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Research Article

NOVEL *IN SILICO* APPROACH OF ANTI-CANCER ACTIVITY BY INHIBITING HEMOPEXIN PROTEINS WITH *INDIGOFERA ASPALATHOIDES* PLANT CONSTITUENTS AT ACTIVE SITE

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ABSTRACT

Objective: The aim of the study was to investigate the anti-cancer activity using the phytoconstituents of *Indigofera aspalathoides*.

Methods: The plant extract has been largely used as cell proliferation inhibitors. In this study, specific phytoconstituent has been targeted towards matrix metalloproteinases (MMPs).

Results: MMPs are group of proteinases that are associated with cell invasion inhibition and also inhibit proliferation. The C-terminal domain of MMPs mimics the serum protein hemopexin (HPX). According to various literatures, a reason for the failure of MMP as anti-cancer agent is the presence of this HPX binding at the active site.

Conclusion: A novel approach was carried to inhibit this binding by Carotal, (-)-Spathulenol, Tau.-Cadinol proteins from the plant *I. aspalathoides*.

Keywords: Hemopexin, Matrix metalloproteinase, *Indigofera aspalathoides*, Molecular docking, Carotal, (-)-Spathulenol, Tau.-Cadinol.

INTRODUCTION

Matrix metalloproteinases (MMPs) are a class of structural and functional related enzymes involved in altering the natural elements of the extracellular matrix. MMP are associated with cell invasion and proliferation, these MMP have been a greater target for anti-cancer agents but have failed continuously. The main reason for this failure may be the presence of hemopexin (HPX) domain present on the protein inhibits this activity. It is stated that integrin binds to the HPX domain present on the MMP2 which further activates the cell invasion activity [1]. It is also found that for angiogenesis, MMP9 requires HPX domain, and the active site. Cell invasion and angiogenesis being the crucial process for cancer cell growth. HPX proteins are found to bind with heme complexes with most affinity among all the proteins These heme complexes are known to produce oxidative stress to the cell and hence HPX proteins acts as scavengers and interacts with the receptors of the liver cell. Thus, these proteins having anti oxidant activity also helps in heme (iron) turn over [2].

In recent years, the drug discovery process has benefited significantly from computational studies on protein-carbohydrate interactions. Plant constituent and nanoparticles have been successfully used in pharmaceutical applications as a system for drug targeting. The present study aims to inhibit this HPX in order to increase the activity of anti-cancer agents in liver cells. HPX inhibitors were isolated and various bioinformatics tools were used to study the binding efficiency of the inhibitors to the proteins.

METHODS

Plant material - gas chromatography-mass spectrometry analysis

The leaves of the plant *Indigofera aspalathoides* Vahl, belong to the family Fabaceae were collected from Trichy district of Tamil Nadu, India. The leaves were allowed to dry for 10 days at room temperature and then powdered, extracted. The extracts were analyzed in GC-MS [3].

Databases used-sequence isolation

Uniprot

Universal protein resource (UniProt.) knowledge database was accessed at http://www.uniprot.org, which had a home page with the query entry tab, where protein HPX was entered and the details of the protein were obtained. The FASTA sequence for the protein of interest was retrieved and further used for basic local alignment search tool (BLAST) analysis [4].

Protein Data Bank (PDB)

The PDB is a worldwide library of all known 3D structures of proteins. It also consists of information about the DNAs, RNAs along with the protein information. This database can be accessed through http://www.rcsb.org/pdb/home.do [5].

Template identification

BLAST

BLAST is a basic sequence similarity search tool, which compares the query sequence to the sequences present in the databases. The public interface of BLAST, http://www.ncbi.nlm.nih.gov/blast, is a most widely used bioinformatics tool for a sequence similarity search. The BLAST analyses give a large amount of results where it provides all the similar sequences available for the query. It also gives specific scores for every alignment and hence based on these scores, the homology between the sequences was determined. These results were further used for multiple sequence alignment and to understand the evolution of a protein [6].

Global alignment - ClustalW

Multiple sequence alignment was carried out by aligning the two closest sequences first and further adding sequences one by one to the list. There are bioinformatics tool that carry out these alignments of which ClustalW is the widely used tool for multiple alignments it also helps in phylogenetic analysis. This database is accessed at http://www.ebi.ac.uk/Tools/msa/clustalw2/with a home page where a set of sequences to be aligned are given in the entry space. From the sequences that have

greater homology (result from BLAST) are retrieved and analyzed. Further, these sequences along with the sequence of HPX were loaded to the ClustalW, the result page has given scores for every alignment. A cladogram was also obtained to understand the evolutionary pattern [7].

Expert Protein Analysis System (ExPASy) server

The ExPASy is a worldwide web compiled by Swiss Institute Bioinformatics. This tool accessed through http://www.expasy.org/ allows access to a wide variety of databases that are solely dedicated to proteins. ExPASy being the main host to SWISS-PROT, SWISS-2D PAGE, ENZYME, SWISS-MODEL, etc., was started to operate in the year 1993. For predicting the secondary structure of proteins self-optimized prediction method (SOPMA) was used from the available plugins [8].

Secondary structure prediction

Self-optimized prediction method (SOPMA)

SOPMA was started in the year 1995 by Geourjon and Delage, which is widely used for prediction of secondary structure of proteins. The most common secondary structures are alpha helices and beta sheets which decide the stability of a protein [9]. In the home page, the sequence retrieved was entered and the detailed explanation of the secondary structure of the protein was obtained.

Molecular modeling tools

Modeler: Version: MOD9.10 platform: Windows XP

Modeler is a homology modeling tool most widely used to model protein structures in its 3-dimensional form. Since HPX lacked a 3D model, modeller.9.10 was used to model with the sequence. The template for the homology modeling was obtained from the BLAST analysis and the modeler was used for modeling. The model was run in Windows platform, where the results are tabulated and gives more than one predicted model. Further, the models were evaluated with the software to get the required model. This model was further visualized using software and may also be used in docking with specific ligands.

Validation method defined in modeler: Discrete optimized protein energy (DOPE)

Every modeled protein has to be evaluated for its nativeness and hence DOPE was used to evaluate the HPX protein. The lower the DOPE value, the better is the modeled protein. Hence of the predicted models, the model with least score is used for further analysis [10].

Ramachandran plot

Ramachandran plot developed by G N Ramachandran is used to visualize the backbone of the protein. Every protein is made up of amino acids; these amino acids have a change in the angles and are represented in Φ psi and Ψ phi angles. These are used to understand the conformation and also the possible empirical distribution of data points [11].

Saves server

Chimera

Chimera (http://www.cgl.ucsf.edu/chimera/) is a molecular graphics program used to visualize the molecules. With the new available option on multiscale, it has the ability to visualize large-scale molecular assemblies, and the modeled protein was visualized using this tool [12].

PubChem

PubChem is a database designed for the chemical molecules to know their functionality in a biological environment. PubChem, https://pubchem.ncbi.nlm.nih.gov/# maintained by NCBI is most widely used tool as it contains detailed information required by the curator. The three compounds, Carotal, (-)-Spathulenol, Tau.-cadinol were studied using PubChem. When these compounds were entered, the 2D/3D structure and the classification of the compound were available. All the information about these compounds were retrieved and used in docking with the protein molecule.

Drug-likeness of the molecules - Lipinski's rule of five

For any molecule to enter into the cytoplasm, the main criteria is to pass through the membrane of the cell, hence Christopher Lipinski postulated 5 rules for a compound to be considered as a lead compound which in case it can permeate through the plasma membrane.

Lipinski *et al.* sorted around 2000 compounds and studied its physiochemical properties and presented that the molecular weight of the compound must be <500, the logP (logarithmic partition coefficient value between the water and 1-octonal) should be <5, the number of groups accepting hydrogen atoms and donating hydrogen atoms to form hydrogen bonds must be <5 [13,14]

Molecular docking tools: PyRX

PyRX is a standalone tool used for visual screening of bioassays. Virtual screening has now become an effective tool in the field of drug discovery as it filters the molecule as it scores these compounds [15]. Before starting with the visual screening, the AutoDock was performed with the protein molecule was docked with compound using AutoDock. The AutoDock software works with two compartments, the AutoGrid was run first, and then the AutoDock was run. After the docking was complete, the results were tabulated, in which the binding energy of at which the compounds docks to molecule becomes the crucial criteria. The confirmation with lowest binding energy is taken as the best docked model. After AutoDock, PyRX was run for visualizing the docked complex.

RESULTS

The GC-MS analysis was carried out and the chromatogram was obtained and nearly 36 peaks were obtained and the phytoconstituents are identified as shown in the Figs. 1 and 2.

Isolation of sequence - HPX - P02790

Protein sequences were isolated from protein knowledge database.

>sp|P02790|HEMO_HUMAN HPX OS=Homo sapiens GN=HPX PE=1 SV=2

MARVLGAPVALGLWSLCWSLAIATPLPPTSAHGNVAEGETKPDPDV
TERCSDGWSFDATT

LDDNGTMLFFKGEFVWKSHKWDRELISERWKNFPSPVDAAFRQGH
NSVFLIKGDKVWVYP

PEKKEKGYPKLLQDEFPGIPSPLDAAVECHRGECQAEGVLFFQGDRE
WFWDLATGTMKER

SWPAVGNCSSALRWLGRYYCFQGNQFLRFDPVRGEVPPRYPRDVRDYFM
PCPGRGHGHRN

GTGHGNSTHHGPEYMRCSPHLVLSALTSDNHGATYAFSGTHYWRLDTSRD
GWHSWPIAHQ

WPQGPSAVDAAFSWEEKLYLVQGTQVYVFLTKGGYTLVSGYPKRLEKEVGT
PHGIILDSV

DAAFICPGSSRLHIMAGRRLWWLDLKSGAQATWTELPWPHEKVDGALCM
EKSLGPNSCSA

NGPGLYLIHGPNLYCYSDVEKLNAAKALPQPQNVTSLLGCTH

Secondary structure prediction

Proteins were subjected to secondary structure prediction. Using various secondary structure prediction tools present in the ExPASy server such as Jpred, GOR, and SOPMA, finally analysis was performed using SOPMA tool.

The result of the secondary structure prediction through the SOPMA tool provides the information about the presence and position of the various

secondary structures of protein, *viz*., helices, sheets, turns, and coils. The number of alpha helices, beta barrels, extended strands, beta turns, bend regions, random coils, and various other states, and their positions along the amino acid sequence length is provided in the results. Furthermore, there is a graphical representation of the frequency and the density of the various secondary structures in the protein. The parameters, window width, similarity threshold, and the number of states, are set as 17, 8, and 4, respectively. A typical SOPMA result page appears as shown below with color codes for various structures (Fig. 3).

Molecular modeling of HPX

The sequence of HPX was collected from UniProt. And its corresponding sequence ID is P02790. It consists of 462 amino acids. This sequence was subjected to BLAST against PDB, using the BLAST tool offered by NCBI. Later, the templates were selected on the basis of structural hits and its alignment pattern against the query sequence. The selected templates were as follows: Chain A of 1GEN, chain A of 1HXN and chain P belonging to 1QHU. All the structures corresponded with the above-mentioned PDB ID`s retrieved from PDB. Templates and their identity with the HPX sequence are defined in Table 1.

Molecular modeling using advanced modeling package provided 5 modeled structures. Among them, the best modeled structure was chosen with the help of a DOPE score. The DOPE score belonging to the best modeled structure was −50059.386719. The stereochemistry qualities of the structures were validated with PROCHECK structural validation tool. PROCHECK results clearly indicated the higher fidelity of modeled HPX structure.

Ramachandran plot plugin is most widely used tool for running the Ramachandran plot. From the plot, modeled structure of hemopexin obtained using chimera. Structure is given with the combination of cartoon and surface model with 60% transparency (Fig. 4 and Table 2).

Table 1: Templates used in molecular modeling

S no.	Template (PDB)	Chain	Length	Identity score with cox-2 sequence%
1	1QHU	A	460	80.00
2	1GEN	A	218	19.00
3	1HXN	A	219	83.00

PDB: Protein data bank

Table 2: Analysis of Lipinski's rule of five for the verification of drug linkages of the compounds using PubMed database

S no.	PubChem compound id	Log p	H-Bond donor	H-Bond acceptor	Molecular weight (dt)
1	4631	2.1	2	4	265.34
2	12389	7.2	0	0	198.38
3	12391	7.7	0	0	212.41
4	12409	15.3	0	0	408.78
5	12412	19.1	0	0	506.97
6	14350	3.6	0	1	220.35
7	19725	4.5	0	0	204.35
8	37839	2.4	0	2	232.31
9	85998	3	1	1	168.27
10	91354	4.7	0	0	204.35
11	101708	4.1	0	0	204.35
12	122544	8.6	1	1	412.69
13	313074	6.4	0	6	490.67
14	403919	5.2	0	0	204.35
15	519329	7	0	0	272.46
16	519960	4.9	0	0	204.35
17	521405	7.1	0	0	272.46
18	522266	3.1	1	1	220.35
19	530816	4.5	0	0	204.35
20	536919	4.3	3	5	436.62
21	541141	5.1	1	4	388.54
22	623309	5.9	1	1	290.48
23	625348	4.9	0	1	286.45
24	5280435	8.2	1	1	296.53
25	5281520	4.5	0	0	204.35
26	5354499	4.4	0	0	204.35
27	5363271	6.9	1	1	394.63
28	5365019	9.4	0	3	498.78
29	5369926	3.8	0	1	218.33
30	6421261	5.9	0	2	316.47
31	6428986	4.8	0	0	204.35
32	6429185	3.3	1	1	222.36
33	6436582	4.7	0	0	204.35
34	6440942	3.2	0	1	218.33
35	10085645	5.9	0	1	290.48
36	10899740	4.7	0	0	204.35
37	10901750	6.9	0	0	272.46
38	53486397	6.5	0	2	318.49
39	44559813	6.9	0	0	272.46
40	56927938	7.2	0	0	272.46

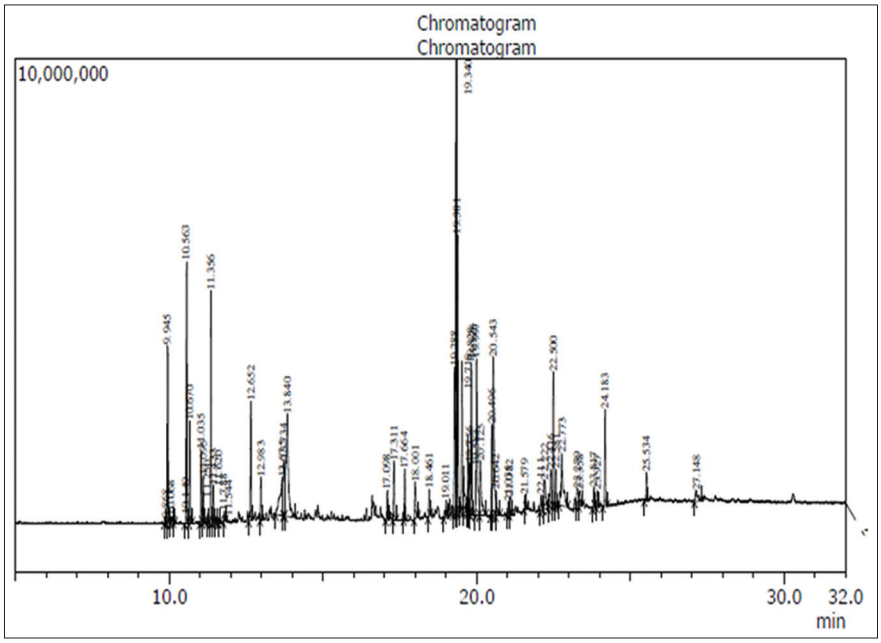

Fig. 1: GC-MS analysis of the plant extract

Peak Report TIC

PEAK#	R.TIME	AREA	AREA%	NAME
1	9.868	197463	0.10	(+)-CYCLOISOSATIVENE
2	9.945	5886795	2.92	Copaene
3	10.068	251740	0.12	.alpha.-Bourbonene
4	10.149	206389	0.10	n-Tetradecane
5	10.563	8728520	4.32	(-)-BETA-CARYOPHYLLEN
6	10.670	3365956	1.67	.alpha.-Bergamotene
7	11.035	2173873	1.08	.ALPHA.-CARYOPHYLLENE
8	11.095	1465590	0.73	ALLOAROMADENDREN
9	11.250	274941	0.14	(-)-.ALPHA.-AMORPHENE
10	11.356	7226463	3.58	GERMACRA-1(10),4(15),5-TRIENE, (-)-
11	11.433	1064672	0.53	PENTADECANE
12	11.544	257400	0.13	AROMADENDRENE
13	11.620	146076	0.07	BETA-BISABOLEN
14	11.788	386050	0.19	tau.-Cadinol
15	12.652	4386104	2.17	Caryophyllene oxide
16	12.983	1598907	0.79	Carotol
17	13.675	5570210	2.76	ETHANOL, 2-(3,3-DIMETHYLBICYCLO[2.2.1]HEPT-2-YLIDENE)-
18	13.734	3426747	1.70	VALERENAL
19	13.840	11645595	5.77	Ethyl iso-allocholate
20	17.098	1062080	0.53	TRACHYLOBANE
21	17.311	2288836	1.13	Manoyl oxide
22	17.664	1798282	0.89	(-)-Kaurene
23	18.001	1668769	0.83	Phytol
24	18.461	993927	0.49	4,6,6-Trimethyl-2-(3-methylbuta-1,3-dienyl)-3-oxatricyclo[5.1.0.0(2,4)]octane
25	19.011	1031462	0.51	TETRAHYDROISOVELLERAL
26	19.288	6979145	3.46	Methyl cis-5,8,11,14,17-Eicosapentaenoate
27	19.340	43096766	21.35	ROSA-5,15-DIENE
28	19.381	8714410	4.32	(-)-16.ALPHA.-KAURANOL
29	19.525	6112457	3.03	Phyllocladene, (-)-
30	19.583	2666072	1.32	KAUR-16-EN-19-OL
31	19.718	3101360	1.54	Pregnane-3,17,20-triol, (3.alpha.,5.beta.,20S)-
32	19.756	1605013	0.80	1-Phenanthrenecarboxaldehyde, 7-ethenyl-1,2,3,4,4a,4b,5,6,7,9,10,10a-dodecahydro-1,4a,7-trimethyl-, [1R-(1.alpha.,4a.b
33	19.828	5911438	2.93	PIMARA-7,15-DIEN-3-ONE
34	19.997	8921947	4.42	Biformene
35	20.125	3685277	1.83	Prasterone
36	20.496	3741448	1.85	Methyl 3,12-bis(acetyloxy)cholan-24-oate
37	20.543	9510064	4.71	(-)-Kaurene
38	20.642	1475103	0.73	Cholan-24-oic acid, 3,12-bis(acetyloxy)-, methyl ester, (3.alpha.,5.beta.,12.alpha.)-
39	21.035	554401	0.27	trans-Retino
40	21.082	624260	0.31	(-)-Kaurene
41	21.579	558716	0.28	(-)-KAUR-16-EN-19-OIC ACID METHYL ESTER
42	22.111	897698	0.44	(-)-Kaurene
43	22.222	2644965	1.31	ANDROST-5-EN-3-OL, 4,4-DIMETHYL-, (3.BETA.)-
44	22.416	2296937	1.14	Androst-5-en-7-one, 3-(acetyloxy)-, (3.beta.)-
45	22.500	5168888	2.56	Rimuen
46	22.581	1749924	0.87	KAUREN-19-YL-ACETATE
47	22.773	5362227	2.66	Cholest-22-ene-21-ol, 3,5-dehydro-6-methoxy-, pivalate
48	23.279	1468561	0.73	ANDROSTA-2,16-DIENE
49	23.358	1011174	0.50	(-)-SPATHULENOL
50	23.817	633182	0.31	3-Acetoxy-bisnor-5-cholenic acid
51	23.927	629274	0.31	3.alpha.,5.alpha.-Cyclo-ergosta-7,9(11),22t-triene-6.beta.-ol
52	24.183	3329218	1.65	NONACOSANE
53	25.534	1164871	0.58	n-Hexatriacontane
54	27.148	1122821	0.56	Stigmasterol
		201840464	100.00	

Fig. 2: List of phytoconstituents isolated from *Indigofera aspalathoides* by using GC-MS

Virtual-screening

Energy minimization with universal force field was done to the modeled structures and small molecules using steepest descent method. These compounds were converted into the input file format, namely PDBQT. During this process, drugs that had not been properly minimized and those not supported for conversion were eliminated from the list. The modeled structure was fixed as a potential target for virtual screening [15]. Finally, virtual screening studies were performed for all the converted drug components against three modeled protein structures, using Vina Wizard available in PyRX-0.8 software. In the end, efficiency of all the ligands was analyzed using binding energy value predicted by PyRX-0.8 software. Binding energy is the sum of the intermolecular energy and the torsional free-energy penalty, with a more negative binding energy representing a stronger inhibition. Virtual-screening results are given in the Table 3 (Fig. 5).

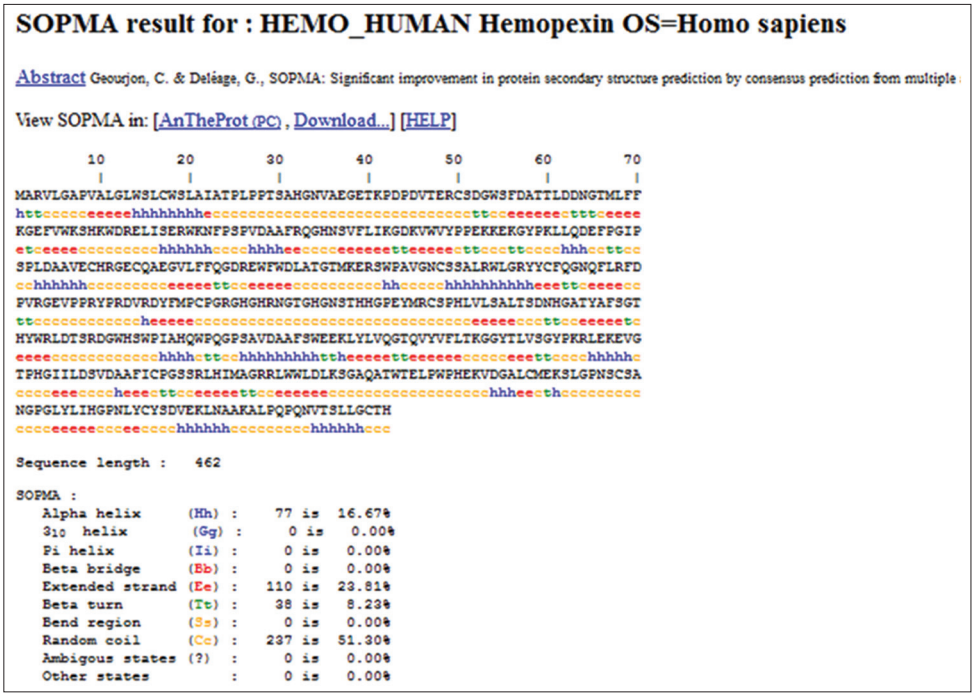

Fig. 3: Secondary structure prediction after self-optimized prediction method analysis

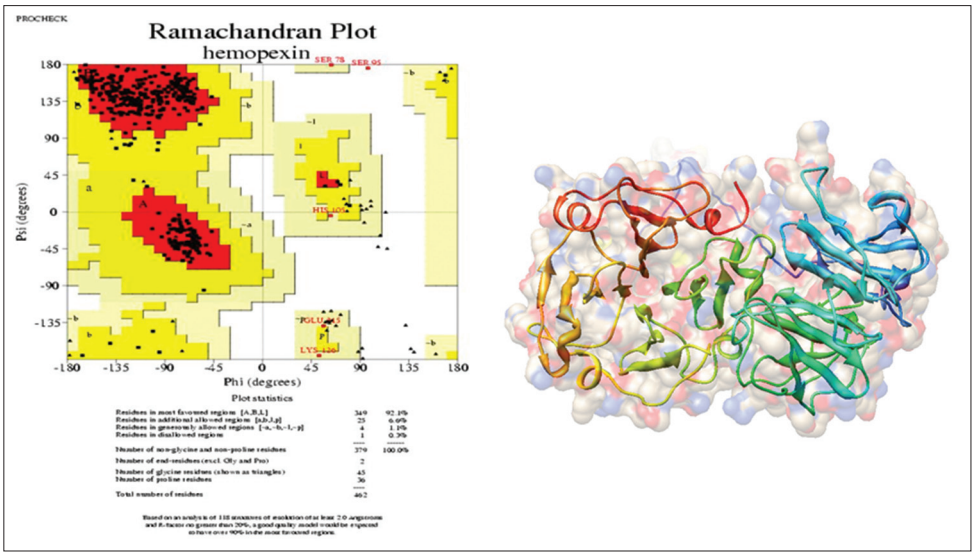

Fig. 4: A model structure of hemopexin using Chimera, Ramachandran plot

Table 3: Virtual screening results

S. No.	Compound	Protein	Binding energy	Ligand efficiency	Inhibitory constant	Reference RMS	Hydrogen bond
1	4631	HPX	−3.53	−0.19	2.58	15.78	ARG 289 TRP 194
2	522266	HPX	−7.72w	−0.48	2.21	17.49	HIS 236
3	6429185	HPX	−7.76	−0.49	2.04	15.27	TYP 199 ARG197

RMS: Root mean square, HPX: Hemopexin

DISCUSSION

MMP are known to be involved in preventing cell proliferation and invasion. However, hugely HPX s proteins that are encoded by HPX gene act as inhibitors of MMP thus are minimizing the antiangiogenic activity of MMP [1]. Thus in this work; we aimed to inhibit the HPX protein using chemical compounds extracted from plant sources. The protein coding 462 amino acid sequence length was retrieved from Uniprot [16] and further used for analysis. Predicting the secondary structure of a protein aids in understanding the hydrogen bonds present in the protein which further indicates the structural and functional efficiency.

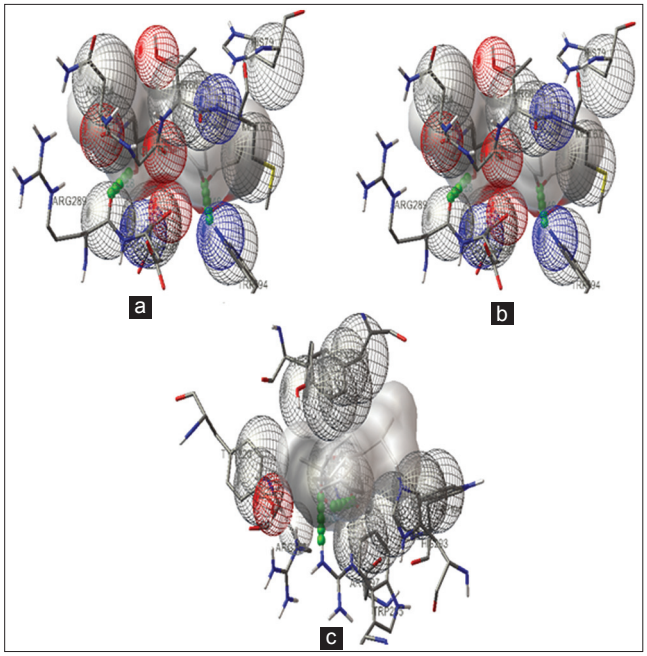

Fig. 5: Structures of hemopexin (HPX) binding. (a) 4631 - HPX, (b) 522266 - HPX, (c) 6429185 - HPX

Secondary structure prediction, though is challenging, can be predicted using advanced bioinformatics tools such as SOPMA, which enable to understand that HPX protein contains about 16.67% and 8% of alpha helices and beta sheaths, respectively. Further, secondary structure also helps in better evolutionary analysis, when a multiple sequence analysis is performed. A BLAST analysis was performed to retrieve homologous sequence for the protein, 3 sequences were available with maximum identity of which 1HXN has an identity of 83% which was used as template in modeling. Modeling was carried out using PyRX modeler and the modeled structure was validated using PROCHECK. A DOPE score of −50059.386719 was gained for the modeled protein.

A Ramachandran plot was obtained using PROCHECK, where about 349 amino acids fall in the most favorable region thus a best model of the protein was prepared. From literature survey, large chemical compounds from plant sources are known to be highly efficient inhibitors for the protein [17]. Thus based on the binding energy, the number of compounds was filtered to three which had the least binding energy. The chemical structure of these isolated compounds were obtained from PubChem and further docked and virtual screening analysis was performed using PYRX-0.8. From the results of virtual screening the least binding energy of - 7.76 was achieved with Tau.-Cadinol and HPX modeled protein. Essential oil isolated from plant species show better microbial inhibitory action, where Tau.-Cadinol is found to be present in most of these essential oils [11] (PMID: 21941915). And also from in silico analysis, we can predict that Tau.-Cadinol may be having a better inhibitory efficiency against HPX protein.

CONCLUSION

MMPs are used as cell proliferation inhibitors where HPX proteins act as inhibitors to this activity, thus leading to failure of anti-cancer activity of these proteases. Thus, further inhibition of these proteins by competitive inhibitory activity may enhance the activity of MMP in inhibiting cell proliferation. Plant compounds are known have an inhibitory effect against HPX, selective identification of the compound present in plant sources may pave the way for better antiangiogenic activity. Thus, in silico analysis has been carried out in discriminating the effective inhibitor. Tau.-cadinol, almost found to be present in most of the essential oils is found to have more binding efficiency toward HPX thereby inhibiting the binding of HPX to MMP and also increasing the anti-cancer activity of MMP.

REFERENCES

1. Van den Steen PE, Van Aelst I, Hvidberg V, Piccard H, Fiten P, Jacobsen C, *et al.* The hemopexin and O-glycosylated domains tune gelatinase B/MMP-9 bioavailability via inhibition and binding to cargo receptors. J Biol Chem 2006;281(27):18626-37.
2. Tudor P, Andrei N, Cristina D, Paul DS, Carmen E. Molecular docking on recomposed versus crystallographic structures of Zn-Dependent enzymes and their natural inhibitors. World Acade Sci Eng Technol 2010;4:8-24.
3. Sermakkani M, Thangapandian V. GC-MS analysis of *Cassia italic* leaf methanol extract. Asian J Pharm Clin Res 2012;5(2):90-4.
4. Magrane M, Consortium U. UniProt Knowledgebase: A hub of integrated protein data. Database (Oxford) 2011;2011:bar009.
5. Helen MB, John W, Zukang F, Gary G, Bhat TN, Helge W, *et al*. The protein data bank. Nucleic Acid Res 2000;28(1):235-42.
6. Johnson M, Zaretskaya I, Raytselis Y, Merezhuk Y, McGinnis S, Madden TL. NCBI BLAST: A better web interface. Nucleic Acids Res 2008;36(Web Server issue):W5-9.
7. Yue F, Shi J, Tang J. Simultaneous phylogeny reconstruction and multiple sequence alignment. BMC Bioinformatics 2009;10 Suppl 1:S11.
8. Gasteiger E, Gattiker A, Hoogland C, Ivanyi I, Appel RD, Bairoch A. ExPASy: The proteomics server for in-depth protein knowledge and analysis. Nucleic Acids Res 2003;31(13):3784-8.
9. Kwok SC, Mant CT, Hodges RS. Importance of secondary structural specificity determinants in protein folding: Insertion of a native beta-sheet sequence into an alpha-helical coiled-coil. Protein Sci 2002;11(6):1519-31.
10. Ward BM, Moss B. Visualization of intracellular movement of vaccinia virus virions containing a green fluorescent protein-B5R membrane protein chimera. J Virol 2001;75:4802-13.
11. Congjin C, Zhangfa T, Dankui L, Yue L, Guoen Y, Mingfei LI. Chemical composition and antimicrobial and DPPH scavenging activity of essential oil of *Toona sinensis* (A. Juss.) Roem from China. Bioresources 2014;9(3):5262-78.
12. Goddard TD, Huang CC, Ferrin TE. Software extensions to UCSF chimera for interactive visualization of large molecular assemblies. Structure 2005;13(3):473-82.
13. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 2001;46(1-3):3-26.
14. Lipinski CA. Lead - and drug-like compounds: The rule-of-five revolution. Drug Discov Today Technol 2004;1(4):337-41.
15. Utomo DH, Widodo N, Rifa'i M. Identifications small molecules inhibitor of p53-mortalin complex for cancer drug using virtual screening. Bioinformation 2012;8(9):426-9.
16. UniProt Consortium. Update on activities at the Universal Protein Resource (UniProt) in 2013. Nucleic Acids Res 2013;41:D43-7.
17. Jain E, Bairoch A, Duvaud S, Phan I, Redaschi N, Suzek BE, *et al.* Infrastructure for the life sciences: Design and implementation of the UniProt website. BMC Bioinformatics 2009;10:136.