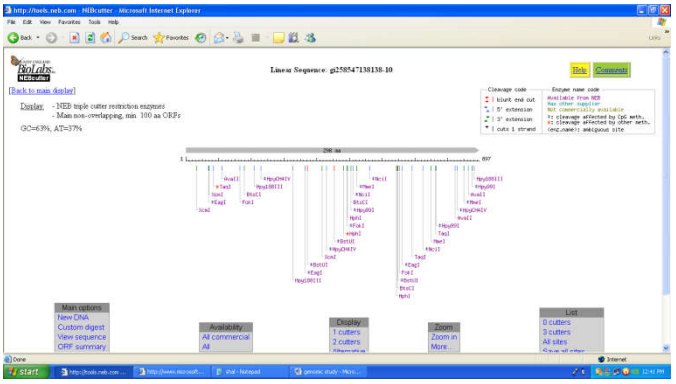


Fig 15: Analysis of Restriction Sites by NEB CUTTER

This fig shows that the restriction sites that are involved in the gene sequence identified by NEB Cutter tool.

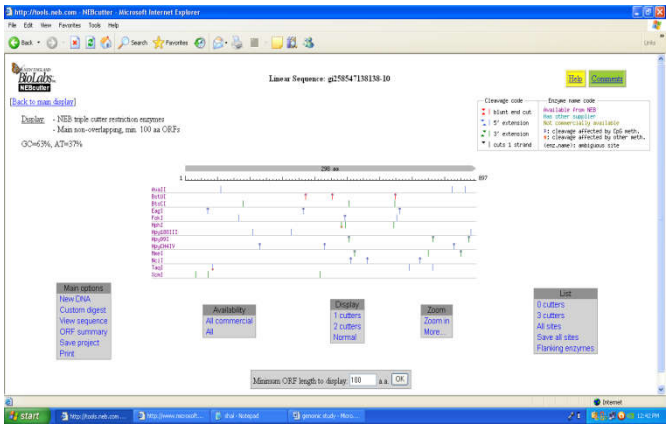


Fig 16: Regions present in the sequence identified by NEB CUTTER

CONCLUSION

This study provides the detailed analysis of phytochemical compounds from *ocimum sanctum* by using GC-MS. It shows the forty three different compounds from the leaves of ocimum sanctum. According to its high peak volume, we choose five compounds for our Studies; they are Caryophyllene, 4-Chlorocholest, 4-en-3-one Germacrene d-4-ol, Phytol and Squalene. The above phytochemical compounds docked with the receptor PB1F2 by Hex tool and calculate the docking scores. We can also focus on Genomic studies to identify the coding regions and predict the structure. The Restriction sites present in the sequence was identified by NEB Cutter tool. Our project will focus that *ocimum sanctum* can act as herbal drug for swine flu. From these observations, we concluded that *ocimum sanctum* act as an anti-viral agent.

REFERENCES

1. Flahault A, Vergu E, Boelle PY. Potential for a global dynamic of Influenza A (H1N1).*BMC Infect Dis.,* 12;(9):129(2009).
2. Le L, Lee E, Schulten, K.Troung. Molecular modeling of swine flu influenza A/H1N1, Spanish H1N1 and avian H5N1 flu N1 neuraminidases bound to Tami flu and Relenza. *PLos Current science.* 27; 1 RRN1015 (2009).
3. Lin CH, Chang TT, Sun MF, Chen HY, Tsai FJ, Chang KL,Fisher M,Chen CY. Potent inhibitor design against H1N1 swine influenza: structure based and molecular dynamics analysis for M2 inhibitors from traditional Chinese medicine database. *J Biomol Struct Dyn.,* 28(4): 471 – 82 (2009)
4. Lin T, Wang G, Li A, Zhang Q, Wu C, Zhang R, Cai Q, Song W, Yuen KY. The hemagglutinin structure of an avian H1N1 influenza A Virus. *Virology.* 15;392 (1):73-81 (2009).
5. Shokeen P, Bala M, Singh M, Tandon V. Invitro activity of eugenol , an active component from Ocimum Sanctum against multiresistant and susceptible strains of Neissseria gonorrhoeae. *Journal of Anti microbial Agents,* 32(2):174-9 (2008)
6. Chen W, Norbury CC, Cho Y, Yewdell JW, Bennink JR.Immunoproteasomes shape immunodominance hierarchies of antiviral CD8(+) T cells at the levels of T cell repertoire and presentation of viral antigens. *J Exp Med.,* 4; 193(11):1319-26.(2001)
7. Geetha RK, Vasudevan DM. Inhibition of lipid per oxidation by botanical extracts of Ocimum Sanctum: in vivo and in vitro studies. *Life Science,* 19; 76(1):218.(2004)
8. Panda S, Kar A.Ocimum Sanctum leaf extract in the regulation of thyroid function in the male mouse.*Pharmacol Res.,*38(2):107-10.(1998)
9. Grienke U, Schmidtke M, Kirchmair J, Pfarr K, Wutzler P, Durrwald R, Wolber G, Liedl KR, Stuppner H, Rollinger JM.Antiviral potential and molecular insight in to neuraminidase inhibiting diarylheptanoids from Alpinia Katsumadai. *Journal of med Chem.,* 28; 53(2):778-86.(2008)
10. Kath RK, Gupta RK. Antioxidant activity of hydro alcoholic leaf extract of Ocimum sanctum in animal models of peptic ulcer. *Indian Journal of Physiol Phramacol., 50* (4):391-6.(2006)
11. Cherkasov A, Ban F, Li Y, Fallahi M, Hammond GL. Progressive docking: a hybrid QSAR docking approach for accelerating in silico high through out screening. *Journal of Med Chem.,* 14; 49 (25):7466-78. (2006)

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