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MOLECULAR DOCKING STUDY TO DISCOVER POTENT DRUG CANDIDATES AMONG COMPOUNDS OF *ZINGIBER OFFICINALE* (*SUNTHI*) FOR MANAGEMENT OF BRONCHIAL ASTHMA- AN INSILICO APPROACH

Ayurveda

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ABSTRACT

Tamaka Swas (Bronchial Asthma) is a well documented disease defined as a chronic inflammatory disease of airways characterized by an increase responsiveness of the tracheo-bronchial tree to a multiple of stimuli clinically manifested by paroxysmal dyspnea, cough and sneezing and chest tightness. Ayurvedic treatment, although is highly effective, proper mode of action, pharmacology, pharmacokinetics, and pharmacovigilance of many important Ayurvedic drugs are still not fully explored. In this study,in silico methods of drug discovery process has been adopted with emphasis on discovery of potent drug candidates among the compounds of. sunthi (*Zingiber officinale*) against Bronchial Asthma. In the molecular docking studies of the screened two ligands out of the 102 phytochemicals of *Zingiber officinale* against the target proteins of Brochial Asthma viz., Il-3, IL-4,IL-5,IL-13,,Eotaxin and TNF alpha, [4]- Shogaol shows a minimum binding affinity of -5.1 kcal/mol with IL-13 whereas Alpha-phellandrene binds with IL-13 at a minimum binding affinity of 5.0 kcal/mol indicating that both the ligands are potent drug candidates against Asthma with [4]- Shogaol being slightly better drug candidate.

KEYWORDS

Bronchial Asthma, Sunthi, In Silico, IL-13, 4-Shogaol

INTRODUCTION

Since ancient times, human race is constantly threatened by disease of respiratory disorders and Bronchial Asthma is one of them[1]. Ayurveda referred to Bronchial Asthma as "*Tamaka Swas*" [2] which is a well-documented disease comparable on the parameters of etio-pathology, risk factors, clinical manifestations and treatment principles. Bronchial Asthma is defined as a chronic inflammatory disease of airways that is characterized by increased responsiveness of the trachea-bronchial tree to a multiplicity of stimuli[3]. It is manifested physiologically by a widespread narrowing of the air passages, which may be relieved spontaneously or as a result of therapy, and clinically by paroxysms of dyspnea, cough, and wheezing, chest tightness, particularly at night or in the morning[4]. According to Global Asthma report 2018, around 1000 people globally die every day owing to Asthma and the disease affects around 339 million individuals every year[5].

Zingiber officinale is a common spice, which is in use for the treatment of various gastrointestinal, pulmonary, cardiovascular and sexual disorders since antiquity in Unani and Ayurvedic medicines[6]. It has anti-inflammatory effect and can reduce coughing and chest pain in patients with tracheitis, and also reduce the adverse gastro-intestinal symptoms induced by bronchodilator and synthetic anti-inflammatory drugs.[7]

Ayurvedic treatment, although is highly effective, proper mode of action, pharmacology, pharmacokinetics, and pharmacovigilance of many important Ayurvedic drugs are still not fully explored. In this study, in silico methods of drug discovery process had been adopted with emphasis on identifying potential drug targets of Asthma and identifying the phytochemicals of ***Sunthi*** (*Zingiber officinale*) along with virtual screening and molecular docking studies for discovery of potent drug candidates among the compounds of *Zingiber officinale* for Bronchial Asthma

AIM OF THE STUDY:

Molecular Docking of compounds of Sunthi with the targeted proteins of Bronchial Asthma.

MATERIALS AND METHODS:

MATERIALS:

The databases used for this study include the KNApSAcK[8] family Database for searching the metabolites based on an accurate mass, molecular formula, metabolite name or mass spectra in several ionisation modes, the KEGG[9] for pathway analysis and identifying drug targets, RCSB[10] (Research Collaboratory of Structural Bioinformatics) for retrieving pdb. format of identified drugs, PUBCHEM[11] for information on chemical substances and their biological activities The softwares used in this study include OPEN BABEL[12] to search, convert, analyze, or store data from molecular modeling, chemistry, solid-state materials, biochemistry, or related areas, DRULITO where drug-likeness descriptors such as Molecular Weight (MW), logP, AlogP, H-Bond Acceptor (HBA), H-Bond Donor (HBD), Total Polar Surface Area (TPSA), Atom Molar Refractivity (AMR), number of Rotable Bond (nRB), number of Atom, number of Acidic group, Rotatable bond Count (RC), number of Rigid Bond (nRigidB), nAtomRing, and nHB for all of the ligands were calculated, AdmetSAR[13] tool for assessment of chemical ADME properties i.e. Absorption, distribution, metabolism, excretion (ADME) properties, PROTOX-II[14] for the prediction of toxicities of small molecules such oral toxicity, organ toxicity (hepatotoxicity), toxicological endpoints (such as mutagenicity, carcinotoxicity, cytotoxicity and immunotoxicity), toxicological pathways (AOPs) and toxicity targets thereby providing insights into the possible molecular mechanism behind such toxic response,AUTODOCK 4-: *Autodock and Autogrid. Autodock* performs the docking of the ligand to a set of grids describing the target protein whereas *Autogrid* pre-calculates these grids, and the PYMOL used for three-dimensional (3D) visualization of proteins, nucleic acids, small molecules, electron densities, surfaces, and trajectories.

METHODS:

Pathway analysis and protein preparation

A thorough study of the Asthma pathway from KEGG pathway database to understand the molecular basis of occurrence of Asthma and the target proteins of Asthma are identified. The 3D protein Structure has been retrieved from RSCB server by using the PDBID (derieved from PDTD database), the structural file has been downloaded in the form of .pdb file (text) format.the Proteins Interleukin-3 (PDB_ID **1jli),** Interleukin-4 (PDB_ID **1bbn),** Interleukin-5 (PDB_ID **3va2),** Interleukin-13 (PDB_ID **3bpo),** Eotaxin (PDB_ID **1eot**), and Tumor Necrosis Factor alpha/ *TNF*- α (PDB_ID 1a8m) had been downloaded in PDB files.

Ligand retrieval and preparation: Using the KNApSAcK Family Database 3D structures of 102 Compounds of *Zingiber officinale* have been retrieved in the form of .mol format.The .mol format of the compounds were viewed in Drulito individually for the calculation of the molecular properties like Molecular weight, LogP, Hydrogen bonds, TPSA,etc.. and drug score (WQed). The 102 phytochemicals of ***Zingiber officinale*** were passed through various drug-like filters to analyse the druggablity of the compounds . The ADME properties of the compounds were assessed using the admetSAR server were the Blood brain barrier, Human Intestinal Absorption, CaCo permiabilty

values of each compound were analysed and toxicity of the Ligands were calculated using the Potox-II server where the hepatoxicity, cytotoxicity, carcinogenicity, immunotoxicity and mutagenicity, LD50 value, toxicity classes were analysed .Out of the 102 ligands, virtual screening was done and the best two filtered compounds have been selected for molecular docking studies. The rest of the compounds have been screened out based on their toxicities and partial drug-likeness.

Molecular docking using Autodock :

Autodock superimpose the three dimensional structure of a potential drug on its possible target site to predict the interaction among them. The cleaned PDB file of the six target proteins were browsed in Autodock and Hydrogen atoms along with Charge were added to the PDB files and saved as PDBQT format.Grid parameters were set up and measured by Autogrid and the config conf.file was created with ligand, protein and exhaustiveness parameters. Bonds that are potentially rotatable but treated as rigid, such as amide bonds and bonds that are made rigid by the user are shown in magenta. Rotation of rotatable bonds may be switched on and off by clicking on bonds based on the user choice and then the file was saved as PDBQT format. Proteins (IL-3, IL-4, IL-5, IL-9, IL-10, IL-13 and TNFα) were optimized for protein ligand interation studies by deleting all hetero atoms, ligands and water molecules and optimized by minimization of energy by using Auto Dock Vina.Ligands were optimized by using AutoDock Vina[14] and later saved in .pdbqt format. The two selected compounds viz. **Alpha-phellandrene** and **[4]-Shogaol** were docked with the proteins using a genetic algorithm and simulated annealing approach to explore wide range of ligand conformational flexibility and rotational flexibility of Auto Dock. The best protein ligand complex was analyzed based on minimum binding affinity. The docked complexes were visualized in Pymol showing how the ligands interact with the IL-3[16], IL-4[17], IL-5[18], IL-13, Eotaxin[19] and TNFα[20].

RESULTS:

In this study, virtual screening of the 102 phytochemicals of Zingiber officinale was performed for their molecular properties, bioactivity properties, drug score, drug likeliness as well as for their possible side effect like tumerogenecity, mutagenicity, irritation and reproductive effect and effect. Then we extended the study by going through an in silico study[21] on the possible molecular interaction of the best druglike compounds and proteins responsible for Asthma and all the proteins are then docked individually with the selected ligands in the molecular docking software- AUTODOCK Vina showing the following results:

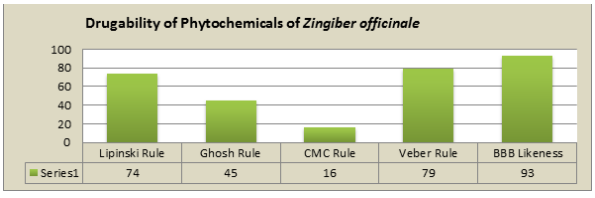


Fig. 1. Graphical representation of Drugability of the phytoche micals of *Zingiber officinale*

Out of the 102 phytochemicals, 11 phytochemicals were found to satisfy all the filters and these are [6]-Gingerdione, (S)-6-Gingerol, [6]-Paradol,[6]- Shogaol, [4]-Shogaol, [6]-Gingerdiol, 1-Dehydro-[6]-gingerdione, Methyl [6]-Shogaol,Dehydrogingerdion, [6]-Dehydroshogaol, 6-Dehydrogingerdione.

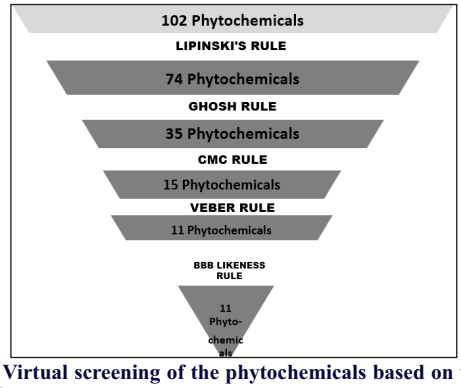
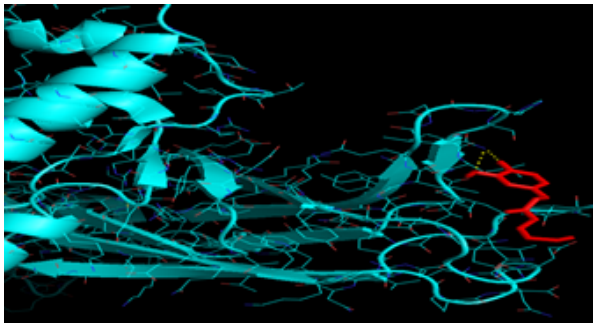


Fig. 2. Virtual screening of the phytochemicals based on various drug filters

On assessment of the ADME properties of the phytochemicals using admeSAR , all the 102 phytochemicals were found to possess good degrees of Blood Brain Barrier, Human Intestinal Absorption and Cell Permeability and all are Non-inhibitors except for Gingerenone A. After analysis of toxicity, a compound named 'p-Cymene' was found to belong to class I toxicity class with LD_{50} value 3mg/kg indicating that it's highly toxic and fatal if swallowed. 56 out of the 102 phytochemicals were found to be Non-toxic and out of the 11 best virtually screened phytochemicals only [4]-Shogaol was found to be non-toxic based on the above toxicities whereas the rest 10 phytochemicals were screened out. Hence, based on the above observations, we have selected the two best compounds viz. **Alpha-phellandrene** (passed Lipinski's rule of 5, Veber rule and BBB likness and Non-toxic with toxicity class VI) and **[4]-Shogaol** (passed all the filters and Non-toxic) for Molecular Docking Studies in the docking software- Autodock Vina. And the results were evaluated based on their number of binding sites and binding affinity of the docked complex. From the docking results it was found that Alpha-phellandrene shows the most minimum binding affinity with IL-13 at a binding energy of -5.0 Kcal/mol whereas [4]-Shogaol shows the most minimum binding affinity with IL-13 with a binding energy of -5.1 kcal/mol.

Table.1.Maximum Binding Affinity(kcal/mol) Of Target Proteins And Ligands In The Docked Complex

SL. NO	LIGANDS	IL-3	IL-4	IL-5	IL-13	EOTAXIN	TNF-α
1	*ALPHA-PHELLANDRENE*	-4.7	-3.6	-4.8	-5.0	-4.4	-4.6
2	*[4]-SHOGAOL*	-4.7	-4.6	-4.7	-5.1	-4.3	-4.4



Molecular Interaction of Alpha-phellandrene with Interleukin-5. Ligand interacts with 3 bonds with THR 133,TRP179,GLU283 in pose 1 with binding affinity -4.8 kcal/mol

Fig.3. Docking results of Alpha phellandrene with Interleukin-13

MODE	BINDING AFFINITY KCal/mol	DISTANCE FROM BEST MODE	
		RMSDL.B	RMSDU.B
1	-5.0	0.000	0.000
2	-4.8	32.455	35.114
3	-4.4	37.067	39.698
4	-4.4	23.528	26.378
5	-4.3	22.454	24.934
6	-4.2	49.470	51.790
7	-4.1	30.369	32.800
8	-4.0	33.959	36.528
9	-4.0	31.971	34.576

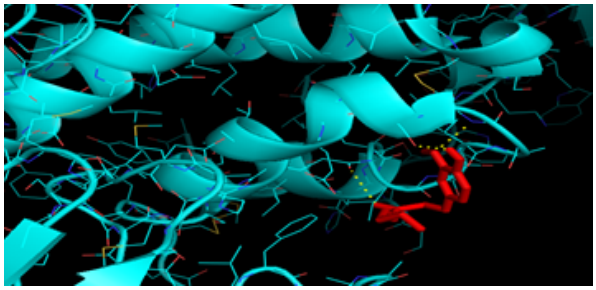
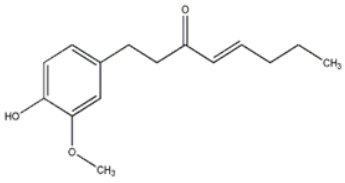
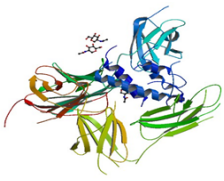


Fig.5. Docking results of [4]- Shogaol with Interleukin-13

Molecular Interaction of [4]- Shogaol with Interleukin-13 Ligand interacts with 3bonds - SER58, CYS57, GLN64 in pose9 with binding affinity -4.4 kcal/mol

MOD	BINDING AFFINITY KCal/mol	DISTANCE FROM BEST MODE	
		RMSDL.B	RMSDU.B
1	-5.1	0.000	0.000
2	-5.0	20.113	22.224
3	-4.9	37.189	40.567
4	-4.6	2.091	3.698
5	-4.5	3.414	4.103
6	-4.5	13.940	16.837
7	-4.5	19.031	21.195
8	-4.4	3.589	5.627
9	-4.4	19.354	22.337





Metabolite: [4]- Shogaol
C_ID : C00035446
Mol. Formula: C15H20O3
Mol. Wt: 248.1412445

Name: IL-13 (PDBID- 3BPO)
Classification: Cytokine/ Cytokine Receptor

Fig.6. [4]- Shogaol and IL-13

DISCUSSION

The Ayurvedic literature has mentioned that *Sunthi* can be used in Bronchial Asthma or *Tamaka Swas* yet the Scientific-based mechanism behind it is not yet known. So a detailed study was made to explore the concept and to find out the pharmacological basis of the functions of the compounds *Sunthi* at the molecular level. In in-silico study, an attempt has been made on virtual screening and molecular docking studies for discovery of potent drug candidates among compounds of *Sunthi* against the potential drug target of Bronchial Asthma.. On virtual screening the best 11 filtered compounds only **[4]-Shogaol** was found to be completely non-toxic and **Alpha phellandrene** which satisfies 3 of the filters was found to belong to class VI toxicity class and non-toxic and hence these two compounds had been selected for the molecular docking analysis[22,10]. Molecular docking studies of the two screened ligand were performed using Autodock and based on binding energy and hydrogen bond formation, the results were analysed using Autodock tools whereas visualization of the docked complex with the target proteins was observed using Pymol. Based on this observation**, [4]- SHOGAOL** was found to bind with IL-13 at the most minimum binding energy of -5.1 Kcal/mol whereas ALPHA-PHELLANDRENE binds with IL-13 at a binding energy of -5.0 Kcal/mol. Hence it can be concluded that both the ligands are potent drug candidates against Asthma with [4]-SHOGAOL as a slightly better potent drug candidate based on its minimum binding affinity..

CONCLUSION

The Ayurvedic literature has mentioned that *Sunthi* can be used in Bronchial Asthma or *Tamaka Swas* yet the Scientific-based mechanism behind it is not yet known. Thus the in-silico study adopted in the present study helped in identifying the ligands which can be considered as potent drugs for the treatment of Bronchial Asthma for further study 'in-vitro' and 'in-vivo' reducing the time and cost in designing a drug in laboratory and subsequently before it enters the clinical trial

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