https://doi.org/10.48047/AFJBS.6.14.2024.5340-5361

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

In Silico Network Pharmacology and Docking studies in identifying molecular targets of Hirsutine in cervical cancer

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Article History
Volume 6, Issue 14, 2024
Received : 02 May 2024
Accepted : 14 Aug 2024
doi: 10.48047/AFJBS.6.14.2024.5340-5361

Abstract
.Cervical cancer (CC) is one of the leading cancers in women worldwide The strategic increase in the frequency of occurrence of cervical cancer is a great challenge for clinical management. HPV infection is the primary cause for cervical cancer. The current therapeutic approaches and treatment methods like surgery, radiation, chemotherapy etc have many side effects. Phytochemicals are abundant in nature but underutilised, one of them is Hirsutine, an indole alkaloid compound from Uncaria rhynchophylla, proven to have anticancer activity. The study involves understanding of molecular mechanisms in vitro anticancer activity. The molecular targets of Hirsutine are predicted by Swiss Target Prediction and SEA databases.The key regulators of cervical cancer were obtained from Genecards and OMIM databases. In Silico network pharmacology analysis of compound target is constructed by using STRING and Cytoscape databases , PPI network is constructed and hub genes were selected based on PPI network and literature studies.The Gene Ontology enrichment and KEGG pathway analysis were obtained from shinyGo database which play important role cancer pathogenesis. Molecular docking studies showed high binding affinity for selected key genes including hydrogen bonding and Vander Waals interaction. In conclusion the in-silico network pharmacology and docking studies of Hirsutine .identified new drug targets cancer progression

Keywords:Hirsutine,Insilico analysis, cervical cancer, Network pharmacology, docking studies

:Introduction

The incidence of cancers has been on a rise globally with more than 19,292,789 new cases and 9.6 to 10 million deaths till 2023 and approximately 26,300 as of 2024 (Report by Global Cancer Observatory). In comparison with other cancers, cervical and breast cancers .have the highest incidence rates of around 2,261,419 and 664,127 new cases respectively

The fourth most common cancer is Cervical cancer, which is the major cause of death in females with a 51.7% mortality rate.According to WHO around 660,000 new cases were recorded in 2022. In 38 developing, low- and middle- income countries, including many in Sub-Saharan Africa, Central America and South- East Asia; cervical cancer is the most common cause of morbidity and mortality in women. In India, every year 122,844 women are diagnosed and 67,477 die from the disease [1]. Cervical cancer is the carcinoma of the uterine cervix, the lower part of the uterus or womb which connects to the vaginal interior. Cervical cancer manifests either as squamous cell carcinoma (75-80% cases) or adenocarcinomas (20% of cases) [2]. Human Papillomavirus (HPV), a small non-enveloped virus which belongs to the papillomavirus family, is the primary cause of malignant cervical cancer. More than 200 HPV genotypes are identified till date and are classified into cutaneous/low-risk HPV and mucosal/high-risk HPV. While low-risk HPV are associated with benign cutaneous lesions that often form warts, high-risk HPV (oncogenic) leads to mucosal infection that progress to cancer. Approximately 99.7% of cervical cancers are caused by high-risk (hrHPV) genital human papillomavirus. HPV-16 is the most prevalent high-risk HPV followed by HPV-18 responsible for approximately 70% of cervical cancers [3]. Healthy epithelial cells of the cervix expand and manifest as tumour upon persistent infection when left untreated or when .host cellular functions are deactivated by the virus [2]

Progression into tumour typically takes 15-20 years and people with infection are often asymptomatic during early stages. The virus is often cleared from the body by the immune ,system but several risk factors such as weakened immune system, infection with HIV smoking, sexual behaviour, repeated pregnancies, use of hormonal contraceptives can lead to persistent HPV infection and cancer progression [4]. Upon infection with HPV, cervical epithelial cells undergo premalignant transformation into cervical intraepithelial neoplasia (CIN). If left untreated in early stages, CIN progresses to carcinoma. Cervical cancer can also metastasize to rectum, vagina, bladder, lungs, liver, abdomen [5]. Current treatment methods .for cervical cancer include surgical excision, chemotherapy, radiotherapy and immunotherapy These methods are used either as monotherapy or combination therapy depending on stage and extent of cancer progression. In case of localised cancers, surgical excision and radiotherapy are preferred; while chemotherapy alone or in a combination with radiation are preferred in treating recurrent and/ metastatic cancer [6]. Recently, targeted immunotherapy in .combination with existing treatment methods has led to improved outcomes in patients

Recently, there has been an increased interest in use of natural products [7] such as polyphenols, a micronutrient of our diet commonly found in fruits, vegetables, legumes, and beverages (tea, coffee, wine) for targeted therapy. Hirsutine (HSN), a primary indole alkaloid of Uncaria plant species, is one such compound of interest. It is mainly found in Uncaria [8] rhynchophylla, a plant genera commonly used as a Chinese herb used to treat symptoms of hypertension and cerebrovascular disorders [9-11]. Recent in vitro and pharmacological studies indicate several therapeutic benefits of Hirsutine. Apart from its use in treating and managing cardiovascular, cerebral, neurodegenerative disorders. [12-15]. Hirsustine also showed migration and invasion properties in breast cancer cell lines.HSN also exerts anti-inflammatory, antiviral, antidiabetic, antihypertensive, antiarrhythmic activity along with .antitumour and anticancer activity [16-20]

Although HSN has shown to have a role in cancer treatment in vitro, the molecular target and its interactions with the target is yet to be elucidated. A new paradigm of drug discovery based on network pharmacological studies and strategies in complex with a biological network analysis can help in identifying the molecular target and understanding crucial drivers to be inhibited for disease control [21-24]. Algorithm-based methodology and

docking respectively can help to determine pharmacokinetics of a compound and its .interaction with target

The literature-based study indicates suppression of cancer by Hirsutine. However, the .molecular targets of the indole alkaloid s in the molecule have not been elucidated

New paradigm of drug discovery [22] is based on the network pharmacological studies and .strategies in complex with a biological network analysis in identification of molecular targets This networking analysis helps in understanding the targets for drug discovery by inhibiting the crucial drivers or hub genes of disease in the pathways. Pharmacokinetics of the compound is determined using algorithm-based methodology. The docking of target and compound .reveals the potential interactions with protein in the drug discovery

Materials and Methods

Data retrieval

Structure of Hirsutine(HSN) in canonical SMILES file format was downloaded in required .format from PubChem website for further studies

(canonical SMILES-CCC1CN2CCC3=C(C2CC1C(=COC)C(=O)OC)NC4=CC=CC=C34)

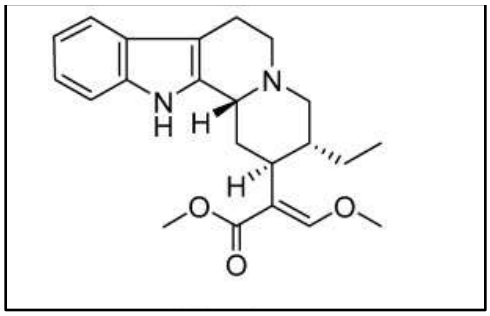


Fig.1 Hirsutine Chemical structure

ADMET profiling and drug likeness studies

ADME profiling -Absorption, distribution,metabolism and excretion , is done using in-silico tool SwissADME tool (http://www.swissadme.ch/index.php)[8] and admetSAR (http://lmmd.ecust.edu.cn/admetsar2/) free online web server .These tools predict physicochemical properties, drug likeness and pharmacokinetic properties which include absorption, distribution, metabolism, elimination of Hirsutine compound .The 2D structure of Hirsutine in canonical SMILES format from the Pubchem database is entered into the .SwissADME

Toxicity studies

ProTox-II tool (http://tox.charite.de/protox_II) online free access web tool for toxicity studies ,which predicts acute & organ toxicity (mutagenicity, immunotoxicity, hepatotoxicity cytotoxicity, carcinogenicity, of Hirsutine Compound. [25].In addition, CarcinoPred (http://112.126.70.33/toxicity/CarcinoPred-EL/about.html) online free web access tool for carcinogenicity study is used in this study [26]. Both the tools are user friendly,self informative with canonical SMILES as input for prediction or drawing box is provided to give compound structure.Protox provides information on acute toxicity and toxicity targets as default if it is .not specified.The user can select single model/ALL models if required

Compound -Target Prediction

The Hirsutine and its biological action were predicted using the Swiss Target Prediction and SEA web servers[27,28,29]. SwissTargetPrediction (http://www.swisstargetprediction.ch/) is a user friendly database for predicting compound targets and generate the biological potential targeted genes of Hirsutine compound .The Similarity Ensemble Approach (SEA) server (https://sea.bkslab. org/), select putative biological target for the Hirsutine. SMILES serve as .the query input for predicting targets which are downloaded and further analysed

Cervical cancer target identification

Cervical cancer targets from the GeneCards database (https://www.genecards.org/) and OMIM (https://www.omim.org/) database are used to retrieve the disease associated genes.The keyword cervical cancer is used for retrieving the cervical cancer genes from the database. The target data of compound and databases are integrated using Venny 2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/)an online tool and are presented in the venn .diagram. The intersected targets of compound and database are further analysed

.Construction of protein-Protein (PPI) network

A Protein protein PPI network was constructed from the intersected genes by uploading the genes to the STRING v_11.0 database (https://string-db.org/) and ShinyGO 0.80 (http://bioinformatics.sdstate.edu/go/).The common targets related to the compound and cervical cancer were manually curated and STRING database is used for mapping[31] The PPI network is constructed in accordance with humans(Homo sapiens) with confidence interval of interaction is set to 0.950. PPI network is built for the uploaded proteins by STRING database and used further for the enrichment analysis where nodes represent the proteins and edges reflect PPI interactions. Furthermore, Cytoscape v 3.9.1 [32] was used for sorting the data based on the topological parameters like degree, closeness, betweenness centrality and clustering. The target hub genes were exported for study of KEGG and Gene ontology .enrichment analysis toShinyGO online tool using Cytoscape software

.Gene Ontology (GO) Enrichment and KEGG analysis

The common targets are exported to ShinyGO 0.77 web server .(http://bioinformatics.sdstate.edu/go/) for KEGG and Gene ontology enrichment analysis[33] In the Kyoto Encyclopedia of Genes and Genomes KEGG pathway analysis top 20 pathways and top10 gene annotations are selected based on FDR<0.05 (False Discovery rate). The bubble scatter plot of GO was constructed and analysed with adjusted P-value to find molecular function, biological function, and subcellular localization relation between the .variables and KEGG pathway with significance value P<0.05

.Molecular docking

The possible interaction was Hirsutine compound with target hub genes KIF11(PDB :ID: 2UYI), AURKA((PDB ID: 1O7L), OPRM1(PDB ID: 8EFO)and ADRA1A (PDB ID 7YMJ) were docked using Autodock-4.software.The Protein PDB ID was retrieved and //:downloaded in the pdb or pdbqt format from Protein Data Bank (https

www.rcsb.org/pages/policies). The compound and target protein interaction is visualised in .the 2 dimensional graphics by using Discovery Studio Visualizer

Results and Discussion

The synthesised prodrug or phytocompounds are screened for the druggability of the ,compound by using SwissADME and admetSAR for predicting physicochemical properties lipophilicity ,pharmacokinetics , druglikeness and medicinal chemistry. Prototox-II and .CarcinoPRED-EL tools are used for determining the toxicity levels of the compound used These two properties are very essential in clinical trials and approval as a drug based on the pharmacokinetics and metabolism data[34] ADME is the software program used in swissADME software for predicting pharmacokinetics of the compound[35] The query input is given in the canonical SMILES format in the box where the result shows bioavailability ,radar where six physicochemical properties were considered1. Lipophilicity 2. size 3.polarity solubility, 5.flexibility 6.saturation.(Fig:3).If the radar plot falls in the pink area is.4 .considered a drug

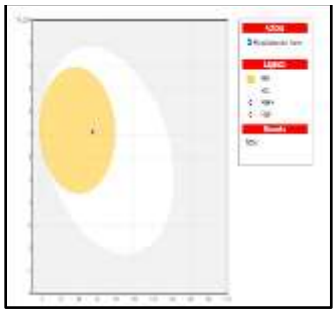


Fig:3 Bioavailability radar

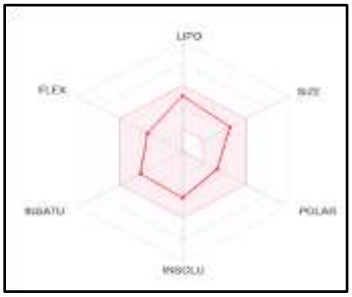


Fig:4 Boiled egg

Physicochemical Properties given in the table for Hirsutine describe ADME properties in a glance .(Table 1a). Lipophilicity (log Po/w) is the partition coefficient between n-octanol ,to water (.There are five predictive models available freely i.e. XLOGP3, WLOGP, MLOGP SILICOS-IT and iLOGP are used for determining partition coefficient and average of the values calculated as consensus logP o/w. Hydrophilicity is one of the important factors determined for oral administration, absorption , formulation. ESOL models were used for predicting water solubility.(Fig:1b).The excretion properties of Hirsutine was analysed bypkCSM (https://biosig.lab.uq.edu.au/pkcsm/prediction_single/excretion_1722347106.91) . with total clearance (0.901 logml/min/kg) and renal OCT2 substrate (yes)

The drug likeness is important in screening of a drug in drug discovery. The acceptance of Lipinski's Rule of Five (Ro5) is criterion for drug likeness.Hirsutine followed all five rules with 0 violations(five rules are molecular weight, XLOGP3 ,no. of hydrogen bond donor and no. of hydrogen bond acceptor).Oral administration of drug is possible if .minimum of two parameters from four basic pharmacokinetic properties satisfied(Fig 1c)[36] Pharmacokinetics properties were predicted by the BOILED-Egg model where a blue dot in yolk indicates it as a safe drug(Table 2). Cytochrome P450 (CYP450) enzymes & its isoforms CYP1A2, 2C9, 2C19, 2D6, and 3A4 plays important role in the metabolism and elimination .of drugs because drug interaction leads to toxic effects in the body[37]

Table:1ADME properties of Hirsutine by SwissADME

a)Physicochemical properties(reference)		b) Lipophilicity		C) Druglikeness	
Molecular weight	g/mol 368.52 (mol/500g ≥)	Log Po/w(iLOGP)	3.55	LipinsKi	Yes,0 violations
Number of heavy atoms	27	Log Po/w(xLOGP3)	3.44 (5 ≥)	Ghose	Yes
Number of aromatic heavy atoms	9	Log Po/w(WLOGP)	3.11	verber	Yes
Number of rotatable bonds	5	Log Po/w(MLOGP)	2.35	Egan	Yes
Number H-bond acceptors	4 (10.6 ≥)	Log Po/w(SILICOS-(IT	3.64	Muegge	Yes
Number H-bond donors	1 (5 ≥)	Consensus Log Po/w	3.22	Bioavailability score	0.55
Molar Refractivity	110.39	Solubility	Moderately soluble		
TPSA	Å2 54.56				

Table:2 ADME Predicted profile of Hirsutine by admetSAR

Model	Result	Probability
Absorption		
Blood–brain barrier	+BBB	0.8507
Human intestinal absorption	+HIA	0.9959
Caco-2 permeability	+Caco2	0.6541
P-glycoprotein substrate	substrate	0.8977
P-glycoprotein inhibitor	Inhibitor	0.9159
P-glycoprotein	Noninhibitor	0.6662
Renal organic cation transporter	Inhibitor	0.5884
Distribution		
Subcellular localization	Mitochondria	0.6820
Metabolism		
CYP450 2C9 substrate	Non-substrate	0.9028
CYP450 2D6 substrate	Non-substrate	0.6706
CYP450 3A4 substrate	Substrate	0.6531
CYP450 1A2 inhibitor	Inhibitor	0.6512
CYP450 2C9 inhibitor	Inhibitor	0.5683
CYP450 2D6 inhibitor	Inhibitor	0.7487
CYP450 2C19 inhibitor	Non -Inhibitor	0.8299
CYP450 3A4 inhibitor	Non -Inhibitor	0.6429
CYP Inhibitory promiscuity	High CYP Inhibitory promiscuity	0.5866
Toxicity		
Human ether-a-go-go-related gene inhibition	Weak inhibitor	0.7098
	Non -Inhibitor	0.5644
AMES toxicity	Non AMES toxic	0.6893
Carcinogens	Non-carcinogens	0.9405
Fish toxicity	High FHMT	0.9834
Tetrahymena pyriformis toxicity	High TPT	0.9884

Honey bee toxicity	Low HBT	0.5962
Biodegradation	Not ready biodegradable	1.0000
Acute oral toxicity	III	0.6614
Carcinogenicity (Three-class)	Non-required	0.6979

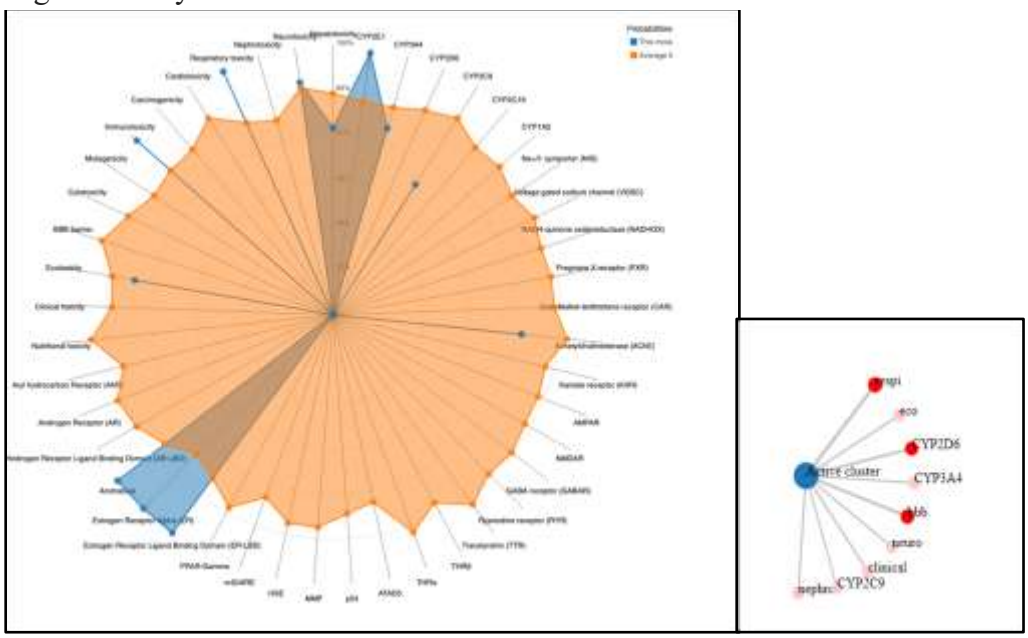
Toxicity studies

ProTox 3.0 is a freely available software tool in the drug designing process .The protoxII provides toxicity analysis on organ toxicity,Toxicity endpoints,Tox21-Nuclear receptor signalling pathways,Tox21-Stress response pathways,Molecular Initiating Events and ,metabolism. This tool accessed many toxicity endpoints like carcinogenicity, hepatotoxicity immunotoxicity, mutagenicity, and cytotoxicity with predicted models from invivo and invitro studies. The criteria used to classify the toxicity levels are given in Table 3. The toxicity predicted class of Hirsutine is class III, Predicted LD50 value is 300 mg/kg.The toxicity ,radar(Fig:5) implies the active cluster for neurotoxicity , nephrotoxicity, respiratory toxicity ,BBB barrier, Ecotoxicity, clinical toxicity, Cytochrome CYP2C9, Cytochrome CYP2D6 Cytochrome CYP3A4 and inactive cluster toxicity for others with different probability scores.[38]

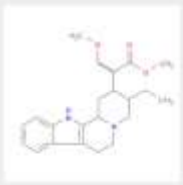
Type	Dosage range of ingestion
Class I	lethal (LD50 ≤ 5 mg/kg)
Class II	lethal (5 mg/kg < LD50 ≤ 50 (mg/kg
Class III	toxic (50 mg/kg < LD50 ≤ 300 (mg/kg
Class IV	≥ detrimental (300 mg/kg < LD50 (mg/kg 2000
Class V	potentially harmful (2000 mg/kg (LD50 ≤ 5000 mg/kg >
Class VI	non-toxic

Table 3.Toxicity analysis

Fig:5 Toxicity radar



The result of the CarcinoPred tool clearly indicates that the hirsutine compound is non carcinogenic based on three ensemble models-SVM, RF, XGBoost predict carcinogenicity of compound using molecular fingerprints and 3 machine learning methods based on a dataset .[39]



Method	CDK	CDKExt	CDKGraph	KR	KRC	MACCS	Pubchem	Average	Class
RF	0.35	0.34	0.26	0.39	0.41	0.34	0.31	0.34	Non-Carcinogen
SVM	0.34	0.33	0.34	0.25	0.30	0.25	0.41	0.32	Non-Carcinogen
XGBoost	0.07	0.19	0.47	0.45	0.42	0.63	0.76	0.43	Non-Carcinogen

Compound -Target Prediction

Swiss Target Prediction and SEA web servers [27,28,29] predict biological activity and potential targets of Hirsutine.Swiss Target Prediction is ligand based target prediction server operational since 2014, to predict and identify protein target for any small molecule synthesized or available bioactive phytomolecule. Predictions for the Hisutine compound were made by assessing 2D and 3D similarity structures with a 0.65 threshold and generating multiple molecular conformations. Target scores were calculated using logistic regression based on similarity scores, and the Swiss Target Prediction tool provided a list of up to 100 potential protein targets with scores from 0 to 1. The Similarity Ensemble Approach (SEA) web server provided a biological target for the query compound Hirsutine based on 2D ligand-based similarity ensemble approach.The SMILES format is the query input format of Hirsutine provide targets. The combined targets of Swiss target prediction and SEA provided targets for the Hirsutine compound which are molecular targets.The duplication of any 123 . target in the both databases is removed and treated as a single target

.Cervical cancer target identification

The cervical cancer targets are downloaded from the GeneCards database (https://www.genecards.org/) and OMIM (https://www.omim.org/) database by searching cervical cancer" as a keyword. The gene cards database provided 9696 targets and OMIM" database with 252 targets for the cervical cancer making total of 9948 targets.These targets of compound and the cervical cancer is integrated in the using the VENNY) and the intersected targets of 73 were/venny/ tools/bioinfogp.cnb.csic.es//2.1.0(https: ,presented in the venn diagram.(Figure 6) BCHE, OPRD1, ADRA1A, ADRA1B, DRD2 ,ADRA2C, HTR1A, ADRA2B, HTR2A, HTR6, ADRA1D, ADORA3, HTR2B, CYP2D6 ,OPRM1, ACHE, KIF11,CYP26A1,CMA1,TACR3,TERT, MDM4, MAPK14, CHEK1 ,OPRL1, TGM2, TGM1, PDE7A, F13A1, CPT1A, SLC27A1, DRD1, SLC6A4, LTA4H ,LCK, PREP, SLC6A9, KCNH2, IMPDH2, FAAH, TRPV1, PPIA, PARP3, MGAT2, NOS1 ,AKT1, TGFBR1, PARP2, RBP4, PSMB5, LGMN, SMO, ABCG2, CASP3, CASP7 ,MAPK11, WNT3A, TNKS2, SLC6A3, MAPK8, SYK, CA12, CA9, IRAK4, MALT1 CACNA1G, NOS3, LRRK2, AURKA, DRD5, KLK7, PDE5A, STAT3.The 73 common protein targets from cervical cancer databases and compound target helps in inhibition of the cancer progression by interaction with these proteins. The 73 targets are sent to Cytoscape for .the protein protein interaction network to find the key regulators in the progression

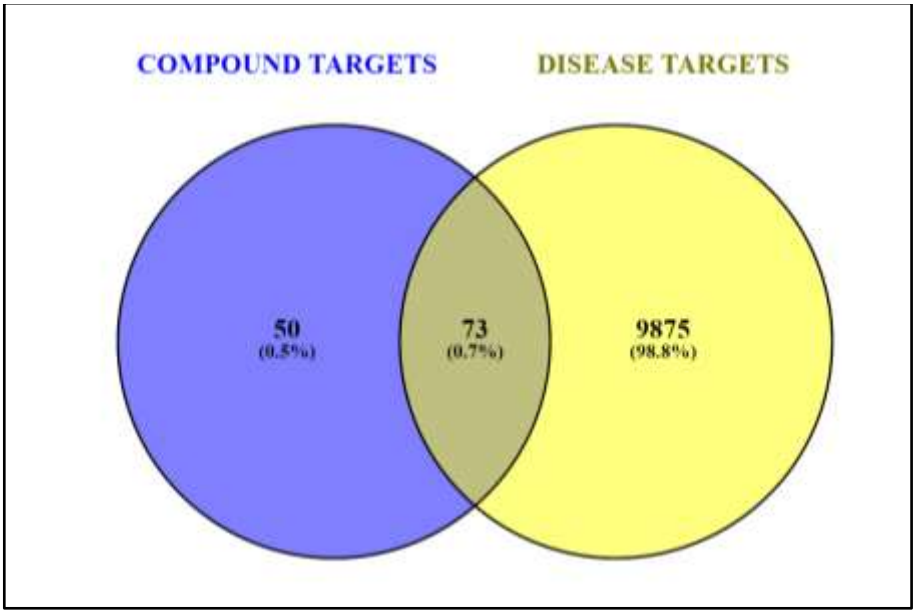
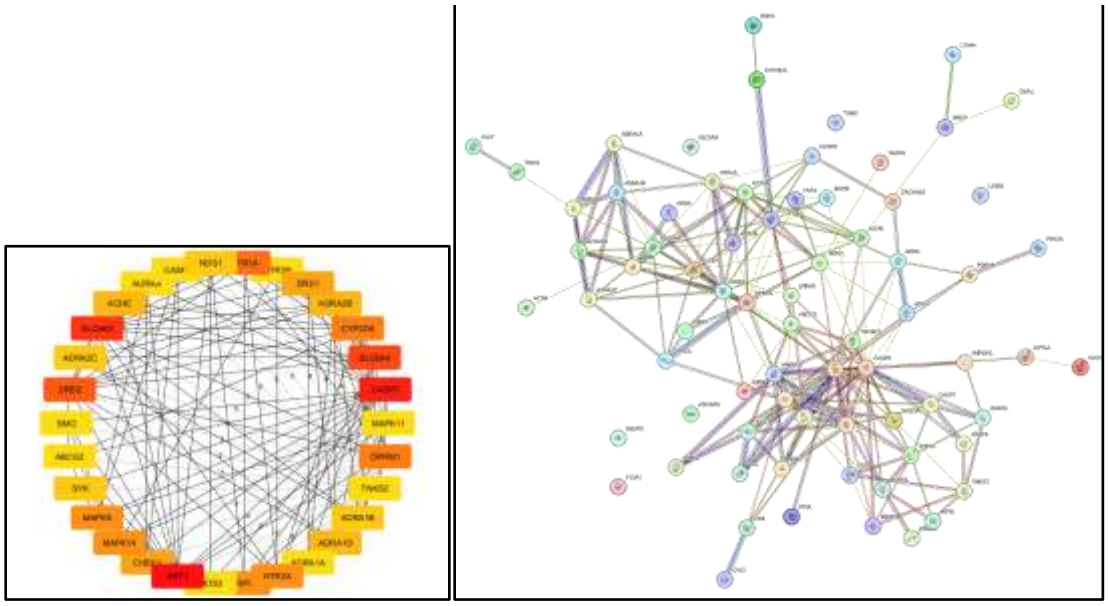


Fig:6 Venn diagram of the compound -(cervical cancer) disease targets

Protein protein interaction

A web based tool STRING (http://stringdb.org; v11.5) is used to construct the protein protein interaction PPI network [41,42] of 73 disease-compound target networks with 22 nodes & 222 edges (Figure) using Cytoscape. The network of the genes from STRING is imported to CytoHubba by Cytoscape tool to test the PPI network and helps in predicting the hub genes in the PPI network and identify the key regulator genes in the network. The genes which are not involved in the interaction are removed. The interaction of hub genes with other

proteins help in understanding the biological importance and role of the proteins involved in it, functional and biological . In the screening process, degree and closeness parameters are used to understand nodes in the network. In the total 73 targeted genes we filtered and selected ,genes is hub genes with key regulatory action by using CytoHubba.The genes are AKT1 30 ,CASP3,SLC6A3, DRD2, OPRM1,SLC6A4,ACHE, HTR1A, TRPV1, CYP2D6, MAPK14 ,NOS1,MAPK8, SMO, CHEK1,HTR2A,ABCG2, NOS3,SYK, BCHE, LRRK2, MAPK11 DRD1,AURKA, HTR2B, CASP7, TERT, MDM4, WNT3A, MALT1.Based on literature study, few other genes cited as druggable hub genes in cervical cancer [43,44] and are also targets of Hirsutine compound predicted by Swiss Target Prediction and SEA server are . selected as hub genes in this study other than hub genes obtained in the PPI network KIF11[45] and AURKA as one of the important genes based on DEG's from GEO datasets and prevalence as pan cancer biomarkers. For docking studies OPRM1,ADRA1A, KIF11,and AURKA are selected to find binding affinity with the Hirsutine compound. The hub genes of compound- disease target and their role in cancer like AKT1-tumour development, MDM4 - Regulator of p53,CASP3 & CASP7 -fragmentation of DNA & morphological changes of apoptosis,(apoptotic death pathway),Trpv1-inflammation & calcium signalling pathway(Tumour suppressor), CYP2D6-association with breast cancer,MAPK - regulate cell proliferation, cell differentiation & cell death, CHEK1-cell cycle progression,BCHE-Prognostic biomarker in endometrial cancer,LRRK2- prognostic biomarker, AURKA-pan cancer expression , TERT -telomerase activity in tumours, WNT3A-activation of Wnt signalling pathway in cancer, MALT1- procaspase and regulate NF- pathway shows the potent .inhibition of hirsutine in different cancers by targeted inhibition of pathways and their proteins

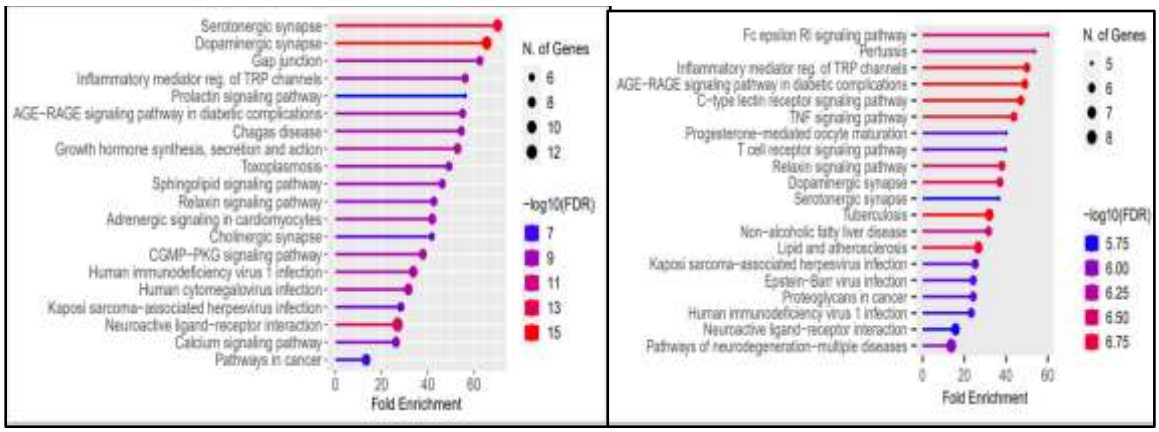


.A .B

Fig 7:Protein protein interaction network (PPI) of compound-(cervical cancer) disease network by String software(A) and hub genes network constructed using Cytohubba plug in of Cytoscape software (B)

.Gene ontology functional enrichment and KEGG pathway analysis

Biological mechanism of Hirsutine against cervical cancer is determined by Gene ontology enrichment studies using ShinyoGo on all 73 targeted genes and 30 hub genes. The gene ontology provides a well organised data with schematic presentation related to molecular . .functions,biological processes and cellular components but the KEGG pathway is considered , The Gene ontology studies are analysed with default setting of threshold of P⩽ 0.05 FDR(false discovery rate) by Benjamini-Hochberg .The Gene ontology enrichment and KEGG pathway analysis high connection with following signalling pathways and their role -in molecular functional pathways of cell of the cell like Fc epsilon RI signalling pathway osteoclast differentiation pathway, inflammatory mediator TRP channels-cancer development and progression, AGE-RAGE signalling pathway-promotes survival pathways in cancer by negative feedback regulation of apoptosis and positive regulation by autophagy, C type lectin receptor signalling pathway- regulate cancer cell invasion, migration, metastasis, TNF signalling pathway- cell proliferation,cell differentiation,apoptosis, induce inflammation ,T ,cell receptor signalling pathway- involves in canonical and noncanonical pathway of NF-k kaposi sarcoma associated herpesvirus infection-associated with many cancers, proteoglycans in Cancer, neurodegenerative disorders, serotonergic synapse, tuberculosis, relaxin signalling pathway, epstein-Barr virus infection, HIV infection, pathways of cancer.In the biological process it is showing significant response to alkaloid ,trans synaptic signalling,cell cell signalling, cell proliferation. In the hub genes molecular function analysis showed correlation with serotonin, amine, cholinesterase, MAPK kinase, serine threonine kinase, tyrosine kinase which has pathogenic role in the cancer and contribute to the tumour development are significant .The cellular component analysis is highly significant in the integral part of synaptic membrane.Based on the study few targets are selected for molecular docking alpha !adrenergic receptor (ADRA1A), cell proliferation (KIF11), cell signalling AURKA, OPRM .Associated with the pain



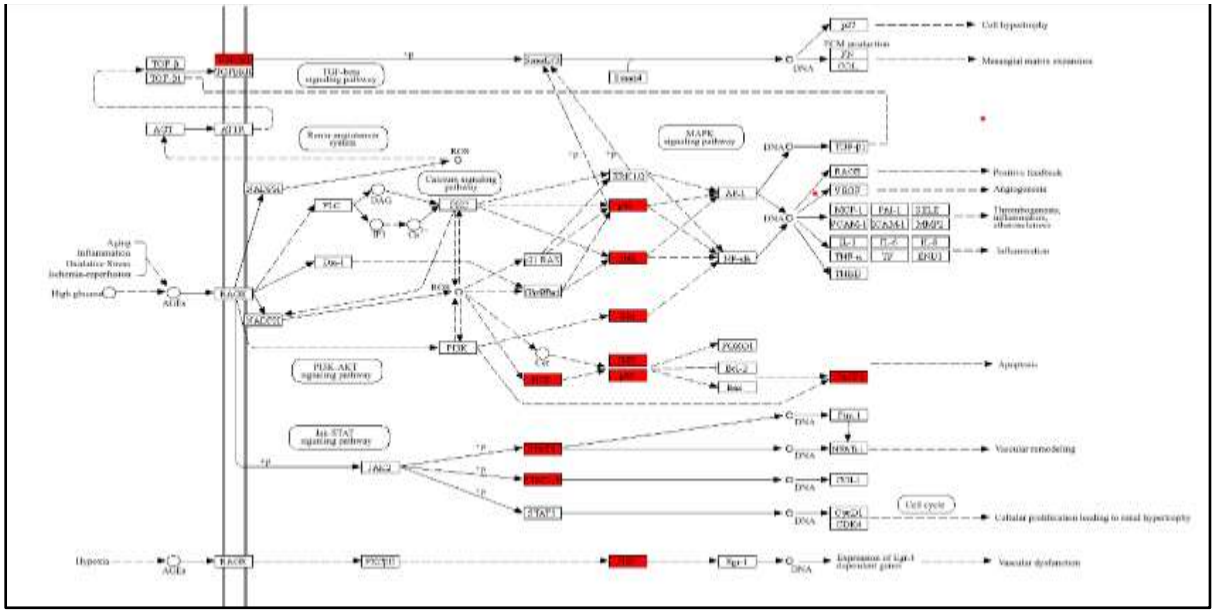


Fig 8: GO and KEGG pathway analysis

: Molecular docking

The molecular docking studies of hirsutine were carried out using Autodock-4.2 software [45]. Crystal structures of KIF11 (PDB ID: 2UYI)is selected based on the literature study, Aurora Kinase A (AURKA) (PDB ID: 1O7L), α1A-adrenergic receptor(ADRA1A) (PDB ID: 7YMJ) and human μ-opioid receptor (OPRM1) (PDB ID: 8EFO) proteins were selected for molecular docking from the hub genes. The hirsutine molecule is downloaded in SDF format or pdbqt format. Molecular docking was carried out with 0.375 Å grid space, KIF11 grid center is x=22.306, y=14.056, z=30.617 and dimensions x=48, y=48, z=52., AURKA grid parameters center is x= 257.186, y=-65.798, z= 107.125and dimensions x=40, y=80, z=50, the ADRA1A grid center is x=106.887, y=112.02, z=127.324 and dimensions x=54, y=58, z= 78,OPRM1 grid parameters set as center is x= 184.67, y=-170.88, z= 174.12 and dimensions are x=60, y=66, z=68 with 0.375 Å grid space.. In present study, input ligand and protein preparation was carried out using MGLtools-1.5.6 [46] and final docking .performed in Autodock-4.2 software [45]and visualised in pymol software

Hirsutine molecule docking into the active site of the KIF11 protein was carried out to understand the affinity of the molecule. The HSN is docked with the active site of KIF11 crystal structure (PDB ID: 2UY) with 2.10 Å resolution and complex with tetrahydro-1-benzothiophene-3-carboxamide analogue [47]. The KIF11 protein has ATP/ADP binding site and another allosteric site, the inhibitor molecule observed in allosteric site which is taken for ,molecular docking. The allosteric site of KIF11 comprises the residue such asGlu116, Gly117 ,Glu118, Arg119, Trp127, Asp130, Leu132, Ala133, Leu160, Lue172, Val210,Tyr211 – Leu214, Glu215, Ala218, Arg221 and Phe239. The binding free energy of the hirsutine is kcal/mol. The hirsutine molecule stabilized in the active site by various non-bonding 9.32 interactions. The Arg221 and Glu116 residues are making cation-π and anion-π (π-interaction with the aromatic ring of hirsutine. The lone pair tertiary ammonium nitrogen(π

,may be involved in hydrogen bond interaction with Gly117 residue (Figure 9A). The Ala211 Ala218 and Leu214 are forming an alkyl interaction with the ligand molecule. The Glu116 is showing some unfavoured interactions with carbonyl oxygen of the inhibitor. The remaining residues in the active site are showing good van der Waal interactions with inhibitor molecules .(Figure 9B)

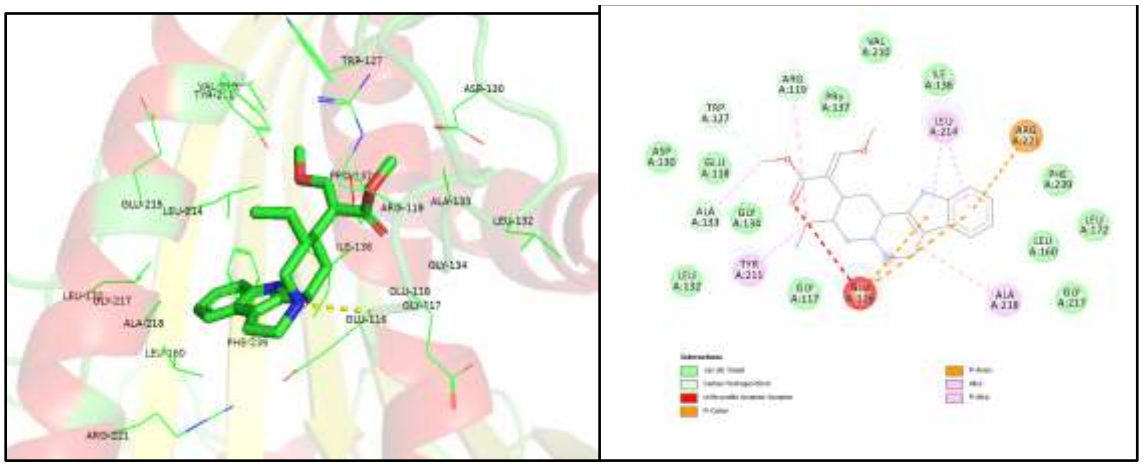


Figure 9: Molecular docking of hirsutine into the active site of the KIF11 protein A) 3D .representation of the docking and the amino acids are shown in lines and hirsutine in sticks B) 2D representation of hirsutine binding in KIF11 and non-bonding interaction are shown in in broken lines

The AURKA crystal (PDB ID: 1O7L) structure with 2.75 Å resolution and bound to an ADP in the active site and in activated form with phosphorylated at Threonine on ,activation loop [48]. The active site residues Leu139, Gly140, Gly142, Lys143, Ala160 ,Lys162,Leu194, Leu210, Glu211, Tyr212, Ala213, Gly216, Thr217, Glu260, Asn261 Leu263, Ala273and Asp274 are making an ATP binding site (Figure 10A) with binding free energy –7.95 kcal/mol. The ester carbonyl oxygen of hirsutine molecule is making a hydrogen bond with the sidechain NH of the Lys162 residue. The Leu139, Ala160, Val147, Ala273 and Ala213 are showing alkyl interaction with the inhibitor molecule. Remaining active site residues are forming van der Waals interactions with the inhibitor molecule and the .interactions are shown in 2D representation (Figure 10B)

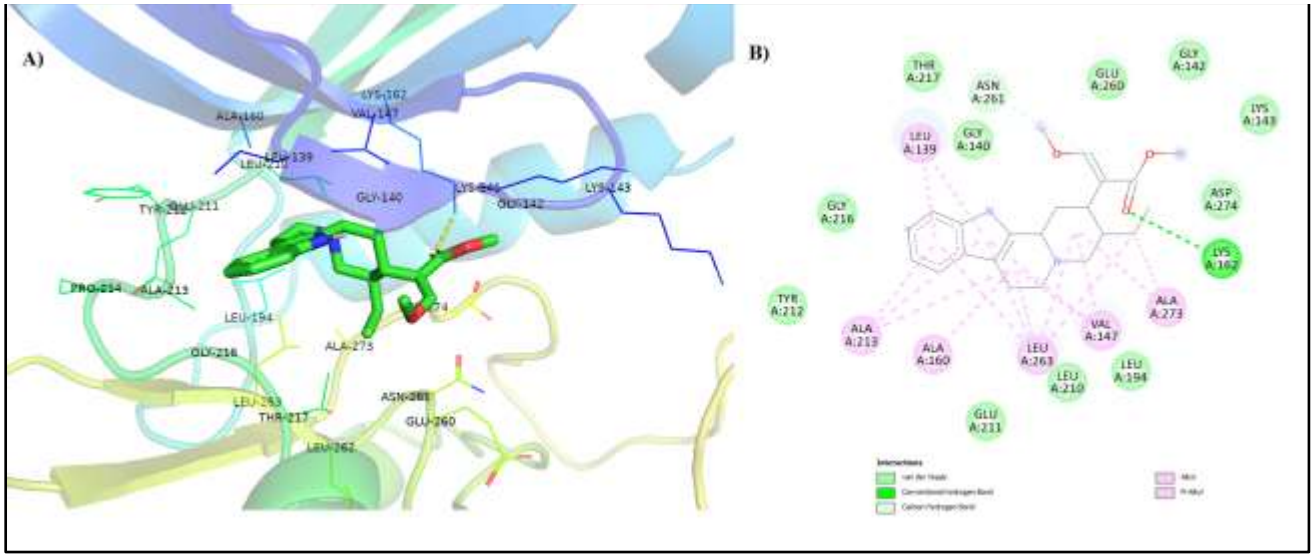


Figure 10: Molecular docking of hirsutine into the active site of the AURKA protein A) 3D .representation of the docking and the amino acids are shown in lines and hirsutine in sticks B)2D representation of hirsutine binding in AURKA and non-bonding interaction are shown in broken lines

The ADRA1A crystal(PDB ID: 7YMJ)structure with 2.20 Å resolution and ,bound to ADP molecule in the active site [49]. The active site comprises Ala103, Asp106 ,Val107,Cys110, Thr111, Ile114, Leu162, Ile178, Asn179, Ser188, Tyr184, Val185, Ser192 Trp285, Phe288, Phe289, Met292, Phe308, Tyr316 residues (Figure 11A) with binding free energy of –8.41 kcal/mol. The Tyr184 side chain OH is making hydrogen bond with the ester oxygen of the inhibitor molecules. The Phe289 and Trp285 residue aromatic rings are forming π-π interaction with inhibitor aromatic rings. The Val107, Ile178, Leu162, Met292 and Cys110 are making alkyl interactions with the hirsutine molecule. The remaining amino acids in the active site are making van der Waal interaction with the docked molecule and .stabilising in the ADRA1A binding site (Figure 11B)

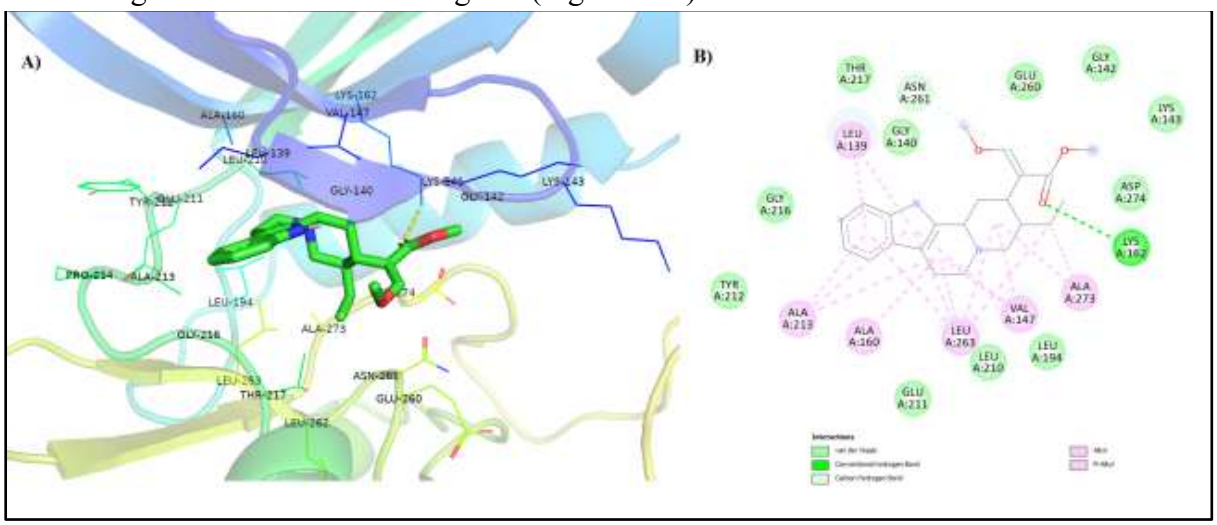
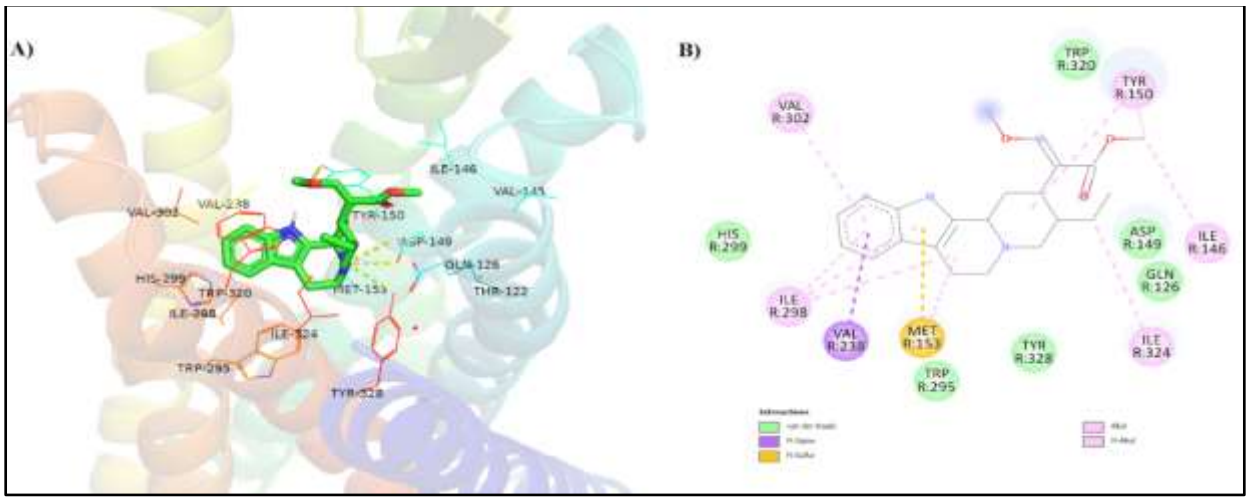


Figure 2: Molecular docking of hirsutine into the active site of the ADRA1A protein A) 3D

.representation of the docking and the amino acids are shown in lines and hirsutine in sticks B)2D representation of hirsutine binding in ADRA1A and non-bonding interaction are shown .in broken lines

OPRM1 Crystal Structure (EM structure) PDB ID: 8EFO was selected for the molecular docking. The OPRM1 crystal structure with 2.80Å resolution and bound to N-[(2S)-(dimethylamino)-3-(4-hydroxyphenyl)propyl]-N'-[(2S)-1-(thiophen-3-yl)propan-2--2 ,yl]urea in the active site [50]. The active site contains residues Thr122,Gln126, Asn129 ,Trp135, Val145, Ile146, Asp149, Tyr150, Met153, Cys219, Lys235, Val238,Trp295, Ile298 His299, Val302, Trp320, Ile324, Tyr328 (Figure 12A). The hirsutine is docked into the ligand binding site of OPRM1 protein and the binding free energy of the hirsutine binding is observed kcal/mol. The ester carbonyl oxygen of hirsutine molecule is making a hydrogen bond 8.5– with the sidechain OH of the Tyr150 residue. The tertiary nitrogen of the molecule may form hydrogen bonds with Tyr328 and Asp149 residues. The Val302, Ile298,Ile324, Ile146 and Tyr150 are showing alkyl interaction with the inhibitor molecule. The Met153 is making a π-Sulfur interaction with the ring of the hirsutine molecule. Remaining active site residues are forming van der Waals interactions with the inhibitor molecule and the non-bonding .interactions are shown in 2D representation (Figure 12B)



So the docking binding affinity of KIF11 is comparatively high than ADRA1, OPRM1and AURKA binding affinity is the least in comparison with the four selected targets.KIF11 is the druggable target with high binding affinity for the hirsutine an indole alkaloid from the natural source of phytochemical. KIF11, a hub gene selected based on literature study and target database is also called EG5or Kinesin-5 and involved in cell mitosis ,Tumour microenvironment,cell differentiation and cell cycle. The inhibition of the KIF11by hirsutine ,In cervical cancer paves a path for a new target for the treatment of Cervical Cancer. AURKA OPRM1, ADRA1A are the selected targets from PPI network are highly relevant in cellular process, centrosome maturation , mitotic spindle formation, metastasis,apoptosis and pain.Network pharmacology aids in drug development and designing but the information

provided in databases on drug and their targets has to more elucidated to understand more .interactions of targets and verified clinically for further studies

:Conclusion

This study on network pharmacology and docking analysis analyse the mechanism of action of Hirsutine for the treatment of cervical cancer.Hirsutine targets many proteins and pathway related protein like AKT, MAPK, CASP3,CASP7,AURKA,STAT3, OPRM1,ADRA hub genes which have role in in cervical cancer proliferation, induces apoptosis ,migration .The targeted genes KIF11, have role in cell proliferation has good binding affinity for Hirsutine. Network pharmacology is a promising way of approach for the treatment of cervical cancer by using a multi target inhibition strategy. This methodology has significant advantages in understanding the mechanism and screening of hub genes with the minimum time and financial input for the pre clinical investigations for the compound in silico analysis . Further this data has to be validator by conducting in vitro and in Vivo clinical studies to to prove the mechanism of action of Hirsutine against cervical cancer

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